Molecular Determinants for the Tissue Specificity of SERMs
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Selective estrogen receptor modulators (SERMs) mimic estrogen action in certain tissues while opposing it in others. The therapeutic effectiveness of SERMs such as tamoxifen and raloxifene in breast cancer depends on their antiestrogenic activity. In the uterus, however, tamoxifen is estrogenic. Here, we show that both tamoxifen and raloxifene induce the recruitment of corepressors to target gene promoters in mammary cells. In endometrial cells, tamoxifen, but not raloxifene, acts like estrogen by stimulating the recruitment of coactivators to a subset of genes. The estrogen-like activity of tamoxifen in the uterus requires a high level of steroid receptor coactivator 1 (SRC-1) expression. Thus cell type- and promoter-specific differences in coregulator recruitment determine the cellular response to SERMs.

Tamoxifen and raloxifene are selective estrogen receptor modulators (SERMs) that bind the estrogen receptor (ER) and modulate ER-mediated gene transcription. Tamoxifen is an effective treatment for all stages of hormone-responsive breast cancer and can prevent breast cancer in high-risk women (J). However, tamoxifen has partial estrogenic activity in the uterus and is associated with an increased incidence of endometrial hyperplasia and cancer. Raloxifene, approved for the prevention and treatment of osteoporosis in postmenopausal women, also appears to prevent

References and Notes
19. The animals received water reward for fixating a pro- jected light-emitting diode (LED), either on the wall in front of the monkey or on the rear-projection screen. Eye position was monitored using the magnetic search coil technique [31, 32]. The room was illuminated by ~1000, small, incandescent lights (1.9 cd/m2 on a black background). The monkey viewed the room-mounted lights. All 44 neuronal studies included a pseudorandom sequence of six repetitions of each trial type. Each trial began with the illumination of the room-mounted light array, and the centered fixation point with recording started after 250 to 500 ms of centered fixation. Movement trials consisted of: 1) iso acceleration at 45°/s2, started after 250 to 500 ms of centered fixation. Movement trials consisted of: 2) acceleration at 45°/s2, followed by 7.5 s of movement at 45°/s2 around 360° at a speed of 47 cm/s. The speed would then decelerate for 1 s at 45°/s2; the room light array was extinguished, and the monkey’s reward was delivered. Stationary position trials required the monkey to maintain centered fixation in front of the room-mounted light array. After 8.5 s, the light array and fixation point were extinguished, and the reward was delivered. The position of the fixation point tracked sled movement so that the monkey maintained neutral, straight-ahead gaze throughout all trials.
20. We recorded 107 neurons from four cerebral hemispheres in two rhesus monkeys. All studies presented are based on data sets that include neurons from both monkeys. All procedures were approved by the University of Rochester Committee on Animal Research and were consistent with Society for Neuroscience policy on the care and use of laboratory animals. Bilateral recording cylinders were placed over trephine holes in the parietal calvarium (stereotaxic coordinates: AP ~2 mm, ML ± 15 mm, angle 90°) above area MST. Microelectrode penetrations were made using epoxy-coated tungsten microelectrodes (FHC, Inc.) that were passed through transcranial guide tubes into cortex (33). The location of recording sites on the anterior bank of the superior temporal sulcus was confirmed by magnetic resonance imaging with selected electrodes in place. MSTD neurons were characterized by using transcranial guide tubes into cortex (33). The location of recording sites on the anterior bank of the superior temporal sulcus was confirmed by magnetic resonance imaging with selected electrodes in place. MSTD neurons were characterized by using transcranial guide tubes into cortex (33).
breast cancer, but it does not increase the incidence of endometrial cancer. The National Cancer Institute supported “Study of Tamoxifen and Raloxifene” (STAR Trial) is currently being conducted to compare the safety and effectiveness of these two agents for the prevention of breast cancer in postmenopausal women (2).

The molecular mechanism underlying the tissue-specificity of SERM action is not clear. The crystal structures of the liganded ER hormone-binding domain (HBD) indicate that both tamoxifen and raloxifene can act as ER antagonists by competing with estradiol (E2) for binding and by inducing conformational changes that block the interaction of ER with coactivator proteins (3, 4). However, this does not explain how SERMs act as agonists or the differences in the spectrum of activity among various SERMs.

