Effect of Prostaglandin E_2 and EP Receptor Agonists on Cell Proliferation and Basic Fibroblast Growth Factor Synthesis of Rheumatoid Synovial Fibroblasts in Vitro

Saura, Ryuichi
Hirata, Soichiro
Ishikawa, Hitoshi
Mizuno, Kosaku

(Citation)
Bulletin of health sciences Kobe,16:1-10

(Issue Date)
2000-12-27

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(JaLCDOI)
https://doi.org/10.24546/00098318

(URL)
https://hdl.handle.net/20.500.14094/00098318

Effect of Prostaglandin E_2 and EP Receptor Agonists on Cell Proliferation and Basic Fibroblast Growth Factor Synthesis of Rheumatoid Synovial Fibroblasts in Vitro

Ryuichi Saura[1], Soichiro Hirata[1], Hitoshi Ishikawa[1], and Kosaku Mizuno[2]

The role of prostaglandin E_2 in inflammatory process is still elusive. We have studied the effect of prostaglandin E_2 on rheumatoid synovial fibroblast proliferation and synthesis of basic fibroblast growth factor in vitro. When synovial fibroblasts were cultured for 72 hours in the presence of prostaglandin E_2, over 10^{-10} M of prostaglandin E_2 has shown significant decrease of tritiated thymidine incorporation in synovial fibroblast monolayer culture. Both EP2 receptor and EP4 receptor agonist also suppressed synovial fibroblast proliferation, whereas EP1 or EP3 receptor agonist failed to affect cell proliferation up to 10^{-6} M. Next, the effect of prostaglandin E_2 on basic fibroblast growth factor mRNA expression of synovial fibroblast was examined. Basic fibroblast growth factor mRNA expression was markedly enhanced when synovial fibroblasts were treated with 10^{-6} M prostaglandin E_2. EP1 receptor agonist has also enhanced basic fibroblast growth factor mRNA induction, whereas EP2, EP3 and EP4 receptor agonist failed to augment mRNA induction of synovial fibroblast. These results suggested that selective EP receptor agonists or antagonists should be utilized in future for treatment of rheumatoid arthritis for preventing pannus formation by EP2/EP4 activation and bone resorption led to joint destruction by EP1 inactivation.

Key Words: Cyclooxygenase (COX), Prostaglandin E_2, EP receptor, Rheumatoid arthritis, Basic fibroblast growth factor.

Introduction

In rheumatoid arthritis (RA), it is characterized that pronounced synovial hyperplasia composed of the extensive proliferation of synovial fibroblasts (SFBs) and inflammatory cell infiltration with neovascularization.[1)] A number of mononuclear cells which infiltrated into the sublining area of synovium through small blood vessels carry out a series of both cellular and humoral immune reactions and are responsible for the spread and persistence of RA synovitis.

Pannus tissue that is the hyperplastic synovium invades both surface of articular cartilage and subchondral bone adjacent to the articular cartilage. It secretes a various kinds of growth fac-

Faculty of Health Science[1] and Department of Orthopaedic Surgery[2], Kobe University School of Medicine, Kobe, Japan.

tors and inflammatory cytokines, which stimulate the growth of pannus tissue itself. Inflammatory cytokines such as Interleukin-1 (IL-1) or tumor necrosis factor α (TNFα) stimulate the prostaglandin E_2 (PGE_2) synthesis of synovial cells through upregulation of inducible form of PGE_2 synthase, cyclooxygenase -2 (COX-2)[2]. PGE_2 is detectable at a high level in the fluid of knee joints in OA[3,4] and RA.[5,6] These findings suppose that PGE_2 might be related to the exacerbation of joint inflammation and involved in the disease process of degenerative arthritis.

On the other hand, PGE_2 is reported to be an antiproliferative molecule. For instance, PGE_2 induces cAMP accumulation and inhibits the growth of the most differentiated breast cancer cells due to loss and probably dysfunction of PGE_2 receptors.[7] PGE_2 also suppressed RA synovial cell proliferation through intracellular cAMP accumulation.[8] Thus, the role of PGE_2 in inflammatory process is still elusive. We have, therefore, studied the effect of PGE_2 on rheumatoid SFB proliferation and synthesis of basic fibroblast growth factor (bFGF), which seems to be one of the potent growth factors related to the pannus formation in RA.

