Increased Susceptibility of Vault Poly(ADP-Ribose) Polymerase–Deficient Mice to Carcinogen-Induced Tumorigenesis

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Abstract

Vault poly(ADP-ribose) polymerase (VPARP) and telomerase-associated protein 1 (TEP1) are components of the vault ribonucleoprotein complex. Vaults have been implicated in multidrug resistance of human tumors and are thought to be involved in macromolecular assembly and/or transport. Previous studies showed that VPARP-deficient mice were viable, fertile, and did not display any vault-related or telomerase-related phenotype, whereas disruption of telomerase-associated protein 1 in mice led to reduced stability of the vault RNA and affected its stable association with vaults, although there were no telomerase-related changes. In this study, we evaluated the susceptibility of *Vparp*$^{-/-}$ and *Tep1*$^{-/-}$ mice to dimethylhydrazine-induced colon tumorigenesis and urethane-induced lung tumorigenesis. Mice received i.p. injections of either 1 g/kg body weight of urethane twice a week for 2 weeks or 20 mg/kg body weight of dimethylhydrazine once a week for 10 weeks and were analyzed after 10 and 60 weeks, respectively. The colon tumor incidence and multiplicity were significantly higher and colon tumor latency was significantly shorter in *Vparp*$^{-/-}$ mice compared with wild-type mice. Increased colon tumor incidence, multiplicity, and reduced tumor latency were also seen in *Tep1*$^{-/-}$ mice, however, these results were statistically not significant. Lung tumor multiplicities were increased in both *Vparp*$^{-/-}$ and *Tep1*$^{-/-}$ mice but were not significant. The increase in carcinogen-induced tumors in VPARP-deficient mice is the only phenotype observed to date, and suggests a possible role for VPARP, directly or indirectly, in chemically induced neoplasia. (Cancer Res 2005; 65(19): 8846-52)

Introduction

Vault poly(ADP-ribose) polymerase (VPARP) and telomerase-associated protein-1 (TEP1) are components of the largest known ribonucleoprotein complex, the vault particle, found in the cytoplasm of mammalian cells (1, 2). Vaults are 13 MDa in size with a distinct barrel-shaped structure resembling arched cathedral ceilings. They are conserved throughout evolution in phylogeny as diverse as mammals, avians, amphibians, and the slime mold *Dictyostelium discoideum* (3). Approximately 70% of the total mass of the vault particle is made of the ~100 kDa major vault protein (MVP) and recombinant vault particles can be purified from *Sf9* insect cells (lacking endogenous vaults) expressing only the MVP using a baculovirus expression system (4). In addition to the three proteins, vaults also contain a unique untranslated RNA, the vault RNA (5).

Vaults or MVP have been shown to be up-regulated in many multidrug-resistant cancer cell lines and in a wide variety of human tumors. Some clinical studies have shown a correlation between MVP expression and poor response to chemotherapy (6). Although the majority of cellular vaults are cytoplasmic, a small population has been localized to the nuclear pore complex (7) and several research groups have suggested a role for vaults in intracellular transport (8, 9). Recently, MVP has been proposed to function as a novel scaffold protein for SHP-2 and Erk, two proteins involved in cell signaling (10). It has been estimated that higher eukaryotic cells contain 10,000 to 100,000 vault particles and an even higher abundance of vaults is found in cells and tissues that have been exposed to xenobiotics (6, 11). These include macrophages, epithelial lining of the gastrointestinal tract and lung. These observations, along with the highly conserved structure of vaults, has led to the hypothesis that vaults are involved in cellular defense against toxic compounds; thereby contributing to the drug resistance phenotype in cancer cells. MVP knock-out mice in which vault particles are absent have been generated. These mice are viable, fertile, and have no obvious phenotype including lack of increased sensitivity to cytotoxic drugs (12). Recent studies involving drug efflux kinetics, intracellular distribution of drugs, and small interfering RNA have indicated that vaults do not play a direct role in the multidrug-resistant phenotype (13, 14).

TEP1 was identified as the mammalian homologue of the *Tetrahymena* p80 telomerase protein and has been shown to interact specifically with vault RNA, telomerase RNA, and the catalytic protein subunit hTERT (2, 15–18). However, purified vaults have no detectable telomerase activity and there is no correlation between vault protein levels and telomerase activity in cancer cells (2, 19). The sharing of TEP1 protein between the two ribonucleoproteins, vaults, and telomerase, suggests that it may play a role in some aspect of ribonucleoprotein structure, function, and/or assembly. Mice deficient in TEP1 do not display any abnormalities and have normal-looking vaults, normal telomerase activity and telomere lengths (20). There is no difference in the level of telomerase RNA or its association with the telomerase complex in these mice compared with wild-type mice. Interestingly, the absence of TEP1 protein in these mice completely disrupts the stable association of vault RNA with vaults and results in decreases in the levels and stability of vault RNA showing that TEP1 is important for the stability of vault RNA and for vault RNA's stable association with the vault particle (21).

