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Sexual dimorphism in cancer

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Abstract

The incidence of many cancer types is significantly higher in the male than female populations, with associated differences in survival. Occupational and/or behavioral factors are well known underlying determinants. However, cellular/molecular differences between the two sexes are also likely to be important. We are focusing here on the complex interplay that sexual hormones and sex chromosomes can have in intrinsic control of cancer initiating cell populations, tumor microenvironment and systemic determinants of cancer development like the immune system and metabolism. A better appreciation of these differences between the two sexes could be of substantial value for cancer prevention as well as treatment.

Introduction (Epidemiology)

Epidemiological studies point to sexual dimorphism as a relevant factor for cancer incidence and survival. Overall, females have a lesser risk and better prognosis than males in a wide range of cancer types unrelated to reproductive function, such as those of colon, skin, head/neck, esophagus, lung and liver [1, 2] (Figure 1). Only very few exceptions exist in which incidence is higher in women, specifically thyroid cancer [3]. Noteworthy, this trend is observed independently to the ethnicity of the studied population.

The generally higher cancer risk in the male population was previously attributed to environmental exposure to chemicals or carcinogens, diet and risk behaviors such as smoking and drinking. However, even after appropriate adjustment for risk factors and statistical evaluation of the data, females still exhibit a higher degree of overall cancer protection than males [4, 5], with significant differences existing also in some childhood malignancies, specifically acute T cell lymphoblastic leukemia and hepatoblastoma [6]. This review investigates the possible cellular/molecular basis of differences in cancer susceptibility between the two sexes, focusing on the complex impact that sexual hormones and sex chromosomes can have in this context. Importantly, a significant interplay is likely to exist between the two, which could be of value for novel forms of cancer prevention and intervention.

General impact of sex-related hormone signaling in cells and organ systems unrelated to reproductive function

Both steroid and protein hormones are involved in sexual development and reproductive functions. Starting around birth, gonadal steroids control production of protein hormones, so called "*somatotrophs*", by the hypothalamus-pituitary axis with a more pronounced impact in puberty and adulthood. Sex-related somatotrophs with a direct sex-related function, such as prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and gonadotropin-releasing hormone (GnRH) are mainly implicated in cancer of reproductive tissues, like prostate, ovarian and breast [7-9]. However, as detailed further below, prolactin impinges also on cancer development in non-reproductive organs, specifically the liver [10, 11]. Among other somatotrophs with a broader function, growth hormone (GH) has been similarly implicated in liver cancer [12]. GH affects liver gene expression differentially in the two genders, possibly in connection with its pulsatile secretion into the plasma in men and constant in women [13]. Beside its action in the liver, GH has been implicated in tumorigenesis of other several tissues, like breast, prostate, colon, skin, endometrium and brain, even if in these cases autocrine GH production by cancer cells may be more important than pituitary secretion [14].

Relative to protein hormones, much more abundant information exists on the impact of sex steroid hormones on cancer development in non reproductive organs. These hormones, being lipid soluble, can enter the plasma membrane of target cells and interact directly with intracellular receptors that can shuttle to the nucleus to

affect gene expression [15]. Action of these hormones extends at the epigenetic level to DNA methylation and chromatin conformation [16, 17]. Analysis of gene expression in different non reproductive tissues indicates the existence of a gender specific influence on transcription [18, 19], which is paralleled at the level of chromatin organization, by specific differences between the sexes [20]. As discussed here below, sex hormone signaling pathways are likely to affect cancer susceptibility through multiple mechanisms, impacting on intrinsic self renewal mechanisms, tumor microenvironment, immune system and metabolism. The vast majority of accrued information relates to the involvement of three major sex steroid hormone receptors, estrogen receptor (ER) α, β and androgen receptor (AR), on which we will be focusing (Fig. 2). It is important to note, however, that the progesterone receptor, under intense investigation in breast cancer [21], may also be involved in cancer of non-reproductive organs, like what recently reported for lung NSCLC [22] and colon cancer [23].

a) Stem cell renewal : Specific ablation of estrogen receptor α, β and androgen receptor affects homeostasis and/or regeneration of organs unrelated to reproductive functions, such as lung [24], colon [25] and skin [26-28]. Sex steroids have an impact on stem cell populations of various types. Estrogens enhance self renewal of embryonic stem cells [29], promote endothelial progenitor cells [30] and, during pregnancy, contribute to the expansion of hematopoietic stem cell populations [31]. Neural stem cells have been reported to fluctuate during the estrous cycle and exogenous estrogen administration is sufficient to increase neural stem cell division

[32]. 17β-estradiol, the most potent form of estrogen, can also counteract senescence through p53-antagonizing mechanisms and/or control of telomerase expression [33, 34].

Like estrogens, androgens are involved in control of hematopoietic cell renewal. Aging men, that experience a reduction in androgen production, are prone to develop normocitic anemia, a condition characterized by a reduced number of red blood cells, that can be corrected with androgen supplementation [35]. Androgen supplementation has also ameliorating effects on acquired aplastic anemia, characterized by a reduced production of all blood cells, and dyskeratosis congenita, a genetic syndrome interesting several parts of the body [36, 37]. These two diseases manifest telomere dis-function, bone marrow failure due to hematopoietic stem cells impairment and are linked to cancer development. Molecularly, in primary hematopoietic cells, androgens induce telomerase expression through aromatase-dependent conversion of testosterone into estrogen and ERα activation [38].

The role of ERα signaling in breast cancer initiating cells is an area of active investigation [39, 40], and so is that of AR in prostate cancer [41, 42]. By contrast, the role that these pathways play in self-renewal of initiating cancer cells of other types is a mostly unexplored topic and important area of future investigation.

b) Cancer stroma : The tumor microenvironment plays a crucial role in both initiation and progression of the disease [43, 44]. Various components of the stromal compartment are under sex hormone control. Still not well depicted is the impact of sex hormones on the behavior of stromal fibroblasts. Cancer Associated Fibroblasts

(CAFs) can be viewed like persistently activated fibroblasts sharing properties with fibroblasts at wound sites [44]. While well known differences exist in skin wound healing between the two sexes [26, 27], where fibroblasts exert an important role in the first phases of the process, studies on the impact of sex hormones on CAF function have mostly focused on prostate [45-47], breast [48, 49] and cervical cancer [50] types. Estrogens were shown to promote mobilization of bone marrow derived precursors to breast cancer stroma [48] and increase stromal density [49]. In cervical cancer, ERα expression in stromal fibroblasts is sustained during the course of the disease [50], pointing to a possibly detrimental role. By contrast, in prostate tumors, expression of ERα is positively associated with survival and restrains cancer associated fibroblast behavior reducing angiogenesis and tumor growth [45]. Such beneficial role contrasts with additional observations that implicate ERα activity as a positive regulator of prostate epithelial cells [51], suggesting that this receptor functions differently in the two compartments. By contrast to ERα, ERβ activation has been reported to restrain prostate hyperplasia and cancer by inducing cell death of both epithelial and stromal cells [52].

Conflicting results exist concerning the role of AR signaling in prostate CAFs. While animal model studies indicate that this receptor in stromal fibroblasts promotes initial stages of the disease [46], clinical evidence points to the opposite possibility that sustained AR expression in this context is linked to less aggressive cancer and better prognosis [47].

Angiogenesis is another key determinant of cancer development. Estrogens have a well established pro-angiogenesis function, best known in the endometrium

during the female reproductive cycle, which is mediated by enhancement of endothelial cell proliferation and migration [30, 53]. This is mediated by diffusible factors like PAF [54] and VEGF [55], which are frequently secreted by cancer cells in response to estrogens. In contrast to ERα, ERβ activation in neoplastic breast cells negatively affects blood vessel formation [56]. Androgens also stimulate endothelial cell migration and new blood vessel formation through VEGF, especially in cells taken from male tissues, due to higher AR expression [57].

