Human SNF5/INI1, a Component of the Human SWI/SNF Chromatin Remodeling Complex, Promotes Nucleotide Excision Repair by Influencing ATM Recruitment and Downstream H2AX Phosphorylation[▽]

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Recent studies have implicated the role of the SWI/SNF ATP-dependent chromatin remodeling complex in nuclear excision repair (NER), but the mechanism of its function has remained elusive. Here, we show that the human SWI/SNF component human SNF5 (hSNF5) interacts with UV damage recognition factor XPC and colocalizes with XPC at the damage site. Inactivation of hSNF5 did not affect the recruitment of XPC but affected the recruitment of ATM checkpoint kinase to the damage site and ATM activation by phosphorylation. Consequently, hSNF5 deficiency resulted in a defect in H2AX and BRCA1 phosphorylation at the damage site. However, recruitment of ATR checkpoint kinase to the damage site was not affected by hSNF5 deficiency, supporting that hSNF5 functions downstream of ATR. Additionally, ATM/ATR-mediated Chk2/Chk1 phosphorylation was not affected in hSNF5-depleted cells in response to UV irradiation, suggesting that the cell cycle checkpoint is intact in these cells. Taken together, the results indicate that the SWI/SNF complex associates with XPC at the damage site and thereby facilitates the access of ATM, which in turn promotes H2AX and BRCA1 phosphorylation. We propose that the SWI/SNF chromatin remodeling function is utilized to increase the DNA accessibility of NER machinery and checkpoint factors at the damage site, which influences NER and ensures genomic integrity.

DNA damage from exposure to environmental agents provokes highly conserved cellular responses essential for maintaining genetic and epigenetic hallmarks of the human genome. The signals emanating from introduction of genomic damage activate checkpoints for arresting the cell cycle, successful completion of DNA repair, or elimination of irreparably injured cells through apoptosis (22, 23). Defects in these processes lead to multiple diseases, including cancer. Chromatin structure modulation is an important regulatory step in DNA damage repair and checkpoint signaling. “Chromatin remodeling” factors incorporate several modifications in chromatin structure, mostly by disruption of histone DNA contacts, and thus facilitate access of proteins to chromatin (19, 31, 47). A number of ATP-dependent chromatin remodeling complexes have been implicated in DNA repair and cell cycle checkpoints. In general, these complexes increase the DNA accessibility of repair proteins, allowing efficient DNA repair (15, 40). Among them, the SWI/SNF complex has been shown to modulate DNA repair in vitro and in vivo after ionizing radiation and UV irradiation (17, 21, 30, 38). The human SWI/SNF complex is composed of a SWI2/SNF2 family ATPase (either BRG1 or BRM), common core subunits (hSNF5/INI1, BAF155, and BAF170), and four to eight addi-

tional subunits (41). The SWI/SNF complex also regulates transcription of several genes (both activation and repression) and is involved in control of proliferation and the mitotic checkpoint (11). Several studies have indicated that the SWI/SNF complex plays an essential role in nucleotide excision repair (NER) of UV damage. For example, the yeast *Saccharomyces cerevisiae* SWI/SNF complex facilitates the removal of 6-4PP [pyrimidine (6-4)pyrimidone photoproduct] lesions in damaged DNA (21), and hSNF5-null mouse embryonic fibroblasts are three- to sixfold more sensitive to UV irradiation than hSNF5 heterozygous mouse embryo fibroblasts (30). Moreover, the depletion of hSNF5 and BRG1 results in defects in cyclobutane pyrimidine dimer repair in HeLa and primary fibroblast cells (17).

NER is a versatile DNA repair pathway that eliminates a wide variety of helix-distorting DNA lesions, e.g., UV-induced cyclobutane pyrimidine dimer and 6-4PP, from the genome of irradiated cells (24). NER occurs by two subpathways: global genomic repair, which removes lesions from the entire genome, and transcription-coupled repair, which eliminates damage from the transcribed strand of actively transcribed genes. NER is mediated by the sequential assembly of repair proteins at the damaged site. UV damage is initially recognized by the DDB1-DDB2-Cul1 4A complex, which binds to lesions and helps recruit the XPC-hHR23B complex (12, 56). The TFIIH complex, containing the XPB and XPD DNA helicases, is recruited by the XPC complex to open the DNA helix around the DNA damage site (13, 62). In transcription-coupled repair, lesions are resected by stalling of RNA polymerase II in coor-

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dination with recognition of stalled transcription by XPG, CSB, and TFIIH (44). Other NER factors, such as XPA and RPA, join the TFIIH complex to verify the DNA structure alteration. Next, two structure-specific endonucleases, XPF-ERCC1 (5' of the lesion) and XPG (3' of the lesion), cut near the junction of single- and double-stranded DNA, releasing a damage-containing 24- to 32-base oligonucleotide (13). The gap-filling DNA synthesis is performed by polymerases δ and ϵ and their coactivators PCNA, RF-C, and RPA.

Checkpoint activation in response to DNA damage is initiated by two related protein kinases, ATR (ATM and Rad3 related) and ATM (ataxia telangiectasia mutated), which are members of the phosphatidylinositol 3-kinase-like kinase family. After UV irradiation, ATR binds directly to UV-induced lesions, or to RPA-coated single-stranded DNA (8, 33). ATR also gets phosphorylated at serine 428 in response to UV, but its role in ATR activation is not known (9, 54). ATM exists as an inactive dimer, but upon ionizing radiation or UV exposure, it gets recruited at the damage site, dissociates to monomers, and becomes autophosphorylated on multiple residues, resulting in its activation (3, 26). While ATR is recognized as the primary transducer in repair and replication of UV-induced DNA damage, a growing body of new evidence also supports the involvement of ATM in response to processed intermediates generated during NER and replication stress (8, 33, 48, 61). ATR and ATM phosphorylate and activate Chk1 and Chk2 protein kinases, respectively, which then promote cell cycle checkpoint arrest through inhibition of CDC25 protein phosphatases (1, 27, 46). DNA damage also results in rapid phosphorylation of the histone variant H2AX on S139 by ATR and ATM. In support of the ATR-dependent ATM activation, it has been shown that the initial UV induction of γ H2AX is dependent on ATR, but double-strand break (DSB) formation at late time points contributes to the ATM activation and increase of γ H2AX (61). Next, γ H2AX recruits MDC1 to chromatin, which also gets phosphorylated by ATM and functions as the docking site for many repair and checkpoint proteins, e.g., 53BP1, the UBC13-RNF8 complex, and BRCA1 (10, 42, 49, 50). Upon recruitment, 53BP1 also gets phosphorylated by ATM and helps recruitment of BRCA1. Recruitment of the MDC1-53BP1-BRCA1 complex and their posttranslational modifications at the damage site ultimately help congregation of other necessary repair proteins. These events at the damage site form megabases of DNA associated with these repair factors to ensure that DNA damage checkpoint signaling is activated, and they also promote DNA repair (43, 51).

