Advances and researches in idiopathic nephrotic syndrome biomarkers

Abstract

In recent years, the incidence of idiopathic nephrotic syndrome (INS) was on the rise year by year. As a important means for diagnosis, staging, prognosis, treatment strategies and efficacy monitoring on INS, biomarkers have become a hot research direction. This review focused on INS biomarkers coming from peripheral blood mononuclear cells (PBMC), cytokines, urine and Cell membrane molecules and cytokine receptors to systematically describe advances and researches in INS biomarkers. Idiopathic nephrotic syndrome (INS) refers to the condition caused by a primary renal lesion, in which kidney histology can reveal minimal podocyte changes (MCNS) or focal and segmental glomerulosclerosis (FSGS). In the previous studies about INS, familial hereditary INS had made great progress in 1990s, which provided the valuable basis for molecular composition of glomerular filtration barrier. But the pathogenesis researches of non-familial INS are still very limited. Shallhub proposed that the pathogenesis of lipidic nephropathy is caused by systemic T cell dysfunction, which induced immune response mediated by chemical secretion of body fluids in 1974. Since then, lots of studies were carried on this basis for researching biomarkers of INS.

Studies of Peripheral Blood Mononuclear Cells (PBMC)

In 1985, through co-culture using PBMC of patients with recurrent primary small change nephropathy (IMLNS) and normal rat glomerular, Garin found that 35sulfate absorption of glomerular was improved and proteinuria was induced. These suggested that the PBMC of patients with relapsed IMLNS may release a factor leading to this result and the muddy pine can reduce 35sulfate absorption of glomerular basement membrane. On this basis, he also observed the synergistic effect of monocytes and lymphocytes. Using co-culture of rat glomerular with PBMC, monocytes and lymphocyte supernatant of MCNS patients, respectively, he suggested that monocytes may play a role of stimulating and amplifying cytokines. Yoshizawa stimulated PBMC of MCNS patients using concanavalin A and injected supernatant into rats. Rats were induced proteinuria and renal pathological changes similar to MCNS. But the other types kidney disease cannot produce this result. Maruyama found that the rats were induced proteinuria and reduced basement membrane anion sites by injecting T-cell culture supernatant of MCNS patients into the rat left renal artery. Heslan thought that vascular permeability factor (VPF) was the product of T cells, which can change glomerular permeability and lead to proteinuria. Koyamal prepared four hybridomas derived from T lymphocytes of MCNS patients to secret glomerular permeability factor (GPF), which was injected into the rat resulting in significant proteinuria. Wang’s study showed that using PBMC supernatant of the active hormone-sensitive nephrotic syndrome (NS) patients to fill the rat kidney, they found glomerular polyanions were decreased and ultrastructural changed like MCNS. Filling PBMC supernatant of MCNS and FSGS patients into the normal renal artery can induce proteinuria and polyanion reduction. In 2006, Garin EH divided PBMC supernatant of IMLNS patients into three parts by liquid chromatography: bovine serum albumin, β-amyelase and ferritin. These were injected into rats, respectively. Only β-amyelase induced podocyte fusion in rats, which may play an important role in IMLNS pathogenesis. The role of humoral immunity in INS pathogenesis is not fully clear. T cell dysfunction has been confirmed by various evidence, but the reliability of the experimental methods need to be improved. Recent studies have shown that the infiltration of monocytes/macrophages in renal tissue was related to the increased expression of adhesion molecules like ICAM-1. CD25+-lymphocytes were also observed in the renal interstitium of animals with NS induced by doxorubicin. Although there are presence reports of macrophages also in glomerulus of animals with NS, most studies have not detected glomerular macrophage infiltration.

Studies of cytokines

The role of cytokines has always been a hot spot in the INS pathogenesis studies. Since 1972, there were a lot of reports about soon recurrence in patients with FSGS after normal donor kidney transplantation. Animal experiments have been carried out validation, which indicate that cytokines play an important role in INS. Japanese scholars found that T cells of MCNS patients can produce a 60-160kDa protein, injecting which into the body can produce proteinuria in rats10. Another scholar group found that stimulating PBMC of MCNS patients using ConA can produce GPF and injecting it into the rat can induce proteinuria and change the glomerular permeability. This phenomenon also can be found in other types of NS. In 2001, Matsumoto found that immunosuppressive factor TGF-β1 (transforming growth factor-β1) can against with VPF. Garin found that IL-8 content and gene expression in PBMC of NS patients had a cumulative amplification effect for VPF. In another experiment, IL-8 antibody was co-cultured with PBMC of MNCS patients. Then the supernatant was injected into the left kidney of rats. Compared with the control group, proteinuria was significantly reduced, which indicated that IL-8 may mediate proteinuria.
addition, atrial natriuretic peptide was increased and insulin-like growth factor was decreased in the blood, increased in urine in when MCNS patients relapse. Matsumoto and Kanmatsuse reported that IL-1 and IL-10 were decreased and IL-12 and IL-18 were increased in PBMC of MCNS patients. The expression of IL-2, γ-interferon and IL-4 mRNA in CD4+ and CD8+ T cells were not significantly different between normal and MCNS patients. But the IL-13 in MCNS patients were elevated, while IL-4 level was normal. The latter authors have shown that podocytes constitutively express functional trans-membrane receptor complexes for IL-4, IL-10, IL-13, and TNF-α. The possible role of IL-13 is also suggested by a rat model of NS. At present, the studies of cytokines in the pathogenesis of NS is complicated. It is still not clear that one or a group of factors play a decisive role in the production of proteinuria and the ultrastructural changes of the cells.

