

An LOH and mutational investigation of the ST7 gene locus in human esophageal carcinoma

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Frequent loss of heterozygosity (LOH) on human chromosome 7q31 has been reported in numerous malignancies. *Suppressor of tumorigenicity 7 (ST7)* has been identified as a candidate tumor suppressor gene in this region. To identify whether 7q31 and genetic alterations of *ST7* were involved in human esophageal carcinogenesis, we performed LOH mapping of a 5.4 cM region at 7q31–q35 in 43 primary esophageal carcinomas, as well as mutational analyses of the *ST7* gene in tumors with LOH in this region. Of 43 tumors, 12 (28%) showed LOH at 7q31–q35. These included four (22%) of 18 squamous cell carcinomas and eight (32%) of 25 adenocarcinomas. The peak LOH locus was D7S480, lying 4.2 Mb telomeric to *ST7* and showing LOH in eight of 37 informative tumors, or 22%. No mutations were found in the entire coding or flanking intronic regions of the *ST7* gene among 12 tumors with 7q-LOH. In addition, quantitative RT-PCR analyses of *ST7* mRNA expression levels in 11/13 normal-tumor pairs failed to show more than a 50% decrease in tumor *ST7* mRNA relative to matched normal tissues. These data suggest that LOH at 7q31–q35 is involved in the origin or progression of at least a subset of esophageal carcinomas, but that *ST7* is not the target gene of this somatic event.

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Esophageal carcinoma is one of the most common cancers worldwide, with a very poor prognosis and a marked increase in prevalence in developed countries

(Miller *et al.*, 1996). To make inroads against this deadly disease, it will be critical to clarify carcinogenic pathways underlying it, including the inactivation of tumor suppressor genes (TSGs). Several abnormalities have been reported in esophageal cancer, notably including frequent *p53* mutation and loss of heterozygosity (LOH) involving numerous chromosomal arms (Hollstein *et al.*, 1990; Shibagaki *et al.*, 1994; Riegman *et al.*, 2001; Roth *et al.*, 2001).

Chromosomal arm 7q31 has been implicated as a TSG site because of frequent LOH at this locus in a variety of tumors of epithelial origin (Liang *et al.*, 1998; van Dekken *et al.*, 1999; Zenklusen *et al.*, 1994b, 1995, 1999). Furthermore, introduction of a single chromosome 7 inhibited the tumorigenicity of a mouse squamous cancer cell line (Zenklusen *et al.*, 1994a). Lately, Zenklusen *et al.* reported that *suppressor of tumorigenicity 7 (ST7)* is a candidate TSG at 7q31, showing frequent mutation in colon and breast cancers. They also demonstrated that the introduction of exogenous wild-type *ST7* cDNA suppresses the *in vivo* tumorigenicity of PC3, a human prostate cancer cell line with LOH at 7q31 (Zenklusen *et al.*, 2001). Since *ST7* is also expressed in the normal esophagus (Zenklusen *et al.*, 2001), we assumed that this gene might be involved in the development of esophageal cancer. Thus, we conducted LOH mapping of 7q31–q35 in esophageal cancers, tested LOH-positive tumors for somatic sequence alterations of the *ST7* gene, and analysed *ST7* mRNA expression levels in a subset of primary tumors.

LOH mapping was performed on 43 paired normal and tumorous esophageal tissues, consisting of 18 squamous cell carcinomas (SCCAs) and 25 adenocarcinomas (ADCAs). Four microsatellite markers localized within a 5.4 cM region at 7q31–q35 including the *ST7* gene were used, i.e., D7S523, D7S480, D7S486, and D7S490 (Table 1). The *ST7* gene is located within a 1.9 cM region between D7S486 and D7S480. LOH was diagnosed when a more than 50% diminution in peak area was observed in tumor compared with its corresponding normal tissue. Of 43 tumors, 12 (28%)

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Table 1 Details of four microsatellite markers used in LOH mapping at 7q31–q35

LOCUS	Position	Size range	Primer sequence
D7S480	7q31–q35	189–206	5'-CTTTGGGGACTGAACCATCTT-3' (sense) 5'-AGCTACCATAGGGCTGGAGG-3' (antisense)
D7S486	7q31	133–146	5'-AAAGGCCAATGGTATATCCC-3' (sense) 5'-GCCCAGGTGATTGATAGTGC-3' (antisense)
D7S490	7q31–q35	92–106	5'-CCTTGGGCAATAAGGTAAG-3' (sense) 5'-AGCTACTTGCAGTGTAAACAGCATTT-3' (antisense)
D7S523	7q31	224–240	5'-CTGATTCATAGCAGCACTTG -3' (sense) 5'-AAAACATTCCATTACCACT-3' (antisense)

Four microsatellite markers (D7S523, D7S480, D7S486, and D7S490) that localize within a 5.4 cM region on chromosome 7q31–35 were used. The *ST7* gene localizes within a 1.9 cM region between D7S486 and D7S480.

