symposium article

doi:10.1093/annonc/mdl963

Apoptosis: a relevant tool for anticancer therapy

A. Russo[1*#], M. Terrasi[1#], V. Agnese[1], D. Santini[2] & V. Bazan[1]

[1]Section of Medical Oncology, Department of Surgical and Oncology, Università di Palermo; [2]Medical Oncology, University Campus Bio-Medico, Rome, Italy

Apoptosis is a form of cell death that permits the removal of damaged, senescent or unwanted cells in multicellular organisms, without damage to the cellular microenvironment. Defective apoptosis represents a major causative factor in the development and progression of cancer. The majority of chemotherapeutic agents, as well as radiation, utilize the apoptotic pathway to induce cancer cell death. Resistance to standard chemotherapeutic strategies also seems to be due to alterations in the apoptotic pathway of cancer cells. Recent knowledge on apoptosis has provided the basis for novel targeted therapies that exploit apoptosis to treat cancer. These new target include those acting in the extrinsic/intrinsic pathway, proteins that control the apoptosis machinery such as the p53 and proteosome pathway. Most of these forms of therapy are still in preclinical development because of their low specifity and susceptibility to drug resistance, but several of them have shown promising results. In particular, this review specifically aims at providing an update of certain molecular players that are already in use in order to target apoptosis (such as bortezomib) or which are still being clinically evaluated (such ONYX-015, survivin and exisulind/aptosyn) or which, following preclinical studies, might have the necessary requirements for becoming part of the anticancer drug programs (such as TRAIL/Apo2L, apoptin/VP3).

Key words: apoptosis, TRAIL/Apo2L, apoptin/VP3, ONYX015, Bortezomib, exisulind, survivin

introduction

An organism uses two main mechanisms for the elimination of cells: necrosis and apoptosis. Necrosi consists of the rupture of the plasmatic membrane and the formation of an inflammatory process that damages the cells and their surrounding tissues. Apoptosis, instead, involves a 'cleaner' type of death, where the chromatin is condensed, the DNA becomes fragmented and vesicles, known as 'apoptotic bodies', are formed. These are rapidly phagocytized by the macrophages with the result that the cell disappears without any inflammatory phenomena [1].

Apoptosis is, therefore, considered to be the most suitable method of anticancer therapy. Its main aim is, in fact, specifically to bring about tumoral cell death while limiting as far as possible cytotoxic effects on healthy tissues. This might be achieved in several ways, for example, by promoting the expression of pro-apoptotic factors while reducing the expression of anti-apoptotic factors only in the tumor cells, or else by means of the infection of viral particles that act specifically within the transformed cells.

Apoptosis is a fundamental physiological process which maintains cell homeostasis; it is a genetically determined mechanism which is, therefore, regulated by cell factors such as proliferation and differentiation [1]. This means that, like all other molecular events, programmed cell death may be compromised by mutations in genes implicated in this intricate process and, in fact, the events regulating the apoptotic pathway are very often altered in tumor cells [2].

The apoptotic pathway is triggered off by two different signals, one extrinsic, which responds mainly to extracellular stimuli, and the other intrinsic, activated by modulators within the cell itself. Although, at least at the beginning, the two pathways are apparently separate from each other, at the end they converge in a single crucial point, i.e. the conversion of pro-caspase into caspase, a protease whose activation is the biochemical event that has the strongest influence on the structural modifications of the apoptotic cell [3].

With regard to the extrinsic pathway, the activation of the receptors belonging to the TNF family (Fas/Apo1, TNFR1, DR3/ TRAMP/Apo3, DR4/TRAILR1/Apo2, DR5/TRAILR2 and DR6/TR7), by means of specific ligands (TNF-α, TNF-β, TRAIL, FasL, etc), bring about the recruitment of the TNFR (Fas associated death domain) and TRADD (TNFR associated DD) family members and the chain activation of the caspases 8, 3 e 7 [4].

In the intrinsic pathway, the mitochondria release a series of molecules, including *cytochrome c*. In cytosol, the association of *cytochrome c* with the adaptor protein Apaf-1 and several pro-caspase 9 molecules, gives rise to the formation of apoptosome, which is responsible for bringing several pro-caspase 9 molecules into close contact with one another in order to allow their self-processing. Caspase 9 is thus able to recruit and activate caspase 3 [5], which is the effector of both pathways.

The mitochondrial apoptotic pathway is negatively modulated by anti-apoptotic factors belonging to the Bcl-2

*Correspondence to: Dr A. Russo, Section of Medical Oncology, Department of Oncology, Università di Palermo, Via del Vespro 127, 90127 Palermo, Italy. Tel: +39-091-6552500; Fax: +39-091-6554529; E-mail: lab-oncobiologia@usa.net

#A. Russo and M. Terrasi have equally contributed to this work.

family; these stop the mitochondria from releasing *cytochrome c*. Furthermore, caspase activity can be blocked by the 'inhibitor of apoptosis proteins' (IAPs), which, in their turn, are inhibited by Smac/DIABLO or OMI/HtrA2, the regulator proteins released by the mitochondria.

The aim of this review is to focus attention on the most promising pro-apoptotic drugs involved in present-day preclinical (such as TRAIL/Apo2L, apoptin/VP3) and clinical trials (such as bortezomib ONYX-015, survivin and exisulind) for the treatment of human tumors.

'death receptors' and 'death ligands'

Considerable progress has been made in the last few years with regard to the understanding of the molecular mechanisms behind programmed cell death activated by 'death receptors' (DR) and 'death ligands' (DL).

DRs are transmembrane proteins which, after binding with a DL, transmit the apoptotic signal to the interior of the cell. The DLs are also transmembrane proteins, which, after being processed by specific proteases, are then transformed into soluble cytochines, which are capable of binding their receptors trimerically. Included in this group are the members of the tumor necrosis factor (TNF) and their receptor superfamily (TNFR) which regulate several biological functions, including cell metabolism, proliferation, cytochine production and apoptosis [6–8].

TNF-α, FasL and TRAIL/Apo2L have aroused a great deal of interest as possible candidates for anti-cancer therapy, since in the form of trimers, they are able to trigger off apoptosis in many transformed cells but not in normal cells. Taken singly, TNF and FasL are extremely efficient in causing the death of a large variety of tumor cells but, unfortunately, their *in vivo* use leads to ischemic phenomena and hemorrhagic lesions [9]. The transfection of the gene TRAIL/Apo2L, unlike TNF and FasL, results in a very low level of toxicity both *in vitro* and *in vivo*, which has led to the hope that it may possibly be useful in the treatment of a large number of human tumors [10].

