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Glucose regulation of CDK7, a putative thiol related gene, in experimental diabetic nephropathy ☆

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Abstract

We previously described the identification of the 3′end of an unknown gene CDK7 using differential display which appeared to be up-regulated in diabetic kidneys [R.A. Page, C.A. Morris, J.D. Williams, C.J. von Ruhland, A.N. Malik, Isolation of diabetes-associated kidney genes using differential display, Biochem. Biophys. Res. Commun. 232 (1997) 49–53]. Here we show that CDK7 is a putative thiol related gene which is regulated by glucose in human and rat renal cells. CDK7 mRNA increased by >threefold in cultured human mesangial cells grown in high glucose for 4 days. In the kidneys of the GK rat, a model of type II diabetes, CDK7 showed a steady age-related increase in mRNA, increasing to >sixfold in 40 week GK rats compared to normoglycemic age-matched Wistar rat kidneys, this increase correlates with progressive hyperglycemia. CDK7 mRNA is widely expressed, showing particularly high levels of expression in rat and human liver, and encodes a putative 338 amino acids highly conserved peptide with several conserved domains, including a cys-pro-arg-cys domain conserved in 15 diverse species which is similar to the catalytic centre of thioredoxin, suggesting a role in oxidative stress.

Keywords: Diabetic nephropathy; Mesangial cells; Hyperglycemia; Glucose regulation; Oxidative stress; Thiol

Diabetic nephropathy (DN) is a major cause of end-stage renal failure and affects between 30% and 50% of all diabetics, developing within 10–30 years of the onset of diabetes [2,3]. There is an urgent need to develop new therapeutic strategies to intervene with the pathways involved in the development of DN, as diabetes currently affects ~170 million people worldwide and both diabetes and associated complications are predicted to rise to epidemic proportions in the near future [3–5]. High glucose has been shown to be the main cause of DN in both clinical and experimental studies through a series of complex overlapping pathways, which include the generation of reactive oxygen species, formation of advanced glycation end products, the activation of protein kinase C and the up-regulation of the cytokine TGF-β1, leading to a pathological accumulation of extracellular matrix in the mesangium and interstitial fibrosis which characterise DN [6,7].

In an attempt to find novel therapeutic targets for DN, a number of novel genes that are regulated by glucose have been identified [8]. For example, connective tissue growth factor [9], gremlin [10], serum glucocorticoid regulated kinase [11], a novel ubiquitin ribosomal fusion protein, UbA52 [12], and beta-defensin-1 [13] are examples of genes which are up-regulated in-vivo in rodent models of diabetes as well as in cultured human mesangial cells grown in high glucose. We used differential display to detect genes that showed transcriptional changes in the kidney during the development of diabetes in the GK rat [1]. A number of

Abbreviations: CDK, candidate diabetes associated kidney genes; GK, Goto Kakizaki; DN, diabetic nephropathy; RT-PCR, reverse transcription polymerase chain reaction; HMC, human mesangial cells.

☆ The rat CDK7 (mg87) nucleotide sequence described in this paper has been assigned the GenBank Accession No. AF095741.

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genes, designated Candidate Diabetes-associated Kidney (CDK) were detected by cloning their 3′ends. [13]. In this paper we describe the cloning and expression of the mRNA corresponding to rat CDK7 (rCDK7), and its human homologue hCDK7, and we test the hypothesis that CDK7 is a glucose regulated gene.

Methods

Tissues and cell lines. The human mesangial cell line (HMCL), and primary human mesangial cells (HMC) cultured from sieved human renal glomerulii at passage 6, obtained from Dr. David Wheeler, Royal Free Hospital, London, were grown as previously described [14]. For the high glucose experiment cells were seeded in triplicate at equal density in 6-well plates, synchronized by growth in reduced serum for 24 h, followed by growth in DMEM (Sigma–Aldrich) containing either normal (5 mM) or high (25 mM) glucose or normal glucose (5 mM) plus mannitol (20 mM), for 4 days, and then used for RNA preparation. A human tissue RNA panel which consists of pooled RNA from three different donors per tissue was used (first choice human total RNA survey panel, Ambion Biotech). The exocrine and islet cDNAs were a gift from Dr. Gua-Cai Huang (King's College London) and the human fetal tissue RNAs and the GK rat kidney and Wistar rat kidney RNAs have been previously described [13,14].

RNA/DNA extraction. Total cellular RNA was extracted from tissues using the Total RNA kit (Ambion) or from cells using RNAqueous-4PCR kit (Ambion) as previously described [14], Genomic DNA was isolated using a Genomic DNA extraction kit (Talent Srl, Med-Bio Enterprises Ltd, NZ).

