Therapeutic Value of Estrogen Receptor α in Hepatocellular Carcinoma Based on Molecular Mechanisms

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Abstract

The incidence of hepatocellular carcinoma (HCC) is significantly lower in women than men, implying that estrogen receptors (ERs) may play an important role in this sex dimorphism. Recently, considerable progress has been made in expanding our understanding of the mechanisms of ERs in HCC. As one of the most important ERs, ERα functions as a tumor suppressor in the progression of HCC through various pathways, such as STAT3 signaling pathways, lipid metabolism-related signaling pathways, and non-coding RNAs. However, the function of ERα was reduced with the changes of some molecules in the liver, which may develop further into HCC and make it difficult to achieve an effective hormone treatment effect. Intriguingly, there are signs that individualized hormone therapy according to the activity of ERα will overcome this challenge. Based on these observations, it is particularly imperative to reassess and extend the function of ERα. In this review, we mainly elucidated molecular mechanisms associated with ERα in HCC and investigated the individualized hormone therapy based on these mechanisms, with the aim of providing new insights for HCC treatment.

Keywords: Hepatocellular carcinoma; Estrogen receptor; Molecular mechanisms; Hormone therapy; Precision medicine.

Introduction

Hepatocellular carcinoma (HCC) is the most common histological type of primary liver cancer, comprising 75–85% of cases. Viral infection, metabolic disorders and alcoholism are the major risk factors for HCC development. In addition, the disparity of incidence and prognosis in HCC suggests that sex is a key factor for HCC. Since it has been reported that oral contraceptives could increase the risk of hepatic neoplasms in the last century (1970s), the role of estrogen and estrogen receptors (ERs) in the progression of HCC has gradually been revealed. Subsequently, a large number of studies explored the therapeutic value of tamoxifen (TMX) or estrogen replacement therapy for HCC, triggering the debate on whether hormone therapy can be used for HCC.

TMX, a selective estrogen receptor modulator (SERM), is used as a first-line treatment for breast cancer through its ability to antagonize estrogen-dependent growth by binding ERs; yet, its efficiency for HCC remains controversial. Early clinical trials with small samples showed that TMX can lower the level of alpha-fetoprotein (AFP) and significantly prolong the survival of patients with inoperable HCC. However, this conclusion was doubted by the subsequent clinical trial which demonstrated that TMX has no efficiency in improving the survival of patients with advanced HCC. TMX treatment of HCC has since reached an impasse. Compared with TMX therapy, the effects of estrogen replacement therapy of HCC are relatively clear. It has been reported that estrogen replacement therapy was mainly used to treat female perimenopausal syndrome, and it has effects on advanced HCC to some extent, especially for certain specific populations. However, estrogen replacement therapy possesses certain side effects, and how to maximize the benefits from this kind of hormone therapy in HCC needs to be further explored.

In addition to the understanding of hormone therapy, the molecular mechanisms of ERs in HCC have been revealed frequently in recent years, with the mechanisms of ERα being the most extensively reported ones. In spite of few studies showing that ERα has tumor-promoting effects on HCC, the estrogen-mediated inhibition of HCC is widely recognized. ERα is considered to regulate inflammation, iron homeostasis, energy metabolism and other processes to protect the liver. Moreover, a multitude of studies have shown that the expression of ERα in primary HCC tissues is decreased compared to normal liver tissues or the adjacent tissues, which indirectly confirmed the suppressive effects of ERα in HCC. In light of this anti-tumor effect of ERα in HCC, reactivating ERα signaling can offer a new therapeutic strategy in HCC prevention and treatment.

Up to now, the therapeutic value of targeting ERα in HCC has not reached a consensus. This review focuses on the regulatory mechanisms of ERα in HCC and seeks out the potential initiative of hormone therapy for HCC.
**Structure and mechanism of ERα**

ERα is the first discovered type of ERs, consisting of 595 amino acid residues. On account of its molecular mass being 66 kDa, it is also called ERα66. ERα is encoded by the ESR1 gene and functionally divided into five parts, including the amino-terminal ligand-dependent transcription activation function 1 (AF-1) domain, DNA binding domain, a hinge region which participates in DNA binding and nuclear localization, ligand binding domain, and carboxyl-terminal ligand-dependent transcription activation function 2 (AF-2) domain (Fig. 1).

