contemporary oncology

współczesna **onkologia**

Indexed in: ISI Science Citation Index Expanded (Sci-Search),
PubMed Central/PubMed, EMBASE, Chemical Abstracts CAS, Scopus,
Index Copernicus (IC), MNiSW, Directory of Open Access Journals (DOAJ),
OpenMed Database and Polish Medical Library (GBL).

TERMEDIA Publishing House

contemporary oncology

współczesna **onkologia**

Journal published under the auspices of the Association for Fight Against Cancer "Genes for Life" (Poznan) and the Foundation of Experimental and Clinical Oncology (Warsaw)

www.termedia.pl
www.onkologia.termedia.pl

Circulation of 2,500 copies

Publisher
Termedia sp. z o.o. Wydawnictwo Medyczne
e-mail: termedia@termedia.pl

Warsaw office
tel./fax +48 22 827 75 14

President of the Management Board of the Termedia Publishing House
Janusz Michalak

Scientific Director of the Termedia Publishing House
Maciej Banach

Production Editor
Marzena Demska
e-mail: m.demska@termedia.pl

Editorial Office
Termedia sp. z o.o.
Kleeberga 2, 61-615 Poznań
tel./fax +48 61 822 77 81, ext. 604
e-mail: contemporaryoncology@termedia.pl

Marketing and Advertising
Małgorzata Prętka
tel./fax +48 61 822 77 81 ext. 507
e-mail: m.pretka@termedia.pl

Distribution and Subscriptions
Iwona Kempa
tel./fax +48 61 656 22 00
e-mail: prenumerata@termedia.pl

contemporary oncology

współczesna **onkologia**

Volume 19, Supplement 1, March 2016

8th International Conference of Contemporary Oncology

Integrated Oncology: Genomics, Immunotherapies, and Molecular Targets

Poznań, 16–18 March 2016

Abstracts

Session 1. Cancer genomics and the TCGA project

Chairs: Jan Lubinski, Maciej Wiznerowicz

The Curse of Big Data. About problems and solutions related to exploration of The Cancer Genome Atlas Data

Przemysław Biecek

Warsaw University of Technology, Warsaw, Poland

The Cancer Genome Atlas Data serves as a rich source of valuable information about genetic profiles of cancer patients. It's a multi platform dataset assembled from multi center study that accumulates information about thousands of patients. Data related to genes expression, exons expression, proteins expression, methylation profiles, genes mutations and various other markers allow to examine very detailed and sophisticated hypotheses.

But, as in most cases, big data means very noisy data. The scale of data and level of noise brings many challenges when it comes to reproducibility of more complex statistical analyses.

During the talk we will point out four issues that should be taken into account in statistical analyses of the TCGA data. We are using this study as an example, but such problems occur also in other multi center, multi platform studies.

- Simple univariate exploration of significant biomarkers may be biased due to problems with lack of homogeneity of the population. We will show computational studies that present how this lack of homogeneity may lead to false positive signals. We will also present stratified models that are more robust.
- Consecutive releases bring another layer of complexity when it comes to correction of the error rate due to number of tested hypotheses. Relations that are significant based on one release of the data may not be significant in the next release. We will show empirical results that present how serious is the problem.
- Large number of potential biomarkers strongly increases the risk of false positives. The strategy of choosing top X significant biomarkers may lead to very strange results. In such settings, a very extensive validation of candidate results, both statistical and graphical, is need. We will present web-based applications that help to validate of TCGA results.
- For large high dimensional data the reproducibility of results is an issue itself. It's quite common to generate a large collection of partial results for different versions of data and different settings. We will show how to use the archivist package for R to manage results and ensure their reproducibility.

Collateral lethality: an emerging therapeutic avenue in oncology

Yu-Hsi Lin, Nikunj Satani, Naima Hammoudi, Pingna Deng, Colla Simona, Chang-Jiun Wu, Alan Wang, Ronald DePinho, Florian Muller

The University of Texas MD Anderson Cancer Center

Genomic deletions are an early and critical driver event in diverse cancer types. Since the first description of chromosomal band deletions over 40 years ago, extensive genomic studies have focused on identifying specific tumor suppressor genes (TSG) targeted by genomic deletions. The pace of knowledge has dramatically accelerated with the advent of economically feasible mass-genetic characterization of tumors and has made the prospect to true genetically personalized cancer therapies a realistic possibility. However, relatively little attention has been paid to the fact that besides TSG "targeted" by genomic deletions, a very large number of non-TSG, playing no role in tumor promotion are often "collaterally" deleted as a result of chromosomal proximity to the "intended" target. This is especially true in the case of large heterozygous scale chromosomal deletions (loss-of-heterozygosity – LOH) but also with homozygous deletions. In the present poster, we discuss the therapeutic possibility offered by collateral genomic deletions for personalized medicine and present proof-of-concept data, both *in vitro* and *in vivo*, for multiple different deleted genes as pharmacologically targetable vulnerabilities.

Key words: glioblastoma, collateral lethality.

Session 2. Oncogenic processes

Chairs: Maciej Żylicz, Cezary Szczylik, Laurence Albiges

Lung and breast cancer patients with mutated TP53 and simultaneous elevation of MDM2 develop more efficiently resistance to chemotherapy

Zuzanna Tracz-Gaszewska[1,2], Bartosz Wawrzynow[2], Marcin Herok[1,3], Milena Wiech[1], Przemyslaw Biecek[4,5], Marcin Kosinski[4,5], Patrycja Czerwińska[1,6], Marta Klimczak[1,6], Maciej Wiznerowicz[6], Maciej Zylicz[1], Alicja Zylicz[1]

[1]International Institute of Molecular and Cell Biology, Warsaw, Poland
[2]Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland
[3]Nencki Institute of Experimental Biology, PAS, Warsaw, Poland
[4]Faculty of Mathematics, Informatics, and Mechanics, University of Warsaw, Warsaw, Poland
[5]Faculty of Mathematics and Information Science, Warsaw University of Technology, Warsaw, Poland
[6]Laboratory of Gene Therapy, Department of Cancer Immunology, the Greater Poland Cancer Center, Poznan, Poland

The roles of mutant p53 protein and increased expression of MDM2 in chemoresistance of cancer cells still remain elusive. Utilizing The Cancer Genome Atlas (TCGA) data sets we show that lung and breast cancer patients with mutated TP53 and simultaneous elevation of MDM2 exhibit statistically decreased survival post treatment. Thus, we hypothesize that in this genetic background, patients develop resistance to chemotherapy more efficiently. In search of a molecular mechanism of how the accumulation of these two key proteins could stimulate the acquisition of chemoresistance, we show that heat shock proteins, apart from stabilizing mut p53, also stabilize mut p53-TAp73α interaction in cancer cells. These events result in a decrease of TAp73α mediated apoptosis. In addition, we show that elevated levels of MDM2 displace molecular chaperones in the mut p53-TAp73α complex, leading to the formation of a three-body complex containing structural mut p53, TAp73α and MDM2, which further augments cancer cell chemoresistance. In view of these results, it is tempting to speculate that the group of cancers, which possess structural p53 mutants and overexpress MDM2 (breast, lung and probably ovarian and head and neck cancers), will be more resistant to classical chemotherapy. The treatment regime for those patients should be supplemented with drug(s), which would release TAp73α from the p53 R175H-TAp73α-MDM2 complex.

Session 3. Cancer microenvironment and immune response

Chairs: Jason Fleming, Bozena Kaminska-Kaczmarek

Tumor heterogeneity and clinical implications

Bozena Kaminska-Kaczmarek

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Tumor heterogeneity can be defined as the presence of subclones of cells, within a given tumor, with different genetic aberrations or features that mediate divergent biology. Within a given tumor several levels of heterogeneity could be distinguished: 1) genetic heterogeneity due to the presence of distinct clones harboring common and unique mutations; 2) cellular heterogeneity due to the occurrence of cancer stem cells; 3) immune cell diversity reflecting differences in host antitumor responses. If tumor subclones exist independently of each other, their elimination is profoundly more difficult and the hope would be to identify "founder mutations" or vulnerable cell subpopulations. Cancer stem cells specifically affect antitumor responses targeting of both cellular (tumor associated macrophages and myeloid derived suppressor cells, MDSC) and sub-cellular (cytokines, chemokines, and PD1/PDL1) components of the tumor microenvironment.

Malignant gliomas attract brain resident microglia and peripheral macrophages, and re-program these cells into pro-invasive, immunosuppressive cells. It results in formation of the tumor supportive microenvironment and evasion of antitumor responses. Gene expression profiling in microglia/macrophages immunosorted from human low and high grade gliomas indicated their switch to the immunosuppressive, proinvasive phenotype. Computational analysis of gene expression networks in gliomas revealed dysfunction of IKKβ-NFκB signaling pathways and generalized immunosuppression in high grade gliomas. Downregulation of IKKβ expression in tumor infiltrating immune cells and deficits in NFκB activation were confirmed by biochemical and immunocytochemical studies of GBM tissues and in animal models. However, signals responsible for polarization of immune cells into protumorigenic phenotype in GBM are poorly known, we found that glioma stem cells are more potent in activation of microglia re-programming into anti-inflammatory, immune cells. Differentiation of those cells reduces their potential to evade immune responses. Cancer stem cells are an attractive target for differentiation and immune therapies because, unlike chemotherapy or radiotherapy, immune effector cells do not specifically require target cells to be proliferating in order to effectively kill them.

Key words: tumor heteregeneity, immune subpopulations, immunosuppression, glioma stem cells, glioblastoma.

Studies were supported by a grant 2012/04/A/NZ3/00630 from the National Science Center.

Immunosuppressive pathways in tumor microenvironment

Viktor Umansky, Carolin Balttner, Christoffer Gebhardt, Jochen Utikal

Skin Cancer Unit, German Cancer Research Center (DKFZ), Heidelberg and Department of Dermatology, Venereology and Allergology, University Medical Center Mannheim, Ruprecht-Karl University of Heidelberg, Mannheim, Germany

Melanoma is known for its fast progression and poor response to current immunotherapies. Insufficient anti-tumor reactivity could be due to chronic inflammation, inducing a profound immunosuppression. Using the *ret* transgenic murine melanoma model that mimics human melanoma, we found in skin tumors and metastatic lymph nodes increased levels of chronic inflammatory factors (IL-1β, IL-6, IFN-γ, TNF-α etc.) associated with the accumulation of Gr1+CD11b+ myeloid-derived suppressor cells (MDSC) that inhibit tumor-infiltrating antigen-specific T cells. The accumulation of MDSC in melanoma lesions was associated with the upregulation of CCR5 expression on these cells. Chemokines CCL3, CCL4 and CCL5 (CCR5 ligands) were significantly elevated in melanoma lesions as compared to their values in serum of tumor-bearing mice. Importantly, CCR5+ MDSC displayed a significantly higher immunosuppressive activity than their CCR5 negative counterparts. MDSC recruitment and activation could be induced also by tumor-derived exosomes. Furthermore, tumor-infiltrating T cells expressed not only markers of activated or memory cells but also ectonucleotidases CD39 and CD73 that can be induced by tumor-derived soluble factors (such as TGF-β). This suggests that adenosine producing by effector T cells can inhibit their anti-tumor reactivity via autocrine signaling as a part of the negative feedback loop.

In melanoma patients with melanoma, we found that the level of serum inflammatory factors and the frequency of circulating monocytic MDSC were strongly increased in advanced melanoma patients that significantly correlated with a decreased progression free survival. Moreover, decreased MDSC frequency and immunosuppressive pattern correlated with the response of melanoma patients to therapy with negative check point inhibitor ipilimumab. Our data suggest that chronic inflammatory mediators, MDSC and adenosine metabolism in melanoma patients are of importance for the pathogenesis and prognosis of melanoma progression and could help to identify patients with high risk of disease progression or those who benefit from the ipilimumab therapy.

Session 5. New approaches to cancer immunotherapy

Chairs: David Fogelman, Cezary Szczylik

Melanoma (MM) Plus

Andrzej Mackiewicz

Chair of Medical Biotechnology, Poznan University of Medical Sciences, Greater Poland Cancer Centre, Poznan, Poland
BioContract Sp. z o.o. Poznan, Poland

Background: Immunotherapy to be effective requires efficient induction and effector phases of anti-tumor response. Despite successful therapeutic vaccination, the treatment usually fails to eliminate tumors due to local tumor and systemic immunosuppression. Blocking of immune checkpoints, normalization of tumor hypoxia changed the strategy of MM immunotherapy. We present combination of therapeutic vaccination with breaking tumor related immunosuppression by surgical removal of metastases.

Clinical trial protocols: Two phase II trials enrolled 198 IIIB-IV MM patients with completely resected metastases. Vaccination was applied in adjuvant setting and comprised of induction and maintenance phases. In progression re-induction was applied alone or combined with surgery then maintenance was continued until patient death. Primary end-point was DSF, secondary OS. **Combination of both modalities was more significant than re-induction alone.**

Therapeutic vaccine (AGI-101H): is allogeneic genetic whole cell vaccine composed of two MM lines, modified with Hyper-IL-6 (H6) cDNA. H6 comprises of IL-6 and its soluble alpha-receptor, which directly activates JAK-STAT pathway. H6 serves as molecular adjuvant, while during vaccine manufacturing it alters AGI-101H cells' phenotype towards MM stem/initiating cells (MMIC). AGI-101H cells show ALDH1A1 isoenzyme activity, what induced of anti-MMIC immune response and MMIC targeting.

Immune-target identification: PBMC were isolated from HLA-A2 positive MM patients before vaccination and after 6 days. Controls were untreated MM and healthy persons. ALDH1A1-CTLs were detected with MHC Dextramer®. The effector functions of CTLs were analysed by degranulation, IL-2, IFN-γ and granzyme-B production. CD4+ responses were analysed using ALDH1A1 protein and ELISpot. ALDH1A1-CTLs were detected in all vaccinated patients. Boosting demonstrated significant increase of ALDH1A1-CTL (4.86-fold increase). In controls no ALDH1A1-specific CD8+ cells were detected.

Biomarker identification: Pretreatment miRNA-221 and miRNA-324 were significantly higher in non-progressing than progressing patients. High baseline miRNA-221 and miRNA-324 was associated with longer DFS and OS ($p = 0.0008$) and over 4-fold lower risk of death ($p = 0.001942$; HR = 0.23). In patients without progression (PD) a decrease of miRNA-221 ($p = 0.0004$), miRNA-7b ($p = 0.0004$) was seen. In patients with PD there was no difference in miRNA-221, miRNA-324.

Conclusions: Proposed combinational treatment especially with personalization using selected miRNAs may provide significant benefit for advanced resected melanoma patients. The possible vaccine mode of action.

Session 6. New emerging therapies, promising preclinical and clinical trials

Chairs: Varsha Gandhi, Piotr Rutkowski

Advances in molecular characterization of gastrointestinal stromal tumors (GIST) – implications for therapy

Piotr Rutkowski

Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland

Gastrointestinal stromal tumors (GISTs) comprise a rare entity of the mesenchymal neoplasms of the gastrointestinal tract. Advances in the understanding of the molecular mechanisms of GIST pathogenesis have resulted in the development of a treatment approach which has become a model of targeted therapy in oncology. It has been demonstrated that a majority of GISTs are associated with activating, somatic, mutually exclusive mutations of two genes, *KIT* and *PDGFRA* (platelet-derived growth factor receptor-alpha), which are the early oncogenic events during GIST development, and result in overexpression and activation of oncoproteins KIT and PDGFR belonging to subclass III family of receptor tyrosine kinase. *KIT* and *PDGFRA* mutational status may also have a prognostic significance in primary GIST. Several studies have indicated a more favorable prognosis for patients carrying exon 11 point mutations or insertions as well as PDGFRA exon 18 mutations, whereas tumors harboring KIT exon 9 duplications as well as KIT exon 11 deletions (especially involving codons 557 and/or 558 or in homozygous state) were associated with more aggressive behavior of GIST. Further developments in molecular analysis (such as inclusion genomic index) may further optimize the individual risk assessment and inclusion criteria for adjuvant therapy after primary tumor resection. The introduction of imatinib mesylate (inhibiting KIT/PDGFRA and their downstream signaling cascade) has revolutionized the therapy of advanced (inoperable and/or metastatic) GISTs. Imatinib has now become the standard of care in the treatment of patients with advanced GIST and its efficacy has been proven recently in adjuvant setting after resection of primary high-risk tumors. However, a majority of patients eventually develop clinical resistance to imatinib. The molecular characterization of primary gain-of-function genes encoding *KIT* or *PDGFRA* correlates strongly with progression-free survival in GIST patients treated with imatinib and the mutational status predicts the probability of achieving response to imatinib. Patients with tumor harboring exon 11 *KIT* mutants have the best response to imatinib, with the highest rate of objective responses (70–85% of patients) and the longest overall and progression-free survivals. On the contrary, approximately 15–30% of cases with tumors with exon 9 *KIT* mutations and 25–50% of patients without detectable *KIT* or *PDGFRA* mutations (wild-type GIST) show primary resistance to imatinib therapy. Clinical and laboratory studies have demonstrated that tumors with exon 18 *PDGFRA* D842V mutations are insensitive to imatinib and sunitinib, whereas other *PDGFRA*-mutant GISTs show variable response. Over the last few years major progress has been made in elucidating the mechanism of disease progression (as secondary mutations in KIT and/or PDGFRA kinase domains) and resistance to imatinib. Currently, the approved second-line drug is multitargeted agent – sunitinib and in n the third-line next generation tyrosine kinase inhibitor – regorafenib).

Mechanism-based targeted combination therapy for chronic lymphocytic leukemia

Varsha Gandhi

MD Anderson Cancer Center, Houston, Texas, USA

During last five years, three targets were exploited for chronic lymphocytic leukemia (CLL). These are PI3 kinase δ and γ (PI3Kδ/γ), Bruton's tyrosine kinase (BTK), and Bcl-2 antiapoptotic protein. Previously it has been established that B-cell receptor (BCR) pathway is critical in survival, proliferation, maintenance, and migration of CLL lymphocytes. Both PI3Kδ and BTK are critical nodes in the BCR axis providing strong rationales to target these molecules. Ibrutinib, an irreversible BTK poison and idelalisib a selective and potent PI3K-delta inhibitor were approved for treatment of CLL while duvelisib, an agent that targets both PI3Kδ and Pi3Kγ is currently in Phase III trials.

