Conclusions: This study suggests that mean plaque size stabilizes at 4 weeks after carotid injury with no further increase at later time points. Furthermore, there appears to be a time-dependent decrease in activated macrophages in the neointima. Potential mechanisms for this observation are currently being investigated.

1200-143

Altered AP-1/Ref-1 Redox Pathway in iNOS Deficient Vascular Smooth Muscle Cells: A Novel Involvement of iNOS in Cellular Signaling

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Background: We previously showed injury-induced medial proliferation and neointimal formation in carotid arteries of norticren nuclear oxode synthase knockout (norkKO) mice were significantly less compared to wild type (WT). iNOS is a source of reactive oxygen species, which could modulate cellular growth and redox signaling molecules such as the AP-1/Ref-1 redox-sensitive pathway.

Hypothesis: SMC deficient in iNOS have altered redox sensitive AP-1/Ref-1 signaling pathway and reduced proliferative response to serum stimulation.

Methods: Confluent aortic SMC isolated from iNOS KO and WT mice were stimulated to proliferate with 20% serum media after 48 hours of quiescence. Cell cycling by FACS analysis and nuclear PCNA and thio-redoxin by Western blot was characterized 24 hours after stimulation. The following assays were performed 30 minutes after stimulation: AP-1 UNA binding activity by gel-shift assay; c-Jun mRNA by semi-quantitative H1-PCR and c-Jun and Ref-1 expression by Western blotting.

Results: Cell cycle analysis showed significantly more iNOS KO cells remained in the G(0)/G1 phase and less in G phase after 24 hours of serum stimulation. c-jun and Ref-1 expression and AP-1 activity were also less in iNOS KO SMC (Table). Thio-redoxin expression was also less in iNOS KO cells.

Conclusion: Our data demonstrated reduced proliferative response and altered AP-1/Ref-1 signaling pathway in iNOS KO SMC, implying a novel signaling mechanism for iNOS involvement in modulating SMC proliferation.

1201

Gene Transfer to Vascular Tissues and Cell Therapies

Poster Session
Tuesday, April 01, 2003, 3:00 p.m.-5:00 p.m.
McNamara Place, Hall A
Presentation Hour: 4:00 p.m.-5:00 p.m.

1201-115

A Combination of Transcriptional Regulatory Elements Increases Transgene Expression 40-Fold In Porcine Coronary Arteries

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SMC are relatively refractory to adenovirus-mediated gene transfer, due in part to poor transgene expression, even from powerful viral promoters. The murine cytomegalovirus promoter (MIEhCMV) significantly improves transgene expression in SMC compared to the widely used human CMV promoter (MIEhCMV). Inclusion of the Woodchuck Hepatitis Virus Post Transcriptional Regulatory Element (WPRE) and a fragment of the rabbit thioredoxin pathway and reduced proliferative response to serum stimulation.

Background: Enhanced extracellular matrix (ECM) accumulation is an important finding in porcine as well as in human coronary stent restenotic tissue, and TGFβ, implicated in ECM formation, is expressed abundantly in these tissues. We assessed the hypothesis that blockade of TGFβ by local delivery of an adenovirus expressing a soluble form of TGFβ type II receptor (AdTβExR) inhibits stent-induced neointima in porcine coronary arteries.

Methods: Two remote coronary arterial segments (n=20) per each pig were randomized to receive 1x10(10) pfu of either AdTβExR or adenovirus expressing β-galactosidase (AdlacZ) using an infiltrator. A stent (n=20) was deployed after gene transfer in each segment in 10 pigs. Localized expression of transgene was confirmed by both reverse transcription-PCR and immunohistochemistry. Computer-based morphometric assessment was performed in stented arteries at 4 weeks after gene transfer.

Results: There were significantly less ratio of intima area (IA)/media area (MA) and higher neointima cell density in stented arteries treated with AdTβExR compared with those with AdlacZ. Neither cell replication rate assessed by PCNA immunohistocchemistry in the intimal pathway was significantly different between two groups.

