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Cell response to genotoxic stress-based anti-cancer therapies

Buněčná odpověď na protinádorové terapie založené na genotoxickém stresu

Doctoral Thesis

Supervisor: Zdeněk Hodný, MD, PhD

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Čestné prohlášení

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Foreword

I completed my doctoral thesis at the Laboratory of Genome Integrity at the Institute of Molecular Genetics of the Czech Academy of Sciences. The main focus of this laboratory is genotoxic stress, while the topic is studied very broadly, from many points of view.

Historically, the main issue has been cellular senescence, especially the connection between senescence and DNA damage and the phenomenon of the bystander effect.

One of the proteins tightly connected to senescence, both as an effector and a marker, is promyelocytic leukemia protein (PML); hence the function of PML in senescent cells was carefully examined in the laboratory. Interestingly, it was noticed that in senescent cells, PML formed distinct structures around the nucleolus, an effect that was recapitulated in non-senescent cells after treatment with several chemotherapeutic drugs. These intriguing interactions remained the focus of further research of this laboratory.

Senescent cells originate, besides others, during radio- and chemo-therapy, as a population of cells resistant to apoptosis. Therefore, another large topic studied at the Laboratory of Genome Integrity is genotoxic stress-induced radio-resistance and chemo-resistance of cancer cells, with a special interest in the population of low-adherent anoikis-resistant cells that were first described and characterized there.

I started my PhD with a project related to these anoikis-resistant cells. I spent my first year by their characterization and the data I obtained were used in a publication on this topic. One part of the publication was also a genome profiling screen; therefore, I continued in the project by trying to elucidate the role of one specific signaling pathway that emerged from the screen. However, the data did not seem promising, so after thorough consideration, we decided to abandon this project and to switch to a different one.

My current topic comprises the PML protein and its interaction with the nucleolus, a phenomenon that is both extremely interesting and extremely complicated. My doctoral thesis is to a large extent based on the results that I got working on this project.

Alongside with this main project, I participated in two other studies, one dealing with the effect of senescent cells on tumor growth and the other one trying to find a new marker of senescent cells. Both of them resulted in publications where I was a co-author.

Changing topic so often and having the interest distributed over so many different areas has taught me a lot. I learned how not to give up, how to abandon old, non-functioning schemes

and try to find better ones, and how to immerse into new topics again and again. Last but not least, I learned that in spite of everything I still love science and the process of revealing the truth, no matter if the result meets my wishes and expectations or not.

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The list of abbreviations

AKT..................AKT serine/threonine kinase 1

ALT..................alternative lengthening of telomeres

AMD................actinomycin D

AMPK..............5' AMP-activated protein kinase

ANT2................adenine nucleotide translocase 2

AP1..................activator protein 1

APBs................ALT-associated PML bodies

APC/C..............anaphase-promoting complex/cyclosome

ARF..................alternative reading frame protein

ATM ....ataxia teleangiectasia mutated

ATR..................ataxia telangiectasia and Rad3-related kinase

B23..................nucleophosmin

Bim..................Bcl-2-interacting mediator of cell death

BLM.................Bloom helicase

Bop1................block of proliferation 1

BRCA1/2...........breast cancer susceptibility protein 1/2

BrdU................5′-bromo-2′-deoxyuridine

C23..................nucleolin

CaP..................prostate carcinoma

CBP..................cyclic adenosine monophosphate (cAMP)-responsive element-binding protein

CD95 ....cluster of differentiation 95

CDC14B...........cell division cycle 14B

Cdk..................cyclin-dependent kinase

cGAS/STING .....cyclic GMP-AMP synthase linked to stimulator of interferon genes

CHK1, 2 ....checkpoint kinase 1, 2

CK2..................casein kinase 2

c-Mos..............Moloney murine sarcoma viral oncogene homolog

c-Myc..............avian myelocytomatosis viral oncogene homolog

COX2................cyclooxygenase 2

CSCs ....cancer stem cells

CXCLs ....chemokine (C-X-C motif) ligands

CXCR-2 ....C-X-C chemokine receptor type 2

DAB2IP.............disabled homolog 2-interacting protein

Daxx ....death domain associated protein

DFC..................dense fibrillary component

DHX9................DExH-box helicase 9, nuclear DNA helicase II (NDHII)

DNA.................deoxyribonucleic acid

DNA-PKcs.........DNA-dependent protein kinase, catalytic subunit

DRB ....5,6-dichlorobenzimidazole riboside

DSBs................double-strand breaks

E2F1 ....E2F transcription factor 1

EdU ....5’-ethynyl-2´-deoxyuridine

EMT.................epithelial-mesenchymal transition

ERC..................extrachromosomal rDNA circle

Erk...................extracellular signal-regulated kinase

FC....................fibrillar center

FUS-CHOP ....fused in sarcoma-C/EBP homologous protein

GC ....granular component

G-CSF ....granulocyte colony-stimulating factor

GFs..................growth factors

GM-CSF............granulocyte-monocyte colony-stimulating factor

GSK3β ....glycogen synthase kinase 3 beta

γH2AX ....gammaH2AX (serine 139-phosphorylated histone H2AX)

HCC-4...............human CC chemokine 4

HDM2...............human homolog of mouse double minute 2

HEATR1............HEAT repeat containing 1

HIF1.................hypoxia inducible factor 1

HIPK2 ....homeodomain interacting protein kinase 2

HIV ....human immunodeficiency virus

HR ....homologous recombination

H-Ras................Harvey rat sarcoma viral oncogene homolog

IFN-γ................interferon gamma

IGF....................insulin-like growth factor

IGS....................intergenic spacer

IL-1..................interleukin-1

IL-12................interleukin 12

IL-6..................interleukin-6

IL-8..................interleukin-8

IR......................ionizing radiation

IRBC................impaired ribosome biogenesis checkpoint

JAK/STAT..........Janus kinase/signal transducer and activator of transcription

K-RAS...............Kirsten rat sarcoma viral oncogene homolog

L1CAM.............L1 cell adhesion molecule

MAPK...............mitogen-activated protein kinase

MCPs................monocyte chemoattractant proteins

MDC1...............mediator of DNA-damage checkpoint protein 1

MDM4.............mouse double minute 4

MIPs................macrophage inflammatory proteins

MMPs.............matrix metalloproteinases

MRE11.............meiotic recombination 11 homolog 1

mRNA...............messenger RNA

mTOR...............mechanistic target of rapamycin

MYBBP1A.........MYB binding protein 1A

NBs..................nuclear bodies

NBS1................Nijmegen breakage syndrome 1

NEDD8.............neural precursor cell expressed, developmentally down-regulated 8

NF-κB...............nuclear factor kappa-light-chain-enhancer of activated B cells

NHEJ................non-homologous end joining

NoLS................nucleolar localization sequence

Nop52.............nucleolar protein 52

NORs...............nucleolar-organizing regions

NRF2................nuclear factor erythroid 2-related factor

OCT4................octamer-binding protein 4

OIS..................oncogene-induced senescence

p16, $p16^{INK4a}$ ..... cyclin-dependent kinase 4 inhibitor A

p21, $p21^{waf1}$ ...... cyclin dependent kinase inhibitor 1A

p38 .... p38 mitogen-activated protein kinase

p53 .... tumor protein p53

PARP1 .... Poly (ADP-ribose) polymerase 1

PDGF .... platelet-derived growth factor

PGE2 .... prostaglandin E2

PI3K phosphatidylinositol-4,5-bisphosphate 3-kinase

PICT1 .... protein interacting with carboxyl terminus 1

Plk1 .... polo-like kinase 1

PML .... promyelocytic leukemia protein

PNAs .... PML nucleolar associations

PML-NDS .... PML nucleolus-derived structures

Pol I .... RNA polymerase 1

Pol III .... RNA polymerase 3

PP1A, 2A .... protein phosphatase 1A, 2A

pRb .... retinoblastoma protein

PTEN .... phosphatase and tensin homolog

RAF .... RAF proto-oncogene serine/threonine-protein kinase

RanBP .... Ran-binding protein

RARα .... retinoid acid receptor alpha

RAS .... rat sarcoma viral oncogene homolog

rDNA .... ribosomal DNA

RFB .... replication fork barrier

RNA .... ribonucleic acid

RNP .... ribonucleoprotein particle

ROS .... reactive oxygen species

RPA .... replication protein A

RPL .... ribosomal protein – component of a large ribosomal subunit

RPS .... ribosomal protein – component of a small ribosomal subunit

RT-qPCR .... quantitative reverse transcription polymerase chain reaction

SAMD .... senescence-associated mitochondrial dysfunction

SASP................senescence-associated secretory phenotype

Sgs1.................slow growth suppressor 1

SIPS ....stress-induced premature senescence

Sir2..................silent information regulator 2

SIRT1, SIRT7 .....sirtuin 1, sirtuin 7

SL1 ....selectivity factor 1

SMARCA5.........switch/sucrose non-fermentable (SWI/SNF)-related matrix-associated actin-dependent regulator of chromatin, subfamily A, member 5

snoRNA ....small nucleolar RNA

SOX2 ....sex determining region Y-box 2

Sp100..............speckled 100 kDa

STK11..............serine/threonine kinase 11

SUMO ....small ubiquitin-like modifier

TERT................telomerase reverse transcriptase

TFIIIA...............transcription factor IIIA

TFIIIB...............transcription factor IIIB

TGF-β ....transforming growth factor beta

TIF-1A..............transcription initiation factor 1A

TIP60...............60 KDa Tat-interactive protein

TNF-α ....tumor necrosis factor alpha

TOP ....5’-terminal oligopyrimidine

TopBP1............DNA topoisomerase II binding protein 1

TRADD.............tumor necrosis factor receptor 1 (TNFR1)–associated death domain

TTF-I................transcription termination factor 1

t-UTP...............transcriptional U three protein

UBC9...............ubiquitin carrier protein 9

UBF ....upstream binding factor

VEGF ....vascular endothelial growth factor

vHL..................von Hippel-Lindau tumor suppressor

Wnt.................wingless-type MMTV integration site

WRN................Werner syndrome helicase

ZEB2................zinc finger E-box binding homeobox 2

I ABSTRACT

The dissertation deals with a cell response to genotoxic stress, specifically to anti-cancer treatments with a genotoxic mechanism of action. In principle, cells can respond to these perturbing stimuli in several ways: in case of severe DNA damage, they usually undergo apoptosis or enter senescence. In case of minor DNA damage, or upon defective checkpoint mechanisms, they may continue the cell cycle, either with successfully repaired DNA or with mutations of various kind. Thanks to selection pressure, the mutations that provide cells with a certain growth advantage under conditions of continuing genotoxic stress, gradually accumulate and render the tumor treatment-resistant. In my thesis, I focus on several aspects of this whole process.

First, I participated in a characterization of a radioresistant and anoikis-resistant population of prostate cancer cells. This population was generated by irradiating cells 35 times by 2 Gy, a regime used in clinics. After this treatment, a population of low-adherent cells emerged that demonstrated increased expression of EMT- and stem cell markers. The low-adherent state of these cells was maintained by Snail signaling and their anoikis resistance by ERK1/2 signaling. Interestingly, after a protracted period of time, these cells were able to re-adhere and restart proliferation, while retaining their tumorigenic potential, as demonstrated by their injection into nude mice. Finally, the survival of these cells was compromised by combined AKT and ERK1/2 inhibition.

Second, I took part in a study where the effects of chemotherapy-induced senescent cells on tumor growth was examined. It was found that docetaxel- or radiation-induced senescent cells accelerate tumor growth, when co-injected with normal proliferating cells into mice with non-compromised immune system. Furthermore, this accelerating effect, as well as the growth of the tumor itself, was reverted by IL-12, a cytokine with known immunostimulatory properties.

Third, I cooperated on a project the aim of which was to find a specific marker of senescent cells. The surface protein L1CAM was identified as a promising candidate, as its mRNA and protein levels were increased in a majority of examined cell lines brought into senescence by various stimuli (serial passaging, γ-radiation, BrdU, IFNγ, TGF-β). Furthermore, the expression of the protein was closely connected to the metabolism, as its expression changed upon cell cultivation in a high-glucose medium, after inhibition of the mevalonate pathway or after downregulation of mitochondrial ATP/ADP translocator ANT2. The role of the protein in cell migration and adhesion was also confirmed. Finally, the reciprocal negative regulation between

L1CAM and the ERK1/2 signaling pathways was described, which explained our previous observation that L1CAM levels were not increased upon H-RAS-induced senescence.

In my own project, I attempted to better understand the genotoxic stress-induced association of the PML protein with the nucleolus. PML has been described as a tumor suppressor and a senescence inducer; and nuclear bodies formed by this protein are specifically present in senescent cells. We found out that a combination of RNA polymerase I (RNAPI) inhibition and topological stress leads to the translocation of PML to the nucleolus and we identified the domains of PML that are important for this interaction, while one of them is also necessary for PML association with SUMOylated proteins. Using super-resolution and time-lapse microscopy, we described how the PML nucleolar associations (PNAs) form, how they evolve and what is their 3D structure. Furthermore, we showed that PNAs contain rDNA, they co-localize with SUMO signal and, in their last stage, they accumulate proteins that are involved in rDNA metabolism. Finally, we described the association of PNAs with γH2AX, a marker of damaged DNA. Therefore, we propose a model when combination of RNAPI inhibition and topological stress leads to a specific type of rDNA damage that is followed by attraction of SUMOylated proteins and formation of PNAs. PNAs then participate on a sequestration and processing of damaged rDNA loci. When unresolved, these damaged loci persist in cells and probably contribute to the onset of senescence.

I ABSTRAKT

Tato práce se zabývá buněčnou odpovědí na genotoxický stres; konkrétně odpovědí na protinádorovou léčbu s genotoxickým mechanismem účinku. Buněčná odpověď může mít některý z následujících průběhů: v případě závažného poškození DNA buňky většinou podstoupí apoptózu nebo vstoupí do senescence. Pokud je poškození méně závažné nebo pokud buňkám nefungují kontrolní mechanismy, mohou tyto pokračovat v buněčném cyklu, ať už s úspěšně opravenou nebo s mutovanou DNA. Díky selekčnímu tlaku se v buňkách hromadí ty mutace, které jim poskytují určitou růstovou výhodu v podmínkách trvajícího genotoxického stresu a buňky se postupně stávají rezistentními. Ve své dizertační práci jsem se zaměřila na několik aspektů celého výše popsaného procesu.

V prvním projektu, kterého jsem se zúčastnila, bylo mým úkolem charakterizovat populaci radiorezistentních a anoikis-rezistentních buněk karcinomu prostaty. Tato populace byla získána po ozáření buněk 35 dávkami 2 Gy, což je režim běžně používaný v klinické praxi. Po takovémto ozařovacím procesu se objevila populace buněk s nízkou adhezí, která vykazovala zvýšenou expresi mezenchymálních a kmenových markerů. Nízká adhezivita těchto buněk byla vyvolána signální dráhou proteinu Snail a jejich resistence vůči anoikis byla umožněna díky signalizaci ERK1/2. Zjistili jsme, že po určité době byly tyto nízce adherentní buňky schopny přisednout na podklad a začít se znovu dělit. Kromě toho si uchovaly schopnost tvořit nádory, což bylo prokázáno jejich injekcí do myší s potlačenou imunitou. Přežití těchto buněk bylo zabráněno kombinovanou inhibicí signálních drah AKT a ERK1/2.

Dále jsem se podílela na studii, která zkoumala vliv buněk, přivedených do senescence pomocí chemoterapie, na růst nádorů. Zjistili jsme, že pokud k nesenescentním nádorovým buňkám přidáme buňky, které byly přivedeny do senescence pomocí docetaxelu nebo vysokými dávkami gama záření, urychlí se růst nádoru v myších s normálně fungujícím imunitním systémem. Jak samotný růst nádoru, tak zjištěný urychlující efekt nádorových buněk, byly navíc potlačeny immunoterapií – interleukinem 12.

Cílem třetího projektu, na kterém jsem spolupracovala, bylo najít specifický marker senescentních buněk. Povrchový protein L1CAM byl vybrán jako vhodný kandidát, protože hladina jeho mRNA i proteinu byla zvýšená u většiny zkoumaných buněčných linií, přivedených do senescence různými způsoby (násobné pasážování, γ-záření, bromodeoxyuridin, interferon γ, transformující růstový factor β). Exprese proteinu L1CAM byla navíc úzce propojena s metabolismem, což bylo patrné díky tomu, že se jeho exprese měnila při kultivaci buněk v médiu

s vysokým obsahem glukózy, při inhibici mevalonátové dráhy nebo při snížení hladiny mitochondriální ATP/ADP translokázy ANT2. Kromě toho byla potvrzena úloha L1CAM v buněčné migraci a adhezivitě. Na závěr byla také popsána reciproká negativní regulace mezi proteinem L1CAM a signální dráhou ERK1/2, což mimo jiné vysvětlilo naše dřívější pozorování, že hladina protein L1CAM není zvýšena u senescence indukované signalizací H-RAS/MAPK.

V rámci svého vlastního projektu jsem se snažila lépe pochopit asociaci mezi proteinem PML a jadérkem, indukovanou genotoxickým stresem. Protein PML byl popsán jako nádorový supresor, který se podílí na indukci senescence, a přítomnost zvýšeného počtu jaderných tělísek tvořených tímto proteinem je zároveň jedním ze znaků senescence. Zjistili jsme, že inhibice RNA polymerázy I (RNAPI) spolu s topologickým stresem vedou k translokaci proteinu PML na jadérkový povrch. Dále jsme identifikovali domény proteinu PML, které jsou pro tuto interakci důležité, přičemž jedna z nich je zároveň nezbytná pro interakci PML se SUMOylovanými proteiny. S využitím super-rezoluční a časosběrné mikroskopie jsme popsali, jak se jadérkové asociace PML (PNAs) tvoří, jak se vyvíjejí a jaká je jejich 3D struktura. Kromě toho jsme ukázali, že PNAs obsahují ribozomální DNA (rDNA), kolokalizují se SUMO a ve své poslední fázi také akumulují proteiny, které se účastní metabolismu rDNA. Nakonec jsme popsali asociaci PNAs s histonem γH2AX, který značí poškozenou DNA. Navrhujeme tedy model, kdy inhibice RNAPI kombinovaná s topologickým stresem vede ke specifickému poškození DNA, které má za následek přitáhnutí konkrétních SUMOylovaných proteinů a tvorbu PNAs. PNAs se pak podílejí na sekvestraci a procesování poškozených lokusů rDNA. Pokud tato místa zůstanou neopravena, přetrvávají v buňkách a pravděpodobně přispívají ke vzniku senescence.

II GENERAL INTRODUCTION

1 Cancer treatment by genotoxic stress-based therapies

The purpose of standard-of-care cancer treatments, radiotherapy and chemotherapy, is to induce genotoxic stress that would lead to cancer cell death (Matt and Hofmann, 2016; Roos and Kaina, 2013). However, this is not always the case. Instead, upon genotoxic stress, cells may activate pathways that enable them to become treatment-resistant (Salehan and Morse, 2013; Swift and Golsteyn, 2014) or proceed to a senescence (Schosserer et al., 2017). The population of treatment-resistant cells can give rise to a new tumor, making treatment ineffective. Senescent cells may reinforce this process by remaining in the tumor tissue and secreting bioactive molecules that further promote tumorigenesis and cancer resistance (Ruhland et al., 2016b). Therefore, in the first part of the thesis I am addressing these two undesired outcomes of genotoxic-based tumor therapy, to better understand how they could be overcome.

In the second part, I look more into how genotoxic stress is sensed by cells; and I focus on the nucleolus as an organelle that has recently been characterized as a hub of cellular stress response, maintenance of genome stability and cancer pathogenesis (Boulon et al., 2010; Lindstrom et al., 2018). It has been demonstrated that after administration of certain chemotherapeutic drugs (actinomycin D, doxorubicin), the tumor suppressor protein PML is attracted to the nucleolus (Janderova-Rossmeislova et al., 2007). I wanted to better understand this association to determine its possible function in cell response to genotoxic stress.

1.1 Current ways of cancer treatment

Cancer is a heterogeneous group of diseases and therefore it is difficult to target it uniformly. Despite continuing advances in more sophisticated strategies (immunotherapy, targeted and personalized medicine), the first-choice therapies remain surgery, radiotherapy and chemotherapy (www.cancer.gov).

1.1.1 Surgery

Surgery is suitable only for solid, localized tumors. The tumor is standardly removed by scalpel; however, it can be also destroyed by laser or extremely low/high temperatures (cryotherapy, hyperthermia, photodynamic therapy). Because it is very unlikely that surgery removes the tumor completely, it is often combined with other treatments like radiotherapy or chemotherapy (www.cancer.gov) (Fisher, 2008; Wyld et al., 2015).

1.1.2 Radiotherapy

The aim of radiotherapy is to damage cell DNA by targeted photon (X-rays, gamma-rays) or particle (electron, proton, neutron) beam (Baskar et al., 2012). Of all these, the proton beam has the advantage of being the most localized and causing minimal damage to the surrounding tissue (Farr et al., 2018). The assumption is that extensive DNA damage will lead to tumor cell death, most often by apoptosis or mitotic catastrophe (Eriksson and Stigbrand, 2010).

1.1.3 Chemotherapy

Chemotherapy is a common name for hundreds of different drugs that target different cellular processes (Table 1). The largest group comprises chemicals that directly damage DNA; other drugs interfere with DNA replication, transcription or unwinding, while the ultimate effect in most cases is also DNA damage (Peters and Raymond, 2016).

Group	Subgroups	Members	Mechanism of action
Alkylating agents	Nitrogen mustard analogues	cyclophosphamide, chlorambucil, melphalan, chlormethine, ifosfamide, trofosfamide, prednimustine, bendamustine, estramustine	Cross-linking of DNA strands which leads to replication inhibition, strand breaks and DNA-damage response activation (Puyo et al., 2014).
	Alkylsulfonates	busulfan, treosulfan, mannosulfan	
	Ethylene imines	thiotepa, triaziquone carboquone	
	Nitrosoureas	carmustine, lomustine, semustine, streptozacin, fotemustine, nimustine, ranimustine, uramustine	
	Epoxides	etoglucid	
	Other alkylating agents	mitobronitol, pipobroman, temozolomide, dacarbazine, altretamine	

Antimetabolites	Folic acid analogues	methotrexate, raltitrexed, pemetrexed, pralatrexate	Analogues of molecules that the cell uses in nucleic acid synthesis. When integrated into nucleic acid metabolism, they inhibit RNA/DNA synthesizing enzymes (Peters, 2014).
	Purine analogues	mercaptopurine, tioguanine, cladribine, fudarabine, clofarabine, nelarabine, rabacfosadine	
	Pyrimidine analogues	cytarabine, fluorouracil, tegafur, carmofur, gemcitabine, capecitabine, azacitidine, decitabine, floxuridine	
	Others	tiazofurin	
Plant alkaloids and other natural products	Vinca alkaloids and analogues	vinblastine, vincristine, vindesine, vinorelbine, vinflunine, vintafolide	Bind tubulin and prevent it from creating microtubules, including mitotic spindle (Martino et al., 2018).
	Podophyllotoxin derivates	etoposide, teniposide	Locks topoisomerase II on a DNA, prevents re-ligation of DNA strands (Gibson et al., 2016; Long, 1992).
	Colchicine derivatives	demecolcine	Binds microtubule + end, to prevent microtubule dynamics (Florian and Mitchison, 2016).
	Taxanes	paclitaxel, docetaxel, paclitaxel poliglumex, cabazitaxel	Bind microtubules, prevent microtubule depolymerization and dynamics (Paier et al., 2018).
	Other plan alkaloids	trabectedin	Blocks DNA binding of the oncogenic transcription factor FUS-CHOP and reverses the transcriptional program in myxoid liposarcoma (Larsen et al., 2016).

Cytotoxic drugs and related substances	Actinomycines	dactinomycin	Inhibits DNA transcription (Gniazdowski et al. 2003).
	Anthracyclines and related substances	doxorubicin, daunorubicin, epirubicin, aclarubicin, zorubicin, idarubicin, mitoxantrone, pirarubicin, valrubicin, amrubicin, pixantrone	Inhibit topoisomerase II, intercalate into DNA, induce ROS, form DNA adducts (Venkatesh and Kasi, 2019).
	Other cytotoxic drugs	bleomycin, plicamycin, mitomycin, ixabepilone	
Other antineoplastic agents	Platinum compounds	cisplatin, carboplatin, oxaliplatin, satraplatin, polyplatillen	Form DNA crosslinks, inhibit DNA synthesis (Dilruba and Kalayda, 2016)
	Methylhydrazines	procarbazine	Alkylating agent (www.drugbank.cz)
	Monoclonal antibodies	32 different monoclonal antibodies	Antibodies targeting different proteins important for tumor progression (Pento, 2017).
	Sensitizers used in photodynamic/radiation therapy	porfimer sodium, methyl aminolevulinate, aminolevulinic acid, temoporfin, efaproxiral, padeliporfin	
	Protein kinase inhibitors	50 different protein kinase inhibitors	
	Antinutrients	asparaginase, pegaspargase	
	Gene transcription modifiers	all-trans retinoic acid, 9-cis-retinoic acid, bexarotene	
	Proteasome inhibitors	bortezomib, carfilzomib	
	Protein-translation inhibitors	omacetaxine mepesuccinate	
	Inhibitors of poly(ADP)-ribose-polymerase (PARP)	olaparib, niraparib, rucaparib, talazoparib	

	Topoisomerase inhibitors	topotecan, irinotecn, etirinotecan pegol, vosaroxin	
	Histone deacetylase inhibitors	vorinostat, romidepsin, panobinostat, belinostat,	
	Inhibitors of signaling pathways	vismodegib, idelalisib, sonidegib, venetoclax, copanlisib	
	Other antineoplastic agents	amsacrine, hydroxyurea, lonidamine, pentostatin, miltefosine, masoprocol, mitoguazone, mitotane, arsenic trioxide, denileukin diftitox, celecoxib, anagrelide, oblimersen, sitimagene ceradenovec, eribulin, aflibercept, ixazomib, talimogene laherparepvec, plitidepsin, epacadostat, enasidenib, tigilanol tiglate	
Hormones and related agents	Estrogens	4 estrogens	
	Progestogens	3 progestogens	
	Gonadotropin-releasing hormone analogues	6 analogues	
	Other hormones		
Hormone antagonists and related agents	Anti-estrogens	3 anti-estrogens	
	Anti-androgens	5 anti-androgens	
	Aromatase inhibitors	6 aromatase inhibitors	
	Other hormone antagonists	3 other hormone antagonists	
Immunostimulants	Colony stimulating factors	11 colony-stimulating factors	
	Interferons	15 interferons	
	Interleukins	2 interleukins	

	Other immunostimulants	19 other immunostimulants	
Immunosuppressants	Selective immunosuppressants	28 selective immunosuppressants	
	Tumor necrosis factor alpha inhibitors	6 inhibitors	
	Interleukin inhibitors	17 inhibitors	
	Calcineurin inhibitors	3 inhibitors	
	Other immunosuppressants	7 other immunosuppressants	

Table 1: Overview of antineoplastic agents.
(based on https://www.whocc.no/atcvet/atcvet_index/?code=QL01)

1.2 Possible outcomes of genotoxic stress-based therapies

A radiotherapy and majority of chemotherapies are the source of an extensive genotoxic stress, meaning they cause a large-scale DNA damage (Goldstein and Kastan, 2015; Roos and Kaina, 2013; Roos et al., 2016; Tian et al., 2015). Generally, in a reaction to DNA damage, any cell triggers a cascade of signaling events, leading to these two major actions:

- DNA repair – the primary response to a genotoxic stress is an attempt to repair the damage. There are various pathways, specific for different types of DNA damage (Jackson and Bartek, 2009; Lord and Ashworth, 2012) (Figure 1).
- Cell cycle arrest – it is initiated together with the repair pathway, in cases when the DNA repair is complicated and requires more time. Halting the cell cycle is important because: 1) the processes of replication, transcription or chromosome separation would impose further stress on DNA and 2) if the damaged DNA was replicated or distributed to daughter cells, it would almost certainly result in a mutation. The most important cycle arrest pathway is the p53 – p21 – cdk/cyclin – pRb – E2F (Barnum and O'Connell, 2014; Chen, 2016; Kastan and Bartek, 2004) (Figure 2).

After successful DNA repair, the cell restarts the cell cycle and none of its functions is affected. However, when the damage is repaired incorrectly or not at all, one of the following scenarios occurs (Khanna, 2015):

- Mutations – when the DNA is not repaired correctly, the information it codes is changed. This might have no phenotype when it happens in the noncoding part of chromatin; however, it might have a mild or dramatic effect when it happens in important coding or regulatory sequences (Goldstein and Kastan, 2015; Tian et al., 2015).
- Senescence – in case of a complicated damage which is not repaired within a certain time period, the cell proceeds from a temporary cell cycle arrest to a permanent one. Permanently arrested cell is called senescent. It cannot proliferate anymore but stays in an organism and influences it in many ways (Ewald et al., 2010; Roninson, 2003; Wu et al., 2012).
- Cell death – a programmed cell death. When the DNA damage is so extensive that it is incompatible with further living, cells commit a programmed suicide called apoptosis. Alternatively, cells can die by anoikis, mitotic catastrophe or necrosis (Galluzzi et al., 2018; Matt and Hofmann, 2016; Roos and Kaina, 2013).

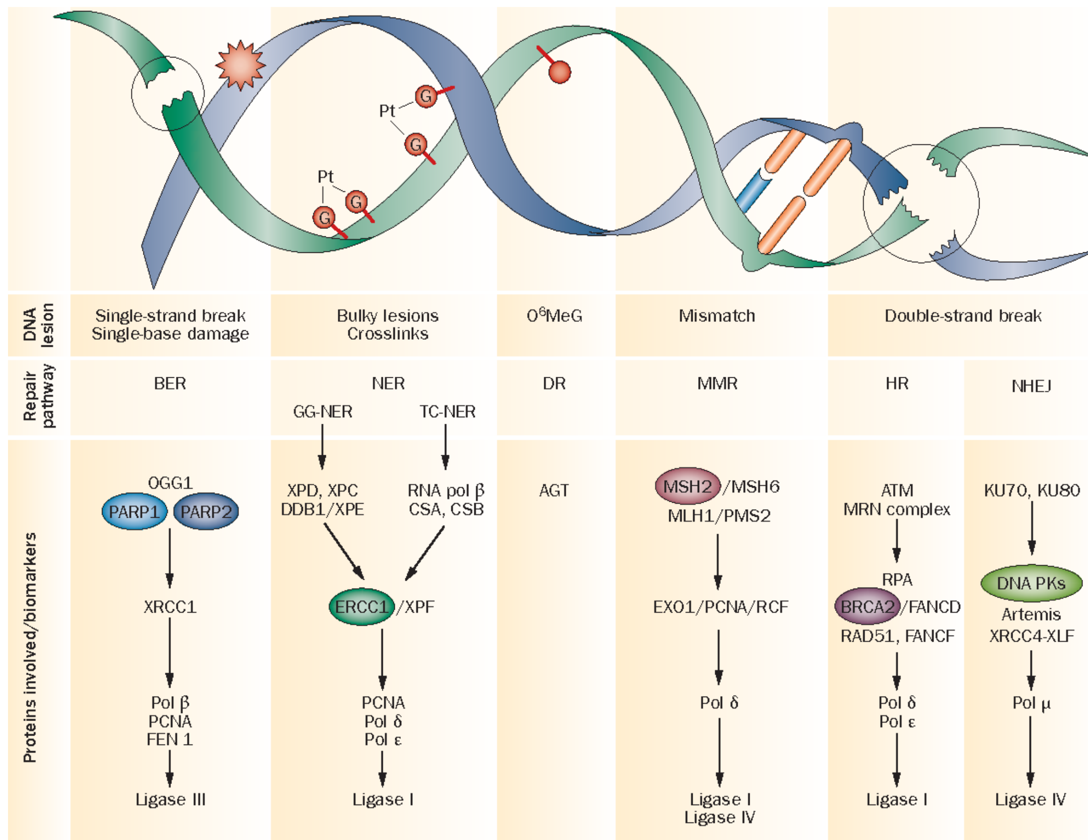


Figure 1: Main DNA lesions and corresponding DNA-damage-repair pathways. (Postel-Vinay et al., 2012)
A single strand break that does not significantly distort the helical structure of DNA is generally repaired by base excision repair (BER), while upon major DNA distorsion the nucleotide excision repair (NER) is used more frequently. Direct repair (DR) deals with changes (usually adducts) that are small and affect a single base, and mismatch repair (MMR) copes with mismatches in the DNA pairing, resulting preferentially from replication errors. Finally, homologous replication (HR) and non-homologous end joining (NHEJ), are involved in the repair of DNA double-strand breaks.
Abbreviations: AGT, O6-alkylguanine-DNA alkyltransferase; ATM, ataxia telangiectasia mutated; GG-NER, global genome NER; 06MeG, O6-methylguanine; MMR, mismatch repair; TC-NER, transcription-coupled NER.

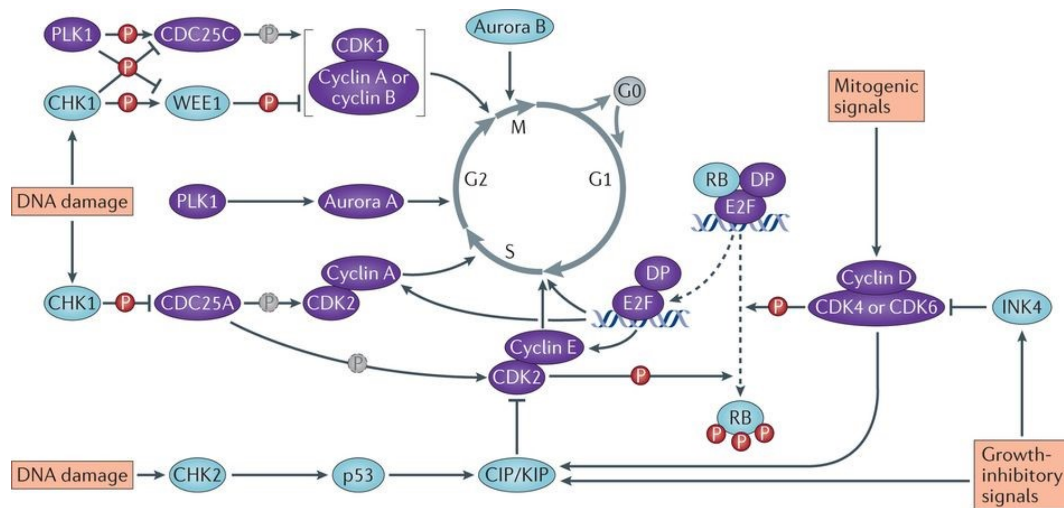


Figure 2: Cell cycle progression and major regulatory proteins. (Otto and Sicinski, 2017) Cyclins and cyclin-dependent kinases (CDKs) are activated by pro-proliferation signals. In response to this signaling, cells phosphorylate (P) several proteins to proceed from the G1 phase into S phase. One of the phosphorylated proteins is the retinoblastoma protein (RB). Upon phosphorylation, RB stops inhibiting the E2F family of transcription factors which in turn become active.

Growth-inhibitory signals antagonize G1–S progression by upregulating CDK inhibitors of the INK4 and CIP/KIP families. The cyclin–CDK complexes control also the progression through S phase and from G2 phase into mitosis (M phase), together with other proteins, for instance Polo-like kinase 1 (PLK1) or Aurora kinases (Aurora A and Aurora B). When cells exit the cell cycle they place themselves into a reversible or a permanent cell cycle arrest (G0 phase).

The cell cycle progression can also be prevented by checkpoint proteins which respond to a DNA damage, like checkpoint kinase 2 (CHK2) and p53 in G1 phase or CHK1 in S or G2 phase. Purple ovals denote positive regulators of cell cycle progression and blue ovals denote negative regulators of cell cycle progression. P in a red circle indicates phosphorylation and in a grey circle dephosphorylation.

2 Mutations in cancer cells

Compared to normal cells, the DNA repair mechanisms of cancer cells are often defective, owing to mutations in DNA repair genes (Hoeijmakers, 2009). These mutations can be inherited, while the carriers are in a high risk of developing cancer (Romero-Laorden and Castro, 2017; Torgovnick and Schumacher, 2015). Alternatively, they can evolve and accumulate with age, as a result of endogenous and exogenous genotoxic stress (Milholland et al., 2015; Risques and Kennedy, 2018; Tubbs and Nussenzweig, 2017). Genotoxic stress-based therapies try to make use of the less effective DNA repair of cancer cells, assuming they will be more susceptible to DNA damage-induced cell death than normal healthy cells (Bartek, 2011; Lord and Ashworth, 2012; Torgovnick and Schumacher, 2015). However, the cell death is not the only possible outcome. Less stringent DNA repair also means that the damaged site is more often repaired incorrectly, giving rise to mutations in other genes (Hanahan and Weinberg, 2011; Loeb, 2001; Negrini et al., 2010).

Mutations are generally an important feature of tumor development. Only thank to accumulation of necessary mutations is the normal cell able to overcome cell cycle checkpoints, adapt to different tissue context (e.g. longer distance from basal lamina), escape the immune system, cope with lower supply of oxygen and nutrients, migrate through extracellular matrix, extravasate into vascular system, survive as a single cell or to get from the vasculature back to the tissues and give rise to secondary tumor (Greaves and Maley, 2012; Hanahan and Weinberg, 2011; Loeb, 2001; Martincorena et al., 2017; McGranahan and Swanton, 2017; Negrini et al., 2010; Shlush and Hershkovitz, 2015; Sidow and Spies, 2015). In addition to it, some mutations favor cell survival in conditions of genotoxic stress, i.e. they render the cells treatment-resistant (Bouwman and Jonkers, 2012; Holohan et al., 2013; Housman et al., 2014; McGranahan and Swanton, 2017).