Materials and Methods

1. Preparation of SFBs derived from RA patients and SFB monolayers

Synovial tissue samples were surgically obtained, after consent, from patients with RA at the time of total knee arthroplasty. Diagnosis of RA was based on the revised criteria for the classification of rheumatoid arthritis by American Collage of Rheumatology in 1987. SFBs were isolated from these tissue samples as described previously with some modifications.[9] Briefly, minced synovial tissue were dissociated using 0.2 % of collagenase (Sigma Chemical Co., St. Louis, MO) and 0.25 % of trypsin-EDTA (DIFCO LABORATORIES, Detroit, MI) at 37 °C for 2 hours, with gentle stirring. The dissociated cells were then suspended in SFB culture medium, consisting of Dulbecco's modified Eagle's medium (DMEM; Gibco BRL., Grand Island, NY) supplemented with 10 % heat-inactivated fetal bovine serum (FBS; Biowhittaker, Walkersville, ML) and antibiotics, and cultured in 75-cm^2 tissue culture flask (Corning Glass Works, Conring, NY) at cell density of 10^5 cell/cm^2. The next day, culture flasks were vigorously rinsed to remove non-adherent lymphoid cells with fresh DMEM, and resultant adherent SFBs were cultured with fresh SFB culture medium.

When the primary cultures reached confluence, cells were trypsinized, resuspended in fresh SFB culture medium, and seeded into 75-cm^2 tissue culture flask at cell density of 10^5 cell/cm^2 for further passage. All the experiments described below were conducted using cells at the second or third passage.

2. Assay for tritiated thymidine ([^{3}H]-TdR) incorporation into SFBs

In order to examine the effect of PGE_2 and EP receptor agonists on DNA synthesis of SFBs, [^{3}H]-TdR incorporation into the SFBs was quantified. For cell proliferation assay, SFBs were obtained from monolayer culture by trypsinization and further cultures were carried out in flat-bottomed 96-

microtiter wells (6.4 mm in diameter). Ten thousand of SFBs were allowed to attach in every well and cultured for 72 hours. Fifteen hours before terminating the assay of incubation time, 37 KBq of [^{3}H]-TdR were added to each well. At the end of this assay, SFBs were washed three times with distilled water. Two hundred microliter of scintillation liquid was added and cell associated [^{3}H]-TdR was determined using scintillation counting (PACKARD INSTRUMENT COMPANY, Mariden, CT, USA).

3. Detection of bFGF mRNA expression of SFBs by RT-PCR

In order to examine the effect of PGE_2 and EP receptor agonists on bFGF synthesis of SFBs, bFGF mRNA expression was detected by RT-PCR in the SFB culture. Briefly, 5.0×10^5 cells were inoculated into the 6-multiwell plate and cultures were carried out in the presence of 10^{-6} M PGE_2 or 10^{-6} M EP receptor agonists. After 6 h-incubation, total RNA was directly isolated from the cell monolayers using RNAzol™B (Tel-test, Friendswood, TX) according to the manufacturer's instructions. Then, first-strand cDNA were synthesized by incubating 1 μg of total RNA with 1.25 μM oligo dT primer in a 40 μl PCR buffer II containing 2.5 mM $MgCl_2$, 0.5 mM dNTP mix, 0.5 units of RNase inhibitor and 1.25 units of MulV reverse transcriptase. The mixtures were incubated for 120 minutes at 37 °C. The PCR reaction volume contained 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 μM sense and antisense primer, 1.5 units of AmpliTaq Gold DNA polymerase, and 1 μl RT reaction in 20 μl of PCR buffer II (Perkin Elmer, Foster City, CA). A temperature profile of 1 min at 94 °C, 1min at 55 °C, 1min at 72 °C was applied for 30 cycles.

