Journal of Nanobiotechnology

REVIEW Open Access

The progress and future of the treatment of *Candida albicans* infections based on nanotechnology

Yang Gao[1], Qinyan Cao[1], Yuyang Xiao[1], Yue Wu[1], Liang Ding[2], He Huang[1], Yanan Li[1*], Jingpeng Yang[1*] and Lingtong Meng[1*]

Abstract

Systemic infection with *Candida albicans* poses a significant risk for people with weakened immune systems and carries a mortality rate of up to 60%. However, current therapeutic options have several limitations, including increasing drug tolerance, notable off-target effects, and severe adverse reactions. Over the past four decades, the progress in developing drugs to treat *Candida albicans* infections has been sluggish. This comprehensive review addresses the limitations of existing drugs and summarizes the efforts made toward redesigning and innovating existing or novel drugs through nanotechnology. The discussion explores the potential applications of nanomedicine in *Candida albicans* infections from four perspectives: nano-preparations for anti-biofilm therapy, innovative formulations of "old drugs" targeting the cell membrane and cell wall, reverse drug resistance therapy targeting subcellular organelles, and virulence deprivation therapy leveraging the unique polymorphism of *Candida albicans*. These therapeutic approaches are promising to address the above challenges and enhance the efficiency of drug development for *Candida albicans* infections. By harnessing nano-preparation technology to transform existing and preclinical drugs, novel therapeutic targets will be uncovered, providing effective solutions and broader horizons to improve patient survival rates.

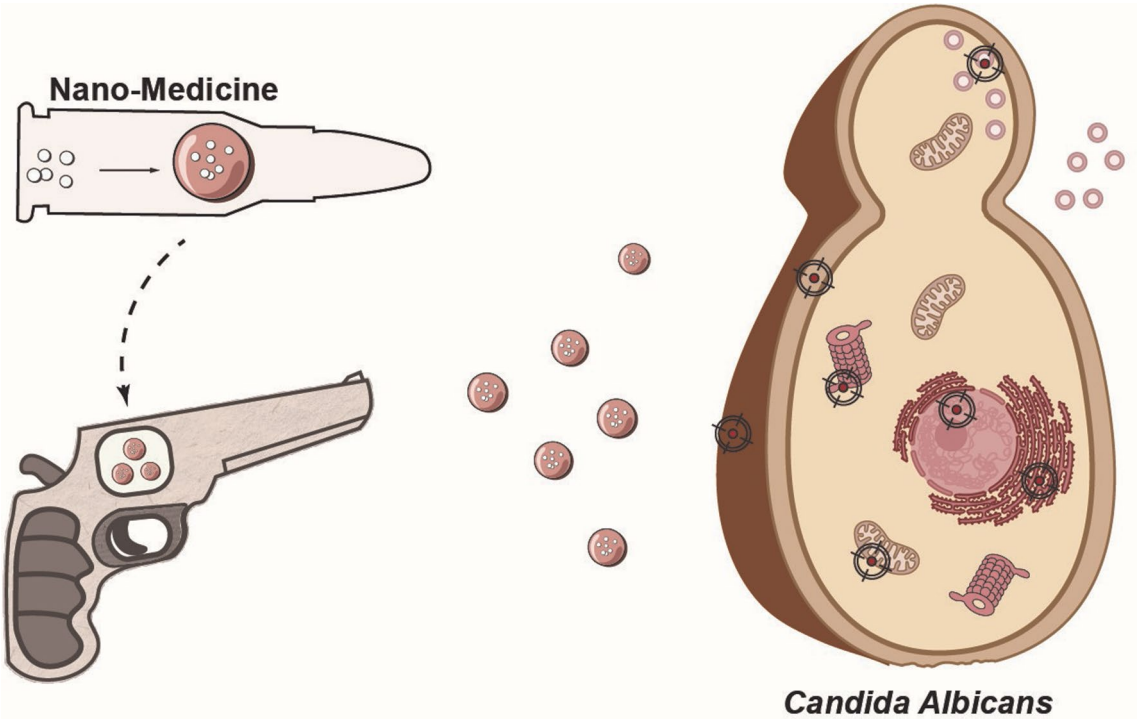
Introduction

Candida albicans is the most widespread fungal species among human pathogenic. *Candida albicans* infections is also a serious problem in intensive care units, where it is the first most common pathogen isolated in patients from Europe and the fourth in the USA [1, 2]. In recent decades, there have been more than 600,000 cases of *Candida* blood infections worldwide each year, resulting in a mortality rate of over 60% [3, 4]. In the United States, *Candida albicans* causes about 60,000 cases of systemic infections each year, and the damage amounts to 2 to 4 billion dollars [5, 6]. *Candida albicans* has a broad spectrum of infections in the human body, ranging from superficial mucosal and skin infections (such as thrush, vaginal infection, and diaper rash) to deep systemic infections (such as lung and spleen). *Candida albicans* is generally harmless as a conditional pathogen in individuals with healthy immune systems and maintains a balance with other members of the microbial community in the body. However, when the microbiota of the host in our body changes (extensive use and abuse of antibiotics) and the immune response of the

*Correspondence:
Yanan Li
liyanan@njnu.edu.cn
Jingpeng Yang
yang_jp008@163.com
Lingtong Meng
mlt_nju@163.com

Full list of author information is available at the end of the article

Graphical abstracts



Keywords Nanomedicine, *Candida albicans*, Hyphae, Subcellular organelles, Drug resistance

host changes (stress, immunosuppressive therapy or chemotherapy), this promotes the excessive proliferation of *Candida albicans*, leading to its transformation from the symbiotic state to the pathogenic state. Nowadays, the probability of opportunistic fungal infection is increasing due to the increasing number of patients with weak immune function who are undergoing organ transplantation, chemotherapy, antibiotic therapy, and immunosuppressive drugs (dexamethasone and IL-6 inhibitors, etc.). AIDS patients, patients in intensive care, and diabetics have an increased risk of *Candida albicans* infection. In the COVID-19 epidemic, many critical patients were also infected with *Candida albicans* [7–9]. At the same time, *Candida albicans* infection exacerbates existing diseases, such as leukemia or AIDS, which led to a mortality rate of 32% for fungal infections [4].

The antifungal drugs that can be used to treat *Candida albicans* infection currently include polyenes, azoles, and echinocandins [10]. Amphotericin B (AmB), which is mainly used as a polyene drug, should be used with caution due to its severe nephrotoxicity and hepatotoxicity although it has a good therapeutic effect [11]. Azoles can be divided into two subcategories according to their chemical structure: Imidazoles, such as clotrimazole, ketoconazole and miconazole; triazoles, such asfluconazole, itraconazole, voriconazole and posaconazole. Local application of imidazole drugs is used to treat mucosal candidiasis, while triazole drugs are generally used to treat mucosal and systemic infections caused by *Candida albicans* [12]. Echinocandins, which mainly include caspofungin, micafungin, and anidulafungin, can inhibit β-(1,3)-D-glucan synthase (an enzyme involved in fungal growth) and then inhibit the production of β-(1,3)-D-glucan, leading to loss of stiffness of the fungal cell wall and cell lysis. In recent years, it is of concern that the resistance of *Candida albicans* to echinocandin is increasing in the clinic (Table 1) [13, 14].

Almost all existing clinical antifungal drugs have some drawbacks, such as drug resistance, off-target effects, high toxicity and side effects, and poor combination efficacy. Antifungal drug resistance is not only caused by clinical drug abuse but also by long-term contact with drugs in daily life. Many antifungal drugs (such as triazoles) are sprayed as pesticides on crops, directly or through meat food, and eventually enter the human body through the food chain [15]. Therefore, the rapid development of drug-resistant strains and the limited number of promising antifungal drugs are two major problems hindering the treatment of infection [16]. In the last forty years, scientific research and pharmaceutical

Table 1 Existing antifungal drugs

Antifungal Agent	Target	Mechanism of Action	Disadvantages	Time to Market
Clotrimazole	CYP51	Impairment of cytochrome P450 activity and decreased ergosterol synthesis	Drug resistance, cross-resistance, hepatotoxicity.	1975
Fluconazole				1989
Itraconazole				1992
Miconazole				1994
Voriconazole				2002
Nystatin	Ergosterol	Extraction of ergosterol from fungal cell membrane	Renal toxicity	1955
Amphotericin B				1963
Capofenjin	1,3-β-D-glucan synthase	Inhibition of synthesis of 1,3-β-D-glucan	Patients with poor adaptability	2001
Micafenjing				2002
Anidulafungin				2006
5-Fluorocytosine	DNA synthetase	Inhibition of fungal DNA synthetase	Serious drug resistance	1971

development have made great progress worldwide, but only echinocandins have entered clinical practice during this time. Compared with the development of antifungal drug resistance, the pace of research and development of new antifungal drugs is really far behind. Although there are some other new drugs against *Candida albicans* in clinical trials, the antifungal activity, side effects and cross-resistance caused by targeting common drug targets have become difficulties for developers [17]. There is a $13 billion market for antifungal drugs worldwide, but due to high drug resistance and the lack of selective targets for antifungal drugs, large pharmaceutical companies have always been skeptical about the commercial viability of developing drugs against *Candida albicans*.

In this case, we urgently need to develop new targets for treatment and improve traditional treatment methods to increase the efficacy of drugs, reduce the side effects of drugs, and figure out the problem of drug resistance. The stagnation in the development of new antifungal drugs also indicates that we need to pay more attention to the modification of the existing dosage forms of antifungal drugs and some physical and biological treatments. The application of nano-agents in anti-*Candida albicans* therapy began in the 1990s, with the best-known products being AmB liposome. AmB encapsulated in liposomes reduces its filtration from the renal tubules and its accumulation in the kidney, effectively reducing the potent nephrotoxicity of AmB [18, 19]. Nano-carrier drug delivery systems can be made of various materials (lipid, polymer, protein, metal, and inorganic materials, etc.). They can realize cross-barrier transport through various mechanisms, which can optimize the delivery of drugs to diseased sites, such as infection. At the same time, they can minimize the distribution of non-target drugs, improve the solubility and stability of drugs, deliver multiple drugs synergistically, improve bioavailability, mediate sustained or controlled release of drugs, and improve treatment efficacy [20]. Given the difficulties encountered by anti-*Candida albicans* drugs, nano-preparation technology could provide us with further ideas and solutions. It can increase the accumulation of drug lesions, specifically identify *Candida albicans*, and specifically distribute them to subcellular organelles in *Candida albicans* cells, providing a breakthrough solution to the current difficulties [21].

Based on the problems of drug resistance, serious side effects and off-target effects of drugs, and the advantages of high efficiency and low toxicity of nano drugs, this paper mainly summarizes the nano preparations against *Candida albicans* and then develops new targets for treatment. This article primarily discusses the possible applications of nano-preparations in *Candida albicans* infections from the following points of view: Nano-preparations for anti-biofilm therapy; innovative dosage forms for conventional drugs targeting cell membranes and cell walls; reverse drug resistance therapy targeting suborganelles (nucleus, mitochondria, endoplasmic reticulum, proteasome, and extracellular vesicles) of *Candida albicans*; treatment based on polyphasic virulence deprivation. We emphasize investigating the intricate interplay between nano-drugs and cells, specifically tailored to the unique characteristics of *Candida albicans* cells. This approach transcends the conventional focus solely on the antifungal properties of nanomaterials, offering a fresh perspective that aligns with cellular dynamics. At the same time, we focus on *Candida albicans* itself rather than all pathogenic fungi. It enables us to uncover superior treatment strategies and insights, advancing our understanding and approach to therapy [22–26]. The nanotechnology has great potential to overcome drug resistance, enhance therapeutic effect, improve targeting of lesions, mitigate side effects and support existing clinical and preclinical experimental drugs to improve the health of patients with *Candida albicans* (Fig. 1).

Anti-biofilm therapy

The biofilm is the outermost structure of fungi in contact with the external environment, and it is also an important reason for the mediation of fungal drug resistance. The biofilm consists of a syntrophic collective of microorganisms, whereby the embedded cells can adhere to each other. These cells within the biofilm produce the extracellular polymeric substances (EPS), which are typically a polymeric combination of proteins, extracellular polysaccharides, lipids and DNA. Extracellular matrix protects the fungi from immune reactions and antifungal agents [27, 28]. A common feature is that biofilm is

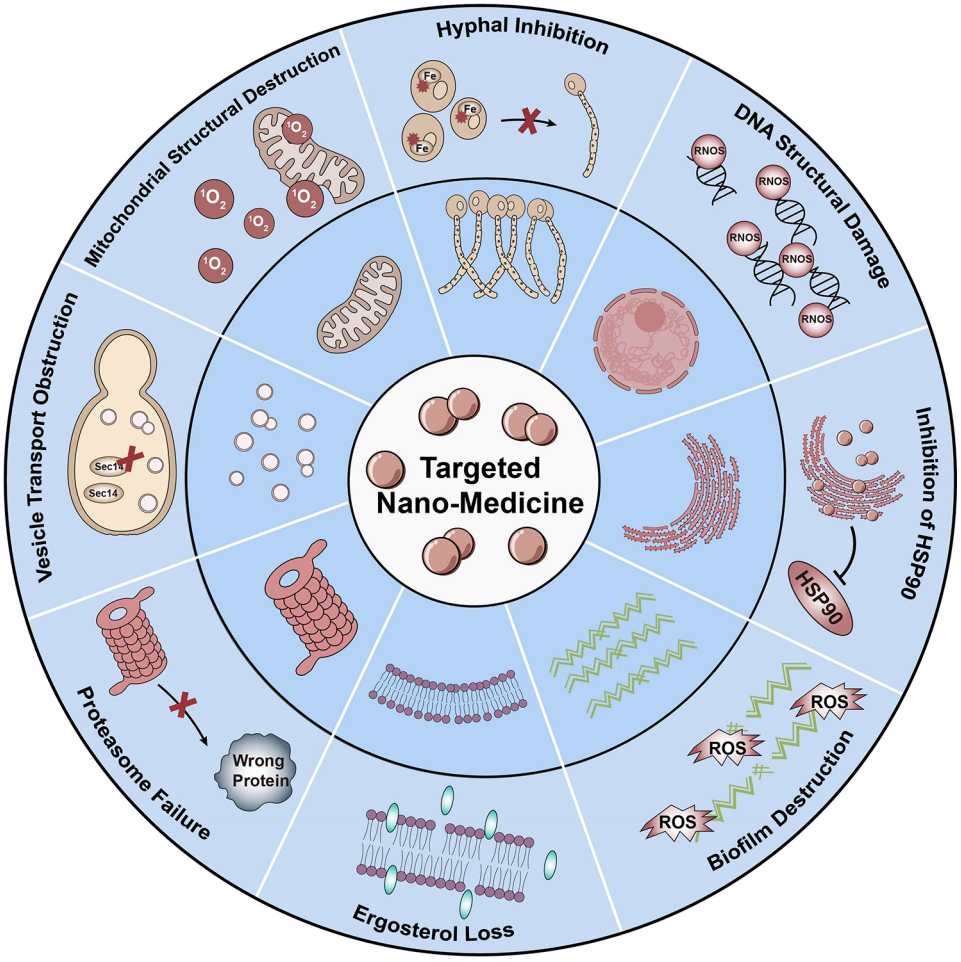


Fig. 1 Schematic overview of the targeted drugs for the treatment of *Candida albicans* infections discussed in this article. These targeted nanomedicines primarily target different organelles within *Candida albicans*. The outermost layer shows the mechanism of action of the individual targeted drugs on the respective organelles

highly resistant to chemical and physical killing by drugs, as the biofilm matrix can limit the penetration of antibiotics and other drugs and prevent them from reaching the embedded cells [29, 30]. The latest research shows that killing bacteria in biofilm requires a 1000 times higher dose of antibiotics than killing bacteria in suspension [31, 32].

The biofilm is also an important structure of *Candida albicans* infection in vivo. *Candida albicans* can form a highly structured biofilm that protects the fungal pathogens from identification and attack by the host's immune system [33]. In addition, *Candida albicans* can also trap drugs in dextran-rich matrix, significantly reducing the effective concentration of drugs [34]. The resistance of *Candida albicans* in biofilm to antifungal drugs such as AmB, fluconazole, itraconazole and ketoconazole is 30 ~ 2000 times higher than that of planktonic cells [35]. Therefore, antifungal drugs in traditional therapy can hardly eliminate the fungal pathogens in the biofilm, and there is limited clinical evidence for biofilm eradication. Achieving an acceptable therapeutic effect by high-dose or long-term antimicrobial chemotherapy usually leads to severe side effects [26, 36].

