Epigenetics

Major finding: In adult GBM, MLL5 reduces H3.3 occupancy, globally reorganizes chromatin, and promotes self-renewal.

Concept: MLL5-induced repression of H3.3 in adult GBM recapitulates the effects of H3.3 mutation in pediatric GBM.

Impact: Small-molecule epigenetic inhibitors may block the self-renewing capacity of GBMs.

MLL5 PROMOTES SELF-RENEWING GLIOBLASTOMA GROWTH VIA H3.3 SUPPRESSION

A large portion of pediatric glioblastomas (GBM) harbor mutations in the histone 3 variant H3.3 (encoded by *H3F3A* and *H3F3B*), which is replication-independent and associated with active chromatin. However, H3.3 mutations are rarely found in adult patients. In order to identify mutation-independent alterations in histone biology in adult GBM, Gallo and colleagues analyzed GBM DNA methylation profiles. Adult GBM self-renewing cells clustered with pediatric samples, and adult GBM cultures had reduced levels of H3.3, suggesting that epigenetic mechanisms may disrupt H3.3 in adult GBM self-renewing cells. Gene expression profiling identified mixed lineage leukemia 5 (*MLL5*, also known as *KMT2E*) as highly expressed in GBM and a putative regulator of H3.3. Expression of *H3F3B*, but not *H3F3A*, was inversely correlated with MLL5 expression, and MLL5 overexpression resulted in more compact chromatin at the *H3F3B* transcription start site, suggesting that MLL5 reduced *H3F3B* expression through chromatin alterations. In addition, MLL5 induced a global reduction in the activating mark histone H3 lysine 4 trimethylation and increased local chromatin condensation. Increased expression of H3.3 was associated with GBM differentiation, whereas MLL5 promoted self-renewal, supporting the idea that MLL5 enhances GBM cell self-renewal by decreasing H3.3 expression. *In vivo*, overexpression of MLL5 in an orthotopic mouse model of GBM increased tumor formation, and adult patients with high levels of H3.3 had better outcomes than patients with lower expression. Small-molecule inhibitors targeting the epigenetic regulators multiple endocrine neoplasia 1 and lysine (K)-specific demethylase 6B, which are coregulated with *MLL5*, reduced the self-renewing capacity of GBM cells. These findings indicate that MLL5 promotes GBM tumorigenesis and self-renewal by reducing H3.3 expression and reorganizing chromatin. This represents an epigenetic mechanism that phenocopies pediatric GBM driven by H3.3 mutations, and suggests that targeting epigenetic regulators may be effective in adult GBM. ■

Gallo M, Coutinho FJ, Vanner RJ, Gayden T, Mack SC, Murison A, et al. MLL5 orchestrates a cancer self-renewal state by repressing the histone variant H3.3 and globally reorganizing chromatin. Cancer Cell 2015;28:715–29.

Neuroblastoma

Major finding: A polymorphism in the *LMO1* super-enhancer protects against neuroblastoma.

Mechanism: The rs2168101 SNP reduces GATA3 binding to the *LMO1* super-enhancer, repressing *LMO1* expression.

Impact: Super-enhancer–mediated *LMO1* expression may be clinically exploitable for neuroblastoma treatment.

A POLYMORPHISM IN THE *LMO1* SUPER-ENHANCER MEDIATES NEUROBLASTOMA RISK

LIM domain only 1 (*LMO1*) is a neuroblastoma oncogene that has been identified as a disease susceptibility locus in neuroblastoma genome-wide association studies. However, the causal DNA sequence variation that underlies the association of *LMO1* with neuroblastoma tumorigenesis has not been defined. Using fine-mapping of germline *LMO1* SNPs, Oldridge, Wood, and colleagues identified a protective single-nucleotide polymorphism (SNP), rs2168101 G>T, as the most significantly associated SNP within the *LMO1* locus. This association was confirmed in additional European neuroblastoma cohorts; however, the rs2168101 SNP is rare or absent in African populations, suggesting that it is a newly evolved polymorphism and may partially explain why high-risk neuroblastoma is more prevalent in African-Americans. The SNP is located in a highly conserved active super-enhancer region as determined by DNase I sensitivity, p300 binding, and acetylation of histone H3 lysine 27, and mRNA sequencing of high-risk neuroblastoma tumors showed that *LMO1* was more highly expressed in G/G homozygous tumors compared with G/T tumors. Allelic imbalance was detected in rs2168101 heterozygous G/T neuroblastoma cell lines, with the G risk allele expressed more highly than the protective T allele. No T/T genotype tumors were observed in the high-risk neuroblastoma mRNA-sequencing cohort, further supporting its protective effect, whereas the G/G genotype was associated with decreased event-free survival in patients with neuroblastoma compared with the G/T and T/T genotypes. The rs2168101 SNP resides in a conserved GATA-binding motif, and chromatin-immunoprecipitation sequencing indicated that the SNP-associated T allele reduced binding of the GATA3 transcription factor to the *LMO1* locus. In G allele–expressing cell lines harboring the intact GATA motif, GATA3 knockdown decreased LMO1 expression and reduced cell growth, suggesting that GATA3 is important in regulating LMO1 expression in neuroblastoma. These findings provide a mechanism to explain the genetic association between the *LMO1* SNP rs2168101 and neuroblastoma risk through disruption of GATA transcription factor binding in the *LMO1* super-enhancer. ■

Oldridge DA, Wood AC, Weichert-Leahey N, Crimmins I, Sussman R, Winter C, et al. Genetic predisposition to neuroblastoma mediated by a LMO1 *super-enhancer polymorphism. Nature 2015;528:418–21.*