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:

Development of the novel tumor cell vaccine carrying oncolytic adenovirus combined with innate immune modulators

2. Term of Research: From 7.1.2009 To 3.31.2011

3. Total Budget

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

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

3rd Year 0 thousand yen

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

4. Project Organization

a. Japanese Principal Researcher	
Name	Shirakawa, Toshiro
Institution / Department Position	Kobe University, Graduate School of Medicine, Associate Professor
b. Korean Principal Researcher	
Name	Lee, Kyung-Mi
Institution / Department Position	Korea University, School of Medicine, Professor

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

Name	Institution/Department	Position
Kawabata、Masato MD, PhD	Kobe University Graduate School of Medicine	Professor
Fujisawa、Masato MD, PhD	Kobe University Graduate School of Medicine	Professor
Kamigaki, Takashi MD, PhD	Kobe University Graduate School of Medicine	Visiting Professor
Hamada, Katsuyuki MD, PhD	Ehime University School of Medicine	Assistant Professor
Yanagi, Daisuke MD	Kobe University Graduate School of Medicine	PhD candidate
Zainal, Adhim MD	Kobe University Graduate School of Medicine	PhD candidate
Saito, Aya DVM	Kobe University Graduate School of Medicine	PhD candidate
Matsuoka, Tadayuki MS	Kobe University Graduate School of Medicine	PhD candidate

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

Name	Institution/Department	Position
Lee, June-Chul PhD	Korea University School of Medicine	Research Professor
Son, Jung-Hee PhD	Korea University School of Medicine	Research Professor
Kim, Sung-Tae PhD	Korea University School of Medicine	Research Professor
Kim, Tae-Jin MS	Korea University School of Medicine	PhD candidate
Kim, Eun-Ok MS	Korea University School of Medicine	PhD candidate

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
Shirakawa, Toshiro Fujisawa, Masato	Kobe University	1.30-1.31.2011	National Cancer Center, Seoul, Korea
Shirakawa, Toshiro	Kobe University	2.28-3.1.2011	Korea University

For Final Fiscal Year(FY2010)	For Final Fiscal Year(FY2010)
Total: 3 persons	Total: 6 man-days

Numbers of Exchanges during the past fiscal years
FY2008: Total 0 persons FY2009: Total 7 persons

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution
Lee, Kyung-Mi Lee, June-Chul Son, Jung-Hee Kim, Tae-Jin Kim, Eun-Ok	Korea University	8.23-8.25.2011	Kobe University
For Final Fiscal Year(FY2010) Total: 5 persons		For Final Fiscal Year(FY2010) Total: 15 man-days	
Numbers of Exchanges during the past fiscal years			
FY2008: Total 0 persons FY2009: Total 5 persons			

6. Objective of Research

Although cancer therapy using replication-selective oncolytic adenoviruses has been available for many years, its anti-tumor efficacy is suboptimal due to low and nonspecific infectivity that depends on Coxsackie Adenovirus Receptor (CAR) expressions of the target cancer and normal cells, and generation of an anti-adenovirus neutralizing antibody. In addition, concerns of triggering a severe innate immune response against the adenovirus limit the systemic administration. We have developed the carrier-cell based adenoviral oncolytic virus system called CBOVS, which can infect cancer cells via CAR-independent pathway. Our preliminary study has eluded that the carrier cells, irradiated A549 cells, also resulted in anti-tumor immunity. These findings shed light on the future potential for combination of oncolytic virus and immunotherapy. Therefore, we will carefully characterize the immune responses of CBOVS in regulating both innate and adaptive immune responses in tumor-bearing hosts and provide the molecular basis underlying these phenomena. Our CBOVS contains irradiated A549 tumor cells as carrier cells and concealing the adenovirus (Ad-IAI.3B) inside to improve the specific infectivity. We investigated the anti-tumor effect of CBOVS in a multiple lung tumor mouse model.

7. Methodology

Experiment 1

First we examined whether CBOVS could protect adenovirus from neutralizing antibody *in vitro*. KLN205, mouse squamous cell carcinoma cells were infected with AdE3-IAI.3B or CBOVS in medium with or without anti-adenovirus antibody for 6 hours. Then KLN205 cells were collected and the amount of infected adenovirus DNA was quantified by Real-Time PCR.

Experiment 2

To examine the capability of CBOVS to deliver AdE3-IAI.3B to the tumor sites, we conducted *in vivo* study. AdE3-IAI.3B or CBOVS was intravenously injected into DBA/2 mice bearing multiple lung tumors of KLN205. Lung, liver, spleen and kidney were removed 12 hours after the injections and the amount of adenovirus DNA was measured.

Experiment 3

Also tumor growth inhibitory effect was assessed by measuring of tumor area.

Experiment 4

To investigate the immune response after CBOVS treatment, splenocytes were cultured and stimulated with KLN205. The levels of INF-γ and IL-12 in supernatant were measured by ELISA.

Experiment 5

Also, the infiltration of lymphocytes (T-cells and B-cells) into the tumor mass was investigated by immunohistochemical study.