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COX Inhibitors Modulate bFGF-Induced Cell Survival in MCF-7 Breast Cancer Cells

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Abstract Basic fibroblast growth factor (bFGF) serves as a modulator of survival in breast cancer cells. The mechanisms by which bFGF transduces the anti-apoptotic signal and interacts with COX inhibitors were investigated. bFGF reduced apoptosis in MCF-7 breast cancer cells and up-regulated the expression of mitocondrial Bcl-2, whereas COX inhibitors meloxicam (selective COX-2) and aspirin (non-selective), induced apoptosis. bFGF up-regulated survivin protein expression and induced cdc-2 phosphorylation moderately at early (2–6 h), and substantially at late (24 h), time-points. Survivin mRNA expression was up-regulated only at the later time-point. COX inhibitors prevented up-regulation of survivin protein expression at both 2 and 24 h and prevented early modest increases in cdc-2 phosphorylation. Up-regulation of survivin mRNA was not found to be modulated by the COX-2 inhibitor meloxicam. bFGF regulation of survivin expression was found to be ERK1/2 kinase dependent and bFGF-induced phosphorylation of c-raf was prevented by the COX-2 inhibitor. bFGF was, however, unable to induce COX-2 protein expression or modulate COX-2 activity in MCF-7 cells as evidenced by unaltered PGE_2 production. These results indicate that bFGF regulates survivin expression in MCF-7 breast cancer cells by signaling through an ERK1/2 dependent pathway. COX-2 inhibitors can modulate bFGF-induced survivin expression in a COX-2 independent manner. J. Cell. Biochem. 91: 796–807, 2004.

Key words: COX inhibitors; basic fibroblast growth factor; cdc2 kinase; survivin

Defects in apoptotic signaling mechanisms contribute to several pathological conditions including carcinogenesis and progression of malignant tumors [Thompson, 1995]. Many cancer cell survival factors, including basic fibroblast growth factor (bFGF), potentiate the dysregulation of apoptosis in an autocrine fashion by up-regulating anti-apoptotic proteins such as Bcl-2. bFGF has a potent mitogenic activity on a wide variety of mesoderm-derived cells. In cancer cells, bFGF serves as a vital modulator of cell survival and is secreted during tumor growth and angiogenesis [Souttou et al., 1994; Bikfalvi et al., 1997]. bFGF activates cancer cells by binding to specific tyrosine kinase receptors on the cell surface that immediately induce transient phosphorylation of their tyrosine residues. The activated receptor phosphorylates a set of cytoplasmic signaling molecules containing Sh2 domains that in turn activates several pathways, in particular, the mitogen activated protein kinase pathway [Vercoutter-Edouart et al., 2000; Liu et al., 2002].

Survivin, a member of the inhibitor of apoptosis protein (IAP) gene family is expressed in various human cancers and is not detectable in terminally differentiated normal adult tissues. Survivin is up-regulated by growth factors and increased expression is associated with a decrease in cancer cell apoptosis and poor disease survival [Lu et al., 1998; Tanaka et al., 2000; Kawasaki et al., 2001]. It is expressed in a cell-cycle dependent manner and found to be up-regulated in the G_2/M phase [Ambrosini et al., 1997; Li et al., 1998]. Over-expression of survivin has been shown to result in an activated Cdk2/cyclinE complex, leading to accelerated S phase shift and resistance to G_1 arrest [Suzuki et al., 2000]. Activation of survivin is dependent on phosphorylation by cdc2 kinase. This activation requires phosphorylation of Thr 34,

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prevention of which results in caspase-dependent cell apoptosis [O'Connor et al., 2000].

The use of cyclooxygenase (COX) inhibitors has been shown to be associated with decreased risk of colonic carcinoma development [Thun et al., 1991]. In animal models, COX inhibitors have been shown to slow the progression of primary mammary tumors and decrease the number of pulmonary metastases [Young et al., 1987; Rozic et al., 2001; Williams et al., 2001].

We hypothesise that bFGF mediates its mitogenic activity in part through the regulation of the anti-apoptotic protein survivin and that COX inhibitors may have the ability to modulate this cell survival pathway. In this report, we provide evidence that COX-2 inhibitors possess a broad negative regulatory effect on the survival of MCF-7 breast cancer cells and can modulate the anti-apoptotic effect of bFGF.

MATERIALS AND METHODS

Cell Culture and Stimulations

Human breast cancer MCF-7 cells were grown in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS), penicillin 100 μg/ml, streptomycin 100 μg/ml (Life Technologies, Inc., Paisley, UK) and incubated in a humidified atmosphere of 5% CO_2 at 37°C. Experiments were carried out when cells reached 90% confluence.

Cells were incubated in a serum free medium for 24 h before treatment with test agents. Cells were incubated in the presence and absence of COX inhibitors Aspirin (100 μM) and Meloxicam (10 μM), 45 min prior to incubation with bFGF (3 ng/ml) for varying time-periods. To inhibit de novo protein synthesis, cells were incubated with cyclohexamide (5 μg/ml) 45 min prior to incubation with bFGF. To inhibit PI3 kinase, p38 MAPK, and MEK1/2 activity, cells were incubated with LY 294002 (10 μM) (Sigma, Mannheim, Germany), SB 203580 (25 μM) and PD 98059 (25 μM) (Sigma), respectively, 45 min prior to stimulation with bFGF.

