Updated Aug. 5, 2019

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（Abroad・~~Domestic~~）Internship report form（Student） 2019/10/02 (Year/Month/Day)

Name	Christida Estu Wastika
Laboratory	Division of Molecular Pathobiology
Year (Grade)	3rd year
Internship institution	Mochtar Riady Institute for Nanotechnology (MRIN), Tangerang, Indonesia
Internship period	Internship period: 09/02/2019 - 09/27/2019 (Departure Date from Sapporo: 08/30/2019, Arrival Date in Sapporo: 09/30/2019)
Purpose	**1. To get the opportunity to begin a professional career in Indonesia.** 2. To gain practical experience in research and working environment of MRIN to support prospective career as researcher. 3. To create a new network to support forthcoming career as researcher and lecturer. 4. To achieve the knowledge to conduct molecular biology research in Indonesia.

- THE REASON WHY YOU CHOSE THIS INSTITUTE

In Indonesia, to find a lecturer position in a university is not so easy, especially if we do not have any network. Furthermore, even though we can get a position as a lecturer, it will be difficult to conduct any experiments related to virology and molecular biology in a short time. After finishing my Ph.D. course at Hokkaido University, I want to conduct some experiments related to virology and molecular biology in Indonesia. Then, while I enhance my laboratory skill, I will make contact with other lecturers and professors to secure a lecturer position.

Our laboratory members have collaborated with Prof. Agus Setiyono who is the vice dean of the Faculty of Veterinary Medicine, Bogor Agricultural University. When he visited our laboratory last October, I had a discussion about how to get a lecturer position in Indonesia and in which institute I can conduct experiments in molecular biology field. He gave me suggestions to apply for a researcher position first, while waiting for an open position as lecturer. Then, he recommended to me to perform internship at the Mochtar Riady Institute for Nanotechnology (MRIN) and give an e-mail address of Dr. Ivet Suriapranata, who is a research coordinator of MRIN, for more detail discussion. To achieve my plan, I would like to do internship at the MRIN which conducts medical science using molecular biology technique at Tangerang, Indonesia.

The MRIN belongs to the Lippo Group which is one of the biggest property developers in Indonesia. As a non-profit organization, research project in MRIN focus on development of therapeutic strategy for cancer patients, improving understanding and control of cancer in Indonesia. This institute is affiliated with the Siloam Hospital and Pelita Harapan University and in collaboration with University of New South Wales, Australia. Recently, the MRIN was looking for researcher who is good at performing experiments for the virology, molecular or cell biology and veterinary background to conduct several experiments, including research on liver cancer, hepatitis and *in vivo* cancer research with small animal models. One research group led by Dr. Teridah Ginting has discovered a potency of Newcastle disease virus-like particles as a strategy for tumor therapy. It will be useful to have a training in this field to improve my skill for conducting experiments which focus on immune expression and my knowledge on the effect of viral infection in various cell lines. **Moreover, this experience will support my future career as researcher and lecturer. In addition, I hope to get an opportunity of working as a researcher in the MRIN through this internship program.**

- RESULT OF THE ACTIVITY

BACKGROUND

Cancer cells are lack of antigen presenting protein, so that our immune cells can not recognized them as an invasive mass. In addition, cancer cells are also deficient of the immune response for its protection against virus. Due to the insufficient of immune response in cancer cells, oncolytic viruses can replicate and trigger the cells to lysis or apoptosis. The molecular process of this phenomenon is still under investigation for several viruses, including Newcastle Disease virus (NDV), an oncolytic virus belonging to *Paramyxoviridae*.

It has been reported that combination therapy of oncolytic virus (NDV) and anti PD-1 or PD-L1 monoclonal antibody in mice was increased their survival rate against melanoma (Zamarin D., et al., J Clin Invest, 2018). Therefore, the MRIN now attempts to examine the effect of NDV infection and treatment of IFN type I and III on immune check point, such as PD-L1. As a part of this project, I have examined whether programmed death-ligand 1 (PD-L1) is upregulated by IFN-γ in human colorectal cancer cells, using *in situ* hybridization and flowcytometric analysis.