The bFGF primers used were 5'-CGGCTGTACTGCAAAAACGGG-3' and 5'-GCTCTTAGCAGACATTGCAAG-3'. These two primers encompass a 375-basepair (bp) nucleic acid region encoding amino acids 31-37 (5'-oligonucleotide) and amino acids 149-155 (3'-oligonucleotide), respectively.[10] As these primers were derived from human sequences, they are specific for human bFGF. The primers specific for human GAPDH as internal control were 5'-GTGAAGGTCGGAGTCAACG-3' and 5'-GAGATGATGACCCTTTTGGC-3'.[11] These primers amplified a 356-bp product. RT-PCR was also performed in the absence of sample RNA and reverse transcriptase as a negative control. A fraction of each amplified DNA fragments were analyzed without further purification by electrophoresis on 1.5 % agarose gels. The separated PCR products were stained by Vistra Green (Amersham International plc, England) and visualized with the fluolomager™ system (Molecular Dynamics Inc., CA, USA). Digital image of the separated PCR products were analyzed with the ImageQuant software (Molecular Dynamics Inc.) and the fluorescence intensity of PCR products amplified from the bFGF mRNA were compared each other after standardized by the intensity of product amplified from the GAPDH mRNA.

4. Statistical analysis

Statistical analysis was carried out on all data points with regard to control by

an unpaired Welch's t-test. Each data point from cell proliferation assay represented the mean of six separate samples with the corresponding standard error of the mean (SEM). *P* values under 0.05 were considered significant statistically.

Results

1. Effects of PGE_2 and EP receptor agonists on [^{3}H]TdR incorporation into SFBs

When SFBs were cultured for 72 hours in the presence of PGE_2, [^{3}H]-TdR incorporation into cells was suppressed in a dose dependent fashion. Over 10^{-10} M of PGE_2 has shown significant decrease of [^{3}H]-TdR incorporation in SFB monolayer culture (Fig. 1). The actions of PGE_2 are reported to be mediated by four distinct classes of EP receptors (EP1 through EP4).[12,13,14,15,16] Therefore, effect of selective agonists of EP receptor on SFB proliferation was examined. Both EP2 receptor agonist, butaprost and EP4 receptor agonist, misoprostol, suppressed SFB proliferation, whereas both EP1 receptor agonist, 17-phenyl-trior PGE_2 and EP3 receptor agonist, sulprostone failed to affect cell proliferation up to 10^{-6} M (Fig. 2).

Increased cAMP generation by PGE_2

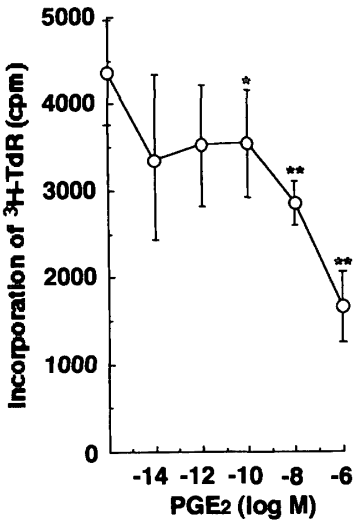

Figure 1. Effects of PGE_2 on [^{3}H]-TdR incorporation into SFBs. The effect of PGE_2 on SFB proliferation was detected as described in materials and methods. SFB obtained from RA patients were cultured in the presence of various concentrations of PGE_2 for 72-hour and incorporation of [^{3}H]-TdR was detected. Statistical analysis was carried out on all data points with regard to control by an unpaired Welch's t-test. Each data point represented the mean of six separate samples with the corresponding standard error of the mean (SEM). *P* values under 0.05 were considered significant statistically.

