Session 15: New Molecular Targets in Personalized Therapy of Cancer

Lectures

L15.1

Grainyhead-like transcription factors as targets of anti-cancer therapy

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Transcription factors have long been believed to be "undruggable". This means that it was considered unfeasible to directly inhibit or activate their function using small molecule modulators. The only exceptions were nuclear receptors, for example, estrogen receptor, which can be successfully inhibited by tamoxifen, a drug used in the treatment of breast cancer. However, most transcription factors are not nuclear receptors, and therefore were regarded as chemically intractable. It was most unfortunate, because transcription factors are promising targets for the development of therapeutics, particularly in oncology. This view was challenged in the 21st century, and nowadays we know of more and more small molecule chemical compounds that can directly modulate protein-DNA and protein-protein interactions of various transcription factors. These findings prove that this important class of proteins can be targeted for the purpose of therapy. In this presentation I will discuss selected examples of such compounds. I will pay particular attention to the well documented Grainyhead-like transcription factor family, as some of its members are oncogenes, while others serve as tumor suppressors. TFCP2 is an oncogene in hepatocellular carcinoma, and inhibitors of TFCP2 are being developed as drugs against this type of cancer.

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L15.2

Natural compounds activating cannabinoid receptors CB1 and CB2: future for cancer treatments

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Cancer cells have the capacity to synthesize their own supply of biologically active compounds of lipid origin (e.g. estrogens, androgens, farnesyl, oxysteroids, fatty acids, and lysolipids), which are linked to the activation of nuclear receptors and signaling pathways involved in carcinogenesis and metastasis. Our laboratory has developed a series of new drugs of natural origin; Gambogic acid (GA), two peanut hairy root-derived isoprenylated analogs of trans-resveratrol (tRes), *trans*-arachidin-1 and -3 (*t*A1 and *t*A3), which exhibits slower metabolism/enhanced bioavailability. Additionally, a series of natural and synthetic cannabinoids, as well as tamoxifen derivatives, were investigated for anti proliferating effects due to their synergistic natures. Our mechanistic studies show that all compounds under investigation, bind to, and activate the cannabinoids receptors (CBRs), CB1 and CB2. Based on these results, we hypothesize that CBRs might serve as novel molecular targets for these natural and synthetic compounds. The binding to CB1 and CB2 with significant affinity, and activation and/or suppression of downstream target genes, might result in regulation of cancer cell proliferation. If our hypothesis is correct, CBRs could constitute a novel molecular target and structural scaffolds for which effective, non-toxic, natural and synthetic cannabinoids might be developed for treatment of various types of cancer.

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Oral presentations

O15.1

The importance of drug type and dose on CLL cells *in vitro* sensitivity to anticancer-agents

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A high diversities in chronic lymphocytic leukemia (CLL) development could be a reason for distinct patient's leukemic cell responses to anticancer agents. Sometimes even anticancer agent doses seem to be very important in anticancer response. Moreover, the diverse cells reactivity toward cells elimination by studied different group of agents (new chemically synthesized agents, natural compounds, approved anticancer drugs) reflecting anticancer potential was observed. The obtained results of anticancer agents sensitivity to anticancer agents revealed the differences for studied cases. Interestingly, the reduction of anticancer agent dose led to necrosis reduction. In current studies, the anticancer response of CLL cells incubations with anticancer agents was analyzed to compare proapoptotic leukemic cells sensitivity to anticancer agents. The distinct CLL cells reactivity was studied during 48h cell incubations with anticancer drugs used in CLL treatment (cladribine + mafosfamide; CM or CM combined with monoclonal antibody – rituximab; RCM), as well as new, directed on molecular targets therapeuticals (idelalisib, ibrutinib, venotoclax), natural compounds displaying anticancer properties (eg. curcumin, quercetin, betulinic acid), as well as a new generation of synthesized roscovitine analogs (BP14 and BP30).
The obtained results revealed that for each CLL patient cells incubated with anticancer agents displayed differences in apoptosis induction potential. Moreover, the reduction of dose value usually led to increase the number of apoptotic cells. The cell viability during expositions to anticancer agent(s) was analyzed using flow cytometry using propidium iodide and Yo-Pro stains.

