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Impacts of chemotherapy and genotoxic stress on the immunological properties of tumour cells

Vliv chemoterapie a genotoxického stresu na imunologické vlastnosti nádorových buněk

Bachelor Thesis

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Abstract

Cancer treatment includes the use of chemotherapeutic agents, which have various effects on tumour cells, such as direct toxicity to cancer cells, immunogenic cell death induction and changes in cancer cells phenotype. Throughout the last decade many researchers have been focusing on the induction of genotoxic stress and cellular senescence, which chemotherapy can trigger. Even though induction of senescence in cancer cells represents an important mechanism for tumour suppression, there has been increasing evidence that shifting cancer cells into a senescent state by chemotherapy is not always beneficial. Senescent cells are associated with a specific secretory phenotype, which allows such cells to alter their microenvironment, modulate anti-tumour immunity, induce tumour suppression and even promote cancer development. Therefore, senescent cells elimination by innate or specific immunity, which can be boosted by immunotherapy, can be an important barrier preventing tumour growth.

Keywords: Cancer, chemotherapy, genotoxic stress, senescence, inflammation, cytokines, interleukins, cell proliferation

Abstrakt

Léčba rakoviny stále zahrnuje užívání chemoterapeutických agens, které mají různé účinky na rakovinné buňky. K těmto patří například přímá toxicita k rakovinným buňkám, indukce imunologické smrti a změny ve fenotypu rakovinných buněk. V posledním desetiletí se mnoho výzkumných týmu zabývalo indukcí genotoxického stresu a buněčné senescence, kterou chemoterapie může navodit. Přestože vyvolání senescence v rakovinných buňkách je důležitým mechanismem pro potlačení nádorů, je neustále poskytováno více a více důkazů, že indukce senescence chemoterapií není vždy pro pacienty výhodná. Senescentní buňky jsou spojeny se specifickým sekrečním fenotypem, který jim umožňuje měnit jejich mikroprostředí, ovlivňovat protinádorovou imunitu, vyvolat potlačení nádorů a dokonce podporovat růst nádorů. Díky tomu eliminace senescentních buněk vrozenou či specifickou imunitou, což lze umocnit imunoterapií, může být důležitou překážkou pro růst nádorů.

Klíčová slova: Rakovina, chemoterapie, genotoxický stress, senescence, zánět, cytokiny, interleukiny, proliferace

List of used abbreviations

5-FU	5-fluorouracil
Activator protein 1	AP-1/a transcriptional factor
Akt	Protein kinase B
ART	ATM- and RAD3-related
ATM	Ataxia telangiectasia mutated, a serine/threonine protein kinase
BrdU	Bromodeoxyuridine
B cells	B lymphocytes
$CD4^+$	Cluster of differentiation 4+
$CD8^+$	Cluster of differentiation 8+
$CD8^+$ alpha	Cluster of differentiation 8+ alpha
C/EBPβ	CCAAT/enhancer binding protein
COX-2	Cyclooxygenase 2
CXCL-1/KC/GROα	Chemokine ligand 1
CXCL2	Chemokine ligand 2
Dcr2	Decoy receptor 2
DNA	Deoxyribonucleic acid
DNAM-1	DNAX accessory molecule 1
EL4	A thymoma cell line
G0	G zero phase/resting phase/quiescent state
G1	Growth 1/Gap 1 phase
GROα/CXCL-1/KC	Growth regulated oncogene-alpha/Chemokine ligand 1
GROβ/MIP-2	Growth regulated oncogene-beta/Chemokine ligand 2
HMGB1	High mobility group box protein 1
HSP-90	Heat shock protein 90
IFN-γ	Interferon gamma
Jak2	Janus kinase 2
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-1R	Interleukin-1 receptor
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IRAK-1	Interleukin-1 receptor-associated kinase 1
MDSC	Myeloid-derived suppressor cells
MIP-2/GROβ	Macrophage inflammatory protein 2/Chemokine ligand 2
miR-146a	Micro RNA 146a
miR-146b	Micro RNA 146b

MMP	Matrix metalloproteinase
MMP-1	Matrix metalloproteinase 1/Interstitial collagenase
MMP-3	Matrix metalloproteinase 2/Stromelysin-1
MMP-10	Matrix metalloproteinase 10/Stromelysin-2
NF-κB	Nuclear factor-kappa B/Nuclear factor-kappa-light-chain-enhancer of activated B cells
NK cells	Natural killer cells
NKG2D	Natural killer group 2, member D
p16/p16^{Ink4A}/CDKN2A	Cyclin-dependent kinase inhibitor 2A/a tumour suppressor
p21/p21^{Waf1}/CDKN1A	Cyclin-dependent kinase inhibitor 1
p53/TP53	Tumour protein p53/ a tumour suppressor protein
p63	Tumour protein p63/transformation-related protein 63
p73	Tumour protein p73
p73α	An isoform of p73
PAI-1	Plasminogen activator inhibitor 1
PAI-2	Plasminogen activator inhibitor 2
PBMC	Peripheral blood mononuclear cell
PCNA	Proliferating cell nuclear antigen
pRb/ Rb	Retinoblastoma protein/A tumour suppressor protein
Ras/p21^{Ras}	Ras protein/Ras protein subfamily
ROS	Reactive oxygen species
SASP	Senescence-associated secretory phenotype
STAT1	Signal transducer and activator of transcription 1
STAT3	Signal transducer and activator of transcription 1
T cells	T lymphocytes
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor alpha
TNFR1	Tumour necrosis factor receptor 1
TLR 4	Toll-like receptor 4

Contents

1 Introduction

Cancer is a cell-autonomous genetic disease (Zitvogel, L. et al., 2008) that results from defects in regulatory circuits providing normal cell proliferation and homeostasis (Hanahan & Weinberg, 2000). These defects are generally caused by modifications in oncogenes, genome-stability genes and tumour-suppressor genes (Zitvogel, L. et al., 2008). While healthy cells precisely control the production of growth promoters and therefore ensure homeostasis, for cancer cells it is necessary to conserve abnormal cell proliferation which leads to the destruction of normal tissue structure and function (Hanahan & Weinberg, 2011).

In 2000 Douglas Hanahan and Robert Weinberg proposed six hallmarks of cancer that are well established and generally accepted today, they compose of six biological capabilities acquired by cells that are in the state of transformation into human tumours (Hanahan & Weinberg, 2000). These capabilities comprise of sustaining proliferative signalling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Intensive cancer research in the last decade suggested four more hallmarks: reprogramming of energy metabolism, genome instability and mutation, tumour-promoting inflammation and escaping immune system (Hanahan & Weinberg, 2011).

Cancer treatment usually includes the combination of surgery, radiotherapy and chemotherapy. Recent research provides evidence that cytotoxic drugs also participate in eliminating tumours by affecting the immune system in several different ways (Zitvogel, L. et al., 2008). Some chemotherapeutic agents ensure the visibility of dying tumour cells to the immune system, others provide momentary lymphocytes and T cells destruction, annihilate immunosuppressive mechanisms or directly or indirectly affect immune effectors (Bracci, L. et al., 2014). Consequently, to successfully treat cancer it is crucial to include both cancer cell destruction through the use of appropriate chemotherapeutic agents and immune system stimulation (Zitvogel, L. et al., 2008).

