**Mycoplasma hyorhinis**: a potential risk factor in gastric cancer progression

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Persistent infection of *Mycoplasma hyorhinis* (*M. hyorhinis*) is associated with various types of cancer. However, the molecular mechanism of *M. hyorhinis* infection and its effect on cancer patients’ prognosis were unknown. Recently, we reported for the first time that *M. hyorhinis* infects mammalian cells via the interaction of its membrane protein p37 and the host protein Annexin A2 (ANXA2). And NF-κB pathway, a downstream of ANXA2, is activated and mediates *M. hyorhinis*-driven cell migration. Furthermore, we demonstrated that *M. hyorhinis* p37 protein expression in gastric cancer tissues positively correlates with tumor metastasis and predicts poor survival. In conclusion, our study uncovers the mechanism by which *M. hyorhinis* infects mammalian cells and promotes cancer cell migration and unveils the effect of *M. hyorhinis* infection on gastric cancer survival.

**Keywords:** Mycoplasma hyorhinis; gastric cancer; p37; Annexin A2; NF-κB


The association of *Mycoplasma hyorhinis* infection with cancer

Mycoplasmas, a genus of bacteria lacking cell wall, could colonize in host tissues for long time without any pathological effects. *Mycoplasma hyorhinis* (*M. hyorhinis*), belonging to mycoplasmas (Class Mollicutes), was first identified in swine in 1962 [1]. It causes respiratory tract infections, arthritis, and inflammation of abdominal cavity in swines [2]. *M. hyorhinis* is well-known as a common contaminant of cell cultures. The association of *M. hyorhinis* with human cancers, such as prostate, gastric, colon and ovarian cancer, has been reported in the past three decades [3-5]. For example, Namiki and colleagues reported the potential of *M. hyorhinis* infection to elicit malignant transformation of benign human prostate BPH-1 cells in cell culture and xenografts [3]. Additionally, *M. hyorhinis* increases the migratory and invasiveness of gastric cancer cells and melanoma cells [5, 6].

In 1980’s, Dong and his colleagues generated monoclonal antibody PD4 through immunizing mouse with gastric cancer cell MGC803 [7]. But the subsequent antigen identification work revealed that the molecule recognized by PD4 is a lipoprotein from *M. hyorhinis*, namely p37, which indicated *M. hyorhinis* being existed in cancer cells [8]. Using this antibody for immunohistochemistry (IHC), the positive rate was 56% (50/90) in gastric cancer, 28% (18/64) in chronic superficial gastritis, 30% (14/46) in gastric ulcer and 37% (18/49) in intestinal metaplasia [4]. In colon cancer, the positive rate was 55.1% (32/58), but 20.9% (10/49) in adenomas or polyp [4]. These results show a step-wise increase of the positive rate following disease progression.
To confirm the result of *M. hyorhinis* infection in cancer tissues, we performed qPCR and IHC with a new cohort of gastric cancer tissues and found that the IHC technique is reliable, whose specificity was 83.0% and the sensitivity was 75.6%. Recently, we found that *M. hyorhinis* infection positively correlated with blood vessel invasion and metastasis, and the patients infected with *M. hyorhinis* had a lower 5-year survival rate than that of uninfected patients. However, the association between *M. hyorhinis* infection and gastric cancer needs to be addressed by multi-centric studies. We also anticipate more interest and the work will be focused on this field in future.

**Identification of the microbial and host factors mediating *M. hyorhinis* infection**

Early studies demonstrated p37 as a major component of high-affinity transport system of *M. hyorhinis* [9, 10]. p37 shares partial homology to the hemagglutinin protein of influenza A [11], but it has no homology to any mammalian proteins. Pro-invasive function of p37 has been documented previously by our lab and others’ [11, 12], but the role of p37 in *M. hyorhinis* infection is unclear. In the recently published work on journal “Cancer Research”, we found that *M. hyorhinis* could infect both cancerous and noncancerous cells, while cancer cells were more prone to be infected. We also demonstrated that antibody anti-p37 could block the binding of *M. hyorhinis* to host cells in qPCR, cell ELISA and flow cytometry assays. Furthermore, *M. hyorhinis*-induced cell migration was also inhibited with the antibody against p37 protein. These results indicate that infection of mammalian cells by *M. hyorhinis* is p37-dependent. The N-terminal region (amino acids 2-23) of p37 was hydrophobic and has limited homology to proteins of other mycoplasma species [10]. We revealed that the peptide of this region could bind to gastric cancer cells, and blocked *M. hyorhinis* infection competitively. These results suggest that p37-mediated *M. hyorhinis* infection rely on its N-terminal region.