Antiapoptotic proteins of Bcl-2 family have been implicated in the survival of CLL lymphocytes. Specifically, Bcl-2 and Mcl-1 are overexpressed in CLL cells. The mechanism for this overexpression is loss of miR15 and miR16 which are located at chromosome 13q14. Deletion 13q is the most common (> 50%) chromosomal abnormality in patients with CLL. Importantly, miR15 and miR16 suppress expression of Bcl-2 and Mcl-1 antiapoptotic proteins and loss of these miRs was primary mechanism for increased production of these two survival proteins in CLL lymphocytes. Venetoclax (ABT-199) is selective and potent antagonist of Bcl-2 protein. Recent preclinical and clinical investigations demonstrated efficacy of this agent in CLL.

During ibrutinib, idelalisib, and duvelisib clinical trials, we collected pre- and post-therapy primary tumor tissue (CLL lymphocytes) from patients. Cellular proteomics followed by immunoblot analyses of these primary CLL cells demonstrated that generally BCR pathway was blunted with these kinase inhibitors. In addition, Mcl-1 protein levels were reduced with these kinase antagonists while Bcl-2 protein levels either remain same or mostly increased. The decline or increase in these survival proteins was also observed at mRNA level. Corollary to these cellular changes, primary CLL cells obtained after kinase inhibitor therapy, when combined *ex vivo* with venetoclax induced a strong apoptotic response presumably due to neutralization of Bcl-2 protein, a survival factor for CLL lymphocytes. These results provided a strong rationale to combine BTK or PI3K inhibitors with venetoclax. To test this rationale in a clinical setting, we have designed protocol to test ibrutinib in combination with venetoclax for patients with poor-prognosis CLL disease.

Drug repurposing in the treatment of brain metastases

Frits Thorsen, Kristian Gerhard Jebsen

Brain Tumour Research Centre and The Molecular Imaging Center, Department of Biomedicine, University of Bergen, Bergen, Norway

Introduction: Around 3.4 million people in Europe will develop cancer every year [1], and up to 40% of these (i.e. 1.5 million people) will develop brain metastases. Untreated, the median survival time is 2–3 months, while aggressive treatment extends survival to 4–12 months. To date, very little has been done to develop drugs specifically targeting brain metastases, and patients with brain metastases have been excluded from clinical trials using drugs to target metastatic disease [2]. Thus, there is a need to find new and more effective drugs that can penetrate the blood-brain barrier to treat this large patient group.

Material and methods: We have developed novel animal models to study brain metastasis, where tumor cells from human melanoma brain metastases or human lung adenocarcinomas are injected into the blood stream of nod/scid mice. We have demonstrated by preclinical, multimodal imaging (MRI, PET/CT, bioluminescence imaging) that the animals develop multiple brain metastases in the brain, similar to what is seen in patients [3–5].

Results and Discussion: From metastatic lesions developing in animal brains, we identified a candidate gene signature list for melanoma brain metastases by deep sequencing. Based on the gene signature list, we found several compounds (already in use on patients for other conditions than cancer), which may improve treatment of brain metastasis.

We showed by multimodal imaging that the cholesterol analogue β-sitosterol effectively inhibited brain metastases and improved survival in our animal models, both on established tumours and in a preventive setting. β-sitosterol broadly suppressed the MAPK pathway via its converging downstream regulators, and effectively reduced mitochondrial respiration through Complex I inhibition. Our results may open new avenues of systemic therapy against metastatic melanoma, as increased mitochondrial respiration is a key mediator of resistance to MAPK-targeted drugs.

Conclusions: This broad-spectrum suppression of melanoma brain metastasis strongly encourages further assessment of β-sitosterol as an adjuvant to established MAPK-targeted therapies.

References

1. Home NCI. SEER Stat Fact Sheets: All Cancer Sites National Cancer Institute Home 2012. Available from: http://seer.cancer.gov/statfacts/html/all.html.
2. Maher EA, Mietz J, Arteaga CL, DePinho RA, Mohla S. Brain metastasis: opportunities in basic and translational research. Cancer Res 2009; 69: 6015-6020.
3. Sundstrøm T, Espedal H, Harter P, Fasmer K, Skaftnesmo KO, Horn S, Hodneland E, Mittelbronn M, Weide B, Beschorner R, Bendner B, Rygh CB, Lund-Johansen M, Bjerkvig R, Thorsen F. Melanoma brain metastasis is independent on LDHA expression. Neuro Oncol 2015; 17: 1374-1385.
4. Sundstrøm T, Daphu I, Wendelbo I, Hodneland E, Lundervold A, Immervoll H, Skaftnesmo KO, Babic M, Jendelova P, Sykova E, Lund-Johansen M, Bjerkvig R, Thorsen F. Automated tracking of nanoparticle-labeled melanoma cells improves the predictive power of a brain metastasis model. Cancer Res 2013; 73: 2445-2456.
5. Thorsen F, Fite B, Mahakian L, Seo JW, Qin SP, Harrison V, Johnson S, Ingham E, Meade T, Caskey C, Sundstrøm T, Meade T, Harter PN, Skaftnesmo KO, Ferrara KW. Multimodal imaging enables early detection and characterization of changes in tumor permeability of brain metastases. J Control Release 2013; 172: 812-822.

Session 7. New emerging therapies, promising preclinical and clinical trials

Chairs: Marek Durlik, Andrzej Mackiewicz

Borderline pancreatic cancer – the benefits of vascular resection

Marek Durlik

Central Clinical Hospital of the Ministry of Interior in Warsaw, Poland

Introduction: Pancreatic cancer remains one of the worst neoplasms characterised by a very poor prognosis. Surgery is the only option for ensuring patients' long-term survival. In contrast to arterial resection, venous resection (portal vein and superior mesenteric vein) is commonly used in high volume pancreatic surgery centres. Borderline pancreatic cancer is a relatively new category of a tumor in between Stage 2 (resectable) and Stage 3 (unresectable).

Aim of the study: The aim of this study was to evaluate the benefits of vascular resection during a pancreatectomy in a high volume pancreatic surgery centre.

Material and methods: Since the beginning of 2013, 474 pancreatectomies were performed (Whipple procedure, distal pancreatic resection and total pancreatectomies) due to pancreatic ductal adenocarcinoma (PDAC). In 87 cases (18%) portal vein and superior mesenteric vein resections were performed. 33 patients (37%) underwent a Gore-tex prosthesis implant – PTFE 10 mm; 28 patients (32%) underwent a simple vein resection; and 26 patients (30%) underwent vascular reconstruction using an autologous left renal vein or iliac vein implantation.

Results: We analysed the short- and long-term effects in patients after venous reconstruction and we compared with the group of patients who underwent a pancreatectomy without vascular resection. The overall survival rate was 16 months of the group without vascular, 13 months of patients with vascular resection regardless of the type of vein reconstruction. Overall survival in the vascular resection group was worse than the one without the venous resection.

Conclusions: The portal and superior mesenteric vein can be safely resected during pancreatic cancer surgery and has become a standard for borderline pancreatic tumors. Neoadjuvant therapy could be proposed preoperatively to better select patients who are more suited to major surgical procedures.

Poster Session

Poster

KW016-00013-2016-01

Efficient methods of human induced pluripotent stem cell differentiation into chondrocyte-like cells

Ewelina Augustyniak[1,2], Tomasz Trzeciak[3], Magdalena Richter[3], Jarosław Pawlicz[3], Wiktoria Maria Suchorska[1,4]

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

The application of stem cells (SCs) in regenerative medicine has recently become a rapidly growing field, holding promise for combating a number of orthopedic disorders including osteodegenerative ones (e.g. osteoarthritis). It has been reported that numerous types of SCs exhibit chondrogenic potential. The development of human induced pluripotent stem cells (hiPSCs) was a turning point in tissue engineering, which has opened up new horizons and generated a great deal of hope. However, the currently used methods for differentiation of hiPSCs into chondrocyte-like cells are not highly efficient and require further improvement.

In the present study hiPSCs obtained from human primary dermal fibroblasts (PHDFs) during reprogramming process were used. The four methods: via embryoid bodies (EBs) with TGF-β3 (10 ng/ml), EBs in the conditioned medium (CM), EBs with TGF-β3 (10 ng/ml) and conditioned medium as well as directed process throughout mesodermal stage of obtaining of hiPSC-derived chondrocytes were evaluated. The CM was obtained by conditioning of chondrogenic medium in the presence of human chondrocytes (HC-402-05a cell line) for 24 hours. The HC-402-05a cell line as a positive control as well as hiPSCs and PHDFs as negative controls served. The evaluation was carried out at the mRNA and protein level.

The results revealed the capacity of hiPSCs to effective chondrogenic differentiation. The obtained cells possess morphology characteristic for mature human chondrocytes. Furthermore, the chondrocyte-like cells reveal the presence of specific markers: type II collagen, Sox6, Sox9 and aggrecan. The differentiated cells show the lack or significantly decreased level of gene expression specific for their parental cells, such as Oct3/4, Nanog (hiPSCs). The dedifferentiation process of hiPSCs into fibroblasts was also excluded. Summing up, hiPSCs can be efficient differentiated into chondrocyte-like cells including directed differentiation without indirect stage such as formation of EBs. The usage of conditioned medium constitutes also a promising and inexpensive approach.

Key words: human induced pluripotent stem cells, differentiation, chondrocytes, cartilage.

The present study was supported by the National Science Centre (grant number 2012/07/E/NZ3/01819).

Poster

KW016-00029-2016-01

TERT promoter mutation and telomere length assessment in peripheral blood leukocytes in head and neck squamous cell carcinoma patients

Wojciech Barczak[1,2,3], Wiktoria M. Suchorska[3,4,5], Agnieszka Sobecka[3], Karolina Bednarowicz[3], Piotr Machczyński[1,2], Paweł Golusiński[1,2], Błażej Rubiś[6], Michał M. Masternak[1,2,7], Wojciech Golusiński[1,2]

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

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 approximately in 80–90% of all cancers, and it is responsible for lengthening of telomeres. Recently, highly recurrent point mutations in hTERT promoter have been reported in multiple human malignancies. The aim of this study was to analyze the frequency of the hTERT promoter C250T mutation, and the telomere length in blood leukocytes of 46 HNSCC patients and 49 healthy individuals. hTERT promoter mutation in 35% of HNSCC patients (42% of mouth cancer (*p*-value = ****), 17% of nose and sinuses cancer, 25% of thyroid gland cancer, and 36% of laryngeal cancer) were identified using qPCR. Furthermore, for the first time, the shorter telomeres in early stage tumors (significantly shorter telomeres in leukocytes from individuals with T2 HNSCC compare to healthy individuals) and longer telomeres in more advanced tumors were reported. Moreover, our results suggest that hTERT promoter mutation (as a common event during cancerogenesis) together with telomere length assessment may be one of the molecular markers of HNSCC progression.

Key words: telomeres, TERT mutation, biomarker, HNSCC.

Poster

KW016-00037-2016-01

Angiotensin II receptor antagonists as renal cell cancer regulators

Klaudia Brodaczewska, Cezary Szczylik, Anna Czarnecka

Department of Oncology with Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland

Renin-angiotensin system (RAS) that regulate blood pressure, but its components also influence proliferation, apoptosis and angiogenesis. These cellular processes are deregulated during tumorigenesis while hypertension was shown to be a risk factor of many cancers, including renal cell cancer (RCC). Angiotensin II up-regulate hypoxia induced factors (HIF) and intratumoral hypoxia promote cancer stem cell phenotype. Clinical studies suggest that inhibition of RAS is prolong survival in RCC patients. Molecular background of this phenomenon is unknown. It is possible that RAS promote survival of CSCs and therefore facilitate disease progression. Aim of the experiment was to evaluate if inhibition of RAS influences RCC cancer stem cells.

Human kidney cancer stem cells (HKCSCs) were pretreated for 24 h with angiotensin II receptor antagonists (sartans) or agonist (angiotensin II) in normoxic or hypoxic conditions. Rate of proliferation was measured with Alamar Blue Assay. Clonogenicity potential was tested in semi-solid agar culture. Expression of stem-related transcription factors Oct4, Sox2 and Nestin was measured with real-time PCR.

Pretreatment of HKCSCs with sartans slightly reduced the rate of proliferation of cells but strongly increased their clonogecity and this effect is dose dependent. Hypoxic conditions (1% O_2) additionally enhance pro-clonogenic effect of sartans. At the same time, receptor agonist, peptide Val5Angiotensin II, reduced cell clonogenicity but only in normoxia. In hypoxia agonist pretreatment does not alter number of colonies. Expression of Nestin and Oct4 – stem-related transcription factors – was increased by drug treatment both in normoxia and hypoxia while agonist treatment had opposite effect. At the same time Sox-2 expression was not up-regulated by angiotensin inhibitors.

Inhibition of Angiotensin II receptor significantly increases stem-related features of RCC. Presented data suggest, that manipulation of renin-angiotesin axis may influence the outcome of anti-tumour treatment and this compounds should be under consideration in hypertensive treatment of RCC patients.

Key words: renal cell carcinoma, angiotensin, hypoxia, sartans, stemness, targeted cancer therapies.

Research was funded by WIM intramural grant nr 1/8863 (355).

Poster

KW016-00004-2016-01

Determination of the immunotherapeutic effect of avirulan parasites in cancer therapy

Ayse Caner[1,2], Dunya Namlıses[1], Alper Erdoğan[1], Seray Toz[2], Cumhur Gunduz[3], Yusuf Ozbel[2], Yuksel Guruz[2], Ayfer Haydaroğlu[1]

[1]Department of Cancer Biology and Immunology, Ege University Institue of Health Sciences, Izmir, Turkey
[2]Department of Molecular Parasitology, Ege University Medical School, Izmir, Turkey
[3]Department of Medical Biology, Ege University Medical School, Izmir, Turkey

Immunologic system is suppressed by a strong immunologic suppression and immunologic response resulted from cancer cells. The main problem is to eliminate any local immunologic suppression produced by the tumor and to discover an agent that may enable formation of a new immunostimulant environment in the tumor location. Intracellular microorganisms activate CD+8 T cells and turn immunologic mechanism toward Th1. Our aim is to discover an agent that may enable formation of a new immunostimulant environment in the tumor location. In this project an intracellular microorganism used in the immunotherapy of the identified local tumors as an immunostimulant. *Leishmania* spp. is a protozoan in the Apicomplexa phylum and is an immunostimulant parasite with rich antigenic structure. There are successful cancer therapy studies carried out with *Leishmania* spp. However, all of these studies were carried out *in vitro* and no *in vivo* studies available.

In this study an *in vivo* breast cancer model was created and the immunologic response stimulated around the microenvironment of the tumor by *Leishmania* spp. injected into tumor tissue and the effects of this response on tumor genesis and therapy were investigated. Primarily breast cancer (4T1 cell line) will be created in BALB/c mice. Three weeks later, as the avirulent *Leishmania* strains injected into the breast cancer tissue. For the analysis of alterations in immunologic system, T lymphocyte types, Th1/Th2 and the dispersion of cytokines examined through flow cytometry. For the pathological follow-up in and around the breast cancer tissue, the immunohistochemical analyses will be performed. At the end of this study the magnitude of the tumor tissue, its invasion on the surrounding, cellular changes and immunologic system responses will be evaluated statistically.

Consequently, the immunotherapeutic effect of *Leishmania* spp. in the experimental mouse model created with breast cancer cells investigated for the first time. Protection against cancer comprises of an increase in CD+8 T cells after stimulation of Th1 immunologic path. As a result, the immunity stimulated by *Leishmania* spp. polarized toward Th1 determined. The avirulan strain of this parasite proliferates slowly and does not cause any disease while invading the live host cells. Despite this, these strains stimulated the immunologic response required in the defense against tumor.

Key words: breast cancer, *Leishmania*, photoimmunotherapy, parasite.

Poster

Cells division, cancer origin, therapeutic possibilities and aging

Vladislav Cizmic[1], Nikola Trifunovic[2]

[1]New Belgrade Hospital
[2]Development Academy of Serbia

Introduction: Everything in biosphere is magnetized. There is no explanation for cells division, aging and death, as well as for healing from cancer.

Aim of the study: To show that Earths magnetic field (EMF) supports division and enables aging and death of cells; prove that in anomalous magnetic fields (AMF), created by urbanisation, Ca is created because immunological system gets out of order, that after moving the patients away from AMF improves healing: explane that methabolism and aging in cells occurs Mo nuclei, organelle and substances; what are the medical risk factors. Present a hospital experiment with 40 patients.

Material and methods: Study of the literature about division, functioning, metabolism and cells aging. Anomalous magnetic fields measurement in Ca patients' beds has lasted for over 30 years. More than 2000 beds were measured with protonic magnetometer 100 nT precise. Results are presented by isolines on sketches. The patients were moved away from AMF. We have elucidated etiopathological data from medical literature, metabolism functioning in cells, why they get old and pass away organisms and functioning of immunology system. A hospital experiment with 40 patients took place.

Results: Mo nuclei of organelle and substances enable division and metabolism. Sequences and nucleotides-genes' parts, during DNA and RNA construction, are in accordance only through magnetic code. Substances accumulation in cells enable intermolecular magnetic foces (Mf) whose separation is enabled by temperature. Gene's Mf enable: crossing over, polimorphism, evolution and immune system functioning. The correlation between AMF – disease location. There are many characteristical Ca examples at various organs. By moving the patients away from AMF there are no recedives. Medical risk factors are not Ca cause.

Conclusions: Cell division is supported by EMF. Anomalous magnetic fields is the Ca cause. Moving patients away from AMF is the greatest help in healing. EMF and cell's Mo have the major role in bioworld evolution processes. By division Mf gets weaker, less water enters cells, toxins accumulate, functions of some organs get weaker, especially hormones activity, calcium salts accumulate in cartilage, immune system gets weaker and it means getting older. We have elucidated some unclearances from etopathology through AMF and natural EMF knowing. The experiment with 40 hospital patients shows that therapy should be innovated by building hospitals without AMF, introduce hyperthermia therapy where diseases are more advanced.

Key words: EMF, AMF, cancer, metastases, aging.

