Mechanisms of Epigenetic Transgenerational Inheritance

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Abstract

Epigenetic processes allow organisms to respond to their environment with changes in gene expression. Epigenetic molecular processes include DNA methylation, histone modifications, non-coding RNAs, RNA methylation and chromatin structure. Epigenetic information and epimutations can be transmitted to subsequent generations through sperm or eggs, and can change gene expression and an organism's phenotype even in transgenerational generations that were themselves never exposed to an inducing factor. All cell types and tissues derived from germ cells carrying epimutations will have the potential for an altered epigenome and altered gene expression. Tissues sensitive to an altered gene expression profile will have a different phenotype and have susceptibility to develop disease or abnormalities as the individual ages. Environmental exposures in past generations could have contributed to the incidence of abnormalities and disease in the current human population.

Glossary

Epigenetics Molecular factors and processes around DNA that regulate genome activity independent of DNA sequence, and are mitotically stable.

Transgenerational epigenetic inheritance Germline (sperm or egg) transmission of epigenetic information between generations in the absence of any continued direct exposures or genetic manipulations.

Key Points

- Mechanisms of epigenetic transgenerational inheritance.

Introduction

Epigenetic processes are the primary mechanisms used by all organisms to respond to their environment with changes in gene expression and phenotypic variation. In addition, it is primarily by epigenetic mechanisms that a stem cell type is able to differentiate and change to develop into all cell types. Since the DNA sequence in all cell types is the same, it is epigenetics that promotes cell specificity and differentiation. The current chapter will review the molecular aspects of environmentally induced epigenetic transgenerational inheritance.

Epigenetics

Epigenetics is defined as: "Molecular factors/processes around the DNA that regulate genome activity independent of DNA sequence, and that are mitotically stable" (Skinner, 2011). There are a variety of epigenetic factors that act around the DNA in a cell to regulate gene expression. In the 1970s DNA methylation was the first epigenetic molecular mark to be characterized. With DNA methylation a small (methyl) chemical group is attached to DNA, primarily at the cytosine base in animals (Holliday and Pugh, 1975; Singer *et al.*, 1979) to produce 5-methylcytosine (5mC). Other chemical modifications of cytosine bases in DNA have since been described. The TET family of enzymes can successively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine

(5fC) and 5-carboxylcytosine (5caC) (Kriaucionis and Tahiliani, 2014). In broad terms, the presence of 5mC often represses DNA transcription, while 5hmC is permissive to transcription (An *et al.*, 2017; Mellen *et al.*, 2017). The functions of other epigenetic modifications to cytosine are under investigation. N(6)-methyladenine is an epigenetic modification to the adenine base of DNA that was once thought to only be present in prokaryotic organisms, but has now been described in mammalian embryonic stem cells (Wu *et al.*, 2016).

The histone proteins that DNA is wrapped around create the nucleosome and can be chemically modified to alter gene expression. There are many different histone post-translational modifications including lysine acetylation, lysine and arginine methylation, arginine citrullination, lysine ubiquitination, lysine sumoylation, ADP-ribosylation, proline isomerization, and serine/threonine/tyrosine phosphorylation (Rothbart and Strahl, 2014). These modifications can change chromatin structure or recruit transcriptional cofactors to DNA in order to regulate gene expression.

Non-coding RNA molecules can act as epigenetic factors (Kornfeld and Bruning, 2014). These are small RNA molecules that do not code for a protein, but rather function as RNA to regulate gene expression. The non-coding RNA molecules that act as epigenetic factors are not DNA sequence dependent, so the majority do not depend on having a nucleotide sequence that is complimentary to a specific DNA or RNA region in order to function. Long non-coding RNAs (lncRNAs) (Wei *et al.*, 2017) and transfer RNA-derived small RNAs (tsRNAs) (Chen *et al.*, 2016a) are examples of RNA classes that are present in sperm and can act as epigenetic factors that affect subsequent generations (Chen *et al.*, 2016b).

The coiling, looping and general structure of DNA, termed chromatin structure, is also an epigenetic factor (Yaniv, 2014). The three-dimensional structure of DNA can make certain regions of the genome accessible to transcription machinery, or bring enhancer regions near to gene promoters, and so affect gene expression. Therefore, epigenetic molecular processes include DNA methylation, histone modifications, non-coding RNAs, RNA methylation and chromatin structure.

