Differences in Expression and Regulation Between Transformed Cells of the Human Gastrointestinal Carcinoma Oncogene Ha-ras and the Untransformed Parent Cells

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An Ha-ras oncogene was isolated from a cell line of gastric carcinoma called BGE-823 in order to elucidate genetic control and the influence of DNA sequences. The oncogene was cloned and identified as a single nucleotide substitution of thymine for guanine in the 12th codon through the sequencing of its first exon. We compared the differences of expression and regulation between the transformed Ha-ras cells and untransformed parent cells. Data indicated that the expression of Ha-ras in the transformed cells was five-fold higher than in the untransformed cells and that the Ha-ras gene in the former was hypersensitive toward DNase I. In addition, a nuclear protein of 35 kilodaltons bound strongly to the 2.5 Kb fragment located upstream of the 6.6 Kb Ha-ras gene and contained a GC rich region. These results suggest that there might be another mechanism of activation for the ras gene besides point mutation.

The ras genes were first characterized as the transforming genes of Harvey and Kirsten sarcoma viruses. The ras gene family (Ha-ras, Ki-ras and N-ras) is conserved in mammalian genomes and encodes structurally related proteins of approximately 21 kilodaltons which are associated with the inner side of plasmic membranes, bind GDP and GTP, and exhibit GTPase activity. Many activated ras genes from human tumors or tumor-derived cell lines have been analyzed and found to contain an amino acid substitution at or near the position of 12 or 61. It is generally believed that there are some promoters and enhancers that control oncogene expression in carcinogenesis. Although indirect data have implied that the expression of ras genes may be under the control of a regulatory region, there is little direct evidence at this time.

In a previous study, mouse and rat fibroblast cell lines NTH 3T3 and Rat-1 were transfected with total DNA extracted from a human gastric carcinoma cell line BGE-823. The malignant phenotypes of the transformed cells were evaluated by the soft agar growth assay and through tumorigenesis in nude mice. A genomic library was constructed using EMBL-3 as the vector. Three BamHI digested fragments, namely 8.8 Kb, 6.6 Kb, and 2.5 Kb were isolated from the clone 120, which was homologous to the proto-oncogene c-Ha-ras. The 6.6 Kb fragment contained four exons of ras. By comparing the sequence of the first exon with that of the proto-oncogene, the activation lesion of the gene was identified as a single nucleotide
substitution of thymine for guanine in the 12th codon. The 2.5 Kb fragment which contains the Alu sequence, was subsequently found to be located upstream to the 6.6 Kb fragment. The present study shows that activation of c-Ha-ras is not only triggered by qualitative changes: point mutation, but also by quantitative changes: overexpression. Data is presented that implies that there might be a possible way to control the quantitative changes in carcinogenesis.

MATERIALS AND METHODS

Rat-1 cell line was a gift of R. Weinberg. Rat3-3 cell line (transformed with BGE-c-Ha-ras gene) was provided by the Laboratory of Genetics of our institute. The BGE-c-Ha-ras fragment (6.6 Kb) and the fragment upstream to it (2.5 Kb) were cloned and prepared in our laboratory. Nude mice were obtained from the Research Center of Experimental Animals, the Chinese Academy of Medical Sciences.

Chemicals and enzymes were purchased as follows: [α-32P]dATP [α-32P]dCTP, from Amesham; deoxyribonucleoside triphosphates, heparin, proteinase K, HEPES, EGTA, PMSF and DTT from Sigma; DNase I and restriction enzymes from Promega Biotec; Ficoll from Pharmacia/P-L; Triton X-100 from ROTH; Salmon sperm DNA from the Shanghai Institute of Biochemistry.

Preparation of mRNA

Freshly dissected tumor tissue was homogenized in cold extracting solution (10 mM sodium acetate, pH 5.0, 3 M LiCl, 0.1% SDS 6 M urea, 2 mg/ml heparin, 10 mM ribonucleoside vanadyl sulfate) at high speed for one minute. The homogenate was stored overnight at -20° C and then centrifuged at 15,000 rpm in a 3N rotor of a TOMY, RS20III centrifuge at 4° C for 25 minutes. To the aqueous phase, 1/10th of 1 volume of 3 M sodium acetate, pH 6.0 and 2.5 volumes of cold ethanol were added. After mixing, the sample was stored overnight at -20° C. This extraction procedure was repeated twice and then the poly (A+) RNA was separated on a column of oligo(dT)-cellulose (0.9 × 2 cm). The column was washed with 0.1 M KOH and 1 M Tris-Cl, pH 7.4 and equilibrated with 10 mM Tris-Cl, pH 7.4, 1 mM EDTA and 0.5 M NaCl. Samples were applied to the column in the same buffer and then eluted with the same buffer. Finally, poly (A+) RNA was eluted with 10 mM Tris-Cl, pH 7.4 and 1 mM EDTA.

Preparation and DNase I Treatment of the Nuclei

Freshly dissected tumor tissue or cells were homogenized in TKM buffer (50 mM Tris-Cl, pH 7.5, 20 mM KCl, 5 mM MgCl2) with 0.5 M sucrose and centrifuged for 10 minutes at 4,000 rpm (3N rotor) in 4° C. The precipitate was suspended in TKM buffer with 0.5% Triton X-100, overlayed on TKM buffer with 0.88 M sucrose and centrifuged as above. The precipitate obtained was resuspended in TKM buffer with 2.0 M sucrose and centrifuged for 40 minutes at 10,000 rpm (all in a 3N rotor if no special indication existed). The pellet was suspended in buffer containing 10 mM Tris-Cl, pH 7.5 and 0.35 M sucrose.