As for cancer initiating cells, the role that ER and AR signaling play in the stromal compartment of tumors of non-reproductive systems is an area of great importance for future studies.

c) Inflammatory/immune system : The microenvironment encompasses different leukocyte populations, with macrophage M1 polarization playing a key role in chronic inflammation-associated cancer development [43]. In macrophages, ERα significantly reduces their pro-inflammatory activity [58] and tumor promoting properties [59]. In contrast, AR favors the migration of macrophages and the release of proinflammatory cytokines delaying wound healing [28]. Mice lacking this receptor in inflammatory cells resolve the wound quicker than wild type mice. Macrophage release of cytokines is dependent on AR and contributes to prostate tumorigenesis [60].

Besides macrophages, other cells of the myeloid and lymphoid lineages like neutrophils or lymphocytes express estrogen and androgen receptors and these sex hormones can modulate the immune system at multiple levels through control of

immune-modulatory genes like *IFN-γ* [61]. Pathogenesis of autoimmune diseases, to which females are much more susceptible to than males, involves an altered balanced between effector T cells, with cytotoxic function, and regulatory (CD4+CD25+) T cells (Treg), a subpopulation of T cells that promotes self tolerance and restrains cancer immune surveillance [62]. The Foxp3 transcription factor is a master regulator of Treg cell number and function [63]. It belongs to the fork-head family of transcription factors and is encoded by the X-linked *foxp3* gene. Polymorphisms and mutations of this gene are associated with a number of diseases with imuno-dysregulation and poly-endochrinopathies. *foxp3* expression and function are under combined control of T cell receptor signaling and multiple cytokines through both transcription and post-transcription regulatory mechanisms [63]. In women, size of Treg cell populations positively correlates with estradiol levels during the luteal cycle and in pregnancy [61], with estradiol treatment inducing both Treg expansion and Foxp3 expression [64]. Testosterone stimulation causes also a strong increase of Treg cells both *in vivo* and *in vitro*, through a mechanism that involves AR recruitment to the *foxp3* locus and its enhanced expression [65].

The fact that both estrogens and androgens are involved in positive regulation of the *foxp3* gene suggests that additional more indirect mechanisms are involved in the differential activity of Treg cells in the female versus male populations. One such mechanism may involve differential production and function of IL6. This cytokine, together with IL-1, promotes degradation of the Foxp3 protein and ensuing Treg suppression [66]. Importantly, IL-6 expression is positively

regulated by estrogens [67] and genetic polymorphisms in the IL6 locus were identified that may be involved in its differential control between the two sexes [68].

Besides a differential impact on various cell populations, sex hormones can also affect the immune system indirectly, through the gut microbiome. In Non Obese Diabetic (NOD) mice, susceptible to autoimmune diabetes, the rise of testosterone after puberty promotes a divergent bacterial commensal population from adult females and prepuberal animals [69]. Such effect, which is reversed upon castration, is associated with reduced susceptibility to autoimmune reaction linked with changes in cytokine expression in lymphoid and myeloid cells.

The X chromosome is also highly enriched in immune-related genes [61] and there are several X-linked microRNAs that may contribute to sex-specific regulation of the immune response by targeting immune-related genes [70, 71]. In one study of an autoimmune disease, Systemic lupus erythematosus (SLE), several X-linked miRNAs were found overexpressed in T cells of female versus male patients [72]. Several of the differentially expressed miRNAs are located within 5000bp of an estrogen response element and, among the predicted targets, was CBL, a negative regulator of T cell receptor activity, which is down-regulated in T cells of the SLE patients [72].

Overall, a complex picture emerges whereby sexual dimorphism in the immune system is the combined result of multiple determinants [61]. Irrespectively of the detailed mechanisms, the greater susceptibility of women to develop autoimmune diseases is likely accompanied by enhanced immune surveillance

against various tumor types. As discussed at the end, further insights into this topic will likely lead to improved forms of cancer immune therapy and prevention.

d) Metabolism : Steroid hormones are also central regulators of systemic metabolism, acting at several levels [73]. Both estrogen and androgen receptors increase glucose tolerance and restrain visceral fat accumulation [74]. Reduced ERα signaling in post-menopausal women and aromatase deficiency, with compromised conversion of androgens to estrogens, are associated with increased adiposity [73], a known risk factor for cancer development [75]. Cancer is often associated with a severe body wasting syndrome called cachexia [76]. Cachexia is a sexually dimorphic trait [77] that is linked to higher levels of autophagy in male hearts and is associated with reduced survival [78]. Androgens have also an important muscle trophic function and the majority of male cancer patients in the seventh decade of life experiences sarcopenia, a condition characterized by the loss of muscle mass and strength, linked to a reduction in testosterone [79]. A decrease in androgen levels has been noticed in multiple studies in male cancer patients [80] that may be a result of chemotherapy [81]. Selective androgen modulators, able to activate AR mainly in the muscle, have been tested for their ability to revert or compensate for body mass wasting with improvement of lean body mass and physical condition [82].

Sex hormone signaling in specific cancer types

A clear example of greater cancer susceptibility of the male versus female populations due to hormonal sex influences is represented by *hepatocellular carcinoma* (HCC), which occurs more frequently in men [1, 2]. A detailed analysis indicates that AR and ERα contribute to hepatocarcinogenesis in an antagonistic way regulating gene expression (Fig. 3): the first stimulates while the second restrains proliferation and nucleotide/ amino acid metabolism [83]. Such estrogen induced resistance and androgen mediated enhancement of tumorigenesis is dependent on FOXA1 and FOXA2 pioneer factors, required for chromatin recruitment of nuclear receptors. Ablation of FOXA proteins eliminates gender differences converting the tumor suppressive role of estrogen into tumor promoting [83]. Estrogens exert a protective function reducing the production of inflammatory mediators like IL6 [84]. However, important estrogen effects on liver physiology can also be mediated by its effects on the gonadal-hypophyseal axis [85, 86]. In rodents, hypophysectomy abolishes sex-dependent differences in HCC [87]. Estrogen can modulate growth hormone signaling acting on pituitary GH secretion [88], as well as on control of GH receptor (GHR) expression [89]. GH in turn targets the liver in a sex-specific manner, which is particularly evident in rodents at puberty, but is also found in humans [90]. GH function is mediated by the transcriptional activator STAT5, which can protect hepatocytes from chronic injuries and malignant transformation [12], providing another possible mechanism for sexual dimorphism in HCC development.

The lesser susceptibility of women to HCC can also result from estrogen stimulated secretion of prolactin, another pituitary-secreted hormone [10, 11]. In this

context, prolactin may protect females from liver tumorigenesis by restricting innate immunity signaling in hepatocytes, which may be of special relevance for the virally-associated forms of the disease [10]. More specifically, prolactin stimulation of hepatic cell lines was shown to suppress response to IL1R, TLF4 and TNFR1 activation by a post-transcriptional mechanism, thereby suppressing c-Myc activation [10].

Several other cancer types exhibit higher incidence and aggressive behavior in the male and post- versus pre-menopausal female populations, with similar gender differences occurring in corresponding animal models. These include *colon cancer*, *gliomas*, *melanoma* and *non melanoma skin cancer*, *head/neck squamous cell carcinoma* (SCC), *esophageal* and *non small cell lung cancer* (NSCLC). Protection in women extends to hematological malignancies, especially lymphomas, even after menopause [1, 2].

In many cases, higher estrogen signaling, leading to ERβ activation, has been implicated as the likely explanation. ERβ expression is often diminished in these various cancer types and sustained ERβ proliferation is a favorable prognostic marker. In colon cancer, a polymorphism in the ERβ promoter, possibly influencing its activity levels, has been associated with increased survival [91]. Polymorphisms in the EGFR gene have also been identified that are positively associated with colon cancer survival in women and negatively in males [92], pointing to a possible interplay between EGFR and ERβ signaling underlying the differences between the two sexes.

