PROGRESS REPORT
Division of Integrated Research

Atsushi Ochiai, M.D., Ph.D.
Members

Faculty members

Professor (concurrent appointment)
Atsushi Ochiai, M.D., Ph.D.

Associate professor
Hiroshi Haeno, Ph.D.

Junior Associate Professor
Shuhei Ogawa, Ph.D.

Research Collaborators

Professors
Shin Aoki, Ph. D.
(Faculty of Pharmaceutical Sciences)

Collaborate company

AGRI SMILE

Hiroshi Haeno, Ph.D.
Members

Faculty members

Associate Professor
Hiroshi Haeno, Ph.D.

Students

Graduate student
Sharafudeen Abubakar Dahiru

Guest graduate student
Mitsuaki Takaki
Qian Yu

Technical staff

Tamao Motoyama

Shuhei Ogawa, Ph.D.
Members

Faculty members

Junior Associate Professor
Shuhei Ogawa, Ph.D.

Guest researchers

Shiho Watanabe, Ph.D.

Division of Integrated Research

Chairman: Atsushi Ochiai, M.D. Ph.D.

Research Institute for Biomedical Sciences (RIBS) plays a role as a hub to promote interdisciplinary collaborative research among life science, medical science and engineering science. Particularly, our division researchers together with other faculties on campus, the outside research institutes, and various industries. To achieve our mission, we maintain an animal facility with high quality and support to create the genetically modified mice. We also provide the various advanced and well-maintained equipment, and the research space in our institute.

Promotion of joint research and operation of joint laboratory

Our institute renovated and is maintaining the joint laboratory space at the 1st floor (about 400m^2) in 2021. Since then, we accepted research proposals for joint collaborative research involved in the faculty members of our institute with that of Pharmaceutical Sciences and a company having collaboration with TUS. To promote these collaborative research projects, we organized the operative committee for joint research and made effective use of conference and common room for lively discussions.

Animal Facility and Research Support of Developmental Engineering.

Our animal facility maintains SPF condition through stringent animal care and breeding. Microbiological quality and health monitoring are carried out six times a year. We continue to maintain over 8,000 mice in the SPF area and 200 mice in an infection experiment area. We have also provided technical assistance to generate gene-modified (knock-out, knock-in and transgenic) mice (8 lines). We introduced 15 mouse lines into the SPF area via an in vitro fertilization (IVF) and have also cryopreserved 5 lines for genetic resources (frozen embryos). We also conducted 12 lines of recovery from frozen embryo. To perform these missions, we employed one guest researcher.

Division of Integrated Research

Hiroshi Haeno, Ph.D.

Our mission is mathematical formulation of diseases (especially cancer) by using mathematical and computational models such as differential equation systems and stochastic models. Recent advancement of measurement technology and computer performance in biological and medical research field enables us to develop verifiable theoretical models based on large and high precision data. We are motivated to propose (i) principles; (ii) drug targets; (iii) prognosis prediction; and (iv) optimal treatment strategies of diseases. As the first year after joining RIBS, we conducted the following research concerning tumor development under immune pressure, emergence of tumor recurrence, and optimal treatment strategies in lung cancer.

1) Computational analysis of cancer immune escape.

It is the well- accepted concept that tumors evolve under the pressure of immune responses and escape from them. Immune checkpoint inhibitors (ICIs) are expected to reactivate antitumor immunity and inhibit tumor progression. However, the durable benefits by ICI treatments are limited to the minority of patients. Therefore, it is important to study the mechanisms of tumor evolution interacting with immune cells and to reveal the condition that ICIs become effective.

To understand the tumor evolution under immune pressure we developed a computational model. A simulation model starts from one tumor cell until the total number of cells reaches 10^6 or 0. We assume that an intrinsic tumor growth rate is common among each tumor cell, which is denoted by r. The cell death rate, however, differs among each tumor cell according to the number of harboring antigenic and escape mutations. The possibility of mutations arises when a cell divides with probability p_A for an antigenic mutation and p_E for an escape mutation. The cell death rate increases with the number of antigenic mutations and decreases with that of escape mutations. Here, we define the cell death rate for i-th clone d_i as follows:

$$d_i = \alpha k_i e^{-\beta n_i},$$

where k_i and n_i are the numbers of antigenic and escape mutations in the i-th clone, respectively, and α and β are the immunoreactive or immunoescape effects per mutation, respectively. A new clone branches from a parental clone once mutation arises. By setting α=0, the cell death rate becomes zero regardless of the mutation profile of a cell. This is the setting of the neutral case in our simulation. The stochastic dynamics of tumor cell populations is simulated by the Gillespie algorithm. To replicate the multiregional sequencing data, our simulation was conducted on a lattice space. Cell migrations are not considered in our simulation.

