**Identifying Autophagy-induced phosphorylation sites on the Hsp70 molecular chaperone**

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Hsp70 is an evolutionarily conserved molecular chaperone responsible for folding proteins, importing proteins into organelles, recovering proteins from aggregations, and assembling multi-protein complexes. Hsp70 overexpression promotes tumor cell proliferation and poor prognosis in cancer patients. Deciphering the regulation of Hsp70 allows us to understand the cell at a basic level and reveal novel anti-cancer therapies.

Autophagy is an important intracellular degradation system whereby cytoplasmic proteins are degraded in the lysosome. There are three independent types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Tumor cells utilize autophagy as a means of survival, suggesting that the inhibition of autophagy could be used to develop anti-cancer treatments. While Hsp70 plays a major role in CMA, there has been *no established function for Hsp70 in macroautophagy*. Recent research from our lab has demonstrated phosphorylation of Hsp70 by the Ulk1 macroautophagy kinase *in vitro.* Further, by using mass spectrometry we identified ten potential Ulk1 phosphorylation sites on Hsp70. This opens the intriguing possibility that *Ulk1 may regulate Hsp70 activity in CMA, connecting macroautophagy and CMA*.

In an effort to validate the Ulk1-mediated phosphorylation sites on Hsp70 detected by mass spectrometry, we expressed and purified recombinant Hsp70 peptides containing putative Ulk1 phosphorylation sites fused to GST. We used these fusions as substrates in kinases assay with Ulk1 and assessed Hsp70 peptide phosphorylation by PhosTag gel analysis. We found that Ulk1 phosphorylates Hsp70 peptide with Threonine-111 and Threonine-120. Future studies will use site-directed mutagenesis to determine the role of these phosphorylation sites *in vivo.*