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TRANSCRIPTOME ALTERATIONS IN HIGH GLUCOSE-INDUCED RENAL TUBULAR CELLS OF BACTRIAN CAMEL

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ABSTRACT

The transcriptome alterations in renal tubular cells of Bactrian camel treated with high glucose were investigated. Primary tubular epithelial cells, obtained from kidney of Bactrian camel were treated with low glucose (LG) or high glucose (HG). The transcriptome gene expression in primary tubular epithelial cells of Bactrian camel was examined using RNA sequencing technology. 1322 genes from HG and LG were significantly altered. The up-regulated genes in the HG group include CAMK2B, CRYAA and VNN1. Several pathways associated with diabetic nephropathy including PI3K-Akt signaling pathway, AGE-RAGE signaling pathway, ECM-receptor interaction and PPAR signaling pathway were activated by HG stimulation. This study provides scientific basis for understanding the mechanism of renal tolerance to hyperglycemia of Bactrian camel.

Key words: Bactrian camel; high glucose; transcriptome; tubular epithelial cells

The Bactrian camel lives in the Gobi Desert or semi-desert regions of the northwest China. Bactrian camel has evolved many distinct abilities to adapt to such environments (Chen *et al,* 2009). The levels of blood glucose in camels are much higher than in sheep and ponies (Elmahdi *et al,* 1997). However, Bactrian camel do not develop diabetes and hypertension (Wu *et al,* 2014). It indicates that Bactrian camel has a unique hyperglycemia tolerance mechanism. High-glucose concentrations trigger various metabolic and cellular dysfunctions, which in the kidney affect many types of cells including mesangial cells and renal tubular cells (Vallon and Komers, 2011). The transcriptome alterations in high glucose-induced mesangial cell model which represents diabetic nephropathy (DN) *in vitro* have been investigated (Li *et al,* 2019). However, there is no reported study on the transcriptome changes in high glucose (HG)-induced renal cells of Bactrian camel. This paper will report our work on transcriptome alterations in HG-induced renal tubular cells of Bactrian camel using RNA-seq approach. This study provides scientific basis for understanding the mechanism of renal tolerance to hyperglycemia of Bactrian camel.

Materials and Methods

All the procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Inner Mongolia Agricultural University (12150000460029509N). Primary tubular epithelial cells (PTECs) were isolated from the kidney cortex segment of healthy Bactrian camel. The cortical fragments were incubated for 30 min at 37°C in a buffer containing 0.1% collagenase type I. The preparation was washed and filtered through a cell strainer. The obtained cells were treated with medium containing 1g/L D-glucose (low glucose, LG) and 10% foetal bovine serum (FBS). Cells were incubated in 5% CO_2 atmosphere at 37°C and grown to confluence. In certain plates, the medium was changed. Cells were treated with medium containing 4.5 g/L D-glucose (HG) and 10% foetal bovine serum (FBS).

After 48 h exposure to HG, Total RNA was extracted from PTECs from LG and HG groups

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using TRIzol reagent. The quality and quantity of the extracted RNA samples were determined with the NanoPhotometer spectrophotometer and the Bioanalyser 2100 system. Pooled RNA from samples was used for library preparation. The mRNA sequencing libraries were constructed by the IlluminaTruSeq RNA preparation kit. The samples were sequenced on the Illumina Hiseq 2500 platform (Novogene, Beijing, China) with125 bp/150 bp paired-end reads. Data quality was checked using the fastq software. The reads were compared with Bactrian camel genome and treated by Hisat2. Differential gene expression analysis was performed by the DESeq2 R package. For statistical analysis, two comparisons (HG vs. LG) were analysed by *p* value and false discovery rate (*q* value). The statistical enrichment of differentially expressed genes (DEGs) in KEGG pathways was tested by clusterProfiler R package. KEGG pathway with corrected p-value and q value less than 0.05 were significantly enriched by DEGs.

Results

To measure the gene expression profiles exposure to HG, we performed RNA-Seq of renal tubular cells of Bactrian camel treated with LG (control) or HG. The genes whose expression differed in the two groups were identified and filtered for corrected p values < 0.05 and $|\log_2$ fold change$|$ ≥0.3. A total of 1, 322 DEGs were identified, of which 758 genes were up-regulated and 564 genes were down-regulated in the HG group. Top 10 genes with the highest $\log_2$ fold change in the HG group were listed in Table 1 and these genes included calcium/calmodulin-dependent protein kinase II beta (CAMK2B), crystallin alpha A (CRYAA), vanin 1 (VNN1), NLR family 2C pyrin domain containing 13 (NLRP13) and bone morphogenetic protein 8a (BMP8A). Analysis of DEGs through KEGG showed that 758 up-regulated genes in the HG group could be significantly enriched in 24 pathways including PI3K-Akt signaling pathway, Cytokine-cytokine receptor interaction, AGE-RAGE signaling pathway in diabetic complications, ECM-receptor interaction and PPAR signaling pathway (Table 2).

