Androgen receptor roles in hepatocellular carcinoma, fatty liver, cirrhosis and hepatitis

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Abstract

Androgen/androgen receptor (AR) signaling plays important roles in normal liver function and in progression of liver diseases. In studies of noncancerous liver diseases, AR knockout mouse models of liver disease have revealed that androgen/AR signaling suppresses the development of steatosis, virus-related hepatitis, and cirrhosis. In addition, studies have shown that targeting AR in bone marrow-derived mesenchymal stem cells (BM-MSCs) improves their self-renewal and migration potentials, thereby increasing the efficacy of BM-MSC transplantation as a way to control the progression of cirrhosis. Androgen/AR signaling is known to be involved in the initiation of carcinogen- or hepatitis B virus-related hepatocellular carcinoma (HCC). However, studies have demonstrated that AR, rather than androgen, plays the dominant role in cancer initiation. Therefore, targeting AR might be an appropriate therapy for patients with early-stage HCC. In contrast, androgen/AR signaling has been shown to suppress metastasis of HCC in patients with late-stage disease. In addition, there is evidence that therapy comprising Sorafenib and agents that enhance the functional expression of AR may suppress the progression of late-stage HCC.

Introduction

Liver function and liver diseases

The liver is the largest visceral organ responsible for systemic homeostasis of blood glucose as well as lipid and protein metabolism. It is also responsible for most xenobiotic clearance even when parts of the liver have been damaged (Rinaldi *et al.* 2011). The liver has extraordinary repair capacity, which partially explains why a diseased/damaged liver is usually asymptomatic. Although the etiologies of liver diseases vary, many share common post-damage healing process pathways. Liver damage can result in the accumulation of matrix proteins, the formation of scars, and the alteration of tissue structure and function. As fibrosis develops, chronic compensatory scar-healing processes in the liver begin to take place. Once irreversible distortion of the hepatic architecture and vascular structure occurs, the cirrhotic liver begins to replace the functional hepatic units (Schiff *et al.* 2003). Progression of cirrhosis can lead to liver failure or malignant transformation of hepatocytes.

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Although cirrhosis is one of the etiological factors contributing to the hepatocarcinogenesis process, a significant number of patients without cirrhosis develop hepatocellular carcinoma (HCC), indicating that the disease process involves oncogenic events and virus-related factors.

Hepatitis B virus (HBV) is a well-known etiological factor contributing to cirrhotic liver progression and early HCC development. Antiviral agents, such as lamivudine and adefovir have been found to improve cirrhotic liver function in some studies (Aspinall et al. 2011), but have not been shown to have anti-carcinogenic effects in other studies (Kwon & Lok 2011, Peng et al. 2012). In addition, there is evidence that these anti-viral agents have little effect on survival of patients with advanced-stage disease (Shin et al. 2012) or on disease recurrence after hepatectomy for HCC (Chan et al. 2011).

Gender differences in liver diseases and their linkage to androgen/androgen receptor

There are four major liver diseases associated with gender (Guy & Yee 2009): steatosis (Wild et al. 2004, Yang et al. 2009), hepatitis (Baig 2009, Yang et al. 2010), cirrhosis (Maheshwari & Thuluvath 2011, Huang et al. 2013), and liver cancer (Yeh & Chen 2010). A study conducted in the United States by Weston et al. (2005) showed that the prevalence of fatty liver and cirrhosis, nonalcoholic fatty liver disease (NAFLD), was 3.5 times higher in men than in women. In addition, Baig (2009) showed that the prevalence of HCC was higher among men than among women (range, 2.5 : 1–7 : 1). Several factors may contribute to the gender difference in liver diseases, including age, alcohol consumption, diabetes, hepatic toxins, virus infection, and variation in sex hormones (Yeh & Chen 2010). This review focuses on androgens and their receptors (androgen receptor, AR), as they may represent the major factors that contribute to the gender difference in various liver diseases.

Androgens are synthesized mainly in the testes, although some androgens form in the adrenal glands (Oshima 1968). Androgens act through the AR, a transcription factor that belongs to the nuclear receptor superfamily. AR exerts physiological and pathological functions in organisms by translocating to the nucleus upon binding to androgens (Chang et al. 1988), where it binds to specific DNA sequences known as androgen response elements (AREs; Claessens et al. 2001) in conjunction with various AR co-factors (Yeh et al. 1999). The AR complex can therefore regulate the expression of

genes that participate in various physiological and pathological functions (Bluemn & Nelson 2012). In certain conditions, androgens can exert biological functions in some diseases through a non-AR signaling mechanism (Miyamoto et al. 2007). The effects of androgen and its receptor can be either transitory or long term and can have either local or systemic impacts on organ function. The most well-known androgen/AR-dependent cancer is prostate cancer. Ablation of androgen/AR actions is currently the gold standard for treating patients with prostate cancer; however, not all patients respond to this treatment (Seruga & Tannock 2011). Although androgen/AR plays a role in liver development in the embryonic stage, the maximum dimorphism of androgen/AR effects seems to occur after puberty (Waxman & O'Connor 2006) via the influence of the activity of the hypothalamus–pituitary–gonad axis. The differential secretion of growth hormone (GH) between men and women may have different impacts on liver function in the adult stage of life. Studies have shown that in men, GH is released in high-amplitude; however, in women there are low frequency bursts while GH is released in low-amplitude but with high-frequency pulses (Lund et al. 1991, Mode et al. 1992).

In this review, we focus on the roles androgen/AR play in the processes of liver disease. In the first part of the review, we discuss androgen/AR signaling in noncancerous liver diseases but include a discussion on the precursors to liver cancer development. In the second part of the review, we discuss the involvement of androgen/AR signaling in liver cancer and potential therapeutic strategies that specifically target AR.

Part I: androgen/AR signaling in noncancerous liver diseases

Roles of androgen/AR signaling in the development of steatosis

Steatosis is the abnormal retention of lipids within hepatic cells. Excess lipid accumulation in vesicles displaces the cytoplasm. When the vesicles are large enough to distort the nucleus, the condition is known as macro-vesicular steatosis, otherwise the condition is known as micro-vesicular steatosis (Hashizume et al. 2007). The most common risk factors associated with steatosis are diabetes mellitus, hypertension, obesity, and alcoholism (Sparks & Sparks 2008). Malnutrition can also cause the over-mobilization of fat from adipocytes to liver where lipid metabolism occurs (Williams 2006). The breakdown of

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large amounts of ethanol in alcoholic drinks produces large amounts of chemical energy in the form of NADH (reduced form of nicotinamide adenine dinucleotide), which signals cells to inhibit the breakdown of fatty acids and, simultaneously, to increase the synthesis of fatty acids. This 'false sense of energy' may then result in more lipids being created than are needed. Failure of lipid metabolism can also lead to impaired lipid breakdown, resulting in the accumulation of unused lipids in the hepatocytes. Finally, certain toxins, such as carbon tetrachloride, aspirin, and diphtheria toxin, can interfere with the cellular machinery involved in lipid metabolism.

