Cytotoxic Effects and Androgen Receptor Expression According to Concentrations of Genistein with Silencing Cyclooxygenase-2 Gene Expression in Prostate Cancer Cells

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Cytotoxic Effects and Androgen Receptor Expression According to Concentrations of Genistein with Silencing Cyclooxygenase–2 Gene Expression in Prostate Cancer Cells

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COX–2 has major roles in inflammatory reaction, and COX–2 inhibitor and genistein have a chemopreventive effect on some cancers such as colorectal, breast, and prostate cancer (PCa). The aims of this study was to investigate combined effect of COX–2 inhibition and genistein treatment. To address this issue, we tested the degree of cell survival and the changes of androgen receptor in PCa cells with silencing of COX–2 according to concentrations of genistein.

DU–145 PCa cells were transfected with COX–2 siRNA. The mRNA expressions of androgen receptor and caspase–3 were detected using reverse transcription polymerase chain reaction in the cells with or without COX–2 siRNA transfection. Immunofluorescent staining was performed on PCa cell with COX–2, androgen receptor and caspase–3 antibody and analyzed with confocal microscopy and image analyzer. Cell cytotoxicity according to concentrations of genistein was analyzed with MTT assay.

The mRNA expression of AR was down–regulated in DU–145 cell line according to concentration of genistein, but caspase–3 expression showed up–regulated pattern in increasing the dosage of genistein. COX–2 siRNA (+) group with genistein was more cytotoxic compared to COX–2 siRNA (–) group. The immunofluorescent staining results were similar with those of mRNA expression. The results of cell survival showed that COX–2 siRNA (+) group with genistein was more cytotoxic compared to COX–2 siRNA (–) group (Repeated Measured ANOVA, p=0.004). There was significant cytotoxic effects in COX–2 siRNA (+) group between 10 μM and 50 μM of concentration of genistein compared to COX–2 siRNA (–) group (p<0.0001).

The effect of genistein and silencing of COX–2 shows that it reduced AR and increased caspase–3 in PCa cells. These results suggest that genistein and silencing of COX–2 might be a role in the inhibition of cell proliferation and induction of apoptosis.

Key words: Androgen receptors, Caspase 3, Cyclooxygenase 2, Genistein, Small interfering RNA

INTRODUCTION

Inflammation is a major reaction of natural immunity against infectious agents such as viruses and bacteria, entrance of antigen like as lipopolysaccharides and cell damages induced by UV irradiation and chemicals. (Kulinsky, 2007). Dubois et al. (1998). reported that osteoarthritis, Crohn’s disease and cancer were related to chronic inflammation and recently more data support their theory that inflammation is closely related to the incidence of rectal, breast, and prostate cancers. (Howe, 2007; Coussens and Werb, 2002; Fujii et al., 2013). As shown by supportive data, Coussens and Werb (2002). reported that the chronic inflammation resulting from infective agents, environmental factors and complex components may induce the malignancies and upwards of 15% of malignancies worldwide can be attributed to infections.

Cyclooxygenase–2 (COX–2) is an inducible enzyme that produced by inflammatory cytokine, endotoxin and growth factor such as TNF α and induced nuclear factor kappa B (NF–kB) in apoptosis pathway. (Khan et al., 2011). Several experimental and clinical studies have established NSAID (Non–steroidal anti–inflammatory drugs) may have a potent anti–cancer activity. Specifically, COX–2 inhibitors have been applied for prevention and therapeutic modality against breast, colorectal, and lung cancer. (Falandry et al., 2009; Eisinger et al., 2007; Mutter et al., 2009). These trials also have been applied in prostate cancer and the factors and mechanisms causing prostate cancer based on the knowledge of epidemiology, histopathology, and molecular biology have been studied. (De Marzo et al., 2004; Platz and De Marzo, 2004).