In skin and head/neck SCC cells in which ERβ expression is down-modulated, increased ERβ expression and/or agonist-specific stimulation promotes

differentiation through induction of NOTCH1 gene transcription and function [19]. Also in melanoma, ERβ expression levels are inversely related to primary cancer progression in both men and women [93] and, in a mouse model of the disease, tamoxifen treatment significantly inhibited metastatic spread in female animals [94]. ERβ function is required for normal lung and colon morphogenesis [24, 25] and its activation exerts beneficial consequences in experimental models of colon [95], glioma [96], mesothelioma [97], renal cell carcinoma (RCC) [98] and T cell lymphoma [99].

In contrast to the above tumor types, the incidence of thyroid cancer is significantly higher in the female than male populations. Even though several studies tried to find associations between this neoplasm and hormonal or dietary factors, the reasons for this discrepancy are still unclear [3]. However, experimentally, it should be noted that AR expression in thyroid follicular cells was reported to reduce proliferation [100], while estrogen treatment induced proliferation and suppressed apoptosis [101], suggesting a possibly opposite role of sex hormones in this context.

Influence of sex chromosomes on oncogenesis

The most significant genetic differences between the two sexes reside in the X and Y chromosomes. As discussed below, mutations and/or epigenetic deregulation of genes on sex chromosomes could contribute to the oncogenetic process. Besides protein-encoding genes, deregulated expression of non-coding

RNAs is likely to be involved, with a likely interplay with sex hormone action, including the fact that the androgen receptor gene is X-linked (Fig. 4).

a) X chromosome

To counteract differences in X chromosome gene dosage, mammals utilize the X inactivation (Xi) process, involving transcriptional silencing and chromatin compaction promoted by the long non coding RNA (lncRNA) Xist [102, 103]. Female tissues are mosaic for most X-linked genes, resulting from Xi in every cell. Although inactivation of one X chromosome over the other is generally random, preferential inactivation of one chromosome (skewed Xi) can protect women against the consequences of X-linked gene mutations [104]. On the contrary the absence of Xi can have detrimental effects: for instance loss of Xist has been reported in breast, ovarian and cervical cancer cell lines [105, 106] and, only in female mutant mice, it caused deregulation of hematopoietic lineages and emergence of myeloid neoplasms associated with genome wide changes in gene expression [107].

In other settings, cancer development has been linked to increased rather than decreased Xist expression. Xist expression can be up-regulated in glioblastoma cells of both sexes and its knockdown results in tumour-suppression [108]. In testicular tumors, increased Xist expression is also frequently observed, which is not coupled with methylation of X linked genes, indicating that its role may be distinct from this process [109].

Despite the X chromosome-wide silencing mechanism, there are regions escaping Xist-dependent inactivation. Besides the pseudoautosomal regions (PAR),

which have corresponding regions of homology on the Y chromosome, other single genes escaping inactivation are spread throughout the chromosome. In particular, UTX (KDM6A) is a H3K27 demethylase expressed from both X chromosomes [110]. In men, UTX has a homolog on the Y chromosome, called UTY (KDM6C), whose catalytic activity is very low or absent [111]. UTX has a tumor suppressing function and inactivating mutations in this gene are not compensated by UTY (Fig. 4). This appears to be of importance in human T-acute lymphocyte leukemia (ALL), a childhood tumor more common in males than in females and a corresponding experimental model of the disease [112-114]. Additional X-linked players altered in T-ALL are the PHF6 and RPL10 genes, which are mutated almost exclusively in male patients [115, 116]. Loss-of-function UTX mutations have also been found in oesophageal SCCs and renal cell carcinomas, which are more frequent in the male population [117, 118]. Group 4 medulloblastomas are also more prevalent in males and it is noteworthy that, besides UTX, several other mutated oncogenes and tumor suppressor genes in these tumors are located on the X chromosome [119] (suppl. Table 1). These include the genes for a component of histone-deacetylase complexes, ZMYM3, and the RNA helicase DDX3X, which has a homologue on the Y chromosome that is not translated. While ZMYM3 is mutated exclusively in male patients' tumors, DDX3X is also found to be mutated in females' tumors, in the allele that escapes X inactivation [120]. Loss of function mutations in DDX3X are also observed in Natural killer/T-cell lymphoma (NKTCL) [121] and NSCLC, where the absence of the helicase promotes malignancy [122].

Other tumor suppressor genes mapping in the X chromosome include WTX, which is involved in pediatric kidney cancer Wilms tumors [123], and KDM5C, coding for a histone demethylase, whose truncating mutation are found in renal carcinoma cells and trigger genomic instability [124] (suppl. Table 1). Of note, KDM5C is also overexpressed in HCCs in which it seems to play a pro-oncogenic function [125].

As mentioned, *foxp3* is a X-linked gene, which plays an important role in the immune system, by controlling transcription of regulatory T cells. Besides Tregs, expression of this gene has been observed in different tumor cells, like lung, melanoma, colon cancer cells [126], as well as pancreatic carcinoma cells [127], which share some Foxp3-dependent immune suppressing functions with Tregs. Furthermore, in breast and prostate cancer Foxp3 inhibits NFkB activation, through a Foxp3-miR-146-NFkB axis, which promotes tumor suppression and apoptosis [128]. We further note that a specific member of the SOX family of transcription factors, SOX3, is encoded in the X chromosome (Fig. 4). This will be discussed below in the context of the Y-encoded SRY gene.

Besides protein-coding genes and lncRNAs like Xist, the X chromosome is also highly enriched in miRNAs, whose density is almost two-fold higher than in the autosomes (suppl. Table 1). Among these are miR-221 and miR-222, which function as oncomiRs through repression of the cell cycle inhibitor p27 [129]. Interestingly, these two miRs map relatively close to KDM6A gene, raising the possibility that they may also escape Xi. Together with miR-222, miR-223 (also X-linked) is up-regulated in gastric cancer, promoting cancer cell migration and metastasis [130]. Expression of

two other X-linked miRs, miR-20b and miR-361, has potential prognostic significance for oropharyngeal carcinoma [131]. There is also a cluster of several miRs (miR-506, miR-507, miR-508, miR-513a-1, miR-513a-2, miR-513b, and miR-513c) that might fine-tune the response of cells to PI3K/Akt activation. Expression of these miRs is under direct control of the Akt-dependent FOXO1 and FOXO3 transcription factors. In turn, the miRs target these transcription factors as well as components of the PI3K and of MAPK cascade [132].

Although all these miRs map to the X chromosome, whether or not their differential expression contributes to gender differences in cancer risk remains to be established.

b) Y chromosome

Broad as well as restricted alterations of the Y chromosome are frequent in cancer development. Loss of the entire Y chromosome (LOY) has been reported with various frequencies in prostate [133-135], pancreatic [136], colorectal [137] and bladder [138], which has also a strong male prevalence. Short arm deletions have also been found in numerous cancer types [139] (http://cgap.nci.nih.gov/Chromosomes/Mitelman). Importantly, Y chromosome loss occurs also in normal tissues; it is frequently observed in normal hematopoietic cells of elderly men and is significantly associated with a greater risk of cancer [140, 141]. Y chromosome loss in blood cells could be used as predictive biomarker and is also induced by smoking [141]. However, it remains to be determined whether or not widespread loss of Y chromosomal regions

promotes cancer development or simply results from elimination of unnecessary genetic material, as proposed during evolution[142].

