SHORT COMMUNICATION

A preliminary study on the potential of *Mycoplasma pneumoniae* to induce dyskaryotic change in respiratory epithelium in adult community-acquired pneumonia

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Background: This study aimed to explore the cellular morphology of respiratory epithelium in *Mycoplasma pneumonia* (MpP) patients. **Materials and Methods:** The cast-off cell morphological findings from bronchoscopic brushings in MpP and community-acquired pneumonia (CAP) caused by typical pathogens were reviewed. **Results:** Compared with the CAP group, cellular dysplasia in respiratory tract epithelial brushings was significantly greater in MpP patients (P = 0.033). **Conclusion:** Unique biological characteristics and mechanisms of pathogenesis of *Mycoplasma pneumoniae* (Mp) may result in dyskaryotic changes in respiratory epithelium in adult MpP.

Key words: Cellular dysplasia, community-acquired pneumonia, *Mycoplasma pneumonia*

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INTRODUCTION

Mycoplasma pneumoniae (Mp) is one of the most common respiratory tract pathogens in community-acquired pneumonia (CAP) patients. As one of the smallest bacterial organisms, Mp is recognized as a capable of existence in both extracellular and intracellular environments which might facilitate the establishment of latent or chronic states.[1] By the first proven link between an infectious agent and cancer came in the 1960s with the discovery of Epstein–Barr virus in Burkitt's lymphoma tissue,[2] it is likely that organisms which can persist at an intracellular level will have the greatest potential to influence oncogenesis. In recent years, the association between malignant cell transformation and infection with *Mycoplasma* has been identified *in vitro*.[3,4] Nevertheless, there have been very few reports focusing on the cell pathology in Mp infection *in vivo*. Therefore, the aim of this study was to identify the degree of cellular dysplasia in respiratory epithelium from MpP in comparison to other CAP infections caused by typical pathogens.

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MATERIALS AND METHODS

All patients were over 18 years old of age with CAP, who underwent both chest computed tomography (CT) and bronchoscopy in two hospitals between April 2013 and August 2015. The acute phase serum samples were tested for IgM antibodies to Mp by enzyme-linked immunosorbent assays test. The bronchoalveolar lavage fluid (BALF) samples were collected by conventional procedure. The definitive diagnosis of Mp infection was confirmed by a positive BALF result using quantitative loop-mediated isothermal amplification (qLAMpP) assays targeting for the P1 operon sequences using the Universal Kit for Bacterial DNA Extraction (Capitalbio Corporation, P.R. China) as described elsewhere.[5,6] A

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presumptive diagnosis of acute Mp infection was made by a positive IgM result. Clinical characteristics of patients were also recorded.

Screening for eight common respiratory bacterial pathogens in BALF was performed using qLAMP assay as described elsewhere.[6] Nine respiratory viruses in BALF were detected using FTD respiratory pathogens 21 plus multiplex real-time polymerase chain reaction kit (Fast-track Diagnostics, Junglinster, Luxembourg). Meanwhile, the assay using conventional sputum culture for each patient was carried out. CAP caused by bacterial pathogens and demonstrating a negative qLAMP assay for Mp gene categorized the Non-Mp (typical CAP) infection group (t-CAP group). The patients with suspected malignant lesions seen under direct vision on bronchoscopy or on CT scan, or patients with CAP caused by respiratory viruses and mixed infection were excluded. This study protocol was approved by the Ethics Committee of our institutions.

The cast-off epithelial cells were obtained via conventional bronchoscopy by brushing the infectious airway mucosa 2–3 times. The samples were analyzed as a cell smear in the fully automatic liquid-based cytology instrument (ThinPrepTM2000, Hologic, Inc., USA). Two senior pulmonary cytopathologists evaluated the specimen blindly under light microscopy by hematoxylin and eosin staining. Cell morphology was graded for cellular dysplasia according to the cellular and nuclei size, nuclear-cytoplasmic (N: C) ratio, the shape and halves of the nucleus, and chromatin pattern as previously established.[7]

Statistical analysis was performed using the SPSS version 16.0 for Windows (SPSS Inc., IBM, USA). Categorical variables and continuous variables were reported as percentages and as the mean ± standard deviation, respectively. Chi-square test and independent-samples Student's *t*-test were employed. A $P < 0.05$ was considered statistically significant.

RESULTS

A total of 15 patients with MpP were included in MpP group and 17 with typical CAP patients caused by other pathogens (t-CAP group). In the MpP group, nine patients were diagnosed based on qLAMP assay alone and six patients based on serology alone. Six patients were positive for both tests. A positive bacterium was detected in only 12% (2/17) of the t-CAP group patients on qLAMP assay, one *Streptococcus pneumoniae,* and one *Klebsiella pneumoniae*. None positive sputum culture assay was obtained in both groups. About 17 of 32 patients had received antibiotic therapy (>3 days duration) prior to admission. The patients in MpP group were younger, more likely to be female, and more likely to have a lower C-reactive protein level than those of the t-CAP group (43.33 ± 16.09 years vs. 57.29 ± 15.89 years, 60% vs. 17.6%, and 55.73 ± 44.63 mg/L vs. 115.95 ± 73.90 mg/L, respectively).

Both epithelial cells and inflammatory cells were found in the brushing samples. A representative high-power photomicrograph of epithelial cellular dysplasia is shown in Figure 1, compared to normal epithelial cells in Figure 2. The identification of total cellular and nuclear dysplasia was significantly higher in the MpP group [Table 1].