ERα commonly refers to nuclear ERα which is recognized by estrogen at the ligand binding domain in cytoplasm. Mechanistically, ERα exposes its DNA binding region after dissociating from heat shock protein, and then directly acts on the estrogen response elements (EREs) to regulate the transcription of target genes or indirectly interact with some transcription factors, such as AP-1, SP1, NF-κB, in a protein-protein manner to regulate the transcription of their target genes.16

Different from nuclear ERα, membrane-localized ERα mainly triggers the protein-kinase cascades to enable gene regulation.17

**Signaling pathways of ERα**

**Upstream signaling pathways**

The activity of ERα is indispensable for its anti-tumor effects and hormone therapy of HCC. However, it has been reduced by the aberration of numerous upstream regulators, such as the weakening of MOF and FOXA1/2, and the enhancement of a series of putative cancer-related microRNAs (miRNAs), metastasis-associated protein 1 (MTA1) and Erbin. These changes together contribute to the low activity of ERα in HCC and the deficiency of hormone therapy (Fig. 1).

**Positive regulators of ERα:** MOF, which was found to have lower expression in HCC tissues, is identified as a liver protective factor.18 It stabilizes ERα by participating in the acetylation of ERα at K266, K268 and K299, hindering ERα ubiquitination and co-activating ERα target genes to augment its anti-tumor effects.19 FOXA1/2 are members of the forkhead box protein family, which bind to the relatively compact chromatin of the ERα target genes, and then enhance the interaction between ERα and its target genes.20 Furthermore, single nucleotide polymorphisms (SNPs) at FOXA2 binding sites and the FOXA1 nonsynonymous variant have frequently been observed in association with the development of HCC, which impairs their interaction with ERα and leads to the loss of ERα protective roles, especially in females.21,22

**Negative regulators of ERα:** Studies have shown that there are many putative upstream cancer-related miRNAs involved in regulating expression of ERα in HCC, such as miR-22, miR-221, miR-18a and miR-939-3p; these miRNAs have been demonstrated as overexpressed in HCC tissues and they can specifically recognize the ERα mRNA 3′-untranslated region and then trigger the degradation and the translation inhibition of ERα mRNA, which ultimately promotes the progress of HCC. It is worth mentioning that this...
interaction between miR-22 and ERα is more pronounced in males. MT1A1 is a co-repressor of ERα, and there is the bidirectional regulation between ERα and MTA1. Not only can ERα decrease the expression of MTA1 to protect liver, but overexpressed MTA1 can reduce the transcriptional activity of ERα to promote the progress of HCC. In addition, Erbin is overexpressed in HCC; transcriptionally, it inactivates ERα nuclear translocation and leads to the proteasomal degradation of ERα by increasing E3 ubiquitin-ligase-mediated ubiquitination, which eventually contributes to the progression of HCC. Intriguingly, the interaction between Erbin and ERα is more pronounced in males, and downregulating Erbin expression enhances the sensitivity of HCC cells to TMX.

**Downstream signaling pathways**

Clinical trials about ERα have been reported sporadically over the last decade, while some basic research about mechanisms of ERα in HCC had been reported successively (Fig. 1). Elucidating these mechanisms will help reveal the targets and screen the beneficiaries of hormone therapy for HCC, pointing towards the direction for individualized therapy and combination therapy.

**STAT3 signaling:** STAT3 is activated by a variety of cytokines, such as IL-6, epidermal growth factor (EGF) and hepatocyte growth factor, and plays an important role in the development of tumors through regulating downstream target genes in the form of a transcription factor. Recently, a number of studies have shown that ERα is an upstream repressor of STAT3 in HCC, which can mediate multiple pathways to inhibit HCC progression. The NF-κB signaling pathway plays an important role in the development of HCC, and its abnormal activation can induce the chronic inflammation that ultimately results in HCC. ERα physically interacts with NF-κB, thereby inhibiting activation of the IL-6/STAT3 pathway. On the foundation of this work, Naugler et al. found that in the HCC mouse models induced by diethylnitrosamine (DEN), the concentration of serum IL-6 and the activities of STAT3 as well as the abilities of tumor cell proliferation and necrosis were at a high level in males, as opposed to those in females. Furthermore, the administration of the ERα-specific agonist 1,3,5-tris(4-hydroxyphenyl)-4-propyl-1H-pyrazole (PPT) in males can block the secretion of IL-6 from Kupffer cells by interacting with NF-κB and thereby reduce the concentration of IL-6 in the circulation, which eventually inhibits the progression of HCC. The above results demonstrate that ERα has the ability to protect liver from damage by inhibiting the NF-κB/IL-6/STAT3 pathway in vivo, and to lower the concentration of IL-6 and eliciting milder underlying liver damage in females who have the higher ERα activity naturally. For this reason, the sexual dimorphism of IL-6 concentration regulated by ERα might be considered as one basis for determining the potential beneficiaries of HCC.

