Fellowship ID：BR240202

2025 年 1 月 21 日
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独立行政法人日本学術振興会理事長　殿
To: President, Japan Society for the Promotion of Science

研究活動報告書
Research Report

１．受入研究者/ Host researcher

受入研究機関・部局・職 Name of Host Institution, Department and Title	京都大学・iPS 細胞研究所・教授
受入研究者氏名 Host Researcher's Name	齊藤　博英

２．外国人招へい研究者/ Fellow

所属研究機関・部局・職 Name of Institution, Department and Title	キュリー研究所・キュリー研究所・研究員
外国人招へい研究者氏名 Fellow's Name	Maria Carla PARRINI

３．採用期間/ Fellowship Period

2024 年　11 月　11 日　～　2024 年　12 月　2 日

４．研究課題/ Research Theme

がん細胞オルガノイドに関する研究

５．研究活動報告/ Research Report

（１）研究活動の概要・成果/ Summary of Research Results

My 3-week stay in Japan had two objectives:
1/ an experimental research activity in the host laboratory of Dr Saito;
2/ a networking activity to visit other Japanese laboratories that perform research in my current research field of tumor-on-chip.

Experimental research activity

We reasoned that the microRNA switch technology of Dr SAITO's laboratory may be an effective and elegant way to solve the problem of isolation of primary patient-derived cells from fresh tumor samples. This would greatly expand the capacity to generate personalized tumor-on-chip models, to be used both in fundamental research and in clinical applications. We discussed how to use microRNA switch technology to efficiently isolate specific cell types composing the tumor microenvironment (TME). We concluded that this will require to realize microRNA sequencing on the various TME cell types, the major challenge being the identification of specific microRNAs which can be used as cell type biomarkers.

（注）採用期間終了後 3 ヶ月以内に提出
※　(Note) Submit the form within 3 months after the expiration of fellowship.
※　様式 1 に記載された情報を元に確認しますので、部局名等の名称含め、内容に誤りが無いか必ずご確認ください。

I learned from Dr Yoshihiko FUJITA how to perform a complete microRNA ON/OFF switch experiment. The procedure required 1-week work.
By a series of PCR reactions, we prepared and purified DNA templates, and verified their quality.
We then performed in vitro transcription to generate two types of EGFP-coding mRNAs: ON switch with miR-21 target sequence after the poly A tail (EGFP is translated only in cells with miR-21), OFF switch with miR-21 target sequence before 5'-UTR (EGFP is not translated in cells with miR-21).
In addition, two controls mRNAs were also prepared: normal EGFP-coding and iRFP-coding mRNAs, with constitutive translation.
After purification and quantification, the mRNAs were transfected in Hela cells (strong miR-21 activity) and in 293 cells (weak miR-21 activity), together with miRNA controls (miRNA mimic and miRNA inhibitor).
The day after transfection the expression of GFP protein was evaluated by fluorescent microscopy and quantified by FACS analysis.
As expected, in both cell lines, we observed i) constitutive expression of normal EGFP-coding and iRFP-coding mRNAs; ii) EGFP expression by ON switch mRNA in presence of miRNA mimic, but not in presence of miRNA inhibitor; iii) EGFP expression by OFF switch mRNA in presence of miRNA inhibitor, but not in presence of miRNA mimic.
Specifically, in Hela cells, we observed stimulation of EGFP expression by ON switch mRNA and inhibition of EGFP expression by OFF switch mRNA, consistently with the fact that these cells have strong miR-21 activity.
Specifically, in 293 cells, we observed inhibition of EGFP expression by ON switch mRNA and stimulation of EGFP expression by OFF switch mRNA, consistently with the fact that these cells have weak miR-21 activity.

Based on these experimental results, we will start a collaborative reflection on how to exploit microRNA ON/OFF switch technology to model tumor ecosystem heterogeneity in tumor-on-chip. We strongly believe that the synergic combination of our competences may bring very innovative ideas and projects.

Networking activity

In addition to the host lab of Dr Hirohide SAITO, I visited 5 more laboratories.
At Tokyo University I visited:

- Laboratory of Prof Shoji TAKEUCHI, Department of Mechano-Informatics, Graduate School of Information Science and Technology, Hongo campus
- Laboratory of Prof Yasuyuki SAKAI, Department of Bioengineering, Graduate School of Engineering, Hongo campus
- Laboratory of Prof Yukiko MATSUNAGA, Institute of Industrial Science, Komaba campus
- Laboratory of Prof Yoshiho IKEUCHI, Institute of Industrial Science, Komaba campus

At Kyoto University I visited:

- Laboratory of Prof Yasuyuki FUJITA, Department of Molecular Oncology, Graduate School of Medicine

During these visits, I gave 4 talks. The title of my seminar was: "Emerging applications of tumor-on-chip technology in fundamental and translational research".

I had plenty of opportunity to discuss with both senior and junior Japanese researchers, during formal scientific meetings, after my talks, but also informally at the time of lunches or dinners.

These interactions led to three outcomes.
First, solid bases for future scientific collaborations were built. With Dr SAITO we plan to apply for grants to financially support a collaborative project. One possible funding agency is the HFSP (Human Frontier Science Program).
Second, the possibility for me to spend a long research period in Japan has been discussed with the direction of LIMMS

(Laboratory for Integrated Micro Mechatronic Systems). LIMMS is a joint laboratory between French CNRS and the University of Tokyo (IIS – Institute of Industrial Science).
Third, the potential of France-Japan exchanges of junior researchers has been expanded. I identified several Japanese laboratories that have the capacity to host French students/postdocs. I promoted among young Japanese researchers the attractiveness of France, by describing the excellence of institutions like Curie Institute and the European culture richness.

（2）主な研究発表（雑誌論文、学会、集会、知的財産権等）/ Main Research Publications
None

（3）その他/ Remarks
I used my Research Support Allowance in part for the business trip to visit laboratories in Tokyo and in part to pay reagents for the experiments realized in the host laboratory during my stay.

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