Biological Pathway Analysis of Mass Spectrometry Results Reveals a Temporally Induced Citrullinome That Regulates Gonadotrope Cell Function

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Peptidylarginine deiminases (PADs or PADIs) catalyze the conversion of positively charged arginine to neutral citrulline, which alters target protein structure and function. Our previous work established that gonadotropin releasing hormone agonist (GnRHa) stimulates PAD2-catalyzed histone citrullination to epigenetically regulate gonadotropin gene expression in the gonadotrope derived LβT2 cell line. However, PADs are also found in the cytoplasm. Given this, we used mass spectrometry (MS) to identify additional non-histone proteins that are citrullinated following GnRHa stimulation and characterized the temporal dynamics of this modification. Our preliminary data reveals temporally citrullinated proteins associated with distinct cellular pathways following 10 and 30 minutes of GnRHa stimulation. We found citrullinated proteins associated in cell proliferation, transcriptional activation, and the RAF/MEK/ERK signaling cascade, all of which are vital to gonadotrope function. Not only do we see signaling cascades, but we also find the cytoskeleton and proteins facilitating vesicle release as targets of citrullination within 10 minutes of GnRHa stimulation. Our previous results in primary pituitaries suggest engagement of the cytoskeleton is necessary for the spatial positioning of gonadotropes towards vasculature and LH release. To confirm specificity, we utilized the pan-PAD inhibitor biphenyl-benzimidazole-Cl-amidine (BB-ClA), which results in decreased levels of citrullination in our identified protein targets. Taken together, our data has identified novel PAD substrates in gonadotropes and provides a temporal spectrum of the collective cellular processes regulated by PADs.