J Appl Physiol 93: 537–545, 2002.
First published April 19, 2002; 10.1152/japplphysiol.00202.2002.

Regenerated *mdx* mouse skeletal muscle shows differential mRNA expression

B. S. TSENG,[1,*] P. ZHAO,[2,*] J. S. PATTISON,[3,*] S. E. GORDON,[3] J. A. GRANCHELLI,[4] R. W. MADSEN,[5] L. C. FOLK,[6] E. P. HOFFMAN,[2] AND F. W. BOOTH[3]
[1]*Division of Child Neurology, Department of Neurology, University of California at San Francisco, San Francisco, California 94143;* [2]*Research Center for Genetic Medicine, Children's National Medical Center, Washington, District of Columbia 20010; Departments of* [3]*Veterinary Biomedical Sciences,* [5]*Statistics, and* [6]*Veterinary Pathobiology, University of Missouri at Columbia, Columbia, Missouri 65211; and* [4]*Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York 14260-1300*

Received 11 March 2002; accepted in final form 15 April 2002

Tseng, B. S., P. Zhao, J. S. Pattison, S. E. Gordon, J. A. Granchelli, R. W. Madsen, L. C. Folk, E. P. Hoffman, and F. W. Booth. Regenerated *mdx* mouse skeletal muscle shows differential mRNA expression. *J Appl Physiol* 93: 537–545, 2002. First published April 19, 2002; 10.1152/japplphysiol.00202.2002.—Despite over 3,000 articles published on dystrophin in the last 15 years, the reasons underlying the progression of the human disease, differential muscle involvement, and disparate phenotypes in different species are not understood. The present experiment employed a screen of 12,488 mRNAs in 16-wk-old mouse *mdx* muscle at a time when the skeletal muscle is avoiding severe dystrophic pathophysiology, despite the absence of a functional dystrophin protein. A number of transcripts whose levels differed between the *mdx* and human Duchenne muscular dystrophy were noted. A fourfold decrease in myostatin mRNA in the *mdx* muscle was noted. Differential upregulation of actin-related protein 2/3 (subunit 4), β-thymosin, calponin, mast cell chymase, and guanidinoacetate methyltransferase mRNA in the more benign *mdx* was also observed. Transcripts for oxidative and glycolytic enzymes in *mdx* muscle were not downregulated. These discrepancies could provide candidates for salvage pathways that maintain skeletal muscle integrity in the absence of a functional dystrophin protein in *mdx* skeletal muscle.

Duchenne muscular dystrophy; dystrophin; GeneChips; microarrays

DYSTROPHIN SERVES AS A membrane-associated protein that interfaces with cytoskeletal actin networks, signaling, and transmembrane proteins (14, 22). Inherited abnormalities of the dystrophin protein have been associated with different phenotypes. For example, patients with Duchenne muscular dystrophy (DMD) (complete loss of function with dystrophin abnormality) lose the ability to walk by the age of 12 yr and eventually succumb to respiratory failure by the second or third decade of life, whereas Becker muscular dystrophy patients (partial loss of function with dystrophin abnormality) are still able to walk at the age of 15 yr and typically do not undergo respiratory failure until after the fourth decade (6). In contrast, the muscles of DMD patients appear to undergo continuous cycles of degeneration and regeneration, with a gradual failure of regeneration. Histological examination of DMD muscle fibers has created the concept that the manifestation of this gene defect triggers a pathological cascade, including the following: *1*) membrane fragility, *2*) aberrant calcium homeostasis, *3*) mechanical susceptibility to injury, *4*) activated degradative mechanisms (e.g., calpain), *5*) fibrofatty replacement, *6*) failure of regenerative and/or repair systems, and *7*) others, including vascular ischemia (6, 14, 22). However, the absence of dystrophin is not always this devastating, especially in other mammalian species.

A genetic homolog model of DMD is the *mdx* mouse, first identified as dystrophin deficient in 1989 (21). The causative mechanism is a point mutation in exon 23, resulting in a premature stop codon (15). The *mdx* mouse has the critical hallmarks of DMD, including loss of dystrophin-associated proteins, a susceptibility to contractile-induced damage, elevated serum creatine kinase, and muscle-fiber degeneration (34). However, whereas the *mdx* mouse shows a complete loss of function of the dystrophin protein, it has a mild clinical course compared with DMD, with an early episode of widespread skeletal muscle necrosis at 3–4 wk of age. The *mdx* muscle then shows subsequent regeneration followed by a relative resistance to further degeneration of skeletal muscle for a period of some months, with only minor physical impairments (decreased voluntary wheel-running) (9, 47), although increased disabilities do appear after 11 mo of age. The progression of the murine disease appears to be slower, with very

*B. S. Tseng, P. Zhao, and J. S. Pattison contributed equally to this work.

Address for reprint requests and other correspondence: F. W. Booth, Dept. of Veterinary Biomedical Sciences, Univ. of Missouri, E102 Vet Med Bldg., 1600 E. Rollins, Columbia, MO 65211 (E-mail: boothf@missouri.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

delayed fibrofatty infiltration (13, 31). It is our hypothesis, previously stated by Infante and Huszagh (22), that the *mdx* mouse staves off severe disability with differential gene effector(s) and/or responder(s) that protect 16-wk-old *mdx* muscle from damage, and that this differential expression will show discordant expression profiles with DMD. The goal of this report was to use a global mRNA expression profiling to compare and contrast the response of human and mouse skeletal muscle to the same biochemical defect (dystrophin deficiency). We focused on *mdx* muscle that had successfully regenerated (e.g., static stable phase of the disease) and compared these findings with a previous report of 6- to 9-yr-old boys with active DMD disease (10). The differentially expressed transcripts are potential candidates for conferring protection to murine dystrophin-deficient muscle and are, therefore, possible therapeutic targets for modulation in the progressive skeletal muscle dystrophinopathies in humans.

METHODS

Animals. Normal [C57Bl10 (black 10)] and *mdx* [C57Bl10 (black 10) *mdx*/*mdx*] mice were bred by Dr. Joseph A. Granchelli (University of New York at Buffalo, New York) from the original breeding pairs supplied by Jackson Laboratories (Bar Harbor, MA).

