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Molecular characterization of the porcine GBP1 and GBP2 genes

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Abstract

Experimental evidence indicated that interferon-inducible guanylate-binding proteins (GBPs) are similar with genes in the myxovirus (Mx) resistance protein subfamily of the large GTPases protein family and play important roles in the resistance to intracellular pathogens. As more diseases exert significant influence on pig industry, it is anticipated that more candidate disease-resistance genes could be found in future strategies aimed at improving genetic resistance to infectious diseases. In this study, we cloned cDNA sequences and analyzed the genomic structure of porcine GBP1 (poGBP1) and GBP2 (poGBP2). The two genes were mapped to SSC4q21-q23 and SSC4q24 by the SCHP panel respectively, further IMpRH panel analysis showed both genes were most closely linked to the marker SWR153. The deduced amino acid sequences of these two genes share the same three classical GTP-binding motifs at the amino terminus and are less conserved at the carboxyl termini except for a CaaX motif, compared with human and mouse counterparts. The reverse transcriptase-polymerase chain reaction (RT-PCR) revealed that poGBP1 and poGBP2 were both widely expressed in many tissues, and transient transfection indicated that poGBP1 and poGBP2 proteins were both located in cytoplasms within Pig Kidney Epithelial cells (PK15). Quantitative real-time PCR (Q-RT-PCR) analyses showed poGBP1 and poGBP2 had very similar expression patterns in PK15 cells at different time points after poly I:C stimulation, suggesting that ISRE (interferon-stimulated response element) plays a crucial role in the transcriptional regulation of these two genes. Four single nucleotide polymorphisms (SNPs) and three SNPs were detected in the cDNA sequences of poGBP1 and poGBP2, respectively. Association analyses revealed that the poGBP1 Eco81I and poGBP2 SspI polymorphisms both had significant associations ($p < 0.05$) with red blood cell count (RBC), haemoglobin concentration (HGB) and hematocrit (HCT) of 17-day-old pigs.

Keywords: Pig; GBP1; GBP2; Genomic structure; Localization; Association analyses

1. Introduction

Interferons are immunomodulatory cytokines that mediate anti-pathogenic and anti-proliferative effects in cells (Stark et al., 1998). The establishment of the antiviral effect by the IFNs is dependent on de novo (neogenesis) RNA and protein synthesis (Friedman and Sonnabend, 1965; Lockart, 1964; Taylor, 1964). By eliciting host antimicrobial programs in nearly all nucleated cells, interferons (IFNs) help to orchestrate the innate immune response of mammals to a diverse array of microbial pathogens. Recent work has highlighted several families of IFN-inducible guanosine 5′ triphosphatases (GTPases)—p47 (with a molecular mass of 47–48 kDa), p65 guanylate-binding protein (GBP) (with a molecular mass of 65–67 kDa), myxovirus resistance proteins (Mx) and very large inducible GTPases (VLIG) (MacMicking, 2004). Their roles on mammalian host defense and potential for pathogen specificity have been demonstrated: (1) murine Mx1 confers resistance to influenza virus (Staeheli et al., 1986), Thogoto virus (Haller et al., 1995), and Dhori virus (Thimme et al., 1995); (2) functional murine Mx2 confers resistance to vesicular stomatitis virus (VSV) (Zurcher et al., 1992); (3) human MxA protein confers resistance to influenza virus and VSV (Pavlovic et al., 1990), measles virus (Schnorr et al., 1993), and Thogoto virus (Frese et al., 1995; Thimme et al., 1995); (4) porcine Mx1 confers resistance to influenza virus, and has differential anti-influenza activity among allelic variants at the Mx1 locus (Palm et al., 2007); (5) human GBP1 confers resistance to VSV and Encephalomyocarditis virus (Anderson et al., 1999).

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The mammalian interferon-inducible guanylate-binding proteins composed a subfamily with the protein family of large GTPases. The p65 GBPs might alternatively restrict viral spread by limiting cell proliferation, such activity might help to limit the cell-to-cell spread of progeny virus, as seen for endothelial cells, an activity that requires the GBP helical domain (Guenzi et al., 2001). Although the overall primary sequence homology is low, the relationship of these proteins is more relevant at the structural level, and by shared biochemical features such as nucleotide dependent oligomerization and high turnover GTPase activity (Prakash et al., 2000).

In addition, there is evidence that human GBP1 is the key mediator of the inhibitory effects of inflammatory cytokines (IC) (such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α and interferon (IFN)-γ, etc.) on endothelial cells (EC) proliferation and invasiveness. hGBP1 may be used as a marker to monitor the IC-induced phenotype of EC in inflammation and may also be exploited as a target to modulate EC activity in inflammatory diseases and tumor angiogenesis (Naschberger et al., 2005). Gene expression profiling in Salmonella Choleraesuis-infected porcine lung using a long oligonucleotide microarray revealed that porcine GBP1 and GBP2 showed significantly increased expression in the top 25 differentially expressed genes (Zhao et al., 2006).

