Department of Oncology[1], Affiliated Nanhua Hospital, University of South China; Department of Obstetrics and Gynecology[2], The 169th Central Hospital of People's Liberation Army, Hengyang, Hunan Province, China

Octreotide inhibits growth of colonic cancer SW480 cells by modulating the Wnt/β-catenin pathway

Jing-song Chen[1], Qing-mo Liang[1], Hua-shu Li[2], Jie Yang[1], Song Wang[1], Jian-wu Long[1]

Received July 23, 2008, accepted August 22, 2008

Qing-mo Liang, Department of Oncology, Affiliated Nanhua Hospital, University of South China, Hengyang, 421002, Hunan Province, P.R. China
hylqm@vip.sina.com, hychenjs@126.com

Pharmazie 64: 126–131 (2009) *doi: 10.1691/ph.2009.8678*

Somatostatin can suppress the growth of various tumor cells including colonic cancer. Activated Wnt/β-catenin signaling pathway plays a critical role in tumorgenesis and development of colorectal cancer. However, the effect of somatostatin on Wnt/β-catenin signaling pathway remains unknown. Thus, we investigated the effect of octreotide on Wnt/β-catenin signaling pathway in human colonic cancer cell SW480. The results of 3-(4,5-imethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and flow cytometric assays showed that octreotide inhibited growth, induced apoptosis and arrested the G_1 cell cycle of SW480 cells in a dose-dependent manner. We demonstrated that octreotide significally up-regulated and down-regulated 13 genes and 17 genes in Wnt/β-catenin signaling using microarray, respectively. Furthermore, as evidenced by western blot, β-catenin protein level decreased, whereas phosphorylated β-catenin protein level increased under octreotide. The present study reveals that octreotide can inhibit human colonic cancer cell growth through inhibition of Wnt/β-catenin signaling pathway.

1. Introduction

Somatostatin is a widely distributed neuropeptide that negatively regulates exocrine, and endocrine functions, neurotransmission, and cell proliferation. Somatostatin and its analogues can inhibit growth of a variety of tumor types including colonic, breast, gastric, hepatocellular cancers, and so on (Tejeda et al. 2006; Wang et al. 2003; Liu et al. 2004). The mechanisms responsible for the inhibitory effects of somatostatin are associated with many signal transduction pathways including MAPK pathway (Wang et al. 2003), phosphatidylinositol 3-kinase signaling pathway (Theodoropoulou et al. 2004), cytosolic free calcium, adenylate cyclase, protein kinase C, and SHP-1 (Petrucci et al. 2000; Hipkin et al. 2000; Thangaraju et al. 1999). However, the exact mechanisms underlying the antineoplastic actions of somatostatin have not been completely elucidated.

The Wnt/β-catenin signaling pathway is a highly conserved pathway that is critical in both cellular proliferation and organismal development. Dysregulation of the Wnt/β-catenin signaling pathway is regarded to play an crucial role in the carcinogenesis and development of colorectal cancer (CRC, Behrens and Lustig 2004; Polakis 2000; Reya and Clevers 2005; Segditsas and Tomlinson, 2006). At present, the effect of somatostatin on the Wnt/β-catenin signaling pathway is still unknown. We conducted this study to test the hypothesis that octreotide inhibits growth of colonic cancer SW480 cells by modulating the Wnt/β-catenin pathway.

2. Investigations and results

2.1. Octreotide inhibited growth and induced apoptosis of SW480 Cells

Octreotide at the concentration of more than 1×10^{-12} mol/L significantly inhibited the growth and induced apoptosis of SW480 cells, and octreotide in the range of $1 \times 10^{-12} - 1 \times 10^{-10}$ mol/L had a concentration-dependent effect ($p < 0.01$) (Fig. 1, Table 1). The effect of octreotide was the most significant at 1×10^{-10} mol/L. However, the inhibitory rates in 1×10^{-9} mol/L -1×10^{-7} mol/L octreotide groups were not higher than that in the 1×10^{-10} mol/L octreotide group ($P > 0.05$).

