Research Article

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ADRB2 signaling promotes HCC progression and sorafenib resistance by inhibiting autophagic degradation of HIF1α

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Background & Aims: Considerable evidence suggests that adrenergic signaling played an essential role in tumor progression. However, its role in hepatocellular carcinoma (HCC) and the underlying mechanisms remain unknown.

Methods: The effect of adrenaline in hepatocarcinogenesis was observed in a classical diethylnitrosamine-induced HCC mouse model. Effects of ADRB2 signaling inhibition in HCC cell lines were analyzed in proliferation, apoptosis, colony formation assays. Autophagy regulation by ADRB2 was assessed in immunoblotting, immunofluorescence and immunoprecipitation assays. *In vivo* tumorigenic properties and anticancer effects of sorafenib were examined in nude mice. Expression levels of ADRB2 and hypoxia-inducible factor-1α (HIF1α) in 150 human HCC samples were evaluated by immunohistochemistry.

Results: We uncovered that adrenaline promoted DEN-induced hepatocarcinogenesis, which was reversed by the ADRB2 antagonist ICI118,551. ADRB2 signaling also played an essential role in sustaining HCC cell proliferation and survival. Notably, ADRB2 signaling negatively regulated autophagy by disrupting Beclin1/VPS34/Atg14 complex in an Akt-dependent manner, leading to HIF1α stabilization, reprogramming of HCC cells glucose metabolism, and the acquisition of resistance to sorafenib. Conversely, inhibition of ADRB2 signaling by ICI118,551, or knockdown ADRB2 expression, led to enhanced autophagy, HIF1α destabilization, tumor growth suppression, and improved anti-tumor activity of sorafenib. Consistently, ADRB2 expression correlated positively with HIF1α in HCC specimens and was associated with HCC outcomes.

Conclusions: Our results uncover an important role of ADRB2 signaling in regulating HCC progression. Given the efficacy of ADRB2 modulation on HCC inhibition and sorafenib resistance, adrenoceptor antagonist appears to be a putative novel treatment for HCC and chemoresistance.

Lay summary: ADRB2 signaling played an essential role in sustaining hepatocellular carcinoma cell proliferation and survival. ADRB2 signaling negatively regulated autophagy, leading to hypoxia-inducible factor-1α stabilization, reprogramming of hepatocellular carcinoma cells glucose metabolism, and the acquisition of resistance to sorafenib. Adrenoceptor antagonist appears to be a putative novel treatment for hepatocellular carcinoma and chemoresistance.

Keywords: Beta-2 adrenergic receptor; Liver cancer; Autophagy; Sorafenib resistance.

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Abbreviations: ADRB2, beta-2 adrenergic receptor; HCC, hepatocellular carcinoma; DEN, diethylnitrosamine; FOR, formoterol; HIF1α, hypoxia-inducible factor α; IHC, immunohistochemistry.

Introduction

Microenvironment played critical roles in regulating tumorigenesis, tumor progression, metastasis, as well as therapeutic response [1]. Recently, the roles of the nervous system and neurotransmitters in the tumor microenvironment have received more attention [2]. Most internal organs/systems are enervated by sympathetic and parasympathetic nerves. These nerves play a central role in tissue homeostasis through direct innervation and release of neurotransmitters such as catecholamines and acetylcholine. Several epidemiologic and experimental animal studies have also showed that psychosocial factors, especially chronic stress, can modulate growth and progression of certain tumors via inducing the release of neurotransmitters and/or hormones [3–5].

Neurotransmitters or receptors for autonomic neurotransmitters can stimulate cell growth or increase their migratory activity through the activation of corresponding signaling pathways. For instance, the activation of β-adrenergic receptors has been implied in multiple processes of cancer initiation and progression [6]. Recently, it has been reported that sympathetic and parasympathetic nervous systems in prostate tumors contribute to prostate cancer development and progression in animal model. Preventing release of neurotransmitter by sympathetic nerve

ablation inhibited early stages of prostate tumorigenesis [7]. Stress-induced release of catecholamines, acting through the β-2 adrenergic receptor (ADRB2), also promoted tumor cell survive and invasion, and played important roles in cancer progression [3].

The liver, too, is innervated by the autonomic nervous system, which has been shown to regulate numerous physiological and pathological events [8]. For instance, the sympathetic nervous system (SNS) may be involved in liver repair, and the β-adrenoceptor agonist, isoproterenol, rescues acetaminophen-injured livers through increasing progenitor numbers by Wnt in mice [9]. Hepatocellular carcinoma (HCC) is among the most common malignancies worldwide [10]. Until recently, the role of psychosocial factors, nerves and neurotransmitters in the liver tumor initiation and progress have received little attention. Recent studies have reported that monoamine oxidase A, a catecholamine neurotransmitter degrading enzyme, was significantly downregulated in clinical HCC samples [11], suggesting that the β-adrenergic signaling may play an important role in HCC development and progression. However, the precise molecular mechanisms by which β-adrenergic signaling may influence HCC development and therapy resistance are not totally understood.

