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Short Communication

Novel Therapeutic Target of Duchenne Muscular Dystrophy: Circulating Exosomal microRNAs

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ABSTRACT

Duchenne Muscular Dystrophy (DMD) is caused by loss-of-function mutations in the dystrophin gene on chromosome Xp21. Disruption of the Dystrophin–Glycoprotein Complex (DGC) on the cell membrane causes cytosolic Ca^{2+} influx, resulting in protease activation, mitochondrial dysfunction, progressive myofiber degeneration, leading to muscle wasting, and fragility. In addition to the function of dystrophin in the structural integrity of myofibers, a novel function of asymmetric cell division in muscular stem cell (satellite cell) has been reported. Therefore, it has been suggested that myofiber instability is not the only cause of dystrophic degeneration, but rather that the phenotype might be caused by multiple factors, including stem cell and myofiber functions. Further, it has been focused functional regulation of satellite cell by intracellular communication of microRNAs *via* exosome in DMD pathology. Recently, novel molecular mechanism of DMD pathogenesis as circulating RNA disease is revealed though study of target pathways modulated by the nSMase2/Smpd3 protein. These findings suggest that circulating exosomal RNAs may be therapeutic targets of DMD.

Keywords: Duchenne muscular dystrophy; Exosome; MicroRNAs; Myofiber degeneration; nSMase2/Smpd3; Satellite cell

DESCRIPTION

Duchenne Muscular Dystrophy (DMD) is a progressive muscular disorder, which is mainly characterized with degeneration and regeneration in skeletal muscles. It is caused by loss-of-function mutations in the dystrophin gene on chromosome Xp21, which codes dystrophin protein formed a complex known as the Dystrophin-Associated Protein Complex (DAPC), with dystroglycan and sarcoglycan, on the cell membrane and transmits forces in muscle [1,2]. The myofiber-specific genetic ablation of dystroglycan in mice does not result in dystrophia-like muscle degeneration, but mice with muscle stem cells (also called satellite cells)-specific loss of dystroglycan markedly delayed muscle regeneration [3]. In addition, satellite cells lacking dystrophin markedly increase abnormal nonpolarized mitotic divisions and reduced asymmetric cell divisions and myogenic progenitors, caused to exacerbation of the dystrophic pathology [4]. These reports suggest that dysfunction of the satellite cells is involved in muscular dystrophy.

Until today, some treatment strategy for DMD, such as 1) AAV-mediated micro/minidystrophin gene delivery, 2) synthetic antisense oligonucleotides for exon skipping, 3) nonsense readthrough, and 4) corticosteroids have been tried [5-7]. On the other hand, non-invasive biomarkers for evaluating properly the improvement of DMD pathology by these therapeutic interventions are paucity. Among some hopeful biomarkers such as proteins, nucleic acids, metabolites, polymorphisms, mutations, RNA splicing, epigenetics, etc., microRNAs have been focused because of 1) high content in body fluids, including serum, plasm, tear, lymph, breast milk, urine, semen, saliva, sweat, etc., 2) high stability in blood and outside the body due to capsulation into exosome and formation of complex with RNA-binding proteins, 3) unique expression profile correlated with pathology progression for monitoring the initial stage of the pathological condition, such as immediately before or after the onset, 4) high sensitivity and specificity to suppress false positives and false negatives, and 5) high throughput and cost reduction [8-13]. As for microRNAs in DMD, three myomiRs, namely, miR-1, miR-133a, and miR-206, were increased in the sera of animal models of muscular dystrophy,

