eGFR60-90 の患者の eGFR30%低下を予測するための早期バイオマーカーとしての有用性は示されなかった。一方、24hrAER は微量アルブミン尿の出現早期あるいは正常アルブミン尿の段階でも腎機能低下を予測する良いマーカーとなる可能性が示された。

今後、さらに高い診断能を有する代謝物バイオマーカーセットを探索していく必要がある。

E. 結論

網羅的メタボローム解析は糖尿病性腎症を早期診断するマーカーを探索するアプローチとして有用である。腎予後を予測するためのマーカーはアルブミン尿に勝るものは得られなかった。今後、メタボローム解析にて探索する必要が再確認された。

F. 健康危険情報

ありません。

G. 研究発表

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H. 知的所有権の出願・取得状況

1. 特許出願

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分担・総合研究報告書

高脂血症による糖尿病性腎症進展に関わる分子病態マーカーに関する研究

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研究要旨

脂質代謝異常は糖尿病性腎症悪化の独立した危険因子である。糖尿病性腎症モデルマウスに高脂肪食を与えるとアルブミン尿が倍増し、糸球体病変も悪化した。また糖尿病性腎症モデルマウスの糸球体においてtoll-like receptor 4 (TLR4)およびその内因性リガンドの一つであるmyeloid-related protein 8 (MRP8またはS100A8)の発現が増加し、高脂肪食の負荷によりさらに増加することを明らかにした。ヒトおよびマウスの糖尿病性腎症の組織において、MRP8は主に糸球体に浸潤するマクロファージに発現していた。マウス骨髄由来マクロファージにおいて高糖濃度および遊離脂肪酸であるパルミチン酸による処理は相乗的にMRP8の発現を増加させ、マクロファージへのMRP8の添加は炎症性サイトカインの発現を誘導した。TLR4ノックアウトマウスでは高脂肪食負荷による糖尿病性腎症の悪化が軽減されていた。糖尿病性腎症、肥満関連腎症、微小変化群、糸球体病変軽微の4群の症例を含む腎生検組織での検討により、糸球体内MRP8陽性細胞数は腎機能低下、蛋白尿と正の相関を示し、1年後の蛋白尿を予測する独立した因子であった。以上の成績より、糸球体内MRP8の発現は高脂血症による糖尿病性腎症悪化のメカニズムの一つであること、予後不良の蛋白尿のマーカーとなることが示唆された。今後さらに、尿中MRP8と糖尿病性腎症の病態との関係を明らかにしていく予定である。

A. 研究目的

糖尿病性腎症は透析導入の最大の原因であり、その病態を解明し新規治療法を見出すことは厚生労働行政上の重要課題の一つと考えられる。高LDLコレステロール血症および高トリグリセリド血症が、それぞれ糖尿病性腎症悪化の独立した危険因子であることは多くの疫学的研究により報告されてきたが、その分子機構は未解明である。

我々は糖尿病性腎症モデルマウスに高脂肪食を与えると腎症が悪化することを確認

し、糸球体内で発現増加する分子の同定を試みた。遺伝子改変動物を用いて病態修飾候補分子の機能的意義を明らかにした。さらにヒト腎生検組織における発現を検討し、分子病態マーカーとしての有用性を検討した。

B. 研究方法

1型糖尿病モデルマウスとしてはストレプトゾトシン(STZ)誘発モデル、2 型糖尿病モデルとしてはdb/dbマウス、脂肪萎縮型糖尿病 A-ZIP/F-1 マウスを用いた(米国 NIH・Charles Vinson 先生より分与)[Moitra et al. Genes Dev 12:3168-3181, 1998]。TLR4 ノックアウト(KO)マウスは大阪大学免疫学フロンティア研究センター・審良静男先生らが開発したものを用いた [Hoshino et al. J Immunol 162:3749-3752, 1999]。マウスマクロファージは骨髄由来細胞を溶血処理後に 50 ng/ml M-CSF を添加して作成した。

(倫理面への配慮)

