SerpinB10 in UV-Induced Cellular Response

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UV-induced DNA damage response and repair are extensively studied processes, as any malfunction in these pathways contributes to the activation of tumorigenesis. Although several proteins involved in these cellular mechanisms have been described, the entire repair cascade has remained unexplored. To identify new players in UV-induced repair, we performed a microarray screen, in which we found SerpinB10 (SPB10, Bomapin) as one of the most dramatically upregulated genes following UV irradiation. Here, we demonstrate that an increased mRNA level of SPB10 is a general cellular response following UV irradiation regardless of the cell type. We show that although SPB10 is implicated in the UV-induced cellular response, it has no indispensable function in cell survival upon UV irradiation. Nonetheless, we reveal that SPB10 might be involved in delaying the duration of DNA repair in interphase and also in S-phase cells. Additionally, we also highlight the interaction between SPB10 and H3. Based on our results, it seems that SPB10 protein is implicated in UV-induced stress as a “quality control protein”, presumably by slowing down the repair process.

SerpinB10 Bomapin serine protease inhibitor replication replication stress UV damage

1. Introduction

Our genome is constantly exposed to endogenous and exogenous sources of damage. One of them is UV radiation, which has many harmful physiological and biological consequences, such as premature aging, immunosuppression, overactivation of inflammatory processes, DNA damage, and even the activation of apoptosis [1][2]. Understanding the mechanism of UV-induced DNA damage and the subsequent cellular response is indispensable, as a malfunction in the DNA repair process often leads to tumorigeneses [1][2]. UV irradiation can induce the formation of 8-hydroxyguanine, DNA-protein crosslinks, or abasic sites or lead to the appearance of cyclobutane-pyrimidine dimers (CPDs) or 6–4 pirimidine-pirimidon photoproducts (6–4 PPs) [3][4][5][6]. The altered DNA structures cause problems during DNA replication, since the replisome quickly and accurately copies billions of DNA bases in each cell cycle, including the damaged bases. Furthermore, altered DNA structures can lead to the formation of mismatches, particularly when the template DNA has been damaged. Therefore, the effectiveness of DNA repair is crucial before the replisome can bypass the damaged nucleotides. To rescue the stalled replication machinery, eukaryotic cells have evolved error-prone translesion (TLS) DNA polymerases, which can pass through the lesions. However, to keep these low-fidelity TLS polymerases away from the undamaged DNA, interaction is required between them and the proliferating cell nuclear antigen (PCNA), which serves as a binding platform for proteins involved in the recognition and repair of damage [7][8][9][10][11][12][13]. One of the main steps in the exchange

of replicative polymerases to TLS DNA polymerases at stalled replication forks is mono-ubiquitylation; hence, the activation of PCNA, mediated by Rad6 and Rad18 ubiquitin ligases, is a crucial step for successful repair [7][10][14][15][16][17]. Any malfunction in these processes can lead to the accumulation of DNA mutations or chromosomal rearrangements or can affect chromosome segregation, resulting in tumorous malformations [18].

Our results and recent studies also showed that UV irradiation induces robust gene expression changes in the Hker E6SFM keratinocyte cell line [19][20][21][22]. Among the upregulated genes, we identified several members of the B clade of the Serpin superfamily, including *SerpinB2*, *SerpinB10*, and *SerpinB13* [19]. The members of the SerpinB (SPB) family are mainly intracellular proteins, and it has been demonstrated that they can regulate various processes, such as inflammation, immune function, mucous production, apoptosis, tumor metastasis, and autoimmunity [23][24][25][26][27]. Nonetheless, only a limited amount of data are available concerning their regulatory role in DNA repair [19][20][28][29][30].

In the last decades, several studies have described proteins with dual, contradictory functions in tumor formation and metastasis progression. Several proteins have been shown to facilitate tumor formation according to some cancer types, and to inhibit it in other tumor classes. SerpinB10 (SPB10), also known as Bomapin, is a redox-sensitive nucleocytoplasmic protein that promotes proliferation of hematopoietic and myeloid leukemia cells under basal conditions, although it also enhances apoptosis following withdrawal of growth factors [31]. The dual function of Bomapin has also been described in several cancer types, such as lung cancer, in which *SPB10* mRNA expression is increased, and breast cancer, in which it is decreased compared with healthy cells [32]. In addition, it has been also described that missense mutation of *Bomapin* can lead to the formation of prostate cancer [33].

