Transgenerational Inheritance of Paternal Neurobehavioral Phenotypes: Stress, Addiction, Ageing and Metabolism

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Abstract

Epigenetic modulation is found to get involved in multiple neurobehavioral processes. It is believed that different types of environmental stimuli could alter the epigenome of the whole brain or related neural circuits, subsequently contributing to the long-lasting neural plasticity of certain behavioral phenotypes. While the maternal influence on the health of offsprings has been long recognized, recent findings highlight an alternative way for neurobehavioral phenotypes to be passed on to the next generation, i.e., through the male germ line. In this review, we focus specifically on the transgenerational modulation induced by environmental stress, drugs of abuse, and other physical or mental changes (e.g., ageing, metabolism, fear) in fathers, and recapitulate the underlying mechanisms potentially mediating the alterations in epigenome or gene expression of offsprings. Together, these findings suggest that the inheritance of phenotypic traits through male germ-line epigenome may represent the unique manner of adaptation during evolution. Hence, more attention should be paid to the paternal health, given its equivalently important role in affecting neurobehaviors of descendants.

Keywords

Transgenerational inheritance; Sperm; Epigenetic modulation; Neurobehavior

Introduction

Epigenetics literally stands for “outside of genetics”. In genetics, alterations in the sequence of genetic DNA result in gene expression and subsequent cellular phenotypes, whereas in epigenetics, the phenotypic trait variations can be caused by external or environmental factors that control gene expression at the transcriptional level, without affecting the DNA sequence per se [1, 2]. Recent studies have suggested that epigenetic modulation participates in various types of neurobehavioral processes through DNA methylation/ demethylation [3, 4], histone acetylation/deacetylation [5], and transcriptional regulators (CREB, MeCP2, noncoding RNAs) [6] to alter synaptic plasticity/transmission, neuronal responses, and finally animal behaviors under both physiological and pathological conditions [7–9]. Epigenetic processes have a crucial role in determining parental imprints, management of gene expression, and regulation of germ cell development [10]. The potential regulation on germ line plasticity by the environment has been mainly based on the observation that external factors (e.g., stress, odors, high-fat diets) can induce epigenetic marks in the germ line [11–13]. Several epigenetic marks were found in sperm including noncoding RNAs, histone modifications, and DNA methylation [14]. These studies helped to understand how dynamic and plastic germs cells can be, although there is a need to further understand how and when these epigenetic marks can develop within the germ cells [10]. Traditionally, there are three types of pathways by which environmental

factors can induce heritable changes in multicellular organisms with a germ line: direct induction, parallel induction, and somatic induction [15, 16]. In direct induction (or gametic induction), challenging conditions affect the germ line directly even if parent organisms do not respond phenotypically. In parallel induction, the same cause independently induces epigenetic changes both in the soma and in the germ line. Thus, germ-line changes are directly induced without somatic mediation, and similar somatic phenotypes are displayed by the parents and its descendants. In contrast, somatic induction is characterized by soma-mediated germ-line changes. Alterations that first occur in the soma are transmitted to the germ line, subsequently inducing the parental phenotypes on the descendants. Small RNAs are able to travel between cells and may be the underlying mechanism to somatic induction [14]. Hormones have also been speculated as possible mediators of information transfer between the soma and the germ line, although the role of the two agents in this process remains poorly understood [14]. Recently, a fourth mechanism designating parallel induction with nonparallel effects has been explored. In this case, there is an induced effect on the soma of the parent which may cause changes in the germ line, with the resulting somatic adjustments of the descents that are different from the ones observed in the parents [15, 16].

