Cloning and characterization of acetyl-CoA:lyso-PAF acetyltransferase. J Biol Chem 2007;282:6532–9.
[29] Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001;294:1871–5.
[30] Hla T, Lee MJ, Ancellin N, Paik JH, Kluk MJ. Lysophospholipids—receptor revelations. Science 2001;294:1875–8.
[31] Watkins BA, Li Y, Seifert MF. Nutraceutical fatty acids as biochemical and molecular modulators of skeletal biology. J Am Coll Nutr 2001;20:410S–6S. discussion 17S–20S.
[32] Jilka RL. Molecular and cellular mechanisms of the anabolic effect of intermittent PTH. Bone 2007;40:1434–46.
[33] Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. J Cell Biochem 2003;88:259–66.
[34] Woolf AD. An update on glucocorticoid-induced osteoporosis. Curr Opin Rheumatol 2007;19:370–5.
[35] Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005;115:3318–25.
[36] Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg Am 2002;84-A:1032–44.
[37] Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev 2004;15:49–60.
[38] Pilbeam CC, Harrison JR, Raisz LG. Prostaglandins and bone metabolism. In: Bilekizian JP, Raisz LG, Rodan GA, editors. Principles of bone biology. San Diego: Academic Press; 2002. p. 979–94.
[39] Klein DC, Raisz LG. Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 1970;86:1436–40.
[40] Hikiji H, Ishii S, Shindou H, Takato T, Shimizu T. Absence of platelet-activating factor receptor protects mice from osteoporosis following ovariectomy. J Clin Invest 2004;114:85–93.
[41] Raisz LG, Pilbeam CC, Fall PM. Prostaglandins: mechanisms of action and regulation of production in bone. Osteoporos Int 1993;3(Suppl. 1):136–40.
[42] Krieger NS, Parker WR, Alexander KM, Bushinsky DA. Prostaglandins regulate acid-induced cell-mediated bone resorption. Am J Physiol Renal Physiol 2000;279:F1077–82.
[43] Suzawa T, Miyaura C, Inada M, Maruyama T, Sugimoto Y, Ushikubi F, et al. The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. Endocrinology 2000;141:1554–9.
[44] Kaji H, Sugimoto T, Kanatani M, Fukase M, Kumegawa M, Chihara K. Prostaglandin E_2 stimulates osteoclast-like cell formation and bone-resorbing activity via osteoblasts: role of cAMP-dependent protein kinase. J Bone Miner Res 1996;11:62–71.
[45] Okada Y, Lorenzo JA, Freeman AM, Tomita M, Morham SG, Raisz LG, et al. Prostaglandin G/H synthase-2 is required for maximal formation of osteoclast-like cells in culture. J Clin Invest 2000;105:823–32.
[46] Kawaguchi H, Nemoto K, Raisz LG, Harrison JR, Voznesensky OS, Alander CB, et al. Interleukin-4 inhibits prostaglandin G/H synthase-2 and cytosolic phospholipase A_2 induction in neonatal mouse parietal bone cultures. J Bone Miner Res 1996;11:358–66.
[47] Onoe Y, Miyaura C, Kaminakayashiki T, Nagai Y, Noguchi K, Chen QR, et al. IL-13 and IL-4 inhibit bone resorption by suppressing cyclooxygenase-2-dependent prostaglandin synthesis in osteoblasts. J Immunol 1996;156:758–64.
[48] Flanagan AM, Chambers TJ. Stimulation of bone nodule formation in vitro by prostaglandins E_1 and E_2. Endocrinology 1992;130:443–8.
[49] Nagata T, Kaho K, Nishikawa S, Shinohara H, Wakano Y, Ishida H. Effect of prostaglandin E_2 on mineralization of bone nodules formed by fetal rat calvarial cells. Calcif Tissue Int 1994;55:451–7.
[50] Wu X, Zeng LH, Taniguchi T, Xie QM. Activation of PKA and phosphorylation of sodium-dependent vitamin C transporter 2 by prostaglandin E_2 promote osteoblast-like differentiation in MC3T3-E1 cells. Cell Death Differ 2007;14:1792–801.
[51] Choudhary S, Kumar A, Kale RK, Raisz LG, Pilbeam CC. Extracellular calcium induces COX-2 in osteoblasts via a PKA pathway. Biochem Biophys Res Commun 2004;322:395–402.
[52] Choudhary S, Wadhwa S, Raisz LG, Alander C, Pilbeam CC. Extracellular calcium is a potent inducer of cyclo-oxygenase-2 in murine osteoblasts through an ERK signaling pathway. J Bone Miner Res 2003;18:1813–24.
[53] Knothe Tate ML, Knothe U, Niederer P. Experimental elucidation of mechanical load-induced fluid flow and its potential role in bone metabolism and functional adaptation. Am J Med Sci 1998;316:189–95.
[54] Mehrotra M, Saegusa M, Voznesensky O, Pilbeam C. Role of Cbfa1/Runx2 in the fluid shear stress induction of COX-2 in osteoblasts. Biochem Biophys Res Commun 2006;341:1225–30.
[55] Xiao G, Jiang D, Thomas P, Benson MD, Guan K, Karsenty G, et al. MAPK pathways activate and phosphorylate the osteoblast-specific transcription factor, Cbfa1. J Biol Chem 2000;275:4453–9.
[56] Franceschi RT, Xiao G. Regulation of the osteoblast-specific transcription factor, Runx2: responsiveness to multiple signal transduction pathways. J Cell Biochem 2003;88:446–54.
[57] Wadhwa S, Godwin SL, Peterson DR, Epstein MA, Raisz LG, Pilbeam CC. Fluid flow induction of cyclo-oxygenase 2 gene expression in osteoblasts is dependent on an extracellular signal-regulated kinase signaling pathway. J Bone Miner Res 2002;17:266–74.
[58] Wadhwa S, Choudhary S, Voznesensky M, Epstein M, Raisz L, Pilbeam C. Fluid flow induces COX-2 expression in MC3T3-E1 osteoblasts via a PKA signaling pathway. Biochem Biophys Res Commun 2002;297:46–51.
[59] Ponik SM, Pavalko FM. Formation of focal adhesions on fibronectin promotes fluid shear stress induction of COX-2 and PGE_2 release in MC3T3-E1 osteoblasts. J Appl Physiol 2004;97:135–42.
[60] Forwood MR. Inducible cyclo-oxygenase (COX-2) mediates the induction of bone formation by mechanical loading in vivo. J Bone Miner Res 1996;11:1688–93.
[61] Tang LY, Cullen DM, Yee JA, Jee WS, Kimmel DB. Prostaglandin E_2 increases the skeletal response to mechanical loading. J Bone Miner Res 1997;12:276–82.
[62] Suda M, Tanaka K, Natsui K, Usui T, Tanaka I, Fukushima M, et al. Prostaglandin E receptor subtypes in mouse osteoblastic cell line. Endocrinology 1996;137:1698–705.
[63] Miyaura C, Inada M, Suzawa T, Sugimoto Y, Ushikubi F, Ichikawa A, et al. Impaired bone resorption to prostaglandin E_2 in prostaglandin E receptor EP4-knockout mice. J Biol Chem 2000;275:19819–23.
[64] Fortier I, Gallant MA, Hackett JA, Patry C, de Brum-Fernandes AJ. Immunolocalization of the prostaglandin E_2 receptor subtypes in human bone tissue: differences in foetal, adult normal, osteoporotic and pagetic bone. Prostaglandins Leukot Essent Fatty Acids 2004;70:431–9.
[65] Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest 2001;108:25–30.
[66] Sakuma Y, Li Z, Pilbeam CC, Alander CB, Chikazu D, Kawaguchi H, et al. Stimulation of cAMP production and cyclooxygenase-2 by prostaglandin E_2 and selective prostaglandin receptor agonists in murine osteoblastic cells. Bone 2004;34:827–34.
[67] Sakuma Y, Tanaka K, Suda M, Yasoda A, Natsui K, Tanaka I, et al. Crucial involvement of the EP4 subtype of prostaglandin E receptor in osteoclast formation by proinflammatory cytokines and lipopolysaccharide. J Bone Miner Res 2000;15:218–27.
[68] Li X, Tomita M, Pilbeam CC, Breyer RM, Raisz LG. Prostaglandin receptor EP_2 mediates PGE_2 stimulated hypercalcemia in mice in vivo. Prostaglandins Other Lipid Mediat 2002;67:173–80.
[69] Li X, Pilbeam CC, Pan L, Breyer RM, Raisz LG. Effects of prostaglandin E_2 on gene expression in primary osteoblastic cells from prostaglandin receptor knockout mice. Bone 2002;30:567–73.

[70] Li X, Okada Y, Pilbeam CC, Lorenzo JA, Kennedy CR, Breyer RM, et al. Knockout of the murine prostaglandin EP_2 receptor impairs osteoclastogenesis in vitro. Endocrinology 2000;141:2054–61.

[71] Alander CB, Raisz LG. Effects of selective prostaglandins E_2 receptor agonists on cultured calvarial murine osteoblastic cells. Prostaglandins Other Lipid Mediat 2006;81:178–83.

[72] Suda M, Tanaka K, Yasoda A, Natsui K, Sakuma Y, Tanaka I, et al. Prostaglandin E_2 (PGE_2) autoamplifies its production through EP_1 subtype of PGE receptor in mouse osteoblastic MC3T3-E1 cells. Calcif Tissue Int 1998;62:327–31.

[73] Fujieda M, Kiriu M, Mizuochi S, Hagiya K, Kaneki H, Ide H. Formation of mineralized bone nodules by rat calvarial osteoblasts decreases with donor age due to a reduction in signaling through EP_1 subtype of prostaglandin E_2 receptor. J Cell Biochem 1999;75:215–25.

[74] Tang CH, Yang RS, Fu WM. Prostaglandin E_2 stimulates fibronectin expression through EP_1 receptor, phospholipase C, protein kinase Cα, and c-Src pathway in primary cultured rat osteoblasts. J Biol Chem 2005;280:22907–16.

[75] Udagawa N, Takahashi N, Jimi E, Matsuzaki K, Tsurukai T, Itoh K, et al. Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor. Bone 1999;25:517–23.

[76] Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci USA 1998;95:3597–602.

[77] Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med 1998;188:997–1001.

[78] Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, et al. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997;89:309–19.

[79] Brandstrom H, Jonsson KB, Ohlsson C, Vidal O, Ljunghall S, Ljunggren O. Regulation of osteoprotegerin mRNA levels by prostaglandin E_2 in human bone marrow stroma cells. Biochem Biophys Res Commun 1998;247:338–41.

[80] Suda K, Udagawa N, Sato N, Takami M, Itoh K, Woo JT, et al. Suppression of osteoprotegerin expression by prostaglandin E_2 is crucially involved in lipopolysaccharide-induced osteoclast formation. J Immunol 2004;172:2504–10.

[81] Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, et al. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 1990;145:3297–303.

[82] Devlin RD, Reddy SV, Savino R, Ciliberto G, Roodman GD. IL-6 mediates the effects of IL-1 or TNF, but not PTHrP or $1,25(OH)_2D_3$, on osteoclast-like cell formation in normal human bone marrow cultures. J Bone Miner Res 1998;13:393–9.

[83] Kozawa O, Suzuki A, Tokuda H, Kaida T, Uematsu T. Interleukin-6 synthesis induced by prostaglandin E_2: cross-talk regulation by protein kinase C. Bone 1998;22:355–60.

[84] Gruber R, Nothegger G, Ho GM, Willheim M, Peterlik M. Differential stimulation by PGE_2 and calcemic hormones of IL-6 in stromal/osteoblastic cells. Biochem Biophys Res Commun 2000;270:1080–5.

