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PROTEOME PROFILE OF THE HUMP OF BACTRIAN CAMEL

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ABSTRACT

Proteome profile of hump of Bactrian camel was identified using a shotgun proteomic approach. GO annotation and KEGG were predicted using bioinformatic tools. As a result, a total of 1077 proteins were identified. We found that the hump of Bactrian camel is equipped with a variety of functional proteins related to cellular process, metabolic process, binding, catalytic activity, cell, cell part and organelle. Three hundred and one different pathways in the hump of Bactrian camel were identified by KEGG analysis. Most of the pathways were associated with signal transduction pathways, metabolic pathways and energy metabolism. The identified proteome profile will help us understand the function of the hump of Bactrian camel.

Key words: Bactrian camel, hump, proteome, shotgun

The camel can survive long periods without feed as it stores energy reserve in a the form of fat in the hump (Kadim *et al,* 2002). The physicochemical properties and the fatty acid composition of fat from the hump of camels has been measured (Kadim *et al,* 2002; Sbihi *et al,* 2013). Proteomic technology has been used for finding differences between different organs in the camel and the rat (Warda *et al,* 2014). There is limited knowledge about detailed proteome of camel hump. To understand functions of the hump of Bactrian camel, it is important to define the molecular constituents of the hump. The aim of the present study was to identify whole proteome profile of the hump of Bactrian camel using shotgun proteomic approach.

Materials and Methods

Transcriptome databases construction

One adult 10-years-old Bactrian camel was used to harvest hump tissue which was collected and frozen in liquid nitrogen, and stored at −80°C. Total RNA from hump tissue sample was extracted according to the manufacturer's protocol. The total RNA quantity and purity were analysed. Sequencing library was constructed and sequenced on an Illumina Hiseq platform. After reads mapping to the reference genome, one-frame translation of our own hump database was constructed.

Shotgun

Sample pretreatment

The sample was added SDT lysis buffer and homogenated. The sample was sonicated and incubated at 100°C for 10 min, then centrifuged at 12000g for 30 min. The supernatant was collected. Protein concentration was determined with BCA method.

FASP

The proteins were added with DTT and incubated at 100°C for 5 min. The sample was added with UA buffer and centrifuged at 14000g for 15 min. Then, IAA buffer was added and incubated for 30 min at room temperature in the dark. After centrifugation, UA buffer was added and centrifuged at 14000g for 15 min. NH_4HCO_3 was added and centrifuged at 14000g for 15 min. Trypsin buffer was added and incubated at 37°C for 16-18 h.

LC-MS/MS

The LC-MS/MS analysis was performed on an Easy nLC system and Q-Exactive mass spectrometer

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system (Thermo Scientific). The sample was loaded onto a trap column (2cm×100µm, 5µm-C18, Thermo Scientific), and then separated onto an analytical column (75µm×100mm, 3µm-C18, Thermo Scientific) at a flow rate of 300 nL/min. Mass spectra were acquired with an Exactive mass spectrometer (Thermo Scientific). MaxQuant software was employed for protein quantitation. Only the protein identifications with false discovery rate (FDR) 1% or less were accepted in the final dataset.

Results and Discussion

A total of 1077 proteins in hump tissue were identified using shotgun proteomic approach. The 27, 12 and 19 catalogs of biological process, molecular function and cellular component were clustered, respectively. In the category of biological process, most annotated proteins were involved in cellular and metabolic processes. In the category of molecular function, most annotated proteins were associated with binding and catalytic activity. In the category of cellular component, most annotated proteins were associated with cell, cell part and organelle (Fig 1).

In present study 301 different pathways were enriched. Signal transduction pathways are the major pathways. Most of the signal transduction pathways involved in lipid metabolism, for example PI3K-Akt signalling pathway (Huang *et al,* 2018), MAPK signalling pathway (Carmen and Victor, 2006), Hippo signalling pathway (Ardestani *et al,* 2018), cAMP signalling pathway (Rogne and Tasken, 2014), Apelin signalling pathway (Bertrand *et al,* 2015), AMPK signalling pathway (Ceddia, 2013), PPAR signalling pathway (Wahli and Michalik, 2012), Sphingolipid signalling pathway (Lambert *et al,* 2018), Insulin signalling pathway (Tencerova *et al,* 2019), TGF-beta signalling pathway (Margoni *et al,* 2012), Wnt signalling pathway (Christodoulides *et al,* 2009) (Fig 2) and Adipocytokine signalling pathway (Lee and Shao, 2014).

