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ORIGINAL INVESTIGATION

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Localization of the human vascular endothelial growth factor gene, *VEGF*, at Chromosome 6p12

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Abstract Using overlapping cosmids representing the vascular endothelial growth factor (VEGF) locus, the VEGF gene was mapped by fluorescence in situ hybridization to chromosome 6p12. This localization permits linkage analysis and the identification of gene interaction in the region, as well as alterations of the VEGF structure or expression in cancer cells with chromosome abnormalities.

Introduction

A large body of recent evidence suggests that the vascular endothelial growth factor (VEGF) is the principal endothelial cell-specific mitogen and an important vascular permeability factor (Senger et al. 1993; Ferrara et al. 1992). Together with its receptors expressed on activated (by transdifferentiation processes) vascular endothelial cells, VEGF may be considered the prime regulator of normal and tumor angiogenesis (Folkman 1995a,b). In tumor angiogenesis the overexpression of VEGF in malignant cells is induced by a general mechanism possibly triggered by hypoxia (Shweiki et al. 1992). The tumor-derived VEGF then stimulates vascular stroma production and expression of corresponding receptors (Fong et al. 1995) on vascular endothelial cells resulting in growth of tumor blood vessels (Folkman 1995a,b; Fong et al. 1995). The principal involvement of VEGF-receptor system in tumor growth suggests new promising approaches to cancer treatment (Folkman 1995a,b; Fidler and Ellis 1994; Kim et al. 1993).

The VEGF gene is a relatively small (about 15 kb) single-copy gene containing eight exons and encoding multiple secreted protein isoforms as a result of alternative splicing (Ferrara et al. 1992; Tischer et al. 1991). This gene is widely expressed during development and overexpressed in adult tissues that require angiogenesis (Ferrara et al. 1992; Risau 1991). In several systems hypoxia upregulates VEGF expression to initiate angiogenesis (Stein et al. 1995).

Here we report the chromosome localization of the VEGF gene to 6p12 by fluorescence in situ hybridization (FISH).

Materials and methods

Molecular biology techniques

All molecular manipulations (screening cosmid library, Southern blotting analysis, PCR) were performed using standard methods (Sambrook et al. 1989).

Isolation and characterization of cosmids

Cosmids were isolated and purified by colony hybridization using VEGF cDNA (Berkman et al. 1993) as a probe. Placental cosmid library in the pWE15 vector was purchased from Stratagene (La Jolla, Calif.).

DNA sequence determination and analysis

Cosmids were partially sequenced on an Applied Biosystem 373 DNA sequencer (Stretch) using *Taq* Dyeoxy Terminator Cycle Sequence Kits and VEGF intron-specific primers (Tischer et al. 1991). DNA analysis was performed using the BLAST network service (Altschul et al. 1990).

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Fluorescent in situ hybridization

Two VEGF cosmid probes labeled with biotin or digoxigenin (Random Primed DNA Labeling Kit, Boehringer-Mannheim) were used for FISH of human chromosomes derived from methotrexate-synchronized normal peripheral lymphocyte cultures. The conditions of hybridization, the detection of hybridization signals, and digital-image acquisition, processing and analysis, as well as the procedure for direct visualization of fluorescence signals to banded chromosomes and rehybridization with whole chromosome painting probes, were performed as previously described (Zimonjic et al. 1994, 1995; Popescu et al. 1994).

Results and discussion

Cosmids containing the VEGF gene were isolated from a placental genomic library by colony hybridization using the VEGF cDNA as a probe. Two cosmids (3 and 4) were further extensively characterized by restriction mapping, PCR, and partial sequencing to show they contain the functional VEGF gene. It was shown previously that VEGF is a small (about 15 kb) single-copy gene (Tischer et al. 1991), but the presence of pseudogenes was not ruled out. Restriction and Southern blot mapping demonstrated that the cosmids contained the same hybridization bands and extensively overlap (data not shown). Comparisons with genomic blots prepared with *Eco*RI, *Hin*dIII and *Taq*I indicated that the cosmids faithfully represented the genomic VEGF locus, but did not contain the 3´ end of the gene (data not shown). This was further demonstrated by PCR of exons 3 and 7 (data not shown), and direct sequencing of the largest exon 3 using intronic primers (Tischer et al. 1991; Fig. 1). This analysis firmly established that the isolated cosmids were not chimeric and contained the VEGF locus except the 3´ end of the gene (exons 6, 7, 8). Therefore, these overlapping cosmids were used to map the chromosome location of the human VEGF gene.

