**Oponentský posudek**

Dizertační práce: Buněčná odpověď na protinádorové terapie založené na genotoxickém stresu
(Cell response to genotoxic stress-based anti-cancer therapies)
Student(ka): Mgr. Terezie Imrichová
Školitel: MUDr.Zdeněk Hodný, PhD.
Posudek vypracoval: Mgr. Vladimír Rotrekl

**General considerations:**

I must say, that despite rather long format (the thesis contains 120 pages and jut the introduction spans over 50 pages) it was my pleasure to read the text and accompanying publications. The thesis deals with a burning topic of cancer plasticity and drug resistance from various angles. In the first part the author describes the MAPK driven plasticity of anoikis-resistant prostate cancer cells, which gain features of stem cells allowing reversible EMT. Further she widens the topic by including the paracrine effect of senescent cell secretome on epithelial lung and prostate cancer cells utilizing syngeneic animal model allowing to study the involvement of the immune system. It is worth mentioning, that each of these parts resulted in practical outcome. In the first part it was identification of the possible target pathways (ERK and AKT) leading to diminished potential for escape from senescence and plasticity leading to stem-like cell potential and EMT. In the second and third part dealing with senescence the thesis offer suppression of cancer promoting paracrine effect and/or suppressing paracrine effect on antitumor immunity stemming from cancer adjacent senescent cells by IL-12 treatment. Furthermore the thesis offers identification of a novel marker L1CAM allowing for senescent cells' phenotyping and it further uncovers molecular changes suggesting negative feedback loop between ERK and L1CAM. In later parts the thesis witnesses the student becoming an expert on PML body formation, function and transition into PNAs and PML-NDS. On the practical note the data allow the authors to derive new hypothesis, that PML is a key player in rDNA stability and possibly serve as a protector of whole genome stability clock.

The thesis is well written in simple and clear English with only few mistakes or typos. The introduction is well structured, each chapter contains clear explanation of the biological process complemented by rather deep insight into the current literature.

The data collected in this thesis contributed and/or served as basis for four publications in very good journals (IF between 5 and 9) which have been already (4 years) 31 times cited. Terezie Imrichova is first author of one article published and coauthor of three others. There seem to be one more publication in Mol Oncology, not included in the thesis where Terezie Imrichova contributed as a coauthor. The commentaries in the thesis also address further manuscript, Terezie Imrichova is first author of "Casein kinase 2 regulates SUMO-mediated interaction of PML with nucleolus during topoisomerase and RNA polymerase I inhibition". However I like the discussion of the author in the thesis I cannot have more in depth comments as neither the text of the manuscript nor data it discusses are part of the thesis.

**Minor issues:**

1. The text is written in a scholarly style offering no doubt about the wide focus and deep orientation of the author in the whole field. However, the introduction part might be little to broad supplementing textbook and not always necessarily focusing on the merit of the thesis. I even dare to say with a bit of exaggeration that introduction from page 40 to 49 would be sufficient to introduce the main topic. The hypertrophy of the introduction is also witnessed by 32 pages! of references (references are not numbered, but if 3 lines are considered as average 1 reference, then it comes to about 500 references!

2. The text is well structured and written in clear English. However, it contains typos/mistakes (such as for example:“ The DNA damage in this case does not have be persistent“ on page 33 or „protein is be recruited to PML NBs“ on pg45 etc.), the frequency of such mistakes is very low and does not hamper the overall good feeling from the text.
3. The too wide field of view the author is pursuing is visible for example on the table 1. However the table on pg.18 contains useful list of most commonly used anticancer drugs (not limited to those used in the thesis), the categories are somewhat confusing. Part of the table seems to divide the compounds according to their effect (alkylating agents, antimetabolites), but then the author opts for dividing according to the origin of the compound (e.g. plant alkaloids) and even further to categories of different compound (e.g. hormones). It would be better to stick to only one way of categorization.
4. Also Fig.1 shows biomarkers involved in DNA repair from Vinay, 2012. The author titled the figure „main DNA lesions and corresponding DNA-damage-repair pathways“, which might be found a bit unfortunate as the list is far from being complete for the mechanisms we can find as influential in cancerogenesis and tumor plasticity (e.g. missing aNHEJ/MMEJ, ssDNA annealing, fork reversal etc.)

The above listed issues are just examples of minor issues, which if addressed would make the thesis easier to digest, but neither any single of them nor their sum is prohibitive in terms of defense recommendation or decreasing the very high standardd of the thesis.

