学位論文内容の要旨

口腔医学専攻口腔病態学専修　　博士（歯学）　　氏　名　Yu Li

学 位 論 文 題 名

The oral bacterium *Streptococcus mutans* promotes tumor metastasis by inducing vascular inflammation

（口腔細菌, *Streptococcus mutans* は、血管炎症を誘発し転移を促進する）

Keywords: endothelial cell, oral bacteria, oral hygiene, tumor metastasis, vascular inflammation

Cancer is the second leading cause of death globally. Many cancers possess the propensity to metastasize to sites of inflammation. Hematogenous metastasis is responsible for 90% of tumor metastasis-associated mortality. Therefore, it is necessary to identify the risk factors that promote hematogenous metastasis.

Streptococcus mutans (*S. mutans*) is a Gram-positive bacterium associated with dental caries. *S. mutans* gains access to the bloodstream during invasive dental procedures, such as tooth extraction, periodontal surgery, or even daily oral hygiene practices to cause systemic diseases. Endothelial cells (ECs) line blood vessels, control the opening and closing of the vascular barrier and provide the nutrients for tumor growth and metastasis. Studies have indicated that *S. mutans* can invade ECs through toll-like receptor 2 to cause inflammation. Inflamed ECs result in hyperpermeability of the blood vessel; however, the role of *S. mutans*-mediated vascular inflammation in the progression of hematogenous metastasis has been unknown.

In our study, we first examined the invasion of *S. mutans* to the ECs using gram staining and colony formation assay. *S. mutans* invade the ECs after 3 h stimulation and the number of intracellular-invading *S. mutans* was significantly increased with the prolonged incubation time. Indicating that *S. mutans* invasion into ECs. In addition, RNA sequencing identified the activation of some pathways related to inflammation and cancer microenvironments in *S. mutans*-stimulated ECs. High levels of inflammatory cytokines such as IL-6, IL-8, IL-1β, and TNF-α were determined in *S. mutans*-stimulated ECs through NF-κB activation. Furthermore, tumor cells migrated more efficiently toward *S. mutans*-stimulated ECs, suggesting that *S. mutans* is involved in inducing vascular inflammation and promoting tumor cell migration toward ECs.

In the process of tumor cell extravasation, tumor cells make contact with ECs. The association of *S. mutans* with tumor cell-EC adhesion was determined using an adhesion assay. The number of tumor cells adherent to the *S. mutans*-stimulated ECs was significantly increased by the *S. mutans* number and stimulation manner. Most importantly, we observed markedly enhanced expression of intercellular adhesion molecule ICAM-1 at the mRNA level and protein level in *S. mutans*-stimulated ECs. These data suggest that *S. mutans* regulates tumor cell adhesion to the endothelium by activating ICAM-1 in the ECs.

Vascular leakage is responsible for tumor cell extravasation across the endothelium for metastasis to secondary organs. Therefore, we compared the expression of the endothelial adhesion molecules, VE-cadherin and ZO-1, in *S. mutans*-stimulated ECs and non-stimulated ECs. Both VE-cadherin and ZO-1 mRNA

expression was significantly decreased in ECs following *S. mutans* stimulation. The transendothelial electrical resistance (TEER) assay revealed a reduction of TEER values in the *S. mutans*-stimulated EC monolayer, indicating enhanced vascular leakage by *S. mutans*.

In vivo, *S. mutans* was intravenously administered to C57BL/6 female mice. The expression of the inflammatory cytokines, IL-6 and TNF-α, was upregulated in the *S. mutans* group compared with the control group. Double immunofluorescent staining of CD31 and CD45 revealed a significant increase in CD45-positive inflammatory cells in the lungs of the *S. mutans* group accompanied by accumulation of CD45-positive inflammatory cells around the CD31-positive area, suggesting that *S. mutans* causes vascular inflammation in the lungs. Consistent with *in vitro* findings, we measured the elevated expression of ICAM-1 and reduction of VE-cadherin in the *S. mutans* group. Lung vascular permeability was studied through intravenous injection of 40-kDa FITC-dextran to the mice. Fluorescent imaging showed higher diffusion of the FITC-dextran in the *S. mutans* group than in the control group. Together, these data indicate that *S. mutans* induces pulmonary vascular inflammation accompanied by enhanced intercellular adhesions and reduced endothelial adherens junctions, as well as vascular hyperpermeability.

The *in vitro* data showed that *S. mutans* promotes the migration and adhesion of tumor cells to ECs. Therefore, we determined whether *S. mutans*-induced vascular inflammation affects tumor metastasis *in vivo*. We injected *S. mutans* intravenously followed by implantation of murine breast carcinoma tdtomato-

Luc2-expressing E0771 cells via the tail vein. The *S. mutans* group exhibited significantly increased lung metastatic tumors as determined by *ex vivo* imaging 1 week following tumor cell injection. In addition, to find *in vivo* evidence that *S. mutans* promotes tumor metastasis by inducing inflammation through NF-κB signaling, an anti-inflammatory drug, aspirin, or NF-κB inhibitor was treated in the pro-inflammatory stage, respectively, significant regression of lung metastasis was observed in the mice along with aspirin or NF-κB inhibitor. These results suggest that *S. mutans* promotes tumor metastasis to the lungs by inducing vascular inflammation.

In this study, we demonstrated that *S. mutans* promotes tumor metastasis by invading blood circulation. To our knowledge, this is the first report demonstrating that oral bacteria can promote tumor metastasis by inducing vascular inflammation at distant organs. This suggests that oral bacteria may represent a risk factor for tumor metastasis. Postoperative pneumonia frequently occurs in hospitalized patients and is intimately associated with postoperative mortality. Oral bacteria increase the risk of postoperative pneumonia and professional oral hygiene practices serve to minimize the risk of postoperative pneumonia in lung and esophageal cancer. Our findings, on the other hand, reveal a novel role of oral bacteria in promoting tumor metastasis. They reinforce the need for professional oral hygiene management in patients with cancer in terms of avoiding postoperative pneumonia and tumor metastasis.