Antitumor activity of the selective cyclooxygenase-2 inhibitor, celecoxib, on breast cancer in Vitro and in Vivo

Zhi-Jun Dai*, Xiao-Bin Ma†, Hua-Feng Kang*, Jie Gao, Wei-Li Min, Hai-Tao Guan, Yan Diao, Wang-Feng Lu and Xi-Jing Wang*

Abstract

Background: Cyclooxygenase-2 (COX-2) promotes carcinogenesis, tumor proliferation, angiogenesis, prevention of apoptosis, and immunosuppression. Meanwhile, COX-2 over-expression has been associated with tumor behavior and prognosis in several cancers. This study investigated the antitumor effects of the selective COX-2 inhibitor, Celecoxib, on breast cancer in vitro and in vivo.

Methods: Human breast cancer MCF-7 and MDA-MB-231 cells were cultured with different concentration (10, 20, 40 μmol/L) of celecoxib after 0-96 hours in vitro. MTT assay was used to determine the growth inhibition of breast cancer cells in vitro. The expression of COX-2 on mRNA was measured by real-time quantitative PCR analysis. Flow cytometry was performed to analyze the cell cycle of MCF-7 cells. Levels of PGE2 were measured by ELISA method. The in vivo therapeutic effects of celecoxib were determined using rat breast cancer chemically induced by 7,12-dimethylben anthracene (DMBA).

Results: The inhibition of proliferation of both MCF-7 and MDA-MB-231 cells in vitro by celecoxib was observed in time and dose dependent manner. Celecoxib effectively down-regulated the expression of COX-2. The cell cycle was arrested at G0/G1, and rate of cells in S phase was obviously decreased. Levels of PGE2 were inhibited by Celecoxib. The tumor incidence rate of the celecoxib group was lower than that of the control group. In addition, the tumor latency period of the celecoxib group was longer than that of the control group.

Conclusions: Celecoxib inhibited the proliferation of breast cancer cell lines in vitro, and prevented the occurrence of rat breast cancer chemically induced by DMBA. Therefore, celecoxib exhibits an antitumor activity and seems to be effective in anti-tumor therapy.

Keywords: Breast cancer, Cyclooxygenase-2, Anti-tumor, DMBA

Introduction

Cyclooxygenases (COX) exists in two isoforms, namely, COX-1 and COX-2. They are rate-limiting enzymes in the formation of prostaglandins from arachidonic acid. COX-1 is considered to be constitutively expressed, while COX-2 is highly inducible by various factors and is associated with tumorigenesis by enhancing angiogenesis [1,2], suppressing apoptosis [3], and promoting invasiveness as well as metastases [4].

COX-2 promotes carcinogenesis, tumor proliferation, angiogenesis, prevention of apoptosis, and immunosuppression [5]. COX-2 over-expression has been associated with tumor behavior and with prognosis in several cancers [6]. The selective inhibition of COX-2 activity in several animal models has been associated with a decrease of new blood vessel production in tumors, a decrease in new vessel formation, and an increase in tumor cell apoptosis [7]. Celecoxib is a paradigmatic selective inhibitor of COX-2. This anti-inflammatory drug has potent anti-tumor activity in a wide variety of human
tumor types, such as colorectal, breast, and lung cancers [8-10]. The over-expression of COX-2 is associated with carcinogenesis, invasiveness, and with the metastasis of malignant tumors [11,12]. The roles of celecoxib in preventing and treating tumors have been attracting broad attention in recent years because of its selective and specific inhibition of COX-2 activity [13-16]. In this study, the inhibitory effect of celecoxib on the proliferation of the human breast carcinoma cell line MCF-7 was investigated in vitro and the breast cancer was chemically induced in vivo.

Materials and methods

Reagents

MCF-7 cell was purchased from the Shanghai Institute of Cell Biology at the Chinese Academy of Sciences (Shanghai, P.R.China); RPMI1640 medium (Gibco, USA); Fetal bovine serum(Gibco, USA); 7,12-dimethylbenzanthracene (DMBA), Dimethyl sulfoxide (DMSO), Propidiumiodide(PI) and 3-(4,5- dimethylthiazol-2-yl) -2.5- diphenytetrazolium bromide (MTT) were purchased from Sigma Chemical (St. Louis, MO); Trizol (Invitrogen, USA);Celecoxib(Pfizer Pharmaceuticals Ltd, USA); Prostaglandin E2(PGE2) ELISA kit(Jingmei Biotech Co., Ltd, China). Rats(Sprague–Dawley rats, female, age 45±5 days, weighting 110±10g) were purchased from the Experiment Animal Center, Medical School of Xi’an Jiaotong University, Xi’an, China (Animal Certificate Number: No.08-005 of Shanxi medical animal test centre).

