

APPROACHES FOR THE INVESTIGATION OF THE FUNCTION OF THE ADAPTER Grb2 IN MEGAKARYOCYTES

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Introduction: Thrombopoiesis is a complex and multistep process that begins with the commitment of pluripotent hematopoietic stem cells (HSCs) to proliferate and subsequently differentiate into the platelet precursor, the megakaryocyte. Differentiation of megakaryocytes is characterised by endomitosis which results in large polyploidy mature MKs and by proplatelets and platelet formation. The molecular mechanisms underlying megakaryocyte differentiation remain largely unknown. Grb2, an adapter protein highly expressed in platelets, is implicated in many signalling pathways such as the mitogen-activated protein kinase (MAPK) pathway (MEK-ERK), PLC γ 2 regulation and cytoskeleton remodelling. The MAPK MEK-ERK pathway has been shown to play a pivotal role in many essential cellular processes such as proliferation and differentiation. Studies using constitutively activated mutants and pharmacological inhibitors in megakaryocyte cell lines have shown a crucial role of MEK-ERK in megakaryocyte differentiation. In this study, we investigate the role of the adapter protein Grb2 and the MAPK MEK-ERK pathway in different aspect of the differentiation process of murine primary megakaryocytes.

Methods: The role of the MEK-ERK pathway in differentiation of megakaryocytes from HSCs isolated from bone marrow and foetal liver is analysed using specific MEK inhibitors (U0126 and PD184161). Inhibitors were added at varying times from the beginning of the differentiation process with the dose repeated every 24 hours. The role of Grb2 in primary megakaryocyte differentiation was analysed using short hairpin RNA (shRNA) to knockdown the level of the protein Grb2 in primary murine megakaryocytes with a good efficiency.

Results: The results demonstrate a critical role for MEK-ERK in events that underlie primary megakaryocyte differentiation. Knockdown of Grb2 demonstrated a critical role in regulating megakaryocyte differentiation and PLC γ 2. These results suggest that Grb2 and MEK-ERK play a pivotal role in the early programming of HSCs to megakaryocytes.

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