Regulation of global genome nucleotide excision repair by SIRT1 through xeroderma pigmentosum C

Mei Ming[a], Christopher R. Shea[a], Xiumei Guo[b], Xiaoling Li[b], Keyoumars Soltani[a], Weinong Han[a], and Yu-Ying He[a,1]

[a]Section of Dermatology, Department of Medicine, University of Chicago, Chicago, IL 60637; and [b]Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709

Edited by C. Thomas Caskey, University of Texas-Houston Health Science Center, Houston, TX, and approved November 22, 2010 (received for review July 16, 2010)

Disruption of the nucleotide excision repair (NER) pathway by mutations can cause xeroderma pigmentosum, a syndrome predisposing affected individuals to development of skin cancer. The xeroderma pigmentosum C (XPC) protein is essential for initiating global genome NER by recognizing the DNA lesion and recruiting downstream factors. Here we show that inhibition of the deacetylase and longevity factor SIRT1 impairs global genome NER through suppressing the transcription of XPC in a SIRT1 deacetylase-dependent manner. SIRT1 enhances XPC expression by reducing AKT-dependent nuclear localization of the transcription repressor of XPC. Finally, we show that SIRT1 levels are significantly reduced in human skin tumors from Caucasian patients, a population at highest risk. These findings suggest that SIRT1 acts as a tumor suppressor through its role in DNA repair.

UVB | PTEN | AKT

Nucleotide excision repair (NER) is a versatile DNA repair pathway that eliminates a wide variety of helix-distorting base lesions, including UV radiation-induced cyclobutane pyrimidine dimers (CPD) and (6-4) photoproducts (6-4PPs) (1, 2), as well as bulky adducts induced by numerous chemical compounds. Defects in NER by mutations cause the autosomal recessive xeroderma pigmentosum (XP) and Cockayne syndromes (3–5). XP patients are clinically characterized by cutaneous sensitivity to sunlight exposure and a predisposition to skin cancer. 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 (3–5).

Mammalian NER consists of two distinct subpathways: global genome NER (GG-NER), which operates throughout the genome, and transcription-coupled NER (TC-NER), which specifically removes lesions on the transcribed DNA strand of active genes. A major difference between these two pathways appears to lie in the strategies for detecting damaged bases. Accumulating evidence indicates that the XP group C (XPC) protein plays an essential role in GG-NER-specific damage recognition (6–8). Although biochemical and genetic analyses have characterized the function of XPC in considerable detail, much remains to be elucidated with regard to its regulation and interaction with other critical cellular pathways.

Sirtuin 1 (SIRT1), a mammalian counterpart of the yeast silent information regulator 2 (Sir2) and a proto member of the sirtuin family, is an NAD-dependent longevity-promoting deacetylase. SIRT1 is crucial for cell survival, metabolism, senescence, and stress response in several cell types and tissues (9–13). Both histone and nonhistone targets of SIRT1 have been identified, including FOXO, p53, PGC-1α, NF-κB, and PPARγ (10, 12, 14). SIRT1 has been implicated as an important player in cancer. However, it remains unclear whether SIRT1 serves as a tumor suppressor or a tumor promoter (13–16). SIRT1 expression is relatively higher in many malignancies, including colon, breast, prostate, and skin cancers and leukemia, as compared with their corresponding normal tissues (17–22). But the specific correlation is not clear-cut. Wang et al. (23) analyzed a public database and found reduced levels of SIRT1 mRNA in ovarian, prostatic, and bladder carcinomas, and glioblastoma, as compared with normal tissues. But they did not find any changes in SIRT1 protein levels in skin cancer compared with normal skin.

In this study, we used an in vitro cell culture model and human skin tumor analysis to study the role of SIRT1 in GG-NER in cells exposed to UVB radiation and in human skin tumors. Our data provide strong evidence that mammalian SIRT1 plays a critical role in tumor-suppressing GG-NER, and that down-regulation of SIRT1 is associated with human skin tumorigenesis.

Results

SIRT1 Is Required for Efficient GG-NER of UVB-Induced DNA Damage. To determine whether SIRT1 plays a role in GG-NER to remove CPD and 6-4PP lesions, we measured the percentage of CPDs or 6-4PPs remaining at different intervals post-UVB, and thereby determined the percentage of repair, in parental and SIRT1-inhibited cells. To genetically delete SIRT1, SIRT1 WT mouse embryonic fibroblasts (MEFs) were used for comparison with SIRT1 KO cells. To inhibit SIRT1 in keratinocytes, which are major targets for human UVB exposure, we transfected human HaCaT cells with siRNA targeting SIRT1. In both MEFs and HaCaT cells, inhibition of SIRT1 significantly inhibited repair of CPDs (Fig. 1 *A* and *B*; $P < 0.05$, two-way ANOVA), as well as 6-4PPs (Fig. 1 *C* and *D*; $P < 0.05$, two-way ANOVA). The inhibition of CPD repair by SIRT1 loss was more profound than that of 6-4PP repair. At these UVB doses (MEFs, 10 mJ/cm^2; HaCaT, 20 mJ/cm^2) and time points (up to 48 h), there was no significant difference in initial DNA damage, growth, or UVB-induced apoptosis between WT and KO MEFs or between NC- and siSIRT1-transfected HaCaT cells (*SI Appendix*, Table S1, Figs. S1 and S2). Our findings demonstrated that loss of SIRT1 results in the inhibition of DNA repair in UVB-induced CPD and 6-4PPs, strongly indicating that SIRT1 is required for efficient GG-NER.

Inhibition of SIRT1 Down-Regulates XPC Levels. XPC is essential for GG-NER of UVB-induced DNA damage (7, 24). To determine the mechanism by which SIRT1 participates in GG-NER, we investigated whether XPC is regulated by SIRT1. When we compared the protein level of XPC from SIRT1 WT MEFs with that from SIRT1 KO MEFs by immunoblotting, we found that the protein level of XPC in SIRT1 KO cells was significantly lower than in SIRT1 WT cells (Fig. 2 *A* and *B*; $P < 0.05$, Student's *t* test). Similarly, the XPC protein level in HaCaT cells

Author contributions: M.M., C.R.S., X.L., and Y.-Y.H. designed research; M.M. performed research; X.G., X.L., K.S., and W.H. contributed new reagents/analytic tools; M.M., C.R.S., K.S., and Y.-Y.H. analyzed data; and Y.-Y.H. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. E-mail: yyhe@medicine.bsd.uchicago.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1010377108/-/DCSupplemental.

