Effects of neonatal diethylstilbestrol exposure on c-fos and c-jun protooncogene expression in the mouse uterus

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Summary. Quantitative and cell-type-specific expression of c-fos and c-jun genes after 17β-estradiol (E2) stimulation, was investigated in the uteri of neonatally diethylstilbestrol (DES)-exposed and ovariectomized adult mice (neoDES-mice), employing Northern blot analysis, immunohistochemistry and in situ hybridization. The c-fos mRNA level before E2 injection (at baseline) was about 2.2-fold higher in neoDES-mice than in vehicle-treated control mice. In controls, E2 treatment transiently increased c-fos mRNA levels, showing a peak value (15.8-fold relative to the baseline) after 2 hours. In neoDES-mice, c-fos mRNA level reached a peak showing a 2.1-fold increase compared with its baseline value 1 hour after E2 injection. Immunohistochemistry and in situ hybridization revealed that c-fos protein (Fos) and mRNA are induced in the epithelium and vascular endothelium in both groups. Most uterine epithelia of neoDES-mice revealed low sensitivity to the c-fos expression after E2 administration compared with those of vehicle-treated controls, whereas few epithelia showed high c-fos mRNA expression even at baseline. The c-jun mRNA concentration in the neoDES-mice uteri at baseline was 70% of that in vehicle-treated controls. At 1 hour after E2 injection, c-jun mRNA levels increased 1.8-fold in controls and 1.3-fold in the neoDES-mice relative to each baseline value. There were no significant differences in the distribution pattern of c-jun protein (Jun) and mRNA in the uteri of either groups; E2 stimulated c-jun mRNA expression in the stromal and myometrial cells but suppressed it in the epithelial cells, whereas intensity of c-jun immunostaining increased in the three cell types. The permanent changes in the expression of estrogen-regulated protooncogenes, c-fos and c-jun genes, by neonatal DES exposure may be responsible for the wide range of abnormalities in the genital tract of mature animals.

Key words: c-Fos, c-Jun, Diethylstilbestrol, Uterus, Mouse

Introduction

It is well known that estrogen administration to prenatal or neonatal animals elicits a wide range of irreversible abnormalities including carcinogenesis in the reproductive organs (Forsberg, 1969; Bern et al., 1975; McLachlan et al., 1975). Takasugi (1963) demonstrated that ovary-independent proliferation and cornification of vaginal epithelium developed in adult mice exposed perinatally to 17β-estradiol (E2). Newbold et al. (1990) reported that uterine adenocarcinoma in mice exposed neonatally to artificial estrogen, diethylstilbestrol (DES), for 5 days occurred in a time- and dose-related manner, and that these DES-induced uterine tumors depended on postpubertally secreted estrogen.

Estrogen stimulates cell proliferation and differentiation in the female reproductive organs. Hormone-occupied estrogen receptors (ERs) bind to the estrogen responsive elements of target genes and modulate the transcription of these genes (Klein-Hitpass et al., 1986; Kumar and Chambon, 1988; Lannigan and Notidis, 1989). ERs also mediate gene transcription from an AP-1 enhancer element that requires ligand and AP-1 transcription factors, dimer of Fos and Jun family proteins, for the transcriptional activation (Gaub et al., 1990; Umayahara et al., 1994; Paech et al., 1997). These two regulatory pathways are thought to be interactive through a signal transducing pathway which is mediated by cell membrane with growth factors such as epidermal growth factor (EGF) and insulin-like growth factor (van der Burg et al., 1990; Katzenellenbogen, 1996).

The expression pattern of Fos and Jun, major components of AP-1 complexes, might play an important role in epithelial proliferation, not only in the uteri of normal mice but also in neonatally estrogen-exposed mice, and might participate in the development of various abnormalities in the uteri of neonatally DES-
exposed mice (Persico et al., 1990; Kirkland et al., 1992; Webb et al., 1993; Kamiya et al., 1996; Yamashita et al., 1996). Recent studies have demonstrated that both c-fos and c-jun protooncogene expression was under direct control of estrogen (Loose-Mitchell et al., 1988; Weisz and Bresciani, 1988). In the uteri of ovariectomized rodents, estrogen transiently activated c-fos expression in the epithelial cells, and stimulated c-jun expression in stromal and myometrial cells but suppressed c-jun expression in epithelial cells (Bigsby and Li, 1994; Nephew et al., 1994; Yamashita et al., 1996).

