International Journal of Molecular Medicine and Advance Sciences 2 (1) : 76-83, 2006

Abnormal Myofibrillogenesis Potentiates Dystrophin Deficiency in Duchenne Muscular Dystrophy

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Abstract: Myofiber hypertrophy occurs in Duchenne muscular dystrophy and appears to predispose specifically to necrosis of affected muscle cells. Hypertrophy would constitute an abnormal response on the part of myofibers in muscular dystrophy that characterizes subsequent progression to necrosis. Aberrant myofibrillogenesis would be implicated in such a process in a manner that would potentiate dystrophin deficiency as the individual myofibers undergo hypertrophy. Abnormally induced gene transcription pathways would, in multiple ways, ultimately result in a complex setting that promotes sarcolemmal disruption. Only in terms of such potentiation can one recognize a tendency for myofiber hypertrophy in the first instance and a subsequent evolution to individual myofiber necrosis. Fibrosis develops as a series of injuries that are propagated by myofiber necrosis and loss, reflecting progressive and variable loss of dystrophin anchorage on sarcolemmae.

Key words: Myofiber, hypertrophy, muscular, dystrophy, myofibrillogenesis

INTRODUCTION

Myofiber necrosis in Duchenne muscular dystrophy results from membrane tears secondary to a defective subsarcolemmal dystrophin network. Such necrosis appears specifically reduced by increasing Insulin Growth Factor-1 within myofibers[1]. Also, cytoskeletal dystrophin in muscle LIM protein gene mutations can lead to dilated cardiomyopathy[2]. Treatment of fetal muscle with adenoviral vectors is attractive in view of the high density of Ad receptors, easy vector accessibility with an immature basal lamina and also the possibility of stem cell therapy[3].

Dystrophic myofibers reproduce cyclic contraction and relaxation phases that alternate in terms of variable parameters involving not only mechanical stress to the sarcolemma but also a full series of underlying pathogenic events that induce hypertrophy and also necrosis. Gene therapy depends on improving both skeletal muscle pathology and also the cardiomyopathy[4]. It is such a scenario of increased protein synthesis concurrently with myofiber degeneration that disrupts the sarcolemma and also disturbs myofibrillogenesis.

Arcolemmal proteins appear to destabilize the sarcolemma[5].

A mechanistic pathway of myofibrillar contraction promotes a dystrophin-deficient sarcolemma and induces loss of anchorage to the extracellular matrix. Adeno-associated virus vector-mediated gene therapy appears a probable means to improve myofiber survival by induced microdystrophin gene therapy[6]. It is nonpathogenic with minimal immunogenicity and also shows considerable cell and tissue tropism[7]. Blocking T cell and antigen-presenting cell interaction diminishes primary induced immunity[8].

Myofibrillogenesis as a source of mechanistic stress might call into focus a series of precipitated events that increase hypertrophy of fibers that evolve as myofiber degeneration. The necrotic myofibers reflect the marked creatine phosphokinase levels that are found in serum and also an increased sarcolemmal permeability. It is significant that in vivo evidence of pathology arising from muscle attachment failure is lacking and that dystrophy is primarily a specific tissue form of pathology[9].

The nature of myofiber permeability would account for increased influx and efflux of ions and enzymes. Myofiber hypertrophy might indicate an engendered predisposition to subsequent mechanistic stress that affects myofibrillogenesis as a central process somehow creating conditions for impending myofiber necrosis.

This should be considered in the context of the majority of boys with Duchenne muscular dystrophy having a deletion in the dystrophin gene[10]. The slowerevolution of Becker's muscular dystrophy correlates with partial functionality of dystrophin in these patients. Also, sarcolemmal (neuronal-type) nitric oxide synthase is absent with very low or absent dystrophin in critical regions of the rod domain[11]. The neuronal nitric

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oxide synthase transgene reduces muscle membrane injury without increasing compensatory expression of structural proteins[12].

CALCIUM ION SEQUESTRATION

Calcium ion sequestration within myofibers appears to implicate a full array of subsequent pathways in signaling and transcription that specifically involve the generation of contraction and relaxation of the myofibrillary apparatus that disrupts the sarcolemma. Increased permeability of the sarcolemma might be a feature that develops relative to a myofibrillogenesis and that developmentally renders the myofiber not only hypertrophic but also prone to aberrant reconstruction and adaptation pathways. This appears true in spite of significant latent regenerative capability of dystrophic muscle[13].