Several recent studies have demonstrated that both ATR and ATM are activated in the G₁ and S phases and phosphorylate H2AX. In the G₁ phase, ATR and ATM activation and H2AX phosphorylation are NER factor dependent, but in the S phase this activation and phosphorylation are not exclusively dependent on NER factors (20, 34–36). Accordingly, in the G₀/G₁ and G₂/M phases, DNA damage checkpoint activation requires recognition and processing of lesions, but in the S phase, checkpoint activation does not depend exclusively on lesion processing by NER, because other kinds of lesions, such as exposed single-stranded DNA at stalled replication forks, activate the checkpoint response.

Although some studies have validated the role of the SWI/SNF complex in NER, the molecular mechanism by which the

SWI/SNF complex influences NER is not well understood. Given that hSNF5 is a core member of a complex that contributes to the regulation of chromatin structure, we knocked down hSNF5 to investigate whether the SWI/SNF complex influences NER by directly regulating the access of NER and checkpoint proteins at the damage site. We have demonstrated that hSNF5 interacts with XPC and localizes at the damage site upon UV irradiation. We revealed that XPC recruitment to the damage site was not affected in the absence of hSNF5. Interestingly, ATM recruitment, but not ATR recruitment to the damage site, was affected in the absence of hSNF5. Accordingly, ATM-mediated H2AX and BRCA1 phosphorylation was also affected in hSNF5-depleted cells. Interestingly, hSNF5-depleted cells were not defective in cell cycle checkpoint arrest, as assayed by Chk1 and Chk2 activation by their phosphorylation. We propose that the chromatin remodeling function of SWI/SNF is required for the assembly of repair and checkpoint proteins at the damage site, which eventually influences NER.

MATERIALS AND METHODS

Cell lines, cell cycle arrest, and hSNF5 inactivation. Normal human fibroblasts, OSU-2 cells, were generated in our laboratory as described previously (55). XP-C cells (GM15983) were purchased from the NIGMS human genetic cell repository. In BO5-1 cells expressing a FLAG-tagged dominant-negative BRG-1, SWI/SNF complexes can be conditionally inactivated by the expression of an ATPase-defective dominant-negative version of BRG-1 under the control of a tet-off system (38). HeLa, OSU-2, and XP-C cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere with 5% CO₂. BO5-1 cells were grown in the presence of G418 (75 μ g/ml), hygromycin B (350 to 400 U/ml), and tetracycline (2 μ g/ml). To induce the dominant-negative BRG-1, cells were washed twice in phosphate-buffered saline (PBS) before trypsinizing and plating. Protein was isolated after 96 h in the presence and absence of tetracycline. To arrest the cells in G₁, OSU-2 cells were grown in serum-free medium for 48 h before UV treatment as described before (63). For small interfering RNA (siRNA) transfection, hSNF5 siRNA (Dharmacon) was used at 60 nM (HeLa) or 100 nM (OSU-2) while BRG-1 siRNA (Dharmacon) was used at 100 nM (HeLa). Cells were seeded at 5×10^4 cells/ml and grown overnight, and siRNA was added for 48 h before processing for Western analysis or immunofluorescence.

Antibodies. Primary rabbit anti-XPC antibody was raised and characterized in our laboratory as previously described (12). Anti-hSNF5/INI1-H9912 (Sigma), Ini1 (C20; sc-16189), ATR (N-19; sc1887), ATM (2C1; sc23921), and histone H2AX (C-20; sc-54606) were all obtained from Santa Cruz Biotechnology. The DNA damage antibody sampler kit 9947 (Cell Signaling) included phospho-ATM (Ser1981), phospho-ATR (Ser428), phospho-BRCA1 (Ser1524), phospho-Chk1 (Ser296), and phospho-Chk2 (Thr68). Phospho-histone H2A.X (Ser139) (2577), Chk2 (2662), and Chk1 (2345) were from Cell Signaling, and anti-BRCA1-OP92 was from Calbiochem.

UV irradiation, localized micropore assay, and immunofluorescence. For UV irradiation, cells were washed twice in PBS and irradiated with various doses of UV. The irradiation was done with a germicidal lamp with UV-C light (254 nm) at a dose rate of 0.8 J/m²/s as measured with a Kettering model 65 radiometer (Cole-Palmer, Vernon Hills, IL). For micropore UV irradiation, a 5- μ m isopore polycarbonate filter (Millipore, Bedford, MA) was placed on top of the cell monolayer before irradiation with UV light. For global exposure, cells were exposed without filter directly under the UV light. UV-irradiated cells were processed immediately or maintained in a suitable medium for the desired period and processed thereafter.

The immunofluorescence staining was conducted according to the method established in our laboratory. Briefly, cells were washed twice with cold PBS and then either pretreated with 0.5% Triton X-100 for 5 min or left untreated, and then cells were fixed with 2% paraformaldehyde in 0.5% Triton X-100 at 4°C for 30 min. The cells were rinsed with PBS, blocked with 20% normal goat serum or normal donkey serum, stained with primary antibody, washed four times for 5 min, and stained with fluorescein isothiocyanate- or Texas Red-conjugated secondary antibodies. Fluorescence images were obtained with a Nikon fluorescence microscope E80i (Tokyo, Japan). The digital images were then captured

FIG. 1. hSNF5 associates with UV damage sites via interaction with XPC. (A) Western blot showing the association of XPC and hSNF5. HeLa cells were UV irradiated or left untreated, and lysates were isolated following chromatin fractionation as described in Materials and Methods. UV irradiation was carried out at 20 J/m², and cells were harvested at the indicated time points postirradiation. An equal amount of nuclear fraction was used, immunoprecipitated with anti-XPC antibody, and detected with anti-hSNF5 and anti-XPC antibodies. Normal rabbit antiserum was used to detect the nonspecific interaction, and input (5%) was used to detect the XPC or hSNF5 on the Western blot. (B) hSNF5 expression levels do not change after UV irradiation. HeLa cells were UV irradiated (20 J/m²) or left untreated and after the indicated time postrepair, whole-cell lysates were isolated. Western blotting was performed using anti-hSNF5 and antilamin antibodies. (C) hSNF5 colocalizes with XPC at the UV damage site. For local UV irradiation, cells were exposed to a 100-J/m² UV dose through a micropore filter, allowed to repair for 1 h, and processed for immunofluorescence using anti-hSNF5 and anti-XPC antibodies as described in Materials and Methods. For global UV irradiation, cells were exposed to UV at 40 J/m² without a filter and processed in a similar manner. (D) hSNF5 does not localize at the UV damage site in XPC-deficient cells. Experiments were done as described for panel C using XPC-deficient cells.

with a cooled charge-coupled-device camera and processed with SPOT analysis software (Diagnostic Instruments, Sterling Heights, MI).

