Fwd: Trends in Endocrinology and Metabolism - Editorial decision TEM-D-20-00037

Cecilia Santos <csantos@fcsaude.ubi.pt>
To: Jose Silva <jfcascalheira@yahoo.com>, Ana Raquel Costa <anarfcosta@outlook.pt>,
Inês Cruz <inesstcruz@gmail.com>, Mariana Lança de Oliveira <mariana.lanca.oliveira@gmail.com>,
Isabel Theriaga Gonçalves <igoncalves@fcsaude.ubi.pt>

Jun 22 at 7:39 PM
Print Raw message

Yesss! This is good news!

---------- Forwarded message ---------
De: Trends in Endocrinology & Metabolism Editorial Office <em@editorialmanager.com>
Date: segunda, 22 de jun de 2020, 17:34
Subject: Trends in Endocrinology and Metabolism - Editorial decision TEM-D-20-00037
To: Cecilia Santos <csantos@fcsaude.ubi.pt>

Dear Dr. Santos,

Thank you for submitting your manuscript entitled "The sex bias of cancer" to Trends in Endocrinology and Metabolism, and for your patience while waiting to hear from us. We sincerely appreciate the time and effort it took to put this work together. Please, also accept my apologies for the delay in communicating a decision on your manuscript. More time than usual was required to obtain reviewer reports for your work.

I have now heard from 2 expert reviewers regarding your manuscript, and their comments are included below. As you will see, they both felt the manuscript was interesting and well written. From an editorial perspective we feel that the piece is informative and timely. I do feel that reviewer comments are very constructive and if addressed will help make this article more complete and authoritative. However, given the main text of the manuscript is curring around 4500 words please try to maintain this without increasing the main text, when addressing the reviewer comments.
****
In closing, we invite resubmission of a paper that has been revised to address the reviewer and editorial comments,

Please ensure that your revised manuscript is accompanied by a separate list of all the major changes made and a detailed response to the comments made by the referees and the editor. For any comments that you did not address through modification of the manuscript, please explain why.

As we work with very tight timelines, I would be grateful if you could submit your revised manuscript by 13 Jul 2020. I hope this deadline is feasible for you. I would also be very grateful if you could acknowledge receipt of this message and confirmation of the suggested re-submission date by sending a brief message to tem@cell.com.

If you have any questions, please don't hesitate to get in contact. I look forward to receiving your revised manuscript.

Regards,
Matthew BEYMER, Ph.D.
Editor, Trends in Endocrinology & Metabolism
Follow TEM on Twitter
Journal metrics| IF2016 10.893 | SJR2015 6.017 | SNIP2015 2.26

Trends in Endocrinology and Metabolism

The sex bias of cancer

--Manuscript Draft--

Manuscript Number:	
Article Type:	Review
Keywords:	gastrointestinal cancers; glioblastoma; kidney cancer; bladder cancer; thyroid cancer; sex hormones; sex hormone receptors; pancreatic cancer; lung cancer
Corresponding Author:	Cecilia Santos, PhD University of Beira Interior Covilha, PORTUGAL
First Author:	Cecilia Santos, PhD
Order of Authors:	Cecilia Santos, PhD
	Ana Raquel Costa
	Mariana Lança de Oliveira
	Inês Cruz
	Isabel Gonçalves
	José Francisco Cascalheira
Abstract:	In cancers of hormone-dependent organs like women breast and reproductive organs, endometrium and ovaries, and men's prostate and testicular cancer, the roles of sex hormones and deregulation of hormone axes are well-documented. More strikingly, epidemiological data highlights significant differences between sexes in the incidence of various cancers in non-reproductive organs, where the role of sex hormones has been less studied. In an era when personalised medicine is gaining recognition, understanding molecular, cellular and biological differences between men and women is timely for developing more appropriate therapeutic interventions according to gender. In this review we show that sex hormones also shape much of the deregulated cellular and molecular pathways leading to cell proliferation and cancer in non-reproductive organs.
Suggested Reviewers:	Jessica Petrick jessica.petrick@nih.gov works in epidemiology of esophagic cancer
	Ignacio Camacho-Arroyo camachoarroyo@gmail.com has developed interesting work in the field of astrocytomas and progesterone receptors
	Leea Jeeyun jyunlee@skku.edu works in gastric cancer and estrogen receptors
	James Amos-Landgraf amoslandgrafj@missouri.edu produced relevant studies on the role of sex hormones in colon cancer
	Rodrigo Fortunato rodrigof@biof.ufrj.br works in thyroid and sex homones and cancer
	Shahrokh Shariat shahrokh.shariat@meduniwien.ac.at works with urothelial cancers and sex hormones
Opposed Reviewers:	

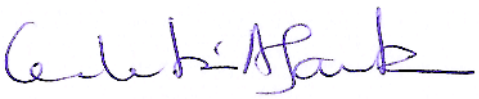
Cover Letter

Covilhã, 25th March 2020

Dear Dr Matthew BEYMER

The authors of the review article entitled *"The sex bias of cancer*" wish that it is considered for publication in *Trends in endocrinology and metabolism*. This review highlights the differences in cancer onset and progression between men and women in non-reproductive organs and attempts to explain these differences in the light of mechanisms regulated the sex hormones in the organs analyzed. Despite the clear differences in cancer prevalence between sexes this is still a largely unexplored subject and hope that this review will raise the interest in the study of the role of sex hormones in carcinogenesis in non-reproductive organs. We also hope that we were able to meet the high standards of TEM to have our work accepted for publication.

Yours sincerely,



Dr Cecília Santos, PhD

Associate Professor of Health Sciences with Habilitation in Biomedicine

Health Sciences Research Centre of University of Beira Interior (CICS-UBI), Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

E-mail: csantos@fcsaude.ubi.pt

Highlights

- Men are more prone to cancer onset and progression in non-reproductive organs than women
- The effects of sex hormones on the hallmarks of cancer depend on the target organs and studies often show divergent findings
- Sex hormone receptors, as well as hormonal levels, often correlate with cancer progression in non-reproductive organs
- Despite the clear sex bias of cancer, the mechanisms regulated by sex hormones that might explain the differences observed in cancer onset and progression between men and women are still poorly understood and upcoming studies should always distinguish between data obtained from men and women, and potentially address the effects of sex hormones on mechanisms known to activate carcinogenesis, either *in vivo* in animal models of disease or *in vitro*.

Title

The sex bias of cancer

Ana Raquel Costa[1], Mariana Lança de Oliveira[1], Inês Cruz[1], Isabel Gonçalves[1], José Francisco Cascalheira[1,2], Cecília R.A. Santos[1,*]

[1]CICS-UBI - Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal

[2]Department of Chemistry, University of Beira Interior, Covilhã, Portugal

*Corresponding author:

Dr Cecília R. A. Santos

CICS-UBI – Health Sciences Research Centre, University of Beira Interior

Av. Infante D. Henrique, 6200-506 Covilhã, Portugal

E-mail: csantos@fcsaude.ubi.pt

Telephone: +351 275329048

Running title

The sex bias of cancer

Keywords: gastrointestinal cancers, glioblastoma, bladder cancer, thyroid cancer, sex hormones

Abstract

In cancers of hormone-dependent organs like women breast and reproductive organs, endometrium and ovaries, and men's prostate and testicular cancer, the roles of sex hormones and deregulation of hormone axes are well-documented. More strikingly, epidemiological data highlights significant differences between sexes in the incidence of various cancers in non-reproductive organs, where the role of sex hormones has been less studied. In an era when personalised medicine is gaining recognition, understanding molecular, cellular and biological differences between men and women is timely for developing more appropriate therapeutic interventions according to gender. In this review we show that sex hormones also shape much of the deregulated cellular and molecular pathways leading to cell proliferation and cancer in non-reproductive organs.

