Dual Role of the Antioxidant Enzyme Peroxiredoxin 6 in Skin Carcinogenesis

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Abstract
The antioxidant enzyme peroxiredoxin 6 (Prdx6) is a key regulator of the cellular redox balance, particularly under stress conditions. We identified Prdx6 as an important player in different phases of skin carcinogenesis. Loss of Prdx6 in mice enhanced the susceptibility to skin tumorigenesis, whereas overexpression of Prdx6 in keratinocytes of transgenic mice had the opposite effect. The tumor-preventive effect of Prdx6, which was observed in a human papilloma virus 8–induced and a chemically induced tumor model, was not due to alterations in keratinocyte proliferation, apoptosis, or in the inflammatory response. Rather, endogenous and overexpressed Prdx6 reduced oxidative stress as reflected by the lower levels of oxidized phospholipids in the protumorigenic skin of Prdx6 transgenic mice and the higher levels in Prdx6-knockout mice than in control animals. In contrast to its beneficial effect in tumor prevention, overexpression of Prdx6 led to an acceleration of malignant progression of existing tumors, revealing a dual function of this enzyme in the pathogenesis of skin cancer. Finally, we found strong expression of PRDX6 in keratinocytes of normal human skin and in the tumor cells of squamous cell carcinomas, indicating a role of Prdx6 in human skin carcinogenesis. Taken together, our data point to the potential usefulness of Prdx6 activators or inhibitors for controlling different stages of skin carcinogenesis. Cancer Res; 73(11): 3460–9. ©2013 AACR.

Introduction
“Tumors are wounds that do not heal” is a famous statement of Harold Dvorak (1), who recognized the remarkable parallels between skin wounds and malignant tumors. In recent years, this hypothesis was supported by various studies and extended to the molecular level. Thus, data obtained by miRNA profiling revealed that many genes are similarly regulated in skin wounds and malignant tumors (2). Therefore, it is of particular interest to identify wound-regulated genes and to determine their roles and mechanisms of action in cancer. A gene that is strongly overexpressed upon skin injury, in particular in the hyperproliferative wound epithelium, encodes Prdx6 (3), a member of the peroxiredoxin family that also includes Prdx1–5. The latter have 2 reactive cysteines and they use predominantly thioredoxin as a substrate (4). Prdx6, which is expressed in most cell types, has only a single redox-active cysteine and uses glutathione and possibly ascorbate as reducing agent (5–7). Remarkably, it can reduce hydrogen peroxide, fatty acid, and phospholipid hydroperoxides, as well as peroxynitrite (8). In addition, it exhibits phospholipase A2 activity (9).

The antioxidant activity of Prdx6 is likely to be of major importance in the skin, which is frequently exposed to harmful insults that induce the formation of reactive oxygen species (ROS), including UV irradiation and toxic chemicals. While low levels of ROS are required for intracellular signaling (10), high levels of these aggressive molecules are deleterious, as they damage cellular macromolecules. As a consequence, skin aging occurs, and the risk of skin carcinogenesis increases (11). Enhanced ROS levels are, at least in part, responsible for the development of UV-induced skin cancer and they are likely to underlie the enhanced frequency of malignant transformation seen in chronically inflamed tissue, for example, in chronic wounds (12). A beneficial role of Prdx6 in the skin was identified in our laboratory, as overexpression of Prdx6 in keratinocytes of transgenic mice enhanced the wound closure rate in aged mice and protected from UV toxicity (13). Vice versa, Prdx6-knockout mice were more susceptible to UV-induced skin damage and they showed severe hemorrhage in healing skin wounds (14). Consistent with these findings, overexpression of Prdx6 protected other cell types and organs from ROS-induced cytotoxicity, whereas knockdown or knock-out of Prdx6 enhanced the sensitivity to the toxicity of ROS induced by several insults (5, 9).

