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Poster

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The Hippo pathway protein LATS1 is involved in progression of melanoma and regulation of EMT phenotype

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Introduction: Melanoma is the most aggressive type of skin cancer. Usually it develops spontaneously and progresses rapidly. Recently increasingly investigated Hippo pathway which is known to control sizes of organs, apoptosis and cells proliferation is also believed to be involved in a tumor biology. One of the Hippo pathway proteins that is still not fully examined is a tumor suppressor LATS1 which is an upstream regulator of an oncogene YAP. LATS1 significance in a melanoma development has not been studied yet just as its influence on expression of EMT (epithelial-to-mesenchymal transition) markers. In our study we used atymic mouse model of human melanoma to analyze influence of LATS1 gene knockdown on a tumor growth and EMT phenotype. We used melanoma cell lines derived from primary lesion and from lymph node metastasis from the same patient what allowed us to examine the significance of LATS1 protein at different stages of a cancer on a tumor progression and EMT marker expression.

Aim of the study: The aim of the study was to examine the influence of LATS1 silencing on tumor growth and progression in atymic mouse model as well as on EMT marker expression.

Material and methods: Different human melanoma cell lines genetically modified to knock down LATS1 gene were injected into atymic mice. Tumor growth kinetics was observed. At the end of experiment mice were sacrificed and tumors were excised, weighted and tumor lysates were prepared for western blot analyses.

Results and conclusions: Silencing LATS1 gene has an influence on a tumor growth and EMT phenotype in atymic mouse model of human melanoma. The most significant differences between LATS1-knocked down cells and control cells were observed in mice injected with a primary melanoma cell line, suggesting that LATS1 is involved in progression of melanoma on early stages of tumor development. We also demonstrated that LATS1 influences EMT biomarker expression. These results together are shedding a new light on the contribution of Hippo pathway in a melanoma development and are showing great potential of LATS1 protein in future studies of melanoma pathogenesis.

Key words: melanoma, EMT, Hippo pathway, LATS1.

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Poster

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Next generation sequencing in comprehensive diagnosis in solid tumors

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Introduction: The STARTRK-2 (Studies of Tumor Alterations Responsive to Targeting Receptor Kinases) trial is a potentially registration-enabling Phase 2 global basket trial of the investigational tyrosine kinase inhibitor compound entrectinib in patients with solid tumors harboring TRK, ROS1, or ALK gene fusions. Phase 1 studies of entrectinib reported a 79% overall rate of response across multiple histology types in patients with gene fusions who were naive to inhibitors of these targets, received an efficacious dose, and had extracranial disease. Patients harboring these gene fusions account for < 3% of cancer population; however, they have been seen in over 40 different tumor types, including gastrointestinal, lung, head and neck, and sarcoma.

Aim of the study: The aim of the study was to identify the TRK, ROS1, ALK and other fusions in patients treated in Cancer Center Institute of Oncology.

Material and methods: The occurrence of TRK, ROS1, and ALK gene fusions in solid tumors was studied in FFPE specimens from 1121 patients. We used 2-step diagnostic test. At first, the IHC screening was performed using a pan-receptor tyrosine kinase cocktail of antibodies targeting those proteins, secondly an RNA-based anchored multiplex-PCR next generation sequencing (NGS) assay was performed in IHC positive specimens.

Results: 1121 out of 480 clinical specimens screened by IHC were positive and further analyzed by NGS. The presence of gene fusions was confirmed in 34 (3%) of them.

Conclusions: The two-step IHC/NGS testing approach is an effective strategy to identify patient populations with low prevalence of molecular alterations and can be included into standard clinical practice. NGS based total nucleic acid analysis enable detection of novel gene fusions as an alternative molecular targets, that extend the population of patients with various solid tumors, who can benefit from treatment. The NGS based analysis of solid tumors will get more important in the context of diagnostics and personal medicine within next years.

Key words: STARTRK-2, gene fusions, TRK, ROS1, NGS.

Poster

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AGI-101H melanoma vaccine enhances humoral response against ALDH1 in patients with advanced melanoma

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Skin melanoma belongs to the most invasive human malignancy with rapidly rising incidence. Whilst surgical treatment for early stage melanoma is relatively successful, there is no cure for progressing disease. It has been reported that subgroups of patients respond variously to different therapeutic approaches. Thus, there is a need for a patient-specific therapy, referred to the personalization of treatment, which allows for the selection of patients that will actually benefit from the treatment. For that purpose, the proper predictive biomarkers must be defined.

In our study we demonstrated that melanoma patients treated with AGI-101H allogeneic whole cell melanoma vaccine genetically modified to display melanoma stem cell-like phenotype, generate specific anti-ALDH1A1 antibodies. Moreover, the antibody titer rises following vaccination course, reaching plateau after a series of AGI-101H doses. ALDH (aldehyde dehydrogenase) isozymes have been considered as melanoma cancer stem cell biomarkers, which makes them especially attractive therapeutic targets for human melanoma. Given their implication in tumorigenesis and the ability to arouse a humoral immunity, ALDH isozymes may emerge as novel predictive factors of clinical responses and the reporters of cancer progression.

Key words: melanoma, AGI-101H, humoral response, ALDH1.

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Poster

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Hippo kinase LATS1 is involved in cell invasion and oxidative stress 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 melanoma cell invasion and in formation of reactive oxygen species (ROS). Using a panel of human melanoma cell lines we demonstrated that LATS1 affects cell migration/invasion through an extracellular matrix (ECM) and is involved in oxidative stress. We propose the mechanisms of those phenomena. Further analysis of Hippo pathway 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.

Key words: melanoma, LATS1, migration, oxidative stress.

This work was supported by NCN grant, OPUS 8 2014/15/B/NZ5/03563.

Poster

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Nrf2 in non-small cell lung cancer – potential role in immune surveillance

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The nuclear factor erythroid 2 related factor 2 (Nrf2) is a relevant basic leucine zipper (bZIP) transcription factor that is essential in the regulation of cell cycle homeostasis, cytoprotection, and innate immunity when cells are under stressful conditions. Nrf2 was shown to be protective against multiple redox-induced and xenobiotic-induced diseases including cancer and its activation is beneficial in terms of prevention of chronic diseases. On the other hand, due to the great potential in protecting cells against different stresses, Nrf2 pathway is activated in many types of tumors, including non-small cell lung cancer. We show here that transcription factor Nrf2 promotes proliferation of non-small cell lung cancer cell lines and its inhibition potentiates action of ROS-inducing anticancer compounds. Interestingly, in normal lung fibroblasts, silencing of Nrf2 expression reduced MHC class I levels, indicating that Nrf2 is important not only for the growth and protection of cells but also in terms of immune surveillance.

Key words: Nrf2, MHC class I, oxidative stress, non-small cell lung cancer (NSCLC).

Poster

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Minocycline reduces production of osteopontin/Spp1 in glioma cells and modulates the immune microenvironment of rat C6 gliomas

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Glioblastoma (GBM) is the common and aggressive primary brain tumour. GBM is resistant to most conventional approaches and with a median survival of 14.6 months carries a dismal prognosis. Glioma-associated microglia and macrophages (GAMs) infiltrate tumor, support tumor cell invasion and contribute to immunosuppression. We demonstrated a critical role of osteopontin (Spp1), a potent immune cell attractant and activator, secreted by glioma cells in GAMs pro-tumorigenic polarization and glioma progression. We found that minocycline (mino) exhibits a prominent effect on glioma grow and could act as an immunotherapy agent. Studying mino effects in several glioma cell lines we found both growth arrest, cell death and reduction of the expression of Spp1 on mRNA and protein levels. Systemic application of mino (30 mg/kg b.w.) to animals implanted intracranially with C6-Luc+ glioma cells reduced tumor volumes at day 14th as determined using an In Vivo-Imaging Xtreme. Immune heterogeneity of glioma microenvironment was analyzed by FACS in controls and mino treated animals. Expression of selected GAM markers in sorted CD11b+ cells from glioma-bearing hemispheres and cytokine production in glioma-bearing hemispheres were measured. Mino treatment did not affect microglia accumulation, but blocked the pro-tumorigenic activation in generated CD11b+ cells and increased the expression of selected GAM markers. The increased accumulation of macrophages and leukocyte subpopulations was detected in mino-treated rats. Profiles of cytokine production in tumor-bearing hemispheres from mino-treated animals showed differences in the production of pro-inflammatory cytokines and macrophage attractants when compared with controls. Mino treatment reduced expression of pro-invasion factors secreted by glioma cells, and stimulated expression of pro-inflammatory-related genes in glioma-bearing hemispheres and immunosorted GAMs. These results validate treatment with mino as a promising strategy to block Spp1 production and microglia/macrophage activation and in consequence tumor growth.

Key words: glioma, minocycline, osteopontin.

Poster

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The effect of 17-aminogeldanamycin on NF-κB activity in patient-derived melanoma cell lines

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Introduction: Melanoma is one of the most aggressive human cancers. Melanoma incidence is increasing, which poses a serious clinical problem worldwide. The V600E BRAF substitution occurs in approximately 50% of melanoma cases, and hence, BRAFV600E has become a target for therapy. However, targeted therapies with RAF inhibitors, alone or in combination with a MEK1/2 inhibitor, result in initial potent response and subsequent relapse with drug-resistant disease. In the search for new therapeutics, HSP90 inhibitors introduce an interesting approach. 17-aminogeldanamycin (AG), an HSP90 inhibitor, was selected for this study based on preliminary results obtained at our Department. NF-κB is a transcription factor regulating the expression of a number of genes involved in the regulation of melanoma cell proliferation, invasion and survival. A constitutive induction of NF-κB has been noted in BRAF-mutant melanoma. In addition, elevated NF-κB activity has been reported in melanoma cells resistant to BRAF inhibitors. The effect of AG on NF-κB activity has not been elucidated so far.

Aim of the study: To assess the influence of 17-aminogeldanamycin (AG) on NF-κB activity and thereby evaluate its anti-melanoma effect.

Material and methods: We used cell lines derived from advanced-stage surgical melanoma specimens named DMBC11, DMBC12, DMBC21 and DMBC22. NF-κB activity was assessed by measuring the level of phosphorylated p65 (a subunit of NF-κB) with the use of Western blotting, subsequent to 4 and 24 hours of incubation with AG. The expression of three NF-kB-dependent genes: BCL2L1 (BCL-XL), CCND1 (cyclin D1) and CXCL8 (interleukin-8) was assessed using real-time PCR, following 6 and 22 hours of incubation. AG concentration of 0.4 μM was used.