Estrogen receptor can regulate gene transcription either by binding directly to the promoter of target genes or by binding indirectly through a mechanism involving other transcription factors such as Sp1 and AP1. Genes regulated through direct ER binding, such as CATD (encoding cathpsin D) (5) (Fig. 1), typically harbor an estrogen responsive element (ERE) with a consensus sequence of 5′-GGTCAnnnTGACC-3′ in their promoters. Genes regulated by binding ER indirectly include c-Myc (8) and insulin-like growth factor 1 (IGF-I) (9), whose promoters do not contain a classical ERE.

We examined transcriptional responses to tamoxifen and raloxifene in the mammary carcinoma cell line MCF-7 and the endometrial carcinoma cell line Ishikawa. In both cell types, estradiol (E2) induced the expression of both the directly bound ER target genes CATD and EBAG9 and the indirectly bound target genes c-Myc and IGF-I (Fig. 1). Neither tamoxifen nor raloxifene stimulated the expression of CATD or EBAG9 in either MCF-7 or Ishikawa cells (Fig. 1). It is noteworthy, however, that in Ishikawa cells, but not in MCF-7 cells, tamoxifen, but not raloxifene, induced the expression c-Myc and IGF-I, whose promoters do not contain a classical ERE. Similar tissue-specific results were also obtained in another endometrial carcinoma cell line ECC-1 and another mammary carcinoma cell line T47-D (10). These observations suggest that promoter context is one of the determinants for tissue-specific activities of tamoxifen.

Estrogen receptor–mediated transcriptional activation is associated with the recruitment of coactivators, such as AIB1, GRIP1, SRC-1, CBP, p300, and pCAF, and subsequent histone acetylation (11–14). In contrast, antagonist-liganded ER is able to recruit corepressors (15–18). Previously, we showed in MCF-7 breast cancer cells that, when bound by tamoxifen, ER recruits the corepressors NCoR and SMRT and a subset of histone deacetylases (HDACs) to target promoters (18). Further examination of the recruitment of ER coregulators to target gene promoters by chromatin immunoprecipitation (ChIP) revealed that, in MCF-7 cells as well as in Ishikawa cells, both tamoxifen and raloxifene induce the recruitment of corepressors and HDACs to the CATD promoter (Fig. 2A, lower panels). In striking contrast, in Ishikawa cells, but not in MCF-7 cells, instead of inducing the recruitment of a core-
pressor complex, tamoxifen, but not raloxifene, induced the recruitment of a coactivator complex including SRC-1, AIB1, and CBP to the c-Myc promoter (Fig. 2B, upper panels, lanes 7, 15, 19). Tamoxifen-stimulated coactivator recruitment was accompanied by histone acetylation (Fig. 2B, upper panels, lane 23) consistent with the current model of gene activation by nuclear receptors. Tamoxifen-induced coactivator recruitment to the c-Myc promoter was also detected in ECC-1 cells and to the IGF-I promoter in both endometrial cancer cell lines (10).

As ER regulates the rate of gene transcription through its association with coregulators, the overall balance of the relative expression levels of coactivators and corepressors may be an important determinant of the tissue-specificity of SERMs. Examination of the expression levels of ERs and a variety of coregulators indicated similar levels of expression in MCF-7 and Ishikawa cells with the exception of SRC-1 (Fig. 3A), whose expression is low in MCF-7 compared with that in Ishikawa cells. The high level of SRC-1 expression in endometrial cells as compared with mammary cells was confirmed in several different cell lines (10). To investigate whether this difference in the level of SRC-1 expression explained the ability of tamoxifen to stimulate c-Myc and IGF-I transcription, we first overexpressed SRC-1 in MCF-7 cells. Remarkably, expression of both c-Myc and IGF-I was stimulated by tamoxifen in SRC-1–transfected MCF-7 cells but not in GRIP1- or AIB1-transfected cells (Fig. 3B). This finding supports our conclusion that a high level of SRC-1 expression is sufficient to support the agonist activity of tamoxifen.

