Insights into the structure and protein–protein interactions of the pro-apoptotic protein ASPP2

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Abstract

ASPP (apoptosis-stimulating protein of p53) 2 is a pro-apoptotic protein that stimulates the p53-mediated apoptotic response. Here, we provide an overview of the structure and protein–protein interactions of ASPP2. The C-terminus of ASPP2 contains Ank (ankyrin) repeats and an SH3 domain (Src homology 3 domain). The Ank–SH3 domains mediate interactions between ASPP2 and numerous proteins involved in apoptosis such as p53 and Bcl-2. The proline-rich domain of ASPP2 is unfolded in its native state, but was not shown to mediate intermolecular interactions. Instead, it makes an intramolecular domain–domain interaction with the Ank–SH3 C-terminal domains of ASPP2. This intramolecular interaction between the unstructured proline-rich domain and the structured Ank–SH3 domains in ASPP2, which is possible due to the unfolded nature of the proline-rich domain, is proposed to have an important role in regulating the intermolecular interactions of ASPP2 with its partner proteins.

Introduction

The tumour suppressor protein p53 is at the centre of a complex protein network, which provides one of the major anticancer defence mechanisms used by the cell. p53 is a transcription factor, which is induced in response to oncogenic stress in order to stop the malignant transformation. This is achieved by the activation of genes that lead to cell-cycle arrest or apoptosis [1–3]. p53 is mutated in more than 50% of human cancers, whereas in most other tumours the p53 pathway is inactivated due to other reasons. A key question in cancer research is how the selection for the p53 response is made. Elucidation of this mechanism could result in novel anticancer therapies that utilize the p53 pathway to selectively stimulate apoptosis of cells even at the early stages of malignant transformation. Recently, the ASPP (apoptosis-stimulating protein of p53) protein family was shown to have a key role in the selection for the apoptotic p53 response [4–6].

The ASPP protein family and its biological functions

ASPP2 is one of the three members of the ASPP family, which also includes ASPP1 and iASPP (inhibitory ASPP). ASPP1 and ASPP2 activate the apoptotic p53 response, but not the cell-cycle arrest response [5,6]. The third member of the family, iASPP, inhibits p53-mediated apoptosis. Depletion of iASPP in *Caenorhabditis elegans* by RNAi (RNA interference) specifically increases apoptosis in germ cells [5].

In the present review, we focus on ASPP2. Human ASPP2 is a 1128-amino-acid protein, whose C-terminal part was originally identified in 1994 as 53BP2 (p53-binding protein 2) by using a yeast two-hybrid system with the DNA-binding core domain of p53 as bait [7]. In a different two-hybrid assay in 1996, with the first 188 amino acids of the anti-apoptotic protein Bcl-2 as bait, another ASPP2 truncated mutant named Bbp (Bcl-2-binding protein) was isolated [8]. In 2001, 53BP2 was found to be the C-terminal 528-amino-acid region of a full-length protein named ASPP2 [6]. Similarly, Bbp was found to be the 1005 C-terminal amino acids of the same ASPP2 protein. ASPP2 and Bbp are encoded by a single copy *TP53BP2* gene that encodes for two alternatively spliced mRNA species and is located in the long arm of chromosome 1 at q42.1 [9]. The ASPP2 protein level is regulated by proteasomal degradation and the protein was found to be ubiquitinated between residues 423 and 848 [4,10].

The mechanism by which ASPP2 induces apoptosis is still under investigation. Initially, it was found that a significant number of cells expressing full-length Bbp fused to GFP (green fluorescent protein) exhibited typical features of apoptosis [11]. ASPP2 was found to specifically stimulate the apoptotic function of p53 in cells by enhancing its transactivation function on the promoters of pro-apoptotic genes, but not of genes involved in cell-cycle arrest. Inhibition of ASPP2 suppresses the apoptotic function of p53 [6]. There is some evidence suggesting a possible relationship of ASPP2 to cancer in cells, as demonstrated by the observation that expression of ASPP2 is down-regulated in some human breast carcinomas [6]. Moreover, low expression of Bbp mRNA in different lung cancer cells makes the cells more resistant to DNA damage caused by UV irradiation, X-ray irradiation and CDDP (*cis*-diamminedichloroplatinum) [12]. There is an increase in the endogenous protein level following DNA damage induced by UV irradiation, and overexpression of

Key words: apoptosis-stimulating proteins of p53 (ASPP), biophysics, natively unfolded protein, p53-mediated apoptosis, proline-rich domain, protein–protein interaction.

