Histopathological study on the prevalence of trichosporonosis among autopsy subjects using peptide nucleic acid probe *in situ* hybridization technique

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Summary

To distinguish *Trichosporon* spp. from *Candida* spp. we underwent *in situ* hybridization (ISH) technique by using peptide nucleic acid (PNA) probe which contain specific nucleotide sequence target of *C. albicans* and *Trichosporon* spp. Finally, 7 cases of trichosporonosis were confirmed, 5 out of which had been diagnosed as candidiasis. In conclusion, some Trichosporonosis may be mingled with cases recoded as candidiasis in past. We also wish to emphasize usefulness of ISH applying to histological examination to diagnose invasive fungal infections.

Introduction

Trichosporon species, which are mostly insusceptible to echinocandins, are some of the most common dimorphic yeasts in Asia including Japan. We sometimes encounter breakthrough trichosporonosis in individuals being treated with echinocandins. Thus, it is therefore important to distinguish between *Trichosporon* spp. and *Candida* spp. through histopathological examination. However, the morphological similarity of these fungi makes it difficult to distinguish between these fungi only by routine histological examination. For choosing optimal antifungal drugs, favorable molecular supplemental procedure is required to identify these fungi for histopathological diagnosis. In our previous studies, we developed peptide nucleic probes (PNA) designed to be specific for *C. albicans* and *Trichosporon* spp. The purpose of this study is to evaluate the utility of an ISH procedure focusing on the histopathological prevalence of trichosporonosis in autopsy subjects by using PNA probe ISH.

Material and Method

Formalin-fixed and paraffin embedded (FFPE) tissue samples used in this study were blood stream yeast infection of autopsy. These autopsy cases were corrected from two major regional hospital in Tokyo during 1975-2012 were used in this study. Three expert pathologists took part in histopathological examination to verify the previous diagnosis of invasive yeast infection and exclude cryptococcosis and mold infections from our cohort. ISH was performed with PNA probes of *C. albicans* and *Trichosporon* spp.

Result

Figure 1 summarize the outline. 7 cases showed a positive signal for the *Trichosporon* spp. probe and 29 cases for the *C. albicans* probe. 5 of the 7 *Trichosporon* spp. probe positive cases had been diagnosed as candiasis in the past autopsy reports.

Figure 1

Nucleotide sequences of the primers and probes used in the study

Probe target	Nucleotide sequence	Probe length and labeling
<i>C. albicans</i> 26S rRNA	ACAGCAGAAGCCGTG	15 mer PNA N terminus: FITC
Oliveira J, et al. J. Clin Microbiol 39: 4138-4141, 2001.		
<i>Trichosporon</i> spp. 28S rRNA	CGGACAATCGAAGAC	15 mer PNA N terminus: FITC
Shinozaki M, Shibuya K, et al. J. Clin. Microbiol 51: 295-298, 2013		

Conclusion

The present study identified 7 cases of invasive trichosporonosis, 5 of which had been misdiagnosed as candidiasis. The misdiagnoses indicate that incidence of trichosporonosis may be higher than previously reported. Our results show that ISH is an useful tool to identify *Trichosporon* spp. in FFPE tissue samples.