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Original contribution

The use of MUC5B antibody in identifying the fungal type of fungal sinusitis

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Summary Fungal sinusitis is an opportunistic fungal infection. *Candida albicans, Aspergillus* spp, and *Mucorales*, the most common pathogenic fungi, differ in both prognosis and therapy. Early diagnosis and differentiation between the subtypes therefore are pivotal for adequate treatment. This report describes a diagnostic immunohistochemical method that is able to distinguish these types of fungi. Formalin-fixed paraffin-embedded blocks of 89 fungal sinusitis specimens (12 *C albicans*, 52 *Aspergillus* spp, and 25 *Mucorales*) and 21 cultures (5 *C albicans*, 11 *Aspergillus* spp, and 5 *Mucorales*) were stained with MUC2, MUC5AC, and MUC5B antibodies. The immunohistochemical staining results of paraffin-embedded samples for MUC5B were successful in 100% and 92.3% of the *C albicans* and *Aspergillus* spp samples, respectively. Only 4.0% of the *Mucorales* parafin sections were found positive, demonstrating a significant difference in detection from *C albicans* and *Aspergillus* spp ($P < .001$). MUC5B expressions for cultures showed that it stained 100% and 90.9% for *C albicans* and *Aspergillus* spp, respectively, but negative for *Mucorales*. The expressions of MUC2 and MUC5AC for both paraffin-embedded samples and cultures were negative.The present study demonstrates the ability of an MUC5B antibody to distinguish *C albicans* and *Aspergillus* spp from *Mucorales* and its use as a diagnostic tool in fungal sinusitis.

1. Introduction

Candida albicans, *Aspergillus* spp, and *Mucorales* are the most common pathogenic fungi and often the cause of fungal sinusitis. The prognosis and therapeutic options for these various opportunistic fungal infections are different; early identification is critical. Proper diagnosis and treatment can prevent the infection progressing from noninvasive state to invasive state [1,2]. Morphological measures such as arrangement and shape do not suffice as a diagnostic particularly when only a small quantity of swollen and anamorphic fungi is available. Thus, there is an important need for sensitive diagnostic assays.

At present, there are many laboratory tests that can identify fungi based on histopathology, culture, serologic differentiation, or molecular techniques. In practice, the organism observed in tissue is the most important diagnostically. To meet the increasing demands for the detection and identification of fungi within tissues, a number of immunohistochemical studies have focused on the search for immunoreagents for the specific identification of fungi in tissues. An important limitation on the widespread application of such immunohistochemical tests lies in the fact that sensitive and specific reagents are usually derived from multiple heterologously absorbed polyclonal antisera, which are not commercially available [3]. The increasing commercial availability of fungal-specific immunoreagents will

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likely aid the clinician in selecting an appropriate therapeutic strategy.

The present study aimed at identifying an appropriate antibody for immunohistochemical use that can identify the fungal type of fungal sinusitis within tissues. A structural organization of α- and β(1,3)-glucans, chitin, galactomannan, and β(1,3),(1,4)-glucan constitutes the cell wall of *Aspergillus fumigatus*. This polysaccharide matrix harbors most of the fungal antigens [4]. The composition of the polysaccharide cell wall differs between different fungi and causes its distinct antigenic character. An antibody that can label the cell walls of *C albicans*, *Aspergillus* spp, or *Mucorales* in human nasal cavity and paranasal sinuses specifically and sensitively could be used as a diagnostic marker. Three antibodies against mucins (MUC2, MUC5AC, and MUC5B) were selected and evaluated as markers for these fungi in tissue samples side by side with periodic acid–Schiff (PAS) stain and Grocott's methenamine silver (GMS) stain for comparison. In addition, these antibodies were used to stain the corresponding fungal cultures and to perform immunoelectron microscopy for detailed analysis.

