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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Loss of Function *MBD4* Mutation Impairs DNA Repair in Association with TET2 in Acute Myeloid Leukemia

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Background: Methyl-CpG binding domain 4 (MBD4) is a known DNA glycosylase involved in critical steps of base excision repair (BER) and DNA mismatch repair (MMR) and is essential for reducing genotoxic stress in normal cells. MBD4 binds to 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) - oxidation products of TET2 dioxygenases - and may initiate a multi-step BER/MMR around mCpG/hmCpG DNA.
Loss of function (LOF) mutations in *MBD4 (MBD4 ^{MT})* result in compromised BER/MMR, leading to genomic instability. Certain germline *MBD4* variants have been reported to be predisposing factors for early onset AML, among other cancers. [1,2] In cases screened at our institution, biallelic *MBD4* p.R431* & p.L563* tracked with AML in brother and sister. [3] This inspired us to hypothesize that partial or complete loss of MBD4 may result in reduced BER/MMR function and ultimately in genomic instability and faster accumulation of somatic lesions favoring malignant evolution.
Methods: We performed NGS and epigenetic studies coupled with detailed biochemical analyses in CRISPR engineered cell lines and primary AML cells and in vivo model systems to dissect the mechanism of clonal evolution of *MBD4 ^{MT}* hematopoietic stem and progenitor cells (HSPCs).
Results: We analyzed WES data from 1,692 patients with myeloid neoplasms (MN, 732 MDS; 960 AML). After adequate filtering based on frequency in healthy population (odds ratio of >1.5), 16 variants in *MBD4* were identified in 24 MN cases (MDS=9, AML=15). Among them, 4 heterozygous variants of unknown significance of suspected germline nature (p.R425Q, p.N461S, p.R486K, p.D504H) mapped in the glycosylase domain. Comparing *MBD4 ^{MT} vs. wild-type (MBD4 ^{WT})* cases, *MBD4 ^{MT}* were found in younger patients (median 55 *vs.* 71 yrs; $p = 0.012$) with *TET2 ^{MT}* co-occurring at a lower frequency (8 *vs.* 24%).
Since MBD4 is known to preferentially bind with 5hmCpG DNA, a stable epigenetic mark induced by TET2, we investigated the biochemical basis of MBD4 dysfunction and its connection to LOF *TET2 ^{MT}*. We show that MBD4 hydrolase activity levels affect the genomic accumulation of 5hmC in a TET2-dependent manner. MBD4 ^{KO} led to more than 2-fold increased 5hmC in *TET2 ^{WT}* cells but not in *TET2 ^{KO}* cells, suggesting a functional connection between MBD4 and TET2 in BER/MMR.
To test the function and effects of the 4 variants (p.R425Q, p.N461S, p.R486K, p.D504H) identified in our cohort on the activity and function of MBD4, we generated these mutations in a pcDNA plasmid using site directed mutagenesis. The mutants were ectopically expressed in isogenic CRISPR-engineered MBD4 ^{KO} HEK293 cells and its effects on 5hmC accumulation were tested. The impact on the functional outcomes were compared to WT controls as described earlier. [4] We found that these 4 variants lead to MBD4 protein instability, and thereby lower MBD4 DNA glycosylase activity resulting in increased (1.6 to 3.3-fold) genomic accumulation of TET2-dependent oxidation products.
To establish the effect of *MBD4 ^{MT}* in primary cells, we generated CRISPR-engineered human CD34 $^{+}$ HSPCs and assessed their clonal evolution via colony forming assay. Loss of MBD4 led to clonal advantage reflected in persistent colonies in *MBD4 ^{KO}* CD34 $^{+}$ HSPCs that demonstrated myeloid differentiation bias similar to what was observed in patients, suggesting that *MBD4 ^{MT}* may favor clonal evolution to fully blown MN.
Finally, transcriptomics analysis of *MBD4 ^{KO}* in K562 cells in the presence and absence of functional TET2 demonstrated loss of BER genes compared to WT. The co-immunoprecipitation of MBD4 followed by western blot analysis identified XRCC1, a key component of the BER complex, as a part of MBD4 interactome. Comprehensive characterization of protein complexes

with LCMS/MS in K562 cells suggests that MBD4 is involved in maintaining genomic integrity. Consistent with our hypothesis, MBD4 ^{KO} in K562 and THP1 cells resulted in a 2- and 3-fold increase in spontaneous mutations respectively, as observed via HPRT assay.

Conclusions: Genomic instability due to MMR/BER dysfunction in *MBD4* ^{MT} cells may result in a higher rate of clonal progression. Interestingly, MBD4 deficiency may provide opportunity for therapeutic targeting of DNA repair pathways in a subset of *MBD4* ^{MT}-associated MN. Indeed, our findings indicate the possibility of leveraging MBD4 as an actionable target to regulate DNA methylation in association with TET2 during leukemogenesis.

Disclosures Maciejewski: *Novartis:* Honoraria, Speakers Bureau; *Omeros:* Consultancy; *Alexion:* Membership on an entity's Board of Directors or advisory committees; *Regeneron:* Consultancy, Honoraria.

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