MATERIALS AND METHODS

Cell lines and viruses

Human colorectal cancer cell lines, HCT116, were maintained in RPMI medium with 10% FBS

and 1% Penicillin-streptomycin, then incubated in 5% CO_2 at 37°C. SW620 cells were maintained in Leibovitz medium with 10% FBS, 1% Penicillin-streptomycin and incubate at 37°C. NDV were purchased from PT. IPB Shigeta Animal Pharmaceutical and propagated in 11 days-old embryonated eggs for 72 h. Thereafter, the viruses were purified and stored at -80°C until use.

Cells preparation and treatments

HCT116 and SW62 0 cells were seeded on the cover slips with 1 x 10^5 cells/cover slip. Then, the cells were infected with NDV at MOI 0.01 or MOI 0.1, or treated with 1:500, 1:250 or 1:125 of IFN-γ (IFNG). The cells were incubated for 24 h in 5% CO_2 at 37°C, for HCT116, and 37°C for SW620. Thereafter, the cells were fixed in 10% normal buffered formalin for 30 min, then dehydrated with 50%, 70% and 100% ethanol. The fixed cells then stored in -20°C in 100% ethanol until used for *in situ* hybridization analysis. All treated cells were maintained in maintenance medium which consist of RPMI medium, 1% BSA and 1% Penicillin-streptomycin for HCT116 cell lines and Leibovitz medium, % BSA and 1% Penicillin-streptomycin for SW620 cell lines.

***In situ* hybridization (ISH)**

Duplex assay

Expression of PD-L1 and major histocompability complex (MHC) class I chain-related protein A (MICA) mRNAs were detected by using RNAscope 2.5 HD Duplex Detection Kit (Chromogenic) (Advanced Cell Diagnostic, Hayward, CA) following manufacture's protocol. PD-L1 and MICA mRNAs were detected by using RNA target oligo probes and visualized in green (GREEN) and red (RED) color, respectively.

Brown assay

The NDV mRNA in HCT116 and SW620 cells were detected using RNAscope 2.5 HD Detection Reagent – Brown (Advanced Cell Diagnostic, Hayward, CA), after infected with NDV (MOI: 0.01) for 24, 48 and 72 hpi, following the manufacture protocols. The viral mRNA was detected by using RNA target HRP-labeled oligo probes and visualized in brown (DAB) color.

Flowcytometry

The 4 x 10^5 cells of HCT116 and SW620 were seeded onto 12-well plates and infected with NDV at MOI 0.01 or MOI 0.1, or treated with 1:500, 1:250 or 1:125 of IFNG, then incubated for 24 h at 37°C for SW620 cells and in 5% CO_2 at 37°C for HCT116 cells. After that, the cells

were collected by accutase, then labeled with 1:50 PD-L1 (B7-H1) antibody and fixed using 2% formalin. The PD-L1 was then detected using BD Accuri C6 flow cytometer (Becton Dickinson, NJ, USA) and the data were analyzed using BD CSampler software (Becton Dickinson).

Data analysis

The expression of PD-L1 (blue dot) and MICA (red dot) in each cell were calculated and scored to identify the H-score based on the manufacture's protocols as followed:

	% Cells	Weighted formula
Bin 0 (0 dots/cell)	A	0*A
Bin 1 (1-3 dots/cell)	B	+1*B
Bin 2 (4-9 dots/cell)	C	+2*C
Bin 3 (10-15 dots/cell)	D	+3*D
Bin 4 (>15 dots/cell)	E	+4*E
H-Score		Total amount of calculated scores

