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Characterization of highly invasive breast cancer cells using novel catulin reporter system

Abstract

Breast cancer is currently the most widely diagnosed cancer in women worldwide. Triple negative breast cancer has the poorest survival ratedue to high metastatic potential and no targeted treatment is available at this time. EMT is a process playing a pivotal role during development, however it is also associated with cancer progression and metastasis. It has been implicated that this phenomenon is non-binary, meaning that there may be some interstates of cells between epithelial and mesenchymal features possessing cancer stem-like properties. In breast cancer progression, loss of intracellular adhesion is crucial for metastatic disease. In the process of EMT, progressive downregulation of apical and basolateral, epithelial specific proteins is accompanied by re-expression of mesenchymal specific proteins. The switch results in increased motility of the cells. It was observed that decrease in the expression of the epithelial protein α-catenin correlated with an increase in the expression of the α-catulin protein and EMT. In addition, the expression of α-catulin was increased in the cells at the invasive front of head and neck squamous cell carcinoma. However, the function of α-catulin in the progression of breast cancer has not been studied so far.In this dissertation, I first focused on reviewing the published data regarding poorly described protein α-catulin. It is a member of the vinculin superfamily proteins. I showed high homology in sequence between α-catulin, α-catenin and vinculin. I described the limited yet representative number of binding partners of α-catulin like Lbc Rho GEF, dystrobrevin, IKK-β, NEK kinase. α-catulin has been shown to be important in inflammation, apoptotic resistance, cytoskeletal reorganization, senescence resistance, cancer progression, and EMT. α-catulin plays a pivotal role during embryonic development as it is necessary for proper neurulation. In cancer progression α-catulin has been shown to be upregulated in highly invasive breast, lung, and prostate carcinoma. Despite multiple reports describing α-catulin as an important factor contributing to cancer cell migration and invasion, the exact molecular mechanism leading to this phenotype remains unclear.

In my second publication, I showed that α-catulin is expressed in human breast cancer samples, and its expression correlates with the cancer progression. Moreover, when I knocked down α-catulin, invasive potential of cells in 3D model was highly decreased. Then I developed a novel reporter catulin system to track highly invasive breast cancer cells, and I was able to isolate those cells from xenograft transplants and perform RNAseq analysis. Expression of catulin in the invasive cancer cells correlated with the expression of genes involved in migration, and also could be important in the modulation of adhesive properties of cancer cells and their interactions with the vasculature. Immunohistochemical staining of xenographic tumors confirmed the results obtained by RNA sequencing. Thus, I demonstrated that the catulin reporter system not only labels invasive tumor cells, but also a rare population of highly plastic tumor cells that express endothelial markers such as MCAM and participate in vascular mimicry.Participation of catulin expressing tumor cells in forming vascular structures enables a flow of nutrients to the tumor mass and may facilitate the entry of cancer cells into the vascular system leading to the metastatic spread. When simultaneously knocking down α-catulin I observed modulation of adhesive properties of tumor cells and a high decrease in the invasive and cancer stemness potential, what resulted in decreased breast cancer stemness marker – CD44. It means that by utilizing a novel catulin reporter system, I could isolate and characterize highly invasive breast cancer cells of high plasticity that participate in vascular mimicry and metastasis.

Concluding, α-catulin may play a pivotal role in the interaction between cancer cells and the microenvironment, modulating the adhesive properties of cancer cells. Increased plasticity of cancer cells highly expressing α-catulin may be of high demand for the cells to change into endothelial like and participate in vascular mimicry and then in metastasis. In this dissertation, I clearly show that α-catulin is crucial in breast cancer tumorigenesis and the invasiveness process. It is clearly a specific marker of plastic cancer cells undergoing different intermediate EMT states. Deciphering the complex nature of α-catulin may lead to finding potentially novel therapeutic targets in various types of carcinomas.