Aberrant differentiation as evidenced by the large hypertrophic fibers might specifically implicate the development of myofibrillary regeneration secondary to a defective sarcolemma.

It is important to recognize a certain phenotypic variability as demonstrated with caveolin 3 associated with dystrophin, dystrophin-associated glycoproteins and dysferlin[14]. Increased permeability might not only develop but also induce an increase in calcium ion influx and efflux within myofibers. Such a process would influence dynamics of Ca2+ sequestration and trafficking via the sarcoplasmic reticulum.

A distinct subpopulation of round cells with stem cell-like properties appears a useful source of donor cells in intramuscular transplantation and holds potential also for successful myogenic cell transfer therapy[15].

It is only insofar as contraction of the myofibrillary apparatus also implicates subsequent relaxation of the myofibers that there would develop dystrophin-deficient anchorage onto the sarcolemma and extracellular domains. Syntrophins regulate the localization of TAPP1, a pleckstrin homolog domain-containing adaptor protein in remodeling the actin cytoskeleton in response to growth factor stimulation[16]. The mdx mouse is often used experimentally to study the dystrophin-deficient state, including quantitative histologic parameters[17]. A major obstacle in studying the disease is the large gap between mouse models and the human disease[18].

CYCLES OF MYOFIBER HYPERTROPHY AND NECROSIS

The actual cycles of myofiber hypertrophy and of necrosis with ensuing fibrosis might implicate a signaling pathway that aberrantly induces operative attempts at myofiber reconstitution linked to increased sarcolemmal permeability to calcium ions. The dystrophin-associated protein complex appears implicated in signal transduction pathways[19]. It might be valid to reconsider how myofibrillogenesis as a developmental process involves the further generation of increasingly dystrophin-deficient anchorage onto the sarcolemma in Duchenne muscular dystrophy. Deficiency of dystrophin would evolve in further characterizing such a cycle of evolving compromise in sarcolemmal anchorage, as supported by lack of subcellular localization of dystrophin demonstrated by immunohistochemistry[20].

It is in terms of ongoing signaling pathways that myofibrillogenesis would also involve the subsequent transformation to a hypertrophic myofiber critically prone to undergo necrosis. In X-linked dilated cardiomyopathy, multiple different pathogenetic pathways are involved that are tissue specific for muscle[21].

In general, many genetically modified organisms do not express the expected phenotype associated with the gene or protein[22].

Actual dynamics of the dystrophic process might implicate in particular a tendency for evolving signaling pathways that on the one hand induce myofiber contraction and on the other promote active myofiber necrosis.

Deflazacort, a subtype corticosteroid, attenuates degeneration of myofibers by upregulating activity of calcineurin, with activation of the calcineurin/NF-AT pathway of gene expression[23].

Understanding such dual pathways would involve aberrant myofibrillogenesis that aberrantly handles calcium ions in a manner that specifically affects the sarcoplasmic reticulum. Large released quantities of calcium from the endoplasmic reticulum would characterize large hypertrophic myofibers that progressively become more deficient in dystrophin-mediated anchorage as aberrant myofibrillogenesis progresses. In hypertrophic feline muscular dystrophy with dystrophin deficiency, there are frequent and widespread electromyographic changes that include myotonic discharges and fibrillation potentials[24].

SIGNALING PATHWAYS

Distinct signaling pathways such as extracellular regulated kinase (ERK 1/2), p70S6 kinase (p70(S6k)) or p38 signaling are differentially activated in skeletal muscle of mdx mice[25].

The signaling pathways involved in contraction of an aberrant myofibrillary apparatus would evolve as

calcium ion hyperpermeability of the sarcolemma and as increased sequestration of the calcium ion within the sarcoplasmic reticulum. Insofar as such dynamic events do develop in relative cooperative fashion with aberrant myofibrillogenesis of the hypertrophic myofiber, there would also evolve a dystrophin deficiency that renders the sarcolemma more prone to disruption. In denervation of muscle, complex molecular changes in the regulation of gene expression and trafficking of the dystrophin-dystroglycan complex may develop[26].

