560f Lineage Switching of Hematopoietic Cells in Response to Changes in Culture Conditions

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The current hierarchical model of hematopoiesis predicts that cells committed to any particular lineage can never become cells of other lineages. A number of reports in recent years, however, have challenged that model. Transdifferentiation among lineages of the hematopoietic system was first reported more than two decades ago when pre-B cells were transformed into macrophages. Since then, other groups have reported producing hematopoietic cells of one lineage from another, such as neutrophils and macrophages from B and T cells. In all of those cases, however, drug treatment or ectopic forced expression of transcription factors or receptors was necessary to induce the switch. Such approaches, however, potentially alter normal cellular regulation and functions. Additionally, in some cases, the cells being switched were progenitor cells that still retain some degree of multilineage potential.

We show that a lineage switch, specifically from megakaryocytes to granuloyctes, within the hematopoietic system can be achieved by simply modifying the culture parameters, employing conditions that more closely mimic the natural *in vivo* environment. Megakaryocytes, derived from $CD34^+$ hematopoietic stem and progenitor cells (HSPCs), were isolated by MoFlo sorting using the megakaryocytic marker CD41. At the time of selection, they were negative for granulocytic markers CD15, CD11b and CD66b. After sorting, they were recultured in a media and cytokine cocktail normally used to induce granulocytic differentiation of $CD34^+$ HSPCs (G media). After two weeks of reculturing, flow cytometric and morphologic analysis revealed that the majority (70%) of cells had switched to become granulocytes, expressing CD15, CD11b and CD66b. Less than 1% of the cells still expressed CD41. Furthermore, approximately 20% of these switched granulocytes were in the segmented, or most mature, stage of differentiation. By comparison, two weeks after $CD34^+$ HSPCs were cultured in G media, 75% of the cells expressed CD15, CD11b and CD66b. However, less than 10% of those cells were in the segmented stage of differentiation. The kinetics and gene expression patterns of this two week reprogramming of megakaryocytes into granulocytes is currently being examined with immunofluorescence microscopy and single cell RT-PCR. Additionally, the conversion of granulocytes into megakaryocytes is also being pursued. Our findings will provide important insights into the transdifferentiation potential of more mature hematopoietic cells and may help redefine the hierarchical model of hematopoiesis. The ability to easily convert committed cells into those of a different lineage by using the appropriate culture conditions will also have enormous potential for clincial applications.