Sirtuin 1-mediated deacetylation of XPA DNA repair protein enhances its interaction with ATR protein and promotes cAMP-induced DNA repair of UV damage

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Running title: SIRT1-deacetylation of XPA mediates cAMP-enhanced DNA repair

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Abstract

Blunted melanocortin 1 receptor (MC1R) signaling promotes melanocyte genomic instability in part by attenuating cAMP-mediated DNA repair responses, particularly nucleotide excision repair (NER) which recognizes and clears mutagenic photodamage. cAMP-enhanced NER is mediated by interactions between the ataxia telangiectasia mutated and Rad3-related (ATR) and xeroderma pigmentosum complementation group A (XPA) proteins. We now report a critical role for Sir2uin 1 (SIRT1) in regulating ATR-mediated phosphorylation of XPA. SIRT1 deacetylates XPA at residues K63, K67, and K215 to promote interactions with ATR. Mutant XPA containing acetylation mimetics at residues K63, K67, and K215 exhibit blunted UV-dependent ATR-XPA interactions even in the presence of cAMP signals. ATR-mediated phosphorylation of XPA on S196 enhances cAMP-mediated optimization of NER, and is promoted by SIRT1-mediated deacetylation of XPA on K63, K67 and K215. Interference with ATR-mediated XPA phosphorylation at S196 by persistent acetylation of XPA at K63, K67, and K215 delays repair of UV-induced DNA damage and attenuates cAMP-enhanced NER. Our study identifies a regulatory ATR-SIRT1-XPA axis in cAMP-mediated regulation melanocyte genomic stability, involving SIRT1-mediated deacetylation (K63, K67, K215) and ATR-dependent phosphorylation (S196) post-translational modifications of the core NER factor XPA.

Introduction

Melanoma is an aggressive and life-threatening malignancy whose incidence has risen steadily over the past several decades (1). UV radiation is the most important environmental risk factor for cutaneous melanoma, as evidenced by the abundance of “UV signature” pyrimidine transition mutations in melanoma (2,3), and the association between such mutations and melanoma progression (4). A major inherited risk factor for UV skin sensitivity and melanoma is loss of signaling function of the melanocortin 1 receptor (MC1R), a G-protein-coupled receptor (GPCR) that signals via the second messenger...
cAMP (5-8). Individuals with germline variant MC1R alleles that diminish cAMP signaling tend to be fair in complexion and burn rather than tan with UV exposure (9,10). Such individuals have a lifetime melanoma risk that averages roughly four-fold higher than MC1R-intact counterparts (11,12). Indeed, somatic and UV signature mutations were higher in melanomas isolated from persons with heterozygous or homozygous MC1R loss as compared to wild-type MC1R individuals (13). Thus, the MC1R is a major determinant of melanocytic responses to UV damage. In addition to its role in promoting melanin synthesis (14-16), a crucial function of MC1R is to enhance nucleotide excision repair (NER) (6,17,18), the principal DNA repair pathway active against UV-induced DNA damage (19).

Genomic integrity is challenged by UV exposure, which generates DNA lesions that, if not repaired can give rise to mutations. ATR (Ataxia Telangiectasia and Rad3-related) is an essential regulator of the DNA damage response (20-23). Upon sensing DNA damage, ATR initiates a signaling cascade via phosphorylation of downstream protein substrates, which ultimately leads to a variety of damage responses including cell cycle arrest (22,24). Recently, ATR has been identified as a direct participant in NER (25,26), a coordinated repair process mediated by the xeroderma pigmentosum complementation group proteins (XPs), which include XPA through XPG. XPA is indispensable in this pathway and has reported functions in DNA damage verification, stabilization of repair intermediates and positioning of NER factors (19,27-29). We and others have documented an NER relevant ATR-XPA interaction in response to UV (17,30-34). We have further linked cAMP signaling to this interaction through a phosphorylation event on ATR at S435, which accelerates repair of UV-induced DNA damage (17).

The silent mating type information regulation 2 homolog 1 (Sir2 homolog 1; SIRT1) is a nuclear-localized member of the sirtuin family. SIRT1 regulates a variety of cellular processes such as metabolism (35), oxidative stress (36), and DNA repair (37). Emerging evidence highlights an important function of SIRT1 in NER by catalyzing the deacetylation of the NER proteins XPA (38-40) and RPA (41). In addition, SIRT1 enhances XPC expression by reducing AKT-dependent nuclear localization of the transcription repressor of XPC (37,42). Despite progress in understanding the role of SIRT1 in NER, the molecular mechanisms by which SIRT1 becomes activated in response to UV and the influence of post-translational modifications (PTMs), such as acetylation in the regulation of ATR-XPA interactions remain to be elucidated.

Herein we present evidence that SIRT1 participates in cAMP-enhanced NER and that SIRT1 deacetylates XPA at the K215 residue which has not been previously shown. UV exposure promotes ATR-directed SIRT1 localization to sites of DNA damage. Mutant XPA containing acetylation mimetics at residues K63, K67, and K215 impair the ATR-XPA interaction and blunt NER. Moreover, SIRT1-dependent deacetylation of XPA enhances the ability of ATR to phosphorylate XPA at S196, a molecular event critical to cAMP-enhanced NER. Our study supports a model of cAMP-DNA repair enhancement that utilizes functional cross-talk between acetylation and phosphorylation and identifies a regulatory ATR-SIRT1-XPA axis in the NER pathway.

**Results**

**ATR promotes SIRT1 localization to sites of UV-induced DNA damage**

Our previous work documented a cAMP-dependent pathway that regulates NER pertinent to MC1R signaling in melanocytes. Briefly, we found that in the context of cell damage and cAMP activation, PKA phosphorylates ATR (17). PKA-mediated ATR phosphorylation on S435 promotes interactions between XPA and ATR and accelerates their recruitment to UV photodamage (17) or platinum adducts (43). Because our earlier work documented that cAMP-enhanced NER was dependent on ATR-XPA interactions (17), we considered whether other post-translational modifications in ATR or XPA might regulate this pathway. Since deacetylation of XPA by SIRT1 was reported to be an important regulator of NER following UV radiation (38), we considered whether SIRT1 is involved in cAMP-enhanced NER. To explore whether an ATR-SIRT1 axis may exist, we tested
whether ATR regulates co-localization of SIRT1 at sites of UV damage. We documented a robust interaction between SIRT1 and cyclobutane pyrimidine dimers (CPDs) in UV-irradiated A375 melanoma cells (Fig. 1A, B). However, these interactions were dramatically decreased with the addition of the expression of a kinase-dead form of ATR compared to wild-type expressing ATR (Fig. 1A) or expression of ATR-P2445L, a clinically-relevant inactive ATR mutant identified from the TCGA database with a base substitution in the kinase domain (44) (Fig. 1A, B). The specificity of the assay was confirmed by showing lack of non-specific staining in the negative controls, displayed by the omission of either CPD or SIRT1 antibody alone (Fig. S1). Together, these results suggest that ATR promotes localization of SIRT1 to sites of UV-induced DNA damage.

