Aquatic Toxicology 183 (2017) 127–134

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Ancestral benzo[a]pyrene exposure affects bone integrity in F3 adult fish (*Oryzias latipes*)

Frauke Seemann[a], Chang-Bum Jeong[b], Ge Zhang[c], Miles Teng Wan[a], Baosheng Guo[c], Drew Ryan Peterson[a], Jae-Seong Lee[b], Doris Wai-Ting Au[a,*]

[a] State Key Laboratory in Marine Pollution, Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Hong Kong
[b] Department of Biological Science, College of Science, Sungkyunkwan University, Suwon 16419, South Korea
[c] Institute for Advancing Translational Medicine in Bone & Joint Diseases, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong

ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form
17 December 2016
Accepted 19 December 2016
Available online 23 December 2016

Keywords:
Transgenerational inheritance
Osterix
Epigenetics
Osteogenic miRNAs
Polycyclic aromatic hydrocarbons
Osteoblast activity

ABSTRACT

Benzo[a]pyrene (BaP) at an environmentally relevant concentration (1 μg/L) has previously been shown to affect bone development in a transgenerational manner in F3 medaka (*Oryzias latipes*) larvae (17 dph). Here, we provide novel histomorphometric data demonstrating that the impaired bone formation at an early life stage is not recoverable and can result in a persistent transgenerational impairment of bone metabolism in F3 adult fish. A decrease in bone thickness and the occurrence of microcracks in ancestrally BaP-treated adult male fish (F3) were revealed by MicroCt measurement and histopathological analysis. The expression of twenty conserved bone miRNAs were screened in medaka and their relative expression (in the F3 ancestral BaP treatment *vs* the F3 control fish) were determined by quantitative real-time PCR. Attempt was made to link bone miRNA expression with the potential target bone mRNA expression in medaka. Five functional pairs of mRNA/miRNA were identified (Osx/miR-214, Col2a1b/miR-29b, Runx2/miR-204, Sox9b/miR-199a-3p, APC/miR-27b). Unique knowledge of bone-related miRNA expression in medaka in response to ancestral BaP-exposure in the F3 generation is presented. From the ecological risk assessment perspective, BaP needs to be regarded as a transgenerational skeletal toxicant which exerts a far-reaching impact on fish survival and fitness. Given that the underlying mechanisms of cartilage/bone formation are conserved between medaka and mammals, the results may also shed light on the potential transgenerational effect of BaP on skeletal disorders in mammals/humans.

1. Introduction

The ubiquitous environmental pollutant benzo[a]pyrene (BaP) has previously been shown to induce compression of the vertebral segments by affecting the notochord sheath and the osteoblast cell population in ancestrally-exposed larval Japanese medaka (*Oryzias latipes*) of the F3 generation (Seemann et al., 2015). Moreover, the deregulation of the bone marker genes *osterix* (Osx), *alkaline phosphatase* (ALP), *SRY-related HMG-box 9a* (Sox9a), *SRY-related HMG-box 9b* (Sox9b) *and bone morphogenetic protein 2* (BMP2) strongly indicated a perturbation in the osteoblast maturation. A compressed vertebral bone structure is likely to increase the risk of fractures and affect the fish's swimming ability, thus compromising the individual's survival fitness (feeding and mating). Therefore, the persistence of the BaP-induced bone impairment in adulthood would have a far-reaching negative impact on the population sustainability.

To investigate if the adult bone integrity can be affected by ancestral BaP-exposure, bone mineral density, bone tissue thickness and the connectivity density, which can be interpreted ambivalently as indicator for microcracks and bone elasticity, were assessed for ancestrally BaP-exposed F3 adults. Changes in the osteoblast numbers and a battery of marker genes involved in bone metabolism and osteoblast differentiation pathways were measured (Fig. 1).

Abbreviations: AcvR1b, activating receptor type 1b; ALP, alkaline phosphatase; APC, adenomatous polyposis coli; ATF4, activating transcription factor 4; BaP, benzo[a]pyrene; BMD, bone mineral density; BMP2, bone morphogenetic protein 2; Col2a1b, collagen 2a1b; Col10, collagen 10a1; CTSK, cathepsin K; dph, days post hatching; miRNA, microRNA; OB, Osteoblast; OP, Osteoprogenitor; OPN, osteopontin; Osx, osterix; PTHrP, para-thyroidhormone-receptor protein; Rankl, receptor activator of nuclear factor-κB ligand; Runx2, runt-related transcription factor 2; Sox9a, SRY-related HMG-box 9a; Sox9b, SRY-related HMG-box 9b.