TUNEL Assay

Apoptotic cells were identified by detecting single- and double-stranded DNA breaks using an in situ Cell Death detection kit (Roche Laboratories, Lewes, UK) according to the manufacturers' instructions. In brief, 1×10^5 MCF-7 cells were plated onto 8-well chamber slides (Lab-Tek, Nunc, Rochester, NY) and maintained in complete medium. Cells were fixed, permeabalized, and then incubated with TUNEL reaction mixture containing TdT and fluorescein-dUTP. The cells were washed and incubated with a peroxidase conjugated anti-fluorescein antibody, washed, stained with DAB, and counterstained with hematoxylin.

Intracellular Nucleosomes Assay

A photometric enzyme immunoassay was used to quantify cytoplasmic histone-associated DNA fragments characteristic of apoptotic cells using a Cell Death Detection ELISA (Roche) according to the manufacturer's instructions. In brief, supernatants from lysed MCF-7 cells were incubated with antibody mixtures containing anti-histone and anti–DNA antibodies in a streptavidin-coated plate. The plates were washed with PBS and substrate solution was added. The plate was read at 405 nm wavelength in a microtiter plate reader.

MTT Proliferation Assay

The tetrazolium salt MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) was used to detect metabolically active cells. In a 96-well plate, cells were incubated in the presence of MTT for 2–3 h at 37°C. Cells were then permeabilized with DMSO and absorbance was read at 540 nm.

Western Blot Immunodetection

Proteins were resolved on a 12% polyacralamide gel (110 V, 120 min) and were transferred to a nitrocellulose membrane (250 mA, 60 min). Membranes were incubated for 1 h in blocking buffer (5% non-fat dry milk, 0.1%Tween in TBS) at room temperature and subsequently with primary antibody raised against Bcl2, c-raf, c-raf-P, survivin, Cdc2, Cdc2-P, or COX-2 (1:1,000) (Cell Signaling, Beverly, MA) in blocking buffer overnight at 4°C. The membrane was washed prior to incubation with the corresponding horseradish peroxidase conjugated secondary antibody (1:2,000) in blocking buffer for 1 h at room temperature. The membrane was washed and developed by chemiluminescence (Santa Cruz Biotechnology, Santa Cruz, CA).

RT-PCR

RT-PCR for the survivin gene was performed using forward primer: 5′-GCA TGG CCC CGA CGA CGT TG-3′ (positions 48–67 Genbank

accession NM 001168) and reverse primer: 5′-GCT CCG AGA GGC CTC AA-3′ (positions 475–494). The house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. Total RNA (1 μg) was reverse transcribed into cDNA in a total volume of 20 μl containing 1 μl oligo-dT, 4 μl of MMLV buffer, 1 μl dNTPs, 2 μl of dTT, 0.2 μl of RNase inhibitor, and 0.5 μl of MMLV-RT (Promega, Southampton, UK). Reverse transcription was performed by incubating for 1 h at 37°C followed by incubation for 5 min at 70°C. cDNA (1 μl) mixture was subjected to amplification in a 25 μl mixture containing 1 U of Taq polymerase 1× PCR buffer, 2 μl of dNTPs, and 5 μl of forward and reverse primers. PCR conditions were an initial denaturing for 2 min at 94°C, followed by 30 cycles of denaturing at 94°C for 30 s, annealing for 1 min at 62°C, extension at 72°C for 1 min, and final extension at 72°C for 5 min. Amplification products (10 μl) were mixed with 2 μl of 6× DNA loading dye and run on 1% agrose gel. The signal was visualized under UV light.

Quantification of PGE_2

Quantification of PGE_2 in the culture medium was performed by ELISA (R&D Systems, Minneapolis, MN) according to the manufacture's instruction. The absorbance was read at 405 nm wavelength in a microtiter plate reader.

Pharmacological Agents Used

Aspirin is a non-selective COX inhibitor. Meloxicam is a member of the oxicam family that selectively inhibits COX-2 (COX-2 $IC_{50} =$ 4.7 μM) [Davies and Skjodt, 1999].

Statistical Analysis

Statistical analysis was performed using the Mann–Whitney U-test with significance accepted at the 5% level.

RESULTS

bFGF Protected Breast Cancer Cells From Apoptosis, Increased Proliferation and Up-Regulated Mitochondrial Protein Bcl-2 Expression

