PTEN Positively Regulates UVB-Induced DNA Damage Repair

Mei Ming¹, Li Feng², Christopher R. Shea¹, Keyoumars Soltani¹, Baohong Zhao², Weinong Han¹, Robert C. Smart³, Carol S. Trempus², and Yu-Ying He¹

Abstract

Nonmelanoma skin cancer is the most common cancer in the United States, where DNA-damaging ultraviolet B (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 with skin lacking these lesions. Likewise, there was a reduction in PTEN levels in human premalignant actinic keratosis and malignant SCCs, 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. Cancer Res; 71(15); 5287–95. ©2011 AACR.

Introduction

Skin cancer is the most common type of cancer in the United States. Each year more than 1 million new cases of skin cancer are diagnosed in the United States alone, accounting for 40% of all newly diagnosed cancer cases (1, 2). The incidence of skin cancers continues to increase each year. The nonmelanoma skin cancers (NMSC) derived from the epidermal basal layer account for approximately 96% of all skin malignancies. The major risk factor for NMSC is ultraviolet (UV) B radiation in sunlight, which causes the formation of 2 major DNA-damaging products [i.e., cyclobutane pyrimidine dimers (CPD) and pyrimidine(6-4)pyrimidone dimers (6-4PP); ref. 3].

Accumulating evidence indicates that the XP group C (XPC) protein plays an essential role in damage recognition 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). Patients with this rare inherited disorder, xeroderma pigmentosum (XP), are highly sensitive to sun exposure and have a risk of developing skin cancer about 1,000 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).

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 1,000 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 squamous cell carcinoma (SCC; ref. 12).

PTEN is a negative regulator of AKT signaling and functions as a tumor suppressor (13). In many human cancers, the functionality of PTEN is lost, placing PTEN in a mechanistically critical position. PTEN has been shown to be a critical tumor suppressor in mouse skin by 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 damage responses of UVB. Here we present data showing that PTEN...
downregulation in epidermal keratinocytes is a predisposing factor for UVB-induced skin tumorigenesis. In humans, PTEN is significantly downregulated 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 University of Chicago 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 by using a solar elastosis index with a scale from 0 to 3 as described previously (15, 16). Absent solar elastosis was classified as having a 0 score, a low level as 1, moderate as 2, and a solid elastotic mass replacing collagen as 3.

Cell culture

Human HaCaT keratinocytes (obtained from Prof. 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% FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin (Invitrogen). HaCaT cell line was cultured for less than 20 passages. Normal human epidermal keratinocytes (NHEK) cells were obtained from Clonetics (Lonza) according to the manufacturer’s instructions. NHEK cells were cultured for less than 4 passages. No authentication was conducted.

UVB radiation

UVB irradiation was carried out 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. K14-Cre and Pten<sup>fl/fl</sup> 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 1 day prior to the initial UVB irradiation and later as needed. Shaved mice were exposed to UVB (30 mj/cm², dose selected to avoid visible sunburn) dorsally or sham-irradiated, 3 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 IHC by the University of Chicago Immunohistochemistry Core Facility by using anti-PTEN (138G6; Cell Signaling). Mice were housed 5 animals per cage, and there was no evidence of dorsal wounds caused by fighting or sunburn.

Statistical analyses

Statistical analyses were done using Prism 5 (GraphPad Software). Data were expressed as the mean of 3 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 BrdUrd-labeled cells in the epidermis was done using ANOVA 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, knockdown, determination of 2 major forms of UVB-induced DNA damage in genomic DNA by ELISA, and in vitro cell proliferation assay can be found in the Supplementary Information.

