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Case Reports

Chromoblastomycosis caused by a meristematic mutant of *Fonsecaea monophora*

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We report the first case of chromoblastomycosis caused by a meristematic mutant of *Fonsecaea monophora* in an 81-year-old Cantonese male. The patient had a seven-month history of a red plaque with thick yellow crust and purulent exudates on the back of his right hand. Microscopic examination of direct smears revealed brown sclerotic cells and black colonies were recovered in culture from samples of the purulent exudantes. The microscopic appearance of this isolate was quite different from that of other *Fonsecaea* species, exhibiting mere meristematic growth. The rDNA ITS sequence data confirmed that this isolate was a mutant of *Fonsecaea monophora*. The patient showed good response to treatment with itraconazole and complete healing was achieved without relapse after long-term follow-up.

Keywords chromoblastomycosis, *Fonsecaea*, meristematic mutant

Introduction

Chromoblastomycosis in China is primarily caused by *Cladophialophora carrionii* in the north and by *F. monophora* and *Fonsecaea pedrosoi* in the southern and eastern parts of the country [1]. We recently recovered a clinical isolate from Guangzhou in the south, which was confirmed to be *Fonsecaea monophora* by rDNA ITS sequence data, but having a quite different, meristematic growth morphology which was similar to *Botryomyces caespitosus* and *Exophiala phaeomuriformis* [2]. The present paper provides a full report of this remarkable case.

Case report

The Cantonese patient was an 81-year-old retired man with chronic bronchitis and rheumatic heart disease, who had a 7-month history of a red plaque with a thick yellow crust and purulent exudates on the back of his right hand. While a history of trauma was denied, a small, itching papule had appeared previously on his middle finger. The papule became larger with purulent exudates after intense scratching to relieve the itching. Staining for acid-fast bacteria, a TB-DNA assay (FQ-PCR) and bacterial culture of exudates were all negative. Despite treatment with several antibiotics, there was no improvement in the lesion and it continued to enlarge. Physical examination on admission on 5 June 2006 revealed an irregular red plaque of about 3×4 cm covered with a thick yellow crust (Fig. 1a) and purulent exudates under the crust. The patient had a history of chronic bronchitis and rheumatic heart disease. A biochemical evaluation, including blood sugar, hepatorenal function, routine blood examination and HIV test revealed no abnormalities.

Purulent exudates from the lesion were used to prepare smears for direct examination and to inoculate fungal cultures. KOH slides showed brown, irregularly septate, thick-walled cells, while hyphae and budding cells were absent (Fig. 1e). After 21 days incubation on Sabouraud's glucose agar (SGA) at 26°C, the exudates

