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**