VPARP was originally identified as a MVP–interacting protein in a yeast two-hybrid screen and has been shown to be a novel PARP. Purified vaults also possess this enzymatic activity and MVP can serve as a substrate (1). In addition to their presence in vaults, TEP1 and VPARP exist in a non–vault-associated state in the cell (21). VPARP has also been localized to the nuclear pore

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(22) and the mitotic spindle (1) in addition to the cytoplasm, suggesting that it is likely to have some function at these sites. Recently, VPARP has been shown to associate with telomerase activity and interact with exogenously expressed TEP1 in mammalian cells (23). However, mice deficient in VPARP appear normal and do not show any changes in telomerase activity or telomere length and vaults purified from these mice look normal (23). These mice do not show any increase in genetic or telomere instability. Double-mutant mice lacking both VPARP and TEP1 have also been generated and these mice show no abnormalities with respect to telomerase activity, telomere length maintenance, and vault structure (23).

Because MVP alone can form vault particles (4, 23), TEP1, VPARP, and the vault RNA may be considered as the functional components of vaults. The association of TEP1 and VPARP with telomerase activity, the localization of VPARP at the mitotic spindle, and the ADP-ribosylation activity of VPARP prompted us to investigate whether *Tep1*$^{-/-}$ and *Vparp*$^{-/-}$ mice are more susceptible to carcinogen-induced tumor development compared with wild-type mice. We used two different carcinogens, dimethylhydrazine (24) and urethane (25), both extensively used for the induction of colon and lung tumors, respectively. Our studies show increased carcinogen-induced tumorigenesis in *Vparp*$^{-/-}$ mice and a trend towards increased tumor multiplicity in both knock-out mice compared with wild-type controls.

Materials and Methods

Animals. The generation of *Tep1*$^{-/-}$ and *Vparp*$^{-/-}$ mice has been described elsewhere (20, 23). The wild-type and *Tep1*$^{-/-}$ mice used in these studies were in a pure C57BL/6 background, obtained by backcrossing seven times, mice in a mixed 129SvJ/C57BL/6 background to mice in a pure C57BL/6 background. Wild-type and *Vparp*$^{-/-}$ mice used for colon tumor studies were in a 129SvJ/C57BL/6 mixed background, whereas the ones used for lung tumor studies were in a pure BALB/c background obtained by backcrossing six times, mice in a 129SvJ(10%)/C57BL/6 (90%) mixed background to pure BALB/c mice. Both male and female mice were used in these studies. All experimental procedures done on mice were in accordance with the guidelines of the Chancellor's Animal Research Committee for the University of California at Los Angeles.

Tumor Induction and Examination

Lung tumors. Nine-week-old mice were injected with 1,000 mg/kg body weight of urethane (dissolved in 0.9% NaCl) i.p. twice a week for 2 weeks. Mice were monitored daily for overall condition and weighed weekly. They were euthanized at either 10 or 20 weeks (as described in Results) after the first injection or earlier if they appeared clinically sick or lost >10% body weight. Following euthanasia, the carcass and body cavities were examined macroscopically for presence of tumors and/or other lesions. Any lesions observed and the presence and number of lung tumors were noted.

Colon tumors. Six-week-old mice were injected i.p. with 20 mg/kg body weight of dimethylhydrazine (dissolved in 0.9% NaCl containing 1 mmol/L EDTA and pH adjusted to 6.5 with NaOH) once a week for 10 weeks. Mice were monitored daily for overall condition and weighed weekly. They were euthanized 60 weeks after the first injection or when they showed signs of sickness/tumors (presence of blood in stools) or >10% body weight loss. Following euthanasia, various organs were examined macroscopically for the presence of tumors and/or other lesions. The entire intestine from the stomach to the anus was removed and the large bowel (from caecum to anus) was isolated. It was then opened longitudinally, washed with PBS, and any lesions/tumors detected were measured. Some of the tumors were dissected for tissue culture. The rest of the tissue was fixed for histopathology. After fixation, the colon was trimmed, routinely processed, embedded, 4 μm sections were cut and stained with H&E.