1. Cancer in non-reproductive organs is sex-biased

Despite the progress in diagnosis and therapy, the global cancer burden is still daunting. Recent reports from the World Health Organization show that cancer new cases and deaths have risen to 18.1 million and 9.6 million in 2018, respectively.

In Europe, the incidence pattern (Figure 1) is dominated by prostate and breast cancers in men and women, respectively, followed by lung and colorectal cancers. Bladder, stomach, and kidney cancers also contribute to these figures, especially in men, while in women cervical and uterine cancers also have incidences greater than 10 cases per 100,000 persons. Breast, prostate and colorectal cancers account for half of all cancers in Europe. These data also show that there are clear gender differences in the incidence and mortality caused by tumours originated in different non-reproductive organs, particularly lung, colorectal, bladder, stomach, kidney, pancreas and thyroid gland cancers. Of these, only thyroid cancer has higher incidence in women [1].

This review correlates the effects of sex hormones (SHs) and their receptors in the regulation of the mechanisms underlying the onset and progression of cancers in non-reproductive organs to explain the cancer sex-bias put in evidence by epidemiological data.

Cancers of the Digestive Tract

Esophageal Cancer

The incidence of esophageal cancer (EC) is 3-4 times higher in men than in women [2]. Large population studies support a protective action of estrogens

against EC. Other population-based studies (Box 1) also analysed the presence and abundance of **estrogen receptors** (ER; see Glossary) α and β in EC biopsies. In a study where EC patients also presented lower levels of **estradiol** (E2) [3], the expression of ERβ was approximately twice that of ERα, and was higher in the primary stages of the lesions. Moreover, the number of ERβ positive cells correlated positively with worst prognosis. In another study, ERα and ERβ mRNA expression were significantly higher in EC tumour tissues and correlated inversely with survival outcome. Upregulation of ERα mRNA correlated with higher pathological T-stage and lymph node metastasis, while ERβ mRNA upregulation correlated with positive vascular invasion [4].

In vitro studies performed in an EC cell line model (ECGI-10) showed that E2 or the ERβ-specific agonist diarylpropionitrile stimulated proliferation of ERβ transfected cells but had no effect on the proliferation of ERα-transfected cells [3]. At first, these findings raise a contradiction with populational studies, where estrogens seem to protect against EC, or they may suggest that the anti-cancer effects of estrogens are not mediated by ER stimulation, as ERβ-expressing EC cells might not be protected from carcinogenesis. Concurrently, in other cell models of EC (OE33 and OE19 cell lines), a concentration-dependent inhibition of proliferation was induced by a highly selective ERα antagonist and an ERβ-specific antagonist through stimulation of apoptotic caspase activity and induced cell death [4]. Thus, the anti-cancer effect of E2 in EC might be independent from ER activation and require that ERs are expressed at lower levels. In line with these findings, other authors have proposed that the anti-proliferative effect of estrogens may rely on 2-methoxy E2 (2-ME), an endogenous E2 metabolite with anti-tumour activities. *In vitro* studies performed in well-differentiated EC9706

cells showed an antiproliferative effect of 2-ME resulting from increased apoptosis, accompanied by cell cycle blockade in the G2/M phase [5]. Other studies with this compound reinforce its pro-apoptotic effect by increasing the Bax/Bcl-2 ratio [6]. In addition, in Barret's esophageal adenocarcinoma, 2-ME also lead to a reduction in the migratory/invasive cell behaviour mediated by β-catenin/E-cadherin signalling pathways [7]. Concurrently, a new water-soluble prodrug of 2-ME2 proved to be effective as antiproliferative and anticancer agent in both *in vitro* and *in vivo* studies and showed great potential in inhibiting the EC xenografts' growth. The anticancer effect of 2-ME2 and its prodrug derivative is likely to result from disruption of the microtubule network [8]. Also, an estrogen analogue compound (ESE-16) has been designed and tested for *in vitro* antiproliferative potential in an EC cell line and was able to decrease cell density, induce metaphase blockage and increase autophagy and activate the intrinsic pathway of apoptosis [9].

On the other hand, the balance between the estrogenic and androgenic environment may also result from disruption of androgenic pathways. Some studies have demonstrated that **androgen receptors** (ARs) are also expressed in esophageal tumours [10], but information on *in vivo* and *in vitro* AR signalling in EC is still very scarce. Even so, the evidences suggest that androgens favour proliferation and that this effect is consistent with receptor expression patterns. The first *in vitro* study showing that EC cell lines respond to androgens was performed using **dihydrotestosterone** (DHT) stimulation. It was shown that lower DHT concentrations lead to the proliferation of AR-expressing cell lines and induced changes in the expression of androgen-responsive genes. These results were dependent on the concentration of DHT or the presence of a permissive

microenvironment, consistent with similar observations in tumoral tissues [11]. However, analysis of the expression of AR in human biopsies have been inconclusive and divergent [12].

Gastric Cancer

The incidence of gastric cancer (GC) is twice as high in men than women. One of the main risk factors is *Helicobacter pilori* infection, which also seems to have a preference for the male stomach [13]. A review paper has attempted to find an explanation for the predominance of the disease among men, considering that it relies on the low estrogen levels, compared to women, as higher long-life exposure to estrogens reduces the risk of GC (Box 1). These observations were corroborated by an ovariectomized female mice study that presented higher risk of GC, which decreased with estrogen treatment [3].

The protective effects of estrogens, however, seem to depend on estrogen receptors, since both ERα and ERβ are present in the gastric mucosa. Although some researchers have shown that ERβ is more abundant in GC tumours, both ERα and ERβ are expressed therein [3]. The mechanism underlying this protection against GC appears to be associated with increased expression of trefoil factor proteins that protect the mucosa, and inhibit c-erb-2 oncogene expression [3]. A study on a Chinese population with GC demonstrated that ERα inhibition reduced GC cell proliferation, migration and invasion by modulating the expression of p53, p21, p27, cyclin D1 and E-cadherin, highlighting a direct association between ERα-positive GC and worse prognosis, which may perhaps be used as a prognostic marker and/or as a therapeutic target [19].

ERα36 is a variant of the full-length 66 kDa ERα66, which lacks the transcriptional activation domains (AF-1 and AF-2) but retains the partial dimerization and ligand-binding domains and DNA-binding domain, mediating non-genomic effects of estrogens important for invasion and metastasis to lymph nodes. In a study of 45 GCs samples analysed by real time PCR, decreased ERα36 expression levels correlated with increased tumour size [14]. Contrastingly, studies using SGC7901 cells, revealed increased cell proliferation associated with non-genomic effects of E2 mediated by ERα36, resulting from the activation of the c-Src signalling pathway [3,15]. Moreover, high ERα36 expression levels in these cells resulted in estrogen hypersensitivity, high growth rates, and high levels of cyclin D1 unlike ERα36-silenced cells [16]. ERα36 silencing in SGC7901 cells reduced the levels of phosphorylated glucose regulated protein 94 and Akt. Therefore, the **Akt signalling pathway** is potentially relevant in ERα36-GRP94-mediated gastric carcinogenesis [15]. Contrastingly, it was shown that ERα overexpression inhibited proliferation, blocked cell entry into G1/G0, promoting apoptosis and reducing invasive capacity of GC cells, perhaps due to a decrease in β-catenin [15].

In a 2014 study, Yi *et al.* demonstrated that E2 increased proliferation in ERα-positive cells but has no effect on ERα-negative cells. When ERα was inhibited by fulvestrant and siRNA, E2-induced proliferation in ERα-positive cells was suppressed, and the ERα suppression by fulvestrant, paclitaxel and siRNA increased the expression of E-cadherin, a crucial factor in diffuse type carcinogenesis [17]. In opposition, E2 treatment in SGC7901 and BGC823 cells expressing ERα36*,* reduced survival rates and viability due to increased apoptosis via caspase-3 activation and decreased in Bcl-2 and Bcl-xl levels [18].

