Minireview

Zebrafish (*Danio rerio*) in deciphering molecular mechanisms of human diseases

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Summary. Biomedical research increasingly exploits zebrafish (*Danio rerio*) for genetic disease modeling and medical genetic research with the main goals of deciphering and understanding disease processes, and identifying new molecular markers and therapeutic targets. Zebrafish has emerged as a high-throughput and low-cost model organism based on the advantages that include the availability and ease of generating mutations in homologous disease-causing genes, the ability of noninvasive imaging for the analysis of phenotypes of different organs in an intact animal, and the suitability of zebrafish larvae for large-scale chemical screens. Identification of causative genes in human diseases and their functional characterization is enabled by forward and reverse genetic manipulation tools including CRISPR/Cas9-based genome editing. Here we review the use of the zebrafish for biomedical research, with a focus on tumors, cardiovascular diseases and myopathies.

Keywords: disease, human, zebrafish.

Introduction

The genetic basis for many human disorders is still unknown and understanding the molecular mechanisms of pathogenesis is crucial to therapy development. Identification of genetic variants in patients with various human genetic diseases is accelerated by genome-wide analysis and next-generation sequencing. To distinguish and validate true pathogenic variants, functional studies in well-established cell or animal models showing phenotypes analogous with human diseases are greatly needed (MacArthur et al. 2014). A small aquatic vertebrate, the zebrafish (*Danio rerio*), turned out to be very suitable for validating and testing newly identified putative human disease genetic variants (Gibbs et al. 2013). Additionally, zebrafish models may be used to identify and study genetic or chemical modifiers of a disease's phenotype.

Currently, there are three primary techniques for generating zebrafish models of human diseases (Ota and Kawahara 2014). Many phenotypes that resemble disease symptoms are uncovered by N-ethyl-N-nitrosourea (ENU) and transposon-based mutational screens. A bank of zebrafish strains at the Wellcome Trust Sanger Institute is the major mutation resource (http://www.sanger.ac.uk/Projects/D_rerio/zmp/), and many of the mutations have not been analyzed yet. Gene knock-down based on the use of morpholinos is rapid but transient. Morpholinos are synthetic short antisense oligonucleotides, injected into embryos. They are usually targeted at the start codon or splice sites of a gene of interest to transiently reduce or eliminate protein expression. Recently, their use has been questioned because approximately 80% of morphant phenotypes were not successfully recapitulated in actual genetic mutants. Transgenic fish express genes with known disease-related mutations that may be introduced by zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas 9 genome editing techniques. Currently, the CRISPR/Cas9 system is the most widely used for its highly efficient induction of targeted gene lesions in somatic cells.

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The Laboratory for Molecular Biology of the IMGGE is involved in the characterization of several biomarkers for cancers of the gastrointestinal tract, including colorectal cancer and pancreatic adenocarcinoma (Nikolic et al. 2011), blood coagulation diseases (Miljic et al. 2017), cardiovascular diseases and myopathies (Kojic et al. 2011). To add a functional characterization of these biomarkers, we recently introduced a zebrafish model system. Thus, the purpose of this review is to describe the use of zebrafish in the modeling of tumors, cardiovascular diseases and myopathies, the human disorders investigated by our group.

Why zebrafish?

Many characteristics of zebrafish biology make it a preferred model for researchers. Compared to frequently used rodent and other mammalian models, zebrafish maintenance and husbandry are inexpensive and relatively easy. Thanks to its small size (2.5-4 cm) (Fig. 1), it is possible to keep thousands of fish in the facilities, but also to perform whole-animal imaging. Zebrafish spawning is controlled by light, they have high fecundity and can produce hundreds of eggs per spawn. Zebrafish eggs are externally fertilized and develop

Fig. 1. Zebrafish embryos at different developmental stages: (**A**) 6 hpf (shield), (**B**) 24 hpf (pharyngula) and (**C**) 48 hpf (long pec). (**D**) Adult female and male are distinguished by body shape. hpf – hours post fertilization.

ex-utero. Genetic manipulation and transplantation are performed by the injection of embryos. Gene editing methods are well-established and further progress in this field will advance medical genetic research and modeling of human diseases in zebrafish. Due to rapid embryonic development, many organs are formed already 24 hours post fertilization (hpf) (Fig. 1). The optical transparency of the embryos allows the visualization of development and monitoring of gene expression *in vivo* in fluorescently labeled transgenes. Visualization in adult fish is possible after chemical removal of pigment or in pigment mutants, such as *Casper*. Generation time is very short; they can reach sexual maturity within 3 months, enabling the rapid transfer of edited genes from the founder lines to next generations. Sequencing of the zebrafish genome revealed that approximately 70% of human genes have functional orthologs in zebrafish, despite the genome duplication that occurred during zebrafish evolution. Zebrafish research is significantly facilitated by the information resource, on-line database ZFIN (Zebrafish Model Organism Database available at http://zfin.org/).

