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REVIEW

Role of sex steroid receptors in pathobiology of hepatocellular carcinoma

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Abstract

The striking gender disparity observed in the incidence of hepatocellular carcinoma (HCC) suggests an important role of sex hormones in HCC pathogenesis. Though the studies began as early as in 1980s, the precise role of sex hormones and the significance of their receptors in HCC still remain poorly understood and perhaps contribute to current controversies about the potential use of hormonal therapy in HCC. A comprehensive review of the existing literature revealed several shortcomings associated with the studies on estrogen receptor (ER) and androgen receptor (AR) in normal liver and HCC. These shortcomings include the use of less sensitive receptor ligand binding assays and immunohistochemistry studies for ERα alone until 1996 when ERβ isoform was identified. The animal models of HCC utilized for studies were primarily based on chemical-induced hepatocarcinogenesis with less similarity to virus-induced HCC pathogenesis. However, recent *in vitro* studies in hepatoma cells provide newer insights for hormonal regulation of key cellular processes including interaction of ER and AR with viral proteins. In light of the above facts, there is an urgent need for a detailed investigation of sex hormones and their receptors in normal liver and HCC. In this review, we systematically present the information currently available on androgens, estrogens and their receptors in normal liver and HCC obtained from *in vitro*, *in vivo* experimental models and clinical studies. This information will direct future basic and clinical research to bridge the gap in knowledge to explore the therapeutic potential of hormonal therapy in HCC.

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Key words: Sex hormones; Estrogen receptor; Androgen receptor; Hepatocellular carcinoma; Hepatocarcinogenesis

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies with limited treatment options. The major risk factors for HCC are chronic liver diseases with cirrhosis that include hepatitis B, hepatitis C, alcoholic liver disease and non-alcoholic steatohepatitis. Epidemiological reports indicate that regardless of etiologies, the incidence of HCC is higher in males than in females with the male: female ratio usually averaging between 2:1 and 4:1[1]. This male predominance is further supported by the clinical observations that chronic liver disease progresses more rapidly to cirrhosis in males than females and therefore cirrhosis that leads to HCC development is largely considered to be the disease of men and postmenopausal women[2]. In view of this remarkable gender disparity, various *in vitro* as well as *in vivo* studies have been initiated from time to time to explore the importance of sex hormones in HCC. However, the precise role of male and female sex hormones and their receptors in HCC remains still poorly understood. Androgens have been suggested to induce and promote HCC[3] and altered androgen metabolism has been reported to be associated with HCC[4]. In contrast, the role of estrogen in HCC has been controversial with evidence suggesting both carcinogenic and protective effects in the liver[3,5-9]. Very limited information is currently available regarding the mechanism of estrogen and androgen action in normal liver as well as in HCC.

It is well known that estrogen and androgen mediate their biological functions by binding with a high affinity to specific receptors, the estrogen receptor (ER) and the androgen receptor (AR). Both ER and AR belong to the family of nuclear receptors that act as transcription factors and regulate the expression of several genes. Our present day knowledge of structure and function of these receptors is primarily attributed to the extensive research on ER and AR in cancer of reproductive organs. However, recent advances in molecular research reveal that sex hormones do play a significant role in normal physiology of various organs other than the organs of the reproductive system. Both androgens and estrogens regulate transcriptional activation of various molecules involved in key cellular processes such as generation of immune responses, cell proliferation and apoptosis through functional receptors localized in various sub-cellular organelles[10-12].

The expression and functional status of AR and ER appear to play a significant role in the carcinogenesis of all hormone sensitive organs. However, liver has remained a less studied organ in the context of sex hormones and their receptors. Differential expression of wild type and variant forms of ER and AR has been reported in normal liver and HCC, indicating a strong link between sex hormones and pathogenesis of HCC[13-19]. Recent *in vitro* studies also provide further evidence in support of AR and ER involvement in various cellular events as well as interaction with viral proteins in hepatitis B virus (HBV) and hepatitis C virus (HCV)-induced HCC[20-25]. This review is focused on the compilation of the information so far available on the significance of AR and ERs in HCC and brings forth wide gaps in the existing knowledge to the notice of scientific world for future research.

SEX HORMONE RECEPTORS IN NORMAL HUMAN LIVER AND HCC

Estrogen receptor

The role of estrogen in modulating morphological and physiological features of liver became evident in early 1970s when a possible correlation between occurrence of hepatic neoplasms and use of oral contraceptives was suggested[26,27]. In the reproductive system, estrogen is known to act by binding to specific cytoplasmic and nuclear receptors. Hence, search began to identify such a receptor in the liver. In 1978, Duffy and Duffy first reported the presence of ER in normal human liver[28]. Subsequently, the presence of ERs in human HCC was demonstrated by Molteni *et al*[29] followed by Friedman *et al*[30] and Iqbal *et al*[31]. Since then, a number of studies have been reported addressing the expression of ERs in normal as well as neoplastic liver tissues. The early studies used indirect methods of receptor detection based on ligand binding assays. Table 1 gives the details of these studies[13,15,17,30-37]. These assays were quantitative and measured the amount of receptor in the samples as well as its affinity for the ligand in terms of dissociation constants. The percentage positivity for ER expression varied significantly among different studies. These variations may be attributed to the differences in sample size, methodologies, ethnicity of the population studied, stage of the disease and underlying etiologies. Earlier studies by Friedman *et al*[30] and Iqbal *et al*[31] showed that ER content is similar in HCC and normal liver. In contrast, later studies consistently showed that the expression of ER is decreased in HCC tissue specimens as compared to normal liver tissue specimens or the non-tumor part of the liver[17,32,38]. However, Eagon *et al*[33] documented elevated levels of cytosolic ER in 3 of the 9 tumors as compared to non-cancerous tissues. Nuclear ER expression was found to be suppressed in all HCC samples as compared to normal samples[33]. The major drawback of these studies was the use of binding assays for detection that do not provide any information on the subtype of ER, i.e. ERα and ERβ as known today. It is important to study the relative expression of both isoforms of ER since ERα and ERβ are known to have overlapping but quite distinct functions. There are few reports on direct detection of ER using specific antibodies. Table 1 gives the details of these studies[32,39-47]. However, all these studies have employed either immunohistochemistry (IHC) or enzyme-immuno assays (EIA) using antibodies specific for only ERα isoform. The ERβ isoform was identified later in 1996[48] and information on the expression of ERβ protein in HCC is lacking though few studies at mRNA levels have been documented.

Since the molecular characterization and cloning of ERα in the mid 1980s[49,50], attempts have been made to determine the expression of ER in liver tumors at mRNA level. Table 2 gives the details of these studies[18,19,40,45,51,52]. *In situ* hybridization using ER specific oligonucleotide sequence probe revealed that 11 out of 15 HCC tissue samples expressed ER mRNA[40]. Interestingly, the same samples were found to be negative for ER protein by IHC, suggesting the use of more sensitive methods and more specific antibodies for detecting ER at protein level. Subsequently, the mRNA expression of ER in HCC tissues was studied in different populations by a highly sensitive method of reverse transcriptase-polymerase chain reaction (RT-PCR). Villa *et al*[51] were the first to demonstrate the presence of wild type ERα in peritumoral and tumoral tissue of HCC patients using this technique. The use of RT-PCR further enabled the authors to detect a splice variant of ERα lacking exon 5 in the hormone binding domain[18,51]. A similar splice variant has been described in breast cancer tissues to be associated with tumor pathogenesis[53]. The significance of the variant ERα (vERα) in pathology, prognosis and treatment of HCC has also been studied. The presence of vER receptor is able to influence the natural history of patients with HCC by regulating tumor growth as well as patient survival. The presence of the liver vERα transcript in the tumor has been described to be the strongest negative predictor of survival in operable HCC patients[54,55]. Furthermore, the presence of vERα was found to correlate with a higher