In this study, we demonstrated that both the mRNA and protein levels of SPB10 are increased as a response to UV radiation, suggesting the role of SPB10 in UV-induced DNA damage repair. From our results, we presume that SPB10 is involved in the mediation of DNA repair upon UV radiation in interphase and also in S-phase cells. Additionally, SPB10 interaction with H3 histone can also be detected, suggesting its chromatin association.

2. Results

2.1. Expression of several members of the SerpinB superfamily, including SPB10, is increased following UV irradiation

Environmental stress factors frequently induce DNA damage, which initiates the activation of DNA repair. These processes are tightly regulated, and post-translational regulation of the participating proteins is required for their proper function. Recently, we demonstrated by microarray experiment that UV irradiation contributes to the overexpression of certain members of the SerpinB family, including *SerpinB2* (*SPB2*), *SerpinB10* (*SPB10*), and *SerpinB13* (*SPB13*), in Hker E6SFM cells, with 1.495-, 4.253-, and 1.180-fold increase ($\log_2$) upon UV irradiation, respectively (Figure S1).

To verify the microarray data, first we performed quantitative PCR (qPCR) on Hker E6SFM cells to measure the mRNA level of *SPB10* in basal conditions and upon UV irradiation. We observed increased expression of *SPB10* 2 and 8 hours after UV irradiation compared to the control. The elevated mRNA level decreased 24 hours after the UV radiation; however, it remained 12.2 times higher compared to the control (Figure 1A).