2.2. Effect of octreotide on cell cycle in SW480 cells

Because of the reported possible interactions between cell cycle deregulation and tumor development (Shapiro and Harper 1999; Jacks et al. 1996), the effect of octreotide

Table 1: Effect of octreotide on apoptosis of SW480 cells (mean ± SD)

Octreotide (mol/L)	Apoptosis rate (%)
0	1.216 ± 0.232
1×10^{-12}	$8.840 \pm 1.025^{**}$
1×10^{-11}	$16.767 \pm 1.105^{**}$
1×10^{-10}	$30.467 \pm 1.953^{**}$
1×10^{-9}	$29.633 \pm 1.907^{**}$

$^{**}P < 0.01$ compared with control

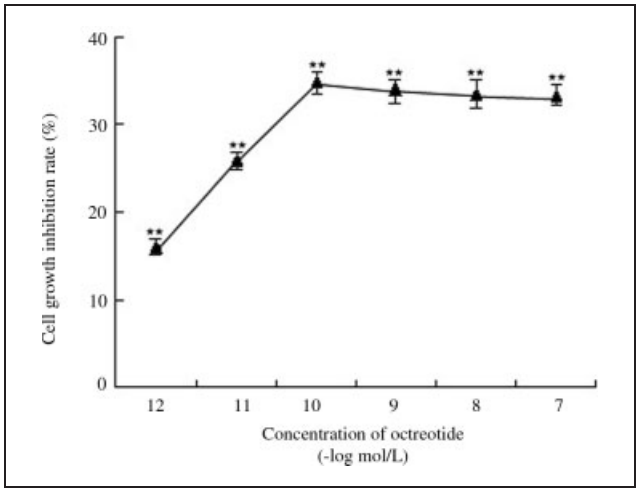

Fig. 1: Effect of octreotide on growth of SW480 cells. Cells were treated with octreotide (1×10^{-12}, 1×10^{-11}, 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7} mol/L) for 48 h. Data are the mean ± SD. $^{**}P < 0.01$ compared with control

on the cell cycle was analyzed. The results (Table 2) demonstrated that the percentage of cells in the G_1 phase significantly increased and the percentage of cells in the S phase significantly decreased after SW480 cells were treated with different concentrations of octreotide for 48 h. However, there seemed to be no alternation in G_2/M phase. This indicated that octreotide could promote G_1 cell cycle arrest and block induction of the S phase of SW480 cells.

2.3. Octreotide altered gene expression in Wnt/β-catenin signaling pathway

Given Wnt/β-catenin signaling pathway is believed to play an important role in the tumorigenesis and development of colorectal cancer, we surveyed changes in gene expression in the SW480 cells using a commercially available gene array system designed to analyse the mRNA levels of genes involved in the Wnt/β-catenin signaling pathway. According to MTT assay results, we performed the experiments with 1×10^{-10} mol/L octreotide, a concentration that was the most significant in inhibiting SW480 cell growth. Microarray analysis revealed profound changes in expression levels of 13 genes being up-regulated and 17 genes being down-regulated in SW480 cells treated by octreotide (Fig. 2, Table 3).

Table 2: Effect of octreotide on cell cycle distribution in SW480 (mean ± SD)

Octreotide (mol/L)	% cells in G1 phase	% cells in S phase	% cells in G2/M
0	45.217 ± 2.042	42.103 ± 2.907	11.876 ± 1.312
1×10^{-12}	53.331 ± 1.550**	34.671 ± 1.351**	12.033 ± 1.201
1×10^{-11}	62.267 ± 1.626**	24.870 ± 1.779**	12.872 ± 1.159
1×10^{-10}	70.667 ± 2.290**	17.833 ± 1.436**	11.534 ± 0.624
1×10^{-9}	68.645 ± 3.063**	18.873 ± 2.055**	12.474 ± 1.445