RNA processing. Sixteen-week-old males were killed by cervical dislocation. Sixteen weeks of age was selected to target *mdx* skeletal muscle in its relative "benign" steady-state condition, after the acute degeneration-regeneration phase at ~3–4 wk of age. Gastrocnemius muscles were excised and flash-frozen in isopentane cooled with liquid nitrogen. Both gastrocnemius muscles from a single mouse formed one observation, with $n = 4$ for control and $n = 4$ for *mdx* groups. All muscle samples were processed in parallel and hybridized such that muscle mRNA from each individual animal was applied to an individual array. Muscles were powdered in liquid nitrogen with mortar and pestle and then in TRIzol (GIBCO BRL, Gaithersburg, MD) by using a Polytron (Kinematica, Lucerne, Switzerland) on setting 7 for three pulses of 15 s. Total RNA was extracted according to the guanidine thiocyanate method of Chomczynski and Sacchi (11). Poly(A)$^+$ mRNA was isolated from total RNA by using OligoTex columns (Qiagen, Valencia, CA). One microgram of mRNA was allowed to hybridize with an oligo T7-(dT)24 primer for cDNA synthesis (Genset Oligos, Huntsville, AL) followed by first- and second-strand synthesis with Superscript Choice (GIBCO BRL). The resulting cDNA was transcribed in vitro with biotinylated nucleotides from a BioArray high-yield kit (Enzo Diagnostics, Farmingdale, NY). A final cleanup of the cRNA was performed with an RNeasy kit (Qiagen). Biotinylated cRNA samples were hybridized to Affymetrix murine genome U74Arev2 arrays and analyzed by fluorescent intensity scanning according to Affymetrix protocols (Affymetrix Expression Analysis Technical Manual). The hybridization and scanning of the arrays was performed in the laboratory of Dr. Eric P. Hoffman at Children's National Medical Center (Washington, DC).

GeneChip array analysis. Of the 12,488 gene sequences offered on the Affymetrix murine genome U74Arev2 GeneChip array, ~6,000 have been functionally characterized in the mouse UniGene database (build 74). Additionally, ~6,000 expressed sequence tag (EST) clusters were also analyzed on these arrays. The probe set for each transcript consists of 16 different, perfectly matched (complementary) 25-base segments corresponding to different regions along the length of a transcript. Similarly, 16 mismatched pairs, which do not complement perfectly the transcript's sequence, containing one incorrect base, are used as a measure of nonspecific binding. The mismatched probes' fluorescence intensity is subsequently subtracted (like background) from the perfect-match intensity to yield a more accurate reading of a transcript's relative expression. All preliminary analyses of each array were carried out with Microarray suite 4.01 (Affymetrix). The average difference intensity information for each gene was exported into Excel or Access (Microsoft, Redmond, CA). Statistical analyses were done in Excel. Some genes did not show measurable expression, and their average-difference intensity values were near or below zero. For those that were less than zero, average intensity values had to be reset to a small, positive value to perform statistics. We chose to reset such values to 20 average difference-intensity units. Others have performed similar corrections (33, 46).

Two scans were taken of each array: a preconjugate antibody scan (S1) and a scan after biotin, streptavidin, and phycoerythrin amplification (S2). If probe sets were deemed to be "saturated" on the S2, then the normalized (postscaling) average difference values of S1 were used for that probe set. We deemed a probe set to show evidence of saturation for seven genes when a comparison analysis of S1 vs. S2 for the same GeneChip showed a significant difference between average difference values (e.g., the normalized average difference value was not reproduced between S1 and S2 for the same probe set). Finally, all remaining calls with a false discovery rate (FDR) of <0.01 and a change greater than twofold were screened for the number of positive probe pairs contributing to the average differences, where at least one-half of the 16 probe pairs for each gene must have been positive to make our final published list in Table 1.

Statistical methods. First, an unequal variance, two-tailed *t*-test was used to compare transcript expression intensities between control and *mdx* groups (values are expressed as means ± SE; $n = 4$) for 12,488 mRNAs. Second, the FDR procedure (3) was used as a criterion for deciding which *t*-test results should be called significant. By setting the FDR criterion at 0.01, 1% (on the average) of the results called significant by the *t*-test may not be true rejections of the null hypothesis.

To determine which mRNAs were differentially expressed, a new statistical method was employed that consists of a simple, sequential Bonferroni-type procedure to account for the large number of tests done by controlling the FDR for independent test statistics (3). Others have previously applied this approach for microarray analysis (8, 12, 45). The FDR is a new approach to multiple-hypotheses testing, such as for thousands of mRNAs. The FDR is the expected proportion of true null hypotheses rejected out of the total number of null hypotheses rejected (3). Multiple-comparison procedures controlling the FDR are more powerful than the commonly used multiple-comparison procedures based on the family wise error rate (3). FDR controlling procedures are especially suited to large multiple-comparison problems, which compensate for the lack of power in existing procedures (3).

RESULTS

The wet weight of the gastrocnemius in *mdx* mice (158 ± 9 mg) did not differ (21% greater, $P = 0.13$) from that in control mice (130 ± 13 mg). Sixteen-week-old *mdx* mice have previously been reported to have 17, 21,

Table 1. *mRNAs differentially expressed in 16-wk-old mdx gastrocnemius muscle compared with age-matched controls*

