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REVIEW ARTICLE

Glucose Transporter Roles in the Development of Glomerulosclerosis

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ABSTRACT

Extensive research has been done over the last several decades in pursuit of mechanisms leading to the glomerular disease and glomerulosclerosis in diabetic nephropathy. Much of the research investigated downstream pathways and terminal pathways to extracellular matrix production in the mesangial cells, after exposure to diabetes mellitus or high extracellular glucose concentrations. More recent research identified glucose transporter proteins expressed in normal and diabetic glomeruli, with the potential to regulate glucose uptake and metabolism in the glomerular mesangial cells. The mesangial cells are directly involved in excess matrix production in both diabetic and nondiabetic glomerulosclerosis. The common finding of excess glomerular glucose transporter expression in the development of both diabetic and nondiabetic glomerulosclerosis, provides a clue to how these glomerular lesions develop. Here, we review the potential roles of glucose transporter proteins, particularly facilitative glucose transporters (GLUTs), in enhancing mesangial cell glucose uptake, metabolism, and signaling to extracellular matrix expression which scars glomeruli. Both diabetes mellitus and glomerular hypertension without diabetes, have been shown to stimulate glomerular GLUT1 expression allowing for increased cellular glucose uptake. The stretch – inducible Mechano-Growth Factor recently identified in mesangial cells has the potential to translate glomerular hypertension with mesangial stretch in both diabetic and nondiabetic glomerular disease, into excess mesangial GLUT1 expression, glucose uptake and matrix production. Future research on this topic will likely be valuable. Positive feedback mechanisms are highlighted which can enhance mesangial GLUT1 expression to perpetuate glucose-induced matrix production and glomerular scarring in vivo.

Keywords: Glucose Transporter, GLUT, diabetic glomerulosclerosis, nondiabetic glomerulosclerosis, mesangial cell.

Introduction

The pathogenesis of diabetic glomerular disease has been investigated for many decades in attempt to understand how diabetic kidney disease develops and progresses to eventually cause end stage renal disease (ESRD.) The kidney disease in diabetes mellitus is a major contributor to morbidity and mortality in these patients, whether Type 1 or Type 2 diabetes[1,2]. A great deal of work and time has been expended in the identification of various glomerular pathways contributing to the scarring of glomeruli in this disease[3]. Transforming Growth Factor beta 1 (TGFß1), Map Kinases (MAPK's), protein kinase C (PKC), reactive oxygen species (ROS), advanced glycosylation end products (AGE's), and other factors have been repeatedly implicated in diabetic glomerular disease[3-7]. More recently, a potential role for glomerular GLUT1, a high affinity facilitative glucose transport protein identified in mesangial cells, endothelial cells, and glomerular epithelial cells, was identified as an important regulator of glomerulosclerosis in diabetic rodents[7-10], and in nondiabetic rodents exhibiting development of glomerulosclerosis[11]. Subsequently, examination of primary culture human mesangial cells from kidney biopsies of Type 2 diabetic patients with diabetic nephropathy, and from nondiabetic patients donating a kidney for transplant, revealed elevated GLUT1 mRNA and protein in the diabetic mesangial cells, associated with increased glucose uptake and extracellular matrix (ECM) expression[12].

GLUT1 is one of multiple facilitative glucose transporters in which glucose transport depends upon a gradient of glucose across the cell membrane[13]. It is a prominent GLUT expressed in mesangial cells. GLUT1 is typically at or near saturation at physiologic glucose concentrations, and tends to be rate-limiting for cellular glucose uptake[13]. It is known to be constitutively expressed in the plasma membrane, for example in muscle and fat[13]. Here we will review the data implicating glomerular glucose transport proteins in the development of diabetic glomerulosclerosis, as well as nondiabetic glomerulosclerosis. It is evident that glucose transporters have the potential to regulate some downstream mesangial cell pathways already implicated in diabetic glomerulosclerosis development[6,7,14,15].