Table 1 Estrogen receptor expression in liver tissue samples from HCC patients

Subcellular localization	Method	ER subtype/Antibody source	*n*	Subjects	Positive cases	Country and area	Yr	Reference
Cytosolic	BA	NA	5	5 M	5 M	United States	1982	[30]
Cytosolic and nuclear	BA	NA	5	3 M, 2 F	3 M, 2 F	United Kingdom	1983	[31]
Cytosolic	IHC	NA, Anti ER Ab Hypolabs, Switzerland	10	NA	1	Singapore	1984	[39]
Cytosolic	BA	NA	30	29 M, 1 F	12	Japan	1986	[13]
Cytosolic and nuclear	BA	NA	8	6 M, 2 F	1 M	Japan	1986	[17]
Cytosolic	BA	NA	13	9 M, 4 F	1 F	Japan	1987	[32]
Cytosolic	EIA	Abbot ER-EIA monoclonal kit	13	9 M, 4 F	3 M, 2 F	Japan	1987	[32]
Cytosolic	BA	NA	19	19 F	7 F	Japan	1989	[15]
Cytosolic	BA	NA	66	52 M, 14 F	23 M, 3 F	Japan	1990	[34]
Cytosolic	BA	NA	6	4 M, 2 F	1 F	Japan	1990	[35]
Cytosolic	BA	NA	21	18 M, 3 F	9 M, 1 F	Japan	1991	[36]
Cytosolic and nuclear	BA	NA	9	6 M, 3 F	6 M, 2 F	Italy, United States	1991	[37]
Cytosolic and nuclear	BA	NA	9	6 M, 3 F	6 M, 2 F	Italy, United States	1991	[33]
NA	IHC	NA, Abott, ER-ICA	15	12 M, 3 F	0	Italy	1993	[40]
Cytosolic	EIA	NA, Abbot anti ER	26	18 M, 8 F	4	Spain	1993	[41]
Cytosolic	EIA	NA, Abbot anti ER	33	20 M, 13 F	8 M, 5 F	Germany	1997	[42]
Cytosolic	IHC	NA, ER monoclonal Ab, Dako	71	59 M, 12 F	15 M, 2 F	Hong Kong	1997	[43]
Cytosolic and nuclear	IHC	ERα, Santacruz	45	37 M, 8 F	21 (cytosolic) 11 (nuclear)	United States, Korea[1]	2004	[44]
NA	IHC	NA	28	NA	11	China	2004	[45]
NA	IHC	NA	66		3	Mexico	2007	[46]
Nuclear	IHC	ERα, Dako (ID5)	31	26 M, 5 F	12 M, 4 F	Spain	2007	[47]

ER: Estrogen receptor; BA: Binding assay; EIA: Enzyme immunoassay; IHC: Immunohistochemistry; *n*: Sample size; M: Male; F: Female; NA: Information not available; Ab: Antibody. [1]Study conducted in USA on patient population from Korea.

Table 2 Messenger RNA (mRNA) expression of estrogen receptor in liver tissue samples from HCC patients

Method	ER subtype	*n*	Subjects	Positive cases	Country	Yr	Reference
ISH	ERα Wt	15	12 M, 3 F	11	Italy	1993	[40]
RT-PCR	ERα Wt	14	7 M, 7 F	1 M, 7 F	Italy	1995	[51]
RT-PCR	ERα delta5 variant	14	7 M, 7 F	7 M, 3 F	Italy	1995	[51]
RT-PCR	ERα Wt	40	25 M, 15 F	16 M, 12 F	Italy	1998	[18]
RT-PCR	ERα delta5 variant	40	25 M, 15 F	20 M, 10 F	Italy	1998	[18]
RT-PCR	ERα Wt	42	35 M, 7 F	20 M, 5 F	Italy	2003	[19]
RT-PCR	ERα delta5 variant	42	35 M, 7 F	37	Italy	2003	[19]
RT-PCR	ERβ Wt	42	35 M, 7 F	12 M, 4 F	Italy	2003	[19]
RT-PCR	ERα Wt	28	NA	25	China	2004	[45]
RT-PCR	ERα delta5 variant	28	NA	27	China	2004	[45]
RT-PCR	ERα Wt	32	23 M, 9 F	23 M, 9 F	Korea	2006	[52]
RT-PCR	ERα delta5 variant	32	23 M, 9 F	21 M, 9 F	Korea	2006	[52]
RT-PCR	ERβ Wt	32	23 M, 9 F	26	Korea	2006	[52]

ER: Estrogen receptor; ISH: *In situ* hybridization; RT-PCR: Reverse transcriptase-polymerase chain reaction; Wt: Wild type; *n*: Sample size; M: Male; F: Female; NA: Information not available.

clinical aggressiveness of the tumor in comparison with the tumors characterized by wild-type ERα transcript. These tumors were responsive to megestrol and unresponsive to anti estrogen tamoxifen. High rates of vERα expression have been shown to be present in men at high risk of HCC development[56,57]. In patients with chronic hepatitis and cirrhosis, the expression of vERα has been associated with higher oxidative stress-induced DNA damage and c-myc mRNA expression, a factor indicating increased genomic instability, augmented cytoproliferation and carcinogenesis[5].

Using RT-PCR, in addition to ERα, the expression of the lately identified isoform of ER, i.e. ERβ, has also been studied in HCC patients. Iavarone *et al*[19] report that both ERβ and ERα wild type receptors either alone or together with vER are co-expressed more frequently in patients with chronic liver disease than in those with HCC. However, both ERs are similarly expressed in tumoral and extratumoral tissues of HCC patients[19]. In this study, HBV-related tumors either expressed wild type ERα and ERβ or expressed variant ER and ER delta 5 more often than HCV-related tumor, and HBV-related tumors showed a tendency towards loss of ERβ expression as the disease progressed from chronic inflammatory liver disease to HCC[19].

Breast cancer studies suggest that ERα:ERβ expression ratio changes during carcinogenesis and is believed to play a role in tumor development[58]. Recently, Wang *et al*[52] studied the expression of ERα and ERβ in HCC tissues of Korean population using RT-PCR, and assessed 32 tumoral and peritumoral tissues from HCC patients with underlying chronic HBV or HCV

Table 3 Estrogen receptor and androgen receptor expression in normal and non-cancerous liver tissue samples