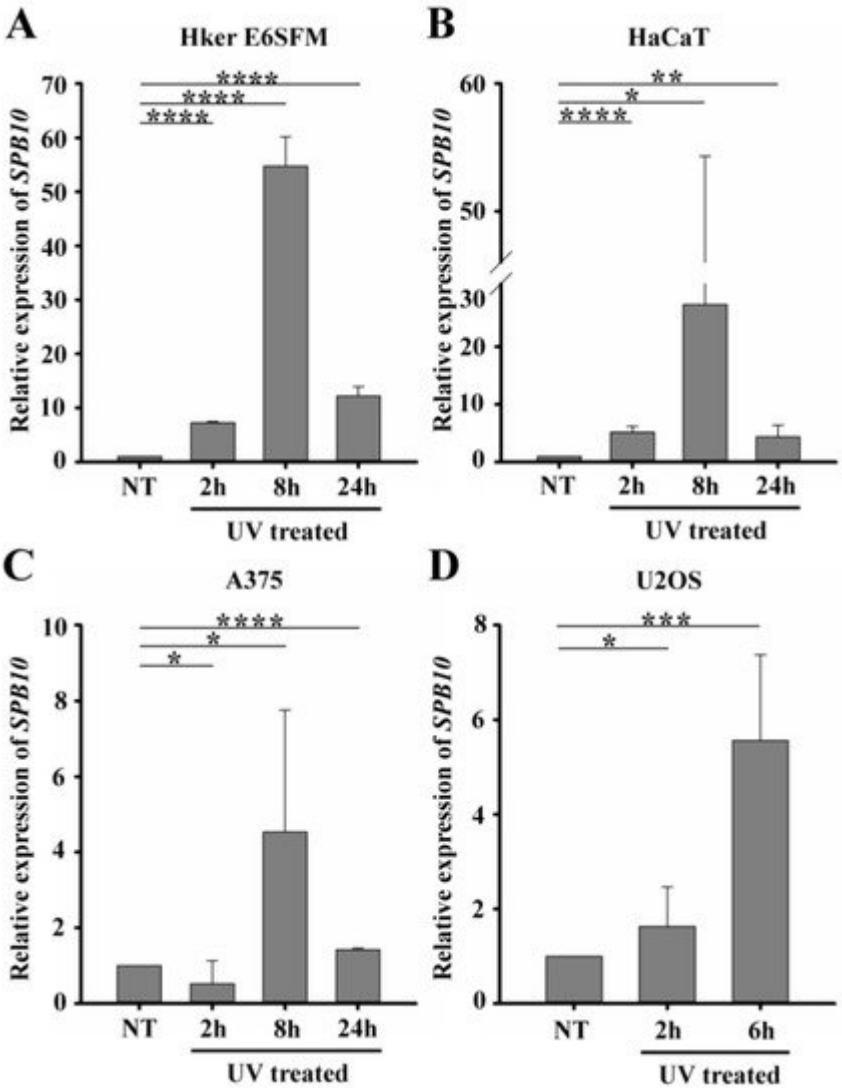

Figure 1. UV irradiation triggers elevation of *SPB10* mRNA: Relative expression levels of *SPB10* gene in (**A**) Hker E6SFM keratinocyte, (**B**) HaCaT keratinocyte, (**C**) A375 melanoma, and (**D**) U2OS osteosarcoma cells analyzed by qPCR. Data were normalized to 18S RNA and compared to control (NT). Means and standard deviations of three independent experimental triplicates are indicated as fold changes. Asterisks indicate statistical significance between datasets (t-test, *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, ****P ≤ 0.0001).

To determine whether elevated gene expression of *SPB10* could be observed in other cell lines as well, we performed qPCR experiments following UV irradiation for 2, 8, and 24 hours and with untreated samples in HaCaT keratinocyte and A375 melanoma cells. Similar to Hker E6SFM, we detected elevated *SPB10* mRNA levels after UV irradiation in both cell lines (Figure 1B, C).

In order to reveal whether SPB10 plays a general role following UV radiation, we involved the U2OS osteosarcoma cell line in our study and performed the above-described experiment, since U2OS is generally used to examine DNA damage-induced cellular responses. We detected elevated *SPB10* mRNA levels 2 and especially 6 hours after irradiation compared to the control samples in this cell line (Figure 1D). All of these results might indicate that the overexpression of *SPB10* upon UV irradiation is not cell-line-specific, but a general cellular response.

2.2. SPB10 is dispensable for cell survival but influences DNA repair kinetics in interphase cells upon UV irradiation

Since our qPCR data suggested that SPB10 plays a role in UV-induced cellular responses, we examined whether it has any effect on cell survival in response to UV irradiation. To examine this hypothesis, we performed a viability assay on non-targeting scrambled siRNA (siSCR) and *SPB10* siRNA-silenced U2OS cells and determined the ratio of live and dead cells 2, 4, 6, and 24 hours after UV radiation using Trypan blue staining (Figure 2A). As expected, we counted a reduced number of cells immediately after UV irradiation. Nonetheless, at later time points (24 hours after exposure), the amount of living cells increased, as by that point, the cells had been already recovered. However, we did not find any significant differences between the numbers of non-targeting (siSCR) and *SPB10* siRNA-silenced cells, which indicates that SPB10 might have no effect on cell survival following UV irradiation.

