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ASPP and iASPP: Implication in cancer development and progression

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Abstract

The well-known guardian of genome, p53 plays critical roles in the induction of apoptosis typically upon DNA damage whereas mutant p53 containing cells are unable to undergo apoptosis which leads to aggressive tumor growth and drug resistance. Moreover, another molecule regulating wild-type p53 function is ASPP (apoptosis stimulating proteins of p53) family. ASPP family consists of ASPP1 and ASPP2, and functions as tumor suppressors whereas the inhibitor of ASPP (iASPP) functions as oncogene. By binding to apoptosis regulating proteins such as p53, p63, p73, Bcl-2, NF-κB p65, etc., ASPP1 and ASPP2 promote apoptosis while overexpression of iASPP inhibits apoptotic cell death typically after DNA damage. In cancer cells, the aberrant expressions of ASPP1, ASPP2 and iASPP have been observed, especially, the high expression of iASPP in cancers is associated with worse disease status, therapy resistance and poor survival of patients with cancers. The molecular interactions between the members of ASPP family and their binding proteins in apoptotic pathway together with other regulators such as miR-124, NF-κB regulated Twist, snail, etc. form a complex signal transduction network to control apoptosis and tumor growth. Therefore, targeting ASPP family could regulate the aberrant communications in the signal transduction network to induce apoptosis and drug sensitivity. Several peptides, miRNAs and natural agents have been used to target ASPP family and show encouraging results in the induction of apoptosis of cancer cells; however, more in vivo animal studies and clinical trials are needed to confirm the true value of targeting ASPP family in the treatment of cancers.

Key words: ASPP1, ASPP2, iASPP, p53, apoptosis.

Introduction

It is well known that p53 (the wild-type p53) plays a critical role in the induction of apoptosis (1). The apoptotic cell death caused by p53 activation is mediated by several cellular signal transduction pathways which interact with p53. One of the important molecules regulating p53 is ASPP (apoptosis stimulating proteins of p53) family (2, 3). ASPP family includes three members named as ASPP1, ASPP2 and inhibitor of ASPP (iASPP). ASPP1 and ASPP2 are p53 activators and play pro-apoptotic roles whereas iASPP is anti-apoptotic (4, 5, 6). All three members of ASPP family have similar sequences in the C-termini of the proteins. The similar sequences contain Ankyrin repeats, SH3 domain and Proline-rich region which are ASPP signature sequences. Therefore, ASPP is also known as Ankyrin repeats, SH3 domain, and Proline-rich region containing Protein.

In 2001, Lu's research group reported the identification of two different ASPP proteins and named them as ASPP1 and ASPP2 (7). ASPP1 is a new protein whereas ASPP2 is a full length p53 binding protein 2 (p53BP2). ASPP2 contains 1128 amino acids and has 123 additional amino acids at the N-terminus of p53BP2 (Figure 1). The sequences of ASPP1 and ASPP2 show about 48% identity. The sequences in the N and C termini of ASPP1 and ASPP2 have higher identity. The C-termini of ASPP1 and ASPP2 have binding sites for other regulatory proteins such as p53, NF-κB p65 and Bcl-2 (7, 8, 9) (Figure 1), which are important molecules in apoptotic pathway. In 2003, the same research group reported that they identified a new member of ASPP family and named it as inhibitory member of the ASPP family (iASPP) because they found that iASPP exerted inhibitory effects on apoptosis (10). The first reported iASPP contains 351 amino acids. The sequences in the C-terminus of iASPP also show high similarity with the sequences of ASPP1 and ASPP2, suggesting that they are the members of the same family. In 2004, a longer form of iASPP isoform with 828 amino acids (Figure 1) was found and it exerts greater regulatory effect on

