Differential Responses of S100A2 to Oxidative Stress and Increased Intracellular Calcium in Normal, Immortalized, and Malignant Human Keratinocytes

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S100A2 is a calmodulin-like, p53-inducible, homodimeric protein that is readily oxidized in keratinocytes subjected to oxidative stress. Here we compare the redox status and subcellular distribution of S100A2 in normal human keratinocytes, immortalized keratinocytes (HaCaT), and malignant keratinocytes (A431) as a function of oxidative stress and intracellular Ca^{2+} levels. Normal human keratinocytes displayed strong nuclear and moderate cytoplasmic S100A2 immunoreactivity. HaCaT and A431 cells, which lack normal p53, expressed S100A2 in similar patterns but in 4- to 8-fold lower amounts. H_2O_2 treatment of normal human keratinocytes caused a reduction of nuclear S100A2 staining accompanied by an increase in cytoplasmic S100A2 staining, with a delayed time course (0.5–1 h) relative to S100A2 oxidative crosslinking (15 min). This phenomenon, consistent with translocation of S100A2 from the nucleus to the cytoplasm, could also be induced in normal human keratinocytes by increasing intracellular Ca^{2+} levels with the ionophore A23187. Sulfhydryl reducing agents blocked these changes, whether induced by H_2O_2 or increased intracellular Ca^{2+} levels. A temporal correlation was identified between S100A2 translocation at 1 h and loss of cell viability at 24 h after H_2O_2 treatment. A431 and HaCaT cells were strongly resistant to H_2O_2-induced S100A2 crosslinking, S100A2 translocation, and cell death. Increased intracellular Ca^{2+} levels caused prominent translocation of S100A2 in normal human keratinocytes and HaCaT, but not in A431 cells. These results identify S100A2 oxidation and translocation as markers for early cellular responses to oxidative stress, which are markedly attenuated in immortalized and malignant keratinocytes. *Key words: carcinogenesis/oxidative stress/p53/S100A2/skin. J Invest Dermatol 119:1196–1201, 2002*

S100A2 is a homodimeric calcium- and zinc-binding protein that is distantly related to calmodulin (Stradal *et al*, 2000). In man, S100A2 belongs to a family of at least 16 genes, encoding products whose functions remain enigmatic despite 35 y of study (Heizmann and Cox, 1998; Donato *et al*, 2001). S100A2 is the only member of the S100 gene family whose expression is downregulated in cancer cell lines relative to their normal counterparts (Donato *et al*, 2001). The recent discovery of positive transcriptional regulation of S100A2 by p53 (Tan *et al*, 1999) may explain the downregulation of S100A2 in cancer cells.

Many p53-inducible genes participate in the cellular response to oxidative stress (Meplan *et al*, 2000). S100A2 contains four cysteine residues, and we found that S100A2 readily surrenders electrons from at least some of these residues, leading to disulfide crosslinking of the S100A2 homodimer in response to oxidative stress (Deshpande *et al*, 2000). We found that S100A2 mRNA is markedly and rapidly induced when cells of normal human skin

or mammary tissue are placed into culture (Xia *et al*, 1997). The concentration of oxygen increases from 4% or less *in vivo* to 20% in standard cell culture (Davies, 2000). Thus, the process of cell culture itself leads to substantial oxidative stress. Our observation of culture-induced upregulation of S100A2 therefore lends credence to the idea that S100A2 might be involved in keratinocyte responses to reactive oxygen species (ROS).

We previously demonstrated that a substantial fraction of the S100A2 present in normal human keratinocytes (NHK) resides in the cell nucleus (Deshpande *et al*, 2000). In this report, we compare patterns of subcellular distribution, expression levels, and oxidative crosslinking in normal, immortalized, and malignant keratinocytes as a function of H_2O_2 treatment and calcium ionophore treatment. We demonstrate marked alterations in each of these responses in immortalized and malignant keratinocytes, relative to NHK. Our results suggest that S100A2 is involved in keratinocyte responses to oxidative stress, and that progressive adaptation to oxidative stress is an important factor in the development of the malignant phenotype.

MATERIALS AND METHODS

Cell culture NHK were isolated by trypsin flotation as previously described (Elder *et al*, 1991) and cultured in modified MCDB 153 medium (M154, Cascade Biologics, Portland, OR) according to the method of

Boyce and Ham (1983). A431 carcinoma cells (Giard *et al*, 1973) were obtained from the American Type Culture Collection and grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS), penicillin, and streptomycin (DMEM/10% FBS). HaCaT keratinocytes (Boukamp *et al*, 1988) were obtained from Professor Norbert Fusenig and grown in the same medium used for A431 cells.

