Visualizing of senescent cells *in vitro* and *in vivo*

Programme and abstracts

Warsaw, Poland, 15-16 December 2012

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PROGRAMME

Saturday, December 15, 2012

8:50-9:50 Registration

9:50-10:00 Invitation

Session I A

VARIES FACES OF CELLULAR SENESCENCE

10:00-11:30 Chairpersons: **Ewa Sikora** and **Jacek Witkowski**

10:00-10:30 L1. **Fabrizio d'Adda di Fagagna**: Molecular mechanisms of cellular senescence

10:30-11:00 L2. **Manuel Serrano**: Tumor Suppressors at the Interface between Cancer and Aging

11:00-11:30 L3. **Krzysztof Książek**: Sudden Senescence Syndrome as a determinant of replicative cell lifespan in vitro

11:30-12:00 Coffee break

Session I B

VARIES FACES OF CELLULAR SENESCENCE

12:00-13:30 Chairpersons: **Fabrizio d'Adda di Fagagna** and **Jiri Bartek**

12:00-12:30 L4. **Jacek Witkowski**: Imaging senescent lymphocytes – beyond SA-β–GAL and towards changed functionalities

12:30-13:00 L5. **Joanna Szczepanowska**: Role of mitochondria in age-related disorders

13:00-13:10 O1. **Andre Ivanov**: Lysosome-mediated processing of chromatin in senescence

13:10-13:20 O2. **Monika Puzianowska-Kuznicka**: Immunosenescence: is there a role for epigenetics?

13:20-13:30 O3. **Anna Lewinska**: Age-related changes in global DNA methylation and repetitive sequences contribute to genomic instability of horses

13:30 – 14:30 Buffet lunch

Session II A

CELLULAR SENESCENCE IN CANCER DEVELOPMENT AND THERAPHY

14:30-16:00 Chairpersons: **Jekaterina Erenpreisa** and **Manuel Serrano**

14:30-15:00 L6. **Jiri Bartek**: Cellular senescence in tumorogenesis and ageing: mechanisms and relevance to human pathologies

15:00-15:30 L7. **Daniel Peeper**: Senescence and melanoma: dissecting mechanism and screening for novel therapeutic targets

15:30-16:00 L8. **Andrzej Składanowski**: Senescing together, senescing apart or how different tumor cell clones respond to antitumor drugs

16:00-16:30 Coffee break

16:30-17:10 Chairpersons: **Joanna Szczepanowska** and **Krzysztof Książek**

16:30-16:40 O4. **Elżbieta Speina**: Helicase and exonuclease activities of Werner protein are modulated by 4-hydroxy-2-nonenal through histidine-, cysteine- and lysine-specific modifications

16:40-16:50 O5. **Mariano F. Zacarias Fluck**: Constitutive Her2 signaling promotes breast cancer metastasis through cellular senescence

16:50-17:00 O6. **Grażyna Mosieniak**: Mitotic disorders induced by curcumin lead to premature senescence and autophagy in human cancer cells

17:00-17:10 O7. **Alexander N. Khokhlov**: Can cancer cells age? Stationary cell culture approach to the problem solution

17:10-18:10 Poster session

18:10 Buffet-style dinner and wine

Sunday, December 16, 2012

Session II B

CELLULAR SENESCENCE IN CANCER DEVELOPMENT AND THERAPHY

10:00-11:40 Chairpersons: **Daniel Peeper** and **Michael Sherman**

10:00-10:30 L9. **Julia Yaglom**: SWING: A novel cellular state on the crossroads between transformation and senescence

10:30-11:00 L10. **Clemens Schmitt**: Visualizing therapy-induced senescence in vivo - imaging novel functionalities

11:00-11:10 O8. **Halina Waś**: Reversible senescence of cancer cells induced by DNA damage

11:10-11:20 O9. **Anna Bielak-Żmijewska:** Replicative and doxorubicin-induced premature senescence of human Vascular Smooth Muscle Cells. Differences and similarities of both types of senescence

11:20-11:30 O10. **Tobias Jung**: Athermal and thermal effects during water-filtered infrared A-irradiation - A Problem of experimental Setup

11:30-11:40 O11. **Thomas R Jackson:** TP53 couples senescence to self-renewal in the response to Etoposide in PA1 embryonal Carcinoma cells

11:40-12:10 Coffee break

12:10-13:10 Chairpersons **Julia Yaglom** and **Andrzej Składanowski**

12:10-12:40 L11. **Jekaterina Erenpreisa**: The trinity: DDR damage, senescence, and self-renewal

12:40-13:10 L12. **Michael Sherman**: Heat shock proteins, cancer and oncogene-induced senescence

13:10-13:20 Closing

13:20-14:20 Buffet lunch

Lectures

L1.

MOLECULAR MECHANISMS OF CELLULAR SENESCENCE

F. D'Adda Di Fagagna

The FIRC Institute of Molecular Oncology Foundation, IFOM, Milan Italy

e-mail: fabrizio.dadda@ifom.eu

Cellular senescence is a powerful mechanism of tumor suppression that also limits the proliferation of healthy cells and thus is associated with organismal ageing. I will propose a unifying mechanism for different types of cellular senescence based on DNA damage generation and persistent DNA damage response (DDR) activation. I will also discuss our most recent results on RNA and DDR modulation.

L2.

TUMOR SUPPRESSORS AT THE INTERFACE BETWEEN CANCER AND AGING

Manuel Serrano

Spanish National Cancer Research Centre (CNIO), Madrid, Spain

For a number of years our research program has focused in trying to understand the connection between cancer protection and healthy lifespan. We have generated a number of mouse cell lines carrying increased gene dosage of important tumor suppressors, such as INK4a/Arf, p53, and, more recently, PTEN. Several of our mouse lines with increased tumor suppression have an extended healthy lifespan that is not accounted for simply because they have less cancer. This is the case of mice with extra gene dosage of INK4/Arf or extra PTEN. From these studies, we have learned that tumor suppressors eliminate damaged cells, by apoptosis or senescence, and in doing so prevent the emergence of cancer and preserve healthy lifespan. Therefore a moderately increased and regulated capacity of cells to undergo apoptosis or senescence, can be beneficial for the organism. This is not in conflict with observations by others that excessive apoptosis or senescence beyond a certain threshold is detrimental and induces premature aging.

In an effort to extend the biological significance of senescence, we have explored the role of senescence during embryonic development. We have found that senescence, indeed, plays a role during particular developmental processes that will be discussed at the conference.

L3.

SUDDEN SENESCENCE SYNDROME AS A DETERMINANT OF REPLICATIVE CELL LIFESPAN IN VITRO

Krzysztof Książek

Poznań University of Medical Sciences, Department of Pathophysiology, Poznań, Poland

e-mail: kksiazek@ump.edu.pl

It has long been hypothesized that the number of divisions cells can endure *in vitro* may be determined by a gradually rising pool of senescent cells rather than by a synchronized loss of proliferative capacity in particular cells composing the culture. This prediction has found a firm support in studies utilizing both computer simulations and experimental data which revealed that the major factor that restricts the replicative lifespan of cells in culture may be the so-called 'Sudden Senescence Syndrome' (SSS). SSS refers to a phenomenon in which a subset of senescent cells emerges abruptly in early-passage cultures in response to stochastic and telomere-independent events. SSS can induce growth arrest within a single division and is thought to be an internal feature of a given culture.

It seems likely that SSS plays a special role in cultures with relatively short replicative lifespan, e.g. in human peritoneal mesothelial cells (HPMCs) which senesce upon reaching 6-10 divisions. It has been found that early-passage HPMC cultures contain a considerable fraction of cells (even up to a half of the population) bearing molecular fingerprint of senescence, including negativity for Ki-67 proliferative antigen, positive staining for senescence-associated β-Gal, excessive DNA damage (γ-

H2A.X foci and 8-OH-dG), and high expression of cell cycle inhibitor, p16(INK4a). Moreover, the experiments employing a co-culture of young with senescent HPMCs revealed an inverse relationship between the size of senescent cell fraction and reproducibility of the culture as a whole.

Despite obvious evidence that SSS affects the kinetics of cell growth and senescence in certain types of cells, the molecular mechanisms underlying this phenomenon are still elusive. It has been suggested that SSS may be associated with some kind of environmental trauma experienced by cells at the very beginning of their lifetime *in vitro* (e.g. oxidative stress) as well as with an autocrine and/or paracrine activity of certain senescence-promoting agents accumulating in culture, such as transforming growth-β1 (TGF-β1). It should be stressed, however, that taking into account literature data, the biological role of SSS in cellular senescence is markedly underestimated.

L4.

IMAGING SENESCENT LYMPHOCYTES – BEYOND SA-B–GAL AND TOWARDS CHANGED FUNCTIONALITIES

Jacek M. Witkowski

Department of Pathophysiology, Medical University of Gdańsk, Poland

E-mail: jawit@gumed.edu.pl

"The lymphocyte is small and round…" this quote from a poem by a seemingly unknown author describing the lymphocyte pinpoints the problem: it is pretty hard to visualize its features using most of currently available techniques, including fluorescent and confocal microscopy. Even at the electron microscopy levels, a resting lymphocyte is rather inconspicuous. Yet here we are to show a difference between an image of a young and an old (senescent) lymphocyte. A golden standard so far is to distinguish young from old cells in vitro by the ability of the latter to exhibit the activity of a galactosidase, aptly named the "senescence-associated β-galactosidase"; it works for most mammalian cell types, but especially well for those equipped with relatively abundant cytoplasm. Probably because of that a PUBMED search using the keywords "senescent lymphocytes" AND "SA-β-GAL" yields exactly zero entries so far. Yet, senescent lymphocytes can be "visualized" based on their inability to divide, loss or gain of certain molecules and functions, including notably apoptosis and cytokine secretion. Some details on these special properties and features of old and senescent human lymphocytes will be discussed in the lecture.

L5.

ROLE OF MITOCHONDRIA IN AGE-RELATED DISORDERS

Joanna Szczepanowska[1], Jarosław Walczak[1], Małgorzata Partyka[1], Grażyna Dębska-Vielhaber[2], Stefan Vielhaber[2], Jerzy Duszyński[1]

[1] Nencki Institute of Experimental Biology, Warsaw, Poland

[2] Department of Neurology, University of Magdeburg, Magdeburg, Germany

e-mail: j.szczepanowska@nencki.gov.pl

Mitochondria are multifunctional organelles that participate in numerous cellular processes. They supply the cell with ATP through oxidative phosphorylation, synthesize key molecules and buffer intracellular calcium ions. Mitochondria are also sites of formation of reactive oxygen species, by-products of the oxidative phosphorylation. Mitochondria continuously undergo cycles of fusion and fission, which impact mitochondrial bioenergetics, ATP production, apoptosis and mitophagy. Dysfunctions of mitochondria are usually associated with numerous multisystem syndromes, neurodegenerative disorders, cancer and ageing. Mitochondrial malfunctions are increasingly recognized as a key component playing fundamental role in the mechanism of cellular ageing and thus may increase the risk of age - related diseases such as Alzheimer disease (AD), Parkinson disease (PD) and amyotrophic lateral sclerosis (ALS) diseases. It was shown that mitochondrial dynamics directly or indirectly is implicated in pathology of these diseases. Because mitochondrial morphology, plasticity or the ability of the mitochondria to fuse and divide in a coordinated manner, appears to be the critical element determining mitochondrial function within the cell we propose a hypothesis

that it could play an important role in neurodegenerative age-related diseases.

In order to understand the importance of the role of mitochondrial dynamics and distribution within the cell in age-related diseases we studied mitochondrial organisation in primary cultures of skin fibroblasts derived from patients diagnosed with AD, PD and ALS. We focused on identification and estimation of expression levels of proteins (Drp1, OPA1, Mfn, Fis1) responsible for the dynamics of mitochondria. The profiles of these proteins in the investigated cells are different. Our observations carried out with a confocal microscope showed also subtle changes in the organization of mitochondria within the cell. In particular, we observed increase of mitochondrial branching in ALS cells and increase of mitochondrial swelling and dissipation in AD cells.

L6.

CELLULAR SENESCENCE IN TUMORIGENESIS AND AGEING: MECHANISMS AND RELEVANCE TO HUMAN PATHOLOGIES

Jiri Bartek[1,2], Martin Kosar[1,2], Jirina Bartkova[1], and Zdenek Hodny[2]

[1] Danish Cancer Society Research Center, Copenhagen, Denmark

[2] Institute of Molecular Genetics, Prague, Czech Republic

e-mail: jb@cancer.dk

Cellular senescence has been implicated in a wide range of pathological conditions as well as ageing, yet there are still numerous open questions about both the mechanisms and the potential to exploit the emerging biomarkers of senescence in diagnostics or treatment of human diseases. The lecture will provide an overview of our recently published and unpublished data on mechanisms that trigger cellular senescence in response to oncogenes and DNA damage (SIPS). Emphasis will be put on DNA damage signaling pathways, and the role of senescence as part of an inducible intrinsic anti-cancer barrier. Both experimental approaches based on cell culture models, and analysis of mouse models and human clinical material (from diverse types of human early lesions and invasive stages, including several types of epithelial cancers, gliomas, myeloid leukemia and germ-cell tumors) will be presented. Furthermore, given the focus of the conference, we will discuss our efforts to introduce and validate various markers of cellular senescence, with particular emphasis on attempts to optimize their detection in routine formalin-fixed, paraffin-embedded tissues. Time permitting, our data on analyses of ageing human cells and tissues from human donors of various ages will be presented, to document

the age-related changes in some candidate senescence biomarkers. Finally, the issue of senescence-associated secretome will be discussed, in view of our data on the phenomenon that we named ‘bystander senescence’.