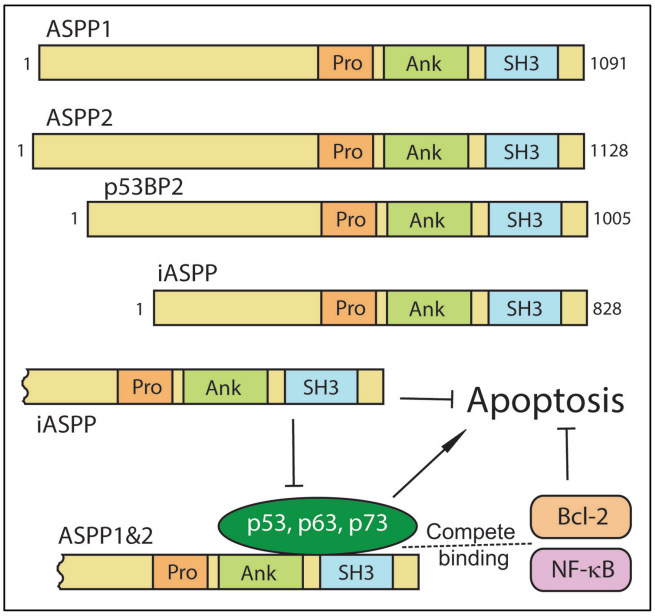

Figure 1. The structure and protein binding of ASPP family.

p53-induced apoptosis (11). In 2007, Zhang et al. identified a new isoform of iASPP and named it as iASPP-SV (iASPP splice variant). The iASPP-SV contains 407 amino acids and inhibits p53 activated Bax and p21 (12).

Because the status of apoptosis in cells and tissues is significantly involved in the processes of carcinogenesis and cancer progression, the role of ASPP regulated p53 in cancer development and progression has become a hot research topic. ASPP family could mediate apoptosis pathway through the regulation of p53 and other apoptosis related molecules. ASPP1 and ASPP2 promote apoptosis through activation of p53 family while iASPP prevents cellular senescence and inhibits differentiation and apoptosis (13). Therefore, targeting ASPP family could be a novel strategy for the prevention and treatment of cancers (4, 14). Here we will restrict our discussion mostly on the function of wild-type p53 and limited discussion on mutant p53 with respect to ASPP family of proteins in apoptosis.

Regulation of ASPP expression

ASPP1 is transcribed from PPP1R13B while ASPP2 is transcribed from TP53BP2. iASPP is encoded by PPP1R13L. The expression level of proteins in ASPP family can be regulated in different stages such as transcription and post-translation. DNA hypermethylation in the promoter region of ASPP could cause decreased transcription of ASPP, leading to lower levels of ASPP protein in cells. ASPP could also be activated by E2F transcription factors. In addition, ASPP could be regulated at the protein level.

Since epigenetic regulation plays important roles in carcinogenesis, DNA methylation appears to be the main epigenetic regulatory process. It has been found that the expression of ASPP1 was significantly down-regulated in acute lymphoblastic leukemia (ALL). Importantly, further analysis of 180 patients with ALL showed that the hypermethylation of ASPP1 promoter was existed in 25% of cases. The expression of ASPP1 mRNA was significantly decreased in these cases (15). Similarly, another study showed hypermethylation of ASPP1 promoter in 34% of 50 patients with T-cell ALL (16). In a study investigating the status of ASPP in various cancer cell lines with wide-type p53, it was found that the CpG island in the promoters of ASPP1 and ASPP2 was hypermethylated and ASPP1 and ASPP2 mRNA expression was reduced in cancer cells compared to normal fibroblasts which showed no such methylation (17). In addition, hypermethylation of ASPP1 and ASPP2 promoters was also observed in hepatitis B virus positive hepatocellular carcinoma (HCC). The expression of ASPP1 and ASPP2 was decreased in HCC cells due to the hypermethylation of ASPP1 and ASPP2 promoters (18). These results suggest that the regulation of ASPP is in part due to DNA hypermethylation.

The expressions of ASPP1 and ASPP2 could also be regulated by E2F1. E2F1 is a transcription factor and could regulate apoptosis through p53 pathway. Molecular experiments showed that E2F family (E2F1, E2F2 and E2F3) interacts with the promoters of ASPP1 and ASPP2 leading to the activation in the transcription of ASPP1 and ASPP2 (19, 20). Further studies showed that E2F increased the expression of ASPP1 and ASPP2 via transcriptional mechanism, leading to the p53-induced apoptosis (21, 22, 23). These results suggest the regulatory effects of E2F on the expression of ASPP1 and ASPP2 in the processes of p53 induced apoptosis.

