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Hexachlorophene suppresses β-catenin expression by up-regulation of Siah-1 in EBV-infected B lymphoma cells

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Abstract

Many studies have shown that the activation of β-catenin signaling can promote oncogenesis, and it is therefore of interest to find agents that modulate this pathway. Recent work has shown using B lymphoma cells that infection by Epstein–Barr virus (EBV) and expression of its latent membrane protein (LMP)-1, cause increases in the expression of β-catenin and cellular transformation. Conversely, results from cell-based small molecule screening studies have shown that the antibiotic hexachlorophene can down-regulate β-catenin in colon cancer cells. Here we report that hexachlorophene also counteracts the elevated β-catenin levels in EBV-infected B lymphomas. This is associated with restoration in levels of Siah-1 (an E3 ubiquitin ligase that is active in β-catenin regulation) which had been diminished by LMP-1. Our results suggest that hexachlorophene also counteracts the elevated β-catenin levels in EBV-infected B lymphomas. This is associated with restoration in levels of Siah-1 (an E3 ubiquitin ligase that is active in β-catenin regulation) which had been diminished by LMP-1. Our results suggest that Siah-1 is targeted by both LMP-1 and hexachlorophene with opposite effects. The hexachlorophene modulation of Siah-1 and β-catenin is independent of p53 and results in reduced expression of cyclin-D1 and c-Myc (target genes of β-catenin), leading to the growth arrest of B lymphoma cells. From these results we propose that hexachlorophene may provide a novel therapeutic strategy for EBV-infected B lymphoma cells by reducing β-catenin levels via the restoration of Siah-1.

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1. Introduction

Many studies have shown that the Wnt/β-catenin signaling pathway plays an important role in cell proliferation, differentiation, and oncogenesis [1,2]. In particular, the abnormal up-regulation of Wnt/β-catenin activity has been frequently detected as an early event in many cancers [3]. The level of β-catenin is normally regulated in part through its targeted decay as mediated by a glycogen synthase kinase-3β (GSK-3β)-dependent pathway [4] and alternatively by a Siah-1-dependent pathway [5]. In the GSK-3β-dependent pathway, β-catenin is phosphorylated by a multi-protein complex composed of APC, Axin, and GSK-3β [6,7], leading to the degradation of β-catenin through a ubiquitin-dependent mechanism [8]. Conversely, 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 [5].

In this report we have focused on the behavior of B cell lymphoma. It is known that infection by Epstein–Barr virus (EBV), a member of the human herpes virus family, enhances transformation of normal resting B cells into indefinitely proliferating lymphoblastoid cell lines. Earlier work...
has shown that latent membrane protein (LMP)-1 of EBV can transform rodent fibroblasts in vitro [9] and induces lymphomas in transgenic mice [10]. Thus, LMP-1 has been considered as an important and multifunctional oncoprotein, causing the dysregulation of cell signaling pathways and inducing a variety of cellular genes that enhance cell survival and adhesive, invasive, and angiogenic potential [11]. For example, LMP-1 oncoprotein appears to mimic cellular CD40 molecules, leading to interaction with TRAF proteins and culminating in the activation of NF-κB and JNK [12,13]. Other studies have shown that LMP-1 also activates Stat molecules through JAK interactions [14], and LMP-1 has recently been reported to elevate β-catenin protein levels through down-regulation of Siah-1 E3 ubiquitin ligase [15].

Screening of chemical libraries has been undertaken to identify small molecule inhibitors of β-catenin activity, yielding as one of the potential candidates hexachlorophene, which is an antimicrobial compound used in disinfectants and surgical scrubs [16]. Hexachlorophene inhibits the activity of enoyl-acyl carrier protein reductase, which is the last enzyme in the fatty acid elongation cycle and a target for antibacterial agents [17]. Hexachlorophene is also an inhibitor of the 3CL protease of SARS-CoV [18], and is reported to suppress β-catenin levels through up-regulation of Siah-1 in colon cancer cells [19].

Thus, we wanted to combine these disparate observations and ask whether hexachlorophene might be effective in diminishing the elevated levels of β-catenin achieved by LMP-1 activity in EBV-infected B lymphoma, to establish by what mechanism this might occur, and to observe any consequences in cell behavior. Here we report that hexachlorophene does indeed suppress the level of LMP-1-induced β-catenin in EBV-infected B lymphoma, apparently through the rescue of Siah-1 E3 ubiquitin ligase. Our results thus raise the possibility that hexachlorophene or similar compounds may ultimately be useful in the treatment of EBV-mediated B lymphoma.