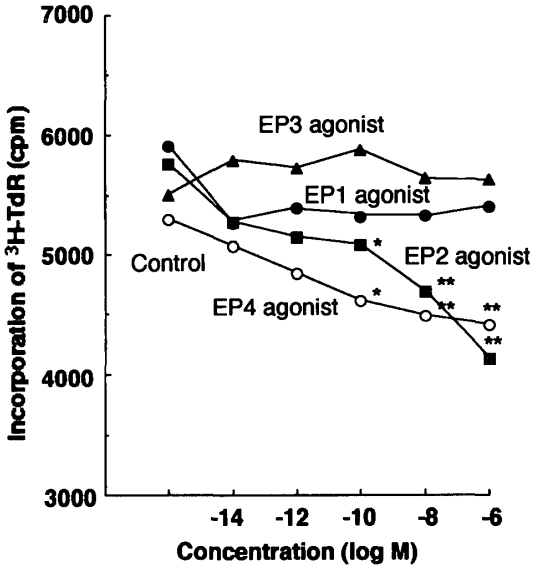

Figure 2. Effects of EP receptor agonists on [^{3}H]-TdR incorporation into SFBs. The effect of EP receptor agonists on SFB proliferation was detected as described in materials and methods. SFB were cultured in the presence of various concentrations of EP receptor agonists for 72-hour and incorporation of [^{3}H]-TdR was detected. Each data point represented the mean of six separate samples. Statistical analysis was carried out on all data points with regard to control by an unpaired Welch's t-test. *P* values under 0.05 were considered significant statistically.

may be due to EP2 or EP4 receptor activation. Activation of EP1 receptor is reported to be associated with a rise in intracellular calcium/protein kinase C (PKC) activation. The effect direct adenylate cyclase activator, forskolin or PKC activator, phorbol 12-myristate 13-acetate (PMA) on SFB proliferation were then examined. When SFBs were cultured for 72 hours with a varying concentration of forskolin, [^{3}H]-TdR incorporation into cells was suppressed in a dose dependent fashion. Over 10^{-7} M of forskolin has shown significant decrease of [^{3}H]-TdR incorporation in SFB monolayer culture (Fig. 3a). However, PMA had no effects on SFB proliferation shown in Fig. 3b. [^{3}H]-TdR incorporation into cells was not affected by addition of PMA up to 10^{-8} M. These results suggested that PGE_2 suppresses SFB proliferation and this antiproliferative effect of PGE_2 were mainly mediated by accumulation of intracellular cAMP through EP2 or EP4 receptor.

2. Effects of PGE_2 and EP receptor agonists on bFGF mRNA expression of SFBs

It is recently reported that PGE_2 increases bFGF mRNA induction through EP_2 receptor activation in rat Müller cells and bovine adrenal medullary cells[17]. Therefore, effect of PGE_2 on bFGF mRNA expression of SFB was examined. As shown in Fig. 4, bFGF mRNA expression was markedly en-