More limited alterations of the Y chromosome can also occur. Of likely importance are those affecting the pseudoautosomal region and the closely located SRY gene, responsible for male sex development [143]. Chromosomal amplifications of the pseudoautosomal region Y11p2 adjacent to SRY (located at Y11p3) have been found in human HCCs [144] possibly contributing to gender disparity in HCC. Indeed SRY ablation can impair development of this cancer type [145], decreasing also chemoresistance [144] (Figure 3). SRY codes for a HMG box transcription factor of the SOX family. Among various members, SRY shares a significant amount of homology with Sox1, 2 and 3, and may have evolved from these [146]. Like Sox2 [147], SRY is likely to play a role in cancer stem cell renewal, as suggested by its control of OCT4 expression (Fig. 4), another key cell reprogramming and stem cell factor [144] and of a c-Myc coactivator, sgf29 [145]. Interestingly, together with these growth promoting functions, SRY has also been reported to negatively regulate AR gene transcription through some as yet unclarified mechanism [148].

TSPY is another Y-linked gene, coding for a SET/NAP (SET proto-oncogene /nucleosome assembly protein), with a known function in male germ cell differentiation, mitosis, and meiosis [149]. Increased TSPY expression occurs in testicular germ cell tumors [149], as well as various types of somatic cancer, including melanoma [150], prostate [151] and liver cancer [152]. Interestingly, while TSPY appears to exert a pro-oncogenic function, it has a homologue on the X chromosome, TSPX, which can be tumour suppressive [153] and protects from hepatitis B viral infection, a

major risk for HCC [154]. In fact, both ectopic TSPY activation and TSPX silencing can contribute to the initiation and progression of hepatocellular carcinoma, which, as discussed in a previous section, is a male-predominant cancer [152] (Fig. 3). In these tumors, there is a positive correlation between TSPY and AR expression, with TSPY promoting AR expression [155]. Conversely, at least in prostate cancer cells, AR stimulation by androgen leads to TSPY up-regulation [156].

Finally, RBMY (RNA binding motif gene on the Y chromosome) codes for a male germ cell-specific regulator of RNA splicing involved in spermatogenesis. RBMY is aberrantly activated in male HCC [157], where, like TSPY, it can enhance AR expression and activity as well as cancer development [158] (Fig. 3).

Conclusions and future perspectives

Sex-specific differences in cancer incidence and mortality across ethnic backgrounds point to significant influences of gender on both cancer initiation and progression (Fig. 1). An important notion is that cancer as a disease is not limited to a group of genetically deranged cells and their immediate environment, but is linked to general alterations in organ homeostasis as a result of perturbed developmental/morphogenetic signals and systemic factors [43, 159]. Selection of cells with cumulative somatic mutations [160] or a "Big Bang" expansion of single cell populations with pre-existing dominant alterations [161] have been implicated in cancer development. However, there can be clonal expansion of cells harboring many cancer driver mutations without any clinical signs of the disease [162]. Indeed, in

most cases, premalignant lesions do not progress into malignancy for reasons that are poorly understood [163, 164]. This led to the view of an "ecological cellular environment" playing a key role in restraining or unleashing tumor growth [164, 165]. As discussed throughout this review, sex hormones and sex chromosome-encode information can impact on all aspects of the cancer process (Fig. 5).

As for the tumor cells themselves, important molecular insights can be provided by recent developments in large-scale genome sequencing of tumors coupled with epigenetic, transcriptomic and proteomic analysis and associated clinical information. For instance, analysis of the TCGA database showed differences in the mutational landscape of Head/Neck SCCs in the male versus female populations, with distinct gene expression profiles [19]. It will be interesting to assess whether similar differences apply to other cancer types, possibly in a cell type or cancer subtype-specific manner, and to determine whether subsets of cancer patients with mutational and/or gene expression profiles closer to those of the other gender can be identified, with distinct clinical behavior.

At the level of the cancer stroma and organ homeostasis, sex hormone regulation of angiogenesis stands out as a key element of divergent cancer progression between the two sexes, as does inflammation. Systemically, the most promising area where understanding sex bias can be of value is at the level of the immune system. Immunotherapy based on immune checkpoint inhibitors is rapidly emerging as a key therapeutic mainstay of cancer types with high gene mutation frequencies [166], most of which exhibit much greater incidence in the male than female populations. As we discussed, sex hormone modulation of the immune

system can be involved as well as X-linked expression of cytokine genes and immune-modulatory miRNAs. It will be important to assess to what extent these elements have an impact on key immune checkpoints, like the PD-1/PD-L1 axis, acting on various T cell populations, neighboring immunomodulatory cells and cancer cells themselves.

Sexual dimorphisms that favour females are also observed in lifespan and aging [167]. Such differences are once again defined by multiple elements. Sex hormones, especially estrogens, can play an important role in control of cellular senescence and aging-associated tissue deteriorations. Concomitantly, at the chromosomal level, it has been suggested that X-linked mutations are more likely to exert detrimental effects in male than female cells, which have the advantageous possibility of selective inactivation of the mutations-carrying X chromosome [104].

Besides its basic value, detailed knowledge of the impact of sex hormones on cancer development in organs of non reproductive function has translational importance, given the available pharmacological tools to either enhance or suppress their function. This is especially true for steroid hormones [168, 169]. Along these lines, a topic of great interest and translational potential is that of the interplay between their influence and epigenetic reprogramming of cells, with a well known interplay between ER and AR function and key histone and DNA modifying enzymes [13], which can be targeted by a large battery of natural and synthetic compounds [170]. Interventions at this level are going to be of great promise for both cancer prevention and treatment.

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Figure legends

Figure 1. Gender differences in cancer incidence and mortality in organs unrelated to reproductive function.

Incidence (A) and mortality (B) rates of various cancer types for the year 2012 in males (M) and females (F) of European, African, Americas and South East Asian regions. Rates (number of cases per 1000) are for all ages and were retrieved from GLOBOCAN 2012 (IARC) (http://globocan.iarc.fr).

Figure 2. Role of sex steroid hormone receptors in non reproductive tissues.

Summary scheme of the complexity of biological functions of the three sex steroid hormone receptors that have been so far implicated. As discussed in the text, activation of ERα elicits hematopoietic stem cell expansion and mobilization, favors skin wound healing enhancing keratinocyte proliferation, promotes blood vessel formation and endothelial cell precursor mobilization, reduces liver cell proliferation and restrains the inflammatory role of macrophages. ERβ also blocks macrophage activation and, contrary to ERα, negatively regulates angiogenesis. ERβ promotes differentiation and suppresses tumor formation in several districts such as the skin (acting on both keratinocytes and melanocytes) and is required for the proper morphogenesis of lung and colon. AR suppresses wound healing, promotes angiogenesis, liver cell proliferation as well as macrophage activation. Androgens activity is not limited to AR activation. In fact, through the action of aromatase, they

are converted into estrogens, thus controlling indirectly hematopoietic stem cell expansion through ERα activity.

Figure 3. Possible mechanisms contributing to gender dimorphism in hepatocellular carcinoma (HCC).

Diagrammatic summary of factors implicated in the greater incidence of HCC in the male versus female populations. At hormonal level, AR and ERα activation have opposing effect on hepatocytes proliferation and nucleic acid metabolism, acting on gene transcription. Estrogen exerts also indirect effects through stimulation of prolactin and growth hormone (GH) secretion by the pituitary gland, with these hormones in turn suppressing inflammatory and proliferative signals in the liver. At the chromosomal level, the X-linked gene TSPX protects from hepatitis B viral infection, a major risk for HCC development and loss of TSPX has been observed in male patients with HCC. On the other hand, TSPY, the TSPX homolog gene on Y chromosome, induces AR expression and is found ectopically expressed in HCC male patients. Amplification of two other Y-linked genes, RBMY and SRY, occurs in HCC, with RBMY enhancing AR expression and SRY promoting tumorigenesis as well as chemo-resistance.

