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HBCD and PCBs Enhance the Cell Migration and Invasion of HepG2 via the PI3K/Akt Pathway

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Abstract

Exposure of hexabromocyclododecane (HBCD) and polychlorinated biphenyls (PCBs) has been proved to result in diversified toxicity including cancerigenesis. The objective of this study was to investigate the influence of HBCD and PCBs on the migration and invasion of HepG2 cells, and to explore the potential underlying mechanism. HepG2 cells were treated with different concentrations of HBCD and/or PCBs, then cell viability, apoptosis, cell migration and invasion were evaluated via cell counting kit-8 (CCK-8) assay, flow cytometry, cell scratch assay, respectively. The results showed that low concentrations of HBCD or PCBs promoted the migration and invasion of HepG2 cells, and enhanced the protein expression level of matrix metalloproteinase 9 (MMP9) and E-cadherin. Further signaling pathway analysis revealed that HBCD and PCBs exposure significantly increased the phosphrylation level of protein kinase B and extracellular signal-regulated kinase (ERK), and expression of mammalian target of rapamycin (mTOR) in the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway. The PI3K/Akt inhibitors LY294002 and MK-2206 attenuated the effects of HBCD and PCBs on the cell migration and invasion. Taken together, low concentrations of HBCD and PCBs can enhance the migration and invasion ability in HepG2 cells through activation of the PI3K/Akt signaling pathway.

***Key Words*:** HBCD, PCBs, Migration, Invasion, PI3K/Akt pathway

Introduction

Hexabromocyclododecane (HBCD) is one of the brominated flame retardants (BFRs) extensively used in polystyrene foams and building materials as thermal insulator. [1-3] As the third dominant BFRs now, HBCD could release into the environment during the processes of production, application and disposal, and enter into organisms mainly through the inhalation and digestive system. [4-6] In fact, HBCD has been detected

globally in abiotic environment and in living organisms with an increasing concentration. [7, 8] Currently, HBCD has been included in the Stockholm Convention on Persistent Organic Pollutants. [9] Polychlorinated biphenyls (PCBs) are another group of structurally related environmental persistent organic pollutants (POPs) that bring adverse influence on environment and human health. [10] Although PCBs have been banned or replaced by other substitutes in most industrialized countries, they are still persistent and widely distributed in the environment because of chemical stability and bioaccumulation capability. In recent years, various PCBs congeners have frequently been detected in human blood, milk, and adipose tissues. [11, 12]

Although the toxicological data have shown that low concentration of HBCD exerts no remarkable acute toxicity in a short-term exposure, a long-term HBCD exposure could cause hepatotoxicity, endocrine disruption, neurotoxicity, and reproductive/developmental toxicity. [2, 13, 14] Results of animal experiments indicated that the liver is one of the major target organs of HBCD exposure. [15, 16] HBCD could result in hepatomegaly, liver nodules, hepatocyte necrosis, and even formation of liver tumor. [8, 13, 14, 17] However, the potential molecular mechanisms for the toxic action of HBCD in the liver are still insufficiently understood.

Considerable studies have documented a broad spectrum of biological effects of PCBs on human health, such as immunotoxicity, neurotoxicity, and carcinogenesis. [18] The epidemiological studies and carcinogenicity tests supported that PCBs can be classified as a carcinogen. [19] Moreover, PCBs have proved to be associated with the tumor recurrence, poor prognosis, and metastatic properties. [20, 21] PCBs participate in the advancing breast cancer progression, and promote invasion and migration of breast cancer cells. [22, 23] The expression of carcinogenic biomarkers cytochrome P450 and glucose-6-phospatase-deficient neoplastic lesions could be induced by PCBs congeners in mouse liver after single or combined treatment. [24]

Our previous study have proved that low dose of HBCD and PCBs could activate the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway in human hepatoma HepG2 cells, [25] which plays an important role in malignant proliferation of tumor cells, angiogenesis, and tumor metastasis. In the present study, the HepG2 cell

line with high sensitivity to toxicants was used as the experimental model to investigate the impact of low concentrations HBCD and PCBs on the cell migration and invasion, as well as the potential molecular mechanism. The concentrations of HBCD (10^{-8} and 10^{-7} mol/L) and PCBs (10^{-8} and 10^{-7} g/mL) used in this study were comparable to the occupational exposure according to the reported documents. [26-28]