Name	Accession No.	I/D	Fold Change	Student's *t*-test *P* Values	FDR-adjusted *P* Values
α-Actin cardiac	M15501	I	6.1	3×10^{-5}	0.0042
ADP-ribosylation-like factor 6 interacting protein	AW122878	I	2.1	4×10^{-5}	0.0049
Amyloid β A4 precursor binding protein	AF020313	I	5.5	9×10^{-6}	0.0030
Annexin A1	M69260	I	3.5	9×10^{-6}	0.0030
Annexin A1	M69260	I	4.4	4×10^{-5}	0.0048
Annexin A1	AV003419	I	3.9	5×10^{-5}	0.0052
Apolipoprotein C1	Z22661	I	~12	2×10^{-4}	0.0096
B-cell leukemia/lymphoma 2-related protein A1b	U23778	I	6.5	1×10^{-4}	0.0090
Calcyclin calcium binding protein A6	X66449	I	2.0	1×10^{-5}	0.0033
Capping protein (actin filament) gelsolin-like	X54511	I	3.4	1×10^{-4}	0.0090
Caspase 3	U54803	I	~21	1×10^{-5}	0.0033
Caspase 11	Y13089	I	~12	8×10^{-6}	0.0028
Cathepsin B*	M65270	I	3.5	4×10^{-5}	0.00063
Cathepsin H*	U06119	I	11	3×10^{-6}	0.0020
Cathepsin K	AJ006033	I	3.3	3×10^{-5}	0.0042
Cathepsin L*	X06086	I	2.3	7×10^{-8}	0.00030
Cathepsin S	AJ223208	I	18	2×10^{-4}	0.0100
CD52/CD80	M55561	I	9.8	1×10^{-7}	0.00045
Cell surface glycoprotein CD53	X97227	I	~75	2×10^{-4}	0.0100
Clathrin light chain 2	U91848	I	2.1	6×10^{-5}	0.0060
CMRF35 leukocyte immunoglobulin-like receptor	AW060457	I	4.9	4×10^{-5}	0.0048
Collagen type VI α3-subunit	AF064749	I	4.5	6×10^{-6}	0.0026
Complement component factor H	M12660	I	2.1	9×10^{-7}	0.0010
Complement component 4 within H-2S	X06454	I	2.3	2×10^{-4}	0.0097
Complement inhibitory protein CD59	U60473	D	2.5	5×10^{-5}	0.0060
Complement receptor 3 β-subunit MAC-1	M31039	I	~8.6	2×10^{-5}	0.0042
Coronin	AW123801	I	~12	3×10^{-5}	0.0044
Cyclin D3	M86183	I	4.4	1×10^{-5}	0.0033
Cyclin-dependent kinase inhibitor 1 AP21	AW048937	I	3.4	1×10^{-4}	0.0084
Cystatin B	U59807	I	2.4	2×10^{-5}	0.0042
Cysteine rich intestinal protein	M13018	I	2.2	3×10^{-6}	0.0020
Cytochrome b_{245}, α-polypeptide	AW046124	I	6.9	2×10^{-8}	0.00013
Dok2, downstream of tyrosine kinase 2	AF059583	I	4.6	2×10^{-4}	0.0096
Ena-vasodilator stimulated phosphoprotein	U72519	I	3.0	2×10^{-5}	0.0042
Endoplasmic reticulum protein 29	AI835644	I	2.6	2×10^{-4}	0.0100
Epithelial membrane protein 1	X98471	I	3.4	2×10^{-4}	0.0097
EST/unknown function	AI848671	I	2.3	1×10^{-5}	0.0033
EST/unknown function	AI849082	I	3.4	5×10^{-5}	0.0057
EST/unknown function	AI854154	I	3.0	7×10^{-5}	0.0064
EST/unknown function	AI465845	I	2.8	7×10^{-5}	0.0065
EST/unknown function	AW060556	I	4.2	7×10^{-5}	0.0066
EST/unknown function	AI853900	I	2.2	1×10^{-4}	0.0079
EST/unknown function	AI504338	D	2.6	1×10^{-4}	0.0092
EST/unknown function	AI509811	I	2.1	2×10^{-4}	0.010
Eukaryotic translation elongation factor 1 α1	M17878	I	3.8	1×10^{-4}	0.00063
Fibronectin 1*	M18194	I	3.5	1×10^{-5}	0.0033
Folate receptor 2	M64817	I	2.8	3×10^{-5}	0.0042
Follistatin-like	M91380	I	3.2	9×10^{-7}	0.0010
Guanidinoacetate methyltransferase	AF010499	I	3.1	1×10^{-5}	0.0033
H19 fetal liver protein	X58196	I	2.9	6×10^{-6}	0.0006
Heme-oxygenase decycling 2	AF054670	I	4.1	1×10^{-4}	0.0090
Histocompatibility 2 class II antigen A α	X52643	I	23	2×10^{-8}	0.00013
Histocompatibility 2 class II antigen β1	M21932	I	~10	6×10^{-6}	0.0026
Histocompatibility 2 class II antigen I–E	X00958	I	5.5	5×10^{-6}	0.0025
Histocompatibility 2 2T region locus 23	Y00629	I	2.4	9×10^{-5}	0.0075
Histocompatibility 2Q region locus 2	X58609	I	2.9	1×10^{-4}	0.0091
Homology of 89% to human actin-related protein 2/3	AW212775	I	2.5	3×10^{-6}	0.0042
Homology of 91% to rat calponin	AW125626	I	2.3	9×10^{-5}	0.0075
Homology of 92% to rat cytochrome *P*-450	AA212964	I	4.1	1×10^{-4}	0.0096
Homology of 82% to human membrane-spanning 4-domains, subfamily A, member 6A	AI504305	I	16.0	7×10^{-6}	0.0028
Homology of 97% to mouse 26S protein regulatory subunit S12	AW120643	I	2.5	1×10^{-4}	0.0077
Homology of 94% to human thyroid hormone receptor-associated protein 240	AI049144	I	2.0	1×10^{-4}	0.0094
HSP cognate 70 (testis)	L27086	D	2.9	1×10^{-5}	0.0033

