Genetically-modified stem cells in treatment of human diseases: Tissue kallikrein (*KLK1*)-based targeted therapy (Review)

MARINA DEVETZI[1], MARIA GOULIELMAKI[1], NICOLAS KHOURY[1], DEMETRIOS A. SPANDIDOS[2], GEORGIA SOTIROPOULOU[3], IOANNIS CHRISTODOULOU[1] and VASSILIS ZOUMPOURLIS[1]

[1]Biomedical Applications Unit, Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 11635 Athens; [2]Laboratory of Clinical Virology, Medical School, University of Crete, 71003 Heraklion; [3]Department of Pharmacy, University of Patras, Rion, 26500 Patras, Greece

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Abstract. The tissue kallikrein-kinin system (KKS) is an endogenous multiprotein metabolic cascade which is implicated in the homeostasis of the cardiovascular, renal and central nervous system. Human tissue kallikrein (KLK1) is a serine protease, component of the KKS that has been demonstrated to exert pleiotropic beneficial effects in protection from tissue injury through its anti-inflammatory, anti-apoptotic, anti-fibrotic and anti-oxidative actions. Mesenchymal stem cells (MSCs) or endothelial progenitor cells (EPCs) constitute populations of well-characterized, readily obtainable multipotent cells with special immunomodulatory, migratory and paracrine properties rendering them appealing potential therapeutics in experimental animal models of various diseases. Genetic modification enhances their inherent properties. MSCs or EPCs are competent cellular vehicles for drug and/or gene delivery in the targeted treatment of diseases. *KLK1* gene delivery using adenoviral vectors or KLK1 protein infusion into injured tissues of animal models has provided particularly encouraging results in attenuating or reversing myocardial, renal and cerebrovascular ischemic phenotype and tissue damage, thus paving the way for the administration of genetically modified MSCs or EPCs with the human tissue *KLK1* gene. Engraftment of *KLK1*-modified MSCs and/or *KLK1*-modified EPCs resulted in advanced beneficial outcome regarding heart and kidney protection and recovery from ischemic insults. Collectively, findings from pre-clinical studies raise the possibility that tissue KLK1 may be a novel future therapeutic target in the treatment of a wide range of cardiovascular, cerebrovascular and renal disorders.

Correspondence to: Dr Vassilis Zoumpourlis, Biomedical Applications Unit, Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vasileos Constantinou Avenue, 11635 Athens, Greece
E-mail: vzub@eie.gr

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Contents

1. Introduction

The tissue kallikrein-kinin system (KKS) is an endogenous multiprotein metabolic cascade which is implicated in a plethora of biological processes such as inflammation, vasodilation, blood coagulation, fibrinolysis, blood pressure control, vascular permeability, cardioprotection, smooth muscle contraction, electrolyte balance and pain induction. Activation of KKS leads to synthesis of the vasoactive peptides kinins by enzymatic hydrolysis of precursor kininogens including high molecular weight kininogen (HMWK) and low molecular weight kininogen (LMWK) (1,2). Kininogens are physiological substrates for proteolytic cleavage by a family of serine proteases consisting of kallikreins (KLKs) originating from plasma (pKLK) and tissue (tKLK) (3). Human plasma kallikrein (KLKB1) is a serine protease synthesized predominantly in the liver that possesses a high affinity binding site for HMWK, which is cleaved to produce the nonapeptide bradykinin. Human tissue kallikrein (KLK1) is a serine protease of the S1 serine protease superfamily which cleaves LMWK to produce the decapeptide Lys-bradykinin (kallidin) that is further processed to bradykinin by a second aminopeptidase cleavage. Bradykinin is the basic vasoactive peptide of the KKS involved in the regulation of blood pressure as well as flow. Bradykinin-related peptides bind to B1 and B2 bradykinin receptors in order to activate a number of downstream targets such as nitric oxide (NO), cGMP, prostacyclin and cAMP, which in turn induce numerous biological processes implicated in angiogenesis

by stimulation of vascular endothelial growth factor (VEGF) formation through binding to B2 receptor, increase of vascular permeability, vasodilation, smooth muscle contraction/relaxation, inflammation and pain (4-8). In addition, KLK1 protease showing both trypsin- and chymotrypsin-like specificity, appears to have many physiological protein substrates including pro-insulin, pro-renin, low-density lipoprotein, and the matrix metalloproteinases (MMPs) pro-gelatinase (MMP-2) and pro-collagenase (MMP-9) (9,10).

