Session 27: Inflammation and Cancer

Lectures

L27.1

RIPK4 maintains epidermal homeostasis and prevents skin cancer by suppressing mitogenic signaling

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The skin is a fast renewing organ with continuous commitment of proliferative progenitor keratinocytes into a terminal differentiation program forming the stratified epidermis. RIPK4, a serine/threonine kinase, is crucial during epidermal development. To elucidate RIPK4 function in keratinocytes, we generated epidermis-specific RIPK4 KO mice (RIPK4EKO). In contrast to RIPK4 full KO epidermis, RIPK4EKO epidermis was normally stratified and the outside-in skin barrier in RIPK4EKO mice was largely intact at the trunk, in contrast to the skin covering the head and the outer end of the extremities. However, RIPK4EKO mice die shortly after birth due to excessive water loss because of loss of tight junction protein claudin-1 localization at the cell membrane, which results in tight junction leakiness. In contrast, mice with tamoxifen-inducible keratinocyte-specific RIPK4 deletion during adult life remain viable. Inducible RIPK4 deletion in adult mouse epidermis caused significant hyperplasia due to the expansion of proliferative basal keratinocytes. Although epidermal keratinocytes eventually commit to differentiation, the barrier is dysfunctional, witnessed by increased transepidermal water loss, coinciding with local immune infiltration. Using ex vivo cultures of primary keratinocytes we found that RIPK4 enables cell cycle exit by suppressing p63 expression in a keratinocyte autonomous manner. Additionally, RIPK4 down-regulates EGFR expression and its downstream ERK and STAT3 mitogenic signaling pathways in a kinase-dependent manner in mouse and human keratinocytes. Loss of RIPK4 led to spontaneous tumor formation of the keratoacanthoma type, with reduced latency by additional deletion of tumor suppressor p53. Furthermore, RIPK4 serves as a brake on tumor growth driven by an oncogenic KrasG12D knockin transgene. Together, our work demonstrates that RIPK4 fulfills a central role in maintaining the homeostatic balance between keratinocyte proliferation and differentiation by suppressing p63 expression and EGFR signaling and providing a tumor suppressive function in clinically relevant genetic squamous skin cancer models.

Oral presentations

O27.1

Epidermal MCPIP1 function is essential to maintain proper skin homeostasis

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Previous studies indicated that monocyte chemotactic protein induced protein 1 RNase (MCPIP1) is expressed in human epidermis and suggested involvement of MCPIP1 in psoriasis-like inflammation. In this study, we show the importance of epidermal MCPIP1 function in maintaining proper physiology of the mouse skin. We generated conditional keratinocyte-specific knock-out mice (Mcpip1^{EKO}) and observed an altered keratinocyte differentiation pattern and increased epidermal proliferation. We performed RNA Sequencing of mouse primary keratinocytes isolated from newborn mice and shortlisted 927 genes to be differentially expressed between the Mcpip1^{EKO} and wild-type (WT) cells. The upregulated transcripts in the Mcpip1^{EKO} epidermis were enriched for those involved in keratinocyte differentiation and positive regulation of immune responses, such as the Il36 cytokines. The upregulation of proinflammatory cytokines is in keeping with the development of spontaneous chronic skin inflammation in adult Mcpip1^{EKO} mice. Furthermore, we show that loss of Mcpip1 in the epidermis leads to aggravated imiquimod (IMQ)-induced skin inflammation. The induction of the Il23/Il17 axis following topical daily application of IMQ on the back of mice skin occurred earlier in the Mcpip1^{EKO} than WT mice, in line with the observed phenotypic features. Taken, we show that epidermal expression of Mcpip1 is crucial to maintain proper function of the mouse skin.

Acknowldgements:
This study was supported by the National Science Centre grant number 2014/13/N/NZ3/00729 (to PK) and 2016/23/B/NZ3/00792 (to JJ).

O27.2

Levels of *NPM1* alternative transcripts in acute myeloid and lymphoblastic leukemia

