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Hexachlorophene Inhibits Wnt/β-Catenin Pathway by Promoting Siah-Mediated β-Catenin Degradation

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ABSTRACT

Aberrant activation of Wnt/β-catenin signaling and subsequent up-regulation of β-catenin response transcription (CRT) is a critical event in the development of human colon cancer. Thus, Wnt/β-catenin signaling is an attractive target for the development of anticancer therapeutics. In this study, we identified hexachlorophene as an inhibitor of Wnt/β-catenin signaling from cell-based small-molecule screening. Hexachlorophene antagonized CRT that was stimulated by Wnt3a-conditioned medium by promoting the degradation of β-catenin. This degradation pathway is Siah-1 and adenomatous polyposis coli-dependent, but glycogen synthase kinase-3β and F-box β-transducin repeat-containing protein-independent. In addition, hexachlorophene represses the expression of cyclin D1, which is a known β-catenin target gene, and inhibits the growth of colon cancer cells. Our findings suggest that hexachlorophene attenuates Wnt/β-catenin signaling through the Siah-1-mediated β-catenin degradation.

Wnts are secreted glycoproteins that play important roles in cell proliferation, differentiation, and oncogenesis (Peifer and Polakis, 2000; Huelsken and Birchmeier, 2001). Central to this pathway is the level of cytosolic β-catenin, which regulates its target genes. The level of β-catenin is regulated by two adenomatous polyposis coli (APC)-dependent proteasomal degradation pathways, a glycogen synthase kinase-3β (GSK-3β)-dependent pathway (Polakis, 2002), and an Siah-1-dependent pathway (Liu et al., 2001). In the GSK-3β-dependent pathway, β-catenin is phosphorylated by a multiprotein complex composed of APC, Axin, and GSK-3β (Hart et al., 1998; Ikeda et al., 1998), leading to the degradation of β-catenin through a ubiquitin-dependent mechanism (Aberle et al., 1997). In the Siah-1-dependent pathway, Siah-1 interacts with the carboxyl terminus of APC, recruits the ubiquitination complex, and promotes the degradation of β-catenin through a pathway independent of both GSK-3β and β-TrCP, an F-box protein in the E3 ubiquitin ligase complex (Liu et al., 2001).

An abnormal regulation of Wnt/β-catenin signaling and the subsequent up-regulation of β-catenin response transcription (CRT) are frequent early events during the development of certain cancers (Giles et al., 2003). Mutations of the APC gene occur in the majority of sporadic colorectal cancers and in familial adenomatous polyposis (Fearnhead et al., 2001). In addition, mutations in the β-catenin gene have been observed in colorectal cancer and melanoma (Morin, 1999). Common sites of β-catenin mutations are the phosphorylation motifs in the N-terminal domain. These mutations lead to the excessive accumulation of β-catenin in the nucleus and to the stimulation of β-catenin target genes, including cyclin D1, myc, matrix metalloproteinase-7, and peroxisome proliferators-activated receptor-δ, which play important roles in colorectal tumorigenesis (He et al., 1998, 1999; Tetsu and McCormick, 1999; Takahashi et al., 2002). Moreover, both Axin and β-catenin are mutated in a certain subset of hepatocellular carcinomas (Miyoshi et al., 1998). The accumulation of β-catenin is also observed in other types

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ABBREVIATIONS: CRT, β-catenin response transcription; APC, adenomatous polyposis coli; GSK-3β, glycogen synthase kinase-3β; Wnt3a CM, Wnt3a conditioned medium; β-TrCP, β-transducin repeat-containing protein; HEK, human embryonic kidney; hFz-1, human Frizzled-1; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; DMSO, dimethyl sulfoxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MG-132, *N*-benzoyloxycarbonyl (*Z*)-Leu-Leu-leucinal.

of cancer, such as ovarian cancer, endometrial cancer, medulloblastoma, pilomatricoma, and prostate cancer (Fearnhead et al., 2001; Karim et al., 2004). Thus, the constitutive activation of CRT is a potential target for chemoprevention and treatment of various cancers.

Hexachlorophene is an antimicrobial compound used in disinfectants and surgical scrubs (Jungermann, 1968). Hexachlorophene inhibits the activity of enoyl-acyl carrier protein reductase, which is the last enzyme in fatty acid elongation cycle and target for the antibacterial drugs (Heath et al., 2000). Hexachlorophene has been identified as an inhibitor of 3CL protease of SARS-CoV (Hsu et al., 2004). In the present study, we identified hexachlorophene as an inhibitor of Wnt/β-catenin signaling using cell-based small-molecule screening. Hexachlorophene may suppress CRT through Siah-1-mediated β-catenin degradation in colon cancer cells.

Materials and Methods

Cell Culture, Transfection, and Luciferase Assay. HEK293, HEK293T, HCT116, LS174T, SW480, DLD-1, WI38, and Wnt3a-secreting L cells were obtained from the American Type Culture Collection (Manassas, VA) and were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 120 g/ml penicillin, and 200 g/ml streptomycin. For Wnt3a-conditioned medium (Wnt3a CM), Wnt3a-secreting L cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum for 4 d. The medium was harvested and sterilized using a 0.22-μm filter. Fresh medium was added, the cells were cultured for another 3 d, and the medium was collected and combined with the previous medium. Transfection was performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The luciferase assay was performed using a Dual Luciferase Assay kit (Promega, Madison, WI).

