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The role of estrogens and estrogen receptor signaling pathways in cancer and infertility: the case of schistosomes

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***Schistosoma haematobium*, a parasitic flatworm that infects more than 100 million people, mostly in the developing world, is the causative agent of urogenital schistosomiasis, and is associated with a high incidence of squamous cell carcinoma (SCC) of the bladder. Schistosomiasis haematobia also appears to negatively influence fertility, and is particularly associated with female infertility. Given that estrogens and estrogen receptors are key players in human reproduction, we speculate that schistosome estrogen-like molecules may contribute to infertility through hormonal imbalances. Here, we review recent findings on the role of estrogens and estrogen receptors on both carcinogenesis and infertility associated with urogenital schistosomiasis and discuss the basic hormonal mechanisms that might be common in cancer and infertility.**

The case of schistosomiasis

Schistosomiasis is a neglected tropical disease transmitted to humans from freshwater snails. It is caused by a blood fluke of the genus *Schistosoma*. Schistosomiasis is considered the most important of the helminthiases and the second most important parasitosis, after malaria, causing high rates of morbidity and mortality. Schistosomes affect at least 76 countries and 200 million people worldwide. From these, 20 million have severe disease and 120 million are considered symptomatic. Risk of infection affects 600 million others including travelers from developed countries [1].

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This opinion focuses on estrogen metabolism and estrogen receptor (ER) signaling pathways associated with cancer induction and female infertility in the context of *Schistosoma haematobium* infection. The present work attempts to integrate a variety of studies and experimental approaches with *S. haematobium* models, while giving particular emphasis to the *in vitro* studies that have contributed to expanding our understanding of the mechanisms of action of estrogen metabolism and ER signaling pathways associated with schistosomiasis. In particular, we suggest that hormonal imbalance resulting from *S. haematobium* may promote cancer and infertility.

Urogenital schistosomiasis

Three major species of schistosomes are the agents of human schistosomiasis – *Schistosoma japonicum* and *Schistosoma mansoni* cause intestinal schistosomiasis in East Asia, Africa, South America and the Caribbean, while *S. haematobium*, occurring widely throughout Africa and the Middle East, causes urogenital schistosomiasis. Recent recalibration of health burdens revealed that in the range of 4.5–70 million disability adjusted life years (DALYs) are lost to schistosomiasis. More people are infected with *S. haematobium* than with the other schistosomes combined. Of ~112 million cases of *S. haematobium* infection in sub-Saharan Africa, 70 million are associated with hematuria, 18 million with major bladder wall pathology, and 10 million with hydronephrosis leading to kidney damage [2–4]. In many patients, deposition of *S. haematobium* parasite ova eventually leads to squamous cell carcinoma (SCC) of the bladder [5,6]. Accordingly, *S. haematobium* has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) [7,8]. In addition, as many as 75% of women infected with *S. haematobium* suffer from female genital schistosomiasis (FGS) of the lower genital tract [3]. FGS results from

deposition of schistosome eggs in the uterus, cervix, vagina, and/or vulva, with ensuing inflammatory responses; it also increases susceptibility of the woman to HIV [9–11]. The resulting FGS is associated with contact bleeding, discharge, pain on intercourse, as well as diminished fertility, besides being a source of shame and stigma [12].

The cellular and molecular mechanisms linking *S. haematobium* infection either with both cancer induction and female infertility remain to be deciphered [12,13]. However, estrogen-derived molecules and estrogen receptor signaling pathways have been described for both associations. Accordingly, we review and discuss the general molecular mechanisms underlying estrogen metabolism, focusing on the hormones and receptors involved.

Molecular mechanism underlying estrogen metabolism

Estrogens are steroid hormones produced in the ovaries, adrenal glands, and placenta during pregnancy. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH and LH induce the production of estrogen in the form of estradiol and estrone by the ovaries. These estrogens bind to ERs in target tissues of the breast, uterus, brain, bone, liver, and heart [14]. When the estrogen molecule binds to its receptor, a conformational change in the ER permits its interaction with a specific regulatory sequence of the ER gene (estrogen responsive element), inducing the transcription of this target coding sequence. The resulting ER protein promotes changes in the cell according to tissue type and underlying conditions. The cycle is completed when high levels of estrogen in the blood send negative feedback to the hypothalamus to suppress the release of GnRH [14].

