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MOLECULAR CLONING AND TISSUE EXPRESSION PATTERN OF PARTIAL HEPATIC GROWTH FACTOR cDNA IN ARABIAN CAMEL (*Camelus dromedarius*)

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ABSTRACT

The present study was performed to clone hepatic growth factor (HGF) in Arabian camel. RT-PCR was conducted on RNA from skeletal muscle using primers designed from the conserved regions in different animal species. The resultant PCR amplicon was subjected to sequencing and bioinformatics analysis. The results revealed that the obtained sequence belongs to HGF gene family. The nucleotides sequence was deposited in the GenBank with accession number KU736793. Furthermore, the data showed base frequencies of A = 32.77%, C = 21.67%, G = 22.06% and T = 23.5%. The nucleotide sequence alignment revealed that *Camels dromedarius* HGF showed 99% identity with *C. bactrianus* and *C. ferus* HGF, while it showed 97% identity with those of either *Bos taurus*, *Capra hircus*, *Ovis aries* and *Equus caballus*. Of the 766 nucleotides analysed, 36 substitutions varied from transitions and transversion were detected. The translated amino acids showed 2 non-synonymous substitutions discriminating camelids from other species; serine (S_{46}) into alanine (A_{46}), alanine (A_{46}) in other species at $T_{136}\rightarrow G_{136}$. The results showed a clear expression of HGF mRNA in a wide variety of the tested tissues; skeletal muscle, spleen, testes, liver, kidney and heart. The obtained results could be useful for more understanding of the structural-function relationship of HGF in Arabian camel and addressing the genetic diversity of the Arabian camel.

Key words: *Camelus dromedarius*, cloning, expression pattern, HGF

Camel has been historically and economically an important species worldwide and especially in Arab Peninsula. Saudi camels comprise 16% of the animal biomass (Al-Swailem *et al,* 2010). Although, dromedary camel has a high economic value in Saudi Arabia, the literatures about its reproduction biology and the molecular studies targeting its genome are limited. It is important to know the nucleotide sequence of various genes as this enables us to correlate between gene sequence and its functions to improve diagnosis of disease and improve drug design to target specific gene products that cause disease.

Many attempts have been carried out to identify some genes for single hump camel, especially in the Arabic regions including putative stress-induced heat-shock protein (Elrobh *et al,* 2011), putative copper-zinc SOD (Ataya *et al,* 2014) and c-Met (El-shazly *et al,* 2016). Growth factors are body proteins act to regulate cell division, differentiation and survival. They can also be produced through recombinant DNA technologies in the laboratory and used in the biological therapeutic purposes. HGF and its c-Met receptor are considered to be of the most important growth factors. HGF has molecular weight of 84 kDa (Nakamura *et al,* 1987; Nakamura and Mizuno, 2010). HGF is a dimeric molecule composed of an α-subunit (69 kDa) and a β-subunit (34 kDa), respectively linked by a disulfide bond. It gives two bands of 69 kDa and 34 kDa under reducing conditions. Thus, it is a heat-labile protein originally was discovered as a mitogen of adult rat hepatocytes (Nakamura *et al,* 1987; Nakamura and Mizuno, 2010). HGF is a multifunctional cytokine derived from stroma. It induces cell proliferation, differentiation and motility in a variety of epithelial cells by binding to the product of the c-Met proto-oncogene (Trusolino and Comoglio, 2002; Birchmeier *et al,* 2003; Yamaji *et al,* 2006). Moreover, HGF and c-Met have been involved in the embryonic and postnatal development of a variety of tissues including those of the mammary gland (Trusolino and Comoglio, 2002; Birchmeier *et al,* 2003; Yamaji *et al,* 2006). Whereas, HGF has a cytotoxic effect on certain tumour cells, such as haematoma, (HepG2.16) with regard to this, Higashio *et al* (1990) found that human lung fibroblasts (IMR-90) secrete a soluble factor named tumour cytotoxic

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factor (TCF) capable of killing Sarcoma-180. This group isolated TCF cDNA from the cDNA library of IMR-90 cells. As a consequence, TCF was found to be molecularly identical to HGF (Nakamura and Mizuno, 2010; Higashio *et al,* 1990).