Results: 17-aminogeldanamycin (AG) substantially reduced the level of phosphorylated p65 after 4 hours. This effect persisted after additional 20 hours of incubation. Such decrease was consistently reported in cell lines harbouring the BRAFV600E variant (DMBC11, DMBC12 and DMBC21) and in NRASQ61R-positive DMBC22 cells. AG significantly reduced transcript levels of both CCND1 and CXCL8 in DMBC12, DMBC21 and DMBC22 cell lines, following a 22-hour incubation. In DMBC11 cells, significant reduction was limited to CCND1. AG did not markedly affect the transcript level of BCL2L1.

Conclusions: 17-aminogeldanamycin (AG) reduces NF-κB activity in melanoma cells representing BRAFV600E and NRASQ61R subtypes.

Key words: melanoma, 17-aminogeldanamycin, NF-κB, patient-derived cell lines, HSP90 inhibitors, BRAF V600E mutation.

Poster

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Circulating EBV DNA in blood of head and neck cancer patients

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Introduction: A viral origin for some head and neck squamous cell carcinomas (HNSCCs) is evident. Many studies have documented the role of Epstain-Barr Virus (EBV) in the pathogenesis of nasopharyngeal (NPC) squamous cell carcinoma. EBV infection is ubiquitously detected in the primary and metastatic tumor cells of almost every patient with NPC, regardless of the geographic origin of the patient or the degree of tumor differentiation. In this work, we presented the clinicopathological results, which focused on detecting the circulating EBV DNA in blood of NPC and non-NPC patients.

Material and methods: Consecutive patients treated definitively with radiotherapy (RT) or radiochemotherapy (ChRT) for HNSCC between 2011 and 2015 at the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology (Gliwice Branch, Poland) were included in our study. QPCR was performed for quantitating EBV DNA in the plasma of head and neck cancer patients. Multivariate analysis using a Cox regression model was performed with the following variables in the model: age, sex, smoke, clinical stage, EBV factor for LRC (locoregional control).

Results and conclusions: The frequency of detecting EBV DNA in plasma samples was 53% (25/47) in the NPC patients. Multiple regression analysis between EBV DNA detection and clinical parameters in NPC patients (n = 47) revealed no important predictors for EBV detection. In multivariate analyses of NPC patients, no independent prognostic factor for LRC was established. The frequency of detecting EBV DNA in plasma samples was 40% (156/395) in the non-NPC patients. Multiple regression analysis between EBV DNA detection and clinical parameters in non-NPC patients (n = 47) revealed no important predictors for EBV detection. In multivariate analyses of non-NPC, overall stage was the independent prognostic factor for LRC (HR = 1.99, 95% CI = 1.1–3.39).

Key words: head and neck squamous cell carcinomas, EBV DNA.

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Poster

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CEACAM-6 CAR-T antitumor efficacy in pancreatic cancer treatment

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Pancreatic cancer is characterized by the lowest survival rate of all cancers in Europe, with estimated over 95,000 deaths every year. With the current therapeutic approaches, the median survival time after the diagnosis is 4.6 months. Immunotherapies with checkpoint inhibitors that have emerged as a novel therapeutic option in many types of malignancies, have shown overall very weak efficacy in pancreatic cancer patients. Therefore, development of effective systematic therapies still remains crucial.

Adoptive transfer of T lymphocytes expressing chimeric antigen receptors is currently considered the most promising anti-cancer therapeutic available to the patients. To generate the appropriate cell therapy product, T cells are collected from patient peripheral blood and redirected to a specific antigen via viral expression of a Chimeric Antigen Receptor (CAR). This therapy is currently used in the treatment of acute leukemias and B-cell malignant lymphomas. Clinical trials are ongoing to assess CAR-T effectiveness in other haematological cancers, as well as in solid tumors. The latter, however, remains challenging.

In our approach, we target carcinocinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6). CEACAM6 is a cell surface protein that is overexpressed in a wide variety of human cancers, including pancreatic adenocarcinoma, and is associated with tumourigenesis, cancer cell adhesion, invasion, and metastasis. CEACAM6 Chimeric-Antigen Receptor T-cells (CEACAM6 CAR-T) were generated using a single-domain camelid monoclonal 2A3 antibody against human CEACAM6. Application of 3rd generation lentiviral vectors allowed for a transduction efficiency of > 25%. CAR-T treatment significantly decreases BxPC3 pancreatic cancer cells growth *in vitro*, what is accompanied by elevated secretion of proinflammatory IL-2 and INF-γ cytokines. The antitumor effect is observed exclusively in CEACAM6 expressing cells, as no viability rate reduction occurred when control breast cancer MDA-MB231 cells, with no detectable antigen expression level, were treated. CEACAM6 CAR-T cells cytotoxicity was confirmed in animal model of human cancer disease. Targeting CEACAM6 antigen with CAR-bearing T-cells significantly decreased BxPC3 xenograft tumor growth, what in few cases resulted in a complete regression. Presented results strongly support the application of CEACAM6 CAR-T cells, manufactured by Helix, as a novel and effective immunotherapy against pancreatic cancer.

Key words: CAR-T, immuno-oncology, pancreatic cancer, adoptive transfer, single-domain antibody, solid tumors.

Poster

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NGS-based identification of cerebrospinal fluid microRNAs as diagnostic markers in primary central nervous system lymphomas

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Introduction: Primary central nervous system lymphomas (PCNSLs), 95% of which are diffuse large B-cell lymphomas (CNS DLBCL), are highly aggressive, extranodal non-Hodgkin lymphomas. In spite of the development of new techniques, differential diagnosis of PCNSLs and non-malignant diseases of the central nervous system (CNS) remains a challenge.

Aim of the study: We aimed at examining cerebrospinal fluid (CSF) miRNome in CNS DLBCL, and distinguishing miRNA expression patterns differentiating CNS DLBCL and non-malignant CNS diseases.

Patients: CSF samples were collected from patients diagnosed with CNS DLBCL (n = 12) and from patients with the initial presentation suggesting PCNSL, but finally diagnosed with non-malignant CNS diseases (n = 13). All patients were consulted at the Maria Skłodowska-Curie Institute and Oncology Center in Warsaw.

Methods: Total RNA was isolated from CSF samples, with mirVana™ PARIS™ RNA (Ambion). miRNA libraries were prepared with Ion Total RNA-Seqv2 and Ion Xpress RNA-Seq BC01-16 Kits, and the generated amplicons were sequenced using the Ion Proton platform (Thermo Fisher Scientific). Read mapping to the human genome hg19, known microRNA quantification and novel microRNA prediction was performed in miRDeep2. Differential microRNA expression was performed with DESeq2.

Results: The NGS analysis of CSFs revealed 411 microRNAs out of 1917 entries of the miRBase 22.1 registry, including 40 microRNAs detected solely in patients with CNS DLBCL and 130 solely in non-malignant diseases. The following 8 microRNAs: miR-15a-5p, miR-21-5p, miR-221-3p, miR-148a-3p, miR-16-5p, miR-19a-3p, miR-423-5p and miR-25-3p, found increased in the CSF of PCNSL patients, significantly differed between the two studied groups. Out of 5 CSF microRNAs of a reference potential, hsa-miR-24-3p, hsa-miR-23a-3p, hsa-miR-101-3p, miR-125b-5p, and miR-145-5p, RT-qPCR verification and NormFinder analysis ultimately identified hsa-miR-24-3p, hsa-miR-23a-3p and hsa-miR-125b-5p as the most stable.

Conclusions: CSFs of patients with CNS DLBCL and of patients with non-malignant CNS lesions are characterized by different microRNA profiles. Eight CSF microRNAs, miR-15a-5p, miR-21-5p, miR-221-3p, miR-148a-3p, miR-16-5p, miR-19a-3p, miR-423-5p and miR-25-3p, present the best potential as CSF biomarkers for differentiating CNS DLBCL from non-malignant CNS lesions. A combination of miR-24-3p, miR-23a-3p and miR-125b-5p was identified as the best reference for cerebrospinal fluid microRNA testing.

Key words: microRNA, next generation sequencing, central nervous system lymphoma, cerebrospinal fluid, DLBCL.

Poster

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Protein analysis of glioblastoma formalin-fixed paraffin-embedded tissues

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Introduction: Glioblastoma (GBM) is the most common and malignant brain tumor in adults and has a poor prognosis with a median survival of 15 months. Protein analysis of GBM tissues is urgently needed to identify potential protein biomarkers that may assist in early disease diagnosis and therapy development. However, simple and reliable protocols that can be implemented in clinics for proteome detection from minute tissue sections remain lacking. We here present methods for protein analysis from small quantities (~1 mm^2) of GBM formalin-fixed paraffin-embedded (FFPE) tissue sections.

Material and methods: Sections of FFPE GBM tissues of different thicknesses (4, 10, 15 and 20 µm) were sliced from tissue blocks and placed on glass slides. FFPE sections were deparaffinized in xylene and hydrated through an ethanol gradient. Several protein extraction buffers varying in composition of detergents (DDM, RapiGest) and chaotropes (urea) were compared. Using different analytical parameters, results were evaluated in terms of number of identified protein groups, number of identified peptides and number of acquired tandem mass spectra, and finally by the physicochemical properties of identified proteins.

Results: Results showed that protein extraction from FFPE tissues blocks was successful producing a high mass spectrometric response for loaded samples. Using a TripleTOF 5600+ (AB Sciex) instrument, we detected more than 700 protein groups, whereas among compared extraction methods buffers containing DDM resulted in the highest number of identified proteins. The method proved sensitive enough yielding a high number of identified proteins from less than 1 mm^2 of FFPE tissue, from which several proteins were identified as biomarker candidates.

Conclusions: In conclusion, we developed efficient and convenient methods for protein analysis from small amounts of GBM FFPE tissues. This is crucial for the development of less invasive procedures for taking patients' tissue samples and allows for the increased use of minute tissues amounts in clinical practice.

Key words: glioblastoma, FFPE, mass spectrometry, cancer, biomarker.

Poster

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microRNA and mRNA signatures differentiate Burkitt-like lymphoma with 11q aberration from Burkitt lymphoma

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Introduction: Burkitt lymphoma (BL) and Burkitt-like lymphoma with 11q aberration (BLL,11q) are highly aggressive B-cell lymphomas. BLL,11q, the new entity recognized by the 2017 WHO classification, is characterized by morphologic, immunophenotypic and clinical features resembling BL, but with no MYC translocation, however with specific 11q aberrations. To date, few studies on BLL,11q are available.

Aim of the study: The aim of the study was to compare transcriptome patterns between BLL,11q and BL. A better molecular characteristics of these rare aggressive B-cell lymphomas will pave the way to improve diagnosis and treatment outcomes.

