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# BOOK OF ABSTRACTS

## CURRENT UNDERSTANDING OF COLORECTAL AND PANCREATIC CANCERS

An international conference organized by the Chaperon project,
Faculty of Medicine in Pilsen, Charles University,
in cooperation with the Institute of Experimental Medicine CAS.

May 29 and 30, 2023
New Town Hall Prague

Novoměstská radnice
Karlovo náměstí 1/23, 120 00, Praha 2, Czech Republic

CHARLES UNIVERSITY
Faculty of Medicine in Pilsen

Czech Academy of Sciences


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# INTRODUCTORY WORDS

Dear participants,

it is our great pleasure to welcome you to the conference titled Current Understanding of Colorectal and Pancreatic Cancers, held in Prague. Both diseases represent serious health problems worldwide and the need for deep understanding of their underlying mechanisms is still high. We believe that this assembly of prominent researchers and clinicians will highlight the current topics of interest. We are very thankful to all of you for contributing to this Conference. We believe that you may also like the historical medieval environment in which the Conference is being held, as well as the unique atmosphere of Prague in spring.

Enjoy the venue!

We have received a kind offer from the journal Mutagenesis (Oxford University Press) to publish full papers from the Conference in a peer-reviewed Special Issue. Please consider this opportunity.

*Kari Hemminki, Pavel Vodicka*

# CONFERENCE PROGRAM

# DAY 1 MONDAY MAY 29 2023

**Ceremonial opening** 8:30 9:00

**Session chairs** **Kari Hemminki, Pavel Vodička**

prof. MUDr. Milena Králíčková, Ph.D., rector of Charles University

prof. RNDr. Eva Zažímalová, CSc., dr.h.c., president of the Czech Academy of Sciences

prof. MUDr. Jindřich Fínek, Ph.D., dean of the Faculty of Medicine in Pilsen, Charles University

Ing. Jan Topinka, CSc., DrSc., chair of the Board of the Institute of Experimental Medicine CAS

MUDr. Pavel Vodička, CSc., DrSc. **History of the New Town Hall**

## Session M1

**Session chairs** **Ludmila Vodičková, Alessio Naccarati**

| Speaker | Title of the talk |
|---|---|
| | 9:00 9:30 |
| Kari Hemminki, Asta Försti | Opening lecture: Epidemiology and familial genetics of colorectal and pancreatic cancers |
| | 9:30 10:00 |
| Ulrike Peters | Keynote lecture: Molecular epidemiological approaches for colorectal cancer |
| | 10:00 10:30 |
| Richarda de Voer | Novel genes predisposing to colorectal cancer and polyposis |

*Coffee break* *10:30 10:55*

## Session M2

| **Session chairs** | **Asta Försti, Ulrike Peters** |
|---|---|
| **Speaker** | **Title of the talk** |
| | 10:55 11:25 |
| Richard Houlston | Using genomics to reveal the mysteries of cancer |
| | 11:25 11:55 |
| Yael Goldberg | Inherited colorectal cancer – genes and clinical insights |
| | 11:55 12:25 |
| Barbara Pardini | Consensus molecular subtyping and other omics in colorectal cancer classification: an integrative approach to improve the classification and its reflection in stool miRNome and metagenome |
| *Lunch* | *12:25 12:55* |
| | *(Lunch continues during the following poster session)* |

## POSTER SESSION

| | |
|---|---|
| Odd poster numbers | 12:55 13:40 |

## Session M3

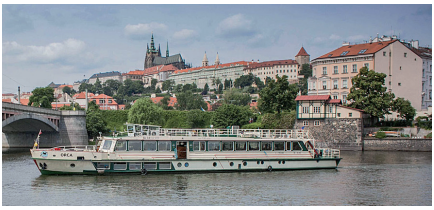
| **Session chairs** | **Barbara Pardini, Richard Houlston** |
|---|---|
| **Speaker** | **Title of the talk** |
| | 13:40 14:10 |
| Jan Lubinski | Management of ovarian and endometrial cancers in women belonging to HNPCC carrier families: review of the literature and results of cancer risk assessment in Polish HNPCC families |

| | | |
|---|---|---|
| | 14:10 | 14:40 |
| Claire Palles | Loss of function mutation in RNF43 is responsible for genetic predisposition in a family with serrated polyposis | |
| | 14:40 | 15:10 |
| Sergi Castellví-Bel | Germline predisposition to serrated polyposis syndrome | |
| *Coffee break* | *15:10* | *15:35* |

## Session M4

| | |
|---|---|
| **Session chairs** | **Claire Palles, Sergi Castellví-Bel** |
| **Speaker** | **Title of the talk** |
| | 15:35 16:05 |
| Alessio Naccarati | A fecal miRNA signature by small RNA sequencing accurately distinguishes colorectal cancers and adenomas: results from a multicentric international study |
| | 16:05 16:35 |
| Pavel Vodička | Genomic instability in adenomas and in colorectal cancer progression |
| | 16:35 17:05 |
| Federico Canzian | Germline genetics of pancreatic cancer risk |
| ***Conference dinner*** | *19:00* *22:00* |

An unconventional dinner with a perfect view of the historic part of Prague. It will be a cruise dinner on the Vltava river accompanied by sightseeing. You can enjoy your dinner with a beautiful scenery while learning about the history and monuments from an experienced guide. The whole experience will be completed by live music on board.



# DAY 2 TUESDAY MAY 30 2023

## Session T1

| **Session chairs** | **Tomáš Büchler, Daniele Campa** |
|---|---|
| **Speaker** | **Title of the talk** |
| | 9:00 9:30 |
| Tom van Wezel | Study on a novel TP53beta mutation in inherited CRC predisposition |
| | 9:30 10:00 |
| Regine Schneider-Stock | How to model peritoneal metastasis in colon cancer? |
| | 10:00 10:30 |
| Pavel Souček | Exome sequencing for precision oncology of metastatic colorectal carcinoma |
| *Coffee break* | *10:30 10:55* |

## Session T2

| **Session chairs** | **Regine Schneider-Stock, Pavel Souček** |
|---|---|
| **Speaker** | **Title of the talk** |
| | 10:55 11:25 |
| Victor Moreno | Microbiome and colorectal cancer: opportunities to personalize screening |
| | 11:25 11:55 |
| David Hughes | The gut-pancreas axis: Association of circulating protein markers of gut barrier integrity and inflammation with colorectal cancer and pancreatic cancer |

| | | |
|---|---|---|
| | 11:55 | 12:25 |
| Ondřej Slabý | A real-world genomics-guided precision medicine in gastrointestinal oncology | |
| *Lunch* | *12:25* | *12:55* |
| | *(Lunch continues during the following poster session)* | |

## POSTER SESSION

| | | |
|---|---|---|
| Even poster numbers | 12:55 | 13:40 |

## Session T3

| | |
|---|---|
| **Session chairs** | **David Hughes, Victor Moreno** |
| **Speaker** | **Title of the talk** |
| | 13:40 14:10 |
| Daniele Campa | Integration of genomic, genetic, clinical and epidemiologic factors to predict IPMNs progression into PDAC |
| | 14:10 14:40 |
| Beatrice Mohelníková-Duchoňová | Individualized treatment in pancreatic cancer |
| | 14:40 15:10 |
| Tomáš Büchler | Treatment deescalation in colorectal cancer |
| **Closing word** | 15:10 15:15 |
| Pavel Vodička, Kari Hemminki | |

# POSTERS

**Poster** **Presenting author**

**P1:** **Štěpán Balatka**
Efficacy of paclitaxel and Stony Brook taxanes in pancreatic cancer in vitro and in vivo

**P2:** **Miroslava Čedíková**
Basic characterization of the groups of „tasters" and „non-tasters" mediated by the receptor TAS2R38 – a questionnaire study

**P3:** **Marie Černá**
Karyotyping is still useful technique in molecular genetics of tumours

**P4:** **Klára Červená**
Plasma KRAS mutations as early diagnostic biomarkers for pancreatic cancer in high-risk group patients

**P5:** **Petr Hanák**
Association of mitochondrial DNA copy number and telomere length with colorectal cancer patient outcomes

**P6:** **Josef Horák**
Inhibition of homologous recombination repair by Mirin ameliorates carboplatin therapy response in vitro

**P7:** **Veronika Iatsiuk**
Regulatory role of Cul4a in the gastrointestinal homeostasis and colorectal cancer progression

**P8:** **Lucie Janečková**
Tcf4-mediated Wnt signaling is required for Paneth cell maintenance and antimicrobial peptide production in the intestinal epithelium and colorectal cancer

**P9:** **Agapi Kataki**
Exploring Caveolin-1 expression profile in neuroendocrine tumours of the gastrointestinal tract and pancreas

**P10:** **Michal Kroupa**
Reduced methylation of olfactory receptor genes and amplification of 6p25.1-p22.3 as specific epigenetic and genetic alterations in colorectal cancer liver metastases

**P11:** **Alice Mášová**
How important is good experimental design if you would like to analyze cfDNA, CTC or miRNA?

**P12:** **Lucie Musilová**
The genetic landscape of metachronous colorectal liver metastases as revealed by whole exome sequencing

**P13:** **Nazila Navvabi**
Alternative splicing deregulation in CRC

**P14:** **Agnieszka Paziewska**
Serum metabolite profiles of pancreatic tumors: neuroendocrine and pancreatic ductal adenocarcinomas – a preliminary study

**P15:** **Agnieszka Paziewska**
Are bacterial metabolites connected with colon cancer pathogenesis?

**P16:** **Marie Rajtmajerová**
MiRNA profiling in primary and metastatic colorectal cancer – preliminary study

**P17:** **Anna Šišková**
Mitochondrial DNA copy number in correlation with telomere length in solid adenomas

**P18:** **Božena Smolková**
Deregulation of EMT genes induced by hypoxia and inflammation independent of DNA methylation changes in human PDAC cell lines

**P19:** **Tereza Tesařová**
The power of bioinformatics in cancer research

**P20:** **Kristýna Tomášová**
Amplification of MALAT1 long non-coding RNA in high-grade colorectal adenoma patients

**P21:** **Jolana Turečková**
Ablation of Atf2 results in highly invasive tumors in an AOM/DSS model of colorectal cancer

# ABSTRACTS

## ORAL PRESENTATIONS

# Treatment deescalation in colorectal cancer


## Tomas Buchler

Department of Oncology, Second Faculty of Medicine, Charles University and Motol University Hospital, V Uvalu 84, 150 06 Prague, Czech Republic

*Presenting author: Tomas Buchler (e-mail: tomas.buchler@fnmotol.cz)*



Most patients with colorectal cancer will receive multimodality treatment consisting of surgery and systemic treatment, with a substantial minority also treated with radiotherapy. This treatment is associated with substantial risk of short- and long-term toxicity. Recent advances enable treatment minimisation in selected patients with colorectal cancer without compromising oncological outcomes and preserving the quality of life. Intensive preparatory regimens consisting of chemotherapy and radiotherapy lead to complete responses in approximately 50% of patients with rectal cancer permitting omission of surgery. Another active area of research are patients with mismatch repair-deficient tumours that usually respond to immunotherapy resulting in profound tumour regressions. Selected biomarkers are studied to improve treatment stratification and tailoring, monitor minimal residual disease, and optimise long-term follow-up.

# Integration of genomic, genetic, clinical and epidemiologic factors to predict IPMNs progression into PDAC

**Daniele Campa**

Department of Biology, University of Pisa, Pisa, Italy

*Presenting author: Daniele Campa (e-mail: daniele.campa@unipi.it)*

Background & Aim: Intraductal papillary mucinous neoplasms (IPMNs) are cystic pancreatic ductal lesions that are increasingly detected and diagnosed and are non-obligatory precursor lesions of PDAC. The management (resection or observation) of IPMNs is very controversial given the fact that some IPMNs progress to invasion while others can remain not invasive for years. The guidelines identify high-risk stigmata (HRS) and worrisome features (WF) as the solely indications for surgical resection of IPMNs. However recent reports show that HRS and WF are inadequate indicators of IPMN progression towards cancer and result in overtreatment in up to 65% of the cases for WF and 30% for HRS. This is a clinically relevant issue considering that pancreatectomy is still a risky operation. Therefore, finding biomarkers that could predict the evolution of IPMN into malignancy is sorely needed.

