In Human Keratinocytes the *Common Deletion* Reflects Donor Variabilities Rather Than Chronologic Aging and can be Induced by Ultraviolet A Irradiation

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Mitochondrial DNA mutations play a major role in human aging processes and degenerative diseases. The most frequently reported marker for mutations of the mitochondrial DNA in human skin is a 4977 bp large-scale deletion, called the *Common Deletion*. Although this deletion is rarely detectable and constitutes only one example of the multitude of about 50,000 known mutations in mitochondrial DNA, it can represent "the tip of the iceberg" of all types of mitochondrial DNA mutations. We established a quantitative real-time polymerase chain reaction assay to detect the *Common Deletion in vitro* as well as *in vivo/ex vivo*. In contrast to previous studies, we were able to demonstrate that the *Common Deletion* is frequently abundant in keratinocytes isolated from various donors. Quantitative analysis of the mutation indicated interperson variations but obviously no relation to the donors' ages. Prolonged proliferation of keratinocytes led to a distinct reduction in the amount of the *Common Deletion*. Single ultraviolet A irradiation (12 J per cm^2 and 15 J per cm^2) neither *in vitro* nor *in vivo* increased the incidence of the mutation in keratinocytes, whereas repetitive irradiation resulted in a clear increase *in vitro*. Again, prolonged cultivation of these irradiated cells caused a significant reduction in the amounts of the deletion. In view of these results, the *Common Deletion* appears to be a useful marker rather for ultraviolet-A-induced alterations than for chronologic aging in human skin keratinocytes. *Key words: mitochondrial DNA/skin/TaqMan-PCR/ultraviolet radiation. J Invest Dermatol 117:892–897, 2001*

The human mitochondrial genome consists of a 16,569 bp circular, double-stranded DNA molecule present in approximately five copies per mitochondrion (Satoh and Kuroiwa, 1991). The mitochondrial DNA (mtDNA) encodes 22 tRNAs, 2 rRNAs, and 13 proteins that are involved in mitochondrial gene expression and oxidative phosphorylation. mtDNA is directly exposed to oxidative stress, due to leakage of reactive oxygen species (ROS) from the electron transport chain. ROS are likely to damage mtDNA because it lacks a protective histone coat and its replication requires long single-stranded DNA regions. Oxidative stress exerts a mutagen potential and is assumed to cause the elevated mutation rate in mtDNA compared to nuclear DNA (Richter, 1995). Oxidized mitochondrial bases and large-scale deletions correlate with oxidative stress (Hayakawa *et al*, 1992; Lezza *et al*, 1999); they accumulate with increasing age in differentiated tissues and are therefore considered to contribute to processes of aging and disease (Wallace, 1992; Wei, 1998).

The most abundant change in mtDNA is the so-called *Common Deletion,* which was originally observed in patients with mitochondrial myopathies (Wallace, 1992). The deleted fragment of 4977 bp comprises five tRNA genes involved in mitochondrial gene expression and seven protein coding genes involved in oxidative phosphorylation. The deletion is considered as the product of an intragenomic recombination event taking place between two 13 bp direct repeats (positions 8470–8482 and 13447–13459) after a single-strand break in mtDNA caused by ultraviolet A (UVA) or ROS (Shoffner *et al*, 1989).

The *Common Deletion* has been thoroughly investigated with respect to its appearance in disease and aging, and its possible use as a biomarker for aging. Several studies on the *Common Deletion* by polymerase chain reaction (PCR) have proven an age-related accumulation in various tissues. In highly differentiated tissues like muscle or nerve, the accumulation is much more distinct than in proliferating tissues like skin or liver (Cortopassi *et al*, 1992; Lee *et al*, 1994; Yang *et al*, 1994). This is presumably caused by energy deficiency of dividing cells carrying numerous copies of deleted mtDNAs (Moraes *et al*, 1989). In proliferating tissues like skin, the absolute amount of the *Common Deletion* remains low, and its detection is complicated by a surplus of nondeleted mtDNA. Therefore, little is known about the *Common Deletion* in cultured normal cells like primary keratinocytes.

Optimized PCR techniques can be useful to measure even low concentrations of deleted mtDNA in mitotic active tissues (von Wurmb *et al*, 1998). In this study, we established a sensitive TaqMan-PCR for quantitative analysis of low copy deleted mtDNA by using a short amplicon and short extension time, in combination with a fluorescence-labeled probe. Using this system, the ratio of mtDNA harbouring the *Common Deletion* to wild-type mtDNA was measured in primary human keratinocytes and in suction blister epidermis.