Here, we demonstrate that ADRB2 activation by adrenaline exerts favorable effects to chemical agent-induced HCC progression. Mechanistically, we propose and verify a novel molecular basis that ADRB2 signaling negatively regulates autophagy by increasing beclin1 homodimer formation in an Akt-dependent manner. The inhibited autophagy activity further leads to HIF1α stabilization, resulting in metabolic reprogramming and enhanced tumor growth. Importantly, blocking ADRB2 signaling also enhanced sorafenib-induced autophagy and improved anti-tumor effects of sorafenib.

Materials and methods

Animal studies

For diethylnitrosamine (DEN)-induced HCC mouse model, healthy pregnant C57BL/6 mice were purchased from Chinese Science Academy (Shanghai, China). After they gave birth, forty 15-day-old male mice of their offspring were chosen and intraperitoneally injected with DEN (20 mg/kg). Two weeks later, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) (3 mg/kg) was given intraperitoneally for once every two weeks lasting for 7 months. After injection of DEN, mice were randomized into 4 groups (n = 10), and were respectively intraperitoneally injected with vehicle, adrenaline (100 μM, 100 μl), ICI118,551 (25 μM, 100 μl) followed by adrenaline (100 μM, 100 μl) or ICI118,551 (25 μM, 100 μl) three times every week lasting for 7 months.

For mouse xenograft model, 6-week-old male nude mice were purchased from Chinese Science Academy (Shanghai, China). 3×10^6 SMMC-7721 cells or indicated cells (Myr-AKT1 transformed liver progenitor cells (LPC) and shNTC or shADRB2 expressing SMMC-7721 cells) were injected subcutaneously (s.c.) in the right flank of nude mice. When the largest tumor was approximately 100 mm^3 in size, mice were randomized into indicated groups (n = 8) and subjected to the indicated treatment. Vehicle, adrenaline (100 μM, 100 μl), ICI118,551 (25 μM, 100 μl) followed by adrenaline (100 μM, 100 μl) or ICI118,551 (25 μM, 100 μl) were all given intraperitoneally every day. Sorafenib (50 mg/kg/d) was also given intraperitoneally. Propranolol (0.1 g/L) was added to drinking water. Tumor volume was measured twice or three times weekly which was calculated by larger diameter × (smaller diameter)^2/2.

Study approval

All animal experiments were performed in accordance with the guidelines for the care and use of laboratory animals and were approved by the Ethical Committee of the Second Military Medical University. All clinical samples were approved for analysis by the Ethical Review Committee of the Eastern Hepatobiliary Surgery Hospital with informed patient consent.

Statistics

Each experiment was performed at least in triplicate and values presented were expressed as mean ± SD. Data analysis was performed by the SPSS software (version 16; SPSS). $\chi 2$ test and Student's t test were applied to determine statistical significance. Kaplan-Meier analysis and log-rank tests were used for survival analysis. A value of $p < 0.05$ was considered significant.

Results

Adrenaline promotes chemically induced hepatocarcinogenesis

Adrenaline is the most studied neurotransmitters for their role in carcinogenesis and tumor progression. To address the role of neurotransmitters in hepatocarcinogenesis, we first evaluated the effect of adrenaline in a classical DEN-induced HCC mouse model. Briefly, male C57BL/6 mice were subjected to DEN treatment at 15 d of age. Afterward, TCPOBOP was given to mice for once ever two weeks lasting for 7 months. During this process, vehicle or adrenaline mimicking the effects of stress was injected to mice three times a week. As expected, all mice developed visible liver tumor foci after 7 months and H&E staining conformed that the liver tumors were HCC (Fig. 1A, B). Intriguingly, injection of adrenaline caused a remarkable increase in hepatocarcinogenesis induced by DEN. Tumor size, tumor number, liver-body weight ratio, and hepatic injury were higher in the adrenaline injection group (Fig. 1A, C–F). These results demonstrate that excessive adrenaline exposure exerts a considerable impact on hepatocarcinogenesis.

Adrenaline actives adrenergic receptors on the cell surface to complete its biological effects. There are at least 8 types of adrenergic receptors expressing on the different cells of human. By examining a database that contains 247 human HCC samples [12], we found that ADRB2 was predominantly expressed in HCC (Supplementary Fig. 1A). We also analyzed the mRNA levels of four frequently expressed adrenergic receptors (namely *ADRA1A, ADRA1B, ADRB1 and ADRB2*) in four different human HCC cell lines. As shown in Supplementary Fig. 1B, *ADRB2* was the predominant receptor in all four tested cell lines, especially in SMMC-7721 and CSQT-2. Therefore, we supposed that adrenaline promoted HCC development mainly through activating ADRB2. To verify this hypothesis, we investigated the effects of adrenaline in the presence of the selective ADRB2 antagonist ICI118,551 in the DEN-induced HCC mouse model. As expected, the promoting effects of adrenaline on DEN-induced HCC development were completely blocked by ICI118,551 (Fig. 1A–F). Together, these findings indicate that adrenaline/ADRB2 signaling promoted DEN-induced hepatocarcinogenesis.

ADRB2 signaling is essential for sustaining HCC cell proliferation and survival

We further examined the role of ADRB2 signaling in HCC cell proliferation and survival. Specific ADRB2 antagonist ICI118,551 and butoxamine (BUTO) significantly inhibited the proliferation and survival of all the HCC cell lines examined (Supplementary Fig. 2A–E). Conversely, epinephrine (EPI) promoted HCC cells proliferation (Supplementary Fig. 2F). Interestingly, following