Methods: Several studies that proposed blood-based, cyst fluid biomarkers and somatic alteration in resected cysts have been evaluated.

Results: Several biomarkers (proteins, RNA, and somatic mutation) have been proposed although many were identified in very small samples and not validated in independent studies, our knowledge on the germline genetic variability is extremely limited. Of particular interest, rs374705585, a stop gain mutation, located in the cystic fibrosis transmembrane conductance regulator (CFTR gene). This variant is strongly associated with CFTR-related disorders including pancreatitis, and therefore, is a highly attractive causative variant.

Conclusion: In conclusion the discovery of reliable biomarkers to predict IPMN evolution into PDAC is still an unmet clinical need, however recent studies have proposed promising candidates.

Acknowledgement & Funding: The research leading to these results has received funding from AIRC under IG 2019 - ID. 23672 project – P.I. Campa Daniele

# Germline genetics of pancreatic cancer risk


## Federico Canzian

Genomic Epidemiology Group, German Cancer Research Center (DKFZ), Heidelberg, Germany

*Presenting author: Federico Canzian (e-mail: f.canzian@dkfz.de)*


I will review the current status of research on germline genetic factors affecting risk of pancreatic cancer (PC), particularly its most common form, pancreatic ductal adenocarcinoma (PDAC).

As for most cancers, recurrence of PC in families has been observed and it is estimated that 3~10% of PC cases have a positive family history. Rare high-penetrance variants co-segregating with the disease have been identified in familial cancer syndromes that include PC, or in families with multiple recurrence of PC alone. The most commonly mutated genes in PC kindreds include BRCA1/2, ATM, CDKN2A and PALB2. Rare variants predicted to have a deleterious effect on function are studied also with a case-control approach, by resequencing candidate genes or whole exomes/genomes.

At the other end of the spectrum, there is a growing number of common polymorphisms associated with PC risk. Most have been identified through genome-wide association studies (GWAS), an endeavor that is still ongoing. Additional loci are being discovered through secondary analyses of existing GWAS data. Polygenic/multifactorial risk scores show much larger risks than individual variants, but their use for stratification of PC risk is not warranted yet. The function of only a handful of risk loci has being thoroughly characterized so far.

Finally, genetics of pancreatic neuroendocrine tumors (PNET), a rarer and heterogeneous form of PC, is still understudied. Likewise, we know very little about the role of germline genetic factors in the risk of developing pre-malignant pancreatic tumors (such as pancreatic intraepithelial neoplasia (PanIN) or intraductal papillary mucinous neoplasm (IPMN)) and of their progression to PDAC.

# Germline predisposition to serrated polyposis syndrome

## Sergi Castellví-Bel

Gastroenterology Department, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Hospital Clínic, Barcelona, Spain

*Presenting author: Sergi Castellvi-Bel (e-mail: sbel@recerca.clinic.cat)*

Colorectal cancer (CRC) is one of the most common cancers worldwide with a significant associated mortality. Most CRC cases develop through an adenoma-carcinoma sequence. In recent years, another carcinogenesis pathway has been identified, the serrated pathway, starting from a different precancerous lesion, the serrated polyp. Although serrated polyps were previously considered indolent, current evidence estimates they are the precursor lesion for up to 30% of CRC cases. Serrated polyposis syndrome (SPS) is a clinical condition characterized by the presence of multiple and/or large serrated polyps in the colon, as well as an associated higher risk of CRC.

CRC, including those cases associated with SPS, are caused by both genetic and environmental factors. Smoking, body mass index, and alcohol intake have been highlighted as important environmental risk factors for serrated polyps. Importantly, twin studies also showed that around 13%-30% of the variation in CRC susceptibility involves inherited genetic factors. APC, MUTYH, and the mismatch repair genes, are among the most relevant genes involved in the main forms of hereditary CRC, familial adenomatous polyposis, MUTYH-associated polyposis and Lynch syndrome. However, SPS is a disease with mostly unknown inherited genetic basis compared to other gastrointestinal polyposis syndromes and it has also been advocated that SPS may not be hereditary but mostly environmental. However, familial clustering and a high CRC risk for first-degree relatives of SPS patients have been described, which supports the involvement of germline predisposition in a subset of cases. The efforts to identify germline predisposition factors for SPS will be presented.

Acknowledgement & Funding: This research was supported by grants from Fundació La Marató de TV3 (2019-202008-10), Fondo de Investigación Sanitaria/FEDER (17/00878, 20/00113), Fundación Científica de la Asociación Española contra el Cáncer (PRYGN211085CAST), STEPUPIORS (HORIZON-WIDERA-2021-ACCESS-03 call, Horizon Europe), COST Action CA17118 (COST, European Cooperation in Science and Technology, www.cost.eu), PERIS (SLT002/16/00398, Generalitat de Catalunya), CERCA Program (Generalitat de Catalunya), Xarxa de Bancs de Tumors de Catalunya (XBTC, Pla Director d'Oncologia de Catalunya) and Agència de Gestió d'Ajuts Universitaris i de Recerca (Generalitat de Catalunya, GRPRE 2017SGR21, GRC 2021SGR01185). CIBEREHD is a research network funded by the Instituto de Salud Carlos III. We are sincerely grateful to Biobanks of Hospital Clínic–IDIBAPS, IDIBELL and Biobanco Vasco, and to the patients.

# Inherited colorectal cancer – genes and clinical insights


## Yael Goldberg[1,2]

[1] Raphael Recanati Genetic Institute, Rabin Medical Center-Beilinson Hospital, Petah Tikva, Israel

[2] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

*Presenting author: Yael Goldberg (e-mail: Yaelgo43@gmail.com)*


Genetic predisposition for colorectal cancer (CRC) should be suspected mainly but not only, in young patients, in patients with significant family history, multiple polyps, mismatch repair-deficient tumors, and in association with malignant or nonmalignant comorbidities. Genetic diagnosis of affected individuals and their at-risk relatives, may allow cancer-preventive potential in these families.

The aim of the talk is to share the insights gained from our translational research. The research involves various steps in diagnostic process of patients with inherited CRC, including gene discovery, raising awareness among caregivers and patients, identification of the suspected patients, providing the optimal genetic tests and clinical interpretation. The research also involves clinical analysis of the gene effect, i.e genotype-phenotype correlation, in order to provide evidence based surveillance protocols.

A 'real world' experience of some of these aspects will be presented, focusing on the nonmalignant f the extracolonic features and comorbidities of polyposis and CRC predisposition syndromes, MCM8/9 associated polyposis; and the clinical features of GREM1 associated HMPS.

# Epidemiology and familial genetics of colorectal and pancreatic cancers

**Kari Hemminki[1], Asta Försti[2]**

[1] Biomedical Center, Faculty of Medicine, Charles University Pilsen, 30605 Pilsen, Czech Republic; Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, D-69120, Heidelberg, Germany

[2] Hopp Children's Cancer Center (KiTZ), Heidelberg, Germany; Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Heidelberg, Germany

*Presenting author: Kari Hemminki (e-mail: k.hemminki@dkfz.de)*

The incidence in colorectal cancer (CRC) and pancreatic cancer (PC) is highest in the developed countries and for both cancers Eastern/Central Europe is a high-risk area. While known environmental factors explain slightly more of the causes of CRC, the proportion is less for PC. Survival in CRC has improved over the years and 5-year survival is currently about 90% in the Nordic countries. PC on the other hand belongs to the most fatal cancers but there has been positive development and in best countries 5-year survival is approaching 20%. About 15% of CRC patients and about 5% of PC patients have first-degree relatives affected by the same malignancy. However, for most families the cause of familial aggregation of cancer is unknown. To identify novel high-to-moderate-penetrance germline variants underlying cancer susceptibility, we performed whole exome (WES) and whole genome sequencing (WGS) in families showing a Mendelian inheritance pattern. After WES or GWS, we used our in-house developed Familial Cancer Variant Prioritization Pipeline to identify novel cancer predisposition variants. In CRC, we identified both nonsense, missense and 5'UTR variants involved in the regulation of innate immune response (SLC15A4), apoptosis and AKT pathway (PTK7), reactive oxygen species and mucus biology (CYBA, TRPM4), Wnt signaling (APCDD1) and histone modification (HDAC5) and in a protooncogene (SRC). Some of the identified variants may show they effect according to a synergistic or polygenic model. Familial PC seems to be highly heterogeneous polygenetic disease caused by low-frequency or rare variants. Our findings contribute to the identification of unrecognized genetic causes of familial CRC and PC.

# Opportunities for precision oncology revealed by whole genome sequencing

**Ben Kinnersley[1,2], Amit Sud[1,3], Alex J. Cornish[1], Daniel Chubb[1], Richard Culliford[1], Andrew Everall[1], Andreas Gruber[4], Adrian Larkeryd[5], Costas Mitsopoulos[6], Genomics England Research Consortium[7], David Wedge[8], Richard Houlston[1]**

[1] Division of Genetics and Epidemiology, The Institute of Cancer Research, Sutton, Surrey, SM2 NG, UK
[2] University College London Cancer Institute, University College London, London, UK
[3] Haemato-oncology Unit, The Royal Marsden Hospital NHS Foundation Trust, Sutton, UK
[4] Systems Biology & Biomedical Data Science Laboratory University of Konstanz, Germany
[5] Division of Molecular Pathology, The Institute of Cancer Research, Sutton, Surrey, SM2 NG, UK
[6] Division of Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey, SM2 NG, UK
[7] Genomics England Research Consortium, Genomics Unit, NHS England and NHS Improvement, London, UK
[8] Manchester Cancer Research Centre, University of Manchester, Manchester, UK

*Presenting author: Richard Houlston (e-mail: richard.houlston@icr.ac.uk)*

The potential promise of precision oncology includes improved treatment efficacy, improved risk-benefit profile and reduction in the administration of ineffective treatments. Underpinning precision oncology is the concept of somatic mutations as the foundation of cancer development. Currently multiple standalone tests or a panel are typically used to capture a set of genomic features for a given tumour type. However, the falling cost makes whole genome sequencing (WGS) a potentially attractive proposition as a single all-encompassing test. In 2012, the UK Government announced funding for WGS of 100,000 genomes from patients in the English NHS to capitalise on the potential of WGS for patient benefit. To assess the potential effect of the WGS on the treatment of cancer in the National Health Service (NHS) in the United Kingdom, we analysed data on 10,983 cancer cases across 35 cancer types enrolled into 100KGP. As well as characterising cancer driver genes we integrate these data with additional features including mutational burden, mutational signatures, copy number, structural variant calls from WGS and the Catalogue of Somatic Mutations in Cancer (COSMIC) and OncoKB databases to quantify the number of individuals who are potentially eligible for a targeted therapy. Finally, we utilise the chemogenomics knowledgebase canSAR and the Cancer Dependency Map (DepMap), to map and pharmacologically annotate the cellular network of cancer driver genes to identify potential novel therapeutic targets.

Acknowledgement & Funding: Funding was provided by the Wellcome Trust (214388), Cancer Research UK (C1298/A8362) and the Medical Research Council. A.S. is in receipt of a National Institute for Health Research (NIHR) Academic Clinical Lectureship, funding from the Royal Marsden Biomedical Research Centre, a starter grant for clinical lecturers from the Academy of Medical Sciences, and is recipient of the Whitney-Wood Scholarship from the Royal College of Physicians. This is a summary of independent research supported by the NIHR Biomedical Research Centre at the Royal Marsden NHS Foundation Trust and the Institute of Cancer Research.

This research was made possible through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health and Social Care). The 100,000 Genomes Project is funded by the National Institute for Health Research and NHS England. The Wellcome Trust, Cancer Research UK and the Medical Research Council also funded research infrastructure. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support.

