ras Gene Mutations in Human Prostate Cancer

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ABSTRACT

Point mutations at codons 12, 13, or 61 of the Ha-, Ki-, and N-ras genes are able to convert these normal cellular genes into activated oncogenes. Previous studies have shown that ras gene mutations occur in a variety of human solid tumors and may be important in the pathogenesis of some of these tumors. In order to test the hypothesis that ras gene mutations may be associated with prostate cancer, we have used an oligodeoxynucleotide hybridization assay to detect wild-type and mutant alleles in genomic DNA from prostate tumors and prostate tumor cell lines amplified using the polymerase chain reaction. Twenty-four primary prostate tumors (23 acinar tumors and one ductal tumor) and five prostate tumor cell lines were examined for mutations at codons 12, 13, and 61 of the Ki-ras, Ha-ras, and N-ras genes. Two mutations were detected: an A → G transition causing a glutamine to arginine amino acid substitution at codon 61 of the Ha-ras gene in a primary prostatic duct adenocarcinoma and a G → T transition causing a glycine to valine amino acid substitution at codon 12 of the Ha-ras gene in a primary prostatic duct adenocarcinoma.

INTRODUCTION

Specific point mutations in the human ras gene family have been implicated in the development of a variety of human tumors (1–6). The ras gene family consists of the human Ha-ras, Ki-ras, and N-ras genes which encode a set of homologous guanosine triphosphate/diphosphate-binding proteins with molecular weights of 21,000 termed p21 (7). The resemblance of the normal p21 protein to G proteins controlling adenylate cyclase has implicated p21 as being involved in signal transduction across cellular membranes (8, 9). Current hypotheses suggest that in some human tumors spontaneous mutations at codons 12, 13, or 61 of the ras gene alter the p21 protein product so that it can stimulate autonomous growth leading to transformation (10). Several assays have been developed to detect ras gene mutations in human tumors including the NIH/3T3 transfection assay and, recently, the more sensitive molecular assays, oligodeoxynucleotide hybridization, and RNase A mismatch cleavage (11–13). As detected by these assays, there is considerable variation in the frequency and specificity of ras gene mutations across tumor types. For example, 95% of human pancreatic adenocarcinomas and 40% of human colon cancers demonstrate point mutations at codon 12 of the Ki-ras gene, whereas ~5% of human breast cancers and renal cancers contain mutations in any of the three ras genes (5, 6, 14, 15). It appears that the role of ras gene mutations in the development of various cancers may be quite different.

Our goal has been to study the extent to which ras gene mutations are found in prostate cancer. One previous study of eight prostatic adenocarcinomas using the NIH/3T3 transfection assay detected one specimen containing an activated Ki-ras gene (16). The NIH/3T3 assay, however, may underestimate the frequency of ras gene mutations especially when screening for mutations in large oncogenes such as Ki-ras which are particularly sensitive to DNA degradation. Also, the admixture of normal tissue with tumor tissue may dilute any positive signal from neoplastic cells (7). In addition, the labor-intensive nature of the NIH/3T3 transfection assay prohibits screening large number of tumors. Recently developed molecular methods for detecting ras gene mutations are more sensitive than the NIH/3T3 assay and are capable of detecting mutations when as few as 5% of the cells in the sample contain the mutation (13, 17). In this study, we describe the use of differential oligodeoxynucleotide hybridization combined with the polymerase chain reaction to assess the frequency of point mutations at codons 12, 13, and 61 of the Ha-ras, Ki-ras, and N-ras genes in 29 primary prostate tumors and prostate tumor cell lines.

MATERIALS AND METHODS

All tumor specimens were obtained from patients undergoing radical prostatectomy for prostate cancer at the Johns Hopkins Hospital between January 1, 1989, and December 31, 1989. The resected prostate was placed on ice and sections of tumor and normal prostate were taken for cryostat examination by a pathologist. Frozen sections of the front and back of each slice were evaluated for the percentage of tumor cells in each slice. Each slice was frozen in liquid nitrogen and stored at −70°C until nucleic acid extraction. Tumor slices in which at least 80% of the cells examined were tumor cells were selected for this study. Five prostate cancer cell lines derived from one primary (PC-82) and four metastatic (TSU-PR1, LNCaP, PC-3, DU 145) prostate tumors were also selected for analysis (18–22).

As positive controls we also analyzed three sources of DNA containing known Ki-ras and Ha-ras mutations. Two of the positive controls were obtained from human colonic adenocarcinomas with mutations at codons 12 and 13 of the Ki-ras gene. The third positive control was DNA obtained from the T24 human bladder cancer cell line containing a G → T transversion at codon 12 of the Ha-ras gene (23). High molecular weight DNA was extracted from frozen tissue and cell lines as previously described (24). The regions of the human Ha-, Ki-, and N-ras genes containing codons 12, 13, and 16 were selectively amplified using the polymerase chain reaction (25). Polymerase chain reaction primer sequences were the following: Ha-ras codons 12 and 13: 3'-CCCAACGCTCACCTTCTATA, 5'-ATGACGGAATATAAGCCCTTGTG; Ha-ras codon 61: 3'-AGGGAAGGCTTCCAGCCTGG, 5'-AGGGTCATCTGATGAGAG; Ki-ras codons 12 and 13: 3'-CTCCTGATTGGTGATATT, 5'-ATGATCAATATATATGCTT; Ki-ras codon 61: 3'-AGAAGGCCCCTCCAGCTCT, 5'-AAGTGAAT-TATGGTGAAGAA; N-ras codons 12 and 13: 3'-CTCCTATG-GGGATATATT, 5'-ATGATCAGTACAAACTGG; N-ras codon 61: 3'-AGAAGGCTTCCAGCTCT, 5'-CAGGTAGATAGATGGTGA. These primers flank codons 12, 13, and 61 of the Ki-, Ha-, and N-ras genes and allow the amplification of an approximately 100-base pair fragment of genomic DNA containing 3' AGGGAAGGCTTCCAGCCTGG; 5'-AGGGTCATCTGATGAGAG;...
the codons of interest. Following amplification, the reaction products were examined on a 4% agarose gel by ethidium bromide staining to verify the presence of a 100-base pair amplification product for each sample. The amplified DNA was slot blotted onto a nylon membrane and tested for point mutations by hybridization with sequence-specific oligodeoxynucleotide probes as previously described by Verlaan-de Vries et al. (13). A panel of end-labeled 20-mer oligodeoxynucleotide probes corresponding to the wild-type sequence and to all known activating mutations at codons 12, 13, and 61 of the Ki-ras, Ha-ras, and N-ras genes was used for analysis of each sample.