Plasmid Constructs. Human Frizzled-1 (hFz-1) cDNA was cloned as described previously (Cho et al., 2005). The cyclin D1 promoter region was amplified by PCR and then inserted into a pRL-null reporter plasmid (Promega). The pTOPFlash and pFOPFlash reporter plasmids were obtained from Upstate Biotechnology (Lake Placid, NY). The ΔSiah-1 expression plasmid was kindly provided by Y. Yang (National Institutes of Health, Bethesda, MD), and the dominant-negative β-TrCP expression plasmid was a gift from M. Davis (Hebrew University-Hadassah Medical School, Jerusalem, Israel). pCMV-RL plasmid was purchased from Promega.

Screening for a Small-Molecule Inhibitor of Wnt/β-Catenin Signaling. The HEK293 reporter cell line was established by selecting HEK293 cells cotransfected with the plasmid expressing hFz-1 and TOPFlash using media containing G418 (1 mg/ml). The cells were inoculated into 96-well plates at 15,000 cells/well in duplicate and grown for 24 h. Wnt3a CM was added, and then the Genesis Plus Collection (MicroSource Discovery Inc., Gaylordsville, CT) chemicals were added to the wells at a final concentration of 10 μM. After 15 h, the plates were assayed for firefly luciferase activity and cell viability.

Western Blotting. The cytosolic and nuclear fractions were prepared as described previously (Dignam et al., 1983). Proteins were separated by SDS-polyacrylamide gel electrophoresis in a 4 to 12% gradient gel (Invitrogen) and transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). The membranes were blocked with 5% nonfat milk and probed with anti-β-catenin (BD Transduction Laboratories, Lexington, KY), anti-p53 (Oncogene Biotechnology, San Diego, CA), anti-cyclin D1 (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-actin antibodies (Cell Signaling Technology, Beverly, MA). The membranes were then incubated with horseradish-peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (Santa Cruz Biotechnology) and visualized using the ECL system (Santa Cruz Biotechnology).

RNA Extraction and Semiquantitative RT-PCR. Total RNA was isolated with TRIzol reagent (Invitrogen) in accordance with the manufacturer's instructions. cDNA synthesis, reverse transcription, and PCR were performed as described previously (Topol et al., 2003). The amplified DNA was separated on 2% agarose gels and stained with ethidium bromide.

Real-Time PCR. Gene expression was determined by real-time quantitative PCR using an iCycler iQ (Bio-Rad). Reactions were carried out in 96-well optical reaction plates in a 20 μl final volume containing 10 μl of the 2X SYBR Green Supermix [100 mM KCl, 40 mM Tris-HCl, pH 8.4, 0.4 mM concentrations of each dNTP (dATP, dCTP, dGTP, and dTTP), iTaq DNA polymerase, 50 U/ml, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein, and stabilizers), 1 μl of each gene-specific primer, 2 μl of diluted sample cDNA, and 6 μl of water. After an initial denaturing step for 10 min at 95°C, conditions for cycling were 40 cycles of 30 s at 94°C, 30 s at 56°C, 30 s at 72°C. The primers used for a real-time PCR of Siah-1 were the same as

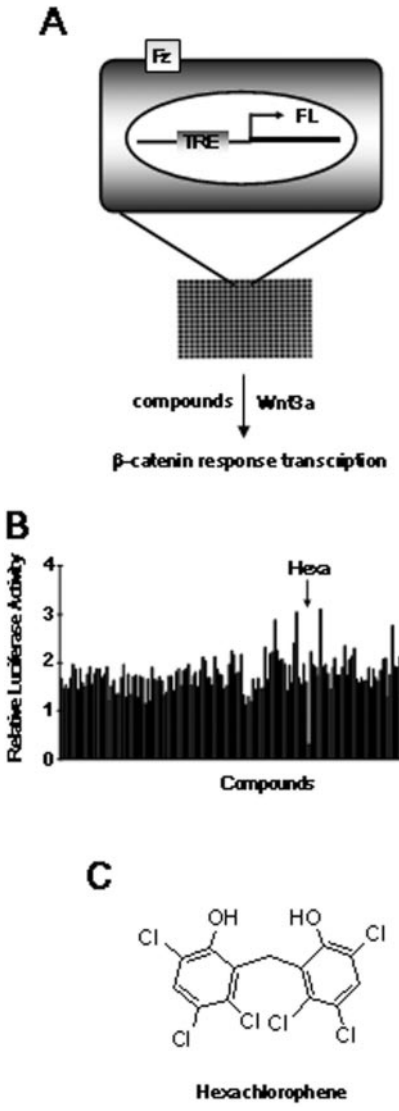

Fig. 1. Identification of hexachlorophene as a small-molecule inhibitor of Wnt/β-catenin signaling. A, a schematic of the screening system. B, screening of compounds that inhibit Wnt/β-catenin signaling. Compounds modulating TOPFlash reporter activity were screened using the HEK293 reporter cells. The controls were assayed in the presence or absence of Wnt3a CM. TOPFlash activities were normalized with Cell-titer-Glo (Promega) activity. C, chemical structure of hexachlorophene.

those used in the RT-PCR analysis. For quantification of the changes in gene expression, we used the comparative C_t method to calculate the relative fold changes normalized against the β-actin.