TRAIL/Apo2L

In spite of the fact that a recombant form of human TRAIL with a polyhistidine tail kills cultured human hepatocytes [11], a more recent study has shown that the native form of TRAIL, not only is non-toxic for *in vitro* human hepatocytes, but that it is also well-tolerated in chimeric mice expressing human hepatocytes [12].

The receptor system for TRAIL/Apo2L includes four specific receptors, two of which, DR4 and DR5, are death receptors containing a cytoplasmatic death domain (DD) able to transduct the apoptotic signal; the other two receptors, DcR1 and DcR2, known as decoy receptors, have no intracellular DD and are therefore unable to induce the apoptotic pathway [13] (Figure 1).

The expression levels of the decoy receptors DcR1 and DcR2 appear to play an important role in the specific induction of apoptosis by TRAIL; these levels are higher in normal cells compared with tumor cells [14–16].

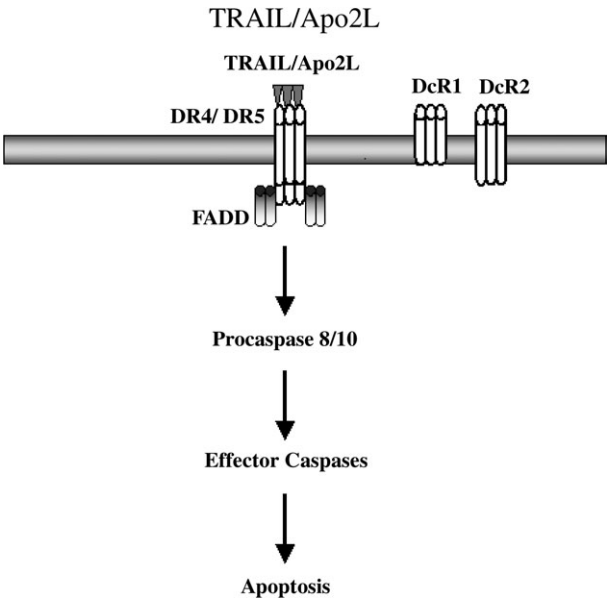

Figure 1. Apo2L/TRAIL and its receptors. Apo2L/TRAIL interacts with four closely related members of the TNF receptor superfamily in a homotrimeric form. DR4 and DR5 contain a cytoplasmic death domain that interacts with FADD (Fas-associated death domain); DcR1 lacks signaling activity; DcR2 has a truncated non-functional death domain. Unlike conventional cancer drugs, DR ligands trigger tumor cell apoptosis independently of the p53 tumor suppressor gene.

preclinical studies. Recent studies have demonstrated that, apart from regulation by the receptors, several other cytosolic factors are able to modulate the apoptosis induced by TRAIL, often giving rise to resistance to the action of this protein [17]. For example, cell lines presenting mutations in FADD (Fas-associated death domain), the molecular adaptor that possesses not only an interaction domain with the death receptor but also an interaction domain with caspase 8/10, prove to be completely resistant to TRAIL-dependent apoptosis [18]. Similarly, cells obtained from infant neuroblastomas, which often have a silent caspase 8 gene, are insensitive to the effects of TRAIL [19].

Apart from the mutations of genes involved in the apoptotic signal, the overexpression of anti-apoptotic proteins such as Bcl-2 and Bcl-xL may also interfere with the action of TRAIL, thus preventing the release of the pro-apoptotic molecules by the mitochondria [20].

The foregoing data suggest that TRAIL alone is not sufficient for the therapy of several forms of cancer. Nevertheless, the treatment of TRAIL-resistant cell lines with chemotherapy agents may convert them into TRAIL-responsive elements [21], although in certain cases the molecular basis of such a synergic action is not clearly understood.

TRAIL-resistant cells from renal, prostate and bladder carcinomas respond to subtoxic concentrations of several chemotherapy agents such as doxorubicin, epirubicin and cisplatin associated with TRAIL [22].

Human U2OS osteosarcoma cells which are resistant to TRAIL-induced apoptosis respond to TRAIL following treatment with doxorubicin and cisplatin, without interfering with either death receptor or decoy receptor expression [23].

Paclitaxel and TRAIL used together bring about a reduction of the tumor *in vivo* and induce apoptosis by means of the interaction of TNF-family death receptors, the activation of caspases and/or the release of cytochrome c from the mitochondria [24]. The sequential treatment of nude mice with chemotherapeutic drugs (paclitaxel, vincristine, vinblastine, etoposide, camptothecin and adriamycin) followed by TRAIL induced caspase-3 activity and apoptosis in xenografted breast tumors. Complete eradication of established tumors and survival of mice were achieved without detectable toxicity [25], which leads to high hopes regarding the treatment of patients with TRIAL associated with chemotherapy agents.

Another *in vivo* study involving nude mice has shown that the use of TRAIL associated with chemotherapy agents not only blocks breast tumor growth, but is also able to cause a reduction in the number of lung metastases, suggesting its possible value in the treatment of metastatic tumors [11].

clinical studies. Although no clinical studies are in progress at the present time, the results obtained until now from *in vivo* and *in vitro* studies lead to the hope that it will soon be possible to produce this new type of anticancer drug therapy for future use.

viral protein and viruses to target apoptosis

In order to complete their life cycle, viruses require actively proliferative cells and for this reason there has been considerable interest regarding the use of viral proteins and attenuated viruses in antitumor therapy.

apoptin

Apoptin or VP3 is a virus protein of avian anemia, which induces apoptosis in a large variety of transformed cells but not in primary cells [26].

preclinical studies. It must be borne in mind, however, that in normal cells, although the co-expression of apoptin with the transforming agent, a large T antigen of SV40, is transient, it leads to susceptibility to apoptin-dependent apoptosis [27].

The mechanism behind the induction of apoptosis by apoptin specifically in transformed cells is still not fully understood. The reason for the phenomenon might be the different site; whereas in tumor cells, apoptin accumulates in the nucleus, in normal cells, it is mainly found in the cytoplasm [28]. Apoptin has a particular nuclear localization sequence, between the 70 and 121 residues, which shows a greater affinity with transformed cells [26] (Figure 2).

Nuclear localization, however, is apparently not the only factor determining the apoptotic action of this protein; in several cases, in fact, the forcing of apoptin into the nucleus of normal cells does not cause apoptosis [29]. Moreover, it has also been demonstrated that the induction of apoptosis requires the phosphorylation of the apoptin threonine residue (Thr-108) [30] in spite of the fact that this event has no effect on the protein localitation, since the abolition of the apoptin phosphorylation site does not lead to any significant reduction in tumor cell nuclei [30].

