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Keratoacanthoma and squamous cell carcinoma are distinct from a molecular perspective

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Keratoacanthoma is a controversial entity. Some consider keratoacanthoma as a variant of squamous cell carcinoma, whereas others see it as a distinct self-resolving squamoproliferative lesion. Our objective is to examine the relationship of keratoacanthoma with squamous cell carcinoma and normal skin by using DNA microarrays. DNA microarray studies were performed on formalin-fixed and paraffin-embedded blocks from ten cases of actinic keratoacanthoma utilizing the U133plus2.0 array. These results were compared with our previously developed microarray database of ten squamous cell carcinoma and ten normal skin samples. Keratoacanthoma demonstrated 1449 differentially expressed genes in comparison with squamous cell carcinoma (>5-fold change: *P*<0.01) with 908 genes upregulated and 541 genes downregulated. Keratoacanthoma showed 2435 differentially expressed genes in comparison with normal skin (>5-fold change: *P*<0.01) with 1085 genes upregulated and 1350 genes downregulated. The most upregulated genes, comparing keratoacanthoma with normal skin included *MALAT1, S100A8, CDR1, TPM4,* and *CALM1*. The most downregulated genes included *SCGB2A2, DCD, THRSP, ADIPOQ, adiponectin,* and *ADH1B.* The molecular biological pathway analysis comparing keratoacanthoma with normal skin showed that cellular development, cellular growth and proliferation, cell death/apoptosis, and cell cycle pathways are prominently involved in the pathogenesis of keratoacanthoma. The most enriched canonical pathways were clathrin-mediated endocytosis signaling, molecular mechanisms of cancer and integrin signaling. The distinctive gene expression profile of keratoacanthoma reveals that it is molecularly distinct from squamous cell carcinoma. The molecular pathways and genes differentially expressed in comparing keratoacanthoma with normal skin suggest that keratoacanthoma is a neoplasm that can regress due to upregulation of the cell death/apoptosis pathway.
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Keratoacanthoma is a crateriform squamous lesion of the sun-exposed skin of elderly patients that typically grows rapidly then involutes. Microscopically, keratoacanthoma shows three distinctive growth phases including: the early growth phase; fully developed/proliferative phase; and senescent/regressive phase.[1] Most keratoacanthoma are biopsied in the fully developed/proliferative phase which is histologically characterized by a symmetric crateriform, exo-endophytic lesion with buttress formation and a prominent central keratin plug. Invasive lobules and nests of tumor cells with low-grade nuclei and abundant, glassy eosinophilic cytoplasm that mature toward the center are characteristic. Keratoacanthoma usually shows a sharp delineation between the tumor nests and stroma and can entrap elastic fibers. A prominent associated mixed inflammatory infiltrate of lymphocytes, eosinophils, and neutrophils is frequent.[1,2]

Keratoacanthoma remains controversial. Some believe that it is a variant of squamous cell carcinoma because of rare reports of metastasis.[3,4] Others believe that keratoacanthoma is a distinct self-resolving benign squamoproliferative lesion.[1,2,5–7] To investigate the relationship of keratoacanthoma to squamous cell carcinoma and normal skin, we analyzed the gene expression profile of over 47 000

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genes using one of the most comprehensive GeneChip microarrays available (human U133 plus 2.0 array) to study differential gene expression between keratoacanthoma and squamous cell carcinoma and keratoacanthoma and normal skin in formalin-fixed and paraffin-embedded tissue.

Materials and methods

Sample Selection

Ten cases of actinic keratoacanthoma in the fully developed/proliferative stage (Figure 1) were identified from the Tamtron database. Slides and formalin-fixed and paraffin-embedded tissue (<1 since excision) were retrieved. The histology was reviewed and their diagnoses coincided. None of these tumors recurred or metastasized. Areas of interest were removed from the paraffin blocks with a sterile surgical scalpel. The ten cases of squamous cell carcinoma that were used for the comparison were previously reported.[8,9] They consisted of well-to-moderately differentiated squamous cell carcinoma arising from skin showing actinic damage with solar elastosis and/or actinic keratosis. The ten cases of keratoacanthoma included sporadic keratoacanthomas and BRAF inhibitor-induced keratoacanthomas. Previous studies demonstrated no induction of MAP kinase pathway expression or significant molecular differences between keratoacanthomas from patients on BRAF inhibitors and patients not on inhibitors.[10] Methods of RNA isolation, quality control, target preparation and microarray hybridization, and data analysis are described below and in our previous studies.[8,9]

