(Abroad・Domestic) Internship report form (Student) 2018/12/18

Name	Benjaporn Kiatpakdee
Laboratory	Molecular Medicine
Year (Grade)	D4
Internship institution	Laboratory of Red Cell Physiology, Lindsley F. Kimball Research Institute (LFKRI), New York Blood Center, New York, USA
Internship period	Internship period: 11/19/2018 - 12/14/2018 (Departure Date from Sapporo: 11/17/2018, Arrival Date in Sapporo: 12/17/2018)
Purpose	1. To learn and gain the experience in molecular hematological research especially the method to isolate erythroid cells in each stage of maturation, to establish models for erythropoiesis study, and to analyze the efficacy of erythroid cell maturation. 2. To understand the strategy of working in professional research institute and obtain information about postdoctoral position. 3. To build the network for research collaboration in the future.

The reason why you chose this institute

Laboratory of Red Cell Physiology in New York Blood Center (NYBC) is headed by Dr. Mohandas Narla who is very expertise in hematological field especially in red cell maturation research. He focused on red blood cell physiology and pathology for better understanding in molecular and structural basis for red blood cell membrane disorders and recently molecular mechanism of red cell maturation in normal and disordered erythropoiesis.

My thesis study focuses on the function of TSPO2 during red blood cell maturation which related to his field. Therefore, I would like to discuss about my research and learn many applicable methods in molecular hematology from the experienced researchers to apply in my study in the future. In addition, I want to learn about the roles of postdoctoral fellows and researchers in the institute and I can realize what should be improved for my knowledge in basic hematology field, research skills, and logical thinking before I finish the doctoral course.

Result of the activity

In the first week, I had done some paper work to get the ID card. Then, I met my research mentor and she explained me about the laboratory and took me around the building. Mainly, New York Blood Center consist of two departments, blood center and research center (Lindsley F. Kimball Research Institute, LFKRI) which including many laboratories related to hematological field. Recently, this laboratory is focusing on cell metabolism during red blood cell maturation using

human blood, bone marrow, and cord blood. They isolated erythroid progenitor cells and culture *in vitro* to induce cell differentiation. Then, they checked cells maturation by flow cytometry, isolated each stage of erythroid maturation by cell sorting machine and cultured with drug treatment to see the effect on maturation.

I've observed and learned about their basic experiments and I also had very good chance to present and discuss with Dr. Narla and his lab members about my research. I got many useful comments to improve my research, some new ideas for my study in the future, and my internship plan for one month.

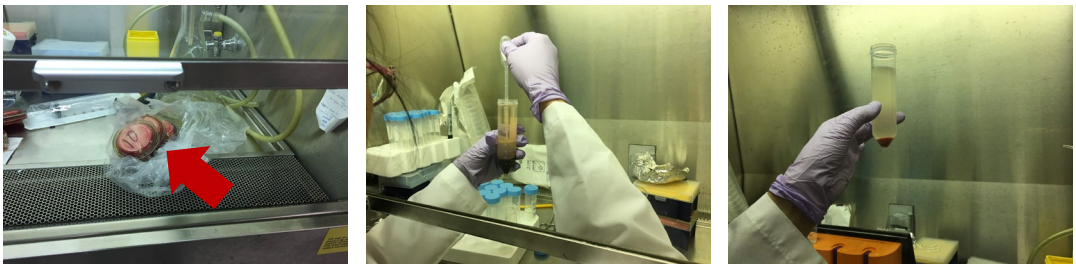
In the second week, I've joined the orientation in NYBC for two days as the visiting scientist. On the first day, they explained about the history of NYBC, organization's policies, how to provide safe and secure working environment, how to correct the document, and how to utilize the working system within this institute such as e-mail and website. The second day was held at Long island branch which is the center for blood processing and transportation. The officer took me around the center and let me see the process after blood donation such as separation of blood ingredients to plasma and packed red cells, blood packaging, and blood transportation to requested hospitals. I also learned about the chemical safety, biosafety level, and how to handle any emergency situations including chemical injury, blood contamination, and even mass shooting. This orientation let me understand the overall and working system of this organization which is very useful for me to know (figure 1).

Fig.1. *Upper*, NYBC building in long Island and central east. *Lower*, inside of the building and laboratory.