Statistical analysis. The significance of differences in tumor incidences was analyzed by χ^2 test and Fishers exact test when sample size was small. Student's t test was used to compare the number of tumors per mouse (tumor multiplicities) and while performing the t test, we did not assume that the variances were equal. Wilcoxon rank sum test was used for tumor volume comparisons. Data presented for tumor multiplicity are mean ± SE. Volume calculations were done using the formula: volume = (width^2 × length / 2). Time until tumor (latency) was determined by the method of Kaplan-Meier and differences between groups were assessed by log-rank test.

Results

Colon tumor development in *Vparp*-deficient mice. A total of 36 (20 female, 16 male) wild-type and 44 (23 female, 21 male) *Vparp*$^{-/-}$ mice were treated with dimethylhydrazine to induce colon tumors and analyzed for tumor incidence (number of mice that developed tumors), tumor multiplicity (number of tumors per tumor-bearing mouse) and tumor latency (time to tumor development). Histopathologic examination of colons of all the mice revealed the presence of tumors in some of the mice that were tumor-negative at autopsy (gross). The data for tumor incidence and tumor multiplicity are summarized in Table 1. The tumor incidence was significantly higher (P = 0.02) in the *Vparp*$^{-/-}$ mice (73%) compared with the wild-type mice (47%). Macroscopic (gross) examination at autopsy revealed that in addition to colon tumors, some of these mice developed lesions in other organs including liver, kidney, uterus, and lymph nodes, most of which turned out to be tumors. The incidence of all the tumors combined (colon and others) was 89% (39 of 44) in the *Vparp*$^{-/-}$ and 67% (24 of 36) in the wild-type mice (P = 0.02).

We then determined the average number of colon tumors per tumor-bearing mouse and found that it was significantly higher in the *Vparp*$^{-/-}$ (1.8 ± 0.1) compared with the wild-type mice (1.3 ± 0.1) as shown in Table 1 (P = 0.04). The tumor volume was also calculated for each tumor. Even though the average tumor volume was higher in the *Vparp*$^{-/-}$ (18 ± 4 mm^3) compared with wild-type mice (13 ± 2 mm^3) and 11% of the *Vparp*$^{-/-}$ tumors had a diameter of >40 mm^3, whereas none of the wild-type tumors were ≥40 mm^3, the differences were statistically not significant.

Not only did the *Vparp*$^{-/-}$ mice have a higher incidence and multiplicity of tumors, they developed tumors earlier than the wild-type mice (Fig. 1, P = 0.02). The average time for tumor development was 39 ± 2 weeks in the *Vparp*$^{-/-}$ mice, and 44 ± 2 weeks in the wild-type mice. In addition, 28% of *Vparp*$^{-/-}$ mice developed tumors within 30 weeks, whereas only 6% of wild-type mice developed tumors in that time.

Table 1. Colon tumor development in *Vparp*$^{+/+}$ and *Vparp*$^{-/-}$ mice

Genotype	Incidence* (%)	Multiplicity† (total)
Vparp$^{+/+}$	17 of 36 (47)	1.3 ± 0.1 (22)
Vparp$^{-/-}$	32 of 44‡ (73)	1.8 ± 0.1§ (56)

*Number of mice bearing tumors per total number of mice analyzed.
† Average number of tumors per tumor-bearing mouse ± SE.
‡ Significantly different at P = 0.02 by χ^2 test.
§Significantly different at P = 0.04 by Student's t test.

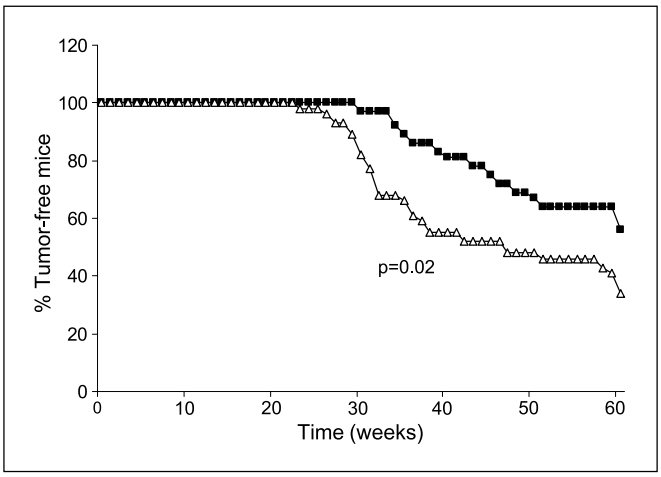

Figure 1. Kaplan-Meier plot of colon tumor incidence in wild-type (■) and *Vparp*$^{-/-}$ (△) mice. The percentage of mice remaining tumor-free with respect to total number of mice at the outset is plotted against time in weeks after the first injection of dimethylhydrazine. Mice were sacrificed when they showed signs of tumors or sickness and autopsied as described in Materials and Methods. All the remaining mice were sacrificed at 60 weeks. The tumor latency was significantly shorter ($P = 0.02$ by log-rank test) in *Vparp*$^{-/-}$ mice compared with wild-type mice.