Protein isolation and Western blotting. HeLa cells were grown to ~80% confluence. Cells were washed with PBS and UV irradiated at 20 J/m² postrepair for the indicated times. Total protein was isolated by using sodium dodecyl sulfate (SDS) lysis buffer (62 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol) with protease and phosphatase inhibitors followed by boiling for 5 min.

Protein fractionation and immunoprecipitation. For fractionation of extracts, we followed the protocol described by Anindya et al. (2). Briefly, HeLa cells were grown to ~80% confluence, UV irradiated or left untreated, and ~3.5 × 10⁷ cells were lysed with 1 ml of cytoplasmic lysis buffer (10 mM Tris-HCl, pH 7.9, 0.34 M sucrose, 3 mM CaCl₂, 2 mM magnesium acetate, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% NP-40, and protease inhibitors). Nuclei were pelleted by centrifugation at 3,500 × g for 15 min. Nuclei were washed with cytoplasmic lysis buffer without NP-40 and lysed with nuclear lysis buffer (20 mM HEPES, pH 7.9, 3 mM EDTA, 10% glycerol, 150 mM potassium acetate, 1.5 mM MgCl₂, 1 mM dithiothreitol, 0.1% NP-40, and protease inhibitors). The nucleoplasmic fraction was cleared by centrifugation at 15,000 × g for 30 min. The chromatin-enriched pellet was resuspended in nuclease incubation buffer (150 mM HEPES, pH 7.9, 1.5 mM MgCl₂, 150 mM potassium acetate, and protease inhibitors). DNA and RNA in the suspension were digested with 25 U/μl Benzonase (Novagen) for 10 to 20 min at room temperature. The sample was cleared by centrifugation at 20,000 × g for 30 min, and the supernatant was collected. For immunoprecipitation, 1 mg of protein was used with 4 μg of antibody and incubated overnight at 4°C on a rocker platform. The next day, 50 μl of protein G Plus/A-agarose beads was added and incubated at 4°C for 3 h. The samples were washed four times with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) and twice with 1× TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The 1× SDS sample buffer was added and boiled for 10 min before loading.

RESULTS

hSNF5 interacts with NER factor XPC in response to UV irradiation. Involvement of the SWI/SNF complex in NER prompted us to examine the mechanism of SWI/SNF function in NER and checkpoint signaling. We investigated whether hSNF5 plays any direct role in NER and checkpoint activation via interaction with the factors operating in these pathways. In yeast, it has been shown that the SWI/SNF subunit SNF5 (ortholog of human SNF5) interacts with the Rad4-Rad23 complex (ortholog of human the XPC-HHR23 complex) and is involved in NER by UV-induced chromatin rearrangement (18). XPC is a crucial NER factor which gets recruited in the early stage of NER and is essential for the recruitment of downstream NER factors to the lesion site (56). Therefore, we examined whether hSNF5 also interacts with XPC and is involved in the early stage of NER. As expected, endogenous XPC and hSNF5 interacted in a coimmunoprecipitation assay. Although a slight association occurred in unirradiated cells, the association between hSNF5 and XPC promptly increased immediately after UV irradiation, reaching a maximum at 1 h and returning to basal levels within 2 h after irradiation (Fig. 1A). As established earlier, XPC is promptly modified and degraded immediately following irradiation, resulting in a gradual decrease in cellular XPC at different postirradiation times

(58). Accordingly, we observed a corresponding increase in the XPC-interacting levels of hSNF5, which dissipates with the initiation of XPC degradation beginning at 2 h postirradiation. To establish whether the hSNF5 interaction with XPC increases due to the increased expression of hSNF5 after UV irradiation, we determined the levels of hSNF5 protein before and after UV irradiation in an immunoblot assay. Expression of hSNF5 did not change after UV irradiation, supporting the contention that the XPC and hSNF5 interaction is not due to an increased level of hSNF5 protein (Fig. 1B). To directly demonstrate whether the SWI/SNF complex is recruited to the UV damage site, we determined the colocalization of hSNF5 with XPC in irradiated cells. Our previous work showed that local UV irradiation triggered an accumulation of XPC and other NER factors at the damage site, forming distinct damage-specific foci (64). hSNF5 is a nuclear protein and homogeneously distributed in the nucleoplasm under normal conditions (Fig. 1C). We observed that hSNF5 unambiguously colocalizes with XPC, forming distinct foci at the UV damage sites upon local UV irradiation (Fig. 1C). Furthermore, hSNF5 also colocalized with XPC as distinct speckles within the nucleus after global UV irradiation (Fig. 1C). Similar hSNF5 localization was not observed in XPC-deficient cells, indicating that hSNF5 recruitment is dependent on XPC (Fig. 1D). Therefore, the interaction of hSNF5 with XPC appears to have a direct modulating effect on the regulation of NER.

