contemporary oncology

współczesna **onkologia**

Volume 22, Supplement 2, March 2018

10th ANNIVERSARY INTERNATIONAL CONFERENCE OF CONTEMPORARY ONCOLOGY

Poznan, 21–23 March 2018

Poster session

Poster

KW018-00056-2018-01

Different levels of let-7d in the head and neck cancers: TCGA data analysis

Tomasz Kolenda[1,2,3], Anna Teresiak[1], Renata Bliźniak[1], Katarzyna Lamperska[1]

[1]Laboratory of Cancer Genetic, Greater Poland Cancer Center, Poznań, Poland
[2]Postgraduate School of Molecular Medicine, University of Warsaw, Warsaw, Poland
[3]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland

Introduction: Let-7d is usually described as a tumor suppressor and regulates genes associated with EMT, cancer stem cells, metastases, cell cycle and drug response and irradiation. In the previous study, we indicated that the different let-7d expression levels have different effects on the cell phenotype and the response to chemical exposure and radiation of the in vitro model. Our observations have shown that depending on the level of expression, let-7d may behave like a suppressor or oncogene. In this study, we used TCGA data from head and neck cancer (HNSCC) to analyze the effect of different let-7d levels on the clinical-pathological parameters of patients and the expression of selected genes.

Material and methods: Gene expression levels and clinical data of 383 HNSCC samples from TCGA project were obtained from available data bases. The let-7d expression level was checked depending on the clinical-pathological parameters. For analysis, the patient groups were divided based on the let-7d expression quartile. The disease-free survival (DFS) and overall survival (OS) were then checked, as well as the expression of EMT-related genes, cancer stem cells, metastases, cell cycle and drug response and irradiation, and compared between groups of patients.

Results: TCGA data analysis shown significant over-expression of let-7d in HNSCC compared to normal samples and ROC analysis shown high discrimination ability of cancer samples based on let-7d expression (Area 0.7303, $p < 0.0001$). No significant differences between expression levels of let-7d and personal gender, age, cancer stage, T-stage were observed. However, the expression level of let-7d depended on the neoplasm histological grade, N-stage, angiolymphatic invasion, HPV status and alcohol consumption. Analysis of patients groups showed significant longer overall survival between patients with the lower and middle level of let-7d expression (< 25 vs. 50-75 quartile; $p = 0.0428$). The analysis of genes connected with EMT, cancer stem cells, metastasis, cell cycle and response to drugs and irradiation reveled distinct phenotype of analyzed groups.

Conclusions: TCGA data analysis confirmed our previously observation. let-7d is frequently up-regulated in HNSCC. Moreover, different levels of let-7d influences in different way and creates unique cell phenotype, which influences on course of disease.

Key words: let-7d, HNSCC, TCGA, biomarker.

This work was supported by Greater Poland Cancer Centre grant No.: 3/2016 (128).

Poster

KW018-00055-2018-01

Hippo pathway protein LATS1 has an impact on paclitaxel response in melanoma cell lines

Maria Zajączkowska, Ewelina Dondajewska, Andrzej Mackiewicz, Urszula Kazimierczak

Department of Cancer Immunology, Poznań University of Medical Sciences, Poland

Introduction: Melanoma is an extremely aggressive type of skin cancer, highly resistant to common therapies. There are several pathways engaged in melanoma development and progression. One of them is the Hippo signaling pathway responsible for organ size control, cell proliferation and apoptosis. Some of its proteins are believed to act as tumor suppressors, including one of the Hippo core kinases – LATS1 (large tumor suppressor kinase 1). LATS1 has an influence on progression and response to therapies of different types of cancer. However, it's significance in melanoma biology remains to be discovered.

Aim of the study: Analysis of response to paclitaxel in 5 different melanoma cell lines after LATS1 silencing.

Material and methods: Human melanoma cell lines: WM-115, WM-266, Mich2, SK-MEL28, MeWo, were transduced with lentiviral vectors expressing shRNAs in order to knock down LATS1 gene. After verification of transduction efficiency and the level of LATS1 silencing, cells were treated with four different paclitaxel concentrations: 5 µg/ml, 10 µg/ml, 25 µg/ml and 50 µg/ml for 48 hour followed by MTT analysis of paclitaxel cytotoxicity.

Results: Silencing of LATS1 gene in 5 different human melanoma cell lines affected response to paclitaxel, as measured with MTT assay. Cells treated with shLATS1 were more sensitive to paclitaxel in comparison to contol cells. The level of paclitaxel response was specific to each cell type.

Conclusions: Our preliminary study showed that the core Hippo pathway kinase - LATS1 has a potential role in response to a chemotherapy in vitro. Our present data confirmed that modifications of LATS1 expression might have an implication in cancer treatment using paclitaxel and might help to improve effectiveness of chemotherapy.

Key words: melanoma, hippo pathway, LATS1, paclitaxel.

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

Poster

KW018-00054-2018-01

Overcoming RT-PCR inhibition caused by high melanin content in melanoma tumor samples

Ewelina Dondajewska, Maria Zajączkowska, Andrzej Mackiewicz

Poznan University of Medical Sciences, Poznan, Poland
Greater Poland Cancer Centre, Poznan, Poland

Melanin is a potent inhibitor of both PCR and reverse transcription PCR (RT-PCR) reactions. It co-precipitates with RNA in standard nucleic acid preparations resulting in high contamination of RNA samples derived from melanin-rich melanomas. This in turn yields low levels of cDNA synthetized in RT-PCR reaction and low levels or no product in PCR as the melanin binds to the thermostable polymerase.

Various methods have been employed to counter this effect. Here we compare the efficacy of different RNA extraction protocols, and melanin removal methods to select the one most suitable for downstream qPCR applications.

Conclusions: Standard fenol-chloroform-based and column-based methods of RNA extractions both result in high melanin contamination. Addition of BSA to either RT-PCR or qPCR only slightly diminishes the effects of melanin inhibition. Further purification of RNA samples by CTAB-Urea protocol is essential for successful RT-PCR and qPCR applications.

Key words: melanoma, melanin, PCR inhibitors.

This work was part of the research project no. 2014/15/B/NZ5/03563 funded by the National Science Center.

Poster

KW018-00053-2018-01

Cancer-associated KRAB-ZNF expression correlates with multiple clinicopathological features in breast and lung cancers

Marta Gładych[1,2], Kornel Kiełczewski[3,4], Rafał Cylwa[3], Katarzyna Zwierzchowska[3], Przemysław Biecek[3,4], Urszula Oleksiewicz[1,2]

[1]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[2]Laboratory of Gene Therapy, Department of Cancer Immunology, The Greater Poland Cancer Centre, Poznan, Poland
[3]Faculty of Mathematics, Informatics, and Mechanics University of Warsaw, Warsaw, Poland
[4]Faculty of Mathematics and Information Science, Warsaw University of Technology, Warsaw, Poland

Epigenetic alterations are frequent drivers in carcinogenesis, however, the exact molecular mechanisms behind these events are still poorly characterized. KRAB-ZNFs genes bind to DNA and act as epigenetic repressors mediating local deposition of heterochromatin marks. However, due to the high complexity of this large family genes, that comprises >800 transcripts including splicing variants and pseudogenes, the function of the majority of KRAB-ZNFs remains unknown. So far, only few KRAB-ZNF genes have been linked to the tumor. Thus, to provide a better description of their potential involvement in tumor biology, we aimed to explore KRAB-ZNF landscape in a pan-cancer setting. Our initial study allowed identification of a small cluster of KRAB-ZNF genes that are commonly overexpressed in multiple cancer types. In the next steps, we narrowed down our analysis to the two most common tumor types, namely breast and lung cancers, and probed the clinical significance of KRAB-ZNF expression using TCGA data and an independent panel of tumor tissues. We found that in both tumor types, KRAB-ZNF expression associates with tumor histology, molecular subtypes, and patient survival. Additionally, in lung cancer, the expression of some KRAB-ZNFs correlated with gender and smoking history. Furthermore, we took advantage of the publically available DNA binding data for ~250 KRAB-ZNF factors. In-depth analysis of the binding sites of the cancer-associated KRAB-ZNFs revealed that these factors might bind close to the transcription start sites of many genes, as well as to the transposable elements. Gene ontology analysis showed that the genes targeted by cancer-associated KRAB-ZNFs participate in proliferation promoting pathways, adhesion, motility, and developmental processes. This indicates that KRAB-ZNFs may play a role in tumor growth, invasiveness and stem cell potential. Altogether, our data suggest that these KRAB-ZNFs may have a crucial role during carcinogenesis and as such may be potentially utilized in cancer management as biomarkers or therapeutic target.

Key words: KRAB-ZNFs, TCGA pan-cancer analysis, breast cancer, lung cancer.

Poster

KW018-00052-2018-01

lncRNA expression in melanoma cell lines after vemurafenib exposure and irradiation

Marcel Ryś, Tomasz Kolenda, Kacper Guglas, Anna Teresiak, Renata Bliźniak, Łukasz Galus, Katarzyna Lamperska, Jacek Mackiewicz

Introduction: Long noncoding RNAs (lncRNAs) are the large family of non coding protein molecules, that are more than 200 nucleotides long. Many of lncRNAs are dysregulated in different types of cancer and act as oncogenes or tumor supressors. It was indicated, that some of them have pivotal role in the regulation and progression of melanoma skin cancer. Despite the great progression in the treatment strategies such as immuno- and targeted therapy, melanoma is still one of the most deadly diseases and causes 80% of deaths among all skin cancers. Probably, the implication of lncRNAs into diagnostic as well as treatment strategies may be beneficial for melanoma patients. In this study we analyzed the possible role of lncRNAs in response of melanoma cell lines to vemurafenib exposure and irradiation.

Material and methods: The different melanoma cell lines: SKMEL28, MeWo, WM115 and WM266 were exposed to vemurafenib (Zelboraf®) and irradiated using different doses (5, 10 and 20 Gy) by Gammacell 1000. Total RNA was isolated from the cell lines 24 hours after exposure and expression of 96 lncRNA was measured using qRT-PCR and compared to the control cell lines. Next, the available data bases were used to check predicted lncRNA targets and their role in melanoma.

Results: Many changes of lncRNA expression after vemurafenib and irradiation compared to controls were observed. The lncRNA expression depended on the type of drug as well as dose of irradiation. Moreover, we observed differences in lncRNA expression depending on BRAF status - the level of PVT1 lncRNA was lower in BRAF positive cell lines than in negative one. The changed lncRNAs are involved in important cellular processes and are connected with patients' clinical and pathological parameters.

Conclusions: Our results show that lncRNAs are dysregulated after vemurafenib exposure and irradiation and give an opportunity to discuss about their role in resistance to therapy. We speculate that knowledge about lncRNA could help to improve therapies, that currently do not provide high successful rate.

Key words: human melanoma, radiotherapy, chemotherapy.

This work was supported by Greater Poland Cancer Centre (13/2016 (128)) and by the National Science Centre, Poland, (2016/21/B/NZ7/01773).

Poster

KW018-00051-2018-01

Melanoma antigen-specific CD8 cells in healthy donors and melanoma patients

Anna Przybyła[1,2], Richard Caspell[1], Andrzej Mackiewicz[2], Paul Lehmann[1]

[1]R&D Department, CTL, Shaker Heights, OH, 44122, USA
[2]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poland

Introduction: For detecting any of the predictable consequences of immune therapies, it is critical to understand the baseline: the activation state of MA-specific CD8 cells in healthy human donors and melanoma patients. Naïve tumor antigen-specific CD8 cells occur in very low frequency in blood and do not secrete IFNγ or Granzyme B (GzB). Effector CD8 cells, capable of cytolysis, secrete GzB in addition to IFNγ, and due to clonal expansion occur with increased frequency in blood. Resting CD8 memory cells secrete IFNγ but not GzB also occurring in increased frequency in blood. Such resting memory cells will re-express GzB within several days upon antigen re-encounter, converting into effector CD8 cells.

Material and methods: Using this basic features of CD8 cell biology, we performed IFNγ and GzB Single-color enzymatic ELISPOT assays to measure the frequency of melanoma antigen-specific CD8 cells secreting these analytes 24 h and 72 h after antigen stimulation. Five melanoma antigen peptide pools were tested on PBMC of healthy donors and untreated melanoma patients. For establishing the type of response, CD4+ or CD8+ T cells were depleted from the general population of PBMC using a magnetic bead positive selection kit.

Results: Of the above melanoma antigens only Tyr triggered relatively high frequency (~1/1000) CD8 cells at 24h ex vivo. At this time point, these CD8 cells did not produce GzB yet. However, they engaged in GzB production by 72 h after antigen stimulation. Therefore, Tyr-specific CD8 cells in healthy controls are clonally expanded resting memory cells (IFNg+/GzB–) that can be reactivated to become effector cells (IFNg+/GzB+) within 72 h.

Conclusions: These MA-specific CD8 cells are resting memory cells at isolation with cytolytic potential. They can be reactivated by antigen within 72h to express and secrete GzB, that is, to convert into cytolytic CD8 effector cells.

Key words: CD8 cells, melanoma antigens, ELISPOT.

This study was supported by The National Centre for Research and Development (Warsaw), project Innomed.

Poster

KW018-00050-2018-01

Analysis of the polarization of macrophages in the microenvironment of three-dimensional (3D) model of breast cancer

Agata Golabek[1,2], Kosma Sakrajda[1,2], Ewelina Dondajewska[3], Andrzej Mackiewicz[1,3], Mariusz Kaczmarek[4], Hanna Dams-Kozlowska[1,3]

1Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland
[2]Chair of Agriculture and Bioengineering, Poznan University of Life Sciences, Poznan, Poland
[3]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Clinical Immunology, Poznan University of Medical Sciences, Poznan, Poland

In recent years, the importance of tumor microenvironment has led to an intensive investigation of the development of in vitro model, which reflect the interaction of cancer cells with neighboring cells and the extracellular matrix. We developed a 3D model of breast cancer, which includes the spatial growth and heterogeneity; it consists of a co-culture of cancer cells and fibroblast on a natural silk scaffold. For further evaluation of this model, we analyzed its influence on a macrophage phenotype.

The aim of the study was the analysis of the polarization of macrophages in the microenvironment of three-dimensional (3D) model of breast cancer.

To generate a 3D breast cancer model the cell lines: EMT6 – murine breast cancer cells, and NIH3T3 – murine fibroblasts were seeded at a ratio of 1 : 9 respectively, on the silk scaffolds and co-cultured for 7 days. The murine macrophages (J774 cell line) were activated by conditioned medium collected from the 3D breast cancer model or the 3D monoculture of EMT6 or NIH3T3. The cytokines content in the conditioned medium was analyzed by Cytometric Bead Array (CBA) assay. The phenotype of J774 cells was examined by flow cytometry. The activity of nitric oxide synthase (NOS) and arginase (ARG) was determined by colorimetric assays.