Poster

KW016-00039-2016-01

Cell growth effects of triiodothyronine and expression of thyroid hormone receptor in renal cancer

Anna Czarnecka[1], Damian Matak[1], Lukasz Szymanski[1], Karolina Czarnecka[2], Sławomir Lewicki[3], Robert Zdanowski[3], Ewa Brzezianska-Lasota[2], Cezary Szczylik[1]

[1]Department of Oncology with Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland
[2]Department of Molecular Bases of Medicine, Medical University of Lodz, Lodz, Poland
[3]Department of Regenerative Medicine, Military Institute of Hygiene and Epidemiology, Warsaw, Poland

Triiodothyronine (T3) is an important regulator of normal and cancer renal cell growth, differentiation and metabolism. In clinical trials renal cell cancer (RCC) patients who develop hypothyreosis during tyrosine kinase inhibitor (sunitinib, sorafenib) treatment have statistically longer progression free survival. In this study we verified the expression of the thyroid hormone receptor in human renal cell cancer cell lines panel from primary and metastatic tumors and human renal cancer stem cells and used *in vitro* model for analysis of T3 on RCC cell growth. Wild type thyroid hormone receptor is ubiquitously express in human renal cancer cell lines, but if mRNA level is normalized against healthy renal proximal tube cells' expression, its level is up-regulated only in selected cases i.e CAKI-2 and RCC6. At the same time thyroid hormone receptor protein was abundant in the cytoplasm of multiple cell lines including CAKI-2 and RCC6 cells but also in the nucleus of cancer stem cells. T3 was shown to promote proliferation most RCC cell lines i.e. Caki-1 or 786-0. Thyroid hormone receptor antagonist CAS 251310-57-3 has little inhibitory effect on RCC proliferation. Renal cancer tumor cells under low T3 may be more responsive to tyrosine kinase toxic activity. At the same time some RCC tumors should be considered as T3-independent and present aggressive phenotype with thyroid hormone receptor activated independently from T3 stimulation.

Key words: renal cell carcinoma, thyroid hormones, receptor, T3, cell proliferation.

This work was supported by Juventus PLUS project IP2011008171 [8736 0081/IP/2011/71].

Poster

KW016-00025-2016-01

Heterotypic culture on silk scaffolds – an innovative tool to study tumor biology *in vitro*

Ewelina Dondajewska[1], Wojciech Juzwa[2], Andrzej Mackiewicz[1,3], Hanna Dams-Kozłowska[1,3]

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

Introduction: Standard, two-dimensional methods of cell culture do not provide enough information about tumor microenvironment. Three-dimensional cultures capture the cell-cell interaction, production of extracellular matrix, cell morphology and proliferation, which are more similar to those observed *in vivo*. This study integrates 3D scaffolds made of silk fibroin with co-culture of cancer cells and stromal fibroblasts to construct functional breast cancer model *in vitro*.

Material and methods: Silk fibroin solution was extracted from Bombyx mori silk cocoons. Porous scaffolds were manufactured by salt-leaching method. Two cell lines were used during the experiments: EMT6 – murine breast cancer cell line and 3T3 murine fibroblasts, both were modified by lentiviral vectors to express fluorescent proteins. Cells were cultured in 2D and on the 3D scaffolds as a monoculture and co-culture in different ratios. Morphology of cells was visualized using confocal and scanning electron microscopy. Total DNA quantity measurement was used to determine cell proliferation. Toxicity of Doxorubicin was measured by AlamarBlue assay. Gene expression profiles of cells cultured in different conditions were examined using fluorescent cell sorting followed by qPCR analyses.

Results: 3D model of breast cancer based on silk scaffold was successfully developed. Difference in cell morphology was observed between 2D and 3D culture conditions. Cells cultured in 2D proliferated faster than those in 3D as observed by total DNA increase and upregulation of ki67 proliferation marker. Cells cultured on 3D silk scaffold showed about ten times higher resistance to cytotoxic agent than cells in 2D conditions. Cells when cultured as monoculture in 3D indicated different pattern of expression of genes related to tumor processes comparing to the corresponding cells cultured in 2D. Moreover, cells grown in 3D co-culture, showed different expression patterns of genes like CD44, CCL24 than corresponding cells in monoculture.
Conclusions: Breast cancer model based on porous silk fibroin scaffolds seeded with fibroblasts and breast cancer cells is a promising tool to study cell interaction, ECM production and drug efficacy. This system allows a better understanding of tumor microenvironment, leading to further progress in oncological therapies.

Key words: cancer microenvironment, silk fibroin, 3D model.

Poster

KW016-00018-2016-01

Application of 3D model of breast cancer to study the polarization of macrophages

Ewelina Dondajewska[1], Mariusz Kaczmarek[2], Agata Golabek[3,4], Karolina Penderecka[3,5], Andrzej Mackiewicz[1,3], Hanna Dams-Kozlowska[1,3]

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

Introduction: Macrophages are one of the largest populations of non-tumorous cells present in tumor microenvironment. Influenced by factors secreted by both tumor and stromal cells, these macrophages undergo an activation process to become Tumor Associated Macrophages (TAMs). To study macrophage activation and polarization process we applied three-dimensional (3D) breast cancer model. The 3D multicellular cell culture models provide better insight into interactions between different cell types, interactions between cells and the extracellular matrix and other processes such as angiogenesis, epithelial to mesenchymal transition, or tumour cell metastasis. In the presented study 3D breast cancer model was used to study tumour-macrophage interactions.

Aim of the study was to analyse macrophage activation and polarization upon stimulation with 3D model of breast cancer.

Material and methods: The following cell lines were used: J774 – murine macrophages, NIH3T3 – murine fibroblasts, EMT6 – murine breast cancer. Fibroblasts and cancer cells were seeded as monoculture or as co-culture in 9 : 1 ratio and incubated for several days on 3D scaffolds. Scaffolds were made of silk fibroin using salt leaching method. Conditioned medium (CM) was collected from 3D cultures of fibroblasts, breast cancer cells or co-culture of the both cell types and then administered to macrophages. The effects of CM from 3D cultures on macrophages phenotype were measured by flow cytometry and real time RT-PCR analyses. The markers characteristic for M2/TAM phenotype were analyzed.

Results: Expression of genes characteristic for M2/TAM macrophage phenotype such as: Itgax (Cd11c), Mrc1 (Cd206), Ccl24 (eotaxin 2), Retla (Fizz-1), Arg1, Il10 and Vegfa increased in response to CM from 3D tumor model. Similar increase was observed for J774 cells treated with CM from 3D cultured cancer cells in mono-culture. No increase in the expression of these genes was observed for macrophages treated with CM from fibroblasts alone. The flow cytometry analysis indicated increased expression level of CD206 and higher percentage of CD11c positive cells upon stimulation with CM from 3D model of breast cancer.

Three-dimensional model of breast cancer is a promising tool to study changes in macrophage phenotype in response to cancer environment. The next step is the integration of J774 cells into a direct triple co-culture in order to study direct, not only paracrine interactions in tumor microenvironment.

Key words: breast cancer, TAM, 3D model.

Poster

KW016-00036-2016-01

Demethylation of MCF7 breast cancer cells after 5-aza-deoxycitidine treatment led to the increase in the CD146 expression and cell morphology changes

Paulina Dudzik, Kinga A. Kocemba, Barbara Ostrowska, Jakub R. Pękala, Joanna Dulińska-Litewka, Piotr Laidler

Chair of Medical Biochemistry, Jagiellonian University Medical College, Krakow, Poland

Activation of epithelial to mesenchymal transition (EMT) in cancer cells leads their most aggresive fenotype, invasion and migration to the distant organs. Recently, the high expression of CD146 has been reported to be associted with tumor progression of several cancers. Despite the significant correlation between a mesenchymal phenotype of cells and overexpression of the protein CD146, the molecular basis for the high level of this protein in tumors, has not been demonstrated so far.

By modulating signaling pathways offen deregulated in tumor cells, we attempted to induce expression of CD146 in epithelial type breast cancer cells (MCF7), that initially presented low expression of CD146 on mRNA and protein level. According to the literature data, EMT could be induced by activation of Wnt pathway (Wnt3A), stimulation of TGF/Smad (TGF), stabilization of HIF-1α (hypoxia and cobalt chloride) and DNA demethylation (5-aza-deoxycytidine). Of all four methods used only 5-aza-deoxycytidine resulted in an increase in the CD146 expression at the mRNA and the protein level. Interestingly, in parallel to the increase of CD146 expression, we observed morphological changes in the treated cells. Analysis of the CD146 promoter by methylation-specyfic PCR (MSP) revealed CpG island methylation in MCF7 cancer cells. Moreover, demethylation after 5-aza-deoxycitidine treatment in CD146 expression led to meaningful increase.

Taken together, our data identified aberrant methylation of the CD146 promoter as a cause of CD146 silencing in breast cancer cell lines.

Key words: methylation, CD146, breast cancer, EMT.

This work was supported by Jagiellonian University Medical College grant: K/ZDS/004540.

Poster

KW016-00048-2016-01

The expression of NFAT transcription factors in human gliomas

Aleksandra Ellert-Miklaszewska, Bartosz Wojtas, Marta Maleszewska, Bozena Kaminska

Nencki Institute of Experimental Biology, PAS, Warsaw, Poland

Brain macrophages (microglia) and peripheral macrophages accumulate in malignant gliomas and are re-programmed into tumor supportive cells that enhance invasion and induce local and systemic immunosuppression. In contrast to other tumors, in which M-CSF (macrophage colony stimulating factor) attracts macrophages into tumor, we found that GM-CSF (granulocyte macrophage colony stimulating factor) is a crucial driver of microglia/macrophage accumulation in experimental gliomas. The mechanism of transcriptional up-regulation of GM-CSF in gliomas is unknown. Our previous studies suggest that expression of CSF2 (coding for GM-CSF) can be transcriptionally regulated by NFAT (nuclear factor of activated T cells) factors. The NFAT family, first described as a regulator of T cell activation and differentiation, is composed of four calcium-responsive members. Emerging evidence suggests a complex and predominant role of NFATc1 and NFATc2 in carcinogenesis. We performed the analysis based on TCGA (The Cancer Genome Atlas) data to compare the expression pattern of NFAT family members in normal brains versus tumor samples from patients with grade II–IV glioblastomas. It revealed significant upregulation of NFATc1, NFATc2 and NFATc3 in human gliomas. We also evaluated the expression of different members of NFAT family using qPCR in human low and high grade gliomas as well as in established and primary glioma cell lines in comparison to non-tumoral brain samples and normal human astrocytes. The results suggest that NFAT proteins may control a new program of cytokine/chemokine expression, which is important for glioma-microglia communication and glioma progression, and thus constitute an plausible target for future therapeutics.

Key words: glioma, NFAT, microglia, GM-CSF.

Poster

KW016-00015-2016-01

Bioengineered silk spheres for targeted drug delivery in Her2-positive breast cancer model

Anna Florczak[1], Tomasz Deptuch[1], Anna Lewandowska[2], Konstanty Korski[2], Hanna Dams-Kozlowska[1], Andrzej Mackiewicz[1]

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

The carriers for systemic cancer treatment have to overcome the challenges of reaching the tumor site and accumulating in the tumor microenvironment. As silk biomaterial is known for its mechanical strength, biocompatibility and biodegradability, it can be used as a drug carrier. Moreover, the bioengineered spider silk vehicles (spheres) may be functionalized with specific tumor-recognizing peptides and applied as drug carrier in cancer treatment. Our *in vitro* results indicated that spheres made of functionalized silk specifically recognized and killed cancer cells when loaded with doxorubicin.

The aim of the present study was the *in vivo* evaluation of functionalized silk spheres efficacy as drug carriers for cancer therapy.

The bioengineered silk proteins MS1 and its Her2-oriented hybrid variant H2.1MS1 were designed based on MaSp1 protein from N. clavipes spider. Stable silk particles were obtained by mixing a soluble protein with potassium phosphate. The Doxorubicin (Dox) loading was analyzed spectrophotometrically. The *in vivo* studies were conducted in a Balb/c mouse using model of Her2-positive breast cancer (D2F2E2/Luc) and control Her2-negative cancer (D2F2/Luc). Spheres were administrated intravenously. *In vivo* silk spheres biodistribution and the therapeutic effect of Dox delivered in silk spheres were investigated by using IVIS Spectrum *in vivo* imaging system. A systemic toxicity was analyzed by histopathological examination.

The increased accumulation of functionalized spider silk spheres at the site of the Her2-positive tumor was demonstrated. Although spheres initially accumulated also in lungs and liver, after 7 days the fluorescent signal from spheres was not detected in these organs in contrast to Her2-positive tumors. Furthermore, Dox delivered in functionalized silk spheres provided the therapeutic effect *in vivo*. In mice with Her2-positive model a tumor growth was inhibited. A histopathological analysis revealed no systemic toxicity after administration of control or drug loaded silk spheres.

The obtained results confirmed that functionalized bioengineered silks can serve as specific drug carriers for cancer treatment.

Key words: silk fibroin, Her2-positive, drug carrier, targeted drug delivery.

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

Poster

KW016-00010-2016-01

Silk spheres loaded with CpG-siRNA: a novel strategy for siRNA delivery into tumor environment

Anna Florczak[1,2], Anna Kozłowska[1], Maciej Smialek[1], Ewelina Dondajewska[1], Andrzej Mackiewicz[1,2], Marcin Kortylewski[3], Hanna Dams-Kozlowska[1,2]

[1]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland
[3]Beckman Research Institute, City of Hope National Medical Center, Duarte, CA, USA

The specific and efficient delivery of siRNAs to the cytoplasm of a target cell remains a challenge to its successful therapeutic application. The CpG-siRNA molecules target TLR9-positive cells and thus overcome a problem of cell-specific delivery. However, systemic delivery of CpG-siRNA requires optimization of molecule stability in body fluids. The bioengineered spider silk is a material with a great potential in biomedical application. Due to the self-assembly property, silk can be processed into several morphological forms. Moreover, its functionalization with poly-lysine domain allows for binding of nucleic acids and enables development of nucleic acid delivery system. Silk spheres loaded with CpG-siRNA may protect from degradation and prolong activity of siRNA in target cells.

The aim of study was analysis of cell uptake of silk spheres loaded with CpG-siRNA and its gene silencing potential.

The hybrid construct (MS2KN) was obtained by adding a poly-lysine nucleic acid binding domain (KN) to spider silk (MS2) based on the MaSp2 from N. clavipes. Spheres loaded with CpG-siRNA were formed by mixing a soluble protein with potassium phosphate at silk: nucleic acid molar ratio of 1 : 1. Obtained spheres were characterized in terms of size, morphology (SEM), Zeta potential and cytotoxicity (MTT). CpG-siRNA loading into particles was examined spectrophotometrically. For stability assay, the CpG-siRNA loaded spheres were analyzed in SDS-PAGE gel after incubation in presence of mouse serum. The uptake of constructs by murine macrophages J774 was assessed using flow cytometry and confocal microscopy. In order to measure the silencing effect of CpG-siRNA on target mRNA (Stat3) and the transcripts related to Stat3 regulation, the real-time quantitative PCR was performed.

Silk spheres improved stability of CpG-siRNA molecules in serum. MS2KN spheres loaded with CpG-siRNA were actively internalized by TLR9-positive macrophages and localized in the cytoplasm of cells. The intracellular localization of CpG-siRNA delivered in silk spheres was prolonged comparing with naked nucleic acid construct. Furthermore, extended silencing effect of Stat3 mRNA was observed for CpG-siRNA confined in silk spheres as compared to naked construct. A correlation was indicated between the Stat3 down-regulation and expression of genes involved in the Stat3 signaling pathway.

Functionalization of silk by adding nucleic acid binding domain enables development of siRNA delivery system.

Key words: bioengineered spider silk, CpG-siRNA, targeted drug delivery, drug carrier, cancer immunotherapy.

Poster

KW016-00019-2016-01

Vaccines based on CSCs and iPSCs as a treatment for malignant melanoma. Immunogenicity and therapeutic effects in mouse models

Agnieszka Gąbka-Buszek[1], Anna Kozłowska[1], Jakub Jankowski[1], Katarzyna Tomela[1], Andrzej Mackiewicz[1,2], Eliza Kwiatkowska-Borowczyk[1,2]

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

Introduction: Melanoma belongs to the immunogenic malignancies. Therefore various immunotherapies are being developed. Limited effectiveness of standard therapies as well as immunotherapies may be related to the lack of elimination of cancer stem cells (CSCs). CSCs are characterized by a low degree of differentiation, capacity for self-renewal, potential for rapid restoration of tumour cells pool and the expression of antigens other than in differentiated tumour cells but similar to those in normal stem cells.

The aim of the study was to analyse and compare the ability to induce a specific immune response and the therapeutic potential of novel tumour vaccines of cancer stem cells (CSCs) derived from B16F10 cells and induced pluripotent stem cells (iPSCs).

Material and methods: C57BL/6 mice were immunized twice by subcutaneous injection using the CSCs and miPSCs mixed with irradiated wild type B16F10 cells modified to secrete Hyper-IL-6 (fusion protein composed of IL-6 and its soluble alpha receptor). For boosting immunization, vaccine cells were dispersed in matrigel to enable analysis of cells infiltrating the vaccine as well as the profile of cytokines produced at the site of matrigel plug. Martigels, draining lymph nodes and spleens were harvested for phenotypic analysis of dendritic cells, granulocytes, macrophages, MDSC, lymphocytes, and for functional analysis of T lymphocytes. To evaluate the anti-melanoma activity of CSCs vaccine, mice were immunized twice and re-challenged with live B16F10 cells 7 days later. Tumour volume was measured 3 times per week.

Results: We observed an increased immune response in mice immunized with melanoma CSCs and miPSCs vaccines compared with control mice immunized with B16F10. *In vitro* stimulation of splenocytes with relevant vaccine or B16F10 cells revealed the presence of antigen specific lymphocytes, producing IL2, IFN-γ, TNF, IL-6 and IL-10, which indicates a mixed Th1/Th2 type immune response. Moreover, CSCs vaccine inhibited tumour growth and prolonged disease-free survival as well as overall survival in tumour-rejection mouse melanoma model.

Conclusions: Obtained results demonstrate high therapeutic potential of cancer vaccines based on melanoma CSCs and iPSCs.

Key words: cancer vaccine, melanoma, CSCs, cancer immunotherapy.

This work was supported by the National Science Centre grant UMO-2012/07/B/NZ6/03495.

