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Interplay of estrogen receptors and FOXA factors in the liver cancer

Yongbing Zhao and Zhaoyu Li

Department of Cancer biology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA

Abstract

Liver cancer is the fifth most common cancer in human with male dominance. Sexual dimorphism of liver cancer is conserved from rodents to humans, which was firstly found in mice in late 1930s and female mice were resistant to liver cancer. Sex hormones were found to affect the incidence of liver cancer in rodents. Estrogen receptor alpha (ER α)-mediated estrogen signaling or androgen receptor-mediated androgen signaling prevents or promotes the growth of rodent liver tumors, respectively. Forkhead box protein A (Foxa) factors, Foxa1 and Foxa2, also known as pioneer transcription factors in liver specification, are essential for both estrogen and androgen signaling by acting as central regulators of sexual dimorphism in liver cancer. This review mainly focuses on the interplay between ER α and FOXA factors in liver cancer, and summarizes recent breakthrough studies in elucidating the mechanisms of sexual dimorphism in liver cancer.

Keywords

estrogen; estrogen receptor (ER); sexual dimorphism; FOXA1; FOXA2; hepatocellular carcinoma (HCC); liver cancer

1. Introduction

Sexual dimorphism has been found in the susceptibility of many cancers (Dorak and Karpuzoglu, 2012). Women show significantly lower incidence of HCC than men (Parkin et al., 2005). Sex hormones, estrogen in females and androgen in males, have been known to modulate sexual dimorphism of liver cancer, primarily hepatocellular carcinoma (HCC) since late 1930s (Andervont and Lorenz, 1937; Burns and Schenken, 1940; Tomita, 1937). Sexual dimorphism in other types of liver cancer has been barely studied, though their incidences showed clear sex difference and male dominance in human (SEER/NCI). Estrogen suppresses the tumorigenesis of HCC, whereas androgen promotes it (Ma et al., 2008; Shimizu et al., 1998; Tsutsui et al., 1992; Wu et al., 2010; Yamamoto et al., 1991). However, the molecular mechanisms underlying sexual dimorphism of liver cancer has been poorly understood. Our recent breakthrough study revealed that forkhead box protein A

Correspondence to: li.zhaoyu@mayo.edu.

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(Foxa) factors, Foxa1 and Foxa2, acted as the central regulators of sexual dimorphism through steroid hormone receptors in a mouse model of carcinogen-induced HCC (Li et al., 2012). In this review, we focus on the estrogen regulation of the liver cancer.

2. Milestones in studying estrogen action in liver cancer

Based on a literature search, we summarized all published research articles regarding estrogen action in liver cancer and found that there were two peak times over the past 70 years. The first boom was a 20-year period from the 1970s to 1990s, and the second began in the late 2000s and has been on the rise to today (Figure 1).

The first study on sexual dimorphism in liver cancer was reported in late 1937, and sex hormones were believed to be the major players (Andervont and Lorenz, 1937; Tomita, 1937). Thereafter, more and more research activities were focused on sex hormone action in liver cancer. After the first estrogen receptor, estrogen receptor alpha (ER α), was identified in 1958 (Jensen and Jacobson, 1960), studies on estrogen regulation in liver cancer started to increase rapidly; the peak of this research interest appeared around the time when the ER α was cloned in 1986 (Green et al., 1986; Greene et al., 1986). But following the finding of the second nuclear estrogen receptor, estrogen receptor beta (ER β) in 1996 (Kuiper et al., 1996) and the discovery of a novel membrane-localized estrogen receptor, G protein-coupled estrogen receptor (GPER), identified in 2000 (Filardo et al., 2000), related studies became less active. Two recent breakthroughs reignited the interest in the study of estrogen action in liver cancer: one was the discovery of the protective role of ER α in HCC found in 2007, which showed that estrogens prevented HCC through inhibition of IL-6 expression (Naugler et al., 2007); the other was the 2012 discovery that ER α -mediated estrogen signaling for the protection against the development of liver cancer in carcinogen-treated mice depended on Foxa factors, Foxa1 and Foxa2 (Li et al., 2012). Based on these recent breakthrough studies, we anticipate that there will be a spike in the investigation and clinical application of estrogen regulation in liver cancer in the coming years (Figure 1).