Many pathways were associated with metabolic pathways, such as amino acid metabolism, carbohydrate metabolism, lipid metabolism and nucleotide metabolism (Fig 3).

Several pathways involved in energy homeostasis, such as glycolysis/gluconeogenesis, fatty acid degradation, citrate cycle (TCA cycle), fatty acid elongation and biosynthesis of unsaturated fatty acids (Fig 4). The results may indicate that part of the energy required by Bactrian camels is derived from the ATP produced by the hump through

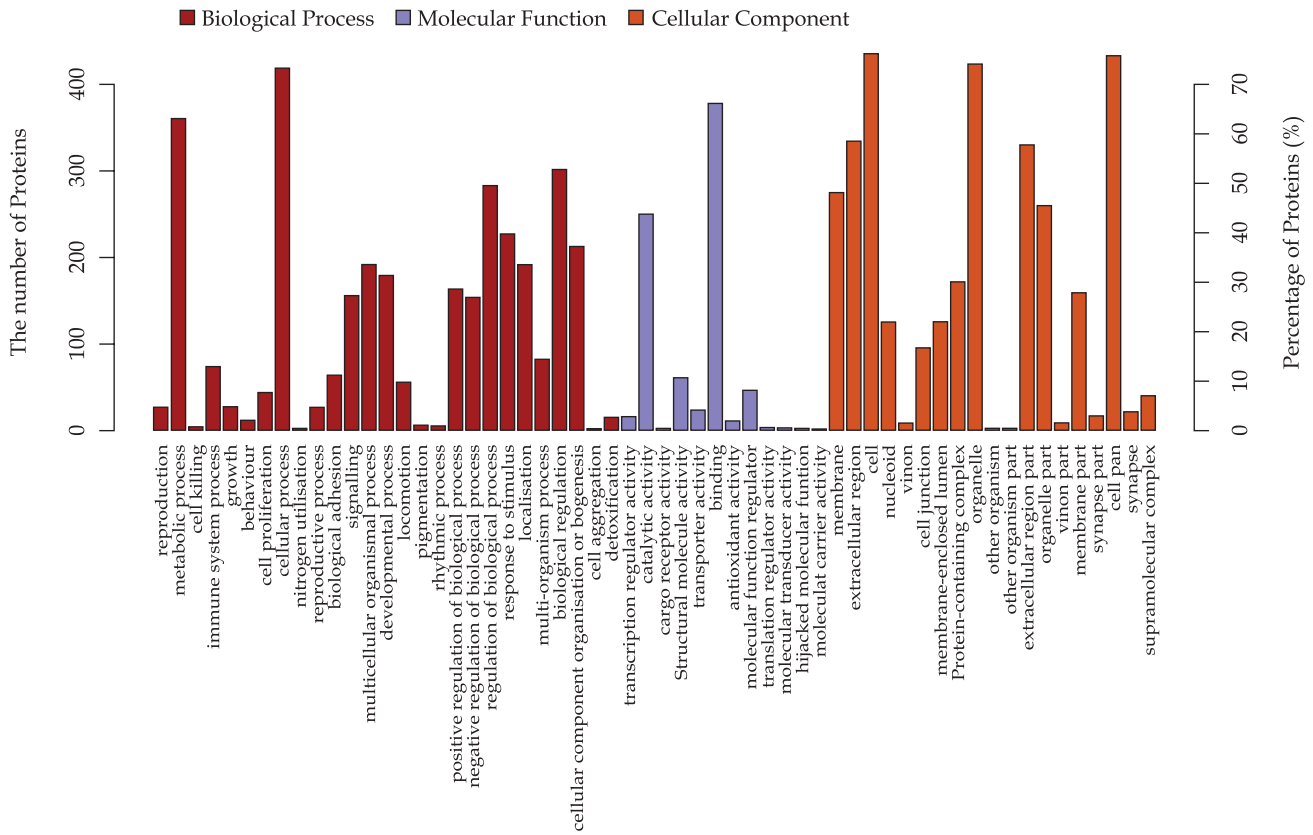

Fig 1. Gene Ontology (GO) categories of proteins identified in the hump of Bactrian camel.