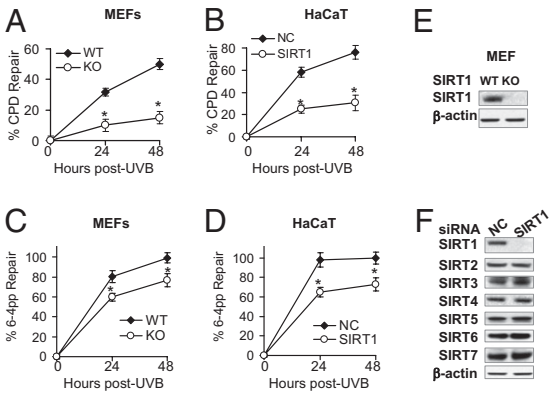


Fig. 1. SIRT1 is essential for efficient DNA GG-NER. ELISA of percent repair of CPDs (*A* and *B*) and 6-4PPs (*C* and *D*) at intervals post-UVB (MEFs, 10 mJ/cm^2; HaCaT, 20 mJ/cm^2). (*A* and *C*) WT or SIRT1 KO MEFs. (*B* and *D*) HaCaT cells transfected with negative control (NC) or siRNA targeting SIRT1. (*E*) Immunoblot analysis of SIRT1 and β-actin (equal loading control) in WT and KO MEFs. (*F*) Immunoblot analysis of SIRT1-7 and β-actin in HaCaT cells transfected with siRNA targeting SIRT1 or NC. Error bars show SE. *$P < 0.05$, significant differences between KO and WT cells, or SIRT1 and NC cells.

transfected with siRNA targeting SIRT1 was significantly reduced as compared with HaCaT cells transfected with negative control siRNA (Fig. 2 *C* and *D*; $P < 0.05$, Student's *t* test).

To determine whether reduction of XPC expression in SIRT1-inhibited cells impacts its recruitment to chromatin upon DNA damage, we compared the chromatin-bound XPC levels in SIRT1 WT MEFs with those in SIRT1 KO MEFs. We found that chromatin-bound XPC levels in SIRT1 KO cells were significantly lower than in SIRT1 WT cells without UVB irradiation (Fig. 2 *E* and *F* and *SI Appendix*, Fig. S3; $P < 0.05$, Student's *t* test). In SIRT1 WT cells, UVB irradiation increased chromatin-bound XPC levels at both 0.5 and 1.5 h post-UVB, consistent with the crucial role of XPC in DNA-damage recognition and subsequent initiation of NER (7, 25). In SIRT1 KO cells, however, the chromatin-bound XPC levels were significantly lower than in SIRT1 WT cells (Fig. 2 *E* and *F* and *SI Appendix*, Fig. S3; $P < 0.05$, Student's *t* test), indicating that down-regulation of XPC proteins in these cells decreases its availability for DNA repair. To further confirm that down-regulation of XPC is responsible for impaired GG-NER in SIRT1-inhibited cells, we overexpressed XPC in siSIRT1 cells and then determined GG-NER by measuring the repair of CPDs and 6-4PPs. Though GG-NER was reduced by inhibition of SIRT1, it was restored by increasing XPC to levels comparable to those in parental cells (Fig. 2 *G*–*J*). These data suggest that SIRT1 loss significantly down-regulates the availability of XPC and its recruitment to chromatin in response to UVB-induced DNA damage, and thus impairs GG-NER.

SIRT1 Regulates XPC Transcription Requiring SIRT1 Deacetylase Activity and E2F Sites in the XPC Promoter. SIRT1 is a well-known deacetylase. To determine whether SIRT1 deacetylase activity is required for SIRT1's effects on XPC expression, KO MEFs were transfected with vector (Vec), wild-type SIRT1 (WT), or inactive mutant SIRT1^{H355Y} (Mut). H355Y mutation of SIRT1 destroys the catalytic activity of its deacetylase (26). As compared with vector-transfected KO cells, WT SIRT1 transfection increased XPC protein levels, whereas mutant SIRT1 had no effect on XPC expression (Fig. 3*A*). These findings suggest that the deacetylase activity of SIRT1 is required for its regulation of XPC.

To further understand how SIRT1 regulates XPC protein levels, we investigated whether SIRT1 loss decreases XPC expression at the mRNA levels and/or at the transcription level. The mRNA level of XPC was significantly lower in SIRT1 KO cells than in SIRT1 WT cells (Fig. 3*B*; $P < 0.05$, Student's *t* test). Using the promoter reporter assay, we found that the transcriptional activity of the 1.5-kb WT mouse XPC promoter (*SI Appendix*, Fig. S4) in SIRT1 KO cells was significantly lower than in SIRT1 WT cells (Fig. 3*C*; $P < 0.05$, Student's *t* test). Transfection of KO MEFs with WT SIRT1 significantly increased the transcriptional activity of the XPC promoter (Fig. 3*D*; $P < 0.05$, Student's *t* test), further demonstrating that SIRT1 is required for XPC transcription.