In conclusion, the role of ERα in mediating the effects of E2 on GC is controversial and deserves further clarification.

Regarding ARs, reduction in their levels suppressed migration and invasion as well as epithelial-mesenchymal transition [19].

Colorectal Cancer

Another example of gender bias are colorectal cancers (CRC), which also are more prevalent in men than women (Box 1). In mice carrying mutations in the adenomatous polyposis coli gene, estrogen protects females from CRC development, an effect that is lost upon ovariectomy. Male rats exposed to **testosterone** after orchiectomy are also at higher risk than those that were orchiectomized but received placebo treatment only. Testosterone also plays similar roles in human CRC [20,21]. Some observations suggest that **progesterone** (P4), rather than E2, appears to reduce the risk of CRC in women [22], however the mechanisms underlying these observations are still poorly studied. E2 treatment reduces cell proliferation by modulating expression of cyclins A and D1 and prevents cell migration by inhibiting expression of plasminogen activators and matrix metalloproteinases in the LoVo cell line [23]. E2 may also increase apoptosis in COLO-205 human CRC cells by decreasing the levels of c-myb and Bcl-2 [24]. In an early CRC animal model (polyposis in the rat colon; Pirc), males develop twice as many adenomas as females. In addition, male wildtype mice injected with the azoxymethane carcinogen also developed more colon adenomas than females. Ovariectomized females showed no differences in comparison to males, even when they received P4 and E2 together or separately. In contrast, the reduction of androgens by castration

protected Pirc mice from the development of adenomas, while testosterone treatments reversed the effects. As the AR could not be detected in either the normal colon or adenomas, these effects were found to be independent of the receptor and suggest that testosterone is the principal responsible for differences in susceptibility between males and females [25].

Central Nervous System Tumours

Men have increased susceptibility to astrocytomas, particularly to primary glioblastoma (GBM), the most aggressive form of astrocytoma (Box 3). The different hormonal backgrounds between men and women might underlie these differences [26]. These tumours are known to express estrogen, progesterone and androgen receptors, and generally higher-grade tumours are associated with decreased ER expression and increased **progesterone receptor** (PR) expression [27,28]. This is consistent with a study where higher PR expression was associated with poorly differentiated tumours like GBM and anaplastic astrocytoma, in contrast to the low levels of PR observed in benign astrocytomas [27,29].

Like other tumours, GBM can also deceive the immune system by facilitating its self-progression, where P4 may also play an important role because of the expression of progesterone-induced blocking factor, an immunomodulatory protein involved in immune response regulation in the reproductive system [30]. Moreover, an *in vivo* study showed that P4 caused an increase in tumours and infiltration area in the cortex of male rats injected with GBM cells (U-87MG cell line), contrarily to animals in which PR was silenced, concluding that progesterone favours GBM growth and invasive capacity via PR in this animal

model [29]. In an *in vitro* study, P4 was combined with temozolomide (TMZ), the most commonly used chemotherapeutic agent for GBM treatment, to assess whether P4 increases the anti-tumour TMZ effect and reduces its side effects. They demonstrated that both P4 and TMZ alone induced tumour cell death, and that the combined administration potentiated the TMZ effect. Moreover, an analysis of intracellular pathways showed that both P4 alone or in combination with TMZ suppressed the PI3K/Akt/mTOR pathway and the expression of O-6-methylguanine-DNA methyltransferase-DNA repair protein in U-87MG cells, suppressing cell proliferation and migration [29]. More recently, three studies carried out in animal models evaluated the effects of P4 or its analogues. Elmaci *et al.* (2019) demonstrated that medroxyprogesterone acetate inhibited the tumour growth in a C6 rat glioma cell model, an effect that was increased by tibolone (P4 analogue) and TMZ [31]. Similar results were obtained in an orthotopic GBM mouse model administrated with different P4 concentrations, leading to a decrease in tumour size as well as to an increase in animal survival [32,33]. In conclusion, the effects of P4 on GBM are still controversial: some studies report pro-tumour effects while others report anti-tumour actions of P4. The differences between these studies might be due to the use of different GBM models, hormone concentrations or other experimental conditions.

Regarding E2, some other evidences suggest that ERα-mediated effects are oncogenic, while ERβ-mediated effects function as tumour suppressors. Both receptors are present at low levels in primary GBM. A pilot study demonstrated that E2 levels were higher in GBM than in grade II and III astrocytoma. However, the study also showed that the number of arotamase- and ER-positive cells inversely decreased with the tumour malignancy [27]. On the other hand, an *in*

vivo study assessing the action of different concentrations of E2 administration to immunodeficient rats previously injected with GBM tumour cells, showed that E2 increased both male and female rats' survival, and this effect was observed since the early stages of tumour progression, possibly by increasing apoptosis [34].

Although the highest incidence of brain tumours occurs in men, studies evaluating the effects of androgens are extremely rare. A study showed that there is a clear AR overexpression in GBM compared to adjacent non-tumour tissues. AR expression was also detected in 8 GBM cell lines, in particular U-87MG, in which the pro-apoptotic and anti-proliferative effect of transforming growth factor beta was reduced by DHT, suggesting that the AR-mediated signalling pathway promotes GBM tumorigenesis [28]. More recently, an increase in cell migration, invasion and proliferation was observed in three GBM cell lines incubated with increasing testosterone concentrations [35].

Thyroid Cancer

Thyroid cancer (TC) is 2.9 times more common in women than in men, although less aggressive subtypes are more common in women (Box 3). Gender disparity in incidence, aggressiveness and prognosis is well-established for this type of cancer, but the underlying causes are still poorly studied. Despite the higher levels of TC found in women, population-based studies show that reduced estrogen exposure favour TC malignancies [40]. These observations suggest that broader exposure to female SHs may prevent the disease, but the underlying mechanisms remain unclear. However, other studies claim that the higher incidence of TC in women may also be related to estrogen signalling. Hydrogen peroxide (H_2O_2) is produced in large quantities in the thyroid and regulates cell

proliferation, migration, survival and death, and there are evidences of sexual dimorphism in the redox balance of thyrocytes characterized by high production of H_2O_2 due to high expression of inflammatory proteins, accompanied by a weakened thyroid antioxidant enzyme, activity in both TC cells (PCCL3) and female adult rats. These data suggest that estrogen-induced redox imbalance may contribute to thyroid dysfunction, including cancer [36]. A recent review summarizes current knowledge of the mechanisms underlying the effects of E2 on TC [12]. Findings supporting a carcinogenic effect of estrogens on TC cells include the hormonal activation of the PI3K pathway and the repression of p27 expression, via the non-genomic pathway associated with the G protein-coupled receptor 30, thus inducing cell proliferation by increasing c-Fos and cyclins A and D1 [12]. However, signalling may also involve nuclear receptors ERα and ERβ. Kumar *et al.* suggested that E2 favours ERα expression over ERβ, contributing to TC cells proliferation and growth [37]. This imbalance can alter the overall behaviour of TC cells, giving them the ability to proliferate and survive by increasing ERK1/2 activity [38]. Therefore, the ERs ratio on TC can provide a better view of prognosis as well as development of new and more efficient therapeutic strategies [12]. Indeed, histological studies on TC show a difference in the expression of ER subtypes and that the response of cells to estrogen is dependent on the specific ER expressed in TC cells. However, what determines tumour-specific SH receptor expression is unclear [39]. Estrogens promote stem cell renovation and as such may be involved in tumour initiation and induction of cell proliferation and stem cell differentiation in thyroid papillary cancers both *in vitro* and *in vivo*. Mice inoculated with papillary TC stem cells develop larger tumours than controls when treated with E2. The cells' own ability to migrate is

stimulated by E2 pre-treatment, allowing these cells to migrate to organs farther than vehicle treated cells [40]. These observations may at least partially justify the higher incidence of TC in women.