The present study was aimed to clone hepatic growth factor (HGF) gene which is a key player in cell growth, differentiation and survival in addition to study its tissue distribution in Arabian camels.

Materials and Methods

Sampling

Different tissue samples were collected from the local slaughterhouse (Taif, Saudi Arabia). These tissue samples included samples from liver, kidney, spleen, heart, skeletal muscle and testes. Samples were exposed to sudden freezing in liquid nitrogen, transferred into laboratory and kept at - 80°C until used.

Primer Design

Two sets of degenerated primers were designed. The first (HGF-1F and HGF-1R) was from the highly conserved regions of HGF gene available in the gene bank for different species including *C. bactrianus* XM_010946631.1, *C. ferus* XM_006194335.2, Equidae (*Equus caballus* XM_014739144.1) and Bovidae (*Bos taurus* NM_001031751.2 and *Capra hircus* XM_018047278.1). The second one (HGF-2F and HGF-2R) was internal primers and was designed according to the obtained sequence and used for studying tissue distribution. Another set of primers (GAPDH-F and GAPDH-R) was for GAPDH and designed from the sequence of *C. dromedarius* GAPDH XM_010975572 (Table 1).

RNA Extraction, cDNA Synthesis and Reverse Transcription PCR

Total RNA was extracted according to the method described by Ahmed *et al* (2014) using Qiazol lysis reagent as per the manufacturer's instructions. Briefly, 100 mg of each tissue sample was homogenised in 1ml QIAzol (QIAGEN Inc., Valencia, CA) then 0.3 ml chloroform was added to the homogenate. The mixtures were shaken for 30 s followed by centrifugation at 4°C and 12,500 rpm for 20 min. The supernatant layer were transferred into a new set of tubes and an equal volumes of isopropanol were added to the samples, shaken for 15 seconds and centrifuged at 4°C and 12500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, briefly dried up then, were dissolved in diethylpyrocarbonate (DEPC) water. The prepared RNA integrity was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm and 280 nm.

For synthesis of cDNA, mixture of 2 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µl sterilised DEPC- water were incubated in the PeX 0.5 thermal cycler (Thermo Electronic Corporation, Milford, Ma) at 70°C for 10 min for denaturing. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U RevetAid Premium reverse transcriptase (Fermentas Canada Inc. Harrington Court, Burlington Ontario) were added and the total volume was completed up to 20 µl by DEPC water. The mixture was then re-incubated in the thermal cycler at 30°C for 10 min, at 42°C for 1 h and at 90°C for 10 min. The resulted cDNAwas preserved at -20°C until used.

Polymerase Chain Reaction ((PCR)

To amplify cDNA of HGF and GAPDH, polymerase chain reaction (PCR) and specific primers for each gene (Table 1) were used. PCR was conducted in a final volume of 50 µl consisting of 2 µl cDNA, 1µl (10 picomoles) of each primer and 25 µl PCR master mix (Promega Corporation, Madison, WI, USA) the volume was brought up to 50 µl using sterilised deionised water. PCR was carried out using a PeX 0.5 thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the cycle sequence at 94°C for 5 min one cycle, followed by 35 (for HGF) and 25 (for GAPDH) cycles each of which consisted of denaturation at 94°C for 1 min, annealing at the specific temperature corresponding to each primer set (Table 1) and extension at 72°C for 1 min with an additional final extension at 72°C for 5 min. PCR products were electrophoresed on 1.5% agarose gel

Table 1. Primers and PCR conditions used for the tested dromedary genes.

Gene	Primer name and sequence (5` - 3`)	Annealing and Cycles	Product size
HGF	HGF1-F:AAAAGAAGAAACACACTTCATGAATTC HGF1-R: GCATTCAGTTGTTTCCATAGGG	54°C, 35 cycles	818 bP
HGF HGF	HGF2-F: CATTCCTCAGTGTTCAGAAG HGF2-R: TTGCCATTCCCACGATAACA	53°C, 35 cycles	454 bP
GAPDH (XM_010990867)	GAPDHF- TGGGAAGCTAACTGGCATG GAPDHR- AGGCAGGGCTCCCTAAGC	53°C, 25 cycles	550 bp

