DNA-array based transcriptional analysis elucidates granulocytic differentiation of human stem cells

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As many as 1 in 3 patients receiving high-dose chemotherapy treatment for cancer suffers a short period of acute neutropenia (low granulocyte levels) lasting on average 17 to 26 days. Neutropenic individuals suffer high rates of morbidity and mortality due to numerous infections. The period of neutropenia can be reduced, and even eliminated, with transplantation of *ex vivo* expanded granulocytic cells and precursors. However, such therapy depends on the generation of a large number of cells spanning various granulocyte differentiation stages. This level of production is difficult to consistently achieve without a better understanding of the molecular mechanisms underlying granulocytic expansion and differentiaton (granulopoiesis).

The initiation and continuation of granulopoiesis consists of a delicate balance of the three processes of expansion, differentiation and apoptosis. The morphological and phenotypical changes associated with each process have thus far provided few clues to their underlying transcriptional program. Of particular interest are the signaling pathways involved in the interactions between and regulation of the three processes. DNA microarray technology provides a panorama of global gene expressions. Thus, we utilized DNA microarrays to capture the temporal gene expression pattern of granulopoiesis, from which signaling pathways and intracellular processes important in different stages of granulopoiesis can be deduced.

DNA microarrays containing over 21,000 human genes were used to profile *ex vivo* granulopoiesis for a two-week period. Cell cultures initiated with human $CD34^+$ cells (which are enriched for and contain the stem-cell compartment) were induced to differentiate into granulocytes by a cytokine combination of SCF, IL-3, IL-6 and G-CSF. Differentiation along the granulocytic lineage was assessed by the pattern of surface marker CD11b and CD15 expression using flow cytometry. By the end of the two-week culture period, over 90% of the cells are committed granulocytes or granulocytic precursors. A subset of genes involved in granulocyte expansion has been identified from the analysis of microarray data of cultures with different expansion rates. Additionally, detailed analysis of the set of differentially expressed genes revealed that many, such as transcription factor C/EBP alpha and the granule protein lactotransferrin, followed known patterns of expression. However, the expression patterns of several primary granule proteins, such as myeloperoxidase and elastase, were different than expected. Furthermore, the expression patterns of the bcl-2 family of apoptosis related genes suggest bcl-2 family members partially regulate the constitutive apoptotic program of granulocytes. Granulocytic functional assays, quantitative (or real time) RT-PCR analysis and flow cytometric analysis of important transcription factors confirmed and enhanced the microarray data. Elucidating the genes and pathways that control the various stages of the granulocytic proliferation, differentiation and apoptotic programs will aid in the development of methods to increase productivity and control of *ex vivo* granulocytic cultures.