Androgen, a group of male steroid hormones, have important role in regulating development of prostate and growing of prostate cancer by binding to androgen receptor (AR) (Taplin and Balk, 2004). Therefore, hormone therapy that reduce androgen level or to prevent it from reaching prostate cancer cells in prostate cancer patient, is frequently selected as a first line treatment. This therapy makes shrink or slow grow of prostate cancers for a while. However, this hormone therapy often failed with appearance of castrate–resistant prostate cancer that
frequently showed increased level of AR expression. Recent study demonstrated that a second-generation antiandrogen enzalutamide inhibited cell growth and induced apoptosis in AR gene amplified prostate cancer cells (Tran et al., 2009). Isoflavone phytoestrogen called genistein found in soybean has several effects such as a weak estrogen effect, (Kuiper et al., 1997), inhibition of 5–α-reductase and aromatase enzymes. (Evans et al., 1995). Furthermore, genistein may have an important role to inhibit angiogenesis required for tumor growth (Harper et al., 1996; Folkman and Shing, 1992), while maintaining the effect of an antioxidant. (Rice–Evans et al., 1995). However, there is no information related the effect of genistein on treated COX–2 silenced prostate cancer cells. In order to demonstrate such an effect, and find more information to understand inflammation and prostate cancer, genistein was chosen for this study. In this study, we evaluated whether silencing of COX–2 in prostate cancer cells affected on apoptosis (change of caspase–3 level) and the expression of AR after treatment with various genistein concentrations. Finally, our data indicate that combination of COX–2 gene silencing and genistein is better treatment option for prostate cancer.

MATERIALS AND METHODS

Culturing prostate cancer cell line

Cell lines DU–145 (KCLB No. 30081) and PC3 (KCLB No. 21435) from the Korean Cell Line Bank were used for this study. Cells were grown in 100×15 mm petri dishes nourished by RPMI 1640 (R8758, Sigma–Aldrich, St. Louis, MO, USA) supplemented with L-glutamine (0.3 g/L), NaHCO 3 (2.0 g/L), and 10% of Fetal Bovine Serum (FBS, Cat No. 16000036, Invitrogen, Carlsbad, CA, USA). Growth conditions with supplement 5% CO 2 were maintained at 37°C with optimal humidity.

COX–2 siRNA transfection

2 ml of cell culture suspension (1×10 6 cells/ml) in absence of antibiotics were distributed to six well plates and were grown under 5% CO 2 , supplied incubator at 37°C for 18–24 hours. The COX–2 siRNA was synthesized as antisense oligonucleotides designed from the human COX–2 gene (GenBank Acc. No. NM_000963.2), and three 40–52% GC content antisense oligonucleotides were used for siRNA transfection (Table 1). Solution A was prepared by mixing 200 μl of OPTI–MEM I (Cat. No. 31985, Invitrogen, Carlsbad, CA, USA) containing 30 pmole siRNA (Invitrogen, Carlsbad, CA, USA), 5 μl of Lipofectamine RNAiMAX Reagent (Cat. No. 13778–075, Invitrogen, Carlsbad, CA, USA), and was maintained under room temperature for 20 min. The 1.8 ml OPTI–MEM I mixed with 0.2 ml of solution A was grown at each well for 48 hours at 37°C with supplement of 5% CO 2 . For control 2 ml of OPTI–MEM was cultured at the same condition. One ml culture mixed with 20% diluted FBS containing 2 times higher concentration of antibiotics was added to the well plate, and new culture media was supplied each 24 hours.

Confirming COX–2 siRNA transfection using immunofluorescence staining

The COX–2 siRNA transfected cells were transferred to chamber slide (177399, Thermo Fisher Scientific Inc., Rochester, NY, USA) which was cultured overnight in order to bring the cells to confluence. After overnight culture cells were washed with PBS and fixed using 3.6% formaldehyde (Samchun Chemical, Seoul, Korea) for 10 min, then washed again in PBS buffer. The fixed chamber slide was treated with 0.1% of Triton X–100 (T–8532, Sigma–Aldrich, St. Louis, MO, USA) for 5 min and was again washed with PBS, prior to blocking with 10% normal donkey serum (sc–2044, Santa Cruz Biotechnology, Inc., Dallas, TX, USA). After 1 hour incubation at 37°C, 400 times diluted antibody against COX–2 (goat polyclonal, sc–1746, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) was reacted with cell embedded chamber slide overnight at 4°C. Slides were washed with PBS twice for 5 min each to remove the COX–2 antibody before adding secondary antibody of anti–goat IgG FITC (1:400, sc–2024, Santa Cruz Biotechnology, Inc., Dallas, TX, USA). In order to stain the nucleus, 400 times diluted 4', 6–diamidino–2–phenyl indole (DAPI, Sigma–Aldrich, St. Louis, MO, USA) was reacted for 1 hour at 37°C, then washed three times for 5 min each time with PBS. The stained chamber slides were scanned by confocal microscopy using a LSM 710 (Carl Zeiss Inc., Jena, Germany).