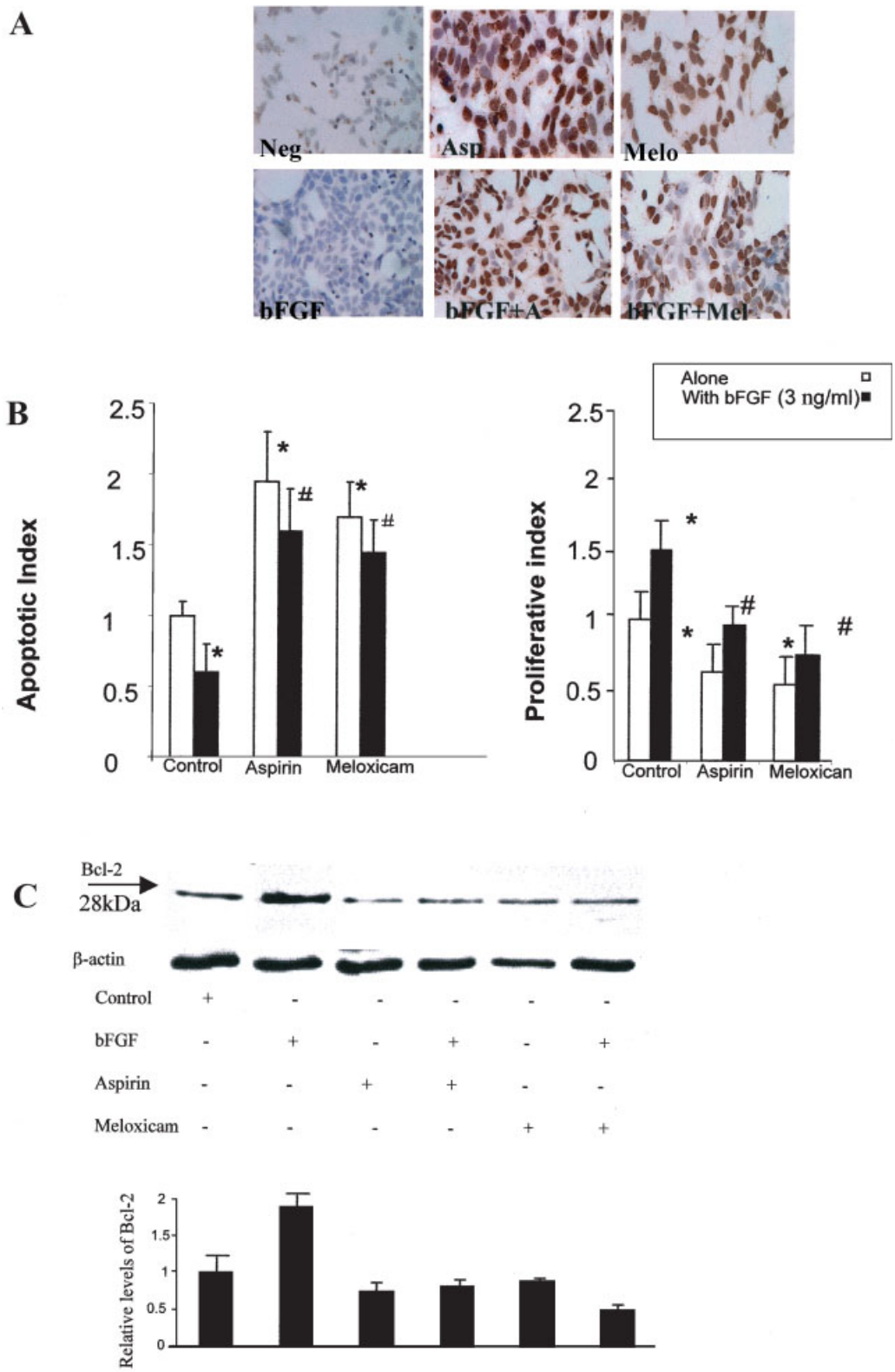
The effect of bFGF on MCF-7 breast cancer cell apoptosis was assessed by DNA fragmentation (TUNEL) and quantified using a total intracellular nucleosome assay. Decreased apoptosis in the presence of bFGF was observed in a dose-dependent fashion (0.3–30 ng/ml), with a maximum effect at 3–10 ng/ml (data not shown). At 3 ng/ml, bFGF decreased the number of cells undergoing apoptosis compared to untreated cells (Fig. 1A). The apoptotic index (AI; defined as the ratio of apoptosis in the treatment group over the control group where the AI of control group is standardized to 1) was significantly reduced in the presence of bFGF compared to control (0.65 ± 0.23, $P < 0.05$). Whereas the proliferation index (PI; defined as the ratio of proliferation in the treatment group over the control group where the PI of the control group is standardized to 1) was significantly increased with bFGF compared to control (1.5 ± 0.26) (Fig. 1B).

The effect of meloxicam (selective COX-2 inhibitor) and aspirin (non-selective COX inhibitor) on quiescent and bFGF stimulated MCF-7 breast cancer cell proliferation and apoptosis was evaluated. Both aspirin and meloxicam induced a decrease in proliferation compared to control (PI of 0.62 ± 0.27 and 0.51 ± 0.28, respectively) and diminished bFGF mediated cell proliferation ($P < 0.05$). Conversely, meloxicam and aspirin alone induced MCF-7 cancer cell apoptosis (AI of 1.78 ± 0.25, 1.95 ± 0.29, respectively, $P < 0.001$), and furthermore pretreatment of cancer cells with meloxicam and aspirin significantly modulated the anti-apoptotic effect of bFGF (Fig. 1B).

