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Genetic Characterization of Vocal Fold Lesions: Leukoplakia and Carcinoma

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Abstract

Objectives/Hypothesis—Malignant transformation of laryngeal keratosis has been reported in a substantial subset of patients, yet reliable criteria for predicting patients most at risk have yet to be determined. Current methods for determining dysplasia ratings are susceptible to errors in biopsy sampling and interpretation. An understanding of the genetic underpinnings of the progression of vocal fold tumorigenesis may contribute to the creation of reliable and predictive diagnostic criteria. We hypothesized that genetic expression markers distinguish patients with keratotic noncancerous vocal fold lesions from invasive carcinoma.

Study Design—Observational cross-sectional study.

Methods—Real-time polymerase chain reaction (RT-PCR) was used to compare expression of 84 cancer pathway genes of patients following histologic diagnosis of nondysplastic keratotic epithelium (ND) ($n = 7$), dysplastic keratotic epithelium (DYS) ($n = 3$), and invasive carcinoma (CA) ($n = 7$). All patients had a clinical diagnosis of leukoplakia, and biopsies were obtained from true vocal fold tissue.

Results—Four genes (IGF-1, EPDR1, MMP-2, S100A4) were significantly upregulated in DYS over the ND group. Seven genes were significantly upregulated in CA over the DYS group, and 31 genes were significantly upregulated in CA over the ND group ($P < .02$). The expression of matrix metalloproteinases (MMP-1, MMP-2, MMP-9) was found to statistically differentiate the groups ($P < .02$) and suggested disease progression associated with extracellular matrix degradation and angiogenesis promotion.

Conclusions—With these preliminary array data, we demonstrate the feasibility of using RT-PCR to identify distinct genetic expression between diagnostic groups. Characterization of genetic changes marking the progression of vocal fold tumorigenesis may lead to robust diagnostic criteria in the future.

Keywords

Leukoplakia; vocal fold tumorigenesis; genetic expression; laryngeal keratosis

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Level of Evidence: 4.

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INTRODUCTION

Early detection has been reported to be the strongest predictor for survival rate in patients with laryngeal squamous cell carcinoma (SCCa),¹ yet rigorous diagnostic markers for the riskiest precancerous lesions are yet undiscovered. Vocal fold leukoplakia has a malignancy transformation rate of approximately 8%.² It presents as a keratinized white patch on the epithelium. Clinical diagnosis is made by imaging the larynx in the office setting, followed by sampling the tissue in the operating room or the office. Histopathology has traditionally been used to determine the presence and degree of dysplasia in tissue samples. Approximately 50% of patients clinically diagnosed with laryngeal leukoplakia have no dysplasia found during histopathologic assessment,³ yet it is known that a subset of these patients will undergo malignant transformation at some point in their lives.² Histopathologic diagnoses are subject to errors of biopsy sampling and interpretation.⁴ As traditional diagnostic methods are imperfect and prognostic categories are unknown, vocal fold leukoplakia presents a unique clinical challenge. If an otolaryngologist adopts a “watchful waiting” approach, at-risk patients may be lost to follow-up and aggressive disease could insidiously develop; if the physician recommends surgical or radiation treatment, unnecessary laryngeal injury could occur in patients that may have otherwise never developed cancer.

Current diagnostic tools are inadequate for identifying patients with the highest risk profiles, partly because of an incomplete understanding of the aberrant cellular and genetic pathways associated with the progression of laryngeal SCCa. As severity of dysplasia determined via histopathology is associated with rate of malignant conversion,² genetic characterization of vocal fold leukoplakia may provide a model to study the promotion of laryngeal tumorigenesis. Across health care, high-throughput gene-based assays able to identify predictive and prognostic gene markers are emerging as viable methods for assisting physicians with clinical decision-making. Importantly, these technologies have yielded gene expression profiles that are better able to predict prognosis than traditional clinicopathologic criteria.⁵ To date, research focused on genetic expression specific to vocal fold SCCa has been limited in the number of genes evaluated and the heterogeneity of tissues used. Further, no investigation of the genetic correlates specific to vocal fold leukoplakia has been reported, per our literature search. Data from this premalignant group may contribute substantially to our understanding of laryngeal tumorigenesis and guide the development of early detection diagnostics. The objective of this study was to identify genetic regulation patterns distinguishing patients diagnosed with vocal fold leukoplakia and varying levels of dysplasia by using real-time polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Subjects