Figure 4. Interplay between sex chromosomes in human cancer.

Representative illustration of X- and Y-linked genes of functional significance discussed in the text. In women, some parts of the X chromosome escape Xist-mediated inactivation, as is the case for the tumor suppressor gene UTX, which is

not compensated by its Y homologue UTY, resulting in differential gene dosage effects with likely cancer-protective consequences. Three other genes on the Y chromosome, SRY, RBMY and TSPY, have the potential of promoting cancer development, which may be mediated, in part, by their modulation of X-linked AR expression. Increased SRY activity may also drive cancer stem cell expansion through increased expression levels of autosomal genes like OCT4.

Figure 5. Hormonal and chromosomal contributions to sexual dimorphism in cancer.

As discussed in the text, a complex interplay exists between sex hormones and X and Y chromosomes, with epigenetic control of gene expression as a link. These combined factors can differentially affect cancer development at multiple levels: i) cancer target cells of origin, through modulation of ubiquitous or cell type specific intracellular processes; ii) cancer stroma and surrounding "field cancerization" tissues, through angiogenesis and inflammation; iii) systemically, through alterations of the immune system and metabolism. It can be further proposed that drug targeting of sex hormone action and associated epigenetic control mechanisms can have beneficial effects for multiple cancer types, well beyond those of organs with reproductive functions.

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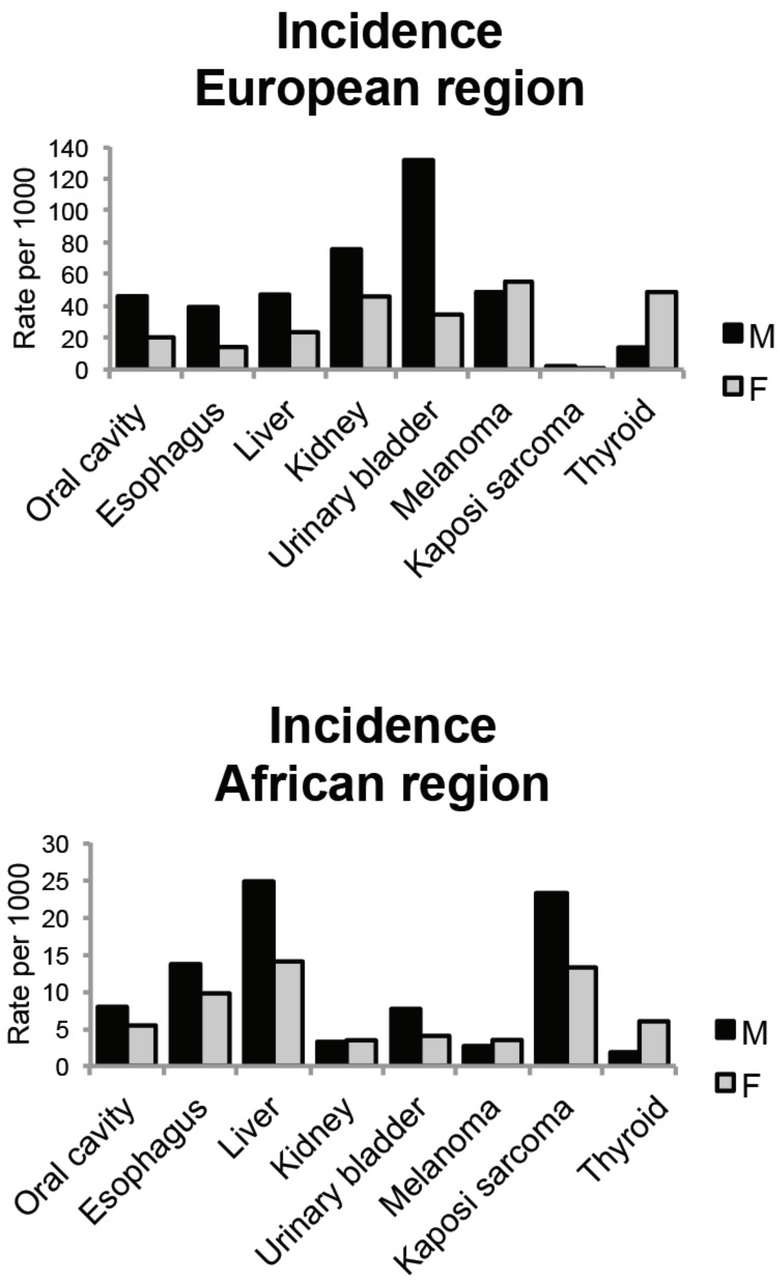
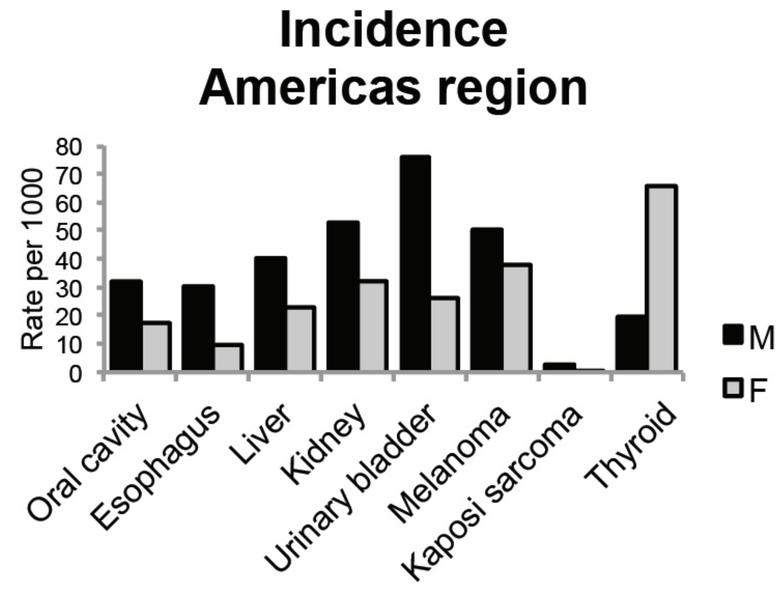
170. Ahuja, N., Sharma, A.R. & Baylin, S.B. Epigenetic Therapeutics: A New Weapon in the War Against Cancer. *Annu Rev Med* **67**, 73-89 (2016).

