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 inducible nitric oxide synthase knockout (iNOS KO) 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/thioredoxin 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 thioredoxin by Western blot was characterized 24 hours after stimulation. The following assays were performed 30 minutes after stimulation: AP-1 DNA binding activity by gel-shift assay; cJun mRNA by semi-quantitative RT-PCR and cJun and Ref-1 expression by Western blotting.
Results: Cell cycle analysis showed significantly more iNOS KO cells remained in the G0/G1 phase and less in S phase after 24 hours of serum stimulation. cJun and Ref-1 expression and AP-1 activity were also less in iNOS KO SMC (Table). Thioredoxin 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.

Cell Type	S-phase	PCNA	AP-1 Gel-shift activity	c-jun mRNA	Nuclear c-Jun	Nuclear Ref-1 (x 10^3)
WT	26.9±18.8 %	8.8±2.7	2.0±0.7	1.79±0.11	6.1±3.8	6±2.5
iNOS KO	8.5±3.7%*	2.0±2.0*	1.0±0.2*	1.08±0.26*	0.7±0.5*	0.09±0.06*

PCNA, AP-1, nuclear c-Jun and c-jun mRNA are expressed as fold change relative to unstimulated cells; Ref-1 is expressed as relative densitometric units; *p<0.05. Each experiment was repeated at least 3 times

POSTER SESSION

1201 Gene Transfer to Vascular Tissues and Cell Therapies

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

1201-117 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 (MIEmCMV) 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 smooth muscle myosin heavy chain promoter (RE) further increases expression of *lacZ* ≈5 fold *in vitro*. We hypothesised that this combination would significantly increase transgene expression *in vivo*. **Methods:** Porcine coronary arteries randomly received 2x10^9iu of the following vectors in 300µl of vehicle (n=4/group) via an Infiltrator™:
1. Ad5-*lacZ*: MIEhCMV
2. RAd36: MIEmCMV
3. Ad5-PP-*lacZ*: MIEmCMV, WPRE
4. Ad5-PREP-*lacZ*: RE, MIEmCMV, WPRE.

After 72 hours β-galactosidase expression and activity was quantified. **Results:** Ad5-PREP-*lacZ* induced 40 times greater β-gal activity than Ad5-*lacZ* (p<0.05) and greater activity than the other MIEmCMV based vectors (both p<0.01). X-gal staining was 7-35 fold greater in Ad5-PREP-*lacZ* infected vessels (all p<0.001). **Conclusion:** Inclusion of WPRE and RE within the cassette of a recombinant adenovirus vector regulated by MIEmCMV substantially improves transgene expression in porcine coronary arteries. By maximizing expression or allowing use of lower virus doses this has important implications for successful gene therapy in the vasculature.

	Ad5-*lacZ*	RAd36	Ad5-PP-*lacZ*	Ad5-PREP-*lacZ*	p (ANOVA)
X-gal staining (µm^2)	1519 ±503	462 ±181	1915 ±507	16381 ±4483	<0.0001
β-gal activity (IU/µg protein/min)	0.05 ±0.02	0.11 ±0.06	0.07 ±0.02	2.05 ±1.46	<0.006

1201-118 Blockade of TGF-Beta by Catheter-Based Gene Transfer of a Soluble TGF-Beta Type II Receptor Inhibits Neointima in a Porcine Coronary Artery After Stenting

Ick-Mo Chung Hikaru Ueno, Donghoon Choi, Yangsoo Jang, Gil-Ja Shin, Youngmi K. Pak, Junwoo Kim, Chan Park, Ewha Women's University, Seoul, South Korea, University of Occupational and Environmental Health, Kitakyusyu, Japan

Background: Enhanced extracellular matrix (ECM) accumulation is an important finding in porcine as well as in human coronary stent restenotic tissue, and TGF-β, implicated in ECM formation, is expressed abundantly in these tissue. We assessed the hypothesis that blockade of TGF-β by local delivery of an adenovirus expressing a soluble form of TGF-β type II receptor (AdTβ-ExR) inhibits stent-induced neointima in porcine coronary arteries.
Methods: Two remote coronary arterial segments (n=20) per each pig were randomized to receive 1x10^9 pfu of either AdTβ-ExR 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 AdTβ-ExR comparing with those with AdLacZ. Neither cell replication rate assessed by PCNA immunohistochemistry nor injury score was significantly different between two groups.
Conclusion: Blockade of TGF-β by local *in vivo* gene transfer of a soluble TGF-β receptor inhibits stent-induced neointima by inhibiting ECM accumulation in porcine coronary arteries, and may provide a therapeutic potential to inhibit restenosis after stenting.