Following a comprehensive examination of the laryngopharyngeal mucosa by an otolaryngologist and obtaining consent for enrollment in the study, true vocal fold biopsy specimens were obtained from 28 individuals according to a protocol approved by the University of Wisconsin-Madison Institutional Review Board. Six specimens were eliminated from the study because of an insufficient amount of tissue available, two were excluded as their histopathology results were outside the scope of the study (i.e., papilloma), and three were excluded because of regulatory discrepancies. Seventeen specimens were used in the final analysis. All seventeen subjects had an initial clinical diagnosis of leukoplakia or suspected malignancy (Table I). None of these patients had undergone previous clinical evaluation or lesion biopsy. Further exclusionary criteria included any coexisting disease or lesions affecting the larynx (e.g., contact ulcers, uncontrolled reflux disease, vocal fold mass lesions), prior irradiation of the larynx, surgery in the head or neck

affecting the larynx, evidence of coexisting head and neck malignancy, and non-SCCa. For each participant, the biopsy specimen was sectioned for histopathologic evaluation, and the remaining tissue was reserved for later genetic analysis. Group membership was determined by the histopathology results and included subjects with nondysplastic keratotic epithelium (ND) (n = 7), dysplastic keratotic epithelium (DYS) (n = 3), and invasive carcinoma (CA) (n = 7). A medical chart review was completed before manuscript submission to identify any patients in the ND and DYS groups that developed cancer in the follow-up period. The follow-up period ranged from 1 month to 3 years.

Tissue

All specimens were collected at University of Wisconsin-Madison Hospital and Clinics by faculty and residents in the Division of Otolaryngology–Head and Neck Surgery. Tissue was placed in RNAlater solution (Ambion, Austin, TX), flash frozen in liquid nitrogen, and stored at -80°C until processing. Tissue disruption and homogenization was completed using a motorized mortar and pestle (Pellet Pestle; Kontes, Vineland, NJ). An RNeasy Mini kit (Qiagen, Valencia, CA) was used to extract total RNA from all samples. The optional on-column DNase digestion using the RNase-Free DNase Set (Qiagen, Valencia, CA) was performed. RNA concentration and quantity was assessed using a Nanodrop 1000 Spectrophotometer (Thermo Scientific, Barrington, IL).

Human Cancer PathwayFinder

Three hundred nanograms of RNA from each sample were reverse-transcribed using an RT² First Strand Kit (SABiosciences, Valencia, CA). The cDNA from each sample was added to the RT² SYBR Green/ROX qPCR Master Mix and aliquoted individually into a 96-well RT Profiler PCR Array-Human Cancer PathwayFinder (SABiosciences, Valencia, CA) according to manufacturer instructions. This assay profiles the expression of 84 genes involved in six tumorigenesis pathways (adhesion, angiogenesis, cell cycle control and DNA damage repair, apoptosis and cell senescence, signal transduction molecules and transcription factors, and invasion and metastasis). RT-PCR was completed in a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). Amplification was performed under the following conditions: 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, 60°C for 1 minute, immediately followed by a melt curve of 95°C for 15 seconds, 60°C for 1 minute, and 95°C for 30 seconds. Relative quantitative analysis was performed. RPL13A was selected as the housekeeping gene, as raw cycle threshold (Ct) values across the samples were consistent. To obtain normalized Ct values for each gene (ΔCt), the raw Ct value of the housekeeping gene (RPL13A) was subtracted from the raw Ct value of the gene of interest. Average ΔCt values for each gene were obtained by pooling the normalized values from each sample in the group. For group comparisons, the difference between the average ΔCt value between groups was calculated ($\Delta\Delta\text{Ct}$), and the fold change was determined using the formula: $2^{-\Delta\Delta\text{Ct}}$.

Validation of our Human Cancer PathwayFinder array results was performed with RT-PCR for four experimental genes and one housekeeping gene using the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA). The specificity of primers was assessed using PCR, which yielded a single DNA band of expected size on agarose gel (Table II) (Integrated DNA Technologies, Coralville, IA).

Statistical Analysis

One-way analysis of variance was used to determine whether the average ΔCt values were different between the groups. Pairwise comparisons were performed using Fisher protected least significant difference tests. $P < .02$ was considered significant. As we were using this as a screening tool to select genes for further study, no corrections were made for multiple

comparisons. Results were obtained using SAS statistical software version 9.1 (SAS Institute, Inc., Cary, NC).

