ウェブサイト公開用

（様式 10-1）
（海外特別研究員事業）
令和　6 年　2 月　5 日

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

採 用 年 度　令和４年度
受 付 番 号　202270021
氏　　名　　杉山奈美

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

1-1. 用務地（派遣先国名）用務地：　チューリッヒ　（国名：　スイス連邦国　）

1-2. 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。

乳がん血中循環腫瘍細胞(CTCs)形成に重要な上皮間葉転換(EMT)の状態の解明

1-3. 派遣期間：令和　4 年　5 月　1 日　～　令和　5 年　7 月　31 日（　457日間）

1-4.　　受入研究機関名及び部局名

受入研究機関名：　チューリッヒ工科大学 (ETH Zurich)

部局名：　Prof. Nicola Aceto Lab

1-5. 海外特別研究員の研究課題の遂行状況…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）**

【記載事項】

・　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

書式任意（A4 判相当 3 ページ以上、英語での記入も可）

Here I report the progress of my project in which I aim to address the roles of cells in distinct stages of EMT spectrum in metastatic breast cancer progression by employing two EMT tracing systems.

The impact of epithelial-to-mesenchymal (EMT) states on the shedding of circulating tumor cells (CTCs) in breast cancer

Introduction:

Most of cancer-related death is caused by metastasis. Local and systemic dissemination and colonization of cancer cells are facilitated by clonal selection and the high phenotypic plasticity of cancer cells. Epithelial-mesenchymal transition (EMT) is a reversible cellular dedifferentiation process, which is associated with phenotypic plasticity of cancer cells. Cancer cells may proceed through an EMT to different degrees and revert into more epithelial states by mesenchymal-epithelial transition (MET), resulted in the generation of heterogenous subpopulations of cancer cells with various degrees of epithelial and mesenchymal features and different biological characteristics within tumors. Since cancer cells acquire migration and invasion abilities through EMT program, EMT has been thought to be required for systemic dissemination of cancer cells. However, circulating cancer cells (CTCs) found in blood derived from breast cancer patients and mouse models mostly express epithelial molecules, while only few studies reported fully mesenchymal-type CTCs. Therefore, the actual impact of the phenotypic states along an EMT continuum on the shedding of CTCs remains elusive.

Achievement of the projects:

1. ***Partial and full EMT lineage tracing systems in transgenic mouse models with metastatic breast cancer***

In the previous study, we have established an EMT lineage tracing system in MMTV-PyMT transgenic breast cancer mouse model to trace breast cancer cell undergoing partial or full EMT during tumor growth and metastasis *in vivo* (published in Lüönd and Sugiyama et al., Dev. Cell, 2021). In this system, by employing dual-color reporter system, only breast cancer cells are labelled by mCherry expression, and cancer cells undergoing either partial or full EMT irreversibly switch to express GFP by $CreER^{T2}$ activity upon tamoxifen administration. Importantly, stromal cells including fibroblasts are not marked (Fig.1). We

found that even though cancer cells in the primary tumor infrequently undergo an EMT, cancer cells in epithelial-mesenchymal hybrid states (partial EMT states) defined by the expression of Tenascin C (Tnc) are highly plastic between epithelial and partial EMT states and critically contribute to metastasis formation in the lungs, while low-plastic N-cadherin (Cdh2) expressing fully mesenchymal cells have lower metastatic potential (reported in Lüönd and Sugiyama et al., Dev. Cell, 2021 and Lüönd et al., 2022, STAR Protocols).

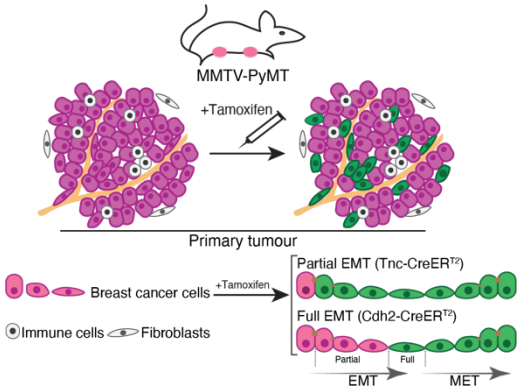

Fig.1 EMT lineage tracing mouse models

In partial EMT (Tnc-CreER^{T2}) and full EMT (Cdh2-CreER^{T2}) mice, all tumour cells are marked by mCherry expression. Upon Tamoxifen administration, mCherry^{+} cancer cells undergoing an EMT irreversibly convert from the expression of mCherry to the expression of GFP (Lüönd and Sugiyama et al., Dev. Cell, 2021).

Given that partial EMT cells encompass multiple subpopulations at different states along the EMT continuum, in this study, I performed orthotopic transplantation of breast cancer subpopulations derived from the partial EMT lineage tracing mouse model. Cells at different EMT states were sorted by flow cytometry based on color (epithelial cells in red, early EMT cells in yellow, and partial or full EMT cells in green), as well as the expression levels of the epithelial marker EpCAM. Each sorted population was then transplanted into the mammary glands of immune compromised NSG mice at 100 cells per mouse. Upon orthotopic transplantation of each population into NSG mice, we observed that cells expressing higher levels of EpCAM, such as red epithelial cancer cells, yellow early EMT cells, and green mesenchymal-epithelial transition (MET) cells, displayed greater tumor propagation ability, while highly mesenchymal populations formed tumors less frequently (Fig. 2a). Interestingly, during tumor growth, cancer cells expressed epithelial molecules (e.g., E-cadherin and EpCAM), even if these cells were sorted as partial EMT states by flow cytometry. Furthermore, yellow early partial EMT cells were predominantly transitioned into green cells over time.