$^{**}P < 0.01$ compared with control

Table 3: Gene expression profiles of SW480 cells treated with octreotide

Genebank	symbol	Gene name	Fold change
NM_005883	APC2	Adenomatosis polyposis coli 2	3.42
NM_003502	AXIN1	Axin 1	8.10
NM_004655	AXIN2	Axin 2 (conductin, axil)	5.71
NM_033637	BTRC	Beta-transducin repeat containing	6.29
NM_001892	CSNK1A1L	Casein kinase 1, alpha 1	6.41
NM_001893	CSNK1D	Casein kinase 1, delta	3.21
NM_022048	CSNK1G1	Casein kinase 1, gamma 1	5.75
NM_001319	CSNK1G2	Casein kinase 1, gamma 2	5.41
NM_001320	CSNK2B	Casein kinase 2, beta polypeptide	2.68
NM_001328	CTBP1	C-terminal binding protein 1	2.36
NM_020248	CTNNBIP1	Catenin, beta interacting protein 1	3.40
NM_033425	DIXDC1	DIX domain containing 1	2.67
NM_012242	DKK1	Dickkopfhomolog1(Xenopus laevis)	2.14
NM_053056	CCND1	Cyclin D1	0.49
NM_001759	CCND2	Cyclin D2	0.49
NM_012164	FBXW2	F-box and WD-40 domain protein 2	0.36
NM_007197	FZD10	Frizzled homolog 10 (Drosophila)	0.46
NM_017412	FZD3	Frizzled homolog 3 (Drosophila)	0.45
NM_012193	FZD4	Frizzled homolog 4 (Drosophila)	0.46
NM_003506	FZD6	Frizzled homolog 6 (Drosophila)	0.31
NM_003507	FZD7	Frizzled homolog 7 (Drosophila)	0.10
NM_031866	FZD8	Frizzled homolog 8 (Drosophila)	0.20
NM_032045	KREMEN1	Kringle containing transmembrane protein 1	0.49
NM_002335	LRP5	Low density lipoprotein receptor-related protein 5	0.27
NM_022825	PORCN	Porcupine homolog (Drosophila)	0.38
NM_021205	RHOU	Ras homolog gene family, member U	0.31
NM_021627	SENP2	SUMO1/sentrin/SMT3 specific protease 2	0.18
NM_022454	SOX17	SRY(sex determining region Y)-box 17	0.48
NM_003882	WISP1	WNT1 inducible signaling pathway protein 1	0.44
NM_004185	WNT2B	Wingless-type MMTV integration site family, member 2B	0.39

Fold change = normalized result of the octreotide-treated group/normalized result of the control group

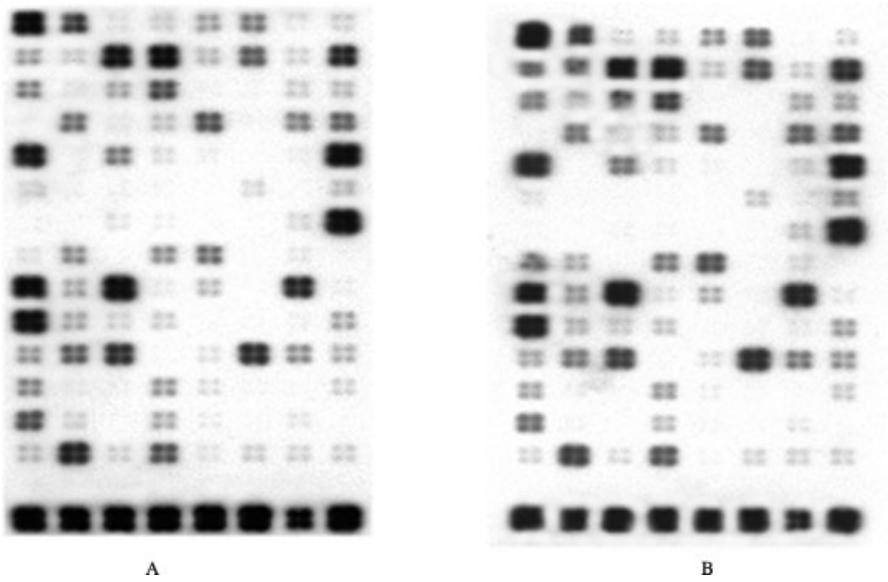

Fig. 2:
Pictures of gene expression of SW480 cells detected by microarray. (A) the control (B) the group treated by octreotide

2.4. Octreotide decreased β-catenin protein level and increased phosphorylated β-catenin protein level in SW480 cells

Based on the fact that β-catenin is a key effector of the Wnt/β-catenin signaling pathway, we asked whether octreotide effected the expression of β-catenin protein in SW480 cells. To investigate β-catenin protein level, we treated SW480 cells with various concentrations of octreotide and harvested at different time points. Western blot analysis showed that octreotide decreased the expression level of β-catenin protein in a concentration- (Fig. 3A) and time-dependent manner (Fig. 3B).

Recent data reported that NH2-terminal serine/threonine phosphorylation of β-catenin is necessary to initiate ubiquitination-mediated degradation (Reya and Clevers 2005). We therefore asked whether the reduced β-catenin is a result of increase in β-catenin phosphorylation. Phosphorylated β-catenin level in SW480 cells was determined by Western blot analysis with antiphospho-Ser33/S37/Thr41 β-catenin. Our results showed that octreotide increased phosphorylated β-catenin protein levels in a concentration- (Fig. 4A) and time-dependent manner (Fig. 4B).

