Head and neck squamous cell carcinoma (HNSCC) that develops from the tissue that is lining the oral, nasal cavity and throat represents the sixth most common cancer in the world, with more than half a million cases diagnosed each year. Mortality from this disease with a 50% 5-year survival rate remains high due to the development of distant metastases and the emergence of eventually inoperable local and regional recurrences that have low responsiveness to radiation or chemotherapy. Why some cancer cells are more resistant or prone to invade surrounding stroma is still unclear. The cancer stem-cell model suggest that within the tumor there is a small population of cells named cancer stem cells (CSC) that have potential to reestablish the tumor and is more resistant to currently available treatments and is responsible for cancer metastasis. The biggest challenge is to isolate this specific population of cancer stem cells in order to better characterize them and develop potential new, specific treatments. So far different combination of cancer stem-cell markers have been used to enrich for cells with higher tumorigenic potential, however difficulty in reproducing cancer stem-cell markers from patients and lack of lineage tracing to delineate the fate of CSCs progenitors has been a com¬mon problem in solid-cancer studies. In addition signaling pathways that govern the phenotypic state of HNSCC remain unclear. In addition some recent discoveries suggest that non-CSCs can acquire CSC-like activity which correlate with epithelial to mesenchymal transition (EMT) emphasizing the greater plasticity of CSCs. In this scenario cell surface markers may not reflect the dynamic changes of CSCs. Therefore in our project we used a self-renewal gene tracking strategy to isolate populations of CSCs from HNSCC upon Oct-4 expression. We showed that Oct4GFP+ population has high tumor initiating potential confirming their CSC potential. Interestingly we also showed that Oct4GFP- cells are capable of plastic transition back to GFP+ state. It is suggested that epithelial to mesenchymal transition (EMT) correlates with the plastic stem cells behavior of cancer cells. However, it’s difficult to observe cells going through EMT in primary tumor due to transient and reversible nature of this process. Therefore on the basis of our recently published data we developed second reporter system Catulin-GFP that allows us for the first time to isolate and characterize in vivo a small population of invasive cancer cells and test their cancer initiating potential and relation to Oct4GFP+ cells.

We propose to use two novel reporter systems developed in our laboratory to unravel biological properties and signaling pathways that govern the highly plastic phenotypic states of oral CSCs. We have identified so far a molecular signature of SCC Oct4 GFP+ cancer initiating cells and Catulin-GFP invasive cancer cells. We will perform series of studies to directly test which of those molecules/pathways are critical for maintenance of oral CSCs, the metastatic spread and the transition between non-CSCs and CSCs. A unique component of our studies is the availability of reporter systems that will reflect the dynamic phenotypic states of CSCs, invasive cancer cells and non-CSCs. From a future therapeutic standpoint, this grant will have a strong impact since the high plasticity within cancer cells suggest that in order to effectively target oral cancer we have to develop strategies that not only eradicate CSCs but also eliminate non-CSCs to CSCs transition.