Isolation of total RNA for assay of COX–2, caspase–3, and AR mRNAs

The 0.5 ml of cell culture suspension was added with 0.8 ml of Trizol (15596–018, Invitrogen, Carlsbad, CA, USA) for 5 min at room temperature. 0.16 ml of chloroform was added to this tube, mixed, and centrifuged for 20 min at 13,200 rpm. The 0.5 ml supernatant was mixed with 1 ml of isopropyl alcohol (1–0398, Sigma–Aldrich, St. Louis, MO, USA) for RNA precipitation. After a 70% ethanol wash, clean RNA was collected by centrifugation (13,200 rpm, 30 min), dissolved in 60 μl of DEPC water, and RNA concentration was measured by Nano spec-trometer (Amersham Biosciences, Buckinghamshire, England).

| Table 1. Sequences of antisense oligonucleotide for silencing of COX–2 gene |
|-----------------------------|-----------|---|
| Sequences                  | GC%       | start location of ORF |
| PTGS2–HSS108792            | 40.0%     | 432          |
| PTGS2–HSS183840            | 44.0%     | 510          |
| PTGS2–HSS183839            | 44.0%     | 1078         |
RT-PCR

Complementary DNA was synthesized by using RT kit (Takara Bio Inc., Shiga, Japan). For amplifying of COX–2, primers F–5’–TTC AAA TGA GAT TGT GGA AAA ATT GCT–3’ and R–5’–AGA TCA TCT CTG CTT GAG TAT CTT–3’ were used. Caspase–3 DNA was synthesized using F–5’–CCG AAA GGT GGC AAC AGA AT–3’ and R–5’–AGA TCA TCT CTG CCT GAG TAT CTT–3’ were used. PCR reaction was performed with SolGent PCR kit (SolGent, Daejeon, Korea) containing 2 μl of 5X buffer, 0.1 μl (SolGent, Daejeon, Korea) containing 2 μl of 5X buffer, 0.1 μl of Taq polymerase, 10 pmole specific primer for each of target genes, and water to fit 20 μl of final volume, and was programmed by three steps consisted of 95°C for 5 min, 35 cycles of 95°C for 30 sec/60°C for 40 sec/72°C for 30 sec, and 72°C for 5 min. The size of PCR products were visualized using 2% agarose electrophoresis gel. UV detected DNA was measured for DNA size using BIO-ID program.

Immunofluorescence staining of caspase–3, AR, and COX–2

The transfected cells were treated using the same procedure indicated above, before adding immunofluorescent antibody for caspase–3, AR and COX–2. Two slides were prepared for staining COX–2 and caspase–3 immunofluorescence, and the other for COX–2 and AR immunofluorescence. Each chamber slide was reacted at 4°C overnight with respect to primary antibodies of AR (1:400, sc–7305, mouse monoclonal, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), caspase–3 (1:400, sc–7148, rabbit polyclonal, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and COX–2 (1:400, sc–1746, goat polyclonal, Santa Cruz Biotechnology, Inc., Dallas, TX, USA), and overnight reacted slide chamber was washed twice with PBS, then secondary antibodies were added. These secondary antibodies as follow; for staining AR and COX–2, donkey anti–mouse IgG Texas Red (TR) (1:400, sc–2785, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and donkey anti–goat IgG fluorescein isothiocyanate (FITC) (1:400, sc–2824, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were used, and for staining COX–2 and caspase–3, donkey anti– goat IgG FITC (1:400, sc–2824, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and donkey anti rabbit TR (1:400, sc–2784, Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were applied. In order to stain the nucleus, DAPI (Sigma–Aldrich, St. Louis, MO, USA) solution was also added to chamber slide. After washing three times with PBS each slide was analyzed by confocal microscopy 710.