Human mesenchymal stem cells (MSCs) are multipotent fibroblast-like somatic cells with the ability to self-renew, proliferate and differentiate in order to give rise to tissue- or organ-specific cells of the mesodermal lineage (e.g., osteoblasts, chondrocytes, adipocytes, stroma cells, skeletal myoblasts and endothelial cells). MSCs are a heterogeneous subset of stromal stem cells that can be isolated from many different adult or fetal tissue sources including bone marrow, adipose tissue, umbilical cord blood, amniotic fluid, synovial fluid, peripheral blood, dermis, liver, skin and skeletal muscle (11-14). As determined by the International Society for Cellular Therapy (ISCT), human MSCs must meet the following minimum criteria: adherence to tissue culture plastic under standard culture conditions, expression of cell surface molecules CD105, CD73 and CD90 and lack of expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA class II and capability of differentiating into adipocytes, osteocytes and chondrocytes under standard experimental conditions *in vitro* (15).

Another stem cell population which has been proposed as remarkable candidate for stem cell therapy is human endothelial progenitor cells (EPCs). EPCs are precursor cells that have the potential to differentiate into mature endothelial cells and can be isolated from bone marrow aspirate or peripheral blood of adult organisms. EPCs participate in the processes of postnatal formation of new blood vessels and recovery of damaged tissues by incorporating into the vasculature and by secreting vasculogenic cytokines and proangiogenic factors such as VEGF, angiopoietin-1 (Ang1), hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1 (MIP-1) (16-20). Vasculogenic cytokines recruit EPCs to the process of healing in response to hypoxia or ischemia, whereas proangiogenic cytokines regulate EPC mobilization, homing, proliferation, and differentiation. The angiogenic potency of EPCs is also demonstrated through their tube formation capacity in *in vitro* assays or when injected to murine models. EPCs also contribute to neovascularization and tissue repair of musculoskeletal and neural tissue including the bone and spinal cord. Transplantation of EPCs has been used to treat ischemic diseases in animal models and clinical trials (20-22).

2. Stem cell properties

Key properties of human MSCs are their immunomodulatory capability and their marked propensity to migrate towards sites of injury or inflammation (tropism). Due to these special characteristics, MSCs have been highlighted as promising tools for clinical use in regenerative medicine as well as targeted cell therapy of various diseases including cardiovascular, cerebrovascular, renal, autoimmune disorders and cancer (13,23,24). MSCs of various origin can be readily extracted from adult tissues and expanded *in vitro* without the loss of their potential for clinical applications or differentiation into multiple cell lineages (14,25).

One of the most intriguing features of MSCs is that they can interact with cells of both the innate and adaptive immune systems and modulate their effector functions by secreting several cytokines. Interleukins 10 (IL-10) and 8 (IL-8) and transforming growth factor-β (TGF-β) produced by MSCs lead to repression of immune responses and promotion of tissue healing. MSC-mediated immunomodulation results in MSC escape from host immunological recognition and rejection in allogeneic injection due to lack of major histocompatibility complex MHC-II and only minimal MHC-I protein expression (13,24,26).

The other crucial feature of MSCs is that they can physiologically perfuse into the peripheral blood and migrate to injured or inflamed tissues (tropism), where they can inhibit the release of pro-inflammatory cytokines and promote the survival of damaged cells (24,27). MSC tropism is mediated through paracrine signaling between the site of injury and corresponding receptor expression on MSCs (23). For example, stromal cell-derived factor-1 (SDF-1) is one of the main chemokines mediating the mobilization and homing of stem cells to damaged tissues and was found to improve repairing efficiency (28). These unique properties render MSCs ideal vehicles for cellular gene transfer.

Interestingly, there is an MSC population that has been particularly highlighted for its unique characteristics: The MSCs derived from the Wharton's Jelly (WJ-MSCs) - an anatomic region within the umbilical cord. WJ-MSCs are primitive cells categorized somewhere between embryonic stem cells (ESCs) and adult stem cells. Due to their immunogenic and functional superiority to other MSCs, a special mention of WJ-MSCs should be made. Similar to ESCs and unlike adult MSCs, they are consistently positive for pluripotency and self-renewal markers (29). Importantly, they are safer to use since they do not form teratomas *in vivo* (in contrast to ESCs) and sustain high proliferation rates for extended periods in culture with no signs of transformation, in contrast to adult MSCs that have been linked to transformation events as a result of replicative senescence (30). The most remarkable feature of WJ-MSCs is their hypo-immunogenic profile (a key requirement for allogeneic transplantation) and their capacity for immunomodulation (31). WJ-MSCs are capable of evading immune recognition due to their lack of co-stimulatory molecule expression, which is normally implicated in activation of T and B cell responses and they can also suppress allogeneically stimulated T cells to a greater extent than adult MSCs (32).

