Molecular characterization and chromosomal assignment of porcine *BAX* and *TAC1*

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Abstract

The BCL-2 associated X protein (*BAX*) is a member of the Bcl-2 protein family and functions as an accelerator of programmed cell death (apoptosis). The preprotachykinin A gene (*TAC1*) encodes two tachykinin peptides (substance P and neurokinin A) that act as neurotransmitters in the central and peripheral nervous systems. To isolate the genes from the porcine genomic PAC library TAIGP714 probes were generated with primers derived from exons of the human orthologs. For *BAX*, a 501-bp long fragment (spanning exons 3 to 4) was amplified on porcine genomic DNA (GenBank Accession no: AM233489). The *TAC1*-specific primers formed a 415-bp long amplicon (spanning exon 7) in pigs (GenBank Accession no: AM233488). Probe sequencing and comparison with the human genes verified sequence identity (*BAX* 94% and *TAC1* 84%). The gene-containing PAC clones were further isolated and sequenced. The chromosomal assignment of the genes was done by analyses of porcine hybrid panels (somatic cell and radiation hybrid panel) and by fluorescent *in situ* hybridization. *BAX* was assigned to SSC6q21 and *TAC1* to SSC9q12-q14. So far, comparative exon sequencing using a panel of 138 animals (Angeln Saddleback, Pietrain, German Landrace, German Edelschwein, Swabian-Haellian swine, Buntes Bentheimer, Thai native pigs, Thai wild pig, Chinese Luchuan, Chinese Rongchang, Chinese Yushanei as well as German and Thai herniated crossbred piglets) identified two SNPs in *BAX* (SNPintron1: C→T, p(C)=0.804 and q(T)=0.196; SNPintron3: T→A, p(T)=0.975 and q(A)=0.025). Up to now, no SNPs have been found in *TAC1*. The chromosomal localization of *BAX* in combination with its known physiology proposes a possible contribution of the gene to the phenotype hernia, but this hypothesis has to be further elucidated.

Keywords: *BAX*, *TAC1*, SSC*6q21*, SSC*9q12-14*, pig

Introduction

The BCL-2 associated X protein (*BAX*) is a member of the Bcl-2 protein family and functions as an accelerator of programmed cell death (apoptosis). Mutation in the promoter regions of the human *BAX* gene has been reported. The presence of a G to A substitution at position (G-248A) in human *BAX* promoter was associated with lower *BAX* protein levels in chronic lymphocytic leukemia patients (Starczynski *et al*., 2005). *Preprotachykinin A (TAC1)* encodes two products of the tachykinins peptide family (substance P and neurokinin A) and the related peptides, neuropeptide K and neuropeptide gamma (Zimmer *et al*., 1998). They play an important role as neurotransmitters and interact with nerve receptors and smooth muscle cells. Mice with disruption of the *TAC1* are resistant to kainate excitoxicity and both necrosis and apoptosis of hippocampal neurons are prevented (Liu *et al*., 1999). The human *BAX* gene has been mapped to chromosome *19q13.3-q13.4* (Bonner *et al*., 1987) and human *TAC1* to chromosome *7q21-q22* (Apte *et al*., 1995). These two genes are considered in apoptosis mechanism. Characterization and mapping of the porcine orthologs might provide valuable information for reveal the controlled machinery of apoptosis processes.

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Materials and methods

Screening of PAC library: The porcine P1-derived artificial chromosome (PAC) library TAIGP714 (Al-Bayati *et al*., 1999) was screened by PCR using primer specific for *BAX* and *TAC1* designed base on the human orthologs. Standard PCR amplification was performed with primer *BAX* For/Rev and *TAC1* For/Rev (Table 1). PCR was conducted using 50 ng of porcine DNA, 100 µM of each dNTP, 20 pmol of each primer, 1.5 mM $MgCl_2$, and 2.5 U of *Taq*-polymerase in 1X PCR buffer as recommended by the manufacturer (Qiagen, Hilden, Germany) in a final reaction volume of 25 μl. The standard PCR profile was as follows: denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, specific annealing temperature of each primer for 45 s, and extension at 72 °C for 60 s. followed by a final elongation step at 72 °C for 10 min. Sequencing and subsequent BLAST (Altschul *et al*., 1997) comparisons was used to confirmed sequence identity to the human orthologs.