However, a few studies have been carried out to demonstrate the expression of c-fos and c-jun protooncogenes in the reproductive organs of perinatally estrogenized animals. Kamiya et al. (1996), using Northern blot analysis, reported that expression of c-fos and c-jun mRNAs was greater (6- and 3-fold) in the uteri of neonatally DES-exposed 50-day-old ovariectomized mice. They also showed that both c-fos and c-jun mRNA expression were stimulated transiently in the uteri of DES-unexposed control mice after single estrogen injection, whereas c-fos and c-jun expression were not changed in the uteri of neonatally DES-exposed mice. By employing immunohistochemistry, Falck and Forsberg (1996) showed that Fos was more highly expressed in the uterine epithelium and stromal cells of 8-week-old mice which were treated with DES neonatally and ovariectomized at the age of 6 weeks, than in those cells of controls.

In this study, we tried to verify the quantitative and cell-type-specific expression of c-fos and c-jun protooncogenes, and to analyze the temporal changes of expression of these genes in the uteri of neonatally DES-treated or untreated 12-week-old mice which were ovariectomized at the age of 10 weeks by employing Northern blot analysis, immunohistochemistry and in situ hybridization.

Materials and methods

Animals and tissue collection

Pregnant CD-1 mice were obtained from Clea Japan Inc. (Tokyo, Japan). Mice were housed at 21 to 22 °C with a 12-hour alternating light-dark cycle at Keio University Animal Facility (Tokyo, Japan). Neonatal mice were given subcutaneous injections of 4 μg of DES (Sigma Chemical Co., St. Louis, MO) dissolved in 0.02 ml sesame oil (neoDES-mice) or vehicle alone for 5 days, starting within 24 hours after birth. The female mice were ovariectomized at the age of 10 weeks. At 2 weeks after ovariectomy, the animals received an intraperitoneal injection of 0.1 ml of 17β-estradiol (E2, 20 μg/kg bw; Sigma) and were killed at 0 (without treatment, baseline), 1, 2, and 3 hours after the injection. For immunohistochemical analysis, small pieces of uterine horn were mounted in OCT compound and frozen in dry-ice-cooled acetone. The tissue blocks for the in situ hybridization were frozen rapidly in isopentane quenched with liquid nitrogen and subsequently freeze-substitution fixed in acetone containing formalin and then embedded in paraffin as previously described (Yamashita and Yasuda, 1992; Yamashita et al., 1996). Uteri collected from 6-7 animals were combined for each time point and processed for immunohistochemistry and in situ hybridization histochemistry. For Northern blot analysis, uteri from 6-8 mice were pooled, cut into small pieces and directly frozen in the liquid nitrogen and stored until isolation of RNA.

Immunohistochemistry

Immunohistochemistry of ERs, and Fos and Jun has been previously reported (respectively: Yamashita and Korach, 1989; Yamashita et al., 1996). Frozen sections, 6-μm thick, were fixed with Zamboni’s fixatives for 30 minutes at room temperature. After incubation with 0.2% glycine in phosphate-buffered saline (PBS), and successively with 10% normal goat serum and 1% bovine serum albumin in PBS, the sections were incubated with anti-Fos rabbit antibody (Oncogene Sciences Inc., MA) or anti-Jun rabbit antibody (Oncogene Sciences), each a 50-fold dilution with the blocking solution, or anti-ER monoclonal rat antibody (Abbott Laboratories, Abbott Park, IL) overnight at 4 °C. They were then treated with horseradish peroxidase-linked F(ab')2 fragments (Amersham Japan, Tokyo, Japan), 100-fold dilution, for 2 hours at room temperature. The peroxidase activity was detected with 3,3'-diaminobenzidine solution containing nickel and cobalt ions (Adams, 1981). For controls, normal rabbit serum or antibodies absorbed with the respective peptides antigens were used as the primary antibodies; S-14-C or T-15-D (Oncogene Sciences) were incubated with anti-Fos or anti-Jun antibodies (Yamashita et al., 1996). Normal rat IgG was used as the primary antibody for the control immunostaining of ER.

