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Review article

REVIEW ARTICLE

Medaka as a model for studying environmentally induced epigenetic transgenerational inheritance of phenotypes

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Abstract

Ability of environmental stressors to induce transgenerational diseases has been experimentally demonstrated in plants, worms, fish, and mammals, indicating that exposures affect not only human health but also fish and ecosystem health. Small aquarium fish have been reliable model to study genetic and epigenetic basis of development and disease. Additionally, fish can also provide better, economic opportunity to study transgenerational inheritance of adverse health and epigenetic mechanisms. Molecular mechanisms underlying germ cell development in fish are comparable to those in mammals and humans. This review will provide a short overview of long-term effects of environmental chemical contaminant exposure in various models, associated epigenetic mechanisms, and a perspective on fish as model to study environmentally induced transgenerational inheritance of altered phenotypes.

Key words: fish; environmental endocrine disrupting chemicals; transgenerational inheritance; epigenetics

Introduction

Environmental stress, a major contributor to evolution, enforces phenotypic variations in organisms that are directly exposed to it or remains as a causative factor for onset of abnormal physical or behavioral health in offspring that had never experienced it. This unique acquisition of phenotypic traits primarily due to the ancestral experience to adverse situation is termed as transgenerational inheritance, and actual mechanisms underlying such unprecedented environmental health outcomes are not clear yet. Emergence of environmentally induced transgenerational phenotypes depends on nature of stressor, window of development of the organism, and affected cell type. Because of organismal variability in reproductive processes and their response to environmental stress, a wide variety of test models have been recommended to study transgenerational inheritance. To date, the transgenerational phenotypes, including those induced by chemical contaminants, have been shown in worms, flies, plants, fish, mice, rats, domesticated farm animals, and humans, of which well-studied models are rodents and plants. Given that parental effects are mediated to offspring via gametes, for a transgenerational trait to develop, environmental stressors should affect germ cells. Species that provide ample opportunities to study biology of germ cells can serve as appropriate models to elucidate mechanisms of transgenerational inheritance at the molecular level. Each model has advantages over the other. Although rodents have been widely used, fish also have been found to be a reliable, economic model to study molecular mechanisms underlying germ cell-mediated epigenetic transgenerational

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inheritance of acquired traits. This review will briefly discuss about current concepts on long-term effects of contaminants in environmental ecotoxicology, evidences of long-term, transgenerational effects of endocrine disruptors (EDs), and the medaka fish as a model to study environmentally induced epigenetic transgenerational inheritance.

Ecotoxicology and Long-Term Effects of Contaminant Issues

During the mid-20th century, organophosphate chemicals were being widely and ambitiously used to free humans from unwanted bugs and pests. Rachel Carson, an environmentalist and a marine biologist at the US Bureau of Fisheries, believed that environmental problems in birds and aquatic wildlife were caused by synthetic pesticides and warned the public of long-term effects of misusing them. She wrote in her 1962 book "*Silent Spring*" [1, 2] asking readers to imagine "the world where all animals and insects are dying, pollination does not occur, greenery turns into brown, and no more birds sing the song for you." The book brought about a change in the US national policy on pesticides, leading to a national ban on DDT and certain other pesticides. Significant efforts have been made since then to address issues in environmental toxicology at all levels. Many comprehensive surveys have been conducted to measure chemical contamination of aquatic resources and human exposures. Many toxic substances have been identified; and their presence in the environment and organisms has been linked to biological processes [3–7]. Many of the chemicals are "-cides," such as herbicides, pesticides, and rodenticides, used to remove unwanted plants, insects, or rodents, respectively. Many others belong to chemicals of everyday household, emergency, or personal use, such as active components of apparels, drugs, cosmetics, firefighting, and food packaging. Majority of these chemicals are ubiquitous in nature and have often been found to interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior [8–11]. Because of their ability to disrupt endogenous hormone function, they are called as ED or endocrine disrupting chemicals (EDCs).