The CBA analysis of the conditioned media indicated that 3D co-culture of EMT6:NIH3T3 secreted higher amount of TNF and IL-10 than a 3D monoculture of EMT6 and NIH3T3 cells. The IL-12 and INF-γwere below the detection level for all 3D cells cultures. Macrophages activated by 3D breast cancer model and 3D monoculture of cancer cells showed increased expression of CD301a and MSR-1 than the negative control macrophages (CN). The decreased expression of MHC class II molecules IA/IE was observed for the J774 cells incubated with medium from 3D breast cancer model than in the CN. The activity of ARG was the highest for J774 cells activated with conditioned medium derived from a 3D model of breast cancer compared with macrophages treated with medium from the 3D monoculture of EMT6 or NIH3T3.The activity of NOS had the lowest level in the J774 macrophages activated by factors present in the 3D cancer model in comparison with CN.

The analyzes indicated that macrophages activated by 3D breast cancer model showed a phenotype characteristic for the M2 subtype. The 3D model of breast cancer is a promising tool to study macrophage - cancer reciprocal interactions.

Key words: breast cancer, cancer microenvironment, tumor-associated macrophages, 3D cancer model.

Poster

KW018-00049-2018-01

Expression and role of let-7d and miR-205 in head and neck cancers patients

Tomasz Kolenda[1,2,3], Maria Zajączkowska[2], Anna Teresiak[1], Renata Bliźniak[1], Katarzyna Lamperska[1]

[1]Laboratory of Cancer Genetic, Greater Poland Cancer Centre, Poznan, Poland
[2]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[3]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland

Introduction: Head and neck squamous carcinoma (HNSCC) is one of the most invasive types of cancer with high mortality. Previous study indicated, that low level of let-7d and miR-205 in HNSCC patients is correlated with worse survival factor. However, the mechanism of their common action remains unclear. Let-7d and miR-205 are described as tumor suppressors and regulators of epithelial-to-mesenchymal transition (EMT). It still unclear whether let-7d and miR-205 together influence on cancer cells in specific way.

Aim of the study: Analysis of let-7d and miR-205 in HNSCC patients.

Material and methods: The TCGA expression data of let-7d and miR-205, their targets as well as clinical data was downloaded from cBioPortal and from the starBase v2.0 for 307 patients. The expression level of let-7d and miR-205 was checked regarding to clinical-pathological parameters. Next, the let-7d and miR-205 high and low expressed groups, disease free survival (DFS) and overall survival (OS) as well as expression of genes connected with EMT, cancer stem cells, metastasis, cell cycle and response to drugs and irradiation were checked.

Results: let-7d and miR-205 are frequently up-regulated in HNSCC compared to normal samples and ROC analysis shows high discrimination ability (area 0.7369 and 0.7739, respectively; $p < 0.0001$). The differences between expression levels of let-7d or miR-205 and grade, N-stage, angiolymphatic and perineural invasion as well as alcohol consumption were indicated. No differences were observed in the case of the tumor localization, gender nor age of patients. Patients with lower level of let-7d and higher level of miR-205 have significantly better OS ($p = 0.0325$) than patients with higher level of let-7d and lower level of miR-205. Moreover, in the group of patients with lower level of let-7d and higher level of miR-205, lower percentage of more advanced cancers was observed. The analysis of genes connected with EMT, cancer stem cells, metastasis, cell cycle and response to drugs and irradiation revealed distinct phenotype of analyzed groups.

Conclusions: Our results show, that the down-regulation of let-7d and over-expression of miR-205 create unique cell phenotype with different behavior compared to cells with up-regulated let-7d and down-regulated miR-205. We concluded, that let-7d and miR-205 seem to be good candidates as new biomarkers for HNSCC.

Key words: let-7d, miR-205, HNSCC, TCGA, biomarker.

This work was supported by Greater Poland Cancer grant No.: 24/2016 (139).

Poster

KW018-00048-2018-01

lncRNA expression in head and neck squamous cell carcinoma cell lines after chemoexposure and irradiation

Kacper Guglas, Tomasz Kolenda, Marcel Ryś, Anna Teresiak, Renata Bliźniak, Izabela Łasińska, Jacek Mackiewicz, Katarzyna Lamperska

Greater Poland Cancer Center in Poznań, Poland

Introduction: Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cause of cancer mortality in the world. Some progress has been made in the therapy of HNSCC, but patients outcome remains unsatisfactory. Recent studies have shown that different types of long non-coding RNAs (lncRNAs) are dysregulated in HNSCC and are connected with many cellular processes. In this study, changes of lncRNA expression in HNSCC cell lines after chemo- and radiotherapy were checked and discussed as potential biomarker.

Material and methods: SCC-40, SCC-25, FaDu and Cal27 cell lines were treated with doxorubicin and cisplatin drugs and irradiated using different doses by Gammacell 1000. Total RNA was isolated from the cell lines 24 hours after exposure and expression of 96 lncRNA was measured using qRT-PCR and compared to the control cell lines. Next, the available data bases were used to check predicted lncRNA targets and their role in HNSCC.

Results: Many changes of lncRNA expression after chemoexposure and irradiation compared to controls were observed. The lncRNA expression depended on the type of drug as well as dose of irradiation. It was indicated, that HOTAIR, PTENP1, HAR1A, HAR1B and Zfhx2as lncRNAs were significantly changed in all analyzed cell lines after irradiation. The predicted targets of these lncRNAs are involved in important cellular processes and are connected with patients' clinical and pathological parameters.

Conclusions: Our results underline important role of lncRNAs in response to chemotherapeutic drugs and irradiation. Probably lncRNAs could be used as biomarker to improve already existing therapies.

Key words: lncRNA, radiation, chemotherapy.

Poster

KW018-00047-2018-01

Prognostic value of erythropoietin and hematological parameters in patients with non-small cell lung cancer

Jolanta Mrochem-Kwarciak, Aleksandra Chmura, Regina Deja, Beata Smolska-Ciszewska, Tomasz Rutkowski, Monika Giglok, Dorota Butkiewicz, Rafał Suwiński

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology Gliwice Branch, Poland

Introduction: In patients with lung cancer anemia is common symptom and usually consist of both components: treatment related anemia and anemia of chronic disease. The disparity between production and elimination of erythrocytes is a direct reason of anemia which is characterized by low levels of circulating red blood cells and hemoglobin, reduced number of reticulocytes (Ret), inadequate secretion of erythropoietin (Epo) and disturbances in iron metabolism. Although the thorough mechanisms of anemia of chronic disease is not fully understood, it causes tissue hypoxia that increases resistance to radiotherapy.

The aim of this study was to assess the clinical utility parameters of the erythrocytic system: reticulocytes (Ret), immature reticulocyte fraction (IRF), reticulocyte hemoglobin content (Ret-He), and the concentration of Epo as a marker of tumor hypoxia.

Material and methods: The study was performed at Center of Oncology, Gliwice Branch. Hundred twenty nine patients qualified to radiotherapy alone (42) or combined with chemotherapy (75) or chemotherapy alone (12) due to non-small cell lung cancer were involved into the study. Clinical stages (cTNM) were as follow: stage IA – 3%, IB – 3%, IIA – 3%, IIB – 4%, IIIA – 37%, IIIB – 35% and IV –15%. The median age was 64 years (range: 41 to 70 years). Epo and parameters of the red blood cell system were estimated in serum or blood before treatment.

Results: Strong negative correlation has been found between initial anemia (Hb < 12 g/ml) and Epo ($p = 0.0001$), Ret ($p = 0.003$), IRF ($p = 0.001$), Ret-He ($p = 0.001$). Significantly longer overall survival (OS) was found for patients with lower Epo ($p = 0.01$) and IFR ($p = 0.001$).

Conclusions: In patients with non-small cell lung cancer, before treatment anemia has the nature of chronic disease which stimulates erythropoiesis. Ret, IFR and Ret-He may indicate increased and ineffective erythropoiesis. Initial high Epo levels and high IFR correlate with increased risk of death.

Key words: lung cancer, Epo.

This study/work was supported/partially supported by the National Science Centre, Poland (NCN), grant no. 2012/05/B/NZ5/01905 to D.B.

Poster

KW018-00045-2018-01

Molecular mechanism of immune cell activation by therapeutic melanoma vaccine

Patrycja Czerwińska[1,2], Marcin Ruciński[3], Katarzyna Gryska[2], Jacek Mackiewicz[1,4], Andrzej Mackiewicz[1,2]

[1]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre in Poznan, Poland
[2]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Biology and Environmental Sciences, Poznan University of Medical Sciences, Poznan, Poland

Melanoma (MM) is a heterogenous disease that belongs to the most invasive human malignancy with rapidly rising incidence. Recently, significant progress in the development of novel therapeutic modalities of advanced MM was observed. However, not all patients benefit from the therapy. Accordingly, markers for treatment personalization are required to increase the effectiveness. Progress and better understanding of immunotherapeutic approaches brought hope to cure MM We have developed therapeutic gene modified allogenic MM vaccine (AGI-101H) that has been tested since 1997 and resulted in a long-term survival of a substantial fraction of immunized patients.

Our goal is to understand the molecular mechanisms of immune cells that determine response to the AGI-101H treatment. Accordingly, T lymphocyte mRNA expression profiling of long-term surviving patients was carried out. Briefly, PBMCs were isolated from immunized patients (n = 18), from untreated MM patients (n = 13) and healthy controls (n = 8). Untouched T lymphocytes were separated with magnetic beads and total RNA was isolated. The quality and quantity of RNA was verified, and the transcriptome profiling was performed with Affymetrix HG U219 microarray (19285 markers). Differential gene expression (DGE) analysis between groups was conducted and validated with RT-qPCR and FACS. The transcriptomic results were further analyzed with Gene Set Enrichment Analysis (GSEA) tool.

DGE analyses comparing AGI-101H vaccinated (AV) and non-immunized (C) patients revealed 538 differentially expressed genes (DEGs), with 373 downregulated and 165 upregulated in AV (adj p-val < 0.05). Among these 538 markers, the expression of 14 (with |log2FC| > 1) is now being validated with RT-qPCR and FACS. Also, GSEA analysis revealed significant enrichment of "TNFa_signaling_via_NFkB", "TGFb_signaling" and "G2/M_checkpoint" hallmark processes (MSigDB Hallmark gene sets) in AGI-101H vaccinated patients, that confirms significant activation of anti-tumor response and indicates activation of T cell differentiation into functionally distinct lineages.

Transcriptome profiling of T lymphocytes in long-term surviving MM patients immunized with AGI-101H vaccine may help in understanding the molecular mechanism of activation of immune cells by AGI-101H vaccine and characterize the features of immune cells that determine the clinical response to the treatment.

Key words: human melanoma, cell vaccine, AGI-101H, hyper IL-6.

Poster

KW018-00044-2018-01

Exosomes – tiny particles with great possibilities. Purification methods

Katarzyna Kluszczyńska, Markus Duechler

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Poland

Exosomes are nano-sized vesicles, of endosomal origin realeased by many cell types, transporting proteins and nucleic acids between cells. They appear to play an important role in physiological and also pathological processes most notably inflammation and cancer, where their eficcient functional delivery of biological cargo seems to contribute to the disease progress. Because of cargo they could cause different effect, propagate cancerogenesis by promoting metastases or develop immunotolerance.

Many hopes are lying in exosomes as a drug carriers, because they have multiple advantages over current artificial drug delivery systems. They are not immunogenic and can penetrate the cellular membrane of target cells easier because of proteins anchored in their membrane. Application of exosomes for this purpose is promising but to use them their purity has to be excellent.

My approach to improve purity of exosomes is to combine different purification methods. There are many ways to obtain exosomes: series of ultracentrifugation, centrifugation in sucrose gradient, precipitation, they differ in harvested yield and purity level. Purity can be improved by additional steps such a size exclusion chromatography, proteolytic digestion, filtering, or immuno - beads. Some were chosen to optimize this process.

The optimal procedure to obtain pure fraction of exosomes will be presented.

Key words: exosomes, vesicles, purification, drug delivery system.

The study was supported by grant no. 2016/21/B/NZ7/02747 from the National Science Centre (NCN, Poland).

Poster

KW018-00043-2018-01

Controlled doxorubicin delivery by functionalized spider silk spheres that target breast cancer cells

Karolina Penderecka[1,2], Tomasz Deptuch[1,2], Kamil Kucharczyk[1,2], Edyta Jakubowska[1], Bogna Juskowiak[1], Anna Florczak[1,2], Katarzyna Jastrzebska[1,2], Andrzej Mackiewicz[1,2], Hanna Dams-Kozlowska[1,2]

[1]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poland
[2]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland

Spider silks are proteins of potential biomedical application due to their biocompatibility, biodegradability and self-assembly. Bioengineered silks can be used to form a drug delivery carriers. Moreover, there is a possibility for the addition of sequence that encodes peptide responsible for a desirable function to the bioengineered silks. Such functionalization can be used to control the binding of silk carriers to cancer cells or to regulate the drug loading and release.

The aim of the study was the development of the system of drug delivery to Her2-positive cancer cells and controlled loading and release of doxorubicin.

The *E. coli* expression system was used to produce the MaSp1- and MaSp2 based bioengineered silk proteins (MS1 and MS2, respectively) and their hybrid functionalized variants (H2.1MS1 with the Her2-recognizing peptide and DOXMS2 comprising doxorubicin binding peptide). After purification of the silks by thermal denaturation method, the high-pressure syringe pumps were used to form the silk nanospheres. Scanning electron microscopy was applied to examine the morphology of the spheres. The nanoparticles were loaded with doxorubicin by the diffusion method. Loading efficiency and release kinetics of the drug was analyzed using spectrophotometry. The MTT assay was used to examine the toxicity of the doxorubicin-loaded spheres on Her2+ cancer and control cells.

Bioengineered proteins MS1, H2.1MS1, MS2, and DOXMS2 were produced, purified and processed into spheres. Doxorubicin loading into DOXMS2 particles was slightly higher than into MS2 spheres. The drug release from all silk nanoparticles was dependent on pH, and the release from DOX functionalized spheres was significantly the lowest in a pH of 7.4. Three types of blended nanospheres were formed: H2.1MS1/DOXMS2, MS1/DOXMS2 and H2.1MS1/MS2 to examine the drug delivery system that targets the cancer cells and controls the drug release. Due to high cytotoxicity towards cancer cells and the lowest release kinetics of doxorubicin in a pH of 7.4, the H2.1MS2/DOXMS2 spheres might be the most promising blend for targeted cancer therapy.

Functionalized silks and their blends are biopolymers that can be used to develop an effective, controllable system for drug delivery. The H2.1MS1/DOXMS2 spheres reduced the release of drug in a pH that corresponds to a pH of blood and efficiently delivered doxorubicin to the cancer cells.

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

Poster

KW018-00042-2018-01

Construction of bioengineered spider silks that contain a metal binding peptides

Aleksandra Smigielska[1], Kamil Kucharczyk[1,2], Andrzej Mackiewicz[1,2], Hanna Dams-Kozlowska[1,2]

[1]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poland
[2]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan

Bioengineered spider silk is a unique material, which can be processed into various morphological forms, such as fibers, gels, scaffolds, micro- and nanospheres and films. Its extraordinary mechanical properties, biocompatibility, and biodegradability enable for wide application in medicine. Moreover, genetic engineering gives an opportunity to design silk of proper function. The aim of this study was the genetic modification of the bioengineered spider silk to obtain hybrid silks that contain the metal binding peptides and to analyze their potential for iron oxide nanoparticles (NPs) binding.

