Genomic Analysis Defines a Cancer-Specific Gene Expression Signature for Human Squamous Cell Carcinoma and Distinguishes Malignant Hyperproliferation from Benign Hyperplasia

Asifa S. Haider[1], Sara B. Peters[2], Helen Kaporis[1], Irma Cardinale[1], Ji Fei[3], Jurg Ott[3], Miki Blumenberg[4], Ann M. Bowcock[5], James G. Krueger[1] and John A. Carucci[6]

Using high-density oligonucleotide arrays, we measured expression of >12,000 genes in surgical excisions of invasive human squamous cell carcinomas (SCCs) versus site-matched control skin. This analysis defined >1,900 genes with altered expression in SCCs that were statistically different from controls. As SCCs are composed of epithelial cells, which are both hyperplastic and invasive, we sought to define gene sets associated with these biologic processes by comparing gene expression to psoriasis vulgaris, which is a condition of benign keratinocyte hyperplasia without invasiveness or pre-malignant potential. Through this analysis, we found genes that were commonly upregulated in both conditions and unique genes with increased expression in SCCs. Differential gene regulation in these two conditions was confirmed by real-time reverse transcription-PCR and immunohistochemistry. We found that benign hyperplasia is associated with upregulation of genes including DEFB4 (defensin B4), SERPINB3 (serine proteinase inhibitor, member 3), STAT1 (signal transducer and activator of transcription 1), K16 (keratin 16), CEACAMs (carcinoembryonic antigen-related cell adhesion molecules), and WNT 5A (wingless-type MMTV integration site family, member 5A). WNT receptor frizzled homolog 6 (FZD6) and prostaglandin-metabolizing enzyme hydroxyprostaglandin dehydrogenase were increased in SCC alone. Growth factor pleiotrophin (PTN) was expressed at higher levels in non-tumor-bearing skin adjacent to excised SCC. SCC was further characterized by upregulation of matrix metalloproteinases 1, 10, and 13, cathepsin L2, cystatin E/M as well as STAT3 and microseminoprotein, beta (MSMB), and downregulation of inducible nitric oxide synthase, granzyme B, CD8, and CD83. The current study defines a unique gene expression signature for cutaneous SCC in humans and suggests potential roles for WNT, FZD, and PTN in the pathogenesis of SCC.

Journal of Investigative Dermatology (2006) **126,** 869–881. doi:10.1038/sj.jid.5700157; published online 9 February 2006

[1]*Laboratory of Investigative Dermatology, Rockefeller University, New York, New York, USA;* [2]*Department of Pathology, Weill Medical College of Cornell, New York, New York, USA;* [3]*Laboratory of Statistical Genetics, Rockefeller University, New York, New York, USA;* [4]*Ronald O. Perelman Department of Dermatology, New York University, New York, New York, USA;* [5]*Department of Genetics, Washington University School of Medicine, St Louis, Missouri, USA and* [6]*Section of Mohs Micrographic and Dermatologic Surgery, Department of Dermatology, Weill Medical College of Cornell, New York, New York, USA*

Correspondence: Dr John A. Carucci, Section of Mohs Micrographic and Dermatologic Surgery, Department of Dermatology, Weill Medical College of Cornell, 525 East 68th Street, Starr 326, New York, New York 10021, USA. E-mail: JAC2015@med.cornell.edu

Abbreviations: CDK, cyclin-dependent kinase; DEFB4, defensin B4; EDC, epidermal differentiation complex; FZD6, frizzled homolog 6; GZMB, granzyme B; HNSCC, squamous cell carcinoma of the head and neck; HPGD, hydroxyprostaglandin dehydrogenase; HARP, human acidic ribosomal protein; iNOS, inducible nitric oxide synthase; MMP, matrix metalloproteinase; PTN, pleiotrophin; RT-PCR, reverse transcription-PCR; SCC, squamous cell carcinoma; STAT, signal transducer and activator of transcription; TIMP, tissue inhibitor of metalloproteinase; WNT 5A, wingless-type MMTV integration site family, member 5A

Received 20 July 2005; revised 3 November 2005; accepted 16 November 2005; published online 9 February 2006

INTRODUCTION

Primary cutaneous squamous cell carcinoma (SCC) is the second most common human cancer and may behave aggressively (Goldman, 1998). As with other invasive cancers, cutaneous SCC, if left untreated, will invade locally, resulting in extensive tissue damage and may eventually metastasize to lymph nodes and distant organs (Veness *et al.*, 1999; Nguyen, 2004). The pathogenesis of SCC is multifactorial. Exposure to ultraviolet radiation, primarily UVB, is a major risk factor owing to DNA damage leading to mutations in tumor suppressor p53 (Brash *et al.*, 1996; Leffell and Brash, 1996). In addition, other factors, including human papilloma virus, have also been implicated in SCC development, indicating the presence of other pathogenic mechanisms and potential regulatory points (Goldman, 1998). Psoriasis (P) is a benign, inflammatory condition characterized by development of cutaneous plaques most commonly located on extensor surfaces (Lowes *et al.*, 2004). Psoriasis is characterized by epidermal hyperproliferation, and altered

expression of ~1,300 genes has been described (Zhou *et al.*, 2003). These include pro-inflammatory cytokines IL-2, IL-6, IL-8, IL-12, IL-23, and IFN-γ (Lew *et al.*, 2004). Psoriasis was chosen for comparison with SCC based on the fact that like SCC, it is characterized by hyperproliferation, but unlike SCC, it is reversible and lacks the capacity for invasion.

Microarray technology allows for global expression profiling of large numbers of genes. Although it has been used to study human cancers (Lu *et al.*, 2001; Leversha *et al.*, 2003), genes contributing to hyperplasia versus malignancy have remained undefined. By defining the gene expression profile for SCC and subtracting genes activated in psoriasis, we were able to define a provisional cancer-specific gene set distinguishing malignant from benign hyperproliferation in human epithelial tissue. Immunohistochemistry was also performed to compare the inflammatory infiltrate of SCC with that of psoriasis.

We found the following: (1) wingless-type MMTV integration site family, member 5A (WNT 5A) was upregulated in SCC and psoriasis, but WNT receptor frizzled homolog 6 (FZD6) was upregulated in SCC alone; (2) expression of pleiotrophin (PTN) was increased in SCC compared with psoriasis but expressed at even higher levels in non-tumor-bearing (N) skin adjacent to excised SCC; (3) SCC was characterized by enhanced expression of proteinases including cathepsin L (CTSL2) and matrix metalloproteinases (MMPs 1, 10, and 13), but decreased expression of protease inhibitor, tissue inhibitor of metalloproteinase (TIMP)3; (4) proliferation-associated genes including cyclin-activating kinase component, cyclin-dependent kinase (CDK)7, and cell cycle kinase, cell division cycle 7 (CDC7), oncogene KRAS, angiogenic factor epiregulin (EREG), and E2F transcription factor 3 were upregulated in SCC, whereas transcription regulators NF-κB2 and NF-κB inhibitor, NF-κBIA, and angiogenic factor vascular endothelial growth factor C were downregulated; (5) cytokine IL-18 was upregulated in SCC, whereas expression of activated T-cell marker CD69 and mature dendritic cell marker CD83 was downregulated and pro-inflammatory inducible nitric oxide synthase (iNOS) was virtually undetected.

RESULTS

We began analysis of human SCC by defining expression of genes in tumor tissue *versus* adjacent (site-matched) normal skin from the same patients using Affymetrix U95 arrays. Statistically significant differences in expression of genes with elevated or reduced expression in SCC tissue were defined by a paired Welch test, with a subsequent correction for multiple gene comparisons by the Benjamini–Hochberg method. This analysis defined 1,048 genes with increased expression and 870 genes with decreased expression in SCC *versus* non-tumor-bearing tissue, as displayed by hierarchical clustering and heat map values in Figure 1a. All genes displayed in this figure are listed in Table S1, whereas the individual *P*-values for expression differences (all are $P<0.05$ after multiplicity correction) are given in Table S6. We noted that many of the genes that were upregulated in SCC were identical to genes previously detected as increased in expression in lesional