Given the major influence of the biofilm on the drug resistance of *Candida albicans*, it is worth paying attention to breaking through the barrier of the biofilm to improve the therapeutic effect of existing antifungal drugs. Photodynamic therapy (PDT) means that the photosensitizer can generate reactive oxygen species (ROS) under light irradiation of a specific wavelength. ROS can oxidize and destroy extracellular polymeric substances and other biological macromolecules such as phospholipids, enzymes, and proteins, effectively inactivating pathogenic microorganisms [37, 38]. PDT has emerged as one of the most promising methods for treating infectious agents, offering remarkable advantages such as negligible drug resistance, high space-time control and low side effects [39]. It has been found that nanoparticle-based photodynamic therapy can effectively destroy biofilm of *Candida albicans* by disrupting the quorum sensing

of albinism. Oligo-chitosan (OC) is normally positively charged, while the lipid layer on the surface of *Candida albicans* is negatively charged. The nanoparticles decorated with chitosan are more easily adsorbed on the surface of *Candida albicans*. Tang et al. found that chitosan-modified photodynamic nanoparticles (NaYF4@NaGdF4@PpIX-OC and BaGdF5@PpIX-OC) exhibited better binding efficiency to planktonic cells and stronger drug penetration ability compared to unmodified chitosan nanoparticles, thus showing a stronger inhibitory effect on early biofilm formation. NaYF4@NaGdF4@PpIX-OC and BaGdF5@PpIX-OC destroy lipids, protein and DNA molecules in the biofilm structure through the production of ROS. On the other hand, NaYF4@NaGdF4@PpIX-OC and BaGdF5@PpIX-OC also showed the ability to regulate the quorum sensing molecules tyrosol and farnesol Fig. 2A-B) [40]. Farnesol and tyrosol are typical quorum-sensing molecules of *Candida albicans* that are secreted into the extracellular microenvironment. Tyrosol can promote the growth of hyphae and the formation of biofilms, while farnesol can inhibit this process [41]. Photosensitizer Rose Bengal (RB) can also produce ROS to destroy the biofilm of *Candida albicans*. Maliszewska et al. synthesized gold nanoparticles (AuNPs) which can be engineered to possess chemical or photothermal functionalities and enhance photodynamic antimicrobial chemotherapy. The AuNPs were synthesized by exposure of $AuCl_4^-$ions to the cell-free filtrate of PenicilliumfuniculosumBL1, were chosen to enhance the efficiency of the photoinactivation of planktonic and biofilm cultures of *Candida albicans*. After 30 min of Xe light irradiation, the killing rate of the RB combined AuNPs group was 97.04% in biofilm, while that of the RB group was 74.73% [42]. Compared to bacteria, fungi have a denser biofilm, which can increase the penetration resistance of nanoparticles. Therefore, nanoparticles of smaller size (10–50 nm) can better deliver drugs to infected sites.

Nanoparticles, especially metal particles, have good adhesion and biocompatibility and can be used as an effective drug delivery system to increase the efficacy of drugs and reduce side effects. Therefore, the load of some drugs against fungal biofilm into metal nanoparticles can achieve synergistic and attenuated side effects and has become a common method for the treatment of microbial pathogens [24]. Nazia et al. formed Fu-AuNPs by loading fucoidan, a marine derivative previously found to have a killing effect on *Candida albicans*, into gold nanoparticles. Gold nanoparticles have good chemical stability, can destroy the cell membrane, release ROS and metal ions. The integration of drug with gold nanoparticles not only plays a synergistic role in antifungal

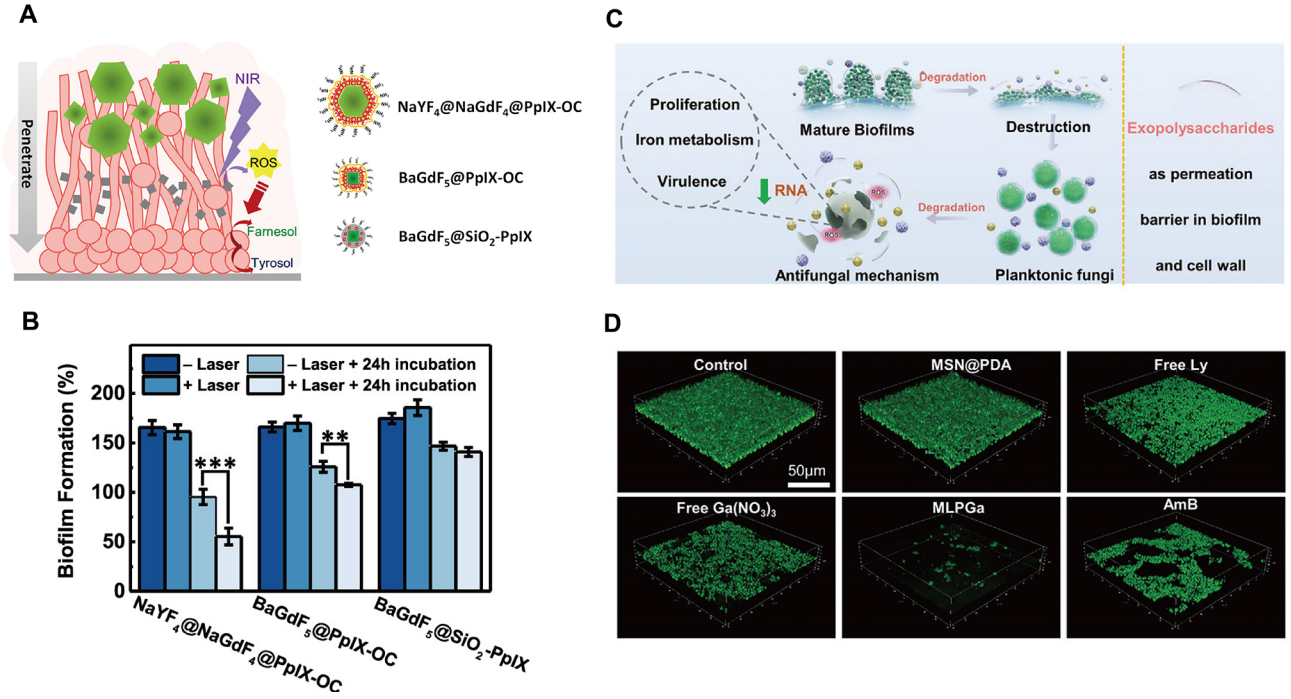


Fig. 2 (A) Schematic illustration of Chitosan-modified photodynamic nanoparticles destroying biofilm. (B) Inhibition efficiency of biofilm formation with PDT. The biomass was measured immediately after PDT (+ Laser) and 21.5 h after PDT (+ Laser + 24 h). Reproduced with permission [40]. Copyright 2023, American Chemical Society. (C) Schematic illustration of MLPGA antifungal strategy assisted by targeted degradation exopolysaccharides in the biofilms. (D) 3D confocal images of SYTO 9 stained mature *Candida. albicans* biofilms after different treatments. Reproduced with permission [46]. Copyright 2022, Wiley-VCH

activity, but also has good surface modifiability, which can enhance the activity of many antibiotics through a multivalent effect [43, 44]. Fu-AuNPs can effectively destroy the biofilm generated by the group interaction and thus effectively enhance the therapeutic index of drugs [45]. He et al. constructed lyticase and gallium ions nano-system (MLPGA), where lyticase can degrade extracellular polysaccharides in mature biofilm, such as 1,3-β-glucan. After the biofilm is destroyed, gallium (III) ions (Ga (III)) can enter the cells of *Candida albicans*. The radius of Ga (III) is almost the same as that of Fe, and it can act as a "Trojan horse" to simulate Fe. Ga (III) has no function of nutrient supply, so the invade of Ga (III) can block the iron metabolism of *Candida albicans*. The abundant ROS produced by MLPGA interfered with the transcription of genes related to antioxidants, extracellular polysaccharides, iron ions related pathway, biofilm development and virulence. Finally, MLPGA effectively eliminated the mature biofilm and killed the planktonic cells (Fig. 2C-D) [46].

Metal nanoparticles have helped many anti-biofilm drugs to improve their efficacy in the treatment of *Candida albicans* infection through their antifungal benefits and unique physical properties. However, the shortcomings of strict preparation conditions and high production costs hinder the large-scale clinical application of metal nanoparticles. At the same time, it should not be overlooked that metal particles also have a certain physical toxicity, so more caution is required when selecting materials. In addition, the complex antifungal mechanism of inorganic nanoparticles needs further investigation.

Nano-preparations can empower many new drugs with satisfactory biofilm inhibition through their strong targeting ability and penetration ability. The collapse of biofilm can attenuate the drug resistance of *Candida albicans*. Nano-agents can integrate various benefits and inhibit biofilm growth in multiple ways. In this way, more promising compounds can become an effective and safe drug.

Innovative formulations of drugs targeting cell membrane and cell wall

The cell wall and cell membrane are the key factors in maintaining the structural integrity of *Candida albicans*, which are currently targeted by three types of clinical antifungal drugs to combat *Candida albicans*. Azoles inhibit the activity of 14-α-demethylase (CYP51) by binding to the heme group in its active site, thus blocking the synthesis of ergosterol, a key component of the fungal cell membrane, and exerting an antifungal effect [47]. The traditional view is that polyene drugs bind directly with ergosterol and form a drug-lipid complex that is introduced into the cell membrane of the fungus, leading to leakage of intracellular components and eventual cell death. However, this theory has also been challenged in recent years by structural and biophysical research. The latest research results show that AmB extracts ergosterol from the fungal cell membrane by forming extracellular aggregates that act like a "sterol sponge" [48]. Echinocandins exert an antifungal effect by destroying the cell wall, which is not present at all in mammalian cells so that they also have good fungal specificity. Echinocandins prevent the synthesis of (1,3)-β-D-glucan by inhibiting (1,3)-β-D-glucan synthase leading to a loss of cell wall integrity and severe cell wall stress [49].

However, the adaptive mechanisms for inactivating these drugs, such as alteration of the drug target, overexpression of the efflux pump and the stress response of the cells, lead to the drug resistance in clinical infections(Fig. 3) [50, 51]. In addition, the serious side effects of antifungal drugs have always limited their use. AmB, for example, can easily bind with cholesterol, resulting in high nephrotoxicity and inability to clear *Candida albicans* [52]. The optimization of existing antifungal drugs with the help of nanotechnology is therefore a timely and feasible way to solve the problem of high toxicity and drug resistance of existing drugs.

The cell membrane module represents an innovative biomaterial and drug delivery system. By decorating nanoparticles, the cell membrane empowers them to evade immune recognition, enhance their systemic circulation lifespan, target recognition of pathogenic fungi and neutralize toxins in combating *Candida albicans* infections. These advantages make this transformational approach a promising strategy for treating *Candida albicans* infections [53–55]. Xie et al. encapsulated AmB with cationic liposomes and decorated with red blood cell (RBC) membrane to obtain RBC-LIP-AmB. Pathogenic fungi can interact with host red blood cells, so RBC-LIP-AmB is able to recognize *Candida albicans* and neutralize the blood toxin (Fig. 4A). Domain of key transmembrane receptors within the cell membrane and the corresponding peptide as a specific ligand, specifically connecting the surface of ligand-modified nanoparticles with the inner side of the cell membrane. The modification ensures that the cell membrane has the correct orientation and improves the targeting effect of AmB while attenuating the toxicity of the drug [56]. Due to their intrinsic expression, biomimetic nanoparticles derive the expression of cell membrane receptors that can perform target recognition, toxin neutralization, and immune regulation [57, 58]. For example, the pattern recognition receptors (PRRs) on immune cells can identify the pathogen-associated molecular patterns (PAMPs) in fungi. PRRs such as dectin-1, dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN) as well as TLR2 and TLR4 interact with PAMPs such as β-glucans, N-mannan and O-mannan, respectively [59, 60]. The targeted

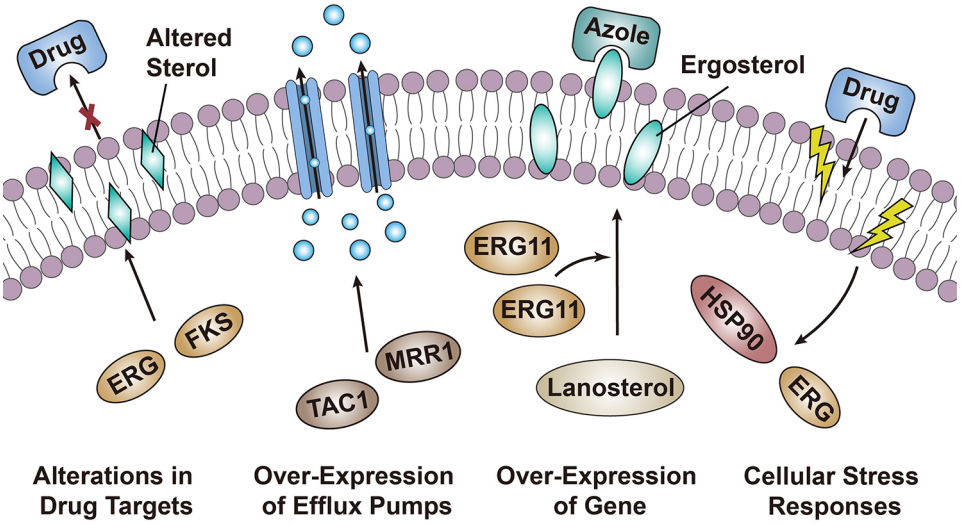


Fig. 3 Schematic illustration of the most important resistance mechanisms of current drugs in *Candida albicans* infections. Mutations in certain genes, including the *ERG* and *FKS* genes, result in alterations to the drug targets encoded by these genes. Additionally, mutations in two specific transcription factors trigger the upregulation of ABC transporters and MF transporters, ultimately leading to the overexpression of the drug efflux pump. Over-expression of genes leads to an increase in the number of targets for drugs, such as ergosterol, thereby diminishing the therapeutic effectiveness of the drugs. When drugs act on the cell membrane, it generates pressure, and this cell membrane stress can also impact certain regulators and upregulate certain genes, thereby promoting drug tolerance

therapy of fungal pattern recognition receptors has also led to encouraging results. The Dectin-1-coated liposome AmB improved the antifungal ability of the drug and attenuated the toxicity of the drug [61]. Existing drugs are meticulously engineered into a precision bullet through the innovative fusion of bio-bionics, advanced material science, and cutting-edge technology. This targeted approach ensures the drugs strike the intended target with unprecedented accuracy, enhancing drug utilization efficiency while safeguarding healthy tissues from unnecessary exposure.

Stimuli-responsive drug delivery systems represent a pivotal strategy in achieving precise drug administration. These systems discern the environmental stimuli signals, such as variations in pH, ionic strength, redox conditions, and the presence of specific proteins or enzymes, and consequently decide on the release of drugs. These signals induce conformational alterations in nano-carriers, modulate the interactions between drugs and their carriers, and even disrupt the structural integrity of the carriers, thereby facilitating precise and controlled drug release [62, 63]. Park et al. have also developed a novel carrier system for AmB based on pH-sensitive and redox-sensitive polymers functionalized by coupling with the antifungal peptide histatin 5. As a new ligand, the antifungal peptide histatin 5 specifically targets fungal pathogens in infected animal models, making it both a targetable ligand and a synergistic antifungal molecule against *Candida albicans* (Fig. 4B). The infection site, characterized by an acidic environment, induces a vertical orientation of the histidine 5 peptide. This structural transformation enables the active form of histidine 5 to target *Candida albicans* with precision. Additionally, the immune cells residing at the site of infection, in response to oxidative stress, produce substantial quantities of glutathione and thioredoxin. These molecules disrupt the redox-sensitive disulfide bonds of particles, ultimately triggering the release of AmB. The pH reaction and the redox-sensitive drug carrier improve the accuracy of drug delivery and achieve lower clinical dosing, which plays a role in improving drug efficacy and reducing toxicity [64, 65].