Recent studies report that the only requirement for the accumulation within the nucleus and the selectivity of cancer cells by apoptin is the protein expression level. Tumor cells, in fact, are often more easily transfected compared with normal cells, which leads to an accumulation of the protein within the cytosol, indispensable for its translocation to the nucleus [29].

Apoptin over-expression may bring about the death of several normal cells. The discordance of the data regarding different cell types may be due to the different techniques used for the insertion of apoptin within the cell. The main aim, therefore, is to identify the most suitable method, either by proteic transduction or the expression of inducible vectors, for an accurate assay of the cell proteins [31].

It is surprising that, in the last few years, spectroscopic studies have reported that the biologically-active form of a recombinant apoptin (recombinant MBP-apoptin) is a multimer made up of about 30–40 monomers [32]. This complex would appear to be produced by the interaction in the hydrophobic regions of the N-terminal (aa 1–69) of each monomer, which contain the nuclear export sequence (NES) (aa 33–46). If this is so, the formation of the multiproteic complex might complicate the exportation of the nucleus [31]. On the other hand, the C-terminal tip of each monomer which contains the nuclear localization sequence (NLS) and the phosphorylation site still remains available.

The molecular mechanism by which apoptin is able to kill is still not fully understood. It acts independently of the p53 status [33] and it has recently been shown that it binds to the anaphase promoter complex (APC/C) with resulting cell cycle block in G2M and p53-independent cell death. This leads to the hope that this viral protein might be useful for the treatment of those tumors which have lost their p53 and are therefore resistant to many forms of anticancer therapy [34].

It has been demonstrated that apoptin interacts with FADD (Fas-associating protein with death domain) and with Bc110, which are both involved in apoptosis mediated by Fas and TNFR [29], although the significance of this is still unknown. On the other hand, MCF7 cells, which have neither FADD or caspase 8 functions, are just as responsive to apoptosis, indicating that in these cells at least apoptin probably acts by means of a pathway which is independent of death receptors.

Apoptin is responsible for the release by the mitochondria of *cytochrome c* and the protein which induces apoptosis (AIP). However, in MCF7 cells, Bcl-2 and Bcl-X_L intervene in order to protect the cell from apoptosis [35]. These results seem to disagree with those obtained in the past on other cell lines, for example, in human cells, Saos-2 transfected with plasmids which codify for apoptin and Bcl-2 undergo apoptosis much more frequently than the same cells expressing apoptin on their

Figure 2. Linear proteic structure of apoptin with nuclear localisation signal (NLS) nuclear export signal (NES)

own account [36]. This might be partially explained by the fact that two different cell types were used in the two studies.

In vivo studies have showed that apoptin is a candidate for safe and effective anti-tumor gene therapy. Toxicity studies with rats showed that recombinant adenovirus expressing apoptin did not result in obvious abnormalities. One single intratumoral infection of nude mice bearing subcutaneous human hepatomas with a single batch of recombinant apoptin-adenovirus, resulted in reduction of tumor-growth and symptoms of regression within 7 days. In contrast, a control adenovirus showed no reduction in tumor growth [37].

In the last few years, studies using apoptin-transgenic mice and other animal models have shown apoptin to be a safe and efficient anti-tumor agent. These *in vitro* and *in vivo* tumor-specific features of apoptin imply that it might form the basis of future anti-tumor strategies [38].

clinical studies. To date no clinical studies are in progress. Only a complete understanding of the mechanism by which apoptin is able to induce programmed cell death and the identification of the proteins that it interacts with will make it possible to decide if apoptin can be used as an anticancer drug or whether its use should be limited to certain specific cases.

ONYX-015

At present, several preclinical and clinical trials involving strategies for the treatment of tumors with mutated p53 are in progress. One of these, ONYX-015, regarding the use of viruses that specifically replicate in deficient p53 cells, has aroused particular interest.

ONYX-015 is an oncolytic virus, which seems to replicate selectively in p53-defective tumor cells. It lacks the E1B-55K gene product and, therefore, fails to degrade p53 during viral replication [39].

preclinical studies. The exact role of p53 in determining ONYX-015 selectivity remains controversial. ONYX-015, in fact, is able to replicate in several tumor cell lines retaining wild-type p53 [40]. This apoptotic response appears to be due to the activity of the viral E1A protein, which is not altered in the ONYX-015 virus and which is capable of activating the host cell p53 via p14/ARF [41]. The selectivity of ONYX-015 for tumor cells has therefore been called into question.

Recently a tumor specific replicative adenovirus vector ZD55 (E1B 55KD deleted Adv.) which is similar to ONYX-015 in targeting function but significantly different in construction has been produced and various single therapeutic genes have been into ZD55 (ZD55-Gene). In mice with xenograft tumors, the ZD55-Gene seems to produce better results [42].

clinical studies. Different phase I and II trials, with intratumoral and peritumoral injections, have been conducted in multiple tumor types with proven safety and evidence of promising clinical activity from several indications [41, 43–45].

A phase II clinical trial reported that ONYX-015 treatment, combined with chemotherapy, was promising in 30 patients with head and neck cancers. Tumors disappeared completely in eight patients and another 19 experienced a dramatic reduction in tumor size [43].

Pilot trials in patients with refractory cancer, have shown that ONYX-015 can be administered safely in combination with CPT11, 5FU or low-dose IL-2 and is able to access malignant tissue following intravenous infusion [46].

Recently, a clinical trial (phase I and II) using ONYX-015 was completed in patients with pancreatic cancer. The phase II trial yielded beneficial results (tumor reduction or stabilization) in about 50% of the patients [47].

proteosome inhibitors

Because of its importance in cell homeostasis, in the past proteosome was studied with a view to using it in anticancer therapy. Particular interest was focused on proteosome inhibitors, which are molecules able to trigger selective apoptosis in tumor cells [48].

Cells can only function correctly when there is a highly regulated turnover of the proteins, brought about, in eukaryotes, by the proteosome complex. The proteins to be eliminated are first 'labeled' by a polyubiquitine tail and then degraded [49]. Proteosome 26S is a multiproteic complex that includes a core (20S) with enzymatic activity and two regulatory complexes (19S), one at each end of the core, responsible for the recognition and for the binding of the polyubiquitine tail [50]. It has been shown that, apart from damaged or mutated proteins, about 80% of the cell proteins are proteosome targets, since they are cell cycle regulators, oncosuppressors and transcription factors [51].

