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Epigenetic effects of environmental chemicals: Insights from zebrafish

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Abstract

Zebrafish have been extensively used for studying vertebrate development and modeling human diseases such as cancer. In the last two decades, they have also emerged as an important model for developmental toxicology research and, more recently, for studying the developmental origins of health and disease (DOHaD). It is widely recognized that epigenetic mechanisms mediate the persistent effects of exposure to chemicals during sensitive windows of development. There is considerable interest in understanding the epigenetic mechanisms associated with DOHaD using zebrafish as a model system. This review summarizes our current knowledge on the effects of environmental chemicals on DNA methylation, histone modifications and noncoding RNAs in the context of DOHaD, and suggest some key considerations in designing experiments for characterizating the mechanisms of action.

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1. Introduction

There is growing evidence from epidemiological and experimental studies that exposure to environmental stressors during critical windows of susceptibility can have long-term consequences [1,2]. Examples of association between exposure to environmental stressors during critical periods of fetal development and increased risk for cardiovascular diseases, obesity and neurological disorders are well documented [3,4]. This is a growing field of research and is collectively termed as the developmental origins of health and disease (DOHaD) [5,6]. The DOHaD hypothesis postulates that early life stressors can cause developmental reprogramming, inducing long-term changes in normal development and physiology [6]. Several studies have demonstrated that developmental exposure to environmental chemicals can cause long-term changes in physiology and behavior of the adults [7]. Some of these effects are shown to be inherited by subsequent generations. The mechanisms involved in developmental reprogramming by toxicants are not thoroughly understood; however, effects on epigenetic landscape during cellular and tissue differentiation are considered to be a potential mechanism of toxicant action [8]. Epigenetic modifications are defined as persistent changes in gene expression that occur without a change in the nucleotide sequence.

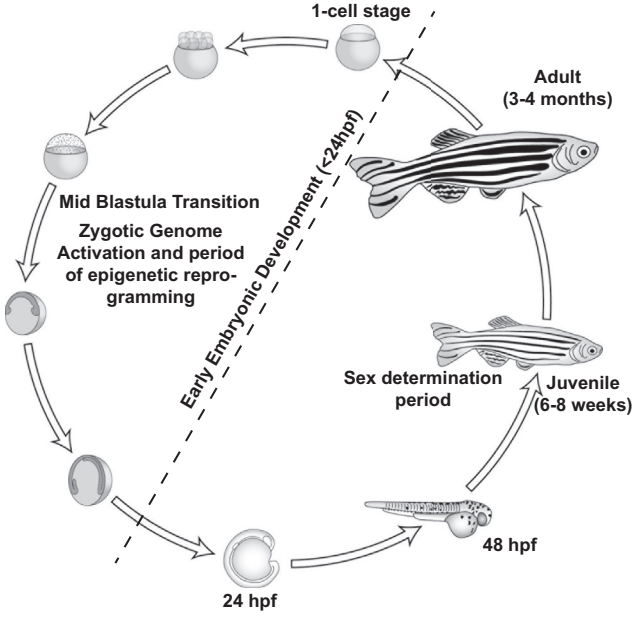
In the past two decades, there has been intense research on the impacts of environmental chemicals on various epigenetic factors using a variety of model systems [9,10]. The majority of the studies were conducted using mammalian models and to a lesser extent in non-mammalian models [11–13]. *Agouti* mouse is one of the well known model systems used to study epigenetic mechanisms of toxicant action [14]. Even though research in mammalian models can be easily translated to humans, conducting *in vivo* studies on a rapidly growing list of chemicals is time consuming and not cost-effective. In addition, studying the mechanisms of developmental reprogramming in embryos during *in utero* development is difficult. Hence, it is increasingly recognized that alternate vertebrate model systems could provide unique advantages in accelerating research in screening toxicants as well as understanding the mechanisms of action. One such model is zebrafish (*Danio rerio*), an established model in toxicology [15], developmental biology and human disease research [16]. More recently, it has been widely used as a model system for DOHaD studies and for understanding the underlying genetic and epigenetic mechanisms of action [17]. This review summarizes our current knowledge on the epigenetic effects of toxicants using zebrafish as a model organism and highlights the challenges and opportunities zebrafish offers for investigating the epigenetic mechanisms of action. Studies conducted so far have mostly focused on the impact of toxicants on the epigenetic machinery and very little is known about the mechanisms by which toxicants alter the epigenetic patterns. As zebrafish are increasingly used as an alternative model for DOHaD studies, this review summarizes the important factors to consider while conducting

studies to characterize the epigenetic basis of DOHaD effects.