Poster

KW016-00052-2016-01

Cancer-associated epigenetic repressors KRAB-ZNFs identified through high-throughput transcriptomic profiling

Marta Gładych[1,2], Barbara Sozanska[3], Przemysław Biecek[3,4], Maciej Wiznerowicz[1,2], 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 and Information Science, Warsaw University of Technology, Warsaw, Poland
[4]Faculty of Mathematics, Informatics, and Mechanics, University of Warsaw, Warsaw, Poland

Carcinogenesis is a complex process driven mainly by aberrant functioning of mutated genes. However, epigenetic changes such as DNA methylation and histone modifications, play a pivotal role in the initiation and progression of cancer. It is well established that cancer genomes become globally hypomethylated, whereas many promoters of tumor suppressor genes are hypermethylated, and thus, transcriptionally repressed. Yet, the exact mechanisms behind this phenomenon are still unclear. KRAB-ZNFs (Krüppel-associated box-zinc finger proteins) comprise the largest family of epigenetic repressors in human genome. Although some KRAB-ZNFs were shown to play a role in cancer, their molecular and physiological functions remain largely uncharacterized. Since KRAB-ZNFs act as potent repressors, we hypothesize that these proteins may participate in the epigenetic regulation of tumor suppressors. Thus, in our study we aim to investigate the involvement of KRAB-ZNFs in the modulation of epigenetic profile in cancer cells. To this end, we analyzed changes in KRAB-ZNFs expression in tumor and normal tissues from TCGA project. Our differential expression analysis revealed that out of 381 KRAB-ZNFs only a fraction is deregulated in tumors. Interestingly, the majority of the KRAB-ZNFs with altered mRNA level exhibited reduced expression, while only a small, but distinct cluster of 11 KRAB-ZNFs showed upregulation in cancer tissues. In our previous studies, one of the overexpressed KRAB-ZNF, ZNF695, manifested involvement in stemness maintenance in pluripotent stem cells. While multiple studies show molecular and functional similarities between pluripotent and cancer stem cells, our observations indicate that ZNF695 may protect stemness properties also in cancer stem cells. Our results suggest that certain KRAB-ZNF factors may play an important role in carcinogenesis. In the next steps, we aim to explore molecular and phenotypic function of selected, cancer-associated KRAB-ZNFs in cancer cell biology and in the modulation of their epigenetic profile.

Key words: cancer epigenetics, KRAB-ZNF transcriptional repressors, TCGA.

Poster

KW016-00016-2016-01

Identification of genes and chromatin topological domains with changed activity, specific to different brain tissues and to response to dietary treatments

***Ilona Grabowicz*[1,2], *Bartosz Wilczyński*[1]**

[1]Institute of Informatics, Warsaw University, Warsaw, Poland
[2]Postgraduate School of Molecular Medicine, Warsaw Medical University, Warsaw, Poland

In the analysis of the data coming from an experiment involving mice being on different dietary regimes (control and high in sugars and fats) we have identified genes exhibiting expression specific to different brain tissues. Moreover, we have identified the chromatin topological domains where these changes occurred and showed that many of the affected genes tend to be co-regulated. Moreover, we have showed that the borders of these domains are very precise and even slight changes can decrease the gene co-regulation. Genes showing significant differences in expression between all investigated tissues show specific 3-stage patterns of regulation.

Besides, we have managed to identify genes which change their expression due to change in the diet of animals. These changes are found to be mainly brain tissue-specific and such genes are also prone to be co-regulated within the chromatin topological domains. Additionally we have investigated the expression of long non-coding RNAs. Despite diet being such a complex stimulus we have managed to find its effects on the animals brain gene expression as well as behaviour.

Key words: diet, gene expression, brain.

Poster

KW016-00022-2016-01

Why new generation technology matters for personalized therapy in cancer diagnostics and treatment?

Kinga Humińska, Marta Kuś, Małgorzata Grzanka, Jacek Wojciechowicz

DNA Research Center

When it comes to cancer diagnosis, speed and accuracy makes all the difference. Thanks to our cancer predisposition panels and somatic mutation tests, the diagnoses is faster and more effective than it was ever before. We are moving forward using molecular biomarkers obtained from peripheral blood sampling or FFPE samples to detect specific germline or somatic mutations, select the best targeted therapy and monitor disease progression, recurrence and stability. All of these can be achieved by using a broad range of the newest technologies.

There is a great potential for integration of NGS and ddPCR in term of targeted resequencing, development and usage of individualized biomarkers and monitoring the response to chemotherapy. Using our ONCO panels based on NGS technology, we are able to screen patients towards a wide range of "hot spot" mutations in 56 tumor suppressor genes and oncogenes. The result allows for selection of the best targeted therapy for individual patient, indicating the most suitable clinical studies that the patient may be eligible for.

Detection of somatic mutations by NGS, allows us an assessment of mutant allele frequency by ddPCR. With this technique we can precisely quantify mutant allele frequency of a rare tumorigenic mutations in a high background of "normal" cells, routinely down to 0.01% and often further. The usage of very sensitive screens for specific mutations that do not inform the user of the mutant allele frequency may be misleading. It is therefore plausible that two important parameters – the percentage of cancer cells that carry a specific druggable mutation and whether a specific allele is amplified as well as mutated – may have a major impact on the response to specific therapies.

Additionally, we encourage to monitor cancer regression by checking the number of circulating tumor cells (CTC) in patient blood. Enumeration of CTCs provides us with an information whether the cells successfully are reduced by the therapy.

Targeted cancer therapies give medical oncologists a better way to customize cancer prevention and treatment. Pipeline that we suggest will shorten the time of diagnosis, help with the choice of individualized treatment what potentially cause less harm to a patient, gives fewer side effects and improves quality of life of a patient.

Key words: next generation sequencing (NGS), cancer diagnosis, circulating tumor cells (CTC), targeted cancer therapies, digital PCR (ddPCR).

Projekt współfinansowany ze środków UE w ramach Europejskiego Funduszu Rozwoju Regionalnego-MAC.

Poster

KW016-00031-2016-01

Pomhex: a high potency enolase inhibitor with *in vivo* anti-neoplastic activity

Yu-Hsi Lin, Nikunj Satani, Naima Hammoudi, Paul Leonard, David Maxwell, Zhenghong Peng, Todd Link, Gilbert Lee, Ananth Bosajou, Duoli Sun, Maria Di Francesco, Barbara Czako, Alan Wang, William Bornmann, Ronald DePinho, Florian Muller

The University of Texas MD Anderson Cancer Center, Texas, USA

Glycolysis inhibition is an active area of investigation in cancer. However, few compounds have progressed beyond the cell culture stage. We have recently demonstrated that genomic passenger deletion of the glycolytic enzyme Enolase 1 (ENO1) leaves gliomas harboring such deletions with less than 10% of normal enzymatic activity, rendering them exquisitely sensitive to enolase inhibitors. However, the compound that we employed for these *in vitro* studies, Phosphonoacetohydroxamate (PhAH), has very poor pharmacological properties and was ineffective *in vivo*. We performed a SAR studies to increase inhibitor specificity towards ENO2 as well as pro-druging to increase cell permeability. The lead compound generated by these efforts, termed POMHEX, is selectively active against ENO1-deleted glioma cells in culture at ~35 nM (versus μM for PhAH). Using an orthotopic intracranial xenografted model where tumor growth and response to therapy are monitored by MRI, we show that POMHEX is capable of eradicating intracranial ENO1-deleted tumors, with mice remaining recurrence-free even after treatment discontinuation. Taken together, these results reinforce that glycolysis is a viable target and provide *in vivo* proof-of-principal for the concept of using passenger deletions as targetable vulnerabilities in cancer therapy.

Key words: Pomhex, enolase, glioblastoma.

Poster

KW016-00026-2016-01

Analyses of TP53 gene in breast cancer patients according to histological factors

Joanna Huszno[1], Ewa Grzybowska[2], Zofia Kołosza[3], Karolina Tęcza[2], Jolanta Pamuła-Piłat[2], Marta Nycz-Bochenek[4], Elżbieta Nowara[1]

[1]Clinical and Experimental Oncology Department, Maria Sklodowska Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland
[2]Center for Translational Research and Molecular Biology of Cancer, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland
[3]Department of Epidemiology and Silesia Cancer Registry, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland
[4]Genetic Outpationt Clinic, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland

Introduction: TP53 tumor suppressor gene is frequently mutated in various solid tumors, including breast cancer. Mutations of TP53 are more frequent in advanced stage or in cancer subtypes with aggressive course (such as triple negative or HER2-amplified breast cancers).

The aim of this study was to evaluate the association between polymorphism TP53 (c.[215G>C]) and histological factors. Authors compare TP53 carriers with other mutations carriers.

Material and methods: We reviewed the medical records of 110 breast cancer patients (mutations carriers) who were diagnosed and treated in COI. Mutation profile was assessed by PCR technique. We evaluated the presence of at least one allele of TP53 (c.[215G>C]) (22 of pts) polymorphism. The control group (88 of pts.) were breast cancer pts with BRCA1 (c.5266dupC), (c.68_69delAG), (c.181T>G), (c.4034delA), BRCA2 (c.9403delC), (c.5946delT), CHEK2 (c.470T>C), (c.1100delC) and NOD2 (c.3016_3017insC) mutations. In statistical analysis Fisher's exact test were used for categorical variables to determine differences between groups.

Results: HER2 overexpression was detected more often in TP53 polymorphism (c.[215G>C]) carriers then in other mutation carriers (55% vs. 8%), $p = 0.0001$. Similarly, there was observed tendency to the presence of lymph node metastases in pts with TP53 polymorphism under study in comparison to pts with other mutations (45% vs. 25%), $p = 0.063$. Negative steroid receptor status (ER–, PR–) was also present insignificantly more often in TP53 carriers (41% vs. 34%), $p = 0.621$. However, triple negative breast cancer was characteristic for other mutation carriers (32% vs. 18%), $p = 0.295$. Higher histological grade (G2 and G3) was detected insignificantly more frequently in TP53 carriers than in control group (100% vs. 87%), $p = 0.351$. Most of TP53 carriers (96%) were diagnosed with early breast cancer.

Conclusions: Carrying at least one less frequent variant of TP53 polymorphism (c.[215G>C]) was associated with negative histopathologic factors such as higher histological grade (G3), HER2 overexpression, negative steroid receptor status and lymph node metastasis. However most of the carriers were diagnosed in early stage of disease. These are preliminary results. Further studies on a larger group of patients are necessary.

Key words: breast cancer, polymorphism TP53, histological factors.

Poster

Predictors of survival in BRCA1-positive breast cancer patients

Huzarski T[1], Byrski T[1], Gronwald J[1], Cybulski C[1], Oszurek O[1], Szwiec M[2], Gugała K[3], Stawicka M[4,5], Morawiec Z[5], Mierzwa T[6], Falco M[7], Janiszewska H[8], Kilar E[9], Marczyk E[10], Kozak-Klonowska B[11], Siołek M[11], Surdyka D[12], Wiśniowski R[13], Posmyk R[14], Domagała P[15], Sun P[16], Lubiński J[1], Narod SA[16] and the Polish Breast Cancer Consortium

[1]Department of Genetics and Pathology, International Hereditary Cancer Center, Pomeranian Medical University, Szczecin, Poland
[2]Regional Oncology Center, Opole, Poland
[3]Department of Pathology, District Specialist Hospital, Olsztyn, Poland
[4]Regional Oncology Center, Poznan, Poland
[5]Department of Oncological Surgery, Regional Oncology Center, Lodz, Poland
[6]Center of Oncology, Franciszek Łukaszczyk Memoriam, Bydgoszcz, Poland
[7]Department of Radiotherapy, Westpomeranian Oncology Center, Szczecin, Poland
[8]Department of Clinical Genetics, Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland
[9]Department of Oncology, Disctict Specialist Hospital, Świdnica, Poland
[10]Department of Oncological Surgery, Regional Oncology Center, Kraków, Poland
[11]Regional Oncology Center, Kielce, Poland
[12]St. John's Cancer Center, Lublin, Poland
[13]Regional Oncology Hospital, Bielsko-Biała, Poland
[14]Regional Oncology Center, Białystok, Poland
[15]Department of Pathology, Pomeranian Medical University, Szczecin, Poland
[16]Women's College Research Institute, Toronto, Ontario, Canada

Other members of the Polish Hereditary Breast Cancer Consortium: Bębenek M, Błasińska-Morawiec M, Breborowicz J, Chosia M, Drosik K, Dziuba I, Fudali L, Gozdecka-Grodecka S, Goździ S, Grzybowska E, Haus O, Jeziorski A, Karczewska A, Kordek R, Kozak A, Kozłowski A, Lamperska K, Lange D, Mackiewicz A, J Menkiszak, Mituś J, Musiatowicz B, Niepsuj S, Rozmiarek A, Ryś J, Sibilski R, Sir J, Sorokin D, Sygut J, Szczylik C, Uciński M, Urbański K, Wachuła E, Waśko B, Wojnar A, Wandzel P

Aim of the study: To identify treatments which predict survival for women with a BRCA1 mutation, including oophorectomy and type of chemotherapy.

Material and methods: 476 women with stage I to stage III breast cancer who carried a BRCA1 mutation were followed from diagnosis until April 2015. Information on treatment was obtained from chart review and patient questionnaires. Dates of death were obtained from the Poland vital statistics registry. Survival curves were compared for different subgroups according to treatment received. Predictors of overall survival were determined using the Cox proportional hazards model.

Results: The ten-year overall survival was 78.3% (95% CI: 74.2–82.6%) and the ten year breast-cancer specific survival was 84.2% (95% CI: 80.5–88.0%). Fourteen patients died of ovarian cancer and two patients died of peritoneal cancer. Oophorectomy was associated with a significant reduction in all-cause mortality in the entire cohort (adjusted HR = 0.41; 95% CI: 0.24–0.69; $p = 0.0008$) and in breast cancer specific mortality among ER-negative breast cancer patients (HR = 0.44; 95% CI: 0.22–0.89; $p = 0.02$).

Conclusions: Among women with breast cancer and a BRCA1 mutation, survival is greatly improved by oophorectomy due to the prevention of deaths from both breast and ovarian cancer.

Key words: breast cancer, BRCA1, survival, prognostic factors.

Poster

KW016-00012-2016-01

Antigen-armed antibodies in the treatment of B-cell lymphoma

Marta Ilecka, Henri-Jacques Delecluse

Deutsches Krebsforschungszentrum, Pathogenesis of Virus Associated Tumors, Heidelberg, Germany

Approximately 90% of lymphoid neoplasms worldwide originate from B cells and their incidence constantly keeps on rising. Diffuse large B-cell lymphoma is the most prominent among B-cell malignancies. Since the discovery of anti-CD20 antibody (rituximab) as an effective treatment, the overall survival of lymphoma patients has dramatically improved. However, lymphoma treatment remains challenging due to heterogeneity among the lymphoma subtypes and the development of resistance to existing therapies.

A promising strategy to combat B-cell neoplasms is to use 'armed antibodies'– i.e. antibodies conjugated to other agents such as radionuclides, drugs or cytokines. Our group has designed a novel antibody arming strategy, which utilizes microbial antigens in order to increase the immune response against B-cell tumors. This approach takes the advantage of an existing persistent viral infection in a cancer patient and T cells that have been primed during the first encounter with the virus. Such antigen- armed antibodies (AgAbs) are specific against B cell surface markers and serve as epitope delivery platform. In detail, this strategy involves introducing T-cell epitopes from Epstein-Barr virus (EBV) into the antibody molecule in order to elicit a specific T-cell response directed against the B-cell lymphoma cell that will present this epitope after AgAbs treatment. The strategy can be extrapolated to other common herpesviruses as for instance human cytomegalovirus or herpes simplex viruses.

Herpesviruses have been recently proven to provoke a cytotoxic response exhibited by CD4+ T cells. Our *in vitro* data strongly supports the idea that different B-lymphoma cell lines are able to internalize, process and present the viral epitopes incorporated into AgAbs. These epitopes are then recognized by cytotoxic CD4+ T cells that have the capacity to kill the tumor cells presenting them as shown in our cytotoxicity assays. It is relatively easy to clone the antigenic sequence into the antibody gene and express the modified immunoglobulin. Moreover, the antibodies can accommodate longer antigenic fragments, which makes it possible to use the therapy in patients with different MHCII haplotypes. We have also shown that stimulation with AgAbs can lead to activation and expansion of the memory EBV-specific CD4 T cells *ex vivo*. All the data shown prove that the therapy we propose has a great potential in lymphoma immunotherapy.

Key words: antigen-armed antibodies, lymphoma, CD4+ cytotoxic T cells.

Poster

KW016-00017-2016-01

NPY system in prostate cancer – preliminary study

Ewa Iżycka-Świeszewska[1,2], Wojciech Wesołowski[1,3], Edyta Szwed[2], Artur Antolak[2], Joanna Kitlińska[4]

[1]Medical University of Gdańsk, Gdansk, Poland
[2]Copernicus Hospital Gdańsk, Gdansk, Poland
[3]EL-PAT Pathology Co Elblag, Elblag, Poland
[4]Georgetown University Washington, USA

NPY and its receptors system are involved in many pivotal metabolic physiological and pathological processes, including cancerogenesis and cancer progression. NPY system elements constitute candidates for diagnostic and therapeutic targets in oncology. This system is activated in few types of cancer.

We present the preliminary results of the immunohistochemical study performed on tissue microarrays built from 50 cases of prostate cancer. The expression of NPY, Y1R, Y2R, and Y5R was examined and correlated with the pathological and basic clinical data of tumors. Our results show that NPY system participates in prostate neoplasia.

Key words: NPY, NPY receptors, prostate cancer.

Poster

KW016-00047-2016-01

The effect of combination therapy (endoglin-based DNA vaccine with interleukin-12) on tumor blood vessels

Magdalena Jarosz-Biej, Natalia Kamińska, Tomasz Cichoń, Sybilla Matuszczak, Ryszard Smolarczyk, Klaudia Kulik, Stanisław Szala

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

The process of tumor blood vascular network development considerably affects tumor growth. The structure of tumor blood vessels is defective and they are functionally abnormal. Slowed-down circulation of blood leads to underoxygenation. Hypoxia stimulates formation of novel microvessels and invasiveness of tumor cells. "Normalization" of irregular tumor vascular network should sensitize cancer cells to chemo- and radiotherapy and should lead to tumor growth regression. Our goal is to investigate the mechanism of action of the drug we constructed: DNA vaccine directed against endoglin overexpressed in the tumor vessels (as a carrier of ENG encoding gene, we used an attenuated strain Salmonella Typhimurium SL7207) together with an immunomodulatory cytokine interleukin 12 (IL-12). In this study, we tested the effect of this combination on tumor blood vessels.

The research was conducted on the B16-F10 murine melanoma model. Condition of tumor blood vessels and tumor cells in both treated and control mice was assessed by several immunohistochemical methods. It was also investigated whether combination of antiangiogenic ENG vaccine with IL-12 increases the sensitivity of cancer cells to doxorubicin.