Previous reports indicated that the XPC gene is repressed by the E2F4-p130 repressor complex (27), and that disrupting this complex by the tumor suppressor ARF (alternative reading frame) stimulates XPC transcription (28). To investigate whether SIRT1 regulates the E2F4-p130 complex, we first determined whether inhibition of SIRT1 alters the nuclear location of E2F4 and p130. E2F4 is a nuclear protein, and p130 is mainly localized in the cytosol (Fig. 3 *E* and *F*). In SIRT1 KO cells, though the nuclear E2F4 levels remained unaltered, the nuclear p130 levels increased as compared with SIRT1 WT cells (Fig. 3 *E* and *F*), suggesting that inhibition of SIRT1 increased the p130 levels in the nucleus. To further examine the role of the E2F site in the XPC promoter, sequences between −51 and +7 positions of the mouse XPC promoter, with either an intact (XPC-WT) or mutated (XPC-Mut) E2F site (*SI Appendix*, Fig. S4), were used to generate luciferase reporter constructs (28). Transcription from the promoter with an intact E2F site was significantly lower than from the promoter with a mutated E2F site (Fig. 3*G*; $P < 0.05$, Student's *t* test), indicating that the E2F site is critical for XPC suppression in SIRT1-deleted cells. Expression of SIRT1 clearly stimulated XPC transcription from the promoter with an intact

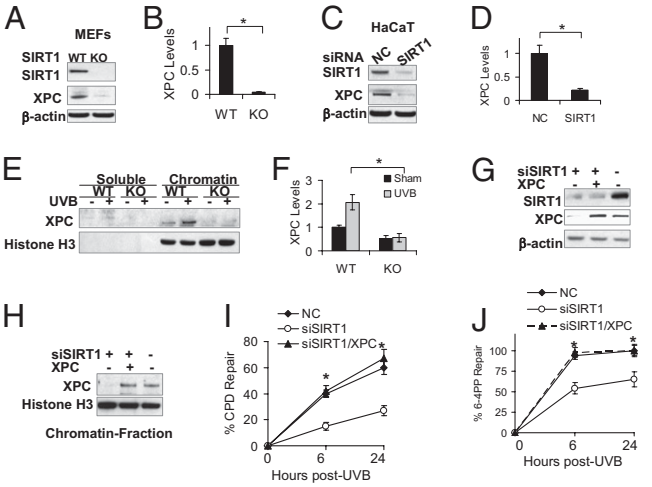


Fig. 2. SIRT1 is required for the expression and chromatin recruitment of XPC post-UVB irradiation. (*A*) Immunoblot analysis of SIRT1, XPC, and β-actin (equal loading control) in SIRT1 WT and KO MEFs. (*B*) Quantification of XPC protein levels from *A*. (*C*) Immunoblot analysis of SIRT1, XPC, and β-actin in HaCaT cells transfected with siRNA targeting SIRT1 or negative control (NC). (*D*) Quantification of XPC protein levels from *C*. (*E*) Immunoblot analysis of XPC and histone H3 (loading control) in soluble and chromatin-bound fractions from WT and KO MEFs at 0.5 h post-UVB (10 mJ/cm^2). (*F*) Quantification of chromatin-bound XPC levels in *E*. (*G*) Immunoblot analysis of SIRT1, XPC, and β-actin in HaCaT cells transfected with or without the siRNA targeting SIRT1 and XPC plasmid. (*H*) Immunoblot analysis of XPC and histone H3 (loading control) in chromatin-bound fractions from cells in *G*. (*I* and *J*) ELISA of percent repair of CPDs (*I*) and 6-4PPs (*J*) from cells in *G* at different times post-UVB (20 mJ/cm^2). *$P < 0.05$ in *A*–*F*, significant differences between KO and WT cells, or SIRT1 and NC cells. *$P < 0.05$ in *I* and *J*, significant differences between XPC and vector-transfected HaCaT cells cotransfected with siRNA targeting SIRT1.

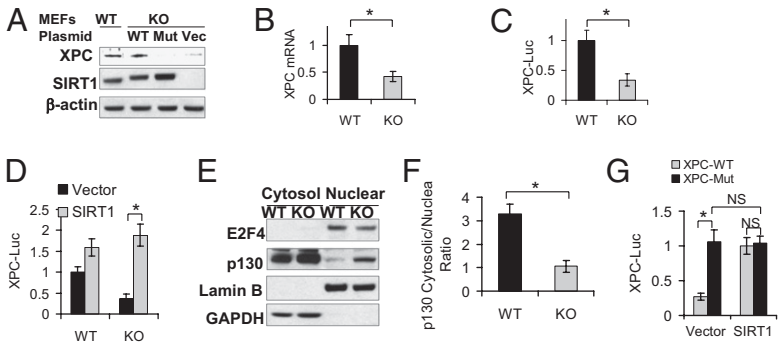


Fig. 3. XPC expression requires the deacetylase activity of SIRT1 and an intact E2F site. (*A*) Immunoblot analysis of XPC, SIRT1, and β-actin of KO MEFs transfected with wild-type SIRT (WT), dominant-negative mutant (Mut) SIRT1, or vector (Vec) plasmids. WT MEFs were used as a comparison control. (*B*) Real-time PCR analysis of XPC mRNA levels in WT and KO MEFs. (*C*) Luciferase reporter assay of the 1.5-kb mouse XPC promoter in WT and KO MEFs. (*D*) Luciferase reporter assay of the 1.5-kb mouse XPC promoter as in *C* in KO MEFs transfected with WT SIRT1 or vector. (*E*) Immunoblot analysis of E2F4, p130, lamin B (nuclear marker), and GAPDH (cytosolic marker) in cytosolic and nuclear protein fractions isolated from SIRT1 WT and KO MEFs. (*F*) Quantification of p130 levels as a ratio of cytosolic/nuclear fractions in *E*. (*G*) Luciferase reporter assay of the XPC promoter with an intact (XPC-WT) or mutated (XPC-Mut) E2F site in SIRT1 KO MEFs transfected with vector or SIRT1. $*P < 0.05$, significant differences between comparison groups. NS, not statistically significant.

E2F site, whereas little stimulation was detected from the promoter with a mutated E2F site (Fig. 3*G*). Taken together, these observations indicate that SIRT1 is required for XPC transcription in a deacetylase-dependent manner, and further suggest an important role for the E2F4-p130 suppressor complex.

