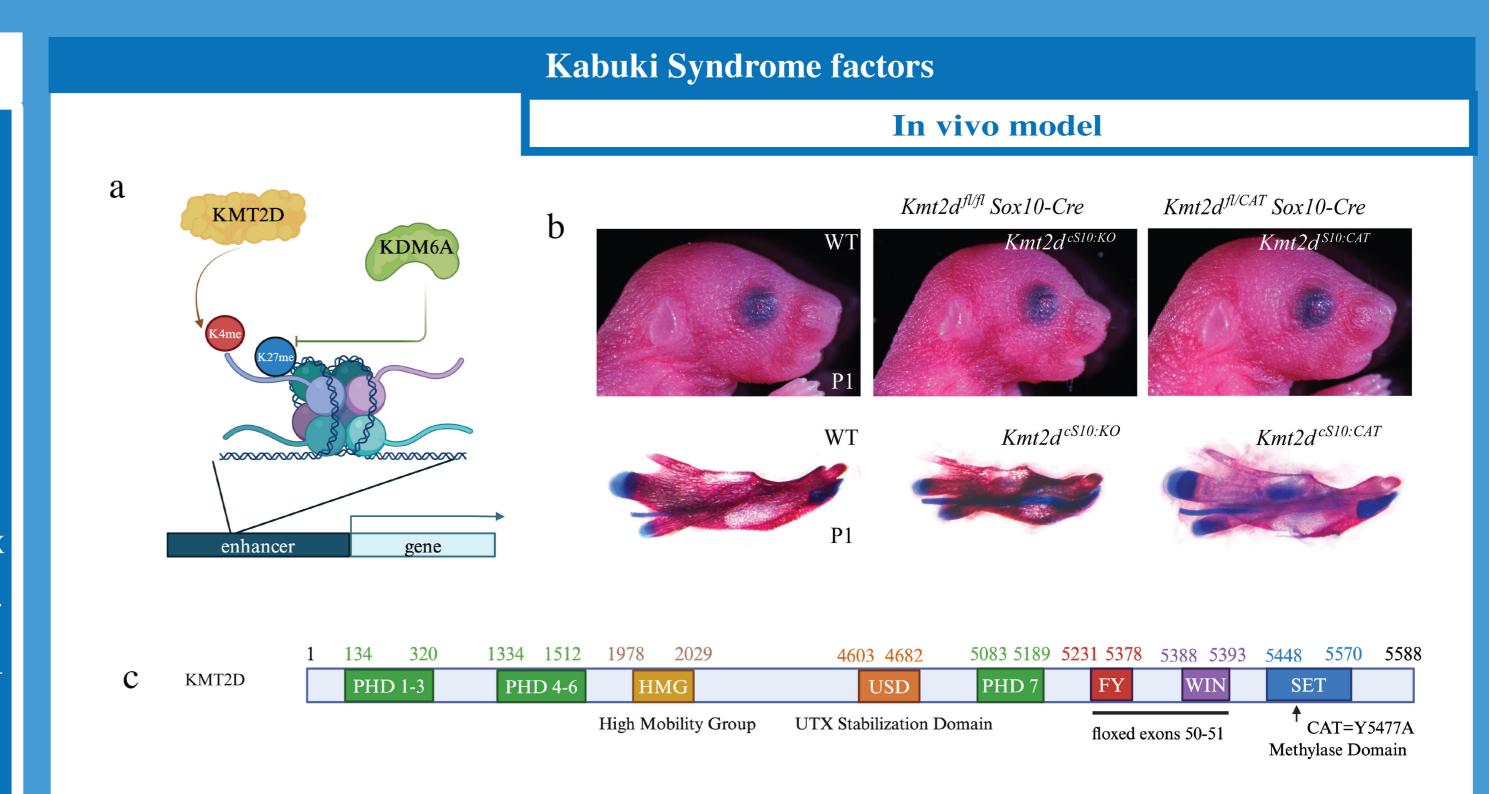


A model system to study Kabuki syndrome utilizing KMT2D histone H3 lysine 4 methylase proteomics during cranial neural crest osteoblast differentiation.

Sara Vardabasso and Karl B. Shpargel, University of North carolina at Chapel Hill, Department of Genetics

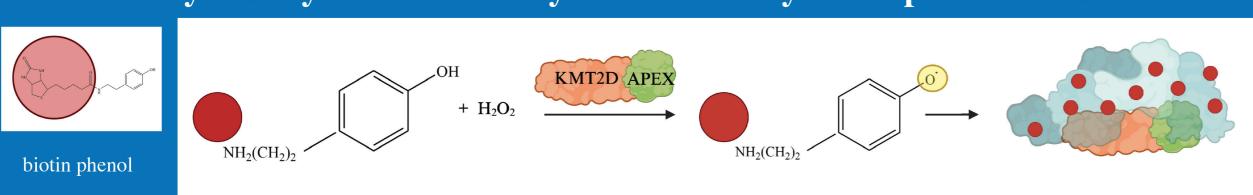
ABSTRACT

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 dentify 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.



a) KMT2D and KDM6 act as histone regulatory factors (methyltransferases and deacetylase, respectively) on the H3 lysine tail. b) From left to right: wild type, Kmt2d neural crest knock out, and point mutation for Kmt2d catalytic site. Point mutation of catalytic site does not prevent KMT2D functionality. c) KMT2D functional domains.

