Transforming growth factor-β1 promotes podocyte migration through impairment of integrin-β1 glycosylation by MAP kinases pathway

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Introduction:

TGF-β1 promotes podocyte migration and glomerulopathy. Cell migration and adhesion may depend on the level of expression of adhesion proteins, and their N-glycosylation that affects receptor-ligand binding. So, the interaction between integrin and extracellular matrix is important in governing cell migration.

Aim:

We evaluate whether TGF-β1 and its downstream pathways regulate integrin-β1 maturation and cell migration.

Methods:

10 ng/ml TGF-β1 was used to stimulate podocytes, then cell migration, mRNA and protein levels of integrin-β1 and downstream pathways of TGF-β1 were analyzed. Inhibitors of downstream pathways of TGF-β1 were used and integrin-β1 expression was analyzed. PNGase F was used to deglycosylation of integrin-β1 to reveal core protein. Monoclonal antibody and siRNA to integrin-β1 were used to decrease integrin-β1 function.

Result:

1. TGF-β1 promoted podocyte migration (Fig 1).
2. mRNA of integrin-β1 did not change under TGF-β1 stimulation (Fig 2A).
3. The mature form of integrin-β1 decreased gradually but precursor form increased gradually under TGF-β1 stimulation (Fig 2B).
4. The core proteins of integrin-β1 after PNGase F treatment were not different between TGF-β1 stimulation and non-TGF-β1 stimulation groups (Fig 2C).
5. The Smad, ERK and p38 pathways were activated after TGF-β1 stimulation (Fig 3).
6. U0126 (inhibitor of p-ERK) and SB20358 (inhibitor of p-p38) prevented, but SIS3 (inhibitor of p-Smad3) did not prevent, the decrease in mature form of integrin-β1 under TGF-β1 stimulation (Fig 4).
7. Down-regulation of integrin-β1 function by monoclonal antibody and siRNA to integrin-β1 promoted podocyte migration (Fig 5).

Conclusion:

TGF-β1 impaired the maturation of integrin-β1 through ERK and P38 pathways, but not through Smad pathway. The decrease in integrin-β1 function may promote podocyte migration (Fig 6).

