Curcumin inhibits tumor epithelial-mesenchymal transition by downregulating the Wnt signaling pathway and upregulating NKD2 expression in colon cancer cells

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Abstract. Tumor invasion and metastasis are closely associated with epithelial-mesenchymal transition (EMT). EMT refers to epithelial cells under physiological and pathological conditions that are specific to mesenchymal transition. Curcumin inhibits EMT progression via Wnt signaling. The Wnt signaling pathway is a conservative EMT-related signaling pathway that is involved in the development of various tumors. In the present study, MTS assays were employed to analyze the proliferation of curcumin-treated cells. Naked cuticle homolog 2 (NKD2), chemokine receptor 4 (CXCR4) and antibodies associated with EMT were examined in SW620 colorectal cancer cell lines using western blot analysis and real-time qPCR. NKD2 small-interfering RNA (siRNA) and CXCR4 expression plasmid was synthesized and transfected into the colorectal cancer cell lines, and NKD2 and CXCR4 expression levels were detected. The results showed that curcumin significantly inhibited the proliferation of colorectal cancer cells and upregulated the expression of NKD2 in SW620 colorectal cells and in the xenograft, resulting in the downregulation of key markers in the Wnt signaling. In addition, the progression of EMT was inhibited due to the overexpression of E-cadherin as well as the downregulation of vimentin. Curcumin also inhibited tumor metastasis by downregulating the expression of CXCR4 significantly. The results suggested involvement of the NKD2-Wnt-CXCR4 signaling pathway in colorectal cancer cells. In addition, curcumin is inhibit this signaling and the development of colorectal cancer.

Introduction

Change to people's lifestyle and dietary habits have led to an increase in the incidence of colon cancer (1-3). Although there have been advances in the study of this type of cancer, colon cancer is a common gastrointestinal malignancy with a high mortality rate (4), owing to metastasis. Therefore, the main challenge is identification of methods to inhibit the metastasis of colon cancer effectively in the clinic. Metastasis occurs due to the interaction of multiple genes and is a complex system (5). However, the exact mechanism underlying metastasis of the disease remains to be elucidated.

Tumor invasion and metastasis are closely associated with epithelial-mesenchymal transition (EMT). EMT refers to epithelial cells under physiological and pathological conditions that are specific to mesenchymal transition (6). EMT has been associated with the initial stage of tumor metastasis, whereby tumor cells have lost the characteristics of epithelial cells and cell polarity, and attained the characteristics of mesothelial and invade adjacent tissues (7). This process is associated with change at the molecular level and the occurrence of cell morphology (8). In the evolution process of tumor growth, EMT includes alteration of cell polarity, reconstruction of the cytoskeleton, loss of intercellular adhesion and destruction of the tumor basement membrane and extracellular matrix between the cells (9), and mesenchymal phenotypes, such as high migration, invasion, resistance apoptosis and degradation of extracellular matrix. Therefore, EMT provides optimal conditions for invasion and metastasis, such as in situ colon, breast, lung, and liver cancer, and plays an important role in tumor invasion and metastasis (12-14).

Chemokine receptor 4 (CXCR4) is a member of the superfamily of the seven-transmembrane G-protein coupled receptors
and is the only receptor of CXCL12. CXCR4 is involved in a variety of physiological and pathological processes. The CXCL12/CXCR4 biological axis structure participates in the infiltration of inflammatory cells and lymphocytes, as well as migration and homing (15,16). It also plays an important role in mediating tumor invasion and metastasis (17-20). Thus, CXCR4 is a common chemokine receptor in tumor cells, and its expression is increased significantly in gastric, lung, and breast cancer, as well as soft tissue sarcoma tumor cells.

In recent years, significant progress has been made in the study of the anti-tumor effects of traditional Chinese medicine, particularly with regard to natural plant-derived anticancer drugs that have become a global hot spot. Curcumin is a plant polyphenol that is extracted from the zingiberaceae plant, *Curcuma longa* root turmeric. Curcumin has various effects including antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-aging, and also eliminates free radicals (21).

Numerous studies have been conducted on the anti-tumor effects of curcumin and its mechanism worldwide. The findings have shown that curcumin obviously inhibits tumor invasion and metastasis in different tumor tissues (22,23).

