Available online at www.sciencedirect.com

ScienceDirect

Biochimica et Biophysica Acta 1783 (2008) 1436–1448

Biochimica et Biophysica Acta BBA

www.elsevier.com/locate/bbamcr

Review

Drug-induced apoptosis in yeast

B. Almeida, A. Silva, A. Mesquita, B. Sampaio-Marques, F. Rodrigues, P. Ludovico *

Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Received 31 October 2007; received in revised form 21 December 2007; accepted 7 January 2008
Available online 17 January 2008

Abstract

In order to alter the impact of diseases on human society, drug development has been one of the most invested research fields. Nowadays, cancer and infectious diseases are leading targets for the design of effective drugs, in which the primary mechanism of action relies on the modulation of programmed cell death (PCD). Due to the high degree of conservation of basic cellular processes between yeast and higher eukaryotes, and to the existence of an ancestral PCD machinery in yeast, yeasts are an attractive tool for the study of affected pathways that give insights into the mode of action of both antitumour and antifungal drugs. Therefore, we covered some of the leading reports on drug-induced apoptosis in yeast, revealing that in common with mammalian cells, antitumour drugs induce apoptosis through reactive oxygen species (ROS) generation and altered mitochondrial functions. The evidence presented suggests that yeasts may be a powerful model for the screening/development of PCD-directed drugs, overcoming the problem of cellular specificity in the design of antitumour drugs, but also enabling the design of efficient antifungal drugs, targeted to fungal-specific apoptotic regulators that do not have major consequences for human cells.

Keywords: Antifungal drug; Antitumour drug; Drug targets; Mitochondria; Reactive oxygen species; Yeast apoptosis

1. Introduction

Throughout the history of mankind, the quest for drugs with direct or indirect impact on our well-being and longevity has been at the cutting edge of human cultural and scientific development. Medicinal consumption has increased to a new level in recent decades, fuelling a constant exploration for new agents that might cure a variety of illnesses, or at least improve life quality. Infectious diseases and cancers, due to their high mortality/morbidity rates and impact on human society, have more recently surfaced as leading target diseases for the design of effective drugs. Interestingly, most of the antitumour drugs used nowadays were first selected as antimicrobial agents; however, after the recognition of their antitumour value, their characterization substantially increased over the following years. Additionally, up to date scientific research has pointed out that the mechanism by which most, if not all, of the antitumour drugs kill tumour cells involves the induction of cell death by apoptosis [1]. In fact, the increase in knowledge on programmed cell death (PCD) itself, particularly apoptosis, as well as its deregulation in tumour cells has dramatically changed the point of view on the pharmacology of antitumour drugs. Consequently, great interest has emerged in developing new strategies that involve the modulation of key molecules that control life and death decisions, thereby offering an exciting multitude of molecular targets and therapeutic options for the future [2,3].

The budding yeast *Saccharomyces cerevisiae* has been successfully used as a model organism for the study of molecular and cellular pathways underlying mammalian diseases. This is in part due to the high degree of conservation of basic cellular processes between yeast and higher organisms, as well as the advantages of yeast genetics [4]. Studies in yeast were the first to reveal the cellular target of rapamycin, an immunosuppressant drug broadly used in human tissue transplants [5,6]. In the last decade compelling evidence accumulated showed that yeasts are valuable for PCD research due to their ability to undergo PCD responses which display a certain degree of conservation with apoptotic mechanisms of higher eukaryotes [7–9]. Specifically, an apoptosis-inducing factor (*AIF1*), cytochrome *c* (cyt *c*) and HtrA/Omi, that play an important role in

* Corresponding author. Tel.: +351 253604812; fax: +351 253604809.
E-mail address: pludovico@ecsaude.uminho.pt (P. Ludovico).

0167-4889/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2008.01.005

the intrinsic pathway of yeast and mammalian systems have been identified [8,10–13]. In contrast, no components of the extrinsic apoptotic regulatory pathway (e.g., death receptors and their ligands) have been described, showing that yeast cells do not completely recapitulate the mammalian apoptotic system [9]. Molecules involved in mammalian PCD but whose counterparts are not known in yeast cells such as Bcl-2 proteins or p53, have been expressed in yeast and studied in more detail in a genetically tractable system [14–18]. A throughout characterization of the yeast conserved and non-conserved PCD regulators and processes may open up new avenues for the evaluation of drug targets and modes of action. DNA damage [19] and defects in DNA replication and cell cycle checkpoints identified in *S. cerevisiae* [20] have been shown to induce cell death resembling apoptosis in metazoans, suggesting that future studies in yeast may provide further valuable input regarding the complex molecular pathways underlying these events or the effects of some antitumour drugs directed against those targets. On the other hand, differences in the architecture of yeast PCD may allow the targeting of non-conserved genes or gene products as novel and specific antifungal drug targets to combat the increasing number of fungal infections seen in immunocompromised individuals (see related article in this issue).

In this article, we aim to review the evidence on drug-induced apoptosis in yeast, stressing the overlapping and distinct elements involved with PCD. To address these issues we have restricted our review to the most commonly tested drugs in yeast cells, which are predominantly antitumour and antifungal drugs.

2. Antitumour drugs

Genetic changes in human tumour cells often include alterations in the control of cell cycle and/or the regulation of the cell death process [21]. Therefore, it is not surprising that most antitumour drugs have, directly or indirectly, apoptotic regulators as targets. According to their mode of action, such drugs may be grouped in several different classes, among which the most relevant promote DNA fragmentation and DNA intercalation, or are microtubule-directed, histone deacetylase (HDAC) inhibitors, phosphatidylcholine (PC) analogues/inhibitors, topoisomerase inhibitors or antimetabolites. Various

Table 1
Overview of the antitumour drugs known to induce apoptosis in yeast and their associated apoptotic phenotypes

Apoptosis-inducing antitumour drugs in yeast				
Antitumour drugs	Apoptotic phenotype	Yeast species	References	References of apoptosis in mammals
Microtubule-directed				
Paclitaxel	ROS accumulation DNA fragmentation Sub-G0/G1 population Arrest in G2/M	*Saccharomyces cerevisiae* AD1-8-tax	[32]	[25–28]
Miscellaneous				
Arsenic	DNA fragmentation Phosphatidylserine exposure Mitochondrial membrane permeabilization ROS accumulation Dependent of metacaspase Dependent of Tim18p	*Saccharomyces cerevisiae* BY4742	[39,40]	[36–38,41]
DNA fragmenting				
Bleomycin	DNA fragmentation Chromatin condensation Sub-G0/G1 population Independent of mitochondrial function at high concentrations	*Saccharomyces cerevisiae* YHP-1	[50]	[48,49]
Histone deacetylase (HDAC) inhibitors				
Valproate	Dependent of metacaspase DNA fragmentation Phosphatidylserine exposure ROS accumulation Dependent of Sir2	*Saccharomyces cerevisiae* W303-1A	[57,58]	[52,53,56]
DNA intercalating				
Doxorubicin	Mitochondrial dysfunction Morphological alterations	*Candida utilis* ATCC 8205	[64]	[62,63]
Phosphatidylcholine (PC) analogues				
Edelfosine	DNA fragmentation Mitochondria-derived ROS accumulation	*Saccharomyces cerevisiae* BY4742	[73]	[65]

Drugs were divided in classes according to their mode of action. Yeast species/strains used in the different studies are listed.

antitumour drugs have been tested in yeast to ascertain their mechanism of action and, representative agents of each of these classes have been shown to induce an apoptotic phenotype (Table 1). However, some antitumour drugs studied in yeast have not been assigned as inducers of PCD, e.g. farnesol, fredericamycin A, camptothecin, etoposide, 5-fluorouracil, selenium, coumarin, and 1,10-phenanthroline. This may be for many reasons, including lack of experiments that directly characterize cell death. Given that, the cytotoxic phenotype and the molecular context of their action are in many cases related to the triggering of a PCD process (Table 2), they will also be covered in this review.

2.1. Apoptosis-inducing antitumour drugs in yeast

Paclitaxel, arsenic, bleomycin and valproate (VPA) represent the most well studied antitumour drugs inducing yeast apoptotic phenotypes (Table 1; Fig. 1). Paclitaxel is a complex diterpene that was initially isolated from the bark of the Pacific yew tree *Taxus brevifolia* [22] and has subsequently been shown to be a fungal metabolite [23]. In mammalian cells, paclitaxel has been shown to bind to β-tubulin, disturbing the equilibrium between the soluble and polymeric forms of tubulin [24], leading to cell cycle arrest at G2/M phases and induction of apoptosis in proliferating cells [25,26]. Paclitaxel has been shown to induce phosphorylation of the anti-apoptotic protein Bcl-2 [27] and, at least *in vitro*, FAS-associated death domain protein (FADD)-dependent apoptosis through activation of caspase-10 [28]. Growth of wild-type yeast cells is not inhibited by paclitaxel, due, most probably, to the differences between yeast and mammalian tubulin residues involved in paclitaxel binding [29–31]. However, mutations in the yeast β-tubulin promote the accumulation of intracellular reactive oxygen species (ROS), DNA fragmentation (detected by terminal dUTP nick-end labeling (TUNEL) assay), and alterations of the cell cycle profile (Fig. 1) characterized by an arrest in the G2/M phases and the appearance of a sub-G0/G1 population [32], consistently with a mitotic blockage as described in mammalian cells [33,34]. Studies with paclitaxel also exemplify how the problem of drug extrusion by yeast (seen as a main constraint in yeast use for antitumour drug target characterization) can be overcome by the modulation of multidrug ABC transporters, thereby facilitating the accumulation of the drug in yeast cells [32].

