Micrrosus cirrosus SZ 2021: A potentially new genotype of Micrrosus cirrosus, which can cause fatal pulmonary infection in acute leukemia patients after haplo-HSCT

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Research Article

Abstract

Background

Uncommon *Microascus cirrosus* (*M.cirrosus*) species have been reported to cause growing subcutaneous and invasive fungal infections worldwide. To our knowledge, no comprehensive clinical characteristics and etiological identification in laboratory tests have been defined till now, notably, for fatal pulmonary infections in the crowd of patients undergoing hematopoietic stem cell transplantation (HSCT).

Methods

We studied the pulmonary infection due to a new *M.cirrosus*, analyzed other related cases retrospectively in our hospital and reviewed other reported cases. This isolated *M.cirrosus* was cultured and determined by morphological observation, antifungal susceptibility, multi-locus sequence analysis (MLST), whole genome sequencing, metagenomic next-generation sequencing(mNGS), and other analysis.

Results

This isolated *M.cirrosus* named *M. cirrosus SZ 2021* was resistant to various therapies and accounted for the fatal pulmonary infection in the HSCT patient. Notably, *M.cirrosus* could be confirmed by mNGS in another two lung infections of HSCT patients. For this new isolated fungus, it had short chains of conidia without apical cysts for the *Micrrosus* genus, whereas growing faster and bigger on chocolate agar plate than Sabouraud's agar. Furthermore, it was not sensitive to fluconazole, amphotericin B, 5-flucytosine, caspofungin and other drugs by drug sensitivity test in vitro, supporting its bad prognosis and a sharp increased colonies across the half month process of insufficient antifungal treatment. This isolate had not been correctly identified until the whole genome sequencing by NGS and MLST were finished, because of the poor knowledge of gene information on the genus. The whole nucleotide sequence (2.693 billion bases) of the genome from *M. cirrosus SZ 2021* have been uploaded in NCBI database for the first time. In addition, its protein fingerprint has been presented by MALDI-TOF-MS.

Conclusions

M. cirrosus SZ 2021 is a potentially new genotype of *M.cirrosus*, which can cause fatal pulmonary infection in immunocompromised patients. These established complete laboratory tests facilitate its correct and rapid etiological diagnosis, especially for the culture independent rapid detection by mNGS.

Background

Nowadays, with the rapid development of medical technology, some hematological malignancies can be cured by transplantation. To effectively avoid graft-versus-host disease (GVHD), calcineurin inhibitors ciclosporin or tacrolimus are often used as long-term preventive drugs after transplantation [1]. As we known, it is inevitable to destroy the normal immunity, inhibiting the proliferation of T cells, the maturation of DCs, and the activation of neutrophils [2, 3]. Therefore, the use of immunosuppressive agents after haplo-HSCT lead to patients susceptible to bacterial and fungal infections, which is the cause of increased non-relapse mortality in patients [4]. The invasive fungal infection has caused serious fatal damage to those patients with haplo-HSCT, including *Aspergillus* spp. and *Pneumocystis*. And those patients with CARD9 variations may be more susceptible to fungal infection after transplantation [5, 6]. In addition to these common pathogens, patients are also vulnerable to some uncommon microorganisms after transplantation. Rare pathogenic microorganism is mainly reported by cases, lack of systematic research, including condition, diagnosis, treatment and traceability [7–10]. As a result, it is challenged by accurate identification of pathogens and optimal management of uncommon disseminated fungal infections.

Some species of the genus Microascus are known to be opportunistic pathogens, mainly causing superficial tissue infections, and they represent some of the principal causes of nondermatophytic onychomycoses. For *Microascus*, morphological and molecular identification of the etiological agent has not been well established. *Microascus cirrosus*(*M.cirrosus*)species account for only 2.1% of the genus and are rare isolates of clinical origin, whereas inducing most human infections among the Microascus genus [11]. To our knowledge, *M. cirrosus* Curzi is reported to cause the first disseminated infection in a pediatric bone marrow transplant recipient in 1994 [12]. Currently, there are less then accepted species of *M.cirrosus* publicly reported to cause human cutaneous and pulmonary infection [10, 12–16]. Because in most of the clinical reports of *M.cirrosus* infections, morphological identification of the etiological agent has not been confirmed at the molecular level, the real prevalence of *M.cirrosus* species in clinical samples is unknown.

In recent years, the development of molecular diagnostic technology has greatly improved the efficiency of pathogen detection in human infections. Remarkably, metagenomics next generation sequencing (mNGS) has been widely used to diagnose lung infections in immunocompromised adults [17–21]. In the present case, extensive conventional microbiologic testing and mNGS failed to identify *Microascus* species, challenging the accurate diagnosis and individualized therapy of pulmonary fungal infection in haplo-HSCT patients. Here, retrospective analysis of the detailed process helps us identify and optimally manage fatal lung infection by *Microascus* species. Pathogenic and microbiological features of a novel *M.cirrosus* species are determined to recognize *Microascus* lung infections in immunosuppressive patients after haplo-HSCT, including growth and morphological findings, extensive drug resistance, the first whole genomic sequences, genetic evolution, pathological characteristics of pulmonary infection. This provides necessary etiological information for investigating real prevalence of *Microascus* infection in immunosuppressive patients, as *Microascus* are saprobes widely distributed in the whole world and China has the largest HSCT crowd.