Isolation of full length cDNA. A λZAP cDNA library from 40 week GK rat kidneys [15,16] was screened with the partial CDK7 insert labelled with [α-^{32}P]dCTP using Ready to Go DNA Labelling beads (Amersham Pharmacia Biotech) and full length cDNAs were purified by secondary and tertiary library screening.

Northern and Southern blot analysis. Genomic DNA (10 μg) was digested with *Eco*RI, electrophoresed on a 0.8% (w/v) agarose gel, and transferred to Hybond-Nfp nylon membrane (Amersham Pharmacia Biotech) for Southern blotting. For Northerns, total RNA was electrophoresed (1% w/v agarose/formaldehyde gel), transferred to Hybond-Nfp membranes (Amersham Pharmacia Biotech) and fixed to the membrane by UV irradiation. Northern and Southern blots were prehybridised for 3 h at 65 °C in 5× Denhardts reagent, 6× SSC, 0.5% SDS (w/v) and 200 μg/ml denatured salmon sperm DNA (Sigma). The blots were probed with the denatured [α-^{32}P]-labelled 770 bp cDNA insert using the Ready To Go DNA Labelling Beads (Amersham Pharmacia Biotech). Unincorporated [α-^{32}P]dCTP was removed using ProbeQuant G-50 microspin columns (Amersham Pharmacia Biotech). Hybridisation was carried out for 16 h at 65 °C, the membranes were washed to high stringency and used for autoradiography (24–72 h).

Quantitative real time PCR. Primers were designed using either Primer3 Input (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) or lightcycler probe design software (Roche Molecular Biochemicals) and synthesized at Sigma-Genosys (Table 1). Quantitative real time PCR was carried out as previously described [14] using the FastStart DNA Master SYBR Green I (Roche Molecular Biochemicals). The reactions were performed in the Roche lightcycler using the following 4-cycle program protocol: pre-incubation at 95 °C for 10 min; amplification at 95 °C for 10 s, annealing at variable temperatures for 20 s (Table 1), and 72 °C for 17 s for 40 cycles. The resulting data was analyzed using the Roche Molecular Biochemicals lightcycler software, version 3.5. Fluorescence detection was carried out immediately at the end of each annealing step and the purity of the amplification was confirmed by analyzing the melting curves. All reactions were performed in duplicate or triplicate in the presence of calibration standards containing a dilution series of 10^7, 10^6, 10^5, 10^4, 10^3, and 10^2 copies per μl of each gene, prepared in the presence of carrier tRNA (Sigma). Statistical analysis was carried out using SPSS using either analysis of variance (ANOVA) with Tukey's test or independent samples *T* test.

Bioinformatics. DNA and protein sequence analysis was performed using the University of Wisconsin Genetic Computer Group software package (GCG) and by using various tools at NCBI (National Centre for Biotechnology and Information, http://www.ncbi.nlm.nih.gov/). Multiple sequence alignments were carried out using Clustal W and Multalign at the European Bioinformatics Institute (EBI) at http://www.ebi.ac.uk/ and http://searchlauncher.bcm.tmc.edu. Alignments were visualised with the BOXSHADE 3.21 program at http://www/ch/embnet.org/software/Box_form.html. Motifs were analysed using PFSCAN, CLUSTALW and MOTIFS at EBI and GCG.

Results

Cloning and elevated expression of rat CDK7 mRNA in diabetic kidneys

The partial cDNA representing the 3′end of an unknown mRNA designated CDK7 [1] was used to probe a cDNA library constructed from the kidneys of 40-week-

Table 1
Primers used in this study

GenBank Accession No.	Primer	Oligonucleotide sequence	Product size (bp)	PCR conditions annealing temp/$MgCl_2$ mM
Rat CDK7 AF095741	r-CDK7F1	AAA GGA GGA CAA CAA AGA A	308	57/3.0
	r-CDK7R1	TCT TTC CTG TCT ATG ATG CC		
Rat CDK7 AF095741	r-CDK7F2	TGA GCT CTT GAT TGG TGA CG	233	62/2.5
	r-CDK7F2	ACA CAG GGT CAC CCA CTC TC		
Rat Actin NM_031144	r-ActinF	ACG GTC AGG TCA TCA CTA TC	299	55/3.0
	r-ActinR	AGC CAC CAA TCC ACA CAG A		
Rat GAPDH X02231	GAPDHF	GTC TAC TGG CGT CTT CA	450	57/3.0
	GAPDHR	GGG TAG GAA CAC GGA AG		
Human Actin NM_001101	h-actinF	TGT GCC CAT CTA CGA GGG GTA TGC	433	55/3.0
	h-actinR	GGT ACA TGG TGG TGC CGC CAG ACA		
Human CDK7 BC011973	h-CDK7F1	GAC ACA TGG TCA CTG CC	187	55/3.0
	h-CDK7R1	TTG CCA CAG TCT CTG C		