Arsenic, a highly toxic metalloid, has been used in a variety of ways over the past 200 years not least as an extremely potent anti-leukemic agent [35]. Cytotoxicity studies have shown that chronic arsenic exposure induces profound cellular alterations including apoptosis characterized by ROS accumulation, mitochondrial aggregation, Bax oligomerization, mitochondrial membrane potential ($\Delta\psi m$) dissipation and caspase activation

Table 2
Overview of the antitumour drugs inducing cytotoxicity in yeast cells

Cytotoxicity of antitumour drugs in yeast				
Antitumour drugs	Phenotype	Yeast species	References	References of apoptosis in mammals
Phosphatidylcholine (PC) inhibitors				
Farnesol	Growth arrest ROS accumulation Repression of cell cycle genes (*CDC9*; *HAT2*)	*Saccharomyces cerevisiae* X2180-1A	[78,79]	[74,162]
Topoisomerases inhibitors				
Fredericamycin A	Arrest in G1 ROS accumulation Aberrant mitochondria	*Saccharomyces cerevisiae* W303-1A	[87]	[82]
Camptothecin	Arrest in G2/M DNA damage	*Saccharomyces cerevisiae* FY250/FY251	[88]	[86]
Etoposide	Arrest in G2/M	*Saccharomyces cerevisiae* JN362acc	[89]	[84]
Antimetabolites				
5-Fluorouracil	Inhibition of growth Arrest in G1/S	*Saccharomyces cerevisiae* BY4741/BY4742 *Candida albicans* Clinical isolate	[92,96,97]	[163]
Miscellaneous				
Selenium	Toxicity exacerbated by glutathione Toxicity exacerbated by thiols Formation of Hydrogen Selenide	*Saccharomyces cerevisiae* DTY7	[93]	[164]
Coumarin	ROS accumulation Nuclear dysfunctions Loss of membrane organelles	*Candida albicans* ATCC 10231	[94]	[98]
1,10-Phenanthroline	DNA degradation Nuclear dysfunctions Mitochondrial function disruption	*Candida albicans* ATCC 10231	[95]	[95,99]

Drugs were divided in classes according to their mode of action. Yeast species/strains used in the different studies are listed.

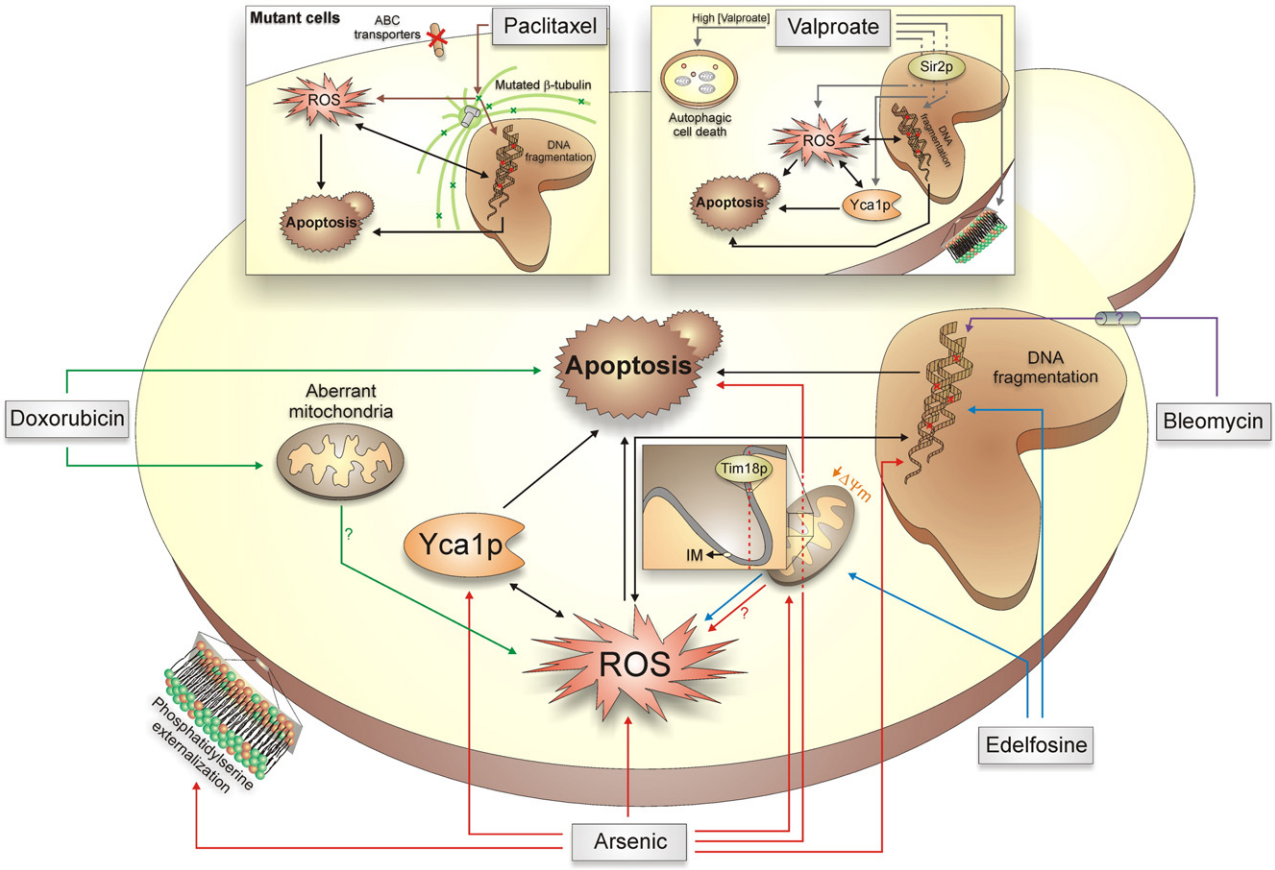

Fig. 1. Schematic representation of yeast apoptotic phenotypes and events induced by antitumour drugs. ROS appears to possess, like in mammalian cells, a central role in the induction/signalling of yeast apoptotic process, with paclitaxel, valproate, arsenic and edelfosine leading to their generation/accumulation. ROS production during edelfosine-induced apoptosis has been demonstrated to be mitochondria-dependent. The crucial involvement of mitochondria on antitumour drug-induced apoptosis is clearly reflected during arsenic-induced apoptosis, with mitochondria suffering a decrease in membrane potential ($\Delta\psi m$) and with the requirement of Tim18p, a mitochondrial translocase located in mitochondrial inner membrane (IM), to act downstream ROS. In addition, mitochondria were described as the targets of doxorubicin. Yeast metacaspase, Yca1p, was also necessary for the execution of apoptosis induced by both arsenic and valproate. Of note, valproate displays a dual effect on yeast cells, with high concentrations inducing cell death with characteristics similar to those of ACD and low concentrations inducing Sir2p-dependent apoptosis. All antitumour drugs seem to induce yeast DNA fragmentation with the exception of doxorubicin where this apoptotic feature was not assessed. The apoptotic events triggered by antitumour drugs are represented by arrows displaying a specific colour for each drug: paclitaxel (brown), valproate (gray), bleomycin (purple), edelfosine (blue), arsenic (red) and doxorubicin (green). Black arrows indicate already known yeast apoptotic events.

[36]. Furthermore, arsenic may directly induce cyt *c* release from isolated liver mitochondria via the mitochondrial permeability transition pore [37,38]. In contrast to paclitaxel, arsenic can exert its toxic effects and trigger apoptosis in wild-type *S. cerevisiae* cells. Recently, arsenic was shown to induce DNA fragmentation, phosphatidylserine exposure, mitochondrial membrane permeabilization and ROS accumulation in *S. cerevisiae* cells [39,40] (Fig. 1). The arsenic resistant phenotype of *rho0* mutant cells, as well as the decrease of DNA fragmentation and cell death in metacaspase (Yca1p) mutant cells supports the involvement of both mitochondria and Yca1p in the cell death process [39]. In addition, Tim18p, a component of the mitochondrial translocator, was implicated as a mediator of arsenic-induced yeast apoptosis, acting downstream of ROS production (Fig. 1) [40]. The deletion of CuZn superoxide dismutase (SOD) also enhances arsenic's toxic effects, further indicating that ROS play an important role in this process [39], in accordance with recent findings by Seok et al. in a zebrafish liver cell line [41]. Arsenic reacts with sulphur containing compounds, such as glutathione (GSH) or cysteine acting as a potent inhibitor of GSH reductase and thioredoxin reductase [42], thereby increasing cellular oxidation levels.

Bleomycin, a compound isolated from *Streptomyces verticillis* [43,44] is primarily used as an antibiotic and is also employed clinically in cancer therapy due to its ability to induce single-strand and double-strand DNA breaks [45,46]. Although not yet characterized, a receptor protein mediating bleomycin internalization has been suggested to exist on the plasma membrane of both mammalian and yeast cells [47]. Upon entering a cell, bleomycin induces cell death through a JNK-dependent mitochondrial death pathway in alveolar epithelial cells [48], and causes apoptosis in lung epithelial cells by increasing ROS generation/accumulation and mitochondrial leakage, which require the participation of caspase-8 and -9, and the Fas/FasL pathway [49]. Bleomycin-induced apoptotic cell death in yeast (Fig. 1) is mainly characterized by the appearance of a sub-G0/G1 population, the generation of DNA double-strand breaks, and, at high bleomycin concentrations,

the induction of a mitochondria-independent cell death process [50].

Similar to other antitumour drugs, VPA, an inhibitor of the class I HDACs [51] can trigger apoptosis in mammalian cells through caspase-dependent and -independent pathways [52,53]. VPA also promotes the down-regulation of pro-survival genes, Bcl-2 and Bcl-XL, and the up-regulation of pro-apoptotic genes such as Bax [54,55]. A recent study also demonstrates that VPA induces caspase-dependent apoptosis in HeLa cells through the blocking of the Akt pathway [56]. Likewise, in yeast cells, VPA triggers a cell death process that is dependent on Yca1p [57] (Fig. 1). Exposure to high concentrations of VPA induces cell death with morphological features similar to those of autophagic cell death (ACD), which is independent of Yca1p [57], while low VPA concentrations result in apoptotic cell death associated with DNA fragmentation, ROS accumulation, phosphatidylserine exposure and morphological alterations such as cell shrinkage [57,58]. Sun et al. showed that Sir2p or sirtuin, a class III HDAC, that is also involved in the DNA damage response and life span extension mediated by caloric restriction [59,60], is required for VPA-induced cell death [58]. Accordingly, Δ*sir2* cells do not produce ROS or accumulate neutral lipids, leading to the conclusion that Sir2p has a role in lipid metabolism, which might be linked to apoptosis [58].

Other antitumour drugs presented in Table 1 and described as inducing apoptosis in yeast cells include doxorubicin (DOX) and edelfosine. DOX is an antibiotic, originally isolated from *Streptomyces peucetius* and currently used as an effective antitumour drug [61] known to induce, among other events, the generation of free radicals, DNA damage and apoptosis, via an inhibition of topoisomerase II [62,63]. In yeast, DOX was shown to induce apoptosis in *Candida utilis*, based merely upon morphological observations, with reported plasma membrane alterations and changes in mitochondrial shape and cristae organization [64] (Fig. 1). Therefore, further studies directed to known yeast apoptotic regulators are needed in order to uncover the mechanism by which DOX kills yeast cells.