Methods

Fungal isolation

Clinicians performed fiberoptic bronchoscopy on patients and collected BALF from left upper bronchus for laboratory examination, including bacterial culture, GM test, mNGS. The BALF was inoculated according to the method of bacterial culture, and cultured on Columbia blood agar plate (Autobio, China) and chocolate agar plate (Autobio, China) at 35°C and 5% CO_2. Dozens of white filamentous-like fungal colonies were formed on the plate. One pure fungal colony was transferred to Sabouraud's agar (Autobio, China) for fungal culture at 28°C and 35°C.

Morphological and physiological assessments

Due to the lack of genomic and protein fingerprint information of uncommon fungi in the database, rapid identification methods such as MALDI-TOF-MS (IVD MALDI Biotyper System, Bruker Daltonik Gmbh, Germany) could not identify the species. We inoculated this fungus on chocolate agar plate, Columbia blood plate, nutrient agar, Sabouraud's agar and LB broth (Sango Biotech, China) respectively, to observe the growth status of the colony every day. The conidia and septate hyphae were observed under the OLYMPUS CX33 microscope (OLYMPUS, Japan) by Lactophenol cotton blue (BASO, China) dyeing.

Resistance to common antifungal drugs

The broth dilution antifungal susceptibility testing was performed according to CLSI M38-A2 [22]. Four kinds of antifungal agents were tested, including amphotericin B (CAS: 1397-89-3, Bio Basic Inc, China), caspofungin acetate (CAS: 179463-17-3, Psaitong, China), fluconazole (CAS: 86386-73-4, Rhawn, China) and fluorocytosine (CAS: 2022-85-7, Rhawn, China). Different reagent grades were tested in the following concentrations according the manufacturers' instructions: amphotericin B 0.03–16 µg/mL, caspofungin 0.03–16 µg/mL, fluconazole 0.12–64 µg/mL, fluorocytosine 0.12–64 µg/mL. The susceptibility of this fungus to each drug was determined.

Multi-site sequence analysis

To accurately identify this fungus, four nuclear DNA regions were amplified and sequenced. Including large subunit ribosomal RNA gene (LSU) and internal transcribed spacer (ITS) regions of the rDNA operon, fragments of the translation elongation factor 1-alpha (EF-1α) and beta-tubulin genes (TUB), following the criteria of Sandoval et al [23]. DNA extraction was conducted with ezup column fungi genomic DNA purification kit (Sango Biotech, China). 2X SanTaq PCR Mix (Sango Biotech, China), primers, fungal nucleic acid and pure water were added into the PCR reaction tube to make a total volume of 50 µl, to amplify on the machine (SLAN-96P, Hongshi, China). The reactions were performed according to the following conditions: 1 cycle at 95°C for 5 min, followed by 40 cycles at 95 °C for 5 s, 52°C-58°C for 30s. The amplification was carried out with the primer described as Brasch Jochen et al [24]. The PCR product was entrusted to Sangong Biotech for sanger sequencing, and the sequencing instrument was Applied Biosystems 3730XL. Consensus sequences obtained for each locus were aligned with sequences of *Microascus* species retrieved from GenBank, using the ClustalW algorithm under MEGA-X v10.0.4 software [25,26]. Phylogenetic reconstructions by maximum likelihood (ML) approaches were performed with MEGA-X v10.0.4.

Genomic DNA extraction and whole genome sequencing

The fungus was cultured in LB broth for about three days, forming a white, pom-shaped fungus. The fungal mass was collected by centrifuge and washing with saline. The cell wall was destroyed by grinding in liquid nitrogen. The ezup column fungi genomic DNA purification kit (Sango Biotech, China) was used to extract genomic DNA for library construction and next-generation sequencing.

A DNA library of 500bp insert size was constructed and sequenced in Illumina's HiSeq platform with a pair-end (PE) 150 bp sequencing strategy. The sequence reads were assembled using SPAdes v3.5.0, then rRNA was predicted. Genome sequencing and analysis were done by the company of Sangon Biotech. The mNGS sequence of patient's sample was aligned with the genome sequence of *M. cirrosus* SZ 2021 by BWA software.

M. cirrosus SZ 2021 detected by MALDI-TOF-MS

For sample preparation, briefly, after the fungi were cultured in liquid for 2 days, the samples were transferred into eppendorf (EP) tubes containing 1 mL of high-performance liquid chromatography (HPLC) water, washed and pelleted by centrifugation. The pellet collected was dissolved in 300 μL of HPLC water. Wash twice using HPLC water. Then, 900 μL ethanol was added and removed by centrifugation and air-drying. Transfer the sample to the grinder for grinding, and inject 100 μL of 70% formic acid into the grinder for about 5 minutes. The homogenate was then transferred to a clean 1.5 ml EP tube, an equal amount of acetonitrile was added and centrifuged again. 1 μL of the supernatant was pipetted onto the MALDI target, overlaid with 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix and analyzed with MALDI-TOF (IVD MALDI Biotyper System, Bruker Daltonik GmbH, Germany), MBT Compass Explorer for results analysis.

