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Patterns of Persistent DNA Damage Associated with Sun Exposure and the Glutathione *S*-transferase M1 Genotype in Melanoma Patients

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Abstract

Solar radiation can lead to changes affecting DNA metabolism resulting in loss of DNA integrity. Skin specimens obtained from melanoma patients treated at the Memorial Sloan-Kettering Cancer Center were used to study patterns of DNA fragmentation using the comet assay and levels of deletions in mitochondrial DNA (mtDNA) using real-time PCR. Skin specimens were classified according to the glutathione *S*-transferase M1 (GSTM1) genotype (either wild type [WT] or null) and patient sunburn history. GSTM1 null individuals with a sunburn history showed increased levels of both DNA fragmentation by comet assays and mtDNA deletions relative to GSTM1 WT patients with little or no sunburn history. Microarray analyses identified a number of genes whose expression was upregulated ≥5-fold in cells from GSTM1-null patients or from those reporting histories of sunburn. These genes encoded small molecule transporters, various growth factor/chemokine receptors, transcription factors and tumor suppressors. Of 17 genes directly involved in DNA repair, three DNA ligases were highly upregulated while the RAD23 UV excision repair gene and the Growth Arrest and DNA Damage gene (GADD45) were downregulated. These findings support the idea that exposure to solar radiation early in life may induce long-term cellular changes that lead to persistent DNA damage and altered patterns of gene expression.

Introduction

The causative association between sun exposure and skin cancer is well established. In *in vitro* models, irradiation of epidermal cells with sunlight, or its UV components, has been shown to lead to oncogenic mutations that result from DNA strand breaks, pyrimidine dimer formation and other types of DNA lesions (1). Because the neoplastic changes induced in skin by solar radiation are generally not seen for long periods of time after the initial DNA damage, oncogenic processes induced by sun exposure are evidently active in the intervening period that lead up to tumor formation. Despite much research, there is still little consensus about the nature of these oncogenic processes. Estimates of the levels of sun exposure that increase cancer risk are surprisingly low; some sources estimate that even a single instance of severe sunburn in childhood is associated with an increased risk of developing skin cancer in adulthood,

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particularly melanoma (2). If so, this suggests that irreversible secondary changes in cellular function that result from exposure to solar radiation in childhood may act synergistically with oncogenic processes that ultimately lead to skin cancers in adulthood. In fact, risk of melanoma increases significantly with exposure to ambient UV mostly when the exposure occurs in the first decade of life (3–5).

Inactivating mutations in genes encoding the group of enzymes that comprise the glutathione *S*-transferase (GSTs) detoxifying system have been associated with the development of various cancers including skin cancer (6,7). In particular, the glutathione *S*-transferase M1 (GSTM1) null genotype (homozygous null) has been associated with lung cancer in smokers (8–10) and with bladder, colon, stomach and breast cancers (11–14). GSTM1 mutations have been related to chromosomal abnormalities (9,10,15,16) and a lowered DNA repair capacity in individuals exposed to adducting carcinogens (8,17) and radiation (15). The GSTs are also responsible for detoxification of superoxides, peroxides and hydroxyl radicals; reactive oxygen species (ROS) and their secondary products that are produced by a variety of oxidizing stimuli including UV radiation (6). For this reason loss of GST activity potentiates the DNA-damaging effects of UV radiation.

Here, we tested the hypothesis that high levels of solar radiation could induce changes in DNA metabolism that might result in loss of DNA integrity over long periods of time subsequent to sun exposure. For this purpose, we examined levels of DNA fragmentation in chromosomal DNA and deletions in mitochondrial DNA (mtDNA) in normal human skin tissue from melanoma patients.

Materials and Methods

Skin specimens

Skin tissues (100–325 mg) were obtained from melanoma patients who underwent wide skin excisions as part of their standard care at the Memorial Sloan-Kettering Cancer Center (MSKCC). Study participants signed an informed consent form before donating their tissue and the study was approved by the Institutional Review Board. Information on age, sunburn history and phenotypic characteristics was extracted from self-administered epidemiological questionnaires.

The phenotypic index was calculated by combining indices for hair color, eye color and tannability/propensity to burn characteristics as indicators of cutaneous phenotype as previously described (18). The phenotypic indices were further grouped into three sunburn risk categories (low, intermediate and high). A total of 67 specimens of skin tissue were collected of which 22 were from patients rated at low risk, 23 at intermediate risk and 22 from high-risk patients.

Germline DNA

DNA from buccal cells was used to test germline polymorphisms in the GSTM1 gene. Buccal cells were collected with mouthwashes and DNA from buccal cells was extracted using Puregene® kits (Gentra Systems, Inc., Minneapolis, MN) according to the manufacturer's recommendations. DNA concentration was measured by spectrophotometry at 260 nm in a Spectramax Plus 384 (Molecular Devices, Sunnyvale, CA). The DNA quality was determined by the ratio A_{260}/A_{280}.

Analysis of germline GSTM1 variants

Multiplex PCR is used to identify GSTM1 genotypes (7,19,20). The null polymorphism in GSTM1 was detected by multiplex PCR, which consisted of the simultaneous amplification

of the GSTM1 fragment and a housekeeping gene fragment (internal control). The PCR products were visualized on agarose gels. The absence of the GSTM1-specific band (273 bp) in the presence of microsomal epoxide hydrolase (EPHX1) used as the internal control band indicated that the sample was indeed homozygous null. The internal control fragments amplified were 322 bp. The PCR reaction included 10–20 ng of DNA which was amplified in a reaction mix containing 200 μM dNTPs, 0.3 μM GSTM1-specific primers (forward 5′-CTGCCCTACTTGATTGATGGG; reverse 5′-CTGGATTGTAGCAGATCATGC); 0.4 μM forward and reverse control EPHX1 primers (forward 5′-TCCCTCTCAACTTGGGGTC; reverse 5′-TTGGGTTCTGAATCTCTCCAA), 2.0 mM $MgCl_2$, 1 M betaine, 0.06 U μL^{-1} Taq polymerase and 1× PCR buffer (Applied Biosystems, Foster City, CA). For PCR an initial denaturation at 95°C for 2 min was carried out. This was followed by a touchdown PCR protocol with denaturation segments of 95°C for 20 s and extension at 72°C for 40 s. The annealing segments were 65°C for two cycles, 63°C for two cycles, 61°C for two cycles and 60°C for 29 cycles (30 s each). A final extension was carried out at 72°C for 10 min.