RESULTS

The Human Cancer PathwayFinder Array was preliminarily used as a high throughput method to detect significant gene expression changes, which were further evaluated by confirmatory RT-PCR in triplicate. Expression data are presented in Figure 1. Fold change refers to the ratio of gene expression found in the group listed first to the group listed second. If fold change is greater than "0," the gene is considered to be upregulated, and if the fold change is less than "0," the gene is considered to be downregulated in the group comparison (e.g., matrix metalloproteinase [MMP]-1 is upregulated 45-fold in the cancer group as compared to the nondysplastic group). Four genes were found to be significantly upregulated in DYS compared to the ND group, seven genes were significantly upregulated in CA compared to the DYS group, and 31 genes were found to be significantly upregulated in CA compared to the ND group ($P < .02$). The significant genes are listed and briefly described in Table III. The genes that were identified as downregulated in the between-group comparisons were not statistically significant at the $P < .02$ level.

Using RT-PCR, we confirmed the expression level of four experimental genes and one housekeeping gene using the original 17 patient samples (Fig. 2). The results showed the same direction and a similar magnitude of change as compared with the original gene expression levels.

A review of the medical records revealed that none of the 10 subjects in the ND and DYS groups developed cancer, although it should be noted that 70% (7 of 10) of these subjects were eventually lost to follow-up (Table I).

DISCUSSION

Genes Distinguishing Keratinized, Dysplastic Epithelium From Keratinized, Nondysplastic Epithelium of the Vocal Fold

Insulin-like growth factor 1 (IGF-1), MMP-2, S100 calcium binding protein A4 (S100A4), and ependymin related protein 1 (EPDR1) were all found to be significantly upregulated in patients with dysplastic lesions as compared to those with nondysplastic lesions. IGF-1 is released when MMP-9 cleaves IGF-binding protein 3, which has been associated with proliferation in a human prostate adenocarcinoma cell line.⁶ IGF-1 and MMP-9 were both upregulated in the DYS group compared to the ND group. In fact, with an 8.53-fold increase in expression in IGF-1, it was the gene that most significantly distinguished patients with dysplasia from patients without dysplasia, marking it as a potential early marker for vocal fold cancer cell proliferation.

MMP-2 was upregulated 4.41-fold in the dysplastic group compared to the nondysplastic group. MMPs are most well known for their role in extracellular matrix degradation. They were originally thought to contribute to cancer invasion by breaking down extracellular structural components thereby allowing for cancer cell migration to local/distal areas. In the past decade, new roles for MMPs in tumorigenesis have emerged, implicating their involvement at earlier stages of disease progression than originally thought. In vitro and animal studies have demonstrated that MMPs are players in signaling cascades regulating tumor cell proliferation, apoptosis, tumor angiogenesis, and immune response.⁷ MMP-2 has also been shown to be upregulated in dysplastic esophageal tissue which has not progressed to SCCa,⁸ demonstrating that it is not merely a gene marking invasion.

Increased expression of S100A4 has been linked to many diseases, including laryngeal, colorectal, and breast cancers.⁹ Although the role of S100A4 in cancer has not been fully discovered or clearly defined, there is evidence that it is involved in regulating cell-cycle progression and intercellular adhesion, both early processes in tumorigenesis.¹⁰ E-cadherin, a molecule responsible for calcium-dependent epithelial cell-cell adhesion, has been reported to be a powerful suppressor of cancer cell invasion. In an animal model, S100A4 was shown to down-regulate E-cadherin.¹¹ In the present study, S100A4 was upregulated 2.76-fold in patients with dysplastic lesions as compared with patients with nondysplastic lesions. In patients with dysplasia, upregulation of S100A4 may provide an opportunity for disease progression via depletion of E-cadherin and eventually epithelial breakdown. In addition to S100A4, EPDR1 was significantly upregulated (i.e., 7.64-fold) in the DYS group compared to the ND group. EPDR1 is also a protein involved in calcium-dependent cell adhesion. It is suggested to have antiadhesive properties and is highly expressed in colorectal tumor cells.¹² Per our literature search, no investigation determining the relationship between S100A4, EPDR1, and malignancy has been completed to date.

Genes Distinguishing Vocal Fold Carcinoma From Dysplastic, Keratotic Epithelium

MMP-1, vascular endothelial growth factor A (VEGF-A), MMP-9, serpin peptidase inhibitor 1 (SERPINE1), plasminogen activator (PLAU), telomerase reverse transcriptase, and cyclin-dependent kinase 2 were significantly upregulated in patients with malignant vocal fold lesions as compared to the patients with dysplastic lesions.