腎生検組織および尿については京都大学内分泌代謝内科・腎臓内科の外来・入院症例および大阪市立総合医療センター腎臓高血圧内科の症例(今西政仁先生との共同研究)のサンプルを用いた。本研究は臨床診断に必要なサンプルの残余部分を用いるものであり、研究対象患者には追加の危険性はないと考えられる。個別の研究結果は、臨床的判断に用いたり対象者に告知することはなく、対象患者への直接的利益はない。臨床研究に参加しないことによる診療上の不利益もない。サンプルは匿名化を行い、人権擁護上の配慮を行う。研究対象者は文書の上でインフォームド・コンセントを示した症例に限定される。本研究は京都大学大学院医学研究科及び大阪市立総合医療センターの医の倫理委員会の承認を受けている。また動物実験委員会、組替え DNA 委員会の承認も得ている。

C. 研究結果

STZ 誘発糖尿病性腎症モデルマウスに脂肪含有量 60%の高脂肪食を与えるとアルブミン尿が倍増し、糸球体病変も悪化した。db/db マウスへの高脂肪食負荷でもアルブミン尿が倍増した。

STZ マウスおよび A-ZIP/F-1 マウスの糸球体より mRNA を抽出し、マイクロアレイにより解析した結果、TLR4 および MRP8 の遺伝子発現が両者で共通して増加していた。STZ マウスへの高脂肪食負荷はさらにこれらの発現を増強した。ヒトおよびマウスの糖尿病性腎症の組織において、MRP8 は主に糸球体に浸潤するマクロファージ(Mac2 あるいは CD68 陽性細胞)に発現していた。

マウス骨髄由来マクロファージにおいて高糖濃度および遊離脂肪酸であるパルミチン酸による処理は相乗的に MRP8 の発現を増加させたが、TLR4 KO マウス由来のマクロファージでは効果が減弱していた。マクロファージへの MRP8 の添加は IL-1β、TNFα および MRP8 自身の遺伝子発現を誘導した。

TLR4 ノックアウトマウスでは高脂肪食負荷による STZ 誘発糖尿病性腎症の悪化が軽減され、アルブミン尿、メサンギウム領域拡大、糸球体内マクロファージ浸潤などが抑制されていた。

糖尿病性腎症、肥満関連腎症、微小変化群、糸球体病変軽微の 4 群の症例を含むヒト腎生検組織での検討により、MRP8 陽性細胞は尿細管間質領域よりも糸球体内に優位に認められ、多いほうから糖尿病性腎症、肥満関連腎症、微小変化群、糸球体病変軽微の順であった。単変量解析では、糸

球体内MRP8陽性細胞数ならびにMRP8陽性尿細管間質面積は、両者ともに収縮期血圧、蛋白尿、血清クレアチニン値、糸球体硬化、尿細管間質線維化と正相関していた。また腎生検 1 年後の蛋白尿を規定する独立した因子を多変量解析にて検討すると、糸球体内MRP8陽性細胞数(β=0.59, P<0.001)、蛋白尿(β=0.37, P<0.005)、収縮期血圧(β=0.21, P<0.05)の３つが選出された。とくに糸球体内 MRP8 陽性細胞数は、単変量解析では腎生検時の蛋白尿(β=0.78)よりもむしろ 1 年後の蛋白尿(β=0.87)と強い相関を示し、治療抵抗性の蛋白尿の指標となると考えられた。

D. 考察

糖尿病性腎症の腎生検組織で糸球体内の CD68 陽性マクロファージおよび尿細管間質領域の萎縮尿細管に MRP8 は強く発現していた。肥満関連腎症における MRP8 発現は糸球体病変軽微・微小変化群よりも強く、糖尿病性腎症よりも弱かった。横断的検討の多変量解析では、尿細管間質 MRP8 陽性面積は蛋白尿と尿細管間質線維化と相関を示し、糸球体内 MRP8 陽性細胞数は尿細管間質線維化と(微小変化群を除いた症例の)蛋白尿と相関を示した。縦断的検討の多変量解析では、腎生検１年後の蛋白尿は糸球体内 MRP8 陽性細胞数・生検時蛋白尿・血圧と関連していた。微小変化群では生検時蛋白尿が多いが、糸球体内のMRP8発現は少なく、１年後の蛋白尿も少なかったため、全体で評価すると、糸球体内MRP8陽性細胞数は、生検時よりもむしろ１年後の蛋白尿と非常に強い相関を示すと考えられた。