hSNF5 depletion does not significantly alter the cellular level or recruitment of XPC to the UV damage site. The association of hSNF5 with XPC suggests that hSNF5 functions in the early stage of NER. To establish this, we assessed whether hSNF5 remodeling activity influences the access and recruitment of XPC to chromatin. DDB2 is recruited at the damage site immediately after UV irradiation (12). Once the damage has been recognized, DDB2 is degraded by the DDB1-Cul4A complex, facilitating the recruitment of the XPC-HHR23 complex. The Cul4-DDB1-DDB2 complex also promotes XPC ubiquitination at the site of damage, which influences recruitment of other components required for NER (12, 37, 52). Therefore, we examined whether the cellular XPC level and its modification were affected in the absence of hSNF5. hSNF5 protein was efficiently depleted when we used 30 to 60 nM hSNF5-specific siRNA (Fig. 2A). After 48 h of transfection with siRNAs, we observed a marked decrease in hSNF5 protein levels compared to that in cells treated with control siRNA. In an immunoblot assay, we noticed small but consistent reductions in the levels and modification pattern of XPC protein in hSNF5-depleted cells compared to the control cells (Fig. 2B). To further establish the role of hSNF5 in NER, we examined whether hSNF5 depletion impaired XPC recruitment at the damage site. Under our experimental conditions, XPC foci were present in ~45% of cells irradiated with a 100-J/m² UV dose, whereas XPC foci were not noticeable at lower doses of UV (5 to 40 J/m²) (see below). Therefore, we investigated whether hSNF5 depletion affected recruitment of XPC at the damage site by using a 100-J/m² UV dose. Cells were treated with hSNF5 and control siRNA, and XPC levels at the damage site were determined by immunofluorescence. XPC foci were present in ~40% of hSNF5-depleted and control cells, indicating that the recruitment of XPC was not affected in the absence of hSNF5 (Fig. 2C). Additionally, we did

not observe any differences in the intensity and quality of the XPC foci in hSNF5-depleted cells compared to the control cells (Fig. 2D). Taken together, the immunofluorescence and the Western analysis data support that hSNF5 depletion has a small effect on XPC protein levels but does not have any effect on XPC localization to the damage site. Collectively, we conclude that hSNF5 does not significantly influence XPC stability, or its accumulation at the damage site, suggesting that hSNF5 might function downstream of XPC in the UV damage response pathway.

hSNF5 depletion does not affect the cellular ATR level or its recruitment but affects the activation and recruitment of ATM at the UV damage site. On the basis of the XPC and hSNF5 interaction, we hypothesized that hSNF5 is recruited to the damage site to facilitate chromatin remodeling at the early stage of NER. Yet, our results showed that hSNF5 does not influence XPC recruitment at the damage site. Therefore, we speculated that the SWI/SNF complex might influence the recruitment of early checkpoint factors at the damage site. This speculation is based on the fact that the SWI/SNF complex influences DSB repair by promoting H2AX phosphorylation at the damage site (38). As H2AX gets phosphorylated upon UV irradiation by ATR and ATM checkpoint kinases (1, 27), we predict that hSNF5 might influence recruitment of ATR and ATM at the damage site. To test this possibility, we first investigated whether the SWI/SNF complex regulates the activation and recruitment of ATR and ATM at the damage site upon UV irradiation. Immunoblot analysis revealed that the total cellular level of ATR or its phosphorylation at S428 was not affected in hSNF5-depleted cells, whereas phosphorylation of ATM at S1981 showed a small but significant reduction (Fig. 3). In these experiments, the phosphorylated form of the protein was compared with the total cellular protein in each lane. The total cellular levels of ATR and ATM were not different in hSNF5-depleted cells compared to the control cells, indicating that hSNF5 is not required for expression of ATR and ATM. These data indicate that hSNF5 might influence ATM activation by phosphorylation. To further confirm our observation, we examined the levels of ATR and ATM at the UV damage site. ATR and ATM recruitment levels at the damage site were determined by their colocalization with XPC and γ H2AX at different doses of UV (5 to 100 J/m²). Both ATR and phospho-ATM were localized at the damage site with XPC and γ H2AX (Fig. 4A, B, C, and D). Under our assay conditions, ATR foci were not noticeable below a 100-J/m² UV dose, whereas phospho-ATM foci were clearly noticeable even at a 5-J/m² UV dose (Fig. 4A and C). Possibly, phospho-ATM is present at a high density along the damage site, enabling its detection with a low dose of UV irradiation. Alternatively, the numbers of ATR and XPC molecules at the damage site might be lower than for ATM and therefore undetectable at low UV doses. Next, we set out to elucidate whether hSNF5 depletion affects ATR and ATM localization at the damage site. Using a 100-J/m² UV dose, we showed that the number of ATR foci was ~24% in hSNF5-depleted cells, compared to ~32% in control cells, whereas the number of ATM foci was ~34% in hSNF5-depleted cells, compared to ~53% in control cells (Fig. 5A). These data showed that hSNF5 depletion has a marginal effect on ATR and phospho-ATM focus formation at a 100-J/m² UV dose. Additionally, we did not observe any noticeable differ-

FIG. 2. hSNF5 depletion does not significantly affect the XPC level or its modification and does not influence XPC recruitment to the UV damage site. (A) Depletion of hSNF5 protein after hSNF5 siRNA treatment. HeLa cells were transfected with 33 or 66 nM hSNF5 siRNA or a nontargeting control siRNA or mock treated (no transfection). At 48 h after the transfection, lysates were isolated. A Western blot assay was performed with anti-hSNF5 and antiactin antibodies. (B) hSNF5 depletion does not significantly affect XPC levels and ubiquitination. HeLa cells were transfected with 60 nM siRNAs. After 48 h of transfection, cells were treated with 20 J/m² UV or left untreated and harvested at the indicated time points postirradiation. A Western blot assay was performed using anti-XPC, anti-hSNF5, and antilamin antibodies. (C) hSNF5 depletion does not affect XPC focus levels at the UV damage site. HeLa cells were transfected with 60 nM siRNAs. After 48 h of transfection, cells were subjected to UV irradiation (100 J/m²) through micropore filters, left untreated, allowed to repair (1 h), and processed for immunofluorescence using anti-XPC antibody as described in Materials and Methods. The XPC foci in hSNF5- and control siRNA-treated cells were quantitated as follows. An average of 200 merged nuclear foci on five independent microscopic fields were used for the quantitation of focus formation for each experiment. The graph shows the average number of XPC focus-containing cells from at least three independent experiments. The error bars indicates means $\pm$ standard deviations. (D) hSNF5 depletion does not affect XPC recruitment at the damage site. Experiments were done as described for panel C. Immunofluorescence was done using anti-XPC antibody. Images from a representative experiment are shown.