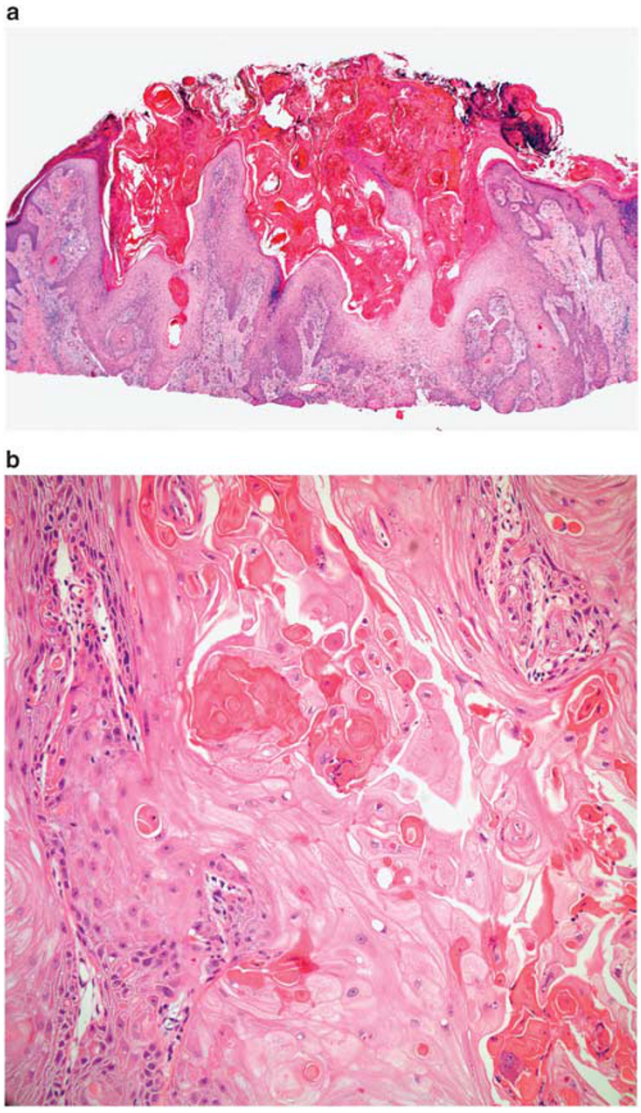

Figure 1 (**a**) Keratoacanthoma with crateriform architecture, central hyperkeratotic plug, and surrounding buttress of squamous epithelium (H&E 20X). (**b**) Keratoacanthoma showing proliferating squamoid cells with abundant pale eosinophilic cytoplasm (H&E 200X).

RNA Isolation and Quality Control

Total RNA was isolated using the Ambion Recover-All (Applied Bio systems/Ambion, Austin, TX, USA) kit according to the manufacturer's instructions. Briefly, formalin-fixed and paraffin-embedded samples were deparaffinized by using a series of xylene and ethanol washes and then subjected to a proteinase K digestion at 50 °C for 16 h to release RNA from covalently linked proteins. Finally, total RNA was purified by capturing on a glass-fiber filter. After washing, the total RNA was eluted. RNA integrity was evaluated by using an Agilent 2100 Bio analyzer (Agilent Technologies, Palo Alto, CA, USA) and purity/concentration was determined by using a NanoDrop 8000 (NanoDrop Products, Wilmington, DE, USA). The RNA samples with RNA integrity number ≥ 5 and 260/280 ratio ≥ 1.7 were selected for the microarray.