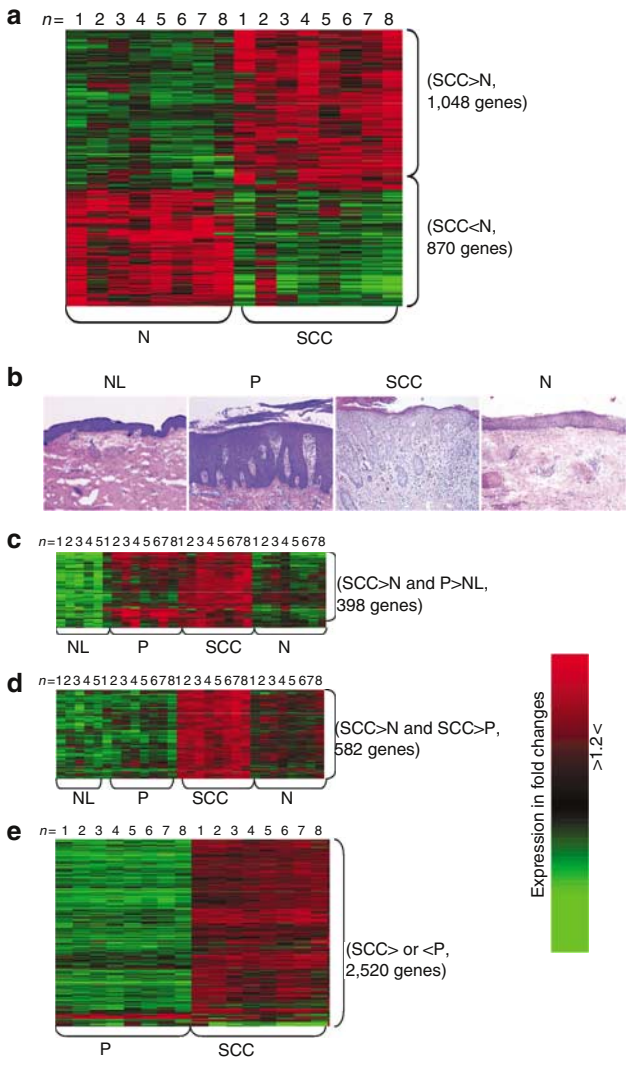

Figure 1. Gene expression profiling of SCC and psoriasis. Gene array analysis of mRNA from eight squamous cell carcinoma (SCC), eight site-matched non-tumor-bearing (N), eight psoriasis (P), and five non-lesional (NL) skin biopsies: mRNA was hybridized to individual oligonucleotide arrays containing ~12,000 human genes (HG-U95A/Av2 chips). Heat maps show unsupervised hierarchical clustering for gene expression differences that are statistically significant ($P<0.05$, after correction for multiplicity). Full tables of gene expression data are given in Tables S1–S5, whereas *P*-values for all expression differences are given in Table S6. (**a**) ''SCC cluster'': heat map of the 1,048 genes upregulated in SCC *versus* N (red area) and 870 genes downregulated in SCC *versus* N (green area). (**b**) Histological comparison of benign *versus* malignant hyperproliferative diseases: representative immunohistochemical hematoxylin and eosin staining of P, SCC, NL, and N. (**c**) ''Common cluster'': heat map of 398 genes commonly upregulated in SCC *versus* N, and P *versus* NL. (**d**) ''SCC-specific cluster'': heat map of 582 genes upregulated in SCC *versus* N but not increased in P. (**e**) Direct comparison of SCC with P: heat map of 2,520 genes up- or downregulated in SCC *versus* P. Expressions in site-matched (N) skin are not considered.

psoriasis tissue. Hence, it seemed that gene expression differences detected in SCC might be a mixture of ''background'' genes altered because of regenerative hyperplasia of keratinocytes and genes altered specifically because of neoplasia. To better characterize and refine gene sets that

are specifically associated with human SCCs *versus* benign hyperplasia, we performed two types of comparative analyses to define similarities and differences in pathologic gene expression in SCC *versus* psoriasis, as described below.

Before presenting genes that are commonly upregulated in SCC and lesional psoriasis tissue, we want to note some overall similarities in epidermal alterations in the two conditions (Figure 1b). Psoriasis is characterized by an ancanthotic epidermis, a marked increase in proliferating keratinocytes, elongated rete, a reduced granular layer, and maturation of squamous keratinocytes with retained nuclei (parakeratosis). SCCs of the type analyzed for this study displayed similar features of keratinocyte growth and differentiation, but nests of malignant keratinocytes were detached from the epidermis in SCCs and formed invading foci in the dermis. A mononuclear inflammatory infiltrate was evident in the dermis/stroma of both psoriasis and SCC. The gene comparison shown in Figure 1c shows heat maps of genes that are upregulated in psoriasis, in comparison to adjacent biopsies of non-lesional (NL) skin, and genes that are upregulated in SCC *versus* site-matched non-tumor-bearing skin. A short list of genes commonly upregulated in both conditions, with associated fold changes and *P*-values, is given in Table 1, whereas a complete list of shared genes is given in Table S2. Many of the shared genes encode proteins involved in epidermal differentiation, for example, desmoglein (DSG3), keratins 6/16, S100 proteins, and small proline-rich proteins (SPRR1) (bottom of Table 1). Other genes that are commonly upregulated in both conditions include the following. SERPINB3 (serine proteinase inhibitor, member 3; SCC antigen), a gene known to be highly expressed in psoriasis (De Pita *et al.*, 1999), cutaneous SCC (Takeda *et al.*, 2002), and carcinomas from organs other than skin (Takeda *et al.*, 2002; Ahmed *et al.*, 2004), was highly expressed in all SCC specimens as well as psoriasis. Carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) was also upregulated in both SCC and psoriasis (Figure 2).

Selected proliferation regulators showed increased expression in psoriasis and SCC. These included WNT 5A, transcription mediator signal transducer and activator of transcription (STAT)1, transforming growth factor-α, and cell cycle regulatory gene cyclin B1 (CCNB1). Genes encoded by the epidermal differentiation complex (EDC) including SPRR1A, 1B, and 2B, S100 A8, A9, and A14, and involucrin (IVL) were increased both in psoriasis and SCC. Finally, keratin 16 (KRT16) expression was also increased in both SCC and psoriasis. Data are summarized in Table 1. Reverse transcription-PCR (RT-PCR) experiments confirmed the

Table 1. Common upregulated genes in psoriasis and SCC

Fold change: P/NL	*P*-value< (corrected)	Fold change: SCC/N	*P*-value< (corrected)	Symbol	Biological process
1.93	0.00001	1.45	0.0001	ABCC1	Transport
2.03	0.00012	1.46	0.0127	ADAM23	Proteolysis and peptidolysis
1.90	0.00461	1.46	0.0002	BLNK	Immune response
1.70	0.00149	1.54	0.00001	CASP4	Apoptosis
3.02	0.00001	1.75	0.00001	CCNB1	Cell cycle
3.54	0.00001	2.01	0.00001	CD24	Immune response
2.78	0.00183	1.50	0.00001	CDH3	Cell adhesion
1.78	0.00001	1.49	0.00001	CDKN3	Cell cycle
4.57	0.00010	2.26	0.00001	CRABP2	Retinoic acid binding
5.51	0.00001	1.56	0.00001	ECGF1	Signal transduction
2.42	0.00001	1.75	0.0021	EVA1	Cell adhesion
3.29	0.01584	1.57	0.0001	G1P3	Immune response
2.46	0.00001	1.49	0.0012	GNA15	Signal transduction
1.62	0.00423	1.63	0.00001	GPR1	Signal transduction
2.73	0.00285	1.54	0.0478	HMOX1	Heme oxidation
5.04	0.00001	2.12	0.0001	IFI27	Immune response
1.33	0.02122	1.56	0.00001	IL12RB2	Cell proliferation
2.32	0.03800	1.53	0.014	IL8RB	Immune response
1.74	0.00001	1.52	0.0011	KIF11	Cell cycle
6.40	0.00001	1.98	0.00001	KLK10	Proteolysis and peptidolysis
9.99	0.00035	3.23	0.00001	KLK13	Proteolysis and peptidolysis
8.27	0.00754	2.81	0.0001	KLK6	Proteolysis and peptidolysis
2.20	0.00011	1.74	0.0034	KNTC2	Mitosis

Table 1 continued on following page

Table 1. continued

Fold change: P/NL	*P*-value< (corrected)	Fold change: SCC/N	*P*-value< (corrected)	Symbol	Biological process
1.78	0.00001	1.51	0.0001	LY6D	Cell adhesion
1.48	0.00868	1.49	0.001	MAD2L1	Cell cycle
2.08	0.00119	1.87	0.00001	MAPK13	Signal transduction
1.57	0.00001	1.55	0.00001	MAPK6	Signal transduction
6.66	0.00381	1.87	0.00001	MX1	Apoptosis
2.20	0.00001	1.53	0.0003	NMI	Transcription
2.62	0.01672	1.56	0.0005	OAS1	Immune response
5.24	0.00008	2.21	0.0013	OASL	Immune response
1.71	0.00109	1.49	0.00001	PAI-RBP1	Transcription
1.44	0.00198	1.53	0.0007	PKP3	Cell adhesion
1.76	0.00769	1.59	0.00001	PPARD	Proliferation
1.85	0.04603	1.72	0.00001	PTGER3	Prostaglandin E receptor
1.82	0.02160	1.47	0.00001	RANBP9	Cell growth
1.70	0.00392	1.30	0.0014	RB1	Cell cycle
2.52	0.02428	2.39	0.00001	RGS20	Signal transduction
1.86	0.00363	1.62	0.0001	RIT1	Signal transduction
24.34	0.00001	3.15	0.00001	SERPINB3	Proteinase inhibitor
77.23	0.00001	3.22	0.0001	SERPINB4	Proteinase inhibitor
1.81	0.01862	1.92	0.00001	SERPINB8	Proteinase inhibitor
5.74	0.00001	1.49	0.00001	STAT1	Signal transduction
1.93	0.03250	1.46	0.00001	TGFA	Cell proliferation
1.93	0.00090	1.77	0.0008	TNFSF10	Apoptosis
1.49	0.00026	1.68	0.00001	TPX2	Mitosis
2.78	0.00001	1.52	0.00001	WNT5A	Signal transduction
Molecules involved in epidermal differentiation (but may also have inflammatory functions)					
3.35	0.00010	1.92	0.00001	ALOX12B	Epidermal differentiation
2.35	0.00001	1.89	0.0098	DSG3	Cell adhesion
2.75	0.00541	2.30	0.00001	IVL	Human involucrin gene
10.24	0.00001	3.12	0.0014	KRT16	Epidermal differentiation
4.28	0.00017	2.19	0.0018	KRT6E	Biogenesis
1.59	0.00133	1.45	0.00001	S100A11P; S100A14	Cell proliferation
7.74	0.00012	1.61	0.0018	S100A12	Immune response
1.82	0.00001	1.61	0.00001	S100A2	Immune response
4.33	0.00084	2.30	0.0001	S100A7	Epidermal differentiation
4.45	0.00321	2.37	0.0013	S100A8	Immune response
13.22	0.00001	3.05	0.0002	S100A9	Immune response
5.02	0.00001	2.33	0.00001	SPRR1A	Epidermal differentiation
5.55	0.00001	1.98	0.00001	SPRR1B	Epidermal differentiation
2.10	0.00001	2.10	0.0067	SPRR2B	Epidermal differentiation
1.59	0.00001	1.59	0.0019	SPRR2C	Epidermal differentiation
4.76	0.00001	2.24	0.00001	TGM1	Keratinocyte transglutaminase

Selected list of genes with commonly upregulated expression in squamous cell carcinoma (SCC) or psoriasis (P) corresponding to heat map in Figure 1c and the upper region of Figure 1a. Fold changes are ratios of gene expressions in SCC/site-matched normal (N) and psoriasis (P)/non-lesional (NL) skin biopsies. Gene symbols and relevant biological processes are described in Gene Ontology annotation.