Since prior work documented UV-dependent interactions between ATR and XPA (17,32-34,45,46), our findings of an ATR-dependent translocation of SIRT1 to UV photodamage prompted us to investigate whether ATR might regulate SIRT1’s association with XPA. XPA is a crucial factor in the repair of UV DNA damage and has been previously identified as an important substrate of SIRT1 (38-40). Therefore, we assessed whether the kinase function of ATR affected interactions between SIRT and XPA (Fig. 1C). We noted robust interaction by co-immunoprecipitation (co-IP) between SIRT1 and XPA in UV-irradiated A375 melanoma cells. However, their interaction was dramatically attenuated by the addition of either a kinase-dead ATR or ATR-P2445L (Fig. 1C). This suggests ATR kinase function is critical to XPA-SIRT1 interaction following UV. In addition, ATR-KD and ATR-P2445L appeared to act in a dominant negative manner to attenuate native ATR function. To characterize this further, we examined the effect of ATR-KD and ATR-P2445L on Chk1 phosphorylation at S345. As expected, expression of ATR-WT resulted in robust Chk1 phosphorylation (Fig. S2). However, levels of Chk1-pS345 were decreased with expression of a kinase-dead form of ATR compared to wild-type expressing ATR, suggesting that kinase-deficient ATR constructs function in a dominant negative manner (Fig. S2).

Realizing that XPA deacetylation by SIRT1 optimizes NER (38,40,47), we evaluated the impact of ATR on the acetylation status of XPA by generating SIRT1 CRISPR/Cas9 deleted A375 melanoma cells and testing the acetylation status of XPA. We observed that in the absence of SIRT1, XPA acetylation was unchanged in response to UV, however with native SIRT expression, UV caused a decrease in acetylated XPA levels (Fig. 1D). Moreover, we conclude that UV-dependent SIRT1-mediated XPA deacetylation is ATR-dependent, since expression of ATR-KD, or ATR-P2445L ablated the SIRT1-dependent deacetylation of XPA following UV-induced DNA damage (Fig. 1D). These data collectively support a UV-induced ATR-SIRT-XPA axis wherein ATR function is needed for SIRT1 localization to sites of photodamage, association with XPA and deacetylation of XPA following UV.

To further investigate whether ATR is the predominant phosphatidylinositol 3′ kinase-related kinase (PIKK), that controls SIRT1 localization to UV-DNA damage, we probed for SIRT1 in UV exposed chromatin fractions in the presence of other PIKK family members ATR, ATM or DNA-PK inhibitors. We determined that SIRT1 localization is reliant upon ATR but does not require ATM or DNA-PK (Fig. S3A). Furthermore, it is unclear how ATR directs SIRT1 to sites of UV DNA damage. To determine whether SIRT1 is a direct phosphorylation target for ATR, we performed co-IPs with an anti-SIRT1 antibody and immunoblotted with an antibody that detects ATR/ATM-phosphorylated SQ sites. Our data indicate SIRT1 is not a direct target of ATR (Fig. S3B).

To provide a greater understanding of the clinical relevance of ATR and SIRT1 in melanoma mutagenesis, we analyzed whole-exome sequence data from melanoma samples obtained from The Cancer Genome Atlas (TCGA). We identified a higher mutational frequency in melanomas that contain mutations in either ATR or SIRT1 compared to melanomas that express their respective wild-type proteins (Fig. S4A,B). Furthermore, mutant ATR-and SIRT1-expressing melanomas are enriched for total mutations at UV-sensitive dipyrimidine sites (i.e. CC-TT), a mutation signature linked to UV exposure (Fig. S4C,D). Together, these data are
consistent in implicating an important role for ATR and SIRT1 in preventing melanoma mutagenesis by UV damage.

The UV-induced XPA-ATR interaction is promoted by SIRT1 and enhanced by cAMP

Having established that SIRT1 deacetylates XPA in an ATR-dependent manner and based on our prior findings documenting cAMP signaling in regulating XPA-ATR interactions (17), we next examined the possibility that cAMP might regulate SIRT1 activity. We first tested whether activation of cAMP signaling would impact intrinsic SIRT1 enzymatic activity. To pharmacologically up-regulate cAMP signaling as we have used previously (17), we treated A375 cells with forskolin, an agent that potently induces cAMP by directly activating adenylyl cyclase. We performed in vitro deacetylation assays using a SIRT1 fluoro-substrate peptide (Fig. S5) with lysate isolated from cells treated with either forskolin or vehicle control. Forskolin treatment increased SIRT1 deacetylase function by roughly two-fold, which was lost in the presence of either the PKA inhibitor H-89 or the selective SIRT1 inhibitor EX 527. In contrast, UV exposure did not significantly modulate SIRT1 activity above baseline levels and did not significantly attenuate forskolin-induced SIRT1 activation (Fig. S5). These data suggests cAMP up-regulates SIRT1 deacetylase activity through a PKA-dependent mechanism independently of UV exposure or ATR-kinase activity.

To assess whether SIRT1 and cAMP signaling impact NER, we investigated the ability of SIRT1 to regulate repair of the major form of UV-induced DNA damage, cyclobutane pyrimidine dimers (CPDs). To do so, we deleted SIRT1 by CRISPR/Cas9 genome editing in A375 cells and measured CPD repair in the presence or absence of SIRT1 in cells exposed to either UV alone or to UV and forskolin. We observed minimal interaction between ATR and XPA in the SIRT1-deleted background basally or with UV and/or forskolin treatments assessed proximity ligation assay (PLA) (Fig. 2B,C). In contrast, we noted robust induction of ATR-XPA association and the effect was enhanced by cAMP stimulation by forskolin. The specificity of the proximity ligation assay was confirmed by showing lack of non-specific staining in the negative controls, displayed by the omission of either ATR or XPA antibody alone (Fig. S8). These experiments confirmed that expression of SIRT1 enhances the physical interaction between ATR and XPA in the context of UV damage and that cAMP signaling augments their association in a SIRT1-dependent manner.

SIRT1-mediated XPA deacetylation promotes the XPA-ATR interaction

To further appreciate how SIRT1 may regulate the interaction between ATR and XPA following UV exposure, we explored XPA acetylation/deacetylation by transfecting either a HA-tagged N- (XPA-Δ2-98) and/or HA-tagged C- (XPA-Δ99-273) terminal domain truncated XPA mutants (Fig. 3A) in XPA CRISPR/Cas9 cells, followed by an immunoprecipitation with anti-HA to obtain the XPA mutant protein. The immunoprecipitated XPA was rinsed with high salt (0.5M) to remove low-affinity binding proteins and then incubated with recombinant
Creb binding protein (CBP), an established XPA acetylating protein (38), followed by incubation with recombinant SIRT1. Acetylation levels were analyzed by western blot with anti-AcK (Fig. 3B,C). Incubation with CBP enhanced acetylation within both N- and C-terminal XPA mutants and addition of SIRT1 resulted in ~20-30% decrease in acetylation in both mutants (Fig. 3C). These experiments demonstrate that XPA has the potential to be acetylated at both the N- and C-terminal domains, and that XPA is a substrate for both CBP-mediated acetylation and SIRT-mediated deacetylation.

To identify important acetylation sites on XPA, we examined specific acetyl-lysine XPA targets using GPS-PAIL 2.0 acetylation prediction analysis. The internal residues of XPA predicted as highly probable reversible acetyl-lysines were K63, K67 and K215. Lysines 63 and 67 were previously identified as substrates for SIRT1-deacetylation (38), however, K215 has yet to be investigated, and the impact of cAMP signaling on modification of these three residues are unknown. To determine the functional impact of these sites in the context of UV-induced DNA damage and cAMP signaling, we deleted XPA from A375 cells by CRISPR/Cas9 genome editing. Using these XPA-null cells, we reconstituted them either with XPA-WT or with K63Q, K67Q or K215Q mutants to mimic acetylation at K63, K67, or K215 respectively. Cells were exposed to either UV or forskolin alone or a combination of UV and forskolin. PLA (Fig. 3D) confirmed that the acetylation-mimicking mutants individually demonstrated reduced interactions between ATR and XPA in response to UV and cAMP signaling. Furthermore, as acetylation/deacetylation K215 pertinent to XPA function has not been previously described, we assessed whether SIRT1 may be a direct deacetylase for the AcK-215 substrate (Fig. S9A). We tested the ability of SIRT1 to deacetylate a short peptide containing an acetylated-K215 and surrounding residues in a cell free-system. The addition of recombinant SIRT1 promoted robust direct deacetylation of K215, which was ablated in the presence of EX 527, a selective SIRT1 inhibitor. Notably, lysine 215 exists as an acetylation consensus sequence and is highly conserved across species (Fig. S9B). Collectively, these results indicate residues K63, K67, and 215 of XPA are direct deacetylation targets of SIRT1 and that interference with the deacetylation of any of the three SIRT1 target lysine residues results in a dramatic reduction in UV-dependent and cAMP-enhanced ATR-XPA interactions.