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Abbreviations: NHEK, normal human epidermal keratinocyte; mtDNA, mitochondrial DNA.

Table I. TaqMan primers and probes for detection of total mtDNA and mtDNA harbouring the *Common Deletion*

Target	Amplicon	Oligonucleotide sequence (5′→3′)
total mtDNA	83 bp	IS_1 forward primer GATTTGGGTACCACCCAAGTATTG IS_2 reverse primer AATATTCATGGTGGCTGGCAGTA IS probe[a] CACCCATCAACAACCGCTATGTATTTCGTACAp
Common Deletion mtDNA	108 bp	CD_1 forward primer ACCCCCATACTCCTTACACTATTCC CD_2 reverse primer AAGGTATTCCTGCTAATGCTAGGCT CD probe[b]ACACAAACTACCACCTACCTCCCTCACCAp

[a]VIC labeled.
[b]FAM labeled.

MATERIALS AND METHODS

Isolation of keratinocytes and preparation of primary cultures Human skin biopsies were obtained from the Universitaetskrankenhaus Eppendorf (Hamburg, Germany) in Dulbecco's modified Eagle's medium (Gibco BRL, Eggenstein, Germany) containing 20 mM HEPES, 20% fetal bovine serum (FBS), and 0.1 mg per ml gentamicin. The underlying fat was trimmed, and the samples were vigorously washed in phosphate-buffered saline (PBS) supplemented with 0.1 mg per ml gentamicin and 2.5 μg per ml amphotericin B. The samples were covered with 2.5 U per ml dispase I overnight at 4°C. The epidermal fraction was separated and subjected to trypsinization in trypsin (1 g per liter)/ethylenediamine tetraacetic acid (EDTA; 400 mg per liter) for 10 min at 37°C, followed by adding keratinocyte growth medium (KGM-2; Clonetics, Remagen, Germany) containing 10% FBS. The resulting epidermal single cell suspension consisting of about 95% normal human epidermal keratinocytes (NHEK) was centrifuged, and the cells were resuspended in KGM-2 without FBS and incubated at 37°C and 7% CO_2 in a humidified atmosphere.

Neonatal NHEK were supplied by CellSystems (St. Katharinen, Germany).

Serial cultivation At a confluency of 50% primary cultures were incubated with trypsin (1 g per liter)/EDTA (400 mg per liter) in PBS for no more than 10 min at 37°C. Trypsinization was stopped with KGM-2 supplemented with 10% FBS, and the detached cells were dispersed into single cells by gentle pipetting. The cells were collected by centrifugation, suspended in KGM-2, and plated in appropriate dilutions.

Cloning procedures Standard plasmids for TaqMan-PCR analysis were generated by cloning standard PCR products of NHEK DNA. Each standard PCR reaction (50 μl) consisted of 50 mM KCl, 10 mM Tris/HCl, 1.5 mM $MgCl_2$, 200 μM each of the four dNTPs, 400 μM of each primer, 1 μg DNA, and 1.25 U Taq DNA polymerase (Perkin Elmer, Norwalk). Reaction mixtures were subjected to the following amplification scheme: 2 min initial denaturation at 97°C, 40 cycles of 1 min denaturation at 97°C, 1 min annealing at 66°C, and 1 min extension at 60°C. Amplification was completed by a final step of 10 min at 72°C. The reactions were carried out in a Perkin Elmer 9600 thermocycler.

The sequence spanning the *Common Deletion* was amplified using the primers 5′-TACTACGGTCAATGCTCTGAAA-3′ and 5′-CTAGGG-TAGAATCCGAGTATGTTG-3′, which were designed to recognize unique sequences found outside of the deletion close to the breakpoints. In deleted mtDNA molecules, these primers come close enough to synthesize the 793 bp PCR product within the given extension time, whereas in nondeleted mtDNA the distance between the primers is too long. The 83 bp fragment serving as internal standard for total mtDNA was amplified with primers IS_1 and IS_2 (**Table I**), each binding to a unique sequence of the mtDNA independently of the presence of the *Common Deletion*. The two amplification products were subjected to cloning into the vector pCR 2.1 (3.9 kb) and transformed into INVaF′ cells using the Original TA Cloning Kit (Invitrogen, Groningen, Germany). Bacteria carrying recombinant plasmids were selected in LB-medium containing 50 μg per ml ampicillin and grown at 37°C to serve for plasmid preparation using the QIAfilter Plasmid Maxi Kit (Qiagen, Hilden, Germany). In order to ensure identity of standards, all inserts were reamplified and subjected to sequencing.

