Inhibitors of Apoptosis Proteins (IAPs): Clinical Significance in Cancer Treatment Research

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Abstract: Apoptosis is a process, which involves a sequence of cellular changes, which ultimately lead to cell death. This programmed cell death is a normal phenomenon required for growth of an organism. Inhibition of apoptosis can result in a number of cancers, inflammatory and autoimmune diseases and viral infections. Inhibitors of apoptosis proteins (IAPs) are a family of structurally and functionally related proteins, which play a crucial role in apoptosis (programmed cell death), proliferation and angiogenesis. Till date 8 IAPs have been identified (Survivin, XIAP, Livin, cellular IAP 1 and 2, ILP-2, NAIP and BRUCE/Apollon). The current review discusses individual protein in details with respect to its structural features, functions and clinical significance. These proteins; especially survivin, XIAP and Livin have been found to express in wide range of malignancies and hence taken as a target of interest by various research groups. The review also highlights the various Phase- 1 and 2 studies of new therapeutic agents that are being developed either as a monotherapy or in combination with existent drugs, which target these IAPs.

Keywords: Apoptosis, Survivin, X-linked inhibitor of apoptosis protein (XIAP), cellular IAPs, NAIP, ILP-2, Livin, BRUCE.

INTRODUCTION

Apoptosis or programmed cell death (PCD) is a process which involves biochemical events leading to characteristic morphological changes in a cell and eventually cell death as against necrosis which is a form of traumatic cell death caused by acute cellular injury. Apoptosis is marked by various stages like blebbing (irregular bulging in the plasma membrane of a cell), cell shrinkage, nuclear fragmentation, chromatin condensation (pyknosis) and chromosomal DNA fragmentation (karyorrhexis). Cell fragments or apoptotic bodies are produced by apoptosis that are flushed out by efferocytosis (a type of phagocytosis) before causing any damage to the surroundings. It's a natural and advantageous biological process in our body that plays a significant role in the life cycle of an organism [1] (Figure **1**).

However the extent of apoptosis is an important factor. Excessive rate of apoptosis can cause tissue destruction or atrophy e.g. in autoimmune disorders, neurodegenerative diseases or AIDS, while low rate of apoptosis is involved in tumor formation [2-4]. Process of apoptosis is initiated by varied types of apoptotic stimuli or signals that bring about sequential changes in the cell. These stimuli can be extrinsic signals (of extracellular origin; like cytokines, toxins, hormones, nitric oxide and growth factors) or in other cases can be initiated by intrinsic signals (of intracellular origin) as a result of cellular stress, which may occur from exposure to radiation/chemicals or viral infection. Extrinsic signals involve binding of death inducing ligand to cell surface receptor/death receptors [5-6] while intrinsic signals in general involve mitochondria [7-8].

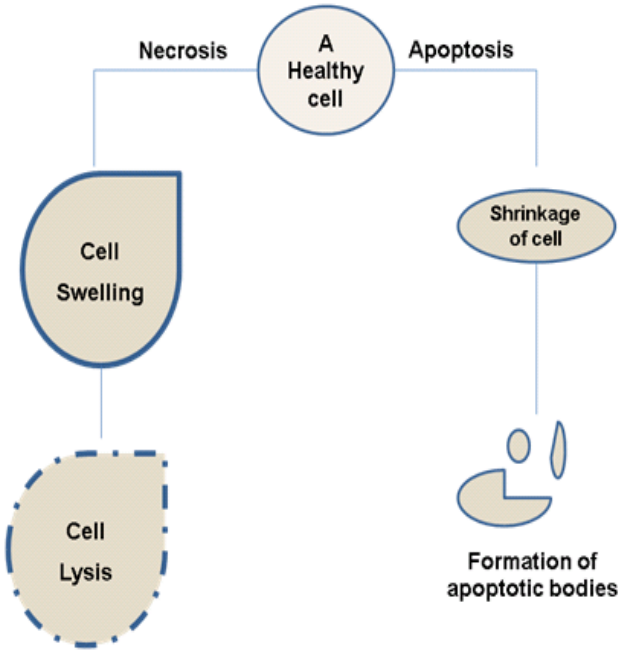

Figure 1: Cellular changes observed during apoptosis and necrosis.

An abnormal signaling plays an important role in various diseases where apoptosis rate is below normal level [9]. Such cases include cancer where low apoptosis results in cell accumulation, resistance to

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drug therapy and failure to monitor tumor by the immune system. Other cases include autoimmunity (failure to eliminate auto-reactive lymphocytes) and persistent infections (Figure **2**).

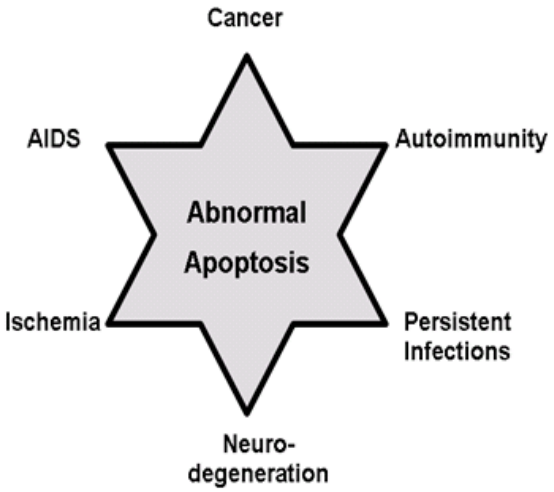

Figure 2: Consequences of dysregulated apoptosis.

