Sustained Expression of Angiogenic Factors Stimulates Vascularized Granulation Tissue Deposition in Diabetic Mice

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Abstract

Introduction
Insufficient vascularization is a limiting factor in the healing of chronic wounds. Strategies to promote the formation of vascularized granulation tissue are promising approaches to promote wound healing. Topical application of individual angiogenic factors is limited by rapid degradation or clearance from the wound environment. This study describes the development of human skin substitutes designed to express sustained, elevated levels of angiogenic factors to promote deposition of highly-vascularized granulation tissue.

Methods
NIKS keratinocytes were stably transfected with non-viral plasmid vectors encoding either a single angiogenic factor (VEGF) or a transcriptional regulator that induces multiple angiogenic factors (HIF-1α). Genetically-modified skin substitutes were evaluated for secretion of angiogenic factors and stimulation of endothelial cell proliferation, as well as the ability to promote deposition of vascularized granulation tissue after engraftment on nude or diabetic mice.

Results
Stably-transfected cells are genetically stable and non-tumorigenic. Skin substitutes modified to express VEGF secreted 100-fold more VEGF than unmodified tissue. Tissue modified to express HIF-1α secreted elevated levels of several pro-angiogenic chemokines. Conditioned medium from tissue expressing VEGF stimulated the proliferation of human microvascular endothelial cells compared to medium from unmodified skin substitutes. Tissue expressing either VEGF or HIF-1α promoted enhanced vascularization compared to unmodified skin substitutes after engrafting on nude mice. Skin substitutes expressing VEGF also enhanced the deposition of vascularized granulation tissue in diabetic mice. Cell banks were produced to support GMP production.

Conclusions
Skin substitutes expressing elevated levels of angiogenic factors enhance vascularization in animal models of impaired wound healing. Uniform, non-viral genetic modification of NIKS keratinocytes offers safety and consistency advantages compared to heterogeneous modification of primary keratinocytes with viral vectors. The ability of skin substitutes expressing elevated levels of angiogenic factors to promote vascularization suggests that these substitutes may accelerate the vascularization and healing of chronic wounds.

Figure 1 Stably-Transfected NIKS® Cells Over-express Angiogenic Factors

Figure 2 Identification of Transgene Integration Site and Copy Number

Figure 3 Comparison of Gene Expression Profiles of ExpressGraft® Tissue

Figure 4 Epidermal Differentiation of ExpressGraft® Tissue

Figure 5 Conditioned Medium From ExpressGraft® Tissue Stimulates Endothelial Cell Growth

Figure 6 Quantitative Analysis of Enhanced Vascularization of ExpressGraft® Tissue in vivo

Figure 7 ExpressGraft® Tissue Promotes Wound Vascularization in Diabetic Mice

Results

Figure 2 Identification of Transgene Integration Site and Copy Number

NIKS cells were transfected with non-viral expression vectors encoding a selectable variant of HIF-1α (CTAD) or VEGF, and selection for stable transfected cells was performed using Bsd. Western blotting confirmed the elevated levels of HIF-1α (CTAD) in the tissue, and immunohistochemical analysis of tissue sections revealed that the HIF-1α (CTAD) protein is localized to the epidermis of the NIKS® tissue.

Transgene integration site was determined by whole-genome sequencing and fluorescence in situ hybridization (FISH) using probes prepared from the vectors used to isolate the stably-transfected VEGF or HIF-1α clone. The VEGF clone contains a single detectable signal localized to the short arm of one copy of chromosome 16. The HIF-1α (CTAD) clone exhibits a single detectable signal localized to the short arm of one copy of chromosome 16.

Conditioned medium from stably-transfected NIKS® tissue or tissue modified by electroporation with plasmid vectors expressing VEGF165 or HIF-1α was tested for the ability to promote angiogenesis. Conditioned medium was collected from tissue expressing VEGF or HIF-1α and used to culture human microvascular endothelial cells (HuMVEC). Conditioned medium from tissue expressing VEGF stimulated the proliferation of HuMVEC compared to medium from unmodified skin substitutes. Conditioned medium from tissue expressing HIF-1α also stimulated the proliferation of HuMVEC.

Conditioned medium from stably-transfected NIKS® skin substitutes or tissue modified by electroporation with plasmid vectors expressing VEGF165 or HIF-1α was tested for the ability to promote angiogenesis. Conditioned medium was collected from tissue expressing VEGF or HIF-1α and used to culture human microvascular endothelial cells (HuMVEC). Conditioned medium from tissue expressing VEGF stimulated the proliferation of HuMVEC compared to medium from unmodified skin substitutes. Conditioned medium from tissue expressing HIF-1α also stimulated the proliferation of HuMVEC.

Conclusions

Stably-transfected clones of genetically-engineered NIKS® keratinocytes secrete multiple pro-angiogenic chemokines and are capable of promoting the growth of human microvascular endothelial cells compared to medium from unmodified skin substitutes. Medium from stably-transfected NIKS® tissue stimulates HuMVEC growth compared to medium from unmodified skin substitutes. Medium from stably-transfected NIKS® tissue stimulates HuMVEC growth more effectively than medium from unmodified skin substitutes.

Acknowledgements

This research was funded by grants R43GM065025, R44GM065025, R44GM062080 and R44GM066303 to ARC and R01DK073426 to LAM.