

## FP08

### Dysbalanced keratins promote steatohepatitis-associated liver carcinogenesis

K. Bettermann<sup>1</sup>, A. Kuldeep Mehta<sup>1</sup>, E.M. Lederer<sup>1</sup>, C. Ernst<sup>1</sup>, S.M. Kessler<sup>1,2</sup>, K. Kojima<sup>3</sup>, X. Cheng<sup>3</sup>, Y. Hoshida<sup>3</sup>, N.M. Bardessy<sup>4</sup>, B.C. Fuchs<sup>4</sup>, K.K. Tanabe<sup>4</sup>, H. Müller<sup>1</sup>, V. Svendova<sup>5</sup>, M.G. Schimek<sup>5</sup>, M. Mach<sup>6</sup>, M.R. Speicher<sup>6</sup>, C. Diwoky<sup>7</sup>, A. Lipfert<sup>7</sup>, V. Mahajan<sup>1</sup>, C. Stumptner<sup>1</sup>, A. Thüringer<sup>1</sup>, L.F. Fröhlich<sup>1</sup>, C. Smole<sup>1</sup>, T. Stojakovic<sup>8</sup>, M. Scheideler<sup>9</sup>, K.P.R. Nilsson<sup>10</sup>, T. Kolbel<sup>11</sup>, T. Rüllicke<sup>11</sup>, T. Longerich<sup>12</sup>, P. Schirmacher<sup>12</sup>, T.M. Magin<sup>13</sup>, P. Strnad<sup>14</sup>, C.D. Fuchs<sup>15</sup>, M. Trauner<sup>15</sup>, R. Spilka<sup>16</sup>, A.K. Kierner<sup>2</sup>, A. Teufel<sup>17</sup>, T. Maass<sup>17</sup>, N. Grabe<sup>18</sup>, K. M. Müller<sup>19</sup>, M. Themanns<sup>19</sup>, R. Moriggl<sup>19</sup>, J.S. Campbell<sup>20</sup>, S. Thorgeirsson<sup>21</sup>, M. Pasparakis<sup>22</sup>, J. Stauffer<sup>23</sup>, M. Karin<sup>24</sup>, J.M. Llovet<sup>25,26</sup>, K. Zatloukal<sup>1</sup>, H. Denk<sup>1</sup>, C. Lackner<sup>1</sup>, J. Haybaeck<sup>1</sup>

<sup>1</sup>Institute of Pathology, Medical University of Graz, Graz, Austria,

<sup>2</sup>Department of Pharmacy, Pharmaceutical Biology, Saarland University, Saarbrücken, Germany, <sup>3</sup>Liver Cancer Program, Tisch Cancer Institute, Division of Liver Diseases, Icahn School of Medicine at Mount Sinai, New York, USA, <sup>4</sup>Center for Cancer Research, Massachusetts General Hospital, Harvard Medical School, Boston, USA, <sup>5</sup>Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, Austria, <sup>6</sup>Institute of Human Genetics, Medical University of Graz, Graz, Austria, <sup>7</sup>Institute of Medical Engineering, Graz University of Technology, Graz, Austria, <sup>8</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria,

<sup>9</sup>Institute for Genomics and Bioinformatics, Graz University of Technology, Graz, Austria, <sup>10</sup>Department of Chemistry, Linköping University, Linköping, Sweden, <sup>11</sup>Biomodels Austria (Biat), University of Veterinary Medicine Vienna, Vienna, Austria, <sup>12</sup>Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, <sup>13</sup>Translational Centre for Regenerative Medicine Leipzig, University of Leipzig, Leipzig, Germany, <sup>14</sup>Department of Internal Medicine III, University Hospital RWTH Aachen, Aachen, Germany, <sup>15</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University Vienna, Vienna, Austria, <sup>16</sup>Laboratory of Pathology, General Hospital Zams, Zams, Austria, <sup>17</sup>Internal Medicine I, University Hospital Regensburg, Regensburg, Germany, <sup>18</sup>Hamamatsu Tissue Imaging and Analysis Center, BIOQUANT, National Center for Tumor Diseases (NTC), Medical Oncology, University of Heidelberg, Heidelberg, Germany, <sup>19</sup>Ludwig Boltzmann Institute for Cancer Research (LBI-CR), Vienna, Vienna, Austria, <sup>20</sup>Department of Medicine Pathology, University of Washington, USA, <sup>21</sup>Center for Cancer Research NCI, NIH, Laboratory of Experimental Carcinogenesis, Bethesda, USA, <sup>22</sup>Institute for Genetics, University of Cologne, Cologne, Germany, <sup>23</sup>Center of Cancer Research, NCI, Mouse Cancer Genetics Program, Genetic Modifiers of Tumorigenesis Section, Frederick, USA, <sup>24</sup>Department of Pharmacology, Laboratory of Gene Regulation and Signal Transduction, School of Medicine, University of California San Diego, La Jolla, USA, <sup>25</sup>Hepatocellular Carcinoma Translational Research Laboratory, Barcelona Clinic Liver Cancer Group, Liver Unit, CIBERehd, Institut d'Investigacions Biomèdiques, August Pi i Sunyer, Hospital Clínic Barcelona, Barcelona, Spain, <sup>26</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