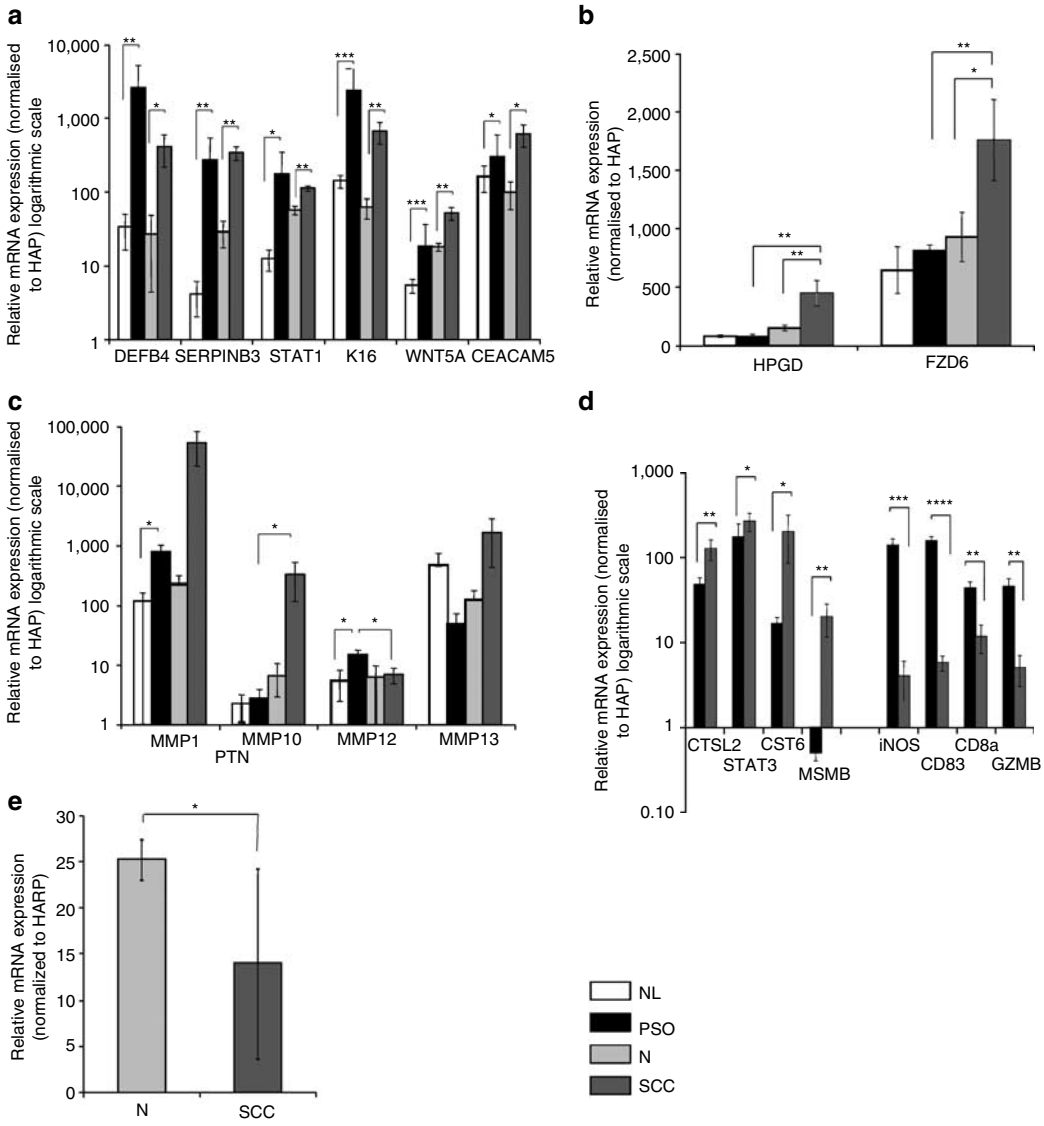

Figure 2. Real time RT-PCR confirmation of selected genes differentially expressed in SCC and psoriasis lesions. Mean values of gene expression for selected genes are graphically represented after normalization of expressions using gene to HARP mRNA. mRNA was analyzed from SCC specimens (SCC), site-matched normal (N), psoriasis (P), and non-lesional (NL) skin biopsies ($n=7$ for each): error bar shown , $^{*}P<0.05$; $^{**}P<0.01$; $^{**}P<0.001$. (**a**) Genes upregulated in both SCC and psoriasis, SCC>N and P>N: DEFB4, SERPINB3, STAT1, K16, WNT 5A, and CEACAM5. (**b**) SCC-specific genes, SCC>N and SCC>P: hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD) and FZD6. (**c**) MMPs in SCC and psoriasis: MMP1, MMP10, MMP12, and MMP13. (**d**) Genes differentially regulated in SCC *versus* psoriasis, SCC> or <P: genes with increased expression in SCC: cathepsin L2 (CTSL2), STAT3, cystatin E/M (CST6), and MSMB; genes with decreased expression in SCC: iNOS, CD83, CD8a, and GZMB. (**e**) PTN is downregulated in SCC.

upregulation of genes for DEFB4 (defensin B4), SERPINB3, STAT1, K16 (keratin 16), WNT 5A, and CEACAM5 in both SCC and psoriasis.

We were able to assign a total of 73 genes in the common clusters to keratinocytes (Table S3) through comparison with a series of cell-specific gene expression maps that we have recently created (Haider A.S. *et al.*, manuscript in preparation). These genes included KRT16, IVL, and other members of the EDC.

Through this comparative approach, we were also able to define genes that were uniquely upregulated in SCC. This group of genes is illustrated in Figure 1d, and a short list of genes, with fold changes and *P*-values and biological processes according to Gene Ontology, is given in Table 2. Key genes were selected as those regulating biological processes important to tumor progression, including invasion, proliferation, differentiation, and immune response, in a manner consistent with the classification scheme used by others in the description of SCC of the head and neck (HNSCC) (Ginos *et al.*, 2004). Some genes with well-characterized mutations associated with SCC were not differentially expressed in our studies. Expression of p53 and CDK4 was unchanged between SCC and site-matched non-tumor-bearing skin, whereas expression of CDKN2A was increased in four of eight patients but did not approach statistical significance (data not shown). Expression of p63

Table 2. Genes upregulated in SCC only

Fold change: P/NL	*P*-value < (corrected)	fold change: SCC/N	*P*-value < (corrected)	Symbol	Biological process
0.8	0.7720	1.3	0.0159	ATR	Apoptosis
1.1	0.1647	1.2	0.0297	CASP8	Apoptosis
1.0	0.2653	1.4	0.00001	CDC7	Cell cycle
1.1	0.1302	1.3	0.00001	CDK7	Cell cycle
0.8	0.9364	1.3	0.0247	COL4A6	Collagen metabolism
1.2	0.9356	1.5	0.0035	DEFB1	Immune response
1.1	0.3593	1.3	0.00001	E2F3	Transcription
1.5	0.8977	1.8	0.00001	EREG	Cell cycle
1.1	0.6890	1.6	0.000001	FZD6	Frizzled signaling pathway
1.6	0.1168	2.5	0.00001	HPGD	Prostaglandin metabolism
0.9	0.0544	1.9	0.00001	IL18	Immune response, angiogenesis
0.9	0.0645	1.2	0.0002	KRAS2	Cell cycle
1.0	0.4117	5.2	0.0418	KRT9	Epidermal differentiation
1.1	0.9480	1.4	0.0003	MAPK14	Kinase
1.4	0.0580	4.0	0.0236	MMP1	Collagen catabolism
0.9	0.5027	3.0	0.0045	MMP13	Collagen metabolism, proteolysis and peptidolysis
1.0	0.2792	1.3	0.0313	RAB11A	Cell cycle
0.8	0.0008	1.2	0.0357	RAB22A	Cell cycle
1.1	0.1155	1.3	0.00001	RAB8A	Cell cycle
1.2	0.9053	1.5	0.0018	RAB9A	Cell cycle
1.2	0.3067	1.2	0.0255	RAD1	DNA damage response
1.2	0.0709	1.3	0.00001	RARS	Cell cycle
1.0	0.002	1.4	0.00008	TP73L	Tumor protein p73-like, p63

Selected list of genes with upregulated expression in squamous cell carcinoma (SCC) only corresponding to heat map in Figure 1d. Fold changes are ratios of gene expressions in SCC/site-matched normal (N) and psoriasis (P)/non-lesional (NL) skin biopsies. Gene symbols and relevant biological processes are described in Gene Ontology annotation.

(TP73L), a p53 homolog, was increased in SCC compared with site-matched non-tumor-bearing skin (Table 2).

