Does HYPE AMPylation Affect BiP Translocation to the Cell Surface?

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My project is an assay of the involvement of HYPE (huntingtin yeast-interacting partner E) in the translocation of BiP (binding immunoglobulin protein) to the cell surface. BiP is the master chaperone of the UPR (unfolded protein response), a pathway that is critical to maintaining the survival of cells, and which is downregulated in cancer cells. In addition, when under cellular stress, such as in those same cancer cells, BiP is translocated to the cell surface. This translocation is likely linked to the regulation of BiP. HYPE is an incredibly important Fic adenylyltransferase which naturally AMPylates BiP in cells, and this modification of BiP is essential to the regulation of the UPR and therefore is also critical to cancers. HYPE is intrinsically inhibited by its 234th residue, however, and in order to test the effect of over-AMPylation on BiP, a constitutively active mutant must be made by mutating the glutamate located there to a glycine (E234G HYPE). As a control, a completely inactive mutant is made by also modifying the catalytically-essential histidine at residue 363 to an alanine (E234G/H363A HYPE). With these mutants, it is possible to test HYPE-mediated AMPylation of BiP in cells. Since under cellular stress, the UPR is downregulated, BiP is likely AMPylated when it is translocated to the cell surface, I propose that transfecting an overactive mutant of HYPE will increase BiP translocation, while transfecting an inactive mutant will not affect or possibly even decrease BiP translocation. This has implications for treating cancer, as if more of the BiP is unmodified, the UPR will be upregulated, leading to far greater mortality in cancer cells. After analyzing the fluorescence images, the preliminary data seem to support my hypothesis.

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