Epigenome dysregulation resulting from NSD1 mutation in head and neck squamous cell carcinoma

Bo Hu
Department of Human Genetics, McGill University, Montreal, QC, H3A 1B1, Canada

Epigenetic dysregulation has emerged as a key mechanism of oncogenesis, thus demanding deeper insights into the epigenomic and transcriptomic consequences of mutations in chromatin modifier genes to develop targeted and effective treatments. We have recently (Papillon-Cavanaugh *et al.*, Nature Genetics 2017) identified mutations in histone methyltransferase gene NSD1 as the defining feature for a molecularly distinct subset of HPV(-) head and neck squamous cell carcinoma (HNSCC) – one of the most common and deadly cancers. Subsequently, our group (Weinberg *et al.* Nature 2019) and others have argued that the epigenetic mechanism underlying the tumorigenicity of NSD1-impaired cancers is a reduction in H3K36me2 that consequently prompts the global loss of DNMT3A-mediated DNA methylation. Here, we used multiple patient-derived primary HNSCC cell lines to generate a controlled tissue- and tumor-relevant setting for introducing NSD1 loss-of-function mutations via CRISPR-Cas9. We then applied (epi)genome-wide profiling approaches to dissect NSD1 mutations' mechanism of action in HNSCC and its downstream effects in an isogenic tumor context.

We demonstrate that loss of NSD1 in HNSCC directly results in drastic changes to the intergenic epigenetic landscape: reduction of intergenic – but not genic – H3K36me2, increased levels of intergenic H3K27me3, and decreased intergenic DNA methylation. We additionally show that the aforementioned changes disproportionally affect regions containing cis-regulatory elements, particularly distal intergenic enhancers, resulting in decreased levels of H3K27ac that presumably corresponds to diminished enhancer activity. Genes that are predicted targets of these compromised enhancers consistently exhibit lowered expression and are linked to pathways including interferon signaling and epithelial-to-mesenchymal transition – bridging the gap between NSD1 loss-of-function mutations and tumor-specific characteristics that have previously been reported in these cancers such as a paucity of immune-cell infiltration. Furthermore, we find strong support for findings from our cell line model through the application of comparable approaches to primary tumors profiled in TCGA**.**

Based on consistencies found across numerous isogenic CRISPR-Cas9 systems and validation from primary tumors, our model describes the loss of intergenic H3K36me2 domains in NSD1-KO cell lines, acting via an attenuation of H3K27ac and enhancer activity of the affected regions, leads to reduced expression of target genes within topologically associating chromatin domains. Considering H3K36me2's growing importance in both health and disease, these results help pave the way towards understanding its relationship with other epigenetic markers as well as higher-level biological functions.