Immunostaining NHK, HaCaT, or A431 cells were plated on Superfrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA) at a density of 5000 cells per cm^2 and incubated in a humidified 5% CO_2 incubator. Cultures were treated and used within 72 h of plating, and the medium was changed 18 h prior to treatment. All fixation and staining steps were performed at room temperature unless otherwise stated. The slides were washed in Tris-buffered saline (TBS) three times between each step. After 4% paraformaldehyde fixation, the slides were permeabilized with 0.5% Triton X-100 in ice-cold 0.1% sodium citrate for 10 min and blocked in 10% FBS in TBS for 1 h, followed by incubation with primary antibodies (Sigma, St. Louis, MO) in TBS/2% FBS for 2 h (or overnight at 4°C). Anti-S100A2 was used at 1 μg per ml; anti-S100A6 was used at 4 μg per ml. The slides were then incubated with fluorescein-isothiocyanate-conjugated goat antimouse IgG (10 μg per ml, Santa Cruz Biotechnology, Santa Cruz, CA) in TBS containing 2% FBS for 45 min, mounted in antifade, and examined under a Zeiss Axiophot fluorescence microscope. Controls for antibody specificity included equivalent molar amounts of the isotype monoclonal antibody MOPC21 (Sigma) and omission of primary antibody. All controls were negative.

Western blotting NHK, HaCaT, or A431 cells were seeded in 100 mm dishes at 10^4 cells per cm^2 in M154 and grown to approximately 50% confluence. All procedures prior to electrophoresis were carried out on ice or at 4°C. Cells were washed twice with ice-cold PBS and subjected to lysis in 0.5 ml 1 × lithium dodecyl sulfate buffer [250 mM Tris–HCl (pH 8.5), 2% lithium dodecyl sulfate, 10% glycerol]. Lysates were harvested and DNA was sheared by passing through a 21G needle. The total protein concentration of the lysates was measured by the DC Protein Assay (Bio-Rad, Hercules, CA). The lysates were divided in half and one half was treated with 37.5 mM dithiothreitol (DTT). DTT-treated and untreated samples were loaded separately onto 4%–12% precast Nu-PAGE gradient gels (Novex, San Diego CA) and run in MES running buffer (20 mM MES [2-(N-morpholino) ethane sulfonic acid], 20 mM Tris base, 3.4 mM sodium dodecyl sulfate (SDS)). After electrophoresis, the gels were transferred to PVDF membranes (Bio-Rad) in 25 mM Bis-Tris, 25 mM bicine, 20% (vol/vol) methanol for 1.5 h at 25 V in constant voltage mode. After blocking in Blotto for 30 min (Blotto is 5% nonfat dry milk in TBST; TBST is 25 mM Tris–HCl pH 8.0, 137 mM NaCl, 11 mM KCl, 0.1% Tween-20), membranes were incubated for 1 h at room temperature in 0.5 μg per ml anti-S100A2 in Blotto. After two washes in TBST, the membranes were incubated with 0.35 μg per ml goat antimouse IgG-HRP (Santa Cruz) in Blotto for 1 h. After two further washes in TBST, chemiluminescent detection was performed using the enhanced chemiluminescence detection kit according to the manufacturer's instructions (Amersham Pharmacia, Piscataway, NJ).

RESULTS

As shown in **Fig 1**, immunofluorescent staining revealed diffuse cytoplasmic and strong nuclear expression of S100A2 in actively proliferating NHK colonies, as previously reported (Deshpande *et al*, 2000). Treatment with 1 mM H_2O_2 for 2 h caused a large decrease in nuclear S100A2 staining, with a concomitant increase in cytoplasmic staining (compare *uppermost panels*, **Fig 1**). This staining pattern is consistent with nuclear-to-cytoplasmic translocation of S100A2, but could also be due to other mechanisms such as epitope masking and unmasking, new synthesis of S100A2, and/or changes in the stability of S100A2 in the nuclear *versus* cytoplasmic compartments. For convenience, we will refer to this phenomenon as S100A2 translocation. This effect could be abrogated by simultaneous treatment with either 3 mM DTT or 10 mM N-acetyl cysteine (NAC) (**Fig 1**, *lower panels*). S100A6 (calcyclin) also displayed prominent nuclear localization in NHK, as previously reported in other cell types (Mandinova *et al*, 1998). Translocation of S100A6 was not observed after H_2O_2 treatment of NHK, however (**Fig 1**, *lower panels*).