Current risk assessment protocols are yet to include examination of long-term, multigenerational, and transgenerational effects of EDCs on human and wildlife health. In many instances, there have been situations that the EDCs were widely used for quite some time, remained in the market, and phased out by the time, or even before, the long-term effects of these chemicals began to appear in organisms. Currently, determining the effective concentration of chemicals that causes adverse health effects in organisms has been a subject of active debate. Highly variable results, mixed opinions from different scientific communities (government, industry, and academia), and lack of comprehensive risk assessment guidelines have restrained decision-making processes and led to the situation of dilemma that eventually may pose humans and wildlife health at risk. Direct and immediate toxic effects of exposure are the prime focus in risk assessment; however, emphasis should be given to exposure effects that are initiated at the critical window of development and manifested as adverse health or behavioral outcomes later in life. Some chemicals that remain below the current level of environmental relevance but surge a few folds higher at certain times in a year can still cause adverse effects when the surge/exposure occurs during the critical stage of life history of the organism [9, 12]. These chemicals are often termed as hit and run chemicals. It is, therefore, advisable to include in the current risk assessment guidelines the screening of long-term, multigenerational and transgenerational effects of chemical of concern using test models with shorter reproductive lifespan. Mechanistically, EDCs not only act as ligands for selective receptors, or alter enzyme activities, but also induce transcriptional activation or repression of genes via epigenetic mechanisms. Differential gene expression is one of the criteria for evaluating adverse outcome in exposed organisms. Epigenetic changes precede transcriptional activation or repression of the target gene and serve as mechanistic links between environment and physiology. Thus, inclusion of epigenetic change as a component in adverse outcome pathway seems necessary.

Environmentally Induced Adverse Health Effects and Phenotypes

Inheritance of acquired traits, a theory postulated by Lamarck, has been a topic of controversy for a long time and of utmost interest since experimental and epidemiological evidence suggested that certain environmentally induced phenotypic traits, including acquired lifetime memory, can be transmitted to subsequent generations [12–19]. The traits that are acquired not as a result of direct exposure to the stressor but due to the lifetime experience of ancestors is called transgenerational traits. Pioneering work of Dr Michael Skinner and co-workers laid the foundation for research in environmental chemical-induced transgenerational diseases [13]. Many of the chemicals tested so far produced multiple disease or behavioral phenotypes at the third or fourth generations [20–28]. To date, several forms of transgenerational phenotypic traits, including those induced by nutritional alterations and environmental chemical toxicants, have been shown in worms [29, 30], flies [31], plants[32], fish [12, 33, 34], mice [14, 19, 35–37], domesticated farm animals [38, 39], and humans [17, 40]. Mechanistic understanding of transgenerational inheritance of adverse health outcomes in model species is gaining momentum, recently. On the other hand, evidence for transgenerational diseases in humans is derived from epidemiological surveys [41], except for multigenerational and/or transgenerational diseases in children and grandchildren of diethylstilbestrol (DES)-prescribed women in the USA (CDC, January 2012, http://www.cdc.gov/des/consumers/daughters/). In the USA, an estimated 5–10 million persons were exposed to DES during 1938–71, including women who were prescribed DES while pregnant and the female and male children born of these pregnancies. More than 30 years of research have confirmed that health risks are associated with DES exposure. However, not all exposed persons have developed health abnormalities. Incidences of diseases such as clear cell carcinoma, reproductive tract deformities, pregnancy complications, and infertility in DES Daughters and non-cancerous epididymal cysts, hypospadias, and infertility in DES sons have been reported to CDC. Interestingly, animal studies mimicking human DES exposure have shown disease phenotypes in mice similar to DES-exposed humans, suggesting that results of the laboratory rodent exposure studies are translational to human health [42, 43]. Molecular studies as to whether DES induces transgenerational diseases in the laboratory rodent models would be interesting and valuable to define mechanisms underlying DES-induced health effects in humans. Alternatively, fish can serve as excellent models to gain mechanistic insights into DES-induced phenotypic defects as fish have shown to develop reproductive phenotypes in response to DES exposure [44].