The (MS1Fe1)2, (MS1Fe2)2 and MS1FE1MS1Fe2 hybrid bioengineered silks were designed. The sMS1 silk was based on the repetitive motif of MaSp1 spidroin from N. clavipes. The functionalized and control sMS1 proteins were produced in a bacterial expression system, purified by thermal denaturation and then analyzed using SDS-PAGE electrophoresis. The silk films were cast on coverslips and subsequently incubated with magnetic iron oxide nanoparticles (NPs) of positive or negative charges. SEM/EDS microscopy was used to analyze the morphology and elemental composition of produced films. The presence of iron oxide nanoparticles was examined using the Prussian blue assay.

The (MS1Fe1)2, (MS1Fe2)2, MS1FE1MS1Fe2 and sMS1 bioengineered spider silks were successfully constructed, produced and purified. SDS-PAGE analysis revealed that obtained proteins were of good quality; showed no impurities and no degradation. The addition of functional peptides did not impede the self-assembly property of hybrid silks and silk films could be formed. The SEM/EDS analysis and Prussian blue staining indicated that films made of silk functionalized with Fe1 binging peptide had higher affinity to NPs comparing with silk functionalized with Fe2 peptide and with the control silk. The SEM/EDS analysis demonstrated that the binding of negatively charged NPs to (MS1Fe2)2 film was faster than it was for positively charged NPs.

The functionalization of bioengineered spider silk with metal binding peptides enabled for the production of composite films with the increased potential for NPs binding. These results indicated the possibility to obtain a composite material which may be potentially applied in medicine.

Key words: silk, Metal binding peptides, Composite material, iron oxide nanoparticles.

Poster

KW018-00041-2018-01

Magnetic spheres made of FeMS1 silk variants for theragnostic applications

Kamil Kucharczyk, Andrzej Mackiewicz, Hanna Dams-Kozlowska

Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland

The property of silk such as superb mechanical strength, biocompatibility and biodegradability made silk an ideal material for biomedical applications. Furthermore, the genetic engineering enables to obtain modified silk of desire function. Moreover, silk can be processed into different morphological forms such as fiber, film, hydrogel, scaffold, capsules, micro- and nanospheres. The iron oxide nanoparticles can be applied in the biosensing, target drug delivery, as contrast agents in MRI, and as therapeutic agents for hyperthermia-based cancer treatments. We genetically modified the bioengineered spider silk with sequences that encode peptides responsible for metal ions binding to develop silk/iron oxide composite material for theragnostic application.

The (MS1Fe1)2, (MS1Fe2)2 and MS1Fe1MS1Fe2 bioengineered spider silks were constructed. The proteins consisted of silk MS1(12mer) (based on the repetitive motif of MaSp1 spidroin from Nephila clavipes) and two peptides capable of binding the metal ions named Fe1 and Fe2. The functionalized and control (sMS1) silks were bacterially produced, purified by thermal method and analyzed in SDS-PAGE gel. For sphere formation, the iron oxide suspension (positively or negatively charged) and silk solution were mixed with 2 M potassium phosphate buffer, pH 8. Morphology and elemental analysis of the spheres were performed using SEM/EDS microscopy.

The functionalized with Fe1 and Fe2 peptides and control sMS1 bioengineered spider silks were successfully constructed, produced and purified. The addition of functional peptides to silk did not influence silk self-assembly property, and spheres were formed. Both functionalized silks increased the binding capacity of iron oxide nanoparticles comparing with control silk as indicated the SEM/EDS analysis of composite silk/NPs spheres. The binding of NPs of negative charge resulted in the formation of spheres with more separated and more spherical morphology than spheres with positive NPs. It was also shown that Fe1 peptide exhibited enhanced binding of NPs comparing to Fe2 peptide.

The addition of metal binding peptides resulted in enhanced affinity of silk to magnetic nanoparticles. The obtained Fe/silk composite nanospheres can be potentially applied for cancer diagnostics and therapy.

Key words: bioengineered spider silk, iron oxide nanoparticles, composite spheres, silk functionalization, cancer therapy.

Poster

KW018-00040-2018-01

The construction of chimeric bioengineered silks that target VEGF receptors for biomedical application

Marta Szmyra[1], Kamil Kucharczyk[1,2], Anna Florczak[1,2], Andrzej Mackiewicz[1,2], Hanna Dams-Kozłowska[1,2]

[1]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poland
[2]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan

Introduction: Bioengineered spider silks are a biopolymers obtained by the expression of an artificial genes that were designed based on the sequences of natural silks. Furthermore, their sequences can be modified by an attachment of a DNA sequence encoding a functional domain. The chimeric, fusion silks obtain a new property that potentially can increase their applicability in the biomedical field i.e. for construction a drug delivery system for cancer treatment. Since cancer is no longer considered as a mass of cancer cells, the cancer treatment strategies should also target tumor microenvironment including blood and lymphatic vessels.

Aim: The design, production, and purification of chimeric spider silk proteins that target VEGF receptors for construction an anticancer drug delivery system.

Material and methods: The nucleotide sequence of peptide VE1 and VE2b that bind to the VEGFR1 and VEGFR2, respectively, were chemically synthesized. The ligation of these fragments was conducted at a 5'-termini of the sequences of the MS1 and MS2 bioengineered silks. Restriction analysis examined the cloning outcome which then was confirmed by sequencing. Chimeric proteins were produced in bacterial expression system. Proteins were purified using thermal denaturation method and analyzed by SDS-PAGE electrophoresis. Spheres were formed by mixing silk with potassium phosphate buffer. The morphology of spheres was analyzed by SEM.

Results: The chimeric constructs VE1/MS1, VE2b/MS1, VE2b/MS2 were designed and constructed. The VE1/MS1 was efficiently produced and then purified. The SDS-PAGE analysis indicated that the protein showed no impurities and no degradation. The functional domain VE1 did not impede the silk's potential to form spheres. The next stage of the research will be obtaining of VE2b protein silk variants and then the examination the binding potential of VE1/MS1, VE2b/MS1, VE2b/MS2 spheres to the cells that overexpress the VEGFR1 and VEGFR2.

Conclusions: Chimeric, functionalized silk proteins, which on the one hand can assemble in a morphological form (e.g., spheres) and on the other hand can recognize a defined cell receptor, have a great potential for biomedical application especially as a drug carrier. Development of the drug delivery system targeting the VEGFR1 and VEGFR2 may be a future strategy for the treatment of cancer.

Key words: bioengineered spider silk, VEGFR, anticancer drug delivery system, chimeric protein, tumor microenvironment.

Poster

KW018-00037-2018-01

Hypoxia modulates global gene expression in ovarian cancer cells treated with mitomycin

Aleksandra Klemba[1,2], Klaudia Brodaczewska[1], Bogdan Lesyng[3,4], Cezary Szczylik[5,6], Rafał Stec[7], Claudine Kieda[1]

[1]Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland
[2]MISMaP College, University of Warsaw, Poland
[3]Bioinformatics Laboratory, Mossakowski Medical Research Institute, PAS, Poland
[4]Faculty of Physics, University of Warsaw, Poland
[5]European Health Centre, Otwock, Poland
[6]Medical University of Warsaw, Poland
[7]Department of Oncology, Military Institute of Medicine, Warsaw, Poland

Introduction: Ovarian cancer (OC), the most aggressive of all gynecological malignancies, is usually detected at late stages when metastates are already settled. Because of the now-admitted concept of the importance of the microenvironment importance together with its direct dependence on the level of oxygen, any study on drug treatment efficacy must be performed in such context. As recent studies have shown that mitomycin can be an effective treatment in some ovarian cancers, the mechanism is worth attention for further therapeutic application. Thus, this work reports the study of the influence of the main microenvironmental factor: hypoxia, on modulating genes expression in ovarian cancer cells under mitomycin treatment.

Aim: To evidence and evaluate the influence of pO2 level on ovarian cancer cells response to mitomycin treatment.

Material and methods: SKOV3 cells were treated in vitro with mitomycin in normoxic and hypoxic (1% pO2) conditions. Viability of cells was investigated with Alamar Blue, while cell cycle and level of Ki67 were evaluated by flow cytometry. Gene expression profiling was performed with HTA 2.0 Affymetrix microarrays.

Results: Mitomycin inhibited ovarian cancer cell growth in both normoxic and hypoxic conditions. Cell cycle was inhibited in S and G2 phases. Mitomycin treatment caused wide transcriptome changes. Over 700 genes were altered upon drug treatment in normoxia. Additionally, when the treatment was applied in hypoxic conditions the modulation of over 60 genes was uncovered. Those genes were mainly related with extracellular matrix organization, including collagen formation. It appeared that expression of IL8, MMP1 and MMP10 genes was significantly altered in all investigated conditions.

Conclusions: Hypoxia significantly modulates ovarian cancer cells response to mitomycin treatment, providing novel information potentially improving its application in OC treatment.

Key words: ovarian cancer, mitomycin, hypoxia, microarray.

The research was funded by National Science Center (UMO-2012/07/N/NZ5/02052) and Military Institute of Medicine statutory funding 1/8912 (412).

Poster

KW018-00035-2018-01

The derivation of chondrocyte-like cells from pluripotent stem cells in serum free conditions

Michał Lach[1,2], Magdalena Richter[3], Tomasz Trzeciak[3], Wiktoria Suchorska[1,4]

[1]Radiobiology Lab, Greater Poland Cancer Centre, Poland
[2]The Postgraduate School of Molecular Medicine, Medical University of Warsaw, Poland
[3]Department of Orthopaedics and Traumatology, Poznan University of Medical Sciences, Poland
[4]Department of Electroradiology, Poznan University of Medical Sciences, Poland

The present techniques of regeneration of damaged joints due to osteoarthritis or mechanical damages are limited and are not long-term solutions, not preventing from total joint replacement. One of the promising sources of cells for regenerative purposes are pluripotent stem cells. Most of the protocols for in vitro differentiation are based on xeno- and serum dependent conditions, which decrease their application into human trials. In our study we used a human embryonic (BGV01) and induced pluripotent stem cells (GPCCi001A, ND*41658) adapted to xeno- and serum free conditions. Differentiation into chondrocyte-like cells was based on embryoid bodies formation protocol with serum free defined medium supplemented with TGF-β (10 ng/ml) for 3 weeks. Additionally, we tested the effect of elongation (up to 5 weeks) in monolayer and 3D conditions to test the improvements of existing protocol. For confirmation of successful chondrogenic differentiation: RT-qPCR, immunofluorescence staining of markers related to chondrocytes was performed. For proteoglycan production alcian blue staining in 2D cell culture were done and semi quantification of staining intensity. For 3D cell culture immunohistochemical staining was performed such as: alcian blue, toluidine blue, o-safranin. As a negative control non-differentiated populations of pluripotent stem cells and for positive control human articular chondrocytes cell line were used.

We obtained chondrocyte-like cells in serum-free conditions. We confirmed that by increased expression or presence of chondrogenesis-related markers by RT-qPCR and immunofluorescence staining. Higher proteoglycan intensity after 5 weeks of differentiation in comparison to controls or standard 3 week approach was notified. Additionally, increased deposition of extracellular matrix (collagen type II and chondroitin sulphate) was observed after 5 weeks in comparison with 3 week approach. Moreover, three dimensional culture enable to obtain chondrocyte-like morphology confirmed by immunohistochemical staining and RT-qPCR. Further analysis such in vivo studies should be performed to indicate their clinical safety and ability to regenerate damaged joints.

Key words: human induced pluripotent stem cells, chondrogenic differentiation.

Poster

KW018-00034-2018-01

The comparison of roscovitine derivatives activity with novel kinase and Bcl-2 inhibitors in CLL cells

Małgorzata Kubczak, Aleksandra Szustka, Tomas Gucky, Vladimir Krystof, Jerzy Błoński, Tadeusz Robak, Małgorzata Rogalińska

Department of Cytobiochemistry, University of Lodz, Poland

Aim: Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in Western countries. Despite advanced methods of treatment this type of cancer still remains incurable. The most common drugs used in CLL therapy, i.e alkylating agents, purine analogs or monoclonal antibodies are often ineffective in case of resistance to treatment. Novel kinase Btk and PI3Kγinhibitors as well as Bcl-2 inhibitor - venetoclax seem to be very promissing anti-leukemic agents for patients who do not respond to conventional therapy. Because of confirmed anticancer activity of CDK inhibitors on cell lines in our studies we compared apoptosis induction potential of new generation of CDK inhibitors (BP14 and BP30) with drugs used in CLL treatment (idelalisib, ibrutinib and venetoclax).

Results: Mononuclear cells obtained from peripherial blood of CLL patients were incubated with venetoclax (50 to 350 nM), idelalisib (20 to 50 μM), ibrutinib (1 to 5 μM) and CDK inhibitors BP14 and BP30 (15 to 50 nM), respectively. We have observed distinct response of CLL cells to anticancer drugs. Both studied anticancer agents (BP14 and BP30) decreased cell viability and increased the level of apoptosis after 24 and 48 hours of cell incubations with anticancer agents. The differences in induction of apoptosis were time- and dose-dependent. The obtained results have shown that nanomolar concentrations of CDK inhibitors were optimal as apoptosis inducers. We have also observed the differences in cell response to applied compounds during experiments. It may suggest that there is a need for personalization of patient's therapy. The ability to induce apoptosis by CDK inhibitors was usually comparable with venetoclax.

Conclusions: The evaluation of apoptosis induction potential could be helpful in monitoring of leukemic cells sensitivity to anticancer agents. Such analysis are useful in searching for new potential drugs for CLL. Obtained results indicate that apoptosis induction caused by CDK inhibitors, when active, was usually faster comparing to other anticancer agents.

Key words: apoptosis, idelalisib, venetoclax, ibrutinib, CDK inhibitors, CLL.

Poster

KW018-00033-2018-01

The effect of PD-1 checkpoint inhibition on the infiltration of myeloid cells into murine intracranial glioblastomas

Paulina Pilanc, Salwador Cyranowski, Natalia Ochocka, Beata Kaza, Bożena Kamińska-Kaczmarek

Nencki Institute of Experimental Biology, Warsaw, Poland

Glioblastoma (GBM) is the most 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. Immunotherapy is a promising alternative strategy in glioblastoma treatment. However, immune cell infiltration and activation of specific immune populations is not fully characterized in GBM. Immune checkpoint inhibition with monoclonal antibodies targeting the programmed cell death-1 (PD-1) protein has yielded significant clinical results in the treatment of several cancer types and has shown a promise in preclinical studies for the treatment of GBM. In order to evaluate the effects of PD-1 checkpoint inhibition in GBM, we utilised a murine syngeneic glioblastoma model induced by intracranial injection of murine GL-261 glioma cells to mice. The cells were engineered to harbour genes coding for luciferase and tdTomato protein to enable monitoring of the tumour growth via bioluminescence and fluorescence, respectively. We treated the mice with an intraperitoneal injection of anti-PD-1 antibody at day 8, 10, 11 and 14. We visualised the tumours in vivo using Xtreme imaging system at days 14 and 21 following tumour implantation. After 21 days of tumour growth with or without anti-PD-1 treatment we processed the brains for histological analysis or isolated immune cells for flow-cytometry to identify major immune cell populations infiltrating the tumours, including lymphocytes and myeloid cells. While we did not detect significant changes in blood from tumour-bearing animals subjected to anti-PD-1 treatment, we found that PD-1 checkpoint inhibition likely promotes the infiltration of CD11b+ cells into murine gliomas. This finding encourages to further investigate the impact of PD-1 checkpoint inhibition on specific immune cell infiltrates in experimental gliomas to fully comprehend its effect on tumour growth and host responses.