By the 1950s, most of the basic actions of the estrogenic hormones were recognized, such as their stimulation on the growth and function of tissues of the female reproductive tract. However, the biochemical processes involved were not entirely clear [15]. The generally accepted hypothesis was that the 17-hydroxyl group of estradiol underwent enzymatic oxidation from a cholesterol molecule using one coenzyme (NADH), and the resulting estrone was reduced using another (NADPH) [15]. The identification of the ER provided a mechanism to describe the target site specificity of estrogen action in the uterus, vagina, pituitary gland, and breast tissue [16]. Most importantly, a test was established to predict the outcome of antihormonal therapy in breast cancer, and a target was identified to develop new drugs for the treatment and prevention of breast cancer [16].

ERs and action of estrogen

Nuclear hormone receptors belong to a family of hormone-activated transcription factors that can initiate or enhance the transcription of genes containing specific hormone response elements [17]. The human ER, which belongs to this family, was cloned and sequenced from MCF-7 human breast cancer cells [17]. The human ER locus is located on chromosome 6q sub-band 25.1 [18] and the mouse ER is located on chromosome 10 [19,20]. The ER consists of 595 amino acids with a molecular mass of 66 kDa and includes six functional domains [20–22]; two of the domains are highly conserved among the members of the nuclear hormone receptor superfamily [20–22]. Two zinc fingers at the DNA-binding domain (DBD) of ER mediate receptor binding to hormone-response elements in the promoter regions of hormone-responsive genes. The hormone-binding domain (HBD), located at the ER C terminus, exhibits two regions of sequence homology with other hormone receptors. These regions confer hormone specificity and selectivity to ER [22–26].

More recently, another sequence belonging to the nuclear hormone receptor superfamily was cloned from a rat prostate cDNA library [27,28]. This sequence was named ERβ (as opposed to ERα). ERβ contains 485 amino acid residues and has a molecular weight of 54.2 kDa (Figure 1). There is a high homology between ERα and ERβ, mainly in the DBD (95%) and the HBD (55%), and both proteins bind estrogen with high affinity, bestowing functional homology. The latter has been determined by the activation of transcription of a vitellogenin A2, an estrogen-response element (ERE)-containing reporter plasmid in the presence and absence of estrogen [20,27].

The mechanism of target site specificity and selectivity seen with anti-estrogens, such as raloxifene, could be explained by the existence of two different ERs [29]. The receptor-specific regions are probably responsible for the differences seen between ERα and ERβ, in spite of the high homology in the conserved regions of both ERs [20].

Estrogen diffuses through the plasma membrane of cells where it binds to the ER. Once estrogen binds to the inactive ER, the receptor is activated, a conformational change and homodimerization occurs, and two receptor–ligand monomers dimerize and bind to the ERE. Once bound to the ERE, the ER uses activation functions (AFs) (AF-1 and AF-2) to stimulate transcription from