**Ideas for discussion**

1. Despite the general idea, that cancer cells have unstable genome, the clones rising from the waves of mutagenesis during carcinogenesis are replaced by the periods when cancer cells tend to cycle while their genome is relatively stable. The author discusses (pg.26) the weakened DNA repair pathways as essential part of tumor development and summarizes that this is due to the mutations in the DNA repair genes. How would author explain the genome stabilization of the nascent, expanding clones?
2. The author lists whole plethora of cell phenotypes leading to cancer on pg.26 and attributes them all just to mutations („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“ and further the list of affected pathways). Cannot at least some of these phenotypes be caused by other means (e.g. epigenetic (mis)regulation)?
3. The author describes the cancer stem cells as cells which divide the asymmetric way (pg. 27). However it is by some authors considered as the hallmark of CSCs (referenced by the author), it turns out that actually symmetric division is associated with less differentiated phenotype in some of the tumors (e.g. breast, glioma, colorectal, lung cancer etc.-summarized for example in Bu, Oncotarget, 2013). This corresponds also to the increased tumorigenic potential of normal stem cells with increased ratio of symmetric divisions compared to asymmetric one. And lastly the asymmetric division in principle leads to less proliferative phenotype due to differentiation of one of the daughter cells in each division which is less useful for further fast proliferation and tumor mass growth.
4. In the commentary to your article no.1 (Kyjacova, 2015) you mentioned the non adherent cells expressing Mesenchymal markers attached and later gradually changed back to epithelial phenotype (including markers) which suggests that this kind of trait is induced by the treatment

rather than being the property of the cell before the treatment. Can you discuss possibilities of phenotype change due to i) plasticity and treatment-induced change of expression of epithelial/mesenchymal genes and/or ii) gradual selective pressure resulting in cell death mediated selection possibly of small fraction of non-proliferating (cancer stem) cells, especially when considering elevated ERK signaling? Did you perform any sort of cell fate tracking? Would it be possible?

5. In the commentary to your second article (Simova, 2016) you tested the effect of senescent cells on lung cancer model in mice. Did you test the senescent cells alone to verify, that elevated tumor incidence in mice treated with mix of proliferating and senescent cells was not due to presence of small fraction of dormant cancer stem cells? I did not see animals treated with TC-1/DTX only. Can you explain the phenotypic characterization ruling out this possibility as the CD80 seems to distinguish only TRAMP from TC-1 cells, but not TC-1 vs TC-1/DCX etc.?
6. (Mrazkova, 2018): „these results showed that L1CAM is substantially involved in cellular metabolism“ but „elevated L1CAM levels have been observed after downregulation of ANT2“ could it be just downstream effect of metabolic change or change associated with metabolism which does not have anything to do with L1CAM being the modulator of metabolism?
7. (Imrichova, 2019 and Imrichova, in press): What is the basis for assumption of the author that PML-NDS deal with ribosomal DNA damage? Is just assumption that they generally deal with repetitions, based on their association with telomeres and association with B23 and DHX9, and from rather vague connection of rDNA damage associated senescence, enough? In this light, does presence of rDNA in PML bodies proven by FISH justify statement „we can infer that PNAs formation is really dependent on rDNA-related processes“. Could the PML body be „just HR factory, where also rDNA might happen to be present especially considering that the repetitive rDNA sequences are prone to DNA damage in presence of torque in the DNA (Topo I inhibition/downregulation)? Could B23 and DHX9 be 2in1 functional proteins and thus their function in the PML body would have nothing to do with rDNA?
8. Interaction of SIM with sumoylated proteins upon phosphorylation by CK2 upon Doxorubicin treatment might be result of topo-mediated DNA damage along the genome, which due to the size of rDNA likely affects also that part of chromosomes. Could than the colocalization of rDNA and SUMO1 increasing in time be just result of persistent foci formation due to presence of repetitive sequences difficult and time consuming to repair by HR?

**Recommendation:**

I recommend the thesis to the defense.

Vladimir Rotrekl, PhD.

Assistant Professor
Department of Biology
Faculty of Medicine Masaryk University
and
ICRC International Clinical Research Center
St.Anne‘s University Hospital Brno
Kamenice 5, building A3
62500 Brno
Czech Republic
Tel.: +420 549 498 002
e-mail: vrotrekl@med.muni.cz