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Prostaglandin $F_{2\alpha}$ receptor (FP) signaling regulates Bmp signaling and promotes chondrocyte differentiation

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ABSTRACT

Prostaglandins are a group of lipid signaling molecules involved in various physiological processes. In addition, prostaglandins have been implicated in the development and progression of diseases including cancer, cardiovascular disease, and arthritis. Prostaglandins exert their effects through the activation of specific G protein-coupled receptors (GPCRs). In this report, we examined the role of prostaglandin $F_{2\alpha}$ receptor (FP) signaling as a regulator of chondrocyte differentiation. We found that FP expression was dramatically induced during the differentiation of chondrocytes and was up-regulated in cartilages. Forced expression of FP in ATDC5 chondrogenic cell line resulted in the increased expression of differentiation-related genes and increased synthesis of the extracellular matrix (ECM) regardless of the presence of insulin. Similarly, $PGF_{2\alpha}$ treatment induced the expression of chondrogenic marker genes. In contrast, knockdown of endogenous FP expression suppressed the expression of chondrocyte marker genes and ECM synthesis. Organ culture of cartilage rudiments revealed that $PGF_{2\alpha}$ induces chondrocyte hypertrophy. Additionally, FP overexpression increased the levels of Bmp-6, phospho-Smad1/5, and Bmpr1a, while knockdown of FP reduced expression of those genes. These results demonstrate that up-regulation of FP expression plays an important role in chondrocyte differentiation and modulates Bmp signaling.

1. Introduction

Chondrogenic differentiation is a key process in endochondral ossification which occurs during the development, growth, and repair of the skeleton. During endochondral ossification, the condensed mesenchymal cells differentiate into chondrocytes to form the cartilage anlagen. Chondrocytes rapidly proliferate and synthesize cartilage extracellular matrix (ECM) such as collagen type II (Col2A1) and aggrecan (Acan1) [1,2]. The chondrocytes then cease proliferation and enlarge by several folds, becoming hypertrophic chondrocytes. Hypertrophic chondrocytes are characterized by the expression of collagen type X (Col10A1) and matrix metalloproteinase 13 (Mmp13) [2–4]. Hypertrophic chondrocytes mineralize the surrounding matrix and secrete vascular endothelial growth factor (VEGF) for blood vessel formation. Eventually, these cells die by apoptosis and are replaced by osteoblasts that form trabecular bone. The differentiation of chondrocytes through the process of endochondral ossification is regulated by many growth factors and signaling molecules. One of the signaling molecules implicated in this process is prostaglandins.

Prostaglandins are a class of bioactive lipid signaling molecules, consisting of prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and prostacyclin (PGI_2). They are generated from arachidonic acid by the sequential actions of cyclooxygenase (COX) and its respective prostaglandin synthases. Prostaglandins exert their effects through their interactions with specific cell surface receptors, which are G protein-coupled receptors (GPCRs). Prostaglandin receptors are classified on the basis of their response to a particular prostaglandin: PGD_2 to DP, PGE_2 to EP, $PGF_{2\alpha}$ to FP, and PGI_2 to IP. They are expressed in variable levels among different tissues and their expression is regulated by various physiological and pathological stimuli [5], providing an additional level of regulation in prostaglandin signaling. For example, LPS treatment highly induced the expressions of EP2 and EP4 receptors [6,7] in macrophages and these inductions were suggested to be involved in the regulation of TNF-α [8]. In addition, the overexpression of the EP2 receptor in the epidermis of EP2 transgenic mice resulted in enhanced skin tumor formation, proliferation, and blood vessel formation [9].

