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Analysis of Sciellin (SCEL) as a Candidate Gene in Esophageal Squamous Cell Carcinoma

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Abstract. *Background: The aim of this study was to investigate whether a candidate gene, Sciellin (SCEL), mapping to the chromosome 13q21-q31 is mutated in esophageal cancer. Materials and Methods: The coding region and intron-exon junctions of SCEL were sequenced in 13 esophageal squamous cell cancers and matching normal esophageal samples to detect mutations. Results: Three single nucleotide polymorphisms were detected in SCEL of which two were silent mutations (L640L and H654H) and one missense mutation (R366K). Conclusion: Single nucleotide polymorphisms were detected in both matching tumor and normal esophageal tissues but no disease-associated mutations suggesting that SCEL is not a major factor in esophageal squamous cell carcinogenesis.*

Worldwide, esophageal carcinomas are the ninth most common malignant cancer (1). Esophageal cancer is fatal in most cases (2). There are two histologic types of esophageal carcinoma: squamous cell carcinoma and adenocarcinoma. Esophageal squamous cell carcinoma affects African-Americans more often than Caucasians. In contrast, 95% of those affected by adenocarcinomas are Caucasian males (3). Epidemiologic data indicates that tobacco and alcohol use increase the risk of squamous cell carcinoma.

The genetic events involved in the initiation and progression of this cancer have not been elucidated. Molecular cytogenetic fingerprinting of esophageal squamous cell carcinoma by comparative genomic hybridization (CGH) has shown chromosomal losses at 2q, 3p, 4, 5q, 13q and 18q and gains in chromosomes 17, 19, 20 and 22, accounting for 95% of the genomic imbalances (4). The frequencies of chromosomal losses associated with this tumor was as follows: 2q (100%), 3p (100%), 13q (100%), Xq (94%), 4 (82%), 5q (82%), 18q (76%), 9p (76%), 6q (70%), 12q (70%), 14q (65%), 11q (59%) and 1p (53%) (4). Most prominently, regions on 2q, 3p and 13q were lost in all the esophageal squamous cell tumors examined. In some chromosomes, short overlapping regions that displayed losses were restricted to 1cen-p22, 2q23-q35, 3cen-p12, 5q13-q23, 6q15-q26, 11q14-q22, 13q22, 14q12-q23 and Xcen-q24 (4). These chromosomal imbalances play a critical role in the complex carcinogenic process leading to esophageal squamous cell carcinoma. A high frequency of LOH at the Retinoblastoma (RB1) on 13q14 has been reported in esophageal cancers (5). Loss of the terminal region of chromosome 13 has been implicated in esophageal squamous cell carcinoma, and may involve loss of the tumor suppressor gene ING1 (6).

We have identified the locus of a novel tumor suppressor gene on chromosome 13q21-q31 in esophageal squamous cell carcinoma (4). Sciellin (SCEL) is a precursor envelope protein of stratified squamous epithelia localized to the cytoplasm of skin, oral cavity, esophagus and vagina and maps to 13q21-q31 (7). Hence, SCEL due to its biological function and chromosomal location obviously becomes an interesting candidate gene for esophageal cancer. SCEL cDNA is 2.35 Kbp and the translated sequence has a central domain containing 16 repeats of 20 amino acids each that is rich in Gln and Lys residues, which are potential transglutaminase susbtrates and a carboxyl domain which contains a single LIM motif (8). We sequenced the exons and intro-exon junctions of the SCEL gene in 13 esophageal squamous cell cancers and matching normal esophageal samples in order to detect mutations. We have identified three single nucleotide polymorphisms but no disease-associated mutations.

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Table I. *Primers for Sciellin mutation analysis.*