We performed simulations with and without selective pressure mimicking the situations of MSI-H and MSS CRCs, respectively (**Figure1**). We additionally executed *in silico* multiregion sequencing and compared the multiregion mutation profiles to those from the real tumors. We further examined the distributions of the VAFs derived from 10 Monte Carlo trials with and without immune selective pressure. Overall, we found that the VAFs of shared mutations of tumors simulated with selective pressure

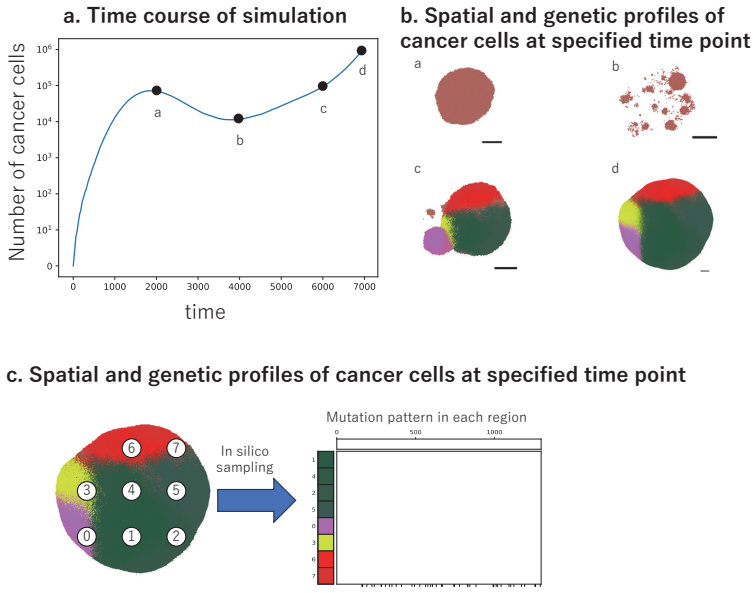

Figure1. Simulation of tumor progression under immune pressure

assuming MSI-H CRCs were significantly higher than those of tumors without selective pressure assuming MSS CRCs, which was consistent with the clinical data [publication 2].

Collaborators:

Koichi Saeki (the University of Tokyo), Yuta Kobayashi (Osaka University), Atsushi Niida (the University of Tokyo), Kazuki Takahashi (the University of Tokyo), Koshi Mimori (Kyushu University)

2) Computational modeling of cancer recurrence based on public recurrence data.

Cancer arises due to sequential non-lethal mutations in cells. Despite advancements in cancer therapy, local and regional recurrence still remains a significant problem in cancer management. Field cancerization - the presence of histologically normal but mutated premalignant lesions surrounding the tumor has been recognized as a significant cause of these recurrences. However, the relationship between tissue dynamics, cancer initiation and cancer recurrence in multistage carcinogenesis is not well characterized.

We have constructed a computational model for cancer initiation and recurrence by combining the Moran and Branching processes in which cells requires 3 or more mutations to become malignant (**Figure2**). In addition, a spatial structure-setting is included in the model to account for positional relativity in cell turnover towards malignant transformation. The model consists of a population of normal cells with no mutation; several populations of premalignant cells with varying number of mutations and a population of malignant cells. The model computes a stage of cancer detection and surgery to eliminate malignant cells but spares premalignant cells and then estimates the time for malignant cells to re-emerge. We have accounts of the cellular conditions that give rise to different patterns of cancer initiation and the conditions favoring a shorter cancer recurrence by analyzing premalignant cell types at the time of surgery.

From this model, we fitted the disease-free clinical data of 8,957 patients in 27 different cancer types. This fitting reveals the relative fitness of several premalignant cells, mutation rates from one cell type to another, the turnover

