304f Megakaryocyte Development Illuminated by Transcriptional Analysis

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Ex vivo culture of hematopoietic stem and progenitor cells (HSPCs) 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 HSPCs, has the potential to provide a future alternative source for platelet transfusions. However, Mks have been one of the least studied hematopoietic lineages due to their low frequency in vivo and the difficulties in establishing effective high-yield Mk-generating culture conditions from HSPCs.

Using DNA microarrays, we have probed the transcriptional program of late-stage Mk differentiation in both maturing Mks derived from ex vivo culture of primary human HSPCs (PrimMk) and a megakaryoblastic cell line (CHRF). A consistent set of upregulated genes was identified which contains a significant fraction of known Mk-related elements. For example, CD41, CD61 and platelet glycoproteins Ib, V, and IX were upregulated, whereas Aurora kinase B is down-regulated. Expression data for hematopoietic transcription factors GATA-1, NF-E2, and PU.1 agreed strongly with intracellular flow cytometry in the primary cell cultures as well as with their accepted roles in hematopoiesis. Surprisingly, several non-Mk genes were found to be transcriptionally upregulated including some erythrocyte genes (e.g. hemoglobin beta and epsilon) – although protein level analysis suggests these are not being translated to proteins. There was also an unexpected upregulation of histone mRNA after cessation of mitosis in both cell types.

Apoptosis plays a key role in terminal megakaryopoiesis. Examination of apoptosis-related genes revealed upregulation in genes involved in both the intrinsic and extrinsic pathways. Because apoptosis is primarily a process mediated by protein level events, it can be challenging to infer information about the apoptotic program from gene expression data. However, we were able to observe certain apoptotic transcriptional events such as the upregulation of PUMA (BBC3), a pro-apoptotic Bcl-2 family member, and FAS (TNFRSF6), an integral membrane receptor which relays extrinsic apoptotic signals. Furthermore, certain apoptotic control and execution proteins such as the BIRC3 and BIRC5 and CARD6 are differentially regulated.

Some observed differences between the apoptotic programs of the CHRF and PrimMk could be explained in part by the transcriptional data. For example, terminally differentiated PrimMk cells, but not apoptotic CHRF cells, undergo chromosomal DNA fragmentation as assayed by TUNEL. Three genes involved in the caspase-directed apoptotic DNA cleavage pathway are downregulated in CHRF compared to PrimMk: the active DNase subunit DFFB (CAD) and two accessory chromosomal proteins (HMGB1/2).

Other differences between the cell sources were only apparent in the transcriptional data. For example, there was a strong and largely transient upregulation of immune and defense response genes in the CHRF cultures which was not observed in the PrimMk system. Many of these genes are known to be interferon (IFN) responsive suggesting IFN signaling may be artificially induced in the CHRF by the phorbol ester differentiation-inducing agent.

This work demonstrates how global transcriptional analysis, when systematically applied to the analysis of ex vivo cell culture systems, can contribute to our understanding of complex biological processes.