Real-time PCR for quantification of mtDNA deletions

QIAamp spin columns (Qiagen, Valencia, CA) were used according to the manufacturer's specifications to extract and purify DNA from about 25 mg of skin adjacent to the excised melanomas. DNA was eluted from the columns and suspended at a final concentration of 100 ng μL^{-1} in 10 mM Tris, 0.1 mM EDTA pH 7.5. The Roche LightCycler for Real Time PCR was employed to measure total mitochondria and the common deletion (CD). Each PCR capillary contained 1× LightCycler® FastStart DNA Master Mix and 300 nM each of the forward and reverse primers, 200 nM of the FAM-labeled probe and 0.1 U heat-labile uracil-DNA glycosylase (Roche Applied Science) in a total volume of 10 μL.

We quantified two types of mtDNA deletions: (1) the CD comprising 4977 bp is located between bp 8470 and bp 13 447 of the revised Cambridge reference sequence (GenBank AC_000021.2) originally published by Anderson *et al.* (21) and (2) the recently characterized 5128 bp deletion called Δ_{uv} (22). The primers and probes for the CD are described by Pogozelski *et al.* (23). The forward primer for the CD spans the mtDNA sequence from bp 8416 to bp 8437 and the reverse primer spans the sequence from bp 13 519 to bp 13 498. The probe for the ensuing amplicon is located at bp 13 461–13 480 of the Cambridge sequence and is tagged with the reporter FAM (6-carboxyfluorescein, λ_{max} 518 nm) at the 5′-end and the quencher BHQ1 at the 3′-end. Similarly, the primers for total mtDNA are located at bp 1307–1328 for the forward primer and bp 1433–1414 for the reverse primer. The total amplicon spans a region of the mtDNA that is rarely deleted (23). The total mitochondria probe is located at bp 1340 to 1359 of the Cambridge sequence. Pogozelski *et al.* (23) also designed two plasmids, one that contains the amplicon for the CD and the other that contains the amplicon for total mitochondria. In each run, as appropriate, the plasmids were run as external controls. The mtDNA copy number per cell (either deleted or intact) was calculated assuming 16 pg of DNA per cell. For the Δ_{uv} deletion, the primers used were MT1A and MT2 described by Soong and Arnheim (24) and a 20 bp probe (5′ TTTGAAATATCCACAACCTT 3′) derived from the sequences flanking the deletion cut site. Threshold cycle (Ct) was used to quantify relative levels of deletions in the various samples. These values were then normalized to the total number of mitochondria in each sample to give the final CD and Δ_{uv} values.

Explant cultures of cells from skin tissues

Keratinocyte monolayers were grown out from tissue explants and used for the comet assays and microarray analyses. For this purpose, fat was trimmed, tissue washed with 70% ethanol and rinsed twice with serum-free DMEM medium with 0.5 μg hydrocortisone and 5× penicillin/streptomycin. Tissue was minced under sterile conditions into 1–2 mm pieces, transferred to a T75 flask and allowed to dry. After an hour, complete medium was added (DMEM with fetal

calf serum, hydrocortisone, 1× penicillin/streptomycin, cholera toxin and EGF). Minced tissue and growing cells were monitored by microscopic examination daily and fibroblasts removed as needed. After 4–6 weeks, keratinocytes were dissociated with 0.25% trypsin and 1.0% collagenase and then stored with complete DMEM media with 10% DMSO in liquid nitrogen for the comet assay.

Alkaline single-cell gel electrophoresis (comet assay)

The comet assay was performed under alkaline conditions as described by Singh *et al.* (25) with minor modifications. Briefly, superfrosted and commercially precleaned microscope slides (Fisher Scientific, Pittsburgh, PA) were immersed in hot 1% normal-melting-point agarose dissolved in PBS (pH 7.4) and allowed to dry at 37°C on a flat surface for 60 min. Cells were quickly thawed and washed with PBS. Five to ten microliters of cell suspension containing approximately 15 000 cells were mixed with 80 μL of 0.5% low-melting-point agarose in PBS, kept at 37°C in a dry-bath incubator, spread on an agarose precoated slide and covered with a coverslip. The agarose was solidified at 4°C for 10 min. The coverslip was taken off and a third layer of 0.5% low-melting-point agarose was added and covered by a new coverslip. After setting the agarose at 4°C for 10 min and taking the coverslip off, the slides were incubated in complete media for 45 min at 37°C and then lysed for 1 h at 4°C in a freshly prepared lysis buffer (pH 10) containing 2.5 M NaCl, 100 mM EDTA, 100 mM Trizma base, 10% dimethyl sulfoxide and 1% Triton X-100. After lysis, the slides were rinsed three times in 0.4 M Tris-HCl (pH 7.5) for 5 min to remove detergents and salts. The slides were immediately placed side by side on a horizontal electrophoretic unit without power for 30 min in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH > 13) at 4°C. Electrophoresis was carried out in a TECA2222 electrophoresis unit (Ellard Instrumentation Ltd., Monrow, WA) for 30 min at 25 V, adjusting the current to 295–300 mA and with a constant recirculating flow of 100 mL min^{-1}. All these steps were carried out in the dark. Finally, the slides were rinsed in neutralization buffer (0.4 M Tris-HCl, pH 7.5) for 5 min ×3 times, fixed in cold 100% ethanol for 15 min and subsequently air dried. The slides were stored overnight in the dark at room temperature.