Cell Viability Assay. Cells were inoculated into 24-well plates and treated with hexachlorophene for 48 h. The cells from each treated sample were counted in triplicate using Trypan blue exclusion and a hemocytometer.

Results

Identification of an Inhibitor of Wnt/β-Catenin Signaling. To screen for small-molecule inhibitors of Wnt/β-catenin signaling, HEK293 cells were stably transfected with a TOPFlash reporter and the hFz-1 expression plasmid to make reporter cells (Fig. 1A). When HEK293 reporter cells were incubated with Wnt3a CM, TOPFlash reporter activity dramatically increased (data not shown). Using this system, we screened a small-molecule library containing 960 bioactive compounds (Fig. 1B). One of the compounds identified from this screen was hexachlorophene (Fig. 1C). As shown in Fig. 2A, treatment with hexachlorophene resulted in a concentration-dependent decrease of the CRT that had been induced by Wnt3a CM (IC_{50} = 7.03 μM). In contrast, hexachlorophene and Wnt3a CM did not affect FOPFlash activity in HEK293 control cells, which stably harbored a FOPFlash reporter and hFz-1 expression plasmids (Fig. 2A). In Wnt/β-catenin pathway, CRT is largely dependent on the level of β-catenin, which regulates its target genes. To investigate the effect of hexachlorophene on the intracellular β-catenin level, we performed Western blotting with anti-β-catenin antibody to analyze the amount of β-catenin in response to hexachlorophene. Consistent with a previous report (Shibamoto et al., 1998), the β-catenin level in cytosolic and nuclear fractions was increased by incubation with Wnt3a

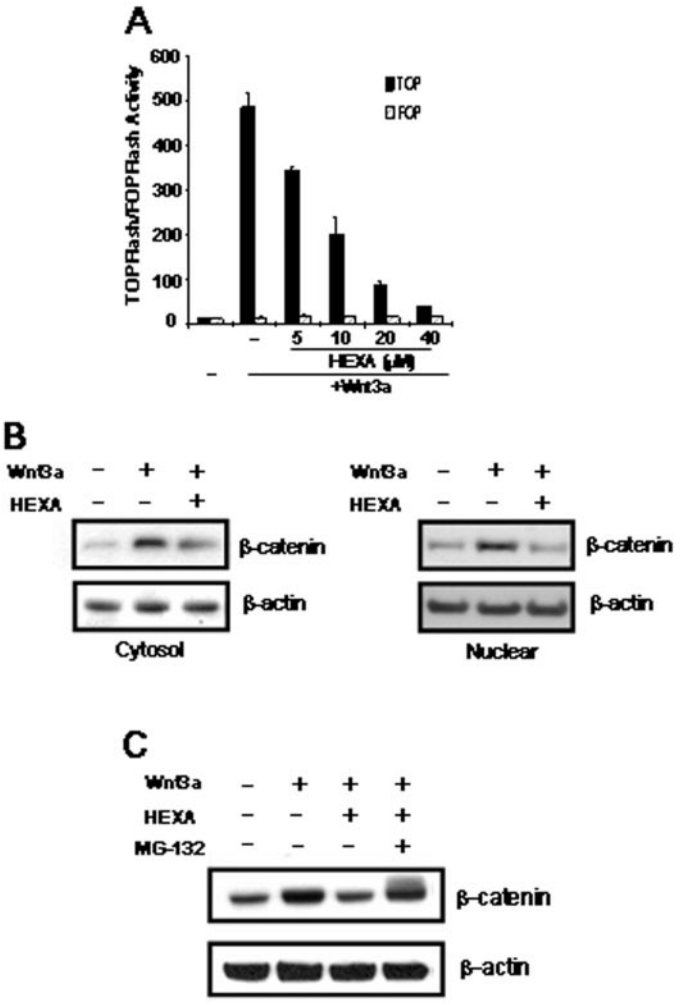

Fig. 2. Hexachlorophene promotes the degradation of β-catenin via a proteasome. A, HEK293 reporter cells were incubated with increasing concentrations of hexachlorophene in the presence of Wnt3a CM. After 15 h, luciferase activity was determined. The results are the average of three experiments, and the bars indicate standard deviations. B, cytosolic and nuclear proteins were prepared from HEK293 reporter cells treated with either vehicle (DMSO) or hexachlorophene (20 μM) in the presence of Wnt3a CM for 15 h and then subjected to Western blotting with β-catenin antibody. C, after incubation of the vehicle (DMSO), hexachlorophene (20 μM), or MG-132 (10 μM) with HEK293 reporter cells, cytosolic fractions were prepared for Western blotting with β-catenin antibody. In B and C, to confirm equal loading, the blot was reprobed with anti-actin antibody.