UVA irradiation *in vitro* NHEK were plated on 9 cm Petri dishes at 200,000 cells and incubated overnight. The following day, the medium was replaced with PBS and the cells were irradiated at room temperature without cover using Dermalight 2020 (Dr. Hoenle, Martinsried, Germany) and the UVB cut filter M-UG2 (Schott Glas, Mainz, Germany). Subsequently, the PBS was removed, fresh medium was added, and the cells were incubated at 37°C for 24 h previous to DNA preparation.

Volunteer study and UVA irradiation *in vivo* Volunteers were exposed to 2 J per cm^2 and 15 J per cm^2 UVA on each arm with UVASPOT 400/K (Dr. Hoenle) through the UVB cut filter M-UG2 (Schott Glas). Suction blisters could be taken 24 h and 72 h postirradiation. In addition, a control was raised from a nonexposed site on both arms; therefore each volunteer served as his or her own control. The blisters were taken using a vacuum manifold with 5 mm diameter chambers and a negative pressure of 300–330 mbar (Goldsmith *et al*, 1991). When a blister filled the 5 mm chamber, the vacuum was released. The blister was disinfected, excised with scissors, and washed in PBS directly followed by DNA extraction.

DNA extraction and analysis DNA extraction was performed using the QIAamp DNA Blood Mini Kit (Qiagen) for cultured cells and suction blister epidermis according to the manufacturer's protocols. These ensure the isolation of total (nuclear and mitochondrial) DNA. Total DNA concentration was determined using the GeneQuant RNA/DNA Calculator (Pharmacia Biotech, Cambridge, U.K.).

TaqMan-PCR The quantitative TaqMan-PCR method provides real-time measurement of target input as PCR accumulation through a dual-labeled probe. This probe anneals between forward and reverse primer, and it is cleaved by the 5′–3′ exonuclease activity of Taq polymerase during the PCR extension phase. Therefore, the 5′-terminal reporter dye FAM (6-carboxyfluorescein) or VIC and the 3′-terminal quencher dye TAMRA (6-carboxy-N,N,N′,N′-tetramethylrhodamine) linked to the probe get separated, resulting in a fluorescence emission of the reporter dye. The probe is not able to serve as primer itself because it is 3′-terminally blocked with a phosphate group.

Amplification reactions were performed as 25 μl triplicates in a 96-well microplate format. Total mtDNA and deleted mtDNA reactions were carried out in separate tubes, each containing 100 ng DNA, 1 × TaqMan Universal PCR Master Mix, 300 μM dATP, dCTP, and dGTP, 600 μM dUTP, 300 μM of each IS primer, or 300 μM of each CD primer. The reactions were completed by adding 225 nM of the specific IS probe or CD probe (**Table I**). Primers and probes were designed using the Primer Express 1.0 software (**Fig 1)**. All reagents were supplied by PE Applied Biosystems (Weiterstadt, Germany). The thermal cycling conditions included a preincubation step of 2 min at 50°C for activation of AmpErase UNG to eliminate carry-over contamination, and 10 min at 95°C for its deactivation. This initial step was followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

Quantification of DNA copies Analysis of the reactions was carried out in an ABI PRISM 7700 Sequence Detector equipped with the Sequence Detection software version 1.6 (PE Applied Biosystems, Foster City). Real-time detection continuously monitored an increase in reporter dye (either FAM or VIC) fluorescent emission during each PCR cycle in every PCR tube. The R_n value is the ratio of the emission intensity of the reporter dye to the emission intensity of the passive reference, a dye included in the TaqMan reaction buffer. ΔR_n is defined as the difference between R_n^+ (R_n of a reaction containing all components including template) and R_n^- (R_n of a no template control). The cycle at which a statistically significant increase in ΔR_n is detected first is called the threshold cycle (C_T). Fluorescence signals are regarded as significant if the fluorescent intensity exceeds 10-fold the standard deviation of the background R_n value to define a threshold. Absolute