NHK are known to suffer irreversible toxicity after > 1 h of exposure to 0.7 mM H_2O_2 (Vessey *et al*, 1992). We confirmed these
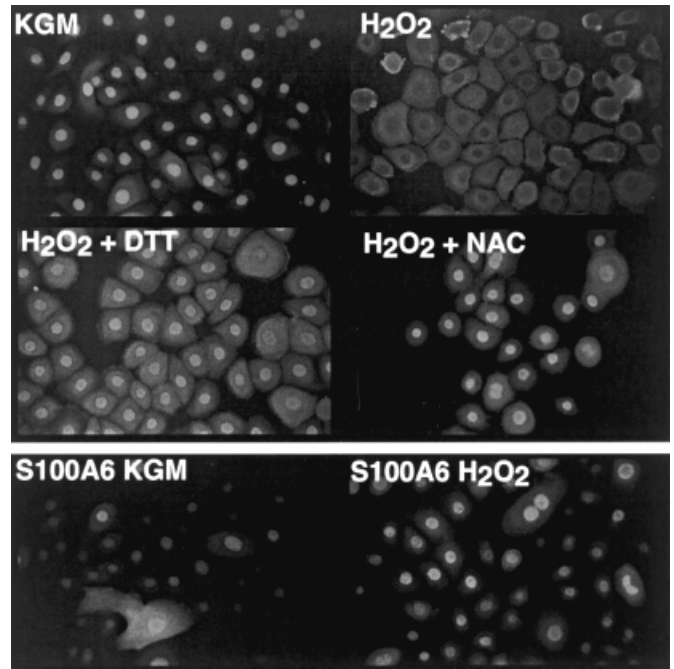

Figure 1. H_2O_2 causes redox-dependent translocation of S100A2 in NHK. NHK were seeded on glass slides at 5000 cells per cm^2. Forty hours later, the cultures were subjected to the indicated treatments for 2 h, followed by fixation and immunostaining for S100A2 or S100A6. The concentration of H_2O_2 was 1 mM, the concentration of NAC was 10 mM, and the concentration of DTT was 3 mM. Note retention of nuclear staining when DTT or NAC are added together with H_2O_2. Also note persistence of S100A6 nuclear staining after H_2O_2 treatment. Results are representative of at least three experiments for each condition shown.

results, and found that S100A2 translocation correlated well with irreversible toxicity to keratinocytes as a function of dose and time of H_2O_2 exposure. Thus, exposure of NHK to 0.2 or 0.5 mM H_2O_2 for up to 24 h did not result in loss of nuclear S100A2 staining, and the treated cells appeared normal and continued to grow well for at least a week (data not shown). As illustrated in **Fig 2**, exposure to 1 mM H_2O_2 for 30 min also did not result in S100A2 translocation. When the H_2O_2 was removed after 30 min and replaced with fresh medium, S100A2 remained in the nucleus and the cells remained fully viable 24 h later. By contrast, exposure of NHK to 1 mM H_2O_2 for 1 h caused pronounced S100A2 translocation. When the 1 mM H_2O_2 was replaced with fresh medium after 1 h, nuclear S100A2 staining was still markedly reduced 24 h later. These cells became markedly flattened and developed blebs and spike-like projections. These effects were even more pronounced if H_2O_2 treatment was continued for 2 h (**Fig 2**, *bottom panels*).

As assessed by trypan blue exclusion, the viable cell count in the cells treated with 1 mM H_2O_2 for 2 h was only 4% that of untreated controls after 24 h, and 0% of control after 48 h. This total loss of keratinocyte viability in response to H_2O_2 was significantly reduced by concomitant treatment with either DTT or NAC (**Fig 3**). Exposure to DTT or NAC alone for 2 h had no significant effects on viable cell counts relative to untreated controls.

Oxidative dimerization of S100A2 was more rapid than its translocation from the nucleus after H_2O_2 treatment. The level of dimerization reached a maximum after 15 min (**Fig 4**), whereas translocation required > 30 min to occur (**Fig 2**). Interestingly, the amount of dimer decreased over time, reaching a level only slightly higher than the pretreatment level after 3 h of continuous 1 mM H_2O_2 treatment (**Fig 4**).