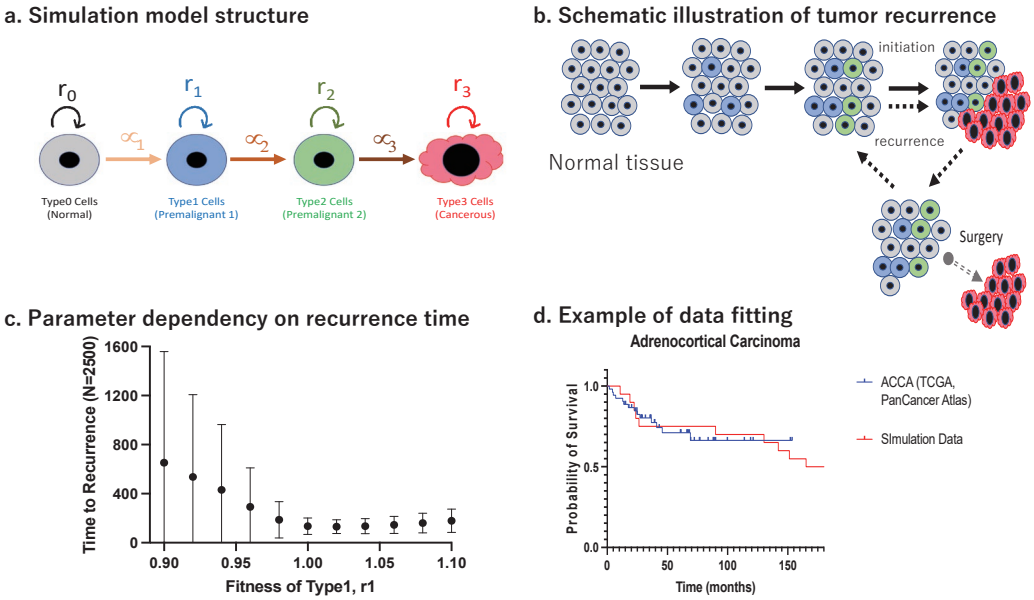

Figure2. Computational modeling of tumor recurrence and data fitting

rate per month, growth rate and death rate of cancer cells in each cancer type. Our study provides a novel tissue-specific carcinogenic profile of different cancer types and can provide key insights into cancer initiation and recurrence [publication 3] .

Collaborators:

Sharafudeen Abubakar Dahiru (Tokyo University of Science), Mitsuaki Takaki (the University of Tokyo)

3) Mathematical analysis of the optimal treatment strategy for epidermal growth factor receptor-mutated non-small cell lung cancer.

In Asians, more than half of non-small cell lung cancers (NSCLC) are induced by epidermal growth factor receptor (EGFR) mutations. Although patients carrying EGFR driver mutations display a good initial response to EGFR-Tyrosine Kinase Inhibitors (EGFR-TKIs), additional mutations provoke drug resistance. Hence, predicting tumor dynamics before treatment initiation and formulating a reasonable treatment schedule is an urgent challenge.

To overcome this problem, we constructed a mathematical model based on clinical observations and investigated the optimal schedules for EGFR-TKI therapy (**Figure3**). Based on published data on cell growth rates under different drugs, we found that using osimertinib that is efficient for secondary resistant cells as the first-line drug is beneficial in monotherapy, which is consistent with published clinical statistical data. Moreover, we identified the existence of a suitable drug-switching time; that is, changing drugs too early or too late was not helpful. Furthermore, we demonstrate that osimertinib combined with erlotinib or gefitinib as first-line treatment, has the potential for clinical application. Finally, we examined the relationship between the initial ratio of resistant cells and final cell number under different treatment conditions, and summarized it into a therapy suggestion map. By performing parameter sensitivity analysis, we identified the condition where osimertinib-first therapy was recommended as the optimal treatment option. This study for the first time theoretically showed the optimal treatment strategies based on the known information in NSCLC. Our framework