I further studied the abilities of cancer cells in different EMT states on the systemic dissemination and metastatic nodule formation in the lungs. To this end, blood samples collected by heart puncture were subjected to assess the color, number, and states of CTCs, and the size and number of metastatic nodules were analyzed by immunohistochemistry. Interestingly, even though the growth rate of primary tumors in mice transplanted with epithelial cancer cells, early EMT cells, and MET cells were comparable to each other, CTCs were barely detected in the liquid biopsies derived from the mice transplanted red epithelial-type cancer cells (2 out of 14 mice). Most of these cells were found as single-CTCs or homotypic CTC clusters composed of 2-5 cells. On the other hand, CTCs were much more frequently detected in the mice

bearing early EMT and MET cell-formed tumors (4/11 and 3/11 mice, respectively), and these cells mostly formed clusters composed of over 5 cells (Fig. 2b). According to the previous report (Aceto et al., Cell, 2014), the number and size of CTCs are associated with the metastatic potential of cancer cells. Consistently, mice bearing early partial and MET cell-formed tumors had significantly greater number of metastatic nodules in the lungs compared with the mice bearing red epithelial cell-formed tumors (Fig. 2b). Interestingly, partial EMT cells had lower tumor propagating ability; however, these cells could have greater metastatic potential compared with the red epithelial cells. Taken together, our results suggest that epithelial-type cells that have ever undergone an EMT could have greater potential for systemic dissemination and metastatic nodule formation in the lungs compared to native epithelial cells.

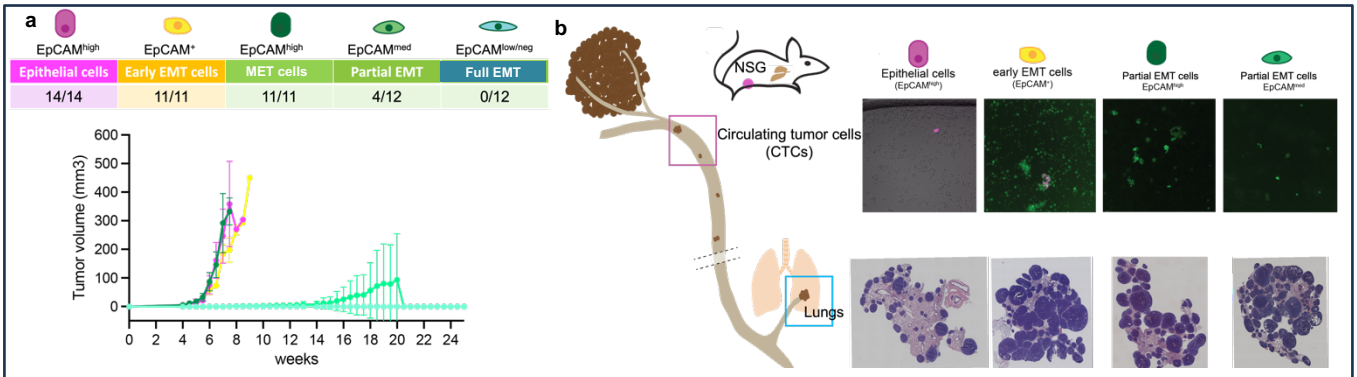

Fig.2 The impact of EMT states on tumor propagation and metastasis

Breast cancer cells derived from partial EMT lineage tracing mouse models were transplanted into NSG mice. a) The tumor propagation ability of cancer cells in different EMT states. The graph shows the growth curve of individual tumors. Color indicates the cells of origin. b) The metastatic abilities of cancer cells in different EMT states. The color, number, and states of CTCs derived from peripheral blood of tumor-bearing mice and the number and size of metastatic nodules in the lungs were analyzed.

2. *ETM/MET tracing system in human breast cancer cell lines*

Since our previous EMT tracing mouse model only labels cancer cells that have ever undergone a partial or full EMT, the real-time cellular states of individual cells cannot be detected. Furthermore, it has remained unclear whether patient-derived cancer cells in distinct EMT states would play similar roles as those found in murine breast cancer *in vivo*. To identify the real-time cellular states of human breast cancer cells during metastasis, I developed a novel EMT/MET tracing system in three different human breast cancer cell lines.

Initially, to identify the specific EMT markers in human breast cancer cells, we analyzed the expression of over 200 epithelial and mesenchymal genes in breast cancer patient-derived CTCs and human breast cancer cell lines, such as the lung metastatic variant of the triple-negative MDA-MB-231 cells (LM2 cells) and breast cancer patient-derived CTC lines (Aceto et al., 2014). Among these genes, we selected four epithelial and mesenchymal markers. Using these markers, I designed EMT/MET tracing reporter systems and transduced them into LM2 and CTC lines. In this system, cancer cells in different states along the EMT/MET spectrum are distinguished by colors. In addition, MET cells are distinguished from epithelial cells with nuclei color.

I have successfully established EMT/MET tracing cell lines. By employing this system, I could distinguish minor epithelial-like populations from LM2 cells, even though these cells mostly exhibit an elongated mesenchymal phenotype. These epithelial-type LM2 cells formed clusters with tight cell-cell contacts in culture, and upon treatment with the EMT inducer TGF-β, some of these cells switched their phenotype into an elongated morphology and obtained new color. Conversely, in long-term culture, some mesenchymal-type LM2 cells changed into an epithelial-like morphology, formed colonies, and changed their colors. EMT/MET tracing cells were transplanted into the mammary glands of NSG mice, and ongoing sample analysis is being conducted in collaboration with my host lab.

Scientific activities:

Since May 2022, I have attended 4 international conferences including 2 invitations. In the recent conference (invited), Symposium TRR 305 organized by German Research Foundation held in Kloster Seeon, Germany (22nd to 25th May, 2023), I presented the results from *Project 1*.