With regard to the specific molecular mechanisms mediating the transfer of epigenetic information between two generations, DNA methylation has been the most popular candidate, although histone modifications and RNA have been also considered as valuable alternatives [17–19]. Prions and selfsustaining loops have also been suggested as possible epigenetic mechanisms, but there is no evidence supporting that they are transmitted between generations through sperm and egg [20]. However, there are studies describing that chromatin marks and RNAs can be transmitted between generations through the germ line, although it is unclear how this occurs [15, 16]. One hypothesis is that the preservation of some partial chromatic marks or histone modification may allow the reconstruction of ancestral epigenetic patterns in the descendants [21]. In male vertebrates, the erasure of histone marks is not total, although there is broad replacement of histones by protamines during gametogenesis [22]. Cells in the germ line also contain small RNAs which are strong candidates to the inheritance process since they guide DNA and histone modification [20]. For example, piRNAs (Piwi-interacting RNAs) have a crucial role in detecting, silencing, or deleting unpaired DNA regions during meiosis [23]. Mammalian spermatocytes and oocytes are filled with large amounts of RNA of all classes, which suggests that they may be transmitted to the next generation and lead to transgenerational effects [18]. Next, we specifically discuss three common types of factors that have been implicated to enable the transgenerational inheritance of paternal neurobehavioral phenotypes, including stress, addictive drugs, and physical or mental changes in fathers (Fig. 1). We also conclude the evidence supporting the involvement of DNA methylation, histone acetylation, and miRNAs (microRNAs) in this biological process.

Stress

Stress-Induced Epigenetic Modulation in the Brain

DNA Methylation

Acute or chronic stress experiences can lead to epigenetically modulated changes of gene expression in stress-responsive brain regions [24–26]. For instance, the early-life stress experience raised the expression of arginine vasopressin (AVP) in the paraventricular nucleus

(PVN) of the hypothalamus due to the hypo-methylation of the DNA binding sites for Methyl CpG binding protein 2 (MeCP2), which mediates the activity-dependent transcription [27]. Chronic social stress demethylated the corticotrophin releasing factor (CRF) Crf gene selectively in the stress-responsive animals rather than their resilient counterparts [28]. Moreover, the expression of DNA methyltransferases 3a (Dnmt3a) was modulated by chronic stress or drug exposure, which then contributed to the changes of spine plasticity in the nucleus accumbens (NAc) and behaviors in the animals [29].

Histone Acetylation

Besides DNA methylation, histone modification is well noted in stress-induced brain changes. Histone deacetylase 6 (HDAC6) has been revealed to be crucial for acute stresselicited potentiation of glutamatergic transmission in the prefrontal cortex [30]. Both siRNA knockdown and pharmacological inhibition of HDAC6 blocked the synaptic changes induced by force-swimming stress in vivo, or by corticosterone treatment in vitro in rats. Likewise, HDAC5 also plays an important role in the pathophysiology and treatment of depression. Chronic administration of imipramine, a tricyclic antidepressant that hyperacetylates histone to promote the transcription of certain splice variant mRNAs of brain-derived neurotrophic factor (BDNF), resulted in a decrease in Hdac5, whereas over-expressing HDAC5 in the hippocampus diminished the antidepressive capacity of imipramine [31]. Chronic social defeat stress in mice enhanced H3 acetylation while lowered HDAC2 levels in the NAc, in contrast to the infusion of HDAC inhibitors into the NAc that was demonstrated to exert antidepressant-like effects [32]. In another study, it was found that the epigenetic modulation of RAS-related C3 botulinum toxin substrate 1 (Rac1) expression in the NAc orchestrated the synaptic remodeling induced by chronic stress in mice. Moreover, the prolonged reduction in Rac1 expression could be rescued with HDAC inhibitors [7].

miRNAs

Last but not least, recent findings have highlighted the indispensability of microRNAs for the therapeutic effects of antidepressants [33]. Specifically, miR135 was reported to be critical to the excitability of serotonergic neurons, mediating the susceptibility and heterogeneity to chronic stress. In a very recent study, Rodgers and colleagues demonstrated that zygotic microinjection of nine microRNAs, whose expression levels have been confirmed to be significantly raised in the sperm of male mice exposed to chronic stress [34], could degrade several important maternal mRNA targets in early zygotes. As a consequence, adult offsprings from these manipulated zygotes exhibited the blunted hypothalamic–pituitary–adrenal stress axis response and altered PVN transcriptome, which together recapitulated the effects of paternal stress [35]. Taken together, these findings raised the possibility to therapeutically fight against stress with agents enabling the epigenetic regulation, particularly those capable of affecting the paternal germ line epigenome.