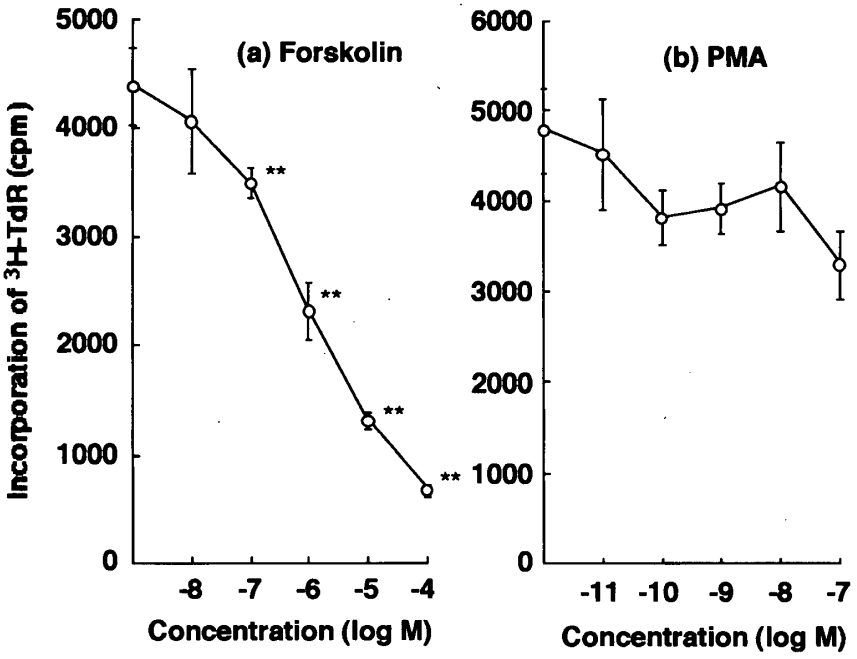

Figure 3. Effects of forskolin or PMA on [^{3}H]-TdR incorporation into SFBs. The effect of forskolin (a) or PMA (b) on SFB proliferation was detected as described in materials and methods. SFB were cultured in the presence of various concentrations of these activators for 72-hour and incorporation of [^{3}H]-TdR was detected. Each data point represented the mean of six separate samples with the corresponding standard error of the mean (SEM). Statistical analysis was carried out on all data points with regard to control by an unpaired Welch's t-test. *P* values under 0.05 were considered significant statistically.

hanced when SFBs were treated with 10^{-6} M PGE_2. Then, effect of EP receptor agonist on bFGF mRNA induction was examined to confirm which type of EP receptor was linked to this mRNA induction of SFB. Surprisingly, EP1 receptor agonist has enhanced bFGF mRNA induction, whereas EP2, EP3 and EP4 receptor agonist failed to augment mRNA induction of SFB as shown in Fig. 5.

When SFBs were treated with 10^{-6} M of calcium ionophore, A-23187 substituting for intracellular signaling pathway by EP1 activation, bFGF mRNA expression of SFB was augmented, whereas forskolin substituting for EP2/EP4 mediated intracellular signal failed to enhance this mRNA expression (Fig. 4). These findings, therefore,

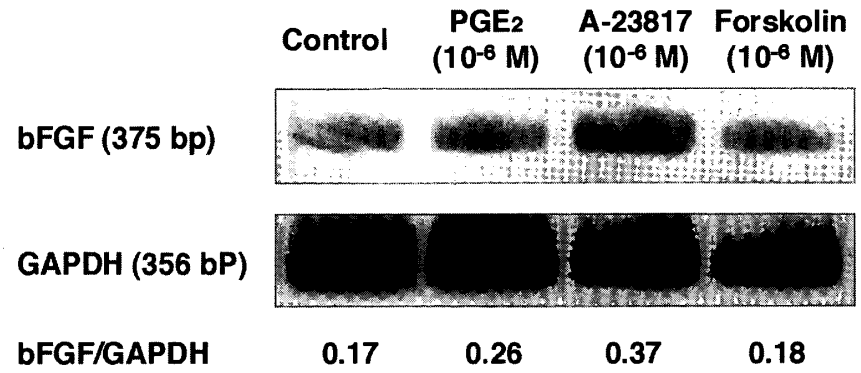

Figure 4. Effects of PGE_2, forskolin or calcium ionophore on bFGF mRNA expression of SFBs. Effect of PGE_2, forskolin or calcium ionophore on bFGF mRNA expression of SFB was examined as described in materials and methods. The first-strand cDNA obtained from total RNA by reverse transcriptation was amplified using specific primers for bFGF or GAPDH. A fraction of each amplified DNA fragments were analyzed by electrophoresis on 1.5 % agarose gels and PCR products stained by Vistra Green was visualized with the fluolomager™ system. The fluorescence intensity of PCR products amplified from the bFGF mRNA were compared each other after standardized by the intensity of product amplified from the GAPDH mRNA.