Sequences are presented from 5′ to 3′, F being the sense strand and R the antisense strand. The primers were designed either using oligo3.0 or LightCycler probe design software. The rat actin and GAPDH primers have been previously described [13].

old GK rats. From a screen of ~100,000 clones, several clones were identified and the five most strongly hybridising clones were sequenced, showing that two clones of 440 bp and two clones of 770 bp were partial cDNAs of the largest clone (1330 bp). Detailed analysis of the largest cDNA clone revealed a 13 bp 5′ UTR, an open reading frame of 1014 bases, encoding a predicted protein of 338 amino acids, and a 300 bp 3′ non-coding region (Fig. 1). We named this mRNA rat CDK7 (rCDK7). Using real time PCR we measured the copy numbers of rCDK7 in various rat tissues and found that rCDK7 is an abundant mRNA expressed in rat brain, heart, kidney, liver, lung, spleen, stomach and urinary bladder. The levels of expression are the highest in liver (>5000 copies), kidney and the urinary bladder (~2000 copies) lower in the heart (~520 copies) and spleen (<115 copies) per 1000 actin copies (data not shown).

The CDK7 mRNA transcript was detected in a 40-week-old GK rat kidney but not in a kidney from a 50 week normoglycemic Wistar rat, suggesting elevated rCDK7 mRNA levels in diabetic kidneys (Fig. 2A). To precisely quantify the mRNA levels of rCDK7, we used real time PCR. We measured the copy numbers of rCDK7 mRNA in kidneys from progressively older GK rats and age-matched Wistar rats (Table 2). GK rats are normoglycemic at 6 weeks and progressively develop hyperglycemia, being hyperglycemic by 26 weeks, whereas Wistar rats, from which the GK rat is derived, remains normolglycemic at all ages [1,13,17]. When rCDK7mRNA levels were expressed as a percentage of the levels seen in age-matched