Cancer cells can be affected by chemotherapy in many ways. Besides direct toxicity to cancer cells, immunogenic cell death and changes in cancer cells phenotype caused by chemotherapy, many researchers have been focusing on the induction of genotoxic stress and senescence, which chemotherapy can trigger. Even though the induction of senescence in tumour cells might sound beneficial for patients battling with cancer, there has been increasing evidence that this is not always the case. It has been proven that DNA-damaging

and stress-inducing chemotherapy can cause an accumulation of senescent cells, which can negatively affect patients with cancer and contribute to the exhaustive impacts of chemotherapy (Coppé, J. P. et al., 2008). Furthermore, senescent cells are associated with a specific secretory phenotype, which allows such cells to alter their microenvironment. They also can affect surrounding cells and influence signal transduction pathways that can result in many diseases, including cancer (Coppé, J. P. et al., 2010). The constant secretion of pro-inflammatory factors by senescent cells can have its negative effects, too. Chronic inflammation has been linked with general ageing and the deterioration of the immune system (Freund, A. et al., 2010).

The objective of this thesis is to review and summarize information on the impacts of chemotherapy on the immunological properties of tumour cells. The main goals are as follows:

(a) Review the information on selected chemotherapeutic agents and their effects on the immune system

(b) Summarize current knowledge about senescence induction in general and by chemotherapy

(c) Describe the properties of senescent cells, focusing on the senescence-associated secretory phenotype and its negative effects

(d) Consider the impacts senescent cells have on the immune system and the possibility of chemotherapy being based on elimination of such cells

2 Immunomodulatory properties of chemotherapeutic agents and their ability to induce senescence

As stated above, in order to successfully defeat cancer it is usually necessary to include several different treatment methods and one of those is the use of antineoplastic drugs. However, they are not only used for cancer therapy (Bouraoui, S. et al., 2011) but also for some rheumatologic and immunologic diseases (Cavallo, D. et al, 2005). In 2010 there were over 50 different antineoplastic drugs in use (Bouraoui, S. et al., 2011).

The purpose of using these antineoplastic drugs is to eliminate cancer cells or irreversibly prevent them from growing, for example through interfering with the deoxyribonucleic acid synthesis, causing direct chemical damage to DNA, hindering topoisomerase II activity (Nitiss, J. 2009), or blocking mitosis by microtubule inhibition (Jordan, M. et al., 1993), eventually leading to tumour cell death (Bracci, L. et al., 2014).

The downside of this treatment is that the anticancer drugs are mutagenic and teratogenic, therefore bringing an ultimate hazard to the health of the medical workers and patients who are exposed to them (Baker and Connor, 1996, cited in Bouraoui, S. et al., 2011).

Chemotherapeutic agents can affect the immune system in several different ways. It is not uncommon for such substances to induce immunosuppression, immunogenic tumour cells death and a change in the immunological phenotype of cancer cells. However, cancer research has also been focused on the induction of senescence, which some chemotherapeutic agents can trigger. Senescence can have both beneficial and deleterious effects and this is discussed in further chapters.

Antineoplastic drugs can be further divided into antimetabolites, alkylating agents, anthracyclines, antimicrotubule agents, free radical generators, platinum compounds, taxanes and topoisomerase inhibitors (Bracci, L. et al., 2014; Bouraoui, S. et al., 2011). For the purpose of this thesis I will go into detail on a few antineoplastic drugs from each of the following categories: antimetabolites, anthracyclines and taxanes.

2.1 Antimetabolites

Antimetabolites are nucleoside and nucleobase analogues essential for anticancer and antiviral chemotherapies (Kodama, T. et al., 2006). They block important steps in the pathways of primarily pyrimidine biosynthesis but also purine biosynthesis which leads to the

absence of elementary units needed to synthetize DNA, therefore inhibiting DNA synthesis and causing the induction of DNA strand breaks (Kinsella and Smith, 1998).

2.1.1 5-fluorouracil (5-FU)

5-flurouracil is one of the most effective chemotherapeutic agents in use. Despite of being developed in 1957 (Zhao, Q. et al., 2010), it is still widely used today in the treatment of systemic and local cancer (Straub, J. O., 2010).

The two major anticancer mechanisms of 5-FU have been ascribed to be the misincorporation of fluoronucleotides into RNA (Yang, L. et al., 2009) and the inhibition of the biosynthesis of deoxythymidine monophosphate through blocking thymidylate synthetase, thus leading to shortage of thymidine and eventually disallowing normal DNA replication (Straub, J. O., 2010).

A recent study reports that 5-FU is capable of inducing senescence in cardiomyocytes and endothelial cells which could be considered as a supplementary mechanism by which 5-fluorouracil impairs cell function (Focaccetti, C. et al., 2015).

It is widely regarded that 5-FU also affects the immune system as several studies have shown this to be the case. It has been proven that 5-FU has immunosuppressive effects while inhibiting primary and secondary immune responses even at low doses (Mitchell, M. and DeConti, R., 1970).

GOLF is a chemotherapeutic multidrug combination consisting of Gemcitabine, Oxaliplatin, Leucovorin and 5-fluorouracil that induces a high rate of cell death caused by necrosis, Ag modulation and high HSP-90 expression in colon carcinoma cells. A study from 2005 showed that cancer cells exposed to this treatment developed dendritic cell-mediated cross presentation of antigens. The same study also suggested that there is a possibility of GOLF treatment to be able to generate more effective multiantigenic cytotoxic lymphocytes in vitro by stimulating peripheral blood mononuclear cells with autologous dendritic cells containing GOLF-treated carcinoma cell lysates (Correale, P. et al., 2005).

Another study executing their experiment with EL4, a thymoma cell line that was injected into mice, reports that the inhibition of tumour growth treated by 5-FU could be caused by its ability to selectively eliminate myeloid-derived suppressor cells (MDSC), therefore blocking their immunosuppressive effects on the T-cell-mediated regulation of the neoplasm. Surprisingly, the effects of 5-fluorouracil on MDSC were stronger than its direct effects on cancer cells (Vincent, J. et al., 2010).

2.1.2 Bromodeoxyuridine (BrdU)

Bromodeoxyuridine, a thymidine analogue that incorporates into DNA chains (Hakala, M., 1959), has been considered a potential antineoplastic drug for a long time. It is generally known that at high doses and in combination with a secondary cancer treatment method, such as ionizing radiation, BrdU is capable of having lethal effects for incorporating cells, thus leading to inhibition of cancer cell proliferation (Levkoff, L. et al., 2008).

Neural stem cells and progenitor cells at various developmental stages exhibit a decrease in proliferation after only one single-pulse of bromodeoxyuridine. The consequence of BrdU incorporation is the activation of p53 and proteins associated with the Rb pathway in primary neural stem and progenitor cells. This eventually leads to a delayed but progressive induction of senescence that discloses over multiple rounds of replication (Ross, H. H. et al., 2008).

2.2 Anthracyclines

Anthracyclines are chemotherapeutic agents that inhibits DNA and RNA synthesis by intercalating between base pairs of either DNA or RNA strands, eventually disallowing rapidly growing cancer cells from any further proliferation (Zitvogel, L. et al., 2008). However, the downside of these chemotherapeutic agents is that cumulative exposures to anthracyclines are associated with cardiomyopathy and congestive heart failure. Due to this, effort has been made to develop newer and less toxic anthracyclines, such as epirubicin, a less cardiotoxic analogue of doxorubicin. Despite their toxicity which also includes myelosuppression, anthracyclines are still frequently used in cancer treatment (Cortés-Funes, H. and Coronado, C., 2007). Anthracyclines have also been proven to increase primary cell-mediated immune response *in vitro* (Orsini, F. et al., 1977).

