A Role for the SIX1 Homeobox Gene in CALM-AF10 Leukemogenesis

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BACKGROUND: The CALM-AF10 translocation is detected in ~10% of T-cell acute lymphoblastic leukemias (T-ALLs), and in some acute myeloid leukemias (AMLs). CALM-AF10 leukemias are characterized by overexpression of proleukemic HOXA genes. Since HOXA genes are difficult to target, we hypothesized that identification of non-HOXA CALM-AF10 effector genes could potentially yield novel therapeutic targets. To this end, we took advantage of our prior observation that the nuclear export factor CRM1/XPO1 tethers CALM-AF10 to HOXA genes by interacting with a nuclear export signal (NES) in CALM. We used RNA-sequencing and microarray to determine that SIX1, similar to HOXA genes, is increased in CALM-AF10 leukemias and decreased in response to CRM1 inhibition.

OBJECTIVE: To evaluate the role of SIX1 in CALM-AF10 leukemias.

DESIGN/METHODS: RT-qPCR and Chromatin Immunoprecipitation were performed using both bone marrow progenitors and murine embryonic fibroblasts (MEFs) transduced with CALM-AF10 or an empty vector, with and without LMB. The ability of SIX1 to enhance self-renewal of hematopoietic progenitors was examined by measuring the colony-forming ability of transduced fetal liver progenitors. CRISPR-Cas9 was used to silence SIX1 in Human Embryonic Kidney 293 (HEK293) cells.

RESULTS: RT-qPCR confirmed overexpression of SIX1 in both CALM-AF10 transduced MEFs and CALM-AF10 leukemias, with decreased SIX1 expression observed in the presence of LMB. ChIP analysis showed that CALM-AF10 binds the SIX1 gene locus. Overexpression of SIX1 in fetal liver cells was sufficient to increase the self-renewal potential of these colony-forming progenitors. SIX1 was successfully knocked out in HEK293 cells, resulting in potentially slowed HEK293 proliferation.

CONCLUSIONS: SIX1 is a homeobox gene that is highly expressed during embryogenesis; expression is normally silenced post-embryogenesis. While increased SIX1 expression has been reported in numerous solid tumors, SIX1 involvement in leukemogenesis is uncertain. We have determined that SIX1 is upregulated in the presence of CALM-AF10, and increases the self-renewal potential of hematopoietic progenitors. Despite decreased proliferation rates in HEK293 cells with SIX1 knocked out, SIX1 is not critical for cell survival, and inhibition could be effective in impairing CALM-AF10 leukemia cell proliferation. Thus, SIX1 may play a pathogenic role in leukemogenesis and could be a novel therapeutic target in CALM-AF10 leukemias.