SIRT1 Negatively Regulates AKT Phosphorylation. Recently, SIRT1 has been implicated as a deacetylase for the tumor suppressor PTEN (29), a known negative regulator for the PI3K/AKT pathway, a key oncogenic pathway that promotes cell growth and survival (30). Acetylation of PTEN has been shown to inhibit its activity and thus activate AKT (31). We used immunoblotting analysis in the same cellular models as shown in Figs. 1 and 2 to determine whether SIRT loss activates AKT and to measure the difference in AKT phosphorylation at serine 473 in parental and SIRT1-inhibited cells. We found that, in both MEFs and HaCaT cells, phosphorylation of AKT was significantly higher when SIRT1 was inhibited (Fig. 4 *A–C*); thus, the loss of SIRT1 increases AKT phosphorylation. To determine the role of PTEN in AKT activation induced by SIRT1 loss, we reexpressed WT or mutant SIRT1 in KO MEFs to examine whether the deacetylase SIRT1 impacts PTEN acetylation and AKT phosphorylation. As compared with vector-transfected KO MEF cells, expression of WT SIRT1 decreased acetylated PTEN levels, whereas mutant SIRT1 had no effect on PTEN acetylation (Fig. 4*D*). WT SIRT1 inhibited AKT phosphorylation, and mutant SIRT1 had no effect (Fig. 4*E*). These findings suggest that SIRT1 decreases PTEN acetylation and inactivates the AKT pathway in a SIRT1 deacetylase-dependent manner.

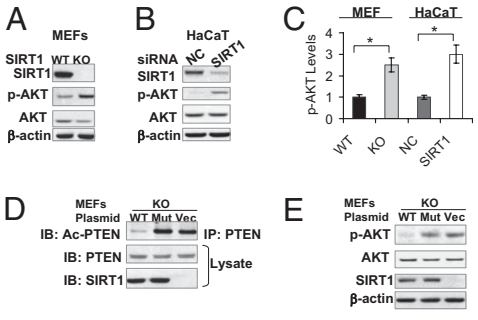


Fig. 4. Loss of SIRT1 activates AKT. (*A* and *B*) Immunoblot analysis of SIRT1, p-AKT (at serine 473), total AKT, and β-actin in WT and KO MEFs (*A*), and HaCaT cells transfected with siRNA targeting SIRT1 or negative control (NC) (*B*). (*C*) Quantification of p-AKT/AKT levels in *A* and *B*. (*D*) Immunoblot analysis of acetylated PTEN (Ac-PTEN) using an antiacetylated lysine antibody following immunoprecipitation (IP) with an anti-PTEN antibody, and PTEN and SIRT1 in total lysates, in KO MEFs transfected with wild-type SIRT (WT), dominant-negative mutant (Mut) SIRT1, or vector (Vec) plasmids. (*E*) Immunoblot analysis of p-AKT, total AKT, SIRT1, and β-actin in cells as in *D*. $*P < 0.05$, significant differences between KO and WT cells, or SIRT1 and NC cells.

The AKT Pathway Is Essential for XPC Suppression Caused by SIRT1 Loss. To determine the role of AKT activation in XPC suppression in SIRT1-inhibited cells, we investigated whether AKT activation in SIRT1-inactivated cells plays an active role in XPC suppression. At 24 h after cells were treated with LY294002 (LY, 10 μM), a PI3K inhibitor that blocks AKT activation (Fig. 5*A*), XPC protein levels were analyzed by Western blotting. Though LY had no effect on XPC protein levels in SIRT1 WT cells, it significantly increased XPC protein levels in SIRT1 KO cells (Fig. 5 *A* and *B*; $P < 0.05$, Student's *t* test). Similarly, LY significantly increased the XPC mRNA levels in SIRT1 KO cells (Fig. 5C; $P < 0.05$, Student's *t* test). AKT knockdown using siRNA targeting AKT1, the dominant AKT isoform in keratinocytes (32), increased XPC protein levels in HaCaT cells transfected with siRNA targeting SIRT1 (Fig. 5*D*).

To determine whether AKT activation plays an important role in the increased p130 nuclear localization seen in Fig. 3*E*, we analyzed the nuclear level of p130 in parental- or AKT-inhibited cells with SIRT1 loss. Both transfection of SIRT1-knockdown HaCaT cells with siRNA targeting AKT1 and treatment of SIRT1 KO MEFs with LY decreased the p130 levels in the nucleus (Fig. 5 *E–G*). Using the promoter reporter assay with XPC promoter with an intact or a mutated E2F site as in Fig. 3*G*, we found that LY treatment significantly increased the transcription of the XPC promoter with an intact E2F site ($P < 0.05$, Student's *t* test), but had no effect on the transcription of the XPC promoter with a mutated E2F site (Fig. 5*H*). These findings suggest that blocking the AKT pathway in SIRT1-inhibited cells suppresses the nuclear location of the XPC transcription repressor p130, and thus restores XPC transcription.

SIRT1 Is Down-Regulated in Human Skin Tumors of Fair-Skinned Patients. To further investigate the specific function of SIRT1 in human skin malignancies, we evaluated SIRT1 protein levels in 94 human skin samples from US patients. We focused on the 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

MEDICAL SCIENCES

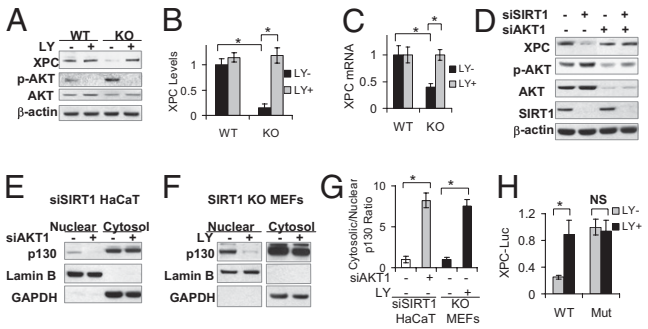


Fig. 5. AKT activation in SIRT1-deleted cells is required for decreased XPC protein levels. (*A*) Immunoblot analysis of XPC, p-AKT, total AKT, and β-actin in WT and KO MEFs treated with or without LY294002 (LY, 10 μM) for 24 h. (*B*) Quantification of XPC protein levels in *A*. (*C*) Real-time PCR analysis of XPC mRNA levels in WT and MEF cells treated with or without LY294002 (LY, 10 μM). (*D*) Immunoblot analysis of XPC, p-AKT, total AKT, SIRT1, and β-actin in HaCaT cells transfected with or without the siRNAs targeting SIRT1, AKT1, or negative control. (*E*) Immunoblot analysis of p130, lamin B (nuclear marker), and GAPDH (cytosolic marker) in cytosolic and nuclear protein fractions isolated from HaCaT cells transfected with siRNAs targeting SIRT1, AKT1, or negative control as in *D*. (*F*) Immunoblot analysis of p130, lamin B (nuclear marker), and GAPDH (cytosolic marker) in cytosolic and nuclear protein fractions isolated from KO MEFs treated with or without LY294002 (LY, 10 μM). (*G*) Quantification of p130 levels as a ratio of cytosolic/nuclear fractions in *E* and *F*. (*H*) Luciferase reporter assay of the XPC promoter with an intact (XPC-WT) or mutated (XPC-Mut) E2F site in SIRT1 KO MEFs treated with or without LY294002 (LY, 10 μM). Error bars show SE. $*P < 0.05$, significant differences between comparison groups. NS, not statistically significant.