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Expression of the *NPM1* gene, encoding nucleophosmin, is upregulated in many cancers. However, in acute myeloid leukemia (AML), the mutational status of the gene seems to play a pivotal role in carcinogenesis and *NPM1* expression has not been widely studied so far. Using droplet digital PCR, we analyzed the levels of three protein-coding *NPM1* transcripts in 66 samples collected from AML and acute lymphoblastic leukemia (ALL) patients and 16 control samples. The levels of the particular transcripts were different but highly correlated with each other. Transcript *NPM1.1*, encoding the longest protein (294 aa), had the highest level of accumulation and was one of the most abundant transcripts in the cell. Comparing to *NPM1.1*, the level of the *NPM1.2* transcript, encoding a 265-aa protein, was 30 times lower whereas the level of the *NPM1.3* transcript, encoding a 259-aa protein, was 3 times lower. All three *NPM1* transcripts were upregulated in both types of acute leukemia compared to control samples, decreased after therapy and increased again with relapse of the disease. At the time of diagnosis, the *NPM1.2* level was decreased in *NPM1*-mutated patients. We found that better prognosis is associated with low levels of at least two transcripts *NPM1.1* and *NPM1.3*. Using RNA-seq data, we detected 8 additional *NPM1* transcripts, including non-coding splice variants with retained introns. However, the contribution of non-coding transcripts to the total level of *NPM1* gene was marginal.

O27.3

The non-canonical poly(A) polymerase *FAM46C* regulates B-cells proliferation and acts as an onco-suppressor in multiple myeloma

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Cytoplasmic RNA polyadenylation carried out by non-canonical poly(A) polymerases (ncPAPs) is important post-transcriptional process affecting genes expression with a broad spectrum of physiological significance [1]. Using a combination of *in vitro* and *in vivo* approaches we have demonstrated experimentally for the first time that *FAM46C* encodes an active non-canonical poly(A) polymerase which enhances mRNA stability and gene expression [2]. To address *FAM46C* gene physiological relevance we have constructed FAM46C-FLAG knock-in mouse strain and showed that the protein level is strongly enhanced during activation of naïve B cells form spleen [2]. Moreover B lymphocytes isolated from generated *FAM46C*$^{-/-}$ mice proliferate significantly faster than those isolated from their WT littermates.

Interestingly, *FAM46C* is one of the most frequently mutated genes in multiple myeloma (MM) cancer of plasma cells, which are lineage of terminally differentiated B cells. Reintroduction of active *FAM46C* into MM stable cell lines, but not its catalytically-inactive mutant, leads to broad polyadenylation and stabilization of mRNAs strongly enriched with those encoding endoplasmic reticulum-targeted proteins and induces cell death [2]. Moreover, silencing of *FAM46C* in MM cells expressing WT protein accelerate their proliferation. Concluding, our data clearly indicate that *FAM46C* works as an B-lymphocyte proliferation regulator and act as onco-suppressor of multiple myeloma cells which is an essential factor for their survival.

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O27.4

(Serum) cancer biomarkers or inflammation biomarkers?

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Inflammation, particularly chronic inflammation, is often linked to cancer development. On one side, immune cells induce inflammation that can remove cancer cells. On the other hand, however, tumor-associated inflammation can enhance tumorigenesis by modifying tumor microenvironment and inducing genome instability. In addition, different factors released by inflammation-related cells could stimulate cancer cells and suppress the anti-tumor activity of immune system. Hence, cancer-associated inflammation is generally known as the tumor enabling feature.
Nevertheless, cancer and chronic inflammation coexist in the body of patients suffering from malignancy. Hence, molecular features of inflammation and cancer disease interfere with each other at the systemic level. As a consequence, several hypothetical "cancer biomarkers" detected in the circulation of cancer patients are mechanistically associated with chronic inflammation rather than cancer itself. These inflammation-bound factors could be used in clinical practice for diagnosis and prognosis of cancer, yet a full understanding of their actual nature would help better interpretation of cancer-directed tests. Examples of inflammation-related factors that appeared in proteomics studies directed on the discovery of biomarkers for detection, classification, and prognosis of cancer are presented and discussed.

Posters

P27.1

Depletion of histone H3K27me3 is an epigenetic stress that induces cellular senescence in fibroblasts

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Cellular senescence is a permanent growth arrest that is triggered by irreparable DNA-damage. Recent evidence suggests that damage to the epigenome can have the same effect and outcome, however, this mechanism is poorly understood. We set out to identify readers, writers, and erasers of epigenetic marks that are capable of inducing a senescence response in the absence of DNA-damage stimuli. Our screening strategy was setup to ectopically overexpress proteins inside proliferating human fibroblasts. Transfection of DNA-constructs, followed by assays for senescence-associated biomarkers, enabled us to screen 58 factors, including bromodomain (BRD)-containing proteins, polycomb-group (PcG) proteins, jumonji containing (JmjC)-domain demethylases, histone deacetylases (HDACs), DNA methyltransferases (DNMTs), and Ten-eleven translocation (TET) dioxygenases. Overexpression of the histone H3K27 demethylase, jumonji domain-containing protein 3 (JMJD3), was found as a prominent inducer of senescence. The removal of histone H3 tri-methyl (H3K27me3) by JMJD3 causes chromatin relaxation, a form of epigenetic stress leading to large-scale changes in chromatin landscape. Paradoxically, the depletion of H3K27me3 contributes to the development of cancer cells. Our findings suggest that upregulation of JMJD3 in senescent cells is an important signaling event that has context-dependent oncogenic and tumor suppressive functions.