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mdx mice as well as in patients that show restoration of their levels by expression of functional dystrophin protein, and these myomiR levels were shown to be inversely correlated with disease severity in DMD patients [14-16]. On the other hand, in the muscle of mdx mice, miR-1 and miR-133a levels have been shown to be downregulated, whereas miR-206 levels are upregulated, which that also recover to wild-type levels by restoration of the dystrophin protein [17-19]. In addition, these myomiRs encapsulated within exosomes, which are membrane microvesicles about 30–100 nm in size generated from multivesicular bodies of the terminal endosomal pathway *via* the biogenesis of ceramide from sphingomyelin are released from cells into circulation, and controlled by the neutral sphingomyelinase 2/ sphingomyelin phosphodiesterase 3 (nSMase2/SMPD3)-regulated secretory machinery of exosomes [20-25]. Thus, to elucidate the relationship between the release of myomiRs *via* exosome and DMD pathogenesis, GW4869 (an inhibitor of nSMase2/SMPD3) was administered into mdx mice. It has been shown that inhibition of ceramide synthesis ameliorates muscular dystrophy in mdx mice [21]. However, there are problems with GW4869 inhibitor such as inhibition of other nSMase2/SMPD3 family and short-term inhibitory effects. Therefore, to investigate the effects of the nSMase2/Smpd3 on dystrophic pathology, we generated mdx mice lacking the nSMase2/Smpd3 gene (mdx:Smpd3 Double Knockout [DKO] mice) [26]. Deletion of the nSMase2/Smpd3 gene in mdx mice reduces inflammation in dystrophic muscles indicated by reduction of infiltration of excess inflammatory cells and decrease of inflammatory cytokine expression levels, such as TNF-alpha, CD68, CD45, Ccr5, IL-1ra, and IL-6 [26]. In addition, disruption of the nSMase2/Smpd3 gene attenuates muscle membrane permeability in dystrophic mdx mice early on, but exacerbates it later. In 6 to 12-week-old mdx:Smpd3 DKO mice, serum Creatine Kinas (CK) levels were significantly lower levels than that of mdx mice. However, at 28 weeks, somewhat or significantly higher serum CK levels in mdx:Smpd3 DKO mice were observed compared with that in mdx mice. Further, at 12 weeks of age, there were significantly fewer evans blue dye (EBD: degree marker of myofiber damage)-positive muscle fibers in the Tibialis Anterior (TA) muscle of mdx:Smpd3 DKO mice than in mdx mice. But, at 20 weeks of age the number of EBD-positive muscle fibers in the TA of some mdx:Smpd3 DKO mice line was higher than in mdx mice. These results suggest that early in life, nSMase2/Smpd3 ablation may have beneficial effects with respect to myofiber membrane degeneration in mdx mice, but that later on it may have adverse effects. Also, genetic ablation of nSMase2/Smpd3 improves muscle performance in mice with dystrophic phenotypes. The grip strength test demonstrated that at 12 weeks of age, the muscle strength of mdx mice was significantly lower than that of wt mice, but that of mdx:Smpd3 DKO mice was significantly recovered. In addition, at an inclined treadmill running test, mdx:Smpd3 DKO mice ran for significantly longer than mdx mice at 16 and 60 weeks of age.

In addition to muscle degeneration of DMD, the loss of dystrophin in the brain has often been associated with nonprogressive cognitive deficits, behavioral disabilities, and enhanced fearfulness [27-29]. Thus, to investigate anxiety, emotionality, and the adaptive stress response to a novel environment in mdx:Smpd3 DKO mice, the hole-board test was performed [26]. The DKO mice exhibited that loss of Smpd3 gene modulates anxiety behavior and stress responses, and the recovery of Brain-Derived Neurotrophic Factor (BDNF) expression through exosomal miRNA in the hippocampus [26]. These findings suggest that DMD is not only muscular disease, but circulation RNA disease through intracellular communication, and signaling pathways modulated by the nSMase2/Smpd3 protein might be novel therapeutic targets for DMD, *via* the combination of regulation of the expression levels of exosomal microRNAs though this nSMase2/Smpd3 protein and AAV-mediated micro/ minidystrophin gene delivery, exon skipping and nonsense readthrough of dystrophin gene, and anti-inflammation drugs (Figure 1).