It was observed that the combination therapy inhibits the growth of tumors significantly better than either of the agents alone. Less necrotic areas, increased infiltration of immune system cells and decreased number of blood vessels (CD31) were observed in tumor sections from mice treated with the combination therapy compared to controls. The structure of tumor vessels in mice treated with combined therapy resemble a regular one: the walls are thick with an increased pericyte coverage (αSMA). Smaller areas of hypoxia and lower level of cancer cells undergoing apoptosis (caspase-3) were also found in tumor sections from treated mice. In addition, suboptimal doses of doxorubicin inhibited the growth of tumors in mice treated with combined therapy, but only slightly inhibited the tumor growth in controls.

In summary, our findings show that the endoglin-based DNA vaccine in combination with IL-12 affects the structure of tumor blood vessels. These data might indicate a "normalization" of tumor blood vasculature that may have occurred following administration of ENG vaccine + IL-12 combination.

Key words: endoglin-based DNA vaccine, IL-12, tumor blood vessels.

This study was supported by Grant No. UMO-2013/11/B/NZ4/04468 from the Ministry of Science and Higher Education (Poland).

Poster

KW016-00038-2016-01

Deregulation of signaling pathways in renal cell cancer lung metastases

Katarzyna Kaminska, Aleksandra Klemba, Cezary Szczylik, Anna Czarnecka

Department of Oncology with Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland

Median progression free patient survival of metastatic renal cancer patients is between 10 to 12 months with first line tyrosine kinase inhibitor treatment and up to 50% patients develop metastases in lung parenchyma. Lung metastasis-associated genes in renal cell cancer reported until now included matrix metallopeptidases MMP7 and MMP9, chemokine receptor CXCR3, differentiation marker MME (CD10), apoptosis inhibitor BCL2 and the cell-surface protein CD44. It was also reported that lung metastases from patients with multiple metastatic loci show an elevated expression of genes associated with cell division and cell cycle including PBK, BIRC5, and PTTG1. Understanding of the molecular basis of lung metastasis development is nevertheless still insufficient in renal cell cancer. Thus, in this study we used renal cell cancer cells and bronchial epithelial cells – representing metastasis target organ cells – co-culture model to identify specific gene expression change responsible for cancer cells viability in metastasis microenvironment. Human renal cancer cell lines from primary (Caki-2, RCC6, 786-O, 769-P, SMKT-R2) and metastatic tumors (ACHN, Caki-1) and human normal cell lines including: lung/bronchus epithelial cells (NL-20/CRL-2503) were studied. For cell co-culture *in vitro* model inserts with a 0.4-um pore size were used. Proliferation was quantified with Alamar blue assay. 44 K Agilent whole genome oligo-microarrays were used for gene expression analysis for ACHN/NL-20 and Caki-2/NL-20 co-cultures. MAPK, EGF-EGFR, and TGFγ signaling pathways have been identified as significantly deregulated upon renal cell cancer and normal lung cell interaction. Moreover upon interaction in ACHN cell line 24 (GeneOntology) to 64 (WikiPathways) pathways were deregulated, while in Caki-2 cell line only 2 (GeneOntology) to 19 (WikiPathways) pathways were deregulated. Identified signaling pathways may be considered as potential therapy targets in metastatic renal cancer.

Key words: renal cell carcinoma, metastasis, gene expression, lung, cell-cell interaction.

Poster

KW016-00007-2016-01

Comparative analysis of tissue expression of different insulin-like growth factor 1 (IGF-1) mRNA isoforms in cancer and in the non-neoplastic diseases of large intestine

Aldona Kasprzak[1], Małgorzata Andrzejewska[1], Witold Szaflarski[1], Jacek Szmeja[2], Tomasz Kościński[2], Elżbieta Siodła[1], Agnieszka Seraszek-Jaros[3], Maria Koczorowska[4], Elżbieta Kaczmarek[3], Maciej Zabel[1]

[1]Chair of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[2]Chair of General Surgery, Endocrinological and Gastroenterological Oncology, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Bioinformatics and Computational Biology, Chair of Clinical Pathomorphology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Molecular Virology, A. Mickiewicz University in Poznań, Poznan, Poland

Introduction: Colorectal cancer (CRC) represents one of the most frequent malignancies worldwide (close to 1.5 million affected patients). It represents a multifactorial disease. Good diagnostic and prognostic markers for the tumour continue to be looked for. Components of insulin-like growth factor (IGF) system play significant roles in mitogenesis, inhibition of apoptosis, augmented proliferation, transformation, survival and formation of metastases. Proofs are available for a relationship between serum concentration of IGF-1 and risk of CRC. The role of alternate splicing of IGF-1 gene in carcinogenesis, including colorectal carcinogenesis is less known.

Aim of the study: Examination of the total IGF-1 gene expression and tissue content of the variant IGF-1 mRNA isoforms (P1, P2, A, B, C), in CRC and in non-neoplastic diseases of the large intestine as related to cell proliferation markers and clinical data.

Material and methods: An advantage was taken of 31 pairs of tissue material isolated from patients with CRC (studied group/control) and from 17 patients with non-neoplastic colorectal lesions (mainly inflammatory polyps/control). The studies included qRT-PCR technique and the immunohistochemistry (IHC).

Results: A significantly lower expression of total IGF-1 mRNA and of all IGF-1 mRNA isoforms was demonstrated in CRC and in non-neoplastic lesions (pseudotumours), as compared to the control, with preserved activity of both promoters of IGF-1 (P1 and P2) and production of the remaining splicing isoforms of the gene (A, B and C) in all studied groups. Our study show the positive correlation between expression of the IGF-1P2 and IGF-1B mRNAs in CRC and production of Ki-67 mRNA. In pseudotumours an increased tissue expression of IGF-1B mRNA isoform was detected as compared to CRC and its positive correlation with expression of the proliferative cell nuclear antigen (PCNA).

Conclusions: Carcinogenesis in human colon and rectum is accompanied by alterations in tissue expression of IGF-1 gene. Role of the two promoters (P1 and P2) of IGF-1 gene seems to be equivalent in formation of IGF-1 transcripts at the tissue level both in the control and on pathological colorectal lesions. Among the studied IGF-1 mRNA isoforms, local production of IGF-1P2 and IGF-1B mRNAs may serve as a marker of an increased cell proliferation in CRC. At early stages of human colorectal carcinogenesis the IGF-1B mRNA isoform seems to play the most important biological role.

Key words: colorectal cancer, inflammatory polyps, IGF-1 mRNA isoforms, qRT-PCR.

Poster

KW016-00053-2016-01

LATS1 tumor suppressor is involved in cancer stem cell formation and chemoresistance in melanoma

Urszula Kazimierczak, Ewelina Dondajewska, Aleksandra Glapa, Aleksandra Nijak

Poznan University of Medical Sciences, Poznan, Poland

Melanoma belongs to the most aggressive human cancers. In metastatic phase it is resistant to systemic treatment. Despite a recent progress in the identification of a number of therapeutic molecular targets or inhibitors of immune checkpoints, metastatic melanoma is still uncurable. Such a high invasiveness and metastatic potential of melanoma result from several mutations and activation of different signal transduction pathways. Two of them- PI3K/Akt (Akt) and ERK/Raf/Ras (MAPK) are particularly involved in melanoma. Effective signal transduction however requires their coordination with other signaling pathways. Our study indicated a relationship between Akt and Ras proteins, and LATS1 kinase, which is one of the key proteins of 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 by affecting epitelial-to-mesenchymal transition (EMT) and cancer stem cells (CSC). Cancer stem cells, or melanoma initiating cells (MIC) regarding melanoma, have recently focused a significant attention. It is believed that this population is responsible for the formation of tumor metastases and resistance to treatment. Our study showed a significant correlation between the level of tumor suppressor LATS1 and the expression of MIC markers. Moreover, LATS1 knocked down cells are more sensitive to chemotherapy. Further analysis of Hippo cascade proteins, their connection with other signaling pathways involved in tumorigenesis, and with melanoma initiating cells, will provide a better understanding of the mechanisms of melanoma pathogenesis and will help to find the new therapeutic targets for more effective treatment.

Key words: melanoma, LATS1, CSC, chemoresistance.

Poster

KW016-00054-2016-01

Gene set enrichment analysis and ingenuity pathway analysis of metastatic clear cell renal cell carcinoma cell line

Mohammed Imran Khan[1], Konrad J. Dębski[2], Michał Dabrowski[2], Anna M. Czarnecka[1], Cezary Szczylik[1]

[1]Molecular Oncology Laboratory, Department of Oncology, Military Institute of Medicine, Warsaw, Poland
[2]Bioinformatics Laboratory, Center of Neurobiology, Nencki Institute of Experimental Biology PAS, Warsaw, Poland

In recent years, genome wide RNA expression analysis has become a routine tool that offers a great opportunity to study and understand the key role of genes that contribute to carcinogenesis. Various microarray platforms and statistical approaches are implemented to identify genes that might serve as prognostic bio-markers and used for anti-tumor therapies in future. Metastatic renal cell carcinoma (mRCC) is a serious life-threatening disease. There are few treatment options for metastatic RCC patients.

We performed one-color microarray gene expression (4X44K) analysis of metastatic RCC cell line Caki-1 and healthy kidney cell line ASE-5063. 1921 genes were differentially expressed in Caki-1 cell line (1023 up-regulated and 898 down-regulated). Gene set enrichment analysis (GSEA) and Ingenuity Pathway Analysis (IPA) approach was used to analyse these differentially expressed data from Caki-1.

The objective of this research is to identify complex biological changes that occur during metastatic development using Caki-1 as a model RCC cell line. Our data suggest that there are multiple de-regulated pathways associated with mccRCC including ILK Signaling, Leukocyte Extravasation Signaling, IGF-1 Signaling, CXCR4 Signaling, and PI3K/AKT Signaling. The IPA upstream analysis predicted top transcriptional regulators which are wither activated or inhibited such as ER, TP53, KDM5B, SPDEF, CDKN1A. The GSEA approach was used to further confirm enriched pathway data following IPA analysis.

Key words: metastatic renal cell carcinoma, gene set enrichment analysis, IPA analysis.

This study was supported by the Foundation for Polish Science TEAM project TEAM/2010-6/8.

Poster

KW016-00002-2016-01

Endocannabinoid system (ECS) in human renal cell carcinoma

Mohammed Imran Khan[1], Anna A. Sobocińska[1,2], Igor Helbrecht[1], Cezary Szczylik[1], Anna M. Czarnecka[1]

[1]Department of Oncology with Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland
[2]Faculty of Biology, Warsaw University, Warsaw, Poland

The therapeutics potential of cannabinoid has been extensively been studied in many *in vivo* and *in vitro* research. Cannabinoid involves in tumor growth suppression and inhibit angiogenesis investigated by many groups. Many of their anti-tumor effects are mediated through endocannabinoid system (ECS) via activation of CB1 and CB2 receptors. These receptors are abundantly expressed in the brain and fatty tissue of human body. Despite all recent development in molecular biology, knowledge about the distribution of CB1 and CB2 receptors in human kidney and data concerning their dysregulation in renal cell carcinoma (RCC) is still not well documented. To address this gap in our knowledge we began to explore the role of ECS in human renal cell carcinoma derived cell lines. In this study, we evaluated expression of cannabinoid receptors (CB1 and CB2) employing different molecular biology techniques like real-time PCR, western blot, immunocytochemistry (ICC) and flow cytometry analysis. Selective cannabinoid receptors agonist and antagonist (separately for CB1 and CB2) were used for cell proliferation using alamar blue cell viability assay. Our result confirm the presence of CB1 and CB2 receptors in RCC cell lines both at mRNA and protein level. Cannabinoid receptor agonists are highly selective for exerting anti-proliferative effects on RCC cells. Furthermore, apoptosis was observed with WIN 55,212-2 (selective agonist for CB1 and CB2 receptors).

Key words: renal cell carcinoma, endocannabinoid system (ECS), CB1, CB2.

This work was funded by National Science Centre (NCN) grant "PRELUDIUM", grant no. UMO-2013/09/N/NZ5/02809.

Poster

KW016-00027-2016-01

Targeting the hypoxic malignant plasma cells by inhibiting the HIF-1 axis

Kinga Kocemba[1], Barbara Ostrowska[1], Przemysław A. Pękala[1], Sonia Trojan[1], Marcin Majka[2], Piotr Laidler[1]

[1]Chair of Medical Biochemistry, Jagiellonian University Medical College, Krakow, Poland
[2]Department of Transplantology, Chair of Clinical Immunology and Transplantology, Jagiellonian University Medical College, Krakow, Poland

Multiple myeloma (MM) is defined as a clonal expansion of malignant plasma cells in the bone marrow (BM). Since MM mainly progresses in the bone marrow cavity, signal from microenvironment play a crucial role in plasma cell growth, survival and migration. Of particular interest is the observation that myelomatous BM environment is hypoxic. According to the current status of knowledge, during MM progression hypoxia significantly contribute to chemoresistance, angiogenesis, invasiveness, metastasis and induction of immature phenotype in malignant plasma cells. Therefore, therapeutic strategies that selectively target hypoxia might be of high importance in the treatment of myeloma patients. In our study we investigated 4 potential HIF1 transcription factor inhibitors upon which hypoxic cell survival depends. Briefly, malignant plasma cells were culture in normoxic, hypoxic and hypoxia/re-oxygenation culture conditions in the presence and absence of HIF1 inhibitors (chetomin, echinomycine, 17AAG and KC7F2). Upon indicated time points the number of living cells was determined by MTT assay adjusted for suspension cells. The proper hypoxic response of malignant plasma cells was confirmed by the HIF1α stabilization and induction of hypoxia target gene expression PFKFB4 and BNIP3. Our study revealed that all four tested HIF1 alpha inhibitors, in the certain range of concentrations, show a selective activity against hypoxic cells with a low or even no activity against normoxic cells. Nevertheless, not all the inhibitors kept high effectiveness during hypoxia-reoxygenation culture conditions. Taken together, our data suggest that HIF1 axis inhibitors may serve as good starting points for developing new anti-MM therapeutic strategies specifically targeting hypoxic malignant plasma cells in MM patients.

Key words: hypoxia, HIF1α, multiple myeloma.

Praca powstała w wyniku realizacji projektu badawczego nr 2013/09/N/NZ1/01873 finansowanego ze środków Narodowego Centrum Nauki.

Poster

KW016-00033-2016-01

lncRNAs as new biomarkers in head and neck cancers

Tomasz Kolenda[1,2], Marta Kapałczyńska[1], Anna Teresiak[1], Kacper Guglas[1], Renata Bliźniak[1], Łukasz Łuczewski[3,4], Wojciech Golusiński[3,4], Katarzyna Lamperska[1]

[1]Genetic Laboratory, The Greater Poland Cancer Centre, Poznan, Poland
[2]Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland
[3]Department of Head and Neck Surgery, The Greater Poland Cancer Centre, Poznan, Poland
[4]Department of Head and Neck Surgery and Laryngological Oncology, Poznan University of Medical Sciences, Poznan, Poland

Introduction: The head and neck squamous cell cancers belong to the group of tumors with poor prognosis and high mortality. In the most cases treatment is based on the surgery and radiotherapy, whereas chemotherapy is seldom applied due to low cancer cells response. lncRNAs are a new class of non-coding RNA which are longer than 200 bp. The human genome possesses about 7000–23 000 lncRNAs. Deregulation of lncRNAs is shown in many diseases, including cancer. Changes in their expression can potentially be used as new biomarkers and hence lead to treatment personalization.

Aim of the study: Analysis of selected lncRNAs in tumour and margin samples obtained from head and neck cancer patients.

Material and methods: Tested samples (cancer, margin and matched healthy tissues) were obtained from 22 patients (7 women and 15 men), who were surgically treated in the Greater Poland Cancer Centre. Total RNA was isolated using TRI reagent, concentrated and purified using the High Pure miRNA Isolation Kit. cDNA library was performed using iScript cDNA Synthesis Kit and taken to qRT-PCR reaction (SYBR Green master mix). All real-time PCR data was analyzed by calculating the ΔΔCt, normalizing against expression of U6.

Results: In the case of Meg3 ($p = 0.0065$), Uca1 ($p = 0.0168$) and Malat1 ($p = 0.0477$) differences in the expression level between the normal and cancer tissues were observed. Other examined lncRNA (HOTAIR, SRA1, LET, SNORD, RRP1B and CDKN2B-AS1) were not changed statistically significant (*t*-test, $p < 0.05$). In margin samples from the same patients diffrent expression of lncRNA (up- or down regulated) were obsrved.

Conclusions: Our observations indicate that lncRNA are involved in the process of tumorigenesis and some of them can be used as indicators for distinction between tumor and healthy tissues. Our results also highlight the need of deeper analysis of lncRNA expression in tumors of the head and neck area including analysis of different localizations separately in order to define the specific diagnostic panel.

Key words: lncRNA, head and neck squamous cell carcinoma, biomarker.

Poster

KW016-00042-2016-01

Biological role of let-7d and miR-18a in cancer cells

Tomasz Kolenda[1,2], Marcin Ruciński[3], Anna Teresiak[1], Marta Kapałczyńska[1], Renata Bliźniak[1], Weronika Przybyła[1], Anna Kowalik[4], Katarzyna Lamperska[1]

[1]Laboratory of Cancer Genetic, Greater Poland Cancer Center, Poznan, Poland
[2]Postgraduate School of Molecular Medicine, University of Warsaw, Warsaw, Poland
[3]Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[4]Medical Physics Department, Greater Poland Cancer Centre, Poznan, Poland

Introduction: Head and neck squamous carcinoma (HNSCC) is one of the most invasive types of cancer with high mortality. In our previous study, the correlation between expression of let-7d and miR-18a in cancer cells was observed. Let-7d is usually described as tumor suppressor whereas miR-18a is known as oncogene, but there are reports suggesting this classification incomplete. We still don't know whether let-7d and miR-18a together influence on cancer cells behavior.

Aim of the study: Analysis of biological role of let-7d and miR-18a in cancer cell models.

Material and methods: Over-expression of let-7d, miR-18a and both of them in the SCC-25 and SCC-040 cell lines was obtained using lentiviral vectors. The proliferation ratio, spheres forming capacity, wound healing ability were checked. The expression of selected genes was measured using qRT-PCR. The models were tested for irradiation and chemical response by clonogenic assay and MTT assay, respectively. Global gene expression of cell line models was analysed by Affymetrix GeneChip.