Edelfosine is a synthetic lipid, analogue of phosphatidylcholine (PC), which induces apoptosis in a wide variety of tumour cells [65]. Edelfosine and its analogues contain ether linked fatty acids, as opposed to the endogenous ester linked fatty acids, rendering them more resistant to cellular phospholipases and, thus, more effective as drugs. Although not as an amplificatory mechanism, like bleomycin [49], edelfosine was found to induce Fas-dependent apoptosis in leukemic cells [66]. Overexpression of Bcl-2 or Bcl-XL was shown to be able to inhibit apoptosis induced by this compound [65,67], which was also shown to be associated with alterations in mitochondrial function, generation of ROS and caspase-3 activation [68,69]. Recently it was suggested that endoplasmic reticulum may also play a major role in edelfosine-induced apoptosis in tumour cells [70]. In addition to its cytotoxic effects [71,72], edelfosine was reported to promote apoptosis in *S. cerevisiae* cells characterized by a TUNEL-positive phenotype and mitochondrial dependent ROS generation [73], presenting similarities with edelfosine-induced apoptosis in human tumour cells, also mediated by mitochondria and correlated with ROS generation [68,69].

The accumulated evidence indicates that the mechanisms of antitumour drug-induced apoptosis in yeast share some homologies with the mammalian system. Particularly predominant are the involvement of mitochondria, DNA fragmentation, and especially ROS production/accumulation. Nevertheless, not all the studies regarding the induction of apoptosis in yeast cells by antitumour drugs explore the knowledge of yeast molecular PCD pathway(s), namely, the precise association of the apoptotic regulators and their hierarchy. Even so, the data herein presented point out the potential value of yeast to study PCD-based therapies and drug targets.

2.2. Cytotoxicity of antitumour drugs in yeast

As a model organism, yeast has long been used as a pharmacological tool in the identification and definition of the molecular context and of critical determinants that confer chemosensitivity to specific cytotoxic injuries induced by drugs. Several of the different antitumour drugs studied in yeast have not been specifically assigned as inducers of PCD, although, the cytotoxic phenotype and the molecular context of their action are suggestive of that. Drugs such as the PC inhibitor farnesol and some topoisomerases inhibitors are worth of further discussion (Table 2). Farnesol is known to induce apoptosis in a wide variety of cell lines [74,75]. Farnesol-induced cell death is attenuated through the addition of exogenous PC or diacylglycerol, but not other lipids [76,77]. In yeast cells, farnesol has been shown to induce growth arrest and cell death, with repression of cell cycle genes encoding a DNA ligase (*CDC9*) and a histone acetyltransferase (*HAT2*), a process that can be inhibited by the addition of a diacylglycerol analogue [78]. Although farnesol induces the generation of ROS [79,80], the farnesol-induced cell death mechanism remains uncharacterized in yeast cells. Nevertheless, farnesol has been described to induce apoptosis in *Aspergillus nidulans* cells characterized by chromatin condensation, a TUNEL-positive phenotype, exposure of phosphatidylserine, and is also dependent on mitochondrial function and ROS generation [81].

Other successful antitumour drugs take advantage of the inhibition of topoisomerases, key enzymes in DNA transcription and replication. Fredericamycin A (FMA), an antibiotic product of *Streptomyces griseus*, camptothecin, an alkaloid derived from the plant *Camptotheca acuminate* and etoposide, a derivative of the podophyllotoxin from *Podophyllum peltatum* are among the antitumour drugs known to induce apoptosis in mammalian cells due to the inhibition of topoisomerases [82–86]. Although the induction of apoptosis in yeast cells by those drugs is not supported by the available data, some lines of evidence do support a link. For example, FMA was shown to induce growth arrest (G1 cell cycle phase) and the appearance of aberrant mitochondria in yeast, just as in mammalian cells, which also results in the generation of high intracellular ROS levels [87]. Furthermore, and even though evidence for apoptosis induced by etoposide and camptothecin in yeast cells is scarce, these topoisomerase inhibitors are known to induce arrest in the G2/M cell cycle phases and DNA damage [88,89], a phenomenon also observed in other drugs inducing apoptosis in yeast [90,91].

Other antitumour drugs, including 5-fluorouracil, selenium, coumarin and 1,10-phenanthroline, have been described as cytotoxic agents that lead to yeast cell death [92–97]. However, the mechanism of the cell death process underlying their cytotoxicity remains unexplored. Nonetheless, treatment with coumarin and 1,10-phenanthroline stimulates ROS generation, changes in nuclear morphology and a loss of membrane organelles [94,95], indicating that apoptotic cell death might take place in yeast as demonstrated in mammalian cells [98,99].

Future studies directed towards the identification of the true nature of the cell death processes that occur upon treatment with these drugs will bring forth important data regarding drug-induced apoptotic phenotype in yeast strengthening its claim as a useful tool for screening the cytotoxic effects of antitumour drugs.

3. Apoptosis-inducing antifungal drugs in yeast

S. cerevisiae represents a practical and conventional system for studying the properties of antifungal compounds, not only against fungal human pathogens with which they are closely related (e.g., *Candida albicans*) [100], but also with those that are evolutionarily more distant (e.g., filamentous fungi). Moreover, the majority of the currently used antifungal drugs are active against *S. cerevisiae* (reviewed in [101]), thus making it a suitable model for both drug development and the elucidation of the mechanisms underlying drug's action. Most antifungal drugs belong to a few structural classes that affect specific fungal cellular targets, such as ergosterol synthesis. However, many of these drugs are associated with a high human toxicity (e.g. amphotericin B) and/or to the selection of resistant fungal pathogens (e.g., azole drugs), two main constraints on the success of antifungal drug therapies. To overcome the changing tide of fungal diseases, novel fungal targets for drug therapy need to be identified. As described above, the possible architectural differences between apoptotic regulators/mechanisms of yeast and mammalian cells may open the door either for the design of new antifungal drugs, or for testing the fungal-specific apoptotic-inducing abilities of the current ones. In fact, some drugs with known antifungal capacities have already been demonstrated to act as yeast-specific apoptotic PCD-inducers (Table 3). Among these, some are currently used in clinics while others are still under development. One of the best characterized and commercially available antifungal drugs is amphotericin B (AmB), a polyene agent efficiently used for treating invasive fungal infections, but generally associated with high toxicity against human cells [102]. AmB binds to sterols, creating pores that increase fungal membrane permeability to small cations, thus promoting the rapid depletion of intracellular potassium and fungal cell death [103]. Phillips et al. assessed the toxic effects of AmB in *C. albicans*, revealing that AmB induces an apoptotic mechanism, with the occurrence of arrest in G2/M cell cycle phases, chromatin condensation, nuclear fragmentation, phosphatidylserine externalization and ROS accumulation [91].

Ciclopirox olamine (CPO), a representative of a quite distinct class of antifungal drugs, was introduced into clinical therapy more than three decades ago. CPO belongs to a group of synthetic antifungal agents, hydroxypyridones that have high affinity for trivalent metal cations [104], that are used effectively in clinical practice since they have a broad spectrum of action against dermatophytes, yeasts, filamentous fungi and bacteria [105]. A remarkable feature of CPO is that no single case of fungal resistance has been reported so far. Work performed by our group has shown that CPO leads to non-apoptotic yeast PCD characterized by chromatin condensation and DNA damage associated with the appearance of a sub-G0/G1 population and arrest in G2/M cell cycle phases [90]. Notably, in contrast to AmB-induced apoptosis, CPO-induced PCD does not involve ROS signalling and is associated with a TUNEL-negative phenotype; CPO-induced PCD also appears to be independent of metacaspase but is associated with unknown protease activities [90].

Besides the described antifungal drugs, other compounds isolated from distinct organisms have proved to display effective antifungal capacities through the induction of apoptosis. Osmotin, a *Tobacco* pathogenesis-related protein, dermaseptins, a family of peptides derived from the tree-frog *Phyllomedusa sauvagii*, and pradimicin (PRM), an *Actinomadura hibisca*-derived antibiotic, were found to induce cell death in yeast with apoptotic features [106–109]. All of these drugs were shown to induce nuclear fragmentation, a TUNEL-positive phenotype and the generation of high intracellular ROS levels [106–109]. However, some differences were detected among the apoptotic cell death processes triggered by these drugs. The mechanism by which osmotin induces apoptosis, relies on the suppression of stress-responsive gene transcription via the RAS2/cAMP pathway, and, upstream from *RAS2*, on the binding of osmotin to the plasma membrane protein Pho36, a homologue of the mammalian receptor for the hormone adiponectin [106,110]. On the other hand, the truncated derivative of dermaseptin S3 [111], which promotes disruption of the yeast cell membrane and a deregulation in the homeostasis of intracellular pH, was shown to induce *S. cerevisiae* PCD associated with ROS generation and nuclear DNA fragmentation [107,108]. Interestingly, the mode of dermaseptin-induced cell death is metacaspase-independent, but dependent on Aif1p, and on the proteasomal substrate, Stm1p [108], which is also involved in yeast apoptosis [112]. In addition to their capacity to induce yeast apoptosis, dermaseptins have very low human cytotoxicity. In fact, the same is true for most of the naturally occurring antimicrobial peptides from amphibian skin, at concentrations that effectively inhibit fungal growth [111,113–115], making them very attractive antifungal drugs. PRM, a mannose-binding antifungal antibiotic that causes membrane permeability dysfunction, is also capable of inducing *S. cerevisiae* apoptotic cell death, characterized by ROS accumulation, DNA damage and nuclear fragmentation [109]. The cell death mechanism seems to be dependent on the sensor kinase, Sln1p, to which PRM can bind [116].

