Role of Extracellular Vesicles in Amphotericin B Resistance in Fungal Infections

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Abstract

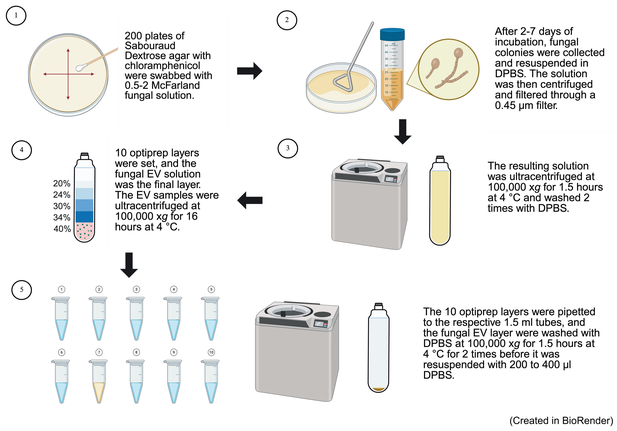
Human fungal pathogens can cause serious infections in immunocompromised patients, especially those who suffer from cancer or acquired immunodeficiency syndrome. Recent studies have shown that fungal extracellular vesicles (EVs) play an important role in fungal pathogenesis and antifungal drug resistance. A previous study from our team proved that a small amount of EVs derived from *Candida auris* increased amphotericin B (AMB) MICs severalfold. It is of particular interest whether other species can cause a similar effect. Hence, this study aims to investigate whether EVs derived from *C. auris*, *Candida albicans*, *Candida parapsilosis*, *Candida krusei*, and *Talaromyces marneffei* can induce AMB resistance. Nanosight results showed that EVs from the above-mentioned species and strains have a mode size between 70 nm and 160 nm, with a mean size between 130 nm and 220 nm. The antifungal drug susceptibility test (AFST) showed that EVs derived from all species and strains can increase AMB MICs to various extents, suggesting that fungal EVs can be a target to ameliorate fungal drug resistance.

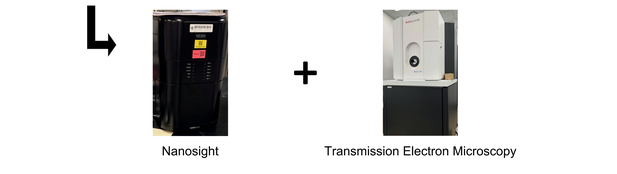
Introduction

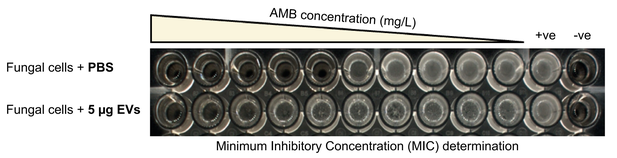
Fungal infections pose a serious health threat worldwide. It is estimated that there are 6.5 million invasive fungal infection cases, with 3.8 million deaths worldwide annually (1). *C. auris*, *C. albicans*, *C. parapsilosis*, *C. krusei*, and *T. marneffei* are common human fungal pathogens listed on the fungal pathogen priority list by the World Health Organization in 2022 (2). Following evaluation by WHO experts based on fungal infectivity and antifungal drug resistance, *C. auris* and *C. albicans* are classified as critical group pathogens (2). *C. parapsilosis* is designated as a high group pathogen, whereas *C. krusei* and *T. marneffei* are categorized as medium group pathogens (2). These fungal species can cause severe bloodstream infection in high-risk populations, including, but not limited to, cancer patients and those who receive immunosuppressive therapy, with a mortality rate ranging from 20% to 50% (2).

Additionally, one of the most concern topic regarding fungal infections is antifungal drug resistance. Antifungal drug resistance is one of the major reasons that leads to treatment failure. Recent studies have suggested that fungal EVs act as a protein cargo that assists in biofilm formation, thereby protecting fungal cells from antifungal drug attack (3). Moreover, our team's published study demonstrated that EVs derived from *C. auris* can induce resistance to AMB, a last-resort drug for treating systemic fungal infection (4). Due to its wide-spectrum properties, it is important to know whether EVs from other strains and species can yield a similar effect (5). However, no study has investigated this phenomenon. Hence, this study aims to demonstrate that fungal EVs are a significant factor that promotes AMB resistance by showing that the addition of EVs from the above-mentioned species increases AMB MICs. Moreover, it can emphasize that fungal EVs are a therapeutic target that can ameliorate antifungal drug resistance.

Methodology



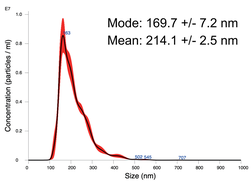


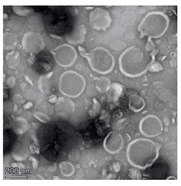


Minimum Inhibitory Concentration (MIC) determination

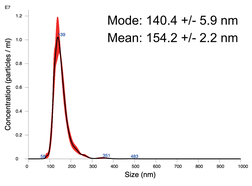
Results

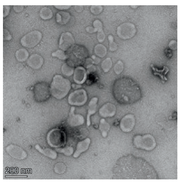
C. auris strain Cau1901



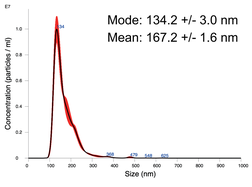


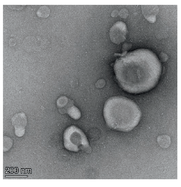
C. auris Type strain



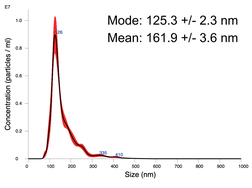


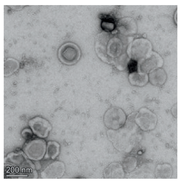
C. auris CBS 12772



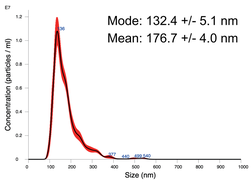


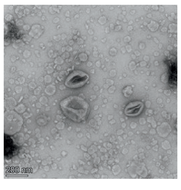
C. albicans ATCC 90028



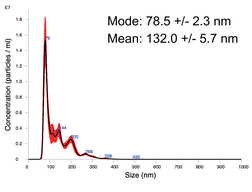


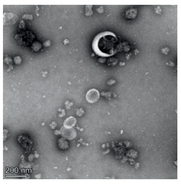
C. parapsilosis ATCC 22019



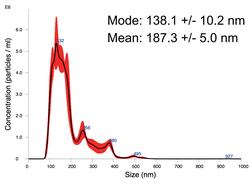


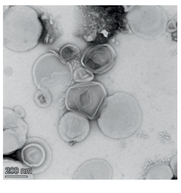
C. krusei ATCC 6258





T. marneffei ATCC 18224





Fungal species or strains	Addition of DPBS (mg/L)	Addition of 5 µg EVs (mg/L)
C. auris isolate Cau1901	1	8
C. auris Type strain	0.25	2
C. auris CBS 12772	1	4
C. albicans ATCC 90028	0.5	1
C. parapsilosis ATCC 22019	0.5	>16
C. krusei ATCC 6258	0.5	2
T. marneffei ATCC 18224	1	>16

Table 1. AMB MIC determination of different fungal species or strains

Conclusion & Acknowledgement with References

To round up, this is the first study to demonstrate that EVs from different fungal species or strains reduce AMB susceptibility, suggesting EVs are a significant contributor to AMB resistance. Hence, inhibiting fungal EV synthesis can be a therapeutic approach to treat the infections caused by AMB-resistant fungal species or strains.

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