# contemporary oncology

# współczesna **onkologia**

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

**TERMEDIA** Publishing House

# contemporary oncology

# współczesna **onkologia**





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

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

Circulation of 2,500 copies

**Publisher**
Termedia Publishing House
e-mail: termedia@termedia.pl

**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**
Małgorzata Kowalska
Termedia sp. z o.o.
Kleeberga 2, 61-615 Poznań
tel./fax +48 61 822 77 81, ext. 602
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**

contemporary oncology
współczesna **onkologia**

Volume 23, Supplement 1, March 2019

# 11th International Conference of Contemporary Oncology

Poznan, 13–15 March 2019

# Oral presentations

Oral presentation

KW019-00045-2019-01

# Markers of progression risk in patients with squamous cell vulvar carcinoma

*Agnieszka Fatalska[1], Natalia Rusetska[2], Elwira Bakuła-Zalewska[2], Artur Kowalik[3], Agnieszka Wroblewska[4], Kamil Zalewski[2], Krzysztof Goryca[2], Dominik Domański[1], Magdalena Kowalewska[2]*

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[2]Maria Sklodowska-Curie Institute – Oncology Center, Warsaw, Poland
[3]Holycross Cancer Center, Kielce, Poland
[4]Holy Family Hospital, Warsaw, Poland

**Objectives:** The application of biomarkers to predict disease outcome for vulvar carcinoma (VC) is obstructed by the limited knowledge on its biology. We aimed to identify protein markers of VC that would be indicative of a tumour that is more likely to progress.

**Method:** Early stage squamous cell VC tumours and normal vulvar tissues were studied using mass spectrometry-based proteomic analysis (iTRAQ). The results obtained for tumour samples of VC patients that progressed (progVC) were compared to those obtained for samples of patients who were disease-free (d-fVC) during a long follow-up. Differentially expressed proteins were validated in solid tissues and blood samples of patients with VC tumours and VC pre-malignant lesions.

**Results:** 5510 proteins were identified in VC samples using iTRAQ. Immune response was the most over-represented Gene Ontology category for proteins differentially expressed in progVC and d-fVC tumours. The top candidate markers were validated using targeted proteomic method (PRM) and immunohistochemistry in VC and VC pre-cancer lesions. Correlation of the results with clinical parameters of the enrolled VC patients indicated that HMGA2 and STAT-1 should be considered as potential protein markers for the prediction of VC progression. Plasma and serum analysis with PRM assays and ELISA tests pointed towards PRTN3 as a potential blood marker of VC progression.

**Conclusions:** Increased HMGA2 and STAT-1 tissue abundance and higher PRTN3 blood levels are associated with aggressive phenotype of VC. Their assessment holds promise as patient stratification tool.

**Key words:** vulvar carcinoma, progression, protein marker, HMGA2, STAT-1, PRTN3.

*This work was supported by the Polish National Science Centre grant No. 2013/10/E/NZ5/00663.*

Oral presentation

KW019-00035-2019-01

# Administration of intraoperative radiotherapy reduces the ability of surgical wound fluids collected from breast cancer patients to induce migration of cancer cells

*Igor Piotrowski[1,2], Katarzyna Kulcenty[1,2], Karolina Zaleska[1], Mateusz Wichtowski[3], Joanna Wróblewska[4,5], Dawid Murawa[6], Wiktoria Suchorska[1,2]*

[1]Radiobiology Laboratory, Greater Poland Cancer Centre, Poznan, Poland
[2]Department of Electroradiology, Poznan University of Medical Sciences, Poznan, Poland
[3]First Clinic of Surgical Oncology and General Surgery, Greater Poland Cancer Centre, Poznan, Poland
[4]Department of Pathology, Poznan University of Medical Sciences, Poznan, Poland
[5]Greater Poland Cancer Center, Poznan, Poland
[6]Department of General and Minimally Invasive Surgery, Poland Baptism Monument Hospital, Gniezno, Poland