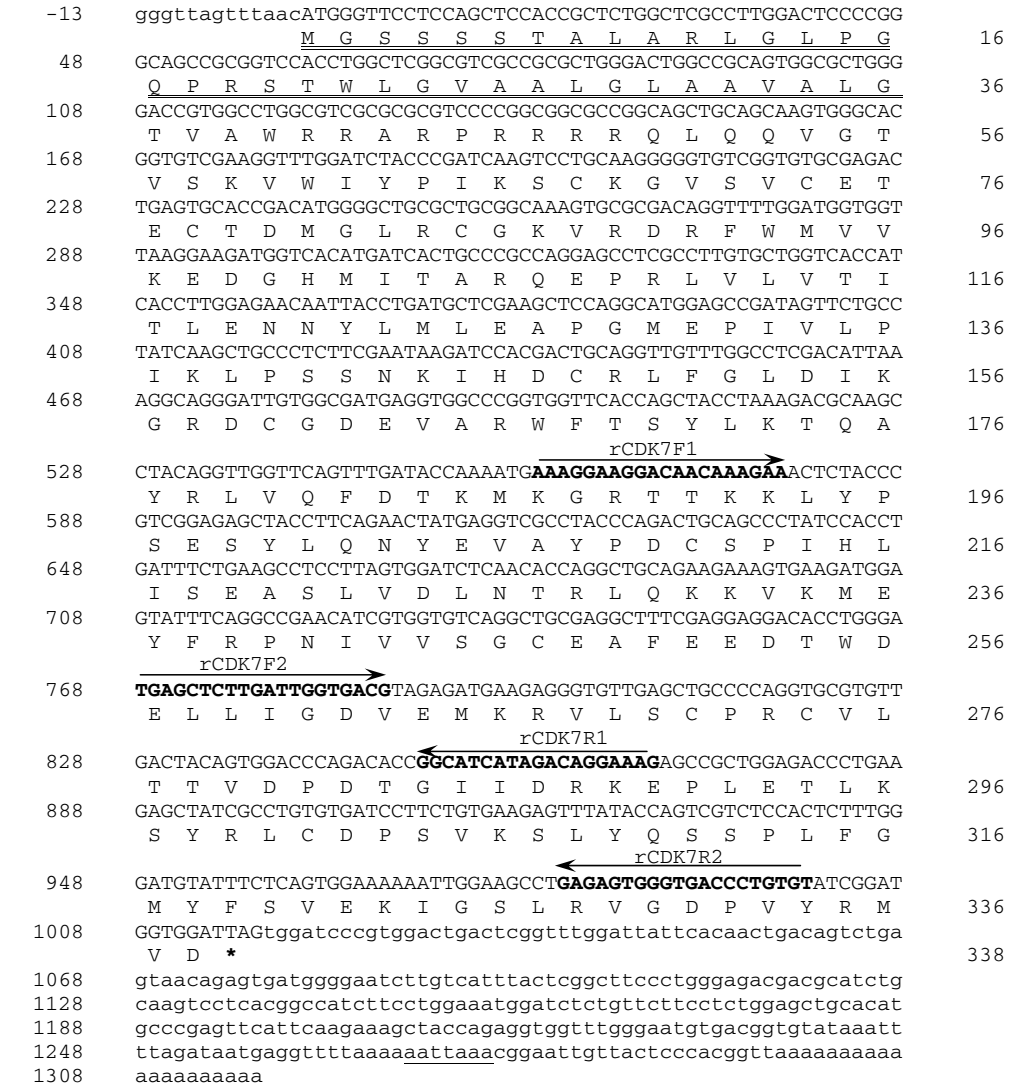

Fig. 1. DNA sequence of rat CDK7 mRNA. Full nucleotide sequence (numbering on left hand side) and deduced amino acid sequence (numbering on right hand side) of rat CDK7 mRNA (Accession No. AF095741). The coding region is shown in upper case, and the 5′ and 3′ UTRs are shown in lower case. The stop codon is represented by an asterisk and the putative polyadenylation signal is underlined. The sense and antisense primers used in PCR (rCDK7F1 and rCDK7F1) and in real-time PCR (rCDK7F2 and rCDK7R2) are as → and ←, respectively, and the sequences in bold type. The putative signal peptide is double underlined.

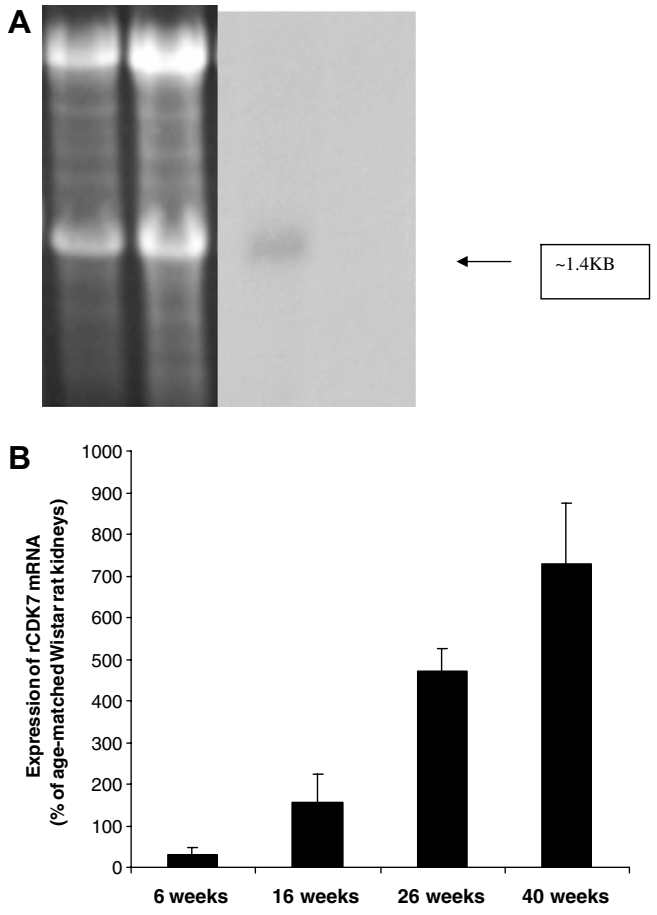

Fig. 2. (A) Rat CDK7 mRNA levels are elevated in diabetic rat kidney. Northern blot showing (i) ethidium bromide staining to show equal loading of Total RNA and (ii) the blot probed with rCDK7. Lane 1, 20 μg of total RNA from 40 week GK rat and lane 2, 20 μg of total RNA from 40 week Wistar rat. (B) Effect of hyperglycemia on expression of rCDK7 mRNA in GK rat kidneys. Kidneys from age-matched normoglycemic Wistar rats (W) and progressively hyperglycemic GK rats were used to examine the effect of hyperglycemia on rCDK7 mRNA levels in the kidney. At 6 weeks GK rats are normoglycemic and progressively develop hyperglycemia, being hyperglycemic by 26 weeks [13]. rCDK7 mRNA levels were quantitated using real time PCR from at least 2 kidneys at each stage, with quantification being carried out in duplicate. The results are expressed as % values relative to those seen in age-matched Wistar rats (normoglycemic controls). The mean copy numbers of rCDK7 in all age groups are shown in Table 2.

normoglycemic Wistar kidneys, a significant increase in rCDK7 mRNA was seen in association with the development of hyperglycemia (Fig. 2B). Analysis of the estimated marginal means showed that rCDK7 mRNA levels are significantly elevated in association with progressive hyperglycemia in the kidneys of the GK rat but in the Wistar rat the levels show a decline (analysis not shown).