Another group of compounds that display antifungal capacities are histatins, histidine-rich cationic peptides secreted by the parotid and the submandibular/sublingual human salivary glands [117]. Histatin 5 has been shown to display potent fungicidal properties against *C. albicans* [117]. Although scarce, evidence for the induction of a histatin 5-mediated apoptotic

Table 3
Overview of the antifungal drugs known to induce apoptosis in yeast and their associated apoptotic phenotypes

Apoptosis-inducing antifungal drugs in yeast				
Antifungal drugs	Apoptotic phenotype	Yeast species	References	References of apoptosis in mammals
Cell permeability disruptor				
Amphotericin B	Arrest in G2/M Chromatin condensation Nuclear fragmentation Phosphatidylserine externalization ROS accumulation	*Candida albicans* CaF-2	[91]	[102]
Metal cation chelator				
Ciclopirox olamine	Chromatin condensation Sub-G0/G1 population Arrest in G2/M Nuclear dysfunction Independent of ROS accumulation Independent of metacaspase Associated with unknown protease(s)	*Saccharomyces cerevisiae* BY4742	[90]	–
Plasma membrane binders/disruptors				
Osmotin	DNA fragmentation ROS accumulation Dependent on the suppression of transcription of stress-responsive genes via RAS2/cAMP pathway Dependent on the binding to Pho36p	*Saccharomyces cerevisiae* BWG7a	[106,110]	–
Dermaseptin	DNA fragmentation ROS accumulation Metacaspase independent Dependent of the apoptosis-inducing factor (Aif1p) and the proteosomal substrate (Stm1p)	*Candida albicans* IP886-65 *Saccharomyces cerevisiae* BY4742	[107,108,111]	–
Pradimicin	DNA fragmentation ROS accumulation Dependent of the sensor kinase, Sln1p	*Saccharomyces cerevisiae* 953	[109,116]	[165,166]
Mitochondrial membrane disruptor				
Histatin	Mitochondrial membrane depolarization Mitochondrial swelling Loss of intracellular ATP and amino acids Arrest in G1 ROS accumulation	*Candida albicans* 10S DS1 31531A	[118–122]	–

Drugs were divided in classes according to their mode of action. Yeast species/strains used in the different studies are listed.

process in yeast exists. It was reported that histatin 5 treatment results in mitochondrial membrane depolarization and mitochondrial swelling, loss of intracellular ATP and amino acids, cell cycle arrest in G1 phase and the generation of ROS, although this latter feature still remains controversial [118–122]. Veerman et al. state that ROS do not play a role in the histatin 5-mediated death of *C. albicans* cells since no effect on survival was observed using the ROS scavenger Tempo (2,2,6,6-tetramethylpiperidine-*N*-oxil) [123]. New studies on the effects of histatin 5 on yeast cells, especially on those focused on the involvement of known apoptotic regulators, may uncover its cell death-inducing mechanism of action.

It is conceivable that the capacity of AmB to trigger yeast apoptotic-PCD underlies its high fungicidal activity. Indeed, AmB also triggers apoptosis in human cells which might explain its high cytotoxicity [102]. Therefore, the design of new antifungal drugs should consider the less evolutionary conserved steps and focus on the yeast-specific regulators of PCD.

Natural compounds isolated from diverse organisms, including humans, are assuming great importance since they possess high antifungal activity and low human toxicity. Interestingly, as described above, most seem to induce apoptosis in yeast revealing that they are potentially targeting fungal-specific cell death pathways and/or regulators. Thereby, the elucidation of unique fungal PCD pathways/regulators would revolutionize the manner in which antifungal drugs are designed.

4. Other drugs inducing apoptosis in yeast

Besides exploiting yeasts in the study of the mode of action of antitumour and antifungal drugs, yeasts have also been used to ascertain the cytotoxicity of many different drug types. In many cases these drugs also have certain antitumour properties although they are not usually used for this purpose. For this review we have selected two such drugs, (i) aspirin, one of the World's safest and least expensive pain relievers with over

100 years of proven and effective treatment against a variety of ailments, and (ii) ricin, a toxin isolated from plants that has the capacity to inhibit protein synthesis by irreversibly inactivating eukaryotic ribosomes.

Aspirin, or acetylsalicylic acid, is a non-steroidal anti-inflammatory drug known to induce apoptosis in mammalian cells by a variety of different mechanisms including caspase activation [124,125], inhibition of NF-KB activation [126], ceramide pathway activation [127] and p38 MAP kinase activation [128]. The effects of aspirin on cell growth and its propensity to induce apoptosis have also been studied in yeast cells. Aspirin was found to commit mitochondrial MnSOD-deficient *S. cerevisiae* cells growing in ethanol to apoptosis [129]. In accordance with aspirin's ROS scavenger properties, it also exhibits a significant antioxidant effect until the onset of overt apoptosis in yeast cells, suggesting that ROS probably do not play a primary role in the apoptosis of cells exposed to aspirin [129]. Instead, the authors suggest that a disruption of the redox balance commits yeast cells to apoptosis upon aspirin treatment [130].

Ricin is naturally synthesized in the seeds of *Ricinus communis* (castor bean). This plant toxin is a type II ribosome-inactivating protein (RIP) that inhibits protein synthesis [131]. It consists of a catalytic A chain (RTA) covalently joined by a disulfide bond to a cell binding B chain (RTB) and is highly toxic to eukaryotic cells [132,133]. The RTB is a lectin that binds galactose or *N*-acetylgalactosamine receptors on the surface of target cells and promotes subsequent endocytosis of the RTA [132,133]. Ricin induces apoptosis in a wide variety of animal cells [134] and recently the effects of ricin were studied in *S. cerevisiae*, using a large-scale mutagenesis screen for variants of the precursor form of RTA (pre-RTA) that were unable to kill yeast cells. Apoptotic markers, such as chromatin condensation, nuclear fragmentation and ROS accumulation were observed for yeast cells expressing the wild-type RTA but not for cells expressing the nontoxic mutants, even though they still depurinated ribosomes and inhibited translation [135]. These results provide evidence showing that similar to the studies in mammalian cells, ribosome depurination and translation inhibition are also not sufficient for the ricin-induced cytotoxicity in *S. cerevisiae*. Moreover, the mechanism of apoptotic cell death seems to be strictly dependent on the early generation of ROS [135].

5. Conclusions and future perspectives

The field of yeast PCD, particularly apoptotic-PCD, has grown rapidly during the last decade [7–9]. The increasing understanding of yeast PCD molecular pathways is crucial either for the basic knowledge or for the application of this knowledge to the use of yeasts as a model for cell death-based therapies. Yeasts have been intensively explored to study a wide range of processes, from the basic cellular and molecular pathways to the implications of their regulation and dysfunction in human diseases. Previously, yeast cells containing mutations in genes associated with a specific disease, e.g. tumour associated alterations in DNA repair, mitotic catastrophe, etc., have allowed the screening of drugs that kill mutant cells more efficiently than wild-type cells [136,137]. These strategies have been used successfully revealing several antitumour agents with a high therapeutic advantage [138,139]. The power of yeast molecular genetics, including the multi-faceted role of yeast in drug discovery is also apparent from yeast two-hybrid and three-hybrid systems that have been employed in target identification and validation; the yeast target-based screenings such as high-throughput screening or cell based assays; phenotype-based screening; gene expression profiling of drug action and drug-induced haploinsufficiency (reviewed in [101,140,141]). Therefore, one may already consider "Yeast as a model in drug target discovery and validation". The question that now arises is, can we now reasonably say that "Yeasts are also a good model in apoptosis or cell death-based therapies and drug targets"? The examples addressed in this review show that some therapeutic agents induce yeast apoptotic-PCD that certainly have some similarities with the cell death processes known in mammalian cells. For most of the cell death scenarios induced by antitumour drugs and discussed herein, ROS and mitochondria appear as crucial yeast and mammalian players. This evidence brings us to a relevant and recurrent theme in tumours and chemotherapy: mitochondria and ROS as therapeutic targets. Indeed, a great variety of drugs can directly be targeted to mitochondria to induce apoptosis [142] or to ROS scavenging, resulting in ROS accumulation and apoptosis (reviewed in [143,144]). Besides ROS, nitric oxide (NO), which reacts with molecular oxygen to form reactive nitrogen species (RNS) and ultimately favours carcinogenesis [145], is also an appealing target. Somewhat paradoxically, both anti-NO and NO-based strategies have been applied in cancer therapy (reviewed in [145,146]) indicating a NO dichotomy and an inevitable need of modulate NO levels according to the specific molecular makeup of each individual tumour cell (reviewed in [145,146]). Recently, we demonstrated that *S. cerevisiae* is able to synthesize NO by an L-arginine-dependent mechanism, controlling the formation of ROS and acting as a crucial apoptotic inducer [147,148]. Following this line of thought, yeast could be employed in the study of the synergistic effects as well as molecular pathways that determine the increased sensitivity of cells to antitumour drugs in the presence of different endogenous NO levels.

Other cellular processes that have been revealed as future therapeutic targets include the proteasome, Heat Shock Protein 90 (HSP90), and non-apoptotic PCD pathways including ACD, all of which could be explored using yeast. Yeast proteasome function has already been linked to apoptotic cell death [112]. As the proteasome is a critical enzymatic complex for fundamental pathways in cell survival and proliferation, its inhibition could be a potential antitumour therapy [149,150]. The established link between proteasome and yeast apoptosis suggests that a proteasome inhibition-based therapy could also be investigated in yeast.

The molecular chaperone HSP90, required to ensure the correct conformation, activity, intracellular localization, and proteolytic turnover of a range of proteins that are involved in cell growth, differentiation, and survival [151,152], is also an attractive target for tumour therapy. It is already known that

inhibition of HSP90's function causes degradation of the so called "client proteins", which are reported to be involved in tumourigenesis [151], via the ubiquitin-proteasome pathway [153,154]. Interestingly, our recent observations point to a protective role of HSP90 members in yeast apoptosis (Almeida, B. et al., unpublished data). Using yeast to assess for HSP90 "client proteins", upon treatment with HSP90 inhibitors, could easily contribute to the understanding of its mode of action and role in tumourigenesis. Regarding non-apoptotic PCD process such as ACD, accumulated evidence has shown that this phenomenon also occurs in yeast cells [155–157]. Since many reports show that antitumour drug-induced cell death may involve non-apoptotic PCD through caspase-independent pathways [158,159] or even through the induction of ACD [160], the yeast system seems promising for revealing clues on the foundation of new opportunities to design targeting therapy to promote non-apoptotic cell death of tumour cells.

An interesting link between HSPs, the proteasome and autophagy relates to the fact that they all act as cellular defenses in neurodegenerative disorders, especially those that involve protein misfolding. Given the fact that yeast is being used as a model to study several neurodegenerative disorders involving protein misfolding and aggregation [161], it seems feasible to also use yeast for screening of drugs that are able to increase survival by acting on these targets.