Target Preparation and Microarray Hybridization

Microarray targets were prepared by using the NuGEN WT-Ovation formalin-fixed and paraffin-embedded RNA Amplification System V2. This system offers the most efficient cDNA amplification powered by Ribo-SPIA technology and is ideal for global gene expression analysis with the small amount of degraded RNA derived from formalin-fixed and paraffin-embedded samples. Fifty nanograms of the total RNA were used for the first-strand synthesis. After the second-strand cDNA synthesis, the double-stranded cDNA was purified using Agencourt RNAClean beads provided with the WT-Ovation kit, followed by SPIA cDNA Amplification. Five micrograms of amplified cDNA were fragmented and labeled by using NuGEN's FL-Ovation cDNA Biotin Module V2 according to the instructions (NuGEN Technologies, San Carlos, CA, USA) and then hybridized to the Affymetrix GeneChip U133

plus 2.0 array (Affymetrix, Santa Clara, CA, USA) according to the manufacturers' instructions. The arrays were washed and stained with streptavidin phycoerythrin in Affymetrix Fluidics Station 450 by using the Affymetrix GeneChip protocol and scanned by using an Affymetrix GeneChip Scanner 3000.

Data Analysis

The acquisition and initial quantification of array images were conducted by using AGCC software (Affymetrix). The subsequent data analyses were performed using Partek Genomics Suite Version 6.6 (Partek, St Louis, MO). We first performed an ANOVA analysis to identify genes between the groups at $P<0.05$ and then calculated relative difference in fold change between the groups. Genes expressed at $\geq$2-fold and $P<0.05$ were considered as differentially expressed between the groups. Cluster analyses were conducted with Partek default settings. Canonical pathway analyses were performed using the Ingenuity Pathway Analysis Version (Ingenuity Systems, Redwood City, CA, USA). Briefly, a differentially expressed gene list containing gene identifiers and corresponding fold changes was first uploaded as an Excel spreadsheet into the software. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. These genes were then used as the starting point for the pathway analysis. Canonical pathway analysis identified the pathways from the Ingenuity Pathways Analysis library of canonical pathways that were most significant to the data set. The significance of the association between data set and canonical pathway was measured in two ways: (1) a ratio of the number of genes from the data set that map to the pathway divided by the total number of genes that map to the canonical pathway was displayed; and (2) a Fischer exact test was used to calculate a *P*-value determining the probability that the association between the genes in the data set and the canonical pathway is explained by chance alone.

Quantitative Real-Time PCR Analyses

After analyzing our DNA microarray data of differentially expressed genes between keratoacanthoma and squamous cell carcinoma, three representative upregulated genes (*CDR1*, *MALAT1*, and *NCRNA00084*) and two representative downregulated genes (*LOC441461* and *CEP170*) were selected for QRT-PCR confirmation. The QRT-PCR confirmation was performed by using the SYBR Green real-time RT-PCR kit (Applied Biosystems) according to the manufacturer's instructions. An additional three cases each of keratoacanthoma and squamous cell carcinoma were selected for the analysis and RNA isolation was performed by using the same protocol as above. The Applied Biosystems 7500 Real-Time PCR System was used for the analyses with the following primers:

CDR1: forward TTTGATGGAAGACCTTGAAATTAT; reverse TGTCTTCCAAGAAGCTCCAAG
MALAT1: forward CCAAAAGAGAACCACACACTACC; reverse GGTTGGTCTGGCCTACTGGG
NCRNA00084: forward TCACTTGAAAGATGGGCGTCA; reverse ACCTAAGTTGCTAAGGGGCAG
LOC441461: forward CGCACAGGCATACAGATTCTC; reverse CAAGGAAGGGCACTGAAGAG
CEP170: forward TCTCCTTGTAAAGCTAAATTCCCCA; reverse AAAAGAGGGAGCAATGCTGA

GAPDH was used as an internal control for normalization. The analysis was performed by comparing CT values (cycle threshold, ie, the cycle where the increase in fluorescence crosses the threshold to be exponential) and the fold change was calculated by using Delta-Delta Ct method.

