THE *IN VITRO* RE-CONSTRUCTED HEPATIC MICROENVIRONMENT BOOSTS UP THE METASTATIC FEATURES OF HCT-116COLORECTAL CANCER CELLS

Alberta Bergamo[1], Sabrina Pacor[2], Emiliana Giacomello[3], Gianni Sava[2]
[1]Callerio Foundation Onlus, Trieste - Italy, [2]Dept. of Life Sciences, University of Trieste, Trieste - Italy, [3]Dept. of Medicine, Surgery and Health Sciences, University of Trieste, Trieste - Italy

Hepatic metastases are the main obstacle to a positive outcome in patients diagnosed with colorectal cancer (CRC). In fact, their eradication is made difficult because of their localization and refractoriness to the pharmacological treatment. This latter aspect is determined in particular by the microenvironment in which the malignant cells coming from the primary tumour take root, develop and grow generating the metastases. To get insights on the interplay between tumour cells and the hepatic microenvironment, we have set up an in vitro model of hepatic metastases from CRC. The model is based on CRC cells (HCT-116or HT-29cell lines) cultured in an hepatic microenvironment constituted by proteins (i.e. fibronectin and/or collagen IV) of the extracellular matrix (ECM) as structural components, and by soluble factors secreted by the IHH hepatic cell line. This re-constructed hepatic microenvironment, besides inducing a metastasis-favouring behaviour, such as tumour cell adhesion on fibronectin and ECM invasion, is able to affect the apoptotic/necrotic events taking place during tumour cell settlement in the liver. Flow cytometry analysis of the cells adhering to the substrate, using the DiOC6(3) probe, sensitive to the membrane mitochondrial potential and propidium iodide (PI), freely entering the damaged cells, clearly indicates a wellness advantage of the cells settled in the re-constructed hepatic microenvironment in comparison to controls and to cells that have not adhered and are still floating in the supernatant medium. Adherent cells, cultured in the hepatocyte-conditioned medium, show the morphological features of malignant and invasive cells with a more elongated "mesenchymal-like" cell shape, as determined by classical and fluorescence microscopy, and a different ability to form colonies, measured with a clonogenic assay. HCT-116cells settled in the hepatic microenvironment display their mitochondria arranged in cords. These organelles are more numerous and occupy a larger space within the cell body in comparison to mitochondria of cells adherent to the non-specific substrate poly-L-lysine. Mitochondria are a pivotal hub in cellular behaviour regulating cell life/death and determining metastatic properties through key signalling mediators, in particular the reactive oxygen species (ROS). ROS production by mitochondria is influenced by the microenvironment also through interactions of the integrin ligands with the corresponding ECM proteins, and we know that a5b1integrin signalling is activated in HCT-116cells adhering to fibronectin in the presence of the hepatocyte conditioned medium. Investigating whether and how ROS levels change in HCT-116cells in the re-constructed hepatic microenvironment would be crucial to clarify this phenomenon. Also, clues obtained from preliminary cell cycle analysis, indicating that the hepatic microenvironment induces a more resting state in CRC cells, noteworthy related to resistance to apoptosis and drug treatments, suggest the need of further experiments.
Overall, the set up of the in vitro model of hepatic CRC metastasis has the potential to explore the features of CRC cells metastasising to the liver at both basic and pharmacological level.

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