Although yeast cells are useful for the study of the cytotoxic effects of a panoply of drugs, their primary relevance might be directed to the design of new antifungal drugs. A new generation of antifungal drugs is urgently needed given the problems associated with ones currently in use and to the increasing number of invasive fungal infections in immunocompromised patients. In this sense, the exploration of yeast PCD processes to identify molecules that allow the specific manipulation of yeast cell death without causing serious side effects on human cells is appealing. Until recently the cumulative knowledge on yeast PCD shows a high conservation of cell death processes and regulators, however substantial differences will necessarily be detected among molecules and/or pathways as the field develops. One good example is the fungal metacaspases which seem to be the main executors of a wide range of apoptotic stimuli. Even though metacaspases are orthologs of caspases, they display enough structural dissimilarity to allow the design/screening of compounds or molecules that selectively activate metacaspases and not caspases. For this to be possible more effort needs to be applied to the study of yeast PCD.

On the other hand, we must not disregard the existence of distinct cellular machineries linked to yeast PCD induction that may be relevant as future therapeutic targets. In fact, one of the main problems regarding the design of antitumour drugs is the cellular specificity; ergo, some drugs are effective only against a particular kind of tumour cells while ineffective for others. As simple eukaryotic microorganisms with less complex PCD regulation without the idiosyncrasies of different cell types, yeasts are undoubtedly important models for the design of therapies directed to basic molecular pathways, thus overcoming the problem of cellular specificity.

The examples presented throughout this review show in very distinct ways the real utility of yeasts in drug-induced cell death discovery. In addition, the plethora of tools available, along with our knowledge of PCD also makes yeast a highly valuable model organism for drug target identification and validation. Future studies are required in order to fully characterize the "ups and downs" of yeast PCD and definitively expose the extent of potential benefits that yeast may present to study these issues.

Acknowledgments

The authors apologize to the researchers whose work was not cited or discussed in this review. The authors would also like to thank Agostinho Almeida for all the helpful suggestions and for the critical reading of the manuscript. This work was supported by a grant from FCT — Fundação para a Ciência e a Tecnologia, Portugal (POCI/BIA-BCM/57364/2004). B.A., A.S., A.M. and B.S.M. have fellowships from FCT (SFRH/BD/15317/2005, SFRH/BD/33125/2007, SFRH/BD/32464/2006 and SFRH/BI/15406/2005, respectively).

References

[1] C.B. Thompson, Apoptosis in the pathogenesis and treatment of disease, Science 267 (1995) 1456–1462.
[2] U. Fischer, K. Schulze-Osthoff, New approaches and therapeutics targeting apoptosis in disease, Pharmacol. Rev. 57 (2005) 187–215.
[3] U. Fischer, K. Schulze-Osthoff, Apoptosis-based therapies and drug targets, Cell Death Differ 12 (Suppl 1) (2005) 942–961.
[4] L.H. Hartwell, Nobel Lecture. Yeast and cancer, Biosci. Rep. 22 (2002) 373–394.
[5] J. Heitman, N.R. Movva, M.N. Hall, Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast, Science 253 (1991) 905–909.
[6] S.L. Schreiber, G.R. Crabtree, The mechanism of action of cyclosporin A and FK506, Immunol. Today 13 (1992) 136–142.
[7] F. Madeo, E. Herker, S. Wissing, H. Jungwirth, T. Eisenberg, K.U. Frohlich, Apoptosis in yeast, Curr. Opin. Microbiol. 7 (2004) 655–660.
[8] P. Ludovico, F. Madeo, M. Silva, Yeast programmed cell death: an intricate puzzle, IUBMB Life 57 (2005) 129–135.
[9] K.U. Frohlich, H. Fussi, C. Ruckenstuhl, Yeast apoptosis—from genes to pathways, Semin. Cancer Biol. 17 (2007) 112–121.
[10] S. Wissing, P. Ludovico, E. Herker, S. Buttner, S.M. Engelhardt, T. Decker, A. Link, A. Proksch, F. Rodrigues, M. Corte-Real, K.U. Frohlich, J. Manns, C. Cande, S.J. Sigrist, G. Kroemer, F. Madeo, An AIF orthologue regulates apoptosis in yeast, J. Cell Biol. 166 (2004) 969–974.
[11] P. Ludovico, F. Rodrigues, A. Almeida, M.T. Silva, A. Barrientos, M. Corte-Real, Cytochrome *c* release and mitochondria involvement in programmed cell death induced by acetic acid in *Saccharomyces cerevisiae*, Mol. Biol. Cell 13 (2002) 2598–2606.
[12] B. Fahrenkrog, U. Sauder, U. Aebi, The *S. cerevisiae* HtrA-like protein Nma111p is a nuclear serine protease that mediates yeast apoptosis, J. Cell Sci. 117 (2004) 115–126.
[13] C.W. Gourlay, W. Du, K.R. Ayscough, Apoptosis in yeast—mechanisms and benefits to a unicellular organism, Mol. Microbiol. 62 (2006) 1515–1521.
[14] W. Greenhalf, C. Stephan, B. Chaudhuri, Role of mitochondria and C-terminal membrane anchor of Bcl-2 in Bax induced growth arrest and mortality in *Saccharomyces cerevisiae*, FEBS Lett. 380 (1996) 169–175.
[15] H. Zha, H.A. Fisk, M.P. Yaffe, N. Mahajan, B. Herman, J.C. Reed, Structure–function comparisons of the proapoptotic protein Bax in yeast and mammalian cells, Mol. Cell Biol. 16 (1996) 6494–6508.

[16] B. Ink, M. Zornig, B. Baum, N. Hajibagheri, C. James, T. Chittenden, G. Evan, Human Bak induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells, Mol. Cell Biol. 17 (1997) 2468–2474.
[17] J. Smardova, J. Smarda, J. Koptikova, Functional analysis of p53 tumor suppressor in yeast, Differentiation 73 (2005) 261–277.
[18] D. Grochova, J. Vankova, J. Damborsky, B. Ravcukova, J. Smarda, B. Vojtesek, J. Smardova, Analysis of transactivation capability and conformation of p53 temperature-dependent mutants and their reactivation by amifostine in yeast, Oncogene (2007).
[19] W.C. Burhans, M. Weinberger, M.A. Marchetti, L. Ramachandran, G. D'Urso, J.A. Huberman, Apoptosis-like yeast cell death in response to DNA damage and replication defects, Mutat. Res. 532 (2003) 227–243.
[20] M. Weinberger, L. Ramachandran, L. Feng, K. Sharma, X. Sun, M. Marchetti, J.A. Huberman, W.C. Burhans, Apoptosis in budding yeast caused by defects in initiation of DNA replication, J. Cell Sci. 118 (2005) 3543–3553.
[21] P. Perego, L. Gatti, N. Carenini, L. Dal Bo, F. Zunino, Apoptosis induced by extracellular glutathione is mediated by H(2)O(2) production and DNA damage, Int. J. Cancer 87 (2000) 343–348.
[22] M.C. Wani, H.L. Taylor, M.E. Wall, P. Coggon, A.T. McPhail, Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*, J. Am. Chem. Soc. 93 (1971) 2325–2327.
[23] A. Stierle, G. Strobel, D. Stierle, Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew, Science 260 (1993) 214–216.
[24] W.B. Derry, L. Wilson, M.A. Jordan, Substoichiometric binding of taxol suppresses microtubule dynamics, Biochemistry 34 (1995) 2203–2211.
[25] C.M. Woods, J. Zhu, P.A. McQueney, D. Bollag, E. Lazarides, Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway, Mol. Med. 1 (1995) 506–526.
[26] K. Torres, S.B. Horwitz, Mechanisms of Taxol-induced cell death are concentration dependent, Cancer Res. 58 (1998) 3620–3626.
[27] V. Ganansia-Leymarie, P. Bischoff, J.P. Bergerat, V. Holl, Signal transduction pathways of taxanes-induced apoptosis, Curr. Med. Chem. Anticancer Agents 3 (2003) 291–306.
[28] S.J. Park, C.H. Wu, J.D. Gordon, X. Zhong, A. Emami, A.R. Safa, Taxol induces caspase-10-dependent apoptosis, J. Biol. Chem. 279 (2004) 51057–51067.
[29] J.V. Kilmartin, Purification of yeast tubulin by self-assembly in vitro, Biochemistry 20 (1981) 3629–3633.
[30] G. Barnes, K.A. Louie, D. Botstein, Yeast proteins associated with microtubules in vitro and in vivo, Mol Biol Cell 3 (1992) 29–47.
[31] C.J. Bode, M.L. Gupta Jr., E.A. Reiff, K.A. Suprenant, G.I. Georg, R.H. Himes, Epothilone and paclitaxel: unexpected differences in promoting the assembly and stabilization of yeast microtubules, Biochemistry 41 (2002) 3870–3874.
[32] T.B. Foland, W.L. Dentler, K.A. Suprenant, M.L. Gupta, R.H. Himes, Paclitaxel-induced microtubule stabilization causes mitotic block and apoptotic-like cell death in a paclitaxel-sensitive strain of *Saccharomyces cerevisiae*, Yeast 22 (2005) 971–978.
[33] P.B. Schiff, J. Fant, S.B. Horwitz, Promotion of microtubule assembly in vitro by taxol, Nature 277 (1979) 665–667.
[34] M.A. Jordan, Mechanism of action of antitumor drugs that interact with microtubules and tubulin, Curr. Med. Chem. Anticancer Agents 2 (2002) 1–17.
[35] K. Alimoghaddam, A. Shariftabrizi, S.M. Tavangar, Z. Sanaat, S. Rostami, M. Jahani, A. Ghavamzadeh, Anti-leukemic and anti-angiogenesis efficacy of arsenic trioxide in new cases of acute promyelocytic leukemia, Leuk. Lymphoma. 47 (2006) 81–88.
[36] N. Haga, N. Fujita, T. Tsuruo, Involvement of mitochondrial aggregation in arsenic trioxide (As2O3)-induced apoptosis in human glioblastoma cells, Cancer Sci. 96 (2005) 825–833.
[37] J. Bustamante, L. Nutt, S. Orrenius, V. Gogvadze, Arsenic stimulates release of cytochrome c from isolated mitochondria via induction of mitochondrial permeability transition, Toxicol. Appl. Pharmacol. 207 (2005) 110–116.
[38] Y. Zheng, Y. Shi, C. Tian, C. Jiang, H. Jin, J. Chen, A. Almasan, H. Tang, Q. Chen, Essential role of the voltage-dependent anion channel (VDAC) in mitochondrial permeability transition pore opening and cytochrome c release induced by arsenic trioxide, Oncogene 23 (2004) 1239–1247.
[39] L. Du, Y. Yu, J. Chen, Y. Liu, Y. Xia, Q. Chen, X. Liu, Arsenic induces caspase- and mitochondria-mediated apoptosis in *Saccharomyces cerevisiae*, FEMS Yeast Res. 7 (2007) 860–865.
[40] L. Du, Y. Yu, Z. Li, J. Chen, Y. Liu, Y. Xia, X. Liu, Tim18, a component of the mitochondrial translocator, mediates yeast cell death induced by arsenic, Biochemistry (Mosc) 72 (2007) 843–847.
[41] S.H. Seok, M.W. Baek, H.Y. Lee, D.J. Kim, Y.R. Na, K.J. Noh, S.H. Park, H.K. Lee, B.H. Lee, D.Y. Ryu, J.H. Park, Arsenite-induced apoptosis is prevented by antioxidants in zebrafish liver cell line, Toxicol. In Vitro 21 (2007) 870–877.
[42] M.F. Hughes, Arsenic toxicity and potential mechanisms of action, Toxicol. Lett. 133 (2002) 1–16.
[43] H. Umezawa, Bleomycin and other antitumor antibiotics of high molecular weight, Antimicrobial Agents Chemother (Bethesda) 5 (1965) 1079–1085.
[44] H. Umezawa, K. Maeda, T. Takeuchi, Y. Okami, New antibiotics, bleomycin A and B, J. Antibiot. (Tokyo) 19 (1966) 200–209.
[45] O. Tounekti, G. Pron, J. Belehradek Jr., L.M. Mir, Bleomycin, an apoptosis-mimetic drug that induces two types of cell death depending on the number of molecules internalized, Cancer Res. 53 (1993) 5462–5469.
[46] O. Tounekti, J. Belehradek, Jr., L.M. Mir, Relationships between DNA fragmentation, chromatin condensation, and changes in flow cytometry profiles detected during apoptosis, Exp. Cell. Res. 217 (1995) 506–516.
[47] M. Aouida, O. Tounekti, O. Belhadj, L.M. Mir, Comparative roles of the cell wall and cell membrane in limiting uptake of xenobiotic molecules by *Saccharomyces cerevisiae*, Antimicrob. Agents Chemother. 47 (2003) 2012–2014.
[48] V.Y. Lee, C. Schroedl, J.K. Brunelle, L.J. Buccellato, O.I. Akinci, H. Kaneto, C. Snyder, J. Eisenbart, G.R. Budinger, N.S. Chandel, Bleomycin induces alveolar epithelial cell death through JNK-dependent activation of the mitochondrial death pathway, Am. J. Physiol. Lung Cell Mol. Physiol. 289 (2005) L521–L528.
[49] S.B. Wallach-Dayan, G. Izbicki, P.Y. Cohen, R. Gerstl-Golan, A. Fine, R. Breuer, Bleomycin initiates apoptosis of lung epithelial cells by ROS but not by Fas/FasL pathway, Am. J. Physiol. Lung Cell Mol. Physiol. 290 (2006) L790–L796.
[50] M. Aouida, H. Mekid, O. Belhadj, L.M. Mir, O. Tounekti, Mitochondria-independent morphological and biochemical apoptotic alterations promoted by the anti-tumor agent bleomycin in *Saccharomyces cerevisiae*, Biochem. Cell Biol. 85 (2007) 49–55.
[51] M. Gottlicher, S. Minucci, P. Zhu, O.H. Kramer, A. Schimpf, S. Giavara, J.P. Sleeman, F. Lo Coco, C. Nervi, P.G. Pelicci, T. Heinzel, Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells, EMBO J. 20 (2001) 6969–6978.
[52] R. Kawagoe, H. Kawagoe, K. Sano, Valproic acid induces apoptosis in human leukemia cells by stimulating both caspase-dependent and -independent apoptotic signaling pathways, Leuk. Res. 26 (2002) 495–502.
[53] A. Angelucci, A. Valentini, D. Millimaggi, G.L. Gravina, R. Miano, V. Dolo, C. Vicentini, M. Bologna, G. Federici, S. Bernardini, Valproic acid induces apoptosis in prostate carcinoma cell lines by activation of multiple death pathways, Anticancer Drugs 17 (2006) 1141–1150.
[54] W.T. Shen, T.S. Wong, W.Y. Chung, M.G. Wong, E. Kebebew, Q.Y. Duh, O.H. Clark, Valproic acid inhibits growth, induces apoptosis, and modulates apoptosis-regulatory and differentiation gene expression in human thyroid cancer cells, Surgery 138 (2005) 979–984 discussion 984–975.
[55] S. Armeanu, A. Pathil, S. Venturelli, P. Mascagni, T.S. Weiss, M. Gottlicher, M. Gregor, U.M. Lauer, M. Bitzer, Apoptosis on hepatoma cells but not on primary hepatocytes by histone deacetylase inhibitors valproate and ITF2357, J. Hepatol. 42 (2005) 210–217.
[56] J. Chen, F.M. Ghazawi, W. Bakkar, Q. Li, Valproic acid and butyrate induce apoptosis in human cancer cells through inhibition of gene expression of Akt/protein kinase B, Mol. Cancer 5 (2006) 71.

