Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis

Yongfeng Shang

Abstract | Endometrial cancer is the most common gynaecological cancer, and is associated with endometrial hyperplasia, unopposed oestrogen exposure and adjuvant therapy for breast cancer using selective oestrogen-receptor modulators (SERMs), particularly tamoxifen. Oestrogen and SERMs are thought to be involved in endometrial carcinogenesis through their effects on transcriptional regulation. Ultimately, oestrogen and SERMs affect the transduction of cellular signalling pathways that govern cell growth and proliferation, through downstream effectors such as PAX2 (paired box 2).

Endometrioid
A tumour containing epithelial or stromal elements resembling endometrial tissue.

SERMs
A group of chemical compounds that are structurally related to oestrogens, biochemically bind to oestrogen receptors, and functionally exert different effects on different tissues.

Department of Biochemistry and Molecular Biology, Peking University Health Science Center, Beijing 100083, China.
e-mail: Jason@bjmu.edu.cn
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Endometrial cancer is the most common gynaecological malignancy and, after breast, colorectal and lung cancer, is the fourth most common cancer in North American and European women[1]. The incidence of endometrial cancer continues to rise, especially in Eastern Asia and some Southern and Eastern European countries such as Slovakia and Slovenia[2]. Mortality from endometrial cancer ranks eighth among cancer deaths in North American women[1], and in Europe nearly 10,000 women die of endometrial cancer each year[3].

Endometrial carcinomas are classified into two types on the basis of biological and histopathological variables. Type I endometrial cancers account for approximately 80% of cases. Type I tumours are usually well-differentiated and endometrioid in histology, and are associated with a history of unopposed oestrogen exposure or other hyperoestrogenic risk factors such as obesity[3]. Patients with this type of endometrial cancer typically exhibit diseases with an early stage and have a favourable prognosis. By contrast, type II endometrial cancers are often poorly differentiated, non-endometrioid and are not associated with hyperoestrogenic factors. These tumours are more likely to be metastatic and can recur even after aggressive clinical intervention.

The pathogenic mechanisms of endometrial cancer are poorly understood. However, as in other malignancies, the transformation of normal endometrium to cancerous tissue is thought to involve a progressive accumulation of genetic abnormalities and epigenetic alterations that ultimately disrupt cellular signalling networks that govern processes such as cell proliferation, apoptosis and angiogenesis. As most endometrial carcinomas are type I oestrogen-associated endometrioid adenocarcinomas, it is important to delineate the molecular mechanisms underlying the roles of oestrogen and selective oestrogen-receptor modulators (SERMs) in endometrial carcinogenesis.

Even though most endometrial carcinomas are sporadic, about 10% of cases have a hereditary basis[4–8] (BOX 1). So far, no specific gene or genes have been linked to the majority of cases of endometrial cancer. However, molecular analyses have implicated several well-characterized oncogenes[9–16] and tumour-suppressor genes[17–22] in endometrial carcinogenesis (TABLE 1). Current data indicate that type I tumours are more commonly associated with abnormalities in the DNA-mismatch repair genes *KRAS*, *PTEN* (phosphatase and tensin homologue) and β-catenin, whereas type II tumours seem to be linked to abnormalities in *TP53* and *ERBB2* (also known as *HER2/neu*). The abnormalities range from mutations, deletions and amplification/overexpression of genes to epigenetic deregulation.

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 oestrogen-only therapy[23]. Subsequently, various clinical and epidemiological investigations, with support from studies in cell culture and animal models, have demonstrated the involvement of oestrogen in the development and/or progression of the disease. Oestrogen is now considered the classic aetiological factor for endometrial carcinogenesis; as previously noted, most endometrial cancers are type I oestrogen-associated endometrioid

At a glance

- Endometrial cancer is the most common gynaecological malignancy.
- Although no specific gene or genes have been linked to the majority of cases of endometrial cancer, several well-characterized oncogenes and tumour-suppressor genes have been implicated in endometrial carcinogenesis.
- Approximately 80% of endometrial cancer cases are type I tumours, which are usually well differentiated and endometrioid in histology, and are associated with a history of unopposed oestrogen exposure or other hyperoestrogenic risk factors such as obesity.
- Oestrogen and selective oestrogen-receptor modulators (SERMs) are implicated in endometrial carcinogenesis through regulation of gene transcription.
- Oestrogen and SERMs exert their carcinogenic roles in the endometrium through their downstream molecular effectors such as PAX2 (paired box gene 2).

adenocarcinomas. In 2002, the US National Toxicology Program listed steroidal oestrogens as carcinogens for the first time. The report cites data from human epidemiological studies that show an association between oestrogen-replacement therapy and an increase in the risk of endometrial cancer, as well as a less consistent increase in the risk of breast cancer.