Selected recent references: Jackson SP, Bartek J. Nature, 461:1071-8 (2009); Takacova S, et al. Cancer Cell 21:517-31 (2012); Bartek J et al., Nature Struct.Mol. Biol., 19:5-7 (2012); Lukas J, Lukas C, Bartek J. Nature Cell Biol. 13:1161-9 (2011); Gudjonsson T. et al., Cell, 150: 697-709.

L7.

SENESCENCE AND MELANOMA: DISSECTING MECHANISM AND SCREENING FOR NOVEL THERAPEUTIC TARGETS

Daniel S. Peeper, PhD

Division of Molecular Oncology, Netherlands Cancer Institute, Amsterdam

d.peeper@nki.nl

http://research.nki.nl/peeperlab/

The BRAF protein kinase is frequently mutated (commonly V600E) in melanoma and a number of other tumor types. Remarkably, BRAFE600 is also found in the vast majority of benign melanocytic nevi (moles). Nevi typically show little proliferative activity and in rare cases progress to malignant melanoma. Previously, we reported that BRAFE600 is associated with the activation of tumor suppressors (including p16INK4A) and induction of long-term, senescence-like cell cycle arrest of nevi (1, 2). Similar results on oncogene-induced senescence (OIS) in vivo have now been reported across a wide variety of model systems, and in the context of several oncogenes and tumor suppressor genes.

In spite of the common long-term arrested state of nevus cells, we observed that nevi often display a mosaic immunopositivity for p16INK4A. This may imply that alongside p16INK4A other factors contribute to the senescent state of BRAFE600-expressing untransformed cells. In addition to taking a candidate gene approach, we are combining gene expression analysis, systematic RNAi and unbiased functional screens to identify such factors. This integrative oncogenomics approach has identified novel signaling networks involved in OIS. For example, we found that the

inflammatory transcriptome is induced in the context of oncogenic stress. This is dependent on the activity of the transcription factor CEPB as well as several of its effector genes, including specific interleukins (3, 4, 5). We also identified a mechanism by which nevi progress to melanomas, involving activation of the PI3K pathway (6). Based on these data, we have begun to build a framework, comprising several transcription factors, which fulfill a central role in melanoma suppression.

Furthermore, we have set out to screen for novel therapeutic targets in melanoma, using function-based screens in combination with next-generation sequencing. Although recently new modes of therapeutic interference have become available, often resistance emerges. The need for, perspective and challenges of combinatorial therapy will be discussed.

1. $BRAF^{E600}$-associated senescence-like cell cycle arrest of human naevi. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. Nature. 2005 Aug 4;436(7051):720-4.
2. Oncogene-induced cell senescence--halting on the road to cancer. Mooi WJ, Peeper DS. N Engl J Med. 2006 Sep 7;355(10):1037-46.
3. Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ, Aarden LA, Mooi WJ, Peeper DS. Cell. 2008 Jun 13;133(6):1019-31
4. Senescence-messaging secretome: SMS-ing cellular stress. Kuilman T, Peeper DS. Nat Rev Cancer. 2009 Feb;9(2):81-94. Epub 2009 Jan 9
5. Kuilman, T., Michaloglou, C., Mooi, W. J. & Peeper, D. S. The essence of senescence. *Genes Dev* 24, 2463–2479 (2010).
6. Vredeveld, L. C. W. *et al.* Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis. *Genes Dev* (2012).doi:10.1101/gad.187252.112

L8.

SENESCING TOGETHER, SENESCING APART OR HOW DIFFERENT TUMOR CELL CLONES RESPOND TO ANTITUMOR DRUGS

Andrzej Skladanowski, Marcin Serocki, Michal Sabisz, Maciej Kujawa

Department of Pharmaceutical Technology and Biochemistry, Gdansk University of Technology, Faculty of Chemistry, Gdansk, Poland

e-mail: as@chem.pg.gda.pl

Cellular senescence is one of the mechanisms that prevent the development of cancer by eliminating cells which acquired deleterious DNA mutations or activated oncogenes. Tumor cells are able to evade cellular senescence by activation of telomere length maintenance mechanisms, mostly by re-expression of telomerase. On the other hand, there are data available in the literature that cancer cells themselves undergo spontaneous senescence as a result of endogenous DNA damage and chromosomal aberrations which accumulate in tumor cells due to defects in cell cycle checkpoints in G2 and mitosis. Premature senescence in tumor cells can also be induced by anticancer agents which produce DNA damage. It was widely believed that tumor cells which become senescent after exposure to antitumor agents are not able to regain their proliferative potential. However, results of several research groups, including our own, revealed that after prolonged post-incubation of drug-treated tumor cells, a small fraction of cells re-starts cell proliferation, which have features of cancer stem cells (CSC).

Based on our previous observations, we proposed that CSC can be less prone to undergo premature senescence after DNA damage. This could

lead to proliferation of these cells after drug exposure and ineffective treatment. More importantly, that may also result in accumulation of tumor cells with additional genetic mutations and drug resistance phenotype. To clarify this important issue, we characterized drug-induced cellular response in sublines of lung adenocarcinoma A549 cells consisting of tumor cells which form only holo- and paraclones, which are proposed to correspond to CSC and non-CSC cells, respectively. Development of this unique cellular model allowed us to characterize the phenotypes and possible differences in cellular response to anticancer drugs. Our results revealed that molecular mechanisms responsible for the maintenance of mixed population phenotype in tumor cells involve cell-cell communication that is based on the secretion of cytokines, and differences in the mitochondrial metabolism. We have also data showing that tumor cells respond to drugs differently when they grow as a mixed population or as selected clones and premature senescence is preferentially induced in holoclones.

Our results may have important implications for the development of new in vitro screening systems that enable testing of drugs and drug/combinations with increased activity toward CSCs.

L9.

SWING: A NOVEL CELLULAR STATE ON THE CROSSROADS BETWEEN TRANSFORMATION AND SENESCENCE

J. Yaglom, V. Gabai, L. Meng, M. Sherman;

Boston University Medical Center, Boston.

Oncogene-induced senescence constitutes a major barrier in the path to neoplastic transformation. Accordingly, the common breast cancer oncogene, Her2, triggers senescence in primary mammary epithelium. Recently, we have shown that expression Her2 in immortalized breast epithelial cells, while promoting various senescence-associated changes, failed to trigger the major senescence end-point, i.e. permanent growth arrest. We have dubbed this novel cellular state senescence with incomplete growth arrest (SWING), and suggested that it constitutes an important step in tumor development. Interestingly, following DNA damaging insults, SWING cells failed to accumulate γH2AX, the major factor in the repair of double-stranded DNA (dsDNA) breaks. Accordingly, SWING phenotype associates with impaired DNA repair that may explain augmented genomic instability commonly observed in many cancers. In fact, SWING cells show increased genomic instability and have increased sensitivity towards DNA damaging insults. Recently, we show that breast cancer cells MCF7 could be reversed back into SWING state by depletion of major heat shock protein Hsp72. These data suggest that inhibition of Hsp72 can sensitize cancer cells towards chemo- and -radiotherapy.

L10.

VISUALIZING THERAPY-INDUCED SENESCENCE *IN VIVO* – IMAGING NOVEL FUNCTIONALITIES

Clemens A. Schmitt and colleagues

Charité-Universitätsmedizin Berlin/ Molekulares Krebsforschungszentrum (MKFZ), and Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Chemoresistance is the most important predictor of poor survival in lymphoma patients, but underlying mechanisms remain poorly understood. Cellular senescence, a DNA damage-initiated terminal cell-cycle block, may be an important component of drug action, but no genetic model exists to assess the specific contribution of therapy-inducible senescence (TIS). The Eμ-*myc* transgenic mouse was previously established as an excellent model to explore the role of candidate genes and candidate programs (such as apoptosis) in response to chemotherapy. Because oncogene-induced senescence is characterized by trimethylated histone H3 lysine 9 marks (H3K9), we aimed to address the impact of TIS on long-term outcome in Eμ-*myc* transgenic mice lacking the H3K9 histone methyltransferase Suv39h1.

Control lymphoma-bearing mice entered TIS and achieved a much better long-term treatment outcome when compared to mice harboring Suv39h1-deficient lymphomas, which did not differ in their apoptotic and proliferative capacity, but lacked a TIS response and rapidly progressed to a terminal disease condition. While Bcl2-protected TIS lymphomas expectedly presented with a sharp decline in ^{18}F-fluoro-deoxythymidine positron emission tomography activity (scanning DNA synthesis as a functionality of dividing cells), they exhibited even enhanced ^{18}F-fluoro-deoxyglucose PET signal intensities (scanning glucose metabolism). *In vitro*, TIS lymphomas showed increased glucose uptake, a higher glycolytic rate and higher ATP levels. They were more sensitive to glucose deprivation or inhibition of glycolysis when compared to equally treated,

but senescence-incapable Suv39h1-deficient lymphoma cells. Mechanistically, we identified proteotoxic stress – due to the overwhelming production of senescence- associated secretory proteins – as the underlying cause of the increased energetic needs of senescent cells, in which we detected massive signs of endoplasmic reticulum stress and the unfolded protein response, ultimately buffered by strongly enhanced (and energy-consuming) autophagy to clear the toxic proteins. As a consequence, TIS lymphomas underwent cell death in an ER stress-specific manner when exposed to autophagy or glycolysis inhibitors. Importantly, the sequential treatment of TIS-capable, Bcl2-overexpressing lymphoma-bearing mice with chemotherapy in the first place followed by either a glycolysis inhibitor or a blocker of autophagy produced lymphoma regression by TUNEL-positive cell death *in vivo*. A long-term trial in matched pairs of lymphoma-bearing mice exposed to either cyclophosphamide alone or cyclophosphamide followed by the autophagy inhibitor bafilomycin resulted in a significantly longer overall survival for the mouse cohort that received the sequential treatment.

Our data demonstrate genetically that TIS significantly improves long-term outcome to anticancer therapy *in vivo*. However, rare senescent cells may eventually resume proliferation, and, thus, give rise to a relapse. The – unexpected – hypermetabolic nature of TIS imposes a therapeutically exploitable cancer liability that can be further exploited by "synthetically lethal (SL)" metabolic targeting strategies to selectively eliminate senescent tumor cells. These findings represent a proof-of-principle how to utilize a cancer-specific condition (not a single gene defect) in a conceptually novel SL strategy that produces little harm to normal tissues.

L11.

THE TRINITY: DNA DAMAGE, SENESCENCE, AND SELF-RENEWAL

Jekaterina Erenpreisa

Latvian Biomedical Research& Studies Centre, Riga, LV1067;
katrina@biomed.lu.lv

Stress-induced cellular senescence (SIS) is poorly understood. On the one hand 'senescence' means decay and is defined as an irreversible arrest of proliferation and a barrier to cancer, however late (Serrano M, Nature 2010). On the other hand, senescent cells have active metabolism and can undergo reverse to proliferation (Sikora E, Exp Gerontol 2012), while senescence as such bears increased risk of cancer. Moreover, the paradoxical findings by Banito and colleagues (2009) and Rudolf Jaenisch lab showed that induction of pluripotency (iPSC) by Yamanaka transcription factors induced senescence as a rule and pluripotency by chance. The data from our laboratory showed that in IMR90 cells pre-senescence is associated with the appearance of self-renewal and senescence markers coupled to DNA damage in the same G2M >4C cells (Huna et al., 2011). Moreover, using Etoposide treated PA-1 (embryonal carcinoma) cell line we showed that this dual response from the G2-arrest is TP53-dependent and safeguards genome stability of self-renewal (Jackson et al, submitted). In this talk, I shall specifically analyse 'senescence' caused by DNA damage in the G2M checkpoint as an adaptive metastable state and prerequisite of self-renewal induction characterised by the expression of opposing regulators. Our hypothesis is that under conditions of genotoxic stress the tumour (stem) cells of the G2

phase supported by macroautophagy (the main component of the sa-β-gal marker of ‘senescence’) and accumulating OCT4 acquire a potential after DNA repair to reach the embryonality attractor by OCT4A-NANOG positive loop. It is a route for initiation of self-renewal and escape from anti-cancer treatment. In fact, our data and interpretation are close to those on the ‘SWING stage’ of incomplete senescence found by J. Yaglom and colleagues (2011). Our studies suggest that self-renewal and senescence are united either by mitotic slippage of the poorly repaired cells or by asymmetric division of the common tetraploid precursor. It follows that SIS can be viewed as another side of self-renewal. Recent finding of senescing cells in embryo (by M. Serrano and colleagues) and adverse prognosis for patients survival with increased sa-b-gal positivity response after neoadjuvant therapy found in further operated small lung cancers (Wu PC et al., Exp Oncol 2012) are also in conformity with this view.

L12.

HEAT SHOCK PROTEINS, CANCER AND ONCOGENE-INDUCED SENESCENCE

Michael Sherman

Boston University Medical School, Boston MA

The major heat shock protein Hsp72 is expressed at elevated levels in many cancers and its expression correlates with tumor progression. We have investigated the role of Hsp72 in tumorigenesis induced by the major breast cancer oncogene Her2. Strikingly, expression of Her2, while causing neoplastic transformation in mammary epithelial cell culture, precipitated senescence in mammary cells depleted of Hsp72. Therefore, in proliferating transformed cells, Hsp72 is needed to suppress oncogene-induced senescence (OIS). In the mouse model of Her2-positive breast cancer, knockout of Hsp72 almost completely suppressed tumorigenesis. In Hsp72 KO mice, expression of Her2 caused massive cell senescence in mammary tissue, and thus instead of mammary hyperplasia led to suppression of duct development and blocked alveolar budding. Therefore Hsp72 plays an essential role in Her2-induced tumorigenesis by regulating oncogene-induced senescence pathways.