[85] Millet I, McCarthy TL, Vignery A. Regulation of interleukin-6 production by prostaglandin E_2 in fetal rat osteoblasts: role of protein kinase A signaling pathway. J Bone Miner Res 1998;13:1092–100.

[86] Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, et al. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 1989;58:573–81.

[87] Udagawa N, Takahashi N, Katagiri T, Tamura T, Wada S, Findlay DM, et al. Interleukin (IL)-6 induction of osteoclast differentiation depends on IL-6 receptors expressed on osteoblastic cells but not on osteoclast progenitors. J Exp Med 1995;182:1461–8.

[88] Palmqvist P, Persson E, Conaway HH, Lerner UH. IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-κB ligand, osteoprotegerin, and receptor activator of NF-κB in mouse calvariae. J Immunol 2002;169:3353–62.

[89] Honda M, Yamamoto S, Cheng M, Yasukawa K, Suzuki H, Saito T, et al. Human soluble IL-6 receptor: its detection and enhanced release by HIV infection. J Immunol 1992;148:2175–80.

[90] Liu XH, Kirschenbaum A, Yao S, Levine AC. Cross-talk between the interleukin-6 and prostaglandin E_2 signaling systems results in enhancement of osteoclastogenesis through effects on the osteoprotegerin/receptor activator of nuclear factor-κB (RANK) ligand/RANK system. Endocrinology 2005;146:1991–8.

[91] Lorenzo JA, Sousa SL, Alander C, Raisz LG, Dinarello CA. Comparison of the bone-resorbing activity in the supernatants from phytohemagglutinin-stimulated human peripheral blood mononuclear cells with that of cytokines through the use of an antiserum to interleukin 1. Endocrinology 1987;121:1164–70.

[92] Linkhart TA, MacCharles DC. Interleukin-1 stimulates release of insulin-like growth factor-I from neonatal mouse calvaria by a prostaglandin synthesis-dependent mechanism. Endocrinology 1992;131:2297–305.

[93] Amano S, Naganuma K, Kawata Y, Kawakami K, Kitano S, Hanazawa S. Prostaglandin E_2 stimulates osteoclast formation via endogenous IL-1β expressed through protein kinase A. J Immunol 1996;156:1931–6.

[94] Park YG, Kang SK, Noh SH, Park KK, Chang YC, Lee YC, et al. PGE_2 induces IL-1β gene expression in mouse osteoblasts through a cAMP-PKA signaling pathway. Int Immunopharmacol 2004;4:779–89.

[95] Akatsu T, Takahashi N, Udagawa N, Imamura K, Yamaguchi A, Sato K, et al. Role of prostaglandins in interleukin-1-induced bone resorption in mice in vitro. J Bone Miner Res 1991;6:183–9.

[96] Tanabe N, Maeno M, Suzuki N, Fujisaki K, Tanaka H, Ogiso B, et al. IL-1α stimulates the formation of osteoclast-like cells by increasing M-CSF and PGE_2 production and decreasing OPG production by osteoblasts. Life Sci 2005;77:615–26.

[97] Jimi E, Ikebe T, Takahashi N, Hirata M, Suda T, Koga T. Interleukin-1α activates an NF-κB-like factor in osteoclast-like cells. J Biol Chem 1996;271:4605–8.

[98] Jimi E, Nakamura I, Ikebe T, Akiyama S, Takahashi N, Suda T. Activation of NF-κB is involved in the survival of osteoclasts promoted by interleukin-1. J Biol Chem 1998;273:8799–805.

[99] Pilbeam CC, Raisz LG, Voznesensky O, Alander CB, Delman BN, Kawaguchi H. Autoregulation of inducible prostaglandin G/H synthase in osteoblastic cells by prostaglandins. J Bone Miner Res 1995;10:406–14.

[100] Murakami M, Kuwata H, Amakasu Y, Shimbara S, Nakatani Y, Atsumi G, et al. Prostaglandin E_2 amplifies cytosolic phospholipase A_2- and cyclooxygenase-2-dependent delayed prostaglandin E_2 generation in mouse osteoblastic cells. Enhancement by secretory phospholipase A_2. J Biol Chem 1997;272:19891–7.

[101] Chambers TJ, Thomson BM, Fuller K. Effect of substrate composition on bone resorption by rabbit osteoclasts. J Cell Sci 1984;70:61–71.

[102] Sakamoto S, Sakamoto M. Osteoblast collagenase: collagenase synthesis by clonally derived mouse osteogenic (MC3T3-E1) cells. Biochem Int 1984;9:51–8.

[103] Holliday LS, Welgus HG, Fliszar CJ, Veith GM, Jeffrey JJ, Gluck SL. Initiation of osteoclast bone resorption by interstitial collagenase. J Biol Chem 1997;272:22053–8.

[104] Kim CH, Park YG, Noh SH, Kim YK. PGE_2 induces the gene expression of bone matrix metalloproteinase-1 in mouse osteoblasts by cAMP-PKA signaling pathway. Int J Biochem Cell Biol 2005;37:375–85.

[105] Blobel CP. Metalloprotease-disintegrins: links to cell adhesion and cleavage of TNFα and Notch. Cell 1997;90:589–92.

[106] Miles RR, Sluka JP, Halladay DL, Santerre RF, Hale LV, Bloem L, et al. ADAMTS-1: a cellular disintegrin and metalloprotease with thrombospondin motifs is a target for parathyroid hormone in bone. Endocrinology 2000;141:4533–42.

[107] Komori T. Requisite roles of Runx2 and Cbfb in skeletal development. J Bone Miner Metab 2003;21:193–7.

[108] Yoshida K, Oida H, Kobayashi T, Maruyama T, Tanaka M, Katayama T, et al. Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. Proc Natl Acad Sci USA 2002;99:4580–5.

[109] Wozney JM, Rosen V. Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. Clin Orthop Relat Res 1998:26–37.

[110] Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 1997;89:747–54.

[111] Arikawa T, Omura K, Morita I. Regulation of bone morphogenetic protein-2 expression by endogenous prostaglandin E_2 in human mesenchymal stem cells. J Cell Physiol 2004;200:400–6.

[112] Garcia AJ, Reyes CD. Bio-adhesive surfaces to promote osteoblast differentiation and bone formation. J Dent Res 2005;84:407–13.

[113] Hakeda Y, Nakatani Y, Kurihara N, Ikeda E, Maeda N, Kumegawa M. Prostaglandin E_2 stimulates collagen and non-collagen protein synthesis and prolyl hydroxylase activity in osteoblastic clone MC3T3-E1 cells. Biochem Biophys Res Commun 1985;126:340–5.

[114] Globus RK, Doty SB, Lull JC, Holmuhamedov E, Humphries MJ, Damsky CH. Fibronectin is a survival factor for differentiated osteoblasts. J Cell Sci 1998;111:1385–93.

[115] Samoto H, Shimizu E, Matsuda-Honjyo Y, Saito R, Nakao S, Yamazaki M, et al. Prostaglandin E_2 stimulates bone sialoprotein (BSP) expression through cAMP and fibroblast growth factor 2 response elements in the proximal promoter of the rat BSP gene. J Biol Chem 2003;278:28659–67.

[116] Wani MR, Fuller K, Kim NS, Choi Y, Chambers T. Prostaglandin E_2 cooperates with TRANCE in osteoclast induction from hemopoietic precursors: synergistic activation of differentiation, cell spreading, and fusion. Endocrinology 1999;140:1927–35.

[117] Kobayashi Y, Take I, Yamashita T, Mizoguchi T, Ninomiya T, Hattori T, et al. Prostaglandin E_2 receptors EP2 and EP4 are down-regulated during differentiation of mouse osteoclasts from their precursors. J Biol Chem 2005;280:24035–42.

[118] Kobayashi Y, Mizoguchi T, Take I, Kurihara S, Udagawa N, Takahashi N. Prostaglandin E_2 enhances osteoclastic differentiation of precursor cells through protein kinase A-dependent phosphorylation of TAK1. J Biol Chem 2005;280:11395–403.

[119] Take I, Kobayashi Y, Yamamoto Y, Tsuboi H, Ochi T, Uematsu S, et al. Prostaglandin E_2 strongly inhibits human osteoclast formation. Endocrinology 2005;146:5204–14.

[120] Lader CS, Flanagan AM. Prostaglandin E_2, interleukin 1α, and tumor necrosis factor-α increase human osteoclast formation and bone resorption in vitro. Endocrinology 1998;139:3157–64.

[121] Chenu C, Kurihara N, Mundy GR, Roodman GD. Prostaglandin E_2 inhibits formation of osteoclastlike cells in long-term human marrow cultures but is not a mediator of the inhibitory effects of transforming growth factor β. J Bone Miner Res 1990;5:677–81.

[122] Merke J, Klaus G, Hugel U, Waldherr R, Ritz E. No 1,25-dihydroxyvitamin D_3 receptors on osteoclasts of calcium-deficient chicken despite demonstrable receptors on circulating monocytes. J Clin Invest 1986;77:312–4.

[123] Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, et al. Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 2000;191:275–86.

[124] Itonaga I, Sabokbar A, Neale SD, Athanasou NA. 1,25-Dihydroxyvitamin D_3 and prostaglandin E_2 act directly on circulating human osteoclast precursors. Biochem Biophys Res Commun 1999;264:590–5.

[125] Suzuki H, Nakamura I, Takahashi N, Ikuhara T, Matsuzaki K, Isogai Y, et al. Calcitonin-induced changes in the cytoskeleton are mediated by a signal pathway associated with protein kinase A in osteoclasts. Endocrinology 1996;137:4685–90.

[126] Mano M, Arakawa T, Mano H, Nakagawa M, Kaneda T, Kaneko H, et al. Prostaglandin E_2 directly inhibits bone-resorbing activity of isolated mature osteoclasts mainly through the EP4 receptor. Calcif Tissue Int 2000;67:85–92.

[127] Sarrazin P, Hackett JA, Fortier I, Gallant MA, de Brum-Fernandes A. Role of EP3 and EP4 prostaglandin receptors in reorganization of the cytoskeleton in mature human osteoclasts. J Rheumatol 2004;31:1598–606.

[128] Li M, Healy DR, Li Y, Simmons HA, Crawford DT, Ke HZ, et al. Osteopenia and impaired fracture healing in aged EP4 receptor knockout mice. Bone 2005;37:46–54.

[129] Akhter MP, Cullen DM, Pan LC. Bone biomechanical properties in EP4 knockout mice. Calcif Tissue Int 2006;78:357–62.

[130] Akhter MP, Cullen DM, Gong G, Recker RR. Bone biomechanical properties in prostaglandin EP1 and EP2 knockout mice. Bone 2001;29:121–5.

[131] Lin BY, Jee WS, Ma YF, Ke HZ, Kimmel DB, Li XJ. Effects of prostaglandin E_2 and risedronate administration on cancellous bone in older female rats. Bone 1994;15:489–96.

[132] Suponitzky I, Weinreb M. Differential effects of systemic prostaglandin E_2 on bone mass in rat long bones and calvariae. J Endocrinol 1998;156:51–7.

[133] Li M, Ke HZ, Qi H, Healy DR, Li Y, Crawford DT, et al. A novel, non-prostanoid EP2 receptor-selective prostaglandin E_2 agonist stimulates local bone formation and enhances fracture healing. J Bone Miner Res 2003;18:2033–42.

[134] Bostrom MP. Expression of bone morphogenetic proteins in fracture healing. Clin Orthop Relat Res 1998:S116–23.

[135] Dekel S, Lenthall G, Francis MJ. Release of prostaglandins from bone and muscle after tibial fracture. An experimental study in rabbits. J Bone Joint Surg Br 1981;63-B:185–9.

[136] Gerstenfeld LC, Thiede M, Seibert K, Mielke C, Phippard D, Svagr B, et al. Differential inhibition of fracture healing by non-selective and cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs. J Orthop Res 2003;21:670–5.

