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Short Communication

Nucleotide sequence of *c-H-ras-1* gene from B6C3F1 mice*

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The *c-H-ras-1* gene of an B6C3F1 mouse was isolated and nucleotide sequence determined. Our study has revealed that this *c-H-ras-1* gene consists of four exons, separated by three introns ranging in size from 150 to 649 bp. The coding parts of the sequence of mouse *c-H-ras-1* gene show no important differences as compared with those of the rat, hamster and human gene. More numerous changes were found in introns. The identity of mouse *c-H-ras-1* gene with rat, hamster and human ones at the nucleotide level is 86.40%, 80.04% and 67.87%, respectively. Comparison of amino acids in protein sequence of *c-H-ras* gene of mouse, rat, hamster and human points to high degree of conservation of the gene.

The *ras* oncogenes form the first link between chemical carcinogens and oncogene activation [1]. Three different *ras* protooncogenes have been identified in mammalian cells: *H-ras*, *K-ras* and *N-ras*. All those protooncogenes code for the highly homologous proteins known as p21. All those proteins can bind guanosine triphosphate and guanosine diphosphate, and show guanosine triphosphatase activity which plays an essential role in cellular signal transduction [1].

It has been well established that specific alterations in members of the *ras* gene family, *H-ras*, *K-ras* and *N-ras*, can convert them into active oncogenes. Those alterations are usually located in the codon for amino acid 12, 13 or 61 [2].

Members of the *myc* and *ras* families are the oncogenes most frequently encountered in human neoplasms, with amplification, rearrangement, overexpression, and single base mutations being the most important mechanisms of their activation [3]. For example alterations of *c-H-ras* may occur in more than one-third of tumors from different tissue origins and may correlate with progression and metastasis of cancer [4].

Activated *ras* oncogenes are frequently found in animal tumors induced by chemical carcinogens [5]. The studies on protooncogene *ras* activity are usually conducted on rats or mice. This paper presents the sequencing of the B6C3F1 mice *H-ras-1* protooncogene. Besides, we compared mouse *c-H-ras-1* PCR sequence products with the sequence of the rat (*Rattus norvegicus*) [6], hamster (Golden hamster) [7] and human (*Homo sapiens*) [8] and also amino-acid sequence in the protein of mouse, rat, hamster, and human encoded by the gene.

MATERIALS AND METHODS

Genomic DNA was extracted from liver of B6C3F1 (C_{57}BL x C3H) male mouse according

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    MetThrGluTyrLysleuValValValGlyAlaGlyGlyValGlyLysSerAlaLeuThr
5'1 ATGACAGAATACAAGCTTGTGGTGGTGGGCGCTGGAGGCGTGGGAAAGAGTGCCCTGACC

    IleGlnLeuIleGlnAsnHisPheValAspGluTyrAspProThrIleGlu
 61 ATCCAGCTGATCCAGAACCACTTTGTGGACGAGTATGATCCCACTATAGAGGTGAGCTCT

121 GCCTACCTGCCAGAGGAGGGCTGTGGCAGTGGTCATGGGTAAATGACCAAACAGCCCAGG
181 AGAAGTAGGTGTTATGGGGTATGATCCATCAGGGTATGAGAGGTGCAAGGGTGTAGGCTG
241 GTTCTGTGGATTCTCTGGTCTGAGGAGAGGTGAGACCCCACCCCCACCCCCCACCCCCAC

                          AspSerTyrArgLysGlnValValIleAspGlyGluThr
301 TAAGCCGTGTTGTTTTGCAGGACTCCTACCGGAAACAGGTGGTCATTGATGGGGAGACAT

   CysLeuLeuAspTyrLeuAspThrAlaGlyGlnGluGluTyrserAlaMetArgAspGln
361 GTCTACTGGACTACTTAGACACAGCAGGTCAAGAAGAGTATAGTGCCATGCGGGACCAGT

   TyrMetargThrGlyGluGlyPheLeuCysValPheAlaIleAsnAsnThrLysSerPhe
421 ACATGCGCACAGGGGAGGGCTTCCTCTGTGTATTTGCCATCAACAACACCAAGTCCTTCG