Expression of human CDK7 mRNA

Using Southern blot hybridisation, we found that the CDK7 gene is conserved in human (data not shown). A blast search was used to identify the human homologue of CDK7, a hypothetical protein FLJ20605 of 335 amino

Table 2
Copy numbers of rCDK7 mRNA in GK and Wistar kidneys at different ages

Age (weeks)	Rat model	Mean values (±SD) of rCDK7 copy numbers
6	GK	704 (±140)
	Wi	2517 (±126)
16	GK	1151 (±511)
	Wi	1545 (±1130)
26	GK	4499 (±800)
	Wi	952 (±570)
40	GK	10269 (±161)
	Wi	1550 (±422)

rCDK7 mRNA copy numbers were quantitated in GK and Wistar rat kidneys from 6, 16, 26, and 40 week rats using real time PCR. Values shown are means expressed relative to 1000 actin. For each biological stage 2 separate kidneys were used to prepare cDNA and the genes were quantitated from each in duplicate.

encoded by a cDNA clone from cervix carcinoma sequenced by the IMAGE consortium (Accession No. BC011973) which shows 84% identity to rCDK7. This high level of identity supports the view the FLJ20605 is the human version of CDK7 and therefore will be referred to as hCDK7 in the following sections. Primers hCDK7F2 and hCDK7R2 (Table 1) were used to amplify a region of the hCDK7 gene from human kidney, the resulting PCR product was sequenced to confirm its identity (data not shown), showing that CDK7 is expressed in human kidney. Using RT-PCR, we detected the expression of CDK7 in both human fetal and adult kidney, as well as human mesangial cells. In addition the transcript was detected in human fetal liver, fetal brain, fetal lung and fetal heart (data not shown). To determine the mRNA expression profile of hCDK7 we carried out real time quantitative PCR on a range of human tissues and found that hCDK7 is widely expressed, showing the highest levels of expression in liver like its rat homologue (Fig. 3A).

Glucose regulation of hCDK7 mRNA in human mesangial cells

To test the hypothesis that hCDK7 may be up-regulated by glucose, primary human mesangial cells (HMCs) and a transformed human mesangial cell line (HMCL) were cultured in triplicate in normal glucose (NG; 5 mM), and high glucose (HG; 25 mM). A mannitol control (NGM; 5 mM glucose + 20 mM mannitol) was included to test for any osmolarity effect. hCDK7 mRNA copy numbers relative to actin were determined using real time PCR (Table 1 and Fig. 3B). Using a post hoc Tukey's test for analysis of variance, we found no significant difference between NG and NGM ($P > 0.5$) showing that there is no osmolarity effect in play. The cells grown in high glucose show a significant increase in hCDK7 mRNA copy numbers when compared to NG or NGM ($P < 0.001$). HMCLs grown in normal glucose contained 17 ± 4 copies of hCDK7, whereas when grown in high glucose contained 44 ± 9 cop-