DISCUSSION

Theories on linkage between *Mycoplasma* and malignancy have been mainly proposed since the 1960s.[8] Laboratory data have demonstrated the potential for some *Mycoplasma* species to induce a karyotypic change and malignant transformation during prolonged or chronic tissue-culture infection *in vitro*.[3,4] Preneoplastic changes in cultured cells may be reversed following eradication by appropriate antibiotic treatment.[4] By a complex and specialized attachment organelle to attach to the respiratory epithelium with ease, *Mycoplasma* is primarily considered a mucosal

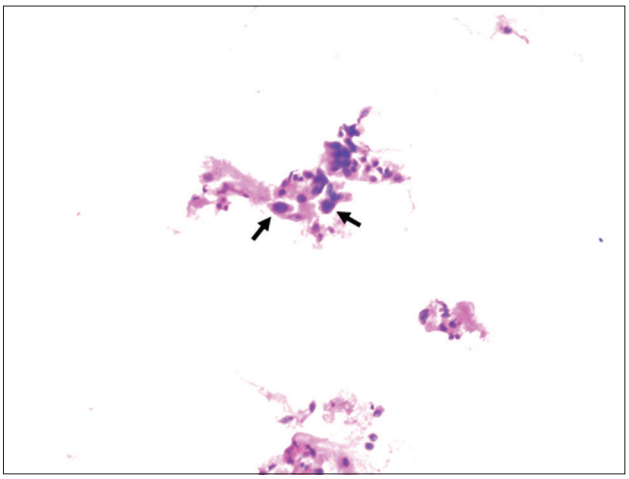

Figure 1: The respiratory epithelial cells of dysplasia (arrow) varied markedly in size and shape, nuclear pleomorphism with a significantly abnormal karyoplasmic ratio (H and E stained smearing sample, ×400 from a patient with *Mycoplasma pneumonia*)

Table 1: Morphological finding of respiratory epithelial cells in patients with *Mycoplasma pneumonia* and other pathogenic pneumonia

Grade of cellular dysplasia	MpP group (*n*=15)	Typical community -acquired pneumonia group (*n*=17)	*P**
Mild	1	1	
Moderate	2	0	
Severe	3	0	
Total	6	1	0.033

*Fisher's exact test

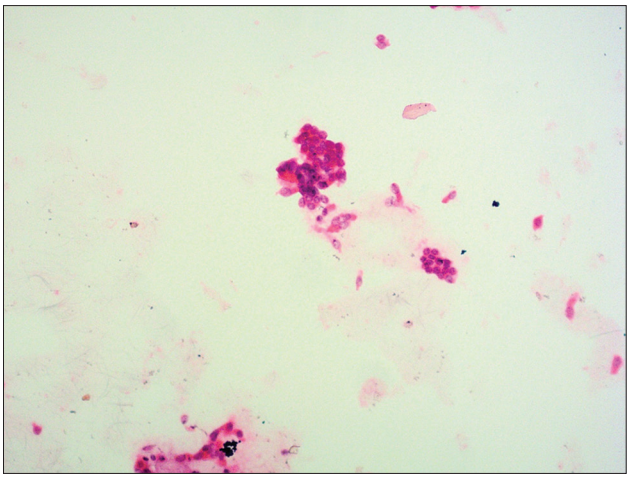

Figure 2: The heaped-up normal respiratory epithelial cells were shown (H and E stained smearing sample, ×400 from a patient with community-acquired pneumonia)

pathogen for parasitic existence.[1] Because it lacks a rigid cell wall, several mycoplasmal species have demonstrated the ability to fuse with and enter host cells which are not normally phagocytic, and the ability to survive, synthesize DNA, and undergo cell replication in host cells *in vitro*.[1,9] In contrast, the common bacteria such as streptococci are generally considered as extracellular pathogens because their polysaccharide capsule significantly attenuates their ability to invade the cytoplasm of respiratory epithelial cells, although occasional intracellular uptake may occur.[10] In addition, the intracellular damage from mycoplasmal enzymes and cytotoxins may be another cause to nuclear atypia.[11] To our knowledge, this is the first study to demonstrate a clear dyskaryotic change in airway epithelial cells in patients suffering from Mp than that in patients suffered by other organisms *in vivo*.

In this study, there was none of patients tested positive bacterial species for routine sputum culture, which was compatible with the findings partly caused by antimicrobial treatment before the collection of specimens as described recently.[12] Due to the application of antibiotics in the majority of candidates before admission and consequent effect on direct bacterial identification from delayed BALF samples, we applied a method of genetic and immunological exclusion to categorize CAP patients. We found that many clinical differences between two groups were similar to those described in other studies.[13,14]

CONCLUSIONS

The special biological characteristics and mechanisms of pathogenesis of Mp may be reflected in the different cytopathological aspects of MpP. This preliminary study identifies a potential ability of Mp to evoke dyskaryotic changes in respiratory epithelium, which may imply an oncogenic potential of latent or chronic Mp infection.

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Conflicts of interest

There are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

SCA carried out the design of the work, conducted the study, performed the statistical analysis and prepared the draft and the final version of the manuscript. ZCG provided assistance in the design of the study. DHY and YS conducted the microbiological laboratory tests and revised the draft. CFL and JZL conducted the radiological study and revised the draft. XC and YW conducted the pathological study and revised the draft. GTL and RQW collected the data and revised the draft. All authors have read and approved the final version of the manuscript.

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