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Cell Biology International 31 (2007) 1420–1427

Cell Biology International

www.elsevier.com/locate/cellbi

Up-regulation of connexin 43 and gap junctional intercellular communication by Coleusin Factor is associated with growth inhibition in rat osteosarcoma UMR106 cells

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Received 11 April 2007; revised 3 June 2007; accepted 12 June 2007

Abstract

Gap junctions, formed by connexin (Cx) family proteins, permit direct exchange of regulatory ions and small signal molecules between neighbouring cells. Gap junctional intercellular communication (GJIC) plays an important role in maintaining the homeostasis and preventing cell transformation. Most of the tumour cells feature deficient or aberrant connexin expression and GJIC level, and restoration of connexin expression and GJIC is correlated with cell growth control. Numerous researches has suggested the possibility of connexins as potential anti-tumour targets for chemoprevention and chemotherapy. We investigated the ability of Coleusin Factor (CF, also named FSK88) to regulate the Cx43 expression and GJIC level in rat osteosarcoma UMR106 cells. The results have demonstrated that CF increased the mRNA and protein expression of Cx43 in both in a dose- and timedependent manner, and concomitant with up-regulation of Cx43, CF treatment up-regulated the diminished GJIC level in UMR106 cells as assayed by dye transfer experiments. In addition, Cx43 distribution at the plasma membrane was also enhanced dramatically by CF treatment. Furthermore, we discovered that CF was potent to inhibit the growth and proliferation of UMR106 cells. These results provide the first evidence that CF can regulate connexin and GJIC, indicating that Cx43 may be a target of CF to exert its anti-tumour effects.

Keywords: Coleusin Factor; Connexin 43; Gap junctional intercellular communication; Cell growth; Osteosarcoma; Retinoic acid

1. Introduction

Most eukaryotic cells are joined with specialised intercellular channels named gap junctions (GJs), which facilitate information to transfer from one cell to neighbouring cells. Small hydrophilic molecules ($M_W < 1000$ Da), such as Ca^{2+}, cAMP, and inositol 1,4,5-trisphosphate, pass freely through GJ channels to maintain the electrical and metabolic tissue homeostasis. This process is known as gap junctional intercellular communication (GJIC) (Valiunas, 2002; John et al., 1999; Kumar and Gilula, 1996). Cell proliferation, differentiation, apoptosis and adaptive responses can be the sequence of up or down regulation of GJIC, and disruption of GJIC may cause lose of cell growth control (Vinken et al., 2006). Each GJ channel is formed by two hemi-channels (connexons) in the apposed membranes of two adjacent cells, and each connexon is composed of six individual trans-membrane proteins called connexins (Cxs) (Martin and Evans, 2004). A single cell type may express one or several kinds of Cxs that form channels with different permeability and ionic selectivity (Steinberg et al., 1994). Approximately 20 members of Cx proteins ranging in size from 26 to 56 kDa have been identified in mice and humans (Evans and Martin, 2002).

A number of studies suggest a suppressing tumour effect of Cx genes, based on the facts that tumour cells are usually

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doi:10.1016/j.cellbi.2007.06.008

accompanied by diminished or entirely missing expression and function of Cxs (Chipman et al., 2003; Yamasaki and Naus, 1996). Cells from the Cx gene knockout mice, which have a much higher tendency for tumourigenesis compared to the wild type mice lacking Cxs genes, are at an increased risk of developing both spontaneous and chemically induced tumours (Zhang et al., 2001; Temme et al., 1997; Fernstrom et al., 2002). The anomaly of Cxs is thought to contribute to the malignant phenotype by disengaging tumour cells from the potential negative growth control signals via abrogated GJIC (Holder et al., 1993). Among all the Cx members, connexin 43 (Cx43) is the most widely expressed and has been invested intensively. Cx43 hemi-channels are involved in diverse roles in cell physiology and pathology, including regulation of cellular volume (Quist et al., 2000), efflux of NAD^+ and ATP (Franco et al., 2001; Stout et al., 2002), and acceleration of cell death during metabolic inhibition (Contreras et al., 2002). Cx43 expression is consistently reduced in various kinds of tumour cells (Huang et al., 1999; King et al., 2000; Hanna et al., 1999; Hossain et al., 1999), and in many tumour-promoting agents and growth factors, as well as in oncogenes, which have been found to inhibit Cx43 (Yamasaki and Naus, 1996). Restoration of GJIC by up-regulation or transfection of Cx43 can lead to the reversal of feather of transformed phenotype induction of differentiation and a reduction in cell growth rate (Naus et al., 1992; Hirschi et al., 1996; Proulx et al., 1997).