Results

Patient

A 40-year-old man was diagnosed with acute myeloid leukemia (AML) in high-risk group in April 18th, 2018. After diagnosis, the patient underwent twice chemotherapy treatments. Unfortunately, the patient later relapsed on July 25, 2018. Laboratory results indicated that he was converted to mixed phenotype leukemia (MPAL) no-remission (NR). On August 14, 2018, the patient received haplo-HSCT, and the transplant was successfully reconstructed on August 29, 2018. Then, the patient underwent post-transplant consolidation: 3 times of decitabine therapy until May 07, 2019. Meanwhile he kept immunosuppressive therapy with Anti-rejection agent (Cyclosporine, 50mg BID) for three years to prevent graft-versus-host disease(GVHD). The patient had never relapsed with any hematonosis until this admission. However, he developed "chest tightness and asthma in the past six months, and worsened half a month ago". Therefore, he was admitted to a local hospital because of the aggravated pulmonary symptoms on February 13, 2021. And he was transferred to our hospital on March 3, 2021 in view of his pulmonary infection symptoms did not improve after anti-infection treatment at the local hospital. After admission, the doctor systematically performed relevant examinations for him. A chest computed tomography (CT) examination presented one infectious cavity in the upper lobe of the left lung (Fig.1). In addition, the scattered small dots and patches in both lungs were new foci. Many routine laboratory examinations also suggested infection. To identify the pathogen, fiberoptic bronchoscopy was performed twice to collect bronchoalveolar lavage fluid (BALF) for culture, 1,3-β-D-glucan (GM) and mNGS. Given these findings, posaconazole and caspofungin were used for antifungal therapy, meropenem and co-sulfamethoxazole for antibacterial, ganciclovir and ribavirin for antiviral. In a word, antifungal drugs had always been used for a long time, as clinical symptoms, CT examination, and laboratory examinations supported fungal infection in the lung. Finally, all these therapies failed to control this fatal pulmonary, and he died of respiratory failure on March 20, 2021. The patient's disease progression is shown in Fig. 2.

Results of laboratory tests for the patient

Blood cell analysis suggested mild anemia (hemoglobin 106g/L) with normal white blood cell count WBC:4.82×10^9/L, NEUT#:4.42×10^9/L, LYM#:0.27×10^9/L↓ . As for inflammatory markers, the level of procalcitonin (PCT) was 0.18 ng/mL, and hypersensitive C-reactive protein (hs-CRP, 59.33mg/L) were increased significantly. No significant abnormalities were found in blood coagulation function. Liver and kidney function were mild abnormality (UA:9.03mmol/L, ALT:82U/L). Sputum had been repeatedly for bacterial culture and no pathogenic microorganisms grew. BALF was collected on February 18, 2021. GM tests were positive GM:5.65 positive and mNGS presented Nocardia nova with 785 reads, cytomegalovirus (CMV) with 109 reads, Epstein-barr virus (EBV) with 26 reads in this BALF. As symptoms worsen, BALF from left upper bronchus on March 10, 2021 was secondly collected for mNGS. Nocardia nova (248 reads), Enterococcus (257reads), Aspergillus (60 reads), Parvovirus (71 reads) and Cytomegalovirus (20reads) were detected by mNGS in the BALF. Some white velvety colonies grew in the BALF after 3 days incubation (Fig. 3), with no evidence of mycobacterial, or additional fungal pathogens isolated. Because the accurate morphology of this fungus had not been established and no *M. cirrosus* were listed in MALDI-TOF-MS library, this fungus had never been correctly identified as a *Microascus* species until a month later. In a word, many results presented some fungal infection in the lung, including GM, mNGS, and pure fungal colonies.

Morphological and physiological characteristics of this fungus

This fungus was detected from the BALF of the patient by growing in both Columbia blood plate and chocolate agar plate for two days. The primary colonies were white and approximately 3-4 mm in diameter (Fig. 3a). Then it was incubated on many media to

observe growing at different temperature. This fungus can grow on Columbia blood plate, chocolate agar plate, nutrient agar, Sabouraud's agar and LB broth (Fig. 3b) respectively, and grow well at 35℃ and 28℃. It could grow faster and bigger on chocolate agar plate than Sabouraud's agar. As the prolonged cultivation, it presented different conies. Overall, white and approximately 3-4 mm colonies could be observed on culture medium for three days (Fig. 3c). Over 12 mm colonies with folds around the periphery and dark pigment in the center could be observed on culture medium for about a week (Fig. 3d). It could also grow at the bottom of a liquid medium. After about one week of cultivation on chocolate agar, the ball-shaped, smooth conidia and septate hyphae could be observed under the microscope by dyeing with Lactophenol cotton blue (Fig. 3e). For about four weeks, in the dark and brown part of the fungal colony, ascoma and ascospores could be formed as the forms of sexual reproduction of the fungus. Microscopically, ascoma were large and spherical-shaped, containing ascospores which were half-moon shaped (Fig. 3f-3h). Based on the presence of branched conidiophores bearing cylindrical annellides in brush-like groups and on the development of small black ascomata (cleistothecia) after four weeks, it was morphologically identified as *Microascus sp*. Compared with the morphological description of *M. cirrosus* from seven clinical cases (Table 1), the *M. cirrosus* presented typical colonies and microscopic morphology. And compared with other filamentous fungi such as Aspergillus, *Microascus* species usually has only short chains of conidia without apical cysts.

Fungal drug sensitivity

The minimum inhibitory concentrations of this fungus against amphotericin B, caspofungin, fluconazole and fluorocytosine were as following: (amphotericin B ≥16 µg/mL, caspofungin ≥16 µg/mL, fluconazole ≥64 µg/mL, fluorocytosine ≥64 µg/mL), according to the criteria [22], these antifungal drugs are all insensitive. This is generally consistent with the results of in vitro drug susceptibility studies on Microascus sp by Sandoval-Denis M et al and Gao L et al [11, 16].

Multi-site sequence analysis

The fungal nucleic acid was amplified by PCR with the primers described as Brasch Jochen et al [24]. and four products were obtained (LSU568bp, ITS618bp, EF-1α898bp and TUB523bp). The analysis revealed that this fungus represented a new genotype in the genus closely related to *M.* cirrosus (Fig.4). Based on its genetic characteristics and on its distinct morphological features, it is proposed below as a new genotype of *M.* cirrosus named by *M. cirrosus SZ 2021*.