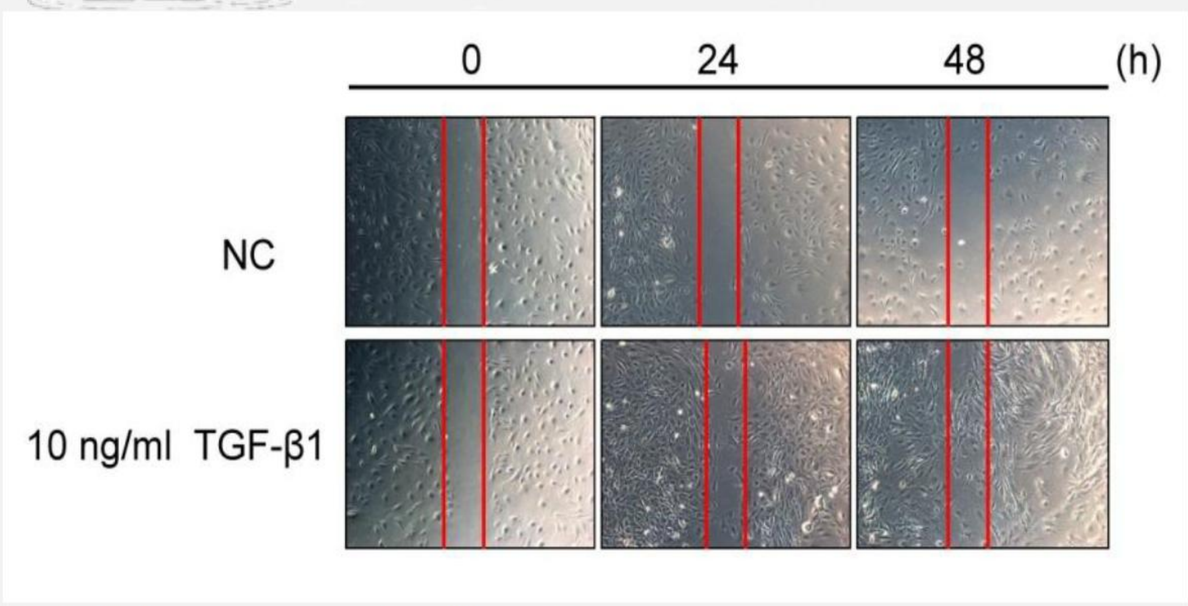

Fig 1. Podocyte migration induced by TGF-β1

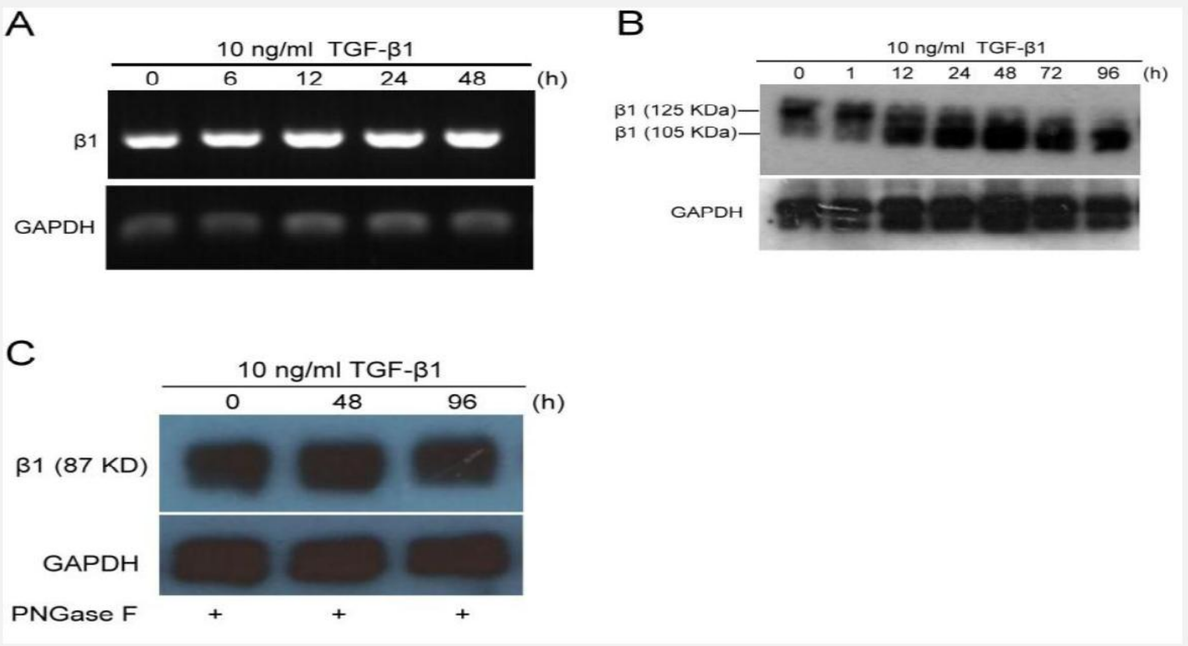

Fig 2. The effect of TGF-β1 on integrin-β1 maturation. (A) TGF-β1 did not influence mRNA of integrin-β1 expression. (B) The mature form of integrin-β1 was decreased and precursor form of integrin-β1 was increased in time-course after TGF-β1 treatment. (C) The core protein of integrin-β1 was not changed after TGF-β1 treatment.