3. Overview of estrogen receptors and estrogen action

In mammals, estrogen is an essential sex steroid hormone involved in many cellular processes, including cell metabolism, cell differentiation, and tissue development. In these processes, estrogen-targeted gene regulation generally requires the interaction between estrogen receptor (ER) proteins and genomic DNA, in which ER acts as a transcription factor (Deroo and Buensuceso, 2010). In addition, the activation of ER α requires the binding to its natural ligand, 17 β -estradiol, which was first reported in rat uteri in 1958 (Jensen and Jacobson, 1960). Estradiol is mainly generated from female ovaries and also synthesized in liver (Yamamoto et al., 1984), fat (Grodin et al., 1973), testicular (Fritz et al., 1976), adrenal (Davies et al., 1970), breast (Miller and Forrest, 1974), and neural (Ryan et al., 1972) tissues. Thus, ER-mediated estrogen signaling has been observed in both males and females. The ER α gene *ESR1* was cloned and sequenced in 1986 (Green et al., 1986; Greene et al., 1986). Ten years later, another isoform of estrogen receptor, ER β , was identified in rat prostates (Kuiper et al., 1996). Both ER α and ER β are ligand-activated transcription factors, belonging to the nuclear hormone receptor protein family. Both of

these receptors have five homologous domains, a highly-conserved ligand-binding domain (LBD, the amino acid identity ~55%), and a DNA-binding domain (DBD, the amino acid identity > 95%), indicating that they could bind to the same *cis*-regulatory elements of genomic DNA (Dahlman-Wright et al., 2006; Kumar et al., 2011). The transcriptional activities of ER α and ER β are mediated by the synergism between two distinct activation function (AF) domains, AF1 and AF2. AF1 is located at the N-terminal, and AF2 is located at the C-terminal of ERs (Dahlman-Wright et al., 2006). In ER α , the AF1 is constitutively active and AF2 is ligand-dependent, while the function of AF1 in ER β is weaker than that in ER α , so the transcriptional activation of ER β depends more on ligand-dependent AF2 domains (Delaunay et al., 2000). Both ER α and ER β can shuttle between the cytoplasm and nucleus (Defranco et al., 1995; Tyagi et al., 1998). In addition, another membrane-associated estrogen receptor, GPER (also known as GPR30), was discovered in 2000 (Filardo et al., 2000), and the *GPER1* gene has been cloned and investigated in several labs (Carmeci et al., 1997; Feng and Gregor, 1997; Kvingedal and Smeland, 1997; O'Dowd et al., 1998). Unlike those nuclear estrogen receptors (ER α and ER β), GPER has seven transmembrane domains, mediates the activation of extracellular signal kinase (Prossnitz and Barton, 2011), and involves in non-genomic estrogen signaling (Olde and Leeb-Lundberg, 2009). GPER widely expresses in liver and many other tissues and systems, such as reproductive system, immune system, and cardiovascular system (Olde and Leeb-Lundberg, 2009; Prossnitz and Barton, 2011). However, the role of GPER in hepatic tumorigenesis remains to be elucidated.

In the estrogen signaling pathway, ERs interact with *cis*-regulatory elements of their target genes by two different types of mechanisms when activated by estrogen. One is the classical pathway in which ERs directly binds to the estrogen response elements (EREs; 5'-GGTCANNNTGACC-3', N means any one of A, C, G or T), and the other is when ERs indirectly associate with other transcription factors, including SP-1 and AP-1 (Klinge, 2001; Paech et al., 1997; Porter et al., 1997; Saville et al., 2000). Genomic studies showed ER α and ER β mostly bind to the same binding sites (Grober et al., 2011). Nuclear expression of estrogen receptors has been detected in many tissues in both rodents and humans. Both ER α and ER β express in the uterus, ovaries, breasts, the immune system, the cardiovascular system, bone, brain, and others, while differential expression of ER α and ER β proteins has been observed in many tissues, e.g., ER β is enriched in cardiovascular system, whereas ER α is enriched in liver, prostate, and breast (Denger et al., 2001; Pinzone et al., 2004). Along with full length ER, many ER splice variants (truncated or selectively modified isoforms of ER) have been found in multiple cells and tissues, and differential expression of these ER variants has been considered as indicators of cancer development (Taylor et al., 2010). Although the sequences for ER α and ER β are quite similar, they have different splice variant profiles (Hirata et al., 2003). These ER splice variants differently express in many tissues, including brain, breast, and prostate (Taylor et al., 2010). Among those ER variants, ER α 2, ER α 5, ER α 5' and ER β 5' have been detected in liver cancer but are barely found in normal liver (Hirata et al., 2003; Taylor et al., 2010; Villa et al., 1995). Thus, hepatic ER variants and their classifications have been considered as biomarkers of HCC (Villa et al., 2003).

