0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 1
Printed in U.S.A.

Tamoxifen-Induced Proto-oncogene Expression Persists in Uterine Endometrial Epithelium*

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ABSTRACT

The use of the antiestrogen tamoxifen for breast cancer management, although generally well tolerated, is linked to an increase in uterine pathologies in a high number of postmenopausal women receiving the drug. This effect is thought to be due to estrogenic stimulation of the uterine endometrium by the antiestrogen; however, the molecular mechanism underlying the uterotrophic activity of tamoxifen and the uterine cellular compartments that respond to the drug have not been clearly established. In this study, we determined which of the several uterine tissues (myometrium, stroma, and luminal and glandular epithelium) demonstrated chronic overexpression of c-*fos* and the *jun* proto-oncogenes in response to tamoxifen. Uteri from tamoxifen-treated, castrated rats were examined histologically, and cell type-specific expression of c-*fos*, c-*jun*, *jun*-B, and *jun*-D was assessed using *in situ* hybridization. Treatment with tamoxifen resulted in uterine luminal and glandular epithelial hypertrophy and basally located nuclei by 36 h. Extreme uterine glandular and luminal epithelial cell hypertrophy persisted 7 days after administration of the drug. Expression of c-*fos* and *jun*-B messenger RNA was first detected in the luminal and glandular epithelial at 12–36 h post tamoxifen injection. Seven days after tamoxifen treatment, c-*fos* and *jun*-B messenger RNA levels were lower but still evident in the uterine endometrial epithelium. Tamoxifen completely repressed constitutive expression of c-*jun* in the uterine luminal epithelial cells by 12 h but, unlike estrogen, did not induce c-*jun* expression in the uterine myometrium. Expression of *jun*-D in the uterine glandular and luminal epithelia was observed at 12 h but not at 24 h post tamoxifen. These results support our working hypothesis that persistent overexpression of cellular oncogenes c-*fos* and *jun*-B in the uterine endometrial epithelium may contribute to the molecular mechanism underlying the uterine toxicity associated with chronic tamoxifen treatment. (*Endocrinology* **137:** 219–224, 1996)

TAMOXIFEN, a nonsteroidal, triphenylethylene derivative, is the most commonly prescribed treatment for breast cancer. Several international Phase III trials are in progress to test the efficacy of tamoxifen as a prophylactic for breast cancer in healthy pre- and postmenopausal women at increased risk for the disease (1). However, the drug does cause side effects, many of which are due presumably to its estrogenic activity (2–5). A central issue regarding the risks and benefits of tamoxifen treatment of healthy women is the increased incidence of endometrial carcinoma in some breast cancer patients receiving tamoxifen therapy. This association has been reported in several clinical trials (reviewed in Refs. 6–8), including the National Surgical Adjuvant Breast and Bowel Project-B14 (9) and Netherlands Cancer Institute (10) trials, which were recently completed. The National Surgical Adjuvant Breast and Bowel Project-B14 trial reported more deaths from endometrial cancer than expected (9). There is the indication that some of the endometrial carcinomas related to tamoxifen treatment might represent the unusually aggressive variant type (11–13). Because most endometrial carcinomas are epithelial in origin (14, 15), an understanding of the effects of tamoxifen on uterine epithelia is of interest and of the utmost relevance to the evaluation of tamoxifen as a chemopreventive regimen for breast cancer. Recently, Friedl and Jordan (8) indicated that the effects of tamoxifen on the human endometrium at the molecular level have not been clearly established. Thus, there is an urgent need to understand the mechanism by which tamoxifen induces changes in uterine compartments.

Tamoxifen stimulates uterine growth and causes histologic changes of the uterus in many species, including humans (7, 8). In fact, a remarkably high number (1/3) of postmenopausal women receiving tamoxifen show estrogenic-like changes in the genital tract (16–18). The uterus is a complex tissue comprised of circular and longitudinal muscle, endometrial stroma, and luminal and glandular epithelium. Of these, the epithelium displays the greatest sensitivity to estrogenic stimulation and potential for transformation to uterine carcinoma by tamoxifen (14, 15). Recently, we (19) and others (20) reported that tamoxifen can augment c-*fos* and *jun* proto-oncogene expression in normal rat uterus. The products of these cellular oncogenes are thought to play important roles in regulating cellular function of many tissues, including uterus (reviewed in Refs. 21 and 22). Members of the Fos and Jun families can dimerize and control transcription of many target genes through the activator protein (AP)-1 elements (23–25).

In this study, we used *in situ* hybridization procedures to establish that tamoxifen induces persistent overexpression of c-*fos* and *jun*-B proto-oncogenes specifically in epithelial cells of the uterine endometrium. Our working hypothesis is that chronic overexpression of these cellular oncogenes could play a role in tamoxifen-induced endometrial carcinoma. These results may have implications about the knowledge of

Received June 12, 1995.
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* This work was supported by NIH Grant HD-22918 (to S.A.K.) and Grant PF-4179 from the American Cancer Society (to K.P.N.).
† National Cancer Institute/National Research Service Award Fellow.
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risk associated with therapeutic and prophylactic use of tamoxifen in the prevention of breast cancer.

