Augmentation of Therapeutic Angiogenesis Using Genetically Modified Human Endothelial Progenitor Cells with Altered Glycogen Synthase Kinase-3β Activity*

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Previously we reported that inhibition of glycogen synthase kinase-3β (GSK3β), a key regulator in many intracellular signaling pathways, enhances the survival and migration of vascular endothelial cells. Here we investigated the effect of inhibition of GSK3β activity on the angiogenic function of endothelial progenitor cell (EPC) and demonstrated a new therapeutic angiogenesis strategy using genetically modified EPC. As we previously reported, two biologically distinct types of EPC, spindle-shaped “early EPC” and cobblestone-shaped “late EPC” could be cultivated from human peripheral blood. Catalytically inactive GSK3β gene was transduced into both EPC. Inhibition of GSK3β signaling pathway led to increased nuclear translocation of β-catenin and increased secretion of angiogenic cytokines (vascular endothelial growth factor and interleukin-8). It enhanced the survival and proliferation of early EPC, whereas it promoted the survival and differentiation of late EPC. Transplantation of either of these genetically modified EPC into the ischemic hind limb model of athymic nude mouse significantly improved blood flow, limb salvage, and tissue capillary density compared with nontransduced EPC. Inhibition of GSK3β signaling of either of these genetically modified EPC augmented the in vitro and in vivo angiogenic potency of these cell populations. These data provide evidence that GSK3β has a key role in the angiogenic properties of EPC. Furthermore, the genetic modification of EPC to alter this signaling step can improve the efficacy of cell-based therapeutic vasculogenesis.

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§§ The abbreviations used are: EPC, endothelial progenitor cells; GSK3β, glycogen synthase kinase-3β; AdGFP, adenoviral vector expressing green fluorescent protein; EC, endothelial cell; PBS, phosphate-buffered saline; T-PBS, PBS containing 0.3% Tween 20; VEGF, vascular endothelial growth factor; IL-8, interleukin-8; TRITC, tetramethylrhodamine isothiocyanate.

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Previously studies have identified that normal adults have a small amount of circulating endothelial progenitor cells (EPC)
which are described above. Recently, it has been shown that a pharmacological inhibitor of GSK3 promotes the self-renewal of embryonic stem cells in vitro (20). GSK3β controls several downstream transcription factors that are crucial in cell survival and function including β-catenin (28), heat shock factor-1 (29), CAMP-response element-binding protein (30), AP-1 (31), Myc (32), NFAT (33), CCAAT/enhancer-binding protein α (34), and cyclin D1 (35). GSK3β has also been shown to be involved in the regulation of angiogenesis through its ability to modulate vascular endothelial cell migration and survival (36). Therefore, we reasoned that it might be possible to genetically manipulate GSK3β activity in EPC with viral vectors to alter their vasculogenic properties in vivo.

**EXPERIMENTAL PROCEDURES**

**Isolation and Culture of EPC from Human Peripheral Blood**—All human projects in this study were approved by the institutional review board of the Seoul National University Hospital. Two kinds of EPC with different biological characteristics, spindle-shaped “early EPC” and cobblestone-shaped “late EPC,” were cultured as previously described (16). In brief, peripheral blood mononuclear cells were isolated from human volunteers and resuspended in the EGM-2 BulletKit system (Clonetics) consisting of endothelial basal medium, 5% fetal bovine serum, human epidermal growth factor, VEGF, human basic fibroblast growth factor, insulin-like growth factor 1, ascorbic acid, and heparin. 1 × 10^5 mononuclear cells/well were seeded on a 2% gelatin (Sigma)-coated 6-well plate and incubated in 5% CO₂ incubator at 37 °C. Under daily observation, the first medium change was done about 6 days after plating. Thereafter, media were changed every 3 days.

**Gene Transfer to Cell**—Replication-defective adenoviral vector expressing catalytically inactive GSK3β (GSK3β-KM) or wild-type GSK3β (GSK3β-WT) was used for transduction. Expression of GSK3β-WT was confirmed by more than 90% of green fluorescence-positive control by counterstaining with anti-GFP antibody (1:750 dilution; Santa Cruz Biotechnology), anti-α-tubulin antibody (1:4000 dilution; Oncogene), and anti-β-catenin (1:500 dilution; Cell Signaling Technology). The secondary antibodies were anti-rabbit IgG/horseradish peroxidase (1:2500 dilution; TSA-Promega). Densitometric quantitative analysis of the immunoblot result was performed using TINA 2.01 (Raytest).