Materials and Methods

Chemicals and Reagents

The HBCD and PCBs samples were purchased from TCI (Tokyo, Japan) and Accustandard (New Haven, United States), respectively. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from GIBCO (Invitrogen Corp., Paisley, UK). Dimethyl sulfoxide (DMSO) was from Sigma (Saint Louis, MO, USA). Cell counting kit-8 (CCK-8) was from Dojindo (Kumamoto, Japan). Matrigel was purchased from BD (San Jose, CA, USA). Transwell permeable supports were purchased from Corning (Tewksbury, MA, USA). Mammalian protein extraction reagent (M-PER) and bicinchoninic acid (BCA) protein assay were purchased from Thermo Fisher Scientific Inc (Waltham, MA, USA). Nitrocellulose membrane was purchased from Millipore (Darmstadt, Germany). PI3K inhibitor (LY294002) and Akt inhibitor (MK-2206) were purchased from Selleck Chem (Houston, Texas, USA). All antibodies were purchased commercially as follows: anti-mTOR, anti-E-cadherin, anti-MMP9 (Epitomics, Burlingame, CA, USA); anti-p-Akt, anti-Akt, anti-p-ERK and anti-ERK, anti-p-JNK and anti-JNK, anti-p-P38 and anti-P38 (Cell Signaling, Beverly, MA, USA)); anti-GAPDH (Multisciences, Hangzhou, China); anti-Rabbit IgG(H+L)/HRP and anti-Mouse IgG (H+L)/HRP(Dingguo, Beijing, China). All other reagents were from Sigma (Saint Louis, MO, USA) and were analytical grade chemicals, if not stated otherwise.

Cell Culture and Treatments

As a widely used model in the toxicological studies and environmental risk assessments, human hepatoma HepG2 cells have similar enzymatic reactions and oxidative responses under chemical stimulation as the primary human hepatocytes. [29, 30] HepG2 cells were cultured in DMEM supplemented with 10% FBS, 0.33% sodium bicarbonate, and antibiotics (100 units/ml penicillin and 0.1 mg/ml streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2.

Two types of treatments were included in this study: HepG2 cells were treated with different concentrations of HBCD (0, 10^{-8}, and 10^{-7} mol/L) or PCBs (0, 10^{-8}, and 10^{-7} g/mL) dissolved in DMSO. Controls were carried out using the vehicle alone with a final concentration of DMSO 0.1% w/w. For the PI3K inhibitors treatments, cells were incubated with HBCD and PCBs, then treated with LY294002 (10 μM) for 30 min, or MK-2206 (5 μM) for 1 h. The concentration and incubation time of LY294002 and MK-2206 were chosen according to the previous studies. [25, 31, 32]

Cell Viability Assay

The cell was measured using CCK-8 assay kit according to the manufacturer's instruction. HepG2 cells were plated in 96-well plates at a density of 3,000 cells per well and cultured for 24 h to allow cell adhesion. Then cells were exposed to HBCD (0, 10^{-8}, and 10^{-7} mol/L) or PCBs (0, 10^{-8}, and 10^{-7} g/mL) for 24 h. After treatment, 10 μL of CCK-8 reagent in 90 μL DMEM per well was added to culture medium and incubated at 37°C for 1 h. The absorbance in each well was measured with a spectrophotometric plate reader (Biorad, iMark, Hercules, USA) at a wavelength of 450 nm. Five replicates were set for each cell sample and each experiment was repeated at least three times.

Flow Cytometric Assessment of Apoptosis

Apoptosis was analyzed using flow cytometry with the Annexin V-FITC apoptosis detection kit (KeyGEN BioTHCH, Nanjing, China) according to the manufacturer's

specifications. After treatment, cells were harvested with 0.25% trypsin (without EDTA) and washed twice with PBS solution. The cell pallets were then re-suspended in 500 μL binding buffer. The cell suspensions were stained with 5 μL of Annexin V-FITC and 5 μL of propidium iodide (PI) for 10 min at 37°C in the dark. The florescence was analyzed by a FACS Calibur flow cytometer (BD FACS Calibur, New Jersey, US) using the Cell Quest Software (BD, New Jersey, US). Three replicates were set for each cell sample and experiment was repeated three times.

Cell Scratch Assay

The cell scratch assay was conducted to measure the cell migration capability of HepG2 in vitro. HepG2 cells were seeded onto 6-well plates at a density of 2×10^5 cells per well and treated with various concentrations of HBCD or PCBs for 2 days. For PI3K/Akt inhibitors treatment, cells exposed to HBCD or PCBs were co-incubated with LY294002 (10 μM) for 30 min, or MK-2206 (5 μM) for 1 h. After treatment, the culture medium was discarded and a scratch was made onto each HepG2 cell-containing well using a 200 μl pipette tip. The cells were then washed twice with D-Hanks and cultured with fresh medium without serum for another 2 d. Finally, images were captured with inverted optical microscope (Olympus, Japan), and the distances of wounds were monitored at 0, 12, 24, and 48 h.

Cell Migration and Invasion Assay

The cells motility was assessed by the transwell migration/invasion assay following the standard protocol. HepG2 were pretreated with HBCD, PCBs and/or PI3K inhibitors as described above, and then seeded onto the upper insert at densities of 5×10^4 cells/24-well in serum-free DMEM. DMEM containing 10% FBS was added to the lower chamber as a chemoattractant. After culturing for 48 h, the non-invading cells were removed from the upper surface with cotton swabs, and the filters (the invading cells) were stained with the crystal violet after cold methanol fixation. The numbers of invading cells in five fields of each triplicate filter were counted using an

inverted microscope (Olympus, Japan). The cell invasion assay was carried out similarly, except that the transwell insert was precoated with 60 μL of PBS-diluted Matrigel 6 h before seeding.

