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**

Editorial Board

Jannik Andersen (USA)
Andrea Ardizzoni (Italy)
Peter Blume-Jensen (USA)
Magdalena Chechlińska (Warsaw)
Thierry Le Chevalier (France)
Salem Chouaib (France)
Tanja Čufer (Slovenia)
Angus Dalgleish (UK)
Andrzej Deptała (Warsaw)
Michał Drews (Poznan)
Soldano Ferrone (USA)
David Fogelman (USA)
Pere Gascon (Barcelona, Spain)
Wojciech Golusiński (Poznan)
Fred R. Hirsch (USA)
Paul Van Houtte (Belgium)
Iwona Hus (Lublin)
Dariusz Iżycki (Poznan)
Jacek Jassem (Gdansk)
Wiesław W. Jędrzejczak (Warsaw)
Claudine Kieda (Orleans, France)
Zygmunt Kopczyński (Poznan)
Marcin Kortylewski (USA)
Beatrix Kotlan (Hungary)
Gabriela Kramer-Marek (United Kingdom)
Andrzej Lange (Wroclaw)
Marek Los (Linkoping, Sweden)
Jan Lubiński (Szczecin)
Jacek Mackiewicz (Poznan)
Julian Malicki (Poznan)
Sergiusz Markowicz (Warsaw)
Andrzej Marszałek (Poznan)
Jan P. van Meerbeeck (Holland)
Piotr Milecki (Poznan)
Paweł Murawa (Poznan)
Dawid Murawa (Poznan)
Jacek Nikliński (Bialystok)
Andrzej Nowicki (Bydgoszcz)
Anna Pawlowska (USA)
Waldemar Priebe (USA)
Jacek Roliński (Lublin)
Stefan Rose-John (Kiel, Germany)
Giovanni Rosti (Italy)
Domenico Rubello (Italy)
Pravin Sehgal (USA)
Branimir I. Sikic (USA)
Janusz Skowronek (Poznan)
Marek Spaczyński (Poznan)
Katarzyna Starska (Lodz)
Stanisław Szala (Gliwice)
Cezary Szczylik (Warsaw)
Maurizio Tonato (Italy)
Maciej Ugorski (Wroclaw)
Maciej Wiznerowicz (Poznan)
Marek Wojtukiewicz (Bialystok)
Theresa Whiteside (USA)
Jan Żeromski (Poznan)

Editor-in-Chief
Andrzej Mackiewicz
e-mail: andrzej.mackiewicz@wco.pl

Co-editors
Piotr Czapiewski
Chu Fuliang
Mirosław Kiedrowski

Editors
Bożena Kamińska-Kaczmarek
Włodzimierz Krzyżosiak
Cezary Szczylik

Associate Editor
Sun Zhi-Gang
Language Editor
Richard Ashcroft

Statistics Editor
Małgorzata Misztal

Publishing Editor
Monika Ślusarska

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 Publishing House
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
tel./fax +48 61 656 22 00
e-mail: prenumerata@termedia.pl

layout
_studio termedia

The advertisers shall be liable for the contents of advertisements placed in Contemporary Oncology. Advertisements of prescription drugs are intended only for physicians licensed to prescribe them

contemporary oncology

współczesna **onkologia**

Volume 21, Supplement 1, March 2017

9th International Conference of Contemporary Oncology

Genome based precision immuno- and targeted therapy

Poznan, 22–24 March 2017

Oral sessions

Session 2. Precision oncology

Chairs: Gabriela Kramer-Marek, Theresa Whiteside

Imaging biomarkers of resistance in breast cancer: useful tools in precision oncology

Gabriela Kramer-Marek

Division of Radiotherapy and Imaging, The Institute of Cancer Research, 123 Old Brompton Road, London, SW7 3RP, UK

The epidermal growth factor receptor 3 (HER3) is a member of the HER family of receptor tyrosine kinases. HER3 has diminished catalytic kinase activity and its phosphorylation depends on heterodimerisation with other receptors. Beyond that, HER3 has 14 tyrosine residues in the C-terminal tail; six of them are docking sites for the recruitment of the p85 subunit of the phosphatidylinositol-3-OH (PI3) kinase. Subsequently, transphosphorylation of HER3 is a key node in the activation of the PI3K/AKT pathway, promoting tumor cell survival, proliferation, and metastasis. Recent studies have highlighted HER3 as a critical driver of carcinogenesis and tumor progression and, above all in the establishment of resistance to targeted therapies. Indeed, it has been shown that EGFR- and HER2-driven cancers invariably become unresponsive to anti-HER therapeutics through compensatory activation of HER3 signaling, bypassing the original treatment. Given the importance of HER3 signaling, it is conceivable to predict that agents targeting this receptor could ultimately provide a more efficient approach towards treatment of such cancers**.** Therefore, several recent studies have triggered major efforts to develop mAbs targeting the receptor ectodomain with some being already in phase I and II clinical trials (i.e. MM-121, U3-1287, and LJM716). Preclinical findings utilizing these mAbs have been very promising. However, in humans the activity of HER3 selective drugs has not been robustly established yet and the overall treatment outcome may be viewed as modest. More recently, patritumab (U3-1287) was used in combination with erlotinib in NSCLC patients who had progressed after at least one course of chemotherapy. But, there was no clear correlation between tumor response and HER3 expression in tumor tissues or serum soluble HER3 levels. In fact, the absence of a suitable biomarker to evaluate HER3 status appears to be the major hurdle in introducing HER3-targeted agents into treatment protocols. Currently, HER3 alterations (e.g. protein overexpression, receptor mutation) are analyzed by methods such as: immunohistochemistry (IHC), proteomics and, next-generation sequencing using tumor tissues derived postoperatively or via biopsies. However, these are invasive methods which might not accurately address inter- and intra-tumor heterogeneity of receptor expression due to the sampling bias and challenges of collecting samples from multiply lesions.

In light of these findings, there is clearly a need for development of imaging biomarker of drug response for targeted therapies that could greatly improve patient management by helping to tailor the treatment regimen to patient-specific biology. Recently developed a low molecular weight (~6.5 kDa) affibody-based positron emission tomography (PET) imaging agent that recognizes HER3 on cell membrane with high binding affinity and specificity. Our findings demonstrate that PET imaging *in vivo* using radiolabeled $Z_{HER3:8698}$ affibody can rapidly provide information about HER3 expression status and monitor acquired therapeutic resistance to agents targeting receptor signaling *in vivo*. We believe this valuable research tool could aid in the future in setting more efficient treatment regiments and bring substantial benefits to patients by improving the efficacy of targeted therapies in HER-driven cancers.

Session 2. Precision oncology

Chairs: Gabriela Kramer-Marek, Theresa Whiteside

KWO17-00037-2017-01

Camelid single domain antibody application in cell based therapies

Heman Chao[1], Marni Uger[1], Wah Wong[1], Baomin Tian[1], Pawel Wisniewski[2]

[1]Helix BioPharma Corp, Toronto, Canada
[2]Helix Immuno-Oncology Sp. z o.o., Warsaw, Poland

Camelids produce antibody that is devoid of lights chains. Their single domain *N*-terminal domain is fully capable of binding antigen with great affinity and specificity without requiring domain paring. The *N*-terminal domain can also be expressed in both bacterial and mammalian systems in high yield. These domains have been applied to research, diagnostic and even therapeutic applications. In this study, two camelid single domain antibodies have been applied to cell based therapies. One of the antibody targets VEGFR2 and the second antibody targets CEACAM6. Both antigens are solid tumors targets. Chimeric-Antigen Receptor (CAR) T cells were engineered to target CEACAM6 antigen or VEGFR2 antigen by transducing respective antibody coupled with appropriate intra-cellular signaling domains. CEACAM6-CAR-T and VEGFR2-CAR-T are cytotoxic to respective antigen carrying tumor cells relative to control T cells. *In vivo* xenograft studies also showed CEACAM6-CAR-T is very potent against BxPC3, a pancreatic cancer model. Given the simplicity of camelid single domain antibody relative to 'classical' antibody, these antibodies would be ideal candidates to be used in cell based therapies.

Key words: CAR-T, CEACAM6, VEGFR2, antibody.

Session 6. Cancer biology and novel therapeutic approaches II

Chairs: Magdalena Chechlińska, Andrzej Lange

KWO17-00016-2017-01

microRNAs as molecular markers in primary CNS lymphomas

Magdalena Chechlinska, Maria Cieslikowska, Grzegorz Rymkiewicz, Pawel Swoboda, Michalina Zajdel, Katarzyna Blachnio, Zbigniew Bystydzienski, Anita Jastrzebska, Krzysztof Goryca, Maria Sromek, Mariusz Kulinczak, Agnieszka Druzd-Sitek, Jan Walewski, Jan Konrad Siwicki

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

Differential diagnosis of central nervous system (CNS) tumors remains challenging, in spite of development of imaging techniques, cytological and flow cytometry examination of cerebrospinal fluid (CSF), and histological examination of stereotactic biopsy material. microRNAs are promising markers for faster and more reliable differential diagnosis of primary lymphomas and nonmalignant lesions in the CNS.

We aimed to assess the diagnostic value of miR-21, miR-19b and miR-92a, miR-155, miR-196b, miR-let-7b, miR-125b and miR-9 expression in CSF and brain biopsies (BB) from patients with primary CNS lymphomas (PCNSL) vs. neurological CNS lesions.

microRNA expression was assessed by RT-qPCR, with miR-24 as a reference, in CSF leftover samples and formalin-fixed paraffin-embedded stereotactic BB samples collected for the routine diagnostic purposes from patients suspected of PCNSL (n = 26) or neurological CNS lesions (n = 59), consulted at the M. Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology in Warsaw.

1. In the CSF, there were significantly higher levels of miR-21, miR-19b and miR-92a in patients with PCNSL than in patients with nonmalignant neurological lesions. CSF levels of the three miRNAs differentiated PCNSL from neurological lesions, with 54% sensitivity and 90% specificity.

2. In BB samples, miR-21, miR-19b and miR-92a expression did not differ between lymphomas and nonmalignant lesions.

3. In BB samples, miR-155 and miR-196b were significantly overexpressed and miR-let-7b, miR-125b and miR-9 were downregulated in PCNSL vs. nonmalignant neurological diseases.

In conclusion, miRs emerge as promising diagnostic markers that may support earlier PCNSL treatment decisions, thus improving patient outcome. Further development will include validation of miRs as markers on independent series of patients and NGS profiling of paired samples (CSF/brain biopsy) from PCNSL patients

Key words: microRNA, CNS lymhoma, differential diagnosis, cerebrospinal fluid.

Session 3. Tumor microenvironment

Chairs: Claudine Kieda, Viktor Umansky

KWO17-00030-2017-01

Advanced three-dimensional co-culture model to study tumor biology *in vitro*

Ewelina Dondajewska, Hanna Dams-Kozłowska, Andrzej Mackiewicz

Poznan University of Medical Sciences, Poznan, Poland
Greater Poland Cancer Center in Poznan, Poland

Recognizing the drawbacks of standard 2D cell culture methods, we constructed a 3D culture model to better represent cell morphology and function *in vitro*. Using silk extracted from Bombyx mori cocoons, a three dimensional, porous scaffolds for cell culture were produced. To further mimic tumor microenvironment, we included stromal cells cultured together with tumor cells to obtain a heterotypic co-culture model. Established cell lines: EMT6 murine breast cancer, and NIH3T3 murine normal fibroblasts were used in the studies and were transduced with lentivarial vectors to express fluorescent proteins. That enabled us to distinguish cells of both types in the co-culture while analyzing cells using CLSM or FACS. We optimized the methods of cell culture on the scaffolds. We compared cell growth, morphology, drug resistance and gene expression profiles of cell cultured as standard 2D monoculture, 3D monoculture and 3D co-culture. Using fluorescent activated cell sorting we separated cells from the co-culture and by RT-PCR we analyzed expression of genes responsible for the process of EMT, CAF transformation, ECM production and tumor aggressiveness. Results showed more epithelial phenotype of cancer cells while cultured on 3D silk scaffolds as shown by the drop in most common EMT markers. Furthermore, we observed significantly higher levels of mRNA for ECM proteins in fibroblasts cultured in 3D as well as some features characteristic for cancer associated phenotype of these cells while co-cultured with tumor cells in 3D. A general increase in MMP9, VEGFa and Vimentin was observed for both cell types. Using cytotoxic agent – doxorubicin we found cells cultured on 3D silk scaffolds to be much more resistant to the effects of the drug than cells cultured in standard monolayer.

In this study we have proven, that silk scaffold – based, three-dimensional culture model, constructed using both cancer cells and fibroblasts, is an advanced tool to study tumor biology *in vitro*. It provides a controlled environment, with the possibility to analyze cell-cell and cell-ECM interactions. Model can be easily expanded by the addition of endothelial cells, immune cells or adipocytes.

Key words: tumor microenvironment, co-culture, breast cancer, silk fibroin scaffold, 3D culture.

Session 6. Cancer biology and novel therapeutic approaches II

Chairs: Magdalena Chechlińska, Andrzej Lange

KWO17-00033-2017-01

Lymphocyte activation and exhaustion in the natural history of chronic lymphocytic leukemia

Ewelina Grywalska, Agata Surdacka, Michal Mielnik, Elzbieta Fitas, Jacek Rolinski

Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Poland

Introduction: Chronic lymphocytic leukemia (CLL) is a disease characterized by the accumulation of morphologically mature monoclonal lymphocytes B with CD19+/CD5+/CD23+ phenotype in lymphoid tissue, peripheral blood and bone marrow. The course of CLL is chronic by default. Of note, however, is its heterogeneity. Programmed cell death protein 1 and its ligand 1 (PD-1, PD-L1) are major inhibitory receptors regulating T cell exhaustion, i.e. a state of T cell dysfunction. The role of lymphocyte activation and exhaustion in the natural history of CLL is still a matter of discussion.

Aim of the study was to determine the percentages and absolute numbers of exhausted and activated lymphocytes B and T in peripheral blood and bone marrow of CLL patients. Moreover, we analyzed relationship between the number of PD-1-positive and PD-L1-positive lymphocytes and established prognostic factors in CLL.

Material and methods: The study included 40 untreated patients with CLL and 20 healthy subjects. The immunophenotype of peripheral blood mononuclear cells (in both groups) and bone marrow cells (solely in the CLL group) was determined by means of flow cytometry.

Results: Patients with CLL showed higher frequencies and absolute number of exhausted B lymphocytes CD19+PD-1+ ($p < 0.0001$), CD19+PD-L1+ ($p < 0.0001$), activated lymphocytes B with phenotypes CD19+CD25+ ($p < 0.0001$) and CD19+CD69+ ($p < 0.0001$), as well as higher frequencies and absolute number of exhausted T lymphocytes CD3+PD-1+ ($p = 0.0021$), CD3+PD-L1+ ($p = 0.0032$), and activated CD3+CD25+ ($p = 0.0027$), and CD3+CD69+ ($p = 0.0062$) lymphocytes T than the controls in the peripheral blood. Similar observations were done in the bone marrow samples ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p = 0.0134$, $p = 0.0183$, $p = 0.0263$, and $p = 0.0169$, respectively). Enhanced exhaustion and activation of peripheral blood and bone marrow lymphocytes was associated with higher Rai stage, increased concentration of lactate dehydrogenase and beta-2 microglobulin and progression of the disease. The number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+PD-1+ B cells and CD3+PD-1+ T cells.

Conclusions: The study confirmed the association between unfavorable prognosis and high expression of exhaustion and activation markers in CLL patients. Determination of PD-1+, PD-L1+, CD25+ and CD69+ lymphocytes T and B constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

Key words: programmed cell death protein 1, Rai stage, Prognostic factors, chronic lymphocytic leukemia.

