(Form4-2)

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:

Investigation of Pathophysiological Mechanisms Related to Hypoxic Micro-environmental Condition in Malignant Tumors Using Molecular Imaging Techniques.

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: thousand yen

4. Project Organization

a. Japanese Principal Researcher	
Name	Yasushi Kiyono
Institution / Department Position	University of Fukui / Biomedical Imaging Research Center Associate professor
b. Korean Principal Researcher	
Name	LEE Jong Doo
Institution / Department Position	Yonsei University, College of Medicine Professor

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

Name	Institution/Department	Position
Yasuhisa Fujibayashi	National Institute of Radiological Sciences / Molecular Imaging Center	Center Head
Hidehiko Okazawa	University of Fukui / Biomedical Imaging Research Center	Center Head / Professor
Tetsuya Mori	University of Fukui / Biomedical Imaging Research Center	Associate
Yukie Yoshii	National Institute of Radiological Sciences / Molecular Imaging Center	Researcher

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

Name	Institution/Department	Position
YUN Mijin	Yonsei University College of Medicine	Associate Professor
KANG Won Joon	Yonsei University College of Medicine	Assistant Professor
AHN Keun Jae	Yonsei University College of Medicine	Research assistant professor
CHOI Min-Ah	Yonsei University College of Medicine	Instructor

5. Number of Exchanges during the Final Fiscal Year*

*Japanese fiscal year begins April 1.

a. from Japan to Korea

Name	Home Institution	Duration	Host Institution
None			
For Final Fiscal Year(FY2011) Total: 0 persons		For Final Fiscal Year(FY2011) Total: 0 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 6 persons FY2010: Total 3 persons			

b. from Korea to Japan

Name	Home Institution	Duration	Host Institution
None			
For Final Fiscal Year(FY2011) Total: 0 persons		For Final Fiscal Year(FY2011) Total: 0 man-days	
Numbers of Exchanges during the past fiscal years			
FY2009: Total 0 persons FY2010: Total 4 persons			

6. Objective of Research

Tumor hypoxia poses a major challenge in cancer treatment due to increased resistance to radiotherapy or chemotherapy. It also induces angiogenesis for distant metastasis, and eventually causes poor patient outcome in malignant tumors. Hypoxia inducible factor-1 (HIF-1) is an important transcriptional regulator related to hypoxia, cellular electron transport system, and energy metabolism. HIF-1 expression is affected by PI3K/Akt/mTOR, or AMPK pathways, and energy metabolic status such as glycolysis. HIF-1 expression also plays an important role in angiogenesis in malignant tumors. For the evaluation of pathophysiological mechanisms related to tumor progression under hypoxic micro-environment, detection of hypoxic cells in the tumor tissue is very important. The Cu-64-ATSM is an ideal positron emission tomography radiotracer to evaluate hypoxic condition and electron transport system dysfunction in the tumor tissue. It was developed by Professor Fujibayashi at University of Fukui, and a number of studies are being done world widely using this radiotracer. Our team is conducting several research projects related to energy metabolism, tumor angiogenesis and tumor hypoxic microenvironment using a recently developed new radiotracer, F-18- RGD compound. In collaboration with Professor Fujibayashi, our team is able to establish the synthesis method of Cu-64 ATSM in our side, and mutual collaboration research studies using various radiotracers such as Cu-64-ATSM, F-18-FMISO, F-18-FDG and F-18-RGD compound with molecular imaging techniques might be able to discover a new pathophysiological mechanism for tumor progression and metastasis under hypoxic micro-environmental condition.

7. Methodology

Uptake mechanism of Cu-64-ATSM in the tumor

Cell line: A mouse colon carcinoma cell line, colon-26 was used in this study. The cells were incubated in a humidified atmosphere of 5% CO2 in air at 37°C. Dulbecco's Modified Eagle's Medium(DMEM) supplemented with 10% fetal bovine serum and antibiotics was used for cell growth.
Animal: BALB/c male mice (6 weeks of age, 20 to 25 g of body weight) were obtained from Japan SLC. Before the experiments, the mice were kept undisturbed for at least 1 week.
Double-tracer autoradiography and immunohistochemistry: Double-tracer autoradiography and immunohistochemistry were performed with colon-26 tumor-bearing mice (n=4). The colon-26 cells suspended in PBS were subcutaneously injected into the flank of the BALB/c mouse. Three weeks after implantation of tumor cells, a mixture of 74 MBq of F-18-FDG and 0.37 MBq of Cu-64-ATSM was injected intravenously into each tumor-bearing mouse; maximum tumor dimension at this point was approximately 1 cm. One hour after the intravenous injection, animals were sacrificed and the tumors were excised. The excised tumors were immediately frozen on crushed dry ice. They were divided into two sections, and the cutting surfaces were flattened with a cryostat and subjected to dual-tracer autoradiography. The frozen blocks used for double-tracer autoradiography were thawed, fixed with 10% neural-buffered formalin and embedded in paraffin for immunohistochemistry. The paraffin blocks were sliced into 2-μm-thick serial sections within 50 μm of the surface used for autoradiography exposure. After waiting for 64Cu decay, immunohistochemical staining to detect expression of CD133 was performed.

Flow cytometory: Cultured colon-26 cells were washed with PBS and labeled with the rabbit anti-mouse CD133 antigen antibody (1:100 dilution) for 30 min at 4°C in the dark. Following the PBS wash, cells were incubated with Alexa Flour 488-conjugated secondary antibody for 30 min at 4°C in the dark. Cells not stained by any antibody were used as negative controls, and cells stained by secondary antibody only were used as controls. For flow cytometry, the labeled cells were analyzed by the Beckman Coulter EPICS XL flow cytometer.

Development of flexible automatic liquid handling modules for Cu-64 production and extraction

The automatic modules are realized to bring reproducibility, reduce exposure and prepare the probe on demand without chemists. Commercially-available automated modules are mechanically stable and reliable, however, they have various limitations such as high cost, low flexibility, professional programming, and so on. Recently, microcomputer technology allows us to assemble miniature humanoid robots in hobby level. Using this technology, we developed flexible modules including automatic three-way valves, syringes and I/O unit for controlling external devises.
The electroplating system was consisted of one syringe, a carbon rod, a power supply and an assembly jig. The purification system consisted of two syringes, 11 three-way valves and three heaters are controlled from temperature controller with timer through I/O unit.