Results: The common over-expression of let-7d and miR-18a caused proliferation ratio decreasing and influenced on ability to closing the wound area. Changes in sphere forming capacity were not observed. Only in the case of SCC-040 models, decreased cell survival after irradiation was observed. The common over-expression of let-7d and miR-18a caused stronger cell lines response to 5-FU, cisplatin and doxorubicin. We observed global changes of genes expression in cell line models.

Conclusions: Expression of let-7d and miR-18a increased or reduced jointly in the same HNSCC cancer tissue. Our results show, that the common over-expression of let-7d and miR-18a creates unique cell phenotype with different behavior compared to cells with up-regulated let-7d or miR-18a separately. Let-7d and miR-18a take part in EMT process, as well as they influence on cancer stem cell populations. However, we did not observe significant changes in gene expression connected with these processes. Only in the SCC-040 cell line models influence of let-7d and miR-18a on radiosensitivity is observed, what indicates the role of genetic background in this phenomenon. We did not observe dramatically changes in cell response after chemoexposure, but the tendency of lower cell survival in let-7d and miR-18a models over-expression is noticeable.

Key words: let-7d, miR-18a, miRNA, gene expression, HNSCC.

Poster

KW016-00050-2016-01

Long-range chromatin looping between enhancer-like elements and the ROD1 promoter is specific to prostate cancer cell lines

Marta Kubiak[1], Marzena Anna Lewandowska[1,2]

[1]Department of Molecular Oncology and Genetics, Innovative Medical Forum, The F. Lukaszczyk Oncology Center, Bydgoszcz, Poland

[2]Department of Thoracic Surgery And Tumors, The Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Torun, Poland

Introduction: ROD1 mediates pre-mRNA alternative splicing and regulates cell proliferation, differentiation and migration. The protein is localized in the perinucleolar compartment (PNC). Our previous studies have shown overexpression of ROD1 in prostate cell lines comparing to Caco2, HT29, CG and VH10.

Aim of the study: Our aim was to evaluate the spatial organization of the ROD1 locus using Chromosome Conformation Capture (3C) and determine if chromatin remodeling regulates ROD1 expression in prostate cancer.

Material and methods: 3C involves formaldehyde crosslinking, digestion with HindIII and intramolecular ligation of the crosslinked fragments with T4 ligase. 3C library was analyzed by real-time PCR using the Taqman probe and primers specific for certain restriction fragments [1]. 3C libraries were prepared for prostate cancer cells lines, differing in the metastatic potential and PNC prevalence (PC3, PC3M), and control fibroblast cell lines (VH10).

Results: Spatial organization of 220 kb locus encompassing the ROD1 gene was evaluated. Two enhancer-like elements localized at –63 kbp and +48 kbp were found. Long-range chromatin looping between them and ROD1 promoter was observed in prostate cancer cell lines but not in fibroblasts. The ROD1 gene spatial conformation and the flanking regions regulate its expression, explaining its overexpression in prostate cancer. Conversely, control fibroblasts had a low ROD1 expression and no chromatin interactions.

Conclusions: Chromatin looping between –63kbp and +48kbp and ROD1 promoter precedes the transcriptional activation of ROD1 in prostate cancer and is not observed in fibroblasts with a low expression of ROD1.

Future directions: To further evaluate results of the spatial chromatin architecture in ROD1, we will determine transcription factors or chromatin remodellers using Genome Browser and Chromatin Immunoprecipitation – PCR.

Key words: gene expression, chromatin conformation, prostate cancer, chromatin looping.

Reference

1. Kubiak M, Lewandowska MA. Can chromatin conformation technologies bring light into human molecular pathology? Acta Biochim Pol 2015; 62: 483-9.

Poster

KW016-00005-2016-01

A model of iron oxide/silk composite material for production of superparamagnetic spheres

Kamil Kucharczyk[1,2], Sylwia Haracz[3], Jakub Rybka[3], Mariusz Ślachciński[4], Michał Krupiński[5], Andrzej Mackiewicz[1,2], Michael Giersig[3,6], Hanna Dams-Kozłowska[1,2]

[1]Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Diagnostics and Cancer Immunology, Greater Poland Cancer Centre, Poznan, Poland
[3]Faculty of Chemistry, Adam Mickiewicz University, Poznan, Poland
[4]Faculty of Chemical Technology, Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Poznan, Poland
[5]Department of Materials Science, Polish Academy of Sciences, The H. Niewodniczanski Institute of Nuclear Physics, Krakow, Poland
[6]Institut für Experimentalphysik, Freie Universität Berlin, Berlin, Germany

The remarkable mechanical properties, biocompatibility and biodegradability make a brief description of spider silk characteristics. Silk can be processed into different morphological forms such as fiber, film, hydrogel, scaffold, capsules, micro- and nanospheres. These properties make silk an ideal material for biomedical applications. Moreover, the genetic engineering enable to obtain silk of modified properties, which may be useful for further production of composite materials.

The aim of the study was to produce and characterize iron oxide/silk sphere composite for cancer diagnostics and therapy.

The bioengineered spider silk – EMS2 was constructed based on the consensus motif of MaSp2 spidroin from Nephilla clavipes spider. The 15 times repeated motif was modified by a glutamic acid addition. The protein was produced in *Escherichia coli* expression system and purified by thermal denaturation method. Iron oxide nanoparticles were prepared by co-precipitation method. To prepare silk nanospheres, the iron oxide suspension and silk solution were mixed with potassium phosphate. The morphology, elemental composition of spheres and distribution of iron oxide nanoparticles were determined using SEM/EDS microscopy. Zeta potential was measured using Zetasizer analyzer. Superconducting quantum interference device (SQUID) was used to investigate the magnetic properties of sphere composites. Secondary structure of spheres was analyzed by FTIR spectroscopy. The cytotoxicity analysis was investigated by MTT assay.

The bioengineered spider silk protein – EMS2 was constructed, produced and purified. Both EMS2 and iron oxide/EMS2 composite formed spheres what was confirmed with SEM/EDS examination. The loading of iron nanoparticles was confirmed by Zeta potential and MIP-OES analysis of spheres. SQUID analysis indicated that the spheres made of iron oxide/EMS2 composite demonstrated superparamagnetic properties. FTIR analysis indicated that secondary structure of EMS2 when mixed with iron oxide was changed in time. The cytotoxicity assay revealed that both EMS2 and iron oxide/EMS2 spheres composite were not toxic.

Magnetic resonance imaging, targeted drug delivery or hyperthermia are the areas in which iron oxide nanoparticles can be applied. Our studies showed that the superparamagnetic composite spheres can be produced by mixing spider silk protein and iron oxide nanoparticles. These composite spheres can be potentially applied for cancer diagnostics and therapy.

Key words: bioengineered spider silk, iron oxide nanoparticles, cancer diagnostics and therapy.

Poster

KW016-00043-2016-01

Analysis of T cell immune response against cancer initiating cells marker ALDH in patients treated with therapeutic genetically modified melanoma vaccine

Eliza Kwiatkowska-Borowczyk[1,2], Katarzyna Tomela[1], Anna Przybyła[1], Katarzyna Gryska[1], Patrycja Czerwińska[1], Anna Kozłowska[1], Andrzej Mackiewicz[1,2]

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

Introduction: Administration of therapeutic cancer vaccine induces T cells specific for melanoma antigens in the vaccine. Due to the phenomenon of epitope spreading, T cells specific to other antigens in the tumour can emerge. Consequently, along with evaluation of immune response driven by vaccine antigens, immune response to tumour cells, including cancer-initiating cells (CISc), should be measured. The unique feature of AGI-101H vaccine is the expression and high activity of ALDH. That ALDH expression may account for direct induction of immune response that target cancer initiating cells in vaccinated melanoma patients.

The aim of the study was to evaluate the specific immune response against CICs elicited by AGI-101H vaccine.

Material and methods: PBMC were isolated and cryopreserved from HLA-A2 positive melanoma patients treated with AGI-101H (before vaccine and after 6 or 11 days). Controls were untreated malignant melanoma patients and healthy persons. To enumerate ALDH1A1-specific CD8+ T cells freshly isolated PBMC were stained with MHC Dextramer® reagents. The effector functions of CTLs were analysed by measuring degranulation and IFN-γ production.

Results: Dextramer staining revealed increase in number of ALDH1A1-specific CD8+ T cells in vaccinated patient compared to the controls. Moreover, there was a significant difference in number of ALDH1A1-specific CD8+ T cells before and after treatment. *In vitro* stimulation of PBMC isolated from vaccinated patients with immunogenic ALDH1A1 peptide induced significant IFN-g response of CD8+ T cells.

Conclusions: ALDH1A1 might serve as a biomarker of patients' immune response to melanoma CICs and for monitoring response to AGI-101H therapy.

Key words: melanoma, immunotherapy, cancer initiating cells, immune response, ALDH.

This work was supported by the National Centre for Research and Development grant INNOMED/I/6/NCBR/2014.

Poster

KW016-00045-2016-01

Analysis of circulating myeloid derived suppressor cells in melanoma patients treated with therapeutic genetically modified vaccine

Eliza Kwiatkowska-Borowczyk[1,2], Anna Tylutka[1], Katarzyna Tomela[1], Anna Przybyła[1], Katarzyna Gryska[1], Patrycja Czerwińska[1], Anna Kozłowska[1], Andrzej Mackiewicz[1,2]

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

Introduction: Advanced cancer is associated with serious disorders of the immune system and the most common manifestation is immunosuppression. Among key mechanism responsible for malfunction of immune system in cancer patients are myeloid-derived suppressor cells (MDSCs). The common feature of this heterogeneous population of cells, that includes immature macrophages, granulocytes, dendritic cells and others, is strong immunosuppressive potential and the ability to suppress T-cell responses. Therefore, MDSCs limit tumour immunity and promote tumour progression in both animal model and cancer patients. The frequency of MDSCs is significantly elevated in melanoma patients and inversely correlate with immune responses to cancer vaccines. Since tumour-induced immunosuppression is one of the major barriers for successful immunotherapy, new strategies that target MDSCs accumulation and function are being developed and tested in animal and *in vitro* models.

Material and methods: Monocytic MDSCs (Mo-MDSC), characterized as Lin-CD11+CD14+HLA-DR- cells, were analyzed by flow cytometry in melanoma patients treated with AGI-101H, untreated melanoma patients and healthy volunteers. Freshly isolated PBMCs (by density gradient using Histopaque®1077) were stained with cocktail of monoclonal antibodies: CD3-FITC, CD19-FITC, CD20-FITC, CD56-FITC, CD57-FITC (referred to as Lin), CD11b-APC, CD14-PerCP-Cy5.5 and HLA-DR-PE. The frequency of circulating Mo-MDSC in vaccinated melanoma patients was examined on the day of vaccination and six days after treatment.

Results: Statistical analysis of cytometric data revealed significant increase in Mo-MDSCs in untreated melanoma patient comparing to healthy controls, that is consistent with published data. Immunization with AGI-101H resulted in a decrease of circulating Mo-MDSCs. Percent of Lin-CD11b+CD14+HLA-DRlow/– in PBMCs and % of HLA-DRlow/– in Lin-CD11b+CD14+ population, measured on the day of vaccination (usually 4 weeks after previous vaccine dose) as well as on day 6th after treatment were significantly reduced comparing to untreated patients. Moreover, paired data analysis indicated reduction of MoMDSC in the peripheral blood of AGI-101H treated patients 6 days after vaccination comparing to % Mo-MDSC on the day of treatment.

Conclusions: Obtained results indicate possible direct impact of AGI-101H vaccine on circulation MDSCs in melanoma patients, that will be further examined.

Key words: melanoma, MDSC, cancer immunotherapy.

Poster

KW016-00040-2016-01

Size (and time) matters: improvement of stem cells differentiation into chondrocyte-like cells

Michał Lach[1,2], Karolina Jankowska[1], Magdalena Richter[3], Jarosław Pawlicz[3], Tomasz Trzeciak[3], Wiktoria Suchorska[1]

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

The osteoarthritis in the future will gain a status of civilization disease in highly-developed countries. The present techniques treatment of articular cartilage lesions are not sufficient due to formation of undesirable fibrocartilage instead of typical hyaline in repaired depletion. According to many studies involving chondrogenesis, the improvement of differentiation protocols is still a challenge. One of the approaches in differentiation of pluripotent cells is taking advantage of their natural ability to form three germ layers via embryoid body (EB) formation. The various studies over controlling the extracellular microenvironment revealed that, size of wells or EB used can results in favouring distinct germ layers.

In present study the human embryonal stem cells (BGV01, ATCC) in the formation of EB consisted of 500–2000 cells per well were used. The homogenous EB were cultured for 5, 10 and 15 days in suspension. Based on the gene expression analysis of germ layer markers, the most prochondrogenic EBs were selected. In order to confirm the increased production of chondrocyte-like cells, EBs from 5th and 15th day were cultured in prochondrogenic medium with addition of transforming growth factor β type 3 (TGF-β3) for 21 days. The assessment of EB transformation into chondrocyte-like cells by real-time polymerase chain reaction (RT-qPCR) and immunofluorescence staining for prochondrogenic and pluripotent markers were performed. Moreover, the production of proteoglycans by obtained cells during differentiation by an alcian blue staining were confirmed. The established cell line of human articular cartilage (HC-402-05a, ATCC) and human embryonic stem cell line (BGV01, ATCC) were used as a positive and negative control, respectively.

The results indicated increased prochondrogenic properties in EBs, which were formed from 500 cells and were picked for differentiation at 5th day of their formation. The prolonged time of suspension culture during EB formation (for 15 days) resulted in decreased expression of markers related to chondrocytes.

Key words: stem cells, regenerative medicine.

Poster

KW016-00041-2016-01

The stem cells response for cancer therapy

Magdalena Łukjanow[1], Ewelina Augustyniak[1,2], Wiktoria Maria Suchorska[1,3]

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

Stem cells (SCs) are undifferentiated cells with long-term self-renewal and the capacity to develop into specialized cells. These features allow to use SCs in the treatment of a wide range of currently unresponsive diseases. In the future, the usage of SCs will be related to their unavoidable exposure to ionizing radiation (IR) during treatment and routinely diagnosis. Radiotherapy is the most often used method in adjuvant treatment of tumorous alterations. It is known that IR causes DNA-damage in cancer cells. However, the knowledge concerning influence of anti-cancer therapies on healthy cells, including SCs is still limited. The issue of the response of SCs on ionizing radiation and genetic integrity is crucial in the context of the application of these cells in clinical practice.

In this study, the investigation the of the response of pluripotent SCs to ionizing radiation was performed. In the experiment two types of pluripotent SCs were used: human embryonic SCs (hESCs)(BGV01, ATCC) and human induced pluripotent SCs (hiPSCs), as well as primary human dermal fibroblasts (PHDF) as a control. The investigated cells were treated with ionizing radiation in the range of low (0; 0,25; 0,5; 1 Gy) and high doses (2; 5; 10; 15 Gy). Then, the analyses of γH2AX by flow cytometry and expression of genes taking part in DNA repair of double strand breaks (p53, BRCA2, RAD51, XRCC4, PRKDC) by quantitative real-time polymerase chain reaction (qRT-PCR) were performed.

We revealed that SCs are more susceptible to DNA damage after IR treatment compared to differentiated cells. The activation of genes involved in DNA damage response (DDR) was observed at higher level in pluripotent SCs than in human fibroblasts. It proves that the DNA repair mechanisms of SCs are much more effective. However, there is worth mentioning that DDR of hESCs and hiPSCs are different, what results in dissimilar gene expression profile.

In conclusion, SCs have enhanced DDR mechanisms. The DNA repair mechanisms in pluripotent SCs are more efficient than those in differentiated cells. The accumulation of DNA damage may contribute to spontaneous differentiation, apoptosis or accumulation of mutations. The powerful and enhanced DDR of SCs avoids the formation of mutations *de novo* and consequently the maintenance of their genetic stability.

Key words: stem cells, DNA damage, DNA repair, ionizing radiation.

The present study was supported by the National Science Centre (grant number 2012/07/E/NZ3/01819) and by The Greater Cancer Centre (grant number1/03/2016/PRB/WCO).

Poster

KW016-00055-2016-01

Liposomal forms of the natural bioactive compounds in pancreatic cancer treatment

Mohamed Mahmud, Nina Filipczak, Jerzy Gubernator

Department of Lipids and Liposomes, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland

The purpose of this study is to design a stable liposomal formulation for the natural compounds exhibiting anticancer activity. Natural compounds are food chemicals present in different natural sources (marine organisms, microorganisms, plants and etc.). They have pharmacological activity to suppress carcinogenesis and inhibits multiple signalling pathways of the cancer cells. The poor aqueous solubility and low bioavailability of some natural compounds restrict its possible clinical application. Liposomes are one of the best carrier system which have been studied to encapsulate different naturals compounds either hydrophilic (in their core) or hydrophobic (in their lipid bilayer) ones. In present study selected natural compounds (curcumin, capsaicin and α-lipoic acid) were encapsulated in a liposomes using lyophilization/extrusion method. The liposomal forms of natural compounds were characterized in terms of basic parameters; as, the size, zeta potential, encapsulation efficiency and stability. The cytotoxicity was assessed using MTT assay for human pancreatic human cell lines AsPC-1 and BxPC-3.

The encapsulation efficiency was higher than 95% for a drug-to-lipid molar ratio of up to 0.05 for all formulations. Liposomes were stable for 45 days in the case of liposomal curcumin and 90 days in the case of liposomal capsaicin and α-lipoic acid. The cytotoxicity results showed that liposomal curcumin had a higher cytotoxicity toward AsPC-1 and BxPC-3 (IC50, 10.4 μM and 4.8 μM respectively) compared to other liposomal formulation containing capsaicin or α-lipoic acid. For liposomal capsaicin, the IC50 was 53.40 μM for AsPC-1 cell line. The liposomal α-lipoic acid was not cytotoxic for both used cell lines.

As conclusion we can ascertain that formulation of liposomal curcumin and capsaicin showed promising results in terms of cytotoxicity toward pancreatic cancer and can be considered as possible chemotherapeutics.

Key words: liposomes, curcumin, natural anticancer compounds, pancreatic cancer, chemotherapy, passive targeting.