Receptor protein/mRNA	Type of liver tissue	Subjects	Positive cases	Method	Country	Yr	Reference
Estrogen Receptor (ER)							
ER protein (cytosolic)	Normal liver tissue	4 F	4 F	BA	United Kingdom	1978	[28]
ER protein	Normal	3 F	3 F	BA	Germany	1978	[59]
ER protein	Normal	2 M	2 M	BA	United States	1982	[30]
ER protein	Normal	1 M, 5 F	1 M, 5 F	BA	Germany	1982	[60]
ER protein	Normal	3 M, 3 F	3 M, 3 F	BA	United States	1983	[61]
ER protein (cytosolic & nuclear)	Normal	2 M, 2 F	2 M, 2 F	BA	United Kingdom	1983	[31]
ER protein (cytosolic)	Surrounding liver tissue	30	13	BA	Japan	1986	[13]
ER protein (cytosolic & nuclear)	Non-cancerous tissue	7	3	BA	Japan	1986	[17]
ER protein (cytosolic & nuclear)	Normal	NA	NA	BA	United States	1987	[62]
ER protein (cytosolic)	Non-cirrhotic liver	5 M, 7 F	5 M, 7 F	BA, EIA	Japan	1987	[32]
ER protein	Normal	2	2		Japan	1988	[63]
ER protein (cytosolic)	Surrounding liver tissue	17	11	BA	Japan	1989	[15]
ER protein	Surrounding non-cancerous tissue	22	14	NA	Japan	1989	[64]
ER protein (cytosolic)	Surrounding normal liver	4 M, 1 F	4 M, 1 F	BA	Japan	1990	[35]
ER protein	Adjacent normal tissue	6 M, 3 F	6 M, 3 F	BA	Italy, United States	1991	[33]
ER protein (cytosolic)	Non-tumoral liver	18 M, 8 F	9 M, 2 F	BA	Spain	1993	[41]
ER mRNA	Non-tumorous liver tissue	13	7	ISH	Italy	1993	[40]
ER mRNA	Peri-tumor tissue	32	28	RT-PCR	Korea	2006	[52]
Androgen Receptor (AR)							
AR protein (cytosolic & nuclear)	Normal	2 M, 2 F	0	BA	United Kingdom	1983	[31]
AR protein (cytosolic & nuclear)	Non-cancerous tissue	6	1	BA	Japan	1986	[17]
AR protein (cytosolic)	Non-neoplastic liver tissues	17	11	BA	Japan	1989	[15]
AR protein	Surrounding non-cancerous tissues	21	7	NA	Japan	1989	[64]
AR protein (cytosolic)	Surrounding liver	9 M, 1 F	7 M, 1 F	BA	Japan	1990	[35]
AR protein	Adjacent normal tissue	6 M, 3 F	6 M, 3 F	NA	Italy, United States	1991	[33]
AR mRNA	Peri-tumor tissue	23 M, 9 F	23 M, 9 F	RT-PCR	Korea	2006	[52]

BA: Binding assay; EIA: Enzyme immunoassay; IHC: Immunohistochemistry; M: Male; F: Female; NA: Information not available.

infection and observed that wild type and variant ERα are expressed in all the samples. However, the expression of vERα is stronger in tumor than in peritumor tissues. Interestingly, ERβ was found to be significantly over-expressed in HCV-infected HCC tissues as compared to HBV-infected HCC tissues. The differences in ER expression in HCV-infected HCC tissues compared to HBV-infected HCC tissues suggest different pathogenetic mechanisms. Overall from these studies there appears to be a change in co-expression pattern of ERα and ERβ from cirrhosis to HCC development in both HBV- and HCV- related tumors. Thus, these studies provide further evidence in support of importance of wild type and variant ERs in HCC, suggesting detailed investigations in this area.

Androgen receptor

Like estrogens, androgens have also been reported to play an important role in liver carcinogenesis. Iqbal *et al*[31] showed the presence of androgen receptors (AR) in HCC in 1983. However, it was not until 1985 that normal human liver was believed to express AR. In 1985, Nagasue and colleagues[14] demonstrated the presence of AR in normal human liver as well as in tumor and non-tumor parts of HCC tissues. Since then, several reports showing protein and mRNA expression of AR in liver have been published. Table 3[13,15,17,28,30-33,35,40,41,52,59-64] and Table 4[14-17,31,33-36,39,41,47,52,65-71] give the details of these studies. In general, AR is found to be over expressed in liver tumor compared to the adjacent normal tissue[15,17,33,36,38]. However, like ER expression studies, majority of the early studies employed indirect binding assays to detect AR in the liver tissues. More precise quantitative methods of direct detection of AR protein are needed. Reports using antibody-based detection of AR in liver tissues are very sparse.

The mRNA levels of AR have been assessed in the non-cancerous and HCC tissues primarily by RT-PCR (Tables 3 and 4). In 1994, Negro and colleagues[16] developed a non-radioisotopic *in situ* hybridization assay specific for human AR mRNA and found that 73% of HCC tissues could express variable amount of AR mRNA. However, normal hepatocytes were stained weakly in 42% of the non-neoplastic tissues. Though initial binding studies demonstrated higher AR levels in tumor tissues than in respective peri-tumoral part[15,17,33,36,38], more recent observations based on mRNA expression do not reveal any significant differences in tumor and peri-tumor tissues. Tavian *et al*[71] found higher AR mRNA levels in tumor than in the corresponding peri-tumoral tissue in a relatively small percentage of HCC samples, suggesting that AR mRNA levels are associated with the histological tumor differentiation showing a lower AR expression in poorly-differentiated HCC than in well-differentiated tumors. In contrast, AR levels in Korean population with HCC do not show differences between tumor and peri-tumor tissues using RT-PCR[52]. Due to these conflicting reports on AR expression in HCC, there is a need for detailed investigation of AR mRNA as well as protein levels using more sensitive and accurate detecting quantitative methods.

Table 4 Androgen receptor protein and mRNA expression in liver tissue samples from HCC patients

	Subcellular Organelle	Method	*n*	Subjects	Positive	Country	Yr	Reference
AR protein	Cytosolic and nuclear	BA	5	3 M, 2 F	3 M, 2 F	United Kingdom	1983	[31]
	Cytosolic	IHC	10	NA	5	Singapore	1984	[39]
	Cytosolic	BA	19	19 M	14 M	Japan	1985	[14]
	Cytosolic and nuclear	BA	5	3 M, 2 F	3 M, 2 F	United Kingdom	1985	[65]
	Cytosolic and nuclear	BA	8	6 M, 2 F	2 M, 2 F	Japan	1986	[17]
	Cytosolic	BA	13	8 M, 5 F	8 M, 5 F	United Kingdom	1988	[66]
	Cytosolic	BA	19	19 F	7 F	Japan	1989	[15]
	Cytosolic	BA	45	31 M, 14 F	25 M, 6 F	Japan	1989	[67]
	Cytosolic	BA	11	9 M, 2 F	6 M, 1 F	Japan	1990	[35]
	Cytosolic	BA	21	18 M, 3 F	18	Japan	1991	[36]
	Cytosolic and nuclear	BA	9	6 M, 3 F	6 M, 3 F	Italy, United States	1991	[33]
	Cytosolic	BA	5	3 M, 2 F	3 M, 2F	Japan	1992	[68]
	Cytosolic	BA	26	18 M, 8 F	14	Spain	1993	[41]
	Cytosolic	BA	43	30 M, 13 F	28	Spain	1995	[69]
	NA	BA	32			China	1998	[70]
	Nuclear	IHC	31	26 M, 5 F	18 M, 3 F	Spain	2007	[47]
AR mRNA		ISH	22	16 M, 6 F	13 M, 3 F	Italy	1994	[16]
		RT-PCR	38	24 M, 14 F	21 M, 13 F	Italy	2002	[71]
		RT-PCR	32	23 M, 9 F	23 M, 9 F	Korea	2006	[52]

AR: Androgen receptor; BA: Binding assay; IHC: Immunohistochemistry; ISH: *In situ* hybridization; RT-PCR: Reverse transcriptase-polymerase chain reaction; *n*: Sample size; M: Male; F: Female; NA: Information not available.

CLINICAL AND PATHOLOGICAL SIGNIFICANCE OF ESTROGEN AND ANDROGEN RECEPTORS IN HCC

Despite a wide variability observed in studies of ER and AR expression in HCC, attempts have been made to determine the significance of these receptors by correlating their levels with clinical and pathological parameters. Table 5[13,15,34,42,55,69,70,72] and Table 6[30,54,73-77] present the salient findings of such clinical studies.