Western Blotting

HepG2 cells treated with HBCD and PCBs for 2 days were collected and lysed with mammalian protein extraction reagent (M-PER). The total protein was isolated and protein concentrations were determined by BCA protein assay. Equal amount of protein samples (60 μg per lane) were separated with the sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto the nitrocellulose membrane. Then the membranes were blocked in a 5% milk containing TNT buffer (Tris-HCl, pH 7.5, 140 mM NaCl, and 0.2% Tween 20), followed by incubation with primary antibodies overnight at 4°C, and blotted with the secondary antibodies for 1 h at room temperature. Finally, blots were visualized using chemiluminescence and analyzed with the Chemi-Imager digital imaging system (Alpha Innotech, San Leandro, USA).

Statistical Analyses

All experiments were repeated three times and quantitative variables are expressed as mean ± SD. Statistical analyses were conducted using SPSS 13 (SPSS Inc., Chicago, IL, USA) SPSS (13.0) software. The differences between groups were evaluated by the post hoc Dunnett's tests based on the homogeneity of variance. A p value less than 0.05 was considered to be statistically significant.

Results

The Effect of HBCD or PCBs on HepG2 Cell Viability and Apoptosis

HepG2 cells were exposed to HBCD (10^{-8} and 10^{-7} mol/L) or PCBs (10^{-8} and 10^{-7} g/mL) for different time (24 and 48 h). CCK-8 assay and Annexin V-FITC double

staining assay were conducted to determine the cell viability and apoptosis, respectively. As shown in Figure 1, compared with the control group, low concentration of HBCD (10^{-8} and 10^{-7} mol/L) or PCBs (10^{-8} and 10^{-7} g/mL) seemed have no obvious effect on the cell viability and apoptosis (p>0.05). Actually, the number of viable cells in the HBCD groups slightly increased as compared with that of controls, although the differences were of no statistical significance.

HBCD and PCBs Enhanced the Migration and invasion of HepG2 cells

HepG2 cells were exposed to different concentrations of HBCD (0, 10^{-8}, and 10^{-7} mol/L) or PCBs (0, 10^{-8}, and 10^{-7} g/mL) for different time (12, 24, and 48 h). The *in vitro* scratch assay results showed that the percentage of gap area covered by migrated HepG2 cells increased in a time-dependent manner. HBCD and PCBs significantly promoted the migration rate of HepG2 cells. HepG2 cells treated with 10^{-8} and 10^{-7} mol/L HBCD for 48 h occupied 16.7±3.4% (p<0.01) and 20±1.8% (p<0.01) of the gap area, respectively (Figure 2A and 2C), compared to 45.6±6.4% in the control group. In the PCBs (10^{-8} and 10^{-7} g/mL) treatment, only 20.8±4.3% and 15.2±3.5% of the gap area was occupied after 48 h, respectively (Figure 2B and 2C).

The transwell migration/invasion assays were performed to further evaluate the cell migration/invasion rate of HepG2 cells exposed to HBCD and PCBs. The results of the transwell-migration system showed that both HBCD and PCBs could promote the migration obviously. In the 10^{-7} mol/L HBCD and 10^{-7} g/mL PCBs groups, the migrating cells more than doubled that of control group (Figure 3A, p<0.01). Furthermore, the transwell-invasion assay revealed that HBCD and PCBs could also significantly promote the invasive potential of HepG2 cells. The invasion rates of the HBCD and PCBs groups were 30-50% higher than that of the control group (Figure 3B, p<0.05).

Expression of Cell Migration Related Proteins in HepG2 Cells Exposed to HBCD and PCBs

HepG2 cells were exposed to HBCD (10^{-8} and 10^{-7} mol/L) and PCBs (10^{-8} and 10^{-7} g/mL) for 48 h, then cells were lysed using mammalian protein extraction reagent (M-PER) and the total protein was isolated. Western blotting results showed that expression level of E-cadherin decreased and MMP9 increased after exposed to both HBCD and PCBs (Figure 4). In the 10^{-7} mol/L HBCD group, the expression of E-cadherin reduced to 48%, and the expression of MMP9 increased more than two times. PCBs had a more significant effect, the expression of E-cadherin reduced to 45%, and MMP9 increased more than 3 folds after 10^{-7} g/mL PCBs treatment.

HBCD and PCBs Activated the PI3K/Akt Signaling Pathway

In order to investigate whether HBCD/PCBs induce the enhancement of cell migration and invasion through activation of PI3K/Akt signaling pathway, the expressions of PI3K pathway molecular were measured with Western Blotting. As shown in Figure 5, both HBCD and PCBs promoted the expression of the mTOR, p-Akt, p-ERK, and p-p38 in a dose-depended manner. In the 10^{-7} mol/L HBCD group, the expression of mTOR nearly doubled that of control group; the expression of p-Akt, p-ERK increased more than 2 times; and the expression of p-p38 increased 1.5 times. Consistently, the expression level of mTOR, p-Akt, p-ERK, and p-p38 increased about 2 times after 10^{-7} g/mL PCBs treatment.