squamous cell carcinoma (SCC)], keratoacanthoma (KA), SCC in situ, and invasive SCC. These lesions were all located in sun-exposed skin, such as the head, face, ears, lips, and hand dorsum. Most samples were from fair-skinned individuals, the population at highest risk for skin cancer. We used immunohistochemical analysis to determine the differences in SIRT1 protein levels in human skin tumors as compared with normal nonlesional skin. Though some SIRT1 was localized in cytoplasm, the majority of the protein was found to be in the nucleus in basal epidermal keratinocytes of normal skin (Fig. 6*A*). The SIRT1 levels were reduced (score 0 or 1) in 84% of AK (21/25), 100% of KA (11/11), 100% of SCC in situ (10/10), and 94% of invasive SCC lesions (30/32) as compared with 13% of normal skin samples (2/16) (Fig. 6 *B* and *C*). This reduction was highly statistically significant as analyzed by the Mann–Whitney *U* test ($P < 0.0001$ for AK, KA, SCC in situ, and invasive SCC vs. normal skin).

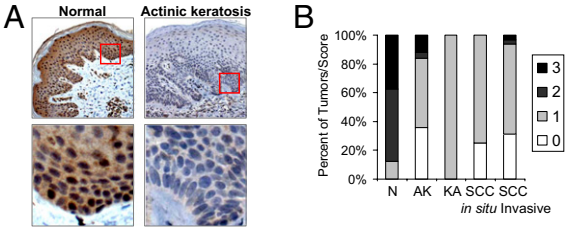
Discussion

Though the role of SIRT1 in metabolism is relatively well defined (13, 14), the function of SIRT1 in cancer is complex, and whether SIRT1 serves as a tumor suppressor or a tumor promoter is still subject to debate. In this study, we showed that SIRT1 is required for efficient GG-NER by enhancing the transcription of the key GG-NER factor XPC, and thus may play an important role in genomic integrity under genotoxic stress. We analyzed the expression of SIRT1 in human skin tumors associated with UV exposure, in which GG-NER capacity is essential for preventing tumorigenesis. In a wide spectrum of UV-associated keratinocyte-derived neoplasms, we demonstrated that SIRT1 expression is significantly reduced as compared with normal skin, implying that loss of SIRT1 may contribute to human skin cancer. Taken together, our findings suggest that SIRT1 is a potential tumor suppressor in cutaneous oncogenesis.

The yeast Sir2 and its mammalian homolog SIRT1 have been implicated in DNA repair. In yeast, Sir2 is known to play an important role in DNA damage repair (33–35). Histone and numerous nonhistone targets have been identified as SIRT1 downstream pathways (12, 14). SIRT1 in mammalian cells has been shown recently to be crucial for repair of double-strand breaks (23, 36), possibly through deacetylating main targets, including Ku70 (37) and NBS1 (38), two proteins involved in the regulation of repair of double-strand breaks. Using genetic and siRNA knockdown methods, we found that SIRT1 loss disrupts the GG-NER pathway for eliminating UVB-induced DNA damage. In SIRT1-inhibited cells, we found that the expression of the key GG-NER factor XPC was significantly down-regulated at the transcriptional level.

Although the direct biochemical mechanisms underlying human GG-NER and the functions of XP proteins, including XPC, have been elucidated to a considerable extent, the in vivo regulation of this repair system has remained elusive (39). Considerable evidence has shown that XPC abundance is critical for efficient GG-NER, and thus for preventing UVB-induced skin carcinogenesis (7, 39, 40). Haploinsufficiency of the *Xpc* gene is a risk factor for UVB-induced skin cancer in mice (41), suggesting a significant biological consequence of XPC down-regulation in increasing skin cancer susceptibility. Induction of XPC facilitates GG-NER (28). We found that the reduced XPC abundance in SIRT1-inhibited cells clearly impairs the capacity of XPC recruitment to chromatin post-UVB damage as compared with parental cells. Increasing XPC expression restored GG-NER capacity of SIRT1-inhibited cells, indicating that XPC is essential for SIRT1 to positively regulate GG-NER. Recent studies using high-resolution mass spectrometry have identified 3,600 lysine acetylation sites in 1,750 proteins, among which are 167 acetylation sites in 72 proteins, including GG-NER proteins DDB1, DDB2, CUL4A, and RAD23A (42). However, it appears that histone deacetylases (HDACs) may play critical roles in regulating GG-NER (43, 44), further supporting the conclusion that XPC is the major target for SIRT1's enhancement of GG-NER. Recent studies have shown that loss of XPC inhibits DSB repair (45). Thus, in addition to GG-NER, regulation of XPC levels by SIRT1 may play an important role in preserving genomic stability by enhancing the repair of double-strand breaks (23).