Session 9: Optimizations in diagnostics and management of cancer family syndromes in Poland

Chairs: Bogdan Kałużewski, Cezary Cybulski

KWO17-00050-2017-01

DICER1 syndrome

Marek Niedziela

Department of Pediatric Endocrinology and Rheumatology, Poznan University of Medical Sciences Poznan, Poland

The DICER1 gene localized on the long (q) arm of chromosome 14 encodes a protein DICER1, an RNase III endoribonuclease, that plays a role in regulating the activity of other genes. The function of DICER1 protein is exerted via microRNA formation. MicroRNAs are involved in cell growth, proliferation and differentiation. Germline mutations in the DICER1 gene cause DICER1 syndrome. Abnormal DICER1 protein disregulates miRNAs production thus leading to uncontrolled tumor formation. Many types of tumors may develop in these patients such as tumors of the lungs (pleuropulmonary blastoma – PPB), kidneys (cystic nephroma), ovaries (Sertoli-Leydig tumors – SLCTs) and thyroid (multinodular goiter – MNG). Cystic and hyperplastic thyroid abnormalities are a common finding in the DICER1 syndrome, particularly prevalent in DICER1 carriers, likely higher than that for neoplasms. Rarely, individuals with DICER1 syndrome develop thyroid cancer. Moreover the other tumors such as embryonal rhabdomyosarcomas, Wilms tumors and other very rare entities, all comprise DICER1 syndrome. DICER1 syndrome is inherited in autosomal dominant pattern but with unknown penetrance. It is a rare condition and its prevalence is unknown. Affected individuals can develop one or more types of tumors, and members of the same family can have different types. Based on the literature it is hypothesized that second somatic "hit" in DICER1 is required in addition to a loss of-function germline DICER1 mutation in order to initiate tumor/cancer development. A genetic counseling and testing should be offered to the family of the affected child/adult.

Key words: DICER1, tumors, children, adults.

Lecturers abstract

KW017-00048-2017-01

Anti-programmed cell death protein 1 in cancer immunotherapy – what do we know so far?

Jacek Rolinski, Ewelina Grywalska

Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Poland

Programmed cell death protein 1, also known as PD-1 and CD279 is a protein expressed on T cells and pro-B cells in humans. It is a cell surface receptor that belongs to the immunoglobulin superfamily. PD-1 is known to be the major inhibitory receptor that functions as an immune checkpoint, playing an important role in down regulating the immune system; by preventing T cell activation it reduces autoimmunity and promotes self-tolerance. However, it has also been proven that T cells with high PD-1 expression lose the ability to eliminate cancer and infectious agents. Antigen presenting, infected by viral agents and cancer cells, have on their surface molecules PD-L1 (B7-H1) and PD-L2 (B7-DC) (programmed cell death ligand protein 1 and 2) are connected to the PD-1 antigen on the surface of cells results in inhibition of their activity. The concept of immunotherapy using antibodies against immune check points is based on their ability to reverse anergy of T cells in the tumor environment and inflammatory processes. Lymphocytes only receive then signals that activate and again therefore lymphocytes are able to act against tumor and infected cells. This strategy is proved to be very effective for some types of cancer and chronic viral infections, and it revolutionized existing immunotherapy methods. The administration of anti-PD-1 or anti-PD-L1 aims to abolish anergy of effector lymphocytes T caused by tumor and viral infected or antigen presenting cells. The anti-PD-1 and anti-PD-L1 therapies are widely used for the treatment or are undergoing the final stages of clinical trials in patients with different types of tumors. Therapies of this kind often provide long-term control of the disease and the development of specific immune response in large variety of tumors. Nivolumab (anti-PD-1) is already used in the treatment of melanoma, non-small cell lung cancer, and renal cancer. Pembrolizumab (anti-PD-1) is used in the treatment of melanoma and non-small cell lung cancer. Atezolizumab (anti-PD-L1) is used in the treatment of clear renal cancer, bladder cancer and non-small cell lung cancer. Moreover there are different case reports, describing positive clinical effect of the inhibitors of PD-1/PD-L1 pathway in the therapy of intracranial meningioma, colorectal cancer, gastric cancer or even penile cancer. They are generally less toxic than chemotherapy, but the treatment complications, i. e. autoimmune phenomena, are not uncommon.

Key words: cancer therapy, chronic viral infections, programmed cell death protein 1, nivolumab, pembrolizumab, atezolizumab.

Session 6. Cancer biology and novel therapeutic approaches II

Chairs: Magdalena Chechlińska, Andrzej Lange

KWO17-00002-2017-01

Adverse drug reactions of voriconazole in relation to CYP2C19 mutations among patients after allogenic hematopoietic stem cell transplantation

Beata Sienkiewicz[1], Donata Urbaniak-Kujda[2], Jarosław Dybko[2], Andrzej Dryś[3], Magdalena Hurkacz[1], Tomasz Wróbel[2], Anna Wiela-Hojeńska[1]

[1]Department of Clinical Pharmacology, Wroclaw Medical University, Wroclaw, Poland
[2]Department and Clinic of Haematology, Blood Neoplasms, and Bone Marrow Transplantation, Wroclaw Medical University, Wroclaw, Poland
[3]Department of Physical Chemistry, Wroclaw Medical University, Wroclaw, Poland

Voriconazole (VCZ) is indicated for treatment of invasive aspergillosis, candidemia, and fungal infections caused by Scedosporium spp. and Fusarium spp. It can also be an alternative for posaconazole in high risk hematological patients' prophylaxis. Pharmacokinetic properties of the drug are influenced by food intake, inter-individual variability and drug-drug interactions. The agent is metabolized by CYP2C19, CYP3A4 and CYP2C9. Only mutations of the first isoenzyme cause variability in voriconazole pharmacokinetics. VCZ treatment may lead to numerous side effects such as pyrexia, nervous system, respiratory, thoracic and mediastinal, gastrointestinal, hepatobiliary and skin disorders. The aim of our study was to determine the influence of CYP2C19 mutations on adverse drug reaction (ADR) occurrence during antifungal prophylaxis conducted with voriconazole in adult patients after allo-HSCT (allogenic hematopoietic stem cell transplantation).

We determined CYP2C19 genotypes in 30 patients after allo-HSCT using PCR-RFLP methods. Biometrical and biochemical data, information on the underlying disease, chemotherapy, prophylaxis failure, adverse drug reactions typical for the use of voriconazole, and probable drug interactions were collected. The observation and reporting of ADR took place from the -1 day before transplantation till the +20th day after transplantation. CYP2C19 genotypes were correlated with observed undesirable effects.

23 patients suffered from at least one side effect during therapy. Most frequent were gastrointestinal disturbances in 15, nervous system disorders in 11 and skin disorders in 7 cases. Patients with CYP2C19*1/*17 suffered mainly from skin, nervous system and gastrointestinal disturbances. CYP2C19*1/*2 and CYP2C19*2/*17 genotype adults showed mainly gastrointestinal, nervous system and skin disorders. Among CYP2C19*17/*17 patients skin disorders and vomiting were observed whereas wild type genotype was connected mainly with swelling. Statistical analysis showed a tendency for patients demonstrating the *2 allele, to experience ADR. No statistical significance was achieved, probably due to a limited number of patients.

In conclusion side effects are common during VCZ treatment. Patients with at least one loss of function allele are more likely to experience adverse drug reactions. Previous determination of CYP2C19 mutations may optimize antifungal treatment in high risk patients.

Key words: adverse drug reactions, voriconazole, CYP2C19, genotyping, hematology.

contemporary oncology

współczesna **onkologia**

Volume 21, Supplement 1, March 2017

9th International Conference of Contemporary Oncology

Genome based precision immuno- and targeted therapy

Poznan, 22–24 March 2017

Poster session

Poster

KW017-00041-2017-01

The response of chondrocytes differentiated from human induced pluripotent stem cells (hiPSCs) treated with ionizing radiation

Ewelina Augustyniak[1,2], Wiktoria M Suchorska[1,3]

[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 Electroradiology, Poznan University of Medical Sciences, Poznan, Poland

The response of stem cell-derived cells to treatment with ionizing radiation (IR) and chemotherapeutics is a questionable issue. It is worth mentioning that un- and differentiated cells possess different radioresistance. It is also unknown, whether their DNA damage response (DDR) mechanisms of stem-derived cells are more similar to those from "parental" stem cells (SCs) or perhaps those from completely differentiated cells. The main obejctive of the project was to evaluate the genetic stability of hiPSC-derived chondrocytes treated with ionizing radiation (IR). The investigations are focused on so far little-known DNA damage response (DDR) in stem cell-derived cells.

In the experiment three types of cell lines were used: human induced pluripotent stem cells (hiPSCs) generated from primary human dermal fibroblasts, stable cell line of human articular chondrocytes and chondrocyte-like cells differentiated from hiPSCs via embryoid bodies. The investigated cells were treated with IR in the range of low and high doses (0; 1; 2; 5 Gy) and collected (1, 5, 9 and 24 h after IR). The analyses of γH2AX, and cPARP by flow cytometry were also performed. Moreover, we investigated the level of senescence in irradiated cells.

These findings demonstrated that kinetics of double strand breaks (DSBs) significantly differ in hiPSCs, chondrocytes, and hiPSC-derived chondrocytes. Nevertheless, the formation of DSBs in chondrocyte-like cells differentiated from hiPSCs is similar to processes occurring in parental hiPSCs rather than in human chondrocytes. The hiPSCs and hiPSC-derived chondrocytes are very prone to DNA damage in comparison with mature adult chondrocytes. However, it is important to point out that hiPSC-derived chondrocytes possess more efficient DNA repair mechanisms resulting in the lower level of DSBs after 24 h in contrast to hiPSCs. Consequently, hiPSC-derived chondrocytes do not easily undergo apoptosis as hiPSCs. Nevertheless, the hiPSC-derived chondrocytes also reveal increased level of cells undergoing senescence.

We demonstrated that induced DDR mechanisms of stem-derived cells remarkably differ from those in "parental" SCs and mature adult chondrocytes. The obtained results contribute to the establishment of reliable, effective and first of all safe hiPSC- based approach for clinical cartilage repair and treatment.

Key words: human induced pluripotent stem cells, chondrocytes, DNA damage response, ionizing radiation.

Poster

KW017-00038-2017-01

Telomerase silencing increases the cell death after radiochemotherapy in HNSCC cells

Wojciech Barczak[1,2], Pawel Golusinski[1], Wiktoria Maria Suchorska[2,3], Michal Masternak[1,4], Wojciech Golusinski[1]

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

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

Material and methods: Knock down of telomerase (TERT subunit) by constructed lentiviral vectors encoding shRNA (designed by author) on cancer cell lines derived from HNSCC tumors (head and neck cancers are squamous cell carcinoma), KB, FaDu, and H103 cells was carried out. The silencing level was performed by qPCR and immunofluorescence staining. The impact of cytostatics (cisplatin and docetaxel) and ionizing radiation on the induction of apoptosis, autophagy, cell cycle, and yH2AX via immunofluorescence staining, cytometer analysis and qPCR was also estimated. The telomere length measurement using a method based on qPCR was assessed.

Results: Silencing of TERT gene by designed shRNA effectively decreases the TERT expression, causes telomeres shortening and changes in molecular profile of genes related with HNSCC cancerogenesis. There was shown that TERT knockdown increase the apoptosis and autophagy in FaDu and H103 cell lines after chemotherapeutics administration. Moreover, TERT silencing causes the cell cycle arrest in G1 phase (FaDu cells) and S/G2 phase (H103 cells). It was also demonstrate that depletion of telomerase in FaDu and H103 cells increase the impact of ionizing radiation alone and with concomitant cisplatin- and docetaxel-based chemotherapy.

Conclusions: The presented results determine increased chemo- and radiosensitivity in HNSCC cell lines after telomerase silencing. Telomerase is likely to play a pivotal role in chemo- and radioresistance of selected HNSCC cell lines, however further studies are needed.

Key words: head and neck cancers, telomerase, RNA interference.

Poster

KW017-00039-2017-01

Transcriptome profiling of peripheral T cells in AGI-101H-treated melanoma patients

Patrycja Czerwińska[1,2], Marcin Ruciński[3], Katarzyna Gryska[1,2], Sylwia Mazurek[1,2], Andrzej Mackiewicz[1,2]

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

Malignant melanoma (MM) belongs to the most invasive human malignancy with rapidly rising incidence. Due to the high molecular and clinical heterogeneity, advanced MM is difficult to treat with homogenous modalities, suggesting a strong need for treatment personalization. To achieve this, specific biomarkers for determining the effectiveness of the treatment should be identified.

Active immunotherapy for advanced MM patients with therapeutic gene modified allogenic MM vaccine (AGI-101H) has been tested in our lab since 1997, resulting in a long-term survival of a substantial fraction of immunized patients. Our goal is to understand the molecular features of the immune cells that determine patients' response to the treatment with AGI-101H. Therefore, T lymphocyte mRNA expression profiling in long-term surviving patients continuously immunized with AGI-101H was performed. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from immunized MM patients (at the day of immunization and 6 days after AGI-101H) and from MM patients not included in the clinical trial and healthy controls. Untouched T lymphocytes were separated with magnetic beads and subjected for further RNA isolation. The quality and quantity of RNA was checked and the transcriptomic profiling was performed with Affymetrix HG U219 microarray. Differential gene expression (DGE) analysis between groups was conducted and is now being validated with RT-qPCR. Also, transcriptomic results are being further analyzed with Gene Set Enrichment Analysis (GSEA) tool.

The expression of 19285 markers was measured with HG U219 microarray. DGE analysis between AGI-101H vaccinated patients at day 0 (before vaccine, BV) and 6 days later (after vaccine, AV) revealed 189 differentially expressed genes (DEGs), with 101 downregulated and 88 upregulated after AGI-101H administration ($|log2FC| > 0.5$). Among 189 BV vs AV DEGs the expression of 32 markers is highly similar in healthy (H) and AV patients (and distinct from non-immunized cancer (C) patients). The expression of selected markers is being validated with RT-qPCR with additional samples from specific groups.

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

Key words: immunotherapy, malignant melanoma, transciptome profiling.

Poster

KW017-00019-2017-01

H2.1MS1 silk spheres are not toxic and can be used for targeted therapy of Her2-positive breast cancer

Tomasz Deptuch, Anna Florczak, Andrzej Mackiewicz, Hanna Dams-Kozlowska

Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poland

Biotechnology can provide many biomaterials suitable for drug delivery for cancer treatment. The drug delivery system based on biopolymers like bioengineered silk can greatly reduce adverse effects of chemotherapeutics in patients. Moreover, thanks to genetic engineering it is possible to incorporate into silk sequence a various peptide motifs for targeted therapy, what additionally can decrease harmful effects of chemotherapeutic. Although silk-based materials are generally regarded as safe and biocompatible, final biotechnological product needs to be tested for possible toxic effects due to contaminations during production and purification process. Herein, we analyzed potential toxic effects and *in vivo* distribution of proposed bioengineered silk carriers for targeted therapy of Her2-positive type cancer.

The main aim of this study was evaluation of maximal tolerated dosage, possible toxic effects and distribution of bioengineered silk spheres in *in vivo* mice model upon systemic administration.

The drug delivery system is based on bioengineered silk MS1 derived from MaSp1 spidroin of N. clavipes spider and its HER2-targeting variant. Both silks were obtained through genetic engineering and production in microbial expression system in bioreactor. The recombinant proteins were self-assembled into spheres in the presence of chaotropic agent. The maximal tolerated dosage was analyzed based on survival and behavior observation of mice up to 24h after systemic administration of spheres. *In vivo* distribution of silk carriers was evaluated using Her2+ cancer model (D2F2E2/luc) in Balb/c mice using IVIS imaging system. The fluorescent labeled spheres were administered intravenously six times at 1, 3, 5, 13, 15 and 17th day of experiment.