2. Materials and methods

2.1. Cell culture and transient transfection

BJAB, IM9, Ramos, and Raji B lymphocytes were cultured in RPMI 1640 supplemented with 10% FBS and 1% penicillin and streptomycin. BJAB or IM-9 B cells were electroporated with 10 μg of DNA at 260 V and 975 μF (Bio-Rad, Hercules, CA). Cells were harvested 48 h after electroporation and the expression of proteins was determined by immunoblotting.

2.2. Reagents and antibodies

Hexachlorophene and LiCl were from Sigma (St. Louis, MO) and MG132 and cyclohexamide were provided from Calbiochem (San Diego, CA). Anti-cyclin-D1, anti-β-tubulin, anti-Sp1, anti-c-Myc, anti-p53, and HRP-conjugated secondary antibodies were purchased from Santa Cruz (Santa Cruz, CA). Anti-β-catenin antibody was obtained from BD Biosciences (San Jose, CA). For the detection of Siah-1 and LMP-1 proteins, anti-Siah-1 (Cosmobio, Tokyo, Japan) and anti-LMP-1 (DAKO, Carpenteria, CA) antibodies were used, respectively. The pTOPFlash and pOPFlash reporter plasmids were obtained from Upstate Biotechnology (Lake Placid, NY).

2.3. RT-PCR analysis

Total RNA was extracted from cells using Trizol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer’s instructions. Three micrograms of total RNA was converted to cDNA using Superscript II reverse transcriptase (Invitrogen), and PCR was performed using specific primers as described elsewhere [15,20]. The cDNAs of each sample were diluted, and PCR was run at the optimized cycle number. β-Actin mRNA was measured as an internal standard. After amplification, the products were subjected to electrophoresis on 1.5% agarose and detected by ethidium bromide staining.

2.4. Luciferase reporter assays

BJAB or IM-9 B cells (1 × 10⁷ cells) were electroporated with TOPFlash-, FOPFlash-, or p53-luciferase reporter vectors. To normalize transfection efficiency, a pGK-βgal vector that expresses β-galactosidase from a phosphoglucokinase promoter was included in the transfection mixture. At 48 h post-transfection, cells were washed with cold PBS and lysed in lysis solution (25 mM Tris [pH 7.8], 2 mM EDTA, 2 mM DTT, 10% glycerol, and 1% Triton X-100). Luciferase activity was measured with a luminometer by using a luciferase kit (Promega, Madison, WI).

2.5. Immunoblotting assays

Cells were harvested and lysed with lysis buffer (0.15 M NaCl, 1% Nonidet P-40, 50 mM Tris [pH 7.5]) containing 0.1 mM Na₂VO₃, 1 mM NaF, and protease inhibitors (Sigma). For immunoblotting, proteins from whole cell lysates were resolved by 10% or 12% SDS– PAGE and transferred to nitrocellulose membranes. Primary antibodies were used at 1:1000 or 1:2000 dilutions, and secondary HRP-conjugated antibodies (Santa Cruz) were used at 1:2000 dilution in 6% nonfat dry milk. After final washing, nitrocellulose membranes were exposed using chemiluminescence assays (GE-Amersham, Piscataway, NJ).

2.6. Cellular fractionation

As described elsewhere [21], cells cultured in 100 mm plates were washed and harvested with ice-cold PBS and cell pellets were lysed with 800 μl of TTN buffer (20 mM Tris–HCl [pH 7.4], 0.05% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 10% glycerol, 0.5 mM PMSF, and 1× cocktail protease inhibitor) on ice for 20 min followed by centrifugation at 10,000g for 15 min. The supernatant was taken as the soluble fraction, and the pellets as insoluble fractions were subsequently solubilized in 800 μl of RIPA buffer (50 mM Tris–HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1 mM DTT, 1% NP-40, 0.5% deoxycholic acid, 0.1% SDS, 10% glycerol, 0.5 mM PMSF, and 1× cocktail protease inhibitor) on ice for 30 min and were centrifuged...
at 12,000g for 15 min. Thereafter, the supernatants were used for the nuclear extracts.