Identification of Glucose Transport Proteins in Glomerular Mesangial Cells:

An early study of the rodent kidney for glucose transport proteins[16] identified facilitative glucose transporters (GLUTs) predominantly in renal tubule segments, by immunofluorescence localization. The GLUT signals in glomeruli were apparently much lower in comparison to brightly labelled tubule segments[16]. Subsequent investigation of rodent kidneys identified low level expression of GLUTs 1, 3 and 4 in normal glomeruli[17-19] using immunogold localization and molecular phenotyping, in addition to higher expression of GLUTs in renal tubule segments[17]. Other GLUTs identified in glomeruli have included GLUT5 and GLUT8[17,20,21]. Expression of GLUTs 1 and 4 in cultured mesangial cells, and also GLUT5 in glomerular mesangial cells, was later confirmed[8,18,20,22] where GLUT1 appeared to dominate. This has potentially important implications for the development of

glomerular scarring. The mesangial cells are believed to play a key role in the glomerular scarring process in general[8,14,23,24]. Data in primary culture human mesangial cells and a preliminary report in a commercial line of human mesangial cells, revealed that they express GLUT1, which increases in response to diabetic levels of extracellular glucose (eg. 20 mM or 360 mg/dl)[25,26]. Recent preliminary reports have also identified Mechano-Growth Factor (MGF), a stretch-induced growth factor in muscle, in normal mouse glomerular mesangium, and it was increased by both Type 1 and Type 2 diabetes mellitus[27]. Mechano-growth Factor (MGF) stimulates mesangial cell GLUT1, glucose uptake and ECM production in preliminary reports[27,28]. Therefore, MGF is a potential link between mesangial stretch from glomerular hypertension, and excess mesangial cell glucose uptake and metabolism leading to ECM production. Further research is needed to investigate a potential role for this mesangial cell growth factor in diabetic and nondiabetic glomerular disease.

In vitro, GLUT1 was identified as the major mesangial cell glucose transport protein[8,23], and this was largely expressed at the cell membrane[10,14,29]. Mesangial cell GLUT1 was then found to respond with increased expression in response to extracellular glucose concentrations in the diabetic range (i.e. 20 mM or 360 mg/dL)[8]. This glucose - induced GLUT1 expression was initially unexpected, since in other tissues, such as vascular smooth muscle, GLUT proteins tend to be suppressed by hyperglycemia or high extracellular glucose concentrations[19,30,31]. Therefore, the glomerular mesangial cells have a relatively unique GLUT1 response, which might play a role in the diabetic damage of these cells and respective glomeruli, if they are not protected from "glucose toxicity"[32]. GLUT5 has also been identified to increase in the diabetic rat mesangium[20], however it is actually a fructose transporter.

Experiments to replicate the high mesangial cell GLUT1 expression of high glucose - exposed mesangial cells and diabetic rat glomeruli were performed in rat 16KC2 mesangial cells[14]. The rat 16KC2 mesangial cell line, a continuous, spontaneously transformed mesangial cell line[33] which maintains the adult phenotype, was used for transduction of human GLUT1, to overexpress this glucose transporter. These cells were then examined for their phenotype. The results of 10-fold overexpression of GLUT1 in these cultured mesangial cells revealed a 5-fold increased glucose uptake rate, with 43-fold increased glucose utilization, and activation of the glycolytic pathway[14]. Evidence for polyol pathway activation was also obtained[6], as well as classic protein kinase C (PKC) protein activations[34]. Specifically, PKCß1 and PKCa were activated, resulting in AP1 transcription factor activation. Chronic, increased expression of Type IV collagen (Col-IV), Type I collagen (Col-I), Fibronectin (FN), and Laminin (LN) ECM proteins was observed in these GLUT1-overexpressing mesangial cells[6,14,35]. So, it became evident that mesangial cell GLUT1 was rate-limiting for glucose entry into the cells, and was an important regulator of mesangial cell matrix protein expression. The latter is important in the development of glomerulosclerosis in vivo, and is demonstrated in the glomerular disease which develops in transgenic mice designed to overexpress GLUT1 in mesangial

cells in vivo[7]. Later experiments in rat mesangial cells transduced with antisense-GLUT1 to reduce mesangial cell glucose uptake, indicated that high glucose-induced ECM expression could be blocked by this maneuver[24]. Preliminary evidence in vivo supports the protective effect of antisense-GLUT1 against diabetes-induced glomerulosclerosis[36]. This has potential implications for future therapeutic prevention of glomerulosclerosis in vivo.