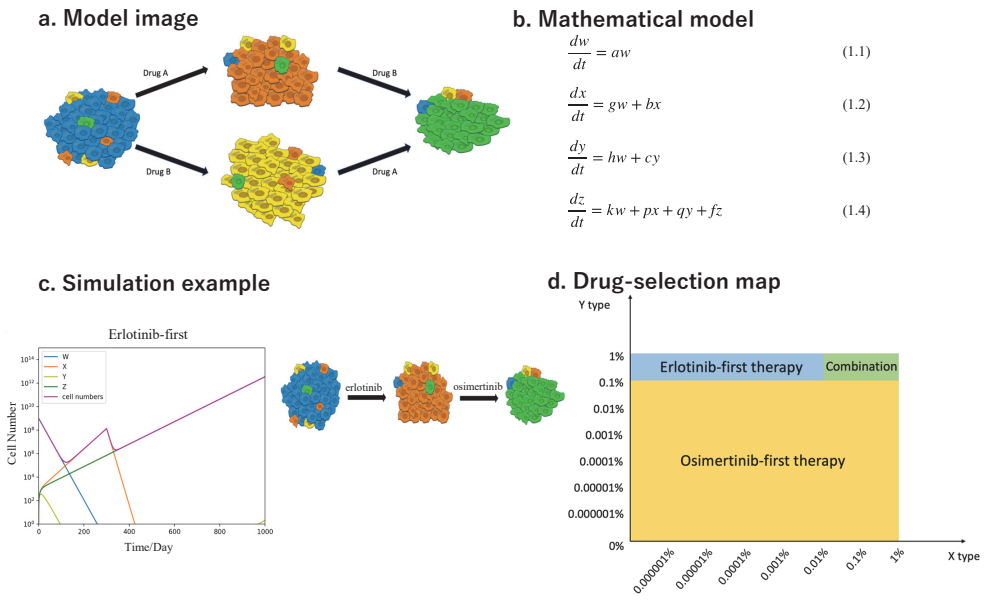

Figure3. Computational modeling of lung cancer progression under molecular targeted drug treatment.

can be applied to other types of cancer in the future [publication 1].

Collaborators:

Qian Yu (the University of Tokyo), Susumu Kobayashi (Harvard Medical School)

Publications (# correspoinding author)

1. Yu Q, Kobayashi SS, **Haeno H#.** Mathematical analysis identifies the optimal treatment strategy for epidermal growth factor receptor-mutated non-small cell lung cancer. ***Front Oncol.*** 13: 1137966. 2023. (doi: 10.3389/fonc.2023.1137966.)
2. Kobayashi Y, Niida A, Nagayama S, Saeki K, **Haeno H,** Takahashi KK, Hayashi S, Ozato Y, Saito H, Hasegawa T, Nakamura H, Tobo T, Kitagawa A, Sato K, Shimizu D, Hirata H, Hisamatsu Y, Toshima T, Yonemura Y, Masuda T, Mizuno S, Kawazu M, Kohsaka S, Ueno T, Mano H, Ishihara S, Uemura M, Mori M, Doki Y, Eguchi H, Oshima M, Suzuki Y, Shibata T, Mimori K. Subclonal accumulation of immune escape mechanisms in microsatellite instability-high colorectal cancers. ***Br. J Cancer.*** 129:1105-1118. 2023. (doi: 10.1038/s41416-023-02395-8.)
3. Abubakar SD, Takaki M, **Haeno H#.** Computational modeling of locoregional recurrence with spatial structure identifies tissue-specific carcinogenic profiles. ***Front Oncol.*** 13: 1116210. 2023. (doi: 10.3389/fonc.2023.1116210.)
4. Shimizu D, Taniue K, Matsui Y, **Haeno H**, Araki H, Miura F, Fukunaga M, Shiraishi K, Miyamoto Y, Tsukamoto S, Komine A, Kobayashi Y, Kitagawa A, Yoshikawa Y, Sato K, Saito T, Ito S, Masuda T, Niida A, Suzuki M, Baba H, Ito T, Akimitsu N, Kodera Y, Mimori K. Pan-cancer Methylome Analysis for Cancer Diagnosis and Classification of Cancer Cell of Origin. ***Cancer Gene Ther.*** 29: 428-436. 2022. (doi: 10.1038/s41417-021-00401-w.)

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Shuhei Ogawa, Ph.D.

A major goal of our group is to understand how the immune system develops and is regulated and how inappropriate immune responses produce local and systemic disease. I believe that the outcome of these efforts will give rise to better tools and strategies to overcome immunological disorders such as autoimmune disease, graft-versus-host disease, and allergy, and lead to the development of efficient immune therapies to treat cancer and infectious disease.

My laboratory has been working on the analysis of the major T cell costimulatory signal transmitted through the CD28 receptor family (CD28, ICOS, CTLA-4, and PD-1) with respect to its role in signal transduction as well as in normal physiological and immunological functions. It has been shown that CD28-mediated costimulation contributes to metabolic reprogramming, thereby regulating functional differentiation of T cells, such as effector helper T cells and memory T cells. We try to investigate the role of CD28 receptor family in T cell activation, effector T cell development, and memory formation.

We are also committed to the maintenance of a high-quality animal facility in RIBS and the services for developmental engineering research, such as clean up and cryopreservation of mice. We also generated genetically modified mice for requests from both inside and outside the University.

The molecular mechanisms of CD28-mediated costimulatory signaling.