Effects of Hsp72 on OIS were due to regulation of p21 levels, most likely via control of the transcription factor FoxM1. In addition to OIS, Hsp72 regulates a number of other pathways that control various stages of tumor development, including cancer cell migration and invasion, angiogenesis and metastases. In regulation of signaling Hsp72 cooperates with its co-factor BAG3, and disruption of this interaction mimics effects of Hsp72 depletion on OIS and other signaling pathways. We have

developed a small molecule that disrupts interaction between Hsp72 and BAG3, and found that it has similar effects. This molecule can be used as lead for anti-cancer drug design.

Oral presentations

O1.

LYSOSOME-MEDIATED PROCESSING OF CHROMATIN IN SENESCENCE

Andre Ivanov*, Jeff Pawlikowski*, John van Tuyn*, Indrani Manoharan*, Taranjit Singh Rai*, David M. Nelson*, Parisha P. Shah^, Hong Wu$, Shelley L. Berger^, Peter D. Adams*.

*Institute of Cancer Sciences, CR-UK Beatson Labs, University of Glasgow.

$Fox Chase Cancer Center, Philadelphia.

^University of Pennsylvania, Philadelphia.

Cellular senescence is a stable proliferation arrest and a potent tumor suppressor mechanism. Here we introduce the concept of senescence "maturation" into "deep senescence". We show that senescent cells *in vitro* exhibit a maturation process, reflected by histone loss coupled to autophagic/lysosomal processing of cytoplasmic chromatin. Lamin A/C negative but strongly γ-H2AX and H3K27Me3 positive cytoplasmic chromatin fragments appear to bud off the interphase nuclei and this process is accompanied by the focal and general loss of nuclear integrity as defined by dextran permeability assay.

In vivo, depletion of histones correlates with nevus maturation, an established histopathologic parameter associated with proliferation arrest and clinical benignancy.

We conclude that the senescent phenotype, as it is typically defined, is not a fixed endpoint. Instead, senescence is a dynamic state, reflected in ongoing progressive histone loss and this process might contribute to irreversibility of senescence and tumour suppression.

O2.

IMMUNOSENESCENCE: IS THERE A ROLE FOR EPIGENETICS?

Monika Puzianowska-Kuznicka[1,2], Monika Budzinska[2], Magdalena Owczarz[2], Eliza Pawlik-Pachucka[1,2], Jacek Połosak[1]

[1] Mossakowski Medical Research Centre, Warsaw, Poland

[2] Medical Centre of Postgraduate Education, Warsaw, Poland

Aging is a multifactorial process resulting from accumulation of a stochastic damage to DNA, proteins, and to lipids. It is accompanied by epigenetic drift – an age-related, tissue-specific change in the pattern of epigenetic modifications. Aging of the immunological system leads to the decreased resistance to infections, increased risk of autoimmunity, and to a chronic, low-grade inflammation. We hypothesized that the age-related alterations of function of blood mononuclear cells might be, in part, a result of epigenetic drift affecting the level of expression of various genes. To verify this hypothesis, we performed a study in human blood mononuclear cells obtained from young (18-45 years old), elderly (65-75 years old), and from long-lived (>90 years old) individuals. We studied the age-associated changes of expression of the *IGF-1R*, *FOXO1*, *SIRT1*, *SIRT3*, *WRN*, *XPD*, and *THRB* – the genes previously indicated as involved in the regulation of the rate of aging. We also evaluated the expression of miRNAs potentially interacting with 3'UTRs of mRNAs of these genes, and age-associated changes in the pattern of methylation of the promoters of the studied genes. We showed that the expression of all these genes significantly decreased with age. In some cases the decrease was initiated at middle age, while in others it started only at young-old age. We also showed that the decreased

expression of the *FOXO1* and *SIRT1* can be, in part, a result of the action of the respective miRNA, expression of which increases with age. In the case of *THRB*, its decreased expression can be a result of the increased methylation of its promoter. In other yet cases (*IGF-1R*, *SIRT3*, *WRN*, *XPD*), the reason for the age-associated decrease of expression remains obscure; however, the role of miRNAs is strongly suggested.

O3.

AGE-RELATED CHANGES IN GLOBAL DNA METHYLATION AND REPETITIVE SEQUENCES CONTRIBUTE TO GENOMIC INSTABILITY OF HORSES

Anna Lewinska[1], Bernadetta Oklejewicz[2,3], Artur Gurgul[4], Tomasz Zabek[4], Leszek Potocki[2,3], Monika Bugno-Poniewierska[2,3,4], Ewa Słota[2,3] Maciej Wnuk[2,3]

[1] Department of Biochemistry and Cell Biology, University of Rzeszow, Poland

[2] Department of Genetics, University of Rzeszow, Rzeszow, Poland

[3] Centre of Applied Biotechnology and Basic Sciences, University of Rzeszow, Kolbuszowa, Poland

[4] Laboratory of Genomics, National Research Institute of Animal Production, Balice n. Cracow, Poland

e-mail: lewinska@univ.rzeszow.pl

Modern biogerontological studies involve comprehensive analysis of age-associated changes of biological processes at both cellular and organismal level. Among biogerontologist topics of interests, issues of fundamental importance are changes in genomic DNA during aging. It was repeatedly reported that age-mediated changes in DNA affect not only genes (induction of point mutations), but predominantly chromatids, chromosomes, and cytogenetic methods can be considered very useful and invaluable to monitor mitotic/meiotic cell cycle abnormalities and aberrant interphase (G1, S and G2). Since horse is a quite long-lived animal with a mean and maximum lifespan of 25 and 40 years, respectively and similarly to old people, old horses suffered for age-related diseases such as cancer, a horse aging model was suggested. With horse aging model, the

mechanisms of immunosenescence and inflamm-aging were intensively studied. In the present study, we evaluated age-associated changes in horse genomic stability and underlying mechanisms. We used two different horse breeds to exclude some breed-dependent phenomena. The level of positive TUNEL cells (both apoptotic and with DNA fragmentation), oxidative DNA damage (8-oxoG immunostaining), sister chromatid exchange and bleomycin-induced chromatid breaks were significantly increased in the combined old group compared to the combined adult group. We showed the global loss of DNA methylation in blood lymphocytes of aged horses. Additionally, we tested a pattern of DNA methylation of ribosomal DNA and selected genes like *IGF2* and found no significant changes during aging. Transcriptional rDNA activity, assessed as the number and size of nucleolar organizer regions, reflecting physiological state of the cell, was decreased during aging. We asked if genetic components such as polymorphisms within DNA methyltransferase genes such as *DNMT1*, *DNMT3a* and *DNMT3b* may contribute to observed changes in global DNA methylation status. The presence of none of 7 analyzed polymorphisms resulted in significant changes in global DNA methylation suggesting an evident correlation between DNA methylation and horse donor age. During horse aging, loss of pericentromeric heterochromatin and telomere shortening were also observed. In conclusion, age-associated changes in global DNA methylation status and repetitive sequences leading to genomic instability may contribute, at least in part, to the aging process in the horse.

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O4.

HELICASE AND EXONUCLEASE ACTIVITIES OF WERNER PROTEIN ARE MODULATED BY 4-HYDROXY-2-NONENAL THROUGH HISTIDINE-, CYSTEINE- AND LYSINE-SPECIFIC MODIFICATIONS

Elżbieta Speina[1], Jolanta Czerwińska[1], Janusz Dębski[1], Vilhelm A. Bohr[2], Barbara Tudek[1,3]

[1] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

[2] National Institute on Aging, National Institutes of Health, Baltimore, USA

[3] Institute of Genetics and Biotechnology, Warsaw University, Warsaw, Poland

e-mail: elasp@ibb.waw.pl

4-Hydroxy-2-nonenal (HNE) is an endogenous product of lipid peroxidation known to play a role in cellular signaling through protein modification and is a major component of the pathogenesis in a spectrum of diseases involving oxidative stress. Werner syndrome arises through mutations in both copies of the *WRN* gene that encode RecQ 3'-5' DNA helicase and exonuclease essential for genomic stability. This hereditary disease is associated with chromosomal instability, premature aging and cancer predisposition. WRN appears to participate in the cellular response to oxidative stress and cells devoid of *WRN* display elevated levels of oxidative DNA damage.

We investigated the direct influence of HNE on purified human WRN protein and demonstrate that both helicase and exonuclease activities of WRN protein are modulated, depending on HNE concentration. Western blot, immunoprecipitation and mass spectrometry were used to identify and

characterize the *in vitro* and *in vivo* covalent modifications of WRN by HNE. Data revealed adduct addition to several cysteines and histidines, including the helicase domain. We will further apply molecular modeling analysis of HNE adducted to His and Cys residues to provide a potential mechanism of deregulation of WRN enzymatic activity.

O5.

CONSTITUTIVE HER2 SIGNALING PROMOTES BREAST CANCER METASTASIS THROUGH CELLULAR SENESCENCE

Mariano F. Zacarias Fluck [a], Pier Davide Angelini [a,c], Kim Pedersen [a], Josep Lluís Parra-Palau [a], Marc Guiu [e], Cristina Bernadó Morales [a], Rocio Vicario [a], Antonio Luque-García [a], Nerea Peiró Novalpotro [b], Jordi Giralt [b], Francesc Canals [a], Roger R. Gomis [d, e], Josep Tabernero [b], José Baselga [b], Josep Villanueva [a] and Joaquín Arribas [a,b,c]

[a] Preclinical Research and [b] Clinical Research Programs; Vall d'Hebron Institute of Oncology (VHIO); Barcelona, 08035, Spain.

[c] Department of Biochemistry and Molecular Biology, Universitat Autonoma de Barcelona, Campus de la UAB, 08193 Bellaterra, Spain

[d] Institució Catalana de Recerca i Estudis Avançats (ICREA), 08010 Barcelona, Spain

[e] Oncology Program, Institute for Research in Biomedicine Barcelona, 08028 Barcelona, Spain

e-mail: mzacarias@vhio.net

Senescence, a terminal cell proliferation arrest, can be triggered by oncogenes. Oncogene-induced senescence is classically considered a tumor defense barrier. However, several findings show that, under certain circumstances, senescent cells may favor tumor progression because of their secretory phenotype. Here we show that the expression in different breast epithelial cell lines of p95HER2, a constitutively active fragment of the tyrosine kinase receptor HER2, results in either increased proliferation or senescence. In senescent cells, p95HER2 elicits a secretome enriched in proteases, cytokines and growth factors. This secretory phenotype is not a mere consequence of the senescence status and requires continuous HER2

signaling to be maintained. Underscoring the functional relevance of the p95HER2-induced senescence secretome, we show that p95HER2-induced senescent cells promote metastasis in vivo in a non-cell autonomous manner.

O6.

MITOTIC DISORDERS INDUCED BY CURCUMIN LEAD TO PREMATURE SENESCENCE AND AUTOPHAGY IN HUMAN CANCER CELLS

Grażyna Mosieniak[1], Małgorzata Śliwińska[1,] Dorota Przybylska[1], Marek Adamowicz[1], Olga Alster[1], Hubert Jaśkowiak[1], Andrzej A.Szczepankiewicz[2], Grzegorz M. Wilczyński[2], Ewa Sikora[1]

[1] Laboratory of the Molecular Bases of Aging, Nencki Institute of Experimental Biology,

[2] Laboratory of Molecular and Systemic Neuromorphology,

[3] Laboratory of Transcription Regulation,

Nencki Institute of Experimental Biology PAS, 02-093 Warsaw, Poland

Pasteura 3 Street, 02-093 Warsaw, Poland

e-mail: g.mosieniak@nencki.gov.pl

In response to chemotherapeutic drugs or irradiation, cancer cells undergo cell death or cell cycle arrest. Thus far apoptosis was considered to be the main mechanism of cell death. More recently, cancer cell senescence was recognized as a common response to chemotherapeutics or irradiation that significantly restricts tumor growth. Curcumin, a natural polyphenol derived from the rhizome of *Curcuma longa,* has anticancer properties both *in vitro* and *in vivo*. Due to its pleiotropic mode of action, curcumin affects carcinogenesis at various stages like tumor initiation, promotion and progression. The main mechanism responsible for anticancer activity of curcumin so far was attributed to the induction of cell death. However in the present study we proved that curcumin, in a relatively low concentration, can induce senescence of human colon cancer HCT116 cells,

both p53+/+ and p53-/- clones, MCF7 human breast cancer cells and U2OS human osteosarcoma cells. Although cells ceased proliferation, they underwent polyploidyzation, showing that increased genomic instability correlated with curcumin-induced senescence. Interestingly, curcumin did not induce DNA damage, as the majority of the senescence-inducing factors do, but it disrupted the mitotic division. Upon curcumins' treatment cells temporally stopped in mitosis due to the improper mitotic spindle formation. A fraction of cells that survived the mitotic slippage and probably underwent senescence. Moreover curcumin-induced senescence was accompanied by autophagy. Using electron microscopy analysis, imunocytochemical detection of the LC3 protein as well as vital staining of acid lizosomes we were able to directly visualize authophagic cells after curcumin treatment. Since autophagy can act as a prosurvival mechanism that protects stressed cells from cell death, we verified the role of autophagy in the process of curcumin –induced senescence. Inhibition of autophagy in curcumin-treated cells via downregulation of the *ATG5* gene led to decreased number of senescent cells, while cell viability remained unaffected. Thus, autophagy observed in our experimental model seemed to a play role in the establishment of cancer cell senescence induced by curcumin treatment.

O7.