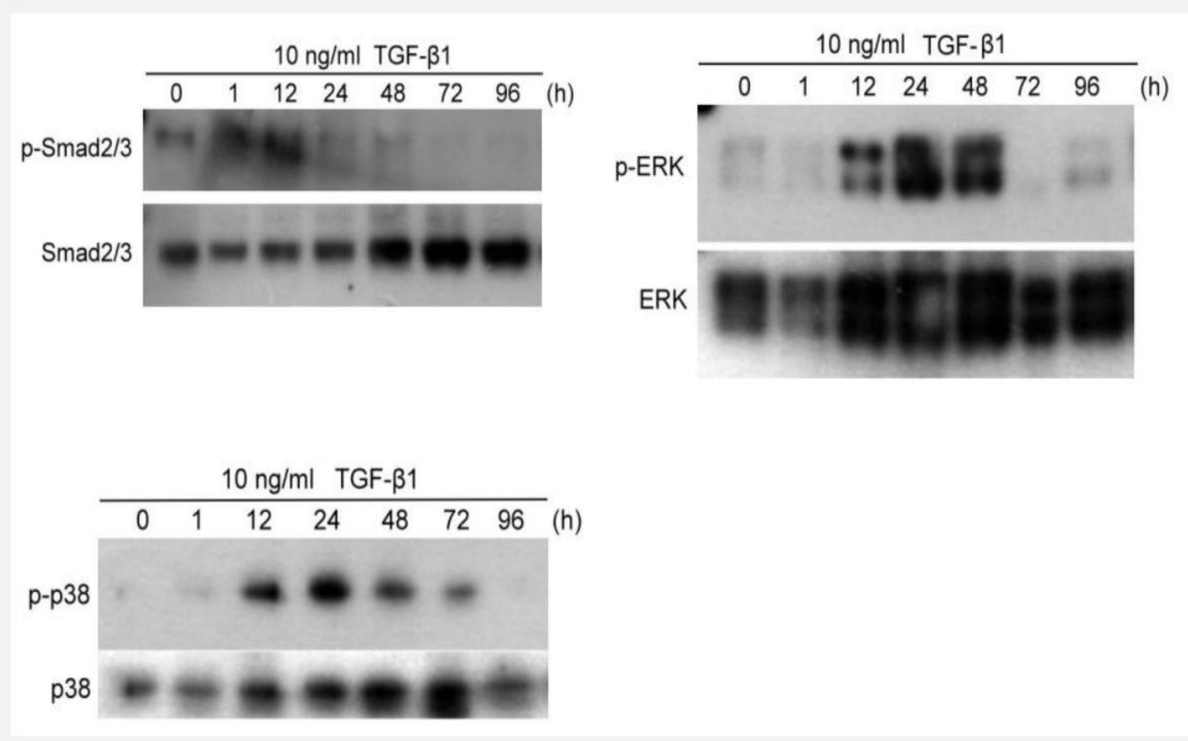

Fig 3. The downstream pathways of TGF-β1.

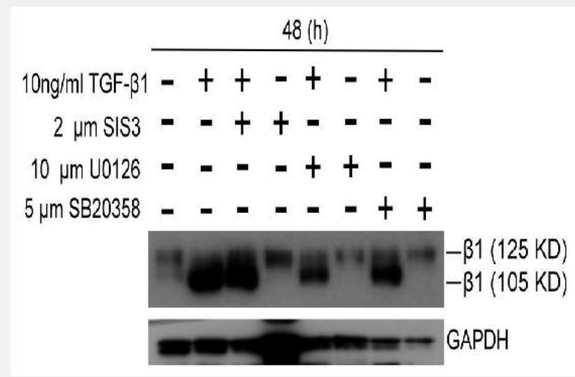

Fig 4. The effects of inhibitors of p-ERK (U0126), p-p38 (SB20358) and p-Smad3 (SIS3) on maturation of integrin-β1.

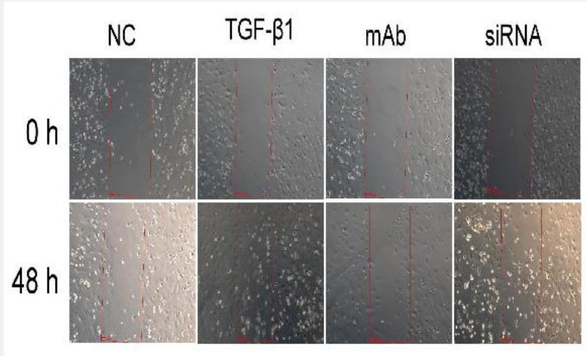

Fig 5. Cell migration after blocking integrin-β1 function by monoclonal antibody and siRNA to integrin-β1.

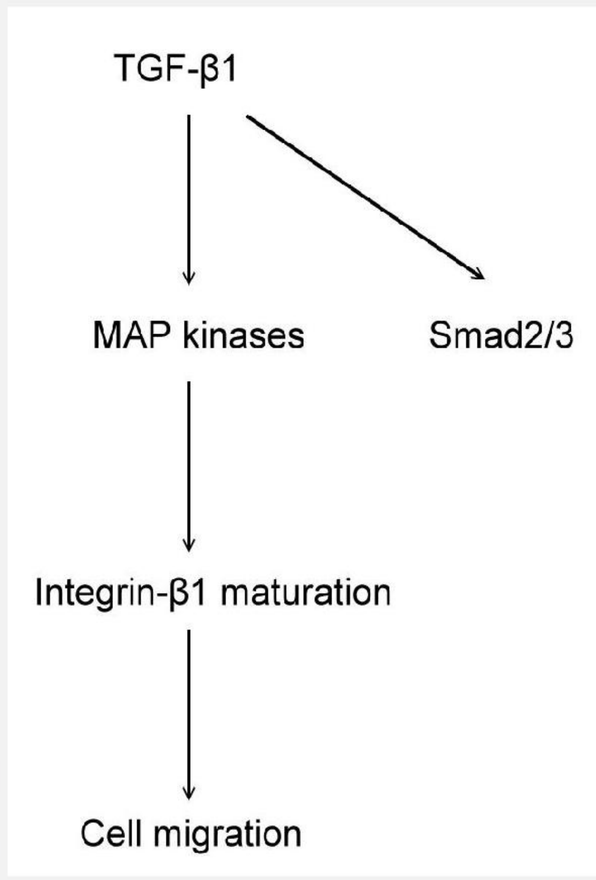

Fig 6. The pathways of TGF-β1 regulate integrin-β1 maturation and cell migration.

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