Epigenetic Transgenerational Inheritance

Two well-studied examples of epigenetic processes are X-chromosome inactivation and imprinted genes (Henckel *et al.*, 2012; Lee and Bartolomei, 2013). Female mammals have two X chromosomes and one must be inactivated for normal biological function. This has been shown to involve DNA methylation, histone modifications and long non-coding RNA (Sado and Ferguson-Smith, 2005). Imprinted genes are a small set of genes that are expressed from either the mother's (maternal) or father's (paternal) contributed DNA (allele), but not both. Imprinting involves DNA methylation, histone modifications, chromatin structure and non-coding RNA to control this parent-of-origin-specific gene expression (Massah *et al.*, 2015). These examples show how epigenetics and genetics cooperate to control genome activity and normal biology.

Altered epigenetic marks at specific DNA sites in response to exposure to an environmental factor are termed "epimutations" (Skinner *et al.*, 2010). Thus, DNA sequence changes are genetic mutations, while environmentally altered epigenetic sites that influence genome activity are epimutations.

Epigenetic information can be transmitted to subsequent generations, and can change gene expression and an organism's phenotype even in transgenerational generations that were themselves never exposed to an inducing factor like an environmental toxicant. The ability to transmit epigenetic information from one generation to the next requires that epigenetic factors be present in sperm or eggs. If the sperm or egg cells are transmitting epigenetic information transgenerationally then epimutations should be detectable in these germ cells. Analysis of the F3 generation (great-grand-offspring) sperm from a line of rats in which the F0 generation pregnant females were treated with the environmental toxicant vinclozolin were found to have sperm epimutations consisting of altered DNA methylation, compared to controls (Guerrero-Bosagna *et al.*, 2010). Interestingly, a variety of different environmental toxicants, each shown to promote transgenerational disease, were each found to promote a unique signature or pattern of epimutations in their F3 generation sperm (Manikkam *et al.*, 2012; Nilsson *et al.*, 2022).

An example of the processes involved in environmentally induced epigenetic transgenerational inheritance is presented in **Fig. 1**. The exposure of an F0 generation gestating female to an environmental factor at the critical window of gonadal (testis or ovary) sex determination in the F1 generation fetus modifies the epigenetic programming of the germ cell that the F1 generation adult animal will transmit to the F2 generation. All cell types and tissues are derived from germ cell derived embryonic stem cells carrying epimutations which will promote cell-type specific alterations in their epigenome and gene expression profiles. For example, isolated transgenerational cells such as Sertoli cells from the testis or granulosa cells from the ovary have been shown to have a transgenerational alteration in gene expression and epigenetics (Guerrero-Bosagna *et al.*, 2013; Nilsson *et al.*, 2012). Tissues sensitive to an altered gene expression profile will have a different phenotype, and may develop disease or abnormalities as the individual ages. For example, the testis and ovary develop pathologies that are associated with somatic cell epigenetic alterations (Guerrero-Bosagna *et al.*, 2013; Nilsson *et al.*, 2012). An adult F2 generation individual will then transmit germ cell epimutations to the transgenerational F3 generation, and the same mechanism and process can occur in all subsequent generations, **Fig. 1**.

More recent aspects of the mechanism of epigenetic transgenerational inheritance have been elucidated (Nilsson *et al.*, 2018). Previous research had suggested that DNA methylation of early embryonic cells is erased and then methylation is re-established as the stem cells initiate development of the blastula embryo (Reik and Surani, 2015). Utilizing a more genome-wide epigenetic analysis and a focus on transgenerational epigenetic DNA methylation sites has recently demonstrated that the majority of the epigenome, having low density CpG sites, shows an increase in DNA methylation during early embryonic development. Regions

