PROGRESS REPORT
Division of Integrated Research

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Collaborate company
AGRI SMILE

Hiroshi Haeno, Ph.D.
Members

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Post-doctoral fellows
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(until Sep. 2023)

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Kouma Shimomura

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Qian Yu (The University of Tokyo)

Secretary
Tamao Motoyama

Shuhei Ogawa, Ph.D.
Members

Faculty members

Junior Associate Professor
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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 400m2) 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. The number of universities and research institutes where RI can be used is decreasing due to the strict radiation control system in recent years. There is an increasing demand for the development of imaging reagents for human medical purposes such as drug discovery using alpha-emitting nuclides such as At211AC225 and PET. To respond to the demand, RIBS applied for the RI laboratory to be a facility where new radioactive nuclides can be used, and immediately began developing alpha-ray imaging equipment together with the Faculty of Science and Technology, the University of Tokyo.

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 second 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) Mathematical analysis of optimal treatment strategies for the immune checkpoint inhibitors combined with radiotherapy in esophageal squamous cell carcinoma

Radiotherapy (RT) is an important component of cancer treatment, and it is administered to approximately 50–60% of all cancer patients. In recent years, the combination of immune checkpoint inhibitors (ICIs) with RT (ICI-RT) has been reported to significantly enhance the therapeutic efficacy of RT in patients with non-small cell lung cancer (NSCLC). The efficacy of ICI-RT has been demonstrated in trials involving esophageal squamous cell carcinoma (ESCC) patients. However, these trials of anti-PD-1/PD-L1 combined with RT showed that more than half of patients experience recurrence and suggested that the effect of the simple combination of anti-PD-1/PD-L1 therapy and RT is inadequate; thus, it is necessary to optimize combination therapies that involve ICIs and RT.

In this study, single-cell RNA-seq and spatial transcriptome analyses were performed to investigate the dynamics of cellular interactions throughout RT. The functional activity of lymphocytes transitioned from innate to adaptive immune activity, with drastic increases in ligand–receptor interactions, such as *PD-1–PD-L1, CTLA4–CD80/86*, and *TIGIT–PVR* interactions. Then, we constructed a mathematical model to predict the impact of treatment on the target interactions and to explore optimal combination regimens by quantitatively assessing the impact of therapeutic agents on ligand–receptor interactions. In this model, an interaction score was calculated for protein expression and ligand–receptor interactions from RNA expression data using a previously reported algorithm [1]. From the information on each drug, the effect of inhibiting the interaction between the ligand and receptor was calculated and considered to be the predicted treatment score. Mathematical model analysis was performed on the ICI targets PD-1, PD-L1, CTLA4, and LAG3, for which targeted therapies have already been approved by the FDA, and on TIGIT antibodies, which are currently under review for approval (Fig. 1). The combination of ICI monotherapy and polytherapy was simulated for the “Before”, “During”, and “Just after” phases. CTLA-4 and TIGIT inhibition showed a weak prediction score at the “Before” time point, while this score was strongest at the “During” time point, and this score persisted at an approximately half-maximal effect at the “Just

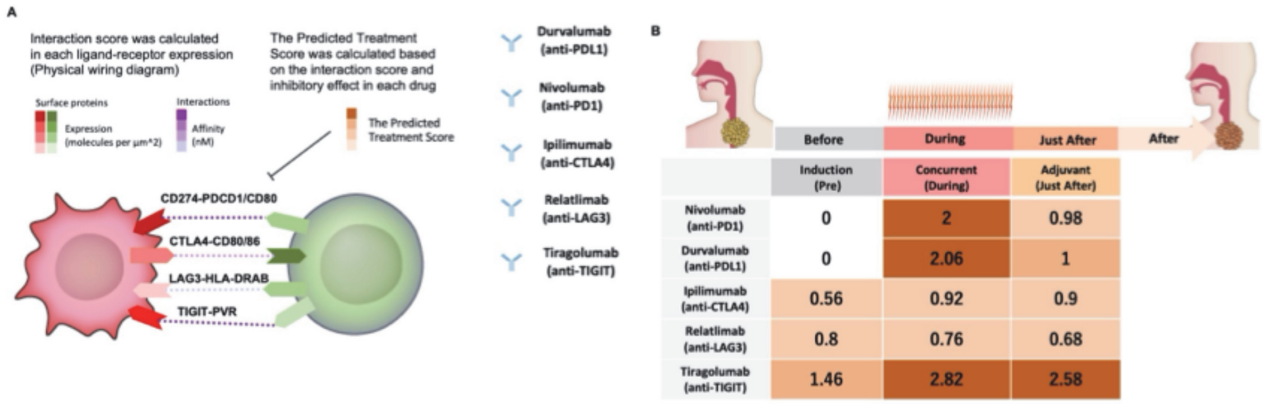

Figure 1. Proposal of optimal treatment based on mathematical model analysis.
(A) Consideration of cellular interaction under molecular targeted drugs.
(B) Calculation of treatment effect relative to one under Durvalumab just after radiotherapy.