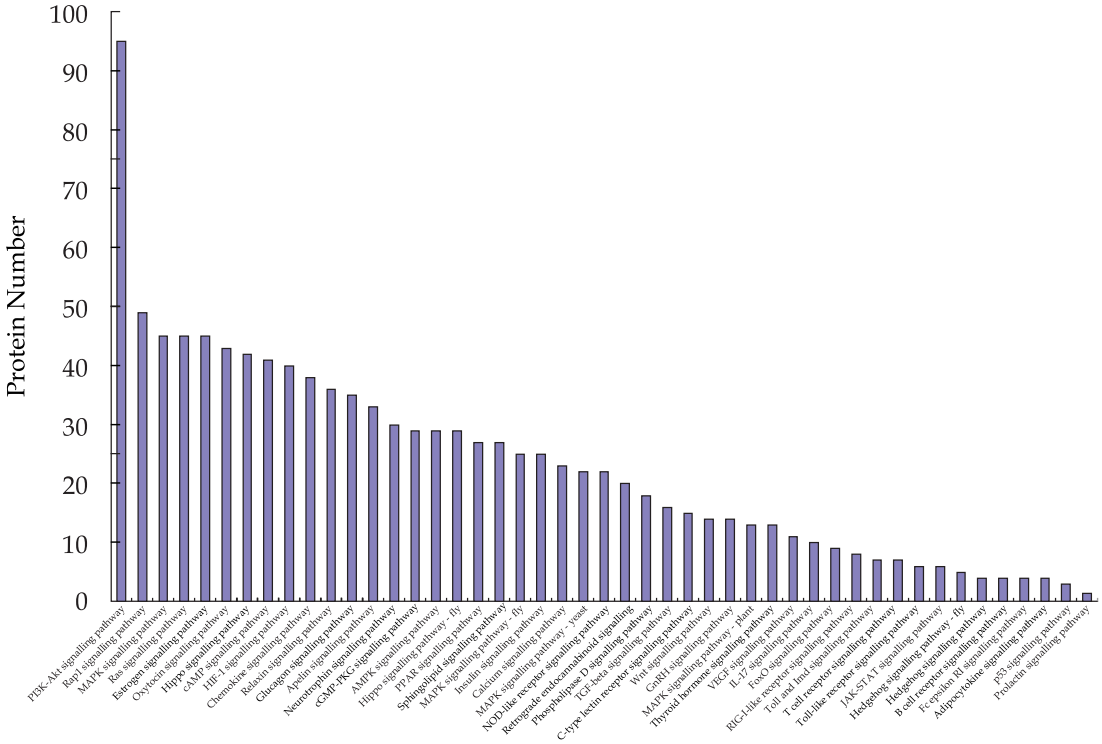

Fig 2. The pathways involved in signal transduction.