Expression of FZD6, a receptor for the WNT family of genes, was increased in SCC but not psoriasis. This was also true for prostaglandin pathway mediator hydroxyprostaglandin dehydrogenase (HPGD). MMP1 and 13 were detected to be significantly upregulated in genomic analysis of SCC (Table 2). MMP12 was more highly expressed in psoriasis and not expressed differentially between SCC and non-tumor-bearing skin. RT-PCR analysis showed a trend toward increased expression of MMP1 and MMP13 and confirmed significant expression of MMP10 in SCC while confirming increased expression of MMP12 in psoriasis (Figure 2).

Because we identified genomic alterations in expression of MMPs in SCCs that were not present in psoriasis, we wanted to confirm that gene expression differences were reflected in expression of these proteins in skin lesions. Immunohistochemical detection of MMP1, 10, and 13 showed MMPs 10 and 13 staining at perivascular areas within the stroma and MMP1 within the dermis (Figure 3).

Increased expression of cyclin-activating kinase component CDK7 was unique to SCC. Cell cycle mediator CDC7 (a cell cycle kinase) was also uniquely increased in SCC, as was DNA damage repair mediator RAD1 and apoptosis mediator CASP8. Increased expression of ATR, another gene regulating apoptosis, was specific to SCC and not observed in psoriasis. Kirsten ras gene homolog (KRAS) was uniquely increased in SCC. Other potential regulators of proliferation unique to SCC included E2F transcription factor 3, transcription mediator mitogen-activated protein kinase 14 (MAPK14), epiregulin (EREG), and IL-18.

We recognized that the approach used to define gene differences between SCC and psoriasis might be limited by differences in gene expression in the site-matched controls. For example, one can appreciate that many genes have higher background expression in skin adjacent to SCCs compared to non-lesional skin of psoriasis patients. Hence, we also performed a direct or ''head-to-head'' comparison of gene expression differences in SCC *versus* psoriasis lesional tissue, without consideration of gene expression in background skin.

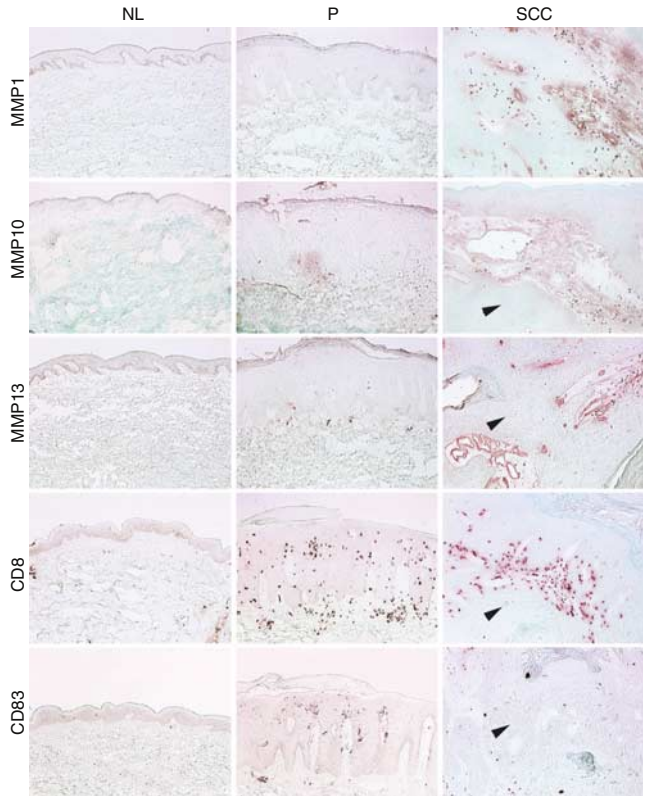

Figure 3. Immunohistochemical analysis of MMPs and CD8+ and CD83+ cells in SCC and psoriasis. Skin biopsies in non-lesional (NL), psoriasis lesions (LS) and SCC tumor specimens. Representative MMPs (MMP1, 10, and 13) stain strongly in SCC. Equivalent numbers of CD8+ cells were seen in SCC and LS, whereas few CD83+ cells were seen in SCC tumor specimens as compared to NL and LS. Black arrows point to the tumor infiltrates. Original magnification: ×10; bar = 100 μm.

In this comparison, expression of 2,520 genes was different between SCC and psoriasis (Figure 1e), based on statistical criteria and correction for multiple gene comparisons. A short list of these genes is given in Table 3, with associated fold changes and *P*-values, whereas a complete list can be found in Table S5.

Enhanced expression of cathepsin L (CTSL), STAT3, cystatin E/M (CST6), and microseminoprotein, beta (MSMB) in SCC was detected by this analysis. These findings were confirmed by RT-PCR (Figure 2d). MSMB has been reported in mice model of adenocarcinoma in the prostate (Gabril *et al.*, 2005). This is the first report to our knowledge that associates this gene with skin cancer. RT-PCR experiments showed that PTN was more highly expressed in site-matched skin adjacent to excised SCC than that in SCC (Figure 2e). Conversely, iNOS, granzyme B (GZMB), CD8, and CD83 were downregulated in SCC compared with psoriasis. Immunohistochemistry studies showed higher numbers of CD83+ cells in psoriasis than in SCC, although there were similar numbers of CD8+ cells in psoriasis and SCC (Figure 3). Furthermore, unlike psoriasis lesions, the CD8+ cells were perivascular and were found in tumor infiltrates. Of interest, TNFSRF6 (APO-1, FAS) was increased in SCC compared with psoriasis (Table 3). Although elevated in SCC *versus* site-matched skin in genomic analyses, this did not reach statistical significance.

Finally, genes with decreased expression in SCC compared to site-matched controls were defined through statistical comparisons. Table 4 contains a short list of these downregulated genes. Of interest, decreased expression of protease inhibitor TIMP3 was unique to SCC. Some proliferation mediators were decreased in SCC. Potential cell cycle mediators cyclin G1 (CCNG1) and ABL1 were specifically downregulated in SCC. Others included fibroblast growth factor 2, oncogenes FOS and JUN, and transcription regulators NF-κB2 and NF-κBIA. A number of immune response genes were decreased in SCC. These included CXC chemokine, cytokine IL-6, activated T-lymphocyte marker CD69, and mature dendritic cell marker CD83 (Figure 2d).

Table 3. Genes differentially regulated in SCC *versus* psoriasis

Fold change: SCC/P	*P*-value < (corrected)	Symbol	Biological process
1.5	0.04781194	CDK2	Cell cycle
5.6	0.00000015	CST6	Morphogenesis
6.0	0.00000080	CTSL2	Proteolysis and peptidolysis
2.3	0.00000080	FZD6	Frizzled signaling pathway
2.0	0.00010055	GATA3	Defense response
4.5	0.00000086	HIST1H2AC	Nucleosome assembly
4.3	0.00003305	HIST1H2BD	Nucleosome assembly
5.2	0.00000080	HPGD	Prostaglandin metabolism
2.0	0.00000080	IL18	Immune response
3.1	0.00000080	IL6	Humoral immune response
2.7	0.00000080	JUN; AP1	Regulation of transcription
3.0	0.00045130	KLK7	Proteolysis and peptidolysis
4.7	0.01879431	MMP1	Collagen catabolism
1.8	0.00022602	MMP15	Proteolysis and peptidolysis
9.4	0.00119212	MSMB	Microseminoprotein, beta-
1.5	0.00000421	PTGER4	Immune response
2.1	0.01334204	PTN	Growth factor activity
1.2	0.00110000	TNFRSF6	Fas gene, apoptosis
2.4	0.01180278	WIF1	WNT receptor signaling pathway
1.9	0.01819798	WNT10B	Frizzled-2 signaling pathway
Genes downregulated in SCC versus psoriasis			
0.5	0.02136103	NOS2A	Inflammatory response
0.6	0.00000004	PTGIS	Prostaglandin biosynthesis

Selected list of genes of direct comparison of genes with regulated expression in squamous cell carcinoma (SCC) with psoriasis (P) corresponding to heat map in Figure 1e. Fold changes are ratios of gene expressions in SCC/psoriasis (P) skin biopsies. Gene symbols and relevant biological processes are described in Gene Ontology annotation.

Table 4. Genes downregulated in SCC

Fold change: SCC/N	*P*-value < (corrected)	Symbol	Biological process
0.73	0.00001	ABL1	Cell proliferation
0.70	0.00080	ARHGEF6	Apoptosis
0.66	0.00340	BIRC3	Apoptosis
0.80	0.00160	CCL17	Immune response
0.60	0.01010	CCNG1	Cell cycle
0.61	0.00001	CD34	Immune response
0.66	0.02900	CD69	Immune response
0.57	0.00001	CX3CL1	Immune response
0.79	0.00460	FGF2	Cell proliferation
0.76	0.00170	FGF7	Cell proliferation
0.70	0.00660	FGF9	Cell proliferation
0.29	0.00100	FOS	Oncogenesis
0.51	0.00070	IGF1	Cell proliferation
0.51	0.02970	IGFBP4	Cell proliferation
0.53	0.04540	IGFBP7	Cell proliferation
0.09	0.01430	IL6	Immune response
0.53	0.01560	JUN; AP1	Oncogenesis, transcription
0.37	0.00740	JUNB	Oncogenesis, transcription
0.73	0.01940	JUND	Transcription
0.78	0.00001	MRAS	Signal transduction, RAS protein
0.74	0.00030	NFKB2	Transcription
0.67	0.00250	NFKBIA	Transcription
0.50	0.00280	PDGFRA	Cell proliferation
0.49	0.01160	PDGFRB	Cell proliferation
0.69	0.00150	PPP1R15A	Apoptosis
0.45	0.00160	PTGDS	Prostaglandin pathway
0.58	0.00001	RARRES2	Retinoid metabolism
0.66	0.00020	RRAS	Oncogenesis, transcription
0.80	0.02160	VEGFC	Angiogenesis
0.59	0.01180	TIMP1	Proteolysis and peptidolysis
0.63	0.00001	TIMP3	Proteolysis and peptidolysis

Selected list of genes with downregulated expression in squamous cell carcinoma (SCC) corresponding to the lower region of heat map in Figure 1a. Fold changes are ratios of gene expressions in SCC/site-matched normal (N) skin biopsies. Gene symbols and relevant biological processes are described in Gene Ontology annotation.