Coleusin Factor (CF, also named FSK88) is a kind of diterpenoid, first isolated and purified from a type of Chinese tropical plants in our laboratory. Previous research had demonstrated that CF has a prominent effect on inhibiting the proliferation of several malignant tumour cells. It can induce human hepatoma HepG2 cells to differentiate by mediating the cell cycle arrested at the G1 phase (Ye and Wang, 2006), conversely, it has the ability to stimulate apoptosis of human gastric BGC823 cells through the mitochondria pathway (Li and Wang, 2006). Prompted by the reports that some anti-tumour agents such as retinoids and carotenoids can up-regulate GJIC and Cx43 levels in a variety of malignant cell types (Stahl and Sies, 1998), rat osteosarcoma UMR106 cells were used as the model to investigate if CF has a similar effect, also compared were the effects of CF and retinoid acid (RA). The study found that CF is potent to up-regulate the expression of Cx43 and in restoring the impaired GJIC of tumour cells, accompanied by the inhibition of cell proliferation. Results suggest that CF may target Cx43 causing it to exhibit the anti-tumour function.

2. Materials and methods

2.1. Cell culture and reagents

The rat osteosarcoma UMR106 cell line was donated by Massachusetts General Hospital. Cells were cultured in DMEM (Gibco, USA), supplemented with 10% heat-inactivated fetal bovine serum (Hyclon, USA), 100 unit/ml benzylpenicillin and 100 μg/ml streptomycin at 37 °C in a humidified incubator with 5% CO_2 in air. CF, a white powder with a molecular weight of 410, was isolated and purified with HPLC. CF and RA (Sigma, USA) were dissolved in absolute ethanol at 100 mM and 10 mM, respectively, as the stock solution and stored in dark at −80 °C. UMR106 cells were treated with the diluted reagents at the indicated concentration in the culture medium. The final concentration of ethanol was always <0.1%, and the possible effects of ethanol were preliminarily excluded by using medium containing 0.1% ethanol as a vehicle control.

2.2. RNA isolation and semi-quantitative RT-PCR

UMR106 cells were treated with CF of different concentrations (25 μM, 50 μM, and 100 μM) or RA (10 μM) for 72 h; or treated with CF of 100 μM for 12, 24, 48, and 72 h. Total RNA was isolated from cells using TRIZOL reagent (Gibco, USA) according to the provider's protocol and stored at −80 °C. cDNA was synthesised from isolated RNA using an $oligo(dT)_{15}$ primer and moloney murine leukemia virus (M-MLV) reverse transcriptase (TaKaRa, Japan). PCR was performed by mixing 1 μg cDNA as template, 2.5 μl of 10× PCR buffer, 10 mmol dNTP, 1 μmol sense primer, 1 μmol anti-sense primer and 2 U *Taq* DNA polymerase in a final volume of 25 μl. Each PCR cycle consisted of a denaturing step for 40 s at 94 °C, an annealing step for 40 s at 57 °C, and an extension step for 40 s at 72 °C. The total cycle is 30 for Cx43 and 25 for β-actin. Primer sequences were as follows. Cx43: sense, 5-GTCTGTGCCCACCCTCCTGT-3, anti-sense, 5-TTGCTCTGCGCT GTAGTTCG-3 (product of 697 bp); β-actin: sense, 5-GATCTTGATCTTCA TGGTGCTAGG-3, anti-sense, 5-TTGTCACCAACTGGGACGATATGG-3 (product of 764 bp). PCR products were visualised by 1% agarose gel and stained with ethidium bromide. β-Actin was used as the loading control, each band was semi-quantified by densitometric scanning, and the data were analysed.

2.3. Western blot analysis

UMR106 cells were treated with CF of different concentration (25 μM, 50 μM, and 100 μM) or RA (10 μM) for 72 h; or treated with CF of 100 μM for 12, 24, 48, and 72 h. After treatment, cells were washed three times with ice cold PBS and were incubated with lysate buffer (20 mM Hepes, pH 7.5, 200 mM NaCl, 0.2 mM EDTA−Na_2, 1% Triton X-100, 0.05% SDS, 0.5 mM DTT, 1 mM Na_3VO_4, 20 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin) at 4 °C for 30 min, centrifuged at 12000 *g* for 5 min and the supernatant was stored at −80 °C. The amount of protein was estimated from the calibration curve of bovine serum albumin as measured by spectrophotometry analysis. Equal amounts of protein (100 μg) were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Hybond, Austria). The membrane was blocked in TBST containing 5% no-fat milk for 2 h at room temperature, incubated with primary mouse monoclonal anti-Cx43 or anti-α-tublin antibody (Sigma, USA) overnight at 4 °C, and incubated with peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz, USA) for 2 h at room temperature. Blots were developed by a chemiluminescence ECL detection kit (Pierce, USA), with α-tublin as the loading control.