[57] K. Mitsui, D. Nakagawa, M. Nakamura, T. Okamoto, K. Tsurugi, Valproic acid induces apoptosis dependent of Yca1p at concentrations that mildly affect the proliferation of yeast, FEBS Lett. 579 (2005) 723–727.
[58] Q. Sun, L. Bi, X. Su, K. Tsurugi, K. Mitsui, Valproate induces apoptosis by inducing accumulation of neutral lipids which was prevented by disruption of the SIR2 gene in *Saccharomyces cerevisiae*, FEBS Lett. 581 (2007) 3991–3995.
[59] L. Guarente, Sir2 links chromatin silencing, metabolism, and aging, Genes Dev. 14 (2000) 1021–1026.
[60] S.J. Lin, M. Kaeberlein, A.A. Andalis, L.A. Sturtz, P.A. Defossez, V.C. Culotta, G.R. Fink, L. Guarente, Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration, Nature 418 (2002) 344–348.
[61] R.B. Weiss, The anthracyclines: will we ever find a better doxorubicin? Semin. Oncol. 19 (1992) 670–686.
[62] D.A. Gewirtz, A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin, Biochem. Pharmacol. 57 (1999) 727–741.
[63] G. Minotti, P. Menna, E. Salvatorelli, G. Cairo, L. Gianni, Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity, Pharmacol. Rev. 56 (2004) 185–229.
[64] E. Keyhani, J. Keyhani, Plasma membrane alteration is an early signaling event in doxorubicin-induced apoptosis in the yeast *Candida utilis*, Ann N Y Acad Sci 1030 (2004) 369–376.
[65] F. Mollinedo, J.L. Fernandez-Luna, C. Gajate, B. Martin-Martin, A. Benito, R. Martinez-Dalmau, M. Modolell, Selective induction of apoptosis in cancer cells by the ether lipid ET-18-OCH3 (Edelfosine): molecular structure requirements, cellular uptake, and protection by Bcl-2 and Bcl-X(L), Cancer Res 57 (1997) 1320–1328.
[66] C. Gajate, F. Mollinedo, The antitumor ether lipid ET-18-OCH(3) induces apoptosis through translocation and capping of Fas/CD95 into membrane rafts in human leukemic cells, Blood 98 (2001) 3860–3863.
[67] O. Cuvillier, E. Mayhew, A.S. Janoff, S. Spiegel, Liposomal ET-18-OCH (3) induces cytochrome c-mediated apoptosis independently of CD95 (APO-1/Fas) signaling, Blood 94 (1999) 3583–3592.
[68] A.S. Vrablic, C.D. Albright, C.N. Craciunescu, R.I. Salganik, S.H. Zeisel, Altered mitochondrial function and overgeneration of reactive oxygen species precede the induction of apoptosis by 1-O-octadecyl-2-methyl-rac-glycero-3-phosphocholine in p53-defective hepatocytes, FASEB J. 15 (2001) 1739–1744.
[69] C. Gajate, A.M. Santos-Beneit, A. Macho, M. Lazaro, A. Hernandez-De Rojas, M. Modolell, E. Munoz, F. Mollinedo, Involvement of mitochondria and caspase-3 in ET-18-OCH(3)-induced apoptosis of human leukemic cells, Int. J. Cancer 86 (2000) 208–218.
[70] T. Nieto-Miguel, C. Gajate, F. Mollinedo, Differential targets and subcellular localization of antitumor alkyl-lysophospholipid in leukemic versus solid tumor cells, J. Biol. Chem. 281 (2006) 14833–14840.
[71] P.K. Hanson, L. Malone, J.L. Birchmore, J.W. Nichols, Lem3p is essential for the uptake and potency of alkylphosphocholine drugs, edelfosine and miltefosine, J. Biol. Chem. 278 (2003) 36041–36050.
[72] V. Zaremberg, C. Gajate, L.M. Cacharro, F. Mollinedo, C.R. McMaster, Cytotoxicity of an anti-cancer lysophospholipid through selective modification of lipid raft composition, J. Biol. Chem. 280 (2005) 38047–38058.
[73] H. Zhang, C. Gajate, L.P. Yu, Y.X. Fang, F. Mollinedo, Mitochondrial-derived ROS in edelfosine-induced apoptosis in yeasts and tumor cells, Acta Pharmacol. Sin 28 (2007) 888–894.
[74] M.L. Anthony, M. Zhao, K.M. Brindle, Inhibition of phosphatidylcholine biosynthesis following induction of apoptosis in HL-60 cells, J. Biol. Chem. 274 (1999) 19686–19692.
[75] J.H. Joo, G. Liao, J.B. Collins, S.F. Grissom, A.M. Jetten, Farnesol-induced apoptosis in human lung carcinoma cells is coupled to the endoplasmic reticulum stress response, Cancer Res. 67 (2007) 7929–7936.
[76] M.M. Wright, A.L. Henneberry, T.A. Lagace, N.D. Ridgway, C.R. McMaster, Uncoupling farnesol-induced apoptosis from its inhibition of phosphatidylcholine synthesis, J. Biol. Chem. 276 (2001) 25254–25261.
[77] M.M. Taylor, K. Macdonald, A.J. Morris, C.R. McMaster, Enhanced apoptosis through farnesol inhibition of phospholipase D signal transduction, FEBS J. 272 (2005) 5056–5063.
[78] K. Machida, T. Tanaka, Y. Yano, S. Otani, M. Taniguchi, Farnesol-induced growth inhibition in *Saccharomyces cerevisiae* by a cell cycle mechanism, Microbiology 145 (Pt 2) (1999) 293–299.
[79] K. Machida, T. Tanaka, K. Fujita, M. Taniguchi, Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial electron transport chain in the yeast *Saccharomyces cerevisiae*, J. Bacteriol. 180 (1998) 4460–4465.
[80] K. Machida, T. Tanaka, Farnesol-induced generation of reactive oxygen species dependent on mitochondrial transmembrane potential hyperpolarization mediated by F(0)F(1)-ATPase in yeast, FEBS Lett. 462 (1999) 108–112.
[81] C.P. Semighini, J.M. Hornby, R. Dumitru, K.W. Nickerson, S.D. Harris, Farnesol-induced apoptosis in *Aspergillus nidulans* reveals a possible mechanism for antagonistic interactions between fungi, Mol. Microbiol. 59 (2006) 753–764.
[82] M.D. Latham, C.K. King, P. Gorycki, T.L. Macdonald, W.E. Ross, Inhibition of topoisomerases by fredericamycin A, Cancer Chemother. Pharmacol. 24 (1989) 167–171.
[83] J.T. Hartmann, H.P. Lipp, Camptothecin and podophyllotoxin derivatives: inhibitors of topoisomerase I and II — mechanisms of action, pharmacokinetics and toxicity profile, Drug Saf. 29 (2006) 209–230.
[84] S.H. Kaufmann, Cell death induced by topoisomerase-targeted drugs: more questions than answers, Biochim. Biophys. Acta 1400 (1998) 195–211.
[85] A. Montecucco, G. Biamonti, Cellular response to etoposide treatment, Cancer Lett. 252 (2007) 9–18.
[86] A. Albihn, H. Mo, Y. Yang, M. Henriksson, Camptothecin-induced apoptosis is enhanced by Myc and involves PKCdelta signaling, Int. J. Cancer 121 (2007) 1821–1829.
[87] Y. Imamura, M. Yukawa, K. Kimura, H. Takahashi, Y. Suzuki, M. Ojika, Y. Sakagami, E. Tsuchiya, Fredericamycin A affects mitochondrial inheritance and morphology in *Saccharomyces cerevisiae*, Biosci. Biotechnol. Biochem. 69 (2005) 2213–2218.
[88] E.A. Kauh, M.A. Bjornsti, SCT1 mutants suppress the camptothecin sensitivity of yeast cells expressing wild-type DNA topoisomerase I, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 6299–6303.
[89] M. Sabourin, J.L. Nitiss, K.C. Nitiss, K. Tatebayashi, H. Ikeda, N. Osheroff, Yeast recombination pathways triggered by topoisomerase II-mediated DNA breaks, Nucleic Acids Res. 31 (2003) 4373–4384.
[90] B. Almeida, B. Sampaio-Marques, J. Carvalho, M.T. Silva, C. Leao, F. Rodrigues, P. Ludovico, An atypical active cell death process underlies the fungicidal activity of ciclopirox olamine against the yeast *Saccharomyces cerevisiae*, FEMS Yeast Res. 7 (2007) 404–412.
[91] A.J. Phillips, I. Sudbery, M. Ramsdale, Apoptosis induced by environmental stresses and amphotericin B in *Candida albicans*, Proc Natl Acad Sci U S A 100 (2003) 14327–14332.
[92] J. Hoskins, J. Scott Butler, Evidence for distinct DNA- and RNA-based mechanisms of 5-fluorouracil cytotoxicity in *Saccharomyces cerevisiae*, Yeast 24 (2007) 861–870.
[93] A. Tarze, M. Dauplais, I. Grigoras, M. Lazard, N.T. Ha-Duong, F. Barbier, S. Blanquet, P. Plateau, Extracellular production of hydrogen selenide accounts for thiol-assisted toxicity of selenite against *Saccharomyces cerevisiae*, J. Biol. Chem. 282 (2007) 8759–8767.
[94] B. Thati, A. Noble, R. Rowan, B.S. Creaven, M. Walsh, M. McCann, D. Egan, K. Kavanagh, Mechanism of action of coumarin and silver(I)–coumarin complexes against the pathogenic yeast *Candida albicans*, Toxicol. In Vitro 21 (2007) 801–808.
[95] B. Coyle, P. Kinsella, M. McCann, M. Devereux, R. O'Connor, M. Clynes, K. Kavanagh, Induction of apoptosis in yeast and mammalian cells by exposure to 1,10-phenanthroline metal complexes, Toxicol. In Vitro 18 (2004) 63–70.
[96] C. Kesavan, A.G. Joyee, 5-fluorouracil altered morphology and inhibited growth of *Candida albicans*, J Clin Microbiol 43 (2005) 6215–6216.
[97] L. Seiple, P. Jaruga, M. Dizdaroglu, J.T. Stivers, Linking uracil base excision repair and 5-fluorouracil toxicity in yeast, Nucleic Acids Res. 34 (2006) 140–151.
[98] B. Thati, A. Noble, B.S. Creaven, M. Walsh, M. McCann, K. Kavanagh, M. Devereux, D.A. Egan, A study of the role of apoptotic cell death and