The highest dosage tested (20 mg/kg of the body weight) was not lethal and did not influence behavior of mice. Observation of fluorescent labeled spheres in IVIS imaging system 24 h post administration reviled that the spheres accumulated mainly in liver and lungs in mice without Her2+ tumors. In D2F2E2/luc tumor model similar accumulation was also observed, but after few days the spheres' signal in those organs diminished and high signal became present at Her2-positive tumor site indicating site-specific accumulation of spheres. Obtained results suggest that bioengineered silk carriers can be safely applied for breast cancer therapy.

Key words: bioengineered spider silk spheres, targeted drug delivery, biotechnology, drug carrier.

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

Poster

KW017-00005-2017-01

Cellular uptake, intracellular distribution and degradation of the functionalized silk spheres used as drug carriers for cancer therapy

Anna Florczak, Andrzej Mackiewicz, Hanna Dams-Kozlowska

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

A major issue in cancer treatment is the development of effective vehicles to deliver a therapeutic to specific target cells for adequate and sustained drug release with minimal toxicity in healthy tissues. Physicochemical properties and addition of ligands for cell-surface receptors play a key role in the cellular uptake of carriers. The bioengineered spider silk is a material with a great potential in biomedical application. Drug-encapsulated biodegradable spheres made of functionalized silk have considerable potential for use as delivery systems.

The objective was to determine the mechanism involved in cellular uptake, intracellular trafficking and degradation of drug carriers made of the functionalized silk.

The hybrid constructs were obtained by adding a Her2-binding peptide (H2.1) to MS1 and MS2 bioengineered silks based on the MaSp1 and MaSp2 proteins from N. clavipes, respectively. The H2.1MS1 and H2.1MS2 proteins were blended at a weight ratio of 8:2. Stable silk particles were formed by mixing a soluble protein with potassium phosphate using a micromixing technique. Obtained spheres were characterized in terms of size and morphology using an SEM. The uptake of spheres by human Her2-positive breast cancer cells SKBR3 in the presence of endocytosis inhibitors was assessed using flow cytometry. The subcellular distribution of silk particles was investigated using CLSM by evaluating the signal colocalization with organelle-specific tracker. CLSM imaging was performed in order to analyze the degradation kinetics of silk particles by employing different lysosomal and exosomal inhibitors.

Functionalized spheres were actively internalized by Her2-positive cells. Silk particles facilitated the entry into cells both through the clathrin- and caveolae-dependent pathway of endocytosis. Silk spheres upon entering the cells localized in lysosomes. Applying various lysosomal inhibitors substantially extended the fluorescence signal from silk particles indicating the intracellular degradation took place in lysosomes. Furthermore, the prolonged presence of silk particles was not observed when using inhibitors of exosomal release, which further proved that the lysosomal function is essential for silk-based carriers removal.

We showed for the first time, that silk spheres could be processed into cells by the endocytosis pathway and their degradation occurred in lysosomes. The degradation of the carrier is of a great importance to develop drug delivery system.

Key words: bioengineered spider silk spheres, drug carrier, cellular uptake, biodegradation, targeted drug delivery, cancer therapy.

Poster

KW017-00027-2017-01

Vaccines based on CSCs as a treatment for malignant melanoma

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]Diagnostic and Immunology Department, 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. **Aim of the study:** The aim of the study was to evaluate the therapeutic potential of novel tumour vaccines based on CSCs derived from B16F10 cells.

Material and methods: C57BL/6 mice were immunized twice by subcutaneous injection using the CSCs 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 vaccines compared with controls immunized with B16F10. We observed systemic immune response in spleens which was revealed by stronger activation of CD8+ and CD4+ lymphocytes and higher number of CD4+ and CD8+ memory T cells. *In vitro* stimulation of splenocytes with relevant vaccine or B16F10 lysate revealed the presence of antigen specific lymphocytes, producing IL2, IFN-γ, IL-6 and IL-10, which indicates a mixed Th1/Th2 type immune response. Morover we observed local immune response at the tumor site which was revealed by increased number of infiltrating dendritic cells, monocytes and mature NK cells and decreased number of regulatory CD4+, Foxp3+ T cells. In addition 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 significant therapeutic potential of cancer vaccines based on melanoma CSCs.

Key words: melanoma, cancer stem cells, cancer vaccines.

Poster

KW017-00029-2017-01

The impact of IDH1/2 mutations on SOCS gene methylation and deregulation of STAT signaling in gliomas

Bartlomiej Gielniewski[1], Bartosz Wojtas[1], Marta Maleszewska[1], Sylwia Krol[1], Tomasz Czernicki[2], Andrzej Marchel[2], Wojciech Kaspera[3], Wojciech Szopa[3], Katarzyna Kotulska[4], Wieslawa Grajkowska[4], Marcin Roszkowski[4], Ryszard Czepko[5], Mariusz Banach[5], Cezary Szczylik[6], Maciej Kawecki[6], Bartosz Czapski[7], Pawel Nauman[7], Iwona Ciechomska[1], Bozena Kaminska[1]

[1]Nencki Institute of Experimental Biology, Warsaw, Poland
[2]Neurosurgery Department and Clinic, Medical University of Warsaw, Warsaw, Poland
[3]Department of Neurosurgery, Medical University of Silesia, Regional Hospital, Sosnowiec, Poland
[4]The Children's Memorial Health Institute, Warsaw, Poland
[5]Department of Neurosurgery, Scanmed S.A. St. Raphael Hospital, Cracow, Poland
[6]Department of Oncology, Military Institute of Medicine, Warsaw, Poland
[7]Institute of Psychiatry and Neurology, Warsaw, Poland

DNA methylation is frequently deregulated in tumors, however there is not clear if this a cause or consequence of tumor transformation. Gliomas, the most common tumors of the central nervous system, represent over 70% of all brain malignancies. Many gliomas have deregulated DNA methylation and some exhibit the hypermethylation phenotype. Whole genome sequencing revealed mutations in the genes coding for metabolic enzymes IDH1/2 that result in generation of an oncometabolite blocking epigenetic enzymes. Some of events (i.e. methylation of the MGMT promoter which silences a gene of DNA repair) are beneficial, while other could be deleterious. We performed an analysis of the occurrence of IDH1/2 mutations by PCR and Sanger sequencing in a panel of 58 low and high grade gliomas and studied methylation of the SOCSs genes coding for negative regulators of cytokine/growth factors signaling. These signaling pathways converging on STAT transcription factors are frequently deregulated in gliomas and contribute to tumorigenesis. The analysis showed correlation between the presence of IDH1 mutation and methylation of SOCS1 and SOCS3 (SOCS2 methylation did not correlate with IDH1/2 status). Methylation of the MGMT gene has been studied in the same samples, as the confirmation of the hypermethylator phenotype. Our results would help to establish a risk group that is particularly vulnerable to glioma development among low grade tumors. Detection of mutations in the IDH1/IDH2 genes and SOCS1, SOCS2 and SOCS3 genes methylation could be a simple diagnostic test allowing classification of patients with increased risk transformation to malignant tumor.

Key words: DNA methylation, STAT signaling, SOCS genes, hypermethylation phenotype, IDH1/2 mutations.

Studies supported by EPTHERON STRATEGMED1/233574/15/NCBR/2015 grant from National Center for Research and Development.

Poster

KW017-00007-2017-01

Knockdown of osteopontin (SPP1) in glioma cells blocks protumorigenic activation of infiltrating myeloid cells and restores activation of cytotoxic T cells

Anna Gieryng, Dominika Pszczolkowska, Michal Dabrowski, Bozena Kaminska

Neurobiology Center, Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Poland

Introduction: Human malignant gliomas are infiltrated with myeloid cells, especially microglia and peripheral macrophages, and lymphocytes. Despite their accumulation and activation in tumor microenvironment, anti-tumor immune responses are defective in these tumors. Tumor-educated immune cells induce an immunosuppressive environment and paralyze cytotoxic T (Tc) cells, support glioma invasion and progression. Osteopontin (Spp1, secreted phopshoprotein 1), a potent immune cell attractant and activator, is secreted and processed by glioma cells. Tumor derived Spp1 induces glioma-associated activation of microglia/macrophages thereby supporting glioma progression. In this study, we analyzed the effects of Spp1 knockdown in glioma cells on the response of immune cells infiltrating intracranial gliomas.

Material and methods: Lentivirally delivered shRNA were used to knockdown Spp1 in glioma cells and Spp1 stably depleted cell lines were developed. Glioma shSpp1 and shNeg cells were intracranially implanted to Wistar rats and 14th or 21st days later CD11b+ and CD8+ cells infiltrating gliomas were sorted and tumor volume was measured. Transcriptome profiling with Affymetrix Rat Gene 2.1 ST microarray and quantitative RQ-PCR were performed.

Results: Spp1 knockdown did not affect accumulation of resident microglia, blood-derived macrophages and leukocytes in gliomas but significantly reduced tumor growth. However, the computational analysis of gene expression revealed different profiles in sorted CD11b+ cells from control and Spp1 depleted gliomas. Significant differences in clusters related to DNA replication, translational regulation, mitosis and defence response, immune response were detected. Moreover, the expression of genes characteristic for protumorigenic activation was reduced in Spp1 depleted gliomas. Detailed analysis of the expression of transcription factors characteristic for T-cell subpopulations and markers of activated Tc cells showed restoring of anti-tumor responses in sorted CD8+ cells from Spp1 depleted gliomas.

Conclusions: Our results confirm a critical role of tumor-derived Spp1 in shaping glioma microenvironment and suggest the impaired acquisition of protumorigenic phenotype in CD11b+ cells from Spp1 depleted gliomas. The presence of activated Tc cells could be a sign of restoration of the antitumor activity of immune responses in SPP1-depleted gliomas, which results in tumor eradication.

Key words: tumor microenvironment, glioma-associated microglia/macrophages, functional reprogramming, myeloid-derived suppressor cells.

Supported by 2012/04/A/NZ3/00630 grant from the National Science Center.

Poster

KW017-00018-2017-01

KRAB-ZNFs – potential cancer epigenome modifiers

Marta Gładych[1],Kornel Kiełczewski[2], Przemysław Biecek[2,3], Urszula Oleksiewicz[1]

[1]Department of Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[2]Faculty of Mathematics and Information Science, Warsaw University of Technology, Warsaw, Poland
[3]Faculty of Mathematics, Informatics, and Mechanics, University of Warsaw, Warsaw, Poland

The role of epigenetic changes in cancer initiation and progression is nowadays intensively studied. Modifications of histones and DNA methylation play essential role in carcinogenesis. Tumor suppressor genes become hypermethylated, thus their transcription is repressed, while cancer genomes become globally hypomethylated. The precise mechanism of cancer-related epigenetic events is still unclear.

KRAB-ZNFs (Krüppel-associated box-zinc finger proteins) are the most abundant transcription factors in the human genome. The family consists of ~700 proteins characterized as potent repressors. The aim of our study was to define the role of selected KRAB-ZNFs in epigenetic regulation of tumor suppressor genes. We took advantage of RNA sequencing data deposited in the TCGA project. We have analyzed changes in expression level of 381 KRAB-ZNF genes in 6272 samples belonging to 14 cancer types and their relative normal samples. We found that expression level of several KRAB-ZNFs is elevated in all cancer samples in comparison to normal tissue. In the next step we wanted to explore the biological role of cancer-associated KRAB-ZNFs. Firstly, we have investigated their expression level in model cancer cell lines and patients tissue samples. We narrowed down further analysis only to the two most common cancer types: lung cancer (adenocarcinoma and squamous cell cancer) and breast cancer (basal and luminal). Using RT-qPCR we have confirmed that selected KRAB-ZNFs are overexpressed in cancer cell lines and tissue panels. What is more we have analyzed clinical data in order to explore the correlation between KRAB-ZNFs expression and histological type or TNM classification in both cancer types and additionally hormone receptor expression and DNA methylation profile in BRCA. In the next step we will explore KRAB-ZNFs influence on cancer cells phenotype and investigate their molecular function in cancer cell biology. Our results suggest that specific KRAB-ZNFs may be involve in epigenetic modifications of cancer genomes and may play an important role in carcinogenesis.

Key words: epigenetics, KRAB-ZNFs, tumor suppressors.

Poster

KW017-00015-2017-01

Development and application of the protocol of histopathological examination of three dimensional breast cancer model

Agata Golabek[1,2], Karolina Penderecka[1,3], Kosma Sakrajda[1,3], Apolonia Kaluzna[4,5], Matthew Ibbs[4,5], Ewelina Dondajewska[6], Andrzej Mackiewicz[1,5,6], Hanna Dams-Kozlowska[1,6]

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

The development of cancer is closely related to the interactions of tumor cells with the cells in the tumor microenvironment and the components of the extracellular matrix (ECM). Standard, two dimensional (2D) cell cultures *in vitro* do not provide information about the complex tumor environment. Three-dimensional cultures reflect better the cell-cell interaction, production of ECM and the impact of microenvironment on tumor progression. We developed a three-dimensional model of breast cancer, which involved spatial co-culture of breast cancer cells and fibroblasts on silk scaffolds. However, 3D model of breast cancer requires further characterization by using different research techniques.

Aim of this study was to develop the protocol of histopathology examination and to use it to analyse the presence of ECM components in the 3D model of breast cancer. Scaffolds were made of silk fibroin extracted from Bombyx mori cocoons. Porous scaffolds were obtained by salt-leaching method. Two cell lines: EMT6 - murine breast cancer cells, and 3T3 - murine fibroblasts were seeded as co-culture at a 9 to 1 ratio on the silk scaffolds. Co-culture was incubated for up to 21 days on 3D scaffolds under static and dynamic condition with agitation. The protocol of histopathology examination included following steps: fixation, dehydration, prestaining, immobilization and paraffin embedding. To analyse the growth of cells and the production of ECM the hematoxylin and eosin (HE) and alcian blue (AB) stainings were used.

The most important steps to obtain histopathological specimen of 3D cancer model were prestaining and immobilization of material before paraffin embedding. The application of these steps allowed to obtain entire cross-section of the 3D cancer model in a controlled position. Functionality of optimized method was confirmed by HE and AB stainings that allowed to analyse the location and the morphology of cells growing on the silk scaffold and the presence of extracellular matrix components such like mucopolysaccharides, hyaluronic acid and sialic acid.

Three-dimensional heterogeneous breast cancer model based on porous silk scaffold is an innovative, promising tool to study tumor biology and potentially may allow to understand the impact of microenvironment on tumor development. The presence of the ECM into 3D cancer model indicates that applied method of culture of cells generates a niche which resembles that observed *in vivo*.

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

Poster

KW017-00049-2017-01

Long non-coding RNAs as new biomarkers in head and neck squamous cell carcinoma

Kacper Guglas, Tomasz Kolenda, Marcel Ryś, Anna Teresiak, Renata Bliźniak, Matthew Ibbs, Małgorzata Wierzbicka, Jacek Mackiewicz, Katarzyna Lamperska

Greater Poland Cancer Center in Poznan, Poland

Long non-coding RNAs (lncRNAs) are a new class of non-coding RNA larger than 200 bp. Deregulation of lncRNAs is shown in many diseases, so changes in their expression can be used potentially as new biomarkers. In this study, expression of 90 lncRNAs was checked in tumour samples as well as in HNSCC cell lines.