Materials and Methods

Animals

Mature (150–200 g) Sprague-Dawley rats (Zivic-Miller, Allison Park, PA) were ovariectomized at least 8 days before use in this study. The rats were given animal chow and water *ad libitum* in animal facilities illuminated between 0500–1900 h. The Department of Laboratory Animal Medicine at the University of Cincinnati maintains American Association for the Accreditation of Laboratory Animal Care accreditation of its animal facilities. All experiments were conducted in accord with NIH standards established by the Guidelines for the Care and Use of Experimental Animals and by the American Veterinary Medical Association. Rats received a sc injection of tamoxifen 1 mg/kg BW (citrate salt; Sigma Chemical Co., St. Louis, MO) in sesame oil or an injection of sesame oil only (control) as we previously described (19). This dose of tamoxifen stimulates rat uterine growth, RNA synthesis, and proto-oncogene expression (19, 20, 26–28). At 0, 3, 6, 12, 24, 36 h, and 7 days after administration of tamoxifen, animals were killed by cervical dislocation.

Synthesis of sense and antisense RNAs

The full-length rat c-*fos* complementary DNA (cDNA) [2.2 kilobases (kb); kindly provided by Dr. T. Curran, Roche Institute of Molecular Biology, Hoffmann-LaRoche, Nutley, NJ], mouse c-*jun* cDNA (1.9 kb; generously provided by Dr. I. Verma, The Salk Institute, La Jolla, CA), mouse *jun*-B cDNA (1.9 kb; American Type Culture Collection, Rockville, MD; clone 465.2), and mouse *jun*-D cDNA (1.7 kb; American Type Culture Collection, no. 63025) were cloned into pBluescript II SK$^+$ (Stratagene, La Jolla, CA) or pGEM (Promega Corp., Madison, WI) transcription vectors. After linearization with the appropriate restriction enzymes, ^{35}S-labeled antisense or control sense RNAs were synthesized using the Promega Riboprobe Gemini Core System II transcription kit, according to the manufacturer's protocol and processed as we previously described (29, 30).

Histology, tissue fixation, and in situ hybridization protocols

Uteri were excised quickly, trimmed of extraneous connective tissue, cryopreserved, and subjected to the *in situ* hybridization procedures we previously described (29, 30). Slides were examined microscopically either under darkfield or brightfield illumination after staining with hematoxylin and eosin. All experiments were repeated three to four times in duplicate. Autoradiography exposure times for sense and antisense sections were identical within a treatment and ranged between 3–5 days for the representative sections illustrated. The 1A RNA probe (sense and antisense) was included in these experiments as a positive control for *in situ* procedures. The 1A messenger RNA (mRNA) is constitutively expressed in rat uterus (31), and our *in situ* localization of 1A demonstrated that it is expressed only in the luminal and glandular epithelial cells of the rat uterus (29). The uterine morphology was further assessed by embedding the tissues in paraffin wax, cutting serial 4-μm transverse sections, mounting on light microscope slides, and staining with hematoxylin and eosin.

Results

Histologic sections from rat uteri at 0, 3, 12, 36 h, and 7 days after tamoxifen administration are shown in Fig. 1. Gradual hypertrophy in uterine luminal and glandular epithelial cells was stimulated by tamoxifen treatment (Fig. 1, D, F, H, and J). By 36 h after tamoxifen administration, marked hypertrophy of glandular and luminal epithelial cells with basally located nuclei was apparent (Fig. 1H). Uterine epithelial cell hypertrophy at 7 days after administration of the drug was extreme (Fig. 1J). Comparisons of the uterine histology after tamoxifen *vs.* 17β-estradiol treatment (3–36 h and 6 days) can be seen in Fig. 1, C, E, G, and I. The marked uterine epithelial cell hypertrophy seen at 7 days after tamoxifen treatment was not apparent at 6 days after estradiol administration (Fig. 1I). These findings are consistent with those of others demonstrating that tamoxifen maintains long-term uterine