Excessive apoptosis leads to neurodegeneration (Alzheimer's disease, Parkinson's disease, Huntington's disease), autoimmunity (uncontrolled apoptosis induction in specific organs), AIDS where depletion of T lymphocytes is noted and ischemia (stroke, myocardial infarction) [10].

Cancer however remains the area of interest from research point of view where defective apoptosis is involved in tumor formation, progression and metastasis as well as the occurrence of multi-drug resistance during cancer therapy [11]. It is now very much clear that formation of tumor is not only because of excessive proliferation due to the activation of oncogenes but also because of impaired apoptosis checkpoints [12-13].

INHIBITORS OF APOPTOSIS (IAPs)

The inhibitors of apoptosis (IAP) are a family of functionally and structurally-related proteins, which serve as endogenous inhibitors of programmed cell death (apoptosis). These proteins contain baculovirus IAP repeat (BIR) domains, 70 amino acid motifs, which are essential for the anti-apoptotic properties of IAPs [14]**.** The primary reason behind this is the interaction between the BIR domains and caspases, which are believed to confer most of the anti-apoptotic activity to IAPs. Of the eight known IAPs; Survivin, Livin, inhibitor of apoptosis protein-like protein-2 (ILP2), cellular inhibitor of apoptosis 1 and 2 (c-IAP1 and c-IAP2), neuronal apoptosis inhibitory protein (NAIP), BIR repeat containing ubiquitin conjugating enzyme system (BRUCE/Apollon), X-chromosome- linked inhibitor of apoptosis protein (XIAP) [15] till date, XIAP, c-IAP1 and c-IAP2 inhibit caspases-3, -7 and 9 directly and possess E3 ubiquitin ligase activity due to highly conserved RING domain at their C-terminal. This family of proteins consists of following three structural components [16] (Figure **3**).

1. The baculoviral IAP repeat (BIR) - The BIR domains consist of approximately 70 amino acids that contain the characteristic sequence CX2CX16HX6C. With both hydrophobic and hydrophilic residues on its surface, the BIR core is theoretically capable of supporting protein-protein interactions. There are three subtypes of BIR domain, BIR1, BIR2 and BIR3, classified by their evolutionary relationship in phylogenesis.

2. RING (RING zinc-finger) - The RING finger domain (C3HC4) exists at the C-terminal in some IAPs. It contains one zinc atom chelated to three cysteine molecules, one histidine molecule and another zinc atom bound to four cysteine molecules.

3. The caspase activating and recruitment domain (CARD).

A. X-Linked Inhibitor of Apoptosis Protein (XIAP)

X-linked inhibitor of apoptosis (XIAP) is a homologous protein, which is encoded by the XIAP gene in humans. The term 'X-linked' has derived from the discovery of a 273 base pair site on the X-chromosome. It is also known human IAP-like protein (hILP). Other parallel terms are: inhibitor of apoptosis protein 3 (IAP3) or baculoviral IAP repeat-containing protein 4 (BIRC) [17-20].

The XIAP gene helps protecting many types of cells including immune cells. It also plays an important role in protecting cells from self-destruction. This is achieved by blocking action of caspases which are necessary for apoptosis. XIAP protein specifically inhibits caspase enzymes 3, 7, and 9 [21-22].

B. Clinical Significance

a. Diabetes

Plesner and co- workers have screened the efficacy of XIAP in protecting β-cells from apoptosis, by using a recombinant adenovirus to overexpress XIAP in transformed murine β-cells and in freshly isolated islets