There exist various molecules, both natural and synthetic, able to inhibit protein degradation through the proteosome, for example the original bacterial compound lactacystin, and

Table 1. Bortezomib: preclinical studies

Tumor type	Study type	Combination	Reference
Bladder	*In vitro/in vivo*	Gemcitabine	Kamat et al. [59]
Breast	*In vitro/in vivo*	Radiotherapy, cyclophosphamide, cisplatin	Teicher et al. [60]
Breast	*In vitro/in vivo*	Doxorubicin	Thornton et al. [61]
Colon	*In vitro/in vivo*	Irinotecan	Cusack et al. [62]
Lung	*In vitro*	Docetaxel	Gumerlock et al. [63]
Ovarian	*In vitro/in vivo*	Docetaxel	Pink et al. [64]
Pancreatic	*In vitro/in vivo*	Irinotecan	Shah et al. [65]
Pancreatic	*In vivo*	Paclitaxel	Sclabas et al. [66]
Prostate	*In vitro/in vivo*	Doxorubicin, Etoposide, Gemcitabine	Williams et al. [67]

pharmacological inhibitors such as synthetic peptidyl aldehydes. These both act by inhibiting the protosome through the binding of the 20S catalytic core to the simil-chemotrypsin, thus imitating the substrate binding to the active site [52].

Since the proteosome possesses a large number of substrates, the inhibition of its function leads to the alteration of several pathways. There is, therefore, an increase of important cell cycle regulators, for instance of the oncosuppressor p53, which acts as a negative transcription regulator and plays an important part in apoptosis induction following DNA damage, and also of cell cycle inhibitors such as p21 and p27, able to induct cell cycle block in G_1, thus making the cell more susceptible to apoptosis.

Considerable interest has been aroused by the inhibition of NF-κB, whose constitutive expression is frequently associated with phenomena of resistance to traditional forms of anticancer therapy [53]. Proteosome inhibitors, in fact, act indirectly on NF-κB, thus stabilizing Ik-B levels and blocking its translocation to the nucleus. NF-κB is a transcription factor that activates the transcription of a whole series of molecules implicated in proliferation and in angiogenesis.

VEGF, a cytochine with angiogenic activity, is also a transcriptional target of NFkB [54]. It has recently been reported that in multiple myeloma, the non-phosphorylation of caveolin-1, a protein implicated in cell motility, by the VEGF, prevents both tumor cell migration and angiogenesis [55].

Proteosome inhibition has also shown its capacity of increasing intracellular levels of c-Jun-NH2 terminal kinases (JNK), which, in response to cell stress and increased levels of misfolded proteins, promote cell death [56]. However, several of these inhibitors are either non-specific for proteosome or else their intracellular kinetics make them unsuitable for clinical use [57] (Figure 3).

bortezomib

It has been seen that if the aldehyde group of the synthetic peptidyl aldehydes is replaced by boronic acid, the selectivity and affinity of these components towards proteosome increases [57]. This occurs with bortezomib or PS-341 or velcade, a modified dipeptyl-boronic acid that has proved to be capable of triggering an irreversible and highly selective 385 inhibition of proteosome 26 S activity (Figure 4).

preclinical studies. Murine studies have shown the antitumoral efficiency and tolerability of bortezomib in a great variety of tumors, both when used alone and when combined with other forms of therapy (Table 1). This drug, in fact, has proved to be efficient for several tumors that had previously shown resistance to other types of traditional treatment. In a study conducted on 60 different cell lines of NCI, bortezomib induced tumoral cell toxicity, thus bringing about a reduction of 50% in cell growth at low concentrations (7 nM). *In vivo* trials on mice implanted with human tumors have shown that if bortezomib is injected directly into the tumor, in 40% of cases the volume of the tumoral mass is reduced by 70% [68].

The association of bortezomib with several other anticancer agents has shown *in vitro* studies synergic effects. Mice implanted with xeno-colon (LOVO), -pancreas (PANC-1), -prostate (PC-3) and -ovary (SKOV-3), were treated either with bortezomic alone or associated with CPT-11, 5-fluorouracil, paclitaxel, docetaxel or cisplatin. In all cases a greater reduction of the tumor was observed with two combined treatments than with bortezomib therapy alone [68].

clinical studies. Due to its high specificity, bortezomib is the first proteosome inhibitor to be approved by the US Food and Drug Administration (FDA). This occurred in 2003 for the treatment of multiple myeloma (MM), but only in those cases where, in spite of the fact that at least two chemotherapy treatments had been concluded, there was disease progression during the final one (third-line therapy). Since April 2005, treatment with Velcade is also permitted in patients who have undergone only one previous treatment (second-line therapy).

A number of phase I and II trials based on preclinical studies in a variety of solid tumor types are currently in process to determine the maximum tolerated dose (MTD), dose limiting toxicity (DLT), and pharmacodynamic and pharmacokinetic effects of bortezomib. Both in NSCLC and SCLC, bortezomib, either singly or in combination regimens, has proved to cause apoptosis via multiple pathways [69]. In a phase II study, however, bortezomib was well-tolerated but showed limited clinical activity against metastatic breast cancer when used as a single agent [70]. Furthermore, bortezomib as a single agent has minimal activity in soft tissue sarcomas [70].

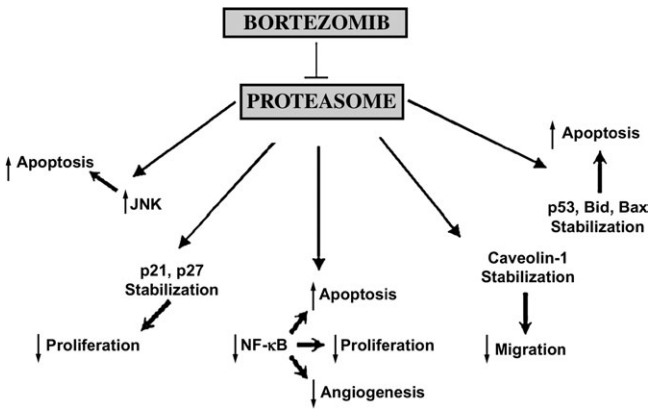

Figure 3. Inhibition of the proteasome by bortezomib results in activations of JNK and stabilization of p53, Bid, Bax, p21, p27, caveolin-1, IkBα. Thus stabilization of Ik-B levels results in inhibition of NF-κB.

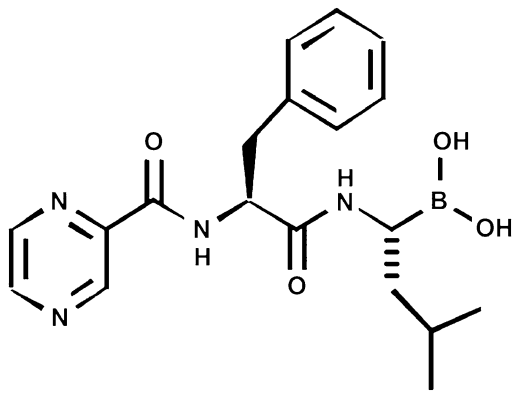

Figure 4. Chemical structure of the proteasome inhibitor bortezomib: pyrazylcarbonyl-Phe-Leu-boronate.