**Deacetylation of XPA enhances ATR-mediated phosphorylation of S196**

As ATR-mediated phosphorylation of S196 in XPA regulates the repair of UV-induced DNA damage (33,46), we next explored whether cAMP signaling impacts ATR-mediated XPA-pS196 generation and assessed whether SIRT1-mediated deacetylation of XPA might affect XPA’s ability to be phosphorylated on S196 by ATR. We used HCT116 ATR<sup>fl<sup>ox</sup>/</sup>-cells (48) which contain one conditional ATR allele that can be deleted by infecting with adenovirus encoding the Cre recombinase and then transfected with ATR-WT and treated with UV and/or forskolin and immunoblotted with a phospho-specific antibody generated against XPA-pS196. UV treatment resulted in an approximately 2-fold increase in XPA-pS196 compared to non-treated cells transfected with ATR-WT; pre-treatment with forskolin before UV exposure further augmented XPA-pS196 roughly 3-fold above baseline levels (Fig. 4A). In contrast, levels of XPA-pS196 induced by forskolin were attenuated when ATR-null cells were reconstituted with ATR-S435A, indicating the importance of cAMP-induced generation of ATR-pS435 in subsequent ATR-mediated XPA phosphorylation on S196. Importantly, the addition of inhibitors for either SIRT1 or ATR each reduced XPA-pS196 levels in ATR-WT reconstituted cells, strongly suggesting that these proteins possess important upstream functions for ATR-mediated phosphorylation of XPA on S196 (Fig. 4A). Moreover, expression of the alanine substitution at the 435 position ablated the cAMP-enhancement in XPA-pS196, suggesting that PKA-mediated phosphorylation of ATR on the S435 residue may facilitate its subsequent ability to phosphorylate (and activate) XPA at position S196. Furthermore, reducing cellular cAMP below basal levels, by treating cells with both a PKA inhibitor and an adenyate cyclase inhibitor, diminished the levels of UV-induced XPA-pS196, below basal levels (Fig. S6D).
As we now had evidence to suggest that SIRT1 activity regulates ATR-mediated XPA phosphorylation in the context of cAMP signaling (e.g. ATR-pS435), we next tested whether SIRT1-mediated deacetylation of XPA on K63, K67, and/or K215 is necessary for ATR-mediated XPA-p196S generation. For these experiments, we used XPA CRISPR/Cas9 deleted A375 cells either complemented with XPA-WT or with one of the following single XPA mutants: K63Q, K67Q or K215Q (Fig. 4B). Cells were exposed to either UV or forskolin alone or a combination of UV and forskolin. Co-IP experiments confirmed that the acetylation-mimicking mutants demonstrated a dramatic reduction in XPA-pS196 accumulation following UV and little-to-no enhancement by cAMP signaling.

As it has been previously shown that ATR phosphorylates XPA at S196 to enhance XPA stability (46), we chose to examine the possibility that XPA-pS196 might also regulate SIRT1 protein stability. To achieve this we utilized CRIPSR/Cas9 deleted XPA cells expressing either XPA-WT or XPA-S916A (Fig. S10A) and measured relative SIRT1 protein levels for 9 hrs after UV treatment, in the presence of cycloheximide. Cells which expressed ATR-S196A demonstrated a reduced half-life of SIRT1 compared to XPA-WT (Fig. S10B), implying that the phosphorylation status of S196 impacts SIRT1 stabilization after UV exposure. Taken together, these results suggest that SIRT1 and ATR are important factors in phosphorylation of XPA at S196, an event known to increase its function in the context of NER. These data further show that cAMP signaling augments XPA-pS196 and that deacetylation of XPA promote phosphorylation of XPA at S196 by ATR. Furthermore, XPA-pS196 enhances the stability of SIRT1 following UV DNA-damage.

**XPA acetylation mimetics K63Q, K67Q and K215Q interfere with cAMP-mediated enhancement of NER**

To assess the functional significance of XPA deacetylation at K63, K67, and 215 and phosphorylation at S196 in cAMP-enhanced DNA repair, we measured the effect of forskolin on clearance of CPDs in XPA CRISPR/Cas9 deleted A375 cells that were transfected with either XPA-WT, single acetylation mimetic mutants (K63Q, K67Q, or K215Q) or a compound mutant of the three lysines (K63/67/215Q) (Fig. S11A). Treatment of XPA-WT expressing cells with forskolin significantly enhanced the repair of CPD at 24 and 48h post-damage compared to vehicle treated cells (Fig. S11B). Each of the deacetylation mutants exhibited some degree of delayed repair of UV-induced damage relative to wild-type XPA and reduced the cAMP benefit in damage removal, as measured by repair kinetics (Fig. S11B-F) and fold change in the time taken to repair half of the initial DNA damage (repair t½) (Fig. S11B-F). These data strongly suggest that deacetylation of XPA’s K63, K67 and K215 sites are important for cAMP-enhanced repair of UV-induced DNA damage.

As shown in figure 5, the XPA-acetylation mimicking mutants (K63Q, K67Q, or K215Q) reduced UV- and cAMP-induced XPA phosphorylation at S196. As this ATR-mediated phosphorylation is an important event in NER (31), we explored the importance of XPA-pS196 in cAMP enhanced repair. We measured the effect of forskolin on clearance of CPDs in XPA CRISPR/Cas9 deleted A375 cells that were either transfected with a compound acetylation mimetic mutant of the three lysines (K63Q/67Q/215Q) acetylation mimetic containing either S196A or S196D. The expression of the phospho-negative construct XPA-K63Q/67Q/215Q/S196A exhibited blunted repair of UV-induced damage relative to wild-type XPA and did not exhibit any benefit from forskolin treatment (Fig. 5A,B and Fig. S11G). In contrast, cells expressing the phospho-mimetic construct XPA-K63Q/67Q/215Q/S196D demonstrated efficient repair of CPDs in both vehicle- and forskolin-treated cells (Fig. 5A,B and Fig. S4H). Furthermore, to test whether XPA-K63Q/67Q/215Q/S196D can bypass SIRT1 or ATR, we treated A375 cells with either vehicle (Fig. S12A) EX-527 (SIRTi) (Fig. S12B) or VE-821 (ATRi) (Fig. S12C) in cells transfected with either XPA-WT or XPA-K63Q/67Q/215Q/S196D. The addition of XPA-K63Q/67Q/215Q/S196D expression enhanced the repair kinetics compared to XPA-WT (Fig. S12A). The addition of EX-527 only inhibited the repair of XPA-WT expressing cells, but did not...
impact the repair kinetics of the XPA-K63Q/67Q/215Q/S196D expressing cells (Fig. S12B). Whereas, treatment with VE-821 impaired the removal of photoproducts in both the XPA-WT and XPA-K63Q/67Q/215Q/S196D expressing cells. This suggests that ATR provides at least another function (in addition to XPA-S196 phosphorylation) involved in regulating the ATR-SIRT1-XPA axis following UV treatment. Taken together, these data indicate that kinase activities of ATR and SIRT1 functionally cooperate to regulate NER by dynamically controlling post-translational modifications within XPA that reduces genomic UV damage downstream of MC1R-cAMP signaling in melanocytes. Moreover, it appears that SIRT1-mediated deacetylation of K63, K67 and K215 promotes ATR-mediated phosphorylation at S196 to enhance XPA’s function in NER.