SERMs were initially envisioned as drugs that would replace oestrogen therapy in alleviating the symptoms that are associated with menopause without the carcinogenic effects of oestrogen in the mammary gland and uterus. The first clinically available SERM was tamoxifen. It was introduced in the 1970s for treatment of advanced breast cancer in postmenopausal women[24,25], and its use was then expanded as an adjuvant therapy for reducing the risk of recurrence of breast cancer following surgery in premenopausal and postmenopausal 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 (NSABP) Breast Cancer Prevention Trial (BCPT)[26]. It was reported by the NSABP-BCPT that the increased rate of endometrial cancer occurred predominantly in women aged 50 or older[26].

Mechanisms of oestrogen action

Oestrogens have 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. Oestrogen mediates its biological effects in target tissues by binding to specific intracellular receptors, oestrogen receptor-α (ERα) and ERβ. Both ERα and ERβ are members of the nuclear hormone receptor superfamily and have modular structures and discrete domains for their specific functions as ligand-regulated transcription factors[27] (FIG. 1a).

ERα and ERβ are highly homologous in their DNA-binding domains (~96%) and have moderate (53%) sequence identity in their ligand-binding domains (LBDs). The main functional difference between ERα

Box 1 | **Familial endometrial cancer**

Even though most cases are sporadic, about 10% of endometrial carcinomas have a hereditary basis. Many of these cases are associated with hereditary non-polyposis colorectal cancer (HNPCC), a dominantly inherited syndrome with germline mutations in one of the DNA-mismatch repair (MMR) genes that can lead to microsatellite instability[4]. HNPCC is characterized by early development of cancer in the colon as well as in the endometrium, stomach, small intestine and ovary. Women with HNPCC have a tenfold higher lifetime risk of endometrial cancer compared with the general population, and that risk (42%) is even higher than that for colorectal carcinoma (30%)[5]. Hereditary endometrial cancer is more likely to occur at a younger age than the sporadic form. Also, it is characterized by a high stage and grade, cribriform growth pattern, mucinous differentiation and necrosis[6].

MMR gene mutations are generally not found in sporadic type I endometrial carcinomas but, instead, MMR genes might be inactivated or silenced in some of these cancers by epigenetic mechanisms such as promoter hypermethylation[149]. The normal function of MMR genes and their association with endometrial cancer are illustrated below. DNA replication leads to occasional errors that are repaired by the MMR proteins MSH2 (mutS homologue 2), MSH3, MSH6, MLH1 (mutL homologue 1), PMS1 (postmeitoic segregation increased 1) and PMS2. Familial defects in the genes that encode these proteins leads to instability of various microsatellites, which can cause endometrial cancer.

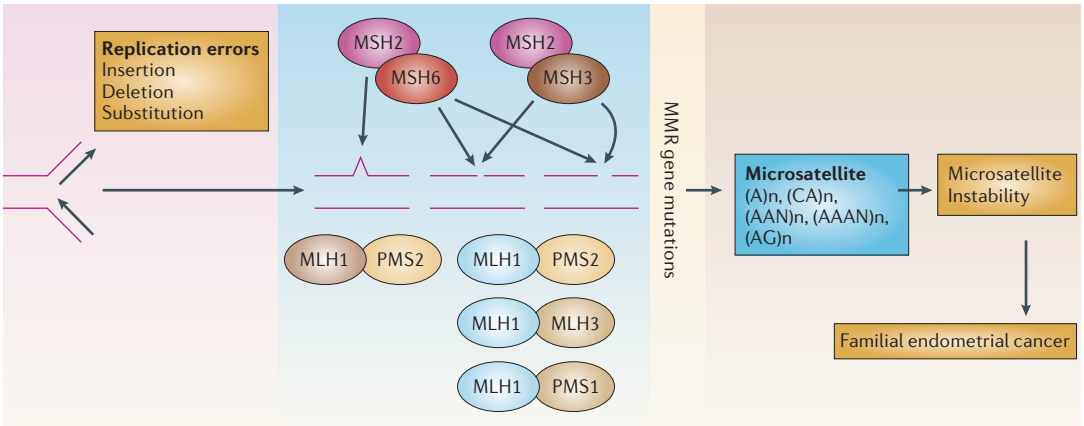

Table 1 | **Genetic and epigenetic abnormalities associated with endometrial carcinomas**

