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Fishbein, I., Alferiev, I. S., Wong, G., Vohs, J. M., Connolly, J. M., Wilensky, R. L., & Levy, R. J. (2003). An Ultra-Thin Polymer Coating for the Tethering of Adenoviral Vector to the Surface of Coronary Stents. *Molecular Therapy, 7* (5, Supplement), S329-S330. http://dx.doi.org/10.1016/S1525-0016(16)41295-5

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An Ultra-Thin Polymer Coating for the Tethering of Adenoviral Vector to the Surface of Coronary Stents

Abstract

Our group has previously demonstrated stent-based gene delivery with either viral or plasmid vectors. However, these previous studies utilized bulky PLGA or collagen stent coatings, known to cause inflammatory reactions in stented arteries. In the present experiments we successfully attached adenoviruses either directly, or via anti-adenovirus antibodies to the steel surface of stents using chemical coordination with biphosphonates.

Disciplines

Biochemical and Biomolecular Engineering | Chemical Engineering | Engineering | Materials Science and Engineering | Polymer Science

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851. Suppression of Chemical Burn-Induced Neovascularization by Subconjunctival Injection of Adeno-Associated Virus Vector Expressing Angiostatin

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Ocular neovascularization (NV) is one of the leading courses of blindness. Many ocular surface diseases such as dry eye syndrome, ocular cicatritial phemphigoid (OCP), limbal insufficiency and sequel of chemical burn frequently lead to cornea NV. The underling mechanisms of these diseases include chronic inflammation, autoimmune response and stem cell loss. This major ocular complication can led to corneal scarring, edema, lipid deposition, and inflammation that may not only significantly alter visual acuity, but also worsen the prognosis of the subsequent penetrating keratoplasty (PKP). Since ocular surface diseases usually have protracted courses, the control of their respective detrimental mechanisms usually depend on long term application of steroid or cytotoxic or immunosuppressive medications such as cyclophosphamide and cyclosporine A. Local and systemic side effects create dilemma for clinician. On the other hand, the recombinant AAV vector is currently considered as one of the most promising viral vectors for gene therapy. In this study, we investigate the efficacy of AAV vectors in a rat model of experimental corneal NV generated by silver nitrate ($AgNO_3$) cauterization. The vectors contained either the mouse angiostatin gene (AAV-angiostatin) or the control green fluorescent protein gene (AAV-GFP). Two weeks after subconjunctival injection the vectors, gene transfer efficiency and therapeutic efficacy were evaluated under slit-lamp biomicroscopy every other day. Vessel length was measured by fluorescent isothiocyanate (FITC)-coupled concanavalin A (ConA) lectin angiography. AAV-angiostatin successfully suppresses the chemical-burn induced corneal NV as shown by Angiography analysis. The areas free from blood vessel invasion in the cornea correlated with the sites of virus injection. The therapeutic effect lasted for the entire 12-week duration of our experiments, while AAV-GFP expression persisted more than 6 months. This is the first report that subconjunctival injection of AAV-angiostatin can significantly inhibit the formation of corneal NV lesion. Importantly, subconjunctival injection can be used as a unique reversible gene therapy method. Therefore, the AAV vector represents a promising gene delivery system for efficient and long-term transduction in ocular tissue. Our study demonstrated the feasibility of gene therapy approaches for the treatment of corneal NV diseases. It may also become clinically useful for a variety of ocular diseases involving NV, such as wound- and inflammation-related corneal angiogenesis with limbal insufficiency, which is still pharmacologically untreatable.

852. Development of a Retroviral Vector Containing the Erythroid Band 3 Promoter Flanked by the Chicken β-Globin 5'HS4 Insulator