Androgen/AR signaling suppresses the development of steatosis Many studies have shown that androgen/AR signaling suppresses the development of steatosis. For example, Jacqueson *et al*. (1978) reported that androgen (19-nortestosterone-phenylpropionate) had a protective effect on *Amanita phalloides*-induced hepatic steatosis and Saint-Aubert *et al*. (1980) found that a dose of testosterone injection for 1 month before subtotal hepatectomy could suppress liver steatosis in rats.

However, there are inconsistencies in the findings from studies on the roles androgens and their receptors play in the development of NAFLD. Although several human clinical studies (Vassilatou *et al*. 2010, Schwingel *et al*. 2011*a*,*b*, Jones *et al*. 2012) and an animal study (Chow *et al*. 2011) have shown that androgens might promote NAFLD, other studies have shown that androgens protect against the development of the disease (Haider *et al*. 2010, Magyar *et al*. 2011, Zhang *et al*. 2013). There is little explanation of such inconsistencies, even the patients with NAFLD in these studies were quite different, with polycystic ovarian syndrome (PCOS) female patients (Jones *et al*. 2012) or people using synthetic anabolic androgens (Schwingel *et al*. 2011*a*, 2011*b*) in studies showing positive roles played by androgens to promote NAFLD vs hypogonadal elderly men (Haider *et al*. 2010) in other studies showing the negative roles played by androgens to suppress NAFLD.

In animal models used to study fatty liver, androgens were demonstrated to play a positive role in an aromatase knockout mouse model (Chow *et al*. 2011); however, androgens were also proven to be suppressors of the disease in rats fed a high-fat diet (HFD; Zhang *et al*. 2013). Furthermore, C57BL/6 mice receiving the anti-androgen hydroxyflutamide were shown to have a higher incidence of NAFLD (Takahashi *et al*. 2012).

In contrast, the effects of AR on steatosis are less controversial. In an HFD-induced NAFLD mouse model,

the loss of AR in the whole body (GARKO) was shown to lead to higher insulin insensitivity and the development of diabetes (Lin *et al*. 2005). Similar results were confirmed in mice that only lacked hepatic AR (L-ARKO), indicating that hepatic AR might play negative roles in HFD-induced NAFLD (Lin *et al*. 2008). Furthermore, aged male mice lacking hepatic AR developed hepatic micro-vesicle steatosis, whereas WT aged mice did not (Lin *et al*. 2008). These two *in vivo* mouse models show that hepatic AR might play a role in the suppression of NAFLD.

Lund *et al*. (1991) found that AR might directly regulate carnitine palmitotyltransferase 1 and that AR might exert its function by phosphorylating 3-hydroxy-3-methyl-glutaryl-CoA reductase. Furthermore, Lin *et al*. (2008) showed that AR might suppress fatty acid *de novo* synthesis by decreasing the expression of sterol regulatory element-binding protein 1c (SREBP1). They also found that AR might induce insulin sensitivity by modulating phosphoinositide-3 kinase activity and by suppressing the expression of phosphenolpyruvate carboxykinase and protein-tyrosine phosphatase 1B (Lin *et al*. 2008).

Potential AR-targeted therapies for steatosis NAFLD can progress to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and HCC (Dima *et al*. 2012). Most treatments for NAFLD focus on lifestyle modifications without intervention (Dima *et al*. 2012). Ongoing clinical trials suggest that statins, fibrates, and other lipid-lowering medicines may lead to improvements in liver biochemistry and histology in patients with NAFLD/NASH, although the potential side effects remain unclear (Dima *et al*. 2012). Metformin, the generally recommended first-line treatment for type 2 diabetes, was also found to be effective in the treatment of NAFLD (Cicero *et al*. 2012). However, Weickert *et al*. (2012) reported that metformin might lead to suppression of serum androgen levels in patients with PCOS. It remains unclear whether this suppressive effect of metformin on androgen observed in patients with PCOS would also occur in men with NAFLD. Importantly, it may be worth testing to see whether therapies that enhance AR signaling, either by increasing AR expression or the expression of its downstream genes, can halt the progression of NAFLD.

Androgen/AR signaling in cirrhotic liver with liver regeneration

Cirrhosis is the 12th leading cause of death in the United States (Heron *et al*. 2009) and is associated with a 10-year mortality rate ranging from 32 to 66% (Sorensen *et al*. 2003),

depending on whether the cirrhosis is alcohol-related cirrhosis (ARC), virus-induced hepatic cirrhosis, or nonalcoholic cirrhosis. Since patients with liver cirrhosis are at risk for developing HCC (Aspinall et al. 2011), early treatment of liver cirrhosis with proper therapy will not only improve cirrhotic symptoms but also prevent HCC incidence.

Androgen/AR signaling is associated with the progression of steatosis to cirrhosis Cirrhosis generally arises from chronic liver injury. During the injury-healing process, the damaged liver can develop fibrotic lesions that may lead to loss of normal hepatic function, impaired liver regeneration, aberrant polarity for cell proliferation, and obstruction of the portal system.

NAFLD/NASH is the most common cause of chronic liver disease in western countries (Weston et al. 2005), and NAFLD/NASH can lead to permanent liver damage with cirrhosis if liver cells are replaced by scar tissue (Swift et al. 2001). Scar tissue blocks the flow of blood through the liver and slows the processing of nutrients, hormones, drugs, and naturally produced toxins (Bradbury 2006).

White et al. (2012a,b) found that high serum testosterone level is associated with advanced steatosis and cirrhosis. Early studies indicated that in males the prevalence of muscular cytochrome P450 enzymes was suppressed during the progression of fatty liver to cirrhosis (Littmann et al. 1973, Murray et al. 1992). Interestingly, it was reported that feminization (e.g., gynecomastia) is an important factor linking the progression of steatosis (Green 1977).

The linkage between male hypogonadism and hypotestosteronemia in patients with ARC suggests that androgen/AR signaling might play negative roles in the development of ARC (Green 1977). In men, cirrhosis manifests as hypogonadism with reduced testicular size and clinical features of inadequate testicular function. Between 50 and 75% of cirrhotic men have both macroscopic and histological evidence of testicular atrophy, and as many as 90% of cirrhotic men show some degree of erectile dysfunction (Green 1977). Furthermore, men with cirrhotic liver have a decreased incidence of benign prostatic hypertrophy and gynecomastia is found in about 40% of cirrhotic men (Yoshitsugu & Ihori 1997).

Androgen/AR signaling suppresses the development of cirrhosis Although transdermal administration of testosterone has been shown to improve symptoms of hypogonadism and gynecomastia (Yurci et al. 2011), most testosterone replacement therapies have little effect on cirrhosis (Nieschlag et al. 1977). Similar results were also reported by Gluud et al. (1987) and Gluud (1988), who showed that oral testosterone treatment yielded little change in liver pathogenesis in men with ARC, even though such treatment significantly reduced the prevalence of gynecomastia. Similarly, a large randomized clinical trial also found that administration of androgens had little effect on ARC (Rambaldi & Gluud 2006).