O15.2

Novel strategies of PD-1/PD-L1 immune checkpoint blockade for cancer therapy: antibodies, peptides and small molecules

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Programmed cell death-1 (PD-1, CD279) protein is an immune receptor expressed mostly on activated T cells. The protein serves as an emergency brake for turning off the cytotoxic activity of these cells. Its natural ligand, PD-L1, is expressed on several types of immune cells. By binding to PD-1, PD-L1 provides so-called *immune checkpoint*, that protects from autoimmune reactions and contribute to T cell homeostasis. Due to its immunosuppressive function PD-L1 is also utilized by a variety of cancer types to evade the destruction provided by the activated T cells.
In the recent years cancer immunotherapy utilizing antibodies targeting PD-1/PD-L1 interaction has been proved to be exceptionally effective in multiple clinical studies. Due to extraordinary clinical outcome the use of both anti-PD-1 and anti-PD-L1 antibodies has been approved for the treatment of several cancer types. Despite great achievements of the use of therapeutic antibodies, a strikingly less progress has been done in the field of small molecules and peptides targeting this interaction.
The immune checkpoint blockade (ICB)-based immunotherapy, especially in combination with classical anti-cancer treatments, is now believed to be the future of oncology. In this report recent discoveries in a field of ICB, utilizing antibodies, peptides, small molecules and combinations will be presented.

O15.3

Telomerase targeting modulates cancer response to therapy

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Telomerase is an enzyme that enables surpassing the Hayflick limit and is one of the most important factors that drives cancer cells immortal. Its main function is telomere restoration but only in a limited group of cells, including cancer cells. Since it is found in a vast majority of cancer cells, it became a natural target for cancer therapy.
The aim of this work was to evaluate the relationship between telomerase regulation and sensitivity of breast cancer cells to anticancer treatment.
Doxorubicin was selected as a model drug. qPCR, western blot, flow cytometry, telomerase activity assay (TRAP) and viability assays were used to conduct the study.
We showed that telomerase inhibition decreased the viability of MCF7 and MDA-MB-231 breast cancer cells. Combination of telomerase inhibitors with doxorubicin caused a stronger cytotoxic effect than the use of each compounds separately. Flow cytometry analysis of breast cancer cells revealed a potential of telomerase inhibitors to block cell cycle that was accompanied by alterations in the expression of cyclins. Interestingly, adhesion properties of breast cancer cells were also modulated.
In conclusion, our results reveal novel non-canonical functions of telomerase that might be used in development of new anticancer strategies.

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Posters

P15.1

Glucose transporters, symporters and sodium/hydrogen exchangers – new targets for clear cell renal cell carcinoma therapy

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Introduction: Clear cell renal cell carcinoma (ccRCC) is a chemo- and immunotherapy resistant cancer. Glucose and *myo*-inositol levels are changed in ccRCC. Expression of metabolite transporters is often disturbed in cancers and is linked with poor survival and resistance to therapy.
Aim: To find changes in expression of glucose transporters, symporters and Na(+)/H(+) exchangers that influence survival of ccRCC patients and to identify potential therapeutic targets.
Material/Methods: RNA from 70 human tissue samples (35 ccRCC tumors and 35 matched-paired non-tumorous controls) was analyzed using qPCR. Survival analysis was performed using SurvExpress platform and TCGA data.
Results: Expression of 6 genes (*SLC2A1, SLC2A3, SLC5A1, SLC5A4, SLC9A5, SLC9A9*) was increased and of 14 (*SLC2A2, SLC2A9, SLC2A11, SLC2A12, SLC2A13, SLC50A1, SLC5A2, SLC5A3, SLC5A10, SLC5A11, SLC5A12, SLC9A2, SLC9A3, SLC9A4*) was decreased in tumor samples compared to controls ($p<0.05$). Altered expression of these genes correlated with poor survival of ccRCC patients (HR=3.3, log rank p=2.7 e−10). Expressions of *SLC2A1, SLC2A12, SLC5A5* correlated with intracellular glucose concentration, while expressions of *SLC2A1, SLC2A12, SLC5A3, SLC9A2, SLC9A4* correlated with levels of *myo*-inositol.
Conclusions: We identified panel of genes involved in transport of glucose and *myo*-inositol that are potential new targets for ccRCC therapy.

