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Molecular mechanisms of 3,3′4,4′,5-pentachlorobiphenyl-induced epithelial-mesenchymal transition in human hepatocellular carcinoma cells

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ABSTRACT

Polychlorinated biphenyls (PCBs) are classic persistent organic pollutants (POPs). Many studies have found a positive association between the progression of hepatocellular carcinoma (HCC) and PCBs exposure. However, the influence of PCBs on epithelial-mesenchymal transition (EMT) of HCC remains to be unclear. In this study, we explored the effect of PCB126 on EMT in HCC cells and its underlying mechanisms. The data showed that PCB126, exposing both Bel-7402 and SMMC-7721 cells for 48 h, promoted EMT that was demonstrated by E-cadherin repression, up-regulation of N-cadherin and vimentin, and morphological alteration. We found that signal transducer and activator of transcription 3 (STAT3)/Snail1 signaling was activated after PCB126 exposure, and the addition of STAT3 inhibitor WP1066 blocked PCB126-induced down-regulation of E-cadherin as well as up-regulation of N-cadherin and vimentin. Moreover, PCB126 exposure increased pyruvate kinase M2 (PKM2) expression and its nuclear translocation, whereas treatment with PKM2 shRNA suppressed the activation of STAT3/Snail1 signaling and the alternation of EMT-related molecules (E-cadherin, N-cadherin and vimentin). Furthermore, this study indicated estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) were involved in PCB126-induced effects on PKM2, STAT3/Snail1 signaling and EMT by according treatment using ER inhibitor ICI and AhR shRNA. Notably, PCB126-increased reactive oxygen species (ROS) production via AhR is associated with activation of PKM2/STAT3/Snail1 cascades and contributes to EMT. Taken together, these results indicated that PCB126 promotes EMT process of HCC cells via PKM2/STAT3/Snail1 signaling which is mediated by ER and AhR.

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of synthetic organic chemicals consisting of 209 congeners. Because of their physical properties, such as thermal stability and low reactivity, PCBs were widely used in many industries as flame retardants, electrical insulators, lubricants, and liquid seals (Safe, 1994; Robertson and Ludewig, 2011). Although PCBs has been banned since 1979, these chemicals are still extensively distributed in the environment due to their high lipophilicity and slow rates of biotransformation (Safe, 1994). PCBs are known to cause a broad range of adverse health effects including immunotoxicity, neurotoxicity, hepatotaxicity, and hormonal disruption (Silberhorn et al., 1990; Crinnion, 2011). Additionally, PCBs has been reported to be related to a variety of cancers, including lung, breast, colorectal cancer and hepatocellular carcinoma (HCC) (Howsam et al., 2004; Liu et al., 2010; Uccello et al., 2012; Zani et al., 2013; Parada et al., 2016). Based on structure and affinity to specific cellular receptors, PCBs are divided into dioxin-like and non-dioxin-like PCBs. 3,3′4,4′,5-pentachlorobiphenyl (PCB126) is the most toxic coplanar congener of dioxin-like PCBs (Liu et al., 2015). PCB126 exerts its toxicity by multiple ways. Similar to most doxin-like PCBs, PCB126 binds to aryl hydrocarbon receptor (AhR) and induces transcription of AhR target genes such as the cytochrome P450 (CYP) enzymes CYP1A1(Lai et al., 2010). In addition, recent study showed that PCB126 can stimulate estrogen receptor (ER) signaling and increase the expression levels of ER-mediated genes (Mortensen and Arukwe, 2008). PCB126 also was proven to induce ROS and thus mediate its toxicity (Hassoun et al., 2002).

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the third leading cause of cancer-related deaths in the world (El-Serag and Rudolph, 2007; Norsa'adah et al., 2013). The development of HCC is closely related to a variety of risk factors including alcoholic liver, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, and so on (Chen et al., 1997). Noteworthily, exposure to

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chemicals, especially carcinogens, has been proven to cause a high risk of HCC (Uccello et al., 2012). PCBs, a probable human carcinogen, are known to induce preneoplastic lesions and HCC in animals (Twaroski et al., 2001; Espandiari et al., 2003; 2006d, 2006c, 2006a, 2006b). Moreover, epidemiological data have suggested that PCBs exposure may increase the risk of HCC (Mallin et al., 2004; Prince et al., 2006), but their precise mechanisms of action remain unclear.

Epithelial-mesenchymal transition (EMT), a pivotal step in cancer progression, is a phenotypic switch that permanently or transiently converts epithelial cells into mesenchymal-like cells. Studies have demonstrated that EMT plays an essential role in invasion and metastasis in various human carcinomas, including HCC (Thiery, 2002; Tsuji et al., 2009). In EMT, epithelial cells lose epithelial properties such as decreased cell adhesive and the expression of adherens junction protein E-cadherin, and acquire mesenchymal properties such as increased cell motility and expression of mesenchymal markers including N-cadherin and vimentin (Lee et al., 2006).