CD28-mediated costimulation is important for full activation of T cells. Crosslinking of CD28 leads to activation of various signaling pathways, such as, PI3K/Akt, Grb2/Gads/MAPK, mTOR, Ca^{2+}/NFAT, and PKCθ/NF-κB pathways. CD28-mediated costimulation also contributes to metabolic reprogramming, and consequently regulates functional differentiation of T cells, such as effector helper T cells and memory T cells. Tyrosine phosphorylation of CD28 is thought to be one of the key events to transduce CD28 specific signal. Previously, we showed that the Y189 but not PYAP motif is critical for tyrosine phosphorylation of CD28. The last decades, we have investigated the interactions between PI3K, Grb2, and Gads to CD28 Y189MNM motif by structural analyses. This year, we found that several compounds (inhibiting CD28 pYMNM – PI3K p85 binding and enhancing CD28 pYMNM – Grb2) showed stimulatory function on T cell activation depending on concentration of compound. We think that these signaling pathways are potential therapeutic targets for cancer immunotherapies, effective vaccines, autoimmune disease, and graft survival by manipulating T cell responses. In this year, we analyzed the effects of compounds on the interactions between CD28 phosphopeptides and SH2 domains using a surface plasmon resonance (SPR) biosensor, Biacore. Several compounds were found to decrease CD28 binding to PI3K cSH2 and increase binding to Grb2 SH2. We also analyzed the effects of trisubstituted carboranes on the function of T cells obtained from C57BL/6 mice and found that they efficiently increased proliferation. We will attempt to evaluate the in vivo function of these compounds using animal models, particularly

preclinical mouse tumor models.

Collaborators:

Watanabe, S., Oda, M (Kyoto Prefectural University)

Several signaling inhibitors of metabolic pathway not only inhibit but also augment CD28-mediated proliferation of T cells.

CD28-mediated costimulation is critical for the activation of T cells. CD28 has no enzymatic activity in the intracellular domain but contains four tyrosine residues and several functional motifs, such as a YMNM motif and two PxxP motifs, which recruit several adaptor proteins and activate PI3K, MAPK, NF-kB, and mTOR signaling pathways. Most inhibitors of signaling pathway inhibit T cell activation and proliferation. However, we found that several signaling inhibitors of metabolic pathway, such as mTOR or mitochondrial Carrier, not only inhibit but also augment CD28-mediated proliferation of T cells depending on the concentration of the inhibitor in the PMA plus anti-CD28 mAb stimulatory condition. In this year, we examined the phenotype of T cells when a signaling inhibitor is added to the above T cell stimulatory conditions. We are now attempting to investigate how one inhibitor both inhibits and enhances T cell proliferation depending on the concentration.

Collaborators:

Watanabe, S.

Development of methods and devices for efficiently recovering CTCs from blood.

Circulating tumor cells (CTCs) are cancer cells that have detached from a primary tumor and entered the bloodstream. The ability to detect and analyze CTCs is important for cancer research and clinical oncology, as these cells can provide valuable information about the status of a patient's cancer, treatment response, and potential for cancer progression.

We have been attempting to capture circulating tumor cells (CTCs) by selectively isolating larger cells from blood using microfluidic devices. However, this method inevitably leads to the inclusion of white blood cells. To address this issue and remove white blood cells, they have explored the use of biological affinity. But capturing white blood cells required stopping the flow and waiting for adsorption. We think that surface irregularities on the cells may be a potential reason for the difficulty in capturing cells. Therefore, we are testing to the hypothesis using microfluidic channels with narrow slits. The ultimate goal is to develop a high-throughput white blood cell capture device that incorporates a mechanism to push cells against the channel walls.

Collaborators:

Hayase, M. (Tokyo University of Science, Faculty of Science and Technology, Department of Mechanical and Aerospace Engineering)

Publications

Ogawa S, Asawa Y, Iiyama M, Yoshimori A, Nakamura H, Oda M. Regulation of CD28 binding to SH2 domains of Grb2 and PI3K by trisubstituted carboranes for T-cell activation. Bioorg Med Chem Lett. 2022 Dec 15;78: 129049. doi: 10.1016/j.bmcl.2022.129049.

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ligament. Development. 2022 Oct 1;149(19):dev201203.doi: 10.1242/dev.201203. Epub 2022 Oct 17.

Ogawa T, Shichino S, Ueha S, **Ogawa S**, Matsushima K. Complement protein C1q activates lung fibroblasts and exacerbates silica-induced pulmonary fibrosis in mice. Biochem Biophys Res Commun. 2022 May 7;603:88-93.