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Short communication

Prostaglandin $F_{2\alpha}$ stimulation of cyclooxygenase-2 promoter activity by the FP_B prostanoid receptor

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Abstract

We have recently shown that the FP_B prostanoid receptor activates β-catenin signaling through the activation of Rho in human embryo kidney (HEK)-293 cells stably expressing the FP_B receptors. We now report that the FP_B receptor can stimulate cyclooxygenase-2 promoter activity and may, therefore, regulate the expression of cyclooxygenase-2. This stimulation of cyclooxygenase-2 promoter activity is blocked by pretreatment with an inhibitor of Rho, but not with an inhibitor of protein kinase C (PKC). Potential up regulation of cyclooxygenase-2 expression by the FP_B receptor would establish a positive feedback loop that would drive β-catenin signaling and could be involved in cancer.

Keywords: FP prostanoid receptor; Cyclooxygenase-2; β-catenin signaling

1. Introduction

Activation of the β-catenin/T-cell factor (Tcf) signaling pathway an important event in the development of colon cancer (Srivastava et al., 2001). For example, mutations in either β-catenin or the adenomatous polyposis coli protein (APCP) that result in unregulated β-catenin/Tcf signaling are responsible for approximately 65% of patients with hereditary nonpolyposis colorectal cancer (Srivastava et al., 2001). Furthermore, one of first markers of the early adenoma is an increase in cytosolic β-catenin and subsequent formation of a β-catenin/Tcf complex. The interaction between β-catenin and Tcf leads to the induction of cyclooxygenase-2 expression and is characteristic of the late adenoma and carcinoma (Srivastava et al., 2001).

In cells stably expressing the ovine FP_B prostanoid receptor, we have recently shown a constitutive phosphatidylinositol 3-kinase (PI3K) activity that leads to an increase in β-catenin expression (Fujino et al., 2002). Another characteristic of colonic cancer cells is a robust expression of PI3K (Petiot et al., 2000). This has also been observed in clinical studies in which PI3K activity was approximately four-fold higher in colorectal tumor tissue as compared with normal tissue from the same patient (Phillips et al., 1998). In our studies, PI3K activity was also approximately four-fold higher in FP_B expressing cells as compared to control cells expressing FP_A receptor (Fujino et al., 2002). Furthermore, the stimulation of FP_B expressing cells with prostaglandin $F_{2\alpha}$ produced a marked activation of β-catenin/Tcf signaling (Fujino and Regan, 2001) that was completely dependent on the activation of the small G protein, Rho (Fujino et al., 2002). To examine the possible induction of cyclooxygenase-2 expression by FP_B receptors, we transiently transfected human embryo kidney (HEK) cells stably expressing either the FP_A or FP_B receptors with a luciferase reporter plasmid under the control of a cyclooxygenase-2 promoter (Inoue et al., 1994). We now report that the FP_B receptor can stimulate cyclooxygenase-2 promoter activity and may, therefore, regulate the expression of cyclooxygenase-2 by the action of prostaglandin $F_{2\alpha}$.

2. Materials and methods

2.1. Stimulation of cyclooxygenase-2 transcriptional activity by FP prostanoid receptors

HEK-293 cells stably expressing either the ovine FP_A or FP_B receptors were cultured in Dulbecco's modified Eagle's

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medium (Life Technologies) containing 10% fetal bovine serum, 250 μg/ml geneticine, 100 μg/ml gentamicin and 200 μg/ml hygromycin B. Cells were transiently transfected with phPES2(−1432/+59), a luciferase reporter plasmid (Inoue et al., 1994) under the control of a cyclooxygenase-2 promoter. The next day, cells were pretreated with either vehicle (water or 0.1% Me_2SO) or 40 μg/ml C3-toxin for 48 h or 10 μM bisindolylmaleimide I for 15 min followed by treatment with either vehicle (sodium carbonate, 0.002% final) or 1 μM prostaglandin $F_{2\alpha}$ for 1 h. The cells were washed three times with media to remove the reagents and 16 h later luciferase activity was measured as previously described (Fujino et al., 2002) using Turner TD-20/20 luminometer. The Dual-Luciferase Reporter Assay System (Promega) was used to control for transfection efficiency.