PI3K/Akt Inhibitors Suppressed the Cell Motility of HepG2 Exposed to HBCD and PCBs

To further clarify the role of PI3K/Akt pathway in HBCD and PCBs-induced biological effects, the PI3K inhibitor (LY294002) and Akt inhibitor (MK-2206) were used to suppress the PI3K/Akt activity. Scratch assay results showed that LY294002 and MK-2206 significantly reduced the cell motility in HepG2 cells exposed to HBCD and PCBs as evidenced by increase of gap area (Figure 6A). The transwell assay results also indicated that LY294002 and MK-2206 treatment could significantly reduced migration induced by HBCD and PCBs. The migration rate in the inhibitor groups decreased 30-60% compared with the non-inhibitor groups

(Figure 6 B). The invasion rate induced by HBCD and PCBs also was inhibited by LY294002 and MK-2206 treatment (Figure 6 C), and the LY294002 groups showed higher suppressing efficiency than MK-2206 groups.

Discussion

The destruction and invasion of malignant cells into the adjacent normal tissues is the underlying pathogenesis mechanism of tumor metastasis. The tumor progression with metastasis is of great importance to the prognosis of cancer patients, since an estimated 90% of cancer deaths are caused by metastasis. [33] Hepatocellular carcinoma, the third leading cause of cancer mortality, is usually diagnosed at an advanced stage with high 5-year recurrence rates approximately 30-40% and overall limited therapeutic efficacy. [34, 35] Therefore, adjuvant therapy need to be explored based on a better understanding of the interplay between hepatocellular carcinoma cells and the microenvironment during tumor metastasis.

The cancer cell invasion and migration is usually initiated by matrix metalloproteinase (MMP) degradation of the surrounding extracellular matrix (ECM). MMP9 is one of molecular markers for tumor metastasis, participating in hydrolyzing the intercellular matrix components and the basement membrane component collagen IV during the invasion and metastasis process. [36] The cancer cell dissemination and metastatic seeding are initiated by the epithelial to mesenchymal transition (EMT) accompanying with the down regulation of the active mesenchymal marker E-cadherin. [37-39] The highly conserved transcription factor Twist can combine with E-box sequence and regulate the expression of E-cadherin. [40]

In the present study, HepG2 cells were exposed to different concentrations of HBCD (0, 10^{-8}, and 10^{-7} mol/L) or PCBs (0, 10^{-8}, and 10^{-7} g/mL) for different time (24 and 48 h). Results of cell viability and apoptosis showed that HBCD and PCBs have no obvious toxicity in HepG2 cells, and low concentration of HBCD seemed have a slight stimulating effect on the cell viability. The *in vitro* cell scratch assay revealed that both HBCD and PCBs could obviously promote the cell migration; and transwell

experiments further confirmed the effects of HBCD and PCBs to enhance the cell migration ability. Moreover, the results of transwell invasion assay displayed that HBCD and PCBs could also significantly promote the invasion ability of HepG2 cell. In brief, the "non-toxic" dose of HBCD and PCBs could promote the migration and invasion ability of hepatoma cells. Western blotting results showed that the expression level of tumor metastasis related protein MMP9 increased, and E-cadherin expression decreased after HBCD and PCBs exposure, which were consistent with the results of migration and invasion.

The PI3K/Akt pathway plays an important role in malignant proliferation of tumor cells, angiogenesis, and tumor metastasis. [41] Akt is the downstream effector molecule of PI3K, and high expression level of p-Akt indicates activation of PI3K/Akt signaling pathway. [42] Mammalian target of rapamycin (mTOR) is also a directly regulated downstream molecule of PI3K/Akt pathway, and is involved tumor angiogenesis. [43] There was close relationship between PI3K/Akt/mTOR signaling pathway and migration/invasion of tumor. [44, 45] Aksamitiene et al. found that activation of PI3K/Akt signaling pathway can increase the level of extracellular signal-regulated kinase (ERK), [46] which consequently promote tumor invasion and metastasis. [47] Our previous study had proved that HBCD (10^{-6} mol/L) and PCBs (10^{-6} g/mL) could activate the PI3K/Akt and NF-κB pathway in HepG2 cells (An et al. 2014). In the present study, we proved that 10^{-8} and 10^{-7} mol/L of HBCD and 10^{-8} and 10^{-7} g/mL of PCBs could stimulate the PI3K/Akt pathway, induce phosphorylation of Akt, and enhance the expression of mTOR and phosphorylated ERK. HBCD and PCBs could also enhance the expression of phosphorylated p-p38 (Figure 5), but have no effect on the phosphorylation of JNK (data not shown). Furthermore, treatment of PI3K/Akt inhibitors LY294002 and MK-2206 effectively countered the increase of cell migration and invasion induced by HBCD and PCBs. These results indicated that HBCD and PCBs could enhance the cell migration and invasion in HepG2 cells through modulation on the PI3K/Akt signaling pathway.

