

# Absence of *ST7* Gene Alterations in Human Cancer

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## ABSTRACT

The *ST7* gene was cloned and mapped to chromosome 7q31.1-q31.2, a region suspected of containing a tumor suppressor gene involved in a variety of human cancers. Subsequent investigation described the presence of *ST7* mutations in human cell lines derived from breast tumors and primary colon carcinoma. Introduction of the *ST7* cDNA into a prostate cancer-derived cell line abrogated *in vivo* tumorigenicity in nude mice. To clarify the role of the *ST7* gene in cancer, we scrutinized primary head and neck squamous cell carcinomas, invasive ductal carcinomas of the breast, and adenocarcinomas of the colon. Loss of heterozygosity of D7S522/D7S677 was detected in 24% (4 of 17) of head and neck squamous cell carcinomas, 17% (2 of 12) of invasive ductal carcinomas of the breast, and 33% (8 of 24) of adenocarcinomas of the colon, but no somatic mutations were found in any of these specimens. We then searched for mutations in breast cancer cell lines and found a complete wild-type sequence in all, including cell lines previously reported to harbor mutations. We believe that the *ST7* gene is not a primary target of inactivation in most human cancers with loss of heterozygosity at 7q31.1-q31.2.

## INTRODUCTION

Genetic alterations of the human chromosomal region 7q are common in human cancer (1). LOH<sup>2</sup> of the 7q31-q32 region has been reported in breast, prostate, pancreatic, ovarian, gastric, colon, and head and neck cancer, as well as uterine leiomyomas and malignant myeloid disease (2–13). Furthermore, the introduction of an intact copy of human chromosome 7 into immortalized human fibroblasts cell lines with LOH/allelic imbalance at 7q31-q32 restore programmed senescence to the cells (14). In addition, transfer of human chromosome 7 inhibits tumorigenicity in most tumor explants and complete suppression in others

(15, 16). These findings suggest the presence of a broad range TSG on human chromosome 7q31-q31.2.

In search of a TSG in the 7q31 critical region, LOH and microcell fusion studies narrowed the region, and a positional cloning strategy identified a candidate suppressor gene, named *ST7*, that is involved in a variety of human cancers (17). Moreover, somatic mutations of *ST7* were reported in human cell lines derived from breast cancer and primary colon carcinomas. Breast tumor cell lines (MDA-MB435s, T-47-D, and MDA-MB231) and 40% of primary colon carcinomas with LOH of D7S522/D7S677 were reported to harbor mutations predicted to yield a truncated *ST7* protein.

Because we (18) and others (12, 19–21) found evidence of LOH 7q31, we investigated a series of primary HNSCCs, invasive DCB, and adenocarcinomas of the colon in search of *ST7* mutations.

## MATERIALS AND METHODS

**Samples and DNA Extraction.** A series of 17 primary HNSCCs, 12 invasive DCB, and 24 colon primary carcinomas were obtained for *ST7* screening. Seventeen primary HNSCCs were selected that showed LOH of 7q22-q31 region from our previous study (18). Twelve primary breast carcinomas were obtained from the Department of Pathology, University campus BioMedico (Rome, Italy), and 24 primary colon carcinomas were obtained from Johns Hopkins Hospital (Baltimore, MD). We also tested three breast tumor-derived cell lines reported to harbor mutations of *ST7* (MDA-MB435s, T-47-D, and MDA-MB231), purchased from American Type Culture Collection. Tumor tissue was selected from an area with greater than 75% malignant cells. DNA was purified by phenol-chloroform extraction and ethanol precipitation and dissolved in 50  $\mu$ l of distilled water, as described previously (22).

**Microsatellite Analysis.** DNA from tumor and normal control was examined for LOH by PCR-based microsatellite analysis. Markers D7S522 and D7S677 were used to identify alterations 7q31.1. PCR conditions and criteria for LOH and homozygous deletion were described previously (23).

**Sequence Analysis.** We carried out manual genomic sequencing. We designed intronic primers that included the intron/exon boundary for sequence analysis of the *ST7* gene [GenBank accession no. AC009152 (cDNA), AC106873 (exon 1), AC002542 (exons 2–15), and AC003987 (exon 16); Table 1]. After detection of a PCR product, direct PCR sequencing reactions were performed using the Amplicycle Sequencing Kit (Perkin-Elmer, Branchburg, NJ) and sequenced on a Genomix electrophoresis apparatus. We carried out both manual and fluorescent DNA sequencing for exons 3, 5, and 12 of *ST7* in the three breast cancer cell lines. We further performed thermal cycling, followed by cloning of PCR products, with The Original TA Cloning Kit (Invitrogen, Carlsbad, CA). After purification of clones containing *ST7* exons, they were analyzed by the Sequence Analysis Facility of The Johns Hopkins University to independently confirm our results in the cell lines.

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<sup>2</sup> The abbreviations used are: LOH, loss of heterozygosity; TSG, tumor suppressor gene; HNSCC, head and neck squamous cell carcinoma; DCB, ductal carcinoma(s) of the breast.