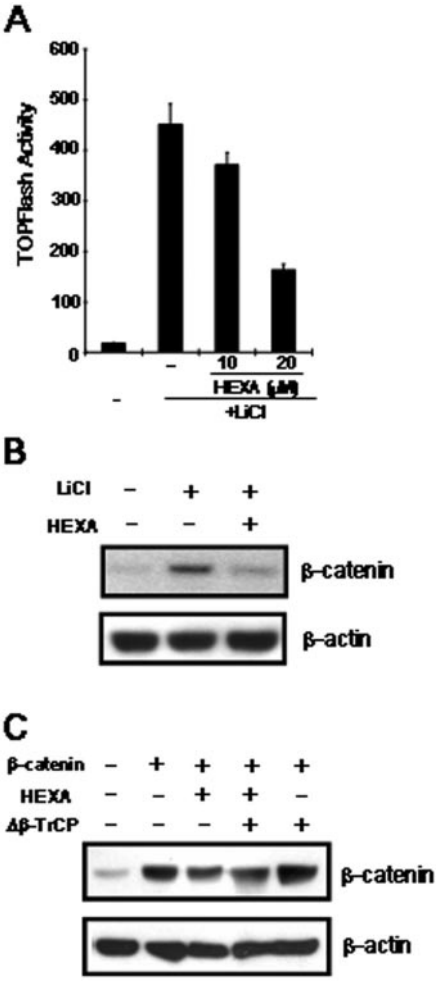

Fig. 3. Hexachlorophene induces β-catenin degradation through a mechanism independent of GSK-3β and β-TrCP. A, HEK293 reporter cells were incubated with hexachlorophene in the presence of 20 mM LiCl. After 15 h, luciferase activity was determined. The results are the average of three experiments, and the bars indicate standard deviations. B, cytosolic proteins were prepared from HEK293 reporter cells treated with either vehicle (DMSO) or hexachlorophene (20 μM) in the presence of 20 mM LiCl for 15 h and then subjected to Western blotting with β-catenin antibody. C, HEK293 cells were cotransfected with the indicated plasmids and then incubated with either the vehicle (DMSO) or hexachlorophene (20 μM) for 15 h. Cytosolic proteins were subjected to Western blotting with β-catenin antibody. In B and C, the blots were reprobed with anti-actin antibody as a loading control.

CM (Fig. 2B). It is interesting that treatment with hexachlorophene led to a down-regulation of the β-catenin level in both fractions (Fig. 2B). It has been reported that the intracellular β-catenin level is regulated by ubiquitin-dependent proteolysis (Aberle et al., 1997). To examine whether the down-regulation of β-catenin by hexachlorophene is mediated by a proteasome, we used MG-132 to block proteasome-mediated protein degradation. Treatment with hexachlorophene consistently induced a decrease of the β-catenin level in HEK293 reporter cells (Fig. 2C). However, the effect of hexachlorophene on the reduction of β-catenin was abrogated by the addition of MG-132 (Fig. 2C), indicating that hexachlorophene induces the degradation of β-catenin in a proteasome-dependent manner. Taken together, these results indicate that hexachlorophene inhibits Wnt/β-catenin signaling via a degradation of the intracellular β-catenin.

Hexachlorophene Down-Regulates the β-Catenin Level through a Mechanism Independent of GSK-3β and β-TrCP. As the phosphorylation of β-catenin by GSK-3β, followed by association with β-TrCP, leads to β-catenin degradation, we examined whether hexachlorophene-mediated CRT inhibition requires GSK-3β and β-TrCP. To this end, HEK293 reporter cells were incubated with hexachlorophene and LiCl, an inhibitor of GSK-3β. This resulted in an accumulation of endogenous β-catenin and the activation of CRT. As shown in Fig. 3A, hexachlorophene inhibited LiCl-induced CRT. Western blot analysis using β-catenin antibody consistently showed that hexachlorophene decreased the level of β-catenin that was accumulated with LiCl (Fig. 3B). Moreover, in the presence of the dominant-negative β-TrCP, which has been observed to interact with phosphorylated β-catenin but is unable to form an $SCF^{\beta\text{-TrCP}}$ ubiquitin ligase complex (Hart et al., 1999), hexachlorophene reduced the β-catenin level (Fig. 3C). These results suggest that CRT inhibition by hexachlorophene is independent of GSK-3β and β-TrCP.

The Effect of Hexachlorophene in Colon Cancer Cells. We tested the effect of hexachlorophene on colon cancer cells because mutations that lead to the accumulation of β-catenin frequently occur in colon cancer. HCT116 colon cancer cells, which contain wild-type APC and Ser45 deletion mutation in β-catenin (Ilyas et al., 1997), were transfected with TOPFlash and then incubated with hexachlorophene. As shown in Fig. 4, A and B, treatment with hexachlorophene resulted in a decrease of CRT and down-regulation of β-catenin level. In contrast, in SW480 colon cancer cells, which have a wild-type β-catenin and truncated form of APC (Ilyas et al., 1997), incubation with hexachlorophene did not lead to an inhibition of CRT or a decrease of β-catenin level (Fig. 4, A and B). We also found similar effect of hexachlorophene on LS174T and DLD-1, which contain mutant β-catenin and mutant APC, respectively (Ilyas et al., 1997) (Fig. 4B). These results indicate that wild-type APC is necessary for hexachlorophene-mediated down-regulation of β-catenin.