Material and methods: The analysis included 17 clinical samples (10 BL and 7 BLL,11q), obtained by fine needle aspiration biopsy of tumors, from patients diagnosed and/or treated at the Department of Lymphoid Malignancies, Maria Skłodowska-Curie Institute – Oncology Center in Warsaw. The diagnoses were made according to the 2017 WHO classification, based on detailed histopathological criteria, immunohistochemical examination, cytological and cytogenetic analyses, flow cytometry immunophenotyping, along with clinical characteristics of the patients. The biopsies were subjected to erythrocyte lysis and kept frozen at –70°C until total RNA was isolated using TriReagent. Next generation sequencing (NGS) was performed on ION Proton Sequencer (ThermoFisher) for microRNA and mRNA profiling of each sample.

Results :NGS indentified 945 microRNAs and revealed 49 microRNAs differentially expressed between BL and BLL,11q. High Area Under the Curve (AUC > 0,8) for 25 microRNAs, including: miR-21-5p, miR-1295a, miR-21-3p, miR-29b-2-5p, miR-4464, miR-34a-3p, suggests their diagnostic potential. Transcriptome sequencing indicated significantly different expression of 2573 transcripts between BL and BLL,11q, including: SIAH2, BEST3, AEBP1, CTTN, CRB2, CHN2, SPOCK1, ETV5, SERPINA11, and STAT3. NGS data need further verification by the RT-qPCR. The analysis revealed differences in 45 molecular pathways at a transcriptome level, e.g., signaling by interleukins, MAPK family signaling cascades, neutrophil degranulation or immunoregulatory interactions between lymphoid and non-lymphoid cells.

Conclusions: Here we identified for the first time significant differences between BL and BLL,11q in mRNA and microRNA expression patterns. A number of microRNAs and mRNAs show high diagnostic potential for the molecular differentiation between BLL,11q and BL.

Key words: BL, BLL,11q, microRNA, mRNA, NGS, lymphoma.

Poster

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Immunogenicity of silk spheres – *in vivo* study

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Drug delivery systems are designed to increase the efficacy and safety of drug-based therapy. Silk is biocompatible and biodegradable material of potential biomedical applications. Herein we present drug delivery system based on nanospheres made of bioengineered spider silk proteins for selective doxorubicin delivery to Her2(+) cancer cells. The system indicated the ability to selectively transport doxorubicin into the Her2(+) cancer cells in the *in vitro* and *in vivo* studies. Moreover, the toxicological studies showed no toxic effects of said carriers on the model organism.

This study aimed to analyze the immunogenic effects of silk carriers after their administration in mice model.

The bioengineered silk proteins MS1 and H2.1MS1 were based on the sequence of major ampullate spidroin 1 from *Nephilia clavipes*. The target-specific variant H2.1MS1 was constructed by adding the oligonucleotide encoding the H2.1 peptide that possesses affinity towards Her2 overexpressed on cancer cells. The silks were produced in a bacterial system, purified by the thermal method and then mixed with 2M potassium phosphate buffer to form the nanospheres. The nanospheres were analyzed in terms of their immunogenic effect in the *in vivo* model. The nanospheres were administered intravenously to Balb/c mouse. As a control PBS was injected. The concentration of proinflammatory cytokines in blood serum was analyzed with cytometric bead assay. The presence of silk specific antibodies in serum after two intravenous injections of carriers was analyzed with immunoenzymatic-based assay.

In the study levels of interleukin-6 (IL-6), interleukin-10 (IL-10), monocyte chemoattractant protein-1 (MCP-1), interferon-γ (IFN-γ), tumor necrosis factor (TNF), and interleukin-12p70 (IL-12p70) were analyzed. Independent of the type of silk (primary or functionalized) the nanospheres did not elicit an increase in the levels of tested proinflammatory cytokines in mice in comparison with the control group that received PBS. The administration of spheres made of primary silk (MS1) did not induce the production of MS1 specific antibodies in the *in vivo* model, however, spheres made of functionalized silk H2.1MS1 with targeting peptide variant induced the moderate production of specific antibodies.

Based on the results the bioengineered silks show great potential as a material for formation of a drug delivery system.

Key words: drug delivery system, bioengineered spider silk, breast cancer.

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Poster

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Somatic mutation history of glioblastoma patients with recurrent tumors

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Glioblastoma (GBM, WHO grade IV) is a common and most lethal primary brain tumor which remains largely resistant to current therapies. Despite tumor resection and the following treatment of GBM patients with radiotherapy and chemotherapy, GBM frequently recurs. Dissection of the GBM genetics and understanding intrinsic mechanisms of tumor recurrence may lead to more targeted and effective treatments. Here we report the results of targeted next-generation sequencing of cancer- and epigenetics-related genes in 37 fresh frozen high grade glioma samples, collected from Polish population. We employed a second generation DNA sequencing target enrichment design comprising a 600 cancer-related gene panel and 100 epigenetics-related genes, comprising the exomes ±the promoter regions. The target region spanning 7 MB (1 × 106 base pairs) was designed to cover meaningful portion of genomic, cancer-related sites with a strong emphasis on epigenetic regulators (histone modifiers, chromatin modelers, histone chaperons). Additionally, RNA sequencing was performed to better understand the transcriptomic profiling in the malignant progression of GBM. Targeted sequencing of GBMs demonstrated different genetic drivers (including well known EGFR, TP53, PIK3R1, IDH1 and PTEN mutations) and numerous genetic alterations in genes responsible for histone modifications, chromatin remodeling, DNA damage repair and gene regulation, like for instance a novel recurrent frameshift deletion in ZN384. In recurring GBMs, we observed an upregulation of genes involved in interferon signaling, cell adhesion, immunoglobulins production, antigen processing and antigen presentation. Moreover, gene signature-based method revealed a significant enrichment in macrophages M2 and immature dendritic cells towards recurrent GBMs.

Key words: glioblastoma, cancer microenvironment, cancer relapse, somatic mutations, transcription profile.

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Poster

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Analysis of cell-cell interactions in the *in vitro* model of 3D breast cancer – development of assays conditions

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It is essential in a tumor biology study to analyze interactions between cell-cell and cell-matrix in the tumor microenvironment. The three-dimensional (3D) environment instead of two-dimensional cells culture better reflects conditions that are present *in vivo*. We developed a three-dimensional breast cancer model. The fibroblast and breast cancer cell line were co-cultured on the scaffold. Scaffolds were made of natural silkworm from *Bombyx mori* cocoons. Besides fibroblast and tumor cells, other types of cells are essential in the tumor microenvironment, and among them, macrophages and endothelial cells can be distinguished. The aim of this work is the development of assays conditions to analyze tumor vs. macrophages and endothelial cells interactions.

The NIH 3T3/635 fibroblast line expressing a red fluorescent protein, EMT6/GFP breast cancer cells expressing green fluorescent protein and J774 macrophages were used. Scaffolds were produced by the salting-out method. Fibroblasts and cancer cells were put on a scaffold in a ratio of 9 : 1, respectively, in an amount of 300,000 cells per scaffold and then grown up to 10 days. On the 7th and 10th day, fresh medium was added, and after 24 hours the conditioned media (CM) was collected. The control 3D cultures consisted of monoculture of fibroblasts and breast cancer cells. The analysis of cells interactions included the following assays: 1) unilateral paracrine interactions 3D cancer model vs. macrophages by using conditioned medium collected above the 3D cell culture, 2) bilateral paracrine interactions 3D cancer model vs. macrophages by simultaneous co-culture with 3D cell culture separated by a semipermeable membrane, 3) analysis of the macrophages migration in the microenvironment of the 3D breast cancer model by using the transwell migration assay, 4) analysis of the endothelial cells activation in the microenvironment of the 3D breast cancer model by using tube formation assay. The analysis was performed using flow cytometry and microscopy. The preliminary results indicated that the obtained three-dimensional model of breast cancer allows studying the interaction of cells that form the microenvironment of breast cancer. However, to generate reproducible cells interactions model, the optimization of assays conditions is needed.

Key words: tumor microenvironment, breast cancer, 3D breast cancer model, cell-cell interactions, silk scaffold.

Poster

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p53 mutant cell line as a model for a neoantigen discovery pipeline

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Aim of the study: Currently, there is great interest in the potential of personalized cancer therapies. The use of a patient's MHC peptides to reawaken killing mechanisms has gained renewed interest due to availability of patient-specific genomes. We used WT and p53 knock out mutant cells as a way to easily generate mutated peptides as surrogates for neoantigens. The data were used to develop an informatic proteogenomic pipeline and separation performance of two different methods were compared for ability to detect mutated peptides.

Methods: A nanoLC coupled to an Orbitrap Q-Exactive was used. Sample was loaded onto the 300µmIDx 5mm C18 trap column and separated either on a µPAC cartridge with 2µm interpillar distance and 2 m separation path operated or on C18 (2µm, 100Å; 75µm ID × 50 cm) at 300 nL/min. Both separations and the trap column were operated in a column oven at 50°C. Data acquisition used a data-dependent process. Database search was conducted using a custom RNAseq derived database in Mascot v2.6.0 with following settings carbamidomethyl was set as fixed modification and trypsin was set as a protease.

Preliminary data: Database searches of tandem MS data identified 87 unique mutated peptides via µPAC and 58 with PepMap. Both columns were operated using identical mobile phases, gradients and settings except for the flow rate 300 nL/min on PepMap and 600 nL/min on µPAC. The µPAC column showed lower backpressure and higher peak capacity with excellent reproducibility. The PepMap column also displayed good peak capacity, but the retention time reproducibility was not comparable to the µPAC column. The µPAC showed improved protein IDs compared to PepMap column and also produced higher individual protein sequence coverage. This comparison suggests that our proteogenomic platform will benefit from the better separation of the µPAC column that will provide better coverage of the mutational landscape in tumor tissues.

Key words: neoantigen, p53, mass spectrometry.

Poster

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In vitro analysis of specific binding of spheres targeting VEGF receptors

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Introduction: The biomedical application of silk is possible due to its unique properties, such as mechanical strength, biocompatibility, and biodegradability. Moreover, a genetic engineering enables to produce bioengineered silk by the expression of artificial genes that are based on natural sequences. Furthermore, the functionalized silk can be bioengineered by adding sequence encoding a homing peptide that provides binding to the target site. Functionalized silk spheres can be applied as a drug delivery system in cancer treatment. Such targeted drug delivery would minimize the adverse effects of chemotherapy. Beside the cancer cells, the cells of blood and lymphatic vessels of tumor can be a target for anticancer therapy.

Aim of the study: The analysis of specific binding of the functionalized silk spheres to cells overexpressing VEGF receptors.