The Inheritance of Stress-Related Neurobehavioral Changes

It has been long known that stress could impair sex-related performances, decrease sperm count and quality, and harm testicular cells, lasting from months to years [36, 37]. Until

recently, it was unveiled that stress-induced behavioral adaptations in male individuals could be transmitted to their offsprings [38, 39] potentially through the sperm epigenome [34]. For example, sperm small-noncoding RNAs (sncRNAs) contain miRNA, piRNA, and rbRNA (ribosomal RNA). In an early-life stress mouse (F0 generation) model of unpredictable maternal separation combined with unpredictable maternal stress (MSUS), the expression levels of different miRNAs were altered in serum, stress-relevant brain regions, as well as sperm RNAs [40, 41], which were correlated to the presence of a series of stress-relevant behaviors. Interestingly, such changes were detected in the brain structures but not sperms of the F1 generation animals, even though both F1 and F2 generation animals exhibited similar behavioral changes as F0 MSUS animals [40]. Of note, injecting the isolated sperm RNAs from F0 animals into fertilized oocytes was sufficient to produce the behavioral phenotype observed in F1 animals [40], suggesting a new way to modulate the parental effect traits or even to treat certain inherited diseases, in a “father-to-son” manner. It will be as well important to understand how stress could impact the sncRNAs in the sperm so as to develop strategies to prevent such changes in people with high-stress professions. In another study from the same group, the behavioral changes in F1 generation animals were attributed to those of different plasticity-related genes in the brain, together with the impaired hippocampal long-term potentiation (LTP) while enhanced long-term depression (LTD) [42]. Moreover, the altered synaptic plasticity was correlated to the decreased methylation level of the promoter region of protein kinase C gamma (PKC-gamma) Prkcc in F1 generation, a neuron-specific form of PKC which is involved in synaptic plasticity [42]. Surprisingly, such methylation decrease was not found in F0 generation animals even in the presence of altered synaptic plasticity and behaviors; therefore, it is highly possible that the F0 sperm miRNA alterations were transferred to the F1 brain structures and coded as DNA methylation changes for the longer-term stability.

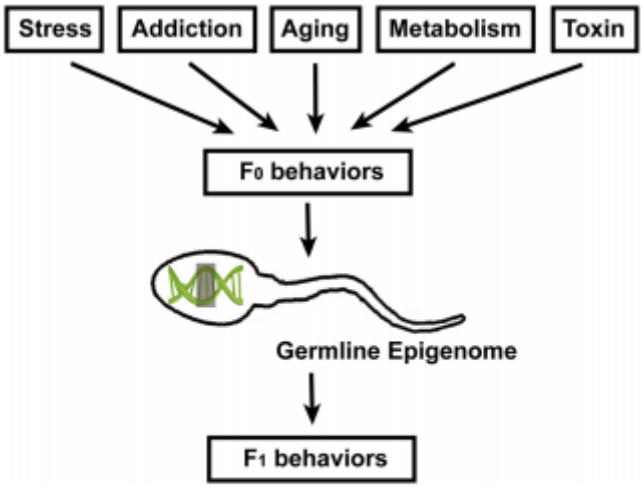

Fig. 1 Transgenerational inherence of neurobehavioral phenotypes through germ-line epigenome. The environment experiences such as stress, addictive drugs, and chemical exposure lead to behavioral adaptations in parents (F0), as well as the offspring (F1) through germ-line epigenome regulation

Reward and Drug Addiction

Epigenetic Mechanisms of Drug Addiction

Addiction represents the drug-induced long-lasting changes in the brain that drive compulsory behaviors of drug seeking. In the past decade, the epigenetic mechanism has been considered as one important player in maintaining these lasting effects, especially through the regulation of synaptic plasticity [43–45]. There were lines of evidence showing that acute or chronic exposure to drugs of abuse resulted in epigenetic changes in reward-related brain regions (e.g., midbrain dopamine neuron and NAc), whereas blockade of these changes could delay or decrease the formation of addiction-related behaviors.

