Glucocorticoid treatment regulates versican pericellular matrix remodelling during myoblast fusion: implications for muscular dystrophy therapies

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Versican is a chondroitin sulphate proteoglycan (CSPG), synthesized as a transitional matrix that functions as a scaffold for mature ECM deposition. V0/V1 versican splice isoforms, the most abundant in skeletal muscle, comprises G1 and G3 globular domains at the N- and C-terminus respectively, and central glycosaminoglycan (GAG) domains to which chondroitin sulphate (CS) groups are covalently bound. CSPGs and CS are implicated in cell signalling and the regulation of growth factor bioavailability. Versican is proteolytically processed by the ADAMTS-1, -4, -5, -8, -9 -15 and -20 proteoglycanases, and its binding partners include hyaluronan. Versican expression is increased during myogenesis and clearance of a versican and hyaluronan rich pericellular matrix by ADAMTS5 and ADAMTS15 is necessary for efficient myoblast fusion (Stupka *et al.*, 2013).

Duchenne muscular dystrophy (DMD) is a fatal hereditary disease caused by a mutation in the dystrophin gene rendering skeletal muscles highly susceptible to degeneration, excessive inflammation and fibrosis. Versican expression is abnormally increased in skeletal muscle biopsies from patients with DMD, but absent in healthy, control muscle. An accumulation of chondroitin sulphate chains in DMD patient muscle biopsies compared to control biopsies has also been reported (Negroni *et al.*, 2014). Here, we used mdx dystrophin deficient mice and control C57BL/10 mice to characterise versican processing in hindlimb and diaphragm muscles. Mice were anaesthetised deeply with sodium pentobarbitone (60mg/ml) and killed by cardiac excision, and the tibialis anterior (TA) and diaphragm muscles were collected for immunohistological analysis. The animal studies were approved by the La Trobe University Animal Ethics Committee, in accordance with NH&MRC guidelines. In dystrophic *mdx* diaphragm and hindlimb muscles high levels of versican and versikine (ADAMTS generated N-terminal G1-DPEAAE versican fragment) were observed, with greater expression in the more severely affected diaphragm muscle.

There is no cure for DMD, although through the activation of various signalling pathways, including those relevant to regeneration and muscle function can be transiently improved by glucocorticoid treatment. Therefore, C2C12 myoblasts were used to examine the effects of glucocorticoids (dexamethasone) on pericellular matrix remodelling during myogenesis. Differentiating C2C12 myoblasts were treated with 0 nM, 25 nM or 100 nM of dexamethasone in DMEM supplemented with 2% horse serum for 3 to 4 days, with or without conditioned media containing exogenous V1 versican, versikine or the empty vector control (Stupka *et al.*, 2013).

Glucocortiocoid treatment enhanced myotube formation in C2C12 cells by targeting pericellular matrix synthesis and remodelling; versican and hyaluronan synthase-2 mRNA transcripts were decreased and ADAMTS-1 mRNA transcripts were increased following glucocorticoid treatment. Addition of exogenous V1 versican or versikine impaired glucocorticoid-mediated fusion demonstrating the effect to be versican-specific. Our study highlights the significance of excessive and persistent expression of versican and subsequent generation of versikine in dystrophic pathology of *mdx* mouse muscles.

Here, we identify a novel mechanism, whereby glucocorticoids regulate pericellular matrix synthesis and remodelling; thus glucocorticoids may mitigate the high levels of versican expression and processing observed in dystrophic muscles. Follow up studies in our laboratory are investigating the effects of a genetic reduction of versican on the dystrophic pathology of *mdx* mice by breeding them with *hdf* (heart defect mice), which are haploinsufficient for the versican allele.

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