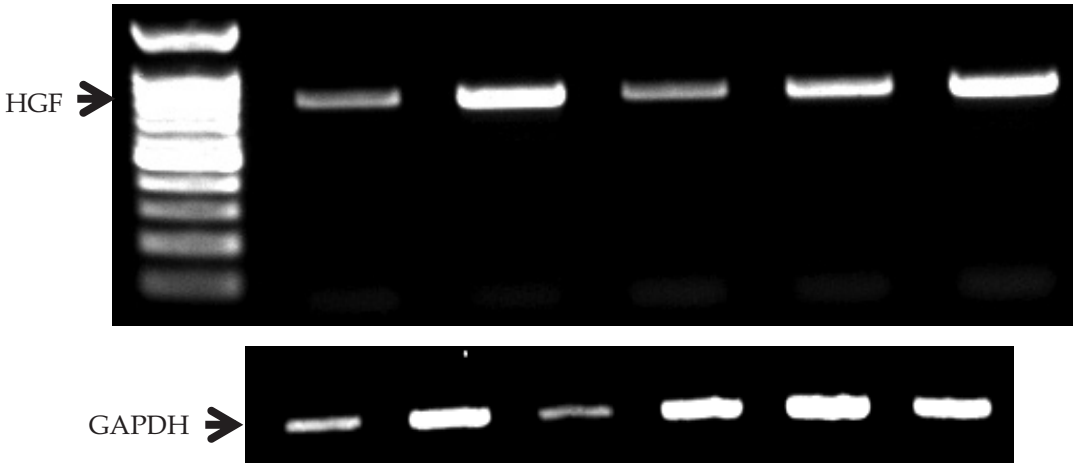

Fig 1. Expression of HGF in skeletal muscle from 5 different Arabian camels.

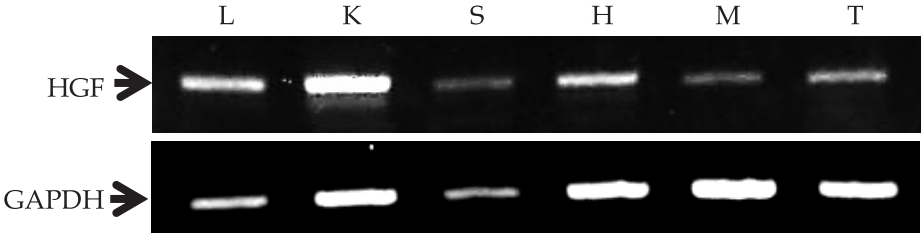

Fig 2. Tissue distribution of HGF mRNA Arabian camels. L: Liver, K: Kidney, S: Spleen, H: Heart, M: Muscle, T: Testis.

(Bio Basic, Konrad Cres, Markham, ON, Canada), stained with ethidium bromide in TAE (Tris–acetate–EDTA) buffer (Sigma–Aldrich, St. Louis, MO, USA). PCR products were visualised under UV light and photographed using gel documentation system (UVP, Upland, CA, USA). Following that, PCR product was purified using FavorPrep PCR Clean-Up mini kit according to the manufacturer's instructions.

Sequence analysis of PCR product

Purified PCR products for HGF were sequenced in an ABI PRISM 3730xl sequencer (Applied BioSystems) and BigDyeTM Terminator Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems) following the protocols supplied by the manufacturer. After reading the targeted genes, the nucleotide sequences have been treated with DNASIS software programs. Amino acid sequence was obtained by translating the sequenced DNA fragment using the DNASIS program and the deduced amino acid sequence was compared with sequences obtained from searches in the NCBI protein database using the BLASTP algorithm.

Results and Discussion

Several studies were conducted to identify some of the Arabian camel genome. Of these studies is Heat-Shock protein (Elrobh *et al,* 2011), putative Copper-Zinc SOD (Ataya *et al,* 2012), putative cytochrome P450s (Saeed *et al,* 2014), heat shock protein 90α (Saeed *et al,* 2015) or even mitochondrial genes (Ahmed *et al,* 2013) and Kappa casein (Minoia *et al,* 1998). The majority of studies performed on camel researches addressed mainly the milk and meat composition and how can it adapt to the harsh, arid climate which could be due to the unique physio-anatomical features of the Arabian camels (Saeed *et al,* 2015). The study of camel genome may give us a clear perception of the mechanism by which camel can adapt himself to hard climate conditions and resist diseases. Moreover, this may be helpful in molecular diagnosis of diseases and drugs designing.

