Name of Student: Elizabeth Bulaeva

Research Supervisor: Dr. Connie Eaves

Title of Presentation: FORCED EXPRESSION OF C-MYC ENHANCES THE GROWTH OF PRIMITIVE HUMAN NORMAL AND CML HEMATOPOIETIC CELLS IN SINGLE-CELL AND BULK CULTURES

Elizabeth Bulaeva, Philip A. Beer and Connie J. Eaves.
Terry Fox Laboratory, BC Cancer Agency, 675 West 10th Avenue, Vancouver, BC, V5Z 1L3.

Abstract

MYC is a well-studied oncogenic transcription factor implicated in the control of self-renewal in studies of mouse hematopoietic stem cells (HSCs) and human cell line models. MYC expression is frequently deregulated in human leukemias, and increased MYC activity has been found to inhibit differentiation in human leukemia-derived cell lines. In particular, increased expression of MYC has been reported in chronic myeloid leukemia (CML) patients and may be associated with the progression of this malignancy. However, the role of MYC in primitive normal human hematopoietic cells or in the pathogenesis of CML has not been previously investigated. The present study was designed to address these questions.

As a first step we compared the effects of lentivirally-mediated overexpression of MYC on the extent and duration of cell production from control (YFP+) and test (GFP+) transduced CD34+ cells. These cells were isolated from 2 separate pooled samples of normal human cord blood (CB) and from a chronic phase CML patient sample in which all primitive cells harbored a *BCR-ABL1* fusion. The transduced cells were co-cultured for up to 12 weeks on mouse stromal feeders engineered to express human FLT3L, Steel factor (SF), IL-3 and G-CSF, and assessed periodically for the number of mature (nonadherent) cells present. All endpoints measured showed that MYC-transduction of both cell sources caused a marked and sustained (at least 12 weeks) competitive enhancement of their growth under these conditions.

Previous studies suggesting that high intracellular levels of MYC may promote cell death prompted us to next examine whether such a phenomenon might be affecting the overall outcomes measured in bulk cultures of transduced human hematopoietic cells. Accordingly, we isolated MYC-high and MYC-low transduced (as inferred from their GFP fluorescence) CD34+ CB cells by fluorescence-activated cell sorting, and incubated them in single-cell cultures containing 50 ng/ml SF, 20 ng/ml GM-CSF, IL-3, IL-6 and G-CSF, and 3 units/mL EPO for 12 days. The efficiency of clone formation was similar in all groups in both experiments performed, but the modal size of the colonies produced by cells with the highest MYC levels was on average ~10-fold larger than that of the controls. The size of the colonies produced by the MYC-low cells appeared intermediate. These findings highlight a marked enhancing effect of MYC expression on the clonal outputs of single progenitors, but speak against a pro-apoptotic effect of MYC in this system.

These findings suggest that the presence of supra-normal levels of MYC in human CML and normal CB CD34+ cells activates pathways that significantly, rapidly and sustainably increase cell outputs from both these sources. Moreover, the levels of MYC achieved when it is expressed under the control of the MND promoter (from our lentiviral vector) do not appear to compromise cell survival.