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PTEN positively regulates UVB-induced DNA damage repair

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Abstract

Non-melanoma skin cancer is the most common cancer in the U.S., where DNA-damaging UVB radiation from the sun remains the major environmental risk factor. However, the critical genetic targets of UVB radiation are undefined. Here we show that attenuating PTEN in epidermal keratinocytes is a predisposing factor for UVB-induced skin carcinogenesis in mice. In skin papilloma and squamous cell carcinoma (SCC), levels of PTEN were reduced compared to skin lacking these lesions. Likewise, there was a reduction in PTEN levels in human premalignant actinic keratosis and malignant SCC, supporting a key role for PTEN in human skin cancer formation and progression. PTEN downregulation impaired the capacity of global genomic nucleotide excision repair (GG-NER), a critical mechanism for removing UVB-induced mutagenic DNA lesions. In contrast to the response to ionizing radiation, PTEN downregulation prolonged UVB-induced growth arrest and increased the activation of the Chk1 DNA damage pathway in an AKT-independent manner, likely due to reduced DNA repair. PTEN loss also suppressed expression of the key GG-NER protein xeroderma pigmentosum C (XPC) through the AKT/p38 signaling axis. Reconstitution of XPC levels in PTEN-inhibited cells restored GG-NER capacity. Taken together, our findings define PTEN as an essential genomic gatekeeper in the skin, through its ability to positively regulate XPC-dependent GG-NER following DNA damage.

Keywords

PTEN; GG-NER; XPC; UVB; Keratinocytes; AKT; tumorigenesis

Introduction

Skin cancer is the most common type of cancer in the United States (US). Each year more than one million new cases of skin cancer are diagnosed in the US alone, accounting for 40% of all newly diagnosed cancer cases (1, 2). The incidence of skin cancers continues to rise each year. The non-melanoma skin cancers (NMSC) derived from the epidermal basal layer account for approximately 96% of all skin malignancies. The major risk factor for NMSC is UVB radiation in sunlight, which causes the formation of two major DNA damage products, i.e. cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidone dimers (6-4PPs) (3).

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Insight into the genetics of skin cancer has come from studies of a rare familial form of early-onset skin carcinogenesis (4-6). Patients with this rare inherited disorder, xeroderma pigmentosum (XP), are highly sensitive to sun exposure and have a risk of developing skin cancer about 1000 times that of the general population with an NMSC onset about 50 years earlier (7, 8). Molecular defects in global genome nucleotide excision repair (GG-NER), a subpathway that removes UVB-induced CPDs and 6-4PPs throughout the genome, in carriers of XP are responsible for increased skin cancer risk under UV challenge. Seven NER-deficient genetic complementation groups of XP (XP-A to -G) have been identified and all of the corresponding genes have now been cloned (4-6). Accumulating evidence indicates that the XP group C (XPC) protein plays an essential role in damage recognition for GG-NER (9-11) and the *XPC* gene is found to be deleted or mutated in human SCCs (12).

PTEN is a negative regulator of AKT signaling and functions as a tumor suppressor (13). In many human cancers, PTEN's functionality is lost, placing PTEN in a mechanistically critical position. PTEN has been shown to be a critical tumor suppressor in mouse skin using a chemical carcinogenesis model (14). Although extensive biochemical and genetic analyses have characterized the function of PTEN in considerable detail, much remains to be elucidated with regard to its direct role in UVB-induced skin tumorigenesis and the precise molecular mechanisms in UVB damage responses. Here we present data demonstrating that PTEN down-regulation in epidermal keratinocytes is a predisposing factor for UVB-induced skin tumorigenesis. In humans, PTEN is significantly down-regulated in both premalignant and malignant skin lesions. PTEN inhibition impairs GG-NER capacity through suppressing the expression of XPC. The PTEN/AKT/p38 axis seems to be critical for regulating XPC levels and thus for affecting GG-NER capacity. These findings support the essential role of PTEN in relevant human skin tumor suppression.