Diet, Transgenerational Effects, and Associated Epigenetic Changes

"You are what you eat is a widely used proverb" but "you are what your grandparents ate" will be an additional proverb in the near future. Nutrition is one of the environmental factors causing lasting transgenerational effects in a wide range of organisms, from worms to humans. For example, in a recent study, nutritional restriction induced transgenerational longevity in worms, *C. elegans* [18]. The study found an epigenetic link to transgenerational inheritance of phenotype. The small RNA (22Gs) levels that follow L1-starvation-induced developmental arrest were passed on for at least three generations. These small RNAs targets included sets of genes involved in nutrition, such as *kin-29*, *gcy-23*, *polg-1*, *coq-3*, and *aco-1* [18]. Malnutrition during gestation and perinatal life has been found to induce metabolic syndromes and alteration in glucose metabolism in rats [45]. Another example of nutrition-induced transgenerational effects is in humans. Epidemiological survey of different famines in the Netherlands during World War II suggested transgenerational effects of malnutrition on health and survival of grandchildren [16, 17, 41]. Understanding the mechanisms underlying nutrition-induced transgenerational health problems and longevity is currently an active area of research. Consumption of food with phytoestrogens, compounds of plant origin with estrogenic activity, has been linked to reproductive adverse outcomes in ewes [46], cattle [47], and laboratory rodents [48]. In addition to the action of phytoestrogens via estrogen receptors, peroxisome proliferator-activated receptors, and the non-classical estrogen receptor GPR30 [49], phytoestrogens have been found to alter epigenetic marks by modulating activities of DNA and histone methyltransferases, NAD-dependent histone deacetylases, and other modifiers of chromatin structure [49–51]. Phytoestrogen (cumestrol and equol) consumption increased DNA methylation levels on the promoter regions of *H-ras*, a protooncogene, causing its silencing in neonatal mice [52], whereas genistein exposure caused activation of tumor suppressor genes in the mouse prostate by modulating histone modifiers, such as histone demethylation or acetylation [53]. Phytoestrogens affect reproduction and behavior in fish. In medaka (*Oryzias latipes*), reproductive problems caused by phytoestrogens include delayed oocyte maturation in female at 0.75 and 30 μg genistein per fish [54, 55], increased egg mortality and larval deformation of brown trout (*Salmo trutta*) at 10 and 20 μg/l mixed phytosterols found in pulp mill effluent [56], and impaired sexual differentiation in mosquitofish [57]. In humans, epidemiological data show a strong association between incidences of reproductive abnormalities and consumption of diet containing phytoestrogens [58, 59]. Despite several individual and species-specific variations in response to exposure to the nature of food, it is concerning that the food has ability to change organism's destiny by modulating non-genetic codes at the molecular level. The nature of phytoestrogen action that is more or less identical to mechanisms of actions of other EDCs and reproductive phenotypes of phytoestrogen exposure in laboratory models suggest that some transgenerational effects could be expected [59], although strong evidence for such effects are still lacking.

Environmentally Induced Transgenerational Inheritance and Epigenetic Mechanisms

Effectiveness of EDC exposure depends on affected cell type and life history stages of the exposed organism. Exposure effects are profound during early development when tissues are still differentiating or during critical life history stages when tissues undergo age specific changes, such as puberty, lactation, and aging. Effects on somatic cells, called somatic effects, are manifested as physiological responses and diseases in the exposed individuals, whereas effects on germ cells can be transmitted to subsequent generations via germ line (sperm or eggs) via epigenetic mechanisms. Understanding epigenetics is essential for studying mechanisms underlying cell fate specification, tissue-specific gene expression, developmental origins of adult disease, and transgenerational inheritance of disease. Most extensively studied epigenetic processes are DNA methylation, histone modifications, and small RNAs.

