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Identification of Free Fatty Acid Receptors GPR40/FFAR1 and GPR120/FFAR4 in a Domestic Cat

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ABSTRACT

G-protein-coupled receptors (GPRs) 40 and 120 are members of the Free Fatty Acid (FFA) receptor group and are termed FFAR1 and FFAR4, respectively. The aim of this study was to clone cat GPR40 and GPR120 cDNAs in several tissues. There was high sequence homology to other mammalian GPR40 and GPR120, with encoding 320 and 361 amino acid residues, respectively. Cat GPR40 encoded extra 21 amino acid residues in the C-terminal cytoplasmic region. Quantitative RT-PCR revealed expression of GPR40 mRNA in the duodenum, liver and pancreas. The GPR120 mRNA was expressed in adipose tissues, cerebral cortex and colon. In conclusion, GPR40 and GPR120 were well conserved and were expressed in cat tissues with different distribution patterns.

Key words: Cat, fatty acid, receptor

INTRODUCTION

The G-protein-coupled receptors (GPRs) 40 and 120 were initially identified as members of the orphan GPR and both genes were sequentially identified in the non-coding human CD22 gene for GPR40 and as a homologue of rhodopsin-like protein for GPR120 (Fredriksson *et al*., 2003; Sawzdargo *et al*., 1997). Both GPR40 and GPR120 were found to be the receptors for long-chain Free Fatty Acids (FFA) and are therefore also known as FFAR1 and FFAR4, respectively (Briscoe *et al*., 2003; Hirasawa *et al*., 2005; Itoh *et al*., 2003; Kotarsky *et al*., 2003). The GPR40 and GPR120 are therapeutic targets for diabetes mellitus because these molecules have important roles in the regulation of glucose and lipid metabolism (Ichimura *et al*., 2014). The FFAs are important energy source and they also act as signaling molecules in various cellular processes, including insulin secretion.

Type 1 diabetes is the most common form of diabetes mellitus in dogs (>50% cases), whereas type 2 diabetes prevails in cats (80-90% cases) (Rand *et al*., 2004). Plasma FFAs concentrations are

commonly elevated in obese and type 2 diabetes (Gordon, 1960; Reaven *et al*., 1988) and a high-fat diet caused lipotoxicity during the early phase of obesity in domestic cats (Mori *et al*., 2014). To understand FFAs and their physiological roles in metabolic regulation, we conducted experiments on cDNA cloning and the tissue mRNA expression profiles of GPR40 and GPR120 in a domestic cat.

MATERIALS AND METHODS

Cloning of cat GPR40 and GPR120 cDNA: The cDNA of cat GPR40 and GPR120 were cloned by a PCR-based method. Total RNA from various tissues was obtained from three-year-old male cat (Zyagen, CA, USA). Tissues were collected in the morning time from unstarved normal and regular cat. Complementary DNA (cDNA) libraries were prepared from total RNA of pancreatic (for GPR40) and adipose tissue (GPR120) using the SMARTer RACE cDNA amplification kit (Clontech, CA, USA). The 5'-ends of GPR40 and GPR120 were amplified by using antisense 40-1 (5'-CCCCGCCGGCCACCCAGGCTGCCCT-3') and 120-1 (5'-GATGGCGCTGGTGAAGAGCAGGT-3') primers. The 3'-ends of GPR40 and GPR120 were amplified by using sense 40-2 (5'-CAGCTCCCTGGGCATCAACACGCC-3') and 120-2 (5'-TCCAGGACCATCACATCACT-3') primers, respectively. The full-ORF region of GPR120 cDNA fragment was amplified with another pair of sense 120-3 (5'-GGGCATGTCCCCTGAGTG-3') and antisense 120-4 (5'-ATTAAACTCAATCATATTACAAACA-3') primers. The cDNA sequence of determined using the ABI Prism 310 Genetic analyzer (Applied Biosystems, CA, USA). Primers were designed from predicted cat GPR40 (NW_004064592 and NW_004065208) and GPR120 (AANG02046668, ti643950600 and ti922610552) genomic sequences from GenBank.