Interestingly, it has been shown that nanomaterials can also act on the cell wall and cell membrane of *Candida albicans* in addition to modifying existing drugs. This has developed into a powerful weapon against *Candida albicans* infection. Chitosan nanoparticles with positive charge can bind to fungal cell walls and cell membranes with negative charge, which will lead to increased permeability of cell envelopes and leakage of cytoplasmic contents from the cells, including ions and protein [66]. Since chitosan is poorly soluble, the solubility in physiological environment can be improved by preparing chitosan into nanoparticles. Adding chitosan to nanoparticles also increases the surface-to-volume ratio, resulting in an increase in positive charge density at the surface, stronger and more frequent association with cell walls and membranes, and increased antifungal activity [67]. Ag^{+} is widely used in many antifungal materials because Ag^{+} can combine with negatively charged inorganic anions, proteins and other molecules to disrupt the growth of *Candida albicans*. Only at a certain concentration can

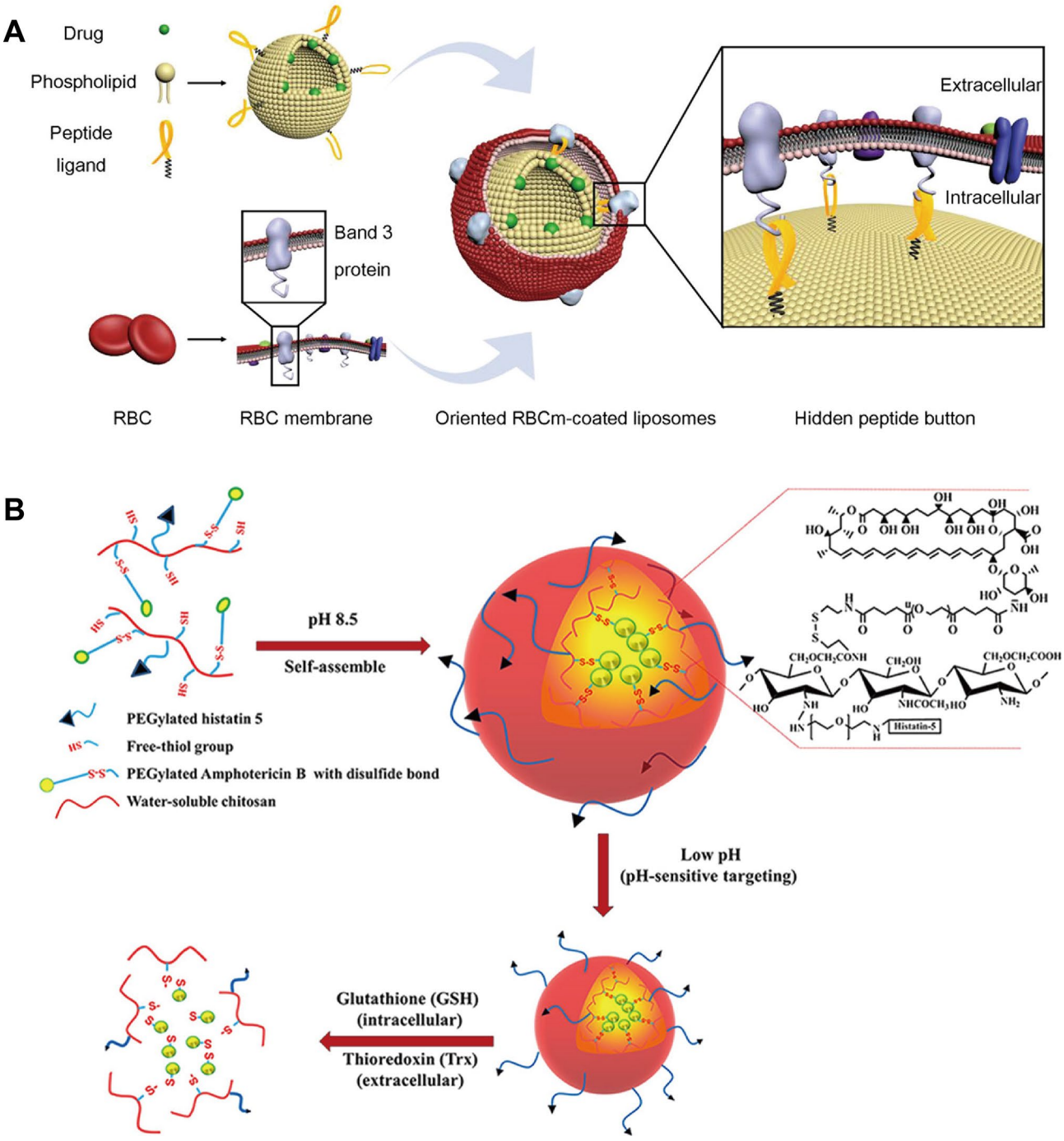


Fig. 4 (A) Schematic illustration of fabrication process of the liposomes and decoration with red blood cell membrane. The transmembrane domain of Band3 on the red blood cell membrane can connect the P4.2-derived peptide on the surface of nanoparticles. Once anchored onto the liposome surface, the nanoparticle engages with the isolated RBC membrane using a "molecular affinity" strategy. This interaction serves to orient the right side outward and enhances AmB targeting capability towards the cell membrane. Reproduced with permission [56]. Copyright 2019, American Chemical Society. (B) Schematic illustration of a nanoparticle conjugated with histatin 5 and AmB. Antifungal peptide histatin 5 as a novel ligand that targets fungal pathogens specifically in infected animal models. pH-sensitive histatin 5 and AmB with a redox-sensitive linker were self-assembled into nanoparticle to enhance ability of targeting infection site. The accuracy of AmB delivery to cell membrane is improved, which can reduce the toxicity and adverse reactions of drugs. Reproduced with permission [64]. Copyright 2017, Elsevier

they exert an antifungal effect, while the strong cytotoxicity also limits their clinical application. Nano silver cannot be easily inactivated due to its particulate properties, and its controlled release also makes it unnecessary to use a large dose at one time. The properties of nano-silver solve the problems of silver ions skillfully, so it is also promising for clinical antifungal treatment. In addition, with the continuous research on nano-silver, it is gradually found that the antifungal effect of nano-silver is antagonistic to *Candida albicans* through various mechanisms. Radhakrishnan et al. found that silver nanoparticles (AgNPs) can produce dose-dependent ROS, which can alter the surface morphology, cell ultrastructure, membrane fluidity, ergosterol content and fatty acid composition, thus damaging the cell membrane of *Candida albicans*. ROS can destroy the cell wall and cell membrane of fungi. In addition to nano-silver, metal nanoparticles such as zinc oxide, gold, iron oxide (Fe_3O_4) and titanium oxide (TiO_2) can also generate ROS, which can be used to combat fungi [68–71]. This destruction leads to an increase in the permeability of the fungi and the loss of cytoplasm contents, which leads to the death of the fungi [72]. Ashraf et al. synthesized silver, copper, and chitosan nanoparticles individually, and then copper-silver-chitosan nanocomposite was synthesized. Chitosan nanoparticles were inserted in the polymer matrix and dispersed on the superficies of the prepared bio-nanocomposites. Chitosan helps nanocomposite adhere to the cell wall and membrane, and then silver nanoparticles and copper nanoparticles play an antifungal role. Compared with other combinations, this nanocomposite has the best anti-*Candida albicans* effect. The therapeutic effects of the investigated nanocomposite are comparable to AmB as a standard material [73, 74].

The lipid bilayer of liposomes containing antifungal drugs can rapidly fuse with the plasma membrane of microbial cells so that a high concentration of drugs can be released into the plasma membrane or cytoplasm of microorganisms at one time. In this way, liposomes can bypass the resistance mechanism of poor drug uptake, resulting in faster drug release and higher drug concentration in the cytoplasm. When present in saturated concentration, the drug can overcome excretion through the transmembrane pump, reducing the loss of the drug from the microbial cells. Therefore, liposomes also overcome the mechanism of drug resistance led by the efflux of the drug [75, 76]. Some materials are used as agents against *Candida albicans*, and their own properties play a major role. These materials can also be processed into nano-sized particles, which help the materials to improve the bioavailability and stability, and overcome their toxicity.

The cell walls and cell membranes are the target of the most drugs against *Candida albicans*. The cell wall, which does not exist in the host cell, is unique to eukaryotes. The emergence of resistance and the complex resistance mechanism pose a major challenge for current anti-*Candida albicans* drugs. If the anti-*Candida albicans* drug is limited to the target at the cell wall and cell membrane, it will bring many unpredictable problems, such as "super fungi". At the same time, some drugs that can attack the cell membrane of the fungus will almost inevitably have an impact on the cell membrane of normal tissues. The optimization of existing drugs in terms of their structure or dose form will improve drug performance or develop new targets on cell walls and membranes. How to use nanotechnology to help drugs overcome obstacles and enter the cell is worthy of our further investigation.

Emerging targets-subcellular organelles

The main difference between *Candida albicans* and common bacteria lies in its eukaryotic properties, and the structural features and physiological functions of fungal pathogens are more similar to those of their hosts [77]. *Saccharomyces cerevisiae* as a eukaryotic model is due, for example, to the fact that many basic biochemical and cellular biological processes have been transferred from fungi to humans, and that many active molecules that have pharmacological effects in yeast are also effective in humans. Therefore, the three types of clinical antifungal drugs are also limited to the unique structure of fungi as mentioned above [78]. The molecular structure and expression level of fungal receptors are easily altered by fungal mutations, which impairs the affinity of antifungal drugs to them. This problem becomes increasingly difficult the longer current clinical antifungals are used [79, 80]. Therefore, it is particularly important to look for some conservative antifungal targets that cannot be easily mutated. Subcellular organelles in fungi are better suited as therapeutic targets for super-fungi, such as fungal mitochondria, nuclei, the endoplasmic reticulum and extracellular vesicles (Fig. 5). When a mutation occurs, the conservative properties of the subcellular organelles (such as structure and composition) are not easily altered [81]. Compared to molecules that are randomly distributed in the cytoplasm, drug molecules concentrated in subcellular compartments are more difficult to excrete, which improves the bioavailability of drugs while reducing the development of drug resistance [82, 83]. Therefore, subcellular organelles are promising targets for the development of organelle-specific antifungal therapy that has a satisfactory killing effect and can reduce the risk of antifungal drug resistance.

Nucleus

Flucytosine is one of the most common drugs used to target fungal nuclei. Flucytosine enters the cell nucleus through a cytosine permease found only in fungal cells and is converted to fluorouracil, which replaces uracil

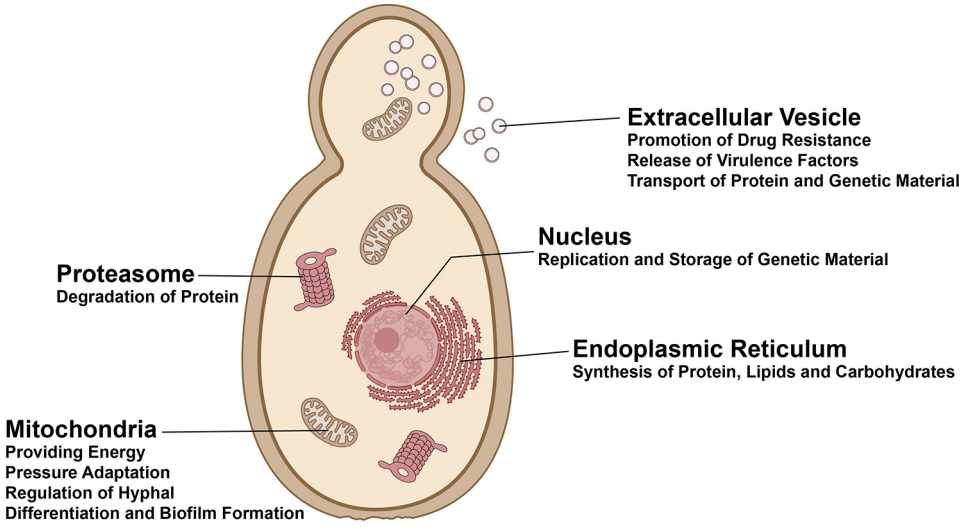


Fig. 5 Schematic illustration of main organelles in *Candida albicans* as antifungal targets and their physiological functions

in the fungal DNA, thus blocking DNA synthesis [84]. However, the alteration of cytosine permease (encoded by *FCY2*) and the mutation of deaminase (encoded by *FCY1*) in *Candida albicans* lead to the increasing drug resistance of flucytosine [85]. If flucytosine is used alone in antifungal therapy, the fungi will acquire a strong resistance to the drug in a short time. Therefore, flucytosine is mainly used in combination with AmB in clinical applications [86]. Faced with the stubborn drug resistance of flucytosine, researchers began to take a fresh look at drugs targeting fungal DNA synthesis and developed the corresponding nano-agents against *Candida albicans*. Among them, nitroxide-releasing nanoparticles take this as the main target and eliminate *Candida albicans* by three mechanisms. Firstly, when the concentration of released NO rises above 1mM, reactive nitroxide categories (RNOS) with antifungal activity are formed, which react with the amino acid residues cysteine, methionine, tyrosine, phenylalanine and tryptophan of the *Candida albicans* protein, especially the plasma membrane protein. Secondly, RNOS causes direct nitrosation damage to DNA, including chain breaks, formation of non-alkaline sites and deamination of cytosine, adenine and guanine. Increased production of H_2O_2 and alkylating agents induced by RNOS in turn destroys DNA. DNA repair enzymes can also be inhibited by RNOS. Finally, when present at a high enough concentration, RNOS irreversibly binds to Fe (II) from heme, resulting in the removal of heme from proteins and depletion of Fe in cells. RNOS can also inactivate zinc metalloproteins and thus inhibit microbial cellular respiration. In addition, NO can stimulate the innate immune response, activate macrophages and promote the production of cytokines, thus inhibiting *Candida albicans* invasion [87, 88].

Mitochondria

Mitochondria play a central role in the morphological changes, virulence and drug resistance of fungi and are considered the fatal weak point of fungi. Compared to the model yeast *Saccharomyces cerevisiae*, the mitochondria of fungal pathogens are different [89]. The function of the electron transfer chain (ETC) in the mitochondria influences the pathogenicity of *Candida albicans* and includes many aspects: the growth and development of the fungus, the sensitivity to drug, the immune response of the host and the transformation of the yeast into hyphae [90]. There are more than 1,000 proteins in the mitochondria, most of which are encoded by the cell nucleus and are highly conserved [91]. Therefore, mitochondria are considered as suitable target organelles for killing fungi [81, 83, 92]. In recent research, Zhou et al. have developed a type of photodynamic therapy using mitochondria-targeted luminophores (AIEgens) with aggregation-induced emission properties. They chose a photosensitizer with a cationic charge and suitable hydrophobicity, which is more likely to target the mitochondria of eukaryotic cells. The cationic AIE photosensitizer is able to distinguish *Candida albicans* cells from normal cells due to the different surface membrane potentials of fungal and mammalian cells. Toxic $^1O_2$ is produced in the mitochondria of *Candida albicans*, which destroys the integrity of the mitochondria and kills *Candida albicans*. Therefore, this photodynamic therapy can target fungal mitochondria without damaging normal mammalian cells [39, 93]. Metal nanoparticles with multiple functions can also eliminate fungi by targeting mitochondria. It has been found that gold nanoparticles can interact with fungal mitochondria, leading to inappropriate reduction of molecular oxygen during adenosine triphosphate (ATP) synthesis through coupled proton and electron transfer

reactions and destruction of mitochondrial membrane [94]. Studies have also found that nano-silver can produce ROS in fungal cells, which can lead to a significant change in the redox situation of fungi and induce the decrease of mitochondrial membrane potential, causing the death of fungi [95].

In the development of antifungal agents, some compounds have also emerged that can act directly on the mitochondria. McLellan et al. found that the compound ML316 can selectively inhibit the mitochondrial phosphate carrier Mir1 in fungi and then reduce mitochondrial oxygen consumption, leading to an unusual metabolic catastrophe in which ATP production is inhibited and fungal death [96]. Arylamidine drug T-2307 accumulates in yeast cells via specific polyamine transporters, inhibits complexes III and IV of the respiratory chain and destroys the mitochondrial membrane potential of yeast. In addition, it can selectively destroy the mitochondria of pathogenic fungi without visible effects on the mitochondrial function of rat liver. Surprisingly, this drug has better efficacy than echinococcin against echinococcin-resistant fungi. The drug is currently in clinical phase II [97, 98]. Currently, the effect of these compounds on drug-resistant fungi is particularly impressive, but general compounds could have some side effects while naturally targeting the mitochondria. Careful safety considerations and appropriate modification of the dosage form are therefore required to ensure that active substances targeting the mitochondria can really be used clinically.