In few earlier studies using binding assays, no correlation was found between ER protein expression and sex, age, alcohol abuse, serum alpha-feto protein, carcinoembryonic antigen, HBV markers or tumor histology[15,34]. However, in subsequent reports, ER mRNA levels were shown to be associated with sex and viral etiology[19,51,52]. Increased vERα expression has been demonstrated more often in males than in females with HCC in Italian population, suggesting a strong link of ER with a higher incidence of HCC in males[19,51]. On the other hand, in Korean subjects no correlation has been found between the expression of vERα and HCC prevalence in males[52]. Interestingly, a distinct difference in ER expression pattern was observed in HBV- and HCV-infected HCC patients. Delta 5 deletion variants of ERα (vERα) and ERβ were found to be more often expressed in HBV-related tumors than in HCV-related tumors (67% *vs* 15%, $P < 0.0007$)[19]. In contrast, Wang *et al*[52] showed no remarkable difference in vERα levels in HCV- and HBV-infected HCC tissues (91.3% *vs* 100%). Nevertheless, a predominant expression of ERβ has been reported in HCV-infected than in HBV-infected patients with HCC (95.7% *vs* 44.4%, $P < 0.05$), suggesting that ERβ may play an important role in HCV-induced liver disease[52].

In addition to gender and etiological factors, tumor size, histopathology, operative mortality, tumor recurrence and survival after curative resection have also been studied in relation to ER expression in HCC[34,42,55]. Nagasue *et al*[34] showed that the large tumors are more commonly found in ER negative HCC patients and therefore the incidence of major hepatic resection is significantly higher in this group than in ER positive HCC patients. However, they did not report significant differences in histopathology of ER positive and ER negative tumors. Rates of mortality, tumor recurrence and long-term survival were also found to be similar in the two groups. In contrast to these observations, Jonas *et al*[42] showed that in patients undergoing curative resection, the 1- and 2-year survival rates in ER positive group are substantially lower than in ER negative group[42], suggesting that ER positive status has a negative effect on patient survival after curative resection of advanced HCC. Further, significantly longer survival rates have been reported in HCC patients with wild type ERs than in those expressing variant ERs[55].

In contrast with ER expression, AR levels are strongly associated with intra hepatic recurrence of tumors. The 5-year survival of recurrence free HCC patients was shown to be 55% for AR negative, 24% for ER negative, 10% for ER positive and 0% for AR positive tumors[67]. Similar findings have been reported by other researchers, suggesting a negative impact of AR positivity on tumor recurrence[69]. It was reported that AR negative patients show significantly better survival than AR positive patients[70]. Considering the tumor size, variable results have been documented in relation with AR expression. Boix *et al*[69] showed that AR expression is significantly related to smaller tumor size while Zhang *et al*[70] found that AR levels are positively correlated to tumor size.

Table 5 Studies on correlation of estrogen receptor and androgen receptor expression with clinical and pathological parameters in HCC patients

Receptor protein/ mRNA expression	Clinical parameter	*n*	Country	Salient findings	Yr	Reference
ER protein	Serum alpha-fetoprotein, carcinoembryonic antigen, HBV profile, tumor histology	30	Japan	No correlation with any parameter	1986	[13]
ER and AR protein	Serum alpha fetoprotein, HBV markers, histopathology	19	Japan	No correlation	1989	[15]
ER protein	Sex, age, alcohol abuse, underlying liver disease, hepatic functions	66	Japan	No correlation	1990	[34]
	Tumor size, hepatic resection Histopathology			Large tumor size and higher rate of resection in ER- No differences in ER+ and ER-		
	Operative mortality, tumor recurrence, long-term survival rate			Similar in ER+ and ER-		
ER and AR protein	Intrahepatic recurrence	78	Japan	AR expression strongly associated with intrahepatic recurrence. Weak association with ER expression	1995	[72]
ER protein	Survival after curative resection	28	Germany	Negative effect of an ER+ tumor on patient survival after curative resection	1997	[42]
Wild type and variant ER mRNA	Survival	96	Italy	Significantly long survival in patients with wild type ERs than variant ERs	2000	[55]
AR protein	Recurrence rate	45	Japan	Significantly higher recurrence rates in AR+ group than AR-	1989	[15]
	Survival rate			Significantly better survival rates in AR- patients than in AR+		
AR protein	Tumor size	43	Spain	AR expression was significantly related to smaller tumor size	1995	[69]
	Tumor recurrence			Higher tumor recurrence rates in surrounding tissues of AR+ than AR-		
AR protein	Tumor size and survival time	32	China	Survival rate correlated inversely with the levels of AR expression	1998	[70]
				AR levels had positive correlation with the tumor size		

CURRENT STATUS OF HORMONAL TREATMENT IN HCC

The association of estrogens and androgens in HCC observed in basic and clinical studies has led to initiation of various clinical trials on hormonal treatment of HCC. Differential clinical outcome was reported in these trials that have resulted in continued debate about the use of hormonal therapy in HCC. Table 6 presents a list of few clinical studies that utilized hormonal therapy in HCC patients. A systematic review of these clinical trials on therapeutic evaluation of anti-estrogen and anti-androgen agents in liver cancer has been recently compiled by Di Maio *et al*[78]. The authors conclude that hormonal treatment should not be a part of the current management of HCC patients[78,79]. However, in most of these clinical studies, various inherent factors may have contributed to the observed inconclusive results. Few of these may include faulty patient subset selection criteria, no monitoring of tumor ER and AR expression at the time of recruitment and also during treatment of these patients and lastly the type of hormonal treatment given to the patient. Therefore, the debatable potential of hormone therapy in HCC may finally be attributed to the lack of complete understanding of ER and AR expression and hormonal responsiveness in the liver and their involvement in development of HCC.

Currently, limited information is available on the functional significance of ER and AR in HCC. In the following sections, we review the *in vivo* animal studies on liver carcinogenesis and *in vitro* studies on cell lines that have been conducted to understand the role of ER and AR in the liver.

ESTROGEN RECEPTOR AND ANDROGEN RECEPTOR STUDIES IN ANIMAL MODELS OF EXPERIMENTAL LIVER CARCINOGENESIS

Estrogen receptor studies

Several attempts have been made to establish the role of estrogen and its receptors in hepatocarcinogenesis using animal models. Rat is the most extensively used model to study liver carcinogenesis. Rat hepatocytes are known to express ERs. ERα is the predominant isoform expressed in rat hepatocytes while cholangiocytes express both ERα and ERβ[80]. However, Inoue *et al*[81] showed that the levels of ERβ are higher than those of ER in cultured rat hepatocytes[81]. Hepatic stellate cells from rats appear to contain mainly ERβ[82]. Due to the lack of information about the existence of various ER isoforms, in most of the earlier studies, hepatic stellate cells did not differentiate ER into ERα and ERβ. In addition, majority of the *in vivo* studies have been conducted in animal models of chemical carcinogenesis. Diethylnitrosamine (DEN) is the most commonly used carcinogen in rat and mouse models of HCC. The pathogenesis of HCC in DEN-induced carcinogenesis in