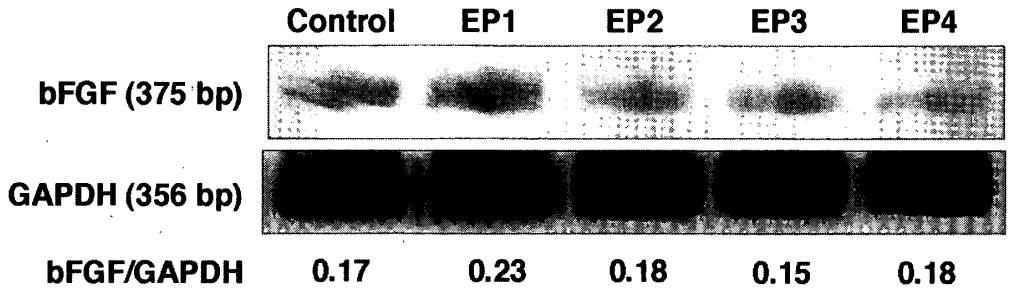

Figure 5. Effects of EP receptor agonists on bFGF mRNA expression of SFBs. Effect of EP receptor agonists on bFGF mRNA expression of SFB was examined as described in materials and methods. The first-strand cDNA obtained from total RNA by reverse transcriptation was amplified using specific primers for bFGF or GAPDH. A fraction of each amplified DNA fragments were analyzed by electrophoresis on 1.5 % agarose gels and PCR products stained by Vistra Green was visualized with the fluolomager™ system. The fluorescence intensity of PCR products amplified from the bFGF mRNA were compared each other after standardized by the intensity of product amplified from the GAPDH mRNA.

suggested that PGE_2 increases bFGF mRNA expression through EP_1 receptor activation in SFB and this effect might be related to the proinflammatory property of PGE_2, which lead to the joint destruction through pannus growth stimulated by bFGF.

Discussion

In this paper we have shown that PGE_2 inhibited the proliferation of synovial fibroblasts obtained from RA patients. This finding is already reported previously and elevation of intracellular cAMP level is suggested to induce the inhibitory effects on the proliferation of synovial cells.[8] It is also reported that PMA stimulated the proliferation of synovial cells. They suggested that PMA is a potent activator of PKC and activation of PKC results in the proliferation of synovial cells. Because the enhancement of proliferation of synovial cells by inflammatory cytokines such as IL-1β, TNFα or granulocytes/macrophage colony stimulating factor is reduced by H7, a potent inhibitor of PKC, the signal for proliferation of synovial cells is supposed to be transmitted through the activation of PKC. However, PMA did not affect the SFB proliferation up to 10^{-7} M this time. The divers response to PMA of SFB may be dependent on the biological property of cells utilized for experiments because the adherent cells in the primary cultures were used for proliferation assay. We use further passaged cells for eliminating the contamination of inflammatory cells such as lymphocytes or macrophages. Another hypothesis that may explain this difference is follows; Normal proliferation of synovial cells is reported not to be significantly affected by the addition of H7. IL-1β stimulates the much amount of PGE_2 synthesis of synovial cells through upregulation of COX-2, and the proliferation of synovial cells at the same time. The enhanced proliferation of synovial cells by IL-1β was further augmented by the addition of indomethacin which inhibited the production of PGE_2 by synovial cells. These observations suggested that PKC may be engaged into signal transduction of cell proliferation by cytokines activation and cell proliferation is well controlled by activation of both protein kinase A (PKA)/ PKC in balance.

Physiological functions of PGE_2 are thought to be mediated through interactions with four distinct prostaglandin receptors, EP1, EP2, EP3, and EP4, which are associated with different signal transduction pathways. Binding with EP2 and EP4 stimulates adenylate cyclase and thus activates PKA. EP3 mediates the inhibition of adenylate cyclase. Interaction of PGE_2 with EP1 activates phospolipase C, which hydrolyses polyphosphoinoisitide lipid, resulting in the second messenger molecule inositol triphosphate and diacylglycerol lipid. The latter activates PKC. Our results show that PGE_2 suppressed SFB proliferation through the PKA pathway and agonist for EP2 and EP4 suppressed [^{3}H]-TdR incorporation into the SFB. These findings imply the effect of PGE_2 on cell proliferation is mediated through EP2, EP4 or both.

We have also revealed that PGE_2 induced bFGF mRNA expression in SFBs through a rise in intracellular calcium/PKC activation and agonist for EP1 receptor induced bFGF mRNA expression in SFBs. These observations imply the effect of PGE_2 on bFGF

mRNA expression is mediated through EP1 receptor neither EP2 nor EP4 because forskolin or EP2, EP4 receptor agonists failed to induce bFGF mRNA expression.

Recently, a number of studies have proposed that some growth factors including transforming growth factor-β, platelet derived growth factor, acidic FGF and bFGF regulate the proliferation of human synovial cells.[18,19,20,21,22,23,24] Among them, bFGF is of particular interest, since the immunostaining of bFGF in the synovium of RA patients was reported to be more extensive and intense than that of OA patients.[25] Cultured synovial cells in vitro express bFGF mRNA and bFGF protein. More over the cells have the functional receptor for bFGF.

