

JOINT RESEARCH PROJECT

FINAL REPORT
For Japan-Korea Joint Research Project

AREA	1. Mathematics & Physics
	2. Chemistry & Material Science
	3. Biology
	4. Informatics & Mechatronics
	5. Geo-Science & Space Science
	6. Medical Science
	7. Humanities & Social Sciences

1. Research Title:

Study on DNA damage responses in infection and oncogenesis

2. Term of Research: From July 1, 2009 To June 30, 2011

3. Total Budget

a. Financial Support by JSPS: Total amount: 2,400 thousand yen

1st Year 1,000 thousand yen 2nd Year 1,200 thousand yen

3rd Year 200 thousand yen

b. Other Financial Support : Total amount: 0 thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Tomohiro Morio
Institution / Department	Tokyo Medical and Dental University Graduate School Department of Pediatrics and Developmental Biology
Position	Associate Professor
b. Korean Principal Researcher	
Name	Hyeyoung Kim
Institution / Department	Yonsei University Dept. of Food & Nutrition College of Human Ecology
Position	Professor

c. List of Japanese-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Shuki Mizutani	Tokyo Medical and Dental University Graduate School, Department of Pediatrics and Developmental Biology	Professor
Masayuki Nagasawa	Tokyo Medical and Dental University Graduate School, Department of Pediatrics and Developmental Biology	Assistant Professor
Masatoshi Takagi	Tokyo Medical and Dental University Graduate School, Department of Pediatrics and Developmental Biology	Instructor
Fumiaki Watanabe	Tokyo Medical and Dental University Graduate School, Department of Pediatrics and Developmental Biology	Graduate student

d. List of Korean-side Participants (Except for Principal Researcher)

Name	Institution/Department	Position
Kyung Hwan Kim	Dept. of Pharmacology, Yonsei University College of Medicine	Professor
Joo Weon Lim	Dept. of Pharmacology, Yonsei University College of Medicine	Post-doctoral fellow
Hye Yun Chung	Dept. of Food & Nutrition, Yonsei University College of Human Ecology	Post-doctoral fellow
Soon Ok Cho	Dept. of Pharmacology, Yonsei University College of Medicine	Graduate student
Sung Hee Jang	Dept. of Food & Nutrition, Yonsei University College of Human Ecology	Graduate student

5. Number of Exchanges during the Final Fiscal Year*

a. from Japan to Korea

*Japanese fiscal year begins April 1.

Name	Home Institution	Duration	Host Institution
Tomohiro Morio	Tokyo Medical and Dental University Graduate School	May 19-21,2011	Yonsei University College of Medicine
For Final Fiscal Year(FY2011) Total: _____ 1 _____ persons		For Final Fiscal Year(FY2011) Total: _____ 3 _____ man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total _____ 1 _____ persons			
FY2010: Total _____ 1 _____ persons			

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution
Hyeyoung Kim	Yonsei University College of Medicine	October 29 - 31, 2010	The 4th Meeting of Japanese Society of alpha-Lipoic Acid
For Final Fiscal Year(FY2011) Total: _____ 1 _____ persons		For Final Fiscal Year(FY2011) Total: _____ 3 _____ man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total _____ 1 _____ persons			
FY2010: Total _____ 1 _____ persons			

6. Objective of Research

Objective of this research is to elucidate the molecular basis of DNA damage responses in infection and in oncogenesis.

Korean principal investigator (PI) has been working on gastric epithelial cell injury induced by various cellular stresses including *Helicobacter pylori* (*H. pylori*) infection as model of gastritis and gastric cancer. They demonstrated the importance of Ku70/80 expression and their nuclear localization in survival of mammalian cells upon DNA damage. Their recent investigation shows that ATM may be essential for Ku activation. Since Ku and ATM are important DNA damage response proteins, the maintenance of both proteins in nucleus is essential for DNA repair process after DNA damage by cellular stress including *H. pylori* infection. The constant DNA damage by *H. pylori* in association with the second hit would lead to the development of gastric cancer. However, we have little knowledge on how *H. pylori*-infected cells respond to DNA damage and transform into malignancy.

Japanese PI will focus on DNA damage responses induced by various cellular stress (*H. pylori*, ionizing irradiation, UV, chemotherapeutic agents) that involves the activation of DNA damage response protein (eg. ATM, ATR, Artemis, Mre11, DNA ligase IV, XLF), cell cycle checkpoint, and chromosomal abnormality. The mechanisms leading to chromosomal abnormality is still unknown. Most of the cells that failed to repair DNA are destined to die via apoptosis, and survived cells need another machinery to have chromosomal abnormality and to acquire oncogenic transformation.

The team of Japanese PI has proposed that defective DNA damage response underlies the development of pediatric malignancy. The group has accumulated data on detailed DNA damage response pathway involving ATM/ATR and Artemis. Our team has also been studying molecular basis of chromosomal translocation and of somatic mutation in p53.

The combination of the data from both side and further collaboration on DNA damage response pathway will lead to depiction of the mechanism that starts from cellular DNA stress and ends in apoptosis or DNA repair. We will obtain a clue on how cellular stress (including *H. pylori* infection) leads to malignancy via analysis on changes in DNA damage response and survival of DNA-damaged cells. The study may result in development of novel strategy to prevent oncogenesis in relation to cellular stress including *H. pylori* and in development of novel therapeutic strategy.

7. Methodology

Stimulation

H. pylori in Korean isolates (HP99), *cagA*⁺, *vacA* s1bm2, *iceA1* *H. pylori* strain, EBV, Mycoplasma, H₂O₂, or ionizing irradiation was used for inducing DNA damage.

Cells

Human gastric epithelial cells: AGS cells, lymphocytes, and monocytes were used for these assays.

Cell viability and DNA fragmentation was determined. In addition *Determination of 8-OH-dG* is done by using Biotrin OxyDNA assay kit. *Neutral comet assay (single-cell gel electrophoresis)* was employed for determination of DNA damage in single cell.

DNA damage response was monitored by Western blotting using polyclonal antibodies for phospho-specific forms of ATM, ATR, Chk1, p53, and H2AX (□-H2AX). We also measured Chk1, Chk2, ATR, ATM, p53, Bax, Bcl-2, or actin. Similarly, the level of ATM phosphorylation, p53 phosphorylation, and H2AX phosphorylation was determined by using a flow cytometry. The detection method was developed in the laboratory of Japanese side and was optimized in this research project.

Cell cycle analysis was done by using a traditional flowcytometric analysis in AGS cells incubated with *H. pylori*.

Measurement of intracellular ROS

Intracellular ROS in AGS cells stimulated with the various agents was detected by using DHR123 staining or by using a luminol assay. counted manually.

Statistical analysis

The statistical differences were determined using one-way ANOVA and Newman-Keul's test. All values are expressed as mean ± S.E. of four different experiments. A value of $p < 0.05$ was considered statistically significant.