Background and Aims: Colorectal cancer (CRC) is the second leading cause of cancer-related deaths, and liver is the most common site of CRC metastasis. None of the current tissue culture models for studying hepatic metastasis mimics the biological, biochemical and structural characteristics of the metastatic microenvironment such as the extracellular matrix (ECM). The aim of this study was to develop a 3D model of colorectal cancer liver metastasis (CRLM) and matched CRC using patient-derived decellularized matrices to recapitulate and study the role of the tissue-specific microenvironment.

Methods: Decellularization of patient-derived samples of matched healthy colon (HC), CRC, healthy liver (HL) and CRLM was performed with a detergent-enzymatic process. A 3D culture model was generated seeding decellularized scaffolds with CRC cell line HT-29.

Results: Decellularization preserved tissue-specific biological and ultrastructural properties of CRC and CRLM ECM. Seeded CRLM and CRC scaffolds supported cancer cell adhesion and, when compared to the respective normal counterpart, they showed increased proliferation and migration. 3D models generated with CRC and CRLM scaffolds also demonstrated induction of epithelial-mesenchymal transition (EMT), supported by protein expression (E-cadherin, Vimentin and SNAI1/2) and gene set enrichment analysis. Cells cultured in the CRLM environment displayed significant differences in their gene expression profile in respect to conventional 2D cultures, with the most represented biological processes involving cellular response to stress metabolic processes, to oxygen level and to starvation, and demethylation and deacetylation. When HT-29 cells grown in CRLM or HL scaffolds were exposed to IC50 of 5-FU and FOLFIRI determined in 2D conditions, the cellular response to the chemotherapy agents was affected by the tissue-specific decellularized scaffolds. HT-29 cells grown in CRLM scaffolds, but not in HL scaffolds, were more resistant to treatment with 5-FU and FOLFIRI determined in 2D conditions, the cellular response to the chemotherapy agents was affected by the tissue-specific decellularized scaffolds. HT-29 cells grown in CRLM scaffolds, but not in HL scaffolds, were more resistant to treatment with 5-FU and FOLFIRI (Figure), which would be the normal situation in man.

Conclusion: We have established a physiologically relevant 3D tissue culture model which is able to mimic in vivo features of CRLM such as proliferation, migration, and chemotherapeutic drug response of CRC cells in liver metastatic tissue and, as such, represents a new powerful tool to investigate formation and progression of liver metastases.
complexity of the in vivo cellular interactions, metabolic activation pathways are largely unknown. We herein aim to elucidate the metabolic asset of both tumoral and non-tumoral primary BECs by profiling both the extra- and endo-metabolome.

**Method:** Primary non-tumoral BECs (NT-BECs) and tumoral iCCA BECs (iCCA-BECs) were isolated from 15 patients surgically resected at the Division of Hepatobiliary and General Surgery, Humanitas Clinical and Research Center. Both tumoral and non-affected BECs from the same donor were cultured until reaching 80% of confluence. Cells and their conditioned medium were analyzed by using mass spectrometry-based untargeted and targeted metabolic approaches to explore the main metabolic processes. Moreover, primary iCCA BECs and HuCC-T1 human iCCA immortalized cell line, were seeded in 96-well plates to perform proliferation assay at different time point with different culture medium to detail the involvement of nutrients in iCCA-BEC proliferation.

**Results:** We observed that iCCA-BECs were characterized by higher mitochondrial activity compared to NT-BECs in all samples, in which glutamine and pyruvate act as metabolic sources to fuel central metabolism for both kind of cells. C) iCCA cells cultured with different metabolic medium composition, were able to exploit the metabolism respectively. Importantly, iCCA-BECs exposed to different nutrient environments were able to reprogram nutrient uptake and utilization to boost central cellular metabolism. Furthermore, the proliferation assay showed that iCCA-BECs, when cultured in a different metabolic medium composition, were able to exploit the different metabolic sources to sustain cell growth.