Morphometric assessment of stented arteries after gene transfer

	IA/MA	MA	stent area	neointima cell density
AdTβ-ExR	0.84±0.44*	2.2±0.8 mm^2	5.4±0.6 mm^2	3663±597/mm^2*
AdLacZ	1.21±0.45	1.9±0.6 mm^2	5.6±0.7 mm^2	2800±190/mm^2

*p<0.05 vs AdLacZ

1201-119 Adenovirus Mediated Prostacyclin Synthase Gene Transfer Inhibits Neointimal Formation by Modulating Peroxisome Proliferator-Activated Receptors Expression

Hajime Imai, Yasushi Numaguchi, Yasuhiro Nishimoto, Hideo Matsui, Toyoaki Murohara, Kenji Okumura, Nagoya University, Nagoya, Japan, Children's Hospital, Boston, MA

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors which regulate cell growth and differentiation by modulating gene transcription. Many data demonstrate that PPARα and γ are expressed in human atherosclerotic lesion and their ligands like fibrates and troglidazone reduce neointimal formation after angioplasty. However, little is known about the role of PPARα in regulatory mechanism of arterial remodeling. Prostacyclin (PGI_2) is a potent ligand of PPARα and we have shown that PGI_2 synthase (PCS) gene transfer accelerates reendothelialization through induction of cyclooxygenase-2 and vascular endothelial growth factor (VEGF) and prevents neointimal formation in balloon-injured arteries. To further explore pathophysiological relationship between PGI_2 overproduction and PPARα expression in balloon-injured arteries, we carried out chronospatial analysis of PPARs (α, γ and δ) and evaluated arterial morphometry after adenovirus-mediated PCS gene transfer(AdPCS). Imunohistochemical analysis revealed that 1)PPARα was positive in endothelium in balloon-injured groups, 2)all PPAR subtypes were expressed in neointimal area at day 7 and 14, while little was found in uninjured vessels, 3)In AdPCS group, PPARδ and γ distribution was limited to the neointima adjacent to endothelium, while, in control, diffuse expression was seen in whole neointima. The concentration of tissue 6-keto-Prostaglandin$F_{1\alpha}$, a metabolite of PGI_2, was significantly increased in AdPCS group (AdPCS vs control; 5.35+/-0.95 vs 2.29+/-0.54 ng/mg tissue, p=0.02). Morphometric analysis at day 14 revealed that AdPCS reduced intima/media ratio up to 40% (AdPCS vs control; 0.83+/-0.05 vs 1.32+/-0.13, p=0.01). In conclusion, PCS gene transfer could inhibit neointimal formation by suppressing smooth muscle cell proliferation and migration partly via PPARδ pathway as well as acceleration of reendothelialization via cyclooxygenase-2-VEGF pathway.

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

Johannes Jacobi, Betty Y. Tam, Uma Sundram, Calvin J. Kuo, John P. Cooke, Stanford University, Stanford, CA

Background: Vascular endothelial growth factor (VEGF) is an important angiogenic growth factor. Since angiogenesis plays a major role in wound repair, we hypothesized that adenovirus-mediated gene transfer of a soluble form of VEGF receptor 2 (Flk-1) might attenuate wound healing in mice.

Methods: In a blinded experiment, genetically diabetic and wildtype control mice (each n=20) were transfected with recombinant adenoviruses encoding the ligand-binding ectodomain of VEGF type 2 receptor Flk-1 (n=10) or cDNA encoding the murine IgG2α Fc fragment (control). Five days after gene transfer two full thickness skin wounds (0.8cm) 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 microspheres were systemically injected for quantitation of wound vascularity. **Results:** In diabetic mice wound healing was markedly impaired compared to control mice ($p<0.001$). Although differences were small, single i.v. injections of viruses encoding soluble Flk-1 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 2.0 fold (non-diabetic) reduction in wound vascularity in Flk-1 treated animals ($p<0.05$). Impairment of angiogenesis was confirmed by CD31 immunohistochemistry. **Conclusion:** Adenoviral gene transfer with the soluble VEGF receptor Flk-1 inhibits wound angiogenesis and delays wound closure in a murine excisional wound model.

1201-121 Hypoxia Upregulates Expression of Periostin, a Novel Extracellular Matrix Protein, in Rat Lung via PI3 Kinase-MAP Kinase Signaling Pathway