In the rat model of experimental adjuvant arthritis, there is a close association between increased bFGF expression and destruction of arthritis joints.[26] As a potent angiogenic and mitogenic polypeptide, bFGF has been implicated in tissue differentiation angiogenesis,[27,28] and synovial proliferation.[29,30] Thus, bFGF is involved in synovial hyperplasia, neovascularization and joint destruction in RA. FGFs also play a critical role in the bone remodeling including bone formation and bone resorption. It is reported that bFGF at low concentrations acts directly on mature osteoclasts to resorb bone moderately, whereas at high concentrations it acts on osteoblastic cells to induce COX-2 and stimulates bone resorption potently. It is also indicated that bFGF induces osteoclast formation by stimulating osteoclast differentiation factor production through COX-2-mediated PG synthesis and by suppressing osteoclastogenesis inhibitory factor production through a mechanism independent of PG synthesis.

We have investigated the effect of PGE_2 on SFB proliferation and bFGF mRNA expression in this paper. PGE_2 seems to be involved in joint destruction physiologically and pathologically due to different receptors activation. Recently, selective COX-2 inhibitors, which may reduce PG synthesis in inflammatory site, are utilized as NSAIDs with less divers effects such as gastric ulcer or renal dysfunction. These effects might result from COX-1 inhibition by conventional NSAIDs such as indomethacin. However, selective EP receptor agonists or antagonists should be utilized in future for RA treatment for preventing pannus formation by EP2/EP4 activation and bone resorption led to joint destruction by EP1 inactivation.

References

1. Harris ED Jr. Pathogenesis of rheumatoid arthritis. Am J Med. 80:4-10, 1986.
2. Knott I; Dieu M; Burton M, et al. Induction of cyclooxygenase by interleukin 1: comparative study between human synovial cells and chondrocytes. J Rheumatol. 21: 462-466, 1994.
3. O'Keefe RJ, Crabb ID, Puzas JE, et al. Influence of prostaglandins on DNA and matrix synthesis in growth plate chondrocytes. J Bone Miner Res 7:397-404, 1992.
4. Atik OS. Leukotriene B_4 and prostaglandin E_2-like activity in synovial fluid in osteoarthritis. Prostaglandins Leukot Essent Fatty Acids 39:253-254, 1990.
5. Balblanc JC, Vignon E, Mathieu P, et al. Cytokines, prostaglandin E_2, phospholipase A and metalloproteases in synovial fluid in osteoarthritis. Rev Rhum Mal Osteoartic