Because of the parallels between wound healing and cancer, a potential role of Prdx6 in the pathogenesis of cancer is of...
Peroxiredoxin 6 and Skin Cancer

particular interest. Recent findings suggest a protumorigenic function of Prdx6 due to its effect on tumor cell proliferation, apoptosis, and invasiveness. Prdx6 interfered with TRAIL- or cisplatin-induced apoptosis in human cancer cells in vitro (15, 16). Furthermore, it enhanced the metastatic potential of breast and lung cancer cells in orthotopic cancer models (17, 18). A potential importance of Prdx6 in cancer is also reflected by the detection of auto-antibodies against this protein in patients with esophageal squamous cell carcinoma (SCC; ref. 19). On the other hand, development of tumors might be reduced by peroxiredoxins, as they detoxify ROS and thereby help maintain genomic integrity. Therefore, peroxiredoxins have been suggested to be tumor preventers rather than tumor suppressors (20). Clearly, there is a strong need to test this hypothesis experimentally and to determine the roles of individual peroxiredoxins at different stages during the development and progression of various cancers. Here, we show a dual role of Prdx6 in skin carcinogenesis, most likely through its ROS-detoxifying activity.

Materials and Methods

Animal experiments

Transgenic mice overexpressing Prdx6 in keratinocytes under control of the keratin 14 (K14) promoter, Prdx6-knockout mice, and their genotyping were previously described (13, 14, 21). Mice expressing the genes of the human papillomavirus-type 8 (HPV8) early region under the control of the K14 promoter (HPV8 mice; ref. 22) were genotyped by PCR using primers 5′-GGATCCCTTCTCTAAAGCAGAACGCGG-3′ and 5′-GGATCCGCCATGCACAAAAATCTTGCACAGTGACCTC-3′.

Prdx6-tg mice in FVB/N background were crossed with HPV8 mice in FVB/N background. Prdx6-ko mice in C57BL/6 background were crossed with K14-HPV8 mice in C57BL/6 background. Single- and double-mutant mice of the F1 generation were observed weekly for the appearance of skin tumors and for progression of existing ones. They were euthanized if a single large tumor (>1 cm), more than one tumor of intermediate size (0.5 cm), or unfavorable localization required their elimination according to animal welfare regulations.

For chemical skin carcinogenesis 25 μg of 7,12-dimethylbenz[a]anthracene (DMBA; Sigma) in 200 μL acetone was applied topically to a shaved area on the back skin of 8- to 10-week-old female mice 2 days after shaving. One week later, 5 μg 12-O-tetradecanoylphorbol 13-acetate (TPA; Sigma) in 200 μL acetone was applied to the same site once weekly for 24 weeks. Tumor number and size were documented every 2 weeks. Termination of TPA treatment, tumor observation was continued until the mice started to develop ulcerated tumors.

Mice were housed under optimal hygiene conditions and maintained according to Swiss animal protection guidelines. All experiments with mice were approved by the local veterinary authorities of Zurich or Lausanne, Switzerland.

Analysis of oxidized phospholipids

Seventy-two hours after the last of 3 TPA treatments, animals were euthanized with CO2, and butylated hydroxyanisole (5 mg in 400 μL acetone) was topically applied to the back skin to inhibit lipoxygenases and to avoid postmortem oxidation of epidermal lipids. Skin was removed, weighed, and epidermis was separated from dermis via heat shock. Tissue was sliced (1 mm thick), frozen in liquid nitrogen. Analysis of oxidized phospholipids was conducted as previously described (23). For details, see Supplementary Information.

Human skin biopsies

Normal human skin from healthy adult volunteers and SCC samples were obtained anonymously from the Departments of Dermatology, University Hospitals of Lausanne and Zurich (in the context of the biobank project), approved by the local and cantonal Research Ethics Committees. SCC was diagnosed by experienced pathologists. Informed consent for research was obtained before routine diagnostic services.

Statistical analysis

Statistical analysis was conducted using Graph Pad Prism 5 Software (Graph Pad Software Inc.). Tumor incidence was analyzed using the log-rank (Mantel–Cox) test. For analysis of tumor multiplicity, the Mann–Whitney test was conducted for single time points. The Mann–Whitney test was used for comparing 2 groups of data. The paired t test was applied for lipid peroxidation data.