Table 6 Clinical outcome of hormonal therapeutic trials in HCC patients

Receptor protein/ mRNA expression	Clinical parameter	Treatment	*n*	Country	Salient Findings	Yr	Reference
ER protein	Tumor growth	Progestin	5	United States	Tumor regression in 2	1982	[30]
NA	Anti-tumor response	Tamoxifen 20 mg twice daily	33	United States	No complete or partial antitumor response	1990	[73]
	Survival time				Long term survival (18+ to 39+ mo) in 4 patients		
NA	Anti-tumoral effect	Tamoxifen 20 mg daily	120 (placebo = 62)	Spain	No-antitumor effect	1995	[74]
	Survival time				No significant differences in survival rate of placebo and treated groups		
Wild type and variant ER mRNA	Tumor size and growth rate	Tamoxifen 80 mg daily or Megestrol 160 mg daily	8	Italy	Growth rate 4 times higher in tumors expression variant ER than wild type ERs. Tumor regression to half size in patients with wild type ER following tamoxifen treatment. Megestrol slowed down tumor growth in tumors with variant ERs	1996	[54]
ER protein	Mortality rates	Tamoxifen	119 (placebo = 58)	China	No difference in 1 mo mortality rates and median survival in treated and control groups	2000	[75]
	Survival				No effect of ER expression on survival		
Variant ER mRNA	Tumor growth, survival	Megestrol 160 mg daily	24 placebo, 21 treated	Italy	Significantly slowed down tumor growth and improved survival in treated patients than placebo group	2001	[76]
NA	Survival rates	Tamoxifen 120 mg daily or 60 mg daily	329	Singapore	No positive effect on survival and increasingly negative impact with increasing doses	2005	[77]

NA: Information not available.

animal models differs from that in humans and therefore may not be directly comparable to human HCC[83]. Nevertheless, the histology and genetic signatures are similar to human HCC and a striking gender disparity with male predominance is also observed in these animal models as seen in humans[84]. In addition to DEN, acetylaminofluorene (AAF), di(2-ethylhexyl) phthalate (DEHP), peroxisome proliferator, arsenic and carbon tetrachloride have been used to induce HCC in various animal models[85-90].

Use of oral contraceptives and synthetic estrogens in women is reported to be a major risk factor for the development of hepatocellular adenoma, a benign liver tumor with malignant potential[26,27]. Shimomura *et al*[91] studied the role of ethinyl estradiol (EE) in inducing HCC in female rats, following EE treatment for 12 mo, 8% of rats developed HCC, revealing that EE causes mutations in hepatocytes leading to DNA adduct formation and induces HCC development in affected cells. The initial events in HCC, i.e. DNA adduct formation by EE, appear to be carried out in an ER independent manner since tamoxifen, a known selective estrogen receptor modulator (SERM), inhibited ER expression and suppressed HCC, but did not affect DNA adduct formation. Exogenous estrogens have also been shown to promote hepatocarcinogenesis induced by other agents[92,93]. Campen *et al*[92] documented that administration of 17-alpha ethinylestradiol in ovariectomized rats promotes DEN-induced carcinogenesis in a dose dependent manner. Further, it was reported that synthetic female hormones act synergistically with ethanol to increase HCC incidence[93]. Alcohol could affect HCC development due to EE by promoting changes in ER kinetics and expression as well as in DNA adduct formation.

Liver is the major site of estrogen metabolism[94]. Alterations in sex hormone metabolism are also considered a critical factor determining the significance of sex hormones in the process of liver carcinogenesis. Eagon *et al*[86,87] reported that the activity of male estrogen-metabolizing enzyme, estrogen 2-hydroxylase and male specific estrogen sequestering protein is reduced in liver, which explains the raised serum estradiol levels but the decreased hepatic activity of cytosolic and nuclear ER observed in DEHP-induced HCC in male Fischer 344 rats. The expression of cytochrome P450 enzymes that play an important role in estrogen metabolism have also been shown to be affected during hepatocarcinogenesis. Waalkes and colleagues[88] have described the feminized pattern of P450 genes in male mice with HCC induced by exposure to arsenic in utero. The expression of female dominant CYP2A4 and CYP2B9 is increased whereas levels of male dominant CYP7B1 gene gets reduced in arsenic treated animals. Recent findings suggest that cytochrome P450 (CYP) is regulated by estrogen itself through the involvement of estrogen receptors[94]. Nonetheless, elucidation of the exact mechanism of regulation of CYP isoforms by estrogen in liver needs further investigation.

In contrast with earlier animal studies that support

estrogens in promoting and inducing carcinogenesis, recent studies highlighted the protective role of estrogens in HCC development. Shimizu *et al*[8] showed that estrogen can suppress chemical hepatocarcinogenesis induced by dimethylnitrosamine (DEN)-2-acetylaminofluorene (AAF) in partial hepatectomy (PH) model of hepatocarcinogenesis. In addition, estrogen has been shown to prevent the progression of liver disease to HCC. Estradiol treatment could reduce hepatic steatosis and restore the impairment in mitochondrial and peroxisomal fatty acid β-oxidation in aromatase-deficient mice which lack intrinsic ability to produce estrogen[95]. Furthermore, estradiol treatment was also shown to result in a dose dependent suppression of hepatic fibrosis in hepatic fibrosis models of male rats[96,97]. The mechanism of protective action of estrogens against progression of chronic liver disease has been recently reviewed by Shimizu and Ito[7].

Recently, using a mouse model of DEN-induced hepatic carcinogenesis, Naugler *et al*[83] described a molecular mechanism explaining the lower HCC susceptibility in females and the anti-inflammatory role of estrogen in preventing HCC development. The authors investigated the relationship between HCC development and gender dependent expression of interleukin-6 (IL-6). IL-6 is a proinflammatory cytokine that plays an important role in chronic hepatitis, the prerequisite for progression to cirrhosis and HCC. The serum IL-6 levels were higher in male mice than in female mice after administration of DEN, leading to a higher rate of liver cell proliferation in male mice. This effect can be further mediated by ERα, suggesting that ERβ plays a little role in modulating the expression of IL-6.

Androgen receptor studies

The role of AR has also been studied in animal models of chemical induced carcinogenesis[87,98-100]. In DEN treated Wistar rats, a 20-fold increase in hepatic AR concentration was reported in females, suggesting that increased hepatic AR concentration is correlated with accelerated tumor development in these animals, in which male rats showed a slower tumor development with no change in AR concentrations[98]. Subsequent studies in the same model revealed that removal of ovary increases AR levels in the liver of female rats but testosterone treatment does not further enhance AR levels[99]. On the other hand, normal adult males with intact testis or testosterone treatment maintain high levels of AR but in castrated rats estrogen treatment reduces AR expression[99]. Animal studies demonstrated that the expression of both AR and ER increases during preneoplastic stages and that progression towards cancer development can suppress ER and maintain AR expression levels[94,100].

Interestingly, anti-androgen treatment has been shown to reduce AR levels in liver as well as the size and number of tumors in male Spargue Dawley rat model of hepatocarcinogenesis[101]. It has been shown that inhibition of AR positive HCC with anti-androgen cypertone acetate in male mice involves cell cycle arrest and to some extent induction of apoptosis due to increased synthesis of transforming growth factor-β 1 (TGF-β1)[102]. In another model of chemical-induced liver carcinogenesis, inhibition of androgens using 5-alpha reductase inhibitors significantly suppressed HCC development in rats[103]. Recent studies in a xenograft model of hepatocarcinogenesis in nude mice suggested that AR expression remains elevated until development of tumor and starts declining as the size of tumor increases[104]. It is therefore proposed that androgen therapy may be ineffective after establishment of the tumor. Nevertheless, for better understanding and rationale design of hormone-based therapies, it is mandatory to study the role of ERs and AR in animal models mimicking the natural course of disease progression to HCC development as in humans. Currently available HBV and HCV transgenic mice depicting features close to human HCC pathogenesis, appear to be promising models for future *in vivo* studies.