Rodent Models of Altered Glomerular Glucose Transporter Expression:

1. Diabetic rodents with increased renal/ glomerular GLUT Expression: Subsequent to identification of the GLUT1 facilitative glucose transporter in mesangial cells[14,22]. D'Agord Schaan in 2001 reported diabetes – induced GLUT1 expression in rat renal cortex[37]. Chen et al in 2003 provided a preliminary report of diabetes-induced glomerular GLUT1 expression in mice[36], and in 2006 Ricci et al described diabetes-induced glomerular GLUT1 in Milan rats[10]. The diabetic Milan Normotensive Strain (MNS) rats exhibited features of diabetic nephropathy along with the increased glomerular GLUT1 expression[10]. In another report, diabetic rats were subsequently shown to upregulate GLUT5 (fructose transporter) in the mesangium as well[20].

2. Nondiabetic Transgenic Mice with Overexpression of Glomerular Mesangial GLUT1 to mimic the effect of diabetes: Subsequent to in vitro experiments defining the role that excess mesangial cell GLUT1 expression has to drive signaling to excessive ECM protein production[14], transgenic mice overexpressing GLUT1 in C57BL6 mice were produced to test the in vivo effects of the excess GLUT1 on glomeruli, in the absence of diabetes mellitus[7]. These mice overexpressed GLUT1 in multiple tissues, however in the glomeruli the overexpression was predominantly in the mesangial cells. The mesangial cells of the transgenic mice were shown to overexpress GLUT1, and in vivo the glomeruli with GLUT1 overexpression revealed activation of PKCa, PKCß1, and NFkß, resulting in excess glomerular Col-IV, Col-I, and FN. The transgenic mice by 26 weeks of age displayed glomerulosclerosis with an elevated serum creatinine level and albuminuria, compared with control nontransgenic mice[7]. The glomeruli of the transgenic mice were also hypertrophied when compared to controls. Therefore, multiple features characteristic of diabetic glomeruli were recreated in nondiabetic mice designed to overexpress GLUT1 in the mesangial cells. This suggested that GLUT1 could play an important role in glomerulosclerosis due to diabetes. Preliminary data in transgenic mice designed to express antisense-GLUT1 in the mesangial cells in vivo supports this idea[36]. The antisense-GLUT1 mice have reduced mesangial cell GLUT1, and when made diabetic with the homozygous db/db genotype, their glomeruli are protected against diabetes - induced PKC and Col-IV, Col-I, and FN expression. Furthermore, when cultured rat mesangial cells were transduced with antisense-GLUT1 in vitro to suppress this glucose transporter, this maneuver was effective at blocking high glucose-induced ECM production[24].

3. In stark contrast to the glomerular disease induced by overexpression of GLUT1 in

mouse glomerular mesangial cells with a modified human ß-Actin promoter[7,38], separate experiments involving transgenic overexpression of GLUT1 in mouse podocytes in vivo with the Podocin promoter did *not* produce detectable glomerular disease, but rather was protective against diabetes – induced glomerulosclerosis[39].

4. Dahl Salt-sensitive vs SHR rats: Dahl Salt-Sensitive (DSS) rats develop glomerular hypertension when fed a high salt diet, which correlates with increased glomerular GLUT1 expression[26]. These mice develop mesangial expansion and glomerulosclerosis, as compared to nonhypertensive control mice (DSR or Dahl Salt Resistant mice)[26,40].

5. Milan Rats: Milan Normotensive rats (MNS) develop age-dependent spontaneous glomerulosclerosis[9,41], and exhibit diabetes-induced expression of glomerular GLUT1, ECM and TGFß when compared to nondiabetic MNS rats and to Milan Hypertensive Strain (MHS) rats[10]. MHS rats have built - in protection against glomerular hypertension and glomerulosclerosis, believed to be due to hypertrophied intrarenal arterioles, and they do not develop diabetes-induced glomerular GLUT1 or ECM[10] The MNS rats differ from MHS rats in that the former are not expected to be protected against diabetes – induced impairment of intrarenal arteriolar autoregulation, and thus could be exposed to development of glomerular hypertension with mesangial cell stretch. Gnudi[26] and Wang[11] have demonstrated that mechanical stretch of human and /or rat mesangial cells increases GLUT1 expression and glucose uptake, an effect expected to induce mesangial cell ECM production[10,14].