2.4. Immunofluorescence staining of Cx43

UMR106 cells cultured on glass cover-slips in the six-well plates were grown to 80% confluence. The cells were treated with 100 μM CF or vehicle control for 72 h and washed thoroughly with PBS before fixing in 4% formaldehyde. Cells were washed again with PBS and incubated with 10% goat serum to block the non-specific sites. The cells were incubated with primary mouse monoclonal anti-Cx43 antibody (Sigma, USA) for 1 h, and incubated with secondary FITC-conjugated goat anti-mouse antibody (Santa Cruz, USA) for 45 m after washing by PBS. The cover-slips were sealed and detected immediately under an inverted fluorescence microscope (Olympus, Japan).

2.5. Scrape loading (SL)/dye transfer (DT) assay

GJIC levels of control and treated cells were determined by using the SL/DT technique. UMR106 cells cultured on the glass cover-slips in the six-well plates were grown to 80% confluence. The cells were treated with CF of different concentrations (25 μM, 50 μM, and 100 μM) or RA (10 μM) for 12, 24, 48, and 72 h, and washed thoroughly with CMF-PBS (PBS without Ca^{2+} and Mg^{2+}). Scrape loading was performed by two cuts on the cell mono-layer with

a razor blade before 500 μl Lucifer Yellow CH (LY) (Sigma, USA) solution of 0.05% concentration was added on the cover-slip to imbue the cells for 5 m. Cells were rinsed three times with CMF-PBS, fixed with 4% formaldehyde in CMF-PBS, and detected by fluorescence emission with an inverted fluorescence microscope (Olympus, Japan). Cells that received the Lucifer Yellow from the scraping-loaded cells were considered to be communicating and the number of cells counted. The number was determined by measurement of six scrapes, and three experiments were carried out with each treatment. GJIC was expressed as a percentage of the vehicle control.

2.6. Cell count

UMR106 cells of the exponential growth phase were seeded into a 24-well plate at 1×10^5 cells per well. The cells were treated with different concentrations of CF (25 μM, 50 μM, and 100 μM), RA (10 μM) or vehicle control for 12, 24, 48, and 72 h. The cells were trypsinised and suspended in 2 ml of medium. The cells were counted by using a hemocytometer and plotted as a growth curve. All experiments were performed using triplicate wells, and the results show the average of three independent experiments.

2.7. Soft agar colony formation assay

UMR106 cells (1.5×10^3) of the exponential growth phase were seeded with the presence of respective reagents in 35 mm^2 dishes containing 0.7% agar. After incubation for 10 days, colonies of more than 50 cells were counted under an inverted microscope. All experiments were performed using triplicate dishes, and the results are the average of three independent experiments.

2.8. Statistical analysis

All the experiments have a minimum of three determinations. The data were expressed as mean ± SEM (standard error mean). The statistical differences between groups were determined by AVOVA and the *t*-Student test, and the difference among groups were considered statistically at $P < 0.05$.

3. Results

3.1. Effects of CF on Cx43 mRNA expression

We examined the effects of CF on Cx43 RNA expression in UMR106 cells using semi-quantitative RT-PCR. As shown in Fig. 1, CF was able to up-regulate Cx43 RNA expression levels in both a dose- and timedependent manner. After treatment for 72 h, the three different concentrations of CF had a significant effect in up-regulating Cx43 mRNA expression, and treatment of 100 μM CF, which is the optimum concentration (Ye and Wang, 2006), resulted in a more than twofold enhancement of the Cx43 mRNA level. RA (10 μM) also significantly increased Cx43 mRNA expression after 72 h treatment, but the increasing extent was less than 100 μM CF and similar to the effect of 50 μM CF. The time course studies demonstrated that significant enhancement of Cx43 mRNA expression by 100 μM CF appeared as early as 24 h into the treatment, although no notable increase was detected when the cells were treated for 12 h. Furthermore, there is no significant difference to the effects of CF treatment after 48–72 h in Cx43 mRNA levels of UMR106 cells.

3.2. Effects of CF on Cx43 protein levels

Western blot analysis was used to determine the relative amount of Cx43 protein expression following the different