Acknowledgments:
This study was supported by grant SONATA 11, 2016/21/D/NZ3/00641 from the National Science Center, Poland.

P27.2

The role of HAX-1 and MCPIP proteins in regulation of transcripts involved in pro-inflammatory response of cancer cells

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HAX1 (HCLS1-associated protein X 1) protein is involved in apoptosis, migration, regulation of calcium ions homeostasis and probably plays a role in innate immune response (Kostmann disease). HAX1 was identified also as an RNA binding protein with known interaction with vimentin (2003) and DNA polymerase beta (2007) transcripts. Those interactions suggest its post-transcriptional regulatory function. HAX1 is a protein engaged in development of cancer but still its role in cancerogenesis stays unknown. RNase MCPIP1 (Monocyte chemoattractant protein-induced protein 1) has a well-established role in inflammation and indirectly, in carcinogenesis. The inflammation is tightly associated with the development of cancer and growing cancer promotes the expression of pro-inflammatory factors. MCPIP1 controls an immune response by destabilization of mRNAs encoding immune related proteins including IL-6 and IL-12p40 *via* their 3' untranslated regions (UTR). MCPIP1 auto-regulates its own mRNA level through interaction with similar stem-loop in the transcript. So far, the molecular basis of MCPIP1 interaction with RNA remains unclear, since no sequence specificity has been found.

To verify the hypothesis that HAX-1 can interplay with MCPIP1 protein we tested this potential interaction using co-immunoprecipitation method. We found out that both proteins co-precipitate. Additionally, to confirm that result, BIFC (BImolecular Fluorescence Complementation) experiments were performed. To show potential role of this interaction, we tested levels of both proteins in clear cell renal carcinoma (ccRCC) clinical samples. Amounts of HAX1 and MCPIP decreased in ccRCC tissues comparing to normal tissue.

CRISPR experiments were also performed to show how the molecular mechanisms in the cell are changed in HAX1 or MCPIP knockout cells (like in ccRCC clinical samples). Results of this project are expected to bring the new knowledge about mechanisms how and where HAX-1 and MCPIP1 proteins cooperate in the cells to maintain the internal homeostasis between cells and inflammation process.

Acknowledgments:
This study was supported by grant PRELUDIUM 9, 2015/17/N/NZ1/00668 from the National Science Center, Poland.

P27.3

Identification of the cellular partners of the proapoptotic HtrA4 protease

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The HtrA (high temperature requirement A) family proteins are serine proteases very well conserved in evolution. Their dysfunction may cause severe diseases, including the neurodegenerative disorders and cancer. There are four human HtrA proteins, HtrA1-4, and changed levels of these proteins have been found in many cancer tissues. The knowledge concerning the function of the HtrA4 protein is limited. It is known that it participates in embryo implantation and formation of placenta. So far, only three HtrA4 cellular partners have been identified: the HtrA1/3 and syncytin-1. The aim of this study was to identify cellular partners of the HtrA4.

Using the pull down and LC/MS techniques we identified a panel of cellular proteins interacting with HtrA4. They include: (1) cytoskeleton structural proteins and those involved in its formation, (2) proteins involved in cellular homeostasis and cell death, (3) participating in cellular transport and (4) connected with DNA replication. Using western blotting and immunoprecipitation we found that HtrA4 forms complexes with actin, β-tubulin, TCP1α, S100A6 and XIAP both *in vitro* and *in vivo*. We showed that HtrA4 protease promotes death of cancer cells treated with a chemotherapeutic drug and cleaves recombinant XIAP. Collectively, our results suggest that HtrA4 is involved in modulation of cytoskeleton stability and regulation of cell death. We believe that these results provide new insight into the function of HtrA4 in the cell.

Acknowledgements:
This work was supported by the National Science Center (Poland), project grant no. UMO-2016/21/N/NZ3/03263 to TW.