Histopathologically, the colon lesions were classified into either (*a*) areas of hyperplasia, or dysplasia, (*b*) adenomas or (*c*) carcinomas. Figure 2 shows typical colon tumors seen in these mice including an adenocarcinoma (Fig. 2*A* and *B*) and a polypoid adenoma (Fig. 2*C* and *D*). Tumors in organs other than the colon were identified as adenoma, hepatocellular carcinoma, hemangioma, and hemangiosarcoma in the liver; hemangiosarcoma in kidney and uterus; lymphosarcoma, follicular lymphoma and carcinoma in the small intestine; lymphosarcoma and lymphoblastic lymphoma in spleen and lymph nodes; Hardenian gland adenoma in eye and squamous cell tumor and carcinoma in anus. There were no significant differences between *Vparp*$^{-/-}$ mice and wild-type mice in the presence of one form of tumor versus another.

Lung tumor development in *Vparp*-deficient mice. Urethane was used to induce lung tumors in wild-type and *Vparp*$^{-/-}$ mice. The results of lung tumor incidence and multiplicity from a total of 12 (10 female, 2 male) wild-type mice and 10 (7 female, 3 male) *Vparp*$^{-/-}$ mice are shown in Table 2. All the mice were sacrificed 10 weeks after the first urethane injection, hence, tumor latency was not evaluated and because most of the mice developed lung tumors, tumor incidences were not compared. However, the total number of tumors per mouse was increased in *Vparp*$^{-/-}$ mice (3.3 ± 0.6) compared with wild-type mice (2.5 ± 0.4), although this difference was not significant (Table 2).

Colon tumor development in *Tep1*-deficient mice. A total of 43 (18 females, 25 males) *Tep1*$^{-/-}$ mice and 39 (21 female, 18 male) background-matched wild-type mice were treated with dimethylhydrazine and analyzed as described above for *Vparp*$^{-/-}$ mice. The colon tumor incidence (Table 3) in dimethylhydrazine-treated *Tep1*$^{-/-}$ mice (37%) was higher than in wild-type mice (23%). However, this difference was statistically not significant. The lower incidence of colon tumors in these mice compared with *Vparp*$^{-/-}$ mice are most likely due to the background strain differences. Histopathology did not find tumors in any more mice other than those observed at autopsy. These mice also showed the presence of lesions in other organs, most of which turned out to be tumors similar in type to the ones seen in *Vparp*$^{-/-}$ mice and their wild-type controls as listed above. The incidence of all the tumors combined was 64% (25 of 39) and 52% (23 of 44) in wild-type and *Tep1*$^{-/-}$ mice, respectively. The average number of colon tumors in