A list of additional clinical studies is given in Table 2. However, further studies of this drug are needed to establish its full spectrum of activity, the ideal regimens for various tumor types, and clinically useful prognostic indicators that predict favorable outcomes.

targeting IAP: survivin

Survivin is highly expressed during embryo development whereas it is more or less absent in a large number of normal differentiated tissues [78]. The only adults tissues where it is found are the thymocytes, the CD34+ bone marrow stem cells and basal colonic epithelium [78–80].

An important feature of survivin is its different expression in tumor cells compared with that of normal differentiated tissues. It is, in fact, overexpressed in a large number of human neoplasias, including those of the lungs, colon, pancreas, bladder, uterus, ovary, breast and liver. Furthermore, it is re-expressed in numerous pre-neoplastic and/or benign lesions, such as colonic polyps and breast adenomas [78]. In tumor cells it has a cytoprotective role by contrasting apoptosis, thus guaranteeing a correct progression by means of mitosis.

In several solid tumors, such as in colorectal and pancreatic cancer [81, 82], the nuclear survivin overexpression was associated with a more favorable prognosis, while its cytoplasmic overexpression proved to be a negative prognostic factor. All these factors have aroused a great deal of interest in the last few years, and have given rise to hope that it might be possible to use this drug as a diagnostic marker and as a target for anticancer therapy.

In order to have an effect on the survivin pathway in tumoral cells, a complete understanding of its mechanism of action in normal cells is required. It is now well-known that survivin induces apoptosis by inhibiting, both directly and indirectly, the activity of caspases 3, 7 and 9 [83] and that a fundamental event for survivin regulation is the phosphorylation of the Threonine 34 residue (Thr34) by the cyclic-dependent p34cdc2 chinase [84].

Several methods have been proposed for the reduction of survivin levels in tumor cells, in order to increase their response to agents inducing apoptosis. One of these proposes the use of survivin-antagonist molecules such as antisense oligonucleotides [85] and small interfering RNA (siRNA) [86] and another is the use of negative dominant mutants [87] and cyclin-dependent chinase inhibitors [88].

preclinical studies

The results obtained from the various *in vivo* and *in vitro* studies have shown that survivin inhibition not only increases the efficiency of traditional chemotherapy drugs, but that it is also able to reduce tumoral angiogenesis [89].

clinical studies

Clinical trials are in progress at present on the use of antisense oligonuceleotides of survivin [90].

synthetic activation of caspases: exisulind

Exisulind (sulindac sulfone, FGN-1, Aptosyn) is a metabolic product of sulindac, a non-steroid, anti-inflammatory drug, belonging to a new group of pro-apoptotic compounds known as selective apoptotic antineoplastic drugs (SAANDS). The pro-apoptotic effects of exisulind differ from the other sulindac derivatives, OSI-461 (formerly CP461) and OSIP486 821 (formerly CP248) in that they do not affect microtubule polymeration [91, 92].

Exisulid is a specific activator of programmed cell death in cancerous and pre-cancerous cells but not of normal cells. It works by means of a pathway which is independent from p53 and Bcl2 [93]. It is able to induce apoptosis through the inhibition of cyclic guanosine monophosphate (cGMP) phosphodiesterases 2 and 5. This inhibition gives rise to an increase in cGMP levels with the resulting activation of PKG (c-GMP-dependent protein kinase G). PKG activation promotes the degradation by the proteosome of β-catenine and the activation of JNK, leading to caspase activation and thus to apoptosis [94].

Table 2. Bortezomib: clinical studies

Solid tumor	Treatment	Phase	Results	Investigator
Advanced solid tumors	Bortezomib and docetaxel	I	MTD: docetaxel 25 mg/m^2 days 1,8 plus bortezomib 0.8 mg/m^2 days 2, 5, 9, and 12 given every 21days	Messersmith [72]
Advanced solid tumors and lymphomas	Bortezomib	I	Well tolerated at doses not exceeding 3.0 mg on day 1 and day 4 every other week	Hamilton [73]
Pediatric refractory advanced solid tumors	Bortezomib	I	Recommended phase II dose: 1.2 mg/m^2/dose twice weekly for 2 weeks followed by a 1-week break	Blaney [74]
Metastatic neuroendocrine tumors	Bortezomib	II	Single-agent bortezomib did not induce any objective responses	Shah [75]
Androgen-independent prostate cancer	Bortezomib	I	Antitumor activity was seen at tolerated doses of bortezomib	Papandreou [76]
Advanced solid tumors	Bortezomib	I	Dose-limiting toxicities on this schedule were diarrhea and sensory neurotoxicity	Aghajanian [78]

preclinical studies

In the murine model it has been demonstrated that exisulind, like sulindac, is able to inhibit the growth of several tumor cells, for example, of the colon, prostate, bladder and breast [95], prostate [96] and lung [97]. Unlike sulindac, however, exisulind does not inhibit Cox-1 and Cox-2 activity.

Pre-clinical *in vitro* studies on prostate and lung tumor cells have reported synergic effects when exisulind is used together with docetaxel or paclitaxel [98], probably because both drugs lead to JNK activation and to the promotion of apoptosis.

clinical studies

Several clinical studies, either already concluded or still in progress, have shown that exisulind, because of its tolerability and activity, could be used for the treatment of solid tumors such as for prostate tumors [99].

A recent phase I study has determined the maximal tolerated dose (MTD) of the combination of weekly docetaxel and exisulind in patients with advanced solid tumors. [100]. However, although preclinical data demonstrate increased apoptosis and prolonged survival for the combination of exisulind and docetaxel, multiple clinical trials do not support further clinical development of this combination regimen in patients with advanced NSCLC [101]. Furthermore, in sporadic colonic adenomas, exisulind causes significant regression of sporadic adenomatous polyps but is associated with toxicity [102].

conclusions

The last decade has seen an extraordinary increase in our understanding of the complexities of apoptosis and the mechanisms evolved by tumor cells to resist engagement of cell death. The activation of alternative pathways by proapoptotic approaches such as death receptors (e.g. TRAIL) or the introduction of exogenous proapoptotic molecules such as apoptin are capable of inducing apoptosis even in a genetically altered context. Although at present there are still many components of the apoptotic pathways that are still not fully understood, the information collected so far has led to a better knowledge of the mechanisms of resistance to standard chemo- and radio-therapy, as well as possible strategies aimed at restoring apoptotic sensitivity. Furthermore, the genetic features of each individual tumor and apoptotic response will make it possible to choose a more suitable therapeutic approach with the aim of overcoming treatment resistance and limiting cytotoxic effects in normal tissues.