3. Therapeutic implications of naïve stem cells

Clinical trials using human MSCs of various origin as well as EPCs are currently underway to treat cardiovascular, cerebrovascular, renal, intestinal and autoimmune diseases.

Implications in cardiovascular disorders. Accumulating evidence from a variety of animal models of acute myocardial infarction (MI) injected or transplanted with MSCs, has

demonstrated that MSCs constitute promising therapeutic tools for repairing and regenerating cardiac cells, interrupting the progress of left ventricular remodeling following acute MI and restoring heart structure and function by reducing infarct size and enhancing angiogenesis and arteriogenesis in the ischemic tissue (11,33-39). Their effects are attributed to their special properties of homing to injured tissues, self-proliferating and differentiating into cardiomyocytes in the damaged area. Indeed, MSCs are able to differentiate into cells that exhibit cardiomyocyte features *in vitro*, however, the proportion of MSCs that differentiate into cardiomyocytes *in vivo* and those that actually survive for a long period is very small (40,41). As known, ischemia induces the production of reactive oxygen species (ROS) and a number of inflammatory molecules, such as tumor necrosis factor (TNF)-α, intercellular adhesion molecule-1 (ICAM-1) and MCP-1 (42). After transplantation into the body, MSCs exhibit paracrine activity by secreting various cytokines including growth factors (e.g., VEGF) that produce anti-inflammatory as well as reparative effects. These molecules can decrease gene expression of inflammatory agents such as TNF-α and IL-1β and IL-6 and they can also promote survival, growth, or differentiation of other cells in the area of the MI, and this is considered the major contribution of MSCs in treatment efficacy (37,41,43,44). The functionally superior WJ-MSCs transplanted by direct injection into the infarcted area of myocardium could survive and differentiate into cardiomyocytes and endothelial cells and also promoted recruitment and differentiation of cardiac stem cells in a porcine model. In addition, WJ-MSC transplantation was shown to reduce apoptosis and fibrosis, enhance viable myocardium, and improve ventricular remodeling and function (45). Scheduled and ongoing clinical trials test the efficacy and safety of these cells in patients with MI (e.g., clinicaltrials.gov; NCT01291329).

Implications in neurological disorders. MSCs have been reported as having significant neural differentiation potential in culture and being neurogenic after transplantation in rodent models, therefore they have gained interest in their potential usefulness in cell-based therapy strategies for neurodegenerative diseases and traumatic injuries of the nervous system (11,46,47). Indeed, prolonged cultured bone marrow-derived MSCs can differentiate into neuron-like cells (48). Transplantation of bone marrow-derived MSCs to animal models of neurodegenerative disorders including Parkinson's disease and ischemic brain injury has been reported to ameliorate functional deficits (49,50). The main challenge to stem cell therapy of central nervous system (CNS) diseases is getting MSCs into the CNS through the blood-brain barrier (51). When transplanted into the brain, MSCs produce neurotrophic and growth factors that protect and induce regeneration of damaged tissue. It has been shown that MSCs can differentiate into neurons and glial cells. Additionally, transplantation of MSCs enables the formation of new blood vessels, thereby increasing blood flow in the ischemic region. It has been shown that intravenous injection of umbilical cord blood-derived MSCs to transgenic mice with Alzheimer's disease results in a decline of cerebral amyloid β (Aβ) peptide and an increase of this peptide in blood plasma due to its excretion from the brain through the blood-brain barrier, as well as a reduction of pro-inflammatory responses in the brain and periphery (52-55). Moreover, WJ-MSCs have been used for induction of neurons and glial cells (56) and they have been shown to promote functional and morphologic recovery of peripheral nerves after axonotmesis and neurotmesis injuries in a rat model (57). WJ-MSCs effectivity in patients with chronic traumatic spinal cord injury is under clinical trial (clinicaltrials.gov; NCT03003364).

Implications in renal disorders. Mounting evidence from ongoing or completed clinical trials indicates that MSC therapy is feasible, safe, well tolerated, and can effectively improve renal pathologies including acute kidney injury (AKI) and chronic kidney disease (CKD), diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), systemic lupus erythematous (SLE), and kidney transplantation. Since regenerative capability of renal cells in humans is very limited, damage in these cells usually lead to devastating diseases. Numerous preclinical studies in various murine models have paved the way for novel therapeutic strategies with the use of MSCs and/or EPCs in a wide range of renal disorders both acute and chronic (58-60).