Results

Loss of Prdx6 enhances development of skin tumors induced by HPV8 oncogenes

To determine a potential role of Prdx6 in skin carcinogenesis, we first used a virus-induced tumorigenesis model to study the consequences of a loss- or gain-of-function of Prdx6 for the development of skin tumors. Prdx6-knockout mice (Prdx6-ko), which do not reveal abnormalities under normal housing conditions (21), were crossed with HPV8 mice. These mice spontaneously develop skin papillomas without physical or chemical insult, as the HPV8 early region encodes the viral oncoproteins (22). By comparing tumorigenesis in single- and double-mutant mice, we determined whether loss of Prdx6 affects HPV8-induced skin tumorigenesis. There was an obvious tumor-preventive effect of endogenous Prdx6; while loss of this enzyme did not affect tumor incidence (number of mice with tumors; Fig. 1A), tumor multiplicity (number of tumors per mouse) was continuously higher in the Prdx6-ko/HPV8 mice after week 55 than in HPV8 single-mutant mice. The difference was statistically significant when the experiment was terminated at week 135 (Fig. 1B). As mice with tumors of a diameter >1 cm or with 2 or more tumors of >0.5 cm had to be eliminated and as their tumor number at the date of sacrifice was included in the graphs at all later time points (cumulative tumor multiplicity), the differences between control and Prdx6-knockout mice are likely to be underestimated. No
Figure 1. Prdx6 protects against HPV8-induced skin carcinogenesis. Kinetics of tumor incidence (A and E) and cumulative tumor multiplicity (B and F) in HPV8 single (n = 17 for tumor incidence; n = 15 for multiplicity) and Prdx6-ko/HPV8 double-mutant mice (n = 26/n = 26; A and B) and in wt/HPV8 single (n = 26) and Prdx6-tg/HPV8 double-mutant mice (n = 33; E and F). C and G, left, histological stainings from representative acanthopapillomas of wt/HPV8 single-mutant mice (C and G), Prdx6-ko/HPV8 double-mutant mice (C), and from Prdx6-tg/HPV8 double-mutant mice (G). Bars, 1000 μm. Right, staining with a Prdx6 antibody and counterstaining with hematoxylin. Asterisks in C indicate background staining of hairs. Bar, 100 μm. D and H, RNA from the skin of wt/HPV8 (n = 6) and Prdx6-ko/HPV8 mice (n = 6) and from wt/HPV8 (n = 6) and Prdx6-tg/HPV8 mice (n = 5) was analyzed for expression of Prdx6 and HPV8 E6. Expression levels of ribosomal protein 29 (rps29) were used for normalization. Mean expression levels in wt/HPV8 mice were arbitrarily set to 1. Graphs show mean values and SEM.
differences were seen in the time periods between appearance of the first and the second tumor or between the second and the third tumor (Supplementary Fig. S1A), in the growth rate of the tumors (Supplementary Fig. S1B), or in their age (Supplementary Fig. S1C). In both single- and double-mutant mice, tumors developed predominantly at the dorsal skin (Supplementary Fig. S1D), and most of them were benign acantho-papillomas (Fig. 1C). The lack of Prdx6 in the tumors and in normal skin of the double mutant mice was confirmed by immunohistochemistry (Fig. 1C) or quantitative real-time reverse transcriptase (RT)-PCR (qRT-PCR; Fig. 1D), respectively. Importantly, loss of Prdx6 did not significantly affect the expression level of the HPV8-E6 transgene (Fig. 1D).