2.2.1 Doxorubicin

Doxorubicin is a chemotherapeutic agent that is known to be capable of inducing immunogenic cell death which is an important mechanism for augmentation of tumour immunity. The induction of cell death by doxorubicin can promote proliferation of $CD8\alpha^{+}$ lymphocytes and IFN-γ generation *in vitro*. Since the immunogenic cell death induction caused by doxorubicin happens *ex vivo*, it is assumable that it does not depend on the host immune system (Inoue, S. et al., 2014).

It has also been proposed that doxorubicin induced immunogenic cell death is dependent on caspase activation and failure to activate caspases may increase the resistance of tumour cells to chemotherapeutic agents in a cell-autonomous way (Casares, N. et al., 2005).

Another protein that plays an important role in the induction of immunogenic cell death is calreticulin. It has been proven that calreticulin is exposed on cells that undergo immunogenic cell death as a response to doxorubicin. Calreticulin exposure appears upstream of apoptosis or necrosis as a part of a specific-danger system and can be partially reduced by caspase inhibition. However, the involvement of caspases in calreticulin exposure seems to be rather indirect as it does not associate with any caspase cleavage (Obeid, M. et al., 2007).

It has been shown that doxorubicin can induce cell death (apoptosis or necrosis) or senescence. When a neuroblastoma cell line was treated with the lowest doxorubicin dose and caspase inhibitor, the cells did not undergo immunogenic cell death but were arrested in their growth and shifted into a senescent state along with increased expression of senescence markers $p21^{Waf1}$ and β-galactosidase and a decrease in caspase-3 activation. However, since senescence occurs at subapoptopic doxorubicin concentrations, it is possible that at pro-apoptopic concentrations cells can undergo either apoptosis or senescence. Although it is not impossible for apoptosis and senescence to compete with each other in an exclusive way, the apoptopic machinery may degrade specific cell cycle regulators and dominate over the senescence pathway. Due to this, senescence would be only able to proceed if apoptosis was inhibited (Rebba, A et al., 2003). Therefore, it is necessary to consider the dosage of chemotherapeutic agents in the treatment of cancer and if it is preferable for cells to undergo senescence rather than apoptosis even though this might bring risks.

2.3 Taxanes

In 2006 there were only two clinically available taxanes in cancer treatment: paclitaxel and docetaxel (de Bree, E. et al., 2006). However, in 2010 a new taxane, cabazitaxel, was approved to treat metastatic prostate cancer which was previously treated with docetaxel (FDA Approves New Treatment for Advanced Prostate Cancer, 2010).

Taxanes hinder cell proliferation by inducing a preserved inhibition of mitosis that occurs at the metaphase/anaphase boundary by stabilizing spindle microtubules. This mitotic block involves a formation of an incomplete metaphase plate of chromosomes and a modified arrangement of spindle microtubules (Jordan, M. et al., 1993).

2.3.1 Docetaxel

Docetaxel has been studied for its immunosuppressive effects for many years. It has been confirmed that this chemotherapeutic agent is capable of hindering the proliferation of lectin-induced peripheral blood mononuclear cells and decreasing the expression of PCNA, the proliferating cell nuclear antigen, in human lymphocytes. However, decetaxel did not seem to inhibit resting PBMC's. Docetaxel can also downregulate expression of T-lymphocyte cell surface activation markers and modify intracellular calcium homeostasis (Si, M. et al., 2003), which can play an important role in calcium induced apoptosis, cell division and differentiation (McKinsey, T. et al., 2002).

Docetaxel treatment is also capable of inducing senescence in cancer cells, but it does not activate a significant reduction in tumour size, nor does it have any major effects on tumour histology which implies that docetaxel therapy results in disease stabilization rather than tumour regression. Furthermore, in tumours exposed to docetaxel treatment, both $CD8^+$ and NK cells were not cytotoxic (Toso, A. et al., 2014) which suggests that docetaxel can affect the function of these cells and therefore suppressing the innate immune system and the interfere with effectiveness of cancer therapy.

3 DNA damage and senescence

There are two essentially different types of cells in multicellular organisms: postmitotic and mitotic. Postmitotic cells are blocked from proliferation and usually predominant in simple organisms. Mitotic cells are capable of dividing and can be found in many somatic tissues in complex organisms such as mammals, thus giving these tissues the ability of renewal, reparation and in many cases regeneration. However, this advantage over postmitotic cells also brings risks. The tissues are repaired by cell proliferation, which can induce somatic mutations, therefore increasing the risks of developing cancer (Campisi, J., 2005).

Another characteristic of mitotic cells is that they can remain in a reversibly arrested state, termed quiescence or G0, for a long period of time. Reversibly arrested cells can resume proliferation upon receiving appropriate signals, such as the need for tissue reparation or regeneration. However, potentially oncogenic events occurring in mitotic cells can trigger senescence, an irreversibly arrested state. Senescent cells are also often immune to cell death signals (Campisi, J. and di Fagagna F. A., 2007) and show changes in expression and regulation of several genes while only some of them are responsible for the inhibition of cell proliferation (Seshadri, T. and Campisi, J., 1990).

Genomic instability, one of the most pervasive origins of carcinogenesis, is connected with oncogene activation and certain tumour suppressor inactivation. Due to this, cells had to develop the protective systems to maintain genomic stability and avoid development of a neoplasm (Tian, H. et al., 2015). Tumour suppressor mechanisms can be widely divided into two categories: gatekeeper tumour suppressor genes and caretaker tumour suppressor genes (Deininger, P., 1999).

Caretaker tumour suppressor genes are responsible for suppressing mutations (Deininger, P. 1999) by preventing DNA damage or optimizing DNA reparation (Davalos, A. et al., 2010). The p53 gene is an example of a tumour suppressor gene with a caretaker ability as its secondary role, however it also plays an important role in a cell cycle checkpoint before replication takes place as a gatekeeper gene (Deininger, P., 1999).

Gatekeeper tumour suppressor genes prevent the development of a neoplasm in two ways. The first of these is the complete elimination of cells that have mutations, thus the potential to transform into tumour cells, provided by apoptosis, the process of programmed cell death. The second mechanism prevents cells from growing, therefore triggering

senescence in those cells. Gatekeeper genes have a definite beneficial anticancer effects, however it has been proposed that they might have been one of the reasons why organisms age (Campisi, J., 2005).

