Enhanced Skin Carcinogenesis in Transgenic Mice with High Expression of Glutathione Peroxidase or Both Glutathione Peroxidase and Superoxide Dismutase

Yao-Ping Lu, You-Rong Lou, Patricia Yen, Harold L. Newmark, Oleg I. Mirochnichenko, Masayori Inouye, and Mou-Tuan Huang

ABSTRACT

Female transgenic mice (C57BL/6 × CBA/J) F1, with a 1-fold increase in expression of glutathione peroxidase (GP) or with a 3-4 fold increase in the expression of GP and a 3-4 fold increase in the expression of superoxide dismutase (SOD) had an enhanced carcinogenic response to initiation by 7,12-dimethylbenz(a)anthracene (DMBA) followed by promotion with 12-O-tetradecanoylphorbol-13-acetate (TPA). GP- or GP+SOD-transgenic mice that were initiated by a single topical application of 200 nmol of DMBA followed by promotion with 8 nmol of TPA twice weekly for 30 weeks developed an average of 10.9 or 11.0 skin tumors per mouse and a 100% tumor incidence in comparison with the corresponding nontransgenic mice, which had 3.9 tumors per mouse and an 83% tumor incidence. After stopping TPA application, partial skin tumor regression occurred more rapidly in nontransgenic mice than in either type of transgenic mouse. At 10 weeks after termination of TPA treatment, 9–11% of the tumor-bearing transgenic mice had 26% of the tumor-bearing nontransgenic mice had complete regression of their tumors. Histopathological examination of 96 skin papillomas revealed that the area, location, degree of tumor dysplasia, bromodeoxyuridine labeling index, and p53 protein levels were closely intercorrelated. Further analysis indicated that papillomas with the same grade of dysplasia had a higher bromodeoxyuridine labeling index and a greater p53 protein level in GP- or GP+SOD-transgenic mice than those in nontransgenic mice. The data indicated that overexpression of skin antioxidant enzymes GP or GP+SOD, which are enzymes that are believed to protect cells from oxidative damage by scavenging reactive oxygen species, lead to the increased, rather than the decreased, tumorigenesis in a DMBA/TPA two-stage skin carcinogenesis model.

INTRODUCTION

Oxygen radicals (ROS)3 have been suggested as causative factors in the carcinogenic processes and are implicated in degenerative diseases such as cancer, aging, and atherosclerosis (cardiovascular diseases; Ref. 1). ROS and other free radicals appear to play an important role in tumor promotion (2–4). Oxygen free radical-generating compounds such as benzoyl peroxide, lauroyl peroxide, and chloroperbenzoic acid have been reported to have tumor-promoting activity on mouse skin (4–6). The generation of oxygen free radicals occurs through the reduction of oxygen (O2) by a single electron pathway and produces the first superoxide anion radical (O2·−), leading to hydrogen peroxide (H2O2) formation. If these are not efficiently scavenged, the resulting hydroxyl radical (OH·) is produced. These ROS are generated in mitochondria, by xanthine oxidase, and NADPH oxidase (in membranes of neutrophils, leukocytes, and macrophages), through arachidonic acid metabolism pathways, catecholamine metabolism, and by myoglobin and iron-mediated radical generation (7). A respiratory burst is noted by the sudden rise of oxygen consumption in phagocytosing neutrophils, and a superoxide anion radical (O2·−) is produced by membrane-bound NADPH oxidoreductase (NADPH oxidase) when bacteria or foreign materials invade organs. This NADPH oxidase is activated by TPA, arachidonic acid, diacylglycerols, or other agonists. Thus, topical application of TPA or arachidonic acid to mouse skin or a skin wound results in the rapid accumulation of inflammatory cells such as neutrophils and macrophages (8), as well as an increase in the release of ROS (2–3). The superoxide anion radical is rapidly converted into hydrogen peroxide by SOD, and hydrogen peroxide may subsequently be converted to water by CT and GP, or the hydrogen peroxide may be converted to a hydroxyl radical, an extremely potent oxygen free radical, the reaction of which is catalyzed by trace metals.