* Corresponding author at: State Key Laboratory in Marine Pollution, Department of Biology and Chemistry, City University of Hong Kong, Tat Chee Avenue, Hong Kong.
E-mail address: bhdwtau@cityu.edu.hk (D.W.-T. Au).

http://dx.doi.org/10.1016/j.aquatox.2016.12.018
0166-445X/

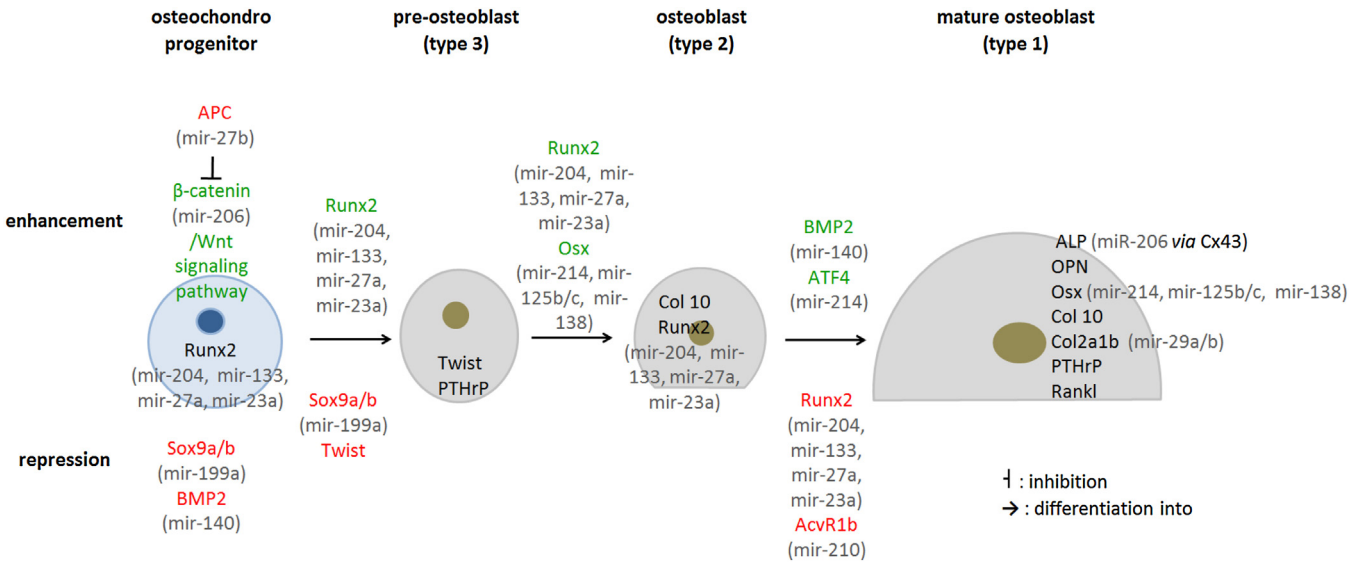

Fig. 1. Hypothetical molecular regulation of osteoblast (OB) differentiation and maturation in adult medaka bone, based on published mammalian pathways*. Designations of type 1–3 osteoblasts are in accordance with the osteoblast populations described for medaka by Inohaya et al. (2007). Osteogenic genes (black: OB activity; red: OB differentiation repressors; green: OB differentiation enhancers) are associated with their regulating miRNAs (grey). Osteochondro progenitor (OP) cells can differentiate into osteoblasts, chondrocytes, myocytes or adipocytes dependent on the microenvironment. In the presence of β-catenin originating from the Wnt-signaling pathway, OP cells can develop into pre-osteoblasts (type 3), which are characterised by twist and parathyroid-hormone receptor protein (PTHrP) expression. Stimulated through runt-related transcription factor 2 (Runx2) and osterix (Osx), pre-osteoblasts can further differentiate into osteoblasts (type 2) expressing collagen 10 (Col10) and Runx2. Bone morphogenetic protein 2 (BMP2) and activating transcription factor 4 (ATF4) are necessary for further development into mature osteoblasts (type 1), which are responsible for the formation of new bone matrix. Dependent on the stage of cell differentiation, the same molecular marker can either enhance or repress the further cell differentiation. For instance, BMP2 inhibits OP cells development towards the osteoblastic lineage, but it is needed for final differentiation from osteoblasts to mature osteoblasts. (AcvR1b: activating receptor type 1b; ALP: alkaline phosphatase; APC: adenomatous polyposis coli; Cx43: connexion 43; Sox9a/b: sry-related HMG-box 9a/b; OPN: osteopontin; Col2a1b: collagen 2a1b; Rankl: receptor activator of nuclear factor kappa-B ligand).
*References: Bialek et al., 2004; Day et al., 2005; Duplomb et al., 2007; Eames et al., 2012; Inohaya et al., 2007; Jing et al., 2015; Komori, 2008; Lian et al., 2012; Long, 2011; Matsushita et al., 2014; Renn et al., 2013; Tonna and Sims, 2014; Zouani et al., 2013.