4. Protective roles of ER α in liver cancer

Earlier studies in rodent models indicate the protective roles of estrogen in liver cancer. Sexual dimorphism in HCC has been observed in mice with chemically induced hepatocarcinogenesis since late 1930s, in which female mice barely developed liver tumors (Andervont and Lorenz, 1937; Burns and Schenken, 1940; Tomita, 1937). Further studies showed that castration of male mice caused a decrease in the incidence of HCC, while castration of female mice led to an increased incidence of HCC, indicating that chemically-induced tumorigenesis in the liver was enhanced by the presence or products of the testes and suppressed by the presence or products of the ovaries (Tsutsui et al., 1992; Yamamoto et al., 1991). Thus, Shimizu et al examined the impact of estradiol or testosterone supplements on castrated male or female mice with carcinogen-induced HCC and found that estradiol prevented and testosterone promoted the hepatic tumorigenesis in either castrated mice, respectively (Shimizu et al., 1998), suggesting that sex hormones are the major players of sexual dimorphism of HCC in mice.

However, later studies in human liver cancer raised the controversy about the roles of ER α -mediated estrogen signaling in liver cancer, unlike the clear roles of androgen in promoting HCC. Hishida et al. reported that 40 out of 48 HCC clinical samples showed hypermethylation at the promoter region of *ESR1* (gene name of estrogen receptor alpha), and the expression level for *ESR1* transcripts was decreased more than 90% in 24 HCC samples (mostly from men) relative to normal liver (Hishida et al., 2013). Jiang et al. showed that ER α protein expression was barely found in human liver cancer cell lines (Jiang et al., 1995); however, Liu et al. showed that ER α proteins were expressed in most of these human liver cancer cell lines and almost all human HCC tumors (Liu et al., 2009). The latter did not mention which ER α antibody they used or which size of ER α bands they blotted (Liu et al., 2009). However, a recent study showed truncated but not full-length *ESR1* mRNA was observed in human HCC tumors (Miceli et al., 2011). Thus, most of these studies have showed that ER α expression is lost or attenuated in human liver cancer cells and liver tumors, indicating the potential protective roles of estrogen signaling in human liver cancer and that defective estrogen signaling might lead to human liver cancer.

Nevertheless, recent mouse studies provided strong evidence to support the protective roles of ER α and deleterious effects of androgen receptor (AR) in liver cancer (Bigsby and Caperell-Grant, 2011; Ma et al., 2008; Wu et al., 2010). Mice lacking ER α lost the resistance to HCC (Bigsby and Caperell-Grant, 2011; Hong et al., 2013; Naugler et al., 2007). Mice lacking AR attenuated the promotion of hepatic tumorigenesis (Ma et al., 2008; Wu et al., 2010). Thus, ER α and AR are the essential mediators for the cellular actions of estrogen and androgen in the hepatocarcinogenesis, respectively. Interestingly, estrogen therapy has never been considered as a potential treatment for HCC patients. In contrast, estrogen was reported to stimulate the proliferation of human liver cancer cells HepG2 (Brandt et al., 2004), thus, anti-estrogen therapy, such as tamoxifen treatment, has been completed in several randomized clinical trials but has failed (Di Maio et al., 2006; Roxburgh and Evans, 2008; Salhab and Canelo, 2011). Considering the fact of defective estrogen signaling in human liver cancer, it is not surprising to see the failure of this type of clinical trials regarding estrogen or anti-estrogen therapy for liver cancer. Thus, detailed

characterizations of estrogen signaling in the livers of liver cancer patients would be promising for future clinical trials of estrogen or anti-estrogen therapy. These data further emphasize the importance of fully understanding the mechanisms of estrogen action in liver cancer in cancer therapy.

A recent breakthrough study illustrated the molecular mechanisms of sexual dimorphism in liver cancer and was the first study to briefly address the protective roles of ER α in hepatic tumorigenesis in mice (Naugler et al., 2007). This study has shown that sex difference in the levels of interleukin 6 (IL-6) produced by Kupffer cells contributed to sex disparity in liver cancer and low levels of IL-6 in female mice were protective from liver cancer (Naugler et al., 2007). Although this study showed that female but not male ER α null mice had increased liver injury upon carcinogen treatment (Naugler et al., 2007), it is still unclear whether the reduction in IL-6 expression is dependent on hepatic ER α -mediated estrogen signaling or what is the direct connection between hepatic ER α regulation and IL-6 expression regarding the resistance to HCC in female mice (Bigsby and Caperell-Grant, 2011; Naugler et al., 2007). More importantly, anti-IL-6 treatments did not show promising results for HCC patients (Di Maio et al., 2006; Kalra et al., 2008; Lawrence et al., 2007). Thus, IL-6-mediated sexual dimorphism in liver cancer is still controversial. We speculate that IL-6-mediated sexual dimorphism in liver cancer probably appear at the initiation stage of hepatic tumorigenesis and may not be a good target for liver cancer therapy. Nevertheless, this study rejuvenated the investigation of sex hormone regulation in liver cancer.