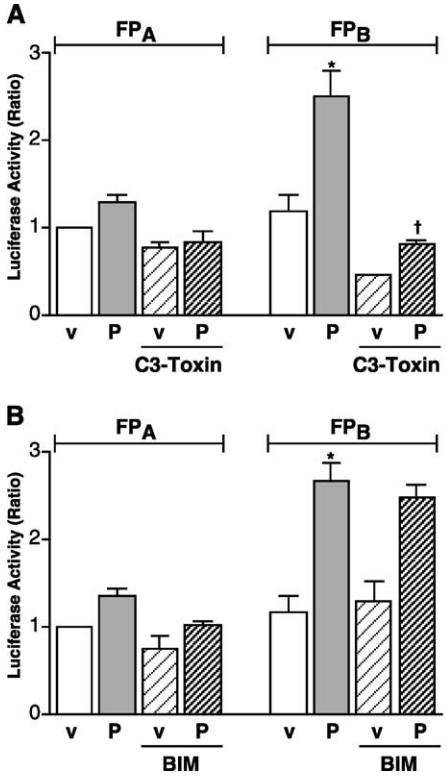

Fig. 1. Effects of C3-toxin and bisindolylmaleimide I (BIM) on prostaglandin $F_{2\alpha}$ stimulation of cyclooxygenase-2 transcriptional activity by FP prostanoid receptors. HEK-293 cells stably expressing either the ovine FP_A or FP_B receptors were transiently transfected with phPES2(−1432/+59), a luciferase reporter plasmid under the control of a cyclooxygenase-2 promoter. The cells were pretreated with either vehicle or 40 μg/ml C3-toxin for 48 h (Panel A) or 10 μM BIM for 15 min (Panel B) followed by treatment with either vehicle (v) or 1 μM prostaglandin $F_{2\alpha}$ (P) for 1 h. The luciferase activity was measured as described in Section 2. Shown are histographs of the pooled luciferase activity (means ± S.E.) from three independent experiments each performed in duplicate. Data are normalized to the vehicle treated FP_A cells. $^*P<0.05$, as compared to vehicle treated FP_B cells. $^\dagger P<0.05$, for the difference between the fold-stimulation in FP_B cells pretreated with C3-toxin (1.79 ± 0.06) as compared with the fold-stimulation in non-pretreated FP_B cells (2.14 ± 0.18).

3. Results

To examine the possible induction of cyclooxygenase-2 expression by FP_B receptors, we transiently transfected HEK cells stably expressing either the FP_A or FP_B receptors with a luciferase reporter plasmid under the control of a cyclooxygenase-2 promoter (Inoue et al., 1994) and then measured luciferase activity following treatment of the cells with 1 μM prostaglandin $F_{2\alpha}$. As shown in Fig. 1, prostaglandin $F_{2\alpha}$ stimulated cyclooxygenase-2 transcriptional activity 2.1-fold in FP_B expressing cells, but only 1.3-fold in FP_A expressing cells. We next examined the effect of the Rho inhibitor, C3-toxin and the protein kinase C (PKC) inhibitor, bisindolylmaleimide I, on prostaglandin $F_{2\alpha}$ stimulated cyclooxygenase-2 promoter activity. As shown in Fig. 1A, prostaglandin $F_{2\alpha}$ stimulated cyclooxygenase-2 promoter activity in FP_B expressing cells was almost completely abolished by C3-toxin pretreatment. On the other hand, Fig. 1B shows that pretreatment of FP_B expressing cells with bisindolylmaleimide I had little effect on prostaglandin $F_{2\alpha}$ stimulated cyclooxygenase-2 promoter activity. Therefore, the stimulation of cyclooxygenase-2 promoter activity by the FP_B receptor is through a Rho signaling pathway and does not require the activation of PKC. Pretreatment with C3-toxin also decreased the basal luciferase activity in FP_B expressing cells; however, this decrease was not observed when the cells were pretreated with bisindolylmaleimide I. We have previously shown that pretreatment of these FP_A and FP_B expressing cells with C3-toxin does not decrease either the basal or prostaglandin $F_{2\alpha}$ stimulated formation of inositol phosphates (Fujino et al., 2002). Therefore, it would appear that the stimulation of cyclooxygenase-2 promoter activity is not through the G_q-mediated pathway, but rather through the activation of Rho and subsequent activation of β-catenin/Tcf signaling.

4. Discussion

Aberrant β-catenin/Tcf signaling in the pathophysiology of colon cancer is well established (Srivastava et al., 2001). What is less clear is the role of cyclooxygenase-2 induction and the increased prostaglandin synthesis, which also characterizes this disease. However, some type of causative relationship is suggested by the observation that nonsteroidal anti-inflammatory drugs are protective in patients with familial adenomatous polyposis (Smalley and DuBois, 1997). As previously noted, 65% of patients with hereditary nonpolyposis colorectal cancer are known to have mutations in either β-catenin or APCP that lead to unregulated β-catenin/Tcf signaling. The underlying basis of the disease in the remaining 35% has not been established although clearly any condition that would promote β-catenin/Tcf signaling would be suspect. For example, mutations in axin have also been associated with

the development of colon cancer (Peifer and Polakis, 2000).

In the present study, we have shown that the FP_B receptor can stimulate cyclooxygenase-2 promoter activity via a Rho dependent pathway that is independent of the activation of PKC. Moreover, pretreatment with C3-toxin, but not bisindolylmaleimide I, decreased the basal luciferase activity in FP_B expressing cells. This is consistent with our previous finding that treatment of FP_B expressing cells with C3-toxin also decreased basal β-catenin/Tcf-mediated transcriptional activation and suggest that the induction of cyclooxygenase-2 promoter activity is through a β-catenin/Tcf signaling pathway. Obviously, the potential consequence of this up regulation of cyclooxygenase-2 promoter activity would be an increased expression of cyclooxygenase-2. Unfortunately, HEK cells do not appear to have inducible cyclooxygenase-2 expression (Murakami et al., 1999) so we were unable to document a corresponding up regulation in the protein. Nevertheless, several interesting parallels exist in colorectal cancer. Thus, in the late adenoma and carcinoma stages, there is a well characterized interaction between β-catenin and Tcf that leads to the induction of cyclooxygenase-2 expression (Srivastava et al., 2001) and increased levels of prostaglandins. In this situation the presence of a human orthologue of the FP_B, or simply a truncated version of the FP_A produced by mutation, would have the potential to set up a positive feedback loop that would drive β-catenin/Tcf signaling. The use of nonsteroidal anti-inflammatory drugs would be expected to interrupt such a feedback loop, which may explain their known protective effect in colorectal cancer.

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