Activating c-Ha-ras Oncogene with a Guanine to Thymine Transversion at the Twelfth Codon in a Human Stomach Cancer Cell Line

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ABSTRACT

The rat fibroblast cell line Rat 1 was transfected with total DNA of a gastrocarcinoma cell line, BGC-823. The transforming gene was cloned from the genomic library of the secondary transfurants using in situ hybridization with a probe of the human Alu repeat sequence. This cloned gene is homologous to the protooncogene c-Ha-ras. The activation lesion of the transforming gene was identified by sequence analysis as a single nucleotide substitution of thymine for guanine in the 12th codon. This results in the substitution of valine for glycine at the 12th amino acid of the M, 21,000 protein.

INTRODUCTION

DNA transfection has been used to identify the transforming genes from human tumors and tumor cell lines (1-4). A number of oncogenes were identified and cloned, such as Ha-ras (5-10), Ki-ras (11-16), N-ras (15, 17, 18), B-lym (19), oncD (20), met (21), and mel (22), from a variety of tumors, tumor cell lines, or induced tumor cell lines.

In this paper, we report that a transforming gene was cloned from a secondary focus of rat fibroblast cell line Rat 1, which was transfected with the high molecular weight DNA of a gastrocarcinoma cell line, BGC-823. The transforming gene is the allele of protooncogene c-Ha-ras and the activation mechanism is the point mutation in the 12th codon, which leads to the substitution of valine for glycine at the 12th amino acid of M, 21,000 protein.

MATERIALS AND METHODS

Cells. Gastrocarcinoma cell lines BGC-823, PACM-82, and MGC 80-3 were obtained from Y. Cai (People's Hospital, Beijing Medical College), S. Li (Stomatological Hospital, Beijing Medical College), and K. Wang (Shandong Teacher's University), respectively. The cell line BGC-823 was established in 1984 through 60 passages of cell culture while that of the negative control was 0.02 foci/µg DNA. Thus the highest transforming potency; the efficiencies for BGC-823, 0.17, 0.14, 0.05, 0.02, and 0.04 foci/µg DNA, respectively, that for these DNA samples, DNA from BGC-823 showed the 0.1% SDS at room temperature and with 0.2× SSC-0.1% SDS at 68°C (stringent condition) or with 2× SSC-0.1% SDS at room temperature and with 0.2× SSC-0.1% SDS at 42°C (less stringent condition).

Southern Transfer and Hybridization. DNA samples were completely digested with restriction endonuclease EcoRI or BamHI, subjected to electrophoresis in 0.8% agarose gel, and transferred to nitrocellulose filters (Schleicher and Schuell) according to the method of Southern (27). The filters were hybridized with 32P-labeled probes overnight at 42°C in 50% formamide-0.9 M NaCl-0.9 M sodium citrate-0.5% SDS-0.1% Ficoll-0.1% polyvinylpyrrolidone-0.1% bovine serum albumin-100 µg/ml denatured salmon sperm DNA. If hybridization was carried out with the probe of human Alu sequence, denatured rat or mouse liver DNAs were added to the mixture to a final concentration of 1 µg/ml to compete with human Alu sequence in hybridizing DNAs of the Rat 1 or NIH 3T3-derived transfurants. After hybridization, the filters were washed with 2× SSC-0.1% SDS at room temperature and with 0.1% Ficoll-0.1% polyvinylpyrrolidone-0.1% bovine serum albumin-100 µg/ml denatured salmon sperm DNA. The DNA fragments to be sequenced were eluted from the gel by electroelution and ligated to the PstI-digested, double stranded M13mp10 DNA. The competent cells of E. coli JM103 [Δ(lac pro), thi, strA, supE, endA, sbcB, hsdR^, hsdM^, gal^, met^, supE] and the genomic library was constructed. The human sequence-containing plaques were obtained by three rounds of screening of the 10^9 plaques of the library with human Alu sequence probe.

DNA Sequence Analysis. DNA from one of the positive clones after the third round screening was digested with restriction enzyme PstI and fractionated by electrophoresis through 4% polyacrylamide gel. The DNA fragments to be sequenced were eluted from the gel by electroelution and ligated to PstI-digested, double stranded M13mp10 DNA. The competent cells of E. coli JM103 [Δ(lac pro), thi, strA, supE, endA, sbcB, hsdR^, F' traD36, prosB, lac^, ZM15] were transformed with the recombiant phage DNAs and grown in X-gal-containing medium. The single stranded DNAs in the white plaques, which were further identified by in situ hybridization with c-Ha-ras probe, were amplified, extracted with phenol/chloroform, and sequenced by using the dideoxynucleotide chain termination procedure (31, 32).

