Session 8. Development and Ageing

Lectures

L8.1

Spindle Assembly Checkpoint in oocytes and zygotes and its importance for human aneuploidy

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The surveillance mechanism called Spindle Assembly Checkpoint (SAC) monitors the status of the division spindle and prevents anaphase entry in the presence of anomalies that could result in erroneous segregation of the chromosomes. Disturbances in SAC activity during female meiosis promotes formation of aneuploid oocytes and results in associated reproductive disorders like pregnancy loss or birth defects. In a similar way embryonic aneuploidy and pregnancy loss may occur due to SAC deficiency in the zygotes and early embryos. The production of aneuploid oocytes rises drastically in females with advancing age due to failure in chromosome cohesion in oocytes from old mice. Whether the decreased SAC efficiency contributes to maternal age-related aneuploidy remains disputable.
Oocytes from LT/Sv mice are unable to timely switch off the SAC activity [1]. As a result the majority of LT/Sv oocytes block at the metaphase of the first meiosis instead to progress to the second meiotic cycle. The defect in the ceasing SAC activity occurs also in LT/Sv zygotes as evidenced by enormously prolonged duration of the metaphase in these cells [2]. Thus, LT/Sv mice represent an valuable model to study the SAC function in the oocytes and at the earliest stages of development. Importantly, the proportion of LT/Sv oocytes which arrest at the first meiosis drops significantly in the older females [3], thus providing the model to study the relationship between SAC activity and maternal-age related aneuploidy.

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L8.2

Mechanisms and players regulating cell differentiation and morphogenesis during both pre and post implantation stages

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The ability to culture and therefore experimentally manipulate and image the development of mouse and human embryos from the zygote to the blastocyst stage has led to an impressive understanding of the mechanisms behind the first cell fate decisions and the plasticity of pre-implantation development. In contrast, development of the embryo as it implants has been hidden from a direct view and experimental manipulations as it occurs within the body of the mother. Yet these "implantation-stages" are critical: this is the time when the embryo acquires a totally different shape and form, the pluripotent population of cells expands and the anterior-posterior axis becomes established. To gain direct and precise that thus far possible insight into this developmental transition we have established a new system that enables embryos to develop, be manipulated and imaged throughout implantation stages outside the mother. This has opened a way to provide a new and unexpected insight into how the mouse embryo develops its form and pattern at this previously inaccessible developmental stage. I will present these new results that led to entirely new way of understanding the morphogenesis throughout implantation stages. I will also show that mouse ES cells can self-organise when culture in the same condition to mimic pre- to post-implantation development.

L8.3

The physiological significance of cellular senescence

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Cellular senescence is a permanent growth arrest that limits proliferation of potentially dangerous cells to prevent tumorigenesis and to restrict short-term tissue damage. Paradoxically, accumulation of senescent cells was suggested to promote tissue aging and tumorigenesis in their microenvironment. However, little is known about the role of senescence under non-pathological conditions. We found that placental syncytiotrophoblast, which serves as the maternal/fetal interface during embryonic development, exhibited the phenotype and also expressed molecular markers of cellular senescence. Endogenous fusion-mediating protein ERVWE1 drives the formation of the syncytiotrophoblast at the placenta. Expression of the ERVWE1 protein caused cell fusion in both normal and cancer cells, and led to the formation of hyperploid syncytia that has cellular senescence features (Chuprin *et al*., 2013, *G&D* **27**: 2356–2366). The fused cells activated the main molecular pathways of senescence, the p53-p21 and p16-pRb-dependent pathways, the senescence-associated secretory phenotype (SASP) and the immune surveillance-related proteins. Mice, deficient in these senescence pathways, might demonstrate alterations in the functionality of the placenta. Altogether, we identify cell fusion as a novel trigger for the induction of senescence and propose that fusion-induced senescence is needed for the proper syncytiotrophoblast function during embryonic development. Similar cell-cell fusion induced senescence program might be utilized later in life in order to protect the organism against pathological expression of endogenous fusogens and fusogenic viral infections.

L8.4

Bad impact of good cells, or how senescent peritoneal mesothelium promotes progression of ovarian cancer

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There is substantial evidence that senescent cells accumulate in tissues during ageing and promote various elements of cancer cell progression, including their proliferation, angiogenesis, and epithelial-mesenchymal transition. This activity, confirmed extensively on breast cancer cells co-cultured with senescent fibroblasts, is primarily attributed to the so-called Senescence-Associated Secretory Phenotype (SASP). This term refers to increased secretion of pro-inflammatory, pro-angiogenic, and extracellular matrix (ECM)-remodelling agents by which senescent cells disrupt normal tissue architecture and facilitate expansion of a malignancy.

Peritoneal cavity is a preferential site of ovarian cancer metastasis. It is believed that the intraperitoneal spread of cancer is associated with a reciprocal interplay between invading cancer cells and peritoneal mesothelium (HPMCs). Despite the fact that the incidence of ovarian cancer rises exponentially with age, the effect of senescent HPMCs on this process was till very recently elusive.

The studies using co-cultures of primary omental HPMCs and three lines of ovarian cancer cells (A2780, OVCAR-3, SKOV-3) revealed that cancer cells subjected to a conditioned medium (CM) from senescent HPMCs proliferated and migrated more vigorously than those exposed to CM from young cells. The same showed the experiments in which cancer cells were seeded on the top of senescent HPMCs. Growth-stimulatory activity of senescent HPMCs towards cancer cells was accompanied by up-regulated (in the latter) expression of genes and proteins involved in regulation of cell growth (Ki67, cyclins A2, B1, B2, D1, E2, G2), ECM remodeling (t-PA, u-PA, TIMP-2), inflammation (MCP-1, IL-1R, IL-6R), and angiogenesis (VEGF, IL-8). Senescent HPMCs secreted, in turn, increased amounts of numerous agents that may promote proliferation and migration of cancer cells, including VEGF, IL-6, IL-8, fibronectin, and GRO-1. Studies with neutralizing antibodies and exogenous recombinant forms of these factors showed that the growth-promoting effect of senescent HPMCs was related to increased secretion of VEGF, IL-6, and fibronectin, while increased migration was promoted by IL-8. Experiments on mice xenografts generated by the i.p. injection of the mixtures of young/senescent HPMCs with ovarian cancer cells (1:1 ratio) showed that the dynamics of tumor growth and tumor size were higher when cancer cells were accompanied by senescent HPMCs.

Collectively, these results show that the age-dependent increase in the peritoneal ovarian cancer aggressiveness may be related, at least to some extent, to the accumulation and deleterious activity of senescent HPMCs.

Acknowledgements

The study was supported by the grant from the National Science Centre (Nr 2011/03/B/NZ3/01214).

Oral presentations

O8.1

Effect of postovulatory aging on developmental competence of mouse oocytes

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Postovulatory aging refers to a period between ovulation and fertilization and may occur in vivo, when mating and fertilization does not follow closely the ovulation, but also in vitro, when isolated oocytes are cultured for several hours before insemination or sperm injection. Postovulatory aging has been indicated previously to decrease developmental competence of mammalian oocytes. In current study, we examined in detail how postovulatory aging in vitro and in vivo affects developmental competence of mouse oocytes, especially pattern of gene expression, Ca^{2+} signalling, energy metabolism and cytoskeleton quality. We analysed mouse oocytes obtained 15 and 24 hrs post hCG administration (i.e. fresh and aged oocytes respectively). We show that postovulatory aging leads to a decrease in mRNA levels of genes involved in Ca^{2+} signalling, such as SERCA2, IP3R1and STIM1 and in result it affects amplitude and frequency of Ca^{2+} oscillations. We also observed that aging causes striking redistribution of active mitochondria in oocytes and leads to a decrease in their membrane potential and oxidation of FADH2. Interestingly, we also find that in vitro aged oocytes are often not able to rotate their spindles during completion of the 2nd meiotic division, which leads to extrusion of both groups of female chromosomes and, in consequence, to a severe aneuploidy. It is very likely that the decreased ability of aged oocytes to develop upon fertilization into healthy embryos, is directly caused by the above-mentioned aberrations.

O8.2

Monitoring the postnatal heart development under the conditional knock-out of one allele of beta-catenin in cardyomyocytes

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Heart is complex and dynamic organ capable of remodeling and proliferation. The functioning of the heart in normal and pathological conditions is controlled by different signaling pathways, canonical Wnt signaling among them. It was shown that the absence β-catenin in embryos leads to lethality, even more ablation of this gene only in embryonic heart causes death at later embryogenesis. The role of this pathway is well-established in embryonic heart development. Though its role in adult heart is not fully understood especially under stress and hypertrophy remodeling and needs more investigations.

We generate mice with embryonic ablation of β-catenin gene in heart tissue using conditional knockout approaches (bacterial Cre-recombinase under a-MHC promotor control). Since earlier in our study the lethality of embryos with knockout of β-catenin gene at later stages of development has been shown we are now focused on signaling and structural role of β-catenin in postnatal heart development under the condition of β-catenin haploinsufficiency. We analyze the heart weight/body weight ratio of β-catenin haploinsufficient mice at different time points (1, 3 and 6 months). With HE, immunostaining and Western-blot analysis we study the influence of β-catenin haploinsufficiency on postnatal heart development, tissue formation, AJ function and formation. In our work we have used the swimming test as model heart adaptation to stress.

Also we are interested in the investigation of β-catenin signaling function under the same conditions as this protein is the main mediator of canonical Wnt signaling. That is why we analyze the level of expression of the stress and hypertrophy response genes and β-catenin target genes as well.

The embryonically induced β-catenin haploinsufficiency in heart was shown to cause the upregulation of foetal genes program and delay in the development and growth of adult heart but without visible morphological abnormalities. We also reviled that cardiospecific β-catenin deficiency leads to canonical Wnt signalling downregulation in our model. The heart development and growth delay could be a result of canonical Wnt-signaling downregulation.