Numerous tests and techniques developed in zebrafish are not easily applicable in other models. For example, in a large-mutagenesis screen approximately 2000 zebrafish mutants were generated, affecting a variety of processes, such as early development, organ formation and behavior to resemble certain human disorders. Zebrafish enable high-throughput genetic and drug screenings. The fact that most zebrafish drug-metabolizing pathways are functionally equivalent to humans makes them an excellent intact *in vivo* system for chemical screens. Toxicity and safety assessments using zebrafish have also been accepted by the US Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) for the approval of newly investigated drugs (Westerfield 2007).

It is important to keep in mind that zebrafish is a non-mammalian species evolutionarily distant from mammals. It lacks some mammalian organs such as lung, prostate and mammary gland. Many genes are present as two copies so the determination of their functional roles is not straightforward. In some cases, phenotypic characteristics of diseases caused by ortholog genes can be very different in fish and humans. Despite these disadvantages, zebrafish has become an attractive model for the study of a variety of human disorders.

Zebrafish and tumors

Zebrafish xenograft models are mainly used for fundamental research in the field of cancer biology and identification of novel anticancer drugs (Ceol and Houvras 2016). Xenotransplantation, the transfer of tissue from one to another animal species, has been employed for many years to study cancer cell proliferation, invasion, migration and induction of tumor angiogenesis in human cancer (Cekanova and Rathore 2014).

Xenograft tumor models are generated by the transplantation of cancer cells (by injection directly into the tissue of interest or into the blood circulation) into embryos at the
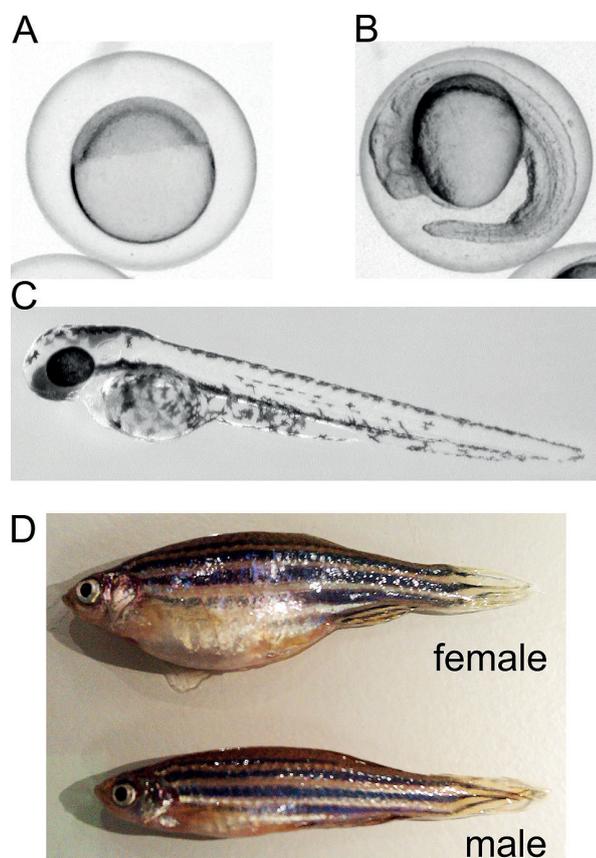
early stage of development. Due to the late onset of zebrafish adaptive immunity, most xenografts are not rejected (Renshaw and Trede 2012).When transplanted cancer cells are fluorescently labeled, their proliferation, death, invasion and metastasis in the transparent zebrafish embryos can be easily visualized (Ignatius and Langenau 2011). If xenotransplantation is performed into different fluorescent reporter fish lines it will facilitate the monitoring of cancer cell progression and their dynamic interactions with the components of microenvironments (Tamplin et al. 2015). Reporter lines are directly available at the Zebrafish International Resource Center (ZIRC; http://zebrafish.org/zirc/home/guide.php) or the European Zebrafish Resource Center (EZRC; http://www.ezrc.kit.edu/). In the widely used fluorescent reporter lines, tg(fli1a-eGFP), tg(mpx:eGFP) and tg(mpeg1:eGFP), expression of fluorescent protein is driven by promoters of the genes specifically expressed in endothelial cells, neutrophils and macrophages, respectively. A cross between the xenograft zebrafish model and transparent mutant enables analysis of the interactions between cancer cells and their living environment (Drabsch et al. 2017). Recently, it was demonstrated that human cancer cells can communicate with the zebrafish host (Tulotta et al. 2016), suggesting well-conserved intercellular communication mechanisms.