Scoring of DNA damage

Immediately before imaging analysis, slides were stained with 60 μL of a 1 μg mL^{-1} ethidium bromide solution for 10 min and covered with coverslips. Evaluations were made at 400× magnification using a fluorescent microscope (Axioskop, Zeiss, equipped with a 515–560 nm excitation filter, a 590 nm barrier filter and a Anchrophan 20/0.45 Ph2 filter) connected to a CoHu 4912 CCD camera. One hundred consecutive cells (50 from each duplicate slide) were randomly selected with care to avoid borders, and quantified with Optomax Comet Assay III image analysis software (Hollis, NH). The extent of the damage was measured quantitatively by the tail moment (TM), defined as the product of the percentage of DNA in the comet tail and the tail length (26), and the tail intensity (TI), defined as the percentage of DNA in the tail. Results were expressed as the mean and standard deviations of TMs and TIs of 200 cells from four slides from each of two separate cultures and two independent assays.

Statistical methods

Data from multiple samples were collected and grouped according to the criteria described in the figures and the standard error of the mean was computed for each group. Statistical significance for paired data points was carried out using Student's *t*-test.

Microarray analysis

To study gene expression we used a total human 1.2 cDNA microarray (BD Biosciences, Clontech, Inc.; no. 7850-1, 1176 genes). RNA was isolated from cells using a standard

guanidine-phenol extraction protocol with reagents supplied as a kit (RNagents; Promega Biotec, Madison, WI). ^{32}P-labeled cDNA probes were created by reverse transcription from the RNA templates using primers, reagents and protocols provided with the cDNA arrays. Hybridizations of the arrays were performed at 68°C at high stringency using protocols and reagents supplied by the manufacturer. For gene expression analysis autoradiographic signals stored on phosphor storage screens were digitized using a Storm 840 phosphoimager (Molecular Dynamics) and analyzed in either TIFF or GEL formats. Relative levels of gene expression from globally normalized hybridization signal intensities for individual gene sequences were then determined using AtlasImage 2.0 software (BD Biosciences-Clontech, Inc.).

Results

DNA damage *vs* GSTM1 status

The comet assay was used to examine the extent of endogenous DNA breaks in epithelial cell outgrowths from the patient skin specimens taken from wide excisions of melanomas and grown in explant culture. As GSTM1 modulates the extent of damaged DNA that needs to be repaired, we compared TM and TI values in skin specimens from patients grouped according to either the GSTM1 WT or null genotypes (Fig. 1). DNA isolated from individuals with the null genotype exhibited a much higher degree of fragmentation than the WT. Specifically, there was at least a two-fold increase in the DNA damage for the null group compared with WT when the TM measures were compared. Similarly, the average TIs indicated that the percentage of DNA in the tail was considerably higher (~50%) in the GSTM null cells than in the WT.

In parallel experiments, levels of mtDNA deletions in the GSTM1 null and WT groups were quantitated by real-time PCR as separate indicators of DNA damage. We used primers for two types of mtDNA deletions: the well-characterized 4977 bp deletion known as the CD and a larger, recently discovered deletion of 5128 bp induced by UVB radiation of human keratinocytes *in vitro* (22). The results of these experiments were similar to those of the comet assays in that both types of mtDNA deletions were more prevalent in the GSTM1 null group compared with WT but the differences (more than six-fold) were much more pronounced for the CD (Fig. 2). The CD is known to accumulate in tissues with age (27–29). Although the patient population varied considerably in age ranging from 16 to 85 years, the average and median ages of patients in the GSTM1 null and WT groups was essentially the same (Fig. 3; mean ages of 58.1 *vs* 56.0 years and median ages of 59.0 *vs* 55.0 years for null and WT, respectively) indicating that the differences seen were related to the GSTM1 genotype and not to differences in age.

History of sun exposure

As we hypothesized that previous exposure to solar radiation might initiate processes that lead to persistent, long-term DNA damage, we used questionnaire responses regarding history of sunburn to categorize patients into low propensity to burn (0–1 severe sunburns) and high exposure to sun exposure (2–7 severe sunburns) histories. As expected, DNA fragmentation measured in the comet assays was found to be greater in the high sensitivity group according to both TM and TI (Fig. 4). There were also substantial differences in the levels of mtDNA deletions (Fig. 5). The CD was found to be more than 10 times as prevalent in the high exposure group as the low exposure group while the levels of the Δ_{uv} deletion were almost 3.5-fold higher in the high exposure group in comparison with the low exposure group. The average age of patients in the two groups (55.3 *vs* 53.9 years for the low and high sunburn groups respectively) was virtually the same (Fig. 6).

A relationship between sun exposures and mtDNA deletions was also borne out when the patient population was separated into subgroups according to the body site location of the melanoma (data not shown). Both types of mtDNA deletion were more prevalent in skin specimens taken from the arm or shoulder area than in skin from either the leg/hip area or trunk. In fact the levels of the CD were more than 46 times higher in skin specimens taken from the arm/shoulder than in skin from the trunk. When compared with the leg/hip regions, the frequency of the CD was about 5.2-fold greater in skin from the arm or shoulder. The Δ_{uv} deletion also showed similar differences in frequency according to body location, although they were not as striking as for the CD. The Δ_{uv} deletions in skin specimens from the arm/shoulder were about twice as prevalent compared with deletions in skin taken from the trunk, and when compared with skin taken from leg/hip areas the ratio was about 2.4.

Microarray studies

RNAs from keratinocytes growing out from skin explant cultures were used to create cDNA probes for microarray analyses of gene expression. To compare patterns of gene expression related to the GSTM1 genotype, relative levels of gene expression in arrays created from patients of each genotype were determined and the expression ratios (null/WT) were calculated. For gene expression related to sun exposure, the ratio of relative levels of gene expression from the high and low sun exposure groups was compared (high/low). Table 1 shows comparison of genes whose expression levels differed by >5-fold according to either GSTM1 genotype or sun exposure history. A number of genes representing a variety of functional classes exhibited wide differences in expression according to GSTM1 genotype or sun exposure. There was a striking concordance in that genes showing the largest differences in expression, when compared according to GSTM1, also showed the largest differences in expression when arrays were grouped according to sun exposure. Of the genes whose expression was modulated five-fold or more, almost all showed higher comparative levels of expression in the GSTM1 null or high sunburn group; only two genes (glucagons and the transcription factor ASH1) showed lower expression in both the GSTM1 null and high sunburn groups.