Acknowledgements

The work was supported by the President of Ukraine Grant for the gifted youth.

O8.3

Reversible senescence of cancer cells subjected to chemotherapeutics is correlated with stemness and autophagy

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A fundamental feature of senescence is an irreversible growth arrest. However, several recent studies, including ours (Sliwinska *et al.*, 2009), indicate that senescence of cancer cells is a transient process that can lead to tumor regrowth.

To answer the question about the mechanism(s) responsible for that phenomenon, we treated human colon cancer HCT116 cells with doxorubicin and 5-fluorouracyl (5-FU). 5-FU is a chemotherapeutic agent commonly used in the clinics to treat colon cancer patients. At first, we established *in vitro* experimental protocols that allowed us to mimic a regime of chemotherapy given to patients. Namely, cancer cells were exposed to drugs given at low dose in repeated cycles for few weeks. In response to the treatment, cells showed all hallmarks of stress-induced premature senescence (SIPS), specifically: changed morphology, increased vacuolization, augmented SA-β-galactosidase activity, elevated expression of cell cycle inhibitors p21 and p53, and senescence-associated secretory phenotype (SASP). In particular, senescent colon cancer cells upregulated secretion of immunomodulatory and proangiogenic cytokines, but downregulated production of growth factors and adhesion molecules. Surprisingly, shortly after drug removal colonies of small and highly proliferative cells appeared. Time-lapse technique revealed that source of escapers could be some of poliploid, senescent cells carring abnormal divisions. Interestingly, outgrowth of descendants correlated with transient upregulation of stem cell-like phenotype, as elevated expression of stem cell marker Nanog and an increase in side population that excludes Hoechsth 33342 were detected. Moreover, epithelial to mesenchymal transition (EMT), another feature of stem cell-like phenotype, seemed to play a role in the process, as levels of E-cadherin and β-catenin were elevated in senescent cells, whereas progeny showed rather mesenchymal phenotype. Finally, changes in lysosomes' number and in expression of autophagy-related proteins: beclin, LC3-II and p62 were observed.

Altogether, we conclude that the cancer cells treated with chemotherapeutics enter the SIPS. In turn, drug removal results in outgrowth of highly proliferative progeny, that correlates with transient appearance of stem cell-like features, EMT and autophagy.

Acknowledgements

Supported by grants no. IP2012 062172 from Ministry of Science and Higher Education and INTER/ Skills from Foundation for Polish Science.

O8.4

p53 protein and insulin resistance in Alzheimer's disease

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Alzheimer's disease(AD) could be perceived as metabolic disorders, in which brain glucose utilization and energy production are impaired. However, development of insulin resistance in neurons remains elusive, and we expose the role of p53 protein in the mechanism. Many factors linked to AD pathogenesis provide stress to neuronal cells, thereby leads to increased level of stabilized, activated p53, which helps to maintain normal cellular metabolism and plays a key role in allowing cells to adapt to various types of stressors. Since the influence of p53 on glucose metabolism is opposite to the effects of insulin, it could be pointed out that p53 evokes insulin resistance. A major role of p53 in regulation of lipid metabolism is a result of transcriptional activation of many genes that enhance lipid catabolism while decrease anabolism of intracellular lipids. Importantly, p53 induces expression of ceramide synthases, while inhibiting sphingosine kinase activity. As a result, elevated intracellular concentration of ceramide, leads to marked inhibition of insulin receptor and insulin receptor transduction pathways. Probably, ceramide action paves the way for insulin resistance. p53 also binds to glycogen synthase kinase-3Beta (GSK 3Beta), and both proteins activate each other in developing insulin resistance and also in driving of apoptotic dealth program, when intracellular level of deleterious metabolites (e.g ROS, avanced glycation endproducts, ceramide) exceeds cellular ability to survive. We think that p53 could be perceived as a hub intergating various causative factors of metabolic disturbances in neurodegeneration, thus future treatment strategies for Alzheimer's disease should comprise also inhibition/blocking of the p53 metabolic activity.

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O8.5

HO-1 deficiency accelerates aging of hematopoietic stem cells

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Heme oxygenase-1 (HO-1) is an enzyme that degrades free heme to carbon monoxide, iron ions and biliverdin. Given that HO-1 possesses potent cytoprotective properties, we hypothesize that HO-1 may protect hematopoietic stem cells (HSC) from premature aging.

We found that HSC were more frequent in HO-1$^{-/-}$ mice (0.011% *vs*. 0.006%, p=0.01), but possessed higher expression of CD150 antigen and lower expression of GCSF receptor. Several observations indicated the myeloid biased differentiation of HO-1$^{-/-}$ HSC: more macrophages in colonies derived from single-sorted HSC and more mature myeloid cells, both monocytes and granulocytes, in peripheral blood of HO-1$^{-/-}$ mice. Interestingly, all these alterations in HSC observed in young HO-1$^{-/-}$ mice, were similar to these observed in two year old wild type mice. Therefore, we concluded that HO-1$^{-/-}$ mice possess HSC that show symptoms of premature aging.

Premature aging of HSC in HO-1$^{-/-}$ mice could be connected with expression of HO-1 in HSC as well as in bone marrow niche cells – important regulator of HSC activity. Using cell sorting, real time PCR and flow cytometry we evidenced that mesenchymal stem cell populations, CAR (*CXCL12-abudant reticular cells,* CD45$^-$Ter119$^-$CD31$^-$Sca-1^{low}CD140a$^+$), PaS (CD45$^-$Ter119$^-$CD31$^-$Sca-1^{high}CD140a$^+$) and endothelial cells, expressed the highest levels of HO-1 (43.9, 7.2 and 27.8 fold increase vs. CD45$^-$Ter119$^-$ control, respectively). High HO-1 expression characterized also bone marrow-derived macrophages (7.0 fold increase vs. CD45$^+$ control). All these high HO-1-expressing cell types compose the bone marrow niche of HSC. In contrast, the expression of HO-1 in hematopoietic stem and progenitor cells (Lin$^-$cKit$^+$Sca-1$^+$) was lower (2.4 fold increase).

To verify role of HSC-extrinsic role of HO-1 in regulation of HSC we performed bone marrow transplantation. HSC from HO-1$^{+/+}$ donor mice were sorted, transduced to express GFP and transplanted into HO-1$^{+/+}$ or HO-1$^{-/-}$ recipients. Initially, peripheral blood chimerism in HO-1$^{+/+}$ and HO-1$^{-/-}$ primary recipients was similar. However, after 32 weeks chimerism in HO-1$^{-/-}$ recipients was lower (6.4% vs. 24.8%, p=0.01). Then GFP$^+$ bone marrow cells from HO-1$^{+/+}$ or HO-1$^{-/-}$ primary recipients was further transplanted to the secondary HO-1$^{+/+}$ recipients. Importantly, only the cells from HO-1$^{+/+}$ primary recipients provided reconstitution of blood in secondary recipients.

Concluding, HO-1 deficiency in HSC bone marrow environment accelerates exhaustion of HSC stemness potential.

O8.6

Hyperhomocysteinemia and bleomycin hydrolase modulate the expression of mouse brain proteins involved in neurodegeneration

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Homocysteine (Hcy) is a risk factor for Alzheimer's disease (AD). Bleomycin hydrolase (BLMH) participates in Hcy metabolism and is also implicated in AD. We found that human BLMH is a major Hcy-thiolactonase that protects cells against Hcy toxicity (Zimny *et al*., 2006, *JBC*) and that Hcy-thiolactonase activity is decreased in brains of AD patients (Suszynska *et al*., 2010, *JAD*), suggesting that the diminished functional activity of BLMH contributes to the pathology of AD. The inactivation of the *Blmh* gene in mice causes accumulation of Hcy-thiolactone in the brain and increases susceptibility to Hcy-thiolactone-induced seizures (Borowczyk *et al*., 2012, *Amino Acids*).

To gain insight into brain-related Blmh function we used two-dimensional IEF/SDS-PAGE gel electrophoresis and MALDI-TOF/TOF mass spectrometry to examine brain proteomes of *Blmh*$^{-/-}$ mice and their *Blmh*$^{+/+}$ littermates fed with a hyperhomocysteinemic high-Met or a control diet. We found that: 1) proteins involved in brain-specific function (Ncald, Nrgn, Stmn1, Stmn2), antioxidant defenses (Aop1), cell cycle (RhoGDI1, Ran), and cytoskeleton assembly (Tbcb, CapZa2) were differentially expressed in brains of Blmh-null mice; 2) hyperhomocysteinemia amplified effects of the *Blmh*$^{-/-}$ genotype on brain protein expression; 3) proteins involved in brainspecific function (Pebp1), antioxidant defenses (Sod1, Prdx2, DJ-1), energy metabolism (Atp5d, Ak1, Pgam-B), and iron metabolism (Fth) showed differential expression in Blmh-null brains only in hyperhomocysteinemic animals; 4) most proteins regulated by the *Blmh*$^{-/-}$ genotype were also regulated by high-Met diet, albeit in the opposite direction; and 5) the differentially expressed proteins play important roles in neural development, learning, plasticity, and aging and are linked to neurodegenerative diseases, including AD.

In conclusion, our findings suggest that BLMH is an important neuroprotective protein and that hyperhomocysteinemia contributes to neurodegeneration by affecting expression of proteins involved in processes important for brain homeostasis, such as cytoskeleton assembly, cell, cycle, energy metabolism, antioxidant defenses, neural development, learning, aging, degeneration, and plasticity.

Acknowledgements

Supported by NCN grants 2011/01/B/NZ1/03417, 2011/02/A/NZ1/00010, 2012/07/B/NZ7/01178, 2013/09/B/NZ5/02794).