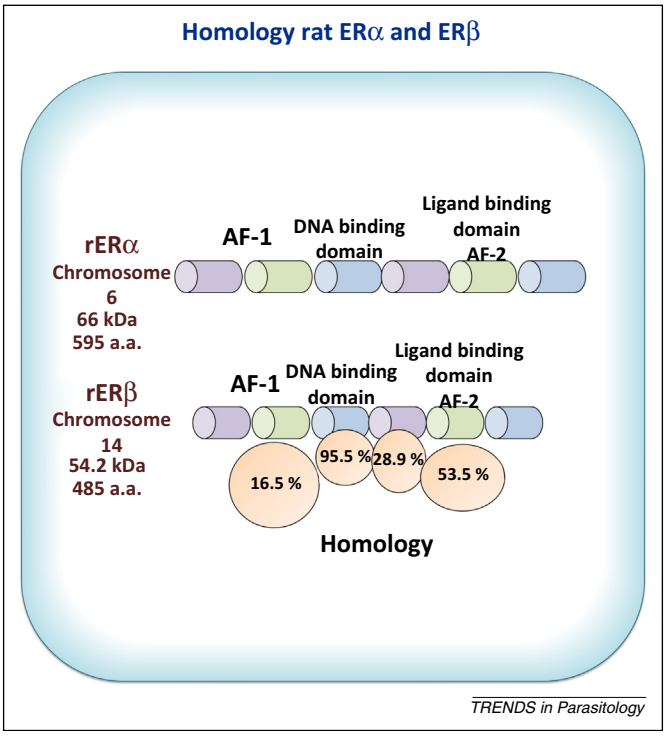

Figure 1. Comparison of the rat (r) ERα and rERβ proteins and percent amino acid homology in the functional regions (Adapted from [20]). Abbreviation: ER, estrogen receptor.

the promoter [20,30]. The ER contains two functional domain areas called AFs: AF-1 is located in the N-terminal region of the ER and AF-2 is located in the C-terminal region in the ligand-binding domain (LBD) of the ER. These are synergistic when the ER is activated by estrogen. Using mammalian cells, it was shown that the AF-1 and AF-2 regions, when expressed as separate polypeptides, functionally interact in response to estrogen and anti-estrogens, thus suggesting that estrogen binding to the ER facilitates a conformational change that brings AF-1 and AF-2 in direct association with one another, leading to synergy that results in transcriptional activation. These findings explained mechanistically the role of the two AFs in mediating hormone-regulated transcription [20,28]. The EREs are constituted by 13-bp palindromic sequences upstream the transcriptional start site. Binding of the ER to the corresponding ERE enhances the transcriptional rate of the gene in the target tissue, for example, breast [20,30].

ER mediates the biological effects of estrogens in a variety of target tissues. Ligand binding to ER stimulates gene transcription via interaction with EREs. ERs are known to mediate important physiological functions, such as reproduction, metabolism, maintenance of bone density and growth of estrogen-responsive tumors, including breast and endometrial cancers. Estrogens are also known to have a mitogenic effect in estrogen-responsive cells [31]. Progress in the regulation of the ER in breast cancer and anti-estrogen therapy has been reviewed [20].

S. haematobium-associated bladder cancer

SCC is a malignant, poorly differentiated neoplasm. SCC is the common form of bladder cancer in rural Africa where *S. haematobium* is prevalent [32,33]. By contrast, the majority of bladder cancer in developing countries and regions not endemic for urogenital schistosomiasis is transitional cell carcinoma (TCC), which arises from the transitional epithelium lining of the bladder. The parasite eggs trapped in the bladder wall release antigens and other metabolites (presumably evolved to expedite egress to the urine, and hence to the external environment). The phenomenon leads to hematuria and to chronic inflammation, in turn increasing the risk of SCC of the bladder. The epidemiological association between SCC of the bladder with schistosomiasis haematobia is based both on case control studies and on the correlation of bladder cancer incidence with prevalence of *S. haematobium* infection within diverse geographic locations. The incidence of urogenital schistosomiasis-associated SCC is estimated in 3–4 cases per 100 000 [34]. Schistosomiasis haematobia is a chronic infection. The adult, egg-producing schistosomes live for many years, re-infections frequently occur, and schistosomiasis associated bladder SCC appears relatively early, often by the mid-decades of life (TCC usually presents in the later decades of life). In its recent monograph, IARC confirmed that chronic infection with *S. haematobium* causes cancer of the urinary bladder [8].

While addressing schistosomiasis-induced hypogonadism in patients infected with *S. haematobium* and *S. mansoni*, Botelho *et al.* observed a noteworthy elevation in serum levels of estradiol, whereas those of LH and FSH remained normal, and hypothesized that the excess estradiol could be external to the host [13]. In fact, we found that the molecule responsible for the effect was an *S. haematobium*-derived estradiol-like molecule that is an antagonist of estradiol and thus repressed the transcriptional activity of the ER. Moreover, new estrogenic molecules were identified in *S. haematobium* total antigen as well as in the serum of infected individuals with this parasitic disease that seem to be produced by this parasite [35]. ER transcriptional activity was suppressed in urothelial cells and ER expression was also suppressed in the bladders of mice in response to *S. haematobium* [36].