3. Results

3.1. LMP-1 induces up-regulation of β-catenin expression in B lymphocytes

To explore whether the infection of B cells with EBV induces up-regulation of β-catenin, we examined IM9 and Raji B lymphoma cells with a persistent infection of EBV, plus BJAB and Ramos lymphoma cells that are free of EBV. The infection with EBV was confirmed by the expression of LMP-1, a primary oncoytic protein in IM9 and Raji B cells (Fig. 1A). This figure also shows that the protein level of Siah-1, an E3 ubiquitin ligase, was decreased whereas the β-catenin protein level was enhanced in the IM9 and Raji cells. By comparison, the Siah-1 level was higher while the β-catenin level was lower in both BJAB and Ramos B cells. To test whether LMP-1 (instead of other viral proteins) is specifically linked to the up-regulation of β-catenin and the down-regulation of Siah-1, we expressed LMP-1 protein in BJAB cells using electroporated pcDNA3- LMP-1 vector. We found that LMP-1 expression alone is sufficient to reduce Siah-1 protein levels, leading to up-regulation of β-catenin (Fig. 1B). In addition, to determine whether the increased β-catenin due to the introduction of LMP-1 is transcriptionally active, we transfected cells with a reporter plasmid containing wild-type (TOPFlash) or mutant (FOPFlash) binding sites for Tcf [22] in the presence or absence of LMP-1. As seen in Fig. 1C, LMP-1 expression specifically induced Tcf reporter activity, indicating that at least some of the LMP-1-induced β-catenin is transcriptionally active. To examine whether LMP-1 also down-regulates Siah-1 mRNA levels, we measured Siah-1 mRNA from BJAB cells in the presence and absence of LMP-1 using RT-PCR. As seen in Fig. 1D, the presence of LMP-1 (instead of other viral proteins) is specifically linked to the down-regulation of Siah-1 mRNA levels in a dose-dependent manner. Furthermore, hexachlorophene treatment reduced LMP-1-induced Tcf transcriptional activity in a dose dependent manner (Fig. 2C). Next, to examine whether proteosomes are involved in the down-regulation of β-catenin following treatment with hexachlorophene, we administered MG132 (a proteosome inhibitor) into hexachlorophene-treated cells and then analyzed the β-catenin level. We found that β-catenin levels were restored by MG132 when compared with cells treated only with hexachlorophene (Fig. 2D), suggesting that down-regulation of β-catenin mediated by hexachlorophene is achieved via proteosome activation. Of interest, the introduction of cycloheximide, an inhibitor of protein synthesis, also partly restored the β-catenin level from hexachlorophene-treated cells (Fig. 2D), suggesting that some aspect of protein synthesis is required for the activity of the β-catenin degradation pathway.

3.2. Hexachlorophene treatment induces the degradation of β-catenin via proteosomes

Hexachlorophene has been identified (by screening from small molecule libraries) as an inhibitor of β-catenin, and is possibly useful for the treatment of colon cancers [19]. We therefore examined whether hexachlorophene can also down-regulate the high levels of β-catenin observed in EBV-infected B lymphoma. As shown in Fig. 2A and B, hexachlorophene significantly down-regulates protein as well as mRNA levels of β-catenin in a dose-dependent manner. Furthermore, hexachlorophene treatment reduced LMP-1-induced Tcf transcriptional activity in a dose dependent manner (Fig. 2C). Next, to examine whether proteosomes are involved in the down-regulation of β-catenin following treatment with hexachlorophene, we administered MG132 (a proteosome inhibitor) into hexachlorophene-treated cells and then analyzed the β-catenin level. We found that β-catenin levels were restored by MG132 when compared with cells treated only with hexachlorophene (Fig. 2D), suggesting that down-regulation of β-catenin mediated by hexachlorophene is achieved via proteosome activation. Of interest, the introduction of cycloheximide, an inhibitor of protein synthesis, also partly restored the β-catenin level from hexachlorophene-treated cells (Fig. 2D), suggesting that some aspect of protein synthesis is required for the activity of the β-catenin degradation pathway.