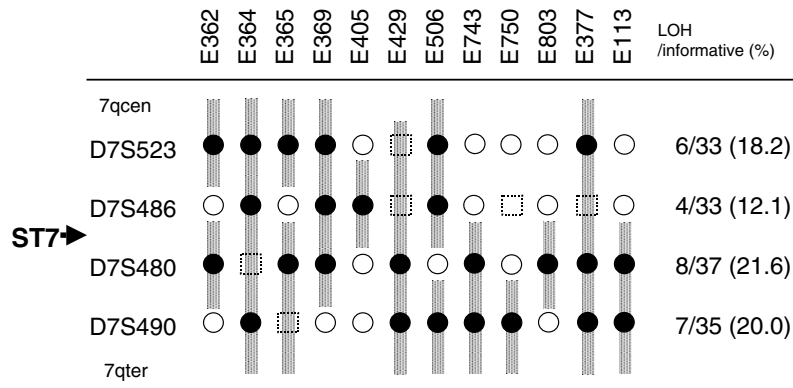


Fig. 1 7q-LOH mapping results. This figure displays 7q-alleleotyping results for the 12 tumors showing LOH at 7q31–q35. Closed circles: LOH; Open circles: retained heterozygosity (no LOH); Dashed squares: uninformative; Dotted bars: regions with LOH. Summarized results for all 43 tumors are shown in the right column. Four microsatellite markers are ordered according to their position on chromosome 7q (i.e., top: centromeric side; bottom: telomeric side). The position of the *ST7* gene is indicated by an arrowhead. LOH was determined by comparing electrophoretic profiles of PCR products from tumor and corresponding normal genomic DNAs on an automated DNA sequencing apparatus (MEGABACE 1000, Molecular Dynamics). PCR conditions were described previously (Mori *et al.*, 2001)

showed LOH at one or more of the four loci analysed (Figure 1). These consisted of four (22%) of 18 SCCAs and eight (32%) of 25 ADCAs. D7S480 was the locus with the highest LOH rate (8/37 informative cases, or 22%). There was no significant difference in LOH frequency between SCCAs and ADCAs at any locus. The most commonly deleted region was between D7S480 and D7S486, within which the *ST7* gene localizes.

Next, we sequenced the *ST7* coding and intronic regions in genomic DNAs from 12 tumors with LOH at 7q31–35, in order to test whether *ST7* is a target of LOH occurring at this locus. Mutation within the entire coding region as well as flanking intronic regions was examined by direct DNA sequencing. Breast cancer cell line MDA-MB231, which was previously reported to carry a frameshift mutation in the *ST7* gene (insertion of a T at nucleotide 1368; Zenklusen *et al.*, 2001), and normal esophageal mucosa were included as positive and negative controls, respectively. All sequencing analyses were performed on both sense and antisense strands at least twice for data verification. No mutations were found in either the entire coding region or flanking intronic regions of *ST7* among any of the 12 tumors studied or in MDA-MB231 cells.

In order to evaluate whether LOH was associated with diminished expression of *ST7* RNA, we performed quantitative real-time RT-PCR. A total of 13 esophageal cancers were examined, including eight SCCAs and five ADCAs, along with corresponding normal esophageal mucosal tissues. Relative expression levels of *ST7* mRNA were measured and normalized by 18S ribosomal RNA. Figure 2 displays relative *ST7* mRNA expression levels after normalization. *ST7* mRNA expression was detected in all normal esophageal tissues, although levels were highly variable. Furthermore, the vast majority of tumors did not show a decrease in *ST7* mRNA levels relative to their corresponding normal esophageal tissues. There was no significant difference between SCCAs and ADCAs at the relative *ST7* mRNA expression level. Three tumors showed greater than 50% upregulated expression compared to their corresponding normal tissues, while only two tumors exhibited more than 50% downregulation of *ST7* mRNA expression.

In this study, we conducted LOH mapping of 7q31–q35, a region found to be deleted frequently in malignancies of multiple organs (Zenklusen *et al.*, 1994b, 1995, 1999; Liang *et al.*, 1998), in primary human esophageal cancers, and found LOH in 28% of cancers tested. The deleted region examined in our study

