Circulating extracellular vesicles with liver-specific RNA species and proteome are potential biomarkers for early liver fibrosis in experimental cholestasis

**Background and Aim:** Biliary diseases represent around 10% of all chronic liver diseases affects both adults and children. While the currently available biochemical tests reflect the surge of the cholestatic disease, there are still no reliable methods to determine the initial phases of liver fibrosis when the latter is still reversible, or to successfully monitor response to therapies, apart from the invasive liver biopsy. Circulating extracellular vesicles (EV) are lipid bilayer-delimited particles released upon tissue injury which may provide a real-time snapshot of the entire organ in a non-invasive way. We thus aimed at searching for novel EV-based biomarkers for cholestasis-induced early liver fibrosis in mice models.

**Method:** Bile duct ligation (BDL, obstructive cholestasis) was performed for different time points in mice in order to detect the initial signs of liver fibrosis. Circulating EVs were enriched, fully characterized by RNA-seq and proteomics, and compared to sham controls. Protein-coding RNA and microRNAs were isolated, and validated by real-time PCR, from EV-enriched serum fractions and from liver specimens of BDL mice, as well as from DDC-treated (drug-induced cholestasis) and MDR2-/- mice (genetic cholestasis).

**Results:** Histopathological analysis of mice livers evidenced early signs of liver fibrosis at 8 days, which progressed by 15 days, after BDL. Whole transcriptome and small RNA-seq analyses revealed the presence of different RNA species in circulating EVs. Unsupervised hierarchical clustering identified a signature that allowed for discrimination between BDL and controls. In particular, 151 microRNAs enriched in BDL were identified, of which 66 were conserved in humans. Interestingly, 5 of the microRNAs selected showed a similar trend in all three cholestatic mice models. Proteomic analysis of blood EVs detected various differentially expressed liver proteins in BDL versus control animals. The liver was an important source of circulating EVs in BDL animals as evidenced by the enrichment in blood with miR-122 and 192, two microRNAs previously described in chronic liver diseases. Analysis of correlation between miRNA levels in EVs with fibrosis (r2 score) is ongoing.

**Conclusions:** These findings suggest a potential for using specific circulating EVs as sensitive and specific biomarkers for the non-invasive diagnosis and monitoring of the appearance of fibrosis in cholestatic diseases.

**References:**
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**Other:** Current interests: *In vitro* differentiation of adult stem cells in functional hepatocytes and assessment of their engraftment *in vivo* in animal models; correction of metabolic diseases of liver with stem cells as platform for gene therapy; Liver diseases and extracellular vesicles: search for new therapies and biomarkers; recently appointed as Chief Editor of *Minerva Biotecnologica* Journal