The gene demonstrating the most dramatic upregulation across the dataset was MMP-1. Confirmatory RT-PCR revealed that the patients with carcinoma had a 46.67-fold increase in MMP-1 expression as compared with the patients with dysplastic lesions. These data identify MMP-1 as having a later role in vocal fold tumorigenesis. Cleavage of collagen type 1 is necessary for endothelial cell invasion and vessel formation.¹³ As a known collagenase I, increased expression of MMP-1 is potentially a marker for invasive vocal fold carcinoma in the present study through its role in angiogenesis. Vascularization has also been associated with increased expression of VEGF-A when tissue inhibitor of metalloproteinase-1 (TIMP-1) is overexpressed in mammary carcinoma cells.¹⁴ In the present study, VEGF-A expression in the carcinoma group was increased 5.27-fold as compared with the dysplastic group, and TIMP-1 expression in the carcinoma group was increased 1.84-fold as compared with the dysplastic group, suggesting that VEGF-1 activity is linked to TIMP-1 activity through an angiogenesis pathway.

MMP-9 is known to promote invasion of tumor cells into surrounding tissue by localizing to invadopodia.¹⁵ Invadopodia are protrusions, or “feet,” on the cell membrane that associate with molecules for proteolysis, transforming the cell into a workhorse for extracellular matrix degradation. In addition to MMP-9, other genes involved in protease activity that were significantly upregulated in the carcinoma group compared to the dysplastic group include SERPINE1 and PLAU. SERPINE1 and PLAU have both been reported to be associated with metastasis in multiple head and neck SCCa sites, consistent with the present results.¹⁶

Genes Distinguishing Vocal Fold Carcinoma From Nondysplastic, Keratotic Epithelium

The 31 genes upregulated in patients with carcinoma as compared to patients with nondysplastic lesions can be found in Figure 1.

MMP-1 was the gene that most significantly differentiated these two groups, with a 36.66-fold increase in expression found in the carcinoma group compared to the nondysplastic group. MMP-2 and MMP-9 also significantly distinguished the groups, potentially acting by

localizing to invadopodia.¹⁵ Of the several MMPs known to be upregulated in head and neck cancer, MMP-1, MMP-2, and MMP-9 are the most frequently reported,¹⁷ a finding that is supported with the present data.

MMP-2 and MMP-9 have been shown to cleave transforming growth factor β 1 (TGF- β 1) at the cell membrane, thereby making it bioavailable.¹⁸ In the present study, TGF- β 1 was significantly upregulated in patients with carcinoma as compared to the patients with nondysplastic lesions. Expression of TGF- β 1 in oral epithelium is associated with keratinocyte hyperproliferation in transgenic mice, a finding the authors suspected was due to inflammation and angiogenesis.¹⁹ Chronic inflammation has not only been observed clinically in many patients with head and neck SCCa, it also has been suggested to contribute to tumorigenesis.¹⁹ One pathway leading to inflammation begins with the recruitment of leukocytes by TGF- β 1 via paracrine signaling. Leukocytes and keratinocytes then encourage a proinflammatory response via many downstream cytokines and transcription factors, such as tumor necrosis factor α (TNF- α).¹⁹ TNF- α expression was significantly upregulated in the patients with carcinoma as compared to the patients with nondysplastic lesions in the present study.

The most well-studied endogenous MMP inhibitors are TIMPs. TIMP-1 expression has been shown to be significantly correlated with advanced T stage in head and neck SCCa.²⁰ In the present study, TIMP-1 was significantly upregulated in the patients with carcinoma as compared to the patients with nondysplastic lesions, but also showed increased expression in the CA:DYS and DYS:ND comparisons. This suggests that there is increased TIMP-1 expression concurrent with disease progression. One mechanism for TIMP-associated disease progression may be due to a coupling effect rather than a causative effect; as the overall MMP activity increases with tumorigenesis, so does the inhibitory activity of TIMP-1 expression.⁷

Limitations

The findings of this study are limited by the small number of participants included, the poor rates of patient follow-up, and the limited number of genes assessed. Despite these limitations, we were able to demonstrate methodologic feasibility for this preliminary project.