# Association of circulating markers of gut barrier damage and inflammation with colorectal and pancreatic cancers

**David J. Hughes[1], Flavia Genua[1], Neil Daniel[1], Petr Holy[2,3,4], Václav Liška[3,4], Simona Susova[4], Beatrice Mohelnikova-Duchonova[5,4], Pavel Soucek[3,4]**

[1] Cancer Biology and Therapeutics Laboratory, Conway Institute, School of Biomedical and Biomolecular Sciences, University College Dublin, Dublin, Ireland
[2] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[4] Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic
[5] Department of Oncology, Palacky University Medical School and Teaching Hospital, Olomouc, Czech Republic

*Presenting author: David Hughes (e-mail: david.hughes@ucd.ie)*

Background & Aim: Microbiome disturbance associated with gut barrier dysfunction and inflammation may influence development of colorectal cancer (CRC) and potentially pancreatic adenocarcinoma (PDAC) though the gut-pancreas axis.

Patients/Methods: Serum biomarkers of gut barrier permeability and inflammation were assessed in case-control cohorts from Ireland (colorectal adenoma (CRA) & CRC) and the Czech Republic (CRC & PDAC). ELISA assays were used to measure circulating concentrations of LBP, CRP, TLR4, ZO1, iFABP, and flagellin, while a panel of 10 inflammatory cytokines and calprotectin were assessed using an MSD MultiSPOT platform and R-plex assay (Mesocale Discovery, Rockville, Md, USA), respectively. Differences between pathology groups were assessed using the Kruskal Wallis or Mann-Whitney U test (Rstudio, version 4.0.0 and GraphPad Prism, version 9.0).

Results: In the discovery Irish cohort after FDR correction, LBP concentrations were significantly higher in patients with CRA than in controls (p=0.02), while concentrations of LBP, TNFα, IL6, calprotectin and CRP were higher in CRCs than in controls (p-values from p=0.003 to 0.04). In the validation Czech cohort, calprotectin, TNFα, IFNγ, and IL10 levels were elevated in CRCs compared to controls (p's= 0.003 to 0.05). Regarding PDAC, concentrations of calprotectin, iFABP, ZO1, cytokines IL6, IL8, and IL10 were higher in cancers compared to controls (p's=0.0003 to 0.05). Biomarker combinations for discriminating between cancer and controls will also be presented.

Conclusions: These results support the hypothesis that increased gut barrier permeability may influence the development of CRC and PDAC. However, further study is needed to assess possible screening utility of gut barrier integrity biomarkers.

Acknowledgement & Funding: This work was supported by the Health Research Board of Ireland project grant ILP-POR-2022-092 (to DJH). Support for this work was also provided by the COST Action CA17118 supported by COST (European Cooperation in Science and Technology, www.cost.eu) to FG, PH, PS and DJH.

# Microbiome and colorectal cancer: opportunities to personalize screening

**Victor Moreno[1,2,3,4], Mireia Obon-Santacana[1,2,3], David Bars[1,2], Elies Gurrea[1,2], Blanca Rius[1,2]**

[1] Oncology Data Analytics Program, Catalan Institute of Oncology (ICO), Barcelona, Spain.
[2] ONCOBELL Program, Institut de Recerca Biomedica de Bellvitge (IDIBELL), Barcelona, Spain
[3] Department of Clinical Sciences, Faculty of Medicine, University of Barcelona (UB), Barcelona, Spain
[4] CIBER of Epidemiology and Public Health (CIBERESP), Madrid, Spain

*Presenting author: Victor Moreno (e-mail: v.moreno@iconcologia.net)*

Background & aim: Colorectal cancer (CRC) is a leading cause of cancer-related death worldwide. It is a complex disease that arises from the interplay between genetic, environmental, and lifestyle factors. In recent years, there has been a growing interest in the role of the gut microbiome in CRC. Several studies have suggested that alterations in the gut microbiome composition and function may contribute to CRC development, highlighting the potential of microbiome-based predictive models for CRC prevention.

Methods: We have developed a colorectal cancer predictive model trained in a meta-analysis of studies with public data on shotgun gut microbiome. The COLSCREN study was used for validation. This study included participants in CRC screening.

Results: A signature of 32 taxa was identified and validated in our independent study. We have mapped the taxa to 16 S and validated the signature in additional samples, though the mapping has been difficult and probably with some degree of misclassification, thus reducing the accuracy. The added value of epidemiological risk factors and the genetic polygenic risk score to improve predictive accuracy and risk stratification was also tested.

Conclusion: These results show the potential of microbiome-based risk stratification and personalized screening for CRC prevention.

# Loss of function mutation in RNF43 is responsible for genetic predisposition in a family with serrated polyposis

**Nathalie Feeley[1], Luke Freeman Mills[2], Edward Arbe Barnes[2], Steve Thorn[1], Anshita Goel[3], Juan Fernandez-Tajes[1], Helen Curley[3], Lai Mun Wang[4], Lynn Martin[3], Hayley Davis[1], Sujata Biswas[1], Laura Chegwidden[3], Richarda de Voer[5], Timothy Maughan[6], Simon Leedham[3], Viktor Koeltzer[7], James East[8], Genomics England Research Consortium, CORGI Consortium, WGS500Consortium, The S:CORT Consortium, Roland Arnold[3], Claire Palles[3], Ian Tomlinson[6]**

[1] Edinburgh Cancer Research Centre, IGMM, University of Edinburgh, Crewe Road, Edinburgh, UK
[2] Wellcome Trust Centre for Human Genetics, University of Oxford, UK
[3] Institute of Cancer and Genomic Sciences, University of Birmingham, UK
[4] Department of Laboratory Medicine, Changi General Hospital, SingHealth, Singapore, Singapore
[5] Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, Netherlands
[6] Department of Oncology, University of Oxford, Old Road Campus Research Building, Roosevelt Drive, Oxford, UK
[7] Department of Pathology and Molecular Pathology, University Hospital Zurich, University of Zurich, Zürich, Switzerland
[8] Translational Gastroenterology Unit, Oxford NIHR Biomedical Research Centre, University of Oxford, Oxford, UK

*Presenting author: Claire Palles (e-mail: c.palles@bham.ac.uk)*

Background & Aim: The genetic causes of serrated polyposis (SPS) are not well understood. We analysed the exomes of patients with multiple serrated polyps with the aim of identifying novel genes associated with SPS.

Patients/Methods: gDNA from patients with SPS was analysed by genome or exome sequencing (exomes=52, genomes=24). Following mapping and variant calling, we identified loss of function and predicted pathogenic missense variants. We also searched for pathogenic germline mutations in RNF43 in WGS from participants of the rare diseases (including 243 SPS patients) and cancer domains of the 100,000 genomes project (100KGP). Tumours were analysed for second hits or loss of heterozygosity.

Results: We identified a frameshift mutation in RNF43 in two related patients (157fs (chr17:56440746 delC)). The mutation leads to the insertion of a premature stop codon in exons 5 of 10. The variant was confirmed by Sanger sequencing and it co-segregated with SPS affection status using DNA from four additional family members. Fresh and FFPE polyps were available for DNA (N=9) and RNA analysis (N=4). All polyps were microsatellite stable, 44% showed BRAF mutation and 11% KRAS mutation. No second RNF43 somatic mutations were identified in the polyps but one polyp showed loss of heterozygosity. In the 100KGP, one p.Arg132* mutation carrier and two p .Arg330* mutation carriers were detected in ~2000 colorectal cancer patients and one p .Arg330* carrier was detected in ~21,000 rare disease controls. No mutation carriers were detected in the SPS patients recruited to the 1000KGP.

Conclusion: Our work identified a novel mutation in RNF43 as the causal mutation in a family with SPS and early onset CRC and confirms that RNF43 germline mutations are a rare cause of SPS.

Acknowledgement & Funding: This research was made possible through access to the data and findings generated by the 100,000 Genomes Project; http://www.genomicsengland.co.uk. CP received project grant funding from Bowel Cancer UK.

# CMS subtyping and other omics in colorectal cancer classification: an integrative approach to improve the classification and its reflection in stool miRNome and metagenome for non-invasive precision medicine

**Barbara Pardini[1,2], Amedeo Gagliardi[1,3], Giulia Francescato[1,4], Giulio Ferrero[3,4], Sonia Tarallo[1,2], Antonio Francavilla[1], Carla Di Battista[1], Enrico Berrino[2,5], Caterina Marchiò[2,5], Francesca Cordero[3], Alessio Naccarati[1,2]**

[1] Italian Institute for Genomic Medicine (IIGM), Candiolo, Turin, Italy
[2] Candiolo Cancer Institute, FPO-IRCCS, Candiolo, Turin, Italy
[3] Department of Computer Science, University of Turin, Turin, Italy
[4] Department of Clinical and Biological Sciences, University of Turin, Turin, Italy
[5] Department of Medical Sciences, University of Turin, Turin, Italy

*Presenting author: Barbara Pardini (e-mail: barbara.pardini@iigm.it)*

Background and Aim: The Consensus Molecular Subtypes (CMS) is a subtyping system used for colorectal cancer (CRC) to facilitate traditional tumor classification and clinical translation. A more in-depth characterization of the genomic/epigenomic background of each subtype with different omics may help in refining this classification, including the missing assignment.

We aim to characterize CMS system by an integrative analysis of target genomic sequencing, RNA-Seq, small RNA-Seq in CRC patients. Differences in stool miRNome and metagenomic profiles will be used to ameliorate classification.

Patients/Methods: Total and small RNA-seq were performed on tumor tissue pairs in RNA later collected from 87 CRC. Tumors were genomically characterized with the TruSight Oncology 500 cancer-panel. Small RNA-seq and shotgun metagenomic analyses were performed on stool samples collected from the same patients.

Results: Tumors were assigned to CMS1(n=8), CMS2(n=27), CMS3(n=26) and CMS4(n=23), with 4 samples not assigned. CMS1 showed the highest number of SNV (n=290) and the highest fraction of frameshift variants. showed A main dysregulation of mRNAs and miRNAs in tissues was found for CMS1-2. In contrast, stool miRNome showed a similar dysregulation among CMS with CMS1 being the only with 7 miRNAs altered in stool and tissues. CRC patients presented lower microbial species richness, with different bacteria abundancies between CMS, such as Fusobacterium nucleatum more prevalent in CMS2.

Conclusions: A more in-depth CMS subtyping by integrating different omics analyses could help in the refining of this classification system and the possible CMS reflection in stool miRNome and metagenomic could shed new light on the host gut-microbiota interactions for a non-invasive classification.

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# How to model peritoneal metastasis in colon cancer?

**Regine Schneider-Stock[1], Kerstin Huebner[1], Corinna Lang-Schwarz[2]**

[1] Experimental Tumorpathology, University Hospital Erlangen, FAU, Germany

[2] Institute of Pathology, University Hospital Bayreuth, Germany

*Presenting author: Regine Schneider-Stock (e-mail: Regine.Schneider-Stock@uk-erlangen.de)*

Despite recent advances in prevention and therapeutics, colorectal cancer (CRC) is still the third most diagnosed cancer world wide. Death from CRC is mostly from metastatic spread to distant organs. Among them peritoneal metastases are associated with the worst prognosis. The molecular mechanisms of peritoneal metastasis are still not well understood. There are many open questions: How do tumor cells disseminate into the peritoneal wall? How do they survive the nutrient restrictive environment in the peritoneal cavity? How do they adhere to the peritoneal wall and how do they generate metastatic foci?

There are a few animal models that address these questions. First I will present molecular and functional data from classical mouse models. Moreover I will introduce two novel ex vivo test systems such as the mesentery model and colon precision cut tissue slices that should be suitable and reliable alternative models for analyzing the complex process of peritoneal metastasis.