Poster

KW016-00009-2016-01

Therapeutic effect of increased calcium delivery using microsecond electroporation in pancreatic cancer – *in vitro* study

Olga Michel[1], Justyna Piłat[1], Katarzyna Bieżuńska-Kusiak[1], Anna Zielichowska[2], Jolanta Saczko[1], Julita Kulbacka[1]

[1]Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
[2]Institute of Experimental Biology; University of Wroclaw, Wroclaw, Poland

In spite of considerable progress in diagnosing and treating cancers, pancreatic ductal adenocarcinoma is still one of the most aggressive and lethal malignancies. Therefore, a continuous search for new therapeutic agents is required. The oldest applied chemotherapeutic is 5-fluorouracil, which is frequently complemented by leucovorin calcium – reduced folic acid. Leuovorin stabilizes the drug binding synthase thereby enhancing cytostatic activity, simultaneously acting as a chemoprotectant. Notwithstanding, high concentration of intracellular calcium may induce apoptotic cell death. Electroporation (EP) represents an innovative technique that enables delivery of therapeutic agents directly into the cell through hydrophilic pores formed under electromagnetic field action. The combination of EP with chemotherapy may result in active drug dose reduction and consequently – limitation of side effects.

The aim of our study was to evaluate the influence of electrochemotherapy with Leucovorin calcium *in vitro* on human pancreatic cancer cell line EPP85-181P.

The cell culture was maintained in modified Leibovitz medium (L-15) in. 37°C and 5% CO_2. The electroporation was performed using the current intensity in the range of 400 and 800 V/cm (8 pulses with a length of 100 µs, interval length 1 s) in EP buffer containing solutions of leucovorin calcium in concentrations 5 µM and 10 µM, respectively. Cellular viability was measured using MTT assay after 24 and 72 hours of incubation. Apoptosis was assessed by immunocytochemical ABC method.

The results suggest that EP may effectively enhance the delivery of calcium into the cells causing a considerable decrease of mitochondrial activity. The best results were obtained after 72 hours incubation with 5 µM leucovorin combined with electric field of 800 V/cm. In this case using the electroporation caused the decrease of the cellular viability by 21% with respect to leucovorin alone, and by 36% comparing to the control.

However, after 24 hours of incubation, the differences between each parameter were minor, therefore further research on different pancreatic cancer cell lines need to be conducted.

Key words: pancreatic cancer, electroporation, leucovorin, calcium.

The study was supported by UMED project PBmn-132 of Wroclaw Medical University (K. Bieżuńska-Kusiak).

Poster

Incidence of brain metastasis from advanced cutaneous melanoma – clinicopathological analysis of patients who failed with local therapy. Single institution experience

Elzbieta Nowara, Jaroslaw Nieckula, Agnieszka Badora-Rybicka, Marcin Rajczykowski, Joanna Huszno

Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Branch Gliwice, Poland

Introduction: Brain metastases (BM) are frequently diagnosed in advanced cutaneous melanoma patients (CM) and indicate poor prognosis with short survival. There is still little known about the impact of BRAF mutations on the incidence of BM in CM patients. Some authors indicate the efficacy of selective BRAF inhibitors in patients with V600 BRAF mutation CM with BM. The aim of this study was to asses the incidence of BM from advanced CM with a special attempt to identify the prognostic factors for BM and overall survival (OS).

Material and methods: There was performed a systematic study of medical records of patients' (pts) with metastatic or inoperable CM treated with systemic therapy in COI. All pts with CM were diagnosed, treated and followed up in COI. 85 unselected pts with metastatic or inoperable CM were included to analysis and treated according to systemic therapy inclusion criteria set by Polish Ministry of Health. All pts received systemic therapy. Median age at the time of diagnosis was 59 years (range 23–80). The most frequent primary tumor localization was skin of the back (36%) and lower limb (21%) with median Clark of 3 and Breslow 3.5 mm. In 65% of pts V600 BRAF mutation was detected.

Results: The most common site of metastases were lung, brain and liver (52%, 28% and 24% respectively). Median time from CM diagnosis to metastases occurrence was 11 months. 82% of pts had multiorgan metastases. V600 BRAF mutation was detected more frequently in pts older than 50 years and in pts with primary tumor localized in lower limb (63% and 43%, respectively). Metastatic brain disease was present in 28% of pts. The majority (70%) of patients had multiple BM. significantly more frequently in pts with V600 BRAF mutation compared to pts without mutation, 45% vs. 16%, $p = 0.025$, but OS was similar, $p = 0.5$. Brain metastases were observed more frequently in pts with normal BMI than in overweight pts, 43% vs 23%, in males and in pts younger than 50 years (54% and 67% respectively). Patients with lung metastases and more likely to have BM (37% vs. 23%, $p = 0.126$).

During the observation time 57% of pts died with median OS of 30 months. Median OS was longer in pts younger than 50 years, $p = 0.04$.

Conclusions: Male, younger age, normal BMI, lung metastases were risk factors for BM. Patients with V600 BRAF mutation had more frequently BM, but overall survival was similar, $p = 0.5$.

The results should be taken into consideration with caution due to small pts sample.

Key words: brain metastases.

Poster

KW016-00011-2016-01

Modulation of Wnt signaling target gene expression by decitabine, panobinostat, PKF118-310 and niclosamide in head and neck squamous cell carcinoma cell lines

Jarosław Paluszczak, Robert Kleszcz, Violetta Krajka-Kuźniak, Wanda Baer-Dubowska

Department of Pharmaceutical Biochemistry, Poznan University of Medical Sciences, Poznan, Poland

The dysregulation of Wnt signaling has been indicated as one of the mechanisms of head and neck carcinogenesis. The induction of the transcriptional activity of β-catenin leads to enhanced expression of pro-tumorigenic genes associated with increased cell proliferation and motility such as CCND1, survivin, c-Myc, MMP7. The activation of Wnt signaling in head and neck carcinomas is most probably associated with the epigenetic silencing of genes which act antagonistically towards the pathway, e.g. DACT2.

The aim of the present study was to test the effect of epigenetic modulators – decitabine and panobinostat and inhibitors of Wnt signaling – PKF118-310 and niclosamide on the expression of β-catenin and GSK3β and the level of expression of catenin target genes: CCND1, survivin, c-Myc, MMP7. Head and neck squamous cell carcinoma cell lines derived from floor of mouth (H314 cell line) and pharynx (BICR6 and FaDu cell lines) were used. Cell viability was assessed using the MTT assay. Cells were incubated with selected doses of the compounds for 48 and/or 72 hours and subsequently fractionated with the Universal DNA/RNA/Protein Purification Kit (EURx, Poland). Total RNA was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit (Thermo) and subsequently amplified in LightCycler 96 (Roche) using HOT FIREPol EvaGreen qPCR Mix (Solis BioDyne, Estonia) with the addition of specific primers. The methylation of DACT2 was assessed with methylation-specific PCR.

The analyzed compounds did not affect the expression of either β-catenin or GSK3β. However, they showed differential modulation of the expression of CCND1, survivin, c-Myc, MMP7 depending on the cell line. Niclosamide and PKF118-310 reduced MMP7 transcript level in FaDu and H314 cells. Decitabine reduced the expression of c-Myc and panobinostat decreased the expression of MMP7 in FaDu cells. Decitabine and panobinostat decreased the level of expression of c-Myc and CCND1 in H314 cells. The expression of survivin was reduced in BICR 6 cells by decitabine and panobinostat. Moreover, decitabine led to the demethylation of the promoter sequence of DACT2 gene in FaDu and H314 cells.

The results indicate that decitabine, panobinostat, niclosamide and PKF118-310 can modulate the transcriptional activity of β-catenin in head and neck cancer cells.

Key words: Wnt pathway, β-catenin, epigenetics, head and neck cancers.

The work was supported by the Polish National Science Centre grant No. 2014/13/D/NZ7/00300.

Poster

KW016-00014-2016-01

In vitro drug sensitivity in canine lymphoma

Aleksandra Pawlak[1], Bożena Obmińska-Mrukowicz[1], Andrzej Rapak[2]

[1]Wrocław University of Environmental and Life Sciences, Wroclaw, Poland
[2]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland

Introduction: Due to high heterogeneity of canine lymphoma, the aim of the present study was to test *in vitro* the chemosensitivity of canine high grade primary lymphoma cells to various cytostatic drugs commonly used to treat dogs: 4-HO-cyclophosphamide, doxorubicin, dexamethasone, prednisolone, vincristine, etoposide, chlorambucil, lomustine, and cytosine arabinoside.

Material and methods: To determine the cell viability and drug ability to induce apoptosis two different tests were used: MTT assay and annexin V/propidium iodide staining.

Results: Both *in vitro* tests were found to be useful tools. Significant differences in the sensitivity, depending on the drug type, between B-, T- and mixed/null type lymphoma cells, were found for the majority of the tested drugs. B-type cells were the most sensitive *in vitro*, whereas T-type cells seemed to be the most resistant. Doxorubicin, chlorambucil, etoposide, and vincristine most strongly reduced the cell viability and induced apoptosis.

Conclusions: The use of *in vitro* assays, such as MTT test and especially Annexin V/PI assay may be a useful tool for predicting a response to the treatment of high grade lymphoma in dogs or improving the treatment outcomes in individual dogs.

Key words: canine lymphoma, chemosenstivity, canine leukemia.

Poster

KW016-00032-2016-01

Aluminum phthalocyanine as a new generation photosensitizer for photodynamic therapy of breast cancer cell lines

Justyna Piłat[1], Jolanta Saczko[1], Julita Kulbacka[1], Olga Wysocka[1], Anna Zielichowska[2]

[1]Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
[2]Department of General Zoology, University of Wroclaw, Wroclaw, Poland

Photodynamic therapy (PDT) is one of intensively developing treatment method in modern oncology. This minimally invasive method is based on phototoxic reaction that integrates action of three main components: the photoactive compound, a light source of appropriate wavelength and oxygen stored in the cells. Effectiveness depend on properties of cells and localization of photosensitizer and irradiation conditions. One of the most important part of therapy is to choose the appropriate, acting photosensitizer. There are ongoing studies that are aimed at selecting light-sensitive compounds, which have the most favorable physicochemical properties. Phthalocyanine dyes have a desirable optical properties and good chemical and thermal stability, therefore they could be suitable for use in PDT.

The aim of the study was to evaluate cytotoxic properties of second-generation photosensitizer (aluminum 1,8,15,22-tetrakis(phenylthio)-29H,31H-phthalocyaninechloride; PS) and its possible implementation in PDT on human breast cancer cell lines. Two cell lines were used: MDA-MB-175 and the high metastatic MDA-MB-231. Both lines were cultured under standard conditions. Cells were incubated with different concentrations of PS for 4 and 24 h. For irradiation set of parameters were used: 780 nm, 10 mW/cm^2 for 10 min. Toxicity assessment (with and without exposure to light) was tested by MTT cell proliferation assay. PS localization was determined by confocal microscopy with fluorescent dyes, for specific cellular structures – Hoechst for nucleus, and LysoTracker-Green for lysosomes.

Cytotoxicity study served for selection appropriate concentrations of cyanine that without the light does not negatively affect the cell. For further step 1 and 5 μM were selected. MTT assay showed significant differences between the amount of PDT-treated cells and the cells not irradiated. Viability of cells treated with PDT decreased to approx. 40% of the control cells. After 4 h incubation PS showed co-staining with LysoTracker revealing co-localization with the endolysosomal compartment, which is consistent with literature.

PDT with phthalocyanine photosensitizer seems to be a promising method for breast cancer treatment. For better results with increase of cell specificity we believe that the next step may be a combination of PS with targeting moiety.

Key words: photodynamic reaction, breast cancer, phthalocyanine.

Poster

KW016-00034-2016-01

The effect of wound fluids collected from the tumor bed after intraoperative radiotherapy (IORT) on stemness of breast cancer cell lines

Igor Piotrowski[1], Katarzyna Kulcenty[1], Dawid Murawa[2], Karolina Zaleska[1], Wiktoria Suchorska[1,3]

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

Breast cancer is the most common cancer in women, accounting for 25% of cases. Breast conserving surgery followed by a fractioned whole breast radiation therapy is the standard therapy for early breast cancer. Intraoperative radiation therapy (IORT) emerged as an alternative to EBRT (external beam radiation therapy) in the therapy of the early breast cancer. IORT is delivered to the tumor bed in a single dose during breast surgery. It was shown, that radiation not only kills residual tumor cells in tumor bed, but also affects tumor microenviroment which might contribute to reducing local recurrence rates. It was previously reported, that IORT alters the microenvironment through the modulation of wound healing response. Thus we wondered, whether wound fluids can change the stemness features of breast cancer cell lines and whether IORT plays inhibitory role in this process.

We collected wound fluids from patients who received IORT (WF-IORT) and from patients who underwent only surgical procedure (WF) 24 hours after the surgery. Two human breast cancer cell lines with different molecular status (basal – MDA-MB-468, luminal – MCF7) were chosen for further experiments. MDA-MB-468 is a basal breast cancer cell line negative for estrogen, progesteron and HER2 receptors (triple negative) with high percent of stem cells. MCF-7 is a luminal A breast cancer cell line positive for estrogen and progesterone receptor and negative for HER2 receptor with low percent of stem cells. The effects of WF-IORT and WF on stemness properties of selected breast cancer cell lines were analyzed by flow cytometry and RT-qPCR.

Our preliminary results show that, unlike WF collected from patients who underwent surgery alone, fluids collected from patients who received IORT suppressed cancer stem cell profile in both cell lines. In summary, surgical wound fluids from both groups (WF and IR-WF) affect the putative stem cell phenotype. In IR-WF group, the lower stem cell phenotype was observed compared to fluids harvested after surgery alone.

Key words: intraoperative radiotherapy, breast cancer, wound fluid, stemness.

Poster

Personalized *versus* directed therapy for CLL

Małgorzata Rogalińska[1], Jerzy Błoński[2], Paweł Góralski[3], Paweł Robak[4], Małgorzata Kubczyk[1], Aleksandra Szóstka[1], Aneta Rogalska[5], Aneta Koceva-Chyła[5], Jan Barciszewski[6], Henryk Piekarski[3], Tadeusz Robak[2], Zofia M. Kilianska[1]

[1]Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland
[2]Department of Hematology, Medical University of Lodz, Lodz, Poland
[3]Department of Physical Chemistry, Faculty of Chemistry, University of Lodz, Lodz, Poland
[4]Department of Experimental Hematology, Medical University of Lodz, Lodz, Poland
[5]Department of Thermobiology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, Poland
[6]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Aim of the study: In chronic lymphocytic leukemia (CLL) the coexistence of two populations of quiescent and cycling cells display a special challenge for choosing the optimal treatment. They differ in cell signaling, expression of factors involved in apoptosis realization, as well as the microenvironment factors, which effect diverse CLL patient's response to therapy. An analysis based on genetic alterations (eg. chromosomal aberrations) only is not enough for prognosis of patient's response to treatment.

To improve the final efficacy of treatment in chronic lymphocytic leukemia, *in vitro* analysis to determine the response of peripheral blood mononuclear cells to drugs before drug(s) administration *in vivo* was performed. The study involve four approaches, i.e. cell viability, apoptosis rate, differential scanning calorimetry (DSC), and Western blot to develop personalized therapy protocol based on patients' cell sensitivity to drug(s).

Results: Simultaneous analyses of cell viability, apoptosis rate, DSC profiles of nuclear fraction preparations, and PARP-1 expression by Western blot were applied to examine the ability of leukemic cells to enter apoptosis after their incubations to drug(s). CLL cells were incubated with fludarabine (F) or cladribine (C) combined with mafosfamide (M) – FM or CM and CM with rituximab (RCM), as well as with new potential anticancer agents: purine analogs or natural plant compounds. All anticancer agents induced apoptosis and a significant decrease of cell viability, an increase of apoptosis rate and decrease or even loss of thermal transition at 95 ±3°C in DSC scans of nuclear preparations were observed.

Conclusions: The evaluation of cell viability, apoptosis rate, the DSC profile's analysis of nuclear preparations and proteolytic cleavage of apoptotic marker (PARP-1) revealed leukemic cell sensitivity to anticancer agents. Such analysis before in vivo drug(s) administration seems to be helpful in selection of an optimal therapy for the CLL patient to avoid a potential resistance to treatment. The obtained results showed that CLL cell incubation with drug(s) has a prognostic value.

Key words: CLL, personalized therapy, anticancer agents, purine analogs, natural anticancer compounds, apoptosis induction.

Poster

KW016-00030-2016-01

Clinical relevance of microRNA expression profile in invasiveness of colorectal cancer

Klaudia Skrzypek[1], Mirosława Püsküllüoglu[2], Elżbieta Wiernasz[1], Aleksandra Ulman[1], Krzysztof Krzemieniecki[2], Marcin Majka[1]

[1]Department of Transplantation, Jagiellonian University Medical College, Krakow, Poland
[2]Department of Oncology, Jagiellonian University Medical College and Department of Clinical Oncology, University Hospital Krakow, Poland

Colorectal cancer (CRC) is one of the most frequently diagnosed malignancies in both men and women. Tumor growth and metastasis may be regulated by microRNAs (miRNAs), which affect gene expression. The aim of our studies was to create the list of potential miRNAs that may act as prognostic factors associated with clinical parameters such as staging, grading, spread of malignancy, and response to treatment.

Small RNA expression profile was analyzed in paraffin-embedded CRC samples from 50 patients by the next generation sequencing on the Illumina NextSeq 500 system. The differential expression analysis was done using the EdgeR statistical software package and TMM normalization.

We selected the list of many miRNAs associated with an increased tumor invasiveness based on histological analysis (grading parameter), such as for examples miR-483-5p or miR-483-3p. Our analysis enabled us to discover miRNAs associated with involvement of local lymph nodes and with different distant metastasis sites, such as distant lymph nodes, peritoneum, bones, lungs, liver and ovaries. For example, metastasis to distant lymph nodes was associated with increased expression of miR-483-5p, whereas to peritoneum with increased expression of miR-31-3p. Since KRAS mutation status affects colorectal cancer prognosis, we discovered many miRNAs associated with the mutation, such as for example miR-31-5p or miR-34a-5p. We selected also miRNAs associated with palliative or radical treatment intention which correlate with TNM cancer staging notation system. We have also discovered miRNAs associated with primary CRC localization: sigmoid, rectum and the rest of colorectal sites. Patients that are more than 60 years old displayed decreased levels of some miRNAs, such as for examples miR-215-5p. Woman displayed decreased expression of miR-483-5p than man. Gene ontology (GO) enrichment analysis enabled to identify potentially significant biological process associated with the differentially expressed miRNAs. Interestingly, we discovered not only miRNAs, but also other small RNAs associated with CRC invasiveness. What is more, we have also identified putative novel miRNAs, which are predicted from the sequences that do not map to any organism found in miRbase, or to other known RNA sequences.

