A Serial Analysis of Gene Expression in Sun-Damaged Human Skin

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To study the phenotypic changes in human skin associated with repeated sun exposure at the transcription level, we have undertaken a comparative serial analysis of gene expression of sun-damaged preauricular skin and sun-protected postauricular skin as well as sun-protected epidermis. Serial analyses of gene expression libraries, containing multiple mRNA-derived tag recombinants, were made to poly(A^+)RNA isolated from human postauricular skin and preauricular skin, as well as epidermal nick biopsy samples. 5330 mRNA-derived cDNA tags from the postauricular serial analysis of gene expression library were sequenced and these tag sequences were compared to cDNA sequences identified from 5105 tags analyzed from a preauricular serial analysis of gene expression library. Of the total of 4742 different tags represented in both libraries we found 34 tags with at least a 4-fold difference of tag abundance between the libraries. Among the mRNAs with altered steady-state[1] levels in sun-damaged skin, we detected those encoding keratin 1, macrophage inhibitory factor, and calmodulin-like skin protein. In addition, a comparison of cDNA sequences identified in the serial analysis of gene expression libraries obtained from the epidermal biopsy samples (5257 cDNA tags) and from both full-thickness skin samples indicated that many genes with altered steady-state transcript levels upon sun exposure were expressed in epidermal keratinocytes. These results suggest a major role for the epidermis in the pathomechanism of largely dermal changes in chronically sun-exposed skin. ***Key words: elastic fibers/epidermis/full-thickness skin/photoaging/SAGE analysis. J Invest Dermatol 119:3–13, 2002***

Aging of the skin is thought to consist of two processes taking place simultaneously. The first process is intrinsic, chronologic aging and similar perhaps to aging of other tissues (Uitto, 1986). The second process is photoaging, an environmentally induced remodeling of the dermis that arises as a result of repeated exposure of skin to sunlight. Although recent studies (Varani *et al*, 1998; 2000) have shown that both intrinsic aging and photoaging share some common characteristics such as decreased procollagen gene expression and increased expression of genes encoding several matrix metalloproteinases, it has been suggested that photoaging is the predominant contributing factor to the prematurely aged appearance of sun-exposed skin (Yaar and Gilchrest, 1998).

Clinically, sun-damaged skin is characterized by wrinkling, loss of resilience, and an altered texture (Kligman, 1989; Taylor *et al*, 1990). Early studies attribute these features primarily to changes in the dermis, as histopathologic analyses have revealed alterations in a variety of extracellular matrix proteins within the dermis of sun-exposed skin. The most prominent of these dermal changes is the marked accumulation of elastic fibers with a clearly altered morphology in the superficial dermis of sun-exposed skin. This accumulation of aberrant dermal elastic fibers following sun exposure has been referred to as solar elastosis (Gilchrest, 1989).

The cellular mechanisms leading to solar elastosis are not understood and, indeed, controversial findings concerning the synthesis of elastic fibers during solar elastosis have been reported. Several reports have demonstrated that elastic fibers deposited during solar elastosis consist of the same components as normal elastic fibers, and these include elastin (the insoluble and crosslinked protein that makes up the amorphous component of elastic fibers) and fibrillin, the major microfibrillar component of elastic fibers. In response to ultraviolet A (UVA) and/or UVB radiation, keratinocytes secrete many mediators that could stimulate fibroblast synthetic activity, and some of them, e.g., tumor necrosis factor β, interleukin-1β, and interleukin-10, have been shown to increase the promoter activity of the elastin gene, steady-state[1] mRNA levels, and elastin accumulation (Kahari *et al*, 1992; Mauviel *et al*, 1993; Reitamo *et al*, 1994). Whereas Bernstein *et al* (1994) have noted increased elastin mRNA levels in sun-damaged skin, Werth and coworkers (Werth *et al*, 1997) have reported no difference in steady-state levels of elastin mRNA during solar elastosis. The latter finding is in agreement with an earlier study that speculated that a post-transcriptional mechanism might lead to an increased transla-

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Abbreviations: CLSP, calmodulin-like skin protein; COMP, cartilage oligomeric matrix protein; EFABP, epidermal fatty acid binding protein; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; MAP kinase, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; SAGE, serial analysis of gene expression.

[1] "Steady state" throughout this paper refers to the levels of mRNAs detected by SAGE or northern analysis as a function of synthesis and degradation of messenger RNA.

tional efficiency responsible for elastin protein accumulation in response to UV irradiation in the absence of increased mRNA levels (Schwartz *et al*, 1995). These results indicate that aberrant expression of genes encoding structural proteins of elastic fibers, as a consequence of UV exposure, could be the basis of solar elastosis. Indeed, several reports have demonstrated changes in steady-state mRNA levels not only of elastin but also of fibrillin (Bernstein *et al*, 1994). Additional observations have also noted changes in the levels of elastic fiber proteins such as lysyl oxidase, the copper-dependent amine oxidase responsible for the catalysis of elastin crosslinking (Smith-Mungo and Kagan, 1998).

Other changes in extracellular matrix proteins in response to UV irradiation have also been demonstrated. For example the amount of collagen fibrils has been shown to be drastically decreased in photoaged skin. This change is not accompanied by a change in collagen mRNA levels, suggesting that degradation of collagen fibrils is associated with UV exposure (Bernstein *et al*, 1996). To explain these changes in collagen deposition, Voorhees and coworkers have proposed that UV irradiation triggers an increase of growth factor and cytokine receptor synthesis in fibroblasts and keratinocytes. This increased receptor synthesis, in turn, leads to an activation of the transcription factor AP-1 (Fisher *et al*, 1996; Fisher and Voorhees, 1998) through a mitogen-activated protein kinase (MAP kinase) signaling cascade, an increase in the expression of genes encoding several collagen-degrading matrix metalloproteinases (Fisher *et al*, 1996), and a decreased expression of the genes encoding type I and III procollagen.

Although an attractive hypothesis, this model for an AP-1 activation of matrix metalloproteinase gene expression does not accommodate the many other changes in extracellular matrix that have been shown to be associated with UV exposure. Moreover it is very likely that the pathobiology of sun-damaged skin arises through a complex interaction of multiple direct and indirect changes in gene expression in the dermis and epidermis, AP-1 activation representing just one of these changes.

To begin to understand this complex cascade of events associated with sun damage, we have initiated a study of the profile of high abundance mRNAs in sun-protected (postauricular) and sun-exposed (preauricular) full-thickness human skin as well as in sun-protected epidermis using serial analysis of gene expression (SAGE). SAGE has been used extensively in recent years to identify comprehensive qualitative and quantitative profiles of mRNAs, within either intact tissue or cultured cells, as a reflection of changes in global patterns of gene expression in a variety of disease states. This method is therefore well suited to identifying changes in transcript profiles in response to sun exposure.

In this report, we describe the use of SAGE to identify a spectrum of genes expressed in human skin and a discrete number of genes differentially expressed in response to sun damage.

MATERIALS AND METHODS

Tissues

Full-thickness skin Skin samples were obtained from female Caucasians with moderately sun-damaged skin undergoing elective facial plasty. Full-thickness skin from the front of the auricle of the ear (preauricular skin) was used as sun-damaged skin in this study. Skin from behind the auricle of the ear (postauricular skin) was used as sun-protected skin.