Kidney Cancer

A potential hypothesis to explain the higher susceptibility of men to kidney cancer (KC) (Box 2) relies on X chromosome-encoded genetic mutations, since these are more prevalent in male-derived tumours [41]. Gender-specific mutations of genes involved in tumorigenesis, like the ubiquitin carboxyl-terminal hydrolase BAP1, that promotes protein degradation important for cell survival and death have been largely described in KC [42,43].

A recent review concluded that the inhibition of most sex-related hormonal pathways could have an important role in supporting therapies against KC. For example, luteinizing and follicle-stimulating (FSH) hormone overexpression seems to promote angiogenesis, while FSH alone promotes metastatic formation [44]. This study also reported that high levels of glucocorticoid and androgen receptors are indicators of tumour suppression. On the other hand, overexpression of ERα increases the transcription of the hypoxia-inducible factor 1-alpha while ERβ has the opposite effect [44]. Concurrently with the predominance of KC in men, there is indication that estrogens may have a protective role against KC via ERβ, while androgens favour KC cell proliferation. Some authors showed that E2 inhibits KC growth through the activation of both apoptosis and autophagy pathways [45], and that it triggers DNA repair and reactive oxygen species production, thus reducing the viability of KC cells [46]. On the other hand, DHT promotes cell proliferation through the activation of signal

transducer and activator of transcription 5 via androgen and glucocorticoid receptors. Concurrently, the inhibition of these receptors showed antitumor activity, highlighting their potential as therapeutic targets in KC [47].

Bladder Cancer

Cigarette smoking is the best-established risk factor for bladder cancer (BC) [48–50], and was considered the major cause of BC in men (Box 2). However, smoking habits are not sufficient to explain the effect of gender on BC and urinary tract cancers incidence and outcomes, and only a minority of BC cases are attributable to environmental exposures [48].

Although the bladder is not a sex organ and BC is not considered an endocrine-related cancer, several authors have agreed that SHs, and particularly their binding to receptors, might also contribute for BC development [48–51]. The disparities between sexes could be explained by the higher levels of circulating androgens in males than females, resulting in increased AR expression and signalling, especially at early stages of tumour development and progression [52]. Moreover, testosterone can be converted to DHT by the enzyme 5α-reductase in BC, although the enzyme expression is lost in higher grade tumours [48].

Several authors described the presence of higher levels of AR in tumours and metastatic lesions than in control tissues [48–51,53]. AR activation has been correlated with chemoresistance in BC cells [51], and AR translocation to the nucleus after androgen stimulation can result in the activation of the PI3K/Akt pathway activation, known by its central role in cancer progression and pro-tumorigenic effects [54,55]. Moreover, AR may also promote carcinogenesis

through the β-catenin, cyclin-D and EGFR signalling pathways, associated with aggressive forms of BC [49].

In vivo evidence highlighting a crucial role for AR in BC development and progression was obtained in an AR knockout (ARKO) mouse model. In their study, only castrated ARKO mice supplemented with DHT and wildtype developed N-butyl-N-(4-hydroxybutyl)nitrosamine-induced tumours, but neither intact male or female ARKO mice developed BC after 12-weeks of exposure to the compound. Moreover, both androgen deprivation and ARKO suppressed cell proliferation and increased apoptosis *in vitro* [48,51,53]. These results were corroborated by other authors, who have shown that the AR antagonists enzalutamide, flutamide and bicalutamide, inhibited BC incidence and/or progression [51]. However, conflicting studies showed no significant correlation between AR expression and tumour grade, or even a decrease in AR expression with increasing stage of BC [48–51,53].

Various single nucleotide polymorphisms have also been associated with increased BC risk. For example, a reduction in AR binding affinity to the androgen response element on the prostate stem cell antigen gene might stimulate tumour growth and metastasis through androgen-independent pathways [48,49]. It has been proposed that an earlier loss of AR reactivity may be caused by lower androgen levels in women, which in turns leads to more aggressive BC tumours [48–50]. These results were corroborated by other studies, where AR expression was lost in more advanced BCs, although there was no association with patient survival [48–51,53].

The overall role of AR in BC remains controversial, and although androgen signalling, rather than AR expression levels, may be primarily responsible for the

men predisposition to BC, AR expression may be less relevant in females [51,53]. Other studies also reported non-androgen-mediated AR activation and non-AR pathway androgen-induced bladder tumorigenesis [48,51,53].

Apart from androgens, estrogens also play important roles in BC development and progression, by exerting both stimulatory and inhibitory actions dependent on the functional activity of ERα and ERβ [48,53]. Most studies refer that ERα loss is associated with BC advanced stages, since it could act as inhibitor of tumour development [49,53]. This result was confirmed by a study performed in an ERα-knockout mouse model, where the loss of ERα in both male and female mice resulted in earlier development and higher incidence of carcinogen-induced BC [53].

ERβ expression is abundant in both normal urothelial and bladder cancer cells, and also in both low- and high-grade tumours, irrespective of gender [48,49,51,53]. Because of the interactions with various growth factors and its key role in several signalling pathways associated with tumorigenesis and cancer progression, there might be a correlation between high expression levels of ERβ and invasive and high-grade tumours and worse prognosis [48–50,53]. However, the role of ERβ in BC remains controversial. Some studies showed an inhibitory effect on cell proliferation while others claim that the expression of this receptor might promote BC [53,56–58]. *In vitro* and *in vivo* experiments showed that ERβ knockdown in non-malignant urothelial cells conferred resistance to carcinogens [53]. Thus, ERβ has been studied as a potential tumour promotor in BC.

Several *in vitro* studies showed that anti-estrogen treatments lead to a reduction on BC incidence [48,49,53]. Furthermore, *in vivo* animal experiments also reported that selective ER modulators, like tamoxifen and raloxifene, could inhibit

BC progression [48,49,51,53]. The effect of tamoxifen treatment was also assessed by two phase II clinical trials (NCT00589017 and NCT02197897), but only inconclusive data are still available. Overall, it appears that estrogens may protect against or inhibit BC development, but afterwards, at more advanced stages they might support tumour progression.

Although the expression of SH receptors seems to vary according to BC grade, they are similarly expressed in males and females, which suggests that gender disparities in BC incidence and prognosis might be associated with other factors. It has been shown that women at older age at menarche, multiparous, and with hormone replacement therapy were at decreased BC risk, contrary to postmenopausal women that seem to be at higher risk of BC development [48–50]. Moreover, gender-specific expression of uridine-5'-diphospho-glucuronosyltransferases at the molecular level, could lead to differences in the degradation of carcinogens, resulting in differential exposure of the urothelium to carcinogens [48–50]. Also, glutathione S-transferase M1 expression contributes to gender-specific differences in BC risk, since a higher susceptibility was found in a null genotype among cigarette-smoking women [48,50]. Sex chromosomal roles also have been investigated. Bladder cancer sex differences are unlikely associated with the Y chromosome, since its loss increases cancer risk, whereas the extra copy of X chromosome among XX individuals is likely to confer better protection against cancer [59,60].

Lung Cancer

The higher incidence of lung cancer (LuC) in men (Box 3) has been largely attributed to higher cigarette smoking habits among men but SHs may also have their contribution [61].

A study performed by Dou *et al.* (2017) showed that ovariectomized mice with decreased hormonal levels as well as significant reduction of protein and mRNA expression of SHs receptors, presented increased tumour growth [62]. Also, PR expression in tumour-surrounding stromal cells has been associated with improved survival for both male and female patients, but the positive PR expression in tumour epithelial cells is an independent and unfavourable predictor for survival in female patients [63]. An international pooled analysis of premenopausal women showed increased risks associated with parity and the number of children, that decreased with breastfeeding. Although menstrual and reproductive factors may play a role in LuC genesis, smoking remains the most important modifiable risk factor [64].