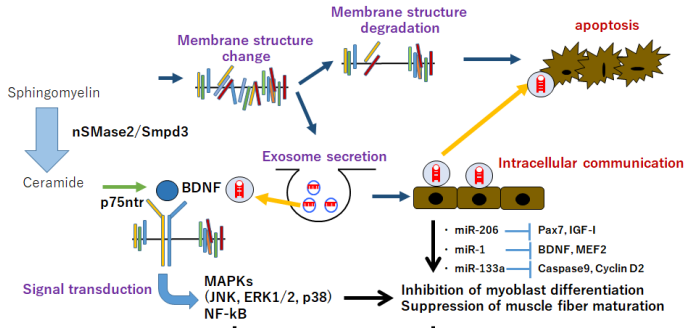

Figure 1: Causal relationship with nSMase2/ Smpd3 pathway with molecular pathology of DMD. p75ntr: nerve growth factor receptor, BDNF: brain derived neurotrophic factor, MAPKs: mitogen-activated protein kinases, JNK:c-Jun N-terminal kinase, ERK1/2; extracellular signal regulated protein kinase, p38: p38 mitogen-activated protein kinase, NF-kB: nuclear factor-κB, PAX7:paired box 7, IGF-1:insulin-like growth factor 1, MEF2: myocyte enhancer factor 2.

REFERENCES

1. Omairi S, Hau KL, Collins-Hooper H, Scott C, Vaiyapuri S, Torelli S, et al. Regulation of the dystrophin-associated glycoprotein complex composition by the metabolic properties of muscle fibres. Sci Rep. 2019; 9: 2770.

2. Gumerson JD, Michele DE. The dystrophin-glycoprotein complex in the prevention of muscle damage. J Biomed Biotechnol. 2011; 2011: 210797.

3. Cohn RD, Henry MD, Michele DE, Barresi R, Saito F, Moore SA, et al. Disruption of DAG1 in differentiated skeletal muscle reveals a role for dystroglycan in muscle regeneration. Cell. 2002; 110: 639–648.

4. Dumont NA, Wang YX, von Maltzahn J, Pasut A, Bentzinger CF, Brun CE, et al. Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. Nat Med. 2015; 21: 1455–1463.

5. Shieh PB. Emerging Strategies in the Treatment of Duchenne Muscular Dystrophy. Neurotherapeutics. 2018; 15: 840-848.

6. Waldrop MA, Flanigan KM. Update in Duchenne and Becker muscular dystrophy. Curr Opin Neurol. 2019; 32: 722-727.

7. Sun C, Shen L, Zhang Z, Xie X. Therapeutic Strategies for Duchenne Muscular Dystrophy: An Update. Genes (Basel). 2020; 11: 837.

8. Salehi M, Sharifi M. Exosomal miRNAs as novel cancer biomarkers: Challenges and opportunities. J Cell Physiol. 2018; 233: 6370-6380.

9. Kacperska MJ, Walenczak J, Tomasik B. Plasmatic microRNA as Potential Biomarkers of Multiple Sclerosis: Literature Review. Adv Clin Exp Med. 2016; 25: 775-779.

10. Arantes LMRB, De Carvalho AC, Melendez ME, Lopes Carvalho A. Serum, plasma and saliva biomarkers for head and neck cancer. Expert Rev Mol Diagn. 2018; 18: 85-112.

11. Barceló M, Mata A, Bassas L, Larriba S. Exosomal microRNAs in seminal plasma are markers of the origin of azoospermia and can predict the presence of sperm in testicular tissue. Hum Reprod. 2018; 33: 1087-1098.

12. Wolenski FS, Shah P, Sano T, Shinozawa T, Bernard H, Gallacher MJ, et al. Identification of microRNA biomarker candidates in urine and plasma from rats with kidney or liver damage. J Appl Toxicol. 2017; 37: 278-286.

13. Rubio M, Bustamante M, Hernandez-Ferrer C, Fernandez-Orth D, Pantano L, Sarria Y, et al. Circulating miRNAs, isomiRs and small RNA clusters in human plasma and breast milk. PLoS One. 2018; 13: e0193527.

14. Mizuno H, Nakamura A, Aoki Y, Ito N, Kishi S, Yamamoto K, et al. Identification of muscle-specific microRNAs in serum of muscular dystrophy animal models: promising novel blood-based markers for muscular dystrophy. PLoS One. 2011; 6: e18388.