The preauricular and postauricular skin samples used to prepare SAGE libraries were obtained from a 55-y-old female. RNA samples for northern analysis were obtained from preauricular and postauricular skin provided by either the above-mentioned donor or three other female subjects (Asian and Caucasian) ranging in age from 49 to 64 y. Most of the tissue sections were frozen for later RNA extraction; a small section of each fresh biopsy was removed prior to freezing for histologic analysis.

Epidermis Ten nick biopsies were obtained from the sun-protected part of the sacral region of the lower back from each of 34 female donors of varied ethnic background with an average age of 26 y.

All tissue samples were snap-frozen in liquid nitrogen immediately upon biopsy and stored at –80°C.

Histology Preauricular and postauricular tissues were fixed in buffered formalin and embedded in paraffin. Five-micron sections were stained for elastic fibers using the Verhoeff–Van Gieson stain.

Extraction of mRNA The multiple nick epidermal biopsies were pooled into a single tissue sample. Total RNA from this epidermal sample and preauricular and postauricular skin samples were extracted using RNA STAT-60 (Tel-Test, Friendswood, TX) following the manufacturer's protocol. Poly(A^+)RNA was isolated from total RNA using oligo-dT cellulose (Gibco/BRL, Rockville, MD) chromatography and quantified by spectrophotometry (Beckman, Fullerton, CA). The integrity of poly(A^+)RNA was confirmed by electrophoresis through 1.2% agarose gels containing formaldehyde.

RNA from preauricular and postauricular skin obtained from a single donor Caucasian female was used to maximize the recovery of mRNA differences between preauricular and postauricular skin that could otherwise be masked if pooled RNA samples from multiple donors had been used. In contrast, an RNA sample was prepared from pooled epidermal biopsies collected from multiple donors for the preparation of the epidermal SAGE library. This epidermal SAGE library was used in this study to qualitatively identify mRNAs of epidermal origin. All donors were females to minimize any gender-specific mRNA differences that would not be relevant to this study.

SAGE analysis SAGE analysis was performed as described previously (Velculescu *et al*, 1995). Essentially, double-stranded cDNA was synthesized from mRNA using a biotinylated oligo-dT primer and then digested with Nla III. The biotinylated 3′ most cDNA fragments were isolated with magnetic streptavidin beads (Dynal, Oslo, Norway) and divided into two separate aliquots. Two different oligonucleotide linkers, containing a Bsmf I recognition site, a Nla III recognition site, and polymerase chain reaction (PCR) priming sites, were ligated to DNA in each sample. Following Bsmf I digestion, the tags were ligated, and the ditag products were PCR amplified, isolated by Nla III digestion, concatamerized, and consecutively cloned into a pZero vector (Invitrogen, Carlsbad, CA). Individual bacterial colonies containing recombinant plasmids were checked for insert sizes by PCR using M13 forward and M13 reverse primers. Insert-derived PCR products of at least 400 bp were then sequenced with the BigDye Terminator Kit (Perkin Elmer, Foster City, CA) and a 310 ABI automated DNA sequencer. Sequences were analyzed by the SAGE 2000 software program (version 4.12), which compares tags to the GenBank/EMBL databases and identifies and excludes duplicate ditags and tags derived from linkers. Tags originating from differentially expressed mRNAs were additionally analyzed with NCBI's SAGEmap ''tag to gene'' software.

Northern blot analysis and probes Between one and two micrograms of poly(A^+)RNA or 10 μg of total RNA was subjected to electrophoresis on 1.2% agarose–formaldehyde gels and then transferred onto Hybond-N+ nylon membranes (Amersham Pharmacia, Piscataway, NJ). Membranes were incubated overnight at 65°C with PCR amplified cDNA fragments or plasmid inserts, which had previously been radiolabeled with α^{32}P dCTP (ICN, Costa Mesa, CA) using the Megaprime DNA labeling kit (Amersham Pharmacia). After incubation, membranes were washed twice with 2 × sodium citrate/chloride buffer (SSC), 0.1% sodium dodecyl sulfate (SDS) for 20 min at room temperature, twice with 0.2 × SSC, 0.1% SDS at room temperature, and three times with 0.2 × SSC, 0.1% SDS at 50°C. A phosphorimager (Molecular Dynamics, Sunnyvale, CA) was used to visualize bound radiolabeled material and quantitate recovered mRNA levels. A radiolabeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was used as an internal control for RNA loading by hybridization to previously incubated membranes that had been stripped of bound radiolabeled material.

RESULTS

Histology of preauricular and postauricular skin We used paired biopsies of sun-damaged preauricular skin and sun-protected postauricular skin obtained from patients undergoing facial plasty to study the changes in gene expression associated with chronic, long-term sun exposure. To confirm solar elastosis in these paired biopsy samples, we examined sections of preauricular and postauricular skin stained for elastic fibers using the Verhoeff–van Gieson stain. Representative histology from a 55-y-old Caucasian female with moderate sun damage is shown in **Fig 1**. The accumulation of morphologically aberrant, thickened elastic fibers within the upper dermis in preauricular skin (*panel A*) is clearly evident by