Cell culture and cell proliferation assay

Cells were cultured in RPMI-1640 medium(Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 1 × 10⁵ U/L penicillin and 100 mg/L streptomycin in a humidified atmosphere with 5% CO₂ incubator at 37°C. The cells were subcultured until reaching logarithmic growth phase. MTT assay was used to determine the effect of Celecoxib on the proliferation of MCF-7 cells. MCF-7 cells were seeded at a concentration of 5×10⁴ cell /well in 96-well plate, and grown at 37°C, 5% CO₂ incubator until adherence. After an overnight incubation in starvation medium containing 0.5% FBS, the cells on the culture plate were divided into groups on the basis of parallel lines, each group had four wells in one line for each group. At the end of the treatment, 20 μl MTT (5 mg/ml) was added and the cells were incubated for another 4 hours. 200 μl of DMSO was added to each well after removing the supernatant. After shaking the plate for 10 mins. in the shaking board, cell viability was obtained by measuring the absorbance at 490 nm wavelength using Enzyme-labeling instrument (Bio-Tek ELX800, USA), this assay was done triplicate. The inhibition rate was calculated using the following formula [17]:

\[
\text{Inhibition rate} = \left(1 - \frac{\text{average absorbance of experimental group}}{\text{average absorbance of blank control group}}\right) \times 100\%.
\]

Real-time quantitative RT-polymerase chain reaction assay for COX-2 expression

MCF-7 cells were seeded in 6-well plates and treated with concentration gradient Celecoxib (0, 10,20,40 μmol/L) separately for 0-96 h. As previously described [18], cells collected at specified time were used to extract total RNA using the Trizol reagent following the manufacturer's instructions. RNA was reverse-transcribed into cDNA using a Primerscript™ RT reagent kit according to the manufacturer’s instructions. Real-time quantitative polymerase chain reaction (PCR) was carried out with the SYBR Green fluorescent dye method, and a Rotor Gene 3000 real-time PCR apparatus. COX-2 primer sequence (Invitrogen CO): 5'- ATCCTTGCTGTCCCAACCA-3' (sense) and 5'-CTT TGACACCCAAGGAGTG-3' (anti-sense). β-actin, its primer sequence was 5'-GTTGCTTACACCCCTTTCTTG-3' (sense), 5'-TGCTGTCCACCCCTTACCC GTTC-3' (anti-sense). β-actin was used as an internal control to evaluate the relative expressions of COX-2. The PCR conditions were as follows: a pre-denaturing at 95°C for 2 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing/extension at 60°C for 20 s. The amplification specificity was checked by melting curve analysis. The PCR products were visualized by gel electrophoresis to confirm the presence of a single product with a correct size. The 2-ΔΔCT method was used to calculate the relative abundance of target gene expression generated by RotorGene Real-Time Analysis Software 6.1.81. For each cDNA, the target gene mRNA level was normalized to β-actin mRNA level. The experiments were performed for three times.

Determination of PGE2 synthesis

As previously described [19], MCF-7 Cells were grown in 12-well plates overnight. 30 min before harvesting of culture media, the culture media of the cells were changed to new media, and then these culture media were centrifuged to remove cell debris. Cell-free culture media were collected at indicated times and PGE2 levels were determined by competitive enzyme-linked immunosorbent assay (ELISA) as described by the kit manufacturer (Cayman Chemical, Ann Arbor, MI, USA) using an ELISA reader (μQuant; Biotek Instruments, Inc, Winooski, VT, USA).

Cell cycle analysis by FCM

MCF-7 cells were incubated at 5 × 10⁵ cells/well in 6-well plates, treated with a homologous drug for 48 h. The detached and attached cells were harvested and
fixed in 70% ice-cold ethanol at -20°C overnight. After fixation, cells were washed with PBS, resuspended in 1 mL PBS containing 1 mg/mL RNase (Sigma) and 50 μg/mL PI (Sigma), and incubated at 37°C for 30 min in the dark. Samples of 10 000 cells were then analyzed for DNA content by FACScan flow cytometry (Beckman, USA), and cell cycle phase distributions were analyzed with the CellQuest acquisition software (BD Biosciences).