after" time point. PD-1 and PD-L1 had the strongest effect at the "During" time point, and LAG3 had the strongest score at the "Before" time point. However, the predicted scores of these three genes were maintained throughout the three phases, unlike those of PD-1/PD-L1.

In conclusion, the model suggested that concurrent anti-PD-1/PD-L1 therapy or concurrent/adjuvant anti-CTLA-4/TIGIT therapy exerts a strong synergistic effect with RT. This study provides a suitable rationale for clinical trials of RT combined with ICIs, and these findings will support future studies to search for more effective targets and timing.

Reference

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Major collaborators:

Koichi Saeki (Tokyo University of Science), Shunsuke Sakai and Shun-ichiro Kageyama (National Cancer Center East).

2) Comprehensive estimation of tissue dynamic parameters for categories of colorectal cancer patients

Colorectal Cancer is the second leading cause of cancer death worldwide and is on course to pose an even more significant death threat worldwide due to rising incidence of early-onset disease. Theoretical and computational models have been widely used to summarize and understand complex disorders like cancer while establishing huge databases and repositories for researchers and clinicians Using a model of cancer initiation and locoregional recurrence, we have previously used clinical recurrence data from The Cancer Genome Atlas (TCGA) to profile unique tissue dynamic parameters for several cancer types and predict unknown number of mutational hits for some cancers. Disease-free survival is a superior survival data and a vital component of cancer research and management. In cancer research, comparing survival among groups is universal and the reliable log-rank tests has proven to be essentially useful. However, it has been shown to suffer from inaccuracy when subjected to multiomics data across several cohorts from TCGA. In the current study, we profiled several categories of patients with colorectal adenocarcinoma using their clinical data from TCGA. We analyzed the distribution and optimal parameter combinations.

Publicly accessible, deidentified data of patients with colorectal adenocarcinoma (COADREAD) was extracted from TCGA PanCancer Atlas. Patients were categorized based

on Race, Sex, Tumor Stage, Cancer Subtype and Tumor Type. To avoid proportion bias, we excluded categories with less than 5% of the whole population and patients with missing data (N/A). From the, we extracted clinical and genetic data obtained using targeted exome sequencing from 594 patients via the cBio Cancer Genomics Portal available at https://www.cbioportal.org. Racial terms used were Asian, Black and White as per updated guidance on the reporting of race and ethnicity in medical and science journals. For genomic alteration data, we extracted only cancer-related genes from OncoKB database available at https://www.oncokb.org because our model is based on driver mutations. Cancer gene hallmarks were obtained from COSMIC database available at https://cancer.sanger.ac.uk/cosmic. All cancer gene hallmarks from COSMIC database are curated from published experimental data. All comparisons were done between groups and the parent COADREAD dataset.