CAN CANCER CELLS AGE? STATIONARY CELL CULTURE APPROACH TO THE PROBLEM SOLUTION

Alexander N. Khokhlov

Evolutionary Cytogerontology Sector, School of Biology, Moscow State University, Moscow, Russia

e-mail: khokhlov@mail.bio.msu.ru

There is a viewpoint that suppression of the proliferative capacity of cells and impairment of the regeneration of tissues and organs in aging are a consequence of specially arisen during evolution mechanisms that reduce the risk of malignant transformation and, thus, protect against cancer. At the same time, senescent cells of the body begin to accumulate a variety of macromolecular defects, which, conversely, increase the likelihood of their transformation into cancer cells. Thus, according to the mentioned viewpoint, the restriction of cell proliferation is a double-edged sword, which, on the one hand, reduces the likelihood of developing cancer at an early age, but on the other hand - limits the life span due to accumulation of "damaged" cells in old age. However, it remains unclear why normal human cells in vitro, with none of the mentioned "anticancer" barriers functioning at the organismal level only, never undergo spontaneous malignant transformation. In addition, it is also unclear how the freshwater hydra which, under certain conditions, has really no postmitotic and senescent cells, escapes both aging and cancer and, at such conditions (excluding the need for sexual reproduction), can live almost indefinitely and have a great regenerative potential. We believe that aging is only a "byproduct" of the program of development, implementation of which in higher organisms suggests the need for the emergence of cell populations with very low or even zero proliferative activity, which determines the limited capacity of relevant organs and tissues to regenerate.

At the same time, it is the presence of highly differentiated cell populations, barely able or completely unable to reproduce (neurons, cardiomyocytes, hepatocytes), that ensures the normal functioning of the higher animals and humans. Our "stationary phase aging" model assumes that restriction of cell culture proliferation by contact inhibition should induce the same "age-related" cell changes as those in aging multicellular organism. And, indeed, we have found the following changes in the stationary cell cultures: single-strand DNA breaks, DNA-protein cross-links, and demethylated bases in DNA accumulate; the frequency of spontaneous sister chromatid exchanges increases; structural changes occur in cell nuclei; lesions arise in cell membranes; the ability of cells to respond to mitogens and form colonies decreases; the content of 8-oxo-2'-deoxyguanosine, known to be a good aging biomarker, increases. It is important that the model can be used with various types of cells including the transformed ones. Besides, our results indicate that, in most cases, the death of the "stationary phase aging" cells follows the Gompertz law i.e., they age in the classical sense of this word. However, it should be emphasized that it is very difficult to register the time of death of a cell. There are many tests to determine cell viability nowadays, but they often yield controversial results. In fact, it is quite common to see that a cell is alive according to one test and dead according to another. We suppose that cancer cells age when their proliferation is restricted but they do not stop proliferating because of their aging. Obviously, cell aging is not exhaustion of proliferative potential but accumulation of some "bad stuff". The transformed cells escape this by permanent dividing when the organism loses its ability to control the cells' proliferative status.

O8.

REVERSIBLE SENESCENCE OF CANCER CELLS INDUCED BY DNA DAMAGE

Halina Waś, Grażyna Mosieniak, Małgorzata Śliwińska, Aneta Babik, Anna Strzeszewska, Ewa Sikora

hwas@nencki.gov.pl

Laboratory of the Molecular Basis of Ageing, Nencki Institute of Experimental Biology,

Polish Academy of Sciences, Pasteura 3, 02-093 Warsaw, Poland

A fundamental feature of senescent cells is an irreversible growth arrest. Importantly, cancer cells preserve capacity to senesce upon induced DNA damage or oncogene overexpression. This type of cellular senescence is telomere-independent, undergoes in a relatively short time and is named stress-induced premature senescence (SIPS). Several recent studies, including ourselves (Sliwinska et al, 2009), indicate that senescence of cancer cells is a transient process and can lead to tumor regrowth. Therefore, we asked the question about the mechanism(s), especially the role of p53/p21 signaling pathway, responsible for that phenomenon. To this end, we cultured $p53^{+/+}$ (p53 WT) and $p53^{-/-}$ (p53 KO) HCT116 cells in medium with doxorubicin or etoposide for one day and afterwards cultivated in a drug-fee medium. Both types of cells underwent senescence displaying changed morphology and granularity as well as SA-β-GAL activity and a high level of p21. Moreover, they revealed senescence-associated secretory phenotype *(*SASP*)*, producing vascular endothelial growth factor (VEGF) and interleukin 8 (Il-8). However, the majority of p53 KO underwent cell death. Of importance, colonies of small cells with a

high proliferative potential and increased genomic instability appeared in cultures of both p53 WT and KO cells. Cell cycle analysis revealed that cells treated with a DNA damaging agents underwent intensive polyploidization, which was followed by depolyploidization. In both cell lines many abnormal divisions were observed such as multipolar divisions, extra numeral centosomes, multinucleation, etc. Additionally, markers of stem cells and EMT (Epitelial to Mesenchymal Transition) were expressed on cells undergoing the process. Finally, cells treated with doxorubicin for seven days and afterwards cultivated in a drug-fee medium have shown the similar way of behavior.

Altogether, we conclude that the cancer cells treated with a DNA damaging agents enter the state which can be described as SIPS, however some polyploid cells with transient features of stem cells can escape senescence by abnormal division leading to selected descendants.

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O9.

REPLICATIVE AND DOXORUBICIN-INDUCED PREMATURE SENESCENCE OF HUMAN VASCULAR SMOOTH MUSCLE CELLS. DIFFERENCES AND SIMILARITIES OF BOTH TYPES OF SENESCENCE

Anna Bielak-Żmijewska[1], Wioleta Grabowska[1], Dorota Przybylska[1], Zbigniew Korwek[1], Olga Alster[1], Anna Ćmoch[2], Sławomir Pikuła[2], Grażyna Mosieniak[1], Ewa Sikora[1]

Laboratories of the Molecular Bases of Ageing[1] and Biochemistry of Lipids [2], Department of Biochemistry, Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

e-mail: a.bielak@nencki.gov.pl

Failure of the cardiovascular system with age and appearance of cardiovascular diseases is partially a consequence of the senescence of cells building the vasculature, e.g., endothelial (ECs) and smooth muscle cells (VSMCs). Two types of cellular senescence were described so far: telomere shortening-dependent replicative senescence (RS) and telomere-shortening-independent, stress-induced premature senescence (SIPS) which occurs much faster than RS. The main hallmark of both RS and SIPS is cessation of proliferation. Some hallmarks of cellular senescence in VSMCs have been already reported, but the molecular mechanisms involved in RS and SIPS of these cells remain so far elusive. It is believed that the main reason of both RS and SIPS could be DNA damage of telomeric regions (shortening in RS and injury in SIPS), followed by DNA damage response (DDR). Despite some common feautures also some differences in signatures of these two types of senescence can be expected. We studied

the senescence markers in *in vitro* growing VSMCs. For SIPS induction we treated cells with doxorubicin (dox), which is a commonly used anticancer agent known to elicit side effects mainly in the cardiovascular system. Dox-treated VSMCs accumulated mainly in the G2/M phase of the cell cycle in contrast to cells undergoing replicative senescence that stopped proliferation after 15-19 passages and accumulated mainly in the G1 phase of the cell cycle. Moreover, in both types of senescence the activity of SA-β-GAL was observed as well as the Senescence Associated Secretory Phenotype (SASP). DNA damage markers were detected in RS and SIPS (p-ATM (ser 1981), γ-H2AX (ser 139) and 53BP1), but the spectacular activation of DDR (p53 and p21) was visible only during SIPS. VSMCs have the so called Cell-type Exclusive Senescent Phenotype (CESP) which is related, among others, to the mineralization process and one of the markers of calcification is activation of alkaline phosphatase. We observed a significant increase of the enzyme activity in cells undergoing RS but not SIPS. Summarizing, we showed for the first time that doxorubicin can induce SIPS in VSMCs and we found some differences between signatures present during RS and SIPS.

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O10.

ATHERMAL AND THERMAL EFFECTS DURING WATER-FILTERED INFRARED A-IRRADIATION - A PROBLEM OF EXPERIMENTAL SETUP

Tobias Jung and Tilman Grune

Institute of Nutrition, Friedrich Schiller University Jena, Jena, Germany

e-mail: tobias.jung@uni-jena.de

Since the water-filtered infrared A (wIRA) irradiation has become a widespread application in medicine, therapy, cosmetics, and even wellness, the biological effects of that spectrum (780 to 1400 nm) are now a focus of experimental research.

In recent publications, direct effects of wIRA were suggested and compared to effects that are induced by UVA/B-irradiation. Some of those effects detected were an induction of the metalloproteinases MMP-1, -2, -3, -9 and -13, of TGF-beta1, activation of the MAPK-pathways was observed, release of cytochrome c and Smac/DIABLO from mitochondria. Furthermore an induction of Bax translocation from cytosol to nucleus, increase of HSP27 and -70, a general increase of ROS formation, decrease in cellular carotenoid concentration and an induction of trypase and p53.

The results of a chronic wIRA-exposure were attributed even to include photooxidation of proteins and long-term effects like premature skin aging, as found after chronic UVA/B-irradiation.

There are currently two main effects discussed: Thermal effects, that are just induced by wIRA-absorption of both the cellular water content

and the aqueous medium surrounding the irradiated sample, as well as supposed athermal wIRA-effects, that may result from a direct interaction of wIRA and cellular structures (except water).

In my lecture I will discuss and compare different experimental setups that are used and present some cellular responses to thermal and athermal wIRA effects, as well as the experimental problems we have to keep in mind if we want to distinguish between them.

O11.

TP53 COUPLES SENESCENCE TO SELF-RENEWAL IN THE RESPONSE TO ETOPOSIDE IN PA1 EMBRYONAL CARCINOMA CELLS

Thomas R Jackson[1], Kristine Salmina[2], Anda Huna[2], Inna Inashkina[2], Eriks Jankevics[2], Paul Townsend[1#], Mark S Cragg[1#], Jekaterina Erenpreisa[2#]

[1] Cancer Sciences Unit, Southampton University Faculty of Medicine, General Hospital, Southampton, SO16 6YD, UK; [2] Latvian Biomedicine Research and Study Centre, Riga, Latvia

Recent studies have highlighted an apparently paradoxical link between self-renewal and senescence triggered by DNA damage in certain cell types. In addition, the finding that TP53 can suppress senescence has caused a re-evaluation of its functional role in regulating these outcomes. To investigate these phenomena and their relationship to pluripotency and senescence we examined the response of the TP53 competent embryonal carcinoma (EC) cell-line, PA1, to etoposide-induced DNA damage. Nuclear POU5F1/OCT4A and P21CIP1 were upregulated in the same cells following etoposide-induced G2M arrest. However, while accumulating in the karyosol, the amount of OCT4A was reduced in the chromatin fraction. Upon release from G2M arrest, cells with repaired DNA entered mitoses, while the cells with persisting DNA damage remained at this checkpoint or underwent mitotic slippage and gradually senesced. Reduction of TP53 using sh- or si-RNA prevented the upregulation of OCT4A and P21CIP1. Subsequently, as confirmed by up-regulation of CDKN2A/P16INK4A and increased sa-□-galactosidase-positivity senescence was also enhanced after

TP53 reduction. TP53 silencing caused mitoses that were shown to be multicentrosomal and multi-polar, containing fragmented and deranged chromosomes, indicating a loss of genome integrity. Together, these data suggest that TP53-dependent coupling of self-renewal and senescence pathways through the DNA damage checkpoint provides a mechanism for how senescence safeguards genome stability and the fidelity of self-renewal in embryonal stem cell-like EC cells.

Posters

P1.

SENESCENCE-PROMOTING ACTIVITY OF SYNTHETIC RESVERATROL ANALOGUE, 3,3',4,4',5,5'-HEXAHYDROXY-*TRANS*-STILBENE

Justyna Mikuła-Pietrasik, Krzysztof Książek

Poznań University of Medical Sciences, Department of Pathophysiology, Poznań, Poland

e-mail: jmikum20@ump.edu.pl

3,3',4,4',5,5'-hexahydroxy-*trans*-stilbene (3,3',4,4',5,5'-HHS) is a synthetic analogue of resveratrol (RVT), highly appreciated for its anti-cancerogenic activity. Moreover, it has been suggested that several biological properties of this stilbene, e.g. its antioxidative capacity, are much more pronounced than in RVT. Because we have recently observed that RVT delays senescence in human peritoneal mesothelial cells (HPMCs) *in vitro*, in this project we wanted to find out of whether 3,3',4,4',5,5'-HHS may act on HPMC proliferation stronger than its natural precursor. To this end, we examined a wide range of parameters associated with cell growth and senescence in primary cultures of omental HPMCs derived from different donors (n=8-12), simultaneously exposed to RVT and 3,3',4,4',5,5'-HHS at 0.5 and 10 μM.

The results showed that 0.5 μM 3,3',4,4',5,5'-HHS, in contrast to RVT, did not improve replicative lifespan of HPMCs. At the same time, 10 μM of stilbene dramatically reduced cell growth potential (decreased PCNA level) and prematurely induced cell growth arrest. This was accompanied by increased activity of senescence-associated β-

galactosidase (SA-β-Gal) and enhanced oxidative DNA damage (8-OH-dG). These negative changes in cells exposed to 3,3',4,4',5,5'-HHS markedly differed activity of this stilbene from the positive (or at least neutral) effects exerted by RVT. The senescence-promoting activity of 3,3',4,4',5,5'-HHS could be related to considerable induction of ROS release by early-passage cells which was followed, in contrast to RVT, by decreased activity of superoxide dismutase (SOD). Last but not least, 10 μM 3,3',4,4',5,5'-HHS increased the percentage of apoptotic cells in late-passage cultures which could also play a role in declined reproducibility of HPMCs *in vitro*.

Altogether, our results imply that the pro-senescence effect of 3,3',4,4',5,5'-HHS —taking into account a well known phenomenon of cancer-promoting activity of senescent cells (including HPMCs)—may strongly jeopardize its clinical usefulness, even despite its anti-proliferative and pro-apoptotic activity towards different kinds of cancer cells.

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P2.