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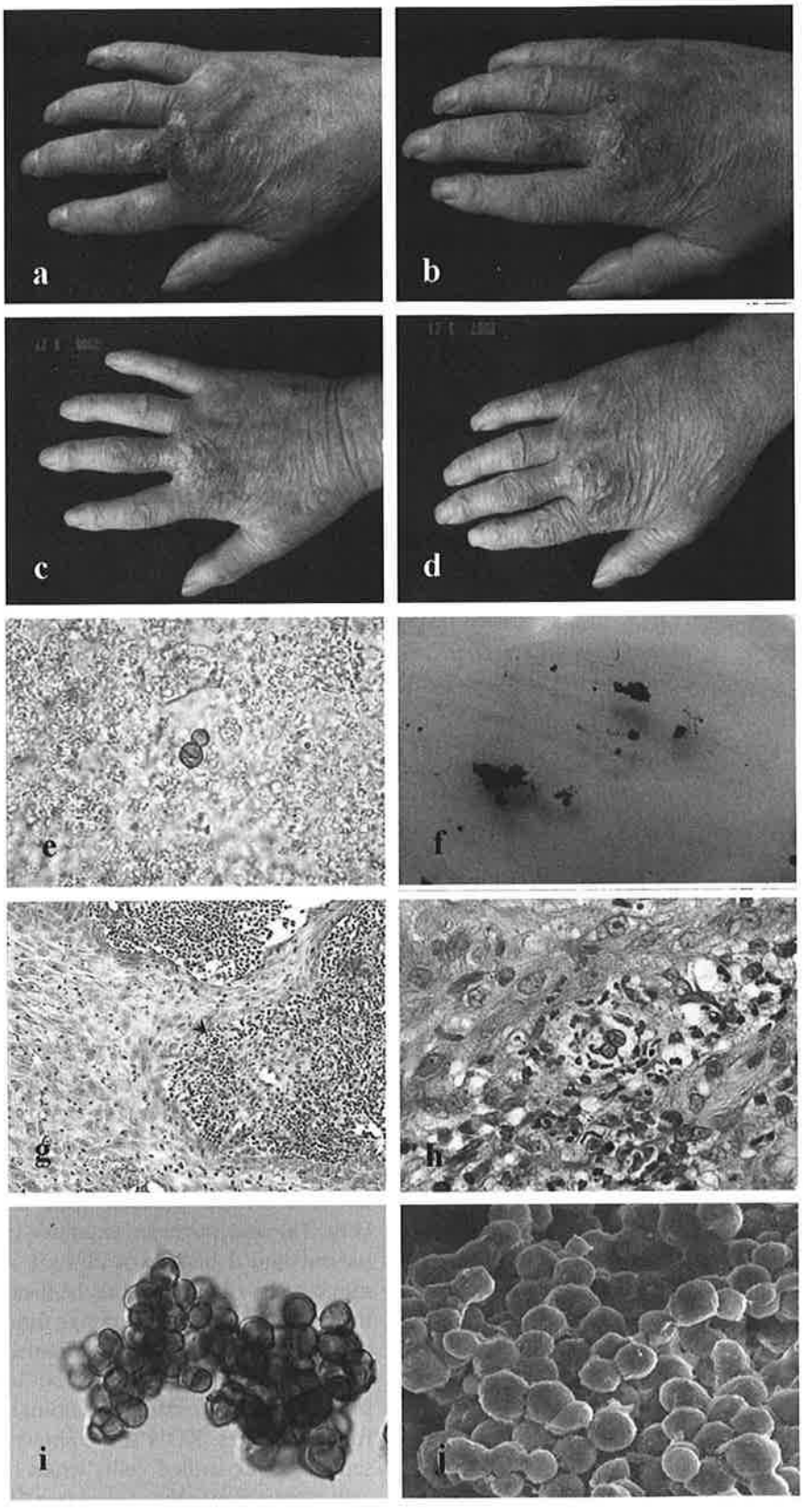

Fig. 1 (*Continued*)

yielded minute, black, smut-like and slow-growing colonies (Fig. 1f). Dry, heaped and fragile mature colonies with loose texture were observed after one month of incubation. The isolate was preserved in our research center of medical mycology with collection number CBS 122845. Examination of haematoxylin and eosin stained sections of the lesion revealed hyperkeratosis, pseudoepitheliomatous hyperplasia and a mixed dermal inflammatory cell infiltrate with sclerotic bodies in the dermis (Fig. 1g). Studies of periodic acid-Schiff (PAS) stained material also showed round, oval brown sclerotic bodies characteristic for chromoblastomycosis (Fig. 1h).

Having established the diagnosis of chromoblastomycosis, oral itraconazole in a dose of 200 mg once daily was administered. After 2 wk of treatment, the inflammation was relieved and the exudates decreased. While direct smear remained positive, cultures for fungi were negative. Further improvement of the lesion, absence of fungal element in direct smear and negative fungal culture was obtained after 5 wk of therapy and treatment with itraconazole was terminated on wk 10. At this time, only a superficial crust with pigmentation remained (Fig. 1b) and both direct smear and fungal culture from crust material were negative. Liver function evaluation showed no abnormalities. Follow-up after one and seven months showed no relapse (Fig. 1c, d).

Microbiology

To observe the colony appearance and growth rate at different temperatures (26, 37 and 40°C), isolate of SUMS0254 was inoculated onto the following media; Sabouraud's Glucose Agar (SGA), Potato Dextrose Agar (PDA), Czapek-Dox Agar (CDA), Cornmeal Agar (CMA) and Brain-heart Infusion Agar (BHI). In addition, scanning electron microscopic (SEM) studies were conducted to observe conidiogenesis. Slow growing, black, dry colonies with friable texture were noted on all media. The microscopic appearance was that of a thallus entirely composed of clumps of irregularly septate, thick-walled dark brown cells, which were subhyaline. However, hyphae and budding cells were not observed. Aggregates of spherical conidia with rough surfaces were occasionally noted (Fig. 1i, j). Maximum growth temperature was 37°C, urea was weakly hydrolyzed and the isolate was cycloheximide tolerant (0.1, 0.05 and 0.01%).