At present, some studies have reported that curcumin inhibits EMT in tumors (24). The Wnt signaling pathway is a conservative EMT-related signaling pathway that plays an important role in the development of a variety of tumors. The β-catenin is the hub of the molecule in the Wnt signaling pathway, which mediates the membrane and facilitates the transfer of molecules from the cytoplasm into the nucleus in the Wnt pathway (25,26). Curcumin also reduced the level of β-catenin gene expression significantly; thus, it has anti-tumor effects through the inhibition of the Wnt signaling pathway (18,27,28). Therefore, the mechanism of action of curcumin with regard to tumor inhibition remains to be determined. Using gene expression profiles, we analyzed the changes of tumor cell expression profiles of curcumin-treated cells, and identified inhibitors of the Wnt pathway naked cuticle homolog 2 (NKD2) (29). NKD2, as the Wnt signaling pathway regulation of gene suppression, significantly delayed the mitosis of HeLa cells (30). Thus, curcumin may inhibit the Wnt signaling pathway by regulation of the expression of NKD2. Previous findings showed that curcumin can also reduce the expression of CXCR4 in tumor cells (31,32). Thus, according to the preliminary experiment, the mechanism of action of curcumin in colon cancer cells is likely to inhibit the Wnt signaling pathway by affecting NKD2 gene expression, EMT and the expression of CXCR4 in tumor cells and eventually inhibiting tumor invasion and metastasis.

**Materials and methods**

**Reagents.** Antibodies purchased for the present study included: axin and TCF4 (Cell Signaling Technology, Boston, MA, USA), β-catenin (Epitomics, San Francisco, CA, USA), NKD2 (Novus International, St. Charles, MO, USA), CXCR4 antibody (Thermo Fisher Scientific, Waltham, MA, USA), and β-actin (BD Biosciences, New York, NY, USA). Curcumin was purchased from Sigma (Beijing, China), and NKD2 small-interfering RNA (siRNA) from Shanghai GenePharma Co., Ltd. (Shanghai, China). The following two pairs of siRNA were designed: NKD2-homo-962 sense, 5'-CAGAUACAC AUGCCGUACATT-3' and antisense, 5'-GUACGGCAU GGUACUGGTT-3'; NKD2-homo-480 sense, 5'-CACGCC CUAUGACUUGACTT-3' and antisense, 5'-GUCAAGUCA UAGAGCGUGTT-3'. NKD2 and CXCR4 primers were purchased from Biosune Biotechnology Co., Ltd. (Shanghai, China). NKD2 primers used were: 5'-ATGCGCTCGTCA ACCACTCC-3' and 5'-TCTGCCAGTTACCCTTCCATC-3' and the length of the product was 151 bp. CXCR4 primers used were: 5'-CCGGTGCAAACCTGTTACTTT-3' and 5'-GAC GCCAACATAGACCACCT-3' and the length of the product was 188 bp.

The CXCR4 expression plasmid (CXCR4 NM_001008540) was purchased from Shanghai GenePharma Co., Ltd. The Wnt pathway activator (WAY 262611) was purchased from Abcam (Shanghai, China).

SW620 human colon cancer cells were provided by the Zhejiang Provincial Key Laboratory of Gastroenterology (Zhejiang, China). The instruments and equipment employed were provided by the Zhejiang Key Laboratory of Biotherapy (Zhejiang, China).

**Cell cultures and transfection.** SW620 human colon cancer cells were routinely cultivated in RPMI-1640 medium containing 10% FBS, at 37°C with 5% CO₂. The cells were passaged at 80% confluency, using 1 mmol/l EDTA with 0.025% trypsin for 3-5 min, and then sub-cultured at a ratio of 1:3-1:5. Cells at the logarithmic growth phase were collected for experiments. Once the cell density had increased to 30-50%, Lipofectamine™ 2000 liposome transfection kit (Invitrogen Life Technologies, Carlsbad, CA, USA) was used to transfect the plasmid into the cells according to the manufacturer's instructions. Following cultivation for 2 days, the original culture medium was discarded and screened using RPMI-1640 culture medium.