Posters

P8.1

The role of nibrin in doxorubicin-induced senescence of primary cells and T lymphocytes derived from Nijmegen Breakage Syndrome patients

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Nibrin is a 95 kDa protein which is involved in DNA damage response (DDR) and DNA repair. A mutation in the gene encoding nibrin causes Nijmegen Breakage Syndrome. Since persistant activation of DDR leads to senescence we wanted to verify whether truncated nibrin (p70), which is present in Nijmegen Breakage Syndrome (NBS) patients, affects the activation of DDR and the induction of senescence in these cells. In our experiments we used two NBS cell lines (S3R and S4). We observed that S3R and S4 cells have the same level of p70 nibrin in non-treated as well as in doxorubicin treated cells. Nevertheless p70 nibrin in S4 cells could form more complexes with ATM and BRCA1 than in S3R cells. Furthermore even though the S3R cells had more damaged DNA than S4 cells, they did not activate the DDR pathway and underwent cell death, but not senescence after doxorubicin treatment. Doxorubicin-induced DDR followed by cell senescence could only be observed in the S4 cells. A time-dependent increase in the level of p21, SA-β-Gal positive cells and cell granularity could be seen. Moreover downregulation of nibrin in human vascular smooth muscle cells (VSMCs) did not affect their ability to activate the DDR and to undergo senescence. Our results indicate that a substantially reduced level of nibrin or its truncated p70 form is sufficient to induce DNA-damage dependent senescence in VSMCs and S4 cells, respectively. Despite the presence of DNA damage in S3R cells the activation of DDR was severely affected, thus preventing induction of senescence.

Acknowledgements

This work was supported by National Science Centre in Poland (grants: 2011/01/M/NZ1/01597, UMO-2011/01/B/NZ3/02137 and 0728/B/P01/2011/40).

P8.2

A major lipid peroxidation product, 4-hydroxy-2-nonenal induces protein PARylation and necrosis in *Ercc1*$^{-/-}$ mouse embryonic fibroblasts

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The ERCC1-XPF protein complex is an endonuclease involved in nucleotide excision repair and processing of DNA interstrand crosslinks. *Ercc1* knockout mice display retarded postnatal growth accompanied by many features of aging and die at about 3–4 weeks of age. Cells from *Ercc1*$^{-/-}$ mice reveal an increased level of reactive oxygen species and are hypersensitive to oxidative stress. One of the consequences of oxidative stress is lipid peroxidation (LPO), that generates numerous reactive aldehydes, e.g. 4-hydroxy-2-nonenal (HNE), crotonaldehyde (CRO), malondialdehyde (MDA) and acrolein (ACR).
We were investigating the role of lipid peroxidation in the phenotype of *Ercc1* deficient mice. Sensitivity of immortalized mouse embryonic fibroblasts (MEFs) derived from wild type (wt) and *Ercc1*$^{-/-}$ mice to HNE, CRO, MDA and ACR was verified. *Ercc1*$^{-/-}$ fibroblasts were more sensitive than wt cells to long chain LPO products: HNE, CRO and MDA, but not to ACR. The most cytotoxic compound was HNE. HNE treatment caused a marked accumulation of *Ercc1*$^{-/-}$ MEFs in G2 phase. We checked three cell death pathways potentially activated by HNE, apoptosis, necrosis and autophagy. Necrosis appeared to be the preferred type of HNE-induced cell death for *Ercc1*$^{-/-}$, while for the wt cells it was apoptosis. In accordance, intensive PARylation of proteins, which is a marker of DNA breaks, was shown in *Ercc1*$^{-/-}$ cells after 2 hours of HNE-treatment, but not in wt MEFs. Intensive protein PARylation can result in NAD^+ depletion, which may trigger necrosis. No activation of autophagy in response to HNE was observed in either *Ercc1*$^{-/-}$ and wt fibroblasts. In both cell lines HNE caused a similar, moderate increase in the frequency of sister chromatid exchanges, which is a marker of homologous recombination (HR). These results suggest that lipid peroxidation may contribute to *Ercc1*$^{-/-}$mouse premature aging phenotype by inducing DNA strand breaks, which are only partially repaired by accurate HR system, inhibiting cell cycle progression, and inducing necrosis.

Acknowledgements

This work was supported by the Polish Ministry of Science and Higher Education, grant No. N N303 819540.

P8.3

Muscle regeneration of HO-1$^{-/-}$ and Nrf2$^{-/-}$ mice after cardiotoxin-induced injury

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Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), encoded by *Nfe2l2* gene, regulates expression of several cytoprotective genes with antioxidant properties. Among them there is *Hmox-1*, encoding heme oxygenase-1 (HO-1) which converts heme to biliverdin, CO and Fe^{2+}. This enzyme was shown by us to strongly affect myoblasts differentiation in *in vitro* conditions (Kozakowska et al, Antioxid Redox Signal, 16: 113-127; 2012), influencing particularly the microRNAs expression. Therefore, in this study we strive to investigate the role of HO-1 and Nrf2 deficiency *in vivo*, in cardiotoxin (CTX) treated mice.
Cohort included 120 mice. The C57BL/6xFVB-HO-1 subgroup was composed of 60 animals. Half of them were HO-1 knockout (KO) while the rest (wild type – WT) had functional *Hmox1* gene encoding HO-1 protein. 25 HO-1 KO and 25 HO-1 WT mice were intramuscularly injected with CTX solution in saline and 5 mice per genotype were sacrificed 1, 3, 7, 14 and 28 days after CTX injury. G*astrocnemius* muscles were collected and expression of mRNAs and microRNAs was analyzed. H&E staining was also performed. Remaining 10 mice that served as control groups were sacrificed one day after intramuscular saline injection. Similar scheme of experiment and number of animals was applied for the second subgroup – C57BL/6/J-NRF2-KO mice.
Collected data indicate that after CTX-induced injury, in the early inflammatory phase, the expression of *IL-1β* and *IL-6* was higher in HO-1 KO than WT mice, while in Nrf2 animals there were no significant changes between genotypes in these regard. Expression of *Pax3* and *Pax7*, genes responsible for regulation of muscle precursor cells proliferation, was higher in HO-1 and Nrf2 deficient mice, however induction of this expression in Nrf2 animals was observed in earlier stages of muscle response to injury. In both subgroups of KO animals, increase in *Pax3* expression preceded an increase in *Pax7* levels. Induction of *MyoD,* one of the myogenic regulatory factors responsible for muscle differentiation, was delayed in HO-1 KO and Nrf2 KO mice when compared to corresponding control animals and Nrf2 KO mice presented higher levels of its expression than WT specimens. In contrast, the expression of *myogenin* and *Myf5* tended to be higher in knockouts of both genotypes than in wild type counterparts. As regards analyzed microRNAs, their expression is more affected by HO-1 deficiency than by the lack of Nrf2. Changes in miR-206 are especially pronounced and HO-1 KO display significantly lower levels of this microRNA when compared to controls, in which level of miR-206 is steadily increased in later phases of muscle regeneration.
Summing up, collected data suggest that HO-1 and Nrf2 play a role in the regulation of proliferation and differentiation of muscle cells.

Acknowledgements

Supported by the MAESTRO grant from the National Science Center (NCN 2012/06/A/NZ1/00004).

P8.4

Curcumin induces cellular senescence of vascular smooth muscle cells: the role of DNA damage and ROS production

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Curcumin is a natural polyphenol used for centuries in natural medicine that has been for many years under scientific and clinical evaluation. It is believed that curcumin has a lot of beneficial properties for the whole organism and among others it is considered an anti-aging factor. It was shown that curcumin, in some concentrations, can induce senescence of cancer cells. We showed that curcumin can induce senescence also in primary cells, namely in vascular smooth muscle cells (VSMCs).
The aim of this study was to elucidate the mechanism of curcumin-induced senescence in VSMCs. We observed transient activation of the components of the DNA damage response (DDR) pathway such as p53 and p21. However, simultaneously, a decreased number of DNA double strand breaks was observed which suggests that curcumin-induced senescence of VSMCs is DNA damage-independent. It is believed that curcumin, apart from its antioxidative properties, can act in some situations as a prooxidant. Therefore we analyzed whether increased production of reactive oxygen species (ROS) is the cause of DDR pathway activation. ROS can influence directly ATM kinase autophosphorylation and in this manner activate DDR pathway and induce senescence. To this end, we pre-treated cells with ROS scavengers, N-acetylcysteine or trolox, followed by curcumin treatment. Subsequently, we analyzed common markers of senescence. We did not observe any increase in viability of cells treated with both curcumin and ROS scavenger in comparison to curcumin-treated only. Supplementation with ROS scavengers also did not cause any decrease in senescent cell number after curcumin treatment. Finally, no changes in activation of DNA damage response pathway were observed. These results suggest that ROS are not involved in the induction of VSMCs senescence after curcumin treatment. What is more, silencing of ATM did not result in a decrease in the number of senescent cells after curcumin treatment.
Summarizing, VSMCs undergo senescence after treatment with cytostatic dose of curcumin. This effect does not depend either on DNA damage or increased ROS production. This suggest that a different mechanism is responsible for DDR pathway activation and senescence induction than direct activation of ATM. The mechanism remains to be elucidated.

Acknowledgements

Financial support: This work was supported by grant 2011/01/B/NZ3/02137 from the National Science Centre.