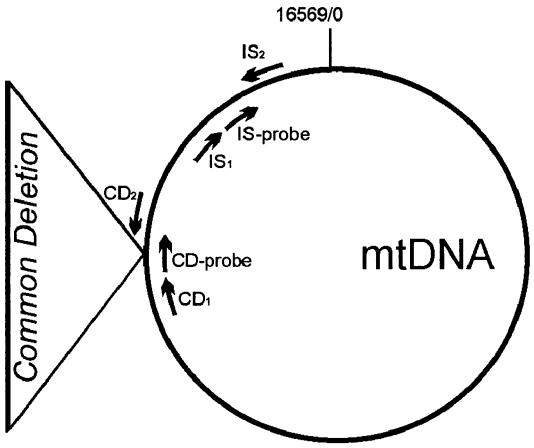

Figure 1. Localization of TaqMan-PCR primers and probes. Schematic representation of the human mtDNA with *Common Deletion* showing the position of oligonucleotide primers and probes. Primers IS_1/IS_2 and IS probe (VIC labeled), annealing to both wild-type and deleted mtDNA, were used to amplify and detect 83 bp total mtDNA fragments as internal standard. Detection of 108 bp DNA fragments originating only from mtDNA harbouring the *Common Deletion* was performed with primers CD_1/CD_2 and CD probe (FAM labeled). The specific CD probe anneals only on deleted mtDNA as it needs to bind exactly to the deletion junction. Furthermore, the deletion brings the CD primers close enough to generate an amplicon under the given PCR conditions.

DNA quantification was performed using the standard curve method. Reactions were carried out with different concentrations of two standard plasmids (see cloning procedures) in parallel to the samples that should be quantified. The standard plasmids, one carrying sequences flanking the *Common Deletion* and one carrying a unique sequence of the mtDNA independently of the *Common Deletion*, allowed the generation of two standard curves showing the number of copies of total mtDNA or mtDNA harbouring the *Common Deletion versus* the measured C_T. The C_T values of samples could then easily be converted to the number of DNA copies by comparing C_T for the sample to C_T for the respective standard plasmid of known concentrations. The amount of mutation corresponds to the ratio of mtDNA with *Common Deletion* to wild-type mtDNA within each sample. If not detected within 40 cycles ($C_T = 40$), DNA is considered absent. $C_T > 36$ and $C_T < 40$ represent measured data, but for absolute quantification only values of $C_T \leqslant 36$ were used as they are much more reliable.

RESULTS

The quantitative TaqMan-PCR detects the 4977 bp *Common Deletion* in mtDNA The 83 bp standard PCR fragment originating from mtDNA and the 793 bp PCR fragment from mtDNA harbouring the *Common Deletion* were cloned and sequenced to confirm the specificity of inserts. The 793 bp fragment contained the characteristic deletion junction created by the *Common Deletion* and therefore carried only one of the two 13 bp repeats that normally flank wild-type mtDNA. Measuring serial dilutions of deleted mtDNA standard plasmids *versus* total mtDNA standard plasmids using TaqMan-PCR, 100 deleted molecules in 10^8 mtDNA copies could be detected with $C_T \leqslant 36$, which is equivalent to a sensitivity of 0.001‰.

The *Common Deletion* shows high interperson variation in keratinocytes isolated from human skin The amount of the 4977 bp *Common Deletion* was analyzed in epidermal keratinocytes derived from areas of chronic sun exposure (head) and nonexposed areas (waist, back, thigh, and paunch) of human skin. The donors' ages ranged from 30 to 78 y. **Table II** shows the incidence of the *Common Deletion* in keratinocytes of 27 independent donors in the first passage. In eight samples the mutation either could only be detected in minimal levels ($C_T > 36$) or could not be detected at all ($C_T = 40$). Nineteen samples showed $C_T \leqslant 36$ revealing proportions of the mutation from 0.001‰ to 0.822‰. Statistical analysis was carried out using Pierson's correlation analysis, which could not show any correlation between the incidence of the *Common Deletion* and either the age of the donors or the site and possible UVA exposure of the sample.

