A model system to study Kabuki syndrome utilizing KMT2D histone H3 lysine 4 methylase proteomics during cranial neural crest osteoblast differentiation.

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Abstract

Kabuki syndrome is a human disorder that presents with characteristic craniofacial phenotypes including facial hypoplasia, a depressed nasal tip, ocular abnormalities, and cleft palate. Kabuki syndrome results from mutations in KMT2D and KDM6A enzymes that regulate histone methylation. KMT2D is a histone H3 lysine 4 methylase involved in enhancer activation. We have previously modeled facial dysmorphism of Kabuki syndrome in the mouse utilizing neural crest specific deletion of KMT2D. Neural crest cell (NCC) specific deletion of KMT2D results in deficiencies in osteoblast differentiation that lead to alterations in the formation of anterior facial bones. We have now developed KMT2D mutations in neural crest cell culture to identify functional mechanisms of histone methylation regulates osteoblast differentiation. KMT2D mutant neural crest cells exhibit strong reductions in H3K4 mono and di-methylation. When placed in osteogenic media, the KMT2D mutant stem cells are deficient in differentiating to osteoblast lineages. To further understand KMT2D mechanisms, we propose to use a knock in system for proximity biotinylation to identify associated KMT2D co-factors during osteoblast differentiation. We have inserted the APEX peroxidase on to the endogenous KMT2D carboxy-terminus. Through oxidation of phenol derivatives, APEX will catalyze the formation of reactive unstable radicals, which have a high affinity for electron-rich amino acids. This will result in proximal protein labeling with biotinylation. Proximity-dependent biotinylation followed by streptavidin capture and mass spectrometry allows for isolation and identification of protein complexes interacting with KMT2D in differentiating neural crest cells. We conclude that KMT2D function is required for H3K4 methylation, NCC osteoblast differentiation, and facial bone formation. These collective proteomics experiments will lead to a better understanding KMT2D cooperation with chromatin and transcription factor complexes during osteoblast differentiation.