Androgen and androgen receptor signals jamming monocyte/macrophage functions in premalignant phase of livers

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Abstract
There is a widely discussed concept that chronic inflammation and repeated damage-repair cycles result in malignant transformation of the liver. Kupffer cells are the major host defending macrophages that reside in the liver. They are also considered as critical in episodes of cancer immune surveillance that wipe out liver malignancies, yet, turn into cancer assisting cells in certain conditions. Monocyte/macrophage population and cytokine profile hierarchization in hepatocellular carcinoma (HCC) has increased the curiosity to search for macrophage modulators. Androgenic signals [androgen/androgen receptors (A/AR)] play an important role in liver function and disease progression. Basic and clinical studies have revealed that A/AR might play a biphasic function in HCC progression. Whether A/AR work with TAM to further manipulate HCC progression is of great interest. In this review article, we will focus on the interaction of hepatocytes and monocytes/macrophages in preneoplastic/inflammatory liver diseases, underlining A/AR actions.

1. Introduction

1.1. Androgen/androgen receptor (A/AR) roles in hepatic host immunity
The liver is the largest visceral organ and is responsible for systemic homeostasis such as blood glucose, lipid and protein metabolism and the clearance of xenobiotics [1]. There are several different cell types found in a liver unit: hepatocytes, Ito cells (lipocyte), Kupffer cells (monocytes), and oval/stellate cells (fibroblast) [1]. The hepatic sinusoids are unique structures that are lined with a thin discontinuous endothelium. Kupffer cells are part of this thin line and are responsible for scavenging hepatic debris for the hepatic host immune response. Liver is under a constant immunological challenge, and the immunological response is dominated by innate immunological components including macrophages, dendritic cells, natural killer cells, natural killer T cells, complement...
components, acute phase proteins, and chemokines [2]. The immune surveillance function (tolerant to daily food absorption to the liver) is largely executed within hepatic reticuloendothelial cells, which are largely composed of Kupffer cells. The cell surface receptors, e.g., TLR, complement receptor and Fc-receptors on Kupffer cells, are known to be largely responsible for sensing innate immunological responses [2,3]. However, little is known in diet-induced liver damage.

During acute illnesses, acute innate immunity could be activated by the activation of macrophages, and by producing certain acute phase proteins. Macrophages and lymphocytes could secrete inflammatory cytokines such as tumor necrosis factor-alpha (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6) when antigens are present in the organs. Interacting with complement pathways, the complex could bring microbes to phagocytes, cause macrophage cytolysis, and chemotactically attract phagocytes to the infected area. Inflammatory cytokines travel through the blood and stimulate hepatocytes in the liver to synthesize and secrete acute phase proteins. This response provides an early defense and enables the body to recognize foreign substances early on in the infection process, prior to the full activation and implementation of the immune responses [2,3].

Using an AR knockout animal model, Lin et al explored the potential roles of A/AR signals in food-induced hepatosteatosis and the relative metabolic syndrome in the mouse [4]. In this report, Lin et al pointed out that AR could act as protein-tyrosine phosphatase 1B (PTP1B) suppressors, which suppress insulin signaling and inhibit β-oxidation of lipids in hepatocytes. However, as previously described, Kupffer cells are host immune confounders against metabolic and chronic steatosis of the liver. Introducing the 5a-reductase inhibitor, 4-hydroxyandrostenedione (4-OHA), suppresses testosterone conversion to 5a-dihydrotestosterone (DHT) in mice and could reduce Kupffer cells cytokine production, but not splenic macrophages [5]. Their studies provide some evidence to suggest that A/AR might also play a role in the overindulgent stress in which gate-keeping by host immune defense in the liver.

1.2. A/AR roles in the hepatic damage/regenerating state

Liver regeneration is the homeostatic machinery to recover the liver structure and function after physical or chemical hazards [6]. Distinguishable from other regenerative organs (skin, bone marrow), the liver regenerates from large populations of mature cells, and not from small amounts of stem/progenitor cells [6,7]. Several distinct cells, such as hepatocytes (the main functional cells of the liver), cholangiocytes (biliary epithelial cells), fenestrated endothelial cells (gates between blood fluid and hepatocytes), Kupffer cells (liver macrophages), and Ito cells (stellate cells; extracellular matrix secretion, growth factors production, and vitamin A storage) [6] are involved in the healing process. Among them, hepatocytes are the primary and most important initiators during the whole liver regeneration process.