Studies supported by the project DIMUNO: 'Development of new cancer therapies based on selective antitumor immunomodulators'—co-financed by the National Centre for Research and Development in the framework of STRATEGMED-3 Prevention Practices and Treatment of Civilization Diseases.

Key words: glioblastoma, checkpoint inhibitor, myeloid cells.

Poster

KW018-00032-2018-01

Estimation of cancer microenvironment in three types of non-small cell lung cancer

Donata Jarmołowska-Jurczyszyn[1], Agnieszka Knopik-Skrocka[2], Andrzej Kluk[1], Nikola Kurzawińska[2], Karolina Weręża[2], Andrzej Lesicki[2]

[1]Department of Clinical Pathomorphology, Medical University of Poznań, Poland
[2]Department of Cell Biology, Faculty of Biology, A. Mickiewicz University of Poznań, Poland

The process of malignancy leads to important changes in lung. It can be formation of different structures like tubes, nests by transformed cells, desmoplastic reaction, vascularization, infiltration with immunological cells in stroma. These changes are the effect of cancer cells`activity and recruitment of microenvironment cells. The aim of the study was to investigate microenvironment of three types of non-small cell lung cancer (NSCLC): squamous cell cancer (SCC, 9 patients, mean age 69 ± 5 years old), adenocarcinoma (AC, 11 patients, 66 ± 1 years old) and large-cell neuroendocrine cancer (LCNC, 8 patients, 62 ± 8 years). In some cases, a proliferation index of cancer cells was also tested. The studies were conducted with material archivized in paraffin blocks. Patients were hospitalized in Centre of Pulmonology and Thoracic Surgery in Poznań. Histological preparations were made in Department of Clinical Pathomorphology of Medical University of Poznań. The procedure of histological preparation included staining with hematoxylin and/or eosin and DAB (3,3'-diaminobenzidine) immunohistochemical reaction to CD3, CD20, CD34, CD68 or Ki67. SCC shows low amount of stroma, low number of blood vessels and poor inflammatory reaction with T, B lymphocytes and macrophages. In AC, a strong infiltration with immunological cells (mainly with T lymphocytes), and desmoplatic reaction is observed. LCNC shows rather poor stroma microenvironment, but high infiltration with immunological cells. There are many necrotic areas with macrophages in cancer nests. A very high proliferation potencial of cancer cells leads to the increase of their number inside the nests. The cells which are not close to blood vessels undergo necrosis. In this type of cancer, the reaction to endothelial cell marker CD34 in stroma is strong and similar to that in AC. Many of capillaries show sprouting-like figures, typical for active process of angiogenesis. The results of the present work indicate evident differences in microenvironment of the three types of lung cancer. A weak response of microenvironment is typical for SCC. Strong response is observed in AC and LCNC, two more aggressive types of NSCLC. A high proliferation index of cancer cells in the latter type can be additional poor prognostic factor.

Key words: lung cancer, immunohistochemistry, infiltration with immunological cells, vascularization, cancer microenvironment.

Poster

KW018-00030-2018-01

Targeting cancer PTEN for hypoxia-related tumor microenvironment

Aleksandra Majewska, Klaudia Brodaczewska, Claudine Kieda

Military Institute of Medicine, Poland

Introduction: Hypoxia is a characteristic feature of the tumor microenvironment which shapes the course of cancer. It causes pathological angiogenesis, which results in metastasis and resistance to treatment. PTEN (phosphatase and tensin homolog deleted on chromosome ten) is a cell cycle regulating protein, which is often mutated in tumors. This leads to extensive cell proliferation and cancer progression. It was also observed that hypoxia influences PTEN activation classifying the gene, as tumor suppressor.

The aim of the study was to investigate how hypoxia affects kidney cancer cells growth and proangiogenic response, which may be related to PTEN activity.

Materials and methods: Murine kidney cancer cells were cultured under conditions of hypoxia (1% pO_2) and normoxia (~19% pO_2). The Alamar Blue test was used to study cell proliferation at various time points (from 24 h to 120 h). PTEN activation was assessed by p-PTEN detection with Western Blot, after 72 h culture. Expression and secretion of VEGF were measured by Real-Time PCR in total RNA and ELISA in culture supernatants, respectively.

Results: The percentage of Alamar Blue reduction increased after 120h of culture in hypoxic conditions. A decrease in the amount of PTEN protein, but an increase in p-PTEN was observed after 72 h. Hypoxia increased the mRNA level of the VEGF gene as well as the amount of protein secreted by kidney cancer cells.

Conclusions: Hypoxia promotes proangiogenic microenvironment as shown by VEGF increase. Upon prolonged culture cell culture in low pO_2 increased proliferation was observed. Simultaneously, hypoxia treatment resulted in the modulation of PTEN expression and activation. Therefore PTEN is a target for future assessment of tumor treatment efficacy through the control of microenvironmental conditions.

Key words: hypoxia, PTEN, renal cancer.

This work was founded by the National Science Centre (NCN) grant "OPUS 12), grant no. UMO-2016/23/B/NZ1/03211.

Poster

KW018-00027-2018-01

The decreased level of SAV1 mRNA expression in colorectal cancer

Anna Kowalczyk, Bartlomiej Krazinski, Janusz Godlewski, Zbigniew Kmiec

Department of Human Histology and Embryology, School of Medicine, Collegium Medicum, University of Warmia and Mazury in Olsztyn, Poland

Introduction: SAV1 (Salvador Family WW Domain Containing Protein 1) gene encodes a protein that contains two WW domains and is a component of the Hippo pathway. This pathway plays an important role in the control of organ size and tumor suppression by restricting proliferation and promoting apoptosis. Expression of the Hippo pathway components is deregulated in various human malignancies, and their level can be considered as a potential prognostic factor.

Aims: The purpose of this study was to determine the expression level of SAV1 mRNA in colorectal cancer (CRC) and to estimate its prognostic value and association with the progression of CRC.

Material and methods: Quantitative real-time PCR was applied to assess the SAV1 mRNA level in paired tumor and unchanged colorectal tissues collected from 120 CRC patients as well as 40 colon biopsies of healthy subjects obtained during screening colonoscopy. The clinicopathological and survival data (median follow-up time was 46.1 months) of the patients were recorded. Expression of SAV1 mRNA was also measured in CRC cell lines with different characteristics (HT-29, SW-480, LoVo) and a control line – CCD 841 CoN (epithelial-like, established from normal colonic tissue).

Results: Among the 120 tumor specimens tested, the relative SAV1 mRNA level (tumor tissue vs. matching unchanged mucosa of CRC patients) was decreased in 110 (91.7%) tumors while it was increased in 10 (8.3%) cases. The SAV1 mRNA level in tumor tissues was significantly lower than in samples of corresponding unchanged tissues and biopsies of healthy colon mucosa. Despite these differences, the SAV1 mRNA level did not correlate with patients' demographic and clinicopathological data as well as their overall survival. Downregulated expression of SAV1 mRNA was also observed in all tested CRC cell lines.

Conclusions: Results of our study suggest that SAV1 may play a role in the pathogenesis of CRC, however did not reveal the prognostic value of SAV1 mRNA expression. Further studies at the protein level are needed to fully determine the prognostic significance of SAV1 in CRC.

Key words: SAV1, colorectal cancer.

Poster

KW018-00026-2018-01

Anticancer activity of natural compounds in CLL cells

Aleksandra Szustka[1], Małgorzata Kubczak[1], Jerzy Błoński[2], Tadeusz Robak[2], Małgorzata Rogalińska[1]

[1]Department of Cytobiochemistry, University of Lodz, Poland
[2]Department of Hematology, Medical University of Lodz, Lodz, Poland

Aim of the study: Chronic lymphocytic leukemia is one of the most common B-cell malignancies in Western world; usually affects elderly. An enormous progress in diagnostic and treatment of CLL has been shown, but this disease is still classified as incurable. There is an increasing number of agents with anticancer potential. World trends move toward agents based on natural medicine which opens new possibilities for anticancer therapies with reduced side effects. Patients are administered usually to purine analogs combined with monoclonal antibody. Such treatment is not always recommended for all patients due to high toxicity of compounds.

Results: Mononuclear cells obtained from peripheral blood of CLL patients and control cells from healthy donors were incubated with natural compounds: curcumin (10 μM, 15 μM), quercetin (350-550 μM), graviola (50 μM,75 μM) and betulinic acid (5-20 μM) as well as with novel anticancer agents used in CLL treatment: venetoclax (50-350 nM), ibrutinib (1-5 μM) and idelalisib (20-50 μM). The viability of CLL and normal cells obtained from healthy donors was measured by flow cytometry (Vybrant Apoptosis Assay #4) to estimate cytotoxicity of all compounds and to compare its activity on healthy and leukemic cells. Simultaneous analysis of cell viability and expression of apoptosis-related proteins by Western blot were applied to examine the ability of leukemic cells to enter apoptosis after their incubations with drug(s). The obtained results revealed that all anticancer agents induced apoptosis with different extent. A significant decrease of CLL cells viability as well as changes in expression of apoptotic related proteins were observed. From the group of studied natural compounds the most effective apoptosis inducers were betulinic acid (20 μM) and curcumin (10 μM and 15 μM).

Conclusions: In the group of studied natural compounds and anticancer drugs used for CLL treatment the differences in cell response to applied agents were observed. Above compounds displayed potential of apoptosis induction on various extent. It might be associated with personal differences in cells response to anticancer agents.

Key words: CLL, novel anticancer agents, natural compounds, apoptosis, curcumin, betulinic acid.

Poster

KW018-00025-2018-01

In vitro activity of novel platinum complex with tris (2-carboxyethyl) phosphine towards human osteosarcoma cell line (U2-OS)

Marta Henklewska[1], Aleksandra Pawlak[1], Hanna Pruchnik[2], Bozena Obminska-Mrukowicz[1]

[1]Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Poland

[2]Department of Physics and Biophysics, Faculty of Life Sciences and Technology, Wroclaw University of Environmental and Life Sciences, Poland

Introduction: Platinum based drugs constitute a very potent class of anticancer agents commonly used in the therapy of malignancies. Due to the fact, that the therapy with cisplatin is often complicated by adverse effects and occurrence of tumor resistance, there are still efforts made to develop new platinum-based drugs with higher effectivity against tumors sensitive and resistant to cisplatin. The aim of the study was to evaluate anticancer activity of the new platinum-based compound, complex of platinum with tris(2-carboxyethyl) phosphine (Pt-TCEP) against human osteosarcoma cell line (U2-OS).

Material and methods: To determine the anti-proliferative activity, the cells were exposed to increasing concentration (0.625-10 μM) of Pt-TCEP for 24, 48 and 72 h and the effect was assessed by MTT assay. Additionally, for comparison, the cells were incubated with similar concentrations of cisplatin. Proapoptotic activity of Pt-TCEP was determined after 24 h incubation with the same concentrations of the studied compound by flow cytometric analysis with propidium iodide (PI) and annexin V staining.

Results and conclusions: The results indicate that Pt-TCEP inhibited cell proliferation in a concentration and time-depended manner. It was far more effective than cisplatin, especially in the short incubation period (24 h). IC50 for Pt-TCEP was 3.45 ± 0.14 μM, 1.74 ± 0.03 μM and 1.53 ± 0.11 μM after 24 h, 48 h and 72 h, respectively. For cisplatin IC50 was not achieved in studied concentrations after 24 h or 48 h. After 72 h, it reached 8.41 ± 0.61 μM. It was also confirmed, that Pt-TCEP induces cell apoptosis in U2-OS cells, with the concentration of 4.17±0.36 μM causing the apoptosis of 50% of cells. These results show that Pt-TCEP has a significant anticancer activity against human osteosarcoma cell line and it can be further tested for its efficacy in this type of tumor.

Key words: osteosarcoma, cisplatin, Pt-TCEP, in vitro study.

Poster

KW018-00023-2018-01

Insulin-like growth factor 2 (IGF2) as a tumor and prognostic marker of colorectal carcinoma

Aldona Kasprzak[1], Małgorzata Andrzejewska[1], Jacek Szmeja[1], Agnieszka Seraszek-Jaros[2], Witold Szaflarski[1], Agnieszka Adamek[3]

[1]Chair and Department of Histology and Embryology, Poznan University of Medical Science, Poland
[2]Department of Bioinformatics and Numerical Biology, Chair of Clinical Pathomorphology, Poznan University of Medical Sciences, Poland
[3]Chair and Department of Infectious Diseases, Poznan University of Medical Sciences, Poland

Introduction: The insulin-like growth factor (IGF) system has an important role in intestinal carcinogenesis. Higher serum levels of IGF2 and IGF2 overexpression in tumor cells are associated with more advanced colorectal carcinoma (CRC) and poor overall survival. Relatively little is known about the IGF2 expression in metastatic and local CRC, also in different histological subtypes of the tumor.

Aim of the study was to analyse of IGF2 immunoexpression as a potential prognostic marker in local CRC and lymph node metastatic carcinoma and to evaluate its expression in mucinous and nonmucinous CRC.

Material and methods: IGF2, Ki-67 and p53 expressions assessed by immunohistochemistry for a total 66 colorectal adenocarcinomas (of which 7 were of mucinous type), matched lymph node metastatic carcinomas and control tissues both from surgical resection and tissue microarray (TMA) samples ($n = 66$). The IRS and Gatter et al. scoring systems were used to semiquantitative analysis. Examination of the immunoexpression of IGF2 as related to cell proliferation markers and clinical data were performed.

Results: IGF2 expression was detected in all control, metastatic and tumor samples. Cytoplasmic pattern of expression (IGF2), and nuclear localization (Ki-67 and p53) were observed. No significant differences were detected in IGF2 immunoexpression between the CRC, lymph node metastatic carcinoma and the control group ($p > 0.05$). Ki-67 expression was significantly higher in CRC than in control group ($p < 0.001$). Higher expression of IGF2 was detected in nonmucinous type of colorectal adenocarcinoma (median = 9.00) as compared with mucinous subtype (median = 6.00) ($p < 0.05$). No significant differences were disclosed in expression of IGF2 of variably advanced histological grading and staging, localisation (colon vs. rectum), and macroscopic type of the tumor (flat vs. protruded) ($p > 0.05$). Significantly higher IGF2 expression was present in CRC patients with N1 (median = 9.00) than in N0 (median = 7.00) TNM staging categories ($p < 0.05$). No significant correlations between IGF2 and Ki-67 and/or p53 expressions in CRC were observed ($r = –0.232$; $r = –0.152$, respectively; $p > 0.05$).