DNA Methylation

Epigenetic alterations, especially DNA methylation and histone modifications, can be mitotically and meiotically stable [60], therefore changes can potentially survive in a cell throughout life. The reprogramming of DNA methylation initially occurs upon fertilization in the zygote and secondly occurs in primordial germ cells (PGCs), which are the direct progenitors of sperm or oocytes [61, 62]. It has, therefore, been believed that abnormal epigenetic modifications in early life are detrimental to an organism's later life health conditions. Any abnormal epigenetic modifications can cause deregulation of the target gene [33]. Such modifications can have deleterious effects when they occur in stem cells or precursor cells since all differentiated cells may carry abnormally programmed epigenome, thereby causing altered patterns of gene expression and molecular interactions in gene networks, consequently leading to an onset of disease states.

Advent of next-generation sequencing has led to a significant advancement in understanding of biological processes. A whole-genome bisulfite sequencing of cells undergoing reprograming, particularly during pre-implantation development in mice, revealed some erasure-resistant genomic regions. These protected genomic regions were intracisternal A particles, long terminal repeats of endogenous retrovirus 1 (LTR-ERV1) elements, and a few single-copy sequences [63]. They may play significant roles in transmission of parental traits to the offspring. Using a similar approach in mice, epigenetic reprogramming of germ line cells has been studied. It has been found that a global erasure of DNA methylation marks gives rise to a stem cell state for PGCs, and *de novo* methylation starts allowing a controlled gene expression pattern in germ cells in a gender specific manner [64]. A small portion (~1%) of the PGC genome has been found to be resistant to global DNA demethylation [65–68], suggesting that these genomic regions might be responsible for inheritance of parental phenotype. In rodent PGCs, DNA demethylation continues 3 days past sex determination and then *de novo* methylation occurs after day 16, coinciding with mitotic arrest of spermatogonia in the developing testis and the start of meiosis in ovaries. This window of germ cell reprogramming has been found to be susceptible to environmental chemical insult [69, 70].

So far, all DNA methylation marks identified in organisms with environmentally induced transgenerational disease traits are correlative; no causative links has been identified yet. Hypotheses are that (i) environmental chemicals interfere with DNA demethylation process in the PGCs during reprogramming or (ii) they establish permanent methylation marks during *de novo* methylation process. Therefore, differentiated germ cells that develop into eggs or sperm also maintain these

differentially methylated regions and these differentially methylated regions are passed on to the next generation via germ line transmission without modification behaving as erasure-resistant imprint-like signatures. The individual with altered germ cell epigenome may exhibit reproductive disease states later in life, whereas the offspring originated by those germ cells may exhibit disease states in both somatic cells and germ cells. Epigenetic alterations, often termed as epimutations, may be detectable in every cell of the offspring. However, yet, neither an exposure-specific nor a phenotype-specific biomarker (epimutations) has been identified to reliably predict exposure history and transgenerational abnormalities. A recent controversial study by Iqbal *et al.* [71] has refuted the dogma that DNA methylation marks are transmitted to subsequent generation via germ line transmission. Mice that received previously shown concentration of environmental contaminants to induce transgenerational phenotypes did not establish the stable DNA methylation marks in germ cells were supposed to be inherited by the offspring germ cells. Although the study was well designed, authors failed to show exposure-induced transgenerational phenotypes at F2 or F3 generations. Absence of phenotype somewhat supported the finding that no stable DNA methylation marks were established in germ cells that could be transmitted to subsequent generation. Moreover, a meta-analysis of diverse data sets related to ED-induced transgenerational gene expression alterations, including the data provided by Iqbal *et al.* [71], suggested that effects of EDCs persist in unexposed generations [72].