Post-translational regulation of ASPP2 expression involves proteasome mediated ASPP protein degradation. It was found that ubiquitin-mediated protein degradation decreased the level of ASPP2 protein, leading to reduced p53-mediated apoptosis (24), and that the inhibitor of proteasome showed increased levels of ASPP2 protein but not the ASPP2 mRNA levels, suggesting the post-translational regulation of ASPP (24).

ASPP binding proteins

The Ankyrin repeats and SH3 domain of ASPP are the binding sites for many proteins which are the regulators of apoptosis. The p53 protein is a major protein which interacts with ASPP and induces apoptosis (Figure 1). The core domain of p53 protein possesses the sequence-specific DNA binding site and the ASPP protein binding site (25). The ASPP Ankyrin repeats and SH3 domain binds to the loops of p53 core domain, leading to the activation of p53. ASPP2 can only bind to the wild-type p53 but not mutated p53 protein (26). In addition, ASPP2 also binds to the p63 and p73 proteins which are the members of p53 family, resulting in the transactivation of these proteins and the induction of apoptosis (27) (Figure 1).

The ankyrin repeats and SH3 domain of ASPP2 also have the ability to bind to the first 188 amino acids of Bcl-2 which is an inhibitor of apoptosis (8). ASPP2 and Bcl-2 are both located in mitochondria. However, ASPP cannot bind to p53 and Bcl-2 simultaneously. Binding of Bcl-2 to ASPP2 prevents p53 from binding to ASPP2, thereby, leading to the inhibition of apoptosis induced by p53 (Figure 1). ASPP2 could also bind to the residues 176-405 of NF-κB p65 protein which is another inhibitor of apoptosis (9, 28). The binding of NF-κB to ASPP2 interferes with ASPP2 binding to p53 protein, resulting in the inhibition of apoptosis.

ASPP1 and ASPP2 also participate in centrosome linker reassembly pathway. ASPP1 and ASPP2 proteins interact with C-Nap1, one of the centrosome linker proteins which control cell mitosis and apoptotic cell death (29). ASPP2 also cooperates with RAS to activate the transcriptional activity of p53 and increased apoptotic cell death induced by p53 (30). ASPP1 and ASPP2 could bind to activated RAS, potentiate RAS signaling transduction and enhance p53 activity in various cancer cell lines (31). Other factors such as those factors inhibiting HIF-1 (FIH-1), protein phosphatase 1 (PP-1), p300, PUMA and Bax have been found to bind and modulate protein interaction activity of ASPP, regulating the transcriptional activity of p53 (32, 33, 34, 35, 36). These studies clearly suggest a complex regulatory process governing the p53-mediated apoptosis.

It has been found that iASPP specifically binds to the proline-rich region of p53 protein and inhibits the induction of apoptosis mediated by p53 (6). Structural analysis showed that an 8-mer peptide of p53 had binding specificity to the C-terminal region of iASPP (37). Unlike ASPP2 binding to the loops of p53 core domain,

iASPP predominantly binds to linker region adjacent to the core domain. The structural analyses of the ASPP family and p53 interactions explain the differential effects of ASPP family members on apoptosis (38). In addition, Pin1 could mediate the dissociation of p53 protein from iASPP, and thereby regulating the induction of apoptosis (39).

ASPP and cancers

Because ASPP family is critically involved in the p53-mediated regulation of apoptosis which is a hallmark of the cancer cell, therefore the members of ASPP family plays important role in controlling the development and progression of cancers. It is well known that ASPP1 and ASPP2 function as tumor suppressors while iASPP has oncogenic effects. But, it is also necessary to note that a study showed that cytoplasmic ASPP1 could also have a oncogenic role in contrast to the tumor suppressive activity of nuclear ASPP1 (40). ASPP1 and ASPP2 bind to nuclear p53 and activate p53 induced apoptosis. However, it has been found that the amino acids in p53 that are required for ASPP2 binding such as His^{178}, Arg^{181}, Met^{243}, Arg^{247}, Arg^{248} and Arg^{273} are frequently mutated in human cancer, suggesting the failure of ASPP2 binding to p53 in the majority of cancers (14, 25). In addition, the relationship between ASPP expression level and the development of various cancers has been investigated. It was found that the levels of ASPP1 and ASPP2 expressions are down-regulated while the expression of iASPP is up-regulated in various cancers (14). Therefore, the ASPP binding to p53 and the levels of ASPP contribute to the control of carcinogenesis mediated through iASPP.