However, Kley (1979) reported that administration of testosterone to male patients with ARC yielded some improvement in ARC symptoms. Importantly, Thole et al. (2004) reported that administration of steroidal or non-steroidal anti-androgens, such as flutamide or cimentidin, might lead to cirrhosis, suggesting that androgen/AR signaling might protect against the progression of cirrhosis.

Potential new therapies via targeting AR for treatment of liver cirrhosis via modulation of liver regeneration Liver regenerative capacity is also associated with the development of cirrhosis (Michalopoulos & DeFrances 1997). Both intrinsic (Cressman et al. 1994, 1996) and extrinsic (El-Ansary et al. 2012, Takami et al. 2012) mechanisms of liver regeneration have been reported. Intrinsic factors include the proliferation of hepatocytes and the self-renewal capacity of oval cells (hepatic stem/progenitor cells) (Sherwood et al. 2005), whereas the extrinsic factors include circulating bone marrow-derived mesenchymal stem cells (BM-MSCs) and infiltrating monocytes/macrophages (Cornell et al. 1990, Seki et al. 2000). The intrinsic repair system, however, is not effective in patients with irreversible chronic liver damage. Therefore, potential approaches to treating cirrhosis should focus on the extrinsic repair system.

BM-MSCs are involved in the liver regeneration process. Chen et al. (2010) revealed that endogenous BM-MSCs could be recruited into fibrotic lesions of mice with injured livers. Moreover, Fang et al. (2004) found that donor transplantation of Flk1 (CD309)-MSCs into mice with CCl4-induced liver fibrosis leads to improvement of liver function. Furthermore, autologous transplantation of BM-MSCs into patients with cirrhotic liver resulted in a temporary halt to disease progression (Vanneaux et al. 2013).

El-Ansary et al. (2012) conducted a Phase II clinical trial using un-differentiated and differentiated MSCs in patients with HCV-induced cirrhosis and found that both types of MSCs resulted in improvement of liver function. In a review of trials of autologous MSC infusion into cirrhotic patients, Takami et al. (2012) found that the

therapy resulted in improvement of liver function and in prolonged survival in some patients.

Using mouse models of CCl4- and thioacetamide-induced liver cirrhosis, Huang *et al.* (2013) revealed that androgen/AR exhibited suppressive effects on the renewal capacity of BM-MSCs and adipose-derived MSCs by modulating the EGFR-mediated Erk and Akt pathways. Targeting AR in BM-MSCs via either AR-siRNA or ASC-J9 (an AR degradation enhancer) (Miyamoto *et al.* 2007, Yang *et al.* 2007) enhanced the self-renewal capacity and migration of BM-MSCs and resulted in reduced inflammation and fibrotic stress. AR-siRNA and ASC-J9 might, therefore, enhance the efficacy of autologous BM-MSC transplantation as treatment for cirrhosis (Huang *et al.* 2013).

Androgen/AR signaling in hepatitis

Hepatitis can be caused by viral infection, chemicals, or drug abuse (Williams 2006). Acute hepatitis is usually defined as inflammation lasting for <6 months and although most patients present with mild symptoms the acute state can present as severe hepatic failure. Chronic hepatitis, however, lasts longer and patients normally are either asymptomatic or mildly symptomatic.

Androgen/AR signaling in cirrhosis and hepatitis In addition to toxic/drug and hepatitis virus contributing to hepatitis (Wright & Lau 1993), cirrhosis may also link to hepatitis since many hepatic patients are also found with cirrhotic liver development (Even *et al.* 1997). A recent study has found that total serum testosterone is associated with increased risk for developing advanced hepatic fibrosis and advanced hepatic inflammatory activity in hepatitis C virus (HCV)-infected men (White *et al.* 2012*a*,*b*). Theve *et al.* (2008) found that the anti-androgen flutamide might prevent the development of cirrhosis and hepatitis in mice. Other studies also found a higher incidence of HBV- or HCV-related cirrhosis and HCC among men (DeLoia *et al.* 1989, Wright & Lau 1993, Schiff *et al.* 2003, Chiu *et al.* 2007). Importantly, direct linkages have been demonstrated between androgen levels (Tanaka *et al.* 2000) and *AR* gene polymorphisms (Yu *et al.* 2000) and the progression of hepatitis and cirrhosis to cancer of the liver.

**Androgen/AR signaling in HBV-induced chronic hepatitis** HBV virus antigens (HBeAg and HBsAg) have been shown to contribute to host immune tolerance to the virus and to be associated with the development of hepatitis. DeLoia *et al.* (1989) found that the expression of HBsAg was higher in pubescent male mice and that testosterone injection promoted the expression of HBsAg in female mice. *In vitro* studies further identified the ARE located in the HBV virus, suggesting that androgens might be able to go through AR to bind to the ARE in the HBV virus to modulate HBV viral titers (Wang *et al.* 2009). Using ARKO mice that lacked AR expression in hepatocytes, Wu *et al.* (2010) reported that AR might play key roles in directly regulating HBV replication to influence HBV viral titers, viral particles, and hepatic viral RNA. Results from *in vitro* cell line studies further confirmed that AR-bound androgen directly binds to ARE in HBV, and that this binding activity is responsible for its transactivation (Wu *et al.* 2010).

Wu *et al.* (2010) revealed that male hormones might promote immune tolerance of HBV in liver. Using HBV transgenic mice, Tian *et al.* (2012) found that male hormones promote HBV virus replication, resulting in higher HBV titers in men than in women and concluded that male hormones but not gender could change the HBsAg and HBx antigens.

Current therapies to improve immune tolerance in patients with HBV-related hepatitis involve administration of interferon to suppress HBV-induced immune activity (Tamori & Kawada 2012) or regimens that boost immune activity to eliminate HBV-infected hepatocytes (Shimizu 2012). Androgen-deprivation therapy with flutamide or cimentidine, however, yielded controversial results. Although Theve *et al.* (2008) found that flutamide prevented the development of cirrhosis and hepatitis in mice, other clinical studies have shown that flutamide can lead to further inflammation of the liver (Thole *et al.* 2004, Manso *et al.* 2006, Matsuzaki *et al.* 2006). Wang *et al.* (2008) reported that the anti-androgen cimentidine resulted in enhanced immune response when administered in conjunction with HBV DNA vaccine by boosting viral clearance. However, Hashimoto *et al.* (1994) found that cimentidine also resulted in exacerbation of liver damage.