CONCLUSIONS

The present data represent quantified genetic expression changes that distinguish patients with ND, DYS, and CA. The data mirror our understanding of the stepwise progression of SCCa. With this project, we demonstrate an ability to obtain true vocal fold lesions, extract high-quality RNA, perform RT-PCR with the RNA, and identify gene-expression changes with biologic significance. A logical next step would be to use cDNA microarray to further define the genomic expression profiles associated with these patient populations to stratify patients into prognostic categories and guide clinical decision making.

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Fig. 1.

Eighty-four gene array data for the three group comparisons. Fold change represents the ratio of gene expression found in the group listed first as compared to the group listed second. Each histogram bar represents a comparison between two groups (e.g., DYS: ND = gene upregulation observed in patients with dysplastic lesions as compared with patients with nondysplastic lesions). $P < .02$. TF = transcription factors; ND = nondysplastic keratotic epithelium; DYS = dysplastic keratotic epithelium; CA = invasive carcinoma. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 2.

Confirmatory real-time polymerase chain reaction (RT-PCR) performed in triplicate validated array findings. Confirmatory RT-PCR yielded fold-change expression levels that were in the same direction (up- or downregulated) and had similar magnitude as was found via arrays. Fold change represents the ratio of gene expression found in the group listed first as compared to the group listed second. Each histogram bar represents the relationship between two groups (e.g., DYS:ND = gene regulation observed in patients with dysplastic lesions as compared with patients with nondysplastic lesions). MMP = matrix metalloproteinase; TGF = transforming growth factor; CDKN2A = cyclin-dependent kinase 2; ND = nondysplastic keratotic epithelium; DYS = dysplastic keratotic epithelium; CA = invasive carcinoma.

TABLE I

Subject Demographic Data.

ID	Sex	Age, yr	Presenting Signs/Symptoms	Histopathology	Follow-up Period
1	M	59	Hoarseness	ND	LTF following biopsy
2	M	57	Hoarseness	ND	FOD 2 mo following biopsy, then LTF
4	M	65	Hoarseness	ND	FOD 4 mo following biopsy, then LTF
9	M	76	Hoarseness	ND	FOD 3 yr
11	M	47	Hoarseness	ND	FOD 2 yr, 7 mo
14	M	48	Hoarseness	ND	LTF following biopsy
18	M	70	Hoarseness	ND	FOD 1 mo following biopsy, then LTF
12	M	57	Hoarseness	DYS	FOD 2 yr, 4 mo
13	M	52	Hoarseness	DYS	LTF following biopsy
24	M	77	Hoarseness	DYS	FOD 5 mo following biopsy, then LTF
6	M	78	Hoarseness	CA	DOD 4 yr following biopsy
10	M	60	Hoarseness	CA	Recurred 3 yr following surgery, FOD after radiation
21	M	68	Hoarseness	CA	FOD 15 mo following surgery
22	M	68	Hoarseness	CA	DOD 4 mo following biopsy; refused treatment
23	M	78	Hoarseness	CA	FOD 14 mo following chemoradiation
25	M	74	Hoarseness	CA	FOD 1 yr following surgery and radiation
28	M	59	Hoarseness	CA	FOD 7 mo following surgery

ND = nondysplastic keratotic epithelium; DYS = dysplastic keratotic epithelium; CA = invasive carcinoma; LTF = lost to follow-up; FOD = free of disease; DOD = died of disease; ID = identification number; M = male.

TABLE II

Primers Used for Real-Time Polymerase Chain Reaction Validation.

Gene	GenBank No.	Forward (5'-3')	Reverse (3'-5')	PCR Product
<i>RPL13A</i>	NM_012423.2	CGCCCTACGACAAGAAAAAG	TGGTACTTCCAGCCAACCTC	121 bp
<i>MMP-1</i>	NM_002421	TGCAACTCTGACGTTGATCCCAGA	ACTGCACATGTGTTCTTGAGCTGC	122 bp
<i>MMP-2</i>	NM_004530	AGAAGGATGGCAAGTACGGCT	AGTGGTGCAGCTGTCATAGGATGT	128 bp
<i>TGF-β1</i>	NM_000660	TGCTCGCCCTGTACAACAGCA	CGTTGTGGGTTTCCACCATTAGCA	126 bp
<i>CDKN2A</i>	NM_000077.4	ACCAGAGGCAGTAACCATGC	CCTGTAGGACCTTCGGTGAC	129 bp

PCR = polymerase chain reaction.

TABLE III

Genes Found to Statistically Differentiate the Groups.

