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POSTER ABSTRACTS

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

CD38 Is a Functional Partner of CD9 in B-Cell Precursor Acute Lymphoblastic Leukemia

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Background: We previously showed that CD9 was expressed in ~80% of pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cases and its positivity was associated with an inferior relapse-free survival (Leung *et al*, *Leukemia*, 2020), suggesting its oncogenic role in this disease entity. Yet, the mechanisms underlying how CD9 affects leukemia progression remain completely unknown. As a prototypic member of the tetraspanin superfamily proteins, CD9 is able to organize with transmembrane or intracellular proteins to form the "tetraspanin web" to exert its modulatory functions. In BCP-ALL, though there has been a standalone study reporting 28 CD9-binding proteins in NALM-6 cells, the results are unlikely representative of the entire disease spectrum due to tumor heterogeneity. Here, we perform an in-depth proteomic study to dissect the CD9 web composition and downstream protein partners to drive disease progression in BCP-ALL.
Methods: Immunoprecipitation coupled mass spectrometry (IP-MS) was performed to map the overall composition of CD9 web in five CD9 $^{+}$ BCP-ALL cell lines with distinct cytogenetic backgrounds, including 697 (*TCF3-PBX1+*), BV-173 (*BCR-ABL1+*), HAL-01 (*TCF3-HLF+*), KASUMI-2 (*TCF3-PBX1+*) and RS4;11 (*KMT2A-AFF1+*). Overlapping protein partners were then validated by IP-Western blot (IP-WB) in 13 patient samples spanning major BCP-ALL subtypes. Shortlisted CD9 partners were further characterized by antibody blockade and CRISPR/Cas9 loss-of-function experiments to evaluate their impact on leukemia proliferation *in vitro* and leukemia progression *in vivo*, coupled with enzymatic and phenotypic assays to evaluate the direct impact of CD9 on partner functions.
Results: A total of 278 CD9-binding proteins comprising 24 known and 254 novel partners were identified by IP-MS. Venn analysis revealed a marked heterogeneity of CD9-binding proteins across leukemia subtypes, with only 10 being intersected representing the "universal CD9 partners" in BCP-ALL. These proteins consisted of 5 established CD9 partners: ADAM10, CD19, CD38, CD81 and IGSF8 as well as 5 novel partners: ANXA11, HBG2, TMEM109, SLC44A1 and STXBP3. Two universal CD9 partners, namely CD38 and CD81, were consistently detected in all pediatric BCP-ALL samples. Neutralizing antibodies against CD9, CD38 or CD81 unanimously inhibited proliferation of 697 cells *in vitro* ($P<0.05$). However, in the background of CD9 neutralization, CD81, but not CD38, offered an additional impact on leukemia suppression ($P<0.05$). Likewise, CD38 knockout cells treated with CD9 antibody did not appear to inhibit cell proliferation in an additive manner. Concordantly, in the 697 xenografts, CD9 or CD38 antibodies alone prolonged animal survival by 2.1- and 1.6-fold, but combinational treatment did not produce the projected additive effect (2.5-fold only). In addition, CD9 neutralization significantly reduced the enzymatic activity of CD38 by 52.5% ($P<0.05$) without altering its expression.
Conclusions: This work presents the composition of the CD9 protein web in BCP-ALL, revealing homogenous and heterogeneous partners across the leukemia subtypes. We also provide definitive evidence showing that CD9 binds to and regulates CD38 activity to mediate leukemia progression, representing the first universal protein partner being identified to have a functional consequence in BCP-ALL, thereby providing a mechanistic basis to support the development of CD9-targeted therapies for this life-threatening malignancy.

Disclosures No relevant conflicts of interest to declare.

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