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Short communication

Identification of a Novel PGE_2 Regulated Gene in SNU1 Gastric Cancer Cell

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Prostaglandin E_2 (PGE_2) plays an important role in the regulation of various gastric functions, and the growth-inhibitory activities on tumor cells are studied *in vitro* and *in vivo*. Although the mechanisms have attracted many researchers in the past decade, the molecular mechanisms of cell cycle arrest, or induction of apoptosis by PGE_2, is unclear. We investigated the effects of PGE_2 on the growth of the human gastric carcinoma cell line SNU1 and genes that are regulated by PGE_2 and isolated them using differential display RT-PCR (DD RT-PCR). FACS analysis suggested that SNU1 cells were arrested at the G1 phase by PGE_2 treatment. This growth inhibitory effect was in a time- and dose-dependent manner. Treatment of SNU1 cells with 10 μg/ml PGE_2, followed by DD RT-PCR analysis, revealed differently expressed bands patterns from the control. Among the differently expressed clones, we found an unidentified cDNA clone (HGP-27) overexpressed in PGE_2-treated cells. The full-length cDNA of HGP-27 was isolated using RACE, which consisted of a 30-nt 5'-noncoding region, a 891-nt ORF encoding the 296 amino acid protein, and a 738-nt 3'-noncoding region including a poly(a) signal. This gene was localized on the short arm of chromosome number 11. Using the MotifFinder program, a myb-DNA binding repeat signature was detected on the ORF region. The COOH-terminal half was shown to have similarity with the NH_3-terminal domain of thioredoxin (Trx). This relation between HGP-27 and Trx implied a potential role for HGP-27 in modulating the DNA binding function of a transcription factor, myb.

Keywords: Differential display RT-PCR (DD RT-PCR), Fluorescence *in situ* hybridization (FISH), Prostaglandin E_2 (PGE_2), Rapid amplification of complementary DNA ends (RACE).

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Introduction

Prostaglandins (PGs) are well known to influence the growth of not only normal cells, such as fibroblasts (Murota *et al.*, 1977) and colonic mucosal cells (Craven *et al.*, 1983), but also various tumor cells. PGs are synthesized in the gastric mucosa (Ahliquist *et al.*, 1982) and are involved in the regulation of various gastric functions, such as mucosal protection (Redfern *et al.*, 1987), enhancement of mucosal blood flow, and the stimulation of mucus secretion (Miller, 1983). Among these actions, accumulating evidence suggests that PGs play an important role in the protection of the gastric mucosa against damaging agents. Nakamura and coworkers reported that PGE_2, as well as $PGF_{2\alpha}$, inhibited the growth of the human gastric carcinoma KATO III cell along with a simultaneous increase of cAMP production, suggesting the involvement of cAMP in the inhibition of cell growth (Nakamura *et al.*, 1991). Ohno *et al.* (1986) found that the PGE_2-induced growth inhibition was exerted by the dehydration product, PGA_2. A specific carrier on the cell surface transported PGA_2 into cells and accumulated in the nuclei, and that are closely related to the growth inhibitory activity. But the precise molecular mechanism by which these PGs exert cytostatic or cytotoxic activity remains unclear, and that makes the search of PGE_2-responsive genes important for the understanding of the molecular mechanism of PGE_2. To identify differentially expressed genes between control and counterpart groups, methods such as differential screening of cDNA libraries, various forms of subtractive hybridization, and most recently, DD RT-PCR have been used (Liang and Pardee, 1992). In our previous studies, we observed the growth inhibitory action of PGE_2 on SNU1, the human gastric cancer cell line. When SNU1 cells were incubated with various doses of PGE_2, growth inhibition was observed in the PGE_2-treated cells with a time- and dose-dependent manner. FACS analysis revealed that this effect might be caused by the cell cycle arrest at G1 phase. Although the exact mechanisms of PGs actions on tumor cell proliferation have not been fully characterized, G1 phase arrest was reported as the major cause for the antiproliferative activities of Δ^{12}-PGJ_2, PGA_2, PGA_1, and