Overexpression of Prdx6 in keratinocytes protects mice against HPV8-induced skin tumorigenesis

To determine whether an increase in the levels of Prdx6 in keratinocytes is beneficial, we conducted an HPV8-induced skin tumorigenesis study with transgenic mice overexpressing Prdx6 in keratinocytes (Prdx6-tg mice). These animals are phenotypically normal under non-challenged conditions (13). In this case, both types of mutant mice were in FVB/N genetic background and, therefore, tumor formation was generally accelerated compared with mice in the C57BL/6 background (22). Tumor incidence was not affected by Prdx6 overexpression (Fig. 1E). However, cumulative tumor multiplicity was reduced at all time points after week 6 in the double transgenic mice, and the difference was statistically significant between week 25 and 100 (Fig. 1F). The time periods between the appearance of the first and the second tumor or the second and the third tumor, respectively, were significantly longer in the double-transgenic mice (Supplementary Fig. S1E), whereas tumor growth, tumor age, and localization of the tumors were not altered (Supplementary Fig. S1F–S1H). Histopathologic features were not obviously affected by Prdx6 overexpression (Fig. 1G). Malignant conversion was rarely observed, and only few early-stage SCCs were seen in mice of both genotypes at the time when the mice had to be eliminated (data not shown). The overexpression of Prdx6 in the tumors and in normal skin of the double-transgenic mice versus HPV8 single-transgenic mice was confirmed by immunohistochemistry (Fig. 1G) or qRT-PCR (Fig. 1H). There was no significant difference in the expression levels of the HPV8-E6 transgene between single- and double-transgenic mice (Fig. 1H). These results show that Prdx6 protects against HPV8-induced skin tumorigenesis in a dose-dependent manner.

Prdx6 overexpression in keratinocytes protects against chemically induced skin carcinogenesis but enhances malignant conversion of existing tumors

The effect of Prdx6 overexpression on skin tumorigenesis was particularly strong and suggested that an increase in the expression/activity of this enzyme could be therapeutically explored to protect skin from malignant transformation. Therefore, we next determined whether Prdx6 overexpression is also protective in a different skin tumorigenesis model. For this purpose, we subjected the Prdx6-tg mice and wt controls to a DMBA/TPA-induced skin carcinogenesis study (24, 25).

Consistent with the results obtained in the HPV8 tumor model, tumor incidence and multiplicity were lower in the Prdx6-tg mice than in wt controls, although the difference was not statistically significant (Fig. 2A and B).

The 2-stage skin carcinogenesis model also allowed us to determine the effect of Prdx6 overexpression on tumor progression. Upon termination of the TPA treatment, appearance of new papillomas ceased within 3 weeks. Most of the tumors regressed, but more than one-third progressed to malignant SCCs. This is consistent with previous findings showing a high rate of malignant conversion in FVB/N mice (26). Interestingly, the progression rate from papilloma to carcinoma was much higher in the Prdx6-tg mice than in wt mice as determined by histopathologic analysis (Fig. 2C and Table 1). This was seen at week 32 (36.6% conversion in wt versus 57.5% in Prdx6-tg mice; n = 41 tumors from wt and 40 tumors from Prdx6-tg mice) and also at week 39 (34.5% conversion in wt mice vs. 58.3% conversion in Prdx6-tg mice; n = 29 tumors from wt mice and 12 tumors from Prdx6-tg mice). Highly malignant tumors were particularly abundant in the transgenic mice (data not shown). These findings reveal a dual function of this enzyme in tumorigenesis: suppression of tumor formation, but acceleration of malignant progression of existing tumors.

Prdx6 overexpression does not affect the response of the skin to DMBA or TPA

As our study focused on the role of Prdx6 in tumor formation, we next characterized the molecular mechanisms underlying the tumor-preventive activity of Prdx6. As the initiation of tumor formation can be more tightly controlled in the DMBA/TPA model compared with the HPV8 model, we analyzed the early events of the tumorigenesis process in this mouse model. We first showed that Prdx6 overexpression does not affect the expression of major enzymes involved in DMBA metabolism in nontreated skin (Fig. 3A), indicating that the response to DMBA is not affected by Prdx6 overexpression. This was verified by staining of skin sections 24 hours after DMBA treatment with an antibody against phosphorylated histone H2AX (γH2AX), a marker of cells with DNA double-strand breaks. The number of γH2AX-positive cells in the epidermis was similar in mice of both genotypes (Supplementary Fig. S2A).

