Recent advances in the molecular mechanism of sex disparity in hepatocellular carcinoma (Review)

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Abstract. Hepatocellular carcinoma (HCC) is more frequently observed and aggressive in men compared with women. Increasing evidence demonstrates that the sex disparity appears to be mediated by the stimulatory effects of androgens and the protective effects of estrogen in the development and progression of HCC. In the past few decades, studies on the sex difference of HCC mainly focused on the effect of sex hormones on the transactivation of hepatitis B virus X protein and the release of inflammatory cytokines, and these studies have further intensified in recent years. Sex hormones are also involved in genetic alterations and DNA damage repair in hepatocytes through binding to their specific cellular receptors and affecting the corresponding signaling pathways. Furthermore, the theory of sex chromosomes participating in HCC has been considered. The present review discussed the recent advances in the molecular mechanisms of sex disparity in HCC, with the aim of improving the understanding of the underlying critical factors and exploring more effective methods for the prevention and treatment of HCC.

Contents

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1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver and the second most common cause of cancer-associated mortality worldwide (1). The associated risk factors for HCC have been established and include viral hepatitis, alcohol consumption, nonalcoholic steatohepatitis, genetic metabolic diseases and environmental exposure (2,3). However, comparative studies and data have identified that a marked feature of HCC is that males have a higher incidence and worse prognosis compared with females in low- and high-incidence areas (4). The American Cancer Society estimated the numbers of new liver cancer cases that occurred in the United States in 2017 to be 40,710 (29,200 males and 11,510 females), with 28,920 mortalities (19,610 males and 9,310 females) (5). In China the most recent statistics indicate an incidence rate of 466,100 (343,700 males and 122,300 females), with 422,100 mortalities (310,600 males and 111,500 in females) (6). The sex disparity of HCC has demonstrated that the ratio of estrogen and testosterone levels may be associated with the initiation and progression of HCC, suggesting that active estrogen- and androgen-mediated signaling pathways may regulate the risk of HCC (7,8). In recent years, increasing attention has been focused on the genetic alterations of sex chromosomes, which may be responsible for the sex disparity in HCC (9-11). Considerable efforts have been exerted in exploring the molecular mechanisms involved in the sex disparity in HCC (7-9). The current article reviewed the molecular mechanisms underlying the involvement of the sex hormones, including androgens and estrogens and their corresponding receptors, as well as of the sex chromosomes in the pathogenesis of HCC.

2. Estrogen may serve an inhibitory role in sex disparity in HCC via miRNAs, DNA repair and obesity associated pathways

In contrast to the tumor-promoting activity of the androgens, the preventive and inhibitory effects of estrogen have been epidemiologically demonstrated by studies revealing an increased incidence of HCC following the menopause (12-14). This is consistent with animal studies in which treatment with estrogen decreased the incidence and metastasis of HCC, and ovariectomy increased susceptibility to HCC in female mice (15). In

past studies, chronic inflammation was a major contributor to tumorigenesis and estrogen modulated inflammatory tumor microenvironment via suppression of pro-inflammatory cytokines (16-20). In addition, the metabolism of 17β-estradiol (E2) is involved in the sex disparity in HCC. Overexpression of liver-specific cytochrome P450 1A2 (CYP1A2) markedly contributed to the inhibitory effect in HCC cells by converting E2 to the cytotoxic 2-methoxyestradiol (21,22).

However, in addition to the frequently reported molecular mechanisms underlining the role of estrogen in the gender disparity of HCC, recent studies have proposed that estrogen may serve an inhibitory role in sex disparity of HCC via micro RNA, DNA repair and obesity associated pathways (23-25).

