Microsatellite Instability at Selected Tetranucleotide Repeats Is Associated with p53 Mutations in Non-Small Cell Lung Cancer

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ABSTRACT

Microsatellite alterations are useful clonal markers for the early detection of cancer. An increase in microsatellite instability has been observed at certain tetranucleotide repeat markers (AAAG)1 in lung, head and neck, and bladder cancer. However, the genetic mechanism underlying these elevated microsatellite alterations at selected tetranucleotide repeat (EMAST) tumors is still unknown. The p53 gene plays an important role in maintaining genome integrity by repairing damaged DNA. Therefore, we tested 88 non-small cell lung cancers with a panel of 13 microsatellite markers previously shown to exhibit frequent instability and also performed p53 sequence analysis in these tumors. Thirty-one of these 88 cancers (35%) demonstrated a novel allele [EMAST(+)] in ≥1 of these 13 microsatellite markers. p53 mutations were detected in 50 of 88 (57%) cancers and were significantly (P = 0.001) more common in EMAST(+) tumors (25 of 31; 81%) than in EMAST(−) tumors (25 of 57; 44%). Among squamous cell cancers, p53 mutations were detected significantly (P = 0.04) more frequently in EMAST(+) tumors (17 of 19; 89%) than in EMAST(−) tumors (10 of 18; 55%). Similarly, among primary adenocarcinomas, p53 mutations were present in 67% of the EMAST(+) tumors and in 35% of EMAST(−) adenocarcinomas. None of the 31 EMAST(+) tumors demonstrated high frequency microsatellite instability when examined with a reference panel of five mono- and dinucleotide markers. Primary lung cancers with microsatellite alterations at selected tetranucleotide repeats have a high frequency of p53 mutations and do not display a phenotype consistent with defects in mismatch repair.

INTRODUCTION

MSI1 was initially identified in colorectal cancer and was immediately clinically significant because of its association with HNPCC (1). MSI is thought to be caused by a failure of the mismatch repair system to repair errors that occur during DNA replication (1). Several mismatch repair genes have been identified (hMLH1, hMSH2, hPMS1, hPMS2, and hMSH6), and their inactivation has been well-characterized in HNPCC and occasionally in other nonpolyposis colon cancer; NSCLC, non-small cell lung cancer; EMAST, elevated microsatellite alterations at selected tetranucleotide repeats; MSI-H, MSI-high frequency.
marker from each primer pair was labeled with T4 polynucleotide kinase (New England Biolabs). PCR amplification was performed in separate reactions with 60 ng of DNA isolated from the tumor, and control non-neoplastic DNA as described above. Products were separated in 8% denaturing urea-polyacrylamide-formamide gels followed by autoradiography.

p53 Sequencing. Mutation analysis of the p53 gene was performed on all 88 lung cancers by both direct dideoxy nucleotide sequencing and using the GeneChip p53 assay as described (7). A 1.8-kb fragment of the p53 gene (exons 5–9) was amplified from primary tumor DNA in all 88 patients by PCR (8, 9). The PCR products were purified and sequenced directly using cycle sequencing (Amplicycle sequencing kit, Perkin-Elmer, Branchburg, NJ), and the products of the sequencing reactions were then separated by electrophoresis in 8% urea and 6% polyacrylamide gels, fixed, and exposed to film. In addition, exons 2–11 of the p53 gene from all 88 tumors were sequenced using the GeneChip p53 assay as described (7).

Statistical Analysis. Comparisons between groups were performed using the Fisher’s exact test (two-tailed).

RESULTS

Pathological Characteristics. Pathological characteristics from 88 patients undergoing pulmonary resection for NSCLC were reviewed. Forty-four patients had stage I disease (26 stage Ia and 18 stage Ib), 24 patients had stage II NSCLC (3 stage IIa and 21 stage IIb), and 20 patients had stage III disease. Mean tumor size was 3.8 cm. The cell type of the 88 NSCLCs included squamous cell cancer (n = 37), adenocarcinoma (n = 44), adenosquamous carcinoma (n = 1), large cell carcinoma (n = 4), and poorly differentiated NSCLC (n = 2).

Microsatellite Alterations. Thirty-one of these 88 cancers (35%) demonstrated a novel allele [EMAST(1)] with at least 1 of 13 microsatellite markers (Fig. 1). Twenty-two of 31 EMAST(+) tumors had MSI at 1 marker, whereas 9 of the EMAST(+) tumors demonstrated microsatellite alterations at ≥2 of 13 markers. These microsatellite “shifts” were seen in 12 tumors (14%) at L17686, 7 tumors (8%) at D20S82, 6 (7%) tumors at UT5320, 4 (4%) tumors at 8S321, 3 (3%) tumors at L17835 and D20S85, 2 (2%) tumors at two markers (UT5307 and D9S242), and in 1 tumor (1%) at four markers (G29028, D11S488, ACTB2, and GO8460). Squamous cell cancers were significantly more likely to be EMAST(+) than adenocarcinoma of the lung (51% versus 20%; P = 0.006). No significant association between patient age, race, gender, tumor size, tumor stage, and tumor differentiation and EMAST status was observed.

All 31 EMAST(+) tumors were examined with a reference panel of five mono- and dinucleotide markers used for determining MSI. Only 1 of 31 EMAST(+) tumors (3%) had instability at a single marker (low frequency microsatellite instability), and the remaining 30 tumors were microsatellite stable.

EMAST and p53. p53 mutations were detected in 50 of 88 (57%) patients with NSCLC. Mutations of the p53 gene were found significantly (P = 0.006) more often in squamous cell cancer (27 of 37 (73%)) than in adenocarcinoma (18 of 44 (41%)). Thirty-three (66%) of the p53 mutant tumors contained a missense mutation, eight (16%) contained a nonsense mutation, five (10%) contained a deletion, and four (8%) contained a splice site mutation.