Of the genes screened, 25 represented genes whose products are directly involved in various aspects of DNA repair. As the comet assays and mtDNA deletion experiments indicated that processes involved in DNA repair might be altered in GSTM1-null individuals or those with histories of exposure to solar radiation, we separately examined the levels of expression of DNA repair genes (Table 2). When microarrays were grouped according to GSTM1 genotype, sunburn exposure was evenly represented within the GSTM1 null and WT subgroups (for the null and WT groups the average numbers of severe sunburns were 1.9 and 1.7, respectively). The pattern of expression of DNA repair genes for the GSTM1 null to WT ratios paralleled that for the ratios of high to low sunburn. The greatest differences in gene expression were seen in the DNA ligases which showed average expression ratios of about seven- and 183-fold. The large majority of the DNA repair genes showed either greater expression in GSTM1 null (*vs* WT) or in high sun exposure (*vs* low sun exposure) or were not significantly different in expression. Perhaps significantly, however, expression of the UV excision repair protein, RAD23A, showed about 70% reduced expression in the GSTM1-null patients and almost 50% reduced expression in the high sunburn group.

Discussion

The studies described here were designed to test the hypothesis that patterns of DNA damage related to solar radiation may persist in sun-damaged skin over long periods of time. The results of the comet assays and real-time PCR quantitation of mtDNA deletions showed a correlation between sun exposure and increased levels of DNA damage. For mtDNA deletion analyses we used primers specific for two separate mtDNA deletions; the CD and a recently described

deletion called Δ_{uv} on the basis of the observation that its formation was found to be induced by UV radiation in a line of keratinocytes (22). It was anticipated that Δ_{uv} would be a better indicator of UV DNA damage than CD. Although relative levels of Δ_{uv} were greater in subjects who experienced more sunburn, the differences were not as great as those seen for the CD. When the occurrence of deletions was compared by body site the CD also showed much greater differences between sun-exposed *vs* unexposed body site than Δ_{uv} (Fig. 5). Eshaghian *et al*. (30) have previously reported that mtDNA deletions in skin accumulate with age. We also observed an age-related increase in mtDNA deletions, although the difference was more striking for the CD than for Δ_{uv}. For example, for patients aged 51–85 years of age *vs* those diagnosed between 16 and 50 years of age, the CD was approximately six times more prevalent in the older population while for Δ_{uv} the age-related increase was only about 71% (data not shown). GSTM1-null subjects also exhibited a higher degree of DNA damage than GSTM1 WT subjects but, again, the differences in levels of the CD were much greater than those seen for the Δ_{uv}, although both deletions were more prevalent in the GSTM1 null population. This observation is consistent with the role of GSTs in detoxifying ROS. ROS can be produced by UV radiation and are known to produce deletions in mtDNA as well as cause single stranded DNA breaks as seen in the comet assays (31–33).

Such long-term effects necessarily involve changes in gene expression in that they must be passed on to daughter cells during the process of cell turnover that occurs over periods of years. The microarray analyses were undertaken with a view toward elucidating relative gene expression levels that may identify important target genes. The tumor suppressors p53 and pRb as well as the cell cycle checkpoint regulator, Wee1Hu were relatively upregulated in GSTM1 null and high sun exposure groups and this is consistent with the role of these proteins as negative regulators of the cell cycle in response to DNA damage. The relative differences in expression of many of the other classes of genes, *e.g*. the transporters and receptors, is much less clear. It is likely that many of these play no causal role in solar-induced oncogenic processes but will turn out to be useful as markers of sun damage. Interestingly, of the array genes directly involved in DNA repair, only the UV excision repair genes RAD23A and GADD45 showed reduced expression in GSTM1 null relative to WT or in high *vs* low sunburn patients. In contrast, expression levels of DNA ligases, which might be expected to act to reduce the DNA fragmentation seen in the comet assays, were relatively enhanced. However, GST gene expression levels were also relatively lower in the high *vs* low sunburn patient group (data not shown), particularly GST theta 1 (GSTT1) which was only about 54% (high *vs* low sunburn). Increased DNA fragmentation might be caused by increased ROS levels when GST activity is lowered. Mitochondrial deletions are thought to reflect increased levels of genotoxic effectors such as ROS rather than loss of DNA repair function (34–37).

Taken together, these findings support the idea that exposure to solar radiation can result in persistent DNA damage and that these long-term effects are paralleled by changes in gene expression.

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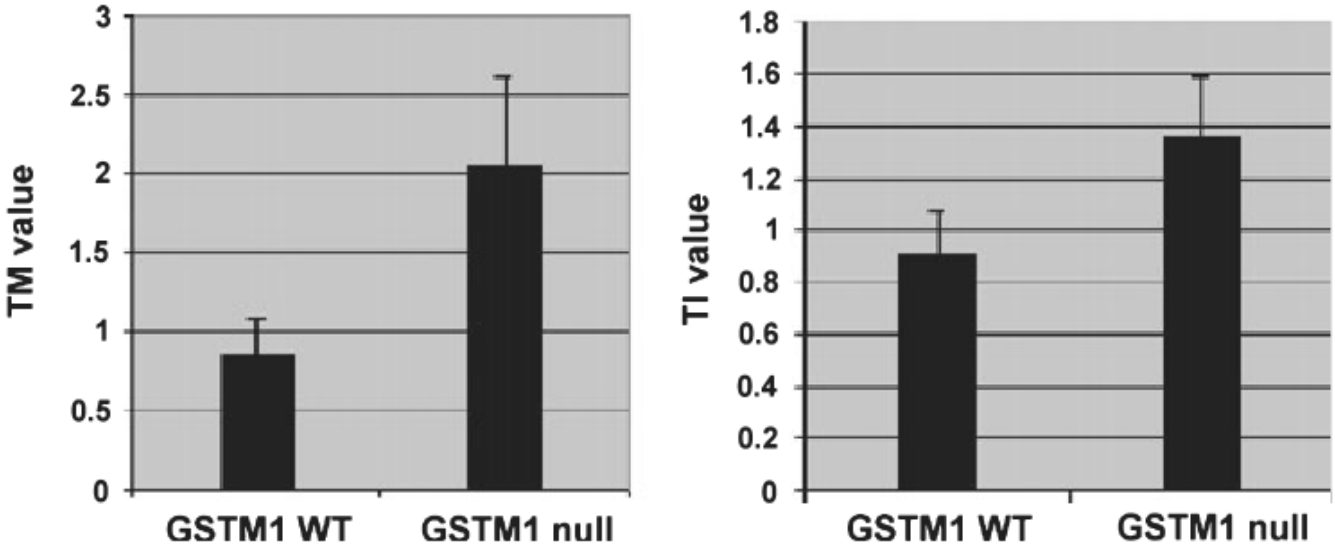

Figure 1.