Abbreviations used: Ank, ankyrin; ASPP, apoptosis-stimulating protein of p53; iASPP, inhibitory ASPP; Bbp, Bcl-2-binding protein; 53BP2, p53-binding protein 2; HCV, hepatitis C virus; NF-κB, nuclear factor κB; PP1, protein phosphatase 1; PRD, proline-rich domain; SH3 domain, Src homology 3 domain; YAP1, Yes-associated protein 1.

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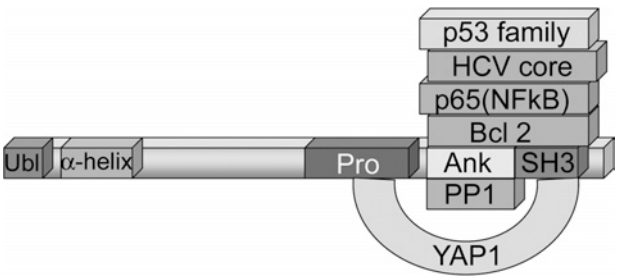
Figure 1 | Domain structure and protein–protein interactions of ASPP2

ASPP2 contains a PRD (Pro), four Ank repeats (Ank) and an SH3 domain. It also contains an α-helical domain and a ubiquitin-like domain (Ubl) at its N-terminus [15]. ASPP2-binding proteins were found to interact mostly with the Ank–SH3 domains. Most of the ASPP2-binding proteins are involved in apoptosis.



Bbp led to increased apoptosis following UV irradiation [13].

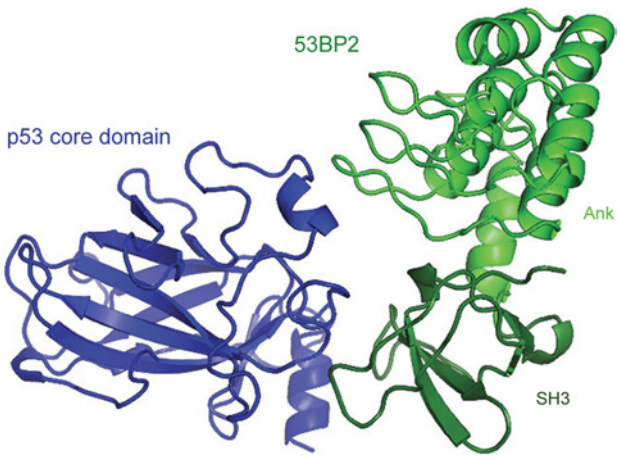
ASPP2 and its truncated isoform Bbp were found to be localized predominantly in the cytoplasm [14]. In 2005, it was shown that Bbp is partly localized in the mitochondria and that it induces cell death associated with the mitochondrial death pathway, suggesting that Bbp is involved in this pathway [14]. However, it was demonstrated that only the truncated form of ASPP2 (residues 882–1128) was localized in the nucleus [11]. This raises the question of how ASPP2 can stimulate the transcriptional activity of p53 in the nucleus while being present mainly in other cellular compartments.

Domain structure of ASPP2

Despite its importance, there is little information about ASPP2 at the structural and molecular levels. ASPP2 and its other family members contain several structural and functional domains, which are also reflected in their names [ASPP is also defined as Ank (ankyrin) repeats, SH3 domain (Src homology 3 domain) and proline-rich protein] [15,16]. The C-terminal part of ASPP2 contains four Ank repeats and an SH3 domain, as revealed from its crystal structure in complex with the p53 core domain (Figures 1 and 2) [17]. ASPP2 also contains a PRD (proline-rich domain) (amino acids 693–918), which we have shown to be unfolded in its native state (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work). The PRD is mainly characterized by the recurrence of the PXXP motif, where X is any amino acid. PXXP motifs are known to mediate protein–protein interactions, particularly by binding to SH3 domains [18]. The ASPP2 PRD contains 40 proline residues, including seven copies of the sequence PXXP (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work). In addition, ASPP2 and ASPP1 are predicted to have an α-helical domain at their N-terminus [8]. Recently, the structure of the N-terminal 83 residues was determined using NMR, showing a β-grasp ubiquitin-like fold [19].

Figure 2 | Crystal structure of the complex between p53 core domain and ASPP2$^{927-1119}$ as solved by Gorina and Pavletich [17] (PDB accession code 1YCS)

The ASPP2 SH3 domain (dark green) binds the L3 loop of p53 core domain (blue), whereas the fourth Ank repeat (light green) binds to the L2 loop of the core domain of p53, overlapping its DNA-binding site [17].



