American Journal of Pathology, Vol. 157, No. 1, July 2000

Commentary

VEGF Expression by Epithelial and Stromal Cell Compartments

Resolving a Controversy

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Vascular endothelial growth factor (VEGF) expression in healing wounds provokes dermal angiogenesis through complex mechanisms involving promotion of endothelial cell proliferation[1,2] and survival,[3–5] specific induction of endothelial cell gene expression,[6–10] and increased microvascular permeability.[11,12] Microvascular hyperpermeability transforms the extracellular matrix by promoting extravasation of fibrinogen,[13] fibronectin,[14] and other proteins from blood plasma.[14]

VEGF expression is normally low in skin[15,16] relative to other more highly vascularized organs such as lung, kidney, and heart.[17] However, during the first few days after tissue injury, VEGF expression is markedly induced, plasma proteins extravasate, and angiogenesis is initiated.[18] Analyses of VEGF mRNA by *in situ* hybridization have implicated the epidermal keratinocyte as the principal source of VEGF during cutaneous wound repair within the first day after excisional wounding.[18] VEGF expression is likely induced by tissue hypoxia[19] which both activates VEGF transcription and promotes VEGF mRNA stability.[20–25] Several cytokines present in the wound bed also probably contribute to the induction of VEGF.[26] Keratinocyte VEGF mRNA is maximal on days 2 and 3; and by day 7, when the epidermis has covered the wound, keratinocyte VEGF mRNA although reduced in comparison with days 2 and 3, still persists above the normally low basal level.[18] Significantly, and in contrast to the epidermis of excisional wounds, VEGF mRNA remains low in the underlying dermis throughout the healing interval.[18] Collectively, these data indicate that the avascular epidermis regulates plasma protein extravasation and angiogenesis in the underlying dermis through a paracrine mechanism involving keratinocyte expression of VEGF.[2]

Consistent with the pattern of VEGF mRNA expression in healing wounds, the epidermal keratinocyte also has been implicated as a principal source of VEGF in other cutaneous lesions including psoriasis,[15] cell-mediated immune reactions,[16] bullous diseases associated with subepidermal blisters,[27] viral warts, and squamous cell carcinomas.[28] In all of these lesions, VEGF mRNA was observed by *in situ* hybridization to be markedly induced in keratinocytes. By contrast, the dermal compartment of these lesions expressed comparatively low levels of VEGF mRNA.

Transgenic mice expressing a reporter under the control of the VEGF promoter offer an alternative strategy for analyses of VEGF mRNA expression in a variety of settings, including healing wounds. To this end, Fukumura et al[29] developed a line of transgenic mice expressing green fluorescent protein (GFP) driven by a portion of the previously reported VEGF promoter sequence.[30] Surprisingly, they found strong GFP expression in dermal fibroblasts throughout the granulation tissue of superficial ulcerative wounds, in contrast to previous studies, which had implicated keratinocytes as the principal source of VEGF expression. There are several possible explanations for the apparent discrepancies with the earlier reports summarized above, including differences in wounding protocols. However, Fukumura et al[29] also found stromal fibroblasts to be the principal source of GFP expression in spontaneously arising mammary carcinomas. Furthermore, they found no GFP expression in mammary epithelial tumor nodules.[29] These findings stand in marked contrast to *in situ* hybridization analyses

Supported in part by National Institutes of Health grants CA77357 (to D. R. S.) and GM56442 (to L. V. D. W.) and by the Shriners Hospital for Children (to L. V. D. W.).

Accepted for publication May 19, 2000.

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performed by others who have documented, with a large human patient sample population, that VEGF mRNA expression predominates in breast carcinoma cells in comparison with stromal cells.[31,32]

In this issue, Kishimoto et al[33] report the derivation of another VEGF-GFP transgenic mouse model. This new model resolves discrepancies between the earlier VEGF-GFP transgenic model and *in situ* hybridization for VEGF mRNA in healing wounds. It also offers insights toward resolution of the controversy surrounding the relative abundance of VEGF mRNA expressed by breast carcinoma cells and associated stromal fibroblasts. As described in their report,[33] GFP expression under the direction of VEGF promoter sequence was strongly induced in the epidermis at the wound edge within 48 hours of wounding. With *in situ* hybridization, these authors also observed a pattern of VEGF mRNA expression identical to that of GFP. Furthermore, they observed low GFP expression in the underlying dermis in comparison with the epidermis. In summary, the observed pattern of GFP expression closely correlated with the previously described pattern of VEGF mRNA expression determined by *in situ* hybridization.[18] However, this pattern of expression contrasted with that observed by Fukumura et al[29] with their transgenic reporter model. A likely explanation for the different findings with the two transgenic mouse lines involves the different human VEGF promoter sequences chosen to drive GFP expression. Whereas Fukumura et al included in their construct 2850 bp immediately upstream (5′) of the translational start site (nucleotides 552-3401, GenBank no. M63971), Kishimoto et al included 2453 bp corresponding to 2362 bp immediately upstream of the transcriptional start site[30] together with an additional contiguous 91 bp containing the transcriptional start site and adjacent downstream (3′) sequence (corresponding to nucleotides 1–2453, GenBank no. M63971). Thus, the promoter sequence chosen by Kishimoto et al and used in the generation of VEGF-GFP transgenic mice described in this issue includes an additional 551 bp at the 5′ end of the VEGF promoter (nucleotides 1–551, GenBank no. M63971). These bases are absent in the promoter construct of Fukumura et al. A comparison of the data from the two transgenic models suggests the possibility that these 551 bp contain key elements required for VEGF expression in epithelial cells. It is also possible that these same 551 bp contain elements that suppress VEGF expression in fibroblasts. Direct comparisons between the different promoter-reporter constructs will be required for definitive testing of these possibilities. Importantly, such comparisons also will clarify the significance of the 551-bp 5′ sequence for regulation of VEGF expression in mammary carcinoma cells and associated stromal fibroblasts.

Thus, the new transgenic mouse VEGF-GFP reporter model described in this issue[33] demonstrates that a 2453-bp fragment containing all of the known promoter sequence of the human VEGF gene[30] directs GFP expression in healing wounds with a similar pattern to that observed previously with *in situ* hybridization for VEGF mRNA. Although the possibility remains that this new model may not contain all transcriptional regulatory elements of the VEGF gene, it properly reflects VEGF mRNA expression by the epidermis as it occurs in cutaneous wounds. Consequently, this model is well-suited for analyzing activation of the VEGF promoter within the epidermis *in vivo*. In addition, keratinocytes and possibly tumor cells derived from this transgenic model may assist in the identification of new drugs for therapeutic regulation of VEGF expression.

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