In two hybridization experiments using VEGF 3 and 4 cosmid probes, 70%–90% of the chromosome spreads had specific fluorescence signals at identical sites on both chromatids of chromosome 6. Chromosome 6 was identified by rehybridization with whole chromosome probe and DAPI-produced banding (Fig. 2). From a total of 200 metaphases examined after hybridization with two probes, 184 had fluorescence signals on chromosome 6, and 151 had both homologues of chromosome 6 labeled. Symmetrical fluorescence label was not observed at any other site and single fluorescence spots were rare and randomly distributed over different chromosomes. A single specific site of hybridization for VEGF overlapping cosmid probes unequivocally demonstrates a single locus for this gene. The location of the fluorescence signal was deter-

Fig. 1 Direct sequencing of VEGF cosmids 3 and 4 by using intronic primers of exon 3. Nucleotide sequence present in cosmids 3 and 4 is *bolded* on the *top lane*; the published nucleotide sequence of exon 3 (Tischer et al. 1991) is given in *bottom lane*; exon 3 nucleotides are indicated by *upper case letters* and intron nucleotides are shown in *lower case letters*; intronic primers are *underlined*

tatgggtatgtctgacaggagaccgtagctgggtggccctggtaagtgtagtgctgagtg

tcactccaccgccctagtctacaccgtctggtacaggggaccctgaactcttgtagcaca

ctagagaccccacatttgtccggaagtggtcacaactaccaccttcgaatccttcacgca

gtgtgtgtcaccctccctgaatgcaatctcaaaccccccctgaacggactcagtcttcgag

gtgtctcacagtaatgtctcgacccacctctctccccgataggtagaaaacacagcgggt

ggcccgagtacacagtagcggagagtacgtcACCACTTCAAGTACCTACAGATAGTCGCG
catcgcctctcatgcagTGGTGAAGTTCATGGATGTCTATCAGCGC

TCGATGACGGTAGGTTAGCTCTGGGACCACCTGTAGAAGGTCCTCATGGGACTACTCTAG
AGCTACTGCCATCCAATCGAGACCCTGGTGGACATCTTCCAGGAGTACCCTGATGAGATC

CTCATGTAGAAGTTCGGTAGGACACACGGGGACTACGCTACGCCCCCGACGACGTTACTG
GAGTACATCTTCAAGCCATCCTGTGTGCCCCTGATGCGATGCGGGGGCTGCTGCAATGAC

CTCCCGGACCTCACACACGGGTGACTCCTCAGGTTGTAGTGGTACGTCcacccgtagaaa
GAGGGCCTGGAGTGTGTGCCCACTGAGGAGTCCAACATCACCATGCAGgtgggcatcttt