Breast cancer is the most common cancer occurring in women. Following the breast-conserving surgery (BCS) the majority of local recurrences occur in the same quadrant as the primary cancer. Wound healing response induced after surgery is thought to play an important role in growth of breast cancer cells remaining after surgery. In order to kill the remaining cancer cells and reduce the risk of recurrence, whole-breast radiotherapy is often administered after surgery. Intraoperative radiotherapy (IORT) allows for high-dose irradiation of tumor bed directly after surgical excision. Several reports show that surgical wound fluids (WF) accumulating in wound cavity after breast conserving surgery stimulate growth of breast cancer cells, and that administration of IORT impairs this effect. The process of epithelial-mesenchymal transition (EMT) is imperative for migration and invasion of epithelial cancer cells, giving them ability to metastasize. Considering the aforementioned data we decided to investigate whether WF collected from patients after IORT changes the migratory capacity of breast cancer cell lines. We speculated, that cells irradiated during IORT secrete soluble factors which mediate radiation-induced bystander effect (RIBE), changing the biological activity of wound fluids. We collected surgical wound fluids from patients after BCS alone (WF) and after BCS followed by IORT (RT-WF). In order to investigate the role of bystander effect we also collected conditioned medium from irradiated breast cancer cell lines (RIBE). We chose two cell lines – MCF7 and MDA-MB-468, and stimulated them with WF, RT-WF, RIBE or WF+RIBE. First we analyzed the changes in cancer stem cell (CSC) phenotype of breast cancer cells. We also measured the expression of genes and the level of proteins involved in the EMT program. Finally, we performed wound healing assay to measure the migratory capacity of breast cancer cells. We observed that incubation with WF significantly increases the CSC population and stimulates the EMT process in both MCF7 and MDA-MB-468 cells. These effects were less pronounced in cells incubated with RT-WF or with WF+RIBE. Our results show the stimulatory effect of WF on CSC phenotype and migratory capacity of breast cancer cells, and prove that this effect is impaired by IORT through radiation-induced bystander effect.

**Key words:** breast cancer, intraoperative radiotherapy, wound healing.

Oral presentation

KW019-00029-2019-01

# Expression of mucins in hepatocellular carcinoma

*Aldona Kasprzak, Joanna Surdyk-Zasada, Małgorzata Andrzejewska*

Katedra i Zakład Histologii i Embriologii Uniwersytetu im. K. Marcinkowskiego w Poznaniu

Currently 21 mucin genes were identified in human, among which 15 are expressed in different regions of the gastrointestinal (GI) system. Mucins are large O-glycoproteins with high carbohydrate content and marked diversity in both the apoprotein and the oligosaccharide moieties. During neoplastic transformation of the GI tract, mucins are considered as diagnostic-prognostic markers, as well as therapeutic targets. It was widely believed that hepatocellular carcinoma (HCC) does not produce mucins, whereas cholangiocarcinoma or combined/mixed hepatocellular-cholangiocarcinoma may produce these glycoproteins. However, a growing number of reports shows that mucins can be produced by HCC cells that do not exhibit, or are yet to undergo, morphological differentiation to biliary phenotypes. In the recent classification of HCC, based on molecular features, three categories of HCC are detailed, including proliferation-progenitor, proliferation-TGF-β, and Wnt-catenin β1. This classification does not take quantitatively or qualitatively altered mucin production into account. The detailed role of tissue expression of mucins in neoplastic cells of HCC in vivo have been poorly recognized, hence, it seems important to study the expression of these proteins in the morphological characteristics of the subtypes of this heterogenous cancer in human, especially when it comes to mixed/combined HCC. In this study we analyzed tissue expression of two mucins, including trans-membrane MUC1 and secreted MUC5AC in tissue microarray (TMA) originated from 40 patients with HCC and 10 normal livers using new polymer-based immunohistochemistry (IHC). Membranous expression of MUC1 and cytoplasmic localization of MUC5AC was detected in the mucosal cells of normal colon (positive control). MUC1 membranous localization was observed also in biliary cells (cholangiocytes) of the control liver. Aberrant expression of both mucins were observed in HCC samples. Cytoplasmic expression of MUC1 in numerous cells was observed in 6 patients, whereas cytoplasmic MUC5AC localization was seen in scattered neoplastic cells of two patients with HCC. Our results have been referenced to literature data. The research on the role of mucins in pathogenesis of HCC, and explaining the mechanisms of their oncogenic action in HCC, requires further studies also in the context of new targeted therapies.

