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

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Background: Cystatins are natural endogenous inhibitors of cysteine proteases universally involved into development of different tumors. Tumor growth and metastazing include increased consumption of these inhibitors, followed by decreased level of cystatins and dysregulation of proteases/protease inhibitors system. However, the biological role of individual cystatins is still not clear. The most known cystatin C was shown to relate to some types of tumor development. Cystatins include a group of intracellular cystatins (belonging to type 1) and extracellular cystatins (type 2), among them cystatins C, D, E/M, F, G, S, SN and SA, which functions were not studied enough. It was suggested that cystatin SN was responsible in regulation of tumor growth locally.
The aim: To investigate cystatin C and cystatin SN concentrations in biological fluids of patients with intraocular melanoma as tumor biomarkers and possible therapy targets.
Materials and methods: The patients with melanoma chorioidea (57 patients; among them woman 36, men 21; aged from 28 to 80, of middle age of 56.6 ± 2.4 years) were under investigation. In all cases the pathological process involved one eye. The control group consisted of 37 healthy persons (volunteers), medical personnel in clinic and students, aged from 20 to 49 years; the middle age 31 ± 4.1 years); 7 patients with age-related cataract aged from 57 to 80 (middle age 71 ± 2.6 years; man 3, woman 4). The biological fluids studied: tears, intraocular fluid (obtained during operation) and blood serum. In all cases investigation was made according to informed agreement of patients and control group members. Cystatins concentration was measured by ELISA kits: for Cystatin C (BioVendor, Chechia) and for cystatin SN with help of Human Cystatin SN (CST1) Elisa Kit Cusabio, China. Statistical analysis was made by non-parametric statistic test of Kruskal–Wallis, for correlations – Spearman test. The difference between groups studied was considered significant $p < 0.05$.
Results: Increased serum level of cystatin C was revealed in patients with melanoma chorioides (1023.5 ± 78.9 ng/ml, $p = 0.019$) compared with the control (809.9 ± 146.8 ng/ml). In tears of patients with melanoma chorioidea, cystatin C concentration (441.7 ± 14.5 ng/ml) had a tendency to increase as compared to the data obtained in tears of the control group (287.5 ± 20.01 ng/ml) as well as the cystatin C level of intraocular fluid of these patients vs the control group ($p < 0.1$). Cystatin SN concentration in serum of patients with melanoma chorioidea (1.45 ± 0.30 ng/ml) was lower vs the control group (3.12 ± 0.32 ng/ml, $p = 0.0038$), as well as Cystatin SN level in intraocular fluid (1.43 ± 0.10 ng/ml) vs the control (2.60 ± 0.60 ng/ml, $p < 0.05$). There was no difference in cystatin SN concentration in tears of patients and control group.
Conclusion: In serum of control (healthy) group, cystatin C concentration is significantly higher than cystatin SN level in serum, tears and intraocular fluid. The reverse correlation was revealed between the level of these inhibitors in serum, that is suggested their possible interaction. In melanoma patients the reciprocal changes in cystatin C and cystatin SN were shown: increased cystatin C and decreased cystatin SN level in all biological fluids studied. On the basis of cystatins distribution in biological fluids of patients one can suggest their involvement in pathological process as system reaction of organism on tumor development.

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Genetic testing in early diagnostics and prevention of gastric cancer

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Background: To determine diagnostic importance of DNA methylation in patients with chronic atrophic gastritis and induction of "Correa" cascade for gastric cancer prevention.
Material and methods: This present study included 80 patients with chronic atrophic gastritis associated with *Helicobacter pylori*. Diagnoses were confirmed by endoscopic, morphologic, serologic examinations. Age of patients varied from 17 to 78 years old. There were 52 (65%) males and 28 (35%) females. The control group consisted of 32 patients with morphological verified diagnosis of stage I–II gastric cancer. Examination with the purpose to determine hypermethylation of DNA was performed simultaneously in biopsy materials and blood plasma. Provoking factors of hypermethylation in 4 tumors' genes, APE, E-Cadherin, T1MP3, hMLHI were determined by quantitative methylation with use of Polymerase Chain Reaction. To evaluate the level of methylation we compared the analysis' results of biopsy and blood plasma tests. Blood serum samples and biopsy specimens were collected at diagnosis until the therapy is started. All patients with chronic atrophic gastritis infected with *H. pylori* underwent anti-*H. pylori* therapy according to the protocol. Chronic atrophic gastritis was found at morphologic examination in 40 (50%) patients according to "Correa" cascade. 36 (45%) patients had intestinal metaplasia, and 4 (5%) patients had dysplasia. Reaction was considered to be positive in cases, when the level of methylation in genes listed above was higher in blood serum than in biopsy materials.
Results: High concentrations of methylated APE, T1MP3 and hMLIH in genes were found in blood serum of 8 (10%) patients. In the control group, all 32 patients with gastric cancer had high methylation level in blood serum. In the remaining 72 (90%) patients, no high concentration of DNA methylation was found. After the 2-nd course of anti-*H. pylori* therapy, patients underwent morphologic and endoscopic examinations according to the protocol. Eradication of *H. pylori* was determined in 86% patients who received therapy. Intestinal metaplasia decreased from 45% to 25% (20 patients). Mild dysplasia was found in 1.2% of cases. Repeated analysis of methylation level showed its decrease after anti-*H. pylori* therapy in 4 (50%) out of 8 patients.
Conclusion: Genetic tests show that DNA methylation in patients with chronic atrophic gastritis has high diagnostic importance. Anti-*H. pylori* therapy at the different stages of