Importantly, in ARKO mouse model studies, Wu *et al.* (2010) demonstrated that AR, but not androgens, play key roles in the promotion of HBV replication and hepatitis. They found that knockout of AR in hepatocytes resulted in reduced HBV replication. The researchers also found that targeting AR with the newly developed AR degradation enhancer ASC-J9 suppresses HBV-mediated viral replication and the development of HCC in mice (Wu *et al.* 2010). Figure 1 illustrates the molecular mechanisms governing the effects of androgen/AR on HBV virus replication as well as the positive feedback loop of AR-HBV virus replication.
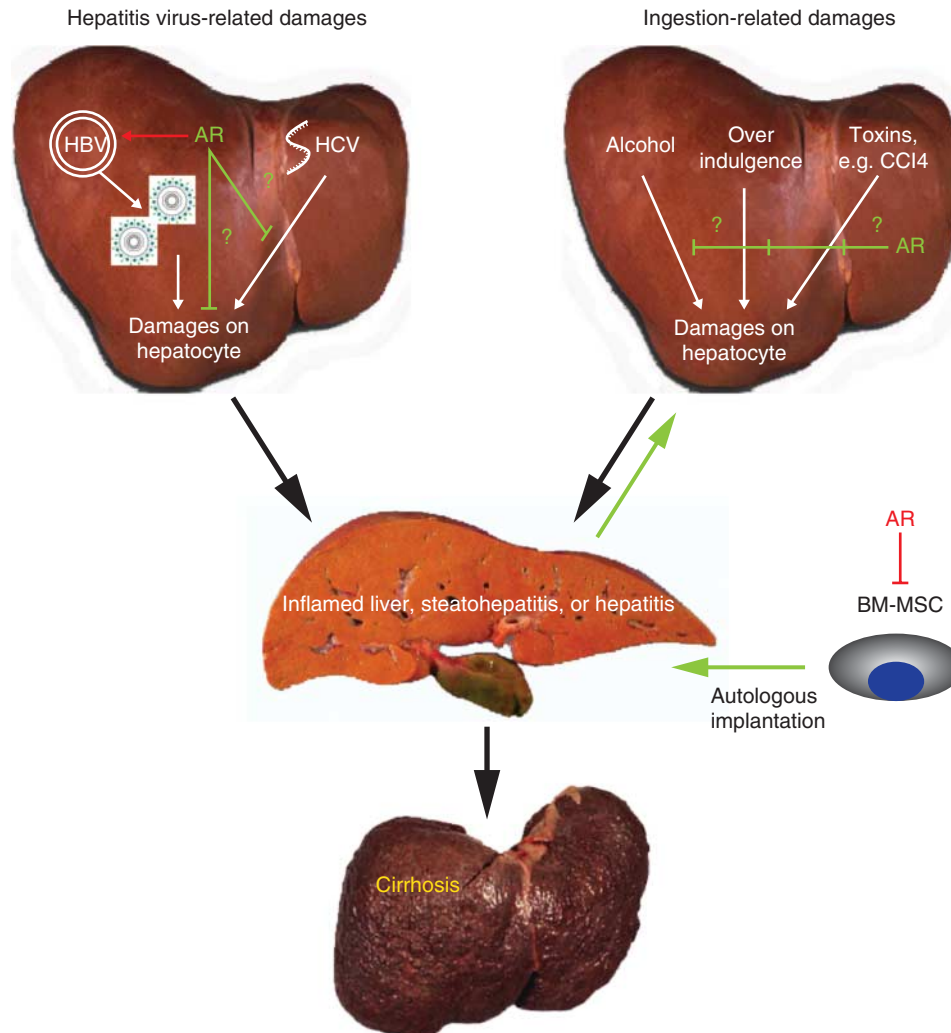
Figure 1

Androgen/AR roles in noncancerous liver disease progression. In hepatitis virus (HBV and HCV)-related hepatic inflammation, androgen/AR promotes HBV virus replication, yet, AR might suppress the hepatitis process. On the other hand, AR also plays a suppressive role in the toxin-related liver inflammation process. Moreover, bone marrow-derived mesenchymal stem cells (BM-MSC) can infiltrate into the damage liver; however, AR suppresses the ability of BM-MSC to repair liver damage.

Androgen/AR signaling in HCV-induced chronic hepatitis

HCV-mediated hepatitis is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis. Chronic HCV infection develops in about 85% of infected patients and Lee et al. (2011) found that female gender was an independent risk factor for HCV infection. Furthermore, Di Martino et al. (2004) reported that hormone replacement therapy was effective at reducing the incidence of HCC development in HCV-infected women. However, other studies have shown that suppression of estrogen signaling reduces the incidence of HCC development in HCV carriers. Using selective estrogen modulators (SERM), researchers have shown that estrogen signaling interferes with the HCV viral cycle (Murakami et al. 2013) and reduces HCV-mediated toll-like receptor 7 (TOLR7) signals (Fawzy et al. 2012), indicating that SERM are potential adjuvant antiviral treatments (Furusyo et al. 2012). Interestingly, Huang et al. (2011) reported that male gender was an independent risk factor for the development of liver disease in patients with HCV-related hepatitis. These studies indicate that while women are at greater risk for HCV infection men are at greater risk of developing HCV-related chronic liver disease.

Kanda et al. (2008) found that HCV viral core antigen could enhance AR transactivation via up-regulation of

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vascular endothelial growth factor (VEGF) and related Stat3 activation. It will be interesting to see in the future whether AR has an effect on HCV virology and related liver diseases by modulating the HCV viral core antigen.

The current therapy for HCV hepatitis is a combination of peginterferon alpha-2a and ribavirin with either boceprevir or telaprevir. However, few studies have linked androgen deprivation therapy or anti-AR therapy to HCV. More studies on how AR influences HCV replication are needed before any potential AR-targeted therapy can be developed.

A summary of the roles that androgen/AR play in noncancerous liver diseases is illustrated in Fig. 1.

Part II: androgen/AR signaling in liver cancer

The American Cancer Society ranked liver cancer as the fifth highest cause of cancer death, with HCC as the most common form of liver neoplasm (Farazi & DePinho 2006). The etiology of liver cancer includes ingestion of related toxins (e.g., alcohol, aflatoxin B1, or AFB1) and infection with hepatic viruses (HBV, HCV, etc.), which are the major oncogenic factors that contribute to the development of HCC.

Part two of this review will focus on the roles that androgens and their receptors play in the development of HCC and cholangiocarcinoma (CC) and potential therapeutic regimens for patients with HCC that target androgen or AR or both.

Androgen/AR signaling in carcinogen-induced HCC

The male predominance in HCC suggests that androgen/AR may promote and that estrogens/ERs may suppress hepatocarcinogenesis (Yeh & Chen 2010). Suppression of carcinogen-induced HCC by estrogen signaling has been shown to regulate MyD88-dependent IL6 production and to trigger cellular innate immunity (Naugler et al. 2007). Although the roles female hormones play in HCC development have been established, that of male hormones are less clear. A number of studies have shown that carcinogen/aflatoxin B1 (AFB1)-induced HCC predominantly affects men (Tejura et al. 1989, Ma et al. 2008, Yeh & Chen 2010, Li et al. 2012), suggesting that androgen/AR may promote the development of AFB1-induced HCC. Although Yu et al. (2001) showed that male gender was a risk factor for AFB1-related cancer development, Nakatani et al. (2001) revealed that AFB1 intake was associated with an increased risk of HCC with little association with AFB1-DNA adducts or testosterone levels in men. The reasons for these inconsistent findings remain to be identified.

