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The importance of MMPs/TIMPs system for impaired degradation of extracellular matrix in chronic kidney allograft injury

Znaczenie układu enzymatycznego MMPs/TIMPs w upośledzonej degradacji macierzy pozakomórkowej w przewlekłym uszkodzeniu aloprzeszczepu nerki

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Summary

Kidney transplantation is a most effective form of renal replacement therapy. Despite increased knowledge about the processes taking place in the transplanted organ and better possibilities of treatment, long-term graft survival is not satisfactory enough. In some renal transplant recipients (RTR), within a short time after kidney transplantation, the processes of interstitial fibrosis and tubular atrophy (IF/TA) develop, resulting in a chronic allograft injury (CAI) and graft loss in more than 50% of RTR after several years. Mechanisms of CAI are complex and not fully understood. The immunological and non-immunological risk factors and the whole sequence of events are taken into account, ranging from an oversecretion of cytokines/chemokines and growth factors, through the excessive accumulation of extracellular matrix (ECM), to impaired degradation of ECM. Dysregulation of enzyme system responsible for degradation of ECM proteins: metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), with advantage of TIMPs activity, indicate the importance of insufficient ECM degradation for CAI development. Tissue inhibitors of metalloproteinases (TIMPs) are new risk factors for CAI and may be useful biomarkers in clinical practice, mainly in the monitoring of transplant recipients at a later period after kidney transplantation, when the chronic allograft injury begins to dominate.

Streszczenie

Zabieg przeszczepienia nerki jest najefektywniejszą metodą leczenia nerkozastępczego z medycznego i ekonomicznego punktu widzenia. Mimo coraz większej wiedzy o procesach zachodzących w przeszczepionym narządzie i lepszych możliwości leczenia, w dalszym ciągu odległe przeżycie przeszczepu nie jest satysfakcjonujące. U części biorców, w krótkim czasie po przeszczepie nerki rozpoczynają się procesy włóknienia podścieliska i zaniku cewek nerkowych, prowadzące do postępującego uszkodzenia i utraty funkcji przeszczepu nerki u ponad połowy biorców w ciągu kilku-, kilkunastu lat. Patogeneza przewlekłego uszkodzenia aloprzeszczepu nerki jest złożona i angażuje wiele czynników powiązanych ze sobą funkcjonalnie. Mechanizmy wywołujące uszkodzenie przeszczepu nie są w pełni poznane. Bierze się pod uwagę czynniki immunologiczne i nieimmunologiczne oraz całą sekwencję zdarzeń, począwszy od nadmiernej sekrecji cytokin, chemokin i czynników wzrostu, poprzez nadmierną depozycję macierzy pozakomórkowej (ECM), do upośledzenia degradacji ECM przez enzymy proteolityczne. Zaburzenia układu białek enzymatycznych degradujących macierz pozakomórkową: metaloproteinaz i tkankowych inhibitorów mataloproteinaz (MMPs/TIMPs), z przewagą aktywności tkankowych inhibitorów metaloproteinaz (TIMPs) wskazują na znaczenie upośledzenia degradacji ECM w procesach przewlekłego uszkodzenia aloprzeszczepu nerki. Tkankowe inhibitory metaloproteinaz (TIMPs) są nowymi wskaźnikami zagrożenia postępującym ubytkiem filtracji i mogą być przydatnymi biomarkerami w praktyce klinicznej do monitorowania biorców w późniejszym okresie po przeszczepie nerki, kiedy zaczyna dominować przewlekłe uszkodzenie przeszczepu.