The whole genome sequence of this fungus by second-generation sequencing

By performing De novo sequencing on the case fungus, we obtained its whole nucleic acid information. The total number of bases after quality control is approximately 2.693 billion and GC Bases Ratio is 53.98%. There is no whole *M. cirrosus* genome available for reference in the NCBI database currently. Thus, we have upload the assembled sequence of *M. cirrosus SZ 2021* to the public database NCBI for research. This second-generation sequencing project was deposited in the NCBI under BioProject: PRJNA835605, BioSample:SAMN28105390 and GenBank: JAMBUN000000000. The version described in this paper is version JAMBUN010000000.

Re-analysis of sequences from BALF detected by mNGS

Because no the whole genomic information of *Microascus* species were available in public databases, no sequences of this fungus had been reported by mNGS. The results of mNGS from BALF had always recommended nocardia and aspergillus for dozens of reads. We reanalyzed the initial mNGS sequence from BALF by comparing the whole genome sequence of *M. cirrosus SZ 2021.* No Aspergillus gene sequence was reported in the former BALF on February 18, 2021 in the local hospital, whereas 4 reads from *M. cirrosus* could be detected by retrospective bioinformatics analysis. In the BALF sample where the *M. cirrosus* colonies were cultured, more than a thousand sequences (1000 reads) could be aligned, including 660 no-human-reads and unclassfiled-reads. We also reanalyzed the initial BALF mNGS sequence from the HSCT patients who had underwent pulmonary infection but failed to cure in our hospital. Different number of sequences of *M.cirrosus* could be detected in another two cases, 3 reads and 13 reads respectively, with no verification of cultural colonies, but favorited by GM tests. In conclusion, the sequences from BALF detected by mNGS demonstrated that *M. cirrosus* could be detected correctly and rapidly by mNGS, as there is correct genomic information in the database.

Distinguishing features of M. cirrosus detected by MALDI-TOF-MS

We obtained the protein fingerprint of *M. cirrsus* SZ 2021 by MALDI-TOF-MS (Fig 5), but did not identify the strain name, probably because the mass spectrometry identification database did not contain strain information. Then, we constructed main spectrum profile (MSP) dendrogram by combining *M. cirrsus* SZ 2021 with other Microascus spp, Aspergillus fumigatus and Nocardia nova. It was found that *M. cirrosus* SZ 2021 was relatively close to *Microascus gracilis*, classified as a different cluster from Aspergillus fumigatus and Nocardia nova (Fig 6).

Discussion

In this case, it is very difficult to discovery the real pathogenic fungus of *M.cirrosus*, because of few knowledges of the *Microascus* genus. No *Microascus* species were involved in the clinical database, although mNGS have been used widespread in China. Only one of repeated culture tests of sputum and BALF presented some pure fungal colonies, what's more, they were not correctly identified as *M.cirrosus*. As followed, their extensive drug resistance was not detected and recognized in time. Thus, effective therapy had not been adjusted until the patient died of fatal lung infection, even though antifungal drugs had been used continuously according to the repeated hints of fungal etiology by GM tests. Indeed, through retrospective reanalysis of the original sequencing of the first BALF sample by mNGS, DNA sequence fragments of *M.cirrosus* were discovered with 13 reads, which indicated that the patient was likely to carry this fungus at the beginning. After anti-fungal drugs lasting for half a month, *M.cirrosus* spread and replicated proficiently within the lung due to its extensive drug resistance, favoring its easy growing in vitro medium. It therefore appears that at times of poor efficacy by routine anti-fungal infection treatment, uncommon fungal pathogens may be misdiagnosed in a single test. Besides studying the characteristics of *M.cirrosus* in detail, timely incorporating *M.cirrosus* into the rapid mass spectrometry identification and gene information database of fungi will be beneficial to improve the correct detection of *M.cirrosus*. Because this fungus is mostly resistant to common antifungal drugs, it is difficult to treat. Till now, most cases were not successfully cured in the crowd of patients after transplantion. Maybe, some uncommon therapies must try, including combined use of many antifungal drugs, surgical resection of localized lesions, and immune regulation therapy (Table 1).

Infection with *M.cirrosus* has been reported in the United States, Belgium, France, Italy, and China. From the geographical distribution point of view, *M. cirrosus* has been reported as a plant-infecting pathogen with no regional limitation [27]. The characteristics of infection are mainly lung and local skin infections, among which lung infections are basically immune-compromised patients, and local skin infections can appear in non-immune-compromised patients. It is of note that, most fatal lung infections by *M.cirrosus* are difficult to be successful cured in transplant population, details are listed in Table 1. Here, we present a case of *M.cirrosus* pulmonary infection, which is known to be the first reported patient undergoing haplo-HSCT in China. Until now, only one case of skin infection of *M.cirrosus* has been reported in China [16]. *M.cirrosus* infection mostly occurs in immunocompromised patients, and China has a huge transplant population. Due to the little knowledge about this fungus, there is no whole genetic information. Based on the whole genome sequence of *M.cirrosus*, we retrospectively reanalyzed the initial BALF mNGS sequence from the HSCT patients who had underwent fatal pulmonary infection but failed to cure in our hospital. Different number of sequences of *M.cirrosus* could be detected in another two cases, 3 reads and 13 reads respectively, favoring the fungal etiological diagnosis by GM tests. Therefore, correct and rapid diagnosis of *Micrrosus* genus in more extensive transplanted populations is required to truly understand the epidemiology of this fungal infection.