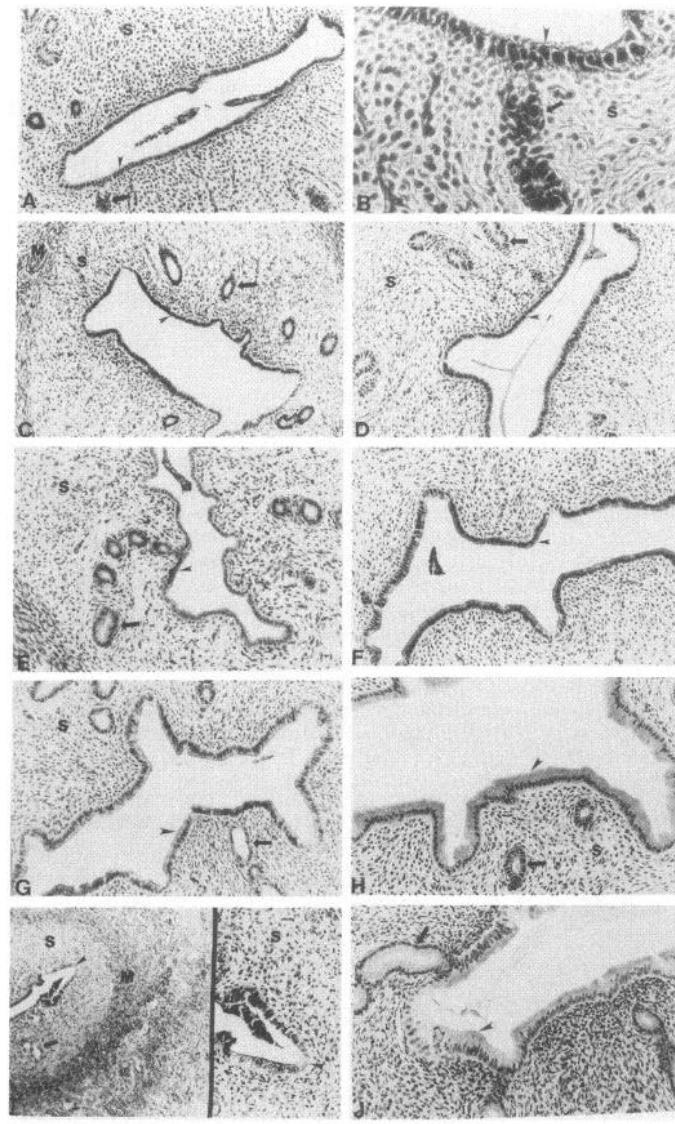

FIG 1. Photomicrographs of transverse sections through the uterus from a mature, ovariectomized rat after treatment *in vivo* with either 1 mg/kg BW tamoxifen for 3 h (D), 12 h (F), 36 h (H), and 7 days (J) or 1 μg of 17β-estradiol for 3 h (C), 12 h (E), 36 h (G), and 6 days (I). Dose of 17β-estradiol used (1 μg/rat) was based on previous studies (19, 22, 30, 41). Beginning 36 h after tamoxifen administration, increase in cell hypertrophy becomes particularly noticeable. Note the difference in appearance of uterine luminal epithelium 6 days after estradiol administration compared with 7 days after tamoxifen treatment (I *vs.* J). Uterine tissues were sectioned at 4 μm, then stained with hematoxylin and eosin. M, Myometrium; S, stroma. *Solid arrows* point to uterine glandular epithelial cells. *Arrowheads* depict uterine luminal epithelial cells. *Open arrow* in *J* points to uterine cells that appear to be apoptotic. Magnification: A and C-J, ~ ×200; B, ~ ×600; I, ×100 (*left*) and ×200 (*right*)

growth and epithelial cell hypertrophy in rodents (2–4, 26–28, 32–40).

In situ hybridization was used to investigate the effect of tamoxifen on uterine cell type-specific pattern of c-*fos*, c-*jun*, *jun*-B, and *jun*-D proto-oncogene expression. Only background levels of sense RNA binding was detected in corresponding uterine sections from control rats or animals treated with tamoxifen (Figs. 2, A and B; Fig. 3A). Uterine sections from adult rats treated with tamoxifen for 0, 3, or 6 h and probed with antisense c-*fos* showed little or no specific binding (Fig. 2). The expression pattern of *jun*-B at 0, 3, and 6 h after tamoxifen was similar to that of c-*fos* (data not shown). However, at 12–36 h after tamoxifen administration, expression of c-*fos* and *jun*-B mRNAs was clearly evident in the epithelial cells of the uterine lumen and glands (Figs. 2, D and E; Fig. 3, B and C). At 7 days post tamoxifen treatment, expression of these proto-oncogenes persisted in uterine luminal and glandular epithelia, but levels appeared to have declined (Fig. 2F and Fig. 3D).

The effect of tamoxifen on the cell type-specific pattern of c-*jun* and *jun*-D mRNA expression is illustrated in Fig. 4. When the c-*jun* antisense probe was used on uterine sections from control rats, silver grains were observed in the luminal epithelial cells and, to a lesser extent, in the uterine stroma

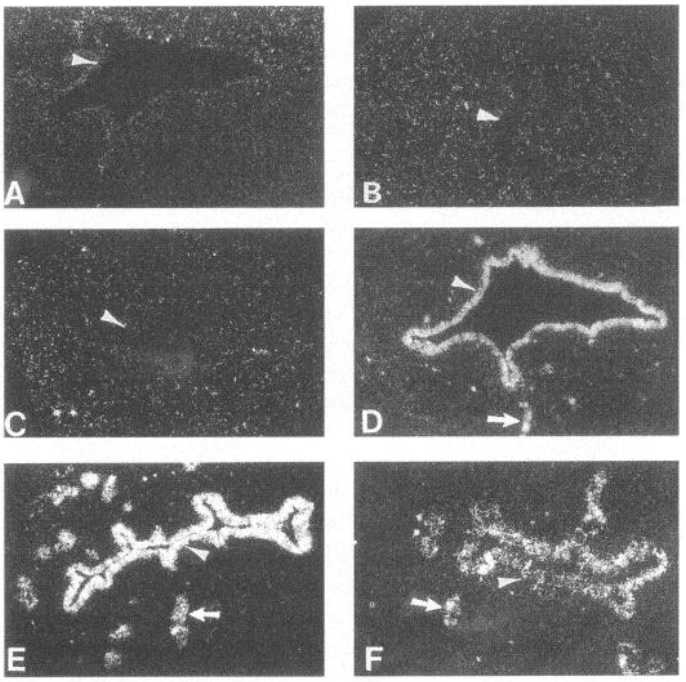

FIG. 2 Expression of c-*fos* mRNA in adult rat uterus. Cryostat sections from untreated control rats (B) or 3–36 h (C-E) and 7 days (F) after tamoxifen administration were hybridized with antisense c-*fos* riboprobe. A, Corresponding adjacent section 12 h post tamoxifen that was hybridized with sense c-*fos* riboprobe. *Arrowheads* depict uterine luminal epithelial cells, and *arrows* point to uterine glands. Magnification: ×100. A, A uterine section 12 h post tamoxifen probed with sense c-*fos*. B, An untreated control uterine section probed with antisense c-*fos*. C, No expression of c-*fos* mRNA is detected at 3 h after tamoxifen administration. D, Expression of c-*fos* in uterine luminal and glandular epithelia is seen 12 h after tamoxifen treatment. E, At 36 h after tamoxifen administration, c-*fos* mRNA is seen in uterine epithelial cells of glands and lumen. F, At 7 days, c-*fos* expression is seen but has declined.