cell cycle events mediating the mechanism of action of 6-hydroxycoumarin-3-carboxylatosilver in human malignant hepatic cells, Cancer Lett. 250 (2007) 128–139.
[99] X. Cai, N. Pan, G. Zou, Copper-1,10-phenanthroline-induced apoptosis in liver carcinoma Bel-7402 cells associates with copper overload, reactive oxygen species production, glutathione depletion and oxidative DNA damage, Biometals 20 (2007) 1–11.
[100] S.M. Barns, D.J. Lane, M.L. Sogin, C. Bibeau, W.G. Weisburg, Evolutionary relationships among pathogenic *Candida* species and relatives, J. Bacteriol. 173 (1991) 2250–2255.
[101] T.R. Hughes, Yeast and drug discovery, Funct Integr Genomics 2 (2002) 199–211.
[102] D.E. Varlam, M.M. Siddiq, L.A. Parton, H. Russmann, Apoptosis contributes to amphotericin B-induced nephrotoxicity, Antimicrob. Agents Chemother. 45 (2001) 679–685.
[103] M. Kleinberg, What is the current and future status of conventional amphotericin B? Int. J. Antimicrob. Agents 27 (Suppl 1) (2006) 12–16.
[104] S.H. Leem, J.E. Park, I.S. Kim, J.Y. Chae, A. Sugino, Y. Sunwoo, The possible mechanism of action of ciclopirox olamine in the yeast *Saccharomyces cerevisiae*, Mol. Cells 15 (2003) 55–61.
[105] K. Kokjohn, M. Bradley, B. Griffiths, M. Ghannoum, Evaluation of in vitro activity of ciclopirox olamine, butenafine HCl and econazole nitrate against dermatophytes, yeasts and bacteria, Int. J. Dermatol. 42 (Suppl 1) (2003) 11–17.
[106] M.L. Narasimhan, B. Damsz, M.A. Coca, J.I. Ibeas, D.J. Yun, J.M. Pardo, P.M. Hasegawa, R.A. Bressan, A plant defense response effector induces microbial apoptosis, Mol. Cell 8 (2001) 921–930.
[107] C.O. Morton, A. Hayes, M. Wilson, B.M. Rash, S.G. Oliver, P. Coote, Global phenotype screening and transcript analysis outlines the inhibitory mode(s) of action of two amphibian-derived, {alpha}-helical, cationic peptides on *Saccharomyces cerevisiae*, Antimicrob. Agents Chemother. (2007).
[108] C.O. Morton, S.C. Dos Santos, P. Coote, An amphibian-derived, cationic, alpha-helical antimicrobial peptide kills yeast by caspase-independent but AIF-dependent programmed cell death, Mol. Microbiol. 65 (2007) 494–507.
[109] F. Hiramoto, N. Nomura, T. Furumai, T. Oki, Y. Igarashi, Apoptosis-like cell death of *Saccharomyces cerevisiae* induced by a mannose-binding antifungal antibiotic, pradimicin, J. Antibiot. (Tokyo) 56 (2003) 768–772.
[110] M.L. Narasimhan, M.A. Coca, J. Jin, T. Yamauchi, Y. Ito, T. Kadowaki, K.K. Kim, J.M. Pardo, B. Damsz, P.M. Hasegawa, D.J. Yun, R.A. Bressan, Osmotin is a homolog of mammalian adiponectin and controls apoptosis in yeast through a homolog of mammalian adiponectin receptor, Mol. Cell 17 (2005) 171–180.
[111] A. Mor, P. Nicolas, The NH2-terminal alpha-helical domain 1–18 of dermaseptin is responsible for antimicrobial activity, J. Biol. Chem. 269 (1994) 1934–1939.
[112] M. Ligr, I. Velten, E. Frohlich, F. Madeo, M. Ledig, K.U. Frohlich, D.H. Wolf, W. Hilt, The proteasomal substrate Stm1 participates in apoptosis-like cell death in yeast, Mol. Biol. Cell 12 (2001) 2422–2432.
[113] A. Mor, M. Amiche, P. Nicolas, Structure, synthesis, and activity of dermaseptin b, a novel vertebrate defensive peptide from frog skin: relationship with adenoregulin, Biochemistry 33 (1994) 6642–6650.
[114] A. Mor, K. Hani, P. Nicolas, The vertebrate peptide antibiotics dermaseptins have overlapping structural features but target specific microorganisms, J. Biol. Chem. 269 (1994) 31635–31641.
[115] M. Zasloff, Magainins, a class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 5449–5453.
[116] F. Hiramoto, N. Nomura, T. Furumai, Y. Igarashi, T. Oki, Pradimicin resistance of yeast is caused by a mutation of the putative N-glycosylation sites of osmosensor protein Sln1, Biosci. Biotechnol. Biochem. 69 (2005) 238–241.
[117] F.G. Oppenheim, T. Xu, F.M. McMillian, S.M. Levitz, R.D. Diamond, G.D. Offner, R.F. Troxler, Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on *Candida albicans*, J. Biol. Chem. 263 (1988) 7472–7477.
[118] R. Isola, M. Isola, G. Conti, M.S. Lantini, A. Riva, Histatin-induced alterations in *Candida albicans*: a microscopic and submicroscopic comparison, Microsc. Res. Tech. 70 (2007) 607–616.
[119] S.E. Koshlukova, T.L. Lloyd, M.W. Araujo, M. Edgerton, Salivary histatin 5 induces non-lytic release of ATP from *Candida albicans* leading to cell death, J Biol Chem 274 (1999) 18872–18879.
[120] D. Baev, X.S. Li, J. Dong, P. Keng, M. Edgerton, Human salivary histatin 5 causes disordered volume regulation and cell cycle arrest in *Candida albicans*, Infect Immun 70 (2002) 4777–4784.
[121] E.J. Helmerhorst, W. van't Hof, P. Breeuwer, E.C. Veerman, T. Abee, R.F. Troxler, A.V. Amerongen, F.G. Oppenheim, Characterization of histatin 5 with respect to amphipathicity, hydrophobicity, and effects on cell and mitochondrial membrane integrity excludes a candidacidal mechanism of pore formation, J. Biol. Chem. 276 (2001) 5643–5649.
[122] E.J. Helmerhorst, R.F. Troxler, F.G. Oppenheim, The human salivary peptide histatin 5 exerts its antifungal activity through the formation of reactive oxygen species, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 14637–14642.
[123] E.C. Veerman, K. Nazmi, W. Van't Hof, J.G. Bolscher, A.L. Den Hertog, A.V. Nieuw Amerongen, Reactive oxygen species play no role in the candidacidal activity of the salivary antimicrobial peptide histatin 5, Biochem. J. 381 (2004) 447–452.
[124] B. Bellosillo, M. Pique, M. Barragan, E. Castano, N. Villamor, D. Colomer, E. Montserrat, G. Pons, J. Gil, Aspirin and salicylate induce apoptosis and activation of caspases in B-cell chronic lymphocytic leukemia cells, Blood 92 (1998) 1406–1414.
[125] L. Klampfer, J. Cammenga, H.G. Wisniewski, S.D. Nimer, Sodium salicylate activates caspases and induces apoptosis of myeloid leukemia cell lines, Blood 93 (1999) 2386–2394.
[126] E. Kopp, S. Ghosh, Inhibition of NF-kappa B by sodium salicylate and aspirin, Science 265 (1994) 956–959.
[127] T.A. Chan, P.J. Morin, B. Vogelstein, K.W. Kinzler, Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 681–686.
[128] P. Schwenger, P. Bellosta, I. Vietor, C. Basilico, E.Y. Skolnik, J. Vilcek, Sodium salicylate induces apoptosis via p38 mitogen-activated protein kinase but inhibits tumor necrosis factor-induced c-Jun N-terminal kinase/stress-activated protein kinase activation, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 2869–2873.
[129] R. Balzan, K. Sapienza, D.R. Galea, N. Vassallo, H. Frey, W.H. Bannister, Aspirin commits yeast cells to apoptosis depending on carbon source, Microbiology 150 (2004) 109–115.
[130] K. Sapienza, R. Balzan, Metabolic aspects of aspirin-induced apoptosis in yeast, FEMS Yeast Res. 5 (2005) 1207–1213.
[131] Y. Endo, K. Mitsui, M. Motizuki, K. Tsurugi, The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. The site and the characteristics of the modification in 28 S ribosomal RNA caused by the toxins, J. Biol. Chem. 262 (1987) 5908–5912.
[132] M.R. Hartley, J.M. Lord, Cytotoxic ribosome-inactivating lectins from plants, Biochim. Biophys. Acta 1701 (2004) 1–14.
[133] S. Olsnes, J.V. Kozlov, Ricin, Toxicon. 39 (2001) 1723–1728.
[134] S. Narayanan, K. Surendranath, N. Bora, A. Surolia, A.A. Karande, Ribosome inactivating proteins and apoptosis, FEBS Lett. 579 (2005) 1324–1331.
[135] X.P. Li, M. Baricevic, H. Saidasan, N.E. Tumer, Ribosome depurination is not sufficient for ricin-mediated cell death in *Saccharomyces cerevisiae*, Infect. Immun. 75 (2007) 417–428.
[136] L.H. Hartwell, Yeast and cancer, Biosci. Rep. 24 (2004) 523–544.
[137] P. Perego, G.S. Jimenez, L. Gatti, S.B. Howell, F. Zunino, Yeast mutants as a model system for identification of determinants of chemosensitivity, Pharmacol. Rev. 52 (2000) 477–492.
[138] L.H. Hartwell, P. Szankasi, C.J. Roberts, A.W. Murray, S.H. Friend, Integrating genetic approaches into the discovery of anticancer drugs, Science 278 (1997) 1064–1068.
[139] J.A. Simon, P. Szankasi, D.K. Nguyen, C. Ludlow, H.M. Dunstan, C.J. Roberts, E.L. Jensen, L.H. Hartwell, S.H. Friend, Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of *Saccharomyces cerevisiae*, Cancer Res. 60 (2000) 328–333.