Based on the present knowledge, the use of these 'biological drugs' in synergistic association with the traditional cytotoxic drugs, might represent an important goal in the treatment of malignant cells.

references

1. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239–257.
2. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995; 267: 1456–1462.
3. Earnshaw WC, Martins LM, Kaufmann SH. Mammalian caspases: structure, activation, substrates and functions during apoptosis. Ann Rev Biochem 1999; 68: 383–424.
4. Dempsey PW, Doyle SE, He JQ, Cheng G. The signaling adaptors and pathways activated by TNF superfamily. Cytokine Growth Factor Rev 2003; 14: 193–209.
5. Li P, Nijhawan D, Budihardjo I et al. Cytochrome *c* and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997; 91: 479–489.
6. Pitti RM, Marsters SA, Ruppert S et al. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 1996; 271: 12687–12690.
7. Wiley SR, Schooley K, Smolak PJ et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995; 3: 673–682.
8. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 2001; 104: 487–501.
9. Havell EA, Fiers W, North RJ. The antitumor function of tumor necrosis factor (TNF), I. Therapeutic action of TNF against an established murine sarcoma is indirect, immunologically dependent, and limited by severe toxicity. J Exp Med 1988; 167: 1067–1085.
10. Kagawa S, He C, Gu J et al. Antitumor activity and bystander effects of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Gene Cancer Research 2001; 61: 3330–3338.
11. Jo M, Kim TH, Seol DW et al. Apoptosis induced in normal human hepatocytes by tumor necrosis factor-related apoptosis-inducing ligand. Na Med 2000; 6: 564–567.
12. Hao C, Song JH, Hsi B et al. TRAIL Inhibits Tumor Growth but Is Nontoxic to Human Hepatocytes in Chimeric Mice. Cancer Research 2004; 64: 8502–8506.
13. Ashkenazi A. Targeting death and decoy receptors in the tumour-necrosis factor superfamily. Nat Rev Cancer 2002; 2: 420–430.
14. Marsters SA, Sheridan JP, Pitti et al. A novel receptor for Apo2L/TRAIL contains a truncated death domain. Curr Biol 1997; 7: 1003−1006.
15. Mongkolsapaya J, Cowper AE, Xu XN et al. Lymphocyte inhibitor of TRAIL (TNF-related apoptosisinducing ligand): a new receptor protecting lymphocytes from the death ligand TRAIL. J Immunol 1998; 160: 3−6.
16. Sheridan JP, Marsters SA, Pitti R et al. Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors. Science 1997; 277: 818−821.
17. Zhang L, Fang B. Mechanisms of resistance to TRAIL-induced apoptosis in cancer. Cancer Gene Ther 2005; 12: 228–237.
18. Sprick MR, Weigande MA, Rieser E et al. FADD/MORT1 and caspase-8 are recruited to TRAIL receptors1 and 2 and are essential for apoptosis mediated by TRAIL receptor 2. Immunity 2000; 12: 599−609.
19. Eggert A, Grotzer MA, Zuzak TJ. et al. Resistance to TRAIL induced apoptosis in neuroblastoma cells correlates with a loss of caspase-8 expression. Med Pediatr Oncol 2000; 35: 603−607.
20. Fulda S, Meyer E, Debatin KM. Inhibition of TRAIL-induced apoptosis by Bcl-2 overexpression. Oncogene 2002; 21: 2283−2294.
21. Gliniak B, Le T. Tumor necrosis factor-related apoptosis-inducing ligand's antitumor activity in vivo is enhanced by the chemotherapeutic agent CPT-11. Cancer Res 1999; 59: 6153–6158.
22. Wu XX, Ogawa O, Kakehi Y. TRAIL and chemotherapeutic drugs in cancer therapy. Vitam Horm 2004; 67: 365–383.
23. Mirandola P, Sponzilli I, Gobbi G et al. Anticancer agents sensitize osteosarcoma cells to TNF-related apoptosis-inducing ligand downmodulating IAP family proteins. Int J Oncol 2006; 28: 127–133.
24. Odoux C, Albers A. Additive effects of TRAIL and paclitaxel on cancer cells: implications for advances in cancer therapy. Vitam Horm 2004; 67: 385–407.
25. Singh TR, Shankar S, Chen X et al. Synergistic interactions of chemotherapeutic drugs and tumor necrosis factor-related apoptosis-inducing ligand/Apo-2 ligand on apoptosis and on regression of breast carcinoma in vivo. Cancer Res 2003; 63: 5390–5400.
26. Poon IK, Oro C, Dias MM, Zhang JP, Jans DA. A tumor cell-specific nuclear targeting signal within chicken anemia virus VP3/Apoptin. J Virol 2005; 79: 1339–1341.