RESULTS

Identification of the Transforming Gene. Rat 1 and NIH 3T3 cells were transfected with high molecular weight DNAs from gastrocarcinoma cell lines BGC-823, PACM-82, and MGC 80-3 and stomach cancer tissues of different patients. We found that for these DNA samples, DNA from BGC-823 showed the highest transforming potency; the efficiencies for BGC-823, PACM-82, MGC 80-3, and tumors from two patients were 0.17, 0.14, 0.05, 0.02, and 0.04 foci/µg DNA, respectively, while that of the negative control was 0.02 foci/µg DNA. Thus we focused our work on BGC-823 DNA transfection. DNAs from the primary foci were used to transfet Rat 1 and NIH 3T3 cells. The transforming efficiencies of the secondary trans-
fection were much higher (0.35–0.77 foci/µg DNA) than those of the first. The transforming phenotypes of both the primary foci and the secondary foci were tested by soft agar growth assay and tumorigenesis of the nude mice. DNAs from four Rat 1-derived secondary foci, which showed transforming characters in soft agar growth assay and tumorigenesis of the nude mice, were digested with restriction endonuclease EcoRI, Southern transferred, and hybridized with Alu sequence as described above. Fig. 1A shows the pattern of the hybridization of DNA samples from four secondary foci, Rat 3-1, Rat 3-2, Rat 3-3, and Rat 3-4. It showed that all of them retained their human DNA sequence and one of the foci, Rat 3-3, kept a single band after hybridization. Therefore only one or a few DNA fragments were incorporated into the genome. Rate 3-3 DNA was then digested with BamHI and subjected to Southern analysis with a c-Ha-ras probe (Fig. 1B). The result showed that after digestion with BamHI, both Rat 3-3 and BGC-823 DNA could be hybridized with the probe at the position of 6.6 kilobase pairs, while Rat 1 DNA did not have the c-Ha-ras fragment at this position. Thus, we presumed that the transforming gene of BGC-823, which has the ability to transform Rat 1, is homologous to protooncogene c-Ha-ras. When DNA samples from the secondary foci were hybridized with the probes of v-Ki-ras, N-ros, and c-myc, no extra bands were detected (data not presented here).

Molecular Cloning of the Transforming Gene. Rat 3-3 DNA was partially digested with BamHI and ligated to EMBL 3 DNA. After in vitro packaging, the packaging efficiency was titrated as $1.3 \times 10^8$ plaque forming units/µg DNA, and $10^6$ plaques were screened with human Alu sequence as the probe. Two clones, λ 120 and λ 151, were obtained, which could hybridize with not only human-specific DNA sequence but also c-Ha-ras probe in further experiments. DNAs were extracted from these two plaques, digested with restriction enzyme BamHI, and subjected to agarose gel electrophoresis. Fig. 2A shows the electrophoresis patterns of λ 120 and λ 151 DNAs digested with BamHI. It is clear that λ 120 contains 8.6-, 6.6-, and 2.5-kilobase pair fragments, while λ 151 contains 6.6- and 6.2-kilobase pair fragments. After these fragments were blotted and hybridized with c-Ha-ras, only 6.6-kilobase pair fragments showed autoradiographic bands (Fig. 2B). In another assay, when these fragments were hybridized with human Alu probe, 8.6- and 2.5-kilobase pair fragments of λ 120 and 6.2-kilobase pair fragments of λ 151 showed autoradiographic bands, while 6.6-kilobase pair fragments of both λ 120 and λ 151 did not (data not shown). Through partial digestion, the order of these four BamHI fragments were determined. Fig. 2C shows the alignment of these fragments in the genome of Rat 3-3 and the cloning spans of λ 120 and λ 151.

The 6.6-kilobase pair fragment from λ 120 was subcloned into pBR322, and the recombinant plasmid was designated as pGC6.6. When 1 µg of pGC6.6 DNA and 74 µg of calf thymus DNA were used to transfect Rat 1 cells, 60 foci/dish were
formed. The efficiency was 480 foci/μg DNA, about 2800 times higher than that in the primary transfection. This indicates that the cloned oncogene has much stronger potency in transforming fibroblast cells than the genomic DNA from the gastrocarcinoma cell line BGC-823. The mutated nucleotides and amino acid are underlined.

Fig. 3. Procedure for sequencing Pstl 371-base pair (bp) and Pstl 299-base pair fragments. In A, 20 μg of λ 120 DNA were digested with Pstl and subjected to electrophoresis in 4% polyacrylamide gel. B, strategy for sequencing Pstl 371-base pair fragments. In A, two fragments from both directions. Fig. 4 is the nucleotide sequence of the Pstl 371-base pair fragment of the transforming gene compared with nontransforming c-Ha-ras in the same area. It is obvious that these two sequences are very similar except two places. The first one is at the 10th nucleotide upstream from the beginning of the reading frame. As pointed by Reddy (35) and Capon et al. (36) that this difference comes from polymorphism of the gene only and it does not produce any impact on the activation of the gene. The second mutation point is located at the 12th codon, it changes the codon GGC of the protooncogene c-Ha-ras to codon GTC of the transforming Ha-ras and leads to the alteration from glycine at the 12th amino acid of M, 21,000 protein in the normal cells to valine in the stomach cancer cells. The Pstl 299-base pair fragment, in which the 61st codon is located, was sequenced as mentioned above. No difference exists between nontransforming c-Ha-ras and the transforming gene.

DISCUSSION

Gastrocarcinoma is the malignant tumor with the highest incidence in China, Japan, and some countries in Europe. Thus, it becomes an important work for these countries to understand the mechanisms of carcinogenesis of stomach cancer at the molecular level. In this report, we showed that the activated form of protooncogene c-Ha-ras had been cloned from a gastrocarcinoma cell line BGC-823. Since this oncogene contains a guanine to thymine transversion at the 12th codon, we concluded that the point mutation at the 12th codon is at least one of the forms of c-Ha-ras activation in this stomach cancer cell line. Besides, the cloning of another oncogene, c-raf-1, from stomach cancer was also reported (37). Furthermore, the amplification of c-myc (38), c-erbB-2 (39), and c-Ha-ras \(^4\) and elevated expression of c-Ha-ras \(^5\) and c-erbB-2 (39) were also found in solid tumors or cell lines of gastrocarcinoma. More data are necessary to illustrate all the changes of oncogenes during the entire process of carcinogenesis of stomach cancer.

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REFERENCES

8. Der, C. J., Krontiris, T. G., and Cooper, G. M. Transforming genes of human human

\(^4\) Y. Andeol, personal communication.

\(^5\) X. Jin, unpublished observation.
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