P27.4

Gain-of-function complement C2 mutant as a supporter of anti-CD20 therapy in lymphoma *in vitro* model

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Introduction of anti-CD20 mAbs has revolutionized the management of lymphoma and leukemia patients, and remains the first line treatment. However, many patients do not respond to treatment or develop a resistance mainly due to overexpression of complement inhibitors by tumor cells. These proteins inactivate particular components of complement cascade and disable pivotal effector mechanism. Due to their activity, the majority of early complement proteins deposited onto the target cells after introduction of anticancer antibodies will not support the terminal stages of the cascade but instead will be unproductively depleted. The problem mostly concerns early complement components (e.g. C2) as their serum concentration can be at least ten times lower than other complement proteins. Thus, C2 emerges as a bottleneck of complement activation pathway and a limiting factor of successful anti-CD20 therapy. We hypothesized that supplementation of anti-CD20 mAbs with complement C2 mutant resistant to cancer-derived complement inhibitors may significantly improve the efficacy of complement-mediated cell killing. We have tested a panel of single and multiple C2 mutants crated based on naturally occurring mutations in homologue protein factor B. Proteins were analysed as the supporters of anti-CD20 cytotoxicity on various lymphoma cell lines. Obtained results clearly indicate that administration of multiple GOF C2 mutant can significantly potentiate an *in vitro* activity of anti-CD20 mAbs.

P27.5

Determination of proteolytic activity in urine samples

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Urine has long been a „favored" biofluid among researchers. It is sterile, easy-to obtain in large volumes, largely free from interfering proteins or lipids and chemically complex. However, this chemical complexity has also made urine a particularly difficult substrate to fully understand. As a biological waste material, urine typically contains metabolic breakdown products from a wide range of foods, drinks, drugs, environmental contaminants, endogenous waste metabolites and bacterial by-products. Many of these compound are poorly characterized and poorly understood [1]. Being an important and easily accessible biological fluid, urine has been the subject of detailed chemical analysis for more than 100 years [2]. In 2016 our research group published a paper where we have described the optimization and synthesis of an internally quenched fluorescent substrate of the 20S proteasome, and investigation of its use as a potential diagnostic test based on urine samples in bladder cancer [3].
Herein, we describe the synthesis and application of novel chromogenic substrate designed to target the proteolytic activity of urine samples collected from patients with diagnosed bladder cancer. We also examined the presence of proteins in selected samples by SDS-PAGE.

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P27.6

Synthesis and biological evaluation of RTD-2 analogues against selected breast cancer cell lines

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RTD-2 (*Rhesus theta-defensin*) peptide belonging to θ-defensins family displays a broad range of antifungal, antibacterial, and antiviral activities [1]. They are relatively non-cytotoxic against mammalian cells, human fibroblast and show little hemolytic activity what makes them promising scaffolds for peptide drugs [2].
In our previous research [3] we were able to show that simplified Ser rich analog of RTD-2 ([Ser3,7,12,16]RTD-2), possessing only one disulfide bridge and head-to-tail cyclisation is cytotoxic to breast cancer cell lines. Taking it a step further, we decided to investigate participation of individual residues or amino acids sequence in regulation of anticancer activity. Therefore, the main aims of our research included:
Design and synthesis of RTD-2 analogues which served as tools to elucidate its anticancer properties. The synthesis was performed using Fmoc/tBu chemistry, with automatic peptide synthesizer or manual synthesis.
In vitro analyses of RTD-2 action in breast cancer cells. Cytotoxic potential of synthesized compounds was determined in two systems: – classical 2D proliferation in 96 well plates and – 3D matrigel cultures.
Cytotoxic properties of RTD-2 analogues were tested against MDA-MB-231, SKBR3 and HB2 cell lines.

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P27.7

Comparison of cellular response induce by unsymmetrical bisacridine derivative, C-2045 and its monomer subunit, C-1311 in HCT116 and H460 cancer cells

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In recent years new promising drugs – unsymmetrical bisacridine derivatives, which exhibited high cytotoxic activity against a lot of experimental cellular and tumor models, were developed in our laboratory. Bisacridine derivative C-2045 is a combination of two monomer acridines previously developed and studied by our team, among them C-1311 derivative. The aim of this study was to compare cytotoxic and cellular effects triggered by C-2045 and C-1311 in human colon HCT116 and human lung H460 cancer cells. Our studies revealed that C-2045 exhibited much more higher cytotoxic activity against both tumor cell lines than C-1311: IC_{90} values for C-2045 was 0.3 μM against both cell lines and 10 μM and 11 μM for C-1311 against HCT116 and H460 cells, respectively. Further studies on cellular response showed that C-2045 at IC_{90} dose induced accumulation of cells in G2/M phase of cell cycle (stronger in HCT116 than in H460 cells), DNA degradation (sub-G1 fraction) and subsequent apoptosis (confirmed by the changes in nucleus morphology and phosphatidylserine externalization). In turn, C-1311 caused cell cycle arrest in G2/M phase of HCT116 cell cycle and weak accumulation in the G1 phase of H460 cells. C-1311 induced necrosis, but in a small amount of cells in both cell lines. In conclusion, bisacridine derivative C-2045 is more effective than parental drug C-1311 against colon and lung cancer cells.