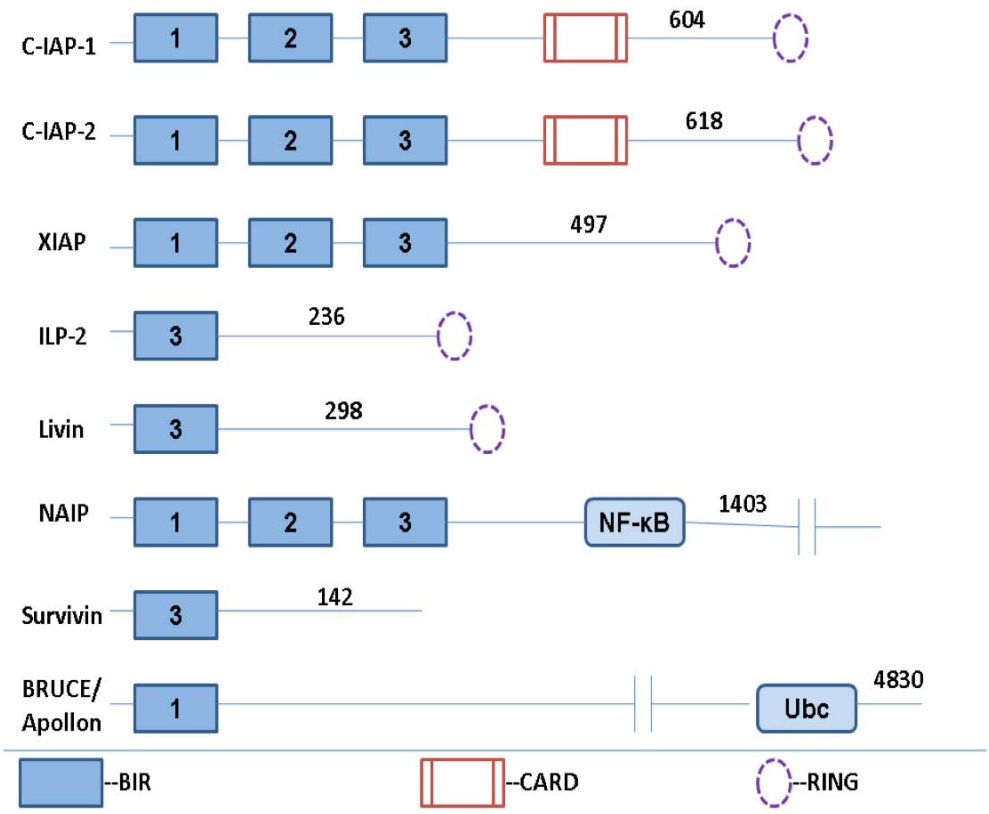

Figure 3: Structural features of different members of IAPs.

in order to establish relation between diabetes and XIAP. They have predicted that long-term protection of islet allografts by XIAP over expression may enhance the survival of islet transplants in diabetes [23].

b. Cancer

Possible role of XIAP in bigger fractions as a useful tumor marker has been studied in prostate cancer in the developing stage. Here XIAP along with three other IAPs are overexpressed in the prostatic epithelium. This raises an important issue that a molecule might have to inhibit all IAPs for effective treatment [24].

c. Inflammatory Bowel Disease

Mutations/ defects in the XIAP gene lead to rare type of conditions such as inflammatory bowel disease and X-linked lymphoproliferative disease [25].

d. Copper Homeostasis

Group of workers have demonstrated a novel role of XIAP in copper homeostasis through its ability to ubiquitinate the copper regulating factor MURR1, which is involved in the regulation of copper homeostasis in mammals [26]. It is thought, to be mediated through high-affinity copper transporters, which mediate copper uptake or efflux from the cell, as well as copper chaperones involved in the transfer of copper ions to certain copper-binding proteins [27]. However, the exact mechanism is unknown [28].

e. Pulmonary Infection

Role of XIAP in experimental pulmonary infection of mice with *C. pneumonia* was studied by Hridayesh and co-workers, by using Wild type and Knock out (KO) mice. Mice were infected with direct non-invasive intra-tracheal application of defined bacterial suspensions. They found that mice, which were deficient in XIAP were sensitized for *C. pneumoniae* pulmonary infection concluding that XIAP plays a crucial role in anti-bacterial host response. However when the same set of experiments were repeated for *salmonella* infection, the results were non-conclusive which showed the specificity of XIAP in certain bacterial infections [29].

C. Livin

Livin is a novel human inhibitor of apoptosis protein (IAP) family, which in high levels is expressed heavily in melanoma. This protein contains a single BIR domain at the N-terminus as well as a C-terminal RING domain. Livin shows its anti-apoptotic activity by its

lone BIR domain. Vucic and co-workers suggested that Livin inhibited caspase-9 through its BIR domain. However in another study there were reports indicating weaker inhibitory effect of Livin on caspase-3 and caspase-9 than that of XIAP [30-32].

D. Survivin

Survivin is a member of the inhibitor of apoptosis (IAP) family and is encoded by the BIRC5 gene in humans. It is usually expressed during the G2/M phase of the cell cycle [33-34].

Structural features

Out of the eight members known in the IAP family, survivin is known to be the smallest of them containing 142 amino acid residues. It exists physiologically as a functional homodimer. Survivin contains a single BIR domain that stretches from amino acid residue 15 to 87 unlike some other IAPs. It comprises a three-stranded anti-parallel β sheet that is surrounded by four α helices. The BIR domain is crucial for dimer formation and other protein– protein interactions, such as with effector caspases [35-37].

Function

Survivin inhibits apoptosis leading to cell proliferation which gives rise to malignancy. This makes malignant cells resistant to various forms of chemotherapy and radiotherapy. Earlier various research groups through their work tried convincing on a common mechanism of apoptosis adopted by Survivin, which is the interaction between initiator and effector caspases. However this was not well supported by another group, which through their *in vitro* studies found that this contact did not result into meaningful inhibition of caspase activity [16, 38-39]. However, recently various other mechanisms have been proposed. These are listed below.

- Antagonism of cell death upstream of effector caspases.
- Interactiion with SMAC/DIABLO, which displaces bound IAPs and these may then bind to and inhibit specific caspases [40-42].