A recent study showed that ER α -mediated estrogen signaling in promoting the growth of breast cancer depended on forkhead box protein A1 (FOXA1) (Hurtado et al., 2011), which sheds light on potential molecular mechanisms of protective roles of ER α and FOXA factors in HCC.

5. A brief history of Foxa factors

Initially, Foxa factors were known as hepatocyte nuclear factor 3 (HNF3), which were identified as a family of pioneer transcription factors for liver specification (Costa et al., 1989; Kaestner, 2010; Kaestner et al., 1993; McPherson et al., 1993). The three HNF3 proteins (HNF3 α , HNF3 β , and HNF3 γ) are known as FOXA1, FOXA2 and FOXA3 in humans and Foxa1, Foxa2, and Foxa3 in mice, respectively (Kaestner, 2000). These Foxa family members are encoded by different genes. All three Foxa factors have an approximate 100 amino acid long DNA-binding domain, known as the forkhead box domain, which is located at the center of the protein sequence and recognizes the same DNA motif (Lai et al., 1990). Foxa factors have been hypothesized to initiate liver specification by de-compacting chromatin and re-positioning nucleosomes (Cirillo et al., 1998; McPherson et al., 1993). Mouse embryonic studies have shown that Foxa factors regulate organogenesis of the liver, lung, neuron, intestine, pancreas, and other tissues (Ferri et al., 2007; Gao et al., 2008; Kaestner, 2010; Kaestner et al., 1993; Lee et al., 2005; Li et al., 2009; Wan et al., 2005; Ye and Kaestner, 2009). Foxa1 and Foxa2 were found to be functionally redundant in liver development, but the hepatic role of Foxa3 is unclear (Kaestner, 2010; Lee et al., 2005; Li et al., 2009).

The studies on the roles of Foxa factors in carcinogenesis have been mainly focused on human prostate and luminal subtype A breast cancers (Barbieri et al., 2012; Bernardo et al., 2013; Carroll et al., 2005; Gao et al., 2003; Jin et al., 2013; Lupien et al., 2008; Mirosevich et al., 2006; Nakshatri and Badve, 2009; Wang et al., 2005; Wang et al., 2009; Yu et al., 2005). Both FOXA1 and FOXA2 are required for AR-mediated androgen signaling in promoting the growth of prostate cancer cells (Barbieri et al., 2012; Gao et al., 2003; Jin et al., 2013; Mirosevich et al., 2006; Yu et al., 2005). Only FOXA1 is required for ER α -mediated estrogen signaling in the promotion of breast cancer cell growth; FOXA2 is undetectable in mammary epithelial cells and breast cancer cells (Bernardo et al., 2013; Besnard et al., 2004; Hurtado et al., 2011). In addition, ER α , FOXA1, and GATA3 may form a transcription factor network to regulate the growth of breast cancer cells (Nakshatri and Badve, 2009). Moreover, FOXA1 has also been shown to modulate the growth of human lung cancer, brain cancer, and endometrial cancer cells (Bernardo and Keri, 2012; Qiu et al., 2014). Genomic distribution analysis showed that FOXA factors and ER α or AR frequently bound to adjacent *cis*-regulatory elements in the genome and the recruitment of ER α or AR to their binding sites was dependent on FOXA factors in breast and prostate cancer cells, respectively (Bernardo et al., 2013; Grasso et al., 2012; Hurtado et al., 2011; Jin et al., 2013). Thus, these studies suggest that FOXA-dependent genomic landscapes of steroid hormone signaling exist in the human genome, which provides a solid foundation for the understanding of sex hormone regulation in liver cancer.