6. Fvb Os/+ Mice exhibit approximately 85% reduction of glomerular numbers compared with Fvb +/+ wild type control mice[11,42] and the Fvb Os/+ mice develop glomerular hypertension[42] plus an increase of glomerular GLUT1 protein early after birth[11,29]. Fvb Os/+ mice developed dramatic albuminuria, already evident at 4 weeks of age, associated with rapid development of glomerulosclerosis and renal failure[11]. The Fvb Os/+ mice die early, at approximately 12 weeks of age in the setting of advanced renal failure. This rapidly progressive renal disease in the absence of diabetes mellitus, developed despite a normal glomerular architecture by TEM and light microscopy at birth[11]. The development of severe, rapidly progressive glomerulosclerosis, renal failure and early death in Fvb mice carrying the Os allele, contrasts sharply with other mouse strains carrying the Os allele: C57BL6 Os/+ mice (minimal glomerulosclerosis) and ROP Os/+ mice (severe glomerulosclerosis without severe functional impairment). The C57BL6 Os/+ mice and ROP Os/+ mice do not develop severe enough disease to cause renal failure or death[43,44]. This suggests the Fvb mice carry susceptibility genes which may predispose them to the severe, sclerosing phenotype that leads to kidney failure and death. A potential contributing factor to the more severe glomerular disease in the Fvb background is that the Os allele caused approximately 85% reduction in the glomerular number in Fvb mice, while the Os allele in C57BL6 and ROP mice caused a less severe, 50% reduction in glomerular numbers[45].

Table 1. Mouse and Mesangial Cell Models of Altered GLUT1 expression, with Parallel Changes in Glomerulosclerosis and Extracellular Matrix

Ref.		Model
Heilig in JCI 1995[14]		Transduced rat MC in vitro with overexpression GLUT1.
Heilig in AJP 2001[24]		Transduced rat MC in vitro with antisense-GLUT1 have suppressed GLUT1
Zhang in AJP 2010[39]		Transgenic mice overexpress GLUT1 in podocytes.
Heilig in Diabetes 1997[8]		High glucose exposure increases MC GLUT1 in vitro.
Wang in AJP 2010[7]		Transgenic overexpression of GLUT1 in mouse glomerular MC in vivo.
Ricci Nephrol Dial Transplant 2006[10]		Milan Normotensive Rat (MNS), GS – prone, exhibits enhanced diabetes – induced glomerular GLUT1 and ECM.
Gnudi in Hypertensin 2003[26]		Glomerular hypertension in DSS rats associated with increased glomerular GLUT1 and glomerulosclerosis.

Abbreviations: Mesangial cells (MC); Glomerulosclerosis (GS); Extracellular Matrix (ECM).

Regulators of GLUT1 in Mesangial Cells, other than High Glucose itself:

FLAVONES AND ISOFLAVONES: Quercetin and Myricetin (flavones), and Genistein plus other isoflavones[46], have been shown to inhibit glucose transport through GLUT1 in HL-60 cells. These agents may bind competitively to an ATP binding site on GLUT1 to inhibit glucose transport[46]. Also, in a preliminary report[47], Quercetin treatment (a potent inhibitor of GLUT1) was protective against high glucose - induced mesangial cell ECM expression.

MESANGIAL CELL STRETCH: Mechanical stretch of rodent and human mesangial cells in vitro has been shown to increase GLUT1 glucose transporter expression and glucose uptake[11,26,48]. The mesangial cells theoretically can become stretched in situations where glomerular hypertension develops, since the mesangial cells are attached to the glomerular capillary basement membranes[49].

GROWTH FACTORS: TGFß, VEGF, IGF1, ATII, and possibly MGF[8,11,25,26]. These growth factors have been shown to increase GLUT1 and/or glucose uptake into mesangial cells. Notably, rat mesangial cells designed to overexpress GLUT1 in the absence of elevated extracellular glucose concentration exhibited persistent excess production of Col-IV, Col-I, FN, and LN in the absence of chronic elevated TGFß expression[35]. Here the

persistently activated PKC pathway may play an important role in the excess ECM production of these mesangial cells.

SIGNALING PROTEINS: (PKC classic isoforms) PKCa and PKCß1 have both been found to be persistently activated in response to increased mesangial and glomerular GLUT1 expression, with enhanced glucose uptake[6,7,14]. In vitro mesangial cells overexpressing GLUT1 in a normal glucose milieu have suppressed Erk ½ MAPK expression[50], which is opposite from the effect of a diabetic milieu[50].