[137] Endo K, Sairyo K, Komatsubara S, Sasa T, Egawa H, Yonekura D, et al. Cyclooxygenase-2 inhibitor inhibits the fracture healing. J Physiol Anthropol Appl Human Sci 2002;21:235–8.

[138] Harder AT, An YH. The mechanisms of the inhibitory effects of nonsteroidal anti-inflammatory drugs on bone healing: a concise review. J Clin Pharmacol 2003;43:807–15.

[139] Zhang X, Schwarz EM, Young DA, Puzas JE, Rosier RN, O'Keefe RJ. Cyclooxygenase-2 regulates mesenchymal cell differentiation into the osteoblast lineage and is critically involved in bone repair. J Clin Invest 2002;109:1405–15.

[140] Paralkar VM, Borovecki F, Ke HZ, Cameron KO, Lefker B, Grasser WA, et al. An EP2 receptor-selective prostaglandin E_2 agonist induces bone healing. Proc Natl Acad Sci USA 2003;100:6736–40.

[141] Nomura S, Takano-Yamamoto T. Molecular events caused by mechanical stress in bone. Matrix Biol 2000;19:91–6.

[142] Stepan JJ, Pospichal J, Presl J, Pacovsky V. Bone loss and biochemical indices of bone remodeling in surgically induced postmenopausal women. Bone 1987;8:279–84.

[143] Ke HZ, Jee WS, Zeng QQ, Li M, Lin BY. Prostaglandin E_2 increased rat cortical bone mass when administered immediately following ovariectomy. Bone Miner 1993;21:189–201.

[144] Li M, Jee WS, Ke HZ, Tang LY, Ma YF, Liang XG, et al. Prostaglandin E_2 administration prevents bone loss induced by orchidectomy in rats. J Bone Miner Res 1995;10:66–73.

[145] Weinreb M, Grosskopf A, Shir N. The anabolic effect of PGE_2 in rat bone marrow cultures is mediated via the EP_4 receptor subtype. Am J Physiol 1999;276:E376–83.

[146] Ke HZ, Crawford DT, Qi H, Simmons HA, Owen TA, Paralkar VM, et al. A nonprostanoid EP4 receptor selective prostaglandin E_2 agonist restores bone mass and strength in aged, ovariectomized rats. J Bone Miner Res 2006;21:565–75.

[147] McClung MR. The menopause and HRT. Prevention and management of osteoporosis. Best Pract Res Clin Endocrinol Metab 2003;17:53–71.

[148] Lane N, Coble T, Kimmel DB. Effect of naproxen on cancellous bone in ovariectomized rats. J Bone Miner Res 1990;5:1029–35.

[149] Gregory LS, Kelly WL, Reid RC, Fairlie DP, Forwood MR. Inhibitors of cyclo-oxygenase-2 and secretory phospholipase A_2 preserve bone architecture following ovariectomy in adult rats. Bone 2006;39:134–42.

[150] Bauer DC, Orwoll ES, Fox KM, Vogt TM, Lane NE, Hochberg MC, et al. Aspirin and NSAID use in older women: effect on bone mineral density and fracture risk. Study of Osteoporotic Fractures Research Group. J Bone Miner Res 1996;11:29–35.

[151] Carbone LD, Tylavsky FA, Cauley JA, Harris TB, Lang TF, Bauer DC, et al. Association between bone mineral density and the use of nonsteroidal anti-inflammatory drugs and aspirin: impact of cyclooxygenase selectivity. J Bone Miner Res 2003;18:1795–802.

[152] Morton DJ, Barrett-Connor EL, Schneider DL. Nonsteroidal anti-inflammatory drugs and bone mineral density in older women: the Rancho Bernardo study. J Bone Miner Res 1998;13:1924–31.

[153] Ono K, Akatsu T, Murakami T, Nishikawa M, Yamamoto M, Kugai N, et al. Important role of EP_4, a subtype of prostaglandin (PG) E receptor, in osteoclast-like cell formation from mouse bone marrow cells induced by PGE_2. J Endocrinol 1998;158:R1–5.

[154] Sato K, Takayanagi H. Osteoclasts, rheumatoid arthritis, and osteoimmunology. Curr Opin Rheumatol 2006;18:419–26.

[155] Martel-Pelletier J, Pelletier JP, Fahmi H. Cyclooxygenase-2 and prostaglandins in articular tissues. Semin Arthritis Rheum 2003;33:155–67.

[156] Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, et al. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999;103:1345–52.

[157] Kojima F, Naraba H, Sasaki Y, Okamoto R, Koshino T, Kawai S. Coexpression of microsomal prostaglandin E synthase with cyclooxygenase-2 in human rheumatoid synovial cells. J Rheumatol 2002;29:1836–42.

[158] Korotkova M, Westman M, Gheorghe KR, af Klint E, Trollmo C, Ulfgren AK, et al. Effects of antirheumatic treatments on the prostaglandin E_2 biosynthetic pathway. Arthritis Rheum 2005;52:3439–47.

[159] Luross JA, Williams NA. The genetic and immunopathological processes underlying collagen-induced arthritis. Immunology 2001;103:407–16.

[160] Honda T, Segi-Nishida E, Miyachi Y, Narumiya S. Prostacyclin-IP signaling and prostaglandin E_2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. J Exp Med 2006;203:325–35.

[161] Hegen M, Sun L, Uozumi N, Kume K, Goad ME, Nickerson-Nutter CL, et al. Cytosolic phospholipase $A_2\alpha$-deficient mice are resistant to collagen-induced arthritis. J Exp Med 2003;197:1297–302.

[162] Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, et al. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. Arthritis Rheum 2000;43:2687–93.

[163] Trebino CE, Stock JL, Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci USA 2003;100:9044–9.

[164] Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, et al. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. J Biol Chem 2004;279:33684–95.

[165] McCoy JM, Wicks JR, Audoly LP. The role of prostaglandin E_2 receptors in the pathogenesis of rheumatoid arthritis. J Clin Invest 2002;110:651–8.

[166] Ghosh P, Smith M. Osteoarthritis, genetic and molecular mechanisms. Biogerontology 2002;3:85–8.

[167] Smith MD, Triantafillou S, Parker A, Youssef PP, Coleman M. Synovial membrane inflammation and cytokine production in patients with early osteoarthritis. J Rheumatol 1997;24:365–71.

[168] Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, et al. Cyclooxygenase 2-dependent prostaglandin E_2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheum 2002;46:1789–803.

[169] Inoue H, Takamori M, Shimoyama Y, Ishibashi H, Yamamoto S, Koshihara Y. Regulation by PGE_2 of the production of interleukin-6, macrophage colony stimulating factor, and vascular endothelial growth factor in human synovial fibroblasts. Br J Pharmacol 2002;136:287–95.

[170] Hilal G, Massicotte F, Martel-Pelletier J, Fernandes JC, Pelletier JP, Lajeunesse D. Endogenous prostaglandin E_2 and insulin-like growth factor 1 can modulate the levels of parathyroid hormone receptor in human osteoarthritic osteoblasts. J Bone Miner Res 2001;16:713–21.

[171] Blanco FJ, Guitian R, Vazquez-Martul E, de Toro FJ, Galdo F. Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. Arthritis Rheum 1998;41:284–9.

[172] Notoya K, Jovanovic DV, Reboul P, Martel-Pelletier J, Mineau F, Pelletier JP. The induction of cell death in human osteoarthritis chondrocytes by nitric oxide is related to the production of prostaglandin E_2 via the induction of cyclooxygenase-2. J Immunol 2000;165:3402–10.

[173] Heitz-Mayfield LJ. Disease progression: identification of high-risk groups and individuals for periodontitis. J Clin Periodontol 2005;32(Suppl. 6):196–209.

[174] Rams TE, Listgarten MA, Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* subgingival presence, species-specific serum immunoglobulin G antibody levels, and periodontitis disease recurrence. J Periodontal Res 2006;41:228–34.

[175] Wada N, Maeda H, Yoshimine Y, Akamine A. Lipopolysaccharide stimulates expression of osteoprotegerin and receptor activator of NF-kappa B ligand in periodontal ligament fibroblasts through the induction of interleukin-1 beta and tumor necrosis factor-alpha. Bone 2004;35:629–35.

[176] Yip KH, Zheng MH, Feng HT, Steer JH, Joyce DA, Xu J. Sesquiterpene lactone parthenolide blocks lipopolysaccharide-induced osteolysis through the suppression of NF-κB activity. J Bone Miner Res 2004;19:1905–16.

[177] Fukushima H, Jimi E, Okamoto F, Motokawa W, Okabe K. IL-1-induced receptor activator of NF-κB ligand in human periodontal ligament cells involves ERK-dependent PGE_2 production. Bone 2005;36:267–75.

[178] Miyauchi M, Takata T, Ito H, Ogawa I, Kobayashi J, Nikai H, et al. Immunohistochemical detection of prostaglandins E_2, $F_{2\alpha}$, and 6-keto-prostaglandin $F_{1\alpha}$ in experimentally induced periapical inflammatory lesions in rats. J Endod 1996;22:635–7.

[179] Tsai CC, Hong YC, Chen CC, Wu YM. Measurement of prostaglandin E_2 and leukotriene B_4 in the gingival crevicular fluid. J Dent 1998;26:97–103.

[180] Miyaura C, Inada M, Matsumoto C, Ohshiba T, Uozumi N, Shimizu T, et al. An essential role of cytosolic phospholipase $A_2\alpha$ in prostaglandin E_2-mediated bone resorption associated with inflammation. J Exp Med 2003;197:1303–10.

[181] Inada M, Matsumoto C, Uematsu S, Akira S, Miyaura C. Membrane-bound prostaglandin E synthase-1-mediated prostaglandin E_2 production by osteoblast plays a critical role in

lipopolysaccharide-induced bone loss associated with inflammation. J Immunol 2006;177:1879–85.

[182] Sakuma Y, Tanaka K, Suda M, Komatsu Y, Yasoda A, Miura M, et al. Impaired bone resorption by lipopolysaccharide in vivo in mice deficient in the prostaglandin E receptor EP4 subtype. Infect Immun 2000;68:6819–25.

[183] Williams RC, Jeffcoat MK, Kaplan ML, Goldhaber P, Johnson HG, Wechter WJ. Flurbiprofen: a potent inhibitor of alveolar bone resorption in beagles. Science 1985;227:640–2.

[184] Williams RC, Jeffcoat MK, Howell TH, Rolla A, Stubbs D, Teoh KW, et al. Altering the progression of human alveolar bone loss with the non-steroidal anti-inflammatory drug flurbiprofen. J Periodontol 1989;60:485–90.

[185] Ramirez-Yanez GO, Seymour GJ, Walsh LJ, Forwood MR, Symons AL. Prostaglandin E_2 enhances alveolar bone formation in the rat mandible. Bone 2004;35:1361–8.

[186] Ramirez-Yanez GO, Seymour GJ, Symons AL. Local application of prostaglandin E_2 reduces trap, calcitonin receptor and metalloproteinase-2 immunoreactivity in the rat periodontium. Arch Oral Biol 2005;50:1014–22.

[187] Feyen JH, van der Wilt G, Moonen P, Di Bon A, Nijweide PJ. Stimulation of arachidonic acid metabolism in primary cultures of osteoblast-like cells by hormones and drugs. Prostaglandins 1984;28:769–81.

[188] Cho MJ, Allen MA. Chemical stability of prostacyclin (PGI_2) in aqueous solutions. Prostaglandins 1978;15:943–54.

[189] Raisz LG, Vanderhoek JY, Simmons HA, Kream BE, Nicolaou KC. Prostaglandin synthesis by fetal rat bone in vitro: evidence for a role of prostacyclin. Prostaglandins 1979;17:905–14.