We next analyzed the response of the skin to TPA. The overexpression of the Prdx6 transgene was even stronger in TPA-treated skin of Prdx6-tg mice (Fig. 3B) than in the skin of Prdx6-tg/HPV8 mice (Fig. 1H) due to the more severe keratinocyte hyperproliferation in DMBA/TPA-treated skin, which activates the keratin 14 promoter. Subsequently, we analyzed several parameters that increase after a single DMBA treatment and/or after 1 or 3 TPA treatments (27). Immunofluorescence staining of sections from DMBA/TPA-treated skin (24 hours after a single TPA treatment) for cleaved caspase-3 revealed a very low number of apoptotic cells in mice of both genotypes (<2 apoptotic cells per cm2 epidermis; data not shown). Epidermal thickness and the rate of keratinocyte proliferation were similar in untreated Prdx6-tg and wt mice (Fig. 3C and data not shown), and the DMBA- or TPA-induced
increase in epidermal thickness was also not affected by the Prdx6 transgene (Fig. 3C). Consistent with this finding, keratinocyte proliferation as determined by staining for proliferating cell nuclear antigen (PCNA) or Ki67 was not affected by the Prdx6 transgene (Fig. 3D and Supplementary Fig. S2B). Furthermore, there was no obvious difference in the inflammatory response, as shown by the similar number of neutrophils (Ly-6G immunohistochemistry; Fig. 3E), mast cells (toluidine blue staining; Supplementary Fig. S2C), and dermal and epidermal T lymphocytes (CD3 immunohistochemistry; Supplementary Fig. S2D) in DMBA- and/or TPA-treated skin of mice of both genotypes. Macrophages were undetectable in the skin after short-term DMBA or TPA treatment (data not shown). Several proinflammatory cytokines and chemokines were hardly detectable in non-treated skin of Prdx6-tg and wt mice (13), but strongly expressed after 3 TPA treatments. However, there was no significant difference in the expression of these cytokines/chemokines between Prdx6-tg and wt mice (Fig. 3F). The lack of alterations in keratinocyte proliferation and expression of proinflammatory cytokines was confirmed for the protumorigenic skin of HPV8 single and Prdx6-tg/HPV8 double transgenic mice (Supplementary Fig. S3A and S3B). These results strongly suggest that the tumor-preventive effect of Prdx6 does not result from alterations in keratinocyte proliferation or apoptosis or from differences in the inflammatory response.

We previously observed severe hemorrhage in wounds of Prdx6-ko mice (14). This was also seen in the tumors, but to a similar extent in wt and of Prdx6-tg mice due to the general leakiness of tumor vessels (Supplementary Fig. S3C). Therefore, alterations in vascular stability are unlikely to contribute to the difference in tumorigenesis in Prdx6-mutant mice.

<p>| Table 1. The percentage of malignant conversion at weeks 32 and 39 after DMBA treatment is shown |
|-------------------------------------------------|-----------------|------------------|--------------------|-----------------|</p>
<table>
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<th><strong>Weeks after DMBA</strong></th>
<th><strong>Group</strong></th>
<th><strong>No. of mice</strong></th>
<th><strong>Total number of tumors</strong></th>
<th><strong>Number of carcinomas</strong></th>
<th><strong>Percentage conversion</strong></th>
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<td></td>
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<tr>
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<td>wt</td>
<td>5</td>
<td>29</td>
<td>10</td>
<td>34.5</td>
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<tr>
<td></td>
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<td>5</td>
<td>12</td>
<td>7</td>
<td>58.3</td>
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*Number of tumors at the day of excision in this group.
Prdx6 protects from lipid peroxidation in vivo