References:
These studies were supported by the National Science Center, Poland, Grant No. UMO-2016/23/B/NZ7/03324.

P27.8

Inefficient repair of oxidative DNA lesions and DNA double strand breaks – the missing link between Rheumatoid arthritis and cancer

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Rheumatoid arthritis (RA) is a systemic, inflammatory disease of the joints and surrounding tissues. RA manifests itself with severe joint pain, articular inflammation and oxidative stress. RA is associated with certain types of cancer. We have assumed that increased susceptibility to cancer of RA patients may be linked with genomic instability induced by disturbed DNA repair.

The aim of this work was to analyze the sensitivity of mononuclear cells isolated from RA patients to DNA damaging agents: tert-Butyl hydroperoxide (TBH) and bleomycin and calculate the repair efficiency. TBH induce oxidative DNA lesions whereas bleomycin mainly DNA double strand breaks. We included 13 rheumatoid arthritis patients and 13 healthy controls and used alkaline version of comet assay to measure sensitivity to DNA damaging agents and DNA repair efficiency.

The cell sensitivity and repair efficiency are statistically different between the groups studied.

The levels of DNA damage induced by bleomycin (RA-48.4 *vs* 24.9 in control) as well as oxidative stress (RA-22.3 *vs* 11.4 in control) was statistically higher in RA patients than in healthy subjects. We also observed inefficient DNA repair in RA patients as compare to control (ROC area curve 0.7931; $p<0.001$ for bleomycin and ROC area curve 0.6216; $p<0.05$ for TBH). These data suggest disturbed DNA repair process in RA patients.

Acknowledgement:
This work was supported by the National Science Center (Poland) - UMO-2017/25/B/NZ6/01358.

P27.9

Antihistaminic and cytotoxic activity of new diarylsulfones

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Diaryl sulfones are a class of organic compounds with a wide range of biological activities that are characterized by the presence of a sulfonic group with various substituents. We have tested 8 new heterofunctional diaryl sulfones for the presence of anti-inflammatory activity on rat mast cells, as well as for presence of a cytotoxic effect on cell lines HeLa and K562 and human fibroblasts. It was established that the compounds Vas557, Vas520 and Yan286 block the release of histamine induced by the substance 48/80 within the concentration range from 0.001 to 0.1 μM. The spontaneous release of histamine was influenced only by the compound Vas557, which caused a 7-fold release of the mediator at a concentration of 0.1 μM. With a decrease in the concentration of this substance, a sharp decrease in the inflammatory effect was observed.

These substances have a pronounced effect on K562 and HeLa cell lines and human fibroblasts only with a preparation concentration of 0.1 μM. When the concentration of compounds is reduced, the cytotoxic activity reduces sharply. The preparation Yan 286 dose-dependently stimulated growth of the HeLa cellular culture.

Based on the results obtained, it can be concluded that compounds Vas520 and Yan286 can be used by basic structures for further synthesis of anti-inflammatory preparations. However, these substances cannot be considered as antitumor drugs, since they do not have a pronounced cytotoxic effect on cancer cell lines in physiologically active concentrations.

P27.10

Designing peptide inhibitors of BTLA-HVEM and CD160-HVEM complexes formation as potential targets for immunotherapy

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Our research is focused on blocking the interaction between immune checkpoint BTLA-HVEM and CD160-HVEM proteins to stimulate immune response. BTLA (B- and T-lymphocyte attenuator) and CD160 (cluster of differentiation 160) are a receptors located on the surface of T lymphocytes which can interact with its ligand, HVEM (herpesvirus entry mediator), present on tumor cells. As a result of the protein complex formation, the immune response is inhibited and it does not recognize the tumor cells as aliens and does not fight with them [1]. The concept of blocking immune checkpoints is highly encouraged by many experts in the field of cancer immunotherapy as this type of approach has already been successfully applied. The idea was introduced for other inhibitory receptors, including PD1 and CTLA-4, with monoclonal antibodies for the receptors blocking. As some currently used antibodies lead to a response in only a fraction of patients, there is a strong need for alternative, non-antibody-based therapies, using small molecule agents such as peptides or peptidomimetics.