Natural and artificial antioxidants inhibit the generation of oxygen free radical enzymes or directly scavenge the reactive oxygen free radicals to effectively remove the excess oxygen free radicals in cells. Many compounds that have antioxidant activity inhibit TPA-induced skin inflammation and tumor promotion in mouse skin (9–14). Butylated hydroxyanisole (9), quercetin (10), α-tocopherol (11), curcumin (12), green tea polyphenols (13), rosemary extract, and its constituent carnosol (14) are examples of compounds that possess antioxidant activity or reactive oxygen-scavenging activity and inhibit tumor promotion and/or certain biochemical events associated with tumor promotion in mouse skin. The biomimetic copper(II)(3,4-diisopropylsalicylic acid), which possesses superoxide dismutase activity, has demonstrated inhibition of TPA-induced ornithine decarboxylase activity and tumor promotion on mouse skin (15, 16). In addition, several inhibitors of arachidonic acid metabolism inhibit TPA-dependent tumor promotion in mouse skin (10, 12, 17, 18). Arachidonic acid metabolism is believed to release oxygen free radicals, and intermediate products of arachidonic acid metabolism may play an important role in the tumor promotion process. Thus, natural and artificial antioxidants as well as inhibitors of arachidonic acid metabolism can inhibit the enzymatic generation of oxygen free radicals or can directly scavenge ROS and inhibit inflammation and tumor promotion in mouse skin. However, the effects are dependent upon continuous antioxidant treatment.

In the present study, we attempt to evaluate the sensitivity of transgenic mice with overexpression of the antioxidant enzymes-human intracellular GP or human GP and Cu,Zn-SOD to skin tumor promotion. Surprisingly, these animals had enhanced tumorigenic response to topical application of DMBA/TPA. In this report, we described the enhancement of skin tumorigenesis in transgenic mice with high expression of antioxidant enzymes, GP or both GP and SOD.
MATERIALS AND METHODS

Materials. TPA was purchased from LC Services Corp. (Woburn, MA). DMBA was obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI). Acetone was obtained from Burdick and Jackson Laboratory (Muskegon, MI).

Transgenic Mice. Transgenic mice with human GP or Cu,Zn-SOD genes were produced as described by Hogan et al. (19). The coding regions of the human GP (20, 21) or Cu,Zn-SOD (22) genes were inserted into the unique BamHI site of the pHMG cassette vector (23). To generate transgenic mice, NotI fragments of recombinant plasmids were microinjected into the pronuclei of the C57BL16 x CBA/J hybrid. Mouse lines GP23 (containing 200 copies of the human GP gene) and SOD42 (containing 70 copies of the human SOD gene) described in previous publications (24, 25) were used for this study. To obtain normal and heterozygous transgenic animals for experiments, transgenic founders were bred with C57BL/6 x CBA/J mice.

Animal Tumor Studies. Mice were fed a Purina Laboratory Chow 5001 diet (Ralston-Purina Co., St. Louis, MO) and water ad libitum and kept on a 12-h light/12-h dark cycle. The dorsal region of each mouse was shaved with electric clippers 2 days before treatment with DMBA or TPA. For tumor studies on mouse skin, the mice were initiated topically with one treatment of 200 nmol of DMBA in 100 μl acetone; 1 week later, the mice were treated topically with 8 nmol (5 μg) of TPA in 200 μl acetone twice weekly for 30 weeks. Skin tumors greater than 1 mm in diameter were counted and recorded every 2 weeks. All of the animals were killed 10 weeks after the last dose of TPA treatment.

Histopathological Classification of Skin Lesions. Histopathological classification of skin lesions and tumors was done according to classification described by Bogovski (26). Skin dysplasias were arbitrarily classified into four grades: grade I, a small and mild hyperplastic lesion on epidermis containing approximately 0—20% of atypical cells; grade II, a middle or moderate hyperplastic lesion on epidermis containing 20—40% of atypical cells; grade III, a large or severe hyperplastic lesion on epidermis containing 40—60% of atypical cells; and grade IV, a large severe hyperplastic lesion with irregular basophilic nuclei on epidermis containing more than 60% of atypical cells including carcinoma in situ.