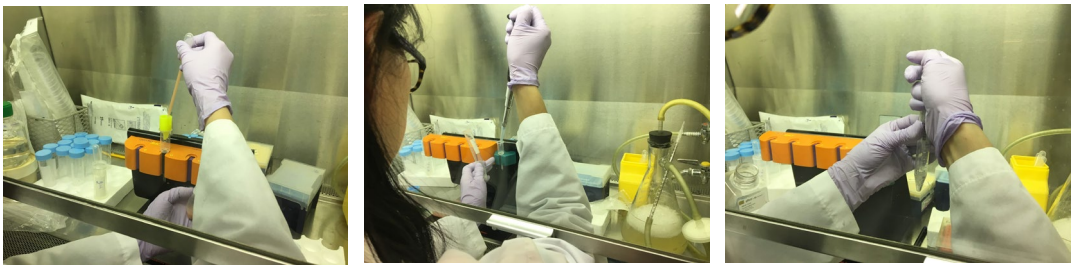
During the internship, I've observed and learned many experiments about molecular hematological research as followings,

1. *Isolation of erythroid progenitor cells (CD 34⁺) from human bone marrows and human peripheral blood.*

Human bone marrow and peripheral blood were collected from the hospital and New York Blood Center, respectively. CD34 positive cells which are hematopoietic progenitor cells were purified using magnetic cell separation (figure 2). Cell purity was checked using flow cytometry analysis. After that, cells were cultured to induce cell differentiation.



Leucocyte filters | Mononuclear cell isolation



CD34 positive cell isolation by magnetic cell separation

Fig.2. Isolation of CD34⁺ cells (hematopoietic stem cell) from human peripheral blood

2. *Flow cytometry analysis of human erythroid cell differentiation*

After cultured for 11 days, cells were stained by fluorochrome-labeled antibodies and analyzed on the flow cytometer to check the maturation stage. Cells were gating with the expression of surface protein after dead cells and aggregated cells were excluded (figure 3).

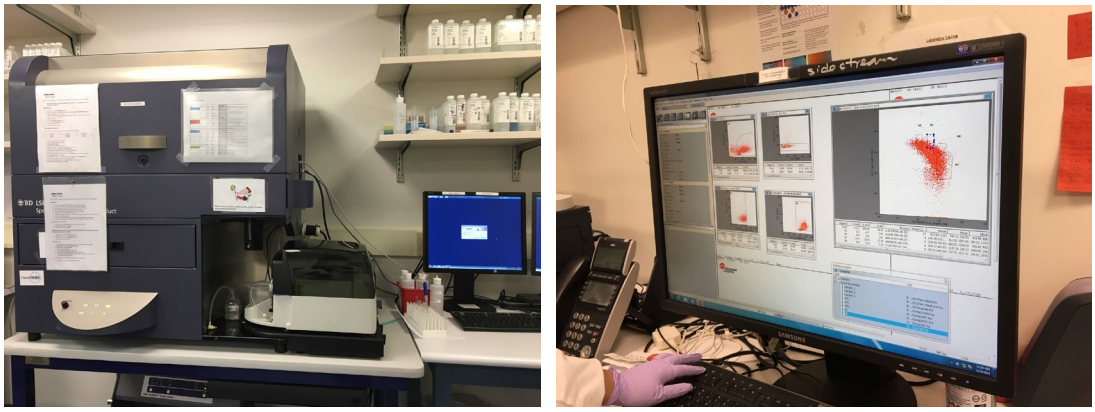


Fig.3. *Left*, flow cytometry machine. *Right*, flow cytometry graph.

3. Isolation of late stage erythroid cells using cell sorting machine.

On day 11 of culture, when erythroid cells undergo maturation to late stage, we isolated erythroid precursor cells from each stage including late basophilic erythroblast, polychromatophilic erythroblast, and orthochromatic erythroblast using erythroid cell marker and cell sorting machine. After that, we checked cell purity by flow cytometry and cell morphology (figure 4)