The PAC clone contained *BAX* gene was isolated by a combination of subcloning, primerwalking and sequencing. Purified plasmid DNAs were bidirectionally sequenced with the ABI Prism® BigDye™ Terminator Cycle Sequencing Kit (version 3.0) and M13fw/M13rev primers. Sequencing of PCR products was done with the respective gene-specific primers and 200 ng DNA- template directly after purification using the QIAquick PCR purification Kit (Qiagen, Hilden, Germany). All sequencing reactions were done on an ABI PRISM® 3100 DNA capillary analyzer (Applied Biosystems, Weiterstadt, Germany). Sequence data was evaluated and overlapping contigs generated with the Seqman software (DNASTAR, Inc, Germany).

Chromosomal assignment: The physical assignment of the PAC clones were performed by Fluorescence *in situ* Hybridization (FISH) on swine metaphase spreads (prepared from peripheral lymphocytes) obtained from a normal, healthy boar. Metaphase preparations and hybridization were carried out essentially as described previously (Lichter *et al*., 1990). The G like banding pattern generated by DAPI staining was used for chromosome identification and for regional assignment of the hybridization signals. FISH experiments were carried out twice, using duplicate slides. A porcine rodent somatic cell hybrid panel (Yerle *et al*., 1996) and a porcine whole-genome radiation hybrid panel (Yerler *et al*., 1998) were screened by PCR using the *BAX* and *TAC1* specific primers. The RH panel results were evaluated using the INRA Toulouse website (http://imprh.toulouse.inra.fr) and the results for somatic cell hybrid panel were analyzed using http://www.toulouse.inra.fr/lgc/pig/hybrid.htm.

Table 1 List of primer used in this study

Primer Name	Primer Sequence (5'→ 3')	Tm (ºC)	Amplicon (bp)
***BAX* For**	AGCTGAGCGAGTGTCTCAA	60.8	501
***BAX* Rev**	CAGTTGAAGTTGCCGTCAG	60.8	501
***TAC1* For**	CAGCTTCATTTGTGTCAATGG	50.9	415
***TAC1* Rev**	CATGAAAATGCTTCAGAGATAC	50.9	415
***BAX* SNP-1 F**	AAATGTAAAACGACGGCCAGTTGCGGATTTGAG GCGTAATG	63	778
***BAX* SNP-1 R**	AAACAGGAAACAGCTATGACCGGTCCTCACAGG TCTGAG	63	778
***BAX* SNP-2 F**	TCAGTTCATCTAGCAGGGAC	54	416
***BAX* SNP-2 R**	CCATGTTACTGTCCAGTTCATC	54	416

Mutation detection and genotyping: Single nucleotide polymorphisms (SNP) in the porcine *BAX* gene were detected with direct sequencing approach. Two single nucleotide polymorphisms (SNPs) were detected in non-coding region of intron 1 (C→T), allele C comprises a restriction site for the enzyme *Ear*I and intron 3 (T→A), allele T comprise a restriction site for the enzyme *BspH*I. Total of 138 animals with different breeds were genotyped (Angeln Saddleback, Pietrain, German Landrace, German Edelschwein, Swabian-

Haellian swine, Buntes Bentheimer, Thai native pigs, Thai wild pig, Chinese Luchuan, Chinese Rongchang, Chinese Yushanei as well as German and Thai herniated crossbred piglets). The RFLP reactions were performed according to the manufacturer (NEB, Schwalbach, Germany) with the respective restriction enzymes (*Ear*I and *BspH*I) (1U each, the supplemented buffer and BSA if recommended) for overnight at 37 °C in a volume of 30 µl. The resulting fragments were separated on a 2% agarose gel stained with ethidium bromide and visualized on an ultraviolet transilluminator.

Result and discussion

Gene characterization: The *TAC1*-specific primers formed a 415-bp (AM233488) long amplicon (spanning exon 7 and 3'UTR) in pigs. For *BAX*, a 501-bp (AM233489) long fragment (spanning exons 3 to 4) was amplified on porcine genomic DNA. Probes were sequence and comparison with the human genes verified sequence identity of 84% and 94% for *TAC1* and *BAX*, respectively. The *BAX*-containing PAC-clone was isolated and sequenced. The partial porcine *BAX* gene isolated sequence consists of the 5'-UTR and exons 1 to 4. Exon-intron boundaries are conserved (Table 2) and ranging in length from 52 bp to 136 bp. A comparison of the porcine *BAX* coding region with mammalian orthologs revealed nucleotide sequence identities of 94% with *Bos taurus* (NM173894.1) and 93% with Human (NM004324.3).

Table 2. Intron-exon boundaries and exon lengths of porcine *BAX* gene.