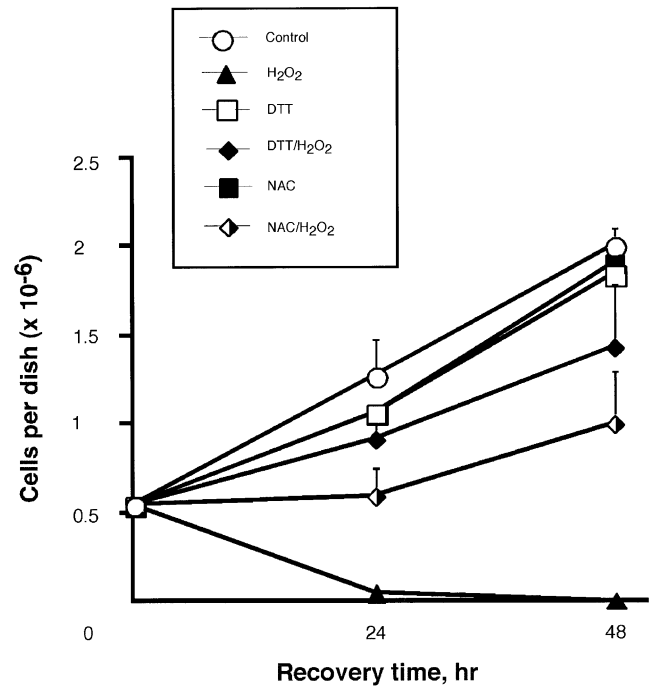The amount of S100A2 was markedly reduced in A431 and HaCaT cells relative to NHK. As determined by densitometry of
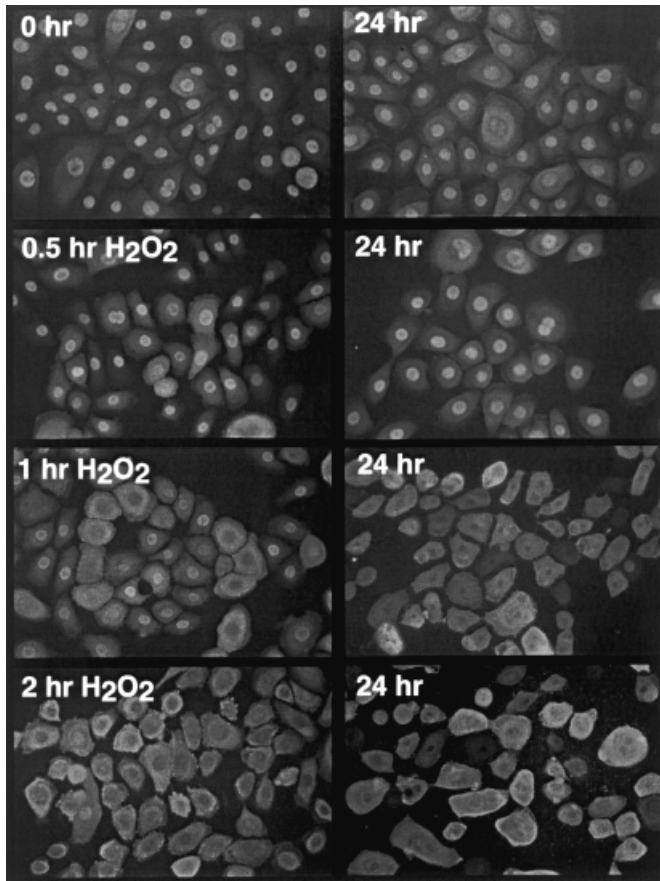
Figure 2. Time-dependent loss of nuclear S100A2 staining after H$_2$O$_2$ treatment correlates with eventual lethality of NHK. Forty hours after seeding NHK on glass slides, cultures were subjected to a change of medium containing no additives or 1 mM H$_2$O$_2$. After 0, 0.5, 1, or 2 h, the cultures were either fixed (*left panels*) or the medium was changed and the cells were incubated for an additional 24 h in fresh medium without H$_2$O$_2$ (*right panels*), followed by immunofluorescent staining for S100A2. Note apparent translocation of S100A2 between 0.5 and 1 h of H$_2$O$_2$ treatment. Translocation was always complete by 2 h of H$_2$O$_2$ treatment. Results are representative of at least three experiments for each time point.

Figure 3. Disulfide reducing agents reduce the toxic effects of H$_2$O$_2$ on NHK survival. NHK survival was determined by trypan blue exclusion 24 and 48 h after H$_2$O$_2$ treatment, in the presence or absence of NAC (10 mM) or DTT (3 mM). Three independent experiments were available and analyzed for all points. Statistical analysis was by ANOVA, with Bonferroni correction applied for two individual tests. The corrected p-values for H$_2$O$_2$ *versus* DTT + H$_2$O$_2$ were 0.003 at 24 h and 0.008 at 48 h. The corrected p-values for H$_2$O$_2$ *versus* NAC + H$_2$O$_2$ were 0.049 at 24 h and 0.009 at 48 h. The corresponding p-values for either DTT or NAC *versus* no treatment were not significant.

the Western blots, S100A2 protein was approximately four times lower in A431 cells, and eight times lower in HaCaT cells, compared to NHK (gels not shown). This reduction was also visible by immunofluorescent staining of each cell type (images not shown).
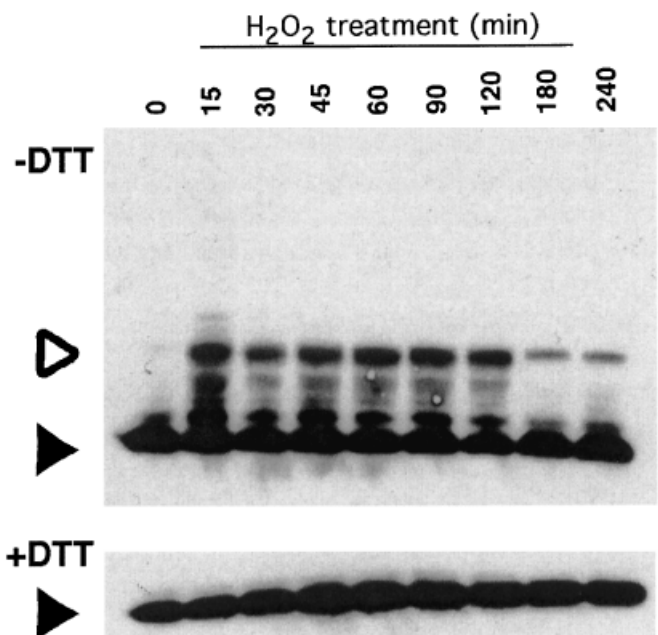
A431 and HaCaT cells were markedly refractory to H$_2$O$_2$–induced oxidative crosslinking of S100A2 (**Fig 5***a*) and to S100A2 translocation after 1 mM H$_2$O$_2$ treatment (data not shown). Both cell lines also survived treatment with much higher concentrations of H$_2$O$_2$ than did NHK (**Fig 5***b*).