Antifungal susceptibility

In vitro susceptibility tests of SUMS0254 to amphotericin B (MIC 0.38 μg/ml), itraconazole (MIC 0.002 μg/ml), fluconazole (MIC 0.002 μg/ml) and ketoconazole (MIC 0.032 μg/ml) were performed by the E-test according to recommendations of the manufacturer (AB-Biodisc, Solna, Sweden).

Sequence analysis

Methods for DNA extraction, sequencing and phylogenetic analyses were those specified by Xi *et al.* [1]. rDNA Large SubUnit (LSU) and Internal Transcribed Spacer (ITS) sequences were compared with GenBank and aligned with voucher strains maintained at CBS including ex-types of *Fonsecaea pedrosoi, F. monophora, Botryomyces caespitosus* and *Exophiala phaeomuriformis*, using a research database maintained under BioNumerics (Applied Maths, Kortrijk, Belgium). D1/D2 of LSU (568 bp) had 99% homology with *Fonsecaea pedrosoi* (IFM 46410), and ITS (669 bp) regions had 100% homology with *Fonsecaea monophora* (CBS 269.37). The sequence data for the isolate were deposited in GenBank with accession numbers EF666992 (LSU) and EF513737.1 (ITS).

Phylogenetic relationship of SUMS0254 and relatives showed near-identity to 20 strains of *Fonsecaea monophora* including CBS 269.37(T). Five Chinese strains of *Fonsecaea pedrosoi* including CBS 272.37(T) differed in six positions, matching with currently accepted criteria for ITS species distinction [1]. *Botryomyces caespitosus* (CBS 177.80T) and *Exophiala phaeomuriformis* (CBS 131.88T) could not be aligned, having only 30% homology.

Discussion

The patient was an 81-year-old retired man without any history of diabetes mellitus or other underlying diseases that would affect his immunologic status, except chronic bronchitis and rheumatic heart problems. The origin of the *Fonsecaea monophora* infection in this case remains obscure. Attempts to isolate the

Fig. 1 (a) Irregular red plaque covered with thick yellow crust on the right hand, (b) Lesion after 10 wk itraconazole treatment, (c) Follow-up after one month, (d) Follow-up after seven months, (e) Sclerotic cell in direct smears (×400), (f) Colonies incubated on SGA for 21 d at 26°C, (g) Hyperkeratosis, pseudoepitheliomatous hyperplasia and a mixed dermal inflammatory cell infiltrate with sclerotic bodies (arrow) in the dermis (HE stain, ×100), (h) Round or ovoidal, brown muriform cells (PAS stain, ×400), (i) Slide culture of SUMS0254 showing clumps of irregularly septate, thick-walled dark brown cells, (j) Scanning electron microscopy (SEM) of SUMS0254 (×2500).

agent from putrid plant material and soil around his living environment were not successful. We presume that the portal of entry of the fungus must have been through minor trauma, even if such history was denied. The patient showed good response to itraconazole treatment and clinical cure was achieved within 10 weeks of antifungal therapy and relapse was not found after 7 months follow-up. Antifungal susceptibility tests also confirmed the efficacy of itraconazole in the treatment of this isolate.

The strain analyzed showed very limited, strictly meristematic growth, as described for *Sarcinomyces phaeomuriformis* [2]. The latter is now known as *Exophiala phaeomuriformis*, due to the fact that in most strains the *Exophiala* synanamorph is prevalent [3]. Since no consistent molecular differences were detected, the strains with meristematic morphology were interpreted to be dysplastic mutants. Similar phenomena are encountered more frequently in black yeasts, e.g. in *Exophiala dermatitidis* [4,5] and in *E. jeanselmei* [6]. In addition, the muriform cells, which are the invasive forms in cases of chromoblastomycosis caused by *Fonsecaea, Cladophialophora* and *Phialophora* species, represents meristematic growth. In addition, some unidentified meristematic strains from Mediterranean rock cluster in the *Chaetothyriales* (G.S. de Hoog, unpublished results). Meristematic morphology is encountered throughout the order *Chaetothyriales* and it can therefore be regarded as a plesiomorphic potential in the entire group. This suggests that one of the ancestral characters determining opportunism in *Chaetothyriales* is extremotolerant growth on rock under water- and nutrient-depletion, and is consistent with supposed shared early evolution of *Chaetothyriales* and lichenized, rock-inhabiting fungi [7].

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