Discussion

Inherited dysfunction of the melanocortin 1 receptor (MC1R), a Gs protein-coupled receptor that signals through cAMP, is a bona fide melanoma risk factor (11,13). We and others have documented that MC1R signaling or cAMP induction promotes clearance of DNA damage by enhancing DNA repair (6,8,17,18,49,50). Our previous work identified that in the context of cellular damage, PKA phosphorylates ATR at S435 through the involvement of the AKAP12 scaffolding protein (17,51). Subsequently, enhanced levels of ATR-pS435 associates with XPA at sites of UV photodamage and promotes NER (17,51). Our study supports a model of cAMP-DNA repair enhancement that involves a functional cross-talk between acetylation and phosphorylation and identifies a regulatory ATR-SIRT1-XPA axis in the NER pathway.

Acetylation at lysine residues and its removal has emerged as a critical post-translational modification that enable fine tuning of UV-induced DNA damage repair response (41,52). We provide evidence SIRT1 acts as a direct positive regulator of NER, supporting previous studies demonstrating SIRT1 in UV (37,38,41) and cisplatin (40) damage/repair responses. It is important to note, however, that SIRT1 may impact NER by more than one mechanism. Work by He and coworkers demonstrated SIRT1 inhibition impairs NER by suppressing transcription of the NER initiating factor XPC (37), and it is possible that SIRT1 may regulate NER proteins using both transcriptional and non-transcriptional mechanisms (37,38,40). SIRT1’s influence on NER may be complex, since others found that SIRT1 negatively regulates the interaction between RPA70 and XPA by deacetylating RPA post-repair (41). The function of SIRT1 in DNA repair may be influenced by multiple factors, including cell type, protein-interactions, repair-context and type or extent of damage (37-40,53). A context-specific role for SIRT1 in NER is not surprising, as tightly regulated control of protein deacetylation is required to prevent incorrect protein-protein binding at inappropriate times, which may obstruct normal DNA repair functions. Our data suggest that in the context of melanocytes, cAMP signaling acts as an activating factor for SIRT1 catalytic activity, which may prime the cell to more effectively deal with UV exposure.

We further identified that SIRT1-mediated deacetylation of XPA promotes the interaction between ATR and XPA. In addition to deacetylation at the K63 and K67 sites (previously reported to alter XPA’s interaction with RPA32 (38)), SIRT1 also deacetylates XPA on the K215 residue. We provide evidence that K215, which is located within the ATR binding region of XPA (54), is relevant to the XPA-ATR association. Our cell-free experiments documented that CBP acetylates XPA at the K215 site, however we cannot rule out the possibility that other acetyltransferases are able to modify K215. A previous study incubating the HAT domain of p300 with XPA, did not detect acetylation past amino acid 97 on XPA (38). This suggests XPA may be a substrate for multiple acetyltransferases and/or a full-length p300 protein maybe required to enable appropriate interactions with XPA. In any case, our observations, taken together with previous studies (38), suggest that XPA acetylation appears to play a negative role in regulating XPA-protein interactions to attenuate NER capacity.

Our data further support previous studies that have demonstrated expression of kinase-dead or reduced-kinase forms of ATR result in a
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dominant-negative phenotype (55-57). In our case, we show a dominant negative impact on the SIRT1 and XPA interaction. Expression of kinase-dead mutant forms of ATR (55,58-61), have been previously shown to have a dominant negative impact on Chk1 phosphorylation (55-57). This phenotype could result from the fact that ATR exists in a multi-protein complex (60). Thus, a mixed complex existing of both mutant ATR and ATR-WT may impart dominant negative effects on kinase activation and/or sequestration of proteins in the ATR-signaling pathway.

Our study provides evidence that ATR has at least two functions involved in regulating the ATR-SIRT1-XPA axis following UV treatment. One is that ATR acts upstream of SIRT1 to enhance SIRT1-DNA damage association and the other is that ATR promotes phosphorylation of XPA at S196. As phosphatidyl inositol 3′ kinase-related kinases (such as ATR and ATM) are activated in response to DNA damage and subsequently phosphorylate target proteins (62), it is plausible that ATR can facilitate a signaling cascade in multiple ways. A previous study described a multi-functional response of ATM in response to DNA damage. SIRT1 was recruited to double strand breaks in an ATM-mediated manner, which in turn facilitated SIRT1 to promote the kinase activity of ATM (63). The signaling events required to localize SIRT1 to UV- and double strand break- damage and repair proteins remain to be fully elucidated. SIRT1-mediated deacetylation of XPA dramatically increased phosphorylation of XPA at S196. ATR has been previously identified as the kinase responsible for this modification (31), and our data support this finding, as in the absence of ATR-WT or in the presence of an ATR-kinase inhibitor, XPA-pS196 levels are severely diminished. We reason that SIRT1-mediated deacetylation of XPA at K63, K67, and K215 may promote conformational changes in XPA to favor phosphorylation of S196 by ATR. In agreement with other studies (31,46,64), we found that the phosphorylation of S196 on XPA enhances NER, and extend their observations by placing the post-translational modification at S196 squarely in the mechanism by which cAMP enhances NER. Interestingly, enhanced repair kinetics afforded by expression of the XPA-K63Q/67Q/215Q/S196D were lost in the presence of an ATR kinase inhibitor, providing evidence that ATR provides other function(s) in addition to phosphorylation of XPA at S196.

We previously linked cAMP signaling to the ATR-XPA interaction through PKA-mediated phosphorylation of ATR at S435 (17). In this study, we find that phosphorylation of ATR at Ser435 facilitates cAMP-enhanced accumulation of XPA-pS196. However, it is unclear how ATR-pS435 enhances levels of XPA-pS196. One explanation is that ATR-pS435 may be able to stabilize interactions with XPA via a conformation change to aid the ability of ATR to phosphorylate XPA. This hypothesis is supported by a recent study that showed phosphorylation at a near-by serine (S428) results in a conformational change in the N-terminal region of ATR (65). It is also possible that S435 phosphorylation may influence the structure of ATR to enable a greater domain accessibility between ATR and XPA. In any case, our findings suggest that phosphorylation of ATR at Ser435 is an important event that facilitates cAMP-enhanced XPA-pS196 accumulation. In addition, our data suggest ATR might, regulate the SIRT1-XPA interaction by providing a larger pool of SIRT1 to be available to interact with XPA.

Our studies support the possibility that pharmacologic cAMP activation may be a useful preventative strategy for enhanced melanocyte genomic stability. Conversely, as NER activity can be impaired through post-translational modifications on XPA (e.g. acetylation of K63, K65 and/or K215), manipulation of these modifications via pharmacological targeting may selectively inhibit DNA repair activities in order to develop novel melanoma therapeutics.

Experimental Procedures

Cell lines, plasmids, pharmaceutical inhibitors, recombinant proteins, antibodies and SIRT1 activity.