DNA Methylation

For instance, the transcription levels of DNMT3a in the NAc were firstly (after 4 h) upregulated and then downregulated (after 24 h) in both acute and chronic exposures to cocaine [29, 46]. The decreased expression or blockade of DNMT3a function was found to increase the behavioral response to cocaine exposure, and vice versa for the overexpression experiment, accompanying changes in spine density and druginduced synaptic plasticity [29]. In addition, chronic cocaine exposure decreased Teneleven translocation methylcytosine dioxygenase 1 (TET1) expression in the NAc, in turn, knocking down TET1 enhanced the addictive behaviors [44]. The dysregulation of methylation was accompanied by altered MeCP2 expression: MeCP2 knockdown promoted the drug-reward behaviors [47]. Future studies are yet required to identify the genomewide methylation changes in animal models of drug addiction and in human postmortem brain samples.

Histone Acetylation

There were rigorous studies linking histone modification to certain phase(s) of drug addiction. It was found that cocaine (or other drugs of abuse) exposure resulted in increased levels of acetylated H3 (or H4) in the NAc, and the manipulation of which could contribute to altered behavioral responses as well [48–54]. Additionally, the NAc-targeted overexpression of HDAC4/5 attenuated the cocaine-relevant behavioral response, whereas the HDAC5 deletion promoted behavioral sensitivity to cocaine [48, 52, 55]. On the other hand, the NAc-selective deletion of HDAC1 mitigated the cocaine response [56], while downregulating HDAC3 facilitated the extinction of cocaine CPP [54]. Chronic cocaine exposure also increased the expression of SIRT1/2 [57]; the molecular targets of these two HDACs are to be investigated. These results collectively pointed out the complexity of histone acetylation regulation during the formation of addictive behaviors and that each step might be controlled by different signaling molecules. The altered histone acetylation was linked to the drug exposure-induced expression of immediate early genes and drug-evoked synaptic plasticity. For instance, H4 acetylation was found to occur at the c-Fos promoter region upon acute drug exposure [58], possibly through G9a [51, 59]. Likewise, H3 acetylation was increased at the BDNF promoter region after chronic cocaine administration [48]. Increases in H3 acetylation were also reported at promoter sites for Cdk5 and CaMKII [48, 60], which have been proven important for the drug-evoked synaptic plasticity well recognized in the NAc [61].

miRNAs

Cocaine exposure resulted in increased expressions of miR181a and miR212 [62–64] and decreased expression of miR124 and let-7d in the striatum [65]. This has been linked to altered CREB activation [63] or glutamate receptor trafficking in the neuron [66].

Transgenerational Susceptibility to Drug Abuse

Parental experience of drug addiction is found to affect the offspring susceptibility to the same drug of abuse. In a rat model of cocaine self-administration (F0), the offspring males (F1) rather than females developed a cocaine-resistant phenotype, including delayed acquisition and reduced maintenance of the self-administration [67]. The drug-resistant behavior was accompanied by the increased association of histone H3 to the BDNF promoter region, and the upregulated BDNF mRNA transcription as well as protein expression in the medial prefrontal cortex of male individuals of the F1 generation. Interestingly, this was attributed to the increased BDNF promoter acetylation in the sperm from the F0 generation (cocaine-exposed animals) [67]. It is yet unknown how sperm BDNF DNA acetylation is selectively modulated by cocaine administration and whether this will be inherited by the F2 generation. In another study, it was reported that maternal exposure to cocaine prior to pregnancy led to altered behavioral responses to cocaine, as well as upregulation of D1 receptor expression selectively in male offsprings [68], suggesting that there might be different effects dependent on the sex of the parent of origin. Other lines of studies demonstrated that parental use of alcohol prior to mating could give birth to offsprings with altered brain structures and functions [69, 70]. In a recent study with two-bottle choice of free alcohol intake, it was found that alcohol consumption was selectively decreased in male offsprings from parents with previous alcohol abuse experience [71]. On the other hand, these animals exhibited increased anxiety and locomotion induced by alcohol. Moreover, these changes were accompanied by BDNF Exon IXa expression in midbrain dopamine neurons, due to the decreased promoter methylation [71]. Taken together, the transgenerational inheritance of the drug susceptibility is to be investigated in details with different animal models, in order to understand the mechanisms of persistence. In addition, many studies have reported the effects of drugs on in utero development of the brain [72–75], with postnatal behavior changes. It will be as well interesting to know if addictive drugs modulate such behaviors through epigenetic mechanisms. In fact, there have already been pilot data showing the altered MeCP2 binding to the BDNF promoter in mice with in utero cocaine exposure [76].