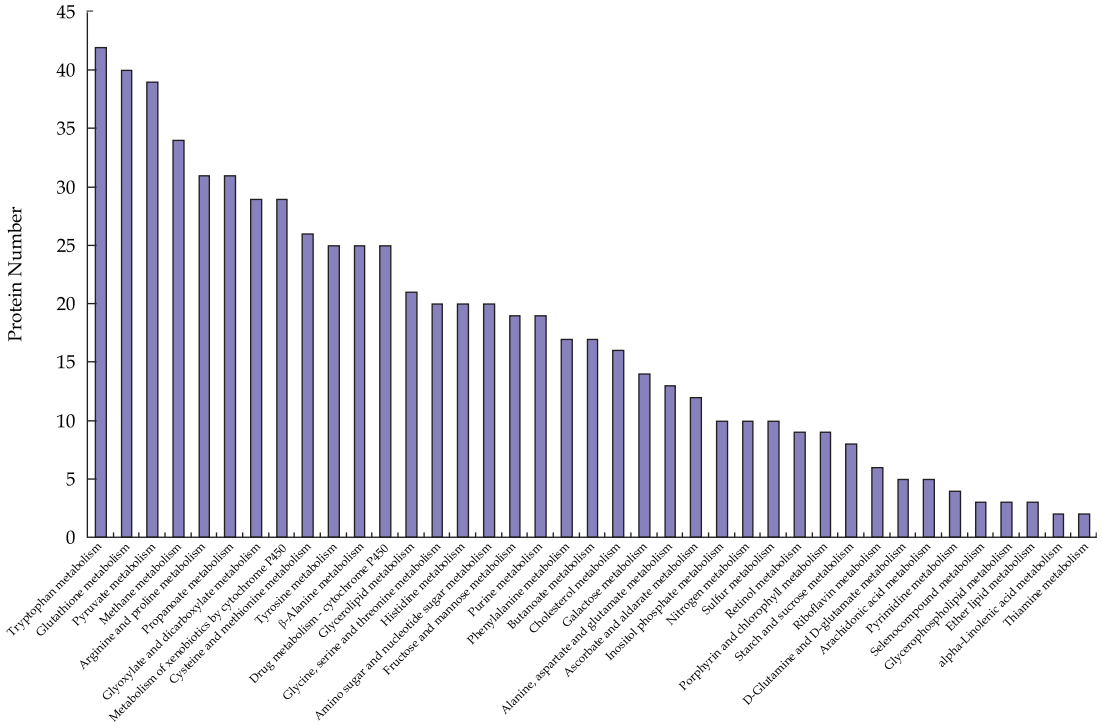

Fig 3. The pathways involved in metabolism.

glycolysis and TCA cycle. The maintenance of blood glucose in Bactrian camel may be due in part to hump gluconeogenesis.

Several pathways were associated with transport and catabolism, such as Endocytosis, Phagosome, Peroxisome, Proteasome and Autophagy (Fig 5). Furthermore, the pathways associated with signalling molecules and interaction, such as ECM-receptor interaction, SNARE interactions in vesicular transport and neuroactive ligand-receptor interaction

were represented. The extracellular matrix (ECM) is essential for tissue architecture and has an important role in adipogenesis (Mariman and Wang, 2010). The results indicate that the accumulation of hump fat is associated with ECM-receptor interaction. The precent study can help us to understand the function of the hump of Bactrian camel.

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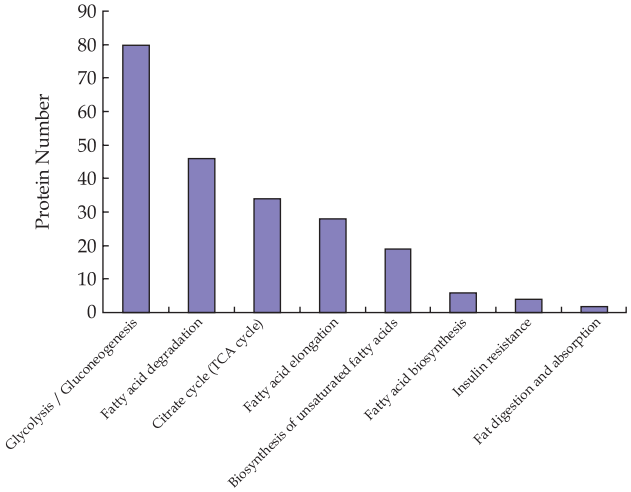

Fig 4. The pathways involved in energy homeostasis.

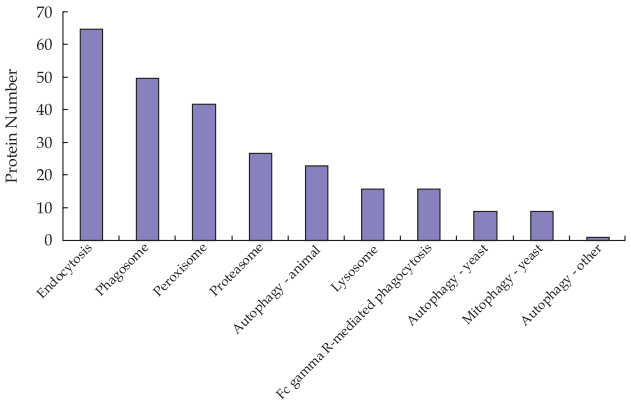

Fig 5. The pathways involved in transport and catabolism.

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