Materials and Methods

Human normal and tumor samples, immunohistochemistry and sun damage

All human specimens were studied after approval by the University of Chicago Institutional Review Board. Formalin-fixed, paraffin-embedded tissue blocks were obtained from the archives in the tissue bank of the Section of Dermatology (Department of Medicine, University of Chicago). PTEN immunohistochemistry (IHC) was performed by the Immunohistochemistry core facility using anti-PTEN (clone 6H2.1, Upstate). The PTEN levels were evaluated in keratinocytes in interfollicular epidermis. For the hematoxylin-eosin (H&E) slides, sun damage was analyzed by a board-certified dermatopathologist using a solar elastosis index with a scale from 0-3 as described previously (15, 16). Absent solar elastosis was classified as a 0 score, a low level was 1, moderate as 2, and a solid elastotic mass replacing collagen as 3.

Cell culture

Human HaCaT keratinocytes (obtained from Professor N Fusenig) were maintained in a monolayer culture in 95% air/5% CO₂ at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units per mL penicillin, and 100 mg per mL streptomycin (Invitrogen, Carlsbad, California). HaCaT cell line was cultured for less than 20 passages. Normal human epidermal keratinocytes (NHEK) were obtained from Clonetics (Lonza) and cultured in KGM Gold BulletKit medium (Clonetics, Lonza) according to the manufacturer's instructions. NHEK cells were cultured for less than 4 passages. No authentication was done.

UVB radiation

UVB radiation was performed as described previously (17). Our UVB radiation was monitored every other week to measure the exposure output and dose. Our UVB system does not emit UVC radiation.

Animal Treatments

All animal procedures have been approved by the University of Chicago Institutional Animal Care and Use Committee. K14Cre and *Pten^{fl/fl}* mice were obtained from Jackson Laboratories. All mice used have a B6 background to minimize variations in mouse background. Littermates were used as comparison controls. Mice (n = 15) were shaved one day prior to the initial UVB radiation and later as needed. Shaved mice were exposed to UVB (30 mJ/cm², dose selected to avoid visible sunburn) dorsally or sham-irradiated, three times a week for 25 weeks and then kept for an additional 20 weeks to monitor tumor formation and growth. The PTEN protein levels were determined by immunohistochemistry by the Immunohistochemistry core facility using anti-PTEN (138G6, Cell Signaling). Mice were housed five animals per cage, and there was no evidence of dorsal wounds caused by fighting or sunburn.

Statistical analyses

Statistical analyses were performed using Prism 5 (GraphPad software, San Diego, CA). Data were expressed as the mean of three independent experiments and analyzed by Student's *t*-test, ANOVA, the Mann-Whitney *U* test and the Kruskal-Wallis test (for data from human specimens). Statistical analysis for BrdU-labeled cells in the epidermis was performed using analysis of variance followed by Scheffe's post-hoc test. Kaplan-Meier survival estimates and log-rank tests were used to evaluate the tumor onset in mice. A *P* value of less than 0.05 was considered statistically significant.

The details for *in vivo* cell proliferation assay, siRNA transfection, retroviral infection and adenoviral infection, Western blotting, Luciferase reporter assays, Chromatin fractionation, Determination of two major forms of UVB-induced DNA damage in genomic DNA by enzyme-linked immunosorbent assay (ELISA), and *in vitro* cell proliferation assay can be found in the supplementary information.

Results

PTEN down-regulation is a predisposing factor for UVB-induced skin tumorigenesis

To analyze whether PTEN down-regulation in epidermal keratinocytes plays an active role in skin tumorigenesis in which UVB is the major risk factor for humans, we compared UVB-induced tumor formation between mice with normal PTEN (+/+) and mice with PTEN hemizygosity (+/-). We deleted one allele of the *Pten* gene in the epidermal keratinocytes by crossbreeding mice expressing Cre recombinase under the control of the keratin 14 promoter (K14-Cre) with mice expressing a LoxP-flanked (floxed) *Pten* gene (*Pten^{fl/fl}*) to generate K14-Cre;*Pten^{fl/+}* (*Pten*+/-). To mimic the common clinical scenario of daily, low-level exposure to UV radiation, we elected to use a suberythemogenic dose of UVB radiation, not exceeding 30 mJ/cm² per exposure (Fig. s1A-B), which did not cause apoptosis. The shaved mice were either exposed to this dose of UVB or protected from UV radiation (Sham).