Signaling of gene transcription pathways appears an integral component[27] that is induced by events leading directly to aberrant myofibrillogenesis. Understanding how calcium ions are often present in normal, free-ion cytoplasmic concentrations within the dystrophic myofiber might implicate active sequestration within such organelles as mitochondria and nucleus.

It is in terms of such abnormal calcium ion sequestration that the dystrophic process proves a relentless progression of signaling pathways via systems of pronounced injury to the sarcolemma enveloping the myofibers perhaps predestined for necrosis.

MYOFIBER NECROSIS

Cell necrosis of myofibers might specifically induce cycles of recurrent damage via transcription gene pathways that disrupt also sarcoplasmic reticulum and mitochondria. Utrophin may function as a substitute for dystrophin and its overexpression ameliorates mdx muscle pathology. Post-transcriptional regulation for utrophin may be implicated[28]. Heregulin ameliorates the dystrophic phenotype in the mdx mouse by mediating kinase dependent activation of the GABP (alpha/Beta) transcription factor complex[29]. In a final analysis, one might indeed view dystrophy as a series of signaling pathways engendered in the first instance by an array of agonists that potentiate or counteract effects of calcium ion sequestration by the sarcoplasmic reticulum.

Myofiber differentiation appears integral to growth as evolving regeneration of the myofibrillary apparatus linked to calcium ion handling. Skeletal muscle proteome expression differentially evolves with growth or aging of the mdx mouse. The actual cascade of events in myofiber degeneration remains undetermined in Duchenne muscular dystrophy[30].

Also, changes in muscle activity appear to influence progression of the dystrophic process, possibly by inducing expression of new genes, or replacement of dystrophin by other molecules[31]. Cytokeratins are expressed in adult striated muscle and contribute to organization of both myoplasm and sarcolemma. Cytokeratin 8 and 19 interact with dystrophin at its actin-binding domain. Cytokeratins are disrupted at the sarcolemma in the mdx mouse[32].

Storage of calcium ion implicates release and signaling pathways ranging from the ryanodine receptor to troponin protein complex mediation and subsequently to the myofibrillar structures. Binding dynamics in calcium handling might involve modes of myofiber necrosis whereby mechanistic stress evolves also as myofibrils accumulate and hypertrophy.

DYNAMICS OF CALCIUM ION BINDING

The actual dynamics of calcium ion binding involves signaling pathways that constitute modes of trafficking implicated in gene transcription. Dystrophin could play a regulatory role in calcium homeostasis and act as one functional link amongst many between calcium signaling and cytoskeleton[33]. It is perhaps in terms of binding dysequilibrium that myofibers undergo progressive dystrophic types of degeneration linked to specific patterns of myofibrillogenesis.

Muscle stretching induces activation of Ca2+ and Na+ channels in dystrophy, and blocking such channels may possibly protect against myofiber degeneration[34]. Reduced expression of key Ca2+ binding proteins affecting Ca2+ buffering in the sarcoplasmic reticulum may disturb uptake and release of Ca2+ during the excitation-contraction-relaxation cycle in dystrophic myofibers. Calsequestrin and sarcalumenin that bind Ca2+ are markedly reduced in cardiac mdx fibers[35]. Specifying such dynamism might necessitate the categorization of calcium ion signaling as a binding receptor process entailing active transcription pathways as evolving myofibrillogenesis.

Hence, one might equate myofibrillogenesis with an ongoing process of receptor calcium ion binding that becomes aberrant as the myofiber progressively hypertrophies.

Myofiber subunits are arranged as separate components and reflected in the mosaic pattern characteristically demonstrated with adenosinetriphosphatase staining at different pH values. It would appear that such structuring of separate myofiber units integrally corresponds to an axonal mode of participation in ongoing maintenance of the myofibrillary apparatus. Changes in distribution of acetylcholine receptors in the neuromuscular junction possibly result from release or response to tropic factors such as calcitonin gene-related peptide[36].

The defective neuromuscular structure in some dystrophin-glycoprotein complex mutants may result from

secondary loss of nitric oxide synthase from muscle[37].