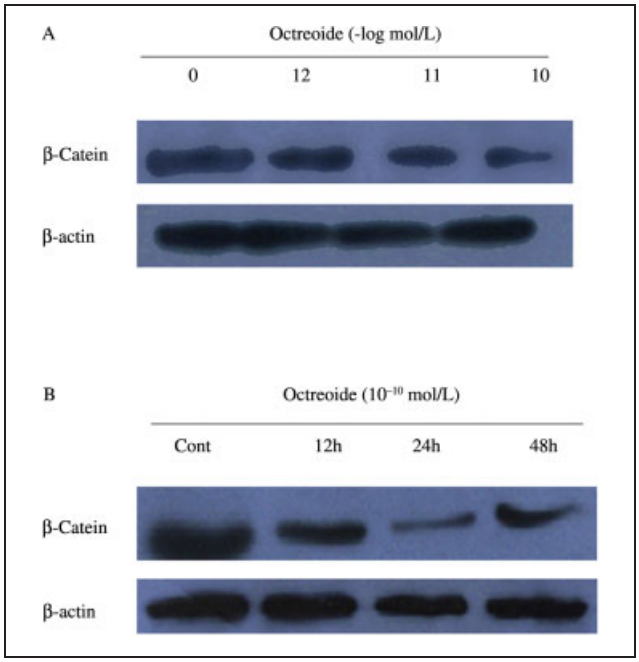

Fig. 3: Effect of octreotide on β-catenin protein levels in SW480 cells. (A) Cells were treated with 0, 1×10^{-12}, 1×10^{-11} and 1×10^{-10} mol/L octreotide for 48 h, respectively. Western blot analysis demonstrated that down-regulation of the β-catenin protein by octreotide was concentration-dependent. (B) Cells were treated with 1×10^{-10} mol/L octreotide for 0, 12, 24 and 48 h, respectively. Western blot analysis showed that down-regulation of the β-catenin protein by octreotide was time-dependent. Results are representatives of 3 independent experiments

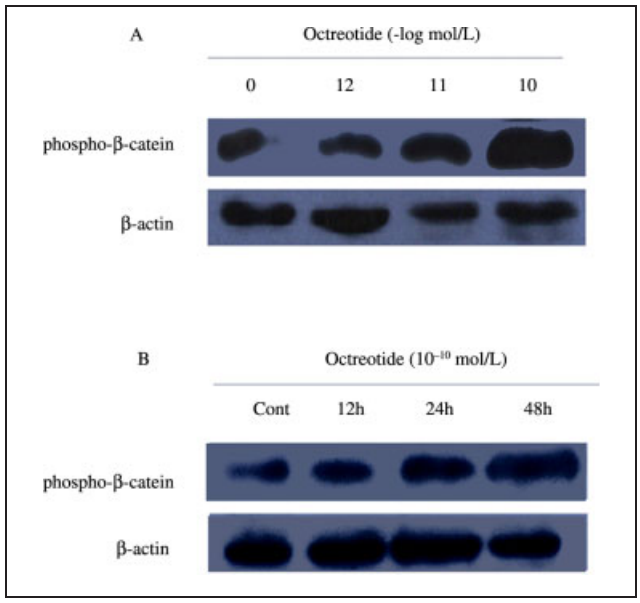

Fig. 4: Effect of octreotide on phospho-β-catenin protein levels in SW480 cells. (A) Cells were treated with 0, 1×10^{-12}, 1×10^{-11} and 1×10^{-10} mol/L octreotide for 48 h. Western blot analysis demonstrated that up-regulation of the phospho-β-catenin protein by octreotide was concentration-dependent. (B) Cells were treated with 1×10^{-10} mol/L octreotide for 0, 12, 24 and 48 h, respectively. Western blot analysis showed that up-regulation of the β-catenin protein by octreotide was time-dependent. Results are representatives of 3 independent experiments

3. Discussion

Octreotide was the first somatostatin analogue introduced for clinical use. Experiments *in vitro* and *in vivo* have revealed that it inhibits the growth of a variety of tumors (Lamberts et al. 1996). The present results show that oc-

treotide potentially inhibits the growth and induced apoptosis of human colonic cancer cell cline SW480 cells *in vitro*. The effect of octreotide on SW480 cells was the most significant at 1×10^{-10} mol/L. Moreover, our results revealed that the control of cell cycle regulation exerted by octreotide specifically targeted the G_1/S transition. Thus, we conclude that the growth inhibitory effect of octreotide on SW480 cells is associated with the induction of apoptosis and cell cycle arrest.