**MTS assay.** SW620 cells in the logarithmic growth phase were collected and the cell concentration was adjusted to 5x10⁴/mL. Subsequently, 200 µl of the abovementioned cell suspension was added into each well of several 96-well plates. When the cells adhered to the wall, curcumin (5-40 µmol/l) was added to the cells and these were cultured for 24 h. Five parallel wells were established for each drug concentration. The supernatant was aspirated and 100 µl MTS was added to the cells and these were cultured for 4 h. The experiments were repeated 3 times. The cell viability was calculated using the formula: viability (%) = [treated d-blank]/(control-blank)] x 100%. The experiments were performed in triplicate.

**Western blot analysis.** For biochemical analysis, the cells were washed with ice-cold phosphate-buffered saline (PBS; Beyotime Institute of Biotechnology, Jiangsu, China) and lysed in radioimmunoprecipitation assay lysis buffer [50 mM Tris, pH 7.4; 150 mM NaCl; 1% Triton X-100; 1% sodium deoxycholate; 0.1% sodium dodecyl sulfate (SDS); Beyotime Institute of Biotechnology]. The lysates were kept on ice for 30 min, followed by centrifugation at 12,000 x g for 25 min at 4°C.
The clear lysate was then collected and β-catenin, axin, TCF4, E-cadherin, vimentin, NKD2, CXCR4 and β-actin proteins were separated by 12% SDS-polyacrylamide gel electrophoresis (30-50 µg protein/lane) and transferred to a polyvinylidene fluoride membrane (Beyotime Institute of Biotechnology). The membranes were incubated in 5% milk for 1.5 h, and then with β-catenin (1:1000), axin (1:1000), TCF4 (1:2000), E-cadherin (1:2000), vimentin (1:1000), NKD2 (1:1000), CXCR4 (1:1500) and β-actin (1:2000) antibodies diluted in non-fat milk overnight. The membranes were then washed with Tris-buffered saline with Tween-20 (Beyotime Institute of Biotechnology) and incubated with the HRP-conjugated secondary antibodies for 2 h. Immunoreactive proteins were visualized using a BeyoECL Plus kit (Beyotime Institute of Biotechnology).

Reverse transcription-qPCR (RT-qPCR) analysis. The cells were washed with ice-cold PBS and total RNA was extracted from the SW620 human colon cancer cells using TRIzol reagent (Invitrogen-Technologies, Carlsbad, CA, USA) and quantified by UV spectrophotometer (Lengguang, Shanghai, China). In addition, the purity and RNA concentration were measured by the UV spectrophotometer. cDNA was obtained by reverse transcription. The PCR conditions were: 10 min at 25°C, 2 h at 37°C, 5 min at 85°C and maintained at 4°C. cDNA was used as a template for PCR amplification of the target genes and GAPDH was used as the standard control. Amplification conditions were: Denaturation for 2 min at 94°C, 30 sec at 94°C, 30 sec at 52°C, 5 sec at 72°C and, following 40 cycles, a total extension of 4 min at 72°C (33).

Statistical analysis. Statistical analysis was conducted using SPSS, version 18.0 (SPSS, Inc., Chicago, IL, USA). Each experiment was performed ≥3 times. Data are indicated as mean values ± standard deviation and any differences were analyzed using a Student's t-test. P<0.05 was considered to indicate statistically significant results.

Results

Effect of curcumin on the viability of SW620 cells. We initially investigated the effect of curcumin on the proliferation of SW620 cells. SW620 cells were treated for 24 h with graded concentrations of curcumin (0-40 µmol/l) and the cell viability was measured using an MTS assay. The results showed the cell viability s was inhibited by curcumin (Fig. 1). An increase in the concentration of curcumin led to a significant change in the rate of inhibition. The results suggested that curcumin effectively inhibited SW620 cell viability.

Curcumin inhibits Wnt signaling and expression of markers of EMT in SW620 cells. The phenotypic alterations occurring suggested that SW620 cells had undergone EMT. Thus, there were two expressions of specific EMT markers, including the epithelial marker E-cadherin and mesenchymal marker vimentin (34). Due to the conservative EMT-related signaling pathway, the β-catenin, axin, and TCF4 genes, which were associated with the Wnt signaling pathway, were selected. The β-catenin is the hub of the molecule in the Wnt signaling pathway and axin a negative regulator of the Wnt signaling pathway (35,36). TCF4 is a Wnt pathway transcription factor and is highly expressed in colorectal cancer. To determine the effect of curcumin on the Wnt signaling pathway and EMT, we used different concentrations of curcumin to treat SW620 cells for 24 h, and measured the protein expression using western blot analysis. We found that β-catenin, TCF4 and vimentin protein expression were significantly reduced, while the axin and E-cadherin protein expression were increased in the curcumin group (Fig. 2). By increasing the concentration of curcumin, the protein expression was more significantly altered. Thus, curcumin is capable of inhibiting the Wnt signaling pathway and EMT in SW620 cells.