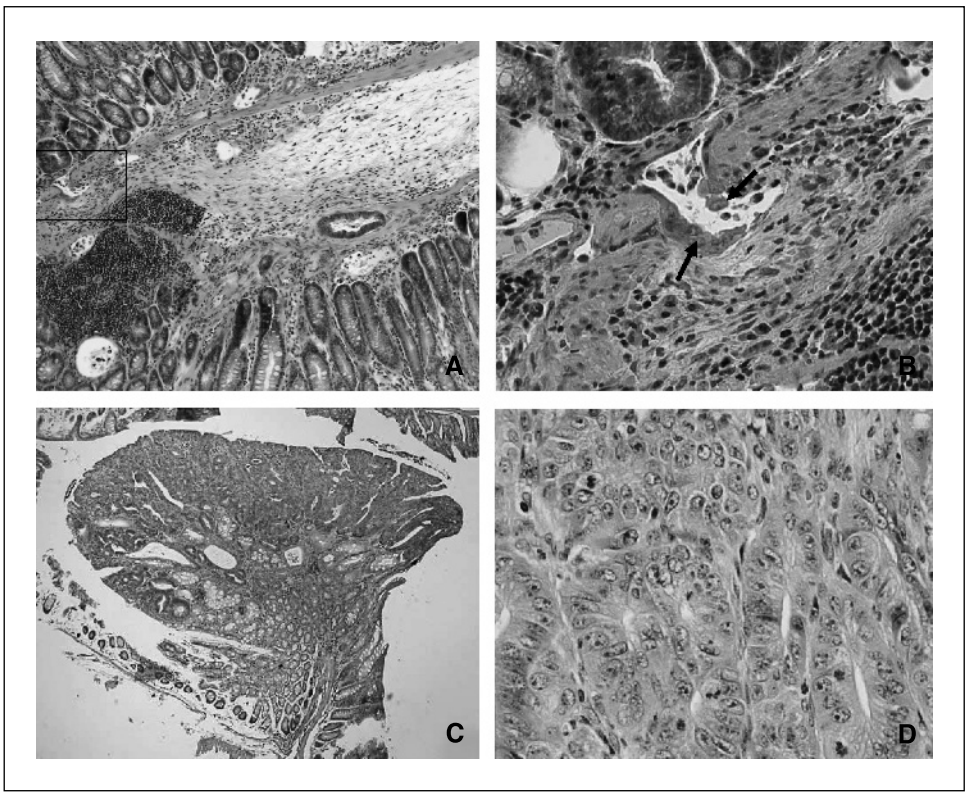

Figure 2. Histologic appearance of representative colon tumors seen in dimethylhydrazine-treated mice. *A,* typical colon adenocarcinoma in a *Vparp*$^{-/-}$ mouse. Several neoplastic glands penetrate the muscularis mucosa into the submucosa. There is a rich chronic inflammatory response with predominance of lymphocytes and plasma cells. H&E (10×). *B,* inset from (*A*) shows a neoplastic gland that has invaded the submucosa. The cells lining the gland (*arrows*) are enlarged with round vesicular nuclei and prominent nucleoli. *C,* adenoma of the colon in a *Vparp*$^{-/-}$ mouse. Many of these tumors were polypoid and contained areas of dysplasia. H&E (4×). *D,* dysplastic glands in a polypoid adenoma in a *Vparp*$^{-/-}$ mouse. The glands show a complex pattern, back to back packing, and goblet cell depletion. Cells are basophilic, show "picket fence" arrangement, have large nuclei, and prominent nucleoli. These glands do not penetrate the muscularis mucosa or the stalk. Also, the neoplastic cells are still contained and do not penetrate the basement membrane of the gland. There are increased numbers of mitotic figures. H&E (40×).

Table 2. Lung tumor development in *Vparp*$^{+/+}$ and *Vparp*$^{-/-}$ mice

Genotype	Incidence* (%)	Multiplicity† (total)
Vparp$^{+/+}$	10 of 12 (83)	2.5 ± 0.4 (25)
Vparp$^{-/-}$	10 of 10 (100)	3.3 ± 0.6 (33)

*Number of mice bearing tumors per total number of mice analyzed.
† Average number of tumors per tumor-bearing mouse ± SE.

Tep1$^{-/-}$ mice (1.4 ± 0.2) was higher than in wild-type mice (1.1 ± 0.1), however, this difference was not significant (Table 3). The average tumor volume in the *Tep1*$^{-/-}$ mice (38 ± 16 mm^3) was higher than the wild-type mice (28 ± 9 mm^3) and 14% of the *Tep1*$^{-/-}$ tumors were >100 mm^3 whereas none of the wild-type tumors attained that size. Here too, these differences were not significant. There was no difference between the tumor latencies of *Tep1*$^{-/-}$ mice and wild-type mice with the average time to tumor development being 51 ± 3 and 47 ± 3 weeks, respectively (Fig. 3). Histopathology showed features similar to those seen in Fig. 2 and revealed no significant differences in the presence of one type of tumor versus another.

Lung tumor development in *Tep1*-deficient mice. The results of urethane-induced lung tumors in *Tep1*$^{-/-}$ mice and their wild-type controls are given in Table 4. We analyzed 27 (9 female, 18 male) *Tep1*$^{-/-}$ mice and 23 (15 female, 8 male) wild-type mice in our first study where all the mice were sacrificed at 20 weeks after the first urethane injection. There was an increase in the tumor multiplicity in the *Tep1*$^{-/-}$ mice (3.8 ± 0.5) compared with the wild-type mice (3.0 ± 0.4), however, this was statistically not significant. During this study, four mice of each genotype were sacrificed at 10 weeks and three of the *Tep1*$^{-/-}$ mice and only one of the wild-type mice showed the presence of tumor-like lesions. Hence, we decided to repeat this study and analyze mice at 10 weeks after the first urethane injection. A total of 16 (7 female, 9 male) wild-type and 16 (8 female, 8 male) *Tep1*$^{-/-}$ mice were analyzed. In this study also, the tumor multiplicity was increased (Table 4) in the *Tep1*$^{-/-}$ mice (4.9 ± 1.0) compared with the wild-type mice (3.5 ± 0.7) but this was statistically not significant.