For this case, the GM test and mNGS suggested that the patient had a pulmonary fungal infection, but the real microorganism was not identified. Finally, the fungus was identified by culture, but it took a long time to delay treatment. The development of mNGS and its wide application in the diagnosis of infectious diseases have broken the existing limitations of conventional diagnostic approaches. Since mNGS does not require live pathogens or cultures, its positive rate can be much higher than conventional diagnostic approaches, and the types of pathogens that can be detected are wider than conventional methods. Thus, mNGS is more suitable for the diagnosis of opportunistic pathogens and mixed infections in immunosuppressed patients. However, metagenomic sequencing technology also has disadvantages such as higher costs, more complicated procedures, the background of human-derived nucleic acids, and the need for continuous updating of databases etc [28, 29]. In this case, we reported a case of *M. cirrosus*, which was not regarded as a pathogen in bioinformatics analysis of mNGS. The reasons may be as follows: (a) *M.cirrosus* is considered to be the Biological Safety Level one grade (BSL-1) environmental microorganism, such as *M. cirrosus CBS217.31* (https://wi.knaw.nl/page//fungal_display/name/ CBS%20217.31), thus, its partial sequence information is not included in the database for mNGS analysis. (b) There is no genome information of *M.cirrosus* in the NCBI database currently, maybe because it is difficult to culture for sequencing, compared with aspergillus[30]. These may cause missed detection or ignored infection in immunosuppressed groups. In this case, we have provided the assembled sequence of *M. cirrosus* and upload it to the public database NCBI for research. In addition, many sequences of this fungus could be detected by retrospective analysis of the mNGS results of BALF. Thus, these data facilitate the rapid detection of *M.cirrosus* by mNGS for well diagnosis of *M.cirrosus* infection.

Compared with other filamentous fungi such as Aspergillus, *M.cirrosus* is identified with only short chains of conidia without apical cysts in this study. Because of the poor information of this fungus, *M.cirrosus* maybe not correctly recognized by morphological identification even if it has been cultivated. MALDI-TOF-MS has been a common method for rapid identification of microbial strains. The cultured *M.cirrosus* can be accurately identified MALDI-TOF-MS by a close the protein fingerprint of *Microascus gracilis*, presenting a very different cluster from *Aspergillus fumigatus* and *Nocardia nova*. Thus, *M.cirrosus* can be rapidly detected by MALDI-TOF-MS.

Conclusion

It is urgent to well investigate the frequency and pathogenicity of *M.cirrosus* in the immunosuppressed patients, because systemic *Microascus* infections are difficult to treat and hence are frequently fatal. For diagnosis, the standard morphologic and growth criteria are necessary to establish. Rapid diagnosis of *M.cirrosus* is reliable by mNGS, if the vital genetic information have been uploaded in the public databases. As its extensive resistance to drugs is frequency, refractory pneumonia due to *M.cirrosus* should be paid special attention when routine antifungal therapy is ineffective. In addition, it is also necessary to explore the effective treatment methods of surgical removal of infection lesions combined with the use of multiple drugs.

Declarations

Ethics approval and consent to participate

This research was conducted in accordance with the ethical standards of the Declaration of Helsinki, and was approved by the Institutional Review Board of Dushu Lake Hospital Affiliated to Soochow University. Informed consent was waived due to its retrospective nature.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. The second-generation sequencing project of *Micrrosus cirrosus SZ 2021* was deposited in the NCBI under BioProject: PRJNA835605, BioSample:SAMN28105390 and GenBank: JAMBUN000000000. The version described in this paper is version JAMBUN010000000.

Competing interests

None conflicts of interests are to declare.

Authors' contributions

QH and JH conceived and designed the study.JC and TZ completed the isolation, culture and morphological observation of the strain.DZ provides cases and data. JC, LZ, XH, TZ, DZ and PZ completed molecular identification and data analysis. JC,QH and LW participated in analysis, or interpretation of the data. JC participated in the drafting of the article. LW, JH and QH contribute to the revision of article. All authors read and approved the final version of the manuscript.

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Tables

Table 1 Summary of patients with *Microascus cirrosus*

Microascus species	Time(year)	Infection Site	Type of transplant or other factors	Age/Gender and area	Antifungal agent or therapeutic methods	Outcome	Author, Reference
Microascus cirrosus	1992	*toenail*	*No*	56/F (*Belgium*)	*imidazole griseofulvin ketoconazole*	*Not cured*	de Vroey C[13]
Microascus cirrosus	1992	*toenail*	*No*	63/F (*Italy*)	*Griseofulvin miconazole*	*Not cured*	de Vroey C[13]
Microascus cirrosus	1995	*lung*	*AML, Auto-BM*	12/M (*America*)	*AMB*	*No recurrence*	Krisher K K[12]
Microascus cirrosus	2006	*Lung*	*AML, BMT*	49/M (*America*)	*VOR, AMB and TER; Surgery*	*Death due to AML relapse during infection*	Ustun Celalettin[14]
Microascus cirrosus	2011	*multiple organs*	*SOT for cystic fifibrosis*	36/M (*France*)	*VOR and CAS*	*Death (9 days)*	Miossec Charline[10]
Microascus cirrosus	2018	*Lung*	*bilateral lung transplant*	60/F (*Belgium*)	*VOR, CAS, TER and AMB*	*Cure*	Taton Olivier[15]
Microascus cirrosus	2018	*left ankle skin*	*Systemic corticosteroids for two months*	17/F (*China*)	*itraconazole*	*Cure*	Gao Lujuan[16]
Microascus cirrosus SZ 2021	2021	*Lung*	*MPAL, haplo-HSCT*	40/M (*China*)	*POS and CAS*	*Death*	Present case