15. Cacchiarelli D, Legnini I, Martone J, Cazzella V, D'Amico A, Bertini E, et al. miRNAs as serum biomarkers for Duchenne muscular dystrophy. EMBO Mol Med. 2011; 3: 258–265.

16. Li X, Li Y, Zhao L, Zhang D, Yao X, Zhang H, et al. Circulating Muscle-specific miRNAs in Duchenne Muscular Dystrophy Patients. Mol Ther Nucleic Acids. 2014; 3: e177.

17. Roberts TC, Blomberg KE, McClorey G, El Andaloussi S, Godfrey C, Betts C, et al. Expression analysis in multiple muscle groups and serum reveals complexity in the microRNA transcriptome of the mdx mouse with implications for therapy. Mol Ther Nucleic Acids. 2012;14:e39.

18. Cacchiarelli D, Martone J, Girardi E, Cesana M, Incitti T, Morlando M, et al. MicroRNAs involved in molecular circuitries relevant for the Duchenne muscular dystrophy pathogenesis are controlled by the dystrophin/nNOS pathway. Cell Metab. 2010; 12: 341–351.

19. Cazzella V, Martone J, Pinnarò C, Santini T, Twayana SS, Sthandier O, et al. Exon 45 skipping through U1-snRNA antisense molecules recovers the Dys-nNOS pathway and muscle differentiation in human DMD myoblasts. Mol Ther. 2012; 20: 2134–2142.

20. Yin X, Zhao Y, Zheng YL, Wang JZ, Li W, Lu QJ, et al. Time-Course Responses of Muscle-Specific MicroRNAs Following Acute Uphill or Downhill Exercise in Sprague-Dawley Rats. Front Physiol. 2019; 10: 1275.

21. Matsuzaka Y, Tanihata J, Komaki H, Ishiyama A, Oya Y, Rüegg U, et al. Characterization and Functional Analysis of Extracellular Vesicles and Muscle-Abundant miRNAs (miR-1, miR-133a, and miR-206) in C2C12 Myocytes and mdx Mice. PLoS One. 2016; 11: e0167811.

22. Cheng M, Yang J, Zhao X, Zhang E, Zeng Q, Yu Y, et al. Circulating myocardial microRNAs from infarcted hearts are carried in exosomes and mobilise bone marrow progenitor cells. Nat Commun. 2019; 10: 959.

23. Zhao H, Li Y, Chen L, Shen C, Xiao Z, Xu R, et al. HucMSCs-Derived miR-206-Knockdown Exosomes Contribute to Neuroprotection in Subarachnoid Hemorrhage Induced Early Brain Injury by Targeting BDNF. Neuroscience. 2019; 417: 11-23.

24. Koutsoulidou A, Photiades M, Kyriakides TC, Georgiou K, Prokopi M, Kapnisis K, et al. Identification of exosomal muscle-specific miRNAs in serum of myotonic dystrophy patients relating to muscle disease progress. Hum Mol Genet. 2017; 26: 3285-3302.

25. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008; 319:1244–1247.

26. Matsuzaka Y, Tanihata J, Ooshima Y, Yamada D, Sekiguchi M, Miyatake S, et al. The nSMase2/Smpd3 gene modulates the severity of muscular dystrophy and the emotional stress response in mdx mice. BMC Med. 2020; 18: 343.

27. Sekiguchi M, Zushida K, Yoshida M, Maekawa M, Kamichi S, Yoshida M, et al. A deficit of brain dystrophin impairs specific amygdala GABAergic transmission and enhances defensive behaviour in mice. Brain. 2009;132:124–135.

28. Remmelink E, Aartsma-Rus A, Smit AB, Verhage M, Loos M, van Putten M. Cognitive flexibility deficits in a mouse model for the absence of full-length dystrophin. Genes Brain Behav. 2016; 15: 558–567.

29. Vaillend C, Chaussenot R. Relationships linking emotional, motor, cognitive and GABAergic dysfunctions in dystrophin-deficient mdx mice. Hum Mol Genet. 2017; 26: 1041–1055.