Investigating the role of introns in the regulation of regenerating gene 1 expression

YURONG CHAI, YUN SUN, LINXIA GUO, DAN LI and YI DING

Department of Histology and Embryology, College of Basic Medicine, Zhengzhou University, Zhengzhou, Henan 450001, P.R. China

Received February 6, 2014; Accepted October 31, 2014

DOI: 10.3892/ol.2014.2712

Abstract. Gastrin is a hormone that physiologically regulates gastric acid secretion and contributes to the maintenance of gastric epithelial architecture by regulating the expression of genes such as regenerating gene 1 (Reg1). Reg1 is involved in gastric carcinogenesis as an antiapoptotic factor. The current study explores the molecular mechanism of gastrin-regulated Reg1 expression in human gastric cancer cells. In total, five intron fragments of the Reg1 gene were cloned by polymerase chain reaction and inserted into luciferase reporter vector pGL3 to construct intron-luciferase reporter vectors. After confirmation by Xho I/Hind III digestion and DNA sequencing, the five constructs were transfected into the SGC7901 gastric cancer cell line. The luciferase activity of the cells transfected with each of the five constructs was detected following incubation without or with gastrin. The five intron fragments of Reg1 were also randomly labeled with digoxin as a probe, and nuclear proteins of gastric cancer cells were extracted following treatment with or without gastrin. Southwestern blotting was subsequently performed to detect transcription factors that bind to the introns. The results indicated that the luciferase activity was significantly higher in cells transfected with recombinant vectors containing introns 2, 3, 4 or 5 than that in the cells transfected with an empty vector (P<0.05). However, no statistically significant difference in luciferase activity was identified between cells transfected with pGL3-intron 1 and those transfected with pGL3-Basic (P>0.05). Following incubation with gastrin, no significant difference was identified (P>0.05). The five introns of Reg1 can bind a number of transcription factors and gastrin may affect this interaction. Introns 2-5 of Reg1 potentially have transcriptional control over gene expression in gastric cancer cells. In conclusion, gastrin may regulate the expression of the Reg1 gene via the interaction of the introns by binding to the transcription factors.

Introduction

Gastric cancer (GC) is a major cause of cancer-related mortalities worldwide (1). There are multiple pathogenic factors that may promote the development and progression of gastric cancer. Gastrin is a type of polypeptide hormone, secreted by G cells in the stomach and upper-section of the small intestines. Its role in the physiological regulation of gastric acid secretion has been well established (2). Additionally, gastrin contributes to the maintenance of gastric epithelial architecture by regulating the expression of genes such as plasminogen activator inhibitor 1 (PAI-1) (3) and regenerating gene 1 (Reg1) (4). Increased expression of gastrin has been demonstrated in the progression of intestinal gastric cancer (5).

Reg1 encodes a β-cell growth factor, Reg1 protein, primarily observed during pancreatitis and pancreatic islet regeneration (6,7). Reg1 has also been referred to as pancreatic stone protein (PSP) (8), or lithostathine (9), according to different studies. The Reg family comprises of four subclasses (Reg1-4) (10) across species, with the majority of the orthologs belonging to the Reg1 and Reg3 groups. The Reg1 gene is approximately 3 kb in size, containing six exons and five introns, and is located at chromosome 2p12. An increasing number of studies have provided evidence of the participation of Reg1 protein in the proliferation and differentiation of diverse cell types (11,12). It has been demonstrated that Reg family members are associated with various pathologies, including diabetes, epithelial inflammation and a number of forms of cancer (13,14). Reg1 is predominantly expressed in enterochromaffin-like (ECL) cells, as well as pepsinogen-secreting chief cells, which are also a target of gastrin within the gastric epithelium (15). It has been proposed that Reg1 and gastrin may synergistically regulate gastric mucosal proliferation during certain pathological settings (16,17). Significantly less is known with regard to the transcriptional mechanisms by which gastrin may regulate genes involved in the maintenance of gastric epithelial architecture. It was previously reported that a C-rich region of the proximal promoter sequence of Reg1 is required for gastrin-stimulated expression in gastric

Correspondence to: Dr Yi Ding, Department of Histology and Embryology, College of Basic Medicine, Zhengzhou University, 100 Science Avenue, Zhengzhou, Henan 450001, P.R. China

E-mail: dingyi@zzu.edu.cn

Key words: human gastric cancer, regenerating gene 1, gastrin, intron, transcription factors
cancer cell line, AGS (15), and that the expression of Reg1 is controlled through separate promoter elements -2111 and -104 bp by gastrin (4).