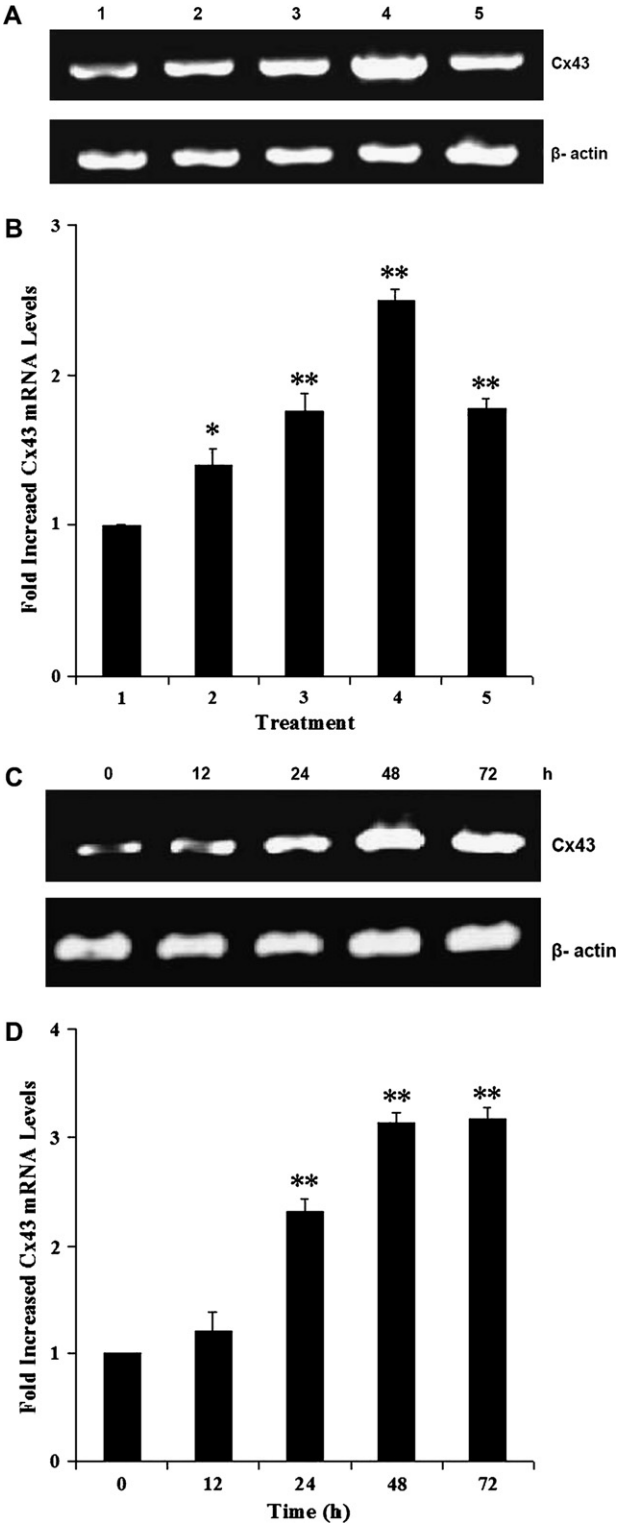

Fig. 1. Effect of CF on Cx43 mRNA expression in UMR106 cells. Total RNA was isolated from the cells with different treatments and the gene expression was assessed by RT-PCR. (A and B) Results of the Cx43 mRNA expression of the cells treated with different concentrations of CF or RA for 72 h. Lane 1, vehicle control; lane 2, 25 μM CF; lane 3, 50 μM CF; lane 4, 100 μM CF; and lane 5, 10 μM RA. (C and D) Results of the Cx43 mRNA expression of the cells treated with 100 μM CF for different time periods. The columns in (B) and (D) represent mean ± SD of three determinations for each treatment condition, showing the fold increased Cx43 mRNA levels. $*P < 0.05$ and $**P < 0.01$ compared with vehicle control.

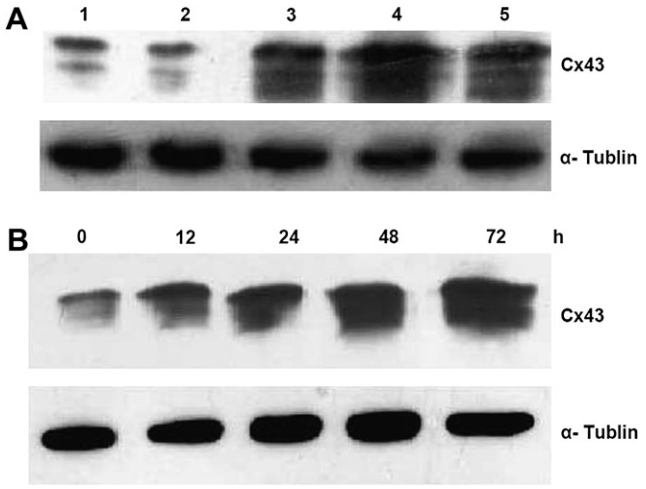

Fig. 2. Effect of CF on Cx43 protein expression in UMR106 cells. Lysate from the cells with different treatments and the protein expression was assessed by Western blot. (A) Results of the Cx43 protein expression of the cells treated with different concentrations of CF or RA for 72 h. Lane 1, vehicle control; lane 2, 25 μM CF; lane 3, 50 μM CF; lane 4, 100 μM CF; and lane 5, 10 μM RA. (B) Results of the Cx43 protein expression of the cells treated with 100 μM CF for different time periods. Three independent experiments were carried out and one representative result was shown.