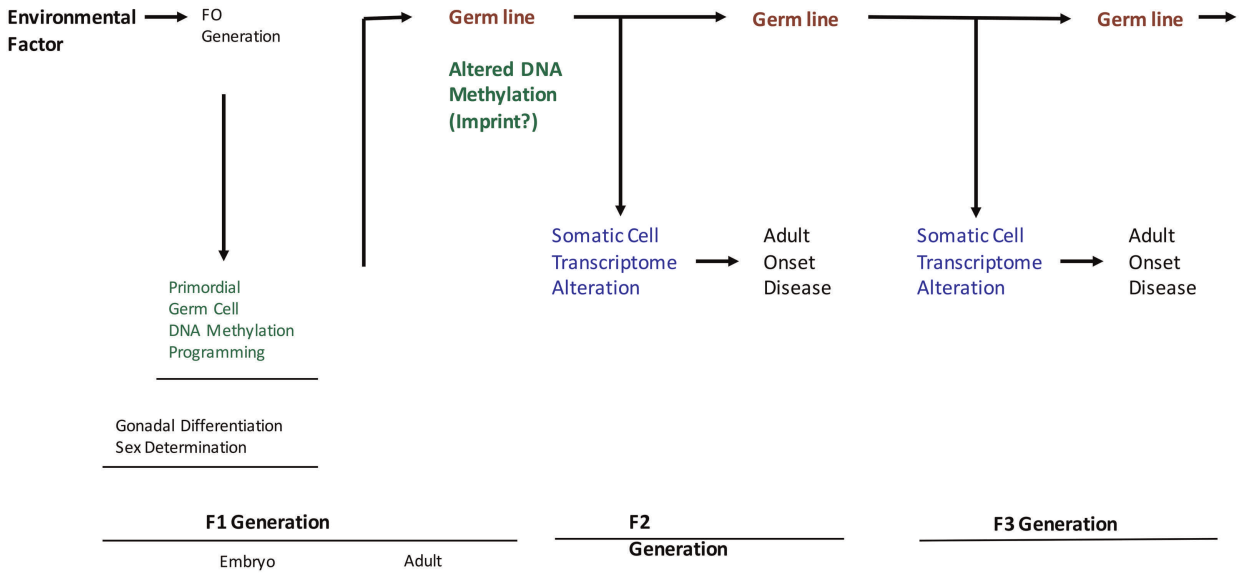

Fig. 1 Role of germline in epigenetic transgenerational inheritance. Summary of environmentally induced epigenetic reprogramming of primordial germ cells that leads to the germline transmission of epimutations, resulting in all somatic cells having altered epigenetics and gene expression. This can result in changes in phenotype or increased disease susceptibility in the F1, F2 or transgenerational F3 generation. Modified from Skinner, M.K., Manikkam, M., Guerrero-bosagna, C., 2010. Epigenetic transgenerational actions of environmental factors in disease etiology. Trends in Endocrinology and Metabolism 21, 214–22.

having a high density of CpG sites, comprising less than 5% of the genome, show methylation erasure, as previously observed (Ben Maamar *et al.*, 2023). Therefore, in the transgenerational epigenetic sites DNA methylation is maintained or increases during this early embryonic development, and is not erased, so is maintained transgenerationally (Ben Maamar *et al.*, 2023). Since numerous epigenetic processes exist, the role of DNA methylation, histone modifications, and ncRNA in the sperm mediating the epigenetic transgenerational inheritance was investigated (Beck *et al.*, 2021). The toxicant induced epigenetic changes in transgenerational sperm were shown to have integrated actions of DNA methylation, ncRNA, and histone modifications to facilitate transgenerational transmission. The integration of epigenetic processes including ncRNA directed DNA methylation and DNA-methylation derived histone retention has been observed (Beck *et al.*, 2021). Transgenerational differential DNA methylation regions (DMRs) were found to be generated throughout the course of development from early primordial germ cells, prospermatogonia, spermatogonia, meiotic spermatocytes, postmeiotic spermatid and spermatozoa stages of development, so are associated with all stages of male germ cell development (Ben Maamar *et al.*, 2021). Therefore, in addition to the transgenerational phenomenon and inheritance phenotypes observed, the molecular processes within the developing germ cell that happen when epigenetic transgenerational inheritance occurs are also being elucidated.

The majority of studies have examined an individual environmental exposure that induced epigenetic transgenerational inheritance. A recent study examined the potential for different exposures at each generation to have a compounding impact on epigenetic transgenerational inheritance (Nilsson *et al.*, 2023). In a rat study, the F0 generation gestating females were exposed to the agricultural fungicide vinclozolin, the F1 generation gestating females were exposed to jet fuel and the F3 generation grand offspring were exposed to DDT (dichlorodiphenyltrichloroethane). This was followed by two more generations breeding to the F5 transgenerational generation with no further exposures. The multiple generation exposure promoted compounded increases in disease for obesity, kidney disease, and multiple disease phenotypes, while other diseases such as testis, ovarian, and prostate disease plateaued and did not show a compounded disease phenotype (Nilsson *et al.*, 2023). Therefore, the distinct exposures of each subsequent generation can generate a greater frequency and occurrence of multiple diseases in subsequent generations. These observations further suggest society needs to consider the environmental justice issues of toxicant exposure impacts on our future generations (Korolenko *et al.*, 2023).

Summary

Environmental exposures can promote non-genetic inheritance through epigenetic transgenerational inheritance mechanisms to promote disease and altered phenotypes in subsequent generations. The potential role of this mechanism in our understanding of disease etiology needs to be considered. For example, environmental exposures in past generations could contribute to the incidence of abnormalities and disease in the current human population. Epigenetic transgenerational inheritance will also play critical roles in other biological processes such as evolutionary biology (Skinner and Nilsson, 2021; Skinner, 2015).

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