Histone Modification

Histone modifications are major carriers of epigenetic information that both reflect and affect the transcriptional states of underlying genes [73]. They include acetylation, phosphorylation, methylation, ubiquitination, and crotonylation [74]. Among histone modifications, histone lysine methylation appears to be the most stable. Among the different histone lysine methylation states, H3K9 and H3K27 methylation appear to be the most likely key regulators of classic epigenetic phenomena [73]. For decades, it was thought that sperm histones are replaced by protamines, and egg histone marks are reset after fertilization and reestablished during preimplantation [75]. Recent studies suggest that histone modifications can be retained in the sperm [76–78] and inherited by offspring [79] and are involved in epigenetic transgenerational inheritance of acquired traits [80, 81]. Hammoud *et al.* [76] unveiled the histone marks that are retained in human sperm. H3K4me2 is enriched at certain developmental promoters, whereas H3K4me3 is localized to a subset of developmental promoters, regions of *HOX* gene clusters, certain non-coding RNAs, and paternally expressed imprinted loci. H3K27me3 is significantly enriched at developmental promoters that are repressed in early embryo. In *C. elegans*, multiple chromatin-modifying factors, including H3K4me1/me2 and H3K9me3 methyltransferases, an H3K9me3 demethylase, and an H3K9me reader have been identified, which either suppress or accelerate the progressive transgenerational phenotypes of spr-5 mutant worms [81]. Recently, Siklenka *et al.* [80] showed that disruption of histone modifications, in the apparent absence of any changes in DNA methylation patterns, can also form the basis for transgenerational transmission of epigenetic programming defects that can modify phenotypic characteristics in subsequent generations [80, 82]. By overexpressing the human KDM1A histone lysine 4 demethylase during mouse spermatogenesis, authors generated a mouse model producing spermatozoa with reduced H3K4me2 within the CpG islands of genes implicated in development and studied the development and fitness of the offspring. KDM1A overexpression in one generation severely impaired development and survivability of offspring. These defects lasted for two subsequent generations in the absence of KDM1A germline expression. No apparent DNA methylation differences were observed in the CpG dense regions and changes in expression and the phenotypic abnormalities observed in offspring correlated with altered histone methylation levels at genes in sperm. These studies provide an evidence for complexity of transgenerational epigenetic inheritance involving multiple molecular factors, including the establishment of chromatin states in spermatogenesis and sperm-borne RNA [80].

Small Non-Coding RNAs

Small non-coding RNAs are potential mediators of gene environmental interactions that can relay signals from environment to the genome and exert regulatory function on gene activity and dysregulation of genes in many diseases [83, 84]. The role of sperm micro RNAs (miRNAs) in transmission of paternal experience and adverse offspring behavior has been emphasized recently. A study by Rodgers *et al.* [85] has found nine specific miRNAs (miR-29c, miR-30a, miR-30c, miR-32, miR-193-5p, miR-204, miR-375, miR-5323p, and miR-698) in the sire's sperm sensitive to paternal stress. By injecting these miRNAs into the single cell stage embryo, the authors examined offspring level of stress and associated molecular changes in paraventricular nucleus of brain using RNAseq. Study found that the paternal sperm miRNAs function to reduce maternal mRNA stores in early zygotes, ultimately reprogramming gene expression in the offspring hypothalamus and recapitulating the offspring stress dysregulation phenotype [86]. This study provides a mechanistic understanding of the transgenerational transmission of paternal lifetime experiences and offer valuable insight into the novel factors influencing offspring disease risk and resilience. In another study, early traumatic stress in early life was found to alter miRNA expression in the sperm (*miR-375-3p, miR-375-5p, miR-200b-3p, miR-672-5p,* and *miR-466-5p*) and behavioral and metabolic responses in the progeny. Injection of these sperm RNAs from traumatized males into fertilized wild-type oocytes reproduced the behavioral and metabolic alterations in the resulting offspring, suggesting that ancestrally acquired traumatic memory can be transgenerationally transmitted and miRNAs play significant roles in mediating inheritance of environmentally induced traumatic stress to offspring [84]. Small RNA-induced gene silencing can persist over several generations via transgenerationally inherited small RNA molecules in *C. elegans* [29, 30]. In an elegant study with *C. elegans*, small RNAs seemed to memorize lifetime experiences that were inherited by subsequent generations [18]. The study found that starvation-induced developmental arrest, a natural and drastic environmental change, leads to the generation of small RNAs that are inherited through at least three consecutive generations. These small, endogenous, transgenerationally transmitted RNAs target genes with roles in nutrition. Additionally, the F3 offspring of starved animals showed an increased lifespan, corroborating the notion of a transgenerational memory of past conditions. It will be interesting to study how these miRNAs are generated by environmental influence, their dynamics during epigenetic reprogramming, and their circulating status to serve as biomarkers of ancestral exposure. Together, transgenerational inheritance is a buffered process that involves coordination of several

epigenetic processes (DNA methylation, histone modifications, and miRNAs) and transport of epigenetic hallmarks (epigenetic memory) across generations finally leading to transcriptional activation or silencing of target genes.