P8.5

Comparison of hFOB1.19 to human mesenchymal stromal cell during differentiation to osteoblasts

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hFOB1.19 (ATCC CRL-11372®) is a cell line derived from human fetal osteoblasts. The cells have been genetically modified by transfection with a gene encoding a temperature sensitive T large antigen mutant of SV40. At a lower temperature (33.5°C) the T-antigen protein promotes cell proliferation. In contrast, at higher temperature (39.5°C) T-antigen is inactivate, and the cells begin differentiation to osteoblasts. Like mesenchymal stem cells, hFOB1.19 cells, also may differentiate to adipocytes and chondrocytes. The aim of this study was to determine at what stage of cell differentiation are hFOB1.19 in comparison to bone marrow mesenchymal stromal cells subjected to osteoblastic differentiation.
The hFOB1.19 cells were cultured in parallel with human mesenchymal stromal cells from bone marrow, and both cell lines were subjected to differentiation toward osteoblasts. The hFOB1.19 were differentiated for 10-days at elevated temperature, whereas the mesenchymal stem cells were differentiated for 4, 7, 14 and 28 days using appropriate differentiating culture medium. After cultivation, expression and activity of alkaline phosphatase, expression of osteopontin and the amount of hydroxyapatite in individual cultures were investigated. The results of measurements were compared to each other.
Obtained results revealed that the hFOB1.19 cells have lower expression level and activity of alkaline phosphatase than the mesenchymal stem cells differentiated for 4 days. However, the amount of calcium deposits in the cultures was comparable to the amount of minerals in deposited in culture of bone marrow mesenchymal stromal cells at this stage of differentiation. In the contrast, the expression of osteopontin was 30 fold higher in the hFOB1.19 cells undergoing differentiation in comparison to differentiating bone marrow mesenchymal stromal cells. Similar range of osteopontin expression in mesenchymal stromal cells was detected after 28 days of their differentiation to osteoblasts. In summary, hFOB1.19 in many ways are similar to mesenchymal stem cells, just shortly after the start of the differentiation process. Therefore, this cells can be considered progenitors of osteoblasts.

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Acknowledgements

The studies were financially supported by Institutional grants: KNW-1-010/D/1/0, KNW-1-013/D/2/0, KNW-2-005/D/3/N, POKL.08.02.01-24-019/11 to DK and KNW-1-041/K/3/0 to ALS.

P8.6

Cell polarization *vs.* cell position: searching for the decisive factor for cell destiny

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Formation of the first cell lineages in the mouse blastocyst: the inner cell mass (ICM) and the trophectoderm (TE) is initiated by two successive waves of asymmetric divisions commencing at the 8- and 16-cell stage. Trophectoderm is an extraembryonic tissue, which during further development gives rise to the embryonic part of the placenta. Shortly before implantation ICM cells differentiate into two subpopulations: the epiblast (EPI) - a source of cells of the fetus and most of the fetal membranes, and the primitive endoderm (PE), which contributes to the endoderm layer of the second extraembryonic tissue - the yolk sac.
Determination of the cell fate in preimplantation mouse embryo has been explored for more than 40 years. The first historical model of establishment of cell lineages considered cell position at the 16- and 32-cell stage as a crucial factor for differentiation of TE and ICM. Further studies indicated the importance of cell polarization in the 8-cell embryo, resulting in generation of polar (outer) and apolar (inner) cells before the late morula stage. Still, the positional information seems to be essential in acquiring different developmental fate in inner and outer cells via the Hippo pathway.
Here we addressed the question about the importance of cell position and cell polarization in determination of the fate of the blastomere in the embryo. To answer this question we constructed aggregates in which a single, inner or outer GFP-positive blastomere from the 16-cell stage embryo was located inside or outside the aggregate composed of five inner and ten outer GFP-negative cells. Reconstructed embryos were then followed by time-lapse recording till the late blastocyst stage.
Our results show that outer blastomeres have a strong tendency to relocate from the inside to the outside of the aggregate, suggesting that polarization of these cells overrides their location and leads to migration into the position occupied in the embryo from which they originated. On the other hand, inner cells placed inside the aggregates tend to stay there and contribute to the ICM, while when put outside the aggregate show greater plasticity than outer, polar blastomeres at the 16-cell stage and their progeny can be found both in the ICM and in the TE.

P8.7

Melatonin and tacrine hybrid evokes autophagy and senescence of breast cancer MCF-7 cells

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Melatonin is present in a variety of animals, plants and microorganisms. In mammals it is a hormone produced mainly by the pineal gland, that plays an important role in such processes as the regulation of the circadian rhythm and antioxidant activity. There is also data showing melatonin's antiproliferative activity in different types of tumors, mainly breast cancer, both *in vitro* and *in vivo*. Tacrine is an acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitor. Cholinesterase inhibitors enhance cholinergic transmission directly by inhibiting AChE which hydrolyses acetylcholine. It has also been demonstrated that both AChE and BuChE play an important role in Aβ-aggregation during the early stages of senile plaque formation. Originally, a melatonin and tacrine hybrid (C10) was synthesized as a potential antineurodegenerative compound possessing strong cholinesterase inhibitory activity. We wanted to know whether it would be able to evoke cytotoxic/cytostatic activity on human breast cancer MCF-7 cells and to eventually disclose cell fate upon C10 treatment. Indeed, we found that C10 showed cytostatic and cytotoxic activity in a dose dependent manner. We have chosen a cytostatic concentration of C10 (4μM) to check whether long-term treatment with this agent would lead to the induction of senescence. Recently cell senescence was recognized to be the most desirable target of anticancer treatment as it can be induced by lower doses of drugs than those which cause cell death. One-day treatment of MCF-7 cells with 4 μM C10-induced cell death of 40% cells. The rest of cells stopped proliferating for several days. We have revealed that the survivors underwent autophagy, which was followed by senescence (20% of cells) and regrowth. Symptoms of cell senescence were analyzed using common markers of this process, such as: cell enlargement, increase of cell granularity, increase of SA-β-galactosidase (Senescence Associated β-galactosidase) activity, increase of the level of the p21 protein and accumulation of DNA damage. The presence of autophagy was shown using the following protein markers: LC3B II, beclin and p62. Additionally, an inhibition of cellular autophagy using Atg5 siRNA prevented C10-induced senescence. Altogether, we showed that C10 induced autophagy which was either followed by the ability of the cells to resume proliferation or permanent cell growth inhibition (senescence), thus being the mechanism of cell survival in response to the insult.

Acknowledgements

The studies were financed by National Center of Science, grant number: 2011/01/M/NZ1/01597.

P8.8

Reproductive capacity and viability of the yeast *Saccharomyces cerevisiae* hypertrophic cells

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The yeast *Saccharomyces cerevisiae* has been useful as a model organism for the studies of cell physiology or cellular response to various environmental stresses and is also used for the studies of aging process. A single yeast cell is able to perform a limited number of buddings which is referred as the replicative lifespan. It is proposed that loss of the proliferative capacity is a consequence of the "senescence factor" accumulation, like carbonyl proteins and rDNA circles. Alternative mechanism explains of this limit is connected with a consequence of hypertrophy. The hypertrophy results from budding as the atypical cytokinesis mechanism which enforces the increase of the yeast cell size. The inverse relationship between the initial cell size and the number of buds produced has been observed. The increase of the cell size may cause a number of changes both in ultrastructure and physiology of cells which make impossible the entry of the hypertrophic cells to the next cell cycle.
The aim of the study was the analysis the parameters which assess the metabolic status and physiological efficiency of the young yeast cells significantly differing in reproductive capacity. We used the cells population consisting mainly of young cells in which the increase in the cell size was obtained by 4 or 8 h incubation with the pheromone (pheromone alpha for the *MAT*a cells). These cells significantly differ in the reproductive capacity. For a detailed characterization the physiological state of the cells with increased size we performed the analysis of the cell viability, cell metabolic activity, ATP content and reactive oxygen species level. Our results show that the increase in the cell size does not cause significant changes of the cell metabolic activity or cell viability. However we observed the size-dependent increase in the ATP content and reactive oxygen species level. These differences appear not so significant if we recalculate obtained value to the size of the individual cells. The higher values but similar dependences were obtained also for Δ*sod1* strain, which is more sensitive to oxidative stress than the wild type strain. These results suggest that the increase in the cell size does not change the physiological status in such extent that it would be the main reason of the decrease the reproductive potential of the hypertrophic cells.

Acknowledgements

This research was supported by grant no 2013/09/B/NZ3/01352 National Science Centre.

P8.9

Response to redox stress in lymphocytes from aging healthy donors and patients with early and late Alzheimer`s disease

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disease. The early stage of AD is often Mild Cognitive Impairment (MCI), a condition in which memory disturbances are slightly greater than in healthy subjects of the same age and education.
In AD alterations in cellular processes occur not only in neurons, but also in peripheral cells. Recently we have demonstrated that sporadic AD (SAD) lymphocytes show G1 phase arrest and increased levels of p21 protein, the key regulator of G1/S cell cycle checkpoint and apoptosis [1]. Therefore we aimed at elucidation of the effects of these changes on the apoptotic response of AD lymphocytes to oxidative stress (OS). We compared apoptotic response to OS evoked by treatment with 2-deoxy-D-ribose (2dRib) in EBV-immortalized B-lymphocytes from 8 patients with familial AD (FAD) bearing 8 different mutations in PS1, and in 16 patients with SAD. Control cells came from healthy individuals in the age corresponding to FAD and SAD, respectively. We found that 24h after 2dRib treatment, the percentage of surviving lymphocytes in MTT assay was significantly decreased in SAD comparing to the age-matched controls and FAD. Accordingly, apoptosis measured by AnnexinV staining and SubG1-phase assessment was higher in SAD cells than in control and FAD. Also measurements of mitochondrial membrane potential (MMP) showed differences in the response to 2dRib between SAD and FAD: MMP of SAD lymphocytes was significantly decreased comparing to control and FAD. We found that the differences in the apoptotic response between SAD and FAD cells are not due to aging. Thus, FAD lymphocytes seem to be significantly more resistant to pro-oxidative apoptotic stimuli than SAD. Moreover, higher apoptotic response in SAD cells correlated with the increased level of p21 protein.
Next we measured selected parameters of apoptotic response in lymphocytes from MCI patients. Our preliminary results have not showed any statistically significant changes in late apoptotic response. However, mitochondrial potential of MCI cells after treatment clearly tended to be decreased, similarly to SAD. These results suggest that changes in the apoptotic response appear early in the AD pathogenesis and gradually increase with the development of the disease. Altogether, our results showed that SAD cells are more vulnerable to 2dRib than age-matched control and FAD and thus mechanism of apoptosis distinguishes cells from SAD and FAD patients. Our data indicate that lymphocytes may be useful for the development of new early AD diagnostic methods based on analyses of the apoptotic response.