Reference:
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Acknowledgments:
The study is supported by the National Science Centre in Poland, Sonata 13, No. UMO-2017/26/D/ST5/00919.

P27.11

Design of inhibitors of BTLA-HVEM complex creation

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Immunotherapy is currently one of the most effective cancer treatments. It is based on stimulation of the immune system to recognize tumor cells and effectively kill them. T- Lymphocytes (T-cells) have receptors on their surface which can stimulate or inhibit an immune response. The B- and T-lymphocyte attenuator (BTLA) is one of the negative receptors which, during interaction with herpes virus entry mediator (HVEM) (present on tumor cells), inhibits T-cell proliferation and cytokine production. Compounds which block the BTLA-HVEM complex formation should stimulate the immune system to eliminate the cancer cells [1,2]. The main goal of the project is to design efficient and selective BTLA blockers that could prevent its interactions with the HVEM protein. In the presented project we synthesized the binding fragment of the UL144 protein, checked its ability to interact with BTLA and inhibit formation of the BTLA-HVEM complex.

References:
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Acknowledgments:
The study is supported by the National Science Centre in Poland, Sonata 13, No. UMO-2017/26/D/ST5/00919.

P27.12

Expression and purification of CD160, protein important for human health

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Control of the immune system's response against body infections is essential to restore normal health. An important role in this regulation is played by the signaling receptors. One of the receptors that has not been fully characterized is member of the immunoglobulin superfamily glycosylphosphatidylinositol-anchored membrane glycoprotein CD160. Currently studies has shown that this protein plays an important role in the development and course of many diseases. CD160 is involved in an immune response against melanoma. As a result of the complex formation with the HVEM protein (Herpesvirus Entry Mediator), activation of $CD4^+$ T cells on the surface of melanoma cells is inhibited [1]. Moreover the increase expression level of CD160 was observed in diseases such as chronic lymphocytic leukemia, atherosclerosis and chronic viral infections (e.g. HIV, HCV).

CD160 expression was performed in two different systems (bacteria and yeast). The oligosaccharide chains added by yeasts were identified by enzymatic deglycosylation followed by SDS-PAGE analysis. CD160 after expression in the bacterial system was recovery from inclusion bodies and refolded by dialysis. Purification was performed using affinity column followed by size exclusion chromatography.

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Acknowledgements:

This work was supported by Polish National Science Center grant no.2016/21/D/NZ1/02777.

P27.13

Medial septal NMDA receptor inhibition affects pro-inflammatory response in rats differing in their stress susceptibility

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Our recent findings suggested that medial septum (MS) NMDA receptor activation modulates blood natural anti – tumor response [1]. The purpose of the present study was to determine the influence of the infusions of NMDA receptor antagonist – D-AP7 into the MS, on plasma interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) concentration in rats. In animals, the locomotor response to novelty has been found to be a particularly useful measure of inter-individual stress susceptibility [2].

Male Wistar rats prior categorized as HRs or LRs in the novelty test (2 h) were exposed to the injection of D-AP7 (DL – 2 – amino – 7 – phosphoheptanoate, 0.1 μg/rat; n=15) or saline (0.5 μl/rat; n=12) *via* implanted cannulae into the MS. Pro-inflammatory activity was measured by the plasma concentration of IL-6 and TNF-α (ELISA) at the baseline and after blocking of the MS glutamate receptors. The data are presented as mean ±standard deviation. Level of significance was set at $p \leq 0.05$.

A single injection of D-AP7 produced alteration in IL-6 and TNF-α concentration. In LRs, a significant decrease, whereas in HRs a significant increase was observed, as compared to the baseline (LRs: $p \leq 0.001$; HRs: $p \leq 0.001$) and SAL group (LRs: $p \leq 0.05$; HRs: $p \leq 0.01$).

The obtained results indicate that the MS NMDA glutamate receptor inhibition differentially modulates peripheral response of pro-inflammatory cytokines such as IL-6 and TNF-α. That could be related to HRs *vs.* LRs differences in stress susceptibility.

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P27.14

Application of ethanol and fatty acids induces Ca^{2+} responses in pancreatic stellate cells *in vitro* and leads to activation of these cells *in vivo*

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Pancreatic fibrosis, a frequent complication of alcoholic pancreatitis, is a result of excessive activation of pancreatic stellate cells (PSCs), which assume a myofibroblast-like phenotype and deposit collagen fibres in the tissue. Although it has been suggested that alcohol may contribute to PSC activation, the exact mechanism of pathology induced by ethanol and its metabolites has not yet been elucidated in PSCs.