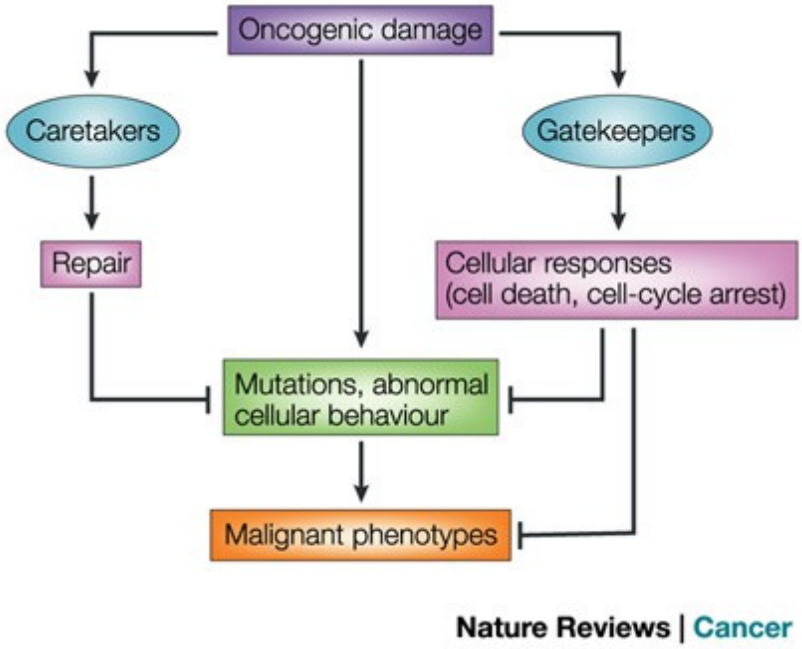

Figure 1. Tumour suppresive mechanisms. There are two groups of genes that protect organisms from developing cancer: Caretakers and Gatekeepers. Caretaker genes act by optimizing DNA reparations, Gatekeeper genes can induce either apoptosis or senescence. For further description see text above. Image was adapted from (Campisi, J., 2003).

4 Senescence Induction

Cells undergo stress of extracellular or intracellular origin continuously during their lifespan. The reactions to cellular stress can range from programmed cell death to irreversible growth arrest. There are several mechanisms that can induce senescence, these are discussed below.

4.1 Internal genotoxic stress mechanisms

The number of cell cycles human cells in culture can undergo is limited. Upon reaching the maximum amount of doublings, they are arrested in their growth and shifted into an irreversibly arrested state called replicative senescence (Hayflick, L., 1965).

The mechanism that controls the number of possible cell cycles prior to senescence is in many cases the lack of telomerase in these cells, leading to telomere loss which triggers a DNA damage response (di Fagagna, F. A. et al., 2003) and eventually disallows the cells from any further proliferation. Due to this, a certain threshold level of telomerase activity is required for cells to have an extended lifespan (Bodnar, M. et al., 1998). However, this is not the only possible cause of senescence. Besides replicative senescence, cells can also undergo accelerated senescence, which is often the response to DNA-damaging chemotherapy (Sidi, R. et al., 2011).

As stated above any DNA damage can trigger the activation of tumour suppressor mechanisms and lead to senescence. There are several sources of endogenous DNA damage, for example reactive oxygen species (ROS) (Rowe, L. et al., 2008), endogenous estrogen activity (Ke, H. et al., 2015) and errors in DNA replication. ROS are produced by cells continuously as a result of metabolic and other biochemical pathways and can lead to several types of DNA damage including single- and double-strand breaks (De Bont, R. and van Larebeke, N., 2004).

The *Ras* protein superfamily has been identified as oncogenes (Der, C. et al., 1982) capable of provoking an irreversible cell cycle arrest in primary fibroblast of rodent and human origin. The arrest occurs at the G1 interval along with increased expression of p53, p21 and p16. It is assumable that premature senescence induction in response to abnormal mitotic signalling is a tumour suppressor mechanism because inactivation of this proliferative inhibition machinery allows cells to divide further (Serrano, M. et al., 1997).

In eukaryotic cells, chromatin, a complex of nucleic acids and histones, is organized into euchromatic and heterochromatic domains. While euchromatin is gene rich and transcriptionally active, heterochromatin is highly condensed during mitotic interphase and thought to be transcriptionally inactive for a long time. Histones, the major chromatin-packaging proteins, are subjects to posttranslational modification and it has been proposed that these modifications play a role in a senescence induction (Li, F. et al., 2008). It has been proven that histone deacetylase inhibitors such as sodium dibutyrate and trichostatin A are capable of inducing a senescent-like state in human cells by a p16-dependent mechanism. Cells treated with sodium dibutyrate inhibitors showed an increased amount of cell cycle inhibitors and the mechanisms of inducing senescence did not include telomere shortening nor any posttranslational modifications of p53 (Munro, J. et al., 2004).

4.2 Extrinsic genotoxic stress mechanisms

Aside from internal factors that cause senescence, there are also extrinsic mechanisms leading to irreversible growth arrest.

It is has been proven that chemotherapy induced DNA damage is capable of activating the tumour suppressive mechanisms and can result in not only apoptosis, but also senescence in tumour cells along with increased expression of PAI-1 senescence marker. It has been proposed that accelerated senescence could be one of the reasons for lack of response to chemotherapy (Sidi, R. et al., 2011).

Exposure to gamma radiation (Di Leonardo, A. et al., 1994), external mitogenic signalling (Hill, R. et al., 2005) and extracellular inflammatory and growth factors have also been known to induce a proliferation arrest (Davalos, A. et al., 2010).

The adaptive immunity hinders cancer cell proliferation by inducing senescence in cancer cells through the action of cytokines IFN-γ and tumour necrosis growth factor. These cytokines drive Tag-expressing cancer cells into senescence by inducing a permanent growth arrest in G0/G1, activation of p16 and downstream Rb hypophosphorylation at serine 795. IFN-γ and TNF also induce characteristic senescence-associated epigenetic and lysosomal changes, such as senescence-associated β-galactosiadase activity. Senescence induction by these cytokines strictly requires TNFR1 and STAT1 signalling (Braumüller, H., et al., 2013).

4.3 Induction of senescence in cancer cells

Induction of senescence in cancer cells is caused by forced overexpression of several different tumour suppressor genes and some other senescence-related growth inhibitory genes (Shay, J. and Ronison, I., 2004).

One of these genes is TP53 which encodes p53 protein and is capable of inducing both cellular senescence and apoptosis in cancer cells (Xue, W. et al., 2007). Apoptosis is not the primary response to p53 activation, but instead it is an induction of cellular senescence which also involves upregulation of inflammatory cytokines. The induction of senescence in cancer cells by p53 might be an important mechanism by which the protein p53 prevents tumorigenesis (Li, T. et al., 2012). Experiments on mouse liver carcinoma cells, which had reactivated p53, showed that reactivation of p53 can trigger an innate immune response that targets tumour cells in vivo and contribute to tumour elimination. Mouse liver carcinoma cells expressing either oncogenic Ras or Akt showed clear signs of senescence along with accumulation of senescence-related-β-galactosidase activity and the expression of several senescence markers (Xue, W. et al., 2007).

Another example of a gene capable of senescence induction is p63, a p53 homologue. P63 isoforms were capable of inducing proliferation arrest in human carcinoma bladder cells that lacked p53 along with senescent-like altered cell morphology and increased activity of β-galactosidase (Jung, M. et al., 2001).

A cellular response leading to an irreversible proliferation arrest and the expression of replicative senescence markers was observed in p53 deficient cells as a reaction to overexpression of either p73α or p73β, alternative products of p73 gene which also belongs to the p53 family gene (Fang, L. et al., 1999).

These studied suggest that common targets of p53 family proteins are capable of triggering a replicative senescence programme. The expression of p21 is induced by all p53 family genes and increased in senescent cells, therefore it is assumable that it plays an important role in p53 family gene proliferation arrest induction (Jung, M. et al., 2001). Even though chemotherapeutic agents are designed to treat cancer, it has been suggested that treatment induced senescence also contributes to tumorigenesis. It has also been shown that senescence-inducing potential and production of treatment-tolerant cells seems to be restricted to some groups of antineoplastic drugs and not all of them (Achuthan, S. et al., 2011).