[190] Glanstchnig H, Varga F, Rumpler M, Klaushofer K. Prostacyclin (PGI_2): a potential mediator of c-fos expression induced by hydrostatic pressure in osteoblastic cells. Eur J Clin Invest 1996;26:544–8.

[191] Turner CH, Forwood MR, Rho JY, Yoshikawa T. Mechanical loading thresholds for lamellar and woven bone formation. J Bone Miner Res 1994;9:87–97.

[192] Joldersma M, Burger EH, Semeins CM, Klein-Nulend J. Mechanical stress induces COX-2 mRNA expression in bone cells from elderly women. J Biomech 2000;33:53–61.

[193] Chambers TJ, Fuller K, Athanasou NA. The effect of prostaglandins I_2, E_1, E_2 and dibutyryl cyclic AMP on the cytoplasmic spreading of rat osteoclasts. Br J Exp Pathol 1984;65:557–66.

[194] Maurin AC, Chavassieux PM, Meunier PJ. Expression of $PPAR_\gamma$ and β/δ in human primary osteoblastic cells: influence of polyunsaturated fatty acids. Calcif Tissue Int 2005;76:385–92.

[195] Brodie MJ, Hensby CN, Parke A, Gordon D. Is prostacyclin in the major pro-inflammatory prostanoid in joint fluid? Life Sci 1980;27:603–8.

[196] Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, et al. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc Natl Acad Sci USA 1993;90:11924–8.

[197] Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. Cytokine Growth Factor Rev 2002;13:357–68.

[198] Hakeda Y, Hotta T, Kurihara N, Ikeda E, Maeda N, Yagyu Y, et al. Prostaglandin E_1 and $F_{2\alpha}$ stimulate differentiation and proliferation, respectively, of clonal osteoblastic MC3T3-E1 cells by different second messengers in vitro. Endocrinology 1987;121:1966–74.

[199] Ma YF, Li XJ, Jee WS, McOsker J, Liang XG, Setterberg R, et al. Effects of prostaglandin E_2 and $F_{2\alpha}$ on the skeleton of osteopenic ovariectomized rats. Bone 1995;17:549–54.

[200] Tokuda H, Harada A, Hirade K, Matsuno H, Ito H, Kato K, et al. Incadronate amplifies prostaglandin $F_{2\alpha}$-induced vascular endothelial growth factor synthesis in osteoblasts. Enhancement of MAPK activity. J Biol Chem 2003;278:18930–7.

[201] Sabbieti MG, Marchetti L, Gabrielli MG, Menghi M, Materazzi S, Menghi G, et al. Prostaglandins differently regulate FGF-2 and FGF receptor expression and induce nuclear translocation in osteoblasts via MAPK kinase. Cell Tissue Res 2005;319:267–78.

[202] Harada S, Thomas KA. Vascular endothelial growth factors. In: Bilesikian JP, Raisz LG, Rodan GA, editors. Principles of bone biology. San Diego: Academic Press; 2002. p. 883–902.

[203] Hurley MM, Marie PJ, Florkiewicz RZ. Fibroblast growth factor (FGF) and FGF receptor families in bone. In: Bilesikian JP, Raisz LG, Rodan GA, editors. Principles of bone biology. San Diego: Academic Press; 2002. p. 825–52.

[204] Ahmed I, Gesty-Palmer D, Drezner MK, Luttrell LM. Transactivation of the epidermal growth factor receptor mediates parathyroid hormone and prostaglandin $F2_\alpha$-stimulated mitogen-activated protein kinase activation in cultured transgenic murine osteoblasts. Mol Endocrinol 2003;17:1607–21.

[205] Sato I, Suzuki A, Kakita A, Ono Y, Miura Y, Itoh M, et al. Stimulatory effect of prostaglandin $F_{2\alpha}$ on Na-dependent phosphate transport in osteoblast-like cells. Prostaglandins Leukot Essent Fatty Acids 2003;68:311–5.

[206] Koshihara Y, Kawamura M. Prostaglandin D_2 stimulates calcification of human osteoblastic cells. Biochem Biophys Res Commun 1989;159:1206–12.

[207] Tokuda H, Kozawa O, Harada A, Uematsu T. Prostaglandin D_2 induces interleukin-6 synthesis via Ca^{2+} mobilization in osteoblasts: regulation by protein kinase C. Prostaglandins Leukot Essent Fatty Acids 1999;61:189–94.

[208] Takagi T, Yamamoto T, Asano S, Tamaki H. Effect of prostaglandin D_2 on the femoral bone mineral density in ovariectomized rats. Calcif Tissue Int 1993;52:442–6.

[209] Gallant MA, Samadfam R, Hackett JA, Antoniou J, Parent JL, de Brum-Fernandes AJ. Production of prostaglandin D_2 by human osteoblasts and modulation of osteoprotegerin, RANKL, and cellular migration by DP and CRTH2 receptors. J Bone Miner Res 2005;20:672–81.

[210] Siddhivarn C, Banes A, Champagne C, Riche EL, Weerapradist W, Offenbacher S. Prostaglandin D_2 pathway and peroxisome proliferator-activated receptor γ-1 expression are induced by mechanical loading in an osteoblastic cell line. J Periodontal Res 2006;41:92–100.

[211] Kakita A, Suzuki A, Ono Y, Miura Y, Itoh M, Oiso Y. Possible involvement of p38 MAP kinase in prostaglandin E_1-induced ALP activity in osteoblast-like cells. Prostaglandins Leukot Essent Fatty Acids 2004;70:469–74.

[212] Harada S, Nagy JA, Sullivan KA, Thomas KA, Endo N, Rodan GA, et al. Induction of vascular endothelial growth factor expression by prostaglandin E_2 and E_1 in osteoblasts. J Clin Invest 1994;93:2490–6.

[213] Kanno Y, Tokuda H, Nakajima K, Ishisaki A, Shibata T, Numata O, et al. Involvement of SAPK/JNK in prostaglandin E_1-induced VEGF synthesis in osteoblast-like cells. Mol Cell Endocrinol 2004;220:89–95.

[214] Hamberg M, Svensson J, Samuelsson B. Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 1975;72:2994–8.

[215] Saito S, Yamasaki K, Yamada S, Matsumoto A, Akatsu T, Takahashi N, et al. A stable analogue of thromboxane A_2, 9,11-epithio-11,12-methanothromboxane A_2, stimulates bone resorption in vitro and osteoclast-like cell formation in mouse marrow culture. Bone Miner 1991;12:15–23.

[216] Shinoda J, Suzuki A, Oiso Y, Kozawa O. Thromboxane A_2-stimulated phospholipase D in osteoblast-like cells: possible involvement of PKC. Am J Physiol 1995;269:E524–9.

[217] Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T. A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. Nature 1997;387:620–4.

[218] Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. A second leukotriene B_4 receptor, BLT2. A new therapeutic target in

inflammation and immunological disorders. J Exp Med 2000;192:421–32.

[219] Yokomizo T, Izumi T, Shimizu T. Leukotriene B_4: metabolism and signal transduction. Arch Biochem Biophys 2001;385:231–41.

[220] Capra V, Thompson MD, Sala A, Cole DE, Folco G, Rovati GE. Cysteinyl-leukotrienes and their receptors in asthma and other inflammatory diseases: critical update and emerging trends. Med Res Rev 2007;27:469–527.

[221] Lynch KR, O'Neill GP, Liu Q, Im DS, Sawyer N, Metters KM, et al. Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature 1999;399:789–93.

[222] Heise CE, O'Dowd BF, Figueroa DJ, Sawyer N, Nguyen T, Im DS, et al. Characterization of the human cysteinyl leukotriene 2 receptor. J Biol Chem 2000;275:30531–6.

[223] Hosoi T, Koguchi Y, Sugikawa E, Chikada A, Ogawa K, Tsuda N, et al. Identification of a novel human eicosanoid receptor coupled to Gi/o. J Biol Chem 2002;277:31459–65.

[224] Gallwitz WE, Mundy GR, Lee CH, Qiao M, Roodman GD, Raftery M, et al. 5-Lipoxygenase metabolites of arachidonic acid stimulate isolated osteoclasts to resorb calcified matrices. J Biol Chem 1993;268:10087–94.

[225] Traianedes K, Dallas MR, Garrett IR, Mundy GR, Bonewald LF. 5-Lipoxygenase metabolites inhibit bone formation in vitro. Endocrinology 1998;139:3178–84.

[226] Meghji S, Sandy JR, Scutt AM, Harvey W, Harris M. Stimulation of bone resorption by lipoxygenase metabolites of arachidonic acid. Prostaglandins 1988;36:139–49.

[227] Garcia C, Boyce BF, Gilles J, Dallas M, Qiao M, Mundy GR, et al. Leukotriene B_4 stimulates osteoclastic bone resorption both in vitro and in vivo. J Bone Miner Res 1996;11:1619–27.

[228] Flynn MA, Qiao M, Garcia C, Dallas M, Bonewald LF. Avian osteoclast cells are stimulated to resorb calcified matrices by and possess receptors for leukotriene B_4. Calcif Tissue Int 1999;64:154–9.

[229] Jiang J, Lv HS, Lin JH, Jiang DF, Chen ZK. LTB4 can directly stimulate human osteoclast formation from PBMC independent of RANKL. Artif Cells Blood Substit Immobil Biotechnol 2005;33:391–403.

[230] Garcia C, Qiao M, Chen D, Kirchen M, Gallwitz W, Mundy GR, et al. Effects of synthetic peptido-leukotrienes on bone resorption in vitro. J Bone Miner Res 1996;11:521–9.

[231] Paredes Y, Massicotte F, Pelletier JP, Martel-Pelletier J, Laufer S, Lajeunesse D. Study of the role of leukotriene B_4 in abnormal function of human subchondral osteoarthritis osteoblasts: effects of cyclooxygenase and/or 5-lipoxygenase inhibition. Arthritis Rheum 2002;46:1804–12.

[232] Ren W, Dziak R. Effects of leukotrienes on osteoblastic cell proliferation. Calcif Tissue Int 1991;49:197–201.

[233] Klickstein LB, Shapleigh C, Goetzl EJ. Lipoxygenation of arachidonic acid as a source of polymorphonuclear leukocyte chemotactic factors in synovial fluid and tissue in rheumatoid arthritis and spondyloarthritis. J Clin Invest 1980;66:1166–70.

[234] Davidson EM, Rae SA, Smith MJ. Leukotriene B_4, a mediator of inflammation present in synovial fluid in rheumatoid arthritis. Ann Rheum Dis 1983;42:677–9.

[235] Ahmadzadeh N, Shingu M, Nobunaga M, Tawara T. Relationship between leukotriene B_4 and immunological parameters in rheumatoid synovial fluids. Inflammation 1991;15:497–503.

[236] Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets. Arthritis Rheum 2001;44:1237–47.

[237] Laufer S. Role of eicosanoids in structural degradation in osteoarthritis. Curr Opin Rheumatol 2003;15:623–7.

[238] Baroukh B, Saffar JS. The effect of leukotriene synthesis inhibitors on hamster periodontitis. Arch Oral Biol 1990;35(Suppl.):189S–92S.

[239] Griffiths RJ, Pettipher ER, Koch K, Farrell CA, Breslow R, Conklyn MJ, et al. Leukotriene B_4 plays a critical role in the progression of collagen-induced arthritis. Proc Natl Acad Sci USA 1995;92:517–21.

[240] Kuwabara K, Yasui K, Jyoyama H, Maruyama T, Fleisch JH, Hori Y. Effects of the second-generation leukotriene B_4 receptor antagonist, LY293111Na, on leukocyte infiltration and collagen-induced arthritis in mice. Eur J Pharmacol 2000;402:275–85.