<sup>9</sup>Institute for Genomics and Bioinformatics, Graz University of Technology, Graz, Austria, <sup>10</sup>Department of Chemistry, Linköping University, Linköping, Sweden, <sup>11</sup>Biomodels Austria (Biat), University of Veterinary Medicine Vienna, Vienna, Austria, <sup>12</sup>Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, <sup>13</sup>Translational Centre for Regenerative Medicine Leipzig, University of Leipzig, Leipzig, Germany, <sup>14</sup>Department of Internal Medicine III, University Hospital RWTH Aachen, Aachen, Germany, <sup>15</sup>Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University Vienna, Vienna, Austria, <sup>16</sup>Laboratory of Pathology, General Hospital Zams, Zams, Austria, <sup>17</sup>Internal Medicine I, University Hospital Regensburg, Regensburg, Germany, <sup>18</sup>Hamamatsu Tissue Imaging and Analysis Center, BIOQUANT, National Center for Tumor Diseases (NTC), Medical Oncology, University of Heidelberg, Heidelberg, Germany, <sup>19</sup>Ludwig Boltzmann Institute for Cancer Research (LBI-CR), Vienna, Vienna, Austria, <sup>20</sup>Department of Medicine Pathology, University of Washington, USA, <sup>21</sup>Center for Cancer Research NCI, NIH, Laboratory of Experimental Carcinogenesis, Bethesda, USA, <sup>22</sup>Institute for Genetics, University of Cologne, Cologne, Germany, <sup>23</sup>Center of Cancer Research, NCI, Mouse Cancer Genetics Program, Genetic Modifiers of Tumorigenesis Section, Frederick, USA, <sup>24</sup>Department of Pharmacology, Laboratory of Gene Regulation and Signal Transduction, School of Medicine, University of California San Diego, La Jolla, USA, <sup>25</sup>Hepatocellular Carcinoma Translational Research Laboratory, Barcelona Clinic Liver Cancer Group, Liver Unit, CIBERehd, Institut d'Investigacions Biomèdiques, August Pi i Sunyer, Hospital Clínic Barcelona, Barcelona, Spain, <sup>26</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

**Background.** Steatohepatitis (SH) is morphologically characterized by steatosis, hepatocyte ballooning and cytoplasmic protein aggregates termed Mallory-Denk bodies (MDBs). SH arises in about 20% of alcoholics and is frequently observed in obese type II diabetics. MDBs are generally composed of misfolded keratin (K) 8, 18 and in part sequestosome 1/p62 and ubiquitin. It is well established that K aggregates are critical for MDB formation, thus Ks are associated with SH which is one of the main preconditions for the development of liver cirrhosis and hepatocellular carcinoma (HCC).

**Methods.** In this study we aimed to clarify the pathophysiological and molecular mechanisms of K18 ablation on hepatocarcinogenesis in mice and its functional implication in human non-alcoholic SH (NASH)-induced liver cancer.

**Results.** 18 month-old Krt18-deficient (Krt18<sup>-/-</sup>/129P2/OlaHsd background) mice were investigated for the incidence of SH and SH-induced liver tumors by radiology, histology, immunohistochemistry, gene expression analysis and immunoblotting.

**Conclusions.** Aged Krt18<sup>-/-</sup> mice developed the whole morphological spectrum of SH whereas aged wild-type mice showed simple steatosis. Aminotransaminase levels were also elevated in aged Krt18<sup>-/-</sup> mice. Of note, 64% of male and 25% of 18 month-old Krt18<sup>-/-</sup> female mice developed liver tumors revealing morphological and genetic features of HCC whereas 45% of male and 16% of female Krt18<sup>-/-</sup> mice developed HCC.