Hepatic growth factor HGF is considered as a multifunctional cytokine with a receptor known as c-Met. They play an important role in the both embryonic and postnatal organ development (Yamaji *et al,* 2006). We have recently cloned the Arabian camel c-Met and studied its tissue expression pattern (El-Shazly *et al,* 2016). When HGF binds to its c-Met receptor, intracellular tyrosine kinase domain of c-Met β-chain undergoes autophosphorylation resulting in a wide range of biological effects including mitogenic, morphogenic and motogenic effects in different types

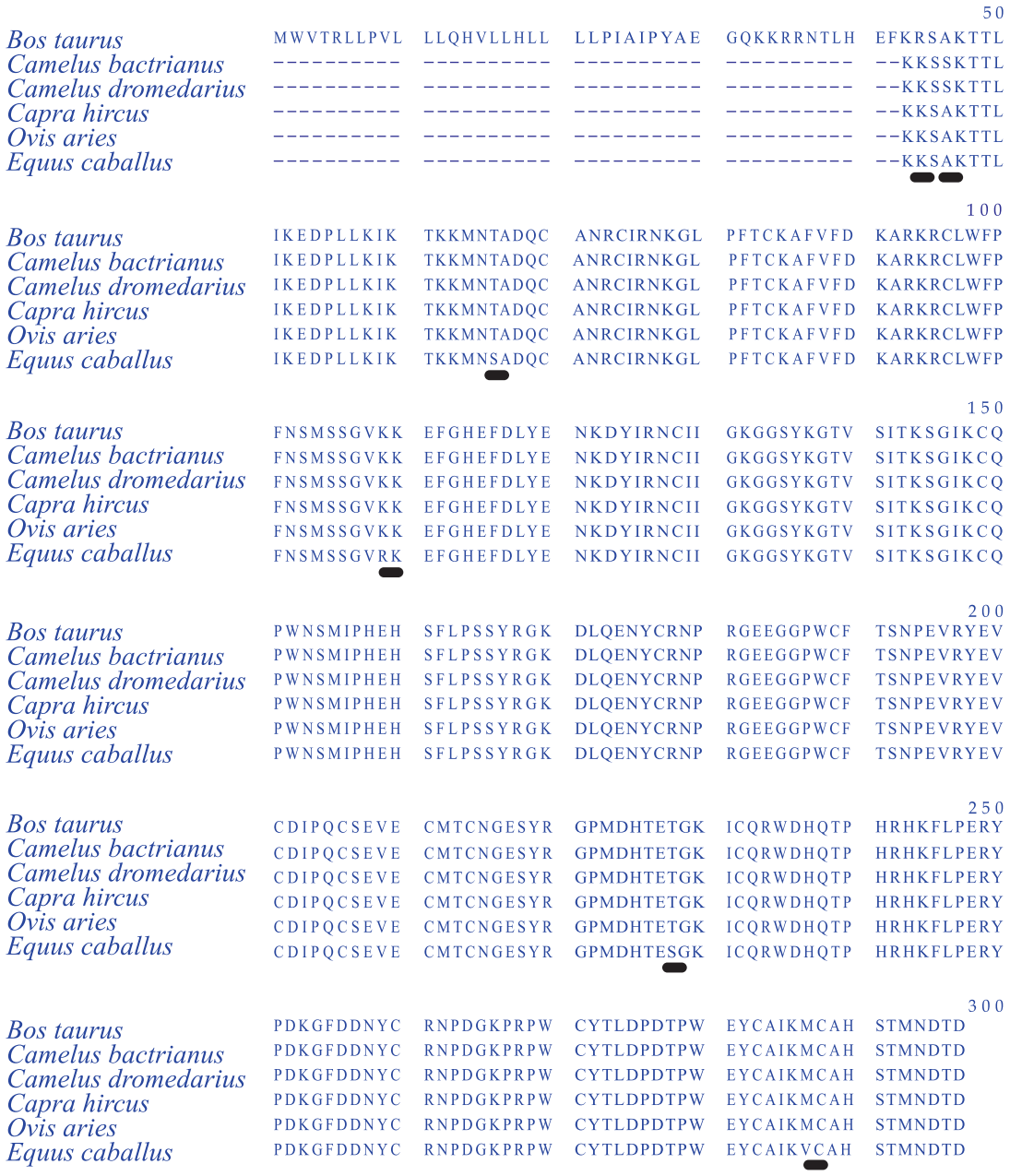

Fig 3. Amino acids alignment in different species.