SEX HORMONE RECEPTORS: GENOMIC AND NON-GENOMIC ACTIONS IN LIVER CELLS MEDIATING HEPATOCARCINOGENESIS

Estrogen receptor

Estrogen action and the role of ERs in carcinogenesis have been well documented in mammary carcinoma and the studies have revealed the involvement of estrogens in key cellular processes such as apoptosis, cell cycle, proliferation, oxidative stress and inflammation. The progress in understanding the role of estrogen in regulating various cellular events in liver carcinogenesis has been rather slow. However, the research conducted over recent years provides key insights in this direction.

The classical mode of estrogen action is the genomic mechanism in which ERs function as ligand-activated transcription factors[105]. Activated ERs translocate to the nuclei and regulate the expression of specific target genes. These transcriptional regulations are achieved through interaction with estrogen responsive element (ERE) sequences located in the promoter region of the target gene[106]. However, one third of the genes regulated by ERs in humans do not contain ERE-like sequences[107]. ERs can also regulate the transcription of such genes without binding to DNA through protein-protein interactions with other transcription factors, such as AP-1 and Sp-1 in the nuclei[108]. In addition, this transcriptional control at alternate response elements is also facilitated by non-genomic actions of estrogen. The non-genomic functions of estrogen are initiated by membrane-localized ERs and are associated with activation of various signaling pathways especially protein kinases[109]. The functions of many transcription factors are regulated through protein kinase-mediated phosphorylation including CREB, NF-κB and AP-1 and these transcription factors may thus be targets

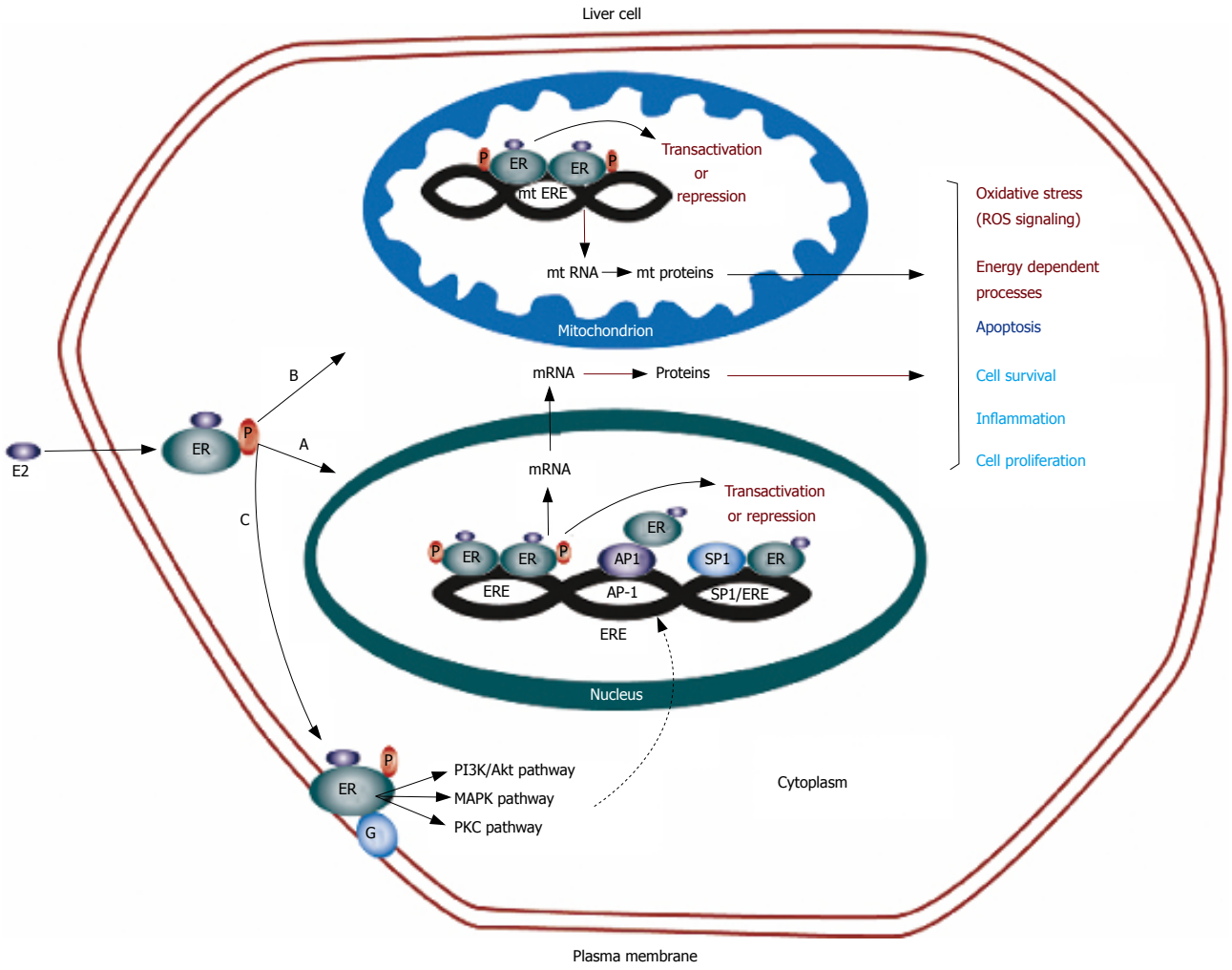

Figure 1 Genomic and non-genomic functions of estrogen mediated by estrogen receptors (ERs) localized in different sub-cellular organelles of a liver cell. A: Genomic actions include translocation of ligand-activated ERs to the nuclei for regulating gene transcription. This transcriptional control is carried out by binding to DNA at a sequence containing either full estrogen response element (ERE) site or an ERE half site adjacent to the binding site for another transcription factor like Sp1. An additional mechanism involves gene regulation at alternate response element through protein-protein interactions with other transcription factors (AP-1, CREB, NF-κB); B: Activated ERs control mitochondrial gene transcription by binding to ERE like sequences (mt ERE) leading to modulation of mitochondrial functions including metabolism, oxidative stress and apoptosis; C: Membrane localized ERs are G-protein coupled receptors that activate various protein kinase pathways. These signal transduction cascades in turn regulate functions of many transcription factors resulting in modulation of expression of a number of genes involved in cell proliferation, survival, apoptosis and inflammation. Genomic and non-genomic actions of estrogen converge to exhibit a fine degree of control for the regulation of transcription by ERs in a liver cell. Modified from: Chen *et al*[110] 2005.

for non-genomic actions of estrogens. This possible convergence of genomic and non-genomic actions at multiple response elements provides an extremely fine degree of control for the regulation of transcription for ERs (Figure 1)[105,110]. In the following section, we discuss the findings of recent *in vitro* studies highlighting the significance of ER in mediating genomic and non-genomic actions of estrogen in liver cells to modulate the expression of a number of genes involved in cellular processes central to carcinogenesis.

Telomerase activation has been implicated in hepatocarcinogenesis and the expression of human telomerase reverse transcriptase (hTERT) that encodes for the catalytic subunit of the multicomponent enzyme telomerase hTERT is the prerequisite for telomerase activation[111,112]. Several studies indicated that estrogen regulates transactivation of the hTERT gene by direct interaction of activated ER with an imperfect ERE sequence in the hTERT promoter[113]. Estrogen treatment has been shown to up-regulate the expression of hTERT mRNA and protein in three normal human hepatic cell lines (hc-cells, hNheps and WRL-68) expressing ERα to varying degrees[90]. Furthermore, estrogen exposure prevents shortening of telomeres and decreases the number of cells undergoing senescence, indicating that estradiol acts as a positive modulator of the hTERT gene in the liver[90]. However, the mechanism of ER-mediated transactivation of hTERT in the liver is not well understood. In contrast, in HepG2 cells, estrogen modulation of telomerase activity has been found to be regulated post-transcriptionally *via* the IP3/PKC pathway[114,115]. IP3 production has been shown to be up-regulated by estrogens in HepG2 cells[114]. Furthermore, estradiol-induced IP3/PKC-alpha production is dependent on either ERα or ERβ expression in both HepG2 and Hela cells[114]. It is

hypothesized that membrane ER-mediated IP3/PKC-alpha pathway represents an alternative signaling pathway utilized by cells when low ER levels are unable to activate classic ER-mediated genomic mechanisms as in HepG2 cells[114].