It has been found that the expression of ASPP1 was significantly down-regulated in acute lymphoblastic leukemia (ALL) due to the DNA hypermethylation in the promoter of ASPP1 (15, 16). In leukemia cell lines HL-60, K562 and Jurkat, the expression of ASPP1 has been found to be reduced (41). In gestational choriocarcinoma, the decreased expression of ASPP2 at mRNA and protein levels has been observed (42). The decreased ASPP2 could activate Src signaling, leading to the development of gestational choriocarcinoma (42). The decreased expression of ASPP1 and/or ASPP2 was also observed in non-small-cell lung carcinoma (NSCLC) (51), leukemia (41), and hepatocellular carcinoma (18). Moreover, the data from Oncomine database also showed lower expression of ASPP in lung cancer and other malignances (Figure 2, www.oncomine.org), suggesting the tumor suppressive role of ASPP.

In contrast to the lower expression of ASPP, the expression of iASPP has been found to be increased in leukemia cell lines HL-60, K562 and Jurkat (41). The expression levels of iASPP in clinical samples were increased in leukemia patients compared to normal subjects (43, 44). The up-regulated expression of iASPP was also observed in lung cancer (45, 46) (Figure 2), prostate cancer (47), bladder cancer (48), gastric cancer (49) and hepatic cancers (50). In prostate cancer cells, both nuclear and cytoplasmic iASPP expressions were found to be significantly up-regulated especially in highly metastatic prostate cancer cells (51). Moreover, nuclear iASPP was correlated with invasion, metasta-

