The overexpression of Ndrg2 promotes C2C12 myoblast proliferation

K. Anderson, A.P. Russell and V.C. Foletta, Deakin University, 221 Burwood Highway, Burwood, VIC 3125, Australia.

N-myc downstream-regulated gene 2 (*Ndrg2*) is a stress-responsive gene that is highly expressed in skeletal muscle. *Ndrg2* expression levels are suppressed following anabolic treatments and following resistance training. Conversely, *Ndrg2* is induced in response to catabolic treatments or to acute exercise effects (Foletta *et al.*, 2009). In C2C12 myoblasts, the knockdown of NDRG2 slows proliferation and impairs differentiation due to early cell cycle exiting with the induction of cell cycle inhibitors, p21 Waf/Cip1 and p27 Kip (Foletta *et al.*, 2009). However, the effect of NDRG2 overexpression on C2C12 myoblast proliferation and differentiation and the protein region controlling the activity of NDRG2 are unknown.

Here, we investigated the overexpression of wild-type NDRG2 (WT) and a phospho-mutant version of NDRG2 (3A) where the amino acids Ser^{332}, Thr^{348}, Ser^{350} in the C-terminus were replaced by alanine residues. These sites were identified previously as potentially phosphorylated by the AGC kinases including PKCθ and Akt (Burchfield *et al.*, 2004). The impact of these overexpressed NDRG2 proteins on mouse C2C12 myoblast proliferation rates and fusion indices were measured in conjunction with protein markers of cell cycle control and myogenesis by western blotting. The stages of myogenesis were measured in sub-confluent myoblasts, confluent myoblasts, and days post differentiation (indicated by the number of days following the addition of differentiation media).

Compared with the empty vector control, increased levels of WT NDRG2 significantly enhanced proliferation rate by 1.4-fold ($p = 0.001$). The phospho-mutant 3A NDRG2 enhanced proliferation 1.2-fold greater than control ($p = 0.026$); however, proliferation rates were significantly less than WT NDRG2 ($p = 0.050$). The positive cell cycle regulator, Cyclin B1, was significantly increased 1.9-fold by WT NDRG2 ($p = 0.049$) while p27 Kip protein expression was significantly down regulated by 0.7-fold ($p = 0.007$) in sub-confluent myoblasts. 3A NDRG2 had no significant effect on Cyclin B1 or p27 protein levels. WT and 3A NDRG2 enhanced the protein expression of an early myogenic marker Myf5 by 1.9- and 1.6-fold ($p = 0.025$ and $p = 0.048$), respectively, in confluent myoblasts while WT NDRG2 also increased MyoD protein expression by 1.5-fold ($p = 0.022$) at day 0 of differentiation. WT NDRG2 overexpression induced a 2.1-fold increase in fusion index at day 2 of differentiation compared with control ($p = 0.002$) and 3A NDRG2 ($p = 0.029$). No differences were observed at later differentiation time-points or during myogenesis in Myogenin or MHC1 protein expression. Therefore, the overexpression of WT NDRG2 enhances the rate of myoblast proliferation. Additionally, amino acids Ser^{332}, Thr^{348}, Ser^{350} in the C-terminus of NDRG2 importantly contribute to the activity of NDRG2 in regulating myogenesis. Together, these results suggest that NDRG2 promotes myoblast growth and may be a future target for the treatment of skeletal muscle atrophy conditions.

Burchfield JG, Lennard, AJ, Narasimhan S, Hughes WE, Wasinger VC, Corthals GL, Okuda T, Kondoh H, Biden TJ & Schmitz-Peiffer C, (2004) *The Journal of Biological Chemistry*, **279**,18623-32.

Foletta VC, Prior MJ, Stupka N, Carey K, Segal DH, Jones S, Swinton C, Martin S, Cameron-Smith D & Walder KR, (2009) *The Journal of Physiology* **587**, 1619-34.