**Analysis of β-Catenin Nuclear Translocation**—The effect of GSK3β inhibition in canonical Wnt pathway was investigated by examining the subcellular localization of β-catenin. Early EPC and late EPC were transduced with multiplicity of infection 50 of adenovirus-GSK3β-KM (AdGSK-KM) and control adenovirus-GFP (AdGFP) in EGM-2 medium with 2% fetal bovine serum for 12 h. Effective gene transfer was confirmed by more than 90% of green fluorescence-positive control EPC transduced with AdGFP, and cells were assayed or harvested.

**Western Blot Analysis**—Western blot analysis was performed to confirm that the transduction of AdGSK-KM into EPC could lead to the sufficient expression of inactive phosphorylated GSK3β (36, 37). Early EPC and late EPC were transduced with multiplicity of infection 50 of AdGK3/AdGFP or AdGSK-KM for 12 h and washed in phosphate-buffered saline (PBS) and harvested by scraping in lysis buffer. After determination of protein concentration with a protein assay kit (Bio-Rad), 20 μg of protein was separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA). The membrane was blocked with PBS containing 0.3% Tween 20 (Sigma)-coated 6-well plate and incubated in 5% CO₂ incubator at 37 °C. Under daily observation, the first medium change was done about 6 days after plating. Thereafter, media were changed every 3 days.

**Analysis of β-Catenin Nuclear Translocation**—The effect of GSK3β inhibition in canonical Wnt pathway was investigated by examining the subcellular localization of β-catenin. Early EPC and late EPC were transduced with multiplicity of infection 50 of adenovirus-GSK3β-KM (AdGSK-KM) and control adenovirus-GFP (AdGFP) in EGM-2 medium with 2% fetal bovine serum for 12 h. Effective gene transfer was confirmed by more than 90% of green fluorescence-positive control EPC transduced with AdGFP, and cells were assayed or harvested.

**Evaluation of in Vivo Angiogenesis Effect of EPC**—A laser Doppler perfusion imager (Moor Instruments), which maps tissue blood flow by thermal dilution of the laser light in the tissue, was used for non-invasive physiological evaluation of neovascularization (5). Each mouse was followed by serial recording of surface blood flow of hind limb on days 0, 3, 7, 14, and 21. After laser Doppler perfusion imager scanning, the digital color-coded images were analyzed to quantify blood flow of the area from the knee joint to toe, and mean values of perfusion were calculated. To avoid data variations due to ambient light and temperature, hind limb perfusion was expressed as the ratio of ischemic to nonischemic limb. Photographs of the limb were also recorded and visually analyzed as “limb salvage” (completely normal status without a sign of ischemia), “foot necrosis” (necrosis of toe or below knee), or “limb loss” (necrosis of tissue above knee).