CORRELATION BETWEEN THE STAGE OF FIBROSIS OF THE ARTERY AND A PATIENT'S AGE

Aleksandra Kuzan[1,2], Agnieszka Chwiłkowska[2], Magdalena Kobielarz[1,3], Celina Pezowicz[1,3], Andrzej Gamian[2]

[1]Regional Specialized Hospital in Wroclaw, Research and Development Centre, Wroclaw, Poland;

[2]Department of Medical Biochemistry, Wroclaw Medical University, Poland

[3]Division of Biomedical Engineering and Experimental Mechanics, Faculty of Mechanical Engineering, Wroclaw University of Technology, Poland

e-mail: aleksandra.kuzan@gmail.com

The aging of the body is associated with numerous biochemical changes, which affect the function of the whole body. One of them is alteration in the metabolism of collagen, which results in loss of elasticity of skin, the arteries and other structures composed primarily of connective tissue. It is reported that collagen fibers are exposed as a result of inflammation occurring during atherosclerosis. These fibers are then bound to the platelets and are more susceptible to non- enzymatic glycosylation.

The research material consisted of fragments of 53 arteries of the deceased sudden death. It was used immunoenzymatic ELISA and immunohistochemical method to investigate the amount of collagen type I, III and IV and it's localization in arterial wall.

In the arteries of the elderly the accumulation of the collagen, large areas of fibrosis in intima and even in media often can be seen. The largest correlation was observed between content of collagen type I and age in women ($r=0,49$, $P \leq 0.05$). Interestingly, for men the same parameter was

significantly less: r=0,33 ($P \leq 0.05$). The correlations between the content of collagen type III and IV and age are statistically insignificantly.

Fibrosis artery is not quite directly proportional to the age, but there is a fairly high correlation between the amount of collagen in the arteries and the age of women. The fact that this association is not so strong in case of men can be interpreted as meaning that a greater number of factors, for example the lipid compounds may be involved in pathological changes in the arteries of men during aging.

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P3.

ANTIPROLIFERATIVE EFFECT OF MELATONIN'S DERIVATIVES ON BREAST CANCER CELL LINE MCF-7

Mikołaj Ogrodnik[1], Magdalena Dudkowska[1], Anna Zawadzka[2], Zbigniew Czarnocki[2], Ewa Sikora[1]

[1] Laboratory of the Molecular Bases of Aging, Nencki Institute of Experimental Biology, Polish Academy of Science, 3 Pasteur Str., 02-093 Warsaw, Poland

[2] Laboratory of Natural Products Chemistry, The Faculty of Chemistry, The University of Warsaw, 1Pasteur Str., 02-093 Warsaw, Poland

Melatonin is present in almost all species. In mammals this hormone is produced mainly by the pineal gland. Melatonin plays an important role in a lot of physiological and pathological processes such as the circadian rhythm regulation, antioxidant function or neoplasmatic growth. There is a lot of data showing its antiproliferative activity in a variety of tumors both in vitro and in vivo. Accordingly, the aim of this study was to answer the question whether synthetic derivatives of melatonin posses an antiproliferative activity in breast cancer cells. It is known that many anticancer drugs not only induce cell death, but also cellular senescence of cancer cells. Thus, we were interested if we could observe such a phenomena in MCF-7 cells treated by melatonin's derivatives. Four melatonin's derivatives, originally synthesized as acetylcholine- and butyrylcholinesterase inhibitors, were tested. All of them were cytostatic or/and cytotoxic in a dose dependent manner. Two of the most powerful inhibitors had the highest antiproliferative effect on MCF-7 cells. It was shown as the decrease of viability measured by the MTT assay and an increase of cell death estimated on the basis of annexin V/ 7 AAD staining measured by flow cytometry. Next the cells were

treated with one of the derivatives for one day and afterwards cultured up to nine days in the agent-free medium to reveal whether they are able to undergo senescence. Indeed, cells enlarged at their size and what was accompanied with an increase of SA-β-galactosidase (Senescence Associated β-galactosidase) activity. Moreover, the treatment led to the accumulation of DNA damage shown by immunocytochemical staining for 53BP1, a marker of double strand breaks. These results suggest that melatonin's derivatives might posses a pro-senescence activity in the MCF-7 breast cancer cells.

P4.

IS HSPA2 A MARKER OF EPIDERMAL UNDIFFERENTIATED KERATINOCYTES?

Anna Habryka[1,2], Monika Byrska[1,2] Agnieszka Gogler – Pigłowska[1], Mariusz Kryj[3], Zdzisław Krawczyk[1,2] , Dorota Ścieglińska[1]

[1] Center for Translational Research and Molecular Biology of Cancer, 3 Oncologic Surgery Clinic, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland;

[2] Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, Poland;

This study was designed to characterise the expression of HSPA2 in human epidermis and during process of HaCaT cells differentiation. HSPA2 was originally described as testis-specific member of the HSPA (HSP70) heat shock protein family, which is crucial for spermatogenesis. However recently we have shown that HSPA2 protein is synthesized in human normal somatic tissues in cell- and tissue-type specific manner. High level of HSPA2 protein was found in basal layer of epidermis and other stratified epithelia. However, possible function of HSPA2 and mechanisms regulating its expression in epidermis are unknown.

In this study, by immunofluorescence analysis we demonstrated that in human epidermis HSPA2 positive cells colocalize with cells expressing markers specific for undifferentiated keratinocytes (CK5 and CK14). Therefore, we assumed that HSPA2 expression can be regulated during keratinocytes' differentiation. We applied the model of calcium-induced differentiation of HaCaT cells to search for realtion between the level of HSPA2 potein and kertinocytes differentiation. HaCaT cells grown in low calcium medium (LCM) showed marker signature specific for

undifferentiated (basal) keratinocytes and reduced proliferation rate. Opossitely, HaCaT cells cultured in high-calcium medium (HCM) showed upregulation of differentiation markers and elevated proliferation rate. The level of HSPA2 transcripts remained stable in cells grown uder HCM, whereas its significant upregulation was detected under LC conditions. Results of HSPA2 protein level analysis partially overlaps with RT-PCR data. In LCM clear, immediate and persistent increase of the HSPA2 protein level was found. In summary our results strongly suggests that HSPA2 gene can be active in undifferentiated HaCaT cell (possibly in epidermal progenitor and/or stem cells).

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P5.

BRAIN-SITE-SPECIFIC PROTEOME CHANGES IN AGING AND AGING-RELATED DEMENTIA BRAINS

Klaus Heese[1], Arulmani Manavalan[2], Manisha Mishra[2], Lin Feng[3], Hiroyasu Akatsu[4]

[1] Department of Biomedical Engineering, College of Engineering, Hanyang University, 222 Wangsimni-ro, Seongdong-gu, Seoul 133-791, Korea

[2] School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore

[3] Department of Obstetrics and Gynecology, University of California Irvine, Irvine, CA 92697, USA

[4] Choju Medical Institute, Fukushimura Hospital, 19-14 Aza-Yamanaka, Noyori, Toyohashi, Aichi 441-8124, Japan

e-mail: klaus@hanyang.ac.kr

To improve our understanding of the contribution of cellular senescence to human brain aging and brain aging-related diseases, such as dementia, it is essential to define an unique brain proteomic signature of cellular senescence that is functionally connected with normal and pathological aging. Our current study aimed at gaining a proteomic signature of the aging brain. In dementia, such as Alzheimer's disease (AD), neuronal death begins in the entorhinal cortex and then gradually spreads to the entire hippocampus and the cortex except the occipital lobe. Besides, the cerebellum is barely affected in AD. Isobaric tags for relative and absolute quantitation (iTRAQ) is an improved proteomic technique which we applied in our brain-site-specific proteome analysis in quest of identifying the alternations of key signaling proteins in the hippocampus, parietal cortex and the cerebellum of aged and aging-related demented

brains. We will discuss our novel findings that could provide potential biomarkers with a strong impact on the diagnosis, staging and prediction of aging-related cellular processes in the brain.

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P6.

ANTIOXIDANT AND PROTECTIVE ACTIVITY OF POMEGRANATE (*PUNICA GRANATUM*) PEEL EXTRACT IN REDOX HOMEOSTASIS DISORDERS.

Szymon Sekowski[1*], Izabela Zukowska[1], Ilya B. Zavodnik[2], Elena Lapshina[2], Saidmukhtar Mavlyanov[3], Maria Zamaraeva[1]

[1] Department of Biophysics, University of Bialystok, Poland

[2] Department of Biochemistry, University of Grodno, Belarus

[3] Institute of Bioorganic Chemistry, Academy of Science, Uzbekistan

*e-mail: s.sekowski@uwb.edu.pl ; sekowski100@vp.pl

The result of cells metabolism and respiration is producing of reactive oxygen and nitrogen species (RONS). Under physiological conditions cells possess different preservation systems (enzymatic and non-enzymatic) that protect them from negative influence of RONS. However many pathological states and process of ageing are accompanied by intensive RONS accumulation. Under these pathological conditions the natural antioxidant defence systems are deregulated and as the consequence disorders of cellular redox homeostasis are developing. In this context natural antioxidants are intensely studied with prospects of their application as prophylactic and curative agents. Tannins, polyphenoles with the molecular mass ranging between 500 and 3000 Da, belong to such compounds. They are classified on the basis of their structure into two groups: the hydrolysable and the condensed tannins.

In this research antiradical activity and protective effects of *Punica granatum* (PG) peel extract, containing hydrolysable tannins, against oxidative stress in human red blood cells (hRBCs) has been studied. The

results showed that PG possesses strong abilities to reduce DPPH radicals (6.25 μg / ml reduce DPPH in about 80%). PG extract was also very effective in inhibition of production of such free radicals like superoxide anion, hydroxyl and nitric oxide radicals and its effect is comparable with Trolox one. We also shown that PG markedly decreased haemolysis and inhibited oxidizing of GSH in hRBCs induced HOCl.

Presented results clearly show that pomegranate tannins has a strong antioxidant activity against different free radicals and can act as red blood cells protectors under redox homeostasis disorder conditions. Peel of pomegranate fruit as a by-product in juice production could be a cheap source of highly effective antioxidants.

P7.

EFFECT OF THE FRACTIONS EXTRACTED FROM THE WINE ON ERYTHROCYTES OXIDATIVE DAMAGE.

Joanna Sajkowska[1,2], João Fernandes[2]

[1] Department of Biochemistry and Clinical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Poland

[2] Department of Biochemistry, Faculty of Pharmacy, University of Porto, Portugal

e-mail: jsajkowska@gmail.com

Oxidative stress plays a major role in the pathogenesis of ischemic heart disease, atherogenesis, Parkinson's, several types of cancer and other chronic diseases, as well as, in the aging process. Consumption of red wine has been reported to decrease risk of atherogenesis and cancer. This has been ascribed in part to antioxidants in red wine inactivating reactive oxygen species (ROS) involved in the initiation or progression of these diseases. In order to further elucidate the antioxidant properties of the red wine in human cells and to identify the molecular mechanisms responsible for its cytoprotective effect, human red blood cells (RBC) were selected as a metabolically simplified model system. RBCs are particularly exposed to endogenous and exogenous oxidative damage. Healthy subjects are equipped with efficient RBC antioxidants, namely glutathione, tocopherol and ascorbate. When ROS are overproduced or the antioxidant defences are impaired, the oxidative stress develops, inducing oxidative damage in RBC membrane, and hemolysis.

To evaluate the antioxidant effects of different components of red wine – carbohydrates, total polyphenols (anthocyanidins and procyanidins fractions were also tested) and polymer content. The antioxidant effects of

the five red wine fractions were assessed by the capacity of each fraction to protect RBCs from oxidative injury induced by hydrogen peroxide and 2,2'-Azobis(2-methylpropionamidine)dihydrochloride (AAPH). In addition, the ability to prevent hemoglobin oxidation was also determined by measuring o*xy-hemoglobin* and *met*-hemoglobin peaks by *spectrophotometry*.

Our results suggest that all tested red wine fractions possess antioxidant effects, since all reduced the hemolysis rate by inhibiting H_2O_2- and AAPH-radicals. However, this capacity showed to vary between fractions and also depended on the concentration level: carbohydrates fraction showed no more than 20% protection rate (the lowest among all of the fractions), while total polyphenols, at 0.50 mg/mL, inhibited 70% the hemolysis caused by AAPH. Among the polyphenol, procyanidins presented higher antioxidant capacity than anthocyanidins against both oxidative agents. In conclusion, all red wine constituents we tested seem to contribute to its claimed antioxidant properties. The polyphenols, mainly the procyanidins seem to be the most responsible for this protective effect.

P8.