2.1 Radio- and chemo-resistance of cancer cells

Radio- and chemo-resistance are brought about by dysregulation of specific signals and pathways. These can either be present in the tumor cell before the treatment and only to be selected for by the insult, or they can be directly induced by the therapy (Figure 3) (Li et al., 2016). In either case, to improve cancer treatment, it is of a high importance to identify those mutations to be able to target them effectively. The following pathways have been many times proven to be responsible for tumor resistance:

- DNA damage repair pathways

- Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/AKT serine/threonine kinase 1 (AKT)/mechanistic target of rapamycin (mTOR) pathway
- Extracellular signal-regulated kinase (ERK) pathway
- Glycolytic pathway
- Vascular endothelial growth factor A (VEGF) pathway
- Wingless-type MMTV integration site (Wnt)/β-catenin pathway
- Notch pathway
- Transforming growth factor beta (TGF-β) pathway
- Hedgehog pathway
- Enhanced protection against reactive oxygen species (ROS)
- Multidrug resistance conferred by increase expression of ABC transporters
- Autophagy

Many of these pathways are upregulated in cancer stem cells (CSCs) (Chang et al., 2016; Krause et al., 2017; Prieto-Vila et al., 2017) and mesenchymal cells (Chang et al., 2013; Stark et al., 2017; Steinbichler et al., 2018; Theys et al., 2011; Zhang et al., 2014), therefore, transition into stem-like state or acquisition of mesenchymal phenotype are generally considered as a processes leading to increased radio- or chemo-resistance. What is more, these two processes are often connected, i.e. CSCs demonstrate mesenchymal characteristics and vice versa.

2.1.1 Cancer stem cells

Cancer stem cells are defined as tumor cells with the ability of unlimited proliferation that can restore the whole tumor (Krause et al., 2017). Like normal stem cells, they are the least differentiated cells within the tissue with the ability of asymmetric division: of the two daughter cells that come out from the CSCs division, one becomes a new stem cell, whereas the other one turns into a more differentiated progenitor cell. During treatment, CSCs demonstrate higher resistance against radiotherapy and chemotherapy and are therefore responsible for the relapse of the disease (Batlle and Clevers, 2017; Yu et al., 2012). However, within the past few years the concept has been slightly revisited, reflecting the newly discovered plasticity inside the tumor. Currently, it is believed that CSCs and non-CSCs are not two distinct cell types but rather two states, between which the cell can switch depending on its microenvironment (Ayob

and Ramasamy, 2018; Batlle and Clevers, 2017). Nevertheless, regardless of their plasticity, an increased treatment resistance of tumor cells with stem cell properties has not been contradicted.

Several studies showed that CSCs are responsible for tumor relapse after chemotherapy. More specifically, it has been documented, for instance, for oxaliplatin treatment in colorectal cancer (Kreso et al., 2013), temozolomide in glioblastoma (Chen et al., 2013), cisplatin in mouse squamous cell carcinoma (Oshimori et al., 2015) and different anti-proliferative drugs in bladder cancer (Kurtova et al., 2015), breast cancer (Creighton et al., 2009) or in models of human glioblastoma (Liau et al., 2017).

Analogically, CSCs demonstrate increased resistance to radiotherapy, as shown in cancer cells of colon and intestine (Asfaha et al., 2015; Sahlberg et al., 2014), breast cancer (Ko et al., 2018; Qi et al., 2017; Rycaj and Tang, 2014; Troschel et al., 2018), head and neck cancer (Cho et al., 2018; Park et al., 2016), glioma (Bao et al., 2006; Jamal et al., 2012; Ong et al., 2017; Rycaj and Tang, 2014; Wang et al., 2019b), cervical cancer (Tyagi et al., 2017), lung cancer (Yun et al., 2016), prostate cancer (Chang et al., 2013), hepatocellular carcinoma (Piao et al., 2012) and others.

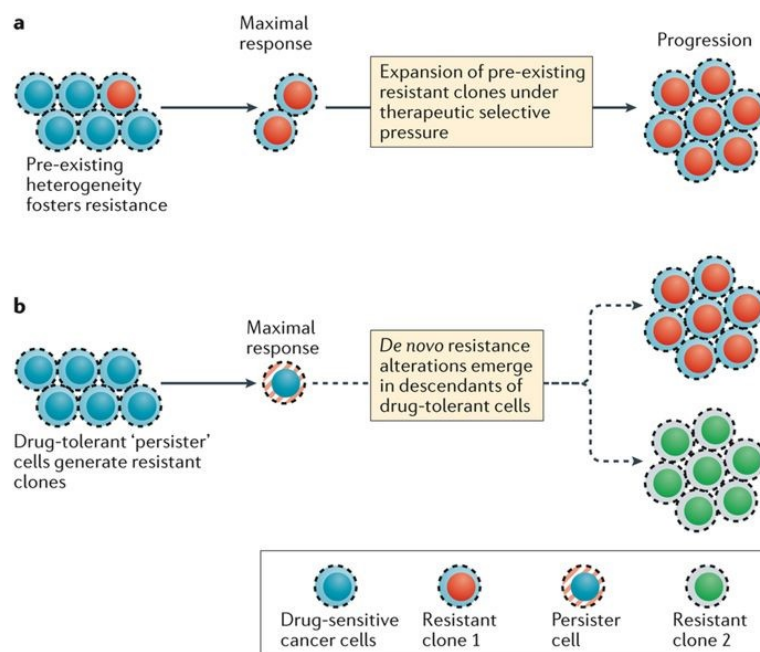
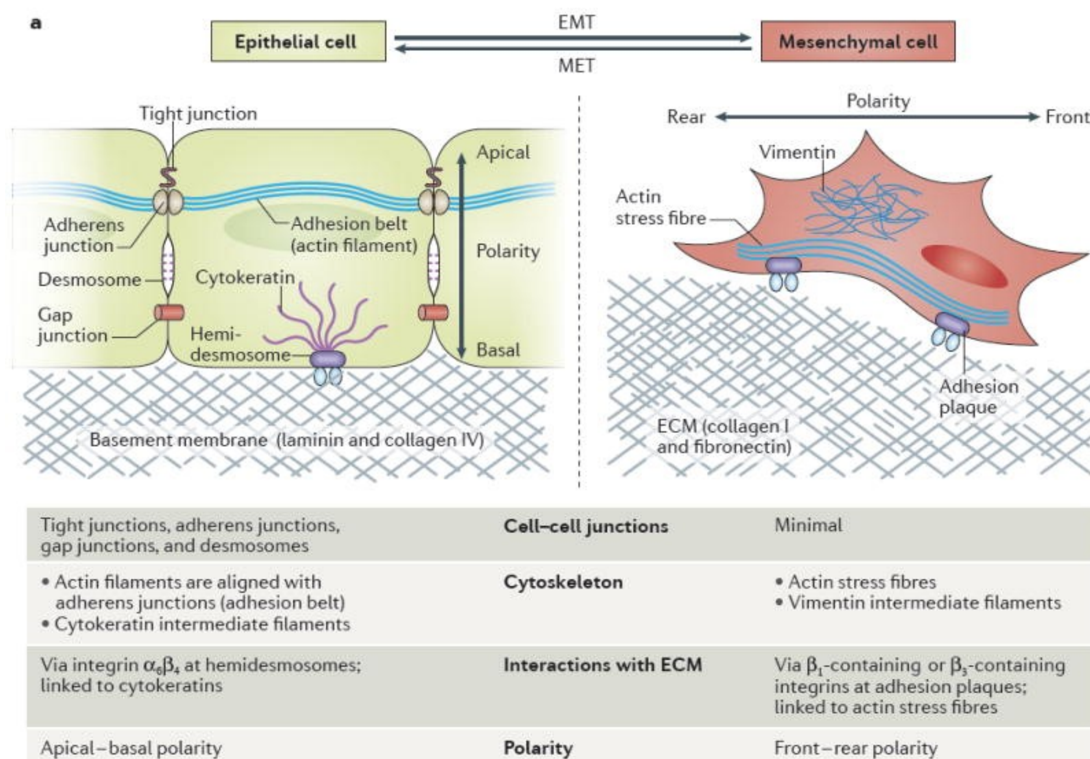


Figure 3: Acquired or pre-existing treatment resistance of cancer cells. (Dagogo-Jack and Shaw, 2018)

2.1.2 Epithelial-to-mesenchymal transition

Epithelial cells differ from mesenchymal cells in many aspects. Epithelial cells are usually tightly interconnected through regular network of adhesions and junctions. They grow in one layer with very limited possibilities of free movement and they tend to be polarized, meaning there is a differential composition of cell membrane components on the inner and outer side of the epithelium. Thanks to their close contact they often serve as a barrier between two different environments (Lee et al., 2006). On the contrary, the connections between mesenchymal cells are weaker and irregular; therefore, the structures they form are usually less organized. The cells are allowed to migrate more, not only as a whole sheet but also individually. That is also reflected in their shape, which is elongated, with front-to-back polarity (Lee et al., 2006). The conversion of epithelial cells into mesenchymal cells is called epithelial-mesenchymal transition (EMT) and requires major changes in cell phenotype and behavior, regarding the cytoskeleton, cell-to-cell contacts and proteolytic activity (Thiery and Sleeman, 2006) (Figure 4).



Epithelial cell		Mesenchymal cell
Tight junctions, adherens junctions, gap junctions, and desmosomes	**Cell–cell junctions**	Minimal
• Actin filaments are aligned with adherens junctions (adhesion belt) • Cytokeratin intermediate filaments	**Cytoskeleton**	• Actin stress fibres • Vimentin intermediate filaments
Via integrin $\alpha_6\beta_4$ at hemidesmosomes; linked to cytokeratins	**Interactions with ECM**	Via β_1-containing or β_3-containing integrins at adhesion plaques; linked to actin stress fibres
Apical–basal polarity	**Polarity**	Front–rear polarity

Figure 4: A schematic overview of EMT-associated changes in cell physiology. (Shibue and Weinberg, 2017)

EMT and complementary mesenchymal-epithelial transition are key events in many physiological processes, such as tissue regeneration or embryonic development (Strauss et al., 2012). In cancer, EMT plays an important role in adoption of more motile phenotype which allows the tumor cells to invade to other tissues, penetrate the vasculature and form secondary tumors (Heerboth et al., 2015). Apart from that, many changes happening during EMT have also been shown to contribute to increased chemoresistance and radioresistance of cancer cells.

More specifically, EMT-driven chemoresistance has been documented for ovarian cancer (Ahmed et al., 2010; Liu et al., 2016a; Qin et al., 2017), hepatocellular carcinoma (Ju et al., 2015; Wu et al., 2013; Zhang et al., 2016), colorectal cancer (Lee et al., 2016; Li et al., 2015), gastric cancer (Dong et al., 2017; Feng et al., 2016), breast cancer (Gu et al., 2016; Jiang et al., 2014), bladder cancer (Amantini et al., 2016), osteosarcoma (Wang et al., 2019a), glioblastoma (Liao et al., 2015; Maciaczyk et al., 2017), prostate (Wang et al., 2017b), pancreatic (Elaskalani et al., 2017; Zheng et al., 2015) and lung cancer (Fischer et al., 2015; Jin et al., 2016),etc.

Similarly, EMT is involved in radioresistance of many cancer types, e.g. esophageal cancer (Jin et al., 2018; Zang et al., 2017; Zhang et al., 2016), prostate cancer (Chang et al., 2013; Stark et al., 2017), nasopharyngeal carcinoma (Lu et al., 2016; Wu et al., 2017), breast cancer (Konge et al., 2018; Zhang et al., 2014), non-small lung cancer (Kang et al., 2013; Yao et al., 2016), gastric cancer (Zhang et al., 2015), colorectal cancer (Bastos et al., 2014), etc.

2.1.3 Resistance to anoikis

Anoikis is a specific variant of intrinsic apoptosis induced upon cell detachment from extracellular matrix (Galluzzi et al., 2018). To become metastatic, any tumor has to overcome this barrier and to acquire anoikis resistance (Paoli et al., 2013). There are many pathways involved in this process (Buchheit et al., 2014; Cao et al., 2016), including EMT and stemness, as resistance to anoikis is one of the defining hallmarks of both EMT and cancer stem cells (Frisch et al., 2013). As such, anoikis can be induced by genotoxic-based therapies on one hand and contribute to increased resistance to these treatments on the other hand. The identification of the pathways conferring resistance to anoikis and their successful inhibition is therefore one of the ways to sensitize cells to radio- and chemotherapy.

In a study performed in our laboratory, I was involved in characterization of a population of anoikis-resistant stem cell-like cells that emerged as a result of fractionated radiation therapy. These cells had activated Notch signaling and expressed stem cell- and EMT markers. Their survival in a low-adherent state was mediated by ERK pathway, as inhibition or knockdown of ERK1/2 resulted in death of this low-adherent population (Kyjacova et al., 2015).

3 Senescent cells in tumors

3.1 Senescence in general

Senescence is defined as a stable cell cycle arrest. Senescent cells are metabolically active but they are not able to proliferate, not even upon mitogenic stimuli, which distinguishes them from quiescent cells (Terzi et al., 2016).

3.1.1 Replicative senescence

The original purpose of a senescence program in living organisms is probably to avoid an indefinite cell proliferation and thus prevent a tumor growth. The term replicative senescence is used for cells growing in a culture without additional apparent stress stimuli. It has been described that such cells can undergo only limited number of cell divisions. After reaching that number, they no more proliferate, but they do not commit apoptosis, either. Instead, they persist in the culture remaining metabolically active and demonstrating changed morphology and physiology (Campisi, 1997; Marcotte and Wang, 2002). This limited cell division was firstly described in human fibroblasts in cell culture by Hayflick and Moorhead in 1960’s and is accordingly called the “Hayflick’s limit” (Hayflick and Moorhead, 1961). At that time, the molecular basis of this phenomenon was attributed to the ends of chromosomes known as telomeres. Each somatic cell starts its life with defined telomere length; and it was proposed that during each cell division the telomere shortens (Harley et al., 1990), until it comes to the point when it is no more protected by specialized protein complexes and starts signaling damage (d'Adda di Fagagna et al., 2003; Takai et al., 2003). This concept might not be entirely wrong; telomeric damage may indeed be responsible for this type of senescence. However, what remains elusive and poorly documented is the regular telomere shortening during each cell cycle as an inherent property of somatic cells. Rather, the signaling leading to this type of senescence is probably caused by sub-optimal cultivating conditions and subsequent stress, which is supported by later studies describing heterogeneity in the rate of telomere shortening (Martin-Ruiz et al., 2004), or manipulation of the Hayflick’s limit by oxygen levels (von Zglinicki, 2002).

In any case, the cascade that is activated upon these events comprises the well-known tumor suppressor p53, a transcription factor that positively regulates expression of the cyclin-dependent kinase inhibitor p21, leading to cell cycle arrest (Fumagalli et al., 2012a). Although the p53-p21-pRb pathway is the most important one in replicative senescence (Campisi, 2013),

the alternative p16-pRb pathway might also play a role, although its association with telomere damage is less understood (Victorelli and Passos, 2017).

3.1.2 Stress-induced premature senescence

Senescence can be brought about or accelerated by additional stress stimuli, a phenomenon known as a stress-induced premature senescence or SIPS (Toussaint et al., 2002). There are numerous inductors of SIPS, including DNA damage, ROS, oncogenic signaling or cytokines from the repertoire of senescence-associated secretory phenotype. Some of these factors, even though in mild levels and not being ectopically induced, may also be responsible for the senescence known as replicative.

3.1.2.1 Senescence induced by ectopic DNA damage

The signaling originating in damaged telomeres does, in principle, not differ from the signaling originating in any other region of damaged DNA; only the telomeric DNA damage foci seem to be, for some reason, mostly irreparable (Fumagalli et al., 2012a; Hewitt et al., 2012) and thus persistent. Therefore, any other DNA damage meeting this requirement should theoretically lead to the same outcome (Zhang et al. 2016). The persistence (or long-lasting presence) of DNA damage signaling and resulting induction of Cdk inhibitors seem to be the key aspect of senescence induction, as short-term DNA damage would only lead to a temporary cell cycle arrest that would be reverted by genotoxic stress recovery. It is therefore believed that senescence is an active additional program that has to be switched on in stressed cells to block cell cycle restart forever. According to Blagosklonny (Blagosklonny, 2011), this switch happens when cell cycle-arrested cells are further exposed to growth-promoting stimuli. The resulting conflict between cell cycle “breaks” and “accelerators” activates senescence program which then remains active even when the “breaks” (cell cycle inhibitors) are removed and “accelerators” (growth-promoting stimuli) continue signaling. Importantly, the crucial molecule, standing on the crossroad between temporary and permanent cell cycle arrest seems to be the cell cycle inhibitor p16 (Rayess et al., 2012).

3.1.2.2 Senescence induced by reactive oxygen species (ROS)

Reactive oxygen species are reactive molecules with a short half-life. They come from various sources, but their main generators are mitochondria. There, passing through electron transport chain, some electrons may escape the regulated transport and react with an oxygen, partially reducing it. The result are molecules such as superoxide ($O_2 \cdot^-$), hydroxyl ($OH \cdot$) or hydrogen peroxide (H_2O_2). Being extremely reactive, these molecules interact with mitochondrial DNA and proteins, damaging them and disrupting mitochondrial integrity, which in turn leads to even

larger production of ROS (Lee and Wei, 2007; Linnane et al., 1989). Major mediators of ROS-induced senescence are NADPH oxidases, proteins that transfer electrons across biological membranes (Abdul-Aziz et al., 2019; Hodny et al., 2016; Hubackova et al., 2016). ROS activate senescence by both DNA damage-dependent and independent pathways, the latter comprising chromatin regulation or cytokine production linked to dysfunctional mitochondria (Pole et al. 2016). The DNA damage in this case does not have be persistent; rather, thanks to continual stress stimulus, the DNA is repeatedly being damaged and repaired, resulting in persistent DNA damage signaling.

3.1.2.3 Oncogene-induced senescence (OIS)

The fact that oncogenic activation can induce senescence was first described by Serrano et al., more than 20 years ago (Serrano et al., 1997). The primary effect of an oncogene activation is an excessive cell proliferation with increased DNA synthesis. Consequently, cells undergo so called replication stress, manifested as stalled replication forks and increased number of DNA double-strand breaks (DSBs) (Bartkova et al., 2006; Di Micco et al., 2006). Additionally, oncogene signaling is accompanied by an accumulation of ROS (Lee et al., 1999) and can therefore induce senescence through their effect in cells. However, it is important to note that not all oncogenes cause DNA damage, just as not all of them increase ROS, so the precise role of both processes in oncogene-induced senescence is still to be better explained (Courtois-Cox et al., 2008; Maya-Mendoza et al., 2015). The oncogenes most often connected with the onset of senescence include rat sarcoma viral oncogene homolog (RAS), RAF proto-oncogene serine/threonine-protein kinase (RAF), AKT, E2F1, Moloney murine sarcoma viral oncogene homolog (c-Mos), avian myelocytomatosis viral oncogene homolog (c-Myc) and cyclin E, as well as inactivation of tumor suppressors PTEN and NF1 (Courtois-Cox et al., 2008).

3.1.2.4 Senescence induced by senescence-associated secretory phenotype (SASP)

Senescent cells develop a specific pattern of molecules they secrete into their surroundings. This so called senescence-associated secretory phenotype (SASP) comprises several cytokines, growth factors, enzymes and ROS (it will be discussed in more detail later). More specifically, ROS in senescent cells activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which then mediates large part of the SASP production (Nelson et al., 2018). What is important, it was found out that these secreted molecules are able to induce senescence in normal cells via DNA damage or ROS pathway (Hodny et al., 2016; Hubackova et al., 2012a; Nelson et al., 2012).

3.2 The role of senescent cells in tumors

Senescence in pre-neoplastic lesions can be triggered directly by activated oncogenes (Braig et al., 2005; Collado et al., 2005; Serrano et al., 1997); or by DNA damage response, reflecting the intrinsic genetic instability of (pre-)malignant cells (Bartkova et al., 2006). Alternatively, it can be brought about by radio- and chemo-therapy, via generated ROS and/or DNA damage (Chang et al., 1999; Gonzalez et al., 2016). Obviously, the primary role of senescence in tumors is beneficial, serving as a barrier to further cell proliferation and disease progression (Rao and Jackson, 2016). However, senescent cells can also affect their surrounding by components of SASP, which can have both positive and negative effect on remaining tumor tissue (Figure 5).

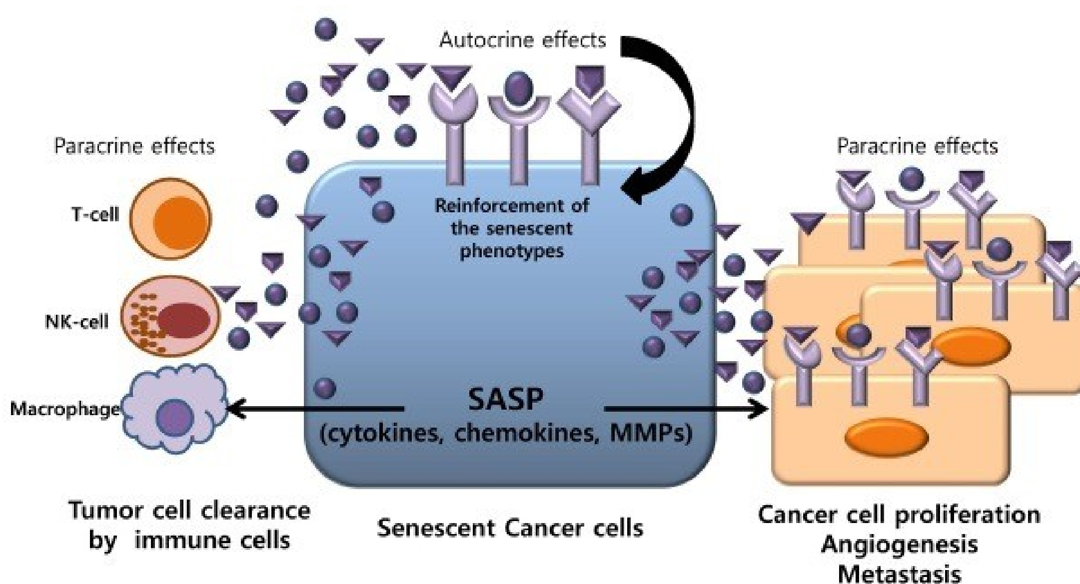


Figure 5: Pleiotropic role of senescent cells in tumors. (Lee and Lee, 2014)

3.2.1 Senescence-associated secretory phenotype and cancer

The pathway that is responsible for SASP production originates in mitochondria. As mentioned in the chapter 4.1.2.2, even in normal cells the mitochondria produce some basal levels of ROS. In senescent cells, however, the mitochondria are often defective, phenomenon known as a senescence-associated mitochondrial dysfunction (SAMD) (Korolchuk et al., 2017). Therefore, the cells are prone to electron leakage from an electron transport chain, leading to constantly high ROS levels. ROS, in turn, not only cause a DNA damage, which further reinforces senescent phenotype in the ROS-producing cell but also activate NF-κB pathway, one of the main orchestrators of SASP production (Nelson et al., 2018; Nelson et al., 2012). The pathway that connects DNA damage with NF-κB activation is the ATM/NEMO/NF-κB pathway. NF-κB is

normally localized in the cytoplasm thanks to its association with NF-κB inhibitor (I-κB). However, I-κB can be phosphorylated by I-κB kinase (IKK), which predestines it for an ubiquitin-dependent degradation. One of the regulatory subunit of IKK is NEMO. NEMO is phosphorylated by ATM in the nucleus in response to DNA damage, and, together with ATM migrates to the cytoplasm, where they activate IKK. Active IKK phosphorylates I-κB, which leads to I-κB degradation and NF-κB activation (Wu et al., 2006). More recently, also the cGAS/STING (cyclic GMP-AMP synthase linked to stimulator of interferon genes) pathway has been implicated in triggering SASP production (Dou et al., 2017; Yang et al., 2017).

The composition of SASP is not uniform, instead it varies a lot with a tissue type, senescence-triggering stimulus and a progression of the disease (Coppe et al., 2008; Dou et al., 2017; Freund et al., 2010; Malaquin et al., 2016; Yang et al., 2017). However, usually it comprises following classes of molecules:

Interleukins and chemokines:

- interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8),
- chemokine (C-X-C motif) ligands CXCL-1 and -2,
- monocyte chemoattractant proteins MCP-1, -2, -3, and -4,
- human CC chemokine HCC-4,
- eotaxin-3,
- macrophage inflammatory proteins MIP-1α and -3α,
- C-X-C chemokine receptor type 2- (CXCR-2) binding chemokines (specific for OIS cells).

Growth factors (GFs) and GFs-binding proteins:

- insulin-like growth factor- (IGF) binding proteins and their regulatory factors,
- platelet-derived growth factors (PDGF),
- vascular endothelial growth factors (VEGF).

Pro-inflammatory cytokines:

- granulocyte-monocyte colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF),
- osteoprotegerin,

- prostaglandin E2 (PGE2),
- cyclooxygenase 2 (COX2).

Other proteins:

- matrix metalloproteinases (MMP-1, -3, -10),
- serine proteases,
- extracellular matrix protein fibronectin.

Other macromolecules:

- ROS,
- lipids,
- carbohydrates,
- nucleic acids (including miRNAs) and proteins enclosed in extracellular vesicles.

(Coppe et al., 2010; Schosserer et al., 2017)

As SASP comprises many molecules with different functions, its effect on surrounding tumor tissue is pleiotropic. Generally, we can talk about these SASP-induced events:

3.2.1.1 Induction of senescent phenotype in neighboring cells

As was mentioned in chapter 4.1.2.4, some members of the SASP family are capable of so called bystander effect, which means that they reinforce senescence in both autocrine and paracrine manner. IL-1, IL-6, TGF-β or IGF-binding proteins are the main mediators of this process (Acosta et al., 2013; Chien et al., 2011; Coppe et al., 2008; Hubackova et al., 2012a; Hubackova et al., 2016; Jing et al., 2011; Kortlever et al., 2006).

3.2.1.2 Transformation of pre-neoplastic cells to malignant and tumor growth promotion

Majority of SASP generates pro-tumorigenic environment by inducing extracellular matrix remodeling and chronic inflammation (Jackson et al., 2012; Krtolica et al., 2001; Lasry and Ben-Neriah, 2015; Ohanna et al., 2011). What is more, SASP has been documented to bring about a stem-like state (Milanovic et al., 2018; Mosteiro et al., 2016) or EMT (Coppe et al., 2010) in cancer cells. The main pro-inflammatory molecules are IL-6, IL-8, PDGF, MMPs or small molecules secreted in extracellular vesicles (Davalos et al., 2010; Krtolica et al., 2001; Lasry and Ben-Neriah, 2015; Ohanna et al., 2011).

3.2.1.3 Attraction and activation of immune system

SASP contains chemokines that attract cells of the immune system (natural killer cells, macrophages and granulocytes, T-cells), as well as cytokines that activate them (Rao and Jackson, 2016). Therefore, presence of senescent cells within a tumor mediates enhanced clearance of tumor cells by immune system (Kang et al., 2011; Lujambio et al., 2013; Toso et al., 2014; Xue et al., 2007).

However, the interplay between senescent cells and immune system is more complex. Firstly, the members of SASP attract not only the immune cells that fight against tumor but also immunosuppressive lineages of lymphocytes that inhibit the activity of cytotoxic T-lymphocytes (Coussens et al., 2013; Rabinovich et al., 2007). Furthermore, the attracted immune cells secrete their own cytokines that can influence the tumor by several ways. For example, TGF-β, interferon gamma (IFN-γ) or tumor necrosis factor alpha (TNF-α) may induce and further reinforce senescence (Braumuller et al., 2013; Reimann et al., 2010; van Riggelen et al., 2010a), whereas other cytokines (or the same cytokines in a different context) may contribute to cell malignant transformation and tumor growth (Balkwill et al., 2005). Sometimes, the role of specific cytokines depends on the stage of disease progression (Eggert et al., 2016).

Last but not least, senescent cells have also been reported to create immunosuppressive microenvironment, inhibiting the tumor infiltration by immune cells (Ruhland et al., 2016b; Toso et al., 2014).

3.2.2 Anti-cancer therapies based on manipulation of senescent cells

As presented in the previous chapter, oncogene- or therapy-induced senescence is an important barrier in tumor development; however, further persistence of senescent cells in tumor tissue might have both positive (senescence induction in neighboring tumor cells, immune system surveillance) and negative (creation of pro-tumorigenic inflammatory environment) consequences. Hence, the ideal strategy targeting senescent cells in cancer would inhibit those functions of senescent cells that promote tumor progression, while preserving those that lead to tumor disappearance (Ruhland et al., 2016a).

In a study I was involved in, it was shown that co-administration of senescent (docetaxel-treated or irradiated) mouse prostate cancer cells accelerates tumor growth in mice. This acceleration was inhibited after injection of cells producing IL-12, a cytokine with known anti-tumor immuno-activatory properties (Simova et al., 2016).

3.2.3 Markers of senescent cells

To be able to better study the role of senescent cells in tumors, selective markers of senescent cells are continuously sought for (Hernandez-Segura et al., 2018). Currently used senescent marker comprise:

- permanent cell cycle arrest,
- activity of senescence-associated β-galactosidase (SA-β-gal),
- expression of $p16^{INK4a}$ cell cycle inhibitor,
- changed morphology and shape,
- change of metabolism,
- senescence-associated secretory phenotype (SASP),
- permanent DNA damage lesions and persistent active DNA damage signaling,
- senescence-associated heterochromatic foci,
- promyelocytic leukemia (PML) nuclear bodies,
- lipofuscin accumulation.

(Figure 6) (Calcinotto et al., 2019; Rodier and Campisi, 2011).

However, none of them seem to fulfill all conditions required for a biomarker to be really useful: to be strongly associated with the condition, to have a known threshold, to be quantifiable (Matjusaitis et al., 2016) and for *in vivo* purposes also to be detectable using non-invasive methods.

3.2.3.1 L1CAM as a new potential marker of senescence

L1 cell adhesion molecule (L1CAM) is a transmembrane glycoprotein from an immunoglobulin family. Its various isoforms are expressed predominantly in neural cells (Rathjen and Schachner, 1984) and proximal tubules in kidneys and to a lesser extent also in skin and intestine (Reid and Hemperly, 1992).

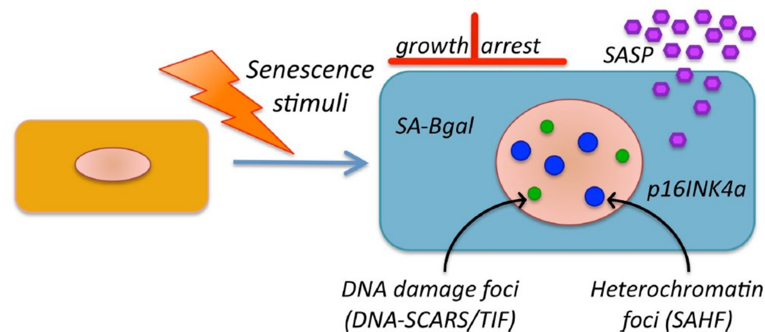


Figure 6: Markers of senescent cells. (Rodier and Campisi, 2011)

Its physiological functions comprise brain development (Fischer et al., 1986; Keilhauer et al., 1985; Lindner et al., 1983; Rathjen and Schachner, 1984) and survival of neural cells (Wood et al., 1990); furthermore, it is responsible for increased cell mobility, invasiveness and adhesion, depending on its binding partner (Gavert et al., 2010; Geismann et al., 2009; Gil et al., 2003; Moos et al., 1988; Rathjen and Schachner, 1984; Silletti et al., 2004).

What is important, L1CAM is aberrantly expressed in cancer, where it has multiple functions in influencing tumor cell phenotype and its susceptibility to anti-cancer treatments. For example, it increases the expression of pro-tumorigenic and anti-apoptotic genes (Gast et al., 2008), stimulates angiogenesis (Friedli et al., 2009), and, just as in normal tissues, increases cell invasiveness and motility, contributing to EMT and metastases (Altevogt et al., 2016). Furthermore, it protects tumor cells against apoptosis, rendering them more resistant to chemotherapy (Sebens Muerkoster et al., 2007) and radiotherapy (Cheng et al., 2011).

In our study, we identified L1CAM as being overexpressed in cells upon $p16^{INK4A}$ expression and in most types of stress-induced premature senescence (the exception was RAS-induced senescence, which was explained by our finding that RAS/MAPK activation inhibits the expression of L1CAM). What is more, senescent cells with the highest levels of L1CAM exhibited increased adhesion and migration, compared to low-L1CAM cells. Therefore, as L1CAM is connected both with senescence and with more aggressive cancer phenotype (see the paragraph above), it could be potentially used as a guide molecule in targeted therapy, to specifically target the most dangerous senescent cells (Mrazkova et al., 2018).

4 The promyelocytic leukemia protein

4.1 PML gene and protein

The promyelocytic leukemia protein (PML) got its name according to the acute promyelocytic leukemia (APL) disease. 97% of APL patients carry translocation between chromosomes 15 and 17, which results in a fusion between the proteins PML (chromosome 15) and retinoid acid receptor α (RARα) (chromosome 17), forming PML/RARα protein. In contrast to RARα, PML/RARα functions as an inhibitor of transcription and inhibits expression of genes important for granulocyte differentiation. Thus, APL is characterized by increased number of less differentiated cells, promyelocytes (de The et al., 2017; Gaillard et al., 2015a; Grignani et al., 1996). Furthermore, PML/RARα disrupts formation of the classical PML nuclear bodies (see below), which might be even more critical for the development of the disease (de The et al., 2017).

In normal conditions, PML is a protein of 48 - 98 kDa, expressed virtually in all tissues (proteinatlas.org). However, its levels in specific tissues or cell types differ a lot (Bernardi and Pandolfi, 2007). The gene, located on chromosome 15, has ~53 kb and the mRNA comprises 9 exons (Guan and Kao, 2015). The alternative splicing of the C-terminus results in 22 splice variants and seven splice families of PML protein. The protein is from 41% intrinsically disordered (Frege and Uversky, 2015) and contains several important domains and motifs (Figure 7):

- The RBCC motif is found in all splice variants (exons 1-3). It consists of a RING domain (zinc finger), which interacts directly with E2 SUMO (small ubiquitin-like modifier) ligase ubiquitin carrier protein 9 (UBC9), then two B-boxes (cysteine-rich, zinc fingers) and alpha-helical, leucine-rich coiled-coil (CC) domain which mediates homodimerization of PML. Interestingly, many proteins of the RBCC family are SUMO ligases (Jensen et al., 2001).
- The SUMO-interacting motif (SIM) is localized to the C-terminus. It is present in PML variants I-V and it can be phosphorylated by casein kinase 2 (CK2) (Kerscher, 2007).
- Furthermore, PML can be SUMOylated, primarily at three lysines: K65, K160 and K490 (Kamitani et al., 1998).
- Nuclear localization sequence is found in PML variants I-VI (exon 6). Therefore, the protein is mainly nuclear, only the isoform VII is cytoplasmic and the isoform I can

shuttle between the cytoplasm and the nucleus (Lallemand-Breitenbach and de The, 2010).

- Putative nucleolar localization sequence (NoLS) is found in variants I, IV and V in the exonuclease III domain and has been described to target PML to nucleolar caps (Condemine et al., 2007). However, the study was performed with very short fragments of PML protein fused to GFP, making the relevance of these findings for full-length isoforms disputable.

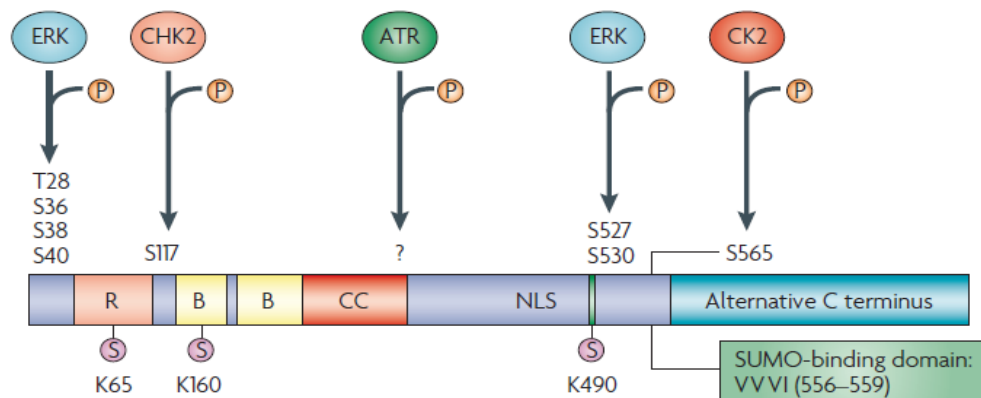


Figure 7: Domain organization of PML protein. (Bernardi and Pandolfi, 2007)

The expression of PML protein is induced on transcriptional level by p53 protein (de Stanchina et al., 2004; Hubackova et al., 2010; Stadler et al., 1995), by interferons and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling (Hubackova et al., 2010; Stadler et al., 1995) and modified by interleukin 6 (Hubackova et al., 2012b) and histone deacetylase (Vlasakova et al., 2007). Furthermore, post-transcriptional regulation by alternative splicing, altering mRNA stability or mRNA translation was demonstrated via herpes simplex virus-1 infection, miR-1246 micro RNA and RAS or p38 pathways (Hsu and Kao, 2018).

PML transcripts are expressed in sperm (PMLII) as well as in oocyte (PMLI, PMLII) (Ebrahimian et al., 2010). During embryonic development, PML nuclear bodies (NBs) first appear in 2-cell embryo in mouse, a process that is probably related to major zygotic genome activation (Ebrahimian et al., 2010).

4.2 PML nuclear bodies

Majority of nuclear PML protein is diffusely localized in the nucleoplasm, only part of it is assembled in so called PML nuclear bodies (NBs) (Lallemand-Breitenbach et al., 2001). PML NBs are small spheres <1 µM, of the ring shape. No DNA or RNA is detectable in the middle, but the periphery is bound to nuclear matrix (Lallemand-Breitenbach and de The, 2010; Sahin et al., 2014a). PML creates a stable, insoluble shell of PML bodies, while the inside contains several hundreds of proteins, some of them constitutively, others only after specific stimuli. The constitutive members comprise, for example, speckled 100 kDa (Sp100, transcriptional activator), death domain associated protein (Daxx, transcriptional repressor), CREB binding protein (CBP, acetylase), SUMO (small ubiquitin-like modifier) and SUMO ligases, DNA damage proteins – ataxia telangiectasia and Rad3-related protein (ATR), checkpoint kinase 2 (CHK2), Bloom helicase (BLM), Rad51 protein, replication protein A (RPA) and meiotic recombination 11 homolog 1 (MRE11) (Bernardi and Pandolfi, 2007). Interestingly, knockout of none of these proteins changes the morphology of PML NBs; only the PML protein itself is indispensable for their formation (Ishov et al., 1999; Zhong et al., 2000a). Notably, it seems that all nuclear PML isoforms co-exist in the PML shell (Condemine et al., 2006; Nisole et al., 2013), however, overexpression of each PML isoform in PML WT background changes the pattern of PML NBs (Beech et al., 2005).

