Examination of Oral Cancer Biomarkers by Tissue Microarray Analysis

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Objective: To validate the DNA microarray results on a subset of genes that could potentially serve as biomarkers of oral squamous cell carcinoma (OSCC) by examining their expression with an alternate quantitative method and by assessing their protein levels.

Design: Based on DNA microarray data from our laboratory and data reported in the literature, we identified 6 potential biomarkers of OSCC to investigate further. We used quantitative real-time polymerase chain reaction to examine expression changes of CDH11, MMP3, SPARC, POSTN, TNC, and TGM3 in OSCC and histologically normal control tissues. We further examined validated markers at the protein level by immunohistochemical analysis of OSCC tissue microarray sections.

Results: Quantitative real-time polymerase chain reaction analysis revealed upregulation of CDH11, SPARC, POSTN, and TNC gene expression and decreased TGM3 expression in OSCC tissue compared with control tissue; MMP3 was not found to be differentially expressed. In tissue microarray immunohistochemical analyses, SPARC (secreted protein, acidic, rich in cysteine), periositin, and tenascin C exhibited increased protein expression in tumor tissue compared with control tissue, and their expression was primarily localized within tumor-associated stroma rather than tumor epithelium. Conversely, transglutaminase 3 protein expression was found only within keratinocytes in control tissue and was significantly downregulated in cancer cells.

Conclusions: Of 6 potential gene markers of OSCC, initially identified by DNA microarray analyses, differential expression of CDH11, SPARC, POSTN, TNC, and TGM3 were validated by quantitative real-time polymerase chain reaction. Differential expression and localization of proteins encoded by SPARC, POSTN, TNC, and TGM3 were clearly shown by tissue microarray immunohistochemical analysis.


HEA ND NECK SQUA Mous cell carcinoma (HNSCC) is the fifth most common cancer worldwide.1 The American Cancer Society estimates that approximately 30,990 Americans were diagnosed as having and 7,430 died of cancer of the oral cavity and pharynx in 2006.2 Despite considerable advances in the treatment of HNSCC during the past 2 decades, overall disease outcomes have only modestly improved.2 Local tumor recurrence affects approximately 60% of patients, and metastases develop in 15% to 25%.3 Less than 30% of patients with HNSCC experience 3 or more years of disease-free survival, and many suffer from impaired speech, swallowing, and/or breathing because of the sensitive location of HNSCC tumors within the upper aerodigestive tract.4 Of the various subgroups of HNSCC, oral squamous cell carcinoma (OSCC) is the most common, representing about 75% of all HNSCC cases.2 High throughput investigations into the molecular characteristics of HNSCC have mainly used DNA microarray technology to search for gene expression profiles associated with disease and disease outcomes. The literature on DNA microarray profiling of HNSCC shows heterogeneity in the specific genes that were found to be upregulated or downregulated in HNSCC. After comparing results from multiple studies, Choi and Chen5 provided a list of genes commonly found to have dysregulated expression in HNSCC tumors. Only a handful of these gene expression alterations have been validated by alternate experimental methods, such as quantitative real-time polymerase chain re-
action (qRT-PCR) and Western blot, Northern blot, and immunohistochemical analyses. Even fewer have been examined for their correlation with disease severity and metastasis status.