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P15.2

The translational aspect of CTCs aggressive phenotype in breast cancer patients

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Presence of circulating tumor cells (CTCs) in breast cancer patients is a hallmark of early tumor spread. CTCs obtain their unique properties as a result of an epithelial-mesenchymal transition (EMT), what plays a critical role in their dissemination and survival during metastatic cascade. In the current study we aimed at performing extended characterization of epithelial-mesenchymal status of CTCs from breast cancer patients and correlate it with expression of other malignancy-related genes and clinico-pathological data of the patients.

Thirty nine CTCs-enriched blood samples collected from operable breast cancer patients were divided into three classes according to epithelial (*CK19, CDH1*) and mesenchymal (*VIM, CDH2, PLS3*) markers expression. We observed significantly different expression of invasion-related genes (*CXCR4, uPAR*) and stemness markers (*CD44, NANOG*) between CTCs classes. Furthermore, patients with mesenchymal CTCs had worse prognosis (50% probability of survival, P=0.02) in comparison to patients without CTCs (100% survival). Unfavorable clinico-pathological statuses, like tumor size and involved lymph nodes, were also overrepresented in mesenchymal CTCs class.

To summarize, presence of mesenchymal markers in CTCs of breast cancer patients translates into negative impact into disease, because of their more aggressive gene expression profile and correlation with worse prognosis.

P15.3

Effect of L- and D-isomers of ascorbic acid on the levels of 5-methylcytosine and its epigenetic derivatives generated by TET family proteins in the genome of established, human cancer cell lines

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Vitamin C is an antioxidant necessary for activity of 2-ketoglutarate-dependent dioxygenases, including TET proteins, responsible for DNA active demethylation. Dynamic interplay between methylation and demethylation of cytosine is crucial for gene expression regulation, and depends on enzymatic, oxidative conversion of 5-methylcytosine to 5-hydroxymethylcysosine and subsequently to 5-formylcytosine and 5-carboxycytosine. As there are two potential mechanisms of regulating TET proteins activity by vitamin C – acting as a cofactor for the enzyme and/or iron ion pool renewal, in our experiments we have cultured K562 and HCT116 cell lines with various concentrations of L- and D-ascorbic acid. The latter has the same antioxidant potential as the L-isomer, but probably due to the different spatial conformation cannot act as a TET cofactor. We have observed a dose-dependent increase in 5-hydroxymethylcytosine, 5- formylcytosine and 5-hydroxymethyluracil levels in DNA of both lines after treatment with both isomers. However, K562 cells had almost twice higher levels of epigenetic derivatives after incubation with L-ascorbic acid, as compared to D-ascorbic acid, whereas in HCT116 cells, we did not observe any significant differences between the effects of both substances. These results indicate that in HCT116 cell line, the regulation of the Fe^{2+} ion level plays a dominant role in regulating the activity of TET proteins whereas in K562 cells both mechanisms are present.