One of the main problems with drugs that attack organelles is that it is difficult for ordinary drugs to penetrate cell walls and cell membranes and act on intracellular organelles at the same time. Many biological medicines can also show their unique advantages in this situation. Wu et al. were inspired by the cell-penetrating peptide octaarginine and expanded it to 28 residues poly (D, L-homoarginine) to achieve effective toxicity against fungi. The positive charge-shielding effect and partial zwitterionic structure induced by a certain amount of L-glutamic acid effectively reduce the normal cytotoxicity of polypeptide polymers and the interaction between polypeptide polymers and plasma proteins to avoid protein aggregation/precipitation. Polypeptide polymers effectively penetrate the membrane structure of *Candida albicans*, accumulate in the cytoplasm, destroy the structure and integrity of the *Candida albicans* nucleus and lead to the release of important contents such as DNA or protein. At the same time, the mitochondria is also destroyed. Polypeptide polymers therefore directly destroy the organelles, show strong antifungal activity and do not easily develop drug resistance [99]. In addition, an antifungal peptide, Histatin5, can also penetrate the membrane along the potential into the cell, act on the mitochondria, cause ATP efflux without cell lysis, inhibit mitochondrial respiration and destroy the integrity of mitochondrial membrane, and finally cause the death of *Candida albicans* [100].

Mitochondria is important sites for the nutrient metabolism of fungi. Starvation therapy has been widely researched in tumor treatment by interrupting the supply of nutrients to the tumor cells [101]. It is also feasible to apply this idea to the treatment of *Candida albicans* infections. Previous studies have shown that there are many antifungal targets in the glucose metabolism of *Candida albicans* [102]. By inhibiting some vital enzymes, the energy intake of *Candida albicans* can be effectively suppressed. Impairment of α-glucosidase, acid trehalase, trehalose-6-phosphate synthase and class II fructose diphosphate aldolase confers antifungal activities to some inhibitors. Energy metabolism plays an important role in the virulence of *Candida albicans*. For example, glycolysis is upregulated for improved virulence during renal infection. During systemic infection in mice, mutants with limited glycolytic flux are not toxic. These results also show that *Candida albicans* adapt better to the environment through metabolism [102, 103]. In most cases, glucose is the preferred carbon source for *Candida albicans*. However, when *Candida albicans* colonizes, there is often a glucose deficiency. In this case, *Candida albicans* adapt to the glucose deficiency by using lactic acid, amino acids and N-acetylglucosamine. Mutants of related genes in these metabolic pathways have been shown to have low virulence [104, 105]. Furthermore, if *Candida albicans* uses oleic acid rather than glucose as a carbon source, its ability to cause a systemic infection is reduced [106]. Ji et al. showed that glucose oxidase (GOX)-modified MNPs (GMNPs) have high activity against *Candida albicans*. GOX consumes oxygen upon contact with *Candida albicans* biofilm and the induced ROS can destroy the dense biofilm matrix. In addition, GOX can block the metabolic pathway by consuming oxygen and glucose, thus "starving" *Candida albicans* [107]. However, when *Candida albicans* successfully invades the host, *Candida albicans* is exposed to blood containing 0.05-0.1%(3-5mmol/L) glucose or phagocytes containing extremely low glucose, both of which are far below the glucose concentration (1-2%) commonly used for yeast cultures in the laboratory [108]. This also shows that *Candida albicans* has a strong ability to adapt to changes in the glucose level in the environment. It is therefore difficult to distinguish whether the increase in drug effect due to the above-mentioned particles is a metabolic obstacle or other reasons. In addition, the iron metabolism of fungi is now also on everyone's lips. VL-2397, a structural analog of the iron carrier, replaced the iron carrier, which contains the element iron, and entered the fungi. It enters the fungi by active transport

via the siderophore iron transporter 1 (Sit1) and thus inhibits the fungi's iron metabolism. Sit1 is not found in mammalian cells, so the drug has good fungal specificity and safety. The drug is currently in clinical phase II research [109, 110]. There are currently few studies on the metabolic blockade of fungi, and all of them generally block only one metabolic pathway. Fungi have an admirable ability to adapt to the environment. Unilateral metabolic blockade is often overcome by metabolic compensation, autophagy and other mechanisms, and the antifungal effect achieved is often limited [111, 112]. The general mechanism of the mutual balance between the metabolic pathways of fungi still needs further research, but the inhibition of fungal metabolism from the perspective of multi-pathway and energy metabolism could bring good results in terms of metabolic balance and prevention of drug resistance.

Endoplasmic reticulum

In fungal cells, the endoplasmic reticulum plays a key role in lipid synthesis, protein folding, modification and secretion from eukaryotic cells, all of which are necessary for the biosynthesis of cell walls (targets for azole drugs). Due to the importance and specificity of the endoplasmic reticulum in fungal cells, this organelle is considered a promising target for antifungal agents [113–115]. The specificity of the endoplasmic reticulum is related to the expression of the enzyme CYP51, which is an antifungal target. Azole drugs can be localized to the endoplasmic reticulum, which can trigger an endoplasmic reticulum stress response and then kill *Candida albicans* [111]. However, the efficiency of localization of some azoles in the endoplasmic reticulum is compromised. Some researchers have developed azoles by combining 7-(diethyl)-aminocoumarin to the pharmacophore of fluconazole to improve the efficiency of location. This new compound is mainly located in the endoplasmic reticulum of cells, and its antifungal activity is 4 to 64 times higher than that of ordinary azoles [116–118]. With the help of nanotechnology, the therapeutic drugs may have the ability to target and thus act on the endoplasmic reticulum to achieve the purpose of treating systemic infection with *Candida albicans*. Many nano-drugs have a targeting effect on the endoplasmic reticulum. Mesoporous silicon is used to load adriamycin, which exerts pressure on the endoplasmic reticulum [119]. Aluminum oxide nanorods are used to load the calcium pump inhibitor of the endoplasmic reticulum thapsigargin and the autophagy inhibitor 3-methyladenine(3-MA) [120]. Quino-1-Fmoc-RACR, which is formed by covalently linking amphiphilic quinoxaline derivatives with Fmoc-modified Racr polypeptides, has self-assembled into nanoparticles that target the endoplasmic reticulum and have imaging functions [121]. After systemic administration, the nanodrugs must undergo the steps of systemic transport in vivo, targeting to the cell membrane, endocytosis, escape from endocytosis, targeting to the endoplasmic reticulum, and so on. All properties that can influence the performance of the above processes determine the final therapeutic effect [122]. The targeting of nano-drugs towards the endoplasmic reticulum encompasses two primary strategies: active targeting and passive targeting. During active targeting, nano-drugs, once inside cells via phagocytosis or reticulum-mediated endocytosis, are liberated from the confines of endosomes or lysosomes. Subsequently, they actively navigate towards the endoplasmic reticulum, facilitated by specific targeting molecules or moieties recognizing and binding to endoplasmic reticulum components. In contrast, passive targeting involves nano-drugs entering cells via selective endocytosis and subsequently undergoing intracellular transport, where they accumulate within the endoplasmic reticulum without specific targeting molecules. This passive approach relies on the inherent properties of the nano-drugs and cellular trafficking mechanisms [123, 124]. Nano-drugs must first precisely target cell walls and membranes before cellular entry, ensuring they avoid infiltrating unintended cells and mitigating the risk of toxicity. The aforementioned cell wall and membrane targeting technologies can be harnessed in this context to enhance specificity. Additionally, the ongoing development of endoplasmic reticulum-targeted moieties and molecules offers promising avenues for addressing this challenge [121, 125]. The creative assembly and design of nanoparticles will bridge this gap, enabling precise delivery of nano-drugs to their intended destinations within the endoplasmic reticulum.

Hsp90 is a molecular chaperone in the endoplasmic reticulum and a highly conserved molecular chaperone in eukaryotes that is involved in protein folding and stabilizes the activity of important protein factors. When pathogenic microorganisms are exposed to stress in the host, Hsp90 becomes an important regulatory factor to adapt to host stress and participate in the morphological development of the cell. It can change the conformation of steroid hormone receptors, regulate the signal transduction pathway of cells, improve the adaptability of fungi to external stimuli and increase the viability of fungi [126]. Hsp90 has multiple regulatory effects on the growth, virulence and drug resistance of pathogenic fungi. The targeting of Hsp90 holds great potential for antifungal therapy, as Hsp90 affects the growth, virulence and drug resistance of pathogenic fungi in a variety of ways [127]. However, it is also highly conserved in all eukaryotes (including human cells), leading to stagnation in the development of specific inhibitors. Recently, research focusing on the nucleotide binding domain of *Candida albicans* Hsp90 has shown that *Candida*

albicans Hsp90 has a unique conformational flexibility. Based on the specific conformational properties, David S. Huang et al. synthesized a series of amipyrazole-substituted isophthalic acid amides. They found that the inhibitor CMLD013075 possesses potent fungus-selective Hsp90 inhibitory activity, which can block the changes in the signaling network caused by the stress response, thereby preventing the development of drug resistance of *Candida albicans* and eliminating drug resistance after development [50, 128, 129].

It is expected that the use of nanomaterials to coat or combine antifungal drugs will better target the endoplasmic reticulum. The stress response of the endoplasmic reticulum has been extensively studied, but the target mechanism of the endoplasmic reticulum is not clear. Compared with tumor treatment and immunotherapy, there are still some problems to be solved in the treatment of fungal infections, such as the lack of targeted drugs and therapeutic mechanism. With further research, there may be more old and new drugs that can be used for this purpose.

Proteasome

The proteasome is a type of organelle that is responsible for the degradation of proteins. It directly affects the renewal of some proteins, including misfolded proteins and many proteins that play an important role in life activities. Proteasome inhibitors are used in the clinical treatment of myeloma, such as bortezomib [130]. In the development and screening of natural products, two new antifungal agents, fellutamides C and D, were isolated and clarified from fungal fermentations, which exhibit proteasome inhibitory activity. These inhibitors can prevent the degradation of false proteins produced during the folding process of fungal proteins and then kill the fungi, and have fungal-specific inhibition. These two compounds are effective against *Candida albicans* and the MIC of fellutamides against *Candida albicans* is less than 4 μg/ml [131–133]. The Venetin-1 nanoparticle, a protein-polysaccharide fraction or complex derived from the coelomic fluid of the earthworm, exhibits potent antifungal activity against *Candida albicans*. Notably, this nanoparticle effectively inhibits the 20 S proteasome without eliciting endocrine toxicity or cytotoxicity toward normal cellular functions. Compared with conventional synthetic inhibitors, natural protease inhibitors like Venetin-1 demonstrate a greater potential in circumventing drug resistance mechanisms, boasting enhanced stability, and reducing toxicity profiles [134, 135].

The eukaryotic properties of *Candida albicans* really limit the understanding of drug targets, but there are still some specific differences that could mean an effective breakthrough for us. Over a period of four years, researchers have identified thousands of bioactive natural product extracts through adaptability testing and analysis to isolate novel compounds with previously uncharacterized antifungal activity. The extracts were found to be active against the translation elongation factor yef3 (yefafungin), cAMP homeostasis (campafungin), the 26 S proteasome (fellutamide C and D), Microtubule dynamics (12-deoxo-hamigerone) and the Fe-S cluster protein dre2p (dretamycin), as well as other inhibitors of basic eukaryotic processes (including fatty acids, ergosterol and ribosome biosynthesis). These findings have created many opportunities for the development of fungi-specific drugs while expanding the ideas for the development of fungi-specific targets [131]. It is also worth noting that the difference in substrate specificity between fungi and human enzymes may also lead to fungal specificity.

Extracellular vesicle

Another subcellular organelle that deserves our attention is the extracellular vesicle. In the process of biofilm growth, vesicles with unique properties play a key role in the transport of substances across the cell wall and release some virulence proteins, such as secreted asparagine protease and lysophospholipase precursors, which strongly promote the development of drug resistance [136–138]. Some studies have investigated an antifungal agent called turbinmicin, which is found in marine microorganisms, and tested its therapeutic effect in the biofilm environment [139]. Zhao et al. found that turbinmicin destroyed the delivery of extracellular vesicles during biofilm growth, which impaired the subsequent build-up of the biofilm matrix. At the same time, the elimination of the biofilm matrix also made drug-resistant *Candida albicans* susceptible to turbinmicin [140]. Sec14 is a cytoplasmic protein in yeast that plays an important role in regulating various cellular functions including intracellular transport. Turbinmicin blocked the function of the Sec14 protein, which meant that yeast fungi such as *Candida albicans* could no longer germinate and multiply. No drug had previously targeted this protein. Since its discovery, some drugs have been able to act specifically on Sec14 of yeast, but not on Sec14 of non-human origin [139].

Developing subcellular organelle targets of *Candida albicans* has great advantages in treating drug-resistant fungal infections. Targeting subcellular organelles is a strategic approach that circumvents common gene mutation targets and evades expulsion by efflux pumps [82]. Consequently, drugs aim at these organelles often exhibit potent effects even against drug-resistant strains. Mitochondrial phosphate carrier Mir1 inhibitor ML316 has played a significant role in fighting fluconazole-resistant fungi. The researchers isolate fluconazole-resistant *Candida albicans* strains from HIV-infected people. The MIC of ML316 to fluconazole-resistant strains can reach

0.5 μg/ml, while the MIC of fluconazole is 128 μg/ml. When ML316 is combined with fluconazole, it can also significantly improve the antifungal ability of the drug. In the mouse model of drug-resistant fungal infection, the fungal load in the ML316 treatment group is reduced by about 100 times, which is equivalent to that in the fluconazole treatment group. Remarkably, when ML316 is administered in combination with fluconazole, the fungal load plummeted by over 1000-fold [96]. CMLD013075, an Hsp90 inhibitor, demonstrates equal growth inhibitory potency against both drug-resistant and wild strains, exhibiting synergistic effects when paired with fluconazole [129]. Given its distinct target mechanism compared to traditional drugs like fluconazole, most of these drugs can exhibit synergistic interactions with traditional drugs.

From a technical perspective in nanoparticle preparation, it is crucial to ensure the delivery of drugs that can efficiently target and destroy fungal subcellular organelles at specific lesion sites. This precise delivery mechanism helps to prevent undesired dispersion, absorption, or hydrolysis by non-targeted organelles, thus enhancing the efficacy of the treatment (Fig. 6). The fluid viscosity of the cytoplasm and its combination with intracellular components are thought to influence the diffusion factors of solutes into the cells. The subcellular fate of drugs is also influenced by the extent to which molecules can interact or even bind with subcellular components (such as cell membranes and organelles) [141, 142]. Due to the particular environment in the cell, we can select the carrier targeting subcellular organelles mainly from two aspects. The first is based on the inherent tendency of nanocarriers to specific compartments, and the second is based on the attachment of subcellular targeting ligands to the surface of nanocarriers to redirect their accumulation into the desired compartments [92]. If we find that the subcellular organelles have specific protein expression or that different molecules have the ability to accumulate in certain subcellular compartments when *Candida albicans* is infected, we might find a method to target subcellular organelles that are suitable for the treatment of *Candida albicans* infections. The problem of cell permeability or prodrug activation can be solved by the application of nano-drugs wrapped in functional materials, due to their controlled release ability. Therefore, the antifungal activity can also be maximally improved. At present, the drug resistance of targeting subcellular organelles is lower, but since there are fewer specific differences from mammalian cells, there is a risk of toxicity. With nanotechnology, this problem can be better avoided and toxicity to the host can be reduced. Some promising drugs listed below are expected to play a better role with the help of nanotechnology (Table 2).

Polymorphism of *Candida albicans*

Antifungal drugs are almost universally applicable in clinical treatment, and the different fungi have differently well adapted to the various broad-spectrum antifungal drugs. The main difference between *Candida albicans* and common fungi is the biphasic nature of *Candida albicans*. *Candida albicans* can transform into yeast and hyphae. The division of labor between the two forms is different (Fig. 7A). When *Candida albicans* is in the blood, *Candida albicans* exists in the form of yeast, which is suitable for *Candida albicans* to spread and multiply in the blood. When *Candida albicans* needs to invade internal organs, it uses hyphae to adhere and penetrate better [149, 150]. At the virulence level, hyphae is an important virulence factor of *Candida albicans*. When *Candida albicans* passes from the yeast stage to the hyphal stage, it releases candidalysin and secretes aspartic protease 4, aspartic protease 5, and aspartic protease 6, and expresses several specific genes encoding virulence factors. For example, *HWP1*, *ALS3* and *RBT5* encode cell wall proteins that are important for attachment to host cells and uptake of nutrients from the host [151, 152]. When *Candida albicans* is phagocytosed by macrophages, *Candida albicans* activates many Candida-specific genes, altering its metabolism and promoting the transformation of forms that help *Candida albicans* to transform from the yeast stage to the hyphal stage in the cells. It activates the inflammation-induced programmed death of macrophages via the caspase-1 pathway to complete phagocytosis and escape, and then escapes from the macrophages and returns to the tissue (Fig. 8A) [153–155]. The process of hyphae formation is also part of biofilm formation, and inhibition of hyphae formation can also effectively inhibit biofilm formation [156, 157]. Therefore, the formation of hyphae is the main cause of systemic infection by *Candida albicans*. The question of how to better inhibit hyphae formation or kill *Candida albicans* in hyphae form is of great importance for the treatment of systemic infections with *Candida albicans* and overcoming drug resistance.