Curcumin increases the expression of NKD2 and inhibits the expression of CXCR4 in SW620 cells. NKD2 acts as an inhibitor of the Wnt signaling pathway, playing an important role in tumor of EMT (37). Chemokine receptor CXCR4 acts as an α-chemokine receptor specific for stromal-derived-factor-1. CXCR4 is highly expressed in various tumors and promotes tumor growth and metastasis (38). Therefore, to clarify whether curcumin significantly affected the genes, we carried out a biological analysis. We used different concentrations of curcumin to treat SW620 cells for 24 h and measured the protein expression of the genes using western blot analysis and mRNA expression RT-qPCR analysis. The NKD2 protein expression was significantly increased, while the CXCR4 protein expression was reduced in the curcumin group (Fig. 3A). The CXCR4 mRNA expression was reduced in the curcumin group (Fig. 3B) while the NKD2 mRNA expression was significantly increased in the curcumin group (Fig. 3C). Thus, curcumin increases the expression of NKD2 in the WNT signaling pathway and inhibits that of CXCR4 in the SW620 cells.

NKD2 siRNA transfection and Wnt pathway activator promotes the Wnt signaling pathway. We demonstrated whether the NKD2 siRNA and Wnt pathway activator affects the Wnt signaling pathway. SW620 cells were transfected with NKD2 siRNA for 48 h and the Wnt pathway activator for 24 h,
respectively, and the Wnt signaling pathway protein expression levels were detected. Western blot analysis (Fig. 4A-B) revealed that the protein expression of β-catenin and TCF4 was significantly upregulated in the NKD2 siRNA and Wnt pathway activator group compared to the control group. The results suggested that NKD2 siRNA can promote the Wnt signaling pathway by silencing the NKD2 gene.

**NKD2 siRNA transfection reverses curcumin inhibition of the Wnt signaling pathway and EMT in the SW620 cells.** Curcumin has been previously found to inhibit Wnt signaling pathways and EMT in 95D cells (39). Therefore, we examined whether curcumin inhibit this pathway by influencing the expression of NKD2. Initially, we transfected the SW620 cells for 48 h with NKD2 siRNA and then treated the cells
with curcumin (10 µmol/l) for 24 h. β-catenin and TCF4 protein expression was significantly increased (Fig. 5A). The E-cadherin protein expression was reduced while the vimentin protein expression was increased in the NKD2 siRNA transfection group (Fig. 5B). Thus, previous results (39) together with those of the present study demonstrated that curcumin inhibits the Wnt signaling pathway and EMT by increasing the expression of NKD2.

**NKD2 siRNA transfection increases the expression of CXCR4 in the SW620 curcumin-treated cells.** CXCR4 is closely associated with EMT and the two induce and promote each other. To confirm whether curcumin inhibited the expression of CXCR4 by influencing the expression of NKD2, we transfected NKD2 siRNA for 48 h, followed by treatment with curcumin (10 µmol/l) for 24 h in SW620 cells. We measured the protein expression using western blot analysis and the mRNA expression by RT-qPCR analysis. The CXCR4 protein expression was significantly increased in the NKD2 siRNA transfection group (Fig. 6A). The CXCR4 mRNA expression was increased in the NKD2 siRNA transfection group (Fig. 6B). Thus, our results demonstrated that curcumin inhibited the expression of CXCR4 by increasing the expression of NDK2 in the SW620 cells.
CXCR4 expression plasmid transfection increases the viability of SW620 curcumin-treated cells. As a chemokine receptor, CXCR4 reflects the invasion and metastasis of tumor cells directly. Therefore, we investigated whether CXCR4 expression plasmid affected the viability of SW620 cells treated with curcumin in vitro. We transfected CXCR4 expression plasmid for 72 h, followed by treatment with the SW620 cells for 24 h with curcumin (10 µmol/l). Cell viability was measured using an MTS assay. As shown in Fig. 7, cell viability was increased by CXCR4 expression plasmid. The results suggested that CXCR4 expression plasmid effectively increased cell viability. Thus, curcumin inhibits the viability of SW620 in vitro by reducing CXCR4 gene expression.