It has been demonstrated that intronic sequences in eukaryotes have the potential to improve gene expression through a variety of mechanisms. Human β-globin (hBG) introns can act as enhancer-like elements for the expression of the human factor IX in cultured Chinese hamster ovary cells, resulting in a higher activity with respect to the second hBG intron compared with the first one. The larger number of transcription factor binding motifs in the second hBG intron accounts for its stronger effect (18). The DNase I-hypersensitive (HSS) sequences in intron 51 of the von Willebrand factor (VWF) gene contain cis-acting elements that are necessary for the VWF gene transcription in a subset of lung endothelial cells in vivo (19). It was demonstrated that Reg1 and gastrin may synergistically regulate gastric mucosal proliferation during certain pathological settings such as wound healing (16). To identify additional cis-acting elements within the Reg1 gene that may participate in transcriptional regulation and the effects of gastrin on expression of Reg1, we investigated whether introns of Reg1 can increase its expression and bind to transcription factors in gastric cancer cells. This also facilitated the exploration of the cellular mechanisms regulating Reg1 expression in gastric cancer cells.

Materials and methods

Cell culture. The human gastric cancer cell line, SGC7901, was provided by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and routinely cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin (Sigma-Aldrich, St. Louis, MO, USA) and 100 µg/ml streptomycin (Sigma-Aldrich), at 37°C in a humidified atmosphere of 5% CO₂.

Cloning of the human Reg1 gene introns. In total, five intron fragments of Reg1 were cloned from genomic DNA of human blood cells by polymerase chain reaction (PCR). PCR was performed in a final volume of 25 µl. The PCR reaction was carried out at 94°C for 3 min, followed by 35 cycles at 95°C for 30 sec, 50-55°C for 45 sec and 72°C for 1 min. PCR primers for the five introns are listed in Table I. The PCR fragments of five introns were inserted into plasmid vector pBluescript SK+, digested by Xho I/Hind III (Takara Biotechnology Co., Ltd., Dalian, China) and subcloned into luciferase reporter vector pGL3-Basic, which was used as a control and digested with the same two enzymes. The five luciferase reporter vectors containing the five introns of Reg1 were constructed, and designated as pGL3-intron 1, pGL3-intron 2, pGL3-intron 3, pGL3-intron 4 and pGL3-intron 5. All constructs were confirmed by Xho I/Hind III digestion and DNA sequencing.

Luciferase assay. The SGC7901 gastric cancer cell line was plated on 96-well plates at a density of 2x10⁵ cells/well. The cells were transiently transfected with the above five luciferase reporter plasmids using Lipofectamine Plus system according to the manufacturer's instructions (Invitrogen Life Technologies, Carlsbad, CA, USA). To evaluate the effect of gastrin (Sigma-Aldrich), the transfected cells were incubated with gastrin (1x10⁻⁷ mol/l) for 24 h after 48 h transfection. The luciferase activity of the transfected cells without or with gastrin incubation was measured by Luciferase Assay System (Promega Corporation, Madison, WI, USA) in a Glomax fluorescence detector (Promega Corporation) according to the manufacturer's instructions. All assays were conducted in triplicate.