Endogenous DNA damage in cultured keratinocytes derived from patients of WT and null GSTM1 genotype. Comet assays were performed on keratinocyte outgrowths from skin samples from patients who were genotyped as either GSTM1 wild type (WT; $n = 11$) or GSTM1 null ($n = 12$) as described in Materials and Methods. Average tail moment (TM; left panel) and tail intensity (TI) values (right panel) are shown (±SEM). The TM values are expressed in arbitrary units. The TI reflects % DNA in the tail. Differences in the mean values were found to be significant at $P < 0.05$ using Student's *t*-test.

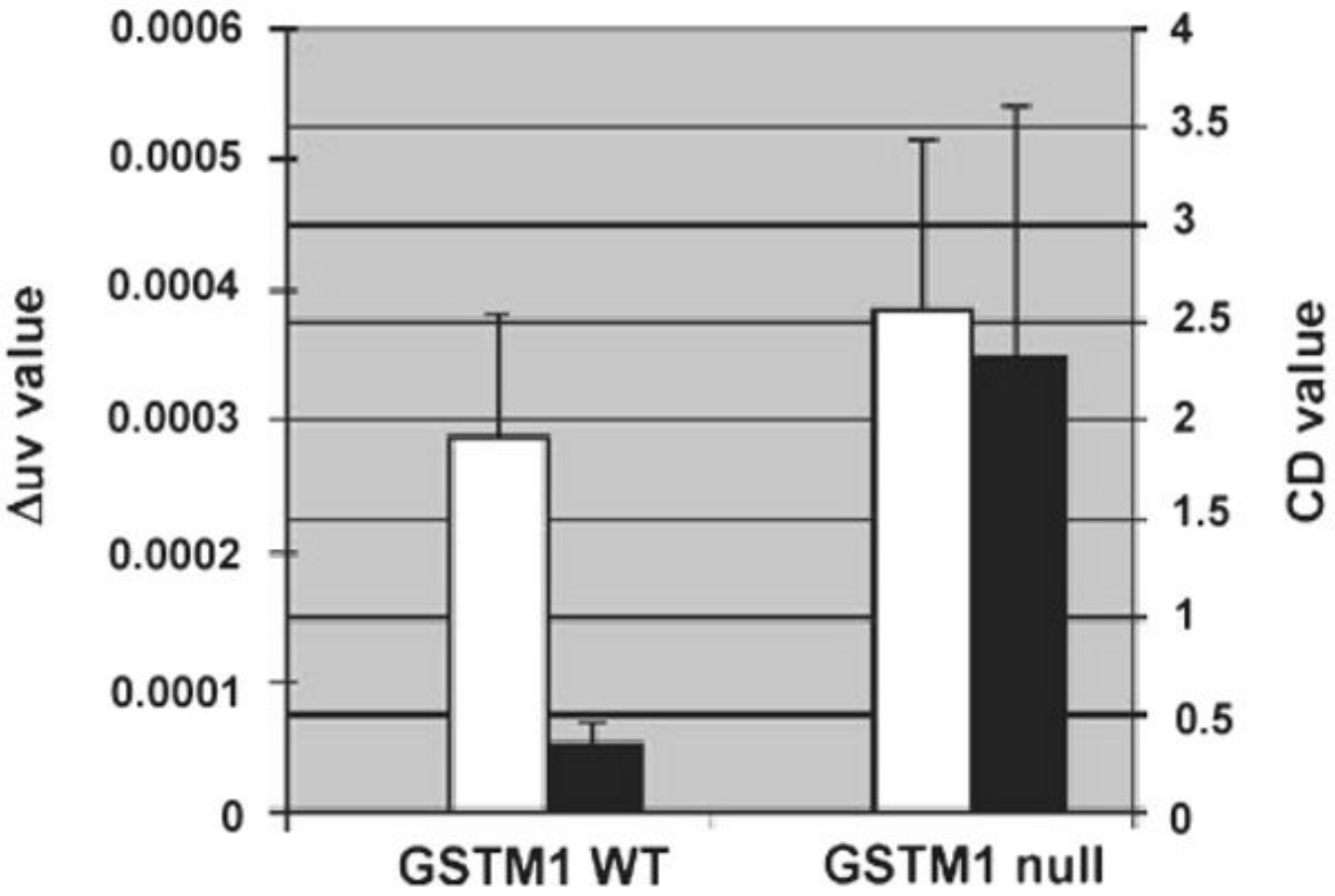

Figure 2.

Mitochondrial DNA deletions in cultured keratinocytes derived from patients of WT and null GSTM1 genotype. Levels of mitochondrial DNA deletions were quantitated by real-time PCR as described in Materials and Methods. The relative levels of the CD (black bars) and Δ_{uv} (white bars) normalized to undeleted genomes in skin samples from patients who were genotyped as either GSTM1 WT (n = 14) or GSTM1 null (n = 17) are indicated (±SEM).