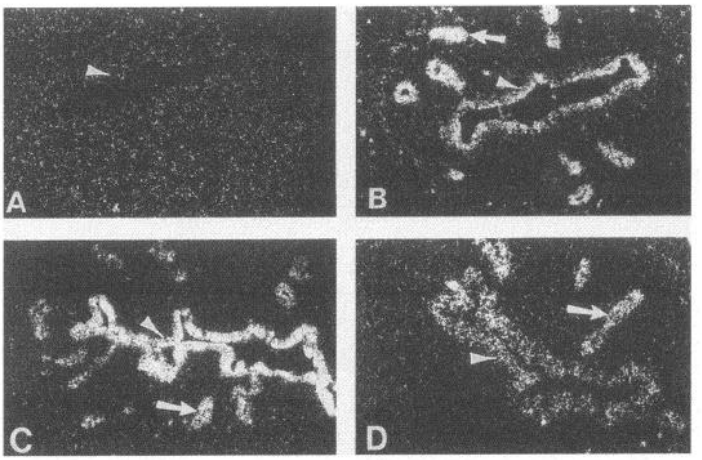

FIG. 3 Expression of *jun*-B mRNA in adult rat uterus. Cryostat sections from rats 12 h (B), 36 h (C), and 7 days (D) after tamoxifen administration were hybridized with antisense *jun*-B riboprobe. A, Corresponding adjacent section 12 h post tamoxifen that was hybridized with sense *jun*-B riboprobe. *Arrowheads* depict uterine luminal epithelial cells and *arrows* point to uterine glands. Magnification: ×100. A, A uterine section 12 h post tamoxifen probed with sense *jun*-B. B, Expression of *jun*-B in uterine luminal and glandular epithelia is seen 12 h after tamoxifen treatment. C, At 36 h after tamoxifen administration, *jun*-B mRNA is seen in uterine epithelial cells of glands and lumen. D, At 7 days, *jun*-B expression is seen but has declined.

and myometrium (Fig. 4A). By 6 h post tamoxifen treatment, silver grains from the uterine luminal epithelium dramatically decreased, and there appeared to be a slight increase in expression of c-*jun* in the uterine stroma and myometrium (Fig. 4B). At 12 h, however, no signal for c-*jun* was apparent in the uterine luminal epithelium, but c-*jun* expression in the uterine stroma and myometrium remained similar to that observed at 6 h post tamoxifen treatment (Fig. 4C). By 7 days after tamoxifen treatment, the c-*jun* signal in the uterine stroma and myometrium remained detectable, and positive silver grains representing c-*jun* reappeared in the uterine luminal epithelial cells (Fig. 4D). The reappearance of c-*jun* in the uterine luminal epithelium is probably due either to metabolism of the drug over time or activity of the tamoxifen metabolites.

No specific signal for *jun*-D transcripts was detected in uterine sections from control rats (Fig. 4E). Silver grains representing *jun*-D were detected in the uterine glandular and luminal epithelial cells at 12 h (Fig. 4F), but by 24 h post treatment and thereafter, no signal for *jun*-D was detected (Fig. 4, G and H). These results are consistent with our Northern blot analysis showing only slight changes in the levels of *jun*-D and c-*jun* mRNA after administration of tamoxifen (19).

Discussion

In the uterus of many species, including the human, tamoxifen displays partial estrogen agonist activity. We now report that tamoxifen-induced overexpression of c-*fos* and *jun*-B cellular oncogenes occurs specifically in the uterine glandular and luminal epithelial cells *in vivo*, and c-*fos* and *jun*-B mRNAs persist for at least 7 days after a single injection