Gene	Function	Abnormalities
MLH1	DNA repair	Mutation, hypermethylation, loss of expression
MSH2	DNA repair	Mutation, hypermethylation, loss of expression
MSH6	DNA repair	Mutation, loss of expression
KRAS	Oncogene	Mutation
ERBB2 (*HER2/neu*)	Oncogene	Amplification
PAX2	Oncogene	Hypomethylation, overexpression
MYC	Oncogene	Amplification, overexpression
β-Catenin	Oncogene	Mutation, overexpression
Survivin	Anti-apoptotic	Overexpression
TERT	Telomere maintenance	Overexpression
RUNX1	Transcription factor	Overexpression
PTEN	Tumour suppressor	Mutation, deletion, hypermethylation, loss of expression
TP53	Tumour suppressor	Mutation, deletion, overexpression
PER1	Circadian-clock control	Hypermethylation, loss of expression
TIG1	Tumour suppressor	Hypermethylation, loss of expression
C/EBPα	Transcription factor	Hypermethylation, loss of expression
CASC2a	Unknown	Mutation, hypermethylation (?), loss of expression

C/EBPα, CCAAT/enhancer binding protein-α; *CASC2a*, cancer susceptibility candidate 2a; *MLH1*, mutL homologue 1; MSH, mutS homologue; *PAX2*, paired box gene 2; *PER1*, period homologue 1; *PTEN*, phosphatase and tensin homologue; *RUNX1*, runt-related transcription factor 1; *TERT*, telomerase reverse transcriptase; *TIG1*, TPA inducible gene 1.

and ERβ seems to be determined by their different hormone-independent transcriptional activation function (AF1) domains in their N terminus. Specifically, the ERα AF1 domain is active in reporter-mediated gene expression, whereas activity of the AF1 domain of ERβ is almost negligible[28]. This difference probably contributes to ligand- and tissue-specific responses to oestrogen and SERMs, as discussed below.

The binding of oestrogen to ERs induces conformational changes in protein structure that allow for receptor dimerization and interaction with co-activator molecules[29,30]. The subsequent transcriptional activation of genes occurs through targeting liganded ER directly to oestrogen response elements (EREs) in gene promoters or indirectly through binding to other transcription factors such as AP1, SP1 or nuclear factor κB (NFκB)[31–38] (FIG. 1b). The efficacy of the activated receptor is regulated by several mechanisms, and the balance of these mechanisms could determine the tissue-specific activity of the receptor. First, although the pathophysiological significance of ERβ in target tissues such as the endometrium is still not established, the relative abundance of ERα versus ERβ might nevertheless have important roles in oestrogen signalling. It has been found that ERα and ERβ have opposite effects on transcription that is mediated through the indirect mode of action, and these divergences might at least partially explain the inhibitory effect of ERβ on the stimulation of cell proliferation by ERα[39]. Second, ERs are subject to a variety of post-translational modifications: ER phosphorylation by several kinases, including the mitogen-activated protein kinase and protein kinase A, enhances ER activity[40]; ER acetylation by histone acetyltransferases regulates ER transactivation and hormone sensitivity[41]; and ER ubiquitylation regulates ER concentration and, therefore, bioavailability[42,43]. Third, the enrichment of co-activator species and their post-translational modifications in a specific tissue are also important for the final transcriptional output of ER by the tissue. In addition, a range of studies revealed 'non-genomic', 'non-nuclear' or 'non-transcriptional' actions of ER[44,45]. Non-transcriptional functions of ER in the cytoplasm are characterized by fast and transient signal outputs that could potentially lead to a convergence of oestrogen signalling with other cellular signalling transduction pathways. These mechanisms could be exploited selectively to amplify tissue-specific responses to oestrogen. Alternatively, survival pathways in cancer could evolve to alter the entire responsiveness to ER signalling.

Expression of ERs in the endometrium

ERα and ERβ have different tissue expression profiles. ERα is predominantly expressed in the breast, uterus and vagina, whereas ERβ is mainly expressed in tissues such as the central nervous system, cardiovascular system, immune system, gastrointestinal system, kidney, lungs and bone[46]. However, although the uterus is thought to predominantly express ERα, increased cell proliferation and exaggerated response to oestrogen in ERβ-knockout mice indicates that ERβ might modulate ERα function in the uterus and have an antiproliferative function[47,48]. Therefore, an imbalance in ERα and ERβ expression