Southwestern blotting. Using genomic DNA from human blood cells as a template, the five intron fragments of Reg1 gene were cloned by PCR using the same primers (Table I) and identified by 1% agarose gel electrophoresis. Subsequently, the PCR products of the introns were randomly labeled with digoxin (Roche Diagnostics Corporation, Indianapolis, IN, USA) as a probe according to the manufacturer's instructions. The sensitivity of the probes was tested with DNA dot blotting by using the DIG High Prime DNA Labeling and Detection Starter Kit I (Roche Diagnostics Corporation). To observe the effect of gastrin, the SGC7901 cells were cultured and incubated without or with gastrin (1x10⁻⁷ or 1x10⁻⁸ mol/l) for 24 h. Their nuclear proteins were subsequently extracted according to the manufacturer's instructions (Fierce Biotechnology, Inc., Rockford, IL, USA). Protein concentration was determined by bicinchoninic acid (BCA) protein assay (BCA assay kit; Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions. Nuclear proteins of the cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% polyacrylamide gel. The proteins were blotted onto the PVDF membrane in a transfer buffer (Sigma-Aldrich). With the five intron fragments of Reg1 as probes, the binding activity of each intron to nuclear proteins was detected as previously described (20). The density of each band was analyzed using Image-Pro Plus Version 6.0.

Statistical analysis. All data are presented as the mean ± standard deviation (SD). Statistical analysis was performed using an unpaired two-tailed t test. P<0.05 was considered to indicate a statistically significant difference. Data analysis was
performed using Statistical Product and Service Solutions software (version 15.0; SPSS, Inc., Chicago, IL, USA).

Results

Effects of introns of Reg1 on luciferase activity. The five luciferase reporter vectors containing introns of the Reg1 gene were identified by enzyme digestion and DNA sequencing. The results demonstrated that the sequences of inserted fragments were consistent with those of GenBank data with the correct direction of transcription. The relative luciferase activities in SGC7901 cells transfected with various recombinant plasmids are shown in Fig. 1.

The relative luciferase activities of cells transfected with pGL3-intron 2, pGL3-intron 3, pGL3-intron 4, and pGL3-intron 5 were significantly higher when compared with that of pGL3-Basic (P<0.05). No statistically significant difference in luciferase activity was identified between cells transfected with pGL3-intron 1 and those transfected with pGL3-Basic, which was used as the control (P>0.05). After gastrin incubation, no significant difference in luciferase activity was identified between cells transfected with any of the above recombinant plasmids (P>0.05).

Transcription factors (Tfs) binding activity to five introns. The five intron fragments were cloned by PCR and labeled with digoxin. The sensitivity of the five intron probes was 1 pg/µl, which was effective for detection. Results of the analysis of transcription factors through Southwestern blotting are shown in Figs. 2 and 3. For the probe of intron 1, 15 major bands of Tfs with different molecular weights were detected. Following gastrin treatment, the density of certain bands was evidently changed. Compared with the control group (without gastrin treatment), the density of bands 1, 2, 3, 4 and 7 was decreased significantly, and that of band 6 was increased significantly (P<0.05). For the cells incubated with 10⁻⁸ mol/l gastrin, the density of bands 1, 3, 4, 6, 9 and 10 was increased significantly when compared with that of the cells incubated with 10⁻⁷ mol/l gastrin (P<0.05).

With the intron 2 fragment as a probe, six different major bands of Tfs were detected. Following gastrin treatment, the density of band 2 was decreased significantly, and bands 3, 4, 5 and 6 were increased significantly (P<0.05). The density of bands 3, 4, 5 and 6 was increased significantly in the cells incubated with 10⁻⁷ mol/l gastrin compared with that of 10⁻⁸ mol/l gastrin (P<0.05).

The results of the intron 3 probe detection identified 13 different major bands of Tfs. Compared with the control group, the density of all 13 bands in the experimental groups was decreased significantly (P<0.05). The density of bands 4, 9, 10, 11, 12 and 13 were increased significantly in the cells incubated with 10⁻⁸ mol/l gastrin compared with that of the cells incubated with 10⁻⁷ mol/l gastrin (P<0.05).