The studies mentioned above indicate that GBPs are important disease-resistance candidate genes. Modern pork production has encountered severe problems in regard to defense against the infectious diseases caused by intensive livestock breeding, and faces a complated array of related pathogens of bacteria and virus (Shinkai et al., 2006). Studies on important immune response genes such as GBPs would be of crucial importance in future strategies aimed at improvement of genetic resistance to infectious diseases. However, in pigs, the information on GBPs is very limited.

In this study, the genomic structures and cDNA sequences of porcine GBP1 and GBP2 were obtained. Transcriptional regulation in poly I:C treated cells of them were analyzed, in addition to their tissue expression patterns, chromosome assignments, subcellular cell localizations, polymorphisms and association analyses. The knowledge of GBPs gained in this study will contribute useful information for breeding from the viewpoint of disease resistance.

2. Materials and methods

2.1. Isolation of full-length coding regions of poGBP1 and poGBP2 genes

Human cDNA sequences of GBP1 (hGBP1) and GBP2 (hGBP2) (GenBank accession nos. NM_002053 and NM_004120) were compared with all sequences available in the expressed sequence tags (EST) and other databases by using BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/). We selected the porcine ESTs that shared more than 80% sequence identity to the corresponding human cDNA to assemble the porcine genes using SeqMan (DNASTAR, Inc., Madison, WI, USA). Two primer pairs (CDS-1F, CDS-1R for poGBP1 and CDS-2F, CDS-2R for poGBP2, Table 1) in both 5′ and 3′ untranslated region covering the entire coding sequences in cDNAs of these two genes were designed from the pig ESTs.

Total RNA was extracted from spleen tissue of an adult Chinese indigenous Tongcheng pig (Hubei Province, China) with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RT-PCR was performed using Taq polymerase (Fermentas, Vilnius, Lithuania), M-MLV reverse transcriptase (Promega, Madison, WI, USA). The predominant PCR product was gel purified and subsequently cloned into the pEGM-T-Easy vector (Promega) prior to sequencing.

2.2. Amplification and analysis of poGBP1 and poGBP2 5′-flanking regions

To obtain 5′-flanking regions of poGBP1 and poGBP2, contigs assembled from ESTs were blasted to pig genomic database. A *Sus scrofa* chromosome 4 clone (GenBank accession no. CU151869) contains genomic DNA sequences of these two genes was isolated. Approximately 3000 bp 5′-flanking fragments upstream from the ATG start codon spanned the exon1 for each gene were amplified by two primer pairs (pro-1F, pro-1R for poGBP1 and pro-2F, pro-2R for poGBP2, Table 1) using genomic DNA of a Tongcheng pig. The 5′-flanking DNA sequences were analyzed using Transcription Element Search System (http://www.cbil.upenn.edu/cgi-bin/tess/tess), CONSITE (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite/) and Promoter Scan (http://www.bimas.cit.nih.gov/molbio/proscan/).

2.3. Poly I:C treatment of PK15 cells and Q-RT-PCR analysis of gene expression

PK15 cells were plated in 6-well plates at a concentration of 2.5×10^5 cell/well (2 mL/well) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented 10% (v/v) fetal bovine serum under humidified air containing 5% CO_2 at 37 °C. When ∼70% confluence was observed, the medium was replaced with fresh challenge media, with or without polyriboinosinic-polyribocytidylic acid (poly I:C) (Amersham/GE Healthcare) at 10 μg/mL. Total RNA was harvested at 0, 6, 12, 24, 48 h by TRIzol, and RT-PCR was performed. Each real-time PCR in 25 μL reaction mixture contained SYBR® Green Real-time PCR Master Mix (ToYoBo), gene-specific primers (exp-1F, exp-1R for poGBP1 and exp-2F, exp-2R for poGBP2, Table 1) and template cDNA. The cycling conditions consisted of an initial, single cycle for 5 min at 95 °C followed by 40 cycles of 20 s at 95 °C, 20 s at 62 °C and 20 s at 72 °C. PCRs were performed and gene expression levels were quantitated relative to the expression of ribosomal protein L32 (RPL32) using Gene Expression Macro software (Bio-Rad, Richmond, CA, USA) employing the comparative Ct ($2^{-\Delta\Delta Ct}$) value method, in which RPL32 was used as an internal control to correct the differences in the mRNA quantities. The $2^{-\Delta\Delta Ct}$ equation is valid only if the amplification efficiencies of control and target genes are approximately equal. Triplicate cell