Conclusions: There was no difference in tissue expression between control samples, metastatic and local CRC patients, suggesting that IGF2 is not a good tumor marker. However, IGF2 expression allows to differentiate patients without (N0) and with lymph node metastasis (N1), and mucinous from nonmucinous subtypes of CRC.

Key words: immunoexpression, Ki-67 proliferating antigen, p53 expression, IGF2, colorectal cancer, mucinous and nonmucinous CRC.

Poster

KW018-00022-2018-01

The usefulness of new laboratory (A/N) index in the assessment of small cell lung cancer prognosis

Ewa Wojcik, Zofia Stasik, Beata Sas-Korczynska, Jadwiga Tarapacz, Urszula Rychlik, Jan Kanty Kulpa

Maria Sklodowska-Curie Institute – Oncology Center, Cracow Branch, Poland

Introduction: In addition to the clinical factors significantly affecting the prognosis of cancer patients, inflammation and malnutrition are also mentioned. The severity of these processes is largely dependent on the performance of the host immune system. It is believed that the changes in the number of neutrophils in the circulating blood as well as the level of serum albumin may be a reflection of the patient's reaction to the presence of cancer. The possibility of using these laboratory parameters to evaluate the prognosis of cancer patients is considered.

The aim of the study was to verify the usefulness of the index calculated as the ratio of serum albumin to neutrophils count in the prognosis of patients with small cell lung cancer.

Material and methods: Blood count and albumin levels were performed before treatment in 163 patients with small cell lung cancer and in the reference group of 67 people considered healthy. In the all persons, the AN index was then calculated.

Results: Compared to the reference group, patients with small cell lung cancer had significantly higher absolute neutrophil counts and significantly lower albumin levels. Neutrophilia (neutrophils count > 7.0/μL), before treatment, was confirmed in 12.9% of patients, and hypoalbuminemia (ALB < 35 g/L) in 18.4%. The A/N values were significantly lower in patients with lung cancer than in the reference group.

The optimal cut-off point for the A/N ratio determined from the ROC curve was 8.32. In 49.7% of patients before treatment, the pathological values of this indicator were found.

Apart from the extensive disease, poor performance status and male gender, a significantly worse prognosis was confirmed in patients with baseline A/N values lower than 9.0 (P = 0.00000). All assessed parameters, except for gender, have been shown to be independent prognostic factors. While the risk of death of patients with extended disease was 3.4 times higher than those with the localized disease, the risk of death of patients with A/N < 9 was 1.5 times higher than those with A/N > 9. Similarly, 1.5- times higher risk of death was found in patients with poor performance status compared to those with better performance status.

Conclusions: The A/N index is helpful in assessing the prognosis of patients with small cell lung cancer. In this study group, the A/N index is also an independent prognostic factor.

Key words: small cell lung cancer, albumin, neutrophils, A/N index.

Poster

KW018-00021-2018-01

TREM2 gene associated with the risk of Alzheimer's disease is upregulated by p53 tumor suppressor protein in cancer cells of different origin

Małgorzata Krześniak, Artur Zajkowicz, Agnieszka Gdowicz-Kłosok, Patryk Janus, Barbara Łasut, Marek Rusin

Maria Skłodowska-Curie Institute – Oncology Center, Gliwice Branch, Poland
Center for Translational Research and Molecular Biology of Cancer, Poland

The genetic variants of TREM2 gene are associated with Alzheimer's disease and other neurodegenerative disorders. In physiological conditions TREM2 protein is expressed exclusively on immune cells and it is located on cell surface. TREM2 is at a top of a signaling pathway, which contains TYROBP – the TREM2 binding partner, SYK kinase and BLNK adapter protein. The gene for SYK kinase was found to be upregulated by tumor suppressor protein p53. It is possible that p53 can also stimulate the expression of the other protein from this pathway. Moreover, the in silico analysis showed a potential p53 binding site close to the TREM2 transcription start site.

The goal of our study was testing the hypothesis that TREM2 is regulated in p53-dependent manner. As the p53-activating stimulus, we employed the co-treatment of cells with actinomycin D and nutlin-3a (A+N). Actinomycin D stimulates p53 by activating kinases that phosphorylate this protein, while nutlin-3a inhibits a negative regulator of p53 – MDM2 protein. These two substances acting together can synergistically activate p53 in various cell types.

The cells in culture were treated with A+N. The protein expression was examined by Western blotting and changes in the levels of mRNA were measured by semi-quantitative real-time PCR. The influence of p53 on the gene regulatory region of TREM2 was measured using dual-luciferase reporter assay system. For this purpose, the gene regulatory region of TREM2 with the potential p53 binding site was cloned into pGL3-Basic reporter vector into the restriction sites generated by PCR primers.

We found strong induction of TREM2 and the other elements of the pathway: TYROBP, SYK and BLNK following co-treatment with actinomycin D and nutlin-3a. Moreover, we demonstrated that expression of these proteins was blocked by p53 knock-down, what confirms that genes for these proteins are regulated in p53-dependent fashion. Moreover, we found TREM2 accumulation following A+N treatment in cells of various origin: A549, U-2 OS and A375. The luciferase reporter assay and ChIP-PCR confirmed that TREM2 sequence includes bona fide p53 response element controlling the expression of this gene. Based on our results we conclude that TREM2 is a novel p53-regulated gene. Thus, we detected a strong link between p53 pathway and Alzheimer's disease.

Key words: p53, TREM2, actinomycin D, nutlin-3a.

This work was supported by grants no. 2013/11/B/NZ5/03190 to MR and 2014/15/D/NZ5/03410 to AG-K from the National Science Centre, Poland (NCN).

Poster

KW018-00020-2018-01

The fibrinogen to albumin ratio in head and neck cancer patients

Zofia Stasik, Ewa Wojcik, Anna Mucha-Małecka, Jadwiga Tarapacz, Urszula Rychlik, Katarzyna Brandys, Jan Kanty Kulpa

Maria Sklodowska-Curie Institute – Oncology Center, Cracow Branch, Poland

Introduction: Elevated concentrations of fibrinogen and decreased levels of albumin have been reported to be markers of systemic inflammation. Elevated fibrinogen to albumin ratio (FAR) was associated with a worse prognosis in breast and esophageal cancer.

The aim of our study was to evaluate the prognostic value of fibrinogen to albumin ratio in head and neck cancer patients.

Material and methods: Serum albumin and plasma fibrinogen concentrations were measured before treatment in 145 patients with head and neck cancer. Disease free survival (DFS) was assessed using the Kaplan-Meier method. Independent prognostic significance was analyzed using the Cox proportional hazards model.

Results: The serum fibrinogen concentration and FAR value were significantly higher in the III+IV stage group than in those of I+II stage ($p < 0.029$; $p < 0.018$; respectively). There were no significant differences in serum albumin level. Patients with T3+4 stage, in comparison to those with T1+2 stage, had significantly higher fibrinogen level ($p < 0.02$) and significantly higher FAR value ($p < 0.02$). There were no significant differences in serum albumin level. In the group of patients with involved lymphatic nodes (N1-3), at the lack of significant differences between albumin and fibrinogen levels, FAR values were significantly higher ($p < 0.043$) in comparison with those without nodal involvement (N0). Univariate analysis showed that III+IV stage ($p < 0.00001$), T3+4 stage ($p < 0.0001$), N1-3 stage ($p < 0.00001$) and high FAR values ($p < 0.013$) were all individually associated with an unfavorable prognosis. However, multivariate analysis showed, that stage of disease [HR = 6.24 (95% CI: 2.74-14.23); $p = 0.000013$] and FAR [HR = 2.09 (95% CI: 1.05-4.18); $p = 0.035$] were significantly associated with disease free survival.

Conclusion: In head and neck cancer patients, apart from stage of disease, the FAR value is an independent, unfavorable predictor of poor disease free survival.

Key words: head and neck cancer, fibrinogen, albumin, FAR.

Poster

KW018-00019-2018-01

hTERT promoter methylation status as a molecular marker of head and neck cancer progression

Agnieszka Sobecka[1,2,3], Wojciech Barczak[1,2,3], Wiktoria Blaszczak[3], Pawel Golusinski[1,2], Blazej Rubis[4], Michal M. Masternak[1,2,5], Wiktoria M. Suchorska[3,6], Wojciech Golusinski[1,2]

[1]Department of Head and Neck Surgery, Poznan University of Medical Sciences, Poland
[2]The Greater Poland Cancer Centre, Poznan, Poland
[3]Radiobiology Lab, The Greater Poland Cancer Centre, Poznan, Poland
[4]Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, Poznan, Poland
[5]University of Central Florida, Burnett School of Biomedical Sciences, College of Medicine, Orlando, FL, United States
[6]Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland

Introduction: The head and neck squamous cell carcinoma (HNSCC) is the sixth leading cause of cancer worldwide, representing over half a million incidents every year. Cancer cells, including HNSCC, are characterized by an increased telomerase activity. This enzymatic complex is active in approximately 80-90% of all malignancies, and is regulated by many factors, i.e. methylation status of hTERT gene promoter. hTERT gene is also surmised to be differentially methylated in cancer patients than in controls.

The aim of this study was to analyze the hTERT gene promoter methylation status in blood leukocytes of HNSCC patients.

Material and methods: DNA was extracted from PBL (Peripheral Blood Leukocytes) of 92 patients with histologically diagnosed HNSCC and 53 healthy volunteers. Methylation status of 19 CpG sites was estimated using bisulfide conversion technique followed by sequencing of PCR products.

Results: Close to the significant (p = 0,0532) differences in the general frequency of hTERT CpG sites methylation was detected between patients and healthy controls. However, it was discovered that some of analyzed positions (CpG sites: 1 [p = 0,0235], 5 [p = 0,0462], 8 [p = 0,0343]) are significantly more often methylated in HNSCC patients than in controls. The opposite finding was observed in case of CpG position 2 (p = 0,0210). Furthermore, closer analysis of single CpG positions revealed differences in methylation status dependent on anatomical site and TNM classification.

Conclusions: hTERT promoter methylation profile (general or in single CpG positions) may be used as a molecular marker of head and neck cancer progression.

Key words: head and neck cancer, telomerase, molecular marker.

Poster

KW018-00018-2018-01

hTERT gene knockdown enhances response to radio- and chemotherapy in head and neck cancer through a DNA damage pathway modification

Wojciech Barczak[1,2,3], Agnieszka Sobecka[1,2,3], Pawel Golusinski[1,2,4], Michal M. Masternak[1,2,5], Blazej Rubis[6], Wiktoria M. Suchorska[3,7], Wojciech Golusinski[1,2]

[1]Department of Head and Neck Surgery, Poznan University of Medical Sciences, Poland
[2]The Greater Poland Cancer Centre, Poznan, Poland
[3]Radiobiology Lab, The Greater Poland Cancer Centre, Poznan, Poland
[4]Department of Biology and Environmental Studies, Poznan University of Medical Sciences, Poznan, Poland
[5]University of Central Florida, Burnett School of Biomedical Sciences, College of Medicine, FL 32827, Orlando, USA
[6]Department of Clinical Chemistry and Molecular Diagnostics, Poznan University of Medical Sciences, Poznan, Poland
[7]Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland

Malignant tumors of the head and neck region differs natural clinical outcome and prognosis depending on the histological diagnosis and location. Despite that the diagnostic and therapeutic problems are similar. The gold standard of therapy these tumors is combined therapy involving the local and systemic treatment. Recently, the great interest arouses application of gene therapy. One of the targets is telomerase, the enzymatic complex participating in immortality of cancer cells.

The aim of the study was to analyze the effect of hTERT gene knockdown in cancer cells in order to develop an improved personalized therapy of head and neck squamous cell carcinoma (HNSCC).

To obtain the most efficient knockdown, transfection with siRNA, or transduction with shRNA-bearing lentiviral vectors were used. The efficiency of hTERT silencing was verified with qPCR, Western blot, and immunofluorescence staining. Subsequently, type of cell death and DNA repair mechanism induction after hTERT knockdown were assessed with the same methods followed by flow cytometry. The effect of a combined treatment (chemotherapy and/or radiotherapy) with hTERT gene knockdown on double strand breaks level was also evaluated by flow cytometry. Results showed that the designed siRNAs and shRNAs were effective tools in silencing hTERT expression in HNSCC cells. Depending on a cell line, hTERT knockdown led to a cell cycle arrest either in phase G1 or S/G2. Induction of apoptosis after hTERT downregulation with siRNA was also observed. Additionally, hTERT targeting with lentiviral system followed by cytostatics administration (i.e. cisplatin and docetaxel) led to induction of both apoptosis and autophagy. Interestingly, an increase in double strand breaks accompanied by activation of the main DNA repair mechanism - NER, was also observed.

Altogether, we conclude that hTERT knockdown significantly contributes to efficacy of HNSCC radiotherapy and chemotherapy. This in turn might significantly reduce the toxicity of chemotherapeutics in patients and thereby increase quality of life.

Key words: telomerase, head and neck cancer, gene silencing, shRNA, RNA interference.

Poster

KW018-00017-2018-01

Relationship between BMI-1 and PHLPP1/2 genes in endometrial cancer

Agnieszka Zaczek, Anna Krześlak

Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland

BMI-1 (B-lymphoma Mo-MLV insertion region 1) as component of Polycomb repressive complex 1, regulates ubiquitin ligase RING1B activity and affects the expression of genes involved in cell cycle control, apoptosis or DNA repair. It has been shown that in many types of cancer BMI-1 contributes to cancer development through activation of PI3K/AKT pathway due to inhibition of PTEN expression. Alterations of PI3K/AKT pathway play essential role in endometrial carcinogenesis however the role of BMI-1 in regulation of this pathway in endometrial cancer is unknown.

The aim of our study was to analyze the effect of BMI-1 silencing by siRNA on expression of several phosphatases directly or indirectly involved in regulation in endometrial cancer cell line HEC1A and to estimate correlation between BMI-1 and PHLPP1/2 as well as PTEN expression in endometrial normal and cancer tissue samples.

The results showed increased PHLPP1 and 2 expression and decreased phosphorylation level of AKT after BMI-1 silencing in HEC1A cells. There was also strong inverse correlation between BMI-1 and PHLPP1/2 in endometrial normal tissue samples. However, in case of cancers the correlation between BMI-1 and PHLPP was observed only in PTEN positive cancer samples.

In conclusion, our results indicate that in endometrial cancer BMI-1 affects on PI3K/AKT pathway via regulation of PHLPP1/2 and this regulation is probably PTEN-dependent.

Key words: endometrial cancer, BMI-1, PHLPP1/2 genes, PTEN.