S100A2 nuclear staining was markedly reduced, and cytoplasmic S100A2 staining was markedly increased, after treating NHK with 3 μM A23187 in conjunction with 1.5 mM CaCl$_2$. This phenomenon was not observed when A23187 or 1.5 mM CaCl$_2$ alone was added to the growth medium (**Fig 6**). Translocation of S100A2 by A23187/1.5 mM CaCl$_2$ could be blocked by treatment with DTT or NAC (**Fig 6**).

A431 and HaCaT cells displayed a pronounced difference in S100A2 translocation after challenge with A23187 in the presence of 1.5 mM CaCl$_2$. This treatment caused no detectable loss of nuclear S100A2 in A431 cells, whereas it caused prominent loss of nuclear S100A2 in HaCaT cells and NHK (**Fig 7**).


Figure 4. H$_2$O$_2$–induced oxidative dimerization of S100A2 is rapid and largely reversible. Western blots of Nu-PAGE gels decorated with anti-S100A2 are shown. Note that oxidative dimerization of S100A2 (*open arrowhead*) is maximal after 15 min, whereas loss of S100A2 nuclear staining (**Fig 2**) requires more than 30 min. Also note the reduction of the disulfide-linked dimer at 180 and 240 min, by which time S100A2 nuclear staining has already been lost and keratinocytes are already destined for cell death.
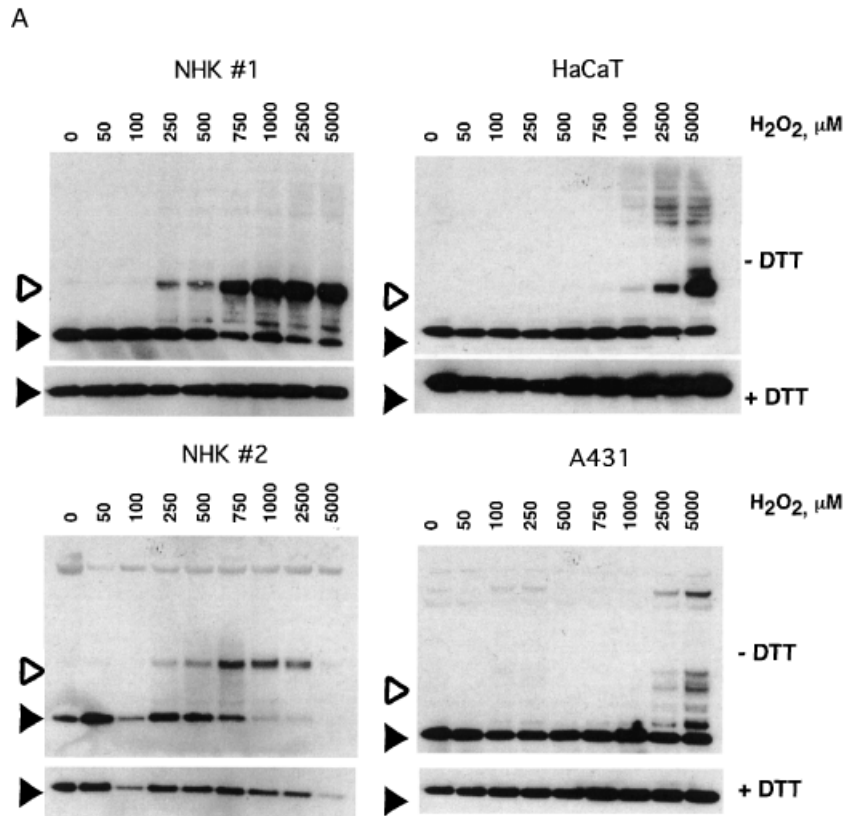
Figure 5. A431 and HaCaT cells are resistant to oxidative dimerization of S100A2 (*A*) and to H₂O₂ toxicity (*B*). (*A*) Western blots. Subconfluent cells were fed 18–24 h before treatment with the indicated concentrations of H₂O₂ for 90 min, and then lyzed as described in *Materials and Methods*. 1% of a 100 mm dish was loaded in each lane. Samples were run with or without 37.5 mM DTT in the loading buffer, as indicated to the right of each panel. Exposure times were longer for A431 and HaCaT cells, due to their lower levels of S100A2 expression. Two strains of NHK are shown to illustrate the reproducibility of the result. Note that both strains are much more sensitive to H₂O₂-induced S100A2 crosslinking than are either HaCaT or A431. (*B*) A431 and HaCaT cells are more resistant to H₂O₂ toxicity than NHK. Cell counts were performed 48 h after H₂O₂ treatment. Error bars indicate SEM ($n = 4$ for HaCaT and A431, $n = 3$ for NHK).
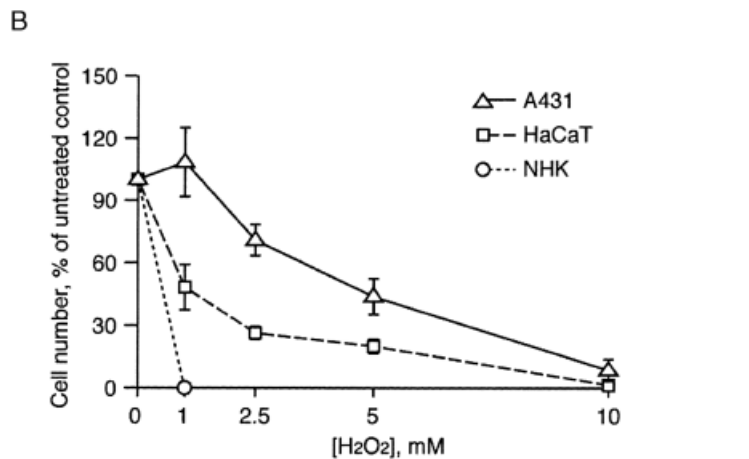
DISCUSSION