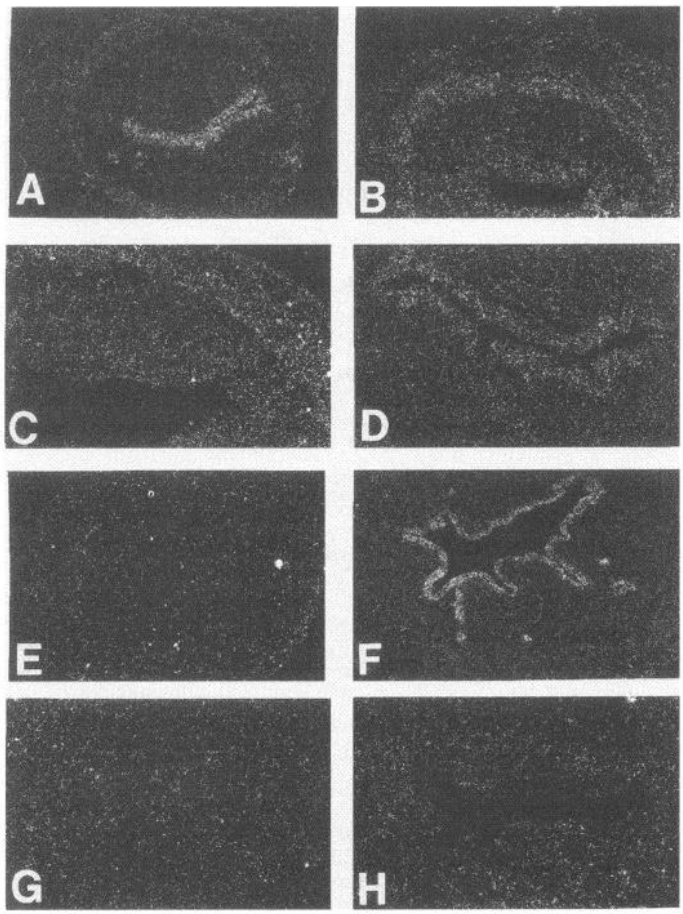

FIG. 4 Expression of c-*jun* and *jun*-D mRNA in adult rat uterus. Cryostat sections from rats at 0 h (A), 6 h (B), 12 h (C), and 7 days (D) after tamoxifen administration were hybridized with antisense c-*jun* riboprobe. E, F, G, and H, Uterine sections at 0 h, 12 h, 24 h, or 7 days after tamoxifen treatment, respectively, that were hybridized with antisense *jun*-D riboprobe. Magnification: A, B, D, E, F, G, and H, ×100; C, ×125. A, A control section probed with antisense c-*jun*. Note constitutive expression of c-*jun* mRNA in uterine luminal epithelium. B, At 6 h after tamoxifen treatment, constitutive expression of c-*jun* in uterine luminal epithelia has declined, and some c-*jun* expression in uterine stroma and myometrium is detected. C, At 12 h after tamoxifen administration, no c-*jun* mRNA is seen in uterine luminal epithelial cells, and c-*jun* expression in uterine stroma and myometrium remains detectable. D, At 7 days, c-*jun* expression in uterine luminal epithelia is again seen but is low. E, A control uterine section probe with antisense *jun*-D. F, Expression of *jun*-D in uterine luminal and glandular epithelia is seen 12 h after tamoxifen treatment. G, By 24 h after tamoxifen, no expression of *jun*-D mRNA is detected. H, Expression of *jun*-D mRNA remains undetectable at 7 days after tamoxifen treatment.

of the drug. Although the cell type-specific pattern of c-*fos* and *jun*-B mRNA expression induced by tamoxifen is similar to that stimulated by estrogen (29, 30), the kinetics of induction contrast markedly to the rapid (within 30 min), and transient (declines after 3 h), and coordinate uterine induction of c-*fos* and all three *jun* genes stimulated by 17β-estradiol (21, 22, 41). Furthermore, we show that, like estradiol (29, 42), the antiestrogen represses the constitutive expression of c-*jun* mRNA in luminal epithelium; however, unlike estradiol (29), tamoxifen does not shift c-*jun* expression to the uterine myometrium. The lack of c-*jun* induction in the myometrium might be because tamoxifen has a preferential effect on the uterine endometrium (35). The observation that both estradiol and tamoxifen suppress the expression of c-*jun* in the epithelial cells, which normally undergo estrogen-initiated mitosis, is intriguing. Does c-*jun* act as a negative regulator of cell division in these cells? It is also intriguing that the kinetic pattern of *jun*-D expression induced by tamoxifen is not similar to that of c-*fos* or *jun*-B, particularly because c-*fos* and the *jun* genes all display similar kinetics in response to estradiol (21, 22, 41). One interpretation of our data could be that expression of *jun*-D and c-*jun* in uterine epithelia is down-regulated by *jun*-B, c-*fos*, or possibly a combination of both. Other possibilities, however, may exist. Ramkumar and Adler (43) used an *in vitro* expression system and reporter gene constructs to document that tamoxifen exerts differential transcriptional regulation, suggesting gene-specific effects in response to the antiestrogen. Recent studies demonstrated that tamoxifen specifically targets AP-1 sites for transcriptional activation of promoters *in vitro*, and that AP-1 proteins, such as Fos and Jun, are needed for tamoxifen stimulation in uterine cell lines (44). Those studies support our *in vivo* observations and the suggestion that Fos and Jun play a significant role in tamoxifen-stimulated uterine growth. Furthermore, Webb *et al.* (44) provided evidence for the existence of a novel pathway for the action of tamoxifen, *i.e.* the tamoxifen-liganded estrogen receptor (ER) specifically targets the AP-1 site and not the estrogen response element. Regulation of *fos* and *jun* genes in the uterus by estrogen appears to be through the interaction of functional estrogen response elements with ER (45–48). However, there may be other mechanisms for tamoxifen regulation of these genes. Interestingly, the number of potential AP-1 elements in the 5′-untranslated regions of c-*fos* and *jun* gene promoters varies; thus, the possible existence of an AP-1 pathway for the tamoxifen-activated ER (44) may offer insight into differential activation of these genes by the antiestrogen.

Our observations are in agreement with previous reports in the rat uterus that show that tamoxifen causes a marked increase in the height of the luminal and glandular epithelial cells (32, 35, 37–40), and we further show that tamoxifen-induced histologic changes persist even at 7 days after administration of one injection of the antiestrogen. It is interesting that the extreme hypertrophy of the uterine epithelia is not associated with similar increases in hyperplasia (32, 34, 35, 40), suggesting that tamoxifen activates some but not all of the genes required to achieve the proliferative response in the majority of uterine cells. Our observation of persistent expression of proto-oncogenes, which are typically expressed during the G_0-G_1 transition, may suggest that tamoxifen causes uterine endometrial epithelial cells to increase the duration of the G_1 phase of the cell cycle, or perhaps undergo a temporary G_1 block, resulting in massive hypertrophy. Unfortunately, the effect of tamoxifen on uterine expression of other cell cycle factors and growth regulators is not well established. The antiestrogen modulates uterine expression of at least two peptide growth factors, insulin-like growth factor I (IGF-I) (49) and transforming growth factor-β-1 and β-2 (50). Interestingly, IGF-I gene expression in the rat uterus was observed at 7 days after administration of the drug (49), the kinetic pattern of which is

markedly different than that stimulated by estradiol (51), but similar to that reported here for c-*fos* and *jun*-B, *i.e.* delayed but persistent. Some of the genes controlled by AP-1 transcription factors have been identified (52), but it is not known if the expression of these growth factors are also under the control of AP-1.