Conclusion: Blockade of TGFβ by local in vivo gene transfer of a soluble TGFβ receptor inhibits stent-induced neointima by inhibiting ECM accumulation in porcine coronary arteries, and may provide a therapeutic potential to inhibit restenosis after stenting.

1201-116

Adenovirus Mediated Prostacyclin Synthase Gene Transfer Inhibits Neointimal Formation By Modulating Peroxisome Proliferator-Activated Receptors Expression

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Peroxisome-proliferator-activated receptors (PPARs) are nuclear hormone receptors which regulate cell growth and differentiation by modulating gene transcription. Many data demonstrate that PPARR are expressed in human atheromatous lesions and their ligands like triamcinolone and trogossolide reduce neointima formation after angioplasty. However, little is known about the role of PPARs in regulatory mechanism of arterial remodeling. Prostacyclin (PGJ(2)) is a potent ligand of PPARα and we have shown that PGJ(2) synthesis (PGF) gene transfer for neointima reduction via induction of cyclooxygenase-2 and vascular endothelial growth factor (VEGF) and prevents neointimal formation in balloon-injured arteries. To further evaluate the role of PPARs in the initiation of neointimal formation, we hypothesized that PPARs activation via PPARα agonists could inhibit VEGF expression in balloon-injured arteries. Therefore, we assessed the expression of PPARα, -δ, and -γ in balloon-injured arteries and evaluated arterial morphology after adenovirus-mediated PGF gene transfer (AdPGF). Immunohistochemical analysis revealed that PPARα was positive in endothelium in balloon-injured groups, and PPARγ was positive in macrophages in balloon-injured groups. In conclusion, PPARs expression in the intima could contribute to neointima formation by reducing VEGF expression and may provide a therapeutic potential to inhibit restenosis after stenting.

1201-120

Inhibition of Angiogenesis and Wound Healing by Adenovirus-Mediated Gene Transfer of a Soluble Form of Vascular Endothelial Growth Factor Receptor in Mice

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Background: Vascular endothelial growth factor (VEGF) is an important angiogenic growth factor. Since angiogenesis plays a major role in wound repair, we hypothesized that overexpression of adenovirus mediated gene transfer of a soluble form of VEGF receptor D (Flk-1) might attenuate wound healing in mice.
Hypoxia Upregulates Expression of Periostin, a Novel Extracellular Matrix Protein, in Rat Lung via PI3 Kinase-MAP Kinase Signaling Pathway

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Periostin, a novel extracellular matrix protein, is expressed in osteoblasts or osteoblast-like cell lines. This study tested the hypothesis that periostin is increased in rat lung and in isolated pulmonary arterial smooth muscle cells (PASMCs) in response to the stress of hypoxia and explored the signaling pathway involved. Sprague Dawley rats were exposed to hypoxia (10% O2) or normoxia (21% O2) for 2 weeks. Growth-arrested PASMCs were incubated under hypoxia (1% O2) for 24 hrs. Hypoxia increased periostin expression 2-fold in lung and 1.8-fold in PASMCs, by Northern analysis. The expression of periostin in PASMCs was stimulated by the treatment (24 hrs) with hypoxia-responsive growth factors, FGF-1, PDGF-BB, endothelin-1 (ET-1), angiotensin II (A II), and the increases were blocked by the receptor tyrrosine kinase inhibitors (PD166866 for FGF-1, AG1295 for PDGF-BB) or receptor antagonists (bosentan for ET-1, isonartan for A II), or by PI3 kinase inhibitors (LY294002 or Wortmannin) or MAP kinase inhibitor (U0126) or DNA transcription inhibitor (actino mycin D), but not by PKC inhibitor (C80) or PKA inhibitor (HI-89) or adenylylate cyclase inhibitor (SQ22536). These data provided the first evidence that hypoxia, alone or through hypoxia-responsive growth factors, upregulates periostin expression in rat lung by a transcriptional mechanism, via PI3 kinase-MAP kinase signaling pathway. Current findings suggest vascular periostin may play a role in hypoxia induced pulmonary vascular remodeling.