In summary, low concentration of HBCD or PCBs significantly enhanced the migration and invasion ability of HepG2 cells, accompanying with elevation of

MMP9 expression and downregulation of E-cadherin. The increased expression level of mTOR, p-ERK and p-Akt in HepG2 cells exposed to HBCD and PCBs indicated that activation of PI3K/Akt pathway might be involved in the promoting effects of HBCD and PCBs on cell migration and invasion, which was further confirmed by the PI3K/Akt inhibitors experiment. To our knowledge, this is the first study to investigate the effect of low dose HBCD on cancer progression, which should be taken into consideration during intervention and prognosis evaluation on the hepatocellular carcinoma.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Figure Legends

Figure 1. Cell viability and apoptosis in HepG2 cells exposed to HBCD or PCBs

HepG2 cells were exposed to different concentrations of HBCD (0, 10^{-8}, and 10^{-7} mol/L) or PCBs (0, 10^{-8}, and 10^{-7} g/mL) for 24 or 48 h. Cell counting kit-8 (CCK-8) assay and Annexin V-FITC double staining assay were conducted to determine the cell viability and apoptosis respectively. Five replicates were set for each cell sample and data were presented as mean ± SD from three independent experiments. (A): The cell viability of HepG2 after HBCD or PCBs treatment for 24 or 48 h. (B): The apoptosis rate analyzed using flow cytometry in HepG2 exposed to HBCD or PCBs.

Figure 2. HepG2 cell migration following treatment with HBCD and PCBs

HepG2 cells were treated with 1 ‰ DMSO or HBCD (0, 10^{-8}, and 10^{-7} mol/L) and PCBs (0, 10^{-8}, and 10^{-7} g/mL) for different time (0, 12, 24 or 48 h). The scratch assay was conducted to determine the cell migration. All images representing cell migration capabilities were taken at the same magnification and time after treatments. A: Representative images of cell scratch migration assay of the HepG2 exposed to HBCD; B: Representative images of cell scratch migration assay of the HepG2 exposed to PCBs; C: Percentage of the gap areas in the HepG2 exposed to HBCD and PCBs after 48 h compared with the baseline level. $^{**}p<0.01$ compared to control.

Figure 3. Effects of HBCD and PCBs on the migration and invasion ability of HepG2 cells

HepG2 cells were exposed to HBCD (10^{-8} and 10^{-7} mol/L) and PCBs (10^{-8} and 10^{-7} g/mL) for 48 h. The transwell migration/invasion assays were conducted to determine the cell migratory ability. Cell migration (A) and cell invasion (B) of HepG2 after

treatment with indicated concentrations of HBCD and PCBs for 48 h. Columns represent the mean of three individual experiments performed in triplicate; error bars represent SD. * p<0.05, ** p<0.01 vs control group (1 ‰ DMSO group).

Figure 4. Expression of E-cadherin and MMP9 in HepG2 Cells Exposed to HBCD or PCBs

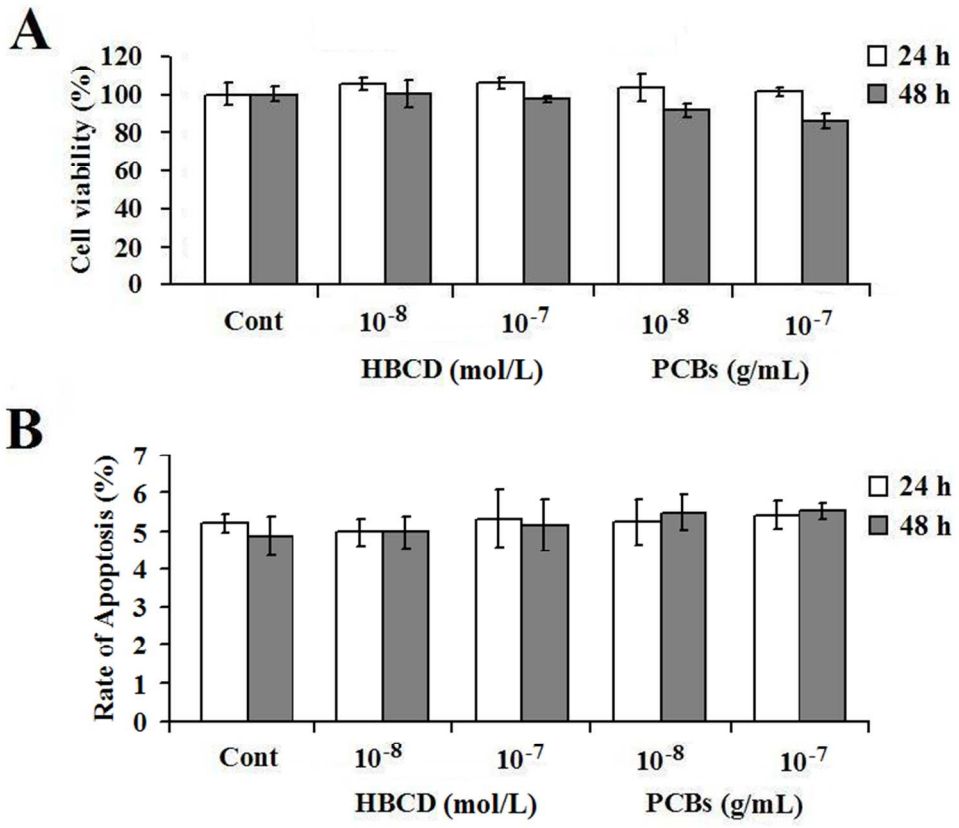
HepG2 cells were exposed to HBCD (10^{-8} and 10^{-7} mol/L) or PCBs (10^{-8} and 10^{-7} g/mL) for 48 h. Western Blotting were performed to evaluate the expression of E-cadherin and MMP9. GAPDH was used as the internal reference. Columns represent the mean of quantization results for blot band density from three independent experiments performed in triplicate; error bars represent SD. * p<0.05, ** p<0.01 vs control group (1 ‰ DMSO group).

