MOLECULAR AND CELLULAR BIOLOGY, Apr. 1986, p. 1349–1351
0270-7306/86/041349-03$02.00/0

Vol. 6, No. 4

Structure of the c-Ki-*ras* Gene in a Rat Fibrosarcoma Induced by 1,8-Dinitropyrene

TOMOKO TAHIRA,[1]* KENSHI HAYASHI,[1] MASAKO OCHIAI,[1] NOBUO TSUCHIDA,[2] MINAKO NAGAO,[1] AND TAKASHI SUGIMURA[1]

National Cancer Center Research Institute, Chuo-ku, Tokyo 104,[1] and Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113,[2] Japan

Received 27 September 1985/Accepted 3 January 1986

Restriction enzyme maps were made of the region around exons 1 and 2 of activated c-Ki-*ras* of a fibrosarcoma (1,8-DNP2) induced in a rat by 1,8-dinitropyrene. Nucleotide sequence analysis revealed that activated c-Ki-*ras* shows a G→T transversion in codon 12 and consequently encodes cysteine instead of glycine in normal rat c-Ki-*ras*.

1,8-Dinitropyrene, a potent environmental mutagen present in diesel exhaust and polluted air, induced fibrosarcomas when injected subcutaneously into Fischer 344 rats (8). Of seven sarcomas examined, one tumor, 1,8-DNP2, contained an activated c-Ki-*ras* oncogene detected by an NIH 3T3 cell transfection assay (7). Studies of transforming c-Ki-*ras* genes in a wide variety of human and rodent tumors revealed that a single point mutation in codon 12 or 61 of the predicted p21 protein was responsible for the activation of the proto-oncogene (4, 6, 10, 12). However, the structure of rat c-Ki-*ras* has not yet been reported. Here we report the structure of a portion of c-Ki-*ras* of the rat fibrosarcoma 1,8-DNP2.

An NIH 3T3 primary transformant (1,8-DNP2-2) containing an activated c-Ki-*ras* gene was obtained by transfection of DNA of the rat fibrosarcoma 1,8-DNP2 (7). Complete digestion of the 1,8-DNP2-2 DNA with *Bam*HI yielded two rat-derived fragments of about 9 and 11 kilobases (kb) that hybridized with HiHi380 (2). These two fragments were ligated to the *Bam*HI site of EMBL3A (3). Three clones that gave a positive signal with the HiHi380 probe were obtained. Clone λDNP2-1 contained two *Bam*HI inserts: an 8.3-kb fragment that hybridized to the probe and a 7.5-kb fragment that did not. The latter fragment may not be related but may be ligated to c-Ki-*ras* during cloning. The other clone, λDNP2-2, had a 10.5-kb *Bam*HI insert. The 8.3- and 10.5-kb *Bam*HI fragments from λDNP2-1 and λDNP2-2, respectively, were recloned into plasmid pSP64, yielding pDNP2-1 and pDNP2-2, respectively.

The restriction enzyme maps of pDNP2-1 and pDNP2-2 are shown in Fig. 1. The hatched boxes indicate the regions with homology to HiHi380 which covers exons 1 and 2 and a 5′ portion of exon 3 of human c-Ki-*ras* (5). A 0.1-kb *Sst*II-*Hin*fI fragment of HiHi380 (2) was used to detect exon 1 of c-Ki-*ras*. Exon 3 was distinguished from the others by use of a 0.2-kb *Xba*I-*Eco*RI fragment of HiHi3 (2). A 0.5-kb *Eco*RI-*Hin*dIII fragment of pDNP2-1 contained exon 1, an

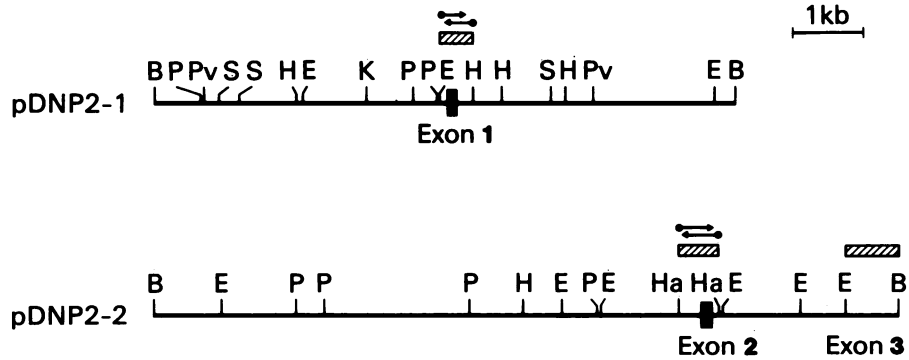

FIG. 1. Restriction maps of pDNP2-1 and pDNP2-2. Hatched boxes indicate the regions that hybridized with HiHi380. Solid boxes indicate the exons that were examined by nucleotide sequencing. Exon 3 has not yet been analyzed. Restriction endonuclease sites were as follows: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I; and Ha, *Hae*III. *Hae*III sites are mapped only in the *Eco*RI-*Eco*RI fragment containing exon 2. *Eco*RI sites and *Hae*III sites in the right half of pDNP2-2 were determined by partial digestion of the end-labeled 5-kb *Hin*dIII-*Bam*HI fragment.