We utilized the Vogelstein 3-step colorectal carcinogenesis model with order of mutation: APC (loss)–KRAS (gain)–TP53 (loss). We model the dynamics of 4 cell types in a tissue. “Type 0”, “Type 1”, “Type S-1” and “Type S” represent normal healthy cells with no mutation; premalignant cells with one driver mutation (APC mutant); premalignant cells with S-1 driver mutations (APC/KRAS mutant) and cancer cells with S driver mutations (APC/KRAS/TP53 mutant) respectively. We assume that malignant cell emergence must be preceded by Type 1 and Type S-1 cells where they undergo cellular turnover with a small probability of mutation. A “healthy” tissue might contain Type 0, Type 1 and Type S-1 cells where total cell number is kept constant with value of N and tissue turnover rate governed by d0. Since the net growth of these cells is zero (cell division=cell death), we compute their dynamics using the Moran process. We adopt tissue turnover rate of Type S cells, dS, to be similar to d0 but since they are highly proliferative (cell division>cell death), their dynamics are computed using the Branching process. Initially, a tissue is comprised of N Type 0 cells arranged in a 2-dimensional lattice structure 〈I x J.〉. Cell death triggers a cell division where a mutation in a cancer-related gene (APC) can transform it to a Type 1 cell at a mutation rate u1. Mutation rate, u, refers to the cumulative effects affecting change form one cell type to another. The cell to divide is selected based on the cell fitness, r, and the spatial structure of the intestinal epithelium. Cell fitness, r, refers to the transcriptional and metabolic potential of a cell type to “out-compete” other cell types. The fitness of Type 0, Type 1, and Type S-1 are denoted by r0, r1, and rS -1, respectively. If a Type 1 cell divides with a mutation at a rate of uS-1, a Type S-1 cell arises. Finally, a Type S cell can emerge with a fitness of rS when a Type S-1 cell divides with a mutation rate of uS. Type S cells are “super-competitors” with outstanding metabolic prowess and assumed to increase exponentially with a net growth rate of rS - dS > 0. The spatial structure governs the positional relativity of cells where only adjacent cells can be chosen to divide to replace a dead cell. For example, If a cell at position $\langle i, j \rangle$ dies, 4 adjacent cells – $\langle i, j-1 \rangle$, $\langle i, j+1 \rangle$, $\langle i-1, j \rangle$ and $\langle i+1, j \rangle$ can possible be chosen to divide and replace the dead cell. The transition probabilities are calculated according to the cell type at those positions. Spatial arrangement of cells in a tissue have been shown to influence tumor evolution as well as the structure and nature of clones at a genomic level.

Disease-free survival of patients’ clinical data is theoretically equivalent to the recurrence data in our model. To deduce parameters for each patient category, we adopted Approximate Bayesian Computation (ABC) through model simulation to fit patient clinical data. Here, our observational data, ϕ, is represented by disease-free intervals - 100 data points extracted from Kaplen-Meier curves of disease-free survival of patients. The prior distribution, θ, is the exact parameter combination that gives the observational data. From our model, we performed 100,000 simulations using random

combination of parameters, θ_n to get ϕ_n. If ϕ_n satisfies the condition of our summary statistic – $\rho(\phi, \phi_n) < \varepsilon$, we accept θ_n into the posterior distribution. The summary statistics employed was the mean squared residuals or mean squared error and optimal parameter combinations were selected using the minimal values of ε. From our analysis, we adopt ϕ_n to serve as the simulation data data which is compared to ϕ, the clinical data.

From the disease-free survival and Approximate Bayesian Computation, the posterior distribution of the tissue dynamic parameters were obtained. These might provide valuable insights into the possible carcinogenic profiles of tumors. From figure 2A, we observe that some subgroups like COAD_GS have a "narrow" posterior distribution possibly indicating "stricter" genetic requirements while others like Tumor_Mucinous have relatively "wide" distributions possibly indicating "loose" genetic requirements. This finding echo recent concerns on clinical classification and the heterogeneity of mucinous colorectal adeno-carcinoma genetics. An analysis of long term recurrence for the subgroups shows the worst outcome for Race_Black and Sub_COAD_MSI where most patients will likely have a recurrence by 15 years. The possibility of this late recurrence have been reported in a 70-year female who had a recurrence after 12 years of apparently healthy living.

From the posterior distribution obtained using Bayesian inference, we proceeded to identify optimal parameter combinations that best fits patient data. Maximum a posteriori Estimation, using the mode of the posterior as optimal parameter combination yielded poor fitting (data not shown). Rather, we used the minimum ε value as the optimal parameter combination. We then used this parameter set to simulate 100 recurrence data points which we subjected to Kaplan-Meier Analysis. Comparing the simulated and clinical data not only displayed a visually well-fitted curve (Figure 2B) but also showed no statistical deviation with the clinical data as seen from the p values obtained using the Mantel-Cox test. Additionally, using these simulated data points, we extended the Kaplan-Meier Analysis for all groups to a 20-year period in order to have insight into their respective long-term disease-free survivals. Here, we see that Race_Black and Sub-COAD_MSI have very poor long-term cancer-free survival hardly reaching 20 years. Sex_Male and Tumor_READ barely reaches the 20-year mark while others scale through.