P15.4

Profile of the products of active DNA demethylation pathways in leukocytes of breast and colon cancer patients

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Aberrant DNA methylation may play significant role in cancer development. Another characteristic feature of malignant cells is a profound decrease in level of 5-hydroxymethylcytosine, product of 5-methylcytosine oxidation by TET enzymes. Our objective was to compare broad spectrum of endogenously generated DNA modifications (5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxycytosine, 5-hydroxymethyluracil and 8-oxoguanine) in leukocytes from three groups: healthy controls, colorectal cancer and breast cancer patients. Leukocytes were isolated from heparinized blood samples with Histopaque 1119 solution, according to the manufacturer's instruction. DNA was extracted from frozen cells by modified phenol method. Isolated material was hydrolysed to deoxynucleosides and analyzed using two-dimensional ultra-performance liquid chromatography with tandem mass spectrometry. Both cancer groups presented with lower levels of 5-methylcytosine and 5-hydroxymethylcytosine than the controls. Similar trend was observed in level of 5-hydroxymethyluracil. Significant differences in levels of 5-methylcytosine, 5-carboxycytosine and 8-oxoguanine was found between two cancer groups. To summarize, this study showed that healthy individuals and patients with colorectal and breast cancer present with distinct specific patterns of epigenetic modifications in leukocyte DNA.

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P15.5

Purification and initial characterization of Nucleobindin-2 protein from *Gallus gallus*

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The Nucleobindin-2 (Nucb2) is multidomain protein which is expressed on high level in central nervous system and variety of peripheral tissues i.e. pancreas, kidney, testis, stomach and heart. This protein is involved in multiple biological processes i.e. calcium homeostasis, biomineralization, food intake inhibition and cancerogenesis. It also has an impact on survival and apoptosis processes by interacting with Necdin protein, the growth suppressor. The molecular characteristic of protein has not been conducted yet. The *in silico* analysis showed that Nucb2 contains inherently disordered regions.

The aim of this study was to obtain a homogenous Nucb2 protein from *Gallus gallus*. We prepared recombinant protein in pQE-80L vector, which contains six histidine tag at amino-terminal end. The expression of recombinant protein was conducted in BL21(DE3)pLysS *Escherichia coli* cells. Nucb2 was purified using immobilized ion metal affinity chromatography (IMAC) and size exclusion chromatography (SEC). The purity of the obtained Nucb2 was at least 95%, which was confirmed by SDS-PAGE. The molecular mass of protein was verified by mass spectrometry. Circular dichroism spectroscopy analysis showed that dominant type of secondary structure is β-sheet. Interestingly, the fluorescence maximum of two Nucb2 Trp residues is located at λ=354 nm which suggests the polar environment of these amino acid residues. The redshifted fluorescence spectrum (i.e. with the maximum at λ>340 nm) is a characteristic feature of inherently disordered proteins.

P15.6

Phenotypic plasticity of primary and metastatic breast cancer cells in the context of epithelial-mesenchymal transition program

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Activation of epithelial-mesenchymal transition (EMT) in cancers might promote disease progression. In the current study we asked if EMT plays a role in induction of stem cell (SC) markers in cancers cells at different stages of metastatic cascade.

From one hundred and seven operable breast cancer patients primary tumours (PTs), lymphatic metastases (LNM) and circulating tumour cells (CTCs) were obtained. SC markers (CD133, ALDH1, CD44, OCT4, NANOG) were tested in all samples, heterogeneity of the markers was additionally assessed in PT and LNM. All samples were profiled for the activation of EMT using E-cadherin, CK19, VIM analysis.

Activation of EMT program was detected most frequently in PTs (44%), then LNM (29%) and CTCs (12%). EMT status was frequently discordant between tested samples, indicating location specific EMT activation. Mesenchymal phenotype correlated with increased expression of SC markers in PTs (OCT-4 and CD44), LNM (OCT-4) and CTCs (OCT-4 and CD44) and higher proliferative index of PTs and LNM. Nevertheless, heterogeneity of the four SCs markers: ALDH1, CD133, OCT-4, CD44 decreased in matched LNM in comparison to PTs. Activation of EMT in CTCs was linked with decreased overall survival, higher tumour stage and lymph node involvement.

EMT phenotypic plasticity is observed among cancer cells from different stages of metastatic cascade, nevertheless, signs of its activation are linked to increased levels of SC and proliferation markers expression as well as poor prognosis.