#### Corresponding author

Assoc. Prof. Johannes Haybaeck, MD PhD

Institute of Pathology  
Medical University of Graz  
Auenbruggerplatz 25  
A-8036 Graz, Austria

Phone: +43 316 385 80594

FAX: +43 316 380 4403

johannes.haybaeck@medunigraz.at

## FP09

### Possible role of Cdx2 in the serrated pathway of colorectal cancer characterized by BRAF mutation, high-level CpG Island Methylator Phenotype and mismatch repair-deficiency

H. Dawson<sup>1,2</sup>, M. Helbling<sup>2</sup>, E. Karamitopoulou<sup>1,2</sup>, V.H. Koelzer<sup>1,2</sup>, A. Lugli<sup>1,2</sup>, I. Zlobec<sup>2</sup>

<sup>1</sup>Clinical Pathology Division and <sup>2</sup>Translational Research Unit, Institute of Pathology, University of Bern, Bern, Switzerland

**Background.** Colorectal cancer is a heterogeneous disease at the histomorphological, clinical and molecular level. Approximately 20% of cases are hypothesized to progress through the “serrated” pathway characterized by BRAF mutation and high-level CpG Island Methylator Phenotype (CIMP). A large subgroup are additionally microsatellite instable (MSI) and demonstrate significant loss of tumor suppressor Cdx2. The aim of this study is to determine the specificity of Cdx2 protein expression and CpG promoter hypermethylation for BRAFV600E and high-level CIMP in colorectal cancer.

**Methods.** Cdx2, Mlh1, Msh2, Msh6 and Pms2 expression were analyzed by immunohistochemistry using a multi-punch tissue microarray (n=220 patients). Additionally, KRAS and BRAFV600E mutation analysis, CDX2 promoter methylation and CIMP were investigated.

**Results.** Among other features, loss of Cdx2 was correlated with larger tumor size (p=0.0154), right-sided location (p=0.0014), higher tumor grade (p<0.0001), more advanced pT (p=0.0234) and lymphatic invasion (p=0.0351). Specificity was 100% for mismatch repair (MMR)-deficiency (p<0.0001), 92.2% (p<0.0001) for BRAFV600E mutation and 91.8% for CIMP-high. Combined analysis of BRAFV600E/CIMP identified Cdx2 loss as sensitive (80%) and specific (91.5%) for mutation/high status. CDX2 methylation correlated with BRAFV600E mutation (p=0.0184) and with Cdx2 protein loss (p=0.0028).

**Conclusions.** These results seem to indicate that Cdx2 may play a role in the serrated pathway to colorectal cancer as evidenced by strong relationships with BRAFV600E mutation, CIMP-high and MMR-

deficiency. Whether this protein can only be used as a “surrogate” marker, or is functionally involved in the progression of tumors through this pathway remains to be elucidated.

#### Corresponding author

PD Inti Zlobec, PhD  
Murtenstr. 31  
CH-3010 Bern, Switzerland  
Phone: 0041-31-6328755  
Fax: 0041-31-6324995  
inti.zlobec@pathology.unibe.ch

#### FP10

### ZEB2 CpG promoter methylation in colorectal cancer is associated with lymph node metastases and invasion in KRAS wild-type patients

I. Zlobec<sup>1</sup>, M. Helbling<sup>1</sup>, H. Dawson<sup>1,2</sup>, V. H. Koelzer<sup>1,2</sup>, E. Karamitopoulou<sup>1,2</sup>, A. Lugli<sup>1,2</sup>

<sup>1</sup>Translational Research Unit (TRU) and <sup>2</sup>Clinical Pathology Division, Institute of Pathology, University of Bern, Bern, Switzerland

**Background.** ZEB2 is thought to be involved in epithelial-mesenchymal transition (EMT) through transcriptional repression of E-cadherin and up-regulation of genes including anoikis-resistance factor neurotrophic tyrosine kinase receptor, TrkB. The aim of this study is to determine the clinical relevance of ZEB2 protein expression and ZEB2 promoter methylation in patients with colorectal cancer and their relationship to E-cadherin, TrkB and molecular features KRAS/BRAF mutation and CpG Island Methylator Phenotype (CIMP).

**Methods.** ZEB2, E-cadherin and TrkB immunohistochemistry was evaluated in 160 colorectal cancer patients using a multi-punch (4–5 tumour punches/patient) tissue microarray. ZEB2 promoter methylation, CIMP, KRAS (codon 12/13) and BRAF (V600E) mutation were determined in 95 patients using pyrosequencing.

**Results.** High ZEB2 expression was associated with advanced pT (p=0.0308), high-grade tumour budding (i.e., detached tumor cell clusters representing an EMT-like process; p=0.0376) and KRAS mutation (p=0.0141), whereas hypermethylation was frequent in KRAS wild-type cases (p=0.0233). In KRAS wild-type cancers, ZEB2 hypermethylation showed marked relationships with lymph node metastasis (p=0.0004), venous invasion (p=0.0247), lymphatic vessel invasion (p=0.0001), unfavourable survival (p=0.0417; 5-year survival: 27 vs. 74 months) and a positive correlation with TrkB (r=0.40). In KRAS mutated cancers, there were no associations of ZEB2 methylation with clinicopathological features, although a strong inverse correlation between methylation and expression (r=-0.45) was observed. Only weak associations with E-cadherin were noted.