APOPTOTIC RESPONSE DISTINGUISHES LYMPHOCYTES FROM FAMILIAR AND SPORADIC ALZHEIMER'S DISEASE PATIENTS

Katarzyna Sawicka[1], Emilia Białopiotrowicz[1], Jacek Kuźnicki[1], Urszula Wojda[1,2]

[1] International Institute of Molecular and Cell Biology, Warsaw, Poland

[2] E-mail: ulawojda@iimcb.gov.pl

Alzheimer's disease (AD) is the most common age-related dementia characterized by complex alterations in cellular processes including changes in the molecular mechanisms regulating cell cycle (CC) and apoptosis. In AD some molecular changes occur not only in neurons, but also in blood cells such as lymphocytes. Recently we have demonstrated disturbances in the CC regulation both in AD neurons and in B-lymphocytes. Moreover, we have found that the CC regulation is different in sporadic (SAD) and familiar (FAD) form of AD. The aim of this study was to compare apoptotic response to oxidative stress in B-lymphocytes from healthy individuals and from FAD and SAD patients. In this study we analyzed B-lymphocytes immortalized with Epstein-Barr virus from patients with SAD and FAD in comparison to immortalized B cells from two age-matched control groups composed of nondemented subjects (S-CTR and F-CTR). We investigated response of lymphocytes to oxidative stress evoked by treatment with reducing sugar 2-deoxy-D-ribose (2dRib) known to lower the glutathione level in cells. Apoptosis was assessed using three different methods. We used flow cytometry and Annexin V-FITC/PI staining to distinguish early apoptotic cells from dead

and live cells, and cationic dye JC-1 staining to investigate the role of mitochondria in response to stress by measuring changes in mitochondrial membrane potential (MMP). We also used MMT assay to check survival rate of cells in different 2dRib concentrations. 24h after 40mM 2dRib treatment, MTT assay showed the highest survival rate of FAD lymphocytes comparing to CTR and SAD cells. Accordingly, after 2dRib treatment FAD lymphocytes have significantly less Annexin V positive cells and showed significantly higher MMP than SAD lymphocytes. In all the assays the results for SAD lymphocytes were not significantly different than for control cells. Moreover, under basal conditions without 2dRib stimulation, each of the assays showed no significant changes between cells from all four groups. These data suggest that FAD cells are more resistant to apoptosis induced by oxidative stress than SAD or control cells. Thus, our data support the notion that molecular mechanisms underlying FAD pathology are different than in SAD cells. Moreover, our data support understanding AD as a systemic disorder and usefulness of human lymphocytes in studies on AD pathogenesis and diagnostics.

P9.

THE USE OF FLOW CYTOMETRY TO STUDY THE CHANGES IN THE MEMBRANES OF Γ-IRRADIATED ERYTHROCYTES FOR TRANSFUSION

Adam Antosik, Magdalena Szejk, Halina M. Zbikowska

Department of General Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143, 90-236 Lodz, Poland

e-mail: adama@biol.uni.lodz.pl

Irradiation of blood and blood components is currently the only accepted methodology to prevent transfusion associated graft-versus host disease (TA-GvHD). It is routinely accomplished by using γ-rays (or X-rays) to inactivate T cells. During storage of red blood cells a loss of enzymatic systems and shortage of various chemical compounds involved in metabolism as well as progressive oxidative processes occur. Ionizing radiation enhances these effects which lead to imbalance and finally a damage of the cell membrane.

The aim of the study was to determine the effect of γ-irradiation and long time storage of human red blood cells on changes in their membranes by using flow cytometry. Erythrocytes were separated from blood, in CPD (citrate-phosphate-dextrose) anticoagulant solution, obtained from the Regional Center for Transfusion Medicine in Lodz. Erythrocyte concentrates with hematocrit of 75% were irradiated (50 Gy) and stored for 20 days in plastic tubes at 4^{o} C. The measurements of phosphatidyloserine externalization with annexin V (FITC) were performed immediately after receiving the concentrates (control), then after 1, 10 and 20 days of storage. At the same time data was collected regarding the cell size (FSC parameter)

and shape (SSC). Additionally microscopic preparations were prepared to evaluate morphology of the stored erythrocytes.

We showed a statistically significant increase in phosphatidylserine exposure both after 10 (by 13%) and after 20 days (39%) of erythrocyte storage. Duration of storage caused significant changes in FSC and SSC parameters. The FSC parameter was noticeable increased relative to the control only after 20 days of storage (15%). The SSC parameter changes were observed already after 1 day of storage (12%) and it increased with time (18% at day 10 and 22% at day 20). The microscopic analysis showed an increase in red blood cell diameter from 7.4 μm (control) to 8.7 μm (20 day) and progressive deformations of the cell membrane.

The results indicate the usefulness of cytometric cell analysis to evaluate the quality of irradiated and stored red blood cell concentrates.

P10.

FISH STUDY OF THE CHROMATIN DIMINUTION IN ETOPOSIDE-TREATED TERATOCARCINOMA PA1 CELL LINE

Kristine Salmina[1], Harry Scherthan[2], Anda Huna[1], Jekaterina Erenpreisa[1]

[1] Latvian Biomedical Research&Study Centre, Riga, Latvia; [2] Bundeswer Institute of Radiation Biology affil. to the University of Ulm, Munich, Germany

DNA and spindle damage of human tumour cell lines can induce different forms of chromatin macroautophagy, a process that is poorly understood and may be associated with stabilisation of the genome (Rello-Varona et al., Cell Cycle 2012; Erenpreisa et al., Autophagy, 2012). Here, we report on the exclusion of γH2AX/DAPI-positive chromatin into perinuclear LC3B-labelled autophagic vacuoles in Etoposide treated PA1 embryonal carcinoma cells. Strong fluorescence of $TTAGGG_n$ telomere and pericentromere 2 as well as TERT specific signals indicated that the degrading chromatin of autophagic vacuoles is enriched with telomerase and telomeric sequences. The frequency of the autophagic chromatin vacuoles with strong telomere FISH signals increased from 0.3% in controls to 7-8% in ETO-treated PA1 cells. This feature was characteristic for the G2 arrested cells and for tetraploid cells in the culture. Enumeration and visualization of telomere FISH signal spots indicates that the cells that extrude chromatin in autophagic vacuoles eliminate numerous telomere repeat clusters. Some telomere signal spots appeared enlarged or as doublets, the latter indicating G2 arrest. Strong TERT fluorescence was contained in the chromatin budded into autophagosomes, while it was reduced in the corresponding mother nuclei. These observations may be

interpreted as elimination of the ETO-damaged chromatin that has received excessive telomeric sequences by telomerase action on ETO-induced DNA double strand-breaks (DSBs). Extensive telomere repeat addition to ETO-induced DSBs may trigger ALT-like recombinogenic interactions that can create extrachromosomal telomere DNA circles which, together with irreparably damaged DNA, may be extruded from the nucleus. In support, nuclear extrusion of circular DNAs has been previously described in senescing mammalian cells (Kunisada et al., Mech Ageing Dev 1985).

P11.

THE EXPRESSION PATTERNS OF THE OPPOSITE REGULATORS OCT4A AND P21CIP1 IN THE ETOPOSIDE-TREATED EMBRYONAL CARCINOMA CELLS

Anda Huna[1], Kristine Salmina[1], Thomas R Jackson[2], Pawel Zayakin[1], Sui Huang[3], Jekaterina Erenpreisa[1]

[1] Latvian Biomedical Research& Studies Centre, Riga, Latvia;

[2] Cancer Sciences Unit, General Hospital, Southampton, SO16 6YD, UK,

[3] Institute for Systems Biology, Seattle WA, USA.

The cells of human embryonal carcinoma PA1 treated with Etoposide undergo 2-3 day long full G2 arrest, death and senescence, however a small proportion of cells recovers clonogenic growth. We found that in the G2-arrest stage most cells simultaneously accumulate in their nuclei the opposite regulators – self-renewal master trascription factor OCT4A and inhibitor of cell cycle and mediator of senescence P21CIP1, the both depend on activation by DNA damage of TP53 (Jackson et al., submitted). Here we report on the preliminary studies of the character of this dual expression in the time course as detected by the two-channel in situ fluorescence measurements using fluorescent microscope, videocamera, and Image Pro Plus 4.1 software. In non-treated cells, the two-dimentional distribution of the intensity of OCT4A and background p21CIP1 staining in individual cells forms rather a compact cluster, while in the treated cells the staining is much enhanced (confirmed by Western blotting) and the variability amplitude increases. Using the hypothesis of ergodicity, we suggest that this pattern reflects the temporal fluctuations of the steady state in individual cells characterising it as bi-potential and

metastable. The bi-potential state probably keeps the both networks alert, however preventing for the time being each other from reaching their destination targets. The ”swing” of the regulators’ levels increases in the time course, with the opposing outliers appearing upon the time of release from the G2-arrest (monitored by the DNA histograms), followed by death of the majority and further dissociation of the remaining minority for proliferating and terminally senescing cells (which die by necrosis). The analysis suggests that the stronger coupling (positive correlation) of the OCT4A and p21CIP1 levels is achieved initially in the DNA damage-induced G2-arrest, the higher chromosome/centrosome stability of the releasing proliferative survivors (as judged adversely by metaphase/anaphase ratio) is obtained. Further studies on the patterns and significance of this temporal collaboration of self-renewal and senescence networks for cell fates decisions are on route.

P12.

SENESCENCE OF DNA-CONTAINING ORGANELLES DURING PROLONGATED CULTIVATION IN EXTRACELLULAR ENVIRONMENT

Tūrs Selga, Daiga Krieviņa

University of Latvia, Faculty of Biology, Kronvalda blv. 4. Rīga LV-1586, Latvia

e-mail: turs.selga@lu.lv

It has been shown that cells of different plant species form plastid-nucleus complexes (PNCs) as permanent structural complexes. Each complex includes nucleus, several plastids, endoplasmic reticulum (ER) and some mitochondria and peroxisomes attached to chloroplasts or ER. PNCs can be separated from plant leaf cells and cultivated in extracellular environment (Selga et al. 2010). Aim of the present work was visualization of activity and senescence of DNA containing organelles of these complexes.

PNCs were mechanically crushed from *Nicotiana tabaccum L.* plant leaves. Isolated PNCs were cultivated in Murishage and Skoog medium with Gamborg vitamins and 2% mannitol in different light conditions during six months. Functional activity was analyzed on the basis of light induced formation of ROS (detected by 2'.7'-dichlorodihydrofluorescein diacetate (H2DCFDA)), GFP fluorescence in endomembranes, DNA and chlorophyll fluorescence.

Senescence of nuclei appeared as fast decrease of size and amount of DNA during 12 days of cultivation. At the same time mitochondria increased their volume, formed clusters and contained high amount of

DNA. Similar mitochondria were observed also after 3 months of cultivation. Cultivation of PNCs in high illumination (1 W/m^2) caused sharp decrease of, chlorophyll fluorescence after 12 days of cultivation in comparison with cultivation in low illumination (0.5 W/m^2). We observed fluorescence of chlorophyll also after 3 and 6 months of cultivation with low illumination, but the number of fluorescing chloroplasts decreased. To visualize functional activity of chloroplasts and mitochondria after 3 and 6 months of cultivation with low illumination we activated photosynthesis and ROS synthesis with 1 h long high illumination. Chloroplasts and mitochondria remained their activity in extracellular environment for 6 months. High illumination caused increase of GFP production during 10 days of cultivation. Low illuminated caused gradual decrease of GFP production after first 4 days of cultivation.

Selga T., Selga M., Gobiņš V., Ozoliņa A. 2010. Plastid-nuclear complexes: permanent structures in photosynthesizing tissues of vascular plants. - Environmental and Experimental Biology, 8:(1/4): 85 – 92.

P13.

ULTRASTRUCTURAL CHANGES INDUCED IN HUMAN COLO 205 COLON ADENOCARCINOMA CANCER CELLS BY CERTAIN CALCIUM HOMEOSTASIS MODULATORS LEAD TO SENESCENCE AND SUBSEQUENT CELL DEATH

[1]Kania E., [1]Pajak B., [1,2]Orzechowski A.

[1] Electron Microscopy Platform, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Pawinskiego 5, 02-106, Poland

[2] Department of Physiological Sciences, Faculty of Veterinary Medicine,Warsaw University of Life Sciences (SGGW), Warsaw, Nowoursynowska 159, 02-776, Poland

e-mail: elakania@gmail.com

In this study we investigated one or two-day effect of several calcium homeostasis regulators as calcium chelators (BAPTA-AM, EDTA, EGTA), calcium ionophore (ionomycin), and calcium channel/pump inhibitors (verapamil, thapsigargin, respectively) in COLO 205 cells. Proliferation and viability dropped upon verapamil (100-200 uM), BAPTA–AM (25-50 uM) and ionomycin (5-10 uM)treatment (6-48 h) as evaluated by CV and MTT assays, respectively. Depending on the agent used, the FACS analysis revealed that after 24 h percentage of apoptotic and necrotic cells has increased. The ultrastructural analysis with transmission electron microcopy (TEM, 24 h) showed some features of senescent cellular phenotype which included irregular and abnormally lobed nuclei, pleomorphic vacuolated mitochondria, decreased ER, and distorted Golgi apparatus. Additionally, we observed electron-dense regions in the nucleoplasm of cells treated with ionomycin (5-10 uM), BAPTA-AM, (25–50 uM) and verapamil (200 uM). Moreover higher

concentrations of these factors led to enlarged and distorted cisterns of trans-Golgi network and RER and pleomorphic mitochondria. We also observed hallmarks of autophagy such as autophagic vacuoles, increased numbers of lysosomes and endosomes and multilamellar bodies (myelin figures) in cells treated with BAPTA-AM (10–50 uM) and verapamil (100–200 uM). Finally, some of the verapamil-treated cells showed also necrotic phenotype with disrupted cell membrane, karyolysis and karyorrhexis. Presumably, changes in calcium homeostasis lead to both impaired protein processing and aggregation of misfolded/unfolded proteins. Consequently, reductive stress (ER-stress) develops and unfolded protein response (UPR) mechanism might be activated. Finally, if the reductive stress is being prolonged it might trigger cell death. Overall, stress induced-premature senescence (SIPS) is apparently attributable to death from apoptosis or autophagy.

P14.