Survivin has become an ideal target for cancer therapy because of many reasons (Figure **4**). Firstly targeting survivin will avoid resistance to various anti-cancer drug therapies for which survivin is responsible. This will thus increase the response to various conventional anti-cancer therapies. Also targeting survivin is highly selective since it is highly expressed in malignant tissue as compared to normal cells. Lastly targeting survivin will not only suppress tumor growth but also block angiogenesis i.e. growth of new blood vessels for tumor expansion [43].

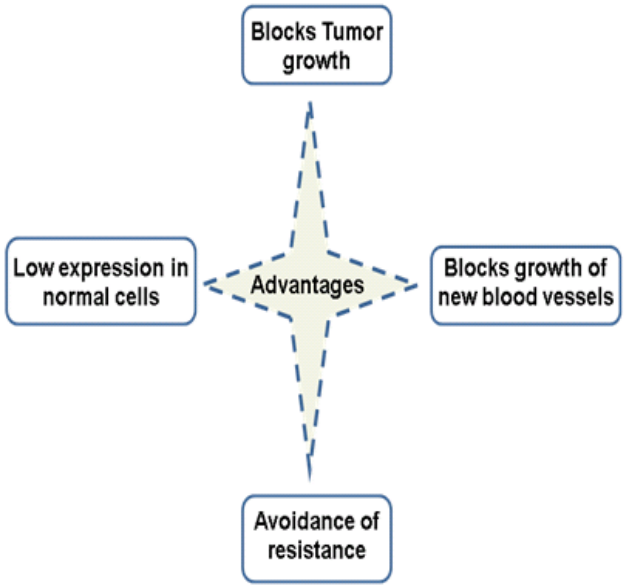

Figure 4: Key advantages of targeting Survivin.

E. cIAP-1 and 2

Structural Features

These are proteins, which contain three BIR domains, a CARD domain and a RING finger domain. cIAP-1 and 2 differ in the amino acid chain length with the former having 604 amino acid, while later having 618 amino acids.

Function

These cellular IAPs inhibit apoptosis by interfering with the activation of caspases. The encoded protein inhibits apoptosis induced by serum deprivation. Studies indicate that cIAP 1 interacts with HtrA serine peptidase 2, Diablo homolog, TRAF1, TRAF2, HSP90B1, RIPK1, Caspase-9, TNFSF14, GSPT1, RIPK2 and Ubiquitin C while cIAP 2 has been shown to interact with TRAF1, TRAF2, RIPK1, Caspase-9 and UBE2D2 [44-54].

F. Other IAPs

BRUCE/Apollon is the largest of the known members of the IAP family, which contains one BIR domain and a C-terminal E2 motif with over 4000 amino acids [55-56]. Decreasing BRUCE content by over expression of Nrdp1 or by RNA interference (RNAi) induces apoptosis in several cell lines. These

findings suggest that BRUCE, unlike other mammalian IAPs, is essential to inhibit apoptosis in certain cell types. Another IAP is the NAIP, which is a complex, containing several tandem copies of NAIP sequences, most representing pseudogenes that vary in number among individuals [57].

ONGOING RESEARCH

Various new compounds have currently been tested for their efficacy against the above- discussed IAPs. Many sponsored agencies have lately started investing heavily in this area taking into account the growing importance of this concept and advantages such as specificity over normal cells and avoidance of drug resistance. Some of these are targeting the proteins directly while rest are being used in combination with known drugs (Figure **5**) to check their efficacy against range of different malignancies like solid tumors, advanced small cell lung carcinoma, Non- Hodgkin's Lymphoma (Table **1**). Till date, Survivin has been the target of choice for many researchers. National Cancer Institute (NCI) has been working on a combination of