Results

PTEN downregulation is a predisposing factor for UVB-induced skin tumorigenesis

To analyze whether PTEN downregulation 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 (+/+), PTEN+/−, and PTEN hemizygosity (+/−). We deleted 1 allele of the Pten gene in the epidermal keratinocytes by crossing mice expressing Cre recombinase under the control of the keratin 14 promoter (K14-Cre) with mice expressing a LoxP-flanked (floxed) Pten gene (Pten<sup>fl/fl</sup>) to generate K14-CrePten<sup>+/−</sup> (Pten<sup>+/−</sup>). 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 (Supplementary Fig. S1A and 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<sup>+/−</sup> and Pten<sup>+/−</sup> mice did not grow any tumors throughout the study period. UVB did not cause tumor formation in Pten<sup>+/−</sup> mice. However, Pten<sup>+/−</sup> mice started to develop skin tumors at 21 weeks of UVB irradiation (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). Pathologic analysis of the tumors by using similar criteria for hairless mice (18) showed that in UVB-exposed Pten<sup>+/−</sup> mice, 40% (8 of 20) of tumors were papillomas and 60% (12 of 20) were SCCs. In both papillomas and SCCs, PTEN levels were reduced as compared with sham-irradiated epidermis (Fig. 1B and C). Thus, PTEN downregulation is a predisposing factor for UVB-induced skin...
tumorigenesis, and further PTEN loss following chronic UVB radiation is correlated with skin tumor formation.

**PTEN downregulation 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 U.S. 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 the 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 developing skin cancer. We used immunohistochemical analysis to determine the differences in PTEN protein levels in human skin tumors as compared with normal nonlesional skin (brown staining; Fig. 2A and B and Supplementary Fig. S2). To exclude the contribution of endogenous brown pigmentation due to melanin, we also carried out H&E 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 of 25) and 94% of invasive SCC lesions (26 of 28) as compared with none of the normal skin samples (0 of 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 downregulation was significantly associated with increasing sun damage ($P < 0.0001$, Kruskal–Wallis test; Supplementary Table S1), suggesting that UV damage specifically downregulates 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 downregulation and UVB irradiation, we investigated the hypothesis that PTEN plays an important role in repairing UVB-induced DNA damage.
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 irradiation.

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 targeting PTEN (siPTEN; Fig. 3A and B). In both models, inhibition of PTEN significantly reduced repair of 6-4PPs at 6 hours post-inhibition of PTEN significantly reduced the repair of 6-4PPs (E) and 6-4PPs (F) at intervals post-shPTEN. D and E, HaCaT cells transfected with NC or siPTEN. Error bars indicate the standard deviation.

PTEN downregulation delayed exit from growth arrest post–UVB irradiation

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 downregulation in DNA damage checkpoint function in vivo, we measured differences in percentage of BrdUrd-labeled (BrdUrd+/−) keratinocytes between Pten+/− and Pten−/− epidermis by IHC. In the absence of UVB treatment, the percentage of BrdUrd+/− cells in Pten+/− epidermis was similar to that in Pten+/− counterparts (Fig. 4A). At 6 hours post–UVB irradiation, the percentage of BrdUrd+/− cells decreased in both Pten+/− and Pten−/− epidermis, showing an arrest in cell proliferation. At 24 to 72 hours post–UVB irradiation, however, the percentage of BrdUrd+/− 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 BrdUrd incorporation post–UVB irradiation (Supplementary Fig. S3A). These findings indicate that PTEN downregulation 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-downregulated keratinocytes. In HaCaT cells, UVB irradiation (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 irradiation (5 mJ/cm²), p-Chk1 in shPTEN cells was lower than in negative control (NC) cells, whereas post–UVB irradiation (20 mJ/cm²), p-Chk1 was similar to that in NC cells at 0.5 hours (Fig. 4B and Supplementary Fig. S3B). At 1.5 and 6 hours post–UVB irradiation (5 or 20 mJ/cm²), however, p-Chk2 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 NHEK cells, 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 and Supplementary Fig. 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 (10 μmol/L) did not reverse the effect of PTEN downregulation (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 whereas the levels of DDB1 and DDB2 were not affected (Fig. 5A). Similarly, the XPC protein level in HaCaT cells transfected with siPTEN was significantly reduced as compared with 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 loss significantly downregulates 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 transcriptional 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 phosphoinositide 3-kinase (PI3K) inhibitor LY294002 (10 μmol/L) increased the XPC protein levels in both HaCaT and NHEK cells (Fig. 5H and I), indicating that AKT activation mediates XPC suppression caused by PTEN inhibition. To determine whether XPC downregulation is essential for impaired GG-NER mediated by inhibition of PTEN, we overexpressed XPC in cells transfected with siPTEN (Fig. 5J) and then determined GG-NER by measuring the repair of UVB damage the chromatin-bound XPC levels were significantly reduced in shPTEN cells as compared with NC cells (Fig. 5D and E; P < 0.05, Student’s t test for each time point and 2-way ANOVA for all time points). These data indicate that PTEN loss significantly downregulates the recruitment to chromatin of XPC in response to UVB-induced DNA damage.

