The contribution of mesangial cell proliferation to progressive glomerular injury

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Abstract: Mesangial cell proliferation is a general characteristic of a variety of glomerular diseases. Therefore, an understanding of the regulatory mechanism is important for treatment of glomerular diseases. The present review shows that the growth arrest gene 6 (Gas 6) is a new autocrine growth factor of mesangial cells and that warfarin inhibits mesangial cell proliferation by inhibiting the γ-carboxylation of Gas 6 *in vitro* and *in vivo*. The present findings also show that a vitamin D analog (22-oxa-calcitriol) is a new growth regulator of mesangial cells *in vitro* and *in vivo*. These findings indicate that these compounds have considerable potential for therapeutic use in the treatment of progressive glomerular disease. J. Med. Invest. 48 : 1-4, 2001

Keywords **:** *mesangial cell, proliferation, Gas 6, growth factor, vitamin D analog*

INTRODUCTION

Diabetic nephropathy and chronic glomerulonephritis represent common causes of end-stage renal disease in Japan and the United States. The characteristic glomerular findings with respect to these lesions are both mesangial cell proliferation and the expansion of the extracellular matrix (ECM) in the mesangial area. Several mechanisms are thought to be involved in the progression of renal injury (1). An epidemiological investigation has shown that only 30-40% of patients with diabetes mellitus progressed to diabetic nephropathy. Recent findings, based on an experimental model and human nephropathy, indicated that a predisposition or resistance to the development of glomerulosclerosis was a genetically determined trait. Hyperfiltration, immune disorders, and metabolic disorders including hyperglycemia may play an important role in the development of glomerulosclerosis (1) (Figure 1).

A comparatively large number of progressive glomerular diseases are characterized by initial mesangial cell proliferation, which is followed by glomerulosclerosis, and finally develops into end stage kidney disease (2). Examples of this process are membranoproliferative glomerulonephritis, diabetic nephropathy and IgA nephropathy. Non-aniticoagulant heparin, a potent inhibitior of mesangial cell proliferation *in vitro*, inhibits the development of glomerulosclerosis in the renal ablation model. Treatment of anti-PDGF also blocks mesangial proliferation *in vivo*, in the model of anti-thy 1 glomerulonephritis (3). These findings suggest that mesangial proliferation may play an important role in the development of glomerular lesions. The search for agents which are capable of favorably regulating mesangial cell proliferation are, therefore, of considerable clinical importance in the area of progressive glomerular change. In the present study, we discuss the pathological role of mesangial proliferation in the progression of glomerular injury.

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Potent Mediator of Glomerular Cell Proliferation

Currently available evidence suggests that a number of growth peptides are involved in the regulation of cell turnover via surface receptors and its intracellular effect (4). Examples of these include insulin-like growth factor I (IGF-I), platelet derived growth factor (PDGF), epidermal growth factor (EGF) and trans-

forming growth factor-β (TGF-β) (Table 1). Although the turnover of glomerular cells in normal subjects is tightly regulated, glomerular cell turnover is markedly increased in the currently used models of glomerulosclerosis, even when the glomerular cell number is slightly increased only in advanced sclerotic lesions (5).

While viewed as leading to glomerular scarring, glomerular cell numbers are regulated by a multiplicity of events, including survival, mitosis, differentiation and cell death. In anti-thy1 glomerulonephritis, apoptotic and mitotic cells are detected in the same glomerulus, indicating that both processes can occur simultaneously. A number of defining factors have been proposed, which might trigger apoptosis in glomerular cells (6) (Table 2). Evidence has accumulated to suggest that direct responsibilities for the rate of progression of glomerular disease is directly controlled by specific positive and negative cell cycle regulators. However, the precise mechanisms which are involved in controlling the glomerular cell number in healthy and diseased conditions are not completely understood.