**Studies of urine**

Because of change of glomerular permeability, the clinical manifestation of NS patients is characterized by selective polymer proteinuria. Hotta reported they can identify a number of glomerular diseases by detection of mature monocytes in urine, such as basement membrane disease, MCNS and FNS. Carraro M et al., reported that normal human serum contains active substance which can block glomerular permeability factor activity. The FSGS patients have imbalance between the inhibitory factor and permeability factor due to increased glomerular protein permeability, which result in proteinuria and glomerular damage. It has been confirmed that these factors were lost in the FSGS patients urine, and this process may have occurred in the initial stage of the disease. Matsumoto focused on IL-17, a newly discovered key factor in regulating noninflammatory response. IL-17 in NS patients urine was significantly elevated, which can reflected the disease active phase. Moreover, to determine urinary nitrite can distinguish MCNS and FSGS in children. The lower apolipoprotein A1 level in urine is suggestive of SRNS. α-2 macroglobulin, retinol binding protein 4 and orosomucoid 2 are markers associated with FSGS. It has been suggested that decreased plasma concentration of zinc in nephrotic syndrome might increase the production of α-2 macroglobulin in the body because α-2 macroglobulin is a carrier for zinc. Urinary RBP was found to predict the histology, specifically FSGS, in a study conducted by Dillon et al.

**Studies of cell membrane molecules and cytokine receptors**

**Podocyte membrane molecules**

Podocytes of NS patients showed significant changes in the foot process fusion and podocyte loss under the electron microscope. These ultrastructural changes are closely related with its membrane protein and skeleton protein, which are found in the basal and apical areas of split diaphragm (SD), including nephrin, nephrin, podocin, CD2AP, Src family protein, WT-1 and actinin-4. Researches in this area have become a hot spot. The possible mechanism of foot process fusion can be summarized as follows:

- The lesion directly disturbs the cytoskeleton of podocytes;
- It interferes with the interaction between foot process and basement membrane;
- It damages negative charge barrier and parietal podocytes;
- The SD complex and its associated lipid rafts are damaged.

These mechanisms are inextricably linked with podocyte membrane protein. Skeleton protein are important molecules to maintain cell morphology and structure. The mature podocytes expresses the intermediate filament protein (Vimontin and Desmin) in somatic and main processes. The most important molecular component of the cytoskeleton of the foot process is F-actinin, which was cross-linked by associated protein, like α-actinin-4 and synaptopodin. SD is the most important functional structure of podocytes, and the abnormal expression of its constituent molecules can affect the integrity of SD. Some molecular changes are closely related to foot process fusion. Professor Din carried out a detailed studies about SD, which showed that there is no direct internal relationship among nephrin, podocin, CD2AP and α-actinin-4. But individual gene knockout caused in podocyte changed and proteinuria.

The podocytes contains cell membrane surface receptors in basal area, including CD151+α3β1 integrin and integrin kinase. These connective receptor complexes are associated with actinin cytoskeletal components, which not only mediates adhesion, but also serve as signal transduction. In addition, podocytes also expresses functional trans-membrane receptor complexes, such as IL-4, IL-10, IL-13 and TNF-α. IL-4/IL-13 receptor complex is expressed in both epithelium and endothelium in vivo. IL-4 and IL-13 directly alter protein typing, ion exchange and lysosomal enzyme activity in podocyte culture experiment. IL-1β and TNF-α also affect nephrin expression and cytoskeletal rearrangement on podocyte.

**Glomerular cell surface receptor**

NS is a noninflammatory response without deposition of immune complexes. Activation of T-cells does not directly contribute to the non-inflammatory response of the glomerular capillary wall because of cytokines and other circulatory factors. The cell surface receptors palythe important role of connecting this interaction. Immune cells, cytokines, as soluble signals associated with cognate receptors, induce a variety of intracellular changes, including cytoskeletal rearrangements, which have been confirmed in other study. The glomerular capillary wall can response to external immune signals because of these cytokine receptors. In the physiological conditions, cytokine level has positive correlation with biological effect, but in pathological conditions, elevated cytokine level can lead to increased glomerular permeability. Binding of cytokines to receptors may stimulate endothelial and/or epithelial to product other factors through paracrine or autocrine cytokine.

In recent years, the incidence of INS was on the rise year by year. Although the specific pathogenesis of the study is still not very clear, some biomarkers changes have been found in the INS pathogenesis. The discoveries of these biomarkers have promoted the diagnosis, staging, prognosis, treatment strategies and efficacy monitoring on INS. Some biomarkers determinations have gradually been widely applied on clinical. However, there is still a gap between the diagnostic specificity of biomarkers and renal biopsy for IMN. Therefore, we need further research in order to find new specific biomarkers to improve the understanding about INS pathogenesis and reduce the invasive examination. These INS biomarkers will have important significance and broad prospects for the clinical diagnosis, prognosis and treatment strategies.

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Conflict of interest

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