and fT4/rT3 distinguished advanced- from mild- fibrosis, even in individuals with similar serum levels of TSH and fT4. Conclusion: Hedgehog-dependent changes in liver stromal cells drive repair-related changes in hepatic deiodinase expression that promote intrahepatic hypothyroidism, thereby limiting exposure to T3, an important factor for hepatic differentiation. Changes in deiodinase expression correlate with reduced serum fT3/rT3 and fT4/rT3 ratios. Thus, increased serum rT3 may serve as a novel biomarker of liver disease severity in humans.

Disclosures:

Manal F. Abdelmalek - Consulting: Islet Sciences; Grant/Research Support: Mochida Pharmaceuticals, Gilead Sciences, NIH/NIDDK, Synageva, Genfit Pharmaceuticals

Anna Mae Diehl - Consulting: Roche; Grant/Research Support: Gilead, Genfit

The following people have nothing to disclose: Brittany Bohinc, Gregory A. Michelotti, Guanhua Xie, Herbert Pang, Ayako Suzuki, Cynthia D. Guy, Dawn L. Piercy, Leandi Kruger, Marzena Swiderska-Syn, Mariana V. Machado, Thiago A. Pereira, Ann Marie Zavacki

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MicroRNA profiling of circulating exosomes during experimental liver fibrosis

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Background: Exosomes arise by inward budding of the limiting membranes of multivesicular bodies which, upon fusion with the plasma membrane, result in their secretion and deposition into body fluids (e.g. blood, urine). Exosomes contain a complex mixture of microRNAs (miRs), mRNAs and proteins that reflect the transcriptional and translational status of the producer cell. Since this molecular payload is a "fingerprint" of the dynamic status of their producer cells, exosomes represent a potentially valuable resource for assessing liver disease or pathology. Our goal was to profile the microRNA content of serum exosomes in experimental liver fibrosis. Methods: PureExo Exosome Isolation Kits were used to isolate serum exosomes. MiR profiling was performed on exosomal RNA from 1ml of pooled serum (5 mice; 200µl/mouse) using a mouse miRnome miR PCR Array. miR profiling was performed for the 940 best characterized miRs in the mouse miRnome on exosomes isolated from the circulation of mice after 1 or 5 weeks of treatment with CCl4, as compared to oil-treated controls, with liver injury/fibrosis confirmed histologically. Differentially expressed miRs were confirmed and/or further evaluated by qRT-PCR of exosomal RNA independently obtained at 1-, 4- or 5-weeks of CCl4 administration (n=5). Results: Isolated exosomes from mice serum were bi-membrane vesicles, 50-200nm in diameter, and positive for the exosome markers, CD9 and flotillin-1. Microarray analysis revealed significant alterations in the expression of many hundreds of miRs after either 1- or 5-wks of CCl4 treatment as compared to their respective oil controls. We then focused on selected miRs previously reported to be altered in fibrotic liver, and confirmed the data by RT-PCR. The exosomal levels of these miRs after 5 weeks of CCl4 (including up-regulation of miR-7a, -21, -22, -24, -34a, -155, or -195, and down-regulation of miR-27a, -192, -214, or -377) reflected their previously documented changes at the tissue level in fibrotic liver. In addition, several exosomal miRs that have not yet to be reported in the literature as being altered during liver fibrosis emerged as potentially novel fibrosis markers (e.g. up-regulation of miR-26b or -122; down-regulation of miR-9 or -196b). As compared to their levels at 5 weeks, many of these miRs exhibited individually distinct patterns of expression during the course of fibrosis progression. Conclusions: Dynamic changes occur in the miR content of circulating exosomes during experimental hepatic fibrosis supporting the concept that fibrosis progression and severity is amenable to minimally-invasive assessment through determination of signature exosomal miRs.

Disclosures:

The following people have nothing to disclose: Li Chen, Ruju Chen, David Brigstock

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Soluble CD146, a novel endothelial marker, is related to the severity of liver disease

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BACKGROUND: Angiogenesis and inflammation have been involved in the progression of fibrosis in patients with chronic liver disease (CLD). Soluble CD146 (sCD146), a biomarker which was recently characterized as a novel component of the endothelial junction is implicated in endothelial proliferation. AIM: To evaluate the performance of sCD146 in assessing liver fibrosis and cirrhosis and determine if its levels are related to the severity of liver disease in patients with cirrhosis. METHODS: sCD146 levels were determined by a commercially available immunoenzymatic technique in sixty-two consecutive patients with cirrhosis, forty-three patients with CLD without cirrhosis and twenty-seven healthy controls. Diagnosis of cirrhosis was based on liver histological findings and/or imaging, endoscopic, clinical findings. The absence of cirrhosis in patients with CLD was based on measurements of liver stiffness by transient elastography and/or liver biopsy. Healthy controls were recruited from the donors attending the Blood Transfusion Centre. RESULTS: The median sCD146 values were significantly higher in patients with cirrhosis [639 ng/ml (interquartile range 421-887)] compared to non-cirrhotic CLD patients [317 ng/ml (267-414), ($P < 0.001$)] or to healthy controls [310 ng/ml (233-345), $P < 0.001$]. Moreover, patients with compensated cirrhosis had higher levels [400 ng/ml (325-533)] than non-cirrhotic CLD patients ($P = 0.016$) but lower than patients with decompensated cirrhosis [848 ng/ml (608-1001), $P < 0.001$]. In receiver operating characteristic (ROC) curve analysis, the cut-off of 412 ng/ml showed a sensitivity of 78% and a specificity of 75% for differentiating cirrhosis from CLD without cirrhosis, offering good diagnostic accuracy (AUROC: 0.838). A cut-off of 534 ng/ml offered a sensitivity of 83% and a specificity of 78% for differentiating compensated from decompensated cirrhosis (AUROC: 0.866). Furthermore, in patients with cirrhosis, ALT, AST, total bilirubin and international normalized ratio (INR) correlated positively with sCD146 levels [$r = 0.324$, ($P = 0.012$), $r = 0.549$, ($P < 0.001$), $r = 0.542$, ($P < 0.001$), $r = 0.648$, ($P < 0.001$), respectively). Most importantly, MELD score correlated significantly with sCD146 [$r = 0.567$, ($P < 0.001$)]. CONCLUSIONS: sCD146 is emerging as a novel, easy to perform, sensitive, non-invasive plasma biomarker, which can reliably detect advanced fibrosis and predict decompensation of cirrhosis. It is well correlated with severity of liver disease in cirrhotic patients.

Disclosures:

The following people have nothing to disclose: Efrossini Nomikou, Alexandra Alexopoulou, Larisa E. Vasilieva, Danai Agiasotelli, Spyros P. Dourakis