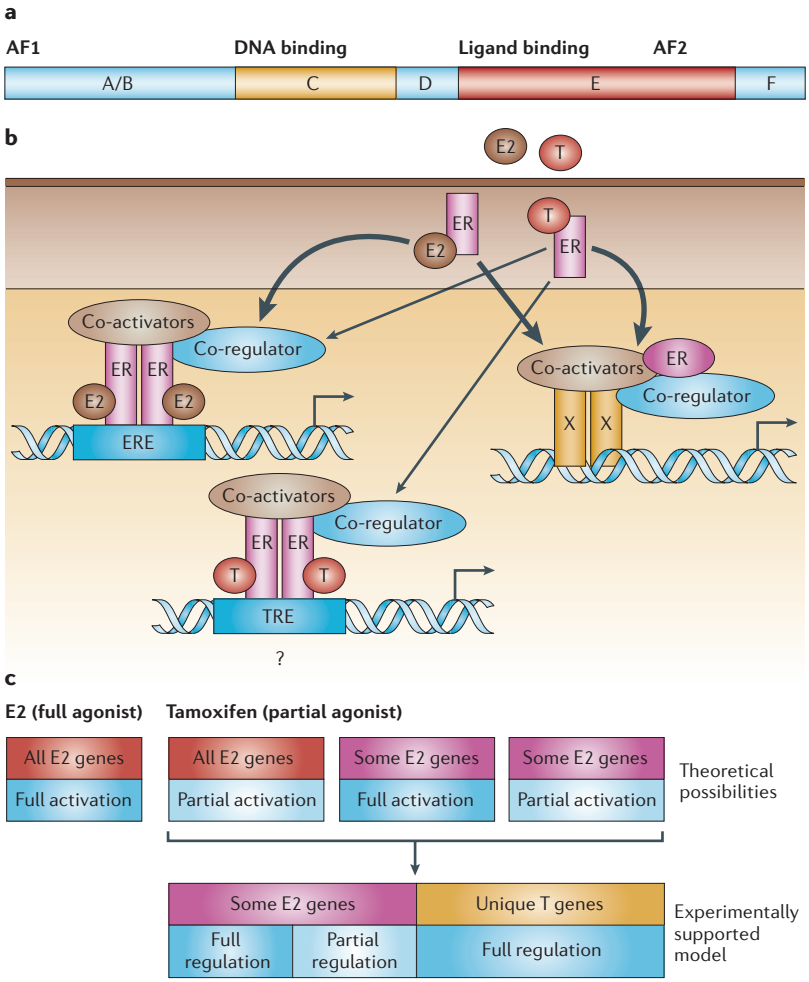

Figure 1 | **Genomic action of oestrogen and tamoxifen in the uterus. a** | The functional domains of the oestrogen receptor (ER). Both ERα and ERβ have six domains (A–F). The amino-terminal A and B domains together encode a hormone-independent transcriptional activation function (AF1) domain. Domain C corresponds to the highly conserved DNA-binding domain that is responsible for specific binding of the receptors to oestrogen response elements (EREs) in the promoter of target genes. Domain D, the hinge region that separates the DNA-binding domain and the ligand-binding domain, is thought to allow conformational changes in the receptor molecule during activation, and it is important in receptor dimerization. The E and F domains encode the ligand-binding domain that is located in the carboxy-terminal portion of the receptors. This region consists of 12 α-helices, which form a hydrophobic pocket allowing for oestrogen or selective oestrogen-receptor modulator binding. Domain E/F also harbours a second transcriptional activation function domain (AF2), which activates transcription in response to oestrogen or synthetic agonists by interacting with co-activators. **b** | Mechanisms of oestrogen and tamoxifen action. Ligand E2 (oestrogen) or T (tamoxifen) diffuses into the cell and binds to the oestrogen receptor (ERα/ERβ). Liganded receptors enter the nucleus and form homodimeric or heterodimeric complexes that bind to EREs or tamoxifen response elements (TREs) on the target-gene promoters. Liganded receptors can also function as a monomer and bind to target gene promoters through interaction with other transcription factors. Promoter-associated ERs initiate gene transcription through interaction with co-regulators and the basal transcriptional machinery. **c** | Theoretical model and experimentally supported model of the genomic action of tamoxifen. Theoretically, if the full agonistic activity of oestrogen is a result of full regulation of oestrogen target genes, the phenotypic manifestation of partial oestrogenic activity of tamoxifen could result from one of the following genotypic behaviours: partial regulation of all E2 target genes, full regulation of some E2 target genes or partial regulation of some E2 target genes. Experimentally, tamoxifen in the endometrium regulates transcription of genes that exhibit an overlapping but distinct profile compared with the target genes of oestrogen. X, other transcription factor (for example, AP1, SP1 or nuclear factor κB).

could be a crucial step in oestrogen-dependent tumorigenesis. However, ERα expression is decreased in grade 3 tumour samples when compared with normal or grade 1 samples, whereas ERβ expression does not correlate with tumour grade. This indicates that a decrease in the ERα/ERβ ratio occurs as the tumour progresses[49,50]. The significance of the relative expression of ER subtypes in endometrial cancer remains to be clarified.