**Key words:** hepatocellular carcinoma, mucins, morphological characteristics of HCC, oncogens.

Oral presentation

KW019-00022-2019-01

# Recurrent loss of ELF1 in classical Hodgkin lymphoma

*Julia Paczkowska[1], Natalia Soloch[1], Magdalena Bodnar[2,3], Katarzyna Kiwerska[1,4], Joanna Janiszewska[1], Julia Vogt[5], Elżbieta Domanowska[2], Jose Ignacio Martin-Subero[6], Ole Ammerpohl[5,7], Wolfram Klapper[8], Andrzej Marszałek[9], Reiner Siebert[5,7], Maciej Giefing[1,7]*

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland
[2]Department of Clinical Pathomorphology, *Collegium Medicum* in Bydgoszcz, Nicolaus Copernicus University in Torun, Bydgoszcz, Poland
[3]Department of Otolaryngology and Laryngological Oncology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Tumor Pathology, Greater Poland Cancer Center, Poznan, Poland
[5]Institute of Human Genetics, Ulm University & Ulm University Medical Center, Ulm, Germany
[6]Insitut d'Investigacions Biomediques August Pi I Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain
[7]Institute of Human Genetics, Christian-Albrechts-University, Kiel, Germany
[8]Department of Pathology, Hematopathology Section and Lymph Node Registry, Christian-Albrechts-University, Kiel, Germany
[9]Department of Tumor Pathology and Prophylaxis Poznan University of Medical Sciences & Greater Poland Cancer Center, Poznan, Poland

**Introduction:** Loss of lymphoid specific transcription factors (TFs) is a key mechanism in the development of classical Hodgkin lymphoma (cHL). Lack of these TFs leads to downstream epigenetic inactivation of their target genes that in turn regulate the maturation of the B-cell lineage. Recently we have reported recurrent downregulation of the ETS1 protein, an important B-cell specific TF in the neoplastic HRS cells of cHL. Here, we have analyzed the status of ELF1 in cHL, another member of the ETS domain containing TF family.

**Material and methods:** To identify putative deregulation of ELF1 in cHL and to decipher the underlying molecular mechanism several methods were applied. We used quantitative real-time PCR and immunohistochemical staining to analyze ELF1 expression on mRNA as well as protein level in cHL and non-cHL cell lines and tissue sections. Furthermore, we screened for ELF1 loss-of-function mutations in cHL cell lines by Sanger sequencing and promoter hypermethylation by DNA bisulfite pyrosequencing. Last, we applied in situ hybridization to identify potential copy number losses of the gene in HRS cells.

**Results:** In result we observed that ELF1 protein is absent or lower abundant in HRS cells of 31/35 (89%) cases in comparison to the bystander cells. In addition, we found significant ($p < 0.01$) downregulation of ELF1 in cHL compared to non-cHL cell lines, both on mRNA and protein level. In search of the putative inactivation mechanism, we have elaborated that in a large proportion of cell lines (4/7 [57%]) ELF1 is targeted by heterozygous deletions combined with hypermethylation of the remaining alleles. In detail, DNA hypermethylation (range 95–99%, mean 98%) upstream of ELF1 transcription start site was observed in 7/7 (100%) cHL cell lines. In line with this findings 5/18 (28%) of the analyzed cHL primary biopsies carried heterozygous deletions of the gene in HRS cells.

**Conclusions:** Our data demonstrate that the expression of B-cell specific TF ELF1 is severely impaired in cHL and thus may be another player contributing to the loss of B-cell phenotype of HRS cells.

**Key words:** ELF1, transcription factors, cHL, loss of B-cell identity.

Oral presentation

KW019-00018-2019-01

# Novel mechanism of metastasis regulated by SNAIL transcription factor in mesenchymal tumors

*Klaudia Skrzypek, Artur Nieszporek, Anna Kusienicka, Małgorzata Lasota, Paweł Konieczny, Marcin Majka*

Department of Transplantation, Jagiellonian University Medical College, Krakow, Poland

Rhabdomyosarcoma (RMS) is a frequent mesenchymal tumor of soft tissue affecting children. For high grade tumors with metastatic involvement, the 5 years overall survival is below 20%. Thus, finding new treatment strategies aiming at new targets is highly necessary. Previously, we have shown for the first time that SNAIL is a key factor regulating RMS development (Skrzypek *et al.* 2018). In our current studies we investigate the mechanistic role of SNAIL in metastasis of mesenchymal tumors, such as RMS.