Based on various criteria, we selected 6 genes to analyze further: CDH11 (OMIM 600023), MMP3 (OMIM 185250), SPARC (OMIM 182120), POSTN (OMIM 608777), TNC (OMIM 187380), and TGM3 (OMIM 600238). CDH11 encodes an integral membrane protein, cadherin-11, which mediates cell-cell adhesion and is thought to be involved in bone cell differentiation and bone formation.\(^4\) MMP3 encodes a secreted protease, matrix metalloproteinase 3, that degrades the major components of the extracellular matrix and is thought to be associated with cervical lymph node metastases in HNSCC.\(^5\) SPARC (secreted protein, acidic, rich in cysteine) encodes the extracellular matrix–associated protein osteonectin, which inhibits cell-cycle progression, causes changes in cell shape, and influences extracellular matrix synthesis.\(^6\) SPARC also has been found to be an independent prognostic marker for short disease-free interval and poor overall survival in patients with HNSCC.\(^7\) POSTN encodes a protein, periostin, that is a ligand for various integrins and, as such, supports adhesion and migration of epithelial cells.\(^8\) Periostin is thought to promote invasion and angiogenesis in OSCC.\(^9\) TNC encodes transglutaminase 3, which crosslinks intracellular structural proteins and is important in cell envelope formation of the epidermis.\(^10\) Transglutaminase 3 is expressed normally in terminally differentiated epithelial cells.\(^11\) It has been shown to be downregulated in esophageal squamous cell carcinoma\(^6-12\) and in the progression of oral leukoplaikia to OSCC.\(^13\) CDH11, MMP3, SPARC, POSTN, and TNC have been shown in gene microarray experiments to be significantly upregulated in cancerous tissues compared with histologically normal control tissues, whereas TGM3 is significantly downregulated.\(^14\)

### METHODS

**BIOMARKER SELECTION**

For the present study, we selected as OSCC markers genes that (1) showed the highest \(z\) scores and greatest expression fold changes between cancerous and histologically normal tissues in our DNA microarray data\(^14,\) (2) have been shown to have significantly altered expression in OSCC when compared with noncancerous tissues in at least 4 other laboratories; and (3) had available antibodies against their encoded protein products for use in immunohistochemical analyses.

**QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION**

Differential gene expression of CDH11, MMP3, SPARC, POSTN, TNC, and TGM3 between control and cancerous tissue specimens was quantified by using SYBR Green I technology and melting-point dissociation curve analyses per manufacturer protocol (Applied Biosystems, Foster City, California). Total RNAs extracted from 6 histologically normal tissue samples and 6 tumor tissue samples (Table 1) were used as templates in qRT-PCR to generate complementary DNA. Each sample was divided into 5 wells for the qRT-PCRs: 3 for the gene of interest and 2 for the endogenous control, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The qRT-PCR analyses were performed on an ABI 5700 Sequence Detector (Applied Biosystems) using 10 ng of complementary DNA and gene-specific primers in 1 \(\times\) SYBR Green I PCR Master Mix in a 50-µL reaction. Cycling parameters were 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and at 60°C for 1 minute. Primer sequences were designed using PE/ABI Primer Express software (Applied Biosystems), checked for specificity against the National Center for Biotechnology Information nucleotide database, and were as follows: CDH11 forward, 5-GCT CAA CCA GCA GAG ACA TTC C-3; CDH11 reverse, 5-AGA ATG CAG CTT GTA CCC CCT-3; MMP3 forward, 5-GGC AAG ACA GCA AGG CAT AGA-3; MMP3 reverse, 5-AGA ATG CAG CTT GTA CCC CCT-3; POSTN forward, 5-ACA GCC ACA GCA AGG CAT AGA-3; POSTN reverse, 5-TGG ATA GGC TGA GCA AAC TGC-3; SPARC forward, 5-CCG CTT TGT GGA GAT CCC ATC-3; SPARC reverse, 5-GGA AGG ACT CAT GAC CTG CAT C-3; TNC forward, 5-ACA GCC CAG CGC TAT TCT GAC-3; TNC reverse, 5-ATC CAA GTG GTG CCA AGC CTG-3; TNC forward, 5-AAG TCG TCC GCC ACA AGC-3; TNC reverse, 5-CTC CCA GAT CAT CCA CCG AAC ACT G-3; TNC forward, 5-GAC AAG GCC ATC ACA CAG ACA-3; and TGM3 reverse, 5-TCT TCG GTT AGA GCC AAG GCC-3. Melting-curve analyses were run immediately after cycling to verify specificity of the reactions. Quantification of the transcripts was determined by choosing a fluorescence threshold at which the amplification of the target gene was exponential in both samples of tumor tissue and histologically normal tissue. The PCR cycle number at which the amplification curve intercepted the threshold is termed the threshold cycle (C\(_t\)). The threshold cycle is inversely proportional to the copy number of the target template. Relative fold changes