3.3. Hexachlorophene induces the degradation of β-catenin protein via up-regulation of Siah-1

Since we observed that hexachlorophene administration caused the decrease in protein levels of β-catenin (Fig. 2), we next investigated the mechanism by which this down-regulation might be achieved. As seen in Fig. 3A, hexachlorophene also induced up-regulation of Siah-1 levels, associated with the decrease of β-catenin in EBV-infected B cells (1st and 2nd lanes). To confirm that this feature is mediated by LMP-1 expression, we also introduced the LMP-1 expression vector into BJAB cells and treated them with hexachlorophene. We found that hexachlorophene

**Fig. 1.** LMP-1 of EBV induces the expression of β-catenin through down-regulation of Siah-1. (A) IM9 and Raji (EBV-infected B cells) and BJAB and Ramos (EBV-negative B cells) were lysed and the expression of Siah-1, β-catenin, and LMP-1 were examined by immunoblotting using each antibody. (B) BJAB B cells (1 x 10⁷ cells) were electroporated with pcDNA3-LMP-1 vector (10 μg) and the expression of Siah-1, β-catenin, and LMP-1 were examined by immunoblotting using each antibody at 48 h after transfection. (C) BJAB B cells (1 x 10⁷ cells) were electroporated with LMP-1 expression vector (5 μg) with pTOPFlash-luciferase (3 μg; black bar) or pFOPFlash-luciferase (white bar). Under the same conditions, pcDNA3 (5 μg) was used as a control vector. To normalize transfection efficiency, a pGK-β-gal vector (2 μg) was included in the DNA mixture. At 48 h post-transfection, cells were harvested and luciferase activity was measured with a luminometer. Error bars indicate standard deviation. (D) Total RNA (2 μg) purified from LMP-1-transfected BJAB cells (1 x 10⁷ cells) was subjected to RT-PCR. Relative ratios of Siah-1/β-actin mRNA levels were expressed after measurements of band intensities using Multi Gauge Ver 2.1 (Fuji Photo, Japan).
treatment rescues the decreased Siah-1 level mediated by LMP-1, resulting once again in up-regulation of $\beta$-catenin (Fig. 3B). Next, since the phosphorylation of $\beta$-catenin by GSK-3$\beta$, followed by association with $\beta$-TrCP, can lead independently to $\beta$-catenin degradation [8], we tested whether GSK-3$\beta$ and Siah-1 might cooperate to regulate $\beta$-catenin. However, we found that GSK-3$\beta$ inhibition using LiCl caused an increase in $\beta$-catenin protein levels without affecting Siah-1 levels in IM9 B cells (Fig. 3A, 3rd lane), suggesting that GSK-3$\beta$ protein is not directly involved in the Siah-1 signaling pathway. However, co-administration of LiCl and hexachlorophene caused the protein level of $\beta$-catenin to be still elevated (Fig. 3A, 4th lane) in IM9 B cells. This suggests that the effect of LiCl on the induction of $\beta$-catenin through GSK-3$\beta$ inhibition overrides that of hexachlorophene through Siah-1 in EBV-infected B lymphoma.

3.4. Hexachlorophene affects Siah-1 protein levels independently from the tumor suppressor p53

It has been suggested that the expression of Siah-1 can be activated by the tumor suppressor p53, leading to the induction of apoptosis and cell-cycle arrest in mammalian cells [5]. In addition, some genotoxic agents, such as doxorubicin, may suppress the $\beta$-catenin response through the p53-inducible transcription pathway involving Siah-1 [5]. To further explore this issue, we investigated whether hexachlorophene treatment might influence p53 transcription in IM9 B cells (Fig. 3A, 3rd lane), suggesting that GSK-3$\beta$ protein is not directly involved in the Siah-1 signaling pathway. However, co-administration of LiCl and hexachlorophene caused the protein level of $\beta$-catenin to be still elevated (Fig. 3A, 4th lane) in IM9 B cells. This suggests that the effect of LiCl on the induction of $\beta$-catenin through GSK-3$\beta$ inhibition overrides that of hexachlorophene through Siah-1 in EBV-infected B lymphoma.

Fig. 2. Hexachlorophene reduces $\beta$-catenin levels via a proteosome-mediated pathway. (A) IM9 B lymphoma cells were treated with hexachlorophene at different concentrations (10, 20, and 30 $\mu$M) and harvested at 12 h after treatment. The expression of $\beta$-catenin was analyzed by immunoblotting using anti-$\beta$-catenin antibody. (B) Total RNA (2 $\mu$g) purified from hexachlorophene-treated IM9 B cells (1 $\times$ 10^6 cells) was subjected to RT-PCR. Relative ratios of Siah-1/$\beta$-actin mRNA levels were expressed after measurement of band intensities as in Fig. 1. (C) IM9 B cells (1 $\times$ 10^6 cells) were electroporated with pTOPFlash-luciferase vector (3 $\mu$g; black bar) or pFOPFlash-luciferase vector (3 $\mu$g; white bar). To normalize transfection efficiency, a pGK-βgal vector (2 $\mu$g) was included in the DNA mixture. The cells were treated with hexachlorophene at different concentrations at 12 h before harvest. At 48 h post-transfection, cells were harvested and luciferase activity was measured with a luminometer. Error bars indicate standard deviation. (D) IM9 B lymphoma cells were treated with hexachlorophene (20 $\mu$M) and then with MG132 (10 $\mu$M), or cyclohexamide (10 $\mu$M) 6 h later. The cells were harvested at 12 h after treatment with hexachlorophene and then the expression of $\beta$-catenin was analyzed by immunoblotting using anti-$\beta$-catenin antibody.

