The Degradation and Subcellular Localization of an Oncogenic protein complex in T-cell Leukemia

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Background and Hypothesis:

LIM domain Only-2 (LMO2) is a key oncogenic driver of human T-cell acute lymphoblastic leukemia. LMO2 functions as part of a large multisubunit complex that regulates gene expression. LMO2 itself does not bind DNA but it does bind class II basic helix loop helix transcription factors, TAL1 or LYL1, which are also part of the LMO2-associated complex. We recently identified LIM domain binding protein 1 (LDB1) as an obligate LMO2 partner that is required for LMO2 protein stability in T-ALL. We believe the interaction between LDB1 and LMO2 is crucial to understanding the pathogenesis of T-ALL and is a potential therapeutic target for this aggressive leukemia. We hypothesize that in the absence of LDB1, LMO2 is rapidly degraded. Nonetheless, the mechanisms for the localization and degradation of LMO2 and its partners have not been fully explored.

Experimental Design or Project Methods:

In this study, we analyzed the first order decay of the LMO2-associated complex including LMO2, SSBP2, SSBP3, TAL1, LYL1, and LDB1. We analyzed the subcellular localization of these same proteins distinguishing between the nucleus and the cytoplasm. Our studies were enabled by cloning the HALO tag to the NH2-terminus of these proteins. Fluorescent small molecules such as HALO ligand, R110, were used for intracellular labeling followed by pulse chase analysis via flow cytometry for half-life and imaging by confocal microscopy.

Results:

We observed a hierarchy of protein stability: LDB1 had the longest half-life, followed by SSBP3, SSBP2, Tal1, LMO2 and Lyl1had the shortest half-lives. Co-expression of LDB1 conferred enhanced staility upon all protein components. All of the protein components studied showed a predominantly nuclear distribution with some in the cytoplasm.

Conclusion and Potential Impact:

The prolonged protein stability of LDB1 allows it to confer enhanced stability to LMO2 and bHLH proteins TAL1 and LYL1. Thus, targeting the assembly of the multisubunit LMO2 complex.