In situ hybridization

Cloning of murine cDNA fragments of c-fos and c-jun has been previously described (Yamashita et al., 1996). The cDNA fragments of c-fos (195 bp) and c-jun (198 bp) were inserted at the Hinc II site of pSPT18 (Boehringer Mannheim, Mannheim, Germany). Digoxigenin (Dig)-labeled RNA antisense and sense probes were prepared employing a Dig-RNA mixture (Boehringer Mannheim) and an AmpliScribe Transcription Kit (Epitectre Technol., Badison, WI). Antisense probes of c-fos and c-jun were synthesized by T7 RNA polymerase using Hind III-digested plasmids as templates, and sense probes were transcribed by SP6 RNA polymerase using Xba I-digested plasmids.

We have previously reported the procedures of the in situ hybridization in detail (Yamashita et al., 1996). After deparaffinization, the sections were treated with 0.2 N HCl, and with protease K (Sigma, P-2308), and
finally fixed with a 4% paraformaldehyde solution. The sections were incubated with Dig-labeled antisense or sense RNA probes at 45 °C overnight. After RNase A (Sigma, R-5250) treatment and washing, the sections were incubated with alkaline phosphatase-labeled anti-Dig Fab fragment (Boehringer Mannheim). The alkaline phosphatase enzyme activity was visualized with 5-bromo-4-chloro-3-indoxyl phosphate and nitroblue tetrazolium.

Total RNA isolation

Total RNA was isolated from the uterine horn. The frozen uteri were homogenized in 2.5 ml of ice-cold RNAzol B (Tel-Test, Inc., Friendswood, TX) using a glass-teflon homogenizer. Total RNA was precipitated by addition of isopropanol, washed with 75% ethanol and finally dissolved in 1 mM EDTA. The concentration of total RNA was determined by measuring the optical density at 260 and 280 nm.

Northern blot analysis

Each sample, 5 μg of total RNA in the buffer containing 45% formamide, 6% formaldehyde and 0.25 mg/ml ethyldium bromide, was separated on a formaldehyde -1% agarose gel and then transferred to nylon membrane by capillary blotting in 10 x SSC buffer. The blotted RNA was cross-linked under UV irradiation. The membrane was incubated with prehybridization solution mixture and single strand DNA for 4 hours at 42 °C, then hybridized with 32P-labeled DNA probes overnight at 42 °C. After hybridization, the membranes were washed with 1% SDS in 2 x SSC twice for 30 minutes each at 65 °C and with 0.1% SDS in 0.1 x SSC twice for 30 minutes each at 65 °C, and they were then exposed to Bas 2000 imaging plates and scanned using a Bas 2000 image analyzer (Fuji Photo Film Co., Tokyo, Japan).

The c-fos and c-jun inserts were PCR-amplified and 32P-labeled by random priming using a Random Primer Extension Labeling System (NEN Life Science Products Inc., Boston, MA) and [32P]-CTP (Amersham Japan).

Results

Morphology

In control 12-week-old mice that were ovariectomized at 10 weeks of age, the uterine luminal epithelia were lined with simple low-columnar epithelium, and glandular epithelia with the narrow uterine lumens were lined with cuboidal cells. In 12-week-old neonatally DES-exposed mice which were ovariectomized at 10 weeks of age (neoDES-mice), endometrial epithelium was composed of simple columnar epithelium but pseudostratified columnar epithelium was also present in some regions. No squamous metaplasia was noted and the lumens of uterine glands were enlarged compared with those of vehicle-treated control mice (Fig. 4C-E). Apoptotic epithelial cells and clusters of epithelial cells being sloughed into the uterine lumens were observed more frequently in the neoDES-mice than in controls. Also, the myometrium was thin in the neonatally estrogenized mice. In particular, the inner circular layers were disordered showing sparse cell density (Fig. 5D), and uterine glands sometimes penetrated into the inner muscular layers.

Localization of ERs

ERs were localized in the nuclei of all major uterine cell-types, i.e., epithelial cells, stromal cells and muscular cells in the vehicle-treated control and neoDES-mice (Fig. 1). In the control mice uteri, glandular epithelium showed stronger ER-immuno-staining than luminal epithelium. In neonatally
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Estrogenized mice, intensity of ER-immunostaining in the stromal and smooth muscle cells was almost the same as that in the controls, although uterine epithelial cells seemed to show the same or slightly weaker reaction than those of control mice.