Fig. 3. Hexachlorophene suppresses the expression of $\beta$-catenin through up-regulation of Siah-1. (A) IM9 B lymphoma cells were treated with hexachlorophene (20 $\mu$M), LiCl (20 mM) and hexachlorophene (20 $\mu$M) plus LiCl (20 mM) for 12 h. The expression of Siah-1 and $\beta$-catenin was analyzed by immunoblotting using anti-Siah-1 and anti-$\beta$-catenin antibody. (B) BJAB cells were electroporated with LMP-1 vector (10 $\mu$g) and treated with hexachlorophene at 12 h before harvest. The cells were harvested at 48 h post-transfection and the expression of LMP-1, Siah-1, and $\beta$-catenin were analyzed by immunoblotting using each antibody.
3.5. Hexachlorophene inhibits the expression of β-catenin-dependent genes, leading to the growth arrest of B lymphoma

We first examined the expression of β-catenin target genes such as cyclin-D1 and c-Myc in nuclear extracts because several lines of evidence have shown that nuclear localization of cyclinD1 or c-Myc is preferentially detected in proliferating and tumorigenic cells [25–28]. As seen in Fig. 5A, the expression levels of cyclin-D1 and c-Myc were diminished in nuclear extracts when compared with Sp-1 expression, in a hexachlorophene dose-dependent manner at 12 h post-treatment. As other groups have reported that shRNA or chemical inhibition of β-catenin induces retardation of tumor cell growth [29–31], we explored the potential biological consequence of the reduced β-catenin levels achieved by treatment with hexachlorophene. When we examined viable cells of EBV positive B lymphoma, we observed a striking dose-dependent decrease in viable cell numbers of IM9 B lymphoma cells 48 h after treatment with hexachlorophene (Fig. 5B). These results suggest that hexachlorophene decreases the expression of β-catenin-dependent genes, leading to the growth arrest of B lymphoma cells.

4. Discussion

Excessive accumulation of β-catenin is frequently detected in various types of cancer, such as colorectal, ovarian, endometrial, and prostate cancers [32,33]. Primary effusion lymphoma cells expressing Kaposi’s sarcoma associated herpes virus (KSHV) also show elevated β-catenin levels. In particular, the LANA protein of KSHV plays a crucial role in up-regulation of β-catenin, by sequestration and down-regulation of GSK-3β [34,35], which might be distinct from the action of LMP-1. Here we show that LMP-1, a primary oncoprotein of EBV also induces up-regulation of β-catenin in B lymphocytes by the decrease of Siah-1 protein and mRNA levels. As it is known that Siah-1 itself is a target for ubiquitination and proteosomal degradation [36], LMP-1
may affect Siah-1 protein destabilization. In addition, LMP-1 down-regulates Siah-1 mRNA levels, which indicates that LMP-1 might inhibit transcription of the Siah-1 gene. Although the promoter region of Siah-1 has been recently identified [37], more work is required to establish in detail how LMP-1 inhibits transcription of Siah-1.

The consequent over-expression of β-catenin target genes, including cyclin-D1, c-Myc, matrix metalloproteinase-7, and peroxisome proliferators-activated receptor-δ, can all play important roles in tumorigenesis [38]. The constitutive activation of β-catenin signaling is therefore a potential target for chemoprevention and treatment of various cancers, including B cell lymphomas.