As a potential solution to overcome antibiotic resistance, a new treatment method, anti-virulence therapy, is currently being applied in the field of killing pathogenic bacteria, which has made great progress in recent years. It is fully accepted as an ideal and practicable method and is increasingly being researched and used in the development of new antibacterial drugs [158, 159]. The hyphae of *Candida albicans* can produce toxicity, which is the main cause of virulence of the systemic infection. Although anti-virulence therapy of *Candida albicans* is not yet very advanced, experience with other pathogenic fungi gives reason to believe that anti-virulence achieved by inhibiting hyphal growth can effectively alleviate systemic infection. Blocking the ideal virulence factor has

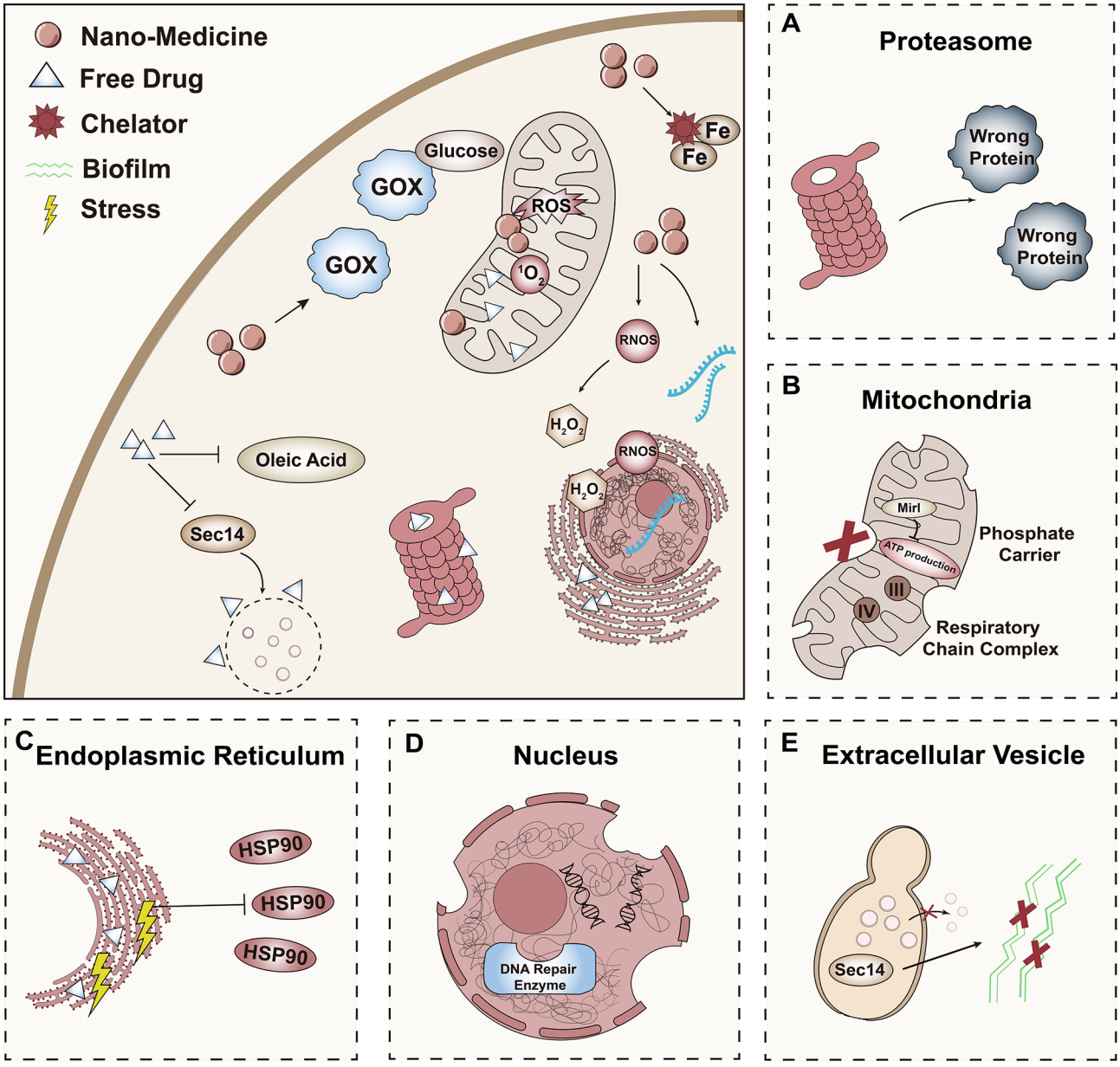


Fig. 6 The process of antifungal drugs targeting specific organelles and their subsequent effect on those organelles. (A) Certain drug molecules inhibit the proteasome, preventing the proteasome from degrading faulty proteins. (B) Many drugs can disrupt the structure of the mitochondria, damage the respiratory chain, and inhibit ATP synthesis, which ultimately leads to depletion of the energy supply. (C) Modified drugs show improved targeting of the endoplasmic reticulum and exert pressure on it. In addition, specific inhibitors of endoplasmic reticulum regulatory factors can attenuate drug resistance. (D) Drugs that target the cell nucleus are primarily aimed at destroying nuclear and DNA structures. They can also inhibit certain DNA-related enzymes, such as DNA repair enzymes. (E) Antifungal drugs hinder the transport of substances via extracellular vesicles and prevent the formation of biofilms by suppressing vesicle-related proteins

no effect on the survival and reproduction of the target flora, so that general selection pressure that controls the evolution of the flora is not altered and the applied selection pressure is lower than that of antibiotics. Since most virulence factors are only controlled by a single gene or several closely related genes, and horizontal gene transfer rarely increases the drug resistance of the receptor, the problem of drug resistance is less serious with antiviral drugs. Anti-virulence therapy can also circumvent harmful and long-known side effects of antifungal drugs that have been ignored. Ordinary antifungal drugs can kill many beneficial symbiotic flora while inhibiting pathogenic fungi. These symbiotic fungi are part of our microbial community that co-evolved with humans. They play a key role in human health by promoting the development of the immune system, improving our

Table 2 Promising antifungal drugs

Antifungal Agent	Targeted organelle	Target	Mechanism of Action	Advantages	Status	Ref
MLPGa	Biofilm	Biofilm matrix	Degradation of extracellular polysaccharides	Attenuated drug resistance	Preclinical	[46]
Fosmanogepix (APX001)	Cell wall	GPI-ankyrin	Inhibition of GPI-ankyrin synthesis	Negligible adverse reaction	Phase III	[143]
Nikkomycin Z		Chitin synthase	Inhibition of chitin synthesis	Synergistic activity with other antifungal drugs	Phase I	[144]
Amphotericin B Cochleate	Cell membrane	Ergosterol	Extraction of ergosterol from fungal cell membrane	Taking orally and minor complications of amphotericin B infusion	Phase II	[145]
RBC-LIP-AmB			Targeting *Candida albicans*	Better targeting ability and attenuated toxicity	Preclinical	[56]
FN7		CYP51	Blockade of ergosterol synthesis and production of oxidative stress	Better drug penetration ability	Preclinical	[146]
Orotomides	Nucleus	Dihydroorotate dehydrogenase	Targeting dihydroorotate and inhibition of fungal pyrimidine biosynthesis	A unique mechanism of action and excellent antifungal activity	Phase III	[144]
Nitric oxide-releasing nanoparticles		Structure of DNA	Release of RNOS and destroyed DNA structure	Attenuated drug resistance and better immune promotion	Preclinical	[88]
Aryldiamidines—T-2307	Mitochondria	Respiratory chain complex	Inhibition of respiratory chain complex	Better antifungal effect	Phase II	[97]
AIEgens		Mitochondria	Production of toxic ROS	Attenuated drug resistance and negligible toxicity	Preclinical	[39]
VL-2397		Iron ion	Disruption of the iron metabolism	Preferable safety	Phase II	[109]
MGCD290	Endoplasmic reticulum	Hsp90	Inhibition of Hos2 histone deacetylase and Hsp90	Adjuvants for antifungal drugs and attenuated drug resistance	Phase II	[147]
Fellutamides C and D	Proteasome	Proteasome	Inhibition of the function of proteasome	Fungal specificity	Preclinical	[132]
Turbinmicin	Extracellular vesicle	Section 14	Inhibition of Sect. 14 protein	High safety and satisfactory curative effect	Preclinical	[140]
AgNPS	Hyphae	Oleic acid	Production of ROS and decreased oleic acid	Preferable safety, stability and multiple antifungal mechanisms	Preclinical	[148]

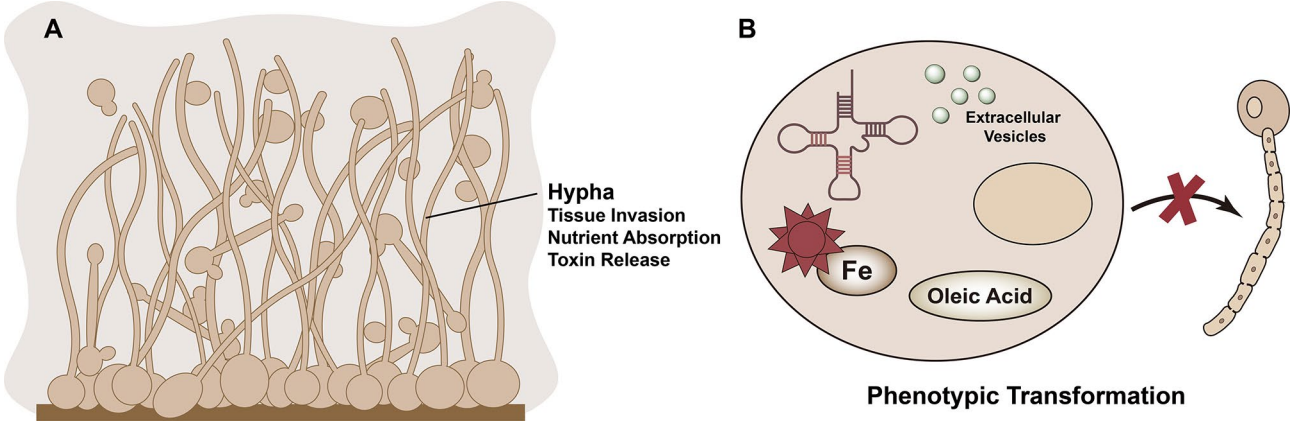


Fig. 7 (A) Schematic illustration of *Candida albicans* hypha and its physiological functions. (B) Inhibition of the phenotypic transformation can deprive *Candida albicans* of virulence by lowering the content of iron ions and oleic acid and controlling the gene expression of hyphae

metabolic balance and resisting colonization by pathogenic fungi. Once the balance of flora is destroyed, the reduction of autoimmunity is not conducive to defense against pathogenic fungi [159, 160]. At the same time, the long-term use of antibiotics leads to a large number of fungal groups that are insensitive to antibiotics, which will lead to new infections and double infections [161]. In the case of superficial infection with *Candida albicans*, anti-virulence therapy may have some limitations, as it cannot inhibit the growth of the pathogenic fungi. However, for systemic infection, antifungal drug therapy combined with anti-virulence therapy can inhibit the growth

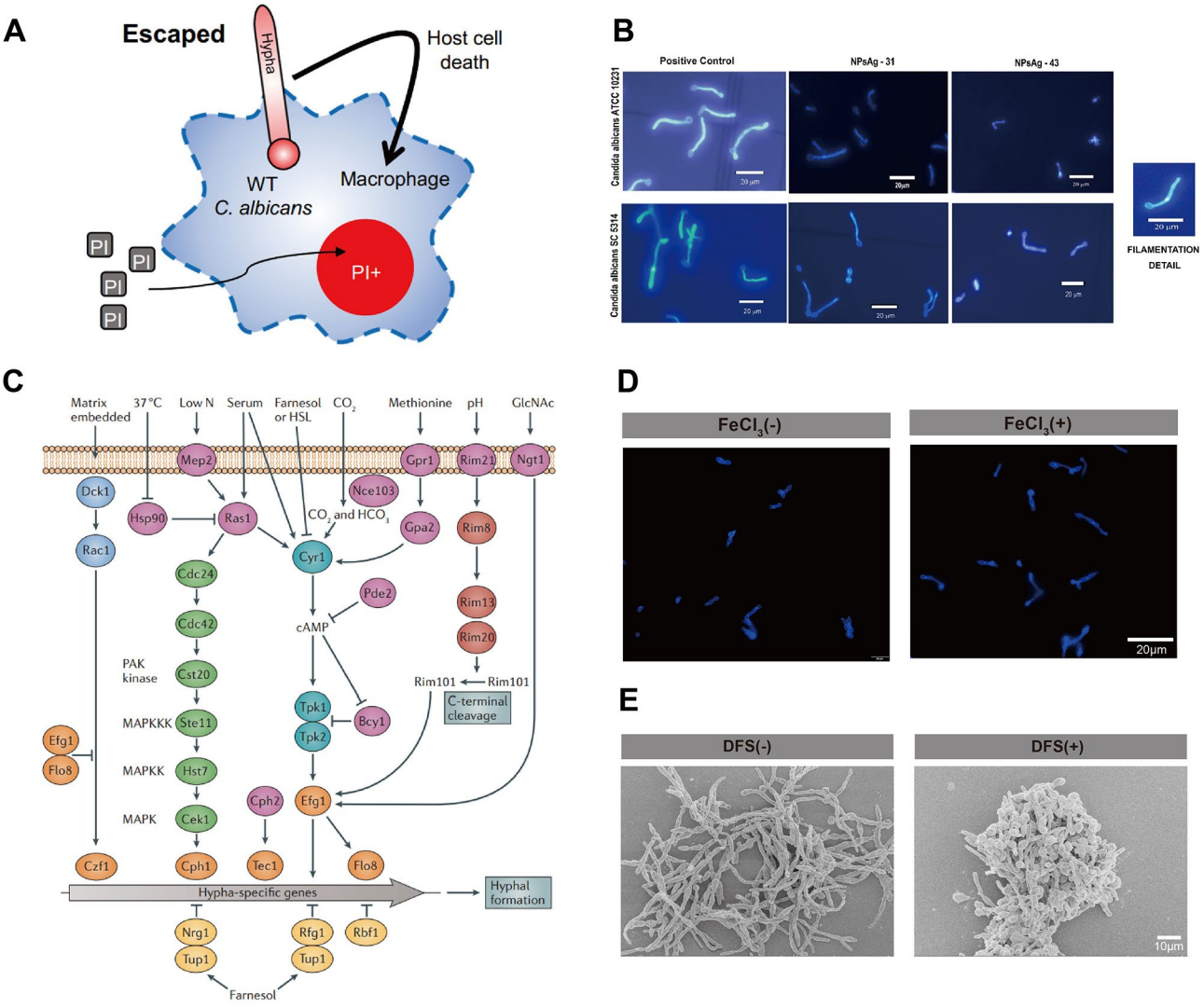


Fig. 8 (A) Schematic diagram illustrating the role of hyphae in mediating macrophage death and facilitating *Candida albicans* escape from macrophages. Reproduced with permission [178]. Copyright 2021, Springer Nature. (B) The filamentation morphology of positive controls, NPsAg-31 and NPsAg-43 of both *Candida albicans* SC 5314 and ATCC 10,231, using fluorescence with Calcofluor white. Reproduced with permission [148]. Copyright 2021, Springer Link. (C) Signal transduction pathways leading to expression of hypha-specific genes. Reproduced with permission [176]. Copyright 2011, Springer Nature. (D) The filamentation morphology of *Candida albicans* after co-incubation with YM medium (control) and $FeCl_3$, using fluorescence with Calcofluor white (unpublished data). (E) SEM images of *Candida albicans* after co-incubation with YM medium (control), and DFS with the concentration of 128 μg mL^{-1} (unpublished data)

of fungi, reduce the release and invasion of toxins, and overcome the negative effects of virulence factors on the immune system, which can achieve better therapeutic effect in infected patients with low immunity.