A375 melanoma cells (ATCC) were cultured in RPMI-10% FBS media. HCT116 ATR\textsuperscript{lox/−} cells were cultured in McCoy\textsuperscript{®}-10% media and Cre recombinase adenovirus (Vector Labs) using at 100pfu per reaction. CRISPR targeted to XPA and SIRT1 was performed using manufacturer’s instructions (Santa Cruz). All
transfections and CRIPSR/Cas9 deletions were confirmed by Western blotting. Cells were transfected with turbofect (Thermo Fisher) using manufacturer’s instructions. Expression of XPA was achieved in a pPM-C-HA vector containing either XPA-WT, or one of the following mutants XPA-Δ2-98, XPA-Δ98-273, XPA-K63Q, XPA-K67Q, XPA-K215Q, XPA-K63/67/215Q or XPA-K63/67/215Q/S196D. Acetylation and phosphorylation mimetics were generated using the Aligent QuickChange II XL mutagenesis kit. Expression of SIRT1-WT was achieved in a pECE vector (Addgene). Forskolin (Sigma) was used at final concentrations of 10uM. Inhibitors for ATR kinase activity (VE-821), SIRT1 kinase activity (EX-527 and Sirtinol) were used at concentrations of 10 µM (Selleckchem). Recombinant SIRT1 (R&D Systems) and XPA (R&D Systems) were used as indicated. Antibodies used were ATR-WT (Amsbio), CPD (Kamiya), XPA (Cell Signaling), XPA-pS196 (Thermo-Scientific), HERC2 (Abcam), SIRT1 (Cell signaling), HA (Cell signaling) and acetyl-lysine (Ac-K) (Cell Signaling). SIRT1 activity was used as directed by the manufactures’ protocol (Abcam).

**UV exposure**

UV radiation was measured via a Model IL1400A handheld flash measurement photometer (International Light) with UV lamps emitting a spectral output in the 290–400 nm range (72 % UVB, 27 % UVA, < 0.01 % UVC) (UVP, Upland, CA). UV exposure was performed when media was removed from the cells. A dose of 10 J/m² of UVB was delivered to cell cultures.

**Sub-cellular fractionation, immunoprecipitation and immunoblotting.**

Sub-cellular fractionation was performed with ~1 × 10⁶ cells. Nuclear extraction was performed using manufacturer’s instructions (Active Motif). Immunoprecipitations were performed with overnight incubations of the primary antibody at 4°C, followed by 3h incubation with protein A beads (GE Healthcare). The precipitates were then washed with PBS and boiled in 2 x SDS loading buffer. Samples were resolved on SDS-PAGE, transferred to PVDF filter membrane and immunoblotted with the indicated antibodies. For Western Blot acquisition analysis, a Storm860 was used and Western blots scanned using channel 2 with blue excitation at 450nm and emission at 520nm, sensitivity was set to normal and PMT voltage set to 400 V.

**Immunofluorescence and proximity ligation assay.**

Following UV-induced DNA damage, cells were either processed immediately, or medium was replaced and DNA repair allowed for indicated periods. Following fixation in 4% paraformaldehyde and cell permeabilization with 0.3% Triton X-100, cells were blocked overnight in 10% donkey serum at 4°C. Proximity ligation assay (DuoLink, Sigma) was performed using the manufacturer’s instructions. All fluorescence images were obtained using a Leica DMI 6000 confocal microscope using x100 objective (1.4 numerical aperture) with LAS AF 2.7.2.9586 software (Leica Application Suite Advanced Fluorescence). Maximum intensity images from focal plane z-stacks (spaced 0.2 µm apart) were acquired and deconvoluted. Fluorescent signals were counted and expressed as either foci number or percent nuclear stain.

**Peptide deacetylation Assay.**

Acetylation assays were performed using a biotinylated peptide substrate which had been acetylated at K215 of XPA and surrounding residues, RQENRRMKQRRF (all other K were changed to R, to confirm specificity to K215) (Genscript) and bound to streptavidin-coated 96 well plates (Thermo-Scientific). Recombinant SIRT1 was incubated for 10 min at 30°C. The reaction buffer consisted of 50 mM Tris-HCl (pH 9.0), 50 mM NaCl, 4 mM MgCl₂, 0.1 mM DTT, 0.01% NP-40, and 100 µM NAD. After indicated treatments, of either recombinant SIRT1 and EX-527, wells were washed with 40 mM Tris-HCl (pH 7.5) containing 0.01% BSA (wash buffer) followed by fixation in 4% paraformaldehyde. After three washes, 2 µg of either anti-Ac-K was added for 1h. Detection was accomplished using an HRP-conjugated anti-rabbit secondary antibody (Abcam) for 1 h followed by the addition of 1-Step Ultra TMB ELISA Substrate (Pierce) and absorbance measured at 400 nm.
Acetylation/Deacetylation interaction Assay.
A375 cells expressing either HA-tagged XPA-WT, or XPAΔ2-98, or XPAΔ99-273 were immunoprecipitated with an anti-HA antibody, and washed with 0.5M NaCl to remove weakly bound proteins. After two PBS washes, recombinant CBP (10ng) was incubated for 30 min at 30°C. The reaction buffer consisted of 50 mM HEPES (pH 8.0), 10% glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 mM sodium butyrate, 1 µM acetyl-CoA. Acetylation was inhibited by the addition of SCG-CBP30 and washed twice with PBS. Recombinant SIRT1 was then incubated for 30 min at 30°C. The reaction buffer consisted of 50 mM Tris-HCl (pH 9.0), 50 mM NaCl, 4 mM MgCl2, 0.1 mM DTT, 0.01% NP-40, and 100 µM NAD. The reactions were resolved on SDS-PAGE and standard immunoblotting procedures and acetylation analyzed using an anti-acetyllysine antibody (anti-Ac-K).

DNA repair kinetics
Cells were exposed to 10 J/m² of UVB and immuno-slot blots or protamine sulfate coated ELISA plates were performed with CPD antibodies as previously described (17) or using protamine sulfate coated ELISA plates and detection was accomplished using an HRP-conjugated anti-rabbit secondary antibody (Abcam) for 1 h followed by the addition of 1-Step Ultra TMB ELISA Substrate (Pierce) to each well and absorbance measured at 400 nm. All repair data is expressed as percent repair compared to initial damage.

Statistical Analysis.
Student’s t tests, and one-way ANOVA were performed with GraphPad Prism 5.0. Data were considered statistically significant if p values were less than 0.05.
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Conflict of Interest
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors declare that they have no conflicts of interest with the contents of this article.
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A

B

C

D

17
FIGURE 1. **SIRT1 localization to sites of UV-induced DNA damage is ATR-dependent.**  

_A_. Proximity ligation assay (PLA) of the SIRT1-CPD interaction in A375 melanoma cells at 1 h after UVB (10 J/m²) or mock treatment. Endogenous ATR was not deleted. Cells were either non-transfected, or transfected with ATR-WT, ATR-kinase dead (KD) or ATR-P2445L. PLA was performed with anti-SIRT1 and anti-CPD antibodies. Green detection events signify juxtaposition between SIRT1 and CPD in maximum intensity projection images. Nuclei were stained with DAPI (blue) Bar represents 50 µm.  

_B_. Quantification of the SIRT1-CPD colocalization shown in panel A. Nuclear foci were counted from at least 50 cells from two separate experiments. Data is expressed as average number of nuclear foci and standard deviation.  

_C_. A375 melanoma cells were either non-transfected or transfected with either ATR-WT, ATR-kinase dead (KD) or ATR-P2445L. At 1 h after UVB (10 J/m²) or mock treatment a Co-IP with anti-SIRT1 and immunoblot with anti-XPA was performed. Input represents 10% of total cellular lysate.  