Abbreviations: AMB, amphotericin; POS, posaconazole; TER, terbinafine; VOR, voriconazole; CAS, caspofungin; AML: Acute myelocytic leukemia; BMT:bone marrow transplant; Auto-BMT: autologous bone marrow transplantation; SOT: solid organ transplant; MPAL: mixed phenotype acute leukemia; haplo-HSCT:Haploidentical hematopoietic stem cell transplantation; M, male; F, female.
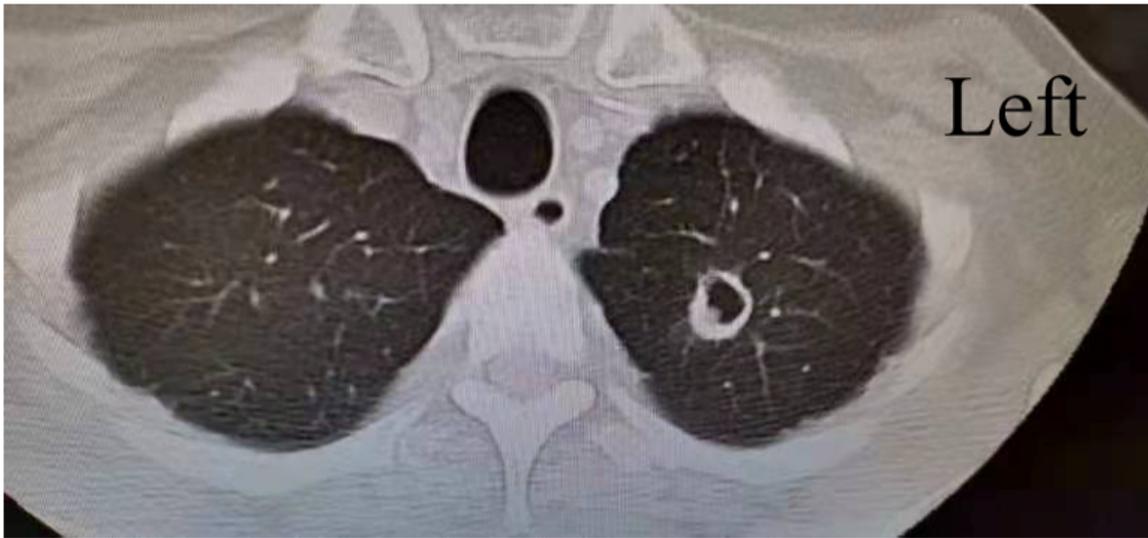
Figures

Figure 1

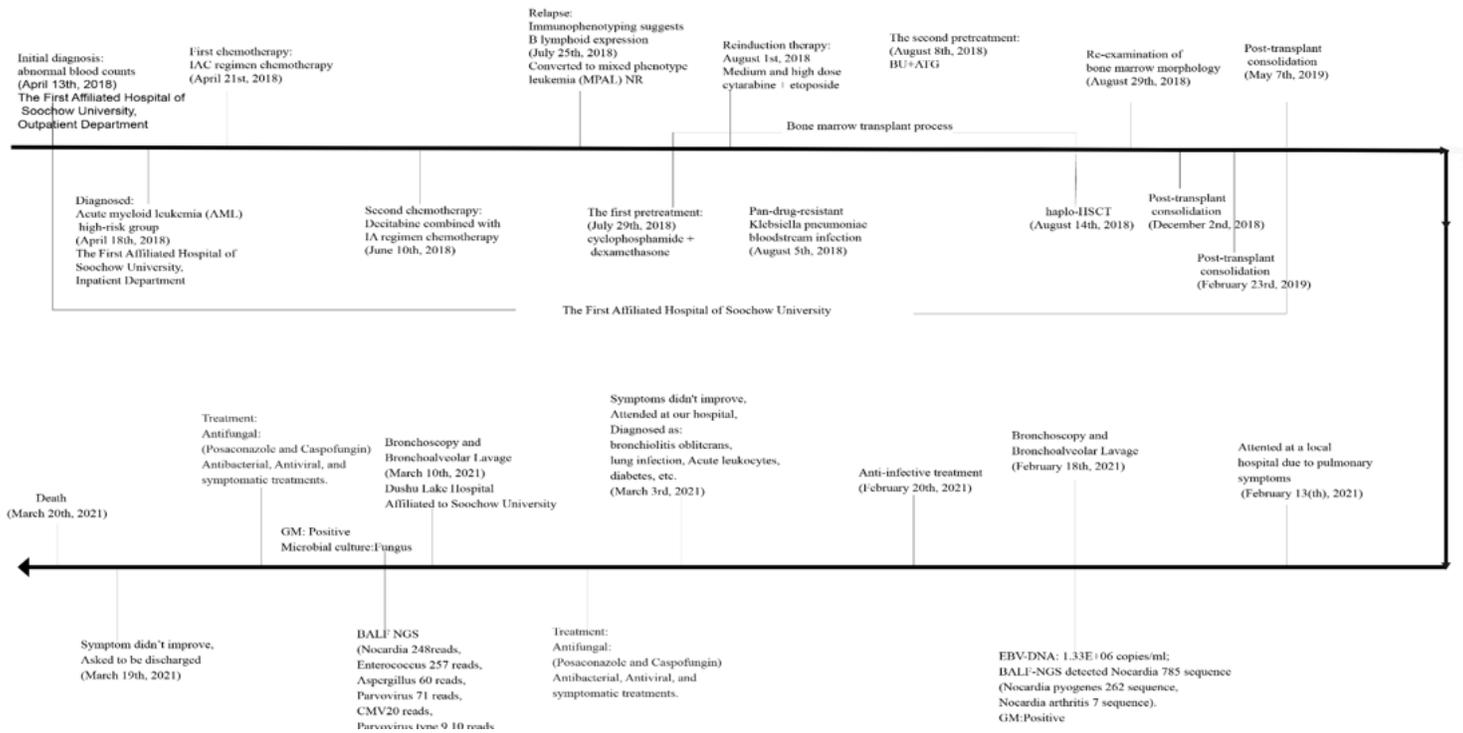Lung computed tomography (CT) of this patient.