Growth Arrest Gene 6 (Gas 6)

Gas 6 was cloned from mouse fibroblasts, which had been grown under serum-starved conditions, and represents growth arrest-specific gene 6. Gas 6 is a homologue of protein S, which contains the epidermal growth factor-like domain and the sex hormone binding domain. Gas 6 also contains 11 to 12 glutamic acids residues, which are post-translationally modified by γ-carboxylase in the presence of vitamin K (7) (Figure 2). This modification is selectively inhibited by warfarin. Gas 6 specifically binds to tyrosine-kinase receptors (Axl, Rse and Mer), of which the affinity for Axl is remarkably high compared with that for other receptors (8). It was recently reported that the

Table 1. Modulators for Mesangial Cell Proliferation

Sources of Factors	
	Endothelial Cell products
	Blood-Borne Cells (Macrophages, T Cells, Platelets)
	Plasma Hormones
	Autocrine or Paracrine Products
	Metabolic Products
Growth Promoters	
	IGF-1
	PDGF
	IL-1
	IL-6
	EGF
	Basic FGF
	TNFα
Growth Inhibitors	
	ANF
	Heparin
	TGF-β
	INF-γ
	Vitamin D Analog

Table 2. Stimulators for Mesangial Cell Apoptosis

Non-specific Factors	
	Serum Starvation
	Detachment
	Shear Stress
	DNA damage
	Reactive Oxygen Species
	Radiation
	Cytotoxic Drugs
Specific Promoters	
	IL-1
	TNF-α
	Gas 6
	C1q
	LDL
	Lovastatin
	NO
	Superoxide
	Cyclic AMP

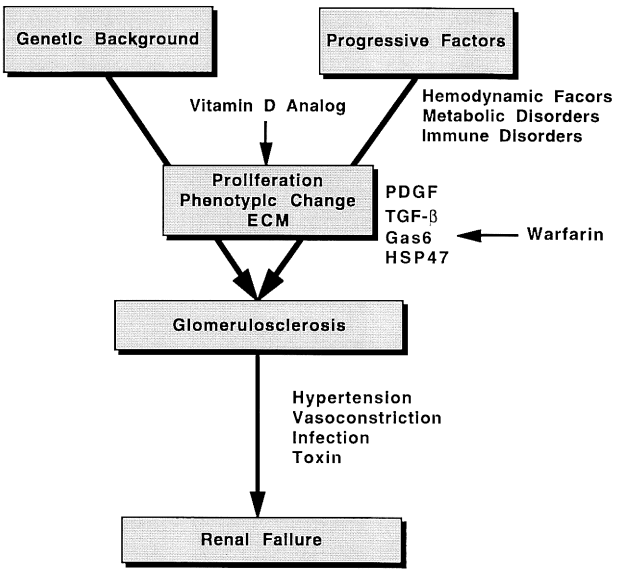


Fig. 1. Pathogenesis of Glomerular Injury

γ-carboxylation of Gas 6 is essential for its receptor-binding and growth-promoting activities in vascular smooth muscle cells.

Gas 6 and its receptor, Axl, were expressed in mesangial cells. The addition of exogenous Gas 6 led to phosphorylation of Axl, activated extracellular signal-regulated kinase (Erk), and [^{3}H]-thymidine incorporation in mesangial cells. Conditioned medium from mesangial cells was able to stimulate total DNA synthesis and the phosphorylation of Erk, whereas the addition of the extracellular domain of Axl inhibited the mitogenic activity of the conditioned medium from mesangial cells. Warfarin inhibited mesangial cell proliferation, which was blocked by the presence of vitamin K. This demonstrates that Gas 6 is a new autocrine growth factor for mesangial cells, and that warfarin inhibits mesangial cell proliferation by inhibiting the γ-carboxylation of Gas 6 (9).

We examined the role of Gas 6/Axl *in vivo* in an experimental glomerulonephritis model, which was induced by anti-Thy1.1 antibody (Anti-thy1 GN) (10). The expression of Gas 6 and Axl was markedly increased in glomeruli in the glomerulonephritis. The administration of Axl-Fc in the model inhibited mesangial proliferation and abolished the induction of PDGF-B. The administration of low doses of warfarin also significantly inhibited mesangial proliferation. These findings indicate that the Gas 6/Axl-pathway played a critical role in glomerulonephritis.