RESULTS

Twenty-four primary tumor samples were obtained from patients undergoing radical prostatectomy for clinically localized prostate cancer. Lymph node metastases were documented in eight of these patients after full pathological review of the cases. Pathological characteristics of the tumors are shown in Table 1. Five prostate cancer cell lines derived from one primary and four metastatic prostate cancers were also studied.

DNA from the 24 primary prostate adenocarcinomas and 5 prostate tumor cell lines as well as three positive control sources with known ras gene mutations was selectively amplified at codons 12, 13, and 61 of the Ki-, Ha-, and N-ras genes using the polymerase chain reaction. The presence of amplified products from each reaction was verified by agarose gel electrophoresis and ethidium bromide staining (data not shown). The amplified DNA was slot blotted onto nylon membranes and screened for point mutations using synthetic oligodeoxynucleotide probes for all known activating mutations at codons 12, 13, and 61 of the Ha-, Ki-, and N-ras genes.

Two mutations were detected in the prostate tumor samples analyzed: one in a primary prostatic ductal adenocarcinoma and one in a metastasis-derived cell line. Fig. 1 shows the detection of an A — G transition resulting in a glutamine to arginine amino acid change at codon 61 of the Ha-ras gene in a prostatic duct adenocarcinoma. The relative strength of the signal from the mutant allele compared to the wild-type allele suggests that this mutation is present in the majority of cells in the tumor sample. Fig. 2 shows the detection of a mutation in the TSU-PR1 cell line which revealed a G — T transversion at codon 12 of the Ha-ras gene causing a glycine to valine amino acid substitution. No mutations were detected in any samples in the Ki-ras or N-ras genes.

DISCUSSION

Models of prostate carcinogenesis have provided some evidence for a role of the ras oncogene in prostate tumor development. Thompson et al. (26) showed that ras and myc oncogenes can cooperate to induce carcinogenesis when introduced into the cells forming a reconstituted mouse prostate gland. Treiger and Isaacs (27) demonstrated that the transfection of the v-Ha-ras gene into Dunning rat prostate tumor cell lines increases the metastatic capability of these cells. In studies of human tumors, Viola et al. (28) reported that ras p21 protein expression was correlated with pathological stage in prostate cancer, although the monoclonal antibody used in the same study was shown to be nonspecific in later work (29). Thus, although data from human studies has been inconclusive, animal studies have provided evidence that the ras oncogene may be involved prostate tumorigenesis and progression.

Using a sensitive assay to detect activating ras gene mutations at codons 12, 13, and 61 of the Ha-, Ki-, and N-ras genes in human prostate tumors, we find an overall prevalence of such mutations of <10%. The low overall percentage of prostate tumors containing these mutations is in contrast to pancreatic and colon cancers which frequently have Ki-ras gene mutations but similar to breast and renal cancers where there is also a low frequency of any ras mutations.

A mutation resulting in a glutamine to arginine amino acid substitution was detected in a prostatic duct adenocarcinoma. Ductal adenocarcinomas are rare (<1% of all prostate cancers).
variants of prostate cancer which are generally more aggressive than ordinary (acinar) prostatic adenocarcinomas. The average survival of patients with prostatic duct adenocarcinoma when treated conservatively is only 36 months; when treated aggressively by radical prostatectomy these patients have tumors with an advanced pathological stage and a much higher short-term failure rate postsurgery compared to acinar cancers (30-32). Only one such tumor was available for analysis in the present study making it difficult to infer that this is a usual feature of prostatic duct adenocarcinomas, although the aggressive nature of these tumors makes this an interesting possibility.

In addition, in the TSU-PR1 prostate cancer cell line, derived from a prostate cancer lymph node metastasis, a mutation in the 12th codon of the Ha-ras gene leading to a glycine to valine amino acid substitution was found. Since this mutation was detected in a cell line, it is unclear whether the mutation was present in vivo or occurred during in vitro passage of the cell line. Three other metastatic prostate cancer cell lines did not have any ras gene mutations. In other tumors where a low overall frequency of ras gene mutations is seen such as breast or renal cancer, any mutations found are usually detected in DNA derived from metastases (14, 19). This may suggest that, while ras gene mutation is a relatively infrequent event in such tumors and is not required for metastatic progression, when ras mutations do occur, they may contribute to the metastatic process.

In summary, the overall frequency of ras gene mutations in prostate cancer is low. The detection of ras gene mutations in a primary prostatic ductal adenocarcinoma and in a metastasis-derived prostate cancer cell line indicates that, when ras gene mutations do occur, they may have a role in the progression of prostate cancer or the development of the unusual ductal variant of prostatic adenocarcinoma.

REFERENCES

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