PGD_2. It has been reported that Δ^{12}-PGJ_2 induced G1 arrest of human neuroblastoma cell line (Marui *et al.*, 1991). PGA_1, A_2, and D_2 also revealed cytotoxic to human melanoma cells accompanied cell cycle arrest from G1 to S (Bhuyan *et al.*, 1986). Therefore, we tried to identify the genes associated with cell growth inhibition induced by PGE_2 using DD RT-PCR. Among the isolated genes, we obtained an interesting unknown gene, which was overexpressed with PGE_2 incubation time, although its exact function has not been characterized yet. To further characterize this gene, we isolated the full-length cDNA by the RACE method, deduced its amino acid sequence and predicted the function. We also showed the localization of the gene by fluorescence *in situ* hybridization assay.

Materials and Methods

Cell culture Human gastric adenocarcinoma SNU1 cells were maintained in RPMI 1640 medium (GibcoBRL, Gaithersburg, USA) containing 10% heat-inactivated fetal bovine serum (Hyclone, Logan, USA), 100 units/ml of penicillin and 100 μg/ml of streptomycin (GibcoBRL, Gaithersburg, USA) at 37°C and in humidified air with 5% CO_2.

DD RT-PCR One-base-anchored oligo-dT primers were used to reverse transcribed total RNA into first-strand cDNA, which were amplified subsequently by PCR using the arbitrary upstream primers. The PCR conditions were the same as described in the RNAimage kit (GenHunter, Nashville, USA). PCR products were labeled with 2 μCi of α-[^{32}P]dCTP (Amersham, Arlington, USA), and analyzed on a 6% polyacrylamide-urea gel using. The cDNA bands that were unique to control, or PGE_2-treated cells, were cut out of the gel, eluted, and reamplified by PCR.

Cloning and DNA sequencing The candidate cDNA was cloned into pGEM-T vector (Promega, Madison, USA) according to the manufacturers instructions. Plasmid with insert was purified and sequenced using an ABI Prism automatic sequencer (ABI PRISM 310 Genetic Analyzer, Perkin-Elmer, Foster city, USA) after performing PCR reactions with an ABI prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, USA).

RNA preparation and northern hybridization Cells were treated with PGE_2 (10 μg/ml) at various time courses and total RNA was isolated using TRI REAGENT (Molecular Research Center, Cincinnati, USA) according to the instructions. Fifteen micrograms of RNA were run on a 1.2% agarose-formaldehyde gel and transferred onto a nylon membrane (Zeta-Probe Blotting Membranes, Bio-Rad, Hercules, USA). After cross-linking RNA to the membrane, the membrane was hybridized to α-[^{32}P]-labeled probes overnight at 42°C.

Isolation of HGP-27 full-length cDNA The full-length cDNA sequence was obtained by the RACE (Rapid amplification of complementary DNA ends) method as described previously (Frohman, 1993). Briefly RACE PCR was carried out using total RNA obtained from PGE_2 (10 μg/ml)-treated SNU1 cells. First-strand cDNA was initiated using a poly(T) primer. The cDNA was amplified by PCR with G-3'SP-1 primer (5'-TACGGAAAGCCATCAACTGGGTGT-3') and AP-1 primer (5'-CCATCCTAATACGACTCACTATAGGGC-3') and PCR reaction conditions were carried out as specified by the manufacturer (Clontech, Palo Alto, USA). GSP-1 primer was designed based on sequence data obtained from the DD RT-PCR and AP-1 primer was supplied by the manufacturer (Clontech, Palo Alto, USA).

Fluorescence *in situ* hybridization To prepare a probe, full-length cDNA of HGP-27 was labeled with biotin 16-dUTP. Metaphase spreads from normal human lymphocytes were prepared according to the method of Dutrillaux and Viegas-Pequignot (1981) and hybridization of FISH probe followed using the previous method (Park *et al.*, 1996). Approximately 100 ng of labeled probe was added to the 10 μl hybridization mixture (50% formamide, 2X SSC, and 5 μg salmon sperm DNA) and denatured at 75°C for 5 min. Hybridization signals were detected by two layers of FITC-conjugated avidin and amplified with one layer of anti-avidin antibody. The slides were counterstained with propidium iodide (PI).