Without radiation, *Pten*+/+ and *Pten*+/- mice did not grow any tumors throughout the study period. UVB did not cause tumor formation in *Pten*+/+ mice. However, *Pten*+/- mice started to develop skin tumors at 21 weeks of UVB radiation (Fig. 1A; n = 15). A log-rank test showed that PTEN hemizygosity significantly accelerates skin tumorigenesis following a low dose of UVB radiation (*P* < 0.0001). Pathological analysis of the tumors using similar

criteria for hairless mice (18) showed that in UVB-exposed *Pten*^{+/-} mice, 40% (8/20) of tumors were papillomas and 60% (12/20) were SCC. In both papillomas and SCCs, PTEN levels were reduced as compared with sham-irradiated epidermis (Fig. 1B-C). Thus, PTEN down-regulation is a predisposing factor for UVB-induced skin tumorigenesis and further PTEN loss following chronic UVB radiation is correlated with skin tumor formation.

PTEN down-regulation in human skin tumors and its association with sun damage

To further investigate the specific function of PTEN in human skin cancer, we evaluated PTEN protein levels in 69 human skin samples from US patients. We focused on types of skin neoplasia that are associated with chronic sun exposure, including actinic keratosis (AK), considered to be either a premalignant lesion or a very early stage in development of SCC and invasive SCC. These lesions were all located in sun-exposed regions of the skin, such as the head, face, ear, and hand dorsum. All samples were from fair-skinned individuals, the population at highest risk for skin cancer. We used immunohistochemical analysis to determine the differences in PTEN protein levels in human skin tumors as compared with normal non-lesional skin (brown staining; Figs. 2A-B and s2). To exclude the contribution of endogenous brown pigmentation due to melanin, we also performed hematoxylin-eosin staining and immunohistochemical analysis using alkaline phosphatase anti-alkaline phosphatase (pinkish staining, data not shown). The PTEN levels were reduced (score 0 or 1) in 92% of AK (23/25) and 94% of invasive SCC lesions (26/28) as compared to none of the normal skin samples (0/16) (Fig. 2C). This reduction was statistically significant as analyzed by the Mann-Whitney U test ($P < 0.0001$ for AK and invasive SCC vs normal skin). When we compared PTEN levels in specimens with different levels of chronic sun damage as measured by solar elastosis (15, 16), we found that PTEN down-regulation was significantly associated with increasing sun damage ($P < 0.001$, Kruskal-Wallis test, Table s1), suggesting that UV damage specifically down-regulates PTEN in human skin during carcinogenesis.

PTEN is essential for efficient GG-NER of UVB-induced DNA damage

To determine the molecular basis for increased tumorigenesis caused by the interaction between PTEN down-regulation and UVB irradiation, we investigated the hypothesis that PTEN plays an important role in repairing UVB-induced DNA damage. To determine the percentage (%) of repair in parental and PTEN-inhibited cells, we measured the percentage (%) of CPDs or 6-4PPs remaining at different intervals post-UVB.

To inhibit PTEN in keratinocytes, we infected human HaCaT cells with a retroviral vector expressing short hairpin RNA targeting PTEN (shPTEN) or transfected the cells with siRNA (PTEN) targeting PTEN (Fig. 3A-B). In both models, inhibition of PTEN significantly inhibited repair of CPDs (Fig. 3C-D; $P < 0.05$, two-way ANOVA). In comparison, inhibition of PTEN significantly reduced the repair of 6-4PPs at 6 h post-UVB (Fig. 3E; $P < 0.05$, Student *t*-test), while complete repair was detected at 24 h post-UVB, indicating that the inhibition of 6-4PP repair by PTEN loss was less profound than that of CPD repair. Our findings demonstrated that loss of PTEN impairs DNA repair in UVB-induced CPDs and to some extent in 6-4PPs, indicating that PTEN is required for efficient GG-NER.