Figure 5. Expression of mTOR, p-ERK and p-Akt in HepG2 Cells Exposed to HBCD or PCBs

HepG2 cells were exposed to HBCD (10^{-8} and 10^{-7} mol/L) or PCBs (10^{-8} and 10^{-7} g/mL) for 48 h. Western Blotting were performed to evaluate the expression of mTOR, p-Akt, p-ERK and p-p38. GAPDH was used as the internal reference. Columns represent the mean of quantization results for the blot band density from three independent experiments performed in triplicate; error bars represent SD. * p<0.05, ** p<0.01 vs control group (1 ‰ DMSO group).

Figure 6. The PI3K/Akt inhibitors reduced the cell motility in HepG2 cells exposed to HBCD and PCBs

A: HepG2 cells were treated with HBCD (10^{-7} mol/L) and PCBs (10^{-7} g/mL) for 48 h. The scratch assay was conducted to determine the cell migration. B and C: HepG2 cells were exposed to HBCD (10^{-8} and 10^{-7} mol/L) and PCBs (10^{-8} and 10^{-7} g/mL) for 48 h. Transwell migration (B) and invasion (C) assay was conducted to determine the cell migration and invasion. For the treatments with inhibitors, cells were incubated with HBCD and PCBs, then treated with LY294002 (10 μM) for 30 min, or MK-2206 (5 μM) for 1 h. * p<0.05, ** p<0.01 vs non-inhibitor groups.



Cell viability and apoptosis in HepG2 cells exposed to HBCD or PCBs
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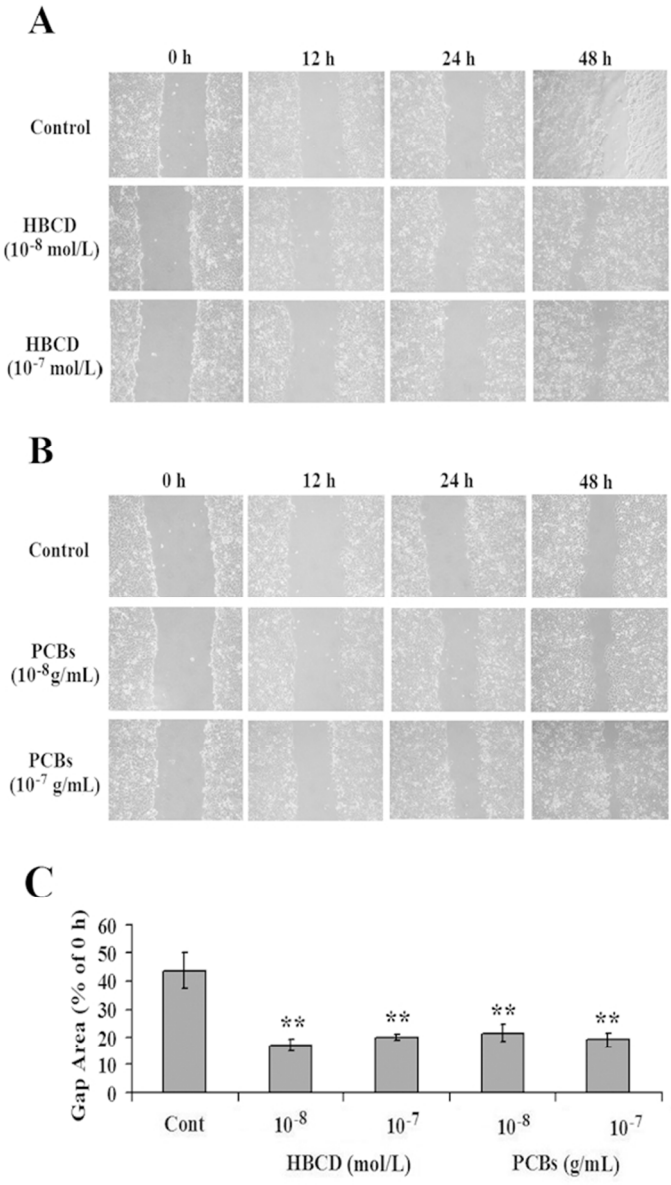

