Bioengineered subcutaneous implantation of megalin-expressing cells for the uremic toxin protein metabolism in renal failure

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Abstract

End-stage renal failure patients on dialysis therapy suffer from serious complications caused by the accumulation of low-molecular-weight uremic toxin proteins in serum and tissues, which are normally metabolized by the kidney. Dialysis-related amyloidosis is one such complication induced by systemic deposition of amyloid proteins derived from a 12-kDa serum protein, β 2-microglobulin. We have developed a new potential therapeutic model for removal of β 2-microglobulin during renal failure using cells expressing megalin, a multi-ligand endocytic receptor that plays a central role in the renal protein metabolism. We identified that cultured rat yolk sac epithelium-derived L2 cells internalized and degraded β 2-microglobulin *in vitro* as well as other low-molecular-weight proteins specifically via megalin. We successfully implanted the cells within the subcutaneous tissues of nude mice using both a type I collagen scaffold and a method for local delivery of basic fibroblast growth factor, an angiogenic factor. After bilateral nephrectomy and intraperitoneal injection with [^{125}I]-labeled β 2-microglobulin, we found that the implanted cells internalized and metabolized the labeled ligand efficiently removing it from the blood. Our data suggest that megalin-expressing cell implantation may represent a new therapy to compensate for the loss of renal protein metabolism during renal failure. We intend to develop a strategy for clinical application of this therapeutic model.

Introduction

Megalin was first identified as an autoantigen (gp330) of rat Heymann nephritis, an experimental model of membranous nephropathy. We elucidated its primary structure by cDNA cloning to reveal that it is a large (~600 kDa) member of the low-density-lipoprotein receptor gene family. Megalin has been recognized as an endocytic receptor for multiple ligands. It is highly expressed at the apical surface of proximal tubule cells (PTCs) that normally reabsorb and degrade low-molecular-weight proteins (LMWPs) filtrated by glomeruli (Fig. 1A). Megalin binds numerous LMWPs *in vitro*, and megalin-deficient mice excrete LMWPs in urine, suggesting that megalin mediates the proximal tubular uptake of such proteins.

Renal failure results in the retention of such LMWPs in serum due to the decrease in glomerular filtration and degradation by PTCs. In patients with end-stage renal disease (ESRD) undergoing long-term dialysis therapy, some LMWPs act as uremic toxins and cause serious complications. β 2-microglobulin (β 2-m) is a well established 12-kDa uremic toxin protein that causes dialysis-related amyloidosis (DRA). It is characterized by osteoarthropathy and variable organ failure due to deposition of β 2-m–derived amyloid proteins. Despite the development of high-flux membrane hemodialysis devices and a direct absorbent column to remove β 2-m, its accumulation is inevitable in afflicted patients because the therapeutic effects are transient and insufficient. Since kidney transplantation is not sufficient for ESRD patients, it is an urgent medical issue to develop a new strategy to overcome the limitations of dialysis therapy.

We have demonstrated for the first time direct evidence that megalin mediates the cellular uptake and metabolism of β 2-m and other LMWPs. Based on these data, we have developed a new therapeutic model for the elimination of β 2-m in renal failure by subcutaneous transplantation of megalin-expressing cells. Cell implantation is carried out using two special techniques: preimplantation of a three-dimensional extracellular matrix scaffold and a local drug delivery system (DDS). The scaffold consists of a type I collagen sponge and is necessary for the implanted cells to attach and grow upon. The DDS was designed for local release of basic fibroblast growth factor (bFGF) to induce angiogenesis in and around the scaffold to promote adequate blood supply to the implanted cells.

Data

We found that cultured rat yolk sac epithelium-originated L2 cells specifically took up and degraded [^{125}I]-labeled β 2-m. We found that anti-megalin antibodies and RAP (a specific competitor of megalin's ligand binding) completely blocked the cell association and degradation of [^{125}I]-labeled β 2-m, i.e., its cellular uptake and degradation are megalin-dependent. We also found that other LMWPs such as lysozyme, cytochrome C, α 1-antitrypsin and retinol binding protein completely blocked the cell association and degradation of [^{125}I]-labeled β 2-m, indicating that megalin also mediates the cellular uptake and degradation of such proteins like β 2-m.

To demonstrate the efficacy of using such cells *in vivo*, we implanted L2 cells subcutaneously in nude mice (Fig. 1B). First, we implanted subcutaneously type I collagen sponges with small gelatin microspheres adsorbing bFGF. The microspheres slowly release bFGF in and around the sponges, inducing angiogenesis. A week later, we injected L2 cells suspended in phosphate-buffered saline (PBS) into the sponges, while we only injected PBS for the control mice. The cells formed an apparent subcutaneous mass in 2 weeks. Then we removed the kidneys of the mice to induce renal failure. We injected [^{125}I]-labeled β 2-m into the peritoneum and sacrificed the animals by systemic perfusion with saline 3, 6 and 14 hours after the injection. The uptake of [^{125}I]-labeled β 2-m (cpm/mg tissue) by the implanted tissues was significantly higher than that of the liver, lung, heart, and skeletal muscle. The trichloroacetic acid-precipitated blood radioactivity of the undegraded [^{125}I]-labeled β 2-m was significantly lower at 6 and 14 hours in the cell-implanted mice compared to the control mice. The uptake of [^{125}I]-labeled β 2-m by the liver, lung, heart, and skeletal muscle was not statistically different between the cell-implanted and control mice. On protein electrophoresis, the implanted cell lysates showed a band of intact [^{125}I]-labeled β 2-m with small degradation products, indicating that the cells took up and degraded the labeled ligand. Autoradiography of the implanted cells confirmed the intracellular uptake of [^{125}I]-labeled β 2-m.

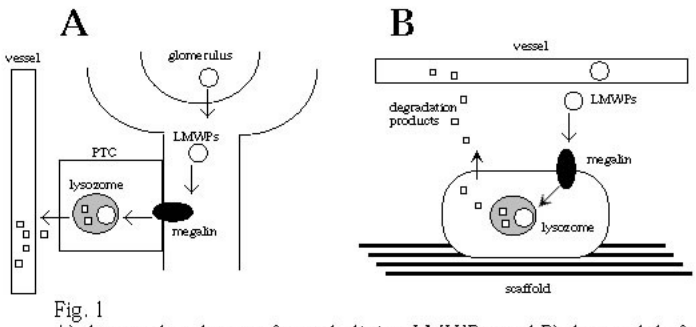

Fig. 1
A) the renal pathway of metabolizing LMWPs, and B) the model of the LMWP metabolism by megalin-expressing cells implanted on the scaffold. LMWP, low-molecular-weight-protein. PTC, proximal tubular cell.

Conclusion

In conclusion, using bioengineered subcutaneous implantation of megalin-expressing cells, we developed a new therapeutic model to compensate for the metabolism of β 2-m, a uremic toxin protein, in renal failure. Also, the cells could metabolize undetermined uremic toxin proteins via megalin that was found to mediate cellular uptake and degradation of multiple LMWPs. This work would make an impact on the field of nephrology, by promoting the development of a new bioengineering cell therapy to compensate for the loss of kidney functions in end-stage renal failure.

Perspective

Our goal is clinical application of this therapeutic model. For this purpose, we will first establish host-derived cells that highly express megalin. This will be achieved by transferring the megalin gene into host's macrophagic cells. Second, we will demonstrate the efficacy of the bioengineered cell implantation in dogs or monkeys with renal failure.