Others

Ageing

Advanced parental age has been associated with increased risks of various neurodevelopmental disorders and psychiatric diseases [77, 78], through, for example, de novo mutation. In one study, it was found that ageing was coincidental with numerous alterations of DNA methylation (F0 generation), which were then transmitted to the offsprings (F1), contributing to a variety of behavioral changes, such as the open-field exploratory activities and the pre-pulse inhibition [79]. In future, it will be interesting to dissect the most relevant genes and neural circuits that are affected by these methylation changes, for the potential rescue of the functional abnormalities.

Olfactory Fear Conditioning

Olfactory sensation and fear conditioning are critical for escaping from the predators and the species survival. The ability of fast learning and extinction to adapt the new environment is therefore with evolutionary importance. It was found that odor fear conditioning in F0 male animals caused the same odorinduced startles in both F1 and F2 offspring generations [12]. Interestingly, the odor acetophenone induced fear conditioning in F0 male animals resulted in the increased innervation area (glomerulus area) of the relevant odorant receptor (Olfr151)-expressing olfactory sensory neurons, in both F1 and F2 generations. Such an effect was due to the decreased Olfr151 gene methylation in F0 and F1 sperm DNA [12]. In this study, the authors did not detect any histone-relevant modifications on the same locus. It is possible that different epigenetic mechanisms are differentially recruited for modulation of distinct neural pathways; yet the "sorting" mechanism is completely unknown.

Endocrine Function and Metabolism

It was reported that administration of antiandrogenic fungicide vinclozolin in parents could result in decreased spermatogenic capacity in male offsprings [80, 81], altered sexual selection behaviors [82], and different behavioral responses to stress in following generations [83, 84]. The mechanisms may involve the altered DNA methylation selectively in the male germ line [85–87], especially the sperm epigenome [88]; yet in certain behavioral aspects, the female offsprings could be more vulnerable as well [89], showing the sexually dimorphic effects. There was evidence suggesting that other endocrinedisrupting agents (e.g., diethylstilbesterol, bisphenol A, and polychlorinated biphenyls) could exhibit transgenerational neuroendocrine modulation as well [90]. Interestingly, parental life experiences that affect the body metabolism could also modulate the neuroendocrine function in offspring generations. For instance, food deprivation in F0 generation mice led to decreased serum glucose levels in both male and female offsprings (F1) [91], whereas the high-fat diet in male rats (F0) selectively resulted in pancreatic betacell dysfunction in female offsprings [13], showing the "father-to-daughter" inheritance through the hypo-methylation of different pancreas-specific genes (e.g., Il13ra2). This implied that the parental lifestyles could significantly impact the metabolic function of offspring kids, and even contribute to certain types of diseases (e.g., diabetes) [92, 93]. Indeed, a largescale investigation in human subjects showed that fathers (even grand-parents) with pre-marriage malnutrition or early smoking experiences influenced the risks in their offsprings to cardiovascular disease or diabetes [94, 95]. The other study examined the offspring generation (F1) of male animals (F0) fed with the low-protein diet from weaning until sexual maturity. The F1 generation exhibited elevated expressions of multiple genes related to lipid and cholesterol metabolism, which might result from the increased methylation (therefore decreased expression) of the key lipid regulator gene—Ppara in F1 F1 offsprings, although the involvement of other epigenetic information carriers like RNA and chromatin could not be excluded [96]. Interestingly, the Ppara expression was also affected by the maternal diet—high-fat maternal diet led to increased Ppara expression at birth and decreased expression at weaning [97]. How the different body metabolism states could selectively affect the epigenome in sperm or ova is yet to be studied. In line with the abovementioned results, malnutrition in F0 pregnancy led to the in utero undernourishment of F1 animals, subsequently altering the sperm DNA methylome of F1 adult males. Interestingly, although persistence of altered methylation was not observed in brain or liver tissues of lategestation (E16.5) F2 offsprings, they also displayed metabolic phenotypes, thereby suggesting a potential

