# COMMENTS

# Understanding Cellular Signaling Pathways and Their Relationship to Genotype and Phenotype of Muscle Disease

GAYLE A. BRAZEAU[1]

*Departments of Pharmacy Practice and Pharmaceutical Sciences, School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, Cooke 126 Dean's Office, Amherst, New York 14260*

With advances in genomics and proteonomics, the development of therapeutic approaches for the treatment of diseases is now being based on understanding cell signaling to identify molecular targets linked to the genetics abnormality or pathophysiology. In Duchenne Muscular Dystrophy (DMD), the absence of dystrophin has been postulated to result in the lack of a mechanical link with the dystrophin-glycoprotein complex (DGC), thus resulting in contraction-induced muscle degeneration as a consequence of a decrease in muscle plasma membrane stability and disruptions in the sarcolemma (1–2). As such, initial therapeutic approaches were targeted at providing this missing protein or reducing the extent of contraction-induced muscle degeneration. However, these approaches have shown limited success in preclinical or clinical studies in animal models or patients (3).

Exploring cellular signaling pathways that may be altered as a consequence of the lack of dystrophin or DGC provides an alternative approach to identifying molecular targets. For example, the lack of dystrophin has been associated with changes in skeletal muscle gene expression in extracellular matrix proteins, signaling proteins, and transporters (4–6). Likewise, DGC disruption has a potential role in altering cellular signaling through the protein kinase B (Akt) pathway (7).

The manuscript by Lang *et al.*, selected for the Best Paper Award in the Experimental Biology Category for 2004, is an excellent example of investigating signaling molecules in muscular dystrophy to identify possible therapeutic targets. Specifically, these investigators hypothesized that different signaling pathways would be distinctly activated depending on the severity of the dystrophic phenotype (8). These studies quantified phosphorylated and total expression of the 70-kd ribosomal S6 kinase ($P70^{S6K}$), the stress-activated protein kinase, SAPK (p38), and the extracellular regulated kinase (ERK1/2) in the costal diaphragm (DIA) and tibialis anterior (TA) muscle in mdx mice (a spontaneous mutation in the X-linked chromosome in inbred C57BL mice) and aged-matched control mice at 3 and 12 months of age.

The critical finding is a signaling response for p38 and $P70^{S6K}$ related to the dystrophic phenotype. In the DIA, which demonstrates a progressive pathology similar to DMD, the percent phosphorylation of p38 was decreased with no changes in the total p38 expression in mdx mice compared with controls at 3 and 12 months. In contrast, the TA, which undergoes extensive myofibrillar degeneration and regeneration at 3–5 weeks of age followed by a limited process of degeneration during the remaining lifespan, showed no changes in the total or phosphorylated p38 levels between mdx and age-matched controls at 3 and 12 months. However, for $P70^{S6K}$, involved with the PI3kinase-Akt-mTOR signaling pathway associated with muscle atrophy, the percent phosphorylation was significantly increased in mdx DIA compared with controls. The increased phosphor-

[1] To whom correspondence should be addressed at University at Buffalo, Cooke 126 Dean's Office, Amherst, New York 14260. E-mail: gbrazeau@buffalo.edu



1535-3702/05/2305-028915.00

ylation was partially attributed to increases at the critical site for $P70^{S6K}$ kinase activity. No significant changes were evident for $P70^{S6K}$ migration or phosphorylation in the mdx TA compared to controls. These findings suggest that p38 and $P70^{S6K}$ are not necessarily linked to the lack of dystrophin, but rather the change in their activity seems associated with the more severe dystrophic phenotype in the DIA.

Finally, the percentage phosphorylation of ERK1/2 between mdx and controls for both muscles and ages was not significant. Yet, total ERK1/2 expression in the mdx mice was increased compared to controls in both muscles. These findings implicate ERK1/2 in the dystrophic genotype but not necessarily the phenotype.

Overall, the importance of this work is that specific kinases, p38 and $P70^{S6K}$, are related to the dystrophic phenotype. The ERK1/2 pathway seems correlated to the genotype, the lack of dystrophin, but not to the disease progression in different muscles in this animal model. By understanding signaling pathways involved in the progression of DMD, rational therapeutic approaches can be developed to specifically stimulate or inhibit particular molecules or pathways to reduce the muscle degeneration, pathological hypertrophy, myofiber necrosis, and fibrosis. Furthermore, identifying target molecules provides a possible biomarker, thus allowing other scientists or clinicians to investigate the pharmacodynamics of existing and future drug therapies.

1. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of Duchenne muscular dystrophy locus. Cell 51:919–928, 1987.
2. Petrof BJ. The molecular basis of activity-induced muscle injury in Duchenne muscular dystrophy. Mol Cell Biochem 179:111–123, 1998.
3. Bogdanovich S, Perkins KJ, Krag TOB, Khurana TS. Therapeutics for Duchenne muscular dystrophy: current approaches and future directions. J Mol Med 82:102–115, 2004.
4. Chen YW, Zhao P, Borup R, Hoffman EP. Expression profiling in the muscular dystrophies: identification of novel aspects of molecular pathophysiology. J Cell Biol 151:1321–1336, 2000.
5. Thatchenko AV, Le Cam G, Léger JJ, Deschesne CA. Large-scale analysis of differential gene expression in the hindlimb muscles and diaphragm of mdx mouse. Biochim Biophys Acta 1500:17–30, 2000.
6. Thatchenko AV, Piétu G, Cros N, Gannoun-Zaki L, Auffray C, Léger JJ, Deschesne CA. Identification of altered gene expression in skeletal muscles from Duchenne muscular dystrophy patients. Neuromuscul Disord 11:269–277, 2001.
7. Langenbach KJ, Rando TA. Inhibition of dystroglycan binding to laminin disrupts the P13K/AKT pathway and survival signaling in muscle cells. Muscle Nerve 26:644–653, 2002.
8. Lang JM, Esser KA, DuPont-Versteegden EE. Altered activity of signaling pathways in diaphragm and tibialis anterior muscle of dystrophic mice. Exp Biol Med 229:503–511, 2004.