[241] Alten R, Gromnica-Ihle E, Pohl C, Emmerich J, Steffgen J, Roscher R, et al. Inhibition of leukotriene B_4-induced CD11B/CD18 (Mac-1) expression by BIIL 284, a new long acting LTB_4 receptor antagonist, in patients with rheumatoid arthritis. Ann Rheum Dis 2004;63:170–6.

[242] Kim ND, Chou RC, Seung E, Tager AM, Luster AD. A unique requirement for the leukotriene B_4 receptor BLT1 for neutrophil recruitment in inflammatory arthritis. J Exp Med 2006;203:829–35.

[243] Chen M, Lam BK, Kanaoka Y, Nigrovic PA, Audoly LP, Austen KF, et al. Neutrophil-derived leukotriene B_4 is required for inflammatory arthritis. J Exp Med 2006;203:837–42.

[244] Shao WH, Del Prete A, Bock CB, Haribabu B. Targeted disruption of leukotriene B_4 receptors BLT1 and BLT2: a critical role for BLT1 in collagen-induced arthritis in mice. J Immunol 2006;176:6254–61.

[245] Marcouiller P, Pelletier JP, Guevremont M, Martel-Pelletier J, Ranger P, Laufer S, et al. Leukotriene and prostaglandin synthesis pathways in osteoarthritic synovial membranes: regulating factors for interleukin 1β synthesis. J Rheumatol 2005;32:704–12.

[246] Wittenberg RH, Willburger RE, Kleemeyer KS, Peskar BA. In vitro release of prostaglandins and leukotrienes from synovial tissue, cartilage, and bone in degenerative joint diseases. Arthritis Rheum 1993;36:1444–50.

[247] Lajeunesse D, Martel-Pelletier J, Fernandes JC, Laufer S, Pelletier JP. Treatment with licofelone prevents abnormal subchondral bone cell metabolism in experimental dog osteoarthritis. Ann Rheum Dis 2004;63:78–83.

[248] Benveniste J, Henson PM, Cochrane CG. Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophils, and a platelet-activating factor. J Exp Med 1972;136:1356–77.

[249] Hanahan DJ. Platelet activating factor: a biologically active phosphoglyceride. Annu Rev Biochem 1986;55:483–509.

[250] Ishii S, Shimizu T. Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. Prog Lipid Res 2000;39:41–82.

[251] Honda Z, Ishii S, Shimizu T. Platelet-activating factor receptor. J Biochem 2002;131:773–9.

[252] Honda Z, Nakamura M, Miki I, Minami M, Watanabe T, Seyama Y, et al. Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 1991;349:342–6.

[253] Ishii S, Kuwaki T, Nagase T, Maki K, Tashiro F, Sunaga S, et al. Impaired anaphylactic responses with intact sensitivity to endotoxin in mice lacking a platelet-activating factor receptor. J Exp Med 1998;187:1779–88.

[254] Ishii S, Nagase T, Shindou H, Takizawa H, Ouchi Y, Shimizu T. Platelet-activating factor receptor develops airway hyperresponsiveness independently of airway inflammation in a murine asthma model. J Immunol 2004;172:7095–102.

[255] Nagase T, Ishii S, Kume K, Uozumi N, Izumi T, Ouchi Y, et al. Platelet-activating factor mediates acid-induced lung injury in genetically engineered mice. J Clin Invest 1999;104:1071–6.

[256] Nagase T, Uozumi N, Ishii S, Kita Y, Yamamoto H, Ohga E, et al. A pivotal role of cytosolic phospholipase A_2 in bleomycin-induced pulmonary fibrosis. Nat Med 2002;8:480–4.

[257] Souza DG, Pinho V, Soares AC, Shimizu T, Ishii S, Teixeira MM. Role of PAF receptors during intestinal ischemia and reperfusion injury. A comparative study between PAF receptor-deficient mice and PAF receptor antagonist treatment. Br J Pharmacol 2003;139:733–40.

[258] Kihara Y, Ishii S, Kita Y, Toda A, Shimada A, Shimizu T. Dual phase regulation of experimental allergic encephalomyelitis by platelet-activating factor. J Exp Med 2005;202:853–63.

[259] Doi K, Okamoto K, Negishi K, Suzuki Y, Nakao A, Fujita T, et al. Attenuation of folic acid-induced renal inflammatory injury in

platelet-activating factor receptor-deficient mice. Am J Pathol 2006;168:1413–24.

[260] Soares AC, Pinho VS, Souza DG, Shimizu T, Ishii S, Nicoli JR, et al. Role of the platelet-activating factor (PAF) receptor during pulmonary infection with gram negative bacteria. Br J Pharmacol 2002;137:621–8.

[261] Talvani A, Santana G, Barcelos LS, Ishii S, Shimizu T, Romanha AJ, et al. Experimental *Trypanosoma cruzi* infection in platelet-activating factor receptor-deficient mice. Microbes Infect 2003;5:789–96.

[262] Rijneveld AW, Weijer S, Florquin S, Speelman P, Shimizu T, Ishii S, et al. Improved host defense against pneumococcal pneumonia in platelet-activating factor receptor-deficient mice. J Infect Dis 2004;189:711–6.

[263] Pacifici R. Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis. J Bone Miner Res 1996;11:1043–51.

[264] Kameda T, Mano H, Yuasa T, Mori Y, Miyazawa K, Shiokawa M, et al. Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. J Exp Med 1997;186:489–95.

[265] Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, et al. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-α. J Clin Invest 2000;106:1229–37.

[266] Pacifici R, Brown C, Puscheck E, Friedrich E, Slatopolsky E, Maggio D, et al. Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc Natl Acad Sci USA 1991;88:5134–8.

[267] Ammann P, Rizzoli R, Bonjour JP, Bourrin S, Meyer JM, Vassalli P, et al. Transgenic mice expressing soluble tumor necrosis factor-receptor are protected against bone loss caused by estrogen deficiency. J Clin Invest 1997;99:1699–703.

[268] Kitazawa R, Kimble RB, Vannice JL, Kung VT, Pacifici R. Interleukin-1 receptor antagonist and tumor necrosis factor binding protein decrease osteoclast formation and bone resorption in ovariectomized mice. J Clin Invest 1994;94:2397–406.

[269] Fuller K, Owens JM, Jagger CJ, Wilson A, Moss R, Chambers TJ. Macrophage colony-stimulating factor stimulates survival and chemotactic behavior in isolated osteoclasts. J Exp Med 1993;178:1733–44.

[270] Suda K, Woo JT, Takami M, Sexton PM, Nagai K. Lipopolysaccharide supports survival and fusion of preosteoclasts independent of TNF-α, IL-1, and RANKL. J Cell Physiol 2002;190:101–8.

[271] Miyazaki T, Katagiri H, Kanegae Y, Takayanagi H, Sawada Y, Yamamoto A, et al. Reciprocal role of ERK and NF-κB pathways in survival and activation of osteoclasts. J Cell Biol 2000;148:333–42.

[272] Lee SE, Chung WJ, Kwak HB, Chung CH, Kwack KB, Lee ZH, et al. Tumor necrosis factor-α supports the survival of osteoclasts through the activation of Akt and ERK. J Biol Chem 2001;276:49343–9.

[273] Wong BR, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, et al. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. Mol Cell 1999;4:1041–9.

[274] Wood DA, Hapak LK, Sims SM, Dixon SJ. Direct effects of platelet-activating factor on isolated rat osteoclasts. Rapid elevation of intracellular free calcium and transient retraction of pseudopods. J Biol Chem 1991;266:15369–76.

[275] Zheng ZG, Wood DA, Sims SM, Dixon SJ. Platelet-activating factor stimulates resorption by rabbit osteoclasts in vitro. Am J Physiol 1993;264:E74–81.

[276] Sato M, Zeng GQ, Turner CH. Biosynthetic human parathyroid hormone (1–34) effects on bone quality in aged ovariectomized rats. Endocrinology 1997;138:4330–7.

[277] Bonnick SL, Shulman L. Monitoring osteoporosis therapy: bone mineral density, bone turnover markers, or both? Am J Med 2006;119:S25–31.

[278] Kimble RB, Matayoshi AB, Vannice JL, Kung VT, Williams C, Pacifici R. Simultaneous block of interleukin-1 and tumor necrosis factor is required to completely prevent bone loss in the early postovariectomy period. Endocrinology 1995;136:3054–61.

[279] Body JJ. Calcitonin for the long-term prevention and treatment of postmenopausal osteoporosis. Bone 2002;30:75S–9S.

[280] Noguchi K, Morita I, Murota S. The detection of platelet-activating factor in inflamed human gingival tissue. Arch Oral Biol 1989;34:37–41.

[281] Pettipher ER, Higgs GA, Henderson B. PAF-acether in chronic arthritis. Agents Actions 1987;21:98–103.

[282] Palacios I, Miguelez R, Sanchez-Pernaute O, Gutierrez S, Egido J, Herrero-Beaumont G. A platelet activating factor receptor antagonist prevents the development of chronic arthritis in mice. J Rheumatol 1999;26:1080–6.

[283] Sun D, Krishnan A, Zaman K, Lawrence R, Bhattacharya A, Fernandes G. Dietary $n-3$ fatty acids decrease osteoclastogenesis and loss of bone mass in ovariectomized mice. J Bone Miner Res 2003;18:1206–16.

[284] Calder PC, Zurier RB. Polyunsaturated fatty acids and rheumatoid arthritis. Curr Opin Clin Nutr Metab Care 2001;4:115–21.

[285] Smith WL. Cyclooxygenases, peroxide tone and the allure of fish oil. Curr Opin Cell Biol 2005;17:174–82.

[286] Yamamoto S. "Enzymatic" lipid peroxidation: reactions of mammalian lipoxygenases. Free Radic Biol Med 1991;10:149–59.

[287] Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 2007;25:101–37.

[288] Kawaguchi H, Raisz LG, Voznesensky OS, Alander CB, Hakeda Y, Pilbeam CC. Regulation of the two prostaglandin G/H synthases by parathyroid hormone, interleukin-1, cortisol, and prostaglandin E_2 in cultured neonatal mouse calvariae. Endocrinology 1994;135:1157–64.

[289] Chen QR, Miyaura C, Higashi S, Murakami M, Kudo I, Saito S, et al. Activation of cytosolic phospholipase A_2 by platelet-derived growth factor is essential for cyclooxygenase-2-dependent prostaglandin E_2 synthesis in mouse osteoblasts cultured with interleukin-1. J Biol Chem 1997;272:5952–8.

[290] Wadleigh DJ, Herschman HR. Transcriptional regulation of the cyclooxygenase-2 gene by diverse ligands in murine osteoblasts. Biochem Biophys Res Commun 1999;264:865–70.

[291] Koide M, Murase Y, Yamato K, Noguchi T, Okahashi N, Nishihara T. Bone morphogenetic protein-2 enhances osteoclast formation mediated by interleukin-1α through upregulation of osteoclast differentiation factor and cyclooxygenase-2. Biochem Biophys Res Commun 1999;259:97–102.