HepG2 cell migration following treatment with HBCD and PCBs
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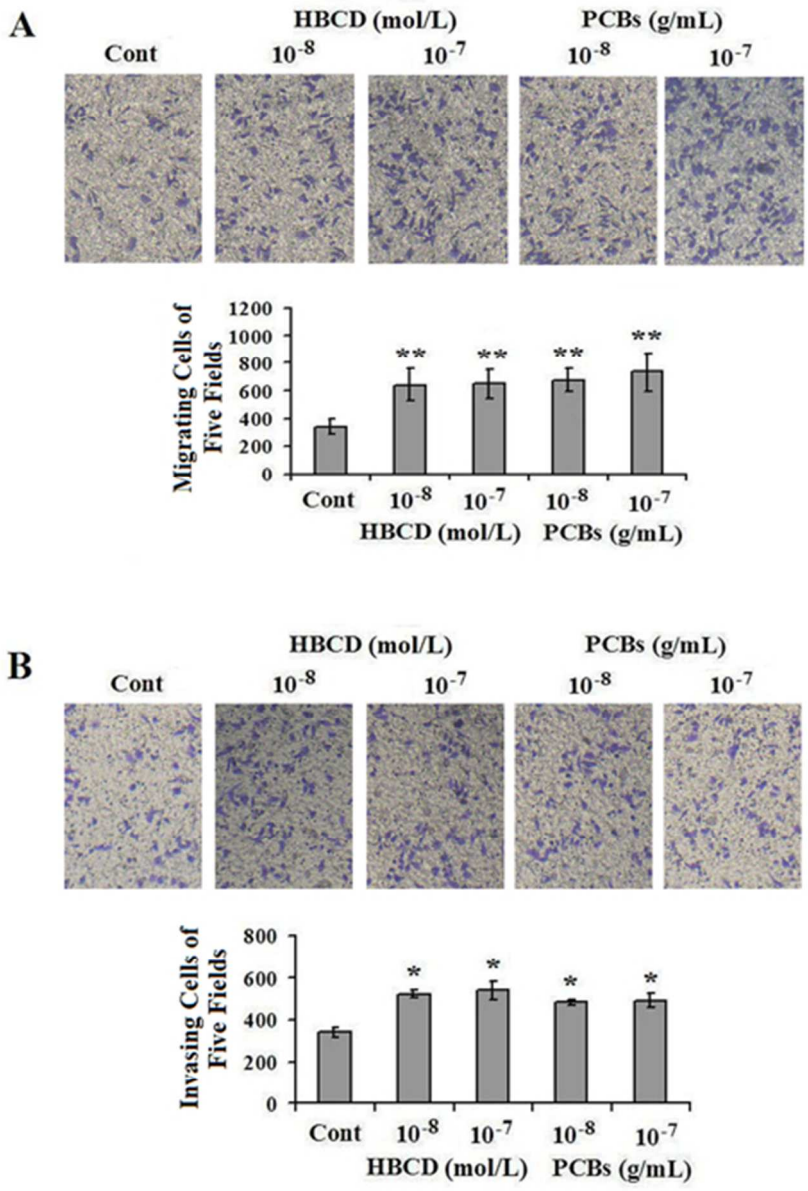

Effects of HBCD and PCBs on the migration and invasion ability of HepG2 cells
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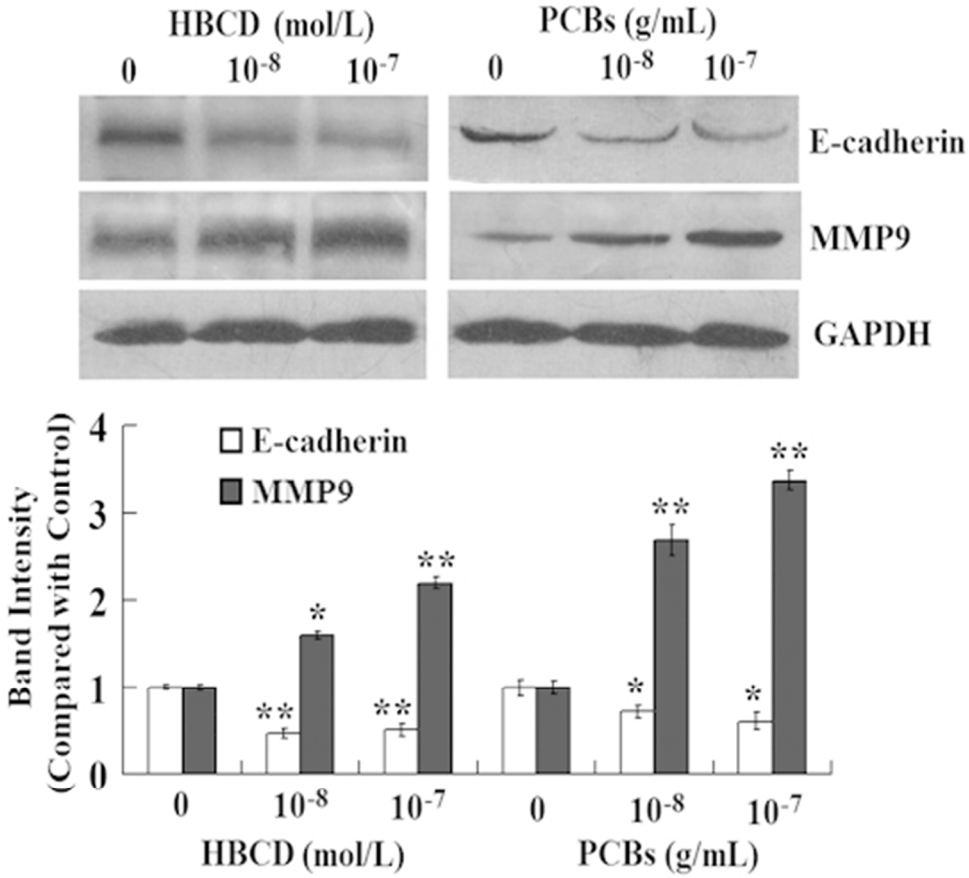

