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IGBP1 protein as a regulator of mtor pathway and its role in haematopoiesis

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Aims: Haematopoiesis is the life-long process of blood cell formation derived from haematopoietic stem cells (HSC). In haematopoietic precursor cells, the PI3K/mTOR pathway plays an important role in controlling the balance between proliferation and differentiation. Grech et al., (2008) found that the mTOR regulatory protein Igbp1 is potent in arresting erythroblast differentiation to mature erythrocytes. Progenitor cells constitutively expressing Igbp1 in fact proliferate exponentially for at least 72 hours without evidence of differentiation.

PI3K activates mTOR through a cascade of phosphorylation events following the activation of PKB. The mTOR targets S6 kinase and 4E-BP1 enhance translation initiation of specific transcripts leading to increased protein synthesis and cell growth.In this study we specifically aim to investigate (1) the activation kinetics of mTOR targets by growth factors that regulate erythroid proliferation and (2) the role of protein phosphatase 2A (PP2A) on mTOR signaling with particular interest in the balance of haematopoietic cell proliferation versus differentiation.

Methods: The methodology mainly focuses on the investigation of the effects of PP2A inhibitor Igbp1 and PP2A activator FTY720 on the control of mTOR signaling in a murine cell culture model developed by Von Lindern et al., (2001). This immortal murine factor-sensitive erythroid progenitor clones are cultured in a serum-free culture medium (Stem Pro 34TM), and can be factor-programmed for renewal or terminal differentiation.

Western blotting is the technique of choice in ultimately establishing and targeting the protein interplay at the levels of phorphorylated p70-S6 kinase and unphosphorylated p70-S6 kinase. This will enable us to track the action of Igbp1 on PP2A, since sequestration of PP2A by Igbp1 will inhibit dephosphorylation of S6 kinase.

Results: Interestingly the synergistic action of erythropoietin and stem cell factor is required to phosphorylate S6K, giving a peak of S6K phosphorylation approximately 30 minutes after initial stimulation. Also, an experiment has been carried out to investigate FTY720 effects on growth of progenitor cells, were it was found that FTY720 attenuates cell growth in a dose-dependant fashion. Evidence of haemoglobin formation in cells dosed with FTY720 has been shown through haemoglobin assays, and thus we can anticipate that the reduced blast cell population was due to progenitor cell differentiation and not apoptosis. This shows that the block of differentiation empirically constituted by erythropoietin and stem cell factor is released by FTY720.

Conclusions: Characterization of the mTOR signaling pathway regulators open up additional possibilities for the development of anticancer drugs. The importance of growth regulation in carcinogenesis has been further supported by the discovery that for example rapamycin, rapamycin analogues and FTY720 modulate mTOR.

Therefore, the outcome of this study will eventually lead to understanding more the fundamental regulatory mechanisms involved in such an important pathway with potential identification of prognostic factors and therapeutic targets.