Estrogen receptors. With different intracellular expression patterns in the nucleus, cytoplasm or membrane, estrogen is involved in various cellular processes including proliferation, survival, apoptosis and differentiation through the estrogen receptors (ERs) (26). ERα and ERβ, two forms of ERs, share significant structural homology and ligand binding properties, and yet function very differently. As in breast cancer, aberrant increases in *ER* gene expression have been reported in liver tumors compared with normal or non-tumorous liver in patients with HCC (27). ERα-mediated inhibition of nuclear factor-κB binding activity is a pivotal event in the process of inhibiting tumor formation (28). A previous study suggested that the malignant behavior of HCC cells is markedly suppressed by treatment with E2 through the E2/ERβ/mitogen-activated protein kinase (MAPK) pathway-mediated increase of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 inflammasome (29). ERα transfection effectively promotes the upregulation of estrogen to protein tyrosine phosphatase receptor type O (PTPRO) in HCC cell lines and it is positively correlated with the expression of ERα and PTPRO in liver tissues (30). It has also been identified that estrogen functions as a suppressor of macrophage alternative activation and tumor progression by preventing ERβ-adenosine triphosphate 5J interaction, thus inhibiting the Janus kinase 1/signal transducer and activator of transcription 6 signaling pathway (Fig. 1) (31). Other studies revealed that ER inhibited the proliferation and invasion of human HCC cells by decreasing the transcription of metastatic tumor antigen 1 and peroxisome proliferator activated receptor γ (32,33).

MicroRNAs (miRNAs). miRNAs are small noncoding RNAs of ~20 nucleotides that bind to conserved 3'-untranslated region sequences of their target mRNAs and induce the inhibition of their translation (34). Thereby miRNAs regulate gene transcription and expression to modulate important physiological functions (35,36). miRNAs serve a vital role in numerous pathological events and in the cell response to various stresses (35). In the hepatocarcinogenic process, numerous miRNAs show abnormal expression in HCC tissues compared with paired adjacent nontumorous tissues. Therefore, miRNAs are recognized as a group of host genetic factors associated with hepatocarcinogenesis (36-38). The cross-linking of some miRNAs with ER is involved in the sex difference in HCC. Zheng *et al* (22) concluded the correlation between some miRNAs and sex disparity in HCC, including miR-23a, miR-545 and miR-221. Other miRNAs associated with sex disparity in HCC will be discussed in the current review (Fig. 1). miR-21 exhibits reduced mRNA binding and silencing activity in healthy mouse liver, but its expression is significantly elevated in HCC (39). Teng *et al* (23) reported that dehydroepiandrosterone, a precursor for adrenal androgen biosynthesis, activates ERβ and androgen receptors and increases miR-21 transcription. On the contrary, E2 inhibits miR-21 expression via ERα (23). The role of circulating miR-22, as an independent prognostic marker of poor clinical outcome, has been demonstrated by Cox regression analysis (40). Jiang *et al* (41) demonstrated that overexpression of miR-22 in male tumor-adjacent tissue was associated with downregulated ERα expression by targeting its 3'-untranslated region. miR-22 suppresses ER transcription and attenuates the protective effect of estrogen, eventually increasing interleukin (IL)-1α expression. The persistently high level of IL-1α may lead to compensatory proliferation and tumorigenesis (41). In addition, by comparing the expression pattern of miRNAs between male and female patients with HCC, miR-18a was identified to be increased in female HCCs. Furthermore, miR-18a targets the estrogen receptor 1 gene, which encodes the ERα protein, and prevents translation of ER, preferentially blocking the protective effects of estrogen and promoting the development of HCC in women (42). In addition, elevated p53 promotes miR-18a processing to decrease the expression level of ERα in female patients with HCC, thereby suppressing the tumor-protective function of the estrogen pathway (43). The production of estrogen is associated with steroidogenesis pathways, including steroidogenesis enzymes (44). However, to the best of our knowledge, there have been no reports regarding the interaction of miRNAs with steroidogenesis genes involved in sex disparity in HCC.