**Northern blot analysis of protooncogenes**

a) c-fos mRNA

Total RNA was extracted from the uteri of control and neoDES-mice which were sacrificed either before (0 hour, baseline) or at 1, 2, and 3 hours after E2 administration. All sample preparations showed single 2.2 kb transcript on Northern blot analysis (Fig. 2A). Very low levels of c-fos mRNA were present in the uterine tissues of vehicle-treated control mice at baseline. A peak expression of c-fos mRNA, 15.8-fold relative to the baseline, was seen at 2 hours after E2 injection, when the signals were analyzed on the basis of total RNA concentration (Fig. 2B). When the amount of c-fos transcripts was normalized on the basis of the β-actin mRNA level in the same blot, the peak of c-fos mRNA expression was at 1 hour after E2 injection; 11.2-fold more than the E2-unstimulated animals. In the uteri of neoDES-mice, the c-fos mRNA level was 2.2-fold higher than that of vehicle-treated control mice at baseline (Fig. 2B). E2 treatment also stimulated c-fos mRNA expression in neonatally estrogenized mice uteri, however, the inductive effect was low compared with controls. At 1 hour after E2 injection, the c-fos mRNA levels reached a peak showing a 2.1-fold increase compared with the value at baseline, even though total RNA or β-actin mRNA concentrations were used for the normalization (Fig. 2B). The c-fos mRNA concentration was about 3-fold higher in the vehicle-treated control animals than in the neoDES-mice at 2 and 3 hours after E2 administration.

b) c-jun mRNA

All specimens displayed 2.7 kb major bands and 3.2 kb minor bands in Northern blotting (Fig. 2A); presence of these two forms of c-jun transcripts in rodents has been reported by many investigators (Persico et al., 1990; Webb et al., 1993; Biggs and Li, 1994). Since it appeared that the ratio of the two bands was almost constant, we analyzed 2.7 kb major bands. The c-jun transcript levels in the uteri of vehicle-treated controls, rapidly increased showing a peak at 1 hour after E2 treatment and returned to the baseline level at 3 hours; c-
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**jun** mRNA peak level was 1.8-fold higher compared to the baseline value (Fig. 2C). When c-jun mRNA concentration was normalized with β-actin transcript levels, it was about 65% and 30% of the baseline value at 2 and 3 hours after E2 injection, respectively. The uterine c-jun mRNA level in the neoDES-mice was 70% of that in vehicle-treated control mice before E2 injection. E2 injection also elicited an increase in c-jun mRNA levels, although the increase was smaller than that of controls. At the 1 hour peak after E2 injection, c-jun mRNA concentration was 1.3-fold higher than compared with that at baseline, when it was normalized on the basis of total RNA or β-actin transcript levels. The concentration then decreased rapidly at 3 hours.

**Expression of c-fos protooncogene**

**a) Immunohistochemistry**

In the control and neoDES-mice, single E2 injection induced c-fos protein in endometrial epithelium and vascular endothelium, but not in stromal and myometrial cells; however, the induction was weak in the neoDES-mice compared to controls. Before E2 injection, very faint immunoreaction was detectable in the nuclei of uterine epithelial cells in vehicle-trated controls (Fig. 3A), and some epithelial cells showed a weak Fos-immunostaining in the neoDES-mice (Fig. 3C). The nuclear Fos-immunostaining gradually increased and peaked at 2 to 3 hours after E2 administration; Fos-immunoreaction was strong in the luminal epithelium and weak to moderate in the glandular epithelium (Fig. 3B). In the uteri of neoDES mice, Fos-immunostaining in the luminal epithelium was slightly stronger than that in the glandular epithelium after 2 or 3 hours. The Fos-immunoreaction was heterogeneous among the epithelial cells (Fig. 3D), whereas it was almost homogeneous in the endometrial epithelium of control animals. No apparent differences in the Fos-immunostaining were observed between pseudostratified epithelium and simple columnar epithelium in the neoDES-mice uteri. In both neonatally vehicle treated-control and estrogenized-mice, vascular endothelium expressed Fos-immunoreaction in the nucleus at 2 hours after E2 injection.

