506d Apoptotic Signaling Pathways in Megakaryocytes

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High dose chemotherapy frequently results in the development of thrombocytopenia, characterized by a decrease in the number of platelets in the blood. Transplantation of lineage restricted progenitor cells may alleviate the state of low platelet levels. Therapies such as this call for the generation of large numbers of cells with the desired state of cell differentiation. In this case, optimal conditions of ex vivo megakaryocyte (Mk) expansion are critical. We propose that investigating the correlation between megakaryocyte maturation, apoptosis, and polyploidization will provide a better understanding of megakaryopoiesis and will aid in optimization of conditions for expansion of Mk cells ex vivo.

Megakaryocyte maturation involves the development of polyploid cells via endomitosis, a modified cell cycle in which several rounds of DNA replication occur without cytokinesis. DNA accumulation is associated with an increase in cytoplasmic volume and therefore indirectly regulates platelet production resulting from fragmentation of the mature megakaryocyte cytoplasm. This process of maturation is further characterized by the induction of apoptosis, which is thought to be correlated to and occur concurrently with platelet formation. We address the question of whether transiently inhibiting apoptosis can lead to a delay in the onset of platelet formation, increasing the number of viable Mk cells in culture. Studies focus on gene targets with known importance in both the extrinsic and intrinsic pathways regulating apoptosis including several caspases and the tumor suppressor protein p53.

The constitutive formation of proplatelet-bearing MKs has been reported by others to be a caspase-dependent process. Here we aim to examine the effect of caspase inhibition on Mk ploidy and percentage of CD41+ cells in culture. Human peripheral blood derived CD34+ cells were cultured in serum-free media in the presence of thrombopoietin (TPO) to induce Mk differentiation. Treatment of cells with the broad acting caspase inhibitor, z-vad.fmk, significantly reduced the number of higher ploidy Mk cells (DNA content >8N) present during the 15 days of culture. Caspase inhibition led to an increase in the percentage of CD41+ cells in culture in addition to reducing the number of proplatelet forming cells. We also plan to investigate whether caspase activation during megakaryopoiesis is dependent on the tumor suppressor protein, p53. Over the course of Mk maturation, protein levels of the various forms of p53 (phosphorylated, acetylated, wild-type) will be characterized along with the effect of p53 inhibition by pifithrin-alpha on megakaryocyte apoptosis and polyploidization.

Additionally, we aim to explore the potential influence of sirtuin protein activity on Mk maturation. Class III histone deacetylases (SIRT1-SIRT7) are members of the silent information regulator 2 (Sir2) family and have been suggested to play a role in lifespan extension. Specifically, SIRT1 deacetylates p53 leading to an inhibition of apoptosis. Our DNA microarray analyses show upregulated expression of both SIRT1 and SIRT7 in Mk cultures. We therefore will study the effect of sirtuin inhibition and activation on Mk expansion, apoptosis, and cell ploidy using the Class III histone deacetylase inhibitor and activator, nicotinamide and resveratrol respectively.

In conclusion, the use of chemical inhibitors provides a method for improving our understanding of the signaling pathways involved in Mk apoptosis. This increased knowledge may aid in the optimization of culture conditions for megakaryocyte expansion.