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Peroxisome proliferator-activated receptor γ (PPARγ) regulates trefoil factor family 2 (TFF2) expression in gastric epithelial cells

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Abstract

Although trefoil factor family 2 (TFF2) plays a critical role in the defense and repair of gastric mucosa, the regulatory mechanism of *TFF2* expression is not fully understood. In this study, we investigated the regulation of *TFF2* expression by peroxisome proliferator-activated receptor γ (PPARγ) in gastric epithelial cells. MKN45 gastric cells were used. *TFF2* mRNA expression was analyzed by real-time quantitative RT-PCR. The promoter sequence of the human *TFF2* gene was cloned into pGL3-basic vector for reporter gene assays. Ciglitazone was mainly used as a specific PPARγ ligand. MKN45 cells expressed functional PPARγ proteins. Endogenous *TFF2* mRNA expression and *TFF2* reporter gene transcription was significantly up-regulated by ciglitazone in a dose-dependent manner. Reporter gene assays showed that two distinct *cis*-elements are involved in the response to PAPRγ activation. Within one of these element (nucleotides −558 to −507), we identified a functional peroxisome proliferator responsive element (PPRE) at −522 (5′-GGGACAAAGGGCA-3′). Electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assay confirmed the binding of PPARγ to this sequence. Another element (nucleotides −407 to −358) appeared to be a composite enhancer element indirectly regulated by PPARγ and a combination of these two *cis*-elements was required for the full response of the human *TFF2* gene expression to PPARγ. These data demonstrate that human *TFF2* gene is a direct target of PPARγ in gastric epithelial cells. Since TFF2 is a critical gastroprotective agent, PPARγ may be involved in the gastric mucosal defense through regulating *TFF2* expression in humans.

Keywords: Trefoil factor; TFF2; PPARγ; Gene regulation; Stomach

1. Introduction

Trefoil factor family (TFF) is a group of small protease-resistant peptides characterized by a conserved three-loop domain, designated as trefoil or P- or TFF domain (Hoffmann & Jagla, 2002; Taupin & Podolsky, 2003; Wong, Poulsom, & Wright, 1999). To date, three TFF peptides have been identified in humans, TFF1 (pS2), TFF2 (spasmolytic polypeptide, SP), and TFF3 (intestinal trefoil factor, ITF) (Wright, Hoffmann, Otto, Rio, & Thim, 1997) of which genes are clustered in chromosome 21q22.3 (Seib et al., 1997). TFF peptides are

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expressed in mucus-secreting epithelial cells, especially in epithelial cells of the gastrointestinal tract (Hoffmann & Jagla, 2002; Taupin & Podolsky, 2003; Wong et al., 1999). Among the three TFF peptides, TFF1 and TFF2 are expressed in gastric epithelial cells, whereas TFF3 expression is found in the goblet cells of the small intestine and colon (Hoffmann & Jagla, 2002). Recent evidence also suggests the expression of TFF3 in normal gastric mucosa (Kouznetsova et al., 2004). TFF expression is also known to be coordinately induced at the site of mucosal ulceration (Wright et al., 1992). Although the functions of TFF peptides are not fully understood at present, TFF peptides are believed to play critical roles in the defense and repair of gastrointestinal mucosa (Hoffmann & Jagla, 2002; Taupin & Podolsky, 2003; Wong et al., 1999).

TFF2, which we focus on in the present study, is expressed in gastric mucous neck cells and antral gland cells (Hanby et al., 1993; Hoffmann & Jagla, 2002). A number of in vitro and in vivo studies revealed an important role of TFF2 in the protection of gastric mucosa (Hoffmann & Jagla, 2002; Taupin & Podolsky, 2003). Farrell et al. (2002) reported characteristics of *TFF2*-deficient mice. Although *TFF2*-deficient mice were viable and fertile without obvious gastrointestinal abnormalities, gastric mucosal thickness and mucosal proliferation rate significantly decreased and gastric acid output significantly increased. Administration of indomethacin caused more severe gastric mucosal damage in the *TFF2*-deficient mice. These results appear to confirm a critical role of TFF2 in the defense and wound healing in the stomach. It should be also noted that recent evidence suggests the involvement of TFF2 in the immune response (Baus-Loncar, Kayademir, Takaishi, & Wang, 2005).

Regulatory mechanisms of TFF2 expression have been investigated by several investigators. Gott et al. (1996) and Beck, Sommer, Blin, and Gott (1998) identified the presence of conserved motifs (Motif I, II, III, and IV) within the promoter regions of human *TFF1* and *TFF2* genes and suggested a role of these motifs in the tissue-specific expression of TFF peptides. GATA-6, a zinc-finger transcription factor, is expressed in gastric epithelial cells and involved in the regulation of the transcription of stomach-specific genes, such as H^+/K^+-ATPase (Yoshida et al., 1997). Al-azzeh, Fegert, Blin, and Gott (2000) identified the presence of the consensus binding sites for GATA within the promoter region of the human *TFF2* gene and demonstrated the increased expression of *TFF2* reporter genes in GATA-6-overexpressed gastric cancer cell lines. Al-azzeh et al. (2002) also showed that upstream stimulatory factor (USF) up-regulates *TFF2* expression by binding to the E-box within the *TFF2* promoter in gastric cancer cell lines. However, many details must to be elucidated for the full understanding of the regulation of *TFF2* expression in gastric epithelial cells.

Peroxisome proliferator-activated receptor γ (PPARγ) is a member of the PPAR family that consists of three nuclear receptors (PPARα, PPARδ, and PPARγ) (Corton, Anderson, & Stauber, 2000; Vamecq & Latruffe, 1999). Upon activation by ligand binding, PPARγ forms a heterodimer with a retinoid X receptor (RXR) and modulates target gene transcription by binding to a peroxisome proliferator responsive element (PPRE). Canonical PPRE is a DR-1 sequence, AGGTCANAGGTCA. Although PPARγ is expressed at a high level in adipocytes and is critically involved in adipocyte differentiation and lipid metabolism (Lemberger, Desvergne, & Wahli, 1996; Mandrup & Lane, 1997), gastrointestinal epithelial cells also express PPARγ at a significant level (Mansen, Guardiola-Diaz, Rafter, Branting, & Gustafsson, 1996). A number of studies showed that PPARγ regulates cell proliferation, differentiation, and cell death in gastrointestinal epithelial cells (Kojima et al., 2002; Sarraf et al., 1998; Sato et al., 2000; Shimada, Kojima, Yoshiura, Hiraishi, & Terano, 2002; Takahashi et al., 1999).

In a previous paper, we found and briefly reported that PPARγ ligands, such as troglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, up-regulate *TFF2* expression in a gastric cancer cell line, MKN45 (Shimada et al., 2003). We also found that some of the non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin and aspirin, up-regulate *TFF2* expression by activating PPARγ in the gastric cancer cell lines, MKN45 and AGS (Shimada et al., 2004). Aspirin-induced up-regulation of *TFF2* expression was also reported by Azarschab, Al-Azzeh, Kornberger, and Gott (2001). However, since there is no canonical PPRE within the human *TFF2* gene promoter, the mode of PPARγ action on *TFF2* expression remains unclear. In this study, we further investigated the detailed mechanism of PAPRγ action on the expression of *TFF2* in a gastric cancer cell line, MKN45.

2. Materials and methods

2.1. Reagents

Ciglitazone, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), and GW9662 were obtained from Cayman Chemical (Ann Arbor, MI). Indomethacin and aspirin were purchased from Sigma–Aldrich (St. Louis, MO). Troglitazone was provided by Sankyo Pharmaceu-