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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Defining Signaling Interactomes Involved in Non-Canonical Wnt Signaling By ROR1 or ROR2 in Hairy Cell Leukemia That Can Influence Cell Morphology and Migration

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Receptor tyrosine kinase-like orphan receptor 1 (ROR1) and 2 (ROR2) are highly conserved oncoembryonic surface proteins expressed on neoplastic cells of patients with a variety of human cancers, but not on most normal postpartum tissues. ROR1 and ROR2 are each a receptor for non-canonical Wnt factors that direct migration and planar cell polarity, which is essential for organ development. We developed humanized monoclonal antibodies (mAbs) each specific for ROR1 (UC-961) or ROR2 (6E6-70) and found the leukemia cells of patients with classic hairy cell leukemia (cHCL) or variant HCL (vHCL) express both ROR1 and ROR2 and that these receptors function in HCL non-canonical Wnt signaling (Widhopf, G. et al). We hypothesized that ROR1 and ROR2 each may associate with overlapping and distinctive sets of proteins, which may contribute to ROR1/2, ROR1, or ROR2 signaling in HCL. To test this hypothesis, we optimized conditions for generating immune precipitates (i.p.) with either UC-961 or 6E6-70, and examined the proteins of each i.p. via data dependent acquisition mass spectrometry. Consistent with the assumed capacity of each of these mAb to disrupt ROR1/2 heteroligomerization, we identified ROR1, but not ROR2, in i.p. generated using UC-961 and ROR2, but not ROR1, in i.p. generated using 6E6-70 on cell lysates of leukemia cells isolated from a patient with cHCL. In the anti-ROR1 i.p. we identified 1,414 proteins, 849 (60%) of which also were found in the anti-ROR2 i.p.. In the anti-ROR2 i.p. we identified 2,523 proteins, a third of which also were found in the ROR1 i.p.. Among proteins common to both anti-ROR1 and anti-ROR2 i.p. we noted hematopoietic cell-specific protein-1 (HS1 or HCLS1), which we found associated a SH3-binding motif that was dependent on a proline residue at position 841 of activated ROR1 in chronic lymphocytic leukemia (CLL); we found this SH3-binding motif was critical for ROR1-dependent Wnt5a-induced chemokine-directed migration (*Leukemia* 12:2615, 2017). Also found common to both ROR1 and ROR2 in HCL were proteins that drive actin polymerization and cytoskeletal dynamics that influence cell morphology and migration. To validate that ROR2 itself was involved in binding HS1 and in enhancing migration, we generated mutant forms of ROR2, each harboring a proline-to-alanine mutation in one of several proline residues found in the proline-rich domain of ROR2. We transduced MEC1 cells, which lack ROR1 or ROR2, with lentivirus vectors encoding wild-type ROR2 or each one of several P-A mutants of ROR2. Flow cytometry confirmed that each of the transduced cells had comparable expression of surface ROR2. We generated anti-ROR2 i.p. using lysates of each and found that all but one of the mutant forms of ROR2 could bind to HS1 by immunoblot analysis. In contrast to wild-type ROR2, or other mutant forms of ROR2, expression of this mutant form of ROR2 could not enhance chemokine-directed migration relative to that of wild-type MEC1 cells. Collectively, our studies define signaling interactomes involved in non-canonical Wnt signaling by ROR1 and/or ROR2 in hairy cell leukemia and define a residue within ROR2 that is necessary for binding HS1 and facilitating cellular migration.

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