Results

Using microarray parameters similar to our prior studies,[8,9] we found that 14 258 genes were differentially expressed between keratoacanthoma and squamous cell carcinoma (>2-fold change (FC): $P<0.05$). Because of the large number of genes, the data was analyzed with the parameters of >5 FC ($P<0.01$) that showed 1449 differentially expressed genes with 908 genes upregulated and 541 genes downregulated.

The most significantly upregulated genes included *CDR1*, *MALAT1*, *TPM4*, *CALM1*, and *TMED2*. The most significantly downregulated genes included *LOC441461*, *TYRP1*, *CEL*, *INTS6*, and *WWOX* (Table 1).

Hierarchical cluster analysis utilizing the most significant differentially expressed genes revealed that keratoacanthoma and squamous cell carcinoma have distinct genetic signatures (Figure 2). To confirm the reliability of the results from microarray analysis, selected upregulated genes (*CDR1*, *MALAT1*, and *NCRNA00084*) and downregulated genes (*LOC441461* and *CEP170*) were verified by QRT-PCR analyses (Table 2).

The keratoacanthoma samples were also compared with normal skin revealing 15 645 differentially expressed (>2 FC: $P<0.05$) genes. Because of the large number of genes, the data were analyzed with the parameters of >5 FC ($P<0.01$) that showed 2435 differentially expressed genes with 1085 genes upregulated and 1350 genes downregulated. The most significantly upregulated genes included *MALAT1*, *S100A8*, *CDR1*, *TPM4*, and *CALM1*. The most significantly downregulated genes included *SCGB2A2*, *DCD*, *THRSP*, *ADIPOQadiponectin*, and *ADH1B* (Table 3). The molecular biological pathway analysis comparing keratoacanthoma with normal skin revealed that the most significantly enriched molecular and cellular functions included cellular development, cellular growth and proliferation, cell

Table 1 Differentially expressed genes distinguishing keratoacanthoma from squamous cell carcinoma

Ref Seq	*Symbol/gene*	*Fold change*
Upregulated genes		
NM_004065	*CDR1:* cerebellar degeneration-related protein 1, 34 kDa	175.70
NR_002819	*MALAT1:* metastasis-associated lung adenocarcinoma transcript 1 (nonprotein coding)	156.64
NM_001145160 /// NM_003290	*TPM4:* tropomyosin 4	86.19
NM_006888	*CALM1:* calmodulin 1 (phosphorylase kinase, delta)	51.38
NM_006815	*TMED2:* transmembrane emp24 domain trafficking protein 2	49.08
NM_001031827	*BOLA2*: BolA homolog 2 (*E. coli*)	48.46
NR_000012	*SNORA68:* small nucleolar RNA, H/ACA box 68	48.01
NM_015313	*ARHGEF12*: Rho guanine nucleotide exchange factor (GEF) 12	45.00
NM_001620 /// NM_024060	*AHNAK:* AHNAK nucleoprotein	43.30
NM_012153	*EHF:* ets homologous factor	42.35
Downregulated genes		
XR_041357 /// XR_041358 /// XR_041359	*LOC441461:* hypothetical LOC441461	−28.80
NM_000550	*TYRP1:* tyrosinase-related protein 1	−24.61
NM_001807	*CEL:* carboxyl ester lipase (bile salt-stimulated lipase)	−22.68
NM_001039937 /// NM_001039938 /// NM_012141	*INTS6:* integrator complex subunit 6	−16.63
NM_016373 /// NM_130791 /// NM_130844	*WWOX*: WW domain containing oxidoreductase	−15.79
NM_001042404 /// NM_001042405 /// NM_014812	*CEP170*: centrosomal protein 170 kDa	−15.63
NM_001146274 /// NM_001146283 /// NM_001146284 /// NM_001146285 /// NM_001146286	*TCF7L2*: transcription factor 7-like 2 (T-cell specific, HMG-box)	−13.43
NM_021188	*ZNF410*: zinc-finger protein 410	−13.42
NM_001047	*SRD5A1*: steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-de	−12.51
NM_001001787 /// NM_001677	*ATP1B1*: ATPase, Na+/K+ transporting, beta 1 polypeptide	−12.49

death/apoptosis, and cell cycle pathways (Table 4). Examination of the top canonical pathways showed prominent upregulation of clathrin-mediated endocytosis signaling, molecular mechanisms of cancer, and integrin signaling (Table 5).