[140] C.D. Armour, P.Y. Lum, From drug to protein: using yeast genetics for high-throughput target discovery, Curr. Opin. Chem. Biol. 9 (2005) 20–24.
[141] M. Menacho-Marquez, J.R. Murguia, Yeast on drugs: *Saccharomyces cerevisiae* as a tool for anticancer drug research, Clin. Transl. Oncol. 9 (2007) 221–228.
[142] L. Galluzzi, N. Larochette, N. Zamzami, G. Kroemer, Mitochondria as therapeutic targets for cancer chemotherapy, Oncogene 25 (2006) 4812–4830.
[143] J.P. Fruehauf, F.L. Meyskens, Jr., Reactive oxygen species: a breath of life or death? Clin. Cancer Res. 13 (2007) 789–794.
[144] R.H. Engel, A.M. Evens, Oxidative stress and apoptosis: a new treatment paradigm in cancer, Front Biosci. 11 (2006) 300–312.
[145] S. Mocellin, V. Bronte, D. Nitti, Nitric oxide, a double edged sword in cancer biology: searching for therapeutic opportunities, Med. Res. Rev. 27 (2007) 317–352.
[146] D.G. Hirst, T. Robson, Nitrosative stress in cancer therapy, Front Biosci. 12 (2007) 3406–3418.
[147] B. Almeida, S. Buttner, S. Ohlmeier, A. Silva, A. Mesquita, B. Sampaio-Marques, N.S. Osorio, A. Kollau, B. Mayer, C. Leao, J. Laranjinha, F. Rodrigues, F. Madeo, P. Ludovico, NO-mediated apoptosis in yeast, J. Cell Sci. 120 (2007) 3279–3288.
[148] N.S. Osorio, A. Carvalho, A.J. Almeida, S. Padilla-Lopez, C. Leao, J. Laranjinha, P. Ludovico, D.A. Pearce, F. Rodrigues, Nitric oxide signaling is disrupted in the yeast model for Batten disease, Mol. Biol. Cell 18 (2007) 2755–2767.
[149] C. Montagut, A. Rovira, J. Albanell, The proteasome: a novel target for anticancer therapy, Clin. Transl. Oncol. 8 (2006) 313–317.
[150] J.P. Spano, J.O. Bay, J.Y. Blay, O. Rixe, Proteasome inhibition: a new approach for the treatment of malignancies, Bull Cancer 92 (2005) E61–E66 945–952.
[151] A. Maloney, P. Workman, HSP90 as a new therapeutic target for cancer therapy: the story unfolds, Expert Opin. Biol. Ther. 2 (2002) 3–24.
[152] L. Whitesell, S.L. Lindquist, HSP90 and the chaperoning of cancer, Nat. Rev. Cancer 5 (2005) 761–772.
[153] P. Connell, C.A. Ballinger, J. Jiang, Y. Wu, L.J. Thompson, J. Hohfeld, C. Patterson, The co-chaperone CHIP regulates protein triage decisions mediated by heat-shock proteins, Nat. Cell Biol. 3 (2001) 93–96.
[154] J. Demand, S. Alberti, C. Patterson, J. Hohfeld, Cooperation of a ubiquitin domain protein and an E3 ubiquitin ligase during chaperone/proteasome coupling, Curr. Biol. 11 (2001) 1569–1577.
[155] E. Ogier-Denis, P. Codogno, Autophagy: a barrier or an adaptive response to cancer, Biochim. Biophys. Acta 1603 (2003) 113–128.
[156] M. Motizuki, S. Yokota, K. Tsurugi, Autophagic death after cell cycle arrest at the restrictive temperature in temperature-sensitive cell division cycle and secretory mutants of the yeast *Saccharomyces cerevisiae*, Eur. J. Cell Biol. 68 (1995) 275–287.
[157] H. Abeliovich, Mitophagy: the life-or-death dichotomy includes yeast, Autophagy 3 (2007) 275–277.
[158] R. Kim, M. Emi, K. Tanabe, Y. Uchida, K. Arihiro, The role of apoptotic or nonapoptotic cell death in determining cellular response to anticancer treatment, Eur. J. Surg. Oncol. 32 (2006) 269–277.
[159] R. Kim, M. Emi, K. Tanabe, S. Murakami, Y. Uchida, K. Arihiro, Regulation and interplay of apoptotic and non-apoptotic cell death, J. Pathol. 208 (2006) 319–326.
[160] Y. Kondo, S. Kondo, Autophagy and cancer therapy, Autophagy 2 (2006) 85–90.
[161] M.Y. Sherman, P.J. Muchowski, Making yeast tremble: yeast models as tools to study neurodegenerative disorders, Neuromolecular Med. 4 (2003) 133–146.
[162] G. Melnykovych, J.S. Haug, C.M. Goldner, Growth inhibition of leukemia cell line CEM-C1 by farnesol: effects of phosphatidylcholine and diacylglycerol, Biochem. Biophys Res Commun 186 (1992) 543–548.
[163] A. Morio, H. Miyamoto, H. Izumi, T. Futagawa, T. Oh, A. Yamazaki, H. Konno, Enhanced induction of apoptosis in lung adenocarcinoma after preoperative chemotherapy with tegafur and uracil (UFT), Surg. Today 34 (2004) 822–827.
[164] L. Zuo, J. Li, Y. Yang, X. Wang, T. Shen, C.M. Xu, Z.N. Zhang, Sodium selenite induces apoptosis in acute promyelocytic leukemia-derived NB4 cells by a caspase-3-dependent mechanism and a redox pathway different from that of arsenic trioxide, Ann. Hematol. 83 (2004) 751–758.
[165] T. Oki, Y. Yamazaki, T. Furumai, Y. Igarashi, Pradimicin, a mannose-binding antibiotic, induced carbohydrate-mediated apoptosis in U937 cells, Biosci. Biotechnol. Biochem. 61 (1997) 1408–1410.
[166] T. Oki, Y. Yamazaki, N. Nomura, T. Furumai, Y. Igarashi, Involvement of Ca2+ ion and reactive oxygen species as a mediator in pradimicin-induced apoptosis, J. Antibiot. (Tokyo) 52 (1999) 455–459.