P15.7

Identification of novel mechanisms of transport of proteins to primary cilia

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Primary cilium is a microtubule-based organelle found on the surface of most mammalian cells. Dysfunctions of primary cilia lead to developmental disorders known as ciliopathies, and cancer. We know that proteins which are components of essential signaling pathways like Hedgehog or TGF is trafficking to this specific cellular antenna. Interestingly, in response to the signal, soluble proteins are transported very efficiently into the primary cilium. We extensively study the still poorly understood mechanism of transport from cytoplasm to the base of primary cilium, which is the first crucial step for many pathways. We use shRNA-based loss-of-function studies coupled with immunofluorescence microscopy and semi-automated image processing to identify novel players in the ciliary transport machinery of mammalian cells.

Our achievements may contribute to the design of targeted therapies in cancer and diseases associated with dysfunctions of primary cilia.

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P15.8

Transcription factors regulating the expression of genes from the Grainyhead-like (*GRHL*) family in the context of human cancer

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Genes from the Grainyhead-like (*GRHL*) family are present in all animal and fungi species that were studied so far. In humans there are three genes belonging to the *GRHL* family, named Grainyhead-like 1 (*GRHL1*), Grainyhead-like 2 (*GRHL2*) and Grainyhead-like 3 (*GRHL3*), respectively. The expression of these genes is observed primarily in various types of the epithelial tissues.

GRHL genes are important factors in protection against cancer. Their silencing in non-tumorigenic cell lines induces tumorigenic features in these cells and, conversely, increasing the expression of these genes in cancer cell lines reverses their tumorigenic phenotype.

Changes in the levels of expression of the genes from the *GRHL* family often brings about the development of many types of cancer. Thus, *GRHL* genes directly influence the process of carcinogenesis. Consequently, changes in *GRHL* gene expression are important for the development and progression of various cancers.

The aim of our project is to perform a systematic analysis of promoter regions of the *GRHL* genes in order to identify and characterize transcription factors binding to these promoters. Our results should thus provide novel and valuable insights into the molecular mechanisms of cancer development.

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P15.9

Regulation of Gli proteins by the ubiquitin-proteasome system

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Protein synthesis and degradation equilibrium is very important for cell homeostasis. The ubiquitin-proteasome system (UPS) is a major pathway responsible for protein degradation in eukaryotic cells. Through complete protein degradation UPS regulates variety of cellular processes such as cell cycle, gene expression or carcinogenesis. Cellular signalling can also be regulated by UPS. One of signalling pathways regulated by UPS is Hedgehog signalling. Hedgehog signalling is necessary for proper development of higher eukaryotes from Drosophila to human. Aberrant pathway activation may lead to development of, among others, medulloblastoma, the most common brain cancer in children.

UPS is know to negatively regulate Hedgehog signalling pathway. In absence of signal, Gli2 and Gli3 transcription factors are partially degraded by the proteasome to their repressor forms (GliR), which are translocated into the nucleus and prevent target gene expression. However, our results suggest that the proteasome may be required for maximal target gene induction by activated Gli2 proteins when the pathway is activated. Thus, the proteasome has a dual role in the Hedgehog pathway – it contributes to Gli repressor formation, but is also critical for Gli activator function.

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P15.10

MLK4 drives aggressiveness of triple-negative breast cancer through NF-κB dependent mechanism

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, characterized by the resistance to available treatments, frequent relapses and poor prognosis. No clinically established targeted therapies have been developed for TNBC so far. Our TCGA cancer genomic data analysis revealed that amplification or mRNA upregulation of Mixed-Lineage Kinase 4 (MLK4) occurred in 23% of invasive breast carcinoma cases. To find the correlation between MLK4 expression and the specific subtype of breast cancer, we performed a transcriptomic analysis of multiple datasets, which showed that MLK4 is highly expressed in triple-negative breast cancer comparing to other molecular subtypes. The knock-down of MLK4 in TNBC cell lines with high endogenous expression level of this kinase impaired proliferation rate and anchorage-dependent colony formation. Moreover, silencing of MLK4 significantly reduced the migratory potential and invasiveness of breast cancer cells as well as the number of spheroids formed in 3D cultures. These *in vitro* findings translate into slower rate of tumor growth in mice upon MLK4 knock-down. Furthermore, we established that MLK4 activates NF-κB signaling and promotes mesenchymal phenotype of breast cancer cells. Immunohistochemical staining of samples obtained from breast cancer patients revealed a strong positive correlation between high expression of MLK4 and metastatic potential of tumors, which was predominantly observed in TNBC subgroup. Taken together, our results show that high expression of MLK4 promotes invasive phenotype of triple-negative breast cancer and represents a novel target for anticancer treatment.