Regarding male hormones, higher testosterone or DHT levels have been associated with increased LuC incidence, especially in older men, where high levels of these hormones predict LuC incidence over the next decade [65]. There has also been recently found that androgens and AR promote M2 macrophage polarization, associated with asthma and prostate cancer, which might also indicate their association with LuC [66]. However, further investigation needs to be performed to confirm this hypothesis.

Concluding Remarks and Future Perspectives

There are clear differences in the incidence of cancers in non-reproductive organs between men and women. Part of these disparities may be attributed to the levels of SHs and to SH receptors (Table 1, Key Table).

Testosterone acts as an inducer of tumour cell proliferation and migration, thus often contributing to the highest incidence of most cancers in men. On the other hand, female sex hormones P4 and E2 may decrease the proliferation and migration of tumour cells, mainly by triggering apoptosis, therefore reducing the risk of tumour development in some settings. In particular, the E2 effects, depending on the organs might be different or even opposite. In digestive system tumours of esophagus, stomach, and colon, E2 decreases cell proliferation, associated with enhanced apoptosis and autophagy. In GBM and kidney tumours, similar anti-cancer effects of estrogens are described, whereas in thyroid and bladder cancers, the effects of estrogens are more controversial (Table 1).

The role of SH receptors has also been studied, and their expression has been assessed in tumours of several non-reproductive organs. Most breast cancers overexpress ERs and PRs. In breast cancer, receptors are also current therapeutic targets that contributed to the increased survival of women with hormone receptor-positive breast cancer [67]. Herein we observed that most of the tumours in non-reproductive organs also present higher levels or overexpression of ERα and ERβ (Table 1). Similar observations were reported for AR expression, whose critical role in the prostate cancer is well-established, since its increased expression is one of the mechanisms that result in cancer cell proliferation [68]. In the non-reproductive organs analysed, ERα appears to be more oncogenic than ERβ, particularly in GC and GBM (Table 1).

Regarding PR, that has been less studied, there are evidences that its expression increases in high-grade tumours. Finally, AR and androgen signalling seem more relevant in colorectal and bladder cancers (Table 1).

Overall, despite the clear differences in the incidence of these cancers between men and women, the reasons underneath are still poorly understood (see Outstanding Questions). The mechanisms underlying the observed differences should be further explored, so that gender-specific targeted therapies can be developed against these cancers taking into account not only the sex of the individuals but also the hormonal status and the levels of sex hormone receptors in these tumours, as it is already done in the treatment of breast and prostate cancers.

Acknowledgements

This work was supported by the Portuguese Foundation for Science and Technology (FCT, Portugal) project grants (PTDC/BIM-ONC/7121/2014, UID/Multi/00709/2013 and UID/Multi/00709/2019), by CENTRO 2020 and Lisboa 2020 project grant (POCI-01-0145-FEDER-016822), and FEDER funds through the POCI – COMPETE 2020 – Operational Programme Competitiveness and Internationalization in Axis I – Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491). Ana R. Costa and Inês Cruz are recipients of a grant from ‘‘Programa Operacional do Centro, CENTRO 2020” through the funding of the ICON project (Interdisciplinary Challenges On Neurodegeneration; CENTRO-01-0145-FEDER-000013)”.

References

1 Bray, F. et al. (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA. Cancer J. Clin. 68, 394–424

2 Malhotra, G.K. et al. (2017) Global trends in esophageal cancer. J. Surg. Oncol. 115, 564–579

3 Chen, C. et al. (2019) The roles of estrogen and estrogen receptors in gastrointestinal disease (Review). Oncology Letters, 18, 5673–5680

4 Al-Khyatt, W. et al. (2018) Selective oestrogen receptor antagonists inhibit oesophageal cancer cell proliferation in vitro. BMC Cancer 18, 121

5 Du, B. et al. (2012) Effects of 2-methoxyestradiol on proliferation, apoptosis and gene expression of cyclin B1 and c-Myc in esophageal carcinoma EC9706 cells. Cell Biochem. Funct. 30, 158–165

6 Joubert, A. et al. (2005) Bax/Bcl-2 expression levels of 2-methoxyestradiol-exposed esophageal cancer cells. Biomed. Res. 26, 131–134

7 Kambhampati, S. et al. (2010) 2-methoxyestradiol inhibits Barrett's esophageal adenocarcinoma growth and differentiation through differential regulation of the β-catenin-E-cadherin axis. Mol. Cancer Ther. 9, 523–534

8 Kambhampati, S. et al. (2013) A Second-Generation 2-Methoxyestradiol Prodrug Is Effective against Barrett's Adenocarcinoma in a Mouse Xenograft Model. Mol. Cancer Ther. 12, 255–263

9 Wolmarans, E. et al. (2014) Novel estradiol analogue induces apoptosis and autophagy in esophageal carcinoma cells. Cell. Mol. Biol. Lett. 19, 98–115

10 Xie, S.H. and Lagergren, J. (2016) The Male Predominance in Esophageal Adenocarcinoma. Clin. Gastroenterol. Hepatol. 14, 338–347.e1

11 Palethorpe, H.M. et al. (2017) Androgen Signaling in Esophageal Adenocarcinoma Cell Lines In Vitro. Dig. Dis. Sci. 62, 3402–3414

12 Hima, S. and Sreeja, S. (2016) Modulatory role of 17β-estradiol in the tumor microenvironment of thyroid cancer. IUBMB Life 68, 85–96

13 Ibrahim, A. et al. (2017) Sex-differences in the prevalence of Helicobacter pylori infection in pediatric and adult populations: Systematic review and meta-analysis of 244 studies. Digestive and Liver Disease 49, 742–749

14 Wang, J. et al. (2012) Expression of ERα36 in gastric cancer samples and their matched normal tissues. Oncol. Lett. 3, 172–175

15 Rahman, M.S.U. and Cao, J. (2016) Estrogen receptors in gastric cancer: Advances and perspectives. World J. Gastroenterol. 22, 2475–2482

16 Wang, X. et al. (2014) Biphasic ER-α36-mediated estrogen signaling regulates growth of gastric cancer cells. Int. J. Oncol. 45, 2325–2330

17 Yi, J.H. et al. (2014) Anti-tumor efficacy of fulvestrant in estrogen receptor positive gastric cancer. Sci. Rep. 4, 1–8

18 Qin, J. et al. (2014) The direct effect of estrogen on cell viability and apoptosis in human gastric cancer cells. Mol. Cell. Biochem. 395, 99–107

19 Tang, W. et al. (2017) Expression of estrogen receptors and androgen receptor and their clinical significance in gastric cancer, Oncotarget. 8, 40765–40777

20 Luo, S. et al. (2016) Diabetes mellitus increases the risk of colorectal neoplasia: An updated meta-analysis. Clin. Res. Hepatol. Gastroenterol. 40, 110–123

21 Roshan, M.H.K. et al. (2016) The role of testosterone in colorectal carcinoma: pathomechanisms and open questions. EPMA J. 7, 22

22 Lin, J.H. et al. (2013) Association between sex hormones and colorectal cancer risk in men and women. Clin. Gastroenterol. Hepatol. 11, 419–424.e1

23 Hsu, H.-H. et al. (2014) Estradiol agonists inhibit human LoVo colorectal-cancer cell proliferation and migration through p53. World J Gastroenterol 20, 16665–16673

24 Wilkins, H.R. et al. (2010) Estrogen prevents sustained COLO-205 human colon cancer cell growth by inducing apoptosis, decreasing c-myb protein, and decreasing transcription of the anti-apoptotic protein bcl-2. Tumor Biol. 31, 16–22

25 Amos-Landgraf, J.M. et al. (2014) Sex disparity in colonic adenomagenesis involves promotion by male hormones, not protection by female hormones. Proc. Natl. Acad. Sci. U. S. A. 111, 16514–16519

26 McKinley, B.P. et al. (2000) The impact of age and sex on the incidence of glial tumors in New York state from 1976 to 1995. J. Neurosurg. 93, 932–939

