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Caldesmon over-expression in type 1 diabetic nephropathy☆

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Abstract

Substantial evidence supports a genetic susceptibility to develop nephropathy in type 1 diabetes and a key pathogenic role of actin cytoskeleton dysfunction in this complication. We previously reported that many cytoskeletal proteins were either up- or down-regulated in fibroblast cells from type 1 diabetic (T1DM) patients with nephropathy. The gene of one of these proteins, caldesmon, lies in a chromosomal region linked to nephropathy and its promoter region contains a single nucleotide polymorphism that is associated with nephropathy. Hence, we analyzed caldesmon gene and protein expression in cultured fibroblasts from T1DM patients with and without nephropathy and from control subjects. Caldesmon gene was studied in cells cultured under normal glucose levels by quantitative real-time RT-PCR. Caldesmon protein isoforms were quantified both under normal and high glucose conditions by two-dimensional electrophoresis. Caldesmon gene was over-expressed in fibroblasts from diabetic patients with nephropathy, in comparison to both those from diabetic patients without nephropathy and those from controls. We quantified six caldesmon protein isoforms, two of them were increased whereas another one was decreased only in fibroblasts from diabetic patients with nephropathy. None of these isoforms showed any difference in their relative abundance in response to high glucose. Variable results in response to high glucose were observed in the expression of other proteins in the three experimental groups. Our data lend further support to an involvement of caldesmon in the susceptibility to diabetic nephropathy in type 1 diabetes, independently from environmental glucose levels.

Keywords: Type 1 diabetes; Nephropathy; Caldesmon

1. Introduction

Diabetic nephropathy (DN) develops in 30–40% of type 1 diabetic (T1DM) patients over their lifespan. A genetic predisposition is thought to play a role in the development of this complication, independently from disease duration, metabolic control, or other factors (Ceriello et al., 2000; Huang et al., 2002; Iori et al., 2003; Lurbe, Fioretto, Mauer, LaPointe, & Batlle, 1996; Schena & Gesualdo, 2005; Trevisan, Fioretto, Barbosa, & Mauer, 1999; Trevisan et al., 1992). Using a proteomic approach, we previously showed that several proteins are differentially expressed in cultured fibroblasts from T1DM patients with (DN+) compare to those without (DN−) established nephropathy (Iori et al., 2008; Millioni et al., 2008; Tessari et al., 2007), indicating that (a) phenotypic differences entailing functionally and structurally important proteins are detectable in vitro and (b) these constitutive traits, which can be genetically determined, are maintained ex vivo under our experimental conditions.

We could also evidence an association between DN and the constitutive expression of some proteins in cultured skin fibroblasts from T1DM(DN+) patients, thus suggesting their involvement in the development of DN.

A typically altered protein class comprised the actin-associated proteins, including caldesmon (Millioni et al.,

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2008). The implication of caldesmon is consistent with results of in vitro and in vivo studies (Clarkson et al., 2002; Cortes et al., 2000; Zhou, Hurst, Templeton, & Whiteside, 1995), and it points to the importance for DN of genes that regulate the actin filament organization. This contention is in line with emerging evidences that actin cytoskeleton is a major target for high-glucose-triggered injury in DN (Clarkson et al., 2002) and entails a contributor to the development of diabetic glomerulosclerosis. In fact, a disassembly of the actin cytoskeleton in mesangial cells after high glucose treatment was reported to be associated with glomerular hyporesponsiveness to vasopressor stimuli (Zhou et al., 1995), which can be partially responsible for afferent arteriole vasodilatation, intraglomerular hypertension, and subsequent renal hyperfiltration, for example, the alterations that are typical of DN early stages (Zatz & Brenner, 1986).

The gene of one such actin-binding protein, caldesmon, is located on chromosome 7q35, a region previously linked to type 2 DN in two independent family-based studies (Fogarty et al., 1999; Imperatore et al., 1998). Moreover, a single nucleotide polymorphism (SNP) in the promoter region of caldesmon gene was found to be associated with type 1 DN in the Northern Ireland population and this was replicated in an independent sample from the Republic of Ireland (Conway et al., 2004). According to a bioinformatic analysis, this SNP would disrupt a transcription factor binding site and thereby might alter expression of the caldesmon gene.

Caldesmon has several actions that can be relevant for DN: it inhibits cell contractility and can promote either assembly or disassembly of actin filaments (Helfman et al., 1999; Yamashiro, Chern, Yamakita, & Matsumura, 2001). It also regulates exocytosis and hence matrix deposition (Matsumura & Yamashiro, 1993). Furthermore, phosphorylation of caldesmon is required for the formation of contractile rings during mitosis and for regulation of cell proliferation (Yamashiro et al., 2001). However, whether an altered caldesmon gene expression is a keynote of DN remains uncertain. Hence, our aims were therefore twofold: firstly, to investigate the caldesmon expression at the trascriptome level in cultured skin fibroblasts from (DN+) and (DN−) patients and, secondly, to assess the effect of hyperglycemia on the expression of this protein. To reach these goals, we took advantage of a study model in which the untoward interferences of environmental factors are removed, thus allowing to analyze only the alterations that derive from a constitutive, genetic predisposition to DN.

2. Materials and methods

2.1. Subjects

Five T1DM(DN+) (AER>200 μg/min) and five T1DM (DN−) patients (AER<20 μg/min) were recruited. Five healthy subjects without a family history of hypertension and diabetes were also studied as controls (Table 1). AER was determined on three timed overnight urine samples by turbidimetric method (Turbiquant Albumin, Dade Behring, Marburg, Germany); the median value of the three samples was used for classification purposes. Serum creatinine (μmol/l; mean±S.D.) was 86±8.5, 85±5.9 and 347.3±160 in healthy, T1DM(DN−), and T1DM(DN+) subjects, respectively. All subjects were of Caucasian origin. Age, male/female ratio, BMI, and mean blood pressure did not differ across the groups. The two T1DM patient groups were also similar for disease duration (~20 years) and glycated hemoglobin (Corning gel electrophoresis, Ciba-Corning, California, USA). The protocol was approved by the Ethical Committee of the Medical Faculty at the University of Padova and was performed according to the Helsinki Declaration (1983 revision).

2.2. Fibroblast cultures

Skin biopsies were taken by excision under local anesthesia from the anterior surface of the forearm. Fibroblasts were cultured in HAM'S F-10 (Sigma-Aldrich, Missouri, USA) supplemented with 10% FBS, 1 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C until confluence. The growth medium was changed with quiescent medium 24 h before protein and RNA extractions. To evaluate the effects of high glucose on the proteomic profile, proteins were extracted from cells cultured in medium containing either 6 or 21 mM D-glucose for 72 h. Cells were used between the seventh and the eighth passage.

Table 1
Clinical features of T1DM patients with and without DN and normal control subjects

	Normal control subjects	T1DM patients without nephropathy	T1DM patients with nephropathy
n (male/female)	2/3	2/3	2/3
Age (years)	37±4.6	39.4±5	35.6±3.8
Body mass index (kg/m^2)	22.9±1.2	24.1±0.4	22.2±1.2
Mean blood pressure (mmHg)	93.3±10	95.2±2.7	99.1±6.8
Serum creatinine (μmol/l) (range)	86±9 (79–94)	85±6 (70–92)	347±160 (73–860)
Duration of diabetes (years)	–	22.8±3.2	21.8±4.5
Glycated hemoglobin (%)	–	9.3±0.8	10.5±1.3
Albumin excretion rate (μg/min) (range)	–	9.9 (0–11.3)	662.5 (625–922)

Values are means±S.E.M., except for albumin excretion rate, which is given as median and range.