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Expression profiling with progression of dystrophic change in dysferlin-deficient mice (SJL)

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Abstract

The SJL mouse is a model for human dysferlinopathy (limb-girdle muscular dystrophy type 2B and Miyoshi myopathy). We used cDNA microarrays to compare the expression profiles of 10,012 genes in control and SJL quadriceps femoris muscles in order to find genes involved in the degeneration and regeneration process and in dysferlin's functional network. Many genes involved in the process of muscle regeneration are observed to be up-regulated in SJL mice, including cardiac ankyrin repeated protein (CARP), Neuraminidase 2, interleukin-6, insulin-like growth factor-2 and osteopontin. We found the upregulation of S100 calcium binding proteins, neural precursor cell expressed, developmentally down-regulated gene 4-like (NEDD4L) with C2 domain, and intracellular protein traffic associated proteins (Rab6 and Rab2). These proteins have the potential to interact with dysferlin. We must reveal some other molecules which may work with dysferlin in order to clarify the pathological network of dysferlinopathy. This process may lead to future improvements in the therapy for human dysferlinopathy.

Keywords: Dysferlin; SJL mouse; Microarray; Muscular dystrophy; Miyoshi myopathy; C2 domain

1. Introduction

Limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM) are caused by mutations in the dysferlin gene (Aoki et al., 2001; Bashir et al., 1998; Illa et al., 2001; Liu et al., 1998). The dysferlin gene product is a member of homologous proteins called the ferlin family that include the *C. elegans* spermatogenesis factor fer-1 (Achanzar and Ward, 1997), otoferlin (Yasunaga et al., 1999), the third human FER-1-like protein (Britton et al., 2000) and myoferlin (Davis et al., 2000). Dysferlin has a single carboxyl-terminal transmembrane domain and six C2 domains that are predicted to reside in a large cytoplasmic domain (Liu et al., 1998). C2 domains are present in many membrane-associated proteins (Nalefski and Falke, 1996). For example, synaptotagmins contain two C2 domains and are implicated in membrane fusion and membrane repair (Chakrabarti et al., 2003; Fernandez-Chacon et al., 2001).

Abbreviations: cDNA, complementary deoxyribonucleic acid; cRNA, complementary ribonucleic acid; CARP, cardiac ankyrin repeated protein; Cspg2, chondroitin sulfate proteoglycan 2; Cxcl, CXC chemokine ligand; EAE, experimental allergic encephalomyelitis; GTP, guanosine 5′-triphosphate; HRP, horse radish peroxidase; IGF2, insulin-like growth factor-2; LGMD2B, limb-girdle muscular dystrophy type 2B; MCP, monocyte chemotactic protein; MHC, major histocompatibility; MIP, macrophage inflammatory protein; MM, Miyoshi myopathy; Mmp3, serine protease inhibitors matrix metalloproteinase 3; Myla, myosin light chain; alkali; cardiac atria; mRNA, messenger ribonucleic acid; NEDD4L, neural precursor cell expressed; developmentally down-regulated gene 4-like; nNOS, neuronal nitric oxide synthase; Neu2, Neuraminidase 2; OSF2, osteoblast specific factor-2; PBS, phosphate buffer saline; Rrad, Ras-related associated with diabetes; RT-PCR, reverse transcriptase polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Scya, Small inducible cytokine A; TGF-beta1, transforming growth factor beta 1; Timp, tissue inhibitor of metalloproteinase; Tnnt2, cardiac troponin T2; VLDLR, very low-density lipoprotein receptor

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An abundance of intracellular vesicles and membrane abnormality are features of dysferlin-deficient muscle (Piccolo et al., 2000; Selcen et al., 2001). The substitution of regions of the plasma membrane with layers of vesicles and membranous projections can be observed by electron microscopy in the majority of fibers (Selcen et al., 2001). Therefore, the function of dysferlin is hypothesized as maintaining the structural integrity of the muscle fiber plasma membrane. Recent data have emerged supporting a role of dysferlin in the repair of the plasma membrane (Bansal et al., 2003).