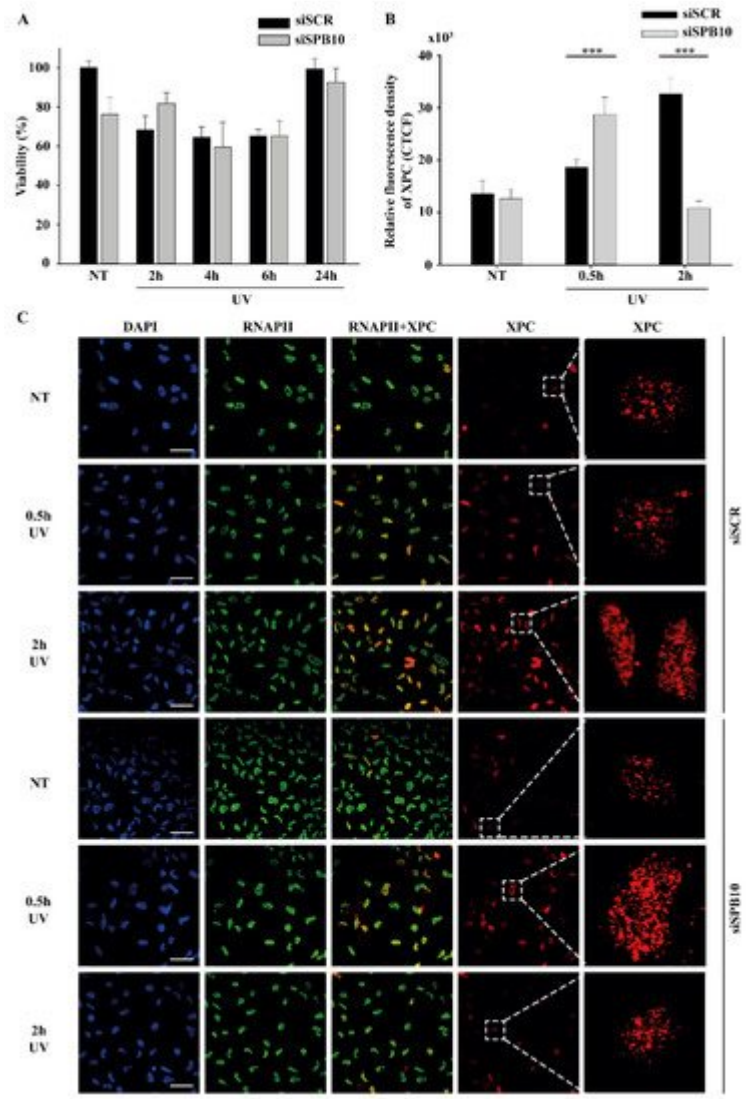

Figure 2. SPB10 is dispensable for cell survival but influences DNA repair kinetics in interphase cells upon UV irradiation: (**A**) Diagram represents cell viability (%) upon UV irradiation (2, 4, 6, and 24 hours) of non-targeting (siSCR) and *SPB10* siRNA-silenced U2OS cells. (**B**) Quantification of relative fluorescence intensity (CTCF) of Xeroderma Pigmentosum C (XPC) protein detected on non-targeting (siSCR) and *SPB10* siRNA-silenced interphase U2OS cells in basal conditions (NT) and following UV irradiation (0.5 and 2 hours). Asterisks indicate statistical significance between datasets (Mann–Whitney test, ***P ≤ 0.001). (**C**) Representative images of XPC protein (red) binding presumably to Nucleotide Excision Repair (NER) foci in non-targeting (siSCR, upstream) and *SPB10* siRNA-silenced (siSPB10, downstream) U2OS cells in basal conditions (NT) and after UV irradiation (0.5 and 2 hours). Only the chromatin-bound proteins were visualized by CSK-immunocytochemistry. DAPI (blue) was used to visualize the nuclei, and RNA Polymerase II (RNAPII) (green) was used as control. For each condition, a higher magnification of a single cell is shown on the right side of the figure.

Although we did not find any changes in cell survival, showing that the SPB10 is not crucial for the UV-induced repair, it can still act as a fine-tune regulator protein in Nucleotide Excision Repair (NER). To test this, we monitored the repair kinetics of NER upon UV irradiation, by following the binding of the Xeroderma Pigmentosum C (XPC) protein presumably to the site of DNA damage by using CSK-immunocytochemistry of non-targeting (siSCR) and

SPB10 siRNA-silenced U2OS cells. As we expected, the number of XPC foci was elevated after 30 minutes and 2 hours UV irradiation compared to the control sample in non-targeting (siSCR) U2OS cells. The repair kinetics was shifted in *SPB10* siRNA-silenced cells 30 minutes following UV irradiation, while it was decreased 2 hours post-UV compared to its control (Figure 2B, C). Based on these results we assumed that SPB10 could influence the XPC binding, and the loss of SPB10 accelerates the repair of UV-induced damage.

2.3. SPB10 influences DNA repair kinetics in S-phase cells upon UV irradiation

Our data suggest that SPB10 may take part in regulating the repair of UV-induced DNA damage. UV-related T-T dimer formation also can results in replication block, which can be resolved by translesion (TLS) DNA polymerases, recruited by the proliferating cell nuclear antigen (PCNA) protein [18,34].

To reveal whether SPB10 is involved in this process, we first performed co-immunoprecipitation (co-IP) to study the association between SPB10 and PCNA. However, we did not detect interaction between these two proteins (data not shown).

Next, we tested whether SPB10 has any influence on the chromatin-bound PCNA recruitment upon UV irradiation. We made CSK-immunocytochemistry of non-targeting (siSCR) and *SPB10* siRNA-silenced U2OS cells and we monitored the level of chromatin-bound PCNA in siSCR cells and 30 minutes after UV treatment. We observed less chromatin-bound PCNA upon UV irradiation in non-targeting (siSCR) siRNA treated U2OS cells, which might be caused by replication fork collapse mechanism. However, in the absence of SPB10, we could not observe this reduction in the PCNA level upon UV radiation (Figure 3A, B). According to these results, we hypothesized that SPB10 might involve in the fine-tuning of UV-induced repair mechanism also in S-phase cells.