Furthermore, we report that hexachlorophene drastically suppresses β-catenin levels through a Siah-1-mediated pathway (rather than the GSK-3β-dependent pathway) in EBV-infected B lymphoma. Hexachlorophene specifically up-regulates Siah-1 mRNA and protein in B lymphoma, counteracting its down-regulation by LMP-1. Furthermore, prolonged exposure of EBV-infected B lymphoma cells to hexachlorophene also induces apoptotic cell death and down-regulation of β-catenin targeted gene expression. However, the up-regulation of β-catenin by LiCl through GSK-3β inhibition overrides that of hexachlorophene through Siah-1 in EBV-infected B lymphoma (Fig. 3). Since previous work showed that hexachlorophene can inhibit the expression of β-catenin induced by LiCl in HEK293 cells [19], this difference in response in the two cell types is currently under investigation. Of interest, we observed that cycloheximide restores β-catenin levels in hexachlorophene-treated cells (Fig. 2D). As we suspect that the stability of proteins that are involved in the regulation of β-catenin expression is also a critical factor, Siah-1 degradation by cycloheximide treatment may contribute to the recovery of β-catenin; this point will be the subject of further studies by our group.

It has been proposed that Siah-1 can be induced by p53 tumor suppressor activation, contributing to cell-cycle arrest, tumor suppression and apoptosis [5]. However, hexachlorophene does not affect p53 at either the transcriptional and translational level in our study: this suggests the existence of another hexachlorophene-responsive and p53-independent pathway for the regulation of Siah-1. A recent report showing that Wnt5a also promotes the degradation of β-catenin through the p53-independent up-regulation of Siah-1 level is consistent with our suggestion [39]. Since hexachlorophene can suppress Wnt/β-catenin signaling in a p53-independent manner, it is possible that this potential therapeutic strategy may be effective against cancer cells with mutant p53, which is commonly found in many cancers that are resistant to genotoxic chemotherapeutics.

Previous searches for small molecule inhibitors of β-catenin activity have revealed candidates that block the interaction between β-catenin and cAMP response element-binding protein [40] or that inhibit the association of β-catenin and the Tcf4 complex [41]. In contrast, hexachlorophene specifically reduces the amount of free β-catenin protein through an independent pathway by Siah-1-mediated degradation. From our results, we propose that hexachlorophene could ultimately provide a potential therapeutic strategy for the treatment of EBV-infected B lymphoma.

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References

[1] M. Peifer, P. Polakis, Wnt signaling in oncogenesis and embryogenesis – a look outside the nucleus, Science 287 (2000) 1696–1699.
[2] J. Huelskens, W. Birchmeier, New aspects of Wnt signaling pathways in higher vertebrates, Curr. Opin. Genet. Dev. 11 (2001) 547–553.
[3] R.H. Giles, J.H. van Es, H. Clevers, Caught up in a Wnt storm: Wnt signaling in cancer, Biochim. Biophys. Acta 1653 (2003) 1–24.
[4] P. Polakis, Casein kinase 1: a Wnt’er of disconnect, Curr. Biol. 12 (2002) R499–R501.
[5] J. Liu, J. Stevens, C.A. Rote, H.J. Yost, Y. Hu, K.L. Neufeld, R.L. White, N. Matsunami, Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenosomatous polyposis coli protein, Mol. Cell 7 (2001) 927–936.
[6] M.J. Hart, R. de los Santos, I.N. Albert, B. Rubinfeld, P. Polakis, Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta, Curr. Biol. 8 (1998) 573–581.
[7] S. Kishida, H. Yamamoto, S. Ikeda, M. Kishida, I. Sakamoto, S. Koyama, A. Kikuchi, Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenosomatous polyposis coli and regulates the stabilization of beta-catenin, J. Biol. Chem. 273 (1998) 10823–10826.
[8] G. Koller, A. Bauer, J. Stappert, A. Kispert, R. Kemler, Beta-catenin is a target for the ubiquitin–proteasome pathway, EMBO J. 16 (1997) 3797–3804.
[9] R.K. Moorthy, D.A. Thorley-Lawson, All three domains of the Epstein–Barr virus-encoded latent membrane protein LMP-1 are required for transformation of rat-1 fibroblasts, J. Virol. 67 (1993) 1638–1646.
[10] W. Kulwicht, R.H. Edwards, E.M. Davenport, J.F. Baskar, V. Godfrey, N. Raab-Traub, Expression of the Epstein–Barr virus latent membrane protein 1 induces B cell lymphoma in transgenic mice, Nat. Med. 5 (1999) 573–581.
[11] S. Kondo, N. Wakisaka, M.J. Schell, T. Horikawa, T.S. Sheen, H. Sato, M. Furukawa, J.S. Pagano, T. Yoshizaki, Epstein–Barr virus latent membrane protein 1 promotes the induction of the metalloproteinase-1 promoter via an Ets binding site formed by a single nucleotide polymorphism: enhanced susceptibility to nasopharyngeal carcinoma, Int. J. Cancer 115 (2005) 368–376.
[12] J. Uchida, T. Yasui, Y. Takaoka-Shichijo, M. Muraoka, W. Kulwicht, N. Raab-Traub, H. Kikutani, Mimicry of CD40 signals by Epstein–Barr virus LMP1 in B lymphocyte responses, Science 286 (1999) 300–303.
[13] M. Luftig, E. Prinarakis, T. Yasui, T. Tschritzis, E. Cahir-McFarland, J. Inoue, H. Nakano, T.W. Mak, W.C. Yeh, X. Li, S. Akira, S. Suzuki, G. Mosialos, E. Kief, Epstein–Barr virus latent membrane protein 1 activation of NF-kappaB through IRAK1 and TRAF6, Proc. Natl. Acad. Sci. USA 100 (2003) 15595–15600.
[14] L. Gires, F. Kohlhuber, E. Kilger, M. Baumann, A. Kieser, C. Kaiser, R. Zeidler, B. Scheffer, M. Ueffing, W. Hammerschmidt, Latent membrane protein 1 of Epstein–Barr virus interacts with JAK3 and activates STAT proteins, EMBO J. 18 (1999) 3064–3073.
[15] E. Jungermann, Soap bacteriostats, J. Am. Oil Chem. Soc. 45 (1968) 345–350.
[16] R.J. Heath, J. Li, G.E. Roland, C.O. Rock, Inhibition of the Staphylococcus aureus NADPH-dependent enoyl–acyl carrier protein reductase by triclosan and hexachlorophene, J. Biol. Chem. 275 (2000) 4654–4659.
[17] J.T. Hsu, C.J. Kuo, H.P. Hsieh, Y.C. Wang, K.K. Huang, C.P. Lin, F.P. Huang, X. Chen, P.H. Liang, Evaluation of metal-conjugated compounds as inhibitors of 3CL protease of SARS-CoV, FEBS Lett. 574 (2004) 116–120.
[18] S. Park, J. Gwak, M. Cho, T. Song, J. Won, D.E. Kim, J.G. Shin, S. Oh, Hexachlorophene inhibits Wnt/beta-catenin pathway by promoting...
Siah-mediated beta-catenin degradation, Mol. Pharmacol. 70 (2006) 960–966.