DNA damage repair. Genetic alterations and genomic instability, possibly resulting from unrepaired DNA lesions, are increasingly recognized as a common feature of human HCC (45,46). In particular, next-generation sequencing technologies have revealed numerous genetic alterations, including recurrently mutated genes and dysregulated signaling pathways in HCC (45). Therefore, timely repair of DNA damage is necessary. However, whether DNA damage repair is involved in the sex disparity in HCC is largely unclear. The potential association between estrogen and genomic instability is worth exploring. Previous studies have reported the development of HCC from the aspects of DNA damage repair-associated genes, including poly (ADP-ribose) polymerase (PARP1), transcription factor IIH (TFIIH) and nicotinamide adenine dinucleotide (NADC) (47-49), which were associated with estrogen signaling pathways (49-51). The present review explored the roles of these DNA repair associated genes in the sex disparity of HCC (Fig. 2).

Poly (ADP-ribose) polymerase (PARP) 1. PARP1, a well-known DNA-binding enzyme, has a potential role in DNA repair, especially in triggering the base-excision repair process in the early stage of oxidative DNA damage repair (52). PARP1 is also involved in a variety of other biological processes, including transcriptional regulation, apoptosis, mitosis and protein degradation (53). The hepatitis B virus (HBV) core promoter region binds to PARP1 and inhibits the DNA repair capacity of

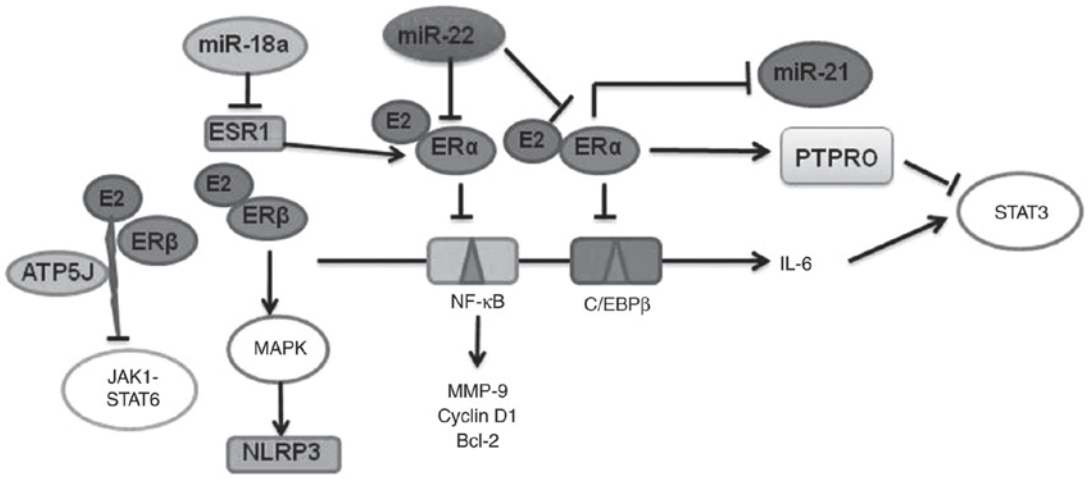

Figure 1. Estrogen serves an inhibitory role in the sex disparity in hepatocellular carcinoma by regulating inflammation and miRNAs. ERα, estrogen receptor α; ERβ, estrogen receptor β; E2, estradiol; NF-κB, nuclear factor-κB; C/EBP β, enhancer-binding protein β; IL-6, interleukin-6; PTPRO, protein tyrosine phosphatase receptor type O; STAT3, signal transducer and activator of transcription 3; MMP-9, matrix metalloproteinase-9; MAPK, mitogen-activated protein kinase; ATP5J, Adenosine triphosphate 5J; JAK1, Janus kinase 1; STAT6, signal transducer and activator of transcription 6; miR-21, microRNA-21; miR-22, microRNA-22; miR-18a, microRNA-18a; Bcl-2, B-cell lymphoma 2.