The retinoblastoma tumour suppressor gene products also play a role in the induction of senescence. The p16/Rb pathways cooperate with mitogenic signals to induce increased

levels of reactive oxygen species, which leads to the activation of protein kinase C-δ. The ROS-PKCδ signalling is followed by an irreversible cytokinesis block and a further reinforced ROS production (Takahashi, A. et al., 2006). The loss of Rb in primary quiescent cells provides the capability of re-entering the cell cycle to such cells and its loss in senescent cells is sufficient for the reversal of the cellular senescence programme, which could suggest that Rb is important for both senescence induction and its preservation (Sage, J. et al., 2003).

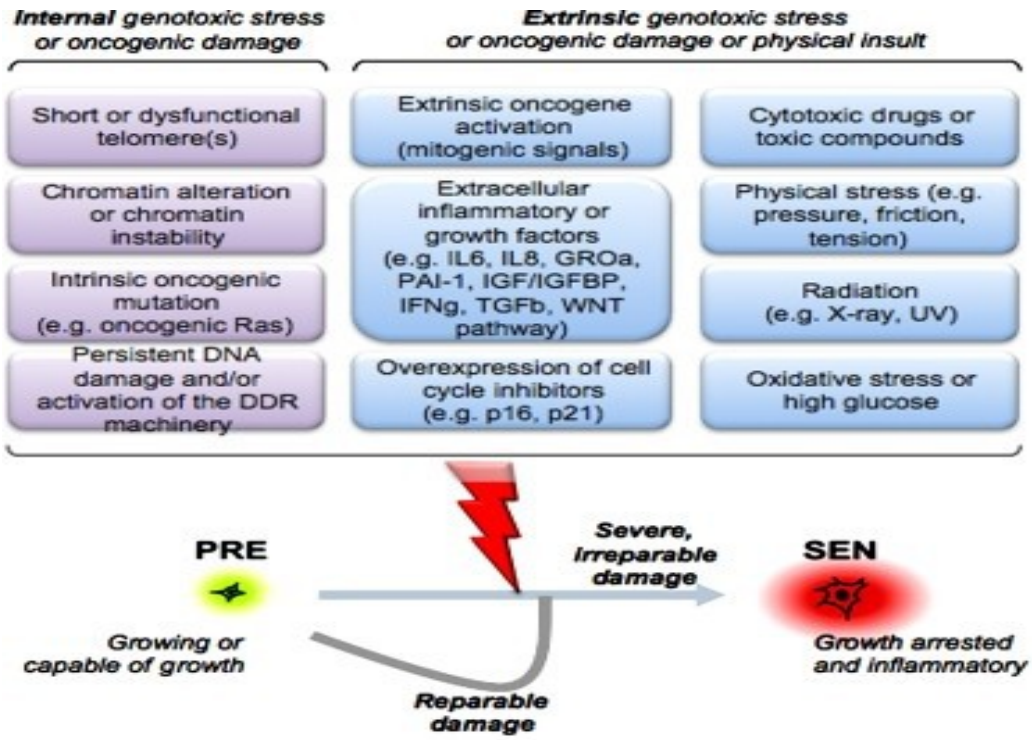

Figure 2. Internal and extrinsic senescence induction mechanisms. Internal genotoxic stress or oncogenic damage includes telomere shortening, chromatin modification, activity of oncogenic Ras and persistent DNA damage. Extracellular genotoxic stress includes mitogenic signals, chemotherapy, radiation, ROS, extracellular inflammatory and growth factors and physical stress. Presenescent cells are capable of growing or are growing. Severe genotoxic stress can induce a proliferation arrest and secretion of inflammatory components in such cells. Image was adapted from (Davalos, A. et al., 2010).

5 Properties of Senescent Cells

Senescent cells are arrested in their growth, although they are not quiescent or terminally differentiated. Not all senescent cells express identified senescence markers and their phenotypes can exist in several different forms. However, there have been outstanding features described in senescent cells which define the senescence state (Rodier, F. and Campisi, J., 2011), these are:

(a) Senescent cells are arrested in their growth through activities of the p53 and pRb tumour suppressor proteins, however it has not been well established what the requirements for maintaining growth arrest in senescent cells are. (Beauséjour, C. et al., 2003).

(b) There are morphological changes following senescence. Senescent cells increase in size (Estrada, J. et al., 2013) and are flattened (Poele, R. et al., 2002).

(c) Senescent cells are often immune to cell death signals (Campisi, J. and di Fagagna F. A., 2007).

(d) Senescent cells express β-galactosidase which is considered to be one of the main senescence markers (Dimri, G. P. et al., 1995) and a specific dysfunctional change of senescent cells. It has been proposed that senescence associated increase in β-galactosidase activity could be entirely explained by an equivalent increase in lysosomal mass. (Kurz, D. J. et al., 2000).

(e) Senescent cells experience a significant increase in the expression of cyclin-dependent kinase inhibitor p16 (Alcorta, D. et al., 1996). Experiments with p16 suggested that replicative senescence is not necessarily irreversible, it has been proven that senescent cells with low levels of p16 can resume robust growth upon p53 deactivation and limited growth upon activation of oncogenic Ras (Beauséjour, C. et al., 2003).

(f) Damaged senescent cells harbour persistent nuclear damage foci that contain DNA damage response proteins. Senescence-associated DNA damage foci are relatively stable structures that are different from transient damage foci and functionally important for both the growth arrest and IL-6 secretion which plays a critical role in SASP. Senescence-associated DNA damage foci lack certain DNA repair proteins and are more likely to allow further procession of DNA lesions (Rodier, F. et al., 2011).

(g) Tissue microenvironment is defined by the phenotypes of the cells that are in the immediate proximity and by the chemical and physical properties of the soluble and insoluble factors ambient to cells within a certain tissue. A senescent cell is metabolically active and

has undergone changes in protein expression and secretion, eventually developing senescence-associated secretory phenotype (SASP), by which senescent cells can affect tumour microenvironment (Coppé, J. P. et al., 2010).

5.1 SASP

The SASP includes several families of soluble and insoluble factors which can affect tumour microenvironment and surrounding cells by interacting with many cell-surface receptors and the appropriate signal transduction pathways that can lead to many diseases, including cancer (Coppé, J. P. et al., 2010). The SASP has many paracrine effects one would expect from a pro-inflammatory stimulus (Davalos, A. et al., 2010).

However, damage, telomere shortening or errors in replication cause the accumulation of senescent cells and their effects, especially the secretory phenotype, which may happen to be significant and deleterious (Krtolica, A. et al., 2001). It has been shown that senescence and SASP can be induced after treatment of patients with cancer undergoing DNA-damaging chemotherapy and that chemotherapy-induced SASP might contribute to the exhaustive effects of DNA-damaging chemotherapy (Coppé, J. P. et al., 2008). If this is considered, it is clear that despite of chemotherapy being invented to treat cancer, there is yet another sign of its ability to cause accumulation of senescent cells and increase the risk of tumorigenesis since factors secreted by senescent cells are believed to be able to promote tumour development (Acosta, J. C. et al., 2013).