To determine whether SRC-1 is required for tamoxifen agonism, we silenced its expression in Ishikawa cells by RNA interference using short interfering RNA (siRNA) molecules (10, 19). Reduction of SRC-1 levels in Ishikawa cells eliminated tamoxifen-stimulated expression of c-Myc and IGF-I (Fig. 4A). It was interesting that SRC-1 silencing had only minimal effects on the E2-stimulated expression of c-Myc and IGF-I. In contrast, silencing of AIB1 expression led to a modest decrease in both E2- and tamoxifen-stimulated expression of c-Myc and IGF-I (Fig. 4A). These results strongly suggest that, although AIB1 plays a role in the maximal activity of both estrogen and tamoxifen, SRC-1 is specifically necessary for the agonist activity of tamoxifen in endometrial cells. These observations also suggest that the specific coactivator requirements for estrogen- and tamoxifen-stimulated gene expression are distinct.

To determine whether SRC-1 expression was required for the growth stimulatory effects of tamoxifen in endometrial cells, we examined the effects of SRC-1 silencing on tamoxifen-stimulated cell-cycle progression in Ishikawa cells (Fig. 4B). As was the case for c-Myc and IGF-I expression, SRC-1 silencing abolished tamoxifen-stimulated cell-cycle progression but had only minimal effects on E2-stimulated cell-cycle progression. These results indicate that SRC-1 is a necessary determinant for the estrogenic effect of tamoxifen in endometrial cells.

In summary, in the breast where tamoxifen and raloxifene are both antagonists, both SERMs induce the recruitment of corepressors and not coactivators to ER target promoters. In contrast, in the endometrium where tamoxifen acts as an agonist and raloxifene as an antagonist, tamoxifen recruits coactivators instead of corepressors to ER target genes that do not contain a classical ERE, such as c-Myc and IGF-I. Finally, SRC-1 is required for the estrogen-like properties of tamoxifen in the endometrium.

It is unclear how coactivators are recruited by tamoxifen-bound ER to promoters that do not contain an ERE. Whether the ER AF-1 domain implicated in the agonist activity of tamoxifen (20–23) or the reported in vitro interactions of SRC-1 with AF-1 (24, 25) are relevant to the recruitment of SRC-1 by tamoxifen-bound ER remains to be shown. It may be that the binding of coactivators to tamoxifen-ligated ER is blocked when ER is directly bound to DNA through a classical ERE, but that when interacting with promoters indirectly, tamoxifen-bound ER adopts a conformation that promotes SRC-1 binding.

These experiments are based on a limited number of ER target genes and coactivators. It remains to be determined if c-Myc and/or IGF-I are the critical genes involved in tamoxifen-stimulated endometrial growth or endometrial cancer. However, c-Myc has been implicated in cell growth, proliferation, apoptosis, and malignant transformation (26). In addition, overexpression of c-Myc and c-Myc gene amplification have been reported in a variety of malignancies including endometrial cancer (27, 28). Likewise, the roles of IGF-I in cell proliferation and survival have also been well established (29).

Finally, our results do not exclude the possibility that other as-yet-undetermined cell-specific factors may contribute to the spectrum of SERM action. Our findings, however, do establish that cell-type– and promoter-specific differences in coregulator recruitment play a critical role in determining SERM function in the breast and uterus and offers a paradigm for understanding SERM action in other important target organs such as the brain, skeleton, and cardiovascular system.

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Television Viewing and Aggressive Behavior During Adolescence and Adulthood

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Television viewing and aggressive behavior were assessed over a 17-year interval in a community sample of 707 individuals. There was a significant association between the amount of time spent watching television during adolescence and early adulthood and the likelihood of subsequent aggressive acts against others. This association remained significant after previous aggressive behavior, childhood neglect, family income, neighborhood violence, parental education, and psychiatric disorders were controlled statistically.

Three to five violent acts are depicted in an average hour of prime-time television and 20 to 25 violent acts are depicted in an average hour of children’s television (1–3). Research has indicated that viewing television violence is associated with aggressive behavior (4–6). However, important questions regarding the nature and direction of this association remain unanswered. Several theories hypothesize that television violence contributes to the development of aggressive behavior (7, 8). An alternative hypothesis is that some or all of the association is due to a preference for violent television programs among aggressive individuals (9). Research has provided support for both hypotheses (10). It has also been hypothesized that certain environmental characteristics, such as living in an unsafe neighborhood and being raised by neglectful parents increase the likelihood of subsequent aggressive behavior and viewing televised violence. This hypothesis has not been extensively investigated.

Experimental and longitudinal studies have provided considerable support for the hypothesis that children's viewing of televised violence is associated with subsequent increases in aggressive behavior (11). However, most of these studies have investi...