**Conclusions.** ZEB2 over-expression and CpG methylation occur frequently in colorectal cancers, but may cause their effects in a KRAS-dependent manner. Among KRAS wild-type patients, methylation of ZEB2 may represent a novel route to tumour cell dissemination.

#### Corresponding author

PD I. Zlobec, PhD  
Head Translational Research Unit (TRU)  
Institute of Pathology, University of Bern  
Murtenstrasse 31, Room L414  
CH-3010 Bern, Switzerland  
Phone: +41 31 632 8755  
Fax: +41 31 632 4995  
inti.zlobec@pathology.unibe.ch

#### FP11

### Influence of KRAS mutations on outcome in patients with curatively resected stage III colon cancer treated with adjuvant chemotherapy

M. Nucifora<sup>1</sup>, S. De Dosso<sup>2</sup>, A. Spitale<sup>3</sup>, N. Sahnane<sup>1</sup>, M.E. Riveiro<sup>4</sup>, V. Bertolini<sup>5</sup>, E. Bucci<sup>5</sup>, S. Crippa<sup>1</sup>, P. Saletti<sup>2</sup>, L. Mazzucchelli<sup>1</sup>, M. Frattini<sup>1</sup>

<sup>1</sup>Institute of Pathology, Locarno, Switzerland, <sup>2</sup>Oncology Institute of Southern Switzerland, Switzerland, <sup>3</sup>Ticino Cancer Registry, Institute of Pathology, Locarno, Switzerland, <sup>4</sup>Beaujon University Hospital, Clichy, France, <sup>5</sup>Istituto Ospedaliero MultiMedica, Castellanza, Italy

**Background.** Postoperative chemotherapy improves survival in curatively resected stage III colon cancer (CC) patients and oxaliplatin in combination with fluoropyrimidine-based regimens has been established as a standard treatment. There is no clear evidence for a predictive marker regarding the effect of adjuvant chemotherapy. KRAS mutations could predict oxaliplatin sensitivity in in vitro experiments, as recently demonstrated. Here, we profiled KRAS and correlated it with outcome in stage III CC patients who underwent adjuvant chemotherapy.

**Methods.** Eligible patients were those with resected stage III CC who underwent 6-months adjuvant chemotherapy, either with single-agent fluoropyrimidine (FP: modulated 5FU or capecitabine) or with oxaliplatin-based regimens (O-FP: FOLFOX or XELOX). DNA extraction was performed on formalin-fixed paraffin-embedded sections, and KRAS mutations were analyzed by direct sequencing. Disease-free survival (DFS) and overall survival (OS) analyses were computed using the Kaplan-Meier method and the Log-rank test.

**Results.** The study population included 261 patients: 115 treated with FP, 146 O-FP. We identified KRAS mutations in 71/261 (27.4%) cases, of which 33 (46.5%) received FP, and 38 (53.5%) O-FP. In wild-type (wt) KRAS cases, DFS and OS group did not significantly differ between the two treatment modalities [in months, FP vs O-FP—median OS: 62.0 vs 49.6, HR: 1.12 (95% CI: 0.65; 1.92); median DFS: 57.1 vs 44.4, HR: 0.93 (95% CI: 0.55; 1.56)]. In patients treated with FP, a worse DFS [in months, mutant vs wt—median DFS: 46.0 vs 57.1, p=0.04; HR: 1.86 (95% CI: 1.01; 3.41)] and a trend toward a worse OS in months, mutant vs wt—median OS: 56.1 vs 62.0, p=0.08; HR: 1.71 (95% CI: 0.94; 3.10)] were observed in KRAS mutated patients. On the contrary, DFS and OS were not statistically different for mutated and wt KRAS patients treated with O-FP [in months, mutant vs wt—median DFS: 43.7 vs 44.4, p=0.62; HR: 1.17 (95% CI: 0.63; 2.18); median OS: 48.9 vs 49.6, p=0.77; HR: 1.11 (95% CI: 0.55; 2.23)].

**Conclusions.** Our results suggest that curatively resected stage III CC patients exhibiting wt KRAS status might benefit from FP alone thus avoiding oxaliplatin-adverse effects. On the contrary, an oxaliplatin-containing regimen should be recommended in KRAS mutated patients.

#### Corresponding author

Martina Nucifora, PhD Student  
Laboratory of Molecular Pathology, Institute of Pathology  
Via In Selva, 24  
6600 Locarno, Switzerland  
Phone: 0041918160803  
Fax: 0041918160719  
martinanucifora85@hotmail.it