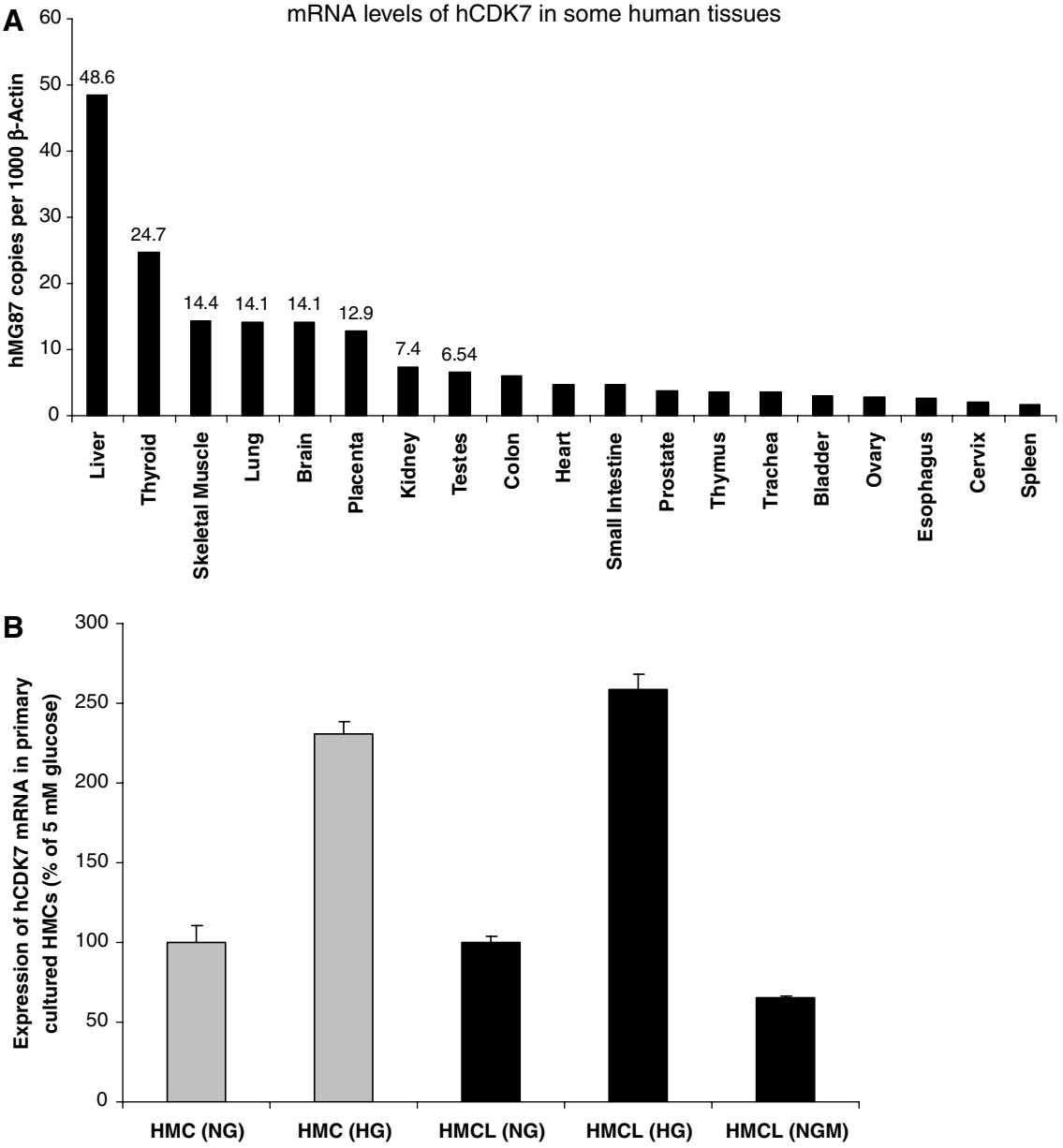

Fig. 3. (A) Expression Profile of hCDK7 mRNA in a range of human tissues. hCDK7 mRNA was quantitated in a range of human tissues from an RNA panel with pooled RNAs using real time PCR. Values shown are copy numbers of hCDK7 mRNA per 1000 copies of actin. (B) Upregulation of CDK7 mRNA in high glucose. Effect of high glucose on expression of hCDK7 mRNA in primary cultures of mesangial cells (HMC) and in a transformed mesangial cell line (HMCL). Cells were incubated in 5 mM glucose (NG), 25 mM glucose (HG) or 5 mM glucose and 20 mM mannitol (NGM). CDK7 mRNA copy numbers were quantitated using real-time PCR ($n = 3$). The results are expressed as values relative to 5 mM glucose.

ies of hCDK7 per 1000 copies of actin. These data show that primary human mesangial cells express CDK7 and that the human CDK7 gene is directly up-regulated by high glucose.

CDK7 homologues in human, mouse and monkey show conservation of key motifs

A BLAST search of the major protein databases identified homologues of this gene in a number of organisms, all of which represent putative predicted proteins of unknown function. Human, monkey, rat and mouse homologues of CDK7 showed the highest identity (>84%) at the protein level, including the conservation of putative MOSC and MOSC_N domains located at residues 35–155 and 169–313, respectively. The highly conserved cysteines found at position 269 and 272 with the motif CPRC are of particular interest as they strongly resemble the catalytic domain found in thioredoxin, an enzyme involved in regulating the redox balance in the cell [21]. These four CDK7 proteins all show conservation of nine putative protein kinase C phosphorylation domains, four *N*-myristilation sites, five casein kinase II sites, and one tyrosine kinase phosphorylation site (Fig. 4).

