Isolation, sequence identification, and tissue expression profile of 3 novel porcine genes: NCF2, BCKDHB and BCKDHA

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Abstract. The complete coding sequences of porcine genes NCF2, BCKDHB and BCKDHA were amplified by using reverse transcriptase polymerase chain reaction (RT-PCR), basing on the conserved coding sequence information of humans or other mammals. These 3 novel porcine genes were then assigned GeneIDs: 100142665, 100142669 and 100142666. The phylogenetic tree analysis revealed that the porcine NCF2, BCKDHB and BCKDHA all are most closely related to the bovine NCF2, BCKDHB and BCKDHA. Tissue expression profile analysis revealed that porcine NCF2, BCKDHB and BCKDHA genes were differentially expressed in tissues, including skeletal muscle, the heart, liver, fat, kidney, lung, small and large intestine.

Keywords: BCKDHA, BCKDHB, NCF2, pig.

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White pigs. Total RNA extraction and first-strand cDNA synthesis for these tissue samples were performed according to the methods described by Liu et al. (2004).

The RT-PCR was performed to isolate these 3 porcine genes by using the pooled cDNAs from the various tissues mentioned above. The 25-μL reaction volume included: 2.0 μL of cDNA, 2.5 μL of 2 mM mixed dNTPs, 2.5 μL of 10 × Taq DNA polymerase buffer, 2.5 μL of 25 mM MgCl₂, 2.0 μL of 10 μM forward primer, 2.0 μL of 10 μM reverse primer, 2.0 units of Taq DNA polymerase (1 U μL⁻¹), and 9.5 μL of sterile water. The primers for porcine NCF2 gene isolation (forward: 5'-ATG TCC CTG GCT GAG GCC-3', reverse: 5'-CTA GAC TCC AGG AGT GCT-3', Ta = 50°C) were designed basing on the conserved coding sequence information from human, rat and mouse NCF2 genes and the highly homologous pig EST sequences: BG895203, DV228478, CJ019790 and EW336216. Similarly, the primers for porcine BCKDHA gene isolation (forward: 5'-ATG GCG GCA GTG GCA GCG -3', reverse: 5'-CTA ATA GTT GAT CAT TTTG-3', Ta = 55°C) were designed basing on the conserved coding sequence information from human, rat and mouse BCKDHA genes and the highly homologous pig EST sequences: DT330393, DT321133 and DY409730. Further BLAST analysis of these proteins revealed that the porcine NCF2 is highly homologous to the neutrophil cytosolic factor 2 (NCF2) of 4 species: domestic cattle (88%), human (87%), mouse (82%), and rat (71%). The porcine BCKDHA is highly homologous to the branched chain keto acid dehydrogenase E1, beta polypeptide (which causes maple syrup urine disease) (BCKDHB) of 4 species: domestic cattle (93%), human (92%), rat (89%), and mouse (89%). The porcine BCKDHA is highly homologous to the branched chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA) of 5 species: domestic cattle (95%), human (93%), chimpanzee (93%), crab-eating macaque (93%), and mouse (91%). Based on the above BLAST results, the phylogenetic tree analysis using the ClustalW software (clustalw) confirmed that the porcine NCF2, BCKDHB and BCKDHA are all more closely related to the bovine NCF2, BCKDHB and BCKDHA.

RT-PCR for tissue expression profile analysis was performed as previously described elsewhere (Liu and Xiong 2007). We selected the housekeeping gene G3PDH (glyceraldehyde-3-phosphate dehydrogenase) as the internal control. The control primers used were: 5'-ACC ACA GTC CAT GCC ATC AC-3' (G3PDH 5' primer) and 5'-TCC ACC CTG TTG CTG TA-3' (G3PDH 3' primer). The primers of porcine NCF2, BCKDHB and BCKDHA genes were then cloned into a PMD18-T vector (TaKaRa, China) and sequenced bidirectionally with the commercial fluorometric method. At least 5 independent clones were sequenced for every gene.

Through RT-PCR with pooled tissue cDNAs, for porcine NCF2, BCKDHB and BCKDHA, the resulting PCR products were 1578 bp, 1191 bp, and 1344 bp (Figure 1). Analysis of these cDNA nucleotide sequences by using the BLAST software at the NCBI server (http://www.ncbi.nlm.nih.gov/BLAST) revealed that these genes were not homologous to any of the known porcine genes, so they were then deposited in the GenBank database (accession numbers: EU442567, EU442568 and EU442569). The sequence prediction was carried out using the GenScan software, and the results showed that the 1578-bp, 1191-bp and 1344-bp cDNA sequences represent 3 single genes that encoded 525, 396 and 447 amino acids, respectively. The theoretical isoelectric point (pI) and molecular weight (Mw) of the deduced proteins of these 3 porcine genes were computed using the Compute pI/Mw Tool. The pI values of porcine NCF2, BCKDHB and BCKDHA genes are 5.40, 5.89 and 8.15, while their molecular weights are 59093.47, 43295.71 and 50860.48 Da, respectively. Finally these 3 novel porcine genes were assigned GeneIDs: 100142665, 100142669 and 100142666.