To validate whether SPB10 indeed played a role in UV-related replication stress we performed post-replication repair (PRR) comet assay under physiological conditions and following UV irradiation of non-targeting (siSCR) and *SPB10* siRNA-silenced U2OS cells and examined the presence of single-stranded DNA fragments generated by incomplete replication or UV light in S-phase cells following 0, 6, and 24 hours of UV treatment. As expected, after UV irradiation, damaged DNA strands (the “tail” of the comet) were accumulated in non-targeting (siSCR) cells, but only a limited amount of DNA damage was detected in *SPB10* siRNA-silenced S-phase cells. Additionally, we observed faster repair, indicated by significantly shorter comet tails in the *SPB10* siRNA-silenced cells (Figure 3 C, D). According to these results, our assumption is that in replicating cells, SPB10 is presumably involved in delaying the DNA repair process, thus facilitating a more precise repair mechanism to eliminate DNA damage.

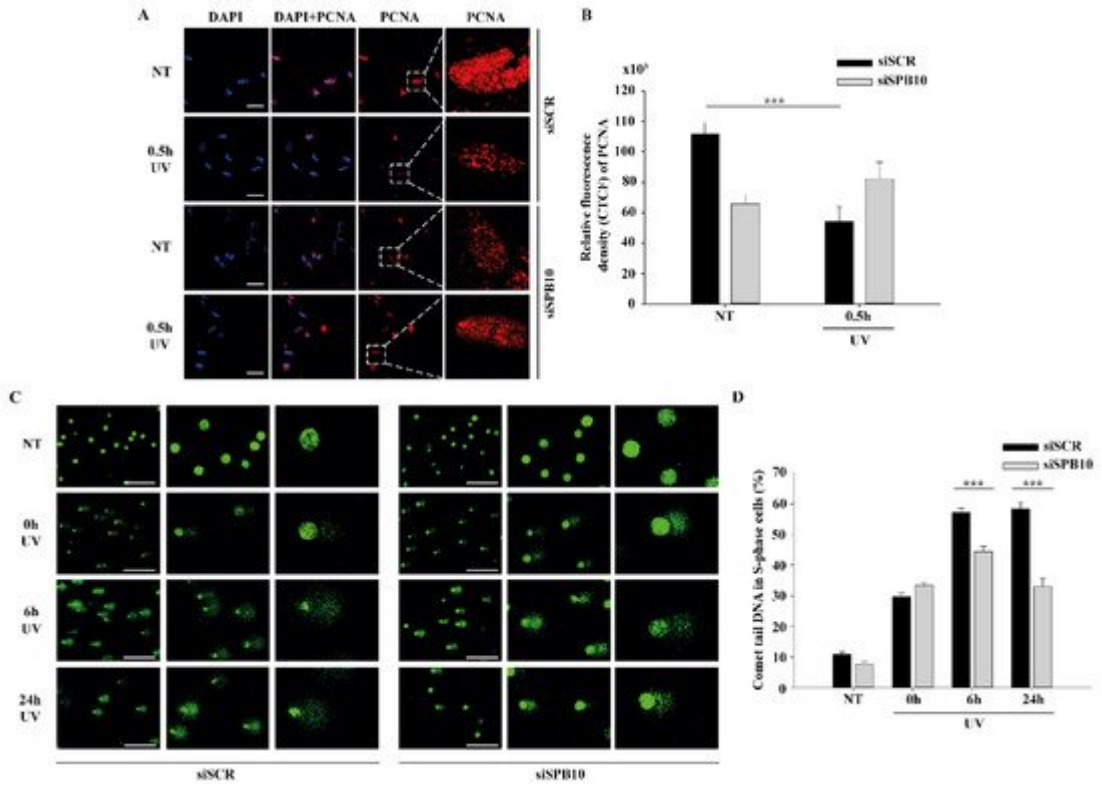

Figure 3 SPB10 influences DNA repair kinetics in S-phase cells upon UV irradiation: (**A**) Representative images of PCNA protein (red) binding in non-targeting (siSCR, upstream) and *SPB10* siRNA-silenced (siSPB10, downstream) U2OS cells in basal conditions (NT) and after UV irradiation (0.5 hour). Only the chromatin-bound proteins were visualized by CSK-immunocytochemistry. DAPI (blue) was used to visualize the nuclei. For each condition, a higher magnification of a single cell is shown on the right side of the figure. (**B**) Quantification of relative fluorescence intensity (CTCF) based on measured foci number of proliferating cell nuclear antigen (PCNA) protein detected of non-targeting (siSCR) and *SPB10* siRNA-silenced U2OS cells in basal conditions (NT) and following UV irradiation (0.5 hour). Asterisks indicate