The role of G-CSF in the growth and development of skeletal muscle cells *in vitro*

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Background: Granulocyte-Colony Stimulating Factor (G-CSF) is a cytokine which stimulates the production of hematopoietic stem cells from bone marrow. Since its discovery and approval for clinical use, various roles for G-CSF outside the hematopoietic system have emerged. Recently, G-CSF treatment has been shown to increase skeletal muscle mass, strength and regeneration in rodent models of muscle disease and damage (Stratos *et al.*, 2007; Pitzer *et al.*, 2008). However, the molecular mechanisms underlining these responses are poorly understood. In cells expressing the G-CSF Receptor (G-CSFR), ligand binding activates several intracellular signalling cascades such as JAK/STAT, Akt, and ERK1/2 (Liongue *et al.*, 2009). These signalling pathways are of vital importance in the regulation of skeletal muscle during hypertrophy, atrophy and regeneration. However, it is unknown whether the G-CSFR is expressed in skeletal muscle, or if these signalling pathways are activated in response to G-CSF treatment.

Methods: *RT-PCR:* mRNA expression for the G-CSFR was determined by RT-PCR. The resulting PCR fragment was separated and purified from a 2% Agarose gel and sequenced. *Western Blotting:* Protein was separated on a polyacrylamide gel and transferred to PVDF membrane. The membrane was probed for the proteins of interest. *Proliferation:* C_2C_{12} proliferation was measured by the BrdU Labelling and Detection Kit III (Roche), according the manufacturers instructions. *Protein Degradation / Synthesis:* Protein synthesis and degradation was determined by the amount of radio-labelled H^3-tyrosine incorporated and released from the cells, respectively.

Results: The expression of the G-CSFR was detected in C_2C_{12} cultures by RT-PCR and western blotting, as well as in mouse and human muscle by western blotting and immunofluorescence. 30 min G-CSF (4ng/ml, 40ng/ml) treatment in C_2C_{12} myotubes increased the phosphorylation of STAT3. Preliminary data showed Akt and ERK1/2 phosphorylation was also increased. However, the rate of proliferation, protein synthesis and protein degradation remained unchanged under basal and catabolic conditions.

Summary/Conclusion: The expression of the G-CSFR in skeletal muscle suggests that G-CSF/G-CSFR may be of importance to muscle physiology. Activation of STAT3 signalling, and the potential activation of Akt and ERK1/2 in C_2C_{12} myotubes, elicits potential signalling pathways for G-CSF/G-CSFR in skeletal muscle. However, a functional outcome remains elusive.

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