



## 2007 Research Grant Program Winning Abstract

### Latexin: A New Tumor Suppressor

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Leukemia is one of the leading causes of death in the US. It is characterized by the accumulation of non-functional, immature hematopoietic cells, which are derived from leukemia stem cells (LSCs). Mechanisms leading to the transformation of LSCs from normal hematopoietic stem cells (HSCs) are largely undefined, and both genetic and epigenetic changes resulting in the inhibition of tumor suppressor gene(s) and/or activation of oncogenes have been shown to play roles. Identification of molecules and mechanisms involved in malignant transformation, especially with respect to cancer stem cells, holds promise for cancer prevention, diagnosis, and therapy.

We have discovered in mice that a gene, Latexin (Lxn), is involved in the regulation of proliferation, apoptosis, self-renewal, and subsequently pool size, of normal hematopoietic stem cells (HSCs) (Liang Y, Nature Genetics, 39, 178-188, 2007). Previous studies showed that Lxn was highly homologous to Tazarotene-Induced Gene 1 (TIG1), which is down-regulated or absent in an extensive list of tumor types. We therefore hypothesize that Lxn is a potential tumor suppressor and plays a role in hematologic malignancies.

The first aim is to determine whether Lxn expression is altered in leukemic cells. Progenitor and stem cell-enriched CD34+CD38<sup>-</sup> cells (BD Pharmingen) in normal individuals and leukemia patients were sorted using a BD FACSVantage (Becton Dickinson), and real-time PCR was performed to measure Lxn mRNA expression. Our preliminary data showed that expression of Lxn was absent or significantly decreased in acute myeloid leukemia (AML) cells from three patients and in leukemia cell lines (K562, HL-60, KG-1, Jurkat, U937, SupB15, CCRF-CEM Molt4 and J45.01), indicating its potential role as a tumor suppressor.

We will next examine Lxn expression in other types of myeloid and lymphoid leukemia samples. We have a large variety of patient samples available including AML, chronic myeloid leukemia (CML), T cell prolympholeukemia (PCTL), plasma cell leukemia (PCL), adult T cell lymphoma (ATLL), acute lymphoid leukemia (ALL, preB phenotype), and myelodysplastic syndromes (MDS). The clinical protocol and consent for sample collection have been submitted to and approved by the Institutional Review Board (IRB) at the University of Kentucky.

By using different combination of cell surface markers (BD Pharmingen) we will be able to determine Lxn expression at various stages of leukemia development. For example, in ALL (PreB phenotype), the LSCs are CD34<sup>+</sup>, but lack expression of the more mature B-lymphoid markers CD10<sup>-</sup> and CD19<sup>-</sup>. In AML samples, LSCs have been refined as CD34<sup>+</sup>, CD38<sup>-</sup>, CD90<sup>-</sup> (T cell), IL-3R<sup>+</sup> (interleukin 3 receptor), CD71<sup>-</sup> (proliferating cell) and HLA-DR<sup>-</sup> (B cell).



The second aim is to investigate the mechanisms leading to the downregulation or absence of Lxn expression in leukemia cells. Promotor methylation has emerged as a novel mechanism for inactivating tumor suppressor genes and thus acts as a contributor to leukemogenesis. Our analysis of the upstream sequence of the Lxn open reading frame revealed a 164bp CpG repeat-enriched region (CpG island). Using bisulfite sequencing PCR, hypermethylation of the CpG island was detected in all cell lines and the methylation content was inversely correlated with Lxn expression. In addition, Lxn gene expression was re initiated or upregulated following addition of the demethylating agent, 5-aza-2- deoxycytidine to cell line cultures, implying that promoter hypermethylation might be a key mechanism repressing Lxn expression.

In order to better understand Lxn regulatory mechanisms in leukemia, we will perform bisulfite sequencing PCR on purified LSCs or progenitor cells (labeled and sorted as described above). The effects of 5-aza-2- deoxycytidine will also be investigated in the invitro culture of LSCs in medium containing growth factors IL-3, SCF and GM-CSF (BD Biosciences). We expect similar results in primary leukemic cells as in the cell lines.

The third aim is to study whether expression of constitutively active Lxn in leukemic cells will inhibit tumor cell growth by decreasing cell proliferation and/or increasing apoptosis. We have found that high level of Lxn expression is associated with decreased proliferation and increased apoptosis. We therefore propose to construct retroviral vector containing Lxn, overexpress Lxn in leukemic cells and study the growth characteristics of the cells infected with either the Lxn or empty vector. Population doubling times will be determined by standard cell counting and by analytical flow cytometry. Cell cycle parameters of each population will be determined using BrdU Flow kit (BD Pharmingen). The fraction of cells in G1, S and G2/M phase of the cell cycle will be identified. The fraction of apoptosis cells will be determined using Annexin V and 7-AAD (BD Pharmingen). All flow cytometric analysis will be performed on BD FACScan or BD FACSVantage. In vivo growth of tumor will also be monitored. Cells containing empty vector or Lxn will be injected subcutaneously into the mice and the cell growth will be monitored 3 times per week by taking perpendicular measurements with a caliper. We expect that, as the potential tumor suppressor, ectopic expression of Lxn will inhibit leukemic cell growth by inhibiting proliferation and enhancing apoptosis.

The role of Lxn in the development of leukemia has not yet been investigated. Therefore, examining its expression profile and methylation status in LSCs, as well as its mode of action, may open a new way for treatment of hematologic malignancies.

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