The relationship between EMAST positivity and mutation of the p53 gene is shown in Table 1. p53 mutations were significantly more common in EMAST(+) than in EMAST(−) NSCLC (81% versus 44%; P = 0.001). Among squamous cell cancers, p53 mutations were also significantly (P = 0.04) more common in EMAST(+) than in EMAST(−) tumors (89% versus 55%). A similar trend was observed with adenocarcinoma of the lung, but this difference did not reach statistical significance (67% versus 35%; P = 0.15). Seven of nine tumors (78%) with MSI at two or more markers contained a p53 mutation.

Eighty % (4 of 5) of the tumors containing a deletion in the p53 gene were EMAST(+), whereas only 47% (21 of 45) of tumors containing a single bp mutation were also EMAST(+). Only 16% of the tumors with wild-type p53 were EMAST(+).

DISCUSSION

Although widespread genome wide MSI is rare in NSCLC, microsatellite alterations at selected tetranucleotide repeats are not uncommon in primary lung cancer. Interestingly, these microsatellite alterations were more common in squamous cell cancer than in adenocarcinoma of the lung. Furthermore, EMAST(+) tumors were significantly more likely to harbor a p53 mutation than EMAST(−) cancers.

Microsatellite alterations have become useful clonal markers for
the early detection of cancer. The generation of novel alleles through the insertion or deletion of a short tandem repeat creates a new DNA marker present only in neoplastic and not in nonneoplastic cells. These markers are easier to detect in clinical samples than subtle changes in loss of heterozygosity and have shown promise in the early detection of bladder, lung, and head and neck cancers in preliminary studies (5, 10–15). The detection of novel microsatellite alleles in the urine has been used to identify both primary and recurrent bladder cancer (11, 12). In several cases, these microsatellite alterations have preceded any clinically detectable (urine cytology, cystoscopy) signs of cancer. In addition, tumor-specific microsatellite alterations have been detected in the bronchoalveolar lavage fluid, sputum, and plasma of patients with lung cancer, suggesting that these alterations may also be potential markers for the early detection of this common malignancy (5, 14, 15).

The association between gene alterations and MSI has been evaluated previously in several tumor types. Frameshift mutations within mononucleotide repeat sequences in several genes (TGFβ-II, BAX, IGFRII, E2F-4, and TCF-4) have been identified in MSI-H colon cancer (16–20). The human BAX gene, which promotes apoptosis, is inactivated by frameshift mutation in ~50% of HNPCC syndrome colon cancers (17, 19). Rampino et al. (17) have suggested that BAX gene inactivation downstream from p53 in the apoptotic pathway would eliminate the selective pressure for p53 gene mutations in colorectal tumorigenesis. Indeed, the incidence of p53 gene mutations is lower in MSI-H colon cancer than in sporadic colon cancer (17, 19).

In contrast to colon cancer, a strong correlation between MSI and p53 mutations has been reported in ovarian cancer (21). MSI was more frequent in tumors containing an insertion or deletion mutation than in tumors with a missense mutation, and 50% of the deletions occurred at iterated sequences or direct repeats (16). Sood et al. (21) concluded that the pattern of p53 mutations (insertions/deletions at iterated bases) observed in ovarian cancer was consistent with DNA strand slippage during replication, and therefore, p53 mutations were likely caused by a generalized genomic instability rather than being the cause of genomic instability. In contrast to these findings, no association between MSI and p53 mutations has been found in gastric cancer, and it was also not observed in the present series of NSCLCs using the Bethesda Conference criteria for defining MSI (22, 23).

MSI attributable to a defect in a DNA repair pathway distinct from mismatch repair has been hypothesized but remains undiscovered. The p53 protein plays a central role in the repair of genetically damaged cells. In response to DNA damage, p53 protein levels rapidly increase by a posttranscriptional mechanism (24). The induction of p53 also results in the transcriptional activation of other cell cycle regulatory genes, such as p21WAF1/CIP1 leading to arrest of the cell cycle at the G1/S checkpoint (24). Loss of functional p53 protein leads to a failure of cell cycle arrest and genomic instability, as evidenced by inopportune gene amplification, aneuploidy, and/or chromosome loss through DNA strand breakage and rejoining (4, 24, 25). The inability to delay cell division and allow adequate DNA repair to take place increases the probability that DNA damage will remain uncorrected during DNA replication, leading to the proliferation of genetically damaged cells (4). The failure to repair deletions or mutations in critical tumor suppressor genes can provide affected clones with a growth advantage. In this study, microsatellite alterations at selected tetranucleotide repeat markers were more commonly present in tumors containing a p53 mutation. These clonal alterations in the noncoding DNA at the microsatellite markers examined are unlikely to provide any growth advantage to the tumor and may simply reflect the defective DNA repair capability of p53 mutant cells. However, an increased mutation rate at tri- or tetranucleotide repeat sequences within genes regulating cell growth may provide a growth advantage to p53 mutant cells. Germ-line studies have documented a higher background rate of instability in larger (tri- or tetranucleotide) microsatellites, which perhaps explains why these particular microsatellites are commonly altered in p53 mutant primary tumors (26, 27).

Microsatellite alterations at selected tetranucleotide markers are common in non-small cell lung, head and neck, and bladder cancer. This study suggests that the underlying mechanism leading to EMAST(+) tumors differs from MSI attributable to defective mismatch repair and is more likely related to abrogation of a p53-dependent repair pathway. A better understanding of this pathway leading to the EMAST phenotype in primary tumors may help further define the role of this diagnostic strategy in cancer screening. The widespread presence of p53 mutations in human cancer suggests that these markers may also be useful in the molecular detection of other tumor types.

REFERENCES


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