Our findings also suggest that the regulation of GG-NER by SIRT1 involves a p53-independent mechanism. p53 is a key tumor suppressor and effector of DNA damage responses as one of the key downstream pathways of DNA damage checkpoints (46, 47). Acetylation of p53 is indispensible for its activation (48, 49), and p53 is a substrate for deacetylation by SIRT1 (14, 50). Therefore, SIRT1 has been proposed as an oncogene. However, the complexity of p53 signaling and the identification of numerous other



C

Lesion type	Normal	AK	KA	SCC *in situ*	Invasive SCC
Low SIRT1 score (0 to 1)					
Low score cases / total cases	2/16	21/25	11/11	10/10	30/32
Low score cases as a %	13%	84%	100%	100%	94%

Fig. 6. SIRT1 expression is significantly reduced in human skin tumors. (*A*) Representative immunohistochemical analysis of SIRT1 protein levels (brown) in normal skin and actinic keratosis. (*Upper*) Low magnification. (*Lower*) High magnification. (*Upper*) Red squares indicate the region shown in *Lower*. (*B*) Percent of tumors (in stacked column format) for each score of SIRT1 expression. (*C*) Tabular summary of SIRT1 immunoreactivity in different diagnosis groups. AK, actinic keratosis; KA, keratoacanthoma.

SIRT1 substrates complicate the function of SIRT1 in tumorigenic pathways (13–16). In the MEFs used in this study, which have WT functional p53 (51), SIRT1 deletion inhibits GG-NER in response to UVB irradiation. Similar inhibition of GG-NER was detected by inhibiting SIRT1 in human HaCaT keratinocytes with UV-type p53 mutations (52), causing defects in transcription from the promoter of the cyclin-dependent kinase inhibitor p21 (53). These data suggest that these UV-type p53 mutations have little effect on the regulation of GG-NER by SIRT1. This finding is consistent with previous findings that, unlike fibroblasts (54, 55), human keratinocytes do not require p53 to maintain basal GG-NER activity (56). Considering the frequency of p53 mutations detected in normal (nonlesional) epidermis (57), the role of p53 in SIRT1 function in skin carcinogenesis needs further elucidation. Similar to p53-independent mechanisms causing the embryonic lethality of SIRT1 mutant mice (23), our findings suggest that p53-independent pathways may play important roles in SIRT1-regulated GG-NER.

We have shown that loss of SIRT1 promotes activation of the oncogenic AKT pathway to mediate XPC down-regulation. AKT activation resulting from SIRT1 inhibition is critical for the increased nuclear abundance of the E2F4-p130 repressor complex (27). Consistent with these findings, mutation of the E2F site in the XPC promoter abolished the effect of SIRT1 inhibition and AKT activation, suggesting that inhibition of SIRT1 down-regulates XPC transcription by activating an AKT-dependent E2F pathway. The deacetylase activity of SIRT1 is required for its positive role in XPC expression. We found that SIRT1 decreases the acetylation of PTEN, a negative regulator of PI3K/AKT activation (58), and thus increases its activity to inhibit AKT activation. SIRT1 has been demonstrated to interact with Rb (retinoblastoma protein), as well as its family members p103 and p130, and to deacetylate Rb (59). It is possible that SIRT1 also acts as a deacetylase for p130, and thus plays an important role in regulating the function of the E2F4-p130 repressor complex in XPC transcription. Considering the critical role of phosphorylation in Rb proteins (60, 61), AKT may phosphorylate p130, and loss of SIRT1 may increase the acetylation of p130, thus increasing nuclear p130 levels. Further investigation is needed to fully elucidate the mechanisms of XPC regulation by SIRT1.

Our data support the conclusion that SIRT1 is a tumor suppressor in human skin cancer, because SIRT1 is significantly down-regulated in a wide range of keratinocyte neoplasia. In fair-skinned patients, SIRT1 loss may be an important event in human skin carcinogenesis. This trend of SIRT1 level reduction in skin tumors in fair-skinned patients was not seen in a Japanese patient group, suggesting that genetic and environmental/geographical factors likely play important roles in SIRT1 expression and function in skin cancer (22). A similarly complex function of SIRT1 has been reported in colon cancer, in which SIRT1 is found to be overexpressed in primary tumors (21), but down-regulated in advanced carcinomas (62). Although SIRT1 has been proposed to be a tumor promoter in prostate and skin cancers in Japanese patients (19, 63), recent studies have shown that SIRT1 expression is reduced in many malignancies other than skin (23). Interestingly, our findings indicate that SIRT1 loss occurs in both the early and late stages of SCC, suggesting that in Caucasian skin, SIRT1 acts as a tumor suppressor.

In conclusion, our findings demonstrate a biologically significant role for SIRT1 and a unique molecular regulation of tumor-suppressing DNA repair pathways by SIRT1, and suggest a rationale for potential use of SIRT1 activators to prevent skin cancer. These modes of regulations by SIRT1 are expected to contribute to a better understanding of skin tumorigenesis in humans, and may lead to a better strategy for skin cancer prevention. Furthermore, our findings may yield unique insights into the precise function of SIRT1 in other types of cancer, as impairing XPC/GG-NER capacity may play a key role in increasing susceptibility to carcinogenesis in various organs, including skin (3–5), lung (64–67), and bladder (68).

Methods

Human Skin Tumor Samples. 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 Section of Dermatology, Department of Medicine, University of Chicago. Non–sun-exposed nonlesional normal epidermis, AK, KA, and SCC were used for immunohistochemical analysis of SIRT1 protein levels.

UVB Irradiation. Cells were washed twice with PBS and then exposed to UVB radiation (UV stratalinker 2400 with UVB bulbs; Stratagene) at different doses. Control samples were sham irradiated under the same conditions. The UVB doses were monitored weekly using a Goldilux UV meter equipped with a UVB detector (Oriel Instruments).

Determination of Two Major Forms of UVB-Induced DNA Damage in Genomic DNA by ELISA. Cells were collected at different time points post-UVB, and DNA was isolated using a QIAamp DNA Mini Kit (Qiagen). DNA concentration was calculated from the absorbance at 260 nm using NanoDrop 1000. The CPD and 6-4pp in DNA were quantified by ELISA with monoantibodies (TDM-2 for CPD and 64 M-2 for 6-4PP; Cosmo Bio Co.) as described previously (69) according to the manufacturer's instructions. For examining repair kinetics, the percentage of repair was calculated by comparing the absorbance at the indicated time to that of the corresponding absorbance at time 0 when there was no opportunity for repair and 100% of the CPDs (or 6-4PPs) were present post-UVB.

