

The effect of hypoxic preconditioning on adult stem cell differentiation, function, and angiogenesis

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INTRODUCTION: This study evaluates the effect of hypoxia on ASC differentiation and function.

METHODS: Human ASCs (CD13+29+90+) isolated from periumbilical fat were cultured in EC-differentiating medium at normoxic or hypoxic conditions for up to 2 weeks. We assessed EC differentiation (expression of CD31, vWF, and eNOS) and stem cell markers (OCT4, SOX2, Nanog, KLF4) by RT-PCR. We evaluated the response to hypoxia (expression of HIF-1alpha) by immunoblot, and quantified VEGF expression by ELISA. Last, we evaluated angiogenic potential by observing capillary-like structure formation on Matrigel.

RESULTS: After differentiation in normoxia, ASCs expressed the EC markers CD31 and vWF. Growth in hypoxia suppressed these changes in a time-related manner, suggesting promotion of stemness rather than differentiation. We did not, however, observe expression of any stem cell markers in hypoxia. We did observe upregulation of HIF-1alpha protein in response to hypoxia by 24 hours, with upregulation of VEGF mRNA by RT-PCR over 2 weeks. VEGF protein showed significant increase with exposure to hypoxia in a time-related manner (19.8 vs 11.9 pg/mL, day 4; P<.01); this was also quantified at the mRNA level where there was a 2.5-fold increase of VEGF at day 4. Conditioned medium from hypoxic cultures of ASC promoted formation of capillary-like structures by EC.

CONCLUSIONS: These data suggest that hypoxia suppresses endothelial differentiation but serves as a potent stimulus of VEGF production likely by upregulation of HIF-1alpha. Given the known pericapillary location of ASCs in vivo, these findings support the hypothesis that ASC plays an important role in angiogenesis in response to tissue ischemia.

Stabilization of hypoxia-inducible factor-1 enhances proangiogenic potential of bone marrow-derived mesenchymal stem cells

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INTRODUCTION: Hypoxia-inducible factor-1 (HIF-1) plays a central role in cell survival during hypoxia by activating multiple downstream proangiogenic genes. However, poly(hydroxylase domain-2 (PHD-2) protein induces degradation of HIF-1 during normoxia. Recent evidence indicates that hypoxia facilitates bone marrow-derived mesenchymal stem cell (bmMSC) response to injury and neovascularization. This study evaluates the effect of normoxic HIF-1 stabilization by silencing PHD-2 on the proangiogenic potential of bmMSC.

METHODS: A self-inactivating lentiviral vector expressing an shRNA sequence against PHD-2 or a scrambled shRNA sequence was generated. Early passage mouse bmMSC were transduced with the lentivirus and subjected to puromycin selection for stable transgene expression. QRT-PCR and Western blot were performed to determine the expression of PHD-2, HIF-1a, and VEGF. The transduced cells were also plated on matrigel in the presence or absence of mouse endothelial bEnd.3 cells and assessed for tube formation.

RESULTS: Compared with the scrambled shRNA as control, transduction with the lentiviral shRNA against PHD-2 significantly abrogated expression of PHD-2 (*p=0.001) and increased expression of HIF-1 and VEGF in bmMSC. When plated on matrigel, the PHD-2 knockdown bmMSC migrated and aligned to create sprouting capillary tubes, in marked contrast to control cells that remained as round cells. In coculture with bEnd.3 cells, complex meshlike structures formed where the HIF-1 stabilized bmMSC appeared to interact and recruit the endothelial cells.

CONCLUSIONS: RNAi-mediated silencing of PHD-2 in bmMSC stabilized HIF-1 under normoxia and increased in vitro angiogenesis. This shRNA interference in bmMSC can improve their potential use in cell-based therapies for vascular disease and wound healing.

Targeting of tumor angiogenesis using engineered Trojan Horse mesenchymal stem cells

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INTRODUCTION: Tumor angiogenesis represents a promising target for the selective delivery of cancer therapeutics.

METHODS: We developed a novel combined gene/stem cell construct composed of engineered, bone marrow-derived mesenchymal stem cells (MSCs) and a differentiation-specific toxic gene product to selectively target exogenous genes to tumor angiogenesis environments. The stem cell component of the construct consisted of engineered, immortalized MSC lines that show remarkable pluripotency and readily generate new vessel growth. When injected into the peripheral circulation, the cells were actively recruited to growing tumor vasculature of a murine orthotopic pancreatic and a spontaneous breast cancer model. The MSCs were engineered to express the herpes simplex virus-thymidine kinase (TK) gene under the control of the Tie2 promoter enhancer, which effectively directs expression of TK when the MSCs develop endothelial-like characteristics. The TK gene product in combination with the prodrug ganciclovir (GCV) produces a potent toxin, which affects replicative cells.

RESULTS: Due to selective stem cell homing into the tumor neoangiogenesis and gene transcription after initiation of differentiation only, this therapy creates a toxic environment that is tumor-specific. Moreover, efficacy of this construct was demonstrated by significant reduction in tumor growth and prolongation of life in both tumor models.

CONCLUSIONS: This Trojan Horse combined stem cell/gene therapy can be a novel treatment strategy for effective patient-tailored therapy of solid tumors.