4.2.1 Formation of PML NBs

The assembly of PML NBs is a multi-step process. First, PML dimers and tetramers are formed, via the N-terminal RBCC motif (de The et al., 2012; Wang et al., 2018). Most likely, oxidation of one cysteine within the B-box domain is important for this process (Jeanne et al., 2010; Niwa-Kawakita et al., 2017; Sahin et al., 2014b; Zhang et al., 2010). Then, PML is SUMOylated by different SUMO ligases, which, together with PML multimerization, was originally thought to be necessary for PML multimerization and shell formation (Cappadocia et al., 2015; Shen et al., 2006). However, other experiments with different PML isoforms suggested that PML NBs form even when PML lost the ability to be SUMOylated or to bind SUMO (Cuchet et al., 2011; Sahin et al., 2014b). Therefore, whether or not PML SUMO modification is necessary for formation of hollow spheres from PML aggregates is still under discussion (Sahin et al., 2014b; Shen et al., 2006). Nevertheless, both PML SUMOylation and non-covalent interaction with SUMO is crucial for the last step of PML NBs assembly – recruitment of partner proteins. Indeed, most of the

NB-resident proteins either can be SUMOylated or contain SIM (Bernardi and Pandolfi, 2007; Sahin et al., 2014a) (Figure 8).

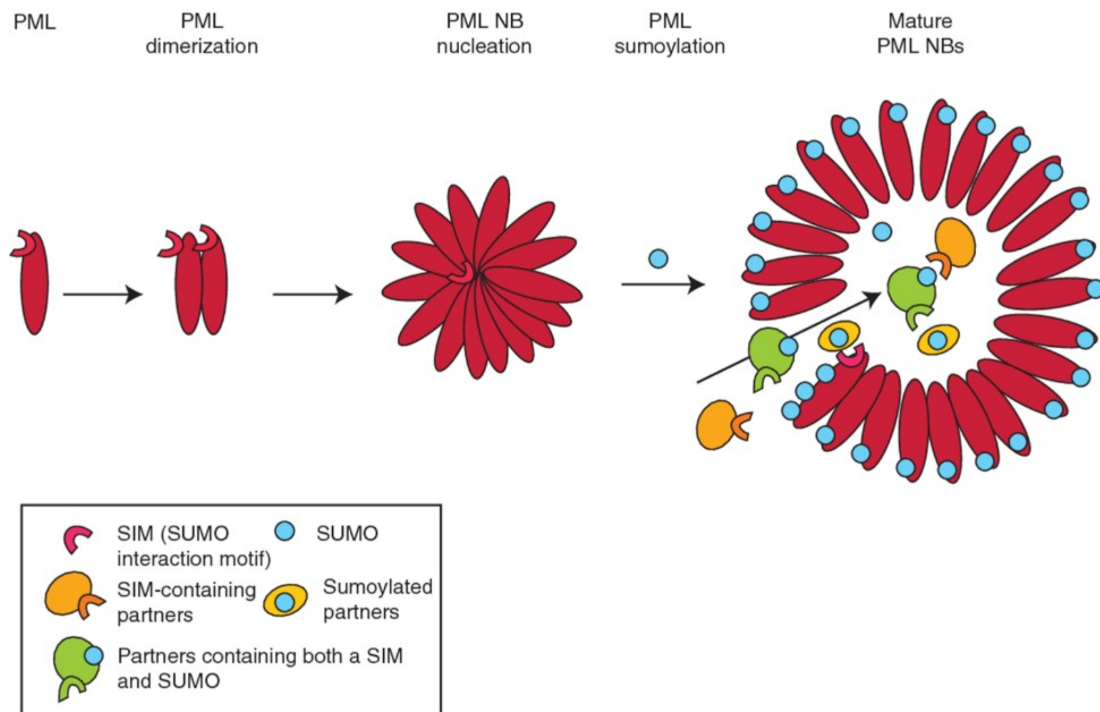


Figure 8: Formation of PML bodies. (adapted from(Lallemand-Breitenbach et al., 2001)).

4.3 The function of PML and PML NBs

Many different functions were assigned to PML protein and PML nuclear bodies. The three most studied and characterized areas encompass:

- anti-viral response,
- DNA damage response, cell cycle arrest, tumor suppression and apoptosis,
- protein storage and modification.

4.3.1 Anti-viral response

Upon viral infection, PML NBs are disrupted and PML expression is induced in response to interferon, which already suggests that PML plays a role in anti-viral defense (Maarifi et al., 2014). The main PML function is to sequester viral DNAs, RNAs or proteins that are necessary for viral replication and particle assembly. For example, PML IV blocks the propagation of encephalomyocarditis virus by sequestering the viral polymerase within PML NBs (Maroui et al., 2011); the early steps of human immunodeficiency virus (HIV) replication are inhibited by PML

binding to the HIV genome in the cytoplasm (Turelli et al., 2001); and the loss of PML renders cells or animals more susceptible to viral infection, as shown for *Pml*$^{-/-}$ mouse embryonic fibroblasts upon rabies viral infection or *Pml* KO mice upon lymphocytic choriomeningitis and vesicular stomatitis viral infection (Bonilla et al., 2002).

4.3.2 DNA damage response

PML participates on DNA damage response by several mechanisms. To mention at least several of them, PML:

- Interacts with Werner helicase and promotes its localization to the sites of DSBs (Liu et al., 2011),
- in S phase, it interacts with Bloom helicase, suggesting its role in the integrity of replication forks (Bischof et al., 2001; Zhong et al., 1999),
- activates the CHK2 kinase by mediating its autophosphorylation (Yang et al., 2006),
- is important for DNA repair by homologous recombination (Boichuk et al., 2011; Vancurova et al., 2019; Yeung et al., 2012),
- is associated with telomeres in cells that maintain their telomeres by a mechanism known as alternative lengthening of telomeres (ALT) and participates in the ALT process (Chung et al., 2011; Yeager et al., 1999),
- is required for telomere stability and preventing telomeric DNA damage (Marchesini et al., 2016).

Above this, PML has been found to associate with many DNA damage repair proteins before and after damage, while the functional consequences of many of these interactions have not been fully elucidated (Dellaire and Bazett-Jones, 2004).

4.3.3 Cell cycle arrest, tumor suppression, senescence and apoptosis

The pathways leading to cell cycle arrest, tumor suppression, senescence and apoptosis are to a large extent intertwined and PML acts as their regulator in both p53-dependent and -independent way. The most well-known examples of these regulation are presented, as follows.

4.3.3.1 Stabilization of p53

- PML mediates various post-translational modifications that stabilize p53, namely:
    - acetylation by CBP or 60 kDa Tat-interactive protein (TIP60) (Cheng et al., 2008; Pearson et al., 2000; Rokudai et al., 2013),

  - phosphorylation by homeodomain interacting protein kinase 2 (HIPK2) and checkpoint kinases 1 or 2 (Chk1, Chk2) (Alsheich-Bartok et al., 2008; D'Orazi et al., 2002; Louria-Hayon et al., 2003; Moller et al., 2003).

- PML also stabilizes p53 by sequestrating HDM2 in the nucleolus (Bernardi et al., 2004; Wei et al., 2003).
- On the other hand, PML facilitates sirtuin 1 (SIRT1)-mediated p53 deacetylation and destabilization (Langley et al. 2002).

4.3.3.2 p53-independent cell cycle arrest

- Via the pRb-E2F1 pathway (Regad et al., 2009; Vernier et al., 2011),
- via inhibiting the PI3K-AKT-mTOR/HIF1 pathway (Song et al., 2008; Trotman et al., 2006).

4.3.3.3 Induction of senescence

- Expression of PMLIV isoform in $PML^{+/+}$ fibroblasts induces senescence in p53-dependent manner (Bischof et al., 2002).
- PMLIV interacts with telomerase reverse transcriptase (TERT) and decreases its activity, leading to telomere shortening and senescence (Oh et al., 2009).
- PML is required for Kirsten rat sarcoma viral oncogene homolog (K-RAS)-induced senescence (Ferbeyre et al., 2000; Scaglioni et al., 2006).

4.3.3.4 Mediating apoptosis

- PML is important for caspase activation after γ-irradiation and CD95/Fas signaling, as $PML^{-/-}$ mice are resistant to the lethal effects of both stimuli (Wang et al., 1998).
- DNA damage-induced, p53-dependent apoptosis is impaired in $PML^{-/-}$ primary cells (Guo et al., 2000).
- c-Jun transcriptional activation and induction of apoptosis in response to DNA damage are induced by PML (Salomoni et al., 2005).
- The adapter protein tumor necrosis factor receptor 1 (TNFR1)-associated death domain (TRADD) protein is be recruited to PML NBs, where it can activate apoptosis by different mechanism than at the membrane (Morgan et al., 2002).

- PML modulates calcium release from endoplasmic reticulum, thus mediating mitochondria-dependent apoptosis (Giorgi et al., 2010).
- PML sequestrates the transcription repressor Daxx, thus activating expression of apoptosis-related genes (Zhong et al., 2000a).

Furthermore, PML has been found to prevent cancer development and progression in various mice models as well as in human cancers (Gambacorta et al., 1996; Gurrieri et al., 2004; Scaglioni et al., 2006; Trotman et al., 2006; Wang et al., 1998; Zhang et al., 2000).

4.3.4 Post-translational modifications of proteins

PML bodies are often referred to as platforms for post-translation modification of proteins, as many protein modification enzymes are localized there (Bernardi and Pandolfi, 2007). The modifications of p53 protein have already been acknowledged; and also many other pathways that have been mentioned here are actually dependent on PML-mediated posttranslational modifications of proteins. For example, the cell cycle arrest through pRb-E2F1 pathway mechanistically relies on dephosphorylation of pRb by protein phosphatases 1A (PP1A) or 2A (PP2A) in PML NBs (Regad et al., 2009). Similarly, PML facilitates PP2A-mediated dephosphorylation of AKT, creating a barrier to AKT-induced tumorigenesis (Trotman et al., 2006).

A large and important area in the PML field are SUMO modifications. PML itself contains SUMO-modified residues and can bind SUMOylated proteins through SIM. What is more, other proteins that reside in PML NBs are also either SUMOylated or contain SIM and this feature is crucial for their recruitment to PML bodies (Bernardi and Pandolfi, 2007; Matunis et al., 2006; Sahin et al., 2014a; Shen et al., 2006). Sometimes, protein SUMOylation and localization to PML bodies happens on certain signal and has important consequences, which is the case e.g. for nuclear factor erythroid 2-related factor (NRF2) (Malloy et al., 2013) or Daxx (Chang et al., 2011). PML itself has been reported to possess E3 SUMO ligase activity (Chu and Yang, 2011; Guo et al., 2014a) and many SUMO ligases are localized to PML bodies, making PML NBs sites of protein SUMOylation (Sahin et al., 2014b; Van Damme et al., 2010).

4.3.5 Other PML functions

Besides all above mentioned functions, PML has been documented to act in:

- regulation of mRNA transcription (Khan et al., 2001; Kumar et al., 2007; Lehembre et al., 2001; Salsman et al., 2017; Suico et al., 2004; Tashiro et al., 2004; Ulbricht et al., 2012; Vernier et al., 2011; Zhong et al., 2000b),

- regulation of mRNA translation (Cohen et al., 2001; Culjkovic et al., 2006),
- chromatin modifications and remodeling (Delbarre et al., 2017; Everett et al., 1999; Kumar et al., 2007; Luciani et al., 2006; Seeler et al., 1998; Shastrula et al., 2019; Spirkoski et al., 2019; Zhang et al., 2005),
- TGF-β signaling (Lin et al., 2004),
- redox homeostasis and mitochondrial function (Guo et al., 2014b),
- autophagy (Missiroli et al., 2016; Morganti et al., 2019),
- suppression of the M2 pyruvate kinase (Shimada et al., 2008),
- promoting cell stemness (Chuang et al., 2011; Ito et al., 2008; Regad et al., 2009),
- fatty acid oxidation (Carracedo et al., 2012),
- circadian rhythms (Miki et al., 2012),
- proteasomal degradation of misfolded, viral and exogenous proteins (Anton et al., 1999; Dino Rockel and von Mikecz, 2002; Lallemand-Breitenbach et al., 2001).

4.4 PML redistribution after stress

PML nuclear organization is sensitive to various types of stress.

Above all, PML reacts to an oxidative stress. As a cysteine-rich protein with 7 free cysteine residues, PML is susceptible to oxidation. Namely two cysteines in the B-box domain seem to be important for PML dimer formation and assembly of PML NBs, following treatment with arsenic or H_2O_2 (Jeanne et al., 2010; Zhang et al., 2010). NBs assembly is induced also by other oxidants, like paraquat or paracetamol (Niwa-Kawakita et al., 2017; Sahin et al., 2014a); and its deacetylation and subsequent nuclear accumulation has been reported after H_2O_2 treatment (Guan et al., 2014). Furthermore, oxidative stress promotes PML degradation by various mechanisms, often through its increased SUMOylation (Erker et al., 2013; Louria-Hayon et al., 2009; Rabellino et al., 2012; Reineke et al., 2008; Weisshaar et al., 2008).

Many kinds of stress cause the dispersal of PML NBs into small dots or even into diffuse pattern. This has been shown after heat shock, heavy metals (Eskiw et al., 2003; Maul et al., 1995; Nefkens et al., 2003), UV radiation (Salomoni et al., 2005; Seker et al., 2003) or alkylating agents (Conlan et al., 2004).

Other stresses, including γ irradiation, cause an increase in PML NBs number (Carbone et al., 2002; Dellaire et al., 2006).

After treatment with doxorubicin (Bernardi et al., 2004; Condemine et al., 2007), mitomycin C (Bernardi et al., 2004) or actinomycin D (Janderova-Rossmeislova et al., 2007; Shav-Tal et al., 2005), after proteasome inhibition (Mattsson et al., 2001) or in senescent cells (Janderova-Rossmeislova et al., 2007), PML has been found to localize to the nucleolus.

To conclude, PML is a protein with diverse functions, among which tumor suppression and regulation of apoptosis has an important place. PML reacts to different stresses and after several treatments it has been found to translocate to the nucleolus. However, so far, this PML redistribution has not been systematically investigated.

In our study we defined several types of nucleolar PML structures, described the process of their formation, showed that rDNA, γH2AX and rDNA-processing proteins are present in the structures and suggested their function in rDNA processing and senescence induction (Imrichova et al. 2019). Furthermore, we characterized PML domains and residues important for the interaction and identified the nature of stress that leads to PML nucleolar redistribution (not published).

5 The nucleolus upon genotoxic stress, in cancer and in senescence

The nucleolus is slowly emerging as an important orchestrator of cell response to genotoxic stress (Lindstrom et al., 2018). Therefore, it is intriguing that after treatment with some (but not all) chemotherapeutic agents, the promyelocytic leukemia protein (PML), which was described - besides others - as a tumor suppressor, associates with the nucleolus (Bernardi et al., 2004; Condemine et al., 2007; Janderova-Rossmeislova et al., 2007).

5.1 The nucleolus

The nucleolus is a membrane-less organelle, the main function of which is ribosome biogenesis. It is assembled around so called nucleolar-organizing regions (NORs), actively transcribed tandem repeats composed of ribosomal DNA (rDNA) and intergenic spacers. The activity of the loci is crucial for the nucleolar assembly, as silent, non-transcribed rDNAs are not included in the nucleoli (Grob et al., 2014; McStay and Grummt, 2008).

5.1.1 Ribosomal DNA

Ribosomal DNA (rDNA) is organized in tandem arrays, consisting of coding regions (about 13 kb) and intergenic spacers (about 30 kb) (Gonzalez and Sylvester, 1995). In humans, approximately 300 of these units are spread on short arms of five acrocentric chromosomes – 13, 14, 15, 21 and 22 (Henderson et al., 1972). The rDNA arrays are flanked by proximal and distal junctions, positioned close to the centromere and telomere, respectively; and surrounded on both sites by perinucleolar heterochromatin (Nemeth and Langst, 2011).

rDNA is transcribed by RNA polymerase I (Pol I) into a 47S RNA precursors, which are subsequently devoid of their external and internal transcribed spacers and further processed, yielding mature 18S, 5.8S and 28S rRNAs (Gonzalez and Sylvester, 1995). What is interesting, even in active NORs, cca 50% of the repeats are silenced, adopting the epigenetic marks of closed chromatin (Goodfellow and Zomerdijk, 2013; McStay and Grummt, 2008). The determining factor for NOR (in)activity is an upstream binding factor protein (UBF), one of the components of Pol I transcription pre-initiation complex (Goodfellow and Zomerdijk, 2013). During mitosis, UBF binds the NORs that were active during previous interphase and allows their rapid re-activation after mitosis, thus establishing them as the seeding elements of new nucleoli (Grob et al., 2014; Grob and McStay, 2014).

UBF binds the rRNA promoter that lies within an intergenic spacer (IGS), which reveals the IGS as an extremely important regulatory region. Apart from the 47S pre-RNA promoter, that serves as a binding site for the UBF and SL1 (selectivity factor 1) transcription factors, the IGS contains: spacer promoters and repetitive enhancer elements that help to control pre-rRNA synthesis; terminator elements downstream and upstream of the pre-RNA transcription unit that can bind transcription termination factor (TTF-I); origins of DNA replication and replication fork barriers (RFBs); tandem repeats; and non-coding RNA genes transcribed by Pol II under certain stress conditions (Figure 9) (Goodfellow and Zomerdijk, 2013).

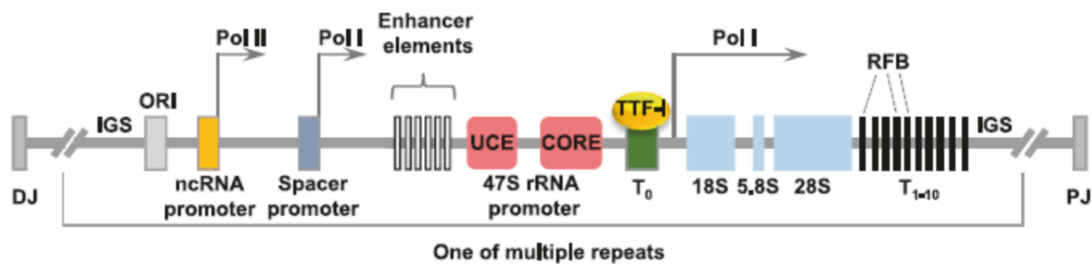


Figure 9: Schematic diagram of the rDNA array. (Lindstrom et al., 2018)
DJ – distal junction, IGS – intergenic spacer, ORI – origin of replication, Pol II – RNA polymerase II transcription start site, Pol I – RNA polymerase I transcription start site, CORE – rDNA core promoter, UCE – upstream control element, T_0 – terminator, TTF-I – transcription termination factor I, RFB – replication fork barrier, PJ – proximal junction.

5.1.2 Nucleolar sub-compartments

The nucleolus consists of three different compartments. The most central part is called fibrillar center (FC), it is surrounded by dense fibrillar component (DFC) and these two are in turn surrounded by granular component (GC). Each step of ribosome biogenesis occurs in one of the compartments, beginning in the center of the nucleolus and proceeding to the outer parts. The transcription of the rDNA takes place at the border between FC and DFC, the nascent pre-RNA is processed in the DFC, and whole 40S and 60S ribosome subunits are assembled in the GC (Figure 10) (Pederson, 2011). Reflecting the different processes that happen within the three sub-compartments, each of them has a distinct protein composition. Thus, FC/DFC are characterized by the presence of UBF and fibrillarin, while GC typically contain nucleolar protein 52 (Nop52) or nucleophosmin (B23) (Savino et al., 2001).

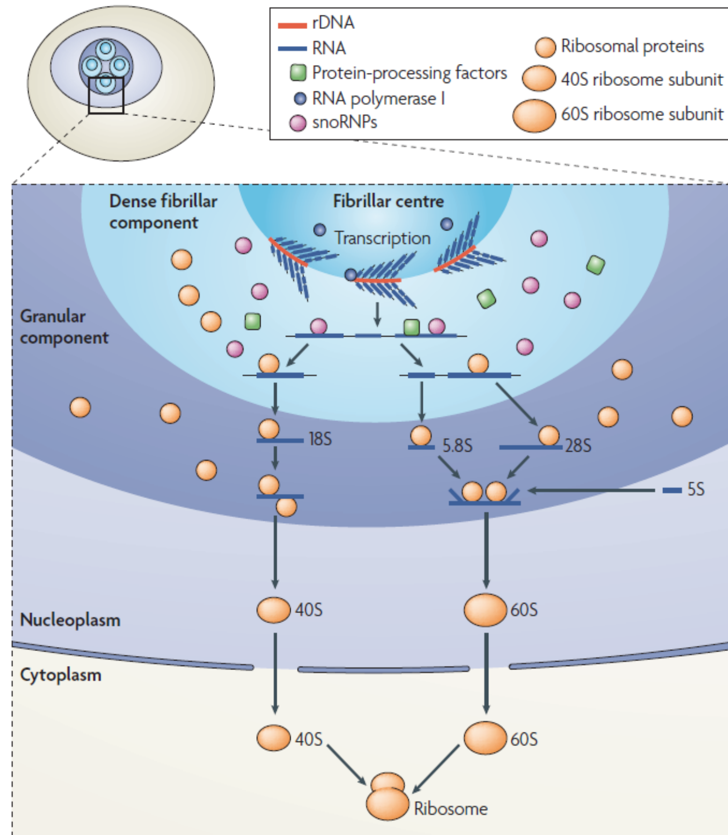


Figure 10: Nucleolar architecture and ribosome biogenesis. (Boisvert et al., 2007)

However, this architecture is solely dependent on ongoing rDNA transcription, as inhibition of Pol I leads to so called nucleolar segregation, during which GC gets to the center of the nucleolus and it is surrounded by "nucleolar caps", containing rDNA and material from both FC and DFC (Floutsakou et al., 2013; Shav-Tal et al., 2005; Sirri et al., 2008).

During cell cycle, the nucleolus demonstrates a great plasticity. It is disassembled before metaphase, while only some of the nucleolar proteins remain associated with metaphase chromosomes. Upon exit from mitosis, pre-nucleolar bodies form, containing pre-RNA processing proteins. During telophase, rRNA transcription is restored and rDNA-bearing chromosomes fuse together, forming the basis of the future nucleolus. This fusion further continues through G1 phase, when the active NORs are already surrounded by nascent FC, DFC and GC, to form several mature nucleoli. Another reorganization of the nucleolus happens in early S phase, when active rDNAs are replicated. During this period, the nucleoli disassemble and transiently lose Pol I machinery, presumably to prevent a collision between replication and transcription (Guan et al., 2017; Hernandez-Verdun, 2011; Smirnov et al., 2016).

5.1.3 The DNA damage repair in the nucleolus

Regarding the unique organization of the nucleolus and the repetitive nature of the rDNA, the DNA damage repair pathway inside the nucleolus has some specific aspects, compared to the repair of general chromatin. From the many types of DNA damage, the double strand breaks (DSBs) are among the most dangerous ones (Hoeijmakers, 2009). In response to nucleolar DSBs, first rDNA transcription is shut down via ATM (ataxia teleangiactasia mutated) signaling (Harding et al., 2015; Korsholm et al., 2019) and subsequently the damaged DNA is relocated to the nucleolar boundary in a form of nucleolar caps (Figure 11) (Harding et al., 2015; van Sluis and McStay, 2015). However, what happens next is a matter of debate. Generally, DSBs can be repaired either by non-homologous end joining (NHEJ), which means that the two ends are directly stuck together without much of a processing; or by homologous recombination (HR), encompassing DNA end resection, strand annealing, synthesis of missing sequences and final ligation (Ciccia and Elledge, 2010; Kanaar et al., 2008). In vast majority of chromatin, NHEJ is the preferred DSB repair pathway, even in G2 phase, when sister chromatid could theoretically be used as a template for HR (Karanam et al., 2012). However, this is not true for repetitive heterochromatin, where the damage is preferably repaired by HR (Chiolo et al., 2011; Murray et al., 2012). rDNA in the nucleolus is repetitive on one hand, but (at least partially) transcriptionally active, on the other hand, which makes the situation more complicated.

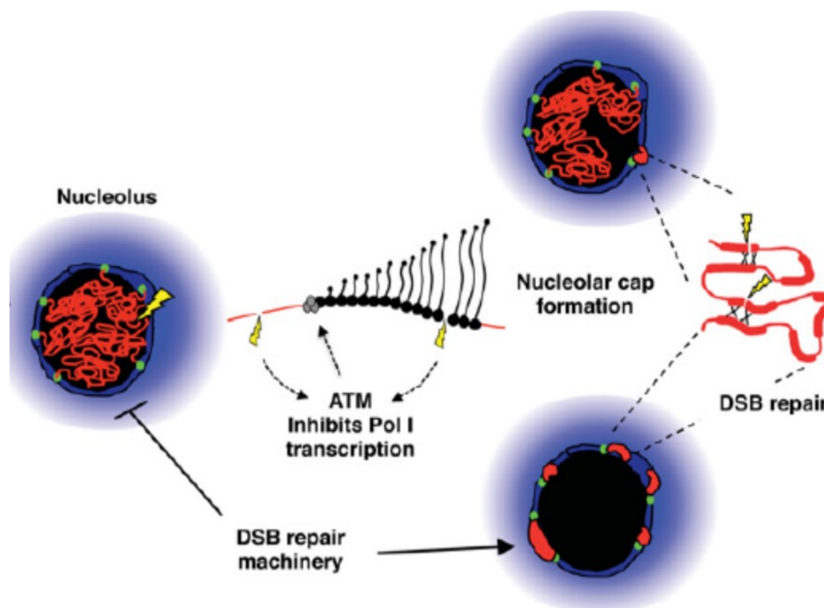


Figure 11: Inhibition of Pol I transcription and segregation of rDNA after double-strand break. (adapted from (van Sluis and McStay, 2015))
Red – rDNA, green – distal junctions, blue – perinucleolar heterochromatin.

Harding et al. suggest that NHEJ is the main pathway used in ribosomal DNA damage response (Harding et al., 2015). On the contrary, van Sluis and McStay showed that rDNA DSBs can be also repaired through HR and, what is more, that this pathway is used throughout the cell cycle including G1 phase, suggesting, that rDNA repeats on the same chromosome or on neighboring chromosomes are the template for HR (van Sluis and McStay, 2015). Importantly, Warmerdam et al. showed that HR in rDNA leads to rDNA instability and shutting down HR results in more efficient DSB repair (Warmerdam et al., 2016).

What is interesting, DNA damage inside the nucleolus leads to Pol I inhibition and segregation only in that particular nucleolus; all other nucleoli remain unaffected (Kruhlak et al., 2007).

The wrongly repaired or unrepaired DNA damage leads to mutations, senescence or apoptosis (see Chapter 1.2). Concerning rDNA, mutations, such as loss or duplication of rDNA copies, are implicated in many pathologies, including cancer, premature aging or neurological impairments (ataxia-telangiectasia and Bloom syndrome) (Diesch, Hannan, & Sanij, 2014). Some data also suggest that thank to its repetitive character, the damage in rDNA is more difficult to repair (Warmerdam et al., 2016), similarly to the DNA damage in telomeres (M. Fumagalli et al., 2012). Such problematic sites often evolve into permanent lesions and are the main cause for the onset of senescence (see Chapter 5.4).

5.2 Nucleolar stress

Having in mind that ribosome biogenesis requires more than 60% of cellular energy (Zhou et al., 2015), it is not surprising that this process is restrained or completely shut down in states of severe cellular stress, when all available energy is needed to overcome the stress conditions (Boulon et al., 2010). What is important, the redistribution of cellular energy is not the only outcome. Perturbation of one or more phases of ribosome production leads to an imbalance of ribosome components; which enables specific protein-protein interactions and results in an activation of pathways involved in cell cycle arrest, cellular senescence and DNA repair (Boisvert et al., 2007; Boulon et al., 2010; Golomb et al., 2014). This phenomenon is referred to as a ribosomal or nucleolar stress.

5.2.1 Ways to impair ribosome biogenesis

The generation of ribosomes comprises several steps: rDNA transcription, early and late pre-rRNA processing and ribosome assembly (Kressler et al., 2017). Even though all of them may be targeted, the inhibition usually happens at the level of rDNA transcription, as it is the most efficient considering energy saving (Sharifi and Bierhoff, 2018). The p53 tumor suppressor interferes with ribosome biogenesis at all levels. However, as p53 stabilization and activation

are supposed to be secondary to ribosomal stress, I deliberately omit the p53-induced pathways from the following overview to come back to them later.

5.2.1.1 Inhibition of RNA polymerase I

There are many conditions upon which RNA polymerase I (Pol I) is inhibited. They range from a DNA damage inside or outside the nucleolus, through multiple cellular stresses, to a treatment with more or less specific Pol I inhibitors (Burger et al., 2010; Grummt, 2003; Sharifi and Bierhoff, 2018). In most cases, the inhibition of Pol I is accompanied by nucleolar segregation (Burger et al., 2010; Grummt, 2003; Sharifi and Bierhoff, 2018).

5.2.1.1.1 DNA damage inside the nucleolus

As discussed in the chapter 5.1.3, in response to nucleolar DSBs, rDNA transcription is shut down via ATM signaling (Harding et al., 2015; Korsholm et al., 2019).

5.2.1.1.2 DNA damage outside the nucleolus

- Kruhlak et al. showed that Pol I is inhibited in response to DSBs anywhere in the nucleus and that the ATM/NBS1 (Nijmegen breakage syndrome 1)/MDC1 (Mediator of DNA-damage checkpoint protein 1) pathway is necessary for this process (Kruhlak et al., 2007).

- More specifically, the ATM-dependent accumulation of Treacle and NBS1 in the nucleolus upon DSBs occuring elsewhere in the nucleus has been described by Larsen et al. They observed that Pol I inhibition in this case is global, i.e. it occurs in all nucleoli of the particular cell, and it is not accompanied by nucleolar segregation and cap formation (Larsen et al., 2014).

- Pol I activity can also be inhibited upon cisplatin-induced DNA damage in DNA-PKcs (DNA-dependent protein kinase, catalytic subunit)- and PARP-1 (Poly [ADP-ribose] polymerase 1)-dependent manner (Calkins et al., 2013).

5.2.1.1.3 Cellular stress of various origin

There are more stress conditions that lead to an inhibition of Pol I. Sometimes, the underlying signaling pathways are not known, but there are many examples when the molecular mechanism has been well characterized:

- Nutrient deprivation – increased AMP/ATP ratio upon cell starvation activates the STK11-AMPK (serine/threonine kinase 11 and 5' AMP-activated protein kinase) pathway (Sengupta et al., 2010) and leads to an inhibition of mTOR kinase. rDNA

transcription is then inhibited via compromised binding of the transcription factors TIF-1A (transcription initiation factor 1A), SL1, and UBF to rDNA promoter (Mayer and Grummt, 2006; Xiao and Grove, 2009). Other proteins that are, together with mTOR, involved in cell response to changes in nutrient levels are the PI3K (phosphatidylinositol 3-kinase), and MAPK (James and Zomerdijk, 2004).

- Replication stress – upon replication stress, the ataxia telangiectasia and Rad3-related kinase (ATR) and DNA topoisomerase II binding protein 1 (TopBP1) are activated. The increase in TopBP1 levels promotes TopBP1 binding to rDNA, inducing a shut-down of rDNA transcription and nucleolar segregation (Sokka et al., 2015).
- Hypoxia – rRNA synthesis is decreased in a process requiring the interaction of the von Hippel-Lindau (vHL) tumor suppressor with the rDNA promoter (Mekhail et al., 2006).
- Heatshock – inhibition of Pol I by various pathways has been demonstrated upon heatshock (Liu et al., 1996; Welch and Suhan, 1985).
- Oncogenic stress – oncogenes, such as RAS or c-Myc increase the levels of alternative reading frame protein (ARF) (Lin and Lowe, 2001; Meyer and Penn, 2008). ARF, in turn, negatively regulates Pol I transcription termination factor TTF-I (Lessard et al., 2010) and inhibits phosphorylation of UBF, thus preventing its binding to the promoter site (Ayrault et al., 2006).

5.2.1.1.4 Knock-out and inhibition of proteins involved in Pol I transcription

In vitro, the inhibition of Pol I transcription has been documented for example after silencing of POLR1A, a gene encoding the catalytic subunit of Pol I (Donati et al., 2011a), knockout of the TIF1-A gene (Yuan et al., 2005) or inactivation of UBF by a monoclonal antibody (Rubbi and Milner, 2003).

5.2.1.1.5 Chemical treatment

Pol I inhibition is the mode of action of many chemotherapeutic drugs, as is discussed more into detail in chapter 5.3.3.

5.2.1.2 Inhibition of pre-rRNA processing

5.2.1.2.1 Knock-out and inhibition of proteins involved in pre-rRNA processing

- As for early pre-rRNA processing, depletion or downregulation of several early processing factors leads to decline of rRNA synthesis, impaired rRNA processing, nucleolar stress and p53 induction. This has been – at least to a certain extent – shown

for: transcriptional U three protein (t-UTP) (Prieto and McStay, 2007), Treacle (Valdez et al., 2004), HEAT repeat containing 1 (HEATR1) (Turi et al., 2018), fibrillarin (Watanabe-Susaki et al., 2014), U3 small nucleolar RNA (snoRNA) or U8 snoRNA (Langhendries et al., 2016).

- Similarly, an expression of dominant negative mutant of Bop1 (block of proliferation 1) protein, involved in late pre-rRNA processing, impairs 28S and 5.8S rRNA processing and 60S ribosome biogenesis (Strezoska et al., 2000).
- Two other multifunctional proteins nucleophosmin (B23) and nucleolin (C23) are also necessary for late rRNA processing, however, at the same time they are the mediators of ribosomal stress (Kurki et al., 2004; Saxena et al., 2006). Therefore, even though several studies have reported p53 induction after B23 or C23 depletion (Qin et al., 2011; Takagi et al., 2005), usually low levels of these proteins rather attenuate the nucleolar stress response that induce it.

5.2.1.2.2 <u>Chemical treatment</u>

Chemical inhibitors of rRNA processing are, again, discussed in chapter 5.3.3.

5.2.1.3 Inhibition of ribosome assembly

The final step of ribosome biogenesis requires adequate amounts of all ribosomal components. The supply of ribosomal RNA relies on functioning 47 pre-rRNA transcription and processing, i.e. processes that have already been discussed above. However, the ribosomes also consist of ribosomal proteins and 5S rRNA, the production of which is an independent process, subject to different regulatory mechanisms (Kressler et al., 2017). One of the pathways involved is the mTOR pathway, which, apart from Pol I transcription, also controls Pol II transcription of ribosomal protein genes and Pol III transcription of 5S rRNA (Mayer and Grummt, 2006), as well as protein translation (Xiao and Grove, 2009). Similarly, MYC and PI3K/AKT/mTOR pathways induce ribosome biogenesis on several levels (Chan et al., 2011; Li et al., 2014; van Riggelen et al., 2010b).

5.2.1.3.1 <u>Impaired production of ribosomal proteins</u>

The activation of p53 after depletion of ribosomal proteins has been reported repeatedly (Barkic et al., 2009; Bursac et al., 2012; Daftuar et al., 2013; Dutt et al., 2011; Fumagalli et al., 2009; Fumagalli et al., 2012b; Jin et al., 2004; Lindstrom and Nister, 2010; Llanos and Serrano, 2010; Sun et al., 2010; Zhou et al., 2013). Interestingly, not all ribosomal proteins are equally

important for maintaining nucleolar integrity and their individual knockouts have varying effect on p53 stabilization (Liu et al., 2016b; Nicolas et al., 2016).

5.2.1.3.2 Inhibition of 5S RNA transcription and processing

Concerning 5S RNA, depletion of one of the RNA polymerase 3 (Pol III) subunits impairs 5S rRNA transcription and leads to cell cycle arrest in a p53-independent manner (Onofrillo et al., 2017), while the same was observed after depletion of 5S RNA-specific transcription factor TFIIIA (Donati et al., 2013).

5.2.1.3.3 Export and import of ribosomal components

Additionally, to keep proportional amounts of all ribosomal components, the nuclear import and export pathways have to be functional. Ribosomal proteins are transported to the nucleus via several transporter proteins, including importin-β, transportin, Ran-binding protein 5 (RanBP5) and Ran-binding protein 7 (RanBP7), importin-11 and importin-7 (Jakel and Gorlich, 1998; Jakel et al., 2002; Plafker and Macara, 2002). The assembled ribosomal subunits 40S and 60S are, in turn, exported with the help of exportin 1 (Thomas and Kutay, 2003). Accordingly, depletion of importin 7 and depletion or inhibition of exportin 1 results in disruption of the nucleolus and activation of ribosomal stress response (Golomb et al., 2012).

5.2.2 The nucleolar stress responses

In reaction to perturbation of ribosome biogenesis, the nucleolar architecture is changed and several pathways are triggered, leading to a cell cycle arrest, DNA repair, or, potentially, to senescence and apoptosis. Most of them act via the p53 tumor suppressor but several are also p53-independent.

5.2.2.1 Nucleolar reorganization upon stress

Nucleolar stress leads to gross rearrangement of nucleolar components, accompanied by changes in the nucleolar proteome. A typical example is the nucleolar segregation, a process when the components of the FC and GC concentrate at the surface of what remains of the nucleolus, in a form of cap-like structures. This is typically observed after inhibition of Pol I transcription by low doses of actinomycin D (Shav-Tal et al., 2005), UV irradiation (Al-Baker et al. 2005) or inhibition of topoisomerase II (Govoni et al., 1994). Another type of nucleolar reorganization is the fragmentation of the nucleolus that may be caused for example by inhibition of Pol II (Haaf and Ward, 1996) or several protein kinases (David-Pfeuty, 1999).