Stimulation of bFGF on cancer cells up-regulated the expression of anti-apoptotic protein, Bcl-2 following 24 h stimulation (Fig. 1C). Aspirin and meloxicam decreased basal expression of Bcl-2 and prevented its up-regulation by bFGF (Fig. 1C).

bFGF-Induced Cdc2 Kinase Phosporylation and Increased Survivin Expression, Which Was Modulated by Meloxicam and Aspirin

To evaluate the potential of bFGF to modulate survivin protein expression, MCF-7 breast cancer cells were incubated with bFGF (3 ng/ml) at varying periods with and without pre-treatment of the cells with aspirin and meloxicam for 45 min. bFGF-induced survivin expression following 2 h of incubation and survivin continued to be up-regulated following 24 h of treatment (Fig. 2A). Meloxicam and aspirin decreased the basal expression of survivin and prevented the up-regulatory effect of bFGF (Fig. 2B). Inhibition of de novo protein synthesis with cyclohexamide down-regulated bFGF-induced survivin protein expression at 24 h,


A
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Relative levels of Bcl-2

Fig. 1.

but not at the earlier 2 h time-period (Fig. 2C). There was no increase in survivin mRNA expression at 2 h in the presence of bFGF, however, when the breast cancer cells were treated with bFGF for 24 h, a marked increase in the expression of mRNA was detected (Fig. 2D). These observations suggest that at short time-periods bFGF can increase survivin protein expression independently of transcription. Meloxicam did not affect the basal expression of survivin, nor did it suppress the up-regulatory effect of bFGF on survivin mRNA (Fig. 2D).

The cytoprotective effect of survivin requires phosphorylation by mitotic cdc2 kinase; the effect of bFGF and COX inhibitors on the expression of total and phospho-cdc2 kinase was examined. Our data demonstrated that phosphorylated cdc2 was moderately up-regulated by bFGF following short incubation periods (2–6 h) and was most marked at longer treatment periods (24 h). The modest increase in expression of phosphorylated cdc2 by bFGF was reduced in the presence of meloxicam and aspirin at short incubation period (2–6 h). bFGF-induced survivin expression at 24 h was not affected by COX inhibitors (Fig. 2E).

bFGF Signals Through ERK1/2 in Breast Cancer Cells

Growth factors, such as bFGF can mediate their down stream effects by signaling through various phosphorylation cascades. To determine whether bFGF-induced survivin expression is mediated through a PI-3–AKT, p38 MAPK, or ERK1/2 signaling pathway in breast cancer cells, MCF-7 cells were pre-treated with specific kinase inhibitors prior to stimulation with bFGF. Following 24 h in the presence of bFGF, neither PI-3 kinase inhibitor LY 294002 (10 μM) or the p38 MAPK inhibitor SB 25380 (25 μM) affected the growth factor-induced survivin expression. The ERK1/2 inhibitor PD 98059 (25 μM), however, prevented bFGF up-regulation of survivin protein expression (Fig. 3A), suggesting that bFGF regulates survivin expression, at least in part through an ERK1/2 kinase dependant pathway.

The MEK-ERK-1/2 pathway is activated by c-raf, a cytoplasmic serine-threonine protein kinase that is recruited by GTP-bound Ras by the occupied tyrosine kinase receptor. In this study, bFGF (3 ng/ml) rapidly increased phosphorylation of c-raf (5–10 min). After longer incubation periods (30 min onwards) in the presence of bFGF, the levels of phospho-c-raf declined (data not shown). Pretreatment of breast cancer cells with meloxican (10 μM) and aspirin (100 μM) decreased phosphorylation of c-raf seen in the presence of bFGF, but did not abolish basal expression (Fig. 3B). bFGF did not modulate the expression of total c-raf. bFGF was found to be unable to induce phosphorylation of AKT, nor did the growth factor alter total AKT protein expression in MCF-7 breast cancer cells (Fig. 3C).

Pro-Apoptotic Effects of COX Inhibitors Were not Dependent on COX Expression or Activity in MCF-7 Breast Cancer Cells

The interaction of COX inhibitors on cellular apoptosis can be mediated through either COX-2 dependent or COX-2 independent mechanisms. To assess whether the cytoprotective effect of bFGF and the apoptotic effect seen with meloxicam and aspirin was mediated through alterations of COX-2 activity in MCF-7 breast cancer cells, COX-2 expression was semi-quantitatively measured by Western blot analysis. MCF-7 breast cancer cells basally expressed a low level of COX-2, which was not found to be inducible in the presence of bFGF (0.3–10 ng/ml) (Fig. 4A). There was no alteration in COX-2 expression detected over several time-points (data not shown). To ensure that COX-2 was not transiently induced by bFGF, we determined COX activity by quantifying the enzymatic products of COX-2 on arachidonic acid, prostaglandin E_2 (PGE_2). PGE_2 was measured in the supernatant of the control

Fig. 1. (*Overleaf*) **A**: Detection of cellular apoptosis by TUNEL assay. Representative results (n = 3): MCF-7 breast cancer cells (control) and cells treated with aspirin (100 μM); meloxicam (10 μM); bFGF (3 ng/ml); aspirin and bFGF and meloxicam and bFGF. Original magnification × 200. **B**: Apoptosis and proliferation of MCF-7 breast cancer cells were quantified by intracellular nucleosome and MTT assays respectively, after 48 h incubation with bFGF alone and in combination with COX inhibitors. Results expressed as mean ± SEM, n = 6, $*P < 0.05$, compared to control; $\#P < 0.05$, compared to bFGF alone. **C**: Western blot immunodetection of Bcl-2 in MCF-7 breast cancer cells after treatment with bFGF (3 ng/ml), aspirin (100 μM), and meloxicam (10 μM) for 24 h. Optical density readings were obtained (Eagle Eye, Stratagene, La Jolla, CA). Control values were normalized to one and experimental groups were expressed as a ratio. Values are expressed as mean ± SEM (n = 3).

