Heat shock proteins, such as Hsp90, are upregulated in Embryonic stem cells (ESCs) to buffer cytotoxic effects, maintain proteostasis, and enhance cell survival. Unsurprisingly, treatment with Hsp90 inhibitors induces apoptosis in ESCs. Recently a significant subpopulation of Hsp90 has been shown to exist in a large, stable multi-chaperone complex in ESCs referred to as the epichaperome. PTMs are known to regulate the function of Hsp90, shown to exist in a large, stable multi-chaperone complex in ESCs referred to as the epichaperome. PTMs are known to regulate the function of Hsp90, however little is known about the major Hsp90 PTMs under stressed states and how these regulate the chaperone cycle. In this study we identified two phosphorylation sites that were enriched in higher-order Hsp90 chaperone complexes and characterized their function in regulating the interaction of cochaperones with Hsp90. E14, a line of mouse embryonic stem cells (mESCs), were used as our model. Cells were cross-linked using DSS before isolating Hsp90 by immobilized inhibitors of Hsp90, both PU-H71 and GA, and LC-MS/MS analysis was used to compare the two experimental conditions. The results were consistent with previous findings showing that PU-H71 has a greater affinity for complexed Hsp90, whereas GA binds greater to the uncomplexed chaperone. Several phosphopeptides were identified from the PU-H71 pull-down with phosphorylation on Ser226 and Ser255 for Hsp90. However, neither of these phosphopeptides were identified in substantial quantity for the GA pull-down samples, possibly indicating that the two inhibitors target Hsp90 subpopulations in distinct posttranslational states. Ion intensity for the phosphopeptides were then compared between the PU-H71 purified Hsp90 and the unmodified ESC lysates, yielding significant enrichment in the PU-H71 pull-down exhibited by sixfold increase in phosphorylated S255 abundance than that of the ESC lysate. Analysis of cross-linked Hsp90 bound to PU-H71 or GA indicates that PU-H71 binds to Hsp90 in a more closed conformation whereas GA binds to Hsp90 in a more open conformation. Effects of phosphorylation on cochaperone and client binding were performed utilizing phosphomimetics or null mutants. Plasmids expressing Wild-Type or mutant Hsp90 tagged with mCherry enabled capture by immunoprecipitation to analyze protein-protein interaction. SILAC quantitation demonstrated upregulation of several binding partners with Hsp90 with the phosphomimetic, such as Ahsa1, Cdc37, and FKBP4.

A.30 Deciphering the mechanisms regulating GLUT4 translocation in skeletal muscle by spatial proteomics

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Insulin-stimulated glucose uptake into skeletal muscle is mediated by the Glut4 transporter. Glut4 resides in specialized vesicles in the cytoplasm and trans-localizes to the plasma membrane to enable glucose uptake by cells. Fusion of Glut4-containing vesicles with the plasma membrane is regulated by stimuli, such changes in glucose concentration. These stimuli signal through kinase systems downstream of the insulin receptor or through AMPK. The mechanisms of Glut4 vesicle generation, translocation and fusion to the plasma membrane are still not well known. A better understanding of this biology is needed for increasing glucose uptake into muscle as a therapeutic strategy to treat diseases, such as insulin resistance and metabolic syndrome. In this study we adapted a proximity-labeling proteomics strategy to determine the Glut4 vesicle composition in skeletal muscle cells. We fused Glut4 to the Apex2 peroxidase and expressed the fusion protein in human skeletal muscle cells. In presence of the Apex2 substrate biotin-phenol and the H2O2 activator, Apex2 biotinylates proteins surrounding Glut4 within a radius of ~20nm. We then isolated biotinylated proteins on streptavidin beads and analyzed them by high resolution mass spectrometry. Using this approach, we detected 90% of the known mediators of the Glut4 translocation machinery (including e.g., Lrp1, Rab11, Rab14, Snap23). We also identified candidate proteins not previously reported to be part of the Glut4 vesicle translocation machinery. Utilizing this system under conditions of AMPK-stimulated glucose uptake in these cells, we identified 120 proteins as candidate targets for glucose uptake modulation. We are now validating these proteomic hits by high content imaging, measuring Glut4 translocation to the plasma membrane after depleting the proteins from cells by siRNA. With the data generated from this approach, we aim to identify new modulators of glucose uptake into skeletal muscle, which may yield new targets for the treatment of insulin resistance and related diseases.