6. Molecular mechanisms of sexual dimorphism in HCC

HCC is a male-dominant cancer in both humans and rodents. Generally, male mice form large tumors after chemically-induced tumorigenesis using varieties of carcinogens, such as dibenzanthracene, o-amido-azotoluene, 2-acetylaminofluorene, or diethylnitrosamine (DEN), whereas females barely develop any tumors (Andervont and Lorenz, 1937; Asamoto et al., 1989; Thomas, 1961; Tomita, 1937). Our recent breakthrough study on sexual dimorphism in liver cancer revealed that Foxa1/2 acted as the central regulator of sex hormone receptor-mediated estrogen and androgen signaling in a mouse model of DEN-induced HCC (Figure 2) (Li et al., 2012). Briefly, by using mouse models with liver-specific ablation of Foxa1/2, we found that sexual dimorphism of HCC was completely reversed in these Foxa1/2-deficient livers. Foxa1/2-deficient female mice had multiple large tumors in their livers, but males had fewer tumors in their livers upon carcinogen treatment. Using genomic sequencing technology, chromatin immunoprecipitation-coupled high-throughput sequencing (ChIP-Seq), we also found that the recruitments of ER α and AR to their *cis*-regulatory elements was dependent on the binding of Foxa1/2 in the genome. More importantly, we observed that genetic mutations at FOXA and/or ER α binding sites caused the loss of estrogen signaling at these loci in human HCC patients. These data indicate that FOXA-dependent ER α -mediated estrogen signaling or AR-mediated androgen signaling prevents or promotes the growth of liver tumors, respectively.

In addition, enhanced co-occupancy of Foxa/ER α or Foxa/AR in female or male control mice after carcinogen treatment indicates that females are responsive to protection from HCC whereas males are not, which would be worthwhile to further investigate in future studies. Interestingly, given that Foxa1/2 are essential to estrogen signaling in protecting

females from HCC and to androgen signaling in sensitizing males to HCC, it can be expected that Foxa1/2-deficient female mice might suffer the same consequences as male control mice. However, more and larger tumors were found in Foxa1/2-deficient female livers, suggesting that estrogen may exert an additional tumor-promoting effect on hepatocytes in the absence of Foxa1/2 (Li et al., 2012). Indeed, Yager et al reported hepatic estrogen metabolites, such as 16 alpha-hydroxy estrone and 2- and 4-hydroxycatechol, led to hepatocarcinogenesis due to their genotoxicities (Yager and Liehr, 1996). Estrogens, including estradiol and diethylstilbestrol, have occasionally been employed to treat cancers, including HCC; however, both preventing and promoting cancer effect were recorded (Di Maio et al., 2006; Kalra et al., 2008; Lawrence et al., 2007). Androgens, including testosterone and dihydrotestosterone, have been shown to promote HCC; however, clinical research employing anti-androgens for HCC treatment had many disappointing consequences, with few beneficial effects or even worse survival for patients (Di Maio et al., 2008; Groupe d'Etude et de Traitement du Carcinome, 2004). Finally, a recent study reported dual yet opposing roles of hepatic AR: promoting HCC initialization and suppressing HCC metastasis (Ma et al., 2012). These studies indicate that there is still a long way to fully understand the detailed mechanisms of sexual dimorphism in liver cancer.

In all, Foxa factors exert a dominant role in determining gender specificity in HCC development, while Foxa-dependent ER α and AR play opposite roles in HCC, in which ER α -mediated estrogen signaling is protective against HCC.

7. Prospects in the research of estrogen action in liver cancer

Genetic mutations at FOXA and/or ER α binding sites are highly correlated with the incidence of human HCC, indicating that individual estrogen targets could play essential roles in hepatic tumorigenesis. Genetic variants at FOXA1 or ER α binding sites that caused loss of estrogen signaling were also found to play a key role in breast cancer (Cowper-Salari et al., 2012; Hsu et al., 2013). Thus, aiming for the downstream targets of ER α could be a better approach than targeting ER α . We are currently working on the elucidation of individual functional ER α target genes in liver cancer and breast cancer by performing a comparative genomics study of FOXA/ER α dual regulation between liver cancer and breast cancer. Full illustration of functional and cancer-specific estrogen signaling would be beneficial for future cancer therapy.

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Highlights

- Milestones of studies on estrogen regulation in liver cancer;
- Protective roles of estrogen receptor alpha in liver cancer;
- Foxa1/2-depedent estrogen signaling in preventing liver cancer.

Figure 1. The annual publication record and milestones for the research of estrogen action in liver cancer

Data were collected from PubMed with key words “estrogen” and (“liver cancer” or “HCC”) in title or abstract, and also manually selected.

Figure 2.
Schematic view of Foxa-dependent androgen receptor (AR)- or estrogen receptor alpha (ERα)-mediated sex hormone signaling in promoting or preventing hepatic tumorigenesis.