Conventional inorganic nanoparticles also have some effect in inhibiting the formation of hyphae. Studies have shown that the AgNPs can reduce the oleic acid content of *Candida albicans*, which is very important for the morphogenesis of hyphae. Targeting fatty acids such as oleic acid can influence toxicity and morphological transformation and thus inhibit the transformation of the yeast into hyphae (Fig. 8B). AgNPs also inhibit the production of virulence factors and biofilms. Targeting virulence could become a new paradigm for the development of nano-silver therapies for the clinical application of fungal treatment [72, 148]. To inhibit the virulence factor of *Candida albicans* hyphae, antisense RNA polymers have also been developed to control the gene expression of hyphae, but one of the problems of antisense polymers is low stability and easy degradation. Cationic liposomes can solve this problem, play a protective role, improve stability and finally achieve an inhibitory effect of more than 60% on the hyphae of *Candida albicans* [162, 163]. Extracellular vesicles secreted by human oral epithelial cells have been shown to inhibit the growth of hyphae. Their nanoscale size allows them to easily penetrate the

cell wall and enter the cell [164]. This also encourages us to pay attention to particle size when developing antifungal drugs in order to achieve better drug delivery.

Toenjes et al. have investigated the biological activities of 480 molecules, of which 53 molecules are cytotoxic to *Candida albicans* and 16 molecules can inhibit hyphal transformation without affecting germination and multiplication. 16 types of hyphal transformation inhibitors affect protein kinase, protein phosphatase, Ras signaling pathway, G protein-coupled receptor, calcium homeostasis, nitric oxide and guanylate cyclase signal transduction and apoptosis of mammalian cells, respectively [165]. This shows that many targets can affect hyphal transformation, and screening of compounds is helpful to find out the parts that can inhibit the hyphae. Plant products, such as resveratrol and curcumin [166, 167], microbial products, such as biatriosporin D, soporolipids, and lichen-derived usnic acid [168–170], animal products, such as ToAP2, centipede oil, have been shown to inhibit hyphae of *Candida albicans* [171, 172]. Among them, curcumin has hindered its practical use due to its poor solubility, acid and enzyme tolerance and other limitations. Sophorolipids (SL) is a biologically derived surfactant that can be used as an effective carrier for hydrophobic molecules. A curcumin-sophorolipid nanocomposite (CU-SL) with stable properties was prepared. The subinhibitory concentration of Cu-SL (9.37 μg/mL) significantly inhibited the adhesion of fungi to substrates and subsequent biofilm development, maturation and filament formation [167]. Ethanolic extract of propolis-loaded poly(lactic-co-glycolic acid) nanoparticles (EEP-NPS) were prepared to solve the problem of poor water solubility of propolis. EEP-NPs mediate effective anti-Candida activity and attenuate virulence factors by reducing the virulence-related proteins, thus destroying the existence of *Candida albicans* hyphae and weakening its virulence [173]. Various plant extracts were loaded into the Ag, Au and ZnO nanoparticles, which caused inhibition of hyphae and attenuation of toxicity [174, 175]. Therefore, many promising compounds can be investigated when considering hyphae as a toxic target of *Candida albicans*. Nano-delivery technology is the right-hand for these new compounds, solving many applicable problems with the physical and chemical properties of the compounds.

The causes for the formation of hyphae are complicated. In the *vitro* model, hyphal growth is influenced by temperature, serum, CO_2 and some other signals (such as starvation) [176]. In the host environment, the internal environment and the immunological environment influence the growth of hyphae, such as pH, CO_2, glucose and ferritin levels of the different organs in the growth environment and phagocytosis of macrophages (Fig. 8C) [152, 176–178]. Previous studies have shown that *Candida albicans* can extract iron from ferritin and that the hyphae have a greater ability to absorb iron [152]. Our research has shown that free iron as a nutrient can promote the growth of *Candida albicans* hyphae (Fig. 8D). We therefore chose an iron chelating agent to specifically inhibit the growth of hyphae. It has been proven that iron chelating agents such as deferasirox (DFS) can inhibit the growth of fungi. In recent years, studies have also shown that they have an inhibitory effect on *Candida albicans*, but the mechanism of action is still unclear(Fig. 8E) [179]. From the viewpoint of promoting hyphal growth by free iron, we found that DFS can inhibit the hyphal growth of *Candida albicans* yeast type by chelating iron ions in the growth environment and then realizing the treatment of *Candida albicans* infection. The nano-lipid vesicles of DFS developed by us have a significant inhibitory effect on hyphae in vivo experiments and considerably improve the survival of infected mice. MLPGA nanoparticles can destroy the biofilm, the Ga (III) ions can penetrate the cells of *Candida albicans*, and the radius of Ga (III) is almost the same as that of Fe, which blocks the iron metabolism of *Candida albicans*. The research focuses on destroying the biofilm and killing the planktonic cells. Further studies of the effect on *Candida albicans* hyphae could broaden the scope of application and increase the value of MLPGA nanomedicine in severe *Candida albicans* infections [46]. In addition, hyphal growth is regulated by many genes and signaling pathways, of which the hyphal G1 cycle 1 (*Hgc1*) is essential for hyphal growth. There are two pathways, namely the MAPK pathway, which is mediated by *Cph1*, and the cAMP-PKA pathway, which is mediated by *Efg1* [180, 181]. The mechanism of hyphae formation in the human body is still unclear, which is a major obstacle to drug development. Future research should focus more on how *Candida albicans* accomplishes hyphal transformation in the human physiological environment and identify the key factors that trigger the process of hyphal transformation and invasion. The problem of systemic infection with *Candida albicans* can be solved more easily by restricting *Candida albicans* to the yeast (Fig. 7B).

Conclusion and prospect

With the increasing use of immunomodulation therapy, the number of patients exposed to the risk of *Candida albicans* infection is rising. We confront a formidable challenge characterized by a surging number of high-risk patients, a growing prevalence of refractory microorganisms, and a sluggish pace in the development of novel drugs. A pivotal reason contributing to this sluggish progress stems from the scarcity of viable targets for drug discovery, primarily attributed to the eukaryotic nature of *Candida albicans*. Another major obstacle to the development of new drugs is that therapeutic agents cannot

be delivered precisely to the target site of anti-*Candida albicans*.

Among the targets of *Candida albicans*, besides the traditional cell wall and cell membrane, the subcellular organelles deserve our attention as they have high antifungal activity and low drug resistance, which has led to the natural antifungal benefits of these targets. Currently, there is a growing body of research focused on antifungal drugs that target mitochondria, many of which demonstrate potent inhibitory effects on drug-resistant fungi. For instance, T-2307 exhibits significant in vivo efficacy against uncommon echinococcosis-resistant strains. Constructing drug-resistant standard *Candida albicans* strains involves inducing them through stress conditions, gene editing, and isolating them from patients. It is crucial to employ diverse methods to construct these strains and validate their efficacy in developing novel clinical drugs to combat drug resistance. While some drugs show promise in mouse infection models, the complexity of fungal drug resistance formation, diverse human environments, and significant individual variabilities often lead to disappointing outcomes in clinical trials. The feasibility of these drugs for clinical application and the potential for nanotechnology to enhance drug delivery and targeted therapy require extensive verification through artificial intelligence and rigorous experimentation.

Currently, there exists a notable dearth of research focusing on the development of novel targets for *Candida albicans*, particularly at the subcellular organelle level. Nevertheless, this domain holds immense potential and significance for applications, underscoring the urgency for heightened investment to expedite research breakthroughs, particularly in the realm of 'super fungi'. Furthermore, the treatment of *Candida albicans* infections is frequently subsumed under generalized clinical fungal therapies, overlooking the distinct characteristics and heightened toxicity of *Candida albicans*, particularly its virulence mediated by hyphal formation, which sets it apart from other fungal species. The superficial infection with *Candida albicans* is quite common in our lives and the public is less concerned about it. However, once *Candida albicans* infection occurs in special people with low immunity, the mortality rate from deep systemic infection is also an unacceptable tragedy. Emerging nano-agents are a promising means of drug delivery in modern pharmaceutical technology. They can refine drug attributes, minimize toxicity while enhancing efficacy, pinpoint individual targets, and drive inherent antifungal properties. We believe novel nanomedicine can hit crucial areas like a rifle bullet to improve the therapeutic prognosis of systemic *Candida albicans* infection.

Acknowledgements
Not applicable.

Author contributions
Y.G wrote original draft . Q.Y.C, Y.Y.X and Y.W prepared figures. L.D revised manuscript. L.T.M, J.P.Y, Y.N.L and H.H wrote review .

Funding
This article was supported by the Natural Science Foundation of China (32200154 to J.Y.), the Natural Science Foundation of China (82203031 to Y.L.).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All authors gave their consent for publication.

Competing interests
The authors declare no competing interests.

Author details
[1]International Center for Synthetic Biology, School of Food and Pharmaceutical Engineering, Nanjing Normal University, Nanjing 210023, China
[2]Nanjing Stomatological Hospital, Nanjing 210008, China

Received: 13 June 2024 / Accepted: 7 September 2024
Published online: 16 September 2024

References

1. Pappas PG, Lionakis MS, Arendrup MC, Ostrosky-Zeichner L, Kullberg BJ. Invasive candidiasis. Nat Reviews Disease Primers. 2018;4:1–20.
2. Koehler P, Stecher M, Cornely OA, Koehler D, Vehreschild MJ, Bohlius J, et al. Morbidity and mortality of candidaemia in Europe: an epidemiologic meta-analysis. Clin Microbiol Infect. 2019;25:1200–12.
3. Hosseini-Moghaddam SM, Ouédraogo A, Naylor KL, Bota SE, Husain S, Nash DM, Paterson JM. Incidence and outcomes of invasive fungal infection among solid organ transplant recipients: a population-based cohort study. Transpl Infect Disease. 2020;22:13250.
4. Denning DW. Global incidence and mortality of severe fungal disease. Lancet Infect Dis. 2024;24:428–38.
5. Liu N, Zhou J, Jiang T, Tarsio M, Yu F, Zheng X, Qi W, Liu L, Tan J, Wei L, et al. A dual action small molecule enhances azoles and overcomes resistance through co-targeting Pdr5 and Vma1: Osimertinib targets Pdr5 and Vma1. Translational Research: J Lab Clin Med. 2022;247:39–57.
6. Liu N-N, Zhou J, Jiang T, Tarsio M, Yu F, Zheng X, Qi W, Liu L, Tan J-c, Wei L. A dual action small molecule enhances azoles and overcomes resistance through co-targeting Pdr5 and Vma1. Translational Res. 2022;247:39–57.
7. Calderone RA, Fonzi WA. Virulence factors of *Candida albicans*. Trends Microbiol. 2001;9:327–35.
8. Wenzel RP. Nosocomial candidemia: risk factors and attributable mortality. Clin Infect Dis. 1995;20:1531–4.
9. Enoch DA, Yang H, Aliyu SH, Micallef C. The changing epidemiology of invasive fungal infections. Hum Fungal Pathogen Identification: Methods Protocols. 2017;10:17–65.
10. Roemer T, Krysan DJ. Antifungal drug development: challenges, unmet clinical needs, and new approaches. Cold Spring Harbor Perspect Med. 2014;4:a019703.
11. Lombardi T, Budtz-Jørgensen E. Treatment of denture-induced stomatitis: a review. Eur J Prosthodont Restor Dent. 1993;2:17–22.
12. Nett JE, Andes DR. Antifungal agents: spectrum of activity, pharmacology, and clinical indications. Infect Disease Clin. 2016;30:51–83.
13. Onishi J, Meinz M, Thompson J, Curotto J, Dreikorn S, Rosenbach M, Douglas C, Abruzzo G, Flattery A, Kong L. Discovery of novel antifungal (1, 3)-β-D-glucan synthase inhibitors. Antimicrob Agents Chemother. 2000;44:368–77.