One of the limitations of the zebrafish xenograft model is that the embryos are maintained at 28 °C, while human cells grow at 37 °C. When cultured at suboptimal temperatures, cell growth and metabolism are altered. Another limitation is that the developmental process of human tumors is much more complex than in xenograft models. It take years for human cancers to grow, while tumor xenografts in zebrafish embryos grow in days (Drabsch et al. 2017).

Zebrafish can be used to model the effects of mutant *KRAS* gene in colorectal cancer. Intestinal hyperplasia was developed in zebrafish expressing mutated human *KRAS* (*KRAS^{G12D}*). Although a suitable model of colorectal cancer in zebrafish has not been developed yet, the genes required for the rapid proliferation of zebrafish intestinal epithelial cells during development have been identified and may represent potential therapeutic targets. Zebrafish research could also add to the study of the intestinal inflammation involved in the initiation and progression of this disease. In the future, the use of zebrafish as hosts for xenografts of human colorectal cancer cells is foreseen (Lobert et al. 2016).

The mutated *KRAS* oncogene is also implicated in several types of pancreatic adenocarcinoma. Zebrafish was used to explore *in vivo* the tumorigenic action of a constitutively active human *KRAS^{G12D}* variant. A model for pancreatic adenocarcinoma was made using transgene fish expressing this variant under the promoter and enhancer elements of *ptf1a*, a transcription factor expressed in the exocrine pancreas (Schiavone et al. 2014). Activity of the Notch pathway during the early stages and the Smad3/TGFβ and Shh pathways during the later stages of pancreatic adenocarcinoma was

impaired. The molecular players in these pathways are candidates for new therapies for pancreatic adenocarcinoma.

Since zebrafish is an excellent *in vivo* platform for screening potential antitumor compounds that can be added directly to the water, there is hope that it will become a preclinical personalized medicine platform for the rapid assessment of the metastatic potential and drug-sensitivity of patient-derived cancers in the near future.

Zebrafish and cardiovascular diseases

Modeling thrombosis and hemostasis in zebrafish

About 300 factors involved in hemostasis and thrombosis are highly conserved in zebrafish and humans (Kretz et al. 2015). Thrombosis models are made by vascular injury induced by chemicals (ferric chloride, phenylhydrazine), laser or mechanically (Gregory et al. 2002). Injuries are induced in embryos 3 to 5 days post fertilization (dpf) and thrombus formation is monitored by microscopy. Thrombin generation may be measured in a drop of non-anticoagulated whole blood (Schurgers et al. 2016). Furthermore, the density and dimensions of the fibrin strands within the fibrin network are visualized by scanning electron microscopy (Schurgers et al. 2016). Relative quantitation of circulating thrombocytes may be performed using a Tg(itga2b:GFP) zebrafish line in which these cells are labeled by the expression of GFP. The mechanisms and physiology of thrombus formation in zebrafish were studied by intravenous injection of FITC-labeled fibrinogen into larvae and subsequent vessel injury. Accumulation of thrombocytes in the arterial thrombosis model was demonstrated by their labeling with DiI-C18 dye (Gregory and Jagadeeswaran 2002) and further confirmed by the use of transgenic zebrafish expressing GFP from the promoter of *CD41*, coding the α subunit of the platelet integrin (Lin et al. 2005).

Using a combination of gene knockdown and laser injury methods, the role of prothrombin, factor VII, factor VIII, hepsin, FSAP, Mlck1a, protein kinase c α, protein kinase c β, G6fl, von Willebrand factor (vWF) and fibrinogen in hemostasis has been investigated so far, but zebrafish can be also used for the identification of novel mediators of hemostasis and thrombosis (Jagadeeswaran et al. 2016). When fibrinogen chains were knocked down by morpholinos, either individually or combined, the obtained phenotypes were strikingly similar to those of patients with hypo- and afibrinogenemia (intraventricular and intramuscular hemorrhage) (Vo et al. 2013).