<table>
<thead>
<tr>
<th>Tissue Specimen</th>
<th>Sex/Age, y</th>
<th>Diagnosis</th>
<th>Stage</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>M/43</td>
<td>Obstructive sleep apnea</td>
<td>T0N0M0</td>
<td>NA</td>
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<tr>
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<td>M/37</td>
<td>Obstructive sleep apnea</td>
<td>T0N0M0</td>
<td>NA</td>
</tr>
<tr>
<td>Control</td>
<td>F/48</td>
<td>Obstructive sleep apnea</td>
<td>T0N0M0</td>
<td>NA</td>
</tr>
<tr>
<td>Control buccal mucosa</td>
<td>M/48</td>
<td>Lateral tongue SCCA</td>
<td>T2N0M0</td>
<td>NA</td>
</tr>
<tr>
<td>Control buccal mucosa</td>
<td>F/54</td>
<td>Lateral tongue SCCA</td>
<td>T2N0M0</td>
<td>NA</td>
</tr>
<tr>
<td>Control palate mucosa</td>
<td>M/64</td>
<td>Tonsil/palate SCCA</td>
<td>T2N2bM0</td>
<td>NA</td>
</tr>
<tr>
<td>Cancerous</td>
<td>M/48</td>
<td>Lateral tongue SCCA</td>
<td>T2N0M0</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cancerous</td>
<td>F/81</td>
<td>Retromolar trigone SCCA</td>
<td>T4N2bM0</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cancerous</td>
<td>M/63</td>
<td>Tonsil/soft palate SCCA</td>
<td>T2N2bM0</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cancerous</td>
<td>M/61</td>
<td>Retromolar trigone SCCA</td>
<td>T4N2bM0</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cancerous</td>
<td>M/76</td>
<td>Gingivobuccal sulcus SCCA</td>
<td>T4N0M0</td>
<td>Moderate</td>
</tr>
<tr>
<td>Cancerous</td>
<td>F/54</td>
<td>Lateral tongue SCCA</td>
<td>T2N0M0</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; qRT-PCR, quantitative real-time polymerase chain reaction; SCCA, squamous cell carcinoma.
were calculated by $2^{-\Delta\Delta C_{T}}$, where $\Delta\Delta C_{T}=(\text{average } C_{j}, \text{gene } j - \text{average } C_{1}, \text{GAPDH})$ tumor tissue − (average $C_{j}$, gene $j$ − average $C_{1}$, GAPDH) control tissue.

**IMMUNOHISTOCHEMICAL ANALYSIS**

Genes that satisfied the following criteria were studied further by immunohistochemical analysis of OSCC tissue microarray sections: (1) gene expression in cancer tissues was significantly different than in normal tissues by each of the two different methods, qRT-PCR and GeneChip (Affymetrix, Santa Clara, California) analysis, and (2) the relative gene expressions determined by the 2 methods were significantly correlated with each other.

The SPARC immunohistochemical analysis was performed using mouse monoclonal anti-SPARC antibody (US Biological, Swampscott, Massachusetts) at a dilution of 1:2000 following antigen retrieval, consisting of 20 minutes of nonpressure steam incubation in 10mM citrate buffer, pH 6.0. Periostin immunohistochemical analysis was performed using rabbit polyclonal antiperiostin antibody (BioVendor, Candler, North Carolina) at a dilution of 1:900. Tenascin C immunohistochemical analysis was performed using mouse monoclonal anti-tenascin C antibody (BioVendor) at a dilution of 1:50 following antigen retrieval, consisting of 2-5 minute microwave incubations in 10mM citrate buffer, pH 6.0. Transglutaminase-3 immunohistochemical analysis was performed using mouse monoclonal antibody (a gift from Kiyotaka Hitomi, PhD, Nagoya University, Nagoya, Japan) raised against purified, recombinant human T-Gase-3, at a dilution of 1:1000. Tissue microarray sections incubated with antibody diluent alone, phosphate-buffered saline containing 1% bovine serum albumin,
CDH11, SPARC, POSTN, TNC, and TGM3 exhibited significant differences in expression between cancerous and normal tissue specimens by qRT-PCR (Table 3). There was a trend toward upregulation of MMP3 expression in cancerous tissue compared with histologically normal specimens, but this did not reach statistical significance.