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human           MGASSSSALARLGLPARPWPRWLGVAALGLAAVALGTVAWRRAWPRRRRRLQQVGTVAKL 60
monkey          MGASSSSALARLGLPAQARPRWLGVAVLGLAAVALGAVAWRRAWPRRRRRLQQVGTVAKL 60
rat             MGSSSSTALARLGLPGQPRSTWLGVAALGLAAVALGTVAWRRARPRRRRQLQQVGTVSKV 60
mouse           MGSSSSTALARLGLPGQPRSTWLGVAALGLAAVALGTVAWRRTRPRRRRQLQQVGTVSKV 60
                **:***:********.:. . *****.*********:*****: *****:*******:*:

human           WIYPVKSCKGVPVSEAECTAMGLRSGNLRDRFWLVIKEDGHMVTARQEPRLVLISIIYEN 120
monkey          WI-PVKSCKGVPVSEAECTAMGLRSGNLRDRFLLVIKEDGHIVTARQEPRLVLVSITYEN 119
rat             WIYPIKSCKGVSVCETECTDMGLRCGKVRDRFWMVVKEDGHMITARQEPRLVLVTITLEN 120
mouse           WIYPIKSCKGVSVCETECTDMGLRCGKVRDRFWMVVKEDGHMVTARQEPRLVLVSITLEN 120
                ** *:******.*.*:*** ****.*::**** :*:*****::**********::*  **

human           NCLIFRAPDMDQLVLPSKQPSSNKLHNCRIFGLDIKGRDCGNEAAKWFTNFLKTEAYRLV 180
monkey          NCLIFKAPDMDQLVLPSKQPSSNKLHNCRIFGLDIKGRDCGNEAAQWFTNFLKTEVYRLV 179
rat             NYLMLEAPGMEPIVLPIKLPSSNKIHDCRLFGLDIKGRDCGDEVARWFTSYLKTQAYRLV 180
mouse           NYLTLEAPGMEQIVLPIKLPSSNKIHNCRLFGLDIKGRDCGDEVAQWFTNYLKTQAYRLV 180
                * * :.**.*: :*** * *****:*:**:***********:*.*:***.:***:.****

human           QFETNMKGRTSRKLLP--TLDQNFQVAYPDYCPLLIMTDASLVDLNTRMEKKMKMENFRP 238
monkey          QFETNMKGRTSRKLLP--TLDQNYQVAYPDCSPLLIMTDASLVDLNTRIEKKMKMENFRP 237
rat             QFDTKMKGRTTKKLYPSESYLQNYEVAYPDCSPIHLISEASLVDLNTRLQKKVKMEYFRP 240
mouse           QFDTSMKGRTTKKLYPSESYLQNYEVAYPDCSPVHLISEASLVDLNTRLKKKVKMEYFRP 240
                **:*.*****::** *  :  **::***** .*: ::::*********::**:*** ***

human           NIVVTGCDAFEEDTWDELLIGSVEVKKVMACPRCILTTVDPDTGVIDRKQPLDTLKSYRL 298
monkey          NIVVTGCDAFEEDTWDELLIGSVEVKKIMACPRCILTTVDPDTGVIDRKEPLDTLKSYRL 297
rat             NIVVSGCEAFEEDTWDELLIGDVEMKRVLSCPRCVLTTVDPDTGIIDRKEPLETLKSYRL 300
mouse           NIVVSGCEAFEEDTWDELLIGDVEMKRVLSCPRCVLTTVDPDTGIIDRKEPLETLKSYRL 300
                ****:**:**************.**:*::::****:*********:****:**:*******

human           CDPSERELYKLSPLFGIYYSVEKIGSLRVGDPVYRMV- 335
monkey          CDPSERELYKLSPLFGIYYSVEKIGSLRVGDPVYRMV- 334
rat             CDPSVKSLYQSSPLFGMYFSVEKIGSLRVGDPVYRMVD 338
mouse           CDPSVKSIYQSSPLFGMYFSVEKLGSLRVGDPVYRMVD 338
                **** :.:*: *****:*:****:*************
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Fig. 4. Sequence alignment of human, monkey, rat and mouse CDK7 peptides. Multiple alignments were carried out using ClustalW (European Bioinformatics Institute) with Human (NP_060368), monkey (XXX), Rat (AF095741), and mouse (NP_598445) CDK7 peptides. The signal peptide was identified using SPScan (GCG), and the motifs were located using MOTIFS programme (GCG). The signal peptide is shown as blue underline at the C-terminus of the CDK7 peptides. The conserved cysteines are shown in red. The protein kinase C phosphorylation site (consensus S/TXR/K) is shown in bold (positions 190/191 overlap and 294 and 297 are adjacent). The casein kinase II phosphorylation sites (S/TXXD/E) are underlined. The tyrosine kinase phosphorylation site (R/KXXD/EXXXY or R/KXXXD/EXXY) is underlined in green. Non conserved motifs are not shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Blast searches with the CDK7 peptide revealed that this protein is conserved in a large number of species from a broad phylogenetic background. We found CDK7 homologous putative proteins from 15 diverse species in data bases, all hypothetical unpublished proteins whose function remains unknown. The majority are approximately 300–350 amino acids long and are found in prokaryotes, insects, fungi, archaebacteria, plants, fish and mammals, showing between 20% identity to 94% identity to hCDK7 and all retaining the MOSC and MOSC N domains, as well as the CPRC domain (data not shown).