0.8-kb *Eco*RI-*Bam*HI fragment of pDNP2-2 contained exon 3, and the remaining 1.7-kb *Eco*RI fragment contained exon 2. pDNP2-2 was digested with *Hae*III, and the resulting 0.5-kb fragment was found to carry exon 2, which hybridized with the HiHi380 probe but not with the *Xba*I-*Eco*RI fragment of HiHi3 (Fig. 1).

For examination of the genetic alterations of exons 1 and 2, we determined their nucleotide sequences. A 0.5-kb *Eco*RI-*Hin*dIII fragment of pDNP2-1 was subcloned into M13mp18 or M13mp19, and a 0.5-kb *Hae*III fragment of pDNP2-2 carrying exon 2 was subcloned into the phospha-

* Corresponding author.

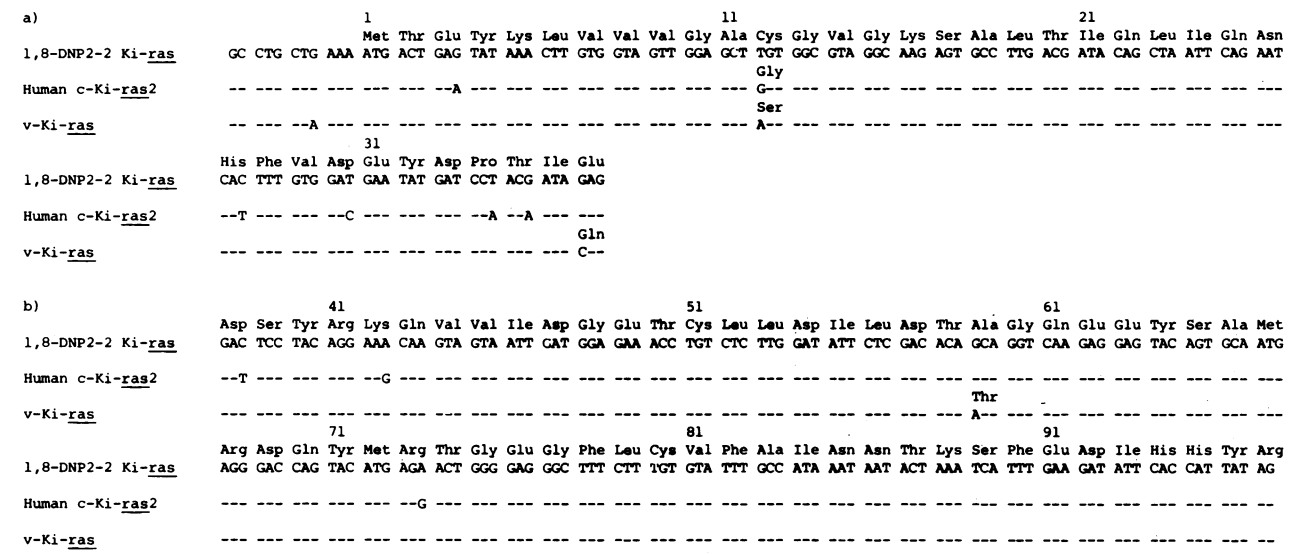

FIG. 2. Nucleotide sequences and predicted amino acid sequences of exons 1 (a) and 2 (b) of c-Ki-*ras* derived from 1,8-DNP2. Corresponding regions of v-Ki-*ras* and normal human c-Ki-*ras*2 determined by others (5, 11) are also shown. Dashes indicate nucleotides that are identical to those in c-Ki-*ras* derived from 1,8-DNP2.

tase-treated *Sma*I site of M13mp10. The nucleotide sequences were determined by the dideoxy method of Sanger et al. (9). Coding regions were assigned by comparison of the DNA sequences with those of the v-Ki-*ras* (11) and human c-Ki-*ras*2 (5) genes. Consensus splicing sequences that were identical to those of human genes were found at intron-exon boundaries (data not shown).

The nucleotide sequences of exons 1 and 2 cloned from 1,8-DNP2-2, encoding a sequence of 97 amino acids at the N terminus, were compared with the corresponding sequences of human c-Ki-*ras* and v-Ki-*ras* (Fig. 2). The nucleotide sequence was more homologous with that of v-Ki-*ras* than with that of human c-Ki-*ras*. In fact, 1,8-DNP2-2 Ki-*ras* and v-Ki-*ras* differed in only three nucleotides in the coding regions, the first letters of codons 12, 37, and 59, although these all resulted in differences in the predicted amino acid sequence. The amino acid sequence encoded by 1,8-DNP2-2 Ki-*ras* was more similar to that encoded by human c-Ki-*ras* than to that encoded by v-Ki-*ras*; the only difference was that at codon 12. Rat c-Ki-*ras* of the primary transformant 1,8-DNP2-2 encoded cysteine, whereas normal human c-Ki-*ras*2 encoded glycine.

