SHORT COMMUNICATION

Chromosome Localization and Genomic Structure of the KiSS-1 Metastasis Suppressor Gene (KISS1)

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The identification and sequence of KiSS-1 (HGMW-approved symbol, KISS1), a human malignant melanoma metastasis-suppressor gene, was recently published. In this report, we present a corrected genomic sequence, genomic structure, and refined chromosomal location for KiSS-1. The genomic organization of the sequence reveals a gene consisting of four exons. The first two exons are not translated; the third exon contains 38 5′ noncoding bases followed by the translational start site and another 100 translated bases. The terminal exon contains a further 332 translated bases, the translational stop codon, and the polyadenylation signal. The gene maps to chromosome 1q32 as determined by radiation hybrid mapping and FISH analysis. The relatively simple organization of this gene will facilitate analyses for mutations and abnormal expression in human tumors.

Metastatic melanoma is a well-recognized and pervasive human neoplasm. As with most human malignancies, progression to the metastatic potential results in a poor prognosis for patients with this disease. However, few of the genetic changes associated with this deadly change in course have been identified. Recently, the isolation of a novel gene involved in the appearance of metastatic melanoma, KiSS-1,[2] was reported (3). Identification of this gene was accomplished by the use of subtractive hybridization of cDNA from metastasis-suppressed neo6/C8161 hybrid cell lines against cDNA of the parent human metastatic melanoma cell line C8161 (5, 7). The resulting cDNA clones were used to screen a neo6/C8161 cDNA library, leading to the identification of the KiSS-1 gene. Following isolation of the a full-length clone, functional tests in athymic nude mice demonstrated that expression of KiSS-1 corresponded to a suppression of melanoma and breast metastasis without affecting establishment of the primary tumor (3, 4). We, therefore, wanted to screen human tumor samples for alterations in KiSS-1 sequence and/or expression. In this report, we have resolved the intron/exon structure of this gene and precisely mapped its chromosomal location. This will allow us to develop the necessary reagents for assessing the role of the KiSS-1 gene in the etiology of melanomas.

To determine whether altered KiSS-1 expression plays a role in the development of metastatic melanoma in human patients, we wished to search for mutations and/or loss of heterozygosity in tumor samples. Therefore, we needed to determine the chromosomal location of this gene and determine its intron/exon structure. To accomplish this task, we initially identified a PAC clone containing the entire KiSS-1 gene. An insert from the plasmid containing the KiSS-1 cDNA was radioactively labeled and used as a probe to screen an arrayed human PAC library (2). A PAC containing approximately 150 kb of genomic sequence, PAC 28A13, was identified.

Our initial report placed the gene in the region of 1q32–q41 (3). We biotin-labeled PAC 28A13 and used it as a probe to determine more precisely the chromosomal location of KiSS-1 by fluorescence *in situ* hybridization (FISH). KiSS-1 mapped to the long arm of chromosome 1, existing as a single locus on band 1q32 (Fig. 1). The localization to chromosome 1 was conclusively determined by the dual hybridization of a digoxigenin-labeled chromosome 1 centromere probe (Oncor). To confirm this and to fine-map the KiSS-1 gene, we screened the GeneBridge 4 radiation hybrid panel (6) by polymerase chain reaction (PCR) using primers B (ACCTGCCTCTTCTCACCAAG) and D (GAGGCCACCTTTTCTAATGG). PCR was performed using a Perkin–Elmer 9600 thermocycler with 20-μl reaction volumes that contained 50 ng of template DNA. PCR conditions were 5 cycles of denaturation at 94°C for 15 s, annealing at 61°C for 30 s, and extension at 72°C

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[2] The HGMW-approved symbol for the gene described in this paper is KISS1.

