Discovering substrates of PRMT5 and CDK4/6 in human melanoma cells with antibody-based PTM peptide specific enrichment strategies.

Mass Spectrometry

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Introduction: The targeting of the cell cycle dependent kinases CDK4/6 with the small molecule inhibitor palbociclib has proven to be a successful anticancer treatment for a number of solid cell cancers. Moreover, inhibition of the protein methyl transferase PRMT5, which catalyzes the formation of symmetric dimethyl arginine (SDMA) with the compound GSK3326595 has also been identified as a promising anticancer treatment. This study identifies and quantifies the reduction in phosphorylation for the targets of MAPK/CDK kinases inhibited by palbociclib, and the SDMA sites down regulated with GSK3326595 inhibition of PRMT5 treatment.

Methods: The human melanoma cell line A375 was treated with the vehicle control (DMSO), the CDK4/6 inhibitor Palbociclib, the PRMT5 inhibitor GSK3326595, or both compounds together for 24 hours in triplicate experiments. The sites of MAPK/CDK phosphorylation and PRMT5 methylation were identified by sequential enrichment of tryptic peptides from the treated cells using antibodies specific for substrates of MAPK/CDK phosphorylation and SDMA containing peptides prior to LC-MS/MS. Changes in peptide abundance between treated and control was determined by label-free quantitation of peptide intensity in the MS1 channel using Skyline.

Results: Following SDMA specific peptide enrichment and LC-MS/MS we identified over 300 SDMA sites on over 250 proteins, with greater than 30 sites showing a four-fold or more reduction in abundance with GSK3326595 treatment. The use of MAPK/CDK substrate specific antibodies combined with label-free quantitation showed that Palbociclib treatment led to the significant reduction in phosphorylation of hundreds of sites of S/T threonine phosphorylation on proteins cell wide.