Continued

Table 1.—*Continued*

Name	Accession No.	I/D	Fold Change	Student's *t*-test *P* Values	FDR-adjusted *P* Values
HSP70	M12571	D	5.1	3×10^{-6}	0.0020
HSPC280	AA833425	I	6.0	2×10^{-5}	0.0035
Integral membrane protein 2B	U76253	I	2.1	2×10^{-5}	0.0042
Interferon-γ induced Mg11	U15635	I	4.3	8×10^{-6}	0.0028
IQ motif containing GTPase activating protein 1	AW209098	I	2.4	8×10^{-5}	0.0069
Kidney cell line derived transcript 1	U13371	I	2.4	2×10^{-5}	0.0034
Kinesin-related protein	AW121381	D	2.2	3×10^{-5}	0.0042
Legumain	AJ000990	I	4.6	5×10^{-5}	0.0052
Leukocyte specific protein 1	D49691	I	5.3	5×10^{-5}	0.0052
Leptin receptor gene-related protein A	AJ011565	I	2.3	2×10^{-4}	0.0098
Long chain fatty acyl-CoA synthetase	U15977	D	2.1	5×10^{-5}	0.0057
Lysosomal thiol reductase IP30 precursor	AI844520	I	17	4×10^{-5}	0.0049
Lysozyme M*	M21050	I	13	5×10^{-6}	0.0025
Mannose-6-phosphate receptor	X56831	I	2.0	9×10^{-7}	0.0010
Mannosidase 2, α1	X61172	I	2.1	2×10^{-5}	0.0042
Mast cell chymase 2	M68899	I	5.1	3×10^{-5}	0.0045
Microsomal glutathione *S*-transferase 3	AI843448	D	2.4	3×10^{-5}	0.0047
Moesin	AI839417	I	2.5	5×10^{-5}	0.0057
Myogenin*	X15784	I	4.0	6×10^{-5}	0.0063
Myoglobin	X04405	I	2.2	2×10^{-4}	0.00059
Myosin-binding protein H	U68267	I	2.7	6×10^{-5}	0.0061
Myosin light-chain cardiac ventricles	X12972	D	3.7	2×10^{-5}	0.0033
Myostatin/growth differentiation factor 8*	U84005	D	4.0	4×10^{-5}	0.0049
Nebulin-related anchoring protein	U76618	D	2.2	5×10^{-5}	0.0057
Neutrophil cytosolic factor 2 $p67^{phox}$	AB002664	I	~25	2×10^{-5}	0.0034
Neutrophil cytosolic factor 4	U59488	I	~11	2×10^{-4}	0.0100
Nuclear matrix attachment DNA binding protein	U05252	D	2.2	1×10^{-4}	0.0092
Ortholog of 98% to rat β-1,2-*N*-acetylglucosaminyltransferase II (Gnt II)	AI117848	I	2.3	9×10^{-5}	0.0076
Ortholog of 92% to human cathepsin B*	AW212271	I	2.3	2×10^{-5}	0.0042
Ortholog of 93% to human calreticulin	AI047107	I	2.3	4×10^{-5}	0.0051
Ortholog of 93% to rat dithiolenthione-inducible gene-1 (DIG-1)	AA596710	I	5.8	7×10^{-6}	0.0028
Ortholog of 96% to rat epididymal secretory protein E1 precursor	AB021289	I	4.0	2×10^{-5}	0.0041
Ortholog of 85% to human fatty acid coenzyme ligase long chain 5	AI838021	I	2.3	3×10^{-5}	0.0042
Ortholog of 95% to human protein tyrosine phosphatase, receptor type C	AW122012	I	3.4	2×10^{-4}	0.010
Ortholog of 93% to human Rab8	AI842492	I	~8.5	8×10^{-6}	0.0028
Orzin	AA790307	I	9.6	2×10^{-5}	0.0034
Paired related homeobox 2	X52875	I	3.4	1×10^{-4}	0.0083
Peroxiredoxin 4/antioxidant enzyme AOE372	U96746	I	3.7	6×10^{-5}	0.0059
Peroxisomal biogenesis factor 11a	AF093669	D	2.2	4×10^{-5}	0.0051
PFTAIRE protein kinase 1	AF033655	I	4.3	9×10^{-5}	0.0077
P glycoprotein 2 (multidrug resistance protein)	J03398	D	2.2	2×10^{-6}	0.0016
Phospholipid transfer protein	U28960	I	4.1	5×10^{-5}	0.0057
Plastin 2 L	D37837	I	~14	7×10^{-5}	0.0066
Procollagen type III, α_1*	X52046	I	7.1	8×10^{-5}	0.0031
Procollagen type VI, α_1	X66405	I	2.3	5×10^{-5}	0.0053
Properdin factor	X12905	I	9.8	2×10^{-5}	0.0042
Prosaposin	U57999	I	2.0	2×10^{-4}	0.0096
Protein kinase C delta binding protein	AW048944	I	1.6	2×10^{-4}	0.0096
Purine-nucleoside phosphorylase	U35374	I	3.3	1×10^{-4}	0.0092
PW1 zinc finger transcription factor	AV353105	I	2.8	3×10^{-5}	0.0042
Ribonuclease RNase A family 4	AI840339	I	2.7	1×10^{-4}	0.0094
Ribosomal protein mitochondrial S7	AW122030	I	5.9	1×10^{-5}	0.0033
S-adenosylmethionine decarboxylase 1*	D12780	D	5.1	5×10^{-5}	0.0017
S-adenosylmethionine decarboxylase 2	Z23077	D	5.5	2×10^{-4}	0.0098
S100 calcium-binding protein A4	M36579	I	3.7	3×10^{-7}	0.00071
S100 calcium-binding protein A11	U41341	I	3.2	5×10^{-5}	0.0057
S100 calcium binding protein A13	AV007820	I	4.6	3×10^{-5}	0.0042
Serine/cysteine proteinase inhibitor clade H Serpinh 1 member 1s	X60676	I	2.5	1×10^{-4}	0.0087
Serping 1 complement component factor 1 inhibitor	AF010254	I	2.5	2×10^{-5}	0.0035
SOX11	AF009414	I	6.0	2×10^{-4}	0.0098
Spermidine/spermine N1-acetyl transferase	L10244	I	3.1	9×10^{-5}	0.0076
Src-associated adaptor protein	AB014485	I	~41	8×10^{-5}	0.0072
STAT 6	L47650	I	3.7	1×10^{-4}	0.0090
TCFCP2-related transcriptional repressor	AA734817	D	4.0	2×10^{-4}	0.0098
Tenascin C*	X56304	I	3.0	2×10^{-4}	0.0100

Continued

Table 1.—*Continued*

Name	Accession No.	I/D	Fold Change	Student's *t*-test *P* Values	FDR-adjusted *P* Values
TGF-β	L19932	I	2.8	9×10^{-5}	0.0076
Thymosin, β4	U38967	I	2.3	5×10^{-7}	0.00063
Tubulin α_2*	M28727	I	3.1	1×10^{-6}	0.0013
Tubulin β_2	M28739	I	4.7	2×10^{-6}	0.0014
Tubulin β_3	AW050256	I	2.0	2×10^{-4}	0.0098
Tum-transplantation antigen P198	X51528	I	2.6	3×10^{-5}	0.0042
Ubiquitin-specific protease UBP41	AF079565	D	2.6	2×10^{-5}	0.0038
VCAM-1	M84487	I	~7.0	8×10^{-6}	0.0028
VLDL receptor	AA408956	D	2.3	1×10^{-4}	0.0089
Williams-Beuren syndrome chromosome 5 homolog WBSCR5	AW125574	I	~8.5	4×10^{-5}	0.0048
Xenotrophic and polytrophic retrovirus receptor 1	AI648965	D	2.8	1×10^{-4}	0.0090

Fold changes are relative to control where control = 1.00, I, fold increase; D, fold decrease. Student's *t*-test results are shown. False discovery rate (FDR) adjusted *P* values are given. MAC-1, membrane attack complex-1; EST, expressed sequence tags; HSP, heat shock protein; TGF, tubuloglomerular feedback; VCAM-1, vascular cell adhesion molecule 1; VLDL, very low-density lipoprotein. *Matches similar directional change reported in the literature for 11 mRNAs or proteins in *mdx* skeletal muscle. No discordances in directionality with previously published *mdx* were found. ~, 1 of the samples had an average difference <10.

and 0% larger extensor digitorum longus, soleus, and plantaris muscles, respectively, than age-matched mice (20).