A similar regulatory mechanism has been observed in case of estrogen modulation of expression of cyclin D1 gene in hepatoma cells. Cyclin D1, important for progression of cells through G1 phase of cell cycle, is a well defined target for estrogen action in mammary carcinoma[116,117], although no detectable estrogen responsive element like sequence in the cyclin D1 gene promoter has been reported in these cells[118]. The cyclin D1 mechanism identified in mammary carcinoma cells involves direct interaction of ERα and Sp1 or ERα and Ap-1[119]. Interestingly, Marino *et al*[23] demonstrated that in HepG2 cells, estrogen-induced activation of cyclin D1 transcription can occur independently of the transcriptional activity of ER. They further showed that the effect of 17-beta estradiol on HepG2 cells is mediated by activation of the MAPK/ERK pathway by membrane-localized ER that increases the expression of cyclin D1 gene through activation of AP-1 transcription factor[23], suggesting that non-genomic signaling pathways play an the pivotal role in estrogen-mediated regulation of gene expression at multiple response elements.

Besides, modulating the molecules involved in cell cycle control and cell proliferation, estrogen has also been shown to regulate the expression of genes crucial for apoptosis of hepatocytes and dysregulation of apoptosis in hepatic cells is reported to be a significant factor in accelerating hepatocarcinogenesis or tumor progression in HCC[120]. The Bcl-2 family of proteins regulates one of the key steps in the conserved apoptotic pathway. Among the members of this family, Bcl-2 and Bcl-xL act as inhibitors of apoptosis where as Bax and Bak promote apoptosis[121,122]. Ethinyl estradiol is known to increase the levels of Bcl-2 protein in cultured female rat hepatocytes[123]. Estradiol and idoxifene, two selective estrogen receptor modulators, are known to induce the expression of Bcl-2 protein in male rat liver tissues[124]. Omoya *et al*[9] and Inoue *et al*[81] also demonstrated that estradiol is able to stimulate the expression of Bcl-2 and Bcl-xL and to suppress Bad expression in oxidative stress-induced early apoptotic rat hepatocytes. Similar findings have been recently documented in response to estradiol treatment of Huh-7 human hepatoma cells describing a dose dependent increase in expression of Bcl-2 and Bcl-xL and a reduction in Bad levels[22]. No change was observed in expression of pro-apoptotic protein Bax. The regulation of Bcl-2 gene expression by estrogen in mammary carcinoma cells has been shown to be mediated indirectly through activation of Sp-1 transcription factor[125]. However, the precise mechanism of Bcl-2 transactivation in hepatocytes has not been clearly understood.

One of the most interesting mechanisms of transcriptional regulation at alternate response elements by estrogen is through inhibition of transcription factor NF-κB. Studies demonstrating a mutually antagonistic cross-talk between these families of transcription factors have been recently reviewed[126]. The ER has been shown to mediate opposition of NF-κB functions at various levels by inhibiting the activation of signaling pathways, preventing nuclear translocation, blocking DNA binding or inhibiting recruitment of co-activators for transcription[126]. Estrogen has been shown to bring about its anti-inflammatory and anti-oxidant effects on liver cells by suppressing the NF-κB activity as evident from the following studies. It was reported that 17 beta-estradiol-bound ERα interferes with cytokine-induced activation of a NF-κB reporter in HepG2 cells, suggesting that estrogen exerts its anti-inflammatory and protective effects on human liver cells[127]. Moreover, in an *in vivo* model, estrogen treatment has been shown to block the induction of hepatic expression of inflammatory vascular cell adhesion molecule-1 (VCAM-1), tumor necrosis factor-α (TNF-α), and regulate normal T-cell expression and secretion upon activation[128]. In a mouse model of DEN-induced HCC, ERα was suggested to suppress IL-6 production, a pro-inflammatory molecule, through the involvement of the NF-κB pathway[83]. Estrogen has also been reported to suppress oxidative stress-induced reactive oxygen species (ROS) generation, lipid peroxidation, activation of AP-1 and NF-κB as well as loss of Cu-Zn SOD activity in cultured rat hepatocytes[2,9].

In addition to genomic and non-genomic actions of estrogen mediated by nuclear and membrane ER, mitochondria have also recently been identified as important targets of estrogen and ERs[110]. Early binding studies on sub-cellular fractions indicated that ER is present in rat liver mitochondria[129]. Both ERα and ERβ have been reported to be present in the mitochondria of human HepG2 cells[130-132]. The mitochondrial genome has been shown to contain sequences that have partial homology to the estrogen responsive elements[132-134]. Both ERα and ERβ bind to mitochondrial DNA and the binding can be increased by estradiol using mobility shift assays and surface plasmon resonance[135]. These results suggest that estradiol is directly involved in the regulation of mitochondrial DNA transcription (Figure 1). Regulation of apoptosis and oxidative metabolism by estrogens in mitochondria may be important in the normal liver and in the development of HCC.

Ethinyl estradiol treatment has been shown to elevate the expression levels of mitochondrial DNA-encoded cytochrome C oxidase subunit Ⅲ (CO Ⅲ) and ATP synthase 6 *in vivo* as well as in HepG2 cells[136]. This increased expression of mitochondrial transcripts is accompanied by increased mitochondrial superoxide production and respiratory chain activity that require cytochrome P450-mediated biotransformation of ethinyl estradiol and 17-beta estradiol to catechol metabolites[136,137]. In addition to CO Ⅲ, the levels of CO Ⅰ and CO Ⅱ encoded by mitochondrial DNA have also been found to be elevated in ethinyl estradiol treated female rat hepatocytes. This effect is accompanied

by increased mitochondrial superoxide production, high ATP levels and increased Bcl2 production, and is suggested to play a role in ethinyl estradiol-mediated inhibition of apoptosis[123]. In contrast, 17-beta estradiol and 17-beta estradiol like compounds, diethylstilbestrol (DES), tamoxifen and genistein, have been found to induce apoptotic effects in human hepatoma Hep3B cell line[138]. These compounds cause the leaking of cytochrome C from mitochondria and activation of caspase-3 in an ER dependent manner. In another study, the two isoforms, ERα and ERβ, showed their opposing actions on apoptosis in a poorly differentiated HCC cell line HA22T[139]. Over-expressed ERβ but not ERα induces the expression of caspase-8 and TNF-α in HA22T cells in response to estradiol treatment, indicating that the death receptor-mediated apoptotic pathway is activated[139].