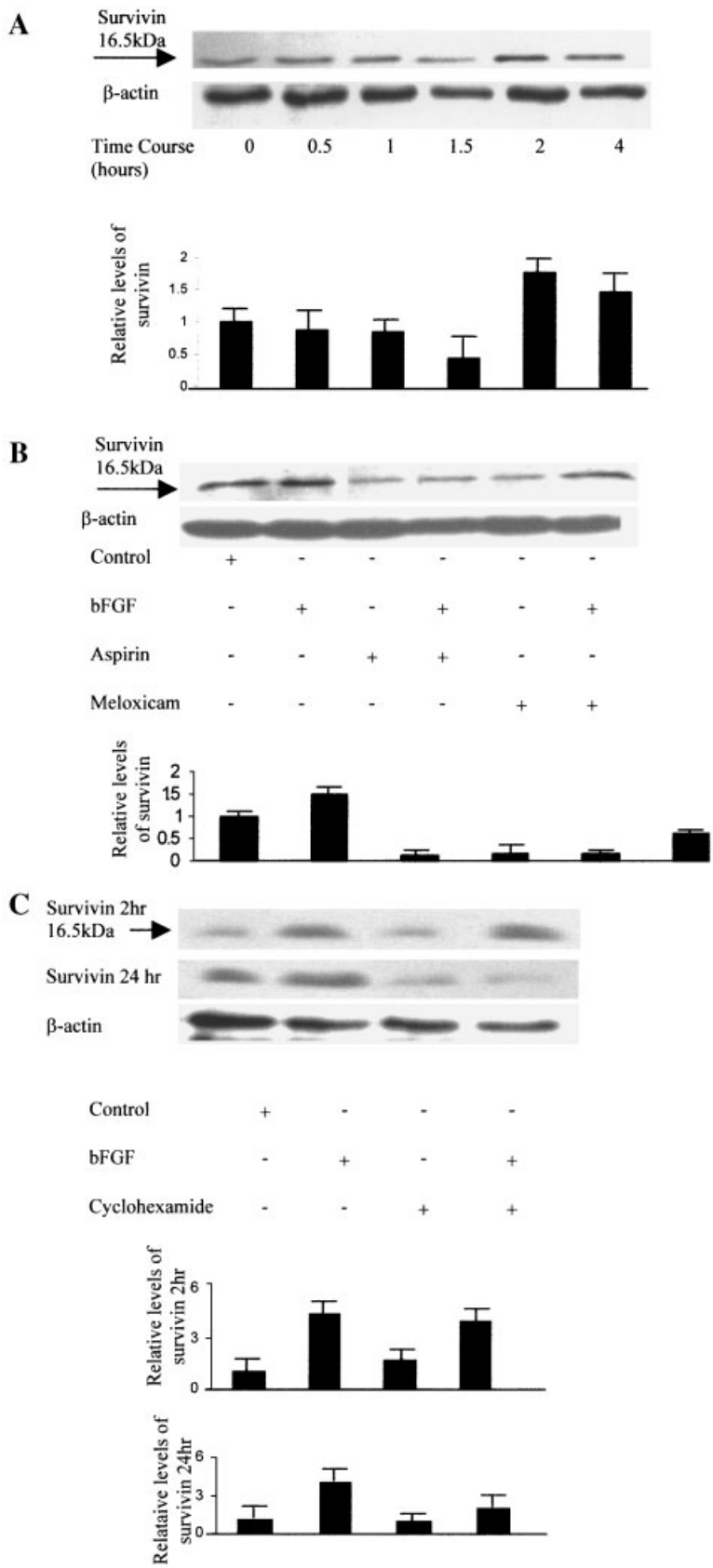
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Fig. 2.