Exon	Primers	Annealing temp (˚C)	Size (bp)
1	5' ACACCTCCTCCCTGAGTGAC		
	5' AAATTTACAGGAAGAAAAGACAAAGA	60	310
2	5' AGGGGCACTTTATGTCATGC		
	5' TGACTAAAAGCTGCGACAAGT	60	378
3	5' TGCCAGCCCAGATTGATG		
	5'GCAACAAAGTGTTTCAGGAGAA	60	350
4	5' AAATGAATTAACCTTGCCAAAAA		
	5' TGATATCACAGATTCTCCTTCACA	60	320
5	5' CCAAGTTGGAAGATTTGGGTA		
	5' CCTCCATCCCTGGGAATAAA	60	364
6	5' TTCTCTTTACTAGAGAAAGGCTATGTT		
	5' CATGAACAGGAGTAATGATAGAGACT	60	318
7	5' AAGAGACAAAATCATGAAATCATCTTA		
	5' TGCAAAAGTCCAGATAGTCACA	60	394
8	5' GAACAGAGAGCAGGCAGGAA		
	5' TCAAGGCCCTACAGGAATTG	60	413
9	5' TTGGAGGAACAGATAGCCTAGAA		
	5' TTACAACCACTGGACACTCTCCT	60	402
10	5' GTGCTCCATGGATTCTGACC		
	5' GCCATTAGTATTCAAAGACAAACA	60	430
11	5' CGCCAACCCAAAGAATTTAAG		
	5' ATTTTGGAGTCAGTTGGAAAAC	60	350
12	5' TTCTGGAGAAAGTGGTGTTGG		
	5' TGCTGCAAGCTTTTATTCAAGA	60	353
13	5' AGCTCATCTTCCCCAACAAG		
	5' GAGAGCACTACCCCATCTGC	60	310
14	5' GGGTAGTGCTCTCAGCATTG		
	5' GCAAAACCAGCACCAAGATA	60	357
15	5' CTGATCCTTTAGGCACTGATTG		
	5' AGGTCTGATGGGTCAAAGCA	60	329
16	5' TTTGGTCATTTGGTCCTGAA		
	5' AGTGAAGATCAGGGCACCAG	60	307
17A	5' CATTTACATATAGCCCAATAAAATCCT		
	5' GGATAAGGAGGGATGGTCGT	60	409
18	5' GCAACATTAAATGGTCTTCAGC		
	5' TGCACAGAATTACTTACTATGAAGTGT	60	400
19	5' TTTCTGCATAGTCACCTTTCACA		
	5' GGGACTTGTTCAAAGACACCA	60	372
20	5' CCAGTGATTGGTAAGAAGGTC		
	5' GCAGGGTTACATGGCTTTTC	60	366
21	5' CCCAGCCCTCTGAGATGAT		
	5' CCGCACAATTTGTCATGTTT	60	271
22	5' GCAGGAAATGAATTGATAGTAGCC		
	5' TCATGTCCCCTTTCAAAATGT	60	319
23	5' TTGATGTCATTGAGTTGTTGTGA		
	5' TCTAGGTTTCCACCTGTGATGA	60	368
24	5' TTCTTCATCCCCTAAATTGGTT		
	5' ATCATTAAGGTTATAAATCTGCTTGAA	60	305
25	5' AAATTTTCACAAACAACCAAAGG		
	5' TGCTAGGTCAATAAAGCAACATT	60	313
26	5' CCTGCAGTAATCAGAAACAATCA		
	5' AAAAGGGAGGAAGGGAGGA	60	292
27	5' TCCCATAGCCTGATAGACCACA		
	5' TTCCCTATTAGCTATTAAGCACCTTG	60	418
28	5' GCTGTGTTAATACCACTCTAACATTCA		
	5' GGTCTTCTATTTGAAAGAAGCACACT	60	336
29	5' GAGGCTTCATGAGGTGGTGT		
	5' TGAGTTGCACTTTGGTCTGC	65	483
30	5' ACATCTTTGTCCCACCCAGA		
	5' GTGGCTGACAGTGCTTCTGA	60	501
31	5' TGCCTCGATGGTAGAGAGATG		
	5' ACAAAAGTTGATTGATTTTACACCATT	60	513
32	5' CCAAAAGCCACAGCGATTAT		
	5' CCACTCACAGCCAACATACG	60	474

Table II. *Clinical stage and the Sciellin genotype of the patients.*

Pt. Number	Stage	Mutation
001	$T_1N_0M_0$	1962 C>T (H654H)
002	$T_2N_0M_0$	1920 A>G (L640L)
003	$T_2N_1M_1$	1962 C>T (H654H)
004	$T_2N_0M_0$	negative
005	$T_2N_0M_0$	1962 C>T (H654H)
006	$T_1N_0M_0$	1097 G>A (R366K)
007	$T_4N_0M_0$	1920 A>G (L640L)
008	$T_1N_0M_0$	1920 A>G (L640L)
009	$T_1N_0M_0$	negative
010	$T_1N_0M_0$	1097 G>A (R366K)
011	$T_1N_0M_0$	1920 A>G (L640L)
012	$T_1N_0M_0$	1920 A>G (L640L)
013	$T_1N_0M_0$	1920 A>G (L640L)

Materials and Methods

The present study includes biopsies from 13 patients undergoing endoscopy for esophageal cancer squamous cell carcinoma. Both normal and tumor tissues were obtained. Histological confirmations of the biopsies were made by the department of pathology service. The tissue specimens were snap-frozen in liquid nitrogen and stored at -80˚C. Genomic DNA was extracted by proteinase-K digestion and phenol-chloroform extraction as described previously (9).