Results and Discussion

DD RT-PCR is a powerful and rapid tool for screening and identifying altered gene expressions at the mRNA level between two or more cell populations (Liang and Pardee, 1992). We prepared RNA from cultured SNU1 cells as a control and 10 μg/ml of PGE_2-treated SNU1 cells for 48 h, and performed DD RT-PCR, as described on the RNAimage kit. Several genes were shown to have different expression patterns after PGE_2 treatment when using a different set of primers. Among these differently expressed genes, we found four genes that had no identical sequences in the GenBank and EMBL databases. Especially, HGP-27, 481bp from DD RT-PCR analysis was one of the most up-regulated genes dependent on PGE_2 treatment. As the result of the Northern

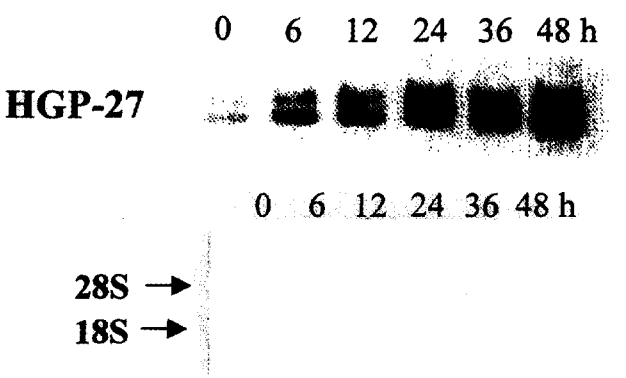

Fig. 1. Northern blot analysis of SNU1 cells treated with 10 μg/ml PGE_2 for various periods. Isolated total RNA was run on a 1.2% agarose-formaldehyde gel and transferred to a nylon membrane. The blot was probed with α-[^{32}P]-labeled HGP-27 fragment obtained from DD RT-PCR.