As a result of exposure to inflammation, ultraviolet light, and oxidizing chemicals, the skin is exposed to substantial levels of ROS. ROS in turn produce DNA damage that leads to mutations or programmed cell death, if the damage is not repaired prior to DNA replication (Marnett, 2000). ROS also generate protein and lipid peroxides, which compromise cellular integrity by a variety of additional mechanisms (Halliwell, 1999). Organisms have evolved a variety of mechanisms to cope with ROS. This work was undertaken to further explore our hypothesis that S100A2 may have evolved to participate in the oxidative stress response. In the course of our studies we uncovered pronounced differences in the oxidative stress and calcium responses of normal *versus* immortalized and malignant keratinocytes, which were reflected in the behavior of S100A2.

Nuclear localization of S100A2 has been noted by others (Mandinova *et al*, 1998); however, this is the first report of the disappearance of S100A2 from the nucleus in response to cellular stimuli. We believe that the loss of nuclear S100A2 immunostaining observed in NHK challenged with 1 mM H₂O₂ (**Figs 1, 2**) reflects translocation of S100A2 from the nucleus to the cytoplasm, because increased amounts of cytosolic S100A2 are readily observed as S100A2 disappears from the nuclei of H₂O₂-treated NHK (**Figs 1, 2**). As mentioned above, our data are consistent with, but do not prove, translocation; additional experiments will be required to clarify this point. For instance, our data cannot exclude the possibility that oxidative stress leads to degradation of nuclear S100A2 coupled with new synthesis of cytoplasmic S100A2. The fact that H₂O₂ promotes translocation of S100A2 but not S100A6 (**Fig 1**) indicates that this apparent translocation of S100A2 is a specific response of this protein to oxidant stress.