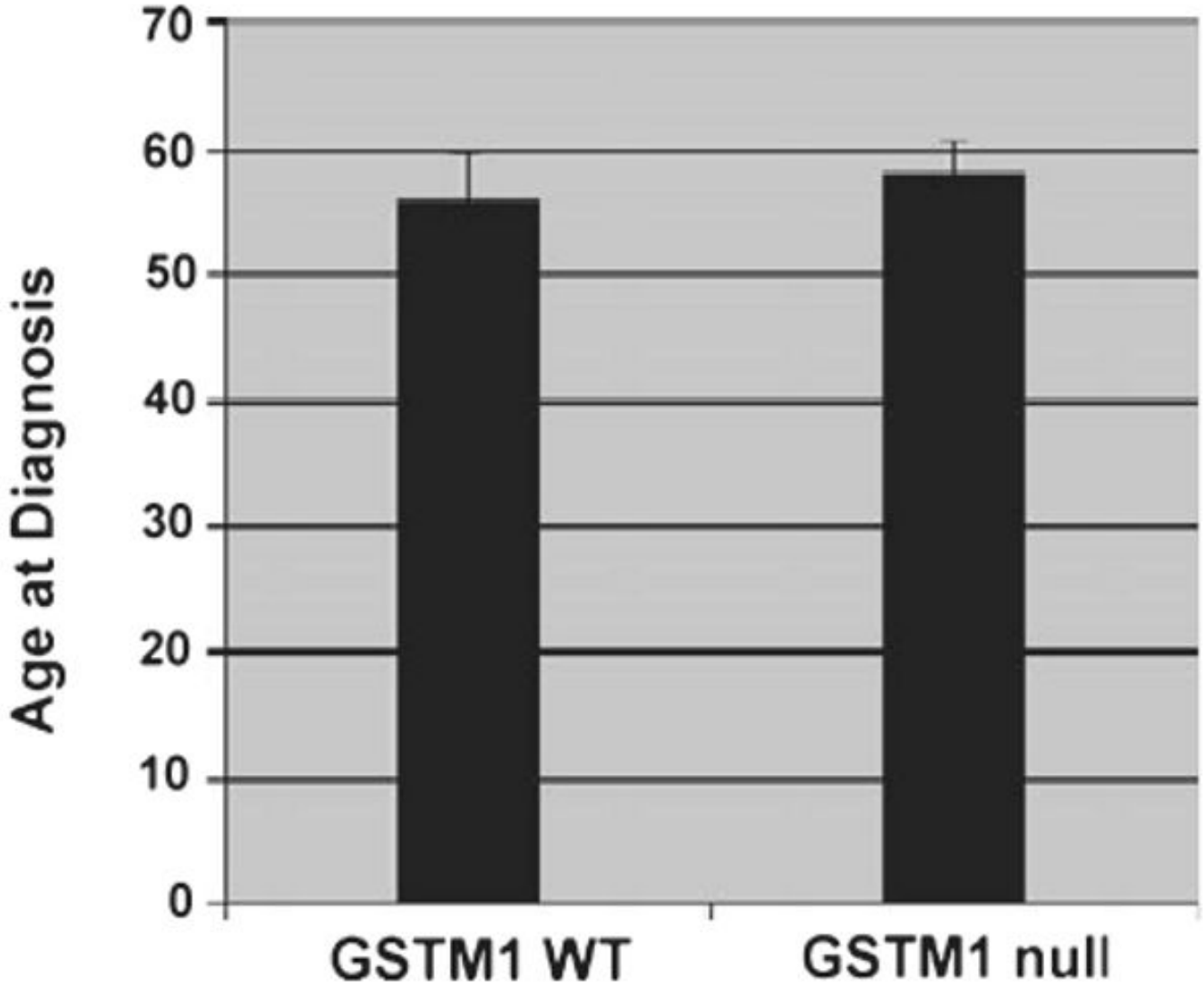

Figure 3.
Average age of patients at diagnosis of melanoma grouped according to WT (*n* = 26) and null (*n* = 34) GSTM1 genotype (±SEM).

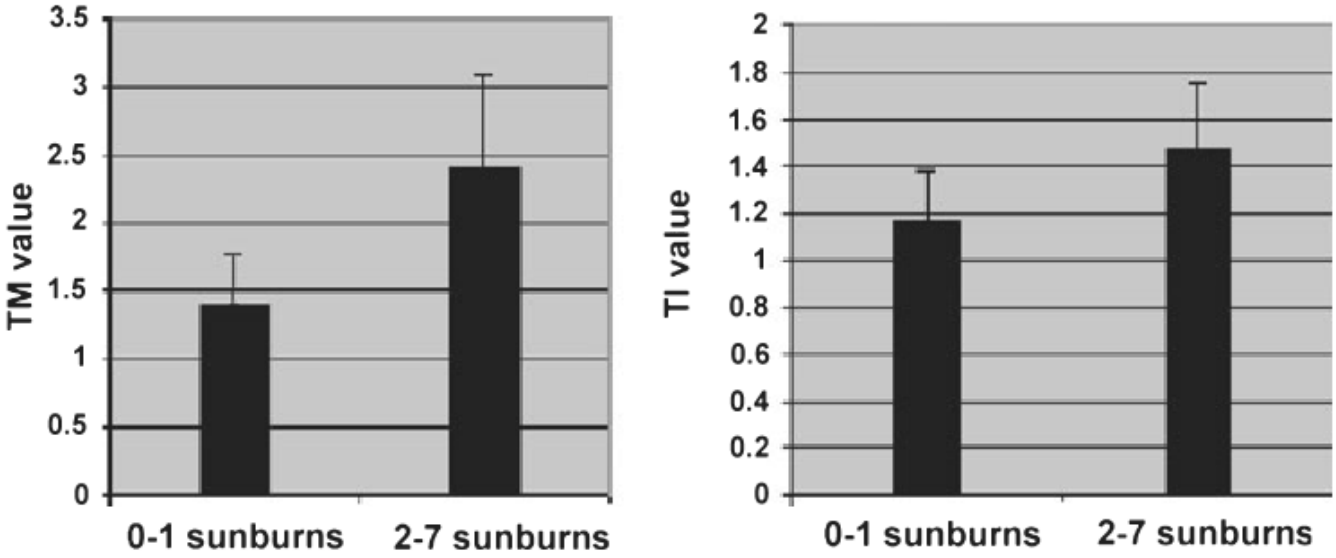

Figure 4.

Comet assays of cultured keratinocytes derived from patients grouped according to high (2–7 severe sunburns; *n* = 11) and low (0–1 severe sunburns; *n* = 7) sunburn history. Average tail moment (TM; left panel) and tail intensity (TI) values (right panel) are shown (±SEM).