Material and methods: The bacterial expression system was used to produce MS1 and MS2 bioengineered silk proteins and their functionalized variants VE1/MS1 and VE2b/MS2, respectively. The VE1/MS1 and VE2b/MS2 variants contain the domains responsible for binding to VEGFR1 and VEGFR2, respectively. The proteins were purified using thermal denaturation method and analyzed by SDS-PAGE electrophoresis. The functionalized and non-functionalized proteins were labeled with FITC. The silk proteins were mixed with potassium phosphate and the silk nanospheres were formed by using high-pressure syringe pumps. The MCF-7 breast cancer cell line was cultured under normoxic and under hypoxic conditions. The cell expression of VEGFR1 and VEGFR2 and the binding of FITC-labeled functionalized silk spheres to the cells was examined by the flow cytometry. The spheres morphology was analyzed by SEM.

Results: The functionalized and non-functionalized silks were produced and purified. The SDS-PAGE analysis did not indicate impurities or major protein degradation. The flow cytometry analysis of VEGFR1 and VEGFR2 expression in cells showed an increase of expression of both receptors in cells cultured under the hypoxic conditions. The flow cytometry analysis showed that the VE1/MS1 functionalized spheres shown about 7,5 times higher binding to VEGFR1 overexpressing MCF-7 cells comparing to non-functionalized MS1. The VE2b/MS2 spheres shown about 1.5 times higher binding to MCF-7 cells compared to non-functionalized MS2 spheres.

Conclusions: Functionalized silk spheres which can recognize VEGF receptors have a great potential for anticancer treatment.

Key words: bioengineered spider silk, VEGFR, silk spheres, drug delivery system, tumor microenvironment.

Poster

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Therapeutic melanoma vaccine displaying cancer stem cells phenotype represses exhaustion and maintains antigen-specific T cell stemness by up-regulation of BCL6

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Aim of the study: We have developed therapeutic gene modified allogeneic melanoma vaccine (AGI-101H), comprising of two cell lines which express cDNA encoding H6 - a fusion protein composed of IL-6 linked with the soluble IL-6 receptor (sIL-6R), that alters vaccine cell phenotype toward melanoma stem cells-like. It has been in clinical trials since 1997 and resulted in a long-term survival of a substantial fraction of immunized patients. Here we present the potential molecular mechanisms behind the long-lasting effect of AGI-101H using transcriptome profiling of peripheral T lymphocytes.

Experimental design: Magnetically separated, untouched peripheral T cells from AGI-101H-immunized long-term survivals, untreated melanoma patients and healthy controls were subjected for transcriptome profiling using HG U219 microarrays. Data were analyzed with bioinformatics tools (DAVID, GSEA) and validated with RT-qPCR.

Results: Substantial transcriptome alterations between peripheral T lymphocytes from healthy controls and melanoma patients either untreated or AGI-101H vaccinated were found. In contrast, AGI-101H immunization triggered similar transcription profile of peripheral T cells as tumor residing in untreated patients. It suggests that the whole stem cells immunization provokes mobilization of analogous peripheral T cells to the natural adoptive anti-melanoma responses. Moreover, AGI-101H administration induced TNF-α (via NFκB) signaling and depleted IL2-STAT5 signaling in peripheral T cells that finally resulted in significant up-regulation of BCL6 transcriptional repressor. Bcl6 amplifies proliferative capacity of central memory T cells and enforces the progenitor fate of antigen-specific T cells that facilitate their longevity and proliferation. Also, high level of BCL6 negatively correlates with exhaustion markers expression, and BCL6 was previously reported to directly suppress exhaustion markers and maintain antygen-specific CD8+ T cell stemness.

Conclusions: The up-regulation of BCL6 expression in peripheral T cells of AGI-101H immunized melanoma patients ultimately provide mobilization of T cells to protect against tumor development by keeping cancer cell dormant in melanoma patients.

Key words: melanoma, whole cell melanoma vaccine, dormancy, transcription profile, microarray, BCL6.

Poster

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The diagnostic role of miR-17 and miR-20a involved in extracellular matrix remodelling in non-small cell lung cancer

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Introduction: Lung cancer is one of the most common cause of death worldwide with relatively high fatality rate. Average 5-year survival is about 18%, which is far from satisfying. One of the hallmarks of cancer is extracellular matrix (ECM) remodelling, which is crucial for metastasis. This process may be regulated by miRNAs. Thus, deep understanding of connection network between miRNAs and genes encoding the most crucial protein for ECM remodelling may lead to develop precise, cheap biomarkers for early detection and more personalized treatment. Search for early biomarkers is essential in non-small cell lung cancer (NSCLC). Using miRTarBase we selected microRNAs: miR-17 and miR-20a targeting MMP2 and TIMP3 genes. Metalloproteinase MMP2 is associated with the ECM breakdown and metastatic process, whereas TIMP3 inhibits the metalloproteinase activity.

Material and methods: Our study group comprised of 48 patients with primary NSCLC, who underwent pulmonecomy or lobectomy. RNA was isolated from tumour tissues and adjacent macroscopically unchanged lung tissue from surgical margin, served as a control. Blood samples for miRNA extraction were collected before and after surgery. The gene and miRNA expression level were evaluated using qPCR. The statistical analysis was performed using Statistica 13.1PL (StatSoft).

Results: The miR-17 expression was higher among patients with SCC compared to AC, both before ($p = 0.02$, UMW test) and after surgery. The opposite dependence was observed for preoperative miR-20a ($p = 0.02$, UMW test). No differences were found regarding pTNM or AJCC staging. Analysis of gene expression revealed lower expression in cancer tissue vs.control tissue for TIMP3 ($p = 0.01$, Wilcoxon test). The positive correlation between TIMP3 and MMP2 expression was observed in the surgical margin ($R = 0.69$, $p < 0.01$; Spearman R correlation).

Conclusions: Obtained data suggests the role of both miR-17 and miR-20a as the biomarkers distinguishing histopathological subtypes of NSCLC. Higher TIMP3 expression level in surgical margin than in tumour underline their role as antimetastatic defence strategy. Strong positive correlation between TIMP3 and MMP2 evaluated in surgical margin, and elevated TIMP3 suggests maintaining the ECM remodelling processes in tissue adjacent to the lesion.

Key words: non-small cell lung cancer (NSCLC), miR-17, miR-20a, MMP2, TIMP3, ECM remodeling.

Poster

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Activation of DNA damage response signaling pathways during chondrogenic differentiation of hiPSCs – gene expression profile analysis

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We examined the mechanisms of DNA damage response (DDR) activated in chondrocyte-like cells differentiated from human induced pluripotent stem cells (ChiPS). High level of DDR mechanisms is considerably associated with the forced chondrogenic differentiation *in vitro*, that may constitute a notable stress for cells. The aims of the study were: a) to investigate gene expression profile of the obtained ChiPS and b) to compare expression of genes involved in DDR process between ChiPS with hiPSCs and mature chondrocytes.

To fulfill those tasks were carried out the following analyses: chondrogenic differentiation of hiPSCs (Suchorska *et al.* 2017) was conducted i), global gene expression microarray was performed and analyzed using GeneAtlasTMWT Expression Kit Assay and AffymetrixGeneAtlasTM Operating Software ii), the Bioconductor and statistical programming language R were used iii), the validation of microarrays was performed by RT-qPCR iv).

All differentially genes engaged in specific biological processes were visualized using gene ontology (GO) plot library. Our latest results indicate that ChiPS possess induced DDR mechanisms acquired during differentiation. In ChiPS, the increased expression of genes classified to the inter alia following GO terms: is "cell cycle arrest", "cell cycle checkpoint", "DNA damage response", "signal transduction in response to DNA damage" and "cellular response to stress" is observed. Moreover, based on the Kyoto Encyclopedia of Genes and Genomes the one of superior pathway regulated during chondrogenic differentiation *in vitro* is p53. The expression of selected genes involved in DDR mechanisms and particularly in p53 signaling pathway was verified with RT-qPCR analysis.

We found that hiPSCs differentiated toward ChiPS may undergo a stress that leads to activation of DDR mechanisms. The differentiated cells probably are also prone to exposure to genotoxic agents.

Key words: hiPSCs, chondrogenesis, DNA damage, p53.

Poster

KW019-00024-2019-01

Effects of radiation on T regulatory cells changes in melanoma patients

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Introduction: Regulatory T cells (Tregs) represent a T cells population showing a role in regulating or suppressing other immune cells. Tregs are able to control the immune response to the antigens as well as can help prevent autoimmune disease. Tregs also can support immunosuppression in cancers and promote local tumor growth. Tregs are the subpopulation of CD4+ cells, expressing nuclear transcription factor Forkhead box P3 (FoxP3) and high level of CD25, which is a component of the IL-2 receptor. FoxP3 expression is the defining property which determines natural Treg development and function. FoxP3 is crucial for maintaining suppression of the immune system. Lymphocytes are considered as radiosensitive cells, however radiotherapy (RTH) can increase the production of Tregs and the Tregs recruitment to local tumor microenvironment. Tregs can attenuate radiation-induced tumor death, which cause the tumor resistance to RTH.

Aim of the study: The aim of this study was the analysis of peripheral blood Tregs level in melanoma patients treated with RTH.

Material and methods: Twenty nine metastatic melanoma patients qualified to RTH and 17 control individuals (with benign or malignant, localized melanocytic tumors, qualified to surgical removal) were included into this study. Tregs level were assessed in peripheral blood samples with flow cytometry, and cells showing CD4+/CD25+/CD127- phenotype were identified as Tregs. In melanoma patients Tregs were analyzed before and 1 month after RTH. In control group Tregs were analyzed before surgery.

Results: Treg level in metastatic melanoma patients before RTH (7.9%) were similar to the control group (9.1%) and significantly increased after RTH (9.8%; $p = 0.0029$ and $p = 0.039$, respectively). Analysis of pair-matched data also showed statistically significant differences ($p < 0.0001$). CD4+ lymphocyte level were the highest in metastatic melanoma patients before RTH (13.5%) and decrease after RTH (9.9%), however this difference were not statistically significant ($p = 0.079$). Nevertheless pair-matched analysis showed statistically significant changes ($p = 0.028$).

Conclusions: Our preliminary results showed an increase of Tregs and slight decrease of CD4+ lymphocytes in melanoma patients treated with RTH. These results seems to confirm the hypothesis of higher Tregs radioresistance or its higher generation after radiation. Further study is required to understand the mechanism of Treg level regulation and function after RTH in melanoma patients.

Key words: melanoma, Treg cells, radiotherapy, immunosuppression, flow cytometry.