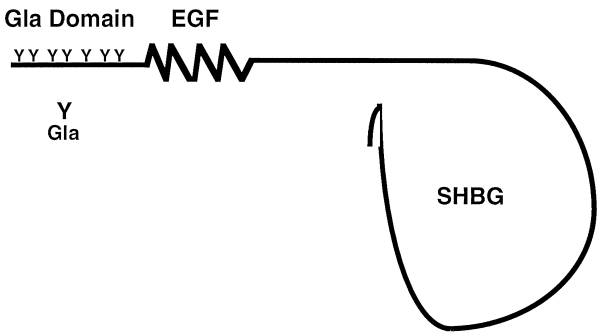
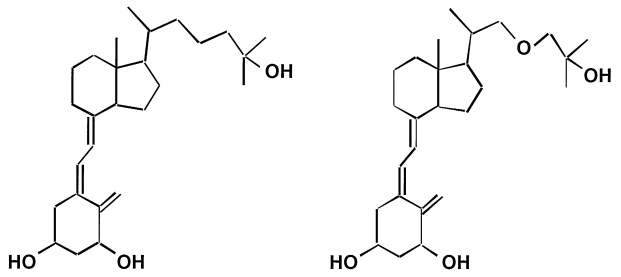


Fig. 2. Gas 6 (product of growth arrest gene 6)
1. Cloned from seum-starved fibroblast
2. It contained 11-12 Gla residues, 4 EGF-like domains and SHBG.
3. Structure similarity to Protein S.
4. Ligands for receptor tyrosine kinases.
Axl, Res, and Mer (Affinity : Axl > Res > Mer)

Gla : γ-carboxyglutamate
SHBG : sex hormone binding globulin
EGF : epidermal growth factor



1,25(OH)$_2$D$_3$ 22-oxa-calcitriol

Fig. 3. Structure of Vitamin D Analogs

Vitamin D Analog

1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) was originally identified as a nutritional factor and a fat-soluble vitamin. Its physiological role is to regulate calcium metabolism. It is generally accepted that the role of 1,25(OH)$_2$D$_3$ is to regulate the proliferation and differentiation of a variety of cells (11). However, effective doses of 1,25(OH)$_2$D$_3$ causes severe side effects including hypercalcemia. To prevent these side effects, a number of analogs of 1,25(OH)$_2$D$_3$ have been examined. One of these, 22-oxa1,25(OH)$_2$D$_3$(OCT), has a more potent differentiation effect than 1,25(OH)$_2$D$_3$, but does not induce hypercalcemia (12) (Figure 3).

Mesangial cell proliferation is significantly inhibited by OCT rather than 1,25(OH)$_2$D$_3$ in a dose-dependent manner. The addition of neutralizing antibody to OCT treated mesangial cells blocked the inhibitory effect of cell proliferation, but OCT does not induce the secretion of active TGF-β. OCT treatment significantly increased the expression of the TGF-β type II receptor. These findings suggest that the vitamin D analog OCT is a new potent modulator for mesangial cell proliferation and that this phenomenon was mediated via the induction of the type II receptor of TGF-β (13).

The effect of OCT on anti-thy 1 glomerulonephritis was examined (14). Both OCT and 1,25(OH)$_2$D$_3$ significantly inhibited mesangial cell proliferation, the degree of glomerulosclerosis, as well as albuminuria. The OCT treated group showed normal calcium levels but the 1,25(OH)$_2$D$_3$ treated group showed even higher levels. These findings demonstrate that OCT inhibits mesangial cell proliferation and extracellular matrix expansion with a low calcemic activity. The suppressive effect of OCT may be mediated by inhibition of TGF-β1. In the present study, we found the different mechanism of TGF-β between the disease state and that in the *in vitro* cell study. The upregulation of TGF-β may play a key role for developing glomerulosclerosis *in vivo*, which is ameliorated by vitamin D analog.

The overall effect is also regulated by the actions of TGF-β, its receptor and the different Smad family. In conclusion, the present findings suggest that OCT has potential for use in therapeutic strategies for the treatment of glomerulonephritis without inducing hypercalcemia.

CONCLUSION

Accumulating evidence suggests that glomerular cell proliferation plays a key role in the progression of glomerular injury. The present findings suggest that the regulation of proliferation is important in the treatment of glomerulonephritis as shown in figure 1.

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