Although there are several explanations regarding the molecular mechanisms by which somatostatin inhibits the growth of tumor (Wang et al. 2003; Theodoropoulou et al. 2006; Petrucci et al. 2000; Hipkin et al. 2000; Thangaraju et al. 1999), they are not well understood. Consequently, it is necessary to investigate whether octreotide effects the Wnt/β-catenin signaling pathway. β-Catenin is the central player of the Wnt/β-catenin signaling pathway. In normal and unstimulated cells, the majority of β-catenin protein is present in cell-cell junctions with very little cytoplasmic or nuclear fractions. Cytosolic β-catenin is degraded through phosphorylation-dependent ubiquitination and subsequent proteosomal clearance (Major et al. 2007). Ubiquitin-dependent degradation of β-catenin strictly depends on NH2-terminal serine/threonine phosphorylation which is triggered by a multiprotein destruction complex composed of Axin, adnomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK-3β) and CK1α (Polakis 2000; Willert and Jones 2006). In the complex, β-catenin is phosphorylated first at Ser-45 by CK1α and then at Ser-33, Ser-37, and Thr-41 by GSK-3β, and subsequently, phosphorylated β-catenin is ubiquitinated by the β-transducing repeat-containing protein (β-TrCP) E3 ubiquitin ligase and is degraded by the proteasome (Willert and Jones 2006). However, in the presence of Wnt signal, phosphorylation of β-catenin by GSK-3β is inhibited, leading to the accumulation of β-catenin in the cytoplasm and subsequent translocation into the nucleus, where it binds to members of the T cell factor (TCF) and lymphoid enhancer factor (LEF) family of transcription factors to activate Wnt/β-catenin responsive genes such as c-Myc (He et al. 1998), cyclin D1 (Tetsu and McCormick 1999; Shtutman et al. 1999), etc. Thus, serine/threonine phosphorylation of β-catenin controls β-catenin protein level and Wnt signaling (Liu et al. 2004). The results presented here demonstrated that in octreotide-treated SW480 cells, the β-catenin protein levels decreased and phosphorylated β-catenin protein levels increased in a concenntration-dependent and time-dependent manner, suggesting that octreotide attenuates Wnt/β-catenin signaling activity and reduced β-catenin levels may be the result of enhanced β-catenin phosphorylation.

To investigate effects of octrotide on gene expression within the Wnt/β-catenin signaling pathway, we used Wnt signaling pathway microarrays. In Wnt/β-catenin signaling pathway, APC gene encodes the protein which antagonizes the transcriptional activity of β-catenin since it plays a key role in the regulation of β-catenin turnover and promoting its nuclear export (Polakis 2000; Tetsu and McCormick 1999). Axin gene family including Axin1 and Axin2 encode proteins which are essential for the degradation of β-catenin and thus are negative regulators of Wnt/β-catenin signaling pathway (Salahshor and Woodgett 2005; Kikuchi 1999). CK1α phosphorylation of β-catenin Ser45 is required for subsequent GSK-3β phosphorylation of Ser33, Ser37, and Thr41 (Liu et al. 2004). Moreover, recent data reported that CK1-dependent phosphorylation inhibited LEF-1/β-catenin transcriptional activity (Hämmerlein et al. 2005). Therefore, CK1 is essential to β-catenin degradation and a negative regulator in the Wnt signaling pathway as well. β-TrCP (BTRC) plays a central role in recognizing phosphorylated β-catenin by GSK-3β and recruiting phosphorylated β-catenin for degradation (Liu et al. 1999). C-terminal binding protein (CtBP) is a transcriptional corepressor, which can inhibit Wnt/β-catenin signaling by binding to TCFs or preventing β-catenin from binding to TCF (Valenta et al. 2003; Hamada and Bienz 2004). Dickkopf 1(DKK1) encodes secreted protein which blocks Wnt/β-catenin signaling pathway by inhibiting Wnt coreceptor – Low density lipoprotein receptor-related protein 5/6 (LRP5/6) (Niehrs 2006). Thus, these genes negatively regulate Wnt/β-catenin signaling pathway. Our microarray results showed that octreotide significantly up-regulated APC2, Axin gene family, BTRC, CSNK1 gene family, CTBP1, DKK1, etc. Thereby, these data suggest that octreotide inhibits the Wnt/β-catenin signaling pathway.

