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Bone biology in animal models, including testing of bone biomaterials

Fish: a suitable system to model human bone disorders and discover drugs with osteogenic or osteotoxic activities

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This review discusses the suitability and advantages of teleost fish for studying underlying mechanisms of normal and pathological development and mineralization of vertebrate skeleton, presents a selection of zebrafish mutants and transgenic lines modeling human skeletal diseases and highlights currently available fish systems for identifying and characterizing novel osteogenic and osteotoxic molecules.

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Introduction

The last decade has seen an exponential interest in the use of model organisms capable of providing suitable alternatives to mammalian models and allowing quicker and less expensive approaches, leading to significant improvements in our knowledge on the molecular basis of human pathologies. In this respect, the study of skeletal diseases has not been an exception but requires the use of vertebrates for obvious reasons. Fish, in particular zebrafish and medaka, have been used with a growing success due to their many similarities with mammals in molecular pathways and mechanisms involved in the onset of patterning and development of skeletal structures. Many reports have confirmed the suitability of fish systems to model many of the pathological defects affecting human skeletal formation, osteoclastic bone resorption, osteoblastic bone mineralization and extracellular matrix maintenance, but also to visualize the onset of normal and abnormal skeletal structures, and the possibility of rapidly detecting drug-induced osteogenic or osteotoxic effects in phenotype-based assays.

Teleost fish and human skeletons are remarkably similar

Despite some small differences that can be attributed to evolutionary distance (their last common ancestor existed approximately 420 million years ago), anatomic and developmental features of teleost fish and mammalian skeletons are remarkably similar, with much of the skull, axial and appendicular skeleton formed by identical bones, and with a high conservation of the developmental events and underlying mechanisms of skeletogenesis, including the early formation of a cartilaginous anlage followed by bone formation through endochondral and dermal ossification [1,2]. Main differences and similarities between fish and mammalian bone are summarized in Table 1 (see [3] for a more comprehensive comparison). The most striking difference is probably the occurrence of acellular bone (devoid of osteocytes) and mononucleated osteoclasts in most teleost fish species, while mammals have exclusively cellular bone and multinucleated osteoclasts. Interestingly, osteocytic bone and multinucleated osteoclast

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Table 1. Features of fish and human bone: similarities and differences

Fish skeleton	Human skeleton	References
Endochondral and membranous ossification		[1,59]
Hydroxyapatite-like calcium-phosphate crystals		[1]
Bone formation by osteoblasts positive for alkaline phosphatase		[5]
Bone resorption by osteoclasts positive for tartrate-resistance acid phosphatase		[5]
Mesenchymal origin of osteoblasts; hematopoietic origin of osteoclasts		[59]
Osteocytic and anosteocytic bone	Osteocytic bone	[5]
Dendritic processes mostly absent and no lacunocanalicular system	Dendritic processes in a well-developed lacunocanalicular system	[60–62]
Mono- and multinucleated osteoclasts	Multinucleated osteoclasts	[63]
Mechanosensing by osteoblasts and lining cells	Mechanosensing by osteocytes	[59,61,64]
Collagen I and II in bone	Collagen I in bone	[1,8]
Perichordal ossification of the vertebral column	Endochondral ossification of the vertebral column	[6]
16 types of cartilage	3 types of cartilage	[8]

occur in zebrafish [4,5]. Also noteworthy is the fact that most teleost fish ossify the vertebrae directly over the notochord, while mammals have a cartilaginous precursor [6,7]. Fish also have a much higher diversity of cartilage types and intermediate tissues than what is found in adult mammals [4,8,9]. Despite those differences, the availability of fish mutants exhibiting features resembling human pathologies has contributed to validate fish as a model organism to study mechanisms underlying skeleton development and skeletal disorders.

Among teleost fishes, zebrafish *Danio rerio* (Hamilton, 1822) has been the target of a growing interest from the scientific community. Zebrafish is a small freshwater fish from tropical regions of South and Southeast Asia for which numerous models of human diseases have been identified or developed during the last decade, leading to its recognition as a suitable model for biomedical research (reviewed in [10]). Zebrafish embryos develop externally and are transparent, allowing for direct observation of embryonic development and organ growth. Additional features such as small size (3–4 cm long), high fecundity (mature females can lay hundreds of non-adhesive eggs every week), short generation time (adulthood attained in 3–4 months) and rapid development (most body structures are visible 48 h after fertilization), robustness (they are easy to manipulate, adapt to a wide range of environments and can be kept in large shoals) and availability of genetic techniques, have reinforced the attractiveness of the zebrafish as a laboratory model. Finally and importantly, the zebrafish genome has been almost entirely sequenced and annotated, with most human genes having orthologs in zebrafish [11]. In addition, it is estimated that approximately 70% of human disease genes have functional homologs in zebrafish [12]. The key regulators of bone formation have been highly conserved, and corresponding zebrafish orthologs share significant sequence similarities and overlapping of expression patterns [13]. For phenotype-based assays related to skeletogenesis, the transparency and external/rapid development of fish embryos is a clear advantage. It is possible to monitor each stage of skeleton development and determine at high resolution the role of single cells in bone and cartilage formation/mineralization. However, zebrafish is not a mammalian species and the lack of some organs (*e.g.* lung or mammary gland) represents a limitation to the modeling of some human diseases. In addition, phenotypic characteristics of diseases caused by orthologous genes can differ between fish and human. Because it suffered the teleost-specific whole genome duplication event, zebrafish genome contains numerous paralogous genes that resulted in gene sub-functionalization and/or neofunctionalization, a situation that may limit, in some cases, the use of zebrafish as a disease model. However, this is also true in studies using the mouse model [11,14]. Still, zebrafish is a relevant model organism to study vertebrate development and human diseases, and is rapidly acquiring standard prominence worldwide as an alternative and complement to rodent models, which remain the standard system in pre-human drug tests.

Fish models of human skeletal disorders

In the last decade, there has been an increasing effort in producing mutant and transgenic fish lines with the objective of modeling human skeletal disorders and improving the visualization of the skeleton. These achievements have decisively contribute to facilitate studies towards uncovering novel drugs with the potential to treat the many skeletal pathologies that affect millions of people worldwide and which incidence is growing due to the increase in human life span. Human diseases such as osteogenesis imperfecta, bone loss (osteoporosis and osteopenia), craniosynostosis, craniofacial defects and spinal deformities (scoliosis, holospondyly) are examples, among many others, of pathologies for which fish mutants are available and being used to unveil the corresponding changes in normal molecular pathways, thus contributing to discover therapeutic molecules capable of rescuing these pathologies (Table 2). Although medaka and guppy have also been used to model human bone disorders,