Total RNA was isolated using TRI reagent from HNSCC samples and matched healthy samples, from DOK, FaDu, Cal27, SCC-25 and SCC-040 cell lines as well as from 6 healthy donors. LncRNA profiling was performed using lncRNA profiler™ qPCR array (System Bioscience). All real-time PCR data was normalized against expression mean of controls and followed by t-test analysis. Additional, expression analysis of selected lncRNA from TCGA data was performed. Comparison of all examined HNSCC samples with adjacent-matched tissues revealed significant up-regulation of Tsix in cancer tissue. For H19, NDM29 and snaR up-regulation was noticed, but not reached statistical significance ($p > 0.05$). In the group of down-regulated lncRNAs in cell lines were: Alpha 280, Alpha 250, anti-NOS2A, lincRNA-p21, lincRNA-VLDLR, NRON, SCA8, Sox2ot, Tsix and Y RNA-1. HOXA6as was the only up-regulated lncRNA in HNSCC cell lines compared to healthy donors without history of cancer.

In the case of HNSCC HVP- tissue samples, the significant up-regulation of anti-NOS2A, HOTAIRM1, KRASP1, lincRNA-ROR, lincRNA-SFMBT2, lincRNA-VLDLR, Nespas, NRON, PRINS, PTENP1, SNHG1, TEA ncRNAs family and Zeb2NAT was observed in T3-stage tumors compared to T1-T2 group.

Expression of EgoA, EgoB, GAS5, HAR1B and HOXA3 was up-regulated in the case of HPV+ tumors as well as in the cancer cell lines infected by lentivirus. However, only significant up-regulation of EgoT transcript (EgoA+EgoB) was confirmed by TCGA data analysis of HNSCC samples.

We observed that the lncRNA expression is changed in tumor samples compared to adjacent-matched tissues. Our analysis suggested, that these changes are connected with more advanced stage of disease and probably are implicated in tumor progression. It is the first report, where changes in the expression of lncRNA EgoT (EgoA+EgoB) were connected with HPV infection in HNSCC. We supposed that EgoT (EgoA+EgoB) may be used as potentially as a new biomarker of virus infection, but it should be verified in large group of patients.

However, the exact biological role of most of the indicated by us lncRNAs is still unknown and *in vivo* studies are necessary.

Key words: biomarkers, long non-coding RNA, head and neck cancers, HNSCC.

Poster

KW017-00014-2017-01

How new generation technologies can support clinical cancer diagnostics?

Kinga Humińska, Marta Kuś-Słowińska, Agata Młodzińska, Anna Brylak, Jacek Wojciechowicz

Genomic Laboratory, DNA Research Center, Poznan, Poland

Introduction: Cancer is a variable disease and the molecular links between cancer susceptibility and progression alteration levels remain mostly unknown. That's why our patients were screened towards germline, somatic mutations and CTCs, looking for the potential of integrating genomic data for a comprehensive and personalized cancer medicine.

Material and methods: Using NGS technology, cancer patients were screened towards targeted panel which consists of 170 genes, suspected to play a role in predisposing to cancer. Analysis included genes associated with both common (e.g., breast cancer, prostate, colorectal, lung) and rare cancers. Content selection was based on expert interpretation of the scientific literature and other high-quality resources. We also screened those patients towards wide range of "hot spot" mutations in 56 tumor suppressor genes and oncogenes annotated in COSMIC database. This test utilizes a 263-amplicon design, covering over 16000 COSMIC mutations to generate targeted libraries compatible with Illumina sequencing platforms. In order to confirm the occurrence of somatic mutation we additionally did a comparison of 3 techniques: Sanger sequencing, qPCR and ddPCR. Additionally, we monitored cancer regression and chemosensitivity by analysis of circulating tumor cells (CTC) from patients' blood.

Results: NGS technology speeds up the identification of causative alterations, proper diagnostics and treatment of patients. Comparison of 3 techniques highlighted the sensitivity of ddPCR, which can precisely quantify mutant allele frequency of a rare tumorigenic mutations in a high background of "normal" cells, routinely down to 0.01%, much deeper than the standard real-time PCR (1-5% limit detection) and Sanger sequencing (often over 30%). Besides, enumeration of CTCs provided us with an information whether the cells successfully are reduced by the therapy.

Conclusions: There is a great potential for integration of new generation technologies looking for genomic links in terms of development, usage of individualized biomarkers and monitoring the response to chemotherapy. Targeted cancer therapies may give medical oncologists a better way to customize cancer prevention and treatment. Next generation sequencing is a tool which gives the clinicians excellent possibilities for the realization of personalized medicine assumptions.

Key words: personalized medicine, germline mutation, somatic mutation, circulating tumor cells, next generation sequencing, cancer therapy.

The project is co-financed by the European Union under the European Regional Development Fund.

Poster

KW017-00044-2017-01

Comparative analysis of drug delivery by carriers made of a blend of hybrid silk proteins

Bogna Juskowiak[1], Tomasz Deptuch[1,2], Anna Florczak[1,2], Kamil Kucharczyk[1,2], Andrzej Mackiewicz[1,2], 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

Introduction: Spider silks due to biocompatibility, biodegradability and self-assembly, can be potentially applied in nanotechnology and medicine. It is possible to modify the silk property by genetic engineering by adding a sequence encoding peptide of designed function. Moreover, the bioengineered spider silk can be processed into different, non-toxic biomaterials such as hydrogels, films, scaffolds or micro- and nanospheres, which can be used as a delivery systems of small molecule drugs, protein and growth factor or nucleic acid based drugs.

Aim of the study is to analyze the effectiveness of loading and release of cytostatic drug form three types of nanospheres made of a blend of hybrid silk proteins.

Material and methods: We designed the MaSp1- and MaSp2-based bioengineered silk proteins (MS1 and MS2) and their hybrid variants:H2.1MS1, DOXMS2. The bacterial systems were used for expression of silk proteins. The protein purification method based on the resistance of spider silk to high temperature was applied to separate silk proteins from bacterial proteins. The blended spider silk nanospheres were formed using high pressure syringe pumps and then spheres were loaded by diffusion method with model drug - doxorubicin. The release of cytostatic drug was examined spectrophotometrically for7 days at three pH: 4.5; 6; 7.4.

Results: Four types of bioengineering spider silk proteins were produced and purified: MS1, H2.1MS1, MS2, DOXMS2and two of them were functionalized by genetic engineering to contain the Her2-recognizing peptide (H2.1) and doxorubicin binding motif (DOX).Three types of blended spider silk nanospheres (H2.1MS1/DOXMS2, MS1/DOXMS2, H2.1MS1/MS2) were formed with similar yield. The efficiency of doxorubicin loading was similar for all variants of blended spheres. The release of cytostatic drug during 7 days was pH dependent: faster at pH of 4.5 and 6 and slower at pH of 7.4.The trend of faster release of doxorubicin from H2.1MS1/MS2 spheres than from MS1/DOXMS2 and H2.1MS1/DOXMS2 particles was observed.

Conclusions: Our results showed, that a blending process can be applied as a controlling factor of particle properties. ObtainedH2.1MS1/DOXMS2 silk spheres can potentially serve as specific delivery vehicles that minimize the side effects of the applied treatment. Next, the cytotoxicity of doxorubicin delivered by these spheres will be examined by MTT assay.

Key words: bioengineered spider silk spheres, doxorubicine, targeted drug delivery.

Poster

KW017-00001-2017-01

Modulation of glycolytic genes expression by targeting the canonical Wnt signaling in head and neck squamous cell carcinoma cell lines

Robert Kleszcz, Jarosław Paluszczak, Wanda Baer-Dubowska

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

The canonical Wnt pathway regulates many physiological processes, but is also involved in the development of various cancers. Activation of Wnt signaling results in the stimulation of TCF/LEF transcription factors and expression of their target genes including the proto-oncogene c-Myc. This protein controls the expression of most of glycolytic enzymes which are over- expressed in cancer cells. Recent data indicate that some of them can be directly targeted by Wnt signaling.

The aim of the present study was to test the ability to change the expression of selected glycolysis-related genes by modulating the canonical Wnt pathway in head and neck squamous cell carcinoma (HNSCC) cells.

Six HNSCC cell lines H314 (floor of mouth), CAL27 and SCC-25 (tongue), BICR6 and FaDu (hypopharynx), BICR18 (larynx) and two non-carcinogenic cell lines OKF4/TERT-1 (keratinocytes – floor of mouth) and DOK (dysplastic keratinocytes – tongue) were used. Wnt signaling was induced by LiCl and inhibited by PKF118-310. Cells were incubated with selected doses of the modulators for 48 hours. Relative expression of glycolytic enzyme genes PKM2, PDHA1, PDK1, LDHA and lactate transporter MCT1 were analyzed using qPCR. The concentration of lactate, the glycolysis end-product was assessed in culture medium.

Treatment with LiCl resulted in a slight increase of the expression of PKM2 gene in BICR18 cell line. In the other cells tested no effect or decrease of expression of genes encoding glycolytic enzymes or MCT1 was observed.

PKF118-310 treatment resulted in a moderate decrease in the expression of all the tested genes in all cell lines, except H314 and BICR18. A tendency to increase the expression of these genes was noted in the latter. The most dramatic decrease was observed in the non-carcinogenic OKF4/TERT-1 cell line.

Significant increase in lactate production upon LiCl treatment was found in BICR18 and SCC-25 cells. Importantly, PKF118-310 treatment led also to a decrease in lactate release into culture medium in SCC-25 cells.

Overall, the results of this study indicate that the canonical Wnt pathway modulation may affect the expression of genes encoding glycolytic enzymes and transporters in HNSCC and non-carcinogenic HN cells. The modulatory effects depend on the cell line. Further studies are required to assess how these changes may affect the Warburg effect in HNSCC.

Key words: Wnt pathway, β-catenin, the Warburg effect, head and neck cancers.

This work was supported by grant from Poznan University of Medical Sciences No. 502-14-03302403-10649.

Poster

KW017-00010-2017-01

The role of large deletions in BARD1 and APOBEC3B as risk factors for breast and/or ovarian cancer

Katarzyna Klonowska, Piotr Kozlowski

Department of Molecular Genetics, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Introduction: Besides BRCA1/2 genes, a considerable fraction of breast and/or ovarian predisposing factors (>50%) is still unknown. One of the intensively studied candidate breast and/or ovarian cancer susceptibility genes is BARD1. Although it was suggested that large mutations (multi-exon deletions or duplications) may contribute substantially to the deleterious variation of BARD1, no systematic study of large mutations in BARD1 was performed so far. Recently, a group of candidate breast cancer genes has expanded by APOBEC3B. A common CNV in APOBEC3 cluster (APOBEC3B deletion) was reported to be associated with breast and/or ovarian cancer.

Aim of the study: The aim of our study was to characterize the role of large deletions in BARD1 and APOBEC3B as risk factors for breast and/or ovarian cancer.

Material and methods: For the study of large deletions in BARD1, we designed a MLPA assay and performed an analysis of 817 women with breast and/or ovarian cancer. Then, we analyzed structure of APOBEC3B deletion using sequencing, MLPA and A3B+ PCR, and performed large association study in three different European cohorts (in total 2972 cases and 3682 controls) using A3B+ PCR. Additionally, we exploited publically available expression data and our qPCR/ddPCR results for the study of a relation between APOBEC3B deletion and expression of the affected genes.

Results: Our study did not reveal any large mutations in BARD1. We observed no association between APOBEC3B deletion and breast and/or ovarian cancer in European population. We elucidated the structure of APOBEC3B deletion what allowed us to confirm the presence of transcriptionally active hybrid gene (APOBEC3A/APOBEC3B; APOBEC3A with 3'UTR of APOBEC3B) and design tests for expression analysis of the affected genes. The analysis showed that APOBEC3B deletion negatively correlates with expression of APOBEC3A and APOBEC3B, and positively correlates with expression of APOBEC3A/APOBEC3B.

Conclusions: Although we cannot exclude presence of large mutations in BARD1, our study indicates that such mutations do not contribute substantially to the risk of breast and/or ovarian cancer. Our case-control study of APOBEC3B deletion does not confirm its association with breast and/or ovarian cancer. We showed that APOBEC3A/APOBEC3B hybrid gene is transcriptionally active and confirmed association of APOBEC3B deletion with the expression of affected genes.

Key words: breast and/or ovarian cancer, BARD1, APOBEC3B, large deletions.

Acknowledgments: NCN2015/19/N/NZ5/02247, NCN2015/17/B/NZ2/01182.

Poster

KW017-00035-2017-01

Role of let-7d and miR-205 in cancer cells

Tomasz Kolenda[1,2], Kacper Guglas[1,3], Marcel Ryś[1,3], Anna Jędrzejak[4], Katarzyna Stefańska[4], Paula Sobieszczańska[4], Anna Teresiak[1], Renata Bliźniak[1], 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 Cancer Immunology, Chair of Medical Biotechnology, Poznan University of Medical Sciences, Poznan, Poland
[4]Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

Introduction: Head and neck squamous carcinoma (HNSCC) is one of the most invasive types of cancer with high mortality. Previous study indicated, that low level of let-7d and miR-205 in HNSCC patients is correlated with worse survival factor. However, the mechanism of their common action remains unclear. Let-7d and miR-205 are described as tumor suppressors and regulators of epithelial-to-mesenchymal transition process. We still don't know whether let-7d and miR-205 together influence in specific way on cancer cells.

Aim of the study: Analysis of biological role of up-regulation of let-7d and miR-205 in a cell model.

Materials and methods: Over-expression of let-7d, miR-205 and both of them (let-7d+miR-205) in the human fibroblast cell line (MSU-1.1) was obtained using lentiviral vectors. The proliferation ratio, spheres forming capacity and wound healing ability were checked by MTT, soft agar and wound healing assays, respectively. The expression of selected genes was measured using qRT-PCR and western blot.

Results: The over-expression of let-7d+miR-205 and let-7d alone caused significantly decreased proliferation ratio of the cells and closing the wound area in contrast to over-expressed miR-205, which did not influence on proliferation and migration compared to control cell line.

The sphere forming capacity assay indicated, that over-expression of let-7d, miR-205 and let-7d+miR-205 significantly influences on sphere size and number. let-7d+miR-205 caused changes in the expression of Vimentin, Beta-catenin, N-cadherin and TJP1 compared to cells over-expressing let-7d or miR-205 alone.

Conclusions: Our results show, that the over-expression of let-7d+miR-205 creates unique cell phenotype with different behavior compared to cells with up-regulated let-7d or miR-205 separately. Let-7d and miR-205 influences on proliferation, EMT process as well as invasion ability. We concluded, that let-7d seems to modify the function of miR-205 and has key function in cellular phenotype changes.

Key words: let-7d, miR-205, HNSCC, EMT, microRNA.

Poster

KW017-00045-2017-01

STARTRK-2 Basket trial for TRK, ROS1 and ALK fusions in patients in Poland

Marzenna Kotarska-Puerto[1], Steven Potts[1], Jason Christiansen[1], Monika Prochorec-Sobieszek[2], Andrzej Tysarowski[2], Michał Wągrodzki[2], Katarzyna Seliga[2]

[1]Ignyta, Inc., San Diego, CA, USA
[2]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw, Poland

Introduction: The STARTRK-2 (Studies of Tumor Alterations Responsive to Targeting Receptor Kinases) trial is a potentially registration-enabling Phase 2 global basket trial of the tyrosine kinase inhibitor entrectinib in patients with solid tumors harboring NTRK1, NTRK2, NTRK3, ROS1, or ALK gene fusions. Phase 1 studies of entrectinib reported a 79% Overall Rate of Response across multiple histology types in patients with gene fusions who were naïve to inhibitors of these targets, received an efficacious dose, and had extracranial disease. Diagnostic testing to identify patient populations with such low prevalence of molecular alterations poses efficiency and cost challenges, as broad profile molecular testing for these gene rearrangements is not yet part of standard clinical practice. In this presentation, we report on the occurrence of TRK, ROS1, and ALK fusions in these tumors. We also discuss the development and validation of an assay for solid tumor samples as well as immunohistochemistry (IHC) screening efficiency rates.