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Experimental Lung Research
Publication details, including instructions for authors and subscription information:
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C-REACTIVE PROTEIN MODULATES HUMAN LUNG FIBROBLAST MIGRATION

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Online Publication Date: 01 February 2009

To cite this Article Kikuchi, Kazuhiko, Kohyama, Tadashi, Yamauchi, Yasuhiro, Kato, Jun, Takami, Kazutaka, Okazaki, Hitoshi, Desaki, Masashi, Nagase, Takahide, Rennard, Stephen I. and Takizawa, Hajime(2009)'C-REACTIVE PROTEIN MODULATES HUMAN LUNG FIBROBLAST MIGRATION',Experimental Lung Research,35:1,48 — 58

To link to this Article: DOI: 10.1080/01902140802404138

URL: http://dx.doi.org/10.1080/01902140802404138

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Experimental Lung Research, 35:48–58, 2009
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ISSN: 0190-2148 print / 1521-0499 online
DOI: 10.1080/01902140802404138

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C-REACTIVE PROTEIN MODULATES HUMAN LUNG FIBROBLAST MIGRATION

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☐ *C-reactive protein (CRP) has been classically used as a marker of inflammation. The aim of this study was to investigate the effect of CRP on migration of human fetal lung fibroblasts (HFL-1) to human plasma fibronectin (HFn). Using the blindwell chamber technique, CRP inhibited HFL-1 migration in a dose-dependent fashion (at 1 μg/mL, inhibition: 32.5% ± 7.1%; P < .05). Western blot analysis showed that CRP inhibited the p38 mitogen-activated protein kinase (MAPK) activity in the presence of HFn. Moreover, the MAPK inhibitors SB202190 (25 μM) and SB203580 (25 μM) inhibited HFn-induced cell migration, suggesting an important role of p38 MAPK in HFn-induced migration. Taken together, these results suggest that the inhibitory effect of CRP is mediated by blocking MAPK. In summary, this study demonstrates that CRP directly modulates human lung fibroblasts migration. Thus, CRP may contribute to regulation of wound healing and may be endogenous antifibrotic factor acting on lung fibrosis.*

Keywords lung fibroblast, C-reactive protein MAPK, migration

Idiopathic pulmonary fibrosis is characterized by excess accumulation of fibroblasts and extracellular matrix in the lung parenchyma. Although it

Received 3 April 2008; accepted 11 August 2008.
The authors thank Miss Makiko Kase for her excellent technical assistance.
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has been believed that alveolitis, alveolar wall inflammation, is the central pathogenic mechanism for the development of pulmonary fibrosis, conventional anti-inflammatory therapy does not improve this disorder. Thus, it is necessary to find new therapeutic strategies for this intractable disease.

C-reactive protein (CRP) is one of the acute-phase proteins that is mostly derived from the liver. It has a molecular weight of 105 kDa, and is induced by inflammatory stimuli, usually in association with increased neutrophils in the peripheral blood. It has been established that CRP is a useful serum marker for evaluation of disease activity and therapeutic response in a variety of conditions, including bacterial infection [1], myocardial infarction [2], and acute respiratory distress syndrome [3, 4].

Recent studies have shown that CRP is not only an acute-phase reactant but also is a modulator of inflammation [4]. CRP inhibits neutrophil chemotaxis through p38 mitogen-activated protein kinase (MAPK) inhibition [5, 6], attenuates nitric oxide production by endothelial cells [7], and up-regulates angiotensin type 1 receptors in vascular smooth muscle [8]

It has recently been demonstrated that CRP is localized in bronchial airways [9]. Moreover, Arase and associates showed that serum levels of CRP are increased prior to the onset of idiopathic pulmonary fibrosis [10]. These findings raise the possibility that CRP may play some roles in the pathogenesis of idiopathic pulmonary fibrosis, although the actual role of CRP in the lung remains unknown.

The current study was designed to explore the hypothesis that CRP could modulate lung fibroblast migration and could contribute to the fibrotic process in the lung.

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MATERIALS AND METHODS

Materials

One of the recombinant CRPs expressed in *Esherichia coli*, SB202190, and SB203580 were purchased from Calbiochem (La Jolla, CA). This recombinant CRP was provided by liquid form (1 mg/mL), which contained 140 mM NaCl, 20 mM Tris-HCl, 2 mM $CaCl_2$, and 0.05% NaN_3. Then we used 0.005% NaN_3as solvent control, which was the same concentration of 100 μg/mL CRP solution (see Figures 2, 5). Another recombinant CRP, which is expressed in the mouse myeloma cell line NS0 and is free from sodium azide and endotoxin, was purchased from R&D Systems (Minneapolis, MN). The azide- and endotoxin-free (<1.0 EU per 1 μg) CRP was dissolved into 0.1% bovine serum albumin–containing phosphate buffered saline (PBS) as a 10 μg/mL of stock solution. As this CRP solution contained 20 mM Tris, 0.2 M NaCl, 5 mM $CaCl_2$, we adjusted the concentration of solvent control to 100 μg/mL CRP (Figure 2) or 10 μg/mL CRP (Figure 3). In this

article, CRP that was free from endotoxin and azide is referred as azide- or endotoxin-free CRP and CRP not free from azide and endotoxin is referred as conventional CRP. SB202190 (25 mM) and SB203580 (25 mM) were dissolved in DMSO and diluted in medium before use. The human plasma fibronectin and lipopolysaccharide (LPS) were purchased from Sigma (St. Louis, MO). LPS was dissolved into saline as stock solution. Tissue culture supplements and media were purchased from Gibco (Life Technologies, Grand Island, NY). Fetal calf serum (FCS) was purchased from Biofluid (Rockville, MD).

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Cell Cultures

Human fetal lung fibroblasts (HFL-1) were purchased from the American Type Culture Collection (Rockville, MD). The cells were cultured in 100-mm tissue culture dishes (IWAKI; Japan) in Ham's F12 medium (F12; Sigma), and supplemented with 10% fetal calf serum (FCS; Biofluid), 50 U/mL penicillin, and 50 μg/mL streptomycin in a 37°C, 5% CO_2 incubator. Subconfluent fibroblasts were trypsinized (0.05% trypsin EDTA solution; Gibco) in order to passage cells. All experiments were conducted between the 9th and 20th passages.

Chemotaxis Assay

Cell migration of HFL-1 was assessed by the modified Boyden blindwell chamber technique [11] using a 48-well chamber (Nucleopore, Cabin John, MD) as previously reported [12]. In general, 50 μL of fetal lung fibroblasts in serum-free F12 (1.0×10^6/mL) were placed in the top wells of the chamber with the desired concentrations of CRP or other reagents. For investigation of priming, HFL-1 cells were preincubated on the culture dishes with the desired concentrations of reagents for 30 minutes in F12 without serum, following which they were trypsinized and used for the migration assay. The chemoattractant (20 μg/mL of HFn) was placed in the bottom chamber. The 2 wells were separated by an 8-μm-pore filter (Nucleopore, Pleasanton, CA) coated with 0.1% gelatin (Bio-Rad, Hercules, CA). The chamber was incubated at 37°C in a moist, 5% CO_2 atmosphere. Except as indicated, chambers were incubated for 6 hours, after which the cells above the filter were removed by scraping. The filter was then fixed, stained with Diff Quik stain (International reagents, Kobe, Japan), and mounted on a glass microscope slide. Migration was assessed by counting the number of cells in 5 randomly selected high-power fields using a light microscope. Triplicate wells were prepared in each experiment for every condition. At least 3 separate experiments were performed with different cell cultures of HFL-1.

Western Blot Analysis

The p38 MAPK activity was determined by using Western blot analysis. After growth to subconfluence in 6-well plates (Becton Dickinson, Franklin Lakes, NJ, USA), HFL-1 cells were washed with PBS 2 times. The cells were then treated with or without various concentrations of CRP with 20 μg/mL of HFn. Because the CRP solutions contained sodium azide, in order to exclude an effect of the solvent, the same concentrations of sodium azide alone were also added in separate cultures without CRP. After 10 minutes' incubation, the cells were solubilized with 250 μL of 2× sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (125 mM Tris-HCl [pH 6.8], 4.6% SDS, 20% glycerol, 10% 2 ME [2-mercaptoethanol], BPB [bromo phenol blue]). The samples were heated in a boiled water bath for 5 minutes to denature the proteins and centrifuged at 12000 revolutions per minute for 10 minutes to remove the debris. The sample protein was separated in a 10% polyacrylamide gel and transferred onto Transfer Membranes (Millipore, Bedford, MA). Each membrane was stained with Ponceau S (Sigma) for protein to verify equal loading and transfer. Membranes were blocked with 3% bovine serum albumin (Sigma) in Tris-buffered saline with 0.1% Tween 20 (Sigma) for 1 hour. Membrane were then reacted with specific antibodies (Cell Signaling Technology, Danvers, MA) against p38 MAPK or phosphorylated p38 MAPK, followed by incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit second antibody (Cell Signaling Technology). The results were visualized by chemiluminescence using enhanced chemiluminescence (ECL) (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions. ECL is a light-emitting nonradioactive method for detection of immobilized specific antigens with HRP-labeled antibodies, as reported previously [13].

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Statistical Analysis

Results were confirmed by repeating experiments on at least 3 separate occasions, each performed in triplicate. Data shown in figures were pooled data for all experiments expressed as mean ± SEM. Samples with multiple comparisons were analyzed for significance using analysis of variance (ANOVA). Where ANOVA indicated significant differences between groups, the Fisher's exact probability test was applied and $P < .05$ was considered as significant.

RESULTS

Because one of the recombinant CRP we used was not free from endotoxin, we checked whether lung fibroblast migration was influenced by LPS, which is known as endotoxin. LPS had no effect on lung fibroblast

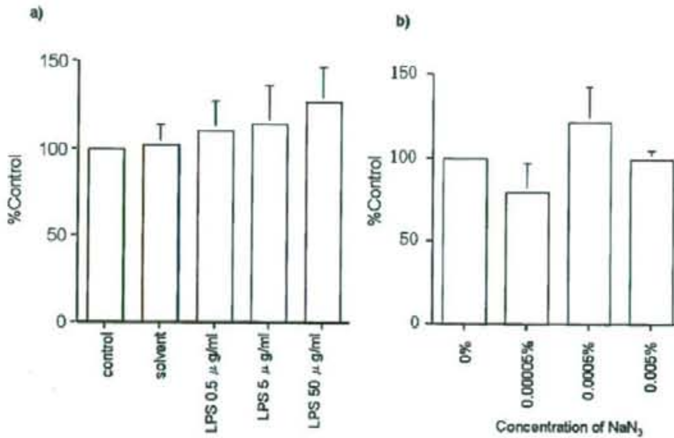

FIGURE 1 The effect of LPS or sodium azide on HFL-1 migration. CRP effect, which was known as endotoxin or sodium azide on HFL-1 migration, were assayed with the Boyden blindwell chamber assay system. HFn (20 μg/mL) was used as the chemoattractant. LPS (*a*) or sodium azide (*b*) at various concentrations were added to the fibroblasts in the top wells of the chemotaxis chamber. Vertical axis: fibroblast migration expressed as percentage of that of control. Horizontal axis: (*a*) LPS (μg/ml); solvent: saline. (*b*) Sodium azide: 0.00005%, 0.0005%, and 0.005% indicate concentration of NaN_3 of 1, 10, and 100 μg/mL CRP solution, respectively. Data are presented as the mean + SEM from 3 separate experiments, each studied in triplicate cultures.

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migration (Figure 1*a*). As there is also the possibility that the effects of commercial CRP are caused by biologically active contaminants such as sodium azide [14, 15], we studied the sodium azide effect on lung fibroblast migration. We put the same concentration of sodium azide without CRP (1 μg/mL of CRP contained 0.00005% NaN_3). There was no significant difference between control and other concentrations of NaN_3 we checked (Figure 1*b*).

Fibronectin led HFL-1 in a concentration-dependent manner as previously reported [16]. In the presence of 20 μg/mL HFn, the number of migrated HFL-1 reached to 175 $\pm$ 28.8 ($n = 5$). We used fibronectin as chemoattractant for HFL-1. Both CRPs (1 to 100 μg/mL) added to the upper wells together with the fibroblasts inhibited the migration. CRP consistently inhibited HFL-1 migration at concentrations ranging between 0 and 100 μg/mL (azide- and endotoxin-free CRP: 32.5% $\pm$ 7.1% inhibition at 1 μg/mL, $P < .05$; conventional CRP: 21.3% $\pm$ 2.5% inhibition at 1 μg/mL, $P < .05$) (Figure 2). Time course studies demonstrated that the number of fibroblasts that accumulated on the bottom side of the membrane was significantly inhibited by 10 μg/mL of azide- and endotoxin-free CRP when incubation periods were over 6 hours (Figure 3).

