Maturation and Death in Terminal Megakaryocytic Differentiation: Phenotypic and DNA-Microarray Characterization

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Ex vivo culture of hematopoietic stem cells (HSCs) under conditions designed to promote megakaryocytic (Mk) commitment, expansion, and differentiation has been proposed as means to produce transplantable megakaryocytes that could supplement stem cell transplants for post-chemotherapy treatment. Furthermore, *ex vivo* production of platelets (the progeny of megakaryocytes) from cultured HSCs, has the potential to provide a future alternative source for platelet transfusions.

Early Mk progenitors have the capacity for division, but as the cells mature, they switch from mitosis to a unique endomitotic cell cycle involving complete replication of DNA without subsequent cytokinesis or cell division. This produces enlarged cells with greater than 4N (polyploid) DNA content which then proceed through the terminal stages of maturation, proplatelet formation, platelet shedding, and constitutive apoptosis. It is of particular interest to be able to predict and control the extent of differentiation obtained in *ex vivo* Mk cultures. To study this process *ex vivo*, we are utilizing the megakaryoblastic cell line CHRF. This cell line is induced to undergo terminal differentiation upon continuous treatment with the protein kinase C-stimulator phorbol 12-myristate 13-acetate (PMA). The unstimulated cells grow almost entirely in suspension. However, within hours of treatment with PMA, nearly 100% of the cells become adherent and cease further expansion. In the first three days of stimulation, cells enlarge, spread, and increase their DNA content, becoming polyploid. After three days, cells extend proplatelet-like processes and begin to undergo apoptosis. Finally, cells release from the surface and rapidly lose viability.

Using commercially available DNA microarrays, supplemented by RT-PCR and protein-level assays, we are developing a global transcriptional picture of terminal Mk differentiation. A particular emphasis will be placed on relating temporal patterns of gene expression to temporal patterns of morphological and phenotypic development in the latter stages of differentiation. These results will also be compared and contrasted to an independent transcriptional data set for cultured megakaryocytes derived from mobilized peripheral blood CD34+ primary cells. In this work, we aim to map the transcriptional state of the CHRF cell line to the corresponding points in primary cell cultures, thus better defining the position of this common model cell line in the pathway of megakaryocytic differentiation.