Evaluating prostate cancer cell survival in proportion to various genistein concentrations

COX–2 siRNA transfected cell suspensions and non–transfected cell suspensions were counted using a hemocytometer and distributed evenly at a concentration of 2×10^4 cells to each of 96 wells, then the plate were incubated at 37°C for 24 hours at 5% CO2. Genistein (G6776, Sigma–Aldrich, St. Louis, MO, USA) dissolved in dimethyl sulfoxide (DMSO) was added to wells at a concentration of 0, 10, 25, 50, 100, and 200 μM respectively. Genistein supplemented plates were cultured at 37°C, and 5% CO2 in an incubator for 24 hours. After 24 hours culture the supernatants were removed from each well and 100 μl of MTT (3–(4,5–Dimethylthiazol–2–yl)–2,5–diphenyl tetrazolium bromide) solution added per well and reacted for 4 hours. After carefully washing the plate with PBS to remove MTT, each well was treated with 200 μl of DMSO and the plate was shaken for 15 to 20 min. The absorbance from each well was analyzed at 450 nm using a plate reader.

Data were analyzed based on repeated measured ANOVA. A p–value <0.05 was considered statistically significant. All the tests in this article were implemented using IBM SPSS Statistics Ver. 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Confirming COX–2 siRNA transfected cell by using immunofluorescence

DU–145 prostate cancer cell line showed higher transfection rate of as much as 60–70% compared to a 40% transfection rate for PC3 prostate cancer cell line (Fig.
1). Green color represented COX–2 antibody by FITC and blue color indicated DAPI staining of nucleus. In COX–2 siRNA transfected cell (Fig. 1B, 1D), green fluorescence area diminished comparing to non–transfected prostate cancer cell line of PC3 and DU–145.

mRNA expression of AR and caspase–3 in proportion to the concentration of genistein

Little effect of increasing genistein concentration was observed on expression levels of AR mRNA in the DU–145 prostate cancer cell line in the absence of COX–2 siRNA treatment, whereas COX–2 siRNA treated cells showed lower mRNA expression of AR than that in absence of siRNA treatment. We also tested expression level of caspase–3 as an indicator for apoptosis. Messenger RNA expression of caspase–3 increased in proportion to concentration of genistein concentration in COX–2 siRNA transfected prostate cancer cell lines (Fig. 2).

Immunofluorescence of caspase–3 and AR resulting from COX–2 siRNA transfection

Caspase–3 production dependence on genistein concentration between COX–2 siRNA treated and non–treated cells was analyzed by confocal microscopy to detect fluorescence binding to caspase–3 antibody. Cells were visualized by green indicating DAPI, red representing caspase–3 and blue where COX–2 antibody bind. The intensity of immunofluorescence of caspase–3 was increased in proportion to concentration of genistein, and it showed higher level of caspase–3 immunofluorescence in COX–2 siRNA transfed prostate cancer cell lines as same as mRNA expression level of caspase–3 (Fig. 3). In addition, in COX–2 siRNA transfed cell line mRNA expression and protein production of AR decreased as concentration of genistein increased (Fig. 4).

Evaluating cell viability rate in proportion to concentration of genistein

In DU–145 prostate cancer cell lines, the effect of COX–2 siRNA transfection on survival rate (Cell viability) was analyzed by MTT assay. Both siRNA treated and non–treated cell lines showed decrease survival rate according to high level of genistein treatment. There was significant difference of survival rate above 50 μM genistein concentration (p <0.05). In case of siRNA treated cell, survival rate of cell line show significantly different above 10 μM (p<0.05). Through these experiments we proved that genistein affect survival rate of cell line and at 10 μM and 25 μM genistein significantly reduced survival rate (p<0.0001) in COX–2 siRNA transfed cell lines. At higher than 50 μM of genistein, COX–2 siRNA treated cells showed relatively low rate of cell survival than control group (without COX–2 siRNA transfection), but the difference was not statistically significant (p >0.05) (Fig. 5).

Fig. 2. mRNA expression levels of AR, caspase–3, and GAPDH in DU–145 prostate cancer cell line according to concentrations of genistein. mRNA expression levels of caspase–3 in COX–2 siRNA (+) group were stronger than COX–2 siRNA (–) group at high concentrations of genistein. A; control, B; DMSO (0 μM), C; 10 μM, D; 25 μM, E; 50 μM, F; 100 μM, AR; androgen receptor, A; AR without COX–2 siRNA, B; AR with COX–2 siRNA, C; caspase–3 without COX–2 siRNA, D; caspase–3 with COX–2 siRNA, E; GAPDH without COX–2 siRNA, F; GAPDH with COX–2 siRNA.