27. Zhang YH, Kooistra K, Pietersen A, Rohn JL, Noteborn MHM. Activation of the tumor-specific death effector apoptin and its kinase by an N-terminal determinant of simian virus 40 large T antigen. J Virol 2004; 78: 9965–9976.
28. Danen-Van Oorschot AA, Zhang YH, Leliveld SR et al. Importance of nuclear localization of apoptin for tumor-specific induction of apoptosis. J Biol Chem 2003; 278: 27729–27736.
29. Guelen L, Paterson H, Gaken J et al. TAT-apoptin is efficiently delivered and induces apoptosis in cancer cells. Oncogene 2004; 23: 1153–1165.
30. Rohn JL, Zhang Y-H, Leliveld SR et al. Relevance of apoptin's integrity for its functional behavior. J Virol 2005; 79: 1337–1338.
31. Tavassoli M, Guelen L, Luxon BA, Gaken J. Apoptin: specific killer of tumor cells? Apoptosis 2005; 10: 717–724.
32. Leliveld SR, Dame RT, Mommaas MA et al. Apoptin protein multimers form distinct higher-order nucleoprotein complexes with DNA. Nucleic Acids Res 2003; 31: 4805–4813.
33. Zhuang SM, Shvarts A, Jochemsen AG et al. Apoptin, a protein derived from chicken anemia virus, induces p53-independent apoptosis in human osteosarcoma cells. Cancer Res 1995; 55: 486–489.
34. Teodoro JG, Heilman DW, Parker AE, Green MR. The viral protein Apoptin associates with the anaphase-promoting complex to induce G2/M arrest and apoptosis in the absence of p53. Genes. Dev. 2004; 18: 1952–1957.
35. Maddika S., Booy E. P., Johar D., Gibson S. B. et al. Cancer-specific toxicity of apoptin is independent of death receptors but involves the loss of mitochondrial membrane potential and the release of mitochondrial cell-death mediators by a Nur77-dependent pathway. J Cell Sci 2005; 118: 4485–4493.
36. Danen-Van Oorschot AAAM, Den Hollander A, Takayama S et al. BAG-1 inhibits p53- induced but not Apoptin-induced apoptosis. Apoptosis 1997; 2: 395–402.
37. Pietersen AM, Van der Eb MM, Rademaker HJ et al. Specific tumor-cell killing with adenovirus vectors containing the Apoptin gene. Gene Ther 1999; 6: 882–892.
38. Noteborn MH. Apoptin acts as a tumor-specific killer: potentials for an anti-tumor therapy. Cell Mol Biol 2005; 51: 49–60.
39. Bischoff JR, Kirn DH, Williams A et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science 1996; 274: 373–376.
40. Turnell AS, Grand RJ, Gallimore PH. The replicative capacities of large E1B-null group A and group C adenoviruses are independent of host cell p53 status. J Virol 1999; 73: 2074–2083.
41. Ries S, Korn WM. ONYX-015: Mechanisms of action and clinical potential of a replication-selective adenovirus. Br J Cancer 2002; 86: 5–11.
42. Liu XY, Gu JF. Targeting gene-virotherapy of cancer. Cell Res 2006; 16: 25–30.
43. Khuri FR, Nemunaitis J, Ganly I et al. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. Nat Med 2000; 6: 879–885.
44. Kirn D. Oncolytic virotherapy for cancer with the adenovirus dl1520 (ONYX-015): Results of phase I and II trials. Expert Opin Biol Ther 2001; 1: 525–538.
45. Rudin CM, Cohen EE, Papadimitrakopoulou VA et al. An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. J Clin Oncol 2003; 21: 4546–4552.
46. Nemunaitis J, Cunningham C, Tong AW et al. Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients. Cancer Gene Ther 2003; 10: 341–352.
47. Kasuya H, Takeda S, Nomoto S, Nakao A. The potential of oncolytic virus therapy for pancreatic cancer. Cancer Gene Ther 2005; 12: 725–736.
48. Orlowski RZ, Eswara JR, Lafond-Walker A et al. Tumor growth inhibition induced in a murine model of human Burkitt's lymphoma by a proteasome inhibitor. Cancer Res 1998; 58: 4342–4348.
49. Wilkinson KD. Ubiquitin-dependent signaling: the role of ubiquitination in the response of cells to their environment. J Nutr 1999; 129: 1933–1936.
50. Zwickl P, Voges D, Baumeister W. The proteasome: a macromolecular assembly designed for controlled proteolysis. Philos Trans R Soc Lond B Biol Sci 1999; 354: 1501–1511.
51. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 2002; 82: 373–428.
52. Piccinini M, Mostert M, Rinaudo MT. Proteasomes as drug targets. Curr Drug Targets 2003; 4: 657–671.
53. Mayo MW, Baldwin AS. The transcription factor NF-κB: control of oncogenesis and cancer therapy resistance. Biochimica et Biophysica Acta 2000; 1470: M55–62.
54. Bharti AC, Aggarwal BB. Nuclear factor-kappa B and cancer: its role in prevention and therapy. Biochemical Pharmacology 2002; 64: 883–888.
55. Podar K, Shringarpure R, Tai YT et al. Caveolin- 1 is required for vascular endothelial growth factor-triggered multiple myeloma cell migration and is targeted by bortezomib. Cancer Res 2004; 64: 7500–7506.
56. Gabai V.L, Meriin AB, Yaglom JA et al. Role of Hsp70 in regulation of stress-kinase JNK: implications in apoptosis and aging. FEBS Lett 1998; 438: 1–4.
57. Kisselev AF, Goldberg AL. Proteasome inhibitors: from research tools to drug candidates. Chem Biol 2001; 8: 739–758.
58. Adams J, Palombella VJ, Sausville EA et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 1999; 59: 2615–2622.
59. Kamat AM, Karashima T, Davis DW et al. The proteasome inhibitor bortezomib synergizes with gemcitabine to block the growth of human 253JB-V bladder tumors in vivo. Mol Cancer Ther 2004; 3: 279–290.
60. Teicher BA, Ara G, Herbst R et al. The proteasome inhibitor PS-341 in cancer therapy. Clin Cancer Res 1999; 5: 2638–2645.
61. Thornton JD, Liu R, Orlowski RZ et al. Doxorubicin-induced NF-kappaB activation in breast cancer is overcome by proteasome inhibition, resulting in enhanced tumoricidal response to treatment. Presented at the 87th Annual Clinical Congress of the American College of Surgeons, New Orleans, LA, 7–12 October 2001.
62. Cusack JC Jr, Liu R, Houston M et al. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS- 341: implications for systemic nuclear factor-kappaB inhibition. Cancer Res 2001; 61: 3535–3540.
63. Gumerlock PH, Moisan LP, Lau AH et al. Docetaxel followed by PS-341 results in phosphorylation and stabilization of p27 and increases response in non-small cell lung carcinoma (NSCLC). Clin Cancer Res 2001; 7: 157.
64. Pink M, Pien CS, Worland P et al. PS-341 enhances chemotherapeutic effect in human xenograft models. Proc Am Assoc Cancer Res 2002; 43: 158.
65. Shah SA, Potter MW, McDade TP et al. 26S proteasome inhibition induces apoptosis and limits growth of human pancreatic cancer. J Cell Biochem 2001; 82: 110–122.
66. Sclabas GM, Dong QG, Fujioka S et al. Drug-elicited apoptosis in pancreatic tumor cells: the role of different complexes between IjB and NF-jB. Proc. Am Assoc Cancer Res 2002; 43: 882.
67. Williams SA, Papandreou C, McConkey D. Preclinical effects of proteasome inhibitor PS-341 in combination chemotherapy for prostate cancer. In Proceedings of the American Society of Clinical Oncology, 37th Annual Meeting 2001; 20: 169b.
68. Lenz H-J. Clinical update: proteasome inhibitors in solid tumors. Cancer Treat Rev 2003; 29: 41–48.
69. Scagliotti G. Proteasome inhibitors in lung cancer. Crit Rev Oncol Hematol 2006 [Epub ahead of print].
70. Yang CH, Gonzalez-Angulo AM, Reuben JM et al. Bortezomib (VELCADE(R)) in metastatic breast cancer: pharmacodynamics, biological effects, and prediction of clinical benefits. Ann Oncol 2006, Jan 10.
71. Maki RG, Kraft AS, Scheu K et al. A multicenter Phase II study of bortezomib in recurrent or metastatic sarcomas. Cancer 2005; 103: 1431–1438.
72. Messersmith WA, Baker SD, Lassiter L et al. Phase I trial of bortezomib in combination with docetaxel in patients with advanced solid tumors. Clin Cancer Res 2006; 12: 1270–1275.
73. Hamilton AL, Eder JP, Pavlick AC et al. Proteasome inhibition with bortezomib (PS-341): a phase I study with pharmacodynamic end points using a day 1 and day 4 schedule in a 14-day cycle. J Clin Oncol 2005; 23: 6107–6116.
74. Blaney SM, Bernstein M, Neville K et al. Phase I study of the proteasome inhibitor bortezomib in pediatric patients with refractory solid tumors: a Children's Oncology Group study (ADVL0015). J Clin Oncol 2004; 22: 4804–4809.