Results of the linear regression analyses show good correlation between relative gene expression as determined by qRT-PCR and that previously determined by DNA microarray on the same specimens for MMP3, SPARC, POSTN, TNC, and TGM3 (Table 3). Correlation between qRT-PCR and gene microarray expression data for CDH11 did not reach statistical significance ($r=0.45, P<.14$).

Representative tissue microarray cores stained for SPARC, peristin, tenasin C, and transglutaminase 3 are shown for both normal mucosa and primary OSCC tumors (Figure). Staining with antibodies to SPARC was predominantly localized to vessels, fibroblasts, and subsets of carcinoma cells. Virtually no epithelial cell staining was observed in normal mucosa (Figure, A). The SPARC immunohistochemical staining in epithelium, fibroblasts, and vessels was significantly higher in tumor specimens compared with normal control specimens (Figure, B, and Table 4). There were no significant differences with regard to epithelial SPARC expression among tumors of different TNM stage.

Staining with antibodies to peristin was predominantly associated with fibroblasts and the extracellular matrix. Expression was significantly higher in cancerous vs control specimens (Figure, C and D and Table 4). In 14 of 24 primary tumors (58%) and 4 of 17 cervical metastatic tumors (24%), epithelial cancer cells exhibited faint staining, whereas the remaining tumors had no epithelial cell staining. Of 14 tumors that exhibited epithelial staining, 13 (93%) were stage III or IV tumors, representing 69% of the 20 stage III and IV tumors examined. Only 1 (7%) was an early stage tumor (case No. 16, a recurrent T2N0M0), representing 1 of 4 stage I and II tumors examined. In addition, all 8 T4 tumors in our tissue microarray analysis exhibited epithelial peristin staining. Nonneoplastic epithelium was virtually negative for staining. Staining of carcinoma cells was, on average, higher in primary tumors compared as negative controls to confirm specificity of immunostaining for all markers.

Staining for all markers.

CDH11 immunoexpression in primary tumors was, on average, higher in specimen compared to normal mucosa using unpaired t test with post hoc multiple comparisons, we believed that a threshold of $P<.05$ was too low and considered comparisons to be statistically significant only if $P<.01$. All statistical analyses were performed using Stata 9.0 software (StataCorp, College Station, Texas).

### Table 3. qRT-PCR Validation of Gene Microarray Data

<table>
<thead>
<tr>
<th>Gene</th>
<th>Method</th>
<th>Cancer-Control Ratio (P Value)</th>
<th>Linear Correlation, r (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH11</td>
<td>Microarray</td>
<td>19.8 (0.003)</td>
<td>0.45 (≤.14)</td>
</tr>
<tr>
<td></td>
<td>qRT-PCR</td>
<td>18.9 (0.03)</td>
<td></td>
</tr>
<tr>
<td>MMP3</td>
<td>Microarray</td>
<td>22.9 (0.09)</td>
<td>0.97 (&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>qRT-PCR</td>
<td>4.3 (10)</td>
<td></td>
</tr>
<tr>
<td>SPARC</td>
<td>Microarray</td>
<td>5.7 (0.001)</td>
<td>0.91 (&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>qRT-PCR</td>
<td>3.9 (0.001)</td>
<td></td>
</tr>
<tr>
<td>POSTN</td>
<td>Microarray</td>
<td>18.8 (0.008)</td>
<td>0.90 (&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>qRT-PCR</td>
<td>4.5 (0.009)</td>
<td></td>
</tr>
<tr>
<td>TNC</td>
<td>Microarray</td>
<td>7.8 (&lt;.001)</td>
<td>0.90 (&lt;.001)</td>
</tr>
<tr>
<td></td>
<td>qRT-PCR</td>
<td>6.7 (&lt;.001)</td>
<td></td>
</tr>
<tr>
<td>TGM3</td>
<td>Microarray</td>
<td>0.06 (&lt;.001)</td>
<td>0.63 (≤.03)</td>
</tr>
<tr>
<td></td>
<td>qRT-PCR</td>
<td>0.07 (0.03)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: qRT-PCR, quantitative real-time polymerase chain reaction.