However, some components positively regulate Wnt/β-catenin signaling. The Wnt gene family including nineteen Wnt genes in mammalian genomes encodes secreted glycoproteins which initiate Wnt/β-catenin signaling by interacting with Frizzled (FZD) cell surface transmembrane receptor together with LRP-5/6 (Reya and Clevers 2005; Nelson and Nusse 2004). Recent data have revealed that Wnt2B and FZD10 are positive regulators of the Wnt/β-catenin signaling pathway (Katoh et al. 2001; Terasaki et al. 2002) and FZD4 expression may play a critical role in responses to Wnt signaling in the tumor microenvironment (Planutis et al. 2007). Our microarray results also demonstrated that octreotide significantly down-regulated the above mentioned genes which positively regulate Wnt/β-catenin signaling pathway. Thus, these results suggest as well that octreotide inhibits the Wnt/β-catenin signaling pathway .

WISP-1 is a β-catenin regulated gene which contributes to tumorigenesis (Xu et al. 2000). CyclinD1 is a target gene modulated by the Wnt/β-catenin signaling pathway (Tetsu and McCormick 1999; Shtutman et al. 1999). The results presented revealed that octreotide significantly down-regulated expression of the WISP-1 and cyclin D1/2 genes, confirming that octreotide can inhibit the Wnt/β-catenin signaling pathway. Interestingly, the present study revealed that, in octreotide-treated SW480 cells, the β-catenin mRNA levels remained constant, but β-catenin protein levels significantly decreased. Thus, the results indicate that octreotide post-transcriptionally regulates β-catenin.

In conclusion, this is the first time that the effect of octreotide on the Wnt/β-catenin signaling pathway has been investigated. The present study demonstrates that octreotide can inhibit growth, induce apoptosis, and arrest G_1/S transition of colonic cancer cells. Furthermore, our data show that octreotide inhibits the growth of colonic cancer SW480 cells by modulating the Wnt/β-catenin pathway. Thus, the findings further interpret the underlying molecular mechanisms of antineoplastic actions of somatostatin and will guide the development of new and more effective somatostatin analogues.

4. Experimental

4.1. Reagents

Human colonic cancer cell line SW480 (In SW480 cells, Wnt/β-catenin signaling pathway is constitutively activated because of high nuclear β-catenin expression levels (Korinek et al. 1997)) was obtained from China Center for Type Culture Collection. RPMI 1640 medium and fetal calf serum were obtained from Gibco (Carlsbad, CA, USA). Octreotide was provided by Novartis Pharma AG (Basel, Switzerland). 3-(4,5-imethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and propidium iodide

(PI) were purchased from Sigma. Rabbit polyclonal anti-human β-catenin and anti-phosph-catenin (Ser33/37Thr41) was provided by Cell Signaling Technology (Beverly, MA). Horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

4.2. Cell culture

Human colonic cancer SW480 cells were grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum, 100 U/ml penicillin, 100 U/ml streptomycin. The cells were maintained at 37 °C in a incubator with a humidified atmosphere containing 5 % CO_2. The culture medium change was performed every 1–2 days.

4.3. MTT assay

The growth inhibitory effect of somatostatin on SW480 cells was determined by MTT assay. Briefly, exponentially growing SW480 cells (5000 cells per well) were seeded in 96-well microtiter plates. After exposure to octreotide (0, 1×10^{-12} mol/L, 1×10^{-11} mol/L, 1×10^{-10} mol/L, 1×10^{-9} mol/L, 1×10^{-8} mol/L, 1×10^{-7} mol/L) for 44 h, 20 μl MTT solution (5 g/L in PBS) was added to each well and cells were incubated for another 4 h at 37 °C. Following careful removal of the supernatant, 100 μl dimethyl sulfoxide (DMSO) was added to each well and plates were vibrated for 10 min. Finally the plates were measured in enzyme-linked immunity implement (Elx800, Bio- TEK,VT,USA) at 570 nm. The inhibitory rate of cell growth (IR) was calculated according to the following equation: IR = [1 -(mean value of treatment group/mean value of control group)] ×100 %. Triplicate wells were analyzed per each concentration and Experiments were carried out triplicately.

4.4. Flow cytometric analysis

Cell apoptosis and cell cycle distribution were measured by flow cytometric assays. Cells treated with octreotide were collected, washed twice with ice-cold phosphate-buffered saline (PBS) (pH 7.4), and fixed in 70% ethanol at 4 °C. overnight. The cells were centrifuged and stained with propdiumiodide (50 mg/L) for 30 min at 4 °C. in the dark. For each sample, at least 10,000 events were collected and analyzed. The apopotic rate and cell cycle distribution were analysed by FACscan flow cytometer (Becton-Dickinson Corp, USA) , and data were analyzed using the CellQuest software (Becton-Dickinson Corp, USA). Experiments were carried out in triplicate for each data point.