2. Zebrafish as a model for DOHaD and epigenetic toxicology

Zebrafish have become an attractive model for DOHaD and transgenerational studies because of high fecundity, short generation time (embryo to adult in 3–4 months), external fertilization and development, and easy maintenance and breeding [16] (Fig. 1). In contrast to murine models where embryonic development occurs *in utero*, in zebrafish it occurs externally. This enables exposure of embryos to stressors immediately after fertilization (2–4 cell stage), in the absence of any maternal influence. Transparent zebrafish embryos allow visualization of any developmental abnormalities associated with exposure. Zebrafish are highly fecund and each female can lay hundreds of eggs at a time. This makes it possible to have relatively high sample size for each experimental condition. There are a number of larval and adult behavioral assays developed to assess the later life effects of developmental exposure to toxicants [18]. Compared to rodent models, rearing and maintenance costs for zebrafish are inexpensive. This is an important consideration for DOHaD and transgenerational studies, because costs associated with raising multiple animals from each treatment condition over a long time period, sometimes over multiple generations, can be expensive. Furthermore, in mammals transgenerational transmission of a phenotype requires assessment of the F_3 generation for embryonic exposure because primordial germ cells of the F_2 generation are exposed in pregnant dams [19]. In contrast, due to external development in zebrafish, studies in the F_2 generation are considered to be transgenerational [17].

Fig. 1



Zebrafish is an ideal model for DOHaD studies. Because of its short life cycle and rapid development they are ideal for conducting long-term studies including multi- and transgenerational studies. Similar to mammals, zebrafish undergo zygotic genome activation as well as epigenetic reprogramming. Exposure to environmental chemicals during sensitive windows of development can have later life consequences.

In addition to these advantages, zebrafish are also ideal for studying the epigenetic mechanisms of action. The availability of numerous transgenic fish strains enables characterization of cell and tissue-specific effects. Zebrafish are also amenable for genetic manipulation, and targeted gene-editing with CRISPR-Cas9 is widely used [20]. The availability of genomic resources [21] and the sequencing methods needed to conduct transcriptomic and epigenomic profiling have garnered enormous attention in the use of zebrafish as a model species in DOHaD studies. In the past few years, there have been several studies characterizing the developmental profiles of DNA methylation [22,23], histone modifications [24] and noncoding RNAs [25–27] providing base line information on the dynamics of epigenetic regulation during embryogenesis. Several studies have demonstrated the long-term effects of developmental exposure to toxicants. Most of these studies have reported later-life effects and in some cases intergenerational or transgenerational effects (Table 1). However, studies aimed at understanding the mechanisms behind DOHaD and multigenerational studies are still in their infancy.

Despite the unique advantages zebrafish offer, there are some distinct differences between zebrafish and mammals in epigenetic programming. Mammals undergo two rounds of reprogramming of DNA methylation, first at the time of fertilization in the zygote, and then in primordial germ cells (PGCs). In zebrafish the second wave of reprogramming has not yet been demonstrated. In addition, the methylomes of sperm and oocytes are significantly different and the paternal genome is resistant to demethylation in zebrafish [22,23]. Furthermore, zebrafish do not have genomic imprinting making them unsuitable for studying parent-of-origin effects.

3. DNA methylation

Similar to mammals, DNA methylation is one of the most well studied epigenetic modifications in zebrafish. Methylation of cytosine residues in CpG islands are generally considered to cause stable silencing of gene expression. Recently, Long et al. [28] empirically demonstrated that CpG islands in gene promoters are conserved among all vertebrates, including zebrafish. DNA methyltransferases (DNMTs) are responsible for the addition of the methyl groups on CpG dinucleotides. Zebrafish possess orthologs of both