Phosphorylation-mediated Regulation of the Rubicon Interactome

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Background and Hypothesis: HER2+ breast cancer uses the human epidermal growth receptor 2 (HER2) protein to grow. As an aggressive form of breast cancer, it accounts for ~20% of all breast cancer diagnoses worldwide. Previous studies show Hormonally Up-regulated Neu-associated Kinase (HUNK) is up-regulated in HER2+ breast cancer. HUNK plays an important role in the cellular process of autophagy, which recycles cellular components into new usable components. Increased autophagy, through HUNK activity, allows for improved survival and proliferation of cancer cells. Autophagy is mediated through a complex of proteins, including Beclin-1, HUNK, Rubicon, UVRAG, and Vps34. In this complex, Rubicon is responsible for the inhibition of autophagy. However, phosphorylation of Rubicon by HUNK inhibits this function, thus promoting autophagy. This phosphorylation of Rubicon is hypothesized to cause its dissociation from the autophagy proteins. To further understand this, we looked at the interaction of Rubicon with UVRAG and SQSTMI in the presence and absence of HUNK, to validate this complex as a means for regulation of the Rubicon interactome.

Experimental Design: 293T cells were cultured and transfected with pcDNA, wt-Rubicon, wt-Rubicon+wt-HUNK, or wt-Rubicon+ kinase-deficient HUNK-K91M plasmids. These cells were lysed to perform whole cell extraction and immunoprecipitation. The proteins of interest were visualized via Western blots.

Results: Autophagy proteins, UVRAG and SQSTM1, were unable to be visualized after multiple attempts to optimize the protocol. However, it was shown that when HUNK kinase activity was eliminated in HUNK-K91M there was a decrease in HUNK interaction with Rubicon.

Conclusion and Potential Impact: Further studies should be done to assess whether HUNK kinase activity is required to stabilize binding to Rubicon. Additionally, alternative techniques, like microscopy or HER2+ cancer cells, should be used to visualize the interaction between Rubicon and autophagy proteins. Understanding the Rubicon interactome and the role of HUNK phosphorylation is imperative for better understanding of HER2+ cancer and effective treatments.