Cytokines and podocyte injury: the mechanism of fibroblast growth factor 2-induced podocyte injury

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Introduction

Recent studies point to an important role for podocytes in the physiology and pathophysiology of the glomerulus. With regard to the pathogenesis of developing segmental glomerulosclerotic lesions, we [1], as well as others [2], previously reported that podocytes initially detached themselves from the glomerular basement membrane (GBM), leading to the development of adhesions between the denuded membrane and Bowman’s capsule. Proliferation of epithelial cells of Bowman’s capsule and an extracellular matrix can be observed in these lesions. Therefore, adhesive lesions show morphological changes in the glomerulus after podocyte injury.

How does this mechanism contribute to adhesive lesions?

Our study is designed to find what promotes the proliferation of epithelial cells in Bowman’s capsule. It has been reported recently that fibroblast growth factor 2 (FGF2) has a proliferative effect on cultured glomerular epithelial cells [3]. Our study focused on the role of FGF2 in the formation of glomerular adhesive lesions. Animal models with glomerular adhesive lesions were prepared by administering puromycin aminonucleoside (PAN). In normal glomeruli, FGF2 staining was detected at the GBM and in the mesangial areas. However, an increase of FGF2 staining in the cytoplasm of podocytes was observed in PAN nephropathy. Furthermore, FGF2 and FGFR 1–4 mRNAs were observed in both podocytes and epithelial cells of Bowman’s capsule. These findings suggest that FGF2 has an important role in the formation of glomerular adhesive lesions.

The role of FGF2 in glomerular adhesive lesions

In our study [4], FGF2 and FGF2-neutralizing antibody were administered to animals with PAN nephropathy. After PAN injection, recombinant FGF2 (group F), FGF2-neutralizing antibody (group M) or the same volume of physiological saline (group C) was injected for 4 days via the tail vein, and the animals were sacrificed on the 25th day after the start of PAN administration. Proteinuria markedly increased in group F. The difference in proteinuria excretion was prominent on day 25. When the cell proliferation was actually assessed by staining with proliferating cell nuclear antigen (PCNA) antibody, PCNA-positive cells clearly increased in group F, while they were suppressed in group M. PCNA-positive cells were made up mainly of podocytes and epithelial cells of Bowman’s capsule. When a comparison was made of podocyte damage using an anti-desmin monoclonal antibody as the marker of podocyte injury, the desmin score was lower in group M than in the other two groups. Adhesive lesions were most common in group F, while they were less prominent in group M. It is assumed that FGF2 was involved in the formation of adhesive lesions due to proliferation of epithelial cells of Bowman’s capsule, since FGF2 administration caused an increase in PCNA-positive epithelial cells of Bowman’s capsule and administration of the neutralizing antibody inhibited the formation of adhesive glomeruli. Furthermore, these findings indicate the possibility that FGF2 will induce the podocyte injury.

Why does FGF2 accelerate podocyte injury?

In contrast to mesangial cells and glomerular endothelial cells, podocytes have little proliferative capacity in vivo. The current consensus is that the mature podocyte is able to carry out limited DNA synthesis, but is unable to undergo cell division [5]. Our study attempted to compare PCNA-positive cells and bromodeoxyuridine (BrdU)-positive cells to ascertain the number of podocytes in PAN nephropathy. Also, mesangial cells were studied with a Thy1 GN model, a model of mesangial proliferative glomerulonephritis.
Labelling with BrdU was done twice a day in the last week before sacrifice. With the Thy1 GN model, the number of BrdU-positive mesangial cells was evidently greater than that of PCNA-positive cells on day 7, during which the mesangial proliferative phase occurs. With PAN nephropathy, the number of podocytes was larger than that of BrdU-positive cells. In FGF2-administered PAN rats, the number of PCNA-positive and BrdU-positive podocytes increased. Furthermore, the difference in the number of these markers was noted. PCNA recognizes cells from the late G1 to the early G2 phase of the cell cycle, and BrdU recognizes cells in the S phase. Using this BrdU labelling method, the findings suggest that podocytes are mostly present in the late G1 phase of the cell cycle even in the proliferative state, and that there is no increase in the number of cells. These findings showed that FGF2-induced podocyte injury might be related to the cell cycle.

What is the mechanism whereby podocytes in PAN rats administered FGF2 remain in the late G1 phase of the cell cycle?

As regards the cyclin kinase inhibitor (CKI) family, which suppresses the shifting of the cell cycle from the G1 to the S phase, p21 and p27 are known [6]. Therefore, in our study, the presence of p21 and p27 was analysed histochemically in PAN nephropathy. With regard to the number of p27-positive cells in podocytes, changes due to administration of FGF2 were not observed. However, an increase in p21-positive podocytes was noted. There was, however, no difference histochemically in p21 expression between the PAN rats which were given FGF2 and those which were not. When the two sets of PAN rats were compared, the number of BrdU-positive cells was clearly greater in the rats administered FGF2. However, the number of podocytes did not increase. This finding suggests that the cause of most podocytes remaining in the late G1 phase might be upregulation of p21. Furthermore, FGF2-induced podocyte injury related to cell cycle transit.

What morphological changes are occurring in BrdU-positive podocytes that entered the S phase of the cell cycle?

When the two sets of PAN rats were compared, the number of BrdU-positive cells was clearly greater in the rats administered FGF2. This finding showed that a few podocytes entered the DNA synthesis phase. The number of bi- or multi-nucleated podocytes was counted in these rats. Some podocytes prominently showed bi- or multi-nucleated cells in PAN rats given FGF2. This suggested an increase in the number of podocytes that entered the M phase. However, it was unclear whether these podocytes tended to become blocked in the M phase.

Conclusions

Podocytes have little ability to synthesize new DNA in vivo, and tend to remain in the late G1 phase of the cell cycle in the proliferative state with FGF2 added. A few podocytes entered the DNA synthesis phase. Furthermore, some podocytes prominently showed bi- or multi-nucleated cells in PAN rats given FGF2 (Figure 1). This finding indicated that some podocytes might have entered the M phase. Our hypothesis is that podocytes entering the S phase are associated with FGF2-induced cell damages. Furthermore, it is necessary to examine the lifetime of bi- or multi-nucleated podocytes, and conduct a more detailed study of the blocking mechanism in the G1/S and M phases of the cell cycle.

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References