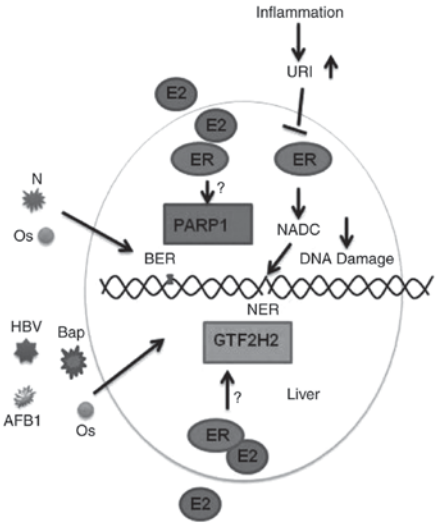

Figure 2. Estrogen may serve an inhibitory role in sex disparity in hepatocellular carcinoma via DNA repair. ER, estrogen receptor; E2, estradiol; PARP1, poly (ADP-ribose) polymerase 1; GTF2H2, general transcription factor IIH subunit 2; BER, base-excision repair; NER, nucleotide excision repair; URI, unconventional prefoldin RPB5 interactor; NADC, nicotinamide adenine dinucleotide; HBV, hepatitis B virus; Bap, benzo(a)pyrene; AFB1, aflatoxin B1; Os, oxidative stress markers.

PARP1, potentially disrupting host DNA damage repair (54). PARP-1 is downregulated in HBV-infected patients compared with uninfected controls (55). It has been reported that the physical interaction of hepatitis B virus X protein (HBX) and PARP1 accelerated DNA damage by inhibiting recruitment of the DNA repair complex to damaged DNA sites, which lead to hepatocarcinogenesis (47). In breast tissue, there is a positive association between PARP1 and ER expression (50). However, there are few studies on the association between ER and PARP1 in HCC, and this merits further exploration.

Transcription factor IIH. Research implies that HBX impedes the DNA repair process via its physical interactions with the helical components of TFIIH, including excision repair cross-complementing rodent repair deficiency, complementation groups 2 and 3 proteins (56). TFIIH is a multiprotein complex of 10 polypeptides and has clearly been shown to be an integral component of the DNA repair pathway (57,58). Lee *et al* (59) reported the interaction of HBX with a probable cellular repair protein UV-damaged DNA-binding protein, which acts as an essential factor in HBV-associated hepatocarcinogenesis. General transcription factor IIH subunit (GTF2H) is located on 5q13.2 and encodes the 44-kDa RNA polymerase II TFIIH protein subunit 2 that interacts with other TFIIH subunits in the nucleotide excision repair pathway. Zhao *et al* (48) identified 30 (36.1%) of 83 HCC cases with loss of heterogeneity at 5q13.2, in which the tumor-associated gene *GTF2H2* was present. *GTF2H2* is an estrogen signaling pathway gene in breast cancer and is downregulated by luteolin (51). Therefore, the sex disparity in HCC partly attributed to *GTF2H2* is increasingly plausible.

Nicotinamide adenine dinucleotide (NADC). In the early stage of many types of cancer, including HCC, oncogene activation induces replication stress, resulting in DNA damage and chromosomal instability and acceleration of tumor development. Tummala *et al* (49) reported that increasing NAD^+ concentration is a critical mechanism in the prevention of HCC. They described that unconventional prefoldin RPB5 interactor (URI) inhibits the aryl hydrocarbon receptor (AhR) and ER-mediated transcription of enzymes implicated in NAD^+ metabolism and synthesis, which causes DNA damage in the early stages of tumorigenesis (49). Djouder (60) proposed boosting NAD^+ as a strategy to prevent and cure HCC and revealed that the activation of AhR and ER was beneficial in HCC. Tummala *et al* (49) reported that AhR and ER could reverse URI-induced transcription of L-tryptophan/kynurenine catabolism and reduce the expression of tryptophan 2,3-dioxygenase through establishing AhR and ER knockout mice and conducting experiments in which AhR and ER were depleted in HepG2 cells.