Enzyme Activities of GP and SOD. GP activity was measured by NADH-nitro blue tetrazolium assay at 540 nm according to the method described by Fried (27) with minor modifications (28, 29). SOD activity was determined according to the method described by Flohe and Gunzler (30) based on NADPH oxidation followed at 340 nm. Protein concentration was determined by the method of Lowry et al. (31) using BSA as the standard.

Immunohistochemical Measurement of GP and SOD Protein Levels. Polyclonal antiserum derived from sheep, which is an anti-human glutathione peroxidase, was purchased from Biodesign International, (Kennebunkport, ME). The samples were stained with Vectastain ABC kit (sheep IgG) from Vector Laboratory, Inc. A monoclonal antibody derived from mouse ascites fluid, which is a specific antihuman SOD, was purchased from Sigma Immuno Chemicals, Inc. (St. Louis, MO), and the samples were stained by the immunoperoxidase method using Histomouse SP kit purchased from Zymed Laboratories, Inc. (South San Francisco, CA). Skin samples were fixed in a 10% sodium citrate buffer (pH 6.0) in a microwave oven at high power for 10 min. They were then incubated with p53 antibody (1:500 dilution) for 1 h at room temperature, then incubated with Link (secondary antibody) for 6 min at 37°C, followed by incubation with Label (conjugated streptavidin solution) for 6 min at 37°C. The samples were treated with New Fuchs Substrate Pack (containing 0.6 mg/ml levamisole solution) for 30 min and counterstained with hematoxylin.

The staining procedure showed a positive reaction as a brown to dark brown (for BrdUrd incorporation) or a pink to red (for p53 protein accumulation) color precipitated in the nuclei of the cells. The appearance of nuclear stain was counted as positive. For BrdUrd incorporation, at least five representative areas per lesion were counted, and the BrdUrd labeling index was calculated from the number of BrdUrd-positive stained cells per 100 cells counted. For p53 protein staining, the reaction was semiquantitatively assessed by estimating the number and intensity of stained cells per lesion. In this way, three levels of reactions were observed: level 1, no reaction or weak reaction; level 2, moderate reaction; level 3, strong reaction.

RESULTS

Increased Expression of GP or SOD Enzyme Activity and GP or SOD Protein Levels in the Skin of Transgenic Mice. The GP activity in skin homogenates of GP-transgenic or GP+SOD-transgenic mice was greater than that in nontransgenic mice by at least 1-fold. The GP activity was 0.046 ± 0.012 unit/mg protein and 0.022 ± 0.04 unit/mg protein in GP-transgenic and nontransgenic mice, respectively (P < 0.01). The SOD activity was 6.04 ± 0.19 units/mg protein and 1.64 ± 0.09 units/mg protein in GP+SOD-transgenic and nontransgenic mice, respectively (P < 0.01). The SOD activity in skin homogenates of GP+SOD-transgenic mice was 3—4-fold higher than that in skin homogenates of nontransgenic mice. No significant difference was observed in the level of SOD activity between GP-transgenic mice and nontransgenic mice. In addition, GP protein was detected in epidermis, sebaceous glands, and hair follicles in GP- and GP+SOD-transgenic mice and nontransgenic mice by the immunohistochemical method. On the basis of the area and intensity of the staining, there was an approximate 1-fold increase of GP protein level in GP- or GP+SOD-transgenic mice compared to that in nontransgenic mice. SOD protein was only detected in the epidermis, sebaceous glands, and hair follicles of nontransgenic mice. A trace or a nondetectable level of SOD protein was found in GP-transgenic mice and nontransgenic mice. The ratio of GP or SOD enzyme activity in the skin homogenates of transgenic mice to the GP or SOD activity in the skin homogenate of nontransgenic mice was comparable to the ratio of GP or SOD protein levels in the skin of transgenic mice to the GP or SOD protein levels in the skin of nontransgenic mice.