Table 1 PCR primer sequences for sequencing

<i>ST7</i>	Sense	Antisense
Exon 1	5'-81948CCGGCTCATCTCTTCACTCT81967-3'	5'-82103AGCAGAGAAGTGGGATCCAT82084-3'
Exon 2	5'-63573TCCTTGTCTTCTCCCTTCTC63594-3'	5'-63722TTTAAATGAGAAGGACTCCACCA63700-3'
Exon 3	5'-83382CCATAAACACGCTTATTTTTCTGT83405-3'	5'-83605GCAAATAATATTGCAAAGTGAAG83582-3'
Exon 4	5'-93561AGGAAGGTGTTTTGCTGAA93580-3'	5'-93729CCATTGTTTCCCAAGGT93710-3'
Exon 5	5'-94292TTGCTTTTCTCTCTCAAAAGTGC94313-3'	5'-94492CCTCCATTTCAGAAGGATGT94472-3'
Exon 6	5'-95699TGGATTGACTTGGTGTCTCTC95720-3'	5'-95832AGCAAGATTTTCCCCACTT95813-3'
Exon 7	5'-97888CCCTGAACTCCGAAAATGACA97907-3'	5'-98065CACCCAACAGGTTCTTGACTT98045-3'
Exon 8	5'-99888CCTTGGCTTGTAAATTGATGG99908-3'	5'-100108CATCAACCTGCAGGAAACCT100089-3'
Exon 9	5'-102211AGGCAATGGGCCTCTGTAT102240-3'	5'-102416AAGCCACTGATCCCAAACAC102397-3'
Exon 10	5'-134691CGTTGGTTCTTCTGCCCTA134710-3'	5'-134884CAGGGAAAATACATCAAAGAGG134862-3'
Exon 11	5'-153117TTGCTCTTTGTACCTGCAAA153127-3'	5'-153276GCATTAGTACCGCGCAAAC153257-3'
Exon 12	5'-154650CCACCTGGATGGTTTTTGTCT154679-3'	5'-154819TAACGAGTCTGTGGGGAT154800-3'
Exon 13	5'-137606AACACAAGTGTGCTCTCTTTT173628-3'	5'-173843CATTTTAGCACCTTTTCATGCTC173821-3'
Exon 14	5'-182872TGCAGTTGGGAAGTTATGACA182892-3'	5'-183069TTTACCACACACCTCACT183050-3'
Exon 15	5'-185752CCCTTTTGGTCTTCTCCACA185771-3'	5'-185972GCTTTTATGCCCTTGGCTTT185955-3'
Exon 16	5'-4997TGGGTGGAGAGTTTGT15016-3'	5'-5195GGTGGGTGAGTGGAGGACA5176-3'

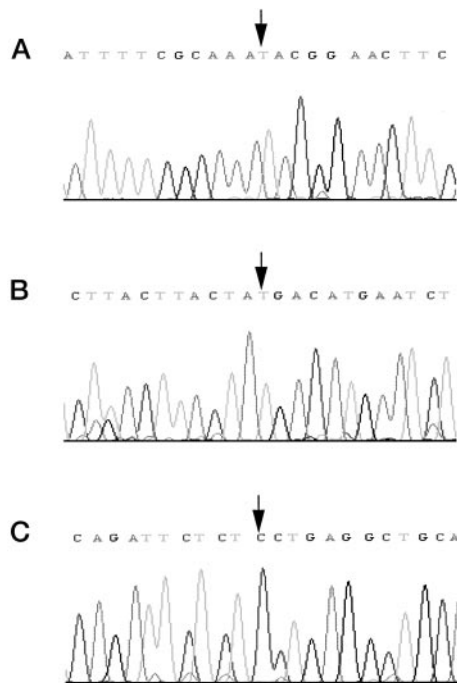


Fig. 1 *ST7* DNA sequence in tumor-derived cell lines. Exons encompassing the reported mutations in these cell lines were PCR amplification from genomic DNA with intron-specific primers. Representative DNA sequence data for AY009152 nt 456–477 of MDA-MB435s (A), nt 683–705 of T-47-D (B), and nt 1354–1375 of MDA-MN231 (C). The arrows indicate the positions of the purported mutations (466insT in MDA-MB435s, 694delT in T-47-D, and 1364insT in MDA-MB231). Although reported to harbor mutations, all of these cell lines contained a wild-type sequence. nt, nucleotide(s).

## RESULTS AND DISCUSSION

We first performed microsatellite analysis on all 53 primary tumors. LOH was found in 24% (4 of 17) of HNSCCs, 17% (2 of 12) of DCB, and 33% (8 of 24) of adenocarcinomas of the colon with markers D7S522 and D7S677 that flank the

*ST7* gene. The frequency of LOH of DCB and HNSCCs was somewhat lower than previous reports (17).

In search of *ST7* gene mutations, we sequenced all of the 53 primary tumors. None of the tumors displayed any changes in the coding regions or intron/exon boundaries. Any of these primary tumors were previously found to harbor point mutations in other TSGs (18, 24, 25). We then investigated the breast tumor cell lines MDA-MB435s, T-47-D, and MDA-MB231 to confirm previous reports of *ST7* gene alterations (17). We did not find any mutations in these cell lines (Fig. 1) by manual sequencing or automated sequencing. We further cloned the PCR products and sequenced 10 clones containing the exons where mutations were previously reported. Again, the wild-type sequence was confirmed in all three cell lines.

The majority of previously reported mutations in the *ST7* gene were identified as single base pair deletions or insertions predicted to form truncated proteins. Little is still known about *ST7* function, but true truncation mutations would be expected to abrogate suppressor function. We have found that automated sequence analysis can both underestimate mutation frequency and also results in false positives, especially if not confirmed by sequence analysis in both directions (26). At this writing, two other recent reports support the absence of *ST7* alterations in 128 human tumors (27, 28).

The complete absence of mutations in our series of primary tumors and absence of putative mutations in breast cell lines argue against a prominent role of *ST7* in these tumor types. However, our work does not exclude a tumor suppressor role for *ST7* based on the reported functional studies. Other mechanisms of inactivation such as promoter hypermethylation, homozygous deletion, or genomic rearrangements were not explored in our study but were not previously described as common mechanisms of *ST7* inactivation. It is likely that other critical TSGs remain to be identified in the commonly deleted 7q31 region.

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