   GluAspIleHisGlnTyrArg
481 AGGACATCCATCAGTACAGGTGAGCCACCTATGGCTAGCCCGTGAGCCCGTGGCACACGA
541 GAGGAAGGTTCCGTGTGCACACTGAGGCTTTATGTCTTTTTTTGAATGTCCTGGACACAG

                                                    GluGlnIle
601 TCATGCCTGCAGCCTGCTAGCTGGCTCATATCCACCCCATCCCCCTCCAGGGAGCAGATC

    LysArgvalLysAspSerAspAspValProMetValLeuValGlyAsnLysCysAspLeu
661 AAGCGGGTGAAAGATTCAGATGATGTGCCAATGGTGCTGGTGGGCAACAAGTGTGACCTG

    AlaAlaArgThrValGluSerArgGlnAlaGlnAspLeuAlaArgSerTyrGlyIlePro
721 GCTGCTCGCACTGTTGAGTCTCGGCAGGCCCAGGACCTTGCTCGCAGCTATGGCATCCCC

    TyrIleGluThrSerAlaLysThrArgGln
781 TACATTGAAACATCAGCCAAGACCCGGCAGTGAGCCTGTTTCCCTCTCCACAGCTAGTCA
841 AGGATTTGCCGCACCCCACCCCAGCCAGGGAGCAGAGCTCATTGCCGCTCTCCTCAACAC
901 AGGGCAGCCGCTCTGGCTCAGTCGACCTCTGGATCCCCCCTCCCCCGGGACCCATGTGAC
961 CCAGCGGCCCTCAAGCTGTAAGTCTCCCAGACCGCAGGCTGTGAGGGAGGCAAGGGCCAG
1021 GGTCTGGGCTTATGCCTGCAGTTCTGAGTTCACATAGCTCTAGGGCAGGATGGGTCCCTG
1081 GAGAGAGCTGCCCTGAGCCAGGCCGGAGCGGCGACCCAGGGGCCTTAGTTCTTCTTGTCC
1141 CCAGTGTCCTGTGATACTGGCTAGTTTTAAGCCCTTCAGTGTTGTTAGGTTGTTCAACTT
1201 GAGACGTACTGGGGGTCTGGGAGAGTCCTGAGTTAAGTGGCTCTGACTTTGAGTGATACT
1261 CAGGAGTGAACCCTATTTCAGGAGAGTGGCCTGTGCCTTTTGGATGGCCAGGGCCAGCTC
1321 CCTATTTGTGTTGGTTTTGCAGCTGAGGAAGGGAGCCTCCAGCGTTGGGGTGTGACCTGA
1381 TCTAGGCAGGGAGTTTCTTACTCAGACAGCACCCCTTTTCTCCTCAGAGTCCTCTCTGAT

                              GlyValGluAspAlaPheTyrTheLeuValArgGluIleArg
1441 CTAGTCCCTCTGTCCCCAGGGCGTGGAGGATGCCTTCTATACACTAGTCCGTGAGATTCG

     GlnHisLysLeuArgLysLeuAsnProProAspGluSerGlyProGlyCysMetSerCys
1501 GCAGCATAAATTGCGGAAACTGAACCCACCCGATGAGAGTGGTCCTGGCTGCATGAGCTG

     LysCysValLeuSer
1561 CAAATGTGTGCTGTCCTGACACCAGGTGAGGCAGGGACCAGCA 3'1603
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Fig. 1. Nucleotide sequence of the c-Ha-ras-1 gene of the B6C3F1 mouse. The coding sequences are shown in bold-face type. The amino acids encoded by the gene are indicated above the nucleotide sequence. The EMBL Nucleotide Sequence Database accession number is: Z50013.