Discussion

HG can induce cell injury, formation of Reactive oxygen species (ROS) has been considered as one of the principal mechanisms of glucose-induced cell toxicity (Vallon and Komers, 2011). Our results showed that HG can change some gene expression level in renal tubular cells of Bactrian camel. CaMKII was reported to as a pathological mediator of ER stress, oxidative stress and mitochondrial dysfunction in a murine model of nephronophthisis. Experiments *in vitro* and *in vivo* demonstrated that CaMKII inhibition relieved endoplasmic reticulum stress and oxidative damage and improved mitochondrial integrity and membrane potential (Bracken *et al,* 2016). The elevated expression of CAMK2B gene indicated that the renal tubular cells of Bactrian camel were damaged by HG stimulation. Vanin-1 (VNN1), another elevated gene which is associated with oxidative stress, was found in the HG group. Studies have showed that urinary vanin-1 could be a useful biomarker for the detection of drug-induced acute tubular necrosis focusing on oxidative stress (Hosohata, 2016). Recent studies have shown that crystallins play an instrumental role in diabetes and its complications. Crystallins specifically α-crystallins had been damonstrated to possess antioxidant, antiinflammatory, antiapoptotic and antiaggregation (chaperone) functions. Crystallins could be exploited as therapeutic targets for diabetic complications (Reddy and Reddy, 2016). The elevated expression of CRYAA in renal tubular cells of Bactrian camel may be one of the reasons why its kidney can tolerate HG.

In the PI3K-Akt signaling pathway, fibroblast growth factor 1 (FGF1), fibroblast growth factor receptor 1 (FGFR1), phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) were upregulated

Table 1. Top 10 upregulated genes in the HG group.

Gene symbol	Gene name	$\log_2$ fold change	*p* value
CAMK2B	calcium/calmodulin-dependent protein kinase II beta	4.568	0.027
CRYAA	crystallin alpha A	4.382	0.019
LOC105079214	putative serine protease 47	4.023	0.009
VNN1	vanin 1	3.599	0.031
NLRP13	NLR family pyrin domain containing 13	2.888	0.048
BMP8A	bone morphogenetic protein 8a	2.854	0.037
INHBC	inhibin beta C	2.546	0.020
LOC105075982	E3 ubiquitin-protein ligase Midline-1-like	2.514	0.018
PCDH11X	protocadherin 11 X-linked transcript variant X2	2.441	0.007
SPRY3	sprouty homolog 3	2.427	0.036

Table 2. Significantly enriched KEGG pathways of upregulated genes in the HG group.

Pathways	*p* value	*q* value
PI3K-Akt signaling pathway	2E-07	4E-05
Human cytomegalovirus infection	4E-04	0.031
Proteoglycans in cancer	9E-04	0.044
Cytokine-cytokine receptor interaction	3E-03	0.044
Focal adhesion	2E-03	0.044
cAMP signaling pathway	3E-03	0.044
Hippo signaling pathway	4E-03	0.049
Adrenergic signaling in cardiomyocytes	4E-03	0.049
Small cell lung cancer	3E-04	0.031
AGE-RAGE signaling pathway in diabetic complications	5E-04	0.031
Thyroid hormone signaling pathway	3E-03	0.044
FoxO signaling pathway	3E-03	0.044
Prostate cancer	2E-03	0.044
Parathyroid hormone synthesis, secretion and action	2E-03	0.044
Cholinergic synapse	3E-03	0.044
ECM-receptor interaction	2E-03	0.044
Longevity regulating pathway	2E-03	0.044
Pancreatic cancer	2E-03	0.044
Insulin secretion	2E-03	0.044
Non-small cell lung cancer	2E-03	0.044
PPAR signaling pathway	3E-03	0.044
Sphingolipid metabolism	1E-03	0.044
Cholesterol metabolism	4E-03	0.044
Glycosaminoglycan biosynthesis	3E-03	0.044

in the HG group. FGF1 has been proved to ameliorate chronic kidney disease via PI3K/AKT mediated suppression of oxidative stress and inflammation (Wang *et al,* 2019). In this study, the elevated expression of FGF1 may contribute to the upregulation of PI3K-Akt signaling pathway. Upon glucose entry into renal cells, there were a number of intracellular events that occurred in the presence of high-glucose ambience. The advanced glycation endproducts (AGEs) were formed. The intracellular AGEs could modify the protein functions and activate PKC, MAPK and transcription factor-like NF-κB and thus modulating the expression of various growth factors, cytokines and consequentially the ECM proteins (Kanwar *et al,* 2005). We identified some pathways that involved in the cellular pathobiology of diabetic kidney disease in the HG group, such as AGE-RAGE signaling pathway in diabetic complications, Cytokine-cytokine receptor interaction and ECM-receptor interaction. These results indicated that HG could cause injury to the renal cells of Bactrian camel. Experimental evidence suggested that PPARα activation attenuates or inhibited diabetic microvascular damage, including lipotoxicity, inflammation, reactive oxygen species generation, endothelial dysfunction and thus might influence the development and pathogenesis of diabetic microvascular complications (Hiukka *et al,* 2010). PPARα may become an important therapeutic target for treating diabetic renal complications (Chung and Park, 2011). Some up-regulated genes in the HG group were enriched in the PPAR signaling pathway, such as peroxisome proliferator-activated receptor α (PPARα) and retinoic acid receptor (RXR) (another nuclear receptor that can heterodimerise with PPARs). These results indicated that elevated expression of PPARα in renal cells of Bactrian camel induced by HG may have protective effects on the cells. This study provided scientific basis for understanding the mechanism of renal tolerance to hyperglycemia of Bactrian camel.

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