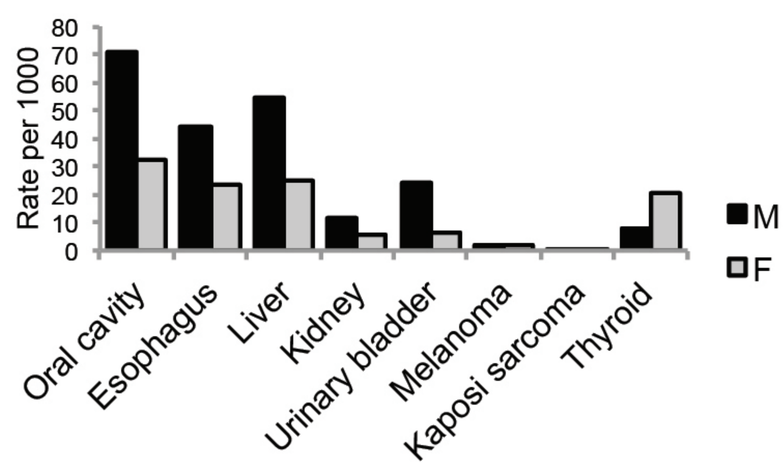
Figure 1

A

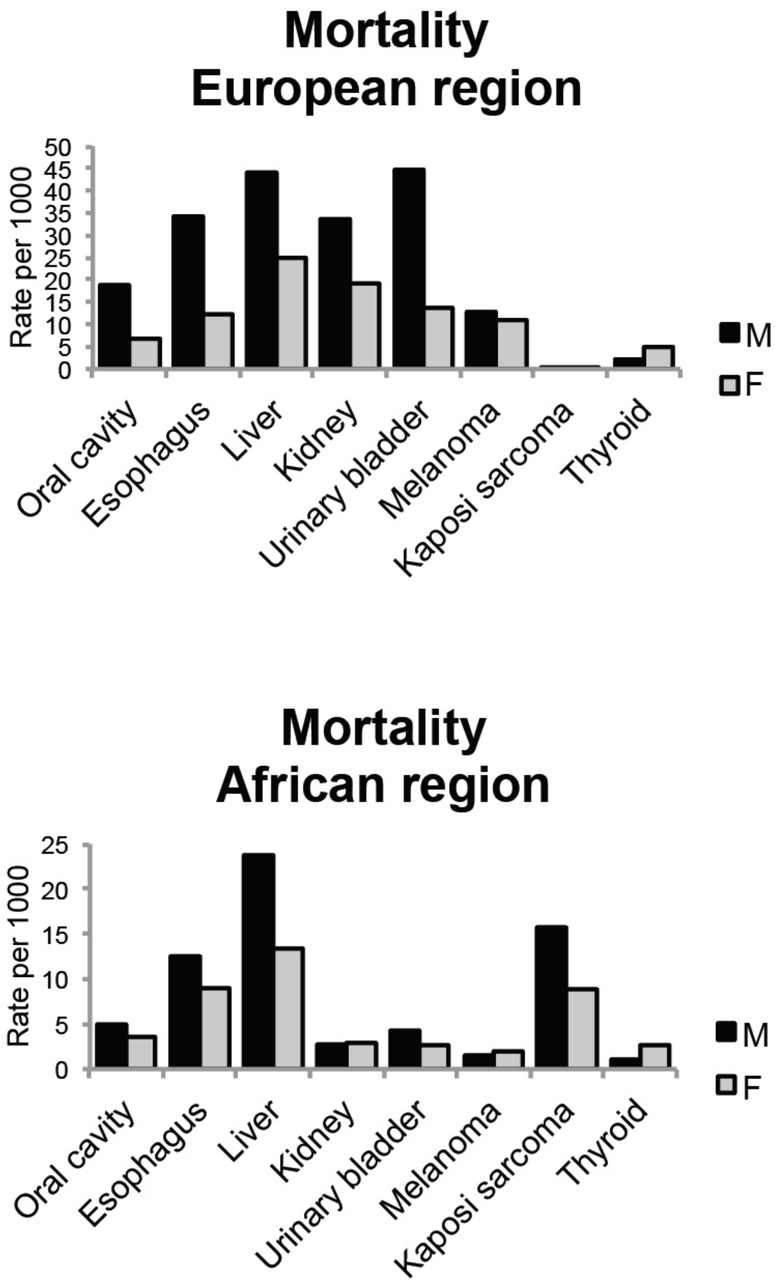

Incidence
European region
Rate per 1000
140
120
100
80
60
40
20
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F

Incidence
African region
Rate per 1000
30
25
20
15
10
5
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F

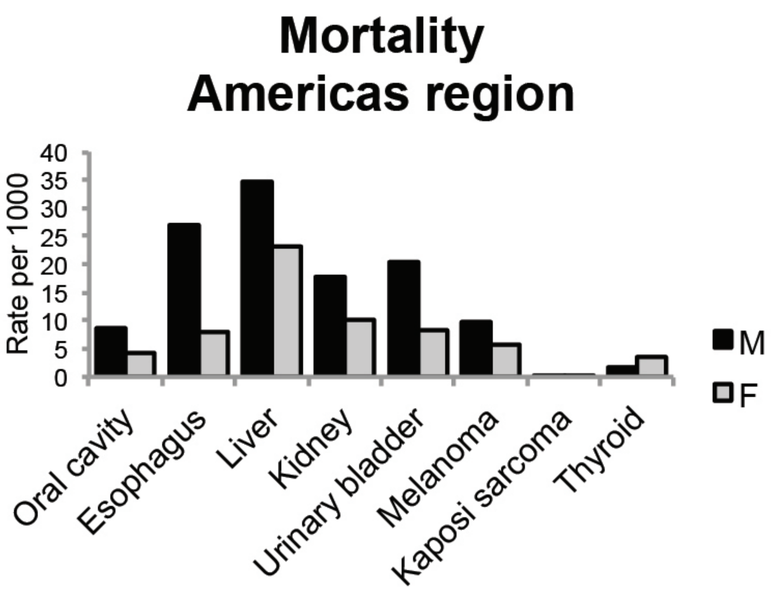

Incidence
Americas region
Rate per 1000
80
70
60
50
40
30
20
10
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F


Incidence
South East Asia region
Rate per 1000
80
70
60
50
40
30
20
10
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F

B


Mortality
European region
Rate per 1000
50
45
40
35
30
25
20
15
10
5
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F

Mortality
African region
Rate per 1000
25
20
15
10
5
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F


Mortality
Americas region
Rate per 1000
40
35
30
25
20
15
10
5
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F

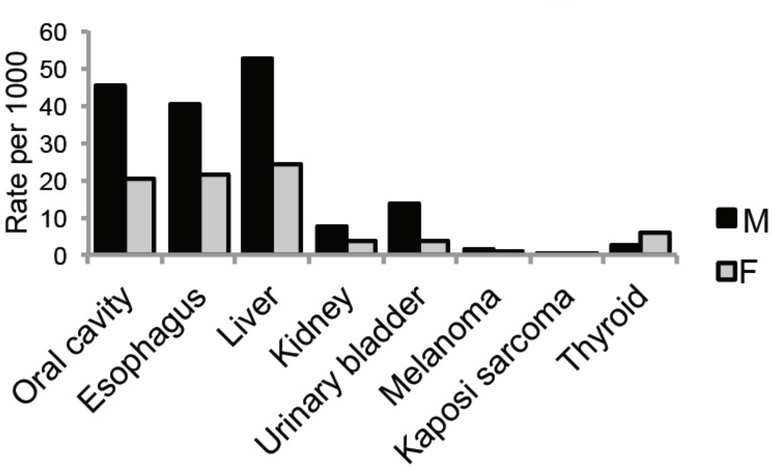

Mortality
South East Asia region
Rate per 1000
60
50
40
30
20
10
0
Oral cavity
Esophagus
Liver
Kidney
Urinary bladder
Melanoma
Kaposi sarcoma
Thyroid
M
F