The SASP reinforces senescence, activates immune surveillance and is capable of inducing senescence in normal cells both in culture and in human and mouse models of oncogene induced senescence *in vivo* (Acosta, J. C. et al., 2013).

It has already been proven that senescent human fibroblasts can stimulate proliferation and progression of preneoplastic epithelial cells and accelerate tumorigenesis by neoplastic epithelial cells. This happens mostly because of the secretion of both soluble and insoluble factors, which function together and can stimulate epithelial cells with oncogenic mutations (Krtolica, A. et al., 2001).

SASP factors can be globally divided into the following main catagories: soluble signalling factors (interleukins, chemokines and growth factors), secreted proteases and secreted insoluble components (Davalos, A. et al., 2010).

5.1.1 Soluble signalling factors

Interleukin-6. IL-6, a pleiotropic proinflammatory cytokine, is the most significant cytokine of SASP (Coppé, J. P. et al., 2010). Secretion of IL-6 is elevated in human cancers and associated with persistent DNA damage (Rodier, F. et al., 2010) and oncogene induced cellular senescence (Kuliman, T. et al., 2008). Through expression of IL-6, senescent cells can directly influence surrounding cells, such as epithelial and endothelial cells (Coppé, J. P. et al., 2010).

Interleukin-1. IL-1 family are one of the most important molecules of the immune system. IL-1 can influence surrounding cells through binding to IL-1/Toll-like receptor superfamily, which plays an important role in a signalling cascade, leading to nuclear factor κB and activator protein 1 activation (Mantovani, A. et al., 2001). IL-1 can also stimulate the expression other SASP proteins, such as IL-6 and IL-8 (Scott, G. et al., 2009).

Other soluble signalling factors produced by senescent cells among many others are IL-8 (Scott, G. et al., 2009), GROα and GROβ. The conserved factor CXCL-1/KC/GROα has the ability to promote the growth of premalignant epithelial cells (Coppé, J. P. et al., 2010).

5.1.2 Secreted proteases

Senescence-associated secretory phenotype proteases have three predominant effects, these are:

(a) Shedding of membrane-associated proteins resulting in soluble versions of membrane-bound receptors.

(b) Cleavage and degradation of signalling molecules.

(c) Degradation or processing of the extracellular matrix.

The activities these proteases provide results in a potent mechanism, by which senescent cells can influence the tissue microenvironment (Davalos, A. et al., 2010).

The MMP family plays a role in the regulation of the SASP. For example, the expression of collagenase (MMP-1), which is regulated by Akt-mediated signals, is increased in senescent human fibroblast (Mawal-Dewan, M. et al., 2002). Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10), the matrix-regulating proteins, have also been shown to have increased expression in senescent cells (Shelton, D. et al., 1999).

Another family of proteases present in SASP comprises of serine proteases and regulators of the plasminogen activation pathway (Coppé, J. P. et al., 2010). For example,

senescent fibroblasts have higher levels of plasminogen activator inhibitors PAI-1 and PAI-2 and urokinase plasminogen activator (Shelton, D. et al., 1999).

5.1.3 Secreted insoluble components

Fibronectin is a large multidomain glycoprotein expressed in connective tissue, on cell surfaces, in plasma and other body fluids and interacts with various molecules, affecting cell adhesion, growth, survival and migration (Coppé, J. P. et al., 2010).

5.1.4 Regulation of the inflammatory components of SASP

Senescence-associated secretory phenotype is at least partly controlled at the transcriptional level, but because the modifications in gene expression in senescent cells are very widespread, the transcriptional regulation might be at the level of chromatin organization, rather than because of specific changes in the transcription factors (Coppé, J. P. et al., 2010). For example the high mobility group box protein 1 has enhanced proinflammatory activity in the presence of IL-1β, IFN-γ and TNF-α, resulting in increased production of MIP-2 and TNF-α (Sha, Y. et al., 2011).

Many elements of the SASP depend on the transcription factors κB (Mantovani, A. et al., 2001) and C/EBPβ, which have increased expression during senescence (Kuliman, T. et al., 2008.

The expression of the SASP is controlled by inflammasome-mediated IL-1 signalling and knockdown of the IL-1 receptor partially prevents oncogene induced senescence (Acosta, J. C. et al., 2013). The inflammatory cytokine IL-1α is the primary interleukin that constitutively induces the nuclear factor κB activation, which in turn increases the expression of IL-1α, making a positive feedback loop. The activity of NF-κB is important for the development of the tumorigenic and metastatic phenotype (Niu, J. et al., 2004).

Both IL-6 and IL-8 are activated by C/EBPβ and depletion of the transcription factor C/EBPβ allows cells to skip senescence along with a loss of expression of both cytokines. It has been proposed that there is a positive feedback loop controlling oncogene induced senescence because a decrease in IL-6 expression also causes a strong depletion of IL-8 and C/EBPβ (Kuliman, T. et al., 2008).

Chemotherapy-induced senescence has been associated with the expression of PAI-1, plasminogen activator inhibitor 1, which is thought to be one of the reasons for lack of response to chemotherapy (Sidi, R. et al., 2011) and both necessary and sufficient for the activation of replicative senescence induced by p53 (Kortlever, R. et al., 2006. The expression

of PAI-1 is regulated by both IL-6 and C/EBPβ, which suggests that there is a possibility of IL-6, C/EBPβ and PAI-1 forming a signalling network that controls cellular senescence (Kuliman, T. et al., 2008).

DNA damage response can independently regulate at least two crucial phenotypes: the p53-depedant proliferation arrest and senescence-associated extracellular inflammatory signalling. DNA damage response regulates only a subset of SASP components, but those include the important inflammatory interleukins IL-6 and IL-8. The cytokine IL-6 is particularly important for senescent cells to be able to promote cancer cell invasion. Mild genotoxic stress can induce DNA damage response, DNA repair and cell cycle arrest, however it does not cause inflammatory cytokine secretion. It takes more severe genotoxic stress for the induction of persistent DNA damage response, which eventually leads to developing SASP (Rodier, F. et al., 2010).

MicroRNAs have also been proven to play a role in the cell non-autonomous effects of cellular senescence and the SASP regulation. It has been demonstrated that two MicroRNAs, miR-146a and miR-146b, negatively modulate the senescence-associated inflammatory molecules IL-6 and IL-8. Both miR-146a and miR-146b were expressed at significantly higher levels in senescent human fibroblast, not depending on the form of senescence induction (Bhaumik, D. et al., 2009). MiR-146a has been found to be NF-κB dependent, which suggests that the regulation of the inflammatory cytokines could be accomplished by modulating the levels of NF--κB (Taganov, K. et al., 2006) When the expression of miR-146a and miR146b is increased in senescent cells, the levels of IRAK-1, which is the established target of these mRNAs (Taganov, K. et al., 2006), are lowered, along with the decreased secretion of IL-6 and IL-8. Since IRAK-1 is an important part of IL-1 receptor pathway, it has been suggested that IL-1R signalling initiates both miR-146a and miR-146b expression and cytokine secretion and that these microRNAs are upregulated in a response to higher levels of IL-6 and IL-8 as a part of a negative feedback loop to ensure there is no immoderate SASP activity (Bhaumik, D. et al., 2009).