Differential roles of ERα and ERβ have also been observed in non-genomic actions of estrogen in the liver[21,23]. There is indirect evidence that membrane ER may exist in human liver as the binding of gold tagged estrogen-BSA conjugate on the surface of clathrin-coated pits in HepG2 cells has been demonstrated by electron microscopic visualization[140]. The non-genomic mechanism of action of sex steroids on the plasma membrane involves the activation of protein kinase cascades (Figure 1). Two major cascades, protein kinase C, and mitogen-activated protein (MAP) kinase are active and important in carcinogenic liver cells. Protein kinase C cascade and its second messenger IP3 are important in cell proliferation and have been discussed in this review in context of transcriptional regulation of hTERT expression by estrogen. The mitogen-activated protein (MAP) kinase cascade is another pathway that is regulated by the action of sex steroids at the plasma membrane. This complex signaling cascade involves three major pathways: ERK, p38, and JNK[141]. In HepG2 cells, estradiol has been found to rapidly increase the phosphorylation of ERK[21,23]. Naringenin, an anti-estrogenic flavonone, induces the activation of p38 in ERα containing HepG2 cells or in ERβ containing human colon adenocarcinoma DLD-1 cells[142], suggesting that naringenin has an antiestrogenic effect only on the ERα expressing cells, whereas it mimicks the estradiol effects on ERβ expressing cells. The role of ERα and ERβ in the regulation of MAP kinase cascade has been further studied in cell lines expressing either ERα or ERβ[21]. It was found that estrogen-bound ERα can rapidly activate the ERK and AKT signal transduction pathways leading to cell cycle progression and inhibition of apoptosis, whereas estrogen-complexed ERβ can induce rapid phosphorylation of p38 leading the cells to the apoptotic cycle and cell death. These studies further support the functional antagonism between ERα and ERβ with respect to estrogen-induced cell proliferation and emphasize the need to study the independent and interactive role of both isoforms in hepatocarcinogenesis.

Androgen receptor

In comparison with ER, there is limited information about genomic and non-genomic functions of AR in the liver. Like ER, AR has also been shown to regulate gene transcription by binding to androgen responsive sequences (ARE)[143,144]. Yoon *et al*[145] demonstrated that androgen can directly regulate the expression of transformation growth factor-beta 1 (TGF-β1) through binding of AR to ARE in TGF-β1 promoter, suggesting that such activation might regulate the progression of HCC in both human and animal models[145]. Furthermore, AR has been shown to interact with a newly identified transcription factor, paternally expressed gene 10 (PEG 10) in hepatoma cell line[146]. PEG 10 has growth promoting properties and is implicated in hepatocarcinogenesis[147,148]. Dihydrotestosterone (DHT) promotes hepatoma formation in nude mice through PEG 10 activation. In addition, DHT treatment is shown to up-regulate hTERT expression in hepatoma cell lines in a PEG-10 dependent manner[146]. These studies indicate that PEG-10-mediated transactivation of target genes by AR has an essential role in hepatocarcinogenesis.

To the best of our knowledge, AR has not been detected in the liver mitochondria. The information about the membrane localization of AR in human liver cells is also lacking. However, AR has been reported to occur in the plasma membranes of male rat liver[149]. Androgens are also involved in the regulation of the MAP kinase signaling pathways as orchiectomy of H-ras 12V transgenic mice decreases phospho-MEK and phospho-ERK in liver tissues. In addition, orchiectomy reduces hepatotumorigenesis in male mice while ovariectomy increases phospho-MEK and phospho-ERK in liver tissue from female mice, but ovariectomy does not affect the incidence of tumorigenesis[150]. Detailed investigations are urgently needed to confirm the existence of non-genomic signaling actions of androgens in liver and the role of AR in mediating these functions.

INTERACTION OF ER AND AR WITH VIRAL PROTEINS IN HBV AND HCV PATHOGENESIS

Chronic infection with HBV and HCV is the major cause of increasing incidence of HCC worldwide. Several reports support the role of HBV and HCV proteins in disturbing cellular homeostasis and causing malignant transformation of hepatocytes[151,152]. Recent studies in hepatoma cell lines suggest the interactive role of ERs and AR with HBV and HCV proteins in viral pathogenesis.

Estrogen receptor and viral proteins

Han *et al*[24] recently reported that HBV protein (HBx) interacts with ERα. HBx is a multifunctional protein involved in neoplastic transformation in cultured cells and can induce HCC in transgenic mice. HBx associates with both ERα and delta 5 deletion variant of ERα (vERα) and inhibits ERα transcriptional activity by recruiting histone deacetylase enzyme, HDAC-1[24].

HDAC-1 belongs to the family of enzymes involved in deacetylation of hyperacetylated histone tails, leading to compaction of chromatin and transcritptional repression[153]. Both HBx and vERα have additive effects on suppression of ERα transactivation[24].

ERα has also been shown to interact with nonstructural (NS) 5B protein of HCV[25]. NS5B is a RNA-dependent RNA polymerase, which plays a central role in viral genome replication[154]. HCV replication takes place in a replication complex consisting of viral RNA and non structural proteins including NS5B[155]. The replication complex forms on the surface of intracellular membranes including endoplasmic reticulum membrane and is associated with lipid rafts rich in caveolin 2 (CAV 2) on these membranes[156-158]. Using chemical biology approach, Watashi *et al*[25] demonstrated that ERα facilitates the interaction of NS5B with CAV 2 in lipid rafts and hence promotes the participation of NS5B in HCV replication complex. However, they did not find that ERβ affects HCV replication in the same study[25]. An important observation of the study is that tamoxifen inhibits ERα actions and suppresses HCV genome replication, further supporting the potential for anti-ER drugs in developing new anti-HCV strategies.

Androgen receptor and viral proteins

Like ER, HBx protein has also been shown to interact with AR. HBx functions as a positive transcriptional co-regulator to increase AR-mediated transcriptional activity. This transcription enhancement is increased in the presence of androgen in a concentration-responsive manner. However, HBx does not physically associate with ligand-bound AR in the nuclei, suggesting that HBx augments AR activity by increasing the phosphorylation of AR through HBx-mediated activation of the c-Src kinase signaling pathway[159]. In contrast, Zheng *et al*[160] demonstrated that HBx can physically bind to AR in the liver and alter the subcellular localization of AR both in the presence and absence of dihydrotestosterone (DHT). Further studies indicated that HBx can enhance the gene transactivation activity of AR by enhancing its DNA binding activity in a DHT-dependent manner.

CONCLUSION

Taken together, studies on hepatoma cell lines, HCC tissues and animal models of hepatocarcinogenesis, highlight the importance of sex hormones and their receptors in HCC pathogenesis. Further investigations are urgently needed to elucidate the precise mechanism of action of estrogens, androgens and their receptors in regulating normal liver physiology and pathophysiology of chronic liver diseases resulting in HCC.

A thorough re-examination of studies conducted so far to detect the expression of ER and AR in liver tissues is needed using newer specific, sensitive and quantitative methods. With the emerging significance of ERβ and the availability of isoform specific antibodies, the relative levels of both ERα and ERβ can be determined. The studies on mRNA expression in liver tissues have demonstrated the presence of deletion mutants (variant forms) that need to be further validated at protein levels for establishing their significance in diagnosis and prognosis of HCC. Considering the male predominance of HCC and the wide gap in the information available on AR in liver, detailed mechanistic studies need to be conducted to reveal the mechanism of androgen function in normal liver and HCC. In addition, evaluation of ER and AR status at premalignant stages of chronic liver disease due to different etiological factors is required for critical understanding of their role in HCC pathogenesis.

Recent *in vitro* studies focusing on molecular interaction of hormonal receptors with viral proteins need to be further confirmed in *in vivo* animal models. Currently available HBV and HCV transgenic mouse models as well as human hepatocyte xenograft models can serve as a valuable preclinical tools to validate the importance of sex hormone receptors in chronic liver disease development and progression to HCC. Thus, with the availability of state of the art technologies, the time is ripe to embark on to move this important field forward. Well designed, systematic studies employing adequate tools to study ERs and ARs in chronic liver disease and HCC may contribute to the development of novel therapeutics or prognostic markers. These studies may also be further helpful in resolving controversies about the use of hormonal therapy for HCC.

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