MSCs have a renoprotective and regenerative action on injured kidney tissues via paracrine mechanisms: anti-fibrotic, anti-apoptotic, pro-angiogenic, proliferative, differentiative, antioxidative, immunosuppressive and immunomodulatory. More specifically, paracrine release of extracellular vesicles including exosomes that contain genetic and protein material by MSCs has been proposed to exert trophic and reparative effects, which can activate mechanisms to ameliorate renal injury (21,60). It has been shown that implantation of bone marrow-derived MSCs after ischemia/reperfusion (I/R)-induced acute renal failure promotes restoring of renal function and morphology, thereby implicating the great therapeutic potential of MSCs in healing damaged kidneys (61-63). Administration of MSCs has demonstrated significant reduction of intrarenal inflammatory infiltrate, decreased fibrosis, and glomerulosclerosis in animal models of CKD (59). Moreover, in animal models of diabetic nephropathy, MSCs reduced glomerulosclerosis and oxidative stress (64-66). Intrarenal delivery of MSCs and EPCs in a porcine model of renovascular hypertension resulted in decreased myocardial injury induced by renovascular hypertension as well as decreased renal injury (67,68). In addition, the identification and characterization of adult renal progenitor cells from rodents as well as humans has provided further insights concerning stem cell regenerative potential in renal tubular injuries (58,69,70). A meta-analysis of studies in animal models by Papazova *et al* demonstrated that cell-based therapy reduced development and progression of CKD by decreasing urinary protein and urea associated with glomerulosclerosis and interstitial fibrosis (71). Although MSC delivery in *in vivo* models of FSGS has been scarcely studied, results were promising as MSCs were shown to stabilize and attenuate the progression of FSGS (72,73). Studies have shown that allogeneic bone marrow or umbilical cord-derived MSC transplantation results in amelioration of disease and could reverse multiorgan dysfunction in SLE (74,75). Treatment with MSCs exhibiting anti-inflammatory and immunomodulatory properties had either beneficial or no adverse effect on autoimmune

lupus nephritis (the major clinical manifestation of SLE) as well as inflammatory bowel disease (IBD), but more research as to whether MSCs could actually benefit these patients is still underway (76,77). Furthermore, WJ-MSCs were shown to improve renal function in a xenogeneic mouse model of acute renal injury by increasing proliferation and decreasing apoptosis of renal tubular cells, a function mediated through the mitochondrial pathway, and through the increase of Akt phosphorylation (78). WJ-MSCs are being clinically tested in patients with diabetic nephropathy (clinicaltrials.gov; NCT03288571).

EPCs are reduced in number and impaired in angiogenic function in patients with atherosclerosis, cardiovascular and chronic renal diseases. Preclinical studies have shown that EPCs are mobilized from the bone marrow to peripheral blood in response to VEGF or other chemotactic molecules, home in at injured or inflamed tissues and differentiate into vessel-forming endothelial cells and/or regulate pre-existing endothelial cells (19,79-81). Moreover, EPCs attenuate vascular inflammation and improve left ventricular function both *in vitro* and *in vivo*, thereby being regarded as promising tools for post-MI therapy. Indeed, post-MI implantation of EPCs into ischemic myocardium of animal models can home to sites of injury and enhance recovery. The number and function of circulating EPCs has been inversely correlated with cardiovascular disease risk as a potential biomarker for patients with hypertension and coronary artery disease (18,80,82-86). Furthermore, EPCs have been proposed as useful tools in cell-based treatment of renal and ischemic cerebrovascular diseases. Studies have shown that mobilization of EPCs contributes to endothelial repair in the kidney immediately after I/R (87,88). EPCs have been also shown to decrease neuronal apoptosis and promote the proliferation and migration of neural stem cells by repairing vascular endothelial cells and inducing neo-vascularization in animal models of cerebral ischemia. Growth factor (e.g., VEGF) secretion by EPCs contributes to post-stroke angiogenesis and neurogenesis, thereby reconstructing the functions and structures of vascular and neural networks (22,89,90).

Stem cell therapy using naïve MSCs and/or EPCs for tissue regeneration confronts many challenges regarding stem cell viability, vitality and functionality. After extensive debate on these issues, research advances could finally provide safer applicable solutions.