Material and methods: The occurrence of TRK, ROS1, and ALK gene fusions in solid tumors was studied, from Ignyta internal and partner collaborations. In order to effectively identify patients eligible for the Phase 2 trial of entrectinib (STARTRK-2), we have developed a 2-step diagnostic test to identify gene fusions in formalin-fixed paraffin-embedded clinical specimens. This test is comprised of IHC screening using a pan-receptor tyrosine kinase cocktail of antibodies targeting these five proteins followed by an RNA-based anchored multiplex polymerase chain reaction next generation sequencing (NGS) assay. The study has been initiated in 5 sites in Poland, and strategies include a mixture of IHC screening and NGS testing.

Results: NTRK fusions have been identified and confirmed in over 40 histologies. Out of several thousand samples, no instances have yet been detected where the IHC was screened negative and gene rearrangements were observed by NGS. A pan-receptor tyrosine kinase IHC cocktail assay enriches detection of the patient population for gene rearrangements over 10 fold, depending on the tumor location, and has a 100% negative predictive value.

Conclusions: The STARTRK-2 trial has been implemented across Poland, and countrywide molecular screening strategies have been developed. Depending on how it is implemented in clinical trials, the novel two-step testing approach can substantially decrease the costs and tissue expended for NGS.

Key words: NTRK, ROS1, ALK, entrectinib, targered therapy.

Poster

KW017-00004-2017-01

Iron oxide nanoparticles increase the loading of chemotherapeutic into EMS2 silk 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
[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

Superb mechanical properties, biocompatibility and biodegradability make spider silk an exceptional material for biomedical applications. Silk can self-assemble and form various morphological forms such as fiber, film, hydrogel, scaffold, micro- and nanospheres. The use of genetic engineering for silk production enables to obtain material of modified properties.

Three bioengineered spider silk proteins were used: MS1, MS2 and EMS2. MS1 protein was constructed based on the consensus repetitive motif of MaSp1 while MS2 and EMS2 proteins on the motif of MaSp2 of dragline silk spidroins of Nephila clavipes spider. To obtain EMS2, the MS2 repeated motif was modified by addition a glutamic acid residue. The proteins were produced in E.coli expression system and purified using thermal denaturation. Silk nanospheres were produced by mixing iron oxide suspension and silks solution with potassium phosphate. MS1/NPs MS2/NPs and EMS2/NPs spheres morphology and elemental composition was analyzed with SEM/EDS microscopy. EMS2/NPs spheres were subsequently subjected to analysis such as: secondary structure content (FTIR), magnetic measurements (SQUID), cytotoxicity assay (MTT) and doxorubicine loading and release profile.

The MS1, MS2 and EMS2 bioengineered were successfully produced, purified and mixed with iron oxide nanoparticles. We obtained composite spheres MS1/NPS, MS2/NPs and EMS2/NPs and the highest magnetite content was observed for EMS2/NPs variant and these particles were further analyzed. FTIR analysis revealed that the addition of Fe3O4 nanoparticles caused change in the structure of EMS2 spheres. SQUID analysis showed that the interaction with silk in EMS2/NPs composite spheres did not influence the superparamagnetic properties of iron oxide. Both EMS2 and EMS2/NPs were not toxic against fibroblast. The drug loading studies exhibited more than two fold higher loading efficiency of doxorubicin for spheres EMS2/NPs than for EMS2. For both EMS2 and EMS2/NPs the drug revealed a pH-dependent release profile.

Our study showed the possibility to produce microspheres composed of silk/iron oxide composite. The new approach resulted in increased particles capacity for incorporated drug which may be a valuable factor for an efficient cancer therapy.

Key words: iron oxide nanoparticles, drug loading, bioengineered spider silk spheres, cancer therapy, doxorubicine.

Poster

KW017-00042-2017-01

Intraoperative radiotherapy impairs breast cancer stem cell phenotype increased by surgical wounding

Katarzyna Kulcenty[1,2], Igor Piotrowski[1], Karolina Zaleska[1], Dawid Murawa[3], Wiktoria Suchorska[1,4]

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

Breast cancer is the most common cancer occurring in women. Conservative breast cancer surgery followed by radiation therapy is currently the standard treatment for this type of cancer. The published data suggest, that the wound healing process after surgery alters the area surrounding the original tumor and the modified microenvironment is more favorable for the tumor to recur. The majority of metastases within scar initiated a series of research and their consequences in clinical trials aimed to assess, whether localized radiotherapy, as intraoperative radiotherapy (IORT), will be more effective in inhibiting formation of local recurrence than the standard postoperative whole breast radiotherapy. It was previously reported, that IORT alters the tumor microenvironment through the modulation of wound healing response. Moreover it was shown, that the growth kinetics of breast cancer micro metastasis were modified by surgery, representing a perturbing factor in the process of relapse or metastasis development. A critical role in promoting metastasis in epithelium-derived carcinoma plays a developmental program termed epithelial-mesenchymal transition. A tumor microenvironment (inflammatory cells infiltrating the tumor) and cancer stem cells (CSC) present in the tumor microenvironment may be the inducers of EMT in tumor cells. Thus we wondered, whether wound fluids can induce the cancer stem cell phenotype and EMT program and whether IORT plays inhibitory role in this process. Wound fluids form patients which underwent IORT (IR-WF), as well as control group without radiotherapy treatment (WF), were collected 24 hours and one week after the surgery. Two human cancer cell lines with different molecular status (basal – MDA-MB-468, luminal – MCF7) were then incubated with wound fluids (WF, IR-WF) in complete culture medium (10%). Flow cytometry and RT-qPCR analysis revealed, that wound fluids from patients who received IORT decreased the phenotype of cancer-stem cells in the basal (MDA-MB-468) and luminal subtype (MCF7) of cancer cell lines compared to IORT-untreated patients. Moreover, we also confirmed, that WF and IR-WF affects the EMT program in analyzed cell lines.

Key words: cancer stem cells, wound fluids, intraoperative radiation therapy.

Poster

KW017-00021-2017-01

Novel roscovitine derivatives as promising drugs for CLL treatment

Małgorzata Kubczak[1], Jerzy Błoński[2], Aleksandra Szustka[1], Tomáš Gucký[3], Vladimir Krystof[3], Tadeusz Robak[2], Małgorzata Rogalińska[1]

[1]Department of Cytobiochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Poland
[2]Department of Hematology, Medical University of Lodz, Poland
[3]Laboratory of Growth Regulators and Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of SciencePalacký University and Institute of Experimental Botany AS CR, Czech Republic

Aim of the study: A coexistence of two populations of cells, i.e quiescent and cycling display a special challenge for choosing the optimal treatment for chronic lymphocytic leukemia (CLL). The differences in cell signaling, expression of factors involved in apoptosis realization, as well as microenvironmental stimuli might lead to diversities in patient's response to therapy. Additionally, some patients display resistance to conventional drugs. It's very important to search for a new compounds which will be effective as apoptosis inducers for CLL patients. New, potent analogs of roscovitine were designed with enhanced anticancer potential in cancer cell lines. In our study we compared the cytotoxity of novel roscovitine analogs on control mononuclear cells isolated from peripheral blood of healthy donors with leukemic cells obtained from CLL patients. **Results:** The potential of apoptosis/necrosis induction for roscovitine derivatives (BA12, BA14, BP14 and BP30) were analysed. The range of anticancer agents concentrations were from 100 mM to 10 nM. Simultaneous analysis of cell viability, and protein expression related to apoptosis by Western blot were performed to examine the ability of leukemic cells to enter apoptosis during their incubations with anticancer agents. BA12 and BA14 displayed strong cytotoxity toward healthy B cells comparing to BP14 and BP30. BA12 and BA14 were rejected from the next phase of research. Both studied anticancer agents (BP14 and BP30) induced in leukemic cells apoptosis, decreased cell viability and increased the level of apoptosis. The differences in induction of apoptosis and necrosis were time- and dose-dependent. The obtained results have shown that nanomolar concentrations were optimal as apoptosis inducers. Low cytotoxic effect was observed in normal B cells. **Conclusions:** The evaluation of cell viability, and changes in expression of protein related to apoptosis is helpful in monitoring of leukemic cells sensitivity to anticancer agents. Such analysis are useful in searching for new potential drugs for CLL. Additional analysis must be performed to choose the optimal concentrations inducing apoptosis in leukemic cells with low level of necrosis.

Key words: anticancer agents, CLL, apoptosis induction, necrosis, novel roscovitine derivatives.

Poster

KW017-00032-2017-01

Comparative genomic analysis of intracranial germ cell tumors – preliminary results focused on SHH pathway elements

Dominika Kuleszo[1], Magdalena Koczkowska[1], Wiesława Grajkowska[2], Elzbieta Drożynska[3], Bożenna Dembowska-Bagińska[4], Beata Lipska-Ziętkiewicz[1], Marcin Roszkowski[5], Katarzyna Czarnota[6], Ewa Iżycka-Świeszewska[7]

[1]Department of Biology and Genetics, Medical University of Gdańsk, Gdansk, Poland
[2]Pathology Department of Children's Health Memorial Institute, Warsaw, Poland
[3]Department of Pediatrics, Oncology and Hematology, Medical University of Gdańsk, Gdansk, Poland
[4]Department of Pediatric Oncology Children's Health Memorial Institute, Warsaw, Poland
[5]Department of Pediatric Neurosurgery, Children's Health Memorial Institute, Warsaw, Poland
[6]Department of Pathomorphology, Copernicus PL, Gdansk, Poland
[7]Department of Pathology and Neuropathology, Medical University of Gdańsk, Gdansk, Poland

Germ cell tumours (GCT) create a heterogenous group of tumors arising from germ cells on different phases of their differentiation and maturation. GCT can be gonadal and extragonadal including intracranial cases constituting 1-3% of all CNS tumors. Intracranial GCT grow most commonly in children and young adults, with midline location especially in the pineal region.

The study was performed on DNA isolated from frozen tumor tissue samples from seven tumors: 3 germinomas, and 4 mature teratomas. Comparative genomic profiling analysis was carried out with microarray-CGH method (Cytosure ISCA UPD 4x180k, Oxford Gene Technology). The results were analyzed with Feature Extraction (Agilent Technologies) oraz Nexus Copy Number 8.0 (BioDiscovery) software.

Chromosomal aberrations were found in two germinomas. These tumors were characterized with complex genomic profiles encompassing chromosomes 7, 8, 9, 10, 11, 12, 16, 17 and 19. Common findings were short arm amplification of 12p13.13p11.1 of 35 Mbp length and long arms of 17q11.1q25.3 of 55 Mbp length. In one tumor also SHH (7q36.3), SMO (7q32.1) and GLI3 (7p14.1) duplication occured together with 9q21.11q34.4 loss including PTCH1, all being elements of SHH signaling pathway. Moreover both tumors showed duplication of genes being ligands, regulators, receptors or target genes of SHH (MTSS1, PRKACA, FKBP8, SFRP1) as well as amplification of genes of SHH coopting WNT pathway (WNT10B, WNT16, WNT2, WNT3, WNT5B, WNT9B).

These are preliminary results. Further studies on bigger group will look for genotype- phenotype relations, and try to establish primary (driver mutations) or secondary (passenger mutations) character in intracranial GCT.

Key words: germ cell tumor, SHH pathway, genomic analysis.

Financial support Narodowe Centrum Nauki 2014/15/B/NZ4/04855.

Poster

KW017-00022-2017-01

In vitro and *in vivo* characterization of slowly dividing murine melanoma cells

Anna Kusienicka, Karolina Bukowska-Strakova, Maciej Cieśla, Witold Nowak, Iwona Bronisz, Monika Żukowska, Józef Dulak, Alicja Józkowicz

Department of Medical Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

One of the features attributed to melanoma aggressiveness is the presence of cells displaying cancer stem cells (CSC) properties. The identification of CSC based on surface markers has many limitations, as function of many markers is still unknown and digestion of tumor samples can lead to antigen cleavage interfering with further analyses. Thus, there is a need for identification of cells with CSC properties that are based on functional features. One of the CSC feature is slow cycling phenotype but the role of slowly dividing cells in melanoma is still largely unknown. Here we identified slowly cycling subpopulation within murine melanoma B16(F10) cell line and characterized these cells *in vitro* and *in vivo*.

Using cell membrane dye PKH26 we identified slowly dividing subpopulation of cells. Slowly dividing PKH+ cells tend to reside in G0 phase. Interestingly, PKH+ cells neither express CSC surface marker CD133 nor display high ALDH activity. Analysis of tyrosinase and MITF gene expression revealed that PKH+ cells do not differ from PKH- subset in terms of differentiation status. Slowly cycling cells have decreased levels of inhibitor of DNA binding 1 (ID1) and increased Bmp7 gene expression. When sorted for clonogenic assay, 43.3% of single PKH+ cells and 61.7% of PKH- cells were able to form clones. PKH+ clones were smaller but displayed higher migratory capabilities than PKH– counterparts. Cell lines that were derived from PKH+ and PKH– clones did not differ in terms of CSC subpopulations constitution (CD20 and ALDH activity). Moreover, PKH+ derived cell lines were not enriched in PKH+ subpopulation, what suggest that slowly cycling phenotype is temporary and reversible. *In vivo* studies showed that only PKH+ subset of cells were able to form tumors after administration of as little as 10 cells (3/22 tumors in PKH+ group vs. 0/22 PKH–). Moreover, tumor cells isolated from primary PKH+ tumors were able to form secondary and tertiary tumors in serial transplantation experiment. To sum up, slowly dividing phenotype facilitates initiation of tumor growth and self-renewal capabilities *in vivo*. It seems that slowly cycling phenotype is reversible and distinct from other CSC subpopulations (CD133+ and ALDHhigh cells). *In vitro*, PKH+ cells do not differ significantly in terms of clonogenic potential but slowly dividing cells form smaller clones with higher migratory properties and upregulate Bmp7, an important mediator of melanoma aggressiveness.

Key words: slowly dividing cells, melanoma, cancer stem cells, B16(F10) cell line.

Poster

KW017-00046-2017-01

The impact of the size of embryoid bodies onto chondrogenic differentiation

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

[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
[4]Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland

Background: Differentiation of pluripotent stem cells into chondrocytes is still a challenge for regenerative medicine. One of the approaches used in differentiation protocols is usage of natural ability of pluripotent stem cells to form three germ layers via embryoid bodies (EB). The major issue of that process is its unification. A tremendous impact onto EBs fate has an amount of cells used for supension culture. Cellular mass determine the accessibility of nutrients, distribution of oxygen concentration, cellular interactions, what affects the spontaneous differentiation process. The aim of the study was to assess the impact of amount of cells onto chondrogenic differentiation.

Methods: Human embryonic stem cells (BGV01) were used to form EB in low-attachment 96 – well. EB were formed form 500, 1000, 1500 and 2000 cells and cultured for 15 days. Analysis of pluripotency, germ layers and prochondrogenic markers gene expression was performed to indicate the more and less mesoderm and prochondrogenic EBs. Further, those variants undergo the prochondrogenic differentiation in chondrogenic medium supplemented with TGF-β3 (10 ng/ml). The genes related to pluripotency and chondrogenesis was analyzed. Additionally to confirm the successful differentiation, immunofluorescence staining for chondrocytes related proteins was tested. Moreover, alcian blue staining was performed to confirm the deposition of proteoglycans.