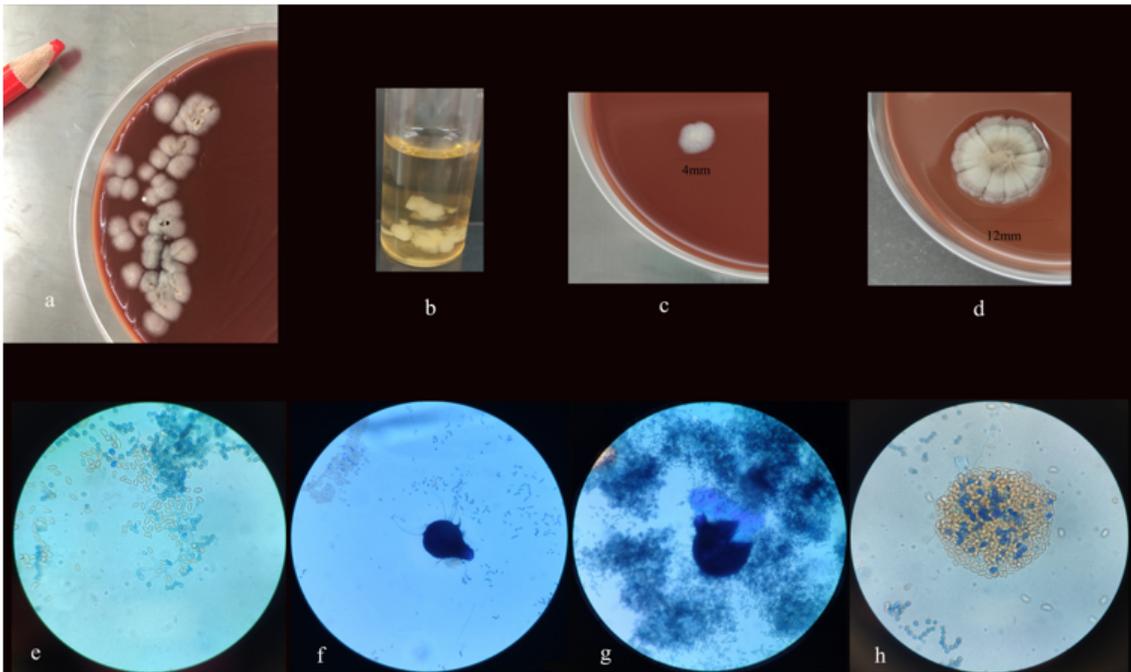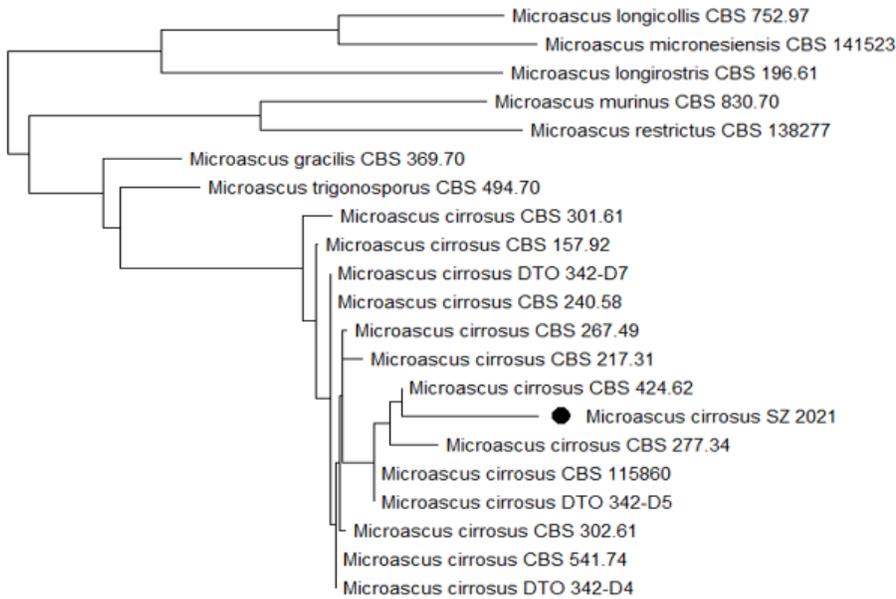
Figure 2

The course record of this case.

Figure 3

Growth and morphological features.

a) Fungal colonies of 3 days' culture for the first time, from the patient's BALF; b) growing in the LB broth; c) Colony morphology of *M. cirrosus SZ 2021* cultured on chocolate medium for three days; d) Colony morphology of *M. cirrosus SZ 2021* cultured on chocolate medium for a week; e) The conidia and septate hyphae under the microscope (1000x) by dyeing with Lactophenol cotton blue; f, g) Morphology of ascoma after lactophenol cotton blue staining under light microscopy (400x); h) Ascospores: pale yellow parts, crescent-shaped, not blue-stained(1000x).

