Editorial

Role of *Mycoplasma* in the Initiation and Progression of Oral Cancer

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Mycoplasma represents a unique group of bacteria measuring about 0.2-0.8 μm in diameter. Due to the absence of a cell wall, *Mycoplasma* varies in size and shape. A number of these *Mycoplasma* species such as *Mycoplasma salivarium, Mycoplasma orale, Mycoplasma faucium, Mycoplasma buccale, Mycoplasma fermentans, Mycoplasma lipophilum,* and *Mycoplasma pneumonia* form a part of the normal microbial flora of the oropharynx.[1-3] Among these *M. salivarium* and *M. orale* are isolated at a higher rate in the mouth including the saliva, gingival sulci, dental plaque, and periodontal pockets. Due to its small genome, *Mycoplasma* lacks the necessary metabolic option for self-survival and replication.[1-3] Thus, it relies on a host cell for its metabolic needs (parasitic organism).

The location of *Mycoplasma* was assumed to be on the surface of cells. However, recent data have confirmed its intracellular location using both *in-vitro* and *in-vivo* techniques. Now the question arises as to the long-term effects of a parasitic organism like *Mycoplasma* on the host cell. The mere presence of *Mycoplasma* in oral cancer tissues does not merit a designation as a carcinogen, as it forms a part of the normal microbial flora. There have been various studies reporting isolation of a greater percentage of *Mycoplasma* species in various infectious, neoplastic tissues, and body fluids than in a healthy individual. Few researchers have even correlated a possible association of *Mycoplasma* with AIDS.[4]

To answer these queries *in-vitro* studies were conducted to observe the effects of *Mycoplasma* on cell lines. Tsai *et al.*[5] in 1995 infected cultured mouse embryo cells, C3H/10T1/2 (C3H) with *M. fermentans* and *Mycoplasma penetrans*. They found that *Mycoplasma* species did not have any detectable effect on the cell morphology or the growth pattern of the cell lines up to five passages (5 weeks). *M. fermentans* and *M. penetrans* had a reversible effect on the cultured cells during the 6th and 15th passages respectively. The changes consisted of the following: The cells lost cell to cell contact, assumed spindle morphology and exhibited growth in multiple layers. The reversible nature of these changes was elicited by treating the cells with three cycles (1 week per cycle) of ciprofloxacin. Eradication of *Mycoplasma* was confirmed by polymerase chain reaction (PCR). Most of the transformed cells reverted back to their original morphology and growth pattern (flat monolayer growth). The cells subjected to more than 18 passages failed to revert back to its previous morphology/growth pattern, indicating an irreversible change. Thus, persistent infection with *Mycoplasma* induced a multistep step carcinogenesis.[5]

Barykova *et al.*[6] in 2011 investigated the role of *Mycoplasma hominis* in prostate cancer. They found that *M. hominis* was detected 3 times higher in patients with prostate cancer in comparison to patients with benign prostatic hyperplasia. Further the prostate specific antigen levels were significantly elevated in subjects positive for *M. hominis*. Correlating these data, they suggested a causal link of *Mycoplasma* in the development of prostate cancer.[6] Logunov *et al.*[7] analyzed the mechanism of *Mycoplasma* induced carcinogenesis. They compared the effects of *Mycoplasma* infection on apoptotic regulators like p53 and nuclear factor (NF)-κB. They infected mouse Balb 3T3 cells with *Mycoplasma arginini.* They noticed a dramatic decrease in activation of p53 with a constitutive activation of NF-κB. This pattern of altered expression was consistent with many human cancers.[7] Thus, *Mycoplasma* infected cells were able to evade apoptosis by inhibiting p53 which in turn rendered the cells susceptible to H-Ras. The control cells not infected with *Mycoplasma* underwent a p53 controlled cell arrest.[7] Feng *et al.*[8] observed that the 32D murine myeloid cell line undergoes apoptosis following withdrawal of interleukin-3 (IL-3). However, when infected with *Mycoplasma* species the 32D cell line evaded apoptosis even in the absence of IL-3.