Poster

KW018-00016-2018-01

Fucus vesiculosus extract inhibits proliferation of head and neck cancer in vitro

Wiktoria Błaszczak[1], Wojciech Barczak[1,2], Wiktoria Suchorska[1,3]

[1]Radiobiology Lab, The Greater Poland Cancer Centre, Poznan, Poland
[2]Department of Head and Neck Surgery, Poznan University of Medical Sciences, The Greater Poland Cancer Centre, Poznan, Poland
[3]Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland

Fucoidans, sulfated polysaccharides derived from marine algae, have been reported to exert anticancer effects with simultaneous low toxicity against healthy tissue. That correlation was observed in several cancer models, however has never been investigated in head and neck cancer, which remains one of the most common causes of death worldwide. To magnify the efficacy of conventional therapy, administration of agents like fucoidan, reducing serious adverse effects incidence in patients, could be beneficial.

The aim of this study was to evaluate for the first time, the anticancer effect of Fucus Vesiculosus (FV) extract's alone and with co-administration of cisplatin in head and neck squamous cell carcinoma (HNSCC).

MTT assay results revealed an FV-induced inhibition of proliferation in all tested cell lines (H103, FaDu, KB). Moreover, fucoidan enhanced the response to cisplatin in all cell lines with HPV-positive one (KB) being the most sensitive. These results have been confirmed by flow-cytometric apoptosis analysis (annexin V/7-AAD staining). Furthermore, a dose-dependent gain in apoptotic fraction was observed. Significant dose-dependent increase in reactive oxygen species (ROS) production (flow cytometry, DCFDA staining) was revealed in H103 cell line, while FaDu cells remained unresponsive. On the contrary, HPV-positive cell line – KB – demonstrated a dose-dependent decrease in ROS synthesis, which suggests that apoptosis induction might have been executed through the extrinsic pathway activation. Flow cytometric (PI staining) cell cycle analysis showed an FV induced, dose-dependent arrest in S/G2 phase in case of H103 cell line and G1 arrest in KB cells.

In conclusion, we confirmed that fucoidan derived from Fucus Vesiculosus exhibits anticancer properties against HNSCC, which are manifested by induction of apoptosis, regulation of ROS production, cell cycle arrest and inhibition of proliferation.

Key words: HNSCC, fucoidan, apoptosis, proliferation.

Poster

KW018-00015-2018-01

Nanoparticles made of functionalized spider silk for targeted cancer therapy – the evaluation of their toxicity and efficacy in the mice model

Tomasz Deptuch[1], Anna Florczak[1], Karolina Penderecka[1], Anna Lewandowska[2], Ewa Leporowska[3], Andrzej Marszalek[2], Andrzej Mackiewicz[1], Hanna Dams-Kozlowska[1]

[1]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Oncologic Pathology and Prophylactics, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Laboratory Diagnostics, Greater Poland Cancer Centre, Poznan, Poland

Efficient and selective drug delivery should significantly reduce potential risks associated with chemotherapy in cancer patients. Herein we present drug delivery system based on the bioengineered silk proteins. The genetic engineering allowed for the introduction to the cDNA sequence of MS1 silk, the cDNA sequence encoding peptide H2.1 for selective targeting of Her2+ breast cancer cells. The obtained nanospheres have been demonstrated to efficiently and selectively transport doxorubicin into Her2+ cells in an in vitro model. This study aimed to determine maximal tolerated dosage, toxic effects, biodistribution of the nanospheres and therapeutic impact of doxorubicin delivered by silk nanospheres in an in vivo model.

The bioengineered silk protein MS1 and its modified for targeted delivery variant – H2.1MS1 were based on the sequence of Major Ampullate Spidroin 1 from Nephilia clavipes. The proteins were overexpressed in a bacterial system. After purification by the thermal method, the silk proteins were mixed with 2M potassium phosphate buffer to form nanospheres. The maximum tolerated dose of nanospheres was examined based on the survival and behavior of mice 24 hours after administration of spheres. The long-term toxic effects of multiple nanospheres administrations were evaluated by a) clinical symptoms, b) morphological and biochemical analysis of the peripheral blood, and by c) histopathologic examination of the internal organs. Distribution of fluorescently labeled nanoparticles was analyzed with IVIS imaging system. The therapeutic effect of doxorubicin delivered by silk nanospheres was based on the measurement of the tumor size in breast cancer mice model.

The maximal tested dosage of silk nanoparticles (20 mg/kg) was not lethal and did not cause the behavioral changes of mice. Based on the clinical symptoms, blood biochemistry and morphology, and histopathological analysis of organs the repeated administrations of silk spheres did not elicit long-term toxicity. The Her2+ oriented particles (H2.1MS1) were observed at the location of the Her2+ tumor, indicating the site-specific accumulation. Moreover, the growth of Her2+ tumor was inhibited by doxorubicin delivered by functionalized silk nanospheres.

The obtained results indicated great potential of the drug delivery system that is based on the functionalized silk in cancer therapy.

Key words: silk, breast cancer, biotechnology, targeted delivery, drug delivery system, recombinant proteins.

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

Poster

KW018-00014-2018-01

Short peptide binding GM-CSF interfers with glioma-microglia environmnent and inhibits glioblastoma progression

Katarzyna Poleszak, Maria Pasierbińska, Paweł Wiśniewski, Bożena Kamińska

Nencki Institute of Experimental Biology

Glioblastoma (WHO grade IV, GBM) is a malignant, very aggressive, primary brain tumor which due to lack of efficient therapy caused by the heterogeneity of genetic abnormalities of tumor cells remains incurable. Tumor microenvironment plays an important role in growth, metastasis and response to treatment in many tumors, also GBM. Therefore, an approach to target tumor microenvironmet gained recently an increased attention. Brain resident immune cells – microglia and peripheral macrophages accumulate in malignant glioma and constitute 30-50% of the tumor mass. Glioma cells overexpress and secrete proteins that reprogram microglia and peripheral macrophages into cells which potentiate tumor invasion and growth, furthermore suppress antitumor immunity. Glioma-derived granulocyte macrophage colony-stimulating factor – GM-CSF (Csf-2) induces accumulation and protumorigenic activation of microglia/macrophages. We designed a humanized peptide that selectively binds to GM-CSF, blocks its binding to respective receptors on microglia, and inhibits activation of the receptors and downstream signaling pathways resulting in inhibition of glioma invasiveness. First, we designed a peptide library containing 26 peptides 14-residue long each. Next, we identified the peptides binding GM-CSF using peptide microarrays, enzyme-linked immunosorbent assay (ELISA) and a technique based on surface plasmon resonance (SPR). Subsequently, we selected peptide (G7) with the most potent capacity for inhibition of U87 MG and LN18 glioma cell invasiveness in the presence of human and mouse microglia cell line using the Matrigel Matrix cell invasion assay. We also confirmed that this peptide blocks binding of GM-CSF to its receptor using a method based on SPR technique and LigandTracer. Antitumor activity of G7 peptide in vivo was confirmed in orthotopic xenograft mouse model.

Key words: microglia, GM-CSF, peptides.

Poster

KW018-00013-2018-01

Targeted sequencing of cancer- and epigenetic-related genes in glioblastoma reveals a deep deregulation of epigenetic mechanisms

Bozena Kaminska[1], Bartosz Wojtas[1], Marta Maleszewska[1], Bartlomiej Gielniewski[1], Paulina Szadkowska[1], Sylwia K. Krol[1], Andrzej Marchel[2], Tomasz Czernicki[2], Andrzej Koziarski[3], Grzegorz Zielinski[3], Andrzej Styk[3], Maciej Kawecki[4], Cezary Szczylik[4], Ryszard Czepko[5], Maciej Banach[5], Wojciech Kaspera[6], Wojciech Szopa[6], Bartosz Czapski[7], Miroslaw Zabek[7]

[1]Laboratory of Molecular Neurobiology at Nencki Institute of Experimental Biology, Warsaw, Poland
[2]Neurosurgery Department and Clinic, Medical University of Warsaw, Warsaw, Poland
[3]Department of Neurosurgery, Military Institute of Medicine, Warsaw, Poland
[4]Department of Oncology, Military Institute of Medicine, Warsaw, Poland
[5]Department of Neurosurgery, Scanmed S.A. St. Raphael Hospital, Cracov, Poland
[6]Department of Neurosurgery, Medical University of Silesia, Regional Hospital, Sosnowiec, Poland
[7]Department of Neurosurgery, Mazowian Brodno Hospital, Warsaw, Poland

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 118 fresh frozen glioma samples of grade II, III, and IV collected from Polish (n = 97) and Canadian (n = 21) populations. We employed a second generation DNA sequencing target enrichment panel comprising 600 cancer-related genes and 100 epigenetic-related genes. The target region spanning 7 MB (1 MB = 1 x 10^6 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). Several filtering steps were used to eliminate variant calling errors: mapping quality > 35, each variant coverage > 20x, the penetration of each variant > 20%. Targeted sequencing of GBMs demonstrated mutations in different genetic drivers (including well known EGFR, TP53, PDGFR and PTEN mutations) and numerous genetic alterations in genes responsible for histone and chromatin modifications, chromatin remodeling and DNA methylation. Newly discovered variants were confirmed by ultra-deep sequencing.
Key words: sequencing, glioblastoma, brain tumor, GBM, Epigenetics.

Funding: Supported by a National Science Centre grant 2013/09/B/NZ3/01402 (MM).

Poster

KW018-00012-2018-01

Impact of hematological parameters on clinical outcome in patients with cancer of larynx and hypopharynx treated with radio- or chemotherapy

Jolanta Mrochem-Kwarciak, Tomasz Rutkowski, Andrzej Wygoda, Aleksandra Chmura, Regina Deja, Agata Kowalska, Krzysztof Składowski

Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland

Introduction: Anemia is associated with a poor outcome in patients treated with radiotherapy (RT), probably, due to a low oxygen level in tumor. Plasma osteopontin (OPN) and hemoglobin (Hb) are parameters associated with tumor hypoxia in HNSCC patients. The number of reticulocytes (Ret), immature reticulocyte fraction (IRF), high fluorescence reticulocytes (HFR), medium fluorescence reticulocytes (MFR) – a new routine hematological parameters, can be used to predict assess erythropoiesis. The aim of this study was to assess the clinical utility parameters of the erythrocytic system and the concentration of OPN as a marker of tumor hypoxia.

Material and methods: Between 01/2009 and 08/2013 93 patients with squamous cell cancer of hypopharynx (27%) and larynx (73%) were treated with RT alone (61%) or combined with chemotherapy (39%). There were 8%, 44%, 30%, and 18% patients with T1, T2, T3 and T4 tumor stage, respectively and 52%, 6%, 34%, and 8% patients with N0, N1, N2 and N3 nodal stage of disease, respectively. OPN and parameters of the red blood cell system were estimated in plasma or blood before and immediately after treatment completion.

Results: Strong negative correlation has been found between initial anemia (Hb < 12 g/ml) and OPN ($p = 0.0001$), IRF ($p = 0.001$), HFR ($p = 0.04$) and MFR ($p = 0.008$). A negative correlation has been found between anemia after treatment and IRF ($p = 0.04$), MFR (.02). Significantly longer overall survival (OS) was found for patients with lower OPN both, before and after treatment ($p = 0.0001$; $p = 0.01$, respectively) and posttreatment: Ret ($p = 0.02$). HFR ($p = 0.0001$) and MFR ($p = 0.0002$).

Conclusions: In patients with cancer of larynx and hypopharynx, anemia is a chronic disease which stimulates erythropoiesis. HFR and MFR may indicate increased and ineffective erythropoiesis which correlates with increased risk of death. Pre- and posttreatment OPN are prognostics determinants of OS in this group of patients.

Key words: osteopontin, reticulocyte.

Poster

KW018-00011-2018-01

Intraoperative radiotherapy alters the radiobiological bystander response induced by the surgical wound fluids in breast cancer cells

Igor Piotrowski[1], Katarzyna Kulcenty[1], David Murawa[2], Wiktoria Suchorska[1,3]

[1]Radiobiology Laboratory, Department of Medical Physics, Greater Poland Cancer Centre, Poznań, Poland
[2]Oncological and General Surgery Department I, Greater Poland Cancer Centre, Poznań, Poland
[3]Department of Electroradiology, University of Medical Sciences, Poznań, Poland

Breast cancer is the most common cancer occurring in women. Currently the standard treatment involves breast-conserving surgery followed by whole breast radiotherapy. The fact that the majority of metastases occur within the surgical scar initiated a series of research and clinical trials which aimed to evaluate the effectiveness of localized intraoperative radiotherapy (IORT) in inhibiting local recurrence formation. IORT was previously reported to modify the biological activity of surgical wound fluids [Belletti *et al*., 2008]. Moreover it has been shown, that soluble factors secreted by irradiated cells can induce DNA damage and mutagenesis in unirradiated bystander cells. This radiation induced bystander effect (RIBE) is likely to contribute to the local tumor control after IORT treatment. The aim of this study was to determine whether the wound fluids collected from patients treated with IORT induce radiobiological response in breast cancer cells and to assess the role of RIBE in this process.

Wound fluids from patients which underwent IORT (RT-WF), as well as from control group without radiotherapy (WF), were collected 24 hours after the surgery. Conditioned medium (CM) was collected from breast cancer cell culture 24 hours after exposure to the dose of 10 Gy. Two human cancer cell lines with different molecular status (basal – MDA-MB-468, luminal – MCF-7) were incubated with wound fluids and conditioned medium (CM, WF, RT-WF, WF+CM) in serum-free culture medium. We used flow cytometry to assess induction of DNA damage and RT-qPCR to analyze the activity of DNA damage repair pathways.

Our results show that both wound fluids from patients who received IORT (RT-WF) and a combination of surgical wound fluids with conditioned medium (WF+CM) induce DNA damage and activate DNA damage repair pathways in breast cancer cells. This effect was not observed after stimulation with control wound fluids (WF).

Our results show that, unlike the control group (WF), wound fluids collected from IORT-treated patients (RT-WF) induce radiobiological response in breast cancer cells. We ascribe this phenomenon to the activity of soluble factors secreted by irradiated cells. Our findings demonstrate that IORT reduces the local recurrence rates not only through the cancer cell killing, but also by altering tumor's microenvironment.

Key words: intraoperative radiotherapy, breast cancer, DNA damage response, apoptosis.