Indeed, myofibrillar maintenance and replacement as differentiation, growth and regenerative processes would enhance susceptibility to an ongoing dystrophy of the myofibers in Duchenne's muscular dystrophy.

The tendency for myofiber regeneration would go hand in hand with a propensity for hypertrophy that subsequently resolves as final necrosis pathways involving the myofibers as functional and anatomic subunits. Dystroglycan spans the cell membrane and is central to stabilization of the myofiber sarcolemma as a component to the dystrophin-glycoprotein complex. As such, it is pivotal in determining ongoing necrosis of dystrophic myofibers in Duchenne muscular dystrophy[38].

ABERRANT TRANSCRIPTION PATHWAYS

Integral events of a process of active degeneration of myofibers would arise from transcription pathways that aberrantly evolve in terms of a dystrophy that renders the myofibers prone to necrosis. The Myo D family of basic helix-loop-helix myogenic regulatory factors determines proper myogenic gene activation and program expression. They may critically modulate myofiber phenotype[39].

It is in terms of such myofiber necrosis arising directly from attributes of aberrant transcription pathways that dystrophin deficiency also evolves in its own right. Molecular recognition by the glycosyl transferase LARGE is essential for expression of functional dystroglycan[40]. Subsarcolemmal networks of dystrophin molecules that connect defectively with cytoskeletal actin and with sarcolemmal dystrophoglycans would render mechanistic pathways a prominent feature in active dystrophy. The exact role of the dystrophin-glycoprotein complex remains unclear however but m utated gene snf-6 induces phenotypes indistinguishable from those of the dystroglycan complex mutants---SNF-6 mediates acetylcholine uptake at neuromuscular junctions in periods of increased synaptic activity. Also dystroglycan complex gene mutations induce SNF-6 loss at neuromuscular junctions with prolonged myofiber excitation[41].

Indeed, multiple pathways would be implicated in calcium ion signaling that lead to a sarcolemmal disruption arising also as aberrant transcription of membrane receptors, including novel functions and signaling roles for dystroglycan[42].

Receptivity binding of calcium linked to a firing frequency of supplying neuronal axons would participate in ongoing dystrophy as an aberrant transcription that paradoxically initiates hypertrophic response and subsequently necrosis of the hypertrophied myofiber.

MECHANISTIC STRESS

Muscular dystrophy should perhaps be recognized as primarily a myofiber necrosis that only subsequently evolves as mechanistic stress-induced injury[43] to the sarcolemma. In such a framework of transcriptionally mediated necrosis of the myofiber the deficiency in dystrophy is only one integral component in accompanying aberrant transcriptional pathways of protein synthesis. The dystrophin muscle enhancer sequences appear important in activating the dystrophin cerebellar Purkinje promoter in skeletal muscle[44] but not in cardiac muscle. Postsynaptically mediated long-term depression is reduced in mdx Purkinje cells leading possibly to the cognitive deficit[45]. Beta-dystroglycan as a transmembrane protein is indeed targeted to the plasma membrane but inaccessible at least to immunolocalization and the usual solubilizing methods[46]. *Ex-vivo* gene therapy would offer potential improvement in Duchenne muscular dystrophy by transfection of the dystrophin gene into the patient's own myogenic precursor cells, followed by transplantation.

Nucleofection and the phiC31 integrins may permit safe autotransplantation[47].

Calcium sparks would be an expression of how sequestered calcium in the sarcoplasmic reticulum becomes an expression of an ongoing endoplasmic reticulum involvement that drives in part the aberrant transcription pathways. Recognition of transcription events as dysregulatory gene activity would implicate calcium ions that enter the nucleus and render the nuclear membrane a feature also of cytoskeletal injury of the myofiber.

Intra-arterial injection of naked plasmid DNA induces stable expression of dystrophin in 1-5% of limb myofibers for at least 6 months in the mdx mouse using either the cytomegalovirus promoter or a mouse-specific human desmin gene control region[48].

LOSS OF CYTOSKELETAL INTEGRITY

Loss of cytoskeletal integrity would specifically characterize the myofiber dystrophic process linked to multiple pathways of calcium ion-mediated injury associated with aberrant gene transcription. Synthetic antisense oligonucleotides (splicomers) may block pre-mRNA splicing at specific exons, affecting constitutively spliced exons in mouse dystrophin RNA[49].