27 Tavares, C.B. et al. (2016) Expression of estrogen and progesterone receptors in astrocytomas: A literature review. Clinics 71, 481–486

28 Yu, X. et al. (2015) Androgen receptor signaling regulates growth of glioblastoma multiforme in men. Tumor Biol. 36, 967–972

29 Altinoz, M.A. et al. (2019) Reproductive epidemiology of glial tumors may reveal novel treatments: high-dose progestins or progesterone antagonists as endocrino-immune modifiers against glioma. Neurosurg. Rev. 42, 351–369

30 Kyurkchiev, D. et al. (2014) Cells isolated from human glioblastoma multiforme express progesterone-induced blocking factor (PIBF). Cell. Mol. Neurobiol. 34, 479–489

31 Elmaci, İ. et al. (2019) Medroxyprogesterone effects on colony growth, autophagy and mitochondria of C6 glioma cells are augmented with tibolone and temozolomide: Cell kinetic and electron microscopical studies with a broad review of the literature. Clin. Neurol. Neurosurg. 177, 77–85

32 Atif, F. et al. (2019) Progesterone Treatment Attenuates Glycolytic Metabolism and Induces Senescence in Glioblastoma. Sci. Rep. 9, 1–12

33 Cheng, Y. et al. (2019) Progesterone Is More Effective Than Dexamethasone in Prolonging Overall Survival and Preserving Neurologic Function in Experimental Animals with Orthotopic Glioblastoma Allografts. World Neurosurg. 125, e497–e507

34 Barone, T.A. et al. (2009) Estrogen increases survival in an orthotopic model of glioblastoma. J. Neurooncol. 95, 37–48

35 Rodríguez-Lozano, D.C. et al. (2019) Testosterone promotes glioblastoma cell proliferation, migration, and invasion through androgen receptor activation. Front. Endocrinol. 10, 16

36 Fortunato, R.S. et al. (2014) Sexual dimorphism and thyroid dysfunction: A matter of oxidative stress?. J. Endocrinol. 221, R31–R40

37 Wu, X. et al. (2009) Genetic variation in the prostate stem cell antigen gene PSCA confers susceptibility to urinary bladder cancer. Nat. Genet. 41, 991–995

38 Tyulmenkov, V. V et al. (2000) Comparison of transcriptional synergy of estrogen receptors alpha and beta from multiple tandem estrogen response elements. Mol. Cell. Endocrinol. 165, 151–161

39 Rahbari, R. et al. (2010) Thyroid cancer gender disparity. Future Oncology, 6, 1771–1779

40 Zane, M. et al. (2017) Estrogen and thyroid cancer is a stem affair: A preliminary study. Biomed. Pharmacother. 85, 399–411

41 Hassler, M.R. et al. (2019) Impact of Patients' Gender on Efficacy of Immunotherapy in Patients With Metastatic Kidney Cancer: A Systematic Review and Meta-analysis. Clin. Genitourin. Cancer

42 Schmidt, L.S. and Linehan, W.M. (2016) Genetic predisposition to kidney cancer. Seminars in Oncology, 43. W.B. Saunders, 566–574

43 Ricketts, C.J. and Marston Linehan, W. (2015) Gender specific mutation incidence and survival associations in Clear Cell Renal Cell Carcinoma (CCRCC). PLoS ONE 10, e0140257

44 Czarnecka, A.M. et al. (2016) Hormone signaling pathways as treatment targets in renal cell cancer (Review). Int. J. Oncol. 48, 2221–2235

45 Chen, K.-C. et al. Dual Roles of 17-β Estradiol in Estrogen Receptor-dependent Growth Inhibition in Renal Cell Carcinoma. Cancer Genomics Proteomics. 13, 219–230

46 Wu, S.T. et al. (2016) Cellular effects induced by 17-β-estradiol to reduce the survival of renal cell carcinoma cells. J. Biomed. Sci. 23, 1–7

47 Pak, S. et al. (2019) Dihydrotestosterone promotes kidney cancer cell proliferation by activating the STAT5 pathway via androgen and glucocorticoid receptors. J. Cancer Res. Clin. Oncol. 145, 2293–2301

48 Dobruch, J. et al. (2016) Gender and Bladder Cancer: A Collaborative Review of Etiology, Biology, and Outcomes. Eur. Urol. 69, 300–310

49 Marks, P. et al. (2016) Female with bladder cancer: what and why is there a difference? Transl. Androl. Urol. 5, 668–682

50 Bilski, K. et al. (2019) Review on gender differences in non-muscle invasive bladder cancer. Transl Androl Urol. 8, 12–20

51 Mizushima, T. et al. (2017) Androgen receptor activation: a prospective therapeutic target for bladder cancer?. Expert Opin. Ther. Targets. 21, 249–257

52 Gil, D. et al. (2019) Dihydrotestosterone increases the risk of bladder cancer in men. Hum. Cell 32, 379–389

53 Godoy, G. et al. (2016) Effects of Androgen and Estrogen Receptor Signaling Pathways on Bladder Cancer Initiation and Progression. Bl Cancer. 2, 127–137

54 Fresno Vara, J.A. et al. (2004) PI3K/Akt signalling pathway and cancer. Cancer Treat. Rev. 30, 193–204

55 Liao, R.S. et al. (2013) Androgen receptor-mediated non-genomic regulation of prostate cancer cell proliferation. Transl. Androl. Urol. 2, 187–96

56 Helguero, L.A. et al. (2005) Estrogen receptors alfa (ERα) and beta (ERβ) differentially regulate proliferation and apoptosis of the normal murine mammary epithelial cell line HC11. Oncogene. 24, 6605–6616

57 Schleipen, B. et al. (2011) ERβ-specific agonists and genistein inhibit proliferation and induce apoptosis in the large and small intestine. Carcinogenesis 32, 1675–1683

58 Pinton, G. et al. (2010) Estrogen receptor β exerts tumor repressive functions in human malignant pleural mesothelioma via EGFR inactivation and affects response to gefitinib. PLoS One 5, e14110

59 Forsberg, L.A. et al. (2014) Mosaic loss of chromosome Y in peripheral blood is associated with shorter survival and higher risk of cancer. Nat. Genet. 46, 624–8

60 Kaneko, S. and Li, X. (2018) X chromosome protects against bladder cancer in females via a KDM6A-dependent epigenetic mechanism. Sci. Adv. 4, eaar5598

61 Gasperino, J. (2011) Gender is a risk factor for lung cancer. Med. Hypotheses 76, 328–331

62 Dou, M. et al. (2017) Reproductive Hormones and Their Receptors May Affect Lung Cancer. Cell. Physiol. Biochem. 44, 1425–1434

63 Skjefstad, K. et al. (2015) The prognostic role of progesterone receptor expression in non-small cell lung cancer patients: Gender-related impacts and correlation with disease-specific survival. Steroids 98, 29–36

64 Ben Khedher, S. et al. (2017) Menstrual and reproductive factors and lung cancer risk: A pooled analysis from the international lung cancer consortium. Int. J. Cancer 141, 309–323

65 Chan, Y.X. et al. (2017) Higher Dihydrotestosterone Is Associated with the Incidence of Lung Cancer in Older Men. Horm. Cancer 8, 119–126

66 Becerra-Díaz, M. et al. (2018) Androgen and Androgen Receptor as Enhancers of M2 Macrophage Polarization in Allergic Lung Inflammation. J. Immunol. 201, 2923–2933

67 Yip, C.H. and Rhodes, A. (2014) Estrogen and progesterone receptors in breast cancer. Future Oncol. 10, 2293–2301

68 Devlin, H.L. and Mudryj, M. (2009) Progression of prostate cancer: Multiple pathways to androgen independence. Cancer Lett. 274, 177–186

69 Petrick, J.L. et al. (2018) Association between circulating levels of sex steroid hormones and esophageal adenocarcinoma in the FINBAR Study. PLoS One 13, 1–13