NUMBER OF APOPTOTIC AND LIFE BLASTODERMAL CELLS IN EMBRYO QUAILS AS A RESULT OF SELECTION

Sawicka Dorota[1,2], Samek Kamila[2], Chojnacka-Puchta Luiza[1], Witkowski Andrzej[3], Knaga Sebastian[3] and Bednarczyk Marek[2]

[1] Institute of Biotechnology and Antibiotics, Department of Bioengineering, Warsaw, Poland

[2] University of Technology and Life Sciences, Department of Animal Biotechnology, Bydgoszcz, Poland

[3] University of Life Sciences, Department of Biological Basis of Animal Production, Lublin, Poland

e-mail: sawickad@iba.waw.pl

Intensive genetic selection over many years has significantly improved the growth rate of broilers and has increased the number of eggs in laying chicken breeds. Eggs from chickens that underwent selection were characterized by a decrease in egg weight, shell coloration, reduced value of Haugh`a unit and an increased percentage of yolk and thickness of the shell. Moreover, chickens selected for growth were characterized by lowered fertility and egg defective syndrome. Furthermore, selection for growth has led to the fast growth of an embryo, and can influence on disturbances of homeostasis and even lead to death of an embryo. We hypothesized that undesired effects of selection can be observed in early embryo development (X stage according to Eyal-Giladi and Kochav 1976). Therefore, the aim of our study was to examine the effects of different selective pressure in three lines of quails: meat, laying and laying selected previously for high yolk cholesterol content on number and percentage of life and apoptotic blastodermal cells (BCs) in X stage of development of

quail embryos. The percentage of life and apoptotic BCs was statistically significantly different ($P \leq 0.01$) for embryos of meat quails (35.82 % and 64.24 %) and for laying quails embryos (65.96 % and 36.38 %), respectively. The number of apoptotic BCs from 3 blastodiscs for meat quails embryos (135 294.12) was statistically varied ($P \leq 0.01$) from laying quails embryos (80 000.00). Selection for high yolk cholesterol content has caused a statistically significant ($P \leq 0.01$) increase in the total number of BCs from 235 208.33 in laying line to 420 416.67 in selected line. The percentage of apoptotic BCs was statistically lower ($P \leq 0.01$) in selected line (17.13%) if compared with laying line (36.38%). Our results have shown that it is possible to evaluate effects of selection in the early stage of embryo development.

P15.

ASSESSEMENT OF AGE-DEPENDENT CHANGES IN THE YEAST *SACCHAROMYCES CEREVISIAE* USING MICROSCOPY TECHNIQUES

Renata Zadrąg-Tęcza[1], Magdalena Kwolek-Mirek[1], Tomasz Biliński[1]

[1] Department of Biochemistry and Cell Biology, University of Rzeszow, Rzeszow, Poland

e-mail: retecza@univ.rzeszow.pl

The yeast *Saccharomyces cerevisiae* has been widely accepted as a simple, eukaryotic model organism for the studies of aging process due to the assumption that basic mechanisms of aging are conserved among all eukaryotic organisms. Aging of the yeast *S. cerevisiae* is measured by the number of daughter cells produced by a mother cell. This is known as replicative lifespan, and is seen also for human cells, which can divide only a limited number of times. The phenomenon of limited cellular division is still not well understood because it is very difficult to directly observe the cellular and molecular events accompanying aging in single yeast cells using the routine procedure based on micromanipulation method. The most accepted view links this limit to the gradual accumulation of molecular damage or rDNA circles in the yeast mother cell. Our observations suggest however, that the existence of reproduction limit in the budding yeast cells may be a consequence of hypertrophy. One of the phenotypic changes that occur during replicative lifespan is the gradual increase of the yeast cell size, resulting from budding as the atypical cytokinesis mechanism.

We examined the size-dependent and age (*number of division*)-dependent morphological changes, accumulation of damaged proteins and metabolic status in the yeast cells of significantly differing in reproductive capacity. We take into consideration the parameters which may have influence on the reproductive potential. Our results show that the increase of the cell size *(without budding*) does not cause significant decrease metabolic cell activity or morphological changes of the cellular structure like mitochondria and actin cytoskeleton but it cause significant decrease of the reproductive potential. Based upon these results we suppose that the morphological changes have a small influence on the reproductive capacity however the up to 10-fold increase of the cellular volume during the lifespan may be leads to lowering the effective concentration of some important regulatory protein molecules and in this way makes impossible the entry of the hypertrophic cells to the next cell cycle.

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P16.

CAN DEREGULATION OF BASE EXCISION REPAIR BY LIPID PEROXIDATION PROMOTE PREMATURE AGING PHENOTYPE OF *ERCC1*$^{-/-}$ MICE?

Jolanta Czerwińska[1], Małgorzata Jeleniewicz[2], Małgorzata Nowak[2], Konrad Kosicki[2], Dominika Skolimowska[2], Laura Niedernhofer[3], Elżbieta Speina[1], Barbara Tudek[1,2]

[1] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

[2] Institute of Genetics and Biotechnology, Warsaw University, Warsaw, Poland

[3] University of Pittsburgh School of Medicine, Pittsburgh, USA

e-mail: jczerwinska@ibb.waw.pl

The ERCC1-XPF endonuclease is involved in nucleotide excision repair (NER) and processing of DNA interstrand crosslinks. Ercc1 knockout mice display retarded postnatal growth and premature aging. They also reveal increased level of reactive oxygen species and are hypersensitive to oxidative stress introducing factors. It was shown that functioning of base excision repair (BER) can be modulated by oxidative stress and lipid peroxidation (LPO) products. To investigate whether the phenotype of *Ercc1*$^{-/-}$ mice can partially result from BER dysfunctions, we investigated its activities in *Ercc1*$^{-/-}$, *Ercc1*$^{+/-}$ and wild type mice tissues (liver, brain, kidney) extracts. Our preliminary results show that DNA cleavage by most DNA glycosylases investigated and AP endonuclease as well as incorporation activities were unchanged in extracts of *Ercc1*$^{-/-}$ and *Ercc1*$^{+/-}$ mice compared to wt ones. However, *Ercc1*$^{-/-}$ mice revealed reduced ability of DNA ligation, especially marked in the liver.

Additionally, we investigated the sensitivity of *Ercc1*$^{-/-}$ and wt mouse embryonic fibroblasts to three lipid peroxidation end products: 4-hydroxy-2-nonenal (HNE), croton aldehyde (Cro) and acrolein (Acr). These reactive aldehydes form adducts to DNA and proteins as well as DNA-DNA and DNA-protein crosslinks. We show that *Ercc1*$^{-/-}$ cells are hypersensitive to HNE and Cro but not to Acr. Overall the results indicate that BER deregulation and LPO products may significantly contribute to the *Ercc1*$^{-/-}$ mice phenotype.

P17.

THE ROLE OF NIBRIN IN DNA DAMAGED INDUCED SENESCENCE

O.Alster[1], A. Bielak-Żmijewska[1], G. Mosieniak[1], Z.Korwek[1], J. K. Siwicki[2], M. Moreno-Villanueva[3], A. Burkle[3], A. Wojtala[1] and E. Sikora[1]

[1] Laboratory of the Molecular Bases of Ageing, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

[2] Department of Immunology, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

[3] University of Konstanz, Molecular Toxicology Group, Konstanz, Germany

e-mail: o.alster@nencki.gov.pl

It is commonly believed that senescence can be induced by treatment with DNA damaging agents, of which the consequence is the activation of the DNA damage response pathway. Besides the well known proteins, such as ATM and p53, nibrin plays an important role in this process. Nibrin is a 95kDa protein, which together with Mre11 and Rad50 forms a multisubunit complex (MRN) involved in the DNA damage response pathway. A mutation in the NBS1 gene causes Nijmegen Breakage Syndrome, a rare autosomal recessive disorder. Among it's characteristic features we can name: genomic instability, immunodeficiency, radiosensitivity and increased susceptibility to cancer development. The aim of my study was to elucidate the role of nibrin in the induction of senescence of spontaneously immortalized human NBS cells. In our experiments we used two NBS cell lines (S3R and S4) and L5-control cells (spontaneously immortalized splenocytes). We treated the

cells with a DNA damaging agent, doxorubicin for 24h and afterwards cultured them in a drug free medium. All of the cell lines were sensitive to doxorubicin treatment what was shown with 53BP1 staining and using the FADU technique. However the DNA damage response pathway was only activated in the S4 and L5 cell lines. Moreover we analyzed several markers of senescence. In the case of the S4 cell line a time-dependent increase in the level of p21 could be observed which was accompanied by the presence of SA-β-Gal positive cells. In the L5 and S3R cell lines a time-dependent decrease in the level of p21 could be found, which correlated with the induction of SA-β-Gal in the case of the L5 cell line. The obtained results encouraged us to further analyze the role of nibrin in the induction of senescence. Our experiments performed using human vascular smooth muscle cells show that the downregulation of nibrin in these cells does not affect their ability to undergo DNA damaged induced senescence, what was shown using the western blotting technique and SA-β-Gal staining. However the level of BrdU incorporation was higher in cells with the dowregulated. level of nibrin in comparison with cells transfected with si neg. Nibrin is not a prerequisite in the induction of senescence.

P18.

CURCUMIN-INDUCED SENESCENCE OF HUMAN VASCULAR SMOOTH MUSCLE CELLS. IS DNA DAMAGE RESPONSIBLE FOR THIS PROCESS?

Wioleta Grabowska, Dorota Przybylska, Ewa Sikora, Anna Bielak-Żmijewska

Laboratory of the Molecular Bases of Ageing, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

e-mail: w.grabowska@nencki.gov.pl

Curcumin is derived from the rhizome of *Curcuma longa* and has been used for millennia for treating a variety of diseases in traditional Indian and Chinese medicine. Recently, curcumin has attracted the attention of researchers and clinicians as an anti-inflammatory and anti-oxidant agent with a potential use in therapy of many diseases with an inflammation constituents, e.g. cancer, cardiovascular diseases, Alzheimer's disease, rheumatoid arthritis and metabolic syndrome. Although it is believed that curcumin is safe for the whole organism, *in vitro* studies have shown that it may be toxic in micromolar concentrations for both normal and cancer cells. Recently, in our laboratory it has been shown that curcumin can induce not only cell death, but also senescence of cancer cells.

The aim of this study was to answer the question whether primary cells can undergo senescence after curcumin treatment. To this end the vascular smooth muscle cells isolated from human aorta were used. First, our study revealed a high sensitivity of these cells to curcumin. Cells treated with 5 μM curcumin ceased to proliferate, while 15 μM curcumin

caused cell death. To induce cellular senescence we chose 5 μM curcumin, this concentration inhibited proliferation and did not induce cell death. Curcumin used in this concentration arrested cells in tha G2/M phase of the cell cycle and slightly increased granularity. After 7 days of curcumin treatment about 80% of cells were senescence-associated-beta-gal-positive (common marker of cellular senescence). We also examined the production of proinflammatory cytokines - Senescence Associated Secretory Phenotype (SASP) and we observed an elevated level of Il-6, Il-8 and VEGF. After curcumin treatment the number of DNA double strand breaks visualized as 53BP1 foci decreased, what can suggest a reduction of DNA damage. However, western blot analysis showed transient activation of components of the DDR pathway such as p53 and p21. To summarize, our results show that primary cells are very sensitive to curcumin and this factor is a potent cellular senescence inducer. These results suggested that curcumin in some range of concentrations can evoke a side effect in the form of the cellular senescence. Whether the cause of the senescence is DNA damage it is yet to be elucidated.

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HOW VASCULAR SMOOTH MUSCLE CELLS UNDERGO SENESCENCE?

Dorota Przybylska, Anna Bielak-Żmijewska, Zbigniew Korwek, Olga Alster, Halina Waś, Wioleta Grabowska, Ewa Sikora, Grażyna Mosieniak

Laboratory of the Molecular Bases of Aging, Nencki Institute of Experimental Biology, Department of Biochemistry, Warsaw, Poland

e-mail: d.przybylska@nencki.gov.pl

Cellular senescence is proposed as one of the potential mechanisms of organismal aging. Till now most research concerning cellular senescence was performed using fibroblasts. That is why we decided to investigate it in other cellular models - in vascular smooth muscle cells.

The senescent cells have the potential to detrimentally affect tissue function. Moreover it was shown that those cells contribute to several age related pathologies. One of them is atherosclerosis which correlates with vascular smooth muscle cells' (VSMCs) senescence. Induction of cellular senescence *in vitro* can be attributed to progressive decrease of the replication potential (replicative senescence), or can be induced by a variety of stress factors leading to stress-induced premature senescence (SIPS). The aim of the study was to check whether replicative and stress-induced senescence of VSMCs can be characterised by the same cellular and molecular markers. To this end we cultured VSMCs till they terminally stop to proliferate or treated early passages of VSMCs with DNA damaging agents – doxorubicin or hydrogen peroxide to induce SIPS. We characterized the process of VSMCs' senescence on the bases of different

markers like SA-β gal activity, BrdU incorporation, expression of p53 and p21. Moreover we estimated the level of DNA damage accumulating in cells during senescence on the bases of immunocytochemical staining of 53BP1. We performed cell cycle analysis and estimated the mitotic index and mitosis disturbances in the cells undergoing replicative and stress-induced senescence. The secretory phenotype, namely the production of IL-6 and IL-8 was also measured. We found that apart from common features of both replicative and stress-induced senescence still some markers are observed only in one but not in the other model of senescence. We observed that cells which underwent replicative senescence were arrested in the G1 phase of cell cycle while stress-induced senescent cells accumulate in G1 and G2/M phases. The differences in the level of expression of the cell cycle regulatory proteins were also revealed. Altogether our results suggest that different types of VSMCs senescence can lead to differences in the senescent phenotype that could affect the role of these cells in the age-related diseases.

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P20.

MOLECULAR MECHANISM OF CELLULAR SENESCENCE AND APOPTOSIS OF HUMAN T CELLS – ROLE OF DNA DAMAGE RESPONSE.