HMGB1 belongs to the family of high mobility group box proteins (Bianchi, M. and Agresti, A., 2005) and is also believed to be a part of the SASP regulation (Sha, Y. et al., 2011). As said above, HMGB1 has been shown to have enhanced proinflammatory activity by binding to cytokines, such as IL-1β, IFN-γ and TNF-α. However, HMGB1 itself has only minimal proinflammatory activity in terms of inducing macrophage- and neutrophil-associated cytokine expression (Sha, Y. et al., 2011). While cells cultured with recomibant

HMGB1 did not stimulate cytokine secretion, it is possible that extracellular HMGB1 may increase the effect of the SASP (Davalos, A. et al., 2010).

HMGB1 can act as an alarmin if it is released extracellulary, initiating the recruitment of antigen presenting dendritic cells and macrophages. HMGB1 also has the capacity to induce phenotypic and functional maturation of dendritic cells and to enhance antigen-specific immune responses, therefore it is assumable that it can contribute to the clearance of senescent cells (reviewed in Yang, D. et al., 2010). Despite these beneficial effect HMGB1 can have, it has been suggested that it also can contribute to tumorigenesis. HMGB1 induced autophagy in a TLR4-mediated pathway in Schwann cells, which eventually promoted neuroblastoma proliferation (Liu, Y. and Song, L. 2015).

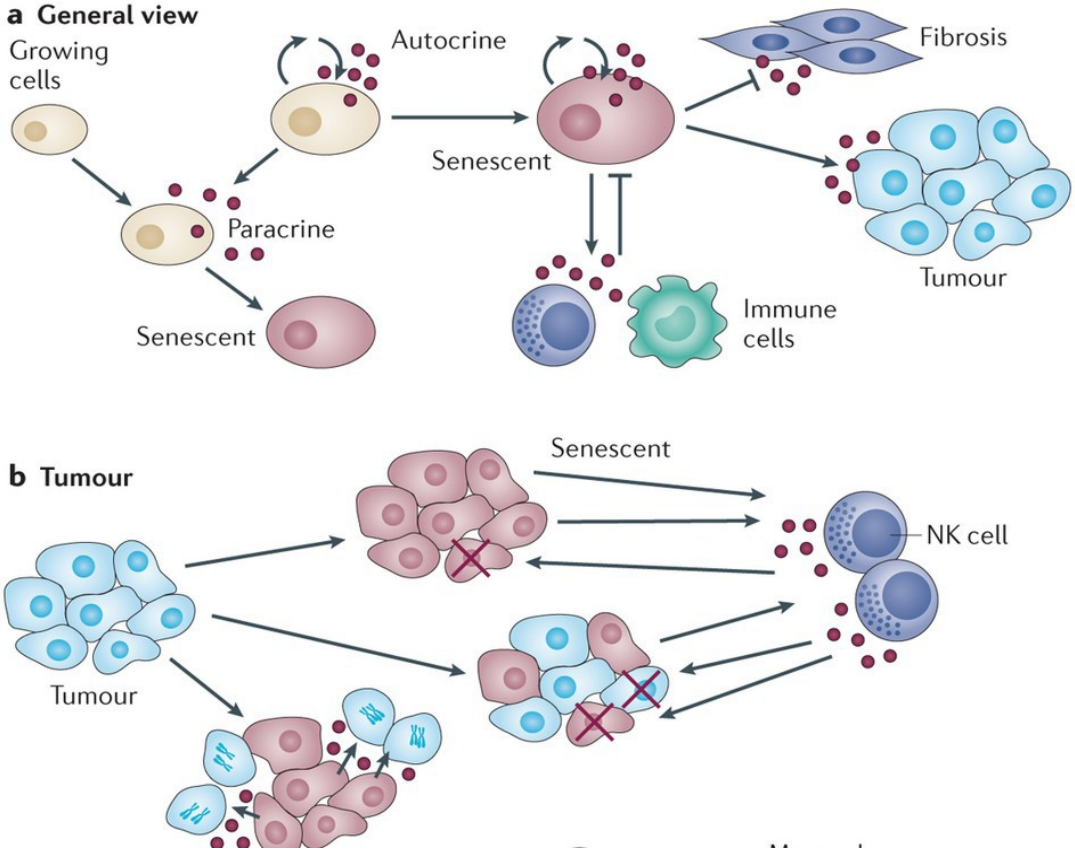

Figure 3. a. SASP enables senescent cells to influence their microenvironment and can also induce senescence in their cells-of-origin due to autocrine signalling. Paracrine signalling can induce senescence in surrounding cells. SASP further enhances senescence and SASP itself and can have deleterious effects, including cancer. **b.** Clearance of senescent cells by NK cells. For further description see text below. Image was adapted from (Pérez-Mancera, P. A. et al., 2014).

6 Senescence and the immune system

Senescent cancer cells can be eliminated by the immune system, which uses several different mechanisms to do so, as discussed below. Elimination of senescent cells seems to be crucial, since they can promote tumour growth and affect the immune system in a deleterious way, inducing a chronic inflammation that eventually leads to immunosuppression.

6.1 Elimination of senescent cells by the immune system

Natural killer cells probably play a major part in the clearance of tumour cells that are shifted into a senescent state (Xue, W. et al., 2007). The elimination of senescent tumour cells by natural killer cells depends on the NKG2D receptor and its ability to recognize its ligands located on the membrane of senescent cells (Iannello, A. et al., 2013). It has been suggested that a constitutive NKG2D ligand expression in tumour cells might be mediated by accelerated loss of genomic stability or other cellular changes that activate the DNA damage response (Gasser, S. et al., 2005). Enhanced expression of NKG2D and DNAM-1 ligands can activate the ATM/ART-senescence programme, which is important in the NK cells degranulation and thus, clearance of senescent cells. Interestingly, some low doses of chemotherapeutic agents, such as doxorubicin, can increase the expression of NKG2D and DNAM-1 ligands and participate in the elimination of senescent cells (Soriani, A. et al., 2008).

It is not the death-receptor-mediated pathway by which natural killer cells execute the destruction of senescent cells, but the cytotoxicity of NK cells caused perforin-mediated granule exocytosis. The reason why death-receptor-mediated pathway is not how NK cells contribute to destruction of senescence cells, is the accumulation of Dcr2 during senescence, which can protect such cells from the death-receptor-ligand-mediated killing (Sagiv, A. et al., 2012).

Another part of the innate immune system is capable of executing the elimination of senescent cells. The SASP programme triggered by p53 can influence the function or polarization of macrophages, therefore contributing to the clearance of senescent hematopoietic stem cells. Tumour-associated macrophages can go through two distinct activation programmes: classic (M1) and alternative (M2). While M1 macrophages increase in numbers as tumours progress and have antitumor activity, M2 macrophages can contribute to tumorigenesis (Lujambio, A. et al., 2013; Movahedi, K. et al., 2010). The activation of p53

in senescent cells shifts their secretory phenotype from supporting M2 to M1 polarization, thus p53 action in senescent cells contributes to an antitumour microenvironment through its impact on macrophage polarization. The presence of macrophages leads to the elimination of senescent HSC's, but also NK cells participate in the process, implying that the activity of p53 in senescent cells triggers surveillance by multiple immune cell types (Lujambio, A. et al., 2013).