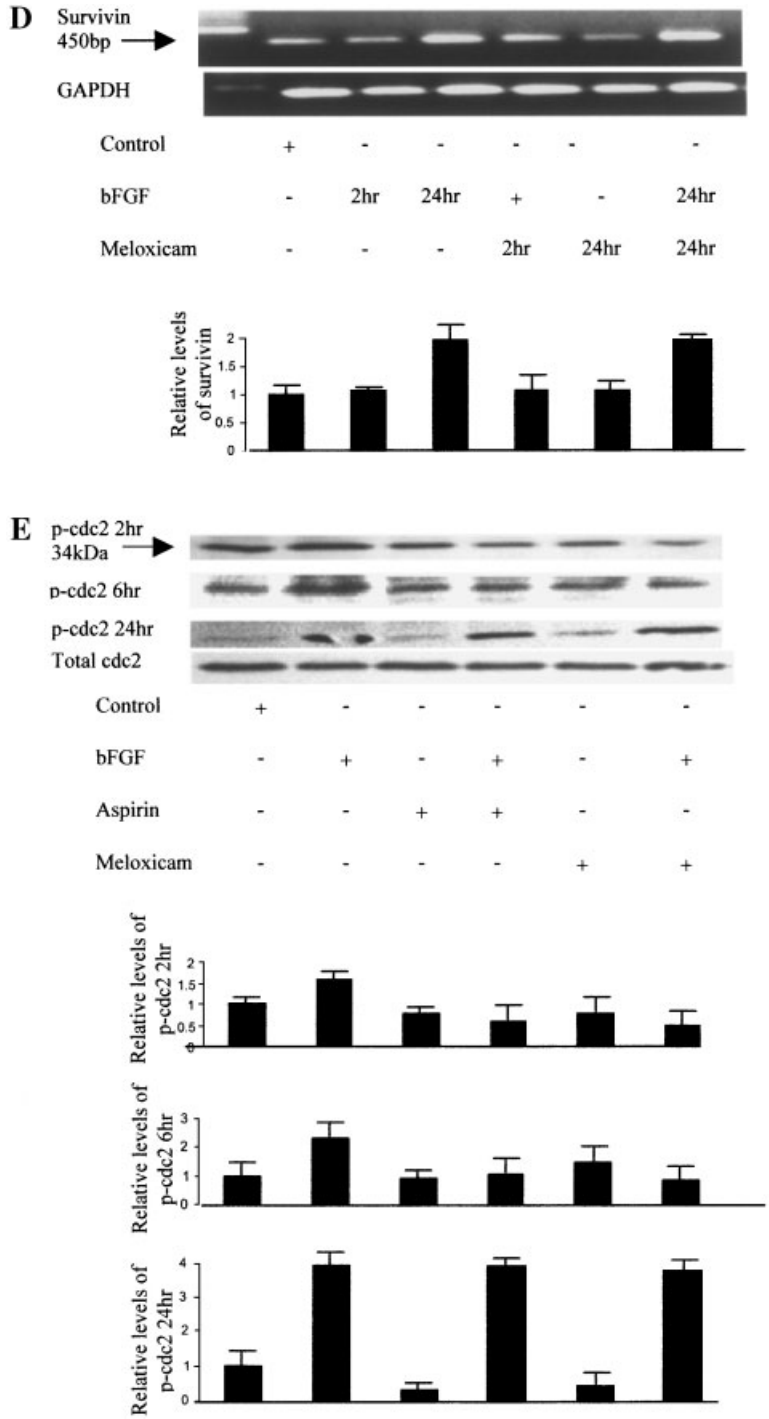

Fig. 2. **A**: Western blot immunodetection of survivin in MCF-7 breast cancer cells after treatment with bFGF (3 ng/ml) for the indicated time-periods. **B**: Survivin protein expression in the presence of bFGF (3 ng/ml), in combination with aspirin (100 μM) or meloxicam (10 μM) for 24 h. **C**: Survivin protein expression in the presence of bFGF in combination with the de novo protein synthesis inhibitor cyclohexamide (5 μg/ml). **D**: RT-PCR detection of survivin mRNA in MCF-7 breast cancer cells following treatment with bFGF for indicated time-periods. **E**: Protein expression of phospho-cdc2 kinase for indicated time-periods and total cdc2 kinase protein expression. Optical density readings of control values were normalized to one and experimental groups were expressed as a ratio. Values are expressed as mean ± SEM (n = 3).