In light of these negative results, we hypothesized that Prdx6 protects from skin tumorigenesis through reduction of oxidative stress, which is essential for the preservation of genomic integrity (28). Indeed, our previous studies showed that Prdx6 protects keratinocytes in vitro from ROS-induced oxidative damage and suppresses the oxidative stress in healing skin wounds (13, 14). As the oxidative stress in TPA-treated skin is lower than in wounded skin (our unpublished data), a sensitive readout for oxidative stress was required to determine potential differences between wt and Prdx6-mutant mice. A lipid peroxidation chain reaction is initiated in cells by oxidation of membrane phospholipids that contain the bulk of cell-associated polyunsaturated fatty acids. Therefore, we analyzed the levels of oxidized phospholipids in the epidermis in vivo using mass spectrometry (23). We focused on phospholipid hydroperoxides, as some of these species had previously been identified as substrates of Prdx6 (29, 30). Furthermore, they are the starting point of the vast majority of nonenzymatic reactions leading to lipid peroxidation. We analyzed the levels of the most abundant phospholipid hydroperoxides, that is, SAPC-OOH, SLPC-OOH, PAPC-OOH, and PLPC-OOH relative to their nonoxidized precursor phospholipids. In addition, we quantified the lyosphospholipids Lyso-PPC and Lyso-SPC.

Figure 3. Overexpression of Prdx6 in keratinocytes does not affect the response of the skin to DMBA or TPA.

A, RNA from nontreated skin of wt (n = 6) and Prdx6-tg mice (n = 6) was analyzed for expression of enzymes involved in DMBA metabolism [cytochrome P4501a1 (Cyp1a1), Cyp1b1, glutathione S-transferase A1 (Gsta1), Gsp1, and NAD(P)H dehydrogenase quinone 1 (Nqo1)]. Mean expression levels in wt mice were arbitrarily set to 1. B, RNA from the skin of wt (n = 6) and Prdx6-tg mice (n = 6) 24 hours after the third TPA treatment was analyzed for expression of Prdx6. C, epidermal thickness of skin treated with DMBA and/or TPA at different time points of treatment. Scatter plots and representative histologic pictures are shown. Bars, 100 μm. D and E, sections from DMBA- and/or TPA-treated skin of wt and Prdx6-tg mice were analyzed by PCNA immunofluorescence (D) or Ly-6G immunohistochemistry (E) at different time points of treatment. Scatter plots and representative stainings are shown. D, dermis; E, epidermis. Bar, 100 μm. F, RNA from skin of wt (n = 6) and Prdx6-tg mice (n = 6) 24 hours after the third TPA treatment was analyzed for expression of proinflammatory cytokines. Mean expression levels in wt mice were arbitrarily set to 1. Rps29 was used for normalization of all qRT-PCR results. All graphs show mean values and SEM.
which represent the terminal oxidation products of phospholipids due to instability of oxidized chains within phospholipid molecules. This results in enzymatic or nonenzymatic cleavage and generation of the lyso-forms. The analysis was conducted 72 hours after 3 TPA treatments, as the levels of oxidized phospholipids were reported to increase after a single TPA treatment and even more after 3 TPA treatments (31), and as a preliminary experiment with wt mice revealed that the levels of PLPC-OOH/PLPC continuously increase within the first 72 hours after application of TPA (Supplementary Fig. S4). As expected, all analyzed phospholipid hydroperoxides increased in the epidermis from wild-type animals after TPA treatment as compared with untreated epidermis (Fig. 4A–F). However, the TPA-induced increase in phospholipid hydroperoxides was lower in the Prdx6-tg mice (left) but more prominent in the Prdx6-ko mice (right), with the single exception of PAPC-OOH/PAPC. Furthermore, the levels of lyso phospholipids were lower in TPA-treated Prdx6-tg mice than in wt controls, whereas they showed the opposite regulation in Prdx6-ko mice (Fig. 4E and F). These findings suggest that both endogenous and over-expressed Prdx6 reduce oxidative stress, thus supporting the notion that Prdx6 controls tumor formation and progression through ROS detoxification.

**PRDX6 is strongly expressed in normal human skin and in SCCs**

Finally, we analyzed PRDX6 expression in several sections from normal human skin (n = 3) and in cutaneous SCCs (n = 10) in parallel, using a highly specific, affinity-purified PRDX6 antiserum (13). Immunohistochemistry revealed strong PRDX6 expression in the epidermis and in blood vessels of normal human skin (Fig. 5A and B). Surprisingly, the PRDX6 staining intensity of human SCCs was highly variable. Some tumors showed only a weak PRDX6 immunoreactivity (Fig. 5C and D), whereas strong PRDX6 staining was observed in the tumor cells and the stroma of other SCCs (Fig. 5E and F). These different expression levels may well affect development and/or progression of human skin tumors.