**Lipid metabolism-related pathways:** Leptin is a peptide hormone secreted by white adipose tissue. It binds to specific receptors and prevents the development of obesity by regulating various physiological and biochemical processes, such as glucose and lipid metabolisms and food intake. Nevertheless, leptin is always at a higher level in obese people, and it is considered to induce HCC by activating JAK-STAT3, PI3K/AKT and other signaling pathways. Shen et al. documented that ERα may be involved in opposing leptin-induced hepatocellular carcinogenesis. In detail, ERβ inhibits HCC mainly through activating the suppressor of cytokine signaling 3 (SOCS3)/STAT3 and p38/MAPK two pathways, while ERα inhibits HCC principally through enhancing ERK signaling and diminishing the leptin-induced STAT3 signaling without affecting SOCS3.

**Noncoding RNAs:** miRNAs, circular RNAs (circRNAs) and long non-coding RNAs (lncRNAs) are the canonical family members of noncoding RNAs that are deficient in protein-coding function. They can be involved in the control of gene expression and affect multiple biological processes in specific ways.

The downstream ERα-mediated signaling pathways with mRNA involvement in HCC have been extensively studied, such as ERα enhancement of the P53-mediated regulation of miR-23a expression and further augmentation of caspase-3/7 activity to induce apoptosis of HCC cells. Besides, ERα can regulate circRNA to exert a tumor suppressor effect. Xiao et al. showed that ERα inhibits HCC through mediating the circRNA-SMGL.72/miR-141-3p/Gelsolin (GSN) signaling pathway. Specifically, ERα binds to the ERE of circRNA-SMGL.72 host gene, thus weakening the expression and sponge role of circRNA-SMGL.72 for miR-141-3p. This indirectly augments the activity of miR-141-3p and ultimately inhibits the invasion of HCC, promoting the degradation of miR-141-3p-regulated GSN mRNA.

Furthermore, in hepatitis B virus (HBV)-related HCC, the transcription factor ERα can bind to the promoter of the lncRNA LINCO1352 by forming a complex with HBx. Functioning as a tumor suppressor, LINCO1352 prevents the miR-135b-mediated suppression of adenosomatous polyposis coli (APC) by sponging miR-135b and then repressing the Wnt/β-catenin signaling pathway. These findings demonstrate that the LINCO1352-miR-135b-APC axis regulated by the ERO/HBx complex acts as an important signaling cross-talk factor for tumor progression, which may offer a theoretical basis for HBV-related HCC. These non-coding RNAs that are involved in the anti-tu-
more attention should be paid to these therapeutic schemes TMX as well as the estrogen replacement therapy in HCC, Considering the current research status and the value of adverse reactions, but there are still controversies in HCC. In some specific tumors, due to its lower price and fewer be desired. Hormone therapy plays an indispensable role therapeutic satisfaction and practical effects still leave much to 4