Prostaglandins regulate cartilage metabolism and are implicated in the pathogenesis of osteoarthritis (OA) and rheumatoid arthritis (RA). In addition to PGE_2, $PGF_{2\alpha}$ is a major prostaglandin in the synovial fluid of joints [10]. However, only a few studies have suggested the role of $PGF_{2\alpha}$ in cartilage metabolism and pathology while many studies investigating the effects of prostaglandins on chondrocyte physiology have focused on PGE_2 signaling. $PGF_{2\alpha}$ stimulated aggrecan synthesis

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in a rat chondrocyte cell line [11] and promoted the expression of Col2A1 mRNA in human articular chondrocytes [12]. Moreover, the levels of PGE_2 and $PGF_{2\alpha}$ are significantly increased in blood and synovial fluid samples from OA or RA patients [10]. The biological effect of $PGF_{2\alpha}$ is mediated by FP. Upon binding to $PGF_{2\alpha}$, FP activates the G_q protein, which in turn increases the levels of inositol triphosphate (IP3)/diacylglycerol (DAG) [13]. FP expression has been recently reported in hypertrophic chondrocytes of growth plate cartilage [14], suggesting that FP signaling may be involved in chondrocyte hypertrophy. However, the expression and the role of FP in cartilage development and diseases have not been clearly determined.

In the present study, we investigated the role of FP signaling in chondrocyte differentiation. We found that FP expression is highly induced during chondrogenesis. We demonstrated that FP signaling stimulates and is required for chondrogenic differentiation. Moreover, our study suggests a possible cross-talk between FP and Bmp signaling pathways.

2. Materials and methods

2.1. Cell culture and treatment

ATDC5 cell line was purchased from Abgent (San Diego, CA) and was maintained in DMEM/Ham's F-12 medium (GIBCO®, Grand Island, NY) with 5% FBS (GIBCO®, Grand Island, NY), 10 μg/ml human transferrin (Sigma Chemical, St Louis, MO), 3×10^{-8} M sodium selenite (Sigma Chemical, St Louis, MO), 50 units/ml penicillin, and 50 mg/ml streptomycin (GIBCO®, Grand Island, NY). For induction of chondrogenic differentiation, the cells were plated in a 2×10^5 cells/60 mm plate. The next day, 10 μg/ml bovine insulin (Sigma Chemical, St Louis, MO,) supplementation was started, as previously described [15]. The ATDC5 cells were treated with 100 nM $PGF_{2\alpha}$ (Cayman chemical, Ann Arbor, MI) for the indicated days and the media with $PGF_{2\alpha}$ was replaced every 2nd day. C3H10T1/2 cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA) and maintained in DMEM medium (GIBCO®, Grand Island, NY) with 10% FBS (GIBCO®, Grand Island, NY), 50 units/ml penicillin and 50 mg/ml streptomycin (GIBCO®, Grand Island, NY) and micromass cultured with recombinant human Bmp2 (Sigma Chemical, St Louis, MO) for the indicated days [16].

2.2. Metatarsal organ culture and treatment

The metatarsal cartilage rudiments were isolated from the hind limb of the E15.5 stage C57BL/6 mouse fetus, and cultured in organ culture medium containing serum free DMEM with 50 μg/ml ascorbic acid, 300 μg/ml L-glutamine, 1 mM β-glycerophosphate, 0.2% BSA Cohn fraction V (Sigma Chemical, St Louis, MO), 50 units/ml penicillin, and