Extracted RNA per milligram of muscle wet weight was twice as high ($P = 0.0004$) in the *mdx* (2.4 ± 0.12 μg RNA/mg muscle) than in the control (1.2 ± 0.11 μg RNA/mg muscle) muscle, verifying an earlier report (29). Equal quantities of mRNA from each mouse's gastrocnemius muscles were applied to each of the GeneChip microarrays. Results in Table 1 are thus the relative amount of mRNA per microgram of RNA extracted from each mouse's muscle and hybridized on an array. Because extracted RNA per whole gastrocnemius muscle was more than twice ($P = 0.0006$) in *mdx* (371 ± 15 μg RNA/whole muscle, $n = 4$) than in the control (155 ± 7 μg RNA/whole muscle, $n = 4$) group, the fold increase of mRNAs for the entire *mdx* muscle would actually be greater than fold increases reported in Table 1. Thus the underestimated fold changes in Table 1 are due to their differences in RNA abundance, which differed between control and *mdx* muscles. In four control and four *mdx* muscles, 5,304 ± 111 and 5,977 ± 430 genes, respectively, were detected as "present" above background with the use of Affymetrix Microsuite 4.01 software on the U74Arev2 GeneChip arrays. One muscle sample in each group was tested in duplicate to verify chip-to-chip reproducibility. All raw data and interpretation files are available on the Children's National Medical Center Microarray website (http://microarray.cnmcresearch.org).

With the use of a FDR of 0.01, 137 transcripts had a $P < 0.0002$ in the unequal-variance *t*-test and a twofold change, which were considered significant (Table 1). Eighty-six percent (i.e., 124) of the significant differences represented in Table 1 were upregulated transcripts in *mdx* muscle, compared with control, and 14% (i.e., 23) were downregulated. With the FDR set at 1%, 137 results were found significant. Therefore, we can expect approximately 1 of these 137 results (on the average) to be wrongly rejected, a true null hypothesis. One hundred fifteen of these mRNAs were associated with known descriptions or attributes indicating cell-type specificity and/or function, whereas 22 were ESTs. Of these ESTs, 14 were homologous or orthologous to known genes in other species, and 8 had no known similarities. No mRNAs in Table 1 were found to have a discordant directional change compared with 13 published analyses for mRNAs and proteins from *mdx* muscles. In agreement with previous reports, the results show increases in mRNAs from *mdx* muscles for myogenin (44), α_2-tubulin (44), H19 (44), lysozyme M (16, 44), α_1(III) procollagen (18, 44), and cathepsin B (16), whereas decreases in mRNAs for *S*-adenosylmethionine decarboxylase (44) and myostatin (44) have also been reported. The results in Table 1 also agree with the immunohistochemical level increase reported for tenascin C (41), fibronectin (26), myogenin (23), cathepsin B (40), cathepsin H (40), and cathepsin L (40). Comparisons to published human DMD mRNA analyses identified novel discordant directional changes in mRNA for β-thymosin, calponin, follistatin-like, myogenin, guanidinoacetate methyltransferase, and mast cell chymase (Table 2). Fold changes for many genes for metabolic proteins were less in 16-wk-old *mdx* than control muscles (Table 3).

DISCUSSION

Despite thousands of publications on dystrophin, the reasons for the progression of the human disease, differential muscle involvement, and different phenotypes in various species are not fully understood. Sander et al. (39) described this dystrophin mechanistic mystery as follows: "Despite a wealth of recent information about the molecular basis of DMD, effective treatment for this disease does not exist because the mechanism by which dystrophin deficiency produces the clinical phenotype is unknown." The strategy employed in the present study was to identify those mRNAs that were differentially expressed in a mouse model (*mdx*) without full-length dystrophin protein, but whose skeletal muscle at 16 wk of age shows successful regeneration. One hundred thirty-seven mRNAs were found to be different from age-matched

Table 2. *Comparison of changes in mRNAs from control in skeletal muscles between* mdx *mice and Duchenne muscular dystrophy patients*

Gene	I/D	*mdx* Mouse, fold change	I/D	Human, fold change	Human Accession No.
Actin-related protein 2/3 (subunit 1B)	I	2.47	I No change	3.5 (subunit 1A) (subunit 4)	AF006084 AF006087
Thymosin, β4	I	2.33	No change		D82345
Calponin	I	2.28	No change No change		D17408 D83735
Follistatin-like	I	3.22	No change		U06863 D89937
Myogenin	I	4.04	No change		X17651
Guanidinoacetate methyltransferase	I	3.08	D	3.45	Z49878
Mast cell chymase 2	I	5.05	No change		M64269

Human fold changes are from Ref. 10.

normal mice of the same strain. A second strategy was then to compare the 137 differentially expressed *mdx* transcripts with those previously found discordant in DMD muscles (10) (i.e., having altered expression in *mdx*, but not in DMD, muscle at a time when a relative rescue from further degeneration was occurring in the *mdx* muscle). The goal was to identify candidate genes that may confer protection against dystrophin-deficiency-induced myofiber damage. Our initial effort may be limited by the comparison of a single-time-point mouse microarray data against human DMD microarray data because of differences in the following: species, age, temporal disease course, muscle specificity, posture, diet, nocturnal habit, and statistical methods.

Dystrophin and its associated proteins function to link the intracellular actin cytoskeleton of muscle to laminins in the extracellular matrix. The actin-filament binding activity of dystrophin has been well characterized, where multiple actin binding sites cause a side-by-side alignment of actin filaments along dystro-

Table 3. *Comparison of fold changes in mRNAs from control for metabolic proteins between 16-wk-old* mdx *and Duchenne muscular dystrophy*

