ELSEVIER

Blood 142 (2023) 4582–4583

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Mutant p53 Drives the Development of Myelodysplastic Syndromes Via Dysregulating Pre-mRNA Splicing in Hematopoietic Stem and Progenitor Cells

Sergio Barajas, BS[1,2], *Sisi Chen, PhD*[3], *Sasidhar Vemula*[2], *Yuxia Yang*[2], *Edward Simpson*[2], *Wenjie Cai, MS*[2,1], *Shiyu Xiao, MS*[1], *Hongxia Chen, MDPhD*[1], *Stephanie Halene, MD*[4], *Madina Sukhanova*[1], *Yunlong Liu, PhD*[2], *Omar Abdel-Wahab, MD*[5], *Yan Liu, PhD*[1]

[1] Northwestern University, Chicago, IL
[2] Indiana University, Indianapolis, IN
[3] Memorial Sloan Kettering Cancer Center, New York, NY
[4] Section of Hematology, Yale Univ. School of Medicine, New Haven, CT
[5] Molecular Pharmacology Program, Memorial Sloan Kettering Cancer Center, New York, NY

Despite the clinical importance of *TP53* mutations, to date there have been no studies on the functional impact of *TP53* mutations on the initiation and progression of MDS. We found that heterozygous p53 mutant mice ($p53^{R248W/+}$) show extended survival and died within 15-20 months after birth. p53 mutant mice had hypercellular bone marrow and displayed cytopenia, thrombocytopenia, and anemia. Approximately 60% of $p53^{R248W/+}$ mice developed MDS based upon pathological analysis of bone marrow (BM) and peripheral blood smears, while the remaining $p53^{R248W/+}$ mice developed lymphoma and sarcoma. We further showed that mutant p53-driven MDS are transplantable. Thus, we have generated a mouse model of MDS that has some features of human MDS with *TP53* mutations.

To understand how mutant p53 drives MDS development, we performed RNA-seq studies to compare gene expression in hematopoietic stem and progenitor cells (HSPCs). GSEA analysis revealed that spliceosome genes were significantly downregulated in middle-aged $p53^{R248W/+}$ HSPCs compared to age-matched $p53^{+/+}$ HSPCs. We confirmed that the expression of splicing factors, such as *Prpf3* and *Prpf4*, was significantly downregulated in middle-aged $p53^{R248W/+}$ HSPCs. We conducted alternative splicing analysis utilizing MISO (Mixture of Isoforms) algorithm to identify differences in exon inclusion ratio between WT and mutant HSPCs. We observed differential splicing of all classes of alternative splicing events, including Cassette Exons (CE), Competing 5′ and 3′ splice sites (A5′ or A3′SS), Mutually Exclusive Exons (MXE), and Retained Introns (RI) in $p53^{R248W/+}$ cells compared to that of the WT HSPCs. Notably, we found that murine p53 mutant HSPCs exhibited aberrant splicing of key regulators of NFκBactivation, such as *USP15*. USP15 overexpression promotes NFκB expression through inhibiting its ubiquitination, whereas NFκB promotes USP15 expression. p53 mutant HSPCs tend to skip exon 7 and express high levels of short isoform of *Usp15*. The short isoform of Usp15, but not the long isoform of Usp15, activates NFκB in HSPCs, manifested by increased phosphorylation of p65. To determine the impact of mutant p53 on pre-mRNA splicing in human HSPCs, we ectopically expressed GFP or $p53^{R248W}$ in human cord blood $CD34^{+}$ cells and performed RNA-seq in transduced cells (GFP^{+}). Human HSPCs expressing mutant p53 displayed alterations in pre-mRNA splicing compared to HSPCs expressing GFP. We found that human HSPCs expressing mutant p53 display aberrant splicing in key regulator of NFκB such as *IKBKE* (Inhibitor of nuclear factor kappa-B kinase subunit epsilon). Human HSPCs expressing mutant p53 tend to include exon 7 and express high levels of long isoform of *IKBKE*. The long isoform of *IKBKE*, but not the short isoform of *IKBKE*, activates NFκB in human HSPCs.

Many proinflammatory cytokine genes are NFκB targets and we observed increased levels of pro-inflammatory cytokines, including IL-1β and IL-6, in the BM of middle-aged p53 mutant mice. Notably, we observed increased levels of IL-1β and IL-6 in the BM of MDS or AML patients with *TP53* mutations.

TP53 mutations co-occur with splicing factor mutations in MDS. To determine whether *TP53* and *SRSF2* mutations cooperate in pathogenesis of MDS, we have generated $p53^{R248W/+}SRSF2^{P95H/+}$ mice. We found that the survival of recipient mice repopulated with $p53^{R248W/+}SRSF2^{P95H/+}$ BM cells was significantly decreased compared to recipients repopulated with WT, $p53^{R248W/+}$, or $SRSF2^{P95H/+}$ BM cells. All recipient mice repopulated with $p53^{R248W/+}SRSF2^{P95H/+}$ BM cells died within 6 months and developed MDS or leukemia. Thus, we demonstrate that *TP53* and *SRSF2* mutations cooperate in MDS or leukemia development.

In summary, we discovered that mutant p53 dysregulates pre-mRNA splicing in key regulators of inflammatory response during aging, thereby generating a chronic inflammatory microenvironment to drive MDS pathogenesis.

Disclosures Abdel-Wahab: *AbbVie, Inc.:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Amphista Therapeutics:* Consultancy; *Nurix Therapeutics:* Research Funding; *Loxo/Lilly:* Consultancy; *AstraZeneca:* Consultancy; *Minovia Therapeutics:* Research Funding; *Harmonic Discovery:* Current holder of *stock options* in a privately-held company.

https://doi.org/10.1182/blood-2023-180382