# A real-world genomics-guided precision medicine in gastrointestinal oncology


## Ondrej Slaby

[1] Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

[2] CEITEC, Masaryk University, Brno, Czech Republic

[3] Department of Pathology, University Hospital Brno, Brno, Czech Republic

*Presenting author: Ondrej Slaby (e-mail: oslaby@med.muni.cz)*



Recent advances in cancer research and the development of modern therapies have significantly extended the therapeutic options of cancer, including gastrointestinal (GIT) cancers. Success has been achieved even in those malignancies that until recently were considered uncontrollable by systemic therapy. Thus, the prognosis of cancer patients is improving, including those with metastatic disease, and the logical goal of clinical research is to transform disseminated disease from a fatal to a chronic disease. Behind this progress and this ambition, besides anti-cancer immunotherapy, one can imagine the application of knowledge from the field of molecular pathology and its use for individualized therapeutic planning. The application of this knowledge takes us from the histopathological evaluation of tumors to the next level, which takes into account the biological behavior of individual malignancies. This enables a higher level of individualization of cancer treatment, where we use technologies that allow comprehensive genomic profiling (next-generation sequencing, NGS), which we refer to as precision oncology. A multidisciplinary approach in the form of a molecular tumor board (MTB) is also essential for precision oncology. Typically, clinical oncologists, pathologists, molecular biologists (molecular pathologists), clinical geneticists, and clinical pharmacologists are represented at MTBs. This indication committee's role is to find an appropriate and highly individualized treatment plan beyond standard treatment based on the evaluation of comprehensive genomic analyses. In addition, deep biological characterization of individual tumors has a huge potential for secondary use in cancer biology and genomics research. In this talk, we will introduce you to the functioning



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# Exome sequencing for precision oncology of metastatic colorectal carcinoma

**Viktor Hlaváč[1], Lucie Musilová[1], Lenka Červenková[2,3], Petr Holý[1,3], Pavel Dvořák[1], Simona Šůsová[1], Václav Liška[2,4], Ondřej Vyčítal[2,4], Ondřej Šorejs[2,5], Ondřej Fiala[2,5], Ondřej Daum[6], Pavel Souček[1]**

[1] Laboratory of Pharmacogenomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2] Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[3] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[4] Department of Surgery, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic
[5] Department of Oncology and Radiotherapeutics, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic
[6] Department of Pathology, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic

*Presenting author: Pavel Souček (e-mail: pavel.soucek@szu.cz)*

Background & Aim: we performed whole exome sequencing of 20 primary-synchronous hepatic metastasis pairs and 41 pairs of metachronous colorectal liver metastasis-healthy liver tissues

Methods: the study utilized onsite established whole exome sequencing methodology, including a bioinformatics pipeline for data processing. The TCGA COAD-READ dataset (n=380) was used for validation in silico, where possible.

Results: the most frequently altered oncodrivers in both cohorts were APC and TP53 with >50% mutational rates in primary or metastasis tissues, followed by the KRAS gene altered in 15% of primary tissues, 25% of synchronous metastases, and 41% of metachronous hepatic metastases. Moreover, harboring variants with a high or moderate predicted functional effect in KRAS in primary tumors was significantly associated with poor relapse-free survival in both our sample set and the TCGA validation dataset. Mutational signatures SBS22 and SBS39 in metachronous metastases were prognostic for progression-free (p=0.026) and overall (p=0.035) survival of patients, respectively, and having SBS93 was prognostic for worse progression-free survival (p=0.001). Due to a lack of suitable validation datasets, these results must be treated with extreme caution. Neither tumor mutational burden nor CNVs seemed to have prognostic potential in the present study, and no profile was significantly associated with response to chemotherapy.

Conclusion: our study provides unique mutation data in hard-to-obtain samples in specific homogeneous cohorts. We underline that hepatic metastases often differ from primary tumors in mutational profiles, which may influence therapy effectiveness, but also provide therapeutic opportunities. The need for larger, high-quality, focused studies is apparent.

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# TP53 beta variants in colorectal cancer predisposition

**Stephanie A. Schubert[1], Dina Ruano[1], Sebastien M. Joruiz[2], Nikolina Hanzic[2], Anna Montali[2], Lia Pinto[2], Daniela Q.C.M. Barge-Schaapveld[3], Maartje Nielsen[3], Thomas H. Sharp[4], Hans Morreau[1], Jean-Christophe Bourdon[2], Noel F.C.C. de Miranda[2], Tom van Wezel[3]**

[1] Department of Pathology, Leiden University Medical Center, The Netherlands
[2] School of Medicine, University of Dundee, Scotland, United Kingdom
[3] Department of Clinical Genetics, Leiden University Medical Center, The Netherlands
[4] Department of Cell and Chemical Biology, Leiden University Medical Center, The Netherlands

*Presenting author: Tom van Wezel (e-mail: tvanwezel@lumc.nl)*

Colorectal cancer (CRC) is a highly heritable malignancy, but known inherited syndromes only account for a small fraction of the genetic risk. In this study, we performed exome sequencing on 100 CRC patients with a familial and/or early-onset disease phenotype to identify novel germline predisposing variants. We discovered a heterozygous variant in the beta exon of TP53 in families with a history of CRC, multiple serrated polyps and other tumor types. Segregation analysis confirmed its association with CRC.

While germline pathogenic variants in the canonical TP53 gene are known to cause Li-Fraumeni syndrome, the clinical significance of germline variation in the alternatively spliced TP53 exons 9β and 9γ, which encode different carboxy-termini, has not been fully explored. We investigated the effect of the TP53 variant in tumors and immortalized B cells from carriers through functional and gene-expression analysis. The TP53beta variant leads to extension of the p53β isoforms, which increases oligomerization to canonical p53 and dysregulates the expression of p53's transcriptional targets. Our work illustrates, for the first time, that p53β mutants can modulate p53 signaling and supports the predisposition to various cancer types, albeit with a slightly later onset and distinct tumor spectrum compared to classical Li-Fraumeni syndrome. These findings highlight the importance of screening for alternative TP53 exons in families with unexplained cancer predisposition.

# Genomic instability in adenomas and in colorectal cancer progression

**Pavel Vodička[1,2,3], Michal Kroupa[1,3], Kristyna Tomasova[1,3], Anna Siskova[1,2], Safaa Andarawi, Katerina Balounova[1], Saba Selvi[1], Veronika Vymetalkova[1,2,3], Sona Vodenkova[1,3], Rajiv Kumar[1], Kari Hemminki[3], Ludmila Vodickova[1,2,3]**

[1] Institute of Experimental Medicine, Czech Academy of Sciences, Prague, Czech Republic
[2] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

*Presenting author: Pavel Vodička (e-mail: pavel.vodicka@iem.cas.cz)*

In solid cancers both impaired DNA repair and disrupted telomere length (TL) homeostasis are key culprits in cancer initiation, progression and prognosis. Altered DNA repair leads through accumulation of mutations into the genomic instability. Telomere attrition resulting in replicative senescence, simultaneously by-passing cell cycle checkpoints, is a hallmark of cellular malignant transformation. Telomerase, ubiquitous in advanced solid cancers, is fundamental to cell immortalisation. Human solid neoplasms often exhibit chromosomal instability (CIN), both structural and numerical. CIN generates either abnormal aneuploid karyotypes, or continually expands phenotypic heterogeneity as tumor cell populations undergoing consecutive cell divisions. We searched for the CIN markers in the adenoma-adenocarcinoma transition and in CRC progression. Understanding the mechanisms and dynamics of tumor genomic diversification, where DNA damage response and telomere homeostasis are important players, is critical in understanding carcinogenesis and overcoming drug resistance. The mitochondrial dysfunction, an another cancer hallmark is linked with DNA repair capacity and compensate for damage by increasing the mitochondrial DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN reported ambiguous and inconsistent results for various cancer types. Telomere shortening has a dual role in tumorigenesis. It promotes cancer initiation by inducing CIN, while TL maintenance characterized by telomerase expression is required for cancer cell proliferation and tumour growth. The reports on TL as a biomarker for cancer risk, patient therapy response and/or survival are contradictory as well. Our investigations were also focused on mtDNA_CN in CRC tissues and adjacent mucosa.

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# ABSTRACTS

## POSTER PRESENTATIONS

## POSTER 1

# Efficacy of paclitaxel and Stony Brook taxanes in pancreatic cancer in vitro and in vivo

**Stepan Balatka[1,2], Tomas Sychra[3,4], Radka Vaclavikova[1,2], Alzbeta Spalenkova[1,2,3], Marie Ehrlichova[1,2], Karolina Seborova[1,2], Kamila Koci[3], Iwao Ojima[5], Pavel Soucek[1,2]**

[1] Laboratory of Pharmacogenomics, Biomedical Centre, Faculty of Medicine, Charles University in Pilsen, Pilsen, Czech Republic
[2] The National Institute of Public Health in Prague, Department of Toxicogenomics, Prague, Czech Republic
[3] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[4] Department of Surgery, University Hospital Kralovske Vinohrady, Prague, Czech Republic
[5] Institute of Chemical Biology & Drug Discovery, State University of New York at Stony Brook, NY, USA

*Presenting author: Štěpán Balatka (e-mail: stepan.balatka@lfp.cuni.cz)*

Taxanes are widely used cytostatics for the treatment of different cancers. One of the conventionally used taxanes is paclitaxel (PTX), but many other experimental taxoid derivatives have been developed in recent years including Stony Brook Taxanes (SB-Ts).

Our study aimed to compare the efficacy between PTX and the second (SB-T-1216) and third (SB-T-121605, SB-T-121606) generation of SB-Ts using in vitro and in vivo models of pancreatic cancer.

The in vitro efficacy of three SB-Ts and PTX was compared using the Paca-44 cell line. The viability of cells was evaluated after 72 hours of incubation with each taxane using CellTiter-Blue Cell Viability Assay. Cell cycle changes were analyzed by flow cytometry. For in vivo experiments, 3 × 106 Paca-44 cells/100 µl were injected subcutaneously into the immunodeficient mice. Treatment by PTX alone (10mg/kg) or in combination with SB-Ts (1-3 mg/kg) was performed by intraperitoneal applications twice a week for two weeks.

In vitro experiments showed 1.7× - 7.4× higher efficacy of SB-T derivatives compared to PTX. The most effective derivative was SB-T-121606, which also blocked the G2/M phase of the cell cycle most effectively. The third generation taxanes SB-T-121605 and SB-T-121606 in combination with PTX were selected for in vivo experiments.

The most effective derivative was SB-T-121606 both individually (in vitro) and in the combination of 9 mg/kg PTX + 1 mg/kg SB-T-121606 (in vivo). This combination regimen slowed down the tumor growth and had no significant side effects. In conclusion, we show that combination regimen of high dose PTX with low dose SB-T present viable option for further preclinical testing.

Acknowledgement & Funding: The study was supported by projects AZV NV19-08-00113, GAČR 21-14082S, MŠMT INTER-ACTION project LTAUSA19032, COOPERATIO

POSTER 2

# Basic characterization of the groups of „tasters“ and „non-tasters“ mediated by the receptor TAS2R38 – a questionnaire study

**Miroslava Čedíková[1,2], Anežka Procházková[1], Kateřina Kabíčková[1], Gabriela Miklovičová[1], Martina Grundmanová[1], Ludmila Vodičková[2,3,4]**

[1] Department of Physiology, Faculty of Medicine in Pilsen, Charles University, Prague, Czech Republic
[2] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Prague, Czech Republic
[3] Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Science, Prague, Czech Republic
[4] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic

*Presenting author: Miroslava Čedíková (e-mail: miroslava.cedikova@lfp.cuni.cz)*

Background & Aim: Colorectal cancer (CRC) is one of the most prevalent and life-threatening cancer types worldwide. Genetic variants in bitter-taste receptor genes have been hypothesized to influence dietary intake and, consequently, could increase the risk of CRC. The aim of our work was to evaluate the phenotype of bitter taste perception mediated by the receptor TAS2R38. This receptor belongs to the TAS2R gene family and its polymorphisms are associated with differences in the bitter taste perception of phenylthiocarbamide.

Patients/Methods: Study participants filled out a questionnaire with questions regarding basic information about their person, diet, lifestyle, and incidence of CRC. Subsequently, their ability to perceive the bitter taste of phenylthiocarbamide was sensory tested.