Hexachlorophene Represses the Expression of β-Catenin-Dependent Genes and Inhibits the Proliferation of Colon Cancer Cells. The cyclin D1 gene is regulated by β-catenin and plays an important role in many tumors (Utsunomiya et al., 2001). To determine the effect of hexachlorophene on the expression of the β-catenin-dependent gene, a reporter construct containing 360 base pairs of the cyclin D1 promoter region was transfected into HCT116 cells, which were subsequently treated with increasing amounts of hexachlorophene. As shown in Fig. 5A, hexachlorophene suppressed cyclin D1 promoter activity. In conjunction with this experiment, we also analyzed the level of endogenous mRNA and protein expressions of cyclin D1 in hexachlorophene-treated cells. Consistent with the reporter assay, hexachlorophene decreased the levels of cyclin D1 mRNA and protein in a concentration-dependent manner (Fig. 5, B and C). Moreover, the expression of Myc, which is also known to be a direct target of Wnt/β-catenin pathway,

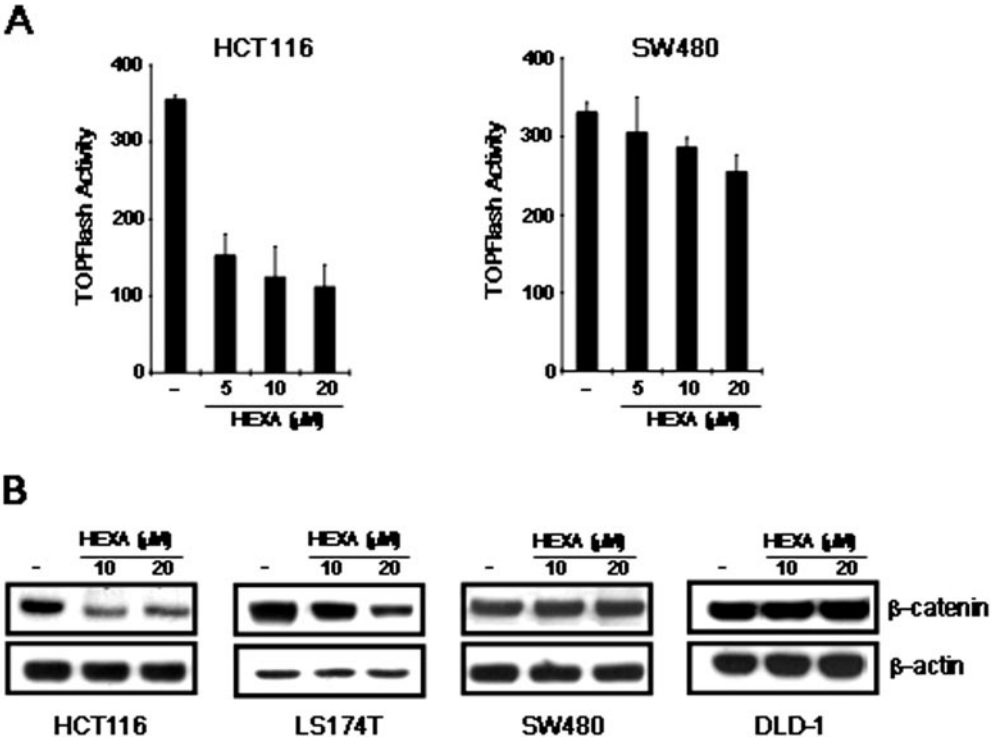

Fig. 4. The effect of hexachlorophene on colon cancer cells. A, HCT116 and SW480 cells were cotransfected with TOPFlash and pCMV-RL plasmids and incubated with hexachlorophene for 15 h. Luciferase activities were measured 39 h after transfection. Results are the average of three experiments, and the bars indicate standard deviations. B, cytosolic proteins were prepared from HCT116, LS174T, SW480, and DLD-1 cells treated with the vehicle (DMSO) or hexachlorophene for 15 h and then subjected to Western blotting with β-catenin antibody. To confirm equal loading, the blots were reprobed with anti-actin antibody.

reduced upon treatment with hexachlorophene (Fig. 5D). Several studies have reported that the disruption of β-catenin function specifically reduced the cell growth of human colon cancer cells (Roh et al., 2001; Verma et al., 2003). Given that hexachlorophene promotes the degradation of β-catenin, we hypothesized that hexachlorophene also reduces the growth of cancer cells containing a mutation in a component of Wnt/β-catenin signaling. To explore this hypothesis, we examined the effect of hexachlorophene on the growth of HCT116 colon cancer cells. The cells were incubated with various concentrations of hexachlorophene, and cell growth was determined. As shown in Fig. 5E, hexachlorophene efficiently inhibited the growth of HCT116 cells in a concentration-dependent manner. In contrast, the growth of WI38 cells, which are nontransformed cells, was less affected by hexachlorophene (Fig. 5E).