Poster

KW018-00010-2018-01

Polymorphisms in angiogenesis-related genes and clinical outcome in patients with laryngeal squamous cell carcinoma

Dorota Butkiewicz[1], Małgorzata Krześniak[1], Agnieszka Gdowicz-Kłosok[1], Tomasz Rutkowski[2], Iwona Matuszczyk[1], Iwona Domińczyk[1], Krzysztof Składowski[2]

[1]Center for Translational Research and Molecular Biology of Cancer, M. Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Poland

[2]I Radiation and Clinical Oncology Department, M. Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Poland

Laryngeal cancer is the most common form of head and neck cancer (HNC) in which radiotherapy and cisplatin-based chemotherapy are commonly used treatment strategies. Angiogenesis together with tumor hypoxia are of great importance for cisplatin and radiation therapy effectiveness. VEGF is a potent stimulator of angiogenesis that acts through its tyrosine kinase receptors VEGFR1/FLT1 and VEGFR2/KDR. Activation of the VEGF/VEGFR pathway leads to an increase in expression of many proteins, e.g. matrix metalloproteinases (MMPs) involved in degradation of basement membrane and ECM components. A special role in angiogenesis play MMP2 and MMP9 gelatinases. In many solid tumors, including HNC, it has been shown that increased levels of VEGF and MMPs constitute a negative prognostic factor. Certain polymorphisms in genes encoding proteins biologically related to angiogenesis, by affecting their expression and functions, may contribute to modulation of angiogenic potential and metastatic propensity that may influence cancer susceptibility, anticancer treatment efficacy, risk of progression and patients survival. The association between angiogenesis gene polymorphisms and prognosis has been observed in many cancers. In HNC, the MMP and VEGF gene variants have been found to affect risk of cancer, whereas studies concerning therapy outcome are scarce, conflicting and conducted in small patient groups.

In this report we examined the possible association between angiogenesis-regulating gene polymorphisms and therapy results in 201 laryngeal squamous cell carcinoma patients treated with radiotherapy and concurrent chemoradiotherapy. Both VEGF gene polymorphisms studied were significantly associated with clinical outcome in the group. The VEGF -634CC genotype was statistically significant predictor of unfavorable overall survival in uni- and multivariate models (p = 0.020 and 0.021, respectively) and a borderline significant indicator of shorter disease-free survival (p = 0.055). The VEGF – 2578C allele showed protective effect on local recurrence in univariate analysis (p = 0.032). The final model revealed the VEGF –634CC genotype to be an independent negative prognostic factor for overall survival (HR 2.36, p = 0.009). These results highlight the importance of host genetics in modulation of response to conventional anticancer treatments and patient survival.

Key words: laryngeal cancer, polymorphism, angiogenesis, therapy outcome, radiotherapy.

This work was supported by the National Science Centre (NCN), Poland, grant no. 2016/23/B/NZ5/03470 to D.B.

Poster

KW018-00009-2018-01

Variation in DNA damage response and repair genes predicts survival in non-small cell lung cancer patients

Agnieszka Gdowicz-Kłosok[1], Małgorzata Krześniak[1], Monika Giglok[2], Małgorzata Marszałek-Zeńczak[3], Iwona Matuszczyk[1], Rafał Suwiński[2], Dorota Butkiewicz[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Poland
[2]II Clinic of Radiotherapy and Chemotherapy, Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Poland
[3]Department of Molecular and Systems Biology, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Radiotherapy (RT) and platinum-based chemotherapy (CT) remain major components of standard care for inoperable and advanced non-small cell lung cancer (NSCLC). Due to the mechanism of action, the efficacy of these treatments may be influenced by individual DNA repair capacity modulated by polymorphisms in DNA damage response genes. DNA damage results in p53 activation leading to cell cycle arrest, DNA repair or apoptosis. The most serious form of damage caused by cytotoxic therapy are DNA double-strand breaks (DSBs) repaired via NHEJ and HR pathways, while single-strand breaks (SSBs) and base damage are removed by BER. NBS1, a member of MRN protein complex, plays a central role in DSB repair by HR and NHEJ. DNA-PKcs is required for NHEJ. EGFR, through interaction with DNA-PKcs, may modulate repair of DNA lesions induced by IR and platinum drugs. Its overexpression is associated with disease aggressiveness and therapy resistance in solid tumors, including NSCLC. PARP1 belongs to the family of proteins being molecular sensors of DSBs and SSBs. PARP1 and APE1 consist important BER pathway elements. One may presume that genetically determined differences in levels and activity of these proteins may influence individual sensitivity to RT and platinum-based CT, that translates into clinical outcome in NSCLC patients.

This study aimed to investigate the association between selected functional or possibly functional polymorphisms in DNA damage response and repair genes and survival in 437 patients with inoperable NSCLC subjected to radiotherapy or platinum-based chemoradiotherapy. The EGFR -191A allele demonstrated protective effect on OS in the whole group ($p = 0.026$ in uni- and 0.033 in multivariate models). The APE1 148GG genotype was significant indicator of poor PFS ($p = 0.031$ in uni- and 0.004 in multivariate analysis). Moreover, EGFR -191A was identified as an independent predictor for better OS, while TP53 72C allele and APE1 148GG genotype were independent risk factors for reduced OS and PFS, respectively. In curative chemoradiotherapy subgroup, EGFR -191A, TP53 72C and NBS1 185C alleles were independent prognostic factors affecting OS. These results emphasize the contribution of common polymorphisms in key genes involved in DNA damage response and repair pathways to clinical outcome in NSCLC treated with DNA-damage inducing anticancer therapy.

Key words: lung cancer, radiotherapy, chemoterapy, gene polymorphism, DNA repair, prognostic and predictive markers.

This work was supported by the National Science Centre (NCN), Poland, grant no. 2012/05/B/NZ5/01905 to D.B.

Poster

KW018-00008-2018-01

Promoter methylation status of circadian genes according to clinical characteristics of breast cancer patients

Monika Lesicka[1], Ewa Jabłońska[1], Edyta Wieczorek[1], Leszek Kalinowski[2,3], Jarosław Skokowski[2,3], Barbara Seroczyńska[2,3], Wojciech Wąsowicz[1], Edyta Reszka[1]

[1]Nofer Institute of Occupational Medicine in Lodz, Poland
[2]Medical University of Gdansk, Poland
[3]Biobanking and Biomolecular Resources Research Infrastructure, Gdansk, Poland

Introduction: Circadian rhythms exist in almost all living organisms and are influenced primarily by exposure to light and darkness. Circadian rhythm allows adjusting numerous internal processes to external environmental factors through manage processes in each cell like genomic stability, DNA repair mechanism, apoptosis. In various types of neoplasms, including breast tumors, these cell functions are frequently disrupted. Thus, it is implied, that one of the molecular causes of the breast cancer is circadian rhythm disorders caused by exposure to light at night (LAN), including an alteration of circadian genes and their epigenetic pattern. Accordingly, the main objective of the study was to search for significant effects of crucial circadian genes in the process of the carcinogenesis in the mammary gland by examining methylation profile of promoter regions of circadian genes in breast cancer.

Methods: Total DNA was isolated from 107 tumor tissues and tumor-adjacent normal tissues in order to determine promoter methylation profile of CLOCK, BMAL1, TIMELESS, PERIOD (PER1, 2, 3) and CRYPTOCHROME (CRY1, 2), according to clinical features of patients. Hypermethylation profiles were evaluated by using sodium bisulfite modification of DNA and quantitative Methylation-Specific PCR analysis.

Results: We observed significantly lower CLOCK, BMAL1, CRY2 promoter CpG methylation (hypomethylation) in tumor tissues of breast cancer patients in comparison to non-tumor tissues ($p < 0.00001$ for all mentioned genes). Hypermethylation was noted for PER1, PER2, PER3 ($p < 0.01$ for all mentioned genes).Molecular subtypes of breast cancer also were associated with an altered epigenetic pattern of PER1 PER2 promoter region with an increased methylation status for triplet negative breast cancer and HER2-over expressed cancer, respectively. We found also associations with an altered epigenetic pattern of PERs genes with histological type of cancer, tumor grading as well as menopausal status and BMI.

Conclusions: Clock genes play a crucial role in many physiological processes like genomic stability, DNA repair mechanism, apoptosis, which are frequently disrupted in breast tumors. Circadian genes disruptions may play an important role (individually or as a result of interactions) in the breast cancer etiology and disease process, including recurrence and progression.

Key words: circadian genes, DNA methylation, circadian rhythm.

This work was supported by the National Science Center (Grant No. 2014/15/N/NZ5/01671) and internal grant IMP 14.4.

Poster

KW018-00007-2018-01

Radiobiological response of human PNT1A cell line to scattered beam outside irradiation field

Wiktoria Suchorska[1,2], Igor Piotrowski[1,2], Natalia Matuszak[1,2], Marta Kruszyna-Mochalska[3], Agnieszka Skrobała[2,3], Katarzyna Kulcenty[1], Julian Malicki[2,3]

[1]Radiobiology Lab, Greater Poland Cancer Centre, Poznań, Poland
[2]Department of Electoradiology, Poznań University of Medical Sciences, Poznań, Poland
[3]Department of Medical Physics, Greater Poland Cancer Centre, Poznań, Poland

Introduction of new techniques to radiotherapy is driven by a need of elevating radiation dose to tumor and to deliver this dose more accurately. The elevation of dose in a target may cause higher distribution of dose in a larger part of body. Absorption of dose in the places that lay outside the beam is caused by scattered radiation. The spectrum and radiobiological response of this scatter radiation remains unknown. The aim of this study was to experimentally determine the in vitro radiobiological effects of scattered radiation in cell located outside the primary bean in a humanoid phantom in a locations corresponding to kidney, lung and thyroid.

Material and methods: A quasi-humanoid phantom which simulates patients' body was designed. It allows to measure the biological response in the radiation field and beyond by designing a special system for locating containers with biological material. Cells from human normal prostate cells were inserted in this phantom in the locations corresponding to AXIS, kidney, lung and thyroid. Cells were irradiated in a fraction dose of 7,25 Gy x 5f = 36,25 Gy. The DNA double strand-breaks (DNA DSBs), apoptosis, DNA repair mechanisms and surviving fraction (SF) were determined.

Results: Out-of-field irradiation of cells located in kidney resulted in slightly increased number of double strand breaks measured by present of γH2AX positive cells after first and second fraction. At this time points an activation of DNA repair mechanisms and decreased proliferation of cells. was also observed. Moreover after first fraction cells located out-of-field exhibited lower SF values comparing to control cells which increased after second fraction to level above control cells. This increased sensitivity of cells to low doses may be explained by low-dose hyper-sensitivity.

Conclusions: Measuring cell response to radiation provides valuable data that can be used to verify dose calculations/measurements made by conventional techniques, particularly in out-of-field regions where measurements are impossible or likely to be inaccurate. Moreover, this technique can be used to accurately assess the effect of irradiation on organs located inside and outside of the primary beam path. These findings suggest that the out-of-field radiation dose from a conventional SBRT dose fractionation scheme is likely to induce a notable radiobiological response in normal prostate PNT1A cells positioned outside the primary beam.

Key words: radiotherapy, low doses, out-of-field.

Poster

KW018-00006-2018-01

Chondrogenic differentiation in vitro leads to induction of DNA damage response signaling pathways in hiPSCs

Ewelina Stelcer[1,2,3], Katarzyna Kulcenty[1,3], Marcin Rucinski[4], Karol Jopek[4], Magdalena Richter[5], Tomasz Trzeciak[5], Wiktoria M. Suchorska[1,3]

[1]Radiobiology Laboratory, Greater Poland Cancer Centre, Poznan, Poland
[2]The Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland
[3]Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[5]Department of Orthopedics and Traumatology, Poznan University of Medical Sciences, Poznan, Poland

Introduction: We investigated the mechanisms of DNA damage response (DDR) activated in chondrocyte-like cells differentiated from human induced pluripotent stem cells (ChiPS) after ionizing radiation (IR) treatment. Importantly, irradiated ChiPS reveal the extremely high level of DNA repair mechanisms. Such high level of DDR mechanisms is considerably associated with the forced chondrogenic differentiation in vitro, that constitutes a meaningful stress for cells. The aims of the study were: a) to investigate gene expression profile of the obtained ChiPS, b) to compare expression of genes involved in DDR process between ChiPS with hiPSCs and mature chondrocytes c) to evaluate the level of activated stress in hiPSCs undergoing differentiation in vitro.

Material and methods: Chondrogenic differentiation of hiPSCs (Suchorska *et al*., 2017) was conducted. Global gene expression microarray was performed and analyzed using GeneAtlasTMWT Expression Kit Assay and AffymetrixGeneAtlasTM Operating Software. The Bioconductor and statistical programming language R were used. 2. The validation of microarrays was performed by RT-qPCR.

Results: The cut-off criteria were based on differences in the gene expression fold change higher than abs. 2 and adjusted p value ≤ 0.05. 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 assume that a noticeable activation of DDR is particularly enhanced during treatment with genotoxic agents like IR.

Conclusions: We found that hiPSCs differentiated toward ChiPS undergo a stress that leads to activation of DDR mechanisms. The differentiated cells are very prone to exposure to genotoxic agents. Thus, they demonstrate extremely high level of members taking part in DNA damage and repair during IR treatment.

Key words: human induced pluripotent stem cells, chondrogenic differentiation, DNA damage response, p53.

Poster

KW018-00005-2018-01

The effect of protein-bound polysaccharides derived from Coriolus versicolor Chinese fungus on SkMel 188 melanoma cells viability

Małgorzata Pawlikowska[1], Jakub Piotrowski[1], Wojciech Jóźwicki[2], Wiesław Kozak[1], Anna A. Brożyna[2]

[1]Department of Immunology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Torun, Poland
[2]Department of Tumor Pathology and Pathomorphology, Oncology Centre – Prof. Franciszek Łukaszczyk Memorial Hospital, Nicolaus Copernicus University Collegium Medicum in Bydgoszcz, Bydgoszcz, Poland

The protein-bound polysaccharides (PBP), isolated from Coriolus versicolor (CV) fungus, are considered as natural compounds with potential therapeutic, mostly anticancer applications. CV-derived products are investigated as potentially adjunctive therapy to standard treatment regimens for various malignancies. However, despite the fact that PBP are widely used as an immunomodifiers, the molecular mechanisms of action of these agents is still poorly understood. Here, we have investigated the PBP effect on the viability of human melanoma SkMel-188 cells with inducible melanogenesis. The results of MTT test have shown that PBP decreased viability of SkMel-188 cells in a time-dependent manner. Significant cytotoxic effect in non-pigmented cells was observed already after 24 hours, and then it was potentiated after 48- and 72 hours, whereas pigmented cells death was observed not sooner than 48- and 72-hours post-treatment with PBP. This cytotoxic effect of the PBP was accompanied by a general decrease of ROS levels (DCFH-DA assayed) in both, melanotic and amelanotic SkMel-188 cells. Interestingly however, we have noticed that untreated cells differ in their intracellular ROS levels. The pigmented cells exhibited almost four times higher level of ROS compared to that of amelanotic ones. One can suggest that elevated ROS level in melanoma cells is responsible for a drug resistance. Therefore, we hypothesize that PBP decreasing the intracellular production of ROS may sensitize melanoma cells to targeted therapy.

Dysregulation of Bcl-2 proteins appears of critical importance for melanoma cell survival and drug resistance. We present that the expression of antiapoptotic Bcl2 protein is increasing with the melanin content in melanoma cells (untreated with PBP). The Bcl2 expression is the lowest in non-pigmented cells. Our study revealed however, that PBP extract has rather minor effect on Bcl2 protein expression in SkMel-188 melanoma cells. Despite initial decrease of Bcl2 level after 24 hours, there was no further change in the expression after 48 hours of stimulation with PBP in both, amelanotic and pigmented cells, when compared to control, untreated cells. These data suggest that the cell death of melanoma cells induced by the PBP extract may be mediated by Bcl-2-independent pathway. Concluding, PBP is promising therapeutic agent for melanoma treatment, however, additional studies are needed to further characterize the mechanisms of its action on melanoma cells.