ence in ATR and phospho-ATM focus intensities in hSNF5-depleted cells compared to the control cells (Fig. 5B and C). Therefore, to ensure that we were observing the differences in the phospho-ATM levels at the damage site between hSNF5-depleted and control cells, we assayed the phospho-ATM focus formation quantitatively, using lower doses of UV. Notably, at a 5-J/m² UV dose, phospho-ATM focus formation was markedly decreased in hSNF5-depleted cells compared to the control cells. The phospho-ATM foci were present in only 7% of hSNF5-depleted cells compared to 29% of control cells (Fig. 5A). Moreover, the amount and intensity of most of the phospho-ATM foci were significantly decreased in hSNF5-depleted cells compared to the control cells (Fig. 5D). As such, these results together with the immunoblotting data demonstrate that hSNF5 is required for efficient ATM phosphorylation and ATM recruitment but is not required for phosphorylation and recruitment of ATR at the damage site. To further understand

how SWI/SNF promotes H2AX phosphorylation, we looked at the interaction of hSNF5 with phospho-ATM in response to UV damage. Our data clearly establish an interaction between endogenous phospho-ATM and hSNF5 in response to UV damage (Fig. 5E). Thus, these data strengthen the contention that hSNF5 influences ATM recruitment and directly interacts with phospho-ATM to promote H2AX phosphorylation. Even though some studies have implicated the roles of ATR and ATM exclusively in the S phase, several recent reports showed that both ATR and ATM are activated in the G₁ phase (due to processing of UV photoproducts or NER) and S phase (due to NER and replication fork stalling) in response to UV irradiation and are responsible for H2AX phosphorylation at all the stages of the cell cycle (20, 36). It has been shown that replication fork stalling results in γ H2AX foci in only 10 to 20% of cells comprising the S phase (48). We observed that phospho-ATM foci were present in $\sim$ 53% of cycling nonar-

FIG. 3. hSNF5 does not affect ATR levels, but it affects phospho-ATM levels upon UV irradiation. ATR or phospho-ATR levels were not affected, but ATM phosphorylation was affected in hSNF5-depleted cells. Cell lysates were immunoblotted with anti-phospho-ATR (phospho-serine 438), anti-phospho-ATM (phospho-serine 1981), anti-ATR, anti-ATM, anti-hSNF5, and antiactin antibodies.

rested cells at 100 J/m² UV (Fig. 5A). Therefore, this most likely represents ATM activation both in the G₁ and S phases. Consistent with this, we also observed ATR and ATM focus formation in G₁-arrested primary fibroblast cells upon UV irradiation (data not shown). To assess whether hSNF5 is required for ATM recruitment at the damage site in the G₁ phase, we determined ATM localization at the damage site in G₁-arrested hSNF5-depleted primary fibroblast cells. At a 5-J/m² UV dose, phospho-ATM focus levels were also affected in G₁-arrested hSNF5-depleted cells, suggesting a role for hSNF5 in recruitment of ATM at the damage site that is dependent on the NER lesions (Fig. 5F).

hSNF5 depletion affects the induction of γ H2AX after UV irradiation. Our finding that the SWI/SNF complex might act on chromatin to allow efficient recruitment of ATM led us to hypothesize that phosphorylation of downstream ATM substrates might be affected in the absence of the SWI/SNF complex. ATR/ATM-dependent phosphorylation of H2AX influences accumulation of various repair proteins required for efficient NER (42, 61). Therefore, we next set out to elucidate

FIG. 4. Colocalization of ATR and phospho-ATM with XPC and γ H2AX at the damage site at various UV doses. Cells were subjected to the indicated UV doses through micropore filters, allowed to repair (1 h), and were processed for immunofluorescence using anti-ATR and anti-XPC antibodies (A), anti-ATR and anti- γ H2AX antibodies (B), anti-phospho-ATM and anti-XPC antibodies (C), and anti-phospho-ATM and anti- γ H2AX antibodies (D).

FIG. 5. hSNF5 depletion does not affect recruitment of ATR but affects recruitment of ATM at the UV damage site. (A) Quantitation of ATR and phospho-ATM foci in hSNF5- and control siRNA-treated cells. Immunofluorescence and quantitation of the foci were performed as described for Fig. 2C, using 100 and 5 J/m² UV irradiation. Immunofluorescence was performed using anti-ATR and anti-phospho-ATM antibodies. (B and C) hSNF5 depletion does not affect the intensities of ATR or phospho-ATM foci at a 100-J/m² UV dose. Images from a representative experiment described for panel A are shown. (D) hSNF5 depletion reduces the intensity of phospho-ATM foci at a 5-J/m² UV dose. Images from a representative experiment described for panel A are shown. (E) hSNF5 physically interacts with phospho-ATM in response to UV damage. Immunoprecipitation was done as described for Fig. 1A by using anti-hSNF5 and anti-phospho-ATM antibodies. Input was 8% of the total protein. (F) hSNF5 depletion reduced the intensity of the phospho-ATM foci at the damage site in G₁-arrested cells with 5 J/m² UV exposure. OSU-2 cells were treated with 100 nM hSNF5 or control siRNA, followed by serum starvation for 48 h for arresting the cells at G₁. The cells were then UV irradiated and allowed to repair for 1 h. Cells were processed for immunofluorescence using anti-phospho-ATM antibody.

whether H2AX phosphorylation is compromised in the hSNF5-depleted cells in response to UV irradiation. To test this possible scenario, cells were treated with different UV doses (1 to 20 J/m²), and the phosphorylation of H2AX was determined by Western analysis. The phosphorylation of H2AX after irradiation was significantly reduced in hSNF5-depleted cells (Fig. 6A and B). There was no difference in the levels of total cellular H2AX protein in hSNF5-depleted and control cells, indicating that the hSNF5 is not required for the efficient induction of H2AX. To strengthen our observation, we also used BRG-1-depleted cells and cells in which a catalytically inactive dominant-negative version of BRG-1 is overexpressed. We observed that H2AX phosphorylation is severely compromised in the absence of BRG-1, or dominant-negative BRG-1, compared to the cells harboring the wild-type BRG-1 (Fig. 6C). Thus, depletion of both hSNF5 and BRG-1 resulted

in significant levels of reduction in H2AX phosphorylation. Next, we determined the level of damage-associated γH2AX in hSNF5-depleted cells. For this, we first determined the dose at which γH2AX foci were clearly noticeable in HeLa cells irradiated with 5- to 100-J/m² UV doses. As previously shown (64), γH2AX foci colocalized with XPC at a 100-J/m² UV dose (Fig. 7). Additionally, similar to the phospho-ATM foci, the γH2AX foci were clearly noticeable at doses as low as 5 J/m² (Fig. 7). Next, we examined γH2AX levels at the damage site in hSNF5- and control siRNA-treated cells. At 100 J/m² of local UV irradiation, we showed that γH2AX foci in hSNF5-depleted cells were reduced to 40%, compared to 60% in the control cells (Fig. 8A). Similar to the phospho-ATM foci, there were no noticeable differences in the quality or intensity of the γH2AX foci in hSNF5-depleted cells at a 100-J/m² UV dose (Fig. 8B). As both the Western analysis and immunofluores-

