Environmentally Induced Epigenetic Transgenerational Inheritance of Disease

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INTRODUCTION

We have investigated the effects of two environmental toxicants (vinclozolin and methoxychlor) following exposure of rats during fetal gonadal sex determination, and the impact on gonadal development and function (*1, 2*). A serendipitous observation was made when F1 generation animals were mistakenly bred to generate the F2 generation offspring: the vast majority of the testes in the F2 generation carried a spermatogenic cell defect that induced apoptosis. This prompted a multiple year study that demonstrated the phenomenon of environmentally induced epigenetic transgenerational inheritance of disease for the first time (*3*). This study showed an increase in apoptosis in testis spermatogenic cells in the F1, F2, F3, and F4 generations, as well as in outcrossed offspring, through non-Mendelian genetic inheritance, affecting 90% of the male population. The causative epigenetic effects were traced to specific DNA methylation sites. The impact of this study on our understanding of the molecular control of inheritance, disease etiology, and environmental toxicology is significant. Over the past ten years a series of studies have further investigated this phenomenon, including environmental factor specificity, germline epigenetics, and disease etiology (*4, 5*).

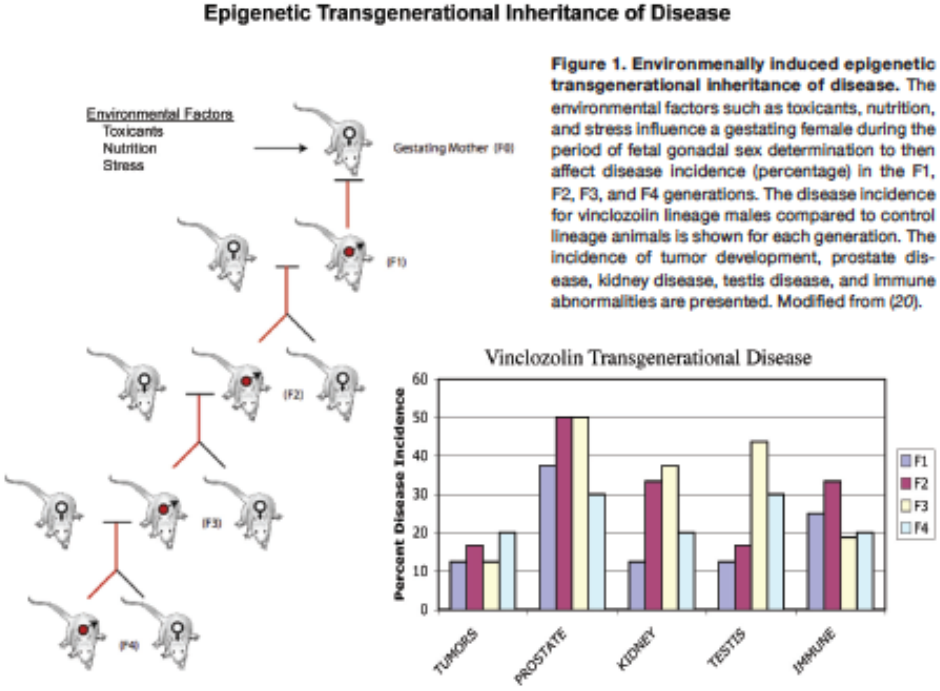

Figure 1. Environmentally induced epigenetic transgenerational inheritance of disease. The environmental factors such as toxicants, nutrition, and stress influence a gestating female during the period of fetal gonadal sex determination to then affect disease incidence (percentage) in the F1, F2, F3, and F4 generations. The disease incidence for vinclozolin lineage males compared to control lineage animals is shown for each generation. The incidence of tumor development, prostate disease, kidney disease, testis disease, and immune abnormalities are presented. Modified from (*20*).

EPIGENETIC TRANSGENERATIONAL INHERITANCE

The definition of epigenetic transgenerational inheritance is "germline mediated inheritance of epigenetic information between generations that leads to phenotypic variation in the absence of direct environmental influences" (*6*). This is in contrast to epigenetic inheritance that involves direct environmental germline or somatic cell exposure, and epigenetic responses during early development that influence phenotypes in later life. An example is the Agouti mouse model, which responds to an environmental signal in utero through a change in an epiallele, thus shifting the coat color of the offspring (*5, 7*). Environmental epigenetic inheritance responses may be corrected in subsequent generations during epigenetic reprogramming of the germline or early embryo, such that the phenotype is lost (*8, 9*).

In order for environmentally induced epigenetic transgenerational inheritance to occur, the external insult must act on a gestating female during fetal gonadal sex determination, influencing the epigenetic programming through altered DNA methylation patterns in the germline (Fig. 1). The epigenetic information is then carried through the epigenome of the germline, into the embryonic stem cell, and thus to all somatic cells of the offspring. Susceptible tissues in offspring will potentially develop disease and the defect will continue to be transgenerationally inherited (Figs. 1 and 2) (*3–5*). Several studies, described below, support this hypothesis of the molecular etiology of epigenetic transgenerational inheritance.

