

DNA Methylation and its Role in a form of Hormone-Dependent Cancer

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Abstract. Diethylstilbestrol (DES), a synthetic estrogen, was widely administered to pregnant women between 1947 and 1971 on account of a misconception that it could prevent miscarriages. The offspring of these women were diagnosed with various reproductive tract abnormalities, including cancer. We focused this study on DES-induced abnormalities in the uterus. We screened for altered DNA methylation patterns in uteri from control vs. neonatally DES-treated hamsters using MSRF. Preliminary results show differential DNA methylation patterns in uteri of control vs. DES-treated hamsters. Next we will determine the nucleotide sequence of the differentially methylated genetic elements.

Introduction

Diethylstilbestrol (DES) was synthesized in 1938 and imitates the action of estradiol-17b (E2), the primary ovarian estrogen [1]. From 1947-1971, it was often prescribed due to the belief that increasing estrogen levels could prevent miscarriages. This practice ceased due to reports of reproductive tract abnormalities (including cancer) in the offspring of DES-treated mothers. At least four million women and their fetuses may have been exposed to DES [2]. The condition gained vast attention and became known as the “DES Syndrome”. DES was then recognized more generally as the prototypical “endocrine disruptor agent” [3].

When hamsters are treated neonatally with DES, reproductive tract abnormalities were always seen in adult animals [4, 5]. Significant to this study, however, was the high incidence of endometrial adenocarcinoma, a form of estrogen-dependent uterine cancer. We are now probing the molecular mechanisms of this phenomenon.

Epigenetics is defined as heritable changes in gene expression that are not accompanied by changes in DNA sequence. Epigenetic events play important roles in the process of gene silencing that allows genetically identical cells to differentiate into functionally diverse tissues and organs.

Methylation of the cytosine within the dinucleotide CpG is the epigenetic modification that is most commonly studied in humans. CpG islands (regions rich in CpG dinucleotides) span the 5' region of many genes. Usually, these cytosines are unmethylated, allowing transcription to occur. Methylation of these regions silences expression of the gene. However, aberrant methylation events can have adverse effects on normal gene expression. For example, hypermethylation events taking place in the CpG islands of tumor-suppressor genes will cause inactivation. These events are critical components of the tumorigenic process. [6]

Neonatal exposure to DES can cause a number of reproductive tract abnormalities, including cancer, in both males and females. How DNA methylation is involved in the progression of these abnormalities is not known. However evidence does suggest that it plays an important role in endocrine disruption [7].

Experiment, Results, Discussion, and Significance

We use Syrian golden hamsters for our animal model system. Within 6 hours of birth, they are injected with either corn oil alone (control), or with corn oil containing 100 ug DES. On day 5 of life, hamsters are sacrificed and uteri are harvested.

DNA is extracted from the uteri and then precipitated and quantified. Methylation Sensitive Restriction Fingerprinting (MSRF) is performed as follows: 2 ug of genomic DNA is digested using the restriction enzyme MseI (a four base cutter, cut site: T/TAA), which cuts the genomic DNA into many pieces, mostly around 200-300 bp long; half of the initial digestion then undergoes a second digestion with the enzyme BstUI, which cuts at the site CG/CG, but only if the C is unmethylated. The result of the digestions are 4 tubes of: control, single digested DNA (C1); control, double digested DNA (C2); DES, single digested DNA (D1);

DES, double digested DNA (D2) - each tube containing 1 µg of digested DNA.

100 ng of each tube then undergoes amplification via PCR using 1 of 10 different arbitrary primer pairs. PCR product is electrophoresed on a 6% polyacrylamide gel at 1600V for approximately 30 min. The bands of interest are determined by comparing the banding pattern to those patterns shown in Table 1.

Table 1.
Possible outcomes of DNA methylation changes detected by MSRF

Methylation condition in DES-treated relative to Control DNA	Control		DES	
	C1	C2	D1	D2
1. No Methylation	—	—	—	—
2. Normal Methylation	—	—	—	—
3. Hypermethylation	—	—	—	—
4. Hypomethylation	—	—	—	—

Preliminary results (Fig. 1) suggest that differential methylation events occur in the uterus of neonatally DES-treated animals compared to control animals. Further studies will involve recovering the bands of interest from the polyacrylamide gels and sequencing the fragments to determine their identity and significance.

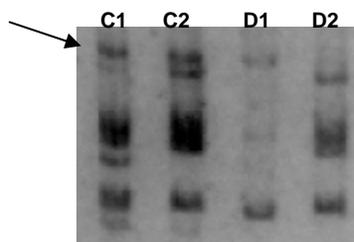


Fig. 1. Hypomethylation in uteri of DES-treated animals compared to control animals

Because methylation causes gene silencing and thereby affects cell differentiation, it is important to study the effects of altering this phenomenon. Methylation events can be altered by a number of factors such as diet and environmental influences. In our animal model, a single dose of DES within 6 hours of birth induces numerous reproductive tract problems in the adult animals. The animals studied here are only 5 days old. Even at that early stage, dramatic morphological changes (DES-exposed uterus is approximately 3 times the size of a normal uterus) do seem to be accompanied by altered DNA methylation patterns.

Epigenetic alterations are now thought to be just as important to the development of cancer as mutations.

Differential epigenetic events are much easier to induce than mutations, meaning that they can be easier to reverse. Understanding how and when epigenetic alterations take place can lead to a better understanding of how to treat, and also how to avoid, the complications that arise from such events.

Conclusions

In this study, we have shown that neo-natal exposure of hamsters to DES can alter the methylation status of the genome. Because DNA methylation is important for biological roles, such as cell differentiation, altering methylation events can be devastating to the development of the organism. Endometrial adenocarcinoma is a clinically important type of estrogen-dependent uterine cancer that we have been able to reliably induce, and therefore study, in our animal model. Studies such as these can lead to a better understanding of the mechanisms involved in such tumorigenic processes.

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