Table II. Amount of the *Common Deletion* in first passage keratinocytes from adult human skin evaluated by TaqMan™-PCR

Sample number	Age [y]	Sex	Site	CD^+ [‰][a]	UV exposure[b]
1	30–40[c]	male	waist	0.822	nonexposed
2	40–50[c]	male	head	0.000[d]	exposed
3	40–50[c]	male	face	0.000[d]	exposed
4	40–50[c]	male	face	0.011	exposed
5	44	male	back	0.009	nonexposed
6	46	female	waist	0.106	nonexposed
7	49	female	thigh	0.000[d]	nonexposed
8	52	female	face	0.000[d]	exposed
9	53	female	ear	0.201	exposed
10	53	female	face	0.000[d]	exposed
11	55	female	forehead	0.013	exposed
12	55	female	paunch	0.004	nonexposed
13	56	female	eyelid	0.000[d]	exposed
14	58	female	face	0.012	exposed
15	58	female	face	0.239	exposed
16	59	female	face	0.200	exposed
17	59	female	face	0.083	exposed
18	60	unknown	unknown	0.734	unknown
19	60–70[c]	female	thigh	0.004	nonexposed
20	61	female	face	0.061	exposed
21	61	female	face	0.002	exposed
22	61	female	face	0.063	exposed
23	68	male	forehead	0.000	exposed
24	73	female	unknown	0.001	unknown
25	73	female	face	0.102	exposed
26	75	female	face	0.000[d]	exposed
27	78	female	thigh	0.002	nonexposed

[a]Results are expressed as copies of deleted DNA out of 1000 total mtDNA molecules.
[b]Possible UVA exposure was estimated with regard to site of sample material.
[c]No precise donor age was available.
[d]$C_T > 36$ were considered background.

Incidence of the *Common Deletion* during cultivation of NHEK As deleted mtDNA molecules are extremely reduced in length without direct alteration of the two replication start points, they were expected to replicate more quickly and therefore accumulate with time. Otherwise the cells must possess an internal control system, which reduces or avoids the replication of deleted DNA in mitochondria. In order to determine the incidence of the *Common Deletion* during cultivation, seven of the keratinocyte cultures, derived from skin biopsies, were cultivated up to passage 4. A portion of the cells of each passage was used to prepare total DNA and to determine the amount of the *Common Deletion*. **Figure 2** shows an immense decrease in the incidence of deleted mtDNA molecules during cultivation in all samples to less than 0.10‰.

Induction of the *Common Deletion in vitro* by UVA irradiation Three independent cultures of different neonatal keratinocytes were each subjected to a single UVA irradiation in passage 2 with 2 J per cm^2, 7 J per cm^2, and 12 J per cm^2 UVA. In addition, the cells were irradiated with the same doses three times at intervals of 10 h. The ratio of the *Common Deletion* to total mtDNA was examined 24 h postirradiation. Single UVA irradiation only resulted in slight changes in the frequency of the *Common Deletion* (**Fig 3*a***). In contrast, triple irradiation led to a clearly dose-dependent increase in all three cell lines (**Fig 3*b***).

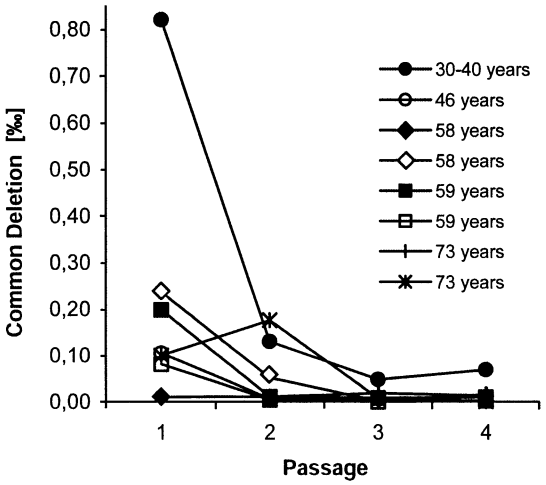

Figure 2. The incidence of the *Common Deletion* in keratinocytes decreases from the first to the fourth passage. Epidermal keratinocytes derived from seven human skin biopsies were cultivated separately and subjected to total DNA preparation. Quantitative TaqMan-PCR using the standard curve method was carried out in order to determine the ratio of deleted mtDNA to total mtDNA. The donors were aged 30–40 y, 46 y, 58 y, 59 y, and 73 y.