Since the coagulation pathways of zebrafish and mammals are quite similar, zebrafish may be the supreme animal model for determining the effect of novel therapeutics on thrombin generation and may serve for mechanistical research in thrombosis and hemostasis.

Zebrafish in modeling cardiomyopathies

Several advantages make zebrafish well suited for the study of cardiovascular disorders. Unlike other vertebrate models, zebrafish embryos and larvae get sufficient oxygen by passive diffusion for the first 7 dpf (Pelster and Burggren 1996). This allows the development and survival of animals with malformed hearts within this time window. In this way, phenotypic analyses of mutations that would in mammals cause early embryonic lethality, are possible. Many of the genes involved in heart development are conserved. Moreover, the development and anatomy of the heart, cardiac contraction, vessels and blood flow are easily observed *in vivo* due to the transparency of embryos. Several cardiomyopathy models have been developed in zebrafish as a result of the identification of mutants with heart defects resembling human diseases (Asnani and Peterson 2014; Bournele and Beis 2016).

The zebrafish heart comprises two chambers (atrium and ventricle), inflow (sinus venosus) and outflow (bulbus arteriosus) tracts, connected in series (Fig. 2). It pumps desaturated venous blood to the ventral aorta leading to the gill arches where oxygenation occurs, and from where it is distributed to the rest of the body. The zebrafish heart shares common structural components with a mammalian heart, making it an appropriate model system for experimental studies of the cardiovascular system (Stainier et al. 1996).

The two most prevalent forms of cardiomyopathies in humans that primarily affect the myocardium are dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). In DCM, one or both ventricles of the heart are enlarged causing diminishing in myocardial contractility, while HCM is characterized by a thickening of the ventricular myocardium. Heart phenotypes of several zebrafish mu-

Fig. 2. Schematic presentation of zebrafish heart comprising two chambers (atrium and ventricle), inflow (sinus venosus) and outflow (bulbus arteriosus) tracts. Direction of blood flow is shown by red dashed arrows (**A**). Optical section of transgene Tg(myl7: ras-GFP); Tg(kdrl: ras-mCherry) zebrafish heart at 78 hpf, obtained by confocal microscopy. Myocardium −green signal, endocardium −red signal (**B**). hpf – hours post fertilization.
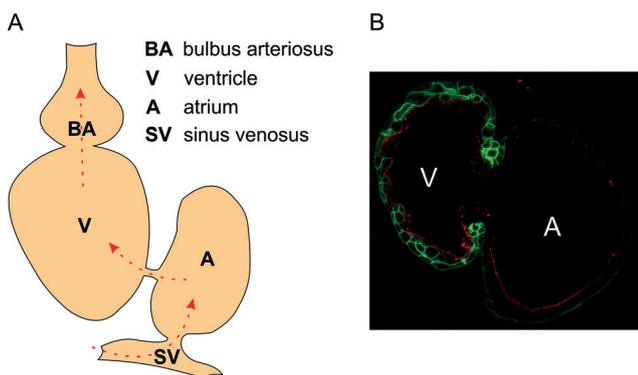
tants resemble the features of human cardiomyopathies. They display pericardial edema, cardiac dysfunction and death of cardiomyocytes. The hearts in DCM zebrafish models develop normally, but the ventricle and/or atrium are enlarged and poor contractility is observed from the first beat (Stainier et al. 1996).

Silent heart has a mutation in the gene *TNNT2*, encoding the cardiac contractile protein troponin T (Stainier et al. 1996). Thin filament proteins, α-tropomyosin and cardiac troponins C and I, are also reduced, resulting in severe sarcomere defects and cardiomyocyte disarray. These ultrastructural changes are similar to those seen in the hearts of patients suffering from familial forms of HCM and DCM. Apart from human HCM and DCM, mutations in human *TNNT2* are also a leading cause of sudden death in young athletes.