Key words: protein-bound polysaccharides (PBP), Coriolus versicolor, human melanoma, pigmentation.

Poster

KW018-00004-2018-01

Quick detection of selectin peaks' differences in Raman spectra of lung cancer cases and healthy controls

Edyta Wolny-Rokicka[1,2], Andrzej Tukiendorf[3], Jerzy Wydmański[4], Agnieszka Zembroń-Łacny[1]

[1]University of Zielona Góra, Faculty of Medicine and Health Sciences, Zielona Góra, Poland
[2]Provincial Multidisciplinary Hospital, Gorzow Wielkopolski, Poland
[3]Department of Biostatistics, Center of Oncology – Maria Sklodowska-Curie Memorial Institute, Branch in Gliwice, Gliwice, Poland
[4]Department of Radiotherapy, Center of Oncology – Maria Sklodowska-Curie Memorial Institute, Branch in Gliwice, Gliwice, Poland

The main goal of study was to detect the intensification of P-selectin in patients with lung cancer before and after surgical procedures and palliative patients compared to healthy volunteer. MATERIAL AND METHOD This prospective study (ClinicalTrials.gov Identifier: NCT02758678; Ethics Committee no. 2/57/2015) was conducted in the Regional Clinical Hospital in Zielona Góra between November 2015 and April 2016. The result were compared between two groups. Group 1: 36 patients who had undergone surgical staging procedures of whom 22 patients had confirmed carcinoma lung cancer and remaining 12 had non-malignant tumor. Blood samples taken just before the procedure were compared to blood samples taken 3 months after. Group 2: 10 palliative patients with disseminated disease were compared with 17 healthy volunteer. Prior to the medical procedure (surgery and radiotherapy) all patients had their condition histologically or cytologically confirmed. Patients with disease without previous chemo/radiotherapy history were included in the study. A set of P-selectin was purchased from Randox Laboratories ltd (UK). The blood samples were analyzed in Raman Spectroscopy methods. STATISTICAL ANALYSES The statistical analysis of registered Raman spectra was conducted [1]. RESULTS Following MALDI computational procedures were identified for 2 groups five probable peaks differentiating the analyzed two sets of samples: before intervention vs. after intervention and palliatives vs. healthy – 853/855; 1005/1007; 1208/1210; 1320/1320; 1449/1449. Based on the very similar peaks estimated in both the spectra analyses, a comparable effect of intervention in cancer cases to a healthy status of patients can be established. However, the estimated diagnostics statistics are not satisfactory because of diagnostics measure: before intervention vs. after intervention – accuracy (0.50); sensitivity (0.57); specificity (0.44) and palliatives vs. health – accuracy (0.66); sensitivity (0.30); specificity (0.88). CONCLUSION It can be seen that the better classification of patients can be done using palliatives vs. healthy spectra, but probably due to a limited number of samples gathered the reported results are not statistically significant in so far and a larger number of samples are required for the future verification.

Key words: P-selectin, lung cancer, Raman spectroscopy, Prognostic factors.

References

1. Gibb S, Strimmer K. Differential protein expression and peak selection in mass spectrometry data by binary discriminant analysis. Bioinformatics 2015; 31: 3156–3162.

Poster

KW018-00003-2018-01

Methoxylated stilbenes – analogs of resveratrol inhibit Wnt/β-catenin signaling, inducing cell cycle arrest and apoptosis in human T98G glioblastoma cells

Aleksandra Majchrzak-Celińska[1], Małgorzata Zielińska-Przyjemska[1], Marcin Wierzchowski[2], Robert Kleszcz[1], Mariusz Kaczmarek[3], Violetta Krajka-Kuźniak[1]

[1]Department of Pharmaceutical Biochemistry; Poznan University of Medical Sciences, Poznań, Poland
[2]Department of Chemical Technology of Drugs; Poznan University of Medical Sciences, Poznań, Poland
[3]Department of Clinical Immunology; Poznan University of Medical Sciences, Poznań, Poland

Introduction: Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a natural polyphenol with plethora of biological activities, including chemopreventive and anticancer effects. However, due to its poor bioavailability and rapid metabolism, its analogs, showing enhanced activity as well as improved pharmacokinetic parameters are the subjects of intensive research and development. Finding such compounds is crucial especially for the most deadly brain tumor – glioblastoma (GBM).

The aim of this study was to evaluate the effect of five new synthetic methoxylated stilbenes (MS): 3,4,4'-tri-MS, 3,4,2'-tri-MS, 3,4,2',4'-tetra-MS, 3,4,2',6'-tetra-MS and 3,4,2',4',6'-penta-MS as compared to resveratrol, on cell viability, the Wnt/β-catenin signaling, apoptosis and cell cycle distribution in human T98G GBM cell line.

Materials and methods: T98G cell line was purchased from ECACC and was grown in standard conditions. The effect of resveratrol and its analogues on cell viability was assessed with the MTT assay, after the treatment of cells with the tested compounds in doses ranging from 1-200 μM. Next, using qPCR we investigated whether the chemicals are capable to inhibit Wnt/β-catenin signaling by modulating the expression of genes regulated transcriptionally by β-catenin: Axin2, c-MYC, CCND1, BIRC5, and NEDD9. Finally, the effect of the tested compounds on cell cycle distribution and apoptosis was tested using FACS and FITC Annexin V/propidium iodide double staining assay, respectively. The p53, Bax, and Bcl-xL level was assessed using Western blot.

Results: All the compounds dose-dependently reduced the viability of T98G cells. 3,4,2',4',6'-penta-MS, 3,4,2',4'-tetra-MS and 3,4,4'-tri-MS displayed an inhibition of viability higher than that of resveratrol. All the three above mentioned compounds downregulated the transcription of β-catenin, and the effect was stronger as compared to resveratrol. The most potent Wnt/β-catenin signaling inhibitor was 3,4,4'-tri-MS. All of the tested compounds affected cell cycle distribution and induced apoptosis, while 3,4,2',4'-tetra-MS and 3,4,4'-tri-MS were the most potent agents, leading to cell cycle arrest in S phase and apoptosis.

Conclusions: These results indicate that 3,4,4'-tri-MS and 3,4,2',4'-tetra-MS are even more effective inhibitors of Wnt/β-catenin signaling in GBM cell, than resveratrol, leading to cell cycle arrest and apoptosis and might potentially be considered as adjuvants in GBM therapy.

Key words: resveratrol, methoxylated stilbenes, GBM, Wnt/β-catenin signaling, apoptosis, cell cycle.

This project was funded by National Science Centre, Poland, grant no. 2017/01/X/NZ7/00673.

Poster

KW018-00001-2018-01

Genetic and epigenetic biomarkers of circadian rhythm disruption in breast cancer

Edyta Reszka, Monika Lesicka

Nofer Institute of Occupational Medicine, Lodz, Poland

Cyclic changes of various processes in metabolism, physiology and behavioral activity are one of the features that characterize living organisms. This intrinsic mechanism plays a crucial role in adaptation to variety of environmental stimuli. Exploring the putative impact of circadian rhythms is a relatively novel approach in the etiology hormone-related breast cancer. Indeed, a paucity of human studies indicates a key role of circadian genes in breast tumor suppression. One of several proposed mechanisms underlying breast cancer risk especially among individuals exposed to LAN involves genetic and epigenetic alteration and deregulation of circadian clock. The major findings from human studies indicate that expression of circadian genes is deregulated in breast cancer. Breast cancer etiology and prognosis-associated PERs, CRYs, CLOCK down-regulation and TIMELESS up-regulation may be related to relevant gene hypermethylation in tumor tissue. Moreover, apart from transcriptional and epigenetic alteration of circadian genes, their polymorphism should be also studied to recognize specific susceptible gene variants among around 20 candidate circadian genes that may be linked with breast cancer etiology. Recent systemic reviews and meta-analysis of 15 molecular epidemiology studies support the hypothesis that circadian BMAL1, BMAL2, CLOCK, NPAS2, CRY1, CRY2, PER1, PER3, RORA, RORB, RORC and TIMELESS gene variants might affect breast cancer risk.

It could be assumed that also diverse clock-controlled genes which can be regarded as tumor suppressor genes may play important role in breast cancer etiology. Therefore, a dual gene- and pathway-based approach appears to offer the greatest advantages in investigating the overall effect of clock-related genes on breast cancer development and disease prognosis. In order to advance the state of the art, it is important to conduct comprehensive and high-throughput studies on multi-pathway SNPs, expression and methylation of core clock and clock-controlled genes, regulatory miRNA expression in relation to clinicopathological features of breast cancer patients.

Key words: breast cancer, circadian genes, gene expression, gene methylation, gene variants, shift work.

This work was supported by Nofer Institute of Occupational Medicine Internal Grant IMP14.4 and the National Science Centre Grant 2014/15/N/NZ5/01671.

Poster

KW018

STARTRK-2 Basket trial for *TRK, ROS1* and *ALK* fusions in cancer patients treated in Cancer Center and Institute of Oncology, Warsaw

Andrzej Tysarowski[1,2], Michał Wagrodzki[1], Katarzyna A. Seliga[2], Anna Szumera-Ciećkiewicz[1], Steven J. Potts[3], Jason H. Christiansen[3], Marzenna Kotarska-Puerto[3], Janusz A. Siedlecki[2], Monika Prochorec-Sobieszek[1]

[1]Department of Pathology and Laboratory Medicine, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
[2]Molecular And Translational Oncology, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
[3]Ignyta, Inc., San Diego, CA, USA

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 are typically rare in the cancer population (< 3%); however, they have been seen in over 40 histologies, including gastrointestinal, lung, head & neck, and sarcoma. Diagnostic testing to identify these gene rearrangements is not yet part of standard clinical practice, due efficiency and cost challenges.

In this presentation, we report on the occurrence of *TRK, ROS1, and ALK* fusions in patients treated in Cancer Center and Institute of Oncology in Warsaw.

The occurrence of *TRK*, *ROS1*, and *ALK* gene fusions in solid tumors was studied in FFPE specimens from 645 patients. In order to identify these fusions and select the patients eligible for the Phase 2 STARTRK-2 trial we used 2-step diagnostic test. The test comprised of IHC screening using a pan-receptor tyrosine kinase cocktail of antibodies targeting those proteins followed by an RNA-based anchored multiplex-PCR next generation sequencing (NGS) assay performed in IHC positive specimens.

221 out of 645 clinical specimens screened by IHC were positive and further analyzed by NGS. The presence of gene fusions was confirmed in 17 of them.

It is of critical importance to develop effective diagnostic testing algorithms that include identifying appropriate patients for targeted therapy.

This the two-step testing approach proved to be an effective strategy to identify patient populations with low prevalence of molecular alterations and can be included into standard clinical practice.

Poster

KW018-00061-2018-01

Hippo kinase LATS1 is involved in epithelial-mezenchymal transition in melanoma

Urszula Kazimierczak[1], Ewelina Dondajewska[1], Maria Zajączkowska[1], Andrzej Mackiewicz[1,2]

[1]Poznan University of Medical Sciences, Poland
[2]Greater Poland Cancr Centre, Poznan, Poland

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. One of the key proteins of Hippo signaling is LATS1 kinase. The role of LATS1 in melanoma remains unknown. The aim of the study was to investigate the influence of LATS1 on tumor growth and epithelial-mezenchymal transition (EMT). Using a xenograft model of human metastatic melanoma we demonstrated that the level of LATS1 expression determines the tumor growth kinetics as well as tumor weight, and is highly connected with the expression of epithelial and mesenchymal markers within a tumor mass. Epithelial-mesenchymal transition (EMT) enables epithelial cells to acquire motility and invasiveness that are characteristic of mesenchymal cells. It plays an important role in development and tumor cell metastasis. Our results indicated a new role of LATS1 in this process. Further analysis of Hippo pathway may provide a better understanding of the mechanisms of melanoma pathogenesis and will help to find the new therapeutic targets for more effective treatment and diagnosis.

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

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

Poster

KW018-00060-2018-01

Comparison of the proteome of exosomes released by HPV(+) or HPV(–) head and neck cancer cells

Monika Pietrowska[1], Łukasz Marczak[2], Agata Abramowicz[1], Marta Gawin[1], Sonja Funk[3,4], Priyanka Sharma[4], Piotr Widłak[1], Theresa L. Whiteside[4]

[1]Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland
[2]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland
[3]Department of Otorhinolaryngology, Otorhinolaryngology, University of Duisburg-Essen, Germany
[4]Department of Pathology, University of Pittsburgh School of Medicine and University of Pittsburgh Cancer Institute, Pittsburgh, PA 15213, USA

Infection with human papilloma virus (HPV) is an important etiological/pathological factor in of head and neck squamous cell carcinoma (HNSCC). Two categories of HNSCC can be distinguished in terms of HPV status: HPV(+) and HPV(–) HNSCCs differ from each other in respect to their biology and response to therapy. HPV+ cancers are associated with favorable responses and have better prognosis. Exosomes are virus-size vesicles which are produced by all living cells. Exosomes mediate intercellular communication. Their protein profiles resemble those of parental cells. Exosomes interact with and reprogram functions of human immune cells. The aim of this study was to examine protein profiles of tumor cell-derived exosomes by mass spectrometry for the presence of proteins which could interact with immune cells and modulate their functions.

We studied the protein profiles of exosomes released by cells of three HNSCC HPV(+) cell lines: SCC-2, SCC-47, SCC-90 and two HNSCC HPV(–) cell lines: PCI-13 and PCI-30. Exosomes were isolated from tumor cell supernatants by min-size exclusion chromatography (mini-SEC). The isolated exosomes were assessed for: (i) morphology and size by transmission electron microscopy (TEM); (ii) number of vesicles by q-Nano; and (iii) the protein content. Molecular profiles were determined using Western blots (WB) and the high-resolution tandem mass spectrometry (LC-MS/MS) technique. The results were confirmed using the on-bead flow cytometry technique.

Exosomes originating from HPV(+) and HPV(–) cancer cells had the same size (30-150 nm) and morphology. However, only HPV(+) exosomes contained the following proteins: E6/E7, Rb and survivin, while HPV(–) exosomes were negative for cyclin D1 and had low levesl of p53. Application of high-resolution mass spectrometry enabled the detection of CD47 and CD276 receptor proteins detected only in exosomes originating from HPV(+) cells. As both these proteins play key roles in exosome interactions with immune cells, the data suggest that HPV(+) cancers modulate the host immune system differently than HPV(–) cancers.

Key words: proteome of exosomes, high-resolution tandem mass spectrometry, human papilloma virus (HPV), head and neck squamous cell carcinoma (HNSCC).

The research was financed in part by the Polish National Science Centre project no. 2013/11/B/NZ7/01512 and 2016/22/M/NZ5/00667.