[20] S.H. Choi, S.E. Jin, M.K. Lee, S.J. Lim, J.S. Park, B.G. Kim, W.S. Ahn, C.K. Kim, Novel cationic solid lipid nanoparticles enhanced p53 gene transfer to lung cancer cells, Eur. J. Pharm. Biopharm. (2007).

[21] H. Habelhah, S. Takahashi, S.G. Cho, T. Kadaya, T. Watanabe, Z. Ronai, Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF-kappaB, EMBO J. 23 (2004) 322–332.

[22] M. van de Wetering, M. Oosterwegel, D. Dooijes, H. Clevers, Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box, EMBO J. 10 (1991) 123–122.

[23] P.Y. Yeh, S.E. Chang, K.H. Yeh, Y.C. Song, L.L. Chang, A.L. Cheng, Phosphorylation of p53 on Thr55 by ERK2 is necessary for doxorubicin-induced p53 activation and cell death, Oncogene 23 (2004) 3580–3588.

[24] J. Ju, J.C. Schmitz, B. Song, K. Kudo, E. Chu, Regulation of p53 expression in response to 5-fluorouracil in human cancer RKO cells, Clin. Cancer Res. 13 (2007) 4245–4251.

[25] A. Radu, V. Neubauer, T. Akagi, H. Hanafusa, M.M. Georgescu, PTEN induces cell cycle arrest by decreasing the level and nuclear localization of cyclin D1, Mol. Cell. Biol. 23 (2003) 6139–6149.

[26] P. Sumrejkanchanakij, M. Tamamori-Adachi, Y. Matsunaga, K. Eto, M.A. Ikeda, Role of cyclin D1 cytoplasmic sequestration in the survival of postmitotic neurons, Oncogene 22 (2003) 8723–8730.

[27] M.A. Gregory, Y. Qi, S.R. Hann, Phosphorylation by glycogen synthase kinase-3 controls c-myc proteolysis and subnuclear localization, J. Biol. Chem. 278 (2003) 51606–51612.