**Statistical Analysis**—All data are presented as mean ± S.E. Statistical significance was evaluated by Student’s t test or analysis of variance. The incidence of limb salvage was evaluated by χ². Proportionality values of two-tailed p < 0.05 were considered to denote statistical significance.
FIG. 1. High transfection efficiencies of adenoviral vectors to EPC were confirmed by immunofluorescence staining following transduction with AdGFP. Modulation of the GSK3β signaling pathway in early EPC and late EPC by GSK3β-KM was investigated by immunoblot analysis. A, subconfluent early EPC and late EPC were transduced with multiplicity of infection 50 of AdGFP for 12 h and examined with fluorescence microscopy. More than 90% of EPC showed green fluorescence. Scale bar, 12.5 μm. B, transduction of the catalytically inactive GSK3β gene significantly increased expression of phosphorylated inactive GSK3β (p-GSK3β) in both early EPC and late EPC. The decrease of GSK3β activity in EPC transduced with AdGSK-KM is reflected by the decrease of phosphorylated β-catenin. Experiments were repeated three times, and representative data are shown. C, the densitometry data of Western blot is normalized to α-tubulin and shown as the graphs. When EPC were transduced with AdGSK-KM, significantly increased expression of total GSK and phosphorylated inactivate GSK3β was identified both in early EPC (total GSK, *p = 0.003; p-GSK3β, *p = 0.046) and late EPC (total GSK, *p = 0.003; p-GSK3β, *p = 0.044). Decrease of GSK activity in EPC transduced with AdGSK-KM was assessed by an immunoblot of phosphorylated β-catenin, in both early EPC (p = 0.003) and late EPC (p = 0.044). β-catenin, phosphorylated β-catenin; GFP, cells transduced with AdGFP; GSK-KM, cells transduced with AdGSK-KM. The asterisks denote statistical significance (*p < 0.05).
FIG. 2. Biochemical aspect of the modulation of GSK3\(\beta\) signaling pathway in EPC with transduction of GSK3\(\beta\)-KM was investigated by examining the subcellular localization of \(\beta\)-catenin and the effect on angiogenic cytokine secretion from EPC. A, transduction with control AdGFP did not cause nuclear localization of \(\beta\)-catenin both in early and late EPC. However, the nuclear \(\beta\)-catenin staining could be clearly identified after transduction with AdGSK-KM in both EPC. The number of \(\beta\)-catenin-positive nuclei was significantly higher in early EPC than late EPC (89.5 ± 5.2 versus 61.3 ± 9.1%, \(p < 0.05\)). The empty arrowheads and filled arrowheads denote negative and positive nuclear \(\beta\)-catenin staining, respectively. Scale bar, 3 \(\mu\)m. B, transduction with AdGSK-KM significantly enhanced VEGF secretion from early EPC, and it also significantly enhanced IL-8 secretion from early EPC and late EPC. *, \(p < 0.05\).
GSK3\(\beta\)-KM transduction enhanced in vitro angiogenic function of both early EPC and late EPC. A, a Matrigel network formation assay was performed to investigate the effect of transduction with GSK3\(\beta\)-KM on the endothelial network formation ability of EPC. Culture of early EPC transduced with either AdGFP or AdGSK-KM showed little endothelial network formation on Matrigel. In contrast, late EPC formed an endothelial network reminiscent of tubules, and the ability of late EPC to form an endothelial network was enhanced when transduced with AdGSK-KM (D) compared with AdGFP (C) (network length: 2.04 ± 0.15 versus 1.11 ± 0.72 mm, respectively, \(p = 0.044\); network circle number: 7.3 ± 3.1 versus 2.4 ± 1.6/mm², respectively, \(p = 0.032\)). Scale bar, 25 \(\mu\)m. B, effect of transduction with GSK3\(\beta\)-KM on the EPC survival and proliferation was evaluated using WST-1 assay in serum-free or growth factor-rich conditions, respectively. In serum-free conditions, the survival of EPC was enhanced when transduced with AdGSK-KM compared with AdGFP (early EPC, 78.1 ± 23.6%, \(p = 0.001\); late EPC, 66.7 ± 25.5%, \(p < 0.001\), respectively). In complete media with serum and growth factors, the proliferation of early EPC was also enhanced when transduced with AdGSK-KM compared with AdGFP (35.7 ± 19.0% increase, \(p = 0.003\)), but the proliferation of EPC was not significantly enhanced (\(p > 0.05\)). *, \(p < 0.05\).
RESULTS

Modulation of GSK3β Signaling Pathway by Dominant Negative GSK3β Transduction into EPC—Early and late EPC cultures were transduced with adenoviral vectors expressing GFP (AdGFP) or a catalytically inactive form of GSK3β (AdGSK-KM). Under the culture conditions, greater than 90% of the cells expressed GFP reporter gene (Fig. 1A). Western blot analysis revealed that the transduction of AdGSK-KM into EPC could lead to increased expression of inactive phosphorylated GSK3β. Both early EPC and late EPC transduced with AdGSK-KM showed a marked increase of total GSK protein (early EPC, 2.5-fold; late EPC, 2.6-fold) and phosphorylated GSK3β (early EPC, 2.2-fold; late EPC, 7.5-fold) compared with control AdGFP-transduced EPC. Inactivated GSK3β signaling pathway in EPC transduced with AdGSK-KM was identified by the decrease of phosphorylated β-catenin (early EPC, 0.4-fold; late EPC, 0.7-fold) (Fig. 1, B and C).

To investigate further the modulation of GSK3β signaling pathway by AdGSK-KM from biochemical perspective, nuclear translocation of β-catenin, a marker for Wnt pathway activation, was investigated at the subcellular level by direct β-catenin immunofluorescence staining. EPC transduced with AdGSK-KM showed profound nuclear translocation of β-catenin, and it was more significant in early EPC than late EPC (89.5 ± 5.2 versus 61.3 ± 9.1%, p < 0.05). EPC transduced with control AdGFP showed no nuclear β-catenin (Fig. 2A).