Figure 4

M.cirrosus SZ 2021 is the fungus described in the case. The tree diagram constructed by four genes [large subunit ribosomal RNA gene (LSU) and internal transcribed spacer (ITS) regions of the rDNA operon, fragments of the translation elongation factor 1-alpha (EF-1α) and beta-tubulin genes (TUB)] shows that the fungus belongs to a new genotype of *M.cirrosus*.
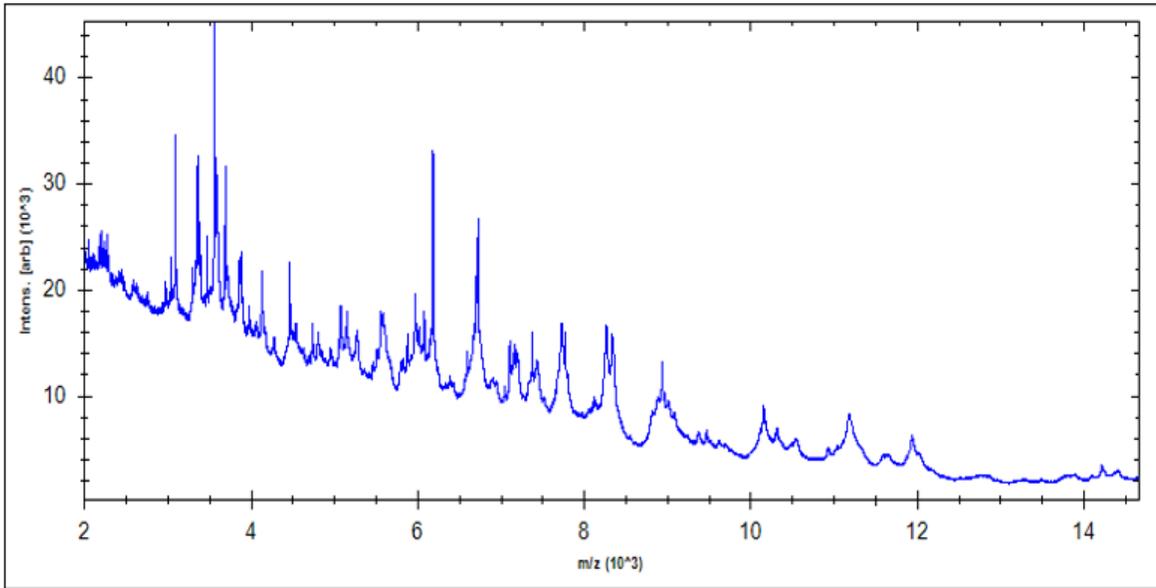

Figure 5

The protein spectra of the *M.cirrosus SZ 2021* examined in the current study
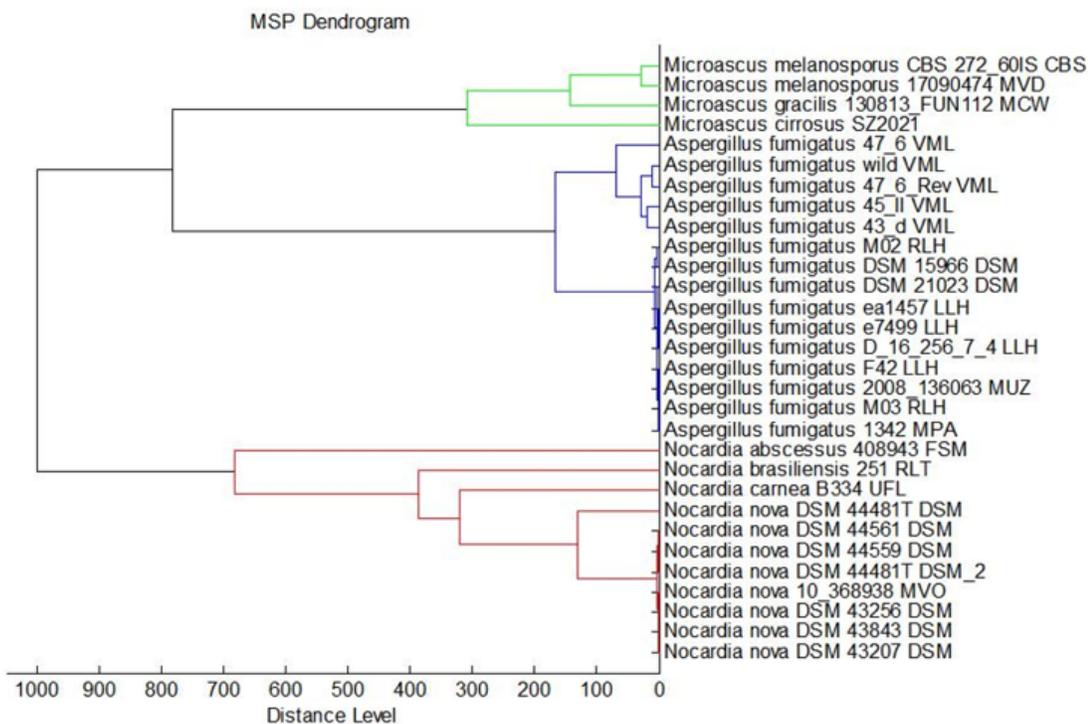

Figure 6

MSP dendrogram constructed from protein fingerprints of *M.cirrosus SZ 2021*, Nocardia nova and Aspergillus fumigatus.