

## Characteristics of invasive tumor cells in clear cell renal cell carcinomas

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The molecular mechanisms that lead to invasion of renal cell carcinoma (RCC) are not clear until now. As about 30% of the patients have metastases even at the time of diagnosis and the five-year survival rate is just about 70%, new insights in early metastatic processes are needed to better understand the conditions for invasion. We investigated the molecular characteristics of invasive tumor cells of clear cell RCC, which were currently invading blood vessels or fat tissue in the environment of the tumor. In a first approach nine cases were selected by an experienced pathologist (E.K.) using hematoxylin / eosin stained sections of formalin-fixed paraffin-embedded (FFPE) tissue. Immunostaining was done using primary antibodies for molecules driving or inhibiting invasion (MMP-2, -9, -14, EMMPRIN, RECK), for markers of the epithelial / mesenchymal phenotype (cytokeratin-19, E-cadherin, N-cadherin, S100A4, ZEB1, beta-catenin) and for renal cancer stem cells (CD105, CD44, C-MET, CXCR4). Depending on the marker, staining of the invading tumor cells was heterogeneous. Some single cells were positive for the epithelial marker cytokeratin-19. The epithelial E-cadherin as well as the mesenchymal N-cadherin congruently stained (often larger) areas of the tissue. S100A4 and ZEB1 showed nuclear staining in many cells which accumulated in distinct areas at the edge of the blood vessels. MMP-2 was expressed at the whole edges of blood vessels, but less in the invading cells. CD105 stained blood vessels and rarely single cells which need to be differentiated from microcapillaries. The other stem cell markers were not detected in the invading cells. The MMP inhibitor RECK appeared in occasional granular spots spread in the tumor cells. Beta-catenin as well as EMMPRIN were widely expressed at the plasma membrane of tumor cells with extremely rare beta-catenin staining in the cytoplasm. Staining of the tumor and invading tumor cells seemed to be equal. In contrary, MMP-9 and MMP-14 levels were higher at the edge of the tumor mass and in the invading tumor cells. Heterogeneous expression of the majority of the markers points to different cell types within the population of invading tumor cells, apart from tumor-supporting cells. Epithelial-to-mesenchymal transition does not seem to play a role in invading clear cell RCC compared to the original tumor, or it is a temporally short phenomenon which could not be recognized in these samples. Elevated expression of MMP-9 and MMP-14, however, could indicate an important regulation which will be further examined.