Translocation of S100A2 was an excellent predictor of ultimate cellular demise after H₂O₂ treatment (**Fig 2**). The morphology of H₂O₂-treated cells was not typical of apoptosis: the cells were flattened, and membrane blebbing, although present, was not prominent. Moreover, we were unable to block H₂O₂-mediated cell death with the broad-spectrum caspase inhibitor ZVAD (data
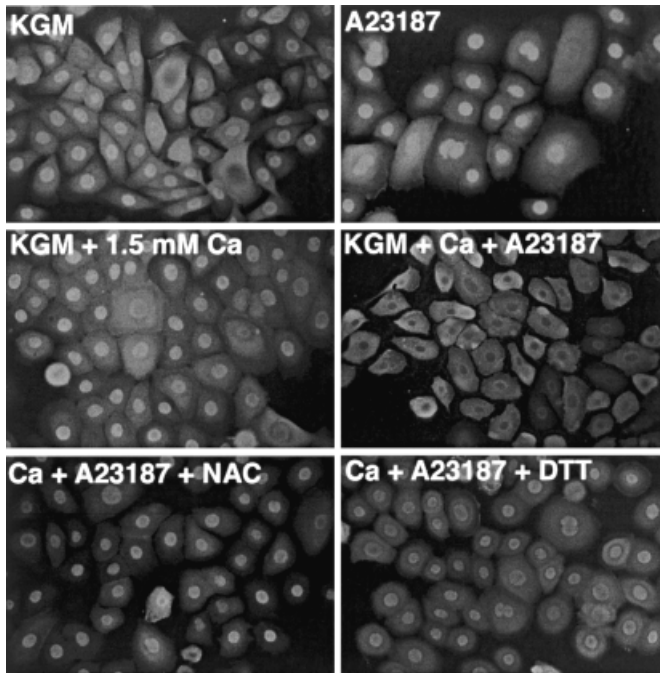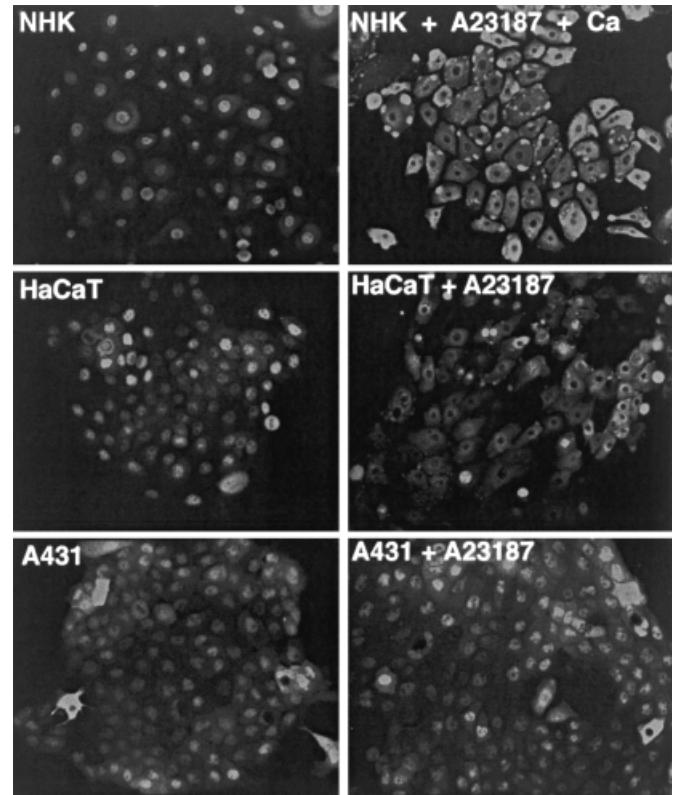
Figure 6. S100A2 translocates from nucleus to cytoplasm in response to increased intracellular Ca^{2+} after A23187 treatment. NHK were plated on glass slides in growth medium containing 0.15 mM Ca^{2+} as described in *Materials and Methods*. After 40 h, cells were treated for 1 h with various combinations of A23187 (3 μM), CaCl$_2$ (1.35 mM, for a total [Ca^{2+}] of 1.5 mM), NAC (10 mM), and/or DTT (3 mM). The experiment shown was repeated with identical results. Note reversal of Ca^{2+}–mediated S100A2 translocation by NAC and DTT.

Figure 7. S100A2 is translocated in NHK and HaCaT but not A431 cells after A23187 + 1.5 mM CaCl$_2$ treatment. NHK, A431, or HaCaT cells were plated in parallel on glass slides as described in *Materials and Methods*. NHK were grown in low Ca^{2+} M154 medium, whereas HaCaT and A431 cells were grown in high Ca^{2+} DMEM (1.5 mM CaCl$_2$). After 40 h, NHK were treated with 3 μM A23187 + 1.35 mM CaCl$_2$ to bring the total [Ca^{2+}] to 1.5 mM, whereas A431 and HaCaT cells, which were already growing in 1.4 mM Ca^{2+} DMEM/10% FBS, were treated with 3 μM A23187 only. After 1 h, cells were fixed and stained for S100A2 as described in *Materials and Methods*. Note persistence of S100A2 nuclear staining after A23187 + CaCl$_2$ treatment in A431 cells, but not in HaCaT or NHK.

not shown). We suspect that lipid peroxidation may be the major cause of cell death after H$_2$O$_2$ treatment (Halliwell, 1999). Consistent with this speculation, cell–permeant disulfide reducing agents (which reduce lipid peroxidation) promoted NHK survival after H$_2$O$_2$ treatment (**Fig 3**) and prevented loss of nuclear S100A2.

S100A2 also was lost from the nucleus to the cytoplasm after treating NHK with 3 μM A23187 in the presence of 1.5 mM extracellular Ca^{2+} ([Ca^{2+}$_o$]), but not in the presence of 0.15 mM [Ca^{2+}$_o$] (**Fig 6**). In cultured NHK, ionophore treatment is known to induce a sustained (>15 min) increase of intracellular free Ca^{2+} ([Ca^{2+}$_i$]) to approximately 1 μM in the presence of elevated (2 mM) [Ca^{2+}$_o$] (Pillai *et al*, 1993). A Ca^{2+} concentration of at least 200 μM, however, is required to provoke conformational changes in S100A2 (Franz *et al*, 1998). Therefore, Ca^{2+}–stimulated alterations in S100A2 conformation cannot readily be invoked to explain Ca^{2+}–mediated nuclear exit of S100A2. Ionophore treatment also increases [Ca^{2+}$_i$], to approximately 0.8 μM, in the presence of low (0.1 mM) [Ca^{2+}$_o$]. The elevation is transient (<2 min), however (Pillai *et al*, 1993). Thus, we would suggest that a prolonged elevation of [Ca^{2+}$_i$] is required to provoke loss of S100A2 from the nucleus.