Structural studies of ASPP2

The Ank–SH3 domains

In 1996, the crystal structure of the complex between p53 core domain and the 229 C-terminal residues of 53BP2 was determined [17]. The structure of 53BP2 revealed that it contains two motifs that are known to mediate protein–protein interactions: Ank repeats and an SH3 domain (Figures 1 and 2). The Ank repeat is one of the most frequently observed structural motifs in protein databases. Each repeat folds into two antiparallel α-helices followed by a β-hairpin or a long loop that points outward at approx. 90° [20]. The ability of the Ank repeats to mediate different protein–protein interactions is due to their non-globular tertiary structure [17]. SH3 domains are composed of 50–70 amino acids forming a structure containing multiple β-sheets [21]. These domains are found in a variety of proteins with important roles in signal transduction and have been shown to mediate protein–protein interactions by binding short proline-rich regions in the partner proteins [22].

The proline-rich domain

Biophysical studies of the PRD performed in our laboratory revealed that this domain is natively unfolded (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work). The CD spectrum of the ASPP2 PRD lacked the typical signatures of secondary structure and was not affected by an increase in the temperature, indicating no structural transition. Analytical gel filtration showed that the protein eluted earlier than expected, a fact that could be attributed either to an oligomeric nature of the protein or to an extended conformation. However, ASPP2$^{693-1128}$, which includes the

Pro–Ank–SH3 domains, was found to be monomeric in solution by AUC (analytical ultracentrifugation). This confirms that the early elution time in the gel filtration experiments is due to an unstructured conformation. Finally, computational prediction by 12 publicly available disorder-predicting servers predicted that the ASPP2-Pro would have the characteristics of a natively unfolded protein. Indeed, 57% of the amino acids in the sequence of $ASPP2^{693-918}$ are typical for disordered segments (glutamic acid, lysine, arginine, glycine, glutamine, serine and proline) [23].

Protein–protein interactions of ASPP2

The Ank–SH3 domains of ASPP2 were found to mediate interactions with numerous partner proteins, most of which are also involved in apoptosis or its regulation (Figure 1). Among these are the following proteins: (i) p53 and its family members, (ii) the Bcl-2 family of anti-apoptotic proteins, (iii) PP1 (protein phosphatase 1), (iv) the pro-apoptotic protein YAP (Yes-associated protein) and (v) HCV (hepatitis C virus) core protein.

p53 and its family members

The crystal structure of the complex between p53 core domain and 53BP2 reveals that the fourth Ank repeat of 53BP2 binds to the L2 loop of p53 core domain by its β-hairpin and that the SH3 domain of 53BP2 binds the L3 loop of p53 core domain (Figure 2). The 53BP2-binding site in the p53 core domain overlaps the DNA-binding site that is frequently mutated in human cancer [7,17]. p53 core domain cannot bind simultaneously to 53BP2 and to its DNA-response elements. The Ank–SH3 domains of 53BP2 bind to the p53 core domain with a K_d of 2.2 μM [24]. ASPP2 also binds the p53 family members p63 and p73 and stimulates their transactivation function on the promoters of pro-apoptotic genes but not on genes involved in cell-cycle arrest [7,25].

The Bcl-2 family of anti-apoptotic proteins

The first 188 amino acids of the anti-apoptotic protein Bcl-2 bind the Ank and SH3 domains of ASPP2, which are both required for this interaction [8]. Bbp ($ASPP2^{123-1128}$) was found to be localized in the mitochondria similar to Bcl-2, but it could not interact with p53 and Bcl-2 simultaneously [8]. Bcl-2 and Bcl-X_L expressed with Bbp in cells inhibit the induction of apoptosis by Bbp [26]. Using peptide arrays, we have shown that ASPP2 Ank–SH3 also binds the Bcl-2 family member Bcl-W (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work).

The transcription factor NF-κB (nuclear factor κB)

ASPP2 binds the p65 subunit of the transcription factor NF-κB, which has a role in regulation of the cellular responses to infectious agents, oxidative stress and chemical agents [27]. Using the yeast two-hybrid system with the NF-κB fragment as a bait, it was found that Bbp binds residues 176–405 of NF-κB p65. This was confirmed by an pull-down assay *in vitro* [11]. Activation of NF-κB by IL-1β (interleukin 1β) in cells or co-transfection of cells with Bbp and p65 inhibited 53BP2-induced cell death [11,26].

PP1

PP1 was found to bind ASPP2 in the Ank repeats (residues 897–1031) but not the SH3 domain, using a yeast two-hybrid assay. Binding was confirmed by co-precipitation of bacterially expressed 53BP2 and PP1. PP1 activity was inhibited by 53BP2 [28].

The pro-apoptotic protein YAP

YAP interacted *in vitro* and *in vivo* with ASPP2. The YAP WW1 domain (protein–protein interaction domain containing two conserved tryptophan residues) binds to the YPPPPY motif in the C-terminus of the PRD of ASPP2, whereas the ASPP2 SH3 domain interacts with the VPMRLR sequence of YAP. The Yes protein, a tyrosine kinase that binds YAP, phosphorylates 53BP2 directly or indirectly. This phosphorylation was suggested to negatively regulate the interactions between YAP and 53BP2 [29].