In the following work, we identified Annexin A2 (ANXA2) as a protein associated with p37 through their N-terminal region interaction. Using antibody to ANXA2 or small interfering RNA (siRNA) technique, we found that ANXA2 was required for *M. hyorhinis* infection. These results indicated that ANXA2 is a host receptor mediating *M. hyorhinis* infection. Several recent studies also reported the relationship between ANXA2 and microbial infection. For example, ANXA2 contributes to HPV16 infection [13], and is also involved in the formation of hepatitis C virus replication complex on the lipid raft [14]. Our findings demonstrate that ANXA2 contributes to *M. hyorhinis* infection through its interaction with p37 at their N-termini. Based on this discovering, the N-termini peptides of p37 or ANXA2 may provide therapeutic options to combat *M. hyorhinis* infection.

Tyr23 phosphorylation of ANXA2 is associated with its membrane localization under stress conditions [15]. We found that *M. hyorhinis* upregulated Tyr23 phosphorylation of ANXA2 and its membrane recruitment. It was reported that EGFR plays a role in regulating ANXA2 phosphorylation and localization [16]. Moreover, our previous work reported that both *M. hyorhinis* infection and recombinant p37 treatment could activate EGFR [5, 12], but the role of EGFR in *M. hyorhinis* infection and *M. hyorhinis*-induced metastasis are not fully elucidated. We noticed that ANXA2-EGFR interaction was increased and EGFR preferred to interact with phosphorylated ANXA2 in *M. hyorhinis*-infected cells. Several recent studies reported that EGFR plays a critical role in pathogen infection. EGFR is a cofactor for HCV entry and is a receptor for HCMV [17, 18], EGFR and HER2 function together as receptors for *C. albicans* [19]. Our findings added more evidence for the important role of EGFR in microbial infection.

**Signaling pathway mediating *M. hyorhinis* induced cancer cell migration**

We found that NF-κB signaling was activated by *M. hyorhinis* exposure and was required for *M. hyorhinis*-induced gastric cancer cell migration. Importantly, both ANXA2 and EGFR were upstream of NF-κB signaling in the context of *M. hyorhinis*. Epithelial-mesenchymal transition (EMT) has been recognized as an important step during cancer metastasis [20]. In our another recent study, we observed that *M. hyorhinis* induced EMT in gastric cancer MGC803, but not in AGS cells, indicating *M. hyorhinis*’ effect on EMT is cell type-dependent for gastric cancer cells [21]. Moreover, molecular markers indicative of EMT was altered upon *M. hyorhinis* infection in MGC803 cells. We found that these changes were TLR4-and NF-κB-dependent. Currently, we are trying to figure out the molecular mechanisms of *M. hyorhinis*-induced EMT and its contribution to cancer development.

**Challenges and Perspectives**

Understanding the molecule mechanisms of gastric cancer and identifying the risk factors are important for its prevention and patient-directed therapy. Our recently published work indicates a link between *M. hyorhinis* infection and the unfavorable outcome of gastric cancer patients. At the molecular level, we uncover the receptor and signaling pathways which mediates *M. hyorhinis* infection and *M. hyorhinis*-promoted malignancies. These data will provide several potential approaches for preventing *M. hyorhinis* infection. However, the role of *M. hyorhinis* infection in
tumorigenesis and the correlated effects of *M. hyorhinis* and *Helicobacter pylori* infection in gastric cancer development are unknown. It is very important to elucidate these issues for developing more potential strategies for prevention and treatment of gastric cancer.

**Conflicting interests**

The authors have declared that no competing interests exist.

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