

PhD Concluding Seminar

Tumor Microenvironment-Driven Reprogramming of Luminal-A Breast Tumors Towards a Metastatic Phenotype

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Luminal A is the most common subtype of breast cancer, and though mostly is considered as the subtype with best prognosis, in some cases patients develop metastases and the prognosis worsens. While Luminal A tumors share some molecular patterns, which define them as Luminal-A, their tumor microenvironment (TME) may differ and have vast implications on disease progression. As part of the TME, we chose to focus on three major pro-metastatic factors that are abundant in Luminal A tumors and represent three different arms of the TME: estrogen, TNF α and EGF (termed herein "TME Stimulation"). In past research done in our lab, we have shown the synergistic effect of these three factors on Luminal A cells, which led to enrichment of cancer stem cells (CSCs) that were predominant elements in metastasis formation.

Our overall aim of the current study was to further elucidate TME Stimulation-driven impacts on pro-metastatic properties and on CSC enrichment in Luminal A breast tumor cells. Furthermore, we aimed to identify the molecular mechanisms involved in these processes and their potential therapeutic implications.

To this end, we investigated pro-metastatic properties promoted by TME Stimulation in Luminal A cell lines and found that TME Stimulation promotes intrinsic tumor phenotypes (CSCs enrichment, and epithelial-to-mesenchymal transition (EMT)-related processes) as well as TME-interacting features (PD-L1 expression and CXCL8 secretion).

Next, following information obtained by RNAseq analyses of TME-stimulated CSCs and Non-CSCs, we found that TME Stimulation has induced the activation of S727-STAT3, Y705-STAT3, STAT1 and p65. Upon TME Stimulation, stattic (STAT3 inhibitor) usage demonstrated that Y705-STAT3 activation negatively controlled CSC enrichment and EMT traits, while inducing CXCL8 and PD-L1 expression. However, STAT3 knock-down (siSTAT3) had no effect on these functions; in terms of CSC enrichment, p65 had down-regulatory roles that compensated for the loss of an entire STAT3 protein. These results were further validated using a Y705A-STAT3 variant alongside p65 knock-down. These findings led to the conclusion that Y705-STAT3 and p65 acted additively in limiting CSC enrichment, in the context of TME Stimulation.

Lastly, we aimed to delineate the functional and clinical relevance of the regulatory roles of STAT3 and p65 on CSC enrichment. We found that Y705A-STAT3 variant + sip65 under conditions of chemotherapy has led to enrichment of chemo-resistant CSCs. Furthermore, Clinical data analyses revealed an inverse correlation between Y705-STAT3 + p65 phosphorylation and CSC signature in Luminal A patients, and connection to improved disease course.

Overall, we find regulatory roles for Y705-STAT3 and p65 in TME-stimulated Luminal A tumors, with the ability to limit CSC enrichment. These findings raise concerns about using inhibitors of STAT3 and p65 as therapeutic strategies in the clinic.