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POSTER ABSTRACTS

617.ACUTE MYELOID LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

Novel Role of DHX15 in Modulating AML/ETO9a Spliced Variant Production in AML/ETO $^{+}$ AML

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AML1/ETO9a(AE9a) is an alternatively spliced isoform of AML1/ETO(AE) fusion protein that coexists with AE and predicts a poor prognosis in AE-positive (AE $^{+}$) AML. The insert of exon9a between exon8 and exon9 of ETO provides a stop codon forming the truncated AE9a lacks ETO domains NHR3/4, and the variant alone can induce leukemia. To date, its production mechanism remains obscure. Our previous studies showed that the splicing factor DHX15 is highly expressed in AML and correlates with poor prognosis (PMID: 29163777). DHX15 is also related to alternative splicing events in AE $^{+}$ AML revealed in an in vitro study (PMID: 27798625). Thus, we further investigated the potential role of DHX15 in AE $^{+}$ AML progression and AE9a splice variant production.

To figure out the DHX15 expression level in AE $^{+}$ AML, bone marrow (BM) cells from 114 AE $^{-}$ AML (Fujian Union Hospital, year 2011-2018) and 104 AE $^{+}$ AML (part from Fujian Union Hospital, n=21, part from Wuhan Kindstar Diagnostics Co., Ltd, n=83, year 2016-2017) were collected. The RT-qPCR results showed that the expression of DHX15 in AE $^{+}$AML is lower than those in AE $^{-}$ AML ($P<0.0001$), which is in line with the previous studies showing that a better prognosis is observed in AE $^{+}$ and DHX15-low expressed AML. The AE9a/AE ratio and DHX15 mRNA level in 98 AE $^{+}$ AML BM samples were further analyzed. The results showed that the AE9a($P<0.0002$) and AE9a/AE ratio($P<0.0001$) were significantly higher in DHX15 ^{low} expression AE $^{+}$ AML group (groups were divided based on medium expression level). Conversely, the DHX15 expression level was higher in AE9a/AE ^{low} group($P<0.0001$), indicating a strong negative correlation between the mRNA level of DHX15 and AE9a/AE ratio in AE $^{+}$ AML.

To further investigate how DHX15 impacts the spliced variant AE9a expression, DHX15 was knockdown (KD) in AE $^{+}$ AML cells. The AE9a/AE ratio was increased ($P=0.0013$) in DHX15-KD group compared to the control group. Gene set enrichment analysis (GSEA) of RNA-seq data revealed suppressed splicing and splicing complex assembly pathways in DHX15-KD Kasumi-1/SKNO-1 cells. These data demonstrated that downregulation of DHX15 increased AE9a expression probably through aberrant alternatively splicing process. RNA immunoprecipitation (RIP) assay showed AE and AE9a mRNA were detected in anti-DHX15 immunoprecipitants. However, DHX15 protein was not exclusively detected in AE9a-RNA pulldown-based Western blot (WB) or mass spectrometry (MS), suggesting DHX15 may not directly interact with AE or AE9a mRNA.

To unravel the association between DHX15 protein and AE9a mRNA, AE and AE9a full-length mRNA pulldown-based MS were performed. We found that AE expression may rely more on spliceosome E complex, while AE9a splicing may rely more on spliceosome A complex. Moreover, the hnRNP protein HNRNPL and spliceosome A complex members SF3A2 and SRSF4 are exclusively combined with exon9a. Clinically, HNRNPL and SF3A2 were highly positively correlated with AE9a and DHX15 revealed in the Pearson analysis on mRNA expression of 16 AE $^{+}$ AML BM samples. Moreover, DHX15 may interact with HNRNPL and SF3A2, which were analyzed by protein-protein interaction analysis from *Pathway Commons Protein-Protein Interactions* and *String*. Collectively, DHX15 may modulate AE mRNA splicing together with HNRNPL and SF3A2, and its downregulation causes increased AE9a spliced variant production in AE $^{+}$ AML.