Fig. 3. Merged images of expression of caspase–3 and COX–2 according to concentrations of genistein on COX–2 siRNA (–) and COX–2 siRNA (+). The expression of caspase–3 in DU–145 prostate cancer cell line was increased in state of increasing dose of genistein and the expression of caspase–3 in DU–145 prostate cancer cell line with silencing of COX–2 was more increased in state of increasing dose of genistein compared to in DU–145 prostate cancer cell line without silencing of COX–2. DAPI (green), COX–2 (blue), caspase–3 (red).
Prostate cancer is a critical public health problem in western countries because it is represented the highest prevalence rate and the second mortality in men's cancer (Siegel et al., 2012). In South Korea, the incidence of prostate cancer has been dramatically increased last 10 years, so active studies of the disease have been significantly expanded (Park et al., 2006). For treatment of prostate cancer, anti–androgen therapy has been used standard therapy in advanced cases, and a recent study showed that prostate cancer incidence is related to inflammation, thus the relationship of these two factors has been examined for cancer treatment (Sobolewski et al., 2010). However, in the study of inflammation and prostate cancer we have not yet found direct evidence for a relationship between cancer and inflammation. The relationship between inflammation and cancer was revealed through clinical data resulting from long–term treatment with nonsteroidal anti–inflammatory drug (NSAIDs) indicating that incidence of colon cancer and rectal cancer were reduced (Yao et al., 2004). Furthermore, DuBois and Smalley (1996) reported that taking aspirin for long period reduces incidence of colon cancer and rectal cancer by as much as fifty percent. Now NSAIDs such as sulindac or celecoxib were approved by the US FDA and had been used for chemoprevention of adenomatous polyposis coli caused by mutation of tumor suppressor gene (Eisinger et al., 2007; Steinbach et al., 2000; Phillips et al., 2002).

Chemoprevention for prostate cancer has been to change dietary habits or inject natural or synthesized substances to inhibit one of the processes involved in cancer development or to reverse existing damage. In the case of prostate cancer, chemical preventive therapy is important because prostate cancer typically has very high incidence rate with the property of very slowly progressing to invasive cancer, and environmental factors contribute to development from latent to clinically diagnosed cancer. One of the environmental factors is isoflavone from a soybean diet and men with a high intake of soybean have a low rate of prostate cancer incidence. Therefore soybean is one of the known chemically preventive foods (Kurahashi et al., 2007). Soybean is a traditional food in Korea and Japan, and it has been thought to be one of the reasons for low incidence rate in Korea comparing to western countries. Genistein in soybean is the main phytoestrogen to prevent not only prostate cancer but also breast cancer and rectal cancer, and it has been reported as a notable preventive chemical. Thus, in this research our goals were to reveal that how inflammation inducing COX–2 and preventive chemical compound genistein function in response to changes in the AR and cell survival in prostate cancer cells.

Since treatment with NSAIDs such as aspirin and sulindac in colorectal cancer was reported to reduce the mortality rate caused by colon cancer in the late 1990’s, it has been attractive significant research goal to suppress COX–2 in order to inhibit cancer incidence. The pathways of tumor induction by COX–2 are through controlling prostaglandin production, apoptosis, and cancer...
cell metastasis (Gallo et al., 2001; Uefuji et al., 2000; Masferrer et al., 2000; Daniel et al., 1999). Also, Uefuji et al. (2000) reported that overexpressed COX–2 was associated with increased prostaglandin E2 biosynthesis and angiogenesis in gastric cancer, and Tsuchi et al. (1997) found that overexpressed COX–2 induces the reactivity of metalloproteinase–2, which caused phenotypic changes altering the metastatic potential of colorectal cancer cells. Furthermore, Chan et al. (2007) showed COX–2 expression interference by siRNA exhibited anti-proliferative effect on gastric cancer cells.

Palayoor et al. (1998) reported the use of NSAIDs (ibuprofen) enhanced the efficacy of radiation in prostate cancer cell and the efficacy was related with the dosage of ibuprofen. And in order to reduce the side effects of NSAIDs, the suppression of COX–2 by siRNA was used in next study but it did not alter radio sensitivity in prostate cancer cells (Palayoor et al., 2005). Previous evidence showing COX–2 inhibition affecting mechanisms of tumor growth supported that efficacy of treatments with either chemotherapy or radiation might be augmented and it could be enhanced prevention of cancer progression.