ERα and ERβ variants that arise from alternative splicing or splicing errors have been described and implicated in cancer. An ERα exon 5 splice variant (Δ5 ERα) has not been detected in normal endometrium but was expressed at significantly increased levels in endometrial carcinomas compared with endometrial hyperplasia[51]. Indeed, Δ5 ERα has been shown to be able to constitutively activate ER-dependent transcription in the absence of hormone[52,53]. This could therefore provide endometrial tumour cells with a proliferative advantage, potentially leading to uncontrolled proliferation. An ERβ exon 8 splice variant, ERβcx (REF.54), has a dominant-negative effect on ERα function and might have an effect on tumour progression in breast cancer[55]. ERβcx is expressed in both normal and malignant endometrium, but its role in the pathogenesis of endometrial cancer is unknown[56,57].

Molecular mechanisms of SERM action

Selective oestrogen-receptor modulators. Three SERMs are currently used in the treatment and/or prevention of breast cancer and osteoporosis (TABLE 2). Tamoxifen is the most prescribed antineoplastic drug worldwide, and it is used to treat patients with all stages of hormone-responsive breast cancer[58]. It has been shown to prevent breast cancer in women who are at high-risk for this disease[26]. Despite having an anti-oestrogenic function in the breast, tamoxifen shows partial oestrogenic effects in other target tissues[26,59–62]. These partial oestrogenic actions have beneficial effects on bones and the cardiovascular system in postmenopausal women[59]. However, in the endometrium it is also associated with an increased incidence of cancer[59–63].

Raloxifene, a second-generation SERM, was initially approved for the prevention of osteoporosis in 1997, and in October 1999 approval was extended to include treatment of patients with existing osteoporosis. Like tamoxifen, raloxifene seems to prevent breast cancer in high-risk women; unlike tamoxifen, it has not been found to increase the incidence of endometrial cancer[64]. The Study of Tamoxifen and Raloxifene was initiated in 1999 to compare the effects of the two agents on breast cancer prevention and endometrial cancer risk; the results are expected in the summer of 2006. Toremifene is another SERM that has anti-oestrogenic activity in the mammary gland. The beneficial effects of toremifene on bone mineral density and lipid profiles are similar to those of tamoxifen, but, like tamoxifen, toremifene also has stimulatory effects on the endometrium and it is presently only used in postmenopausal women with metastatic breast cancer.

Table 2 | **Generation and effect of selective oestrogen-receptor modulators (SERMs)**

SERMs	Generation	Activity				Application
		Mammary	Uterus	Bone	Blood vessels	
Oestrogen		+++	+++	+++	+++	
'Ideal SERM'		–	–	+++	+++	
Tamoxifen	I	–	++	+	+	Breast cancer
Raloxifene	II	–	–	++	+	Osteoporosis
Toremifene	II	–	+/–	+	+	Breast cancer
Idoxifene	II	–	+/–	+	+	
Droloxifene	II	–	+/–	+	Unknown	
GW-5638	II	–	+/–	+	Unknown	
Arzoxifene	III	–	–	++	+	
EM-652	IV	–	+/–	+	Unknown	

In addition to tamoxifen, raloxifene and toremifene, several new SERMs are currently being investigated for the treatment of breast cancer and for potential chemoprevention of breast and endometrial cancers (TABLE 2). Arzoxifene, a third-generation SERM, is a long-acting raloxifene analogue. Arzoxifene can protect against bone loss, and it reduces serum cholesterol levels in animal models. It is also highly effective for the prevention of mammary cancer induced in the rat by the carcinogen nitrosomethylurea. Arzoxifene is even more potent in these respects than raloxifene, but it is devoid of the uterotrophic effects of tamoxifen[65]. EM-652 (acolbifene) and GW-5638 are fourth-generation SERMs that exert complete anti-oestrogenic effects on the breast and uterus. At the same time, they have also been shown to have oestrogenic effects, preventing bone loss and reducing serum cholesterol in ovariectomized rats and having minimal negative effects on the endometrium[66–68]. Another novel SERM, SP500263, was discovered in a screen that used a small-molecule compound library to identify oestrogen agonists in bone[69]. It has high affinity for both ERα and ERβ, but functions through ERα only. SP500263 functions as an anti-oestrogen in human mammary carcinoma cells (MCF-7 cells), and effectively reduced oestrogen-stimulated tumour growth in a murine breast cancer xenograft model. The efficacy of SP500263 was comparable to tamoxifen and superior to raloxifene. Importantly, SP500263 did not promote uterine wet weight in immature rats or in adult ovariectomized rats.