HCV core protein

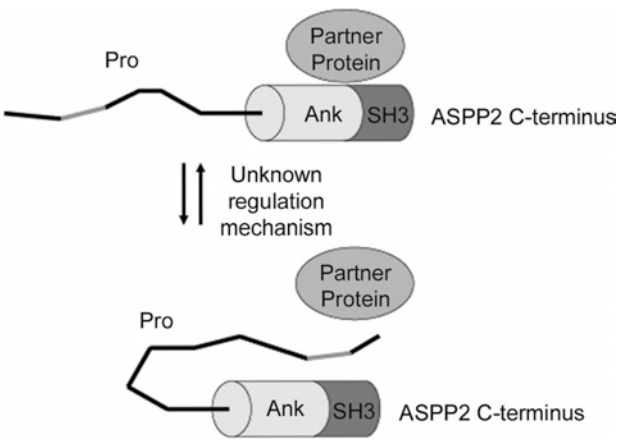
The N-terminal 46 residues of HCV core protein, which affects cellular growth and apoptosis, were found to bind the Ank and SH3 domains of ASPP2 using the yeast two-hybrid and pull-down assays. Neither the Ank nor the SH3 domains alone interacted with the core protein. It was suggested that the HCV core domain inhibits p53-mediated apoptosis by preventing the interaction between ASPP2 and p53 [30].

Domains in ASPP2 that mediate its protein–protein interactions

As described above, the results presented in the literature show that the Ank–SH3 domains almost exclusively mediate the protein–protein interactions of ASPP2. On the other hand, the PRD is not likely to be required for this purpose in ASPP2 (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work). This is despite the fact that such domains in other proteins often mediate protein–protein interactions, especially with SH3 domains [18]. We have tested which domains in ASPP2 mediate interactions with its target proteins using peptide arrays. An array of cellulose-bound overlapping peptides derived from the ASPP2-binding proteins NF-κB, PP1, HCV core protein, YAP and BclW was screened for binding the Ank–SH3 domain and PRD of ASPP2 separately and in combination. Our results showed that ASPP2 Ank–SH3 domains bound peptides derived from all these proteins. However, the PRD did not bind any of the peptides derived from the proteins known to bind the Ank–SH3 domains. The existence of a PRD in proximity to an SH3 domain within the same protein led us to raise the hypothesis that an intramolecular interaction between these two domains might occur. To test this idea, we studied the binding of

Figure 3 | A proposed mechanism for the regulation of ASPP2 binding to its partner proteins

The unstructured PRD (Pro) binds the Ank–SH3 domains and masks them, blocking the protein–protein interactions they mediate. When the PRD is not bound to the SH3 domain, the partner protein can bind to the Ank–SH3 domains. It remains to be explored how the domain–domain interaction is regulated.



the ASPP2 Ank–SH3 domains (amino acids 893–1128) to the PRD (amino acids 693–918) using fluorescence spectroscopy and pull-down assays. Indeed, we discovered that there is an intramolecular domain–domain interaction between the proline-rich and the Ank–SH3 domains, with the K_d for the interaction *in vitro* being approx. 35 μM. By screening an array of peptides derived from ASPP2 for binding ASPP2 Ank–SH3, we have mapped the precise areas in the PRD that bind the Ank–SH3 domains. The major binding site is located between amino acids 723 and 737, and a weaker binding site is located between amino acids 693 and 712. Using fluorescence anisotropy, we found that ASPP2 Ank–SH3 domains bound ASPP2 amino acids 723–737 with a K_d of 15 μM. The other peptides showed weaker binding to ASPP2 Ank–SH3. It is possible that in the context of the full-length domain, residues 723–737, which have a positive charge, mediate the initial domain–domain interaction and the other proline-rich segments bind the Ank–SH3 domains at a second stage (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work).

Conclusions: implications for regulation of ASPP2

The intramolecular domain–domain interaction in ASPP2 is possible only due to the flexible unstructured conformation of the natively unfolded PRD. This intramolecular interaction may have an important role in the regulation of the intermolecular protein–protein interactions of ASPP2. According to our proposed model, the intramolecular interaction with the PRD masks the Ank–SH3 domains and inhibits the binding of the partner protein (Figure 3). Following some yet unknown regulation mechanism, the PRD is released and the Ank–SH3 domains are available again to bind their target proteins (S. Rotem, C. Katz, C. Benyamini, M. Lebendiker, D. Veprintsev, S. Rüdiger, T. Danieli and A. Friedler, unpublished work). How the intramolecular domain–domain interaction in ASPP2 is regulated remains to be explored.

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