![Fig. 3. Immunohistochemistry of Fos in the uterus of mouse. Fos is localized in the uteri of control (A and B) and neoDES-mice (C and D). Animals sacrificed before E2 injection (A and C) and 2 hours after injection (B and D). Arrows indicate Fos-immunoreaction in vascular endothelium. x 300.](image-url)
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E2 treatment induced c-fos mRNA rapidly and transiently in the uterine epithelia and vascular endothelia of vehicle-treated control and neoDES-mice (Fig. 3). Expression of c-fos transcript was not evident in any of the uterine cell types of control mice before E2 stimulation (Fig. 4A) but it reached a maximum in the epithelial cells at 2 hours (Fig. 4B) and in the endothelial cells at 1 hour after E2 injection, respectively. In the neoDES-mice at baseline, most uterine epithelia showed negative or very faint c-fos mRNA signals (Fig. 4C), while some epithelia displayed strong c-fos mRNA expression (Fig. 4D). Endometrial epithelium of neoDES-mice showed either the same or slightly stronger c-fos mRNA signals at 1 hour after E2 administration and weaker signals at 2 hours than those of vehicle-treated control animals. Glandular epithelium showed a weaker reaction than luminal epithelium in the uteri of both groups. Faint c-fos hybridization signals were present in the epithelium of both control and neoDES-mice 3 hours after E2 injection.

Expression of c-jun protooncogene

E2 elicited differential c-jun expression responses in all uterine cell types: Jun-immunoreaction was localized in the nuclei of uterine cells (Fig. 5). There were no significant differences in the expression pattern of Jun between control and neoDES-mice. Epithelial cells displayed weak to moderate Jun-immunoreaction at baseline (Fig. 5A, C). The intensity of Jun-immunostaining increased in the epithelial, stromal and myometrial cells from 2 to 3 hours after E2 treatment. Weak Jun-immunostaining was present in the endothelial cells of blood vessels at 2 hours.

b) In situ hybridization

Uteri of control and neoDES-mice provided almost the same c-jun mRNA expression pattern after E2 administration. Strong c-jun mRNA signals were present

![Image of in situ localization of c-fos mRNAs in the mice uteri. Uteri of control (A and B) and neoDES- mice (C-E). The c-fos mRNAs are detected in the uterine sections before E2 injection (A, C and D) and at 2 hours after E2 injection (B and E), by employing in situ hybridization histochemistry with digoxigenin (Dig)-labeled RNA probes. x 250](image)
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in the luminal epithelium and a weak reaction was seen in the glandular epithelium in the uteri of vehicle-treated control and neoDES-mice before E2 injection (Fig. 6A,B). The amount of c-jun transcripts in the epithelial cells decreased at 1, 2 and 3 hours after E2 treatment. No detectable c-jun mRNA was seen in the stromal or muscular cells at baseline and the mRNA was transiently induced and peaked at 1 to 2 hours after E2 injection (Figs. 6C,D). Vascular endothelium displayed c-jun mRNA signals at 1 hour.

Discussion

We investigated temporal and quantitative expression of c-fos and c-jun protooncogenes by E2 stimulation in the uteri of neoDES-mice. In Northern blot analysis, the c-fos mRNA concentration reached a peak at 2 hours after E2 injection in the uteri of vehicle-treated control mice, when the mRNA levels were compared with the total RNA concentration of each sample preparation (Fig. 2). However, c-fos mRNA expression peaked at 1 hour in neoDES-mice, when normalized on the basis of the β-actin mRNA levels; a similar result was shown when compared with rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels (data not shown). Results of in situ hybridization revealed that the strongest c-fos mRNA signals were in the uterine epithelia at 2 hours after E2 administration. This finding agreed with our previous data obtained in adult mice, which were fed without neonatal DES-exposure and were ovariectomized at 12 week of age (Yamashita et al., 1996). Postpubertal estrogen treatment also stimulated the expression of β-actin and GAPDH mRNAs in the mouse uterus. Therefore, the use of total mRNA levels seems to be reliable for the normalization of not only c-fos but also c-jun mRNA concentrations in the uteri of E2-stimulated mice by the Northern blot analysis.