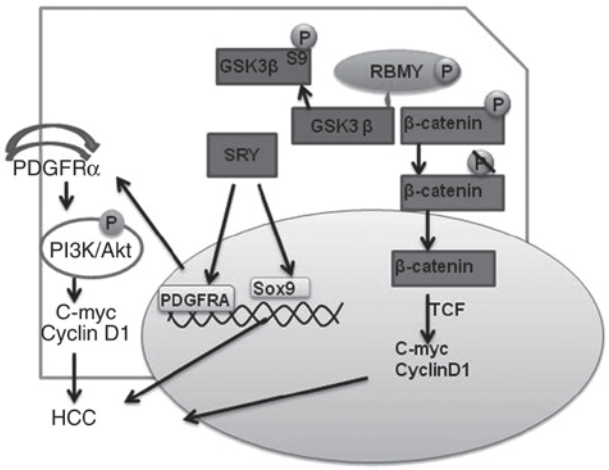

Figure 3. Y chromosome associated protein-coding genes are involved in HCC. SRY, sex-determining region on the Y chromosome; PDGFRα, platelet-derived growth factor receptor α; PI3K, phosphoinositide 3-kinase; RBMY, RNA-binding motif gene on the Y chromosome; GSK3β, glycogen synthase kinase 3β; TCF, T-cell factor; HCC, hepatocellular carcinoma.

Obesity. Unhealthy lifestyles including smoking and alcohol consumption are more prevalent among males compared with females, and are also speculated to be susceptibility factors for sex disparity of HCC (61). Obesity is a significant risk factor for certain types of cancer, including HCC (61,62). Park *et al* (63) described that both dietary and genetic obesity enhance the inflammation-dependent increase in IL-6 and tumor necrosis factor expression and promote liver inflammation and tumorigenesis. Leptin, a 16 kD protein hormone secreted by white adipose tissue, participates in the regulation of numerous physiological functions including atherosclerosis and carcinogenesis (64). Abnormal regulation of leptin-signaling serves a crucial role in obesity-associated liver cancer (64,65). Shen and Shi (25) investigated the function of E2 in opposing oncogenic actions of leptin in HepG2 cells, which are poor host cells for supporting the replication of HBV or hepatitis C virus. The researchers used small interfering-RNAs specific for ER-α, ER-β and G protein-coupled ER (GPER) to verify that E2 decreased activation of the leptin-signaling pathway through its receptors (25). E2 enhanced the activity of extracellular signal-regulated kinase via activation of ER-α and GPER and upregulated p38/MAPK via activation of ERβ. These responses reversed leptin-induced alterations, eventually inhibiting cell proliferation and stimulating cell apoptosis (25).

3. Androgen/AR serves a role in promoting sex disparity in HCC

Androgens are male hormones that have been increasingly reported in male-predominant HCC (66,67). They are mainly involved in various physiological and pathological activities by combining with androgen receptors (ARs) (68,69). A study by Wu *et al* (70) identified that overexpression of ARs enhanced HCC cell growth and invasion *in vitro*, and HCC initiation *in vivo*. Previous studies have reported higher androgen levels and more active androgen response elements (AREs) in liver tumor tissues, compared with control tissues (8,71). Further investigation revealed that when male mice with AR knockout were induced by diethylnitrosamine (DEN), fewer tumors formed compared with wild-type mice (72). Androgen binding directly to AREs in the enhancer I of HBV genes activated the androgen-signaling pathway and increased the rate of HBV-induced hepatocarcinogenesis (8,73). AR binding to ARE of the cell cycle related kinase promoter region controls activation of the β-catenin/T-cell factor signaling pathway, and has been identified as a major carcinogenic event and described in animal models and up to 90% of HCC cases (74). Ligand-stimulated AR upregulated miR-216a, resulting in tumorigenesis, and AR and miR-216a were concordantly over-expressed in clinical specimens (38). Both activity and secretion of aromatase, an enzyme which converts androgens to estrogens, was markedly increased in human HCC tissues and HepG2 cells (75,76). This contradicts the protective effect of estrogen and promoting effect of androgen, and further studies are required to verify this observation.

4. Sex chromosomes are involved in sex disparity in HCC

Previous studies have revealed that genetic alterations of chromosomes X and Y are frequently observed in patients with HCC, including chromosome-specific gene change, oncogene and/or tumor suppressor gene expression and structural rearrangements of chromosomes (9,11,77). This indicates that genes located on sex chromosomes may be responsible for HCC (78,79).