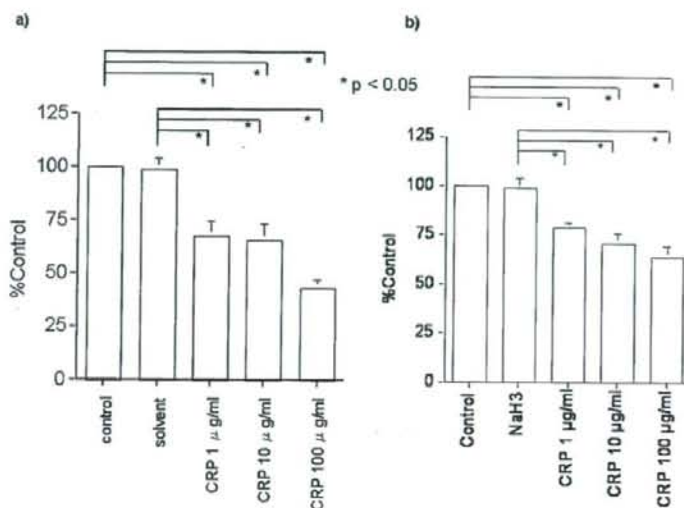

FIGURE 2 Concentration-dependent inhibition of fibroblast migration by azide- and endotoxin-free CRP (*a*) and conventional CRP (*b*). Migration of HFL-1 was assayed with the Boyden blindwell chamber assay system. HFn (20 μg/mL) was used as the chemoattractant. The azide- or endotoxin-free CRP at various concentrations was added to the fibroblasts in the top wells of the chemotaxis chamber. Vertical axis: fibroblast migration expressed as percentage of that of control. Horizontal axis: CRP concentration (μg/mL). NaN_3: 0.005% sodium azide solvent for conventional CRP. Data are presented as the mean + SEM from 3 separate experiments, each studied in triplicate cultures. $^*P < .05$

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Because there is a report that shows that neutrophil migration driven by fMLP is mediated by the p38 MAPK pathway, we next tried to determine if the fibroblast migration driven by HFn is also mediated by the p38 MAPK pathway and if the CRP effect is related to this mechanism. To accomplish this, we examined the effect of the specific p38 MAPK inhibitors SB203580 or SB202190 on HFn-mediated migration. Both inhibitors inhibited fibroblast migration to HFn to a degree similar to that observed with CRP (Figure 4*a*, *b*). In addition, we examined the activation of p38 MAPK by Western blot analysis. Sodium azide, at the concentrations used, had no effect on p38 MAPK activity. In the current studies, 20 μg/mL of HFn for 10 minutes' incubation was used according to preliminary evaluation. As shown in Figure 5, CRP inhibited the phosphorylated p38 MAPK in the presence of HFn (Figure 5).

DISCUSSION

In this report, we showed that CRP was capable of inhibiting lung fibroblast migration to human fibronectin in a concentration-dependent fashion

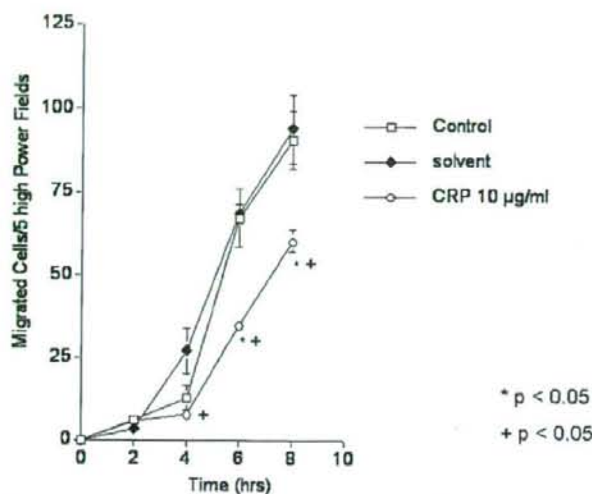

FIGURE 3 Time course effect of azide- and endotoxin-free CRP on HFn-induced fibroblast migration. Migration of HFL-1 was assayed with the Boyden blindwell chamber assay system. HFn (20 μg/mL) was used as chemoattractant. The azide- and endotoxin-free CRP was added to the fibroblasts in the top wells at 10 μg/mL. Solvent: solvent for azide- and endotoxin-free CRP. Vertical axis: fibroblast migration expressed as number of cells migrated per 5 high-power fields; horizontal axis: incubation time in hours. The data are from a single experiment representative of triplicate assays. Data are presented as the mean ± SEM from triplicate wells. *, compared with control; +, compared with solvent.

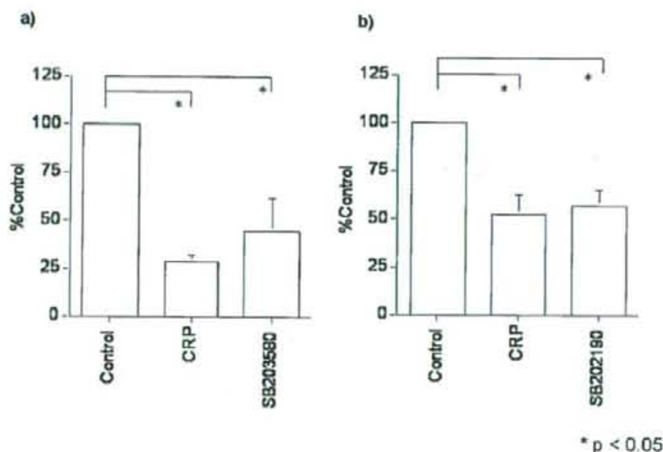

FIGURE 4 Effect of the MAPK inhibitors and CRP on inhibition of fibroblast migration. MAPK inhibitors or CRP (10 μg/mL) were added to the upper wells of the chemotaxis chambers together with HFL-1 cells. (*a*) SB203580, (*b*) SB202190; HFn (20 μg/ml) was used as the chemoattractant. Vertical axis: fibroblast migration expressed as percentage of control. Data are presented as the mean + SEM from triplicate cultures each assayed for migration in triplicate. *$P < .05$

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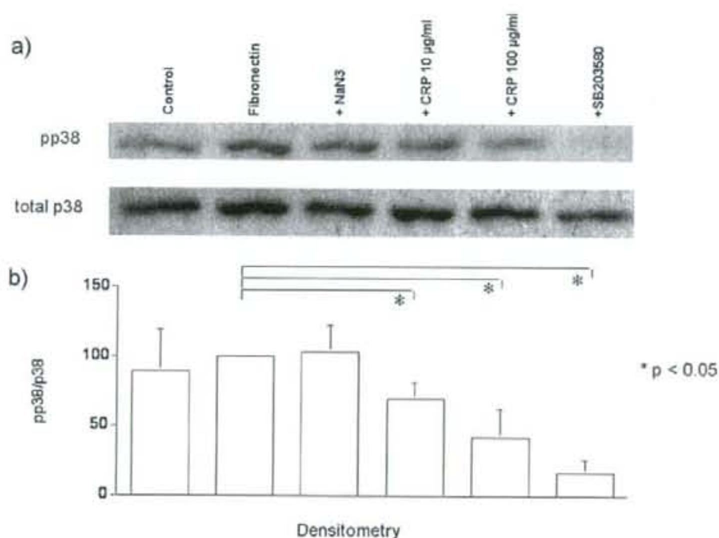

FIGURE 5 Western blot analysis of phosphorylated p38 MAPK. The protein samples were prepared after the 10-minute incubation at described conditions. (*a*) The representative result from 3 separate experiments. (*b*) The densities of phosphorylated p38 were divided by the density of total p38. Data are presented as the mean ± SEM from separate triplicate experiments. $^{*}P < .05$

(Figure 2). The concentration used in this study was similar to in vivo concentrations, suggesting that this pathway has relevance in the clinical events. The inhibitory effect of the CRP increased with time (Figure 3). Furthermore, our data suggested that this biological effect of CRP was mediated through inhibition of p38 MAPK (Figure 5).

In the process of wound repair, fibroblast migration, proliferation, and contraction are essential events. If these fibroblast functions do not work exactly, inaccurate repair remodeling would be proceeded. Remodeling of the lung structure is a major part of the pathogenesis of several lung diseases, including pulmonary fibrosis [17], chronic obstructive lung disease [18, 19], and bronchial asthma [20, 21]. It is believed that the fibrosis that often characterizes lung remodeling results from excess migration, proliferation and activation of fibroblasts. Thus modulation of fibroblast migration may be an option for controlling the excess development of fibrosis.

C-reactive protein is an acute phase protein and is clinically utilized as a nonspecific marker of inflammation. Over the past few years, several reports have demonstrated that the level of CRP in the serum reflects the severity of several diseases, including atherosclerosis [22] and cardiac vascular disease [23]. Moreover, CRP induces release of a number of mediator proteins from

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target cells [24], attenuates nitric oxide (NO) production [7], and inhibits migration of neutrophils [5, 6]. These reports suggest that CRP is not only an inflammatory marker but is potentially also an important mediator of disease. However, it remains largely unknown if CRP modulates any biological processes in the lung.

p38 MAPK activation is involved in HFn-induced migration. Fibronectin is believed to plays an important role in the regulation of fibroblast recruitment and accumulation [25, 26]. However, it remains unclear whether or not p38 MAPK activation is involved in HFn-induced migration. In the present study, we showed that (1) pharmacological inhibition of p38 MAPK significantly blocked cell migration, and that (2) CRP inhibited p38 MAPK activation as well as cell migration. These findings strongly suggest that p38 MAPK plays a pivotal role in HFn-induced fibroblast migration, and a possible role in CRP-reduced cell migration. CRP-modulated fibroblast migration may occur, at least in part, via inhibition of the p38 MAPK pathway. We have no direct evidences to support this hypothesis, because the effects of both CRP and p38 MAPK inhibitors showed an inhibitory effect on cell migration. The report that CRP inhibits chemotactic peptide–induced p38 MAPK activity in neutrophils [6] might support our hypothesis.

p38 MAPK inhibition might be a candidate of novel therapeutic strategy in fibrotic processes of the lung. Rousseau and associates [27] reported that fibroblasts derived from p38 MAPK–lacking mice showed a markedly reduced migratory response to chemoattractants, showing an inevitable role of p38 MAPK in cell migration. Inhibition of p38 MAPK reduces airway inflammation of patient in cystic fibrosis [28]. Therefore, it might be a good treatment for fibrotic lung disease to inhibit p38 MAPK activation.

C-reactive protein effect was not through the protein kinase A pathway. Our previous studies indicate that the mediator that decreases the lung fibroblast migration are related to the protein kinase A pathway [12, 29, 30]. We have also tried to confirm whether CRP effect was by way of the protein kinase A pathway. The protein kinase A inhibitor KT5720 did not block the CRP effect.

It is CRP itself that decreases the movement of lung fibroblast. Several in vitro studies have suggested that CRP induces the inflammatory response directly. However, there is the possibility that the effects are caused by biologically active contaminants of commercially available CRP such as endotoxin and sodium azide [14, 15, 31]. First of all, we examined the effect of LPS, which was known as endotoxin on lung fibroblast migration. LPS had no effects on the HFL-1 migration (Figure 1*a*). Second, we examined the effect of sodium azide. Even at the highest concentration of sodium azide examined, there was no effect on fibroblast migration (Figure 1*b*). Furthermore, we compared the effects of azide- and endotoxin-free CRP and conventional CRP, which is not free from azide and endotoxin. We could demonstrate similar results (Figure 2). These results indicated that the decreased the

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fibroblast migration was not due to endotoxin or sodium azide but CRP itself.