FIG. 6. hSNF5-depleted cells are compromised in the induction of γ H2AX after UV damage. (A) hSNF5-depleted cells show a decrease in γ H2AX levels at different doses of UV irradiation. Lysates from HeLa cells were transfected with siRNA as described for Fig. 2B and UV irradiated at the indicated doses. After 1 h of UV irradiation lysate was immunoblotted with anti- γ H2AX, anti-H2AX, anti-hSNF5, and antilamin antibodies. (B) hSNF5-depleted cells show decreases in γ H2AX levels at different times postirradiation. Experiments were performed as described for panel A, using 20 J/m² UV irradiation. (C) Both BRG-1-depleted cells and cells expressing dominant-negative BRG-1 show decreases in γ H2AX levels. Experiments were performed as described for panel A, using 20 J/m² UV irradiation.

cence data indicated attenuation of H2AX phosphorylation in hSNF5-depleted cells, we further evaluated γ H2AX focus formation by quantitative analysis at lower doses of UV. The number of γ H2AX foci was reduced to ~25% in control cells and 11% in hSNF5-depleted cells at a 5-J/m² UV dose (Fig. 8A). Additionally, the majority of the γ H2AX foci appeared to be much smaller in size and less intense relative to the control cells (Fig. 8C). We further extended the analysis by monitoring focus formation as a function of time in control and hSNF5-

FIG. 7. Formation of γ H2AX foci at the UV damage site at various UV doses. Experiments were performed as described for Fig. 4. Cells were UV irradiated at the indicated doses and processed for immunofluorescence using anti- γ H2AX and anti-XPC antibodies.

depleted cells. The defect in focus formation in hSNF5-depleted cells manifested as early as 1 h postirradiation and was apparent throughout the tested time course up to 8 h (Fig. 8C). Our data showed that γ H2AX foci appear to spread and become more pronounced with time in control cells, but such spreading is compromised in hSNF5-depleted cells. Additionally, upon global UV irradiation (5 J/m²), γ H2AX foci were seen in 42% of hSNF5-depleted cells compared to 63% of control cells (Fig. 8A). At this lower UV dose, the intensity of most of the γ H2AX foci in hSNF5-depleted cells was much lower than in the control cells (Fig. 8D). In a similar assay, BRG-1-depleted cells also showed distinct differences in the number and intensity of γ H2AX foci, further emphasizing the role of the SWI/SNF complex in H2AX phosphorylation (Fig. 8E and data not shown). For example, upon 5 J/m² global irradiation, γ H2AX foci were seen in 33% of BRG-1-depleted cells compared to 63% of control cells. At lower doses, γ H2AX focus formation exhibited a dose-dependent reduction in control cells, while BRG-1-depleted cells showed a consistent reduction at all doses. Collectively, these studies in cell-free and cultured cell systems support a direct role for the SWI/SNF complex in facilitating phosphorylation of H2AX following UV damage.

Consistent with the higher levels of phospho-ATM focus formation (~53%), γ H2AX foci were observed in ~60% of cells at 100 J/m² UV. These data further supported the probability of H2AX phosphorylation and ATR and ATM activation in both the G₁ and S phases. Indeed, γ H2AX foci were also present in G₁-arrested primary fibroblast cells, supporting the previous observation by others (35, 36) that γ H2AX foci are formed in G₁ due to the presence of UV lesions (Fig. 9A). Similar to the phospho-ATM foci, the quality of the γ H2AX

FIG. 8. hSNF5-depleted cells showed reduced levels of γ H2AX at the UV damage site. (A) hSNF5-depleted cells showed a decrease in the number of γ H2AX foci compared to control cells. Immunofluorescence and quantitation of the foci were done as described for Fig. 2C, except that UV irradiation was done by using micropore filters or cells were globally irradiated without filters at the indicated doses. Immunofluorescence was performed using anti- γ H2AX antibody. (B) hSNF5 depletion does not affect the intensity of γ H2AX foci at a 100-J/m² UV dose. Images from a representative experiment, described in panel A, are shown. (C) hSNF5 depletion reduces the intensity and spreading of γ H2AX foci at different times postirradiation (1 to 8 h). Cells were treated with a 5-J/m² UV dose. Representative images from each time point are shown. Experiments were done as described for panel A. (D) hSNF5 depletion reduces the number of γ H2AX foci. Representative images from the experiment described for panel A using global UV irradiation are shown. (E) BRG-1 deficiency reduces the number of γ H2AX foci. Experiments were done as described for panel A, using control-treated and BRG-1 siRNA-treated cells.

FIG. 9. (A) hSNF5 depletion does not affect the intensity of the γ H2AX foci at the UV damage site in G_1 -arrested cells after 100 J/m² UV irradiation. Experiments were performed as described for Fig. 5F. Immunofluorescence was performed using anti- γ H2AX antibody. (B) hSNF5 depletion reduced the intensity of the γ H2AX foci at the UV damage site in G_1 -arrested cells at 5 J/m². Experiments were performed as described for Fig. 5F, using anti- γ H2AX antibody.

foci was also affected in G_1 -arrested hSNF5-depleted cells compared to the control cells at a 5-J/m² UV dose (Fig. 9B). Therefore, our data revealed that H2AX phosphorylation is dependent on hSNF5 in the G_1 phase, further supporting the fact that hSNF5 can influence ATM recruitment and H2AX phosphorylation when only the NER lesions are present.