P15.11

Import-export business: nuclear trafficking of Gli proteins

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Gli proteins play a key role in many developmental processes and in cancer. They are transcription factors activated as a result of cellular signal transduction through the Hedgehog protein (Hh) cascade. As transcription factors, they require an efficient and reliable mechanism that transports them in and out of the cell nucleus. While we know more and more about the process of activating Gli proteins, their subsequent transition to the nucleus remains poorly studied. We hypothesize that the transport of Gli proteins depends on the interaction between specific regions of these proteins with the nuclear import/export machinery. The transport capacity can be a crucial factor in determining the outcome of signaling.

Our goal is to analyze the role of selected shuttle proteins in Gli proteins localization and pathway activity using protein-protein interaction analysis and immunofluorescence combined with the knock-down approach. Our results suggest that some members of nuclear export machinery are involved in the active removal of Gli proteins from the nucleus. We also show that the accumulation of Gli proteins in the nucleus upon defective nuclear export may limit the ability of Gli transcription factors to induce target genes.

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P15.12

Ghrelin and GHS-R receptor in colorectal cancer

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Ghrelin is a short 28 aa O-octanoylated peptide secreted mainly by neuroendocrine cells in human gastrointestinal tract. It works as a "hunger-satiety" hormone. Its serum level rises during fasting and decreases rapidly after food intake. Ghrelin has been also found to take part in neuronal development, bone metabolism, and cardiovascular system. In addition, ghrelin plays a significant role in the regulation of glucose homeostasis, lipid profiles, and body composition. Ghrelin exerts its actions through GHS-R receptor which belongs to a superfamily of G protein-coupled receptors. GHS-R is expressed in multiple tissues including brain, mainly in the hypothalamus, liver, skeletal muscle, and heart. Ghrelin was found to be generally up-regulated in different types of cancer and suggested to be involved in the Ras-PI3K-Akt-mTOR pathway. GHS-R receptor was also noted to have higher expression in many cancers. Moreover, it was reported that GHS-R has the ability to induce intracellular pathways even in absence of its ligand. We have studied mRNA and protein expression of ghrelin and GHS-R in human colorectal cancer and in colonic mucosa distal from tumor lesion. It has been demonstrated a significant increase in ghrelin and GHS-R expression in studied tumor samples which appeared to correlate with progression of the disease.

P15.13

Vemurafenib-resistant melanoma cells demonstrate altered invadopodia and changes in sensitivity to EGFR and c-Met inhibitors

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Melanoma is highly aggressive cancer characterized by increased incidence of metastases. Mutations and elevated levels of receptor tyrosine kinases (RTKs) significantly contribute to its invasive abilities. We previously showed the synergistic cytotoxic effect of pairs of anti-RTKs agents – foretinib (c-Met inhibitor) with lapatinib or gefitinib (EGFR inhibitors) on melanoma cells. We also observed that pairs of these drugs decrease invasiveness of examined cells. However, all cell lines used in our experiments exhibit mutation in Braf kinase (Braf V600E), which is part of signal transducing pathway activated a.o. by c-Met and EGFR. We noted that vemurafenib (inhibitor of Braf V600E) used alone majorly diminishes level of pErk and moderately decreases viability of examined cells. To imitate *in vivo* conditions we tested previously used drugs in cell lines resistant to vemurafenib. Obtained cells were less sensitive to RTKs inhibitors used independently, simultaneously showing elevated response to combination therapy. Resistant cells also exhibited abnormal cortactin localization, decrease in number of invadopodia, altered motile abilities and changes in actin cytoskeleton organization, including occurrence of more pronounced stress fibres. Acquired results are crucial for development of efficient anti-melanoma therapy directed towards patients demonstrating resistance to vemurafenib.