DISCUSSION

This is the first report contrasting gene expression profiles of malignant hyperproliferation and benign hyperplasia in a stratified squamous epithelium that would form a carcinoma upon malignant transformation. These experiments facilitate insight into human cancer biology through an attempt to separate factors that are specific to malignancy from those that are attributable to hyperplasia.

Defects or mutations in p53, RAS, CDK4, and CDKN2A have been described for SCC in humans (Kubo *et al.*, 2002; Green and Khavari, 2004; Tsai and Tsao, 2004), but little is known about the genetic basis of SCC in human skin. In our studies, expression of p53 (SCC/N: 0.97) and CDK4 (SCC/N: 1.06) was unchanged between SCC and site-matched non-tumor-bearing skin, whereas expression of CDKN2A was increased in four of eight patients. This is consistent with functional mutations in these genes rather than over- or underexpression leading to SCC. As many but certainly not all SCCs harbor mutations in these genes, other factors are likely to be involved in its pathogenesis. Expression of p63, a p53 homolog that identifies keratinocyte stem cells (Pellegrini *et al.*, 2001), was increased in SCC compared to site-matched non-tumor-bearing skin. Reis-Filho *et al.* (2002) report variable presence of p63 (TP73L; Table 2) in grade 1 SCC but increased immunoreactivity in undifferentiated SCC. Increased expression of p63 in our studies supports the potential role of maintenance of keratinocyte stem cells in early SCC development.

The WNT signaling pathway regulates normal development and cancer progression (Logan and Nusse, 2004). However, little is known about the role of WNT signaling in human cutaneous SCC. WNT/β-catenin signaling inhibited death receptor-mediated apoptosis in nude and promoted invasive growth of HNSCC in nude mice (Yang *et al.*, 2005). In our studies, WNT 5A was upregulated in both SCC and psoriasis. WNT 5A has not been previously described in human cutaneous SCC; however, our results are consistent with Taki *et al.* (2003), who showed upregulation of WNT 5A by epithelial mesenchymal transition by human SCC cells in culture. That WNT 5A was upregulated in both SCC and psoriasis raises the possibility that differential receptor expression may play a role in the ultimate determination of biological behavior. WNT inhibitory factor (WIF1) is more highly expressed in SCC than in psoriasis in our studies. Although not previously described in skin cancer, WIF is suppressed in lung cancer (Mazieres *et al.*, 2004).

The FZD family of WNT receptors comprises a group of proteins with a large, cysteine-rich extracellular domain, a seven-transmembrane spanning domain, and a cytoplasmic tail (Wodarz and Nusse, 1998). In our studies, FZD6 was increased in SCC compared to site-matched skin and was not increased in psoriasis. Golan *et al.* (2004) showed that FZD6 acts as a negative regulator of canonical WNT signaling in cell lines. This was supported by Lyons *et al.* (2004), who further demonstrated a role of FZD6 in non-canonical signaling in kidney epithelial cells. Based on unique expression in SCC compared with site-matched skin and a lack of expression in benign hyperplasia, it is possible that FZD6 is involved in WNT-mediated signaling in cutaneous SCC.

MMPs are involved in extracellular matrix degradation, which is key to tumor invasion (Kerkela and Saarialho-Kere, 2003). MMP1 degrades collagens I, II, and III (Aznavoorian *et al.*, 2001), and has been reported to mediate invasiveness and survival of keratinocytes (Nagavarapu *et al.*, 2002). MMP13 degrades type II collagen most efficiently and has

also been implicated in malignant transformation of keratinocytes (Ala-aho *et al.*, 2002). MMP10 degrades fibronectin and proteoglycans and has been implicated as a tumor promoter in lymphoma (Van Themsche *et al.*, 2004). Induction of MMPs 1, 10, and 13 and suppression of protease inhibitors TIMP1 and TIMP3 as seen in our studies suggest an environment supporting dermal invasion in SCC (Kerkela and Saarialho-Kere, 2003). Protease inhibitors are likely to play an important role by inhibiting tumor invasion. Previously, TIMPs including TIMP3 were observed to be upregulated in invasion and metastasis from oral SCC but not in pre-malignant lesions (O'Donnell *et al.*, 2005). This may indicate that TIMP3 induction, in the skin, might not be observed until the most biologically aggressive tumors are analyzed.

Cathepsin L was increased in SCC in our studies. Macabeo-Ong *et al.* (2003) demonstrated an association between expression of cathepsin L and progression from oral dysplasia to oral cancer. MMP12 was not highly expressed in SCC *versus* non-tumor-bearing skin or in SCC *versus* psoriasis in our studies. Impola *et al.* (2004) report that expression of MMP12 correlates with aggressive behavior in oral carcinoma. Kerkela *et al.* (2002) report that MMP12 expression by tumor cells correlates with invasiveness in vulvar SCC, whereas expression of MMP12 by macrophages predicts better prognosis.

Our findings differ from the findings of others concerning invasion and differentiation determined by genomic analysis of HNSCC. Ginos *et al.* (2004) describe a set of invasion-related genes specifically increased in HNSCC. These included ras, type IV collagen, laminin $\gamma 2$, and integrins $\alpha 3$, $\alpha 6$, $\beta 4$, and $\beta 6$. In our study, KRAS was increased as was collagen IV $\alpha 6$, whereas laminin 2 was downregulated (SCC/N: 0.4) and laminin 5 remained unchanged. However, integrins $\beta 4$ (P/NL: 1.2) and $\beta 6$ (P/NL: 1.4) were not increased in invasive SCC but were increased in psoriasis, indicating that enhanced expression of integrins $\beta 4$ and $\beta 6$ may be required for hyperplasia rather than malignancy. Enhanced expression of KRAS is consistent with the findings of Vitale-Cross *et al.* (2004), who report that expression of KRAS in an epithelial compartment containing stem cell is sufficient for squamous cell carcinogenesis in mice.

Poorly differentiated cutaneous SCCs (Rowe *et al.*, 1992) are more likely to develop distant and nodal metastases. It is therefore not surprising that the EDC genes, including involucrin (IVL), small proline-rich proteins (SPRR1A, 1B, 2B, 3), and S100 1 and 6 were reported to be decreased in HNSCC (Ginos *et al.*, 2004). In contrast, in our studies, EDC-derived genes for IVL, SPRR, and S100 A8, A9, and A14 were increased in both SCC and psoriasis. This indicates that de-differentiation is not an obligate feature of early malignant transformation of epithelial cells and that differentiating epithelial cells can invade. In one study, $\sim$60% of in-transit metastases occurred after treatment of well-differentiated although otherwise high-risk, primary cutaneous SCC (Carucci *et al.*, 2004). Analysis of more deeply invasive or metastatic skin cancers may likely show de-differentiation and decreased expression of EDC genes.

Prostaglandin metabolism has been implicated in the development of SCC, particularly through cyclooxygenase-2 (An *et al.*, 2002; O'Grady *et al.*, 2004). Whereas cyclooxygenase (PTGS2, cyclooxygenase-2) was present in higher levels in SCC *versus* psoriasis, the levels in site-matched normal skin were higher as compared to SCC. Inhibition of UVB-mediated skin tumors by cyclooxygenase-2 inhibition has been demonstrated (Pentland *et al.*, 1999; Wilgus *et al.*, 2003). Several genes of the downstream pathway of PTGS2 were modulated in our study. Prostaglandin E receptor 4 and prostaglandin E2-metabolizing enzyme, HPGD, were up-regulated in SCC *versus* non-tumor-bearing tissue. Prostacyclin synthase was downregulated in SCC *versus* site-matched non-tumor-bearing skin. HPGD has been reported to be suppressed in colon cancer (Yan *et al.*, 2004), whereas Gee *et al.* (2003) report that prostaglandin dehydrogenase was detected in 64% of transitional cell carcinoma of the bladder but in only 10% of bladder SCC. In their study, expression correlated with higher tumor stage. All cutaneous tumors in our study were stage 1. Inactivation of prostacyclin synthase has been associated with colorectal carcinogenesis (Frigola *et al.*, 2005), and chemoprevention of lung carcinogenesis by expression of prostacyclin synthase has been described (Keith *et al.*, 2004). It is possible that prostacyclin synthase expression might be key in maintaining non-aggressive biological behavior in benign hyperproliferative processes in the skin.

Analysis of SCC *versus* psoriasis allowed us to consider potentially genes that might not be differentially regulated in SCC *versus* non-tumor-bearing skin adjacent to excised SCC but that might nonetheless be important in determining biological behavior. PTN encodes a 136-amino-acid heparin binding cytokine that accelerates tumor growth and angiogenesis (Deuel *et al.*, 2002), and has been implicated in the pathogenesis of melanoma (Wu *et al.*, 2005); however, its potential role in the pathogenesis of SCC has been previously undefined. In our studies, PTN was more highly expressed in SCC than in psoriasis and even more highly expressed in non-tumor-bearing skin adjacent to SCC. This might indicate that perilesional skin acts to induce SCC through PTN.