PTEN down-regulation delayed exit from growth arrest post-UVB

UVB-induced damage to DNA activates checkpoint pathways and thereby induces cell cycle arrest (19, 20), a critical mechanism for facilitating proper DNA repair. To determine the role of PTEN down-regulation in DNA damage checkpoint function *in vivo*, we measured differences in percentage (%) of BrdU-labeled (BrdU+) keratinocytes between *Pten*^{+/+} and *Pten*^{+/-} epidermis by immunohistochemistry. In the absence of UVB treatment, the percentage of BrdU+ cells in *Pten*^{+/+} epidermis was similar to that in *Pten*^{+/-} counterparts

(Fig. 4A). At 6 h post-UVB, the percentage of BrdU⁺ cells decreased in both *Pten*^{+/+} and *Pten*^{+/-} epidermis, demonstrating an arrest in cell proliferation. At 24-72 h post-UVB, however, the percentage of BrdU⁺ cells remained decreased in *Pten*^{+/-} epidermis but not in its *Pten*^{+/+} counterparts, implying that *Pten*^{+/+} cells but not *Pten*^{+/-} cells exited from UVB-induced growth arrest (Fig. 4A). Similarly PTEN knockdown reduced BrdU incorporation post-UVB irradiation (Fig. s3A). These findings indicate that PTEN down-regulation impairs the exit from UVB-induced growth arrest.

DNA damage-mediated growth arrest operates through the activation of Chk1 and Chk2, and subsequently p53 accumulation (19, 21, 22). Recent studies have shown that in cells treated with ionizing or UVC radiation, PTEN loss inhibits Chk1 activation through the AKT pathway and thus causes genomic instability (23, 24). To determine the role of PTEN in UVB-induced checkpoint function at the molecular level, we examined the phosphorylation of Chk1 and Chk2, and the formation of γ -H2AX, a marker for persistence of DNA damage (25-27), in parental and PTEN-down-regulated keratinocytes. In HaCaT cells, UVB radiation (5 or 20 mJ/cm²) dramatically induced Chk1 phosphorylation at serine 345 (p-Chk1) and Chk2 phosphorylation at threonine 68 (p-Chk2) at 1.5 and 6 hours (Fig. 4B). Post-UVB (5 mJ/cm²) p-Chk1 in shPTEN cells was lower than in NC cells, while post-UVB (20 mJ/cm²) p-Chk1 was similar to that in NC cells at 0.5 h (Fig. 4B and s3B). At 1.5 and 6 h post-UVB (5 or 20 mJ/cm²), however, p-Chk1 in shPTEN cells was significantly higher than in NC cells. In comparison, p-Chk2 in shPTEN cells was similar to that in NC cells (Fig. 4B). In normal human epidermal keratinocytes (NHEK), PTEN knockdown increases Chk1 phosphorylation, γ -H2AX formation and p53 accumulation, but not Chk2 phosphorylation similar to the response of HaCaT cells and primary mouse keratinocytes (Fig. 4C, s3B-D). However, activating AKT by expressing constitutively active AKT (A⁺) in NHEK cells did not resemble the effect of PTEN knockdown (Fig. 4D). Inhibiting AKT signaling by LY294002 (LY, 10 μ M) did not reverse the effect of PTEN down-regulation (Fig. 4E). These data indicated that, following UVB irradiation, the increased checkpoint activation caused by PTEN loss is independent of AKT activation.

