Cell Research (2007) 17: 277-279

www.nature.com/cr

COMMENTARY

Hormones and cancer

Yongfeng Shang[1]

[1]*Department of Biochemistry and Molecular Biology, Peking University Health Science Center, 38 Xueyuan Rd, Beijing 100083, China*

Cell Research (2007) **17:**277-279. doi:10.1038/cr.2007.26; published online 10 April 2007

Estrogen exhibits a broad spectrum of physiological functions ranging from regulation of the menstrual cycle and reproduction to the modulation of bone density, brain function, and cholesterol mobilization. However, estrogen is also associated with pathological complications particularly with the onset of gynaecological malignancies including breast cancer and endometrial cancer; estrogen is now considered to be a classical etiological factor for breast cancer and endometrial cancer.

In the early 1970s, it was reported that there was a 20-35% increase in incidence of endometrial cancer in Western Caucasian women who had undergone estrogen therapy [1]. Subsequently, a variety of clinical and epidemiological investigations, with support from studies in cell culture and animal models, have demonstrated the association of estrogen with the development and/or progression of the disease. In 2002, the US National Toxicology Program listed steroidal estrogens as carcinogens for the first time. The report cites data from human epidemiologic studies that show an association between estrogen replacement therapy and an increase in the risk of endometrial cancer, as well as a less consistent increase in the risk of breast cancer.

Selective estrogen receptor modulators (SERMs) were initially envisioned as drugs that would replace estrogen therapy in alleviating the symptoms associated with menopause without the carcinogenic effects of estrogen in the mammary gland and uterus. The first clinically available SERM was tamoxifen. It was introduced in the 1970s for the treatment of advanced breast cancer in post-menopausal women, and its use was then expanded as an adjuvant therapy for reducing risk of breast cancer in high risk pre- and post-menopausal women. However, in the mid-to-late 1980s, a series of reports documented an association between tamoxifen therapy in women with breast cancer and the development of endometrial carcinoma. The observation was subsequently substantiated in 1998 by the National Surgical Adjuvant Breast and Bowel Project's (NSABP) P-1: Breast Cancer Prevention Trial (BCPT) [2]. It was reported by the NSABP-BCPT that the increased rate of endometrial cancer occurred predominantly in women aged 50 years or older [2].

Estrogen action at target sites around the body is mediated through related but distinct estrogen receptors (ERs) designated ERα and β. Estrogens bind to the ligand binding domain of the ER to induce a conformational change in protein structure that permits the subsequent receptor dimerization and interaction with coactivator molecules [3]. The sequential activation of genes occurs via multiple mechanisms either through direct binding at estrogen response elements in the promoter region of estrogen-responsive genes or through a tethering protein-protein interaction with sites that are canonical to other well-known transcription factors [3]. Various cellular signal transduction pathways can potentially be exploited to influence tissue response specificity. Alternatively, survival pathways in cancer could evolve to alter the entire responsiveness to ER signaling.

The stimulation of endometrial carcinogenesis by tamoxifen is of great interest in both clinical medicine and basic research. Based on the crystal structures of the ligand-bound hormone-binding domains of ERs, it is believed that tamoxifen acts as an ER antagonist in the mammary gland by binding to ERs and inducing a conformational change that block the interaction of ERs with coactivator proteins. However, this molecular mechanism is not compatible with the partial estrogenic activity of tamoxifen in the uterus. Among the theories being investigated is the possible genotoxicity of tamoxifen, but the detection of endometrial

Correspondence:Yongfeng Shang
Tel: 86-10-82805118; Fax: 86-10-82801355
E-mail: yshang@hsc.pku.edu.cn

tamoxifen-DNA adducts in exposed women is still controversial. In contrast, biochemical and animal experiments as well as genetic studies strongly favour an estrogen receptor-dependent model, implicating gene regulation as the mechanism of tamoxifen action in the uterus [3]. In 2002, we showed that in endometrial cells, tamoxifen-liganded ERα is able to recruit coactivator proteins and to initiate gene transcription, and that differential recruitment of a coactivator contributes to the tissue specificity of the function of tamoxifen-liganded ERα [4]. Thus, there is a strong possibility that, mechanistically, endometrial carcinogenesis proceeds from alterations in gene expression due to tamoxifen-activated gene transcription.

Based on this hypothesis and by applying genomic approaches and using immunomagnetically-purified endometrial cells from Type I endometrial carcinomas, we recently have demonstrated that tamoxifen regulates gene transcription in endometrial carcinoma cells, and that genes targeted by tamoxifen are largely different from those targeted by estrogen [5]. Our experiments suggest that gene transcriptional regulation could dictate tamoxifen's role in endometrial carcinogenesis. Our observations also indicate that tamoxifen is a compound with distinct genomic activity rather than simply a partial ER agonist as traditionally described.