Figure 2

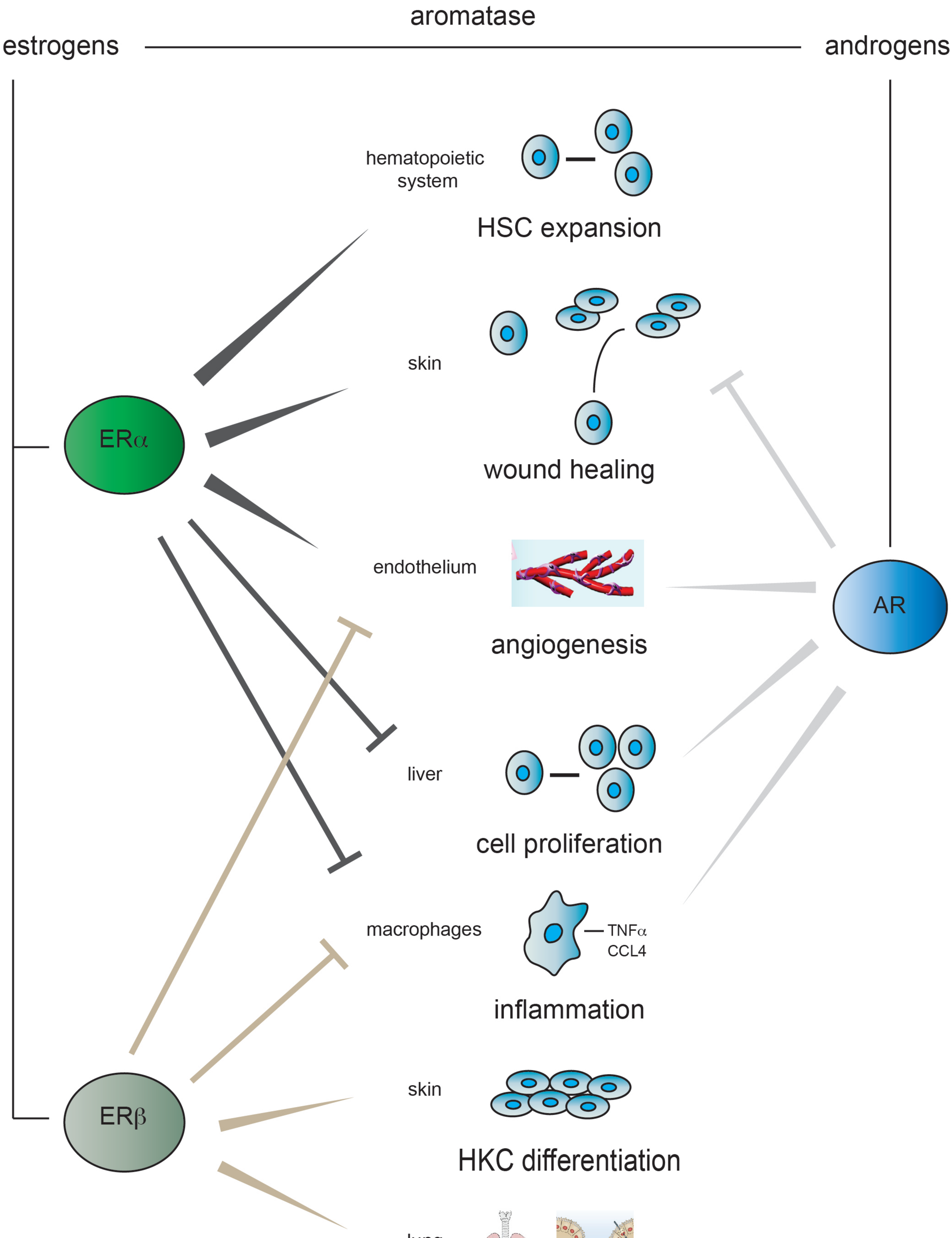

aromatase
estrogens
androgens
hematopoietic system
HSC expansion
skin
wound healing
ERα
endothelium
angiogenesis
AR
liver
cell proliferation
macrophages
TNFα
CCL4
inflammation
ERβ
skin
HKC differentiation
lung
colon
morphogenesis
organ development

Figure 3

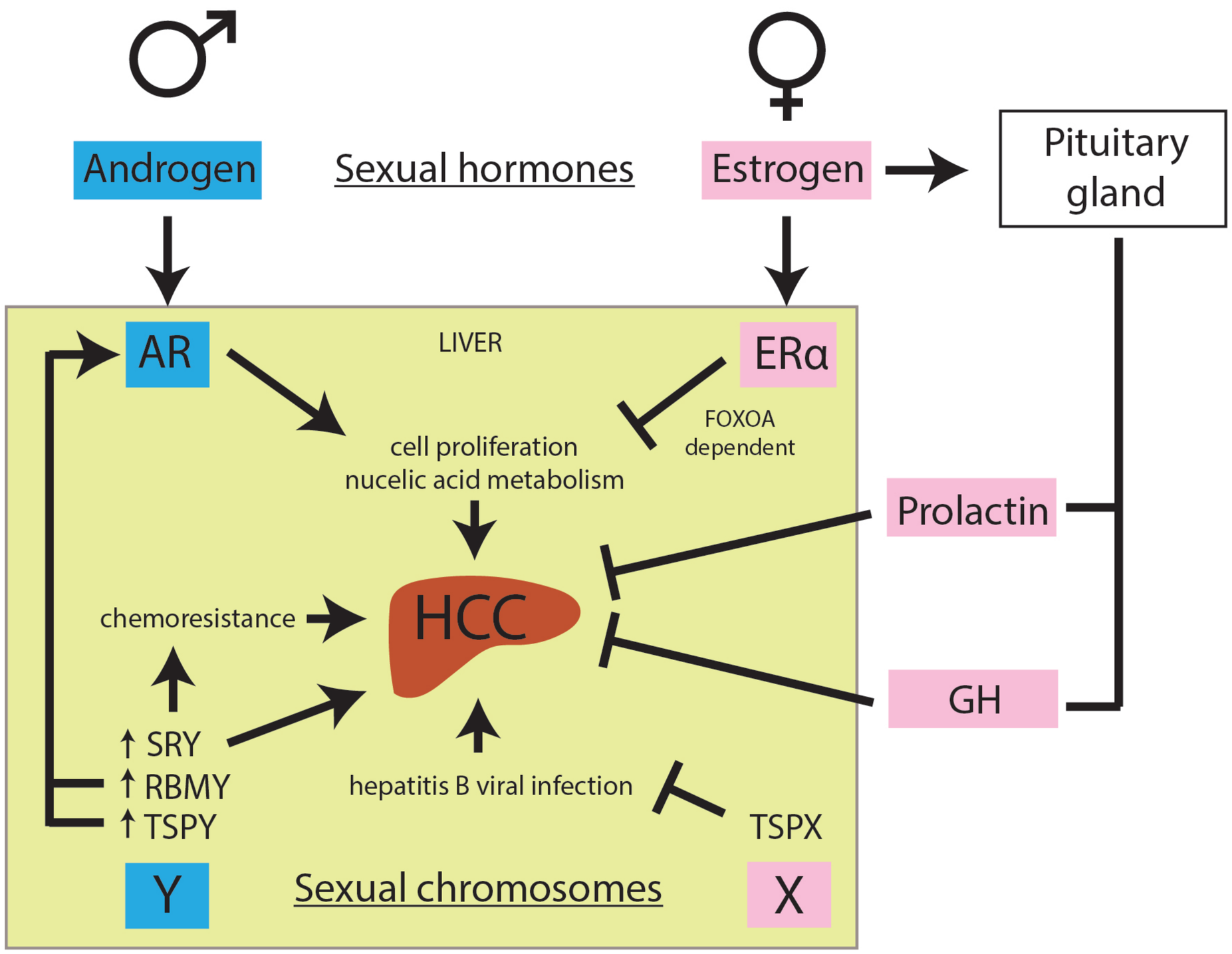

Androgen
Sexual hormones
Estrogen
Pituitary gland
AR
LIVER
ERα
FOXOA dependent
cell proliferation
nucelic acid metabolism
Prolactin
chemoresistance
HCC
GH
SRY
RBMY
TSPY
hepatitis B viral infection
TSPX
Y
Sexual chromosomes
X