Statistical analysis
All values were expressed as the mean ± standard deviations (SD). Statistical analysis was performed with one-way analysis of variance (ANOVA) and student t test using the statistical software SPSS 13.0. P<0.05 was considered as statistically significant.

Results and discussions
Celecoxib inhibited the proliferation of breast cancer cells in vitro
The anti-proliferative effect of celecoxib on two breast cancer cell lines, MCF-7 cells (ER–positive) and MDA-MB-231 (ER-negative), were examined using MTT assays. Cells were treated with medium and different doses of celecoxib, and the inhibition rate was evaluated after 0 h to 96 h. Celecoxib in the high dose and medium dose groups could significantly inhibit the proliferation of both MCF-7 cells and MDA-MB-231 cells. As shown in Figure 1, the inhibitory rates of celecoxib on cell growth were (62.6±4.5)%, (67.5±4.8)%, (78.7±6.3)% in MCF-7 cells, and 53.5±3.7)%, (62.3±4.5)% (70.9±7.1)% in MDA-MB-231 cells, when the cells were treated with different doses of celecoxib for 96 hours. Thus, Celecoxib inhibited breast cancer cell proliferation in a dose- and time-dependent manner.

Celecoxib is known to induce apoptosis [23], but its effect on proliferative is not conclusive. For example, some researches reported that celecoxib did not affect tumor cell proliferation in primary adenocarcinomas and ductal carcinoma in situ of the breast in vivo [24,25]. However, celecoxib could prevent the development of breast cancer with ER-negative and HER-2-positive status [26]. These results indicate that the effect on proliferative of celecoxib is related with tumor’s molecular phenotype possibly. In this study, we choose a ER–positive breast cancer cell line (MCF-7) and a ER –negative breast cancer cell line (MDA-MB-231), and found celecoxib could significantly inhibit the proliferation of both MCF-7 cells and MDA-MB-231 cells in a dose- and time-dependent manner.

COX-2 mRNA expression detected by real-time qPCR
Real-time qPCR assay was used for COX-2 mRNA expression. It revealed that COX-2 mRNA was highly expressed in normal breast cancer cells. What’s more, as celecoxib concentration increased, the mRNA expression of COX-2 gradually decreased. As shown in Figure 2, the amount of COX-2 mRNA in both MCF-7 cells and MDA-MB-231 cells after celecoxib treatment were significantly decreased in a dose-dependent manner.

It was previously reported that COX-2 is expressed in most human cancers including those of the breast, and administration of selective COX-2 inhibitors in humans may reduce the risk of cancer development [27]. Our date indicated that cell viability was gradually declined as celecoxib concentration increased. As can been seen, the lowest COX-2 mRNA was in 40 μmol/L group. Therefore, celecoxib could suppress expression of COX-2 to
inhibit cell proliferation. This results points out the efficacy of celecoxib against breast cancer growth.

**Effects of celecoxib on the PGE2 level of MCF-7 cells by ELISA**

Prostaglandin E2 (PGE2) is an important mediator in tumor-promoting inflammation [28]. The major mechanism of COX-2 in stimulating tumorigenesis is its product, PGE2. PGE2 promotes tumor cell proliferation, induces VEGF up-regulating, and inhibits tumor cell apoptosis as well as immune function [29]. Tari et al. [30] reported that COX-2 induced PGE2 to stimulate the activities of protein kinases A and C and induced tamoxifen resistance in ER alpha-positive breast cancer cells selectively. However, the COX-2 selective inhibitor celecoxib can inhibit tumorigenesis and tumor development through these ways.

In this study, the PGE2 level of MCF-7 cells was determined with ELISA analysis. As shown in Figure 3, the PGE2 level of MCF-7 cells in the control group and in the 10 μmol/L to 40 μmol/L celecoxib groups were (75.32 ±8.73), (58.15±6.56), (42.84±6.12) and (28.65±4.33) pg/mL, respectively. PGE2 levels in the celecoxib therapy groups were significantly lower than that in the control group. Furthermore, the PGE2 level gradually decreased in a dose-dependent manner (P <0.01).