A pickwickm171 mutation in zebrafish causes a weakly contractile heart and pericardial edema (Driever et al. 1996). This mutation is attributed to an alternatively spliced exon of the titin gene *ttn*. Titin is necessary for proper sarcomere assembly in cardiomyocytes and variants in *TTN* cause human idiopathic DCM. It is interesting that the severe phenotype in DCM patients with titin truncation mutations at the C-terminus, in contrast to the milder phenotype associated with mutations located at the N-terminus, was explained using zebrafish (Zou et al. 2015). Zou and coauthors (2015) identified a conserved internal promoter in the titin gene that is placed at the distal I-band region and gives rise to the titin isoform Cronos. The location of this promoter and position of titin truncations in patients with the most severe forms of DCM overlap. The resulting protein is capable of partially rescuing N-terminal truncations, with more severe disease occurring if both the full-length titin and Cronos are disturbed.

The main squeeze mutant displays ventricle dysfunction starting at 60-70 hpf. In addition to titin and cardiac troponin T, laminin α-4 and integrin-linked kinase mutations, which are linked to familial DCM in humans, produce heart failure in zebrafish by affecting the cardiomyocytes and endothelial cells (Knöll et al. 2007).

Cardiotoxic chemotherapeutics cause cardiomyopathy in zebrafish, as they do in humans, leading to heart failure. Anthracyclines such as doxorubicin are used to treat a number of common malignancies, including breast cancer, lung cancer, leukemia and lymphoma. Although extremely effective, exposure to doxorubicin causes dose-dependent cardiotoxicity, which can lead to clinically significant cardiomyopathy and congestive heart failure. It was found that doxorubicin leads to heart failure in zebrafish larvae, resulting in decreased myocardial contractility and increased cardiomyocyte apoptosis (Han et al. 2015). Targeted anticancer drugs sunitinib and sorafenib, tyrosine kinase inhibitors, also have cardiovascular side effects. They cause ventricular dilation and impaired cardiac function in zebrafish larvae

(Cheng et al. 2011).These specific models may be used to support preclinical screening of chemotherapies, and to assist in the design of cardioprotective cancer treatment.

The adult human heart exhibits a limited degree of regeneration, and is incapable of counteracting severe loss of cardiac muscle tissue after myocardial infarction (MI). In contrast to mammals, zebrafish can fully regenerate the myocardium after MI via the orchestrated action of inflammation, matrix deposition and remodeling and cardiomyocyte proliferation, which is followed by intense neovascularization (Kikuchi 2014). It is important to understand why adult mammals develop extensive scarring instead of regeneration. In order to increase healing of an injured human heart, one promising strategy is to re-activate the endogenous regenerative potential of neonatal mammals. The barriers to human heart regeneration after cardiac injuries are still not fully understood and current research is directed towards establishing why zebrafish have remarkable capacity to regenerate injured heart, while mammals dramatically lose cardiac regenerative potential.

MI has been recently modeled in fish using cryo injury, which caused substantial cell death. Necrotic tissue that remains in the heart after cryo injury resembles the pathogenesis after MI (Chablais et al. 2011). During post-MI recovery, the activated epicardium contributes to the neovascularization of injured tissue. By limited dedifferentiation, differentiated cardiomyocytes acquire a more embryonic identity characterized by structural changes and the expression of cell-cycle progression genes (Jopling et al. 2010; Kikuchi et al. 2010). Additionally, a variety of growth factors and cytokines are being studied for their efficacy to stimulate cardiomyocyte proliferation. It is hoped that studies on regeneration of injured zebrafish hearts will help in the discovery of novel drug targets and therapeutic strategies for heart failure.

Zebrafish and myopathies

Skeletal muscle represents a significant portion of the zebrafish body. It becomes functional very early during development; the first contractions in the zebrafish embryo are observed at 17 h post fertilization. Many histological and ultrastructural features are similar between zebrafish and human skeletal muscle. Zebrafish research has substantially enhanced the identification of new muscle disease genes and the investigation of novel therapeutic strategies (Li et al. 2017).

Zebrafish axial muscles derive from the presomitic mesoderm which becomes segmented in an anterior-to-posterior fashion. Reiterated embryonic segments, called somites, subsequently differentiate into the myotomes (Fig. 3). Zebrafish fast and slow myofibers are spatially segregated in the morphologically distinct territories; the multinucleated fast (white) muscle fibers form the bulk of the zebrafish myotome and are located medially to the superficial layer of

slow (red) muscle fibers (Fig. 3). Striated myofibers, which span each myotome, are interconnected at a myotendinous junction (MTJ), also called the vertical myoseptum (Gibbs et al. 2013).

