4h Leveraging Large-Scale Transcriptional Analysis in Cell Culture Engineering

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The advent of global gene expression profiling offers an exciting means to develop better understanding of ex vivo cell culture systems. By focusing attention on cells' transcriptional responses to in vitro culture conditions, we aim to develop improved culture conditions for generation of mature platelet-producing megakaryocytes (Mk) from hematopoietic stem and progenitor cells (HSPC) – a goal which is driven by the need to produce these rare cells to supplement stem cell transplants. We are generating and analyzing high quality global gene expression microarray data from time-course biological experiments to develop an understanding of the pathways governing key facets of Mk maturation: polyploidization, apoptosis, and proplatelet formation. Specifically, we performed transcriptional analysis on a megakaryoblastic cell line (CHRF) as a highly reproducible and scalable model system for terminal megakaryopoiesis. Analysis of these data reveals which genes are temporally regulated during phorbol ester-induced differentiation. As an alternative analytical strategy, we compared those results to the expression signature of fresh human hematopoietic stem and progenitor cells and to primary cells cultured to induce Mk differentiation. The latter is a novel approach to gain insights into which aspects of normal megakaryopoiesis are conserved in the transformed cell line and can help with interpretation of past and future cell line experiments. The regulated gene set in the CHRF cultures was strongly enriched in genes typically associated with megakaryopoiesis and platelets: e.g. CD61 and platelet glycoproteins Ib, V, and IX were upregulated, whereas Aurora kinase B is down-regulated. Unexpectedly, we observed a delayed but persistent increase in histone gene mRNA levels and a transient upregulation of defense- and immune-associated genes. While some of the latter are expected in light of platelets' role in innate immunity, several, including granzyme A and CD244, likely represent either novel expression patterns, artifacts of cell line immortalization, or PMA induction. Comparison with independent transcriptional data from primary HSPC cultures revealed clear conservation among many of the megakaryocyte-specific genes and also among the previously unexpected histone genes. Notable differences between the cell types include expression among apoptosis and cell cycle-related genes. Also, the primary cells do not upregulate many of the immune-response genes observed in the CHRFs, suggesting the presence of an interferon response artifact in the cell line model which must be considered when interpreting the cell line results. Along with an improved understanding of human megakaryopoiesis, these studies have offered direction to our research into improving ex vivo Mk culture conditions.