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The potential role of the homeobox gene, Hhex in haematopoietic progenitor expansion

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Background: The decision of an erythroid progenitor to proliferate or differentiate is regulated at the level of (i) transcription; (ii) recruitment of transcripts to polysomes for protein synthesis and (iii) signal transduction activating functional effectors. We utilized a factor sensitive erythroid progenitor cell model to study the gene expression profile of cells under proliferative signals. We have shown previously that translation control is an extremely important level of regulation that controls the balance between proliferation and differentiation of erythroid progenitors. This led us to investigate those transcripts that are shifted to polysomes in cells stimulated by erythropoietin (Epo) or stem cell factor (SCF).

Aim: to investigated the effect of growth factors on the expression of Hhex in erythroid progenitors.

Methodology: We utilized a factor sensitive erythroid progenitor cell model (I11; murine foetal liver derived) to study the gene expression of cells under proliferative signals using SCF and Epo. In addition to total RNA, we isolated polysome bound mRNA transcripts. Sucrose gradients were used to centrifuge cell lysates at high speeds, separating free RNA from polysome bound RNA. Microarray experiments revealed a subset of transcripts that are regulated at transcription and those loaded on polysomes. Data was validated in a separate experiment using Real time PCR.To assess the function of Hhex, the coding sequence was cloned in a mammalian expression vector and overexpressed in

primary bone marrow cells. The transduced cells were plated in semi-solid media and colonies were counted after 5 days and replated to assess clonogenic potential.

Results: The transcription factor, Hhex is 15-fold upregulated upon addition of SCF. In addition, Hhex transcript is selectively recruited to polysomes upon SCF addition in a PI3K- dependent manner. Secondary plating showed a significant increase in colony number in the Epo sensitive and GM-CSF sensitive cells transduced with Hhex. Of interest the colony size was significantly increased when compared to control cells.

Conclusion: Our results show a potential role of Hhex in haematopoeitic progenitor expansion, supported by the enhanced clonogenicity of primary murine cultures in both erythroid and myeloid lineages. To understand the mechanisms of Hhex deregulation, it is imperative to study its role at different maturity stages of lineage commitment and maturation. Hence, the expression and mechanism of Hhex function will be studied in human cellular models targeting various maturity stages, and in a cohort of Acute Myeloid Leukaemia (AML) patients representing different stages of maturity.