Role of SerpinB2 and SerpinB10 in DNA repair and tumorigenesis

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Ph.D. thesis summary

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Introduction

Our cells are constantly exposed to various DNA damaging agents originated from both endogenous and exogenous sources. Ultraviolet radiation (UV) is one of the most deleterious exogenous DNA damaging agents (targeting mostly the skin cells), which is the major causative factor of several destructive physiological and biological effects, such as immunosuppression, inflammation, DNA damages or initiation of apoptotic processes.

In mammalians, approximately 10^4-10^6 DNA damages are formed in each cell per day. These DNA damages have to be repaired quickly and precisely, since the improper or delayed repair of the errors can lead to cancerous malformations. Cells have developed several repair mechanisms to eliminate these DNA damages. Nucleotide excision repair (NER) is an exclusive pathway for the recognition and elimination of a wide-range of structurally diverse DNA damages, such as UV-induced cyclobutene-pyrimidine dimers (CPDs), 6-4 pyrimidine-pyrimidone photoproducts (6-4 PPs).

Since our skin is the first defence layer against exogenous agents, it is the most UV-exposed organ. Two types of malignant skin tumours have been known: melanocytic and non-melanocytic skin cancers (NMSC). NMSC tumour has the

highest occurrence rate among the malignant human cancer types in the world. The two most important and common NMSC tumour types are the basal cell carcinoma (BCC) and the squamous cell carcinoma (SCC).

SCC tumours are highly invasive tumour types with metastatic predisposition. Although in case of early diagnosis the survival rate increases.

The progression of BCC tumours is very slow, and they are just rarely fatal. In case of late diagnosis, they can invade the surrounding tissues and bones, but the occurrence of distant metastasis is infrequent. The BCC is one of the most frequent tumour types in the world. UV radiation strongly initiates the progression of BCC tumours.

The serine protease inhibitor superfamily, called Serpins, is one of the largest and functionally the most diverse protein family. According to recent findings, Serpins consists of 1,500 proteins possessing similar domain structure. The role of Serpins has already been described in several processes, such as tumorigenesis, blood clotting, hormone transport, inflammation or immune function. Based on the sequence similarities, the Serpins superfamily is divided into 16 different clades (A-P).

SerpinB2 (SPB2) protein was first isolated from human placenta by Uemura et al. in 1970. Initially, the protein was named PAI-2 (Plasminogen activator protein), because it was

originally identified as an extracellular inhibitor of the plasminogen activator uPA (urokinase plasminogen activator) and tPA (tissue plasminogen activator) enzymes during pregnancy. Since then, the upregulation of *SPB2* mRNA and protein levels has been evaluated in several cell types and it has been also reported that SPB2 is involved in numerous cellular processes, such as signal transduction, inhibition of apoptosis, macrophage survival, monocyte and keratinocyte differentiation, regulation of inflammatory and immune processes.

According to the literature, the SPB2 is involved in the progression of various tumour types. Its plasminogen inhibitor function has been described in gastrointestinal, breast and lung cancerous patients.

These results suggest that SPB2 could play a role in the regulation of homeostasis upon different kind of injuries, errors, and stress factors.

SerpinB10 (SPB10) also known as Bomapin, is a redox-sensitive hematopoietic and myeloid leukaemia specific nuclear protein belonging to the SerpinB family. The protein plays role in the activation of the proliferation and also in the apoptosis induction of myeloid leukaemia cells depending on the presence of specific growth factors. Furthermore, *SPB10* mRNA

expression is increased in lung cancer patients, while it is decreased in case of breast cancer patients.

According to already published data, SerpinB clade is a functionally diverse family. It is assumed that many functions of the SPB clade remain unknown, and it is likely that proteins with a well-characterized function may be involved in other, yet uncharacterized processes.

Aims

The main goal of my research was the characterization of the two most dramatically upregulated SerpinB2 (SPB2) and SerpinB10 (SPB10) genes following UV irradiation. During the experiments our questions were the followings:

SPB2:

- Could the UV-induced increase in *SPB2* mRNA level (observed by microarray experiment) be confirmed by qPCR?
- Does the UV-induced activation of *SPB2* show cell line or tissue type specificity?
- Does the UV irradiation affect the SPB2 protein level?
- Does the subcellular localization of SPB2 change upon UV irradiation?
- Does the SPB2 protein bind to the damaged DNA after UV irradiation?
- Could additional stress factors also lead to SPB2 activation?
- Can the SPB2 protein form discrete repair foci together with NER proteins?