treatments. Fig. 2 shows that Cx43 protein levels increased with the CF concentration rise and reached a significant amount after treatment of 100 μM CF for 72 h. RA up-regulated Cx43 protein expression, but as with the effects to the mRNA level, the cells treated with 100 μM CF expressed more Cx43 protein than the cells treated with 10 μM RA for the same length of time. We detected the effects of CF to Cx43 protein expression level in time course by treating UMR106 cells with 100 μM CF for 12, 24, 48, and 72 h. Results showed that the Cx43 protein increased by CF was consistent with the length of treatment time. The studies indicated that CF was also a potential to up-regulate Cx43 protein expression in the dose- and timedependent manner.

3.3. Effects of CF on Cx43 expression on the cell membrane

To further validate the influence of CF to Cx43 level, immunofluorescence staining was performed to determine the distribution and expression of Cx43. As shown in Fig. 3, Cx43 was localised to the plasma membrane of UMR106 cells. Compared with the vehicle control treated cells, which only exhibited limited expression of Cx43, treatment with 100 μM CF for 72 h resulted in an apparent increase in level and a wider distribution of the Cx43 protein. The increased immuno-staining involved both the extent and the size of highly fluorescent zones, suggesting a possible up-regulation in the number and size of gap junction plaques.

3.4. CF enhances GJIC

To determine if the up-regulation of Cx43 by CF is related to GJIC level, we performed SL/DT assays using the gap

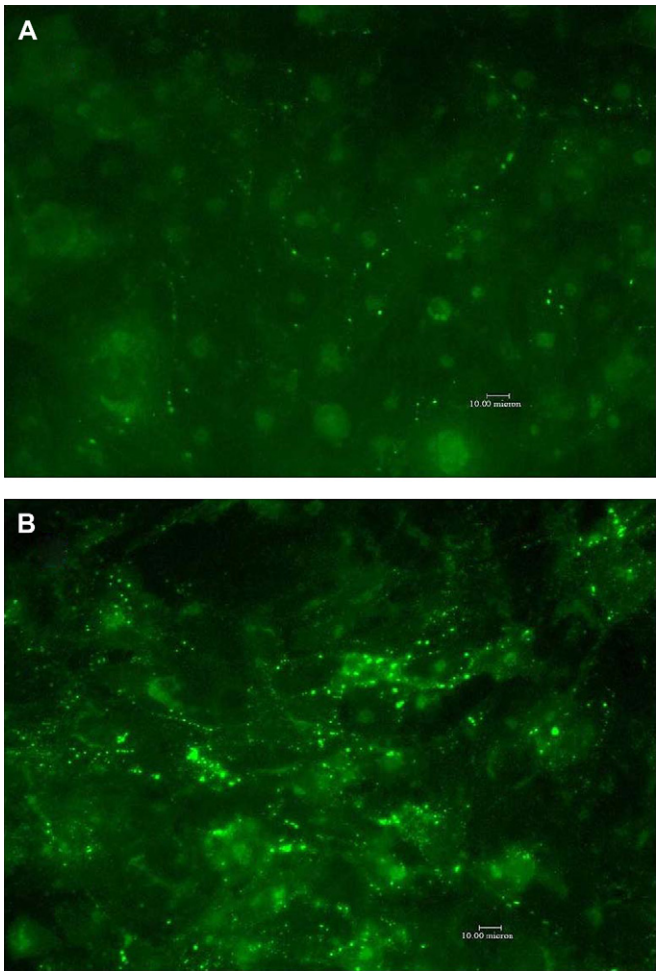

Fig. 3. Cx43 expression and localisation following 100 μM CF treatment for 72 h, assessed by using immunofluorescence staining analysis. The images were visualised by fluorescent microscope (×100). The results demonstrated that Cx43 distribution at the plasma membrane of UMR106 cells was dramatically increased by CF treatment. (A) Vehicle control and (B) 100 μM CF. Three independent experiments were carried out and one representative result was shown.

junction permeable fluorescent dye LY (Fig. 4). The GJIC level was detected after UMR106 cells were treated with 25 μM, 50 μM, and 100 μM CF plus 10 μM RA, respectively, as demonstrated for CF-induced increases of Cx43 mRNA and protein levels. Cells treated with all three concentrations of CF exhibited a much larger LY transfer than the control cells, and this effect was especially significant after 24 h. Similar to its action on Cx43 expression, CF enhanced GJIC of UMR106 cells in both a dose- and timedependent manner. The treatment with 100 μM CF for 72 h caused a 350% increase in the number of cells showing LY fluorescence. RA enhanced the GJIC level of UMR106 cells, however, 10 μM RA was less potent than 100 μM CF.