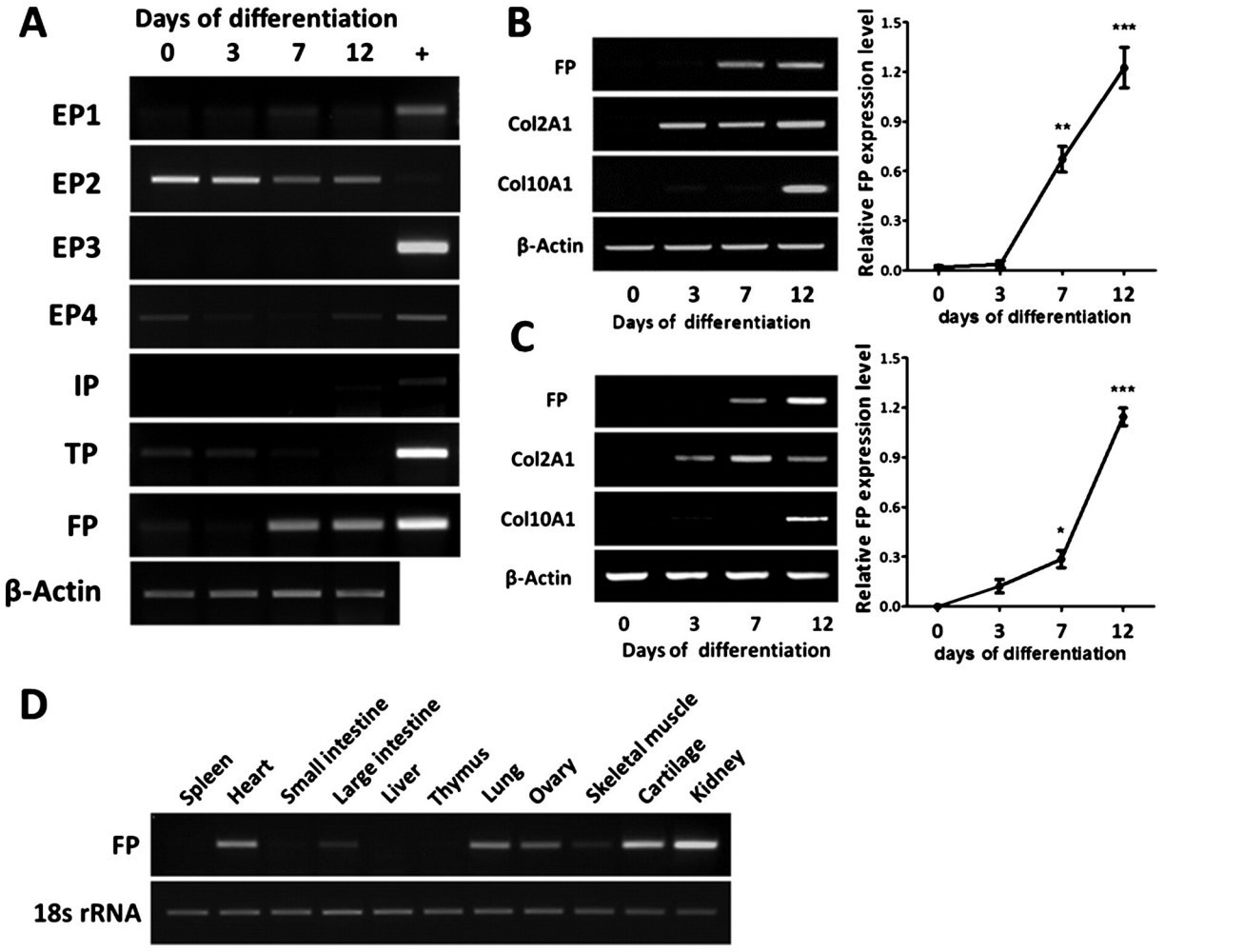

Fig. 1. Prostaglandin $F_{2\alpha}$ receptor (FP) mRNA is highly induced during chondrogenic differentiation of the ATDC5 cell line. (A) Expression of prostaglandin receptors during chondrogenic differentiation of ATDC5 cell line. ATDC5 cells were induced to differentiate in the presence of 10 μg/ml insulin. RNA was isolated at the indicated time points and RT-PCR analysis was conducted. RNA from the mouse kidney and colon were used as a positive control (+). DP was not detected in any sample. (B) RT-PCR analysis of FP, Col2A1, Col10A1, and β-actin during chondrogenic differentiation of ATDC5 cells (left panel) ($n = 3$, **: $p < 0.01$, ***: $p < 0.001$). The level of FP mRNA at each time point was quantified and normalized to β-actin mRNA using ImageJ software. The relative level of FP was plotted (right panel) ($n = 3$, *: $p < 0.05$, ***: $p < 0.001$). (C) RT-PCR analysis of FP, Col2A1, Col10A1, and β-actin during Bmp2-induced chondrogenic differentiation of C3H10T1/2 cell line (left panel). The expression level of FP at each time point was normalized to the β-actin level and the relative level of FP was plotted (right panel). (D) RT-PCR analysis for FP expression in various tissues of 4 weeks old C57BL/6 mouse. 18 s rRNA served as a loading control.