Diethylnitrosamine (DEN) was used for induction of hepatic tumorigenesis. The male mice, 2 weeks old, were injectedintraperitoneally with 20 mg/kg body weight of DEN. The liver tissue samples were collected 4, 6, 8, 12 months after injection. We performed a comprehensive histological analysis of all kinds of liver samples: tumors, tumor surrounding tissue and normal tissue. Dysplasia was observed 4 months after injection, at hepatocellular adenomas were identified 6–8 months after injection. In the experimental group, 100% of mice had multifocal HCC 12 months after injection. Evaluation of samples from the control group showed normal liver architecture and histology.

Western blot analysis showed the presence the HCC tumor marker alfa-fetoprotein only in tumor samples. High levels of Igf-1R and FoxO3 proteins were observed in tumor tissue in contrast to the surrounding and normal tissues 12 months after injection. The expression profiles of Igfbp-1,-2,-3,-4,-5,-6,-7, Igf-1R, FoxO3 were analyzed by real time PCR. The most significant results were observed 8 and 12 months after DEN injection. RT-PCR showed the dynamic increase in Igf-1R expression in tumor. Expression pattern of Igfbps has shown a high level of Igf-1,-2,-5,-6,-7 mRNA in surrounding tissue and Igfbp-1,-3,-5 in tumor compared to control. The expression of Igfbp-2,-4,-5,-6,-7 was up-regulated, whereas expression of Igfbp-1,-3 was down-regulated in surrounding tissue. The expression of Igfbp-4 was decreased in tumor samples. The expression levels of Igfbp-3,-4 in surrounding tissue and Igfbp-7 in tumor were the same as in the control. We detected high level of mRNA of FoxO3 in tumor.

In conclusion, these data confirm previously existing assumption about paracrine effect of Igfbps on tumor growth and progression. Phosphorylation of FoxO3 protein may play an important role in survival of cancer cells. Our results showed up-regulation of Igfbp-2 and Igfbp-5 in tumor and tumor surrounding tissue. Igfbp-2 and Igfbp-5, well known activators of Igfs, can be pro-tumorigenic factors, which are important for hepatocarcinogenesis. Hence, therapeutic targeting of these proteins may offer options for intervention in human HCC.

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A141

Quantitative alterations of phospholipids in peripheral blood mononuclear cells in patients with breast and cervical cancer

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The aim of this study was to investigate the quantitative changes in the phospholipid (PL) content of peripheral blood mononuclear cells (MNC), plasma membrane (PM), fraction in breast (BC) and cervical cancers (CC) compared to normal levels. Eight PL fractions were identified by TLC method in the PM of MNC, namely: lysophosphatidylcholines (LPC), sphingomyelins (SPM), phosphatidylcholines (PC), phosphatidylinositols (PI), phosphatidylserines (PS), phosphatidylethanolamines (PE), phosphatidic acids (PA) and diphosphatidylglycerols (DPG). Data obtained indicate that all PLs, quantified in this study, were significantly altered in blood MNC of cancer patients compared to healthy individuals. It was shown that compared to norm levels of LPC, PC, PE fractions were reliable increased in BC and CC, when PI, PS, PA – decreased. Notably, regular disturbances reveled in BC and CC were identical with those observed earlier in chronic lymphocytic leukemia and also distinctly individual for each patient. We conclude that alterations in PLs content of crude MNC PMs have been associated with disease pathology and similarly involved in the onset and evolution of diverse forms of cancer. These data can be useful for prospective biomarkers selection and cancer definition as well as for discovery of new personalized treatment modes.

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Metabolic profiling of bile juice from the patients with cholangiocarcinoma-associated diseases

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Background: *Opisthorchiasis* is a form of foodborne trematodiasis which is caused by liver flukes. It has been shown that a chronic *Opisthorchiasis* infection increases a risk of cholagiocarcinoma of liver. It is commonly believed that a gradual change of homeostasis in a parasite microenvironment (bile) leads to liver fluke-induced cancer. Nevertheless, no systematic, analytically driven studies confirming this hypothesis have been published yet. The restricted access to clinical material and extreme complexity of the biological matrix (bile) both are the important "rate limiting factors" for a progress in the field. Here we present for the first time a cross-platform mass spectrometric analysis of bile juice collected from the patients with cholangiocarcinoma-associated diseases. We show that an effective analysis of such complex biological matrix as bile juice requires a combination of orthogonal analytical platforms (e.g. RPLC–MS and HILIC–MS) maximizing coverage of the metabolic space.