Pyruvate kinase M2 (PKM2), a rate-limiting enzyme that catalyzes the process of transferring phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP, is overexpressed in tumor cells and essential for aerobic glycolysis in tumors (Tamada et al., 2012). PKM2 has been reported to be upregulated in HCC, which is correlated with the progression of malignant phenotypes in HCC cells (Tanaka et al., 2013; Fan et al., 2014; Chen et al., 2015; Dong et al., 2015). Recently, studies found PKM2 promotes EMT in various cancers including HCC (Fan et al., 2014; Hamabe et al., 2014). Signal transducer and activator of transcription 3 (STAT3), a member of the STAT family of transcription factors, plays an important role in EMT. Besides participating in aerobic glycolysis, PKM2 is localized in the nucleus and thus acts as a protein kinase to regulate gene transcription (Lee et al., 2008; Yang et al., 2012). Gao et al. found nuclear PKM2 can activate STAT3 (Gao et al., 2012). Accumulated evidences have indicated that activated STAT3 induces HCC progression by mediating EMT (Zhang et al., 2015). It has been reported that activated STAT3 (p-STAT3) translocates to the nucleus and regulates the transcription of

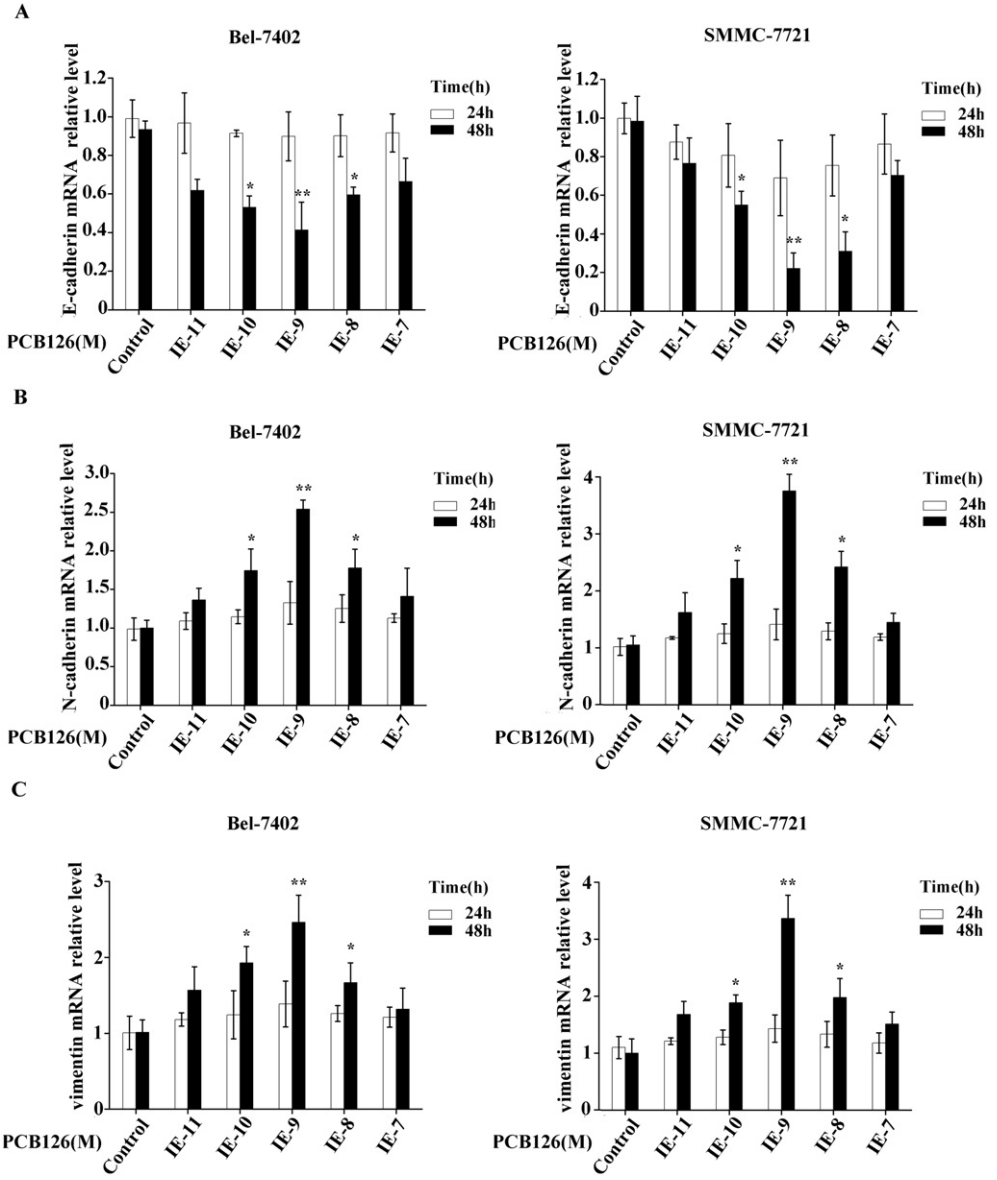

Fig. 1. Effects of PCB126 exposure on the expressions of EMT markers in both Bel-7402 and SMMC-7721 cells. The expression levels of EMT markers including E-cadherin (A), N-cadherin (B) and vimentin (C) were determined by qRT-PCR after 24 or 48 h of treatment with 10^{-11} to 10^{-7} M PCB126. Relative mRNA levels were normalized with control mRNA. The data from triplicate experiments were shown as the means ± SD. Asterisks represented significant differences from control ($^{*}p < 0.05$, $^{**}p < 0.01$).