4. Genetically-modified stem cells in treatment of human diseases

Genetic modification of human stem or progenitor cells (e.g., MSCs and/or EPCs) for targeted delivery of specific therapeutic agents or genes has been proven to be a very significant advancement in regenerative medicine, since it can improve viability, proliferative capability and metabolic features of these cells which are sensitive to the hypoxic and inflammatory environment in ischemic tissue. For example, MSCs overexpressing the anti-apoptotic gene *Akt1* (*Akt-MSCs*) were shown to be more resistant to apoptosis *in vitro* and *in vivo* (91). Moreover, the efficacy of MSCs for clinical use can be optimized by pre-treatment with drugs, cytokines, and growth factors (92,93).

MSCs can be genetically modified by viral and non-viral methods. Non-viral physical and chemical methods of gene transfer are able to deliver larger transgenes than viral methods, but their main drawback is the low transfection efficiency and transient gene expression (94). MSCs can be efficiently transduced with viral vectors such as lentiviruses, retroviruses, baculoviruses and adenoviruses without affecting their stem cell properties. Viruses can be useful as delivery vectors after being considerably modified in order to become replication incompetent with attenuated cytopathic effects and immunogenicity. Viral vectors are particularly appealing because they can enable high transduction efficiency and, depending on the type of virus used, can deliver long-term stable transgene expression. The safety of cell-based therapy with the use of viral vectors is a crucial issue that has not been resolved yet, but advances in vector design have helped towards this direction (23,95). For example, MSCs genetically modified to express VEGF have been shown to enhance the cardioprotective effects of MSCs followed by angiogenesis effects for the treatment of acute MI, whereas *Akt* gene- or heme oxygenase-1 (*HO-1*) gene-modified MSCs have been shown to dramatically improve ischemic cardiac function and MSC viability and prevent ventricular remodeling and apoptosis of cardiomyocytes and endothelial cells, thus restoring the function of infarcted hearts (91,96-98). In addition, MSCs genetically modified with HGF or VEGF ameliorated acute renal damage, inflammation and apoptosis (99,100).

Genes such as tissue *KLK1* that have been shown to inhibit inflammation, apoptosis, fibrosis and ROS formation, would be a choice for the genetic modification of MSCs and/or EPCs that are intended against organ or tissue injury in human diseases. Indeed, tissue *KLK1*-modified MSCs (*KLK1*-MSCs) have been reported to play a protective role in cardiovascular, cerebrovascular and renal disorders *in vivo* as well as *in vitro* (63). In this review we discuss the advances in the use of *KLK1*-MSCs and/or *KLK1*-EPCs in cell-based therapy of human diseases.

5. Tissue *KLK1*-modified stem cells in cardiac and vascular diseases

The KKS through its components is a crucial regulator of homeostasis of the cardiovascular system throughout the life of an individual and has been implicated in blood pressure regulation (vasodilation) as well as pathogenesis of hypertension (6,101). Since the discovery of tissue KLK1 localization in cardiac and vascular tissues (102-104), multiple experimental studies have investigated its role and potential therapeutic application both *in vitro* and *in vivo*. Tissue KLK1 has been demonstrated to exert pleiotropic beneficial effects in cardiovascular system function by reducing hypertension, attenuating cardiac inflammation and myocardial fibrosis, increasing NO formation, restoring coronary blood flow, decreasing infarct size, promoting neo-vascularization and capillary density and preventing restenosis after acute MI through the VEGF and kinin B2 receptor-Akt-glycogen synthase kinase (GSK)-3β signaling pathways (105-110). Many of these beneficial effects of KLK1 are mediated by the activation of NO signaling pathways, which are responsible for a decrease in oxidative stress in animal models. *In vivo* studies with gene delivery using adenoviral vectors that contained the human *KLK1* gene or

with KLK1 protein infusion have shown that KLK1 reduces cardiac inflammation, hypertrophy, fibrosis and apoptosis of cardiomyocytes in animal models of MI (105-108,111-113). *In vitro* studies on cardiomyocytes and endothelial cells showed that tissue KLK1 expression inhibited hypoxia-induced ROS formation as well as cardiomyocyte apoptosis via activation of Akt-mediated signaling cascades (Akt-GSK-3β and Akt-Bad-14-3-3) (4,108,109,114). Moreover, *KLK1* gene delivery increases the population of cardiac progenitor cells (CPCs) and promotes viability, increases the regional blood flow and neo-vascularization in the peri-infarct myocardium (115). Adenovirus-mediated *KLK1* gene delivery in rodent models was also found to induce endogenous angiogenesis in response to ischemia, and to reduce neointima formation in injured vessels or after balloon angioplasty via activation of Akt and NO-cGMP signaling pathways (116-119). These data suggest that *KLK1* gene therapy might be applicable to peripheral occlusive vascular diseases.

