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Editorial

Duchenne Muscular Dystrophy from a Zebrafish's Perspective

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Editorial

Musculature plays a crucial role for essential body functions such as movement, breathing, or heartbeat. Hence, diseases associated with muscle can be devastating; not only are they debilitating and life-threatening for patients, they also have a high cost-of-illness and are an economic burden [1]. In general, muscle diseases are distinguished between Muscular Dystrophies (MD) that are characterized by progressive myofibre degeneration accompanied by fibrosis and myopathies that are diagnosed by muscle hypotonia and weakness without dystrophic features. Duchenne muscular dystrophy is one of the most frequent and severe forms of MD. Duchenne MD results from null mutations in the dystrophin gene (*DMD*) that lead to complete abrogation of DMD protein synthesis [2]. If the dystrophin function is only partially lost, for instance by in-frame deletions, patients generally suffer from Becker MD, which shows milder symptoms than Duchenne MD. Within skeletal muscle, dystrophin connects the actin cytoskeleton to the extracellular matrix by binding N-terminally to actin and C-terminally to the dystrophin-associated glycoprotein complex, which spans through the myofibre membrane and integrates into the extracellular matrix. These observations have led to the hypothesis that muscle breakdown in Duchenne MD patients is caused, at least in part, by the mechanical stress provoked by myofibril contraction not being transferred efficiently to the extracellular matrix, causing failure of sarcolemma integrity and subsequent fibre loss. Live imaging of translucent dystrophin-deficient zebrafish demonstrated that myofibre detachment is triggered upon muscle contraction [3]. Though several other functions of dystrophin have also been discovered, this life imaging analysis suggests that the mechanical features of dystrophin have a substantial contribution to the pathology of Duchenne MD. In over 3 decades of dystrophin research many animal models for Duchenne MD have been generated, with the dystrophin null mutant mouse, named *mdx*, being the first and still most widely used model [4]. Despite their dystrophin deficiency, *mdx* mice lack many aspects of the human DMD pathology and undergo a relatively mild dystrophic response [2]. Only the diaphragm and skeletal muscle of aged *mdx* mice show robust dystrophic features of degeneration, fibrosis and functional deficits [5,6]. Other mammalian model systems, such as the dystrophin-deficient dog reflect the human condition more closely [7], but have other disadvantages such as phenotypic variability [8], small litter size, prohibitive expense, and limited genetic tractability. Also dystrophin-deficient Zebrafish closely match many aspects of Duchene MD [3]. Similar to dystrophin in humans, zebrafish dystrophin initially localizes to the peripheral ends of the myofibres at the myotendinous junction and gradually shifts to non-junctional sites. Dystrophin deficiency in zebrafish is characterized by extensive muscle degeneration, fibrosis, muscle progenitor proliferation, and greater variation in myofibre cross-sectional areas [3]. The only marked difference to Duchenne MD is the decreased level of new myofibres with centralised nuclei. The muscle of wild type zebrafish larvae mainly grows through hyperplasia, which is in contrast to the hypertrophic muscle growth of post-natal mammals [9]. Therefore the discrepancy might be explained by a preferential loss of new myofibres during the dystrophic response.

The zebrafish animal system is well suited for high-throughput small molecule screens that aim to identify compounds with therapeutic potential from large libraries of chemicals [10]. Zebrafish combine effective breeding with cost-efficient husbandry and the embryos' yolk enables rapid development without the need for feeding in the first week. More importantly, the translucent embryos are amenable for microscopic observation and the birefringent muscle readily enables assessment of the muscle integrity under polarized light [11]. Small molecule screens have also been performed with dystrophin-deficient zebrafish and several novel compounds have been identified that ameliorate the dystrophic pathology [12,13]. In a subsequent study, the potential of six identified compounds to up-regulate heme oxygenase 1 protein (Hmox1) has been discovered, revealing heme oxygenase signaling as a novel target for treatment of Duchenne MD [14]. However, rigorous examination of the metabolic and pharmacokinetic properties of identified compounds needs to be performed to explore their value as lead drugs. The most advanced drug for treatment of Duchenne MD to date is Ataluren, which is currently in clinical phase III [15]. Initially published as PTC124, Ataluren was reported to suppress premature stop codon mutations generated by nonsense mutations without affecting endogenous termination codons [16], a finding challenged by other studies [17]. Whereas the molecular function of Ataluren might not have fully been established, beneficial effects of Ataluren for the function of dystrophic muscle have been demonstrated in dystrophin-deficient zebrafish [18].