Poster

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Proteomic profiles of melanoma-derived exosomes (MTEX) from the plasma of melanoma patients – a preliminary study

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Tumors can form immunosuppressive microenvironment that „turns off" immune cells while promoting the development of cancer, hence „tumor escape" from the immune system control still remains a major unsolved problem of cancer treatment. It is proposed that exosomes released by tumor cells mediate signals that inhibit the immune system. Moreover, the molecular composition of exosomes reflects that of parental cells, hence exosomes are an emerging source of cancer markers. Here we hypothesize that the unique molecular protein profile of exosomes produced by melanoma cells (MTEX) and released to patient's plasma is similar to proteome if parental melanoma cells, therefore these vesicles can serve as an equivalent to „liquid biopsy".

In the plasma of patients diagnosed with cancer, there are both exosomes released by normal cells and cancer cells, hence separation of vesicles produced by tumor (TEX) is a prerequisite of their successful characterization. We use a CSPG4-specific 225.28 mAb, which selectively recognizes an epitope overexpressed on melanoma cells in at least 80% of melanoma patients but is not detected in normal tissues. Here we used a combination of size-exclusion chromatography (SEC) and immunoaffinity-based capture using the 225.28 mAb to purify exosomes from patients' plasma. MTEX and not-MTEX were separated from the plasma of 10 melanoma patients. Exosomes were characterized by transmission electron microscopy (TEM) and Western-blotting for exosome markers, then their proteomics profiles were further analyzed using the shotgun LC-MS/MS proteomics approach.

In general, there were about 3,700 proteins identified in not-MTEX and about 3,600 proteins identified in MTEX. This is noteworthy, that about 300 proteins were identified exclusively in the MTEX. These included proteins previously reported in the development of melanoma and other malignancies, also in the context of immune-modulation. Moreover, quantitative analysis revealed individual differences between patients, which will be further characterized when a larger group of melanoma patients is included in the study.

Key words: melanoma, plasma, proteomics, mass spectrometry, exosomes.

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Poster

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Spontaneous senescence of primary epithelial ovarian cancer cells

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According to classic paradigm, cellular senescence restricts the mitotic lifespan exclusively in normal somatic cells. Last two decades, however, provided evidence that senescence may also be induced in cancer cells in response to various kinds of chemotherapy or ionizing radiation (so-called "therapy-induced senescence"). Also, some reports suggest that senescence of cancer cells may occur spontaneously, albeit the knowledge regarding mechanism(s) of this process is elusive.

Recently, we observed that a significant fraction of epithelial ovarian cancer cells (EOCs), either in tumors *in vivo* or in culture *in vitro*, display signs of cellular senescence, such as positive staining for senescence-associated β-galactosidase (SA-β-Gal) and excessive DNA damage foci (histone γ-H2A.X). Experiments on primary EOCs showed that they divide *in vitro* approximately 3.3 ±1.2 times, and then they degenerate morphologically (become hypertrophic) and lose their ability to replicate. The fraction of cells displaying SA-β-Gal increases from 6.6 ±4.6 in early-passage cultures to 83.9 ±11.6% in senescent cultures. The percentage of cells bearing γ-H2A.X foci increases, in turn, from 11.2 ±8.5 in early-passage cultures to 71.5 ±1.2 in senescent pEOCs. When the expression of SA-β-Gal was colocalized with the presence of γ-H2A.X foci, the fraction of cells displaying this phenotype increased from 1.6 ±1.2 to 67.1 ±6.6%. Analysis of telomere length using qPCR showed that senescence of EOCs is associated with shortening of these structures from 4.6 ±1.8 to 2.8 ±1.1 kbp, and this effect was accompanied by reduction of telomerase (hTERT) activity from 3.6 ±2.5 to 1.0 ±0.0 TPG units. As for the effectors at the level of cell cycle, senescence of EOCs was associated with an up-regulation of p16 (from 4.2 ±2.7 to 52.6 ±25.43% of cells), p21 (from 1.9 ±1.5 to 36.4 ±26.2% of cells), and p53 (from 3.6 ±1.6 to 30.8 ±24.8% of cells).

Collectively, our findings indicate that primary EOCs undergo replicative senescence *in vitro* similar to normal somatic cells. The mechanism of this process, despite the short replicative lifespan of these cells, seems to be mainly telomere-dependent. Some component of telomere-independent, p16-related senescence cannot be, however, excluded.

Key words: ovarian cancer, cellular senescence.

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Poster

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The study of biologically synthesized Au-CuO and CuO-ZnO nanoparticles against glioma cells and microorganisms

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In the current study, the synthesized Au-CuO and CuO-ZnO nanoparticles using *Cnici benedicti* were analyzed in terms of their antibacterial activity, as well as their influence on malignant cell viability, using two specific cell lines: C6 rat brain glioma (ATCC® CCL-107™) and T98G human glioma (ATCC® CRL-1690™). The studies carried out using AFM helped to determine the presence Au-CuO nanoparticles size about 13 nm. In contrast, the size of CuO-ZnO nanoparticles was about 28 nm. The obtained nanoparticles showed cytotoxic activity against glioma cells depending on the concentration of the substance and the time of culture. In the first stage, the nanoparticles limited the ability to divide cells; then, they blocked the cell cycle in the G2-M phase, and finally led to massive cell death.

Key words: nanoparticles, green synthesis, glioma, cytotoxicity.

Poster

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The influence of microenvironment generated by pleural macrophages on lung cancer cells

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Macrophages constitute a link between various elements of the immune system involved in defense mechanisms against external and internal danger. However, in certain circumstances, they can support cancer development, directly and indirectly.

The study aimed to assess the impact of soluble factors presented in conditioned media obtained from short term culture of macrophages isolated from malignant and non-malignant pleural effusions on the established cell line of human non-small cell lung cancer A549.

The use of RT-PCR and real-time qPCR served for molecular evaluation with of the expression of selected genes, as ITGAV, ITGB, BCL-2, BAX, PTK, ERK, TTF-1, and IL-6, involved in the cancer development and progression.

Based on the obtained results, it was found that macrophages which are an essential part of the microenvironment of pleural effusion, affects cancer cells. Used conditioned media affected the level of expression of studied genes in different ways. In most cases, conditioned media derived from malignant pleural effusion favored the development of cancer cells, while non-malignant conditioned media inhibited it. The increase of expression of BCL2 and IL6 genes suggest the pro-malignant properties of the used conditioned media. These observations correspond with our previous studies in which we noted the influence of macrophage-derived conditioned media on proliferation activity and apoptosis of cancer cell lines *in vitro*.

Key words: pleural effusion, cancer microenvironment, pleural macrophages, lung cancer.

Poster

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UV radiation influences ferroptosis in human colorectal cancer

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Aim of the study: Aim of the study was to investigate the way which the human colorectal cancer cells (HCT116) die after exposure to ultraviolet radiation. Study was focused on ferroptosis cell death which results from oxidative stress and accumulation of lipid peroxidation products in cells. Nevertheless, both normal or cancer cells can response to ferroptosis with different level of resistance what generally depends on the type of cells affected. The HCT 116 cells are ferroptosis sensitive cells and for that reason Erastine and RSL-3, ferroptosis specific inducers, were used to initiate ferroptosis in tested cells.

Material and methods: HCT116 cancer cells were exposed to three different wave ranges of UV radiation (A = 10 kJ/m^2; B = 5 kJ/m^2; C = 100 J/m^2) and were collected after 1 h, 3 h, 6 h, 12 h and 24 h for further investigation. First cells were stained with 2'7'-dichlorodihydrofluorescein and DAF-FM to measure with flow cytometry the level of reactive oxygen species (ROS) and nitrogen oxygen species (NOS), respectively. Secondly RT-PCR reaction was applied to measure gene expression level of NOX4 and eNOS enzymes responsible for production of free radicals and also GPX4 the ferroptosis marker gene. Finally, MTT assay was performed to test cellular proliferation and viability after UV exposure and/or Erastine and RSL-3 treatment.

Results and conclusions: UV radiation reduced cell proliferation and viability by 30%. Exposure to UVA and UVB radiation increased NO levels in cells after 12 h but only after UVB, gene responsible for NO production, eNOS was elevated. For UVA exposure especially, tested cells intensified the production of superoxide anion at 3rd and 12th hour after irradiation what correlated with higher NOX4 gene expression at 12th hour. However, NOX4 gene expression after UVB radiation at the same time point was downregulated. GPX4 gene expression profile analysis showed that cells exposed to UVA irradiation upregulated GPX4 gene expression at 6th, 12th, 24th hour but those exposed to UVB only at 24th. These results suggest that the HCT 116 cells in order to resist ferroptosis over expressed GPX4 gene. HCT116 sensitivity to ferroptosis was confirmed by survival fraction decrease in MTT assay after treatment with ferroptosis inducers Erastine and RSL3, and also all types of applied UV wave ranges.

Key words: ferroptosis, GPX4, Cell death, ROS, UV radiation.

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Poster

KW019-00016-2019-01

lncRNAs as a new biomarkers of HPV infection in head and neck squamous cell carcinomas

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is a malignancy characterized by high level of patients' mortality. Numerous studies show that human papillomavirus (HPV) infection is one of the important risk factors in the progress of the disease. HPV influence on the expression of many different genes such as long non-coding RNA molecules (lncRNA). The expression level of lncRNA may be connected with clinical parameters and patients' overall survival and could serve as new biomarkers.

Aim of the study: This study is focused on the influence of HPV infection on the expression of long non-coding RNA molecules (lncRNA).

Methods: The TCGA expression data for selected lncRNAs and their targets as well as clinical data were downloaded from cBioPortal. The expression levels of lncRNAs were verified according to clinicopathological parameters. The high- and low-expression groups of lncRNAs as well as overall survival (OS) and expression levels of targets' genes were investigated.

Results: The expression of lncRNAs CDKN2B-AS1, TTTY14, TTTY15, PRINS, MEG3 and H19 are significantly different among HPV positive and HPV negative patients. Further analyses indicated a few parameters in the HPV positive group which are essentially distinct depending on the expression level of the particular lncRNA: smoking category, gender, N stage, lymph node dissection indicator and grade. It was also found that the OS for HPV positive patients with high expressions of PRINS is significantly better in comparison to lower levels. Moreover expression levels of viral and inflammation response genes among patients with high and low PRINS expression were analyzed. The results revealed that patients with high PRINS have significantly higher expression of genes of these groups.

Conclusions: The influence of HPV on selected lncRNAs expression was indicated. Moreover their utility as potential positive biomarkers of HPV HNSCCs have revealed. Analyzed data referring to lncRNA PRINS indicate its important role in the biology of HPV HNSCCs.

Key words: HNSCC, lncRNA, biomarker.