involvement of changes in methylation during the early developmental stage [98]. Collectively, environmental stimuli can impact the sperm methylome even before maturation of the individual; this may be due to the epigenetic changes in spermatogonium cells. Accordingly, the spermatogonium acts as a candidate target to prevent such transgenerational inheritance of certain diseased phenotypes.

Epigenetic Modulation in Different Developmental Phases

Taking into account the previous findings, it is clear that epigenetic processes produce a wide range of developmental variability, which can be induced by environmental factors and then transmitted to the following generations. However, for a transgenerational epigenetic inheritance via the gametes to occur, an epigenetic mark must be present in the germ cell and endure epigenetic reprogramming [17–19]. Hence, epigenetic marks have to be reprogrammed and reestablished in the absence of reexposure to the environmental stimuli. In sexually reproducing organisms, epigenetic variations have to survive the complex process of meiosis and be transmitted to the next generation; in multicellular organisms, they also have to survive early embryogenesis and gametogenesis, two developmental stages that involve significant restructuring of both cells and chromatin [20, 99, 100]. The first phase occurs soon after fertilization, where the paternal genome undergoes a wave of genome-wide DNA demethylation [99]. However, despite the severe reprogramming process, some epigenetic modifications escape this remodeling and allow for some information to be maintained until adulthood [101, 102]. The second and last period of major epigenetic reprogramming occurs during the developing of the male and female germ line, more specifically, in the post-migratory primordial germ cells (PGCs) [103]. This phase is a major barrier to transgenerational epigenetic inheritance, during which histone variants and their modifications, as well as small RNAs and DNA methylation, are all reset in order to give rise to functional gametes [104, 105]. After this phase, the epigenome is at its most “naive” state and prepared for the acquisition of new epigenetic information and genomic imprints that will be transmitted to the next generation through mature gametes [99]. The complex processes previously described have led researchers to postulate that transgenerational epigenetic inheritance can be displayed by several patterns, where epigenetic information can be more or less closely reconstructed across generations [15]. The most commonly addressed possibility is the epigenetic recall, characterized by a partial reconstruction of epigenetic material. There is a partial inheritance of the epigenetic pattern that can be induced on the parent, without modified morphology in progeny. However, for the fullepigenetic pattern to be utterly re-established, the progeny will need a reduced intensity of the environment inducer [106]. Other possibilities relate to reactive but dissimilar epigenetic effects, where the faithful inheritance of the epigenetic marks followed by an exposure of the progeny to different environments provides an entry point for new phenotypes [107]. There is also the possibility of directional changes across generations, which can be accumulative (inducing conditions persist leading to more extreme phenotypes) or lingering-fading (noninducing conditions for the offspring generations reduce the epigenetic marks and respective phenotypes) [108]. The preceding options represent only a small fraction of the possibilities, as transgenerational epigenetic inheritance involves rather complex mechanisms.

Summary

The transgenerational inheritance of epigenetic traits, together with the neurobehavioral adaptions to previous environmental stimuli, serves as an important mechanism during evolution. The epigenome lasts for only several generations, and the rapid removal of such "memory" allows further "writing" of new environmental conditions. The epigenome examinations also permit the prediction and potential therapy against certain birth defects resulted from environmental toxin/stress.

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