CXCR4 expression plasmid transfection reverses curcumin inhibition of EMT and promotes the expression of CXCR4 in the SW620 cells. In the process of tumor invasion and metastasis, CXCR4 is highly expressed in metastatic tissues, and tumor invasion and metastasis are closely associated with EMT. Previous experiments (40) confirmed that curcumin is capable of inhibiting EMT in HCT116 cells. Thus, we transfected CXCR4 expression plasmid to observe whether it is able to reverse curcumin inhibition of EMT. We transfected CXCR4 expression plasmid for 72 h in SW620 cells, followed by treatment with curcumin (10 µmol/l) for 24 h. We measured the protein expression of EMT and CXCR4 expression using western blot analysis and RT-qPCR analysis. E-cadherin protein expression was reduced while the protein expression of vimentin and CXCR4 was increased in the CXCR4 expression plasmid transfection group (Fig. 8A). The CXCR4 mRNA expression was significantly increased in the CXCR4 expression plasmid transfection group (Fig. 8B). Thus, CXCR4 expression plasmid is capable of inducing EMT and promote the expression of CXCR4 in SW620 cells.

Discussion

Colon cancer, the second most frequent cancer, is a common gastrointestinal malignancy that occurs in the junction of the rectum and sigmoid colon. The highest incidence of colon cancer occurs in middle-aged and elderly individuals (41). Patients with advanced colon cancer are prone to distant metastases, particularly in the liver, lungs, and peritoneal metastasis (40). The present study indicate that EMT plays a key role in tumor metastasis. EMT refers to epithelial cells that acquire mesenchymal, fibroblast-like phenotypes with reduced cell-to-cell adhesion, and loss of cell polarity, with...
increased migration and invasiveness (42). Furthermore, the Wnt signaling pathway, one of the important pathways in EMT, is important in embryogenesis and human diseases including various types of cancer. Wnt signals can be transduced to the canonical Wnt pathway for cell-fate determination or to the non-canonical Wnt pathway for the regulation of tissue polarity and cell movement (43). In a previous study, Deng et al reported that celecoxib inhibited the Wnt signaling pathway in colon cancer (44).

Curcumin is an antioxidant polyphenol derived from several curcuma species, commonly known as turmeric (Curcuma longa), which has been shown to inhibit carcinogen activation and angiogenesis, modulate cell survival and apoptosis, with anti-invasive and anti-metastatic effects on lung, breast and prostate cancer (45,46). Curcumin induces anti-migratory activity, which functions via the Wnt signaling pathway (28,47). In the current study, different concentrations of curcumin were used for 24 h in SW620 cells. We found that curcumin has a strong inhibitory effect on the Wnt signaling pathway and EMT. However, these anti-metastatic effects remain to be elucidated. Nevertheless, to the best of our knowledge, the present is the first study to identify that curcumin upregulates the expression of NKD2. NKD2, a secreted-type Wnt signaling inhibitor (48), suppressed the Wnt signaling pathway significantly. Hu et al reported that myristoylated NKD2 antagonizes the Wnt pathway by degrading dishevelled-1 at the plasma membrane (49). Therefore, it can activate the Wnt signaling pathway following transfection of NKD2 siRNA into SW620 cells. We found curcumin inhibited the Wnt pathway and EMT by increasing the expression of NKD2.

The chemokine superfamily plays multifaceted roles in the regulation of tumor development and progression. Chemokines induce leukocyte infiltration to tumors and regulate immune functions, direct the homing of tumor cells to specific metastatic sites, and regulate antigenicity at the tumor milieu (50). Thus, CXCR4 was considered an important factor in tumor cell metastasis and is a potential therapeutic target for the treatment of cancer. Recent findings have indicated that CXCR4 expression is closely associated with the Wnt signaling pathway (51,52). Hu et al identified that CXCR4 promoted CRC progression and EMT was regulated by the Wnt/β-catenin signaling pathway in colorectal cancer (33). Additionally, Wang et al identified that CXCR4 is crucial in the metastasis of human ovarian cancer possibly by modulating the Wnt/β-catenin pathway (53). To the best of our knowledge, this is the first study to demonstrate that the anti-metastatic effects of curcumin are associated with NKD2, the Wnt signaling pathway and CXCR4 in colon cancer cells. Curcumin can also inhibit the Wnt signaling pathway through the upregulation of NKD2 gene expression, suppression of EMT and the expression of CXCR4, and inhibition of tumor invasion and metastasis. Results of the present study therefore offer a new perspective on the role of curcumin in preventing the progression of cancer.