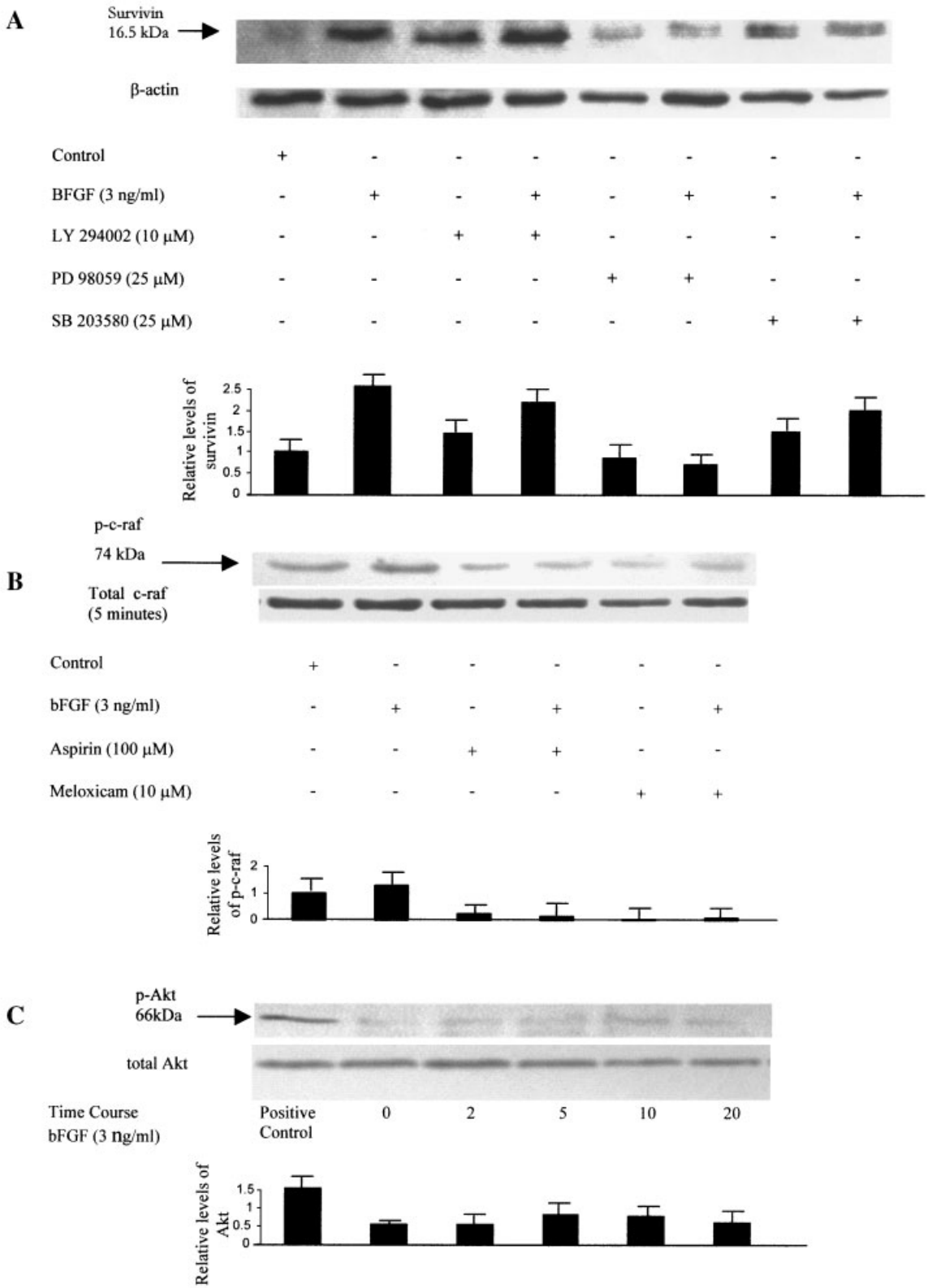

Fig. 3. **A**: Immunodetection of survivin protein expression in cells treated with bFGF following 45 min incubation in presence of LY294002 (10 μM) (PI3 kinase inhibitor), SB 203580 (25 μM) (p38 MAP kinase inhibitor), or PD98059 (25 μM) (MEK1/2 inhibitor). **B**: Protein expression of phospho-c-raf and total c-raf following treatment with bFGF (3 ng/ml) in the presence of aspirin (100 μM) or meloxicam (10 μM). **C**: Protein expression of phospho-AKT and total AKT following treatment with bFGF (3 ng/ml) for indicated time-periods. Positive control NIH/3T3 cell extracts. Optical density readings of control values were normalised to one and experimental groups were expressed as a ratio. Values are expressed as mean ± SEM (n = 3).

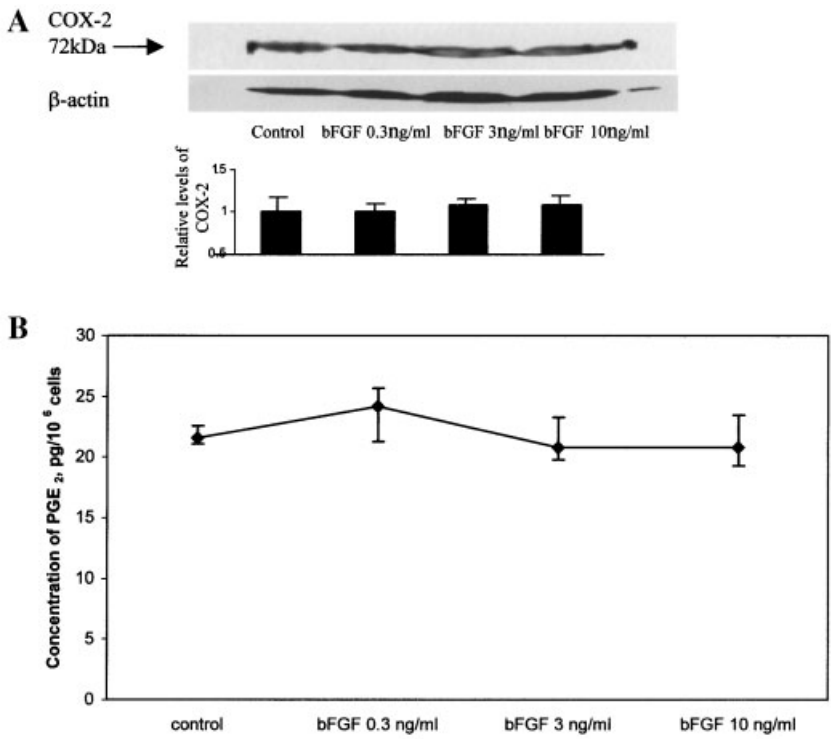

Fig. 4. **A**: Immunodetection of COX-2 protein expression in MCF-7 cells treated with bFGF (0.3, 3, 10 ng/ml). Optical density readings of control values were normalized to 1 and experimental groups were expressed as a ratio. Values are expressed as mean ± SEM (n = 3). **B**: Detection of PGE_2 by ELISA in MCF cell lysates treated with bFGF (0.3, 3, and 10 ng/ml) for 24 h. Data represent the mean ± SEM of triplicate determinations from three separate experiments.

and bFGF treatment groups after 24 h incubation. PGE_2 levels were low in the control group (23.0 ± 1.8 pg/mL/10^6 cells) and did not significantly differ in bFGF (0.3, 3, and 10 ng/ml) treated groups 23.8 ± 1.7 pg/mL/10^6 cells, 22.5 ± 1.6 pg/mL/10^6 cells and 22.0 ± 1.7 pg/mL/10^6 cells, respectively (Fig. 4B). To assess the significance of this physiological concentration of PGE_2 on MCF-7 breast cancer cell apoptosis, we treated the cells with range of PGE_2 concentrations (1–50 μg/ml) seen in culture medium of the cells following bFGF stimulation and apoptosis was assessed as above. At these physiological concentrations, PGE_2 did not show any apoptotic effect. Only treatment of cancer cells with much higher doses of PGE_2 (1 ng/ml) demonstrated a decrease in apoptosis (data not shown).