RESULTS

PD-L1 and MICA detection

The expression of PD-L1 and MICA, which expressed in green and red dots (Figure

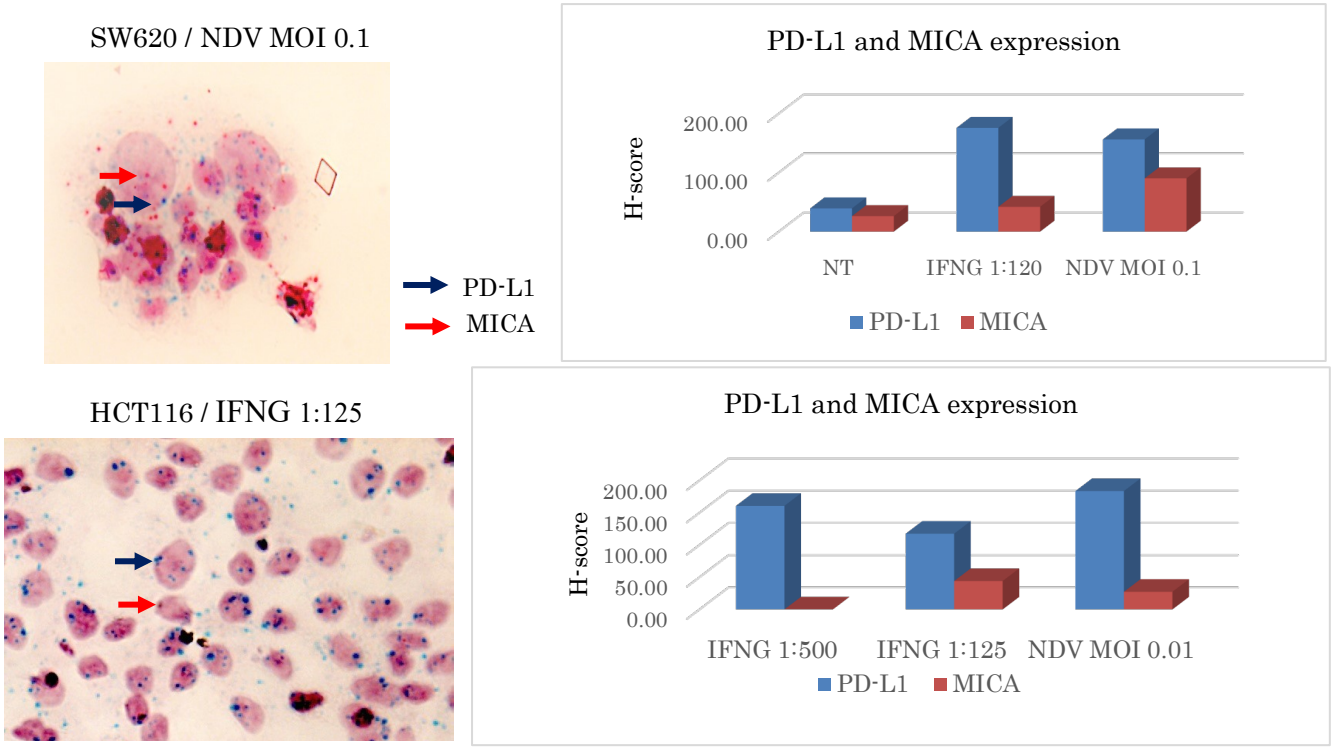

Figure 1. PD-L1 and MICA expression in SW620 and HCT116-cells infected with NDV or treated with 2 µl, 4 µl or 8 µl of IFNG. NT: no-treatment;

1, left), in IFNG-treated and NDV-infected SW620 cells were increased compared to the non-treatment cells. In HCT116 cells, the expression of PD-L1 in IFNG-treated and NDV-infected cells was higher than those in HCT116 cells (Figure 1, bar graph).

Flowcytometry

PD-L1 was expressed in all treated- and infected-cells (Figure 2). Compared to the non-treatment cells, PD-L1 expression was increased in IFNG-treated cells and NDV-infected cells (Figure 3).

