ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
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海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

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受 付 番 号　201960693
氏　　名　[redacted]

（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：シカゴ　（国名：アメリカ合衆国）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
癌遺伝子異常と抗腫瘍免疫回避の関係に着目した免疫療法に対する耐性の克服

3. 派遣期間：平成　31 年　4 月　1 日　～　令和　2 年　9 月　6 日

4. 受入機関名及び部局名
シカゴ大学医学部 病理学部門

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Since we achieved a quite interesting preliminary results in terms of the relationship between Batf3+ cells and CD8+ cells, I focused on this using patient surgical or biopsy samples of various cancer types.

Patients

- Bladder cancer

A total of 68 patients with bladder cancer were included. Forty seven of them underwent surgery without neoadjuvant chemotherapy and their surgically resected samples had been previously submitted to the Cancer Genome Atlas (TCGA) project, which means the DNA alteration data and the RNA expression data is available. Meanwhile, twenty one of them underwent immunotherapy for the recurrent disease after surgery.

- Melanoma

A total of 45 patients with unresectable or metastatic melanoma were included. They underwent biopsy just before treatment with immunotherapy. These patients were enrolled in a biobank project of the University of Chicago Medical Center and had multi-omics data such as whole exon sequencing and RNA sequencing as well as

clinical data including response to immunotherapy.

- Breast Cancer

A total of 46 patients with triple negative breast cancer (TNBC) who received neoadjuvant chemotherapy at The University of Chicago and had pretreatment tissue available for study were included.

Multiplex fluorescence IHC and Multispectral scanning

Full-section 5-μm slides of surgically resected formalin-fixed paraffin-embedded (FFPE) bladder cancer specimens were stained using Opal multiplex kits (Akoya Bioscience).

- Panel for bladder cancer: Batf3, CD8, Foxp3, PD-1, PD-L1, Pancytokeratin
- Panel for melanoma: Batf3, CD8, Sox10
- Panel for breast cancer: Batf3, CD8, Foxp3, PD-L1, Pancytokeratin

Then the slides were imaged using the Vectra® Polaris™ Automated Quantitative Pathology Imaging System (PerkinElmer). On each scanned image, five regions of interest (ROI) that have the most abundant CD8+ T cell infiltration with the least background staining and autofluorescence was selected. Those selected ROIs were scanned at 20x resolutions to make .im3 format image files for the following image analysis.

Image processing

The scanned .im3 format image files were analyzed using inForm® Cell Analysis software (Perkin Elmer). Using the inForm® software, both tissue and cell compartments were identified and segmented. Tissue segmentation were performed by highlighting examples of pancytokeratin+ tumor area, pancytokeratin- stromal area, and non-tissue area, allowing the algorithm to learn each tissue type. Cell segmentation were performed using DAPI counterstain, and x and y coordinate were assigned to each cell. Cells were phenotyped by using the phenotyping function of the software and highlighting examples of each cell type, allowing the algorithm to learn each cell type. Finally, batch analysis using the trained algorithm were performed for all the ROIs, outputting information including tissue area, phenotype, location (x, y), and intensity of each fluorophore for each cell.

Analysis/Key findings

- Bladder Cancer

- The number of immune cells such as Batf3+, CD8+, Foxp3+, and PD-L1+ in IHC was positively and significantly correlated with the RNA expression of each corresponding gene. In terms of relationship between the numbers of immune cells, the focus of this study, the number of Batf3+ cells and CD8+ cells showed positive correlation.

- Based on the RNA expression of 160 immune-related genes, patients were classified into three T cell-inflamed phenotypes: high, medium, and low. When the patients were divided into two groups based on the median of the numbers in IHC (high and low), each the high group for Batf3, CD8, Foxp3, and PD-L1 included significantly higher proportion of hot tumors. When high and low of Batf3+ cells and CD8+ cells were combined, Batf3highCD8high population was nearly universally hot or medium tumor except for one case.
- Spatial relationship between CD8+ T cells and other immune cells, especially focusing on Batf3+ DCs, based on phenotype and location information (x, y) was analyzed. The data indicated that those two types of immune cells tend to make clusters rather than distribute randomly.

These preliminary results were presented at American Association for Cancer Research Annual Meeting 2019 (Hatogai K, Kim D, Zha Y, et al. Multichannel immunofluorescence imaging to assess the immune composition of tumor microenvironment in bladder cancer (Abstract#3193): American Association for Cancer Research Annual Meeting 2019 (Mar 29-Apr 3, Atlanta, GA)).

The following analyses are undergoing or completed and ready for publication:

- Analyzing the correlation between the infiltration of immune cells or spatial analysis and the expressions of oncogenes and immune-related genes
  FGFR3 expression has shown to be related to non-inflamed tumor microenvironment. The relationship between FGFR3 mRNA expression and the infiltration of each type of immune cells is being studied.
  Chemokines such as CXCL9, CXCL10, and CXCL11 have been reported to be produced by dendritic cells and play crucial role on the recruitment of effector T cells. The relationship between mRNA expression of these chemokines and the infiltration of each type of immune cells is being studied.
- Analyzing the relationship between the infiltration of immune cells or spatial analysis and the survival outcomes
- Analyzing the relationship between the infiltration of immune cells or spatial analysis and the response to immunotherapy

Review of literatures concerning tumor microenvironment of bladder cancer as well as our preliminary data was submitted as a review article and accepted (Hatogai K and Sweis RF, Advances in Experimental Medicine and Biology).

Some of the preliminary data lead to achievement of research funding in collaboration with another faculty in the University of Chicago.

- Melanoma

The following analysis using biobank samples will be published in combination with

data from mouse models.

- The spatial relationship between the infiltration of Batf3+ cells and CD8+ cells
- The genes whose expression levels is positively or negatively correlated with tumors with abundant Batf3+ cells and CD8+ cells are being studied.
- How the infiltration of these immune cells is related to chemokine expressions is being studied using RNAscope in combination with multiplex IF.
- The relationship between the infiltration of Batf3+ cells and CD8+ cells and clinical outcomes including response to immunotherapy.

- Breast Cancer

The work was done in collaboration with a Breast cancer laboratory.

- The relationship between glucocorticoid receptor expression and the infiltration of immune cells was studied.
- The relationship between the infiltration of immune cells and response to neoadjuvant chemotherapy was studied.
- The relationship between the infiltration of immune cells and multi-omics data is being studied.

The relationship between glucocorticoid receptor expression and tumor microenvironment will be presented at the San Antonio Breast Cancer Symposium 2020.

Also, these data will be combined with genomic data of tumor samples and clinical data, and will be published.