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Review

Drug-induced apoptosis in yeast

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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

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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.

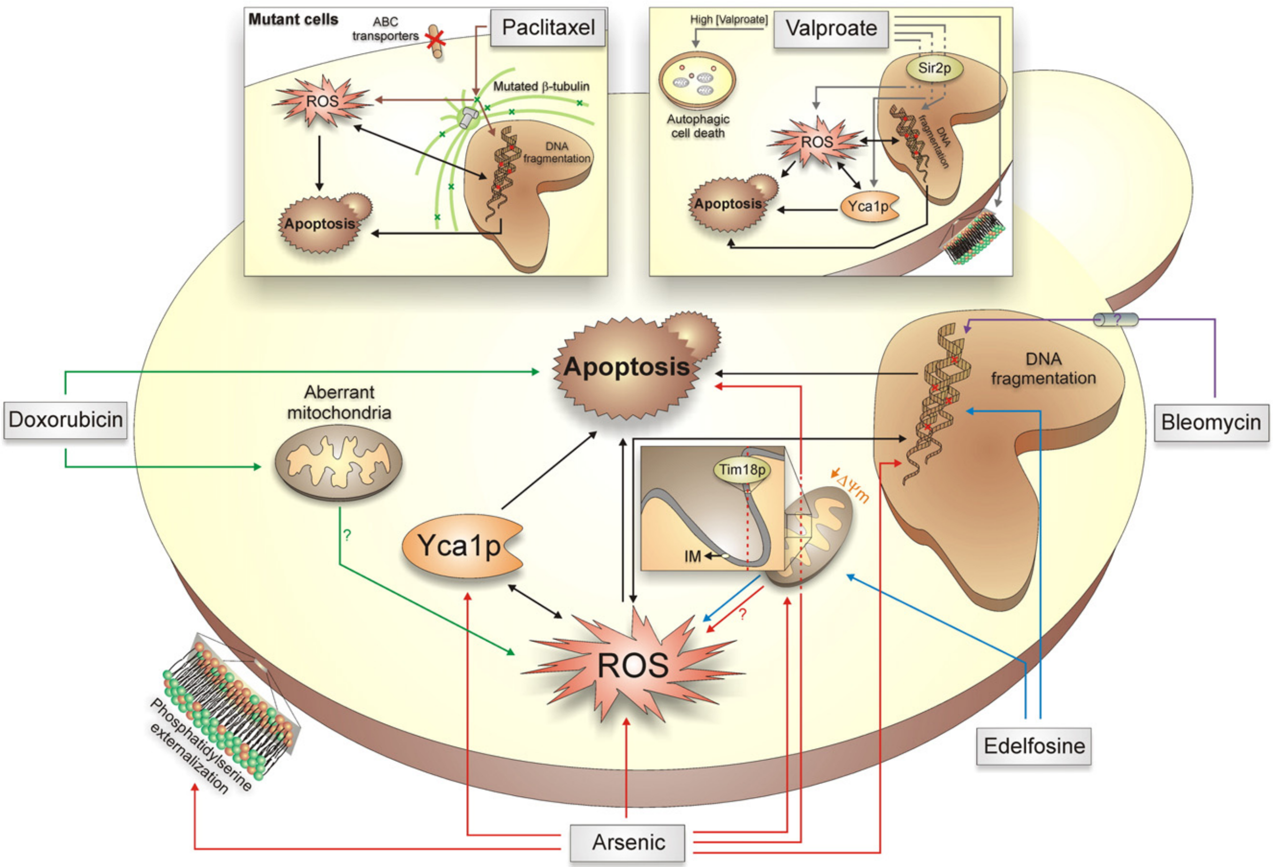

Fig. 1. Schematic representation of yeast apoptotic phenotypes and events induced by antitumour drugs. ROS appears to possess, like in mammalian cells, a central role in the induction/signalling of yeast apoptotic process, with paclitaxel, valproate, arsenic and edelfosine leading to their generation/accumulation. 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.

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