75. Shah MH, Young D, Kindler HL et al. Phase II study of the proteasome inhibitor bortezomib (PS-341) in patients with metastatic neuroendocrine tumors. J Clin Oncol 2005; 23: 6107–6116.
76. Papandreou CN, Daliani DD, Nix D et al. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. J Clin Oncol 2004; 22: 2108–2121.
77. Aghajanian C, Soignet S, Dizon DS et al. A phase I trial of the novel proteasome inhibitor PS341 in advanced solid tumor malignancies. Clin Cancer Res 2002; 8: 2505–2511.
78. Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med 1997; 3: 917–921.
79. Carter BZ, Milella M, Altieri DC, Andreeff M. Cytokine- regulated expression of survivin in myeloid leukemia. Blood 2001; 97: 2784–2790.
80. Gianani R, Jarboe E, Orlicky D et al. Expression of survivin in normal, hyperplastic, and neoplastic colonic mucosa. Hum Pathos 2001; 32: 119–125.
81. Ponnelle T, Chapusot C, Martin L et al. Cellular localisation of survivin: impact on the prognosis in colorectal cancer J Cancer Res Clin Oncol 2005; 131: 504–510.
82. Tonini G, Vincenzi B, Santini D et al. Nuclear and cytoplasmic expression of survivin in 67 surgically resected pancreatic cancer patients.Br J Cancer 2005; 92: 2225–2232. [Erratum in: Br J Cancer 2005; 93: 1084.]
83. Deveraux QL, Reed JC. IAP family proteins-suppressors of apoptosis. Gen Develop 1999; 13: 239–252.
84. O'Connor DS, Wall NR, Porter ACG, Altieri DC. A p34cdc2 survival checkpoint in cancer. Cancer Cell 2002; 2: 43–54.
85. Olie RA, Simõ{}es-Wüst AP, Baumann B et al. A novel antisense oligonucleotide targeting survivin expression induces apoptosis and sensitizes lung cancer cells to chemotherapy. Cancer Res 2000; 60: 2805–2809.
86. Kappler M, Bache M, Bartel F et al. Knockdown of survivin expression by small interfering RNA reduces the clonogenic survival of human sarcoma cell lines independently of p53. Cancer Gene Ther 2004; 11: 186–193.
87. Kanwar JR, Shen W-P, Kanwar RK, Berg RW et al. Effects of survivin antagonists on growth of established tumors and B7-1 immunogene therapy. J Nat Cancer Inst 2001; 93: 1541–1552.
88. Sausville EA. Complexities in the development of cyclin-dependent kinase inhibitor drugs. Trends Mol Med 2002; 8: S32–S37.
89. Nicholson DW. From bench to clinic with apoptosisbased therapeutic agents. Nature 2000; 407: 810–816.
90. Zaffaroni N, Pennati M, Daidone MG. Survivin as a target for new anticancer interventions J Cell Mol Med 2005; 9: 360–372.
91. Yoon JT, Palazzo AF, Xiao D et al. CP248, a derivative of exisulind, causes growth inhibition, mitotic arrest, and abnormalities in microtubule polymerization in glioma cells. Mol Cancer Ther 2002; 1: 393– 404.
92. Moon EY, Lerner A. Benzylamide sulindac analogues induce changes in cell shape, loss of microtubules and G2-M arrest in a chronic lymphocytic leukemia (CLL) cell line and apoptosis in primary CLL cells. Cancer Res 2002; 62: 5711–5719.
93. Piazza GA, Rahm AK, Finn TS et al. Apoptosis primarily accounts for the growth inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxigenase inhibition, cell cycle arrestand p53 induction. Cancer Res 1997; 57: 2452–2459.
94. Soh JW, Mao Y, Kim MG et al. Cyclic GMP mediates apoptosis induced by sulindac derivatives via activation of c-Jun NH2-terminal kinase 1. Clin Cancer Res 2000; 6: 4136–4141.
95. Thompson HJ, Jiang C, Lu J et al. Sulfone metabolite of sulindac inhibits mammary carcinogenesis. Cancer Res 1997; 57: 167–171.
96. Goluboff ET, Shabsigh A, Saidi JA et al. Exisulind (sulindac sulfone) suppresses growth of human prostate cancer in a nude mouse xenograft model by increasing apoptosis. Urology 1999; 53: 440–445.
97. Malkinson AM, Koski KM, Dwyer-Nield LD et al. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced mouse lung tumour formation by FGN-1 (sulindac sulfone). Carcinogenesis (Lond) 1998; 19: 1353–1356.
98. Soriano AF, Helfrich B, Chan DC et al. Synergistic effects of new chemopreventative and conventional cytotoxic agents against humanlung cancer cell lines. Cancer Res 1999; 59: 6178–6184.
99. Goluboff ET, Prager D, Rukstalis D et al. UCLA Oncology Research Network. Safety and efficacy of exisulind for treatment of recurrent prostate cancer after radical prostatectomy. J Urol 2001; 166: 882–886.
100. Garcia AA, Iqbal S, Quinn D et al. Phase I clinical trial of weekly docetaxel and exisulind, a novel inducer of apoptosis. Invest. New Drugs 2006; 24: 79–83.
101. Jones SF, Kuhn JG, Greco FA et al. A phase I/II study of exisulind in combination with docetaxel/carboplatin in patients with metastatic non-small-cell lung cancer. Clin Lung Cancer 2005; 6: 361–366.
102. Arber N, Kuwada S, Leshno M et al. Exisulind Study Group Sporadic adenomatous polyp regression with exisulind is effective but toxic: a randomised, double blind, placebo controlled, dose-response study. Gut 2006; 55: 367–373.