Several simple and noninvasive techniques are used for the analysis of muscle structure and function in zebrafish. Within the first days of life, developing zebrafish embryos rhythmically coil inside the chorion. The movements can be easily measured, enabling the monitoring of the early func-
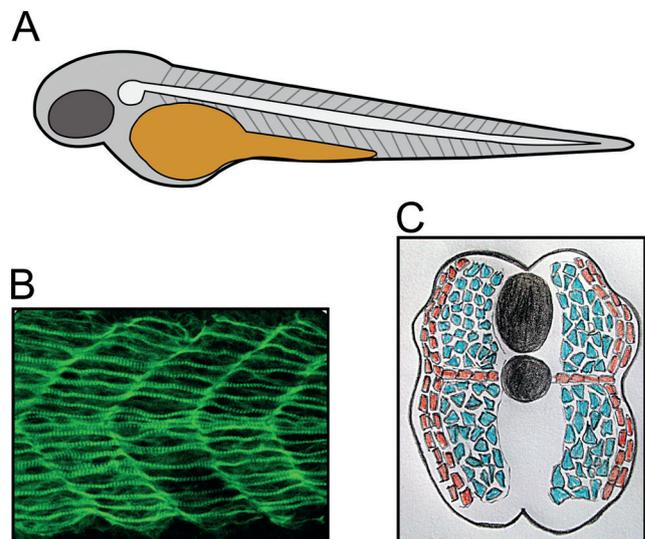
Fig. 3. Schematic presentation of zebrafish axial skeletal muscles that are positioned along the trunk and tail of the larvae and consist of 30 segmental blocks of muscle, called myotomes (**A**). Whole mount immunostaining of zebrafish striated myofibers with anti-actin antibody (**B**). Fast (red) and slow (blue) myofibers are spatially segregated in the morphologically distinct territories (**C**).

tion of slow muscle (Dowling et al. 2009). The touch-evoked escape assay could be performed 2 dpf, when zebrafish are able to rapidly swim away from mechanical stimuli (escape response), which can be recorded and subsequently analyzed. In contrast to wild-type larvae, mutant larvae frequently display a weak escape contraction followed by slow swimming to a short distance (Smith et al. 2013). Using special imaging systems, it is possible to quantify swim behaviors such as velocity and turn frequency in larvae that begin to swim at approximately 3 dpf. There are several techniques for the evaluation of muscle function that measure parameters of muscle contractions (Dowling et al. 2009). The integrity of developing zebrafish muscle can be easily monitored using birefringence, a simple and noninvasive approach for observing disorganization of sarcomeres (Smith et al. 2013). If quantified, it is a direct measure of loss of muscle integrity and particularly useful in the functional characterization of novel muscle mutants, as well as for testing the efficiency of therapies in dystrophy models. It is possible to rapidly and ef-

ficiently isolate and maintain myofibers from the developing zebrafish *ex vivo* (Horstick et al. 2013), which allows more precise measurements of myofiber size, characterization of protein subcellular localization, identification of important histopathological features in validation studies of newly developed disease models, live imaging and electrophysiological studies.

Zebrafish models for muscle diseases are powerful tools in understanding disease pathogenesis and accelerating the discovery of therapeutics. The *sapje* (*dmd*) mutant was molecularly characterized in 2003 as the first zebrafish mutant model for Duchenne muscular dystrophy (DMD) (Bassett and Currie 2003). The disease is caused by mutations in the dystrophin gene, it is lethal due to respiratory or cardiac failure, incurable and characterized by severe wasting of muscle tissue. Zebrafish *sapje* mutants display progressive degeneration of skeletal muscle resembling a phenotype similar to the human disease (Bassett and Currie 2003). Cell detachment and death are accompanied by inflammation and fibrosis. Regeneration cannot compensate for the massive cell death, and *sapje* mutants die at an early larval stage. Maximal force production in this mutant, as an indicator of general muscle strength, is significantly reduced, reflecting the loss of functional myofibers and contractile units. The *sapje* mutants have been used in a chemical suppressor screen aimed at identifying potential compounds able to correct the disease's pathology (Kawahara et al. 2011a) and a number of compounds that appear to effectively reduce dystrophic symptoms in zebrafish were identified. Exon skipping by antisense oligonucleotides (AOs) has also been tested in the *sapje* model (Berger et al. 2011). The main advantage in testing novel therapies is that dystrophin-deficient zebrafish exhibit muscle degeneration early during development, while dystrophin-deficient mdx mice have a very mild phenotype, probably due to partial compensation of dystrophin loss by utrophin.