to Maniatis [9]. Two fragments of *H-ras* gene were amplified using primers HR1 (5'-GAAGCTATGACAGAATACAA 3'), HR3 (5'-CGAGACTCAACAGTGCGAGC 3') and RAS1(5'-GAAGAATTCAGATGATGTGCCAATG 3'), RAS2 (5'-GCTGGATCCACTGCCCCAGATGTCT 3'), respectively. After polyacrylamide gel electrophoresis the amplified fragments had the size — 723 bp (from 1 to 723 bp) and 968 bp (from 693 to 1782 bp). The termini of the first fragments' were filled with Klenow polymerase and cloned in phagemide vector PBS (+) (Stratagene) after hydrolysis with *Hin*cII restriction nuclease (Boehringer). A recombined plasmid was obtained. After digestion with *Eco*RI and *Hin*dIII restriction nucleases the inserts possessed the appropriate size (about 700 bp). The second fragment was digested with *Eco*RI and *Bam*HI restriction nucleases (Boehringer). Since an additional site (in position 1076) was recognized by *Bam*HI, two fragments were obtained (*Eco*RI/*Bam*HI and *Bam*HI/*Bam*HI). Both fragments were cloned in pBS (+) and pBluescript SK (–) (Stratagene). Besides, the whole 968 bp fragment was treated with Klenow polymerase and digested by *Eco*R1. Target DNA was cloned in pBS (+) plasmid after digestion with *Eco*RI/*Sma*I.

The sequencing was carried out by method of Sanger *et al.* [10] with synthetic M13/pUC forward sequencing primer, M13/pUC reverse sequencing primer (Boehringer) and specific primers HR1, HR3, RAS1, RAS3 (5'-CCCATGTGACCCATGTG 3'), and RAS4 (5'-CCCTTAAGTGTTGTTAG 3').

RESULTS AND DISCUSSION

The B6C3F1 mice are used worldwide to gauge the carcinogenic hazard posed by chemicals to humans. We examined the sequence of mouse *H-ras-1* gene which is linked with tumorigenicity [2, 5] and compared it with the sequences of rat, hamster and human *H-ras-1* genes. The *H-ras* gene in mouse includes 1603 nucleotides [11], in rat 2169 [6], in hamster 2070 [7], and in human 1687 [8]. The coding region of the mouse *H-ras* gene is organized into four exons separated by three introns ranging in size from 150 bp to 649 bp (Fig. 1). Comparison of the mouse gene sequence with that of the rat and hamster revealed an identity between the genes equal to 86.40%, and 80.04%, respectively and between mouse and human, 67.87%.

Two highly homologous regions were found when we compared the exons of mouse and rat. One of them covers the protein encoding portion of exon 1 (1 to 80 bp), and the second, a portion of exon 3 (651 to 810 bp in mouse and 631 to 781 in rat). Some differences in the sequence of DNA are visible in exons 2 and 4, and larger differences are noticed in introns.

The organization of the mouse gene with that of the hamster showed a similarity in size and location of exons site as in the case of mouse

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  1 MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET A
    MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET B
    MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET C
    MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET D

 51 CLLDYLDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQI A
    CLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQI B
    CLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQI C
    CLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQI D

101 KRVKDSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTRQ A
    KRVKDSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTRQ B
    KRVKDSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTRQ C
    KRVKDSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTRQ D

151 GVEDAFYTLVREIRQHKLRKLNPPDESGPGCMSCKCVLS A
    GVEDAFYTLVREIRQHKLRKLNPPDESGPGCMSCKCVLS B
    GVEDAFYTLVREIRQHKLRKLNPPDESGLGCMSCKCVLS C
    GVEDAFYTLVREIRQHKLRKLNPPDESGPGCMSCKCVLS D
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Fig. 2. Comparison of the amino-acid sequence of the c-H-ras-1 gene protein of the mouse (A), rat (B), hamster (C) and human (D). The amino acids that differ from those of the mouse are shown in bold-face letters.

and rat, but larger differences in nucleotides, especially in introns, were detected. As shown in Fig. 2, the amino-acid sequences of the protein encoded by the mouse, rat, hamster and human *c-H-ras-1* gene are highly homologous. The differences were noticed only in one place when we compared the sequence of mouse proteins with rat and human ones and in two places when we compared the respective sequences of mouse and hamster. The comparison revealed a high degree of conservation of *c-H-ras-1* gene.

Activation of *c-H-ras-1* genes occurs most often by point mutations which usually are located in codons 12, 13 and 61 [4]. The substitution of a nucleotide within codon 12 is the most frequent mutation identified in human tumors, and this substitution at the first or second base of codon 12 may precede the development of malignancy [12, 13]. Considering that the structure of mouse, rat and hamster *H-ras* gene show a high homology and is very similar to the human one, we can state that all those animals seem to be equally valuable for *H-ras-1* gene activation studies.

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