PTEN regulates GG-NER through XPC

XPC is essential for repairing UVB-induced DNA damage (10, 28). To examine the mechanism by which PTEN participates in GG-NER, we investigated the role of PTEN in XPC regulation. We compared the protein level of XPC in NC cells with that in shPTEN cells by immunoblotting and found that the protein level of XPC in HaCaT cells transfected with shPTEN was significantly lower than in NC cells, while the levels of DDB1 and DDB2 were not affected (Fig. 5A). Similarly the XPC protein level in HaCaT cells transfected with siRNA targeting PTEN (PTEN) was significantly reduced as compared to HaCaT cells transfected with negative control siRNA (NC) (Fig. 5B). Furthermore, XPC levels were lower in the *Pten*^{+/-} mouse epidermis than in its *Pten*^{+/+} counterparts (Fig. 5C). These data indicate that PTEN positively regulates XPC protein levels.

To determine whether reduced XPC expression in PTEN-inhibited cells impacts its recruitment to chromatin upon DNA damage, we determined the protein levels of XPC that bound to chromatin with or without UVB radiation in NC cells and shPTEN cells. We found that the basal levels of chromatin-bound XPC in shPTEN cells were significantly lower than those in NC cells without UVB irradiation (Fig. s4A-B; $P < 0.05$, Student *t*-test). In NC cells, UVB irradiation increased chromatin-bound XPC levels at 10 min (Fig. s4A-B; $P < 0.05$, Student's *t*-test), consistent with the crucial role of XPC in DNA-damage recognition and initiation of NER (10, 29). In shPTEN cells, however, XPC levels bound to chromatin were significantly lower than in NC cells, while the levels of chromatin-bound DDB1 and DDB2, two other critical recognition factors for GG-NER (30), were not affected (Fig. s4A-B). Kinetic analysis further demonstrated that at 5, 10 and 30 min post-UVB damage the

chromatin-bound XPC levels were significantly reduced in shPTEN cells as compared with NC cells (Fig. 5D-E; $P < 0.05$, Student's *t*-test for each time point and two-way ANOVA for all time points). These data indicate that PTEN loss significantly down-regulates the recruitment to chromatin of XPC in response to UVB-induced DNA damage.

To further investigate how PTEN regulates XPC protein levels, we asked whether PTEN loss decreases XPC expression at the transcription level. Using the promoter reporter assay, we found that the transcriptional activity of the XPC promoter in shPTEN cells was significantly lower than in NC cells (Fig. 5F; $P < 0.05$, Student's *t*-test). XPC immunoblot analysis showed that infection with an adenoviral vector expressing functional PTEN (Ad-PTEN) in shPTEN cells increased the protein levels of XPC (Fig. 5G). Similarly, inhibiting AKT activation by the PI3K inhibitor LY294002 (LY, 10 μ M) increased the XPC protein levels in both HaCaT and NHEK cells (Fig. 5H-I), indicating that AKT activation mediates XPC suppression caused by PTEN inhibition. To determine whether XPC down-regulation is essential for impaired GG-NER mediated by inhibition of PTEN, we overexpressed XPC in cells transfected with siRNA targeting PTEN (Fig. 5J) and then determined GG-NER by measuring the repair of CPDs. While inhibition of PTEN reduced GG-NER, increasing XPC to levels comparable to those in parental cells restored GG-NER (Fig. 5K). These data indicate that PTEN loss impairs GG-NER through down-regulating the expression of XPC at the transcriptional level.

