Lineage plasticity and determinism in *ex vivo* differentiation of hematopoietic stem cells examined by large-scale transcriptional analysis

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A fundamental question in developmental biology is how and when lineage commitment of stem cells occurs. Lineage commitment and differentiation involve alterations in gene expressions, as evidenced by the varying patterns of surface markers displayed by stem cells, less primitive progenitor cells and mature terminal cells. However, the exact changes in gene expression that occur and the period over which they occur remain unclear. The hematopoietic system is an attractive system in which to study these questions because it is readily accessible. Hematopoietic stem cells (HSC) give rise to several distinct lineages, including granulocytes and megakaryocytes, that together function as the body's circulatory and immune systems. How and when commitment of the granulocytic (G) and megakaryocytic (Mk) lineages occurs is of particular interest because these cell types are crucial lineages for successful hematopoietic (bone marrow) transplantation.

DNA microarray technology allows for the rapid and simultaneous monitoring of the expression of tens of thousands of genes. As such, it is an ideal tool for uncovering the transcriptional programs underlying the complex and multigenic process of lineage plasticity, commitment and determinism. We utilized DNA microarrays containing over 21,000 human genes to study the transcriptional programs of human HSC and early G and Mk differentiation from human HSC ($CD34^+$ cells) in response to lineage-specific cytokines. The degree of respective lineage commitments were assessed by detection of lineage associated surface markers (CD11b & CD15 for G cells and CD41 for Mk cells) using flow cytometry.

Our results show the expected decrease in expression of HSC related genes and proteins in both G and Mk cultures. However, several G-related proteins and genes were highly expressed in Mk cultures and vice versa. In Mk cells, the expression levels of granule proteins myeloperoxidase (MPO), cathepsin G (CTSG) and Charot-Leyden crystal protein (CLC) were comparable to or higher than those in G cells. Conversely, G-cells displayed a high mRNA level of GATA-1, an important Mk transcription factor. Thus, G and Mk cells may possess a longer period of interlineage plasticity than previously thought. Real time (or quantitative) RT-PCR and flow cytometric analysis of intracellular and surface proteins are used to verify and enhance the information from microarray analysis and to provide additional insights into HSC lineage commitment. These findings are of fundamental importance in stem-cell biology, but, significantly, also provide important information for the development of methods to improve *ex vivo* manipulation of HSC for potential clinical applications of cellular and gene therapies.