Medaka Fish (*Oryzias latipes*, Hd-rR Strain): A Model to Study Environmentally Induced Transgenerational Inheritance of Altered Phenotypes

Studies from model organisms have significant translational values as human subjects, or non-model wild species of concern, cannot be directly used to study these mechanisms. Obviously, environmental chemicals that are capable of producing a phenotype in the model organisms should produce similar effects in humans and domestic or wild animals but to varying degrees given that the genetic make-up, body size, food habits, and life history are different. Human exposure data predominantly represent epidemiological survey-derived correlative incidences of health and disease. However, information on health of descendants of DES-prescribed pregnant women in the mid-20th century and phenotypes in laboratory rodent models with similar exposure history suggest that mechanism of action of EDCs is conserved across taxa [42, 43].

In the recent years, fish models, especially zebrafish, medaka, and fathead minnow, have been increasingly popular for use in biomedical, ecological risk assessment, and environmental toxicology research. Genome sequences of zebrafish and medaka have been annotated, whereas that of fathead minnow is in progress. Characterization of epigenetic processes has been advanced in zebrafish and medaka mainly because of cutting edge molecular tools, such as Crisper/Cas system, available for gene manipulation, specific peptides and antibodies, and relevance to human health and disease [87–91]. High-throughput sequencing of zebrafish germ cell epigenome (DNA methylation) has identified that epigenetic germ cell programming events between mice and zebrafish are common [92, 93]. The role of histones in regulation of key developmental genes has been described in medaka [91] and studies underlying epigenetic reprogramming of cells during early development and germ cell sex determination in medaka are currently in progress (Bhandari *et al.*, unpublished). Both medaka and zebrafish are equally useful model organisms. Each model has advantages over the other. Use of medaka (*Oryzias latipes*), which is also biomedical research model [94–98], may overcome the technical limitations in understanding germ-cell-mediated transgenerational effects in mammals. Medaka sex determination occurs between days 5 and 8 after fertilization. Germ cell reprogramming events are believed to be complementary to mouse [99]. Medaka have advantages over mice, including external fertilization and embryo development, daily spawning, availability of large numbers of eggs and sperm, and virtually unlimited number of embryos produced by the same two parents to assign to different treatment groups, along with a short generation time (2 months) and easy, low-cost culture [96, 100]. In fish, transgenerational effects are manifested at F2 generation as F0 embryo, and its F1 germ cells are directly exposed to test chemicals. Zebrafish, however, may not be suitable for the study that focuses on epigenetic modifications in sex-specific germ cells because of gonadal hermaphroditism in early embryonic development and undefined sex determination in germ cells at the stage of interest.