Comparison of DMBA/TPA Two-Stage Tumorigenesis in Nontransgenic, GP-Transgenic, or GP+SOD-Transgenic Mice. Mice were initiated with a single dose of 200 nmol of DMBA and promoted with 8 nmol TPA twice weekly for 30 weeks. The first skin tumor appeared in both GP- and GP+SOD-transgenic mice at 10 weeks after TPA promotion, but it appeared in nontransgenic mice at 14 weeks after TPA treatment. Tumor latency is 4 weeks longer in nontransgenic mice than in both types of transgenic mice. An average of 3.9,
10.9, or 11.0 skin tumors per mouse developed in nontransgenic, GP-transgenic, or GP+SOD-transgenic mice, respectively. At 30 weeks after TPA promotion, 100% of GP- or GP+SOD-transgenic mice had skin tumors, whereas only 83% of nontransgenic mice had skin tumors (Fig. 1 and Table 1). The average tumor volume per tumor was 69, 102, or 115 mm³, respectively, in nontransgenic, GP-transgenic, or GP+SOD-transgenic mice.

After termination of TPA treatment, skin tumors regressed gradually. Ten weeks after the last dose of TPA, the mice were sacrificed, and skin tumors were examined histopathologically. An average of 0.14, 0.56, or 0.55 carcinomas per mouse; 1.4, 5.3, or 3.1 papillomas per mouse; and 1.5, 5.9, or 3.6 total skin tumors per mouse were found in nontransgenic, GP-transgenic, or GP+SOD-transgenic mice, respectively. Skin carcinoma incidence, papilloma incidence, and total skin tumor incidence were all lower in nontransgenic mice than in GP- or GP+SOD-transgenic mice. These data indicated that GP- or GP+SOD-transgenic mice were more sensitive to DMBA/TPA two-stage skin tumorigenesis than the corresponding nontransgenic mice.

Comparison of Skin Tumor Regression in Nontransgenic, GP-Transgenic, or OP+SOD-Transgenic Mice after Discontinuation of TPA Administration. Ten weeks after termination of TPA treatment, the number of tumor-bearing mice in nontransgenic mice decreased by 32%, whereas the number of mice with skin tumors in GP- or GP+SOD-transgenic mice only decreased by 9–11% (data calculated from Table 1). During this period, some animals died, and the death rate was higher in GP- or GP+SOD-transgenic mice than in nontransgenic mice. Twenty-two % of the nontransgenic mice died, whereas 40–42% of the GP- or GP+SOD-transgenic mice died during this 10-week period after discontinuation of TPA administration. The average tumor volume per tumor decreased by 55% in nontransgenic mice, whereas the average tumor volume per tumor in GP- or GP+SOD-transgenic mice was only reduced by 19 or 25%, respectively (data not shown). These results indicated that the skin tumor regression was slower in the GP- or GP+SOD-transgenic mice than in that in the corresponding nontransgenic mice.

**Comparison of Growth Rates of Skin Papillomas in Nontransgenic, GP-Transgenic, or GP+SOD-Transgenic Mice at 10 Weeks after Termination of TPA Treatment.** The skin tumor growth rate was estimated by the BrdUrd labeling index. Fig. 2A shows a skin papilloma with mild to moderate dysplasia (H&E stain) but with a high BrdUrd labeling index (Fig. 2B). In contrast, another papilloma with moderate to severe dysplasia (Fig. 2E with H&E stain) had a low BrdUrd labeling index (Fig. 2F). These observations suggested that the skin papilloma in Fig. 2, A and B, grew rapidly, although the tumor was only mildly dysplastic (accelerated growth), whereas the skin papilloma in Fig. 2, E and F, grew slowly, although the tumor was moderately to severely dysplastic (deaccelerated growth). These results and similar results with other tumors indicated that the grade of dysplasia in papillomas did not appear to be closely related to the proliferation rate; and in fact, some papillomas with severe dysplasia had a low proliferation rate, whereas other papillomas with a very low extent of dysplasia had a high proliferation rate. A total of 18 papillomas in nontransgenic mice, 47 skin papillomas in GP-transgenic mice, and 34 skin papillomas in GP+SOD-transgenic mice were analyzed for their grades of dysplasia and the BrdUrd labeling index. Unfortunately, we were unable to distinguish the difference in terms of the grade of dysplasia between nontransgenic, GP-transgenic, or GP+SOD-transgenic mice. However, studies at the molecular level have showed that the average ratio of BrdUrd labeling index to dysplasia grade in nontransgenic, GP-transgenic, or GP+SOD-transgenic mice was 17, 32, or 44, respectively (Fig. 3). These results indicated that based on the same grade of dysplasia, the papillomas had a significantly higher BrdUrd labeling index in transgenic mice than that in nontransgenic mice (Fig. 3). Skin papillomas with a high ratio of BrdUrd labeling index to dysplasia grade had a rapid growth rate. Comparably, skin papillomas with a low ratio of BrdUrd labeling index to dysplasia grade had a slow growth rate.

