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Myeloproliferative disorders

CD9 DEFINES THE DISEASE-PROPAGATING HEMATOPOIETIC STEM CELL POPULATION IN MYELOFIBROSIS

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Introduction: Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders, among which myelofibrosis (MF) represents the most severe form. MF is marked by progressive bone marrow fibrosis, worsening cytopenia, and a median overall survival of approximately five years. The disease originates from the expansion of a single hematopoietic stem and progenitor cell (HSPC) that has acquired somatic driver mutations in *JAK2*, *CALR*, or *MPL*, followed by additional cooperating mutations that promote clonal dominance over wild-type HSPCs. Although current therapies, including JAK inhibitors, provide symptomatic benefit, they fail to eradicate the malignant HSPC pool or induce molecular remission. Therefore, identifying and characterizing the stem cell population that sustains the MF clone is essential for developing curative strategies.

Methods: HSPCs were purified from peripheral blood samples, collected from MF patients with *JAK2*, *CALR*, or *MPL* driver mutations and healthy donors (HDs). We performed RNA sequencing on purified HSPCs from 13 MF samples and 5 HDs, focusing on surface molecules with higher expression in MF. Candidate markers were then assessed by flow cytometry in an expanded cohort (30 MF, 15 HDs). To explore whether CD9 expression identified the neoplastic stem cell population, we performed single-cell mutational profiling combined with surface protein analysis using the Tapestri platform (Mission Bio) in 8 MF patients. CD9+ and CD9- HSCs from both MF and HD samples were compared in clonogenic and differentiation assays *in vitro*. Finally, the disease-propagating potential of each population was tested through patient-derived xenografts, generated by transplanting sorted cells into sub-lethally irradiated NSGS mice.

Results: Transcriptomic profiling highlighted CD9 as one of the most upregulated surface molecules in MF HSPCs. Flow cytometry confirmed a clear expansion of the CD9+ HSPC compartment in patient samples. Single-cell proteogenomics data then revealed that the majority of mutated HSPCs clustered within the CD9+ fraction, supporting CD9 expression as a phenotypic marker of the malignant stem cell population. In functional assays performed *in vitro*, MF CD9+ HSPCs exhibited a more primitive phenotype and enhanced clonogenic potential unlike healthy and MF CD9- HSPCs. Our *in vivo* data further indicate that CD9+ MF HSPCs displayed higher engraftment levels relative to their CD9- counterparts, supporting their role as key disease-propagating cells.

Conclusions: Across MF patients with distinct driver mutations, CD9 marks an expanded, mutation-enriched HSPC population with enhanced clonogenic activity and disease-propagating potential. These findings support CD9 as a promising surface marker of MF HSPCs, with potential implications for future therapeutic targeting aimed at eradicating the malignant clone.