Interestingly, cystatin M (CST6) was increased in SCC compared with psoriasis. Cystatin M, an endogenous protease inhibitor, has been implicated as a potential tumor suppressor for breast cancer (Zhang *et al.*, 2004), but its role in human skin cancer has not yet been defined. It is possible that loss of CST6 may be involved in deeply invasive or metastatic SCC.

Similarly, CTSL2 expression was increased in SCC *versus* psoriasis. CTSL2, along with other cathepsins, is associated with the plasma membrane of malignant cells and degrade the extracellular matrix during tumor progression (Nomura and Katunuma, 2005). It may be that CTSL2 plays a role in early invasion by SCC in human skin.

STAT3 was increased in SCC *versus* psoriasis in our studies. STAT3 activation has been implicated in multistage skin carcinogenesis in mice (Chan *et al.*, 2004). STAT3 activation has also been described as a key regulator of keratinocyte proliferation following UV radiation (Sano *et al.*,

2005b). Quadros *et al.* (2004) demonstrated EGFR-mediated STAT3 activation is restricted to malignant kertinocytes in culture. Mice deficient in STAT3 have been shown to be resistant to developing skin cancer (Pedranzini *et al.*, 2004). The role of STAT3 in benign proliferation is supported by a STAT3 transgenic mouse that develops psoriasis-like lesions (Sano *et al.*, 2005a). It may be that STAT3 is required but not sufficient for development of SCC in human skin.

Conversely, iNOS, GZMB, CD8, and CD83 were downregulated in SCC compared with psoriasis. Decreased expression of CD83 (McLellan *et al.*, 1995) and low numbers of CD83+ cells indicate a lack of mature dendritic cells associated with SCC, consistent with a relatively immunosuppressed microenvironment. The pro-inflammatory mediator iNOS, possibly dendritic cell-derived (unpublished observation), although highly expressed in psoriasis, was virtually undetected in SCC. We found high numbers of CD8+ cells surrounding SCC despite decreased expression of CD8 in genomic analyses. This is consistent with Terao *et al.* (1992), who found that T lymphocytes predominated and natural killer cells, B cells, and monocytes were rarely detected surrounding SCC. Lack of correlation might indicate that the CD8+ cells are homing to tumor sites. Low expression of the product of cytotoxic T cells, GZMB, key in tumor immunity (Pardo *et al.*, 2002), and downregulation of T-cell activation marker, CD69, are also consistent with a relatively immune-suppressed microenvironment.

The present studies define a genetic signature for primary cutaneous SCC that accounts for components specific to malignancy and those related to hyperplasia. Further characterization of mechanisms governing these and other regulatory and inflammatory pathways involved with SCCs at various stages may reveal novel control points leading to development of rational molecular and/or immune-based therapies for cutaneous SCC and for SCC originating from sites other than skin.

MATERIALS AND METHODS

Samples used in study

Institutional review board approval was obtained before inviting patients to participate in the study. Informed consent was obtained from patients before their participation, and the study was performed with strict adherence to the Declaration of Helsinki Principles. Paired samples of tumor and site-matched normal skin were obtained from eight patients with SCC. Tumor samples were obtained at surgery. Site-matched uninvolved skin was similarly obtained from site-matched uninvolved skin at the time of repair after clear margins were achieved. Samples were obtained and processed in identical fashion to psoriasis samples. Mean age of patients with SCC in this study was 72 years (range 57–83 years). Mean tumor size was 0.9 cm (range 0.5–2.0 cm); however, final defect after clearing tumors by Mohs micrographic surgery was 1.8 cm in the largest dimension (range 1.0–2.6 cm). Average duration before diagnosis was 6 months (range 3–12 months). All tumors were well-differentiated, locally invasive cancers with excellent prognosis following excision with clear margins. All tumors were located on sun-exposed areas. All patients had fair skin with predisposition to sunburn with Fitzpatrick skin type 2.

Paired samples of lesional psoriasis and non-lesional skin from eight patients with psoriasis were obtained by skin biopsy. Samples were processed in identical fashion to SCC samples (Bowcock *et al.*, 2001).

Lists of genes present exclusively in cultured human keratinocytes were kindly provided by Banno *et al.* (2004). A list of genes of interest found in keratinocytes and lesional psoriasis or SCC is given in Table S3.

Target preparation

The microarrays used for this study were U95A-set GeneChip probe arrays (Affymetrix Inc., Santa Clara, CA) that contain probe sets representing approximately 12,000 genes.

Fragmentation, array hybridization, scanning, and quality control

The labeled target was fragmented and hybridized to probe arrays as described elsewhere (Zhou *et al.*, 2003). The probe arrays were then washed, stained, and scanned. Briefly, total RNA was extracted from tissues frozen in liquid nitrogen using the RNeasy Mini Kit (Qiagen, Valencia, CA). DNA was removed with on-column DNAse digestion by using Quiagen RNAse-free DNAse Set. Total RNA ($\sim 4\,\mu g$) was reverse-transcribed, amplified, and labeled as described previously (Zhou *et al.*, 2003). Briefly, mRNA was isolated and converted to double-strand cDNA and then to biotinylated cRNA (BioArray High Yield RNA Transcription Labeling Kit, Enzo Biochem Inc., Farmingdale, NY). After fragmentation and quality confirmation with the Affymetrix Test-3 Array, $15\,\mu g$ of the biotinylated cRNA was hybridized to Affymetrix Human Genome U95A GeneChips (12,000 probe sets) (Affymetrix Inc., Santa Clara, CA). The chips were washed, stained with streptavidin–phycoerythrin, and scanned with a probe array scanner (HP GeneArray Scanner, Hewlett-Packard Company, Palo Alto, CA). On each chip, the human housekeeping genes β-actin and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) served as controls. When comparing the data, the Suite 5.0 software normalized the values of expression level using all these controls. Chips with 3′-to-5′ ratios for GAPDH less than 3 and scaling factor within three-fold of each other were compared in the study.

GeneChip data analysis

Data were analyzed with Affymetrix Microarray Suite 5.0 software (Affymetrix Inc., GeneSpring 7.0 software, Silicon Genetics, Redwood City, CA).

Gene expression values. The Suite 5.0 software uses the one-sided Wilcoxon's signed-rank test to generate a detection *P*-value and a detection call to decide statistically whether a transcript is expressed on a chip. The software generates the detection call based on the detection *P*-value for each transcript: present ($P<0.04$), marginal ($0.04<P<0.06$), or absent ($P>0.06$).

Gene expression analysis. Using GeneSpring 7.0, RMA (Robust Multi-Chip Average) algorithm was applied for normalizing and summarizing probe-level intensity measurements. Briefly, starting with the probe-level data from a set of GeneChips, the perfect-match values are background-corrected, normalized, and finally

summarized resulting in a set of expression measures. Each gene was divided by the median of its measurements in all samples. If the median of the raw values was below 10, then each measurement for that gene was divided by 10 if the numerator was above 10, otherwise the measurement was thrown out. Fold changes were calculated using the formula $2^{\text{signal log ratio}}$.

Principle component analysis. Principle component analysis as quality control was performed with the normalized raw data using GeneSpring 7.0 software.

Hierarchical clustering and heat maps. In order to organize gene expression, genes with a similar pattern of expression are grouped together as hierarchical clusters and presented as heat maps. Unsupervised hierarchical clustering builds the hierarchy by establishing which two genes are the closest together and then combining these into a single node and repeating until the tree is complete. The gene trees were computed based on significantly regulated genes and distances between samples are computed using Pearson correlation as similarity measures, that is, a distance close to 0 indicates very similar data, a distance close to 1 indicates that there is no linear correlation between the samples, and a distance close to 2 indicates a strong negative correlation between the samples.

For individual transcripts, their fold change in expression was the ratio of the mean expression level of tumor-bearing *versus* site-matched non-tumor-bearing skin and lesional (P) *versus* non-lesional skin biopsies. The heat map of the computed tree was presented as red and green lines. Each line presents genes with relative upregulated (red) or downregulated (green) expression values in fold changes.

Statistical comparisons. To obtain a list of biologically most interesting genes used in this study, multiple statistical analyses were performed (Table S6).

Statistical comparisons of expression levels pairwise between each condition (SCC, N, P, and NL) and unpaired between SCC and P were performed by using the Data pre-processing available in the statistical package R at http://www.r-project.org/>http://www.r-project.org. Based on the full data set, paired Welch tests (*t*-test for unequal variance) were performed to assess the interesting genes for the distinction. Owing to the multiple testing problem encountered in the analysis of microarray data, we chose to declare those genes with a false discovery rate of at most 5% as having a significant effect. Different methods may be applied to determine the false discovery rate (Benjamini–Hochberg, Benjamini–Yekutieli) and family-wise error rate (maxT) and SAM (significance analysis of microarray data). In this study, we considered genes that passed the Benjamini–Hochberg criterion as biologically most interesting. A complete list of significantly regulated genes (Benjamini–Hochberg false discovery rate $\leqslant 5\%$) is presented as up- or downregulated by 1.2-fold based on the average of eight patients (P *vs* NL, SCC *vs* N, and P *vs* SCC) in Tables S1–S5.

Description of relevant functions of genes. Gene Ontology annotations of differentially expressed genes were collected from LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink).