The human CDK7 gene is located at chromosome 1q42.11, approximately 8 Mb in the centromeric direction from a region of 1q42 which has been shown to carry a Type 1 and Type 2 diabetes susceptibility locus [18–20]. The human CDK7 gene spans 35,917 bp and comprises of eight exons. The rat CDK7 gene localises to rat chromosome 13q26. Based on Southern analysis we would predict that the rat CDK7 gene spans less than 70 kb (data not shown), and by searching the rat genome databases we have identified six exons of the gene (not shown).

Discussion

In this paper we describe the cloning and characterisation of a novel glucose regulated renal transcript, rCDK7, from diabetic rat kidneys which encodes a highly conserved putative peptide of 335 amino acids of unknown function. Both rCDK7 and hCDK7 mRNAs are transcriptionally up-regulated in experimental DN by >600% and >300%, and are located at rat chromosome 13q26 and human chromosome 1q42.11, respectively. Human chromosome 1 band q42 has been proposed to carry an unknown diabetes gene in several independent studies [18–20]. Although a considerable distance, 8 Mb is close enough in genetic distances to suggest that the inheritance of CDK7 in diabetes needs to be further investigated.

Hyperglycemia is an important aetiological factor in the development of microvascular diseases in diabetic patients [37], and induces renal injury through several complex and overlapping biochemical pathways [22] involving various key molecules, for example TGF-β1 [23], advanced glycation end products [24], and protein kinase C [25,26]. There is growing evidence that reactive oxygen species (ROS)

play a central role in the initiation and progression of DN by affecting the intracellular redox balance of the cell, which in turn can lead to abnormal activation of numerous pathways [27,28]. Schulze et al. [21] showed that hyperglycemia can promote oxidative stress through the inhibition of thioredoxin function by a thioredoxin interacting protein. Thioredoxin reductase and thioredoxin provide a ubiquitous and highly conserved oxidoreductase system with antioxidant and redox regulatory roles [21,29]. Thioredoxin is a small 12 kd protein with reactive cys-sulfhydryl groups located close to each other with the motif cys-gly-pro-cys. This motif is the catalytic centre of the enzyme and is also found in thioredoxin reductase. Within the MOSC and MOSC_N domains of CDK7 protein described in this paper, there is a remarkable conservation of specific cysteines. Interestingly a highly conserved cys-pro-arg-cys domain is present in all CDK7 homologues and is reminiscent of the catalytic domain in the enzyme thioredoxin [21]. The remarkable conservation of the cys-pro-arg-cys domain in all CDK7 homologues and its likeness to the thioredoxin catalytic domain are suggestive of a functional link between thioredoxin like proteins and CDK7. The involvement of CDK7 in some aspect of regulating the intracellular redox potential in the cell could explain why the gene is up regulated in hyperglycemia.

TGF-β induced cytoskeletal alterations in endothelial cells have been shown to be mediated by ROS production via NADPH oxidase [26]. Inoguchi et al. [26] demonstrated that vascular cells cultured in high glucose led to generation of ROS through the activation of protein kinase C dependent NADPH oxidase. Interestingly the CDK7 protein sequences contain 9 highly conserved protein kinase C phosphorylation sites (Fig. 4). There is strong evidence linking high glucose-induced ROS, activation of PKC signaling cascade and increased TGF-β activity to thickening of the mesangium in diabetic kidney [30].

The CDK7 protein is conserved across a diverse range of species, including mammals, plants, insects, fungi and prokaryotes. All CDK7 homologues show a characteristic presence of the MOSC (MOCO sulfurase C-terminal) and the MOSC_N domain in the same orientation. The MOSC domain is a superfamily of β-strand-rich domains identified in the molybdenum cofactor sulfurase and several other proteins from both prokaryotes and eukaryotes [31,32]. The proteins containing MOSC and MOSC_N are believed to be derived from the promitochondrial endosymbiont and have been proposed to function as sulfur carrier proteins in the synthesis of metal-sulfur clusters in the eukaryotic mitochondrion. A second function for the MOSC domain in the thiol-dependent redox pathway has also been proposed [31].

The thiol group, found in cysteines, can be oxidized to form disulphide. The oxidation and reduction of thiol groups found in many cellular proteins play an important role in the cellular antioxidant defence system as well as in intracellular signaling. [33]. The cellular antioxidant response has been shown to be defective in DN [34,35] whilst up-regulation of certain genes involved in the thiol pathway has also been previously described [33,36]. The up-regulation of CDK7 in high glucose in two experimental models of DN and the presence of highly conserved cysteines in the protein are consistent with the idea that this is a novel thiol protein involved in the cells' response to oxidative stress.

Acknowledgments

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