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Abstract Title : Mechanisms and biological roles of allele-specific DNA methylation in multiple myeloma

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Review Category: 651. Multiple Myeloma and Plasma Cell Dyscrasias: Basic and Translational

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Abstract Body

Background: Using whole-genome bisulfite sequencing (WGBS) we previously described gains of allele-specific DNA methylation (ASM) in cases of multiple myeloma, compared to peripheral blood mononuclear cells (PBMC) and normal B cells, linked to allele-specific losses of methylation in differentially methylated regions (ASM DMRs). These DMRs were often centered on SNPs that disrupt transcription factor (TF) binding sites, and sometimes co-localized with genome-wide association study (GWAS) peaks for MM risk. Here we utilize Oxford Nanopore long-read sequencing (Nanopore-SEQ) applied to an expanded case series to address mechanisms and biological roles of ASM in MM.

Methods: To map ASM while also assessing net methylation, chromosomal copy number aberrations (CNAs) and chromosomal translocations relevant to MM clinical risk categories, we carried out Nanopore-SEQ in 28 primary MM cases, with parallel analysis of paired PBMC samples from 23 of these cases and normal peripheral blood B cells from 6 individuals. For cross-validations, we utilized Illumina EPIC Beadchips to profile net methylation at 800K CpGs in a series of 50 MM cases, 16 of which were included in the Nanopore case series. Using a custom pipeline for visualizing ASM and associated SNP genotypes in genome browser tracks we assessed whether gains of ASM at MM GWAS loci show a consistent directionality among MM cases, and whether DNA methylation signatures for hyperdiploidy and for clinical high risk included loci affected by ASM. Lastly, we utilized CRISPR-Cas9-mediated site-directed mutagenesis to test the functional role of an MM GWAS SNP in producing an ASM DMR.

Results: The Nanopore series included 6 ultra-high risk (UHR) and 19 standard risk (SR) cases, while the EPIC cohort included 6 UHR and 37 SR cases. The Nanopore and EPIC cohorts comprised 14 hyperdiploid vs 14 non-hyperdiploid cases, and 21 hyperdiploid vs 29 non-hyperdiploid cases, respectively. Genome-wide analysis revealed a marked increase in the number of ASM DMRs in MM, with a median of 101,621 ASM-DMRs per case (range, 7,939–332,542), compared with 815 in PBMCs (range, 477–6,224) and 1,229 in normal B cells (range, 537–3,185). The ASM DMRs in MM cases were generally compact, with a median length of 283 bp, emphasizing their value for localizing functional SNPs in post-GWAS applications. Among 73 published supra-threshold MM GWAS loci, 70 harbored one or more strong ASM DMRs in one or more MM case in a 10-kb window centered on the GWAS lead SNP, and 28 of these DMRs directly overlapped the GWAS lead SNP. We next sorted the GWAS peaks to prioritize those that contained ASM DMRs seen in multiple cases of MM. Using heterozygous SNPs to distinguish alleles, recurrent ASM DMRs at rs4487645 (chr7, *DNAH11/CDCA7L*), rs877529 (chr22, *CBX7*), and other examples, showed consistent directionality of the epigenetic changes, with the informative cases showing losses or gains of methylation on the same alleles. In contrast, another group of loci, also analyzed using single heterozygous SNPs, showed apparently random directions of the acquisition of ASM among MM cases. However, our ongoing analysis of extended haplotypes suggests that some of these loci in fact have a nonrandom directionality of gains of ASM, dictated by the haplotypes in which they are embedded. Next,

by integrating EPIC array and Nanopore sequencing data, we derived methylation signatures for hyperdiploid and UHR MM and found that many of the top methylation marker loci for these clinically relevant subgroups, including *CDKN2A-CDKN2B* in UHR cases and *PARD3* and *CDKN1C* in hyperdiploid MM, also contained strong ASM DMRs. Lastly, CRISPR-Cas9-directed mutagenesis of the MM GWAS SNP rs4487645 in *DNAH11/CDCA7L* demonstrated that this variant, which disrupts a PU.1/IRF4 binding site, is causally linked to formation of the ASM DMR at this locus.

Conclusions: Gains of ASM are pervasive in MM, with ASM DMRs frequently forming at germline MM risk loci as well as in genes that are components of clinically relevant DNA methylation signatures. Functional analysis confirms that inherited genetic variants in TF binding motifs can be sufficient to nucleate gains of ASM in MM cells.

Keywords: Research, Diseases, Lymphoid Malignancies

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