In addition, at the protein level, we found the RecA1 domain of DHX15 can directly interact with the AE fragments containing RHD and NHR4 domains shown in immunofluorescence(IF) and co-immunoprecipitation (CO-IP) assays in vitro, which may contribute to the full-length production of AE. Moreover, DHX15 knockdown significantly decreased cell proliferation and cell

cycle arrest through ATM/AKT-p53-p21 pathway in AE-positive AML cell lines demonstrated by cell proliferate, GSEA, and immunoblotting assays. These results suggest that DHX15 also plays a unique role in AML/ETO $^{+}$ AML oncogenesis.
In conclusion, our studies focused on the AE $^{+}$ AML and revealed DHX15 regulates AE9a expression by alternative splicing together with HNRNPL and SF3A2. Since AE-positive AML is a heterogeneous disease with different phenotypes and varied prognoses based on expression patterns of the other four genes, our study provides new insights into DHX15 upon AML1/ETO9a splice variant production and DHX15 might be served as a therapeutic target in AE9a $^{+}$ AML.

Disclosures No relevant conflicts of interest to declare.

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Abstract Figure

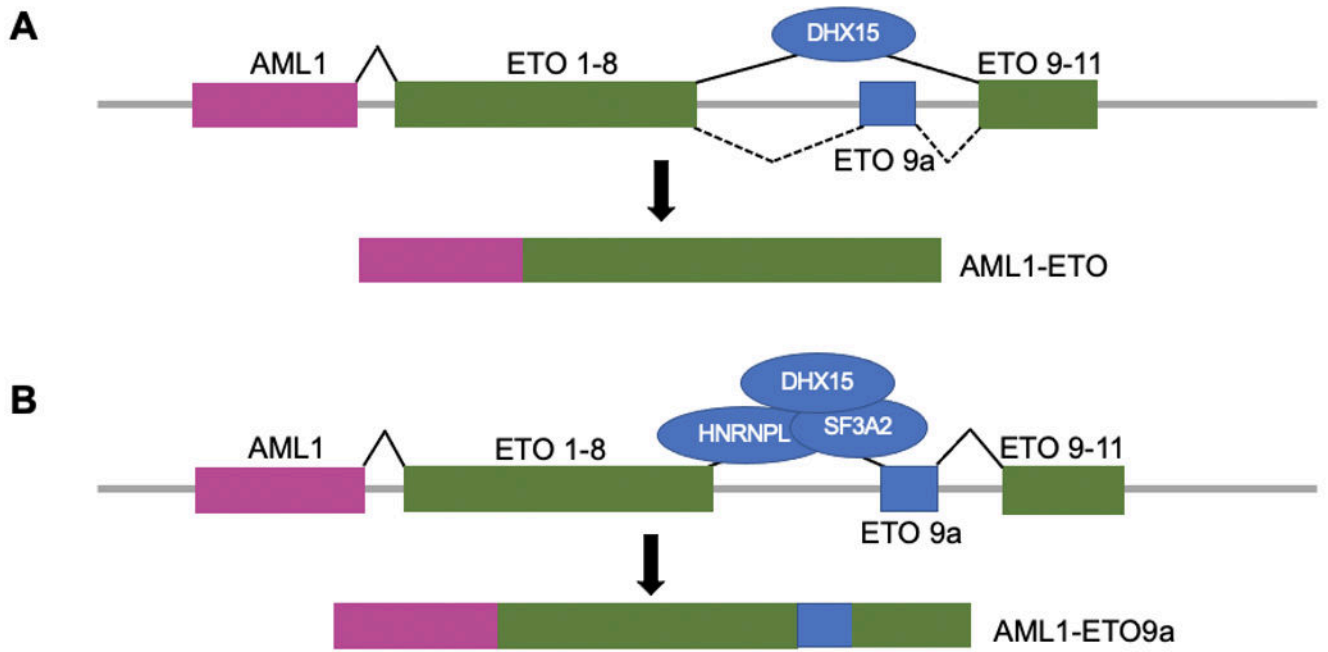

Fig. Abstract Mechanism of DHX15 regulating AE and AE9a production. A) Without HNRNPL and SF3A2, DHX15 promotes the formation of AE mRNA. B) With HNRNPL and SF3A2, DHX15 interact with them to promote the formation of AE9a.

Figure 1