Here, the effects of acute administration of ethanol and palmitoleic acid (EtOH/POA) on intracellular Ca^{2+} signals were investigated in human PSCs *in vitro*. EtOH/POA induced global and sustained cytosolic Ca^{2+} rises in PSCs, partially dependent on extracellular Ca^{2+}. After the ER store was depleted with cyclopiazonic acid (CPA), POA/ethanol failed to trigger Ca^{2+} responses in PSCs.

Further, EtOH/POA induced acute pancreatitis *in vivo* (mouse model), which was characterised by mild to moderate pancreatic oedema, necrosis, and immune cell infiltration. Importantly, EtOH/POA increased the expression of α-SMA in between pancreatic acini, which has been attributed to the increased activation of PSCs.

Our study sheds new light on the current understanding of mechanisms underlying alcohol metabolite-induced pancreatic pathology, which involves Ca^{2+} responses in PSCs, likely leading to their activation *in vivo*.

P27.15

Selective inhibition of Bcl-2 by venetoclax (ABT-199) does not induce intracellular Ca^{2+} responses or cell death in pancreatic acinar cells

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Many cancers depend on the mitochondrial function of anti-apoptotic B cell lymphoma 2 (Bcl-2) proteins for their survival. Therefore Bcl-2 antagonism through BH3 mimetics emerged as a precision anti-cancer therapy. We previously showed that early Bcl-2 inhibitors elicit sustained Ca^{2+} responses in pancreatic acinar cells (PACs) inducing cell death. Because of this, BH3 mimetics could potentially be toxic for the pancreas. Venetoclax (ABT-199) is a recently developed selective Bcl-2 inhibitor which was introduced into the clinic for the treatment of leukaemia patients. Although venetoclax was shown to kill Bcl-2-dependent cancer cells without affecting intracellular Ca^{2+} signalling, its effects on PACs have not yet been determined. Therefore, it becomes essential and timely to assess whether the recently approved drug might have potentially pancreatotoxic effects. In this study single-cell real-time Ca^{2+} measurements and cell death analysis were performed on mouse PACs. We show here that selective inhibition of Bcl-2 via ABT-199 neither elicited intracellular Ca^{2+} signalling on its own nor altered Ca^{2+} signalling induced by physiological/pathophysiological stimuli in PACs. Also, cell death was unaffected by ABT-199. In contrast, selective inhibition of Bcl-X_L or inhibition of multiple anti-apoptotic Bcl-2 family proteins potentiated pathophysiological Ca^{2+} responses in PACs, but without exacerbating cell death. In conclusion, our results demonstrate that ABT-199 does not alter intracellular Ca^{2+} homeostasis in PACs and thus should be safe for the pancreas when used for treating cancer.

P27.16

Lichen-derived depsides and depsidones modulate the STAT3 signaling pathway in colorectal cancer cells

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Lichens are symbiotic organisms producing unique secondary metabolites – depsides and depsidones which possess anti-inflammatory and cytotoxic activity. The molecular mechanisms of the activity of lichen-derived compounds remain largely unknown, although the anticancer effects of lichen extracts were shown in many tumors types, including colorectal cancer.

The aim of this study was to evaluate the effects of depsides (atranorin, lecanoric acid, squamatic acid) and depsidones (physodic acid, salazinic acid) and a fatty acid – caperatic acid, on the level of STAT3, Bcl-xl and c- MYC in colorectal cancer cell lines. The compounds were extracted from samples of several lichen species and their purity was verified. HCT116 and DLD-1 colorectal cancer cell lines were treated with the compounds at the concentration of 50 μM or 25 μM (physodic acid). Gene transcript and protein level was assessed by RT-PCR and Western blot, respectively.

The results showed that the tested depsides and depsidones significantly decreased the level of *STAT3* transcript in HCT116 and DLD-1 cells. Moreover, all the compounds reduced the nuclear accumulation of STAT3. Furthermore, all the studied compounds decreased Bcl-xl and c-MYC protein level in both studied cell lines, and the effects in DLD-1 cells were much stronger. These results indicate that the repression of Bcl-xl, c-MYC and STAT3 by lichen compounds, especially physodic and salazinic acids, may partly explain their anti-cancer effects.

P27.17

Modulation of NF-κB signaling pathway by oleanolic acid derivatives and its conjugates with non-steroidal anti-inflammatory drugs in hepg2 hepatocellular cells line

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Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer and one of the leading cause of cancer-related deaths. Chronic inflammation is considered as important risk factor of this form of cancer. The key role in the inflammatory response play transcription factor NF-κB and thus it is a natural target of anti-inflammatory agents and drugs.