Figure 4. PTEN inhibition delays exit from growth arrest post-UVB irradiation. A, quantification of percentage of BrdUrd− epidermal keratinocytes in Pten+/+ or Pten−/− mouse epidermis at different times post–sham irradiation or UVB irradiation. Error bars show SE. *, P < 0.05, significant difference between Pten+/+ and Pten−/− mouse epidermis. B, immunoblot analysis of PTEN, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, and β-actin at 0.5, 1.5, and 6 hours post–UVB irradiation (5 or 20 mJ/cm²) in HaCaT cells stably infected with a retroviral vector expressing NC or shPTEN. C, immunoblot analysis of PTEN, p-AKT (serine 473), AKT, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, p53, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in NHEK cells transfected with 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 μmol/L)-treated NHEK cells transfected with siPTEN.

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 irradiation in NC and shPTEN cells. We found that the basal levels of chromatin-bound XPC in shPTEN cells were significantly lower than in NC cells, UVB irradiation increased chromatin-bound XPC levels in both NC and shPTEN cells, however, XPC levels bound to chromatin were significantly lower than in NC cells whereas the levels of chromatin-bound DDB1 and DDB2, 2 other critical recognition factors for GG-NER (30), were not affected (Supplementary Fig. S4A and B). Kinetic analysis further showed that at 5, 10, and 30 minutes post–UVB damage the chromatin-bound XPC levels were significantly reduced in shPTEN cells as compared with NC cells (Fig. 5D and E; P < 0.05, Student’s t test for each time point and 2-way ANOVA for all time points). These data indicate that PTEN loss significantly downregulates the recruitment to chromatin of XPC in response to UVB-induced DNA damage.

Figure 5. PTEN regulates XPC protein levels by modulating XPC expression at the transcriptional and translational levels. A, quantification of percentage of BrdUrd− epidermal keratinocytes in Pten+/+ or Pten−/− mouse epidermis at different times post–sham irradiation or UVB irradiation. Error bars show SE. *, P < 0.05, significant difference between Pten+/+ and Pten−/− mouse epidermis. B, immunoblot analysis of PTEN, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, and β-actin at 0.5, 1.5, and 6 hours post–UVB irradiation (5 or 20 mJ/cm²) in HaCaT cells stably infected with a retroviral vector expressing NC or shPTEN. C, immunoblot analysis of PTEN, p-AKT (serine 473), AKT, p-Chk1, Chk1, p-Chk2, Chk2, γ-H2AX, p53, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in NHEK cells transfected with 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 μmol/L)-treated NHEK cells transfected with siPTEN.
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 irradiation, we infected HaCaT cells with adenoviral vector expressing constitutively active AKT (Myr-AKT; refs. 33, 34). Expression of active AKT inhibited p38 phosphorylation post–UVB irradiation (Fig. 6B). To further examine the regulation of p38 by AKT in the keratinocyte response to UVB, we investigated the effect of repeated UVB irradiation on p38 as it relates to PTEN inhibition and AKT activation, because our recent studies indicate that UVB radiation activates AKT and suppresses PTEN expression (17). Compared with cells without previous UVB exposure, 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 LY294002 increased phosphorylation of p38 in shPTEN cells (Fig. 6D). Consistent with this finding, inhibiting p38 with its specific inhibitor SB203580 (10 μmol/L) in parental HaCaT cells reduced the XPC levels (Fig. 6E). The presence of SB203580 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 showed that mice with a targeted PTEN downregulation in their epidermis are predisposed to skin tumorigenesis. These findings support the conclusion that a 50% reduction in the PTEN level increases susceptibility to skin tumorigenesis following UVB radiation. In human skin malignancies, PTEN is significantly downregulated 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. Downregulation of XPC transcription caused by PTEN downregulation 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, a known negative regulator of the P53/AKT pathway (13). PTEN downregulation 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 shown 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-downregulated 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).
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 showed that PTEN downregulation 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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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In Memoriam

This article is dedicated to the memory of Dr. Colin F. Chignell.

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