Though sufficient studies have demonstrated the malignant potential of *Mycoplasma* in prostate cancer, myeloid cell lines, and mouse embryo cells, there was no substantial evidence linking *Mycoplasma* to oral cancer.[8] Mizuki was the first to formulate a correlation between oral leukoplakia and *M. salivarium.*[9] They observed a small granular bisbenzimide stained fluorescent structures in the cytoplasm of oral leukoplakia cells. Based on its morphology and size they presumed it to represent a *Mycoplasma* species. Following the observation of Mizuki *et al*. several light and electron microscopic studies were conducted to reveal the presence of *Mycoplasma* in the oral leukoplakia cells. None of the studies revealed their presence spiraling debate as to Mizuki *et al*. findings. To confirm their earlier results Mizuki *et al*. performed an immunohistochemical analysis to detect *Mycoplasma* in oral leukoplakia cell.[10] They utilized both a polyclonal and a monoclonal antibody specific for *M. salivarium*. The result showed a substantial increase in the presence of *Mycoplasma* within the cytoplasm of oral leukoplakia in comparison to control (normal oral mucosa). Immunoelectron microscopy demonstrated electron dense particles in the cytoplasm of oral leukoplakia cells confirming their intracellular location. The presence of *M. salivarium* was reconfirmed by PCR. PCR produced a fragment of about 150 bp. The NCBI/BLAST databases indicated a 100% match to *M. salivarium.*[10]

Based on the consistent demonstration of *Mycoplasma* in oral leukoplakia, Mizuki *et al*. proposed a causal role for *M. salivarium* in the initiation of oral leukoplakia. The proposal was based on the following data: (a) Salivary IL-6 and tumor necrosis factor alpha are reported to be expressed at a higher level in oral leukoplakia,[11] (b) several studies including that of Drexler and Uphoff have found that *Mycoplasma* induced increased cytokine expression (as noticed in oral leukoplakia) in addition to various chromosomal alterations,[12] (c) further the intensity of the polyclonal and monoclonal antibody of *M. salivarium* was higher in areas with thicker cornified layer (hyperkeratosis, representing the initial stage of oral leukoplakia). At present, there is a lack of *in-vivo* studies illustrating the pathogenesis of *Mycoplasma* in oral potentially malignant disorders. *In vivo* studies using animal models like the Syrian hamster cheek pouch may aid us in understanding the molecular pathway of *Mycoplasma* induced oral carcinogenesis, which in turn will provide us with vital diagnostic markers and potential therapeutic targets.

References

1. Kumagai K, Iwabuchi T, Hinuma Y, Yuri K, Ishida N. Incidence, species, and significance of *Mycoplasma* species in the mouth. J Infect Dis 1971;123(1):16-21.
2. Blanchard A, Bebear CM. *Mycoplasma*s of humans. In: Razin S, Herrmann R, editors. Molecular Biology and Pathogenicity of *Mycoplasma*s. New York: Kluwer Academic/Plenum Publishers; 2002. p. 45-71.
3. Watanabe T, Matsuura M, Seto K. Enumeration, isolation, and species identification of *mycoplasma*s in saliva sampled from the normal and pathological human oral cavity and antibody response to an oral *mycoplasma* (*Mycoplasma salivarium*). J Clin Microbiol 1986;23(6):1034-8.
4. Lo SC. *Mycoplasma*s and AIDS. In: Maniloff J, McElhaney RN, Flinch LR, Baseman JB, editors. *Mycoplasma*s, Molecular Biology and Pathogenesis. Washington, DC: American Society for Microbiology; 1992.
5. Tsai S, Wear DJ, Shih JW, Lo SC. *Mycoplasma*s and oncogenesis: Persistent infection and multistage malignant transformation. Proc Natl Acad Sci U S A 1995;92(22):10197-201.
6. Barykova YA, Logunov DY, Shmarov MM, Vinarov AZ, Fiev DN, Vinarova NA, *et al*. Association of *Mycoplasma* hominis infection with prostate cancer. Oncotarget 2011;2(4):289-97.
7. Logunov DY, Scheblyakov DV, Zubkova OV, Shmarov MM, Rakovskaya IV, Gurova KV, *et al*. *Mycoplasma* infection suppresses p53, activates NF-kappaB and cooperates with oncogenic Ras in rodent fibroblast transformation. Oncogene 2008;27(33):4521-31.
8. Feng SH, Tsai S, Rodriguez J, Lo SC. *Mycoplasma*l infections prevent apoptosis and induce malignant transformation of interleukin-3-dependent 32D hematopoietic cells. Mol Cell Biol 1999;19(12):7995-8002.
9. Mizuki H. *In situ* staining with DNA-binding fluorescent dye, Hoechst 33258, to detect microorganisms in the epithelial cells of oral leukoplakia. Oral Oncol 2001;37(6):521-6.
10. Mizuki H, Kawamura T, Nagasawa D. *In situ* immunohistochemical detection of intracellular *Mycoplasma salivarium* in the epithelial cells of oral leukoplakia. J Oral Pathol Med 2015;44(2):134-44.
11. Brailo V, Vucicevic-Boras V, Cekic-Arambasin A, Alajbeg IZ, Milenovic A, Lukac J. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. Oral Oncol 2006;42(4):370-3.
12. Drexler HG, Uphoff CC. *Mycoplasma* contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology 2002;39(2):75-90.