Alongside with the structure, also the composition of the nucleolus changes after stress. Many quantitative mass spectrometry-based studies have been performed, to monitor the changes

of nucleolar proteome after stresses such as treatment with actinomycin D, Pol II inhibitor 5,6-dichlorobenzimidazole riboside (DRB), proteasome inhibitor MG132 (Andersen et al., 2005), etoposide-induced DNA damage (Boisvert et al., 2010; Boisvert and Lamond, 2010), after viral infection (Emmott et al., 2010; Lam et al., 2010) or treatment with topoisomerase I inhibitor, camptothecin (Cohen et al., 2008). To mention at least several examples, ARF, B23 or C23 are released from the nucleolus upon stress (Gjerset and Bandyopadhyay, 2006; Scott and Oeffinger, 2016), while NF-κB translocates into the nucleolus (Thoms et al., 2010).

5.2.2.2 Stabilization of p53 by nucleolar stress-induced mechanisms

5.2.2.2.1 The IRBC checkpoint and involvement of other ribosomal proteins

- One of the results of impaired ribosome biogenesis is the disproportion of individual ribosomal components. Most excess ribosomal proteins are subject to degradation (Lam et al., 2007), but ribosomal proteins RPL5 and RPL11, together with 5S rRNA, are specifically stabilized and maintained for further signaling, as so called 5S ribonucleoprotein particle (RNP) (Bursac et al., 2012; Donati et al., 2013; Sloan et al., 2013). The signaling cascade they trigger has been recently termed the impaired ribosome biogenesis checkpoint (IRBC) and it is exerted by binding of the 5S RNP to HDM2, the E3 ubiquitin ligase. Normally, HDM2 ubiquitinates p53 and thus targets it for a degradation. Upon 5S RNP binding, HDM2 is inhibited and p53 is stabilized (Bhat et al., 2004; Dai and Lu, 2004; Fumagalli et al., 2012b; Lohrum et al., 2003; Nishimura et al., 2015; Zhang et al., 2003) (Figure 12).

- From the total number of 79 eukaryotic ribosomal proteins, 14 others have been found to function similarly to RPL5 and RPL11 – i.e. to bind HDM2 and inhibit its ubiquitination activity. Namely, these are: RPL6, RPL23, RPL26, RPL37, RPS3, RPS7, RPS14, RPS15, RPS20, RPS25, RPS26, RPS27/RPS27L, and RPS27a (Liu et al., 2016b).

Maintaining relatively high levels of these proteins upon stress is enabled, besides other, thanks to presence of so called TOP (5’-terminal oligopyrimidine) sequence at 5’ terminus of their mRNAs. mRNAs with this sequence are selectively stabilized and their proteins increase in abundance (Caldarola et al., 2009).

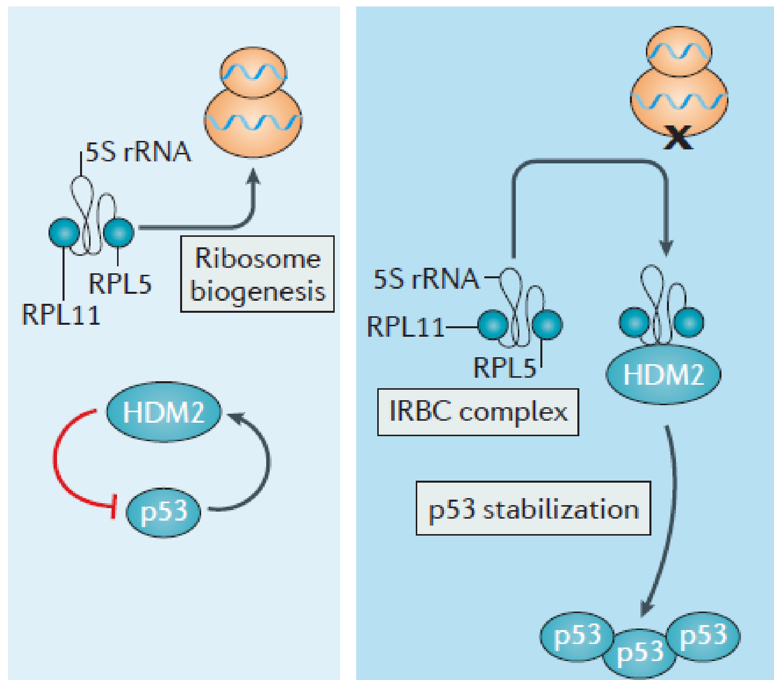


Figure 12: Scheme of the impaired ribosome biogenesis checkpoint. (Pelletier et al., 2018)

5.2.2.2.2 The B23- and C23-dependent interactions

Nucleophosmin (B23) and nucleolin (C23) are nucleolar proteins participating on ribosome biogenesis.

- B23 is a protein with double nucleo-cytoplasmic localization (Borer et al., 1989). In the nucleolus, it sequesters the tumor suppressor ARF and stabilizes it (Bertwistle et al., 2004; Chen et al., 2010; Colombo et al., 2005; Korgaonkar et al., 2005). Upon ribosomal stress, the B23-ARF binding is disrupted and ARF is released to the nucleoplasm where it can bind HDM2, thus inhibiting HDM2-mediated p53 ubiquitination and degradation (Gjerset and Bandyopadhyay, 2006).

  In addition to it, B23 directly binds HDM2 in the nucleoplasm, boosting the inhibitory effect (Kurki et al., 2004).

- Analogically to B23, another rRNA processing protein, C23, is able to bind HDM2 and to stabilize p53 (Saxena et al., 2006).

5.2.2.2.3 Involvement of other proteins

- The ARF protein is specifically induced upon oncogenic signaling mediated by RAS or c-Myc oncogenes (Lin and Lowe, 2001; Meyer and Penn, 2008). Apart from HDM2 inhibition, ARF acetylates p53, leading to its stabilization (Mellert et al., 2007).
- Similarly, MYB binding protein 1A (MYBBP1A) has been found to translocate to the nucleoplasm upon ribosomal stress and to facilitate p53-p300 interaction to enhance p53 acetylation and stabilization (Kuroda et al., 2011).
- Besides, other proteins, such as MDM4 (mouse double minute 4), PML (promyelocytic leukemia), nucleostemin, PICT1 (protein interacting with carboxyl terminus 1) and NEDD8 (neural precursor cell expressed, developmentally down-regulated 8) modulate the RP/HDM2/p53 pathway in response to ribosomal stress (Holmberg Olausson et al., 2012).

5.2.2.3 p53-independent nucleolar stress responses

Alongside with p53-dependent response, several p53-independent pathways leading to cell cycle arrest are activated upon ribosomal stress:

- In response to genotoxic stress in G2, the phosphatase CDC14B (cell division cycle 14B) translocates from the nucleolus to the nucleoplasm and activates the ubiquitin ligase APC/C (anaphase-promoting complex/cyclosome), leading to a degradation of a mitotic kinase Plk1 (polo-like kinase 1). This results in stabilization of claspin and Wee1 kinase, which together induce G2 cell cycle checkpoint (Bassermann et al., 2008).
- In cells lacking p53, other functions of RPL11 and HDM2 leading to cell cycle arrest have been described: in normal conditions, HDM2 is able to stabilize the E2F1 transcription factor, necessary for S-phase entry. Upon ribosomal stress, RPL11 translocates to the cytoplasm, where it inactivates the HDM-2-E2F1-stabilising function, which leads to E2F1 downregulation and cell cycle arrest (Donati et al., 2011b).
- Furthermore, deficiency of ribosomal proteins results in destabilization of Pim1 kinase, which in turn leads to an increase of the $p27^{Kip1}$ cell cycle inhibitor and cell cycle arrest, even in the absence of p53 (Iadevaia et al., 2010).
- Recently, it has been described that upon senescence-induced diminished ribosome biogenesis, the ribosomal protein S14 (RPS14) accumulates in the nucleoplasm, where

it binds and inhibits Cdk4, promoting pRb hypo-phosphorylation and cell cycle arrest (Lessard et al., 2018).

- Finally, p53-independent effects of HDM2 have to be taken into consideration (Ganguli and Wasylyk, 2003).

5.2.2.4 p53 can further reinforce ribosomal stress

The pathways described above all lead to cell cycle arrest and/or senescence and apoptosis. p53 protein, which is the main orchestrator of majority of them, can additionally inhibit ribosome assembly and induce ribosomal stress in a positive feedback loop:

- p53 can function as an inhibitor of RNA Pol I transcription by disrupting SL1-UBF interactions, which results in a decrease in ribosome subunit biogenesis (Zhai and Comai, 2000).

- By binding to TFIIIB, a transcription factor important for transcription by RNA polymerase III (Pol III), p53 also inhibits Pol III transcripts, including 5S rRNA (Chesnokov et al., 1996).

- The canonical p53 target, p21, activates the retinoblastoma tumor suppressor (pRb), a recognized inhibitor of Pol I and Pol III rRNA transcription (Gjidoda and Henry, 2013; Voit et al., 1997).

- Furthermore, p53 inhibits the expression and activity of the mTOR and c-Myc oncogenes, both of which are potent inducers of ribosome biogenesis on multiple levels (Feng et al., 2005; Ho et al., 2005; Levy et al., 1993; Stambolic et al., 2001).

5.3 The role of the nucleolus in cancer

5.3.1 <u>Nucleolar defects connected with cancer development</u>

5.3.1.1 rDNA damage and cancer

The nucleolar DNA is generally prone to instability, owing to some of its inherent properties, such as organization into repeats, distribution on five different chromosomes or high transcription rate (Durkin and Glover, 2007; McStay, 2016). The latter one is especially dangerous in the context of DNA replication, as the two processes may collide, leading to single- or double-strand breaks and R-loop formation (Brambati et al., 2015; Branzei and Foiani, 2010; D'Alessandro and d'Adda di Fagagna, 2017; Helmrich et al., 2013). If wrongly repaired, they can ultimately result in recombinations, mutations and chromosome re-arrangements (Burrell et

al., 2013; Gaillard et al., 2015b), all events that potentially lead to tumor development (Shen, 2011; Tubbs and Nussenzweig, 2017).

Vice versa, it is well established that activation of many oncogenes (including c-Myc, PI3K/AKT, mTORC1, RAS/MAPK, and cyclin E), as well as loss of tumor suppressors (such as phosphatase and tensin homolog [PTEN], pRb, p53, ARF, glycogen synthase kinase 3 beta [GSK3β]), boost the rRNA transcription (Grummt, 2010; Turi et al., 2019), which may further enhance the risk of collision between the transcription apparatus and other DNA-processing machineries.

5.3.1.2 The balance between rRNA and ribosomal proteins in cancer

As stated above, activated oncogenes and diminished tumor-suppressors are direct causes of a high rate of rRNA transcription, ribosome production, proteosynthesis and cell growth, all characteristics of cancer cells (Ruggero, 2013; Ruggero and Pandolfi, 2003; Zhou et al., 2015). The nucleolar hypertrophy (prominent, enlarged nucleolus) is even considered a reliable parameter to estimate tumor aggressiveness (Derenzini et al., 2009).

Of note, the side-effect of an enhanced ribosome biogenesis is, that major pool of ribosomal proteins is engaged in this process; and their availability for HDM2 binding (i.e. exerting their tumor-suppressive function) is therefore reduced. That was nicely shown in an experiment, when synthesis of ribosomes was induced by IL-6, and the consequence, indeed, was HDM2-mediated downregulation of p53 and more aggressive cancer phenotype (Brighenti et al., 2014).

On the other hand, decreased rate of proteosynthesis and, more specifically, low amount of ribosomal proteins, are also connected with cancer predisposition. It is illustrated on a group of diseases called ribosomopathies, which are characterized by reduced levels of ribosomal proteins and which have been identified as a risk factor for tumor progression (Lessard et al., 2019; Liu et al., 2016b; Narla and Ebert, 2010). There are more explanations for this phenomenon, generally, they belong to two categories:

1. Considering the potential tumor-suppressive role of selected ribosomal proteins in HDM-2 binding and p53 stabilization, their lack recognized as a reason why ribosomal stress response cannot be activated and pro-proliferative pathways may freely progress.

    - This is illustrated in an experiment when downregulation of rRNA synthesis and following nucleolar stress was able to induce nuclear stress and p53 response but only in a situation when high levels of ribosomal proteins were present.

Simultaneous downregulation of ribosomal protein production reverted this effect (Donati et al., 2011a).

- Furthermore, the deficiency of different ribosomal proteins (RPL5, RPL10, RPL22) have been documented in several cancers (Penzo et al., 2019).

2. The reduced amount of ribosomal proteins may also be interpreted as a cause of ribosomal stress. The remaining hypotheses are based on this presumption.

   - First, reduced number of ribosomal proteins leads to different composition of ribosomes. These altered ribosomes might preferentially translate the mRNAs of some oncogenes and neglect those of some tumor suppressors, such as p53 and p27 (Ferretti et al., 2017; Shi et al., 2017).

   - Second, lower number of ribosomes, arising from lack of ribosomal proteins, may result in a feedback loop, allowing even defective ribosomes to participate on the translation process, which might reprogram the proteome to the cancer-prone phenotype (Sulima et al., 2014).

   - Third, nucleolar stress and activation of p53 creates a selective pressure for suppressor mutations in the p53 pathway (Ajore et al., 2017; Sulima et al., 2014).

5.3.2 Nucleolus as a mediator of tumor-suppressive response

Various genotoxic and non-genotoxic stress stimuli can lead to a transformation of a tumor cell to a cancerous one. As described in Chapter 4.2.2, the nucleolus is able to sense these stresses and to trigger a stress response, involving activation of several tumor suppressors, thus presenting a barrier to tumor development.

5.3.3 Targeting the nucleolus as an anti-cancer strategy

The presumption that cancer cells are addicted to high rate of ribosome biogenesis initiated development of drugs targeting this process. This strategy is justified, for instance, by the finding that inhibition of translation is critical for cancer cells (Boussemart et al., 2014; Hsieh et al., 2010; She et al., 2010). As explained above, even non-genotoxic treatments can be used to inhibit ribosome biogenesis, raising the possibility of less deleterious cancer cure (Quin et al., 2014). Furthermore, as several pathways triggered by ribosomal stress are p53-independent, they may be functional also in cancers with inactivated p53, which is almost 50 % of all cases (Hollstein et al., 1991).

However, inducing ribosomal stress might not always lead to a satisfactory result. As shown on the example of ribosomopathies, decreased ribosome biosynthesis may even lead to a cancer development by several mechanisms (chapter 5.3.1.2).

5.3.3.1 Inhibitors of rRNA transcription

Many chemicals that inhibit rRNA transcription have been specifically tested for their effect on cancer cells.

- The agents that preferentially intercalate into GC-rich sequences and thus inhibit the Pol I movement on rDNA – actinomycin D (Perry and Kelley, 1970), BMH-21 (Peltonen et al., 2014), aminacrine, ethacridine (Morgado-Palacin et al., 2014).
- Relatively new inhibitors, CX-3543 (Drygin et al., 2009) and CX-5461 (Drygin et al., 2011), that stabilize the G-quadruplex structures on rDNA and block the binding of nucleolin (C23) or selectivity factor 1 (SL1), respectively, to the rDNA genes.

Actinomycin D is already approved chemotherapeutic drug, CX-5461 is in now in phase I clinical trial for patients with BRCA1/2 deficient tumors, CX-3543 in phase I/II in solid and neuroendocrine tumors and lymphomas.

From the new inhibitors, CX-5461 has been the most extensively studied, and its anticancer activity has been reported in multiple cancer backgrounds (Catez et al., 2019). Thanks to stabilization of G-quadruplexes, both these drugs also interfere with DNA replication, which leads to replication fork stalling and strand breaks (Xu et al., 2017). Therefore, at least part of their effects has to be attributed also to DNA damage, which was further supported by the fact that both these inhibitors are the most effective in cells with impaired DNA repair pathways (Bywater et al., 2012; Drygin et al., 2011; Peltonen et al., 2010; Peltonen et al., 2014; Xu et al., 2017). What is promising, both the CX inhibitors and BMH-21 show increased specificity for cancer cells, leaving normal cells less unaffected (Bywater et al., 2012; Drygin et al., 2009; Peltonen et al., 2010; Peltonen et al., 2014).

Besides specific inhibitors of pre-rRNA transcription, there are several chemicals that primarily target other cellular processes, but Pol I inhibition has been identified as one of their side effects. The mechanism of inhibition is not always known and it is not excluded that the DNA damage response pathway or any of the stress response pathways may be involved. The following chemicals belong to this group:

- cisplatin, oxaliplatin, doxorubicin, mitomycin C, mitoxantrone, methotrexate (Burger et al., 2010) and ellipticine (Andrews et al., 2013).

5.3.3.2 Inhibitors of rRNA processing

Targeting other steps of ribosome biogenesis may also contribute to cancer vulnerability. Small nucleolar RNAs (snoRNAs) are important for rRNA processing. It was shown in mouse xenograft models that tumors in which two snoRNAs (U3 and U8) were silenced, were smaller and less aggressive than tumors with functioning snoRNAs (Langhendries et al., 2016). Similar effect was observed for a knock down of an rRNA-processing protein, fibrillarin (Su et al., 2014).

Several chemical substances have been designed to target rRNA processing. For example, the chemical NSC348884 binds B23, weakening B23-mediated ARF sequestration, thus inducing p53 in ARF-dependent way (Qi et al., 2008). Furthermore, C23-specific aptamer AS1411 prevents C23 binding to RNA and induces apoptosis in cancer cells (Bates et al., 2009; Soundararajan et al., 2008). This compound has already entered several phase I and phase II clinical trials (Bates et al., 2017).

Additionally, as in case of rRNA transcription, targeting rRNA processing has been found as a side effect of several already known and used chemotherapeutic drugs:

- Inhibitors of early rRNA processing – camptothecin, 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), flavopiridol, roscovitine, etc. (Burger et al., 2010).
- Inhibitors of late rRNA processing – 5-fluorouracil, MG-132, cycloheximide, homoharringtonine, etc. (Burger et al., 2010).

5.4 The role of the nucleolus and rDNA in senescence

rDNA both in yeast and in mammalian cells is especially prone to instability. Not only is it repetitive, but it is also highly transcribed, which together poses a serious challenge for a replication machinery. Replication fork stalling often proceeds into a DSB, which in the repetitive environment frequently results in erroneous recombination events, ending in gross rearrangements, deletions or duplications (Warmerdam and Wolthuis, 2019).

5.4.1 rDNA and senescence in yeast

In yeast, the recombination between rDNA repeats is often accompanied by formation of extrachromosomal rDNA circles (ERCs), consisting of rDNA repeats that recombine out of the genome (Sinclair and Guarente, 1997). The presence of ERCs, as well as rDNA instability in general, are then connected with yeast senescence and aging (Ganley and Kobayashi, 2014). Several proteins are known to maintain rDNA integrity, while they also possess a protecting role against yeast aging. The examples include the slow growth suppressor 1 (Sgs1) helicase that is known to function in HR (Sinclair et al., 1997); or the silent information regulator 2 (Sir2), a

histone deacetylase that contributes to chromatin silencing in repetitive loci (Fritze et al., 1997; Kobayashi and Ganley, 2005).

5.4.2 rDNA and senescence in mammals

The mammalian rDNA shares many features of the yeast rDNA, such as its repetitive character and a high transcription rate, which makes it equally susceptible to undesired replication events. DSBs and rearrangements of rDNA loci are often found in solid tumors (Stults et al., 2009; Tchurikov et al., 2015). However, rDNA instability is also documented in patients with Bloom syndrome (deficiency of the Bloom syndrome helicase, BLM) and ataxia teleangiectasia (deficiency of the ataxia teleangiectasia mutated kinase, ATM), two syndromes associated with premature aging (Hallgren et al., 2014; Killen et al., 2009); and rDNA replication stress is responsible for aging of hematopoietic stem cells (Flach et al., 2014). Interestingly, homologues of the yeast proteins Sgs1 (human Werner syndrome helicase, WRN) and Sir2 (human sirtuin 7, SIRT7) participate on the rDNA maintenance in humans, suggesting that the processes dealing with rDNA integrity are vitally important and highly conserved. The deficiency of WRN in humans is a direct cause of Werner syndrome, generally known as progeria or premature aging. On a molecular level, the cells of progeria patients demonstrate rearrangements of rDNA repeats, occurrence of extrachromosomal DNA and a propensity to undergo senescence (Caburet et al., 2005). Concerning SIRT7, it has been found recently that it is crucial for maintenance of heterochromatic state of rDNA, via preserving high levels of SMARCA5 protein (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily A, member 5) at rDNA loci. SIRT7-deficient cells show rDNA instability and develop acute senescence, while both these effects can be reverted upon exogenous expression of SMARCA5. This provides a first direct evidence for the role of rDNA (in)stability in senescence development in human cells (Paredes et al., 2018).

Interestingly, BLM helicase is localized both to PML bodies (Ishov et al., 1999) and PML nucleolar structures (Imrichova et al. 2019), and WRN helicase localizes to PML bodies upon DNA damage (Blander et al., 2002), pointing to a possible role of PML in the entangled processes comprising DNA damage, rDNA instability and senescence.

To sum up, the nucleolus is an important organelle, the primary function of which is ribosome biogenesis. Various conditions can disrupt this process and bring about so called ribosomal or nucleolar stress, which results in cell cycle arrest in p53-dependent or -independent way. One of such stress conditions is DNA damage. DNA damage both outside and inside the nucleolus

can trigger nucleolar stress response, however, it is important to point out that ribosomal DNA is especially prone to instability and genomic rearrangements. As those events often lead to cancer development, ribosomal stress-induced tumor suppressive pathways are an important barrier against this process. Vast majority of ribosomal stress pathways are triggered either by selected ribosomal proteins that are stabilized upon stress conditions or by nucleolar proteins that are released from the nucleolus during nucleolar stress-induced nucleolar reorganization. In cancer, the transcription of pre-rRNA is boost up and few ribosomal or nucleolar proteins are available for nucleolar stress execution. Hence, despite several drawbacks of this approach, targeting ribosomal biogenesis in cancer, especially at the level of pre-rRNA transcription, might be a promising future strategy to cure cancer. Recently, the instability of rDNA loci has been indentified as a possible cause of senescence.

In our study, we showed that PML associates with the nucleolus after treatment with doxorubicin, a chemotherapeutic drug that causes DNA damage, inhibits RNAPI, brings about nucleolar stress and induces senescence. This PML nucleolar association (PNA) is dynamic and in its last stage it leads to PML-directed sequestration of part of the nucleolar material into a new compartment called PML nucleolus-derived structure (PML-NDS). In the moment of their formation, all PML-NDS are positive for γH2AX, a marker of DNA damage, which implies their role in rDNA processing. What is more, PML-NDS specifically accumulate proteins that are known to function in DNA metabolism and they can persist in senescent cells for very long time periods. This suggests that the PML compartment described by us might be somehow involved in resolving rDNA damage that, when unresolved, can contribute to senescence development (Imrichova et al. 2019). Furthermore, in our unpublished data we present direct microscopic evidence that rDNA is really present in PML-NDS.

III AIMS OF THE STUDY

The broader aim of this thesis was to better understand cell response to cancer therapies based on genotoxic stress; more specifically, I was interested in the processes of therapy-induced radioresistance, cellular senescence and the behavior of PML protein in the context of nucleolar stress.

The specific aims were:

1. To characterize the population of radiation-induced anoikis-resistant cells in terms of mesenchymal properties and stemness, to identify the pathways responsible for their survival and to propose a mechanism of their eradication.

2. To examine the effect of therapy-induced senescent cells on tumor growth in mice with non-compromised immune system and to verify possible role of IL-12 in tumor elimination.

3. To find a specific marker of senescent cells and to elucidate whether and how it contributes to the senescent phenotype.

4. To better understand the interaction between the PML protein and the nucleolus under stress conditions.

IV COMMENTS ON PRESENTED PUBLICATIONS

Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, ERK signaling-dependent cells

Kyjacova L, Hubackova S, Krejcikova K, Strauss R, Hanzlikova H, Dzijak R, **Imrichova T**, Simova J, Reinis M, Bartek J, Hodny Z: Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, ERK signaling-dependent cells. Cell Death Differ. 2015 Jun;22(6):898-911

In the first study, we aimed to elucidate one of the mechanisms of genotoxic stress-induced radioresistance of cancer cells. As a model for our experiments, we chose metastasis-derived prostate carcinoma (CaP) cell lines. Since ionizing radiation is routinely used to treat prostate cancer (presently, the first-choice therapy for CaP is represented by prostatectomy, followed by localized fractionated ionizing radiation, consisting of 35 doses of 2 Gy (Heidenreich et al., 2014)), our results would offer a clinically relevant insight into this topic.

Prostate carcinoma is the most prevalent cancer type in men, with the second highest incidence and the sixth highest mortality worldwide (Bray et al., 2018). The main problem concerning this type of treatment is acquired radiotherapy resistance of some cells, followed by development of secondary tumors and cancer dissemination. At the time of our study, several mechanisms regarding CaP radioresistance were described, including downregulation of disabled homolog 2-interacting protein (DAB2IP) (Kong et al., 2010), IL-6 and NF-κB signaling (Sakai et al., 2011) or expression of activator protein 1 (AP-1) transcription factors (Kajanne et al., 2009). Many of these effects have been attributed to enhanced activity of pro-survival mitogen-activated protein kinase MAPK, PI3K/AKT and JAK/STAT pathways (Skvortsova et al., 2008).

However, most of the studies dealing with CaP radioresistance at that time displayed two major limitations: 1) they were performed using only one or a few doses of ionizing radiation, while 35 cumulative doses are used in clinics; 2) they were focusing only on the adherent population of irradiated cells, neglecting the non-adherent one. On the contrary, in our laboratory, we noticed that after cell irradiation with 35 doses of 2 Gy, a non-adherent population of cells appeared. Most cells in this population were dead, however, several were able to survive in this non-adherent state, as documented by their ability to re-attach and resume proliferation certain time after the end of the irradiation. Therefore, together with the adherent surviving

population, we decided to characterize also this minor non-adherent sub-population of cells and to identify the pathways that render them radioresistant.

In our study, the adherent radiation-surviving cells adopted senescent phenotype as expected and some of them resumed proliferation at around 2 weeks after the end of irradiation.

The occurrence of an anoikis-resistant non-adherent fraction was not expected and came as a surprise. We observed that these cells persisted in the non-adherent state for protracted period of time (10 – 21 days), which was followed by their re-attachment and proliferation restart. Several transcription factors responsible for induction of mesenchymal phenotype were increased in this non-adherent population, including Snail, Slug, Twist1 and ZEB2 (zinc finger E-box binding homeobox 2). The elevation of Snail was the most consistent between the cell lines and radiation regimes used and the knockdown of Snail resulted in decrease in the number of non-adherent cells. Additionally, the expression of selected stem cell markers was enhanced, namely cluster of differentiation 133 (CD133), sex determining region Y-box 2 (SOX2), octamer binding protein 4 (OCT-4), Nanog and members of the Notch signaling pathway. Interestingly, shortly after re-attachment, the cells exhibited more mesenchymal phenotype than the controls or the irradiated adherent cells and they gradually switched back to epithelial phenotype during serial passaging. Together, these data confirmed the previous observations that radioresistance and anoikis resistance are tightly connected with mesenchymal and stem-like cell characteristics (Bensimon et al., 2013; Cao et al., 2016; Chang et al., 2013; Dave et al., 2012; Easwaran et al., 2014; Facompre et al., 2012; Frisch et al., 2013; Hambardzumyan et al., 2006; Lamb et al., 2013; Panaccione et al., 2016; Stark et al., 2017; Steinbichler et al., 2018; Theys et al., 2011; Wang et al., 2017a; Zhang et al., 2016).

Interestingly, the cells in the non-adherent fraction did not proliferate but they were able to restart proliferation after re-attachment and their tumorigenicity in immunocompromised mice was preserved.

Trying to decipher which factors are responsible for anoikis resistance of the non-adherent fraction, we found out, that inhibition of ERK1/2 signaling or ERK1 and/or ERK2 downregulation impaired cell detachment and survival. This was accompanied by an increased expression of pro-apoptotic protein Bim (Bcl-2-interacting mediator of cell death) and a decreased expression of anti-apoptotic protein Bcl-XL, both of which are regulated by ERK1/2 pathway. Furthermore, combined inhibition of AKT and ERK1/2 not only suppressed the survival of the non-adherent fraction but also prevented escape from senescence in the adherent population.

Altogether, in this study we characterized previously unnoticed population of radioresistant anoikis-resistant cells arising from fractionated ionizing radiation of prostate cancer cells. In concert with previous reports, we demonstrated that these cells activated their mesenchymal and stem-like program.

Concerning resistance to genotoxic anti-cancer treatments, the discussion is going on whether the traits conferring treatment-resistance are already present in cells prior to treatment or whether they are treatment-induced. Our study rather supports the latter model, given that the adherent cells demonstrated epithelial, non-stem cell phenotype and only after irradiation they switched into more mesenchymal phenotype with stem-like characteristics. The cells kept this phenotype even several days after re-attachment and several passages were needed so that they would return back to the epithelial state. What is more, this process of radiation-induced loss of adhesion and consequent re-adhesion, accompanied by respective phenotypic changes, could be repeated several times, confirming that the cell plasticity is indeed triggered by the radiation treatment.

What was intriguing, the anoikis-resistant cells were able to persist in their non-adherent state for several days to weeks, neither proliferating, nor dying, which was remarkable, given that all the examined cell lines were originally adherent. Therefore, it is possible that what we observed, was an in vitro analogy for radiation-induced metastasis, when a cell first needs to separate from its parental tissue, then to acquire anoikis resistance, prevail in a non-adherent state, travel through vasculature to a novel site and there to re-adhere and resume proliferation, giving rise to a secondary tumor. If that was the case, the pathways identified by us as crucial for radiation-induced anoikis resistance would be of a high clinical importance.

Tumor growth accelerated by chemotherapy-induced senescent cells is suppressed by treatment with IL-12 producing cellular vaccines

Simova J, Sapega O, **Imrichova T**, Stepanek I, Kyjacova L, Mikyskova R, Indrova M, Bieblova J, Bubenik J, Bartek J, Hodny Z, Reinis M: Tumor growth accelerated by chemotherapy-induced senescent cells is suppressed by treatment with IL-12 producing cellular vaccines. Oncotarget. 2016 Aug 23;7(34):54952-54964.

In the second study, we characterized the onset of senescence induced by genotoxic-based chemotherapy in epithelial lung and prostate cancer cells of mouse origin; and we performed series of experiments to determine the effect of these senescent cells on the growth of lung carcinoma-derived tumors in mice. Furthermore, we suggested how the detrimental effect of senescent cells could be overcome by immunotherapeutic approach and we managed to verify this concept.

In tumors, senescence can occur either as an anti-tumor barrier, activated by cells in a response to an oncogenic signaling, or as a consequence of radio- and chemotherapy-induced genotoxic stress (Bartkova et al., 2006; Di Micco et al., 2006; Ewald et al., 2008; Gewirtz et al., 2008). In either case, senescent cells influence the surrounding tumor tissue by many ways, secretion of bioactive molecules known together as SASP (senescence-associated secretory phenotype) being the most prominent of them (Coppe et al., 2008; Davalos et al., 2010). The unfavorable role of SASP in tumor progression has been described repeatedly; however, conflicting data has been obtained concerning co-administration of senescent and proliferating tumor cells in mouse model (Ewald et al., 2008; Krtolica et al., 2001). Therefore, we first aimed to dissect the effect of senescent cells on proliferating tumor cell growth.

To obtain senescent cells, we treated two mouse cancer cell lines with docetaxel and we verified their senescent status by assessing several commonly used senescent markers (lack of proliferation, increased senescence-associated-β-galactosidase activity, characteristic cell morphology, increased expression of $p16^{INK4A}$ and $p21^{waf1}$, persistent serine 139-phosphorylated histone H2AX (γH2AX) foci or decreased incorporation of EdU). Next, we analyzed the changes of the secretory phenotypes of these cells and we found out that the mRNA levels of several pro-inflammatory and immunomodulatory cytokines were increased.

To test the effect of these senescent cells on the growth of proliferating tumor cells in mice, we injected the mice either only with proliferating cells or with the mixture of proliferating and senescent cells. Importantly, since the injected cells were of a mouse origin, we were able to perform the experiments in mice with fully functioning immune system. In all cases, we saw accelerated tumor growth after co-injection of proliferating and senescent tumor cells compared to proliferating cells alone.

One potential strategy to eradicate tumors are treatments stimulating immune system to more effectively fight cancer. Interleukin 12 (IL-12) is one of the cytokines successfully boosting anti-tumor immunity by enhancing the expression of interferon gamma and activating NK cells and T cells (Grufman and Karre, 2000; Tugues et al., 2015). IL-12 may be administered to the tumor site by various means, while one of them is so called IL-12 vaccine, when lethally irradiated IL-12-producing cells are injected into the tumor (Bubenik, 1996, 2011). We decided to make use of this approach to test the potential of IL-12-based therapy to diminish tumor growth in our experimental setting.

Interestingly, the IL-12 vaccine significantly attenuated the tumor growth in all tested conditions, i.e. in the tumors originating from the proliferating cells alone, as well as in the tumors originating from co-injection of proliferating and senescent cells.

Of note, by explanting the cells from the arising tumors and their phenotypic characterization, we determined that the tumors really originated only from the non-senescent proliferating cells, which proves that in our experimental settings the senescent cells are not able to bypass senescence and return to the cell cycle and that the accelerated tumor growth is solely the result of paracrine signaling of senescent cells.

To summarize, we established a model system to test the effect of genotoxically-induced senescent cells on a tumor growth in vivo. The major advantage of our experimental setting was the use of a syngeneic model allowing us to observe the tumor growth in mice with functioning immune system, in contrast with vast majority of studies performed in immunocompromised mice. Thus, we could take into account the interaction between the tumor and the immune system, which has been ascribed to play a major role in the progression of the disease.

Several studies have aimed to abolish the unfavorable effects of senescent cells on a tumor tissue, usually by means targeting preferentially or exclusively the senescent tumor cells (Zhang et al., 2019). It is therefore intriguing that our single-treatment IL-12 therapy not only abrogated

the growth-accelerating effects of senescent cells but also restricted the growth of the tumor itself.

Induction, regulation and roles of neural adhesion molecule L1CAM in cellular senescence

Mrazkova B, Dzijak R, **Imrichova T**, Kyjacova L, Barath P, Dzubak P, Holub D, Hajduch M, Nahacka Z, Andera L, Holicek P, Vasicova P, Sapega O, Bartek J, Hodny Z: Induction, regulation and roles of neural adhesion molecule L1CAM in cellular senescence. Aging (Albany NY). 2018 Mar 28;10(3):434-462.

One of the outcomes of cell exposure to genotoxic stress is cellular senescence. Senescent cells do not proliferate but they persist in an organism and influence it by many ways, most of them being detrimental (Baker et al., 2016; Munoz-Espin and Serrano, 2014). Therefore, it is of a high importance to study the interplay between normal and senescent cells in benign tissues, as well as in tumors, and to explore the possible beneficial effects of senescent cells elimination. The main complication concerning the research in this area is the lack of a strictly specific biomarker of senescent cells (Sharpless and Sherr, 2015). Hence, in our third study, we sought to find an unambiguous senescent marker. As we wanted our potential marker to be easily accessible and thus suitable for non-invasive methods, including possible clinical applications, we restricted our search to the proteins that are localized on the outer side of the plasma membrane.

To determine which surface proteins are differentially expressed in replicatively-senescent BJ fibroblasts compared to proliferating controls, we performed biotin labeling of surface proteins, followed by quantitative proteomic analysis. From the list of differentially expressed proteins, thirty-six most upregulated were subject to RT-qPCR analysis; and for five of them the protein levels were also confirmed by immunoblotting. Finally, L1 cell adhesion molecule (L1CAM) was selected for further analysis.

Apart from replicative senescence, cells can undergo so called stress-induced premature senescence, when they enter a permanent cell cycle arrest in response to external stimuli (Toussaint et al., 2002; Toussaint et al., 2000). To decipher whether L1CAM expression is increased also in prematurely senescent cells, we treated the BJ fibroblasts by BrdU or IFNγ, irradiated them by high doses of γ-radiation or let them overexpress H-Ras oncogene; all treatments known to induce premature senescence (Kim et al., 2009; Michishita et al., 1999; Robles and Adami, 1998; Serrano et al., 1997). Intriguingly, L1CAM mRNA and protein level was enhanced after all tested treatments, with the exception of H-Ras-induced senescence.

To rule out the possibility that increased L1CAM expression is a cell type-specific phenomenon, we measured its mRNA and protein levels in proliferating versus senescent cells also in several other cell lines. And, indeed, in most cases, L1CAM expression was increased in IR-induced and BrdU-induced senescent cells compared to proliferating controls, even though the exact expression pattern was specific for each cell line. What is more, L1CAM transcript levels were also increased in three mouse cell lines that were driven to senescence by docetaxel treatment.

Enhanced expression of the p16 cyclin-dependent kinase inhibitor is a shared feature of senescent cells (Rayess et al., 2012). To dissect whether p16 signaling is involved in increased L1CAM expression, we measured L1CAM mRNA and protein levels in cells with p16 ectopic expression. As expected, p16 overexpression drove cells into senescence and, interestingly, their L1CAM mRNA level was increased. Unfortunately, this did not translate into enhanced protein level, suggesting, that p16 signaling is a condition necessary but not sufficient for increased L1CAM protein expression and that other downstream mechanisms are involved in its regulation.

Then we wanted to map further consequences of increased L1CAM expression for cell signaling pathways and cell metabolism. Since it has been described that activation of L1CAM influences the activity of ERK1/2 (Schaefer et al., 1999; Silletti et al., 2004), we were keen to know whether we would see such an effect also in our model. And, indeed, we detected increased ERK1/2 activity in cells with low expression of L1CAM, an effect that was even stronger after L1CAM knockdown. Interestingly, inhibition or downregulation of ERK1/2 pathway reciprocally led to an increase in L1CAM mRNA and protein level, suggesting negative feedback loop between these two signaling pathways; even though this mutual interaction seems to be cell type-specific. What is important – as ERK1/2 is a direct mediator of H-Ras-induced oncogenic transformation, the finding that ERK1/2 signaling negatively regulates L1CAM levels explains our initial results showing the lack of L1CAM upregulation in H-RAS-induced premature senescence.