The fact that tamoxifen causes a long-lasting pattern of cellular oncogene expression invites discussion about the potential ramifications of this persistent overexpression. The *fos* and *jun* proto-oncogenes encode the AP-1 transcription factor, a key component of the signal transduction networks that regulate cell growth and differentiation (23–25). In uterine cells stimulated by tamoxifen, the balance of dimers could be shifted toward formation of c-Fos:Jun-B heterodimers or Jun-B:Jun-B homodimers. Differential activity of AP-1 dimers exists (53–55), suggesting that Fos:Jun and Jun:Jun dimers have different target genes or differentially control the same target gene. The net result of altered AP-1 levels or activity could be altered normal transcriptional controls and multiple changes in downstream gene expression programs, such as increased expression in growth-promoting genes, decreased expression of growth-attenuating genes, or possibly a combination of both. Although it has not been established if perpetual overexpression of cellular oncogenes in uterine cells stimulated by tamoxifen can develop into an oncogenic event, inappropriate expression or mutation of c-*fos* (56) and the *jun* genes (57) has been shown to result in abnormal signal transduction leading to oncogenesis in experimental systems. Overexpression of *jun*-B elicited phenotypic changes associated with tumor progression in fibrosarcoma cell lines (58). Progressive elevation in levels of cellular oncogenes, particularly c-*fos*, was observed in estrogen-induced rodent kidney tumors (59). Expression of c-*fos* in human endometrial carcinoma transplanted into mice was increased by tamoxifen (60), suggesting a role for this proto-oncogene in growth promotion of endometrial carcinoma. Miao and Curran (61) recently demonstrated that when a critical threshold level of Fos and Fos target gene products was reached in rat fibroblasts, the cells became fully transformed. Interestingly, morphologic transformation by c-*fos* overexpression was not linked to cell proliferation in that study (61).

The results reported here are clinically relevant. The uterine epithelium is a primary target for tamoxifen, and women treated with tamoxifen frequently exhibit uterine changes that include increased uterine volume, thickening of the endometrium, endometrial polyps, and endometrial hyperplasia (6–8). Our observations suggest that aberrant proto-oncogene expression could provide a molecular mechanism underlying the phenomenon of uterine pathologies reported in women undergoing tamoxifen therapy. A better understanding of the molecular action of tamoxifen may directly impact on its efficacy as a chemopreventive agent and use in the therapy of women with breast cancer. It would be worthwhile to consider evaluating proto-oncogene expression in endometrial biopsies from women taking the agent, and to determine if tamoxifen-induced overexpression of c-*fos* and *jun*-B renders uterine epithelial cells more prone to becoming tumorigenic. We are currently pursuing such possibilities.

Acknowledgments

We express our appreciation to Dr. Wally Ip and Emma Lou Cardell for helpful discussions and Moli Tang for expert technical assistance.

References

1. **Kelloff GJ, Crowell JA, Boone CA, Steele VA, Lubet RA, Greenwald P, Alberts DS, Covey JM, Doody LA, Knapp GG, Nayfield S, Parkinson DR, Prasad VK, Prorok PC, Sausville EA, Sigman CC** 1994 Clinical development plans for cancer chemopreventive agents. J Cell Biochem Suppl 20:252–267
2. **Harper MJK, Walpole AL** 1967 A new derivative of triphenylethylene: effect on implantation and mode of action in rats. J Reprod Fertil 13:101–119
3. **Clark JH, Anderson JN, Peck, Jr, EJ** 1973 Estrogen receptor antiestrogen complex: atypical binding by uterine nuclei and effects on uterine growth. Steroids 22:707–718
4. **Martin L, Middleton E** 1978 Prolonged oestrogenic and mitogenic activity of tamoxifen in the ovariectomized mouse. J Endocrinol 78:125–129
5. **Nayfield SG, Karp JE, Ford LG, Dorr A, Kramer BS** 1991 Potential role of tamoxifen in prevention of breast cancer. J Natl Cancer Inst 83:1450–1459
6. **Neven P, de Muylder X, van Belle Y, Campo R, Vanderick G** 1994 Tamoxifen and the uterus. Br Med J 309:1313–1314
7. **Jordan VC, Morrow M** 1994 Should clinicians be concerned about the carcinogenic potential of tamoxifen? Eur J Cancer 11:1714–1721
8. **Friedl A, Jordan VC** 1994 What do we know and what don't we know about tamoxifen in the human uterus. Breast Cancer Res Treat 31:27–39
9. **Fischer B, Constantino J, Redmond C, Fisher E, Wickerham L, Cronin W** 1994 Endometrial cancer in tamoxifen-treated breast cancer patients: findings from the national surgical adjuvant breast and bowel project (NSABP) B-14. J Natl Cancer Inst 86:527–537
10. **van Leeuwen FE, Benraadt J, Coebergh JWW, Kiemeney LALM, Gimbrere CHF, Otter R, Schouten LJ, Damhuis RAM, Bontenbal M, Diepenhorst FW, van den Belt-Dusebolt AW, van Tinteren H** 1994 Risk of endometrial cancer after tamoxifen treatment of breast cancer. Lancet 343:448–452
11. **Magriples U, Naftolin F, Schwartz PE, Carcangiu ML** 1993 High-grade endometrial carcinoma in tamoxifen-treated breast cancer patients. J Clin Oncol 11:485–490
12. **Silva EG, Tornos CS, Follen-Mitchell M** 1994 Malignant neoplasms of the uterine corpus in patients treated for breast carcinoma: the effects of tamoxifen. Int J Gynecol Pathol 13:248–258
13. **Atlante G, Pozzi M, Vincenzoni C, Vocaturo G** 1990 Four case reports presenting new acquisitions on the association between breast and endometrial carcinoma. Gynecol Oncol 37:378–380
14. **Gurpide E** 1991 Endometrial cancer: biochemical and clinical correlates. J Natl Cancer Inst 83:405–416
15. **Murphy LJ** 1994 Growth factors and steroid hormone action in endometrial cancer. J Steroid Biochem Mol Biol 48:419–423
16. **Cohen I, Rosen DJD, Shapira J, Cordoba M, Gilboa S, Altaras MM, Yigael D, Beyth Y** 1993 Endometrial changes in postmenopausal women treated with tamoxifen for breast cancer. Br J Obstet Gynecol 100:567–570
17. **Kedar RP, Bourne TH, Powles TJ, Collins WP, Ashley SE, Cosgrove DO, Campbell S** 1994 Effects of tamoxifen on uterus and ovaries of postmenopausal women in a randomised breast cancer prevention trial. Lancet 343:1318–1321
18. **Cohen I, Rosen DJD, Shapira J, Cordoba M, Gilboa S, Altaras MM, Yigael D, Beyth Y** 1994 Endometrial changes with tamoxifen: comparison between tamoxifen-treated and nontreated asymptomatic, postmenopausal breast cancer patients. Gynecol Oncol 52:185–190
19. **Nephew KP, Polek TC, Akcali KC, Khan SA** 1993 The antiestrogen tamoxifen induces c-*fos* and *jun*-B, but not c-*jun* and *jun*-D, protooncogenes in the rat uterus. Endocrinology 133:419–422
20. **Kirkland JL, Murthy L, Stancel GM** 1993 Tamoxifen stimulates expression of the c-*fos* proto-oncogene in rodent uterus. Mol Pharmacol 43:709–714
21. **Khan SA, Nephew KP, Wang H** 1994 The *jun* protooncogene family