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Acknowledgements

This research was supported by the Polish National Science Centre grant NN401 596840 and the JPND grant 2/BIOMARKAPD/JPND/2012.

P8.10

Curcumin induces oxidative-dependent cell cycle arrest mediated by SIRT7 inhibition of rDNA transcription in human aortic smooth muscle cells

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It is widely accepted that abnormal accumulation of vascular smooth muscle cells (VSMCs) may promote atherosclerosis and post-angioplasty restenosis and the use of some plant polyphenols with potent antiproliferative activities may be considered a therapeutic intervention to diminish/prevent the development of cardiovascular pathologies. In the present study, VSMCs response to curcumin treatment was evaluated. 5 μM curcumin stimulated a cytostatic effect, which was accompanied by protein carbonylation, oxidative DNA damage and changes in the nucleolar activity (the size and number of nucleolus, nucleolar protein levels and their localization). The levels of p53 and p21 were elevated. However it was independent from DNA DSB. Curcumin-mediated inhibition of rDNA transcription may be executed by both SIRT7 downregulation and site-specific methylation of *RNA18S5* promoter region. Curcumin-induced DNA methyltransferase 2 (DNMT2) upregulation was also shown. Perhaps, DNMT2-mediated RNA methylation may promote RNA stabilization upon curcumin treatment. In conclusion, nucleolus-focused cytostatic action of curcumin at low micromolar range, which could be feasibly achieved through dietary means, and its novel underlying mechanisms in VSMCs were established. We believe that our results may contribute to better understanding of the biological and pharmacological effects of curcumin on the human cardiovascular system.

Acknowledgements

This work was supported by Grant 2011/01/B/NZ3/02137 from the National Science Centre.

P8.11

Proliferative capacity and the metabolic rate of the yeast *Saccharomyces cerevisiae*

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The yeast *S. cerevisiae* has been used as a model in studies on replicative aging. The age of yeast cell is measured as a number of buds produced by single cell during its life. One of the proposed causes which explain the yeast cell reproductive capacity limit is hypertrophy as a consequence of inevitable increase of the cell size during consecutive cell cycles. The hypertrophy hypothesis assumes the existence of maximum volume that prevents further cell cycles. Furthermore our earlier results suggest that the reproductive potential in most cases negatively correlates with the increase of cell size per generation. The growth rate per generation can be modulated by genetic (deletion specific genes) or environment factors for example availability of the energy sources. Glucose is the preferred carbon and energy source for yeast and has a significant influence on the growth rate and size of yeast cells. In this study we applied the growth conditions (different glucose concentration in the medium) that modify the rate of biosynthetic processes.
The aim of this study was investigation the relationship between growth rate on the medium with different glucose concentration and proliferative capacity of cells. We used three wild-type yeast strains which represent different genetic backgrounds: SP4, BY4741 and BMA64-1A. Analyses were concentrated on measurements of cells diameter, their vitality and viability and the degree of glucose consumption by single cell. The data was analysed at strictly defined periods of time (0, 4, 8, 12, 16, 24, 36, 48 h) in the strains grown cultures on the medium with different glucose concentrations (0.1, 0.5, 2, 3, 4%). We demonstrate that the level of glucose consumption by a single cell depends on its content in the medium. Cells growing on the medium with high glucose concentration show the highest rate of its consumption especially at the beginning of growth. We observed strong correlation between the growth rate of cell and glucose concentration. Culturing of cells in medium containing higher concentrations of glucose (3%; 4%) resulted in larger cell size in comparison with cells grown on low-glucose medium (0.1%; 0.5%). The observed differences in the growth rate do not cause a significant differences of cells activity (measured by their vitality and viability).
The obtained data confirm the significance important role of glucose metabolism on the mechanism of cells growth and regulation their proliferative potential.

Acknowledgements

This research was supported by grant no 2013/09/B/NZ3/01352 National Science Centre.

P8.12

3,3',4,4'-tetrahydroxy-*trans*-stilbene exerts stronger anti-senescence properties than its natural precursor, resveratrol

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Resveratrol (Res), a natural phytoestrogen abundant in grapes and red wine, is known for its anti-cancer properties. It has been found that it can delay an onset of replicative senescence in cultured somatic cells as well as to expand the average and maximal lifespan of model organisms in vivo. Because the availability of Res is relatively low and its clearance from an organism fast, the extensive studies on its synthetic, additionally hydroxylated or methoxylated, derivatives are in progress. In this project we explored the effect of a new Res analogue, 3,3',4,4'-tetrahydroxy-trans-stilbene (3,3',4,4'-THS), on growth potential and replicative senescence of primary cultures of omentum-derived human peritoneal mesothelial cells (HPMCs) in vitro. The results showed that the cells subjected to 3,3',4,4'-THS proliferated more vigorously and senesced and a slower rate than their counterparts exposed to Res. In both these contexts, 3,3',4,4'-THS appeared to be most effective at the concentration of 0.5 μM. Besides, the lifespan-extending activity of 3,3',4,4'-THS was accompanied by the suppression of senescence-associated activities of SA-β-Gal and p38 MAPK. In addition, 3,3',4,4'-THS restored the functionality of mitochondria, in particular it increased an inner membrane potential and prevented senescence-related induction of mitochondria biogenesis. Moreover, it significantly stimulated the production of mitochondrial superoxides and non-specific ROS which was followed by the hyper-induction of antioxidants, including superoxide dismutase, catalase, and reduced glutathione. The magnitude of DNA damage (8-hydroxy-2'-deoxyguanosine, histone γ-H2A.X) in cells exposed to 3,3',4,4'-THS was decreased which coincided with the suppression of 53BP1, a transducer of DNA damage response.Collectively, our study proves that 3,3',4,4'-THS is more potent anti-senescence factor than Res, and that this effect may result from a hormetic-like stimulation of ROS release leading to a compensatory hyper-induction of mechanisms protecting cells against oxidative stress and DNA damage.

Acknowledgements

The study was supported by the grant from the National Science Centre (2011/03/N/NZ7/06277).

P8.13

Acetic acid as a potential factor, which limits chronological lifespan of yeast *Saccharomyces cerevisiae*

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The model of chronological aging determines the survival time of cells population in a stationary phase. According to literature it is considered as a model for studying aging of post-mitotic cells of higher organisms (MacLean *et al*., 2001). There are many controversies connected with this kind of model, which is used for studying mammals' aging, including humans. Experiments are conducted in the stationary growth phase, that is why, after running out of nutrients, the cell dies not only due to hunger, but also as a result of a negative effect of metabolites e.g. the ones of acetic acid, which gradually accumulate in the cell growth environment. Acetic acid has become a potential factor, leading to shorten chronological lifespan (CLS) of cells (Burtner *et al*., 2009). Starvation and poisoning are not factors associated with aging. The aim of this thesis was to define CLS of SP-4 wild-type strain and three deletion mutants: *fob1*⊿, *rpl20b*⊿ and *sfp1*⊿. For quantitative measurement of survival, the CFU assay and the kinetics of growth assay were used. For semi-quantitative measurement of survival, the spot assay was used. Subsequently with the use of gas chromatography methods (GC), the content of metabolites was determined, among others acetic acid in post – breeding fluid. Studies prove assumptions that the factor, which is responsible for aging in this model is acetic acid, which appears in post – breeding fluid. Substantially increase CLS could act by either reducing the amount of acetic acid present in the environment or increasing the intrinsic resistance of the cells to acetic acid. In our case we deal with reducing the amount of acetic acid present in the environment as far as longevity strain is concerned. Studies have shown that cells of none of the strains, which have been analyzed, are more acetic acid-resistant.

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Acknowledgements

This work was supported by Grant no. 2013/09/B/NZ3/01352 National Science Centre.

P8.14

Chronic stress: functions of pivotal regulatory and energy brain systems

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The extent of development of present-day biochemistry of stress gives but a rough idea of the mechanisms of CNS cells adaptation to chronic stress as well as stress markers role in early brain ageing. It is sufficiently well-known that catecholamines and neurosteroids, along with functions of AOS, play an important protective role in the CNS. CNS functions considerably depend on the functional activity of mitochondria responsible for providing neuronal cells with ATP molecules. Progressing mitochondrial dysfunction is a leading determinant of brain ageing. This is accompanied by changed expression of genes related to inflammation, enzymes of protein processing, oxidative stress and neuronal growth. The most important prerequisite of brain ageing is believed to be impaired processes of neurogenesis which is regulated through neurotransmitter receptors expressed by neorotrophic and growth factors.
Aim of the work: to assess effects of chronic emotional stress on the functional activity of mitochondria and free amino acid metabolism in the rat brain.
Chronic emotional stress was simulated using the techniques of Desiderato O. and Tolmachev D.A. Animals were stressed daily during 20 min over 4 weeks. Mitochondria (MH) were isolated from rat large hemispheres and hippocamp by differential centrifugation. Concentrations of amino acids and biogenic amines were determined by HPLC in homogenates (1:10) after deproteinisation.
In contrast to acute stress, characterized by activation of mitochondrial functions, chronic stress was distinguished by either absence of decrease of MH functional response. The multiple exposure to 20-minute stress resulted in a decreased rate of succinate-stimulated respiration and elevated oxidation and phosphorylation conjugation. Nevertheless, after 24 hours of a post-stress period, large hemisphere MH showed activated tricarboxylic acid cycle enzymes (isocitrate dehydrogenase, aconitase) while hippocamp MH demonstrated decreased aconitase and MDH activities. The absence of changes in GSH and GPO concentrations along with decreased SOD activity in the large hemispheres (27%) and hippocamp (25.4%) and the tendency to LPO activation can be regarded as tension of defense systems. Effects of chronic emotional stress in the brain large hemispheres were marked by a considerable amino acid imbalance which can include impaired transport of aromatic amino acids into the brain and mediator synthesis in monoaminergic systems. The relationship of the stress effects found and their ranking in different brain regions require further studies. However, these data indicate a possible dysfunction of both energy metabolism and neurotransmitter systems.