Duchenne Muscular Dystrophy		*mdx*		
Name	Fold change	Fold change	Accession no.	Name
NADH-ubiquinone oxidoreductase (51-kDa)	−6	−1.33	AI837493	NADH-ubiquinone oxidoreductase (49-kDa subunit)
NADH-ubiquinone oxidoreductase flavoprotein	−3	−1.42	AI835847	NADH-ubiquinone oxidoreductase (13-kDa subunit)
		−1.49	AI853863	NADH-ubiquinone oxidoreductase (9-kDa subunit)
		−1.71	AI835847	NADH-ubiquinone oxidoreductase (13-kDa subunit)
Mitochondrial NADH dehydrogenase-ubiquinone Fe-S protein 8	−5	−1.74	AA590675	Mitochondrial NADH dehydrogenase-ubiquinone Fe-S protein 8
Phosphoglycerate mutase	−4	−1.30	AF029843	Phosphoglycerate mutase, muscle
Mitochondrial aldehyde dehydrogenase I	−4			
		−1.16	AI647493	Mitochondrial aldehyde dehydrogenase 2
		−1.40	AV329607	Mitochondrial aldehyde dehydrogenase 2
		−1.77	U07235	Mitochondrial aldehyde dehydrogenase 2
Glutamine oxaloacetate transaminase (M37400)	−4	−1.02	X07302	Glutamine oxaloacetate transaminase
Glutamine oxaloacetate transaminase (M22632)	−2			
Glycogen phosphorylase	−3	−1.70	AI850363	Glycogen phosphorylase
Phosphorylase kinase-1	−3	−1.58	J03293	Phosphorylase kinase,
Succinyl CoA synthetase	−3	−1.29	AF058955	Succinyl CoA synthetase, β-subunit
Cytochrome-*c* oxidase subunit Va	−2			
		−1.32	V53157	Cytochrome-*c* oxidase subunit Vb
		−1.56	U08439	Cytochrome-*c* oxidase subunit VIaH
		1.53	U08440	Cytochrome-*c* oxidase subunit VIaL
		−1.50	AF037370	Cytochrome-*c* oxidase subunit VIIa-H
		−1.26	AF037371	Cytochrome-*c* oxidase subunit VIIa-L
		−1.80	X52940	Cytochrome-*c* oxidase subunit VIIc
Aconitase	−2	1.26	X61147	Aconitase
Lipoprotein lipase	−2	−2.86	M63335	Lipoprotein lipase
		−2.00	AA726364	Lipoprotein lipase
3,2-CoA-isomerase	−2	−1.37	Z14050	3,2-CoA-isomerase
Somatic cytochrome-*c*	−2	−1.54	X01756	Somatic cytochrome-*c*
Glycogenin	−2	−1.52	AW049730	Glycogenin
		−1.90	AV357306	Glycogenin

Duchenne muscular dystrophy fold changes are from Ref. 10.

phin and protect actin filaments from depolymerization in vitro (35, 36). This interaction leads to a strong association of γ-actin filaments with the plasma membrane, but this association is completely lost with dystrophin deficiency (37). Thus our observations of the differential upregulation in the more benign *mdx* than in the more devastating DMD of some actin-associated mRNAs whose proteins regulate actin polymerization suggest a new hypothesis of a potential rescuing role by the cytoplasmic actin remodeling, which can be subsequently tested. Transcripts for actin-related protein 2/3 (subunit 4), β-thymosin, and calponin were all increased in the *mdx* muscles but, although present on the human microarray, were not increased in the DMD muscle (Ref. 10; Table 2). The actin-related protein 2/3 complex is the cellular factor that generates new actin filaments (branching) in a site-directed, signal-controlled fashion at the leading edge of motile cells (4, 28) and forms identical branches in vitro (32). Alteration of the bimodal spatial stability by cytoskeletal actin network remodeling with branching processes near the cell membrane has been proposed by Sambeth and Baumgaertner (38) to be essential for the induction of a spontaneous breaking of isotropic cell motion observed in processes such as the amoeboid crawling of animal cells in advancing neural growth cones. Supporting this actin remodeling postulate is the 2.3-fold increase in β-thymosin mRNA, as β-thymosin binds to actin monomers, facilitating their polymerization into filaments (14). Future experiments at the protein level would be required to test this hypothesis of whether these mRNA differences are reflections of the differences in the severity of the phenotype in the absence of intact dystrophin protein or due to differences between species.

Myostatin mRNA in *mdx* muscle was only 25% of the level found in controls (Table 1). Tkatchenko et al. (44) previously detected myostatin mRNA downregulation in the *mdx* mouse using suppression subtractive hybridization but made no mention of its possible significance. We have also observed decreased myostatin mRNA in DMD skeletal muscle (unpublished observations). Myostatin is a transforming growth factor-β family member that acts as a negative regulator of skeletal muscle mass, because mice without this gene exhibit hypertrophy (27). This adaptation might play some role in the sporadic vs. widespread fiber hypertrophy and/or maintenance of muscle mass and functional rescue of *mdx* muscle, which is known to be a factor in the compensatory strength of *mdx* mice. The enhanced regenerative capacity of *mdx* muscle is in concordance with the upregulation of myogenin mRNA, a key myogenic differentiation gene for skeletal muscle fiber development (23). Future experiments should test the candidate genes identified here at the protein level and should functionally test their relative impact on dystrophic muscle.

Other mRNAs were differentially expressed between *mdx* and DMD muscles. For example, mast cell chymase mRNA was increased fivefold in *mdx* muscle (Table 2) but was unchanged in DMD muscle (10). At the 16-wk-old age selected, *mdx* muscle essentially has no fibrosis (7) compared with the 6- to 9-yr-old subjects with DMD. Mast cell chymase activates matrix metalloproteinases (which degrade the extracellular matrix) and processes precollagenases, whose product degrades collagen and cleaves fibronectin (43). Mast cell inhibitors resulting in extracellular matrix degradation provide a potential mechanism for improving *mdx* muscle strength (19). Whereas guanidinoacetate methyltransferase mRNA increased 3.1-fold in *mdx* muscle, it was decreased fourfold in DMD muscle (10). As guanidinoacetate methyltransferase catalyzes the last step in creatine biosynthesis, we speculate that its increase in *mdx* muscle could reflect a crucial cellular response that increases at an mRNA level (and potentially at the protein level) in an attempt to compensate for the leak and loss of creatine kinase (30). Markers of apoptosis (increased caspase, decreased 70-kDa heat shock protein) and increased protein degradation (cathepsins, lysosomal proteins) occurred in the 16-wk-old *mdx* muscles, suggesting continuing remodeling.

In muscle biopsies from male 6- to 9-yr-old Duchenne patients, Chen et al. (10) observed a greater than twofold downregulation of 26 mRNAs for proteins that are involved in mitochondrial function and energy metabolism, which they suggested indicated a generalized mitochondrial dysfunction and "metabolic crisis." Mitochondrial dysfunction has been reported previously by using a variety of assays in both human dystrophy patients and animal models (1, 17, 25). Another difference between *mdx* and DMD muscles is the amplitude of decrease of those transcripts for mitochondrial and metabolic enzymes changed in the 16-wk-old *mdx* muscle (Table 3). Previously in DMD muscle, mitochondrial and metabolic transcripts have been reported to decrease two- to sixfold (10), whereas many of the same mRNAs showed less than a twofold decrease in *mdx* muscle (Table 3 and Ref. 17). However, because RNA per gram of 16-wk-old *mdx* muscle was twice that of age-matched controls, the estimated concentration of mRNA for mitochondrial transcripts per gram of 16-wk-old *mdx* muscle is essentially unchanged (½ mRNA/RNA times 2× RNA/g = unchanged mRNA/g), in contrast to the decrease found in DMD muscle (10).