Genomic action of tamoxifen. The stimulation of endometrial carcinogenesis by tamoxifen is of great interest both in clinical medicine and to research scientists. Tamoxifen and raloxifene bind to ERs. The crystal structures of ERα in the presence of its hormone agonists or liganded with tamoxifen or raloxifene indicate that the receptor LBD interaction surfaces are composed of amino-acid residues belonging to α-helices 3, 4, 5 and 12 (REFS 70,71). When the LBD of ERα is complexed with 17β-estradiol (E2 — a type of oestrogen), the α-helix 12 is positioned over the ligand-binding pocket and forms an interaction surface for the recruitment of co-activators[71]. By contrast, when the LBDs of either ERα or ERβ are liganded with tamoxifen or raloxifene, α-helix 12 is displaced from its agonist position and occupies the hydrophobic groove adjacent to α-helices 3, 4 and 5, causing α-helix 12 to block the co-activator interaction surface[72,73]. The positioning of the α-helix 12 following ligand binding has been proposed as an important mechanism for full oestrogen action on ERα and ERβ[74,75]. Accordingly, the binding of the anti-oestrogen ICI 182,780 to ERα causes an alteration of the geometry of α-helix 12, preventing co-activator association, whereas raloxifene induces a unique conformational change in the ER structure, favouring its interaction with a specific subset of co-activators[76]. The conformation that ER adopts in the presence of raloxifene enables the engagement of tissue-specific cofactors that allow raloxifene to have a positive effect in bone[77,78]. In summary, based on the crystal structures of the ligand-bound LBDs of ERs[70,71], it is believed that tamoxifen and raloxifene function as ER antagonists in the mammary gland by inducing conformational changes that block the interaction of ERs with co-activator proteins. However, this molecular mechanism is not compatible with the partial oestrogenic activity of tamoxifen in the uterus. Among the theories being investigated is the possible genotoxicity of tamoxifen, but the detection of endometrial tamoxifen–DNA adducts in exposed women is still largely controversial[79–85].

By contrast, biochemical and animal experiments[70,86–97] as well as genetic studies[98–101] strongly favour an oestrogen-receptor-dependent pathway in all tissues, implicating gene regulation as the mechanism of tamoxifen differential action. Experimental evidence supports a transactivation activity for tamoxifen-liganded ERs, and the association of tamoxifen-liganded ERs with co-activator proteins has been well documented[36,37,93,102–105] and is an established model for the role of nuclear receptors in gene regulation. For example, it has been shown that differential recruitment of a co-activator contributed to the tissue specificity of tamoxifen-liganded ERα[37]. So, there is a strong possibility that, mechanistically, endometrial carcinogenesis proceeds from alterations in gene expression due to tamoxifen-activated gene transcription.

Second-, third- and fourth-generation SERMs
Chemical compounds that are developed in different stages and are intended to improve the beneficial effects and reduce the harmful effects of original SERMs.

Genotoxicity
Effects that cause genetic mutations and/or changes in chromosome structure and number.

Canonical
Standard or well-established and recognized DNA sequences to which transcription factors bind.

If tamoxifen does function to regulate gene transcription in the endometrium, and if we assume that the full agonistic activity of oestrogen is a result of full regulation of oestrogen target genes, the phenotypic manifestation of partial oestrogenic activity of tamoxifen could result from one of the following genotypic behaviours: partial regulation of all oestrogen target genes, full regulation of some oestrogen target genes, or partial regulation of some oestrogen target genes (FIG. 1c). Recent studies using human genome microarrays have demonstrated that tamoxifen regulates gene transcription in endometrial epithelial cells isolated from type I endometrial carcinoma samples[106]. Furthermore, the genes that are targeted by tamoxifen are different from the genes that are targeted by oestrogen. A comparison of the gene-expression profile in cancerous versus normal endometrial epithelial cells indicates that there are more upregulated genes and fewer downregulated genes in the cancer cells. The ability of tamoxifen and raloxifene to regulate gene transcription in the endometrium has also been demonstrated by other laboratories[107–109], and, consistent with the pathobiology of type I endometrial cancer, it has been reported that transcriptional responses were identified in epithelial cells but not in stromal cells[107]. These data indicate that the ability to regulate gene transcription could dictate the role of tamoxifen in endometrial carcinogenesis. It will be important to fully study the genomic activities of SERMs such as tamoxifen and raloxifene, in addition to the roles of these compounds as partial ER agonists.