Estrogenic molecules present in the egg extract of *S. haematobium* were also identified and characterized by liquid chromatography-mass spectroscopy (LC-MS). The majority of these compounds are catechol estrogens [1]. Catechol estrogens are formed by hydroxylation on the steroid aromatic ring A. Hydroxylation of both C-2 and C-3 on a steroid ring was apparent and suffered further oxidation into an estradiol-2,3-quinone. The genotoxic effects of estrogen metabolites might be attributed to oxidation of catechol estrogens to quinones, followed by redox cycling and formation of reactive oxygen species that in turn react with DNA [37,38] (Figure 2).

Given the context of the unarguable link between *S. haematobium* infection and bladder cancer, the presence of putative carcinogenic molecules in *S. haematobium* eggs hopefully may have practical consequences for new approaches to disease control [1,35]. Metabolism of estrogens and the production of depurinating estrogen–DNA adducts can be implicated in a pathway underlying *S. haematobium*-promoted host cell DNA damage, leading eventually to cell transformation. The carcinogenic effect of this estrogen–DNA adduct-mediated pathway could explain the link between chronic schistosomiasis haematobia and SCC of the bladder [1]. We anticipate that these findings will contribute to understanding how schistosomiasis haematobia leads to SCC of the bladder.

Schistosoma haematobium and infertility

Infertility is a common medical condition, affecting one in six couples (15–20%) worldwide [39]. Human and animal models have unraveled an association between estrogen insufficiency with abnormal spermatogenesis and male infertility [40–42]. Animal models reveal that ERα knockout (ERαKO) and double ERα/ERβ knockout (ERα/βKO) mice are infertile from puberty, and display atrophy of the testes and seminiferous tubule dysmorphogenesis, likely to lead to decreased spermatogenesis and sperm motility [43]. By contrast, ERβ knockout (ERβKO) mice are fertile and have no apparent reproductive alterations or morphologic changes [40,43].

Hormonal disturbances in women with FGS may be linked to infertility and suboptimal fecundity [11,44]. Recently, estrogen-like metabolites were detected by LC–MS in urine of *S. haematobium*-infected women. These metabolites are similar to those identified previously in the adult worm and egg stages of *S. haematobium* [1]. The presence of estrogen-like metabolites during FGS was statistically associated with self-reported infertility [11]. These electrophilic compounds can react with DNA