**STATISTICAL ANALYSIS**

The qRT-PCR results were analyzed by an unpaired t test to compare gene expression between cancerous and histologically normal tissue specimens. Relative gene expression values determined by qRT-PCR analyses were then compared with those previously determined by gene microarray analyses by linear regression analysis. Pairwise comparisons of immunohistochemical analysis scores were made between primary tumor tissue and normal mucosa, tumor tissue and lymph nodes, tumor tissue and carcinoma in situ, and carcinoma in situ tissue and normal mucosa using unpaired t tests. Because of multiple comparisons, we believed that a threshold of $P<.05$ was too low and considered comparisons to be statistically significant only if $P<.01$. All statistical analyses were performed using Stata 9.0 software (StataCorp, College Station, Texas).

**RESULTS**

Images of the immunohistochemically-stained tissue microarray sections were digitized using the BLISS Tracer imaging system and visualized with WebSlide Server software (Bacus Laboratories, Lombard, Illinois). Marker immunoreactivity was scored using a validated, modified H-score system by a board-certified pathologist (C.D.J.) blinded to all characteristics of the cases and controls. Immunohistochemical analysis scores were determined by taking the product of the estimated staining intensity ([2], 1, +, 2, +, 3+) and area of tissue (tumor or normal; epithelial or stromal) stained ([0]=0, [25%]=1, [25%-75%=2, [>75]=3), giving a range of possible scores between 0 and 9. Scores for replicate cores were averaged to determine a composite score for each case.

Staining of carcinoma cells was, on average, higher in primary tumors compared as negative controls to confirm specificity of immunostaining for all markers.

Staining with antibodies to peristin was predominantly associated with fibroblasts and the extracellular matrix. Expression was significantly higher in cancerous vs control specimens (Figure, C and D and Table 4). In 14 of 24 primary tumors (58%) and 4 of 17 cervical metastatic tumors (24%), epithelial cancer cells exhibited faint staining, whereas the remaining tumors had no epithelial cell staining. Of 14 tumors that exhibited epithelial staining, 13 (93%) were stage III or IV tumors, representing 69% of the 20 stage III and IV tumors examined. Only 1 (7%) was an early stage tumor (case No. 16, a recurrent T2N0M0), representing 1 of 4 stage I and II tumors examined. In addition, all 8 T4 tumors in our tissue microarray analysis exhibited epithelial peristin staining. Nonneoplastic epithelium was virtually negative for staining. Staining of carcinoma cells was, on average, higher in primary tumors compared as negative controls to confirm specificity of immunostaining for all markers.

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pared with cervical lymph node metastases (Table 4). Average extracellular matrix staining of periostin also was greater in primary tumors compared with metastatic lymph nodes (Table 4).

Staining with antibodies to tenascin C was predominantly localized to the extracellular matrix (Figure, E and F). In normal mucosa, only the region of extracellular matrix immediately adjacent to the basal epithelium showed moderate staining. Tenascin C staining in the extracellular matrix was significantly higher in OSCC tumors compared with histologically normal mucosa (Table 4). Staining was occasionally associated with carcinoma cells, particularly in tumor regions directly adjacent to desmoplastic stroma.