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Expression of genes in retroviral vectors is subject to the epigenetic effects of surrounding heterochromatin after integration, resulting in transgene silencing or position effect variegation (PEV). It has been shown that an insulator element from the chicken β-globin locus (5'HS4) serves as a barrier against such position effects and thereby promotes long-term, uniform expression of a transgene but the mechanism by which an insulator suppresses PEV has not been elucidated. The erythroid anion exchanger 1 (AE1 or band 3) promoter is active only in erythroid cells. Using a transgenic mouse assay, we have analyzed human γ-globin gene expression using promoter elements from the erythroid form of the AE1 gene. Transgenic mice containing 2 different AE1 promoter sequences, -1738 to +22 (9 lines) or -357 to +22 (9 lines), fused to the γ-globin gene coding region 5 bases upstream of the initiation codon showed erythroid specific expression of the transgene with an average AE1/γ-globin mRNA level of 13.5±0.07% and 7.3±0.04% of total mouse α-globin mRNA per transgene copy, respectively. With both promoters, γ-globin expression was position independent and copy number dependent. However, γ-globin distribution in circulating erythrocytes was variegated in 14/18 lines. We hypothesized that flanking the 5' and 3' ends of the -1738 to +22 AE1/γ-globin gene construct with the chicken 5'HS4 would suppress PEV, resulting in more uniform expression of the transgene. In transgenic mice containing the insulated, long AE1/γ-globin construct, expression of the transgene was erythrocyte specific, position independent (6/6 lines express) and copy number dependent ($p = 0.0001$). Transgenic mice had an average AE1/γ-globin mRNA level of 19.8±0.03% of total mouse α-globin mRNA per transgene copy. AE1/γ-globin mRNA from the non-insulated construct initiated at cryptic sites upstream of the start site used by the endogenous gene. In contrast, the insulated AE1/γ-globin construct initiated transcription at the start site used by the endogenous gene. Distribution of γ-globin in all 6 lines was also uniform, with 100% of erythrocytes containing human γ-globin. We conclude that the erythroid AE1 promoter contains an insulator element upstream of position -1738. Furthermore, the chicken 5'HS4 insulator suppresses PEV by directing transcription initiation to the correct start site. We have constructed an oncoretroviral vector containing the -357 to +22 AE1/γ-globin gene with the chicken 5'HS4 insulator in the 3'LTR. Stable transduction of the AE1/γ-globin gene and transfer of 5'HS4 to the 5'LTR was demonstrated by Southern blot analysis of transduced K562 cell DNA. We hypothesize that an insulated AE1/γ-globin vector will express γ-globin at relatively high levels in primary erythroid cells.

CARDIOVASCULAR I

853. An Ultra-Thin Polymer Coating for the Tethering of Adenoviral Vector to the Surface of Coronary Stents

Ilia Fishbein,[1] Ivan S. Alferiev,[1] Gordon S. Wong,[2] John M. Vohs,[2] Jeanne M. Connolly,[1] Robert L. Wilensky,[3] Robert J. Levy.[1]
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Our group has previously demonstrated stent-based gene delivery with either viral or plasmid vectors. However, these previous studies utilized bulky PLGA or collagen stent coatings, known to cause inflammatory reactions in stented arteries. In the present experiments we successfully attached adenoviruses either directly, or via anti-adenovirus antibodies to the steel surface of stents using chemical coordination with bisphosphonates. Bisphosphonate derivatives of polyallylamine possessing primary amines (PAA-BP), or both primary amines and latent thiols (PrSSPAA-BP) were custom synthesized. The dynamics of PrSSPAA-BP adsorption to the

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surface of 316L stainless steel was studied using a compound comprised of a fluorescent marker, dansyl, attached to the polymer backbone through a cleavable bond. Fluorometric studies showed fast (less than 1 hour) saturation of the steel surface with PrSSPAA-BP. Estimation of the bound polymer amount (10^{-12} M/cm²) corresponds to monolayer deposition of the polymer. Additionally, XPS analysis confirmed the presence of the polymer and showed that the thickness of polymer coating does not exceed 5 nm. Reaction between primary amines in the polymer coating and the bifunctional cross-linker, SPDP, was employed to introduce thiol-reactive, pyridyldithio (PDT) groups on the stent surface. A panel of reduced anti-adenoviral antibodies (anti-hexon, anti-fiber and anti-knob) was used for the virus-tethering purposes. Monoclonal anti-knob antibody (AkAb) was found to be the most effective in terms of the amount of tethered AdV (10^8-10^9/cm²). The attachment of AdV to AkAb-primed surface was profoundly inhibited (87%) by a 1000-fold excess of knob protein. Alternatively, vector immobilization was achieved by SPDP modification of AdV with subsequent covalent tethering to deprotected thiols in the PrSSPAA-BP coating. Disulfide bridges emerging during the latter immobilization scheme may be cleaved by endogenous reducing compounds, regenerating viable AdV particles. However, the direct tethering procedure is far less effective than Ab-mediated immobilization both in terms of bound vector amount, and overall transduction success. Stainless steel meshes with tethered AdV-GFP placed in confluent cultures of A10 cells have driven 90% transduction of cells within 50 μm of the mesh borders, while the same amount of free virus (MOI=1) does not result in measurable cell transduction. Moreover, meshes recovered from the A10 culture and transferred to new cultures retained their transducing potential up to a fourth round of relocation. Stents bearing AkAb-tethered AdV-GFP were deployed in rat carotid arteries (n=8). The extent of transgene expression in the stented arteries was determined by immunohistochemistry 1, 3 and 7 days after deployment. GFP-positive cells were abundant in arterial media and adventitia at 7 days. In conclusion, we studied new strategies for AdV immobilization on the stent surface, which allow site-specific tethering of a clinically significant amount of the recombinant AdV. *In vitro* and *in vivo* studies demonstrated high transduction efficacy and superb localization of the reporter transgene expression.