Acknowledgements

The work was supported by the BRFFR, grant M13MC-0-34.

P8.15

Single chromosome comet assay-based analysis of chromosome abnormalities using selected yeast mutant strains

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Single-cell organism yeast *Saccharomyces cerevisiae* is considered a model to study molecular mechanisms underlying cytophysiological processes of higher eukaryotes. In a haploid state, the genome of budding yeast consists of sixteen chromosomes, which can be separated using pulsed-field gel electrophoresis (PFGE). PFGE-based approach allows for chromosome polymorphism and structural aberration analyses (e.g. translocations, deletions). However, cell populations are heterogeneous and large-scale karyotyping techniques provide us with a lot of average values, which reflects the average genetic characteristics of the population but does not give us information on the individual cell status. To address cellular heterogeneity, the development of single-cell level approaches is needed. More recently, we have modified the single cell electrophoresis protocol to evaluate DNA breaks at the chromosomal level (Lewinska A *et al.,* 2014, *Fungal Genet Biol* **63**: 9–16). After PFGE separation, yeast chromosomes have been subjected to the single chromosome comet assay, and DNA breaks have been investigated. The mutants lacking *BUB1*, *BUB2*, *MAD1*, *TEL1*, *RAD1* and *TOR1* genes, both in haploid and diploid hemizygous states, and their corresponding wild types have been analyzed and mutant- and chromosome-specific susceptibility to DNA breaks has been revealed.

Acknowledgements

This work was supported by Project No. IUVENTUS PLUS 0607/IP1/2011/71 from the Ministry of Science and Higher Education.

P8.16

The impact of NOX4 NADPH oxidase on proliferation and senescence of vascular smooth muscle cells

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Cellular senescence is proposed as one of the mechanisms that drives organism aging as well as age-related diseases. This process was shown to participate in pathogenesis of cardiovascular diseases since senescent vascular smooth muscle cells (VSMCs) were found in atherosclerotic plaques. Cellular senescence is related to unrepairable DNA double strand breaks and the activation of the DNA damage response (DDR) pathway as well as increased reactive oxygen species (ROS) production. Although defective mitochondria are considered to be the main source of ROS, a number of studies prove that ROS-generating enzymes like NADPH oxidases (NOX) could also play an important role in this process. Data suggests that NOX4 could be involved in the regulation of both proliferation and aging. The aim of this study was to elucidate the role of NOX4 in the senescence of human VSMCs (hVSMCs). We observed increased ROS production in hVSMCs undergoing stress-induced and replicative senescence. Treatment of the cells with DPI – NOX family inhibitor, decreased the level of ROS in proliferating and senescent hVSMCs. Suprisingly DPI did not affect senescence. In contrary, it caused permanent cell cycle arrest and appearing of the other senescence markers in proliferating hVSMCs. The DPI-induced senescence was not correlated with activation of the DDR pathway. To address the NOX4 exclusive role in these process we downregulated its expression with siRNA. Cells with reduced *NOX4* transcript accumulated in mitosis due to abnormal mitotic spindle formation. What is more, we observed activation of p53 and after a few days of culture cells underwent senescence. Majority of those cells were multinucleated proving that they underwent improper mitotic divisions on the road to senescence. What is interesting, disturbed nuclei morphology was also observed in replicatively senescing hVSMC what corresponded with gradual decrease of *NOX4* mRNA level. These findings suggest that NOX4-derived ROS play an important role in regulation of the cell cycle in hVSMCs; decreased level of this enzyme could be responsible for senescence induction.

Acknowledgements

This work was supported by grant 0728/B/P01/2011/40 financed by the Polish Ministry of Science and Higher Education and 2011/01/B/NZ3/02137 from the National Science Centre.

P8.17

p-53 independent senescence of cancer cells in response to DNA damage

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Cellular senescence is a state of irreversible growth arrest. Interestingly, cancer cells, which are immortal, preserve the capacity to senesce – upon DNA damage they can undergo so named stress-induced premature senescence (SIPS). In the process of senescence the key protein is p53, which transactivates *CDKN1A* encoding for cdk's inhibitor p21. We were interested whether other proteins than the p53 transcription factor can activate *CDKN1A* and induce senescencein cells treated with a DNA damaging agent. To this end we used cancer cell lines lacking p53 (HCT116 p53KO, H1299, H358) and one cancer cell line with mutated, non-functional p53 (A431), which we treated with Topo2 inhibitor, doxorubicin. We used the HCT116 p53 WT cells as a control. Cells were treated for 5 days with cell-line dependent cytostatic doses of doxorubicin (50nM to 100nM). After treatment with doxorubicin both p53 WT cells and cells without functional p53 displayed hallmarks of senescence, such as increased p21 expression, increased size and granularity, senescence-associated β-galactosidase (SA-β-GAL) activity as well as arrest in the G2/M phase of the cell cycle. Cells with both wild-type p53 and without functional p53 exhibited senescence-associated secretory phenotype (SASP) – they produced vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). Firstly, we examined the possible role of NF-κB in the induction of senescence. Western blot analysis of the total and phosphorylated key proteins from this pathway, such as IκBα, IKKα and IKKβ, showed no activation of NF-κB. Also, western blots performed on nuclear and cytoplasmic cell fractions of HCT116 p53KO and p53WT cells, did not show an increase in nuclear translocation of p65 after doxorubicin treatment. Moreover, silencing of p65 with siRNA in HCT116 p53KO and p53WT cells had no impact neither on SA- β-GAL activity nor on p21 expression. However, silencing of p65 unexpectedly caused a decline in the level of secreted IL-8, indicating the crucial role of NF-κB in SASP, which needs to be further elucidated. As the decline in IL-8 was not accompanied by a decrease in other markers of senescence, we can conclude that SASP is not inevitable in senescence of HCT116 cells. To further elucidate the p53-independent induction of p21 in HCT116 p53KO, H1299, H358 and A431 cells, we are currently investigating other possible pathways.

Acknowledgements

This work was supported by grant UMO2011/01/M/NZ1/01597 from the National Science Centre.

P8.18

Molecular mechanisms of neuronal senescence. Is DNA damage involved?

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Neuronal ageing has been linked to stress and DNA damage. One of the consequences of DNA damage is so-called stress-induced premature senescence (SIPS), whose main feature is irreversible growth arrest of mitotic cells. Our research hypothesis is that stress induced by DNA damaging compounds can also result in the acceleration of neuronal aging/senescence with its specific characteristics that could conform with the post-mitotic phenotype of neurons.
In order to test this hypothesis a model of primary rat neuronal cell culture was established and using viability assays (MTT and LDH release) low doses of doxorubicin were selected which did not cause massive cell loss but were sufficient to cause DNA damage (as observed by the γH2AX and p53BP staining). For the identification of senescent cells a common marker: an intercellular level of beta-galactosidase (SA-β-GAL) activity was applied. Importantly, due to significant presence of glial cells in the neuronal culture, senescent neurons were specifically identified by performing colocalization study of SA-β-GAL with a neuronal marker MAP2 protein. Firstly, it was observed that both neurons and glial cells gradually senesce in in vitro cortical culture and that about 50% of neurons with higher SA-β-GAL activity are present in 18div (days in vitro) culture. Moreover, the majority of these neurons had comparable to non-senescent neurons level of double-strand break foci in their nucleus. In both groups it was very low. Secondly, treatment of cultures at 14div with doxorubicin for 4 days caused DNA damage in both groups; however it did not change significantly the percentage of senescent neurons. Currently a long-term exposition of cultures to the compound is being tested. Finally, characterization of neuronal cultures in terms of the presence of other features, which could correlate with the augmented SA-β-GAL activity and the progress of senescence, is carried out. So far observations suggest that neuronal aging involves: changed morphology, lysosomes' number and alterations in the expression of cell cycle inhibitors.
In conclusion neuronal cells, as opposed to mitotic cells, treated with one of the DNA damaging compounds for short time do not enter senescence (identified by augmented SA-β-GAL activity). Moreover, it seems that chronological senescence of neurons in primary cultures is not associated with accumulation of DNA damage.

Acknowledgements

Supported by grant no. 2012/07/B/NZ3/02180 from National Science Centre.