In a group of 158 patients SNAIL level is significantly increased in RMS samples from patients displaying higher stages of the disease. To investigate the mechanism of SNAIL action, we downregulated its level with siRNA or shRNA in different RMS cell lines, what resulted in reorganization of actin cytoskeleton. Analysis of mRNA transcriptome in SNAIL deficient cells revealed regulation of extracellular matrix organization and degradation, as well as collagen formation. In vivo, stable SNAIL silencing resulted in an impaired engraftment of RMS cells into murine lungs after intravenous implantation. This process may be regulated by SDF1 expressed in murine lungs. In vitro, when SNAIL deficient RMS cells were treated with SDF1, adhesion of those cells to the activated endothelial cells was diminished. Ezrin and p44/42 MAPK are important mediators of SNAIL effects. Ezrin acts as linker between the plasma membrane and the cytoskeleton. Its promoter has several SNAIL binding sites. Both SNAIL and ezrin silencing in RMS cells resulted in decreased motility and diminished chemotaxis to SDF1 due to the diminished RHO activity. The other important mediators of SNAIL action are miRNAs. Analysis of miRNA transcriptome revealed that SNAIL-miRNA axis regulates processes associated with reorganization of actin cytoskeleton. One of SNAIL-dependent miRNAs – miR-28-3p diminishes motility of the cells and downregulates ezrin. Ezrin may be regulated either directly or indirectly by SNAIL. Moreover, in RMS samples from patients, SNAIL level positively correlates with ezrin expression.

Our data show novel mechanism of rhabdomyosarcoma metastasis, that may be also significant in other mesenchymal tumor types. SNAIL may be a new promising target for future RMS therapies.

**Key words:** SNAIL, tumor metastasis, rhabdomyosarcoma, ezrin, microRNA, mesenchymal tumor.

*The project was supported by grants from the National Science Centre in Poland: 2013/09/B/NZ5/00769 and 2015/17/D/NZ5/02202.*

Oral presentation

KW019-00011-2019-01

# Epigenetically mediated CEACAM6 downregulation in laryngeal squamous cell carcinoma is correlated with tumor stage

*Kinga Bednarek[1], Magdalena Kostrzewska-Poczekaj[1], Marcin Szaumkessel[1], Katarzyna Kiwerska[1,2], Julia Paczkowska[1], Ewa Byzia[1], Adam Ustaszewski[1], Joanna Janiszewska[1], Anna Bartochowska[3], Reidar Grenman[4], Malgorzata Wierzbicka[1,3], Krzysztof Szyfter[1], Maciej Giefing[1,3] Malgorzata Jarmuz-Szymczak[1,5]*

[1]Institute of Human Genetics, Polish Academy of Sciences, Poznan, Poland
[2]Department of Tumor Pathology, Greater Poland Cancer Center, Poznan, Poland
[3]Department of Otolaryngology and Laryngological Oncology, Poznan University of Medical Sciences, Poznan, Poland
[4]Department of Otorhinolaryngology – Head and Neck Surgery and Department of Medical Biochemistry, Turku University Hospital and University of Turku, Turku, Finland
[5]Department of Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznan, Poland

Deregulated expression of CEACAM6 was shown in different types of tumors. Its overexpression is observed in pancreatic adenocarcinoma, gastric and head and neck cancers. In contrast, we found significant downregulation of CEACAM6 in laryngeal squamous cell carcinoma (LSCC). Therefore, the aim of presented study was to identify the molecular mechanism responsible for downregulation of CEACAM6 in LSCC.

The study was performed on LSCC cell lines ($n$ = 25) analyzed with reference to non-tumor control samples from head and neck ($n$ = 15). Preliminary microarray-based gene expression data for CEACAM6 were validated with RT-qPCR. Expression data were correlated with clinicopathological characteristics of tumors used to establish the analyzed cell lines (U-test). To identify the molecular mechanism leading to gene downregulation we have analyzed such known gene inactivation mechanisms like (i) DNA copy number of the gene with the use of array-CGH (ii) in silico search for inactivating mutations using cBioPortal and COSMIC databases, (iii) gene promoter DNA methylation level using bisulfite pyrosequencing. To verify the role of DNA methylation, two LSCC cell lines were treated with DNA methyltransfrerase inhibitor (DAC) and the resulting changes of DNA methylation and gene expression were evaluated.