また我々は動物実験において、高脂血症合併による糖尿病性腎症の悪化に伴い、糸球体内MRP8発現は増加するが、MRP8受容体である TLR4 欠損マウスでは高脂血症による腎症悪化が軽減されることを見出しており、糸球体内 MRP8 陽性細胞は、単なる腎病変のマーカーのひとつに留まらず、病理学的に重要なプレーヤーであると考えられた。培養マクロファージを高糖濃度と遊離脂肪酸で刺激すると MRP8 の発現が増加した。一方、尿細管細胞における MRP8 の発現は尿細管間質の線維化を悪化させることも報告されている[Fujiu et al. J Clin Invest 121:3425-3441, 2011]。

さらに骨髄由来マクロファージに MRP8 蛋白を添加すると MRP8 自身および IL-1β や TNFα などの炎症性サイトカインの発現が TLR4 依存的に誘導された。マウスの糖尿病性腎症では糸球体内と尿細管間質領域の両方で Mac2 陽性マクロファージが増加したが、MRP8 は主に糸球体内のマクロファージに発現していたことからマクロファージと糸球体構成細胞との間の相互作用の存在が示唆される。マクロファージを炎症誘発性 M1 マクロファージと、抗炎症性・線維化誘発性 M2 マクロファージに大別すると、高脂血症合併糖尿病性腎症マウスでは MRP8/TLR4 のシグナルは主に M1 マクロファージを誘導すると考えられた。また 1 型糖尿病患者の末梢血単核球の MRP8 の発現は、腎症を含めた糖尿病合併症を有する症例で高値を示すことも示されている[Jin et al. Diabetes Care 36:2794-2802, 2013]。

今後は尿中 MRP8 濃度と糖尿病性腎症、あるいは他の慢性腎臓病の病態との関係を明らかにしていく予定である

E. 結論

重症の糖尿病性腎症、とくに高脂血症、高血圧症、高度蛋白尿などの合併に伴う糖尿病性腎症の悪化において MRP8/TLR4 系

シグナルは増悪因子として重要な役割を果たしていることが示唆された。

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G. 知的所有権の出願・取得状況

特記事項なし

研究成果の刊行に関する一覧

研究成果の刊行に関する一覧表

雑誌 （謝辞があるものを以下に記載する）

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REVIEW ARTICLE

Clinical impact of albuminuria in diabetic nephropathy

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Abstract Patients suffering from diabetic nephropathy, resulting in end-stage renal failure, are increasing in number. The pathophysiology of diabetic nephropathy remains to be fully investigated. In the clinical setting, the presence of albuminuria/overt proteinuria and a low glomerular filtration rate may predict poor renal prognosis, but the prognosis of the normoalbuminuric renally insufficient diabetic patient remains controversial. In addition to the measurement of urinary albumin excretion, biomarker studies to detect diabetic nephropathy more specifically at the early stage have been performed worldwide. There is a growing body of evidence for remission and/or regression of diabetic nephropathy, which may be an indicator for cardiovascular and renal risk reduction. Deeper insights into the pathological characteristics as well as the clinical impact of albuminuria on renal and cardiovascular outcome are required.

Keywords Diabetic nephropathy · Albuminuria · Proteinuria · Glomerular filtration rate · Cardiovascular disease · Renal outcome

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Introduction

Based on the annual report of the Japanese Society for Dialysis Therapy (JSDT), diabetic nephropathy is a leading cause of end-stage renal failure in Japan [1]. The number of dialysis patients had increased to 297,126 by the end of 2010. According to the annual report of the JSDT, diabetic nephropathy has been a leading primary disease of new patients who have been started on dialysis since 1998 [1]: the number of such patients with diabetic nephropathy has increased to 43.5%. In addition, cardiovascular diseases and deaths in patients with diabetes and underlying renal disease before and after dialysis has increased [2, 3]. Therefore, preventing and halting the progression of diabetic nephropathy is important if we are to prolong the survival of such patients.