hSNF5 depletion affects cellular phospho-BRCA1 levels and phospho-BRCA1 foci at the UV damage site. To further corroborate the role of hSNF5 in the DNA damage response pathway, we determined whether the downregulation of γ H2AX influences activation and recruitment of downstream proteins in the DNA damage response pathway. Several studies have demonstrated that γ H2AX recruits the MDC1-53BP1-BRCA1 complex at the damage site to accomplish the damage repair (49, 50). Moreover, ATM also influences BRCA1 phosphorylation directly at the damage site (16). As hSNF5 depletion impairs ATM recruitment at the damage site and affects γ H2AX levels on the chromatin, we speculated that it would affect the recruitment and phosphorylation of the MDC1-53BP1-BRCA1 complex. As BRCA1 is the most downstream component in this pathway and also gets phosphorylated by ATM, we examined whether the cellular phospho-BRCA1 level was affected in hSNF5-depleted cells. Western analysis showed that BRCA1 phosphorylation was affected in hSNF5-depleted cells, lending further credence to the potential role of hSNF5 in the DNA damage response pathway (Fig. 10A). Indeed, consistent with the γ H2AX result, phospho-BRCA1 foci at the UV damage site were also detectable at a very low dose (5 J/m²) of UV (Fig. 10B). Next, we compared phospho-BRCA1 foci levels in hSNF5-depleted and control cells. At 100 J/m², phospho-BRCA1 focus levels were reduced to 26% in hSNF5-depleted cells, compared to 51% in control cells (Fig. 10C). Additionally, at 5 J/m² the effect on focus formation was more dramatic, and phospho-BRCA1 foci were reduced to 10% in hSNF5-depleted cells compared to 26% in control cells, a 2.5-fold reduction (Fig. 10C). As expected, phospho-BRCA1 foci did not show substantial differences in the intensity and quality of hSNF5-depleted cells compared to control

cells at a 100-J/m² UV dose (Fig. 10D). However, phospho-BRCA1 foci intensity was significantly lower at a 5-J/m² UV dose in the majority of the hSNF5-depleted cells compared to that of control cells (Fig. 10E). Therefore, these data showed that the defect in ATM recruitment affects γ H2AX and phospho-BRCA1 levels at the damage site.

hSNF5 depletion does not affect Chk1 and Chk2 activation by phosphorylation and maintains the cell cycle checkpoint. Our data showing that hSNF5 affects activation and recruitment of ATM at the damage site led us to examine whether the ATM/ATR-mediated Chk2/Chk1 phosphorylation and downstream checkpoint activation are affected in the absence of hSNF5. Therefore, we detected the Chk1 and Chk2 phosphorylation levels by Western analysis, using extracts from hSNF5-depleted and control cells after UV irradiation. The cellular levels of phosphorylated Chk1 were not affected in hSNF5-depleted cells compared to the control cells. This would be expected if the ATR level and its recruitment at the damage site were not affected by hSNF5 depletion. In contrast, there was a small reduction in the cellular levels of phosphorylated Chk2 in hSNF5-depleted cells (Fig. 11). This was unexpected, because defects in ATM activation and recruitment would affect Chk2 phosphorylation. It is possible that Chk2 might also be phosphorylated by ATR in the absence of ATM recruitment at the damage site, as Wang et al. (59) have shown that ATR also phosphorylates Chk2 after UV irradiation in the absence of ATM. Therefore, the remaining phosphorylation of Chk2 might be mediated by ATR which results in its activation in parallel with Chk1 activation. Together, from these data we concluded that the cell cycle checkpoint is intact in hSNF5-depleted cells.

DISCUSSION

Even though the SWI/SNF complex plays a role in NER, a detailed analysis of its function has been lacking. In this study, we have uncovered the role of the SWI/SNF complex in the NER pathway. This is the first evidence, so far, to demonstrate

FIG. 10. hSNF5-depleted cells are compromised in the induction of phospho-BRCA1 after UV damage. (A) hSNF5 depletion affects the levels of phospho-BRCA1 after DNA damage. Experiments were performed as described for Fig. 2B. Lysates were immunoblotted using anti-phospho-BRCA1, anti-BRCA1, anti-hSNF5, and antiactin antibodies. (B) Localization of phospho-BRCA1 at the damage site. Micropore UV irradiation assays were performed as described for Fig. 4, using 100 and 5 J/m² UV irradiation. Immunofluorescence was done using anti-phospho-BRCA1 antibody. (C) hSNF5-depleted cells show a decrease in the number of phospho-BRCA1 foci compared to control cells. Immunofluorescence and quantitation of the foci were done as described for Fig. 2C, using anti-phospho-BRCA1 antibody. (D) hSNF5 depletion does not affect the intensity of phospho-BRCA1 foci at 100 J/m² UV. Images from a representative experiment, described for panel C, are shown. (E) hSNF5 depletion reduces the intensity of phospho-BRCA1 foci at a 5-J/m² UV dose. Representative fluorescence images of phospho-BRCA1 foci from the experiment described for panel C are shown.

FIG. 11. The DNA damage-induced cell cycle checkpoint is intact in hSNF5-depleted cells. Experiments were done as described for Fig. 2B. Total extract was immunoblotted for anti-phospho-Chk1, anti-phospho-Chk2, anti-Chk1, anti-Chk2, anti-hSNF5, and antiactin antibodies.

the physical association of the SWI/SNF complex at the UV damage site in cultured cell systems. Our data strongly support that the SWI/SNF activity is required for the recruitment of ATM to the damaged DNA, which influences H2AX phosphorylation and recruitment of the MDC1-53BP1-BRCA1 complex. Defects in this pathway eventually affect the assembly of downstream repair and checkpoint proteins, which influences NER. Thus, the SWI/SNF complex is integral to maintaining genomic integrity.

Our findings that hSNF5 associates with XPC in response to UV irradiation and gets recruited to the damage site suggest a direct role for the SWI/SNF complex in NER. While no overt defects in the protein levels and modification of XPC or in its recruitment to the damage site were observed, ATM activation by phosphorylation and its recruitment to the damage site were defective in the absence of hSNF5. Interestingly, we observed that phospho-ATM, γH2AX, and phospho-BRCA1 fo-

cus formation levels were detectable at a very low dose of UV, whereas ATR and XPC were not detectable at lower UV irradiation doses, showing a possible difference in the number of these molecules at the damage site.