There are several animal models using rodents for studying liver regeneration. The most common is the removal of two-thirds of the liver (partial hepatectomy; PHx) to induce liver regeneration. Another is the injection of the hepatic toxin, CCl4, to facilitate hepatic proliferation. Results from these animal studies suggested that proliferation within the hepatic parenchyma starts from the portal triad to the pericentral area within 48 hours. Several immediate early response genes, such as NFκB and STAT3, could be activated rapidly (within 60 minutes) after PHx [8,9]. Other factors involved in liver regeneration after PHx are the growth factors and cytokines, such as hepatocyte growth factor (HGF), tumor necrosis factor alpha (TNFα), interleukine-6 (IL-6), epidermal growth factor and transforming growth factor alpha [10]. Nuclear receptor signals, such as thyroid hormone [11], retinoid acids [12], and glucocorticoid [13] as well as estrogens and androgens, also play important roles in liver regeneration.

In general, the liver regenerative response is better in females than in males [14], suggesting that A/AR signals may play a negative role in regenerating the liver. The decreased serum androgen levels, accompanied by down-regulation of AR protein in the liver during liver regeneration, also implies the negative role of A/AR signals using either androgen or estrogen. However, other reports also suggested that A/AR signals might play a small role in the liver regeneration. For example, the antiandrogen, cimetidine, showed little effects on PHx rat liver [22], and administration of tamoxifen in male hepatotomized rats resulted in increased AR activity, yet had little effect on liver regeneration [18]. Similarly, the addition of the antiandrogen, flutamide, resulted in initial overexpression of AR, yet had little effect on liver regeneration in PHx rats [23].

Kahn et al [22] also demonstrated that in the rat liver, hepatic injury with a portacaval shunt results in minor effects on AR activity, and partial clamp of the portal vein or clamp of the hepatic artery yields little effect on AR activity. However, using CCl4 toxicity in the liver, Smirnova et al [19,20] and Shchelkunova et al [25] found that A/AR signals might indirectly facilitate liver regeneration through modulation of a specific protein, unusual estrogen-binding protein (UEBP), in the hepatocytes that can increase the uptake of estrogen.

The above contradictory effects (suppression vs. promotion vs. little influence) of A/AR signals using either androgen administration or surgical castration to test the liver regeneration ability in different animal models (PHx or CCl4 toxicity) implied the complexity of A/AR signals during the liver regeneration process. There is no perfect explanation for the contradictory effects of A/AR signals between liver regeneration models. Whether the differential secretion of growth factors or cytokines that were induced by surgical hepatectomy, or chemical toxicity in different animal models, results in such diverse A/AR responses, is an interesting question for further study.
1.3. A/AR signals and hepatitis B virus (HBV) antigen expression and replication

The evidence that A/AR signals influence HBV replication was based on HBV transgenic mice studies. Although HBV virions, HBsAg and HBeAg, can be detected in HBV transgenic mice, the mice did not exhibit pathological changes because of the immune tolerance. The high HBV DNA amount is associated with the occurrence of human HCC, so it is important to know how A/AR signals affect HBV antigen expression and replication in mice. It has been found that serum HBsAg concentration is higher in male than in female HBV transgenic mice. Castration of male mice could eliminate this sexual dimorphism, while supplementation with testosterone could restore the difference [26–28]. Furthermore, in order to determine the effects of AR on the HBV virus, Breidbart et al [26], using testicular feminization mutation (Tfm) mice, in which 90% of AR was reduced, found that the serum HBsAg concentration is higher in wild type mice compared with XYTfm mice. Except for direct A/AR signals on regulating HBV antigen expression [29], more evidence is required for the effect of A/AR signals in HBV-related HCC. A previous bottleneck of HBV-HCC studies was due to the lack of proper animal models in which HCC spontaneously develops from hepatitis B. Woodchuck hepatitis virus (WHV) is similar to the human HBV, both in structure and replicative viral life cycle. WHV infection can cause acute and chronic hepatitis. Chronic WHV infection in woodchuck will develop into HCC within the first 2 to 4 years [30]. Therefore, the WHV model might allow us to know more about the effects of A/AR in the progression of HBV infection and the related liver disease. Recent studies showed that androgenic signals promote HBV viral replication through direct regulation of HBV replication [31–34]. Furthermore, Wu et al, found that HBV transgenic mice, supplemented with a subminimal dose of carcinogen, can induce spontaneous HCC development, while knockout hepatic AR could reduce HBV-related hepatocarcinogenesis through direct regulation of HBV virus replication [31].