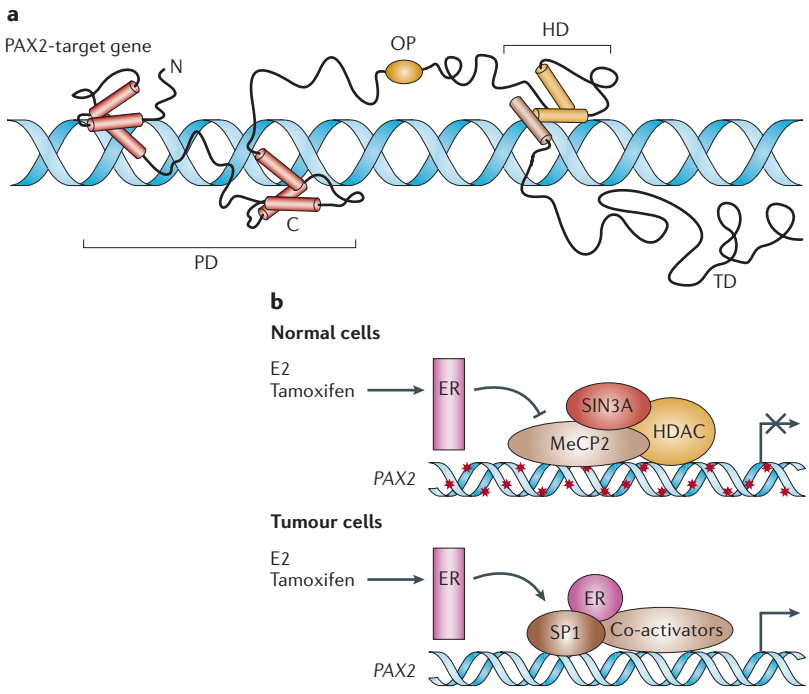

Figure 2 | **PAX2 in the endometrium. a** | The functional domains in the PAX2 (paired box 2) protein include: a paired domain (PD) that is composed of N- and C-terminal subdomains, each of which is composed of three α-helices and the third helix of each subdomain (red) makes contact with the major groove of DNA; an octapeptide motif (OP); a homeodomain (HD) with a helix-turn-helix structure; and a C-terminal transactivation domain (TD). **b** | *PAX2* is a developmentally regulated gene that is switched off by methylation-mediated binding of transcription-repressive complexes containing methyl-CpG binding protein 2 (MeCP2), SIN3A and histone deacetylase (HDAC). However, in endometrial cancer, loss of the methylation mark leads to dissociation of the transcription-repressive complexes, the association of the transcription-activation complex, and gene reactivation under conditions such as oestrogen and tamoxifen stimulation. Part **a** is modified, with permission, from REF. 117 © (2002) Elsevier Science.

Transcriptional regulation by tamoxifen. The observation that tamoxifen target genes are different from oestrogen target genes is intriguing. After all, 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[86,94,96,110,111]. Second, previous studies by several laboratories have demonstrated that the transcription activation of tamoxifen-bound ERs is promoter context-specific[32,37,86,88,94,96,110,112]. ERs can target gene promoters that harbour either a classical ERE, a half ERE site, or sites that are canonical for other transcription factors such as AP1, SP1 and NF-κB[31–37]. It is reasonable to speculate that oestrogen-liganded ERs and tamoxifen-liganded ERs, owing to their different conformations[70] and different co-activator associations, possess different affinities for different gene promoters. As a result, oestrogen and tamoxifen could regulate different sets of genes. Some of the gene promoters could accommodate both oestrogen-liganded ERs and tamoxifen-liganded ERs. In this situation, the gene would be a target for both oestrogen and tamoxifen. Moreover, it is well documented that the transactivation function of tamoxifen-liganded ERs largely resides in AF1 in the N terminus of the ERs[93,94,97,112–115]. This is in contrast to the transactivation action of oestrogen-liganded ERs, which mainly depend on the AF2 domain in their C terminus. Differential association of co-activator proteins with AF1 versus AF2 could determine the affinity of oestrogen-liganded ERs and tamoxifen-liganded ERs for different gene promoters.

PAX2 and endometrial carcinogenesis. PAX2 (paired box 2) has been identified as an important effector of oestrogen- and tamoxifen-stimulated proliferation of endometrial cells[106]. *PAX2* is one of the nine related genes in the Pax family of transcription factors. Pax genes possess a 128-amino-acid DNA-binding domain along with a paired-box domain, which makes sequence-specific contacts with DNA[116,117] (FIG. 2a). The expression of Pax genes is tightly regulated in both a temporal and a spatial manner — they are primarily expressed during fetal development and are switched off during later phases of terminal differentiation in most structures[117,118]. *PAX2* is normally expressed in the developing urogenital tract, spinal cord, midbrain, hindbrain, ear and optic nerve[117,119–121]. *Pax2*-null mice fail to develop the urogenital tract and have anomalies in the optic nerve, central nervous system and inner ear[122–124].