70 Brusselaers, N. et al. (2017) Menopausal hormone therapy and the risk of esophageal and gastric cancer. Int. J. Cancer 140, 1693–1699

71 Huang, F.-Y. et al. (2015) Estradiol induces apoptosis via activation of miRNA-23a and p53: implication for gender difference in liver cancer development. Oncotarget 6, 34941–34952

72 Rawla, P. et al. (2019) Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. World J. Oncol. 10, 10–27

73 Iversen, L. et al. (2017) Lifetime cancer risk and combined oral contraceptives: the Royal College of General Practitioners' Oral Contraception Study. Am. J. Obstet. Gynecol. 216, 580.e1-580.e9

74 Tang, B. et al. (2015) Relationship between female hormonal and menstrual factors and pancreatic cancer. Med. (United States) 94, e177

75 Andersson, G. et al. (2018) Hormonal factors and pancreatic cancer risk in women: The Malmö Diet and Cancer Study. Int. J. Cancer 143, 52–62

76 Scelo, G. et al. (2018) Variability of Sex Disparities in Cancer Incidence over 30 Years: The Striking Case of Kidney Cancer. Eur. Urol. Focus 4, 586–590

77 Lucca, I. et al. (2015) Gender differences in incidence and outcomes of urothelial and kidney cancer. Nat. Rev. Urol. 12, 585–592

78 Cordina-Duverger, E. et al. (2017) Hormonal and reproductive risk factors of papillary thyroid cancer: A population-based case-control study in France. Cancer Epidemiol. 48, 78–84

79 Barta, J.A. et al. (2019) Global epidemiology of lung cancer. Ann. Glob. Health. 22, 85(1)

Figure legends

Figure 1. Incidence and mortality of the most prevalent cancers in Europe in men and women in 2018 [1]. Data is presented as age-specific rates per 100.000 person (ASR).

Text Boxes

Box 1. Cancers of the digestive system

A large population-based study (FINBAR study) showed that higher testosterone:estradiol ratios were associated with increased odds of esophagus cancer and poorer prognosis [69]. Conversely, women subjected to menopausal replacement therapy whether estradiol alone or estradiol plus progestin had decreased odds ratio of esophageal carcinomas than non-users, further sustaining a protective action of estrogens against esophageal cancer [70].

Women with a longer fertile life who have undergone menopausal hormone replacement therapy and men with prostate cancer treated with estrogens appear to have a decreased risk of gastric cancer [3]. Conversely, women with breast cancer treated with tamoxifen, an anti-estrogenic agent, have higher gastric cancer risk. Contrastingly, in a study on a Chinese population with gastric cancer, ERβ was the most abundant receptor identified, followed by AR and ERα, but only ERα and AR expression were associated with decreased patient survival [16].

Men develop colon adenomas and carcinomas at a younger age and at a higher rate than women [25], and the existing data suggests that men are at higher risk of developing colorectal cancer due to the influence of testosterone [22].

Liver cancer is the third most common type of cancer and the second leading cause of death in men, who are more susceptible to this cancer than women. One of the few sex hormone mediated mechanisms associated with liver cancer progression identified, shows that estrogens can have a protective role in the incidence of liver cancer by transcriptionally activation of miR23a and p53 via

ERα. The expression of miR23a is inversely correlated with the expression of the target gene X-linked inhibitor of apoptosis protein, and positively correlated with caspase-3/7 activity [71].

Incidence of pancreatic cancer is usually higher in men, but the reasons behind the prevalence differences observed between sexes are still poorly understood [72]. Studies addressing the effects of hormonal contraception did not find any significant correlations with the risk of developing pancreatic cancer [73]. Conversely, a meta-analysis also concluded that exogenous hormone use, age at menarche and/or menopause, hysterectomy, oophorectomy, hormone replacement therapy or the oral contraceptives used do not correlate with pancreatic cancer [74]. So far, only a Swedish population-based study found a significant association between hormone replacement therapy and decreased pancreatic cancer risk, especially in women subjected to estrogen-only treatment. The same study also showed that higher age at menarche was correlated with increased pancreatic cancer risk, suggesting that estrogens have a protective role against pancreatic cancer as well [75].

Box 2. Cancers of the urinary system

Kidney, urothelial carcinomas and bladder cancer are more prevalent in men [48,50,51,53]. Men have twice the risk of developing kidney cancer than women. This sex disparity has been studied and remained solid across all ages, regions, sociocultural habits and health behaviours, suggesting that the incidence differences might only be explained by biological differences between genders [76]. Despite the higher prevalence of urothelial carcinomas and bladder cancer in men, women have lower cancer-specific survival and worse prognosis [49],

along with diagnosis at advanced stages of tumours [49,77]. This may be due to misdiagnosed symptoms with urinary tract infections, i.e. haematuria, in women [48,49]. Several contributors, including genetics, chronic irritation and environmental exposures to carcinogens have been proposed for this gender disparity, with cigarette smoking being the best-established risk factor for bladder cancer [48-50].

Box 3. Other cancers

Men's increased susceptibility to astrocytoma, the most common **brain tumour**, and particularly to primary glioblastoma, the most aggressive form of astrocytoma, have been well-established since the 1990s. The most recent epidemiological data show that the incidence of primary glioblastoma in men is 50% higher than in women [27,28].

Thyroid cancer is the only cancer that affects more women than men. A French case-control population study named CATHY analysed the association of reproductive and hormonal factors with thyroid cancer among women. This study which included 430 papillary thyroid cancer cases and 505 controls demonstrated that increased age at menarche and the postmenopausal phase increased the risk of thyroid cancer incidence, particularly in cases where menopause resulted from an ovariectomy or when women reached menopause before the age of 55. Oral contraceptive use and hormone replacement therapy reduced thyroid cancer association by about one third and breastfeeding by about 27% [78].

Lung cancer has been more incident in men for years. However, over the past decades, the number of cases has exponentially increased among women and, in turn, cases among men have been declining. There has also been a rapid

increase in lung cancer mortality in women compared to men [60]. Both clinical and basic research studies support the hypothesis that E2 and cigarette smoking are cofactors in lung carcinogenesis in women [79].

Glossary

Androgen Receptor (AR): type of nuclear receptor that is activated by binding any of the androgenic hormones in the cytoplasm and then translocating into the nucleus. AR function as a DNA-binding transcription factor that regulate gene expression, whose regulated genes are critical for the development and maintenance of the male sexual phenotype and skeletal integrity, and for female sexual, somatic, and behavioral functions.

Dihydrotestosterone (DHT): endogenous androgen sex steroid hormone, whose formation from testosterone is catalyzed by the enzyme 5α-reductase. It is the most potent known endogenous ligand of the androgen receptor. Unlike other androgens, DHT cannot be converted by the enzyme aromatase into estrogen, thus it is frequently used to distinguish between the effects of testosterone and those caused by testosterone's conversion to estradiol.

17β-Estradiol (E2): estrogen steroid hormone and the major female sex hormone, affecting target tissues mainly by interacting with the two nuclear estrogen receptors α and β. It is involved in the regulation of the male and female reproductive systems, in the neuroprotection of the nervous system, and in other tissues like the bone, skin, liver and blood vessels. E2 has also been tied to cancer development and progression.

Estrogen Receptor (ER): steroid hormone receptor found in cells, important in sexual maturation and gestation. Two classes of ER exist: nuclear estrogen receptors (ERα and ERβ), and membrane estrogen receptors (GPER, ER-X, and Gq-mER). In the cytoplasm, once activated by 17β-estradiol, nuclear ERs are

able to translocate into the nucleus and bind to DNA to regulate the activity of different genes.

PI3K/Akt Pathway: signal transduction pathway that promotes survival and growth in response to extracellular signals. Impairment of PI3K/Akt pathway has been linked to a range of diseases, such as cancer, where its upregulation promotes cell survival, migration and angiogenesis, cell cycle progression, and increased glucose metabolism.