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References

1. Noreen F, Röösli M, Gaj P, Pietrzak J, Weis S, Ufer P, Regula J, Schär P and Truninger K: Modulation of age- and cancer-associated DNA methylation change in the healthy colon by asparagus and lifestyle. J Natl Cancer Inst 106: 106, 2014.

2. Pelser C, Arem H, Pfeiffer RM, Elena JW, Alfano CM, Hellenbeck AR and Park Y: Prediagnostic lifestyle factors and survival after colon and rectal cancer diagnosis in the National Institutes of Health (NIH)-AARP Diet and Health Study. Cancer 120: 1540-1547, 2014.

3. Winkels RM, Heine-Bröring RC, van Zutphen M, van Harten - bröring RC, van Zutphen M, van Harten -

4. Huang W, Liu Z, Zhou G, Tian A and Sun N: Magnetic gold nanoparticles for cancer therapy. Adv Healthc Mater 5: 1061-1068, 2016.

5. Pelser C, Arem H, Pfeiffer RM, Elena JW, Alfano CM, Hellenbeck AR and Park Y: Prediagnostic lifestyle factors and survival after colon and rectal cancer diagnosis in the National Institutes of Health (NIH)-AARP Diet and Health Study. Cancer 120: 1540-1547, 2014.

6. Winkels RM, Heine-Bröring RC, van Zutphen M, van Harten -

7. Wang Y and Zhou P: Epithelial-mesenchymal transition (EMT)-mediated tumor progression in colorectal cancer. World J Gastroenterol 14: 3792-3797, 2008.

8. Hay ED: The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev Dyn 233: 706-720, 2005.

9. Dobbs T, Baraghi S and Hu GF: Epithelial-mesenchymal transition and cell cooperation in metastasis. Cancer Res 69: 7135-7139, 2009.

10. Hay ED: The mesenchymal cell, its role in the embryo, and the remarkable signaling mechanisms that create it. Dev Dyn 233: 706-720, 2005.

11. Zheng H and Kang Y: Multilayer control of the EMT master regulators. Oncogene 33: 1755-1763, 2014.

12. Guarino M: Epithelial-mesenchymal transition and tumour invasion. Int J Biochem Cell Biol 39: 2153-2160, 2007.

13. Talalawa A, Spychal R and Tselepis C: Epithelial-mesenchymal transition mediated tumourigenesis in the gastrointestinal tract. World J Gastroenterol 14: 3792-3797, 2008.

14. Wang Y and Zhou BP: Epithelial-mesenchymal Transition-A Hallmark of Breast Cancer Metastasis. Cancer Hallm: 1-38, 2013.

15. Gonzalez EJ, Arns L and Vizzard MA: The role(s) of cytokines/chemokines in urinary bladder inflammation and dysfunction. BioMed Res Int 2014: 120552, 2014.

16. Sallusto F and Greguolto M: Chemokines and leukocyte traffic. Nat Immunol 9: 949-952, 2008.

17. Hu XM, Liu YN, Zhang HL, Cao SB, Zhang T, Chen LP and Sheng W: CXCL12/CXCR4 chemokine signaling in spinal cord: a potential therapeutic target in cancer. J Neurochem 132: 452-463, 2015.

18. Batsi O, Giannopoulou I, Nesseris I, Valavanis C, Gakiopoulou H, Patsouris ES, Arapondoni-Dadioti P and Lazaris AC: Immunohistochemical evaluation of CXCL12-CXCR4 axis and VEGFR3 expression in primary urothelial cancer and its recurrence. Anticancer Res 34: 3537-3542, 2014.

19. Colm C, Hetzsch T, Trautmans F, Polischuk L, Teleguee GD and Dubrovskova A: Emerging targets in cancer management: Role of the CXCL12-CXCR4 axis. Onc Targets Ther 6: 1347-1361, 2013.