Thus, our results indicate that *Vparp*$^{-/-}$ mice are more susceptible to carcinogen-induced tumorigenesis. There is an increase in the number of tumors in *Tep1*$^{-/-}$ mice and although not statistically significant, this may warrant further investigation.

Table 3. Colon tumor development in *Tep1*$^{+/+}$ and *Tep1*$^{-/-}$ mice

Genotype	Incidence* (%)	Multiplicity† (total)
Tep1$^{+/+}$	9 of 39 (23)	1.1 ± 0.1 (10)
Tep1$^{-/-}$	16 of 43 (37)	1.4 ± 0.2 (22)

*Number of mice bearing tumors per total number of mice analyzed.
† Average number of tumors per tumor-bearing mouse ± SE.

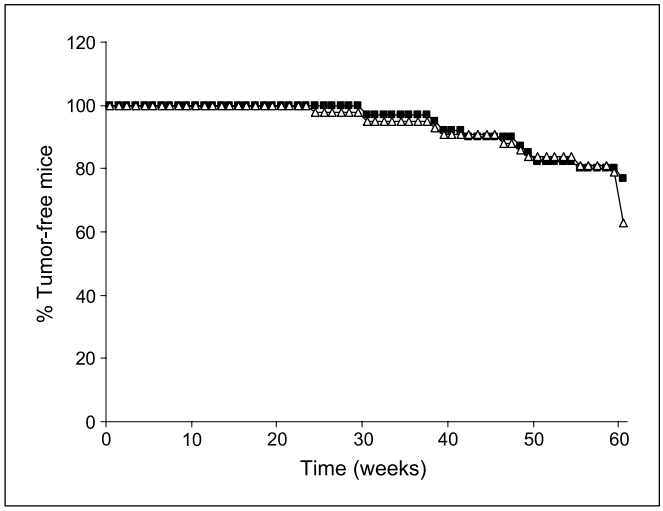

Figure 3. Kaplan-Meier plot of colon tumor incidence in wild-type (■) and *Tep1*$^{-/-}$ (△) mice. The percentage of mice remaining tumor-free with respect to total number of mice at the outset is plotted against time in weeks after the first injection of dimethylhydrazine. Mice were sacrificed when they showed signs of tumors or sickness and autopsied as described in Materials and Methods. All the remaining mice were sacrificed at 60 weeks. There was no significant difference between the two groups.

Discussion

Knockout mice have proven to be good model systems for deciphering the function(s) of a gene/protein of interest. In some instances, there are obvious phenotypic changes associated with the loss of a particular gene, whereas in many others, there are no obvious phenotypic manifestations and often the mice have to be challenged in various ways, including stress and administration of specific chemicals to reveal the phenotype. *Vparp*$^{-/-}$ mice show no obvious phenotype and appear perfectly normal (23). *Tep1*$^{-/-}$ mice appear overtly normal, however, lack of TEP1 completely disrupts the stable association of vault RNA with vaults and leads to a decrease in stability of vault RNA (20, 21). Our present studies show that loss of VPARP causes increased tumorigenesis in carcinogen-treated mice. Although the results attain statistical significance only in the case of colon tumorigenesis in *Vparp*$^{-/-}$ mice, there is an increase seen in lung tumorigenesis in *Vparp*$^{-/-}$ mice and also in both colon and lung tumorigenesis in *Tep1*$^{-/-}$ mice. Different mouse strains are

Table 4. Lung tumor development in *Tep1*$^{+/+}$ and *Tep1*$^{-/-}$ mice

Genotype	Incidence* (%)	Multiplicity† (total)
Tep1$^{+/+}$ ‡	12 of 16 (75)	3.5 ± 0.7 (42)
Tep1$^{-/-}$	14 of 16 (88)	4.9 ± 1.0 (68)
Tep1$^{+/+}$§	21 of 23 (91)	3.0 ± 0.4 (62)
Tep1$^{-/-}$	23 of 27 (85)	3.8 ± 0.5 (87)