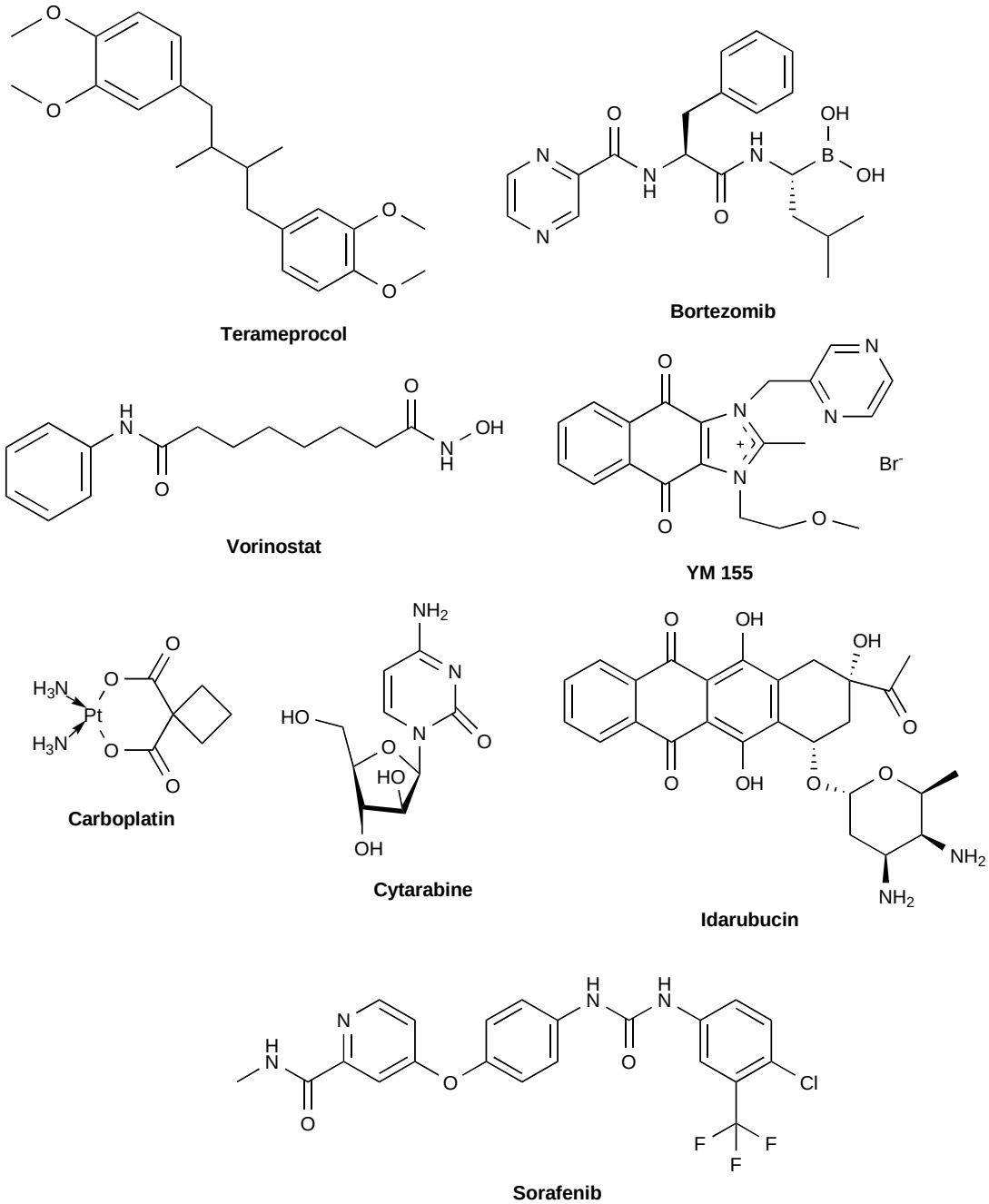

Figure 5: Drugs under clinical trials being tested against different malignancies.

Table 1: Clinical Trials Involving Different IAPs

Name of agent	Primary owner	Type of cancer	Trial status
SURVIVIN AS TARGET			
LY2181308 sodium, Idarubicin and Cytarabine (NCT00620321)	Eli Lilly and Company	Acute myeloid leukemia	Completed Phase-II
LY2181308 and Docetaxel (NCT01107444)	Eli Lilly and Company	Non-small cell lung cancer	Started Phase-II
LY2181308 and Docetaxel (NCT00642018)	Eli Lilly and Company	Hormone- refractory prostate cancer	Phase-II will run until early 2012.
YM155, Paclitaxel and carboplatin (NCT01100931)	National Cancer Institute	Solid tumor	Phase-1 active
		Advanced non-small cell lung carcinoma	Phase-2 active
YM155 and Rituximab (NCT01007292)	Astellas Pharma US	Non-Hodgkin's Lymphoma	Phase-2 active
YM155 and Docetaxel (NCT01009775)	Astellas Pharma US	Advanced Hormone Refractory Prostate Cancer	Phase 2 completed
		Other solid tumors	Phase 1 completed
		Stage III (Unresectable) or Stage IV Melanoma	Phase 2 and closed
Terameprocol	Erimos pharmaceuticals	Refractory solid tumors	Phase 1 completed
		Refractory leukemias	Phase 1 completed
		Refractory gliomas	Phase 1 completed
Multipeptide Vaccine (NCT00573495)	University of Pennsylvania	Metastatic Breast cancer	Phase 1
XIAP AS TARGET			
AEG35156 (NCT00363974)	Aegera Therapeutics	Refractory/Relapsed AML	Phase 1, 2
AEG35156 and Sorafenib (NCT00882869)	Aegera Therapeutics	Advanced Hepatocellular Carcinoma (HCC)	Phase 1, 2
AEG35156 and Docetaxel (NCT00372736)	NCIC Clinical Trials Group	Locally Advanced, Metastatic, or Recurrent Solid Tumors	Phase1
Vorinostat (MK0683) and Bortezomib (NCT00773838)	Merck	Relapsed or Refractory Multiple Myeloma	Phase 2

YM155 and Paclitaxel-Carboplatin. YM155 is a drug that targets a type of chemical often found in cancer cells. It has been investigated in several phase I and phase II clinical trials, and it has been shown to be well tolerated and moderately effective in treating advanced non small cell lung cancer (NSCLC) in patients who had not responded well to one or two standard treatments. The objective of the study was to determine the efficacy of this combination. YM155 is also been tested in combination with rituximab by Astellas Pharma, US to evaluate response rate, survival, safety and tolerability in subjects with Non-Hodgkin's Lymphoma. Astellas Pharma is also working on a combination of YM155 with docetaxel with similar objective in subjects with stage III (Unresectable) or stage IV Melanoma. LY2181308 sodium is another new molecule, which is been tested in combination with, idarubicin and cytarabine by Eli Lilly and company to understand the safety profile of this combination as well as to establish the proof-of-concept of survivin knockdown in AML blast cells. Eli Lilly and company is also conducting a phase-II study of LY2181308 in combination with docetaxel versus docetaxel in patients with NSCLC. Some other emerging developments are multipeptide vaccine for advanced breast cancer by University of Pennsylvania and terameprocol by Erimos Pharmaceuticals for refractory tumors, refractory leukemia and refractory gliomas.