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P15.14

In research of molecular mechanism of gastric cancer cell response to FGFR inhibitor

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Gastric cancer is still posing a major challenge. Surgery and chemoradiation are routinely used as a treatment. More than half of radically resected gastric cancer patients relapse locally or with distant metastases and 5-years overall survival is 10–15%. Therefore, there is an extensive need to search for biological therapies for gastric cancer. Fibroblast growth factor receptors family (FGFR1-4) is involved in regulation of cell growth, differentiation, apoptosis and survival. *FGFR2* gene is amplified in approx. 20% of gastric cancer patients.

We aimed to find proteins markers of gastric cancer cell response/resistance to FGFR inhibitor (CPL-304-110) designed by Celon Pharma. Of the five tested gastric cancer cell lines, Snu-16 and Kato-III lines, strongly responded to the inhibitor with IG50 0.03 μM and 0.04 μM respectively. To look for markers of sensitivity/resistance to the drug we derived resistant variants of these cells and analysed expression/activation of proteins which belong to FGFR-triggered signalling (FRS2, PLCy, AKT, ERK), positive/negative regulators of FGFR pathway (Spry2, Shp2), and those involved in gastric cancer progression (ErbB family. HGFR, cadherins) and so on. So far we did not manage to identify a universal marker indicating sensitivity/resistance to FGFR inhibitor. It is highly possible that several pathways might be responsible for emergence of gastric cancer cell resistance to inhibition of FGFR signalling.

P15.15

TRIM28 protein domains in self-renewal process in human induced pluripotent stem cells (hiPSC)

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Tripartite motif protein 28 (TRIM28) is a transcription regulator and plays role in regulation of cancer stem cells (CSC) populations and tumorigenesis, and according to our results, is responsible for self-renewal capabilities and maintenance of pluripotency state in human induced pluripotent stem cells. TRIM28 is a multidomain protein and, as a result of its structural complexity and enzymatic activity, exhibits diversified biological functions in cells. TRIM28 triggers the formation of heterochromatin by recruiting nucleosome remodeling complexes, heterochromatin protein 1 (HP1), histone modifying enzymes: histone deacetylase-containing complex NuRD and histone methyltransferase SETDB1 through the activity of particular domains. Here we indicate the essential role of RING, PHD and HP1-binding domain in self-renewal of hiPSC. With the application of lentiviral vectors and RNA interference we replaced the expression of endogenous TRIM28 by exogenous structural mutants in RING domain (C91A), PHD domain (C628R), and Bromodomain (N773G). We also examined three different phosphorylation mutants: Ser 437 located near HP1-binding domain, triple tyrosine phosphorylation mutant which ablates tyrosine phosphorylation Y449F/ Y458F/Y517F (3YF), and C-terminal serine – Ser824, located near Bromodomain.

P15.16

On the Quest of Therapeutic Opportunities – filtering "big data" to find novel oncogenic drivers

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Cancer is one of the most lethal diseases worldwide. Current methods of treating cancer, such as surgery or chemo- and radiation therapies have limited efficacy and serious side effects. In the recent decade, much hope has been placed in combining molecular diagnosis with targeted therapies. Identifying specific biomarkers and the associated "weak spots" of the tumor should in principle guide successful treatment of cancer with minimal side effects. The challenge is to find the right biomarker for the right cancer subtype.

We searched through data from two recently published large-scale loss-of-function screens that tested the sensitivity of cancer cells to the loss of different genes. We found several previously unknown cancer type-specific factors that determine the growth capacity of cancer cells. This allowed us to focus our studies on a new potential target for melanoma treatment.