The extensive signaling and cell receptor mRNAs (29 different transcripts) altered in *mdx* muscle call attention to far more complex signaling changes to produce these differences in mRNA responses due to the loss of functional dystrophin expression than heretofore appreciated. A number of these mRNAs showed largefold changes. For example, the mRNA of the cell-surface glycoprotein CD53 increased 71-fold in *mdx* muscle. CD53 is a transmembrane-4 superfamily (TM4SF) protein (see Ref. 48 for references). TM4SF proteins can regulate cell signaling, motility, and tumor cell metastasis. TM4SF proteins also tend to assemble into protein complexes at the plasma membrane, where they may recruit growth factor ligands and phosphatidylinositol 4-kinase into proximity with integrins. The Src-associated adaptor protein (RA70) mRNA increased 41-fold in *mdx* muscle. RA70 is highly homol-

ogous to human Src kinase-associated phosphoprotein (SKAP55) and, according to Kouroku et al. (24), may play an essential role in the Src signaling pathway in various cells. The p67^{phox} mRNA increased 25-fold in *mdx* muscle. The assembly of a membrane-associated flavocytochrome b_{559} with the cytosolic proteins p47^{phox} and p67^{phox} and the small GTPase Rac (1 or 2) activate the superoxide (superoxide anion)-generating NADPH oxidase of phagocytes (1). These changes in signaling transcripts may provide new directions for investigation.

The present approach identified mRNAs differentially expressed in only *mdx* or DMD muscles. Because both lack appropriate expression of dystrophin protein with different phenotypes, mRNA differences between *mdx* and DMD muscles provide the basis for testable hypotheses as to how *mdx* muscle is salvaged from the early deleterious fate of the DMD muscle. The number and varied gene function of the identified mRNAs differentially expressed in *mdx* muscle suggest that there may be a complex interplay of groups of genes that may provide key insights elucidating the more benign and less devastating pathological mechanisms involved with mouse *mdx*, unlike human dystrophin-deficient muscular dystrophy.

We thank Drs. Yi-Wen Chen and Marina Bakay in Dr. Eric P. Hoffman's laboratory for allowing us to cite the DMD myostatin data as an unpublished observation.

This study was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-19393 (to F. W. Booth), a Program in Genomic Applications grant (HOPGENES; to E. P. Hoffman), and the Muscular Dystrophy Association (to E. P. Hoffman).

REFERENCES

1. **Alloul N, Gorzalczany Y, Itan M, Sigal N, and Pick E.** Activation of the superoxide-generating NADPH oxidase by chimeric proteins consisting of segments of the cytosolic component p67 (phox) and the small GTPase Rac1. *Biochemistry* 40: 14557–14566, 2001.
2. **Barbiroli B, Funicello R, Ferlini A, Montagna P, and Zaniol P.** Muscle energy metabolism in female DMD/BMD carriers: a ^{31}P-MR spectroscopy study. *Muscle Nerve* 15: 344–348, 1992.
3. **Benjamini Y and Hochberg Y.** Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodological* 57: 289–300, 1995.
4. **Borisy GG and Svitkina TM.** Actin machinery: pushing the envelope. *Curr Opin Cell Biol* 12: 104–112, 2000.
5. **Bosca L and Lazo PA.** Induction of nitric oxide release by MRC OX-44 (anti-CD53) through a protein kinase C-dependent pathway in rat macrophages. *J Exp Med* 179: 1119–1126, 1994.
6. **Brooke MH.** *A Clinician's View of Neuromuscular Diseases* (2nd Ed.). Baltimore, MD: Williams & Wilkins, 1986, p. 117–157.
7. **Carnwath JW and Shotton DM.** Muscular dystrophy in the mdx mouse: histopathology of the soleus and extensor digitorum longus muscles. *J Neurol Sci* 80: 39–54, 1987.
8. **Carson JA, Nettleton D, and Reecy JM.** Differential gene expression in the rat soleus muscle during early work overload-induced hypertrophy. *FASEB J* 16: 207–209, 2002.
9. **Carter GT, Wineinger MA, Walsh SA, Horasek SJ, Abresch RT, and Fowler WM Jr.** Effect of voluntary wheel-running exercise on muscles of the mdx mouse. *Neuromuscul Disord* 5: 323–332, 1995.
10. **Chen YW, Zhao P, Borup R, and Hoffman EP.** Expression profiling in the muscular dystrophies: identification of novel aspects of molecular pathophysiology. *J Cell Biol* 151: 1321–1336, 2000.
11. **Chomczynski P and Sacchi N.** Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159, 1987.
12. **Clement K, Viguerie N, Diehn M, Alizadeh A, Barbe P, Thalamas C, Storey JD, Brown PO, Barsh GS, and Langin D.** In vivo regulation of human skeletal muscle gene expression by thyroid hormone. *Genome Res* 12: 281–291, 2002.
13. **Cullen MJ and Jaros E.** Ultrastructure of the skeletal muscle in the X chromosome-linked dystrophic (mdx) mouse. Comparison with Duchenne muscular dystrophy. *Acta Neuropathol (Berl)* 77: 69–81, 1988.
14. **De La Cruz EM and Pollard TD.** Structural biology. Actin' up. *Science* 293: 616–618, 2001.
15. **De La Porte S, Morin SS, and Koenig J.** Characteristics of skeletal muscle in Mdx mutant mice. *Int Rev Cytol* 191: 99–148, 1999.
16. **Fang J, Shi GP, and Vaghy PL.** Identification of the increased expression of monocyte chemoattractant protein-1, cathepsin S, UPIX-1, and other genes in dystrophin-deficient mouse muscles by suppression subtractive hybridization. *J Cell Biochem* 79: 164–172, 2000.
17. **Gannoun-Zaki L, Fournier-Bidoz S, Le Cam G, Chambon C, Millasseau PH, Leger JJ, and Dechesne CA.** Down-regulation of mitochondrial mRNAs in the mdx mouse model for Duchenne muscular dystrophy. *FEBS Lett* 375: 268–272, 1995.
18. **Goldspink G, Fernandes K, Williams PE, and Wells DJ.** Age-related changes in collagen gene expression in the muscles of mdx dystrophic and normal mice. *Neuromuscul Disord* 4: 183–191, 1994.
19. **Granchelli JA, Avosso DL, Hudecki MD, and Pollina C.** Cromolyn increases strength in exercised mdx mice. *Res Commun Mol Pathol Pharmacol* 91: 287–296, 1996.
20. **Hayes A and Williams DA.** Beneficial effects of voluntary wheel running on the properties of dystrophic mouse muscle. *J Appl Physiol* 80: 670–679, 1996.
21. **Hoffman EP, Brown RH Jr, and Kunkel LM.** Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 51: 919–928, 1987.
22. **Infante JP and Huszagh VA.** Mechanisms of resistance to pathogenesis in muscular dystrophies. *Mol Cell Biochem* 195: 155–167, 1999.
23. **Jin Y, Murakami N, Saito Y, Goto Y, Koishi K, and Nonaka I.** Expression of MyoD and myogenin in dystrophic mice, mdx and dy, during regeneration. *Acta Neuropathol (Berl)* 99: 619–627, 2000.
24. **Kouroku Y, Soyama A, Fujita E, Urase K, Tsukahara T, and Momoi T.** RA70 is a src kinase-associated protein expressed ubiquitously. *Biochem Biophys Res Commun* 252: 738–742, 1998.
25. **Kuznetsov AV, Winkler K, Wiedemann FR, von Bossanyi P, Dietzmann K, and Kunz WS.** Impaired mitochondrial oxidative phosphorylation in skeletal muscle of the dystrophin-deficient mdx mouse. *Mol Cell Biochem* 183: 87–96, 1998.
26. **Lagrota-Candido J, Canella I, Savino W, and Quirico-Santos T.** Expression of extracellular matrix ligands and receptors in the muscular tissue and draining lymph nodes of mdx dystrophic mice. *Clin Immunol Immunopathol* 93: 143–151, 1999.
27. **Lee SJ and McPherron AC.** Regulation of myostatin activity and muscle growth. *Proc Natl Acad Sci USA* 98: 9306–9311, 2001.
28. **Machesky LM and May RC.** Arps: actin-related proteins. *Results Probl Cell Differ* 32: 213–229, 2001.
29. **MacLennan PA and Edwards RH.** Protein turnover is elevated in muscle of mdx mice in vivo. *Biochem J* 268: 795–797, 1990.
30. **McArdle A, Edwards RH, and Jackson MJ.** Release of creatine kinase and prostaglandin E_2 from regenerating skeletal muscle fibers. *J Appl Physiol* 76: 1274–1278, 1994.
31. **Muller J, Vayssiere N, Royuela M, Leger ME, Muller A, Bacou F, Pons F, Hugon G, and Mornet D.** Comparative evolution of muscular dystrophy in diaphragm, gastrocnemius