Incidence of the *Common Deletion* during cultivation of UVA-irradiated neonatal keratinocytes In order to compare the level of the *Common Deletion* between irradiated cells in passages 2 and 4, three different neonatal keratinocyte cultures were cultivated to passage 4 after triple exposure to 2 J per cm^2, 7 J per cm^2, and 12 J per cm^2 UVA. Keratinocytes with a relatively high amount of the *Common Deletion* in passage 2 showed a significant decrease of the mutation during prolonged proliferation (**Fig 4*a*–*c***). Cells with low abundance of the *Common Deletion* remained at this stage during cultivation. These data are consistent with the findings in nonirradiated keratinocytes isolated from adult human skin after prolonged cultivation.

Detection of the *Common Deletion* in suction blister Suction blister epidermis served as a system for measuring the *Common Deletion in vivo*. Five volunteers, aged from 24 to 41 y, were exposed to 2 J per cm^2 and 15 J per cm^2 UVA radiation on each inner forearm. As the deletion needs a DNA replication step to arise, suction blister epidermis was taken 1 and 3 d after irradiation sessions. The amount of the *Common Deletion* did show interperson variations with levels from 0.02‰ to 1.39‰ irrespective of irradiation dose. In addition, relatively high intraperson variations were obtained, even on control areas without UVA exposure (**Fig 5*a*, *b***).

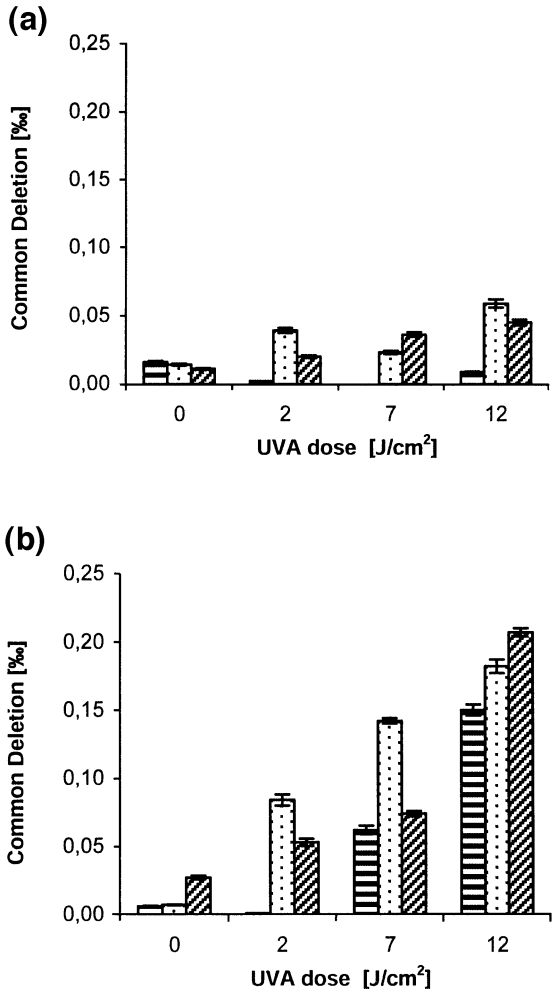

Figure 3. Triple UVA irradiation, in contrast to single irradiation, induces the *Common Deletion* in neonatal human keratinocytes. The ratio of the *Common Deletion* to total mtDNA was determined in second passage neonatal keratinocytes treated with 2 J per cm^2, 7 J per cm^2, and 12 J per cm^2 UVA. The cells were subjected to either (*a*)single irradiation or (*b*)triple irradiation at intervals of 10 h. Total DNA was prepared 24 h after irradiation sessions and analyzed using TaqMan-PCR. Reactions were carried out in triplicate; results are mean values ± SD.

DISCUSSION

The incidence of the mitochondrial 4977 bp *Common Deletion* has been investigated most intensely in slowly dividing tissues like skeletal muscle and brain tissues where levels seem to be highest (Corral-Debrinski *et al*, 1992; Bogliolo *et al*, 1999). In this study, we developed a sensitive TaqMan-PCR system that allowed us to detect low concentrations of the *Common Deletion* in human keratinocytes that show relatively high mitotic activity. The TaqMan-PCR is superior to standard PCR techniques due to its suitability for quantitative analysis. It benefits by very short amplicons and extension times and avoids nested PCR, applied by other groups to measure deleted mtDNA in proliferating cells (Gattermann *et al*, 1995; Berneburg *et al*, 1999). The serial dilution method was used to perform absolute quantification of DNA copy number, and a sensitivity of 0.001‰ could be reached.