DISCUSSION

Dysregulation of programmed cell death is a universal feature of cancer. In this study, we have shown that bFGF exerts an anti-apoptotic effect and COX-2 inhibitors a pro-apoptotic effect, on MCF-7 human breast cancer cells. bFGF is associated with an inhibition of p53 and an up-regulation of anti-apoptotic proteins, such as Mdm2, which serves as an important intracellular survival protein [Shaulian et al., 2001; de Jong et al., 2001]. We and others have demonstrated bFGF up-regulation of the downstream mitochondrial anti-apoptotic protein Bcl-2 [Karsan et al., 1997; Hosokawa et al., 1998], which is consistent with the observation that bFGF confers vital survival advantages for tumor cell growth [Peyrat et al., 1992; Korah et al., 2000; Bairey et al., 2001]. Moreover, elevation of circulating bFGF in patients with breast cancer correlates with a poor disease prognosis and a more angiogenic aggressive tumor [Smith et al., 1999; Pichon et al., 2000].

The mitogenic effect of bFGF requires binding of the growth factor to its high affinity tyrosine kinase receptor, resulting in altered down stream effector expression, including PI3-Akt, p38 MAPK, and ERK1/2 [Burgering and Coffer, 1995; Janecki et al., 2000; Chaudhary and Hruska, 2001; Tortorella et al., 2001; Zubilewicz et al., 2001]. Many of these second messengers

exert their anti-apoptotic effect through the modulation of cell survival proteins. Survivin initiates cell-cycle entry by interacting with and subsequently being phosphorylated by cdk2/cyclin E [Suzuki et al., 2000]. In the present study, survivin protein expression was up-regulated 2 h following incubation with bFGF and remained elevated for at least 24 h. Inhibition of de novo protein synthesis with cyclohexamide modulated bFGF-induced protein expression only at the later time-point. Increases in survivin mRNA were detected at 24 h following bFGF treatment. There was no increase in survivin mRNA seen at the earlier time-period. Taken together, these results suggest that rapid elevation of survivin occurs at the translational level, whereas later regulation by bFGF can occur at the level of transcription. bFGF was also found to induce an up stream phosphorylation of the cdc2 kinase, which was moderately increased following 2 h incubation and most marked following longer incubation periods.

AKT upstream signaling is known to mediate an anti-apoptotic signal by several mitogens [Burgering and Coffer, 1995; Hsu et al., 2000; Chaudhary and Hruska, 2001]. In endothelial cells, survivin expression has been shown to be AKT dependent [Tran et al., 1999; Papapetropoulos et al., 2000]. Recently, it has been suggested, however, that PI-3-AKT signaling is not crucial in transducing the survivial signal in cells involving individual mitogens [Pardo et al., 2001]. The MAP kinase ERK1/2 cascade is thought to provide an alternative pathway for some mitogens to exert their survival activity [Liu et al., 1999; Jost et al., 2001]. Here we have shown that in MCF-7 breast cancer cells bFGF induces survivin expression in an ERK1/2 dependent manner through the phosphorylation of c-raf, independently of AKT.

The chemopreventive mechanism of cyclooxygenase-2 inhibitors has received considerable attention and has been proposed to be due to alterations in cell-cycle regulatory proteins such as p21, waf1, p70S6, and p21Kip1 kinase [Grosch et al., 2001; Harada et al., 2001; Hung et al., 2001; Zahner et al., 2002]. We have demonstrated that both aspirin and meloxicam induce human breast cancer cell apoptosis. We hypothesized that COX-2 inhibitors may interact with the intra-cellular pathways involved in bFGF-induced cell survival observed in MCF-7 cells. The non-specific COX inhibitor (aspirin) and the COX-2 inhibitor (meloxicam) were found to down-regulate two distinct anti-apoptotic proteins that regulate both cell-cycle independent (mitochondrial Bcl-2) and dependent (survivin) pathways. These COX inhibitors also prevented the increased expression of Bcl-2 and survivin seen in the presence of bFGF. Furthermore, COX inhibitors prevented the modest increases in cdc2 phosphorylation seen at early time-points, but did not, however, affect later bFGF-induced cdc2 phosporylation. COX modulation of early bFGF-induced cdc2 phosphorylation may be due in part to the inhibition of bFGF-induced c-raf phosphorylation.

The mechanism by which COX inhibitors exert their pro-apoptotic effects is unclear. Previous works have reported both a COX-dependant and a COX-independent role for these inhibitors [Tegeder et al., 2001]. In this study, aspirin and meloxicam exerted their apoptotic effect on MCF-7 cells independently of COX-2 expression and activity. Furthermore, the concentration of PGE_2 required to exert an anti-apoptotic effect on MCF-7 cells was found to be 50-fold higher than the levels of PGE_2 found in the culture medium of bFGF-treated cells.

In conclusion, these data support a role for bFGF in cancer cell survival through the induction of cell-cycle dependant and cell-cycle independent survival proteins. We have demonstrated that in MCF-7 breast cancer cells, bFGF regulates survivin expression in an ERK1/2 dependent manner, at both the level of transcription and translation. This work provides new evidence that COX inhibitors can modulate survivin expression independently of COX activity. The anti-survivin properties of aspirin and meloxicam in our in vitro model may in part explain the chemo-preventative role of COX inhibitors.

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