Validation of expression changes with real-time RT-PCR analysis of tissue mRNA gene expression

The primers and probes for the TaqMan RT-PCR assays were generated with the Primer Express algorithm, version 1.0, using published genetic sequences (NCBI-PubMed) for each gene. Primer sequences were as follows: CD83-forward GAGCTATTTAATGGC CGGCTG, CD83-reverse CAAGTGGGCGAGCACCC, CD83 probe 6FAM-AAATGCTGGGCTGACGGTGCAGTC-TAMRA (GenBank accession number NM_004233); DEFB4-forward CCAGTC TTTTGCCCTAGAAGGTATAA, DEFB4-reverse GGCTTTTTGCA GCATTTTGTT, DEFB4 probe 6FAM-CAAATTGGCACCTGT GGTCTCCCTGK (GenBank accession number NM_00492); GZMB-forward GAGGCCCTCTTGTGTGTAACAAG, GZMB-reverse CAGGCTCGTGGAGGCATG, GZMB-probe 6FAM-CCAGGGCATT GTCTCCTATGGACGAA-TAMRA (GenBank accession number NM_004131); iNOS-forward CCTCAAGTCTTATTTCCTCAACGTT, iNOS-reverse CCGATCAATCCAGGGTGCTA, iNOS probe 6FAM-CCCCATCAAGCCCTTTACTTGACCTCC-TAMRA (GenBank accession number AF068236), K16-forward GCGAGGATGCCCACCTTT, K16-reverse GAAGACCTCGCGGGAAGAAT, K16 probe 6FAM-CCCAGCAAGCATCTGGCCAATCC-TAMRA (GenBank accession number AF061809); MMP12-forward AGCACTTCTTGGGTCT GAAAGTG, MMP12-reverse CGAGGTGCGTGCATCATCT, MMP12 probe 6FAM-CCGGGCAACTGGACACATCTACCC-TAMRA (GenBank accession number NM_002426); STAT1-forward AAGA GAGGGCCCACCAGA, STAT1-reverse ACTGGACCCCTGTCTTCAA GAC, STAT1 probe 6FAM-AACGCACCCTCAGAGGC CGCT-TAMARA (GenBank accession number M97935), and STAT3-forward AGGAGGAGGCATTCGGAAA, STAT3-reverse AGCGCCTGGGT CAGCTT, STAT3 probe 6FAM-CGGCCAGAGAGCCAGGAGCA-TAMRA (GenBank accession number BC000627). The primers and probes for CD8A (assay ID Hs00233520), CEACAM5 (assay ID Hs00237075), CST6 (assay ID Hs00154599), CTSL2 (assay ID Hs00426731), FZD6 (assay ID Hs00171574), HPGD (assay ID Hs00168359), MMP1 (assay ID Hs00233958), MMP10 (assay ID Hs00233987), MMP13 (assay ID Hs00233992), MSMB (assay ID Hs00159303), PTN (assay ID Hs00383235), SERPINB3 (assay ID hCG34089), and WNT 5A (assay ID Hs00180103) were designed by Applied Biosystems (Foster City, CA). All primers and probes were purchased from Applied Biosystems. The RT-PCR reaction was performed using EZ PCR Core Reagents (Applied Biosystems) according to the manufacturer's directions. The samples were amplified and quantified on an Applied Biosystems PRISM 7700 using the following thermal cycler conditions: 2 minutes at 50°C; 30 minutes at 60°C; 5 minutes at 95°C; and 40 cycles of 15 seconds at 95°C followed by 60 seconds at 60°C. The human acidic ribosomal protein (HARP) gene, a housekeeping gene, was used to normalize each sample and each gene. Primer sequences HARP-forward CGCTGCTGAACATGCTCAA, HARP-reverse TGTCGAACACCTGC TGGATG, HARP-probe 6FAM-TCCCCCTTCTCCTTTGGGCTGG-TAMRA (GenBank accession number NM-001002) were used. The data were analyzed and samples quantified by the software provided with the Applied Biosystems PRISM 7700 (Sequence Detection Systems, ver. 1.7).

Statistics. Statistical comparisons of expression level, pairwise (SCC *vs* N and P *vs* NL) or unpaired (SCC *vs* P), between each condition were performed using a two-tailed, Student's *t*-test.

Immunohistochemistry

Tissue sections of all patients with SCC and psoriasis were stained with hematoxylin (Fisher, Fair Lawn, NJ) and eosin (Shandon, Pittsburgh, PA) and with purified mouse anti-human monoclonal antibodies to CD83 (Becton Dickinson, San Jose, CA), CD8 (BD Pharmingen, San Diego, CA), and MMP1, MMP10 and MMP13 (Abcam Inc., Cambridge, MA). Biotin-labeled horse anti-mouse antibody (Vector Laboratories, Burlingame, CA) was amplified with avidin–biotin complex (Vector Laboratories) and developed with chromogen 3-amino-9-ethylcarbazole (Sigma Aldrich, St Louis, MO).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

These studies were supported in part by the Dermatology Foundation and by NIH Grants RO1-A149572, A149832, and MO1-RR00102.

SUPPLEMENTARY MATERIAL

Table S1. Genes upregulated (1,048 genes) and downregulated (870 genes) in SCC (SCC *vs* N: 1.2-fold, $P<0.05$, BH corrected).

Table S2. Genes upregulated (398 genes) in SCC and psoriasis (P > NL, SCC > N: 1.2-fold, $P<0.05$, BH corrected).

Table S3. Genes upregulated (73) in SCC and psoriasis and found in keratinocytes (SCC > N, LS > NL: 1.2-fold $P<0.05$, BH corrected).

Table S4. Genes upregulated (582 genes) in SCC (SCC *vs* N: 1.2-fold, $P<0.05$, BH corrected) but not significantly changed in psoriasis.

Table S5. Genes regulated (2,520 genes) in SCC *vs* psoriasis (P) (1.2-fold, $P<0.05$, BH corrected).

Table S6. Statistical analysis of complete data set with different methods to determine the false discovery rate, UNI-Gene ID and *P*-values. The significant differences are color-coded: tumor *versus* normal (orange), psoriasis *versus* NL (green), and tumor *versus* psoriasis (yellow).

REFERENCES

Ahmed MI, Salahy EE, Tawfiq H, Khalifa A, Hassan MM (2004) Matrix metalloproteinase-2, squamous cell carcinoma antigen, and tissue polypeptide-specific antigen expression in Egyptian patients with cervical carcinoma: relationship with prognosis. *Dis Markers* 20:333–43

Ala-aho R, Grenman R, Seth P, Kahari VM (2002) Adenoviral delivery of p53 gene suppresses expression of collagenase-3 (MMP-13) in squamous carcinoma cells. *Oncogene* 21:1187–95

An KP, Athar M, Tang X, Katiyar SK, Russo J, Beech J *et al.* (2002) Cyclooxygenase-2 expression in murine and human nonmelanoma skin cancers: implications for therapeutic approaches. *Photochem Photobiol* 76:73–80

Aznavoorian S, Moore BA, Alexander-Lister LD, Hallit SL, Windsor LJ, Engler JA (2001) Membrane type I-matrix metalloproteinase-mediated degradation of type I collagen by oral squamous cell carcinoma cells. *Cancer Res* 61:6264–75

Banno T, Gazel A, Blumenberg M (2004) Effects of tumor necrosis factor-alpha (TNF alpha) in epidermal keratinocytes revealed using global transcriptional profiling. *J Biol Chem* 279:32633–42

Bowcock AM, Shannon W, Du F, Duncan J, Cao K, Aftergut K *et al.* (2001) Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* 10:1793–805

Brash DE, Ziegler A, Jonason AS, Simon JA, Kunala S, Leffell DJ (1996) Sunlight and sunburn in human skin cancer: p53, apoptosis, and tumor promotion. *J Investig Dermatol Symp Proc* 1:136–42

Carucci JA, Martinez JC, Zeitouni NC, Christenson L, Coldiron B, Zweibel S *et al.* (2004) In-transit metastasis from primary cutaneous squamous cell carcinoma in organ transplant recipients and nonimmunosuppressed patients: clinical characteristics, management, and outcome in a series of 21 patients. *Dermatol Surg* 30(4 part 2):651–5

Chan KS, Carbajal S, Kiguchi K, Clifford J, Sano S, DiGiovanni J (2004) Epidermal growth factor receptor-mediated activation of Stat3 during multistage skin carcinogenesis. *Cancer Res* 64:2382–9

De Pita O, Frezzolini A, Cianetti A, De Sanctis G, Fontana L, Bottari V (1999) Squamous cell carcinoma-related antigen (SCCr-Ag), sICAM-1 and beta 2-microglobulin are useful markers of disease activity in psoriasis. *Acta Derm Venereol* 79:132–5

Deuel TF, Zhang N, Yeh HJ, Silos-Santiago I, Wang ZY (2002) Pleiotrophin: a cytokine with diverse functions and a novel signaling pathway. *Arch Biochem Biophys* 397:162–71

Frigola J, Munoz M, Clark SJ, Moreno V, Capella G, Peinado MA (2005) Hypermethylation of the prostacyclin synthase (PTGIS) promoter is a frequent event in colorectal cancer and associated with aneuploidy. *Oncogene*

Gabril MY, Duan W, Wu G, Moussa M, Izawa JI, Panchal CJ *et al.* (2005) A novel knock-in prostate cancer model demonstrates biology similar to that of human prostate cancer and suitable for preclinical studies. *Mol Ther* 11:348–62

Gee JR, Montoya RG, Khaled HM, Sabichi AL, Grossman HB (2003) Cytokeratin 20, AN43, PGDH, and COX-2 expression in transitional and squamous cell carcinoma of the bladder. *Urol Oncol* 21:266–70