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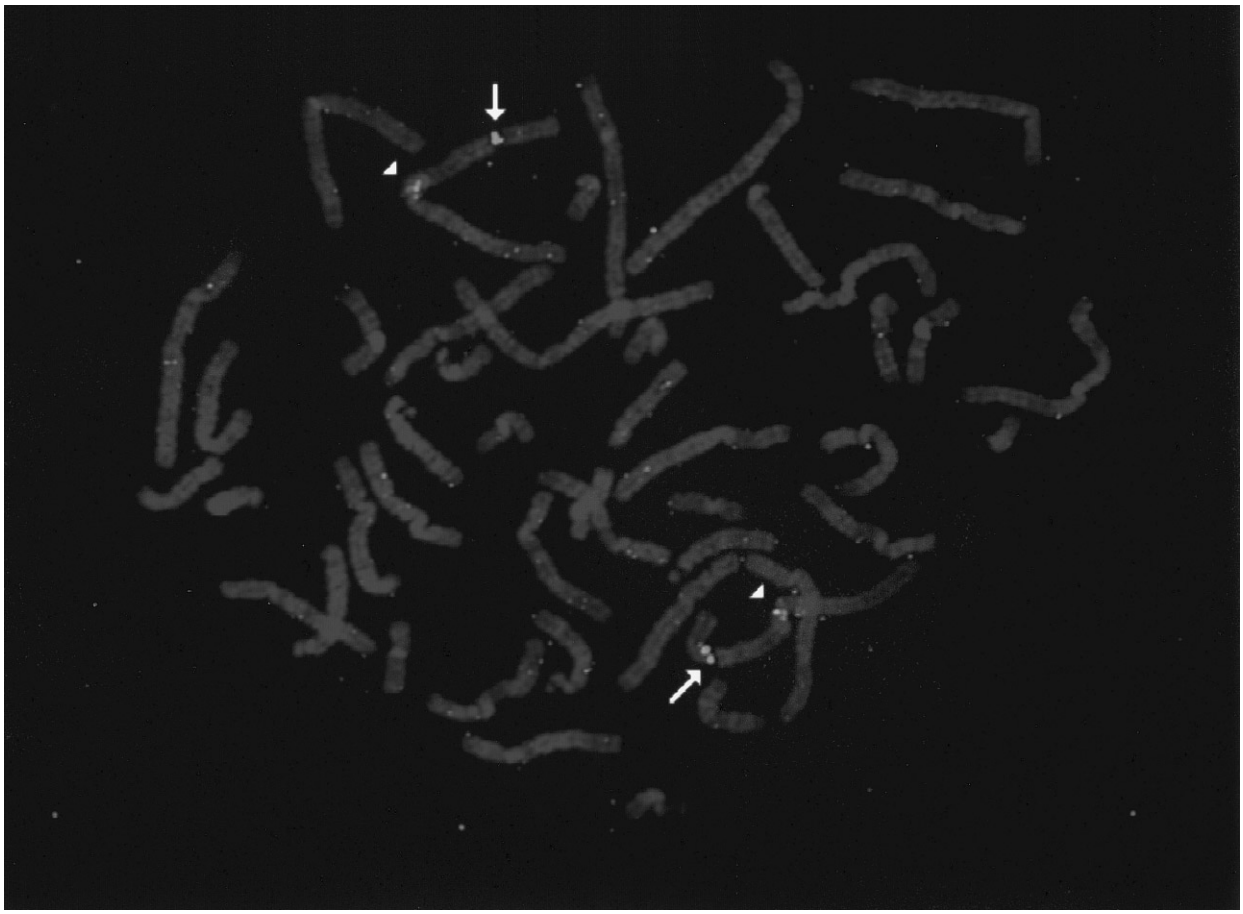

FIG. 1. Fluorescence *in situ* hybridization of biotinylated PAC 28A13 to human metaphase chromosomes. Arrows indicate the location of PAC 28A13 on the long arm of chromosome 1. The metaphase spread was cohybridized with a digoxigenin-labeled chromosome 1 centromere probe, which is identified by the solid arrowheads.

for 30 s followed by a further 36 cycles with a change of annealing temperature to 56°C. PCR products were analyzed on 1.5% agarose gels. Screening data were submitted via the World Wide Web to the Whitehead Institute/MIT Center for Genome Research radiation hybrid mapping server (http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map). Resultant data analysis placed the KiSS-1 cDNA within the radiation hybrid framework map (1) to an interval flanked by microsatellite markers D1S504 (proximal) and WI-9641 (distal), 3.05 cR from WI-9641 (LOD $>$ 3.0). Thus, both methods clearly place this gene on the long arm of chromosome 1.