The mRNA expression profile of cat GPR40 and GPR120 in tissues: Total RNA (1 μg) was reverse-transcribed using the QuantiTect Reverse Transcription Kit (Qiagen, Tokyo, Japan). The genomic DNA was removed by DNase treatment and cDNA was synthesized. One microliter of the cDNA product was subjected to quantitative PCR (Q-PCR) according to the user's instructions for the Real-Time PCR System 7300 (Applied Biosystems). The PCR was performed at 95°C for 5 sec and 60°C for 35 sec in 20 μL of buffer containing 1×Premix EX Taq II (Takara, Shiga, Japan), 1×ROX reference dye and 0.4 μM each of primers. Specific primers were designed for GPR40 (primers 40-2 and 40-3 (5'-GCCAGTGCCCGGAGGCAGCCCACGTA-3')) and GPR120 (primers 120-5 (5'-TCGTTTGTTACTTTGAACTTCTTG-3') and 120-6 (5'-ATGGGGCTCCACATAATGAA-3')). Quantitative measurement was performed by establishing a linear amplification curve from serial dilution of corresponding-region cDNAs of cat GPR40, GPR120 and $-actin. Each value of GPR40 and GPR120 mRNA were normalized to that of $-actin mRNA (primers $-actin-S (5'-GCCAACCGTGAGAAGATGACT-3') and $-actin-A (5'-CCCAGAGTCCATGACAATACCAG-3')).

RESULTS AND DISCUSSION

The complete sequence data of cat GPR40 and GPR120 cDNAs has been submitted to DDBJ/EMBL/GenBank databases under accession number LC014925 and LC014926, respectively. Cat GPR40 cDNA consisted of 338 bp of 5'-untranslated region (UTR), 960 bp of the coding region and 296 bp of the 3'-UTR. Cat GPR120 cDNA consisted of 56 bp of 5'-UTR, 1083 bp of the coding region and 317 bp of 3'-UTR. A computer assisted search of cat GPR40 cDNA sequences in the cat genome (www.ncbi.nlmnih.gov/BLAST) showed that cat GPR40 consists with three exons. In contrast, GPR40 cDNA consists of a single exon in human, mouse, rat and pig. Cat GPR120 cDNA consisted of three exons and it was same with other mammalian GPR120 cDNAs. Although the long

splice variant of GPR120 (GPR120-L) was only detected in the human colon, the human GPR120-L cDNA had four exons (Galindo *et al*., 2012). We successfully cloned cat GPR120 that corresponded to the short splice variant of human GPR120 (GPR120-S). The alternative splicing exon consists 48 bp (16 amino acid residues) in human GPR120-L and is inserted into third intercellular loop region. We tried unsuccessfully to clone the alternative splicing exon in cat using RT-PCR with primer 120-3 and primer 120-4, or primer 120-5 and primer 120-6 and were able to find the alternative splicing sequence in cat genomic DNA sequence by BLAST search. It is unknown if there is an alternative exon, however it is likely that the GPR120-S isoform dominates in cat.