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INTRODUCTION

Kidney transplantation is a form of renal replacement therapy that gives the greatest benefits for patients with chronic kidney disease (CKD). Transplantation is more effective (medically and economically) than chronic dialysis therapy, with lower mortality rate and twice longer life expectancy, and improved quality of life (1). The clinical goal of transplantation is long-lasting patients' and grafts' survival. Despite improvements in immunosuppression and increased knowledge about the processes taking place in the transplanted organ, long-term graft survival is not satisfactory. In some patients after kidney transplantation, within a short time, develop processes of interstitial fibrosis and tubular atrophy (IF/TA), resulting in a chronic allograft injury (CAI) and graft loss in more than 50% of renal transplant recipients (RTR) after several years. In 10 years these patients need dialysis treatment and re-transplantation. Chronic allograft injury (CAI) remains the most important single cause of late graft loss after kidney transplantation (2-4). In two last decades of 20th century graft survival improved significantly and 1-year renal allografts survival rates are over 80% for cadaveric and 90-95% for living related donors (5). Despite reducing the frequency and severity of acute rejection episodes, calcineurin inhibitors (Tac and CsA) have no protective effect on the development of chronic allograft dysfunction (2), with inconsiderable improvement of the half-life of renal allografts, and renal allografts continue to be lost at the rate of 2 to 4% per year due to CAI (6, 7). More than 1 million of renal transplant recipients live over the world. The mechanisms of fibrosis are complex, and may involve excess synthesis of collagen with decreased degradation, in association with interstitial injury and loss of functional tubules and glomeruli. Better understanding of mechanisms of interstitial fibrosis and tubular atrophy (IF/TA), as the morphological surrogate of renal allograft deterioration may improve outcome after renal transplantation (8).

MECHANISMS OF CHRONIC ALLOGRAFT INJURY

Chronic allograft injury (CAI) is a multifactorial clinical and pathological entity with progressive decline in glomerular filtration rate (GFR) and not fully understood etiology (9). Both immune (antigen dependent) and nonimmune (antigen independent) events may promote graft injury (10). Wide repertoire of factors is involved: chemokines, profibrotic cytokines, growth factors, pro-angiogenic factors and proteolytic enzymes. The whole sequence of events is taken into account, ranging from the excessive accumulation of extracellular matrix (ECM) to reduced degradation of ECM proteins by proteolytic enzymes. One of the possible hypotheses of CAI is irreversible disruption of three-dimensional structure of ECM. Besides ECM expansion also occur significant changes in kidney allografts' architectonics, with myofibroblasts accumulation and fibrosis induced by epithelial-to-mesenchymal transition, glomerular hypertrophy and sclerosis, as well as tubular atrophy and loss of peritubular capillars. The inflammatory cells (macrophages, various T-cells, dendritic cells, plasma cells and granulocytes) infiltrate is present in acute phase of injury. These findings give the histopathological picture of interstitial fibrosis and tubular atrophy (IF/TA). The term IF/TA is reserved for unclear and unspecific etiology of graft dysfunction. Changes in serum creatinine levels and proteinuria occur late and may not represent the actual state of allograft damage. In protocol biopsies it was shown that structural injury develops early and the presence of IF/TA occurs before functional dysfunction. The presence of IFTA has a predictive impact, independent from other classic factors of graft injury (11). More advanced fibrosis correlate with progressing allograft dysfunction (12).

Physiologically ECM is a balanced network of proteins and proteoglicans, but in pathological conditions increased protein synthesis or decreased protein degradation lead to ECM accumulation and fibrosis (4). The predominance of protein synthesis over degradation leads to an ECM remodeling, and the presence of ongoing interstitial inflammation, even in areas of fibrosis and atrophy, is considered as active injury and worsen prognosis (13-16). In the kidney with interstitial fibrosis, matrix synthesis is no longer in balance with matrix degradation as a result of increased synthesis, decreased degradation, or a combination of both (10).

The incidence of fibrosis varies. Stegall et al. showed moderate to severe fibrosis in 13% of biopsies after 1 year after transplantation and in 17% after 5 years with no significant progression between 1th and 5th year (17). Also Baboolal et al. find chronic allograft nephropathy in 4% of biopsies after 3 months and 12% after 6 months (18) and up to 23% at 5 yr after transplantation in study by Harris et al. (19). In protocol biopsies of kidney allograft after 10 years the presence of IF/TA is almost universal phenomenon – probably due to calcineurin inhibitor (CNI) nephrotoxicity or to non diagnosed or inadequate treated borderline rejection (20, 21). Even after living donor transplantation fibrosis, mostly mild, is present in 71% of recipients after 2 years (22). Chronic allograft injury (CAI) with the picture of IF/TA is the most important cause of late kidney allograft loss (23).

Various proteolytic enzymes are involved in ECM proteins degradation with important role of metalloproteinases (MMPs) (4). The imbalance between MMPs and tissue inhibitors of metalloproteinases (TIMPs) may predispose to progressive fibrosis, because decreased degradation favors fibrosis development more than increased ECM accumulation (24).