**Discussion**

We identified Prdx6 as a novel preventer of skin carcinogenesis in 2 different tumor models. In the DMBA/TPA model tumor formation is mainly driven by mutations in the *ha-ras* proto-oncogene that are induced by DMBA (24, 25), whereas HPV8 induces tumor formation via its own oncogenic proteins, in particular E6 (22, 32). This involves microRNA-mediated downregulation of the tumor suppressors RB and PTEN (33). In contrast, ras mutations are rarely found in these tumors (22, 32). The similar effect of Prdx6 on skin tumorigenesis in these 2 models therefore suggests that the effect is not dependent on specific oncogenic events.

A hallmark of both skin tumor models is the chronic cutaneous inflammation (25, 27, 29). As inflammatory cells are major producers of ROS, oxidative damage of macromolecules progressively occurs during the tumorigenesis process (34). ROS produced upon DMBA metabolism further contribute to oxidative stress (35). Enhanced ROS levels covalently modify DNA and therefore increase the rate of mutations that contribute to tumorigenesis. In addition, ROS can modify...
proteins and lipids, thereby affecting cell proliferation, apoptosis, epithelial–mesenchymal transition, and/or inflammation (2, 12). Therefore, ROS are thought to be key players in carcinogenesis of different tissues and organs including the skin (36). However, this general picture is probably oversimplified, as DMBA/TPA-induced skin carcinogenesis was enhanced in transgenic mice overexpressing the selenoenzyme glutathione peroxidase alone or in combination with Cu/Zn-superoxide dismutase (SOD; ref. 37). In contrast, overexpression of Mn-SOD reduced DMBA/TPA-induced skin carcinogenesis, whereas heterozygous knockout of this enzyme had no effect (38, 39). Thus, the roles of individual ROS detoxifying enzymes are likely to depend on the type of ROS that they detoxify, on the intracellular localization of the enzyme, and on the expression level. Therefore, the tumor-suppressive effect of Prdx6 is particularly remarkable, as it was observed for the endogenous protein and also for overexpressed Prdx6. The capacity of Prdx6 to detoxify hydrogen peroxide and to directly reduce oxidized phospholipids provides a likely explanation for the beneficial effect and for the nonredundant functions of this enzyme in the prevention of skin tumorigenesis. The capability of Prdx6 to detoxify peroxynitrite is likely to further enhance the protective effect, as this aggressive molecule damages various types of cellular macromolecules (40). Our hypothesis that Prdx6 protects against skin carcinogenesis through enhanced ROS detoxification (Fig. 5G) is supported by our findings that proliferation, apoptosis, DMBA detoxification, and inflammation were not obviously affected by Prdx6 during skin tumorigenesis. Rather, we previously showed that Prdx6 overexpression protects keratinocytes from menadione- and UVA-induced cell damage and apoptosis (13). Furthermore, UV-induced apoptosis was reduced in Prdx6-tg and enhanced in Prdx6-ko animals (13, 14). Most importantly, a reduction in major types of oxidized phospholipids was observed in the TPA-treated skin of Prdx6-tg mice compared with wild-type controls (this study), providing evidence for reduced oxidative stress in these animals. This result also revealed that Prdx6 indeed affects the levels of oxidized phospholipids in vivo. Taken together, all these data point to an important role of the peroxidase activity of Prdx6 in skin carcinogenesis, although a role of its phospholipase A2 activity cannot be excluded.
Our data also revealed that overexpression of Prdx6 promotes malignant conversion of existing tumors in the DMBA/TPA model. Unfortunately, tumor progression could not be analyzed in the HPV8 model, as the rate of malignant conversion is generally low in these mice (22) and as the animals had to be eliminated due to the tumor size before malignant conversion had occurred. The result seen in the DMBA/TPA model is consistent with results obtained with mice lacking the antioxidant enzyme heme oxygenase-1, which were characterized by enhanced skin tumorigenesis, but reduced tumor progression upon application of the same tumorigenesis protocol (41). The most likely explanation is a protection from ROS-induced apoptosis of tumor cells by the overexpressed Prdx6 (Fig 5G) as previously shown for ovarian cancer cells (16) and for other peroxiredoxins in different cancer cells in vitro (20). Consistent with this hypothesis, Prdx6 is highly expressed or even overexpressed compared with normal tissue in several human cancers (5). Our immunohistochemical data revealed that PrDX6 is strongly expressed in keratinocytes and blood vessels of normal human skin. This is consistent with its function in skin tumor prevention that we identified in this study. Strong staining of PRDX6 was also seen in the tumor cells as well as in the stroma of some cutaneous human SCCs. Surprisingly, however, PRDX6 expression was strongly down-regulated in some other SCCs. These results are consistent with data obtained with other Prdx6 antibodies (published in The Human Protein Atlas database; http://www.proteinatlas.org/ENSG00000117592/cancer/skin/cancer), where obvious differences in the expression levels of PRDX6 were detected between different SCC samples and also between normal skin of different individuals. We confirmed this finding using a highly specific and affinity-purified Prdx6 antiserum, which we used to stain sections from normal skin and from tumors at the same time to directly compare differences in expression. In the future, it will be interesting to analyze PRDX6 expression in a larger number of normal skin and tumor samples and to monitor these patients with regard to skin tumor development and malignant progression. This will reveal if a differential expression of PRDX6 correlates with different susceptibility to skin carcinogenesis and if upregulation of this enzyme correlates with malignancy of the tumor and clinical outcome. Independent of these open issues, our results support the concept that Prdx6 is a tumor preventer rather than a tumor suppressor in the epidermis and suggest that activation or inhibition of Prdx6 could be useful for controlling different stages of skin carcinogenesis. Finally, they highlight the importance of wound-regulated genes in the pathogenesis of cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: S. Werner
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Analysis and interpretation of data (e.g., statistical analysis, bios-statistics, computational analysis): F. Rolfs, M. Huber, F. Gruber, V.N. Bochkov, E. Tscharchter, D. Hohl, M. Schärer
Writing, review, and/or revision of the manuscript: F. Rolfs, M. Huber, H.J. Pfister, V.N. Bochkov, R. Dummer, D. Hohl, M. Schärer, S. Werner
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Dummer, D. Hohl
Study supervision: D. Hohl, M. Schärer, S. Werner