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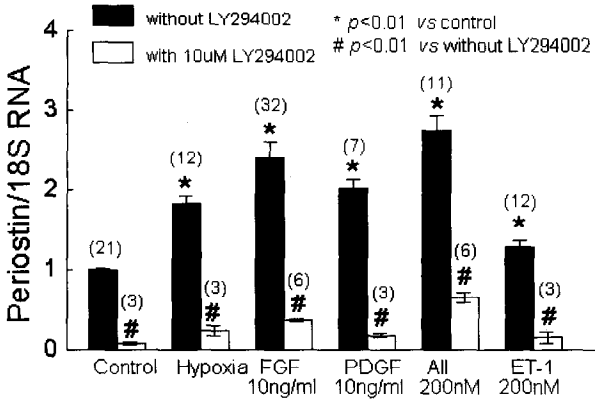
Peroistin, a novel extracellular matrix protein, is expressed in osteoblasts or osteoblast-like cell lines. This study tested the hypothesis that periostin is increased in rat lung and in isolated pulmonary arterial smooth muscle cells (PASMCs) in response to the stress of hypoxia and explored the signaling pathway involved. Sprague Dawley rats were exposed to hypoxia (10% O_2) or normoxia (21% O_2) for 2 wks; growth-arrested rat PASMCs were incubated under hypoxia (1% O_2) for 24hrs. 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 (24hrs) with hypoxia-responsive growth factors, FGF-1, PDGF-BB, endothelin-1(ET-1), angiotensin II(AII), and the increases were blocked by the receptor tyrosine kinase inhibitors (PD166866 for FGF-1, AG1295 for PDGF-BB) or receptor antagonists (bosentan for ET-1, losartan for AII), or by PI3 kinase inhibitors (LY294002 or Wortmannin) or MAP kinase inhibitor (U0126) or DNA transcription inhibitor (actinomycin D), but not by PKC inhibitor (Calphostin C) or PKA inhibitor (H-89) or adenylate 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.



1201-122 Transplantation of Autologous Endothelial Cells Induces Angiogenesis in a Sheep Model of Chronic Ischemia

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Objective: The objective of the study was to investigate the feasibility and efficacy of autologous endothelial cell transplantation using a fibrin matrix in the ischemic myocardium of sheep.

Methods: Four weeks after placing an ameroid constrictor in the second branch of the circumflex artery of 24 adult sheep, six animals (EC-group) were subjected to endothelial cell transplantation. In 6 (saline group) saline with added inactivated cells were injected, in other 6 (fibrin group) fibrin sealant was injected, and 6 animals served as control. Eight weeks after treatment the animals were sacrificed to assess myocardial blood flow, left ventricular function, and neovascularization were 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.

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

1201-152 Angiogenic Potential of Subcutaneous Adipose Stromal Cells for Autologous Cell Therapy

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Background: The delivery of autologous pluripotent 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 pluripotent 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 angiogenic 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 cultured. 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. Transfectability 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 10297 ± 4724 pg / 10^6 cells of VEGF, 8698 ± 3459 pg / 10^6 cells of HGF and 976 ± 439 pg / 10^6 cells of G-CSF over a period of 72 hours. These cells manifest greater than 50% transfection with pGFP 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 synergistically acting pro-angiogenic growth factors, in vitro tube or cord formation and successful transfection with a plasmid. These findings suggest that autologous delivery of either native or transduced subcutaneous adipose stromal cells may be a novel therapeutic option to enhance angiogenesis.

1201-153 Tumor Necrosis Factor Signaling Not Important for Arteriogenesis but for Angiogenesis

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Background: The restoration of flow after femoral artery (FA) occlusion in mice is predominantly due to the growth of proximal collateral arteries (arteriogenesis). Tumor Necrosis Factor (TNF) α is a cytokine that has been found to be expressed by macrophages around growing collateral arteries and capillaries. There are conflicting reports about the role of TNF in angiogenesis *in vivo* and one study suggested that TNF signaling is important for arteriogenesis in mice.

Methods: We performed right FA ligations in rats, C57BL/6 mice (WT)(n=10), TNF Receptor 1 (TNFR1)-/- (n=10), TNFR2-/- (n=10), and TNFα -/- mice (n=8)(backcrossed to C57BL/6 background). Furthermore, we studied WT mice (n=6) and littermates with endothelial-specific overexpression of a membrane-spanning precursor of TNF (tmTNF)(n=10). In rats immunofluorescent staining on growing collaterals with an antibody specific for tmTNF was performed. Mice were serially examined up to 21 days after surgery, perfusion was measured by laser Doppler imaging, hemoglobin oxygenation by transcutaneous spectrometry, and calf blood flow by MRI (MRflow). As angiogenesis assay, subcutaneous matrigel plugs (containing FGF-2 and VEGF) were implanted in mice endothelial tmTNF overexpressing mice(n=6) and controls (n=7); capillaries staining positive for CD31 were counted in the matrigel plugs 14 days after implantation (blinded).

Results: Already 12 hours after arterial occlusion tmTNF was expressed by endothelial cells in growing rat collateral arteries, but not on the non-ligated side. However, there were no significant differences in relative (right-to-left ratio) hindlimb perfusion, oxygenation, or calf blood flow between the transgenic/knock-out mice and their controls at rest. Capillary counts per HPF in matrigel plugs of tmTNF overexpressing mice were higher than in controls, 1.183±0.412 vs. 0.092±0.025 (mean±SEM, $p<0.01$).

Conclusion: TNF is expressed in the endothelium of growing collaterals and by surrounding macrophages. However our data do not suggest any significant involvement of TNF on arteriogenesis in a mouse hindlimb ischemia model. On the other hand, endothelial overexpression of TNF promotes angiogenesis *in vivo*.