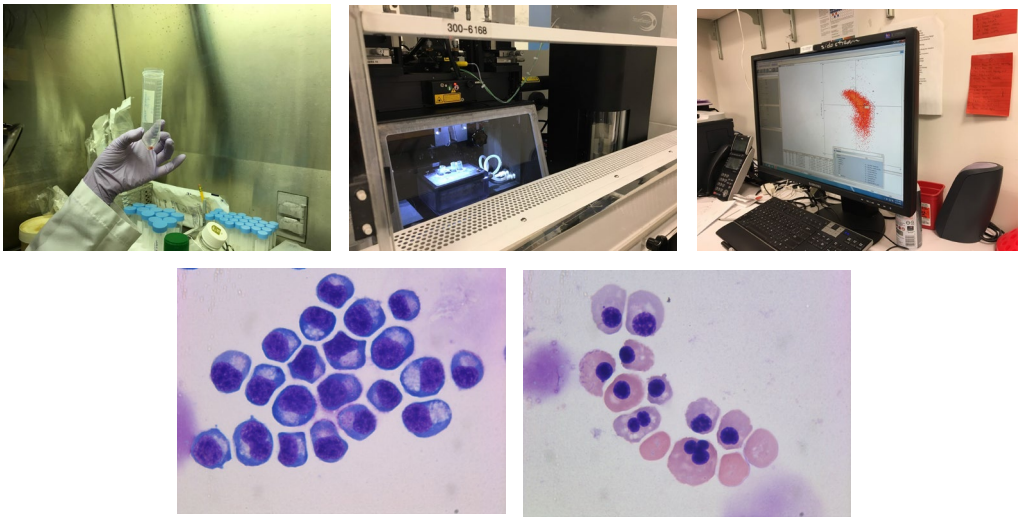


Fig.4. *Upper*, cell sorting process. *Lower*, cell morphology of isolated erythroid cells

4. Analysis of enucleation rate using DNA marker and flow cytometry

Orthochromatic erythroblast (late stage) must exclude nuclear to become reticulocyte and red blood cell which called enucleation process. Using DNA marker, enucleated cells (reticulocyte and red blood cell) will show clearly low DNA expression than late erythroblast. After we isolated late stage erythroblast, cells were cultured for 2-3 days and stained with DNA marker to examine the

enucleation rate (figure 5).

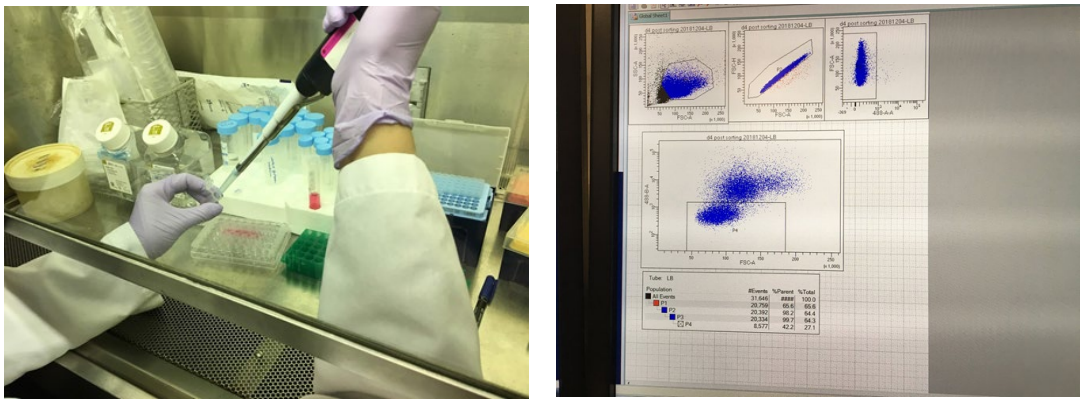


Fig.5. *Left*, collection of cell culture. *Right*, flow cytometry analysis of enucleation rate

In addition, I also joined the seminar by invited speaker to update my knowledge about this field. His topic is about mitochondria dynamics and calcium signaling regulate hematopoietic stem cell function. I realized that now many researchers are focusing on the metabolism of hematopoietic stem cell, myeloid and lymphoid lineages of blood cells. It's very interesting since this laboratory is also study on this issue in erythroid cells as well.

In summary, this internship provides the great chance for me than I expected. I will not be able to complete my goals in this internship without kindly support from everyone. I would like to express my gratitude to Graduate school of Veterinary Medicine Hokkaido University, Leading program, my laboratory and laboratory of Red Cell Physiology, NYBC.

What do you think the positive impact of the activity will have on your further career path?

1. My English communication skill was improved, and I've learned to adapt myself in new environment.
2. I've updated knowledge in hematological field and learned the advanced techniques in molecular hematological research which will become my advantage to work as a postdoctoral fellow in this field.
3. I've understand the overall and working system of the research institute which is useful for me to figure out the difference between research institute and laboratory in University and give me the choice to consider my career path.
4. I've built the connection with supervisor and lab members there, so, I can

keep discussing and collaborating with them in the future.

5. I can include this internship as a good profile within my resume for job hunting.

Advice for your junior fellows

1. Since the period of internship is just one month, please clearly think about your goals for internship and find the place that match with yours.
2. Please check your visa condition carefully especially when you have to transit during your flight.
3. Provide some time to take a rest before starting internship if you travel through many time zones.
4. Be active and be nice to everyone but don't forget to be careful.

Approval of supervisor	Institution・Official title・Name

※1 Send the electronic file to the Leading School section, International Affairs Office
※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
※3 The Steering Committee of the Leading Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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