PTEN enhances NER through suppressing AKT-dependent inhibition of p38 pathway

Activation of p38 plays an important role in UVB responses and has been implicated in regulating NER in human fibroblasts and HeLa cells (1, 31, 32). To determine the mechanisms by which PTEN/AKT regulates XPC function, we examined the hypothesis that in keratinocytes PTEN/AKT regulates NER through modulating the p38 pathway. To determine the effect of PTEN loss on p38 activation post-UVB radiation, we determined p38 phosphorylation at different times post-UVB in NC and shPTEN cells. In NC cells, p38 phosphorylation increased at 5 to 30 min post-UVB, while in shPTEN cells it was abolished (Fig. 6A). To determine whether AKT is necessary to inhibit UVB-induced p38 activation, we infected HaCaT cells with an adenoviral vector expressing constitutively active AKT (Myr-AKT) (33, 34). Expression of active AKT inhibited p38 phosphorylation post-UVB (Fig. 6B). To further examine the regulation of p38 by AKT in the keratinocyte response to UVB, we investigated the effect of repeated UVB radiation on p38 as it relates to PTEN inhibition and AKT activation, since our recent studies indicate that UVB radiation activates AKT and suppresses PTEN expression (17). Compared to cells without previous UVB exposure, both basal and UVB-induced p38 phosphorylation was inhibited in cells with previous UVB exposure, in parallel with increased AKT phosphorylation and decreased PTEN expression (Fig. 6C). Inhibition of the AKT pathway by the PI3K inhibitor LY increased phosphorylation of p38 in shPTEN cells (Fig. 6D). Consistent with this finding, inhibiting p38 with its specific inhibitor SB203580 (SB, 10 μ M) in parental HaCaT cells reduced the XPC levels (Fig. 6E). The presence of SB significantly inhibited CPD repair (Fig. 6F). As compared with normal skin, human SCC exhibited increased AKT phosphorylation and decreased p38 phosphorylation and XPC protein levels (Fig. 6G). Taken together, these findings suggest that AKT-dependent inhibition of p38 signaling plays an active role in compromising GG-NER caused by PTEN loss.

Discussion

In this study, we investigated the specific function of PTEN in UVB damage responses and skin tumorigenesis. Using low suberythemal UVB radiation, we demonstrated that mice with a targeted PTEN down-regulation in their epidermis are predisposed to skin tumorigenesis. These findings support the conclusion that a 50% reduction of the PTEN

level increases susceptibility to skin tumorigenesis following UVB radiation. In human skin malignancies PTEN is significantly down-regulated in association with sun damage. Our findings further support the conclusion that the PTEN dose is critical for tumor suppression under genotoxic stress.

Our findings clearly indicate that PTEN positively regulates GG-NER by promoting XPC transcription in keratinocytes. Down-regulation of XPC transcription caused by PTEN down-regulation provides a previously unrecognized mechanism in inhibiting XPC function in addition to deletion and mutations in the *XPC* gene in human SCCs (12). Considering that failure to repair CPD is the principal cause of skin cancer, the role of PTEN in GG-NER through XPC is critical for the tumor-suppressing action of PTEN for the skin.

Our current studies identified the AKT/p38 pathways as important actors in the regulation of XPC by PTEN. PTEN is known as a negative regulator of the PI3K/AKT pathway (13). PTEN down-regulation increases AKT activation and enhances cell survival after UVB damage (17). Indeed, the regulation of the p38 pathway by AKT and its impact on cell survival have been reported in several other models, including endothelial cells (35), β -cell (36), and E1A-induced apoptosis (37). Although the role of the p38 pathway in UVB responses is complex (1, 31), in human fibroblasts and HeLa cells p38 has recently been shown to promote GG-NER by stabilizing DDB2 (32). In line with these findings, our studies have demonstrated that the reduction in the p38 pathway through increased AKT activation upon PTEN inhibition can be mimicked by the effect of biochemical inhibition of p38 in regulating XPC levels and GG-NER, suggesting that in PTEN-down-regulated keratinocytes, suppression of p38 by AKT signaling reduces XPC levels and thus impairs GG-NER. In SIRT1-inhibited cells, however, PTEN inhibition and AKT activation due to increased PTEN acetylation suppress XPC expression through increased nuclear translocation of a transcription repressor p130 (38), but not the p38 pathway (data not shown). Further investigation is needed to reveal these different molecular mechanisms depending on SIRT1 status. Taken together, these results imply that the PTEN/AKT/p38 axis is critical for GG-NER and regulates tumor susceptibility.