Involvement of Siah-1 in β-Catenin Degradation by Hexachlorophene. Previous studies have demonstrated that Siah induces GSK-3β-independent and β-TrCP-independent β-catenin degradation (Liu et al., 2001). Our results also suggested that hexachlorophene promotes β-catenin degradation through a GSK-3β-independent and β-TrCP-independent mechanism. Thus, we tested whether Siah activity was required for hexachlorophene-induced β-catenin degradation. HEK293 and HCT116 cells were treated with increasing amounts of hexachlorophene, and Siah-1 mRNA levels were examined by quantitative reverse transcriptase-PCR. As shown in Fig. 6A, treatment with hexachlorophene led to an increase of Siah-1 mRNA in both cells. We also confirmed hexachlorophene-mediated Siah-1 up-regulation with real-time quantitative reverse transcriptase-PCR (Fig. 6B). In addition, dominant-negative Siah-1 (ΔSiah-1) suppressed the degradation of β-catenin induced by the hexachlorophene

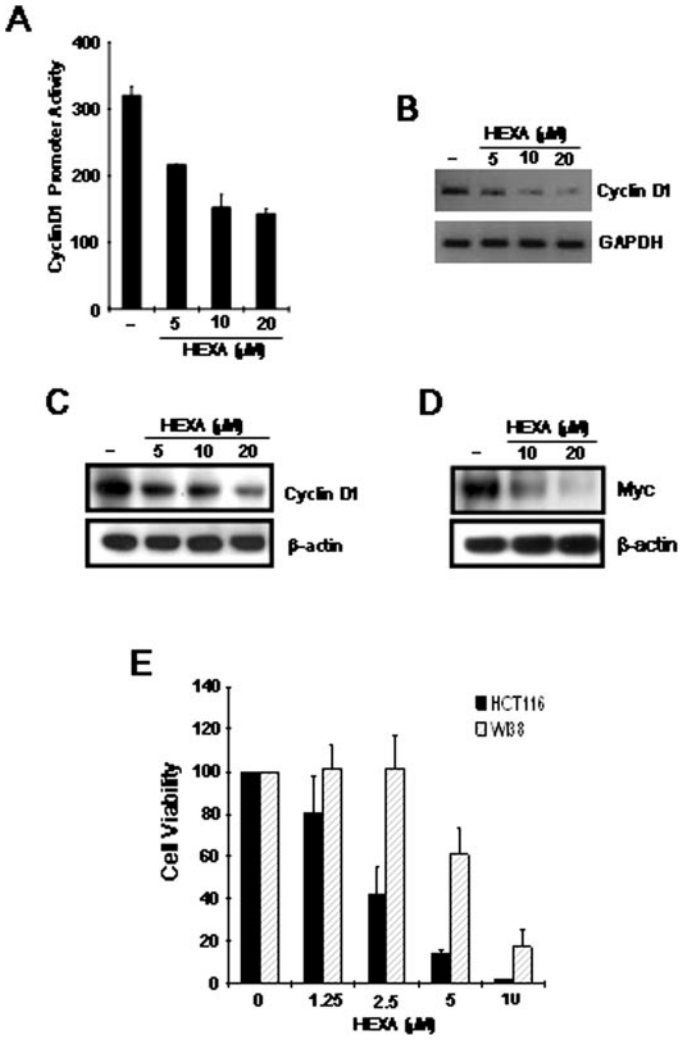

Fig. 5. Hexachlorophene inhibits the expression of the TCF/β-catenin target gene. A, HCT116 cells were cotransfected with cyclin D1-RL and pSV40-FL, and then incubated with increasing of hexachlorophene for 15 h. Luciferase activities were measured 39 h after transfection. The results are the average of three experiments, and the bars indicate standard deviations. B, semiquantitative RT-PCR for cyclin D1 and GAPDH was performed with total RNA prepared from HCT116 treated with the vehicle (DMSO) or hexachlorophene for 15 h. C and D, HCT116 cells were incubated with the vehicle (DMSO) or hexachlorophene for 15 h. Whole-cell extracts was prepared for Western blotting with anti-cyclin D1 and anti-myc antibodies. To confirm equal loading, the blots were reprobed with anti-actin antibody. E, cells were incubated with the increasing amounts of hexachlorophene for 48 h in 24-well plates, and cell viability was determined as described under *Materials and Methods*. The results shown are the average of three experiments, and the bars indicate standard deviations.