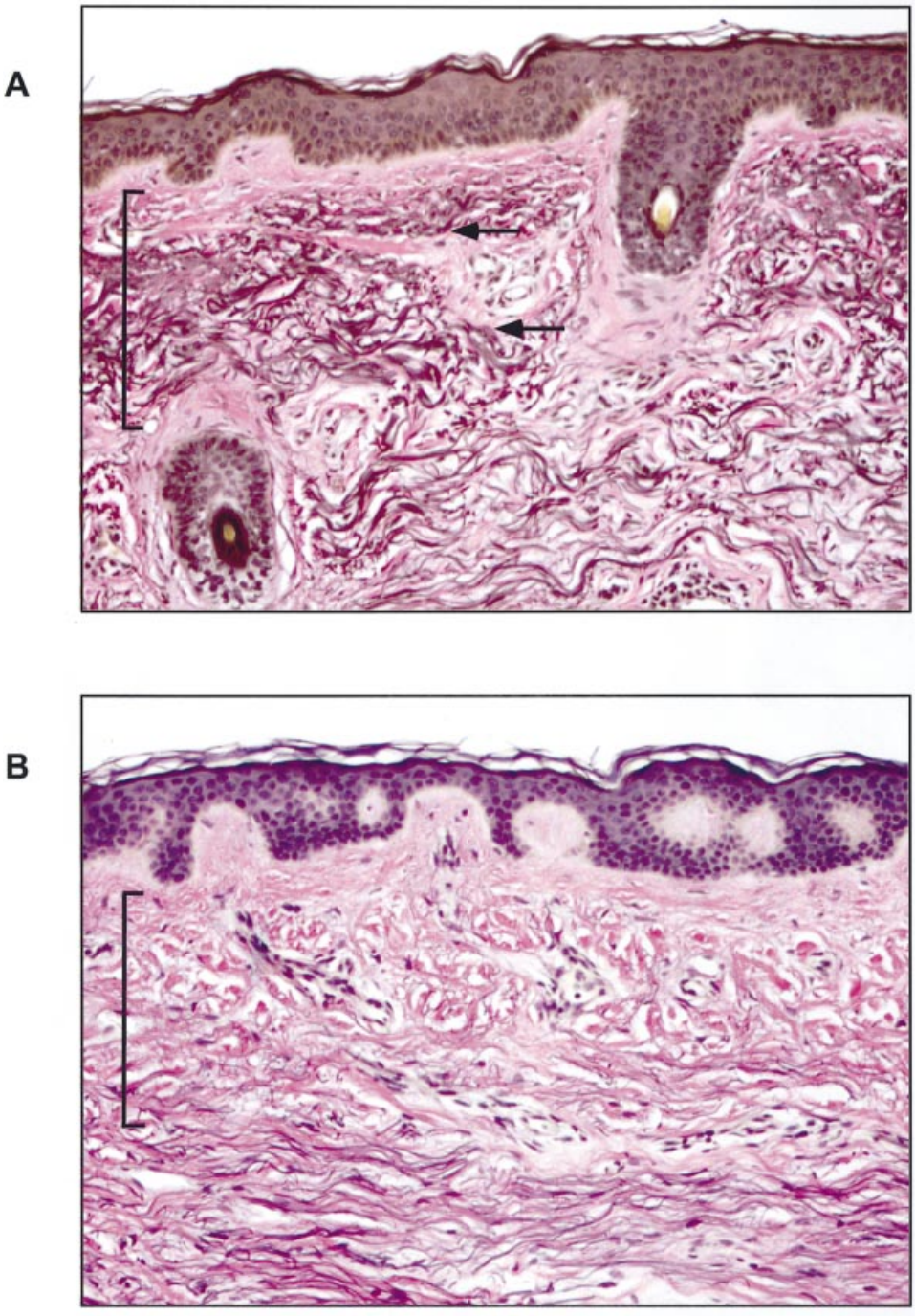

Figure 1. Solar elastosis in sun-exposed preauricular skin. Five-micron full-thickness paraffin-embedded skin sections from sun-exposed preauricular skin (*A*) and sun-protected postauricular skin (*B*) were stained for elastic fibers using the Verhoeff–Van Gieson stain. The region of the upper dermis is indicated with square brackets and the solar elastotic material deposited in the upper dermis in sun-exposed skin is indicated with *arrows*. Both preauricular and postauricular skin sections were obtained from the same patient and these skin samples were the same samples used for the preparations of poly(A^+)RNA for the development of preauricular and postauricular SAGE libraries. *Scale bar*: 100 μm.

comparison with sun-protected, postauricular skin (*panel B*) and entirely consistent with earlier reports of solar elastosis (Chen *et al*, 1986). Although some previous reports have documented changes in thickening within the epidermis in sun-exposed skin (Lavker *et al*, 1995), no such consistent changes within the epidermis were noted between sections from our paired preauricular and postauricular skin biopsies.

SAGE libraries We constructed three different SAGE libraries using poly(A^+)RNA isolated from preauricular skin and postauricular skin from a single donor and pooled epidermal nick biopsies. Upon subtracting tags derived from linkers, we generated 4830 SAGE tags derived from preauricular skin and 4990 tags derived from postauricular skin. In addition, we generated 5215 SAGE tags derived from human epidermis. Collectively, these 15,035 tags represented 6598 unique genes.

Analysis of SAGE tags from postauricular skin Of the 2858 unique tags obtained from postauricular skin, 127 tags were observed at least five times and the total number of these repetitive

Table I. The 50 most abundant tags from a human postauricular skin SAGE library

	Tag sequence	Tag count[a]	Accession no.[b]	UniGene match[c]
1	CCCGTCCGGA	63	AI291979	ribosomal protein L13
2	TGCACGTTTT	45	X03342	ribosomal protein L32
3	CGCCGCCGGC	38	U12465	ribosomal protein L35
4	CGCTGGTTCC	34	L05092	ribosomal protein L11
5	GAGGGAGTTT	31	U14968	ribosomal protein L27a
6	GGACCACTGA	31	M90054	ribosomal protein L3
7	AGGCTACGGA	30	AA045770	ribosomal protein L13a
8	GCCCCTGCTG	30	M21389	keratin 5
9	GTGAAACCCC	29		multiple matches
10	GGCAAGCCCC	27	NM007084/NM007104	SRY-box 21/ribosomal protein L10a
11	ACGCAGGGAG	23	AF187554/AF130111	glucose phosphate isomerase/histone deacetylase 3
12	TTGGTCCTCT	23	AF026844/NM001007	ribosomal protein L41/ribosomal protein S4
13	ACTTTTTCAA	22		no reliable matches
14	CCTGTAATCC	22		multiple matches
15	CTTCCTTGCC	21	X05803	keratin 17
16	TGTGTTGAGA	21	M27364/L141490	elongation factor 1-alpha 1/elongation factor 1-alpha 1-like14
17	GCAGCCATCC	20	U14969/BC004230	ribosomal protein L28/triosephosphate isomerase 1
18	GTGGAGGGCA	20	U81233/U62800	cystatin E/cystatin M
19	CACAAACGGT	19	L19739	ribosomal protein S27
20	GATGTGCACG	19	AA583889	keratin 14
21	GGATTTGGCCT	19	M17887	ribosomal protein P2
22	GGGCTGGGGT	18	U10248/BC011934	ribosomal protein L29/sperm associated antigen 7
23	TCACCCACAC	18	AI268626	ribosomal protein L23
24	CGCCGGAACA	17	X73974/BC004532	ribosomal protein L4/H19, imprinted maternally exp. untransl, mRNA
25	TGGTGTTGAG	17	X69150	ribosomal protein S18
26	GCCGAGGAAG	16	X53505/AK025643	ribosomal protein S12/hypothetical protein
27	GTTGTGGTTA	16	AB021288	beta 2-microglobulin
28	AGGTCAGGAG	15		multiple matches
29	CTAAGACTTC			no reliable matches
30	GCCTGTATGA	15	AA324873	ribosomal protein S24
31	GTGAAGGCAG	15	M77234/D14710	ribosomal protein S3a/ATP synthase, H+ transporting, alpha subunit
32	TAGGTTGTCT	15	NM03295/AK000037	translationally-controlled tumor protein 1/ hypothetical prot. FLJ20030
33	ACCTCCACTG	14	AA582988	keratinocyte differentiation associated protein
34	GTGGCCACGG	14	AA128515	calcium-binding protein S100 A9 (calgranulin B)
35	TAAACCTGCT	14	L07769	galectin 7
36	TGGGCAAAGC	14	M55409	elongation factor-1-gamma
37	AGCACCTCCA	13	Z11692	eukaryotic translation elongation factor 2
38	GCATAATAGG	13	L38826	ribosomal protein L21
39	GCCGTGTCCG	13	M20020	ribosomal protein S6
40	TCAGATCTTT	13	M22146	ribosomal protein S4
41	GAAAACAAAG	12	M77663	keratin 10
42	TTGGCCAGGC	12		multiple matches
43	AAGACAGTGG	11	X66699	ribosomal protein L37a
44	CCACTGCACT	11		multiple matches
45	GCGAAACCCC	11		multiple matches
46	GGAGGGGGCT	11	X03444	lamin A/lamin C
47	AAGGTGGAGG	10	L05093	ribosomal protein L18a
48	AATAGGTCCAA	10	M64716	ribosomal protein S25
49	GAACACATCCA	10	S56985	ribosomal protein L19
50	GCAAAACCCC	10		multiple matches