Zbigniew Korwek, Anna Bielak-Żmijewska, Grażyna Mosieniak, Olga Alster and Ewa Sikora

Laboratory of the Molecular Bases of Ageing, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

e-mail: z.korwek@nencki.gov.pl

Eukaryotic cells are permanently exposed to various factors, which cause DNA damage. To protect genetic information eukaryotic cells evolved a surveillance system called the DNA damage response (DDR) signalling pathway, activation of which determines the cell fate. Based on the level of DNA damage and it's location the cells can undergo: transient cell cycle arrest (repair), stable cell cycle arrest (senescence) or cell death (apoptosis). The key protein is p53, which can lead to cell cycle arrest and senescence by the induction of p21 or to apoptosis by the induction of the proapoptotic proteins from the BCL-2 family. The aim of our study was to investigate the molecular mechanism of cellular senescence and apoptosis of normal human T cells and the role of DNA damage in these processes. First, we checked the response of quiescent T cells to DNA damage induced by etoposide. We observed the expression of the marker of DNA double strand breaks γH2AX, and an upregulation of the phosphorylated proteins involved in the DDR, such as: ATM and p53. This was followed by the induction of a proapoptotic protein PUMA and the cleavage of caspases. Altogether, in quiescent T cells DNA damage induced by

etoposide activated the DDR leading to apoptosis. To answer the question whether DNA damage can be involved in T cell senescence we stimulated cells with a unspecific mitogen PHA, which mimics TCR activation. PHA stimulation caused hyperproliferation, which induced replication stress and DNA damage which could be observed in the form of γH2AX foci. Moreover, an upregulation of p53 and p21 could be seen, as well as senescence associated β-galactosidase activity, a marker of senescent cells, was found. Summing up, replicative stress just like direct DNA damage leads to DDR, but only hyperproliferation causes both cell death and cellular senescence.

P21.

DISSECTING PATHWAYS LEADING TO SENESCENCE OF CANCER CELLS

Anna Strzeszewska, Halina Waś, Grażyna Mosieniak, Małgorzata Śliwińska, Ewa Sikora

astrzeszewska@nencki.gov.pl

Laboratory of the Molecular Bases of Aging, Department of Biochemistry, Nencki Institute of Experimental Biology, Warsaw

Senescence, which is a cessation of proliferation, can occur not only to normal cells but also to cancer cells as a result of stress. So called stress-induced premature senescence (SIPS), induced for example by DNA-damaging agents, is a much more acute response than replicative senescence. It is now clear that senescence of cancer cells is an important outcome in chemotherapy. Interestingly, it was shown, that anticancer agents induced senescence even in neoplastic cells that lack senescence-associated tumor supressors such as p53.

The main aim of this study was to dissect crucial pathways involved in p53-independent activation of p21 and induction of senescence of cancer cells. Aforementioned p21 upstream signaling of our interest accounts mainly for ATM-NFκB pathway.

Experiments were performed using p53 wild type (WT) and p53 knock-out (KO) human HCT116 colon cancer cells. We showed that both lines cease proliferation upon treatment with low dose of DNA-damaging agent, namely doxorubicin or etoposide. Treatment with doxorubicin as well as with etoposide lead to polyploidization of HCT116.

Induction of senescence was more pronounced in WT line, as characterized by SA-β-gal staining. In contrast, more p53 KO than WT cells underwent apoptosis, which we showed by flow cytometry analysis and detection of a cleaved form of poly (ADP-ribose) polymerase (PARP). Senescent cells enlarged their size and increased their granularity. Additionally, both lines exhibited senescence-associated secretory phenotype (SASP) - after treatment with DNA-damaging agent, cells produced vascular endothelial growth factor (VEGF) and interleukin 8 (IL-8). Moreover, we observed enhanced expression of other common senescence marker- p21 and - in p53 WT cells also activation of p53. Induction of senescence was reflected in activation of NFκB.

Our preliminary studies with permanent doxorubicin treatment scheme confirm results obtained with 24-hour drug treatment, regarding activated pathways and cell cycle status.

Altogether, our data suggest that etoposide as well as doxorubicin is capable of inducing senescence in colon cancer cells independently of p53 status.

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P22.

CURCUMIN IN CONCENTRATION CLOSE TO THE BIOAVAILABLE ONES IN THE ORGANISMS MAY INDUCE SENESCENCE OF ENDOTHELIAL CELLS.

Karolina Kucharewicz, Ewa Sikora, Anna Bielak-Żmijewska

Laboratory of the Molecular Bases of Aging, Nencki Institute of Experimental Biology, 3 Pasteur Street, Warszawa, Poland

e-mail:kkuchare@nencki.gov.pl

Curcumin is an orange-yellow organic compound obtained by solvent extraction from dried turmeric roots. Turmeric is a spice commonly used in South Asia for the preservation of food, as a yellow dye and for health care. Research within the last century has documented its beneficial activities and, recently, curcumin has became an atractive phytochemical for medicinal use because of its antioxidative and anti-inflammatory properties (it is able to decrease the activity of transcription factor NF-κB and of some proinflammatory cytokines e.g. IL6 and IL8). There are data about curcumin's beneficial effects in many complaints, diseases and about its protective role in heart injury. Increased ROS production and chronic low grade inflammation are characteristic for age-related diseases. Furthermore, they are it is important events accompanying cellular senescence. The aging process of an organism is closely related to the cellular senescence and is considered as one of the main risk factors for cardiovascular diseases (CVDs) such as atherosclerosis and hypertension. Senescent cells were found in atherosclerotic plaques which may prove that senescence of vascular cells plays a crucial role in CVDs. According to the recent data curcumin could potentially play a protective role in the

cardiovascular system during the aging process by anti-oxidant and anti-inflammatory activities and its interaction with hemeoxygenase – 1 (HO-1), which improves the functioning of the circulatory system. However there is no data about the anti-senescent role of this agent. It is believed that addition of curcumin to the diet even in high doses (8g/day) for several months is safe. Although the bioavailability and stability of curcumin are very poor the concentration found in blood is about 2 μM. Curcumin, transported by the cardiovascular system, has direct contact with cells building the vasculature. Endothelial cells (ECs) are the first layer exposed to the contact with blood and its ingredients. The aim of our study was to analyze the influence of curcumin on ECs isolated from human aorta. We showed that endothelial cells are very sensitive to curcumin. The concentrations above 5 μM were cytotoxic and caused cell death. Surprisingly we also observed that this agent could induce premature senescence of these cells. The 2,5μM concentration was sufficient to inhibit proliferation but did not induce massive cell death. At this concentration of curcumin, some markers of cellular senescence were observed, such as SA-β-galactosidase activity and cell cycle arrest in the G2/M phase. But we did not observe the accumulation of DNA damage foci whicht usually accompanied cellular senescence. Our results suggested that curcumin at certain concentrations closed to those detected in the organism by dietary consumption, in high doses may have side effects for endothelial cells. It is important to consider whether the approaches aiming to increase the bioavailability of curcumin are justified taking into account its hazardous effects for ECs.

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P23.

PROAPOPTOTIC ACTIVITY OF WP 631 IN OV-90 (OVARIAN CANCER CELLS)

Arkadiusz Gajek, Aneta Rogalska, Agnieszka Marczak

Department of Thermobiology, Faculty of Biology and Environmental Protection, University of Lodz, Pomorska 141/143 st., 90-236 Lodz, Poland

e-mail: arekgajek86@wp.pl

Ovarian cancer is the leading cause of death from gynecological malignances. The lack of specific symptoms and efficient methods of screening causes that the most ovarian cancer cases are detected only in the III or IV clinical stage, which means that most women die from this type of cancer, despite the fact that it is not the most common disorder of female genital. For this reason, better treatment regimens are needed, including the use of new anticancer drugs.

Anthracycline antibiotics are well-known antitumor agents, with the best-characterized members of this group being daunorubicin (DNR) and doxorubicin (DOX). They are used in the treatment of many kinds of cancer (including ovarian cancer) but are often inefficient because of multidrug resistance (MDR). WP 631 is a novel anthracycline analog composed of two monomeric units of daunorubicin, symmetrically linked with a p-xylenyl linker which have been designed to overcome these limitations.

In the present study, we investigated the apoptotic activity of WP 631 in OV-90 cell line. The effect of WP 631 was compared with the activity of DOX, the best known first-generation anthracycline.

Methods Cytotoxicity of DOX and WP 631 was determined spectrophotometrically by MTT method. The type of cell death induced by WP 631 and DOX was analyzed using flow cytometry method by determination of phosphatidylserine (PS) externalization and double staining with Hoechst 33258 and propidium iodide (PI).

Results The data showed that WP 631 was considerably more cytotoxic towards OV-90 cells than DOX. IC_{50} concentration of new anthracycline analog (125±2,2 nM) was repeatedly lower than that of DOX (591,4±24,7nM). Phosphatidylserine externalization and staining with Hoechst 33258/PI indicate that WP 631 is able to induce timecourse – dependent apoptosis more effectively than DOX.

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P24.

IS SENESCENCE THE MAIN MECHANISM RESPONSIBLE FOR GLIOBLASTOMA CELL LINE STABILIZATION FAILURE?

Ewelina Stoczynska-Fidelus[1], Michal Bienkowski[2], Sylwester Piaskowski[2], Mateusz Banaszczyk[1], Marta Winiecka[1], Jolanta Zieba[1], Krystyna Hulas-Bigoszewska[2], Anna Radomiak-Zaluska[3], Piotr Rieske[1]

[1] Department of Tumor Biology, Medical University of Lodz, Poland

[2] Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, Poland

[3] Neurological Surgery, The Maria Sklodowska-Curie Regional Specialist Hospital in Zgierz, Poland

e-mail: ewelina.stoczynska-fidelus@umed.lodz.pl

It is well known that glioblastoma cells with *EGFR* amplification may not be cultured by means of classical *in vitro* conditions. Although the difficulties in the culturing of these cells is a well-known issue, the mechanisms responsible therefor remain vague. Several glioma cases were tested and the following molecular techniques were used: microarray analyses, LOH, Real-Time PCR (for the EGFR gene dosage and the CDKN2A gene deletion), FISH (for EGFR and CEP7), TP53 sequencing, immunocytochemical staining for various markers and statistical analyzes. In addition, BrdU incorporation assay and senescence-associated β-Gal assay were used. Our results indicate that only 10% of glioblastoma cases enables the cell line stabilization. In the remaining cases an enhanced growth of normal cells (infiltrating tumor sample) and the proliferation arrest of neoplastic cells was observed. Moreover, a similar situation was

observed in oligodendroglioma primary cell cultures. In the majority of cases, glioblastoma and oligodendroglioma cells became quickly non-proliferative (did not incorporate BrdU) and were β-Gal positive in early passages of cell culture. Standard monolayer conditions positively selected normal, most likely glioma associated stromal cells, *versus* glioma cells. In passage 2, only single GFAP expressing neoplastic cells were observed. Intriguingly, all of these cells were β-Gal positive, however, they did not show SAHF. Moreover, microarray analyses comparing glioma samples enabling and not-enabling the cell line stabilization did not show any differences in the senescence markers expression. Thus our results did not allow to determine whether the senescence is the mechanism responsible for glioma cell culture failure. Moreover, they generate the question whether the β-Gal staining is a specific marker precisely identifying senescent cells. It is not clear if SAHF are observed in the senescence of all types of cells (including neoplastic cells) and which markers are present in case of stress- or oncogene-induced senescence of neoplastic cells.

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P25.

"AGE-RELATED" CHANGES OF THE POLY(ADP-RIBOSYL)ATION SYSTEM IN CULTURED CHINESE HAMSTER CELLS

Inna V. Vladimirova[1], Gregory A. Shilovsky[2], Alexander N. Khokhlov[2], Stanislav I. Shram[1]

[1] Institute of Molecular Genetics, Russian Academy of Sciences, Moscow, Russia

[2] School of Biology, Lomonosov Moscow State University, Moscow, Russia

e-mail: shram@img.ras.ru

During long-term cultivation of transformed cells in the stationary growth phase they accumulate various deleterious changes similar to those in the cells aging *in vivo*. We have called the process "stationary phase aging". Its main feature is "age-dependent" accumulation of DNA damages. That is why it is tempting to study the possible changes in protein poly(ADP-ribosyl)ation system which plays an important role in maintaining genome integrity. We investigated the "stationary phase aging" effect on the poly(ADP-ribosyl)ation status in cultured B11-dii FAF28 Chinese hamster cells (CHC): the poly(ADP-ribose) (PAR) level and the cell ability to intensify PAR synthesis in response to H_2O_2. All the measurements were made at the 3rd –15th day after subcultivation. Number of dead cells was determined by trypan blue staining. To assess the cell ability to intensify PAR synthesis in response to DNA damage, CHC were incubated for 5 min in a medium containing 1 mM H_2O_2. PAR was detected by immunofluorescent staining technique. A well-known biomarker of aging – senescence-associated beta-galactosidase (SA-b-Gal) activity was

determined as described by Dimri G.P. et al. *(PNAS USA. 1995. 92:9363)*. Under the experimental conditions used CHC cultures reached the saturation density (about 5×10^5 cells/cm^2) in 6 days of cultivation, and for the next 9 days it has not changed. An abrupt increase in the number of dead cells after the 10-day cultivation was observed. It is interesting that PAR level increased immediately after the formation of confluent monolayer. In the 8-13-day cultures the PAR level was roughly two-fold higher than in the 3-day culture. However, a longer cultivation paradoxically resulted in a decrease of PAR level. On the contrary, H_2O_2-induced PAR synthesis has gradually decreased with "age". It was shown that H_2O_2 did not cause any increase in PAR synthesis in the 13- and 15-day-old cultures that clearly indicated a decrease in the DNA repair systems' potential of senescent cells. The SA-b-Gal activity was not detected in "young" CHC, but became well detected in the cells at the stationary growth phase. We concluded that transition of cell culture to the stationary growth phase leads to an increase in cellular PAR level and simultaneously to a decrease in the cell ability to synthesize PAR in response to DNA damage. These changes are accompanied with an increase in the number of cells with detectable SA-b-Gal activity and precede the onset of massive cell death in "stationary phase aging" culture. The study was partially supported by Russian Foundation for Basic Research (Project No 10-04-01770).