We examined 27 human skin samples from various donors aged between 30 and 78 y and observed high interperson variation in the amount of the *Common Deletion* from 0.001‰ to 0.822‰. In addition, human dermal fibroblasts revealed similar results (data not shown). There was no detectable accumulation of the *Common Deletion* in keratinocytes with increasing age *in vitro*. This finding is in contrast to previous studies that determined the frequency of the *Common Deletion* in different tissues (Cortopassi *et al*, 1992; Yang *et al*, 1994). In skeletal muscle, brain tissue, and blood cells the incidence of the *Common Deletion* clearly increases with age of the donors (Corral-Debrinski *et al*, 1992; Gattermann *et al*, 1995; Bogliolo *et al*, 1999). Therefore, the *Common Deletion* seems to be useful as a biomarker for aging in slowly dividing tissues but not in fast dividing cells like keratinocytes.

Prolonged cultivation of keratinocytes derived from seven skin biopsies was carried out to examine the incidence of the *Common Deletion* during proliferation. As the mutation does not directly affect the two replication starting points of mtDNA, deleted mtDNA molecules were expected to show wild-type or even higher replication rates due to their reduced length. Conversely, we observed a significant decrease in the relative amount of the *Common Deletion* of up to 90% in all samples within two passages. Our results suggest a mechanism that either reduces or blocks

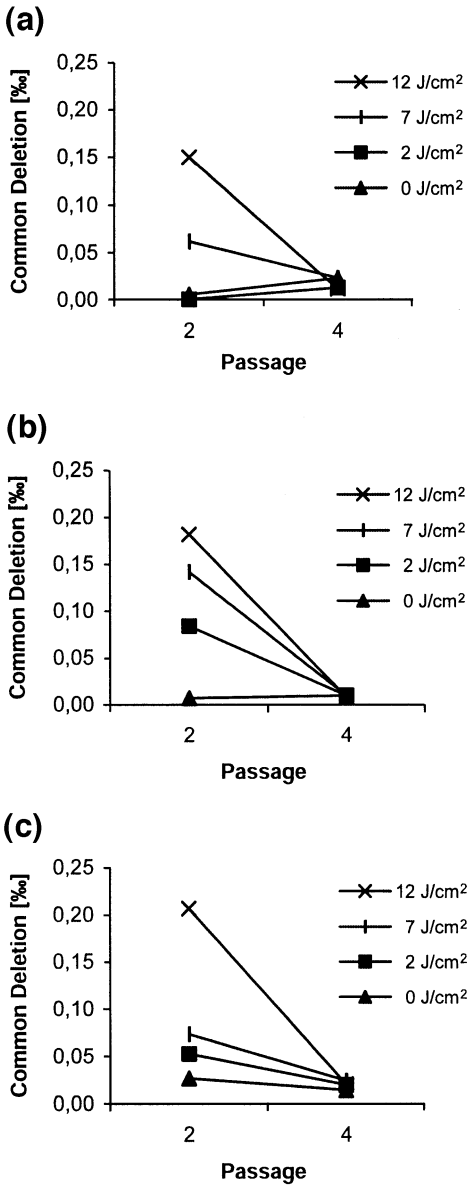

Figure 4. The frequency of the *Common Deletion* in UVA-irradiated neonatal keratinocytes reaches the level of the unirradiated control in passage 4. (*a–c*) Three independent cultures of neonatal keratinocytes were cultivated after triple exposure to 0 J per cm^2, 2 J per cm^2, 7 J per cm^2, and 12 J per cm^2 UVA in passage 2. The amount of the *Common Deletion* was evaluated by TaqMan-PCR after DNA isolation in passages 2 and 4.