In men, the testes are the major source of systemic androgen levels with the adrenal gland contributing a small amount. In women, ovarian-derived androgen is an important source of systemic androgen levels. Tejura et al. (1989) reported an increase in AR expression in ovariectomized rats after injection of hepatic carcinogen, suggesting a potential female source of androgen in the development of HCC. Early studies found that the change in endogenous or exogenous androgens in male rodents could lead to altered HCC progression (Nakatani et al. 2001). In vitro HCC cell line studies also found that androgen/AR-facilitated cell growth was governed by transcriptional regulation of TGFβ1 (Yoon et al. 2006).

Using an ARKO mouse model, Ma et al. (2008) found that AR, but not androgens, plays major roles in hepatocarcinogenesis. They also found that the incidence of carcinogen-induced HCC in L-ARKO mice lacking AR in hepatocytes was lower than that in WT mice, even though the serum testosterone levels in both showed little difference (Ma et al. 2008). Using siRNA to knockdown AR or AR-cDNA to overexpress AR, Ma et al. (2008) also found that targeting AR could alter HCC cell growth. Based on their findings, they suggested that hepatic AR might influence the degree of cellular oxidative stress by modulating superoxide dismutase expression and that it might influence the extent of DNA damage repair by modulating p53 and GADD45α/β expression.

Feng et al. (2011) reported that AR might be able to enhance hepatocarcinogenesis by modulating cell cycle-related kinase-β-catenin activation signaling. Using systems biological approaches to illustrate the interplay between androgen and estrogen signaling in hepatocarcinogenesis, Li et al. (2012) found that Foxa1 and Foxa2 were the bona fide molecules that governed gender disparity in HCC.

The data suggest, therefore, that androgen/AR signaling promotes hepatocarcinogenesis and HCC development, at least in the early stages. Figure 2 illustrates the androgen/AR roles in carcinogen-induced hepatocarcinogenesis.

Androgen/AR signaling in HBV-induced HCC

In a nested case–control study, Yuan et al. (1995) concluded that high androgen level is a risk factor for developing HCC in men with chronic HBV infection. Other studies found that higher serum testosterone, increased number of steroid 5α-reductase type II (SRD5A2) V89L polymorphisms, and fewer AR gene CAG repeats in exon 1 (<23 repeats) in HBV patients were
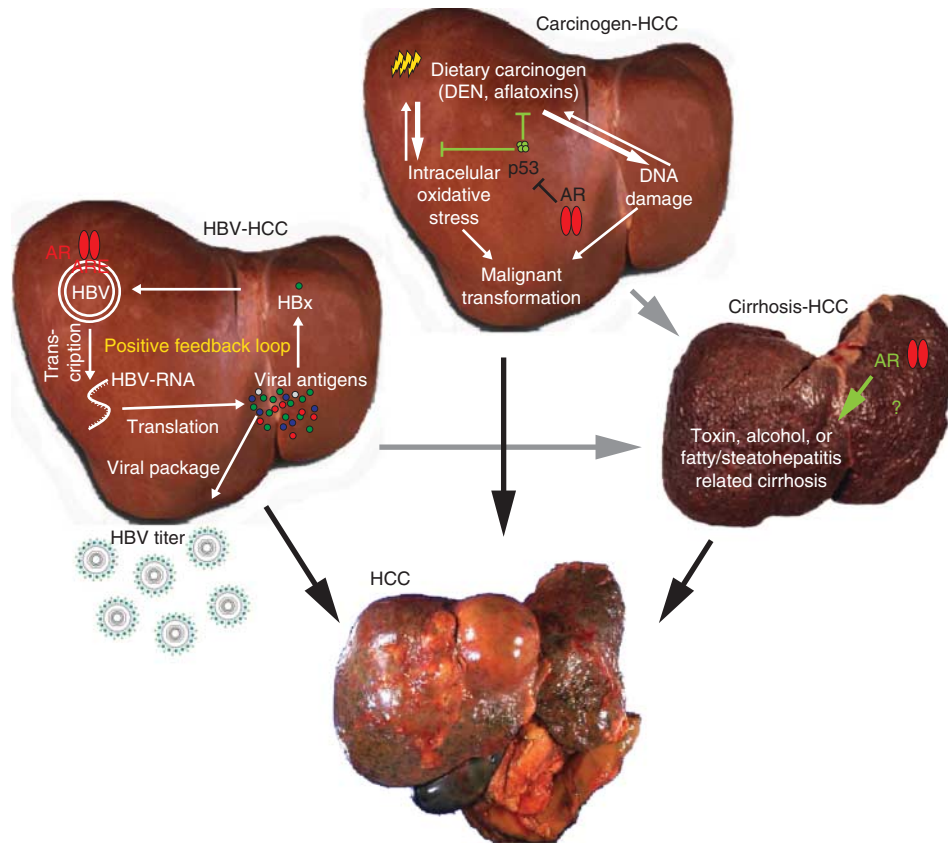
Figure 2

Androgen/AR signaling in hepatocarcinogenesis. There are three major factors that contribute to the development of liver tumors, e.g., HBV, carcinogens, and cirrhosis. In HBV-related HCC, AR promotes HBV virus replication to increase viral titers, as well as viral antigens to positively feedback to AR transactivation function on HBV replication.

In carcinogen- and DEN-related HCC, AR suppresses p53 and related cellular oxidative stress and DNA damage repair, thereby promoting hepatocyte transformation. However, it is still unknown how AR is involved in cirrhosis-related HCC.

correlated with high risk of HCC (Yu *et al*. 2000, 2001), suggesting that more active androgen/AR signaling might lead to higher risk of HBV-induced HCC.

Using HBV transgene mice lacking hepatic AR, which were fed sub-minimal doses of the carcinogen DEN, Wu *et al*. (2010) found that the incidence of HBV-induced HCC was lower among mice with loss of hepatic AR than in WT mice. They revealed that AR enhanced HBV virus replication by binding directly to the HBV core promoter region, which up-regulated the expression of the HBV X antigen (HBx). AR-induced HBx expression subsequently promotes AR transactivation, thereby amplifying its role in hepatic cell transformation. Importantly, AR was also shown to enhance HBV-mediated cell proliferation and to suppress apoptosis (Wu *et al*. 2010).

Other studies have reported that HBV antigen interacts with AR to promote HCC initiation and that HBx enhances hepatic cell transformation by interacting

with AR signaling (Chiu *et al*. 2007, Zheng *et al*. 2007). The mechanism of action governing the effects of HBx on enhanced AR transactivation might involve an increase in phosphorylation of AR rather than binding to ARE sites (Chiu *et al*. 2007). In contrast, androgen/AR signaling might promote HCC development by enhancing HBx function.

Although some clinical and animal studies have provided evidence that androgen/AR signaling promotes HBV-induced HCC development, other studies have shown that inhibition of androgens has little effect on preventing the progression of hepatitis. Using the LHRH analog triptorelin to suppress testosterone production in male patients with chronic HBV infection, Jilma *et al*. (1998) found no significant effect on serum HBsAg and HBV-DNA concentrations. Using murine *Helicobacter hepaticus* infection to study the effects androgen has on chronic hepatitis and HCC progression in mice, Arlin

et al. (Rogers et al. 2007) also found little effect of castration on hepatitis and HCC incidence when compared with control mice. Supplementation with dihydrotestosterone also failed to change the outcome. They concluded that an imprinting effect of endogenous androgens might be responsible for chronic hepatitis and HCC in men.