Statistical Analyses. Data were expressed as the mean of three independent experiments and analyzed by Student's *t*-test, ANOVA, and Mann–Whitney *U* test (for data from human specimens). A two-sided value of $P < 0.05$ was considered significant in all cases.

The details for cell culture, immunohistochemical analysis, siRNA and plasmid transfection, isolation of chromatin-bound fraction, immunoprecipitation, cell growth and apoptosis, cytosolic and nuclear fractionation, Western blotting, real-time PCR, and luciferase reporter assays can be found in *SI Appendix*.

ACKNOWLEDGMENTS. We thank Terri Li for SIRT1 immunohistochemistry. We are grateful to Dr. Pradip Raychaudhuri (University of Illinois, Chicago) for kindly providing the XPC-Luc plasmids and to Dr. Ann Motten for critical reading of the manuscript. This work was supported by National Institutes of Health (NIH) Grant ES016936 (to Y.Y.H.), NIH Grant Z01 ES102205 (to X.L.), University of Chicago Comprehensive Cancer Center Grant P30 CA014599, Clinical and Translational Science Award NIH Grant UL1RR024999, and the University of Chicago Friends of Dermatology Endowment Fund.

1. Niggli HJ, Röthlisberger R (1988) Cyclobutane-type pyrimidine photodimer formation and induction of ornithine decarboxylase in human skin fibroblasts after UV irradiation. *J Invest Dermatol* 91:579–584.
2. Vink AA, Berg RJ, de Gruijl FR, Roza L, Baan RA (1991) Induction, repair and accumulation of thymine dimers in the skin of UV-B-irradiated hairless mice. *Carcinogenesis* 12:861–864.
3. Cleaver JE (2005) Cancer in xeroderma pigmentosum and related disorders of DNA repair. *Nat Rev Cancer* 5:564–573.
4. Cleaver JE, Lam ET, Revet I (2009) Disorders of nucleotide excision repair: The genetic and molecular basis of heterogeneity. *Nat Rev Genet* 10:756–768.
5. Hoeijmakers JH (2001) Genome maintenance mechanisms for preventing cancer. *Nature* 411:366–374.
6. Volker M, et al. (2001) Sequential assembly of the nucleotide excision repair factors in vivo. *Mol Cell* 8:213–224.
7. Sugasawa K, et al. (1998) Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol Cell* 2:223–232.
8. Riedl T, Hanaoka F, Egly JM (2003) The comings and goings of nucleotide excision repair factors on damaged DNA. *EMBO J* 22:5293–5303.
9. Haigis MC, Guarente LP (2006) Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction. *Genes Dev* 20:2913–2921.
10. Blander G, Guarente L (2004) The Sir2 family of protein deacetylases. *Annu Rev Biochem* 73:417–435.
11. Guarente L (2000) Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev* 14:1021–1026.