Codon 12 of the genomes from normal rats and the original tumors were analyzed further by oligodeoxynucleotide hybridization. Two synthetic 19-mers spanning the region from nucleotide 25 in codon 9 to nucleotide 43 in codon 15 of the human c-Ki-*ras* gene (Pharmacia P-L Biochemicals), one containing GGT (glycine) and the other containing TGT (cysteine) at codon 12, were used as probes after being labeled with ^{32}P in a polynucleotide kinase reaction. Consistent with the restriction map shown in Fig. 1, in double digests with *Sac*I and *Eco*RI of DNAs of normal rat kidney, sarcoma 1,8-DNP2, and the primary transformant 1,8-DNP2-2, exon 1 of rat c-Ki-*ras* was found in a 1.5-kb fragment. The 1.5-kb *Eco*RI-*Sac*I fragments of normal rat kidney and of 1,8-DNP2 but not of 1,8-DNP2-2 hybridized with the probe of 19-mer containing GGT (glycine) at codon 12 by direct gel hybridization (1). The 19-mer containing TGT (cysteine) at codon 12 hybridized with the DNAs of 1,8-DNP2 and of 1,8-DNP2-2 but not of normal rat kidney (data not shown).

From the results on nucleotide sequencing and oligodeoxynucleotide hybridization, we conclude that glycine is encoded at codon 12 of normal rat c-Ki-*ras*, that a G→T transversion has occurred at the first letter of codon 12 in 1,8-DNP2 c-Ki-*ras*, and that 1,8-DNP2 is a heterozygote of normal and activated c-Ki-*ras*. The same mutation has been observed in activated c-Ki-*ras*2 of human tumors (6, 12), and the transforming activity of c-Ki-*ras* encoding cysteine as amino acid 12 was first determined by using chimeric genes (10). From these results, it is conceivable that the somatic mutation (G→T) in 1,8-DNP2 is responsible for the activation of c-Ki-*ras*.

This work was supported by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture and by grants from the Princess Takamatsu Cancer Research Fund and the Hishinomi Cancer Research Fund.

LITERATURE CITED

1. **Bos, J. L., M. Verlaan-de Vries, A. M. Jansen, G. H. Veeneman, J. H. van Boom, and A. J. van der Eb.** 1984. Three different mutations in codon 61 of the human N-*ras* gene detected by synthetic oligonucleotide hybridization. Nucleic Acids Res. **12**:9155–9163.
2. **Ellis, R. W., D. DeFeo, T. Y. Shih, M. A. Gonda, H. A. Young, N. Tsuchida, D. R. Lowy, and E. M. Scolnick.** 1981. The p21 *src* genes of Harvey and Kirsten sarcoma viruses originated from divergent members of a family of normal vertebrate genes. Nature (London) **292**:506–511.
3. **Frischauf, A.-M., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
4. **Guerrero, I., A. Villasante, V. Corces, and A. Pellicer.** 1984. Activation of a c-K-*ras* oncogene by somatic mutation in mouse lymphomas induced by gamma radiation. Science **225**: 1159–1162.
5. **McGrath, J. P., D. J. Capon, D. H. Smith, E. Y. Chen, P. H. Seeburg, D. V. Goeddel, and A. D. Levinson.** 1983. Structure and organization of the human Ki-*ras* proto-oncogene and a related processed pseudogene. Nature (London) **304**:501–506.
6. **Nakano, H., F. Yamamoto, C. Neville, D. Evans, T. Mizuno, and M. Perucho.** 1984. Isolation of transforming sequences of two human lung carcinomas: structural and functional analysis of the activated c-K-*ras* oncogenes. Proc. Natl. Acad. Sci. USA

81:71–75.

7. **Ochiai, M., M. Nagao, T. Tahira, F. Ishikawa, K. Hayashi, H. Ohgaki, M. Terada, N. Tsuchida, and T. Sugimura.** 1985. Activation of K-*ras* and oncogenes other than *ras* family in rat fibrosarcomas induced by 1,8-dinitropyrene. Cancer Lett. **29**:119–125.
8. **Ohgaki, H., C. Negishi, K. Wakabayashi, K. Kusama, S. Sato, and T. Sugimura.** 1984. Induction of sarcomas in rats by subcutaneous injection of dinitropyrenes. Carcinogenesis (London) **5**:583–585.
9. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
10. **Shimizu, K., D. Birnbaum, M. A. Ruley, O. Fasano, Y. Suard, L. Edlund, E. Taparowsky, M. Goldfarb, and M. Wigler.** 1983. Structure of the Ki-*ras* gene of the human lung carcinoma cell line Calu-1. Nature (London) **304**:497–500.
11. **Tsuchida, N., T. Ryder, and E. Ohtsubo.** 1982. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. Science **217**:937–939.
12. **Varmus, H. E.** 1984. The molecular genetics of cellular oncogenes. Annu. Rev. Genet. **18**:553–612.