In this study, the effect of conjugates of the new synthetic derivatives of oleanolic acid (OA) with two nonsteroidal anti-inflammatory drugs (NSAIDs) on the expression of NF-κB active subunits and the COX-2 enzyme gene, controlled by this transcription factor was evaluated.

HepG2 cells were incubated with OA and NSAIDs conjugates at the doses of 20 and 30 μM for 24h. Genes expression was evaluated by R-T PCR and the level of proteins in cytosolic and nuclear fractions by Western blot, while NF-κB binding to DNA by ELISA test.

All investigated conjugates decreased expression of *NF-κB* subunits and their translocation from cytosol to nucleus. As result of NF-κB expression and activation inhibition, reduced transcript and protein level of COX-2 was observed. The most pronounced effect was observed after treatment with ketoprofen and ibuprofen OA conjugates with chemical structure of benzyl ester and morpholide.

These results indicate that the conjugates of NSAIDs with OA derivatives with this structure may potentially enhanced the effect of NSAIDs and thus reduced the risk of HCC.

Acknowledgements:
This work was supported by grant no 2016/21/B/NZ7/01758 from the National Science Centre, Poland.

P27.18

The cellular response of human colon and lung cancer cells to treatment with unsymmetrical bisacridine derivatives

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The search for potential anticancer drugs conducted at Gdańsk University of Technology led to the development new promising compounds, unsymmetrical bisacridine derivatives (UAs), which exhibited high cytotoxic and antitumor activity, preferentially against human pancreatic, colon and lung cancers. Here we studied the influence of three bisacridine derivatives: C-2028, C-2041, C-2053 on the cell cycle progression as well as the ability of these drugs to induce cell death in human colon HCT116 and lung H460 cancer cells. Our studies demonstrated that both cancer cell lines were very sensitive to unsymmetrical bisacridines (with IC_{90} values 0.04 μM for C-2028 and C-2041, 0.2 μM for C-2053). Treatment of the cells with UAs at IC_{90} caused transient G2/M arrest which was accompanied by an appearance of hypodiploid cells (sub-G1fraction), corresponding to apoptotic cell death. The morphological examination of the cells confirmed apoptosis commitment (chromatin condensation, apoptotic bodies). Percentage of Annexin-V and PI positive cells increased gradually upon treatment with UAs and after 72 h it reached about 40% and 20% for HCT116 and H460 cells, respectively, what indicated late stages of apoptosis and/or necrosis. In conclusion, unsymmetrical bisacridines, at biologically relevant concentrations, induced apoptosis in human colon and lung cancer cells. This cellular effect was more profound in HCT116 cells.

Acknowledgements:
These studies were supported by the National Science Center, Poland, Grant No. UMO-2016/23/B/NZ7/03324.

P27.19

The impact of extracts from cranberrybush (*Viburnum opulus*) leaves on the growth of human colon cells

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Introduction: Cranberrybush (*Viburnum opulus* L.) is a plant rich in phenols. Chlorogenic acid is a main compound responsible for its antioxidant activity. This plant also has antibacterial and anti-inflammatory properties, for this reason it is used for prevention, as well as for the treatment of many diseases, mainly those of the genitourinary system, but also of the heart or lungs. So far, there are few studies that suggest *V. opulus* has anti-cancer activity and could become a supplement in such therapies.
Aim: The aim of the study was to assess and compare the effect of both leaf extract (ELK) and phenol-rich leaf extract from cranberrybush (FELK) on the growth of colon cancer and normal colon cells.
Materials and methods: The HPLC method allowed to estimate the chemical composition of ELK and FELK. In addition, two colon cancer cell lines HT29 and SW480 were used, as well as normal epithelial cells CCD841CoN. The cells were treated with various concentrations of *V. opulus* extracts. The cell viability was assessed by MTT test after 24 h, 48 h and 72 h, respectively.
Results: ELK and FELK extracts had moderate effect on the inhibition of HT29 cell growth. The cell viability of SW480 was much more pronounced after ELK and FELK treatment. The FELK extract inhibited the growth of HT29 and SW480 more significantly compared to ELK. The growth of CCD841CoN cells was higher after FELK treatment than ELK.
Conclusions: The purified leaf extract of *V. opulus* (FELK), richer in phenolic compounds than the unpurified ELK, more significantly inhibited the growth of HT29 and SW480 colon cancer cells as well as had a more gentle influence on the growth of normal epithelial CCD841CoN colon cells.

Acknowlegdements:
The reaserch was supported by a grant from the Medical University of Lodz (No. 503/1-156-04/503-11-001).