P8.19

The impact of curcumin on the level of sirtuins 1 and 6 and DNA damage in endothelial cells derived from human aorta

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Aging of the vascular system is accompanied by senescence of cells building the vasculature. One of the most common pathologies observed in cardiovascular diseases (CVD) and which also belongs to age-related diseases, is atherosclerosis. In atherosclerotic plaques cells with markers of senescence, such as increased SA-β-Gal activity, inhibitors of the cell cycle and increased number of DNA double strand brakes (DNA DSB) have been found. Curcumin, a polyphenol used for millennia in natural medicine and food industry, for some time has also been studied in laboratories and clinics. This compound possesses anticancer, anti-inflammatory properties and up till now they are it's mostly appreciated and studied features. But for some years the anti-aging properties have been taken into consideration and today curcumin is a promising anti-aging factor. It has been shown that curcumin elongates the lifespan of some organisms like mouse or *C. elegans* and *D. melanogaster*.
The aim of this study was to establish the mechanism of potential modulatory activity of curcumin on cellular senescence. DNA damage is one of the most important causes of cellular senescence. Sirtuins, NAD^+-dependent deacetylases, are involved in DNA repair, telomere maintenance and increase lifespan of the model organisms. Therefore we decided to analyze the impact of curcumin on DNA damage and the level of sirtuins in endothelial cells. Moreover it is documented that some sirtuins are indispensable for homeostasis of the cardiovascular system. Curcumin belongs to hormetins, it means that it is harmful in high concentrations and beneficial in low ones. We show that curcumin in cytostatic concentrations induced cellular senescence and in senescent cells the level of sirtuins 1 and 6 temporarily increased what was followed by a decrease in the level of these proteins on the 7th day. However, the concentrations not affecting proliferation elevated the level of sirtuins in long term culture. Surprisingly the number of DNA DSB decreased in cells treated with all of the used concentrations of curcumin.
To summarize, our results suggested that curcumin can protect from the accumulation of DNA damage and increased the level of sirtuins 1 and 6, what could correlate with the protection of the cardiovascular system and could be responsible for its anti-aging properties.

Acknowledgements

Financial support: This work was supported by grant 2011/01/B/NZ3/02137 from the National Centre for Science.

P8.20

Inactivation of the paraoxonase 1 gene affects the expression of mouse brain proteins involved in neurodegeneration

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Elevated homocysteine (Hcy) is a risk factor for Alzheimer's disease (AD). Paraoxonase 1 (Pon1), named for its ability to hydrolyze and inactivate the organophosphate paraoxon, is synthesized in the liver and circulates in the blood attached to high-density lipoproteins (HDL). Pon1 participates in Hcy metabolism by hydrolyzing the toxic metabolite Hcy-thiolactone and is also linked to AD. The inactivation of the *Pon1* gene in mice causes the accumulation of Hcy-thiolactone in the brain and increases the susceptibility to Hcy-thiolactone-induced seizures (Borowczyk *et al.*, 2012, *JAD*).
To gain insight into the brain-related Pon1 function we used two-dimensional IEF/SDS-PAGE gel electrophoresis (2DE) and MALDI-TOF/TOF mass spectrometry to study brain proteomes of $Pon1^{-/-}$ mice and their $Pon1^{+/+}$ littermates fed a standard chow diet. We also studied how brain proteome is affected by hyperhomocysteinemia induced by a high-Met diet and examined the interaction between *Pon1* genotype and hyperhomocysteinemia. We found that: *1*) proteins involved in brain-specific function (Nrgn), antioxidant defenses (Sod1, DJ-1), and cytoskeleton assembly (Tbcb, CapZa2) were differentially expressed in brains of Pon1-null mice; *2*) proteins involved in brain-specific function (Ncald, Nrgn, Stmn1), antioxidant defenses (Prdx2, DJ-1), energy metabolism (Ak1), cell cycle (GDI1, Ran), cytoskeleton assembly (Tbcb), and unknown function (Hdhd2) showed differential expression in brains of Pon1-null fed with a hyperhomocysteinemic high-Met diet; *3*) most proteins regulated by the $Pon1^{-/-}$ genotype were also regulated by the high-Met diet; 4) the proteins differentially expressed in Pon1-null mouse brains play important roles in neural development, learning, plasticity, and aging and are linked to neurodegenerative diseases, including Alzheimer's.
Taken together, our findings suggest that Pon1 interacts with diverse cellular processes – from energy metabolism and anti-oxidative defenses to cell cycle, cytoskeleton dynamics, and synaptic plasticity – essential for normal brain homeostasis and that these interactions are modulated by hyperhomocysteinemia and account for the involvement of Hcy and Pon1 in AD.

Acknowledgements

Supported by NCN grants 2011/01/B/NZ1/03417, 2011/02/A/NZ1/00010, 2012/07/B/NZ7/01178, 2013/09/B/NZ5/02794).

P8.21

Endogenous factors responsible for the fenotype of human cells defective in Fanconi anemia pathway

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Fanconi anemia (FA) is complex inherited disorder characterized by increased incidence of cancer and premature senescence. The disease is caused by mutations in distinct genes, which encode proteins engaged in DNA metabolism. Cells derived from FA are characterized by genomic instability and display increased sensitivity to DNA crosslinking agents and oxidative stress. However, it is unknown whether endogenous DNA damage may constitute a substrate for FA proteins. It was shown that during oxidative stress secondary products of reactive oxygen species – lipid peroxidation (LPO) products are intensively generated. The aim of our study was to determine the role of LPO products in etiology of the FA. For this purpose human fibroblasts and lymphoblasts derived from patients with FA complementation group D2 (*FANCD2*$^{-/-}$) and fibroblasts with reversed wild type phenotype (*Micro hybrid,* MH) or lymphoblasts from healthy individuals (WT) were used. We investigated the sensitivity and proliferation of *FANCD2*$^{-/-}$ and MH or WT cells to three major aldehydes – end products of LPO: 4-hydroxy-2-nonenal (HNE), crotonaldehyde (CRO) and acrolein (ACR). These compounds are very reactive and can form adducts to DNA and proteins as well as DNA-DNA and DNA-protein crosslinks. Our results demonstrated hypersensitivity of *FANCD2*$^{-/-}$cells to all the compounds but the biggest difference in viability between *FANCD2*$^{-/-}$ and WT cells was in the case of HNE treatment. Next, we examined DNA damage response by Western blot analysis of signaling kinases. In response to HNE and CRO, we found increased phosphorylation of ATM, ATR and Chk2 kinases in FA cells; however, activation of Chk1 kinase was inhibited. To investigate how LPO products may influence DNA replication and whether FA cells are more or less prone to stalling/collapse of replication forks in response to HNE and CRO, we used DNA fiber technique. Our results demonstrated decreased velocity of replication both in *FANCD2*$^{-/-}$ and WT cells but effect was stronger in case of mutated cells. The number of stalled replication forks was increasing linearly with concentration of aldehydes. We also measured the cell cycle of *FANCD2*$^{-/-}$ fibroblasts after treatment with HNE by flow cytometry and found accumulation of cells in subG1 and G1 phase. These results suggest that lipid peroxidation may contribute to FA phenotype by inducing DNA damage, which is not repaired by accurate HR system, inhibiting replication and cell cycle progression.

Acknowledgements

This work was supported by the grant from National Science Center UMO-2012/05/B/NZ5/01392.

P8.22

DNA repair in retinal pigment epithelial cells arrested in G0/G1 checkpoint

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Age-related macular degeneration (AMD) is a leading cause of vision loss in developed countries. Oxidative stress, which is strongly linked to AMD pathogenesis and progression, causes DNA damage, which may induce DNA repair and/or cell cycle arrest. Retinal pigment epithelial cell (RPE) are arrested in G0 phase, however, little is known about the DNA repair in cells arrested in G0/G1 checkpoint. The aim of our research was to investigate the kinetics of DNA repair in cells arrested in G0/G1 checkpoint in response to oxidative stress. We induced acute stress to check the cell ability to immediately overcome damage, and chronic stress to analyse whether cells adapt to the insult.
We employed ARPE-19 cell line, derived from RPE and induced cell cycle arrest through a combination of contact growth inhibition, restriction of growth factors and the presence of retinoic acid (RA). For the generation of acute oxidative stress we used hydrogen peroxide or *tert*-butyl peroxide and for chronic oxidative stress – glucose oxidase – which continuously produce hydrogen peroxide in the presence of medium. The cell viability was assessed using trypan blue exclusion assay and DNA damage by comet assay.
Increasing cell density along with RA treatment induced cell cycle arrest associated with morphological changes in APRE-19 typical for terminally differentiated cells *in vivo.* We observed that there was no difference in the level of DNA damage after the generation of oxidative stress and at the late stage of DNA repair (from 30' to 60') between ARPE-19 cells arrested in G0/G1 and cycling cells. Analysis of repair kinetics in ARPE-19 cells arrested in G0/G1 showed a clear defect at the intermediate (from 5' to 15') time points following generation of acute oxidative stress. In contrast, there was no repair defects at any time point in ARPE-19 cells arrested in G0/G1 after induction of chronic oxidative stress.
In summary, we suggest that RPE cells may have impaired DNA repair in response to acute oxidative stress due to their arrest in G0 phase, because all RPE cells *in vivo* are in this phase, and this association may contribute to the pathogenesis of AMD.

Acknowledgements

This work was supported by the The National Science Centre, decision nr DEC-2012/07/N/NZ3/01755.

P8.23

Nanoparticle health risk assessment: Pro-senescent activity of silver, silica and diamond nanoparticles in selected normal and cancer cell lines

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Nanoparticles and nanomaterials are widely used in nanomedicine and nanotechnology. Nanoparticle-based biomedical applications include biosensors/biochips and biomedical nanorobots, drug, gene and protein carriers, protein purification, implant coating and imaging probes, which is particularly useful for e.g. tumor targeting, intravascular bioimaging, diagnostic and therapeutic purposes. Biocompatibility is the capability of being biologically compatible by not producing a cytotoxic (systemic and local), genotoxic, mutagenic, carcinogenic, immunogenic and other responses in living systems (cells, organisms) and nanoparticles are considered relatively biocompatible. Nevertheless, further studies are needed for better understanding of hazards of occupational or environmental exposure to nanomaterials and to design biologically inert, no-inflammatory and biocompatible nanoparticle-based devices for nanomedicine. In the present study, pro-senescent activity of silver, silica and diamond nanoparticles in normal human dermal fibroblasts (HDFs), breast (MCF7), kidney (ACHN), lung (A549) and cervical (HeLa) cancer cell lines have been analyzed. Nanoparticle-mediated cytotoxicity, cell proliferation, changes in the cell cycle, apoptosis and genotoxicity have been investigated. Moreover, the nanoparticle ability to promote stress-induced premature senescence (SIPS) has been studied, namely senescence-associated beta-galactosidase activity, oxidative stress parameters, p53, p21 and p16 levels, DNA damage and repair, and telomere status: telomere length, telomerase activity and TRF1 and TRF2 levels. Results will be presented and discussed.