**Comparison of p53 Protein Levels of Skin Papillomas in Nontransgenic, GP-Transgenic, or GP+SOD-Transgenic Mice.** The ratio of p53 protein level to dysplasia grade was 0.9, 1.8, or 2.6 in nontransgenic, GP-transgenic, or GP+SOD-transgenic mice, respectively (Fig. 3). Compared with the same grade of dysplasia, skin papillomas in GP- or GP+SOD-transgenic mice had higher ratios of p53 protein level to dysplasia grade than that in nontransgenic mice. Recently, Nakano et al. (34) also reported that the expression of human Mn-superoxide dismutase protein level correlates with p53 protein status.

The accumulation of p53 protein is an independent marker of prognosis and is associated with rapid cell proliferation in certain tumors (35). The risk potential of skin papilloma progression to carcinoma can be determined by p53 protein accumulation using immunohistochemical assay. Fig. 2A shows a skin papilloma with mild to moderate dysplasia (H&E stain) but with a high p53 protein accumulation (Fig. 2C, and its negative control in Fig. 2D). In contrast, another papilloma with moderate to severe dysplasia (Fig. 2E, H&E stain) had a low p53 protein accumulation (Fig. 2G, and its negative control in Fig. 2H). These observations suggested that the skin papilloma in Fig. 2, A and C, may have high risk potential to malignancy, although the tumor was only mildly dysplastic (high risk tumor), whereas the skin papilloma in Fig. 2, E and G, may have low risk potential.
risk potential to malignancy, although the tumor had moderate to severe dysplasia (low risk tumor). Skin papillomas that have a higher p53 protein level may have a poorer prognosis.

DISCUSSION

The present results demonstrated that treatment of transgenic mice with overexpression of antioxidant enzymes GP or GP+SOD with DMBA/TPA led to increased papillomas per mouse and carcinomas per mouse as well as a higher papilloma and carcinoma incidence. Compared with the same grade of dysplasia, the papillomas showed a higher BrdUrd labeling index as well as a higher p53 protein level in OP- or OP+SOD-transgenic mice than the papillomas in nontransgenic mice. These results suggested that the transgenic mice with overexpressed antioxidant enzymes GP or GP+SOD were more sensitive to the DMBA/TPA two-stage skin tumorigenesis than the nontransgenic mice, and the papillomas in GP- or GP+SOD-transgenic mice had a higher proliferation rate and a higher risk potential to malignancy than that in nontransgenic mice.

It has been generally accepted and believed that antioxidants inhibit the carcinogenic process (9–14, 40). Most of the evidence for this has been obtained by studies involving the addition of antioxidants exogenously, which, in most cases caused direct extracellular scavenging of ROS. Activities of different antioxidant enzymes in already established tumors have been reported and reviewed (36–38). Altered enzymatic activities of different antioxidant enzymes were frequently found, although no clear patterns were established. At least in human tumors, there was a tendency of decreased Cu,Zn-SOD, Mn-SOD, and CT activities and increased GP and glutathione reductase (39, 40). These data are, unfortunately, not sufficient to answer the fundamental question of whether changes in activities of these enzymes are one of the many causes of cancer, or if they are, instead, the consequence of already established neoplastic abnormalities. The efficiency of transformation in vitro after introduction of excess antioxidant enzymes or their inhibition has also been analyzed (41). Contradictory data were obtained, dependent on cell type, carcinogen nature, and path of enzymatic activity modulation. Also, this does not facilitate understanding of the process of carcinogenesis in vivo. In this respect, transgenic mice with overproduction of antioxidant enzymes represent a unique model system, enabling us to test the process of induced carcinogenesis in the presence of increased antioxidant enzymes in the whole animal.