Ginos MA, Page GP, Michalowicz BS, Patel KJ, Volker SE, Pambuccian SE *et al.* (2004) Identification of a gene expression signature associated with recurrent disease in squamous cell carcinoma of the head and neck. *Cancer Res* 64:55–63

Golan T, Yaniv A, Bafico A, Liu G, Gazit A (2004) The human Frizzled 6 (HFz6) acts as a negative regulator of the canonical Wnt. beta-catenin signaling cascade. *J Biol Chem* 279:14879–88

Goldman GD (1998) Squamous cell cancer: a practical approach. *Semin Cutan Med Surg* 17:80–95

Green CL, Khavari PA (2004) Targets for molecular therapy of skin cancer. *Semin Cancer Biol* 14:63–9

Impola U, Uitto VJ, Hietanen J, Hakkinen L, Zhang L, Larjava H *et al.* (2004) Differential expression of matrilysin-1 (MMP-7), 92 kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. *J Pathol* 202:14–22

Keith RL, Miller YE, Hudish TM, Girod CE, Sotto-Santiago S, Franklin WA *et al.* (2004) Pulmonary prostacyclin synthase overexpression chemoprevents tobacco smoke lung carcinogenesis in mice. *Cancer Res* 64:5897–904

Kerkela E, Saarialho-Kere U (2003) Metalloelastase (MMP-12) expression by tumour cells in squamous cell carcinoma of the vulva correlates with invasiveness, while that by macrophages predicts better outcome. *Exp Dermatol* 12:109–25

Kerkela E, Ala-aho R, Klemi P, Grenman S, Shapiro SD, Kahari VM *et al.* (2002) Metalloelastase (MMP-12) expression by tumour cells in squamous cell carcinoma of the vulva correlates with invasiveness, while that by macrophages predicts better outcome. *J Pathol* 198:258–69

Kubo Y, Murao K, Matsumoto K, Arase S (2002) Molecular carcinogenesis of squamous cell carcinomas of the skin. *J Med Invest* 49:111–7

Leffell DJ, Brash DE (1996) Sunlight and skin cancer. *Sci Am* 275:52–3, 56–9

Leversha MA, Fielding P, Watson S, Gosney JR, Field JK (2003) Expression of p53, pRB, and p16 in lung tumours: a validation study on tissue microarrays. *J Pathol* 200:610–9

Lew W, Lee E, Krueger JG (2004) Psoriasis genomics: analysis of proinflammatory (type 1) gene expression in large plaque (Western) and small plaque (Asian) psoriasis vulgaris. *Br J Dermatol* 150:668–76

Logan CY, Nusse R (2004) The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20:781–810

Lowes MA, Lew W, Krueger JG (2004) Current concepts in the immunopathogenesis of psoriasis. *Dermatol Clin* 22:349–69, vii

Lu J, Liu Z, Xiong M, Wang Q, Wang X, Yang G *et al.* (2001) Gene expression profile changes in initiation and progression of squamous cell carcinoma of esophagus. *Int J Cancer* 91:288–94

Lyons JP, Mueller UW, Ji H, Everett C, Fang X, Hsieh JC *et al.* (2004) Wnt-4 activates the canonical beta-catenin-mediated Wnt pathway and binds Frizzled-6 CRD: functional implications of Wnt/beta-catenin activity in kidney epithelial cells. *Exp Cell Res* 298:369–87

Macabeo-Ong M, Shiboski CH, Silverman S, Ginzinger DG, Dekker N, Wong DT *et al.* (2003) Quantitative analysis of cathepsin L mRNA and protein expression during oral cancer progression. *Oral Oncol* 39:638–47

Mazieres J, He B, You L, Xu Z, Lee AY, Mikami I *et al.* (2004) Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. *Cancer Res* 64:4717–20

McLellan AD, Starling GC, Williams LA, Hock BD, Hart DN (1995) Activation of human peripheral blood dendritic cells induces the CD86 co-stimulatory molecule. *Eur J Immunol* 25:2064–8

Nagavarapu U, Relloma K, Herron GS (2002) Membrane type 1 matrix metalloproteinase regulates cellular invasiveness and survival in cutaneous epidermal cells. *J Invest Dermatol* 118:573–81

Nguyen TH (2004) Mechanisms of metastasis. *Clin Dermatol* 22:209–16

Nomura T, Katunuma N (2005) Involvement of cathepsins in the invasion, metastasis and proliferation of cancer cells. *J Med Invest* 52:1–9

O'Donnell RK, Kupferman M, Wei SJ, Singhal S, Weber R, O'Malley B *et al.* (2005) Gene expression signature predicts lymphatic metastasis in squamous cell carcinoma of the oral cavity. *Oncogene* 24:1244–51

O'Grady A, O'Kelly P, Murphy GM, Leader M, Kay E (2004) COX-2 expression correlates with microvessel density in non-melanoma skin cancer from renal transplant recipients and immunocompetent individuals. *Hum Pathol* 35:1549–55

Pardo J, Balkow S, Anel A, Simon MM (2002) Granzymes are essential for natural killer cell-mediated and perf-facilitated tumor control. *Eur J Immunol* 32:2881–7

Pedranzini L, Leitch A, Bromberg J (2004) Stat3 is required for the development of skin cancer. *J Clin Invest* 114:619–22

Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S *et al.* (2001) p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci USA* 98:3156–61

Pentland AP, Schoggins JW, Scott GA, Khan KN, Han R (1999) Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis* 20:1939–44

Quadros MR, Peruzzi F, Kari C, Rodeck U (2004) Complex regulation of signal transducers and activators of transcription 3 activation in normal and malignant keratinocytes. *Cancer Res* 64:3934–9

Reis-Filho JS, Torio B, Albergaria A, Schmitt FC (2002) p63 expression in normal skin and usual cutaneous carcinomas. *J Cutan Pathol* 29:517–23

Rowe DE, Carroll RJ, Day CL Jr (1992) Prognostic factors for local recurrence, metastasis, and survival rates in squamous cell carcinoma of the skin, ear, and lip. Implications for treatment modality selection. *J Am Acad Dermatol* 26:976–90

Sano S, Chan KS, Carbajal S, Clifford J, Peavey M, Kiguchi K *et al.* (2005a) Stat3 links activated keratinocytes and immunocytes required for development of psoriasis in a novel transgenic mouse model. *Nat Med* 11:43–9

Sano S, Chan KS, Kira M, Kataoka K, Takagi S, Tarutani M *et al.* (2005b) Signal transducer and activator of transcription 3 is a key regulator of keratinocyte survival and proliferation following UV irradiation. *Cancer Res* 65:5720–9

Takeda A, Kajiya A, Iwasawa A, Nakamura Y, Hibino T (2002) Aberrant expression of serpin squamous cell carcinoma antigen 2 in human tumor tissues and cell lines: evidence of protection from tumor necrosis factor-mediated apoptosis. *Biol Chem* 383:1231–6

Taki M, Kamata N, Yokoyama K, Fujimoto R, Tsutsumi S, Nagayama M (2003) Down-regulation of Wnt-4 and up-regulation of Wnt-5a expression by epithelial–mesenchymal transition in human squamous carcinoma cells. *Cancer Sci* 94:593–7

Terao H, Nakayama J, Urabe A, Hori Y (1992) Immunohistochemical characterization of cellular infiltrates in squamous cell carcinoma and Bowen's disease occurring in one patient. *J Dermatol* 19:408–13

Tsai KY, Tsao H (2004) The genetics of skin cancer. *Am J Med Genet C* 131C:82–92

Van Themsche C, Alain T, Kossakowska AE, Urbanski S, Potworowski EF, St-Pierre Y (2004) Stromelysin-2 (matrix metalloproteinase 10) is inducible in lymphoma cells and accelerates the growth of lymphoid tumors in vivo. *J Immunol* 173:3605–11

Veness MJ, Quinn DI, Ong CS, Keogh AM, Macdonald PS, Cooper SG *et al.* (1999) Aggressive cutaneous malignancies following cardiothoracic transplantation: the Australian experience. *Cancer* 85:1758–64

Vitale-Cross L, Amornphimoltham P, Fisher G, Molinolo AA, Gutkind JS (2004) Conditional expression of K-ras in an epithelial compartment that includes the stem cells is sufficient to promote squamous cell carcinogenesis. *Cancer Res* 64:8804–7

Wilgus TA, Koki AT, Zweifel BS, Kusewitt DF, Rubal PA, Oberyszyn TM (2003) Inhibition of cutaneous ultraviolet light B-mediated inflammation and tumor formation with topical celecoxib treatment. *Mol Carcinogen* 38:49–58

Wodarz A, Nusse R (1998) Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 14:59–88

Wu H, Barusevicius A, Babb J, Klein-Szanto A, Godwin A, Elenitsas R *et al.* (2005) Pleiotrophin expression correlates with melanocytic tumor progression and metastatic potential. *J Cutan Pathol* 32:125–30

Yan M, Rerko RM, Platzer P, Dawson D, Willis J, Tong M *et al.* (2004) 15-Hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF-beta-induced suppressor of human gastrointestinal cancers. *Proc Natl Acad Sci USA* 101:17468–73

Yang F, Zeng Q, Yu G, Li S, Wang CY (2005) Wnt/beta-catenin signaling inhibits death receptor-mediated apoptosis and promotes invasive growth of HNSCC. *Cell Signal*

Zhang J, Shridhar R, Dai Q, Song J, Barlow SC, Yin L *et al.* (2004) Cystatin m: a novel candidate tumor suppressor gene for breast cancer. *Cancer Res* 64:6957–64

Zhou X, Krueger JG, Kao MC, Lee E, Du F, Menter A *et al.* (2003) Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* 13:69–78