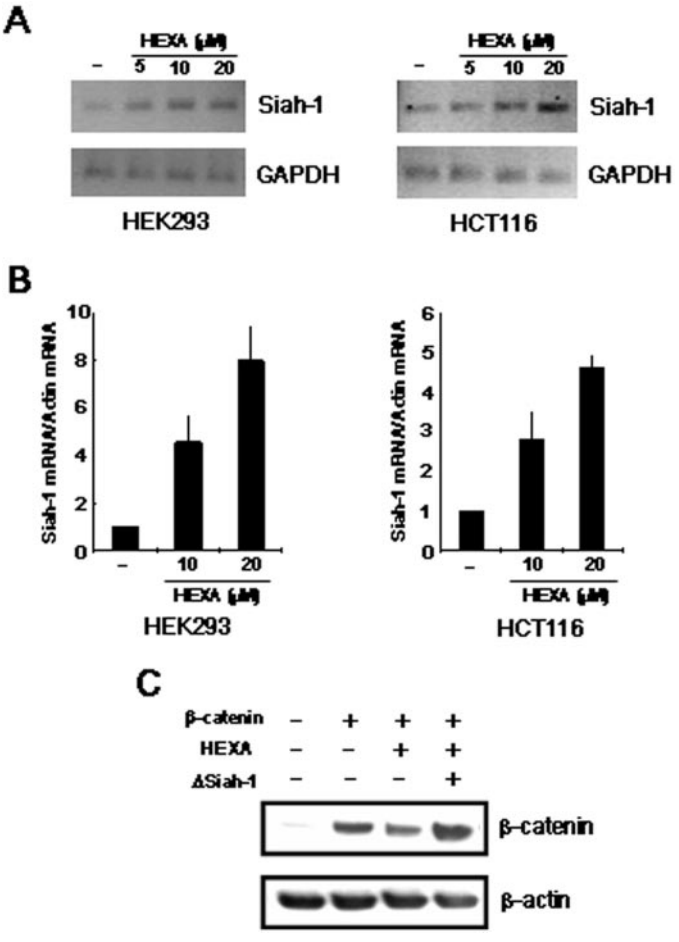

Fig. 6. Involvement of Siah-1 in hexachlorophene-induced β-catenin degradation. Hexachlorophene induces Siah-1 expression. A, semiquantitative RT-PCR for Siah-1 and GAPDH was performed with total RNA prepared from HEK293 and HCT116 cells treated with the vehicle (DMSO) or hexachlorophene for 15 h. B, real-time quantitative RT-PCR for Siah-1. C, dominant-negative Siah-1 inhibits hexachlorophene-induced β-catenin degradation. HEK293 cells were cotransfected with the indicated plasmids and then incubated with either the vehicle (DMSO) or hexachlorophene (20 μM) for 15 h. Cytosolic proteins were subjected to Western blotting with β-catenin antibody. To confirm equal loading, the blot was re-probed with anti-actin antibody.

treatment (Fig. 6C). These results suggest that hexachlorophene promotes β-catenin degradation through an Siah-1-dependent mechanism.

Hexachlorophene Does Not Induce the Accumulation of p53. It has been suggested that the expression of Siah-1 is induced by tumor suppressor p53, resulting in the induction of apoptosis and cell-cycle arrest in mammalian cells (Liu et al., 2001). In addition, some genotoxic reagents, such as doxorubicin (Adriamycin; Pfizer, New York, NY), suppress CRT through the p53-inducible pathway involving Siah-1 (Liu et al., 2001). Therefore, we investigated whether hexachlorophene activates p53-dependent transcription in HCT116 cells, which contain wild-type p53. As shown in Fig. 7A, Adriamycin up-regulated p53-dependent transcription. In contrast, hexachlorophene did not induce p53-dependent transcription. We also examined the p53 protein level in hexachlorophene-treated cells. Western blot analysis showed that hexachlorophene did not increase p53 protein level (Fig. 7B). Moreover, in HEK293T cells, which lack functional p53 via an interaction between adenovirus E1B protein and p53 (Shirata et al., 2005), hexachlorophene induced the degradation of β-catenin (Fig. 7C). These results suggest that hexachlorophene induces the expression of Siah-1 independently of the p53 pathway.

