2005 SCIENCE REPORT STUDY SUMMARY

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Abstract:
Transgenerational effects of environmental toxins require an epigenetic alteration of the germ-line. Transient exposure of a gestating female rat during the period of sex determination to the endocrine disruptors vinclozolin (i.e. anti-androgenic compound) or methoxychlor (i.e. estrogenic compound) induced an adult phenotype in the F1 generation of decreased spermatogenic capacity and increased incidence of male infertility. These effects were transferred through the male germ-line to nearly all males of all subsequent generations examined (i.e. F1-F4). The epigenetic effects of the endocrine disruptors appear mediated by altered DNA methylation patterns in the germ-line. An epigenetic re-programming of the germ-line to promote a reproductive disorder in a transgenerational manner has significant evolutionary biology and disease etiology implications.

Observation:
A pregnant female was exposed to an environmental toxin for a short period at a critical period of sex determination for the embryo. The male progeny had reduced sperm production and subfertility. This phenotype/disease state was passed to all subsequent generations examined. Only the original F0 generation mother was exposed to the toxin. Nearly all males of all generations had the disease state and passed it on to their progeny. No known DNA sequence mutation mechanism can cause this type of transgenerational (i.e. heritable) disease phenotype. An epigenetic mechanism was identified in that the male germ-line (i.e. sperm) developed abnormal DNA methylation of specific genes. The environmental toxin permanently reprogrammed the sperm that passed the disease state on to all subsequent generations. This epigenetic transgenerational phenotype was found to induce other disease states in addition to male infertility. This is the first transgenerational effect of an environmental toxin identified, and the first indication that epigenetic mechanisms can permanently alter the germ-line and genetic traits of all subsequent generations and progeny of an exposed individual.

Impact:

Toxicology:

Indicates that a class of environmental toxins known as endocrine disruptors can induce a permanent transgenerational effect on an individual. The exposure your pregnant grandmother had could induce a disease state in you and you will pass this on to your grandchildren. Therefore, the potential hazard of environmental toxins is dramatically increased, in particular for pregnant women in mid-gestation.

Evolutionary Biology:

Darwinian evolution is based on the appearance of genetic mutations (i.e. DNA sequence alterations) that promote a natural selection process and competitive biological advantage. This evolution process is the basis for our understanding of biology and the relationship of ecosystems. The concept that an environmental factor (i.e. toxin) could induce an epigenetic effect that could induce a genetic trait and permanent reprogramming of the germ-line (i.e. sperm), impacts our concept of evolutionary biology. This suggests environmental impacts can be a critical variable in evolution. The current observations suggest new variables and factors in evolution that need to be considered and explains some unexplained rapid evolutionary events previously observed.

Molecular Basis of Heritable Disease:

Previously we have realized that fetal and embryonic development events can impact disease states in the adult. A number of environmental toxins have been shown after an embryonic exposure to cause an adult disease. The concept that these induced disease states could be transgenerational and permanently inherited has not been appreciated. The current study demonstrates an effect on male fertility and sperm production, however, other disease states (e.g. prostate, kidney and cancer) have been suggested. This indicates that an epigenetic transgenerational mechanism could be involved in some heritable diseases. Many diseases have increased in frequency of occurrence but faster than can be explained from normal genetic (i.e. DNA sequence mutation) mechanisms. This epigenetic transgenerational phenomenon could explain the rapid onset of these diseases and would suggest an environmental factor in the process. This information provides new mechanistic insights into the molecular basis of disease and new therapeutic strategies to potentially treat the disease states.

Summary:

The transient exposure of a pregnant female at the time of embryonic sex determination to an environmental toxin (endocrine disruptor) can induce an epigenetic transgenerational disease phenotype in all subsequent generations. This has a significant impact on our understanding of factors that influence human disease and the basic concepts of evolutionary biology.

BULLET POINTS

New Results

- We exposed mid-gestation pregnant rats to an environmental toxin (endocrine disruptor) at the time of embryonic gonadal (testis) sex determination. The offspring, or first generation males, had lower sperm counts and abnormal spermatogenesis (sperm production) in the testis. Approximately 10% of the animals were completely infertile.

- When this first generation was mated, the males passed down the same male low fertility disease state to the second-generation males, and so on. We found this disease state passed on through

the four generations we examined. This transgenerational disease condition occurred in over 90% of all males in all the generations we examined.

- The frequency of disease transmission cannot be explained with a genetic DNA sequence mutation that would occur at less that 1% of progeny. Analysis suggested an epigenetic mechanism involving abnormal methylation of specific genes. Two genes were identified that had altered methylation for multiple generations.

- Therefore, a human analogy would be if your grandmother was exposed to an environmental toxin during mid-gestation, you may develop a disease state even though you never had direct exposure, and you may pass it on to your great grandchildren. This is an epigenetic transgenerational phenomena that impacts: 1) the potential hazards of environmental toxins, 2) provides a new variable for consideration in the development of disease, and 3) is a new factor to consider in evolutionary biology as it pertains to environmental influence on adaptive mutations and natural selection.

Epigenetics:

- Epigenetics does not involve DNA sequence changes but chemical modification of the DNA. This can alter gene expression and if genes are turned on or off. A subset of genes called imprinted genes can transfer their epigenetic pattern, methylation of DNA to the next generation and affect activity of DNA. The frequency of an epigenetic effect is high compared to that of genetic sequence mutations. The effect observed in the current study appears to be epigenetic, which is a new concept for disease transmission.

Next Steps:

- The dose of endocrine disruptors used in our research are higher than those expected in the environment. Dose curves are needed to see if environmental levels can cause the effect and to screen additional compounds and classes of environmental toxins.

- Our preliminary results suggest other disease states develop in older animals, so further examination of other diseases influenced by this transgenerational phenomena are needed

- The phenomenon requires a permanent mutation of the male germ line, sperm, thus we are now identifying the specific genes with altered methylation status that result in this permanent mutation.

Clinical Implications:

- Disease etiology and development mechanisms could involve this epigenetic transgenerational phenomenon and be a factor in disease development not previously appreciated. The question now is what aspect of disease are due to DNA sequence mutations versus epigenetics involving chemical modification of the DNA.

- Since this is an environmental effect that is multigenerational, it could explain why different sub-populations in different regions may develop different diseases. For example, sperm numbers have been shown to be reducing in the UK but remain constant in Japan.

- This new phenomena may provide alternate approaches for disease diagnosis and therapy that have not been previously considered. This should be considered in future therapeutic development strategies.

- The influence of environmental toxins on disease development needs to be considered in the future and caution for mid-gestation pregnant mothers provided.

Highlights:

- In the past we have been concerned with the influence environmental toxins may have on our offspring and us if we are exposed during embryonic development. Based on our analysis we should be concerned with the impact of these toxins on our grandchildren and subsequent progeny.

- If this phenomena is involved in a variety of other diseases then we need to consider this mechanism and factor it into our understanding of how diseases develop and how to treat diseases.