- Could the SPB2 play role in the ubiquitin mediated fine-tune regulation of the NER pathway?
- Is it possible that the malfunction of SPB2 protein is implicated in the progression of basal cell carcinoma (BCC)? Does the subcellular localization or the level of the protein change in the tumorous part of the BCC tissues compared to the normal part?

SPB10:

- Could the UV-induced increased *SPB10* mRNA level (observed with microarray experiment) be confirmed by qPCR? Does the UV-induced activation of *SPB10* show cell line specificity?
- Does the siRNA silencing of SPB10 have any effects on cell viability upon UV irradiation?
- Does SPB10 play role in the UV-induced cellular response processes?
- Does SPB10 bind to chromatin during the restoration of UV lesions?

Methods

- Trypan blue staining was performed to investigate the toxicity of UV irradiation and H_2O_2 treatment on human cell lines.
- Microarray technique was used to investigate the effect of UV irradiation on *SPB2* and *SPB10* genes expression levels of Hker E6SFM cells.
- Quantitative real time PCR (qPCR) technique was implemented in order to investigate the changes in gene expression level of *SPB2* and *SPB10* upon UV irradiation.
- Western blot analysis was performed to detect the changes in SPB2 protein levels and subcellular localizations of this protein upon UV irradiation.
- CSK-Immunohistochemistry staining was used to detect the localization of SPB2 protein upon various DNA damaging agents.
- CSK-Immunohistochemistry staining was performed to investigate the co-localization of SPB2 and the proteins implicated in NER pathway.
- Co-immunoprecipitation was used to examine the interaction between SPB2 and XPB, elongating RNA Polymerase II (S2P RPB1), or H3 protein as well.

- "LacO-tethering" technique was performed to study whether the SPB2 could be observed at nucleotide excision repair foci.
- Immunohistochemistry staining was used to detect the level and subcellular localization of SPB2 protein in tissue samples of basal cell carcinoma.
- SPB10 siRNA silencing was performed to investigate the role of SPB10 on cell viability upon UV irradiation.
- Comet-assay was used to evaluate the role of SPB10 in the UV-induced DNA repair process.

Results

Recently we have found and published that in an immortalized keratinocyte cell line (Hker E6SFM) several members of the Serpin protein family showed elevated expression upon UV irradiation. The results of this high-throughput screen prompted us to hypothesize a novel, so far undescribed, potential role of Serpin type proteins during the process of DNA repair. Based on these preliminary data, during my PhD work, I have characterized the function of the two Serpin genes (*SerpinB2* (*SPB2*) and *SerpinB10* (*SPB10*)), which were found to be the most dramatically upregulated following UV irradiation.

Our experimental data demonstrated that UV irradiation resulted in an increase of both *SPB2* mRNA and protein levels in various skin-derived cell types. U2OS cells also showed similar, but milder increase in the mRNA level of *SPB2*. These data might indicate that the activation of SPB2 in response to UV irradiation is a common mechanism among different cell types. In addition, we also proved that UV irradiation triggered transport of SPB2 into the nuclei, where it appeared at discrete nuclear foci at the sites of DNA damage. This phenomenon was also observed following H_2O_2 treatment. This raised the possibility that SPB2 protein might play role in DNA repair.

In accord with this assumption our data showed that SPB2 co-localized with XPB protein upon UV irradiation. We showed that XPB tethering resulted in the recruitment of SPB2 protein to the repair foci. In addition, we detected interaction between SPB2 protein and ubiquitin residues following UV treatment. These results suggest that SPB2 can play an important role in the ubiquitin-mediated fine-tune regulation of the NER pathway.

We investigated the subcellular localization and the possible changes in the level of SPB2 protein in human basal cell carcinoma tissues. Although we did not observe any significant differences in the protein level of SPB2 in the tumorous part of the tissue compared to its normal part, we found differences in the subcellular localisation of the protein. In the tumorous part of the tissue, SPB2 was mainly detected in the cytoplasm, while in the normal part it was found in both cellular compartments. In summary, we conclude that SPB2 protein is a potential player in UV-induced NER pathway.