2. Materials and methods

2.1. Immunohistochemistry of formalin-fixed paraffin-embedded specimens

2.1.1. Samples

Formalin-fixed paraffin-embedded blocks of 89 fungal sinusitis specimens were obtained from the Department of Pathology of Tongren Hospital in Beijing. Two pathologists reviewed all hematoxylin and eosin–stained slides to confirm the diagnosis and ensure the presence of representative tissue before immunostaining was performed. All diagnoses in this research were established using current standard criteria and were confirmed by culture. The cases included 46 fungal balls, 15 allergic fungal sinusitis, and 28 invasive fungal sinusitis. The types of fungi were *C albicans* (12), *Aspergillus* spp (31 *A fumigatus*, 7 *A flavus*, 6 *A terreus,* 6 *A niger*, and 2 *A nidulans*), and *Mucorales* (14 *Mucor*, 8 *Rhizopus*, and *3 Absidia*).

2.1.2. Immunohistochemistry

Sections cut at a thickness of 5 μm were deparaffinized twice in xylene for 5 minutes and rehydrated through graded ethanol solutions to distilled water. Antigen retrieval was performed by heating sections in citrate buffer. After a 30-minute cooling period, slides were incubated in 3% H_2O_2 for 15 minutes and placed in phosphate buffered saline (PBS) for 5 minutes, followed by primary antibodies (MUC2, Neomarkers, Fremont, CA, Clone M53, prediluted; MUC5AC, Zymed, Carlsbad, CA, Clone 45M1, prediluted; MUC5B, Santa Cruz, CA, Clone G-16, 1:100 dilution) overnight at 4°C. After a PBS wash, biotinylated rabbit antimouse IgG (Zymed, PV-6000) was applied to slides of MUC2 and MUC5AC, whereas rabbit-antigoat IgG (Zymed, SP-9003) was applied to slides of MUC5B for 15 minutes at room temperature (24°C). After a PBS wash, slides of MUC5B needed another step for S-A/HRP for 15 minutes. After another PBS wash, all slides were incubated in DAB solution (DAKO, Carpinteria, CA) for 5 minutes, washed in distilled water, counterstained with hematoxylin, and dehydrated with xylene before mounting.

The positive controls of MUC2, MUC5AC, and MUC5B were human ileum mucosa, gastric carcinoma tissue, and colon carcinoma tissue, respectively. PBS replaced primary antibodies as the negative controls.

2.2. PAS and GMS stain

PAS and GMS stain were performed according to the methods of Gilks et al [5] and Churukian and Schenk [6].

2.3. Immunohistochemistry of cultures

Cultures were kindly provided by the clinical laboratory of Tongren Hospital. The fungi included *C albicans* (5), *Aspergillus* spp (3 *A fumigatus*, 3 *A flavus*, 2 *A terreus,* 2 *A niger*, and 1 *A nidulans*), and *Mucorales* (1 *Mucor*, 2 *Rhizopus*, and 2 *Absidia*). Cultures were grown for 4 days at 25°C on Vogel's glucose agar. Isolates from cultures were attached to slides and the slides were incubated with MUC2, MUC5AC, and MUC5B (1:100) overnight at 4°C. The following processes were similar to that of formalin-fixed paraffin-embedded specimens. PBS replaced primary antibodies as the negative controls.

2.4. Immunoelectron microscopy

Immunoelectron microscopy was performed by the preembedding technique. Isolates from cultures were attached to slides and then the slides were immersion fixed in a mixture of 4% paraformaldehyde and 1% glutaraldehyde for 2 hours. After rinsing several times in PBS, the slides were incubated with MUC2, MUC5AC, and MUC5B (1:500) overnight at 4°C. The following processes until DAB dyeing were similar to that of formalin-fixed paraffin-embedded specimens. PBS replaced primary antibodies as the negative controls. After washing 3 times in distilled water, the slides were postfixed for 30 minutes at room temperature in 1% acid chromic, dehydrated, and embedded in Epon 812 (Merck, Whitehouse Station, NJ). Ultrathin sections were placed on nickel net and stained by lead citrate. All sections were examined by a JEOL 1200 EX2 electron microscope (JEOL, Tokyo, Japan).

2.5. Data analysis

Statistical evaluations were done by using the SPSS 11.5 software (SPSS, Chicago, IL). The significance of the results