[a]Tags have been ranked by abundance, as indicated by tag count.
[b]The accession number or numbers indicate a representative expressed sequence tag derived from the corresponding UniGene cluster.
[c]No accession number has been provided for tags with either multiple matches or an expressed sequence tag match.

tags represented 32% of the total tag number. 2254 tags of the remaining low abundance tags were detected only once. **Table I** lists the 50 most abundant postauricular SAGE tags that we detected, together with the frequency of these tags, reliable UniGene matches, and a corresponding GenBank accession number. Tags originating from mitochondrial DNA were excluded. Whenever available, the fifteenth base in the SAGE tag sequence (CATG + 11 bp) was used to discriminate between multiple matches for the same tag. All tags except for two (tag 13 and tag 29) could be assigned to at least one gene. Seventeen tags had multiple assignments; 10 of these tags matched sequences derived from two different genes and seven originated from more than two different genes. Many of these abundant tags were derived from genes that are known to be widely expressed in various cell types, especially genes encoding ribosomal proteins, genes involved in protein synthesis (elongation factor 1), cytoskeletal genes (lamin A/C), and genes active in energy metabolism (glucose phosphate isomerase). Tags matching mRNAs derived from genes known to be specifically expressed in skin were also found. Among the most highly abundant of these skin-specific SAGE tags, we detected tags

Table II. The 50 most abundant tags from a human preauricular SAGE library[a]

	Tag sequence	Tag count	Accession no.	UniGene match
1	CCCGTCCGGA	50	AA010823	ribosomal protein L13
2	TAAACCTGCT	40	L07769	galectin 7
3	CGCCGCCGGC	34	U12465	ribosomal protein L35
4	GTGAAACCCC	34		multiple matches
5	GATGTGCACG	26	AA583889	keratin 14
6	TTGGTCCTCT	26	AF026844/NM001007	ribosomal protein L41/ribosomal protein S4
7	GCCCCTGCTG	25	M19723	keratin 5
8	GCAGCCATCC	24	U14969/BC004230	ribosom al protein L28/triosephosphate isomerase 1
9	TGTGTTGAGA	24	M27364/L141490	elongation factor 1-alpha 1/elongation factor 1-alpha 1-like14
10	ACGCAGGGAG	23	AF187554/AF130111	glucose phosphate isomerase/histone deacetylase 3
11	GAAAACAAAG	23	J04029	keratin 10
12	TGCACGTTTT	23	X03342	ribosomal protein L32
13	AGGCTACGGA	21	AA045770	ribosomal prot. L13a
14	CGCTGGTTCC	21	L05092	ribosomal protein L11
15	GCCGAGGAAG	21	X53505/AK025643	ribosomal protein S12/hypothetical protein
16	GGCAAGCCCC	21	AF107044/AL022721	SRY-box 21/ribosomal protein L10a
17	CCTGTAATCC	20		multiple matches
18	GAGGGAGTTT	20	U14968	ribosomal protein L27a
19	GGGCTGGGGT	20	U10248/BC011934	ribosom al protein L29/sperm associated antigen 7
20	GTGGAGGGCA	19	U81233/U62800	cystatin E/cystatin M
21	TGGTGTTGAG	19	X69150	ribosomal protein S18
22	ACCTCCACTG	18	AA582988	likely ortholog of rat keratinocyte differentiation associated protein
23	GCCGTGTCCG	18	M20020	ribosomal protein S6
24	CGCCGGAACA	17	X73974/BC004532	ribosomal protein L4/H19, imprinted maternally exp. untransl. mRNA
25	GGACCACTGA	17	X73460	ribosomal protein L3
26	TGGGCAAAGC	17	M55409	elongation factor -1-gamma
27	AGGTCAGGAG	16		multiple matches
28	ATCCGCGAGGC	16	Af172852	calmodulin-like skin protein
29	GGATTTGGCC	16	M17887	ribosomal protein P2
30	CTAAGACTTC	15		no reliable match
31	AAGGTGGAGGA	14	AB007175	ribosomal protein L18a
32	GTGGCCACGG	14	M26311	S100 calcium-binding protein A9
33	AAAAAAAAAA	13		multiple matches
34	CTTCCTTGCC	13	X05803	keratin 17
35	GCATAATAGG	13	U14967	ribosomal protein L21
36	TCACCCACAC	13	AI268626	ribosomal protein L23
37	TTCAATAAAA	13	M17886/AK025203	ribosomal protein P1/FLJ21550 fis, clone COL06258
38	CACAAACGGT	12	L19739	ribosomal protein S27
39	CCACTGCACT	12		multiple matches
40	CCCATCCGAA	11	L07282	ribosomal protein L 26
41	GTGAAACCCT	11		multiple matches
42	GTTGTGGTTA	11	AB021288	beta 2-microglobulin
43	AAGGAGATGG	10	AB024057/X15940	vascular Rab-GAP(TBC-containing)/ribosomal protein L31
44	AGAAAAAAAA	10		multiple matches
45	CTGGGTTAAT	10	M81757	ribosomal protein S19
46	GACGACACGA	10	L05091	ribosomal protein S28
47	AGGCTCCTGGC	9	AF106911	member 14 (BRAK) of the small inducible cytokine subfamily B
48	ATGGCTGGTAT	9	X17206	ribosomal protein S2
49	CCAGTGGCCCG	9	U14971	ribosomal protein S9
50	CTCCTGGGCGC	9	M58026	calmodulin-like 3

[a]Footnotes are as given in **Table I**.

derived from mRNAs encoding several keratins as well as galectin 7 and S100calcium-binding protein A9(calgranulin B), all of which are typically found in full-thickness skin.

Analysis of SAGE tags from preauricular skin Among the 4830 tags generated from preauricular skin, 2931 were found to be unique. Of these, 127 unique tags (4%) appeared more than five times; 30% of the total amount of tags were represented by these repetitive tag sequences. Almost 50% or 2393 of all tags appeared only once. **Table II** provides a summary of the 50 most abundant tags detected in our SAGE library constructed from preauricular skin. As for postauricular skin, all but one tag (CTAAGACTTC, tag 30 in preauricular skin, tag 29 in postauricular skin) could be matched to at least one gene and multiple tags could be assigned to more than one gene. Nearly all of the most abundant preauricular skin tags were also found to be of high copy number in postauricular skin. Except for tag 28 [ATCCGCGAGGC, calmodulin-like skin protein (CLSP)] all tags in the list of the 50 most abundant preauricular tags either were found among the 50 most abundant tags in postauricular skin or were detected in similar

Table III. The 50 most abundant tags from a human epidermal SAGE library[a]