Discussion

The DNA microarrays showed that actinic keratoacanthoma and cutaneous squamous cell carcinoma are distinct entities with unique molecular signatures (Figure 2). Our study identified differentially expressed genes and enriched molecular pathways when keratoacanthoma is compared with squamous cell carcinoma and normal skin, laying a strong basis for their separation at the molecular level. Our data showed 1449 differentially expressed genes between keratoacanthoma and squamous cell carcinoma (>5 FC: $P<0.01$). The large number of genes differentially expressed suggests that keratoacanthoma is not only a distinct lesion but also is markedly different molecularly from squamous cell carcinoma. Our previous microarray study of the closely related precursor lesion, actinic keratosis[8] showed only nine genes that were differentially expressed from squamous cell carcinoma (>2 FC: $P<0.05$). Additional entities that are molecularly distinct from squamous cell carcinoma include normal skin[8] and pseudoepitheliomatous hyperplasia:[9] 382 and 703 differentially expressed genes, respectively (>2 FC: $P<0.05$) in our previous microarray studies.

Other studies have similarly shown that keratoacanthoma and squamous cell carcinoma are molecularly distinct. One of the earliest molecular studies utilizing loss of heterozygosity showed multiple differences between keratoacanthoma and squamous cell carcinoma.[11,12] The frequency of loss of heterozygosity for keratoacanthomas was low with isolated losses at 9p, 9q, and 10q. These results were in contrast to squamous cell carcinoma where loss of heterozygosity was common on chromosome arms 3p, 9p, 9q, 13q, 17p, and 17q.[11,12] More recent array CGH studies showed significant differences between keratoacanthoma and squamous cell carcinoma in the distribution of numbers of aberrant clones and recurrent aberrations between them.[13,14] Li *et al*[14] showed recurrent aberrations in keratoacanthomas on chromosomes 17, 19, 20, and X in about a third of cases. Recurring aberrations in squamous cell carcinomas were found in 40% of squamous cell carcinomas on chromosomes 7, 8, 10, 13, 17, and X, with losses on certain regions of 17p and 17q recurring in 50% of samples. Also, a recent FISH studied showed that EGFR and MYC gene copy number aberrations were more common in squamous cell carcinoma than keratoacanthoma.[15]

There has been much debate as to whether keratoacanthoma is a reactive/hyperplastic or neoplastic