3.5. CF inhibits the growth and proliferation of UMR106 cells

Cell counts were performed to determine if CF treatment suppressed growth of UMR106 cells (Fig. 5A). A significant

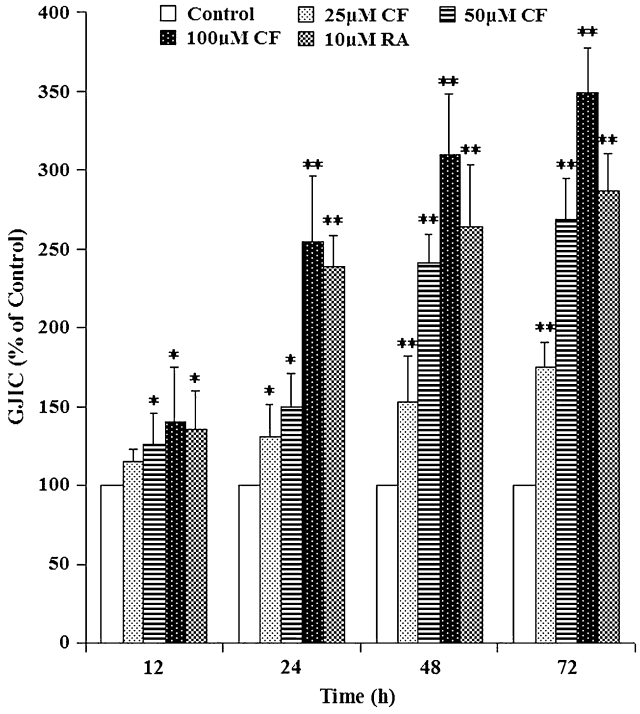

Fig. 4. CF enhances GJIC level. UMR106 cells were treated with vehicle control, CF of different concentrations (25 μM, 50 μM, and 100 μM) or RA (10 μM) for 12, 24, 48, and 72 h. After that, GJIC level was assessed by SL/DT technique as described in Section 2. The results are demonstrated as the columns representing mean ± SD of three determinations for each treatment condition. Values are expressed as the percentage of those obtained after treatment with vehicle control. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle control.

decrease of cell number between the vehicle control and the CF treatment was noted, and all three concentrations of CF (25 μM, 50 μM, and 100 μM) caused a dose dependent suppression effect on cell growth during 72 h treatments. Cell proliferation was measured by soft agar colony formation assay. The number of colonies in vehicle control and various treatment groups was counted and summarised in Fig. 5B. From the results, it is evident that CF had an obvious effect on inhibiting the colony formation in UMR106, and 100 μM CF caused approximately a 95% inhibition of the colony number, indicating that CF is a potent inhibitor of cell proliferation. RA (10 μM) decreased the cell growth rate and colony formation, but its effects were less potent than 100 μM CF.

4. Discussion

Direct communication between cells by gap junctions is considered an indispensable mechanism in the maintenance of cellular homeostasis. More importantly, GJIC mediated by Cxs is actively involved in almost all aspects of the cell life cycle, from cell growth and cell differentiation to cell death (Vinken et al., 2006). A number of studies have demonstrated that tumour cells are deficient in GJIC as a result of the down-regulated expression of connexins, and the consistent lack of normal GJIC in tumour cells suggests that developing tumour cells must isolate themselves from the influence of surrounding normal cells in order to progress. Consequently, Cxs function has the majority of tumour suppressor genes whose expression and function must also be diminished during carcinogenic progression (Vine and Bertram, 2002; Weinberg, 1991; King and Bertram, 2005). Cx43 is one of the most widely distributed and well studied Cxs, which plays a critical role in the inhibition of tumour development. The impaired Cx43 expression level is directly related to numerous kinds of tumorigenesis, and the up-regulation of Cx43 expression is correlated with cell proliferation control both in vitro and in vivo (King and Bertram, 2005). For example, in human osteosarcoma U2OS cells transfected with cDNA of rat Cx43 exhibited dramatic inhibition of cell proliferation and restoration of GJIC (Zhang et al., 2001). The study demonstrated that over expression of Cx43 rather than Cx45 in rat osteosarcoma UMR106 cells resulted in the formation of gap junctions with high permeability, which may benefit the intracellular message molecules distribution (Steinberg et al., 1994). Cx43 has been suggested as a potential anti-oncogenic target for chemotherapy. Some chemical agents are able to up-regulate the level of Cx43 and to promote the formation of GJ with effects to suppress the neoplasia as well as the tumour cell growth, including retinoids (Rogers et al., 1990), carotenoids (Zhang et al., 1991, 1992), compounds of green tea (Sigler and Ruch, 1993), etc.