The apoptotic epithelial cells and clusters of swollen epithelial cells being sloughed into the uterine lumens were observed more frequently in the uteri of neoDES-mice than in controls. Mori (1975) and Iguchi et al. (1985) reported that steady-state mitotic rates are higher in the uterine epithelia of ovariectomized adult mice that were exposed to DES neonatally than in those of vehicle-treated controls. These results indicate that the uterine epithelia of neonatally-estrogenized mice turn

![Fig. 5. Immunohistochemistry of Jun in control (A and B) and neoDES-mice (C and D). Localization of Jun is demonstrated in the uterine sections before (A and C) and 2 hours after E2 injection (B and D). m: myometrium. x 300.](image-url)
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over more rapidly compared with control animals even in the absence of estrogen stimulation. Hendry III et al. (1997) revealed that hyperplastic epithelium is also an active site of cell death by apoptosis in the uteri of neonatally DES-exposed and postpubertally estrogen-stimulated hamsters. The present data from Northern blot analysis revealed that the baseline level of c-fos mRNA was higher and c-jun mRNA was lower in the uteri of neoDES-mice than in those of vehicle-treated controls. Therefore, the persistent high c-fos and low c-jun mRNA levels might be responsible for the high-mitotic rates in the uterine epithelia of neonatally-estrogenized mice.

Kamiya et al. (1996), employing Northern blot analysis, showed that the steady-state concentrations of both c-fos and c-jun mRNA were higher in the neonatally DES-exposed mice than in the vehicle-treated control animals. The reason for the inconsistency concerning the c-jun mRNA expression is unclear, and may result from the differences in the strain and age of mice. In the present study, nuclear Jun-immunostaining increased in the uterine epithelium of both control and neoDES-mice after E2 injection but c-jun mRNA levels decreased in these cells. The reason for the difference in the results by immunohistochemistry and in situ hybridization may be due to that accessibility of antibody to Jun in the nuclei became easy because of a change of nuclear structure after E2 injection, or that translation of c-jun mRNA may be activated in the E2-stimulated epithelial cells.

The c-fos gene expression appeared heterogeneous in the uterine epithelium of neoDES-mice. The c-fos mRNA and protein were synthesized more rapidly in some populations of the uterine epithelia in the neoDES-mice than in the vehicle-treated controls. Some epithelium showed strong and others exhibited faint immunostaining and in situ hybridization signals at 2 hours after E2 injection, while the epithelium of control animals showed almost homogeneous c-fos expression (Figs. 3, 4). In addition, few epithelial cells displayed high c-fos mRNA expression in the uteri of neonatally-estrogenized mice without E2 injection (Fig. 4D). These results suggest that the uterine epithelia of neonatally DES-treated mice are composed of a heterogeneous

Fig. 6. In situ localization of c-jun mRNAs in the mice uteri in control (A and B) and neoDES-mice (C and D). Expression of c-jun mRNAs is seen in the uteri before (A and C) and 2 hours after E2 injection (B and D), by employing in situ hybridization with Dig-labeled RNA probes. m: myometrium. x 250
population in relation to their responses to postpubertal estrogen stimulation. Mori (1975) pointed out that uterine epithelia of ovariectomized neonatally DES-exposed mice consisted of a mixed population of cells; total mitotic rate of uterine epithelia in the neonatally DES-exposed mice was about 30% of that for control mice after a single injection of E2, but the epithelia rapidly entered the mitotic stage.

Nelson et al. (1994) demonstrated that neonatal DES exposure results in the persistent ovary-independent activation of estrogen-regulated genes, such as lactoferrin and EGF genes in the mouse uterus and vagina. Iguchi et al. (1993) showed that EGF receptor levels in neonatally DES-treated mice were almost the same in the uterus and oviduct but significantly lower in the vagina compared with those of control animals. The present study also revealed that the baseline levels of estrogen-regulated protooncogenes alter in the uteri of neoDES-mice. One of the mechanisms of alteration of estrogen-regulated gene expression may be based on the demethylation or methylation of DNA in the genital tract expression patterns of estrogen-regulated nuclear receptors.

Erk et al. (1997) demonstrated that c-fos is expressed primarily in epithelial cells of prostatic urethra and ducts, which are sites that show the most pronounced morphological changes in neoDES-mice. Long-term expression of Fos was shown to cause loss of epithelial polarity irreversibly and trigger epithelial-fibroblastoid cell conversion (Reichmann et al., 1992). A function of Jun in the epithelium appears to modulate epithelial polarity and tissue organization; expression of Jun elicits a loss of mammary epithelial polarity involving a destabilization of adherens junctions (Fialka et al., 1996). The persistent changes in the expression of estrogen-regulated genes such as c-fos, c-jun and EGF which regulate the cell differentiation and proliferation, should be responsible for the wide ranges of abnormalities in the genital tract of perinatally-estrogenized animals.

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References


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