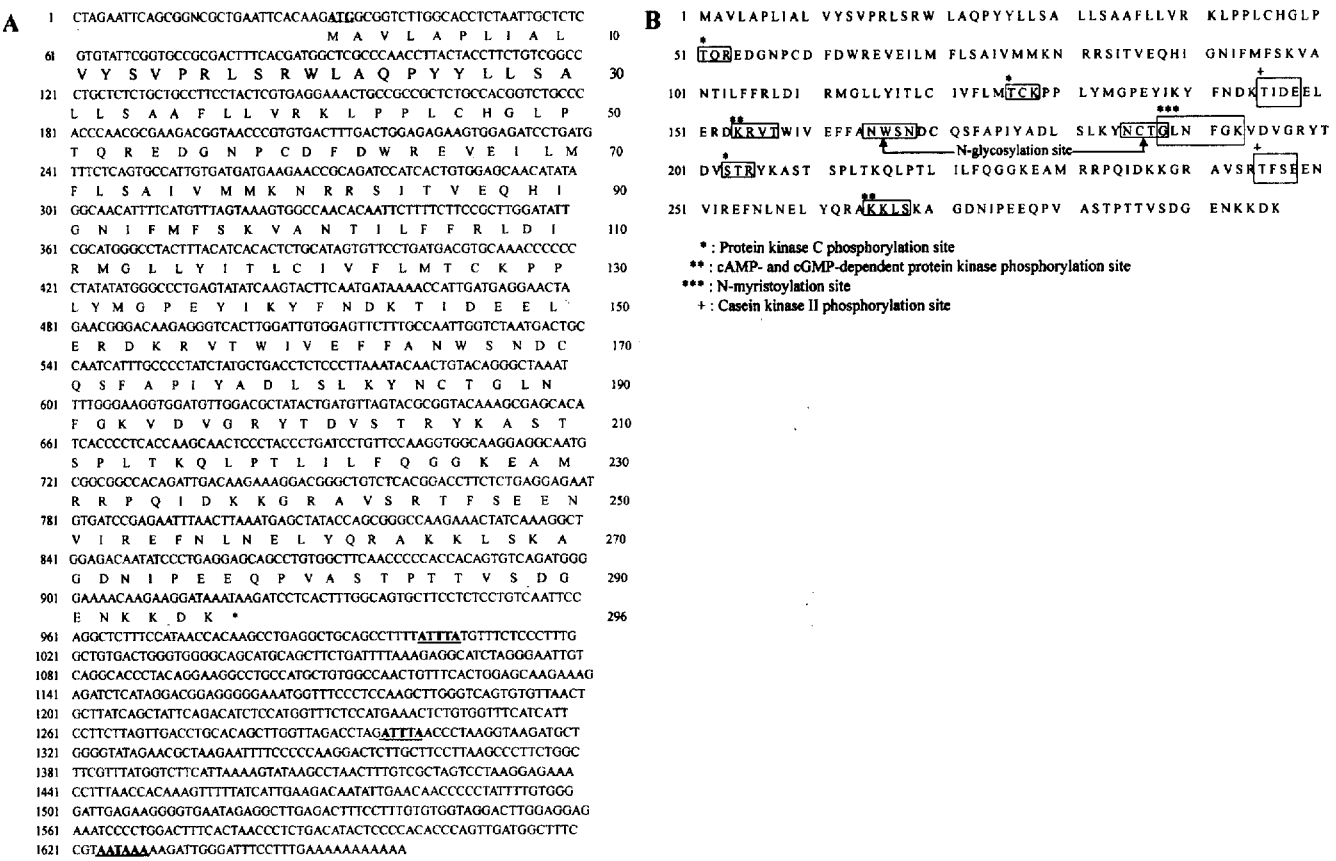

Fig. 2. Nucleotide sequence of HGP-27 cDNA and its deduced amino acid sequence. A; The full-length cDNA was isolated using the RACE method. The stop codon is shown with an asterisk. The Myb DNA-binding domains repeat signature is shown in the box. The underlined nucleotides indicate the polyadenylation signal sequence and AUUUA motif. The numbers on the left denote the nucleotides and the numbers on the right refer to the amino acid residues. B; Ten posttranslational sites predicted by MotifFinder are indicated by boxes.

Fig. 3. Chromosomal localization of HGP-27 was ascertained by fluorescence *in situ* hybridization analysis. Full-length cDNA of HGP-27 probe was labeled with biotin dUTP by nick translation. The labeled probe was hybridized to normal metaphase chromosomes from phytohemagglutin-stimulated PBLs. Specific hybridization signal (arrow) was observed on the short arm of chromosome 11.

blot analysis, we confirmed that about 1.6 kb-sized HGP-27 was overexpressed by PGE_2 in a time-dependent manner (Fig. 1). The FASTA program used to search the GenBank and EMBL database revealed that HGP-27 had a 98% similarity to human CGI-31 protein mRNA. Although the CGI-31 protein was identified as a novel human gene isolated by using *C. elegans* proteome as a template, the exact function of CGI-31 has not been defined or characterized (Lin, 1999; unpublished). To further characterize the novel expressed gene sequence, we obtained the full-length cDNA by the RACE method, and sequenced it. As shown in Fig. 2, the 1659 bp cDNA consists of a 30-nt 5'-noncoding region, a 891-nt ORF encoding the 296 amino acid protein, and a 738-nt 3'-noncoding region including a poly(A) signal. Using the MotifFinder program, ten posttranslation modification sites were found in the predicted polypeptide (Fig. 2B) and one myb-DNA binding repeat signature also existed (Fig. 2A). When this cDNA was used as a probe of FISH analysis, we identified that this gene was localized on the short arm of a chromosome number 11 (Fig. 3). Although the exact function

of this gene was not characterized, there were interesting alignments at the structural protein level (PSI-BLAST search). One potentially important region was at the COOH-terminal half that revealed a sequence identity of over 30% with the NH_3-terminal domain of thioredoxin (data not shown). Thioredoxin (Trx) is a 12-kDa protein, known to be present in many prokaryotes and eukaryotes and appears to be truly ubiquitous in all living cells (Holmgren, 1985). Among the variety of its functions, it can also modulate the DNA binding activity of some transcription factors either directly (Bannister *et al.*, 1991) or indirectly (Matthews *et al.*, 1992) through the nuclear factor Ref-1, which in turn is reduced by Trx (Abate *et al.*, 1990). This relation between HGP-27 and Trx implied a potential role for HGP-27 in modulating the DNA binding function of transcription factors, especially myb. Ongoing studies, examining the interaction of HGP-27 with myb and other molecules, will help elucidate the mechanisms through which it inhibits cell growth. Finally, evaluating the amplification and expression of HGP-27 in a range of human tumors will further delineate its contribution to tumor development.