14. Beyda ND, Lewis RE, Garey KW. Echinocandin resistance in Candida species: mechanisms of reduced susceptibility and therapeutic approaches. Ann Pharmacother. 2012;46:1086–96.
15. Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bignell EM, Bowyer P, Bromley M, Brüggemann R, Garber G, Cornely OA. Tackling the emerging threat of antifungal resistance to human health. Nat Rev Microbiol. 2022;20:557–71.
16. Donnelly RF, McCarron PA, Tunney MM. Antifungal photodynamic therapy. Microbiol Res. 2008;163:1–12.
17. Butts A, Krysan DJ. Antifungal drug discovery: something old and something new. PLoS Pathog. 2012;8:e1002870.
18. Stone NR, Bicanic T, Salim R, Hope W. Liposomal amphotericin B (AmBisome®): a review of the pharmacokinetics, pharmacodynamics, clinical experience and future directions. Drugs. 2016;76:485–500.
19. Jarvis JN, Lawrence DS, Meya DB, Kagimu E, Kasibante J, Mpoza E, Rutakingirwa MK, Ssebambulidde K, Tugume L, Rhein J. Single-dose liposomal amphotericin B treatment for cryptococcal meningitis. N Engl J Med. 2022;386:1109–20.
20. Liu Q, Zou J, Chen Z, He W, Wu W. Current research trends of nanomedicines. Acta Pharm Sinica B. 2023;13:4391–416.
21. Leenders A, Marie S. The use of lipid formulations of amphotericin B for systemic fungal infections. Leukemia. 1996;10:1570–5.
22. Cheeseman S, Christofferson AJ, Kariuki R, Cozzolino D, Daeneke T, Crawford RJ, Truong VK, Chapman J, Elbourne A. Antimicrobial metal nanomaterials: from passive to stimuli-activated applications. Adv Sci. 2020;7:1902913.
23. Araujo VHS, Duarte JL, Carvalho GC, Silvestre ALP, Fonseca-Santos B, Marena GD, Ribeiro TC, dos Santos Ramos MA, Bauab TM, Chorilli M. Nanosystems against candidiasis: a review of studies performed over the last two decades. Crit Rev Microbiol. 2020;46:508–47.
24. Li B, Pan L, Zhang H, Xie L, Wang X, Shou J, Qi Y, Yan X. Recent developments on using nanomaterials to combat *Candida albicans*. Front Chem. 2021;9:813973.
25. Huang Y, Guo X, Wu Y, Chen X, Feng L, Xie N, Shen G. Nanotechnology's frontier in combatting infectious and inflammatory diseases: prevention and treatment. Signal Transduct Target Therapy. 2024;9:34.
26. Choi V, Rohn JL, Stoodley P, Carugo D, Stride E. Drug delivery strategies for antibiofilm therapy. Nat Rev Microbiol. 2023;21:555–72.
27. DeQueiroz G, Day D. Antimicrobial activity and effectiveness of a combination of sodium hypochlorite and hydrogen peroxide in killing and removing Pseudomonas aeruginosa biofilms from surfaces. J Appl Microbiol. 2007;103:794–802.
28. Berman J, Krysan DJ. Drug resistance and tolerance in fungi. Nat Rev Microbiol. 2020;18:319–31.
29. Costerton JW, Cheng K, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. Bacterial biofilms in nature and disease. Annual Reviews Microbiol. 1987;41:435–64.
30. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284:1318–22.
31. Davis SC, Martinez L, Kirsner R. The diabetic foot: the importance of biofilms and wound bed preparation. Curr Diab Rep. 2006;6:439–45.
32. Smith AW. Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems? Adv Drug Deliv Rev. 2005;57:1539–50.
33. Chandra J, Kuhn DM, Mukherjee PK, Hoyer LL, McCormick T, Ghannoum MA. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J Bacteriol. 2001;183:5385–94.
34. Nett JE, Crawford K, Marchillo K, Andes DR. Role of Fks1p and matrix glucan in *Candida albicans* biofilm resistance to an echinocandin, pyrimidine, and polyene. Antimicrob Agents Chemother. 2010;54:3505–8.
35. Al-Fattani MA, Douglas LJ. Penetration of Candida biofilms by antifungal agents. Antimicrob Agents Chemother. 2004;48:3291–7.
36. Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, Sobel JD, Pappas PG, Kullberg BJ. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. Clin Infect Dis. 2012;54:1110–22.
37. Liu C, Wu H, Xu K. Photodynamic therapy and the application of antitumor immunotherapy for tumor treatment. Zhongguo Zhong Liu Lin Chuang. 2021;48:35–9.
38. Jia Q, Song Q, Li P, Huang W. Rejuvenated photodynamic therapy for bacterial infections. Adv Healthc Mater. 2019;8:1900608.
39. Zhou C, Peng C, Shi C, Jiang M, Chau JH, Liu Z, Bai H, Kwok RT, Lam JW, Shi Y. Mitochondria-specific aggregation-induced emission luminogens for selective photodynamic killing of fungi and efficacious treatment of keratitis. ACS Nano. 2021;15:12129–39.
40. Tang N, Yuan S, Luo Y, Wang A-J, Sun K, Liu N-N, Tao K. Nanoparticle-based photodynamic inhibition of *Candida albicans* Biofilms with Interfering Quorum sensing. ACS Omega. 2023;8:4357–68.
41. Kovács R, Majoros L. Fungal quorum-sensing molecules: a review of their antifungal effect against Candida biofilms. J Fungi. 2020;6:99.
42. Maliszewska I, Lisiak B, Popko K, Matczyszyn K. Enhancement of the efficacy of photodynamic inactivation of *Candida albicans* with the use of biogenic gold nanoparticles. Photochem Photobiol. 2017;93:1081–90.
43. Madkhali OA. A comprehensive review on potential applications of metallic nanoparticles as antifungal therapies to combat human fungal diseases. Saudi Pharm J. 2023;31:101733.
44. Zhao X, Tang H, Jiang X. Deploying gold nanomaterials in combating multidrug-resistant bacteria. ACS Nano. 2022;16:10066–87.
45. Tabassum N, Khan F, Kang M-G, Jo D-M, Cho K-J, Kim Y-M. Inhibition of Polymicrobial Biofilms of *Candida albicans–Staphylococcusaureus/Streptococcus* mutans by Fucoidan–Gold Nanoparticles. Mar Drugs. 2023;21:123.
46. He J, Ye Y, Zhang D, Yao K, Zhou M. Visualized Gallium/Lyticase-Integrated Antifungal Strategy for Fungal Keratitis Treatment. Adv Mater. 2022;34:2206437.
47. Perfect JR. The antifungal pipeline: a reality check. Nat Rev Drug Discovery. 2017;16:603–16.
48. Anderson TM, Clay MC, Cioffi AG, Diaz KA, Hisao GS, Tuttle MD, Nieuwkoop AJ, Comellas G, Maryum N, Wang S. Amphotericin forms an extramembranous and fungicidal sterol sponge. Nat Chem Biol. 2014;10:400–6.
49. Letscher-Bru V, Herbrecht R. Caspofungin: the first representative of a new antifungal class. J Antimicrob Chemother. 2003;51:513–21.
50. Lee Y, Puumala E, Robbins N, Cowen LE. Antifungal drug resistance: molecular mechanisms in *Candida albicans* and beyond. Chem Rev. 2020;121:3390–411.
51. Gao J, Wang H, Li Z, Wong AH-H, Wang Y-Z, Guo Y, Lin X, Zeng G, Liu H, Wang Y. *Candida albicans* gains azole resistance by altering sphingolipid composition. Nat Commun. 2018;9:4495.
52. Lemke A, Kiderlen A, Kayser O. Amphotericin b. Appl Microbiol Biotechnol. 2005;68:151–62.
53. Rao L, Tian R, Chen X. Cell-membrane-mimicking nanodecoys against infectious diseases. ACS Nano. 2020;14:2569–74.
54. Dash P, Piras AM, Dash M. Cell membrane coated nanocarriers-an efficient biomimetic platform for targeted therapy. J Controlled Release. 2020;327:546–70.
55. Liu Y, Luo J, Chen X, Liu W, Chen T. Cell membrane coating technology: a promising strategy for biomedical applications. Nano-Micro Lett. 2019;11:1–46.
56. Xie J, Shen Q, Huang K, Zheng T, Cheng L, Zhang Z, et al. Oriented assembly of cell-mimicking nanoparticles via a molecular affinity strategy for targeted drug delivery. ACS Nano. 2019;13:5268–77.
57. Li B, Wang W, Zhao L, Wu Y, Li X, Yan D, Gao Q, Yan Y, Zhang J, Feng Y. Photothermal therapy of tuberculosis using targeting pre-activated macrophage membrane-coated nanoparticles. Nat Nanotechnol. 2024;19:834–45.
58. Zhu J, Xie R, Gao R, Zhao Y, Yodsanit N, Zhu M, et al. Multimodal nanoimmunotherapy engages neutrophils to eliminate Staphylococcus aureus infections. Nat Nanotechnol 2024;19:1032–43.
59. Deerhake ME, Shinohara ML. Emerging roles of Dectin-1 in noninfectious settings and in the CNS. Trends Immunol. 2021;42:891–903.
60. Brown GD. Dectin-1: a signalling non-TLR pattern-recognition receptor. Nat Rev Immunol. 2006;6:33–43.
61. Ambati S, Ferarro AR, Kang SE, Lin J, Lin X, Momany M, Lewis ZA, Meagher RB. Dectin-1-targeted antifungal liposomes exhibit enhanced efficacy. MSphere. 2019;4:e00025–00019.
62. Krishnan N, Fang RH, Zhang L. Engineering of stimuli-responsive self-assembled biomimetic nanoparticles. Adv Drug Deliv Rev. 2021;179:114006.
63. Fatima M, Almalki WH, Khan T, Sahebkar A, Kesharwani P. Harnessing the power of Stimuli-Responsive nanoparticles as an effective therapeutic drug delivery system. Adv Mater. 2024;36:2312939.
64. Park S-C, Kim Y-M, Lee J-K, Kim N-H, Kim E-J, Heo H, Lee M-Y, Lee JR, Jang M-K. Targeting and synergistic action of an antifungal peptide in an antibiotic drug-delivery system. J Controlled Release. 2017;256:46–55.
65. Wang Z, Liu X, Duan Y, Huang Y. Infection microenvironment-related antibacterial nanotherapeutic strategies. Biomaterials. 2022;280:121249.
66. Friedman AJ, Phan J, Schairer DO, Champer J, Qin M, Pirouz A, Blecher-Paz K, Oren A, Liu PT, Modlin RL. Antimicrobial and anti-inflammatory activity of

chitosan–alginate nanoparticles: a targeted therapy for cutaneous pathogens. J Invest Dermatology. 2013;133:1231–9.
67. Khalaf EM, Abood NA, Atta RZ, Ramírez-Coronel AA, Alazragi R, Parra RMR, Abed OH, Abosaooda M, Jalil AT, Mustafa YF. Recent progressions in biomedical and pharmaceutical applications of chitosan nanoparticles: a comprehensive review. Int J Biol Macromol. 2023;231:123354.
68. Mohamed M, Fouad S, Elshoky HA, Mohammed GM, Salaheldin TA. Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*. Int J Veterinary Sci Med. 2017;5:23–9.
69. Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhori SKM, Hasan H, Mohamad D. Review on Zinc Oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-Micro Lett. 2015;7:219–42.
70. Arakha M, Pal S, Samantarrai D, Panigrahi TK, Mallick BC, Pramanik K, Mallick B, Jha S. Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Sci Rep. 2015;5:14813.
71. Kubacka A, Diez MS, Rojo D, Bargiela R, Ciordia S, Zapico I, et al. Understanding the antimicrobial mechanism of TiO2-based nanocomposite films in a pathogenic bacterium. Sci Rep 2014;4:4134.
72. Radhakrishnan VS, Mudiam MKR, Kumar M, Dwivedi SP, Singh SP, Prasad T. Silver nanoparticles induced alterations in multiple cellular targets, which are critical for drug susceptibilities and pathogenicity in fungal pathogen (*Candida albicans*). Int J Nanomed. 2018;13:2647.
73. Ashrafi M, Bayat M, Mortazavi P, Hashemi SJ, Meimandipour A. Antimicrobial effect of chitosan–silver–copper nanocomposite on *Candida albicans*. J Nanostructure Chem. 2020;10:87–95.
74. Youssef A, Abd El-Aziz M, Abd El-Sayed ES, Moussa M, Turky G, Kamel S. Rational design and electrical study of conducting bionanocomposites hydrogel based on chitosan and silver nanoparticles. Int J Biol Macromol. 2019;140:886–94.
75. Zhang L, Pornpattananangkul D, Hu C-M, Huang C-M. Development of nanoparticles for antimicrobial drug delivery. Curr Med Chem. 2010;17:585–94.
76. Huh AJ, Kwon YJ. Nanoantibiotics: a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Controlled Release. 2011;156:128–45.
77. Vincent BM, Langlois J-B, Srinivas R, Lancaster AK, Scherz-Shouval R, Whitesell L, Tidor B, Buchwald SL, Lindquist S. A fungal-selective cytochrome bc1 inhibitor impairs virulence and prevents the evolution of drug resistance. Cell Chem Biology. 2016;23:978–91.
78. Rex JH, Walsh TJ, Nettleman M, Anaissie EJ, Bennett JE, Bow EJ, Carillo-Munoz A, Chavanet P, Cloud GA, Denning DW. Need for alternative trial designs and evaluation strategies for therapeutic studies of invasive mycoses. Clin Infect Dis. 2001;33:95–106.
79. Fisher MC, Hawkins NJ, Sanglard D, Gurr SJ. Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science. 2018;360:739–42.
80. Healey KR, Zhao Y, Perez WB, Lockhart SR, Sobel JD, Farmakiotis D. Prevalent mutator genotype identified in fungal pathogen Candida glabrata promotes multi-drug resistance. Nat Commun. 2016;7:11128.
81. Satori CP, Henderson MM, Krautkramer EA, Kostal V, Distefano MM, Arriaga EA. Bioanalysis of eukaryotic organelles. Chem Rev. 2013;113:2733–811.
82. Jhaveri A, Torchilin V. Intracellular delivery of nanocarriers and targeting to subcellular organelles. Expert Opin Drug Deliv. 2016;13:49–70.
83. Chen WH, Luo GF, Zhang XZ. Recent advances in subcellular targeted cancer therapy based on functional materials. Adv Mater. 2019;31:1802725.
84. BENNETT JE: Flucytosine. Ann Intern Med. 1977;86:319–22.
85. Hope WW, Tabernero L, Denning DW, Anderson MJ. Molecular mechanisms of primary resistance to Flucytosine in *Candida albicans*. Antimicrob Agents Chemother. 2004;48:4377–86.
86. Hsiao CR, Huang L, Bouchara J-P, Barton R, Li HC, Chang TC. Identification of medically important molds by an oligonucleotide array. J Clin Microbiol. 2005;43:3760–8.
87. Blecher K, Nasir A, Friedman A. The growing role of nanotechnology in combating infectious disease. Virulence. 2011;2:395–401.
88. Schairer DO, Chouake JS, Nosanchuk JD, Friedman AJ. The potential of nitric oxide releasing therapies as antimicrobial agents. Virulence. 2012;3:271–9.
89. Qin Y, Wang J, Lv Q, Han B. Recent progress in Research on Mitochondrion-targeted antifungal drugs: a review. Antimicrob Agents Chemother. 2023;67:e00003–00023.
90. Murante D, Hogan DA. New mitochondrial targets in fungal pathogens. Mbio. 2019;10:e02258–02219.
91. Kummer E, Ban N. Mechanisms and regulation of protein synthesis in mitochondria. Nat Rev Mol Cell Biol. 2021;22:307–25.
92. D'Souza GG, Weissig V. Subcellular targeting: a new frontier for drug-loaded pharmaceutical nanocarriers and the concept of the magic bullet. Expert Opin Drug Deliv. 2009;6:1135–48.
93. Zhang D, Wen L, Huang R, Wang H, Hu X, Xing D. Mitochondrial specific photodynamic therapy by rare-earth nanoparticles mediated near-infrared graphene quantum dots. Biomaterials. 2018;153:14–26.
94. Piktel E, Suprewicz Ł, Depciuch J, Cieśluk M, Chmielewska S, Durnaś B, Król G, Wollny T, Deptuła P, Kochanowicz J. Rod-shaped gold nanoparticles exert potent candidacidal activity and decrease the adhesion of fungal cells. Nanomedicine. 2020;15:2733–52.
95. Hwang Is, Lee J, Hwang JH, Kim KJ, Lee DG. Silver nanoparticles induce apoptotic cell death in *Candida albicans* through the increase of hydroxyl radicals. FEBS J. 2012;279:1327–38.
96. McLellan CA, Vincent BM, Solis NV, Lancaster AK, Sullivan LB, Hartland CL, et al. Inhibiting mitochondrial phosphate transport as an unexploited antifungal strategy. Nat Chem Biol. 2018;14:135–41.
97. Yamashita K, Miyazaki T, Fukuda Y, Mitsuyama J, Saijo T, Shimamura S, et al. The novel arylamidine T-2307 selectively disrupts yeast mitochondrial function by inhibiting respiratory chain complexes. Antimicrob Agents Chemother. 2019;63:e00374-19.
98. Wiederhold NP, Najvar LK, Fothergill AW, Bocanegra R, Olivo M, McCarthy DI, et al. The novel arylamidine T-2307 maintains in vitro and in vivo activity against echinocandin-resistant *Candida albicans*. Antimicrob Agents Chemother. 2015;59:1341–3.
99. Wu Y, Jiang W, Cong Z, Chen K, She Y, Zhong C, Zhang W, Chen M, Zhou M, Shao N. An effective strategy to develop potent and selective antifungal agents from cell penetrating peptides in tackling drug-resistant invasive fungal infections. J Med Chem. 2022;65:7296–311.
100. Helmerhorst EJ, Breeuwer P, van't Hof W, Walgreen-Weterings E, Oomen LC, Veerman EC, Amerongen AVN, Abee T. The cellular target of histatin 5 on *Candida albicans* is the energized mitochondrion. J Biol Chem. 1999;274:7286–91.
101. Martinez-Outschoorn UE, Peiris-Pagés M, Pestell RG, Sotgia F, Lisanti MP. Cancer metabolism: a therapeutic perspective. Nat Reviews Clin Oncol. 2017;14:11–31.
102. Wijnants S, Vreys J, Van Dijck P. Interesting antifungal drug targets in the central metabolism of *Candida albicans*. Trends Pharmacol Sci. 2022;43:69–79.
103. Chen X, Zhang Z, Chen Z, Li Y, Su S, Sun S. Potential antifungal targets based on glucose metabolism pathways of *Candida albicans*. Front Microbiol. 2020;11:296.
104. Vesely EM, Williams RB, Konopka JB, Lorenz MC. N-Acetylglucosamine metabolism promotes survival of *Candida albicans* in the phagosome. Msphere. 2017;2:00357–00317.
105. Alves R, Mota S, Silva S, Rodrigues F, Brown CP, Henriques AJ, Casal M, Paiva M. The carboxylic acid transporters Jen1 and Jen2 affect the architecture and fluconazole susceptibility of *Candida albicans* biofilm in the presence of lactate. Biofouling. 2017;33:943–54.
106. Ene IV, Adya AK, Wehmeier S, Brand AC, MacCallum DM, Gow NA, Brown AJ. Host carbon sources modulate cell wall architecture, drug resistance and virulence in a fungal pathogen. Cell Microbiol. 2012;14:1319–35.
107. Ji Y, Han Z, Ding H, Xu X, Wang D, Zhu Y, An F, Tang S, Zhang H, Deng J. Enhanced eradication of bacterial/fungi biofilms by glucose oxidase-modified magnetic nanoparticles as a potential treatment for persistent endodontic infections. ACS Appl Mater Interfaces. 2021;13:17289–99.
108. Rodaki A, Bohovych IM, Enjalbert B, Young T, Odds FC, Gow NA, Brown AJ. Glucose promotes stress resistance in the fungal pathogen *Candida albicans*. Mol Biol Cell. 2009;20:4845–55.
109. Dietl A-M, Misslinger M, Aguiar MM, Ivashov V, Teis D, Pfister J, et al. The siderophore transporter Sit1 determines susceptibility to the antifungal VL-2397. Antimicrob Agents Chemother. 2019;63:e00807-19.
110. Nakamura I, Yoshimura S, Masaki T, Takase S, Ohsumi K, Hashimoto M, Furukawa S, Fujie A. ASP2397: a novel antifungal agent produced by Acremonium Persicinum MF-347833. J Antibiot. 2017;70:45–51.
111. Yu Q, Jia C, Dong Y, Zhang B, Xiao C, Chen Y, Wang Y, Li X, Wang L, Zhang B. *Candida albicans* autophagy, no longer a bystander: its role in tolerance to ER stress-related antifungal drugs. Fungal Genet Biol. 2015;81:238–49.
112. Brown AJ, Brown GD, Netea MG, Gow NA. Metabolism impacts upon Candida immunogenicity and pathogenicity at multiple levels. Trends Microbiol. 2014;22:614–22.
113. Mora-Montes HM, Bates S, Netea MG, Díaz-Jiménez DF, López-Romero E, Zinker S, Ponce-Noyola P, Kullberg BJ, Brown AJ, Odds FC. Endoplasmic