The result of the intron 4 probe detection showed 10 different major bands of Tfs. Compared with the control group, the

| Introns | Forward primer 5’-3’ | Reverse primer 5’-3’ | PCR products length, bp | Tm, °C |
|---------|---------------------|---------------------|------------------------|-------|
| Intron1 | CCAACTCAGACTCAGCCAAC | CATGCTGAGCTGCAATGAAT | 376 | 50 |
| Intron2 | GCTGATCTCCTGCCTGATGT | AACTCTGTCTGGGCCTCTTG | 700 | 55 |
| Intron3 | TGCCATATCGCTCTACTGTCT | AGTTGCCCGAATTCTCATGT | 400 | 55 |
| Intron4 | CTGTGAGCCTGAGCCTCAA | CAAGGCACATCCTCCCATT | 245 | 55 |
| Intron5 | CTGTGAGCCTGAGCCTCAA | CAAGGCACATCCTCCCATT | 245 | 55 |

Tm, annealing temperature.

Figure 2. Analysis of transcription factors by Southwestern blotting with the five introns of the Reg1 gene as probes: (A) intron 1, (B) intron 2, (C) intron 3, (D) intron 4 and (E) intron 5. Lane i, SGC7901 cells without gastrin incubation, as a control; lane ii, SGC7901 cells incubated with 1x10⁻⁸ mol/l gastrin; lane iii, SGC7901 cells incubated with 1x10⁻⁷ mol/l gastrin.
density of bands 2, 4, 6 and 11 were decreased significantly, and the density of bands 7 and 8 were increased significantly (P<0.05). When compared with those incubated with 10⁻⁸ mol/l gastrin, the density of bands 1, 3 and 4 was increased significantly for cells incubated with 10⁻⁷ mol/l gastrin, and that of band 11 was decreased significantly (P<0.05).

The result of the intron 5 probe detection showed 14 different major bands of Tfs. Compared with the control group, the density of band 12 was decreased significantly, and that of bands 1, 2, 5, 7, 8, 9 and 13 was increased significantly (P<0.05). The density of band 1, 7, 9 and 12 was increased significantly in the cells incubated with 10⁻⁸ mol/l gastrin compared to those incubated with 10⁻⁷ mol/l gastrin (P<0.05).

Discussion

Human Reg1 gene is a single copy gene that is located on chromosome 2 and is composed of 6 exons and 5 introns (21). One of the most well-documented effects of Reg1 is on the proliferation of acinar and islet cells of the pancreas. The expression of Reg1 is increased in regenerating or hyperplastic islets. In addition to its islet proliferation- and regeneration-promoting effects, tumor-promoting activity of Reg1 protein has also been reported (22). Aberrant Reg expression has been detected in tissues from colorectal carcinoma and gastric cancer (23,24). In gastric cancer tissues, expression of Reg1 is associated with patient survival and numbers of metastatic lymph nodes (25). Reg1-deficient mice have normal gastric development, however Reg1 promotes gastric mucosal growth and restoration with gastrin. Reg1 and gastrin may synergistically regulate gastric mucosal proliferation during certain pathological settings, such as wound healing (16). The proliferative efficiency of gastric cancer cell line SGC7901 decreases significantly following Reg1 knock-down and incubation with gastrin (26). It has been suggested that Reg1 may be a critical downstream gene in the process of gastrin stimulated gastric cancer development. To further understand the molecular mechanism by which gastrin stimulates the expression of Reg1 in gastric cancer cells, the cis-regulatory function of the introns of Reg1 and the relationship with gastrin were explored.

In recent years, studies have shown that introns of eukaryotic genes can promote transcriptional efficiency (27). The ability of introns to stimulate gene expression is an extensively investigated subject area for a wide range of organisms, including mammals and nematodes. Introns may act as transcriptional enhancers or alternative promoters, depending on cis-elements located within the intron spanning sequence (28). For example, uncoupling protein (Ucp) 2 and 3 expression is activated by the peroxisome proliferator-activated receptors (PPARs). The most prominent PPARγ binding site in the Ucp2 and Ucp3 loci was identified in intron 1 of the Ucp3 gene and was the only site that facilitates PPARγ transactivation of a heterologous promoter. The transactivation of Ucp2 and 3 is mediated through this novel enhancer in Ucp3 intron 1 (29). A conserved Mad-binding element (SBE1) in intron 1 of the follistatin gene can also regulate the expression of this gene (30).

