

The influence of hypoxia on podoplanin expression in cancer-associated fibroblasts (CAF) and its role in the progression of breast cancer

Tumor is a pathologic tissue including, in addition to cancer cells, a modified extracellular matrix, endothelial cells, blood and lymphatic vessels, immune and inflammatory cells as well as activated fibroblasts called cancer-associated fibroblasts (CAFs). Tumor microenvironment plays an important role in both tumor development and metastasis. However, the knowledge about individual components of the microenvironment is very limited. Podoplanin (PDPN), mucin-type transmembrane glycoprotein is expressed in tumor cells and CAFs, depending on the tumor type. Podoplanin induces platelet aggregation, which promotes metastasis. In vivo, its role in metastasis promotion has been demonstrated for breast cancer cells into lymph nodes. Here we show that it modulates the activity of the CCL21/CCR7 chemokine/receptor axis in a hypoxia-dependent manner. In the present model, breast cancer MDA-MB-231 cells express CCR7 surface receptor for CCL21 which is a potent chemoattractant able to bind to podoplanin. The role of CCL21/CCR7 axis in the adhesion of MDA-MB-231 breast cancer cells was reduced by hypoxia, as in the tumor environment. Cancer progression is strongly affected by the tumour stroma in which CAFs are characterized by distinct gene expression and properties from normal fibroblasts. They promote tumour growth, recruitment of endothelial progenitor cells and angiogenesis via secretion of stromal cell-derived factor-1. In breast cancer up to 80% of fibroblasts display the CAF phenotype. Here a podoplanin expressing model of CAFs made it possible to demonstrate the involvement of CCL21/CCR7 axis in the tumor cell-to-CAF recognition through podoplanin binding of CCL21. Podoplanin was induced by hypoxia and its overexpression undergoes reduction of adhesion, making it an anti-adhesion molecule in the tumor, in the absence of CCL21. Little is still known about podoplanin influence on cancer cells. In this view, microRNA, which control gene expression at post-transcriptional level are good candidates. MiR-21 is a key regulator of the oncogenic process, through its downstream target proteins among which the tumor suppressor phosphatase and tensin homologue deleted on chromosome ten, PTEN. We analysed the effect of miR-21, but also oncogenic and hypoxia dependent miRs: miR-210 and miR-29b, on podoplanin expression in fibroblasts in conditions mimicking the intra tumor microenvironment, i.e. in hypoxia. This points to crucial differences as compared to normoxia. Moreover we uncover the effect of podoplanin on

angiogenesis by endothelial cells colocalizing with CAFs expressing podoplanin and expression of most prominent proangiogenic factors. Podoplanin on CAFs has a direct impact on pseudo-tube formation into aberrant vascular network. It also increased migration of fibroblasts and endothelial cells in normoxia but this effect is annihilated by hypoxia while it does not impact breast cancer cells motility.