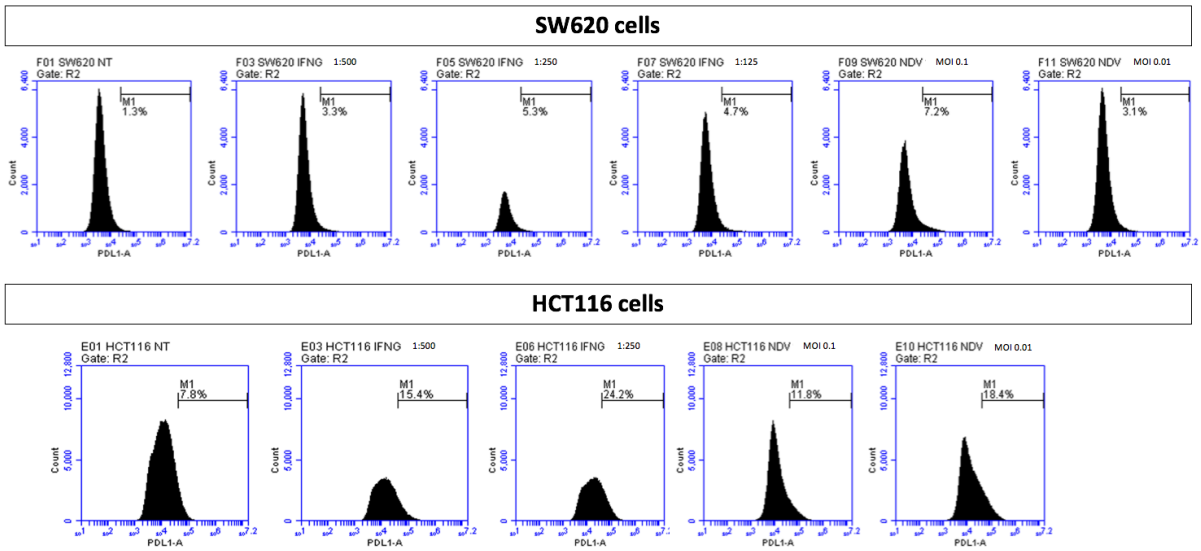

Figure 2. The histogram of PD-L1 expression in HCT116 and MHC620 cells detected using flowcytometer. NT: no-treatment.

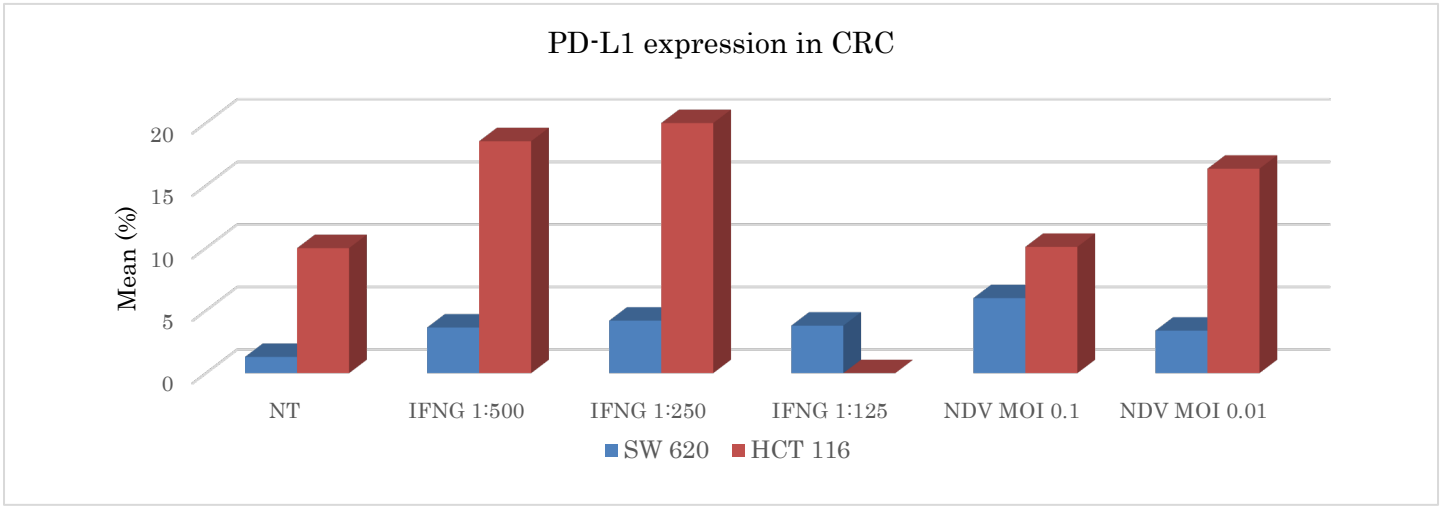

Figure 3. PD-L1 expression in HCT116 and MHC620 cells detected using flow cytometer

Brown assay

Due to a reagent was not labelled properly, we could not get a clear result. The cells were damaged because of high concentration of PBS that we used for washing step. As shown in Figure 4, almost all cells were shrinking. In the positive control, small part of HCT116 cells'

housekeeping gene (*PPIB*) were stained as brown dots, meanwhile, all SW620 cells were stained brown.