One study also linked L1CAM to metabolic changes (Zhang et al., 2012) and we managed to confirm this connection between L1CAM expression and cellular metabolism. First, cells growing in a medium with high concentration of glucose showed decreased expression of L1CAM. Second, L1CAM levels increased after inhibition of the mevalonate pathway. Last but not least, elevated L1CAM levels have been observed after downregulation of ANT2, an ADP/ATP translocase that has been suggested in a tumor cells glucose metabolism (Chevrollier et al., 2011). On the other hand, the conditions that lead to increased levels of L1CAM (i.e. the

inhibition of ERK1/2 pathway, treatment with TGF-β or IFNγ) caused a decrease in the levels of ANT2. Together, these results showed that L1CAM is substantially involved in cellular metabolism.

Finally, the L1CAM is known to control cell adhesion and migration (Li and Galileo, 2010; Valiente et al., 2014). In agreement with this, our cells demonstrated impaired migratory properties in wound healing assay after downregulation of L1CAM. Similarly, senescent cells with low L1CAM levels migrated slower in 2D wound healing assay, as well as in 3D migration essay. Concerning the adhesive properties, the senescent cells with low levels of L1CAM, or the cells in which L1CAM was downregulated, exhibited lower adhesion to selected proteins of extracellular matrix.

To summarize, we identified L1CAM to be overexpressed on a surface of replicatively and prematurely senescent cells of various origin; even though, unfortunately, this did not apply to all cell lines tested. Since the mRNA levels, total protein levels and surface protein levels of L1CAM protein did not always correlate, we inferred that the regulation of L1CAM surface expression is a complicated process, controlled on multiple levels. For instance, ERK1/2 signaling and glucose metabolism that have been described here as factors manipulating L1CAM levels, could vary between individual cell lines and thus to be responsible for the cell line-specific differences in L1CAM expression.

Furthermore, we confirmed and better described here, how L1CAM expression is implicated in ERK1/2 signaling, cellular metabolism, migration and adhesiveness. These findings can be increasingly important, as the role of senescent cells in tumorigenesis and aging is gradually emerging and better understanding of senescence-associated proteins and processes is highly needed.

Dynamic PML protein nucleolar associations with persistent DNA damage lesions in response to nucleolar stress and senescence-inducing stimuli

Imrichova T, Hubackova S, Kucerova A, Kosla J, Bartek J, Hodny Z, Vasicova P: Dynamic PML protein nucleolar associations with persistent DNA damage lesions in response to nucleolar stress and senescence-inducing stimuli. Aging (Albany NY) (*in press*).

Senescence is a protracted cell cycle arrest, brought about in most cases by persistent DNA damage signaling and subsequent activation of cell cycle inhibitors. Most frequently, this type of damage has been related to telomeres, as repetitive sequences that are difficult to repair (d'Adda di Fagagna et al., 2003; Hewitt et al., 2012). However, a new piece of evidence suggests that also rDNA repeats and their instability may be a direct cause of senescence (Paredes et al., 2018).

PML protein is tightly connected to senescence in many ways. Most importantly, PML overexpression (specifically that of PMLIV) is able to induce senescence (Bischof et al., 2002; Ferbeyre et al., 2000; Pearson et al., 2000; Pearson and Pelicci, 2001) and PML bodies associate with permanent DNA damage lesions that are characteristic for senescent cells (Boe et al., 2006; Carbone et al., 2002; Dellaire et al., 2006; Seker et al., 2003).

Several proteins of PML bodies are known to participate in rDNA maintenance and repair (Bischof et al., 2001; Ishov et al., 1999; Rapkin et al., 2015) and PML itself has been associated with DNA repair by homologous recombination (HR) (Bischof et al., 2001; Vancurova et al., 2019); a pathway that is especially relevant for repetitive sequences, such as rDNA or telomeres. The association between PML and HR at telomeres is well documented in cells that maintain their telomeres by alternative lengthening of telomeres (ALT) mechanism, which is based on a DNA replication and HR. In these cells, PML forms special ALT-associated PML nuclear bodies (APBs) that contain the components of classical PML bodies and telomeric DNA (Yeager et al., 1999) and that are functionally involved in the ALT process (Chung et al., 2011; Osterwald et al., 2015; Yong et al., 2012). A direct link between PML and HR at rDNA has yet not been provided.

Upon specific stress stimuli (including treatment with the chemotherapeutic drug doxorubicin), PML has been found to localize to the nucleolus but this interaction has not been systematically

examined. Therefore, in our fourth study, we wanted to better describe these PML nucleolar associations (PNAs), mainly regarding the process of their formation and evolution.

We found out that there are more types of PNAs, named by us PML caps, forks, circles and PML nucleolus-derived structures (PML-NDS). Using time-lapse microscopy, we showed that all these structures are only different stages of one process; when diffuse PML concentrates on the border of the nucleolus forming a PML cap, the cap transforms into a fork and the fork progresses into a PML-NDS in a way that involves stripping the PML shell off the nucleolus, while taking with it some nucleolar material into the newly formed PML-NDS. Furthermore, we showed that the individual stages are linked to the activity state of the nucleolus; more specifically, PML caps form around the nucleoli where the inactivation begins, PML forks and circles can be found around completely inactive nucleoli and PML-NDS are associated with nucleoli that are being re-activated. Using super-resolution microscopy, we also reconstructed 3D models of PNAs in respect to the nucleolus and we showed that PML wraps the whole nucleolus, forming PML bowls, funnels and balloons.

Interestingly, as detected by confocal and super-resolution microscopy, all stages of PNAs co-localize with γH2AX, a marker of DNA damage. This was most intriguing concerning the last stage of PNAs, PML-NDS. We showed that PML-NDS are characteristic for senescent cells, as they are the only type of PNAs present in senescent cells at least 19 days after doxorubicin treatment. While almost 100% of PML-NDS co-localized with γH2AX at the time of their formation, in later time-points the co-localization was found only in a portion of PML-NDS. This suggests that PML-NDS participate in some process dealing with damaged nucleolar DNA, possibly rDNA, that involves sequestration of the DNA into separate compartment and its subsequent degradation or repair, which is mirrored by disappearance of the γH2AX signal in later stages of PML-NDS.

Furthermore, PML-NDS not only associate with γH2AX but also specifically accumulate two proteins that are known to be involved in rDNA metabolism, B23 and DHX9. The concrete function of these proteins inside PML-NDS remains to be fully elucidated; however, their presence further supports the hypothesis that DNA processing occurs within this compartment. What is important, B23 and DHX9 accumulations are not present in PML-deficient cells, demonstrating that these structures, as well as their function, are fully dependent on PML.

Together, the data indicate that PML is summoned to the nucleolus that contains damaged DNA, likely rDNA, and participate on its processing. This is especially relevant, given the facts

that 1) PML-NDS containing rDNA-processing proteins and γH2AX lesions persist in senescent cells for a very long time, and 2) rDNA rearrangements have been found to induce senescence. What is the exact role of PML in the whole process remains to be further investigated. However, it is known that the fate of damaged rDNA is to a large extent dependent on the repair pathway used, while in case of DSBs the choice lies between non-homologous end joining (NHEJ) and HR (van Sluis and McStay, 2019; Warmerdam and Wolthuis, 2019). PML, as a protein known to function in DDR and, more specifically, in HR, could be somehow involved in the decision making, for example by facilitating one of the pathways. Our finding that BLM, a member of HR pathway, co-localizes with PNAs, would further support such a notion (Imrichova et al. 2019).

Casein kinase 2 regulates SUMO-mediated interaction of PML with nucleolus during topoisomerase and RNA polymerase I inhibition

Imrichova T, Hubackova S, Kucerova A, Kosla J, Bartek J, Hodny Z, Vasicova P: Casein kinase 2 regulates SUMO-mediated interaction of PML with nucleolus during topoisomerase and RNA polymerase I inhibition. (unpublished)

The association of the PML protein with the nucleolus (PNAs) has been previously shown by us and others (Bernardi et al., 2004; Condemine et al., 2007; Janderova-Rossmeislova et al., 2007; Mattsson et al., 2001). However, PNAs formation has been observed after various stimuli, while the molecular basis for their formation, as well as their function, have remained largely unknown. Therefore, we aimed to specify the signal that is necessary for PNAs formation, which would hopefully also bring us closer to their function.

First, we focused on γH2AX, a marker of DNA damage, since we showed previously that PNAs co-localize with this marker after doxorubicin treatment (Imrichova et al. 2019). Hence, we treated RPE-1^{hTERT} cells with various chemicals or irradiated them with gamma-radiation and assessed number of PNAs together with γH2AX signal. However, we could see no clear correlation, suggesting that DNA damage or DNA damage response are not the primary causes for induction of PNAs formation.

Then we concentrated on nucleolar stress, as one of the processes that lead to nucleolar rearrangement after various treatments, and might but do not have to involve DNA damage (Rubbi and Milner, 2003). During nucleolar stress, ribosome biogenesis is perturbed, which results in an activation of multiple nucleolus-associated molecular pathways that cause p53-dependent and -independent cell cycle arrest (Boisvert et al., 2007). Multiple chemicals have been reported to induce nucleolar stress by disrupting rDNA transcription or early and late rRNA processing (Burger et al., 2010), including doxorubicin and AMD, known inducers of PNAs. However, when we tested several chemicals that cause nucleolar stress, we got very variable results, ranging from very high amount to absence of PNAs, so we concluded that nucleolar stress in general is not responsible for PNAs formation. Furthermore, we found out that neither p53 is necessary for PNAs formation, since we could detect PNAs in p53$^{-/-}$ cells after treatment with doxorubicin or downregulation of topoisomerase I.

However, we noted that most treatments that cause high number of PNAs, also 1) inhibit Pol I, 2) inhibit or downregulate topoisomerases. Intriguingly, when only one of these conditions is fulfilled, the number of PNAs is significantly lower. Therefore, it seems that combination of Pol I inhibition and topological stress is the real signal for formation of PNAs. We confirmed this hypothesis by a series of experiments when we challenged the cells with topological stress (low dose of doxorubicin, downregulation of topoisomerase I). The number of PNAs after these treatments was relatively low but increased dramatically when the cells were pre-treated with Pol I inhibitors AMD or CX-5461. Nevertheless, it is still not known, what happens with the nucleolar DNA upon these conditions. We suppose that it might adopt a complicated conformation, the resolution of which requires distinct signaling and repair pathways; while one of the intermediates might be DSBs, explaining the presence of γH2AX in doxorubicin-induced PNAs. We argue for HR as the preferential pathway used in this process, as it is often associated with repetitive DNA sequences including rDNA and it is generally used in case of more complicated damage. During HR, rDNA is translocated to the nucleolar periphery (van Sluis and McStay, 2015) and, importantly, PML is known to participate in HR (Boichuk et al., 2011; Vancurova et al., 2019), which creates a link between HR and PNAs formation.

It is vital that we managed to detect rDNA within PNAs directly, using the immune-FISH technique. Therefore, we can infer that PNAs formation is really dependent on rDNA-related processes. Interestingly, the mechanisms of rDNA topology resolution could involve segregation of part of the rDNA from the nucleolus to a new compartment. We have previously described such a compartment, stemming from the nucleolus upon topological stress, being decorated with γH2AX at early time-points, and accumulating proteins functioning in DNA metabolism (Imrichova et al. 2019). We have called it PML nucleolus-derived structure (PML-NDS), since its formation is fully dependent on the presence of PML. Our current notion that PML-NDS also contains rDNA, makes our observation even more significant.

Furthermore, with the use of deletion and substitution mutants of PML, we identified two domains and several residues that are important for PML interaction with the nucleolus. One of them is exon 8b that contains motif interacting with the p14/ARF protein. However, as we could not detect p14/ARF in our model cells, we estimate that the interaction partner of exon 8b on the nucleolus will be different, yet unknown protein. The other domain is exon 7a which contains two important motifs: the SUMO-interacting motif (SIM) and motif that can be phosphorylated by CK2 (CK2 phospho-motif). Interestingly, phosphorylation by CK2 promotes PML interaction with SUMO and SUMOylated proteins via its SIM. By substitution of individual amino acids, we revealed that both the presence of SIM and the presence and phosphorylation

of CK2 phospho-motif are important for PML nucleolar translocation. Therefore, we can speculate that at least one of the nucleolar PML binding partners is SUMOylated.

To further support this, we monitored rDNA co-localization with SUMO-1 signal after doxorubicin treatment and we could observe that it increases with time. The additional piece of information that SUMO modification is an important player in HR pathway (Takahashi et al., 2008), further interconnects rDNA topology, HR and PNAs formation.

Therefore, our study suggests involvement of PML in resolving topological problems on rDNA that are accompanied by rDNA segregation.

V CONCLUSIONS

The main conclusions of this dissertation are as follows:

1. One of the sources of genotoxic stress is γ-radiation. Fractionated irradiation of metastasis-derived prostate cancer cells generates a low-adherent population that is resistant to anoikis and that possesses mesenchymal-like and stem cell-like properties. After some time, these cells are able adhere to the surface again and restart proliferation, as demonstrated *in vitro* as well as *in vivo* in immunocompromised mice. Importantly, the maintenance of the cells in a non-adherent state is enabled by Snail and ERK1/2 signaling, and combined inhibition of AKT and ERK1/2 signaling pathways compromises their survival.

2. Senescent cells in tumors might arise, apart from other reasons, as a result of genotoxic stress-based therapies. Their presence in tumors has multifaceted effects that largely depend on the interaction of the tumor with the immune system. Interestingly, when the chemotherapy-induced and radiation-induced senescent cells are co-injected with non-senescent proliferating cells into mice with normally functioning immunity, they accelerate tumor growth. Administration of IL-12 diminishes the growth of the tumors including those accelerated by senescent cells.

3. A better understanding of the role of senescent cells in physiological and pathophysiological conditions depends on finding their specific markers. L1CAM is a promising candidate, as its levels are upregulated upon senescence in many cell lines. Functionally, L1CAM is involved in cell metabolism, migration, and adhesiveness; and a negative feedback loop between L1CAM and ERK signaling pathways has been described.

4. The nucleolus is an important hub of a stress response. After specific types of treatment, topological stress is imposed on rDNA, Pol I is segregated and some proteins in the vicinity of rDNA are probably modified by SUMO-1. Upon these conditions, the PML protein interacts with the nucleolus; while the casein kinase 2-dependent phosphorylation of PML phospho-SIM domain, and the presence of exon 8b are

necessary for the interaction. PML interaction with the nucleolus is a dynamic process, comprising several morphologically distinct stages. The last stages, PML nucleolus-derived structures (PML-NDS), originate as a γH2AX-positive compartments containing rDNA, they accumulate proteins engaged in DNA processing and they persist in senescent cells for protracted period of time. We suggest that PML is involved in rDNA metabolism upon rDNA segregation and that unresolved conflicts on rDNA may contribute to senescence onset.

VI AUTHOR CONTRIBUTION TO PRESENTED PUBLICATIONS

Publication 1

- Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, ERK signaling-dependent cells.

Terezie Imrichova detected the stem cell and EMT markers of low-adherent cells by flow cytometry and immunofluorescence, performed the proliferation assays using cell proliferation dye and helped with the preparation of the publication.

Publication 2

- Tumor growth accelerated by chemotherapy-induced senescent cells is suppressed by treatment with IL-12 producing cellular vaccines.

Terezie Imrichova analysed cell proliferation by cell counting (growth curves) and EdU incorporation (measured by flow cytometry and immunofluorescence), assessed DNA damage by immunofluorescence detection of γH2AX and helped with the preparation of the publication.

Publication 3

- Induction, regulation and roles of neural adhesion molecule L1CAM in cellular senescence.

Terezie Imrichova performed majority of flow cytometry measurements.

Publication 4

- Dynamic PML protein nucleolar associations with persistent DNA damage lesions in response to nucleolar stress and senescence-inducing stimuli.

Terezie Imrichova performed majority of the experiments and, together with the other co-authors, participated on the data analyses and preparation of the publication.

Publication 5 – unpublished

- Casein kinase 2 regulates SUMO-mediated interaction of PML with nucleolus during topoisomerase and RNA polymerase I inhibition

Terezie Imrichova performed majority of the experiments and, together with the other co-authors, participated on the data analyses and preparation of the publication.

VII THE LIST OF REFERENCES

Abdul-Aziz, A.M., Sun, Y., Hellmich, C., Marlein, C.R., Mistry, J., Forde, E., Piddock, R.E., Shafat, M.S., Morfakis, A., Mehta, T.*, et al.* (2019). Acute myeloid leukemia induces protumoral p16INK4a-driven senescence in the bone marrow microenvironment. Blood *133*, 446-456.

Acosta, J.C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J.P., Athineos, D., Kang, T.W., Lasitschka, F., Andrulis, M.*, et al.* (2013). A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nature cell biology *15*, 978-990.

Ahmed, N., Abubaker, K., Findlay, J., and Quinn, M. (2010). Epithelial mesenchymal transition and cancer stem cell-like phenotypes facilitate chemoresistance in recurrent ovarian cancer. Current cancer drug targets *10*, 268-278.

Ajore, R., Raiser, D., McConkey, M., Joud, M., Boidol, B., Mar, B., Saksena, G., Weinstock, D.M., Armstrong, S., Ellis, S.R.*, et al.* (2017). Deletion of ribosomal protein genes is a common vulnerability in human cancer, especially in concert with TP53 mutations. EMBO molecular medicine *9*, 498-507.

Alsheich-Bartok, O., Haupt, S., Alkalay-Snir, I., Saito, S., Appella, E., and Haupt, Y. (2008). PML enhances the regulation of p53 by CK1 in response to DNA damage. Oncogene *27*, 3653-3661.

Altevogt, P., Doberstein, K., and Fogel, M. (2016). L1CAM in human cancer. International journal of cancer *138*, 1565-1576.

Amantini, C., Morelli, M.B., Nabissi, M., Cardinali, C., Santoni, M., Gismondi, A., and Santoni, G. (2016). Capsaicin triggers autophagic cell survival which drives epithelial mesenchymal transition and chemoresistance in bladder cancer cells in an Hedgehog-dependent manner. Oncotarget *7*, 50180-50194.

Andersen, J.S., Lam, Y.W., Leung, A.K., Ong, S.E., Lyon, C.E., Lamond, A.I., and Mann, M. (2005). Nucleolar proteome dynamics. Nature *433*, 77-83.

Andrews, W.J., Panova, T., Normand, C., Gadal, O., Tikhonova, I.G., and Panov, K.I. (2013). Old drug, new target: ellipticines selectively inhibit RNA polymerase I transcription. The Journal of biological chemistry *288*, 4567-4582.

Anton, L.C., Schubert, U., Bacik, I., Princiotta, M.F., Wearsch, P.A., Gibbs, J., Day, P.M., Realini, C., Rechsteiner, M.C., Bennink, J.R.*, et al.* (1999). Intracellular localization of proteasomal degradation of a viral antigen. The Journal of cell biology *146*, 113-124.

Asfaha, S., Hayakawa, Y., Muley, A., Stokes, S., Graham, T.A., Ericksen, R.E., Westphalen, C.B., von Burstin, J., Mastracci, T.L., Worthley, D.L.*, et al.* (2015). Krt19(+)/Lgr5(-) Cells Are Radioresistant Cancer-Initiating Stem Cells in the Colon and Intestine. Cell stem cell *16*, 627-638.

Ayob, A.Z., and Ramasamy, T.S. (2018). Cancer stem cells as key drivers of tumour progression. J Biomed Sci *25*, 20.

Ayrault, O., Andrique, L., Fauvin, D., Eymin, B., Gazzeri, S., and Seite, P. (2006). Human tumor suppressor p14ARF negatively regulates rRNA transcription and inhibits UBF1 transcription factor phosphorylation. Oncogene *25*, 7577-7586.

Baker, D.J., Childs, B.G., Durik, M., Wijers, M.E., Sieben, C.J., Zhong, J., Saltness, R.A., Jeganathan, K.B., Verzosa, G.C., Pezeshki, A.*, et al.* (2016). Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. Nature *530*, 184-189.

Balkwill, F., Charles, K.A., and Mantovani, A. (2005). Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer cell *7*, 211-217.

Bao, S., Wu, Q., McLendon, R.E., Hao, Y., Shi, Q., Hjelmeland, A.B., Dewhirst, M.W., Bigner, D.D., and Rich, J.N. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature *444*, 756-760.

Barkic, M., Crnomarkovic, S., Grabusic, K., Bogetic, I., Panic, L., Tamarut, S., Cokaric, M., Jeric, I., Vidak, S., and Volarevic, S. (2009). The p53 tumor suppressor causes congenital malformations in Rpl24-deficient mice and promotes their survival. Molecular and cellular biology *29*, 2489-2504.

Barnum, K.J., and O'Connell, M.J. (2014). Cell cycle regulation by checkpoints. Methods in molecular biology (Clifton, NJ) *1170*, 29-40.

Bartek, J. (2011). DNA damage response, genetic instability and cancer: from mechanistic insights to personalized treatment. Molecular oncology *5*, 303-307.

Bartkova, J., Rezaei, N., Liontos, M., Karakaidos, P., Kletsas, D., Issaeva, N., Vassiliou, L.V., Kolettas, E., Niforou, K., Zoumpourlis, V.C.*, et al.* (2006). Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature *444*, 633-637.

Baskar, R., Lee, K.A., Yeo, R., and Yeoh, K.W. (2012). Cancer and radiation therapy: current advances and future directions. International journal of medical sciences *9*, 193-199.

Bassermann, F., Frescas, D., Guardavaccaro, D., Busino, L., Peschiaroli, A., and Pagano, M. (2008). The Cdc14B-Cdh1-Plk1 axis controls the G2 DNA-damage-response checkpoint. Cell *134*, 256-267.

Bastos, L.G., de Marcondes, P.G., de-Freitas-Junior, J.C., Leve, F., Mencalha, A.L., de Souza, W.F., de Araujo, W.M., Tanaka, M.N., Abdelhay, E.S., and Morgado-Diaz, J.A. (2014). Progeny from irradiated colorectal cancer cells acquire an EMT-like phenotype and activate Wnt/beta-catenin pathway. Journal of cellular biochemistry *115*, 2175-2187.

Bates, P.J., Laber, D.A., Miller, D.M., Thomas, S.D., and Trent, J.O. (2009). Discovery and development of the G-rich oligonucleotide AS1411 as a novel treatment for cancer. Experimental and molecular pathology *86*, 151-164.

Bates, P.J., Reyes-Reyes, E.M., Malik, M.T., Murphy, E.M., O'Toole, M.G., and Trent, J.O. (2017). G-quadruplex oligonucleotide AS1411 as a cancer-targeting agent: Uses and mechanisms. Biochimica et biophysica acta General subjects *1861*, 1414-1428.

Batlle, E., and Clevers, H. (2017). Cancer stem cells revisited. Nature medicine *23*, 1124-1134.

Beech, S.J., Lethbridge, K.J., Killick, N., McGlincy, N., and Leppard, K.N. (2005). Isoforms of the promyelocytic leukemia protein differ in their effects on ND10 organization. Experimental cell research *307*, 109-117.

Bensimon, J., Altmeyer-Morel, S., Benjelloun, H., Chevillard, S., and Lebeau, J. (2013). CD24(-/low) stem-like breast cancer marker defines the radiation-resistant cells involved in memorization and transmission of radiation-induced genomic instability. Oncogene *32*, 251-258.

Bernardi, R., and Pandolfi, P.P. (2007). Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. Nat Rev Mol Cell Biol *8*, 1006-1016.

Bernardi, R., Scaglioni, P.P., Bergmann, S., Horn, H.F., Vousden, K.H., and Pandolfi, P.P. (2004). PML regulates p53 stability by sequestering Mdm2 to the nucleolus. Nature cell biology *6*, 665-672.

Bertwistle, D., Sugimoto, M., and Sherr, C.J. (2004). Physical and functional interactions of the Arf tumor suppressor protein with nucleophosmin/B23. Molecular and cellular biology *24*, 985-996.

Bhat, K.P., Itahana, K., Jin, A., and Zhang, Y. (2004). Essential role of ribosomal protein L11 in mediating growth inhibition-induced p53 activation. The EMBO journal *23*, 2402-2412.

Bischof, O., Kim, S.H., Irving, J., Beresten, S., Ellis, N.A., and Campisi, J. (2001). Regulation and localization of the Bloom syndrome protein in response to DNA damage. The Journal of cell biology *153*, 367-380.

Bischof, O., Kirsh, O., Pearson, M., Itahana, K., Pelicci, P.G., and Dejean, A. (2002). Deconstructing PML-induced premature senescence. The EMBO journal *21*, 3358-3369.

Blagosklonny, M.V. (2011). Cell cycle arrest is not senescence. Aging *3*, 94-101.

Blander, G., Zalle, N., Daniely, Y., Taplick, J., Gray, M.D., and Oren, M. (2002). DNA damage-induced translocation of the Werner helicase is regulated by acetylation. The Journal of biological chemistry *277*, 50934-50940.

Boe, S.O., Haave, M., Jul-Larsen, A., Grudic, A., Bjerkvig, R., and Lonning, P.E. (2006). Promyelocytic leukemia nuclear bodies are predetermined processing sites for damaged DNA. Journal of cell science *119*, 3284-3295.

Boichuk, S., Hu, L., Makielski, K., Pandolfi, P.P., and Gjoerup, O.V. (2011). Functional connection between Rad51 and PML in homology-directed repair. PloS one *6*, e25814.

Boisvert, F.M., Lam, Y.W., Lamont, D., and Lamond, A.I. (2010). A quantitative proteomics analysis of subcellular proteome localization and changes induced by DNA damage. Molecular & cellular proteomics : MCP *9*, 457-470.

Boisvert, F.M., and Lamond, A.I. (2010). p53-Dependent subcellular proteome localization following DNA damage. Proteomics *10*, 4087-4097.

Boisvert, F.M., van Koningsbruggen, S., Navascues, J., and Lamond, A.I. (2007). The multifunctional nucleolus. Nat Rev Mol Cell Biol *8*, 574-585.

Bonilla, W.V., Pinschewer, D.D., Klenerman, P., Rousson, V., Gaboli, M., Pandolfi, P.P., Zinkernagel, R.M., Salvato, M.S., and Hengartner, H. (2002). Effects of promyelocytic leukemia protein on virus-host balance. Journal of virology *76*, 3810-3818.

Borer, R.A., Lehner, C.F., Eppenberger, H.M., and Nigg, E.A. (1989). Major nucleolar proteins shuttle between nucleus and cytoplasm. Cell *56*, 379-390.

Boulon, S., Westman, B.J., Hutten, S., Boisvert, F.M., and Lamond, A.I. (2010). The nucleolus under stress. Molecular cell *40*, 216-227.

Boussemart, L., Malka-Mahieu, H., Girault, I., Allard, D., Hemmingsson, O., Tomasic, G., Thomas, M., Basmadjian, C., Ribeiro, N., Thuaud, F.*, et al.* (2014). eIF4F is a nexus of resistance to anti-BRAF and anti-MEK cancer therapies. Nature *513*, 105-109.

Bouwman, P., and Jonkers, J. (2012). The effects of deregulated DNA damage signalling on cancer chemotherapy response and resistance. Nature reviews Cancer *12*, 587-598.

Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A.H., Schlegelberger, B., Stein, H., Dorken, B., Jenuwein, T., and Schmitt, C.A. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. Nature *436*, 660-665.

Brambati, A., Colosio, A., Zardoni, L., Galanti, L., and Liberi, G. (2015). Replication and transcription on a collision course: eukaryotic regulation mechanisms and implications for DNA stability. Frontiers in genetics *6*, 166.

Branzei, D., and Foiani, M. (2010). Maintaining genome stability at the replication fork. Nat Rev Mol Cell Biol *11*, 208-219.

Braumuller, H., Wieder, T., Brenner, E., Assmann, S., Hahn, M., Alkhaled, M., Schilbach, K., Essmann, F., Kneilling, M., Griessinger, C.*, et al.* (2013). T-helper-1-cell cytokines drive cancer into senescence. Nature *494*, 361-365.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians *68*, 394-424.

Brighenti, E., Calabrese, C., Liguori, G., Giannone, F.A., Trere, D., Montanaro, L., and Derenzini, M. (2014). Interleukin 6 downregulates p53 expression and activity by stimulating ribosome biogenesis: a new pathway connecting inflammation to cancer. Oncogene *33*, 4396-4406.

Bubenik, J. (1996). Cytokine gene-modified vaccines in the therapy of cancer. Pharmacology & therapeutics *69*, 1-14.

Bubenik, J. (2011). Interleukin 12 in cancer treatment. Folia biologica *57*, 1-2.

Buchheit, C.L., Weigel, K.J., and Schafer, Z.T. (2014). Cancer cell survival during detachment from the ECM: multiple barriers to tumour progression. Nature reviews Cancer *14*, 632-641.

Burger, K., Muhl, B., Harasim, T., Rohrmoser, M., Malamoussi, A., Orban, M., Kellner, M., Gruber-Eber, A., Kremmer, E., Holzel, M.*, et al.* (2010). Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. The Journal of biological chemistry *285*, 12416-12425.

Burrell, R.A., McClelland, S.E., Endesfelder, D., Groth, P., Weller, M.C., Shaikh, N., Domingo, E., Kanu, N., Dewhurst, S.M., Gronroos, E.*, et al.* (2013). Replication stress links structural and numerical cancer chromosomal instability. Nature *494*, 492-496.

Bursac, S., Brdovcak, M.C., Pfannkuchen, M., Orsolic, I., Golomb, L., Zhu, Y., Katz, C., Daftuar, L., Grabusic, K., Vukelic, I.*, et al.* (2012). Mutual protection of ribosomal proteins L5 and L11 from degradation is essential for p53 activation upon ribosomal biogenesis stress. Proceedings of the National Academy of Sciences of the United States of America *109*, 20467-20472.

Bywater, M.J., Poortinga, G., Sanij, E., Hein, N., Peck, A., Cullinane, C., Wall, M., Cluse, L., Drygin, D., Anderes, K.*, et al.* (2012). Inhibition of RNA polymerase I as a therapeutic strategy to promote cancer-specific activation of p53. Cancer cell *22*, 51-65.

Caburet, S., Conti, C., Schurra, C., Lebofsky, R., Edelstein, S.J., and Bensimon, A. (2005). Human ribosomal RNA gene arrays display a broad range of palindromic structures. Genome research *15*, 1079-1085.

Calcinotto, A., Kohli, J., Zagato, E., Pellegrini, L., Demaria, M., and Alimonti, A. (2019). Cellular Senescence: Aging, Cancer, and Injury. Physiol Rev *99*, 1047-1078.

Caldarola, S., De Stefano, M.C., Amaldi, F., and Loreni, F. (2009). Synthesis and function of ribosomal proteins--fading models and new perspectives. The FEBS journal *276*, 3199-3210.

Calkins, A.S., Iglehart, J.D., and Lazaro, J.B. (2013). DNA damage-induced inhibition of rRNA synthesis by DNA-PK and PARP-1. Nucleic acids research *41*, 7378-7386.

Campisi, J. (1997). The biology of replicative senescence. European journal of cancer (Oxford, England : 1990) *33*, 703-709.

Campisi, J. (2013). Aging, cellular senescence, and cancer. Annual review of physiology *75*, 685-705.

Cao, Z., Livas, T., and Kyprianou, N. (2016). Anoikis and EMT: Lethal "Liaisons" during Cancer Progression. Crit Rev Oncog *21*, 155-168.

Cappadocia, L., Mascle, X.H., Bourdeau, V., Tremblay-Belzile, S., Chaker-Margot, M., Lussier-Price, M., Wada, J., Sakaguchi, K., Aubry, M., Ferbeyre, G.*, et al.* (2015). Structural and functional characterization of the phosphorylation-dependent interaction between PML and SUMO1. Structure (London, England : 1993) *23*, 126-138.

Carbone, R., Pearson, M., Minucci, S., and Pelicci, P.G. (2002). PML NBs associate with the hMre11 complex and p53 at sites of irradiation induced DNA damage. Oncogene *21*, 1633-1640.

Carracedo, A., Weiss, D., Leliaert, A.K., Bhasin, M., de Boer, V.C., Laurent, G., Adams, A.C., Sundvall, M., Song, S.J., Ito, K.*, et al.* (2012). A metabolic prosurvival role for PML in breast cancer. The Journal of clinical investigation *122*, 3088-3100.

Catez, F., Dalla Venezia, N., Marcel, V., Zorbas, C., Lafontaine, D.L.J., and Diaz, J.J. (2019). Ribosome biogenesis: An emerging druggable pathway for cancer therapeutics. Biochem Pharmacol *159*, 74-81.

Chan, J.C., Hannan, K.M., Riddell, K., Ng, P.Y., Peck, A., Lee, R.S., Hung, S., Astle, M.V., Bywater, M., Wall, M.*, et al.* (2011). AKT promotes rRNA synthesis and cooperates with c-MYC to stimulate ribosome biogenesis in cancer. Science signaling *4*, ra56.

Chang, B.D., Broude, E.V., Dokmanovic, M., Zhu, H., Ruth, A., Xuan, Y., Kandel, E.S., Lausch, E., Christov, K., and Roninson, I.B. (1999). A senescence-like phenotype distinguishes

tumor cells that undergo terminal proliferation arrest after exposure to anticancer agents. Cancer research *59*, 3761-3767.

Chang, C.C., Naik, M.T., Huang, Y.S., Jeng, J.C., Liao, P.H., Kuo, H.Y., Ho, C.C., Hsieh, Y.L., Lin, C.H., Huang, N.J.*, et al.* (2011). Structural and functional roles of Daxx SIM phosphorylation in SUMO paralog-selective binding and apoptosis modulation. Molecular cell *42*, 62-74.

Chang, L., Graham, P., Hao, J., Ni, J., Deng, J., Bucci, J., Malouf, D., Gillatt, D., and Li, Y. (2016). Cancer stem cells and signaling pathways in radioresistance. Oncotarget *7*, 11002-11017.

Chang, L., Graham, P.H., Hao, J., Ni, J., Bucci, J., Cozzi, P.J., Kearsley, J.H., and Li, Y. (2013). Acquisition of epithelial-mesenchymal transition and cancer stem cell phenotypes is associated with activation of the PI3K/Akt/mTOR pathway in prostate cancer radioresistance. Cell death & disease *4*, e875.

Chen, C., Xu, T., Lu, Y., Chen, J., and Wu, S. (2013). The efficacy of temozolomide for recurrent glioblastoma multiforme. European journal of neurology *20*, 223-230.

Chen, D., Shan, J., Zhu, W.G., Qin, J., and Gu, W. (2010). Transcription-independent ARF regulation in oncogenic stress-mediated p53 responses. Nature *464*, 624-627.

Chen, J. (2016). The Cell-Cycle Arrest and Apoptotic Functions of p53 in Tumor Initiation and Progression. Cold Spring Harbor perspectives in medicine *6*, a026104.

Cheng, L., Wu, Q., Huang, Z., Guryanova, O.A., Huang, Q., Shou, W., Rich, J.N., and Bao, S. (2011). L1CAM regulates DNA damage checkpoint response of glioblastoma stem cells through NBS1. The EMBO journal *30*, 800-813.

Cheng, Z., Ke, Y., Ding, X., Wang, F., Wang, H., Wang, W., Ahmed, K., Liu, Z., Xu, Y., Aikhionbare, F.*, et al.* (2008). Functional characterization of TIP60 sumoylation in UV-irradiated DNA damage response. Oncogene *27*, 931-941.

Chesnokov, I., Chu, W.M., Botchan, M.R., and Schmid, C.W. (1996). p53 inhibits RNA polymerase III-directed transcription in a promoter-dependent manner. Molecular and cellular biology *16*, 7084-7088.

Chevrollier, A., Loiseau, D., Reynier, P., and Stepien, G. (2011). Adenine nucleotide translocase 2 is a key mitochondrial protein in cancer metabolism. Biochim Biophys Acta *1807*, 562-567.

Chien, Y., Scuoppo, C., Wang, X., Fang, X., Balgley, B., Bolden, J.E., Premsrirut, P., Luo, W., Chicas, A., Lee, C.S.*, et al.* (2011). Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. Genes & development *25*, 2125-2136.

Chiolo, I., Minoda, A., Colmenares, S.U., Polyzos, A., Costes, S.V., and Karpen, G.H. (2011). Double-strand breaks in heterochromatin move outside of a dynamic HP1a domain to complete recombinational repair. Cell *144*, 732-744.

Cho, K.J., Park, E.J., Kim, M.S., and Joo, Y.H. (2018). Characterization of FaDu-R, a radioresistant head and neck cancer cell line, and cancer stem cells. Auris Nasus Larynx *45*, 566-573.

Chu, Y., and Yang, X. (2011). SUMO E3 ligase activity of TRIM proteins. Oncogene *30*, 1108-1116.

Chuang, Y.S., Huang, W.H., Park, S.W., Persaud, S.D., Hung, C.H., Ho, P.C., and Wei, L.N. (2011). Promyelocytic leukemia protein in retinoic acid-induced chromatin remodeling of Oct4 gene promoter. Stem cells (Dayton, Ohio) *29*, 660-669.

Chung, I., Leonhardt, H., and Rippe, K. (2011). De novo assembly of a PML nuclear subcompartment occurs through multiple pathways and induces telomere elongation. Journal of cell science *124*, 3603-3618.

Ciccia, A., and Elledge, S.J. (2010). The DNA damage response: making it safe to play with knives. Molecular cell *40*, 179-204.

Cohen, A.A., Geva-Zatorsky, N., Eden, E., Frenkel-Morgenstern, M., Issaeva, I., Sigal, A., Milo, R., Cohen-Saidon, C., Liron, Y., Kam, Z., *et al.* (2008). Dynamic proteomics of individual cancer cells in response to a drug. Science (New York, NY) *322*, 1511-1516.

Cohen, N., Sharma, M., Kentsis, A., Perez, J.M., Strudwick, S., and Borden, K.L. (2001). PML RING suppresses oncogenic transformation by reducing the affinity of eIF4E for mRNA. The EMBO journal *20*, 4547-4559.