Loss of nuclear S100A2 after A23187/Ca^{2+} treatment could be blocked by disulfide reducing agents (**Fig 6**). A23187 is known to promote formation of H$_2$O$_2$ in HaCaT keratinocytes (Goldman *et al*, 1998), suggesting that A23187 and H$_2$O$_2$ might promote S100A2 translocation via a common mechanism. The amount of ROS generated by A23187 is quite low, however, being equivalent to that produced by micromolar quantities of exogenous H$_2$O$_2$ (Goldman *et al*, 1998). In contrast, millimolar quantities of H$_2$O$_2$ are required to provoke nuclear exit of S100A2 (**Fig 1**). Also, treatment with 1 mM H$_2$O$_2$ causes both oxidative crosslinking and loss of nuclear S100A2, whereas A23187/Ca^{2+} treatment causes loss of nuclear S100A2 (**Fig 6**) but does not lead to oxidative crosslinking of S100A2 (Deshpande *et al*, 2000). Thus, the

mechanisms of S100A2 nuclear loss appear to differ in some respects after H$_2$O$_2$ *versus* A23187/Ca^{2+} treatment. It will be interesting to explore the relative effects of these two agents on lipid peroxidation in keratinocytes.

ROS are known to be a very significant driving force in carcinogenesis (Kovacic *et al*, 2001). Tumor cells would be expected to gain an important advantage by elimination of mechanisms that normally trigger cycle withdrawal in response to oxidative stress. We found that S100A2 is expressed at 4- to 8-fold lower levels in immortalized HaCaT and malignant A431 keratinocytes, relative to NHK (data not shown). These findings are in good agreement with our earlier studies of S100A2 mRNA levels in A431, HaCaT, and NHK (Xia *et al*, 1997), and are probably explained by the fact that both A431 and HaCaT cells lack normal p53 (Lehman *et al*, 1993; Kwok *et al*, 1994). Several genes involved in oxidative stress responses are p53–inducible (Meplan *et al*, 2000). The fact that the S100A2 gene is also strongly induced by p53 (Tan *et al*, 1999) would be consistent with a role for S100A2 in oxidative stress responses.

We found that HaCaT and A431 cells were markedly resistant to H$_2$O$_2$–induced cellular toxicity, relative to NHK (**Fig 5*b***). To our knowledge, this is the first report of resistance of immortalized and malignant keratinocytes to H$_2$O$_2$, relative to NHK. We also observed that immortalized HaCaT and malignant A431 cells are highly resistant to H$_2$O$_2$–induced crosslinking and translocation of S100A2 (**Fig 5*a*** and data not shown). Recalling that H$_2$O$_2$–induced S100A2 crosslinking is very rapid (**Fig 3**), the

refractoriness of A431 and HaCaT cells to oxidative crosslinking of S100A2 indicates that their resistance to H_2O_2 is exerted at an early point in the cellular response. Additional studies will be required to determine whether S100A2 is functionally involved in H_2O_2-mediated cellular toxicity.

Although A431 and HaCaT cells were both refractory to H_2O_2-induced translocation of S100A2 (**Fig 5**), they differed markedly in their translocation responses to A23187/Ca^{2+} (**Fig 7**). HaCaT cells demonstrated S100A2 translocation very similar to that manifested by NHK, whereas no translocation could be observed in A431 cells. This difference provides further evidence that the mechanisms of S100A2 translocation may not be identical for H_2O_2 and A23187/Ca^{2+} treatments. HaCaT cells, when immortalized, are not malignant, and will not form tumors in immunosuppressed mice unless other transforming oncogenes are added and/or tumor suppressor genes are inactivated (Fusenig and Boukamp, 1998). By contrast, A431 cells readily form invasive tumors without any additional modifications (Giard *et al*, 1973). How these differences in biologic aggressiveness relate to the behavior of S100A2 remains to be determined.

Our laboratory was the first to study the expression of S100A2 in actual human tumors, rather than in cell lines derived from them (Xia *et al*, 1997). By means of *in situ* hybridization, we showed that S100A2 mRNA was indeed markedly downregulated in most basal cell carcinomas. Squamous cell carcinomas of the skin and oral cavity yielded a more complex picture, however, with several examples of strong S100A2 expression in primary and locally invasive tumors. More recently, increased (rather than decreased) expression of S100A2 has also been reported in ovarian carcinomas (Hough *et al*, 2001). These findings suggest that downregulation of S100A2 is not a universal feature of epithelial cell tumor. Based on our findings in this work and in previous work (Deshpande *et al*, 2000), we would suggest that the primary role played by S100A2 could lie in the realm of oxidant defense, rather than suppression of tumor cell proliferation.

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