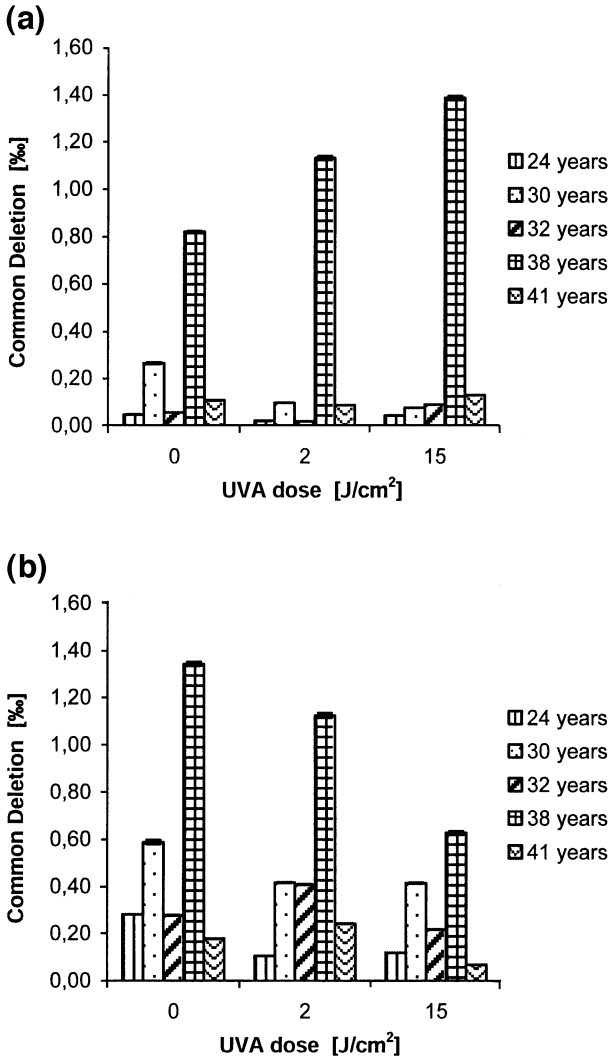

Figure 5. Incidence of the *Common Deletion* in suction blister epidermis of five volunteers. Volunteers were irradiated with 2 J per cm^2 and 15 J per cm^2 UVA on each inner forearm and three suction blisters were taken (*a*) 24 h postirradiation from the right arm and (*b*) 72 h postirradiation from the left arm. The donors' ages were 24 y, 30 y, 32 y, 38 y, and 41 y. Suction blister epidermis directly served for DNA preparation, followed by TaqMan analysis. Reactions were carried out in triplicate; results are mean values ± SD.

replication of deleted mtDNA or ensures degradation of mitochondria bearing the *Common Deletion*. As mitochondria carrying deleted mtDNA molecules might concentrate due to mitotic segregation, affected cells could die as a consequence of reduced activity of the respiratory chain. A decrease in the amount of the *Common Deletion* related to a regeneration of respiratory activity in human fibroblasts has already been described by Bourgeron *et al* (1993).

The present model describes the *Common Deletion* as a product of an intragenomic recombination event that requires a single-strand break in mtDNA. This damage might be induced by UVA radiation or ROS. In our study, a single UVA irradiation of human neonatal keratinocytes with 2 J per cm^2, 7 J per cm^2, and 12 J per cm^2 did not result in a distinct increase in the relative amount of the *Common Deletion*. A considerable induction could only be observed after triple exposure to the same UVA doses. Berneburg *et al* (1999), who investigated the effect of UVA on human dermal fibroblast cultures using nested PCR, were able to detect the induction of the *Common Deletion* after repetitive irradiation with 8 J per cm^2 UVA. As the effect was dependent on the presence of ROS, the single-strand break seems to be mediated by UVA-generated reactive oxygen.

Triple-irradiated neonatal keratinocytes were cultivated up to passage 4. The incidence of the *Common Deletion* in all samples reached the level of the nonirradiated control after prolongation. This finding is consistent with the data on cultivated adult keratinocytes, which indicated a selective mechanism eliminating deleted mtDNA molecules or keratinocytes carrying the *Common Deletion*.

The TaqMan assay established in this study is applicable to experiments *in vitro* as well as *in vivo* due to the relatively small amount of sample material required. We investigated irradiated suction blister epidermis as an example of *in vivo* samples. All sites

irradiated with 2 J per cm^2 and 15 J per cm^2 and nonirradiated control areas showed high interperson variations in the incidence of the *Common Deletion* irrespective of irradiation dose. This observation is in accordance with the data on cultivated NHEK after a single irradiation. Additional intraperson variations are most probably caused by the mosaic distribution of cells during mitosis.

The improved detection method described brings a new perspective for further research concerning the amount of deleted mtDNA depending on mitotic activity (Cortopassi *et al*, 1992) and the involvement of the *Common Deletion* in photoaging of slowly dividing cells, posted by various groups (Yang *et al*, 1994; Berneburg *et al*, 1999).

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