Collado, M., Gil, J., Efeyan, A., Guerra, C., Schuhmacher, A.J., Barradas, M., Benguria, A., Zaballos, A., Flores, J.M., Barbacid, M., *et al.* (2005). Tumour biology: senescence in premalignant tumours. Nature *436*, 642.

Colombo, E., Bonetti, P., Lazzerini Denchi, E., Martinelli, P., Zamponi, R., Marine, J.C., Helin, K., Falini, B., and Pelicci, P.G. (2005). Nucleophosmin is required for DNA integrity and p19Arf protein stability. Molecular and cellular biology *25*, 8874-8886.

Condemine, W., Takahashi, Y., Le Bras, M., and de The, H. (2007). A nucleolar targeting signal in PML-I addresses PML to nucleolar caps in stressed or senescent cells. Journal of cell science *120*, 3219-3227.

Condemine, W., Takahashi, Y., Zhu, J., Puvion-Dutilleul, F., Guegan, S., Janin, A., and de The, H. (2006). Characterization of endogenous human promyelocytic leukemia isoforms. Cancer research *66*, 6192-6198.

Conlan, L.A., McNees, C.J., and Heierhorst, J. (2004). Proteasome-dependent dispersal of PML nuclear bodies in response to alkylating DNA damage. Oncogene *23*, 307-310.

Coppe, J.P., Desprez, P.Y., Krtolica, A., and Campisi, J. (2010). The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol *5*, 99-118.

Coppe, J.P., Patil, C.K., Rodier, F., Sun, Y., Munoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., and Campisi, J. (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol *6*, 2853-2868.

Courtois-Cox, S., Jones, S.L., and Cichowski, K. (2008). Many roads lead to oncogene-induced senescence. Oncogene *27*, 2801-2809.

Coussens, L.M., Zitvogel, L., and Palucka, A.K. (2013). Neutralizing tumor-promoting chronic inflammation: a magic bullet? Science (New York, NY) *339*, 286-291.

Creighton, C.J., Li, X., Landis, M., Dixon, J.M., Neumeister, V.M., Sjolund, A., Rimm, D.L., Wong, H., Rodriguez, A., Herschkowitz, J.I., *et al.* (2009). Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proceedings of the National Academy of Sciences of the United States of America *106*, 13820-13825.

Cuchet, D., Sykes, A., Nicolas, A., Orr, A., Murray, J., Sirma, H., Heeren, J., Bartelt, A., and Everett, R.D. (2011). PML isoforms I and II participate in PML-dependent restriction of HSV-1 replication. Journal of cell science *124*, 280-291.

Culjkovic, B., Topisirovic, I., Skrabanek, L., Ruiz-Gutierrez, M., and Borden, K.L. (2006). eIF4E is a central node of an RNA regulon that governs cellular proliferation. The Journal of cell biology *175*, 415-426.

d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N.P., and Jackson, S.P. (2003). A DNA damage checkpoint response in telomere-initiated senescence. Nature *426*, 194-198.

D'Alessandro, G., and d'Adda di Fagagna, F. (2017). Transcription and DNA Damage: Holding Hands or Crossing Swords? Journal of molecular biology *429*, 3215-3229.

D'Orazi, G., Cecchinelli, B., Bruno, T., Manni, I., Higashimoto, Y., Saito, S., Gostissa, M., Coen, S., Marchetti, A., Del Sal, G., *et al.* (2002). Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. Nature cell biology *4*, 11-19.

Daftuar, L., Zhu, Y., Jacq, X., and Prives, C. (2013). Ribosomal proteins RPL37, RPS15 and RPS20 regulate the Mdm2-p53-MdmX network. PloS one *8*, e68667.

Dagogo-Jack, I., and Shaw, A.T. (2018). Tumour heterogeneity and resistance to cancer therapies. Nature reviews Clinical oncology *15*, 81-94.

Dai, M.S., and Lu, H. (2004). Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. The Journal of biological chemistry *279*, 44475-44482.

Davalos, A.R., Coppe, J.P., Campisi, J., and Desprez, P.Y. (2010). Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev *29*, 273-283.

Dave, B., Mittal, V., Tan, N.M., and Chang, J.C. (2012). Epithelial-mesenchymal transition, cancer stem cells and treatment resistance. Breast cancer research : BCR *14*, 202.

David-Pfeuty, T. (1999). Potent inhibitors of cyclin-dependent kinase 2 induce nuclear accumulation of wild-type p53 and nucleolar fragmentation in human untransformed and tumor-derived cells. Oncogene *18*, 7409-7422.

de Stanchina, E., Querido, E., Narita, M., Davuluri, R.V., Pandolfi, P.P., Ferbeyre, G., and Lowe, S.W. (2004). PML is a direct p53 target that modulates p53 effector functions. Molecular cell *13*, 523-535.

de The, H., Le Bras, M., and Lallemand-Breitenbach, V. (2012). The cell biology of disease: Acute promyelocytic leukemia, arsenic, and PML bodies. The Journal of cell biology *198*, 11-21.

de The, H., Pandolfi, P.P., and Chen, Z. (2017). Acute Promyelocytic Leukemia: A Paradigm for Oncoprotein-Targeted Cure. Cancer cell *32*, 552-560.

Delbarre, E., Ivanauskiene, K., Spirkoski, J., Shah, A., Vekterud, K., Moskaug, J.O., Boe, S.O., Wong, L.H., Kuntziger, T., and Collas, P. (2017). PML protein organizes heterochromatin domains where it regulates histone H3.3 deposition by ATRX/DAXX. Genome research *27*, 913-921.

Dellaire, G., and Bazett-Jones, D.P. (2004). PML nuclear bodies: dynamic sensors of DNA damage and cellular stress. BioEssays : news and reviews in molecular, cellular and developmental biology *26*, 963-977.

Dellaire, G., Ching, R.W., Ahmed, K., Jalali, F., Tse, K.C., Bristow, R.G., and Bazett-Jones, D.P. (2006). Promyelocytic leukemia nuclear bodies behave as DNA damage sensors whose response to DNA double-strand breaks is regulated by NBS1 and the kinases ATM, Chk2, and ATR. The Journal of cell biology *175*, 55-66.

Derenzini, M., Montanaro, L., and Trere, D. (2009). What the nucleolus says to a tumour pathologist. Histopathology *54*, 753-762.

Di Micco, R., Fumagalli, M., Cicalese, A., Piccinin, S., Gasparini, P., Luise, C., Schurra, C., Garre, M., Nuciforo, P.G., Bensimon, A*., et al.* (2006). Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature *444*, 638-642.

Dilruba, S., and Kalayda, G.V. (2016). Platinum-based drugs: past, present and future. Cancer chemotherapy and pharmacology *77*, 1103-1124.

Dino Rockel, T., and von Mikecz, A. (2002). Proteasome-dependent processing of nuclear proteins is correlated with their subnuclear localization. Journal of structural biology *140*, 189-199.

Donati, G., Bertoni, S., Brighenti, E., Vici, M., Trere, D., Volarevic, S., Montanaro, L., and Derenzini, M. (2011a). The balance between rRNA and ribosomal protein synthesis up- and downregulates the tumour suppressor p53 in mammalian cells. Oncogene *30*, 3274-3288.

Donati, G., Brighenti, E., Vici, M., Mazzini, G., Trere, D., Montanaro, L., and Derenzini, M. (2011b). Selective inhibition of rRNA transcription downregulates E2F-1: a new p53-independent mechanism linking cell growth to cell proliferation. Journal of cell science *124*, 3017-3028.

Donati, G., Peddigari, S., Mercer, C.A., and Thomas, G. (2013). 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint. Cell reports *4*, 87-98.

Dong, J., Wang, R., Ren, G., Li, X., Wang, J., Sun, Y., Liang, J., Nie, Y., Wu, K., Feng, B., *et al.* (2017). HMGA2-FOXL2 Axis Regulates Metastases and Epithelial-to-Mesenchymal Transition of Chemoresistant Gastric Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research *23*, 3461-3473.

Dou, Z., Ghosh, K., Vizioli, M.G., Zhu, J., Sen, P., Wangensteen, K.J., Simithy, J., Lan, Y., Lin, Y., Zhou, Z., *et al.* (2017). Cytoplasmic chromatin triggers inflammation in senescence and cancer. Nature *550*, 402-406.

Drygin, D., Lin, A., Bliesath, J., Ho, C.B., O'Brien, S.E., Proffitt, C., Omori, M., Haddach, M., Schwaebe, M.K., Siddiqui-Jain, A., *et al.* (2011). Targeting RNA polymerase I with an oral small molecule CX-5461 inhibits ribosomal RNA synthesis and solid tumor growth. Cancer research *71*, 1418-1430.

Drygin, D., Siddiqui-Jain, A., O'Brien, S., Schwaebe, M., Lin, A., Bliesath, J., Ho, C.B., Proffitt, C., Trent, K., Whitten, J.P., *et al.* (2009). Anticancer activity of CX-3543: a direct inhibitor of rRNA biogenesis. Cancer research *69*, 7653-7661.

Durkin, S.G., and Glover, T.W. (2007). Chromosome fragile sites. Annual review of genetics *41*, 169-192.

Dutt, S., Narla, A., Lin, K., Mullally, A., Abayasekara, N., Megerdichian, C., Wilson, F.H., Currie, T., Khanna-Gupta, A., Berliner, N., *et al.* (2011). Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. Blood *117*, 2567-2576.

Easwaran, H., Tsai, H.C., and Baylin, S.B. (2014). Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Molecular cell *54*, 716-727.

Ebrahimian, M., Mojtahedzadeh, M., Bazett-Jones, D., and Dehghani, H. (2010). Transcript isoforms of promyelocytic leukemia in mouse male and female gametes. Cells, tissues, organs *192*, 374-381.

Eggert, T., Wolter, K., Ji, J., Ma, C., Yevsa, T., Klotz, S., Medina-Echeverz, J., Longerich, T., Forgues, M., Reisinger, F., *et al.* (2016). Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. Cancer cell *30*, 533-547.

Elaskalani, O., Razak, N.B., Falasca, M., and Metharom, P. (2017). Epithelial-mesenchymal transition as a therapeutic target for overcoming chemoresistance in pancreatic cancer. World J Gastrointest Oncol *9*, 37-41.

Emmott, E., Rodgers, M.A., Macdonald, A., McCrory, S., Ajuh, P., and Hiscox, J.A. (2010). Quantitative proteomics using stable isotope labeling with amino acids in cell culture reveals changes in the cytoplasmic, nuclear, and nucleolar proteomes in Vero cells infected with the coronavirus infectious bronchitis virus. Molecular & cellular proteomics : MCP *9*, 1920-1936.

Eriksson, D., and Stigbrand, T. (2010). Radiation-induced cell death mechanisms. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine *31*, 363-372.

Erker, Y., Neyret-Kahn, H., Seeler, J.S., Dejean, A., Atfi, A., and Levy, L. (2013). Arkadia, a novel SUMO-targeted ubiquitin ligase involved in PML degradation. Molecular and cellular biology *33*, 2163-2177.

Eskiw, C.H., Dellaire, G., Mymryk, J.S., and Bazett-Jones, D.P. (2003). Size, position and dynamic behavior of PML nuclear bodies following cell stress as a paradigm for supramolecular trafficking and assembly. Journal of cell science *116*, 4455-4466.

Everett, R.D., Earnshaw, W.C., Pluta, A.F., Sternsdorf, T., Ainsztein, A.M., Carmena, M., Ruchaud, S., Hsu, W.L., and Orr, A. (1999). A dynamic connection between centromeres and ND10 proteins. Journal of cell science *112 (Pt 20)*, 3443-3454.

Ewald, J., Desotelle, J., Almassi, N., and Jarrard, D. (2008). Drug-induced senescence bystander proliferation in prostate cancer cells in vitro and in vivo. British journal of cancer *98*, 1244-1249.

Ewald, J.A., Desotelle, J.A., Wilding, G., and Jarrard, D.F. (2010). Therapy-induced senescence in cancer. Journal of the National Cancer Institute *102*, 1536-1546.

Facompre, N., Nakagawa, H., Herlyn, M., and Basu, D. (2012). Stem-like cells and therapy resistance in squamous cell carcinomas. Advances in pharmacology (San Diego, Calif) *65*, 235-265.

Farr, J.B., Flanz, J.B., Gerbershagen, A., and Moyers, M.F. (2018). New horizons in particle therapy systems. Med Phys *45*, e953-e983.

Feng, S., Zheng, Z., Feng, L., Yang, L., Chen, Z., Lin, Y., Gao, Y., and Chen, Y. (2016). Proton pump inhibitor pantoprazole inhibits the proliferation, selfrenewal and chemoresistance of gastric cancer stem cells via the EMT/betacatenin pathways. Oncology reports *36*, 3207-3214.

Feng, Z., Zhang, H., Levine, A.J., and Jin, S. (2005). The coordinate regulation of the p53 and mTOR pathways in cells. Proceedings of the National Academy of Sciences of the United States of America *102*, 8204-8209.

Ferbeyre, G., de Stanchina, E., Querido, E., Baptiste, N., Prives, C., and Lowe, S.W. (2000). PML is induced by oncogenic ras and promotes premature senescence. Genes & development *14*, 2015-2027.

Ferretti, M.B., Ghalei, H., Ward, E.A., Potts, E.L., and Karbstein, K. (2017). Rps26 directs mRNA-specific translation by recognition of Kozak sequence elements. Nature structural & molecular biology *24*, 700-707.

Fischer, G., Kunemund, V., and Schachner, M. (1986). Neurite outgrowth patterns in cerebellar microexplant cultures are affected by antibodies to the cell surface glycoprotein L1. The Journal of neuroscience : the official journal of the Society for Neuroscience *6*, 605-612.

Fischer, K.R., Durrans, A., Lee, S., Sheng, J., Li, F., Wong, S.T., Choi, H., El Rayes, T., Ryu, S., Troeger, J.*, et al.* (2015). Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature *527*, 472-476.

Fisher, B. (2008). Biological research in the evolution of cancer surgery: a personal perspective. Cancer research *68*, 10007-10020.

Flach, J., Bakker, S.T., Mohrin, M., Conroy, P.C., Pietras, E.M., Reynaud, D., Alvarez, S., Diolaiti, M.E., Ugarte, F., Forsberg, E.C.*, et al.* (2014). Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature *512*, 198-202.

Florian, S., and Mitchison, T.J. (2016). Anti-Microtubule Drugs. Methods in molecular biology (Clifton, NJ) *1413*, 403-421.

Floutsakou, I., Agrawal, S., Nguyen, T.T., Seoighe, C., Ganley, A.R., and McStay, B. (2013). The shared genomic architecture of human nucleolar organizer regions. Genome research *23*, 2003-2012.

Frege, T., and Uversky, V.N. (2015). Intrinsically disordered proteins in the nucleus of human cells. Biochemistry and biophysics reports *1*, 33-51.

Freund, A., Orjalo, A.V., Desprez, P.Y., and Campisi, J. (2010). Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med *16*, 238-246.

Friedli, A., Fischer, E., Novak-Hofer, I., Cohrs, S., Ballmer-Hofer, K., Schubiger, P.A., Schibli, R., and Grunberg, J. (2009). The soluble form of the cancer-associated L1 cell adhesion molecule is a pro-angiogenic factor. The international journal of biochemistry & cell biology *41*, 1572-1580.

Frisch, S.M., Schaller, M., and Cieply, B. (2013). Mechanisms that link the oncogenic epithelial-mesenchymal transition to suppression of anoikis. Journal of cell science *126*, 21-29.

Fritze, C.E., Verschueren, K., Strich, R., and Easton Esposito, R. (1997). Direct evidence for SIR2 modulation of chromatin structure in yeast rDNA. The EMBO journal *16*, 6495-6509.

Fumagalli, M., Rossiello, F., Clerici, M., Barozzi, S., Cittaro, D., Kaplunov, J.M., Bucci, G., Dobreva, M., Matti, V., Beausejour, C.M.*, et al.* (2012a). Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nature cell biology *14*, 355-365.

Fumagalli, S., Di Cara, A., Neb-Gulati, A., Natt, F., Schwemberger, S., Hall, J., Babcock, G.F., Bernardi, R., Pandolfi, P.P., and Thomas, G. (2009). Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpL11-translation-dependent mechanism of p53 induction. Nature cell biology *11*, 501-508.

Fumagalli, S., Ivanenkov, V.V., Teng, T., and Thomas, G. (2012b). Suprainduction of p53 by disruption of 40S and 60S ribosome biogenesis leads to the activation of a novel G2/M checkpoint. Genes & development *26*, 1028-1040.

Gaillard, C., Tokuyasu, T.A., Rosen, G., Sotzen, J., Vitaliano-Prunier, A., Roy, R., Passegue, E., de The, H., Figueroa, M.E., and Kogan, S.C. (2015a). Transcription and methylation analyses of preleukemic promyelocytes indicate a dual role for PML/RARA in leukemia initiation. Haematologica *100*, 1064-1075.

Gaillard, H., Garcia-Muse, T., and Aguilera, A. (2015b). Replication stress and cancer. Nature reviews Cancer *15*, 276-289.

Galluzzi, L., Vitale, I., Aaronson, S.A., Abrams, J.M., Adam, D., Agostinis, P., Alnemri, E.S., Altucci, L., Amelio, I., Andrews, D.W.*, et al.* (2018). Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. Cell death and differentiation *25*, 486-541.

Gambacorta, M., Flenghi, L., Fagioli, M., Pileri, S., Leoncini, L., Bigerna, B., Pacini, R., Tanci, L.N., Pasqualucci, L., Ascani, S.*, et al.* (1996). Heterogeneous nuclear expression of the promyelocytic leukemia (PML) protein in normal and neoplastic human tissues. The American journal of pathology *149*, 2023-2035.

Ganguli, G., and Wasylyk, B. (2003). p53-independent functions of MDM2. Molecular cancer research : MCR *1*, 1027-1035.

Ganley, A.R., and Kobayashi, T. (2014). Ribosomal DNA and cellular senescence: new evidence supporting the connection between rDNA and aging. FEMS yeast research *14*, 49-59.

Gast, D., Riedle, S., Issa, Y., Pfeifer, M., Beckhove, P., Sanderson, M.P., Arlt, M., Moldenhauer, G., Fogel, M., Kruger, A.*, et al.* (2008). The cytoplasmic part of L1-CAM controls growth and gene expression in human tumors that is reversed by therapeutic antibodies. Oncogene *27*, 1281-1289.

Gavert, N., Ben-Shmuel, A., Lemmon, V., Brabletz, T., and Ben-Ze'ev, A. (2010). Nuclear factor-kappaB signaling and ezrin are essential for L1-mediated metastasis of colon cancer cells. Journal of cell science *123*, 2135-2143.

Geismann, C., Morscheck, M., Koch, D., Bergmann, F., Ungefroren, H., Arlt, A., Tsao, M.S., Bachem, M.G., Altevogt, P., Sipos, B.*, et al.* (2009). Up-regulation of L1CAM in pancreatic duct cells is transforming growth factor beta1- and slug-dependent: role in malignant transformation of pancreatic cancer. Cancer research *69*, 4517-4526.

Gewirtz, D.A., Holt, S.E., and Elmore, L.W. (2008). Accelerated senescence: an emerging role in tumor cell response to chemotherapy and radiation. Biochem Pharmacol *76*, 947-957.

Gibson, E.G., King, M.M., Mercer, S.L., and Deweese, J.E. (2016). Two-Mechanism Model for the Interaction of Etoposide Quinone with Topoisomerase IIalpha. Chemical research in toxicology *29*, 1541-1548.

Gil, O.D., Sakurai, T., Bradley, A.E., Fink, M.Y., Cassella, M.R., Kuo, J.A., and Felsenfeld, D.P. (2003). Ankyrin binding mediates L1CAM interactions with static components of the cytoskeleton and inhibits retrograde movement of L1CAM on the cell surface. The Journal of cell biology *162*, 719-730.

Giorgi, C., Ito, K., Lin, H.K., Santangelo, C., Wieckowski, M.R., Lebiedzinska, M., Bononi, A., Bonora, M., Duszynski, J., Bernardi, R.*, et al.* (2010). PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. Science (New York, NY) *330*, 1247-1251.

Gjerset, R.A., and Bandyopadhyay, K. (2006). Regulation of p14ARF through subnuclear compartmentalization. Cell cycle (Georgetown, Tex) *5*, 686-690.

Gjidoda, A., and Henry, R.W. (2013). RNA polymerase III repression by the retinoblastoma tumor suppressor protein. Biochim Biophys Acta *1829*, 385-392.

Goldstein, M., and Kastan, M.B. (2015). The DNA damage response: implications for tumor responses to radiation and chemotherapy. Annual review of medicine *66*, 129-143.

Golomb, L., Bublik, D.R., Wilder, S., Nevo, R., Kiss, V., Grabusic, K., Volarevic, S., and Oren, M. (2012). Importin 7 and exportin 1 link c-Myc and p53 to regulation of ribosomal biogenesis. Molecular cell *45*, 222-232.

Golomb, L., Volarevic, S., and Oren, M. (2014). p53 and ribosome biogenesis stress: the essentials. FEBS letters *588*, 2571-2579.

Gonzalez, I.L., and Sylvester, J.E. (1995). Complete sequence of the 43-kb human ribosomal DNA repeat: analysis of the intergenic spacer. Genomics *27*, 320-328.

Gonzalez, L.C., Ghadaouia, S., Martinez, A., and Rodier, F. (2016). Premature aging/senescence in cancer cells facing therapy: good or bad? Biogerontology *17*, 71-87.

Goodfellow, S.J., and Zomerdijk, J.C. (2013). Basic mechanisms in RNA polymerase I transcription of the ribosomal RNA genes. Sub-cellular biochemistry *61*, 211-236.

Govoni, M., Farabegoli, F., Pession, A., and Novello, F. (1994). Inhibition of topoisomerase II activity and its effect on nucleolar structure and function. Experimental cell research *211*, 36-41.

Greaves, M., and Maley, C.C. (2012). Clonal evolution in cancer. Nature *481*, 306-313.

Grignani, F., Testa, U., Rogaia, D., Ferrucci, P.F., Samoggia, P., Pinto, A., Aldinucci, D., Gelmetti, V., Fagioli, M., Alcalay, M.*, et al.* (1996). Effects on differentiation by the promyelocytic leukemia PML/RARalpha protein depend on the fusion of the PML protein dimerization and RARalpha DNA binding domains. The EMBO journal *15*, 4949-4958.

Grob, A., Colleran, C., and McStay, B. (2014). Construction of synthetic nucleoli in human cells reveals how a major functional nuclear domain is formed and propagated through cell division. Genes & development *28*, 220-230.

Grob, A., and McStay, B. (2014). Construction of synthetic nucleoli and what it tells us about propagation of sub-nuclear domains through cell division. Cell cycle (Georgetown, Tex) *13*, 2501-2508.

Grufman, P., and Karre, K. (2000). Innate and adaptive immunity to tumors: IL-12 is required for optimal responses. European journal of immunology *30*, 1088-1093.

Grummt, I. (2003). Life on a planet of its own: regulation of RNA polymerase I transcription in the nucleolus. Genes & development *17*, 1691-1702.

Grummt, I. (2010). Wisely chosen paths--regulation of rRNA synthesis: delivered on 30 June 2010 at the 35th FEBS Congress in Gothenburg, Sweden. The FEBS journal *277*, 4626-4639.

Gu, X., Lu, C., He, D., Lu, Y., Jin, J., Liu, D., and Ma, X. (2016). Notch3 negatively regulates chemoresistance in breast cancers. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine *37*, 15825–15833.

Guan, D., and Kao, H.Y. (2015). The function, regulation and therapeutic implications of the tumor suppressor protein, PML. Cell & bioscience *5*, 60.

Guan, D., Lim, J.H., Peng, L., Liu, Y., Lam, M., Seto, E., and Kao, H.Y. (2014). Deacetylation of the tumor suppressor protein PML regulates hydrogen peroxide-induced cell death. Cell death & disease *5*, e1340.

Guan, X., Jiao, Y., Chen, L., Li, X., Shang, G., Wang, F., Feng, Y., and Jiao, M. (2017). Disruption and restoration of nucleolar FC and DFC during S phase in HeLa cells. Cell biology international *41*, 258-266.

Guo, A., Salomoni, P., Luo, J., Shih, A., Zhong, S., Gu, W., and Pandolfi, P.P. (2000). The function of PML in p53-dependent apoptosis. Nature cell biology *2*, 730-736.

Guo, L., Giasson, B.I., Glavis-Bloom, A., Brewer, M.D., Shorter, J., Gitler, A.D., and Yang, X. (2014a). A cellular system that degrades misfolded proteins and protects against neurodegeneration. Molecular cell *55*, 15-30.

Guo, S., Cheng, X., Lim, J.H., Liu, Y., and Kao, H.Y. (2014b). Control of antioxidative response by the tumor suppressor protein PML through regulating Nrf2 activity. Molecular biology of the cell *25*, 2485-2498.

Gurrieri, C., Capodieci, P., Bernardi, R., Scaglioni, P.P., Nafa, K., Rush, L.J., Verbel, D.A., Cordon-Cardo, C., and Pandolfi, P.P. (2004). Loss of the tumor suppressor PML in human cancers of multiple histologic origins. Journal of the National Cancer Institute *96*, 269-279.

Haaf, T., and Ward, D.C. (1996). Inhibition of RNA polymerase II transcription causes chromatin decondensation, loss of nucleolar structure, and dispersion of chromosomal domains. Experimental cell research *224*, 163-173.

Hallgren, J., Pietrzak, M., Rempala, G., Nelson, P.T., and Hetman, M. (2014). Neurodegeneration-associated instability of ribosomal DNA. Biochim Biophys Acta *1842*, 860-868.

Hambardzumyan, D., Squatrito, M., and Holland, E.C. (2006). Radiation resistance and stem-like cells in brain tumors. Cancer cell *10*, 454-456.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646-674.

Harding, S.M., Boiarsky, J.A., and Greenberg, R.A. (2015). ATM Dependent Silencing Links Nucleolar Chromatin Reorganization to DNA Damage Recognition. Cell reports *13*, 251-259.

Harley, C.B., Futcher, A.B., and Greider, C.W. (1990). Telomeres shorten during ageing of human fibroblasts. Nature *345*, 458-460.

Hayflick, L., and Moorhead, P.S. (1961). The serial cultivation of human diploid cell strains. Experimental cell research *25*, 585-621.

Heerboth, S., Housman, G., Leary, M., Longacre, M., Byler, S., Lapinska, K., Willbanks, A., and Sarkar, S. (2015). EMT and tumor metastasis. Clin Transl Med *4*, 6.

Heidenreich, A., Bastian, P.J., Bellmunt, J., Bolla, M., Joniau, S., van der Kwast, T., Mason, M., Matveev, V., Wiegel, T., Zattoni, F.*, et al.* (2014). EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. European urology *65*, 124-137.

Helmrich, A., Ballarino, M., Nudler, E., and Tora, L. (2013). Transcription-replication encounters, consequences and genomic instability. Nature structural & molecular biology *20*, 412-418.

Henderson, A.S., Warburton, D., and Atwood, K.C. (1972). Location of ribosomal DNA in the human chromosome complement. Proceedings of the National Academy of Sciences of the United States of America *69*, 3394-3398.

Hernandez-Segura, A., Nehme, J., and Demaria, M. (2018). Hallmarks of Cellular Senescence. Trends Cell Biol *28*, 436-453.

Hernandez-Verdun, D. (2011). Assembly and disassembly of the nucleolus during the cell cycle. Nucleus (Austin, Tex) *2*, 189-194.

Hewitt, G., Jurk, D., Marques, F.D., Correia-Melo, C., Hardy, T., Gackowska, A., Anderson, R., Taschuk, M., Mann, J., and Passos, J.F. (2012). Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. Nature communications *3*, 708.

Ho, J.S., Ma, W., Mao, D.Y., and Benchimol, S. (2005). p53-Dependent transcriptional repression of c-myc is required for G1 cell cycle arrest. Molecular and cellular biology *25*, 7423-7431.

Hodny, Z., Reinis, M., Hubackova, S., Vasicova, P., and Bartek, J. (2016). Interferon gamma/NADPH oxidase defense system in immunity and cancer. Oncoimmunology *5*, e1080416.

Hoeijmakers, J.H. (2009). DNA damage, aging, and cancer. The New England journal of medicine *361*, 1475-1485.

Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C.C. (1991). p53 mutations in human cancers. Science (New York, NY) *253*, 49-53.

Holmberg Olausson, K., Nister, M., and Lindstrom, M.S. (2012). p53 -Dependent and -Independent Nucleolar Stress Responses. Cells *1*, 774-798.

Holohan, C., Van Schaeybroeck, S., Longley, D.B., and Johnston, P.G. (2013). Cancer drug resistance: an evolving paradigm. Nature reviews Cancer *13*, 714-726.

Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., and Sarkar, S. (2014). Drug resistance in cancer: an overview. Cancers (Basel) *6*, 1769-1792.

Hsieh, A.C., Costa, M., Zollo, O., Davis, C., Feldman, M.E., Testa, J.R., Meyuhas, O., Shokat, K.M., and Ruggero, D. (2010). Genetic dissection of the oncogenic mTOR pathway reveals druggable addiction to translational control via 4EBP-eIF4E. Cancer cell *17*, 249-261.

Hsu, K.S., and Kao, H.Y. (2018). PML: Regulation and multifaceted function beyond tumor suppression. Cell & bioscience *8*, 5.

Hubackova, S., Krejcikova, K., Bartek, J., and Hodny, Z. (2012a). IL1- and TGFbeta-Nox4 signaling, oxidative stress and DNA damage response are shared features of replicative, oncogene-induced, and drug-induced paracrine 'bystander senescence'. Aging *4*, 932-951.

Hubackova, S., Krejcikova, K., Bartek, J., and Hodny, Z. (2012b). Interleukin 6 signaling regulates promyelocytic leukemia protein gene expression in human normal and cancer cells. The Journal of biological chemistry *287*, 26702-26714.

Hubackova, S., Kucerova, A., Michlits, G., Kyjacova, L., Reinis, M., Korolov, O., Bartek, J., and Hodny, Z. (2016). IFNgamma induces oxidative stress, DNA damage and tumor cell senescence via TGFbeta/SMAD signaling-dependent induction of Nox4 and suppression of ANT2. Oncogene *35*, 1236-1249.

Hubackova, S., Novakova, Z., Krejcikova, K., Kosar, M., Dobrovolna, J., Duskova, P., Hanzlikova, H., Vancurova, M., Barath, P., Bartek, J.*, et al.* (2010). Regulation of the PML tumor suppressor in drug-induced senescence of human normal and cancer cells by JAK/STAT-mediated signaling. Cell cycle (Georgetown, Tex) *9*, 3085-3099.

Iadevaia, V., Caldarola, S., Biondini, L., Gismondi, A., Karlsson, S., Dianzani, I., and Loreni, F. (2010). PIM1 kinase is destabilized by ribosomal stress causing inhibition of cell cycle progression. Oncogene *29*, 5490-5499.

Imrichova, T., Hubackova, S., Kucerova, A., Kosla, J., Bartek, J., Hodny, Z., Vasicova, P.: Dynamic PML protein nucleolar associations with persistent DNA damage lesions in response to nucleolar stress and senescence-inducing stimuli. Aging (Albany NY) (*in press*).

Ishov, A.M., Sotnikov, A.G., Negorev, D., Vladimirova, O.V., Neff, N., Kamitani, T., Yeh, E.T., Strauss, J.F., 3rd, and Maul, G.G. (1999). PML is critical for ND10 formation and recruits the PML-interacting protein daxx to this nuclear structure when modified by SUMO-1. The Journal of cell biology *147*, 221-234.

Ito, K., Bernardi, R., Morotti, A., Matsuoka, S., Saglio, G., Ikeda, Y., Rosenblatt, J., Avigan, D.E., Teruya-Feldstein, J., and Pandolfi, P.P. (2008). PML targeting eradicates quiescent leukaemia-initiating cells. Nature *453*, 1072-1078.

Jackson, J.G., Pant, V., Li, Q., Chang, L.L., Quintas-Cardama, A., Garza, D., Tavana, O., Yang, P., Manshouri, T., Li, Y.*, et al.* (2012). p53-mediated senescence impairs the apoptotic response to chemotherapy and clinical outcome in breast cancer. Cancer cell *21*, 793-806.

Jackson, S.P., and Bartek, J. (2009). The DNA-damage response in human biology and disease. Nature *461*, 1071-1078.

Jakel, S., and Gorlich, D. (1998). Importin beta, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. The EMBO journal *17*, 4491-4502.

Jakel, S., Mingot, J.M., Schwarzmaier, P., Hartmann, E., and Gorlich, D. (2002). Importins fulfil a dual function as nuclear import receptors and cytoplasmic chaperones for exposed basic domains. The EMBO journal *21*, 377-386.

Jamal, M., Rath, B.H., Tsang, P.S., Camphausen, K., and Tofilon, P.J. (2012). The brain microenvironment preferentially enhances the radioresistance of CD133(+) glioblastoma stem-like cells. Neoplasia *14*, 150-158.

James, M.J., and Zomerdijk, J.C. (2004). Phosphatidylinositol 3-kinase and mTOR signaling pathways regulate RNA polymerase I transcription in response to IGF-1 and nutrients. The Journal of biological chemistry *279*, 8911-8918.

Janderova-Rossmeislova, L., Novakova, Z., Vlasakova, J., Philimonenko, V., Hozak, P., and Hodny, Z. (2007). PML protein association with specific nucleolar structures differs in normal, tumor and senescent human cells. Journal of structural biology *159*, 56-70.

Jeanne, M., Lallemand-Breitenbach, V., Ferhi, O., Koken, M., Le Bras, M., Duffort, S., Peres, L., Berthier, C., Soilihi, H., Raught, B.*, et al.* (2010). PML/RARA oxidation and arsenic binding initiate the antileukemia response of As2O3. Cancer cell *18*, 88-98.

Jensen, K., Shiels, C., and Freemont, P.S. (2001). PML protein isoforms and the RBCC/TRIM motif. Oncogene *20*, 7223-7233.

Jiang, L., He, D., Yang, D., Chen, Z., Pan, Q., Mao, A., Cai, Y., Li, X., Xing, H., Shi, M.*, et al.* (2014). MiR-489 regulates chemoresistance in breast cancer via epithelial mesenchymal transition pathway. FEBS letters *588*, 2009-2015.

Jin, A., Itahana, K., O'Keefe, K., and Zhang, Y. (2004). Inhibition of HDM2 and activation of p53 by ribosomal protein L23. Molecular and cellular biology *24*, 7669-7680.

Jin, Y., Xu, K., Chen, Q., Wang, B., Pan, J., Huang, S., Wei, Y., and Ma, H. (2018). Simvastatin inhibits the development of radioresistant esophageal cancer cells by increasing the radiosensitivity and reversing EMT process via the PTEN-PI3K/AKT pathway. Experimental cell research *362*, 362-369.

Jin, Z., Guan, L., Song, Y., Xiang, G.M., Chen, S.X., and Gao, B. (2016). MicroRNA-138 regulates chemoresistance in human non-small cell lung cancer via epithelial mesenchymal transition. European review for medical and pharmacological sciences *20*, 1080-1086.

Jing, H., Kase, J., Dorr, J.R., Milanovic, M., Lenze, D., Grau, M., Beuster, G., Ji, S., Reimann, M., Lenz, P.*, et al.* (2011). Opposing roles of NF-kappaB in anti-cancer treatment outcome unveiled by cross-species investigations. Genes & development *25*, 2137-2146.

Ju, B.L., Chen, Y.B., Zhang, W.Y., Yu, C.H., Zhu, D.Q., and Jin, J. (2015). miR-145 regulates chemoresistance in hepatocellular carcinoma via epithelial mesenchymal transition. Cellular and molecular biology (Noisy-le-Grand, France) *61*, 12-16.

Kajanne, R., Miettinen, P., Tenhunen, M., and Leppa, S. (2009). Transcription factor AP-1 promotes growth and radioresistance in prostate cancer cells. International journal of oncology *35*, 1175-1182.

Kamitani, T., Kito, K., Nguyen, H.P., Wada, H., Fukuda-Kamitani, T., and Yeh, E.T. (1998). Identification of three major sentrinization sites in PML. The Journal of biological chemistry *273*, 26675-26682.

Kanaar, R., Wyman, C., and Rothstein, R. (2008). Quality control of DNA break metabolism: in the 'end', it's a good thing. The EMBO journal *27*, 581-588.

Kang, J., Kim, E., Kim, W., Seong, K.M., Youn, H., Kim, J.W., Kim, J., and Youn, B. (2013). Rhamnetin and cirsiliol induce radiosensitization and inhibition of epithelial-mesenchymal transition (EMT) by miR-34a-mediated suppression of Notch-1 expression in non-small cell lung cancer cell lines. The Journal of biological chemistry *288*, 27343-27357.

Kang, T.W., Yevsa, T., Woller, N., Hoenicke, L., Wuestefeld, T., Dauch, D., Hohmeyer, A., Gereke, M., Rudalska, R., Potapova, A.*, et al.* (2011). Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature *479*, 547-551.

Karanam, K., Kafri, R., Loewer, A., and Lahav, G. (2012). Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase. Molecular cell *47*, 320-329.

Kastan, M.B., and Bartek, J. (2004). Cell-cycle checkpoints and cancer. Nature *432*, 316-323.

Keilhauer, G., Faissner, A., and Schachner, M. (1985). Differential inhibition of neurone-neurone, neurone-astrocyte and astrocyte-astrocyte adhesion by L1, L2 and N-CAM antibodies. Nature *316*, 728-730.

Kerscher, O. (2007). SUMO junction-what's your function? New insights through SUMO-interacting motifs. EMBO reports *8*, 550-555.

Khan, M.M., Nomura, T., Kim, H., Kaul, S.C., Wadhwa, R., Shinagawa, T., Ichikawa-Iwata, E., Zhong, S., Pandolfi, P.P., and Ishii, S. (2001). Role of PML and PML-RARalpha in Mad-mediated transcriptional repression. Molecular cell *7*, 1233-1243.

Khanna, A. (2015). DNA damage in cancer therapeutics: a boon or a curse? Cancer research *75*, 2133-2138.