GERMLINE EPIMUTATIONS AND EXPOSURE (TOXICANT) SPECIFICITY

The environmental compound (toxicant) administered in the initial study was vinclozolin, one of the most widely used agricultural fungicides (*3*). The outcross information from this work indicated that

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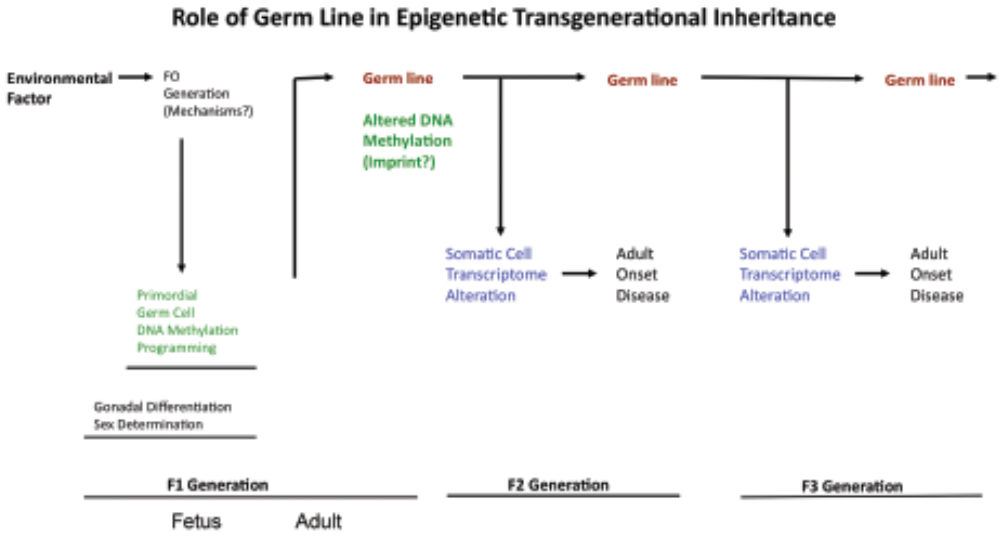

Figure 2. Role of germline in epigenetic transgenerational inheritance. An environmental factor acts on the F0 generation gestating female (left) to influence the developing F1 generation fetus, resulting in reprogramming of the methylation state of the primordial germ cell DNA. This altered DNA methylation pattern becomes fixed, similar to an imprinted gene, and is transferred through the germline to subsequent generations. Somatic cells in the offspring in later generations also carry the altered epigenome, generating an aberrant transcriptome, and promoting adult-onset disease. Modified from (*4*).

the transgenerational phenotype was transmitted through the male sperm (*3*). A genome-wide promoter analysis identified approximately 50 differentially methylated regions (DMRs) in the sperm of the F3 generation from vinclozolin-treated animals when compared with sperm from matched control (vehicle exposed) F3 rats (*10*). The DMRs carry epimutations that can transmit this epigenetic information transgenerationally (*4*).

A critical question was whether this phenomenon was unique to vinclozolin. A series of studies investigated the actions of dioxin (*11*), a pesticide and an insect repellent (permethrin and DEET) (*12*), plastics (BPH and phthalates) (*13*), and hydrocarbons (JP8 jet fuel) (*14*). All were found to promote the transgenerational inheritance of sperm epimutations and of disease (*15*). Interestingly, each toxicant promoted a unique set of epimutations with negligible overlap (Fig. 3) (*15*). These studies did not measure true environmental risk, but provide a good foundation for future analysis to determine the environmental hazards of exposure. Others have now shown transgenerational inheritance of disease as a result of exposure to various environmental factors including nutrition (*16*), stress (*17*), and other toxicants (*18*). Combined observations suggest that epigenetic biomarkers for ancestral toxicant exposure and adult onset disease may exist.

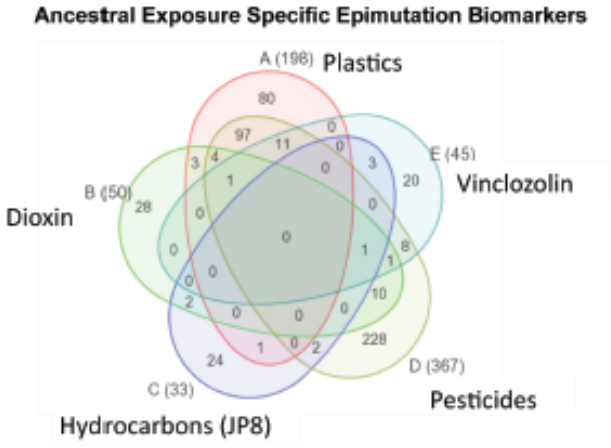

Figure 3. Venn diagram of transgenerational epimutations associated with different exposure groups showing a number of common epimutations in the F3 generation of rats due to ancestral exposure of F0 generation gestating females to dioxin, pesticide, plastics, or hydrocarbons. Modified from (*15*).