The effect of GSK3β inactivation on the secretion of angiogenic cytokines from EPC, which is regarded as one of the major angiogenic mechanisms of EPC, was also investigated (10, 16). The concentrations of VEGF and IL-8, which is under the regulation of the GSK3β signaling pathway (41, 42), were significantly higher in the supernatant of early EPC than in late EPC. In early EPC, transduction of AdGSK-KM led to significantly increased secretion of VEGF and IL-8 (VEGF, 132.3 ± 9.3 versus 247.5 ± 26.1 pg/10^5 cells, p = 0.002; IL-8, 401.7 ± 21.6 versus 516.7 ± 11.7 pg/10^6 cells, p = 0.001). In late EPC also, transduction of AdGSK-KM led to a significant increase of IL-8 secretion (265.7 ± 23.4 versus 334.4 ± 10.1 pg/10^6 cells, p = 0.01), although it did not affect the amount of VEGF that was barely secreted from late EPC (Fig. 2B).

Effect of AdGSK-KM on the in Vitro Angiogenesis Model—A Matrigel network formation assay was performed to investigate the ability of EPC to form endothelial cell networks reminiscent of tubules. Both AdGFP- and AdGSK-KM-transduced early EPC in Matrigel showed little evidence of endothelial network formation. In contrast, late EPC formed an endothelial network reminiscent of tubules, and the network formation was more evident when late EPC was transduced with AdGSK-KM than with control AdGFP (network length: 2.04 ± 0.15 versus 1.11 ± 0.72 mm, respectively, p < 0.05; network circle number: 7.3 ± 3.1 versus 2.4 ± 1.6/mm², respectively, p < 0.05) (Fig. 3A).

Fig. 1. Administration of EPC transduced with GSK3β-KM increased limb perfusion following femoral artery resection. A, serial analysis of laser Doppler perfusion imaging revealed a greater increase of limb perfusion in the ischemic limb of mice injected with EPC that were genetically modified with AdGSK-KM than with AdGFP. Representative images were shown. B, computer-assisted quantitative analysis of hind limb perfusion showed a significantly improved ischemic-normal limb blood perfusion ratio in mice injected with EPC that were genetically modified with AdGSK-KM than AdGFP at day 21 (early EPC-AdGSK-KM versus early EPC-AdGFP, 57.8 ± 18.5 versus 29.2 ± 14.8%, p = 0.004; late EPC-AdGSK-KM versus late EPC-AdGFP, 54.9 ± 14.2 versus 31.0 ± 17.0%, p = 0.02). *, p < 0.05.

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FIG. 5. Administration of EPC transduced with AdGSK-KM increased capillary density of ischemic skeletal muscle. A, the endothelial morphology of a histological murine skeletal muscle specimen was identified using various staining of transverse-sectioned muscle with the classic histological method (hematoxylin-eosin), using biochemical characteristics (alkaline phosphatase), and the immunologic method using surface marker (CD31). All images showed similar morphological characteristics, dots scattered on a background. Staining using mouse endothelial cells-specific B. simplicifolia lectin 1 was performed in a tilted section of skeletal muscle, showing the longitudinal aspect of vascular endothelial cells. Incorporation of human EPC, which was marked with human endothelial cell-specific U. europaeus lectin 1 (UEA-1), into sites of neovascularization, was identified in the merged image. B, representative photographs of histological evaluation of neovascularization in ischemic tissue at day 21. Capillary densities of mice given EPC transduced with AdGSK-KM were markedly increased compared with control EPC. Areas enclosed by dotted lines are shown in insets located in the lower right quadrant of each photograph with double magnification. Quantitative analysis revealed a significantly increased capillary density in mice given EPC genetically modified with adenovirus GSK3β-KM than EPC with control adenovirus (early EPC-AdGFP versus early EPC-AdGSK-KM, 285.7 ± 97.0 versus 584.0 ± 151.1 mm², p < 0.001; late EPC-AdGFP versus late EPC-AdGSK-KM, 361.4 ± 124.4 versus 570.3 ± 142.6 mm², p = 0.006). C, anatomic localization of U. europaeus lectin 1-labeled human EPC (green, arrowheads) into foci of neovascularization in ischemic mouse muscle stained with B. simplicifolia lectin 1 (BS-1)-labeled mouse EC (red), which were observed at day 7. Quantitative analysis revealed a greater number of human-derived EPC in mice given EPC that were genetically modified with adenovirus GSK3β-KM than EPC with control adenovirus (early EPC-AdGFP versus early EPC-AdGSK-KM, 5.2 ± 2.9 versus 28.0 ± 2.9%, p = 0.001; late EPC-AdGFP versus late EPC-AdGSK-KM, 13.1 ± 4.6 versus 29.3 ± 2.7%, p = 0.022). Scale bar, 100 μm. *, p < 0.05.