Significant downregulation of CEACAM6 expression on mRNA level in LSCC cell lines versus control samples was detected with microarray-based gene expression analysis and confirmed with RT-qPCR in 14/25 (56%) samples. This change was significant in cell lines derived from recurrent ($p$ = 0.048), highly advanced ($p$ = 0.049) and less differentiated tumors ($p$ = 0.036). Heterozygous deletion of the gene was identified in 1/13 LSCC cell lines, and no further CEACAM6 deletion was indicated from the database search. In line, missense mutation of the gene was observed in only one case in the two databases. However, using bisulfite pyrosequencing we have found significant difference ($p$ = 0.0001) in gene promoter DNA methylation in LSCC cell lines versus control samples, shoving hypermethylation of the gene in 9/25 (36%) LSCC samples. As a result of DAC-induced inhibition of DNA in two analyzed cell lines, decreased CEACAM6 DNA methylation resulted in restoration of its expression.

We have demonstrated, that CEACAM6 is specifically downregulated in advanced LSCC tumors and most probably this deregulation is caused by aberrant DNA promoter hypermethylation.

**Key words:** CEACAM6, cancer, HNSCC, tumor suppressor gene.

Oral presentation

KW019-00040-2019-01

# Molecular characteristics of Burkitt-like lymphoma with 11q aberration: Meeting the classification and diagnostic challenges

*Magdalena Chechlińska[1], Michalina Zajdel[1], Agnieszka Paziewska[1], Michalina Dąbrowska[1], Zbigniew Bystydzieński[1], Maria Kulecka[2], Mariusz Kulińczak[1], Maria Sromek[1], Katarzyna Błachnio[1], Beata Grygalewicz[1], Anita Borysiuk[1], Renata Woroniecka[1], Jolanta Rygier[1], Barbara Pieńkowska-Grela[1], Łukasz Szafron[1], Joanna Miłoszewska[1], Jan Walewski[1], Jerzy Ostrowski[1], Jan Konrad Siwicki[1], Grzegorz Rymkiewicz[1]*

[1]Maria Sklodowska-Curie Institute – Oncology Center, Warsaw, Poland
[2]Centre of Postgraduate Medical Education, Warsaw, Poland

Burkitt-like lymphoma with 11q aberration (BLL,11q) is a new entity of a germinal center (GCB)-derived, highly aggressive B-cell lymphoma. Clinically and pathomorphologically BLL,11q resembles Burkitt lymphoma (BL), but it lacks MYC rearrangements and presents proximal gains and distal losses in chromosome 11. BLL,11q has been recognized in the updated 2017 WHO classification, but as a provisional entity, because its ultimate classification as a variant of BL, of diffuse large B cell lymphoma (DLBCL), of high-grade B-cell lymphoma (HGBL), or as a distinct form of highly aggressive B-cell lymphoma remains controversial. Still, the recognition of BLL,11q entity is clinically relevant because favorable patients' outcomes are observed after BL-directed therapy. Unfortunately, the routine diagnosis of BLL,11q is difficult, and BLL,11q is often misdiagnosed by histopathology, and because of the lack of MYC rearrangements, it is inappropriately treated. Molecular studies of BLL,11q are expected to add to our understanding and managing of this very rare malignancy.

Our initial RT-qPCR-based studies on miR-155, miR-21 and mir-26a expression showed similarities of BLL,11q ($n$ = 12) to MYC-positive BL ($n$ = 34), but not to DLBCL ($n$ = 49). Next, a detailed immunohistochemical and flow cytometry analysis of 10 BLL,11q and 23 MYC-positive BL cases revealed similarities along with subtle but essential differences in BLL,11q and BL immunophenotypes. Most recently, by next-generation sequencing of 10 BL and 7 BLL,11q, we identified 49 microRNAs and 2573 mRNA transcripts differentially expressed between BL and BLL,11q. Accumulating data not only show significant differences between BLL,11q and BL in mRNA and microRNA expression patterns, but also point to a different chromosomal and mutational profile of BLL,11q from BL, but also from other aggressive GCB-lymphomas, such as DLBCL and HGBL. Thus, molecularly, BLL,11q seems indeed to be a distinct category. Still, BLL,11q patients, if treated with BL-directed regimen, have a relapse-free outcome similar to BL patients.

We proposed an original and practical flow cytometry- and immunohistochemistry-based diagnostic algorithm for the differential diagnosis of BLL,11q vs. BL, DLBCL and HGBL, which enables BLL,11q diagnosis within 1.5 hours following fine needle aspiration biopsy procedure. Hopefully, further studies will explain the pathogenesis, resolve the classification dilemma and help to set-up the optimal treatment for BLL,11q patients.

**Key words:** Burkitt-like lymphoma with 11q aberration (BLL,11q), Burkitt lymphoma (BL), aggressive B-cell lymphoma, differential diagnosis, DLBCL, HGBL.