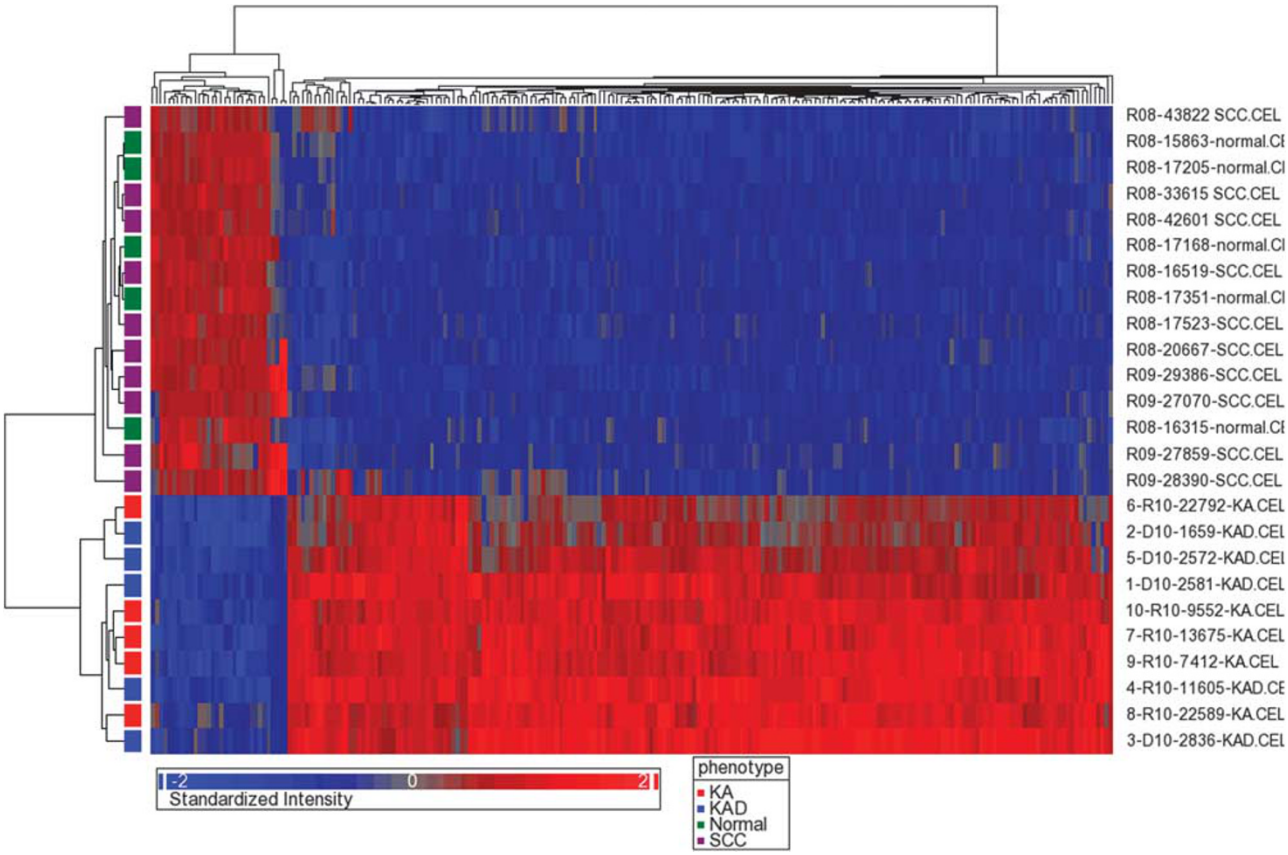

Figure 2 Hierarchical cluster analysis using the most significant differentially expressed genes (*y*-axis) revealed that keratoacanthoma and squamous cell carcinoma demonstrated distinct genetic signatures. Downregulated genes are in blue and upregulated genes are in red (at 10-fold FDR001-224 genes cluster).

Table 2 QRT-PCR confirmation of the microarray data

Gene	*Average fold change (KA vs. SCC)*
CDR1	2.416 (upregulated)
MALAT1	2.557 (upregulated)
NCRNA00084	2.220 (upregulated)
LOC441461	0.156 (downregulated)
CEP170	0.290 (downregulated)

lesion. In order to gain insight into the pathogenesis of keratoacanthoma, we focused on the altered molecular biologic functions and canonical pathways in comparing keratoacanthoma with normal skin, and the results suggest that keratoacanthoma is neoplastic. Two of the three top canonical pathways: molecular mechanisms of cancer and integrin signaling are well known to be involved in neoplasia.[8] We have previously reviewed the integrin signaling pathway and its purported role in squamous cell carcinoma carcinogenesis.[8] Four of the five most significant molecular biological pathways deregulated in keratoacanthoma are related to tumorigenesis and include cellular development, cellular growth and differentiation, cell cycle, and cell movement. In additional support of keratoacanthoma as a neoplastic lesion, the previously discussed molecular studies showed molecular aberrations including loss of heterozygosity and copy number aberrations by CGH that would be expected in a neoplastic lesion.[11–14] These molecular findings would be unusual in a reactive or hyperplastic process.