*Number of mice bearing tumors per total number of mice analyzed.
† Average number of tumors per tumor-bearing mouse ± SE.
‡ Mice analyzed at 10 weeks.
§ Mice analyzed at 20 weeks.

known to display varying susceptibilities to spontaneous and carcinogen-induced tumors (26, 27) and this effect could explain the absence of significance in the increase in urethane-induced lung tumors in *Vparp*$^{-/-}$ mice (pure BALB/c background). Likewise, it could also be true for *Tep1*$^{-/-}$ mice because they were in a pure C57BL/6 background. The absence of significant changes in urethane-induced tumorigenesis in *Vparp*$^{-/-}$ mice might also be due to the small number of mice used in the experiment. We did not observe any sex-related changes in these studies.

Mechanism of carcinogenesis. The differences in susceptibilities of *Vparp*$^{-/-}$ and *Tep1*$^{-/-}$ mice to carcinogen-induced lung and colon tumorigenesis also suggest that the function of VPARP and TEP1 may be tissue-and cell type–dependent and/or could also reflect the differences in their individual functions because each of the carcinogens has a different mechanism of action, although they both lead to formation of DNA adducts. It has been suggested that two compounds, vinyl carbamate and its epoxy derivative, may be the proximate and ultimate electrophilic metabolites responsible for genotoxicity and carcinogenicity of urethane, also known as ethyl carbamate. Epoxyethyl carbamate interacts with DNA to form 7-(2-oxoethyl)guanine adducts (28– 30). Dimethylhydrazine is an alkylating agent and causes methylation of DNA generating O^6-methylguanine (31, 32), which has been shown to induce GC→AT transition mutations in the absence of DNA repair (33). This would suggest a role of VPARP in DNA repair and although it has been shown that VPARP is not activated by damaged DNA *in vitro* and its cellular localization does not change upon UV treatment of cells (1), the cellular signals that activate VPARP have not been determined. It should be noted that VPARP has a very weak ADP-ribosylation activity *in vitro* (1).

Poly(ADP-ribose) polymerase, telomerase, and tumorigenesis. PARP-1 and PARP-2 are involved in genomic stability, DNA repair, and cell death (34). *Parp-1*$^{-/-}$ mice are acutely sensitive to alkylating agents (35, 36) and several reports have documented the relevance of PARP in tumorigenesis and carcinogenesis (37). The incidence of colon tumors and the average number of colon tumors per mouse were significantly higher in azoxymethane-treated *Parp-1*$^{-/-}$ mice than in wild-type mice (38). Azoxymethane is a metabolite of dimethylhydrazine (39) and hence the two are very similar in inducing tumors (40). The numbers of hemangiomas and hemangiosarcomas in liver and adenomas in lung were markedly higher in *Parp-1*$^{-/-}$ mice than in wild-type mice after *N*-nitrosobis (2-hydroxypropyl)amine (also an alkylating agent) administration (41).

Telomeres stabilize the genome and play an important role in tumor development (42). Loss of telomere function in *Terc*$^{-/-}$ mice (lacking the telomerase RNA component TERC) was associated with increased incidences of spontaneous tumors and reduced tumor latency in addition to shortened life span, a reduced capacity to respond to stresses, increased incidences of hair graying, hair loss, and severe ulcerative skin lesions (43). However, decreased incidence of methylnitrosourea-induced tumors was seen in second-generation *Terc*$^{-/-}$ mice compared with wild-type mice and enhanced sensitivity to alkylating agent methylnitrosourea was seen in fifth-generation *Terc*$^{-/-}$ mice with critically shorter telomeres (44). Second-generation *Terc*$^{-/-}$ mice showed intermediate sensitivity to methylnitrosourea toxicity. Similarly, telomerase-deficient mice with short telomeres were resistant to papilloma formation after chemical carcinogenesis of the skin (45). *Parp-1*$^{-/-}$ mice and *Terc*$^{-/-}$ mice are also hypersensitive to γ-radiation (35, 36, 46, 47). It remains to be seen if *Vparp*$^{-/-}$ and *Tep1*$^{-/-}$ mice are more sensitive to γ-radiation.