To conclude, our comprehensive analysis discovered novel miRNAs, which may play roles as promising therapeutic or diagnostic targets. They need to be validated in further studies *in vitro*.

Key words: microRNA, colorectal cancer, metastasis.

Poster

KW016-00046-2016-01

Mesenchymal stromal cells (MSC) as carriers of IL-12 cDNA in treatment of mice bearing B16-F10 melanoma

Ryszard Smolarczyk[1], Natalia Kułach[1,2], Tomasz Cichoń[1], Magdalena Jarosz-Biej[1], Ewelina Pilny[1,3], Marek Rusin[1], Stanisław Szala[1]

[1]Center for Translational Research and Molecular Biology of Cancer, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland
[2]Department of Animal Physiology and Ecotoxicology, University of Silesia, Katowice, Poland
[3]Department of Organic Chemistry, Biochemistry and Biotechnnology, Silesian University of Technology, Gliwice, Poland

Mesenchymal stromal cells (MSC) are multipotent cells present in diverse animal tissues. MSC have the ability to differentiate into multiple cell lines, exhibit regenerative and immunoregulatory properties. MSC *in vivo* are an important functional element of hematopoietic stem cells niche and provide reservoir of a variety of cytokines and growth factors. MSC migrate to the site of injury and exert an exceptionally high tropism towards cancer cells.

Interleukin-12 is a cytokine with a broad spectrum of action: it exerts both immunostimulatory and anti-angiogenic properties. It activates CD4+, CD8+ and NK cells as well as triggers release of IFN-γ, all of which induce a strong immune response against cancer cells.

In the study MSC were used as specific IL-12 carriers in treatment of mice bearing B16F10 melanoma. This strategy allows the therapeutic cells to reach hard to access areas of tumors and facilitates their elimination. During migration, MSC release active IL-12 in the nearest vicinity of cancer cells. The local release of the cytokine in tumors leads to the stimulation of the immune system and destroys tumor cells.

Mesenchymal stromal cells (MSC) with Sca-1+ CD105+ CD90+ CD29+ CD44+ CD106– CD45– phenotype were isolated from murine bone marrow and the potential of isolated cells to differentiate into adipocytes and osteocytes was confirmed. MSC migration capacity towards tumor cells was evaluated in Boyden chamber. cDNA encoding the two subunits of murine IL-12 was cloned into the adenoviral vector. The transduction of murine MSC was conducted and an amount of IL-12 secreted by the cells was determined by ELISA. Modified MSC were administered at the site of the tumor.

Few days after administration of the modified cells a significant reduction in B16F10 tumor volume was observed. The therapeutic approach with modified MSC appears to be effective in eliminating tumor growth.

Key words: B16F10 melanoma, mesenchymal stromal cells, IL-12.

Poster

KW016-00044-2016-01

Insulin signaling in renal cancer cells

Wojciech Solarek[1], Michał Koper[2], Cezary Szczylik[1], Anna Czarnecka[1]

[1]Department of Oncology with Laboratory of Molecular Oncology, Military Institute of Medicine, Warsaw, Poland
[2]Institute of Genetics and Biotechnology, Faculty of Biology, University of Warsaw, Warsaw, Poland

Renal cell carcinoma (RCC), with renal pelvis cancer is predicted to be diagnosed in 62,700 new cases and to cause 14,240 deaths in United States in 2016. RCC in Poland is diagnosed in about 4600 new cases and leads to 2500 deaths. The pathological conditions like obesity and diabetes are related to disturbances in insulin and probably insulin-like growth factors (IGFs) signaling, what may also influence RCC tumorigenic processes. Insulin regulates carbohydrate and fat metabolism on whole organism level. Insulin acts through the Insulin Receptor (IR), but in high concentration it inhibits autophagocytosis, proteasome activity and apoptosis. IGFs are produced mainly in liver and regulates processes connected with cells growth and proliferation. It induce two crucial intracellular signaling pathways: PI3K-Akt-mTOR pathway and Ras-MAPK pathway, which affect processes connected with cancer development and progression. In our study, human renal cancer cell lines (Caki-2, 786-O, 769-P, ACHN, Caki1) and control healthy kidney and embryonic kidney cell lines (PCS-400-010, HEK293) were studied. Viability was measured with AlamarBlue assay. Total and phosphorylated IGF1R and IR as well as Insulin, IGF1 and IGF2 concentrations were detected with multiplexing Magpix instrument. RT-qPCR was performed using Human Insulin Signaling Pathway PCR Array. We demonstrate in renal cancer cells that insulin and IGFs are stimulatory factors for cancer growth and migration despite the lack of insulin receptors. We also checked whether IGF1 and insulin stimulation leads to expression of insulin signaling pathway. Our data together are consistent with the conclusion that IGFs and insulin in high concentration may play a stimulatory role in renal cancer tumorigenesis and progression.

Key words: renal cell carcinoma, insulin, insulin-like growth factors.

This work was supported by the National Science Centre projects no. UMO-2012/05/D/NZ5/01844.

Poster

Circulating miR-9, miR-16, miR-205 and miR-486 in the diagnosis and monitoring of non-small cell lung cancer patients

Maria Sromek, Maciej Glogowski, Magdalena Chechlinska, Mariusz Kulinczak, Lukasz Szafron, Michalina Zajdel, Pawel Swoboda, Maria Cieslikowska, Robert Wlodarczyk, Mariusz Zmijewski, Maciej Turski, Klara Zakrzewska, J. Owczarek, Piotr Wisniewski, Jan K. Siwicki

Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland

Introduction: Non-small cell lung cancer (NSCLC) accounts for 80% of lung cancers, the leading cause of cancer mortality. microRNAs (miRs) have emerged as important components of carcinogenesis and promising cancer biomarkers.

Aim of the study: We aimed to asses the potential of plasma miR-9, miR-16, miR-205 and miR-486 in the diagnosis and monitoring of NSCLC patients.

Material and methods: Plasma was collected from the peripheral blood of 40 healthy donors and of NSCLC patients before surgery (n = 62), 1 month after surgery (n = 37) and 1 year after surgery (n = 14). Patients were diagnosed with adenocarcinoma (n = 23), squamous cell carcinoma (n = 23), large cell carcinoma (n = 5), and miscellaneous carcinoma (n = 11). microRNA expression was quantified by RT-qPCR by the ΔCt, 2- ΔCt method with the use of TaqMan MicroRNA Assays and TaqMan® MicroRNA Reverse Transcription Kit. miR-24 served as a reference.

Results and conclusions: 1) Plasma levels of miR-16, miR-486 and miR-205 in NSCLC patients before surgery, significantly exceeded those in healthy donors ($p < 0.0001$, $p < 0.0001$, $p = 0.0036$, respectively). A combination of the three differentially expressed miRs discriminated NSCLC from healthy persons with a specificity of 65% and sensitivity of 77%. miR-9 levels tended to be higher in NSCLC patients than in controls ($p = 0.069$).

2) One month after surgery plasma miR-9 and miR-205 levels significantly decreased ($p = 0.0001$, $p = 0.0002$, respectively) down to normal levels.

3) miR-16 and miR-486 concentrations decreased after surgery slower than miR-9 or miR-205, and also reached the normal levels one year after surgery.

4) Regarding the histopathological type, adenocarcinoma patients presented significantly lower plasma miR-205 levels than those with squamous cell carcinoma ($p = 0.0171$).

5) Tumour stage was found not to influence plasma levels of miR-16 and miR-486, but miR-205 level was significantly elevated in stages II and III/IV, as compared to controls ($p = 0.0025$ and $p = 0.0008$, respectively).

6) Plasma levels of miR-16, miR-205 and miR-486, but not miR-9, strongly correlated with each other in healthy donors ($p < 0.001$). In patients with NSCLC, there was a strong correlation between the levels of all the studied miRs (miR-9, miR-16 miR-486 and miR-205, $p < 0.001$).

Key words: microRNA, NSCLC, circulating biomarkers.

Poster

KW016-00020-2016-01

Osteopontin/SPP1 is overexpressed in glioblastoma and through distinct domains participates in glioma stem cell renewal and glioma-microenvironment interactions

Karolina Stępniak[1], Marta Kocyk[1], Marta Maleszewska[1], Anna Gieryng[1], Piotr Przanowski[1], Bartosz Wojtaś[1], Paweł Nauman[2], Katarzyna Kotulska[3], Wiesława Grajkowska[3], Bożena Kamińska[1]

[1]Nencki Institute of Experimental Biology PAS, Warsaw, Poland
[2]Department of Neurosurgery, Institute of Psychiatry and Neurology, Warsaw, Poland
[3]The Children's Memorial Health Institute, Warsaw, Poland

Osteopontin/SPP1 (a secreted phosphoprotein 1) is involved in immune responses, bone/tissue remodeling and biomineralization. SPP1 is overexpressed in many cancers, including glioblastoma (GBM) and contributes to tumor progression by regulating migration, invasion and cancer stem cell self-renewal. There are five SPP1 splice variants generated by alternative splicing and posttranslational protein modifications. We found that the expression of specific SPP1 isoforms is upregulated in resected GBMs and GBM cell lines, correlates with poor prognosis in GBM patients. Tumor-derived SPP1 interacts with cell surface receptors of microglia, myeloid cells residing in the central nervous system that infiltrate and support glioma invasion. Glioma-derived SPP1 induces a pro-tumoral, M2 activation. Knockdown of Spp1 in C6 glioma cells with lentivirally delivered shRNA reduced the number of tumor spheres and diminished expression of M2 markers in primary microglial cultures. To assess the role of functional domains of Spp1 in glioma stem cell self-renewal and glioma-microglia interactions, shRNA resistant constructs coding for a wild type and mutated Spp1 were created. A rescue experiment with Spp1 variants lacking *C*-terminal, CD44 binding domain or with point mutations in specific functional domains was performed. Our results show that the CD44 binding domain of Spp1 is necessary for a sphere forming activity, while mutations in a thrombin cleavage site and a integrin binding site disturb interactions with microglia. Altogether, we demonstrate that tumor-derived Spp1 supports glioma stem cell self-renewal and shapes glioma microenvironment.

Key words: glioma, glioblastoma, osteopontin, microglia, glioma stem cells.

Studies supported by a 2012/04/A/NZ3/00630 grant from the National Science Center.

Poster

KW016-00024-2016-01

Electro-photodynamic reaction in human breast cancer cells – *in vitro* study

Joanna Weżgowiec[1,2], Małgorzata Kotulska[1], Jolanta Saczko[3], Grzegorz Chodaczek[4], Julita Kulbacka[3]

[1]Faculty of Fundamental Problems of Technology, Wroclaw University of Technology, Wroclaw, Poland
[2]Department of Dental Materials, Wroclaw Medical University, Wroclaw, Poland
[3]Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
[4]Wroclaw Research Centre EIT+, Wroclaw, Poland

Introduction: Photodynamic therapy (PDT) is a low-invasive method of local cancer treatment. The effectiveness of PDT may be limited due to insufficient uptake of a photosensitizer by cells. A phenomenon of multidrug resistance (MDR) of cells is another potential impediment of anti-cancer therapies.

A possible method to overcome the barrier of cell membrane and to improve drug delivery is electroporation (EP). It is a process of formation of transient, nano-scale pores in the cell membrane subjected to pulsed electric field of sufficient energy. So far, EP has been combined with chemotherapy in a clinically applied procedure, called electrochemotherapy.

Aim of the study: The aim of this study was to investigate the possibility of using electroporation for the enhancement of photodynamic reaction (PDR) in human breast cancer cells *in vitro*, particularly in the cells resistant to standard chemotherapy.

Material and methods: Experiments were conducted on two human breast adenocarcinoma cell lines: sensitive (MCF-7/WT) and resistant (MCF-7/DX) to doxorubicin. Two photosensitive agents were selected: Photofrin and the cyanine dye IR-775. Eight electric pulses with the duration of 100 µs were delivered with the repetition frequency of 1 Hz by the ECM 830 Square Wave Electroporation System. The obtained strengths of the electric field were 800 V/cm and 1200 V/cm. Confocal microscopy was used for the observation of cellular uptake of Photofrin and IR-775. Changes of cellular morphology were evaluated via bright-field microscopy. The immunochemistry (ABC method) was used to report the expression of two multidrug resistance proteins (MDR1 and MRP7) and glutathione S-transferase (GST).

Results: The results showed that EP and PDR alone induced no significant morphological changes in the cells. However, the combination of EP with PDR significantly improved the treatment effectiveness in both cell lines. EP enabled the increase of the uptake of Photofrin and IR-775. EP-PDR resulted in the increase of the expression of GST, MDR1 and MRP7. Microscopic observations of cellular morphology indicated the death of cells subjected to EP-PDR.

Conclusions: EP enhanced the transport of Photofrin and IR-775 into the drug-sensitive and drug resistant types of breast cancer cells, resulting in the improvement of the effectiveness of PDR.

Key words: electroporation, photodynamic therapy, electro-photodynamic therapy, breast cancer, multi-drug resistance.

This work was supported by National Science Centre of Poland, grant No. 2014/15/N/NZ7/02983 (J. Wezgowiec).

Poster

Detection of molecular signatures of oral squamous cell carcinoma and normal epithelium – application of a novel methodology for unsupervised segmentation of imaging mass spectrometry data

Piotr Widłak[1], Monika Pietrowska[1], Magdalena Kalinowska[1], Mykola Chekan[1], Janusz Wierzgoń[1], Marta Gawin[1], Grzegorz Mrukwa[2], Grzegorz Drążek[2], Joanna Polańska[2]

[1]Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland
[2]Silesian University of Technology, Gliwice, Poland

Intra-tumor heterogeneity is a vivid problem of molecular oncology that could be addressed by imaging mass spectrometry. Here we aimed to assess molecular heterogeneity of oral squamous cell carcinoma and to detect signatures discriminating normal and cancerous epithelium. Tryptic peptides were analyzed by MALDI-IMS in tissue specimens from five patients with oral cancer. Novel algorithm of IMS data analysis was developed and implemented, which included Gaussian mixture modeling for detection of spectral components and iterative k-means algorithm for unsupervised spectra clustering performed in domain reduced to a subset of the most dispersed components. About 4% of the detected peptides showed significantly different abundances between normal epithelium and tumor, and could be considered as a molecular signature of oral cancer. Moreover, unsupervised clustering revealed two major sub-regions within expert-defined tumor areas. One of them showed molecular similarity with histologically normal epithelium. The other one showed similarity with connective tissue, yet was markedly different from normal epithelium. Pathologist's re-inspection of tissue specimens confirmed distinct features in both tumor sub-regions: foci of actual cancer cells or cancer microenvironment-related cells prevailed in corresponding areas. Hence, molecular differences detected during automated segmentation of IMS data had an apparent reflection in real structures present in tumor.

Key words: proteomics, imaging mass spectrometry, tumor heterogeneity, head and neck cancers.

This work was supported by National Science Centre of Poland, grant No. 2014/15/N/NZ7/02983 (J. Wezgowiec).

Poster

Cannabinoids as a potential source of active pharmaceutical ingredients to use in drugs for the treatment of pain in cancer patients

Anna Krause[1], Marek Milewski[1], Hanna Kierońska[1], Karolina Wielgus[2], Milena Szalata[2], Judyta Cielecka-Piontek[3], Ryszard Słomski[4]

[1]PozLab sp. z.o.o., Poznan, Poland
[2]Department of Pharmaceutical Chemistry, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Biotechnology, Institute of Natural Fibres & Medicinal Plants, Poznan, Poland
[4]Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poznan, Poland

Cannabinoids are a group of C_{21} terpenophenolic compounds, typically present in *Cannabis sativa L.* From among of them, the Δ^9-tetrahydrocannabitol (THC) and cannabidiol (CBD) are the most important. THC is the main psychoactive ingredient while CBD is a non-psychoactive component. CBD is an especially promising compound from the point of view of its pharmacological properties and may be considered as a candidate for the management of medical conditions where emesis and pain are prevalent [1]. Moreover, combination of CBD and THC has significantly reduced patients' pain scores of acute pain and chronic pain associated with central neuropathic, also induced by cancer disorders [2].

The aim of this study is to develop the innovative mucoadhesive pharmaceutical dosage form containing the cannabinoid extract modified to achieve lower level of THC and higher level of CBD.

The subsequent stages of the studies involved (i) obtaining of cannabinoid extract modified in regards to advantageous ratio between CBD and THC, (ii) evaluation of pharmacological activity of modified cannabinoid extract, including the studies of metabolic polymorphism, (iii) development of mucoadhesive pharmaceutical dosage form containing the modified cannabinoid extract.

During the first stage of the studies the cannabinoid extracts were obtained in the result of maceration with the use of different solvents. The influence of extraction parameters such as temperature, shaking, ultrasounds and darkness on the efficiency of applied method was tested. The content of CBD and THC in obtained extracts was determined.

Next, the modified cannabinoid extracts were subjects of studies aimed at: evaluation of their analgesic, anti-inflammatory, antiemetic properties by using *in vivo* model as well as polymorphism and expression of genes involved in metabolism of cannabinoids in Polish population.

The final, currently ongoing stage of this work is dedicated to the preformulation and formulation studies of mucoadhesive pharmaceutical dosage form containing the modified cannabinoid extract. The following crucial stages can be distinguished in technological studies of mucoadhesive pharmaceutical dosage form: (i) physicochemical characterization of cannabinoid extract properties, (ii) compatibility studies between the extract and excipients dedicated for mucoadhesive pharmaceutical dosage form, (iii) stability studies of systems of extract and excipients, (iv) preparation of the mucoadhesive pharmaceutical dosage form and quality control of the obtained mucoadhesive pharmaceutical dosage form.

References

1. Parter L, Mechoulam R, Schlievert C. Cannabidiol, a non-psychoactive component of cannabis and its synthetic dimethylheptyl homolog suppress nausea in an experimental model with rats. NeuroReport 2002; 13: 567-70.
2. Zajicek J, Fox P, Sanders H, Wright D, Vickery J, Nunn A, Thompson A; UK MS Research Group. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. Lancet 2003; 362: 1517-26.

This research was supported by the National Centre for Research and Development, Poland INNOMED Sector Programme (subsidy contract No: INNOMED/ I/11/NCBR/2014).