Effect of AdGSK-KM on EPC Proliferation and Survival—Transduction of GSK3β-KM significantly increased survival of both early and late EPC exposed to serum-free condition (early EPC, 1.8-fold; late EPC, 1.7-fold). Transduction of GSK3β-KM also increased proliferation of early EPC (1.4-fold) cultured in medium with growth factors but did not affect the proliferation of late EPC cultures in the same condition (Fig. 3B).

Effect of AdGSK-KM on the in Vivo Angiogenesis Model—The in vivo angiogenic effect of GSK3β-KM-transduced EPC in a murine model of hind limb ischemia was evaluated.Serial evaluation of hind limb blood perfusion by laser Doppler perfusion imager revealed significant improvement in vascular perfusion at 21 days using either early or late EPC transduced with GSK3β-KM compared with control EPC transduced with GFP or media (Fig. 4, A and B). Capillary densities were also evaluated in tissue sections retrieved at day 21 from lower calf muscle of ischemic hind limb. We confirmed that the endothelial morphology of histological murine skeletal muscle specimen is appropriate, by various staining methods including the classic histological method (hematoxylin-eosin), using biochemical characteristics (alkaline phosphatase), and the immunologic method using surface marker (CD31) (Fig. 5A). Higher capillary densities were observed in mice that received either early or late EPC transduced with GSK3β-KM, corroborating the vascular perfusion measured by laser Doppler perfusion imager (early EPC, 2.0-fold increase; late EPC, 1.6-fold increase) (Fig. 5B). The incorporation of human EPC into the skeletal capillary system was assessed using immunofluorescence staining with human-specific fluorescein isothiocyanate-conjugated U. europaeus lectin 1 at day 7, the time at which the proliferation of endothelial cell is maximal (39). A greater number of human-derived EPC were detected in mice given early or late EPC transduced with GSK3β-KM (early EPC, 5.4-fold increase; late EPC, 2.2-fold increase) (Fig. 5C).
Some athymic nude mice develop extensive necrosis of ischemic hind limb and eventually lose their limbs by autoamputation due to severe limb ischemia. Administration of either early or late EPC transduced with AdGSK-KM resulted in a significantly lower rate of limb loss (necrosis of tissue above knee or autoamputation) compared to EPC transduced with control AdGFP (early EPC-AdGSK-KM versus early EPC-AdGFP, 18.8% (3/16) versus 58.8% (10/17); p = 0.032; late EPC-AdGSK-KM versus late EPC-AdGFP, 0% (0/10) versus 50% (9/18); p = 0.010). The rate of complete limb salvage was also higher in early EPC-AdGSK-KM (50.0% (8/16)) than early EPC-AdGFP (17.6% (3/17)), although statistical significance was marginal (p = 0.07). Administration of late EPC-AdGSK-KM also salvaged more limbs (70% (7/10)) than late EPC-AdGFP (27.8% (5/18)) (p = 0.008). The outcomes in mice given early EPC and late EPC transduced with vectors carrying same gene were comparable (p > 0.05). *, p = 0.07; †, p < 0.05.

**FIG. 6.** Administration of EPC transduced with GSK3β-KM improves ischemic limb salvage. A, photographs of mice showing three outcomes. Left, complete limb salvage; middle, foot necrosis; right, autoamputation due to severe limb ischemia. B, administration of either early or late EPC transduced with AdGSK-KM resulted in a significantly lower rate of limb loss (necrosis of tissue above knee or autoamputation) compared to EPC transduced with control AdGFP (early EPC-AdGSK-KM versus early EPC-AdGFP, 18.8% (3/16) versus 58.8% (10/17); p = 0.032; late EPC-AdGSK-KM versus late EPC-AdGFP, 0% (0/10) versus 50% (9/18); p = 0.010). The rate of complete limb salvage was also higher in early EPC-AdGSK-KM (50.0% (8/16)) than early EPC-AdGFP (17.6% (3/17)), although statistical significance was marginal (p = 0.07). Administration of late EPC-AdGSK-KM also salvaged more limbs (70% (7/10)) than late EPC-AdGFP (27.8% (5/18)) (p = 0.008). The outcomes in mice given early EPC and late EPC transduced with vectors carrying same gene were comparable (p > 0.05). *, p = 0.07; †, p < 0.05.