Drug resistance and response to genotoxins. The increased tumorigenesis in VPARP-deficient mice and increased number of tumors in TEP1-deficient mice suggests that the normal function(s) of these proteins may be impaired in these animals. This function could be dependent or independent of their association with vaults because each of them exists in a non–vault-associated form (21). The fact that vaults are enriched in tissues and cells that encounter xenobiotics such as the gastrointestinal tract, make it plausible that the function of VPARP and TEP1 is vault-related. This argument is supported by the following observations. Overexpression of MVP is seen in a variety of multidrug-resistant cell lines treated or selected with a wide range of chemotherapeutic drugs, both classical and non–multidrug-resistant–related, including doxorubicin, mitoxantrone, etoposide, cytarabine, cisplatin, vincristine, and methotrexate, with different mechanisms of action (48). In addition to MVP, levels of VPARP and TEP1 proteins are also increased in some of the drug-resistant cell lines (19, 49). Induction of MVP mRNA and protein is seen upon treatment of cells with the tumor promoter phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate or the carcinogen benzo(*a*)pyrene (50, 51). We have observed simultaneous increases in MVP, VPARP, and TEP1 in cells treated with 12-*O*-tetradecanoylphorbol-13-acetate (Raval-Fernandes and Rome, data not shown). Thus, MVP can be visualized as the structural component of the vault particle and VPARP, TEP1, and vault RNA as the functional components. This could explain the absence of a drug-sensitive phenotype in *mMvp*$^{-/-}$ mice if the function of these vault components is preserved in the absence of MVP. No studies on the susceptibility of the *mMvp*$^{-/-}$ mice to carcinogens have thus far been carried out.

It is interesting to note that *Parp-1*$^{-/-}$ mouse embryonic fibroblasts display increased resistance to anticancer drugs including doxorubicin (52). *Vparp*-deficient embryonic stem cells did not display increased resistance to doxorubicin or etoposide and *Tep1*$^{-/-}$ embryonic stem cells did not yield any conclusive results (Raval-Fernandes and Rome, data not shown). On the other hand, using *Terc*$^{-/-}$ mice on either an *INK4a*-null background or *p53*-null background, it has been shown that telomerase dysfunction increases chemosensitivity to doxorubicin, an agent that induces double-stranded DNA breaks (53). *p53* is the key molecular component of the DNA damage response and is a major determinant of cellular response to chemotherapy and telomere dysfunction (54–57). Loss of *p53* enhances the initiation of malignant transformation in *Terc*$^{-/-}$ mouse embryonic fibroblasts (58). *Parp-1* deficiency results in a high frequency of *p53* loss of heterozygosity in tumor cells and in mouse embryonic fibroblasts during immortalization (59) and facilitates loss of *Rb*, as well as the gain of a chromosome encompassing the c-*Jun* oncogene (60). *Parp-1* deficiency has been shown to cause telomere shortening in mammalian cells (61). However, normal telomere lengths were reported in *Parp-1*-deficient mice and primary cells in another study (62). It would be interesting to see whether VPARP and TEP1 knock-out mice show higher tumor incidence or spontaneous tumor development in a *p53*-deficient background.

Complex network (or cross-talk between) poly(ADP-ribose) polymerases and telomerase-associated proteins. The multitude of proteins that associate with and/or regulate telomeres include TRF1, TRF2, TIN2, POT1, PTOP, and RAP1, which form a multisubunit complex (63) and L22, Staufen, Hsp90, p23, DKC, hEstA, hEstB, hEstC, hnRNPs, and La (64). Interestingly, the La

autoantigen has been shown to copurify with vaults, and like TEP1, it interacts with the vault RNA, both *in vivo* and *in vitro* (65).

Thus far, 18 different putative PARP-1 homologues have been identified and at least 7 of these have been studied in some detail (34). Several of these associate with centrosomes (PARP-1 and PARP-3), telomeres (Tankyrase, which also localizes to nuclear pores, Golgi vesicles, and pericentriolar material during mitosis and has been shown to be involved in telomere regulation), and centromeres (PARP-1 and PARP-2). PARP-1 and PARP-2 interact with proteins involved in the kinetochore structure and in the mitotic spindle checkpoint and PARP-2 has been recently implicated in maintenance of telomere integrity (66). VPARP has been colocalized with microtubules at the mitotic spindle (1) and to the nuclear pores (22) and it interacts with TEP1 and telomerase activity independent of its association with vaults (23). The complexity of interactions between the PARP family members and those associated with telomeres suggest that VPARP and TEP1 could be yet another set of proteins involved in telomere maintenance, chromosomal stability and/or DNA repair. It would be interesting to investigate tumorigenesis in double knock-out mice lacking both VPARP and TEP1.

Our present studies support the hypothesis that vault component proteins may be important under certain cellular stresses such as exposure to genotoxins.

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