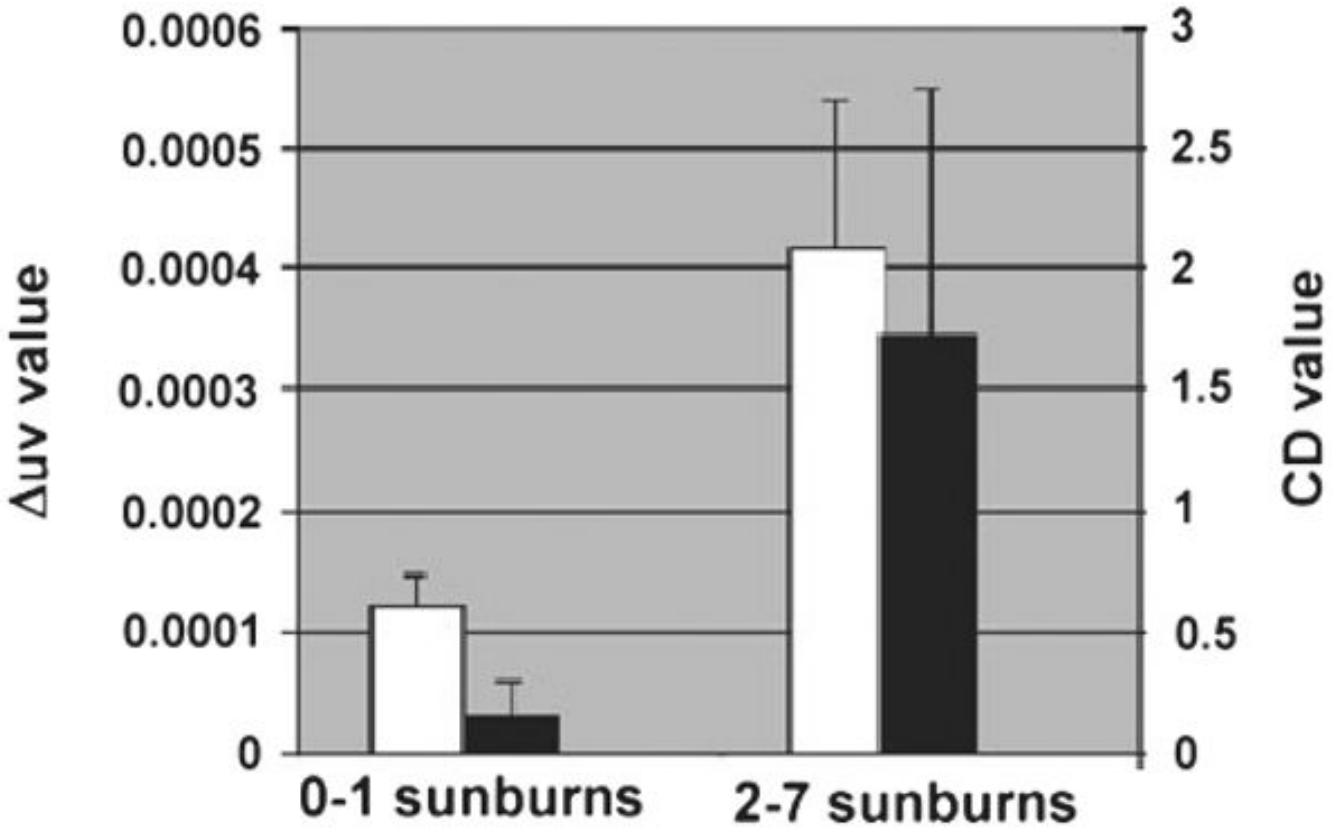

Figure 5.
Mitochondrial DNA deletions in cultured keratinocytes derived from patients grouped according to high and low sunburn history. The relative levels of the CD (black bars) and Δ_{uv} (white bars) normalized to undeleted genomes in skin samples from patients who reported either 0–1 severe sunburns (n = 7) or 2–7 severe sunburns (n = 19) are indicated (±SEM).

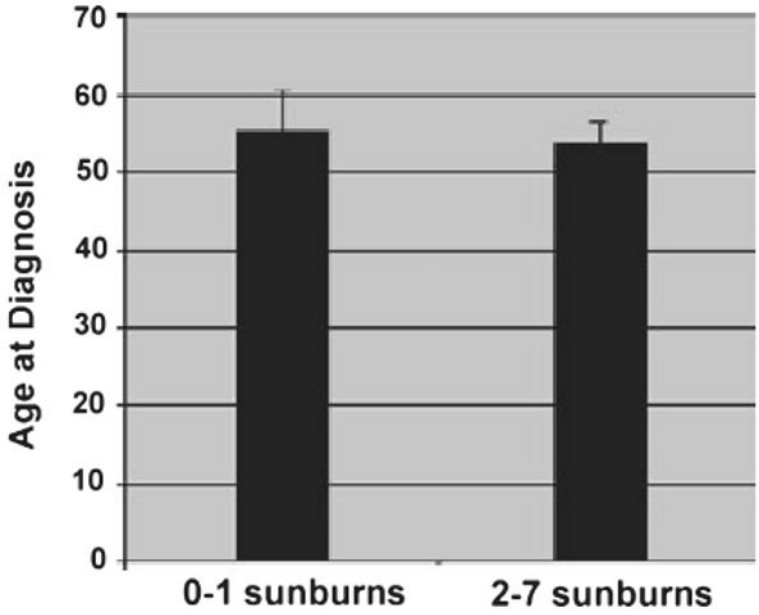

Figure 6.
Average age of patients at diagnosis of melanoma grouped according to high (n = 28) or low (n = 12) history of severe sunburn as in Fig. 5 (±SEM).

Table 1

Relative expression levels of genes in patients grouped according to GSTM1 genotype and sunburn history.