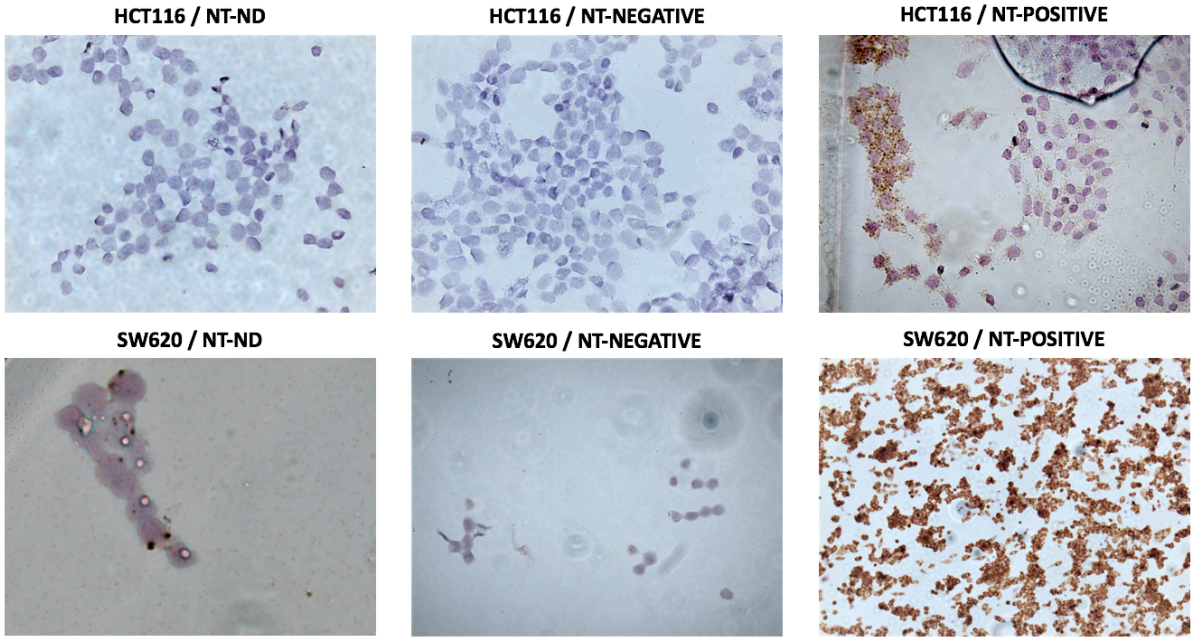

Figure 4. Control samples for NDV mRNA detection in HCT116 and SW620 cells. NT: no treatment; ND: NDV probe; Negative: negative control; Positive: positive control

DISCUSSION AND CONCLUSION

IFNG treatment was enhanced expression of PD-L1 in human colorectal cancer (CRC) cell lines (SW 620 and HCT 116). It has been reported by Song M., et al. (PLoS One, 2013) that upregulation of PD-L1 in human CRC was induced by IFNG and loss of phosphatase and tensin homolog deleted on chromosome ten (PTEN). Besides, the upregulation of PD-L1 in human CRC cell lines were induced by NDV infection. Previously, Epstein-Barr virus (EBV) was reported to be a predisposition factor for the malignancy of gastric cancer due to its ability to increase PD-L1 expression.

The upregulation of MICA mRNA in NDV infected- and IFNG- treated cells were coherent with the previous study described by Castro F., et al. (Front Immunol, 2018) and Bommareddy P.K. et al. (Nat Rev Immunol, 2018). They have demonstrated that IFNG treatment and oncolytic virus infection could increase the expression of MHC class I in cancer cells.

In conclusion, IFNG treatment in human CRC cell lines can induce the upregulation of PD-L1. Further study needs to be conducted to identify the optimum condition for IFNG treatment in human colorectal cancer cell lines as a positive control for PD-L1 expression.

- WHAT DO YOU THINK THE POSITIVE IMPACT OF THE ACTIVITY WILL HAVE ON YOUR FURTHER CAREER PATH?

At the beginning, I have a plan to continue my research in Indonesia and start my career there. During my internship in the MRIN, I explained my plan to continue my research there after finishing my Ph.D. to my supervisor in the MRIN. She gave me a suggestion that it will be better for me to go abroad first to gain more experience, knowledge and techniques, thereafter, I can back to Indonesia. I had a chance to visit the National Institute of Health Research and Development of Indonesia and discussed research condition in Indonesia. Thereafter I have obtained some updates what their achievement in recent year is. In short, it is not easy to do a continuous basic research as a part of government.

After consider many aspects, I decide find a place to continue my research abroad.

- ADVICE FOR YOUR JUNIOR FELLOWS

Be a long-life learner. Knowledge is everywhere and they are waiting for you.

Approval of supervisor	Institution・Official title・Name Division of Molecular Pathobiology, Research Center for Zoonosis Control Professor Hirofumi Sawa

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