4.5. Oligonucleotide microarray analysis

GEArray Q series human oligonucleotide Wnt signaling pathway microarrays (Cat No: OHS-043, SuperArray Inc, MD, USA) were employed for analysis of gene expression change in SW480 cells treated with octreotide. The microarrays employed contained 128 genes in Wnt signaling pathway. Total RNA of SW480 cells was isolated using TRIzol Reagent (Invitrogen Life Technologies). There were two isolated RNA samples used for this experiment: (A) control RNA was isolated from cells treated by normal saline, whereas (B) octreotide -treated RNA was isolated from cells treated by 1×10^{-10} mol/L octreotide for 4 h. The biotin-16-dUTP-labeled cRNA (complementary RNA) probes were synthesized according to instruction from the manufacturer. Hibridization and the signal detection steps were perfomed according to the manufacturer's instructions as described previously (Zhang et al. 2007). Data analysis was completed by the web-based completely integrated GEArray Expression Analysis Suite. Genes were identified as differentially expressed if their fold change was ≥ 2 or ≤ 0.5.

4.6. Western blot analysis

Cell lysates were prepared for western blot analysis of β-catenin and phosph-β-catenin using whole cellular protein extraction kits (Beyotime Institute of Biotechnology, China). The concentration of protein in each cell lysate was determined using a BCA protein assay kit (Beyotime Institute of Biotechnology, China). An identical amount of protein (40 μg) from each sample was loaded onto a 6% sodium dodecyl sulfate-polyacrylamide gel and electrotransferred to polyvinylidene difluoride membranes (0.45 μm, Millipore, USA). The membranes were blocked with the blocking buffer (5% nonfat milk in PBS containing 0.1% Tween 20) for 2 h at room temperature and then incubated with anti-β-catenin, anti-phosph-β-catenin or anti-actin specific polyclonal IgG primary antibody overnight at 4 °C. The membranes were washed three times, then incubated with horseradish peroxidase (HRP)-conjugated IgG secondary antibody for 1 h at room temperature. The antibody-associated protein bands were developed using LumiGLO® Reagent and Peroxide detection system (Cell Signaling Technology), according to the manufacturer's instructions, and were then visualized by exposure to X-ray film. Blots were scanned and analyzed using the AlphaImager 2200 (Alpha Innotech) for the measurement of the band intensities. Experiments were performed triplicately.

4.7. Statistical analysis

All data were presented as means ± SD (standard deviation). Differences between the groups were assessd with one-way ANOVA and Student's t test using the SPSS 13.0 for Windows (SPSS Inc,Chicago,IL,USA). Differences are considered significant at $P < 0.05$.

Acknowledgments: This work was supported by grants from the natural science Foundation of Hunan province (07JJ6158) and the Foundation of Hunan provincial Department of Eduction. We thank Kangchen Biotechonology Company (Shanghai, China) for technical support on microarray technical service.

References

Albanese C, D'Amico M, Pestell R, Ben-Ze'ev A (1999) The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. Proc Natl Acad Sci USA 96: 5522–5527.

Behrens J, Lustig B (2004) The Wnt connection to tumorigenesis. Int J Dev Bio 48: 477–487.

Hamada F, Bienz M (2004) The APC tumor suppressor binds to C-terminal binding protein to divert nuclear beta-catenin from TCF. Dev Cel 7: 677–685.

Hämmerlein A, Weiske J, Huber O (2005) A second protein kinase CK1-mediated step negatively regulates Wnt signaling by disrupting the lymphocyte enhancer factor-1/beta-catenin complex. Cell Mol Life Sci 62: 606–618.

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW (1998) Identification of c-MYC as a target of the APC pathway. Science 281:1509–1512

Hipkin RW, Wang Y, Schonbrunn A (2000) Protein kinase C activation stimulates the phosphorylation and internalization of the sst2A somatostatin receptor. J Biol Chem 275: 5591–5599.

Jacks T, Weinberg RA (1996) Cell-cycle control and its watchman. Nature 381: 643–644.

Katoh M, Kirikoshi H, Terasaki H, Shiokawa K (2001) WNT2B2 mRNA, up-regulated in primary gastric cancer, is a positive regulator of the WNT- beta-catenin-TCF signaling pathway. Biochem Biophys Res Commun 289: 1093–1098.

Kikuchi A (1999) Roles of Axin in the Wnt signaling pathway. Cell Signal 11: 777–788.

Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, Vogelstein B, Clevers H (1997) Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC-/-colon carcinoma. Science 275: 1784–1787.

Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ (1996) Octreotide. N Engl J Med 334: 246–254.