Acknowledgements

This study was supported by the Operational Programme – Innovative Economy 2007-2013 MNISW 19/POIG/GP/2013.

P8.24

Alterations of intracellular structures and function of the yeast *Saccharomyces cerevisiae* hypertrophic cells

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The yeast *Saccharomyces cerevisiae* has been proposed as a simple, eukaryotic model organism for the studies of aging process especially due to its limited proliferative capacity and the assumption that basic mechanisms of aging are conserved among eukaryotic organisms. Aging of the yeast *S. cerevisiae* is measured by the number of buds produced by a mother cell and it is referred as replicative life span. This phenomenon is observed also for some kind of human cells, which can divide only a limited number of times. The phenomenon of limited reproductive capacity is still not well understood however the most accepted view links this limit to the gradual accumulation of molecular damage in the yeast mother cell. Our observations suggest however, that the potential cause of the reproduction limit in the budding yeast cells may be a hypertrophy and its consequences on the cellular and molecular level. The gradual increase in the yeast cell size which occurs during replicative lifespan and leads to hypertrophy, results from budding as the atypical cytokinesis mechanism.

The aim of the study was to analyse whether the increase in the cell size leads to the morphological and structural changes of cells, which could explain the differences in their reproductive capacity. We took into consideration especially these parameters which may have influence on the reproductive potential. These analysis were performed on the population of young (virgin) cells in which the increase in the size was induced by the pheromone (pheromone alpha for the *MAT*a cells). We applied the fluorescence technique and specific fluorescent dyes to examine the metabolic status and alterations of intracellular structures i.e. nucleus, mitochondria, vacuoles, actin cytoskeleton in the young yeast cells significantly differing in cell size and reproductive capacity. Our results show that the increase in the cell size does not cause the significant decrease of the cell metabolic activity or essential intracellular alterations but it causes the significant decrease of the reproductive potential. Based upon these results we suppose that other consequences especially on the molecular level, whose are connected with increase in the cell size, may have more essential influence on the cell ability to reproduce than the structural alterations.

Acknowledgements

This research was supported by grant no 2013/09/B/NZ3/01352 National Science Centre.

P8.25

DNAtraffic — a new database for systems biology of DNA dynamics during the cell life with the direct links to human diseases

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DNAtraffic (http://dnatraffic.ibb.waw.pl/) is dedicated to be a unique comprehensive and richly annotated database of genome dynamics during the cell life.
The main goal of the DNAtraffic database is to collect data from other well known and commonly used databases (such as: UniProt, OMIM, NCBI, KEGG, PDB, DrugBank, and other) and organize them. The DNAtraffic database contains extensive data on the nomenclature, ontology, structure and function of proteins related to the DNA integrity mechanisms such as chromatin remodeling, histone modifications, DNA repair and damage response from eight organisms: *Homo sapiens, Mus musculus, Drosophila melanogaster, Caenorhabditis elegans, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Escherichia coli* and *Arabidopsis thaliana*. DNAtraffic contains comprehensive information on the diseases related to the assembled human proteins. DNAtraffic is richly annotated in the systemic information on the nomenclature, chemistry and structure of DNA damage and their sources, including environmental agents or commonly used drugs targeting nucleic acids and/or proteins involved in the maintenance of genome stability. One of the DNAtraffic database aim is to create the first platform of the combinatorial complexity of DNA network analysis. Database includes illustrations of pathways, damage, proteins and drugs. Since DNAtraffic is designed to cover a broad spectrum of scientific disciplines, it has to be extensively linked to numerous external data sources. Our database represents the result of the manual annotation work aimed at making the DNAtraffic much more useful for a wide range of systems biology applications.
DNAtraffic can be queried by the name of pathway, damage, protein, disease and some useful keywords, and provides the links to internal data sources and external databases.
The DNAtraffic database is freely available and could be accessed at http://dnatraffic.ibb.waw.pl.

Reference:

Kuchta *et al* (2012) DNAtraffic — a new database for systems biology of DNA dynamics during the cell life. *Nucleic Acids Res* 40 (Database issue): D1235-40; doi: 10.1093/nar/gkr962.

Acknowledgements

This work was supported by the grant (nr. DZP/POL-NOR/251/2013) from Norway Grants through the Polish-Norwegian Research Programme operated by The National Centre for Research and Development (NCBiR).

P8.26

Functional characterization of proteins involved in DNA repair and genome stability

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The variety of bacterial homologues in higher organisms raise a question for discussion about the biological function of gene amplification. Such multiplications of genes underlie their pivotal role in fundamental metabolism concerning DNA. Understanding the alterations of the DNA damage recognition by the active site among DNA repair proteins is crucial for development of fundamental knowledge about functioning of the genome integrity network.
Our innovative research approach is focused on structural and functional (*in silico* and *in vitro*) analysis of DNA damage and DNA repair proteins in order to annotate their function (*in vitro* and *in vivo*) in nucleic acids repair pathways. We are able to explain the gene/protein multiplication by simple bacterial or yeast study of the null gene(s) strains versus its mammalian, bacterial, and yeast's replacement (structural or functional). We collected data about DNA lesions and analyzed them as three-dimensional (3D) as well as flat structures (2D). Surprisingly, well known DNA damage showed us its unknown face. Our pilot study and preliminary results showed that some type of DNA lesions which form base dimers (UV-induced damage) or adducts (cisplatin-induced crosslinks) adopt similar 3D structure in dsDNA and mimic other type of DNA lesions which can be recognized by the AlkB-like proteins.
Bacterial dioxygenase AlkB protein oxidatively demethylates 1meA and 3meC in DNA resulting in the recovery of the natural adenine (A) and cytosine (C) bases. Among human homologues of bacterial AlkB, ALKBH1-9, there are two proteins, ALKBH3 (PCA, prostate cancer antigen) and ALKBH9 (FTO, fat mass- and obesity-associated), known to affect human health.
In our study we focus on bacterial AlkB and its human homologues (ALKBH2 and ALKBH3) towards their recognition, binding and *in vivo* and *in vitro* removal in a direct manner of anti-cancer drug and UV-induced DNA lesions.

Acknowledgements

This work was supported by the grant (nr. DZP/POL-NOR/251/2013) from Norway Grants through the Polish-Norwegian Research Programme operated by The National Centre for Research and Development (NCBiR).

P8.27

How low concentrations of Bisphenol-A can alter human implantation and pregnancy: an in vitro study on decidualized endometrial stromal cells and placenta chorionic villous explants

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Introduction. Bisphenol A (BPA) is an environmental pollutant which may influence human reproductive health. This chemical is able to interfere with the delicate balance of the endocrine system by mimicking the action of steroid hormones. BPA can be transferred from the mother to the embryo and cause reproductive and developmental toxicity. We investigated the effects of BPA on *in vitro* models, representative of tissues at the feto-maternal interface, namely of human endometrium and placenta.
Methods. Firstly, stromal cells were isolated from healthy endometrial tissues and *in vitro* decidualized by priming with steroid hormones (17β-estradiol and progesterone). Decidualized stromal cells were exposed to 1 nM BPA for 24 hours. ontrol cultures were exposed to vehicle (0.1% ethanol). The protein and mRNA expression of Estrogen (ERα, ERβ), Progesterone (PRA, PRB), and human Chorionic Gonadotropin/ Luteinizing Hormone (hCG/LH) receptors was investigated by western blot and qrt-PCR.
Secondly, villous explants were isolated from human placenta and cultured on matrigel. Villous explants were exposed to 1 nM BPA for 24 and 48 hours. Control cultures were exposed to vehicle (0.1% ethanol). The secretion of β-hCG was measured by ELISA.
Finally, in order to study the role of the endometrium in fetal contamination, placental cultures were exposed to the cell-conditioned medium derived from BPA-treated endometrial cells. The effect of endometrial-conditioned medium on β-hCG secretion was investigated by ELISA assay, while the content of free-BPA in the culture media was detected by GM-MS analysis.
Data and Conclusions. BPA was able to up-regulate the expression of ERs and PRs (P0.05) and to down-regulate the expression hCG/LHR in endometial cells (P0.05), resulting in an altered endometrial environment. Furthermore, BPA riggered the secretion of β-hCG by the placenta (P0.05). The exaggerated secretion of β-hCG observed in placental cultures after BPA exposure was abolished when the placentl cultures were exposed to endometrial-conditioned medium. This effect was probably due to an accumulation of BPA inside endometrial cells, as shown by GM-MS analysis. Endometrial segregation of BPA made the pollutant less available to the placental tissue. On the other hand, endometrial cells were able to release the stored BPA into the surrounding environment.
Altogether, these data show that low BPA concentrations, that can be easily detected in the population, are able to influence endometrial and placental physiology. Even though a protective effect of the endometrial cells was identified, the negative outcomes of a long – term exposure to BPA cannot be easily predicted. BPA exposure could lead not only to negative effects on pregnancy establishment and development, but also trigger or exacerbate pathologies of the reproductive tract.

Supported by the NSC Grant Preludium 2013/09/N/NZ5/03062