Table 1. Landmark trials of hormone therapy of HCC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Main population</th>
<th>Comparison group</th>
<th>Salient findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMX (10 mg bid)</td>
<td>Patients with advanced HCC</td>
<td>No treatment</td>
<td>TMX improved survival in patients with advanced HCC and was well tolerated</td>
<td>Martínez 1994⁶</td>
</tr>
<tr>
<td>TMX (10 mg tid) plus triptorelin (3.75 mg monthly)</td>
<td>Patients with HCC</td>
<td>Flutamide plus triptorelin or placebo</td>
<td>TMX significantly prolongs survival and the TVDT in unresectable HCC</td>
<td>Manesis 1995⁵⁶</td>
</tr>
<tr>
<td>TMX (60 mg bid)</td>
<td>Patients with inoperable HCC</td>
<td>TMX (30 mg bid) or placebo</td>
<td>TMX does not prolong survival in patients with inoperable HCC and has an increasingly negative impact with increasing dose</td>
<td>Chow 2002⁷⁷</td>
</tr>
<tr>
<td>TMX (20 mg daily)</td>
<td>Patients with advanced HCC</td>
<td>Symptomatic treatment</td>
<td>TMX is not effective in prolonging survival of patients</td>
<td>Barbare 2005⁴⁸</td>
</tr>
<tr>
<td>TMX (80 mg daily)</td>
<td>Patients with inoperable HCC, wild-type ER</td>
<td>Megestrol for patients with inoperable HCC, variant ER</td>
<td>Treatment with TMX is strongly dependent on the type of ER present in the HCC</td>
<td>Villa 1996⁵⁰</td>
</tr>
<tr>
<td>TMX (20 mg bid) plus octreotide (0.6 mg daily)</td>
<td>Patients with inoperable HCC, ER-positive</td>
<td>5-Fluorouracil plus mitomycin C</td>
<td>TMX plus octreotide is superior to 5-fluorouracil plus mitomycin C in the patients with ER-positive status</td>
<td>Pan 2003⁵¹</td>
</tr>
<tr>
<td>TMX (40 mg daily) plus sorafenib (400 mg daily)</td>
<td>Patients who are intolerant to or progressed during sorafenib therapy</td>
<td>Full-dose sorafenib therapy (800 mg daily)</td>
<td>TMX could produce some clinical benefit at sorafenib progression in advanced HCC</td>
<td>Ottaviano 2017⁵²</td>
</tr>
<tr>
<td>HRT</td>
<td>Women with HCC (mostly menopausal)</td>
<td>Healthy women with no treatment</td>
<td>HRT reduced risk of HCC and increased overall survival times of patients</td>
<td>Hassan 2017⁸⁸</td>
</tr>
<tr>
<td>HRT</td>
<td>Women with HCC (most menopausal)</td>
<td>Patients' female relatives without HCC</td>
<td>Use of HRT was associated with a lower risk of HCC</td>
<td>Yu 2003⁴⁹</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; HCC, hepatocellular carcinoma; HRT, hormone replacement therapy; TVDT, tumor volume doubling time; TMX, tamoxifen.

There are various therapeutic approaches for HCC, but therapeutic satisfaction and practical effects still leave much to be desired. Hormone therapy plays an indispensable role in some specific tumors, due to its lower price and fewer adverse reactions, but there are still controversies in HCC. Considering the current research status and the value of TMX as well as the estrogen replacement therapy in HCC, more attention should be paid to these therapeutic schemes (Table 1).⁶,⁸,⁴⁶–⁵²

Tamoxifen – growing in doubts

In the past decades, researchers held different views about the clinical use of TMX for HCC. On the one hand, according to a small sample-sized TMX clinical study, TMX significantly prolonged the life cycle of patients with advanced HCC than that without any interventions.⁸ A further controlled trial evaluated the effectiveness of TMX in HCC patients, and found that compared with the placebo group, the survival and tumor volume doubling time of TMX-treated patients were significantly prolonged.⁴⁶ Moreover, basic research disclosed that TMX suppresses HCC by downregulating survivin at the transcriptional and post-transcriptional levels, as well as by inhibiting volume-activated chloride currents and the activity of cyclin-dependent kinase 5.⁵³–⁵⁵ Therefore, TMX is considered effective for HCC patients. On the other hand, the beneficial therapeutic potential of TMX for HCC patients has been questioned. A multicenter randomized controlled study containing 329 patients was developed to assess the effect of high-dose TMX compared with placebo, and showed that even a moderate dose of TMX has a negative impact on the survival of advanced HCC patients.⁴⁷ Another investigation showed that TMX seems to benefit patients without severe liver insufficiency, but for patients with advanced HCC, TMX is almost ineffective.⁴⁸ However, it is noteworthy that the above studies did not incorporate ER expression into the standard of patient selection, which may have resulted in the above different outcomes.

Hormone replacement therapy (HRT)

HRT is another alternative of hormone therapy, and the effectiveness of estrogen replacement therapy in HCC has been proven to some extent. Estrogen is at a high level in women during pregnancy or before menopause. In order to verify the influence of reproductive factors on HCC, a large sample-sized case-control study has been conducted between HCC patients and their relatives, and showed that the longer the period of the administration of estrogen, full-term pregnancies and age at natural menopause, the lower risk of adult women suffering from HCC.⁴⁹ Recently, another case-control study of female patients with HCC showed that estrogen replacement therapy effectively inhibited hepatitis C virus- or HBV-related HCC, and long-term application of
Individualized treatment – breaking through the bottleneck of HCC hormone therapy potentially

In view of the mechanisms of ERs in HCC, ERα-selective stimulation was postulated to achieve better results in the treatment of HCC. RNA sequencing was established in a human HCC-derived HepG2 cell line following treatment of control, estradiol (E2) and the ERα-specific agonist PPT; the results indicated that E2 or PPT suppressed the HepG2 cell transcriptome involving cellular and metabolic processes, which provided insight into the protective effects of an ERα-specific agonist in HCC development. However, agents selectively agonizing ERs for HCC are limited to the basic research realm, neither TMX nor estrogen replacement therapy can precisely and arbitrarily regulate ERs. Therefore, it is especially indispensable to accurately classify HCC patients underlying ERα expression and molecular mechanisms in order to find the potential candidate of hormone therapy.