Staining with antibodies to transglutaminase 3 localized only within epithelial cells; no stromal expression was observed (Figure, G and H). Within nonneoplastic epithelium, the suprabasal layers stained intensely, whereas the basal layer of epithelial cells did not exhibit staining. Staining in invasive carcinomas was patchy, and when present, was typically associated with areas of increased differentiation and keratinization. Many carcinomas had no staining. There were statistically significant differences in transglutaminase 3 immunoreactivity between different specimen groups; the highest expression was seen in nonneoplastic epithelium, with significantly lower expression in carcinoma in situ specimens, and stepwise significantly lower expression in primary tumors (Table 4).

We selected 6 potential biomarkers of OSCC for the present validation study by examining DNA microarray data from our laboratory as well as that published in the literature.2 The SPARC, POSTN, TNC, and TGM3 microarray expression differences were validated by qRT-PCR and immunohistochemical analysis of tissue microarray sections. The qRT-PCR results for MMP3 and CDH11 did not reach statistical significance. In contrast with another immunohistochemical study reporting high levels of periostin expression within oral carcinoma epithelium,12 we noted periostin staining to be localized primarily to the stroma and did not see robust staining within tumor cells themselves (Figure, D). The reason for this disparity is unclear. Nonoverlapping antibody epitopes may partially explain the disparity of periostin staining patterns. The antibody we used for periostin was raised in rabbits against recombinant human periostin containing 648 amino acid residues (corresponding to amino acids 679-692 of full-length periostin) with an N-terminal HisTag fusion (available at http://biovendor.com/pdf/RD181045050.pdf), whereas periostin immunohistochemical experiments by Siriwardena et al.12 used a polyclonal antibody generated by immunizing rabbits with a specific peptide (EGEPFRLIKEGETC) corresponding to amino acids 679-692 of full-length periostin.

Despite the strong stromal predominance of periostin expression, the percentage of OSCC tumor specimens that exhibited epithelial periostin in our study (38%) was comparable to the 69% reported by Siriwardena et al.12 Of the stage III and IV OSCC tumors, including 100% of T4 tumors we examined, 65% were positive for epithelial periostin immunostaining, compared with only 25% of the stage I and II tumors. These findings suggest that epithelial expression of periostin may be associated with a more aggressive tumor phenotype in OSCC. This is supported by other studies, which show that subsets of HNSCC cells expressing periostin, or cells engineered to overexpress periostin, exhibit enhanced tumor growth and invasiveness, and tumors that express periostin have a more invasive phenotype.13,12
We found the proportion of metastatic lymph node tumors positive for epithelial peroxidase expression (24%) was less than half that of primary tumors (58%). However, each lymph node tumor specimen that exhibited staining was associated with a primary tumor that also had epithelial peroxidase expression, suggesting that presence of peroxidase in the epithelium of primary tumors may be necessary, but not sufficient, for its presence in metastatic tumors.

In laryngeal and OSCC, increased levels of tenascin C immunostaining have been found to correlate with malignancy and invasion. Abundant expression of tenascin C in our OSCC tissue microarray sections was localized primarily to the stroma, although some minor staining of tumor cells was also observed, particularly at tumor edges adjacent to desmoplastic stroma. This observation is consistent with reports in the literature showing tenascin C localization along the invasive fronts of carcinomas of the lung, liver, bladder, and skin.

Roepman et al identified TGM3 to be significantly downregulated in metastatic HNSCC compared with both nonmetastatic tumors and normal epithelium. O’Donnell et al similarly found significant downregulation of TGM3 gene expression in metastatic primary OSCC tumors compared with nonmetastatic primary tumors. Our cross-sectional immunohistochemical data show that the levels of transglutaminase 3 protein expression were seen to decrease in a stepwise fashion from normal to premalignant to malignant specimens. This suggests that the loss of transglutaminase 3 activity might be associated with the progression of squamous cell carcinoma.