**Effects of celecoxib on the cell cycle distribution by flow cytometry**

Celecoxib may exert an inhibitory effect on the enhanced radiation-induced G2/M arrest in the COX-2-overexpressing cells. This effect may allow the arrested cells to enter mitosis and die after radiation [31]. It has been found that a low dose of celecoxib (5 μM to 10 μM) could induce G2/M arrest, followed by the induction of apoptosis in the transformed cells but not in the normal cells. Growth inhibition was related to the COX-2 function with 90% to 95% reduction in PGE2 production [32]. However, Liu et al. [33] holds a different opinion that celecoxib can induce apoptosis and cell-cycle arrest at the G0/G1 checkpoint in the nasopharyngeal carcinoma cell lines, which is associated with a significantly reduced STAT3 phosphorylation.

In the present study, the effects of celecoxib on cell cycles were analyzed using flow cytometry. The percentage of cells in the celecoxib therapy groups significantly
decreased at the S phase and increased at the G0/G1 phase. These results suggest that celecoxib can induce cell cycle arrest at the G0/G1 phase in MCF-7 cells (Figure 4). Besides, the cells at the G2/M phase significantly decreased in the 40 μmol/L celecoxib group compared with that in the control group.

Anti-tumor effects of celecoxib on DMBA-induced breast cancer

Celecoxib has a striking chemopreventive activity. It can inhibit preneoplastic lesions during hepatocarcinogenesis in vivo, which suggests that celecoxib effects are mediated by PGE2-independent mechanisms [34]. A low
A dose of celecoxib can augment CDDP-induced growth inhibition of Tca8113 cells and its xenograft in Balb/c nude mice [35]. It is reported by Nakatsug et al. [21] that 400 ppm of nimesulide could degrade tumor incidence rate, volume and multisitus rate. This study found that celecoxib could inhibit rat carcinogenesis and cancer development. With a dosage of 1000 ppm, celecoxib decreased the incidence rate, average tumor number and tumor volume with statistical significance ($P < 0.05$), compared with that of tumor control group. As in vitro, celecoxib inhibited MCF-7 cell proliferation in a dose- and time-dependent manner, while in vivo, celecoxib could inhibit rat carcinogenesis and cancer development in a dose-dependent manner. Abou Issa et al. [9] observed the preventive effect of celecoxib on 7,12-dimethylbenzanthracene (DMBA)-induced rat breast cancer. When treated with 250 ppm, 500 ppm, 1000 ppm and 1500 ppm celecoxib, the incidence rate was 80%, 50%, 45% and 25% respectively, compared with that of 100% incidence rate in control group ($P < 0.001$).

In the present study, celecoxib was found to be capable of inhibiting rat carcinogenesis and cancer development. The tumor incidence rate of each group was 85.71% (24/28) in the control group, 50.00% (14/28) in the celecoxib group, and 48.15% (13/27) in the tamoxifen group. As shown in Figure 5, the tumor latency period of the celecoxib group was significantly longer than that of the control group ($P < 0.05$). In addition, no significant difference was found between the celecoxib and tamoxifen groups ($P > 0.05$).

The average tumor numbers of each group were as follows: 3.50±1.62(1-7) pieces in the control group; 1.77±0.73 (1-3) pieces in the tamoxifen group; and 1.71±0.61(1-2) pieces in the celecoxib group. The average tumor numbers of the celecoxib and tamoxifen groups were less than that
of the control group ($P < 0.05$). No significant difference was found between the two treatment groups ($P > 0.05$).

The average tumor volumes of each group were $6.42 \pm 3.96 \text{cm}^3$ in the control group, $1.78 \pm 0.71 \text{cm}^3$ in the tamoxifen group, and $2.05 \pm 1.04 \text{cm}^3$ in the celecoxib group. No significant difference was found between the tumor volumes of the celecoxib and tamoxifen groups ($P > 0.05$). The tumor volumes of the two treatment groups decreased significantly compared with the tumor control groups ($P < 0.05$).

