2006 ENDOCRINOLOGY PAPERS SUMMARY

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Paper #1 – Publication Online September 7, 2006
Matthew Anway, Charles Leathers and Michael K. Skinner. (2006) Endocrine Disruptor Vinclozolin Induced Epigenetic Transgenerational Adult Onset Disease. Endocrinology [Epub ahead of print].

Paper #2 – Publication Online September 7, 2006
Hung-Shu Chang, Matthew Anway, Stephen Rekow and Michael K. Skinner. (2006) Transgenerational Epigenetic Imprinting of the Male Germ-Line by Endocrine Disruptor Exposure During Gonaldal Sex Determintation. Endocrinology [Epub ahead of print].

Abstract -
An epigenetic transgenerational phenotype is described involving the induction of adult onset disease for all progeny exposed to an environmental compound, as well as the molecular mechanism involved. Transient exposure of a gestating female rat during the period of sex determination to the endocrine disruptor vinclozolin (ie anti-androgenic endocrine disruptor used as a fungicide in the fruit industry) induced an adult phenotype in the first F1 generation of breast tumors, prostate disease, kidney disease, immune abnormalities and premature aging. These adult onset diseases were transferred through the male germ-line to 85% of all males of all subsequent generations examined (ie F1-F4). The frequencies of diseases are similar to those observed in the human population. The mechanism involved is an epigenetic one involving an alteration in DNA methylation of sperm and the induction of new imprinted-like genes that modify the epigenome. This reprogramming of the epigenome becomes permanent and allows the abnormal pathology to be transferred transgenerationally to all subsequent progeny. Therefore, this epigenetic re-programming (i.e. imprinting) of the germ-line and transgenerational phenotype has significant impacts on disease etiology considerations and areas of biology such as evolution.

Observation –
A pregnant female was exposed to an environmental compound (ie endocrine disruptor) for a short period at a critical period of sex determination for the embryo. The male progeny had breast tumors, prostate disease, kidney disease, tesitis defects and immune abnormalities. This phenotype/disease state was passed to all subsequent generations examined. Only the original F0 generation mother was exposed to the environmental toxicant. Nearly all males of all generations had a 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 toxicant

permanently reprogrammed the sperm and induced new imprinted-like genes that passed the disease state on to all subsequent generations. This epigenetic transgenerational phenotype was found to induce adult onset disease states relevant to humans. This is one of the first transgenerational effects of an environmental toxicant 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 compounds 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 toxicants is dramatically increased, in particular for pregnant women in mid-gestation.

Molecular Basis of Heritable Disease and Adult Onset Disease
Previously we have realized that fetal and embryonic development events can impact disease states in the adult. A number of environmental compounds 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 effects on male fertility tumor development, prostate disease, kidney disease, immune abnormalities and premature aging. All at frequencies similar to that found in the human population. 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. The current study demonstrates the ability to promote new imprinted-like genes and a change in the epigenome. 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 toxicant (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.

BULLET POINTS

New Results:

- We exposed mid-gestation pregnant rats to an environmental compund (endocrine disruptor) at the time of embryonic gonadal (testis) sex determination. The offspring, or first generation males, had a variety of major diseases from cancer to kidney disease. Approximately 85% of the animals had some disease state.

- When this first generation was mated, the males passed down the same disease states 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 85% of all males and females 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 demonstrated an epigenetic mechanism involving abnormal methylation of specific genes. Twenty-five DNA sequences were identified that became an imprinted-like gene with an altered methylation for multiple generations.

- Therefore, a human analogy would be if your grandmother was exposed to an environmental toxicant 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 toxicants, 2) provides a new mechanism for consideration in the development of disease (ie adult onset 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 determine 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. A set of new imprinted-like genes were identified. The frequency of an epigenetic effect is high compared to that of genetic sequence mutations. The epigenetic effect observed in the current study is a new concept for disease transmission.

Next Steps:

- The dose of endocrine disruptor (ie vinclozolin) 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 toxicants.

- Our results demonstrate disease states develop in older animals, so further examination of human diseases influenced by this transgenerational phenomena are needed.

- The phenomenon requires a permanent epigenetic re-programming of the male germ line, sperm, how the new imprinted DNA sequences identified effect the genome and gene expression needs to be determined and related to disease development.

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, prostate disease is shown to be high in the USA but remain low 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 toxicant exposures on disease development needs to be considered in the future and caution for mid-gestation (ie 6 weeks to 5 months) pregnant mothers provided.

Highlights:

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

- If this phenomena is the mechanism involved in the development of diseases, then we need to consider this as an opportunity to design prevention diagnostics and therapies.