Low levels of ROS have been shown to influence cell growth, whereas high levels of ROS have direct cytotoxic effects. On the basis of these properties, we have been able to characterize the cancer cell of nontransgenic as well as transgenic mice. Comparison of the rate of skin papilloma growth in nontransgenic mice to that in GP- or GP+SOD-transgenic mice for a 10-week interval after termination of TPA treatment as determined by tumor size and BrdUrd labeling index revealed that an average papilloma volume per mouse or the BrdUrd labeling index was greater in GP- or GP+SOD-transgenic mice than in nontransgenic animals. The ratio of BrdUrd labeling index to dysplasia was significantly higher in GP- or GP+SOD-transgenic mice (Fig. 3). These data suggested that papillomas with similar grades of dysplasia in GP- or GP+SOD-transgenic mice grew more rapidly than papillomas in nontransgenic mice. Similar dysplastic papillomas in GP- or GP+SOD-transgenic mice also regressed more slowly than that in nontransgenic mice.

It is known that the intracellular oxidation/redox status is a critical regulator of cytokines and growth factor signal transduction pathways through the activity modulation of protein kinases, phosphatases (42), transcription factors, and proto-oncogenes (43). The exact mechanisms of enhancement by DMBA/TPA-induced skin tumorigenesis in these transgenic mice are unknown at present, but several considerations should be taken into account. GP is a key enzyme in the cellular defense mechanisms against oxidative stress. Several reports indicated that this enzyme is more efficient than SOD or CT (44). The ability of this enzyme to use a wide range of hydroperoxides enables it to play, not only a major protective role, but also a regulatory one. This regulation, for example, occurs at least in part through modulation of the arachidonic acid metabolic pathway. Several studies indicated the presence of an “endogenous peroxide tone” necessary for eicosanoid production (45). GP activity alters the profile of the lipoxygenase and cyclooxygenase products (46, 47) ultimately involved in tumorigenesis (48).

The development of malignant tumors may depend not only on neoplastic transformation but also on the failure of host resistance to eliminate aberrant cells. Several mechanisms may be suggested. Cells with increased activity of antioxidant enzymes (GP) should be highly resistant to activated neutrophil-mediated cytotoxicity. If such cells already have genetic mutations, they will be able to overcome immune protection systems that may ultimately lead to increased malignancy. Down-regulation of MHC class I expression has frequently been reported in breast, colon, urinary, and kidney tumors (49). Loss of MHC class I antigen expression has been related to mutations and down-regulation of the MHC class I gene products. Most importantly, it was demonstrated recently that H2O2 is able to selectively activate

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Table 1 Comparison of DMBA/TPA-induced skin tumorigenesis in nontransgenic, GP-transgenic, and GP+SOD-transgenic mice

<table>
<thead>
<tr>
<th>Groupa</th>
<th>No. of mice per group</th>
<th>Papilloma</th>
<th>Carcinoma</th>
<th>Total tumors</th>
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<td></td>
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<td>Papillomas per mouse</td>
<td>% of mice with papillomas</td>
<td>% of mice with carcinomas</td>
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<tr>
<td>A</td>
<td>Nontransgenic mice</td>
<td>18</td>
<td>3.9 ± 0.4</td>
<td>83</td>
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<td>GP-transgenic mice</td>
<td>15</td>
<td>11.0 ± 0.8*</td>
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<td>GP+SOD-transgenic mice</td>
<td>19</td>
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<td>B</td>
<td>Nontransgenic mice</td>
<td>14</td>
<td>1.4 ± 0.6</td>
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<td>GP-transgenic mice</td>
<td>9</td>
<td>5.3 ± 1.6f</td>
<td>89d</td>
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<td>GP+SOD-transgenic mice</td>
<td>11</td>
<td>3.1 ± 0.7f</td>
<td>82f</td>
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*Statistically different from the nontransgenic mouse group P < 0.01 as determined by the Student’s t test.