The observation that tamoxifen target genes are largely different from estrogen target genes is intriguing. After all, both oestrogen and tamoxifen are believed to bind the same ERs. However, it is well documented that different ligands bind to different ER subtypes with different affinities; this could result in differential gene regulation. Secondly, previous studies by us as well as by others have demonstrated that the transactivation activity of tamoxifen-bound ERs is promoter context-dependent. It is known that ERs can target gene promoters harbouring at either a classical oestrogen response element (ERE), a half ERE site, or sites canonical to other transcription factors such as AP-1, Sp-1, and NF-κB. It is reasonable to speculate that estrogen-liganded ERs and tamoxifen-liganded ERs, due to their different conformations and different cofactor associations, possess different affinities for different gene promoters. As a result, estrogen and tamoxifen could regulate different sets of genes. Some of the gene promoters could accommodate both estrogen-liganded ERs and tamoxifen-liganded ERs. In this situation, the gene would be a target for both oestrogen and tamoxifen. Finally, it is well documented that the transactivation function of tamoxifen-liganded ERs largely resides in the activation function 1 (AF-1) in the N-terminus of the ERs as compared to that of estrogen-liganded ERs, which is more dependent on AF-2 in the C-terminus. Differential association of cofactor proteins by AF-1 versus AF-2 could also influence the affinity of oestrogen-liganded ERs and tamoxifen-liganded ERs toward different gene promoters.

In our study, we found that *PAX2* was activated by estrogen and tamoxifen in endometrial cancer-derived cells and endometrial cancer cell lines but not in the normal endometrium [5]. Both gain of function and loss of function experiments demonstrated that PAX2 was able to promote the growth of endometrial cancer cells and endometrial carcinogenesis. In addition, our experiments showed the co-expression of PAX2 and ERα in endometrial cancer samples and demonstrated that the transactivation of PAX2 expression in cancerous endometrial cells but not in normal endometrial cells. Collectively, all these evidence strongly indicates that PAX2 is a molecular effector for oestrogen and tamoxifen in endometrial carcinogenesis.

PAX genes are developmentally regulated and are silenced in adulthood. Our observation that PAX2 expression was not regulated by estrogen and tamoxifen in normal endometrium is consistent with this idea. Silencing of gene expression is often accompanied by epigenetic modifications including DNA methylation. Although hypomethylation was the originally characterized epigenetic alteration in cancer [6], it was under-appreciated for many years over hypermethylation. Recently, however, gene re-activation by cancer-linked hypomethylation has been re-discovered [7]. In our study, we found hypomethylation of the *PAX2* promoter in endometrial carcinomas but hypermethylation of the promoter in normal endometrium, suggesting that the activation of PAX2 expression in endometrial carcinoma cells is associated with *PAX2* promoter hypomethylation in these cells and indicating that *PAX2* is a cancer-linked hypomethylated gene [5].

It will be interesting to investigate in future studies the mechanism involved in the loss of PAX2 methylation mark in endometrial carcinomas in the future study. Perhaps more relevant to our findings, despite its essential role in development and diseases, exactly how PAX2 functions to promote cell proliferation is not known and there are few genes known to be directly regulated by PAX2. For example, early in the metanephric mesenchyme, PAX2 can transactivate glial-derived neurotrophic factor and WT1 genes [8]; and in embryonic kidney cells, it was shown that the secreted frizzled-related protein 2 (SFRP2) gene was a direct target of PAX2 [9]. Future studies are warranted to delineate the mechanism for PAX2's involvement in development and carcinogenesis.

Acknowledgments

The work in the author's lab was supported by grants (30621002, 30393110, and 30470912) from National Natural Science Foundation of China and grants [Na-

tional Basic Research Program of China (973 Program): 2005CB522404 and Hi-Tech Research and Development Program (863 Program): 2006AA02Z466] from the Ministry of Science and Technology of China.

References

1 Shapiro S, Kaufman DW, Slone D, *et al*. Recent and past use of conjugated estrogens in relation to adenocarcinoma of the endometrium. N Engl J Med 1980; **303**:485-489.
2 Fisher B, Costantino JP, Wickerham DL, *et al*. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst 1998; **90**:1371-1388.
3 Shang Y. Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. Nat Rev Cancer 2006; **6**:360-368.
4 Shang Y, Brown M. Molecular determinants for the tissue specificity of SERMs. Science 2002; **295**:2465-2468.
5 Wu H, Chen Y, Liang J, *et al*. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. Nature 2005; **438**:981-987.
6 Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 1983; **301**:89-92.
7 Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer 2004; **4**:143-153.
8 Dressler GR. The cellular basis of kidney development. Annu Rev Cell Dev Biol 2006; **22**:509-529.
9 Brophy PD, Lang KM, Dressler GR. The secreted frizzled related protein 2 (SFRP2) gene is a target of the Pax2 transcription factor. J Biol Chem 2003; **278**:52401-52405.