Fish models have been proven to be useful for studying long-term, transgenerational effects of environmental stressors [12, 33, 34] and proposed as an alternative to mice especially with regards to ease of their use, husbandry, economy, and resource availability [33, 101]. Some species specificity of response to chemicals exists. Issues such as life history, route of exposure, rate of metabolism of chemical contaminants, mechanisms of absorption and excretion, and genetic make-up are important points to consider. However, the common mode of action and conserved mechanism of cellular processes across taxa allow scientists to interpret cross-species data from exposure studies. In all species examined so far, the period of early embryonic development or the stage in the life history when further differentiation of tissues occurs has been found to be a susceptible window to environmental stressors. For example, exposure of medaka embryos to 100 μg/l bisphenol A (BPA) or 0.05 μg/l ethinylestradiol (EE2) only during the first 7 days of embryonic life and never thereafter did not cause any gross phenotypic abnormalities in F0 and F1 generation adults, but the F2 and F3 adults showed significant reduction in fertilization capacity and resultant F3 and F4 embryos showed a reduced rate of survival [12]. The transgenerational phenotype was induced just because of exposure to these chemicals at the critical window of embryonic development. Although the concentrations used were above the level of current environmental relevance but were within the level of annual surge in the natural environment [9, 102, 103], indicating that irrespective of exposure to environmental relevant levels of chemicals exposure to hit and run concentration of chemicals at the critical window of development can cause a significant damage to a population via transgenerational effects. Characterization of epigenetic processes (histone modifications, DNA methylation, and circulating miRNA profiles) associated with transgenerational inheritance of altered phenotypes, these fish is currently in progress (Bhandari *et al.*, unpublished). In zebrafish, exposure to waterborne dioxin (50 pg/ml) at 3 weeks postfertilization (wpf) and again at 7 wpf stage caused a reduction in egg release and fertilization success, a female skewed sex ratio, and skeletal kinks at F1and F2 generation [33]. Paternal exposure to BPA during spermatogenesis increased rate of heart failures of progeny up to F2 generations in zebrafish [104]. Parental exposure of zebrafish to a varying concentration of Benzo[A]Pyrene (BaP) for 21 days resulted in body morphology deformities (shape of body, tail, and pectoral fins) that continued to F2 and F3 generations, whereas craniofacial structures (length of brain regions, size of optic and otic vesicles, and jaw deformities), although not significantly affected in the F1 generation, emerged as significant deformities in the F2 generation [34], suggesting human health risks of exposure to BaP. In a live bearing fish guppy, *Poecilia reticulate*, maternal exposure to 20 ng/l EE2 until birth caused a transgenerational anxiety phenotype in the F2 generation offspring [105]. Studies showing occurrence of environmentally induced transgenerational inheritance of phenotypes in fish are accumulating rapidly and similar phenotypes in other species are anticipated. These increasing numbers of studies in fish and other model species indicate that environmental EDCs can induce transgenerational effects relevant to human and ecosystem health. Although molecular mechanisms underlying transgenerational inheritance of environmental phenotype in these fish are not yet clarified, unraveling of associated genetic and epigenetic changes is, however, expected.

Irrespective of the route of exposure, it is important to consider how much of the effective chemical was taken by the exposed organism. In a study with medaka, uptake concentration

of both BPA and EE2 was measured by using radiolabeled chemicals (^{3}H-BPA, ^{3}H-EE2) [12]. Out of 100 μg/l ^{3}H-BPA, medaka embryo's uptake was only 178 pg/mg of egg and ^{3}H-EE2 was 4 pg/mg egg out of 0.05 μg/l. F2 and F3 generation fish from the exposure lineage developed transgenerational abnormalities. It is, therefore, possible that an exposure to very small amount of chemical is likely sufficient to establish chemical signatures on the genome (i.e. epigenome) that subsequently lead to adverse health and disease in subsequent generations. Effects in cells that proliferate slowly would take comparatively longer to appear than that in cells that proliferate rapidly. Some effects seem to be persistent across multiple generations and some detectable only in the third or fourth generation. The investigation on mechanisms mediating such transgenerational effects are currently of the best interest to scientific community and government agencies.

Conclusions

The basic molecular mechanisms of gene regulation are highly conserved in all eukaryotic organisms. Diseases such as metabolic syndromes, neurological disorders, infertility, cancer, and increased susceptibility to microbial diseases have developmental origins and epigenetic links. Given that the mode of action of environmental EDCs is common in organisms across taxa, the downstream molecular pathways and gene networks associated with development of disease and behavioral phenotypes, although highly variable and organism-specific, are likely to share some common routes. Epigenetics links environment with physiology. It seems crucial to find chemical-specific epigenetic signatures and the common downstream routes to phenotypes to develop the biomarkers that are reliably predictive of exposure and disease phenotype. Fish can serve as economic, easy, and convenient model to study environmentally induced epigenetic transgenerational inheritance of phenotypes.

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