Androgen/AR signaling in HCV-induced HCC

Although the prevalence of HCV-induced HCC is highest among men, the gender difference in prevalence is not as obvious for HBV-induced HCC. Huang et al. (2011) conducted a prospective cohort study of newly developed HCC and studied the cumulative lifetime incidence rates of HCC in men and women who were positive for both HBV surface antigen (HBsAg) and antibodies against HCV (anti-HCV). They found that there was a significant male predominance in the incidence of HCC for chronic HBV carriers but not for chronic carriers of HCV (Lee et al. 2011). Epidemiological studies have shown polymorphisms of three enzymes involved in androgen and estrogen biosynthesis among anti-HCV positive patients, namely SRD5A2, cytochrome P450c17α (CYP17), and catechol-O-methyltransferase (Rossi et al. 2003). The researchers found that the frequency of the CYP17 C/C polymorphism in female patients with hepatitis or HCC was higher than that in asymptomatic carriers (Rossi et al. 2003). Kanda et al. (2008) reported that HCV core protein, but not NS5A oncogene, up-regulated AR target genes in Huh7 human HCC cells by augmenting androgen/AR activity. In addition, they found that the HCV-induced increase in AR activity also up-regulated VEGF expression and tube formation in human coronary microvascular endothelial cells (Kanda et al. 2008). Interestingly, Vizoso et al. (2007) examined AR expression in HCV-related HCC lesions and found little evidence for a linkage between AR or ER expression and HCC progression. Similar negative results also occurred in a study by Wang et al. (2006), who showed that AR expression was not linked to HCV-related HCC. These controversial results indicate that more studies, especially those on HCV mice lacking hepatic AR, are needed to better understand the roles AR plays in HCV-induced HCC.

Androgen/AR signaling in cirrhosis-induced HCC

Regardless of the etiological factors, cirrhosis and HCC progress at unequal rates in the two sexes, with more frequent disease in men than in women (Giannitrapani et al. 2006). In addition, Tanaka et al. (2000) found that elevated serum testosterone, together with decreased serum estrogens, may promote the development of HCC in patients with cirrhosis.

Although an increase in HCC incidence was reported in patients with NAFLD and cirrhosis (White et al. 2012a), the evidence of direct linkage of androgen/AR signaling in NAFLD with cirrhosis-induced HCC remains unclear.

A direct positive linkage between androgen/AR signaling and the progression of HBV-cirrhosis to HCC is still lacking (Iloeje et al. 2012). Interestingly, Gong et al. (2010) found that loss of an X chromosome might result in altered transformation of hepatocytes in patients with HBV-induced cirrhosis, suggesting the potential linkage between AR loss and the initiation of HCC. Furthermore, Rossi et al. (2003) reported that higher CYP17 activity might increase serum androgen levels and that these increased levels might be associated with risk for HCV-related cirrhosis.

The androgen/AR roles in HBV-, carcinogen-, and cirrhosis-related hepatocarcinogenesis are summarized and illustrated in Fig. 2.

Androgen/AR signaling in HCC progression and invasion

Although there is sound evidence that androgen/AR promote hepatocarcinogenesis and HCC development in the early stages, there is less evidence showing a direct link between androgen/AR signaling and HCC progression in advanced stages. Yuan et al. (1995) reported that testosterone levels were higher in men than in women with advanced HCC, indicating that androgen might play positive roles in advanced HCC. In addition, Kew et al. (1977) found that testosterone levels were positively correlated with survival in patients with HCC.

However, the correlation between AR expression and progression of HCC remains controversial (Kalra et al. 2008). Nagasue et al. (1995) reported up-regulation of AR protein in peripheral tumor lesions, while Tavian et al. (2002) reported downregulation of AR mRNA in poorly differentiated HCC lesions. The reason for the inconsistencies in results is unknown. It could be due to small sampling size and different measurement methods. However, the reason is most likely misclassification of samples. Most of the studies failed to differentiate HCC samples by stage, tumor size, and malignant cellularity. Ma et al. (2012b) found that AR was up-regulated only in tumors smaller than 3 cm. They also found little AR expression in severe HCC lesions, which was consistent with results from Zhu et al. (2011), which showed that AR

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was expressed only in the tumor margins and not in the tumor centers.

In a study using L-ARKO mice lacking hepatic AR with carcinogen-induced HCC, Ma et al. (2012b) demonstrated that mice with loss of hepatic AR developed more malignant tumors, had poorer survival, and had a higher incidence of metastatic lung tumors. They further used in vitro HCC cell lines to investigate whether knock down of hepatic AR or overexpression of AR altered HCC cell migration and invasion and found that AR alters HCC invasion by indirectly suppressing NFκB. These results indicate that AR might suppress HCC metastasis in the advanced stage of HCC.

HCC cell survival in the detached environment (circulation) and the ability to metastasize to distant organs or micrometastasize to neighboring liver tissues is critical for malignant progression of HCC in the advanced stage. A recent report revealed that AR enhanced HCC cell anoikis by suppressing p38 phosphorylation (Ma et al. 2012b), raising the possibility that detecting AR expression in circulating HCC-tumor cells could be a diagnostic marker for HCC recurrence/invasion after curative surgery.

Taken together, the ability of AR to enhance HCC cell anoikis and suppress HCC cell invasion suggests that AR might function as a suppressor of metastasis in late-stage HCC.

Cholangiocarcinoma

Cholangiocarcinoma (CC), a liver tumor arising from the epithelial cells (cholangiocytes) lining the biliary tree, is characterized by poor prognosis and poor response to current therapies (Marsh Rde et al. 2012a,b, Valero et al. 2012). The incidence of and mortality associated with CC are increasing worldwide and an etiological study found that the daily ingestion of germs, including Helicobacter spp., and parasites might contribute to the development of CC (Samaras et al. 2010). Liossi et al. (1988) found 17β-estradiol (E$_2$), but not testosterone, immunoactivity in CC lesions, suggesting potential protective/redundant roles of male hormones in CC progression. Other studies found that estradiol levels were higher among patients with CC than among patients with HCC or cirrhosis (Kuper et al. 2001a). Further studies found that estrogenic signaling might promote CC progression via the estrogen receptor alpha (ERα)-AKT pathway (Alvaro et al. 2006) and up-regulation of VEGF (Mancino et al. 2009), and that E$_2$ and ERα levels were increased in male CC patients.

Potential AR-targeted therapies for HCC

Dual and opposite roles of AR in HCC progression Hepatic AR plays positive roles in hepatocarcinogenesis and in the early development of HCC (Nagasue et al. 1995, Tavian et al. 2002), but have been shown to play negative roles in the advanced stage of HCC (Nagasue et al. 1995, Tavian et al. 2002). A clear understanding of the mechanisms governing these opposite and dual functions of AR in HCC progression might lead to the development of different therapeutic approaches for HCC at different stages (see sections 'ASC-J9, a new AR-targeted therapy for early-stage HCC' and 'AR-targeted therapy in combination with other therapeutic agents as treatment for late-stage HCC').