In summary, the inhibitory effect of CRP on cell migration and p38 MAPK activation in human lung fibroblasts shown here suggest that CRP might be an endogenous modulator of lung fibrosis. Further studies are necessary for better understanding the role of CRP in lung fibrosis and wound healing.

Declaration of interest: This work was supported in part by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan, a grant to the Respiratory Failure Research Group from the Ministry of Health, Labour and Welfare, Japan, and grants-in-aid for Comprehensive Research on Aging and Health from the Ministry of Health, Labour and Welfare, Japan. The authors thank Makiko Kase for her excellent technical assistance.

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REFERENCES

[1] Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340;448–454.

[2] Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, Pepys MB: C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. J Exp Med. 1999;190;1733–1740.

[3] Kew RR, Hyers TM, Webster RO: Human C-reactive protein inhibits neutrophil chemotaxis in vitro: possible implications for the adult respiratory distress syndrome. J Lab Clin Med. 1990;115;339–345.

[4] Heuertz RM, Webster RO: Role of C-reactive protein in acute lung injury. Mol Med Today. 1997;3;539–545.

[5] Zhong W, Zen Q, Tebo J, Schlottmann K, Coggeshall M, and Mortensen RF: Effect of human C-reactive protein on chemokine and chemotactic factor-induced neutrophil chemotaxis and signaling. J Immunol. 1998;161;2533–2540.

[6] Heuertz RM, Tricomi SM, Ezekiel UR, Webster RO: C-reactive protein inhibits chemotactic peptide-induced p38 mitogen-activated protein kinase activity and human neutrophil movement. J Biol Chem. 1999;274;17968–17974.

[7] Verma S, Wang CH, Li SH, Dumont AS, Fedak PW, Badiwala MV, Dhillon B, Weisel RD, Li RK, Mickle DA, Stewart DJ: A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation. 2002;106;913–919.

[8] Wang CH, Li SH, Weisel RD, Fedak PW, Dumont AS, Szmitko P, Li RK, Mickle DA, Verma S: C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. Circulation 2003;107;1783–1790.

[9] Gould JM, Weiser JN: Expression of C-reactive protein in the human respiratory tract. Infect Immun. 2001;69;1747–1754.

[10] Arase Y, Ikeda K, Tsubota A, Saitoh S, Suzuki Y, Kobayashi M, Suzuki F, Someya T, Akuta N, Hosaka T, Kobayashi M, Kumada H: Usefulness of serum KL-6 for early diagnosis of idiopathic pulmonary fibrosis in patients with hepatitis C virus. Hepatol Res. 2003;27;89–94.

[11] Boyden S: The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J Exp Med. 1962;115;453–466.

[12] Kohyama T, Liu XD, Wen FQ, Kim HJ, Takizawa H, Rennard SI: Prostaglandin D2 inhibits fibroblast migration. Eur Respir J. 2002;19;684–689.

[13] Yamauchi Y, Okazaki H, Desaki M, Kohyama T, Kawasaki S, Yamamoto K, Takizawa H: Methotrexate induces interleukin-8 production by human bronchial and alveolar epithelial cells. Clin Sci (Lond). 2004;106;619–625.

[14] Liu C, Wang S, Deb A, Nath KA, Katusic ZS, McConnell JP, Caplice NM: Proapoptotic, antimigratory, antiproliferative, and antiangiogenic effects of commercial C-reactive protein on various human endothelial cell types in vitro: implications of contaminating presence of sodium azide in commercial preparation. Circ Res. 2005;97;135–143.

[15] VanUffelen BE, Van der Zee J, de Koster BM, VanSteveninck J, and Elferink JG: Sodium azide enhances neutrophil migration and exocytosis: involvement of nitric oxide, cyclic GMP and calcium. Life Sci. 1998;63;645–657.

[16] Kohyama T, Liu X, Wen FQ, Kim HJ, Takizawa H, Rennard SI: Potentiation of human lung fibroblast chemotaxis by the thromboxane A(2) analog U-46619. J Lab Clin Med. 2002;139;43–49.

[17] Parra ER, David YR, da Costa LR, Ab'Saber A, Sousa R, Kairalla RA, de Carvalho CR, Filho MT, Capelozzi VL: Heterogeneous remodeling of lung vessels in idiopathic pulmonary fibrosis. Lung 2005;183;291–300.

[18] Chung KF: The role of airway smooth muscle in the pathogenesis of airway wall remodeling in chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2005;2;347–354.

[19] Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Pare PD: The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004;350;2645–2653.

[20] Lavigne MC, Thakker P, Gunn J, Wong A, Miyashiro JS, Wasserman AM, Wei SQ, Pelker JW, Kobayashi M, Eppihimer MJ: Human bronchial epithelial cells express and secrete MMP-12. Biochem Biophys Res Commun. 2004;324;534–546.

[21] Karagiannidis C, Hense G, Martin C, Epstein M, Ruckert B, Mantel PY, Menz G, Uhlig S, Blaser K, Schmidt-Weber CB: Activin A is an acute allergen-responsive cytokine and provides a link to TGF-beta-mediated airway remodeling in asthma. J Allergy Clin Immunol. 2006;117;111–118.

[22] Sun H, Koike T, Ichikawa T, Hatakeyama K, Shiomi M, Zhang B, Kitajima S, Morimoto M, Watanabe T, Asada Y, Chen YE, Fan J: C-reactive protein in atherosclerotic lesions: its origin and pathophysiological significance. Am J Pathol. 2005;167;1139–1148.

[23] Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, Lowe GD, Pepys MB, Gudnason V: C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med. 2004;350;1387–1397.

[24] Cermak J, Key NS, Bach RR, Balla J, Jacob HS, Vercellotti GM: C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. Blood 1993;82;513–520.

[25] Kawamoto M, Matsunami T, Ertl RF, Fukuda Y, Ogawa M, Spurzem JR, Yamanaka N, Rennard SI: Selective migration of α-smooth muscle actin-positive myofibroblasts toward fibronectin in the Boyden's blindwell chamber. Clin Sci. 1997;93;355–362.

[26] Mattoli S, Colotta F, Fincato G, Mezzetti M, Mantovani A, Patalano F, Fasoli A: Time course of IL1 and IL6 synthesis and release in human bronchial epithelial cell cultures exposed to toluene diisocyanate. J Cell Physiol. 1991;149;260–268.

[27] Rousseau S, Dolado I, Beardmore V, Shpiro N, Marquez R, Nebreda AR, Arthur JS, Case LM, Tessier-Lavigne M, Gaestel M, Cuenda A, Cohen P: CXCL12 and C5a trigger cell migration via a PAK1/2-p38alpha MAPK-MAPKAP-K2-HSP27 pathway. Cell Signal. 2006;18:1897–1905.

[28] Raia V, Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Auricchio S, Cimmino M, Cavaliere M, Nardone M, Cesaro A, Malcolm J, Quaratino S, Londei M: Inhibition of p38 mitogen activated protein kinase controls airway inflammation in cystic fibrosis. Thorax. 2005;60;773–780.

[29] Kohyama T, Ertl RF, Valenti V, Spurzem J, Kawamoto M, Nakamura Y, Veys T, Allegra L, Romberger D, Rennard SI: Prostaglandin E(2) inhibits fibroblast chemotaxis. Am J Physiol Lung Cell Mol Physiol. 2001;281;L1257–L1263.

[30] Kohyama T, Liu X, Kim HJ, Kobayashi T, Ertl RF, Wen FQ, Takizawa H, Rennard SI: Prostacyclin analogs inhibit fibroblast migration. Am J Physiol Lung Cell Mol Physiol. 2002;283;L428–L432.

[31] Taylor KE, Giddings JC, van den Berg CW: C-reactive protein-induced in vitro endothelial cell activation is an artefact caused by azide and lipopolysaccharide. Arterioscler Thromb Vasc Biol. 2005;25;1225–1230.

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British Journal of Pharmacology (2009), ••, ••–••
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RESEARCH PAPER

Eicosapentaenoic acid inhibits voltage-gated sodium channels and invasiveness in prostate cancer cells

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Background and purpose: The voltage-gated Na^+ channels (Na_v) and their corresponding current (I_{Na}) are involved in several cellular processes, crucial to metastasis of cancer cells. We investigated the effects of eicosapentaenoic (EPA), an omega-3 polyunsaturated fatty acid, on I_{Na} and metastatic functions (cell proliferation, endocytosis and invasion) in human and rat prostate cancer cell lines (PC-3 and Mat-LyLu cells).

Experimental approach: The whole-cell voltage clamp technique and conventional/quantitative real-time reverse transcriptase polymerase chain reaction analysis were used. The presence of Na_v proteins was shown by immunohistochemical methods. Alterations in the fatty acid composition of phospholipids after treatment with EPA and metastatic functions were also examined.

Key results: A transient inward Na^+ current (I_{Na}), highly sensitive to tetrodotoxin, and Na_V proteins were found in these cells. Expression of $Na_V1.6$ and $Na_V1.7$ transcripts (SCN8A and SCN9A) was predominant in PC-3 cells, while $Na_V1.7$ transcript (SCN9A) was the major component in Mat-LyLu cells. Tetrodotoxin or synthetic small interfering RNA targeted for SCN8A and SCN9A inhibited metastatic functions (endocytosis and invasion), but failed to inhibit proliferation in PC-3 cells. Exposure to EPA produced a rapid and concentration-dependent suppression of I_{Na}. In cells chronically treated (up to 72h) with EPA, the EPA content of cell lipids increased time-dependently, while arachidonic acid content decreased. Treatment of PC-3 cells with EPA decreased levels of mRNA for SCN9A and SCN8A, cell proliferation, invasion and endocytosis.

Conclusion and implications: Treatment with EPA inhibited I_{Na} directly and also indirectly, by down-regulation of Na_v mRNA expression in prostate cancer cells, thus inhibiting their metastatic potential.

British Journal of Pharmacology (2009) doi:10.1111/j.1476-5381.2008.00059.x

Keywords: voltage-gated sodium channels; prostate carcinoma cell; eicosapentaenoic acid; SCN9A; SCN8A; RT-PCR; invasion; endocytosis; proliferation

Abbreviations: EPA, eicosapentaenoic acid; FBS, fetal bovine serum; I_{Na}, Na^+ current; Na_v, voltage-gated Na^+ channel; NMDG, N-methyl-D-glucamine; PUFA, polyunsaturated fatty acid; RT-PCR, reverse transcriptase polymerase chain reaction; siRNA, synthetic small interfering RNA; TTX, tetrodotoxin

Introduction

The therapy of prostate cancer including the use of hormone-based drugs has progressed over recent years, but prostate cancer is still one of the leading causes of cancer deaths in the world. Therefore, new therapeutic strategies to prevent prostate cancer, to inhibit its progression and metastasis are needed. Epidemiological studies show clear geographical variations in the incidence of this disease (Parkin *et al.*, 2005), with it being more common in the developed countries such as North America and Europe, compared with developing countries. And, within the developed countries, considerable differences of the mortality rates exist; North America and Europe have high mortality rates as compared with Japan and other Asian countries (Breslow *et al.*, 1977). However, the frequency of latent prostate carcinoma diagnosed at autopsy is as common in Asian countries as in Western countries (Dunn, 1975). In addition, the consumption of a diet rich in fat tends to increase the risk of developing prostate carcinoma (Shennan and Bishop, 1974) and immigrants from Poland and Japan to the Western countries show a significant increase in the risk of developing prostate cancer (Haenszel and Kurihara, 1968; Armstrong and Doll, 1975). These findings implicate environmental factors, probably diet, as significant

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Received 21 July 2008; revised 22 September 2008; accepted 30 September 2008