Characteristic pathologic changes associated with diabetic nephropathy are accumulation of extracellular matrix (ECM) and the infiltration of inflammatory cells into glomeruli and tubulointerstitial regions [4, 5]. These pathologic abnormalities are induced by alterations in ECM production or degradation [6]. Generally speaking, the occurrence of albuminuria is a reflection of increased matrix deposition, leading to glomerular and tubulointerstitial lesions. Diabetic nephropathy is a clinical entity in

which the presence of persistent albuminuria and declines in renal function and glomerular filtration rate (GFR) are the major characteristic findings, which are closely associated with end-stage renal diseases, enhanced cardiovascular morbidity and eventual mortality [7]. The incidence of albuminuria, which currently contributes to the diagnosis of diabetic nephropathy, is well correlated with a decrease in GFR and the incidence of cardiovascular diseases.

Here, we focus on the clinical impact of albuminuria along with GFR levels on the progression of diabetic nephropathy and the incidence of cardiovascular diseases, which is closely related to the mortality of patients with diabetic nephropathy in this manuscript.

Albuminuria in the diagnosis of diabetic nephropathy

The definitive diagnosis of diabetic nephropathy is based on pathological findings such as the presence of diffuse mesangial lesions and nodular lesions. However, renal biopsy is not performed for all patients with diabetic nephropathy. In the clinical setting, the presence of persistent proteinuria as well as other complications such as diabetic retinopathy and renal dysfunction is important in the diagnosis of diabetic nephropathy. However, early detection of the presence of diabetic nephropathy is clinically required for the best prognosis. The measurement of urinary albumin excretion is currently crucial to the detection of early diabetic nephropathy. The increased excretion of albumin (albuminuria) is an early diagnostic indicator of diabetic nephropathy. Thus, Mogensen et al. [8] proposed a classification of diabetic nephropathy in patients with type 1 diabetes based on increased urinary albumin excretion once diabetic nephropathy was diagnosed. Diabetic nephropathy is also staged in Japan [9, 10], and the staging was described by Yokoyama et al. [11] as follows: stage I: urinary albumin-to-creatinine ratio (ACR) <30 mg/g creatinine; stage II: ACR $\geq$ 30 and <300 mg/g creatinine (i.e., albuminuria); stage III: ACR $\geq$ 300 mg/g creatinine and/or persistent proteinuria with serum concentration of creatinine <2 mg/dl; stage IV: serum concentration of creatinine $\geq$2 mg/dl with proteinuria; and stage V: being treated with dialysis. The Japan Diabetes Clinical Data Management Study Group (JDDM) reported that the prevalence of albuminuria (stage II) in Japanese type 2 diabetic patients was 32%, which is very similar to the 39% observed in the DEMAND study [12]. These results suggest that albuminuria is common, and that 76% of patients with diabetic nephropathy are categorized as stage II, as evidenced by the presence of albuminuria. Further, 58% of the patients enrolled were at stage I, 7% were at stage III, 2.6% were at stage IV, and 0.4% were at stage V [11]. A very recent study from the Japan Diabetes Complications Study (JDCS) revealed that the annual transition rate to proteinuria (ACR $\geq$ 300 mg/g creatinine) was 0.67%, and that this was substantially higher for the low-albuminuric group (defined as a urinary ACR of 30–150 mg/g creatinine) than for the normoalbuminuric group (defined as a urinary ACR of <30 mg/g creatinine) [13]. In this sense, UKPDS 64 reported that the progression to albuminuria occurred at 2.0% per year, and from albuminuria to macroalbuminuria at 2.8% per year [14]. However, about 40% of the diabetic patients had no urinary albumin excretion measurements, regardless of the recommendation for the JDDM cohort [11]. Therefore, the measurement of urinary albumin excretion is required for the early detection of diabetic nephropathy in Japan.