The present study demonstrated that the introns of the Reg1 gene exhibited cis-regulating function, with the exception of intron 1, indicating that introns 2, 3, 4 and 5 of Reg1 may contain cis-regulatory elements. It has been reported that a C-rich region of the rat Reg1 promoter is critical for gastrin-stimulated Reg expression (15). It is hypothesized that cis-regulatory elements in introns and promoters of Reg1 may synergistically regulate the gene expression. The present study also showed that, following gastrin incubation, the luciferase activity was not significantly different, which appeared to indicate that gastrin had no effect on regulating Reg1 gene expression.
expression. However, in physiological conditions, gene expression is regulated by interaction of promoter and intron. The luciferase activity was detected in the cells transfected with only a single intron, which may contribute to the negative results obtained in the current study.

In eukaryotic cells, transcriptional regulation is executed by the interaction between trans-acting factors and cis-acting regulatory elements. Trans-acting factors are also known as transcription factors, and can recognize and bind to specific cis-acting elements. For example, the cis-elements of human ubiquitin C, able to bind in vitro the ubiquitous Spl and YY1 transcription factors, are involved in the stimulation of reporter gene transcription (31). In the present study, the effect of gastrin on Tfs that bind to the cis-acting regulatory elements in introns of the Reg1 gene in gastric cancer cells was also explored by Southwestern blotting. The results revealed multiple Tfs binding to the five introns of Reg1, which suggested that the introns may function via binding to their Tfs.

The direct effect of intron-mediated transcriptional regulation is often referred to as intron-mediated enhancement (IME) (28). IME requires the presence of an intron close to the 5′ end of the gene. It has been hypothesized that a promoter proximal to the 5′ splice site facilitates the recruitment of transcriptional machinery to the promoter, which includes transcription factors such as c-Jun and activating transcription factor 2 binding to the cyclic adenosine monophosphate response element site and, therefore, aids in the initiation of transcription (32-34). The precise mechanism of intron-dependent enhancement of transcription, however, remains unclear. It was recently reported that the inclusion of an intron in INOI, which is a nonintronic gene, resulted in the constitutive activation of the gene. In the presence of the intron, the promoter of INOI interacted with its terminor region to form a gene loop in yeast (35). The intronic cis-acting elements of the cystic fibrosis transmembrane conductance regulator gene (CFTR) interact with the CFTR promoter and contribute to the regulation of CFTR gene expression (36). In Reg1, the C-rich region in the gene promoter was found to be critical for the response to gastrin (15,37), and the expression of Reg1 was controlled through separate promoter elements by gastrin (4).

The present study found that gastrin may alter the density of certain Tfs bands, and that the density of some Tf bands was altered at different gastrin concentrations. This indicates that gastrin can alter the ability of Tfs to bind to the recognition sequences in introns to affect the formation of the transcriptional complex, and may be significant in the interaction between the promoter and introns to regulate the expression of Reg1. Although the first intron of Reg1 has no cis-regulatory function, it can bind to at least 14 types of Tf. It was reported that the control of human Ucp3 transcription in skeletal muscle is not solely conferred by the promoter, but depends on several cis-acting elements in intron 1, suggesting a complex association between the promoter and intronic sequences (38). Intron removal, or replacement with a heterologous chimeric intron, caused a significant reduction in promoter activity (27). The results of the current study suggest that intron 1 of the Reg1 gene may function only as a mediator in the formation of multiple molecule complexes for regulating gene expression.

In conclusion, our data demonstrate that introns of Reg1 can bind to many transcription factors and enhance gene expression. However, we still did not identify the precise transcription factors. The hormone gastrin can influence the ability of Tf binding to introns. Gastrin may regulate Reg1 gene expression by binding to the transcription factors to form a multiple molecule complex. Future research on the interaction of the promoter and introns of the Reg1 gene and identifying the transcription factors that bind to the introns of Reg1 gene will be useful in elucidating the mechanism of Reg1 gene expression.