Results: The questionnaire was filled out by 170 people, of which 135 were tasters and 35 were non-tasters. The group of tasters consisted of 102 women and 33 men. Their mean height was 170.66 cm, weight 71.25 kg, and BMI 24.46. The most common blood group was group A (39.3%), 18 individuals (13.3%) did not drink alcohol and 117 individuals (86.7%) were non-smokers. There were 26 women and 9 men among the non-tasters, the average height was 173.76 cm, weight 78.10 kg, and BMI 25.87. The most represented blood type was 0, there were 5 abstainers (14.3%) and 31 non-smokers (88.6%). When comparing the occurrence of CRC among individuals from the monitored groups, a higher frequency of occurrence of this disease was found in non-tasters (0.7% vs 5.7%). The same was true when comparing the incidence of CRC in the family (14.0% vs 22.9%).

Conclusion: The perception of taste affects the composition of our food, which can affect the development of various diseases, including CRC.

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## POSTER 3

# Karyotyping is still useful technique in molecular genetics of tumours

**Marie Černá[1], Markéta Pokorná[1], Michael Hudec[1], Zdeňka Polívková[1]**

[1] Department of Medical Genetics, Third Faculty of Medicine, Charles University, Ruská 87, Prague

*Presenting author: Marie Černá (e-mail: marie.cerna@lf3.cuni.cz)*

Background & Aim: The human derived cell line U87MG was originally obtained from a 44-year-old female patient in 1966 at Uppsala University, Sweden. A comparison of gene-expression profiles between the U87MG cell line distributed by American Type Culture Collection (ATCC) cell repository and the original tumor tissue began after a higher proliferation rate was noted in the former as well as the identification of a Y chromosome. In recognition of the discrepancy, ATCC currently marks U87MG with the HTB-14TM and the description that the cell line is likely to be a male origin. Our aim was to study chromosomal anomalies in U87MG cell line after confirming its female origin sourced from Europe.

Methods: U87MG (ECACC; cat. #89081402) was grown in Dulbecco modified eagle medium, fetal bovine serum, penicillin and streptomycin. Following cell suspension attachment, microscope slides were heated to 95 C for 25 min. Then slides were immersed for 20 s in Sörensen phosphate buffer (SPB) containing trypsin. After washing with distilled water, slides were drained and allowed to air dry. At least 10 metaphase spreads of chromosomes per passage were viewed under an Olympus BX43F light microscope at 1000x magnification.

Results: Microscopic evaluation of chromosomal spreads revealed varying susceptibility to aberrations across the 22 autosomes and X gonosome. Frequent chromosomal anomalies included potential inter-chromatid interchanges, interstitial deletions, break discontinuities and dicentric occurrence in U87MG passaged 10-16 times. Monosomy was evident at passage 10 with chromosomes 11, 13, 14 and 21 represented as single no paired chromosomes.

Conclusion: Passaging U87MG revealed the presence of chromosomal anomalies reflective of structural genomic alterations.

Acknowledgement & Funding: This study was financially supported by the Charles University research program COOPERATIO: the scientific project "Medical Diagnostics and Basic Medical Sciences" (the field "Medical Genetics")

POSTER 4

# Plasma KRAS mutations as early diagnostic biomarkers for pancreatic cancer in high-risk group patients

**Klara Cervena[1,2], Giulia Rossini[3], Alzbeta Hujova[4], Richard Palek[5], Ludmila Vodickova[1,2,5], Veronika Vymetalkova[1,2,5], Stefano Landi[3], Pavel Vodicka[1,2,5]**

[1] Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Videnska 1083, 142 00 Prague, Czech Republic
[2] Institute of Biology and Medical Genetics, 1st Medical Faculty, Charles University, Albertov 4, 128 00 Prague, Czech Republic
[3] Department of Biology, University of Pisa, Pisa, Italy
[4] Department of Gastroenterology and Hepatology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[5] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University in Prague, 323 00 Pilsen, Czech Republic

*Presenting author: Klara Cervena (e-mail: klara.cervena@iem.cas.cz)*

Pancreatic cancer (PanCa) has one of the lowest 5-years survival rates – less than 10%. To improve the prognosis of PanCa, it is necessary to develop tools that will enable earlier diagnosis. More than 90% of PanCa cases are characterized by the presence of KRAS mutations in tumor tissue that play a critical role in the initiation of PanCa. Recently, attention has been focused on liquid biopsy which can better reflect the whole genetic profile of the tumor and may help with early diagnosis.

Our study aims to discover whether KRAS gene mutations can be detected in the plasma isolated from the PanCa risk group patients - diabetes mellitus II (DMII) and chronic pancreatitis (CP). The KRAS mutation was analyzed in 34 PanCa, 68 DMII, and 26 CP patients by droplet digital PCR. Plasma cell-free DNA was investigated for three mutations hotspots: KRAS p.G12D c.35G>A, p.G12V c.35G>T, p.G12R.

Detailed results of the study will be presented during the meeting.

We believe that these results discover whether plasma analysis of KRAS mutation can serve as a biomarker for early diagnosis in risk group patients.

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POSTER 5

# Association of mitochondrial DNA copy number and telomere length with colorectal cancer patient outcomes

**P. Hanak[1], K. Tomasova[1,2], A. Siskova[1,3], J. Horak[1, 5], N. Danesova[1], M. Gentiluomo[4], D. Campa[4], P. Vodicka[1,2,3], S. Vodenkova[1,2]**

[1] Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic
[2] Biomedical Center of the Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[3] Institute of Biology and Medical Genetics of the First Faculty of Medicine, Charles University, Prague, Czech Republic
[4] Unit of Genetics, Department of Biology, University of Pisa, Italy
[5] Third Medical faculty UK, 100 00 Prague 10, Czech Republic

*Presenting author: Petr Hanak (e-mail: petr.hanak@iem.cas.cz)*

The dysfunction of mitochondria (mt) is one of the cancer hallmarks. Mt evince a limited DNA repair capacity and compensate for damage by increasing the mt DNA copy number (mtDNA-CN). Current studies on the mtDNA-CN in cancer report ambiguous results. Telomere shortening promotes cancer initiation by inducing chromosomal instability, while telomere length (TL) maintenance characterized by telomerase expression is required for cancer cell proliferation and tumour growth. The reports on TL in cancer risk, therapy response and/or survival are contradictory.

MtDNA-CN and TL are highly variable across cell types but stable within a constant range according to the specific tissue type. Mt biogenesis and energy production were decreased in telomerase-deficient mice with severe telomere dysfunction. It has been hypothesized that telomere alteration affects both oxidative defence mechanisms and mt functions. The deregulation of the telomere-mt axis, (caused by ageing or other physiological factors) triggers carcinogenesis. We investigated mt and telomere changes in colorectal cancer (CRC), one of the leading causes of cancer-related deaths. Our study looked closely at mtDNA-CN, mtDNA damage, TL, and the expression of mt transcription factor A and telomerase reverse transcriptase in association with CRC patient outcomes.

Our cohort included tumour and adjacent non-tumour tissues, and blood from 163 untreated sporadic CRC patients. After collecting the experimental data, comprehensive statistical analysis using patient clinical and follow-up data has been performed. We expect that the results may contribute to the current understanding of CRC, by identifying the role of mtDNA-CN and TL in CRC pathogenesis.

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POSTER 6

# Inhibition of homologous recombination repair by Mirin ameliorates carboplatin therapy response in vitro

**Josef Horak[1,2], Dominika Vallusova[3], Andrea Cumova[1,4], Petr Holy[2,5,6], Pavel Vodicka[1,4,5], Alena Opattova[1,4,5]**

[1] Department of Molecular Biology of Cancer, Institute of Experimental Medicine Czech Academy of Sciences (CAS), Prague, Czech Republic
[2] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Faculty of Science, Charles University, Prague, Czech Republic
[4] First Faculty of Medicine, Charles University, Prague, Czech Republic
[5] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[6] Toxicogenomics Unit, National Institute of Public Health, Prague, Czech Republic

*Presenting author: Josef Horak (e-mail: josef.horak@iem.cas.cz)*

Cancer chemotherapy resistance poses one of the most significant challenges of cancer therapy. Cancer chemotherapy resistance is the ability of cancer cells to blunt and counteract the effects of chemotherapeutics. The important mechanism in cancer therapy and chemoresistance is an alteration of DNA repair pathways. The overexpression of DNA repair genes in the tumor may confer more efficient repair of chemotherapy-induced damage, thus contribute to the chemoresistance. Carboplatin (CbPt) is one of the most used chemotherapeutics in ovarian cancer (OVC) treatment. MRE11 constitutes a part of homologous recombination (HR), which is responsible for the repair of CbPt-induced DNA damage, particularly DNA crosslinks. The study's main aim was to address the role of HR in CbPt chemoresistance in OVC and to evaluate the possibility of overcoming CbPt chemoresistance by Mirin-mediated MRE11 inhibition in an OVC cell line.

Lower expression of MRE11 was associated with in better overall survival in a cohort of OVC patients treated with platinum drugs (TCGA dataset, $p < 0.05$). Using in vitro analyses, we showed that the high expression of HR genes drives the CbPt chemoresistance in our CbPt-resistant cell line model. Moreover, the HR inhibition by Mirin not only increased sensitivity to carboplatin ($p < 0.05$) but also rescued the sensitivity in the CbPt-resistant model ($p < 0.05$).

Our results suggest that MRE11 inhibition with Mirin may represent a promising way to overcome OVC carboplatin resistance. More therapy options will ultimately lead to better-personalized cancer therapy and improvement of patients' survival.

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POSTER 7

# Regulatory role of Cul4a in the gastrointestinal homeostasis and colorectal cancer progression

**Veronika Iatsiuk[1], Jolana Tureckova[1], Kerstin Huebner[6], Katharina Erlenbach-Wuensch[7], Petra Baranova[3], Vendula Novosadova[2], Pavel Talacko[4], Karel Harant[4], Cyril Barinbka[3], Inken Beck[2,5], Regine Schneider-Stock[6,7], Radislav Sedlacek[1,2], Jan Prochazka[1,2]**

[1] Laboratory of Transgenic Models of Diseases, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2] Czech Centre for Phenogenomics, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[3] Laboratory of Structural Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Vestec, Czech Republic
[4] BIOCEV proteomics core facility, Faculty of Science, BIOCEV, Charles University, Vestec, Czech Republic
[5] Animal Research Centre, Ulm University, Ulm, Germany
[6] Experimental Tumorpathology, Institute of Pathology, University Hospital Erlangen, Friedrich Alexander University Erlangen-Nuremberg, Erlangen, Germany
[7] Institute of Pathology, FAU Erlangen-Nürnberg, Erlangen, Germany

*Presenting author: Veronika Iatsiuk (e-mail: veronika.iatsiuk@img.cas.cz)*

Background: Cancer development is facilitated by various alterations, including protein ubiquitination. However, the full complexity of the ubiquitinating network in cancerogenesis remains to be elucidated. Cul4a is a scaffold protein for the multicomponent Cullin4-RING E3 ubiquitin ligase (CRL4), which aberrant expression was found in many tumor types, including colorectal cancer (CRC). In the current study we aimed to uncover a role of the ubiquitin ligase Cul4a in the alteration of regulatory pathways, resulting in the gastrointestinal homeostasis disorders and tumor expansion.

Methods: To access the Cul4a involvement in the colorectal cancer progression, we used a Cul4a knockout mouse model on the ApcMin/+ background and human CRC samples. Followed investigation of molecular mechanism, involving a pull-down assay, mass spectrometry analysis and co immunoprecipitation, helped to identify possible interaction partners of Cul4a in the gastrointestinal tract.

Results: Here, we revealed novel interaction partners of ubiquitin ligase Cul4a in colorectal cancer expansion – Huwe1 and Smad3. Simultaneous loss of Cul4a and hyperactivation of Wnt pathway, mediated by Apc mutation, promote tumor expansion from small intestine to the distal colon, which indicates changes in the tumor etiology. We discovered that tumor expansion is caused by changes in the intracellular trafficking of Smad3, an essential mediator of TGF-β pathway, and stabilization of Huwe1 ubiquitin ligase.

Conclusions: Collectively, we identified Cul4a as a pivotal element in regulation of Smad3 and Huwe1, and demonstrate a critical role of the cooperative interaction between ubiquitination and the Wnt and TGF-β signaling pathways in gastro-intestinal homeostasis.