in uterine growth and differentiation. In: Khan SA, Stancel GM (eds) Protooncogenes and Growth Factors in Steroid Hormone Induced Growth and Differentiation. CRC Press, Boca Raton, FL, pp 105–124
22. **Stancel GM, Boettger-Tong H, Hyder SM, Kirkland JL, Loose-Mitchell DS** 1994 Uterine c-*fos*: a paradigm for control of gene expression during estrogen stimulated target tissue growth. In: Khan SA, Stancel GM (eds) Protooncogenes and Growth Factors in Steroid Hormone Induced Growth and Differentiation. CRC Press, Boca Raton, FL, pp 87–104
23. **Curran T, Franza, Jr, BR** 1988 Fos and Jun: the AP-1 connection. Cell 55:395–397
24. **Bravo R** 1990 Growth factor-responsive genes in fibroblasts. Cell Growth & Differ 1:305–309
25. **Angel P, Karin M** 1991 The role of Jun, Fos and the AP-1 complex in cell proliferation and transformation. Biochim Biophys Acta 1072: 129–157
26. **Waters AP, Knowler JT** 1980 A comparison of the effects of oestrogen and tamoxifen on the synthesis of uterine RNA in immature rats. J Steroid Biochem 14:625–630
27. **Wakeling AE, Bowler J** 1988 Novel antioestrogens without partial agonist activity. J Steroid Biochem 31:645–653
28. **Robinson SP, Langan-Fahey SM, Johnson DA, Jordan VC** 1990 Metabolites, pharmacodynamics, and pharmacokinetics of tamoxifen in rats and mice compared to the breast cancer patient. Drug Metab Dispos 19:36–43
29. **Nephew KP, Tang M, Khan SA** 1994 Estrogen differentially affects c-*jun* expression in uterine tissue compartments. Endocrinology 134: 1827–1834
30. **Nephew KP, Peters GA, Khan SA** 1995 Cellular localization of estradiol-induced c-*fos* messenger ribonucleic acid in the rat uterus: c-*fos* expression and uterine cell proliferation do not correlate strictly. Endocrinology 136:3007–3015
31. **Hsu CYJ, Komm BS, Lyttle CR, Frankel F** 1988 Cloning of estrogen-regulated messenger ribonucleic acids from rat uterus. Endocrinology 122:631–639
32. **Jordan VC, Dix CJ** 1979 Effect of oestradiol benzoate, tamoxifen and monohydroxytamoxifen on immature rat uterine progesterone receptor synthesis and endometrial cell division. J Steroid Biochem 11:285–291
33. **Katzenellenbogen BS, Bhakoo HS, Ferguson ER, Lan NC, Tatee T, Tsai TS, Katzenellenbogen JA** 1979 Estrogen and antiestrogen action in reproductive tissues and tumors. Recent Prog Horm Res 35:259–292
34. **Dix CJ, Jordan VC** 1980 Subcellular effects of monohydroxytamoxifen in the rat uterus: steroid receptors and mitosis. J Endocrinol 85:393–404
35. **Holinka CF, Bressler RS, Zehr DR, Gurpide E** 1980 Comparison of effects of estetrol and tamoxifen with those of estriol and estradiol on the immature rat uterus. Biol Reprod 22:913–926
36. **Lyman SD, Jordan VC** 1985 Metabolism of tamoxifen and its uterotrophic activity. Biochem Pharmacol 34:2787–2794
37. **Branham WS, Sheehan DM, Zehr DR, Medlock KL, Nelson CJ, Ridlon E** 1985 Inhibition of rat uterine gland genesis by tamoxifen. Endocrinology 117:2238–2248
38. **Galman MS, Sundstrom SA, Lyttle CR** 1990 Antagonism of estrogen- and antiestrogen-induced uterine complement component C3 expression by ICI 164,384. J Steroid Biochem 36:281–286
39. **Branham WS, Zehr DR, Sheehan DM** 1993 Differential sensitivity of rat uterine growth and epithelium hypertrophy to estrogens and antiestrogens. Proc Soc Exp Biol Med 203:297–303
40. **Rumpel E, Michna H, Wolfgang K** 1995 PCNA-immunoreactivity in the uterus of rats after treatment with the antiestrogen tamoxifen. Ann Anat 177:133–138
41. **Weisz A, Bresciani F** 1988 Estrogen induces expression of c-fos and c-myc proto-oncogenes in rat uterus. Mol Endocrinol 2:816–824