Much hope has been placed in gene replacement therapy to cure Duchenne MD, but many obstacles still need to be overcome, including the host immune responses to the therapeutic proteins or the viral capsid proteins [19,20]. A more promising strategy

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to restore dystrophin function in Duchenne MD patients is gene repair therapy. Eteplirsen, currently in phase II of clinical trails, is an antisense oligonucleotide that targets exon 51 of dystrophin and mediates its exclusion from the mature dystrophin transcript [21]. In a process named exon, antisense oligonucleotides sterically block the splice motifs of a targeted exon, which leads to exclusion of the exon from the mature dystrophin transcript. If skipping of the targeted exon does not disrupt the open reading frame, the resulting altered dystrophin transcript can encode for a slightly shorter but largely functional dystrophin protein and, in case the skipped exon harbors a disease-causing mutation, restore dystrophin function [22]. Also in dystrophin-deficient zebrafish exon-skipping has been reported to restore dystrophin function and rescue the dystrophic phenotype [23]. In addition, this study has shown that about 30% to 40% of dystrophin transcript needs to be restored in dystrophin-deficient zebrafish to significantly improve muscle function and levels of about 10% to 20% only partially restores the function of the dystrophic muscle [23]. This correlates well with studies with the *mdx* mice demonstrating that approximately 20% of dystrophin significantly mitigates muscle pathology [24]. Similarly, patients suffering from moderate to severe Duchenne MD symptoms show levels of 15% or less and individuals with dystrophin levels above 30% suffer from milder Becker MD [25]. However, the challenge of the exon-skipping strategy is to effectively deliver antisense oligonucleotides to all tissues affected by the lack of dystrophin and current research is analyzing various chemistries for antisense oligonucleotides to optimize repair of mutant dystrophin. In conclusion, an abundant array of animal models for Duchenne MD, including dystrophin-deficient zebrafish, has been generated and contributed to a better understanding of dystrophin function and how MD is provoked by mutations in dystrophin. This research has opened and explored novel therapeutic pathways, which in future might be able to provide patients suffering from Duchenne MD a resolutive therapeutic treatment.