	Tag sequence	Tag count	Accession no.	UniGene match
1	GAAAACAAAG	77	M77663	keratin 10
2	CCCGTCCGGA	66	AA010823	ribosomal protein L13
3	GCCCCTGCTG	52	M21389	keratin 5
4	GATGTGCACG	48	AA583889	keratin 14
5	ACTTTTTCAA	42		no reliable matches
6	ACAGCGGCAA	40	M77830	desmoplakin I
7	ACATTTCAAAG	39	AA024512	keratin 1
8	CGCCGCCGGC	39	U12465	ribosomal protein L35
9	TAAACCTGCT	39	L07739	galectin 7
10	GGATTTGGCCT	33	M17887	ribosomal protein P2
11	GTGAAACCCC	33		multiple matches
12	GTTGTGGTTAA	32	AB021288	beta 2-microglobulin
13	GGGCTGGGGTC	30	U10248/AF047437	ribosom al protein L29/sperm associated antigen 7
14	ACCTCCACTGG	25	AA582988	keratinocyte differentiation associated protein
15	CCACAGGAGAA	25	AJ251830	p53-induced protein PIGPC1
16	ATCCGCGAGGC	24	Af172852	calmodulin-like skin protein
17	CCTGTAATCC	23		multiple matches
18	GCAGCCATCCG	21	U14969/BC004230	ribosom al protein L28/triosephosphate isomerase 1
19	GGACCACTGAA	20	M90054	ribosomal protein L3
20	TGTGTTGAGA	20	M27364/L141490	elongation factor 1-alpha 1/elongation factor 1-alpha 1-like 14
21	AGAAAAAAAAA	19		multiple matches
22	CGCTGGTTCC	19	L05092	ribosomal protein L11
23	GAGGGAGTTTC	19	U14968	ribosomal protein L27a
24	GGCCGCGTTCG	19	M13932	ribosomal protein S17
25	GCCGAGGAAG	18	X53505/AK025643	ribosomal protein S12/hypothetical protein
26	GTGTGGGGGGC	18	Z68228	junktion plakoglobin
27	AAGGTGGAGGA	17	L05093	ribosomal protein L18a
28	GAGAGCTAACT	17	M60502	filaggrin
29	GCCGTGTCCG	17	M20020	ribosomal protein S6
30	GGCAAGCCCCA	17	AF107044/AL022721	SRY-box 21/ribosomal protein L10a
31	ATGGCTGGTAT	16	AL031671	ribosomal protein S2
32	GCCTTCTGGAT	16		no reliable matches
33	AAAAAAAAAA	15		multiple matches
34	CAGGTTTCATA	15	AF106911	member 14 (BRAK) of the small inducible cytokine subfamily B
35	CCACTGCACT	13		multiple matches
36	CCAGAACAGAC	15		multiple matches
37	TTCAATAAAAA	15		multiple matches
38	ATTTGAGAAGC	14		no reliable matches
39	TTGGTCCTCTG	14	AF026844/NM001007	ribosomal protein L41/ribosomal protein S4
40	AGGCTCCTGGC	13	AF106911	member 14 (BRAK) of the small inducible cytokine subfamily B
41	TAGGTTGTCTA	13	NM03295/AK000037	translationally-controlled tumor protein 1/hypothetical prot. FLJ20030
42	GGAGGCTGAGG	12		multiple matches
43	AATCTTGTTT	11	BC004493	ESTs/hypothetical gene ZD52F10
44	ACCTGGAGGGG	11		ESTs
45	ATAATTCTTT	11	AK021540/AA147325	cDNA FLJ11778 fis, clone HEMBA1005911/ribosomal protein S29
46	CTGGGTTAAT	11	M81757	ribosomal protein S19
47	CCAGTGGCCC	10	AI064904	ribosomal protein S9
48	GCGAAACCCC	10		multiple matches
49	TCAGATCTTT	10	M22146	ribosomal protein S4
50	ACGCAGGGAG	9	AF187554/AF130111	glucose phosphate isomerase/histone deacetylase 3

[a]Footnotes are as given in **Table I**.

tag numbers. The majority of the most abundant tags in preauricular skin were derived from mRNAs encoded by housekeeping genes, consistent with previous SAGE studies using other tissues (Velculescu *et al*, 1997; Chen *et al*, 1998).

Analysis of SAGE tags from epidermis We generated 5215 SAGE tags from epidermal nick biopsies, representing 2982 unique genes. Tag distribution in this library was similar to the distribution we had observed in SAGE libraries from preauricular and postauricular skin libraries. The most abundant tags in the epidermal library (five or more tags) represented 4% of the unique tags. Single tags represented 80% of the unique tags and 45% of all tags present in this SAGE library. The 50 most frequent epidermal tags are listed in **Table III**. Of these 50 tags, two tags did not show any reliable gene match by comparison to the UniGene database, nine tags could only be assigned to a multitude of UniGene clusters, and furthermore 10 tags could not be attributed to a single gene but to two genes. Among the seven most highly expressed genes, we found four different keratins (keratin 1, 10, 5, and 14), typically expressed in epidermal keratinocytes. The genes for the intermediate filament proteins keratins 5 and 14 are known to be highly expressed in the basal layer of the epidermis, whereas

Table IV. Genes downregulated in preauricular skin

Tag sequence[a]	Post	Pre	Post/Pre[d]	UniGene match[b] (Accession no.)[c]
CAAAAAAAAAA	7	1	7.0	multiple matches
ACGTTAAAGA	6	1	6.0	no reliable matches
ACATCATCGAT	5	1	5.0	ribosomal protein L12 (L06505)
ACTCCAAAAAA	5	1	5.0	ribosomal protein S15 (AA079663)/IMAGE clone 3840457 (BC012990)
GAAATACAGTT	5	1	5.0	cathepsin D (M11233)
GCCAGGAGCTA	5	1	5.0	ladinin 1/ESTs, Highly similar to ATIC (U42408/AI214479)
CTCCTCACCTG	9	2	4.5	ribosomal protein L13a/BCL2-antagonist (NM012423/U16811)
CAATAAACTGA	4	1	4.0	putative translation initiation factor (AA009621)
CAGCTCACTGA	4	1	4.0	ribosomal protein L14 (D87735)
CAGGACCTGGT	4	1	4.0	no reliable matches
CCCAACGCGCT	4	1	4.0	hemoglobin alpha 1 and alpha 2 (J00153)
CCCTGGCAATG	4	1	4.0	uncharacterized hematopoietic stem/progenitor cells protein MDS027 (Af161418)
CTGCCAAGTTG	4	1	4.0	zyxin (U15158)
GCAAAACCCCG	4	1	4.0	multiple matches
GGAAAAAAAAA	4	1	4.0	multiple matches
GGGGCAGGGCC	4	1	4.0	eukaryotic translation initiation factor 5A (AW505485)
GTGCACTGAGC	4	1	4.0	major histocompatibility complex, class I A and I C (M11887; M11886)
TCTCCCACACC	4	1	4.0	calcium-binding protein S100 A3 (N002960)
CGGGGTGGCCG	4	0	4.0	cartilage oligomeric matrix protein (L32137)

[a]Tags have been ranked by fold downregulation, as indicated by a post/pre ratio.
[b]The accession number or numbers indicate a representative expressed sequence tag derived from the corresponding UniGene cluster.
[c]No accession number has been provided for tags with either multiple matches or an expressed sequence tag match.
[d]In order to avoid division by zero, we used a tag value of one for tags that were not detected at all.

keratins 1 and 10 are predominantly found in the differentiating keratinocytes of the suprabasal layers of the epidermis. Tags from mRNA encoding filaggrin, which crosslinks keratin, and also plakoglobin, a crosslinker of intermediate filaments and the dense plaques of desmosomes, are also among the 50 most abundant tags. The remainder of the tags in this table are largely derived from genes known to be expressed in many different tissues.