High expression levels of putative hepatic stem/progenitor cells biomarkers related to tumor angiogenesis and poor prognosis of hepatocellular carcinoma

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INTRODUCTION: It has been reported that cases of hepatic stem cell-like hepatocellular carcinoma (HCC) subtypes had poor prognosis. More studies are still needed to investigate the prognostic values and the precise mechanisms of putative hepatic stem/progenitor cells (HSCs/HPCs) in HCC patients.

METHODS: Fourteen biomarkers related with HSCs/HPCs or tumor angiogenesis were assessed by qRT-PCR and then validated by tissue microarrays (TMAs) in 3 independent cohorts of HCC patients who underwent curative resection (n = 67, 314, and 73).

RESULTS: Most of the biomarkers were found overexpressed in recurrent HCC patients by qRT-PCR. HSCs/HPCs biomarkers cytokeratin 19, ABCG2, CD133, Nestin, CD44 and angiogenesis agents CD34, VEGF, and PD-ECGF were confirmed as significant predictors for overall survival (OS) and/or relapse-free survival (RFS) in TMAs analysis. Compared with the low HSCs/HPCs profile group, patients with high HSCs/HPCs profile had significantly lower OS and RFS ($p < 0.0001$) and expressed higher VEGF levels ($p = 0.012$) and microvessel density (MVD; determined by CD34 immunostaining; $p = 0.030$). Based on Cox regression, a simplified model including CD133, CD44, Nestin, and MVD was constructed and confirmed as an independent predictor for OS ($p < 0.0001$) and RFS ($p < 0.0001$), regardless of alpha-fetoprotein level, tumor stage, and recurrence time ($p < 0.0001$ for all).

CONCLUSIONS: High expression levels of HSCs/HPCs biomarkers are related to tumor angiogenesis and poor prognosis of HCC. The simplified model based on HSCs/HPCs and tumor angiogenesis profile can be used to classify HCC patients with high risk of tumor recurrence after operation.

Potential of adipose-derived stem cells harvested from diabetic mice on wound healing

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INTRODUCTION: Adipose-derived stem cells (ASCs) have potential applications for repair and regeneration of damaged tissues. We hypothesized that ASCs harvested from diabetic mice (db-ASC) may accelerate wound healing and participate in differentiation and neovascularization.

METHODS: ASCs were harvested from inguinal fat pads of 2 male, 25-week-old diabetic mice (C57BL/KsJ-Lepr^{db}). Following 3 pas-

sages, cells were analyzed by FACS and CD31⁻/CD45⁻/CD29⁺/CD90⁺ cells were used for transplantation. Following a 1 × 1-cm full-thickness skin wound, 5 × 10⁵ cells diluted in 0.5 mL of saline were injected into overlying muscle in each wild-type (C57BL/6) (n = 8) and diabetic (n = 7) mouse wound. DiI staining was used for cell tracking. Saline-injected groups served as controls (n = 6). 7 and 14 days following transplantation, digital photographs of wounds were taken, and tissues were harvested for histological and gene expression evaluation.

RESULTS: DiI-positive db-ASCs were distributed throughout both the diabetic and wild-type wounds, and DiI-PECAM double-positive cells were detected in the wound. Real-time RT-PCR revealed VEGF, VEGFr, PDGF, and PDGFr levels were significantly higher in wild-type wounds treated with db-ASCs. Diabetic wounds treated with db-ASCs had decreased VEGF but increased TGF, VEGFr, and PDGFr levels. After 7 days, digital photographs showed db-ASC-treated diabetic and wild-type wounds had significantly higher wound area closure compared with their control groups.

CONCLUSIONS: db-ASCs may accelerate wound healing and actively participate in neovascularization, although further investigation is needed on safety and efficacy of db-ASCs.

A novel single cell gene expression analysis identifies critical gene transcription deficits in diabetic murine mesenchymal stem cells

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INTRODUCTION: While mesenchymal stem cells (MSCs) have shown promise for regenerative medicine, diabetic MSCs exhibit impaired stem cell function in response to tissue injury. Traditional transcriptional analysis cannot define differences among subpopulations. We applied a novel microfluidics-based single cell gene expression analysis across 48 gene targets to characterize the differences between diabetic and wild-type MSCs. We identified a unique MSC subpopulation with a highly pluripotent transcriptional profile that is more rare in diabetes, which may explain the observed dysfunctions in diabetic MSCs.

METHODS: Total bone marrow was harvested from wild-type and diabetic (streptozotocin-induced and db/db) C57BL/6 mice. Cells were expanded in culture, and FACS was used to sort individual MSCs (defined as Sca-1⁺/CD45⁻/Lin⁻). Single cell mRNA was converted into cDNA by low-cycle preamplification and loaded onto microfluidics chips for quantitative-PCR analysis.

RESULTS: Our analysis identified a subpopulation that expressed Sca-1, a murine stem cell surface marker, as well as Klf4, ID2, and REST, which function to maintain pluripotency. Taken together, the expression of these genes strongly suggests that these unique cells represent a highly pluripotent subpopulation of murine MSCs. This subpopulation was 10% of normal MSCs, 1% of type I diabetic MSCs, and not found in type II diabetic MSCs ($p < 0.01$).