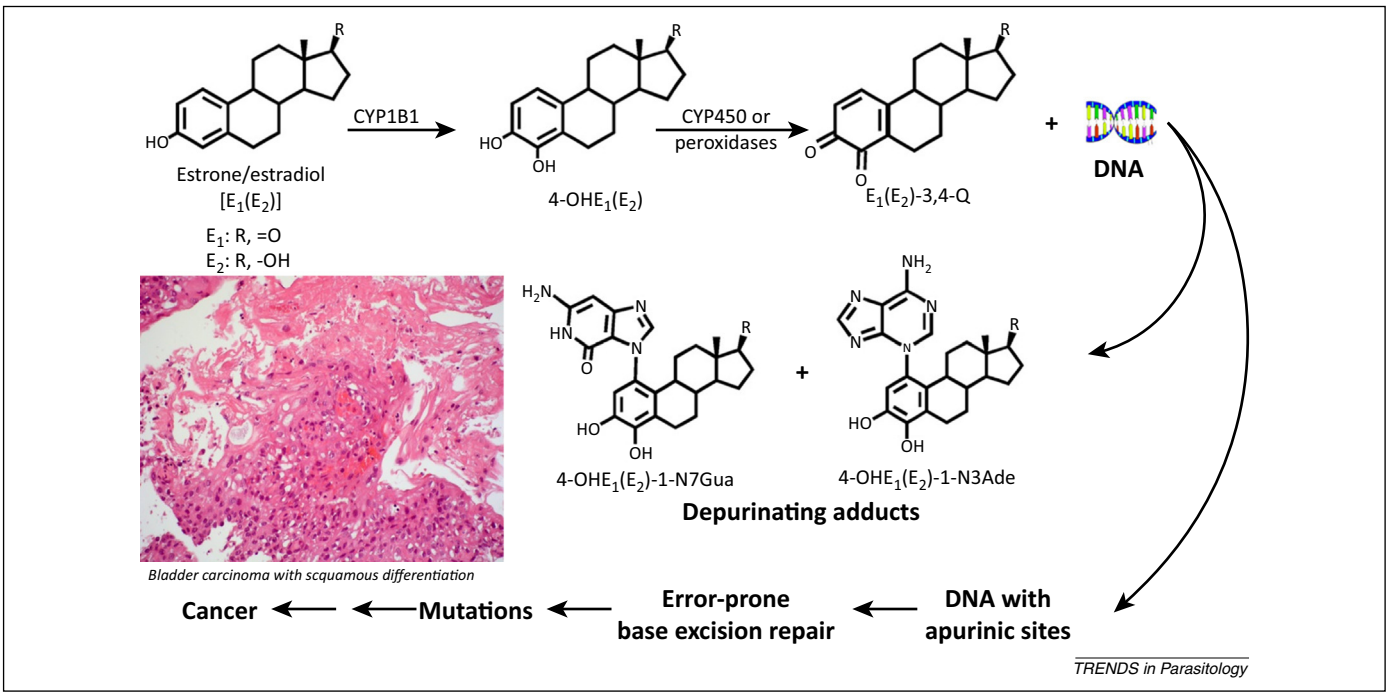

Figure 2. Major metabolic pathways in cancer initiation influenced by estrogens. The formation of catechol estrogens, that is, 2-hydroxy(OH)E1(E2) and 4-OHE1(E2) can lead through oxidation to semiquinones and quinones, for example, E1(E2)-3,4Q that eventually react with DNA to form depurinating adducts. Error-prone repair of the apurinic sites may lead to mutations that can initiate bladder carcinoma with squamous differentiation (microphotograph). (Adapted from [40]).

to form depurinating adducts. It is not inconceivable that apurinic sites in chromosomal DNA that result from this reaction generate mutations that might underlie infertility [11,44].

Concluding remarks and future perspectives

Studies are necessary to identify and characterize production of these estradiol-like moieties in schistosomes and ascertain the functions of the hormone in the developmental cycle of the blood fluke. Indeed, blocking the binding of this molecule to its receptor, with the use of antihormonal therapy such as ICI 182,780, a potent anti-estrogen with the ability to inhibit and downregulate ER [45], could be explored as a complementary therapy to schistosomiasis.

A functional estrogen transmembrane receptor, G protein-coupled receptor (GPR)30, modulates both rapid non-genomic events and genomic transcriptional events of estrogen. GPR30 promotes the progress of estrogen-related tumors through mitogen-activated protein kinase (MAPK) signaling pathways. Effects mediated by GPR30 are maintained when classic ERs are absent or blocked. In addition, GPR30 is involved in drug resistance, which is often occurring during cancer treatment. Hence, simultaneous blocking both GPR30 and classic ERs may be a better strategy for the treatment of schistosomiasis-related cancer and infertility [46].

It will be informative to assess the specific effects of the estrogenic molecules identified in the lysates of schistosomes and to evaluate activities of specific catechol estrogens identified in the schistosome eggs, either by using catechol estrogens purified from eggs of *S. haematobium* and/or synthetic versions of these putative carcinogens. In addition, given that the genome and transcriptome of eggs, female and male adult worms of *S. haematobium* are available, studies utilizing RNAi to silence components of estrogen catabolism pathways such as schistosome estradiol 17-β dehydrogenase and other candidate genes should be informative [47–49].

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