Biomarkers for diabetic nephropathy and disease progression

Further studies to detect diabetic nephropathy more specifically at the early stage in addition to urinary albumin excretion are needed. In this sense, biomarker studies to identify the presence and predict the progression of diabetic nephropathy have been performed worldwide [15]. Recently, Kamijo-Ikemori et al. [16] reported that urinary levels of liver-type fatty acid-binding protein (L-FABP) accurately reflected the severity of diabetic nephropathy in type 2 diabetes. Importantly, urinary L-FABP levels were high in patients with normoalbuminuria, suggesting its usefulness for detecting early nephropathy in these patients. Further, an increase in urinary Smad1—a key transcriptional factor for mesangial matrix expansion in diabetic nephropathy—at the early stage was correlated with subsequent development of glomerulosclerosis in experimental rodent models [17]. Regarding renal function, serum cystatin C was reported to be a good marker for nephropathy [18]. Notably, cases of early renal dysfunction, defined by a loss of cystatin C GFR exceeding −3.3%/year, occurred in 9% of the normoalbuminuria group and 31% of the albuminuria group [19].

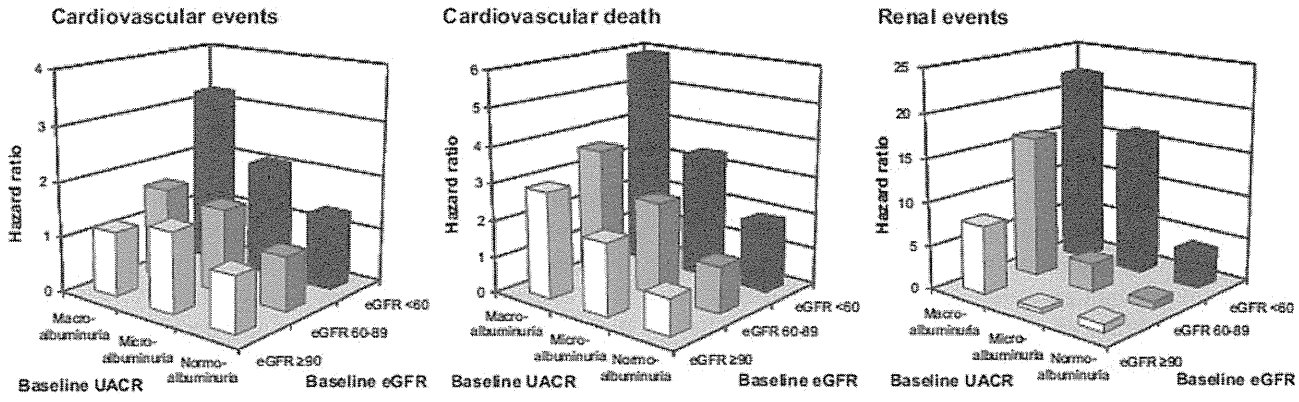
Prevalence of albuminuria and low GFR in type 2 diabetic patients in Japan

As previously described, diabetic nephropathy is diagnosed through the detection of albuminuria. Recently, Kidney Disease: Improving Global Outcomes (KDIGO) reported the definition, classification and prognosis of chronic kidney disease based on both estimated GFR and urinary levels of albumin excretion [20]. In this sense, there are diabetic patients with decreases in GFR and normoalbuminuria. Is diabetic nephropathy observed in such patients? In fact, the percentage of diabetic patients with normoalbuminuria and low estimated GFR is believed to be relatively high. Importantly, Yokoyama et al. [21] described