Two significant pathways induce cell death, of which one is caused by an internal pathway involved in mitochondria induced by damaged cells and the other by external pathway with TNF (tumor necrosis factor)–induced and the Fas–Fas ligand–mediated pathway. Meanwhile, suppression of COX–2 played a role in both pathways to induce cell death. Liu et al. (1998) found that an androgen–sensitive prostate cancer cells which highly expressed COX–2 could be changed to apoptotic cell by COX–2 suppressor NS–398 inducing inhibition of Bcl–2. Cell death and caspase activation was inhibited by Bcl–2 family inhibiting the production of the factor such as cytochrome c to turn on cell death (Marcelli et al., 2000; Marcelli et al., 1999). Based on Bcl–2 gene, Hsu et al. (2000) revealed that COX–2 is constitutively expressed in androgen–sensitive LNCaP and androgen–insensitive PC3 cells and that COX–2 inhibitor, celecoxib, induced apoptosis by blocking Akt phosphorylation independently of Bcl–2. The inhibition of COX–2 and Bcl–2 might affect the initiating of apoptosis therefore we evaluated whether COX–2 affect the programmed cell death in androgen insensitive DU–145 prostate cancer cells using siRNA of COX–2. And we included caspase–3 in our study in order to investigate not only the apoptosis mechanism but also to evaluate cell death efficiency as chemical preventive in COX–2 siRNA suppressed prostate cancer cells. Through our research we found that siRNA treated cells showed high expression of caspase–3, and as genistein dosage increased the expression of caspase also increased. Therefore we assumed suppression of COX–2 enhanced efficiency of cell death with co–treatment of genistein.

Androgen has an important role in development and proliferation of prostate and it is converted dehydroxytestosterone (DHT) by 5–alpha reductase. DHT–AR complex induced transcription of DNA and produce several substances such as TGF–beta, Insulin–like growth factor, epidermal growth factor, PDGF and so on that affect proliferation and development in prostate cell (Rizzo et al., 2005).

The findings in this research that inhibition of COX–2 suppress the expression of AR was in accordance with the results of Narayama et al. (2006) indicating that treatment with COX–2 siRNA in mouse prostate cancer cell lines (TR–75) and human PC3 prostate cancer cell stops the cell growth caused by high activity of cell division in G1–S phase and expression of the AR decreased in TR–75 cell but the change of AR expression was not identified in PC3. We thought one of this reasons why AR expression did not change in PC3 cells that it was poorly differentiated cancer cell than DU–145 cell and another reason was different activity of 5–alpha reductase in PC3 cells.

Peterson and Barnes (1993) reported that genistein inhibited cell growth of both hormone dependent and independent cells with respect to LNCaP and DU–145. In addition, Gellar et al. (1998) showed that the concentration of genistein in the range of 1.25–10 μg/ml has the effect of suppressing growth of both benign prostatic hyperplasia and prostate cancer in histoculture. Newly introduced genistein effect on prostate cancer treatment has been frequently reported, however the mechanism by which genistein inhibits growth was not clearly revealed except that it is not through the EGF–receptor tyrosine kinase. Recent data from Li et al. (2008) and Oh et al. (2010) gave the clues that inhibiting cell growth and suppressing expression of AR resulted from genistein inhibition of AR expression, and regulated Akt/PKB serine/threonine kinase including nuclear factor kappa B and EGFR–Akt/p70S6k. At the same time we showed that survival rate of COX–2 siRNA treated prostate cancer cell significantly decreased compared to non–treated cells, and genistein treated cells depending on its concentrations (25 μM–50 μM) were affected on survival rate; however higher concentration of genistein (100 μM) had no benefit on cell survival. From these results, we assume that genistein contributes to cell death rather than having an effect by inhibition of COX–2, but decrease of AR expression may result from both COX–2 suppression and genistein effect.

In summary, through our research data indicating COX–2 suppression and genistein effect on AR expression and cell vitality rate, we suggest that genistein may be valuable as an alternative cancer treatment, specifically in prostate cancer for clinical purposes.

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