Results: EBs formed from 500, 1000 cells indicated higher expression of mesodermal, ectodermal and prochondrogenic markers in comparison to 1500 and 2000 cells EB. The differentiation of EBs indicated higher expression of genes related to chondrocytes and mesenchyme condensation. Additionally, more condensed cartilage-like nodules were observed in EBs differentiated from 500 cells instead of 2000 cells.

Conclusions: EBs formed from 500 cells possess the highest mesodermal and prochondrogenic properties. The chondrogenic differentiation of 5th day 500 cells EBs was more efficient in comparison with larger and 15th day EBs.

Key words: human embryonic stem cell, regenerative medicine.

Poster

KW017-00009-2017-01

Database of breast cancer-related BARD1 sequence variants – searching for founder mutations in Polish population.

Sylwia Łuczak, Katarzyna Klonowska, Piotr Kozłowski

Instytut Chemii Bioorganicznej PAN w Poznaniu

In all breast cancer cases about 10% constitute familial breast cancer. It is estimated that germline mutations, mostly in BRCA1/2 genes and in some others, e.g., TP53, PALB2, CHEK2, PTEN, can be responsible for ~ 45% of familial breast cancer cases. Additionally, a recent GWAS study identified 67 new and previously reported SNPs associated with breast cancer. Altogether, only about 50% of familial breast cancer cases can be induced by known genetic factors.

One of the suspected and studied genes is BARD1 (BRCA1 associated RING domain 1). Product of this gene is essential for tumor-suppressor function of BRCA1 protein. BARD1 and BRCA1 share many structural and functional similarities. Both have RING-finger motif and two BRCT repeats. These two proteins form heterodimer which is crucial for a number of cellular processes. Recent data suggest that germline mutations in the BARD1 gene predispose to breast and/or ovarian cancer. Also, our recent study of large deletions in this gene led to the identification of 3 mutations in 7 different breast and/or ovarian cancer patients. Presumably, two of them are founder mutations in Polish population. Till now, several dozen of various BARD1 sequence variants have been described. Among them are deleterious and potentially deleterious variants which lead to premature termination of translation, disruption of protein structure/function or alternative splicing, and even large deletion. Also, GWAS analysis identified some BARD1 SNPs associated with different types of cancer. However, most of them need confirmation of functional significance.

To characterize spectrum of genetic variation in BARD1, we created an open database of all known changes of sequence of this gene. It will not only include information about mutations but also about unconfirmed variants and polymorphisms, identified in patients with different types of cancer and associated with cancer. The collected information will be mostly based on so far released publications presenting sequence variants in BARD1. This database not only helps in interpretation of function of identified mutation but also in understanding the role of this gene in predisposition to different cancers.

Key words: BARD1, database, sequence variants, founder mutations, Polish population.

Acknowledgements: NCN 2015/17/B/NZ2/01182.

Poster

KW017-00023-2017-01

The application of lentiviral and RNAi vectors for stable transgene expression in induced pluripotent stem cells.

Sylwia Mazurek[1,2], Patrycja Czerwińska[2,3]

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

Introduction: It is postulated that cancer stem cells (CSC) are a population of progenitor cells with unlimited potential for replication, and may generate tumors through the stem cell processes of self-renewal and differentiation into multiple cell types. It is also assumed that the CS cells are resistant to therapeutic agents and have the ability to cause relapse and metastasis after treatment. These cells are, however, a sparse subpopulation of tumor cells (less than 1%), which hinders their direct examination. Because of the relative ease of preparation of iPSC cells and a many common features with cancer stem cells, such as the capacity for self-renewal, differentiation, avoiding programmed cell death, resistance to toxic agents causing DNA damage and a low proliferative activity provide a good experimental model to determine the molecular basis of these processes. Future research may direct new therapeutic strategies in cancer therapy specifically targeting a population of cells having a phenotype of cancer stem cells.

Aim of the study was to develop a system of stable and efficient transgene expression in induced pluripotent stem cells using lentiviral vectors (LV) and based on the mechanism of RNA interference (RNAi).

Materials and methods: In order to select the best expression system for iPS cells, lentiviral vectors carrying green fluorescent protein (GFP) expression under control of various promoters were used.

For the production of vectors carrying GFP expression, plasmids containing eukaryotic EF1-short, EF1 alpha and PGK promoters were used. Lentiviral vectors were produced in human embryonic kidney 293T cells (HEK-293T) by calcium phosphate precipitation and then introduced to eukaryotic cells, HEK-293T cells, primary human dermal fibroblasts (PHDF) and iPS cells. Depending on the genetic background of given cell line, a different level of transcription is achieved.

Effectivity of particular vectors was examined by flow cytometry analysis of GFP fluorescence intensity in obtained cell lines. The fluorescence intensity was measured in cells containing one or more copies of the transgene. Basing on literature reports it was assumed that these conditions are met, when < 10% of the cells have become transduced.

Key words: cancer stem cells, induced pluripotent stem cells, RNA interference, lentiviral vectors.

Poster

KW017-00026-2017-01

Electrochemotherapy with cisplatin against metastatic pancreatic cancer – *in vitro* study on primary cells

Olga Michel[1], Justyna Mączyńska[1], Piotr Błasiak[2], Adam Rzechonek[2], Jolanta Saczko[1]

[1]Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland
[2]Department of Thoracic Surgery, Wroclaw Medical University, Wroclaw, Poland

Electroporation (EP) is a technique based on a delivery of short electric pulses to the cells in order to provoke their permeabilization. Electrochemotherapy (ECT) is a combination of EP and chemotherapy which is currently applicable to many types of cancer as an experimental treatment. Pancreatic cancer is characterized by late diagnosis and drug resistance. Hence, there is a clear need to expand experimental regimens, especially for patients burdened with metastasis.

The aim of our study was to evaluate the effectiveness of ECT with cisplatin on cells derived from pulmonary metastasis of pancreatic cancer. Primary cell culture was established from tissue fragment collected from patient during surgery. Cells were maintained in standardized conditions and only early passages were used for experiments. Following chemotoxicity tests we performed electroporation in EP buffer with 5 and 10 μM concentration of cisplatin, using the electric field strength of 600 and 1000 V/cm. Cell viability was measured via MTT assay after 24 and 48 hours of incubation. Additionally, we evaluated the expression of mitochondrial superoxide dismutase (SOD-2) and caspase-3 (Casp3).

The obtained results show that microsecond EP may enhance cisplatin effectiveness in cells from PDAC pulmonary metastases. The most notable difference in cell's viability was observed following application of low cisplatin concentration and electric field strength of 1000 V/cm after 24hour of incubation. Overexpression of SOD-2 was observed in cells subjected to therapy, which may suggest the occurrence of posterior oxidative stress in cells. Furthermore, the slight increase of Casp3 expression indicates the apoptosis which may be attributed to ECT. Noteworthy, increased SOD-2 expression and changes in cells morphology were observed following application of higher voltage.

The use of electrochemotherapy seems to be promising experimental treatment for pancreatic cancer metastasis. However, our results indicate that cells derived from metastatic lesions exhibit relatively high sensitivity to increased voltage therefore EP parameters have to be chosen carefully to selectively support the cisplatin transport directly into the cancer cells.

Key words: pancreatic cancer, pulmonary metastasis, primary cell culture, electroporation, cisplatin.

Financing: This work was supported by grant of Wroclaw Medical University STM.C010.16.007 (Piotr Błasiak).

Poster

KWO17-00011-2017-01

Static and dynamic culture conditions implemented for 3D heterogeneous cancer model - comparative study

Karolina Penderecka[1,2], Agata Gołąbek[1,3], Kosma Sakrajda[1,2], Apolonia Kałużna[4,5], Matthew Ibbs[4,5], Ewelina Dondajewska[6], Andrzej Mackiewicz[1,6], Hanna Dams-Kozłowska[1,6]

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

Silk fibroin is a protein obtained from the silkworm Bombyx mori cocoons. It is a biocompatible material and it has potential for wide biomedical applications. Is can be processed into different morphological forms such as films, hydrogels, micro- and nanospheres and scaffolds. In this study the silk scaffold was used to co-culture the cancer cells and stromal fibroblast to provide more relevant in vitro breast cancer model as a tool to study tumor biology.

The aim of this study was to compare the growth of the cells under static and dynamic conditions in 3D model of breast cancer.

Silk scaffolds were prepared using salt leaching method using silk solution extracted from B. mori cocoons. Murine breast cancer cells EMT6 and murine fibroblasts NIH3T3 expressing respectively green (GFP) and red (turboFP635) fluorescent proteins were seeded on silk scaffolds at a ratio of 1 to 9. The experiment was performed under static and dynamic conditions with the use of agitation platform. After 7, 14 and 21 days of cultivation cells were analyzed quantitatively and qualitatively. Flow cytometry was conducted to measure the percentage content of each cell line. The quantity and viability of the cells were measured by trypan blue exclusion test. The cells distribution into the scaffold and the morphology of the cells were examined by histopathology methods.

The cells exhibited high viability during mono- and co-culture in both static and dynamic model. However, in the dynamic conditions their amount was significantly larger than in static conditions. During culture the proportion between fibroblast and cancer cells were changed in favor of the cancer cells, however the kinetics of these changes was more pronounced under dynamic culture conditions. The penetration of the scaffolds by the cells was different depending on the culture model. Cells cultured under constant agitation overgrown quickly and evenly the entire volume of the scaffold, while under static conditions cells initially overgrown the edges and then gradually the internal part of the scaffold.

Silk scaffolds can be used to co-culture both breast cancer and fibroblast cells to imitate the *in vivo* environment of tumor. The implication of dynamic co-culture conditions into 3D breast cancer model provides quick and evenly distribution of cells into entire scaffold. 3D breast cancer model can be used to study tumor biology.

Key words: 3D cancer model, static cell culture, dynamic cell culture, silk fibroin scaffold, breast cancer.

Poster

KW017-00017-2017-01

Molecular profiles of thyroid cancer subtypes: classification based on features of tissue revealed by mass spectrometry imaging

Monika Pietrowska[1], Hanna C. Diehl[2], Grzegorz Mrukwa[3], Magdalena Kalinowska-Herok[1], Marta Gawin[1], Mykola Chekan[1], Julian Elm[2], Grzegorz Drazek[3], Anna Krawczyk[3], Dariusz Lange[1], Helmut Meyer[2], Joanna Polanska[3], Corinna Henkel[2], Piotr Widlak[1]

[1]Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland
[2]Medizinisches Proteom-Center, Ruhr-University Bochum, Bochum, Germany
[3]Faculty of Automatic Control, Electronics and Computer Science, Silesian University of Technology, Gliwice, Poland

Introduction: Determination of the specific type of thyroid cancer is crucial for the prognosis and selection of treatment of this malignancy. However, in some cases appropriate classification is not possible based on histopathological features only, and it might be supported by molecular biomarkers.

Aim of the study: Here we aimed to characterize molecular profiles of different thyroid malignancies using mass spectrometry imaging (MSI) which enables direct annotation of molecular features with morphological pictures of an analyzed tissue.

Material and methods: Fifteen formalin-fixed paraffin embedded tissue specimens corresponding to five major types of thyroid cancer were analyzed by MALDI-MSI after in-situ trypsin digestion, and the possibility of classification based on the results of unsupervised segmentation of MALDI images was tested. Novel method of semi-supervised detection of the cancer region of interest (ROI) was implemented.

Results: We found strong separation of medullary cancer from malignancies derived from thyroid epithelium, and separation of anaplastic cancer from differentiated cancers. Reliable classification of medullary and anaplastic cancers using an approach based on automated detection of cancer ROI was validated with independent samples. Moreover, extraction of spectra from tumor areas allowed the detection of molecular components that differentiated follicular cancer and two variants of papillary cancer (classical and follicular).

Conclusions: We concluded that MALDI-MSI approach is a promising strategy in the search for biomarkers supporting classification of thyroid malignant tumors.

Key words: classification, FFPE tissue, mass spectrometry imaging, molecular signature, thyroid cancer.

Poster

KW017-00012-2017-01

Metastatic potential of selected factors presented in serum of patients with pancreatic cancer

Agata Poniewierska-Baran[1,2], Marta Tkacz[2], Karol Serwin[2], Krzysztof Dąbkowski[3], Teresa Starzyńska[3], Mariusz Z. Ratajczak[4]

[1]Katedra Immunologii, Wydział Biologii, Uniwersytet Szczeciński
[2]Katedra Fizjologii, Pomorski Uniwersytet Medyczny w Szczecinie
[3]Katedra i Klinika Gastroenterologii, Pomorski Uniwersytet Medyczny w Szczecinie
[4]James Graham Brown Cancer Center, Department of Medicine, University of Louisville, KY, USA

Introduction: Pancreatic cancer is a serious clinical problem and a challenge for doctors and scientists. Despite the new possibilities in the diagnosis and treatment of cancer, the 5-year survival rate applies to less than 5% of patients, also many studies of pancreatic cancer pathogenesis, as well as any other cancers, remains unknown and needs further investigation. The process of metastasis is a cascade of mechanism, involving proteolysis, cell motility/migration, proliferation and neoangiogenesis. Cancer cells released from the primary tumor penetrate into the lymphatic vessels and/or blood vessels, which occurs by spreading. Circulating cells can migrate through the walls of blood into surrounding tissues, where they settle, proliferate and form a secondary tumors. Hypothesis. Understanding cellular processes responsible for cancer spread can be useful to improve diagnosis and prognosis of patients with pancreatic cancer.

Aim of the study was to supplement knowledge about the process of metastasis in pancreatic cancer, as a very aggressive and dangerous type of cancer. The influence of blood factors, such as SDF-1, VEGF or HGF in the metastasis process of pancreatic cancer had to be checked.

Material and methods: We evaluated the effect of serum collected from patients with pancreatic cancer and serum from control group, to pancreatic cancer cell line CRL1687™ (ATCC® cell bank BxPC-3 ™). To determine the prometastatic condition we compare the following parameters: cell migration, adhesion to plate coated with fibronectin, MAPK44/42 and Aktser473 signaling and we compare the concentration of blood factors, such as HGF – hepatocyte growth factor, VEGF – vascular endothelial growth factor and SDF-1 – stromal cell-derived factor-1 in patients and control group sample, by ELISA test.

Results: The results showed: 1) that serum from patients and control group mobilized migration, adhesion and proliferation of pancreatic cancer cell line CRL1687, as well as SDF-1, HGF, VEGF factors; 2) we observed higher metastatic potential of serum from patients with pancreatic cancer, compared to control serum; 3) we observed higher concentration of SDF-1, HGF/SF, VEGF in the serum of patients with pancreatic cancer, compared to control serum.

Conclusions: The results prove a strong metastatic activity of blood serum and a significant role of HGF/SF, VEGF and SDF-1 factors, as a potential biomarkers in pancreatic cancer cell metastasis.

Key words: cancer metastasis, pancreatic cancer.

Poster

KW017-00034-2017-01

Phase I/II dose escalation study of immunoconjugate L-DOS47 as a monotherapy in non-squamous non-small cell lung cancer patients

Rodryg Ramlau[1], Dariusz Kowalski[2], Cezary Szczylik[3], Aleksandra Szczesna[4], Elzbieta Wiatr[5], Heman Chao[6], Steve Demas[6], Kazimierz Roszkowski-Śliż[4]

[1]Department of Oncology, Poznan University of Medical Sciences, Poznan, Poland
[2]The Maria Sklodowska-Curie Institute of Oncology, Warsaw, Poland
[3]Military Institute of Health Institute, Warsaw, Poland
[4]Mazovian Centre of Pulmonary Diseases and Tuberculosis in Otwock, Poland
[5]National Tuberculosis and Lung Diseases Research Institute, Warsaw, Poland
[6]Helix BioPharma Corp.