Killen, M.W., Stults, D.M., Adachi, N., Hanakahi, L., and Pierce, A.J. (2009). Loss of Bloom syndrome protein destabilizes human gene cluster architecture. Human molecular genetics *18*, 3417-3428.

Kim, K.S., Kang, K.W., Seu, Y.B., Baek, S.H., and Kim, J.R. (2009). Interferon-gamma induces cellular senescence through p53-dependent DNA damage signaling in human endothelial cells. Mechanisms of ageing and development *130*, 179-188.

Ko, Y.S., Jin, H., Lee, J.S., Park, S.W., Chang, K.C., Kang, K.M., Jeong, B.K., and Kim, H.J. (2018). Radioresistant breast cancer cells exhibit increased resistance to chemotherapy and enhanced invasive properties due to cancer stem cells. Oncology reports *40*, 3752-3762.

Kobayashi, T., and Ganley, A.R. (2005). Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. Science (New York, NY) *309*, 1581-1584.

Kong, Z., Xie, D., Boike, T., Raghavan, P., Burma, S., Chen, D.J., Habib, A.A., Chakraborty, A., Hsieh, J.T., and Saha, D. (2010). Downregulation of human DAB2IP gene expression in prostate cancer cells results in resistance to ionizing radiation. Cancer research *70*, 2829-2839.

Konge, J., Leteurtre, F., Goislard, M., Biard, D., Morel-Altmeyer, S., Vaurijoux, A., Gruel, G., Chevillard, S., and Lebeau, J. (2018). Breast cancer stem cell-like cells generated during TGFbeta-induced EMT are radioresistant. Oncotarget *9*, 23519-23531.

Korgaonkar, C., Hagen, J., Tompkins, V., Frazier, A.A., Allamargot, C., Quelle, F.W., and Quelle, D.E. (2005). Nucleophosmin (B23) targets ARF to nucleoli and inhibits its function. Molecular and cellular biology *25*, 1258-1271.

Korolchuk, V.I., Miwa, S., Carroll, B., and von Zglinicki, T. (2017). Mitochondria in Cell Senescence: Is Mitophagy the Weakest Link? EBioMedicine *21*, 7-13.

Korsholm, L.M., Gal, Z., Lin, L., Quevedo, O., Ahmad, D.A., Dulina, E., Luo, Y., Bartek, J., and Larsen, D.H. (2019). Double-strand breaks in ribosomal RNA genes activate a distinct signaling and chromatin response to facilitate nucleolar restructuring and repair. Nucleic acids research *47*, 8019–8035.

Kortlever, R.M., Higgins, P.J., and Bernards, R. (2006). Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. Nature cell biology *8*, 877-884.

Krause, M., Dubrovska, A., Linge, A., and Baumann, M. (2017). Cancer stem cells: Radioresistance, prediction of radiotherapy outcome and specific targets for combined treatments. Adv Drug Deliv Rev *109*, 63-73.

Kreso, A., O'Brien, C.A., van Galen, P., Gan, O.I., Notta, F., Brown, A.M., Ng, K., Ma, J., Wienholds, E., Dunant, C.*, et al.* (2013). Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. Science (New York, NY) *339*, 543-548.

Kressler, D., Hurt, E., and Bassler, J. (2017). A Puzzle of Life: Crafting Ribosomal Subunits. Trends in biochemical sciences *42*, 640-654.

Krtolica, A., Parrinello, S., Lockett, S., Desprez, P.Y., and Campisi, J. (2001). Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proceedings of the National Academy of Sciences of the United States of America *98*, 12072-12077.

Kruhlak, M., Crouch, E.E., Orlov, M., Montano, C., Gorski, S.A., Nussenzweig, A., Misteli, T., Phair, R.D., and Casellas, R. (2007). The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks. Nature *447*, 730-734.

Kumar, P.P., Bischof, O., Purbey, P.K., Notani, D., Urlaub, H., Dejean, A., and Galande, S. (2007). Functional interaction between PML and SATB1 regulates chromatin-loop architecture and transcription of the MHC class I locus. Nature cell biology *9*, 45-56.

Kurki, S., Peltonen, K., Latonen, L., Kiviharju, T.M., Ojala, P.M., Meek, D., and Laiho, M. (2004). Nucleolar protein NPM interacts with HDM2 and protects tumor suppressor protein p53 from HDM2-mediated degradation. Cancer cell *5*, 465-475.

Kuroda, T., Murayama, A., Katagiri, N., Ohta, Y.M., Fujita, E., Masumoto, H., Ema, M., Takahashi, S., Kimura, K., and Yanagisawa, J. (2011). RNA content in the nucleolus alters p53 acetylation via MYBBP1A. The EMBO journal *30*, 1054-1066.

Kurtova, A.V., Xiao, J., Mo, Q., Pazhanisamy, S., Krasnow, R., Lerner, S.P., Chen, F., Roh, T.T., Lay, E., Ho, P.L.*, et al.* (2015). Blocking PGE2-induced tumour repopulation abrogates bladder cancer chemoresistance. Nature *517*, 209-213.

Kyjacova, L., Hubackova, S., Krejcikova, K., Strauss, R., Hanzlikova, H., Dzijak, R., Imrichova, T., Simova, J., Reinis, M., Bartek, J.*, et al.* (2015). Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, Erk signaling-dependent cells. Cell death and differentiation *22*, 898-911.

Lallemand-Breitenbach, V., and de The, H. (2010). PML nuclear bodies. Cold Spring Harbor perspectives in biology *2*, a000661.

Lallemand-Breitenbach, V., Zhu, J., Puvion, F., Koken, M., Honore, N., Doubeikovsky, A., Duprez, E., Pandolfi, P.P., Puvion, E., Freemont, P.*, et al.* (2001). Role of promyelocytic leukemia (PML) sumolation in nuclear body formation, 11S proteasome recruitment, and As2O3-induced PML or PML/retinoic acid receptor alpha degradation. The Journal of experimental medicine *193*, 1361-1371.

Lam, Y.W., Evans, V.C., Heesom, K.J., Lamond, A.I., and Matthews, D.A. (2010). Proteomics analysis of the nucleolus in adenovirus-infected cells. Molecular & cellular proteomics : MCP *9*, 117-130.

Lam, Y.W., Lamond, A.I., Mann, M., and Andersen, J.S. (2007). Analysis of nucleolar protein dynamics reveals the nuclear degradation of ribosomal proteins. Current biology : CB *17*, 749-760.

Lamb, R., Ablett, M.P., Spence, K., Landberg, G., Sims, A.H., and Clarke, R.B. (2013). Wnt pathway activity in breast cancer sub-types and stem-like cells. PloS one *8*, e67811.

Langhendries, J.L., Nicolas, E., Doumont, G., Goldman, S., and Lafontaine, D.L. (2016). The human box C/D snoRNAs U3 and U8 are required for pre-rRNA processing and tumorigenesis. Oncotarget *7*, 59519-59534.

Larsen, A.K., Galmarini, C.M., and D'Incalci, M. (2016). Unique features of trabectedin mechanism of action. Cancer chemotherapy and pharmacology *77*, 663-671.

Larsen, D.H., Hari, F., Clapperton, J.A., Gwerder, M., Gutsche, K., Altmeyer, M., Jungmichel, S., Toledo, L.I., Fink, D., Rask, M.B.*, et al.* (2014). The NBS1-Treacle complex controls ribosomal RNA transcription in response to DNA damage. Nature cell biology *16*, 792-803.

Lasry, A., and Ben-Neriah, Y. (2015). Senescence-associated inflammatory responses: aging and cancer perspectives. Trends Immunol *36*, 217-228.

Lee, A.C., Fenster, B.E., Ito, H., Takeda, K., Bae, N.S., Hirai, T., Yu, Z.X., Ferrans, V.J., Howard, B.H., and Finkel, T. (1999). Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. The Journal of biological chemistry *274*, 7936-7940.

Lee, H.C., and Wei, Y.H. (2007). Oxidative stress, mitochondrial DNA mutation, and apoptosis in aging. Experimental biology and medicine (Maywood, NJ) *232*, 592-606.

Lee, J.M., Dedhar, S., Kalluri, R., and Thompson, E.W. (2006). The epithelial-mesenchymal transition: new insights in signaling, development, and disease. The Journal of cell biology *172*, 973-981.

Lee, M., and Lee, J.S. (2014). Exploiting tumor cell senescence in anticancer therapy. BMB reports *47*, 51-59.

Lee, T.Y., Liu, C.L., Chang, Y.C., Nieh, S., Lin, Y.S., Jao, S.W., Chen, S.F., and Liu, T.Y. (2016). Increased chemoresistance via Snail-Raf kinase inhibitor protein signaling in colorectal cancer in response to a nicotine derivative. Oncotarget *7*, 23512-23520.

Lehembre, F., Muller, S., Pandolfi, P.P., and Dejean, A. (2001). Regulation of Pax3 transcriptional activity by SUMO-1-modified PML. Oncogene *20*, 1-9.

Lessard, F., Brakier-Gingras, L., and Ferbeyre, G. (2019). Ribosomal Proteins Control Tumor Suppressor Pathways in Response to Nucleolar Stress. BioEssays : news and reviews in molecular, cellular and developmental biology *41*, e1800183.

Lessard, F., Igelmann, S., Trahan, C., Huot, G., Saint-Germain, E., Mignacca, L., Del Toro, N., Lopes-Paciencia, S., Le Calve, B., Montero, M.*, et al.* (2018). Senescence-associated ribosome biogenesis defects contributes to cell cycle arrest through the Rb pathway. Nature cell biology *20*, 789-799.

Lessard, F., Morin, F., Ivanchuk, S., Langlois, F., Stefanovsky, V., Rutka, J., and Moss, T. (2010). The ARF tumor suppressor controls ribosome biogenesis by regulating the RNA polymerase I transcription factor TTF-I. Molecular cell *38*, 539-550.

Levy, N., Yonish-Rouach, E., Oren, M., and Kimchi, A. (1993). Complementation by wild-type p53 of interleukin-6 effects on M1 cells: induction of cell cycle exit and cooperativity with c-myc suppression. Molecular and cellular biology *13*, 7942-7952.

Li, F., Zhou, K., Gao, L., Zhang, B., Li, W., Yan, W., Song, X., Yu, H., Wang, S., Yu, N.*, et al.* (2016). Radiation induces the generation of cancer stem cells: A novel mechanism for cancer radioresistance. Oncol Lett *12*, 3059-3065.

Li, J., Liu, H., Yu, J., and Yu, H. (2015). Chemoresistance to doxorubicin induces epithelial-mesenchymal transition via upregulation of transforming growth factor beta signaling in HCT116 colon cancer cells. Molecular medicine reports *12*, 192-198.

Li, P., Wang, D., Li, H., Yu, Z., Chen, X., and Fang, J. (2014). Identification of nucleolus-localized PTEN and its function in regulating ribosome biogenesis. Molecular biology reports *41*, 6383-6390.

Li, Y., and Galileo, D.S. (2010). Soluble L1CAM promotes breast cancer cell adhesion and migration in vitro, but not invasion. Cancer cell international *10*, 34.

Liao, H., Bai, Y., Qiu, S., Zheng, L., Huang, L., Liu, T., Wang, X., Liu, Y., Xu, N., Yan, X.*, et al.* (2015). MiR-203 downregulation is responsible for chemoresistance in human glioblastoma by promoting epithelial-mesenchymal transition via SNAI2. Oncotarget *6*, 8914-8928.

Liau, B.B., Sievers, C., Donohue, L.K., Gillespie, S.M., Flavahan, W.A., Miller, T.E., Venteicher, A.S., Hebert, C.H., Carey, C.D., Rodig, S.J.*, et al.* (2017). Adaptive Chromatin Remodeling Drives Glioblastoma Stem Cell Plasticity and Drug Tolerance. Cell stem cell *20*, 233-246.e237.

Lin, A.W., and Lowe, S.W. (2001). Oncogenic ras activates the ARF-p53 pathway to suppress epithelial cell transformation. Proceedings of the National Academy of Sciences of the United States of America *98*, 5025-5030.

Lin, H.K., Bergmann, S., and Pandolfi, P.P. (2004). Cytoplasmic PML function in TGF-beta signalling. Nature *431*, 205-211.

Lindner, J., Rathjen, F.G., and Schachner, M. (1983). L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum. Nature *305*, 427-430.

Lindstrom, M.S., Jurada, D., Bursac, S., Orsolic, I., Bartek, J., and Volarevic, S. (2018). Nucleolus as an emerging hub in maintenance of genome stability and cancer pathogenesis. Oncogene *37*, 2351-2366.

Lindstrom, M.S., and Nister, M. (2010). Silencing of ribosomal protein S9 elicits a multitude of cellular responses inhibiting the growth of cancer cells subsequent to p53 activation. PloS one *5*, e9578.

Linnane, A.W., Marzuki, S., Ozawa, T., and Tanaka, M. (1989). Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. Lancet (London, England) *1*, 642-645.

Liu, J., Song, Y., Qian, J., Liu, B., Dong, Y., Tian, B., and Sun, Z. (2011). Promyelocytic leukemia protein interacts with werner syndrome helicase and regulates double-strand break repair in gamma-irradiation-induced DNA damage responses. Biochemistry Biokhimiia *76*, 550-554.

Liu, S., Sun, J., Cai, B., Xi, X., Yang, L., Zhang, Z., Feng, Y., and Sun, Y. (2016a). NANOG regulates epithelial-mesenchymal transition and chemoresistance through activation of the STAT3 pathway in epithelial ovarian cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine *37*, 9671-9680.

Liu, Y., Deisenroth, C., and Zhang, Y. (2016b). RP-MDM2-p53 Pathway: Linking Ribosomal Biogenesis and Tumor Surveillance. Trends in cancer *2*, 191-204.

Liu, Y., Liang, S., and Tartakoff, A.M. (1996). Heat shock disassembles the nucleolus and inhibits nuclear protein import and poly(A)+ RNA export. The EMBO journal *15*, 6750-6757.

Llanos, S., and Serrano, M. (2010). Depletion of ribosomal protein L37 occurs in response to DNA damage and activates p53 through the L11/MDM2 pathway. Cell cycle (Georgetown, Tex) *9*, 4005-4012.

Loeb, L.A. (2001). A mutator phenotype in cancer. Cancer research *61*, 3230-3239.

Lohrum, M.A., Ludwig, R.L., Kubbutat, M.H., Hanlon, M., and Vousden, K.H. (2003). Regulation of HDM2 activity by the ribosomal protein L11. Cancer cell *3*, 577-587.

Long, B.H. (1992). Mechanisms of action of teniposide (VM-26) and comparison with etoposide (VP-16). Seminars in oncology *19*, 3-19.

Lord, C.J., and Ashworth, A. (2012). The DNA damage response and cancer therapy. Nature *481*, 287-294.

Louria-Hayon, I., Alsheich-Bartok, O., Levav-Cohen, Y., Silberman, I., Berger, M., Grossman, T., Matentzoglu, K., Jiang, Y.H., Muller, S., Scheffner, M.*, et al.* (2009). E6AP promotes the degradation of the PML tumor suppressor. Cell death and differentiation *16*, 1156-1166.

Louria-Hayon, I., Grossman, T., Sionov, R.V., Alsheich, O., Pandolfi, P.P., and Haupt, Y. (2003). The promyelocytic leukemia protein protects p53 from Mdm2-mediated inhibition and degradation. The Journal of biological chemistry *278*, 33134-33141.

Lu, Y., Li, T., Wei, G., Liu, L., Chen, Q., Xu, L., Zhang, K., Zeng, D., and Liao, R. (2016). The long non-coding RNA NEAT1 regulates epithelial to mesenchymal transition and radioresistance in through miR-204/ZEB1 axis in nasopharyngeal carcinoma. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine *37*, 11733-11741.

Luciani, J.J., Depetris, D., Usson, Y., Metzler-Guillemain, C., Mignon-Ravix, C., Mitchell, M.J., Megarbane, A., Sarda, P., Sirma, H., Moncla, A.*, et al.* (2006). PML nuclear bodies are highly organised DNA-protein structures with a function in heterochromatin remodelling at the G2 phase. Journal of cell science *119*, 2518-2531.

Lujambio, A., Akkari, L., Simon, J., Grace, D., Tschaharganeh, D.F., Bolden, J.E., Zhao, Z., Thapar, V., Joyce, J.A., Krizhanovsky, V.*, et al.* (2013). Non-cell-autonomous tumor suppression by p53. Cell *153*, 449-460.

Maarifi, G., Chelbi-Alix, M.K., and Nisole, S. (2014). PML control of cytokine signaling. Cytokine & growth factor reviews *25*, 551-561.

Maciaczyk, D., Picard, D., Zhao, L., Koch, K., Herrera-Rios, D., Li, G., Marquardt, V., Pauck, D., Hoerbelt, T., Zhang, W., *et al.* (2017). CBF1 is clinically prognostic and serves as a target to block cellular invasion and chemoresistance of EMT-like glioblastoma cells. British journal of cancer *117*, 102-112.

Malaquin, N., Martinez, A., and Rodier, F. (2016). Keeping the senescence secretome under control: Molecular reins on the senescence-associated secretory phenotype. Experimental gerontology *82*, 39-49.

Malloy, M.T., McIntosh, D.J., Walters, T.S., Flores, A., Goodwin, J.S., and Arinze, I.J. (2013). Trafficking of the transcription factor Nrf2 to promyelocytic leukemia-nuclear bodies: implications for degradation of NRF2 in the nucleus. The Journal of biological chemistry *288*, 14569-14583.

Marchesini, M., Matocci, R., Tasselli, L., Cambiaghi, V., Orleth, A., Furia, L., Marinelli, C., Lombardi, S., Sammarelli, G., Aversa, F., *et al.* (2016). PML is required for telomere stability in non-neoplastic human cells. Oncogene *35*, 1811-1821.

Marcotte, R., and Wang, E. (2002). Replicative senescence revisited. The journals of gerontology Series A, Biological sciences and medical sciences *57*, B257-269.

Maroui, M.A., Pampin, M., and Chelbi-Alix, M.K. (2011). Promyelocytic leukemia isoform IV confers resistance to encephalomyocarditis virus via the sequestration of 3D polymerase in nuclear bodies. Journal of virology *85*, 13164-13173.

Martin-Ruiz, C., Saretzki, G., Petrie, J., Ladhoff, J., Jeyapalan, J., Wei, W., Sedivy, J., and von Zglinicki, T. (2004). Stochastic variation in telomere shortening rate causes heterogeneity of human fibroblast replicative life span. The Journal of biological chemistry *279*, 17826-17833.

Martincorena, I., Raine, K.M., Gerstung, M., Dawson, K.J., Haase, K., Van Loo, P., Davies, H., Stratton, M.R., and Campbell, P.J. (2017). Universal Patterns of Selection in Cancer and Somatic Tissues. Cell *171*, 1029-1041.

Martino, E., Casamassima, G., Castiglione, S., Cellupica, E., Pantalone, S., Papagni, F., Rui, M., Siciliano, A.M., and Collina, S. (2018). Vinca alkaloids and analogues as anti-cancer agents: Looking back, peering ahead. Bioorganic & medicinal chemistry letters *28*, 2816-2826.

Matjusaitis, M., Chin, G., Sarnoski, E.A., and Stolzing, A. (2016). Biomarkers to identify and isolate senescent cells. Ageing Res Rev *29*, 1-12.

Matt, S., and Hofmann, T.G. (2016). The DNA damage-induced cell death response: a roadmap to kill cancer cells. Cell Mol Life Sci *73*, 2829-2850.

Mattsson, K., Pokrovskaja, K., Kiss, C., Klein, G., and Szekely, L. (2001). Proteins associated with the promyelocytic leukemia gene product (PML)-containing nuclear body move to the nucleolus upon inhibition of proteasome-dependent protein degradation. Proceedings of the National Academy of Sciences of the United States of America *98*, 1012-1017.

Matunis, M.J., Zhang, X.D., and Ellis, N.A. (2006). SUMO: the glue that binds. Developmental cell *11*, 596-597.

Maul, G.G., Yu, E., Ishov, A.M., and Epstein, A.L. (1995). Nuclear domain 10 (ND10) associated proteins are also present in nuclear bodies and redistribute to hundreds of nuclear sites after stress. Journal of cellular biochemistry *59*, 498-513.

Maya-Mendoza, A., Ostrakova, J., Kosar, M., Hall, A., Duskova, P., Mistrik, M., Merchut-Maya, J.M., Hodny, Z., Bartkova, J., Christensen, C., *et al.* (2015). Myc and Ras oncogenes engage different energy metabolism programs and evoke distinct patterns of oxidative and DNA replication stress. Molecular oncology *9*, 601-616.

Mayer, C., and Grummt, I. (2006). Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. Oncogene *25*, 6384-6391.

McGranahan, N., and Swanton, C. (2017). Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell *168*, 613-628.

McStay, B. (2016). Nucleolar organizer regions: genomic 'dark matter' requiring illumination. Genes & development *30*, 1598-1610.

McStay, B., and Grummt, I. (2008). The epigenetics of rRNA genes: from molecular to chromosome biology. Annual review of cell and developmental biology *24*, 131-157.

Mekhail, K., Rivero-Lopez, L., Khacho, M., and Lee, S. (2006). Restriction of rRNA synthesis by VHL maintains energy equilibrium under hypoxia. Cell cycle (Georgetown, Tex) *5*, 2401-2413.

Mellert, H., Sykes, S.M., Murphy, M.E., and McMahon, S.B. (2007). The ARF/oncogene pathway activates p53 acetylation within the DNA binding domain. Cell cycle (Georgetown, Tex) *6*, 1304-1306.

Meyer, N., and Penn, L.Z. (2008). Reflecting on 25 years with MYC. Nature reviews Cancer *8*, 976-990.

Michishita, E., Nakabayashi, K., Suzuki, T., Kaul, S.C., Ogino, H., Fujii, M., Mitsui, Y., and Ayusawa, D. (1999). 5-Bromodeoxyuridine induces senescence-like phenomena in mammalian cells regardless of cell type or species. Journal of biochemistry *126*, 1052-1059.

Miki, T., Xu, Z., Chen-Goodspeed, M., Liu, M., Van Oort-Jansen, A., Rea, M.A., Zhao, Z., Lee, C.C., and Chang, K.S. (2012). PML regulates PER2 nuclear localization and circadian function. The EMBO journal *31*, 1427-1439.

Milanovic, M., Fan, D.N.Y., Belenki, D., Dabritz, J.H.M., Zhao, Z., Yu, Y., Dorr, J.R., Dimitrova, L., Lenze, D., Monteiro Barbosa, I.A.*, et al.* (2018). Senescence-associated reprogramming promotes cancer stemness. Nature *553*, 96-100.

Milholland, B., Auton, A., Suh, Y., and Vijg, J. (2015). Age-related somatic mutations in the cancer genome. Oncotarget *6*, 24627-24635.

Missiroli, S., Bonora, M., Patergnani, S., Poletti, F., Perrone, M., Gafa, R., Magri, E., Raimondi, A., Lanza, G., Tacchetti, C.*, et al.* (2016). PML at Mitochondria-Associated Membranes Is Critical for the Repression of Autophagy and Cancer Development. Cell reports *16*, 2415-2427.

Moller, A., Sirma, H., Hofmann, T.G., Rueffer, S., Klimczak, E., Droge, W., Will, H., and Schmitz, M.L. (2003). PML is required for homeodomain-interacting protein kinase 2 (HIPK2)-mediated p53 phosphorylation and cell cycle arrest but is dispensable for the formation of HIPK domains. Cancer research *63*, 4310-4314.

Moos, M., Tacke, R., Scherer, H., Teplow, D., Fruh, K., and Schachner, M. (1988). Neural adhesion molecule L1 as a member of the immunoglobulin superfamily with binding domains similar to fibronectin. Nature *334*, 701-703.

Morgado-Palacin, L., Llanos, S., Urbano-Cuadrado, M., Blanco-Aparicio, C., Megias, D., Pastor, J., and Serrano, M. (2014). Non-genotoxic activation of p53 through the RPL11-dependent ribosomal stress pathway. Carcinogenesis *35*, 2822-2830.

Morgan, M., Thorburn, J., Pandolfi, P.P., and Thorburn, A. (2002). Nuclear and cytoplasmic shuttling of TRADD induces apoptosis via different mechanisms. The Journal of cell biology *157*, 975-984.

Morganti, C., Missiroli, S., Lebiedzinska-Arciszewska, M., Ferroni, L., Morganti, L., Perrone, M., Ramaccini, D., Occhionorelli, S., Zavan, B., Wieckowski, M.R.*, et al.* (2019). Regulation of PKCbeta levels and autophagy by PML is essential for high-glucose-dependent mesenchymal stem cell adipogenesis. International journal of obesity (2005) *43*, 963-973.

Mosteiro, L., Pantoja, C., Alcazar, N., Marion, R.M., Chondronasiou, D., Rovira, M., Fernandez-Marcos, P.J., Munoz-Martin, M., Blanco-Aparicio, C., Pastor, J.*, et al.* (2016). Tissue damage and senescence provide critical signals for cellular reprogramming in vivo. Science (New York, NY) *354*.

Mrazkova, B., Dzijak, R., Imrichova, T., Kyjacova, L., Barath, P., Dzubak, P., Holub, D., Hajduch, M., Nahacka, Z., Andera, L.*, et al.* (2018). Induction, regulation and roles of neural adhesion molecule L1CAM in cellular senescence. Aging *10*, 434-462.

Munoz-Espin, D., and Serrano, M. (2014). Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol *15*, 482-496.

Murray, J.M., Stiff, T., and Jeggo, P.A. (2012). DNA double-strand break repair within heterochromatic regions. Biochemical Society transactions *40*, 173-178.

Narla, A., and Ebert, B.L. (2010). Ribosomopathies: human disorders of ribosome dysfunction. Blood *115*, 3196-3205.

Nefkens, I., Negorev, D.G., Ishov, A.M., Michaelson, J.S., Yeh, E.T., Tanguay, R.M., Muller, W.E., and Maul, G.G. (2003). Heat shock and Cd2+ exposure regulate PML and Daxx release from ND10 by independent mechanisms that modify the induction of heat-shock proteins 70 and 25 differently. Journal of cell science *116*, 513-524.

Negrini, S., Gorgoulis, V.G., and Halazonetis, T.D. (2010). Genomic instability--an evolving hallmark of cancer. Nat Rev Mol Cell Biol *11*, 220-228.

Nelson, G., Kucheryavenko, O., Wordsworth, J., and von Zglinicki, T. (2018). The senescent bystander effect is caused by ROS-activated NF-kappaB signalling. Mechanisms of ageing and development *170*, 30-36.

Nelson, G., Wordsworth, J., Wang, C., Jurk, D., Lawless, C., Martin-Ruiz, C., and von Zglinicki, T. (2012). A senescent cell bystander effect: senescence-induced senescence. Aging cell *11*, 345-349.

Nemeth, A., and Langst, G. (2011). Genome organization in and around the nucleolus. Trends in genetics : TIG *27*, 149-156.

Nicolas, E., Parisot, P., Pinto-Monteiro, C., de Walque, R., De Vleeschouwer, C., and Lafontaine, D.L. (2016). Involvement of human ribosomal proteins in nucleolar structure and p53-dependent nucleolar stress. Nature communications *7*, 11390.

Nishimura, K., Kumazawa, T., Kuroda, T., Katagiri, N., Tsuchiya, M., Goto, N., Furumai, R., Murayama, A., Yanagisawa, J., and Kimura, K. (2015). Perturbation of ribosome biogenesis drives cells into senescence through 5S RNP-mediated p53 activation. Cell reports *10*, 1310-1323.

Nisole, S., Maroui, M.A., Mascle, X.H., Aubry, M., and Chelbi-Alix, M.K. (2013). Differential Roles of PML Isoforms. Frontiers in oncology *3*, 125.

Niwa-Kawakita, M., Ferhi, O., Soilihi, H., Le Bras, M., Lallemand-Breitenbach, V., and de The, H. (2017). PML is a ROS sensor activating p53 upon oxidative stress. The Journal of experimental medicine *214*, 3197-3206.

Oh, W., Ghim, J., Lee, E.W., Yang, M.R., Kim, E.T., Ahn, J.H., and Song, J. (2009). PML-IV functions as a negative regulator of telomerase by interacting with TERT. Journal of cell science *122*, 2613-2622.

Ohanna, M., Giuliano, S., Bonet, C., Imbert, V., Hofman, V., Zangari, J., Bille, K., Robert, C., Bressac-de Paillerets, B., Hofman, P.*, et al.* (2011). Senescent cells develop a PARP-1 and nuclear factor-{kappa}B-associated secretome (PNAS). Genes & development *25*, 1245-1261.

Ong, D.S.T., Hu, B., Ho, Y.W., Sauve, C.G., Bristow, C.A., Wang, Q., Multani, A.S., Chen, P., Nezi, L., Jiang, S.*, et al.* (2017). PAF promotes stemness and radioresistance of glioma stem cells. Proceedings of the National Academy of Sciences of the United States of America *114*, E9086-e9095.

Onofrillo, C., Galbiati, A., Montanaro, L., and Derenzini, M. (2017). The pre-existing population of 5S rRNA effects p53 stabilization during ribosome biogenesis inhibition. Oncotarget *8*, 4257-4267.

Oshimori, N., Oristian, D., and Fuchs, E. (2015). TGF-beta promotes heterogeneity and drug resistance in squamous cell carcinoma. Cell *160*, 963-976.

Osterwald, S., Deeg, K.I., Chung, I., Parisotto, D., Worz, S., Rohr, K., Erfle, H., and Rippe, K. (2015). PML induces compaction, TRF2 depletion and DNA damage signaling at telomeres and promotes their alternative lengthening. Journal of cell science *128*, 1887-1900.

Otto, T., and Sicinski, P. (2017). Cell cycle proteins as promising targets in cancer therapy. Nature reviews Cancer *17*, 93-115.

Paier, C.R.K., Maranhao, S.S., Carneiro, T.R., Lima, L.M., Rocha, D.D., Santos, R.D.S., Farias, K.M., Moraes-Filho, M.O., and Pessoa, C. (2018). Natural products as new antimitotic compounds for anticancer drug development. Clinics (Sao Paulo, Brazil) *73*, e813s.

Panaccione, A., Chang, M.T., Carbone, B.E., Guo, Y., Moskaluk, C.A., Virk, R.K., Chiriboga, L., Prasad, M.L., Judson, B., Mehra, S.*, et al.* (2016). NOTCH1 and SOX10 are Essential for Proliferation and Radiation Resistance of Cancer Stem-Like Cells in Adenoid Cystic Carcinoma. Clinical cancer research : an official journal of the American Association for Cancer Research *22*, 2083-2095.

Paoli, P., Giannoni, E., and Chiarugi, P. (2013). Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta *1833*, 3481-3498.

Paredes, S., Angulo-Ibanez, M., Tasselli, L., Carlson, S.M., Zheng, W., Li, T.M., and Chua, K.F. (2018). The epigenetic regulator SIRT7 guards against mammalian cellular senescence induced by ribosomal DNA instability. The Journal of biological chemistry *293*, 11242-11250.

Park, Y.M., Lee, S.Y., Park, S.W., and Kim, S.H. (2016). Role of cancer stem cell in radioresistant head and neck cancer. Auris Nasus Larynx *43*, 556-561.

Pearson, M., Carbone, R., Sebastiani, C., Cioce, M., Fagioli, M., Saito, S., Higashimoto, Y., Appella, E., Minucci, S., Pandolfi, P.P.*, et al.* (2000). PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. Nature *406*, 207-210.

Pearson, M., and Pelicci, P.G. (2001). PML interaction with p53 and its role in apoptosis and replicative senescence. Oncogene *20*, 7250-7256.

Pederson, T. (2011). The nucleolus. Cold Spring Harbor perspectives in biology *3*.

Pelletier, J., Thomas, G., and Volarevic, S. (2018). Ribosome biogenesis in cancer: new players and therapeutic avenues. Nature reviews Cancer *18*, 51-63.

Peltonen, K., Colis, L., Liu, H., Jaamaa, S., Moore, H.M., Enback, J., Laakkonen, P., Vaahtokari, A., Jones, R.J., af Hallstrom, T.M.*, et al.* (2010). Identification of novel p53 pathway activating small-molecule compounds reveals unexpected similarities with known therapeutic agents. PloS one *5*, e12996.

Peltonen, K., Colis, L., Liu, H., Trivedi, R., Moubarek, M.S., Moore, H.M., Bai, B., Rudek, M.A., Bieberich, C.J., and Laiho, M. (2014). A targeting modality for destruction of RNA polymerase I that possesses anticancer activity. Cancer cell *25*, 77-90.

Pento, J.T. (2017). Monoclonal Antibodies for the Treatment of Cancer. Anticancer research *37*, 5935-5939.

Penzo, M., Montanaro, L., Trere, D., and Derenzini, M. (2019). The Ribosome Biogenesis-Cancer Connection. Cells *8*.

Perry, R.P., and Kelley, D.E. (1970). Inhibition of RNA synthesis by actinomycin D: characteristic dose-response of different RNA species. Journal of cellular physiology *76*, 127-139.

Peters, G.J. (2014). Novel developments in the use of antimetabolites. Nucleosides, nucleotides & nucleic acids *33*, 358-374.

Peters, G.J., and Raymond, E. (2016). Contemporary reviews on cancer treatment. Cancer chemotherapy and pharmacology *77*, 3-4.

Piao, L.S., Hur, W., Kim, T.K., Hong, S.W., Kim, S.W., Choi, J.E., Sung, P.S., Song, M.J., Lee, B.C., Hwang, D.*, et al.* (2012). CD133+ liver cancer stem cells modulate radioresistance in human hepatocellular carcinoma. Cancer letters *315*, 129-137.

Plafker, S.M., and Macara, I.G. (2002). Ribosomal protein L12 uses a distinct nuclear import pathway mediated by importin 11. Molecular and cellular biology *22*, 1266-1275.

Postel-Vinay, S., Vanhecke, E., Olaussen, K.A., Lord, C.J., Ashworth, A., and Soria, J.C. (2012). The potential of exploiting DNA-repair defects for optimizing lung cancer treatment. Nature reviews Clinical oncology *9*, 144-155.

Prieto-Vila, M., Takahashi, R.U., Usuba, W., Kohama, I., and Ochiya, T. (2017). Drug Resistance Driven by Cancer Stem Cells and Their Niche. Int J Mol Sci *18*.

Prieto, J.L., and McStay, B. (2007). Recruitment of factors linking transcription and processing of pre-rRNA to NOR chromatin is UBF-dependent and occurs independent of transcription in human cells. Genes & development *21*, 2041-2054.

Puyo, S., Montaudon, D., and Pourquier, P. (2014). From old alkylating agents to new minor groove binders. Critical reviews in oncology/hematology *89*, 43-61.

Qi, W., Shakalya, K., Stejskal, A., Goldman, A., Beeck, S., Cooke, L., and Mahadevan, D. (2008). NSC348884, a nucleophosmin inhibitor disrupts oligomer formation and induces apoptosis in human cancer cells. Oncogene *27*, 4210-4220.

Qi, X.S., Pajonk, F., McCloskey, S., Low, D.A., Kupelian, P., Steinberg, M., and Sheng, K. (2017). Radioresistance of the breast tumor is highly correlated to its level of cancer stem cell and its clinical implication for breast irradiation. Radiother Oncol *124*, 455-461.

Qin, F.X., Shao, H.Y., Chen, X.C., Tan, S., Zhang, H.J., Miao, Z.Y., Wang, L., Hui, C., and Zhang, L. (2011). Knockdown of NPM1 by RNA interference inhibits cells proliferation and induces apoptosis in leukemic cell line. International journal of medical sciences *8*, 287-294.

Qin, S., Li, Y., Cao, X., Du, J., and Huang, X. (2017). NANOG regulates epithelial-mesenchymal transition and chemoresistance in ovarian cancer. Bioscience reports *37*.

Quin, J.E., Devlin, J.R., Cameron, D., Hannan, K.M., Pearson, R.B., and Hannan, R.D. (2014). Targeting the nucleolus for cancer intervention. Biochim Biophys Acta *1842*, 802-816.

Rabellino, A., Carter, B., Konstantinidou, G., Wu, S.Y., Rimessi, A., Byers, L.A., Heymach, J.V., Girard, L., Chiang, C.M., Teruya-Feldstein, J.*, et al.* (2012). The SUMO E3-ligase PIAS1 regulates the tumor suppressor PML and its oncogenic counterpart PML-RARA. Cancer research *72*, 2275-2284.

Rabinovich, G.A., Gabrilovich, D., and Sotomayor, E.M. (2007). Immunosuppressive strategies that are mediated by tumor cells. Annu Rev Immunol *25*, 267-296.

Rao, S.G., and Jackson, J.G. (2016). SASP: Tumor Suppressor or Promoter? Yes! Trends in cancer *2*, 676-687.

Rapkin, L.M., Ahmed, K., Dulev, S., Li, R., Kimura, H., Ishov, A.M., and Bazett-Jones, D.P. (2015). The histone chaperone DAXX maintains the structural organization of heterochromatin domains. Epigenetics & chromatin *8*, 44.

Rathjen, F.G., and Schachner, M. (1984). Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. The EMBO journal *3*, 1-10.

Rayess, H., Wang, M.B., and Srivatsan, E.S. (2012). Cellular senescence and tumor suppressor gene p16. International journal of cancer *130*, 1715-1725.

Regad, T., Bellodi, C., Nicotera, P., and Salomoni, P. (2009). The tumor suppressor Pml regulates cell fate in the developing neocortex. Nature neuroscience *12*, 132-140.

Reid, R.A., and Hemperly, J.J. (1992). Variants of human L1 cell adhesion molecule arise through alternate splicing of RNA. Journal of molecular neuroscience : MN *3*, 127-135.

Reimann, M., Lee, S., Loddenkemper, C., Dorr, J.R., Tabor, V., Aichele, P., Stein, H., Dorken, B., Jenuwein, T., and Schmitt, C.A. (2010). Tumor stroma-derived TGF-beta limits myc-driven lymphomagenesis via Suv39h1-dependent senescence. Cancer cell *17*, 262-272.

Reineke, E.L., Lam, M., Liu, Q., Liu, Y., Stanya, K.J., Chang, K.S., Means, A.R., and Kao, H.Y. (2008). Degradation of the tumor suppressor PML by Pin1 contributes to the cancer phenotype of breast cancer MDA-MB-231 cells. Molecular and cellular biology *28*, 997-1006.