that the proportion of subjects with low estimated GFR (<60 ml/min/1.73 m^2) and normoalbuminuria was 11.4% of the type 2 diabetic patients examined (262/2298). In this manuscript, 63.4% of the 262 patients studied had neither diabetic retinopathy nor neuropathy. On the other hand, these patients were older and included a higher proportion of women and patients with hypertension, hyperlipidemia and cardiovascular disease, as well as fewer smokers compared with those with normoalbuminuria and preserved GFR. In contrast, the proportion of type 2 diabetic patients with preserved GFR but albuminuria or overt proteinuria was 27% (755/2791). Most importantly, the lack of histologically proven diabetic nephropathy should be discussed. In type 1 diabetes patients with normoalbuminuria and low GFR, renal biopsy specimens revealed more advanced diabetic glomerular lesions. It is worth noting that a reduced GFR was found much more often among female patients, particularly if retinopathy and/or hypertension were also present [22]. Deep insight into the prevalence and prognoses of these patients with proven pathological characteristics and grading is required to understand the pathophysiology of diabetic nephropathy in greater depth, together with future perspectives.

Clinical impacts of albuminuria and GFR on the prognoses of diabetic patients

Obviously, diabetic patients who had both albuminuria/overt proteinuria and low GFR were at risk of adverse outcomes, including cardiovascular events, cardiovascular death, and renal events, as reported by the Action in Diabetes and Vascular Disease: Preterax and DiamicroN MR Controlled Evaluation (ADVANCE) study [23] (Fig. 1). Do normoalbuminuric renally insufficient diabetic patients have a poor prognosis? Rigalleau et al. [24] reported that the risks of renal progression and death in these patients with type 1 or type 2 diabetes are lower. Concomitantly, in type 2 diabetic patients, the Casale Monferrato study revealed that macroalbuminuira was the main predictor of mortality, independently of both estimated GFR and cardiovascular risk factors, whereas the estimated GFR provided no further information on all-cause mortality and cardiovascular mortality in normoalbuminuric patients [25]. Supporting this notion, regarding renal end-points, there was also a progressive increase in risk associated with declined renal function, which was mainly observed in the albuminuric group in Chinese type 2 diabetic patients [26]. Interestingly, those with a reduced estimated GFR were at high risk of developing cardiovascular end-points (cardiovascular death, new admissions due to angina, myocardial infarction, stroke, revascularization or heart failure) and all-cause mortality, independent of albuminuria [26]. In contrast, as previously described, in the ADVANCE study, patients with normoalbuminuria and estimated GFR <60 ml/min per 1.73 m^2 had a 3.95-fold higher risk of renal events, a 1.33-fold higher risk of cardiovascular events, and a 1.85-fold higher risk of cardiovascular death [23] (Fig. 1). Moreover, Vlek et al. reported that an estimated GFR <60 ml/min/1.73 m^2 without albuminuria was



Cardiovascular events

Baseline UACR	Baseline eGFR (ml/min/1.73 m^2) GFR ≥90	GFR 60-89	GFR <60
Normoalbuminuria	1.00 (Reference)	0.98 (0.78-1.22)	1.33 (1.02-1.75)
Microalbuminuria	1.48 (1.09-2.01)	1.54 (1.20-1.98)	2.04 (1.54-2.69)
Macroalbuminuria	1.19 (0.52-2.69)	1.67 (1.09-2.57)	3.23 (2.20-4.73)

Cardiovascular death

Baseline UACR	Baseline eGFR (ml/min/1.73 m^2) GFR ≥90	GFR 60-89	GFR <60
Normoalbuminuria	1.00 (Reference)	1.22 (0.91-1.64)	1.85 (1.17-2.92)
Microalbuminuria	1.96 (1.16-3.32)	2.52 (1.65-3.84)	3.37 (2.15-5.30)
Macroalbuminuria	2.87 (1.01-8.18)	3.61 (2.02-6.43)	5.93 (3.45-10.20)

Renal events

Baseline UACR	Baseline eGFR (ml/min/1.73 m^2) GFR ≥90	GFR 60-89	GFR <60
Normoalbuminuria	1.00 (Reference)	0.99 (0.31-2.58)	3.95 (1.38-11.34)
Microalbuminuria	0.45 (0.05-3.83)	3.17 (1.15-8.74)	16.19 (6.16-42.54)
Macroalbuminuria	7.82 (1.51-40.53)	16.13 (5.49-47.42)	22.20 (7.62-64.72)