of cells (Bottaro *et al,* 1991; Nakamura and Mizuno, 2010). Using cell-free cloning strategy, HGF gene of Arabian camel was partially cloned through PCR amplification technique. Primers HGF-1F and HGF-1R were used and a fragment of 818 bp was resulted (Fig 1). The PCR product was sequenced and a clear 766 bp peaks were analysed. The obtained nucleotide sequence (766 bp) of partial camel HGF cDNA was deposited in the GenBank data base under accession number of KU736793.

To analyse the obtained nucleotides sequences data, 766 nucleotides from Arabian camel HGF gene were aligned with their counterparts from in the Genbank database for the previously mentioned species. The data showed base frequencies of A = 32.77%, C = 21.67%, G = 22.06% and T = 23.5%. The nucleotide sequence alignment revealed that *C. dromedarius* HGF showed 99% identity with *Camelus bactrianus* and *C. ferus* HGF while it showed 97% identity with those of either *Bos taurus*, *Capra hircus*, *Ovis aries* or *Equus caballus* (Table 2). In addition, to 766 nucleotides analysed, 36 substitutions varied from transitions and transversion were detected. Of these 36 substitutions, only 6 were non-synonymous

resulted in amino acids substitution while the remaining 30 SNP were synonymous resulted in no amino acids substitutions among the aligned species (Fig 3). Among these substitutions, the translated amino acids showed one non-synonymous substitution at position 46 (Fig 3) discriminating the camelids from other species. These substation included change of serine (S_{46}) in the genus *Camelus* into alanine (A_{46}) in other species at $T_{136}\rightarrow G_{136}$. In addition, there were also two substitutions discriminating the non-ruminant, *Equus caballus* from the ruminants (*Bos taurus*, *Capra hircus*, *Ovis aries*) and pseudo-ruminants (*Camelus bactrianus*, *C. dromedarius*). That is arginine (R_{109}) at position 109 in *Equus caballus* was changed into lysine (K109) in the other species as a result of substitution of $G_{326}\rightarrow A_{326}$ and valine (V_{287}) at position 287 in *Equus caballus* was changed into methionine (M_{287}) in the other species as a result of substitution of $G_{859}\rightarrow A_{859}$. On the other hand, camelids (pseudo-ruminant) shared the non-ruminant (*Equus caballus*) in amino acids at positions 197, serine (S_{66}); serine (S_{228}) which was substituted by threonine (T_{197}) and (T_{228}) in other species (ruminants) as a result of change of $G_{197}\rightarrow C_{197}$ and $T_{682}\rightarrow A_{682}$, respectively (Fig 3). The numbers below the letters referred to the corresponding positions of either the amino acid or the nucleotide inside the complete gene sequence. To investigate the mRNA expression pattern of HGF tissue distribution, RT-PCR analysis using internal primers was conducted on different tissue samples. The obtained results revealed a wide range of HGF expression in different tissue including kidney, spleen, heart, liver testis and muscle.

Table 2. Identity percentage of *Camelus dromedarius* HGF with other species.

Animal species	HGF	
	Accession number	Identity
Camelus bactrianus	XM_010946631.1	99%
Camelus ferus	XM_006194335.2	99%
Bos taurus	AB110822.1	97%
Ovis aries	XM_012176561.2	97%
Equus caballus	XM_014739144.1	97%
Capra hircus	XM_018047278.1	97%

In conclusion, in the per cent study, we succeeded to partially clone HGF cDNA from Arabian camel tissue and investigate the mRNA expression pattern in some different tissues. Further studies are required to clone full length HGF gene from dromedaries which could be useful for more understanding of the structural-function relationship of HGF in Arabian camel.

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