cccttcaccgcgttgcccccctatccctccccccagagg
ggga

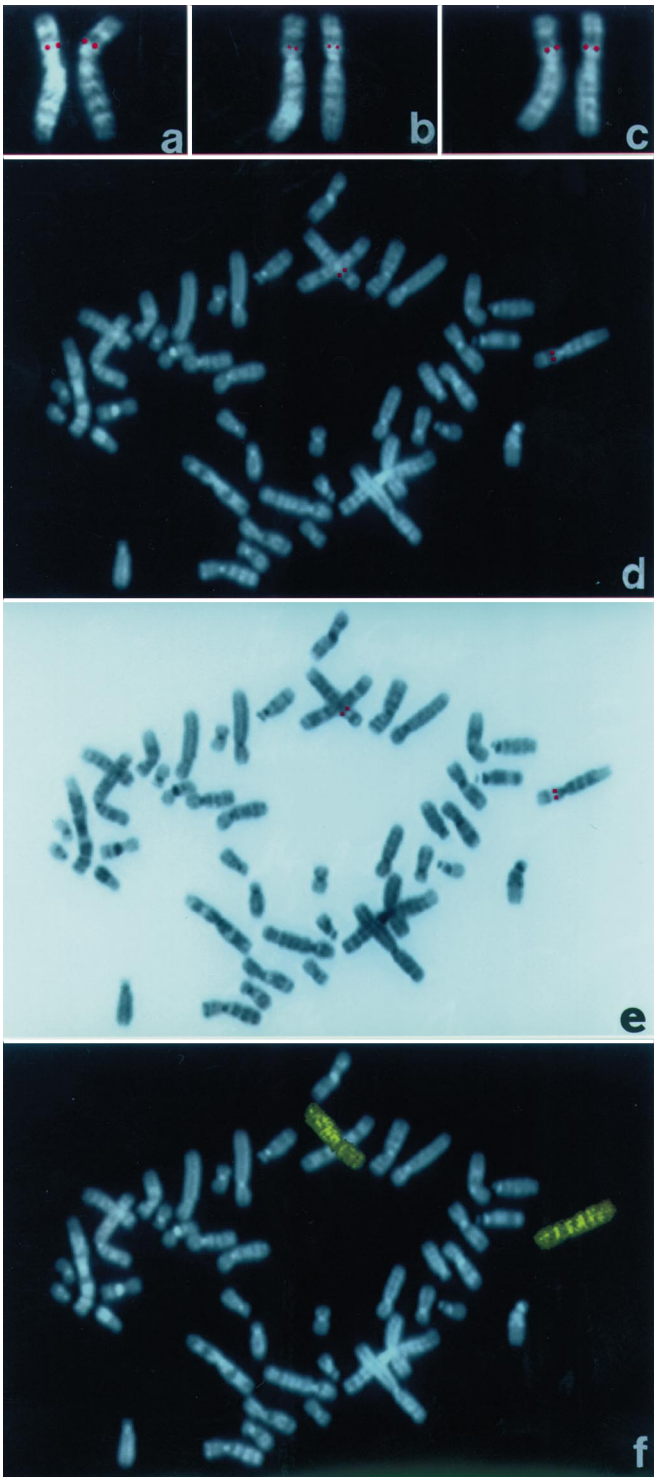

Fig. 2 a–c Digital images of partial karyotypes from normal human metaphases hybridized with a digoxigenin-labeled VEGF 3 (**a**) and VEGF 4 (**b, c**) cosmid probe and counterstained with DAPI. All three chromosome-6 pairs exhibit symmetrical rhodamine signals at the same site on sister chromatids. **d** Digital image of a complete metaphase with both chromosomes 6 labeled with fluorescent rhodamine doublets. **e** The same metaphase showing G-like banding pattern after the digital image of the DAPI-counterstained chromosomes was contrast-enhanced and look-up-table-inverted. The distinct banding pattern of individual chromosomes permits direct localization of the hybridization signal to band 6p12. **f** Rehybridization of the same spread with a chromosome-6 painting probe provides unequivocal confirmation of the identity of the labeled chromosome

mined directly in 50 metaphases with DAPI-enhanced G-like banding at region 6p12, where we assign the locus of the VEGF gene (Fig. 2).

In recent years several positive mitogenic factors were identified and shown to play a principal role in normal and pathological angiogenesis (Folkman 1995a,b). Among these, VEGF, which is a mitogen specific for vascular endothelial cells, is considered a direct key regulator of blood vessel growth in cancer and vascular diseases (Folkman 1995a; Plate et al. 1992; Shweiki et al. 1992; Berkman et al. 1993; Ferrara 1993). The switch to an angiogenic phenotype that precedes the onset of rapid tumor growth is usually accompanied by augmented expression of VEGF in tumor cells (Folkman 1995a,b; Fidler and Ellis 1994; O'Reilly et al. 1994). At least, in the case of glioblastoma multiforme, this dramatic upregulation of VEGF levels did not result from amplification of the gene (Berkman et al. 1993). However, it is tempting to speculate that rearrangements or some sort of amplification of the gene might be causally associated with increased expression of VEGF in other tumor cells.

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