†Statistically different from the nontransgenic mouse group P < 0.05 as determined by the Fisher’s exact test.

aStatistically different from the nontransgenic mouse group P < 0.1 as determined by the Fisher’s exact test.
Fig. 2. Comparison of skin tumor dysplasia, BrdUrd incorporation into DNA, and p53 protein expression between transgenic mice and nontransgenic mice. A. skin papilloma with mild dysplasia; H&E staining (×200). B. skin papilloma with high BrdUrd incorporation, rapidly (accelerated) growing (×200). C. skin papilloma with high p53 protein accumulation—high risk potential to malignancy (×200). D. normal rabbit IgG (negative control for p53). E. skin papilloma with moderate to severe dysplasia; H&E staining (×200). F. skin papilloma with low BrdUrd incorporation, slowly (deaccelerated) growing (×200). G. skin papilloma with low p53 protein accumulation, low risk potential to malignancy (×200). H. normal rabbit IgG (negative control for p53).

MHC class I gene expression (50). If this mechanism takes place in vivo, scavenging peroxides by excessive GP in transgenic mice might help cancer cells escape recognition by CTLs. Measurement of MHC class I expression in papillomas from normal and transgenic animals, as well as comparison of induction of these molecules in primary fibroblasts under oxidative stress conditions, is under way.

Our preliminary data indicated that SOD transgenic mice also developed more tumors than nontransgenic mice in experiments similar to ones described here. More importantly, we also demonstrated that these animals possessed abnormalities in their immune system (25). Overexpression of the human Cu,Zn-SOD enzyme in SOD transgenic mice altered microbicidal and fungicidal activity of phagocytes. These cells showed altered endogenous and exogenous ROS production and signal transduction pathway. Changes in the properties of immune cells and in the pro-oxidant state of other cell types in SOD transgenic mice, as reported by several groups (51), may underlie the increased sensitivity to tumorigenesis, possibly related to reduced surveillance of tumor cells by the immune system.

The GP+SOD-transgenic mouse strain also showed increased incidence of induced tumor formation compared to normal animals; therefore, it can be proposed that at the existing ratio of SOD to GP activity, there is still an unbalanced production of ROS. If we theorize that the levels of both enzymes increase at similar ratios in the cells, the altered enzyme levels could influence the process of tumorigenesis via mechanisms discussed above: by modulating cell growth; increasing resistance of cells with mutations to ROS; or by altering immune function. We did not observe an additive effect on the efficiency of tumor formation, even by overproduction of both enzymes, suggesting...
that one enzyme does not dominate the other. Detailed analysis of specific ROS production at different stages of multistep carcinogenesis in these transgenic animals should help to make a final conclusion concerning the mechanism of their increased susceptibility.

An interesting similarity was observed in the work of Machlemater et al. (52), who have shown that the difference in sensitivity of two clones of mouse epidermal JB6 cells to tumor promotion may lie in the 2-3-fold higher Cu,Zn-SOD and CT activity in a promotable clone in comparison to a nonpromotable clone. Cu,Zn-SOD and CT transfectants revealed different cell growth response dependent on the relative level of overproduction of these enzymes in the clones. The authors’ conclusion was that under oxidative conditions, only the promotable clone was protected. However, the results do not provide a complete and multistage carcinogenesis in mouse skin. Carcinogenesis (London), 8: 889—889, 1987.


29. Amstad P., Peskin, A., Shah, G., Minault, M.-E., Moret, R., Zhbden, I., and Cerutti, P. The balance between Cu,Zn-superoxide dismutase and catalase affects the sensi-
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