# Doctoral thesis/dissertation Digest Form

Thesis/dissertation Title

**Analysis of H. pylori-induced gastric pathology using mouse ES cells-derived gastric organoids**

Student's Name

**Trinh Thi Thuy Linh**

Approved Digest

[**Background**] Chronic infection with *H. pylori* is associated with the development of gastric diseases such as gastritis, gastric ulcer, and gastric cancer. One of the major virulence factors of *H. pylori* is cytotoxin associated gene A (*CagA*). Organoids that mimic *in vivo* tissue complexity and cellular diversity have been used as potential models to analyze diseases *in vitro*. By using the stomach organoids derived from mouse embryonic stem cells, my project aimed to generate a *H. pylori*-infected stomach disease model that overexpresses *CagA* gene to investigate CagA-dependent pathogenesis in the stomach.

[**Method**] Mouse ES cells were sequentially differentiated to definitive endoderm, stomach precursors, and mature gastric organoids. To efficiently induce the differentiation of initial definitive endoderm to stomach precursors, various combinations of growth factors and chemical compounds were examined. Using the optimized conditions, the stomach precursors were further differentiated to the mature gastric organoids and *CagA* was overexpressed in these gastric organoids by Tet-off inducible system. The effects of CagA on the gastric organoids were analyzed by co-immunostaining of CagA and several marker proteins related to cell proliferation, cell differentiation, and cell morphology.

[**Results**] The best culture condition for the differentiation of stomach precursor was the combination of FGF4, a Wnt pathway agonist CHIR99021, a BMP pathway inhibitor LDN193189, and retinoic acid. This combination highly induced the expression of stomach precursor marker, *Sox2*, and suppressed the expression of intestinal precursor marker, *Cdx2*. With the optimized culture conditions, the organoids terminally differentiated to gastric organoids with glandular epithelial cells surrounded with multiple layers of

mesenchymal cells. These epithelial cells were positive with a mucus-secreting pit cell marker, GKN2, a hormone-producing gastric endocrine cell marker, Chromogranin A, a mucus-secreting neck cell marker Agr2, or proliferating isthmus stem cell marker, Ki67. Next, gastric organoids were differentiated with optimized protocol and CagA was overexpressed in the mature organoids. These CagA+ gastric organoids induced DNA double strand breaks (DSBs) and suppressed gastric epithelial cell proliferation. [Discussion] The induction of DNA DSBs may suppress the cell proliferation in the CagA+ gastric epithelial cells. Although the detailed mechanisms of these observations remain elucidated, I have established an *in vitro* model system to analyze how a pathogenic protein CagA affects stomach epithelium *in vitro*.