Engineered MSCs can be used as vehicles to deliver therapeutic agents such as tissue KLK1 to injured end-organs. It has been shown that MSCs transduced with adenovirus containing the human tissue *KLK1* gene (*KLK1*-MSCs) acquire improved properties that augment their protective role in cardiovascular diseases. *KLK1*-MSCs secrete KLK1 which may contribute to the reduction in myocardial fibrosis via proteolytic activation of pro-MMP-2 and -9. MMPs are known to degrade the physiological collagen scaffold of the myocardium and other extracellular matrix (ECM) proteins. *KLK1*-MSCs also express higher levels of VEGF and VEGF-R compared to unmodified MSCs (39,107,120,121). The upregulation of VEGF and its receptor could partly account for KLK1-induced neo-vascularization in the infarct myocardium (107). The *in vivo* pro-angiogenic effects of *KLK1*-MSCs exhibiting augmented VEGF secretion were additionally confirmed *in vitro* in cultured endothelial cells wherein a significant increase of proliferation, migration and tube formation was observed (39). KLK1 can also inhibit collagen synthesis and promote collagen breakdown. Notably, administration of *KLK1*-MSCs to rats reduces cardiac collagen deposition and cardiomyocyte hypertrophy (39,122). Moreover, *KLK1*-MSCs show reduced caspase-3 activity compared to controls and their administration to rats decreased myocardial apoptosis after MI. Cultured *KLK1*-MSCs are more resistant to hypoxia-induced apoptosis compared to control MSCs and this resistance possibly enables engraftment of *KLK1*-MSCs to the infarct area in larger amount than control MSCs. Administration of *KLK1*-MSCs to rats also resulted in significant decrease of inflammatory cell (neutrophil and monocyte/macrophage) accumulation in the myocardium and parallel downregulation of TNF-α, ICAM-1 and MCP-1 after MI (39). It has also been suggested that *KLK1*-MSCs could provide significant cardioprotection possibly due to the ability of KLK1 to activate the kinin B2 receptor to either form kinins or not, thereby attenuating myocardial damage through Akt signaling and NO production (39,106). Taken together, the aforementioned findings converge to the notion that MSCs modified with human tissue *KLK1* gene constitute appealing therapeutics with multifaceted potential in cell-based therapy of myocardial ischemia.

EPCs have also been transduced with adenovirus containing the human tissue *KLK1* gene and studied *in vitro* and *in vivo* for the effects of their implantation in animal models of ischemia. Yao *et al* reported that genetic modification of EPCs with the human *KLK1* gene induces Akt phosphorylation and VEGF expression in response to oxidative stress. *KLK1*-EPCs provided enhanced cardioprotection in rats by preventing cardiomyocyte apoptosis, reducing infarct size, restoring left ventricular function and increasing therapeutic angiogenesis and arteriogenesis after acute ischemia-induced MI (85,123). The angiogenic activity of cultured *KLK1*-EPCs is promoted by increased expression of endothelial NO synthase (eNOS) and integrin αvβ3 on the surface of EPCs (19). Furthermore, the increased *KLK1* expression levels lead to enhanced cell proliferation, adhesion, migration, invasion, and tube formation and decreased hypoxia-induced apoptosis in cultured *KLK1*-EPCs (19,85). Importantly, *KLK1*-EPCs exhibited significant retention and viability in ischemic heart which is an important factor for preservation of cardiac function. Fu *et al* also found that the administration of *KLK1*-EPCs into the caudal vein of ischemic rats results in a more effective increase of muscular capillary density, blood flow and myofiber number in an induced hindlimb ischemia rat model in comparison to administration of unmodified or control EPCs (19).

6. Tissue *KLK1*-modified stem cells in renal diseases

The renal KKS is involved in electrolyte and water homeostasis, blood pressure regulation and inflammation. Historically, tissue KLK1 was discovered in human urine at the beginning of the twentieth century as a substance exerting hypotensive action. KLK1 is localized in the collecting segment of the renal distal tubule and its release into the tubules can be induced by the electrolyte balance (low sodium levels, high potassium levels) and antidiuretic hormone (114,124). KLK1 renal excretion in urine is decreased in hypertensive rodents and humans to an extent that is proportional to the severity of renal failure. This decrease might result from a decrease in kinin generation (e.g., bradykinin) in hypertensive conditions, since kininogen levels and kinin-forming factors are reduced in essential and malignant hypertension. It has been suggested that the role of renal bradykinin is to excrete the excess sodium. Therefore, decrease in renal bradykinin generation may lead to sodium accumulation in the body which in turn could result in the development of hypertension (124-126).