Fig. 1 Combined effects of albuminuria and eGFR levels at baseline on the risk of an adverse outcome. The estimates are adjusted for baseline covariates, including age, gender, duration of diabetes, SBP, history of currently treated hypertension, history of macrovascular disease, HbA1c, LDL cholesterol, HDL cholesterol, log-transformed triglycerides, BMI, electrocardiogram abnormalities, current smoking, and current drinking. (From Ref. [23] reproduced with permission from the American Society of Nephrology)

the strongest risk factor in the occurrence of vascular events (hazard ratio 1.50; 1.05–2.15) [27]. Recently, the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study revealed that normoalbuminuric patients with eGFR 30–59 ml/min per 1.73 m^2 were at higher risk of a cardiovascular event, cardiovascular death, noncoronary heart disease death, and death from any cause than normoalbuminuric patients with eGFR ≥60 ml/min per 1.73 m^2 [28]. Interestingly, high normal levels of albuminuria (≥5 μg/min) predicted the development of micro- and macroalbuminuria and increased mortality in Brazilian type 2 diabetic patients [29]. Furthermore, in Japanese patients with type 1 and type 2 diabetes, even within the normal range (≤30 mg/g), ACR ≥10 mg/g in women and ≥5 mg/g in men was associated with a significantly greater rate of decline in eGFR relative to subjects with ACR ≤5 mg/g [30]. It is of interest that the risk of cardiovascular events in individuals with diabetes increases with the ACR, starting well below the microalbumin cutoff [31]. Taken together, an evaluation of the clinical impact of albuminuria along with an evaluation of the effect of GFR on the prognoses of diabetic patients is required.

Remission/regression of albuminuria in patients with diabetic nephropathy

Fioretto et al. [32] reported that pancreas transplantation reversed the lesions of diabetic nephropathy in patients with type 1 diabetes mellitus, but that reversal required more than 5 years of normoglycemia. A growing body of evidence since then has pointed to the possibility of remission and/or regression of diabetic nephropathy, especially in patients treated with renin-angiotensin system blockade drugs. However, there is a lack of data on pathological findings in these patients. In the clinical setting, Perkins et al. [33] stated that regression of albuminuria was frequent in patients with type 1 diabetes mellitus, with a 6-year cumulative incidence of 58%. In this context, the definition of regression of microalbuminuria is a 50% reduction in albumin excretion from one 2-year period to the next. In addition, Hovind et al. [34] at the Steno Diabetes Center reported that the total number of patients who obtained remission was 92 (31%), with a duration of remission of 3.4 years, and regression occurred in 67 (22%) of 301 consecutive type 1 diabetic patients with diabetic nephropathy. Remission was defined as albuminuria <200 μg/min sustained for at least 1 year and a decrease of at least 30% from pre-remission levels, and regression as a rate of decline in GFR equal to the natural aging process: ≤1 ml/min/year during the investigation period in this report. Moreover, remission of nephrotic-range albuminuria in type 1 diabetic patients was also reported at the Steno Diabetes Center [35]. In this report, remission was induced in 28 of 126 (22%) patients; 21 were predominantly treated with angiotensin-converting enzyme (ACE) inhibitors, and 7 with non-ACE inhibitor medications. Remission lasted 3.6 years. In particular, more women (37%) than men (16%) obtained remission. In addition to type 1 diabetic patients, recent studies have revealed that remission is induced in type 2 diabetic patients. Araki et al. [36] reported that a reduction in urinary albumin excretion rate was frequent, with a 6-year cumulative incidence of 51% for remission, defined as a shift to normoalbuminuria, and 54% for regression, defined as a 50% reduction in the urinary albumin excretion rate. Interestingly, in this particular study, the frequency of progression to overt proteinuria was 28%, and albuminuria of short duration, the use of renin-angiotensin system-blocking drugs, and lower titers for HbA1c and systolic blood pressure were independently associated with remission or regression. More recently, JDCS revealed that a return from low microalbuminuria to normoalbuminuria was observed in 137 out of 452 patients (30.3%) [13].