A comparison of postauricular and preauricular skin SAGE libraries In order to identify genes that were differentially expressed in sun-exposed skin, we compared the SAGE libraries for preauricular and postauricular skin. A small but significant fraction of the analyzed SAGE sequence tags showed marked differences in copy number between preauricular and postauricular skin. Nineteen unique tags were found at significantly lower levels (at least 4-fold lower) in sun-exposed preauricular skin, whereas 15 showed at least 4-fold higher levels in preauricular skin. **Tables IV** and **V** list these tags with notably different copy numbers. Of these tags, 25 could be uniquely matched to the UniGene database and nine tags had either multiple matches or no matches. Three of these unmatched tags have sequences that consist primarily of multiple deoxyadenosine residues [tag 1 (CAAAAAAAAAA) and tag 15 (GGAAAAAAAAA) in **Table IV**; tag 7 (TAAAAAAAAAA) in **Table 5**]. Another three tags reliably matched two different genes; two tags showed no significant similarity with any UniGene cluster sequence.

Of the 25 uniquely matched tags, we observed a 7-fold higher keratin 1 tag number in preauricular skin. We also found elevated copy numbers for tags derived from several other genes in sun-damaged skin (**Table V**) and these include the following.

1 The psoriasin gene encodes a member of the S100 calcium binding protein family and tags derived from this gene were found in a 4-fold higher level in preauricular skin. Psoriasin protein and mRNA levels have been reported to be raised in UVB-exposed skin *in vivo* up to 10 d postexposure (Di Nuzzo *et al*, 2000). Furthermore, psoriasin has previously been shown to be present in all layers of psoriatic epidermis and has been shown to be associated with epidermal fatty acid binding protein (EFABP), and both the genes encoding psoriasin and EFABP are known to be upregulated in psoriasis (Hagens *et al*, 1999). EFABP gene expression has also been shown to be induced in human skin by topical application of retinoic acid (Larsen *et al*, 1994). Our SAGE data revealed a 6-fold higher tag count for EFABP mRNA in sun-exposed skin compared to normal skin.

2 The mRNA encoding insulin-like growth factor binding protein 6 (IGFBP-6) is represented by six tags in preauricular skin and by one tag in postauricular skin; IGFBP-6 binds insulin-like growth factor II (IGF-II) with high affinity and this binding inhibits IGF-II action. Three groups of IGFBP proteases (matrix metalloproteinases, kallikreins, and cathepsins) cleave the IGFBP–IGF complex and have been shown to release a functional IGF from its binding protein. IGFBP-6 has been associated with quiescent, nonproliferating cells, suggesting that IGFBP-6 acts as an autocrine growth inhibitor (Kato *et al*, 1995). Kelley *et al* (1996) have suggested that IGFBPs may also have additional intrinsic biologic activities, independently of IGFs.

3 The mRNA for CLSP is represented at a 16:4 tag ratio between sun-damaged and sun-protected skin. CLSP is a recently identified protein that was reported to be particularly abundant in the epidermis. CLSP gene expression moreover has been shown to be directly associated with keratinocyte differentiation (Mehul *et al*, 2000).

4 Macrophage migration inhibitory factor (MIF) mRNA was 4-fold upregulated in preauricular skin. MIF, originally reported be released by activated T cells, inhibits the migration of macrophages and activates macrophages at inflammatory loci. In addition, a previous study implicated MIF as a regulator in epidermal immunity and cell differentiation (Shimizu *et al*, 1996). UVB irradiation has been shown to induce MIF production in human epidermal keratinocytes *in vivo* and *in vitro* (Shimizu *et al*, 1999). In addition MIF is also thought to be involved in psoriasis as MIF levels are elevated in psoriatic plaques (Steinhoff *et al*, 1999). MIF appears therefore to function as an inhibitor of anti-inflammatory action by coordinating several pro-inflammatory cytokines, as well as regulation of the immunosuppressive effects of steroids on immune cell activation and cytokine production.

5 The number of tags representing cellugyrin mRNA increased 4-fold in preauricular skin. Cellugyrin is a ubiquitously expressed member of the synaptic vesicle protein family of synaptogyrins,

Table V. Genes upregulated in preauricular skin

Tag sequence[a]	Pre	Post	Pre/Post[d]	UniGene match[b] (Accession no.)[c]
ACATTTCAAAG	7	1	7.0	keratin 1 (AA024512)
CAGCTATTTCA	6	1	6.0	fatty acid binding protein 5 (AF181449)
GGCCCCTCACC	6	1	6.0	insulin-like growth factor binding protein 6 (M69054)
ATCCGCGAGGC	16	4	4.0	calmodulin-like skin protein (AF172852)
AACGCGGCCAA	8	2	4.0	macrophage migration inhibitory factor (L10612)
GAGCAGCGCCC	8	2	4.0	S100 calcium-binding protein A7 (psoriasin 1) (M86757)
AAGAAGATAGA	4	1	4.0	ribosomal protein L23a (NM_002952)
TAAAAAAAAAA	4	1	4.0	multiple matches
TCAGACTTTTG	4	1	4.0	diacylglycerol O-acyltransferase (NM 032564)
TTGGTGAAGGA	4	1	4.0	beta 4 thymosin (M17733)
AACTAACAAAA	4	0	4.0	ribosomal protein S27a (X63237)
CAATAAATGTT	4	0	4.0	ribosomal protein L37 (D23661)
GCTCCCAGACT	4	0	4.0	synaptogyrin 2 (AJ002308)
GGAAGTTTCGA	4	0	4.0	mitochondrial ribosomal protein 64 (AB049959)
TCAAAAATATA	4	0	4.0	mitochondrial ribosomal protein S31 (NM005830)

[a]Tags have been ranked by fold upregulation, as indicated by a pre/post ratio.
[b,c,d]As given in **Table IV**.

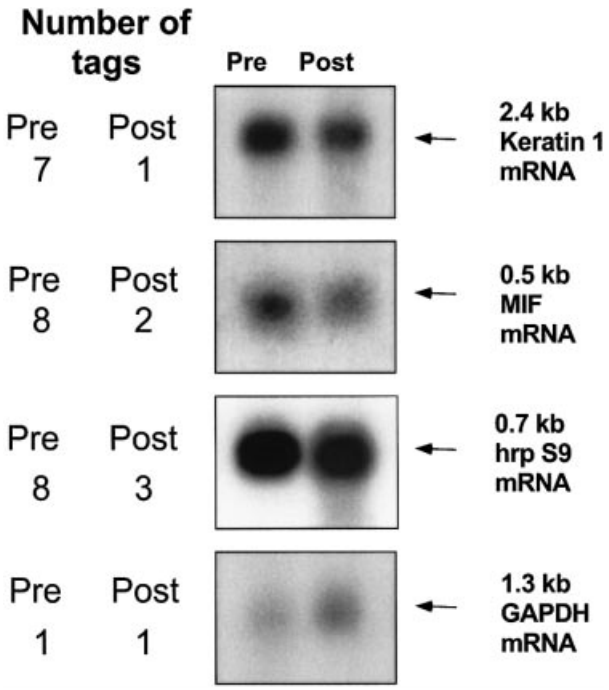

Figure 2. Northern blot analysis of preauricular and postauricular poly(A^+)RNA. Between 1 and 2 μg of messenger RNA from preauricular and postauricular skin, from which the SAGE libraries were constructed, was used per lane. Northern blot analysis was performed as described in *Materials and Methods*. The steady-state levels of these mRNAs observed by SAGE (in tag numbers) are listed in the columns on the left of the autoradiograms. GAPDH mRNA was used as a control mRNA. K1, keratin 1; hrp S9, human ribosomal protein S9.

which are essential for the regulation of synaptic vesicle trafficking (Janz and Sudhof, 1998). In adipocytes, for example, insulin activates the translocation of glucose-transporter-4-containing membrane vesicles from intracellular compartments to the plasma membrane, which ultimately leads to an increased glucose uptake. As insulin stimulation does not initiate a redistribution of cellugyrin-positive glucose transporter 4 vesicles to the plasma membrane, it is believed that these vesicles do have unique functional properties, independent of those glucose transporter 4 vesicles that translocate to the plasma membrane (Kupriyanova and Kandror, 2000).