TRANSCRIPTION FACTORS: (AP-1, NFkß). AP-1 Transcription Factor activation was described in mesangial cells overexpressing GLUT1[15]. AP-1 activation was linked to stimulation of FN expression in these mesangial cells[35]. NFkß activation was described in glomerular mesangial cells of transgenic mice overexpressing GLUT1[7,11], and in Fvb Os/+ mice with high glomerular GLUT1 expression and rapidly developing glomerulosclerosis[11]. The GLUT1 - induced AP-1 activation and NFkß activation can promote ECM gene expression downstream[14,15].

POSITIVE FEEDBACK ON GLUT1: Experiments in rat mesangial cells in vitro revealed that both an isolated increase in GLUT1 expression, and exposure to 20 mM high glucose medium, could stimulate GLUT1 gene transcription[24]. Furthermore, suppression of mesangial cell GLUT1 protein with antisense prevented 20 mM high glucose stimulation of GLUT1 gene transcription[24]. The effect of increased mesangial cell GLUT1 expression to increase glucose uptake also induces classic PKC signaling[14], which could increase GLUT1 gene expression via TPA-responsive elements (TRE's) in two enhancers of the GLUT1 gene[51]. TGFß1, and potentially other glucose – induced growth factors such as VEGF and MGF, might also increase GLUT1 gene transcription via the same serum/growth factor/oncogene - responsive enhancers in the GLUT1 gene, 5' and 3' to the promoter[51]. This could be tested in vitro in mesangial cells.

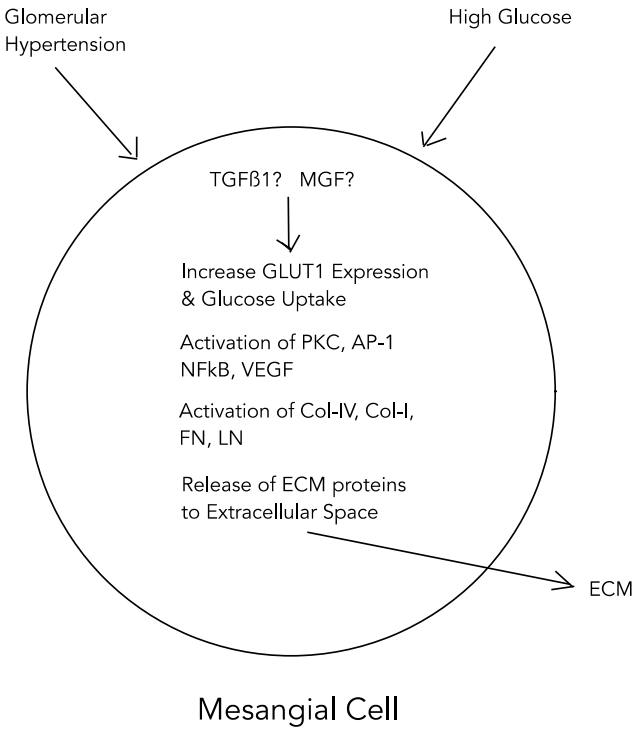

Figure 1.

Conclusions:

Elevated glomerular GLUT1 expression is observed in multiple rodent models where glomerulosclerosis or excess ECM production develops, both diabetic and nondiabetic: Milan mice, DSS mice, transgenic GLUT1-overexpressing mice; diabetic mice, and Fvb Os/+ nondiabetic mice. Positive feedback mechanisms on GLUT1 expression in mesangial cells appear to promote persistent stimulation of mesangial cell GLUT1 expression and attendant glucose uptake via glucose-induced PKC and TGFß1 expression, and possibly also VEGF and MGF expression.

Glomerular hypertension without diabetes also appears to stimulate glomerular GLUT1

expression, and consistent with this, in vitro experiments have described the induction of mesangial cell GLUT1 expression by mechanical stretch. GLUT1 has been found to be expressed by human mesangial cells, and as in the diabetic rodents, high glucose exposure in the diabetic range stimulates increased expression of the GLUT1 protein. Suppression of GLUT1 in mesangial cells is protective against high glucose - induced ECM protection.

The data therefore suggests the glomerular mesangial GLUT1 glucose transporter may be a valuable target for future therapies designed to prevent glomerulosclerosis, whether it is being induced by diabetes mellitus, or by glomerular hypertension unrelated to diabetes. Further examination of glucose transporter involvement in both diabetic and nondiabetic human glomerular disease will be needed to more extensively characterize the role of these transporters in the scarring process.

Conflict of Interest:

Technology licensing of an in vitro mesangial cell model for glomerulosclerosis, allowing initial screening of potential drugs to inhibit the glomerular scarring process.

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