reticulum α-glycosidases of *Candida albicans* are required for N glycosylation, cell wall integrity, and normal host-fungus interaction. Eukaryot Cell. 2007;6:2184–93.
114. Lecomte F, Ismail N, High S. Making membrane proteins at the mammalian endoplasmic reticulum. Biochem Soc Trans. 2003;31:1248–52.
115. Almeida C, Amaral MD. A central role of the endoplasmic reticulum in the cell emerges from its functional contact sites with multiple organelles. Cell Mol Life Sci. 2020;77:4729–45.
116. Elias R, Benhamou RI, Jaber QZ, Dorot O, Zada SL, Oved K, Pichinuk E, Fridman M. Antifungal activity, mode of action variability, and subcellular distribution of coumarin-based antifungal azoles. Eur J Med Chem. 2019;179:779–90.
117. Benhamou RI, Jaber QZ, Herzog IM, Roichman Y, Fridman M. Fluorescent tracking of the endoplasmic reticulum in live pathogenic fungal cells. ACS Chem Biol. 2018;13:3325–32.
118. Benhamou RI, Bibi M, Berman J, Fridman M. Localizing antifungal drugs to the correct organelle can markedly enhance their efficacy. Angew Chem. 2018;130:6338–43.
119. Zhang W, Yu M, Xi Z, Nie D, Dai Z, Wang J, Qian K, Weng H, Gan Y, Xu L. Cancer cell membrane-camouflaged nanorods with endoplasmic reticulum targeting for improved antitumor therapy. ACS Appl Mater Interfaces. 2019;11:46614–25.
120. Wang Y, Kaur G, Chen Y, Santos A, Losic D, Evdokiou A. Bioinert anodic alumina nanotubes for targeting of endoplasmic reticulum stress and autophagic signaling: a combinatorial nanotube-based drug delivery system for enhancing cancer therapy. ACS Appl Mater Interfaces. 2015;7:27140–51.
121. Shi L, Gao X, Yuan W, Xu L, Deng H, Wu C, Yang J, Jin X, Zhang C, Zhu X. Endoplasmic reticulum–targeted fluorescent nanodot with large Stokes Shift for Vesicular Transport Monitoring and long-term bioimaging. Small. 2018;14:1800223.
122. Ma X, Gong N, Zhong L, Sun J, Liang X-J. Future of nanotherapeutics: targeting the cellular sub-organelles. Biomaterials. 2016;97:10–21.
123. Shi Y, Wang S, Wu J, Jin X, You J. Pharmaceutical strategies for endoplasmic reticulum-targeting and their prospects of application. J Controlled Release. 2021;329:337–52.
124. Yang J, Griffin A, Qiang Z, Ren J. Organelle-targeted therapies: a comprehensive review on system design for enabling precision oncology. Signal Transduct Target Therapy. 2022;7:379.
125. Wang J, Fang X, Liang W. Pegylated phospholipid micelles induce endoplasmic reticulum-dependent apoptosis of cancer cells but not normal cells. ACS Nano. 2012;6:5018–30.
126. Cowen LE, Lindquist S. Hsp90 potentiates the Rapid Evolution of New traits: Drug Resistance in Diverse Fungi. Science. 2005;309:2185–9.
127. Schell R, Mullis M, Ehrenreich IM. Modifiers of the genotype–phenotype map: Hsp90 and beyond. PLoS Biol. 2016;14:e2001015.
128. Huang DS, Leblanc EV, Shekhar-Guturja T, Robbins N, Krysan DJ, Pizarro JC, Whitesell L, Cowen LE, Brown LE. Design and synthesis of fungal-selective resorcylate aminopyrazole Hsp90 inhibitors. J Med Chem. 2019;63:2139–80.
129. Whitesell L, Robbins N, Huang DS, McLellan CA, Shekhar-Guturja T, Leblanc EV, Nation CS, Hui R, Hutchinson A, Collins C, et al. Structural basis for species-selective targeting of Hsp90 in a pathogenic fungus. Nat Commun. 2019;10:402.
130. San Miguel JF, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, Kropff M, Spicka I, Petrucci MT, Palumbo A, Samoilova OS. Bortezomib plus Melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med. 2008;359:906–17.
131. Roemer T, Davies J, Giaever G, Nislow C. Bugs, drugs and chemical genomics. Nat Chem Biol. 2012;8:46–56.
132. Xu D, Ondeyka J, Harris GH, Zink D, Kahn JN, Wang H, Bills G, Platas G, Wang W, Szewczak AA. Isolation, structure, and biological activities of fellutamides C and D from an undescribed Metulocladosporiella (Chaetothyriales) using the genome-wide *Candida albicans* fitness test. J Nat Prod. 2011;74:1721–30.
133. Roemer T, Xu D, Singh SB, Parish CA, Harris G, Wang H, Davies JE, Bills GF. Confronting the challenges of natural product-based antifungal discovery. Chem Biol. 2011;18:148–64.
134. Gutierrez-Gongora D, Geddes-McAlister J. From naturally-sourced protease inhibitors to new treatments for fungal infections. J Fungi. 2021;7:1016.
135. Wójcik-Mieszawska S, Lewtak K, Skwarek E, Dębowski D, Gitlin-Domagalska A, Nowak J, Wydrych J, Pawelec J, Fiołka MJ. Autophagy of *Candida albicans* cells after the action of earthworm Venetin-1 nanoparticle with protease inhibitor activity. Sci Rep. 2023;13:14228.
136. Zarnowski R, Sanchez H, Covelli AS, Dominguez E, Jaromin A, Bernhardt J, Mitchell KF, Heiss C, Azadi P, Mitchell A. *Candida albicans* biofilm–induced vesicles confer drug resistance through matrix biogenesis. PLoS Biol. 2018;16:e2006872.
137. Rodrigues ML, Franzen AJ, Nimrichter L, Miranda K. Vesicular mechanisms of traffic of fungal molecules to the extracellular space. Curr Opin Microbiol. 2013;16:414–20.
138. Zarnowski R, Noll A, Chevrette MG, Sanchez H, Jones R, Anhalt H, Fossen J, Jaromin A, Currie C, Nett JE. Coordination of fungal biofilm development by extracellular vesicle cargo. Nat Commun. 2021;12:6235.
139. Zhang F, Zhao M, Braun DR, Ericksen SS, Piotrowski JS, Nelson J, Peng J, Ananiev GE, Chanana S, Barns K. A marine microbiome antifungal targets urgent-threat drug-resistant fungi. Science. 2020;370:974–8.
140. Zhao M, Zhang F, Zarnowski R, Barns K, Jones R, Fossen J, Sanchez H, Rajski SR, Audhya A, Bugni TS. Turbinmicin inhibits Candida biofilm growth by disrupting fungal vesicle–mediated trafficking. J Clin Investig. 2021;131:e145123.
141. Lukacs GL, Haggie P, Seksek O, Lechardeur D, Freedman N, Verkman A. Size-dependent DNA mobility in cytoplasm and nucleus. J Biol Chem. 2000;275:1625–9.
142. Seksek O, Biwersi J, Verkman A. Translational diffusion of macromolecule-sized solutes in cytoplasm and nucleus. J Cell Biol. 1997;138:131–42.
143. Hoenigl M, Sprute R, Egger M, Arastehfar A, Cornely OA, Krause R, Lass-Flörl C, Prattes J, Spec A, Thompson GR. The antifungal pipeline: fosmanogepix, ibrexafungerp, olorofim, opelconazole, and rezafungin. Drugs. 2021;81:1703–29.
144. Larwood DJ. Nikkomycin Z—ready to meet the promise? J Fungi. 2020;6:261.
145. Lipa-Castro A, Nicolas V, Angelova A, Mekhloufi G, Prost B, Chéron M, Faivre V, Barratt G. Cochleate formulations of Amphotericin b designed for oral administration using a naturally occurring phospholipid. Int J Pharm. 2021;603:120688.
146. Fatima I, Rasul A, Shah S, Saadullah M, Islam N, Khames A, Salawi A, Ahmed MM, Almoshari Y, Abbas G. Novasomes as nano-vesicular carriers to enhance topical delivery of fluconazole: a new approach to treat fungal infections. Molecules. 2022;27:2936.
147. Pfaller M, Messer S, Georgopapadakou N, Martell L, Besterman J, Diekema D. Activity of MGCD290, a Hos2 histone deacetylase inhibitor, in combination with azole antifungals against opportunistic fungal pathogens. J Clin Microbiol. 2009;47:3797–804.
148. Gómez-Garzón M, Gutiérrez-Castañeda LD, Gil C, Escobar CH, Rozo AP, González ME, Sierra EV. Inhibition of the filamentation of *Candida albicans* by Borojoa patinoi silver nanoparticles. SN Appl Sci. 2021;3:1–8.
149. Antinori S, Milazzo L, Sollima S, Galli M, Corbellino M. Candidemia and invasive candidiasis in adults: a narrative review. Eur J Intern Med. 2016;34:21–8.
150. Thomson DD, Wehmeier S, Byfield FJ, Janmey PA, Caballero-Lima D, Crossley A, et al. Contact-induced apical asymmetry drives the thigmotropic responses of *Candida albicans* hyphae. Cell Microbiol. 2015;17:342–54.
151. Staab JF, Bradway SD, Fidel PL, Sundstrom P. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. Science. 1999;283:1535–8.
152. Almeida RS, Brunke S, Albrecht A, Thewes S, Laue M, Edwards JE Jr, Filler SG, Hube B. The hyphal-associated adhesin and invasin Als3 of *Candida albicans* mediates iron acquisition from host ferritin. PLoS Pathog. 2008;4:e1000217.
153. Jimenez-Lopez C, Lorenz MC. Fungal immune evasion in a model host–pathogen interaction: *Candida albicans versus* macrophages. PLoS Pathog. 2013;9:e1003741.
154. Saville SP, Lazzell AL, Bryant AP, Fretzen A, Monreal A, Solberg EO, Monteagudo C, Lopez-Ribot JL, Milne GT. Inhibition of filamentation can be used to treat disseminated candidiasis. Antimicrob Agents Chemother. 2006;50:3312–6.
155. Brand A, MacCallum DM, Brown AJ, Gow NA, Odds FC. Ectopic expression of URA3 can influence the virulence phenotypes and proteome of *Candida albicans* but can be overcome by targeted reintegration of URA3 at the RPS10 locus. Eukaryot Cell. 2004;3:900–9.
156. Pinto AP, Rosseti IB, Carvalho ML, da Silva BGM, Alberto-Silva C, Costa MS. Photodynamic antimicrobial chemotherapy (PACT), using Toluidine blue O inhibits the viability of biofilm produced by *Candida albicans* at different stages of development. Photodiagn Photodyn Ther. 2018;21:182–9.
157. Carvalho ML, Pinto AP, Raniero LJ, Costa MS. Biofilm formation by *Candida albicans* is inhibited by photodynamic antimicrobial chemotherapy (PACT), using chlorin e6: increase in both ROS production and membrane permeability. Lasers Med Sci. 2018;33:647–53.
158. Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. The biology and future prospects of antivirulence therapies. Nat Rev Microbiol. 2008;6:17–27.

159. Dickey SW, Cheung GY, Otto M. Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. Nat Rev Drug Discovery. 2017;16:457–71.
160. Allen RC, Popat R, Diggle SP, Brown SP. Targeting virulence: can we make evolution-proof drugs? Nat Rev Microbiol. 2014;12:300–8.
161. Henderson J, Nickerson JF. Bacterial endocarditis with *Candida albicans* superinfection. Can Med Assoc J. 1964;90:452.
162. Araújo D, Gaspar R, Mil-Homens D, Henriques M, Silva BF, Silva S. Cationic lipid-based formulations for encapsulation and delivery of anti-EFG1 2′ O MethylRNA oligomer. Med Mycol. 2021;60:myac030.
163. Barbosa A, Araújo D, Henriques M, Silva S. The combined application of the anti-RAS1 and anti-RIM101 2′-O MethylRNA oligomers enhances *Candida albicans* filamentation control. Med Mycol. 2021;59:1024–31.
164. Zhao M, Zhang M, Xu K, Wu K, Xie R, Li R, Wang Q, Liu W, Wang W, Wang X. Antimicrobial effect of extracellular vesicles derived from human oral mucosal epithelial cells on *candida albicans*. Front Immunol. 2022;13:777613.
165. Toenjes KA, Stark BC, Brooks KM, Johnson DI. Inhibitors of cellular signalling are cytotoxic or block the budded-to-hyphal transition in the pathogenic yeast *Candida albicans*. J Med Microbiol. 2009;58:779–90.
166. Okamoto-Shibayama K, Sato Y, Azuma T. Resveratrol impaired the morphological transition of *Candida albicans* under various hyphae-inducing conditions. J Microbiol Biotechnol. 2010;20:942–5.
167. Rajasekar V, Darne P, Prabhune A, Kao RY, Solomon AP, Ramage G, Samaranayake L, Neelakantan P. A curcumin-sophorolipid nanocomplex inhibits *Candida albicans* filamentation and biofilm development. Colloids Surf B. 2021;200:111617.
168. Zhang M, Chang W, Shi H, Zhou Y, Zheng S, Li Y, Li L, Lou H. Biatriosporin D displays anti-virulence activity through decreasing the intracellular cAMP levels. Toxicol Appl Pharmcol. 2017;322:104–12.
169. Haque F, Alfatah M, Ganesan K, Bhattacharyya MS. Inhibitory effect of sophorolipid on *Candida albicans* biofilm formation and hyphal growth. Sci Rep. 2016;6:23575.
170. Nithyanand P, Shafreen RMB, Muthamil S, Pandian SK. Usnic acid inhibits biofilm formation and virulent morphological traits of *Candida albicans*. Microbiol Res. 2015;179:20–8.
171. do Nascimento Dias J, de Souza Silva C, de Araújo AR, Souza JMT, de Holanda Veloso Junior PH, Cabral WF, et al. Mechanisms of action of antimicrobial peptides ToAP2 and NDBP-5.7 against *Candida albicans* planktonic and biofilm cells. Sci Rep. 2020;10:10327.
172. Kim Y-G, Lee J-H, Park JG, Lee J. Inhibition of *Candida albicans* and Staphylococcus aureus biofilms by centipede oil and linoleic acid. Biofouling. 2020;36:126–37.
173. Iadnut A, Mamoon K, Thammasit P, Pawichai S, Tima S, Preechasuth K, et al. In vitro antifungal and antivirulence activities of biologically synthesized ethanolic extract of propolis-loaded PLGA nanoparticles against *Candida albicans*. Evid Based Complementary Altern Med. 2019;2019:3715481.
174. Jalal M, Ansari MA, Ali SG, Khan HM, Rehman S. Anticandidal activity of bio-inspired ZnO NPs: effect on growth, cell morphology and key virulence attributes of Candida species. Artif Cells Nanomed Biotechnol. 2018;46:912–25.
175. Jalal M, Ansari MA, Alzohairy MA, Ali SG, Khan HM, Almatroudi A, Siddiqui MI. Anticandidal activity of biosynthesized silver nanoparticles: effect on growth, cell morphology, and key virulence attributes of Candida species. Int J Nanomed. 2019;14:4667–79.
176. Sudbery PE. Growth of *Candida albicans* hyphae. Nat Rev Microbiol. 2011;9:737–48.
177. Han T-L, Cannon RD, Villas-Bôas SG. The metabolic basis of *Candida albicans* morphogenesis and quorum sensing. Fungal Genet Biol. 2011;48:747–63.
178. Ding X, Kambara H, Guo R, Kanneganti A, Acosta-Zaldívar M, Li J, Liu F, Bei T, Qi W, Xie X. Inflammasome-mediated GSDMD activation facilitates escape of *Candida albicans* from macrophages. Nat Commun. 2021;12:6699.
179. Puri S, Kumar R, Rojas IG, Salvatori O, Edgerton M. Iron chelator deferasirox reduces *Candida albicans* invasion of oral epithelial cells and infection levels in murine oropharyngeal candidiasis. Antimicrob Agents Chemother. 2019;63:e02152–02118.
180. Sun W, Zhang L, Lu X, Feng L, Sun S. The synergistic antifungal effects of sodium phenylbutyrate combined with azoles against *Candida albicans* via the regulation of the Ras–cAMP–PKA signalling pathway and virulence. Can J Microbiol. 2019;65:105–15.
181. Cao C, Wu M, Bing J, Tao L, Ding X, Liu X, Huang G. Global regulatory roles of the c AMP/PKA pathway revealed by phenotypic, transcriptomic and phosphoproteomic analyses in a null mutant of the PKA catalytic subunit in C andida albicans. Mol Microbiol. 2017;105:46–64.

Publisher's note