Reduced tag numbers from mRNAs encoding known proteins in sun-exposed skin (**Table IV**) include the following.

1 Cathepsin D showed the most significant decrease (5-fold less) in mRNA steady-state levels in preauricular skin. Cathepsin D is a lysosomal aspartic proteinase known to be present in the epidermis as well as many other tissues. The chronology of activation and degradation of this protein has been shown to be connected with stages of cellular differentiation and the expression of cathepsin D in the epidermis resembles that of other structural proteins such as keratin 10, involucrin, and transglutaminase, in response to calcium concentration changes (Horikoshi *et al*, 1998).

2 Ladinin mRNA, which showed a 5-fold decrease of representative SAGE tags in our preauricular library in comparison to the postauricular library, is an anchoring-filament-associated protein and is one of several basement-associated proteins that contribute to autoimmune disorders such as linear IgA disease (Moll and Moll, 1998).

3 The SAGE tag GCCAGGAGCTA was found nine times in postauricular skin and only twice in preauricular skin. This tag matched two different UniGene database entries, one for an mRNA encoding ribosomal protein L 13a and a second entry for an mRNA encoding Bcl-2 antagonist. Bcl-2 antagonist is a proapoptotic protein that shares a high sequence homology with bax. Both proteins are thought to oligomerize in mitochondrial membranes, forming pores that facilitate cytochrome c efflux (Korsmeyer *et al*, 2000) and trigger an apoptosis cascade.

4 A 4-fold lower mRNA level for the mRNA encoding zyxin was detected in sun-damaged skin compared to normal skin. Zyxin is a focal adhesion phosphoprotein reported to be expressed in all layers of the epidermis (Leccia *et al*, 1999). Moreover zyxin is also found in fibroblasts where the protein has been shown to be colocalized both with cell–substratum and also with cell–cell adherens junctions. Zyxin shares architectural characteristics (such as LIM domains, a double zinc-finger motif) with signal transducers involved in developmental regulation and previous work has suggested that zyxin may also be involved in the regulation of cell proliferation and differentiation (Beckerle, 1997).

5 An mRNA encoding the calcium ion binding protein S100 A3 showed decreased levels in the SAGE library derived from sun-damaged skin. As with psoriasin, S100 A3 is a member of the S100 calcium binding gene family. Significant expression of the gene encoding S100 A3 in mouse is limited to the hair follicle and the timing of expression of this gene is synchronized with the neonatal and adolescent phases of the hair growth cycle (Kizawa *et al*, 1998).

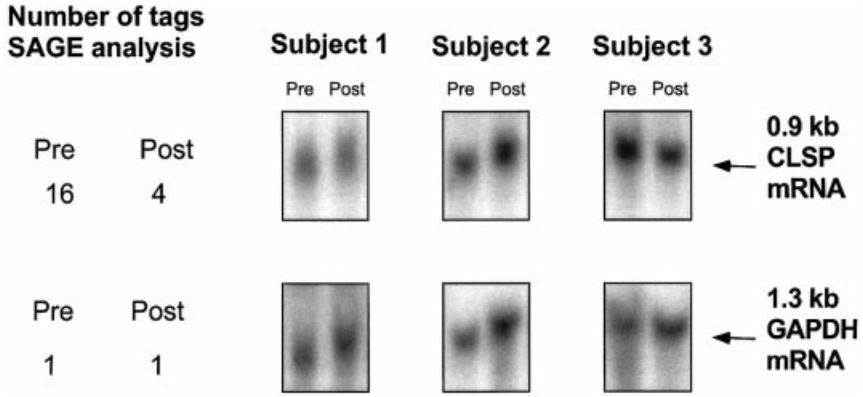

Figure 3. Northern blot analysis of preauricular and postauricular total RNA from three different subjects. Ten micrograms of total RNA from preauricular and postauricular skin from three different Caucasian female subjects was used per lane and probed with ^{32}P-labeled CLSP cDNA. A radiolabeled GAPDH cDNA probe was used as a control for RNA loading. The steady-state levels of these mRNAs observed by SAGE analysis (in tag numbers) are listed in the columns on the left of the autoradiograms.

6 A reduced number of tags in sun-exposed skin was observed for another Ca^{2+} binding protein called cartilage oligomeric matrix protein (COMP). COMP is an extracellular matrix glycoprotein expressed not only in cartilage and ligaments but also in human dermal fibroblasts *in vitro* (Dodge *et al*, 1998) and cultured human vascular smooth muscle cells (Riessen *et al*, 2001). Mutations in COMP have been shown to result in decreased calcium binding ability, which ultimately leads to the skeletal disorder pseudoanchodroplasia (Maddox *et al*, 2000). In other respects, however, very little is known about the function of COMP.

We also observed a reduction in the number of tags derived from ribosomal RNAs (ACATCATCGAT and CAGCTCACTGA) and from mRNAs encoding unknown proteins (CAGGACCTGGT and ACGTTAAAGAC) in sun-exposed preauricular skin.

Northern blot analysis We confirmed by northern blot analysis that the differences in tag numbers between our preauricular and postauricular skin SAGE libraries correlated with differences in steady-state levels for the mRNAs from which these tags were derived (**Fig 2**). Radiolabeled cDNA complementary to mRNAs for keratin 1, MIF, and human ribosomal protein S9 were used to determine the levels of these mRNAs in poly(A^+)RNA from the same preauricular and postauricular skin samples used to construct the SAGE libraries. Relative to the levels of a control GAPDH mRNA, the levels of keratin 1 mRNA, MIF mRNA, and human ribosomal protein S9 in preauricular and postauricular skin (**Fig 2**) were consistent with our SAGE tag recovery data.

We have also used northern hybridization to analyze the steady-state levels of mRNA encoding CLSP and GAPDH in total RNA isolated from preauricular and postauricular skin from three unrelated individuals, each of which was also different to the donor of preauricular and postauricular skin that was used to construct our SAGE libraries. The results presented in **Fig 3** confirmed that the increased steady-state levels of CLSP mRNA that we had observed in our SAGE analysis are paralleled by increased CLSP mRNA levels as seen by northern hybridization.