Exon	Length (bp)	Splice acceptor	Splice donor	Intron	Length (bp)
1	86		AGGCGGGG*gt*gaggcg	1	563
2	52	tcctct*ag*GGCCCACC	CTTCAGGG*gt*gagtgt	2	91
3	149	cactct*ag*TTTCATCC	GCAGAGGT*gt*ggcccc	3	351
4	136	ccctgc*ag*GATGATCG	TGCTCAAG*gt*gggcga		

Chromosomal assignment: The chromosomal localization of the porcine *TAC1* and *BAX* genes were done by *FISH* and showed a signal on *SSC9q12-q14* and *SSC6q21*, respectively (Fig 1). Analysis of the somatic cell hybrid panel revealed a significant correlation of 1.00 between *TAC1* and *SSC9,* 0.86 between *BAX* and *SSC6* (error risk lower than 0.5% and 0.1%, respectively). Radiation hybrid panel analysis showed that *TAC1* linked to marker *SW915* on *SSC9q* (67 cR distance and LOD score 5.79) and *BAX* linked to marker *S0220* on *SSC6q21* (18 cR distance and LOD score 16.35). The chromosomal localization of *BAX* in combination with its known physiology proposes a possible contribution of the gene to the phenotype hernia, but this hypothesis has to be further elucidated.

SNPs detection: SNPintron1, A 416 bp PCR fragment was amplified by *BAX*-SNP2 primer combination with *EarI* digestion. Two alleles could be distinguished. Allele T has no recognition site for *EarI* and shows an undigested PCR product, where as allele C has recognition site for *EarI* and shows the fragments 120 and 296 bp. Figure 2 showed the PCR product after digested with *EarI* and the related sequence. SNPintron3, 778 bp PCR fragment was amplified by *BAX*-SNP1 primer combination with *BspHI* digestion. Allele T has recognition site for *BspHI* and showed 2 bands after digestion (fragments 324 and 454 bp), where as allele A has no recognition site for *BspHI* and showed an undigested PCR product. Allele frequency estimation of SNPintron1 are p(C)=0.804, q(T)=0.196 and SNPintron3 are p(T)=0.975,q(A)=0.025).

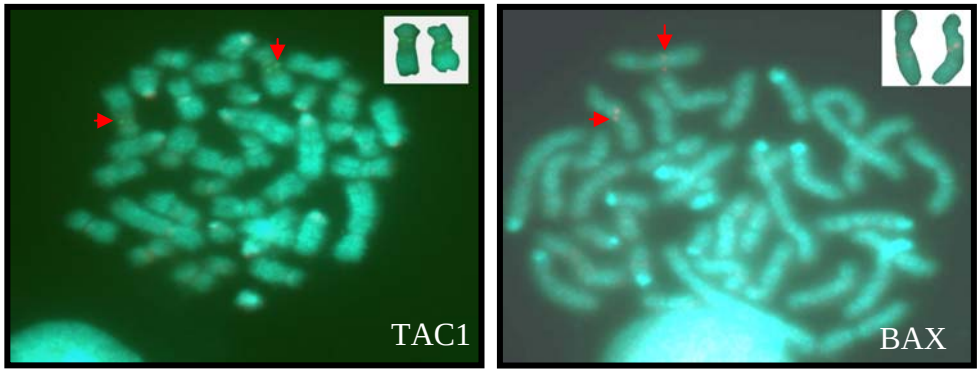

Figure 1. FISH mapping results of PAC-clones contain *TAC1* and *BAX* gene. Signals are marked by arrows.

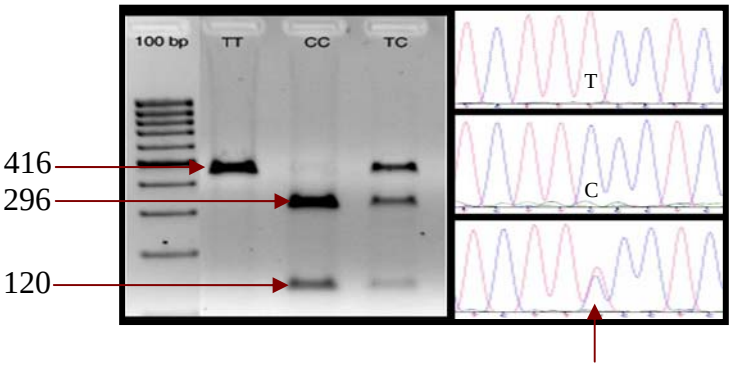

Figure 2. Restriction pattern and sequencing chromatograms of the SNPintron1 of the *BAX* gene.

Acknowledgments:

The authors would like to thank Viola Raupach for expert technical assistance. And special thank to Ratchaneewan Wongprachan for statistical analyses.

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