POSTER 8

# Tcf4-mediated Wnt signaling is required for Paneth cell maintenance and antimicrobial peptide production in the intestinal epithelium and colorectal cancer

**Lucie Janečková[1], Monika Šťastná[1], Dušan Hrčkulák[1], Tereza Müllerová[1,2], Stela Dostalíková[1,2], Vladimír Kořínek[1]**

[1] Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2] Faculty of Science, Charles University, Prague, Czech Republic

*Presenting author: Lucie Janečková (e-mail: lucie.janeckova@img.cas.cz)*

Background & Aim: Alpha defensin 6 (Defa6) is expressed specifically in Paneth cells, providing antimicrobial protection of the gut. Mutations in the gene encoding T-cell factor 4 (Tcf4), an effector of canonical Wnt signaling, are associated with Defa6 decay in ileal Crohn's disease. Conversely, aberrant activity of the Wnt pathway underlies the development of colorectal cancer. Defa6-expressing cells were found in human colorectal tumors. We aimed to clarify the role of Wnt signaling in antimicrobial peptide production and to reveal the function of Defa6 cells in the intestinal tumors. Methods: Mouse Defa6-expressing cells were visualized using a reporter allele yielding a red fluorescent protein. A conditional knockout of the gene encoding Tcf4 was introduced into Defa6-expressing cells in the healthy gut and multiple intestinal neoplasia mice carrying a mutation in the adenomatous polyposis coli gene (Apc-MIN). We employed immunohistochemical staining and gene expression analysis to evaluate the impact of Tcf4 loss in Defa6 cells on the morphology and function of the intestinal epithelium and tumor tissue.

Results: Tcf4 loss in mature Paneth cells resulted in defensins and lysozyme depletion, altered cell morphology and loss of positional signal. Epithelium lacking functional Paneth cells showed higher proliferation with earlier exhaustion of stem cells during aging. Defa6 cells in colorectal tumors exhibited secretory precursor characteristics. We observed reduced proliferation and quantity of Tcf4-deficient Defa6 cells, which nevertheless persisted in the tumors.

Conclusion: Wnt signaling drives the production of multiple antibacterial peptides. Defa6 cells likely mediate the immune response in colorectal cancer and may represent a beneficial therapeutic target.
Acknowledgement & Funding: This research was funded by the Czech Science Foundation, grant no. 18-26324S.

POSTER 9

# Exploring Caveolin-1 expression profile in neuroendocrine tumours of the gastrointestinal tract and pancreas

**A. Kataki[1], I. Angelioudaki[2], E. Koniaris, A. Mitrousias[2], A.G. Tzingounis[2], L. Stoupis[2], I. Papakonstandinou[2], N. Dafnios[2], G. Giogkas[2], G. Zografos[1], M.M. Konstadoulakis[2]**

[1] First Department of Propaedeutic Surgery, ""Hippokratio"" General Hospital, Athens, Greece
[2] Second Department of Surgery, Aretaieion Hospital, Medical School, NKUA, Greece
[3] Department of Pathology, "Hippocratio" General Hospital of Athens, Greece

*Presenting author: Agapi Kataki (e-mail: akataki@med.uoa.gr)*

Background & Aim: Neuroendocrine neoplasms (NENs) include distinct clinical entities which are classified as carcinomas (NECs), and tumours (NETs) and can be developed in different sites along the gastrointestinal tract(GI). Since understanding their carcinogenesis process is crucial for their clinical management, the present study is focusing on exploring the expression profile of caveolin-1 protein in these rare neoplasms, as caveolin-1 plays a critical role in various cellular processes.

Patients/Methods: Study's cohort consisted of 29 NETs (mean age 61.93±2.09), of which 20 are located in pancreas (pNET) and the others in appendix/stomach/ small intestine & duodenum (giNEC); 20 NECs (mean age 65.3±2.39): 11 in pancreas (pNEC) and 9 along GI (giNEC); and 7 MANEC (mean age 67.56±2.62) of which 2 are located in pancreas (pMANEC). Caveolin-1 expression was detected immunohistochemically, and both percentage of positive cells and intensity were scored in both lesions and normal adjacent epithelium and stroma. Their multiplication score was used in statistical analysis which was performed using SPSSv28.0.

Results: No caveolin-1 expression was detected in NET lesions epithelium. Expression in tumour stroma was present and significant increased in giNETs (p=0.007). In contrast, within NECs group, protein expression was only detected in cancer epithelium of pNECs (p=0.05), whereas no statistical difference was found in cancer stroma expression between the two groups. Similarly, caveolin-1 expression was only detected in pMANEC lesions epithelium (p=0.016).

Conclusion: The herein presented data indicates that these rare neoplasms present unique site-specific traits and they further support a relation between caveolin-1 expression and tumour's aggressiveness.

Acknowledgement & Funding: This project has received funding from the European Union H2020 Research and Innovation Programme under Grant Agreement No857381

POSTER 10

# Reduced methylation of olfactory receptor genes and amplification of 6p25.1-p22.3 as specific epigenetic and genetic alterations in colorectal cancer liver metastases

**Katerina Saskova[1,5], Mattias Landfors[2], Josef Horak[1,3], Katerina Honkova[4], Kristyna Tomasova[1,7], Jana Drabova[5], Fernanda Schäfer Hackenhaar[2], Jitka Pavlikova[4], Ludmila Vodickova[1,6,7], Torbjörn Nilsson[2], Sanja Farkas[8,9], Veronika Polakova Vymetalkova[1,6,7], Pavel Vodicka[1,6,7], Sofie Degerman[2], Michal Kroupa[1,7]**

[1] Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic
[2] Department of Medical Biosciences, Pathology, Umeå University, Umeå, Sweden
[3] Charles University, Third Faculty of Medicine, Prague, Czech Republic
[4] Department of Genetic Toxicology and Epigenetics, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic
[5] Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic
[6] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic
[7] Faculty of Medicine and Biomedical center in Pilsen, Pilsen, Czech Republic
[8] School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
[9] Clinical Pathology and genetics, Örebro University hospital, Örebro, Sweden

*Presenting author: Michal Kroupa (e-mail: michal.kroupa@iem.cas.cz)*

Aberrant DNA methylation and chromosomal instability (CIN) both play a pivotal role in the pathogenesis of CRC. Mapping both phenotypes during the disease progression can help to identify potential targets for pharmacological intervention and improve the management of CRC metastasis.

In the discovery set of nine patients diagnosed with advanced CRC, high-throughput DNA methylation and DNA somatic copy number alterations (SCNAs) analyses within primary tumors and corresponding liver metastases were performed. Methylation profiles and the most frequent SCNAs identified in the DNA were verified on a validation set of eight CRC patients by whole-exome sequencing, genome-wide DNA methylation measurement, and gene expression profiling.

In the discovery set, promoter regions of 2395 genes were methylated differently between tumors and metastases. All the metastases had an increase in hypomethylated and a decrease in hypermethylated CpGs. Enrichment analysis of the differently methylated gene promoters showed the olfactory receptors pathway as the most frequently represented (P=1.06E-8), with all the identified promoters (n=120) less methylated in metastases. Irrespective of the tissue, the degree of CIN correlated with the hypomethylation level (P=0.026). DNA regions amplified in primary tumors were highly concordant with those in liver metastases. Gain unique for metastases was located on Chr6 (p25.1-p22.3).

Taken together, herein, we identified a decrease in methylation within promoters of odorant receptors as the most prominent epigenetic difference occurring in metastatic tissues. Furthermore, several genomic hot-spot regions with SCNAs possibly important for the metastatic process were identified. Validation of the results is currently underway.

Acknowledgement & Funding: The study was funded by the Ministry of Health of the Czech Republic (NU22J-03-00028)

POSTER 11

# How important is good experimental design if you would like to analyze cfDNA, CTC or miRNA?

**Alice Mášová, Eva Rohlová, Lucie Langerová, Martin Mokrejš**

Institute of Biotechnology of the Czech Academy of Sciences, Vestec, Czech Republic

*Presenting author: Alice Mášová (e-mail: alice.masova@ibt.cas.cz)*

GeneCore is a service provider specialized in gene expression analysis and its application in the experiments. We have extensive experience in the design of various types of experiments based on the long-term practice in the areas of qPCR, digital PCR and NGS.

In the past we helped to develop guidelines for standardization sampling of biological materials. We focused on the analysis of the different biomarkers in human plasma as cfDNA, CTC (circulating tumor cells) and miRNA for early detection of cancer development.

Quality control during the entire experiment is often neglected by researches. We know that QC is crucial to correctly interpret obtained data and prevents misleading conclusions. In cooperation with the Laboratory of gene expression (IBT CAS, Mikael Kubista), we constantly develop and introduce new QC techniques to our service procedures, we are familiar with RNA sequencing, from experiment design, library preparation and data analysis. We also offer miRNA analysis using an ultra-sensitive technique (Two-tailed PCR). We introduced methods enabling transcriptome analysis at the single cell level (scRNA-seq).

We co-organized several local and international conferences. In 2016 we organized CTC meeting in Prague. We had great success with Single cell Europe conference in 2018. International conference Precision diagnostic Europe was arranged online in 2020.

We provide different courses focused on analysis miRNA, RNA or DNA using quantitative PCR or NGS.

Our goal is to help researchers and medical doctors with their projects. You have the rarest material and a multidisciplinary approach will help you clarify other biological questions.

POSTER 12

# The genetic landscape of metachronous colorectal liver metastases as revealed by whole exome sequencing

**Lucie Musilová[1], Viktor Hlaváč[1], Petr Holý[1,2], Václav Liška[1,3], Ondřej Vyčítal[1,3], Ondřej Fiala[1,4], Pavel Dvořák[1,5], Pavel Souček[1]**

[1] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Department of Surgery, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic
[4] Department of Oncology and Radiotherapeutics, Faculty of Medicine and University Hospital in Pilsen, Charles University, Pilsen, Czech Republic
[5] Department of Biology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

*Presenting author: Lucie Musilová (e-mail: lucie.musilova@lfp.cuni.cz)*

Aberrant DNA methylation and chromosomal instability (CIN) both play a pivotal role in the pathogenesis Background & Aim: Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the world. Due to its high progression rate, the prognosis of the patients remains dismal. Treatment of CRC mostly involves surgery followed by chemotherapy; however, the stage III 10-year recurrence rate still exceeds 50%. The knowledge of genetic landscape of metastatic processes might help establish a more tailored treatment and improve the prognosis of patients. Therefore, we aimed to investigate the genetic background of CRC liver metastasis and its associations with progression-free (PFS) and overall survival (OS).

Patients/Methods: Whole exome sequencing was performed in 41 pairs of metachronous CRC liver metastases and adjacent liver tissue. All patients were diagnosed and treated in one centre. For preparation of samples and data analysis, we utilized on site developed procedures.

Results: The most frequently altered genes were TP53 and APC (altered in 76% and 66% of patients, respectively) followed by KRAS (41%) suggesting these genes act as oncogenic drivers. The presence of alterations in TP53 was mutually exclusive with alterations in FREM2 and PIK3CA. Carriage of variants in TP53, KRAS, and UNC80 was associated with shorter PFS while patients bearing variants in RYR1 had significantly longer PFS. A lower share of mutational signature SBS22 and higher share of SBS93 were significantly associated with shorter PFS. Shorter OS was associated with alterations in VIPR2 and higher share of SBS39.

Conclusion: Our results provide unique genomic data associated with metastatic processes. Further validation is needed to support our results and more effort is required to reveal the mechanisms of metastasizing and fully explain their potential for targeted therapy.