All of the upregulated gene markers we identified by reviewing gene microarray reports, validated by qRT-PCR, and subsequently studied with immunohistochemical analysis revealed protein expression to be localized primarily within the stroma and modestly or not at all within tumor cells. This finding illustrates an important prediction regarding the interpretation of gene microarray data based on the methods used for specimen processing. The methods used by different laboratories for tumor specimen processing vary significantly. Although some investigators isolated relatively homogeneous populations of tumor cells for microarray analysis via laser capture microdissection, others established arbitrary thresholds for minimum tumor cell content in surgical specimens, as assessed by histologic evaluation of adjacent tissue, before RNA extraction and microarray analysis. The latter method clearly results in varying amounts of stromal cells contributing to the final pool of extracted RNA, and thus the variability of the resultant microarray data. Notably, even laser capture microdissection does not ensure isolation of a purely homogeneous population of tumor cells, as varying degrees of leukocytosis and neovascularization within tumors exist and correlate with survival, tumor stage, metastases, and presence of extracapsular spread in HNSCC.

Upregulation of SPARC, POSTN, or TNC was not reported by any of the DNA microarray studies that examined expression of HNSCC cell lines or by others that used laser capture microdissection to isolate tumor cells from stroma. Presumably this is because of the relative absence of stromal cells within the analyzed specimens in these studies, although absence of 1 or more of these markers on the microarrays used by these studies may also contribute. These findings, together with the immunohistochemical data we report herein, suggest that upregulation of SPARC, POSTN, and TNC is caused by (1) upregulation within stromal cell populations vs carcinoma cells and/or (2) stroma-induced transcriptional upregulation of these markers in cancer cells. In any case, these observations underscore the importance in examining tumor and stroma in the pathogenesis of OSCC.

The “seed and soil” hypothesis of tumor-stromal interaction was originally proposed by Paget in 1889, but only recently have researchers examined how tumor microenvironments influence the growth and spread of cancers. Carcinoma-associated fibroblasts, extracellular matrix macromolecules, neovascularization, and inflammatory and immune cell infiltration within the stroma adjacent to tumors can have profound effects on tumor progression in breast, prostate, and skin carcinomas. The situation in OSCC is less well understood, but studies of carcinoma-associated fi-
Our observations indicate that significant changes in the expression of 4 genes, SPARC, POSTN, TNC, and TGM3, initially identified by gene microarray studies, are associated with similar changes in protein expression based on immunohistochemical analyses. The localization of SPARC, peristin, and tenasin C predominantly within the stroma of OSCC tumors supports the idea that stromal elements are important in OSCC pathogenesis. Our observations indicate that significant changes in the expression of 4 genes, SPARC, POSTN, TNC, and TGM3, initially identified by gene microarray studies, are associated with similar changes in protein expression based on immunohistochemical analyses. The localization of SPARC, peristin, and tenasin C predominantly within the stroma of OSCC tumors supports the idea that stromal elements are important in OSCC pathogenesis.

Submitted for Publication: June 13, 2007; accepted September 16, 2007.

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Author Contributions: Drs Choi, Mendez, and Chen had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Choi, Jordan, Mendez, Farwell, and Chen. Acquisition of data: Choi, Jordan, Mendez, Houck, and Futran. Analysis and interpretation of data: Choi, Jordan, Mendez, Yueh, and Chen. Drafting of the manuscript: Choi. Critical revision of the manuscript for important intellectual content: Choi, Jordan, Mendez, Houck, Yueh, Farwell, Futran, and Chen. Statistical analysis: Choi and Yueh. Obtained funding: Choi, Farwell, Futran, and Chen. Administrative, technical, and material support: Mendez, Yueh, and Chen. Study supervision: Yueh, Farwell, Futran, and Chen. Financial Disclosure: None reported.

Funding/Support: This review was supported by grant R01 CA 095419-01A1 from the National Cancer Institute, National Research Service Award T32 DC00018 from the National Institute on Deafness and Other Communication Disorders, and in part by Fred Hutchinson Cancer Research Center funds.

Role of the Sponsors: The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

CONCLUSIONS