Another target that has been explored is the XIPA. Aegera Therapeutics is working on AEG35156 for refractory/relapsed acute myeloid leukemia (AML), which targets XIAP mRNA thus lowering XIAP levels and the apoptotic threshold of cancer cells. Also in the pipeline is the combination of AEG35156 and sorafenib to determine the recommended dose and efficacy of this combination whereas NCIC Clinical Trials Group is conducting a phase-I trial of AEG35156 and docetaxel, for relapsed or refractory multiple myeloma. Merck is working on the combination of vorinostat (MK0683) and bortezomib to evaluate the clinical activity of this combination in patients with multiple myeloma.

CONCLUSION

Inhibitors of apoptosis proteins are a group of important proteins, inhibiting cell death and are preferentially expressed in malignancy. Exhaustive literature study has made it evident that there is strong correlation between IAP and cancer. Scientists have taken up this as the starting tool to look for the overexpression of the same in various types of cancer. Many new drugs in combination with existing anti-cancer agents are currently at various phases of clinical trials using these proteins as targets to understand the clinical significance of these proteins from diagnostic and therapeutic point of view.

REFERENCES

[1] Keith A, Alexander J, Julian L, Martin R, Walter R. In Apoptosis: Programmed Cell Death Eliminates Unwanted Cells. Molecular Biology of the Cell. 5th ed. Garland Science 2008; p. 1115.

[2] Fulda S, Debatin KM. Targeting Inhibitor of Apoptosis Proteins (IAPs) for Diagnosis and Treatment of Human Diseases. Recent Patents on Anti-Cancer Drug Discovery 2006; 1: 81-9. http://dx.doi.org/10.2174/157489206775246539

[3] Hengartner MO. The biochemistry of apoptosis. Nature 2000; 407: 770-6. http://dx.doi.org/10.1038/35037710

[4] Lowe SW, Lin AW. Apoptosis in cancer. Carcinogenesis 2000; 21: 485-95. http://dx.doi.org/10.1093/carcin/21.3.485

[5] Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2002; 2: 420-30. http://dx.doi.org/10.1038/nrc821

[6] Naismith JH, Sprang SR. Modularity in the TNF-receptor family. Trends Biochem Sci 1998; 23: 74-9. http://dx.doi.org/10.1016/S0968-0004(97)01164-X

[7] Bernardi P, Scorrano L, Colonna R, Petronilli V, Di Lisa, F. Mitochondria and cell death. Mechanistic aspects and methodological issues. Eur J Biochem 1999; 264: 687-701. http://dx.doi.org/10.1046/j.1432-1327.1999.00725.x

[8] Loeffler M, Kroemer G. The mitochondrion in cell death control: certainties and incognita. Exp Cell Res 2000; 256: 19-26. http://dx.doi.org/10.1006/excr.2000.4833

[9] Fadeel B, Orrenius S, Zhivotovsky B. Apoptosis in human disease: a new skin for the old ceremony? Biochem Biophys Res Commun 1999; 266: 699-17. http://dx.doi.org/10.1006/bbrc.1999.1888

[10] Reed JC. Apoptosis-based therapies. Nat Rev Drug Discov 2002; 1: 111-21. http://dx.doi.org/10.1038/nrd726

[11] Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. Cell 2002; 108: 153-64. http://dx.doi.org/10.1016/S0092-8674(02)00625-6

[12] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70. http://dx.doi.org/10.1016/S0092-8674(00)81683-9

[13] Wang XW. Role of p53 and apoptosis in carcinogenesis. Anticancer Res 1999; 19: 4759-71.

[14] Takahashi R, Deveraux Q, Tamm I, Welsh K, Assa-Munt N, Salvesen GS, *et al.* A single BIR domain of XIAP sufficient for inhibiting caspases. J Biol Chem 1998; 273: 7787-90. http://dx.doi.org/10.1074/jbc.273.14.7787

[15] Nachmias B, Ashhab Y, Ben-Yehuda D. The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer. Semin Cancer Biol 2004; 14: 231-43. http://dx.doi.org/10.1016/j.semcancer.2004.04.002

[16] Salvesen GS, Duckett, CS. IAP proteins: blocking the road to death's door. Nat Rev Mol Cell Biol 2002; 36: 401-10. http://dx.doi.org/10.1038/nrm830

[17] Liston P, Roy N, Tamai K, Lefebvre C, Baird S, Cherton-Horvat G et.al. Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. Nature 1996; 379: 349-53. http://dx.doi.org/10.1038/379349a0

[18] Duckett CS, Nava VE, Gedrich RW, Clem RJ, Van Dongen JL, Gilfillan MC. A conserved family of cellular genes related to the baculovirus iap gene and encoding apoptosis inhibitors. EMBO J 1996; 15: 2685-94.