Acknowledgments
The authors thank Mario Gysi, Christiane Born-Berclaz, and Nicole Hall-Schmid for excellent technical assistance; Drs. Tamara Ramadan and Katharina Birkner for help with the mouse experiments; Jennifer Helm for help with the short-term TPA treatment experiments; Dr. Beverly Paigen, Jackson Laboratories, for kindly providing Prdx6-knockout mice; Dr. Sigrun Smola, Saarland University (Homburg, Germany) for helpful suggestions; and Dr. Gertrude Beer, Zurich, for providing samples of normal human skin.

Grant Support
This work was supported by grants from the Wilhelm Sander-Stiftung (S. Werner), Cancer Research Switzerland (KFS 2822-08-2011 to S. Werner), the Promedica Foundation Chur (S. Werner), and the Swiss National Science Foundation (310030_132884/1 to S. Werner and 3100A3-138416 to M. Huber).

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Received November 28, 2012; revised March 20, 2013; accepted March 25, 2013; published OnlineFirst April 10, 2013.

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www.aacrjournals.org Cancer Res; 73(11) June 1, 2013 3469

34. Marcuzzi GP, Huftbauer M, Kasper HU, Weissenborn SJ, Smola S, Pfister H. Peroxiredoxin 6 and Skin Cancer
# Dual Role of the Antioxidant Enzyme Peroxiredoxin 6 in Skin Carcinogenesis

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