The predicted sequences of cat GPR40 and GPR120 showed high similarity between human and other animals (Fig. 1a-b). The seven transmembrane (TM1-7) regions are conserved in cat GPR40 and GPR120 with those of other animals (Fredriksson *et al*., 2003; Sawzdargo *et al*., 1997). There are two N-glycosylation sites (N-X-S/T) in the second extracellular loop of cat GPR40 (N155, N165), N-(N21) and in the C-terminal (N322) regions of cat GPR120 (Fredriksson *et al*., 2003; Sawzdargo *et al*., 1997). An E/DRY motif, which is known to be involved in the modulation of ligand binding and G-protein activation, is present at the junction of TM3 and the second cytoplasmic loop in GPR40 (as GRY motif) and GPR120 (as ERM motif) in cat and other species (Strader *et al*., 1994). However, low sequence similarity and 21 unique amino acid residues (300-QQPPLERRGTEQGRPAALAGP-320) were observed in the C-terminal region of cat GPR40. We cloned cat GPR40 full-cDNA sequence by RACE-PCR. Moreover, the unique 21 amino acid sequence (from Q300 to P320) was identified in cat genomic sequence and computer-based predicted sequence (GenBank accession number: NC_018737). The 63 bp of unique cat cDNA sequence was conserved with human GPR40 genomic sequence. The presence of 21-amino acid residues may be explained as a result of changing from “t” to “c”, in the stop codon [nucleotide position 947-tag-949 in porcine GPR40 (NM_001278783); nucleotide position 911-taa-913 in human GPR40 cDNA (NM_005303); nucleotide position 1435-tag-1437 in mouse GPR40 (AF539809)] was translated to glutamine (Q300) amino acid residue in cat GPR40 (nucleotide position 1236-cag-1238 in cat GPR40 cDNA). These 21 amino acid residues can also be detected in the predicted amino acid sequence of Amur tiger GPR40 (XP_007097010), of which 19 amino acids matched with cat GPR40 sequence. In the case of GPR120, phosphorylation of amino acid residues in C-terminal tail is essential for association with $-arrestin-3 independently from $G_{q/11}$ coupling (Butcher *et al*., 2014). In addition, it may difficult to speculate about the absence or presence of 21 unique amino acid residues to receptor function without functional assays (such as measuring intracellular Ca^{2+} response and $-arrestin-3 interaction). Further investigations will need to clarify the question in cat.

To examine the mRNA expression profiles in cat tissues, we conducted an experiment using tissues from male adult cat GPR40 and GPR120 mRNA expression by quantitative RT-PCR. The mRNA expression profile of cat GPR40 revealed higher levels tendency in pancreas and spleen (Fig. 2). Similar mRNA expression profiles of GPR40 were observed in humans, rats and mice (Briscoe *et al*., 2003; Hirasawa *et al*., 2005; Itoh *et al*., 2003), but mRNA expression was not detected in pig (Colombo *et al*., 2012). In the pancreas, greater mRNA expression of GPR40 was observed in rat islets and some cell lines established from $-cell (Itoh *et al*., 2003). The GPR40 mediates insulin secretion and is activated by medium-chain and long-chain FFAs in humans and mice (Briscoe *et al*., 2003; Itoh *et al*., 2003). The GPR120 mRNA was predominantly expressed in the adipose tissue, cerebral cortex and colon (Fig. 2). Similar mRNA expression profiles of GPR120