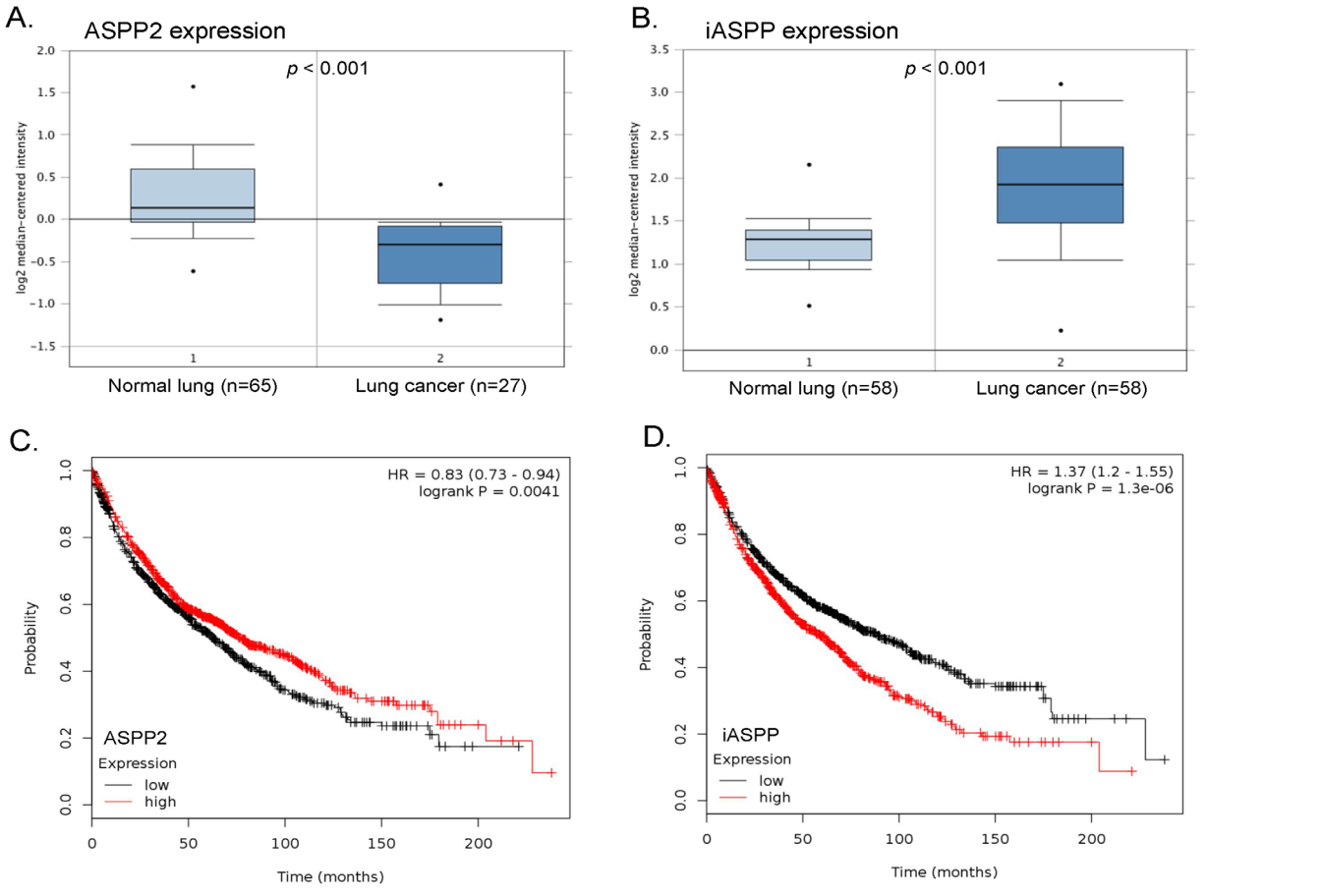

Figure 2. The aberrant expressions of ASPP2 and iASPP in lung cancers and their association with survival of patient with lung cancer. The data analyzed is from Oncomine database (www.oncomine.org).

sis, and poor treatment outcome of prostate cancer (51). In oral tongue squamous cell carcinoma (OTSCC), the expression of iASPP was significantly higher in OTSCC tissues and the up-regulated iASPP expression was found to be associated with poor differentiation and lymph node metastasis (52). Similarly, in oral cavity squamous cell carcinomas (OSCC), the expression of iASPP in both cytoplasm and nucleus were up-regulated. Moreover, the high expression of iASPP in the cytoplasm of OSCC cells was associated with poor survival and recurrence of patients with OSCC (53). The similar phenomena showing high levels of iASPP with poor patient outcome has also been observed in cervical cancer (54), head and neck squamous cell carcinoma (HNSCC) (55), ovarian cancer (56), and hepatocellular carcinoma (57). Moreover, the data from Oncomine database also showed that the high expression of iASPP was associated with poor survival of patients with lung cancer (Figure 2, www.oncomine.org), suggesting the oncogenic role of iASPP.

In addition to the alteration of iASPP level, the phosphorylation status of iASPP also plays an important role in the inhibition of p53 induced apoptosis. It was found that cyclin B1/CDK1 could phosphorylate iASPP, resulting in the inhibition of iASPP dimerization. Therefore, the iASPP monomer could easily enter nucleus and expose binding sites for p53, leading to the suppression of p53 induced apoptosis (58).

It is also important to note that ASPP proteins play important roles in the control of cellular sensitivity to chemotherapeutics and radiation therapy. A study showed that in lung, breast and colon cancers, higher expression of ASPP2 was associated with the sensitivity of cancer cells to UV irradiation, X-ray irradiation and cisplatin (59). It was also found that the NSCLC cells with higher ASPP1 and ASPP2 expression were more sensitive to cisplatin compared with the NSCLC cells with lower expression of ASPP1 and ASPP2 (45). In contrast, higher expression of iASPP has been found to be related to chemoresistance in hepatocellular carcinoma, breast cancer and osteosarcoma (10, 57). Inhibition of iASPP expression showed increased cell sensitivity to cisplatin and radiation, leading to a significant induction of apoptosis (10). These findings suggest that targeting ASPP family especially targeted inactivation of iASPP could be a novel strategy for the regulation of cancer cell response to cytotoxic (DNA damaging) therapeutics.

Targeting ASPP for the prevention and treatment of cancer

In targeting ASPP family for the prevention and treatment of cancer, the molecules or agents that can induce the activities of ASPP1 and ASPP2 or reduce the abilities of iASPP would likely allow to design novel approach for the cancer prevention and treatment of human malignancies. A peptide (CDB3) containing 9 amino acids from ASPP2 was designed and synthesized showing that CDB3 could bind to p53 core domain and stabilize p53. Importantly, the peptide could restore DNA binding ability of mutant p53, leading to the induction of apoptosis and the elimination of cancer cells with mutant p53 (60).