42. **Bigsby RM, Aixin L** 1994 Differentially regulated immediate early genes in the rat uterus. Endocrinology 134:1820–1826
43. **Ramkumar T, Adler S** 1995 Differential positive and negative transcriptional regulation by tamoxifen. Endocrinology 136:536–542
44. **Webb P, Lopez GN, Uht RM, Kushner PJ** 1995 Tamoxifen activation of the estrogen receptor/AP-1 pathway: potential origin for the cell-specific estrogen-like effects of antiestrogens. Mol Endocrinol 9:443–456
45. **Hyder SM, Stancel GM, Loose-Mitchell DS** 1991 Presence of an estradiol response region in the mouse c-*fos* oncogene. Steroids 56:498–504
46. **Hyder SM, Stancel GM, Nawaz Z, McDonnel DP, Loose-Mitchell DS** 1992 Identification of an estrogen response element in the 3′-flanking region of the mouse c-*fos* protooncogene. J Biol Chem 267: 18047–18054
47. **Hyder SM, Stancel GM** 1994 In vitro interaction of uterine estrogen receptor with the estrogen response element present in the 3′-flanking region of the murine c-*fos* protooncogene. J Steroid Biochem Mol Biol 48:69–79
48. **Hyder SM, Nawaz Z, Chiappetta C, Yokoyama K, Stancel GM** 1995 The protooncogene c-*jun* contains an unusual estrogen-inducible enhancer within the coding sequence. J Biol Chem 270:8506–8513
49. **Huynh HT, Pollak M** 1993 Insulin-like growth factor-I gene expression in the uterus is stimulated by tamoxifen and inhibited by the pure antiestrogen ICI 182780. Cancer Res 53:5585–5588
50. **Sartor BM, Sartor O, Flanders KC** 1995 Analogous tamoxifen and estrogen effects on transforming growth factor-betas 1 and 2 in the rat uterus. Reprod Toxicol 9:225–231
51. **Murphy LJ, Ghahary A** 1990 Uterine insulin-like growth factor-I: regulation of expression and its role in estrogen-induced uterine proliferation. Endocr Rev 11:443–453
52. **Busslinger M, Bergers G** 1994 Identification of AP-1-regulated genes. In: Angel PE, Herrlich PA (eds) The Fos and Jun Families of Transcription Factors. CRC Press, Boca Raton, FL, pp 133–150
53. **Halazonetis TD, Georgopoulos K, Greenberg ME, Leder P** 1988 c-Jun dimerizes with itself and with c-Fos, forming complexes of different DNA binding affinities. Cell 55:917–924
54. **Ryseck RP, Bravo R** 1990 c-Jun, Jun-B and Jun-D differ in their binding affinities to AP-1 and CRE consensus sequences: effect of Fos proteins. Oncogene 6:533–542
55. **Curran T, Vogt PK** 1992 Dangerous liaisons: Fos and Jun, oncogenic transcription factors, p. 797–831. In: McKnight SL, Yamamoto KR (eds) Transcriptional Regulation. Cold Spring Harbor Press, Cold Spring Harbor, NY, vol 2:797–831
56. **Muller R** 1994 Role of Fos proteins in the induction of transformation and DNA synthesis. In: Angel PE, Herrlich PA (eds) The Fos and Jun Families of Transcription Factors. CRC Press, Boca Raton, FL, pp 193–202
57. **Vogt PK** 1994 Oncogenic transformation by Jun. In: Angel PE, Herrlich PA (eds) The Fos and Jun Families of Transcription Factors. CRC Press, Boca Raton, FL, pp 203–219
58. **Bossy-Wetzel E, Bravo R, Hanahan D** 1992 Transcription factors Jun-B and c-Jun are selectively up-regulated and functionally implicated in fibrosarcoma development. Genes Dev 6:2340–2351
59. **Liehr JG, Chiappetta C, Roy D, Stancel GM** 1992 Elevation of protooncogene messenger RNA in estrogen-induced kidney tumors in the hamster. Carcinogenesis 13:601–604
60. **Sakakibara K, Kan NC, Satyaswaropp PG** 1992 Both 17β-estradiol and tamoxifen induce c-*fos* messenger ribonucleic acid expression in human endometrial carcinoma grown in nude mice. Am J Obstet Gynecol 166:206–212
61. **Miao GG, Curran T** 1994 Cell transformation by c-*fos* requires an extended period of expression and is independent of the cell cycle. Mol Cell Biol 14:4295–4310.