Bone metabolism in mammals is tightly orchestrated by designated microRNAs (miRNA) (He et al., 2009; Lian et al., 2012; Zhao et al., 2014). These 'osteomiRs' comprise a group of miRNAs expressed in osteoblast lineage cells. They regulate bone formation by repressing inhibitors or promoting osteogenic gene expression (Lian et al., 2012). MiRNAs silence their target genes either through translational repression or mRNA destabilisation (Jonas and Izaurralde, 2015). Together with DNA methylation and histone modification, miRNA deregulation is considered an important epigenetic mechanism attributing to transgenerational inheritance (Castel and Martienssen, 2013).

Despite major breakthroughs in the discovery and description of the genetic regulation of bone metabolism in medaka (Inohaya et al., 2007; Kudo, 2011; Renn and Winkler, 2010, 2012, 2014; Shanthanagouda et al., 2014; Takeyama et al., 2014), knowledge of bone-regulating miRNAs remains scarce in fish. Based on published research from mammals and fish a hypothetical pathway of mRNAs and miRNAs involved in the differentiation of the bone forming osteoblasts is proposed for the medaka model (Fig. 1). Osteoblasts in fish originate from the scelorotomal stem cells (Inohaya et al., 2007). In the presence of β-catenin, produced by the Wnt-signalling pathway, differentiation of osteochondro-progenitor cells into pre-osteoblasts is induced. This step is promoted by runt-related transcription factor 2 (Runx2) (Duplomb et al., 2007; Long, 2011). Maturation of the osteoblasts is initiated through Runx2 and Osx (Day et al., 2005; Komori, 2008; Renn et al., 2013). Activating transcription factor 4 (ATF4) and BMP2 stimulate the final differentiation step into mature bone forming osteoblasts (Zouani et al., 2013). During osteoblast differentiation, Sox9a, Sox9b and activing receptor type 1b (AcvR1b) exert a solely repressing function, while Runx2 and BMP2 regulate osteoblast differentiation ambivalently depending on the developmental stage (Eames et al., 2012). Adenomatous polyposis coli (APC) is an indirect inhibitor of osteoblast differentiation, acting through the repression of the β-catenin/Wnt-signalling (Lian et al., 2012; Matsushita et al., 2014). The majority of osteoblast differentiation enhancing and repressing genes and osteoblast markers have been identified as targets for miRNA silencing in mammals (Ell and Kang, 2014; Fang et al., 2015; Jing et al., 2015; Kapinas and Delany, 2011; Lian et al., 2012; Titorencu et al., 2014). The miRNAs for screening have been selected due to (i) their presence during bone development in medaka or (ii) their susceptibility to BaP-exposure and their implication in osteoblast differentiation in mammals. MiR-7, miR-23a, miR-27a, miR-27b, miR-125b, miR-133, miR-140, miR-199a, miR-206 and miR-214 have been localized by whole mount in-situ hybridization during early development of the pharyngeal arches in medaka (Ason et al., 2006). Changes of miR-26, miR-29, miR-125b/c, miR-133, miR-138, miR-140, miR-204, miR-210 and miR-214 expression have been shown in mammals subsequent to BaP/cigarette smoke exposure (Brevik et al., 2012a; Brevik et al., 2012b; Izzotti et al., 2009; Lizarraga et al., 2012), and these miRNAs have been described for mammalian osteoblast differentiation (Lian et al., 2012; Wang et al., 2013).

Notwithstanding the evidence of transgenerational toxicity of BaP in the bone tissue of fish larvae (Corrales et al., 2014; Seemann et al., 2015), the persistence of skeletal impairment during adulthood remains unknown. The present study aims to link potential abnormal vertebral phenotypes (microarchitecture and cellular pathologies) observed in the ancestrally BaP-exposed adult F3 generation with potential candidate miRNAs and target genes (the molecular pathways). This novel knowledge of miRNA expression in medaka will shed light on the epigenetic regulation of osteogenic genes expression in the ancestrally BaP-exposed F3 fish. The investigation of the genetic network controlling bone formation may reveal potential candidate genes (i) responsible for impaired bone