_D_. A375 melanoma cells or SIRT1 CRISPR/Cas9 deleted A375 melanoma cells were either non-transfected or transfected with either ATR-WT, ATR-kinase dead (KD) or ATR-P2445L. At 1 h after UVB (10 J/m²) or mock treatment a Co-IP with anti-acetylation (Ac-K) and immunoblot with anti-XPA was performed. Input represents 10% of total cellular level.
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A

CRISPR-SIRT1 + SIRT1-WT

CRISPR-SIRT1 + empty vector

% CPD Repair

Time after UVR (Hr)

Forskolin

Vehicle

B

CRISPR-SIRT1 + empty vector

UV

Forskolin

PLA: ATR-XPA

DAPI

CRISPR-SIRT1 + SIRT1-WT

UV

Forskolin

PLA: ATR-XPA

DAPI

C

CRISPR-SIRT1 + empty vector

Foci/Nucleus

Forskolin

UV

CRISPR-SIRT1 + SIRT1-WT

Foci/Nucleus

Forskolin

UV
FIGURE 2. SIRT1 promotes the cAMP-enhancement of NER and the XPA-ATR interaction. A, CRISPR/Cas9 deleted A375 melanoma cells transfected with empty vector or SIRT-WT and pre-treated with either vehicle or forskolin (10 μM) for 30 min and mock treated or UVB irradiated (10 J/m²). DNA repair was measured using an anti-CPD antibody at the indicated time points. Inset shows expression levels of SIRT1. B, SIRT1 CRISPR/Cas9 deleted A375 melanoma cells or CRISPR/Cas9 deleted A375 melanoma cells complemented with either empty vector or SIRT-WT were pretreated with vehicle or forskolin (10 μM) for 30 min and mock treated or UVB irradiated (10 J/m²). Proximity ligation assay of the ATR-XPA interaction in A375 melanoma cells at 1 h after UVB (10 J/m²) or mock treatment. PLA was performed with anti-ATR and anti-XPA antibodies. Green detection events signify juxtaposition between ATR and XPA in maximum intensity projection images. Nuclei were stained with DAPI (blue). Bar represents 50 μm. C, Quantification of the ATR-XPA interaction shown in panel B. Nuclear foci were counted from at least 50 cells from two separate experiments. Data is expressed as average number of nuclear foci and standard deviation.
SIRT1-deacetylation of XPA mediates cAMP-enhanced DNA repair

A

B

C

D

XPA-WT

PLA: ATR-XPA

Foci/Nucleus

0 50 100 150 200

Forskolin

- + - +

UV

- - + +

XPA-K63Q

PLA: ATR-XPA

Foci/Nucleus

0 50 100 150 200

Forskolin

- + - +

UV

- - + +

XPA-K67Q

PLA: ATR-XPA

Foci/Nucleus

0 50 100 150 200

Forskolin

- + - +

UV

- - + +

XPA-K215Q

PLA: ATR-XPA

Foci/Nucleus

0 50 100 150 200

Forskolin

- + - +

UV

- - + +
FIGURE 3. XPA deacetylation promotes the XPA-ATR interaction. A, schematic representation of XPA-WT and the XPA-N-terminal (XPA$^{Δ2-98}$) and XPA-C (XPA$^{Δ99-273}$)-terminal truncation mutants. B, flow chart outlining the protocol to analyze acetylation of XPA-WT and XPA$^{Δ2-97}$ and XPA$^{Δ99-273}$, in panel C. C, XPA CRISPR/Cas9 deleted A375 melanoma cells complemented with either HA-tagged XPA-WT, XPA$^{Δ2-98}$, or XPA$^{Δ99-273}$ were analyzed for acetylation. Co-IP with anti-acetylation (Ac-K) and immunoblot with anti-HA. Input represents 10% of total cellular level. D, XPA CRISPR/Cas9 deleted A375 melanoma cells complemented with either XPA-WT, XPA-K63Q, XPA-K67Q, or XPA-K215Q and were pretreated with vehicle or forskolin (10 μM) for 30 min and mock treated or UVB irradiated (10 J/m²). Proximity ligation assay of the ATR-XPA interaction in A375 melanoma cells at 1 h after UVB (10 J/m²) or mock treatment. PLA was performed with anti-ATR and anti-XPA antibodies. Green detection events signify juxtaposition between ATR and XPA in maximum intensity projection images. Nuclei were stained with DAPI (blue). Bar represents 50 µm. Quantification of the ATR-XPA interaction were from nuclear foci were counted from at least 50 cells from two separate experiments. Data is expressed as average number of nuclear foci and standard deviation. Values not sharing a common letter were significantly different as determined by one-way ANOVA; p ≤ 0.05.
SIRT1-deacetylation of XPA mediates cAMP-enhanced DNA repair

A

| Treatment       | - | - | - | - | - | - | + | - |
|-----------------|---|---|---|---|---|---|---|--|
| EX 527          |   |   |   |   |   |   |   |   |
| VE-821          |   |   |   |   |   |   |   |   |
| ATR-S435A       |   |   |   |   |   |   |   |   |
| ATR-WT          | + | + | + | + | + | + |   |   |
| UV              | - | + | + | + | + | + |   |   |
| Forskolin       | - | + | - | + | + | + | + | + |
| I.B XPA-pS196   | -50| -25|   |   | |   |   |   |
| 1 1.8 4.2 5.9 3.5 0.7 0.9 |

B

| Treatment       | - | - | + | + | + | - |
|-----------------|---|---|---|---|---|---|
| UV              |   |   |   |   |   |   |
| Forskolin       |   |   |   |   |   |   |
| I.B XPA-pS196   | -50| -25|   |   |   |   |
| 1 1.8 2.8 3.6  |

| Treatment       | - | - | + | + | + | - |
|-----------------|---|---|---|---|---|---|
| UV              |   |   |   |   |   |   |
| Forskolin       |   |   |   |   |   |   |
| I.B Total XPA   | -50| -25|   |   |   |   |

| Treatment       | - | - | + | + | + | - |
|-----------------|---|---|---|---|---|---|
| UV              |   |   |   |   |   |   |
| Forskolin       |   |   |   |   |   |   |
| I.B Total XPA   | -50| -25|   |   |   |   |

| Treatment       | - | - | + | + | + | - |
|-----------------|---|---|---|---|---|---|
| UV              |   |   |   |   |   |   |
| Forskolin       |   |   |   |   |   |   |
| I.B Total XPA   | -50| -25|   |   |   |   |

| Treatment       | - | - | + | + | + | - |
|-----------------|---|---|---|---|---|---|
| UV              |   |   |   |   |   |   |
| Forskolin       |   |   |   |   |   |   |
| I.B Total XPA   | -50| -25|   |   |   |   |

| Treatment       | - | - | + | + | + | - |
|-----------------|---|---|---|---|---|---|
| UV              |   |   |   |   |   |   |
| Forskolin       |   |   |   |   |   |   |
| I.B Total XPA   | -50| -25|   |   |   |   |
FIGURE 4. Deacetylation of XPA increases ATR-mediated phosphorylation of XPA-S196. A, HCT116 ATR^flox/^- cells were either transfected with ATR-WT or ATR-435A and were treated with forskolin, VE-821, and/or EX-527, as indicated in panel A. Cells were mock-treated or exposed to UVB (10 J/m²). Nuclear levels of XPA-pS196 were determined by immunoblotting. B, XPA CRISPR/Cas9 deleted A375 melanoma cells complemented with either XPA-WT, XPA-K63Q, XPA-K67Q, or XPA-K215Q were pretreated with vehicle or forskolin (10 μM) for 30 min and mock treated or UVB irradiated (10 J/m²). Nuclear levels of XPA-pS196 were determined by immunoblotting.
SIRT1-deacetylation of XPA mediates cAMP-enhanced DNA repair