References

1. Landfeldt E, Lindgren P, Bell CF, Schmitt C, Guglieri M, Straub V, et al.The burden of Duchenne muscular dystrophy: an international, cross-sectional study Neurology . 2014; 83: 529-536.
2. Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus Cell. 1987; 51: 919-928.
3. Berger J, Berger S, Hall TE, Lieschke GJ, Currie PD. Dystrophin-deficient zebrafish feature aspects of the Duchenne muscular dystrophy pathology Neuromuscul Disord. 2010; 20: 826-832.
4. Bulfield G, Siller WG, Wight PA, Moore KJ. X chromosome-linked muscular dystrophy (mdx) in the mouse Proc Natl Acad Sci USA. 1984; 81:1189-192.
5. Stedman HH, Sweeney HL, Shrager JB, Maguire HC, Panettieri RA, Petrof B, et al. The mdx mouse diaphragm reproduces the degenerative changes of Duchenne muscular dystrophy Nature. 1991; 352: 536-539.
6. Pastoret C, Sebille A. mdx mice show progressive weakness and muscle deterioration with age J Neurol Sci.1995; 129: 97-105.
7. Cooper BJ, Winand NJ, Stedman H, Valentine BA, Hoffman EP, Kunkel LM, et al. The homologue of the Duchenne locus is defective in X-linked muscular dystrophy of dogs Nature.1988; 334 :154-156.
8. Zucconi E, Valadares MC, Vieira NM, Bueno CR Jr, Secco M, Jazedje T, et al. Ringo: discordance between the molecular and clinical manifestation in a golden retriever muscular dystrophy dog Neuromuscul Disord. 2010; 20: 64-70.
9. Patterson SE, Mook LB, Devoto SH. Growth in the larval zebrafish pectoral fin and trunk musculature Dev Dyn. 2008; 237: 307-315.
10. Berger J, Currie P.The role of zebrafish in chemical genetics Curr Med Chem.2007; 14: 2413-2420.
11. Berger J, Sztal T, Currie PD. Quantification of birefringence readily measures the level of muscle damage in zebrafish Biochem Biophys Res Commun. 2012; 423: 785-788.
12. Kawahara G, Karpf JA, Myers JA, Alexander MS, Guyon JR Kunkel LM. Drug screening in a zebrafish model of Duchenne muscular dystrophy Proc Natl Acad Sci USA. 2011;108: 5331-5336.
13. Waugh TA, Horstick E, Hur J, Jackson SW, Davidson AE, Li X, Dowling JJ. Fluoxetine prevents dystrophic changes in a zebrafish model of Duchenne muscular dystrophy Hum Mol Genet. 2014; 23: 4651-462.
14. Kawahara G, Gasperini MJ, Myers JA, Widrick JJ, Eran A, Serafini PR, et al. Dystrophic muscle improvement in zebrafish via increased heme oxygenase signaling Hum Mol Genet. 2014; 23: 1869-1878.
15. Bushby K, Finkel R, Wong B, Barohn R, Campbell C, Comi GP,et al. Ataluren treatment of patients with nonsense mutation dystrophinopathy Muscle Nerve. 2014; 50: 477-487.
16. Welch EM, Barton ER, Zhuo J, Tomizawa Y, Friesen WJ, Trifillis P,et al. PTC124 targets genetic disorders caused by nonsense mutations Nature. 2007; 447: 87-91.
17. Auld DS, Lovell S, Thorne N, Lea WA, Maloney DJ, Shen M, et al. Molecular basis for the high-affinity binding and stabilization of firefly luciferase by PTC124 Proc Natl Acad Sci USA. 2010; 107: 4878-483.
18. Li M, Andersson-Lendahl M, Sejersen T, Arner A. Muscle dysfunction and structural defects of dystrophin-null sapje mutant zebrafish larvae are rescued by ataluren treatment FASEB J. 2014; 28: 1593-1599.
19. Wang Z, Storb R, Halbert CL, Banks GB, Butts TM, Finn EE, et al. Successful regional delivery and long-term expression of a dystrophin gene in canine muscular dystrophy: a preclinical model for human therapies Mol Ther. 2012; 20: 1501-1507.
20. Mendell JR, Campbell K, Rodino-Klapac L, Sahenk Z, Shilling C, Lewis S,et al. Dystrophin immunity in Duchenne's muscular dystrophy N Engl J Med. 2010; 363: 1429-1437.
21. Kole R Krieg AM. Exon skipping therapy for Duchenne muscular dystrophy Adv Drug Deliv Rev. 2015.
22. Mann CJ, Honeyman K, Cheng AJ, Ly T, Lloyd F, Fletcher S, et al. Antisense-induced exon skipping and synthesis of dystrophin in the mdx mouse Proc Natl Acad Sci USA. 2001; 98: 42-47.
23. Berger J, Berger S, Jacoby AS, Wilton SD, Currie PD. Evaluation of exon-skipping strategies for Duchenne muscular dystrophy utilizing dystrophin-deficient zebrafish J Cell Mol Med. 2011; 15: 2643-2651.
24. Chamberlain JS. Dystrophin Levels Required for Genetic Correction of Duchenne Muscular Dystrophy Basic Appl Myol.1998; 7: 251-255.
25. Angelini C, Fanin M, Pegoraro E, Freda MP, Cadaldini M, Martinello F. Clinical-molecular correlation in 104 mild X-linked muscular dystrophy patients: characterization of sub-clinical phenotypes Neuromuscul Disord. 1994; 4: 349-358.

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