Poster

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The polyamine analog verlindamycin promotes differentiation and cell death in neuroblastoma

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Neuroblastoma (NB) is the second most common solid tumor in childhood, accounting for 8-10% of the total number of pediatric cancers and 15% of deaths. Deregulated polyamine biosynthesis is a common feature of NB and drugs targeting this metabolic pathway, such as difluoromethylornithine (DFMO), are in clinical and preclinical development. The polyamine analogue 2d (verlindamycin) inhibits the histone demethylase LSD1, as well as homologous enzymes involved in polyamine biosynthesis such as spermine oxidase and N1-acetylpolyamine oxidase. We previously demonstrated that 2d cooperated with all-trans-retinoic acid (ATRA) to promote differentiation of acute myeloid leukemia and reasoned that this drug might be effective in NB. Consistent with this notion, we found that treatment of a panel of NB cell lines with 2d and ATRA strongly induced differentiation that was associated with reduced growth and colony formation, as well as induction of G0 arrest and apoptosis. We found that 2d/ATRA treatment targeted NB cells regardless of MYCN status and biochemical analysis revealed that when expressed, MYCN protein was strongly downregulated. This process was not transcriptionally regulated but was due to increased turnover of MYCN protein, at least in part via proteasome-dependent destruction. Here we report that in addition to its established activities, 2d effectively induces, via ribosomal frame-shifting, expression of functional antizyme (Az). Consistent with previous results describing the function of Az tumor suppressor, we found that 2d treatment led to the selective targeting of ornithine decarboxylase (the rate-limiting enzyme for polyamine biosynthesis) as well as key oncoproteins such as cyclin D and Aurora A kinase. The finding that 2d treatment diminished Aurora A levels is notable in the context of MYCN expressing NB as we have previously shown that Aurora A binds MYCN, preventing its degradation. Furthermore, transcriptomic analysis revealed that treatment of NB cells with 2d/ATRA led to upregulation of genes associated with neuronal differentiation and downregulation of genes linked to a stem cell phenotype and NB pathogenesis, including EZH2. Retinoid-based multimodal differentiation therapy is one of few interventions that extends relapse-free survival in MYCN-associated high-risk NB and the results presented here strongly argue that the potential inclusion of verlindamycin in this regimen merits further investigation.

Key words: neuroblastoma, differentiation, polyamine.

Poster

KW019-00014-2019-01

Synthesis methods and effect of gold nanorods on prostate cancer cells

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Nanoparticles are widely studied for biomedical applications in particular as materials for medical engineering, pharmaceutical industry (drug transportation) and medical imaging (contrast agents). Within nanopharmacy special attention is put on the use of nanoparticles for cancer treatment. Considering synthesis techniques and properties, the application of rod-shaped gold nanoparticles have become the new interesting topic in nanomedicine.

In this work, we focused on two objectives, namely the synthesis of gold nanorods (GNRs) and investigating their effect on two prostate cancer cell lines LNCaP and PC-3. The GNRs were synthesized by modified two-step wet chemistry, seed-mediated method. The morphology of synthesized nanorods was determined using transmission electron microscopy (TEM). Their effect on cell viability was tested using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. After selecting appropriate concentrations, the cell cycle distribution was measured using flow cytometry. A wide range of concentrations was tested, which allowed us to determine the half maximal inhibitory concentration (IC50) of GNRs for chosen cell lines.

Increasing concentration determined the linear cytotoxic effect of nanoparticles. Both the cell viability and proliferation decreased with increasing concentration. Gold nanorods also affected the cell cycle of chosen cell lines. It can be concluded that gold nanorods can become a promising tool in cancer therapy. It is worth to emphasize that uncomplicated synthesis method is an important aspect of nanoparticles as well.

Key words: gold nanoparticles, nanorods, cancer treatment, prostate cancer, cytotoxicity.

Poster

KW019-00013-2019-01

Targeted sequencing of cancer- and epigenetic-related genes combined with RNA sequencing revealed novel mutations in the *TOP2A* gene as an important factor in glioblastoma pathogenesis

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Recent whole genome studies demonstrated that epigenetic enzymes, histones and chaperone proteins harbor mutations that may result in gross alterations of the epigenome leading to genome instability. Such mutations are common in pediatric hematopoietic and solid tumors, and are targets of innovative treatments with epigenetic enzyme inhibitors. Glioblastoma (GBM, WHO grade IV) is a common and most lethal primary brain tumor in adults, and remains incurable by conventional therapies. Greater understanding of GBM genetics may lead to more targeted and effective treatments. Here we report the results of targeted next-generation sequencing of cancer- and epigenetics-related genes in 203 fresh frozen glioma samples of grade II, III, and IV collected from Polish and Canadian populations.

The analysis has revealed a new somatic variant in the *TOP2A* gene encoding DNA topoisomerase II alpha, which is involved in many important processes, such as chromosomes condensation and chromatid separation. The mutation in the *TOP2A* gene was found in four WHO grade IV glioblatoma patients, only in Polish population. For the samples with the *TOP2A* mutation we performed mRNA sequencing and RNA sequencing after ribosomal depletion (to reveal also non-coding RNAs). Bioinformatic analyses of both types of RNAseq data in wild-type and mutated *TOP2A* glioblastoma samples revealed several interesting findings and differences in transcriptomes of wt or mutated *TOP2A* GBM samples. Altogether, our targeted NGS supported analysis of genomic alterations revealed a new potential pathogenic mechanisms for GBM pathology.

Funding: TEAM TECH CORE FACILITY FNP: Development of comprehensive diagnostics and personalized therapy in neuro-oncology.

Key words: next generation sequencing, glioblastoma, TOP2A, cancer.

Poster

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Evaluation of the effective dose of doxorubicin delivered by the functionalized silk spheres in a mouse model of breast cancer

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Chemotherapy is one of a therapeutic approach for the treatment of cancers. Nonspecific delivery of chemotherapeutic agents leads to undesired side effects to normal tissues. Targeted drug delivery systems can potentially resolve the problem of drug off-targeting. A silk biomaterial, which is known for its mechanical strength, biocompatibility, and biodegradability, can be applied as a drug carrier.

The aim of the present study is the *in vivo* evaluation of the dose of doxorubicin (Dox) delivered in the functionalized silk spheres to obtain its therapeutic efficacy.

The bioengineered MS1 and H2.1MS1 silks that were based on the MaSp1 protein from N. clavipes were used to obtain nanoparticles. Spheres were formed by mixing silk with potassium phosphate and then loaded with Dox by the diffusion method. The Her2-positive (D2F2E2/LUC) and control Her2-negative (D2F2/LUC) breast cancer cells were used to develop cancer in a BALB/c mouse. Biodistribution of the MS1 and H2.1MS1 spheres was investigated by using IVIS Spectrum *in vivo* imaging system. To analyze the therapeutic effect of Dox, the tumor-bearing mice received the Dox-loaded spheres in different treatment schedules, and then the tumor volume was measured. Systemic toxicity was analyzed by histopathological examination.

The accumulation of functionalized spider silk spheres at the site of the Her2(+) tumor was demonstrated. The treatment with Dox-loaded H2.1MS1 nanoparticles significantly suppressed Her2(+) tumor growth in comparison with treatment with Dox-loaded MS1 spheres at an equivalent dose of Dox. On the contrary, in mice that developed Her2(–) tumors and received Dox-loaded H2.1MS1 particles, the drug did not suppress the tumor growth. Moreover, the study significantly demonstrated the impact of the quantity of the first dose and the frequency of spheres administration on the therapeutic efficacy. Histopathological analysis revealed no systemic toxicity of drug when administrated as incorporated into silk spheres.

The obtained results indicated that functionalized bioengineered silks are great candidates as drug carriers for cancer treatment.

Key words: cancer treatment, bioengineered spider silk, targeted therapy, drug delivery, nanomaterials, biodistribution.

The study was supported by grant from The National Science Centre (UMO-2014/15/B/NZ7/00903).

Poster

KW019-00010-2019-01

lncRNA expression after irradiation and Zelboraf exposure of melanoma cell lines

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Introduction: Melanoma develops from melanoma cells and is responsible for approximately 2.3% of all skin cancers. Main risk factor of developing this type of cancer is exposure to ultraviolet radiation. The two most common mutations occur in BRAF V600E and BRAF V600K genes. These mutationts activate MAPK signaling pathway causing continous cell proliferation and apoptosis inhibition. One of commonly used drug in melanoma treatment is vemurafenib (Zelboraf), which is used in patients with BRAF V600E mutation and stops its activity. Radiotherapy is used as adjuvant therapy in melanoma. Long, non-coding RNAs (lncRNA) are at least 200 bp long and do not translate into proteins, however they carry on many regulatory functions in cells. Many previous studies have discovered that a vast number of lncRNAs are dysregulated in melanoma patients and have influence on patients' treatment outcome.

Aim of the study: The aim of this study is to determine lncRNAs expression profiles in melanoma cell lines after exposure to ionizing radiation and vemurafenib.

Material and methods: For the study we have chosen 4 melanoma cell lines: MeVo, Skmel-28, WM266 and WM115. Each cell line was treated with different doses of ionizing radiation (5 Gy, 10 Gy and 20 Gy). Other set of examined cell lines was treated with vemurafenib. Next, the RNA was isolated and the lncRNA expression was examined using qRT-PCR method. The results were compared with nontreated control cell lines.

Results: Exposure to ionizing radiation showed different lncRNA expression panels in examined melanoma cell lines. The dose of 5 Gy caused dysregulation of 4 lncRNAs, 10 Gy caused dysregulation of 2 lncRNAs and 20 Gy caused dysregulation of 5 lncRNAs. Vemurafenib inhibited proliferation in all of examined melanoma cell lines and caused dysregulation of 12 lncRNAs, also the lncRNA expression panels differed between cell lines.

Conclusions: Ionizing radiation as well as vemurafenib exposure have influence on lncRNA expression in melanoma cell lines. Different doses of ionizing radiation caused different expression panels of lncRNA showing that it depends strictly on dose as well as type of a cell line. As for the exposure to vemurafenib, we have confirmed its function as proliferation inhibitor. Many of dysregulated lncRNAs were previously correlated with different cancer types, treatment response and cellular processes.

Key words: lncRNA, melanoma, ionizing radiation, Zelboraf.

Poster

KW019-00009-2019-01

Oncogenic role of ZFAS1 lncRNA in head and neck squamous cell carcinomas

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is very heterogeneous disease with high mortality. The identification of specific biomarkers will increase the treatment efficacy and limit toxicity of current therapeutic strategies. Long non-coding RNAs (lncRNAs) are promising biomarkers. Accordingly, the biological role of ZFAS and its potential as a biomarker in HNSCC was studied.