The sensitivity of TMX for HCC is believed to have a positive relationship with the expression of ERα. Considering this positive relationship and anti-HCC effect of ERα, it is plausible that TMX and estrogen can be used to cure HCC to a certain extent. Nevertheless, considering the impacts of ER variants and upstream negative regulatory molecules, ERα is found only in a limited percentage of HCC patients, and this may contribute to the non-response to hormone therapy. In the work of Villa and colleagues, 50 HCC patients were included in these studies is limited, the conception of classified treatment opens the possibility for developing individualized precision treatments for HCC patients.

Conclusions

In the work of Villa and colleagues, 50 HCC patients were distinguished by wild-type ERα and ERα5 phenotypes, and the treatment effectiveness of TMX was confirmed in the wild-type phenotype patients. Therefore, the application of hormone therapy may be mostly dependent on the classification of ERα, and screening or expanding HCC patients with higher ERα expression may be conducive to improving the hormonal therapy sensitivity. Besides, the targets of hormone therapy are not only ERα (ERα66) but also ERβ, ERα36 and others, and the therapy effects of these receptors are not completely identical. For instance, ERβ can inhibit HCC by blocking the oncogenic actions of leptin and transcription of PPARα, promoting pyroptosis mediated by the NOD-like receptor pyrin domain containing-3 inflammasome. Unlike ERβ, ERα36, one variant of ERα, was found to be highly expressed in HCC tissues and can promote the EGF receptor/Src/ERK signaling pathway to exert anti-tumor effects after E2 treatment. The G protein-coupled estrogen receptor (GPER) should also be mentioned, although its functions are still highly controversial. Thus, in the condition of the present understanding for ERs in HCC, the hormone therapy for HCC patients with higher expression of ERβ and lower expression of ERα36 should be considered into the ER classification system likewise to develop a comprehensive individualized hormone therapy regimen of HCC.

Identifying the target population who will benefit most from this hormone treatment is another important approach. The impacts caused by the changes of IL-6, FOXA1, miR-22 and Erbin showed that distinct sex morphism in HCC tissues. Higher circulating concentrations of IL-6 which can be reduced by ERα presenting in males, rather than females, suggests that TMX or estrogen mimics may carry more potential for benefit in males. FOXA1/2 protect females from HCC and promote HCC in males following hepatocarcinogenesis, which seems to indicate the importance of the hormone therapy for females; yet, it is also noteworthy that mutations of the FOXA1 and FOXA2 binding sites are significantly frequent in HCC tissue and have little effect on the attenuation of ERα transcriptional activity in males (as opposed to females); so, treating male patients who carry the above-described mutations with hormone therapy is worth investigating. miR-22 as well as Erbin exert stronger negative regulatory effects on ERα in male mice (vs. female mice), giving the opportunity to enhance the efficiency of hormone therapy for males after their inhibition. The G protein-coupled pathways and PPARα transcriptional activity are most common in obese HCC patients, which emphasizes the potential value of hormone therapy for them. In sum, we hypothesized that males or obese HCC patients may be the population most likely to benefit from TMX and estrogen replacement therapy. Besides, based on the fact that a slice of upstream factors can negatively regulate ERα activity, trying hormone therapy in combination with inhibiting aberrations of ERα upstream regulators may be an entry point to expand the potential-benefit population.

It is important to acknowledge the limitations of hormone therapy alone for HCC. Early research tried the combination of hormonal therapy with other agents and partly confirmed the superiority of it (vs. monotherapy). Sorafenib, the first-line targeted therapy for unresectable HCC patients, combined with TMX, enabled patients to achieve a substantial overall disease control rate regarding. Whether the drugs from among the present first-line treatment spectrum, e.g. oxaliplatin, can achieve the additive effect with the combination of individualized hormonal therapy is worth investigating. Although the current status of hormone therapy of HCC is not optimistic, the further research on combination therapy that we postulated above will aid in providing a novel therapeutic strategy for the treatment of patients with HCC.

Considering the aforementioned molecular mechanisms and potential therapeutic strategy, we believe that hormone
therapy under the guidance of precision medicine and combination therapy will help to address the limitation of hormone therapy alone for HCC patients.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Conceptualization and writing of the manuscript (XM, XL).

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Meng X. et al: Therapeutic value of ERα for HCC