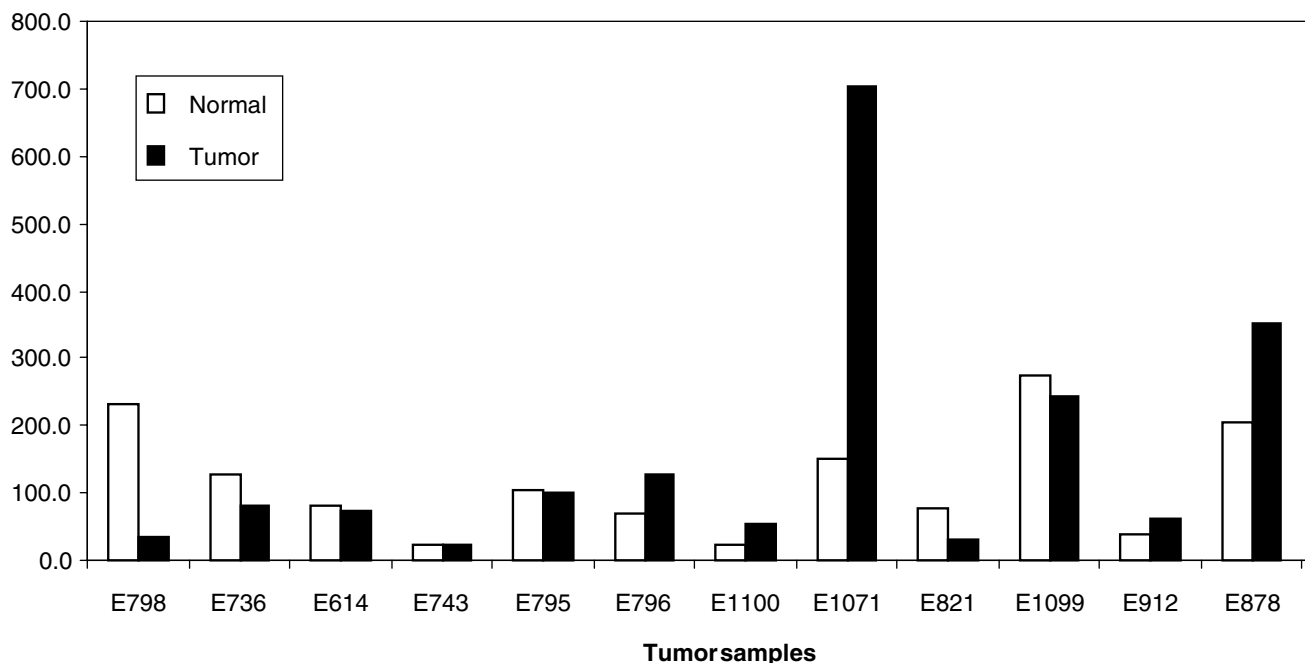


Fig. 2 *ST7* mRNA expression levels in paired primary esophageal tumors and normal esophageal tissues. Open and solid bars represent the relative expression levels of *ST7* mRNA normalized by 18S ribosomal RNA in normal and corresponding tumor tissues, respectively. Quantitative PCR was performed on an ABI 7700 (TaqMan) apparatus (Applied Biosystems) and PCR conditions were described previously (Zou *et al.*, 2002).

included a recently described candidate TSG, *ST7* (Zenklusen *et al.*, 2001). Since it was already known that *ST7* mRNA expression was detectable in normal esophagus (Zenklusen *et al.*, 2001), we hypothesized that *ST7* might also be a TSG at this locus in esophageal carcinogenesis, and examined somatic alterations of this gene in esophageal cancers. However, we did not find mutations or diminished expression of *ST7* in these primary cancers. Tumors in our study were selected for mutational studies based on LOH at the *ST7* flanking regions, which should have ensured a higher prevalence of mutation if *ST7* was a target of LOH events in these tumors. Quantitative real-time measurement of mRNA expression levels also suggested that *ST7* is not likely to be inactivated by epigenetic events such as methylation or haploinsufficiency, since these events would have been expected to result in diminished or absent *ST7* expression. Altogether, these data imply that *ST7* does not play a dominant role in esophageal carcinogenesis.

To our knowledge, two additional mutational analyses of the *ST7* gene have been published (Hughes *et al.*, 2001; Thomas *et al.*, 2001). Both of these studies failed to find any mutations in primary tumors, suggesting that *ST7* is not the responsible TSG at this locus, at least in ovarian, breast, and colorectal cancers. Moreover, in agreement with our results, these two studies did not

find mutations in the cell lines reported by Zenklusen *et al.* to carry mutations. The reason for these contradictory findings of *ST7* mutations in the cell lines remains unclear. As stated by Thomas and Hughes, the mutation described by Zenklusen *et al.* may have been acquired during cell culturing passages.

The existence of a TSG at 7q31 is supported by the LOH rate that we found in esophageal cancers, although its identity was not determined in this study. There are potential TSGs other than *ST7* that localize in this region including *testis-derived transcript (TES)* and *inhibitor of growth family member three (ING3)*. Loss of expression and methylation at the 5' CpG island of *TES* are found frequently in various human cancers (Tatarelli *et al.*, 2000; Tobias *et al.*, 2001). *ING3* belongs to a TSG family, and decreased mRNA expression of *ING3* is frequent among head and neck cancers (Gunduz *et al.*, 2002). Further investigation is required to clarify the identity of any mutative target gene at 7q31.

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