CHAPTER 1

Epigenetic Transgenerational Actions of Endocrine Disruptors through the Male Germ-Line

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1.1 Review

Embryonic exposure to environmental factors has been shown to cause adult onset disease,\(^3\) but few have looked at the second F2 generation. Examples include the late embryonic and early postnatal exposure to cyclophosphamide causing embryonic defects,\(^4\) embryonic nutritional defects causing immune defects,\(^5,6\) diethylstilbestrol (DES) causing female reproductive tract abnormalities,\(^7,8\) and other endocrine disruptors causing male reproductive defects.\(^9,10\) Studies suggest effects of environmental factors on the first generation. Any transgenerational phenotype would require transmission through the germ-line.

A recent observation demonstrated that the exposure of a pregnant rat transiently to endocrine disruptors caused a spermatogenic cell defect and subfertility in the F1 generation and all subsequent generations examined (F1–F4).\(^11\) The endocrine disruptors used were the anti-androgenic fungicide vinclozolin used in the fruit (e.g., wine) industry\(^12\) and the pesticide methoxychlor used to replace DDT.\(^13\) The critical exposure period was at the time of sex determination and the transgenerational phenotype was transmitted through the male germ-line.\(^11\) The phenotype of increased spermatogenic cell apoptosis, decreased sperm numbers and sperm motility was observed in greater than 90% of all males of all the generations examined. When the animals were allowed to age up to 1 year additional diseases developed including cancer, prostate disease, kidney disease, and immune cell defects.\(^14\) A high frequency of transmission was observed in all generations examined for all the disease states.

The frequency of the transgenerational phenotype was such that a DNA sequence mutational event could not be involved. The random nature of a DNA sequence mutation has a phenotype typically less than 1% and this often
declines in subsequent generations. An epigenetic mechanism is found to be involved due to the frequency of the phenotype. To support these conclusions, two genes were identified in the sperm that had altered methylation patterns associated with the transgenerational phenotype discussed. Therefore, the endocrine disruptors appear to induce an epigenetic transgenerational disease condition for four generations through the male germ-line. The epigenetics appears to involve altered DNA methylation. Although most genes are re-set in early embryonic development, a subset of genes called imprinted genes maintains their DNA methylation pattern which appears to be permanently programmed. In contrast to all somatic cells the primordial germ cells undergo a de-methylation during migration and early colonization of the embryonic gonad, followed by a re-methylation starting at the time of sex determination in a sex-specific manner. The exposure of the pregnant mother at the time of sex determination appears to have altered the re-methylation of the germ-line and permanently re-programmed the imprinted pattern of DNA methylation. This provides a unique epigenetic mechanism to promote a transgenerational phenotype induced by an environmental factor.

Altered methylation of imprinted genes has been shown to promote disease states. Cancer and tumor development has also been shown to be involved in epigenetic alteration of DNA methylation. Therefore, the epigenetic reprogramming of the male germ-line causes numerous transgenerational disease states that can be explained by this epigenetic mechanism. The identification of the altered DNA methylation sites and associated genes will provide more insight into the proposed epigenetic transgenerational phenotype.

The level of endocrine disruptors used in the recent studies is higher than levels anticipated in the environment, such that conclusions regarding the toxicology of these endocrine disruptors are not possible. However, the important factor is the identification of this novel phenomenon, that an environmental factor can promote an epigenetic transgenerational phenotype. Due to this observation the potential hazards of environmental factors need to be carefully evaluated. If the exposure of your grandmother at mid-gestation to environmental toxins can cause a disease state in you with no exposure, and you will pass it on to your grandchildren, the potential hazards of environmental toxicants must be rigorously assessed. Transgenerational studies need to be performed in evaluating the toxicology of environmental compounds.

The epigenetic transgenerational phenotype also provides critical insights into disease etiology. Since a number of common disease states were induced, an epigenetic component of disease now needs to be seriously considered. In the event a major epigenetic component exists, the epigenetic background of an individual may be a major factor in susceptibility to disease development. Therefore, identification of the genes involved with altered methylation may provide essential new diagnostics to assess future onset of disease. This will allow new therapeutic targets and therapies to potentially prevent the onset of disease. This is a new paradigm in disease etiology that needs to be considered.

In a broader biological perspective, the ability of an environmental factor to cause a permanent genetic trait in all subsequent progeny of an affected
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individual can significantly impact our understanding of evolutionary biology. Currently, a DNA sequence mutation event that allows an adaptation and natural selection is considered the driving factor in evolutionary biology. However, the frequency of specific evolutionary events and regional influences on evolution suggests that an additional epigenetic mechanism should be considered. Although a DNA sequence mutational event will be important for evolutionary biology, an epigenetic component influenced by an environmental factor needs to be considered as an alternate factor that will help explain some aspects of evolutionary biology.

The epigenetic transgenerational actions of endocrine disruptors observed provide novel insights into several areas of biology. The ability of an environmental compound to promote a transgenerational phenotype suggests toxicology studies need to consider transgenerational elements of the actions of potential toxic agents. Future studies need to investigate the types of compounds that can induce the epigenetic effect. Currently we know anti-androgenic compounds can, but need to assess if other factors can as well. The toxicology studies need to be done to assess the minimum required dose to obtain a phenotype and compare this to potential environmental levels. This information will reveal if the levels in our environment are a problem. The epigenetic effects on the methylation state of specific genes needs to be determined to provide insights into the mechanisms of action of the environmental factors. In addition, these genes will provide potentially critical diagnostic markers and therapeutic targets for a variety of common diseases. The basic elements of disease etiology now need to consider epigenetic factors as markers and/or causal factors. In a broader context, the epigenetic transgenerational impact of environmental factors needs to be considered in the mechanisms involved in evolutionary biology. Epigenetics will likely be a much more important factor in biology than currently appreciated. Epigenetics is the next layer of complexity beyond the DNA sequence.

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References