References

Abate, C., Patel, L., Rauscher, F. and Curran, T. (1990) Redox regulation of fos and jun DNA-binding activity *in vitro*. *Science* **249**, 1157-1161.

Ahliquist, D. A., Duenes, J. A., Madson, T. H., Romerro, J. C., Dozois, R. R. and Malagelada, J. R. (1982) Prostaglandin generation from gastroduodenal mucosa: regional and species differences. *Prostaglandins* **24**, 115-125.

Bannister, A. J., Cook, A. and Kouzarides, T. (1991) *In vitro* DNA binding activity of Fos/Jun and BZLF1 but not C/EBP is affected by redox changes. *Oncogene* **6**, 1243-1250.

Bhuyan, B. K., Adams, E. G., Badiner, G. J., Li, L. H. and Barden, K. (1986) Cell cycle effects of prostaglandins A_1, A_2, and D_2 in human and murine melanoma cells in culture. *Cancer Res.* **46**, 1688-1693.

Craven, P. A., Saito, R. and Derubertis, F. R. (1983) Role of local prostaglandin synthesis in the modulation of proliferative activity of rat colonic epithelium. *J. Clin. Invest.* **72**, 1365-1375.

Dutrillaux and Viegas-Pequignot (1981) High resolution R- and G-banding on the same preparation. *Hum. Genet.* **57**, 93-95

Frohman, A. (1993) Rapid amplification of complementary DNA ends for generation of full-length complementary DNAs: thermal RACE. *Methods Enzymol.* **218**, 340-356.

Holmgren, A. (1985) Thioredoxin. *Annu. Rev. Biochem.* **54**, 237-271.

Liang, P. and Pardee, A. B. (1992) Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. *Science* **257**, 967-971.

Marui, N., Nishino, H., Sakai, T., Aoike, A., Kawai, K. and Fukushima, M. (1991) Delta 12-prostaglandin J_2 mimics heat shock in inducing cell cycle arrest at G1 phase. *Biochem. Biophys. Res. Commun.* **179**, 1662-1669.

Matthews, J. R., Wakasugi, N., Virelizier, J. L., Yodoi, J. and Hay, R. T. (1992) Thioredoxin regulates the DNA binding activity of NF-kappa B by reduction of a disulfide bond involving cysteine 62. *Nucleic Acids Res.* **20**, 3821-3830.

Miller, T. A. (1983) Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. *Am. J. Physiol.* **245**, G601-623.

Murota, S., Morita, I. and Abe, M. (1977) The effects of thromboxane B_2 and 6-etoprostaglandin $F_{1\alpha}$ on cultured fibroblasts. *Biochim. Biophys. Acta.* **479**, 122-125.

Nakamura, A., Yamatani, T., Fujita, T. and Chiba, T. (1991) Mechanism of inhibitory action of prostaglandins on the growth of human gastric carcinoma cell line KATO III. *Gastroenterol.* **101**, 910-918.

Ohno, K., Fujiwara, M., Fukushima, M. and Narumiya, S. (1986) Metabolic dehydration of prostaglandin E_2 and cellular uptake of the dehydration product: correlation with prostaglandin E_2-induced growth inhibition. *Biochem. Biophys. Res. Commun.* **139**, 808-815.

Park, S. H., Kim, H., Kim, H. S. and Chun, Y. H. (1996) Construction of band-specific painting probes from a single microdissected chromosome. *Mol. Cells* **6**, 51-55.

Redfern, J. S., Blair, A. J., Lee, F. and Feldman, M. (1987) Gastrointestinal ulcer formation in rabbits immunized with prostaglandin E_2. *Gastroenterol.* **93**, 744-752.