DISCUSSION

In this SAGE analysis of mRNA profiles in human skin, we have identified over 15,000 tags representing mRNAs from more than 6500 different genes. These mRNAs in full-thickness skin were identified in poly(A^+)RNA isolated from chronically sun-exposed preauricular skin and sun-protected postauricular skin, obtained from a patient with sun damage undergoing elective facial plasty. By choosing this model of preauricular and postauricular skin we were able to circumvent at least some of the limitations of other model systems, including the inherent difficulties of using mice to study solar elastosis. Using sun-exposed human skin also allows us to study the effects of natural sunlight, rather then having to differentiate between the effects of the different components of sunlight. A recent study by Brown *et al* (2000) reported, for example, that common fluorescent sunlamps are inadequate substitutes for natural sunlight. Moreover, cell culture models rarely give a satisfactory representation of the different cell types as well as the three-dimensional structure of tissues with the consequence of, for example, neglecting the interactions between cell types. Additionally, the skin biopsies in our study were taken from adjacent sites of the face in an attempt to minimize phenotypic differences in skin from different regions of the body. Using preauricular and postauricular human skin, however, the possibilities of controlling and influencing experimental conditions such as total duration of sun exposure are limited. Furthermore, we are aware that our model reflects changes in mRNA steady-state levels that are due to many years of sun exposure rather than representing alterations caused by a controlled and limited exposure to UV light.

Full-thickness human skin contains a variety of different cell populations, the most abundant cell type in human skin being epidermal derived keratinocytes. We would therefore expect that an mRNA profile obtained from full-thickness skin would reflect a spectrum of mRNAs derived largely from keratinocytes, and this is indeed the result we have obtained. In comparing the 50 most abundant mRNAs in both preauricular and postauricular skin to the mRNA profile we have identified in a SAGE library from epidermal nick biopsies, it is clear that many of these tags are derived from mRNAs encoding proteins typically found in epidermis. These include keratins, galectin 7, and CLSP.

A similar analysis of tags obtained from a human skin fibroblast SAGE library (data not shown) revealed several mRNAs expected to be among the most abundant in skin fibroblasts. These include the mRNAs encoding proα1(I), proα2(I), and proα1(III) collagen and several matrix metalloproteinases. Tags derived from these mRNAs were not observed in our full-thickness skin libraries, supporting the conclusion that most of the abundant mRNAs that we have observed in this SAGE analysis of full-thickness skin are derived from epidermal keratinocytes.

We have noted, in **Tables IV** and **V**, tags derived from 34 different genes that are either increased or decreased in abundance between preauricular and postauricular skin. These changes in tag numbers are a direct reflection of changes in steady-state levels for mRNAs from which these tags were derived. This SAGE study does not address a direct alteration in gene expression associated with chronic sun exposure but rather a direct measurement of altered mRNA steady-state levels, which indirectly reflects changes in gene expression and assumes that, in most cases, altered mRNA levels will be reflected in changes in the amount of proteins these mRNAs encode.

The changes in mRNA levels that we have noted between preauricular and postauricular skin were measured using RNA from a single donor. In a preliminary northern blot analysis using RNA from preauricular and postauricular skin from three unrelated donors, it is clear that at least the steady-state level of one mRNA known to be increased in our SAGE analysis is also increased in the preauricular skin from three other different donors. This reprodu-

cible increase in CLSP mRNA therefore represents an mRNA difference functionally related to sun damage and probably reflects a significant number of such changes in mRNA levels that are directly related to sun damage. It is possible and indeed very likely that at least some of the mRNA differences between preauricular and postauricular skin that we have identified in a single donor will reflect interindividual changes in mRNA levels that are unrelated to the development of solar elastosis in sun-damaged skin.

Of the 34 different mRNAs that are represented by at least a 4-fold difference in abundance between preauricular and postauricular skin, seven of these mRNAs encode ribosomal proteins (either cytoplasmic or mitochondrial) and two mRNAs encode translation initiation factor proteins. We have assumed in this study that these changes in ribosomal and initiation factor protein mRNA reflect overall changes in protein synthesis associated with chronic sun exposure. Tags derived from mRNAs encoding ribosomal proteins are commonly present in most SAGE studies and most authors attach no particular functional significance to the appearance of these tags.

Four tags in **Tables IV** and **V** were shown to have multiple matches. Three of these tags correspond to stretches of poly(A). As SAGE tags are constructed from the 3′-end of mRNAs, these poly(A) sequences almost certainly represent either stretches of poly(A) within the 3′-untranslated region of one or more mRNAs, or poly(A) tails added post-transcriptionally to the 3′-end of the 3′-untranslated region of mRNAs. In either example, the tag match to these common sequences in most mRNA precludes a more definite identification of the mRNA from which these tags were derived. The two tags in **Table IV** that were not identified in UniGene as a reliable match represent either an mRNA not previously identified as an expressed sequence tag (ACGTTAAAGA) or an mRNA that could not be assigned to any UniGene cluster (CAGGACCTG-GT).

Of the remaining tags encoding 18 different mRNAs, it is striking that the proteins these mRNAs encode represent a functionally diverse group of proteins, largely confined to the epidermis. Taken together, these changes suggest a defense mechanism of skin and specifically of the epidermis against chronic exposure to UV irradiation that includes a sustained inflammatory reaction, as indicated by the elevated levels of MIF. Furthermore IGFBP-6, CLSP, and EFABP, all of which have been implicated in keratinocyte differentiation, showed increased mRNA steady-state levels, and additionally the increased level of apoptosis inhibition by the Bcl-2 antagonist implies an altered keratinocyte proliferation–differentiation cycle in sun-damaged skin. Moreover, as Ca^{2+} levels are known to be an important factor for this cycle switch in keratinocytes, it is not surprising that the mRNA levels encoding several Ca^{2+} binding proteins are also altered in sun-damaged skin.

The preauricular and postauricular SAGE libraries that we have described in this paper were constructed from skin samples obtained from a 55-y-old female donor at the time she was undergoing elective facial plasty. This preauricular skin sample therefore represented skin subject to many decades of repeated sun exposure. Few studies have addressed the biosynthetic consequences of chronic and repeated sun exposure. Voorhees and colleagues, for example, have proposed an attractive hypothesis of UV-induced, MAP-kinase-mediated activation of matrix metalloproteinases as the underlying mechanism for the aberrant remodeling of collagens and other components of dermal connective tissue during repeated sun exposure. These authors have suggested that both epidermal keratinocytes and dermal fibroblasts may play a role in releasing a variety of growth factors and cytokine receptors in response to UV exposure that could initiate a MAP-kinase-mediated release of metalloproteinases. Most of the studies in support of this hypothesis, however, have been conducted using UV-irradiated sun-protected skin. The relationships therefore between our SAGE analysis on chronically exposed skin and these studies on short-term UV exposure of sun-protected skin is unclear. It is tempting to speculate, however, that the altered biosynthesis of one or more of the 18 proteins identified in our SAGE analysis might be part of the initiating events that lead eventually to matrix metalloproteinase remodeling of dermal connective tissue, including elastic fibers.

In summary, the SAGE analysis reported in this paper is the first attempt to obtain a comprehensive profile of biosynthetic changes in full-thickness human skin associated with chronic sun exposure. We have identified 18 different mRNAs from a total of 6500 unique transcripts analyzed that have significantly altered steady-state levels associated with chronic sun exposure. Although the functional significance of these mRNA level differences has yet to be clarified, most of these changes are, in all likelihood, specific to keratinocytes. Moreover, the variety of different mRNAs altered in steady-state levels in sun-exposed skin suggests multiple biosynthetic changes in chronic sun exposure, and we speculate that several different pathomechanisms are responsible for the dermal connective tissue changes in sun-exposed skin and that keratinocytes play a major role in initiating these pleiotropic events.

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