Conclusion: Heterotopic transplantation of endothelial cell within a fibrin matrix enhances neovascularization, increases myocardial blood flow, and improves left ventricular function.

Methods: In a blinded experiment, genetically diabetic and wildtype control mice (each n=20) were transplanted with recombinant adenoviruses encoding the ligand-binding ectodomain of VEGF (scFv 200 nM) or cDNA encoding the mature IgG scFv fragment (control). Five days after gene transfer two full thickness skin wounds (0.8 cm) were created on the dorsum of each mice. Wound closure was measured over 9-14 days after which wounds were resected for histological analysis. Prior to sacrifice fluorescent microscopy was performed to verify transfection. Results: In diabetic mice wound healing was markedly impared compared to control mice (p<0.001). Although differences were small, single i.v. injections of viruses encoding soluble scFv were significantly attenuated wound closure compared to Fc treated animals. In both diabetic and non-diabetic mice (p<0.05). Fluorescence microscopy revealed a 2.9 fold (diabetic) and 1.6 fold (non-diabetic) reduction in wound vascularity in Fc1 treated animals (p<0.05). Impairment of angiogenesis was confirmed by CD31 immunohistochemistry. Conclusion: Adenoviral gene transfer with the soluble VEGF receptor Fc1 inhibits wound angiogenesis and delays wound closure in a murine excisional wound model.

Methods: The objective of the study was to investigate the feasibility and efficacy of autologous endothelial cell transplantation in a fibrin matrix in the ischemic myocardium of sheep. Objective: Adequately vascularized muscle, which has been shown to improve myocardial function, was evaluated.

Results: Eight weeks after injection, ventricular function was markedly improved in the EC transplant and fibrin groups, but had deteriorated in the saline and control groups. Myocardial blood flow was also increased in the EC-group. Histology and electron microscopy revealed extensive neovascularization after endothelial cell transplantation and improved myocardial appearance.

Angiogenic Potential of Subcutaneous Adipose Stromal Cells for Autologous Cell Therapy

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Background: The delivery of autologous progenitor cells to increase angiogenesis is emerging as a novel treatment option for patients with coronary artery disease. Autologous delivery of such cells is limited by the fact that the required cell numbers often exceed the number of available progenitor cells. It has recently been shown that the subcutaneous adipose tissue contains large numbers of pluripotent stem cells in the non-adipocyte stromal fraction and may thus be a source for autologous delivery. We therefore examined the anecological potential of adipose stromal cells.

Methods: Subcutaneous adipose tissue biopsies were obtained from obese volunteers. The stromal fraction was separated from the adipocyte fraction by centrifugation and cultivation. Conditioned media was assayed after 72 hours for Vascular Endothelial Growth Factor (VEGF), Hepatocyte Growth Factor (HGF) and Granulocyte Colony Stimulating Factor (G-CSF) by ELISA; data are reported as mean pg per 10^6 cells ± standard error of mean. The ability of adipose stromal cells to form tubes in vitro was evaluated using a Matrigel® assay. Transdifferentiability of the adipose stromal cells was assessed using Green Fluorescent Protein (GFP) plasmid to determine whether the cells could be used as autologous cell vectors.

Results: Subcutaneous adipose stromal cells secreted 10.93 ± 2.74 pg / 10^6 cells of VEGF, 6269 ± 345 pg / 10^6 cells of HGF and 376 ± 43 pg / 10^6 cells of G-CSF over a period of 72 hours. These cells manifested greater than 50% transfection with GFP vector following electroporation. Adipose stromal cells were able to form cord-like structures in the Matrigel® assay similar to those formed by endothelial cells.

Conclusions: Our experiments delineate the angiogenic potential of the easily accessible subcutaneous adipose stromal cells by demonstrating the secretion of multiple synergistic angiogenic factors, in vitro and in vivo and successful transduction with a plasmid. These findings suggest that autologous delivery of native adipose tissue could be a novel treatment option for angiogenesis in vivo.