Interestingly we found that, in contrast to the response to ionizing or UVC radiation (23, 24), PTEN loss increases UVB-induced Chk1 activation in keratinocytes in an AKT-independent manner. It seems that AKT plays differential roles in DNA repair and checkpoint response. Therefore the effect of PTEN on checkpoint pathways may depend on the type of DNA damage, the repair efficiency, the waveband-specific signaling pathways between UVB and UVC (31, 39), and/or the cell-type specific response to PTEN inhibition, similar to the different findings on the regulation of Rad51 expression (40, 41). Much more investigation is needed to elucidate these important differences at the molecular level. Nevertheless, our findings indicate that normal PTEN expression and AKT activation are required for proper XPC expression and thus better DNA repair. These data also suggest that, in keratinocyte response to UVB-induced DNA damage, the checkpoint response is passive, associated with the levels of unrepaired DNA damage, and depends on the PTEN levels but not AKT activation, further underscoring the importance of constitutive PTEN levels in reducing susceptibility to UVB tumorigenesis.

In summary, we demonstrated that PTEN down-regulation is a predisposing factor for UVB-induced skin carcinogenesis *in vivo*, and negatively regulates UVB-induced DNA damage repair through limiting XPC expression. Taken together, our findings suggest that the interaction between PTEN levels and UVB is critical for early tumorigenesis and tumor progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

AK	actinic keratosis
AKT	a serine-threonine kinase, downstream of PI3K, also called protein kinase B
ATM	ataxia-telangiectasia mutated
ATR	ATM and RAD3-related
LY	LY294002, a specific PI3K/AKT activation inhibitor
NMSC	non-melanoma skin cancer
PI3K	phosphoinositide 3-kinase
PTEN	phosphatase and tensin homologue deleted on chromosome 10
SCC	squamous cell carcinoma
UVB	Ultraviolet B
XP	xeroderma pigmentosum
XPC	xeroderma pigmentosum group C

Fig. 1.

PTEN hemizyosity is a predisposing factor for skin tumorigenesis following low-level UVB radiation. A, percent (%) of tumor-free mice ($n = 15$). B, immunoblot analysis of PTEN and β -actin in normal +/+ and +/- skin, papillomas (PAP), and SCCs. C, immunohistochemical analysis of PTEN in sham-irradiated normal skin and UVB-induced tumor samples from *Pten*+/- mice using an anti-PTEN antibody. Scale bar: 20 μ m.

Fig. 2. PTEN expression is significantly reduced in human skin tumors. A and B, representative immunohistochemical analysis of PTEN protein levels (brown) in normal skin and SCC. EP, epidermis; HF, hair follicle; T, tumor. Scale bar: 100 μm. C, Percent of tumors (in stacked column format) for each score of PTEN expression.

Fig. 3.

PTEN is required for efficient GG-NER. A and B, immunoblot analysis of PTEN and β -actin (equal loading control) in NC and shPTEN cells (A) and in NC and siPTEN cells (B). C-E, Enzyme-linked immunosorbent assay (ELISA) of percent (%) repair of CPDs (C and D) and 6-4PPs (E) at intervals post-UVB (20 mJ/cm²). C, HaCaT cells infected with a retroviral vector expressing negative control (NC) or shRNA targeting PTEN (shPTEN). D and E, HaCaT cells transfected with negative control (NC) or siRNA targeting PTEN (siPTEN). Error bars show standard error (SE). *, $P < 0.05$, significant differences between shPTEN and NC cells, or PTEN and NC cells.

Fig. 4.

PTEN inhibition delays exit from growth arrest post-UVB. A, quantification of percent (%) of BrdU positive (BrdU+) epidermal keratinocytes in *Pten*^{+/+} or *Pten*^{+/-} mouse epidermis at different times post-sham- or UVB-irradiation. Error bars show standard error (SE). *, $P < 0.05$, significant difference between *Pten*^{+/+} and *Pten*^{+/-} mouse epidermis. B, immunoblot analysis of PTEN, p-Chk1 (serine 345), Chk1, p-Chk2 (threonine 68), Chk2, γ-H2AX, and β-actin at 0.5, 1.5 and 6 h post-UVB (5 or 20 mJ/cm²) in HaCaT cells stably infected with a retroviral vector expressing negative control (NC) or shRNA targeting PTEN (shPTEN). C, immunoblot analysis of PTEN, p-AKT, AKT, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, p53, and GAPDH in NHEK cells transfected with negative control (NC) or siPTEN. D, immunoblot analysis of p-AKT, AKT, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, p53, and GAPDH in NHEK cells infected with an adenoviral vector expressing empty vector (EV) or constitutively active AKT (A+). E, immunoblot analysis of p-AKT, AKT, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, p53, and GAPDH in vehicle or LY294002 (LY, 10 μM)-treated NHEK cells transfected with siPTEN.