statistical significance between datasets (Mann–Whitney test, ***P ≤ 0.001). (**C**) Representative images of comet tail formation in non-targeting (siSCR, left) and *SPB10* siRNA-silenced (siSPB10, right) bromodeoxyuridine (BrdU)-labelled S-phase U2OS cells in basal conditions (NT) and after UV irradiation (0, 6, and 24 hours). (**D**) Quantification of comet DNA tails detected on non-targeting (siSCR) and *SPB10* siRNA-silenced S-phase U2OS cells in basal conditions and following UV irradiation (0, 6, and 24 hours). Asterisks indicate statistical significance between datasets (Mann–Whitney test, ***P ≤ 0.001).

2.4. SPB10 is associated with chromatin

The above-described data suggest that SPB10 might have an effect in the early steps of the UV-induced repair processes. To investigate whether this function takes place on the chromatin, we tested a possible association of SPB10 with the chromatin-related histone H3 protein following UV irradiation by co-IP in U2OS cells. For this, we transiently expressed SPB10-GFP fusion protein in untreated and UV-irradiated (2 and 4 hours) U2OS cells and performed an IP experiment using GFP antibody. Immunoprecipitated samples were analyzed by Western blot in order to detect co-immunoprecipitated H3 protein. We found that regardless of the cellular conditions, SPB10

showed interaction with H3 histones (Figure 4A and Figure S2A). A verifying reciprocal co-IP experiment using H3 specific antibody confirmed the interaction between H3 and SPB10 (Figure 4C and Figure S2B).

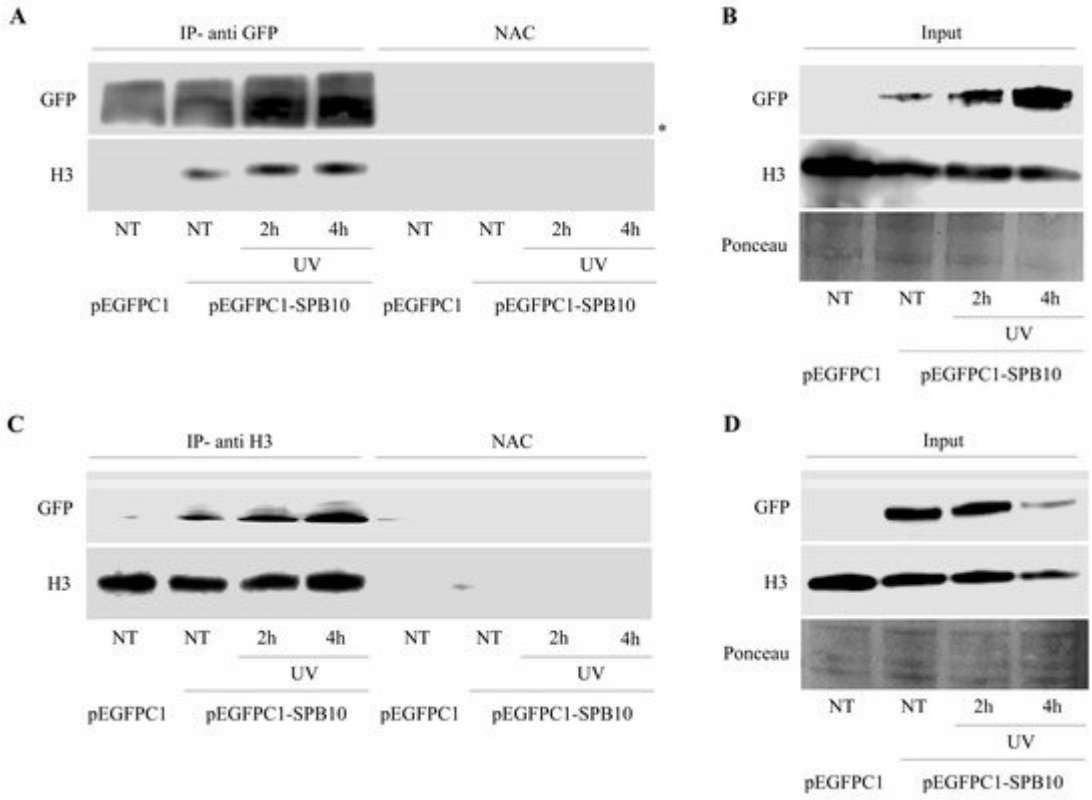

Figure 4. SPB10 shows interaction with H3: Immunoblot detection to reveal interaction between SPB10 and H3 in U2OS cells. Efficiency of immunoprecipitation experiment was controlled with (**A**) anti-GFP antibody (since cells were transfected with plasmid encoding SPB10-GFP fusion protein) and (**C**) anti-H3