Risques, R.A., and Kennedy, S.R. (2018). Aging and the rise of somatic cancer-associated mutations in normal tissues. PLoS genetics *14*, e1007108.

Robles, S.J., and Adami, G.R. (1998). Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. Oncogene *16*, 1113-1123.

Rodier, F., and Campisi, J. (2011). Four faces of cellular senescence. The Journal of cell biology *192*, 547-556.
Rokudai, S., Laptenko, O., Arnal, S.M., Taya, Y., Kitabayashi, I., and Prives, C. (2013). MOZ increases p53 acetylation and premature senescence through its complex formation with PML. Proceedings of the National Academy of Sciences of the United States of America *110*, 3895-3900.
Romero-Laorden, N., and Castro, E. (2017). Inherited mutations in DNA repair genes and cancer risk. Curr Probl Cancer *41*, 251-264.
Roninson, I.B. (2003). Tumor cell senescence in cancer treatment. Cancer research *63*, 2705-2715.
Roos, W.P., and Kaina, B. (2013). DNA damage-induced cell death: from specific DNA lesions to the DNA damage response and apoptosis. Cancer letters *332*, 237-248.
Roos, W.P., Thomas, A.D., and Kaina, B. (2016). DNA damage and the balance between survival and death in cancer biology. Nature reviews Cancer *16*, 20-33.
Rubbi, C.P., and Milner, J. (2003). Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. The EMBO journal *22*, 6068-6077.
Ruggero, D. (2013). Translational control in cancer etiology. Cold Spring Harbor perspectives in biology *5*.
Ruggero, D., and Pandolfi, P.P. (2003). Does the ribosome translate cancer? Nature reviews Cancer *3*, 179-192.
Ruhland, M.K., Coussens, L.M., and Stewart, S.A. (2016a). Senescence and cancer: An evolving inflammatory paradox. Biochim Biophys Acta *1865*, 14-22.
Ruhland, M.K., Loza, A.J., Capietto, A.H., Luo, X., Knolhoff, B.L., Flanagan, K.C., Belt, B.A., Alspach, E., Leahy, K., Luo, J.*, et al.* (2016b). Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. Nature communications *7*, 11762.
Rycaj, K., and Tang, D.G. (2014). Cancer stem cells and radioresistance. Int J Radiat Biol *90*, 615-621.
Sahin, U., de The, H., and Lallemand-Breitenbach, V. (2014a). PML nuclear bodies: assembly and oxidative stress-sensitive sumoylation. Nucleus (Austin, Tex) *5*, 499-507.
Sahin, U., Ferhi, O., Jeanne, M., Benhenda, S., Berthier, C., Jollivet, F., Niwa-Kawakita, M., Faklaris, O., Setterblad, N., de The, H.*, et al.* (2014b). Oxidative stress-induced assembly of PML nuclear bodies controls sumoylation of partner proteins. The Journal of cell biology *204*, 931-945.
Sahlberg, S.H., Spiegelberg, D., Glimelius, B., Stenerlow, B., and Nestor, M. (2014). Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells. PloS one *9*, e94621.
Sakai, I., Miyake, H., Terakawa, T., and Fujisawa, M. (2011). Inhibition of tumor growth and sensitization to chemotherapy by RNA interference targeting interleukin-6 in the androgen-independent human prostate cancer PC3 model. Cancer science *102*, 769-775.
Salehan, M.R., and Morse, H.R. (2013). DNA damage repair and tolerance: a role in chemotherapeutic drug resistance. Br J Biomed Sci *70*, 31-40.
Salomoni, P., Bernardi, R., Bergmann, S., Changou, A., Tuttle, S., and Pandolfi, P.P. (2005). The promyelocytic leukemia protein PML regulates c-Jun function in response to DNA damage. Blood *105*, 3686-3690.
Salsman, J., Stathakis, A., Parker, E., Chung, D., Anthes, L.E., Koskowich, K.L., Lahsaee, S., Gaston, D., Kukurba, K.R., Smith, K.S.*, et al.* (2017). PML nuclear bodies contribute to the basal expression of the mTOR inhibitor DDIT4. Scientific reports *7*, 45038.
Savino, T.M., Gebrane-Younes, J., De Mey, J., Sibarita, J.B., and Hernandez-Verdun, D. (2001). Nucleolar assembly of the rRNA processing machinery in living cells. The Journal of cell biology *153*, 1097-1110.

Saxena, A., Rorie, C.J., Dimitrova, D., Daniely, Y., and Borowiec, J.A. (2006). Nucleolin inhibits Hdm2 by multiple pathways leading to p53 stabilization. Oncogene *25*, 7274-7288.

Scaglioni, P.P., Yung, T.M., Cai, L.F., Erdjument-Bromage, H., Kaufman, A.J., Singh, B., Teruya-Feldstein, J., Tempst, P., and Pandolfi, P.P. (2006). A CK2-dependent mechanism for degradation of the PML tumor suppressor. Cell *126*, 269-283.

Schaefer, A.W., Kamiguchi, H., Wong, E.V., Beach, C.M., Landreth, G., and Lemmon, V. (1999). Activation of the MAPK signal cascade by the neural cell adhesion molecule L1 requires L1 internalization. The Journal of biological chemistry *274*, 37965-37973.

Schosserer, M., Grillari, J., and Breitenbach, M. (2017). The Dual Role of Cellular Senescence in Developing Tumors and Their Response to Cancer Therapy. Frontiers in oncology *7*, 278.

Scott, D.D., and Oeffinger, M. (2016). Nucleolin and nucleophosmin: nucleolar proteins with multiple functions in DNA repair. Biochemistry and cell biology = Biochimie et biologie cellulaire *94*, 419-432.

Sebens Muerkoster, S., Werbing, V., Sipos, B., Debus, M.A., Witt, M., Grossmann, M., Leisner, D., Kotteritzsch, J., Kappes, H., Kloppel, G.*, et al.* (2007). Drug-induced expression of the cellular adhesion molecule L1CAM confers anti-apoptotic protection and chemoresistance in pancreatic ductal adenocarcinoma cells. Oncogene *26*, 2759-2768.

Seeler, J.S., Marchio, A., Sitterlin, D., Transy, C., and Dejean, A. (1998). Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment. Proceedings of the National Academy of Sciences of the United States of America *95*, 7316-7321.

Seker, H., Rubbi, C., Linke, S.P., Bowman, E.D., Garfield, S., Hansen, L., Borden, K.L., Milner, J., and Harris, C.C. (2003). UV-C-induced DNA damage leads to p53-dependent nuclear trafficking of PML. Oncogene *22*, 1620-1628.

Sengupta, S., Peterson, T.R., and Sabatini, D.M. (2010). Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. Molecular cell *40*, 310-322.

Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D., and Lowe, S.W. (1997). Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell *88*, 593-602.

Sharifi, S., and Bierhoff, H. (2018). Regulation of RNA Polymerase I Transcription in Development, Disease, and Aging. Annual review of biochemistry *87*, 51-73.

Sharpless, N.E., and Sherr, C.J. (2015). Forging a signature of in vivo senescence. Nature reviews Cancer *15*, 397-408.

Shastrula, P.K., Sierra, I., Deng, Z., Keeney, F., Hayden, J.E., Lieberman, P.M., and Janicki, S.M. (2019). PML is recruited to heterochromatin during S phase and represses DAXX-mediated histone H3.3 chromatin assembly. Journal of cell science *132*.

Shav-Tal, Y., Blechman, J., Darzacq, X., Montagna, C., Dye, B.T., Patton, J.G., Singer, R.H., and Zipori, D. (2005). Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition. Molecular biology of the cell *16*, 2395-2413.

She, Q.B., Halilovic, E., Ye, Q., Zhen, W., Shirasawa, S., Sasazuki, T., Solit, D.B., and Rosen, N. (2010). 4E-BP1 is a key effector of the oncogenic activation of the AKT and ERK signaling pathways that integrates their function in tumors. Cancer cell *18*, 39-51.

Shen, T.H., Lin, H.K., Scaglioni, P.P., Yung, T.M., and Pandolfi, P.P. (2006). The mechanisms of PML-nuclear body formation. Molecular cell *24*, 331-339.

Shen, Z. (2011). Genomic instability and cancer: an introduction. Journal of molecular cell biology *3*, 1-3.

Shi, Z., Fujii, K., Kovary, K.M., Genuth, N.R., Rost, H.L., Teruel, M.N., and Barna, M. (2017). Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide. Molecular cell *67*, 71-83.

Shibue, T., and Weinberg, R.A. (2017). EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nature reviews Clinical oncology *14*, 611-629.

Shimada, N., Shinagawa, T., and Ishii, S. (2008). Modulation of M2-type pyruvate kinase activity by the cytoplasmic PML tumor suppressor protein. Genes to cells : devoted to molecular & cellular mechanisms *13*, 245-254.

Shlush, L.I., and Hershkovitz, D. (2015). Clonal evolution models of tumor heterogeneity. Am Soc Clin Oncol Educ Book, e662-665.

Sidow, A., and Spies, N. (2015). Concepts in solid tumor evolution. Trends in genetics : TIG *31*, 208-214.

Silletti, S., Yebra, M., Perez, B., Cirulli, V., McMahon, M., and Montgomery, A.M. (2004). Extracellular signal-regulated kinase (ERK)-dependent gene expression contributes to L1 cell adhesion molecule-dependent motility and invasion. The Journal of biological chemistry *279*, 28880-28888.

Simova, J., Sapega, O., Imrichova, T., Stepanek, I., Kyjacova, L., Mikyskova, R., Indrova, M., Bieblova, J., Bubenik, J., Bartek, J.*, et al.* (2016). Tumor growth accelerated by chemotherapy-induced senescent cells is suppressed by treatment with IL-12 producing cellular vaccines. Oncotarget *7*, 54952-54964.

Sinclair, D.A., and Guarente, L. (1997). Extrachromosomal rDNA circles--a cause of aging in yeast. Cell *91*, 1033-1042.

Sinclair, D.A., Mills, K., and Guarente, L. (1997). Accelerated aging and nucleolar fragmentation in yeast sgs1 mutants. Science (New York, NY) *277*, 1313-1316.

Sirri, V., Urcuqui-Inchima, S., Roussel, P., and Hernandez-Verdun, D. (2008). Nucleolus: the fascinating nuclear body. Histochemistry and cell biology *129*, 13-31.

Skvortsova, I., Skvortsov, S., Stasyk, T., Raju, U., Popper, B.A., Schiestl, B., von Guggenberg, E., Neher, A., Bonn, G.K., Huber, L.A.*, et al.* (2008). Intracellular signaling pathways regulating radioresistance of human prostate carcinoma cells. Proteomics *8*, 4521-4533.

Sloan, K.E., Bohnsack, M.T., and Watkins, N.J. (2013). The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress. Cell reports *5*, 237-247.

Smirnov, E., Hornacek, M., Kovacik, L., Mazel, T., Schrofel, A., Svidenska, S., Skalnikova, M., Bartova, E., Cmarko, D., and Raska, I. (2016). Reproduction of the FC/DFC units in nucleoli. Nucleus (Austin, Tex) *7*, 203-215.

Sokka, M., Rilla, K., Miinalainen, I., Pospiech, H., and Syvaoja, J.E. (2015). High levels of TopBP1 induce ATR-dependent shut-down of rRNA transcription and nucleolar segregation. Nucleic acids research *43*, 4975-4989.

Song, M.S., Salmena, L., Carracedo, A., Egia, A., Lo-Coco, F., Teruya-Feldstein, J., and Pandolfi, P.P. (2008). The deubiquitinylation and localization of PTEN are regulated by a HAUSP-PML network. Nature *455*, 813-817.

Soundararajan, S., Chen, W., Spicer, E.K., Courtenay-Luck, N., and Fernandes, D.J. (2008). The nucleolin targeting aptamer AS1411 destabilizes Bcl-2 messenger RNA in human breast cancer cells. Cancer research *68*, 2358-2365.

Spirkoski, J., Shah, A., Reiner, A.H., Collas, P., and Delbarre, E. (2019). PML modulates H3.3 targeting to telomeric and centromeric repeats in mouse fibroblasts. Biochemical and biophysical research communications *511*, 882-888.

Stadler, M., Chelbi-Alix, M.K., Koken, M.H., Venturini, L., Lee, C., Saib, A., Quignon, F., Pelicano, L., Guillemin, M.C., Schindler, C.*, et al.* (1995). Transcriptional induction of the PML growth suppressor gene by interferons is mediated through an ISRE and a GAS element. Oncogene *11*, 2565-2573.

Stambolic, V., MacPherson, D., Sas, D., Lin, Y., Snow, B., Jang, Y., Benchimol, S., and Mak, T.W. (2001). Regulation of PTEN transcription by p53. Molecular cell *8*, 317-325.

Stark, T.W., Hensley, P.J., Spear, A., Pu, H., Strup, S.S., and Kyprianou, N. (2017). Predictive value of epithelial-mesenchymal-transition (EMT) signature and PARP-1 in prostate cancer radioresistance. Prostate *77*, 1583-1591.

Steinbichler, T.B., Alshaimaa, A., Maria, M.V., Daniel, D., Herbert, R., Jozsef, D., and Ira-Ida, S. (2018). Epithelial-mesenchymal crosstalk induces radioresistance in HNSCC cells. Oncotarget *9*, 3641-3652.

Strauss, R., Hamerlik, P., Lieber, A., and Bartek, J. (2012). Regulation of stem cell plasticity: mechanisms and relevance to tissue biology and cancer. Mol Ther *20*, 887-897.

Strezoska, Z., Pestov, D.G., and Lau, L.F. (2000). Bop1 is a mouse WD40 repeat nucleolar protein involved in 28S and 5. 8S RRNA processing and 60S ribosome biogenesis. Molecular and cellular biology *20*, 5516-5528.

Stults, D.M., Killen, M.W., Williamson, E.P., Hourigan, J.S., Vargas, H.D., Arnold, S.M., Moscow, J.A., and Pierce, A.J. (2009). Human rRNA gene clusters are recombinational hotspots in cancer. Cancer research *69*, 9096-9104.

Su, H., Xu, T., Ganapathy, S., Shadfan, M., Long, M., Huang, T.H., Thompson, I., and Yuan, Z.M. (2014). Elevated snoRNA biogenesis is essential in breast cancer. Oncogene *33*, 1348-1358.

Suico, M.A., Yoshida, H., Seki, Y., Uchikawa, T., Lu, Z., Shuto, T., Matsuzaki, K., Nakao, M., Li, J.D., and Kai, H. (2004). Myeloid Elf-1-like factor, an ETS transcription factor, up-regulates lysozyme transcription in epithelial cells through interaction with promyelocytic leukemia protein. The Journal of biological chemistry *279*, 19091-19098.

Sulima, S.O., Patchett, S., Advani, V.M., De Keersmaecker, K., Johnson, A.W., and Dinman, J.D. (2014). Bypass of the pre-60S ribosomal quality control as a pathway to oncogenesis. Proceedings of the National Academy of Sciences of the United States of America *111*, 5640-5645.

Sun, X.X., Wang, Y.G., Xirodimas, D.P., and Dai, M.S. (2010). Perturbation of 60 S ribosomal biogenesis results in ribosomal protein L5- and L11-dependent p53 activation. The Journal of biological chemistry *285*, 25812-25821.

Swift, L.H., and Golsteyn, R.M. (2014). Genotoxic anti-cancer agents and their relationship to DNA damage, mitosis, and checkpoint adaptation in proliferating cancer cells. Int J Mol Sci *15*, 3403-3431.

Takagi, M., Absalon, M.J., McLure, K.G., and Kastan, M.B. (2005). Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. Cell *123*, 49-63.

Takahashi, Y., Dulev, S., Liu, X., Hiller, N.J., Zhao, X., and Strunnikov, A. (2008). Cooperation of sumoylated chromosomal proteins in rDNA maintenance. PLoS genetics *4*, e1000215.

Takai, H., Smogorzewska, A., and de Lange, T. (2003). DNA damage foci at dysfunctional telomeres. Current biology : CB *13*, 1549-1556.

Tashiro, S., Muto, A., Tanimoto, K., Tsuchiya, H., Suzuki, H., Hoshino, H., Yoshida, M., Walter, J., and Igarashi, K. (2004). Repression of PML nuclear body-associated transcription by oxidative stress-activated Bach2. Molecular and cellular biology *24*, 3473-3484.

Tchurikov, N.A., Fedoseeva, D.M., Sosin, D.V., Snezhkina, A.V., Melnikova, N.V., Kudryavtseva, A.V., Kravatsky, Y.V., and Kretova, O.V. (2015). Hot spots of DNA double-strand breaks and genomic contacts of human rDNA units are involved in epigenetic regulation. Journal of molecular cell biology *7*, 366-382.

Terzi, M.Y., Izmirli, M., and Gogebakan, B. (2016). The cell fate: senescence or quiescence. Molecular biology reports *43*, 1213-1220.

Theys, J., Jutten, B., Habets, R., Paesmans, K., Groot, A.J., Lambin, P., Wouters, B.G., Lammering, G., and Vooijs, M. (2011). E-Cadherin loss associated with EMT promotes radioresistance in human tumor cells. Radiother Oncol *99*, 392-397.

Thiery, J.P., and Sleeman, J.P. (2006). Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol *7*, 131-142.

Thomas, F., and Kutay, U. (2003). Biogenesis and nuclear export of ribosomal subunits in higher eukaryotes depend on the CRM1 export pathway. Journal of cell science *116*, 2409-2419.

Thoms, H.C., Loveridge, C.J., Simpson, J., Clipson, A., Reinhardt, K., Dunlop, M.G., and Stark, L.A. (2010). Nucleolar targeting of RelA(p65) is regulated by COMMD1-dependent ubiquitination. Cancer research *70*, 139-149.

Tian, H., Gao, Z., Li, H., Zhang, B., Wang, G., Zhang, Q., Pei, D., and Zheng, J. (2015). DNA damage response--a double-edged sword in cancer prevention and cancer therapy. Cancer letters *358*, 8-16.

Torgovnick, A., and Schumacher, B. (2015). DNA repair mechanisms in cancer development and therapy. Frontiers in genetics *6*, 157.

Toso, A., Revandkar, A., Di Mitri, D., Guccini, I., Proietti, M., Sarti, M., Pinton, S., Zhang, J., Kalathur, M., Civenni, G.*, et al.* (2014). Enhancing chemotherapy efficacy in Pten-deficient prostate tumors by activating the senescence-associated antitumor immunity. Cell reports *9*, 75-89.

Toussaint, O., Dumont, P., Remacle, J., Dierick, J.F., Pascal, T., Frippiat, C., Magalhaes, J.P., Zdanov, S., and Chainiaux, F. (2002). Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? TheScientificWorldJournal *2*, 230-247.

Toussaint, O., Medrano, E.E., and von Zglinicki, T. (2000). Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Experimental gerontology *35*, 927-945.

Troschel, F.M., Bohly, N., Borrmann, K., Braun, T., Schwickert, A., Kiesel, L., Eich, H.T., Gotte, M., and Greve, B. (2018). miR-142-3p attenuates breast cancer stem cell characteristics and decreases radioresistance in vitro. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine *40*, 1010428318791887.

Trotman, L.C., Alimonti, A., Scaglioni, P.P., Koutcher, J.A., Cordon-Cardo, C., and Pandolfi, P.P. (2006). Identification of a tumour suppressor network opposing nuclear Akt function. Nature *441*, 523-527.

Tubbs, A., and Nussenzweig, A. (2017). Endogenous DNA Damage as a Source of Genomic Instability in Cancer. Cell *168*, 644-656.

Tugues, S., Burkhard, S.H., Ohs, I., Vrohlings, M., Nussbaum, K., Vom Berg, J., Kulig, P., and Becher, B. (2015). New insights into IL-12-mediated tumor suppression. Cell death and differentiation *22*, 237-246.

Turelli, P., Doucas, V., Craig, E., Mangeat, B., Klages, N., Evans, R., Kalpana, G., and Trono, D. (2001). Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. Molecular cell *7*, 1245-1254.

Turi, Z., Lacey, M., Mistrik, M., and Moudry, P. (2019). Impaired ribosome biogenesis: mechanisms and relevance to cancer and aging. Aging *11*, 2512-2540.

Turi, Z., Senkyrikova, M., Mistrik, M., Bartek, J., and Moudry, P. (2018). Perturbation of RNA Polymerase I transcription machinery by ablation of HEATR1 triggers the RPL5/RPL11-MDM2-p53 ribosome biogenesis stress checkpoint pathway in human cells. Cell cycle (Georgetown, Tex) *17*, 92-101.

Tyagi, A., Vishnoi, K., Kaur, H., Srivastava, Y., Roy, B.G., Das, B.C., and Bharti, A.C. (2017). Cervical cancer stem cells manifest radioresistance: Association with upregulated AP-1 activity. Scientific reports *7*, 4781.

Ulbricht, T., Alzrigat, M., Horch, A., Reuter, N., von Mikecz, A., Steimle, V., Schmitt, E., Kramer, O.H., Stamminger, T., and Hemmerich, P. (2012). PML promotes MHC class II gene expression by stabilizing the class II transactivator. The Journal of cell biology *199*, 49-63.

Valdez, B.C., Henning, D., So, R.B., Dixon, J., and Dixon, M.J. (2004). The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. Proceedings of the National Academy of Sciences of the United States of America *101*, 10709-10714.

Valiente, M., Obenauf, A.C., Jin, X., Chen, Q., Zhang, X.H., Lee, D.J., Chaft, J.E., Kris, M.G., Huse, J.T., Brogi, E.*, et al.* (2014). Serpins promote cancer cell survival and vascular co-option in brain metastasis. Cell *156*, 1002-1016.

Van Damme, E., Laukens, K., Dang, T.H., and Van Ostade, X. (2010). A manually curated network of the PML nuclear body interactome reveals an important role for PML-NBs in SUMOylation dynamics. International journal of biological sciences *6*, 51-67.

van Riggelen, J., Muller, J., Otto, T., Beuger, V., Yetil, A., Choi, P.S., Kosan, C., Moroy, T., Felsher, D.W., and Eilers, M. (2010a). The interaction between Myc and Miz1 is required to antagonize TGFbeta-dependent autocrine signaling during lymphoma formation and maintenance. Genes & development *24*, 1281-1294.

van Riggelen, J., Yetil, A., and Felsher, D.W. (2010b). MYC as a regulator of ribosome biogenesis and protein synthesis. Nature reviews Cancer *10*, 301-309.

van Sluis, M., and McStay, B. (2015). A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. Genes & development *29*, 1151-1163.

van Sluis, M., and McStay, B. (2019). Nucleolar DNA Double-Strand Break Responses Underpinning rDNA Genomic Stability. Trends in genetics : TIG.

Vancurova, M., Hanzlikova, H., Knoblochova, L., Kosla, J., Majera, D., Mistrik, M., Burdova, K., Hodny, Z., and Bartek, J. (2019). PML nuclear bodies are recruited to persistent DNA damage lesions in an RNF168-53BP1 dependent manner and contribute to DNA repair. DNA repair *78*, 114-127.

Venkatesh, P., and Kasi, A. (2019). Anthracyclines. In StatPearls (Treasure Island (FL): StatPearls Publishing.

Vernier, M., Bourdeau, V., Gaumont-Leclerc, M.F., Moiseeva, O., Begin, V., Saad, F., Mes-Masson, A.M., and Ferbeyre, G. (2011). Regulation of E2Fs and senescence by PML nuclear bodies. Genes & development *25*, 41-50.

Victorelli, S., and Passos, J.F. (2017). Telomeres and Cell Senescence - Size Matters Not. EBioMedicine *21*, 14-20.

Vlasakova, J., Novakova, Z., Rossmeislova, L., Kahle, M., Hozak, P., and Hodny, Z. (2007). Histone deacetylase inhibitors suppress IFNalpha-induced up-regulation of promyelocytic leukemia protein. Blood *109*, 1373-1380.

Voit, R., Schafer, K., and Grummt, I. (1997). Mechanism of repression of RNA polymerase I transcription by the retinoblastoma protein. Molecular and cellular biology *17*, 4230-4237.

von Zglinicki, T. (2002). Oxidative stress shortens telomeres. Trends in biochemical sciences *27*, 339-344.

Wang, D., Qian, G., Wang, J., Wang, T., Zhang, L., Yang, P., and Lin, F. (2019a). Visfatin is involved in the cisplatin resistance of osteosarcoma cells via upregulation of Snail and Zeb1. Cancer Biol Ther, 1-8.

Wang, M., Han, J., Marcar, L., Black, J., Liu, Q., Li, X., Nagulapalli, K., Sequist, L.V., Mak, R.H., Benes, C.H.*, et al.* (2017a). Radiation Resistance in KRAS-Mutated Lung Cancer Is Enabled by Stem-like Properties Mediated by an Osteopontin-EGFR Pathway. Cancer research *77*, 2018-2028.

Wang, P., Benhenda, S., Wu, H., Lallemand-Breitenbach, V., Zhen, T., Jollivet, F., Peres, L., Li, Y., Chen, S.J., Chen, Z.*, et al.* (2018). RING tetramerization is required for nuclear body biogenesis and PML sumoylation. Nature communications *9*, 1277.

Wang, W., Wang, L., Mizokami, A., Shi, J., Zou, C., Dai, J., Keller, E.T., Lu, Y., and Zhang, J. (2017b). Down-regulation of E-cadherin enhances prostate cancer chemoresistance via Notch signaling. Chin J Cancer *36*, 35.

Wang, Y., Hou, Y., Zhang, W., Alvarez, A.A., Bai, Y., Hu, B., Cheng, S.Y., Yang, K., Li, Y., and Feng, H. (2019b). Lipolytic inhibitor G0S2 modulates glioma stem-like cell radiation response. Journal of experimental & clinical cancer research : CR *38*, 147.

Wang, Z.G., Ruggero, D., Ronchetti, S., Zhong, S., Gaboli, M., Rivi, R., and Pandolfi, P.P. (1998). PML is essential for multiple apoptotic pathways. Nature genetics *20*, 266-272.

Warmerdam, D.O., van den Berg, J., and Medema, R.H. (2016). Breaks in the 45S rDNA Lead to Recombination-Mediated Loss of Repeats. Cell reports *14*, 2519-2527.

Warmerdam, D.O., and Wolthuis, R.M.F. (2019). Keeping ribosomal DNA intact: a repeating challenge. Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology *27*, 57-72.

Watanabe-Susaki, K., Takada, H., Enomoto, K., Miwata, K., Ishimine, H., Intoh, A., Ohtaka, M., Nakanishi, M., Sugino, H., Asashima, M.*, et al.* (2014). Biosynthesis of ribosomal RNA in nucleoli regulates pluripotency and differentiation ability of pluripotent stem cells. Stem cells (Dayton, Ohio) *32*, 3099-3111.

Wei, X., Yu, Z.K., Ramalingam, A., Grossman, S.R., Yu, J.H., Bloch, D.B., and Maki, C.G. (2003). Physical and functional interactions between PML and MDM2. The Journal of biological chemistry *278*, 29288-29297.

Weisshaar, S.R., Keusekotten, K., Krause, A., Horst, C., Springer, H.M., Gottsche, K., Dohmen, R.J., and Praefcke, G.J. (2008). Arsenic trioxide stimulates SUMO-2/3 modification leading to RNF4-dependent proteolytic targeting of PML. FEBS letters *582*, 3174-3178.

Welch, W.J., and Suhan, J.P. (1985). Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. The Journal of cell biology *101*, 1198-1211.

Wood, P.M., Schachner, M., and Bunge, R.P. (1990). Inhibition of Schwann cell myelination in vitro by antibody to the L1 adhesion molecule. The Journal of neuroscience : the official journal of the Society for Neuroscience *10*, 3635-3645.

Wu, P.C., Wang, Q., Grobman, L., Chu, E., and Wu, D.Y. (2012). Accelerated cellular senescence in solid tumor therapy. Exp Oncol *34*, 298-305.

Wu, Q., Wang, R., Yang, Q., Hou, X., Chen, S., Hou, Y., Chen, C., Yang, Y., Miele, L., Sarkar, F.H.*, et al.* (2013). Chemoresistance to gemcitabine in hepatoma cells induces epithelial-mesenchymal transition and involves activation of PDGF-D pathway. Oncotarget *4*, 1999-2009.

Wu, S.L., Li, Y.J., Liao, K., Shi, L., Zhang, N., Liu, S., Hu, Y.Y., Li, S.L., and Wang, Y. (2017). 2-Methoxyestradiol inhibits the proliferation and migration and reduces the radioresistance of nasopharyngeal carcinoma CNE-2 stem cells via NF-kappaB/HIF-1 signaling pathway inactivation and EMT reversal. Oncology reports *37*, 793-802.

Wu, Z.H., Shi, Y., Tibbetts, R.S., and Miyamoto, S. (2006). Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. Science (New York, NY) *311*, 1141-1146.

Wyld, L., Audisio, R.A., and Poston, G.J. (2015). The evolution of cancer surgery and future perspectives. Nature reviews Clinical oncology *12*, 115-124.

Xiao, L., and Grove, A. (2009). Coordination of Ribosomal Protein and Ribosomal RNA Gene Expression in Response to TOR Signaling. Current genomics *10*, 198-205.

Xu, H., Di Antonio, M., McKinney, S., Mathew, V., Ho, B., O'Neil, N.J., Santos, N.D., Silvester, J., Wei, V., Garcia, J.*, et al.* (2017). CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. Nature communications *8*, 14432.

Xue, W., Zender, L., Miething, C., Dickins, R.A., Hernando, E., Krizhanovsky, V., Cordon-Cardo, C., and Lowe, S.W. (2007). Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature *445*, 656-660.

Yang, H., Wang, H., Ren, J., Chen, Q., and Chen, Z.J. (2017). cGAS is essential for cellular senescence. Proceedings of the National Academy of Sciences of the United States of America *114*, E4612-e4620.

Yang, S., Jeong, J.H., Brown, A.L., Lee, C.H., Pandolfi, P.P., Chung, J.H., and Kim, M.K. (2006). Promyelocytic leukemia activates Chk2 by mediating Chk2 autophosphorylation. The Journal of biological chemistry *281*, 26645-26654.

Yao, Y.H., Cui, Y., Qiu, X.N., Zhang, L.Z., Zhang, W., Li, H., and Yu, J.M. (2016). Attenuated LKB1-SIK1 signaling promotes epithelial-mesenchymal transition and radioresistance of non-small cell lung cancer cells. Chin J Cancer *35*, 50.

Yeager, T.R., Neumann, A.A., Englezou, A., Huschtscha, L.I., Noble, J.R., and Reddel, R.R. (1999). Telomerase-negative immortalized human cells contain a novel type of promyelocytic leukemia (PML) body. Cancer research *59*, 4175-4179.

Yeung, P.L., Denissova, N.G., Nasello, C., Hakhverdyan, Z., Chen, J.D., and Brenneman, M.A. (2012). Promyelocytic leukemia nuclear bodies support a late step in DNA double-strand break repair by homologous recombination. Journal of cellular biochemistry *113*, 1787-1799.

Yong, J.W., Yeo, X., Khan, M.M., Lee, M.B., and Hande, M.P. (2012). Stable expression of promyelocytic leukaemia (PML) protein in telomerase positive MCF7 cells results in alternative lengthening of telomeres phenotype. Genome integrity *3*, 5.

Yu, Z., Pestell, T.G., Lisanti, M.P., and Pestell, R.G. (2012). Cancer stem cells. The international journal of biochemistry & cell biology *44*, 2144-2151.

Yuan, X., Zhou, Y., Casanova, E., Chai, M., Kiss, E., Grone, H.J., Schutz, G., and Grummt, I. (2005). Genetic inactivation of the transcription factor TIF-IA leads to nucleolar disruption, cell cycle arrest, and p53-mediated apoptosis. Molecular cell *19*, 77-87.

Yun, H.S., Baek, J.H., Yim, J.H., Um, H.D., Park, J.K., Song, J.Y., Park, I.C., Kim, J.S., Lee, S.J., Lee, C.W.*, et al.* (2016). Radiotherapy diagnostic biomarkers in radioresistant human H460 lung cancer stem-like cells. Cancer Biol Ther *17*, 208-218.

Zang, C., Liu, X., Li, B., He, Y., Jing, S., He, Y., Wu, W., Zhang, B., Ma, S., Dai, W.*, et al.* (2017). IL-6/STAT3/TWIST inhibition reverses ionizing radiation-induced EMT and radioresistance in esophageal squamous carcinoma. Oncotarget *8*, 11228-11238.

Zhai, W., and Comai, L. (2000). Repression of RNA polymerase I transcription by the tumor suppressor p53. Molecular and cellular biology *20*, 5930-5938.

Zhang, B., Lam, E.W., and Sun, Y. (2019). Senescent cells: A new Achilles' heel to exploit for cancer medicine? Aging cell *18*, e12875.

Zhang, H., Luo, H., Jiang, Z., Yue, J., Hou, Q., Xie, R., and Wu, S. (2016). Fractionated irradiation-induced EMT-like phenotype conferred radioresistance in esophageal squamous cell carcinoma. J Radiat Res *57*, 370-380.

Zhang, H., Wong, C.C., Wei, H., Gilkes, D.M., Korangath, P., Chaturvedi, P., Schito, L., Chen, J., Krishnamachary, B., Winnard, P.T., Jr.*, et al.* (2012). HIF-1-dependent expression of angiopoietin-like 4 and L1CAM mediates vascular metastasis of hypoxic breast cancer cells to the lungs. Oncogene *31*, 1757-1770.

Zhang, P., Chin, W., Chow, L.T., Chan, A.S., Yim, A.P., Leung, S.F., Mok, T.S., Chang, K.S., Johnson, P.J., and Chan, J.Y. (2000). Lack of expression for the suppressor PML in human small cell lung carcinoma. International journal of cancer *85*, 599-605.

Zhang, P., Wei, Y., Wang, L., Debeb, B.G., Yuan, Y., Zhang, J., Yuan, J., Wang, M., Chen, D., Sun, Y.*, et al.* (2014). ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. Nature cell biology *16*, 864-875.

Zhang, R., Poustovoitov, M.V., Ye, X., Santos, H.A., Chen, W., Daganzo, S.M., Erzberger, J.P., Serebriiskii, I.G., Canutescu, A.A., Dunbrack, R.L.*, et al.* (2005). Formation of MacroH2A-

containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. Developmental cell *8*, 19-30.

Zhang, X., Zheng, L., Sun, Y., Wang, T., and Wang, B. (2015). Tangeretin enhances radiosensitivity and inhibits the radiation-induced epithelial-mesenchymal transition of gastric cancer cells. Oncology reports *34*, 302-310.

Zhang, X.W., Yan, X.J., Zhou, Z.R., Yang, F.F., Wu, Z.Y., Sun, H.B., Liang, W.X., Song, A.X., Lallemand-Breitenbach, V., Jeanne, M., *et al.* (2010). Arsenic trioxide controls the fate of the PML-RARalpha oncoprotein by directly binding PML. Science (New York, NY) *328*, 240-243.

Zhang, Y., Wolf, G.W., Bhat, K., Jin, A., Allio, T., Burkhart, W.A., and Xiong, Y. (2003). Ribosomal protein L11 negatively regulates oncoprotein MDM2 and mediates a p53-dependent ribosomal-stress checkpoint pathway. Molecular and cellular biology *23*, 8902-8912.

Zheng, X., Carstens, J.L., Kim, J., Scheible, M., Kaye, J., Sugimoto, H., Wu, C.C., LeBleu, V.S., and Kalluri, R. (2015). Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. Nature *527*, 525-530.

Zhong, S., Hu, P., Ye, T.Z., Stan, R., Ellis, N.A., and Pandolfi, P.P. (1999). A role for PML and the nuclear body in genomic stability. Oncogene *18*, 7941-7947.

Zhong, S., Muller, S., Ronchetti, S., Freemont, P.S., Dejean, A., and Pandolfi, P.P. (2000a). Role of SUMO-1-modified PML in nuclear body formation. Blood *95*, 2748-2752.

Zhong, S., Salomoni, P., and Pandolfi, P.P. (2000b). The transcriptional role of PML and the nuclear body. Nature cell biology *2*, E85-90.

Zhou, X., Hao, Q., Liao, J., Zhang, Q., and Lu, H. (2013). Ribosomal protein S14 unties the MDM2-p53 loop upon ribosomal stress. Oncogene *32*, 388-396.

Zhou, X., Liao, W.J., Liao, J.M., Liao, P., and Lu, H. (2015). Ribosomal proteins: functions beyond the ribosome. Journal of molecular cell biology *7*, 92-104.

VIII PUBLICATIONS

Kyjacova L, Hubackova S, Krejcikova K, Strauss R, Hanzlikova H, Dzijak R, Imrichova T, Simova J, Reinis M, Bartek J, Hodny Z: Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, ERK signaling-dependent cells. Cell Death Differ. 2015 Jun;22(6):898-911.

Simova J, Sapega O, Imrichova T, Stepanek I, Kyjacova L, Mikyskova R, Indrova M, Bieblova J, Bubenik J, Bartek J, Hodny Z, Reinis M: Tumor growth accelerated by chemotherapy-induced senescent cells is suppressed by treatment with IL-12 producing cellular vaccines. Oncotarget. 2016 Aug 23;7(34):54952-54964.

Mrazkova B, Dzijak R, Imrichova T, Kyjacova L, Barath P, Dzubak P, Holub D, Hajduch M, Nahacka Z, Andera L, Holicek P, Vasicova P, Sapega O, Bartek J, Hodny Z: Induction, regulation and roles of neural adhesion molecule L1CAM in cellular senescence. Aging (Albany NY). 2018 Mar 28;10(3):434-462.

Imrichova T, Hubackova S, Kucerova A, Kosla J, Bartek J, Hodny Z, Vasicova P: Dynamic PML protein nucleolar associations with persistent DNA damage lesions in response to nucleolar stress and senescence-inducing stimuli. Aging (Albany NY) (*in press, uncorrected version*).

Imrichova T, Hubackova S, Kucerova A, Kosla J, Bartek J, Hodny Z, Vasicova P: Casein kinase 2 regulates SUMO-mediated interaction of PML with nucleolus during topoisomerase and RNA polymerase I inhibition (unpublished).