References
1. Ferlay J, Shin HR, Bray F, et al.; Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893-2917, 2010.
2. Dockray G, Dimalone R and Varro A; Gastrin: old hormone, new functions. Pflugers Arch 449: 344-355, 2005.
3. Norett KG, Steele I, Duval C, et al.; Gastrin stimulates expression of plasminogen activator inhibitor-1 in gastric epithelial cells. Am J Physiol Gastrointest Liver Physiol 301: G446-G453, 2011.
4. Steele IA, Dimalone R, Pritchard DM, et al.; Helicobacter and gastrin stimulate Reg1 expression in gastric epithelial cells through distinct promoter elements. Am J Physiol Gastrointest Liver Physiol 293: G347-G354, 2007.
5. Henwood M, Clarke PA, Smith AM and Watson SA; Expression of gastrin in developing gastric adenocarcinoma. Br J Surg 88: 564-568, 2001.
6. Okamoto H; The Reg gene family and Reg proteins: with special attention to the regeneration of pancreatic beta-cells. J Hephatoibiliary Pancreat Surg 6: 254-262, 1999.
7. Terazono K, Yamamoto H, Takasawa S, et al.; A novel gene activated in regenerating islets. J Biol Chem 263: 2111-2114, 1988.
8. De Curo A, Lohse J and Sarles H; Characterization of a protein isolated from pancreatic calculi of men suffering from chronic calcifying pancreatitis. Biochim Biophys Res Commun 87: 1176-1182, 1979.
9. Sarles H, Dagorn JC, Giorgi D and Bernard JP; Renaming pancreatic stone protein as ‘lithostathine’. Gastroenterology 99: 900-901, 1990.
10. Graf R, Schiesser M, Reding T, et al.; Excocrine meets endocrine: pancreatic stone protein and regenerating protein - two sides of the same coin. J Surg Res 133: 113-120, 2006.
11. Miyaoka Y, Kadowaki Y, Ishihara S, et al.; Transgenic overexpression of Reg protein caused gastric cell proliferation and differentiation along parietal cell and chief cell lineages. Oncogene 23: 3572-3579, 2004.
12. Sanchez D, Mueller CM and Zenilman ME; Pancreatic regenerating gene 1 and acinar cell differentiation: influence on cellular lineage. Pancreas 38: 572-577, 2009.
13. Parikh A, Stephan AF and Tzanakakis ES; Regenerating proteins and their expression, regulation and signaling. Biomol Concepts 3: 57-70, 2012.
14. van Beelen Granlund A, Östvik AE, Bremna O, et al.; REG gene expression in inflamed and healthy colon mucosa explored by in situ hybridisation. Cell Tissue Res 352: 639-646, 2013.
15. Ashcroft FJ, Varro A, Dimalone R and Dockray GF; Control of expression of the lectin-like protein Reg-1 by gastrin: role of the Rho family GTPase RhoA and a C-rich promoter element. Biochem J 381: 397-403, 2004.
16. Peterson AJ, Nguyen N, Okamoto H, et al.; Loss of Reg1 in conjunction with gastrin deficiency in mice facilitates efficient gastric ulcer healing but is dispensable for hyperplasia and tumourgenesis. Regul Pept 160: 9-18, 2010.
17. Fukuhara H, Kadwaki Y, Öse T, et al.; In vivo evidence for the role of Reg in gastric regeneration: transgenic overexpression of Reg accelerates the healing of experimental gastric ulcers. Lab Invest 90: 556-565, 2010.
18. Haddad-Mashadrizeh A, Zomorodipour A, Izadpanah M, et al.; A systematic study of the function of the human beta-globin introns on the expression of the human coagulation factor IX in cultured Chinese hamster ovary cells. J Gene Med 11: 941-950, 2009.
19. Kleinschmidt AM, Nassiri M, Stitt MS, et al.; Sequences in intron 51 of the von Willebrand factor gene target promoter activation to a subset of lung endothelial cells in transgenic mice. J Biol Chem 283: 2741-2750, 2008.