Histopathological observation

The tissues of the control group presented an infiltrating ductal carcinoma, showing cancer nest, obvious nuclear atypia and nuclear division. A few gland-like structures and stroma were also observed (Figure 6A). The medullary carcinoma was composed of cancer cells and had ductless glands. The specimens without tumorigenesis showed different degrees of lobuli mammae hyperplasia and glandular epithelium atypical hyperplasia. The slight degree of hyperplasia showed an expanded or increased intralobulus and interlobulus fibrous tissue and gland alveolus. However, acinous cells were still in the monolayer. The atypical hyperplasia showed acinous cells that were arranged disorderly and in a multilayer, an increased karyoplasmic ratio, and changed nuclear atypia (Figure 6B).

In the experimental groups, the infiltrating ductal carcinoma had more gland-like structures and stroma wherein the cells were dispersed. A few nuclear atypia and nuclear division were also observed (Figure 6C). The specimens that did not cancerate showed no or a slight degree of glandular epithelium hyperplasia, increased intralobulus and interlobulus fibrous tissue, and lesser gland alveolus and ducts (Figure 6D).

Conclusions

In conclusion, celecoxib inhibited the proliferation of breast cancer cell lines in vitro. Furthermore, the inhibitory effect of celecoxib on the proliferation of breast cancer cells in vitro was observed in a dose- and time-dependent manner. The cell cycle was arrested at G0/G1, and the rate of cells in the S phase was obviously decreased. In addition, celecoxib could prevent the occurrence of rat breast cancer in vivo. Therefore, celecoxib exhibits an antitumor activity and seems to be effective in anti-tumor therapy. However, further studies are needed to clarify the detailed mechanism involved in the antitumor effects of celecoxib.

Competing interest

The authors declare that there are no conflicts of interest in relation to this article.

Authors’ contributions

DZJ and WXJ designed the research. DZJ, MXB, GJ, MWL and DY performed the experiments throughout this research. LXX, KHF and GHT contributed to the reagents, and participated in its design and coordination. DZJ and GJ analyzed the data; DZJ and MXB wrote the paper. Co-first authors: DZJ and MXB. All authors have read and approved the final manuscript.

Acknowledgements

This study was supported by the Fundamental Research Funds for the Central Universities, China; Tackle Key Problems in Science and Technology Foundation of Shaanxi Province, China [NO. 2011K13-03-08]. The funding sources had no role in the study design, data collection, analysis and interpretation, or in the writing of this manuscript.

Received: 5 September 2012 Accepted: 18 December 2012
Published: 19 December 2012
13. Reddy BS, Hirose Y, Lubet R, Steele V, Kelloff G, Paulson S, Seibert K, Rao CV: Cyclooxygenase-2 expression in human colon cancer cells: implications for chemoprevention. Cancer Res 1998, 58:362–366.

14. Jang TJ, Jung HG, Jung KH, O MK: Cyclooxygenase-2 expression in the lung. Cancer Control 2003, 10:160–166.

15. Koki AT, Masferrer JL: Celecoxib: a selective COX-2 inhibitor with anticancer potential. J Natl Cancer Inst 2000, 92:35–36.

16. Hilmi I, Goh KL: The contributions of cyclooxygenase-2 to tumor angiogenesis. J Exp Clin Cancer Res 2005, 24:453–462.

17. Dai ZJ, Gao J, Li ZF, Ji ZZ, Kang HF, Guan HT, Diao Y, Wang BF, Wang XJ: Cyclooxygenase-2 inhibitors: a rational advance? Cancer Control 2003, 10:160–166.

18. Sugiyama S, Sugimura T, Wakabayashi K: Induction of apoptosis and Bcl-2 expression by prostanoid E2 in human colon cancer cells. Cancer Res 1998, 58:362–366.

19. Tsujii M, Kawano S, Kuwano H, Tsuboi T, Inoue Y, Ikuhara T, Miyazaki H, Hori M, Dubois RN: Chemoprevention of mammary carcinogenesis by celecoxib, a selective cyclooxygenase-2 inhibitor. Cancer Epidemiol Biomarkers Prev 2006, 15:1598–1606.

20. Abou-El-Magd HM, Al-Shehri NA, El-Serafi A, El-Nashar H: Enhanced radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance? J Natl Cancer Inst 2003, 95:1440–1452.

21. Choy H, Milas LS: Enhancing radiotherapy with cyclooxygenase-2 enzyme inhibitors: a rational advance? J Natl Cancer Inst 2003, 95:1440–1452.