Several studies have shown that KLK1 improves renal function by increasing glomerular filtration rate and renal blood flow via its anti-inflammatory, anti-oxidative, anti-fibrotic and anti-apoptotic actions in animal models of renal injury. For example, *KLK1* gene delivery using an adenovirus vector or KLK1 protein infusion in hypertensive Dahl salt-sensitive rats has been shown to attenuate renal dysfunction, induce NO production and reverse the process of renal inflammation and fibrosis in bradykinin B2 receptor-mediated manner (127-131). Liu *et al* confirmed the previous findings by blocking endogenous KLK1 activity in a rat model of CKD which resulted in increased inflammatory cell (macrophages/monocytes) infiltration and myofibroblast and collagen deposition in kidneys (132). Specifically, endogenous KLK1 was shown to inhibit angiotensin II-induced ROS and superoxide formation as well as renal NADH oxidase activity through NO production in deoxycorticosterone acetate (DOCA)-salt

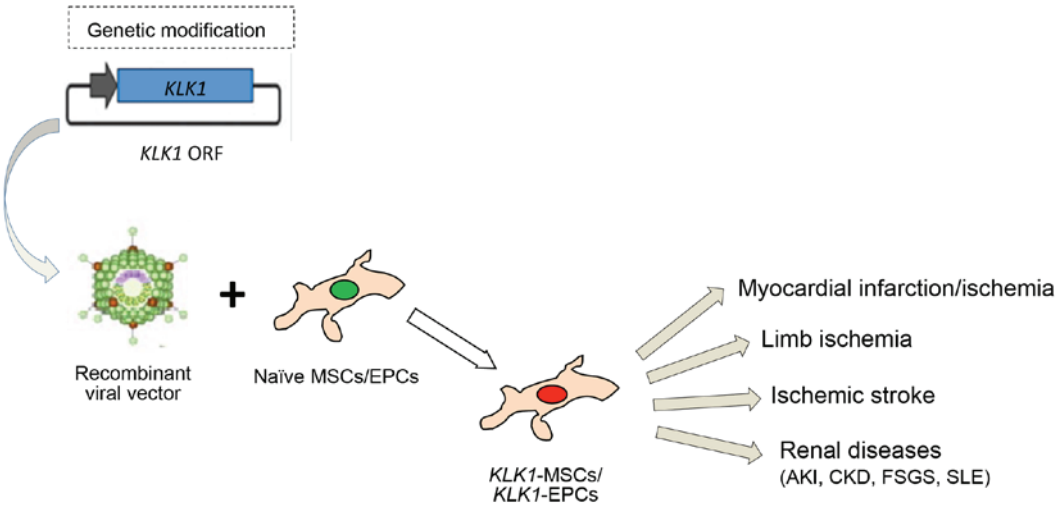

Figure 1. Genetic modification strategy for the insertion of *KLK1* gene into naïve stem cells and possible therapeutic applications. MSCs, mesenchymal stem cells; EPCs, endothelial progenitor cells; AKI, acute kidney injury; CKD, chronic kidney disease; FSGS, focal segmental glomerulosclerosis; SLE, systemic lupus erythematous.

hypertensive rats, which exhibit high renal KLK1 levels. Moreover, KLK1 significantly increased MMP-2 activity and inhibited synthesis of tissue inhibitor of MMP-2 (TIMP-2) as well as plasminogen activator inhibitor-1 (PAI-1), thereby promoting degradation of ECM protein components (e.g., collagen I and fibronectin) as demonstrated both *in vivo* and in cultured renal cells (63,132,133). Most likely, KLK1 exerts its anti-fibrotic effect by increasing ECM degradation which leads to a decrease of mesenchymal fibroblast accumulation in the interstitium of the cortex and medulla. Moreover, KLK1 was demonstrated to decrease renal hypertrophy, namely kidney weight, glomerular size, and proliferation of epithelial tubular cells. KLK1 protein infusion in rats also promoted the recovery of gentamicin-induced nephrotoxicity by inhibiting apoptosis and caspase-3 activity and increasing Akt phosphorylation in proximal tubular renal cells. Therefore, endogenous tissue KLK1 can attenuate and reverse renal injury by reducing oxidative stress, apoptosis, inflammation and fibrosis *in vivo* through activation of bradykinin B2 receptor (63,114,133-135). Overall, these studies outline the beneficial role of tissue KLK1 in the preservation of kidney structure and function by promoting tissue repair and regeneration in AKI and modulating the progression of CKD.