and masseter muscles from old male mdx mice. *J Muscle Res Cell Motil* 22: 133–139, 2001.
32. **Mullins RD, Heuser JA, and Pollard TD.** The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments. *Proc Natl Acad Sci USA* 95: 6181–6186, 1998.
33. **Notterman DA, Alon U, Sierk AJ, and Levine AJ.** Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. *Cancer Res* 61: 3124–3130, 2001.
34. **Pagel CN and Partridge TA.** Covert persistence of mdx mouse myopathy is revealed by acute and chronic effects of irradiation. *J Neurol Sci* 164: 103–116, 1999.
35. **Rybakova IN, Amann KJ, and Ervasti JM.** A new model for the interaction of dystrophin with F-actin. *J Cell Biol* 135: 661–672, 1996.
36. **Rybakova IN and Ervasti JM.** Dystrophin-glycoprotein complex is monomeric and stabilizes actin filaments in vitro through a lateral association. *J Biol Chem* 272: 28771–28778, 1997.
37. **Rybakova IN, Patel JR, and Ervasti JM.** The dystrophin complex forms a mechanically strong link between the sarcolemma and costameric actin. *J Cell Biol* 150: 1209–1214, 2000.
38. **Sambeth R and Baumgaertner A.** Autocatalytic polymerization generates persistent random walk of crawling cells. *Phys Rev Lett* 86: 5196–5199, 2001.
39. **Sander M, Chavoshan B, Harris SA, Iannaccone ST, Stull JT, Thomas GD, and Victor RG.** Functional muscle ischemia in neuronal nitric oxide synthase-deficient skeletal muscle of children with Duchenne muscular dystrophy. *Proc Natl Acad Sci USA* 97: 13818–13823, 2000.
40. **Sano M, Wada Y, Ii K, Kominami E, Katunuma N, and Tsukagoshi H.** Immunolocalization of cathepsins B, H and L in skeletal muscle of X-linked muscular dystrophy (mdx) mouse. *Acta Neuropathol (Berl)* 75: 217–225, 1988.
41. **Settles DL, Cihak RA, and Erickson HP.** Tenascin-C expression in dystrophin-related muscular dystrophy. *Muscle Nerve* 19: 147–154, 1996.
42. **Tanabe Y, Esaki K, and Nomura T.** Skeletal muscle pathology in X chromosome-linked muscular dystrophy (mdx) mouse. *Acta Neuropathol (Berl)* 69: 91–95, 1986.
43. **Tchougounova E, Forsberg E, Angelborg G, Kjellen L, and Pejler G.** Altered processing of fibronectin in mice lacking heparin. A role for heparin-dependent mast cell chymase in fibronectin degradation. *J Biol Chem* 276: 3772–3777, 2001.
44. **Tkatchenko AV, Le Cam G, Leger JJ, and Dechesne CA.** Large-scale analysis of differential gene expression in the hindlimb muscle and diaphragm of mdx mouse. *Biochem Biophys Acta* 1500: 17–30, 2000.
45. **Tusher VG, Tibshirani R, and Chu G.** Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 98: 5116–5121, 2001.
46. **Watson MA, Perry A, Budhjara V, Hicks C, Shannon WD, and Rich KM.** Gene expression profiling with oligonucleotide microarrays distinguishes World Health Organization grade of oligodendrogliomas. *Cancer Res* 61: 1825–1829, 2001.
47. **Wineinger MA, Abresch RT, Walsh SA, and Carter GT.** Effects of aging and voluntary exercise on the function of dystrophic muscle from mdx mice. *Am J Phys Med Rehabil* 77: 20–27, 1998.
48. **Zhang XA, Bontrager AL, and Hemler ME.** Transmembrane-4 superfamily proteins associate with activated protein kinase C (PKC) and link PKC to specific beta(1) integrins. *J Biol Chem* 276: 25005–25013, 2001.