22. Gately S: The contributions of cyclooxygenase-2 to tumor angiogenesis. J Exp Clin Cancer Res 2005, 24:453–462.

23. Barnes NL, Flint PJ, Knox WF, Clarke RB, Bundeb NR: Celecoxib decreases COX-2 protein expression and increases apoptosis in ductal carcinoma in situ of the breast in vivo. Meeting Abstracts: AACR 2005, 532.

24. Basu GD, Pathangey LB, Tindel TL, Gendler SJ, Mulhern PG: Celecoxib, a selective COX-2 inhibitor, induces apoptosis and reduces VEGF levels in a preclinical model of metastatic breast cancer. Meeting Abstracts: AACR 2004, 317.

25. Ding H, Han C, Zhu J, Chen CS, D’Ambrosio SM: Celecoxib derivatives induce apoptosis via the disruption of mitochondrial membrane potential and activation of caspase 9. Int J Cancer 2005, 113:803–810.

26. Susan L, Sheldon M, John F, Kathleen G, Constantine D, Nasleifer J, Ben SZ, Harjeet S, Irma HR: The Cyclooxygenase-2 Inhibitor, Celecoxib, Prevents the Development of Mammary Tumors in HER-2/neu Mice. Cancer Epidemiol Biomarkers Prev 2003, 12:1486–1491.

27. Howe LR, Subbaramaiah K, Patel J, Masferrer JL, Deora A, Hudis C, Thaler HT, Muller WI, Du B, Brown AM, Dannenberg AJ: Celecoxib, a Selective Cyclooxygenase 2 Inhibitor, Protects against Human Epidermal Growth Factor Receptor 2 (HER-2)/neu-induced Breast Cancer. Cancer Res 2002, 62:5405–5407.

28. Rasmussen A, Kock A, Fuskedag OM, Kunspig B, Smim-Santamaria J, Gogvdace V, Johnsen K, Kogner P, Sveninjörsön B: Autocrine prostanoid E2 signaling promotes tumor cell survival and proliferation in childhood neuroblastoma. PLoS One 2012, 7:e29331.

29. Pockaj BA, Basu GD, Pathangey LB, Gray RJ, Hernandez JL, Gendler SJ, Mulhern PG: Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 overexpression and prostanoid E2 secretion in patients with breast cancer. Ann Surg Oncol 2004, 11:328–339.

30. Tari AM, Simeone AM, Li YJ, Gutierrez-Puentey Y, Lai S, Symmans WF: Cyclooxygenase-2 protein reduces tamoxifen and N-(4-hydroxyphenyl) retinamide inhibitory effects in breast cancer cells. J Exp Clin Cancer Res 2005, 24:453–462.

31. Shin YK, Park JS, Kim HS, Jun HJ, Kim GE, Suh CO, Yun YS, Pyo H: Radiosensitivity enhancement by celecoxib, a cyclooxygenase (COX)-2 selective inhibitor, via COX-2-dependent cell cycle regulation in human cancer cells expressing differential COX-2 levels. Cancer Res 2005, 65:9501–9509.

32. Devy-Sobol H, Cohen-Noyman E, Kazanov D, Figer A, Birkenfeld S, Madar-Shapiro L, Benamouzig R, Arber N: Celecoxib leads to G2/M arrest by induction of p21 and down-regulation of cyclin B1 expression in a p53-independent manner. Eur J Cancer 2006, 42:422–426.

33. Liu DB, Hu GY, Long GY, Qiu H, Mei Q, Hu GQ: Celecoxib induces apoptosis and cell cycle arrest in nasopharyngeal carcinoma cell lines via inhibition of STAT3 phosphorylation. Acta Pharmacol Sin 2012, 33:662–670.

34. Márquez-Rosado L, Trejo-Solís MC, García-Cuéllar CM, Villa-Treviño S: Celecoxib, a cyclooxygenase-2 inhibitor, prevents induction of liver preneoplastic lesions in rats. J Hepatol 2005, 43:653–660.

35. Li WZ, Wang XY, Li ZG, Zhang JH, Ding YQ: Celecoxib enhances the inhibitory effect of cisplatin on Tca8113 cells in human tongue squamous cell carcinoma in vivo and in vitro. J Oral Pathol Med 2010, 39:579–584.