Proximity Biotinylation to identify KMT2D catalytic indipendent mechanism



Biotin phenol is oxidized by APEX KMT2D. The reactive radical interacts with proteins in proximity of KMT2D.

Switch to an exogenous Turbo ID system dox Turbo ID HMG-USD biotin

System design transfection G418 select 226 bp guide RNA target

a P2A

System design 1978 2029 4603 4682

KMT2D^{HMGUSD}

HMG-USD-no dox

10³

99.44%

 10^{3}

Turbo-GFP-H

(10³)

10⁵

GFP+

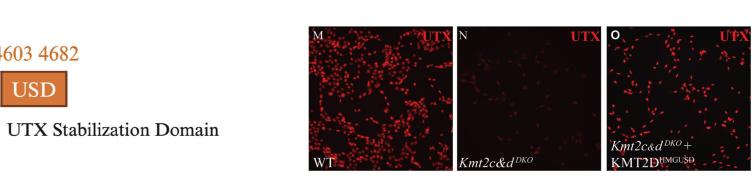
0.56%

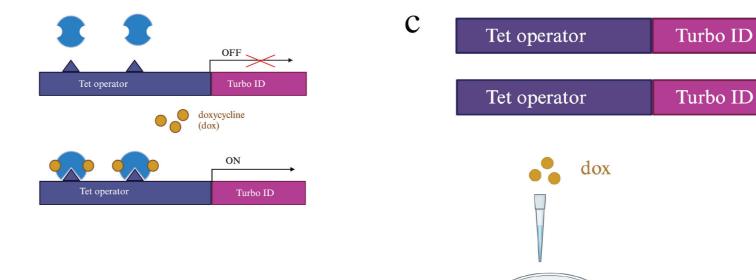
10⁵

Turbo-GFP-H

High Mobility Group

Turbo ID HMG-USD catalyzes the covalent attachment of biotin to the proteins in proximity with KMT2D





HMG-USD-1µg/mLdox

10³

47.33%

10¹

Turbo-GFP-H

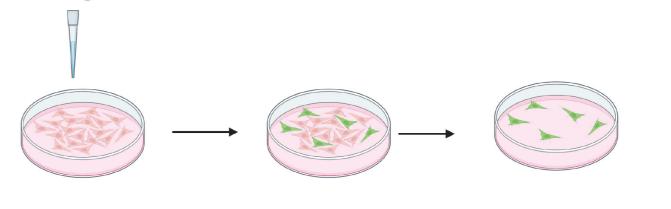
10¹

10⁵

52.67%

10⁵

Turbo-GFP-H



No bio

NLS HMG-USD

Dox +

30 min bio

NLS

GFP

GFP

a) UTX levels are restored when HMG-USD regions are re-inserted after Kmt2c&d DKO. b) Doxycyline binds to the reverse variant of the repressor, promoting gene expression when presenT c) Model for Turbo KI with HMG-USD and GFP control. Experimental design: dox injection and gene activation promotes GFP expression, that is used for cell selection.

30 min bio

Turbo-HMG-USD

647-streptavidin

KMT2D **APEX** biotin phenol no biotin phenol SA-beads KMT2D APEX KMT2D APEX **APEX**

System test

a) Design for Apex knock in through CRISPR in NCC Kmt2d gene and final product obtained. b) NCC transfection, and selectrion through drug resistance and mCherry expression levels c) PCR test for KI of 8 isolated cell lines. d) Immunofluorescence anti mCherry, anti Flag (in KI), and anti BRG1 (control) of WT VS APEX KMT2D e) Fluorescent streptavidin beads interacts with biotin-targeted proteins in KMT2D APEX and WT + biotin thynamide VS KMT2D APEX without biotin thynamide. f) Western blot anty ASH2L and anti RBBP5 (on input and streptavidin beads known component of protein complex in which KMT2D acts. KMT2D APEX did not enrich known interacting partners.

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usd-hi usd-hi ctl-hi usd-low □usd-neg usd-7hr 10³ 10⁵ 10³ 10⁵ 10³ 10⁵ Turbo-GFP-H Turbo-GFP-H Turbo-GFP-H

a) Quantitative analysis for GFP fluorescence in Turbo-HMG-USD-GFP treated with 1 µg/mL VS negative control b) Percent of GFP+ in Turbo-HMG-USD-GFP dox treated group VS controls. Left plott: 1 µg/mL dox 24 hr(usd-hi) VS no dox(usd-neg). Middle plot: 1 µg/mL dox 24 hr (usd-hi) VS 0.5 µg/mL 24 hrs (usd-low) VS 1 µg/mL ' hrs. Right plot: 1 μg/mL dox 24 hrs VS Turbo-GFP-control treated in the same conditions