Gene	GenBank #	Chromosomal Locus	Description
<i>ATM</i> [‡]	NM_000051.3	11q22-q23	Cell cycle checkpoint kinase
<i>BAD</i> [‡]	NM_004322.3	11q13.1	Regulator of apoptosis
<i>BAX</i> [‡]	NM_004324.3	19q13.3-q13.4	Activator of apoptosis
<i>CDC25A</i> [‡]	NM_001789.2	3p21	Phosphatase required for cell cycle progression
<i>CDK2</i> ^{‡,‡}	NM_001798.3	12q13	Protein kinase necessary for cell cycle progression
<i>CDK4</i> [‡]	NM_000075.2	12q14	Protein kinase necessary for cell cycle progression
<i>CHEK2</i> [‡]	NM_001005735.1	22q12.1	Cell cycle checkpoint regulator
<i>E2F1</i> [‡]	NM_005225.2	20q11.2	Transcription factor involved in cell cycle control
<i>EPDR1</i> ^{*,‡}	NM_017549.3	7p14.1	Type II transmembrane protein
<i>GZMA</i> [‡]	NM_006144.3	5q11-q12	T cell- and natural killer cell-specific protease
<i>IFNβ1</i> [‡]	NM_002176.2	9p21	Interferon β 1, involved in tumor angiogenesis
<i>IGF1</i> ^{*,‡}	NM_000618.3	12q23.2	Protein involved in regulation of growth and development
<i>ITGA4</i> [‡]	NM_000885.4	2q31.3	Integrin, α 4 involved in adhesion
<i>ITGA5</i> [‡]	NM_001144999.1	2q31-q32	Integrin, α 5 involved in adhesion
<i>ITGB3</i> [‡]	NM_000212.2	17q21.32	Integrin chain β 3 involved in cell adhesion/signaling
<i>MMP-1</i> ^{‡,‡}	NM_001145938.1	11q22.3	Collagenase-1; interstitial, fibroblast, tissue collagenase
<i>MMP-2</i> ^{*,‡}	NM_001127891.1	16q13-q21	Gelatinase A, 72-kDa gelatinase, neutrophil gelatinase
<i>MMP-9</i> ^{‡,‡}	NM_004994.2	20q11.2-q13.1	Gelatinase B, 92-kDa gelatinase
<i>MTA1</i> [‡]	NM_004689.3	14q32.3	Protein involved in metastasis, role unclear
<i>NME4</i> [‡]	NM_005009.2	16p13.3	Kinase that regulates transfer of gamma-phosphates
<i>PDGFA</i> [‡]	NM_002607.5	7p22	Platelet-derived growth factor protein α
<i>PDGFB</i> [‡]	NM_002608.2	22q13.1	Platelet-derived growth factor protein β
<i>PLAU</i> ^{‡,‡}	NM_001145031.1	10q24	Plasminogen activator involved in ECM breakdown
<i>PLAUR</i> [‡]	NM_001005376.1	19q13	Plasminogen activator receptor
<i>S100A4</i> ^{*,‡}	NM_002961.2	1q21	S100 calcium binding protein A4
<i>SERPINE1</i> ^{‡,‡}	NM_000602.3	7q21.3-q22	Serpin peptidase inhibitor 1
<i>TERT</i> [‡]	NM_001193376.1	5p15.33	Telomerase reverse transcriptase
<i>TGF-β1</i> [‡]	NM_000660.4	19q13.1	Regulates proliferation, differentiation, adhesion
<i>THBS1</i> [‡]	NM_003246.2	15q15	Adhesive glycoprotein
<i>TIMP1</i> [‡]	NM_003254.2	Xp11.3-p11.23	Inhibitor of MMPs, promotes cell proliferation
<i>TNFα</i> [‡]	NM_000594.2	6p21.3	Tumor necrosis factor proinflammatory cytokine
<i>TWIST1</i> [‡]	NM_000474.3	7p21.2	Transcription factor involved in cell lineage determination
<i>VEGFA</i> [‡]	NM_001025366.2	6p12	Vascular endothelial growth factor A

Upregulation found for group relationships:

* dysplastic keratotic epithelium compared to nondysplastic keratotic epithelium (DYS:ND);

† invasive carcinoma compared to dysplastic keratotic epithelium (CA:DYS);

‡ invasive carcinoma compared to nondysplastic keratotic epithelium (CA:ND).

ECM = extracellular matrix; MMPs = matrix metalloproteinases.