Liu C, Kato Y, Zhang Z, Do VM, Yankner BA, He X (1999) beta-Trcp couples beta-catenin phosphorylation-degradation and regulates Xenopus axis formation. Proc Natl Acad Sci U S A 96: 6273–6278.

Liu C, Li Y, Semenov M, Han C, Baeg GH, Tan Y, Zhang Z, Lin X, He X (2002) Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108: 837–847.

Liu HL, Huo L, Wang L (2004) Octreotide inhibits proliferation and induces apoptosis of hepatocellular carcinoma cells. Acta Pharmacol Sin 25: 1380–1386.

Major MB, Camp ND, Berndt JD, Yi X, Goldenberg SJ, Hubbert C, Biechele TL, Gingras AC, Zheng N, Maccoss MJ, Angers S, Moon RT (2007) Wilms tumor suppressor WTX negatively regulates WNT/beta-catenin signaling. Science 316: 1043–1046.

Nelson WJ, Nusse R (2004) Convergence of Wnt, beta-catenin, and cadherin pathways. Science 303: 1483–1487.

Niehrs C (2006) Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 25: 7469–7481.

Pagotto U, Stalla GK (2006) Octreotide, a somatostatin analogue, mediates its antiproliferative action in pituitary tumor cells by altering phosphatidylinositol 3-kinase signaling and inducing Zac1 expression. Cancer Res 66: 1576–1582.

Petrucci C, Cervia D, Buzzi M, Biondi C, Bagnoli P (2000) Somatostatin-induced control of cytosolic free calcium in pituitary tumour cells. Br J Pharmacol 129: 471–484.

Planutis K, Planutiene M, Moyer MP, Nguyen AV, Pérez CA, Holcombe RF (2007) Regulation of norrin receptor frizzled-4 by Wnt2 in colon-derived cells. BMC Cell Biol 8: 12.

Polakis P (2000) Wnt signaling and cancer. Genes Dev 14: 1837–1851.

Reya T, Clevers H (2005) Wnt signaling in stem cells and cancer. Nature 434: 843–850.

Salahshor S, Woodgett JR (2005) The links between axin and carcinogenesis. J Clin Pathol 58: 225–236.

Segditsas S, Tomlinson I (2006) Colorectal cancer and genetic alterations in the Wnt pathway. Oncogene 25: 7531–7537.

Shapiro GI, Harper JW (1999) Anticancer drug targets: cell cycle and checkpoint control. J Clin Invest 104: 1645–1653.

Shtutman M, Zhurinsky J, Simcha I, Valenta T, Lukas J, Korinek V (2003) HMG box transcription factor TCF-4's interaction with CtBP1 controls the expression of the Wnt target Axin2/Conductin in human embryonic kidney cells. Nucleic Acids Res 31: 2369–2380.

Tejeda M, Gaál D, Hullán L, Hegymegi-Barakonyi B, Kéri G (2006) Evaluation of the antitumor efficacy of the somatostatin structural derivative TT-232 on different tumor models. Anticancer Res 26: 3477–3483.

Terasaki H, Saitoh T, Shiokawa K, Katoh M (2002) Frizzled-10, up-regulated in primary colorectal cancer, is a positive regulator of the WNT – beta-catenin – TCF signaling pathway. Int J Mol Med 9: 107–112.

Tetsu O, McCormick F (1999) Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398: 422–426.

Theodoropoulou M, Zhang J, Laupheimer S, Paez-Pereda M, Erneux C, Florio T, Thangaraju M, Sharma K, Liu D, Shen SH, Srikant CB (1999) Interdependent regulation of intracellular acidification and SHP-1 in apoptosis. Cancer Res 59: 1649–1654.

Wang CH, Tang CW, Liu CL, Tang LP (2003) Inhibitory effect of octreotide on gastric cancer growth via MAPK pathway. World J Gastroenterol 9: 1904–1908

Willert K, Jones KA (2006) Wnt signaling: is the party in the nucleus? Genes Dev 20: 1394–1404.

Xu L, Corcoran RB, Welsh JW, Pennica D, Levine AJ (2000) WISP-1 is a Wnt-1- and beta-catenin-responsive oncogene. Genes Dev 14: 585–595.

Zhang Y, Hu Z, Ye M, Pan Y, Chen J, Luo Y, Zhang Y, He L, Wang J (2007) Effect of poly(ethylene glycol)-block-polylactide nanoparticles on hepatic cells of mouse: low cytotoxicity, but efflux of the nanoparticles by ATP-binding cassette transporters. Eur J Pharm Biopharm 66: 268–280.