The primary screen of UV induced genes also suggested that beside SPB2, SPB10 could also play a role in the UV-induced cellular processes. As a first step, we verified the results of the microarray experiment by using qPCR method and we found that the mRNA level of *SPB10* was elevated upon UV

irradiation in Hker E6SFM and also in two additional skin-derived cell lines (A375 melanoma and HaCaT keratinocytes). Using Comet assay, we showed that the process of DNA repair was accelerated in SPB10-silenced S-phase cells compared to the control. We conclude that SPB10 influences the repair of the UV-induced DNA damages, presumably by slowing down that process in replicating cells, which facilitates a more precise, but time-consuming repair mechanism by further reducing the mutation rate. Finally, we demonstrate that SPB10 interacts with H3 and this physical interaction is enhanced following UV irradiation.

In conclusion, we demonstrated involvement of several members of the SerpinB protein family might have been involved in the UV-induced cellular response and we further characterized the regulatory role of SPB2 and SPB10. Based on experimental evidences we established that the expression of these two Serpins was increased upon UV irradiation and we also showed that both proteins were enriched in the chromatin-bound fraction after UV irradiation. Eventually, our results suggest that SPB2 and SPB10 are involved in the fine-tune regulation of the DNA repair processes. We assume that SPB2 protein can play a pivotal role in the regulation of NER pathway after the activation of XPB protein, whereas SPB10 can participate in the resolution of UV-induced replication stress.

Publications

MTMT identifier: 10048472

Articles related to this Ph.D. thesis:

SerpinB2 is involved in cellular response upon UV irradiation, **Hajnalka Majoros**, Zsuzsanna Ujfaludi, Barbara Nikolett Borsos, Viktória Vivien Hudacsek, Zita Nagy, Frederic Coin, Krisztina Buzas, Ilona Kovács, Tamás Bíró, Imre Miklós Boros, Tibor Pankotai; Sci.Rep. 2019 Feb 26; 9 (1): 2753. doi: 10.1038/s41598-019-39073-w

***SCIENTIFIC REPORTS* 9:** 2753. (2019)

IF: 4,525

Coordinated activation of a cluster of MMP genes in response to UVB radiation, Zsuzsanna Ujfaludi, Agota Tuzesi, **Hajnalka Majoros**, Balint Rothler, Tibor Pankotai, Imre Miklós Boros; Sci.Rep. 2018 Feb 8; 8(1):2660. doi: 10.1038/s41598-018-20999-6

***SCIENTIFIC REPORTS* 8:** 2660. (2018)

IF: 4,525

Other publications:

Quantification of DNA damage induced repair focus formation via super-resolution dSTORM localization microscopy, Dániel Varga*, **Hajnalka Majoros***, Zsuzsanna Ujfaludi, Miklós Erdélyi, Tibor Pankotai; Nanoscale 2019 doi: 10.1039/C9NR03696B

***NANOSCALE*:** 2019.06.30.

IF: 7,233

Human p53 interacts with the elongating RNAPII complex and is required for the release of actinomycin D induced transcription blockage, Barbara Nikolett Borsos, Ildiko Huliák, **Hajnalka Majoros**, Zsuzsanna Ujfaludi, Péter Pukler, Imre Miklos Boros, Tibor Pankotai; Sci.Rep. 2017 Jan 19; 7:40960. doi:10.1038/srep40960.

***SCIENTIFIC REPORTS* 7:** p. 40960. (2017)

IF: 5,228

Detection of invasive fungal pathogens by real-time PCR and high-resolution melting analysis, Ferenc Somogyvari, Adam Horvath, Julianna Serly, **Hajnalka Majoros**, Csaba Vagvolgyi, Zoltan Peto; In Vivo 2012 Nov-Dec; 26(6):979-83

***IN VIVO 26*:** 979-984 (2012)

IF: 1,219

Total impact factor: 22,73

Other article:

DNS-hibák és ami mögöttük van. Borsos B, **Majoros H**, Újfaludi Zs, Páhi Z, Pankotai T; ÉLET ÉS TUDOMÁNY LXXI:(6) pp. 180-182. (2016)

ÉLET ÉS TUDOMÁNY **LXXI:**(6) pp. 180-182. (2016)