Further, the clinical impact of remission/regression on renal outcome and cardiovascular events is still to be fully investigated. Importantly, Araki et al. [37] have reported that a reduction in albuminuria in patients with type 2 diabetes is an indicator of cardiovascular and renal risk reduction. In this study, the cumulative incidence of mortality from and hospitalization for renal and cardiovascular events was significantly lower in patients with a 50% reduction. Collectively, remission/regression in patients with diabetic nephropathy is relatively frequent, and insight into the pathological characteristics as well as the clinical impact on renal and cardiovascular outcomes when remission/regression is induced is needed.

Hematuria in diabetic nephropathy

Hematuria, the other major characteristic finding aside from albuminuria/overt proteinuria, was reported in 14 out of 34 Japanese patients with biopsy-proven diabetic nephropathy [38]. Patients with hematuria had significantly lower renal function, and the prevalences of nephrotic syndrome and retinopathy were significantly higher than in patients without hematuria. Interestingly, based on a logistic regression analysis, the presence of nephrotic syndrome and a known duration of diabetes were identified as significant predictors for hematuria with diabetic nephropathy.

Concluding remarks and future directions

Deep insights into the onset and progression of albuminuria along with GFR may elucidate the pathogenesis of

progressive kidney complications and associated cardiovascular diseases. Further studies of the clinical characteristics and the pathological findings of kidney involvement in patients with diabetes are required for a better understanding of diabetic nephropathy and the benefits of therapy for it.

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REVIEW ARTICLE

Nodular lesions and mesangiolysis in diabetic nephropathy

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Abstract Diabetic nephropathy is a leading cause of end-stage renal failure all over the world. Advanced human diabetic nephropathy is characterized by the presence of specific lesions including nodular lesions, doughnut lesions, and exudative lesions. Thus far, animal models precisely mimicking advanced human diabetic nephropathy, especially nodular lesions, remain to be fully established. Animal models with spontaneous diabetic kidney diseases or with inducible kidney lesions may be useful for investigating the pathogenesis of diabetic nephropathy. Based on pathological features, we previously reported that diabetic glomerular nodular-like lesions were formed during the reconstruction process of mesangiolysis. Recently, we established nodular-like lesions resembling those seen in advanced human diabetic nephropathy through vascular endothelial injury and mesangiolysis by administration of monocrotaline. Here, in this review, we discuss diabetic nodular lesions and its animal models resembling human diabetic kidney lesions, with our hypothesis that endothelial cell injury and mesangiolysis might be required for nodular lesions.

Keywords Diabetic nephropathy · Nodular lesion · Mesangiolysis · Glomerulosclerosis · Extracellular matrix · Matrix metalloproteinase

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Introduction

The number of patients with chronic kidney disease (CKD) on dialysis due to diabetic nephropathy is increasing worldwide. In Japan, the annual incidence of starting dialysis due to diabetic nephropathy has been in first place since 1998, indicating diabetic nephropathy as one of the most important causes of end-stage kidney disease. In clinical settings, insights for functional–pathological relationships in diabetic nephropathy are required for better prognosis. Generally speaking, it appears that in patients with type 2 diabetes, kidney structural changes are more heterogenous and diabetic glomerulopathy lesions are less severe than in type 1 diabetes patients with similar urine albumin levels [1]. The presence of persistent albuminuria is the hallmark of clinical diagnosis of diabetic nephropathy [1, 2]. However, some type 2 diabetic patients with microalbuminuria and proteinuria have lesions showing strikingly normal glomerular appearance or less advanced glomerular diseases than those observed in type 1 diabetes with similar urine albumin excretion [3, 4].

Characteristic pathologic changes of diabetic nephropathy are accumulation of extracellular matrix (ECM) in

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