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Gene imprinting in developmental toxicology: a possible interface between physiology and pathology

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Abstract

Gene imprinting is an epigenetic mechanism for accomplishing persistent change in gene expression. In this brief paper, we explore the mechanisms for imprinting genes and present data showing that the synthetic estrogen, diethylstilbestrol (DES) can developmentally imprint genes by changing the pattern of DNA methylation. We further discuss the implications of this and other findings for non-mutagenic aspects of developmental toxicology, and suggest ways to use this concept in modifying in vitro screening for developmental toxicants. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Genomic imprinting refers to a very specific phenomenon, 'the non-Mendelian inherited epigenetic form of gene regulation that results in monoallelic expression' or, put another way, 'genomic imprinting is an epigenetic form of gene regulation that results in the expression of only one parental allele' (Jirtle, 1999).

The elements of genomic imprinting are the persistent change in gene expression that is accomplished in an epigenetic fashion, and the silencing of a gene from one parent, but not the other.

The process of cell differentiation is based on differential gene expression, or the sequential turning on and off of different genes to establish a stable phenotype. While this process may not be monoallelic, it is both epigenetic and inherited.

In developmental toxicology, the term 'gene imprinting' refers to persistent changes in gene expression that occur through non-mutagenic mechanisms.

The same conundrum has appeared in the molecular biology of cancer, in which 'silencing' of tumor suppressor genes has been associated with the cancer process. Since tumor suppressor

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genes are, in many cases, genomically imprinted monoallelic genes, the process fits the specific definition. However, in cases where the neoplastic transformation process involves persistent activation or repression of non-genomically imprinted monoallelic genes, the term 'gene imprinting' is useful.

2. Hormonal carcinogenesis

The terminology is especially useful when considering mechanisms of hormonal carcinogenesis. It is well known that treatment of various species, including humans, with exogenous estrogen is associated with tumors in different organs (Hertz, 1976). Estrogens have been largely assumed to function in the tumorigenic process as a secondary stimulus or promoter. This is, to a large extent, due to the fact that there is a paucity of evidence that estrogens or estrogenic chemicals are point mutagens (Degen and Metzler, 1987). In spite of the failure to demonstrate conclusively that estrogenic chemicals, unlike other carcinogenic chemicals, neither form covalent adducts to DNA, nor induce structural DNA mutations, it has been shown that estrogens of various structures and biological potencies can function as carcinogens. This evidence includes neoplastic transformation of cells in culture in the absence of enhanced cell proliferation (Barrett et al., 1981; McLachlan et al., 1982; Tsutsui et al., 1983); carcinogenic effects in the adult hamster kidney (Li and Li, 1990); and in the neonatal mouse (Newbold et al., 1990) and hamster (Leavitt et al., 1981) uterus.

Estrogens are considered reversible cellular signals. Administration of estrogen to an ovariectomized mouse results in organ growth, cell proliferation and target gene expression in the uterus (Nelson et al., 1991). When estrogen is withdrawn, uterine size and weight, as well as expression of estrogen-regulated genes, returns to close to the unstimulated state.

On the other hand, when estrogens are given to newborn mice, at least one gene under estrogen control is expressed persistently, even in the absence of estrogen (Nelson et al., 1994).

The estrogen treatment that results in persistent expression of lactotransferrin also results in epithelial cancers of the uterus in a majority of the mice (Newbold et al., 1990). This leads to the question, 'How does a reversible signal become irreversible in the absence of detectable gene mutation?'

As described earlier, during the process of cell differentiation, genes are differentially turned on or off. For the most part, a key event in establishing the pattern of gene expression in a cell is the methylation or demethylation of the gene's regulatory elements.

3. Effects of diethylstilbestrol (DES)

Thus, we have studied the methylation pattern of the promoter of the lactoferrin gene in mice treated developmentally with DES (a treatment resulting in persistent expression of lactotransferrin and epithelial cancers in the uterus). The pattern of DNA methylation in developmentally treated mice was compared to those treated as adults (neither persistent gene expression, nor cancers are seen in the uterus after treatment as adults).

Five CpG sites available for methylation occur in a region upstream from the ERE (estrogen response element) in the mouse lactotransferrin promoter. In the developmentally estrogenized mouse, two sites remain unmethylated, while in the corresponding control, only one CpG site remains unmethylated (Li et al., 1997). It has been shown previously that a one base pair change in methylation pattern can have a strong effect on expression of the gene (Yokomori et al., 1995).

Adult mice treated with the same dose of DES for the same time did not have a change in the DNA methylation pattern of the lactotransferrin gene. This is consistent with the inability of such treatment in the adult to persistently change expression of the gene.

The altered methylation pattern associated with estrogen treatment during differentiation of uterine epithelial cells provides a mechanism for irreversible expression of a normally reversible signal or provides one possible route for the change

from epigenetic to genetic in hormonal carcinogenesis.

4. Estrogens and gene imprinting in carcinogenesis

The generalization of this mechanism to hormonal carcinogenesis requires studies on methylation and expression of different genes that are important to cell differentiation or proliferation. It has been shown that breast cancer cells have silenced genes that function as tumor suppressor genes and that the silencing is a function of altered methylation (Ferguson et al., 2000). It is not known whether estrogens play any role in the methylation or demethylation of these genes.

In fact, very little is known regarding the role of estrogens or other hormones in gene imprinting. A case can be made, however, that estrogen-associated signaling pathways may contribute to DNA methylation or demethylation.

For example, we earlier showed that estrogen-like effects were induced in uterine cells in culture (Tomooka et al., 1986) or in the mouse uterus in vivo (Nelson et al., 1991) by epidermal growth factor (EGF). Subsequently, we demonstrated that the EGF signal required the presence of a functioning estrogen receptor alpha (ERα) in cell culture for the expression of the growth factor effect (Ignar-Trowbridge et al., 1992). This study was subsequently confirmed in mice lacking the ERα gene (Curtis et al., 1996).

5. Regulation of imprinting

The demonstration of cross talk between membrane receptors and steroid hormone receptors has also been shown for insulin-like growth factor 1 (IGF-1) and ER (estrogen receptor), dopamine and PR (progesterone receptor), and both IGF-1 and EGF (epidermal growth factor) and ER in cell culture. Studies of receptor cross talk have established ER as an important signal transduction molecule in peptide hormone signaling systems. We have recently obtained additional evidence for this signaling convergence by showing that the cell survival pathway associated with PI (phoshatidylinositol) 3-kinase and protein kinase B (Akt) also requires a functioning ER for its action (Burow et al., submitted). Thus, pathways involved in cell proliferation, differentiation and survival apparently converge on the ER.

Finally, it has been shown previously that both EGF and estrogen increase the levels of c-fos and c-jun in target cells or tissues (Kamiya et al., 1996). The response of the immediate early genes to estrogen also proceeds, apparently, via an ER. In the case of developmental estrogenization, c-fos and c-jun were persistently expressed. Recently, a demonstration that cells constitutively over-expressing c-fos upregulated cytosine methyltransferase, the enzyme involved in DNA methylation, suggested to the authors that c-fos may be one regulator of the process.

6. Conclusions

In summary, we suggest a mechanism whereby estrogen, either directly or through related signaling pathways, plays a role in programming or imprinting genes involved in cell proliferation, differentiation or survival. Further, the genes are either persistently over- or under-expressed, or they will respond atypically to a later cue, such as another hormone, leading to altered cell function and ultimately disease. Given that the process of gene imprinting is a normal one seen in cell differentiation and genomic allelic silencing, one may speculate that, for estrogens or estrogenic chemicals, the distinction between physiology and pathology is blurred.

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