An STS in a 5' untranslated exon of the human acidic fibroblast growth factor (aFGF) gene

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Human acidic fibroblast growth factor (aFGF), also known as heparin-binding growth factor 1 (HBGF-1), is one member of an expanding family of growth factors that share a high affinity for the glycosaminoglycan heparin. Members of this family also have in common significant amino acid sequence homology and a similar exon/intron structure in the protein coding region. Localized to chromosome 5q31.3-32 (1), the aFGF gene consists of three protein-coding exons with a long 3' untranslated region (2, 3). We and Crumley *et al.* have previously reported the isolation of two different cDNA clones which result from alternative splicing of upstream, untranslated exons (4, 5). A novel aFGF cDNA clone was subsequently isolated from a human glioblastoma cell line, U1242MG (6). Using a pair of nucleotide primers derived from the sequence of this cDNA in a PCR reaction, an 89 bp fragment was amplified from human genomic DNA (Figure 1) as well as from phage and YAC clones spanning the upstream region of the human aFGF gene. This has enabled us to localize this previously uncharacterized exon 29.3 kbp upstream from the first protein coding exon (6).

PCR primers:

Forward primer:

5'AGACTCTTGGGGGATTCCCTTAGTGAGTGAGT3';

reverse primer:

5'CTGCTGGCCCGTGGCTGGCTTCCCTGCAGA3'.

PCR components:

1 µg, 500 ng, 100 ng, 50 ng, and 25 ng of human genomic DNA, 25 pmol of each oligonucleotide, 200 µM dNTPs, and 2.5 U Taq polymerase (Perkin-Elmer Cetus) in 100 µl of PCR buffer [100 mM Tris-HCl (pH 8.3 at 25°C), 500 mM KCl, 15 mM MgCl₂, 0.01% (w/v) gelatin].

PCR profile:

Prior to the addition of 2.5 U of Taq polymerase, 95°C for 5 minutes then cooled to 72°C. After Taq polymerase addition, 40 cycles of 1 minute at 94°C, 1 minute at 55°C, and 2 minutes at 72°C, followed by a 15 minute extension at 72°C.

The sequence (accession no. X62586) of the amplified product with underlined primers is the following:

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AGACTCTTGGGGGATTCCCTTAGTGAGTGAGTCACTGCTCAAAGA 45
AGGGCTTTGCCACTTCTGCAGGGAAGCCAGCCACGGGCCAGCAG 89
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Figure 1 A. PCR products were electrophoresed on a 3% NuSieve and 1% Seakem agarose gel and photographed after ethidium bromide staining. Lane M contains 500 ng of lambda DNA digested with HindIII and 250 ng of ϕ X174-RF DNA digested with HaeIII. Lane 1 contains 10 µl of amplified DNA using a control template and primers supplied by Perkin-Elmer Cetus. Lanes 2 to 6 contain 10 µl each of amplified DNA using 1 µg, 500 ng, 100 ng, 50 ng and 25 ng of genomic DNA isolated from human white blood cells. Arrow corresponds to the expected 89 bp amplified product. Arrowhead corresponds to a primer-dimer-product. B. Lanes 1-3 contain 10 µl of PCR product using 500 ng, 100 ng and 25 ng human WBC genomic DNA. PCR conditions were identical to that in panel A except that the PCR buffer included 5% formamide to enhance the specificity of amplification. Arrow indicates the 89 bp expected product. Notice the absence of primer-dimer formation.

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