Skipping of the nonsense mutation-encoding exon may be achieved using antisense oligonucleotides composed of chimeric RNA/ethylene bridged nucleic acids[50]. Electroporation-assisted plasmid-based gene

transfer is a potential alternative to viral transfer vectors of the dystrophin gene[51]. Hematopoietic stem cell transplantation intramuscularly does not restore dystrophin expression[52]. Inclusion of vascular endothelial growth factor/vascular permeability factor may enhance systemic gene transfer to striated muscle using adenovirus-related vectors[53,54].

In muscular dystrophy ongoing events of macrophage infiltration of the myofibers and the subsequent necrosis would implicate fibrogenesis due to a loss of the integral subunit organization of muscle tissue. Realized evolution of myofiber necrosis appears in part mediated by macrophage action that disrupts further the sarcolemma and organelles in a manner conducive to further necrosis afield.

Macrophage propagation of myofiber dystrophic necrosis would specifically participate in terms of the abnormally differentiated myofiber and its transcription machinery.

A distinguishing feature of dystrophic necrosis appears to involve a cytoskeletally mediated series of pathways that induce further evolution of the characteristic fibrosis that subsequently disrupts normal structural and functional attributes underlying contraction/relaxation of surviving myofiber units.

There is deposition of extracellular matrix protein components, type 1 collagen and laminin. This process appears influenced by sex hormones with increased expression in male mdx mice[55].

CONTRACTION/RELAXATION OF MYOFIBERS

It is in terms particularly of relaxation of myofibers that one might view the dynamics of myofibril disorganization that entail both hypertrophy and subsequent necrosis. Changes in Ca2+ may probably be more significant in inducing myofiber degeneration than stretch-induced injury with increased membrane permeability[56]. Aberrant differentiation pathways that specifically affect possible regenerative attempts of dystrophin-deficient myofibers would render cytoskeletal injury a persistent stimulus for ongoing myofiber necrosis in the face of macrophage infiltration of weakly regenerative myofibers.

CALCIUM ION-MEDIATED DYSREGULATION

Abnormal ion handling appears to render dystrophin-deficient myofibers more susceptible to necrosis. There is reduced Ca2+ buffering capacity in the sarcoplasmic reticulum and decreased calsequestrin expression[57]. Increased Ca2+ concentration in the cytosol promotes myofiber necrosis in muscle dystrophy. The inositol 1,4,5-trisphosphate receptors are involved in calcium release in the excitation and contraction of Duchenne dystrophic myofibers, with consequent induced myofiber degeneration[58]. Intracellular Ca2+ is elevated in Duchenne muscular dystrophy. The dystrophin glycoprotein complex appears to be a matrix laminin, G-protein-coupled receptor[59].

Crucial pathways of calcium ion-mediated dysregulation of gene transcription might implicate cytokine production in terms of Tumor Necrosis Factor alpha and Interleukins that induce macrophages to ingest myofibers.

Nuclear Factor kappaB would perhaps serve together with S100 proteins in terms of an aberrant myofibrillogenesis that ultimately involves necrosis as an endpathway in myofiber loss. The vigorous fibrogenesis implicates blood delivery of cytokines to the myofiber bed.

The inflammatory components are influenced by prednisolone.that reduces cellular adhesion molecule expression[60]. Inflammation-induced transcription pathways execute both hypertrophy and necrosis of myofibers. These two distinct processes would subsequently interact and potentiate each other.

CONCLUSIONS

The recognition of dystrophic processes strongly implicates aberrant transcriptional pathways in differentiation of the dystrophic myofiber as a separate subunit of the skeletal muscle and also as a reflection of potential plastic response to injury. The whole array of events that propagate in positive feedback fashion a series of calcium-ion mediated effects ranges from sarcolemmal disruption with deficiency of dystrophin to mechanistic pathways of induced stress implicating possibly variable axonal frequency discharge. It is in terms of an aberrant myofibrillogenesis that deficiency of dystrophin would prove a crucial factor determining the progression of a dystrophic process that is primarily fibrogenic as an ultimate consequence to myofiber necrosis.

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