[28] M. Tamamori-Adachi, H. Ito, P. Sumrejkanchanakij, S. Adachi, M. Hiroe, M. Shimizu, J. Kawachi, M. Sunamori, F. Marumo, S. Kitajima, M.A. Ikeda, Critical role of cyclin D1 nuclear import in cardiomyocyte proliferation, Circ. Res. 92 (2003) e12–e19.

[29] W.S. Huang, J.P. Wang, T. Wang, J.Y. Fang, P. Lan, J.P. Ma, ShRNA-mediated gene silencing of beta-catenin inhibits growth of human colon cancer cells, World J. Gastroenterol. 13 (2007) 6581–6587.

[30] M. Wieczorek, A. Paczkowska, P. Guzenda, M. Majorek, A.K. Bednarek, M. Lamparska-Przybysz, Silencing of Wnt-1 by siRNA induces apoptosis of MCF-7 human breast cancer cells, Cancer Biol. Ther. 7 (2008) 268–274.

[31] J. Pannequin, N. Delaunay, M. Buchert, F. Surrel, J.F. Bourgaux, J. Ryan, S. Boireau, J. Coelho, A. Pelegrin, P. Singh, A. Shulkes, M. Yim, G.S. Baldwin, C. Pignodel, G. Lambeau, P. Jay, D. Joubert, F. Hollande, Beta-catenin/Tcf-4 inhibition after progastrin targeting reduces growth and drives differentiation of intestinal tumors, Gastroenterology 133 (2007) 1554–1568.

[32] N.S. Fearhead, M.P. Britton, W.F. Bodmer, The ABC of APC, Hum. Mol. Genet. 10 (2001) 721–733.

[33] R. Karim, G. Tse, T. Putti, R. Scioley, S. Lee, The significance of the Wnt pathway in the pathology of human cancers, Pathology 36 (2004) 120–128.

[34] M. Fujimuro, F.Y. Wu, C. Aptherys, H. Kajumbula, D.B. Young, G.S. Hayward, S.D. Hayward, A novel viral mechanism for dysregulation of beta-catenin in Kaposi's sarcoma-associated herpesvirus latency, Nat. Med. 9 (2003) 300–306.

[35] M. Fujimuro, J. Liu, J. Zhu, H. Yokosawa, S.D. Hayward, Regulation of the interaction between glycogen synthase kinase 3 and the Kaposi's sarcoma-associated herpesvirus latency-associated nuclear antigen, J. Virol. 79 (2005) 10429–10441.

[36] G. Hu, E.R. Fearon, Siah-1 N-terminal RING domain is required for proteolysis function, and C-terminal sequences regulate oligomerization and binding to target proteins, Mol. Cell. Biol. 19 (1999) 724–732.

[37] G. Fiucci, S. Beaucourt, D. Duflaut, A. Lespagnol, P. Stumptner-Cuvelette, A. Geant, G. Buchwalter, M. Tuynder, L. Susini, J.M. Lassalle, C. Wasylyk, B. Wasylyk, M. Oren, R. Amson, A. Telerman, Siah-1β is a direct transcriptional target of p53: identification of the functional p53 responsive element in the siah-1β promoter, Proc. Natl. Acad. Sci. USA 101 (2004) 3510–3515.

[38] M. Takahashi, T. Tsunoda, M. Seiki, Y. Nakamura, Y. Furukawa, Identification of membrane-type matrix metalloproteinase-1 as a target of the beta-catenin/Tcf/beta-catenin complex in human colorectal cancers, Oncogene 21 (2002) 5861–5867.

[39] R. Topol, X. Jiang, H. Choi, L. Garrett-Beal, P.J. Carolan, Y. Yang, Wnt5a inhibits the canonical Wnt pathway by promoting GSK-3 independent beta-catenin degradation, J. Cell Biol. 162 (2003) 902–908.

[40] K.H. Emami, C. Nguyen, H. Ma, D.H. Kim, K.W. Jeong, M. Eguchi, R.T. Moon, J.L. Teo, H.Y. Kim, S.H. Moon, J.R. Ha, M. Kahn, A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected], Proc. Natl. Acad. Sci. USA 101 (2004) 12682–12687.

[41] M. Lepourcelet, Y.N. Chen, D.S. France, H. Wang, P. Crews, F. Petersen, C. Bruseo, A.W. Wood, R.A. Shivdasani, Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex, Cancer Cell 5 (2004) 91–102.