1.4. A/AR signals in cirrhotic livers

Cirrhosis is the pathological feature that can be observed in diverse liver diseases, generally arising from chronic liver injury. During the injury healing process, the liver could develop fibrotic lesions that may lead to the loss of normal hepatic function, impaired liver regeneration, aberrant polarity for cells to proliferate, and obstruction of the portal system’s ability to secrete bile acid. Based on the population epidemiology, liver cirrhosis can be classified into three categories: (1) alcoholic-related cirrhosis (ARC); (2) virus-induced hepatic cirrhosis; and (3) non-alcoholic cirrhosis.

The linkage of male hypogonadism and hypotestosteronemia with ARC victims [35] suggests that A/AR signals might play some role in the ARC. Reduced testicular size and the clinical features of inadequate testicular function are manifested as clinical hypogonadism in cirrhotic men. Between 50% and 75% of cirrhotic men have both macroscopic and histological testicular atrophy. Associated with this, 80%–90% of cirrhotic men are impotent, and seminal fluid characteristics are grossly abnormal in the small minority of patients who are able to produce an ejaculation [35]. Furthermore, cirrhotic men have a decreased incidence of benign prostatic hypertrophy, and gynecomastia could also be found in about 40% of cirrhotic men [24].

Testosterone administration can improve both hypogonadism and gynecomastia syndrome effectively, however, it may have little effect in improving cirrhosis [36]. This suggests that the decrease of testosterone, due to irreversible damage of the liver, may be a consequence of cirrhosis. Interestingly, Kley [37] found that administration of testosterone to male ARC might yield some improvement. In agreement with this, Thole et al [38] also reported that administration of steroidal or non-steroidal antiandrogens, such as cimetidine or flutamide, might lead to cirrhosis [24], although the potential toxicity of these antiandrogens might also contribute to such cirrhosis.

In contrast, Glud C et al [39,40] found that oral testosterone treatment yielded little change in the liver pathogenesis of ARC men, even when such treatment could significantly reduce the prevalence of gynecomastia. Similarly, a large population in a randomized clinical trial also found androgens, such as testosterone and oxandrolone administration could not improve ARC patients.

2. Concluding remark: Illustrating the relations within Kupffer cells, A/AR and hepatocarcinogenesis

Recent reports on A/AR signals in hepatocarcinogenesis have gained advances regarding carcinogen- and HBV-related liver malignancy [31,41,42]. However, the bimodal function of A/AR has also been described in HCC progression [43]. Although there is little information regarding A/AR signals in the immune surveillances of hepatocarcinogenesis, A/AR signals did have documented in prostatic malignancy in terms of innate and adaptive immunities. Published by Lai et al, in the immune cells specific AR knockout mice described, A/AR is pivotal for both innate and adaptive immune regulations [50,51].

It has been reported that innate immunity plays a pivotal role in hepatocarcinogenesis. By removing NF-κB signals in the hepatocytes, the mouse became resistant to carcinogen-induced HCC [44]. This result clearly demonstrated the linkage of innate immunity to HCC. The Kupffer cells population is not increased in the cancer lesion. The Kupffer cell number may even be decreased [45]. These conflicting findings could lead to the conclusion that innate immunity might be an important factor, however, the innate immunity does not necessarily require invasive macrophages into the tumor lesion. Previous studies, in both humans and rodents, have shown that tumor necrosis factor alpha (TNFα) is an important mediator of liver injury [46–48]. Although many types of cells in the liver are capable of producing TNFα, Kupffer cells are thought to be the main hepatic source of TNFα. They also produce factors such as IL-12 and IL-18 and interferon gamma, which enhance TNF activity, as well as those that inhibit TNF-α, such as IL-10 [49]. The role of A/AR in the whole process of HCC development and progression is still a mystery. Whether A/AR and TAM participate in the hepatocarcinogenesis process is yet to be explored.
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