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## POSTER 13

# Alternative splicing deregulation in CRC

**Nazila Navvabi[1,4,6], Frantisek Zitricky[1,5], Petr Hosek[1], Azita Navvabi[2], Ondrej Vycital[1,3], Jan Bruha[1,3], Richard Palek[1,3], Jachym Rosendorf[1,3], Saba Selvi[6], Vaclav Liska[1,3], Pavel Pitule[1], Ludmila Vodickova[1,6], Pavel Vodicka[1,6]**

[1] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2] Biological Center, Faculty of Marine Sciences and Technologies in Bandar Abbas, Hormozgan University, Hormozgan, Iran
[3] Department of Surgery, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[4] Department of Biology, Faculty of Medicine in Pilsen
[5] Laboratory of Translational Cancer Genomics in Pilsen
[6] Department of Molecular Biology of Cancer, Institute of Experimental Medicine Czech Academy of Sciences (CAS), Prague, Czechia

*Presenting author: Nazila Navvabi (e-mail: nazila.navvabi@gmail.com)*

Background: Colorectal cancer, one of the most frequent malignancy of digestive tract, remains the third leading cause of cancer-related death worldwide. AS is a versatile and powerful mechanism which is tightly regulated by splicing regulatory proteins. One of the trans - acting splicing regulatory factors are MBNL family, consisting of three members MBNL1, MBNL2 and MBNL3. MBNL proteins function as key regulators of AS during mRNA maturation and their disruption has been identified in cancer progression.

Aim: We focused on gene expression level of MBNL family as key regulators of alternative splicing in CRC. In addition to MBNL genes, we analyzed selected alternatively spliced isoforms that were confirmed to be regulated by MBNL to evaluate change in MBNL activity, and expression of cancer-related CD44 variants 3 and 6 as a relevant model of alternative splicing.

Methods: Samples were collected within 20 min after the removal of the tumor tissue from the patient, and biopsies of tumor samples and adjacent mucosa were immediately frozen and archived. Relative gene expression was tested by quantitative real-time PCR.

Results: In the present study, we analyzed the expression of selected gene set on 108 patients. All genes show statistically significant deregulation between tumor and healthy tissue. Our data suggest MBNL1, MBNL3, and alternative splicing of FOXP, CD44 and EPB41L3 could be deregulated in tumor tissue.

Conclusion: It is estimated that the expression profile of three MBNL paralogs and their correlated effect with the set of transcription factors might alter multiple splicing event. Our data show the change in the MBNL expression and corresponding changes in expression of splice-variants.

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POSTER 14

# Serum metabolite profiles of pancreatic tumors: neuroendocrine and pancreatic ductal adenocarcinomas – a preliminary study

**Karolina Skubisz[1,2], Krzysztof Dąbkowski[3], Emilia Samborowska[4], Anna Deskur[3], Filip Ambrożkiewicz[5], Jakub Karczmarski[4], Mariusz Radkiewicz[4], Katarzyna Kusnierz[6], Beata Kos-Kudła[7], Tadeusz Sulikowski[8], Patrycja Cybula[1,9], Teresa Starzyńska[3], Agnieszka Paziewska[1]**

[1] Institute of Health Sciences, Faculty of Medical and Health Sciences, Siedlce University of Natural Sciences and Humanities, Siedlce, Poland
[2] Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Pediatric Hospital of Medical University of Warsaw, Warsaw, Poland
[3] Department of Gastroenterology, Medical Pomeranian University in Szczecin, Szczecin, Poland
[4] Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[5] Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Alej Svobody 1665/76, 32300 Pilsen, Czech Republic
[6] The Department of Gastrointestinal Surgery Medical University of Silesia, Katowice, Poland
[7] Department of Endocrinology and Neuroendocrine Tumours, Department of Pathophysiology and Endocrinology, Medical University of Silesia, Katowice, Poland
[8] Department of General, Minimally Invasive, and Gastroenterological Surgery, Pomeranian Medical University in Szczecin, Poland
[9] Molecular Biology Laboratory, Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

*Presenting author: Agnieszka Paziewska (e-mail: agnieszka.paziewska@uph.edu.pl)*

Background & Aim: Pancreatic cancer (PC) is a malignant tumor, still in the majority diagnosed at an advanced clinical stage, when therapeutic possibilities are very limited and not satisfactory. Moreover, there is a need to improve knowledge about the pathogenesis of pancreatic tumors, and to identify reliable prognostic biomarkers.

The aim of our study was to search for metabolites in serum, related to pancreatic ductal adenocarcinomas (PDAC) and pancreatic neuroendocrine tumors (PNET) pathogenesis, which could be potential biomarkers of tumor development and progression.

Patients/Methods: In our research, we used serum samples from patients with PDACs (n=15), PNETs samples (=16) and healthy individuals (n=10). We have analysed the level of 188 serum metabolites using AbsoluteIDQ® p180 kit with liquid chromatography-mass spectrometry (LC-MS).

Results: We identified metabolites, whose concentrations were statistically significantly different (p.adj<0.05) between the control and tumor samples. Glutamine, acetylcarnitine (C2), and citrulline present a lower concentration in PDAC compared to control serum, while phosphatidylcholine aa C32:0, sphingomyelin C26:1, and glutamic acid higher. Additionally, C2 and serotonin reached higher concentration values in the PNET serum samples compared to PDAC, while phosphatidylcholine aa C34:1 had a higher concentration in the PDAC samples, with a very good diagnostic power to discriminate pancreatic tumor patients.

Conclusion: The presented results allow for a better understanding of the development and progression of pancreatic tumors related to metabolism of PNETS and PDAC. Identified disturbed metabolic profiles and serum circulating metabolites may help clinicians in the diagnosis of pancreatic tumor.

Acknowledgement & Funding: The project was financed by Institute of Health Sciences, Faculty of Medical and Health Sciences, Siedlce University of Natural Sciences and Humanities. The equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT), a project co-sponsored by European Regional Development Fund and Innovative Economy, The National Cohesion Strategy of Poland.

POSTER 15

# Are bacterial metabolites connected with colon cancer pathogenesis?

**Jakub Radziszewski[1,2], Patrycja Cybula[1,3], Emilia Samborowska[4], Jakub Karczmarski[4], Paweł Piotrowski[2], Krzysztof Kicki[2], Grzegorz Mikiciuk[2], Michał Kołodziejak[2], Mariusz Radkiewicz[4], Karolina Skubisz[1,5], Filip Ambrozkiewicz[6], Agnieszka Paziewska[1]**

[1] Institute of Health Sciences, Faculty of Medical and Health Sciences, Siedlce University of Natural Sciences and Humanities, Siedlce, Poland
[2] Department of Oncologic Surgery, Jan Paweł II Memorial Provincial Mazovia Hospital, 26, Księcia Józefa Poniatowskiego, Siedlce, Poland
[3] Molecular Biology Laboratory, Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland
[4] Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
[5] Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Pediatric Hospital of Medical University of Warsaw, Warsaw, Poland
[6] Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic

*Presenting author: Agnieszka Paziewska (e-mail: agnieszka.paziewska@uph.edu.pl)*

Background & Aim: Colorectal cancer (CRC) is one of the leading causes of death in the world. Despite many well-known risk factors for CRC including obesity or a diet high in fats, a growing number of studies indicate the relationship between the intestinal microbiome, dysbiosis, and in consequence the development of CRC. Additionally, bacterial metabolites affect various metabolic processes, may contribute to inflammation, and influence cancer pathogenesis. To define the metabolic profiles of CRC, we measured the level of metabolites in tissue from patients with CRC using liquid chromatography-mass spectrometry (LC-MS). As a result, we were able to investigate which metabolites may be involved in the initiation and progression of CRC.

Patients/Methods: The study involved 55 patients diagnosed with CRC. The material for the study consisted of tumor tissue and fragments of tissue without the tumor cells taken during a surgical procedure. Using liquid chromatography-mass spectrometry (LC-MS) we have measured the concentration of 16 bacterial metabolites (including TMAO metabolite panel and 10 of SCFA) in tissue samples.

Results: The concentrations of two metabolites: betaine and carnitine differed statistically significantly (p-val <0,05) between tumor tissue and tissue without neoplastic infiltration. Concentrations of these metabolites were higher in the fragments of tumor tissue, and correlated with localization and histological classification of tumor.

Conclusion: Our research presents differences in bacterial metabolic profiles between cancer tissue and tissue without neoplastic infiltration. It may contribute to a better understanding of cancer pathogenesis and provide the basis for further research on the role of bacterial metabolites in carcinogenesis.

Acknowledgement & Funding: The project was financed by the Institute of Health Sciences, Faculty of Medical and Health Sciences, Siedlce University of Natural Sciences and Humanities.

POSTER 16

# MiRNA profiling in primary and metastatic colorectal cancer – preliminary study

**V. R. Mallela[1], M. Rajtmajerová[1], A. Trailin[1], L. Červenková[2,5], V. Liška[2,3], R. Pálek[2,3], K. Hemminki[1,4], F. Ambrozkiewicz[1]**

[1] Laboratory of Translational Cancer Genomics, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Alej Svobody 1665/76, 323 00 Pilsen, Czech Republic
[2] Laboratory of Cancer Treatment and Tissue Regeneration, Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Alej Svobody 1665/76, 323 00 Pilsen, Czech Republic
[3] Department of Surgery, University Hospital in Pilsen and Faculty of Medicine in Pilsen, Charles University, Alej Svobody 80, 323 00 Pilsen, Czech Republic
[4] Department of Cancer Epidemiology, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[5] Department of Pathology, Third Faculty of Medicine, Charles University, Ruská 87, 100 00, Prague, 10, Czech Republic

*Presenting author: Marie Rajtmajerová (e-mail: marie.rajtmajerova@lfp.cuni.cz)*

Background & Aim: As major transcriptional regulators, microRNAs (miRNAs) have recently gained scientific attention. Specific profiles of miRNAs are associated with different tissues and conditions. In our study we focus on miRNA differential expression in different stages of colorectal cancer (CRC). CRC is the second leading cause of cancer-related death worldwide and Czech Republic belongs to countries with its highest incidence. Whilst driver mutations are well studied in CRC, other ways of transcriptome regulation are still not sufficiently described in literature. In order to understand CRC progression, it is necessary to investigate how miRNA signature evolves throughout different tumor stages.

Patients/Methods: We have obtained primary tumor, metastatic liver and non-tumor adjacent tissue FFPE samples of patients with metastatic CRC who underwent resection in Pilsen University Hospital. RNA isolation and small RNA sequencing was then performed.

Results: We have found 79 significantly dysregulated miRNAs in primary tumor and 81 miRNAs in metastatic liver compared to non-tumor tissue. 71 miRNAs exerted significantly different expression when comparing metastatic liver against primary tumor (Qval2). Unique miRNA were also found between non-tumor and metastatic samples (23 miRNAs), non-tumor versus primary (27 miRNAs) and non-tumor versus metastasis (38 miRNAs).

Conclusion: Significant differences in terms of miRNA expression are found between primary and metastatic tumor. Especially the unique miRNAs may provide important information about stage-specific biomarkers. Further verification and functional studies are needed to explore their impact and role in CRC progression.

Acknowledgement & Funding: This study was supported by European Union's Horizon 2020 research and innovation program under grant agreement N°856620, grant of Ministry of Health of the Czech Republic AZV NU21–03-00506, project National Institute for Cancer Research—NICR (Programme EXCELES, ID Project No. LX22NPO5102), funded by the European Union—Next Generation EU and grant of Grant Agency of the Czech Republic 23-05609S.

POSTER 17

# Mitochondrial DNA copy number in correlation with telomere length in solid adenomas

**Anna Siskova[1,2], Kristyna Tomasova[1,3], Michal Kroupa[1,3], Petr Hanak[1], Josef Horak[1,4], Sona Vodenkova[1,3], Jiri Jungwirth[5,6], Jan Kral[7], Tomas Hucl[7], Pavel Kohout[8], Sandra Summerova[8], Pavel Vodicka[1,2,3], Veronika Vymetalkova[1,2,3]**

[1] Department of Molecular Biology of Cancer, Institute of Experimental Medicine Czech Academy of Sciences (CAS), Prague, Czech Republic
[2] Institute of Biology and Medical Genetics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic
[3] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[4] Third Faculty of Medicine, Charles University, Prague, Czech Republic
[5] Institute of Physiology, 1st Faculty of Medicine Charles University, Prague, Czech Republic
[6] Department of Gastroenterology, Libera Scientia, Prague, Czech Republic
[7] Clinic of Hepatogastroenterology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[8] Department of Internal Medicine, 3rd Faculty of Medicine University and Faculty Thomayer Hospital Prague, Prague, Czech Republic

*Presenting author: Anna Siskova (e-mail: anna.siskova@iem.cas.cz)*

Background & Aim: Colorectal adenomas are aberrantly growing tissue from intestinal epithelium. They are precursors of colorectal cancer (CRC), second cancer leading in mortality worldwide. To reduce CRC mortality, we need to identify new biomarkers standing behind the development of already pre-cancerous stages.