[19] Holcik M, Korneluk RG. Functional Characterization of the X-Linked Inhibitor of Apoptosis (XIAP) Internal Ribosome Entry Site Element: Role of La Autoantigen in XIAP Translation. Mol Cell Biol 2000; 20: 4648-57. http://dx.doi.org/10.1128/MCB.20.13.4648-4657.2000

[20] Deveraux QL, Reed JC. IAP family proteins - suppressors of apoptosis. Genes Dev 1999; 13: 239-52. http://dx.doi.org/10.1101/gad.13.3.239

[21] Duckett CS, Li F, Wang Y, Tomaselli KJ, Thompson CB, Armstrong RC. Human IAP-Like Protein Regulates Programmed Cell Death Downstream of Bcl-xL and Cytochrome c. Mol Cell Biol 1998; 18: 608-15.

[22] Eckelman BP, Salvesen GS, Scott, FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. EMBO Rep 2006; 7: 988-94. http://dx.doi.org/10.1038/sj.embor.7400795

[23] Plesner A, Liston P, Tan R, Robert G, Korneluk C, Verchere B. The X-Linked Inhibitor of Apoptosis Protein Enhances Survival of Murine Islet Allografts. Diabetes 2005; 54: 2533-40. http://dx.doi.org/10.2337/diabetes.54.9.2533

[24] Watson RW, Fitzpatrick JM. Targeting apoptosis in prostate cancer: focus on caspases and inhibitors of apoptosis proteins. BJU Int 2005; 96: 30-4. http://dx.doi.org/10.1111/j.1464-410X.2005.05944.x

[25] Worthey EA, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, *et al.* Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. Genet Med 2011; 13: 255-62. http://dx.doi.org/10.1097/GIM.0b013e3182088158

[26] Burstein E, Lakshmanan G, Robert DD, Bart VDS, John CW, Leo WJK, *et al*. A novel role for XIAP in copper homeostasis through regulation of MURR1. EMBO J 2004; 23: 244-54. http://dx.doi.org/10.1038/sj.emboj.7600031

[27] Puig S, Thiele DJ. Molecular mechanisms of copper uptake and distribution. Curr Opin Chem Biol 2002; 6: 171-80. http://dx.doi.org/10.1016/S1367-5931(02)00298-3

[28] Tao TY, Liu F, Klomp L, Wijmenga C, Gitlin JD. The copper toxicosis gene product Murr1 directly interacts with the Wilson disease protein. J Biol Chem 2003; 278: 41593-6. http://dx.doi.org/10.1074/jbc.C300391200

[29] Hridayesh P, Marco A, Daniel B, Tanja K, Thomas R. Deficiency of XIAP Leads to Sensitization for Chlamydophila pneumoniae Pulmonary Infection and Dysregulation of Innate Immune Response in Mice. J Biol Chem 2010; 285: 20291-302. http://dx.doi.org/10.1074/jbc.M109.096297

[30] Vucic D, Deshayes K, Ackerly H, Pisabarro MT, Kadkhodayan S, Fairbrother WH, *et al*. SMAC negatively regulates the anti-apoptotic activity of melanoma inhibitor of apoptosis (ML-IAP). J Bio Chem 2002; 277: 12275-9. http://dx.doi.org/10.1074/jbc.M112045200

[31] Vucic D, Stennicke HR, Pisabarro MT, Salvesen GS, Dixit VM. ML-IAP, a novel inhibitor of apoptosis that is preferentially expressed in human melanomas. Curr Biol 2000; 10: 1359-66. http://dx.doi.org/10.1016/S0960-9822(00)00781-8

[32] Vucic D, Franklin MC, Wallweber HJ, Das K, Eckelman BP, Shin H, *et al*. Engineering ML-IAP to produce an extraordinarily potent caspase 9 inhibitor: implications for Smac-dependent anti-apoptotic activity of ML-IAP. Biochem J 2005; 385: 11-20. http://dx.doi.org/10.1042/BJ20041108

[33] Colnaghi, R, Connell CM, Barrett RM, Wheatley SP. Separating the anti-apoptotic and mitotic roles of survivin. J Biol Chem 2006; 281: 33450-6. http://dx.doi.org/10.1074/jbc.C600164200

[34] O'Driscoll L, Linehan R, Clynes M. Survivin: role in normal cells and in pathological conditions. Curr Cancer Drug Targets 2003; 3: 131-52. http://dx.doi.org/10.2174/1568009033482038

[35] Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 38: 917-21. http://dx.doi.org/10.1038/nm0897-917

[36] Verdecia MA, Huang H, Dutil E, Kaiser DA, Hunter T, Noel JP. Structure of the human anti-apoptotic protein survivin reveals a dimeric arrangement. Nat Struct Biol 2000; 77: 602-8.

[37] Chantalat L, Skoufias DA, Kleman JP, Jung B, Dideberg O, Margolis RL. Crystal structure of human survivin reveals a bow tie-shaped dimer with two unusual alpha-helical extensions. Mol Cell 2000; 61: 183-9.