UMR106 cell lines were established from a ^{32}P-induced, transplantable osteosarcoma in the rat, extensively characterised as the counterpart of the osteoblast (Ng et al., 1985; Martin et al., 1976). UMR106 cells have a very low expression level of Cx43, so UMR106 cells were used as the model to examine the effect of CF to Cx43 by semi-quantitative RT-PCR and Western blot. The results demonstrated that CF up-regulated Cx43 mRNA and protein expression in both a dose- and timedependent manner. Hundred micrometres of CF had the most effective activation of Cx43, in line with our previous study result that 100 μM is the optimal concentration of CF (Ye and Wang, 2006). The mechanism by which CF elicits Cx43 up-regulation requires further study. One of the possibilities is CF exerts this function through a cAMP pathway, as CF can result in cAMP accumulation in UMR106 cells immediately after the treatment (unpublished work), and increased levels of cAMP induced up-regulation of Cx43 and enhancement of GJ in osteoblast cells (Romanello et al., 2001).

In the conventional creed, connexins only exert anti-tumour effects via forming GJ channels and restored GJIC (Holder et al., 1993). However, since 1995 when the study indicated that connexins may have non-GJIC-related functions (Mesnil et al., 1995), more evidence is emerging that suggests GJIC independent function of connexins in cell growth, tumorigenicity, differentiation and apoptosis (Jiang and Gu, 2005). The study has demonstrated that transfection of Cx43 gene into human glioblastoma cells reversed the transformed phenotype without enhancing activity of GJIC (Huang et al., 1998). Furthermore, Cx43 was found to regulate cell growth independent of GJ channel formation (Moorby and Patel, 2001). Cx43 suppressed cellular proliferation associated with an increased