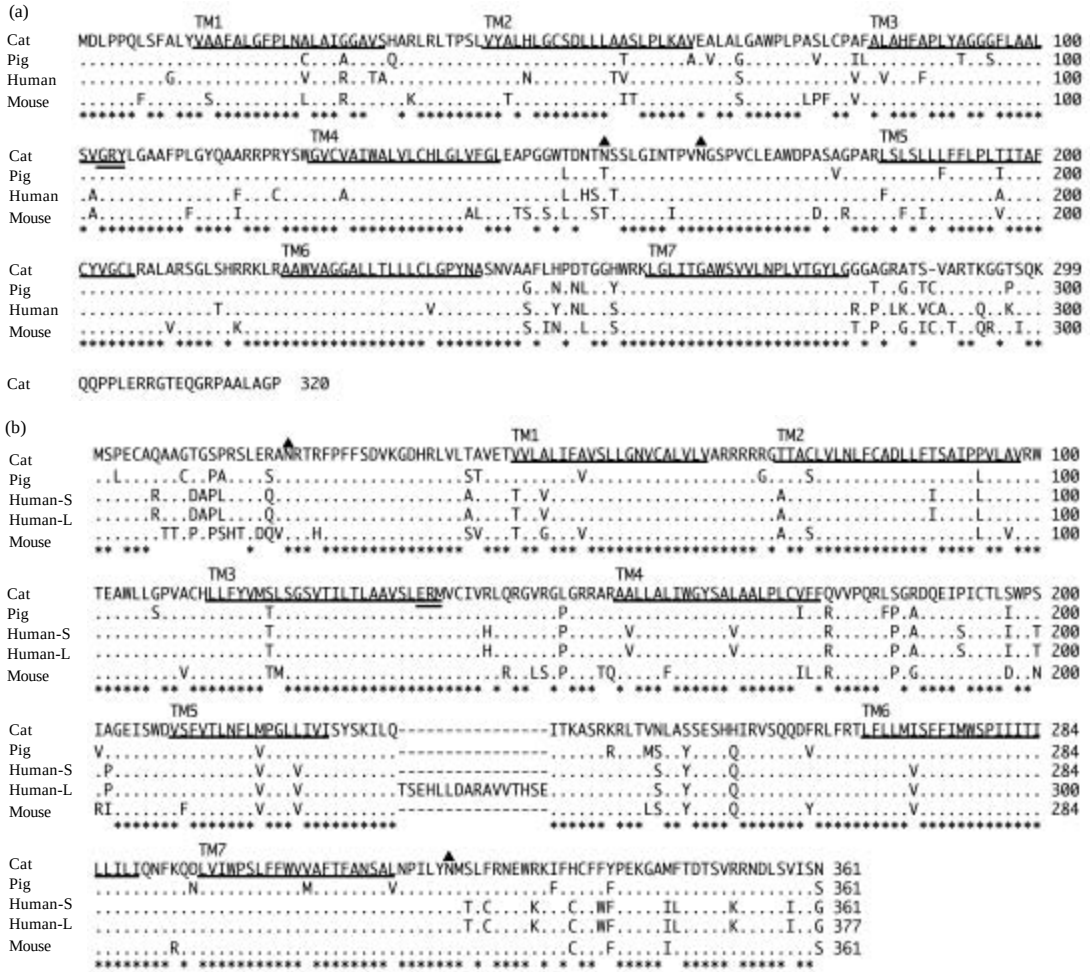

Fig. 1(a-b): Alignment of the amino acid sequences of cat (a) GPR40 and (b) GPR120 to other mammalian GPRs. The amino acid sequences of cat GPR40 (LC014925) are aligned with those of Pig (NM_001278783), Human (NM_005303), Mouse (AF539809) GPR40. The amino acid sequences of cat GPR120 (LC014926) are aligned with those of Pig (HQ662564), Human-S (NM_001195755), Human-L (NM_181745), Mouse (NM_181748) GPR120. The seven transmembrane (TM1-7) are underlined. Identical amino acid residues represented as dot. Asterisks indicate amino acid residues matched to all GPR40 and GPR120, respectively. The putative N-glycosylation sites are indicated by black-triangles. The E/DRY motifs (GRY in GPR40 and ERM in GPR120) are indicated by double-underline

were observed in humans, mice and pigs (Colombo *et al*., 2012; Gotoh *et al*., 2007; Hirasawa *et al*., 2005). We succeeded in cloning and measuring cat GPR120 expression as short-form (GPR120-S) cDNA but not as GPR120-L cDNA. The GPR120-L cDNA was cloned in human colon and the expression level was very low (Galindo *et al*., 2012; Hirasawa *et al*., 2005). Molecular characterization for these two isoforms gave differences in signaling responses by

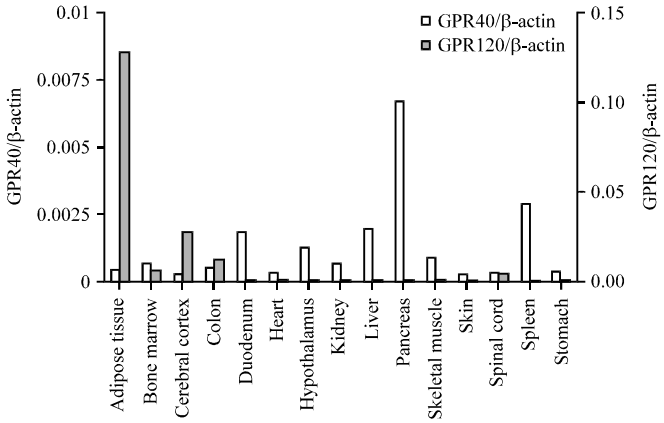

Fig. 2: GPR40 and GP120 mRNA expression in cat tissues

challenging various short-chain and medium-chain FFA (Galindo *et al*., 2012). These data suggested that GPR120-S was dominantly expressed in cat tissues and might play important roles in lipid metabolism.

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