Progesterone (P4): endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis. P4 has a variety of important functions in the body, and it is also a crucial metabolic intermediate in the production of other endogenous steroids, playing an important role in brain function as a neurosteroid.

Progesterone Receptor (PR): protein found inside cells that is activated by the steroid hormone progesterone. In humans, PR has two isoforms PR-A and PR-B. Once activated upon progesterone binding, the complex enters the nucleus and binds to DNA, regulating gene transcription of target genes.

Testosterone: is the primary male sex hormone and anabolic steroid. In men, plays a key role in the development of reproductive tissues, as well as a promoter of secondary sexual characteristics. In addition, it is involved in health and well-being, and in the prevention of osteoporosis. It exerts its action through binding to and activation of the androgen receptor. Free testosterone is transported into the cytoplasm of target tissue cells or can be reduced to 5α-dihydrotestosterone, which binds to the androgen receptor more strongly than testosterone.

Outstanding Questions Box

- Why cancers in non-reproductive organs have different incidences between men and women?
- What is the impact of sex hormones in cancers of non-reproductive organs?
- How sex hormone receptors correlate with cancer in non-reproductive organs?

Key Figure

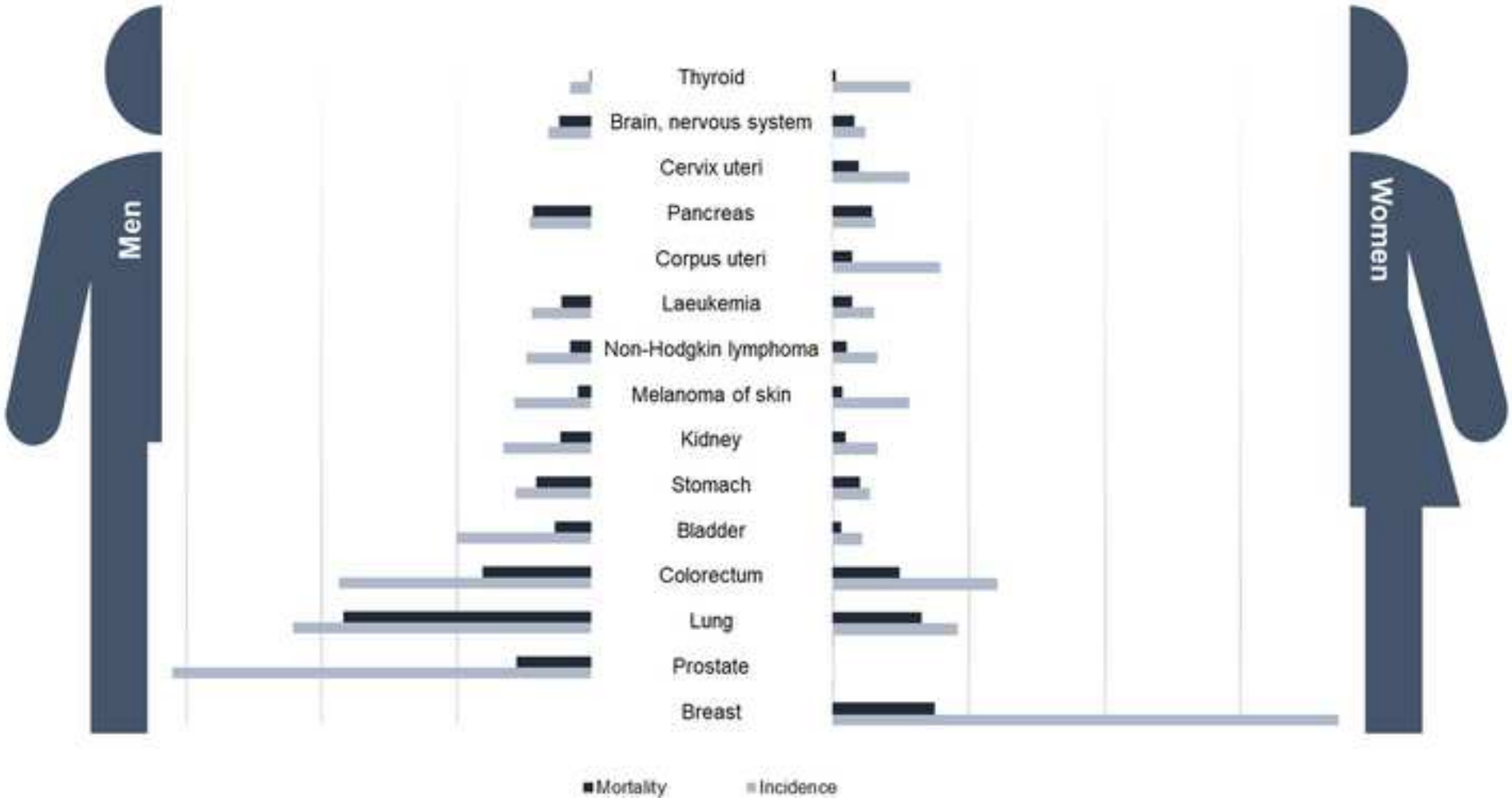


Table 1. Effects of sexual steroid hormones in cancers of non-reproductive organs.

Tissue	***Incidence Ratio**** [1]	***Estradiol***	***Progesterone***	***Testosterone***	***Receptors***				***Mechanisms***	***Refs***
					ERα	ERβ	PR	AR		
Digestive Tract										
Oesophagus	3-4x	↓proliferation ↓migration ↑apoptosis		↑proliferation	+	++		+	↑Bax:Bcl-2	[3-11]
Stomach	2x	↓risk ↑apoptosis ↓proliferation			+	+++		++	↑trefoil, ↓c-erb-2 ↓Bcl-2, ↓Bcl-xL ↑Casp-3	[3,15,18,19]
Colon	1-2x	↓proliferation ↓migration ↑apoptosis	↓risk in women	↑risk				-	↓Cyclin-A, -D1 ↓Bcl-2, ↓c-myb	[20-24]
CNS Tumours										
Astrocytoma	1.5x				+	+	--	+		[26;27]
Glioblastoma	1.5x	↑survival ↓proliferation	↑apoptosis ↓proliferation ↓migration	↑proliferation ↑migration	--- higher grades		++ higher grades	+++		[27-29,31-35]
Thyroid	-3x	↑proliferation			++	--			Redox imbalance ↑PI3K, ↓p27 ↑c-Fos, ↑ Cyclin-A, -D1 ↑ERK1/2	[12,36-40]
Kidney	2x	↓proliferation ↑apoptosis ↑autophagy		↑proliferation				++		[44-47]
Bladder	4-5x	↑proliferation		↑proliferation	-- +++ higher grades	+		++	↑PI3K/Akt ↓ UGTs ↑ 5α-reductase	[48,49,51,53-55]

									↑β-catenin, cyclin-D1 and EGFR	
Lung	2-3x			↑incidence			+		M2 macrophage polarization	[62,63,65,66]
Liver		↓proliferation							↑miR23a, ↑p53	[71]
Pancreas	1.5x		↓risk in women			++				[73,74]

*Incidence Ratio Men:Women - Not detected -- Rarely expressed --- Underexpressed + Abundant ++ Highly expressed +++ Overexpressed

Abbreviations: ER – estrogen receptor; PR – progesterone receptor; AR – androgen receptor; c-erb-2 – human epidermal growth factor receptor 2; Bax – Bcl-2-associated X protein; Bcl-2 – B-cell lymphoma 2; Casp3 – caspase 3; miR23a – microRNA 23a; PI3K – phosphoinositide 3-kinase; ERK1/2 – extracellular signal-regulated protein kinases 1 and 2; UGT – uridine-5'-diphospho-glucuronosyltransferase; EGFR – epidermal growth factor receptor.