12. Michan S, Sinclair D (2007) Sirtuins in mammals: Insights into their biological function. *Biochem J* 404:1–13.
13. Haigis MC, Sinclair DA (2010) Mammalian sirtuins: Biological insights and disease relevance. *Annu Rev Pathol* 5:253–295.
14. Brooks CL, Gu W (2009) How does SIRT1 affect metabolism, senescence and cancer? *Nat Rev Cancer* 9:123–128.
15. Deng CX (2009) SIRT1, is it a tumor promoter or tumor suppressor? *Int J Biol Sci* 5: 147–152.
16. Lim CS (2006) SIRT1: Tumor promoter or tumor suppressor? *Med Hypotheses* 67: 341–344.
17. Nayagam VM, et al. (2006) SIRT1 modulating compounds from high-throughput screening as anti-inflammatory and insulin-sensitizing agents. *J Biomol Screen* 11: 959–967.
18. Zhang Y, et al. (2009) Deacetylation of cortactin by SIRT1 promotes cell migration. *Oncogene* 28:445–460.
19. Huffman DM, et al. (2007) SIRT1 is significantly elevated in mouse and human prostate cancer. *Cancer Res* 67:6612–6618.
20. Bradbury CA, et al. (2005) Histone deacetylases in acute myeloid leukaemia show a distinctive pattern of expression that changes selectively in response to deacetylase inhibitors. *Leukemia* 19:1751–1759.
21. Stünkel W, et al. (2007) Function of the SIRT1 protein deacetylase in cancer. *Biotechnol J* 2:1360–1368.
22. Hida Y, Kubo Y, Murao K, Arase S (2007) Strong expression of a longevity-related protein, SIRT1, in Bowen's disease. *Arch Dermatol Res* 299:103–106.
23. Wang RH, et al. (2008) Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. *Cancer Cell* 14:312–323.
24. Sugasawa K, et al. (2005) UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. *Cell* 121:387–400.
25. Sugasawa K (2006) UV-induced ubiquitylation of XPC complex, the UV-DDB-ubiquitin ligase complex, and DNA repair. *J Mol Histol* 37:189–202.
26. Lan F, Cacicedo JM, Ruderman N, Ido Y (2008) SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. *J Biol Chem* 283:27628–27635.
27. Cam H, et al. (2004) A common set of gene regulatory networks links metabolism and growth inhibition. *Mol Cell* 16:399–411.
28. Dominguez-Brauer C, Chen YJ, Brauer PM, Pimkina J, Raychaudhuri P (2009) ARF stimulates XPC to trigger nucleotide excision repair by regulating the repressor complex of E2F4. *EMBO Rep* 10:1036–1042.
29. Ikenoue T, Inoki K, Zhao B, Guan KL (2008) PTEN acetylation modulates its interaction with PDZ domain. *Cancer Res* 68:6908–6912.
30. Datta SR, Brunet A, Greenberg ME (1999) Cellular survival: A play in three Akts. *Genes Dev* 13:2905–2927.
31. Okumura K, et al. (2006) PCAF modulates PTEN activity. *J Biol Chem* 281:26562–26568.
32. Han W, Ming M, He TC, He YY (2010) Immunosuppressive cyclosporin A activates AKT in keratinocytes through PTEN suppression: Implications in skin carcinogenesis. *J Biol Chem* 285:11369–11377.
33. Tsukamoto Y, Kato J, Ikeda H (1997) Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*. *Nature* 388:900–903.
34. McAinsh AD, Scott-Drew S, Murray JA, Jackson SP (1999) DNA damage triggers disruption of telomeric silencing and Mec1p-dependent relocation of Sir3p. *Curr Biol* 9:963–966.
35. Mills KD, Sinclair DA, Guarente L (1999) MEC1-dependent redistribution of the Sir3 silencing protein from telomeres to DNA double-strand breaks. *Cell* 97:609–620.
36. Oberdoerffer P, et al. (2008) SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* 135:907–918.
37. Jeong J, et al. (2007) SIRT1 promotes DNA repair activity and deacetylation of Ku70. *Exp Mol Med* 39:8–13.
38. Yuan Z, Zhang X, Sengupta N, Lane WS, Seto E (2007) SIRT1 regulates the function of the Nijmegen breakage syndrome protein. *Mol Cell* 27:149–162.
39. Sugasawa K (2008) Xeroderma pigmentosum genes: Functions inside and outside DNA repair. *Carcinogenesis* 29:455–465.
40. Berg RJ, Ruven HJ, Sands AT, de Gruijl FR, Mullenders LH (1998) Defective global genome repair in XPC mice is associated with skin cancer susceptibility but not with sensitivity to UVB induced erythema and edema. *J Invest Dermatol* 110:405–409.
41. Cheo DL, et al. (2000) Ultraviolet B radiation-induced skin cancer in mice defective in the Xpc, Trp53, and Apex (HAP1) genes: Genotype-specific effects on cancer predisposition and pathology of tumors. *Cancer Res* 60:1580–1584.
42. Choudhary C, et al. (2009) Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science* 325:834–840.
43. Toyooka T, Ibuki Y (2009) Histone deacetylase inhibitor sodium butyrate enhances the cell killing effect of psoralen plus UVA by attenuating nucleotide excision repair. *Cancer Res* 69:3492–3500.
44. Kim MS, Baek JH, Chakravarty D, Sidransky D, Carrier F (2005) Sensitization to UV-induced apoptosis by the histone deacetylase inhibitor trichostatin A (TSA). *Exp Cell Res* 306:94–102.
45. Despras E, et al. (2007) Long-term XPC silencing reduces DNA double-strand break repair. *Cancer Res* 67:2526–2534.
46. Cimprich KA, Cortez D (2008) ATR: An essential regulator of genome integrity. *Nat Rev Mol Cell Biol* 9:616–627.
47. Zhou BB, Elledge SJ (2000) The DNA damage response: Putting checkpoints in perspective. *Nature* 408:433–439.
48. Luo J, et al. (2004) Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. *Proc Natl Acad Sci USA* 101:2259–2264.
49. Tang Y, Zhao W, Chen Y, Zhao Y, Gu W (2008) Acetylation is indispensable for p53 activation. *Cell* 133:612–626.
50. Lain S, et al. (2008) Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. *Cancer Cell* 13:454–463.
51. Guo X, Williams JG, Schug TT, Li X (2010) DYRK1A and DYRK3 promote cell survival through phosphorylation and activation of SIRT1. *J Biol Chem* 285:13223–13232.
52. Lehman TA, et al. (1993) p53 mutations in human immortalized epithelial cell lines. *Carcinogenesis* 14:833–839.
53. Datto MB, et al. (1995) Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc Natl Acad Sci USA* 92:5545–5549.
54. Smith ML, et al. (2000) p53-mediated DNA repair responses to UV radiation: Studies of mouse cells lacking p53, p21, and/or gadd45 genes. *Mol Cell Biol* 20:3705–3714.
55. Zhu Q, Wani MA, El-Mahdy M, Wani AA (2000) Decreased DNA repair efficiency by loss or disruption of p53 function preferentially affects removal of cyclobutane pyrimidine dimers from non-transcribed strand and slow repair sites in transcribed strand. *J Biol Chem* 275:11492–11497.
56. Ferguson BE, Oh DH (2005) Proficient global nucleotide excision repair in human keratinocytes but not in fibroblasts deficient in p53. *Cancer Res* 65:8723–8729.
57. Jonason AS, et al. (1996) Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc Natl Acad Sci USA* 93:14025–14029.
58. Di Cristofano A, Pandolfi PP (2000) The multiple roles of PTEN in tumor suppression. *Cell* 100:387–390.
59. Wong S, Weber JD (2007) Deacetylation of the retinoblastoma tumour suppressor protein by SIRT1. *Biochem J* 407:451–460.
60. Weinberg RA (1995) The retinoblastoma protein and cell cycle control. *Cell* 81: 323–330.
61. Mittnacht S (1998) Control of pRB phosphorylation. *Curr Opin Genet Dev* 8:21–27.
62. Kabra N, et al. (2009) SirT1 is an inhibitor of proliferation and tumor formation in colon cancer. *J Biol Chem* 284:18210–18217.
63. Jung-Hynes B, Nihal M, Zhong W, Ahmad N (2009) Role of sirtuin histone deacetylase SIRT1 in prostate cancer. A target for prostate cancer management via its inhibition? *J Biol Chem* 284:3823–3832.
64. Marín MS, et al. (2004) Poly (AT) polymorphism in intron 11 of the XPC DNA repair gene enhances the risk of lung cancer. *Cancer Epidemiol Biomarkers Prev* 13: 1788–1793.
65. Hollander MC, et al. (2005) Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis. *Proc Natl Acad Sci USA* 102:13200–13205.
66. Lee GY, et al. (2005) XPC polymorphisms and lung cancer risk. *Int J Cancer* 115: 807–813.
67. Hu Z, et al. (2005) DNA repair gene XPC genotypes/haplotypes and risk of lung cancer in a Chinese population. *Int J Cancer* 115:478–483.
68. Zhu Y, et al. (2007) Genotypes, haplotypes and diplotypes of XPC and risk of bladder cancer. *Carcinogenesis* 28:698–703.
69. Nakagawa A, et al. (1998) Three-dimensional visualization of ultraviolet-induced DNA damage and its repair in human cell nuclei. *J Invest Dermatol* 110:143–148.