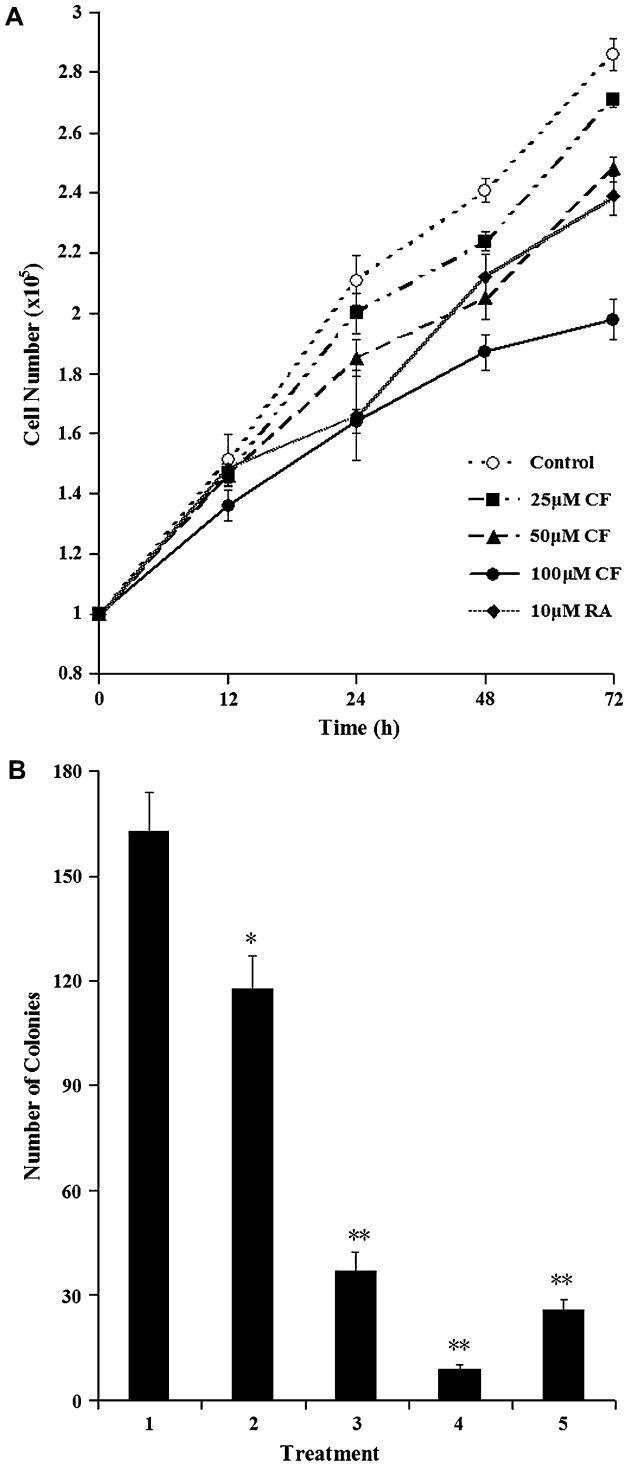

Fig. 5. The inhibition effects of CF on the growth rate and proliferation ability of UMR106 cells. (A) Cell growth curve of UMR106 cells. The cells were treated with vehicle control, CF of different concentrations (25 μM, 50 μM, and 100 μM) or RA (10 μM) for 12, 24, 48, and 72 h. After that, cell numbers were counted and cell growth curve was used to assess the growth rate affected by different treatments. (B) Results of soft agar colony formation assay. UMR106 cells were cultured in the media containing different reagents, and after incubation for 10 days, colonies of more than 50 cells were counted under an inverted microscope. Lane 1, vehicle control; lane 2, 25 μM CF; lane 3, 50 μM CF; lane 4, 100 μM CF; and lane 5, 10 μM RA. All the results are represented as mean ± SD of three determinations for each treatment condition. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with vehicle control.

level of p27 and the degradation of Skp2, a protein involved in p27 ubiquitination (Zhang et al., 2001, 2003a,b). Further evidence demonstrates that connexins directly control the expression of some genes either coding for growth enhancing or repressing proteins (Flachon et al., 2002; Zhang et al., 2003a,b; Koffler et al., 2000; Yano and Yamasaki, 2001). In this regard, connexins are thought to exert their regulatory function both as the building blocks of GJ channels and as molecules with growth control and tumour suppressing effects independent of GJ activity. To examine if the up-regulation of Cx43 by CF will lead to the activation of GJIC, the localisation and expression of Cx43 were detected by using immunofluorescence staining, GJ formation requires that connexin proteins be located at the plasma membrane (Conklin et al., 2007). The results showed that after treatment with 100 μM CF for 72 h, UMR106 cells expressed punctuate Cx43 staining at the plasma membrane, suggesting a possible up-regulation in the number and size of gap junction plaques. We examined the effect of CF on GJIC by using SL/DT assay, the results demonstrating that CF also up-regulated GJIC in a dose- and timedependent manner. The findings suggest that Cx43 up-regulated by CF can result in the restoration of GJIC level, which is always diminished in tumour cells.

We previously reported the ability of CF to inhibit the growth of some kinds of tumour cells by inducing cell differentiation or apoptosis (Ye and Wang, 2006; Li and Wang, 2006). Here CF treatment was proved to reduced cell number and the proliferative ability of UMR106 cells detected by cell counting and soft agar colony formation assay, 100 μM CF induced the greatest number of reduced cells and colonies. The growth suppressive effects of CF have been established, but the anti-tumour effects of CF through gap junctional mechanism have not been investigated. These results provide evidence that cellular growth suppression by CF may be dependent on up-regulation of Cx43 and GJIC. We have found that CF can induce UMR106 cells to differentiation through increasing the expression level of some differentiation marker genes (unpublished work), other researchers have reported that Cx43 and GJIC are potent to modulate the expression of osteoblastic differentiation markers (Li et al., 2005; Lecanda et al., 1998). Taken together, these findings provide some evidence that Cx43 may be one of the CF's targets to exert its anti-tumour function.

Retinoic acid (RA), a biologically active metabolite, is able to regulate the reversion of transformed phenotype and induce the growth arrest of many kinds of tumour cells (Abu et al., 2005; Yen et al., 1998; Ng et al., 1985). Several studies have demonstrated that RA has the ability to up-regulate both the Cx43 and GJIC levels, which may be one of its mechanisms to inhibit tumours (Stahl and Sies, 1998; Tanmahasamut and Sidell, 2005). In previous research, RA was used as a positive control and comparison with CF of anti-tumour effect (Ye and Wang, 2006; unpublished work). Therefore, in this study, 10 μM RA was also used to make comparison with CF. The results demonstrated that RA could up-regulate the Cx43 expression and GJIC level as well as inhibit the growth of UMR106 cells. However, 10 μM RA has a less potent effect than 100 μM CF.

In conclusion, this study provides the first evidence that CF up-regulates the Cx43 expression, both in mRNA and in protein levels, and the Cx43 distribution at the plasma membrane in rat osteosarcoma UMR106 cells. Cx43 increased by CF treatment results in the restoration of GJIC accompanied by the inhibition of cell growth and proliferation. These new findings suggest that Cx43 plays an important role in mediating the anti-tumour effects of CF.

Acknowledgements

This work was supported by a grant from the National Natural Sciences Foundation of China (No. 30171071). We thank Ran Lu for her valuable technical assistant and we are very grateful to Zhaozhe Liu and Xia Liu for their discussion during the research.

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