There are zebrafish mutant models for several types of congenital muscular dystrophy (CMD) including CMD1A (merosin-deficient muscular dystrophy).The dominant pathological feature of the zebrafish laminin 2 mutant *candyfloss* (*lama2*, *caf*) larvae is fiber detachment from the MTJ, causing fiber retraction, while motor neuron development and early myogenesis progress normally (Hall et al. 2007). Muscles of *candyfloss* mutants contract with significantly reduced force, while mechanical stress, applied as immobilization, can rescue the disease phenotype (Li and Arner 2015). Mutations in the human integrin gene *ITGA7*, which codes for the striated muscle-specific integrin α7 subunit, lead to integrin α7-related congenital muscular dystrophy which affects basal lamina integrity and function. *itga7* morphants have dystrophic phenotype and muscles display fiber retraction and detachment at an early larval stage (3 dpf) (Postel et al. 2008).

Zebrafish models are also used to investigate pathogenic mechanisms of limb-girdle muscular dystrophy, including

dysferlinopathy (LGMD2B, Miyoshi myopathy) and sarcoglycanopathies (LGMD 2C-F). Dysferlinopathy is muscular dystrophy with milder symptoms, developing in adulthood and characterized by a loss of the functional transmembrane protein dysferlin. Muscle injury leads to an accumulation of dysferlin in the membrane compartment in sarcolemmal lesions, which subsequently recruits annexins to the repair patch. The larval muscles of a zebrafish dysferlin morphant are severely disorganized (Kawahara et al. 2011b). Myofibers are misaligned and curved, with many gaps (Roostalu and Strähle 2012). Dysferlin morphants were used to study the mechanism of muscle cell repair (Roostalu and Strähle 2012). Sarcoglycans (α, β, γ, δ and ε) are transmembrane proteins of the dystrophin-associated glycoprotein complex (DGC) family. Mutations in sarcoglycans are associated with several forms of LGMD characterized by clinical manifestations comparable to DMD. Like the human patient phenotype, zebrafish δ-sarcoglycan morphant larvae have disorganized muscles and loose muscle activity, but at an earlier developmental stage. In addition, heart looping and asymmetry are also altered (Cheng et al. 2006).

Dystroglycan, an essential, highly conserved component of DGC, connects dystrophin to the extracellular matrix and transduces signals in the neuromuscular junction. Loss of *dag1* expression in zebrafish knockout disrupted the DGC, affected muscle cell integrity and led to necrosis (Parsons et al. 2002). The muscles of *patchytail* (*dag1*) mutant exhibit structural abnormalities such as destabilization of fiber attachment and disorganization of T-tubules at the early stages (Gupta et al. 2011).

The zebrafish model for nemaline myopathy, characterized by severe muscle weakness, impaired locomotion, early death and the presence of rod-like (nemaline) bodies, is called *träge* (*trg*). Myofibers of this mutant, which has a nonsense mutation in *tropomodulin 4* (*tmod4*), are disorganized. Thin filaments are dispersed throughout the myofibers, they have various lengths, widened Z-disks, undefined H-zones and electron-dense aggregations. Nemaline rods, the hallmark of disease, are also present. It is interesting that mutations in TMOD4 have not been associated with myopathy, so it represents a novel candidate for unresolved nemaline myopathy cases (Berger et al. 2014). Overexpression and loss-of-function zebrafish models for skeletal muscle α-actin (*ACTA1*) and *nebulin* (*NEB*) were generated to investigate the formation of nemaline bodies, their dynamics *in vivo* and their role in pathogenesis (Sztal et al. 2015).

Perspectives

In the last few decades, the zebrafish has been developed into a very powerful tool to study the mechanisms leading to human diseases and to model them. This field is growing rapidly due to the yet unexplored potential of the zebrafish models for clinical and basic biomedical research.

The combination of the CRISPR-Cas9 system and zebrafish is of great promise for studying human genetic diseases, since they enable relatively easy and fast validation of the functions of candidate disease genes which are continuously being identified. Apart from deciphering the molecular mechanisms of diseases, zebrafish models will be beneficial to the discovery of new therapeutic agents for a wide range of disorders.

Acknowledgments

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