Some of the most upregulated genes in comparing keratoacanthoma with normal skin are implicated in neoplasia and include *MALAT-1, S100A8*, and *EHF* (FCs = 216.93, 156.65, and 69.28). *MALAT-1* is a long noncoding RNA that is thought to induce tumor migration and growth possibly through the modulation of caspase-3, -8, Bax, Bcl-2, and Bcl-xL.[16] Its overexpression has been shown to be significantly associated with metastasis and poor prognosis in colorectal, nonsmall cell lung, prostate, pancreas, and cervical carcinomas.[16–18] *EHF* is part of the ETS family of transcriptions factors that have been shown to participate in a variety of cellular functions including development, differentiation, proliferation, apoptosis, migration, tissue remodeling, invasion, and angiogenesis.[19] Fusions of ETS genes with other targets have been described in Ewing's sarcoma, chronic myelomonocytic leukemia,

Table 3 Differentially expressed genes distinguishing keratoacanthoma from normal skin

Ref Seq	*Symbol/Gene*	*Fold Change*
Upregulated genes		
NR_002819	*MALAT1:* metastasis-associated lung adenocarcinoma transcript 1 (nonprotein coding)	216.93
NM_002964	*S100A8: S100* calcium-binding protein A8	156.65
NM_004065	*CDR1:* cerebellar degeneration-related protein 1, 34 kDa	146.93
NM_001145160 /// NM_003290	*TPM4:* tropomyosin 4	109.70
NM_006888	*CALM1:* calmodulin 1 (phosphorylase kinase, delta)	73.16
NM_012153	*EHF:* ets homologous factor	69.28
NM_006815	*TMED2:* transmembrane emp24 domain trafficking protein 2	66.98
NM_001031827	*BOLA2*: BolA homolog 2 (*E. coli*)	63.00
NR_000012	*SNORA68:* small nucleolar RNA, H/ACA box 68	55.07
NM_001114978 /// NM_001114979 /// NM_001114980 /// NM_001114981 /// NM_001114982	*TP63*: tumor protein p63	46.38
Downregulated genes		
NM_002411	*SCGB2A2:* secretoglobin, family 2A, member 2	– 132.56
NM_053283	*DCD:* dermcidin	– 109.61
NM_003251	*THRSP:* thyroid hormone responsive (SPOT14 homolog, rat)	– 64.04
NM_004797	*ADIPOQadiponectin:* C1Q and collagen domain containing	– 60.42
NM_000668	*ADH1B:* alcohol dehydrogenase 1B (class I), beta polypeptide	– 58.21
NM_000517 /// NM_000558	*HBA1 /// HBA2:* hemoglobin, alpha 1 /// hemoglobin, alpha 2	– 49.82
NM_006551	*SCGB1D2:* secretoglobin, family 1D, member 2	– 48.13
NM_020692	*GALNTL1:* UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase	– 42.47
NM_173833	*SCARA5:* Scavenger receptor class A, member 5 (putative)	– 37.36
NM_001143981 /// NM_001143982 /// NM_001143983 /// NM_145234	*CHRDL1:* chordin-like 1	– 35.17

Table 4 Molecular biological pathway analysis

Molecular and cellular functions	P-*value*	# *Genes*
Cellular development	2.91E-15—1.92E-03	425
Cellular growth and proliferation	8.94E-13—1.47E-03	471
Cell death	4.90E-10—1.79E-03	446
Cell cycle	1.99E-09—1.92E-03	220
Cellular movement	8.01E-09—1.88E-03	268

Table 5 Top canonical pathways

Name	P-*value*
Clathrin-mediated endocytosis signaling	1.10E-08
Molecular mechanisms of cancer	9.99E-07
Integrin signaling	2.23E-06
Glucocorticoid receptor signaling	2.89E-06
Neuregulin signaling	3.56E-06

and prostate carcinoma.[20] EHF has been shown to be differentially regulated in prostate, serous ovarian, and breast carcinomas.[19–21] *S100A8* is a calcium-binding protein that was also upregulated in our study comparing squamous cell carcinoma to pseudoepitheliomatous hyperplasia and squamous cell carcinoma to normal skin.[8,9] It is involved in the regulation of various cellular processes, and its role in tumorigenesis was previously discussed.[9]

Many believe that keratoacanthomas are benign regressing neoplasms. It has been hypothesized that these lesions may be follicle-derived and undergo apoptosis akin to catagen involution of the hair follicle.[1,2,5–7] Our data supports this hypothesis by showing prominent upregulation of the molecular biological cell death/apoptosis pathway and the canonical clathrin-mediated endocytosis signaling pathway in comparing keratoacanthoma to normal skin (Table 3). Several of the most upregulated differentially expressed genes (*CALM1*, *TP63*, *YWHAZ*, *CALR*, *MMP1*, *S100A8*, and *ARHGEF12*) are purported to be involved in cell death/apoptosis. The upregulation of these pathways may account for the benign behavior of keratoacanthoma.