58:343-347, 1991.
6. Dayer JM, Robinson DR, Krane SM. Prostaglandin production by rheumatoid synovial cells: stimulation by a factor from human mononuclear Cells. J Exp Med 145:1399-1404, 1977.
7. Planchon P, Veber N, Magnien V, et al. Alteration of prostaglandin E receptors in advanced breast tumour cell lines. Mol Cell Endocrinol 111:219-223, 1995.
8. Yamamoto M, Yasuda M, Shiokawa S, et al. Intracellular signal transduction in proliferation of synovial cells. Clin Rheumatol. 11:92-96, 1992.
9. Sakurada S, Kato T, Okamoto T. Induction of cytokines and ICAM-1 by proinflammatory cytokines in primary rheumatoid synovial fibroblasts and inhibition by N-acetyl-L-cysteine and aspirin. Int Immunol. 8:1483-1493, 1996.
10. Emoto A; Nakagawa M; Wakabayashi Y, et al. Induction of tubulogenesis of microvascular endothelial cells by basic fibroblast growth factor from human SN12C renal cancer cells. J Urol. 157:699-703, 1997.
11. Adler H; Frech B; Thony M, et al. Inducible nitric oxide synthase in cattle. Differential cytokine regulation of nitric oxide synthase in bovine and murine macrophages. J Immunol. 154:4710-4718, 1995.
12. Funk CD; Furci L; FitzGerald GA, et al. Cloning and expression of a cDNA for the human prostaglandin E receptor EP1 subtype. J Biol Chem. 268:26767-26772, 1993.
13. Regan JW; Bailey TJ; Pepperl DJ, et al. Cloning of a novel human prostaglandin receptor with characteristics of the pharmacologically defined EP2 subtype. Mol Pharmacol. 46:213-220, 1994.
14. Adam M; Boie Y; Rushmore TH, et al. Cloning and expression of three isoforms of the human EP3 prostanoid receptor. FEBS Lett. 338:170-174, 1994
15. Bastien L; Sawyer N; Grygorczyk R, et al. Cloning, functional expression, and characterization of the human prostaglandin E_2 receptor EP2 subtype. J Biol Chem. 269:11873-11877, 1994.
16. Regan JW; Bailey TJ; Donello JE, et al. Molecular cloning and expression of human EP3 receptors: evidence of three variants with differing carboxyl termini. Br J Pharmacol. 112:377-385, 1994.
17. Cheng T; Cao W; Wen R, et al. Prostaglandin E_2 induces vascular endothelial growth factor and basic fibroblast growth factor mRNA expression in cultured rat Muller cells. Invest Ophthalmol Vis Sci. 39:581-591, 1998.
18. Wilder RL; Lafyatis R; Roberts AB, et al. Transforming growth factor-beta in rheumatoid arthritis. Ann N Y Acad Sci. 593:197-207, 1990
19. Goddard DH; Grossman SL; Williams WV, et al. Regulation of synovial cell growth. Coexpression of transforming growth factor beta and basic fibroblast growth factor by cultured synovial cells. Arthritis Rheum. 35:1296-1303, 1992.
20. Remmers EF; Sano H; Lafyatis R, et al. Production of platelet derived growth factor B chain (PDGF-B/c-sis) mRNA and immunoreactive PDGF B-like polypeptide by rheumatoid synovium: coexpression with heparin binding acidic fibroblast growth factor-1. J Rheumatol. 18:7-13, 1991.
21. Butler DM; Leizer T; Hamilton JA. Stimulation of human synovial fibroblast DNA synthesis by platelet-derived growth factor and fibroblast growth factor. Differences to the activation by IL-1. J Immunol. 142:3098-3103, 1989.
22. Reuterdahl C; Tingstrom A; Terracio L, et al. Characterization of platelet-derived growth factor beta-receptor expressing cells in the vasculature of human rheumatoid synovium. Lab Invest. 64:321-329, 1991.
23. Melnyk VO; Shipley GD; Sternfeld MD, et al. Synoviocytes synthesize, bind, and respond to basic fibroblast growth factor. Arthritis Rheum. 33:493-500, 1990.
24. Sano H; Forough R; Maier JA, et al. Detection of high levels of heparin binding growth factor-1 (acidic fibroblast growth factor) in inflammatory arthritic joints. J Cell Biol. 110:1417-1426, 1990.
25. Nakashima M; Eguchi K; Aoyagi T, et al. Expression of basic fibroblast growth factor

in synovial tissues from patients with rheumatoid arthritis: detection by immunohistological staining and in situ hybridisation. Ann Rheum Dis. 53:45-50, 1994.
26. Qu Z; Huang XN; Ahmadi P, et al. Expression of basic fibroblast growth factor in synovial tissue from patients with rheumatoid arthritis and degenerative joint disease. Lab Invest. 73:339-346, 1995.
27. Friesel RE; Maciag T. Molecular mechanisms of angiogenesis: fibroblast growth factor signal transduction. FASEB J. 9:919-925, 1995.
28. Bikfalvi A. Significance of angiogenesis in tumour progression and metastasis. Eur J Cancer. 31A:1101-1104, 1995.
29. Goddard DH. Regulation of synovial cell growth by polypeptide growth factors. DNA Cell Biol. 11:259-263, 1992.
30. Remmers EF; Lafyatis R; Kumkumian GK, et al. Cytokines and growth regulation of synoviocytes from patients with rheumatoid arthritis and rats with streptococcal cell wall arthritis. Growth Factors. 2:179-188, 1990.