Amplification of genomic DNA was performed in a 30 ml reaction volume, using 60-100 ng of DNA template, 50 mM dNTP, 0.25 mM of each primer, 3 ml of 10x PCR amplification buffer (Invitrogen, CA, USA), 1.5 ml 10X Enhancer buffer (Invitrogen), 0.9 ml of 50 mM $MgSO_4$ and 1 U AmpliTaq (Perkin Elmer, CA, USA). All reactions were performed using a PTC-225 thermocycler (MJ Research, CA, USA). Primer pairs, annealing temperature and their respective fragment size are described in Table I. Typical PCR cycling parameters were 95˚C for 4 min followed by 30 cycles at 95˚C for 30 sec, annealing at the indicated temperatures for 30 sec, extension at 72˚C for 1min, and a final extension step of 72˚C for 5 min. A small aliquot of the amplified DNA was analyzed on 2% agarose gel as a part of the quality control procedure. The amplified PCR products were purified using a Qiagen PCR purification kit (Qiagen, CA, USA) and bi-directionally sequenced using the BigDye™ terminator cycle sequencing kit according to the manufacturer's protocol (Applied Biosystems, CA, USA). Sequencing reactions were analyzed on the ABI 3100 automated sequencer.

The gene mutation nomenclature used in this study follows the recommendations of den Dunnen and Antonarakis (10). The gene symbols are those following the recommendations of the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk).

Results

The genomic structure of human Sciellin gene was determined by comparison of cDNA and genomic sequences available in the public genomic database. Primers were designed flanking the intron-exon junctions. Initial amplification products of the Sciellin exons with normal control DNA were sequenced,

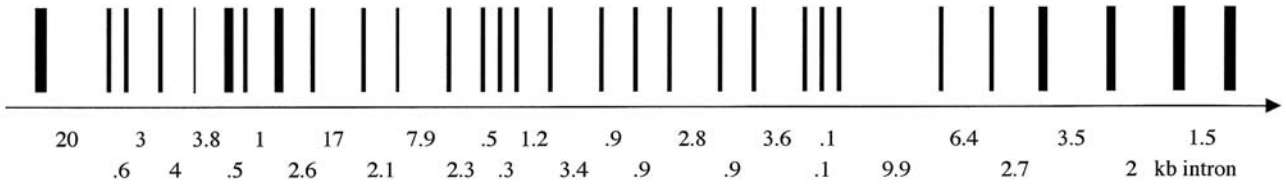

Figure 1. *Genomic structure of human Sciellin. Exons, introns and splicing sites were determined by aligning the reported sequence of the human Sciellin. The exons of the gene are depicted as boxes, and the introns as lines. The length of the introns is denoted in the bottom of each intron in kb.*

analyzed against the sequences obtained from the genomic database and confirmed that all the primers successfully amplified the *bona fide* human Sciellin gene.

In this panel of esophageal squamous cell carcinomas only two were normal for Sciellin but in the other 11 samples we detected single nucleotide polymorphisms. The two identified silent mutations, 1920 A>G (L640L) and 1962 C>T (H654H) seem to be common polymorphisms whereas the 1097 G>A (R366K) missense mutation was detected only in two samples and exclusively in the tumor DNA but not in the matched normal DNA. The R366K found in two samples is the last residue in the stretch of 20 amino acids that is rich in Gln and Lys residues. There are 16 such repeats of approximately 20 residues, which are potential transglutaminase substrates.

Discussion

Our previous CGH data of esophageal squamous cell carcinoma samples strongly indicated the chromosomal loss of 13q and the critical region was around 13q21-q31(4). Furthermore, the presence of the Sciellin gene on 13q21-31 and the tissue specific expression as a precursor envelope protein of stratified squamous epithelia localized to the cytoplasm of skin, oral cavity, and esophagus suggested that Sciellin deserved to be examined as an esophageal carcinoma candidate gene (7). In this study, we investigated the sequence variation of Sciellin in esophageal carcinomas and found no evidence for its role as a common genetic determinant of this carcinoma in our panel of samples. The utilization of a DNA sequencing approach to mutation screening is extremely sensitive as compared to the traditional single-stranded conformation polymorphism (SSCP). Even though our mutation screening did not suggest loss or gain of function of Sciellin as a common denominator of esophageal squamous cell carcinoma, nonetheless, we cannot formally exclude the possibility that rare cases could be caused by this mechanism.

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