TRANSGENERATIONAL INHERITANCE OF DISEASE AND PHENOTYPIC VARIATION

The first transgenerational abnormality observed was an increase in spermatogenic cell apoptosis (*3, 19*). Other abnormal pathologies seen (Fig. 1) included prostate disease (*11–15, 20, 21*), kidney disease (*11–14, 20*), mammary tumors (*20*), immune system abnormalities (*14, 20*), and behavioral effects such as anxiety (*22*). Other laboratories have shown transgenerational effects on reproduction (*23, 24*), stress response (*25*), and obesity (*14, 26*). Some transgenerational diseases were induced by a variety of different environmental exposures (*15*), including polycystic ovaries and reduction of primordial ovarian follicle pool size, which were found in the majority of females from all exposure groups examined (*27*).

Epigenetic transgenerational inheritance may also impact other areas of biology. One study found that the F3 generation of vinclozolin-treated rats had significant altered mate preference behavior (*28*). Since sexual selection is a major determinant in evolutionary biology, this work suggests that transgenerational epigenetics may play a critical role in evolution (*4*).

TRANSGENERATIONAL SOMATIC TRANSCRIPTOMES AND THE ETIOLOGY OF REPRODUCTIVE DISEASE

Transgenerational germline transmission of epimutations results in all somatic cells in offspring carrying an altered methylation pattern and transcriptome (*4, 6*). Building upon earlier transgenerational transcriptome studies in fetal rat testis (*29*), we examined the

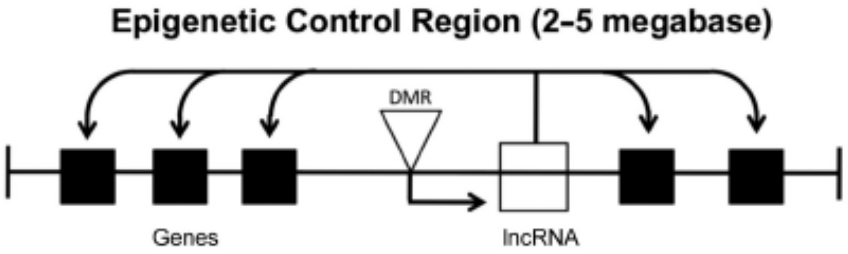

Figure 4. Schematic showing a 2–5 Mbp epigenetic control region containing multiple distal gene regulatory units (black boxes) under the control of a differentially methylated region (white triangle, DMR) and a long noncoding RNA (white box, lncRNA). Modified from (*30*).

transgenerational transcriptomes of 11 different tissues in male and female vinclozolin-treated versus control lineage rats (*30*). All tissues had a transgenerational transcriptome that was unique to the specific tissue, with negligible overlap between tissues. It is intriguing that a relatively small number of epimutations can produce such a large number of specific transcriptome changes (*30*). The identification of epigenetic control regions—areas of 2–5 megabases with an overrepresentation of regulated genes close to DMRs and long noncoding RNA regions—may provide a clue (Fig. 4) (*30*), suggesting unique molecular regulatory mechanisms that will require further investigation.

To further elucidate the role of epimutations in adult onset disease, the molecular etiology of ovarian diseases were studied (*27*). Follicular somatic granulosa cells were found to have an altered epigenome and transcriptome that suggested specific signaling pathways were affected. Similarly, somatic Sertoli cells in the testis were found in a separate study to have transgenerational alterations in their epigenomes and transcriptomes, affecting genes previously shown to be involved in male infertility (*31*).

IMPACT AND FUTURE STUDIES

Our original publication (*3*) described the phenomenon of environmentally induced epigenetic transgenerational inheritance of a disease. Subsequent publications confirmed and clarified the molecular and physiological parameters involved. The research shows the existence of a non-genetic (i.e., epigenetic) form of transgenerational inheritance that impacts the etiology of certain diseases, as well as a potential molecular mechanism describing how environmental factors could directly influence gene expression and therefore disease (*4*, *5*). Further studies clearly are needed to clarify the role of epigenetics and transgenerational inheritance in disease etiology, evolutionary biology, and other areas of cell and developmental biology. The specific next steps needed include investigation into why specific sites are more susceptible to becoming transgenerationally programmed and the mechanism by which this occurs, as well as the translation of this work from animals to humans. These and other studies will undoubtedly have significant impact on our understanding of normal biology and disease etiology.

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