[38] Kobayashi K, Hatano M, Otaki M, Ogasawara T, Tokuhisa T. Expression of a murine homologue of the inhibitor of apoptosis protein is related to cell proliferation. Proc Natl Acad Sci USA 1999; 964: 1457-62. http://dx.doi.org/10.1073/pnas.96.4.1457

[39] O'Connor DS, Grossman D, Plescia J, Li F, Zhang H, Villa A et.al. Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc Natl Acad Sci USA 2000; 9724: 13103-7. http://dx.doi.org/10.1073/pnas.240390697

[40] Dohi T, Beltrami E, Wall NR, Plescia J, Altieri DC. Mitochondrial surviving inhibits apoptosis and promotes tumorigenesis. J Clin Invest 2004; 1148: 1117-27.

[41] Verhagen AM, Ekert PG, Pakusch M, Silke J, Connolly LM, Reid GE, *et al*. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 2000; 1021: 43-53. http://dx.doi.org/10.1016/S0092-8674(00)00009-X

[42] Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000; 1021: 33-42. http://dx.doi.org/10.1016/S0092-8674(00)00008-8

[43] Chiou SK, Jones MK, Tarnawski AS. Survivin - an anti-apoptosis protein: its biological roles and implications for cancer and beyond. Med Sci Monit 2003; 9: 25-9.

[44] Ramesh H, Srinivasa MS, Pinaki D, Muniswamy M, Richard W, ZhiJia Z, *et al*. The polypeptide chain-releasing factor GSPT1/eRF3 is proteolytically processed into an IAP-binding protein. J Biol Chem 2003; 278: 38699-706. http://dx.doi.org/10.1074/jbc.M303179200

[45] Verhagen AM, Silke J, Ekert PG, Pakusch M, Kaufmann H, Lisa CM, *et al*. HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins. J Biol Chem 2002; 277: 445-54. http://dx.doi.org/10.1074/jbc.M109891200

[46] Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. EMBO J 1997; 16: 6914-25. http://dx.doi.org/10.1093/emboj/16.23.6914

[47] Shu HB, Takeuchi M, Goeddel DV. The tumor necrosis factor receptor 2 signal transducers TRAF2 and c-IAP1 are components of the tumor necrosis factor receptor 1 signaling complex. Proc Natl Acad Sci USA 1996; 93: 13973-8. http://dx.doi.org/10.1073/pnas.93.24.13973

[48] Li X, Yang Y, Ashwell JD. TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. Nature 2002; 416(6878): 345-7. http://dx.doi.org/10.1038/416345a

[49] Thome M, Hofmann K, Burns K, Martinon F, Bodmer JL, Mattmann C, *et al*. Identification of CARDIAK, a RIP-like kinase that associates with caspase-1. Curr Biol 1998; 8: 885-8. http://dx.doi.org/10.1016/S0960-9822(07)00352-1

[50] Uren AG, Pakusch M, Hawkins CJ, Puls KL, Vaux DL. Cloning and expression of apoptosis inhibitory protein homologs that function to inhibit apoptosis and/or bind tumor necrosis factor receptor-associated factors. Proc Natl Acad Sci USA 1996; 93: 4974-8. http://dx.doi.org/10.1073/pnas.93.10.4974

[51] Yoneda T, Imaizumi K, Maeda M, Yui D, Manabe T, Katayama T, *et al*. Regulatory mechanisms of TRAF2-mediated signal transduction by Bcl10, a MALT lymphoma-associated protein. J Biol Chem 2000; 275: 11114-20. http://dx.doi.org/10.1074/jbc.275.15.11114

[52] Bertrand JM, Milutinovic S, Dickson KM, Chi HW, Boudreault A, Durkin J, *et al*. cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 2008; 30: 689-700. http://dx.doi.org/10.1016/j.molcel.2008.05.014

[53] Sekine K, Takubo K, Kikuchi R, Nishimoto M, Kitagawa M, Fuminori A, *et al*. Small molecules destabilize cIAP1 by activating auto-ubiquitylation. J Biol Chem 2008; 283: 8961-8. http://dx.doi.org/10.1074/jbc.M709525200

[54] Mace PD, Linke K, Feltham R, Schumacher FR, Smith CA, Vaux DL, *et al*. Structures of the cIAP2 RING domain reveal conformational changes associated with ubiquitin-conjugating enzyme (E2) recruitment. J Biol Chem 2008; 283: 31633-40. http://dx.doi.org/10.1074/jbc.M804753200

[55] Kelliher MA, Grimm S, Ishida Y, Kuo F, Stanger BZ, Leder P. Immunity 1998; 8: 297-303. http://dx.doi.org/10.1016/S1074-7613(00)80535-X

[56] Lee TH, Shank J, Cusson N, Kelliher MA. J Biol Chem 2004; 279: 33185-91. http://dx.doi.org/10.1074/jbc.M404206200

[57] Roy N, Mahadevan MS, McLean M, Shutler G, Yaraghi Z, Farahani R, *et al*. The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. Cell 1995; 80: 167-78. http://dx.doi.org/10.1016/0092-8674(95)90461-1

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