3. Discussion

In this study, we investigated a novel, yet-to-be-characterized function of the SPB10 protein following UV irradiation. Our preliminary microarray data indicated that members of the SerpinB family could play a role in UV-induced cellular responses [19][20]. We revealed an increase in the mRNA level of *SPB10* upon UV irradiation in three skin-derived cell lines (Hker E6SFM, HaCaT, and A375). Additionally, we demonstrated that the UV-triggered enhanced expression of *SPB10* is a general cellular response since we also observed this phenomenon in the U2OS osteosarcoma cell line, which is physiologically not exposed to UV light. This might indicate that the overexpression of *SPB10* upon UV irradiation is a common mechanism among different cell types possessing tissue-specific repair mechanisms [34][35]. In addition, we investigated the role of SPB10 in cell survival upon UV irradiation. We did not find a significant difference in the survival rate between non-targeting (siSCR) and *SPB10* siRNA-silenced U2OS cells. Although Przygodzka et al. found that under optimal growth conditions SPB10 has survival-promoting activity, this phenomenon is specific to myeloid cells, since ectopic expression of this Serpin in HT1080 fibroblasts did not change the proliferation rate of cells [31].

Although the *SPB10* mRNA level increased upon UV irradiation, suggesting its role in UV-induced cellular response, it does not influence cell viability. To examine this hypothesis, we studied the effect of SPB10 in UV-induced DNA damage repair in interphase and S-phase cells. We observed accelerated Nucleotide Excision Repair (NER) kinetics in interphase *SPB10* siRNA-silenced U2OS cells by detecting the XPC foci formation upon UV irradiation. By using post-replication repair (PRR) comet assay, we found accelerated UV-induced DNA repair in *SPB10* siRNA-silenced replicating cells. We also found that SPB10 influences the PCNA exchange on the chromatin, therefore presumably affecting the loading of the translesion polymerases. All these results suggest that SPB10 may have a function in the fine-tuned regulation of UV-triggered DNA repair. We suspect that SPB10 is involved in slowing down DNA repair, thus facilitating a more precise but prolonged repair mechanism both in interphase and in S-phase cells. Our assumption may also be supported by another example of the role of a serine protease in fine-tuning of DNA replication. Kirillova et al. demonstrated that TNF induces DNA replication in growth-arrested cells through NFκB, but this activation could be inhibited by N-tosyl-L-phenylalanine chloromethyl ketone (TPCK) serine protease inhibitor [36]. In addition, SPB10 is known to be an inhibitor of TNFα-induced cell death [37]. Furthermore, Kojima et al. reported that the serine protease FAM111A plays an important role in the removal of protein obstacles from DNA, thereby supporting the replicating fork progression. The knockout, or mutation, of *FAM111A* promotes replication fork stalling, leading to DNA damage accumulation, cell-cycle arrest in the G2/M phase, and eventual cell death [38].

Our assumption is that SPB10, as a serine protease inhibitor, may also take part in the regulation of FAM111A-mediated protein obstacle removal from the replication fork. Nevertheless, all of these data presume that SPB10 plays a role in UV-induced DNA damage repair, which is also supported by our finding of an interaction between SPB10 and the H3 core histone. Based on our data showing that the loss of SPB10 results in faster DNA repair, our assumption is that SPB10 could delay chromatin decondensation by interacting with H3. These results are in accordance with several studies demonstrating that regulatory proteins taking part in the early steps of the repair process exert their activity on the chromatin structure by binding to the nucleosomes. Among these proteins, 53BP1, RNF169, RAP80, and RAD18 were shown to bind to the ubiquitylated H2A histone, thereby participating in the DNA damage response and in the pathway choice between Non-homologous end joining and Homologous Recombination [39][40][41][42][43].

In this study, we analyzed the yet-uncharacterized function of the SPB10 protein in cellular UV response. Our findings suggested that SPB10 is a possible mediator involved in the UV-dependent modulation of the DNA repair rate.

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