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P15.17

CD151 regulates activity of ERBB receptors

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Breast cancer (BCa) is the leading cause of women death worldwide. The members of ErbB family of transmembrane receptor tyrosine kinases (RTKs) have been implicated in several human carcinomas. Overexpression of ErbB2 has been reported in 18-25% breast cancer patients and is associated with poor clinical outcome. Herceptin (Trastuzumab), a monoclonal antibody, is used for the treatment of ErbB2-positive BCa. However, approximately 60% of patients do not respond to this therapy and most of the initial responders develop resistance within one year. The mechanism underlying of drug resistance is not fully understood. Increasing evidence suggests that tetraspanin CD151, one of the best characterized members of the transmembrane protein family, is involved in invasiveness of human cancers. Recent studies show that CD151 plays a crucial role in ErbB2-dependent signaling and sensitizes cells to Herceptin treatment. Therefore we further investigate a role of tetraspanin CD151 in regulations of ErbB2-mediated signal transduction in breast cancer and its impact on cell response to Herceptin. We found that CD151 i) impairs heregulin-dependent cell growth, ii) inhibits heregulin-triggered signalling pathways, iii) attenuates heterodimerization ErbB2/ErbB3 and cell response to Herceptin. These results support previous findings and show that CD151 is involved in ErbB2/ErbB3 mediated signaling pathway which has implication for breast cancer progression and resistance to therapy.

P15.18

LATS1 HIPPO kinase is involved in cancer stem cell formation and EMT in melanoma

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Melanoma belongs to the most aggressive human cancers. In metastatic phase it is resistant to common therapies. Such a high invasiveness and metastatic potential result from several mutations and activation of different signal transduction pathways. One of them is the Hippo signaling. Hippo pathway is responsible for a growth control and differentiation of tissues and organs. It is also largely involved in tumor formation and metastasis. LATS1 is a core kinase of Hippo signaling. The role of LATS1 in melanoma remains unknown. The aim of the study was to investigate the role of LATS1 in tumor growth, its connection with cancer stem cells (CSC) and epithelial-mezenchymal transition (EMT). Using an *in vitro* as well as a xenograft models of human melanoma we demonstrated that LATS1 affects tumor growth, and is highly connected with the expression of melanoma initiating cell and EMT markers. Further analysis of Hippo pathway regarding CSC formation and EMT will provide a better understanding of the mechanisms of melanoma pathogenesis and will help to find new therapeutic targets for more effective treatment and diagnosis.

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P15.19

The influence of quantum dots Ag-In-Zn-S conjugated with unsymmetrical bisacridine derivatives on cytotoxicity and cell cycle distribution in lung and colon cancer cells

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Quantum dots (QDs) are tiny nanoparticles which have a huge potential for biological application, including drug delivery system. Unsymmetrical bisacridine derivatives (UAs), synthesized in our laboratory, are a new class of promising antitumor agents demonstrating high cytotoxic and antitumor activity against many cancers, including: colon, lung, pancreatic, breast and prostate. In the presented work we investigated whether QDs conjugated with UAs affect their cytotoxicity and have an influence on the cell cycle distribution in human lung and colon cancer cells.

The studies were performed using $QD_{red/green}$ (Ag-In-Zn-S/MUA nanocrystals) conjugated with two bisacridines: C-2028 and C-2045. UAs conjugated with QD_{red} and QD_{green} decreased IC_{50} values of both compounds (QD_{red}: 12.3 and 4.7 fold, QD_{green}: 2.6 and 2.1 fold, respectively) in H460 cells. In contrary, conjugation UAs with $QD_{red/green}$ did not influence the cytotoxicity of these compounds in HCT116 cells. Moreover, UAs conjugated with $QD_{red/green}$ were less active against HCT116 than H460 cells. Importantly, QDs alone did not influence cancer cells proliferation. Cell cycle analyses revealed that there was no obvious difference in cell cycle distribution in H460 and HCT116 cells after the treatment with UAs conjugated with QDs. Further studies are needed to examine the cellular effects of QDs conjugated with UAs in cancer cells.

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