A

Vehicle

Faster Repair

Slow Repair

Fold-change in Repair t½

WT  K63Q  K67Q  K215Q  K63/Q725Q  K63/Q725Q/S196D  K63/Q725Q/S196A

B

Forskolin

Faster Repair

Slow Repair

Fold-change in Repair t½

WT  K63Q  K67Q  K215Q  K63/Q725Q  K63/Q725Q/S196D  K63/Q725Q/S196A

C

1) UV

2) cAMP

3) Increased Repair

ATR → SIRT1 → XPA → SIRT1 → XPA → ATR

K63  K215  K67  S196

Ac

Ac

Ac

Ac
FIGURE 5. XPA acetylation mimetics K63Q, K67Q and K215Q interfere with cAMP-mediated enhancement of NER. XPA CRISPR/Cas9 deleted A375 melanoma cells complemented with either XPA-WT, XPA-K63Q, XPA-K67Q, XPA-K215Q, XPA-K63/67/215/215Q-S196A or XPA-K63/67/215/215Q-S196D were pretreated with A, vehicle or B, forskolin (10 μM) for 30 min and mock treated or UVB irradiated (10 J/m²). CPD levels were measured at 24 and 48 h post-damage using anti-CPD antibodies. Repair is expressed as the fold-change in the time taken to repair half of the initial DNA damage (repair t½) (17,51) of the mutant XPA expressing cells compared to XPA-WT expressing cells. C, schematic diagram of our proposed ATR-SIRT-XPA axis; 1) ATR promotes SIRT1 localization to UV-induced DNA damage, 2) cAMP enhances SIRT1-mediated deacetylation of XPA, 3) Deacetylation of K63, 67 and 215 on XPA promotes ATR phosphorylation of XPA at S196. XPA-pS196 enhances the repair of UV-induced DNA damage.
Sirtuin 1-mediated deacetylation of XPA DNA repair protein enhances its interaction with ATR protein and promotes cAMP-induced DNA repair of UV damage
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Supporting Information

FIGURE S1. **Confirmation of the specificity of anti-CPD and -SIRT1 antibodies for proximity ligation.** Proximity ligation assay (PLA) using A, SIRT1-CPD antibodies, B, only single SIRT1, C, only single CPD antibodies or D, no amplification controls. A375 melanoma cells were exposed to UVB (10 J/m²) and allowed to repair for 1 h or mock treated. Green detection events signify juxtaposition between SIRT1 and CPD in maximum intensity projection images. Nuclei were stained with DAPI (blue). Note: the omission of each primary antibody or all primary antibodies did not generate any non-specific signals.

FIGURE S2. **Expression of kinase-deficient forms of ATR diminish UV-induced Chk1 phosphorylation.** A375 melanoma cells were either not transfected or transfected with ATR-WT, ATR-kinase dead (KD) or ATR-P2445L and exposed to UVB (10 J/m²) or mock treated. At 1hr post-UV lysates were immunoblotted with anti-Chk1 and anti-Chk1-pS345.

FIGURE S3. **SIRT1 is localized to damage DNA in an ATR-dependent manner but is not directly phosphorylated by ATR.** A, A375 melanoma cells were treated with vehicle or either 10µM of the following inhibitors for 30 min, ATRi (VE-821), ATMi (Ku-55933) or DNA-PKi (KU-7026). Cells were exposed to UVB (10 J/m²) or mock treated and allowed to repair for 1 h. Chromatin extract was used to immunoblot with anti-SIRT1. The levels of H2A was used as a loading control. B, Immunoprecipitation of SIRT1 with anti-SIRT1 antibodies in A375 cells either mock treated or 1h post UVB exposure (10 J/m²). Lysates were immunoblotted with an ATR/ATM phosphorylation-specific antibody that detects phosphorylated SQ/TQ motifs. The phosphorylation of Chk1 by ATR was used as a positive control. Immunoblot inputs represent 10% of total cellular lysate.

FIGURE S4. **Mutations in ATR and SIRT1 are associated with a higher mutational burden in melanoma.** Comparison of the total mutational burden in primary melanomas either containing A, mutated ATR versus a wild-type ATR or B, mutated SIRT1 versus wild-type SIRT1. Comparison of the mutational burden in primary melanomas at CC-TT sites either containing C, mutated ATR versus a wild-type ATR or D, mutated SIRT1 versus wild-type SIRT1. All analyses were performed by Wilcoxon rank sum tests using The Cancer Genome Atlas human skin cutaneous melanoma dataset (n=470). P<0.05 was considered as statistically significant. Note that all values in the figure were added by 1 before taking the log-transformation due to the presence of zeros.

FIGURE S5. **cAMP enhances SIRT1 activity.** A, A375 melanoma cells were pretreated with vehicle or forskolin (10 µM) for 30 min and mock treated or UVB irradiated (10 J/m²) as indicated. In addition, cells were either treated with 10µM of H-89, EX-527 or VE-821 as indicated. At 1hr post-damage, cell lysates were used to measure SIRT1 activity using a fluorophore peptide containing an acetylated lysine, as described in the manufacturer’s protocol. Values not sharing a common letter were significantly different as determined by one-way ANOVA; p ≤ 0.05.

FIGURE S6. **Basal cAMP levels impact DNA repair, XPA-deacetylation and XPA-pS196 levels.** A, basal levels of cAMP in A375 cells were measured with a combination of 10µM of H-89 (PKAi) and 2’,5’-Dideoxyadenosine (ACi). B, A375 cells were pretreated with vehicle, forskolin (10 µM) or a combination of 10µM of H-89 (PKAi) and 2’,5’-Dideoxyadenosine (ACi) for 30 min and mock treated or UVB irradiated (10 J/m²). CDP levels were measured at 24 and 48 h post-damage using anti-CPD antibodies. The * on the treated (either forskolin treated or PKAi and ACi treated) cells repair kinetics indicate a significant difference in amount of damage at the indicated time point compared to the amount of damage in non-treated cells (vehicle-treated). *p ≤ 0.05. C, A375 cells were pretreated with vehicle, forskolin (10 µM) or a combination of 10µM of H-89 (PKAi) and 2’,5’-Dideoxyadenosine (ACi) for 30 min and mock treated or UVB irradiated (10 J/m²). Co-IP with anti-acetylation (Ac-K) antibody and immunoblot with anti-XPA antibody was performed. Input represents 10% of total cellular level. D, A375 cells were pretreated with...
vehicle, forskolin (10 μM) or a combination of 10μM of H-89 (PKAi) and 2′,5′-Dideoxyadenosine (ACi) for 30 min and mock treated or UVB irradiated (10 J/m²). Nuclear levels of XPA-pS196 were determined by immunoblotting.

FIGURE S7. **UV and cAMP promote the XPA-ATR interaction.** A, A375 melanoma cells were pretreated with vehicle or forskolin (10 μM) and mock treated or UVB irradiated (10 J/m²). Proximity ligation assay of the ATR-XPA interaction in A375 melanoma cells at 1 h after UVB (10 J/m²) or mock treatment. PLA was performed with anti-ATR and anti-XPA antibodies. Green detection events signify juxtaposition between ATR and XPA in maximum intensity projection images. Nuclei were stained with DAPI (blue). Bar represents 50 μm.