X chromosome. X-chromosome-coupled zinc finger protein is abundantly expressed in HCC cells, and is associated with the proliferation and survival of tumor cells (77). In addition, mRNA and protein levels of dosage-sensitive sex reversal adrenal hypoplasia congenital critical region on X chromosome, gene 1 (DAX-1), are downregulated in HCC tissues and cell lines (10). DAX-1 is known for its fundamental roles in sex steroid-dependent neoplasms and interacts with β-catenin to attenuate its transcriptional activity (80,81). Jiang *et al* (10) first reported the role of the DAX-1/β-catenin molecular network in controlling HCC development. Furthermore, it was revealed that DAX-1 is regulated by androgens (82).

Y chromosome. Due to a lack of *in vivo* models, the impact of a small number of protein-coding genes in the Y chromosome remains largely unknown. Dysregulation of certain Y chromosome-specific genes, including RNA-binding motif gene on the Y chromosome and testis-specific protein Y-encoded (83,84), have been identified in male HCC. Y chromosome associated protein-coding genes responsible for HCC are briefly illustrated in Fig. 3.

Sex-determining region on the Y chromosome (SRY). SRY has been recognized as an oncogene and cancer stem cell promoter in male HCC in *in vitro* studies (85,86). Liu *et al* (9) reported overexpression of SRY in ~84% of male patients with HCC. A liver-specific transgenic murine model with overexpression of SRY was susceptible to DEN-induced hepatocarcinogenesis compared with age- and sex-matched wild-type mice (9). SRY activates its downstream target SOX9 and the platelet-derived

growth factor receptor α/phosphoinositide 3-kinase/protein kinase B pathway, which stimulates the expression of proliferation-associated genes *MYC* and cyclin D1 (*CCND1)*, eventually accelerating tumorigenesis (9).

RNA binding motif protein Y-linked (RBMY). Oncogenic activation of RBMY is an important factor in hepatocarcinogenesis, and a marked increase of cytoplasmic and nuclear RBMY has been noted in HCC tissues (11,87). The cytoplasmic expression of RBMY is associated with poor prognosis and decreased survival rate in patients with HCC (11). Cytoplasmic RBMY competes with the β-catenin destruction complex for binding GSK3b and enhancing the phosphorylation of glycogen synthase kinase 3β Ser9 residue, which eventually induces nuclear entry of β-catenin for transcription of downstream oncogenes (11). The tumorigenicity of RBMY has also been demonstrated though its ability to induce cell transformation and tumor formation in nude mice, and RBMY transgenic mice exhibited an increased DEN-induced liver cancer incidence (83).

Y chromosome loss and other genomic alterations. The genomic imbalances in HCC tissues have been studied mostly by comparative genomic hybridization (CGH) (88,89). Y chromosome loss and other genomic alterations in HCC cell lines were analyzed by CGH and CGH array by Park *et al* (78). Park *et al* (78) detected the karyotypes of 21 male HCC cell lines and identified 18 HCC cell lines with Y chromosome loss, which may be responsible for the male preponderance in HCC. In addition, increased copy number of several genes, *CCND1* and fibroblast growth factor 3/4 at 11q13, sarcoma amplified sequence/cyclin-dependent kinase 4 at 12q13, telomerase RNA component at 3q26, *MET* at 7q31, and *MYC* at 8q24, were identified in 20 primary HCC tissues (90).

5. Conclusions

HCC is characterized by an apparent sex disparity for which there lacks a clear mechanistic understanding. This current review summarized the recent research exploring the role of sex hormones and sex chromosomes in this process. Sex hormones and their receptors constitute two tumor-promoting and inhibiting axes through different channels. Genetic alterations in sex chromosomes could also contribute to the underlying mechanism of the sex disparity in HCC. In summary, the sex disparity in HCC is attributed to multiple mechanisms, and the targeting of both sex hormones and sex chromosomes is a novel and promising therapeutic approach for patients with HCC.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

JH designed the study and revised it. YL, AX and SJ drafted the manuscript and revised the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This review does not contain any studies with human participants or animals performed by any of the authors.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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