Since iASPP functions as an oncogene which is up-regulated in cancers, inhibition or blockage of iASPP would be a promising approach to overcome the anti-apoptosis effects of iASPP mediated by the interruption of iASPP binding to p53. To that end, a p53-derived peptide containing 37 amino acids which targets iASPP was synthesized (61, 62). The peptide consists of conserved regions II and III (amino acids 118-142 and 171-181) in the DNA-binding domain of p53. This peptide did not transactivate p53 target genes; however, it induced apoptosis by competitively binding to iASPP, leading to the interruption of iASPP binding to p73 and the abrogation of anti-apoptosis effects of iASPP (61, 62). Furthermore, systemic nanoparticle delivery of the peptide caused apoptotic cell death and tumor regression (61), suggesting its therapeutic effect for the treatment of cancer. A similar study showed that a small peptide containing 34 amino acids from p53 linker region was synthesized and used to competitively bind to iASPP and thereby release p53 from iASPP (63). This peptide promoted the transactivation activity of p53 on target gene Bax and PUMA, resulting in increased apoptosis and decreased tumor growth (63).

In gastric cancer, siRNA against iASPP could significantly decrease the expression of iASPP, leading to the induction of apoptosis and the inhibition of proliferation, colony formation and tumor growth in vivo (49). Similarly, siRNA against iASPP also induced apoptosis of breast cancer cells and leukemic cells (64, 65). Moreover, endogenous interference RNAs such as miRNAs could also regulate the expression of iASPP. It was found that miR-124 could bind to the 3'UTR of iASPP and reduce the expression levels of iASPP mRNA (66). Lentivirus mediated miR-124 overexpression inhibited the proliferation of prostate cancer cells through down-regulation of iASPP (66). Several studies also showed that miR-124 could target iASPP and significantly reduce the expression of iASPP protein, leading to the induction of apoptosis and inhibition of proliferation on colorectal cancer (67) and glioblastoma cells (68). A similar study also showed that enforced expression of miR-124 significantly inhibited the expression of iASPP in neuronal cells, leading to apoptotic cell death (69).

It was also found that the molecule RITA (reactivation of p53 and induction of tumor cell apoptosis) was able to disrupt p53 protein binding to iASPP and MDM2, resulting in the reactivation of p53 and induction of apoptosis in cancers (70). Simvastatin is a drug for lowering cholesterol; however, it also exerts anti-cancer activity. It was found that simvastatin had inhibitory effects on choroidal melanoma OCM-1 cells through the induction of p53 and Bax expressions and the reduction of Bcl-2 and iASPP expressions (71). Resveratrol is a natural agent with anti-cancer activity and it was found that resveratrol increased the levels of ASPP1, Bax and p21 expressions, leading to the induction of apoptosis in breast cancer cells (72), suggesting that natural agents could indeed be useful in retaining the p53 mediated apoptotic function in cancer.

Conclusion and perspectives

In the summary of this brief review article, we conclude that ASPP family includes tumor suppressors

and oncogenic function. By binding to apoptosis regulating proteins such as p53, p63, p73, Bcl-2, NF-κB p65, etc, ASPP1 and ASPP2 promote apoptosis while iASPP inhibits apoptotic cell death. In various cancer cells, the aberrant expressions of ASPP1, ASPP2 and iASPP have been observed. Importantly, high expression of iASPP in tumors has been found to be associated with disease status, therapy resistance and poor survival of patients with cancers. The molecular interactions between the members of ASPP family and their binding proteins including p53, p63, p73, Bcl-2 and NF-κB p65 in the apoptosis pathway together with other regulators such as miR-124, NF-κB regulated Twist, snail, etc. form a complex signal transduction network to control apoptosis and tumor growth. Therefore, targeting ASPP family could normalize the aberrant communications in the signal transduction network to induce apoptosis and drug sensitivity. Several peptides, siRNA, miRNA, synthetic and natural agents have been used to target ASPP family and showed some encouraging results in the induction of apoptosis of cancer cells, which clearly justify our perspectives that targeting ASPP could become a promising strategy for the prevention and treatment of cancers. However, further in-depth in vivo animal studies and clinical trials are warranted to benefit the true value of targeting ASPP in the treatment of human malignancies especially through the inhibition of iASPP.

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Other articles in this theme issue include references (73-84).

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