FIGURE S8. **Confirmation of the specificity of anti-ATR and -XPA antibodies for proximity ligation.** Proximity ligation assay (PLA) using A, ATR-XPA antibodies, B, only single ATR, C, only single XPA antibodies or D, no amplification controls. A375 melanoma cells were exposed to UVB (10 J/m²) and allowed to repair for 1 h or mock treated. Green detection events signify juxtaposition between SIRT1 and CPD in maximum intensity projection images. Nuclei were stained with DAPI (blue). Note: the omission of each primary antibody or all primary antibodies did not generate any non-specific signals.

FIGURE S9. **Acetylated-lysine 215 is deacetylated by SIRT1 in a cell free system.** A, Recombinant SIRT1 together with NAD, as described in experimental procedures, were incubated with 10 μM peptide (RQENRERMK(AcK)QRKF) containing K215 of XPA and surrounding residues (with other lysine residues modified to arginine). The peptide was acetylated by CREB, as described in experimental procedures. Deacetylation was measured using anti-acetyl-lysine antibody coupled with fluorescence detection. B, Lysine 215 is highly conserved.

FIGURE S10. **XPA-S196 enhances SIRT1 protein stability.** XPA CRISPR/Cas9 deleted A375 melanoma cells were complemented with either A, XPA-WT or XPA-S196A. B, XPA CRISPR/Cas9 deleted A375 melanoma cells expressing either XPA-WT or XPA-S196A were exposed to UVB (10 J/m²) and allowed to repair for the indicated times in the presence of cycloheximide (20 μg/ml). Nuclear extracts were probed with anti-SIRT1 levels and immunoblot. Equal loading was confirmed by probing for Lamin B1.

FIGURE S11. **XPA acetylation mimetics K63Q, K67Q and K215Q interfere with cAMP-mediated enhancement of NER.** A, Protein expression levels of XPA in CRISPR-edited A375 cells and subsequent expression of XPA plasmids. XPA CRISPR/Cas9 deleted A375 melanoma cells complemented with either B, XPA-WT, C, XPA-K63Q, D, XPA-K67Q, E, XPA-K215Q, F, XPA-K63/67/215Q, G, XPA-K63/67/215Q-S196A or H, XPA-K63/67/215Q-S196D were pretreated with vehicle or forskolin (10 μM) for 30 min and mock treated or UVB irradiated (10 J/m²). CPD levels were measured at 24 and 48 h post-damage using anti-CPD antibodies. The red * (forskolin) on the mutant XPA graphs indicate a significant difference in amount of damage at the indicated time point compared to the amount of damage in the XPA-wild-type cells (Panel A). *p ≤ 0.05.

FIGURE S12. The **XPA acetylation mimetic K63Q/K67Q/K215Q/S196D bypasses SIRT1 but not ATR.** XPA CRISPR/Cas9 deleted A375 melanoma cells complemented with either A, XPA-WT or XPA-K63Q/K67Q/K215Q/S196D were pretreated with either A, vehicle, B, EX-527 (10 μM) or , C, VE-821 (10 μM) for 30 min and UVB irradiated (10 J/m²). CPD levels were measured at 24 and 48 h post-damage using anti-CPD antibodies.
SIRT1-CPD Antibodies, SIRT1 Antibody Only, CPD Antibody Only, No Amplification Control

A  B  C  D

SIRT1-CPD Antibodies
- UV  + UV
SIRT1 Antibody Only
- UV  + UV
CPD Antibody Only
- UV  + UV
No Amplification Control
- UV  + UV

PLA

DAPI
**UV - + - + - + - +**

|        | Empty Vector | ATR-WT | ATR-KD | ATR-P2445L |
|--------|--------------|--------|--------|------------|
| UV     | -            | -      | -      | -          |

**I.B Chk1-pS345**

| KDa    | Empty Vector | ATR-WT | ATR-KD | ATR-P2445L |
|--------|--------------|--------|--------|------------|
| -75    | 1            | 1.9    | 1      | 2.1        |
| -50    | 1            | 1      | 1.3    | 1.5        |

**I.B CHK1**

| KDa    | Empty Vector | ATR-WT | ATR-KD | ATR-P2445L |
|--------|--------------|--------|--------|------------|
| -75    | 1            | 1.9    | 1      | 2.1        |
| -50    | 1            | 1      | 1.3    | 1.5        |

Jarrett et al, Figure S2
A

P = 0.0000012

B

P = 0.0317

C

P = 0.0000091

D

P = 0.0172

Jarrett et al, Figure S4
| Compound     | H-89 | EX 527 | VE-821 | UV    | Forskolin |
|--------------|------|--------|--------|-------|-----------|
|              | -    | -      | -      | -     | +         |
|              | -    | -      | -      | +     | -         |
|              | -    | +      | +      | +     | -         |
|              | -    | +      | +      | +     | +         |
|              | +    | +      | +      | +     | +         |

**SIRT1 Deacetylase Activity**

**Fold change compared to basal conditions**

(Bar chart showing different treatments with fold change values indicated.)

Jarrett et al, Figure S5
### A375 cells

|        | UV | Forskolin |
|--------|----|-----------|
| PLA:   | -  | +         |
| ATR-XPA|    | -         |
| DAPI   |    | +         |

Experiment conditions:
- **UV**: - (negative), + (positive)
- **Forskolin**: - (negative), + (positive)

Images show PLA:ATR-XPA and DAPI staining patterns for different conditions.
ATR-XPA Antibodies, ATR Antibody Only, XPA Antibody Only, No Amplification Control

A

- UV + UV

B

- UV + UV

C

- UV + UV

D

- UV + UV

PLA

DAPI

Jarrett et al, Figure S8
**A**

Absorbance (AU)

- Ack
- SIRT1
- SIRT1 [10nM]
- SIRT1 [100nM]
- SIRT1 [10nM] + EX 527
- SIRT1 [100nM] + EX 527

**B**

Homo sapiens (human) QENREKMKQKKFDKK
Pan troglodytes (common chimpanzee) QENREKMKQKKFDKK
Equus caballus (horse) QENREKMKQKKFDKK
Bos taurus (cow) QKNREKMKQKKFDKK
Canis lupus (gray wolf) QENREKMKQKKFDKK
Sus scrofa (wild boar) QKNREKMKQKKFDKK
Rattus norvegicus (brown rat) QENREKMKQKKFDKK
Mus musculus (house mouse) QENREKMKQKKFDKK
Oryctolagus cuniculus (European rabbit) QENREKMKQKKFDKK
Condylura cristata (star-nosed mole) QENREKMKQKKFDKK
Gallus gallus (chicken) RDSREKMKQKRFDKK
Xenopus laevis (African clawed frog) KDNRDKMQKKFDKK
Drosophila melanogaster (fruit fly) RKYNKKMKQLRMEVR
### A

**CRISPR-XPA A375 Cells**

| Transfection | XPA-WT | XPA-S196A |
|--------------|--------|-----------|
| I.B XPA      | -      | 1         |
| I.B Lamin B1 | -      | 1         |

**KDa**

- 50
- 25
- 75
- 50

### B

| CHX (h) | XPA-WT | XPA-S196A |
|---------|--------|-----------|
| 0       | 3      | 9         |
| 1       | 0.8    | 0.4       |
| 0.5     | 0.2    | 0.1       |
| 0.05    |        |           |

**I.B SIRT1**

- 1
- 0.8
- 0.7
- 0.4
- 0.5
- 0.2
- 0.1
- 0.05

**I.B Lamin B1**

- 1
- 0.8
- 0.7
- 0.4
- 0.5
- 0.2
- 0.1
- 0.05

*Jarrett et al, Figure S10*
Jarrett et al, Figure S11