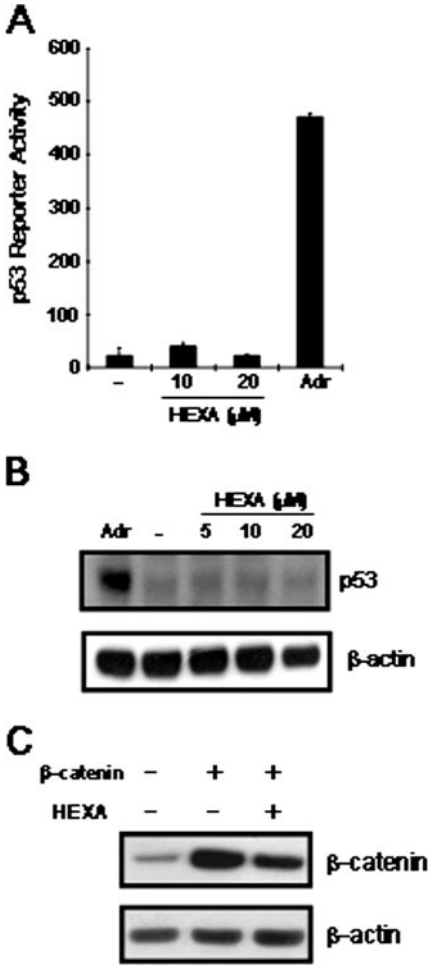

Fig. 7. Hexachlorophene does not induce the accumulation of p53 protein. A, HCT116 cells were cotransfected with p53-reporter plasmid and pCMV-RL and then incubated with increasing amounts of hexachlorophene or Adriamycin (1 μM) for 15 h. Luciferase activities were measured 39 h after transfection. The results are the average of three experiments, and the bars indicate standard deviations. B, HCT116 cells were treated with different amounts of hexachlorophene or Adriamycin (1 μM) for 15 h. Whole-cell extracts were prepared for Western blotting with anti-p53 antibody. C, HEK293T cells were transfected with β-catenin expression plasmid and then incubated with either the vehicle (DMSO) or hexachlorophene (20 μM) for 15 h. Cytosolic proteins were subjected to Western blotting with β-catenin antibody. In B and C, the blots were reprobed with anti-actin antibody.

Discussion

A number of studies have demonstrated that mutations in components of Wnt/β-catenin signaling are associated with the development of several cancers, including colon cancer and melanoma (Fearnhead et al., 2001; Karim et al., 2004). In this report, we used a small-molecule-based chemical screening to identify hexachlorophene as an inhibitor of Wnt/β-catenin signaling, and we revealed its possible mechanism of action. Hexachlorophene inhibited CRT through the promotion of β-catenin degradation. There are two pathways to regulate intracellular β-catenin level, GSK-3β/β-TrCP and Siah/APC pathways (Liu et al., 2001; Polakis, 2002). Several lines of evidence in this study indicated that hexachlorophene induced the degradation of β-catenin through the Siah/APC pathway rather than the GSK-3β/β-TrCP pathway. First, in the presence of LiCl, which is an inhibitor of GSK-3β, hexachlorophene was still able to decrease the β-catenin level. Second, overexpression of dominant-negative β-TrCP failed to block the hexachlorophene-mediated β-catenin degradation. Third, hexachlorophene decreased the level of β-catenin in HCT116 and LS174T, which express wild-type APC, but not in SW480 and DLD-1, which express truncated APC. Fourth, hexachlorophene induced the expression of Siah-1 in HCT116 and HEK293, leading to the decrease of β-catenin. Fifth, overexpression of dominant-negative Siah-1 abrogated β-catenin degradation mediated by hexachlorophene.

In mammals, Siah-1 is a gene induced by p53 and is involved in cell-cycle arrest, tumor suppression, and apoptosis (Amson et al., 1996; Nemani et al., 1996; Linares-Cruz et al., 1998; Matsuzawa et al., 1998; Roperch et al., 1999). Siah-1 targets the deleted in colon cancer and nuclear receptor corepressor genes and c-Myb for ubiquitin-dependent proteolysis (Hu et al., 1997; Zhang et al., 1998; Tanikawa et al., 2000). Siah-1 protein is normally maintained at a relatively low level through ubiquitin-dependent proteolysis (Hu and Fearon, 1999). However, activation of p53 by genotoxic reagents or radiation would cause Siah-1 induction and subsequent destruction of the target proteins. In this study, we found that hexachlorophene induced Siah-1 expression without activation of p53-dependent transcription and stabilization of the p53 protein, indicating that another pathway could also induce Siah-1 expression, possibly through a parallel pathway to p53, which remains to be elucidated. A possible explanation comes from a recent observation that Wnt5a promotes the degradation of β-catenin through p53-independent up-regulation of Siah expression (Topol et al., 2003). Because hexachlorophene can inhibit Wnt/β-catenin pathway without p53 activation, it can be applicable for the cancer therapeutic agent against cancer cells containing the mutant type of p53, which can be found in most of cancer cells that are resistant to the genotoxic chemotherapeutics.

Previous attempts to inactivate the function of β-catenin

through antisense or small interfering RNA strategies (Roh et al., 2001; Verma et al., 2003) have demonstrated the important role of β-catenin in colon cancer development. Small-molecule inhibitors that show antagonistic effects on the activity of CRT have been discovered by high-throughput screening (Emami et al., 2004; Lepourcelet et al., 2004). Small molecules that inhibit an association between Tcf4 and β-catenin antagonize cellular effects of β-catenin-dependent activities, such as cell proliferation and duplication of the *Xenopus laevis* embryonic dorsal axis (Lepourcelet et al., 2004). Another small molecule, which blocked the β-catenin/cAMP response element-binding protein interaction, specifically induces apoptosis in colon cancer cells (Emami et al., 2004). In contrast to previous small molecules, hexachlorophene specifically reduces the amount of free β-catenin protein, which is up-regulated in various cancers, via an Siah-1-mediated degradation, leading to a decreased expression of cyclin D1 that plays a pivotal role in the G_1-to-S phase cell-cycle transition. Thus, hexachlorophene exhibits a noteworthy advantage with a respect of inhibiting the cell proliferation through not only a repression of cyclin D1 expression but also an activation of Siah-1 that has been known as a tumor suppressor. Consistent with these observations, we found that hexachlorophene inhibits cellular proliferation of colon cancer cells (Fig. 5C). Taken together, these results show that hexachlorophene could be developed into therapeutic agents against cancers, which abnormally contain active CRT.

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