Several immunohistochemical studies have also shown that apoptosis has a role in the regression of keratoacanthoma. One study distinguished keratoacanthoma from squamous cell carcinoma by showing that keratoacanthoma expressed markers associated with the initiation (the cytolytic receptor P2X7) and completion phases of (TUNEL) of apoptosis.[22] Others have shown that keratoacanthoma strongly expresses the proteins bax and bak, which are considered essential for apoptosis execution.[23] Another study examined keratoacanthomas showed lower expression of the anti-apoptotic protein Bcl-xL that is consistent with a possible role of apoptosis in

keratoacanthoma regression.[24] BCL-2 is a proto-oncogene involved in protecting cells from undergoing apoptosis and has been shown to have decreased expression in regressing keratoacanthomas.[23,25]

We hypothesize that prominent enrichment of the clathrin-mediated endocytosis signaling pathway may be because of granzyme-mediated apoptosis. Cytotoxic T cells and natural killer cells utilize lytic effector proteins of perforin and granzymes to eliminate target cells. Cytotoxic T cells have been shown to play an important role in the regression of keratoacanthoma.[26] Thiery *et al.*[27] showed that perforin released by the cytotoxic T cells activates clathrin- and dynamin-dependent endocytosis to facilitate the internalization of perforin and granzyme by target cells after their release. This is the critical first step initiating target cell apoptosis and may explain the upregulation of clathrin-mediated endocytosis signaling.

The granzyme-mediated apoptosis model also may account for the prominent upregulation of the calcium-binding proteins *CALM1* (FC = 73.16). *CALM1* encodes calmodulin which is a member of the EF-hand calcium-binding protein family. It encodes a calcium-binding protein that is one of the four subunits of phosphorylase kinase. In the model of granzyme-mediated apoptosis, the action of perforin creates pores in the target cell plasma cell membrane, transiently allowing Ca^2 into the cell. The influx of Ca^2 leads to a wounded membrane repair response, where lysosomes and endosomes fuse to the plasma membrane to reseal the damaged membrane. This response protects the target cells from necrosis and allows them to undergo the slower, ATP-dependent process of granzyme-mediated apoptosis.[27] Calmodulin is a calcium-binding protein that may have a role in this response. Increased intracellular calcium in conjunction with calmodulin-associated Fas-mediated apoptosis has been shown in the human B-cell line FMO, Jerkat cells, osteoclasts, and cholangiocarcinoma cells.[28]

The role of S100A8 in tumorigenesis has been previously discussed. However, S100A8 through a complex with S100A9 has also been shown to have a role in tumoral apoptosis in mouse lymphoma and human leukemia cell lines, cervical carcinoma cell lines, and colon carcinoma cells lines.[29–31] The S100A8/A9 complex has been shown to induce inhibitory effects on the proliferation and invasiveness of tumor cells[30] through zinc exclusion from target cells and through binding to target cells' surface receptors.[31]

We believe keratoacanthoma is a distinct entity, separate from squamous cell carcinoma. We and others have shown that keratoacanthoma and squamous cell carcinoma have unique molecular signatures.[11–14] In addition, there is a preponderance of evidence that keratoacanthoma and squamous cell carcinoma have distinct clinicopathological features.[1,2,5–7] The differentially expressed genes and enriched molecular biological pathways that separate keratoacanthoma from normal skin suggest that keratoacanthoma is a neoplasm that may regress due to upregulation in the cell death/apoptosis pathway. Furthermore, we believe that actinic keratoacanthomas should be treated conservatively and hope that our findings will help prevent radical treatments including wide excision, radiotherapy, and neck dissection for this benign involuting squamous neoplasm.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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