In this report we thoroughly examined SJL mice which show a deficiency of dysferlin (Bittner et al., 1999) and inflammatory changes in muscle. The spontaneous myopathy of SJL mice begins at 4–6 weeks of age and is nearly complete by 8 months of age. The gene abnormalities of patients with dysferlinopathy consist of stop codon or frame shift mutations. On the other hand, the mutation in SJL mice is an in-frame deletion of 171 base pairs. This is probably one of the reasons why the phenotype of the SJL mice is milder than that of patients with dysferlinopathy, as in the case of Becker type muscular dystrophy as compared to Duchenne type (Chelly et al., 1990; Monaco et al., 1988). Assuming that the structure of the gene is conserved between mice and human, this in-frame deletion predicts the removal of 57 amino acids, including most of the fourth C2 domain. This C2 domain extends from amino acids 1582–1660, whereas the deletion spans amino acids 1628–1685.

Campanaro et al. (2002) performed expression profiling in dysferlinopathy patients and pointed out an upregulation of proteins interacting with calcium. Recently, Lennon et al. (2003) reported the expression pattern of the SJL mice and focused on annexins, widely expressed Ca^{2+} and phospholipid binding proteins. They proposed a central role for dysferlin in patch fusion events that comprise a novel wound healing model in skeletal muscle sarcolemma. However, there are probably many more genes involved in intracellular protein traffic and calcium binding which may interact with dysferlin. And not much was done to clarify the aspects of inflammation and regeneration in these reports. This study was carried out to find other factors involved in the pathogenesis of dysferlinopathy, especially in membrane repair and the regeneration process.

2. Materials and methods

2.1. Animals

All mice were handled according to approved animal protocols in our institution. Quadriceps femoris were dissected from 2-month-old male control (C57BL/6NCrj), 2-month-old male SJL (SJL/JOrllcoCrj) mice and 9-month-old male SJL mice (Charles River Japan Inc., Yokohama, Japan). We previously examined muscles of the two strains of mice to investigate the muscle pathology and the progression of myopathy (Hinuma et al., in preparation). In 2-month-old SJL mice, there were slight or no muscle changes compared to control mice. On the other hand, 9-month-old SJL mice had an increased variety of fiber diameters, degenerating fibers, regenerating fibers, and an increased inflammatory response. Therefore, we used these two time points for the early and progressive stage. To minimize variability, each group of nine mice was divided into three groups of three mice. RNA was extracted from one quadriceps from each mouse using TRIzol reagent (Invitrogen, Carlsbad, CA). The remaining muscles were retained for subsequent immunohistochemical and western blot analysis.

2.2. Target preparation

Samples were prepared and labeled for use on CodeLink Uniset Mouse I Expression Bioarray (Amersham, Piscataway, NJ). The UniSet Mouse I Expression Bioarray used in these experiments contains an array of 10,012 probes within a single reaction chamber on a single slide. All oligonucleotide probes are 30 bases long. The details of the process have been described elsewhere (Ramakrishnan et al., 2002). Briefly, total RNA was extracted from the quadriceps femoris. Then we synthesized and purified cDNA. Then, the cRNA was synthesized and purified and was assessed for the concentration, yield and quantity. A set of bacterial control mRNAs is included in this procedure as a control for the cDNA and the in vitro transcription reaction.

2.3. Array hybridization

Fragmented target cRNA was used for the hybridization of each chip. The hybridization solution was heated at 90 °C for 5 min to denature the cRNA and chilled on ice. Then the slides were incubated for 18 h at 37 °C while shaking. After hybridization each slide was washed in buffer. The signal was developed using streptavidin. Processed slides were scanned using a GenePix Scanner (Axon Instruments, Foster City, CA). Slides were scanned using CodeLink. Expression Scanning Software (Amersham Biosciences, Piscataway, NJ) and images of each slide were analyzed using the same software.

2.4. Data analysis

For the comparison of muscle expression data between SJL mice and controls, values for each transcript across all muscle samples were imported into Excel (Microsoft, Redmond, WA), and the output was then analyzed statistically with Cyber T. Cyber T is a statistical program based on Student's *t*-test that is designed for output data from large-scale microarray experiments (Baldi and Long, 2001; Long et al., 2001; Winokur et al., 2003). Transcripts were considered to be significantly dysregulated if they displayed a two-fold or greater fold change and had a Cyber T generated P-value < 0.05.