METALLOPROTEINASES (MMPs) AND TISSUE INHIBITORS OF METALLOPROTEINASES (TIMPs)

Matrix metalloproteinases (MMPs, matrixins) are a large family of proteinases able to remodel extracellular matrix (ECM) components. Originally it was

thought that MMPs cleave only ECM proteins, but now other substrates are known like signaling molecules (growth factor receptors) and cell adhesion molecules (25). Both gelatinases (MMP-2 and MMP-9) have the ability to degrade collagen IV and V (26). Glomerular epithelial cells may produce both MMPs (MMP-2 and MMP-9), but mesangial cells can synthesize only MMP-2 (26, 27). Active form of MMP-2 have the molecular mass of 72 kDa, so is not physiologically filtrate into urine, but is often release into urine in case of proteinuria (28). In contrast to MMP-2, which is widespread, MMP-9 has much lower expression. MMPs after synthesis are rapidly released from cells into the blood stream. Higher concentrations of pro-MMP-2 were detected in patients with CAI and correlated with proteinuria and higher serum creatinine (29).

MMPs activity is regulated via a number of mechanisms, including expression and secretion of enzymes, proteolytic activation of pro-enzymes or inhibition by tissue inhibitors of metalloproteinases (TIMPs), which form complexes with MMPs and inhibit latent and active form of enzymes. TIMP-1 inhibits all latent MMPs but have not expression in normal kidneys in contrast to TIMP-2, with constitutive kidney expression of mRNA (4). Physiologically there is the balance between MMPs and TIMPs, because all TIMPs form with all MMPs noncovalent complexes in ECM (30). It has been shown, however, that TIMP-2 has a higher affinity to MMP-2 (and pro-MMP-2) and TIMP-1 to MMP-9 (and pro-MMP-9) (10, 31). Complexes of MMPs/TIMPs can dissociate and re-releasing enzyme and inhibitor. Imbalance between MMPs and TIMPs was demonstrated in many pathological conditions. But TIMPs activities are not limited only to regulatory properties, but also have the role in apoptosis and cell growth (26). ECM expansion may be a result not only insufficient proteolytic activity of MMPs, but rather excessive TIMPs activity. TIMPs are mediators of fibrosis via inhibition of proteolytic activity of MMPs (32). As a result, there is a massive interstitial fibrosis and progressive loss of kidney function.

Mazanowska et al. show that patients had significantly higher plasma and urine concentrations of MMP-9, TIMP-1, and TIMP-2, as well as decreased plasma MMP-2, compared with healthy volunteers (control group). Recipients with good allograft function (serum creatinine < 1.5 mg/dl) showed lower plasma TIMP-1 ($p < 0.001$) and TIMP-2 ($p = 0.003$) and estimated glomerular filtration rate (eGFR aMDRD) negatively correlated with plasma TIMP-1 and TIMP-2 levels ($r_s = -0.43$; $p < 0.0001$ and $r_s = -0.42$; $p < 0.0001$), respectively. Multivariate and receiver operating characteristic (ROC) analyses showed that plasma TIMPs concentrations may be useful to estimate of CAI (33). Probably insufficient degradation of ECM may have more significant impact on fibrosis than excessive synthesis, and imbalance between MMPs and TIMPs, with advantage of TIMPs, are important molecular mechanisms of fibrosis development (33).

CONCLUSIONS

Chronic allograft injury remains the leading cause of renal allograft loss after the first year following transplantation. Late allograft loss is high despite remarkable reductions in acute rejection rates, and no direct therapeutic strategies are known yet. The pathogenesis is unclear and involves multifactorial injuries. Recent knowledge about the underlying molecular mechanisms suggest increased secretion of cytokines and growth factors with change in fibroblast phenotype leading to the excessive deposition of extracellular matrix. Impaired degradation of ECM by proteolytic enzymes, mainly metalloproteinases (MMPs) is one of the reasons, but probably repeated insults trigger upregulation of the tissue inhibitors of matrix metalloproteinases (TIMPs), favoring accumulation of ECM. Impairment in ECM degradation by inhibition of MMPs may be the leading cause of progressive fibrosis, and TIMPs may be the early biomarkers of IF/TA.

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