In adult tissues, constitutive expression of several Pax genes, either as part of a fusion gene or as a whole gene, promotes tissue hyperplasia and malignant transformation[117,125–129]. Deregulation of Pax genes owing to gene mutation, translocation, rearrangement or amplification has been found in several tumours, and Pax gene products can undergo cellular transformation[117,129–139],

indicating that Pax genes can be classified as oncogenes. *PAX2* expression has been detected in Wilms' tumour[140–142], a childhood renal tumour of embryonic origin, as well as in a high proportion of primary tumours, including breast, ovarian, lung, colon and prostate tumours as well as lymphoma[135,138,139]. Typically, it has been found that *PAX2* expression accompanies high rates of cell division[136–139]. In endometrial cells, gain-of-function and loss-of-function experiments have both demonstrated that PAX2 promotes cell proliferation and tumour growth[106]. PAX2 and ERα are co-expressed in endometrial tumour samples, and PAX2 expression is activated in cancerous endometrial cells but not in normal endometrial cells. Collectively, these data indicate that PAX2 is a molecular effector for oestrogen and tamoxifen in endometrial carcinogenesis.

***Epigenetic deregulation of* PAX2.** As mentioned above, Pax genes are developmentally regulated and are silenced in adulthood. Why is *PAX2* activated by oestrogen and tamoxifen in endometrial cancer? Studies of the *PAX2* promoter reveal that it is methylated, and therefore silenced, in normal adult endometrial cells, but that this methylation is lost in 75% of endometrial carcinomas[106]. Therefore, the activation of *PAX2* expression in endometrial carcinoma cells through promoter hypomethylation is one possible mechanism for its upregulation in endometrial tissue. This might occur through demethylation of CpG sites within the *PAX2* promoter. CpG methylation is mediated by methyl-CpG binding proteins (MeCPs), which, in turn, are associated with SIN3A–histone deacetylase (HDAC)-containing transcription repression complexes[143]. The association of MeCP2, SIN3A and HDAC1 with the *PAX2* upstream regulatory region was reported in normal endometrial epithelial cells, but not in cancer cells[106]. Loss of association of the MeCP–SIN3A–HDAC1-containing protein complex with the *PAX2* promoter could therefore be a mechanism for the upregulation of this gene in cancer cells (FIG. 2b).

Future perspectives

Future studies are needed to investigate the mechanisms by which the *PAX2* promoter loses its methylation mark and to determine how *PAX2* expression, once reactivated, promotes cell proliferation in endometrial carcinoma cells. It is unclear at present whether there are any causative effects of oestrogen and tamoxifen on the loss of *PAX2*-promoter methylation in endometrial cancer. However, it is possible that oestrogen and tamoxifen, through their capacity for gene-transcription regulation, alter the cellular milieu that maintains the silenced state of the developmentally regulated genes that include *PAX2*. PAX2 is a transcription factor, and once expressed, it regulates other downstream genes, the products of which could promote the cell proliferation and oncogenic transformation. So far, only a few downstream target genes of PAX2 have been identified[144–147], and the effector or effectors that are downstream of PAX2 in mediating the carcinogenic effect of oestrogen and tamoxifen in endometrium will be a subject for future investigations. In addition, it will be interesting to determine whether or not the loss of methylation in the *PAX2* promoter is endometrial specific. It is possible that deregulation of *PAX2*-promoter methylation might not differ between endometrial and mammary carcinomas. Rather, the consequence of the hypomethylation could differ between these two types of carcinoma in responding to tamoxifen stimulation, and this difference could be associated with the difference in co-activator/co-repressor balance in these two types of tissue, as reported previously[37].

There is considerable interest in developing new SERMs as multifunctional agents for improving women's health. In particular, new SERMs could have chemopreventive potential for breast cancer and endometrial cancer. The recent success of breast cancer prevention trials with tamoxifen[26] and raloxifene[148] in high-risk populations indicates that chemoprevention is possible and worth pursuing. An ideal SERM would be one with tissue-specific beneficial effects coupled with lack of harm to other tissues. The key to such a SERM relies on not only an expanded delineation of the oestrogen signalling pathway, but also a better understanding of the tissue specificity of SERM functions.

Biophysical studies to determine how the structures of ERs and their ligands change when they interact, and how these configuration changes affect transcription, will facilitate our studies of SERM function and oestrogen signalling. Currently, new anti-oestrogens, including the SERMS (for example, toremifene, droloxifene, idoxifene and arzoxifene), the selective ER downregulators (for example, fulvestrant), and the new steroidal (for example, exemestane) and non-steroidal (for example, anastrozole and letrozole) aromatase inhibitors, are being investigated for the treatment of patients with breast cancer and hormonally sensitive tumours of the uterine body. The optimal approach for future research includes delineation of the molecular modulation mechanisms that are involved in ER signalling.

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Competing interests statement
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