To develop sets of PCR primers to distinguish intron/exon boundaries, we confirmed the primary sequence of the full-length KiSS-1 cDNA clone. The previous publication had predicted the protein sequence of the KiSS-1 gene as containing GCC CGG GGA at positions 133–141, corresponding to Ala Arg and Gly, respectively (2). Since this publication, two of our laboratories (B.E.W. and D.W.) have found a sequencing error that introduces two additional cytosine residues at positions 137 and 138. This change alters the reading frame of the DNA sequence, leading to a different amino acid sequence following the previously identified Ala at amino acid position 45. The corrected protein translation is presented in Fig. 2. The new open reading frame is shorter than the previously reported 164 amino acids: it is 145 amino acids long (Fig. 2). Although original reports show an incorrect protein sequence for the KiSS-1 gene, the functional assays demonstrating the metastatic suppression activity of the KiSS-1 cDNA remain uncompromised.

The new protein encoded by KiSS-1 cDNA was analyzed using PROSITE and was found to contain the following protein homology motifs. A tyrosine kinase phosphorylation site was located at amino acid positions 105 to 112 with a sequence of REKDLPNY. A further pattern entry showed protein homology at amino acid positions 118 to 123 to an N-myristoylation

FIG. 2. (**A**) Graphic representation of exons contained. (**B**) Sequence of the KiSS-1 cDNA. Partial intron sequence is denoted in lowercase letters; uppercase letters indicate exon sequence. The symbol "++" indicates the sequence correction from that previously published. Underlined sequence indicates the position of oligos used in sequencing and determination of intron–exon sites. Protein motifs are indicated by double-underlined proteins: *a* indicates a PKC phosphorylation site, *b* indicates a PKA phosphorylation site, and *c* indicates a tyr-phosphorylation site.

A

B

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-238  CCCGGGCTGC AGGAATTCGG CACGAGTCTC TCTCTCTCTC TCTCTCTCTC

-188  TCTCTCTCTC TCTCTCTCTC TCTCTCTCTC TCTCTCTCCT CGTGCCGAAT

-138  TGGCACGAG ...ggctttataa aagggatgtg atcagggagc tggggagaac

-129  tcttgagacc gggagcccagGCTGCCCA CCCTCTGGAC ATTCACCCAG
        Primer A
-101  CCAGGTGGTC TCGTCACCTC AGAGGCTCCG CCAGACTCCT GCCCAGGCCAG

-51   GGACTGAGGC AAGgtaggcacac tgcattgtcc acccctggga gggggtctgc

      cctgacctgg ggatgctctg  ... ctcagcaccc agcccagatc ctgtgcctga

-38   cctagtcttt gttccctctc tctgtctcagCCTCAAGG CACTTCTAGG

            Primer B/C          *
-20   ACCTGCCTCT TCTCACCAAG ATG AAC TCA CTG GTT TCT TGG CAG CTA
                              M   N   S   L   V   S   W   Q   L

28    CTG CTT TTC CTC TGT GCC ACC CAC TTT GGG GAG CCA TTA GAA
       L   L   F   L   C   A   T   H   F   G   E   P   L   E
      Primer D
70    AAG GTG GCC TCT GTG GGG AAT TCT AGA CCC ACA Ggtatgtatcc
       K   V   A   S   V   G   N   S   R   P   T

      tctggggaaa ggagtgggag ggagcaagtg ggttgttgca aaatgagctt ...

      ggtgttgcaa agccatcttt cccggacccc ggcctcatct ttctgtgccc

                                              Primer E    ++
104   tctgtcctagGC CAG CAG CTA GAA TCC CTG GGC CTC CTG GCC CCC
                 G   Q   Q   L   E   S   L   G   L   L   A   P
                                                  Primer F
139   GGG GAG CAG AGC CTG CCG TGC ACC GAG AGG AAG CCA GCT GCT
       G   E   Q   S   L   P   C   T   E   R_a  K   P   A   A

181   ACT GCC AGG CTG AGC CGT CGG GGG ACC TCG CTG TCC CCG CCC
       T   A   R_a L   S   R   R   G   T_b  S   L   S   P   P

223   CCC GAG AGC TCC GGG AGC CGC CAG CAG CCG GGC CTG TCC GCC
       P   E   S   S   G   S   R   Q   Q   P   G   L   S   A

265   CCC CAC AGC CGC CAG ATC CCC GCA CCC CAG GGC GCG GTG CTG
       P   H   S   R   Q   I   P   A   P   Q   G   A   V   L

307   GTG CAG CGG GAG AAG GAC CTG CCG AAC TAC AAC TGG AAC TCC
       V   Q   R   E   K   D   L   P   N   Y_c  N   W   N   S