The adaptive immune system is also capable of contributing to the elimination of senescent cells. $CD4^+$ T-cell-mediated immune response is crucial for destroying pre-malignant senescent hepatocytes with well-established SASP. (Kang, T. et al., 2011). However, $CD4^+$ T lymphocytes depend on monocytes and freshly replenished macrophages to successfully destroy senescent cells (Hoenicke, L. and Zender, L., 2012). Since damaged immune surveillance leads to the development of cancer, it is clear that clearance of senescent cells is important for tumour suppression *in vivo* (Kang, T. et al., 2011).

6.2 Induction of immunosenescence by chemotherapy

Immunosenescence is the deterioration of the immune system caused by age-associated changes in function in a variety of cells. This phenomenon includes a decrease in function of immune system cells and changes in both innate and adaptive immunity (Pawelec, G. et al., 2010).

Immunosenescence is not unusual to observe along with age-related changes, however, a recent study reports that chemotherapy is also capable of inducing immunosenescence. Results showed that there was a consistent decrease in absolute numbers of leukocytes and lymphocytes, especially $CD8^+$ lymphocytes during DNA-damaging chemotherapy in patients with breast cancer. Since the normal condition was not restored after six months of chemotherapy, it is assumable that chemotherapy impairs the immune system and can result in immunosuppression (Onyema, O. O. et al., 2015).

6.3 Induction of immunosuppression and immunosenescence by senescent cells

As stated above, even though the immune system plays a major role in regulating the levels of pro- and anti-inflammatory factors, it is not the only source of such factors (Freund, A. et al., 2010). Senescent cells have increased secretion of many pro-inflammatory molecules, therefore being capable of altering their microenvironment and promoting tumour development (Acosta, J. C. et al., 2013). In addition to these cancer promoting effects, there

has been evidence that senescent cells and their secretory phenotype can contribute to age-related pathologies that are unrelated to cancer (Freund, A. et al., 2010), such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (Sarkar, D. and Fisher, P., 2006). Chronic inflammation is associated histologically with the presence of lymphocytes and macrophages, the proliferation of blood vessels, fibrosis and tissue necrosis (Sarkar, D. and Fisher, P., 2006). One of the causes of chronic inflammation among many is cancer (Chew, V. et al., 2010).

Chronic inflammation is not only the source of many age-related diseases, but can also induce telomere dysfunction *in vivo* by increasing oxidative stress at least partially through COX-2 activation (Jurk, D. et al., 2014). Telomere shortening is a key factor in inducing replicative senescence, therefore it is assumable that chronic inflammation can cause even higher accumulation of senescent cells and significantly intensify its own effect.

Chronic inflammation has also been associated with general ageing. Since interleukin-6 is upregulated in senescent cells, playing a major role in the SASP (Coppé, J. P. et al., 2010), it has been proposed IL-6 can that IL-6 pathways can participate in the pathophysiology of physical function decline and ageing (Maggio, M. et al., 2009).

One of the ways chronic inflammation might contribute to ageing is the persistent presence of circulating proinflammatory factors, which would lead to the immune system being in a state of chronic low-level activation. Eventually, this sustained activation of the immune system would lead to the development of immunosenescence, therefore decreasing the functional effectivity of the adaptive immune system (Freund, A. et al., 2010). Immunosenescence is caused mainly by a reduction of the immunological space and a reduction of T lymphocytes population, which could cause the inability to respond to antigens (Franceschi, C. et al., 1999). It has also been proven that both age and and health status can contribute to the accumulation of Treg cells, which can impair the function of both NK and CTL cells, therefore reducing their cytotoxicity (Trzonkowski, P. et al., 2006).

Pten-null senescent tumour cells create an immunosuppressive tumour environment, which could impair the immune surveillance of senescent cells and contribute to tumour growth and chemoresistance in a murine experimental model (Toso, A. et al., 2014). The immunosuppressive tumour environment is established by the activation of Jak2/Stat3 pathway and characterized by the absence of $CD4^+$, $CD8^+$ and NK cells. Stat3 transcriptionally regulates several cytokines secreted by Pten-null senescent cells, such as CXCL1, CXCL2, IL-6 and IL-10. However, treatment with docetaxel and Jak2 inhibitor is capable of reprogramming the SASP and increase the efficiency of docetaxel induced

senescence due to the activation of a strong immune response in Pten-null senescent tumour cells (Toso, A. et al., 2014).

7 Protumorigenic impacts of tumour senescent cells

Chemotherapy is still one of the key parts of cancer treatment and has obvious beneficial effects for patients battling this disease. However, although the induction of cellular senescence in tumour cells might be desired for its tumour suppressive effect (Chen, Z. et al., 2005), there has been increasing evidence that SASP can also have deleterious impacts. It has been proven that the activity of SASP can promote the development of age-related cancer by modifying the tissue microenvironment (Coppé, J. et al., 2008).

Senescent human fibroblast are capable of stimulating the growth of preneoplastic and neoplastic cells. This happens mostly due to both soluble and insoluble factors senescent cells continuously produce (Krtolica, A. et al., 2001). Nonetheless, the mechanisms of senescent cells stimulating the proliferation of premalignant and malignant cells are not completely understood (Coppé, J. et al., 2008).

Breast cancer. Senescent fibroblasts have been shown to create an oncogenic microenvironment, which disrupts glandular morphogenesis in untransformed mammary epithelial cells, enforces mitogenic effects on cells with dysregulated cell death pathways and facilitates invasive growth of advanced malignant mammary epithelial cells. The reason this happens is due to the dysregulated cell-cell and cell-matrix interactions promoted by senescent fibroblasts (Tsai, K. et al., 2005).

Prostate cancer. Human prostate gland fibroblasts, which had induced senescence by oxidative stress, DNA damage and replication exhaustion, have also been demonstrated to be able to create an environment that promotes the growth of preneoplastic and neoplastic cells (Bavik, C. et al., 2006). It is also possible that chemotherapy can cause an accumulation of senescent cells, which leads to patients with prostate cancer relapsing after their treatment (Coppé, J. P. et al., 2008).

Furthermore, senescent cells mediated by SASP have the ability to induce epithelial-mesenchymal transition in nonaggressive carcinoma cells and the ability to affect their invasion through a basement membrane. IL-6 and IL-8 are the two cytokines mainly responsible for these biological activities (Coppé, J. et al., 2008).

8 Conclusion

Cancer is still one of the leading causes of death, therefore it is necessary to give the patients battling the disease the best treatment possible. Chemotherapy is a crucial part of cancer treatment and its ability to induce genotoxic stress and senescence is valued for its tumour growth suppressive effects. However, the negative effects chemotherapy-induced senescence can bring should not be ignored since creating an inflammatory environment can alter the tissue microenvironment and promote tumorigenesis. Chronic inflammation can develop after chemotherapy-induced senescence and bring many problems to patients, including immunosuppression and age-related diseases. Senescent cells can be specifically targeted by many components of the immune system, therefore it is necessary to consider antitumour therapy, such as immunotherapy, being based on the elimination of senescent cells. Patients undergoing such therapy could benefit from lower doses of chemotherapeutic agents, since it has been proven that lower doses induce senescence. Lower doses of chemotherapeutic agents could also mean that the exhaustive effects would not be as severe if senescent cells were fully eliminated. Furthermore, it is crucial to discover the mechanics of senescent cells stimulating the proliferation of premalignant and malignant cells and the targeting of senescent cells by the immune system. Once we fully understand this, we will be able to determine how beneficial antitumor therapy based on the elimination of senescence cells can be.

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