**DISCUSSION**

In this study, EPC were isolated from human peripheral blood and transduced with catalytically inactive GSK3β (GSK3β-KM). Collectively, the genetically modified EPC displayed enhanced proliferation, survival, and differentiation in vitro. These cells also displayed augmented vasculogenic potential in vivo, suggesting that GSK3β is a regulator of angiogenic function of EPC.

In our previous study, we demonstrated that two biologically distinct types of EPC emerge sequentially from a single source of adult peripheral blood, although the in vivo vasculogenic potential was not appreciably different between these two populations (16). In the current study, we observed that transduction of GSK3β-KM into early and late EPC significantly enhance the in vivo vasculogenic potential of the early and late EPC to comparable degrees. The modulation of GSK3β signaling pathway by transduction of catalytically inactive GSK3β gene, GSK3β-KM, was proved at the subcellular level by the increase of phosphorylated GSK3β and nuclear translocation of β-catenin. However, we could observe differences in the effect of GSK3β-KM on the in vitro angiogenic function of the two EPC populations. These in vitro results might shed light on the possible mechanisms for the increased in vivo vasculogenic potential of GSK3β-KM-transduced EPC.

In our study, the survival of both early and late EPC was improved by transduction of GSK3β-KM. This can be speculated from the prosurvival effect of GSK3β-KM on mature endothelial cells (36), because EPC is a vascular endothelium-committed stem cell (1, 7). However, transduction of GSK3β-KM enhanced the proliferation of early EPC, whereas the proliferation of late EPC was not significantly influenced. The weak effect of GSK3β-KM on late EPC can be explained by the relatively smaller amount of β-catenin nuclear translocation in late EPC than early EPC, which was shown in immunoblot analysis and immunofluorescence staining. In addition, the proliferation of late EPC may not be significantly influenced by transduction of GSK3β-KM, because late EPC exhibits a high exponential growth rate innately, contrary to the limited proliferative capacity of early EPC (14, 16, 17). The high growth rate might also lead to the less nuclear translocation of β-catenin observed in late EPC, because β-catenin subcellular localization can be affected by cell density (43).

Early EPC contributes to neovascularization mainly by secreting the angiogenic cytokines that help recruit resident mature vascular endothelial cells and induce their proliferation and survival, whereas late EPC enhances neovascularization by providing a sufficient number of endothelial cells based on their high proliferation potency (10, 16). Transduction of GSK3β-KM enhanced secretion of VEGF in early EPC. GSK3β phosphorylates the HIF-1α oxygen-dependent degradation domain. Therefore, the inactivation of GSK3β by GSK3β-KM increases VEGF probably through HIF-1α accumulation, which increases VEGF transcription (41). Transduction of GSK3β-KM also enhanced secretion of IL-8 in early and late EPC. This could be explained by up-regulation of IL-8 by β-catenin, which is downstream of GSK3β (42). Conversely, GSK3β-KM promoted the differentiation of late EPC, but early EPC did not exhibit this property. These data suggest that early and late EPC may be subject to different but overlapping regulatory controls and that the effect of GSK3β signaling on phenotype may be influenced by the status of EPC differentiation.

A potential problem with cell-based therapy for vasculogenesis is insufficiency of EPC numbers for clinical applications. Based upon preclinical findings, it has been estimated that more than 10 liters of autologous peripheral blood would be required to harvest sufficient EPC to induce angiogenesis in a...
the potency of EPC for therapeutic vasculogenesis. Here, we targeted the GSK3β signaling step. Under conditions of these assays, control EPC salvaged ~20–30% of the ischemic limbs. However, when EPC were modified with GSK3β-KM, salvage improved to ~50–70%. Furthermore, in these experiments, we used smaller numbers of EPC than in our previous study (16), suggesting further that genetic modification with GSK3β-KM can overcome the limitation of EPC number.

In conclusion, our data show that vasculogenic potentials of both spindle-shaped early EPC and cobblestone-shaped late EPC can be augmented by transduction of catalytically inactive GSK3β gene. Diminished GSK3β signaling was shown to increase the proliferative potential of early EPC, promote the differentiation potential of late EPC, and exert an antiapoptotic action on both. In vitro, GSK3β-KM-transduced EPC exhibited a more potent vasculogenic response than control EPC. These data show that GSK3β signaling is an important determinant of EPC behavior, and they support the hypothesis that the administration of biologically modified EPC may be a strategy for increasing the potency of EPC for therapeutic vasculogenesis.

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