

# characterization of cis-regulatory elements of the vascular endothelial growth inhibitor gene promoter

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## Abstract

VEGI (vascular endothelial growth inhibitor), a member of the tumour necrosis factor superfamily, has been reported to inhibit endothelial cell proliferation, angiogenesis and tumour growth. We identified and cloned approx. 2.2 kb of the VEGI promoter from mouse cerebral endothelial cells. The promoter contained an atypical TATA-box-binding protein sequence TAAAAAA residing at -32/-26 relative to the transcription initiation site (+1), 83 bp upstream from the ATG start codon. To investigate critical sequences in the VEGI promoter, a series of deleted and truncated segments were constructed from a 2300 bp promoter construct (-2201/+96) linked to a luciferase reporter gene. Transient transfection of cerebral microvascular cells (bEND.3) and rat C6 glioma cells demonstrated that a 1700 bp deletion from the -2201 to -501 did not significantly affect promoter activity; however, a truncated construct (-501/+96) lacking the region between -312 and -57 resulted in nearly 90% loss of promoter activity. A consensus NF- $\kappa$ B (nuclear factor  $\kappa$ B) and several SP1 (specificity protein-1)-binding sequences were identified within the deleted segment. Supershift analysis revealed that NF- $\kappa$ B subunits, p50 and p65, interacted with the VEGI promoter. Exposure of cerebral endothelial cells to the proinflammatory cytokine, tumour necrosis factor- $\alpha$ , increased VEGI mRNA levels and DNA-binding activities, whereas an NF- $\kappa$ B inhibitor attenuated this increase. In addition, p65 overexpression enhanced, whereas p50 overexpression decreased, the luciferase activity. Furthermore, mutation of the NF- $\kappa$ B DNA binding site blocked this p65- and tumour necrosis factor- $\alpha$ -induced luciferase activity. These findings suggest that the transcription factor NF- $\kappa$ B plays an important role in the regulation of VEGI expression.