Figure 4

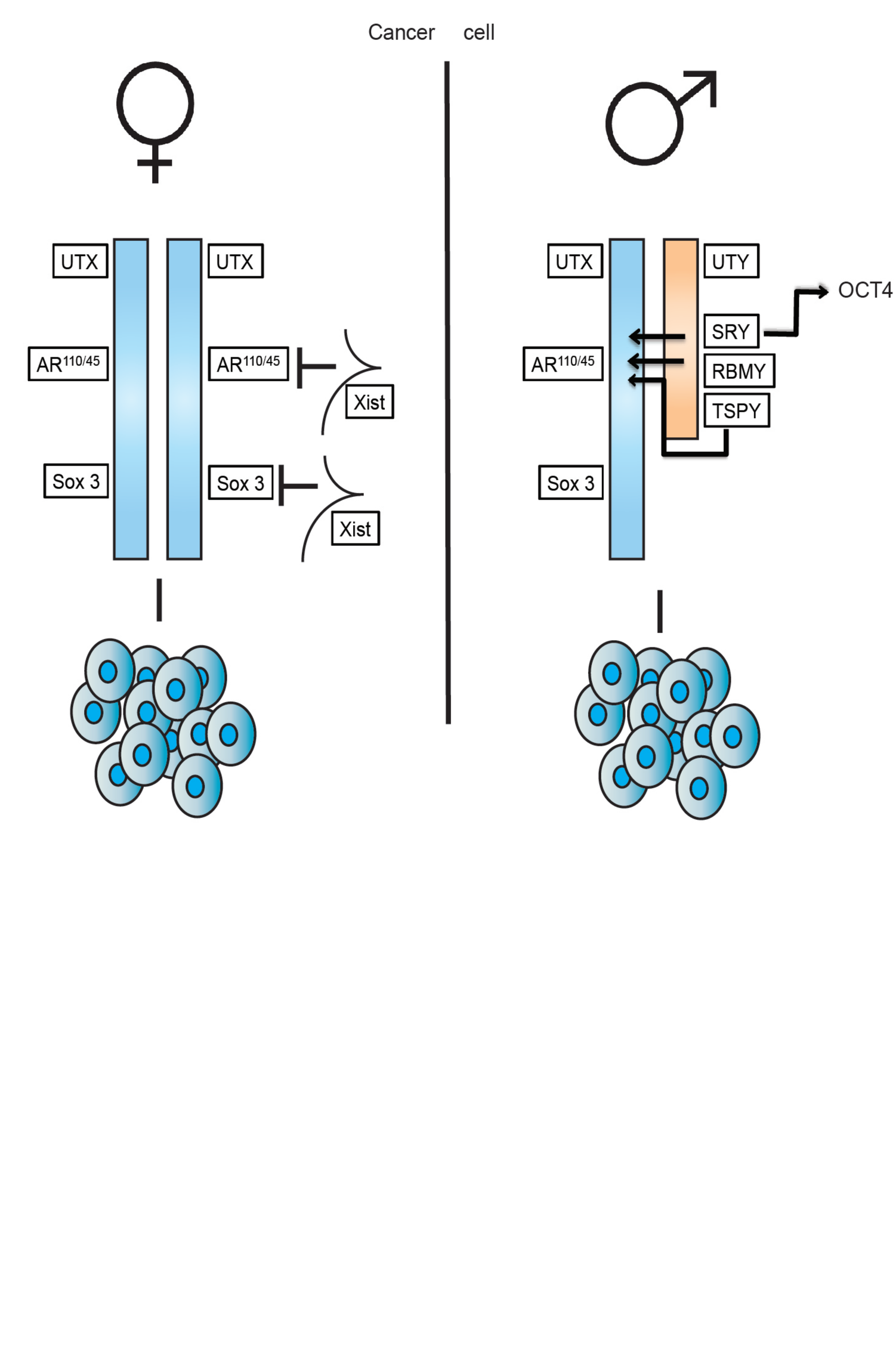
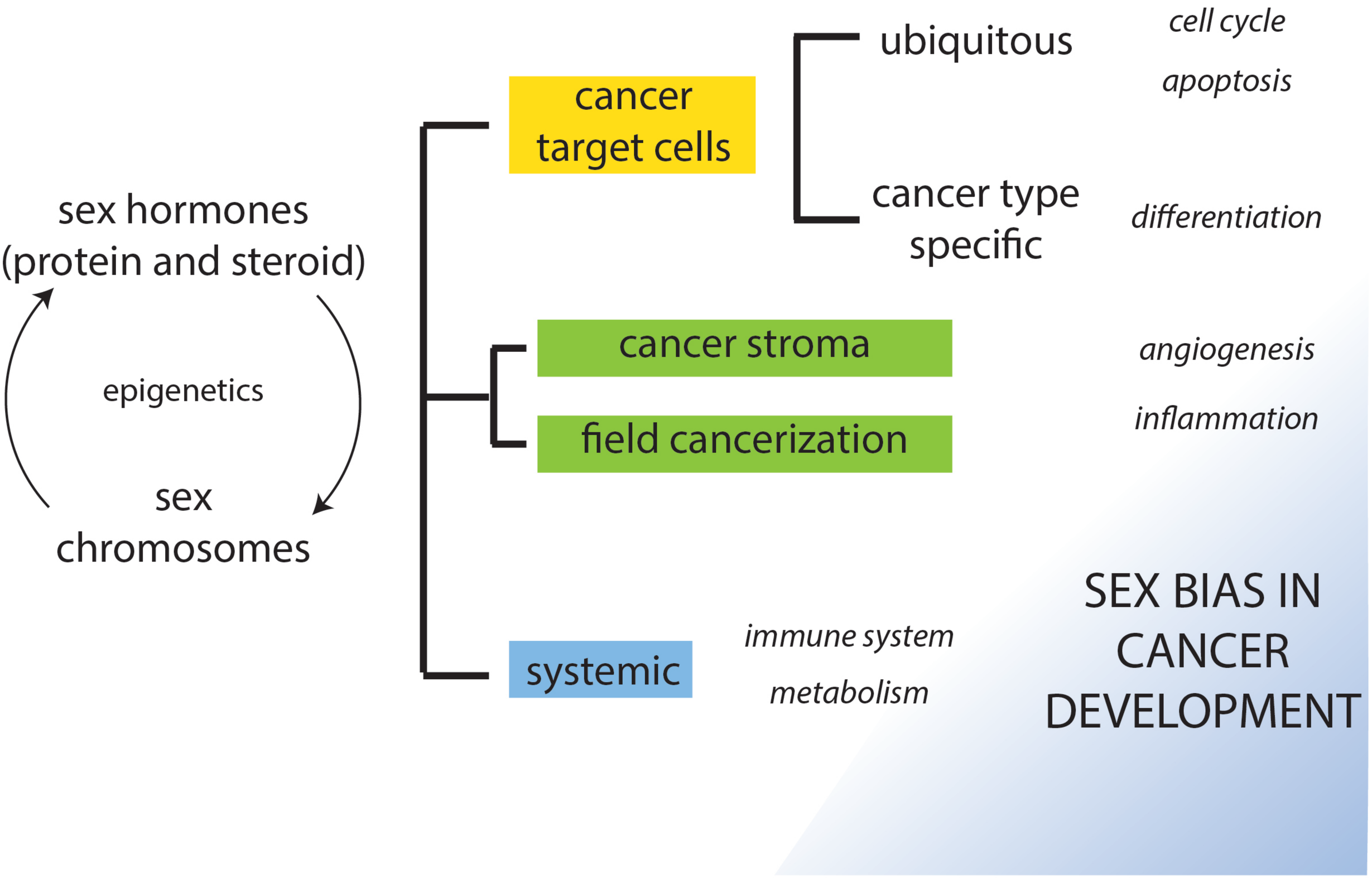

Cancer cell
UTX
UTX
AR110/45
AR110/45
Xist
Sox 3
Sox 3
Xist
UTX
UTY
OCT4
SRY
AR110/45
RBMY
TSPY
Sox 3

Figure 5


sex hormones
(protein and steroid)
epigenetics
sex
chromosomes
cancer
target cells
ubiquitous
cancer type
specific
cancer stroma
field cancerization
systemic
cell cycle
apoptosis
differentiation
angiogenesis
inflammation
immune system
metabolism
SEX BIAS IN
CANCER
DEVELOPMENT