Methods: Expression level of ZFAS1 in HNSCC cell lines was analyzed using qRT-PCR. Based on the HNSCC TCGA data ZFAS1expression profile, clinico-pathological features and expression of correlated and targets of ZFAS1 genes, lncRNA were analyzed in patients' tissue samples. The selected genes were classified into groups premised on their biological function through PANTHER tool. All statistical analyses were accomplished using Graphpad Prism 5.

Results: The expression of ZFAS1 in most cases were up-regulated in cell lines compared to the control one and up-regulated in HNSCC samples. The ZFAS1 levels generally considerably differ depending on the cancer stage and T-stage. Patients with a lower expression of ZFAS1 present longer disease-free survival and overall survival. The analysis of genes correlated with ZFAS1 as well its targets indicated that they are associated with crucial cellular processes. Moreover, in the group of patients with low expression of ZFAS1 the up-regulation of suppressors and down-regulation of genes associated with epithelial-to-mesenchymal transition (EMT) process, metastases and cancer-initiating cells were observed.

Conclusions: In HNSCC ZFAS1 displays oncogenic properties, regulates important processes associated with EMT, cancer-initiating cells and metastases, and may affect the patients' clinical outcome. The ZFAS1 following validation may prove a new valuable biomarker.

Key words: ZFAS1, ZNFX1 antisense RNA 1, lncRNA, non-coding RNA, HNSCC, biomarker.

Poster

KW019-00008-2019-01

Overweight is associated with better prognosis in metastatic colorectal cancer patients treated with bevacizumab plus FOLFOX chemotherapy

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Introduction: Previous studies showed that high and low body mass index (BMI) was associated with worse prognosis in early-stage colorectal cancer (CRC), and low BMI was associated with worse prognosis in metastatic CRC (mCRC). Whether BMI is a prognostic or predictive factor in mCRC is unclear. We aimed to assess efficacy outcomes according to BMI in patient with metastatic colorectal cancer treated with bevacizumab plus FOLFOX chemotherapy regimen.

Material and methods: The retrospective analysis of 122 patients with metastatic colorectal cancer treated with bevacizumab plus FOLFOX in the second line chemotherapy treated between 2014–2018 at four reference oncological centers. 84 patients with complete data were enrolled for BMI analysis.

Results: The median overall survival (OS) and progression-free survival (PFS) of the all 122 patient was 14,6 and 8,5 months, respectively. The rate of BMI ≥ 25 was in 51.2% of patients. Comparison of overweight patients versus normal BMI range patients revealed a significant improvement of median overall survival (OS) (17.2 vs. 12.4 months, $p = 0.01041$) and median progression-free survival (PFS) (10.5 vs. 9.0 months, $p = 0.02597$). Neutrophil-to-lymphocyte ratio (< 3.5), platelet-to-lymphocyte ratio (< 150), objective response (CR, PR, SD) were independent positive predictors for OS and PFS.

Conclusions: Overweight patients had a prolonged OS and PFS compared with normal weight patients with mCRC cancer treated with bevacizumab plus FOLFOX chemotherapy regimen.

Key words: overweight, colorectal cancer, bevacizumab, chemotherapy, BMI.

Poster

KW019-00007-2019-01

Normal peritoneal mesothelial cells and fibroblasts undergo premature senescence in response to carboplatin and paclitaxel

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Carboplatin (CPT) and paclitaxel (PCT) constitute the gold standard in the treatment of epithelial ovarian cancer, particularly in patients with the advanced disease encompassing the peritoneal cavity. At the same time, despite reports showing that both these drugs may affect the functionality of various kinds of normal cells (e.g., tubule epithelial cells, neurons, endothelial cells), very little is known about their effects on the biology of normal peritoneal cells. This project was aimed at examining whether CPT and PCT may accelerate senescence in the primary, omental mesothelial cells (PMCs) and fibroblasts (PFBs), a process known to stimulate various aspects of ovarian cancer cell progression *in vitro* and *in vivo*. The drugs were applied to the PMCs and the PFBs at the concentrations at which they affect the viability of less than 15% of cells (50 μM CPT + 25 nM PCT, and 25 μM CPT and 10 nM PCT for the PMCs and PFBs, respectively). Experiments showed that PMCs and PFBs subjected for 3 days to the drugs display increased percentage of senescent cells, as evidenced according to staining of senescence-associated β-galactosidase and the activation of DNA damage response (increased level of histone γ-H2A.X and 53BP1 foci). This effect coincided in PMCs with maintained telomere length, whereas in PFBs the telomeres shortened significantly. At the level of the cell cycle, the PMCs exposed to CPT and PCT displayed up-regulated expression of p16, p21, and p53 inhibitory proteins, whereas the PFBs had increased level of p21. These changes were accompanied by elevated production of mitochondrial superoxides and cellular peroxides, decreased mitochondrial membrane potential, and increased mitochondrial mass, suggesting the activation of mitochondrial retrograde signaling response. Significantly, effects generated by the combination of the drugs were consistently more pronounced than those elicited by the drugs applied separately.

Taken together, our results indicate that CPT and PCT may accelerate senescence in the normal peritoneal cells in the mechanism involving mitochondria-related oxidative stress. This may, in turn, indicate that the two commonly used chemotherapeutics may paradoxically support ovarian cancer spread within the peritoneum.

Key words: chemotherapy, ovarian cancer, cellular senescence.

The study was supported by the grant from the National Science Centre, Poland (# 2017/25/B/NZ3/00122).

Poster

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The H2.1MS1:DOXMS2 spheres for targeted and controlled doxorubicin delivery to Her2-positive cancer cells

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The mechanical properties, biocompatibility, and biodegradability make silk the material of a potential biomedical application. Genetic modification enables the functionalization of bioengineered spider silk by the addition of a sequence that encodes the peptide responsible for the desired function. Functionalized silks can be processed into spheres as drug delivery carriers. In this study we modified silk by addition: i) H2.1 domain, responsible for binding to Her2+ cells and ii) DOX domain with affinity to doxorubicin, to obtain controlled delivery of drug to Her2 positive cancer cells.

The *E. coli* expression system was used to produce the bioengineered spider silk proteins MS1, MS2, and their hybrid variants H2.1MS1 and DOXMS2. The proteins were purified by thermal denaturation method. Silk blends H2.1MS1:MS2, H2.1MS1:DOXMS2 and MS1:DOXMS2 were prepared at the weight ratio 8 : 2, respectively. The spheres were formed by using high-pressure syringe pumps upon the process of salting out the proteins. Flow cytometry was implemented to study the binding of the particles to the breast cancer and control cells. The loading and release of doxorubicin from spheres were measured using spectrophotometry. The localization of doxorubicin in cells was analyzed using confocal microscopy. The cytotoxicity of the drug loaded in spheres was examined by the MTT test.

Five variants of spheres were formed: MS2, DOXMS2, H2.1MS1:MS2, H2.1MS1:DOXMS2 and MS1:DOXMS2. The most efficient binding to the Her2+ breast cancer cells was observed for the H2.1MS1:DOXMS2 spheres comparing with other blended variants. Moreover, the doxorubicin loading to these spheres was the most efficient as compared to other particles. The confocal microscopy analysis indicated that the delivery of the drug by H2.1MS1:DOXMS2 and H2.1MS1:MS2 particles into the Her2+ cancer cells was higher than into control cells. Moreover, the release of the drug inside the cells from H2.1MS1:MS2 spheres was faster than from H2.1MS1:DOXMS2 particles. The MTT analysis showed the highest cytotoxic effect of the drug delivered by H2.1MS1:DOXMS2 spheres comparing with other spheres variants.

This study demonstrated, that blending of functionalized silks with a domain responsible for binding to the Her2-positive cells and with the affinity to doxorubicin allows to obtain spheres that deliver the drug to targeted cells in a controlled manner.

Key words: bioengineered spider silk, drug delivery, targeted therapy, blended silk spheres, cancer treatment.

Poster

KW019-00005-2019-01

Composite spheres comprised of functionalized silks and iron oxide nanoparticles for drug delivery and hyperthermia treatment of cancer

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Spider silk is a material that combines superb mechanical properties, biocompatibility, and biodegradability. Genetic engineering of silk enables the addition of sequences encoding functional domains. Moreover, variously functionalized silks can be blended to obtain complex material. The application of iron oxide nanoparticles includes drug delivery, contrast imaging in MRI and cancer treatment via hyperthermia. The study aimed to obtain the composite spheres that comprise two functionalized silks and iron oxide nanoparticles. H2.1MS1 silk contains a peptide that recognizes Her2, and MS1Fe1 silk contains peptide for binding metal ions. Composite spheres were formed for drug delivery and hyperthermia treatment of cancer.

The MS1Fe1 and H2.1MS1 bioengineered silks were previously constructed, produced in bacterial expression system and purified using thermal denaturation method. For formation of composite spheres, the functionalized silks were combined at a different weight ratio, added to iron oxide nanoparticles (IONPs) suspension and mixed with 2 M potassium phosphate, pH 8. SEM/EDXS microscopy was used for the morphology and elemental analysis of obtained particles. The binding of blended spheres to cells was assessed using flow cytometry. The loading and release of Doxorubicin from spheres were analyzed spectrophotometrically. Magnetic hyperthermia was performed by using the heating induction system on composite spheres.

The blending of functionalized silks did not influence their ability to sphere formation. The increased IONPs content and decreased binding of spheres to cells were observed with increasing content of MS1Fe1 silk in spheres. The H2.1MS1: MS1Fe1/IONPs composite particles blended in the 8 : 2, respectively, exhibited higher Dox loading efficiency comparing to the blended spheres. The release of drug from composite spheres was higher at acidic and lower at neutral pH, as compared with the blended spheres. After magnetic treatment, the composite spheres demonstrated a greater temperature increase comparing with the blended spheres without IONPs.

The obtained composite spheres can potentially increase the effectiveness of anticancer therapy by combining targeted drug delivery and hyperthermia treatment.

Key words: bioengineered spider silk, iron oxide nanoparticles, cancer theranostics, composite spheres, cancer hyperthermia, drug delivery.

Poster

KW019-00003-2019-01

Tumor microvesicles interactions with endothelial cells of target metastatic organs – understanding tumor metastasis

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Introduction: Tumor-derived microvesicles (TMVs) induced angiogenesis within the tumor microenvironment to support tumor progression and probably are important for endothelial cell (EC) modification at distant site to form pre-metastatic niche. To assess the role of TMVs in the cancer metastasis we investigated the biological activity of non-malignant endothelial cells isolated from different target organs, after activation with TMVs.