Materials and methods: 28 patients with *O. felineus* infection and 30 negative controls were included in the study. The infection status was confirmed using microscopy analysis of the bile. Bile samples were collected from the gallbladder using sterile puncture, directly frozen and stored at −80 °C until analysis. The samples were randomized and organized into the acquisition blocks consisting of the samples and quality controls (QC). Experiments were carried out with a Dionex Ultimate 3000 LC system (Thermo Scientific/Dionex, The Netherlands) equipped with a Dual Gradient Separation pump allowing for parallel LC analysis, and

hyphenated to an Impact UHR-qTOF mass analyzer (Bruker Daltonics, Germany). Reversed-phase experiments (RPLC) were performed with an UHPLC BEH Shield RP18 column 100 × 2.1 mm, 1.7 μm (Waters) and HILIC experiments with a Luna HILIC column (Phenomenex, The Netherlands) of 100 × 2.00 mm, 3 μ m. RPLC data were acquired in ESI positive mode and HILIC in negative mode, respectively. The data acquisition rate was set to 1 Hz over a mass range of *m/z* 50–1000. The LC–MS data files were aligned by using the in-house developed alignment algorithm MS-Align 2 tool (www.ms-utils.org/msalign2).

Results: After the data prepressing, which includes alignment, noise filtering and peak picking two data matrixes costing of 412 features (metabolites) for RPLC and 428 ones for HILIC were generated. To evaluate a degree of similarity between the two data matrixes the RV coefficient (a multivariate extension of correlation coefficient) was used. The coefficient has flattened at 0.58 showing that despite a strong overlap between the datasets there is a substantial number of the "platform specific" metabolites. Those structures will certainly be missed if a single platform strategy is applied.

Conclusion: Here we present for the first time a cross-platform mass spectrometric analysis of bile juice collected from the patients cholangiocarcinoma-associated diseases. We show that a combination of the two platforms greatly improves the coverage of the metabolome and as such should be a firstchoice for exploratory studies of the complex biological matrixes.

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Intratumor morphological heterogeneity in breast cancer and distant metastasis: Expression analysis of genes involved in cell motility and pre-metastatic niche formation

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Background: Breast cancer, particularly invasive carcinoma of no special type (IC NST), demonstrates considerable intratumor morphological heterogeneity. Five types of morphological structures representing different architectural arrangements of tumor cells – tubular, alveolar, trabecular, solid structures, and discrete groups have been described in IC NST. Previous studies reported the contribution of intratumor morphological heterogeneity of IC NST to chemotherapy efficiency and lymph node metastasis (Zavyalova et al., 2013; Denisov et al., 2014); however, its role in distant metastasis remains unidentified. Aim: to study the contribution of intratumor morphological heterogeneity of IC NST to distant metastasis and to identify gene expression features of metastatic behavior of different morphological structures.

Materials and methods: 358 IC NST patients (age range 29–90, mean age 49.8 ± 9.5, T1-4N0-3M0-1) treated with neoadjuvant chemotherapy (NAC) have been enrolled in this study. Chi-square test and Kaplan–Meier analysis were used to estimate the association between the presence of certain morphological structures in breast tumors and the frequency of distant metastasis and metastasis-free survival. qRT-PCR was applied for measurement of the expression levels of genes involved in cell motility (CDH1, CDH2, CDH3, CTNNA1, CTNNB1, ITGA6, ITGAV, ITGB1, ITGB3, ITGB4, SNAIL, MMP14, ROCK2, L1CAM, MMP2, MMP9, PDPN) and pre-metastatic niche formation (TNFa, TGFb, VEGFa, LOX, M-CSF, GM-CSF, HIF1A, SDF2) in different morphological structures isolated from breast tumors ($n = 4$) by laser microdissection.

Results: Patients with alveolar structures in breast tumors more frequently displayed distant metastasis than cases without this morphological variant (71.9% vs. 56.5%; $p = 0.004$). The association between alveolar structures and high frequency of hematogenous metastasis was found only in patients with poor response to NAC ($p = 0.003$), but not in cases with good chemotherapy efficiency ($p = 0.377$). Increased distant metastasis was also shown in patients with trabecular structures as compared to cases without this morphological type (88.3% vs. 70.0%; $p = 0.0001$). Kaplan–Meier analysis demonstrated a significantly higher probability of developing metastasis in patients with alveolar or trabecular structures in breast tumors ($p = 0.011$). No significant association between other morphological structures and distant metastasis was found. Expression analysis showed the presence of cell motility phenotype in all morphological structures. In particular, we found changes in cell adhesion gene expression, which declined in the row: solid–alveolar–trabecular structures–discrete groups of tumor cells ($p < 0.05$). In addition, almost all structures demonstrated SNAIL and ROCK2 gene expression, and there were differences in expression of other cell migration genes between morphological structures. For example, PDPN was observed to be expressed in solid and alveolar structures, whereas L1CAM – in trabecular, tubular structures and discrete groups of some breast tumors. The expression of pre-metastatic niche genes also varied between distinct structures and, in general, declined in the row: alveolar–solid–trabecular structures–discrete groups of tumor cells ($p < 0.05$).

Conclusion: Intratumor morphological heterogeneity of IC NST contributes to distant metastasis probably by variations in expression of genes involved in cell motility and pre-metastatic niche formation between different morphological structures.

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Endogenous inhibitors of cysteine proteases cystatin C and cystatin SN in biological fluids of patients with intraocular melanoma as possible biomarkers and therapy targets