Controversial results of using anti-androgens to battle HCC The dual and opposite roles of AR during HCC progression might also explain the controversial results of past clinical studies involving the targeting of androgens (but not AR) to battle HCC. Results from several studies on the use of anti-androgens to manage HCC remain controversial (Hépatocellulaire 2004, Di Maio et al. 2008). For example, in a large cohort-nested case–control study, Yuan et al. (1995) found that serum testosterone levels in HCC patients were significantly higher than those in non-HCC controls. Ex vivo studies using human HCC primary cells also demonstrated a positive correlation between androgen/AR signaling and HCC progression (Yu et al. 1997). Recent studies have also suggested that HBx protein might function as a coactivator to promote AR-mediated anchorage-independent cell growth via AR-HBx protein interaction (Chiu et al. 2007, Zheng et al. 2007). Importantly, clinical studies using the anti-androgen cyproterone acetate (300 mg daily) showed some positive improvement in HCC cell growth (Forbes et al. 1987). Similarly, the results of an ex vivo study using the anti-androgen flutamide showed that flutamide suppressed androgen-induced HCC cell growth (Jie et al. 2007). In contrast, other studies found that serum testosterone levels were lower in HCC patients than in non-HCC control patients (Lampropoulou-Karatzas et al. 1993, Kuper et al. 2001b). A small-scale phase II clinical trial of flutamide failed to show improvement in HCC patient survival (Chao et al. 1996). A similar result was found in a large-scale population study using leuprorelin and flutamide (Hépatocellulaire 2004).

There are several possible explanations for the controversial findings among clinical trials and basic studies. One of the explanations is that most of the anti-androgen trials included patients with advanced stage, unresectable HCC. Anti-androgens only suppress a small

portion of fast growing cancer cells, but not poorly differentiated lesions. Another explanation is that AR expression is low in advanced HCC; therefore, anti-androgens no longer exert tumor suppressive effects. The controversial results of the effects of androgens or anti-androgens on HCC progression suggest that targeting androgens to suppress HCC progression might have limited value. Therefore, targeting AR may represent a better therapeutic approach to battle HCC progression.

ASC-J9, a new AR-targeted therapy for early-stage HCC   Using testicular feminized mice that lack AR in the whole body with little serum testosterone, Kemp & Drinkwater (1989) found that AR expression in liver tumors was lower than that in the surrounding normal liver tissue. They concluded that testosterone might function through AR to promote HCC progression, and suggested that either testosterone or AR might be able to promote HCC progression. Using GARKO mice that lack AR in the whole body with little serum testosterone,

Ma et al. (2008) also showed a reduced incidence of HCC development, cancer growth, and apoptosis.

However, since it was impossible to distinguish between the effects of androgen and the effects of AR in the two mouse models mentioned earlier, Ma et al. developed a new mouse model that only lacks hepatic AR (L-ARKO) with little change in serum testosterone. They found that the suppressive effects of AR on hepatocarcinogenesis in mice without hepatic AR were similar to those observed in GARKO mice, suggesting that AR, and not androgens, might play key roles in the promotion of hepatotumorigenesis and the development of HCC (Ma et al. 2008).

These results provide a new therapeutic approach for treating patients with early-stage HCC. Using a newly developed AR degradation enhancer, ASC-J9, which has been shown to degrade AR in selected cells with few side effects (Miyamoto et al. 2007, Yang et al. 2007, Lai et al. 2012, 2013), Ma et al. (2008) found that targeting AR could suppress HCC progression in a carcinogen-induced HCC mouse model and in an HBV-lowDEN-induced HCC
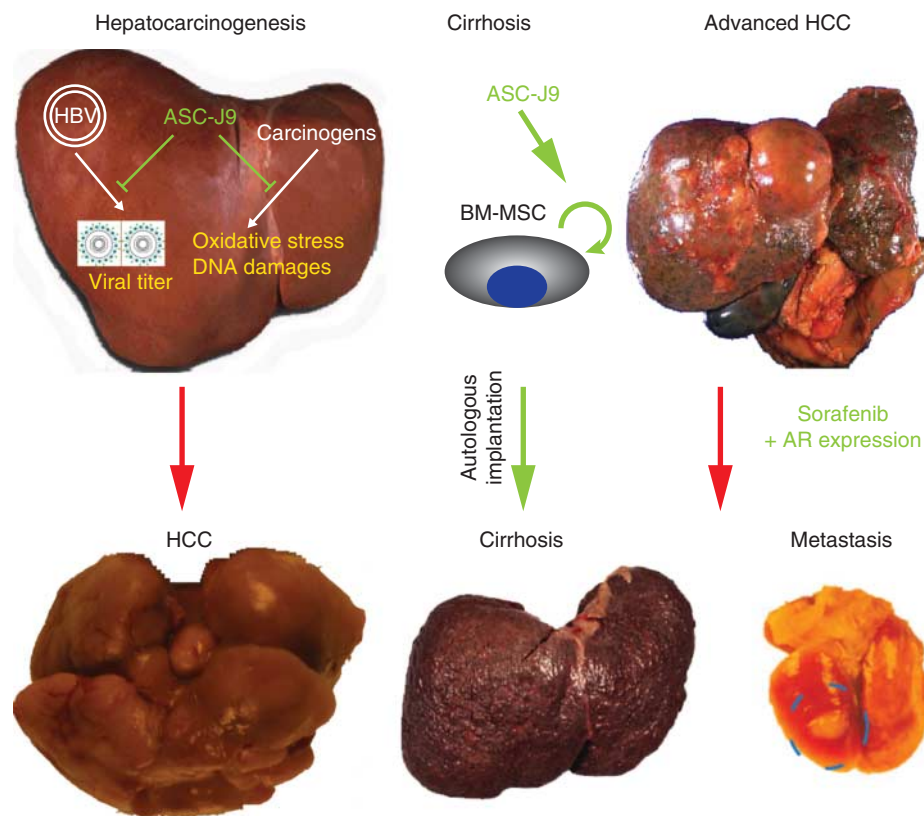
Figure 3

Novel therapeutic strategy targeting AR to battle liver diseases. In the early stage of HCC development, targeting AR using ASC-J9 might suppress early cancer progression. And in the cirrhotic liver, ASC-J9 applied to BM-MSC for autologus implantation might be effective against cirrhosis. At last, treatment of patients with advanced-stage HCC with Sorafenib and expressing AR in the liver might improve the therapeutic efficiency of Sorafenib at reducing the incidence of metastasis or recurrence.

mouse model (Wu *et al.* 2010). These findings suggest that ASC-J9 might be an effective therapy for stage I or grade 1 HCC. Considering the fact that the rate of recurrence of HCC is as high as 60% in patients with HCC stage I–II or grade 1–2 disease who are treated with preventive chemotherapy or transcatheter arterial chemoembolization (Lim *et al.* 2012, Jung *et al.* 2013), targeting the remaining AR-induced HCC cells via ASC-J9 at the early stage of HCC may prevent or delay the recurrence of tumors after treatment. The impact of using ASC-J9 to suppress HBV-lowDEN-induced HCC may be huge, especially in countries with a high rate of HBV infection, as there have been around 522 400 new liver cancer patients yearly in the USA (Jemal *et al.* 2011) and Jemal *et al.* (2011) also reported that 60% of over 1 million newly identified liver cancer patients yearly were from HBV infections in the Eastern and South-Eastern Asia, especially in China, considering the HBV-infected population in China is around 8% of the over 1.3 billion people. The value of any successful therapy to prevent or delay the development of HCC from HBV hepatitis in these HBV-hepatitis patients can be also huge.