20. Hu XM, Liu YN, Zhang HL, Cao SB, Zhang T, Chen LP and Sheng W: CXCL12/CXCR4 chemokine signaling in spinal cord: a potential therapeutic target in cancer. J Neurochem 132: 452-463, 2015.

21. Batsi O, Giannopoulou I, Nesseris I, Valavanis C, Gakiopoulou H, Patsouris ES, Arapondoni-Dadioti P and Lazaris AC: Immunohistochemical evaluation of CXCL12-CXCR4 axis and VEGFR3 expression in primary urothelial cancer and its recurrence. Anticancer Res 34: 3537-3542, 2014.

22. Cojoc M, Hetzsch T, Trautmans F, Polischuk L, Teleguee GD and Dubrovskova A: Emerging targets in cancer management: Role of the CXCL12-CXCR4 axis. Onc Targets Ther 6: 1347-1361, 2013.

23. Hu XM, Liu YN, Zhang HL, Cao SB, Zhang T, Chen LP and Sheng W: CXCL12/CXCR4 chemokine signaling in spinal cord: a potential therapeutic target in cancer. J Neurochem 132: 452-463, 2015.

24. Batsi O, Giannopoulou I, Nesseris I, Valavanis C, Gakiopoulou H, Patsouris ES, Arapondoni-Dadioti P and Lazaris AC: Immunohistochemical evaluation of CXCL12-CXCR4 axis and VEGFR3 expression in primary urothelial cancer and its recurrence. Anticancer Res 34: 3537-3542, 2014.

25. Cojoc M, Hetzsch T, Trautmans F, Polischuk L, Teleguee GD and Dubrovskova A: Emerging targets in cancer management: Role of the CXCL12-CXCR4 axis. Onc Targets Ther 6: 1347-1361, 2013.
44. Deng Y, Su Q, Mo J, Fu X, Zhang Y and Lin EH: Celecoxib downregulates CD133 expression through inhibition of the Wnt signaling pathway in colon cancer cells. Cancer Invest 31: 97-102, 2013.

45. Jagtap S, Meganathan K, Wagh V, Winkler J, Hescheler J and Sachindis A: Chemoprotective mechanism of the natural compounds, epigallocatechin-3-O-gallate, quercetin and curcumin against cancer and cardiovascular diseases. Curr Med Chem 16: 1451-1462, 2009.

46. Chen QY, Jiao DM, Wang LF, Wang L, Hu HZ, Song J, Yan J, Wu LJ and Shi JG: Curcumin inhibits proliferation-migration of NSCLC by steering crosstalk between a Wnt signaling pathway and an adherens junction via EGR-1. Mol Biosyst 11: 859-868, 2015.

47. Leow PC, Bahety P, Boon CP, Lee CY, Tan KL, Yang T and Ee PL: Functionalized curcumin analogs as potent modulators of the Wnt/β-catenin signaling pathway. Eur J Med Chem 71: 67-80, 2014.

48. Katoh M and Katoh M: WNT signaling pathway and stem cell signaling network. Clin Cancer Res 13: 4042-4045, 2007.

49. Hu T, Li C, Cao Z, Van Raay TJ, Smith JG, Willert K, Solnica-Krezel L and Coffey RJ: Myristoylated Naked2 antagonizes Wnt-beta-catenin activity by degrading Dishevelled-1 at the plasma membrane. J Biol Chem 285: 13561-13568, 2010.

50. Ben-Baruch A: The multifaceted roles of chemokines in malignancy. Cancer Metastasis Rev 25: 357-371, 2006.

51. Choe Y and Pleasure SJ: Wnt signaling regulates intermediate precursor production in the postnatal dentate gyrus by regulating CXCR4 expression. Dev Neurosci 34: 502-514, 2012.

52. Zhao S, Wang J and Qin C: Blockade of CXCL12/CXCR4 signaling inhibits intrahepatic cholangiocarcinoma progression and metastasis via inactivation of canonical Wnt pathway. J Exp Clin Cancer Res 33: 103, 2014.

53. Wang J, Cai J, Han F, Yang C, Tong Q, Cao T, Wu L and Wang Z: Silencing of CXCR4 blocks progression of ovarian cancer and depresses canonical Wnt signaling pathway. Int J Gynecol Cancer 21: 981-987, 2011.