349   TTC GGC CTG CGC TTC GGC AAG CGG GAG GCG GCA CCA GGG AAC
       F   G   L   R   F   G   K   R   E   A   A   P   G   N

391   CAC GGC AGA AGC GCT GGG CGG GGC TGG GGC GCA GGT GCG GGG
       H   G   R   S   A   G   R   G   W   G   A   G   A   G

433   CAG TGA ACTTCAGACC CCAAAGGAGT CAGAGCATGC GGGGCGGGGG
       Q   .

479   CGGGGTGGGG GGGACGTAGG GCTAAGGGAG GGGGCGCTGG AGCTTCCAAC

529   CCGAGGCAAT AAAAGAAATG TTGCGTAACT CAAAAAAAAA AAAAAAAAAA

579   CTCCAGGGG
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site with a protein sequence of GLRFGK. Also identified were a consensus PKC phosphorylation site [S/T]-X-[R/K] at amino acids 54–56 and 61–63 as well as a consensus PKA phosphorylation site $[R/K]_2$-X-[S/T] at amino acid position 66–69.

The intron/exon boundaries of the KiSS-1 gene were determined by use of the PAC 28A13 genomic DNA as template in various automated sequencing reactions performed using an Applied Biosystems, Inc. Model 377 DNA sequencer. Analysis of the sequence results revealed that the KiSS-1 gene is encoded by 145 amino acids. The gene consists of four exons. The first two exons of the gene are not translated. Exon I consists of 109 noncoding bases and is separated from exon II, which contains 91 noncoding bases, with an intron of undetermined size. Exon III contains the translational start site preceded by 38 noncoding bases and 103 translated bases. The fourth exon is the largest, consisting of 335 translated and 121 nontranslated bases. Sequence results using primers A (CCAGCCAGGTGGTCTCGT) and C (CTTGGTGAGAAGAGGCAGGT) revealed the presence of an intron 37 bases upstream of the methionine start site in the untranslated upstream sequence. Another intron/exon boundary was identified within the coding region at position 103 of the cDNA using primers B and F (TAGCAGCTGGCTTCCTCTC) in sequencing reactions.

To determine the size of the intervening introns, we attempted to isolate them by PCR amplification using PAC 28A13 genomic DNA as well as three different sources of normal human genomic. We were unsuccessful in designing primers that would amplify the first intron. PCR amplification, using primers A and D that flank the intron/exon boundaries, revealed a product of 4 kb in the 5′ upstream untranslated region. PCR in this case was performed on a Perkin–Elmer Model 4500 thermocycler using a 50-μl reaction volume with the following PCR conditions: 94°C for 5 min for initial denaturation followed by 5 cycles of 94°C for 30 s of denaturation, 55°C for 1.5 min of annealing, and 72°C for 2 min of extension followed by another 5 cycles with a change of annealing temperature to 56°C, and finally 25 cycles with a change of annealing temperature to 52°C. To determine the size of the third intron contained within the coding region of the KiSS-1 cDNA, primers B and E (CAGGAGGCCCAGGGATTC), which flank the exon junction site, were used for amplification. The reaction for these primers was run using the following PCR conditions: 94°C for 5 min for initial denaturation followed by 5 cycles of 94°C for 30 s of denaturation, 62°C for 1.5 min of annealing, and 72°C for extension followed by another 5 cycles with a change of annealing temperature to 60°C, and finally 25 cycles with a change of annealing temperature to 56°C. In each case the products were analyzed on a 1% agarose gel, which revealed one major product. To confirm further that this was indeed the intron, the gels were subsequently transferred to nylon where the product was used in a Southern blot analysis using the entire cDNA as a probe. A single band of 4 kb was detected with primers A and D and a band of 2 kb for primers B and E in each of the template DNA sources studied (data not shown). While these data are suggestive of the intron sizes, further characterization of the PCR products is required for confirmation.

In summary, the intron/exon structure of the metastasis suppressor gene KiSS-1 has been determined. KiSS-1 appears to be a gene containing two 5′ untranslated exons and two partially translated exons. The entire coding sequence of the gene is coded for by 438 bp. FISH and hybrid panel mapping have conclusively localized KiSS-1 to the long arm of chromosome 1. While the originally published sequence was found to contain an error, the conclusions as to the biological activity of metastasis suppression have not been compromised.

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