20. Sui FK, Lee LT and Chow BK; Southwestern blotting in investigating transcriptional regulation. Nat Protoc 3: 51-58, 2008.
21. Watanabe T, Yonekura H, Terazono K, Yamamoto H and Okamoto H: Complete nucleotide sequence of human reg gene and its expression in normal and tumoral tissues. The reg protein, pancreatic stone protein, and pancreatic thread protein are one and the same product of the gene. J Biol Chem 265: 7432-7439, 1990.
22. Yamaoka T, Yoshino K, Yamada T, et al: Diabetes and tumor formation in transgenic mice expressing Reg I. Biochem Biophys Res Commun 278: 368-376, 2000.
23. Zheng HC, Sugawara A, Okamoto H, et al: Expression profile of the REG gene family in colorectal carcinoma. J Histochem Cytochem 59: 106-115, 2011.
24. Fukui H, Fujii S, Takeda J, et al: Expression of reg I alpha protein in human gastric cancers. Digestion 69: 177-184, 2004.
25. Dhar DK, Udagawa J, Ishihara S, et al: Expression of regenerating gene I in gastric adenocarcinomas: correlation with tumor differentiation status and patient survival. Cancer 100: 1130-1136, 2004.
26. Cheng Sh, Ding Y, Zhang QX: Role of RegI in the pathway of gastrin stimulating proliferation of gastrin cancer cells in vitro. Acta Anatomica Sinica 43: 63-67, 2012 (In Chinese).
27. Bianchi M, Crinelli R, Giacomini E, Carloni E and Magnani M: A potent enhancer element in the 5'-UTR intron is crucial for transcriptional regulation of the human ubiquitin C gene. Gene 448: 88-101, 2009.
28. Rose AB: IntermEDIATE regulation of gene expression. Curr Top Microbiol Immunol 326: 277-290, 2008.
29. Bugge A, Siersbaek M, Madsen MS, et al: A novel intronic peroxisome proliferator-activated receptor gamma enhancer in the uncoupling protein (UCP) 3 gene as a regulator of both UCP2 and -3 expression in adipocytes. J Biol Chem 285: 17310-17317, 2010.
30. Blount AL, Vaughan JM, Vale WW and Bilezikjian LM: A Smad-binding element in intron 1 participates in activin-dependent regulation of the follistatin gene. J Biol Chem 285: 7016-7026, 2008.
31. Bianchi M, Crinelli R, Giacomini E, et al: Yin Yang 1 intronic binding sequences and splicing elicit intron-mediated enhancement of ubiquitin C gene expression. PloS One 8: e65932, 2013.
32. Kamo K, Kim AY, Park SH and Joung YH: The 5'UTR-intron of the Gladiolus polyubiquitin promoter GUBQ1 enhances translation efficiency in Gladiolus and Arabidopsis. BMC Plant Biol 12: 79, 2012.
33. Damgaard CK, Kahns S, Lykke-Andersen S, et al: A 5' splice site enhances the recruitment of basal transcription initiation factors in vivo. Mol Cell 29: 271-278, 2008.
34. Tanabe A, Konno J, Tanikawa K and Sahara H: Transcriptional machinery of TNF-α-inducible YTH domain containing 2 (YTHDC2) gene. Gene 535: 24-32, 2014.
35. Moabbi AM, Agarwal N, El Kaderi B and Ansari A: Role for gene looping in intron-mediated enhancement of transcription. Proc Natl Acad Sci USA 109: 8505-8510, 2012.
36. Ott CJ, Suszko M, Blackledge NP, et al: A complex intronic enhancer regulates expression of the CFTR gene by direct interaction with the promoter. J Cell Mol Med 13: 680-692, 2009.
37. O'Hara A, Howarth A, Varro A and Dimaline R: The role of proteasome beta subunits in gastrin-mediatd transcription of plasminogen activator inhibitor-2 and regenerating protein1. PloS One 8: e59913, 2013.
38. Girousse A, Tavernier G, Tiraby C, et al: Transcription of the human uncoupling protein 3 gene is governed by a complex interplay between the promoter and intronic sequences. Diabetologia 52: 1638-1646, 2009.