Glucocorticoids regulate extracellular matrix gene expression of the ADAMTS5-versican enzyme-substrate axis in C2C12 skeletal muscle cells

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Versican is an extracellular matrix proteoglycan whose binding partners include hyaluronan, which is invariably linked to CD44, and tenascin C. It is remodelled (cleaved) by the ADAMTS proteoglycanases of which ADAMTS5 is the most prominent. Versican cleavage by ADAMTS enzymes results in a bioactive G1-DPEAAE fragment that can affect cell behaviour directly (*e.g.*, apoptosis (McCulloch *et al.*, 2009) or promote further remodelling of the extracellular matrix (*e.g.* accretion of hyaluronan; Murasawa *et al.*, 2013). Versican expression is increased during myogenesis and clearance of a versican and hyaluronan rich pericellular matrix by ADAMTS5 is necessary for efficient myoblast fusion (Stupka *et al.*, 2013). ADAMTS5 expression and activity is increased in regenerating muscle from dystrophic *mdx* mice and following myotoxin injury (*unpublished data*). The effects of versican remodelling by ADAMTS proteoglycanases on myoblast proliferation and migration are not well understood. Versican expression is greatly increased in fibrotic skeletal muscle from patients with Duchenne muscular dystrophy (DMD) but is absent in healthy, control muscle (Chen *et al.*, 2000). DMD is a fatal hereditary disease caused by a mutation in the dystrophin gene rendering skeletal muscles very susceptible to degeneration, excessive inflammation and fibrosis due to inadequate regenerative responses. There is no cure for DMD, although muscle function can be transiently improved by glucocorticoid treatment. The expression and activity of the versican-ADAMTS axis has been shown to be regulated by glucocorticoids in other diseases with a heightened pro-inflammatory and fibrotic state.

Using C2C12 cells, the aim of this study was to investigate the biological signficance of versican remodelling by ADAMTS proteoglycanases during myoblast proliferation and migration, and whether during myogenesis this axis is regulated by glucocorticoids. Scratch wound assays were performed on confluent C2C12 cells to assess the effects of adding exogenous full length V1 versican, the ADAMTS generated G1- DPEAAE versican fragment, and ADAMTS5. Full length V1 versican and ADAMTS5 had no effect on myoblast migration rate following 6 h and 11 h of treatment. However, the G1-DPEAAE fragment significantly reduced migration rate ($p < 0.05$); suggesting that excess production of the G1-DPEAAE fragment by ADAMTS enzymes may compromise myoblast migration and whose alignment is needed for myotube formation. To characterise the regulation of the versican–ADAMTS axis by glucocorticoids, proliferating C2C12 myoblasts were treated with 0 nM, 25 nM or 100 nM of dexamethasone in DMEM supplemented with 10% FBS for 48 hours, and differentiating C2C12 myoblasts were treated with 0 nM, 25 nM or 100 nM of dexamethasone in DMEM supplemented with 2% horse serum for 72 hours. In proliferating myoblasts, dexamethasone treatment reduced myoblast number ($p < 0.001$) as assessed using a WST-1 mitochondrial viability assay, and in differentiating myoblasts, dexamethasone enhanced myoblast fusion in a dose dependent manner ($p < 0.05$). Proliferating and differentiating myoblasts were also harvested for gene expression analysis of the versican-ADAMTS axis using quantitative RT-PCR. Preliminary findings indicate that the expression of *Adamts5* (*Vcan*) was suppressed by dexamethasone in differentiating myoblasts, whilst the expression of versican (*Vcan*) and another ADAMTS versicanase (*Adamts15*) was not altered. Thus, the versican-ADAMTS axis is targeted by glucocorticoids and represents a novel pathway for understanding the mode of action of these drugs in DMD. Follow-up *in vivo* studies using appropriate mouse models are now required to further investigate how these drugs regulate myogenesis in the presence of inflammation through the versican-ADAMTS axis.

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