Introduction: L DOS47, a cancer therapeutic designed to exploit the acidic tumour extracellular environment, is a protein conjugate consisting of a urease conjugated to a camelid monoclonal antibody (AFAIKL2) that is targeted to the CEACAM6 antigenic tumour marker. The AFAIKL2 antibody serves as a targeting agent to deliver the enzyme to the tumor sites while the urease enzyme converts urea, an abundant natural metabolite, into ammonia and generates a local pH increase. The combined effect of ammonia toxicity and pH increase is cytotoxic to cancer cells in culture and in xenograft models. This first in human study of L DOS47 was designed to define the maximum tolerated dose of multiple doses of L-DOS47 administered intravenously to patients with non-squamous NSCLC when given as a monotherapy.

Material and methods: Stage IIIb or IV histologically confirmed non-squamous NSCLC patients (aged ≥18 years, ECOG PS ≤ 2) receive multiple cycles of L-DOS47 during the study treatment period. L-DOS47 is administered once weekly over 14 days followed by 7 days rest in each treatment cycle. Patients are recruited into cohorts and received the same dose of L-DOS47 on Days 1 and 8 of each treatment cycle. Dose levels of L-DOS47 are escalated in further cohorts following a review of safety data by the Trial Steering Committee.

Results: Fifty-five (55) pts (median age 61, 53% male) were enrolled in sixteen cohorts (dose levels: 0.12 to 13.55 µg/kg) in four Polish centers. L-DOS47 was well tolerated at the dose levels reviewed. One (1) DLT was reported in a cohort 13 patient (spinal pain). Forty-seven (47) of the 55 patient dosed in the Phase I component of the study contributed to the response evaluable population.

A dose response trend was observed when comparing the percentage of patients who were progression free at 16 weeks across dose ranges. A similar trend was observed when comparing the percentage of patient who had Stable Disease (as defined in RECIST v1.1) and had a reduction in target lesions.

Conclusions: L-DOS47 may be effective in treatment of CEACAM6 expressing tumors and may be more efficacious in combination with other therapies that may benefit from the pH-modulating effects of L-DOS47.

Key words: tumor microenvironment, CEACAM6, pH-modulating effects, non-squamous NSCLC.

Poster

KW017-00003-2017-01

Molecular signatures based on serum lipids and metabolites discriminate patients with early lung cancer and healthy participants of lung cancer screening

Małgorzata Roś-Mazurczyk[1], Anna Wojakowska[1], Karol Jelonek[1], Monika Pietrowska[1], Łukasz Marczak[2], Krzysztof Polański[3], Michał Marczyk[4], Joanna Polańska[4], Rafał Dziadziuszko[5], Jacek Jassem[5], Witold Rzyman[5], Piotr Widłak[1]

[1]Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland
[2]Polish Academy of Science, Institute of Bioorganic Chemistry, Poznań, Poland
[3]University of Warvick, Coventry, Great Britain
[4]Silesian University of Technology, Gliwice, Poland
[5]Medical University of Gdańsk, Gdańsk, Poland

Introduction: The role of a low-dose computed tomography (LD-CT) lung cancer screening remains a matter of controversy due to its low specificity and high cost. Screening complementation with blood-based biomarkers may allow a more efficient pre-selection of candidates for imaging tests or discrimination between benign and malignant chest abnormalities detected by LD-CT. We searched for a molecular signature based on a serum metabolome profile distinguishing individuals with early lung cancer from healthy participants of the lung cancer screening program.

Material and methods: Blood samples were collected during a LD-CT screening program performed in the Gdansk district (Northern Poland). The analysis involved 100 patients with early stage lung cancer (including 31 screen-detected cases) and the pair-matched group of 300 healthy participants of the screening program. MALDI-ToF mass spectrometry was used to analyze the molecular profile of lipid-containing fraction of serum samples in the 320-1000 Da range. The GC/MS approach was used to identify and quantify small metabolites present in serum.

Results: Several components of the serum lipidome were detected, with abundances discriminating patients with early lung cancer from high-risk smokers. An effective cancer classifier was built with an area under the curve of 0.79 and 0.72 in the training and test groups, respectively. Corresponding negative predictive values were 100% and 92%, and a positive predictive value was 28% each. The downregulation of a few lysophosphatidylcholines (LPC18:2 and LPC18:1) in samples from cancer patients was confirmed using a complementary LC-MS approach. Moreover, several metabolites were detected in the sera which abundances discriminated patients with lung cancer (31 screen-detected cases) from matched controls (92 healthy individuals). Majority of differentiating components were downregulated in cancer samples, including amino acids, carboxylic acids and tocopherols, whereas benzaldehyde was the only compound significantly upregulated. A classifier including nine serum metabolites allowed separation of cancer and control samples with 100% sensitivity and 95% specificity.

Conclusions: Metabolome-based serum signatures showed potential usefulness in discriminating early lung cancer patients from healthy individuals. These signatures, though not validated in an independent dataset, deserves further investigation in a larger cohort study.

Key words: lung cancer screening, metabolomics, serum biomarkers.

This work was supported by the NCN grant 2013/11/N/NZ7/00770 and NCBiR grant PBS3/A7/29/2015 (MoltestBIS). The computation analyses were carried out using the GeCONiI infrastructure (NCBiR grant POIG.02.03.01-24-099).

Poster

KW017-00036-2017-01

Quantification of long non-coding RNAs using qRT-PCR method – comparison of different cDNA synthesis methods and RNA stability

Marcel Ryś, Tomasz Kolenda

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

Introduction: Long non-coding RNAs (lncRNAs), a new class of regulatory RNA molecules, are more than 200 nucleotides long. They play important role in cellular processes such as proliferation, apoptosis, response to stress and regulation of cell metabolism or phenotype. Disturbances in lncRNA expression were linked with cancer processes and growing number of studies focused on lncRNAs as a new potential biomarker. One of the most popular methods used in lncRNA research is qRT-PCR, but there is lack of standardization of this method and different approaches to prepare cDNA are used. Moreover, influence of RNA degradation on quantification of lncRNA is not clear.

Material and methods: In this study, different cDNA synthesis kits based on: i) random hexamer primers preceded by polyA-tailing and adaptor-anchoring steps, ii) simple reaction using blend of random hexamer primers and oligo(dT), iii) using only random hexamer primers, as well as iv) only oligo(dT) were checked and three degradation degrees of RNA samples were compared.

Results: The lower Ct values of lncRNAs after qRT-PCR quantification were observed for cDNA synthesized using random hexamer primers preceded by polyA-tailing and adaptor-anchoring steps compared to the other cDNA synthesis methods. In the case of 75/90 (83%) tested lncRNA, the RNA degradation weakly influenced on Ct values of lncRNAs and lack of differences ($p > 0.05$) was observed between high quality RNA and degraded samples. However, 17% of examined lncRNAs showed significantly different Ct values depending on RNA degradation.

Conclusions: Using of cDNA synthesis kits with random hexamer primers preceded by polyA-tailing and adaptor-anchoring steps allows to enhance specificity and sensitivity of lncRNA quantification. Moreover, in the most cases degradation of RNA samples does not affect on lncRNA quantification due to quite good stability of these molecules, but high integrity of RNA is still recommended for some of lncRNA transcripts.

Key words: lncRNA, cDNA synthesis, qRT-PCR, RNA stability and degradation.

Poster

KW017-00028-2017-01

Polarization of macrophages under paracrine stimulation by 3D model of breast cancer

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

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

Introduction: The three-dimensional (3D) breast cancer model is an *in vitro* model of mammary carcinoma composed of cancer cells and stromal cells i.e. fibroblasts. Cells are co-cultured on the three dimensional scaffolds made of silkworm silk. This heterogeneous model containing both cancer cells and fibroblast has already been proven as the innovative tool to study the cells interactions in tumor microenvironment. The significant population among the non-tumoric cells forming the tumor's microenvironment are macrophages. Macrophages interact with a wide range of growth factors, cytokines and chemokines in the tumor microenvironment which modify their phenotype. The activated macrophages, depending on the phenotype, can be pro-tumorogenic or anti-tumorogenic.

Aim of the study: Aim of this research was to analyze the phenotypic changes of macrophages upon stimulation by the 3D model of breast cancer.

Material and methods: The following cell lines were used: J774 – murine macrophages, EMT6 - murine mammary carcinoma, NIH 3T3 – murine fibroblast. Scaffolds were prepared using salt leaching method. Fibroblast and cancer cells were seed at a 9 to 1 ratio as co-culture on the silk fibroin scaffolds and incubated up to 10 days. The macrophages were treated with the conditioned medium (CM) collected from the 3D co-cultures of fibroblast and cancer cells. Medium collection were performed at the 7th and 10th day of culture and from the single, double and triple scaffolds to optimize the condition for the activation of macrophages. Medium containing murine interleukin 4 (mIL-4) and interleukin 6 (IL-6) were used as a positive control, and fresh culture medium were used as a negative control. The macrophages were incubated with the CM for 12 and 24 hours. The effects on the macrophages phenotype were analyzed using flow cytometry.

Results: The optimal conditions to study activation of macrophages paracrine stimulation by 3D model of breast cancer were: incubation of macrophages for 24 hours with CM collected at the 10th day of co-culture from double scaffold. These conditions induced the most pronounced changes in the expression of markers characteristics to the phenotype of M2 macrophages.

Conclusions: Three-dimensional model of breast cancer is a promising tool to study changes in macrophage phenotype in response to cancer environment.

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

Poster

KW017-00013-2017-01

Mucin expression profiles in colorectal carcinoma

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

[1]Chair and Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Bioinformatics and Numerical Biology, Chair of Clinical Pathomorphology, Poznan University of Medical Sciences, Poznan, Poland
[3]Chair and Department of General Surgery, Gastroenterological Oncology and Plastic Surgery, Poznan University of Medical Sciences, Poznan, Poland

Introduction: Colorectal cancer (CRC) is the third most common cancer and the fourth most common cancer cause of death globally. It is one of the most intensively studied cancer types. Altered mucin expression may be correlated with biological behaviour and the prognosis of CRC. However, the contradictory results make difficult to interpret its clinical significance.

Aim of the study: Immunohistochemical analysis (IHC) of tissue expression of selected mucins in CRC histological subtypes as a potential markers of tumour progression.

Material and methods: Selected membranous mucins (MUC1, MUC4) and secreted mucins (MUC2, MUC5AC) expressions assessed by immunohistochemistry for a total 34 CRC cases (of which 10 were of mucinous type) and control tissue both from surgical resection. The quantitative morphometric analysis (Filtr HSV software), and semiquantitative IRS scale were used. We correlated tissue expression of mucins with selected clinical data and expression of Ki-67 proliferation antigen. Results: All examined mucins were detected in the control and the neoplastically transformed mucosa of large intestine. MUC1 and MUC4 were detected in cell membranes, MUC2 and MUC5AC in cell cytoplasm. Number of immunopositive neoplastic cells varied extensively between individual patients. The frequency of immunopositivity was demonstrated as follows: for MUC1 and MUC2 (100%), MUC4 (56%), and MUC5AC (29%). Quantitative expression of MUC2 and MUC4 manifested a significantly lower expression in CRC than in control. In contrast, expression of MUC5AC and MUC1/MUC2 ratio was significantly higher in CRC as compared to control tissue. A significantly higher expression of MUC-2 and MUC4 was demonstrated in mucinous adenocarcinoma as compared to other subtypes of CRC. Higher expression of MUC5AC was observed in proximally located CRC. Only in case of MUC4 we demonstrated significant differences in this mucin expression profile and grading. Moreover, expression of MUC-2 showed negative correlation with intensity of cell proliferation (expression of Ki-67) in CRC.

Conclusions: Colorectal cancer has changed mucin tissue expression as compared with control. Examination of mucin expression profile in CRC allows more complete evaluation of cellular proliferation of cancer (MUC2), tumor histological subtype (MUC2 and MUC4), grading (MUC4), staging (MUC4) and anatomic localization of the tumor.

Key words: colorectal carcinoma, mucins, Immunohistochemical analysis.

Poster

KW017-00043-2017-01

hTERT C250T promoter mutation and telomere length as a molecular markers of cancer progression in patients with head and neck cancer

Agnieszka Sobecka[1,2], Wojciech Barczak[1,2], Karolina Bednarowicz[3], Piotr Machczynski[1], Pawel Golusinski[1], Blazej Rubis[4], Michal Masternak[1,5], Wiktoria Suchorska[2,6], Wojciech Golusinski[1]

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

Introduction: The head and neck squamous cell carcinoma (HNSCC) is the sixth leading cause of cancer worldwide, representing over half a million incidents every year. Cancer cells, including HNSCC, are characterized by an increased telomerase activity. This enzymatic complex is active 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 61 HNSCC patients and 49 healthy individuals.

Material and methods: DNA was extracted from PBMC (Peripheral Blood Mononuclear Cells) of 61 patients with histologically diagnosed HNSCC and 49 healthy volunteers. Telomere length was assessed using quantitative PCR-based technique with two pairs of primers (telomere-specific and a single copy gene-specific). To identify C250T hTERT promoter mutation, the High Resolution Melting analysis was performed. Statistical analysis of the results was performed using the Student's, ANOVA, Chi-square, and Fisher's exact tests.

Results: The average relative telomere length in the studied and control groups was evaluated, however no significant difference was observed ($p = 0.787$). Telomeres in leukocytes from individuals with T2 HNSCC cancer were significantly shorter compared with telomere length in leukocytes of healthy individuals (6.329 ±1.864 and 19.06 ±1.801, respectively; $p = 0.0001$). There was also significant difference of telomere length between T2 and T3 patients (6.329 ±1.864 and 16.94 ±3.301, respectively $p = 0.0063$), and T2 and T4 (6.329 ±1.864 and 26.3 ±7.615, respectively $p = 0.0028$). hTERT promoter mutation was identified in 36% of HNSCC patients and in 27% of healthy individuals. There was significant correlation between frequency of mutation and grade of tumor (T1 = 27%; T2 = 36%; T3 = 35% T4 = 46%; $p \leq 0.0001$).

Conclusions: C250T hTERT promoter mutation represents common event during cancerogenesis in HNSCC patients, and together with telomere length assessment may be one of the molecular markers of HNSCC progression. The finding of long or short telomeres in PBMC of HNSCC patient does not necessarily indicate the presence or absence of hTERT promoter mutation, and both parameters should be considered to characterize patients status.

Key words: head and neck cancers, TERT promoter mutation, telomere length, molecular markers.

Poster

KW017-00047-2017-01

Quantitative morphometry of the breast cancer vascularity

Agata Stanek-Widera, Magdalena Biskup-Frużyńska, Mirosław Śnietura, Dariusz Lange

Department of Tumor Pathology, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland

Background: During the last 20 years a system of factors regulating the process of neovasculogenesis has been identified. Methods of immunohistochemical labeling of the pathologic vessels are widely used in practice. The goal of the present study is identification and quantitation of morphometric characteristics of tumor and lymph nodes vascularity and its predictive value for treatment of breast cancer patients. Multifactor morphometric evaluation of vascular network in primary tumor and regional lymph nodes in patients with breast cancer comparing two antibodies: podoplanin and CD34 to identify and assess prognostic features and factors. Methods 60 cases of breast cancer in stage pT1-pT4 were enrolled in the study. Samples of tumors and positive and negative lymph nodes were used as direct materials for immunohistochemical assays. Using CD34 and podoplanin assays morphometric parameters of: vessel density, average surface area, perimeter, elongation ratio, compactness and fullness were estimated for tumors (T-N(0) versus T-N(+)) and lymph nodes (N(0), N(–) versus N(+)). Vessels characteristics assessed using CD34 staining were compared with podoplanin estimates and correlated with T and N stage.