To explain these data, we propose that the phospho-ATM- γ H2AX-phospho-BRCA1 complex might spread along the chromatin to encompass megabases of DNA, as seen with damaged chromatin at the DSB sites. Such a complex would influence the repair of DSBs generated upon processing of UV lesions. This explanation is supported by the fact that in mammalian systems γ H2AX spreading in the vicinity of DSB is dependent on ATM (5, 6, 32, 45). Based on these studies, one of the most accepted models in the DSB signaling pathway predicts that activated ATM is initially recruited to the damage site and it phosphorylates nearby H2AX. The γ H2AX then influences further recruitment of more ATM molecules (4). Therefore, we speculate that the SWI/SNF-dependent chromatin remodeling facilitates the exposure of a specific H2AX motif to ATM, or promotes the translocation of the ATM along the flanking chromatin to phosphorylate more H2AX molecules, and that this leads to the recruitment of the MDC1-53BP1-BRCA1 complex. This model is supported by the fact that γ H2AX focus maintenance is dependent on sustained recruitment of ATM molecules at the damage site via MDC1 (49, 50). Consistent with this, we found that phosphorylation of H2AX and BRCA1 is also affected in hSNF5-depleted cells. Furthermore, the defects in the levels of phospho-ATM, γ H2AX, and phospho-BRCA1 are not due to the defects in their expression levels in hSNF5-depleted cells, as SWI/SNF inactivation does not affect ATM, ATR, H2AX, and BRCA1 cellular protein levels in response to UV irradiation. At this point, we cannot rule out the possibility of the involvement of ATR in γ H2AX spreading near the UV damage site, as Kim et al. clearly demonstrated that in yeast γ H2AX spreading surrounding a DSB is dependent on ATR, but not ATM (28). It is possible that ATR association is limited at or near the damage site in response to UV irradiation, or ATR is not detectable at the lower doses of UV under our assay conditions. As the nature of the processed intermediates after UV damage is still not clear and may not be exclusively DSBs, there might be multiple mechanisms in regulating chromatin structure to cope with different types of DNA lesions, with repair depending on the organism's chromatin organization. It is possible that processed intermediates of UV damage generate signals different from the DSB repair pathway. Interestingly, recent studies by Peng et al. (39) showed that BRIT1/MCPH1, an early DNA damage response protein, interacts with the human SWI/SNF complex in response to DNA DSB, and this interaction is dependent on ATM/ATR. Moreover, the SWI/SNF component BAF170 is phosphorylated by ATM/ATR, and SWI/SNF chromatin remodeling activity influences DSB repair (39). Our results showing that the SWI/SNF complex physically interacts with phospho-ATM also establish the role of SWI/SNF in DNA damage repair through ATM signaling.

Our quantitative analysis demonstrated that the level of impairment in γ H2AX and BRCA1 focus formation in hSNF5-depleted versus control cells is relatively lower than phospho-ATM focus formation, e.g., 1.5-fold versus 4-fold for γ H2AX and 2.5-fold versus 4-fold for phospho-BRCA1. As ATM recruitment, but not ATR recruitment, is affected by hSNF5

inactivation, it is possible that the remaining γ H2AX and phospho-BRCA1 foci might be contributed by ATR activity. In support of this view, it has been shown that upon UV irradiation ATR significantly phosphorylates H2AX, and H2AX phosphorylation is affected in ATR-depleted Seckel cells and ATM-depleted AT cells (20, 60). After phosphorylation by ATR, γ H2AX molecules can recruit BRCA1 molecules at the damage site. Furthermore, after recruitment, these BRCA1 molecules might also be phosphorylated by ATR in the absence of ATM, as BRCA1 has also been shown to be an ATR substrate (7, 16).

From several studies, it is now increasingly evident that DSB can arise through processing of the UV lesion, which is then repaired by single-strand annealing and recombination (14, 42). We showed that hSNF5 influences ATM recruitment, H2AX phosphorylation, and assembly of the ATM- γ H2AX-MDC1-53BP1-BRCA1 complex at UV lesions in the G₁ phase. As it has been well established that the ATM- γ H2AX-MDC1-53BP1-BRCA1 complex assembled in a similar manner at the site of collapsed replication forks generated in S phase, we predict that SWI/SNF also influences the assembly of this complex in S-phase cells and helps recovery of collapsed replication forks by recombination-mediated DNA repair.

We observed that hSNF5 depletion did not affect Chk1 phosphorylation and did not have a substantial effect on Chk2 phosphorylation. Chk2 can be phosphorylated in the absence of ATM recruitment by ATR, as Chk2 is also an ATR substrate (59). Another possibility is based on the fact that major portions of Chk1 and Chk2 stay soluble, and a small portion (10% to 20%) is in an insoluble nuclear fraction, presumably bound to chromatin (51). Therefore, the activated form of Chk1/Chk2 might not be exclusively chromatin associated, and thus their activation by phosphorylation might not be affected by the accessibility of ATR/ATM to the damaged chromatin. Collectively, these data showed that checkpoint activation is intact in hSNF5-depleted cells. These results support the most recent concept in the DSB repair pathway, in which the ATM- γ H2AX-MDC1-53BP1-BRCA1 complex is involved in damage repair, whereas the ATM-Chk2-Cdc25 and the ATR-Chk1-Chk2-Cdc25 checkpoint complexes are involved in cell cycle arrest in response to DNA damage (22). Thus, our data revealed that the SWI/SNF complex is involved in the NER pathway and does not influence the checkpoint pathway.

Based on our data, we propose a model (Fig. 12) where the SWI/SNF complex associates with XPC and gets recruited at the UV damage site. Next, the SWI/SNF chromatin remodeling activity facilitates ATM recruitment by making the DNA damage sites more accessible. Recruitment and spreading of ATM along the chromatin further influence H2AX phosphorylation and MDC1-53BP1-BRCA1 recruitment at the UV damage site, which promotes NER (42, 61). Additionally, the interaction between phospho-ATM and hSNF5 indicates that this complex might move along the chromatin to facilitate the assembly of repair and checkpoint proteins, further supporting our proposed model. Collectively, our data elucidate a mechanism of SWI/SNF function that involves directly regulating the access of NER and checkpoint factors at the damage site.

Several lines of evidence support that hSNF5 is a tumor suppressor and hSNF5 deficiency results in mitotic defects and cancer development (25, 29, 57). In addition, recent studies

FIG. 12. Model depicting the mechanism of action of SWI/SNF in NER and checkpoint signaling. UV irradiation results in immediate binding of XPC and the SWI/SNF complex at the damage site, which facilitates ATM activation and recruitment, H2AX phosphorylation, and BRCA1 recruitment and phosphorylation. Recruitment and subsequent phosphorylation of these factors are required for successful completion of NER.

suggest that DNA damage-induced H2AX phosphorylation is the best-characterized histone modification and is required for suppressing cancer development (53). Our observations integrate these two phenomena in delineating the potential role of SWI/SNF complex in cancer control. Overall, our study provides crucial insights for the tumor suppressor function of this evolutionarily conserved chromatin remodeling complex with implications in cancer therapy.

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