Patients/Methods: The study focused on telomere length and mitochondrial DNA copy number (mtDNA CN) in solid colorectal adenomas from 145 patients as a potential biomarker for adenoma formation. In addition, we decided to follow in the same time the gene expression of enzyme telomeric reverse transcriptase (TERT), and mitochondrial gene Transcription Factor A, Mitochondrial (TFAM), a regulator of mitochondrial genome copy number. We performed multiplex RT-qPCR using specific Taqman probes.

Results: The results suggest that telomere length in healthy tissue is longer than in adenoma tissue. This is also related to the fact that telomerase expression was significantly higher in healthy tissue than in adenomas, although only at low expression levels in both tissues. The results of mtDNA CN in correlation with TFAM gene expression will be presented at the conference.

Conclusion: Differences in telomere length between healthy tissue and adenoma were found and relation with TERT expression was confirmed. These data will be further correlated with CN mtDNA and TFAM expression.

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POSTER 18

# Deregulation of EMT genes induced by hypoxia and inflammation independent of DNA methylation changes in human PDAC cell lines

**Maria Urbanova[1,2], Sabina Strapcova[3,4], Eliska Svastova[3], Viera Horvathova Kajabova[1], Verona Buocikova[1], Alena Gabelova[1], Sona Ciernikova[1], Jozef Bizik[1], Bozena Smolkova[1]**

[1] Cancer Research Institute, Biomedical Research Center of the Slovak Academy of Sciences, Dubravska Cesta 9, 845 05 Bratislava, Slovakia
[2] Department of Genetics, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovicova 6, 841 04 Bratislava, Slovakia
[3] Institute of Virology, Biomedical Research Center of the Slovak Academy of Sciences, Dubravska cesta 9, 84005 Bratislava, Slovakia
[4] Department of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava, Ilkovicova 6, 841 04 Bratislava, Slovakia

*Presenting author: Bozena Smolkova (e-mail: bozena.smolkova@savba.sk)*

Background & Aim: The 5-year survival rate in pancreatic ductal adenocarcinoma (PDAC) is less than 5%. Inflammation is a key mediator of PDAC development and inducer of epithelial-to-mesenchymal transition (EMT), which reversibility can be explained by epigenetic plasticity. We investigated mechanisms connecting inflammation and hypoxia with EMT, focusing on EMT-associated DNA methylation changes.

Materials and Methods: We established a fibro-inflammatory pancreatic cancer model by indirect co-cultivation of PDAC cell lines with activated stromal fibroblasts. The BxPC-3, MIA PaCa-2, and SU.86.86 cells were cultivated with or without conditioned media for 48 hours and six days in normoxic or hypoxic (1% O2) conditions. The expression pattern of inflammatory and EMT genes was assessed using the RT² Profiler PCR Arrays. DNA methylation of 15 top-ranked genes was evaluated by pyrosequencing. DNA methyltransferase inhibitor decitabine was used to confirm the epigenetic regulation of studied genes.

Results: Inflammation, mainly combined with hypoxia, induced a significant shift in gene expression, BxPC-3 cells being the most sensitive, with significant deregulation of 44 inflammatory and 41 EMT-associated genes. The highest changes were found in VIM, ITGA5, BMP2, and FN1 expression. However, these changes were only rarely associated with corresponding DNA methylation changes.

Conclusion: Although short-term cultivation under inflammatory and hypoxic conditions altered EMT-associated genes' expression, these changes did not correlate with corresponding DNA methylation. We hypothesize that other mechanisms, rather than DNA methylation alone or additional epigenetic modifications occurring in malignant cells, may contribute to detected gene expression regulation changes.

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POSTER 19

# The power of bioinformatics in cancer research

**Tereza Tesarova[1,2], Thomas Mohr[3], Karolina Seborova[1,2], Ondrej Fiala[1,4], Vaclav Liska[1,5], Pavel Soucek[1,2], Radka Vaclavikova[1,2]**

[1] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, 323 00 Pilsen, the Czech Republic
[2] Toxicogenomics Unit, National Institute of Public Health, 100 00 Prague 10, the Czech Republic
[3] Center for Cancer Research, Medical University of Vienna, 1090 Vienna, the Republic of Austria
[4] Department of Oncology and Radiotherapeutics, Faculty of Medicine in Pilsen and University Hospital, Charles University, 301 00 Pilsen, the Czech Republic
[5] Department of Surgery, Faculty of Medicine in Pilsen and University Hospital, Charles University, 301 00 Pilsen, the Czech Republic

*Presenting author: Tereza Tesařová (e-mail: tereza.tesarova@lfp.cuni.cz)*

Background & Aim: Next-generation sequencing is becoming the main diagnostic and research tool across the world laboratories in the last decade. With the amount of sequencing data comes an issue of mathematically and statistically analyzing them in a reasonable timeframe. Therefore, bioinformatics is lately coming to the fore of any biochemistry research field.

Patients/Methods: In our laboratories, we established a bioinformatics workflow to maximize the outcome of the RNA sequencing (RNASeq) results. Firstly, the RNASeq data needs to be quantified and its quality needs to be checked. To do that we use the FastQC package for quality control and the kallisto for quantification. Using the R programming language operated in the R Studio we run multiple analyses with the help of various packages for visualization of mostly affected pathways by gene deregulation or co-expression gene networks – e.g. ClusterProfiler, WGCNA, and more.

Results: We successfully created a pipeline for deep RNASeq data analyses. We were able to identify differentially expressed genes and pathways and perform gene ontology analyses. Additionally, we were able to visualize the data using heatmaps and volcano plots, which allowed us to identify patterns in gene expression. All performed analyses are allowing us to put the information about gene dysregulation into a relevant biological context and thus better understand the changes in transcriptome profile.

Conclusion: In conclusion, to analyze deep RNASeq data effectively, utilizing proper bioinformatics tools is essential. Our established workflow enabled efficient analyses and extraction of valuable information, providing a comprehensive understanding of gene dysregulation within a more meaningful biological context.

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POSTER 20

# Amplification of MALAT1 long non-coding RNA in high-grade colorectal adenoma patients

**Kristyna Tomasova[1,2], Katerina Saskova[1,3], Anna Siskova[2,4], Jana Drabova[5], Pavel Vodicka[1,2,4], Michal Kroupa[1,2]**

[1] Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic
[2] Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic
[3] Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic
[4] Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic
[5] Department of Biology and Medical Genetics, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic

*Presenting author: Kristyna Tomasova (e-mail: kristyna.tomasova@iem.cas.cz)*

Background & Aim: Chromosomal instability (CIN) refers to structural chromosome changes appearing through defective mitotic segregation or DNA repair and is a characteristic attribute for 70-80% of colorectal cancer cases. Considering the high prevalence of CIN and its impact on colorectal cancer formation, the study aimed to investigate the presence of CIN in precancerous colorectal adenoma tissue samples and find common copy number variations (CNVs), which might be connected to tumor development.

Patients/Methods: Thirteen high-grade adenomas were selected for the study and analyzed using a Comparative genomic hybridization array (aCGH) compared to the commercial reference DNA.

Results: Nearly all high-grade adenoma samples (85%) displayed a certain degree of CIN. A frequent CNV was identified in a small ~4500bp-long region on chr11 (q13.1) encoding MALAT1/TALAM1 genes. The locus was either amplified or deleted in 46% of the adenomas and posed for one of them the only alternation. Amplification of the whole long arm of chr13 (q11-q34) was often identified along with the trisomy of whole chr20 and appeared both in 46% of the patients. Frequent changes were located on chrX; q27.1-q28 and q22.33-p11.1 were amplified or lost in 31% of the patients.

Conclusion: We analyzed CNV profiles of adenomas detected by aCGH. The CNVs are now being verified by TaqMan Copy Number Assays and MALAT1/TALAM1 expression analysis. In addition, we confirmed the MALAT1/TALAM1 gains in a validation set consisting of nine metastatic colorectal cancer patients. The amplification occurred in three primary tumors and four liver metastases.

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POSTER 21

# Ablation of Atf2 results in highly invasive tumors in an AOM/DSS model of colorectal cancer

**Jolana Turečková[1], Kerstin Huebner[2], Jan Procházka[1], Ilir Sheraj[3], Katharina Erlenbach-Wuensch[4], Veronika Iatsiuk[1], Inken M. Beck[5], Fulvia Ferrazzi[4,6], Susanne Merkel[7], Robert Grützmann[7,8], Sreeparna Banerjee[3], Arndt Hartmann[4,8], Radislav Sedláček[1], Regine Schneider-Stock[2,8]**

[1] Czech Center for Phenogenomics, Institute of Molecular Genetics of the ASCR, v.v.i., 252 50 Vestec, Czech Republic
[2] Experimental Tumor Pathology, Institute of Pathology, University Hospital Erlangen, Friedrich Alexander University Erlangen-Nürnberg (FAU), 91054 Erlangen, Germany
[3] Department of Biological Sciences, Middle East Technical University, 06800 Ankara, Turkey
[4] Institute of Pathology, University Hospital Erlangen, Friedrich Alexander University Erlangen-Nürnberg (FAU), 91054 Erlangen, Germany
[5] Animal Research Center, Ulm University, 89081 Ulm, Germany
[6] Department of Nephropathology, Institute of Pathology, University Hospital Erlangen, Friedrich Alexander-University Erlangen-Nürnberg, 91054 Erlangen, Germany
[7] Department of Surgery, University Hospital Erlangen, Friedrich Alexander University Erlangen-Nürnberg, 91054 Erlangen, Germany
[8] Comprehensive Cancer Center Erlangen-EMN (CCC ER-EMN), University Hospital Erlangen, Friedrich Alexander University Erlangen-Nürnberg, 94054 Erlangen, Germany

*Presenting author: Jolana Turečková (e-mail: turejo@img.cas.cz)*

Aim: Activating transcription factor 2 (Atf2) is involved in many physiological and developmental processes including colorectal cancer (CRC). Yet, the role of Atf2 in CRC is poorly understood. Therefore, we aimed to unravel a role of Atf2 in tumor growth using a novel conditional Atf2KO mouse model.

Method: We established conditional Atf2fl/fl mouse strain bred with tamoxifen-inducible villin-CreERT2 mice to delete Atf2 transcription in intestinal mucosa. The latter mice were injected with one dose of AOM and then underwent chronic colitis induced by two runs of treatment with 2% DSS. Tamoxifen was injected either before DSS or after its second application. After additional 4-week period, we compared tumor development in vil-CreERT2- and vil-CreERT2+ animals. Colons were pre-stained with Alcian blue to estimate tumor numbers. Then, tissues were fixed and paraffin-embedded for immunohistochemical analysis.

Results: We performed a detailed histological analysis of colorectal tumors formed in our Atf2KO model. Both early and late tamoxifen-induced ablation of Atf2 led to a significantly early onset of invasive tumor growth. Importantly, while most of vil-CreERT2- mice developed sessile serrated lesions of low- and high-grade dysplasia, vil-CreERT2+ mice displayed invasive serrated adenocarcinoma with infiltrations, desmoplastic stroma and mucinous areas. We also found differences in number and size of the lesions in both genotypes. According to in silico analysis of Atf2KO gene signature it resembles that of the CMS3 CRC subtype. We evaluated some of genes of the signature by IHC in the AOM/DSS-derived tumors.

Conclusion: Our results show that ablation of Atf2 accelerates colorectal cancer. This Atf2KO mouse might present a suitable model for therapeutic strategies.

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