**Conclusion:** This observation raises the prospect that interfering with mitochondrial activity of iCCA cancer cells could make them more susceptible to cytotoxic drugs, opening new possibility to improve the outcomes of the iCCA patients.

**FRI500**
Mitochondrial oxidative metabolism contributes to maintain a cancer stem cell phenotype in cholangiocarcinoma

Chiara Raggi1, Marina Letizia Taddei1, Elena Sacco2, Nadia Navari1, Margherita Correnti2, Benedetta Piombanti1, Mirella Pastore1, Jessica Iorio1, Giulia Lori1, Clelia Peano1, Javier Gibella1, Monika Lewinska4, Giovanni Di Maira4, Matteo Ramazzotti1, Ivan Orlandi2, Paola Chiarugi1, Fabio Marra1.

**1University of Florence; 2University Milan-Bicocca; 3Humanitas Institute; 4University of Copenhagen**

Email: chiara.raggi@unifi.it

**Background and Aims:** Accumulating evidence indicates cancer stem cells (CSC) as a key target in cancer. Although metabolic reprogramming is considered an important feature of cancer cells, little is known about metabolic regulation in CSC derived from cholangiocarcinoma (CCA). This study investigated the role of mitochondria-dependent metabolism and of the related signaling pathways in the maintenance of a stem-state in CCA.

**Method:** Stem-like subset was enriched by sphere culture (SPH) in established human intrahepatic CCA cells (HUCCT1, CCLP1). Extracellular flux analysis was examined by Seahorse technology. Mitochondrial membrane potential and mitochondrial mass were assessed by MitoTracker Red and MitoTracker Green, respectively. Glucose uptake was quantified by incorporation of (U-14C)-D-Glucose. Gene set enrichment analysis (GSEA) and correlation with overall survival (OS) (log rank/Mantel-cox statistics) and time to recurrence (TTR) (Gehan-Breslow Wilcoxon test) were carried out from a transcriptome database of 104 CCA patients.

**Results:** In contrast to parental cells grown as adherent monolayers (MON), metabolic analyses by Seahorse revealed a more efficient respiratory phenotype in CCA-SPH, due to mitochondrial oxidative phosphorylation. In addition, CCA-SPH retained high mitochondrial membrane potential and elevated mitochondrial mass, as well as over-expression of PGC-1α, a master regulator of mitochondrial biogenesis. In vitro targeting of mitochondrial complex I by metformin impaired the ability to form SPH, expression of CSC-associated genes, and genes related to pluripotency and epithelial mesenchymal transition. In an in vivo model in immunocompromised mice, growth of tumors derived from CCA-SPH was suppressed by metformin. Furthermore, PGC-1α silencing highly reduced the expression of stem-like markers in CCA-SPH, and reduced sphere-formation and cell invasion. Notably, GSEA analysis showed that patients with with high levels of mitochondrial complex II had a worse prognosis in terms of OS (p = 0.036) and TTR (p = 0.029). In addition, PGC-1α was significantly correlated with mitochondrial complex II and stem-like genes in CCA patients.

**Conclusion:** Our data indicate a pivotal role of mitochondrial oxidative metabolism in the biology of the stem-like subset in CCA.

**FRI502**
NEDD8 specific protease 1 (NEDP1) as a tumor suppressor in hepatocellular carcinoma

Marina Serrano-Macia1, Aymeric Bailly2, Aurelien Perrin2, Maghames Chantali1, Órsolya Leidecker1, Helene Trauchsesse1, Narao Goikotxea1, Rubén Rodríguez Agudo1, Sofía Lachiondo-Ortega1, Anton Gartner2, Teresa Cardoso Delgado1, Dimitris Xiromidas2, María Luz Martínez-Chantar1. 1CIC bioGUNE, Liver disease lab, Spain; 2CRBBM, CNRS, Univ. Montpellier, France; 3University of Dundee, Centre for Gene Regulation and Expression, College of Life Sciences, United Kingdom

Email: mlmartinez@cicbiogune.es

**Background and Aims:** Neddylation is a reversible posttranslational modification similar to ubiquitination that conjugates to target