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Influence of tumor hypoxia on autophagy, epithelial to mesenchymal transition and invasive potential of pancreatic cancer stem cells

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Pancreatic adenocarcinoma is the deadliest cancer and currently the fourth most frequent cause of cancer-related deaths. The disease is characterized by the lack of early symptoms, extensive metastasis and high resistance to chemotherapy and radiation. Enhanced activity of NF-κB is commonly found in pancreatic cancer and dysregulated autophagy is suggested to be involved in cancer growth and progression. Increasing evidence suggests the presence of a rare population of highly tumorigenic cancer stem cells (CSCs) within pancreatic tumors. They are inherently resistant to conventional cytotoxic therapies. Pronounced hypoxia is present in pancreatic cancer tumors and contributes to aggressiveness, invasiveness and metastasis. Due to their high therapy resistance CSCs may survive hypoxic conditions and and start to metastasis in contrast to sensitive, more differentiated cancer cells, which undergo apoptosis under these conditions. Prior to metastasis, tumor cells need to undergo a process called epithelial to mesenchymal transition (EMT). It is a physiological process, in which epithelial cells lose their characteristics and gain mesenchymal properties. Recent reports have implicated EMT in a malignant conversion of transformed cells, which represents invasive or metastasizing properties in a variety of cancers and it could be induced by hypoxia. EMT and hypoxia are considered as crucial events favoring migration, invasion and metastasis of many cancer cells including pancreatic cancer. However, the effects of hypoxia on cancer stem-like cells are not clear. Thus, I attempted to identify molecular mechanisms leading to the progression of pancreatic cancer and analyzed strategies for therapeutic intervention.

I found that expression of hypoxia marker HIF-1α was associated with expression of EMT-related proteins (E-cadherin, Vimentin, Slug) in human pancreatic cancer

tissues derived from patients. In addition, there was co-expression of the hypoxia marker CA IX with Vimentin and Twist2 in tumor tissues from pancreatic, breast, kidney, lung, ovarian and prostate cancer patients. In vitro I used pancreatic cancer cell lines with a high (CSC^{high}: MIA-PaCa2, AsPC-1, Capan-1) or low amount of CSC characteristics (CSC^{low}: BxPc-3, Capan-2) to investigate the effects of hypoxia on autophagy, the expression of EMT genes (E-cadherin, Slug, Snail, Twist2, Vimentin, ZEB-1), cell migratory properties and NF-κB activity. My results reveal that hypoxia-starvation (H/S) enhanced clonogenic survival and migration of established pancreatic CSC^{high} cells, while more differentiated pancreatic CSC^{low} did not survive these conditions. Modulation of autophagy by inhibitors (G3-Methyladenine, Monensin) and activators (Rapamycin, Sulforaphane) re-sensitized CSC^{high} prevented migratory activity. Inhibition of lysosomal degradation by the antibiotic bafilomycin A1 (BAF) indicates that CSC^{high} cells lines have higher basal and H/S-induced autophagy than CSC^{low} cells. I also detected that low oxygen of 1% not only induced morphological changes with a typical fibroblastoid spindle-shape phenotype but also up-regulated EMT-related protein expression in both CSC^{high} and CSC^{low} cell lines. However, the morphological changes induced by hypoxia were less pronounced compared to the effects of TGF-β, which was characterized as a strong inducer of EMT. Next, I analyzed cancer cell migration in three migration assays (scratch assay, 3D extracellular collagen matrix and transwell assay) and detected that hypoxia stimulated migratory properties of pancreatic cancer stem-like cells and the stimulating effect was higher in CSC^{high} AsPC-1 than in CSC^{low} BxPc-3 cells. Furthermore, I examined whether CSC characteristics are enriched by hypoxia. I observed a slight increase in CSC characteristics after culture in stem cell medium and long-time exposure to hypoxia, while culture in normal growth media and short-time hypoxia had no effect. This result indicates that CSCs may be selected by a hypoxic tumor microenvironment. Recent studies have shown that the transcription factor NF-κB is essential for EMT in a highly relevant, metastasis-prone human cancer. Therefore, I examined whether induction of NF-κB by hypoxia is associated with EMT and plays a role in the migratory response of pancreatic cancer stem-like cells. I found enhanced expression of the NF-κB subunits c-Rel and Rel-A. Inhibition of NF-κB activity by PG490 (Triptolide) from Traditional Chinese Medicine or by specific siRNA toward

c-Rel reduced morphological changes in cells exposed to hypoxia, down-regulated the hypoxia-induced EMT and migration of pancreatic cancer stem-like cells. Thus, my study identified NF-κB as a pivotal regulator of the EMT process, which by itself is a critical prerequisite for metastasis. I further investigated whether the conventional anti-inflammatory agent aspirin and bioactive dietary substances with known inhibiting activities against NF-κB could also modulate hypoxia-induced EMT. I found that aspirin, Sulforaphane enriched in broccoli and EGCG enriched in green tea, inhibited hypoxia-induced NF-κB activity and reduced EMT marker expression.

In conclusion, my present work suggest up-regulated autophagy in CSC^{high} cells and this is involved in survival of pancreatic cancer stem-like cells in a condition of low oxygen and lack of nutrition. My data also indicate that tumor hypoxia induces EMT and migration of pancreatic cancer cells and this process is associated with NF-κB activity. Down-regulation of NF-κB by anti-inflammatory agents (Triptolide, Aspirin, Sulforaphane and EGCG) could overcome NF-κB-mediated EMT. The present work provides new insights into molecular mechanisms of hypoxia-induced metastasis in pancreatic cancer and suggests the use of anti-inflammatory agents or autophagy-modulating drugs for therapeutic intervention