854. A Novel Adenovirus Vector Producing Multiple Isoforms of VEGF Reverses Hind Limb Ischemia in the Mouse at Lower Doses Than Equivalent Vectors Expressing Only a Single VEGF Isoform

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Alternate splicing is a process that permits the expression of multiple protein isoforms with overlapping biological properties from a single gene. As an example of an alternately spliced gene we have used human vascular endothelial growth factor (VEGF) whose pre-mRNA is spliced to give seven isoforms of which three, VEGF121, VEGF165 and VEGF189, represent >99% of the VEGF expressed. Physiological angiogenesis occurs in the context of multiple isoforms determined by alternate splicing, resulting in VEGF proteins with overlapping properties. By contrast, as presently predicted therapeutic angiogenesis uses only one of these isoforms. The hypothesis of this work is that angiogenic gene therapy with the genomic form of VEGF expressing the three major isoforms, could be more potent than a vector expressing a single isoform. An adenovirus vector (AdVEGF-All) expressing a cDNA/genomic hybrid gene capable of expressing multiple human VEGF isoforms was constructed and shown to express VEGF121, VEGF165 and VEGF189 in a 2:2:1 ratio. The AdVEGF-All vector was given by intramuscular injection in a model of mouse hind limb ischemia, involving excision of a 0.6 cm portion of the iliac artery. Assessment of blood flow recovery was then monitored up to 21 days using a laser Doppler scanner. At 3 wk, tissues were harvested and stained for vonWillebrand factor. Initial post-operative scans showed blood flow in the ischemic limb to be 9 ± 1% of that in the non ischemic limb. At a dose of 10^8 particle units, flow recovered to 92 ± 9% in AdVEGF-All treated animals by day 21, compared with recovery to 95 ±8% in AdVEGF121 treated animals and 50 ± 4% in AdNull controls ($p>0.5$ comparing AdVEGF-All to AdVEGF121; $p < 0.05$ comparing AdVEGF-All and AdVEGF121 to AdNull). For groups treated with 10^8 particle units the ratio of vessel numbers assessed by histology were 1.42 ± 0.21 for VEGF-All, 1.39 ±0.22 for VEGF121, and 0.67 ±0.17 for AdNull. Strikingly, however, at a dose of 10^5 particle units, the recovery was 79 ± 8% in AdVEGF-All treated animals by day 21, compared to only 56 ± 3% in AdVEGF121 treated animals and 47 ± 3% in AdNull controls ($p<0.05$ comparing AdVEGF-All to AdVEGF121 and AdNull). Interestingly, a mixture of vectors coding individually for VEGF 121, 165, and 189 made up to a total dose of 10^5 particle units in a ratio of 2:2:1, mediated recovery to 87 ±7% ($p>0.5$ comparing AdVEGF-All to mixture). Histological analysis showed that the ratio of vessel numbers in the ischemic compared to the control limb in animals treated at a dose of 10^5 particle units was 0.61 ± 0.01 for AdNull, 0.63 ±0.21 for AdVEGF121, and 1.43 ± 0.39 for AdVEGF-All. We conclude that a mixture of VEGF isoforms provides a more potent angiogenic response than a single isoform, suggesting that the individual isoforms function synergistically, an observation with important implications for gene and recombinant protein therapy.

Dr. Crystal has equity in, is a consultant to, and receives sponsored research funds from, GenVec, Inc., Gaithersburg, Maryland, a publicly-traded biotechnology company.

855. A Genetically Engineered Plasmid Encoding a Zinc Finger VEGF-Activating Transcription Factor Induces Angiogenesis in the Rabbits with Hind-Limb Ischemia

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BACKGROUND: Peripheral arterial disease (PAD) afflicts hundreds of thousands of patients and is major cause of morbidity and mortality. Therapeutic angiogenesis seeks to treat PAD via the growth and proliferation of new blood vessels. Unfortunately this approach has met little success when single agents have been employed. The purpose of this study was to investigate the role of intra-muscular injection of plasmid-DNA encoding zinc finger-based vascular endothelial growth factor (VEGF)-activating transcription factors (ZFP-VEGF) that will enhance multiple VEGF isoforms in TA muscle following surgically induced hind-limb ischemia. **METHODS:** In total, 23 New Zealand White rabbits underwent femoral artery ligation and excision and were divided into two groups at 10 days post-operatively. One group received a single unilateral (ischemic limb TA muscle) intra-muscular injection of ZFP-VEGF (500mg) (n=13) and the other (control) group (n=10), included b-galactosidase injection (500mg) (n=7) and sham treatment (n=3). The ischemic and contra-lateral TA muscles of all animals were harvested 21 days post-operatively. In a subgroup of these rabbits, blood flow in ischemic and non-ischemic limbs (contra-lateral limb)

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