Results: Presence of metastases in lymph nodes was accompanied by statistically significant increase in average density of lymphatic vessels. Intensified lymphangiogenesis was also noted in metastasis-free lymph nodes in patients with N1/2-stage disease. The examined vessel shape factors showed statistically significant differences between particular groups. This may reflect deformation of lymphatic vessels in cases with spread of metastatic cells. Also, this indicates possibility of using the discussed vessel morphometric profiles as a prognostic factor. Use of precision devices (tablets) in computerized image analysis of chromogene-stained vessels makes this method fast and reliable.

Conclusions: Present study shows that podoplanin assay provides more precise and significant estimates of vascular morphometric parameters than CD34 and therefore is recommended for further studies. The use of precise devices (tablets) in computerized image analysis of chromogene-stained vessels makes the method fast and reliable. Finally, tumor and lymph nodes vascularity characteristics might be used as prognostic and predictive factors in breast cancer combined treatment strategy planning and in treatment outcomes, respectively. Studies in this field are in progress.

Key words: lymphangiogenesis, breast cancer, morphometry, podoplanin, CD34.

Poster

KW017-00040-2017-01

Qualitative and Quantitative Impact of Scattered Out-of-Field Radiation on MDA-MB-231 Cell Lines.

Wiktoria Suchorska, Agnieszka Skrobała, Małgorzata Skórska, Anna Kowalik, Marta Kruszyna, Weronika Jackowiak, Julian Malicki

Greater Poland Cancer Centre, Poznan, Poland

Introduction: The study presented here is the third of three inter-related papers that together form a three part study whose primary aims were to determine a) the out-of-field radiation doses at varying distances from the primary beam (Part I), b) the properties of the scattered radiation responsible for these out-of-field doses (Part II), and c) the impact of these doses on biological response of in vitro cells (Part III). Each segment of this three-part study contains an experiment that uses a specific technology (dosimetry, calculation algorithms, or cell studies, respectively).

Aim of the study: Patients who undergo external beam radiotherapy are at risk of developing second tumours due to scattered radiation outside the path of the primary beam. The aim of this study was to experimentally determine the in vitro radiobiological effects of scattered radiation in cells located outside the primary photon beam and to compare this to the effects that occur in cells inside the primary beam. The comparison was performed by assessing cell viability, DNA damage, and apoptosis.

Material and methods: Cells from the human breast cancer line MDA-MB-231 were inserted in a water phantom and irradiated at varying doses (1.5, 2.0, 2.5, and 3.0 Gy). The cells were placed at two geometrical points: in the central beam axis (CAX) and at 10 cm out-of-field. Survival fraction (SF), number of DNA double strand-breaks (DNA DSBs), and cleaved PARP levels were determined by clonogenic assay and flow cytometry.

Results: A slight, non-significant decrease of 3-5% in cell SF was observed in cells irradiated outside the primary field. The number of PARP-positive cells and DNA DSBs both increased after out-of-field irradiation.

Conclusions: Scattered irradiation appears to induce an in vitro biological response on out-of-field cells that is stronger than the effect of primary radiation on in-field cells, independent of the bystander effect. These findings suggest that the biological response of healthy tissues outside the primary beam might be higher than previously believed.

Key words: out-of-field radiation.

Poster

KW017-00020-2017-01

Natural compounds as a potential anticancer agents for CLL

Aleksandra Szustka[1], Jerzy Błoński[2], Małgorzata Kubczak[1], Paweł Góralski[3], Tadeusz Robak[2], Małgorzata Rogalińska[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

Aim of the study: Chronic lymphocytic leukemia (CLL) belongs to the group of hematological diseases with unknown etiology; mainly occurs older people. There is an increasing number of compounds with anticancer potential used in preclinical and clinical studies. The most desirable are agents which have a potential to induce apoptosis. The best results are reported using chemotherapy based on purine analogs combined with rituximab. However, the cytotoxicity of those agents is relatively high; it opens a new possibilities for searching natural compounds with proapoptotic potential.

Material and methods: To improve the efficacy of CLL patient's treatment, the in vitro analysis of mononuclear cells incubations with anticancer agents before drug(s) administration in vivo should be performed. The present studies involve three approaches, i.e. cell viability analysis by Vybrant Apoptosis Assay #4 (flow cytometry), differential scanning calorimetry (DSC), and Western blot to choose the optimal therapy for CLL patients based on leukemic cell sensitivity to drugs.

Results: CLL cells or control cells obtained from peripheral blood of CLL patients or healthy donors were incubated with cladribine (C) combined with mafosfamide (M) – CM, and CM combination with rituximab (RCM), as well as with natural plant compounds (curcumin, graviola, quercetin). Simultaneous analysis of cell viability, DSC profiles of nuclear fraction preparations, and expression of apoptosis-related proteins by Western blot were applied to examine the ability of leukemic cells to enter apoptosis after their incubations with drug(s). The obtained results revealed that all anticancer agents induced apoptosis with different extend. The significant decrease of cell viability were observed when anticancer agent was active. Moreover, the decrease or loss of thermal transition at 95 ±5°C in DSC profiles of nuclei isolated from CLL cells as well as proteolytic cleavage of PARP-1 confirmed induction of apoptosis.

Conclusions: In the group of analyzed natural compounds the personal differences in cell response for anticancer agents were observed. Such analysis seems to be helpful in searching for new anticancer compounds.

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

Poster

KW017-00025-2017-01

Is NPY system involved in crosstalk between prostate cancer and peripheral nervous system?

Wojciech Wesolowski[1,2], Agnieszka Gruszecka[3], Joanna Kitlińska[4], Dawid Sigorski[5], Edyta Szwed[6], Ewa Iżycka-Świeszewska[2]

[1]EL-PAT Pathology Co Elblag, Poland
[2]Department of Pathology and Neuropathology, Medical University of Gdansk, Gdansk, Poland
[3]Department Nuclear Medicine and Radiological Informatics, Medical University of Gdansk, Poland
[4]Department of Biochemistry and Molecular and Cellular Biology, Georgetown University Washington, USA
[5]Clinical Department of Oncology and Immunooncology, Hospital with Oncology Center MSW, Olsztyn, Poland
[6]Department of Pathomorphology, Copernicus Hospital, Gdansk, Poland

Ability to infiltrate is one of essential hallmarks of cancer. Some of tumors are more prone to neuroinvasion then others. This phenomenon may be explained by presence or absence of specific adhesion molecules on cell surface, but there is growing evidence of dynamic influence of nervous system on cancer behavior. Nerve ablation or suppressing sympathetic system delays cancer progression and metastases in several animal models.

In this study we evaluated Neuropeptide Y (NPY) and its receptors system in prostate cancer (CaP), neoplasm that very often infiltrates peripheral nerves and/or ganglia. The study was conducted on 51 archival clinical cases of CaP, six cases of benign prostate hyperplasia (BPH) and ten CaP bone metastases. Tissue microarrays were manually constructed including 150 tissue cores. Immunohistochemical analysis of NPY system (NPY, Y1R, Y2R, and Y5R) expression comprised qualitative and quantitative assessment by two independent pathologists. Moreover, patho-clinical data such as patients' age, pTNM stage, Gleason grade, as well as ERG status and Ki67 index were studied. The staining of NPY system within central CaP tumor portion and in neuroinvasion areas was compared. Analysis was carried out on BPH, PIN areas and bone metastases also.

Both NPY and its receptors ashowed higher expression in cancer then in BPH. NPY system presented statistically significant higher expression in areas of neuroinvasion then in central parts of cancer mass (Wilcoxon test for NPY, Y1R, Y2R and Y5R with $p < 0,001$ in all). Expression of NPY system elements in extraprostatic extension foci was also higher then in central part of tumors. PIN areas revealed similar NPY reaction to surrounding invasive cancer. CaP bone metastases revealed no significant differences versus primary CaP.

Our observations show role of NPY in CaP progression and infiltration pattern, as well as in connection between nervous system and CaP.

Key words: NPY, neuroinvasion, prostate cancer, Immunohistochemical analysis.

Poster

KW017-00006-2017-01

Correlation between BMI-1 expression and phosphorylation level of AKT in endometrial cancer

Agnieszka Zaczek, Paweł Jóźwiak, Anna Krześlak

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

BMI-1 (B-lymphoma Mo-MLV insertion region 1) as a component of Polycomb Repressive Complex 1, by regulation of ubiquitin ligase RING1B affects the expression of genes involved in cell cycle control, apoptosis or DNA repair. Alteration in expression of BMI-1 contribute to development of many cancers. It is suggested that one of the possible mechanisms by which BMI-1 promotes tumorigenesis is the activation of PI3K/AKT pathway due to inhibition of PTEN expression.

The aim of our study was to analyze expression of BMI-1, AKT, PTEN and the level of phosphorylated at Ser 473 AKT (pAKT) in relation to clinicopathological characteristics of endometrial cancers. The correlation of BMI-1 protein with PTEN mRNA and protein expressions as well as phosphorylation level of AKT was studied.

The results showed lower expression of BMI-1 in more advanced tumors, classified as stage III and IV than in tumors less advanced, corresponding to the first and second stage according to FIGO. The lower expression of this protein was observed also in patients with lymph node metastasis compared to patients with no lymph node metastasis. Ratio pAKT to AKT was higher in tumor than normal samples but lower in more advanced cancers compared to less advanced tumors. Expression of PTEN was significantly lower in tumors than normal samples. There was no significant correlation between BMI-1 and PTEN protein and mRNA levels, but we observed positive correlation between BMI-1 and pAKT.

In conclusion, our results indicate that decreased PTEN and increased phospho-AKT levels are more likely associated with an early events in endometrial tumorigenesis, while low expression of BMI-1 and low level of AKT phosphorylation may be involved in endometrial cancer progression.

Key words: BMI-1, PI3K/AKT pathway, PTEN, endometrial cancer.

Poster

KW017-00024-2017-01

Influence of LATS1 silencing on oxidative stress, apoptosis and CSC phenotype in melanoma cell lines

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

[1]Department of Cancer Immunology, Poznan University of Medical Sciences, Poznan, Poland
[2]Diagnostic and Immunology Department, Greater Poland Cancer Center, Poznan, Poland

Introduction: Melanoma is one of the most aggressive cancers. As such, the understanding of its mechanisms of developement and progression are especially important for the development of effective therapies. Hippo pathway with one of its main kinases – LATS1 protein, was thought to act as a tumor suppresor. Recently new evidence emerged questioning its role in various cancers. We decided to analyze its function in melanoma, focusing on LATS1 role in ROS production, apoptosis and the aquisition of CSC phenotype.

Aim of the study: Analysis of the influence of LATS1 silencing on oxidative stress, apoptosis and cancer stem cell phenotype in various melanoma cell lines.

Material and methods: LATS1 gene was silenced using shLATS1 delivered by a lentiviral system. Oxidative stress and apoptosis were measured by flow cytometry with the use of CellROX Green Reagent and FITC Annexin V Apoptosis Detection Kit I, respectively. CSC phenotype was analyzed by studying the expression levels of Oct4, Sox2 and Nanog, measured by RT-PCR and CD271 expression by FACS.

Results: Analysis of 5 melanoma cell lines showed that the effects of LATS1 silencing in melanoma are cell line specific. Changes in both ROS production and CSC phenotype were observed between analyzed cell lines and corresponding controls transduced with mock lentivirus. No significant impact of LATS1 silencing on apoptosis was observed.

Conclusions: Our initial results show that hippo pathway plays an important role in melanoma development. There is possible correlation with aggressiveness of melanoma and the expression levels of LATS1 protein. Further studies are under way to determine the mechanism of cell line – specific LATS1 function in melanoma.

Key words: melanoma, Hippo pathway, oxidative stress, apoptosis, CSC.

Poster

Near-infrared photoimmunotherapy targeting EGFR in glioblastoma

Thomas A. Burley[1], Anna Wilczkiewicz-Mrozek[2], Wojciech Szopa[3], Maria Vinci[4], Piotr Czekaj[5], Gabriela Kramer-Marek[1], Wojciech Kaspera[3]

[1]Division of Radiotherapy and Imaging, The Institute of Cancer Research, London, UK
[2]A. Chelkowski Institute of Physics, University of Silesia, Katowice, Poland,
[3]Department of Neurosurgery, Medical University of Silesia, Regional Hospital, Sosnowiec, Poland
[4]Division of Molecular Pathology, The Institute of Cancer Research, London, UK
[5]Department of Cytophysiology, Chair of Histology and Embryology, Medical University of Silesia, Katowice, Poland

Introduction: High grade gliomas appear to be both preferentially and differentially driven by alterations (amplification, deletion, or missense mutations) in the epidermal growth factor receptor (EGFR). However, despite the fact the blockade of EGFR-dependent processes has resulted in experimental and clinical treatment success, cells capable of using alternative signalling have ultimately escaped this strategy. Therefore, combination of interventions targeting tumour-specific cell surface regulators along with convergent downstream signalling pathways will likely enhance treatment efficacy. Therefore, against this background, there is an urgent need to develop effective companion diagnostics for targeting agents and test them in animal models that closely resemble key features of the primary tumour.

Aim of the study: 1) To establish primary patient-derived brain tumour cell lines and animal models which reflect the heterogeneity of the disease and are amenable to preclinical testing of novel therapeutic strategies. **2)** To evaluate whether an affibody-infrared light activated conjugate, specifically targeting both EGFRwt and EGFRvIII could be used for the treatment of EGFR+ve glioma models.

Material and methods: Patients (n = 5) **w**ith primary glioblastoma underwent surgical resection. Tumour samples were collected for routine histopathological assessment and for initiation of primary cell cultures which were established from fresh tumours or minced-cryopreserved tissues. Cells were cultured adherently on laminin-coated flasks under stem cell culture conditions. Additionally, U251, U87, U87vIII glioma cell lines were cultured in the presence of fetal bovine serum (FBS) either as adherent monolayers or three-dimensional (3D) spheroids. The expression of EGFR in selected cell lines was confirmed by Western blot (WB) and IHC. EGFR-specific affibody molecules were conjugated to the IRDye 700DX. Afterwards, $Affi_{EGFR}$-IR700 conjugate was thoroughly characterised by in vitro binding assays using fluorescence activated cell sorting (FACS) and confocal microscopy. Cell proliferation after photoimmunotherapy (PIT; fluence rate of 4, 8, 16 J/cm^2) was evaluated using the CellTiter-Glo viability assay. The in vivo studies of the immunoconjugate were performed using subcutaneous glioma xenografts. The tumour growth inhibitory properties after PIT (100 J/cm^2) were evaluated at different time points. In addition, mice were imaged before and during the PIT course using the IVIS/Spectrum/CT to monitor treatment response (theranostic approach). Post-treatment, tumours were dissected for subsequent *ex vivo* analysis (IHC, WB).

Results: Two primary cell lines were successfully established and the EGFR expression level was confirmed by IHC, WB and FACS. $Affi_{EGFR}$-IR700 binding of the probe correlated with the expression level of EGFR in all tested cell lines and more importantly, blocking the cells with 100x fold excess of non-labelled affibody effectively reduced the median fluorescence. The cellular retention profile of $Affi_{EGFR}$-IR700, investigated using confocal microscopy at 1 and 6 h, further confirmed the specific binding of the probe. Phototoxicity studies *in vitro* using adherent cell lines as well as 3D spheroids have shown significantly reduced cell proliferation in a dose dependent manner. A significant reduction in EGFRvIII+ve tumour size was observed following administration of the immunoconjugate and irradiation, importantly, this response was not seen in EGFR-ve tumours.

Conclusions: The $Affi_{EGFR}$-IR700 allowed for the imaging of EGFR receptor spatial distribution and more importantly showed therapeutic efficacy *in vitro*, in models that closely replicate the human GBM, and in glioma xenografts.