	Ratio null/WT*	Ratio high/low sunburns*	Protein (gene)
Transporters	1360	34.9	Erythrocyte glucose transporter 1 (GLUT1)
	218	183	Sodium- and chloride-dependent GABA transporter 3
	45.4	18.9	Copper-transporting ATPase 2
	28.8	39.0	Vesicular acetylcholine transporter (VAChT)
	5.46	15.5	High-affinity glutamate transporter
	36.0	25.7	Organic cation transporter 1
	23.8	15.1	Cardiac muscle sodium channel alpha subunit; HH1
	10.4	11.2	Zinc transporter 4
Cell cycle control	673	3820	wee1Hu CDK tyrosine 15-kinase; wee-1-like protein kinase
	107	129	Cyclin-dependent kinase inhibitor 1C (CDKN1C); p57-KIP2
	6.58	5.21	G1/S-specific cyclin D3 (CCND3)
Receptors	304	91.0	High-affinity nerve growth factor receptor precursor; trk-1
	111	97.4	Transforming growth factor beta receptor III
	69.3	59.9	CC chemokine receptor type 1 (CC CKR1; CCR 1)
	37.0	24.3	Colon carcinoma kinase 4 precursor (CCK4)
	28.6	12.2	Angiopoietin 1 receptor precursor
	23.7	17.4	Macrophage-stimulating protein receptor precursor
	20.4	27.1	Vascular endothelial growth factor receptor 2 precursor (VEGFR2)
	18.2	11.1	N-sam; fibroblast growth factor receptor 1 precursor (FGFR1)
	17.9	16.6	ERBB4 receptor protein-tyrosine kinase; Her4
	13.1	5.84	Triiodothyronine receptor (THRA1)
	12.3	9.79	ERBB2 receptor protein-tyrosine kinase
	10.9	7.47	Insulin-like growth factor 1 receptor (IGF1R)
	6.03	6.78	Vascular endothelial growth factor receptor 1 (VEGFR1)
	5.10	8.49	Vascular endothelial growth factor receptor 3 precursor (VEGFR3)
Signaling intermediates	162	49.7	cAMP-dependent 3′,5′-cyclic phosphodiesterase 4D (PDE43); DPDE3
	35.2	11.4	Dual-specificity protein phosphatase 2; PAC-1
	24.2	8.56	Ribosomal protein kinase B (RSKB)
	23.1	6.14	Myotubularin
	20.7	11.0	Proto-oncogene tyrosine-protein kinase lck; p56-lck
	20.4	13.7	Tyrosine-protein kinase lyn
	9.53	5.22	Anaplastic lymphoma kinase GN (ALK)
	6.74	5.73	T-lymphoma invasion and metastasis inducing TIAM1

	Ratio null/WT*	Ratio high/low sunburns*	Protein (gene)
	5.53	8.90	Myosin light chain kinase (MLCK)
Transcription factors	140	68.8	Breast cancer type 2 susceptibility protein (BRCA2)
	43.7	46.1	Signal transducer and activator of transcription 1 alpha/beta (STAT1)
	13.3	11.4	Transcription factor DP2 (Humdp2); E2F dimerization partner 2
	9.37	5.36	Signal transducer and activator of transcription 2 (STAT2); p113
	9.19	8.29	Human immunodeficiency virus type I enhancer-binding protein 2 (HIV-EP2)
	8.90	9.11	Homeobox protein hLim1; LHX1
	8.05	6.34	Endothelial transcription factor GATA2
	7.63	11.6	Homeobox protein HOX-A5; HOX-1C
Growth factors	28.0	46.8	Brain-derived neurotrophic factor (BDNF)/NT-3
	9.66	6.11	Macrophage colony-stimulating factor I receptor precursor (CSF-1-R)
	8.89	7.92	Transforming growth factor beta2 precursor (TGF-beta2; TGFB2)
Intermediary metabolism	43.1	21.0	Lactotransferrin precursor; lactoferrin
	29.5	19.4	Pyruvate dehydrogenase kinase kinase precursor
Tumor suppressors	58.5	24.6	p53 cellular tumor antigen
	34.8	21.6	Retinoblastoma-like protein 2 (RBL2; RB2)
	19.6	11.3	Retinoblastoma-associated protein (RB1); PP110; P105-RB
	9.53	8.01	LUCA15 putative tumor suppressor
Oncogenes	19.5	6.46	C-fes proto-oncogene
	15.9	8.65	H-ras proto-oncogene; transforming G protein
	7.31	16.7	C-mos proto-oncogene serine/threonine-protein kinase
Apoptosis	50.1	33.8	DAXX
	14.9	13.3	bcl-2 interacting killer (BIK); NBK apoptotic inducer protein; BP4; BIP1
Hormones	5.61	10.2	Leptin precursor; obesity factor; obese protein
Translation factors	26.0	42.9	NIP1 (NIP1)

*Values are the averaged relative expression levels of genes in microarrays for keratinocyte outgrowths from skin specimens from patients grouped by GSTM1 genotype (for GSTM1 WT the values shown represent the average of six microarrays; for the null genotype values represent the average of seven microarrays) or sunburn history as indicated. Only ratios of five-fold or greater in magnitude are shown.

Table 2

Averaged expression levels of DNA repair genes in patients grouped according to GSTM1 genotype and sunburn history.

Protein (gene)	Ratio null/WT	Ratio high/low sunburns
DNA ligase IV (LIG4)	145	183
DNA ligase I (LIG1)	32.0	35.4
DNA ligase III (LIG3)	7.71	6.78
Uracil-DNA glycosylase (UNG1)	6.46	6.34
Growth arrest and DNA damage-inducible protein 153 (GADD153)	3.55	4.83
recA-like protein HsRad51	3.08	1.5
DNA repair protein XRCC1	1.48	1.02
DNA lyase; AP endonuclease 1	1.20	2.02
DNA mismatch repair protein PMS1	1.20	0.62
DNA mismatch repair protein MLH1; COCA2	0.89	0.97
DNA excision repair protein ERCC2	0.84	2.08
Repair complementing protein p58/HHR23B	0.82	1.22
DNA excision repair protein ERCC5	0.64	0.9
Growth arrest and DNA damage-inducible protein (GADD45)	0.59	0.89
DNA excision repair protein ERCC3	0.58	0.93
HHR23A; UV excision repair protein RAD23A	0.53	0.3
Growth arrest and DNA damage-inducible protein 45 beta (GADD45 beta)	0.17	0.58