Fig. 5.

PTEN loss suppresses XPC expression through increasing AKT signaling. A, immunoblot analysis of XPC, DDB2, DDB1, PTEN, and β -actin (equal loading control) in NC and shPTEN cells. B, immunoblot analysis of XPC, PTEN, and β -actin in HaCaT cells transfected with siRNA targeting PTEN (siPTEN) or negative control (NC). C, immunoblot analysis of XPC, PTEN and GAPDH in +/+ and +/- mouse epidermis. D, immunoblot analysis of XPC, DDB1, DDB2, and Histone H3 (loading control) in soluble and/or chromatin-bound fractions from NC and shPTEN cells at different times (5, 10, and 30 min) post-UVB (20 mJ/cm²). E, quantification of XPC levels in D. Error bars show standard error (SE). * in E, $P < 0.05$, significant difference between NC and shPTEN cells. F, luciferase reporter assay of XPC promoter in NC and shPTEN cells. G, immunoblot analysis of XPC, PTEN, and β -actin in shPTEN cells infected with an adenoviral vector expressing wild-type PTEN at different multiplicity of infection (MOI). H, Immunoblot analysis of XPC, p-AKT, AKT, and β -actin in shPTEN cells treated with vehicle or LY294002 (LY, 10 μ M), as well as in NC HaCaT cells. I, Immunoblot analysis of XPC, PTEN, p-AKT, AKT, and GAPDH in siPTEN-transfected cells treated with vehicle or LY294002 (LY, 10 μ M), as well as in NC-transfected NHEK cells. J, immunoblot analysis of XPC, PTEN, and β -actin in HaCaT cells transfected with or without the siRNA targeting PTEN and the XPC plasmid. K, enzyme-linked immunosorbent assay (ELISA) of percent (%) repair of CPDs from cells in J at different times post-UVB (20 mJ/cm²).

Fig. 6.

Inhibition of p38 signaling by AKT is critical for inhibiting GG-NER by PTEN down-regulation. A, immunoblot analysis of p-p38, p38, PTEN, p-AKT (serine 473), and AKT in NC or shPTEN cells at different times post-UVB (20 mJ/cm²). B, immunoblot analysis of p-p38, p38, p-AKT (serine 473), AKT, Myr-AKT (by HA), and β-actin in HaCaT cells infected with an adenoviral vector expressing constitutively active AKT (Myr-AKT) or empty vector at 1.5 h post-sham or -UVB. C, immunoblot analysis of p-p38, p38, p-AKT, AKT, PTEN, and β-actin at 6 h post-UVB in cells that have not been exposed to UVB (First, 1st) and in cells that have been exposed to UVB (20 mJ/cm²) once one week before. D, immunoblot analysis of p-p38, p38, p-AKT, AKT, PTEN, and β-actin in shPTEN cells treated with vehicle or LY (10 μM) at 1.5 h post-UVB, in comparison with NC cells. E, Immunoblot analysis of XPC and β-actin in HaCaT cells treated with vehicle or SB203580 (SB, 10 μM). F, enzyme-linked immunosorbent assay (ELISA) of percent (%) repair of CPDs from cells in E at different times post-UVB (20 mJ/cm²). G, immunoblot analysis of PTEN, p-AKT, AKT, p-p38, p38, XPC, and GAPDH in normal human skin and human SCCs.