Material and methods: The organ-specific non-malignant ECs lines were: human brain (HBrMEC) and lung (HLMEC) microvascular ECs. TMVs were isolated from human breast cancer MDA-MB-231 and human melanoma Hs294T cell lines using sequential centrifugation. After characterization and enumeration of TMVs the proliferations of ECs exposed to TMVs were examined by MTT assay. Next, adhesion (flow cytometry) and migration (transwell assay) of breast and melanoma tumor cells towards ECs activated by TMVs were assessed. Finally, the secretion profile of ECs treated with TMVs was investigated using protein membrane array analysis.

Results: TMVs secreted by breast and melanoma cancer cells stimulate ECs proliferation of brain and lung origins at the day 4th compared to control of unstimulated ECs. Adhesion of examined tumor cells was more efficient to ECs of lung and brain-origin treated with TMVs compared to unstimulated ECs. The most effective migration was observed for MDA-MB-231 cells towards ECs of brain origin after TMVs stimulations. ECs after stimulation by TMVs secreted and expressed more chemotactic and inflammatory factors ICAM-1, RANTES, SDF-1, VEGF-D, MCP-1 and MCP-2 compared to control. Additionally, non-malignant ECs treated with TMVs expressed TGF-β1.

Conclusions: It was demonstrated that MVs of breast cancer-origin preferentially activated ECs of brain origin, and may participate in remodeling of distal metastatic niche. Moreover, secretion profile of non-malignant ECs stimulated by TMVs suggests that TMVs are responsible not only for tumor angiogenesis (augmentation of VEGF-D, angiogenin-2) but also may lead to endothelial mesenchymal transition (induction of TGF-β1 expression).

Key words: organ-specific endothelial cells, tumor derived-microvesicles, tumor metastasis.

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Poster

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Identifying drug combinations for the treatment of resistant acute myeloid leukemia patients

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Acute myeloid leukemia (AML) is the most common form of acute leukemia in adults and is classified into 14 different groups depending on its genetic makeup. The heterogeneity of AML is defined by a diverse genetic landscape and is one of the major challenges in finding an effective treatment option for patients that do not respond to current standard treatment, which remained unchanged for the past 20 years and is applied across all 14 different groups not considering the genetic diversity of this disease.

Therefore, we aim to identify the characteristics properties behind the response and resistance of individual patients, who might not only develop resistance to standard treatment, but also to targeted therapy. The ability to predict the potential risk of resistance to a treatment and identify strategic and personalized treatment options for a group of individual patients is of substantial significance.

We have screened a group of patients by assessing the sensitivity of primary patient-derived cancer cells *ex vivo* from individual AML patients and healthy donors to a panel of anticancer drugs. We observed that only a group of patient-derived cancer cells showed response to a number of drugs. However, the other group did not show any therapeutically relevant response to the most effective drugs indicating the potential of resistance in an eventual treatment. We are approaching this therapeutic issue through a systematic screening of synergizing drug combinations. We have been able to develop computational methods for personalized single and/or combinatorial drug-sensitivity screens from high-throughput experiments including randomized dispensing and automated deconvolution of big data for further qualitative downstream analysis.

We further aim to map potential genetic alterations to drug response or resistance and to identify potential biomarkers through multidimensional data together with the implementation of automated high-throughput screening methods in the hope not only to predict response and resistance, but also identify strategic treatment options for individual AML patients.

Key words: drug sensitivity screen, drug combinations, leukemia, AML, personalized medicine.

Poster

KW019

Oncolytic myxoma virus shielded by mesenchymal stem cells destroys melanoma tumors

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Introduction: Oncolytic viruses are a great promise of anticancer therapy as they are able to target hard-to-reach aggressive or disseminated tumor beds, trigger oncolysis and elicit antitumor immune response. One of challenges still facing oncolytic therapy is the success of systemic delivery of the benevolent virus. Bearing in mind that human bone marrow-derived mesenchymal stem cells (MSCs) are capable of homing *in vivo* to various tumors we previously characterized MSC-mediated transfer (Trojan horse) of oncolytic myxoma virus (MYXV) to cultured melanoma cells. Next, we investigated kinetics of *in vivo* distribution of MYXV in murine tissues and treated mice bearing experimental lung melanoma lesions.

Methods: MSCs isolated from healthy human bone marrow donors were infected with vMyx-Fluc/tdTr (MYXV encoding firefly luciferase protein and tomato red fluorescent protein under poxvirus synthetic early/late promoter) or vMyxIL15Rα-tdTr (MYXV encoding IL-15 complex with subunit α of its receptor and tdTr). *In vivo* studies using competent melanoma-challenged (+MET) or unchallenged (–MET) C57BL6 mice were performed with *in vitro* MYXV-preinfected MSCs.

Results: Animal studies demonstrated that B16-F10 cells preloaded *in vitro* with MYXV-infected MSC can effectively inhibit melanoma foci formation in lungs. The MSC-shielded MYXV constructs upon iv. infusion quickly accumulated in murine lungs (as opposed to naked virus) and their level there remained high enough to warrant a successful therapeutic attempt to destroy experimental melanoma lesions in murine lungs.

Conclusions: We show that human bone marrow mesenchymal stem cells are suitable cell carrier (i.e. Trojan horse) to ferry advanced therapeutic MYXV constructs to sites of disseminated cancer such as metastatic lung melanoma.

Key words: mesenchymal stem cells, myxoma virus, melanoma, oncolytic virotherapy.

Poster

KW019

Micro RNA hsa-miR-6510-3p inhibits cell proliferation and migration in head and neck cancer cells

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Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, representing over half a million incidents every year. Currently, the treatment of choice for head and neck cancer is surgery, followed by postoperative chemo- and/or radiotherapy. Despite advances in conventional methods, the 5-year mortality rate of HNSCC patients has not improved. With progress in technologies and molecular genetics, there is a growing potential of gene therapy as a powerful tool for HNSCC treatment. One of the promising therapeutic agents for this approach appear to be small molecules of micro RNA that function as post-transcriptional regulators of genes expression. There is now growing evidence that dysregulation of miRNAs expression may participate in cancer progression. In our preliminary studies we identified micro RNA hsa-miR-6510-3p, which is nearly 6-fold downregulated in tumor cells compared to healthy tissue. Moreover, our analysis of level3 miRNA-Seq data from 497 HNSCC patients (TCGA HNSC dataset) revealed significant association between hsa-miR-6510-3p downregulation, HNSCC patients' standardized mortality and cancer T stage, suggesting that micro RNA 6510-3p may act as suppressor of head and neck carcinogenesis.

Aim of the study: The aim of this study was to investigate the effect of has-miR-6510-3p on the cell cycle, cell proliferation, cell motility and induction of cell death in two established head and neck cancer cell lines.

Material and methods: Established HNSCC cell lines (FaDu, H103) were transfected with 20 nM of miR-6510-3p mimic using Lipofectamine RNAiMAX transfection reagent and Opti-MEM medium. Cell proliferation and cell motility were evaluated using MTT assay and *wound healing assay* respectively. Subsequently, analyses of cell cycle and cell death mechanism were performed with RT qPCR and flow cytometry.

Results: Micro RNA hsa-miR-6510-3p decreases cell proliferation in and inhibits cell migration in FaDu and H103 cell lines compared to control cells transfected with non-specific, random sequence miRNA. Moreover, transfection with miR-6510 led to cell cycle arrest and induction of apoptosis in HNSCC cells.

Conclusions: MicroRNA hsa-miR-6510-3p plays an important role in the carcinogenesis, acting as a potential tumor suppressor in head and neck squamous cell carcinoma.

Poster

KW019

Proapoptotic activity of novel dicarboximides in leukemia cells

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Aim of the study: Evaluation of the anticancer activity and identification of the mechanism of action of the new derivatives of dicarboximides. Selection of lead dicarboximides with anticancer activity.

Material and methods: cytotoxicity assay, cleavage assay of caspase 3, 7, 8, 9 and PARP, flow cytometry, Annexin V apoptotic assay, immunoblotting, DNA microarray, real time RT-PCR.

Results: Novel derivatives of dicarboximides were found to be selectively toxic towards human chronic myelogenous (K562), acute myelogenous (HL-60) and acute lymphoblastic (MOLT-4) leukemia cells while non-toxic to normal primary human endothelial cells (HUVEC) [1, 2]. The reported IC_{50} values for dicarboximides in K562 and HL-60 cells were similar or lower to IC_{50} of registered drugs, as cytarabine, sorafenib or irinotecan. Dicarboximides 7, 9 and 10 induced apoptosis in K562 and MOLT-4 cells via receptor and mitochondrial pathways. Specifically, compound 9 induced cleavage of caspase 8 and 9 while compound 7 increased the expression of several proapoptotic genes involved in both, receptor (e.g. *TNFRSF 10A, TNFRSF 10B, CASP8*) and mitochondrial (e.g. *BAX, BID, NOXA, APAF1*) pathways of apoptosis.

Conclusions: New, lead dicarboximides with potent anticancer activity were identified. They showed significant cytotoxicity against leukemia cells and induced apoptosis via receptor and mitochondrial pathways.

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Poster

KW019

ALA-mediated photherapeutic effect on apoptosis induction and secretion of macrophage migration inhibitory factor (MIF) and of monocyte chemotactic protein (MCP-1) by colon cancer cells in normoxia and in hypoxia-like conditions *in vitro*

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Introduction: Photochemotherapy reveals immune modulatory effect. The aim of the study was to evaluate the influence of 5-aminolevulinic acid (ALA) mediated photodynamic effect on secretory activity (MIF, MCP-1) of colon cancer cells *in vitro* both in normoxia and in hypoxia-like conditions.

Methods: Two colon cancer cell lines differing in malignancy potential: SW480 (lower grade) and SW620 (higher grade) were used. MCP-1 and MIF concentrations in supernatant of cells cultures after pretreatment with ALA at concentrations of 500, 1000 and 1500 μM and irradiation within coherent light (λ = 600–720 nm) at fluences of 10, 30 and 60 J/cm^2, using Bio-Plex ProTM Assay kit and Bio-Plex Suspension Array System apparatus, were measured.

Results: The SW620 cells were more susceptible to ALA-mediated phototoxic effect than SW480 one, however this effect may be partly abolished in hypoxia-like condition. In the case of SW480 cell line, no influence of hypoxia-like conditions on cell susceptibility to ALA-mediated photochemotherapeutic effect was found. The MIF concentration increased, contrary to MCP-1 one which decreased after ALA-mediated photodynamic action in both cell lines. No difference between cytokine concentrations in supernatant from cells cultures in normoxia or hypoxia-like conditions was observed.

Conclusions: Detected reduction in MCP-1 secretion appears to be advantage because of tumor's growth limiting but an increase in the secretion of MIF, which is responsible for stimulation of tumor cells proliferation, is an unfavorable effect. These results may be explained by the fact that the used cancer cell lines differ from each other in cancer stage.