AR-targeted therapy in combination with other therapeutic agents as treatment for late-stage HCC In the advanced stages of HCC, chemotherapy with Sorafenib has been shown to have beneficial effects in selected patients (Llovet *et al.* 2008, Yau *et al.* 2009). Increasing the effectiveness of therapies that benefit more

HCC patients is challenging and needed. Ma *et al.* (2012*b*) found that AR could suppress HCC metastasis by modulating p38. They also found that the addition of functional AR in SKhep1 and HepG2 HCC cells to decrease p38 enhanced the effectiveness of Sorafenib against HCC cells in later stages of development. Importantly, results from tail-vein injection cancer metastasis mouse model showed using a much lower Sorafenib dosage (30 mg/kg per mouse) with combined therapy to increase AR expression could lead to similar therapeutic effects as compared with the higher dosage used in the Bayer preclinical trial (40–60 mg/kg per mouse) (Hoshino-Yoshino *et al.* 2011). This suggests that pre-screening for higher expression of hepatic AR in patients with advanced HCC may help to increase the efficacy of treatment with Sorafenib. Alternatively, a new combinational therapy with Sorafenib and some compounds to enhance the functional AR expression may be developed to battle HCC at later advanced stages in the future.

Together, the findings of differential androgen/AR roles in early HCC development vs advanced HCC progression provided insights for targeting AR in these two stages of cancers, which is illustrated in Fig. 3.

Summary and future prospects

Androgen/AR signaling plays different roles in various liver diseases. Androgen/AR signaling suppresses the development of steatosis and may also suppress the development of cirrhosis. In HBV-induced hepatitis,

Table 1 Summary of the androgen/AR roles in liver diseases

Liver diseases	Etiological factor(s)	Androgen/AR roles
Inflamed liver (hepatitis)	HBV/HCV	Promoter (Jacqueson *et al.* 1978, Kanda *et al.* 2008, Theve *et al.* 2008, White *et al.* 2012*a,b*)
	Hepatic toxins (chemical/alcohol.)	Protective effect (Jacqueson *et al.* 1978)
	Diet (metabolic)	Promoter (Vassilatou *et al.* 2010, Schwingel *et al* 2011*a,b*, Jones *et al.* 2012)
Fatty liver/NASH/steatosis	HBV/HCV	Suppressor (Lin *et al.* 2005, 2008)
	Hepatic toxins (chemical/alcohol)	Promoter (Chow *et al.* 2011)/suppressor (Zhang *et al.* 2013)
Cirrhosis	HBV/HCV	Promoter/suppressor (DeLoia *et al.* 1989, Wright & Lau 1993, Tanaka *et al.* 2000, Yu *et al.* 2000, Schiff *et al.* 2003, Chiu *et al.* 2007, Wang *et al.* 2009, Wu *et al.* 2010)
	Hepatic toxins (chemical/alcohol)	Inconclusive (Thole *et al.* 2004, Rambaldi & Gluud 2006)
	Diet (metabolic)	Unknown
Hepatic carcinogenesis	HBV/HCV	Promoter/suppressor (Yu *et al.* 2000, 2001, Chiu *et al.* 2007, Zhang *et al.* 2007, Kanda *et al.* 2008, Wang *et al.* 2008, Wu *et al.* 2010, Huang *et al.* 2011)
	Hepatic toxins (chemical/alcohol)	Promoter (Tejura *et al.* 1989, Rossi *et al.* 2003, Ma *et al.* 2008, Li *et al.* 2012)
	Diet (metabolic)	Unknown
HCC progression/ cancer metastasis	HBV/HCV	Unknown
	Hepatic toxins (chemical/alcohol)	Unknown
	Diet (metabolic)	Suppressor (Tavian *et al.* 2002, Zhu *et al.* 2011, Ma *et al.* (2012*b*))

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androgen/AR signaling enhances HBV replication and promotes HBV- and carcinogen-induced hepatotumorigenesis and HCC development. However, in the advanced stages of HCC progression, androgen/AR signaling suppresses HCC metastasis. In Table 1, we have summarized the overall androgen/AR signaling effects in liver disease.

There is mounting evidence that the effects of androgen are not equal to those of AR on HCC progression and that targeting AR instead of targeting androgens may lead to better therapies for patients with HCC. Additional studies are needed to uncover how AR influences individual infiltrating cells and their interaction within the HCC microenvironment (Ma et al. 2012a).

Although hepatocytes comprise more than 85% of all cells in normal livers, the cancerous liver presents with an increased number of infiltrating immune cells (Doumba et al. 2013, Guo et al. 2013), including dendrocytes (Butterfield 2004), monocytes/macrophages (Shirabe et al. 2012), CD4/8+T cells (Guo et al. 2013), neutrophils (Motomura et al. 2013), B cells (Chen et al. 2012), and mast cells (Ju et al. 2009). Other cells in the liver, such as endothelial cells (Sato & Mori 2011) or BM-MSCs (Garcia et al. 2011), may also be altered due to chronic inflammation or tumorigenesis. Ma et al. found that infiltration of monocytes/macrophages might alter the growth and invasion patterns of HCC and that targeting AR in HCC might reduce the infiltration of monocytes/macrophages, thereby suppressing monocyte/macrophage-mediated cancer growth (Wen-Lung Ma, Yin-Yi Chen, Chawnshang Chang, 2013, unpublished observations). Huang et al. (2013) also revealed that infiltrating BM-MSCs in the cirrhotic liver might result in enhanced liver repair and subsequent improvement in liver function. It will be interesting to see whether targeting AR in BM-MSCs suppresses or prevents the progression of cirrhosis to HCC.

Many studies have focused on sex hormone signaling to try to explain the gender difference associated with HCC. In a recent review article, Ruggieri et al. (2010) provided an abundance of evidence that estrogens inhibit IL6 production by suppressing NFκB/MyD88 and that they also reduce cellular oxidative stress. However, they also found that androgen signaling promotes the VEGF/Stat3 pathway and suppresses p53-mediated antitumor effects. Therefore, there appears to be a crossover of estrogen and androgen signals in the regulation of cellular oxidative stresses.

In summary, the dual yet opposite roles of AR in early HCC initiation vs later advanced stages and findings developing new and differential therapeutic approaches via targeting AR to battle HCC at different stages may help us to better battle the HCC.

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