As already mentioned, *KLK1* modified MSCs secrete recombinant human KLK1 as well as increased VEGF levels into culture medium, and exhibit augmented survival under oxidative stress conditions compared to control MSCs. After systemic injection in rats, *KLK1*-MSCs migrated to the injured kidney and KLK1 overexpression was detected in rat glomeruli after I/R injury. *KLK1*-MSCs implantation in rat kidney provided advanced protection against ischemia-induced kidney injury by suppression of apoptosis and interstitial inflammatory cell accumulation. The engraftment of *KLK1*-MSCs reduced blood urea nitrogen, serum creatinine levels, and tubular injury. VEGF secretion from *KLK1*-MSCs may be partly responsible for the improvement of renal injury, as well (114,120). Moreover, the KKS has been shown to be involved in the acute manifestations of lupus nephritis which occurs when antibodies and complement components accumulate and cause inflammation of the kidney in SLE patients (136). KLK1, among other members of the KLK family, was found to exert a protective role against SLE and anti-glomerular basement membrane (anti-GBM) antibody-induced nephritis in rodents as well as humans (137). Li *et al* studied the effects of *KLK1*-MSC administration into murine injured kidneys and confirmed that KLK1 attenuated spontaneous lupus nephritis in mice (138). *KLK1*-MSCs displayed a remarkable protective effect against anti-GBM induced-nephritis and lupus nephritis compared to control MSCs by inhibiting oxidative stress, renal cell apoptosis and inflammatory cell infiltration into the kidneys of nephritic mice, in line with findings of Hagiwara *et al* (120). Taken together, the above presented data suggest that *KLK1*-modified MSCs have the potential to be used as therapeutic agents in a wide variety of renal diseases.

7. Tissue *KLK1*-gene delivery in neurological diseases

The KKS is capable of dilating cerebral arterial vessels partly because of the release of endothelium-derived relaxing factor NO which plays a complex role in cerebral ischemia. Ischemic conditions trigger an excessive activation of neuronal NO synthase (NOS), which results in production of NO that is toxic to surrounding neurons, but critical in maintaining cerebral blood flow and reducing infarct volume. The KKS through participation in NOS activation and following NO formation is implicated in endothelial cell function in the setting of ischemic stroke (139).

Despite the lack of studies using *KLK1* modified MSCs in models of cerebrovascular or neurodegenerative diseases, we review current knowledge on the effects of adenovirus-mediated *KLK1* gene delivery in animal models of cerebral ischemia/ischemic stroke. Zhang *et al* have demonstrated that *KLK1* gene transfer attenuates the blood pressure rise and cerebral damage in hypertensive Dahl salt-sensitive rats leading to a decrease in the stroke-induced mortality rate (140). Subsequent studies have shown that intracerebroventricular injection of adenovirus carrying the *KLK1* gene prevents

stroke-induced ischemic brain injury (cerebral infarction) by inhibiting neuronal and glial apoptosis and inflammation while promoting neurogenesis and angiogenesis in the ischemic brain of a rat model as well as *in vitro* (139,141,142). Moreover, administration of tissue KLK1 can specifically stimulate the proliferation of murine neural stem cells only (no other neural cell types) independent of kinin formation, but without inducing their differentiation into neurons or glial cells (143). Tissue KLK1 administration can also suppress glutamate- or acidosis-mediated neurotoxicity *in vitro* and protect from hypoxia/reoxygenation-induced neuronal injury by promoting neuron viability at least partially through the KLK1-B2R-ERK1/2 signaling pathway (144). Collectively, these findings raise the possibility that tissue KLK1 may be a novel therapeutic target in the treatment of ischemic stroke-induced brain injuries paving the way for *KLK1*-MSC administration research in neurological diseases.

8. Conclusion

Numerous studies have outlined the pleiotropic beneficial effects of tissue KLK1 protease, component of the KKS, in the protection of cardiovascular, renal and central nervous systems from tissue injury. Genetic modification of stem cells or progenitor cells with *KLK1* gene enhances their viability and proliferative, migratory and functional properties, thus increasing their tissue healing effects in various human diseases (Fig. 1). Engraftment of *KLK1*-MSCs and/or *KLK1*-EPCs into animal models provided advanced protection against vascular and organ damage. Aforementioned findings reveal the KLK1 relevance to human diseases and pave the way for further research on the potential therapeutic perspectives of *KLK1*-MSCs and/or *KLK1*-EPCs that could lead to the translation of preclinical studies into effective and safe targeted therapies for cardiovascular, cerebrovascular and renal diseases.

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