Expression of E-cadherin and MMP9 in HepG2 Cells Exposed to HBCD or PCBs
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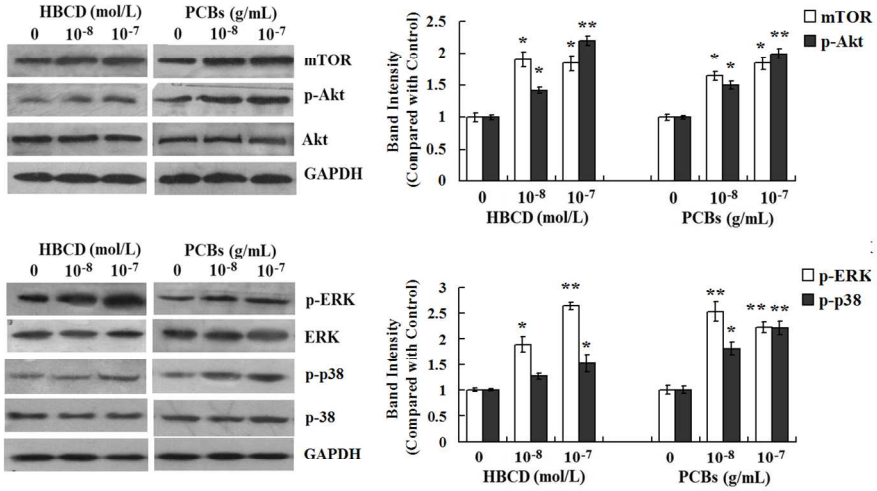

Figure 5
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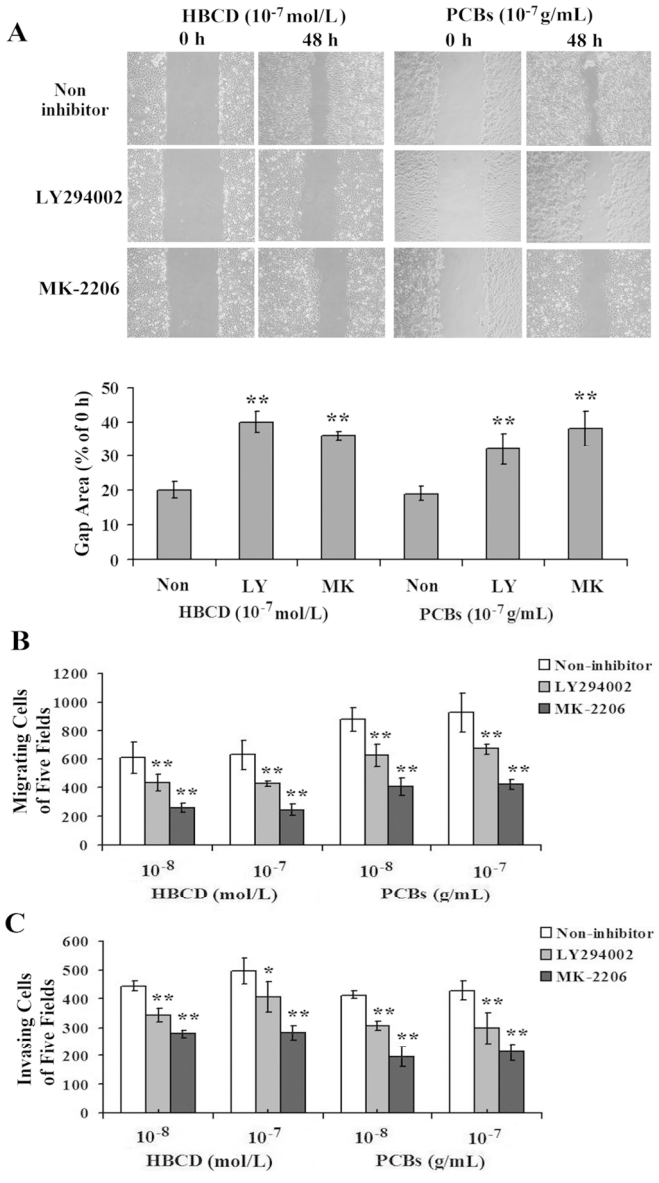

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