We have successfully profiled carcinogenic profiles of subgroups of colorectal adeno-carcinoma patients using data from recurrence

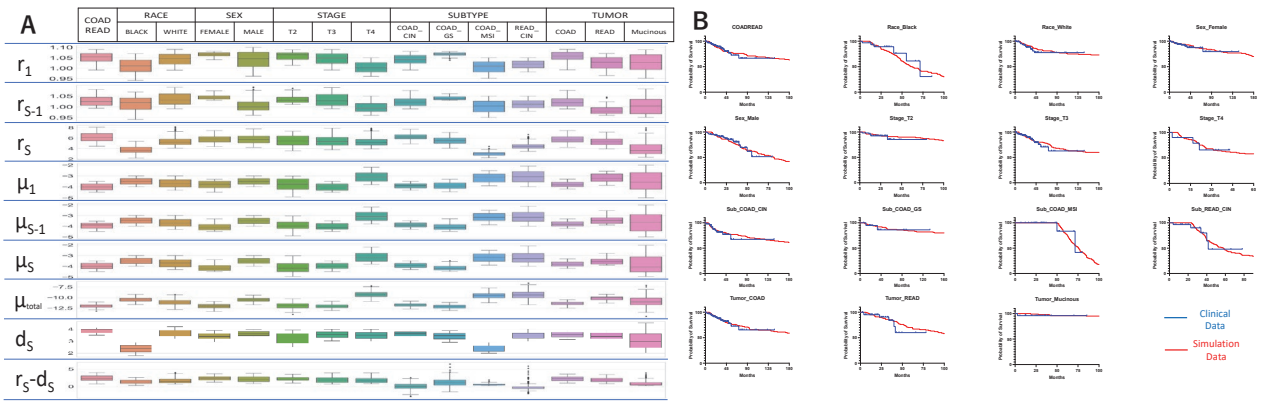

Figure 2. Parameter distribution and disease-free survival curves of COADREAD groups.
(A) Comprehensive estimation of tissue dynamic parameters in subgroups of COADREAD.
(B) Comparison of disease-free survival curves between simulation and clinical data.

data from the TCGA. We deduced fitness, mutation rate and tissue turnover parameters for each group and investigated how genetic alterations in driver genes and other cancer genes could affect these parameters by extracting similarities in their cancer gene hallmarks. We propose an expansion of carcinogenesis to accommodate pathways and cancer gene hallmarks rather than the "rigid" driver mutation pathway only. Furthermore, we predict long-term recurrence survival for each category with potential for extension as far as possible. Our model could serve as a valuable tool to discern additional genetic information from data where these details are absent or lost.

Collaborator:

Sharafudeen Abubakar Dahiru (Memorial Sloan-Kettering Cancer Center)

3) Single cell RNA-Seq analysis identifies cell fate of aneuploidy in cancer cells to facilitate adaptation overcoming aneuploid-mediated intracellular stress

More than 90% of solid tumors exhibit aneuploidy of aberrant karyotype, which exerts an important role in cancer proliferation. In contrast, aneuploidy is also known to cause various intracellular stresses, termed aneuploid stresses, which intensively suppress cellular proliferation or survival. These paradoxical contexts of aneuploidy in cancer proliferation remain unsolved. The objective of this study is to identify key signaling pathways for cell survival under aneuploidy stresses. We also determined that how cancer cells overcome the aneuploid stress and facilitate adaptation to the stressful conditions in our aneuploid models.

To this end, we developed a unique aneuploid model using pseudo-diploid HCT116 in treatment with an Aurora-B inhibitor (AZD1152), which promotes aneuploidy in HCT116 through failed cytokinesis and chromosomal miss segregation. In this study, we treated HCT116 with AZD1152 for 24 h (on Day1) to initiate aneuploidy, and then cultured the aneuploid cells for additional 12 days in AZD1152-free medium (on Day13). More than 90% of AZD1152-induced HCT116 cells were killed in ~10 days posttreatment, while ~10% of the cells were still survived with comparable proliferative activity to the untreated HCT116. More importantly, the survived HCT116 successfully acquired aneuploidy (pseudo-tetraploid formation), suggesting these cells would overcome the aneuploid stress and facilitate adaptation.

To understand adaptive mechanism to aneuploid stress more comprehensively, we conducted a time-course experiments in AZD1152-treated HCT116 for integrative analyses of cell biology and single cell RNA-seq (scRNA-seq). We collected the cells on 1, 3, 7, and 13 days after AZD1152 treatment as well as untreated control cells. Microscopic and cell cycle analyses revealed that, following aneuploidy formation on Day1, the aneuploid cells were extensively killed on Day3-7, and then the surviving aneuploid cells expanded form Day7 to 13.

In scRNA-seq, a high correlation was observed between a gene set that characterizes proteasomal degradation and the strength of the E2F targets signaling at days 3 and 7 (cor = 0.63, Figure. 3A). This result is consistent with an increase in the S phase proportion of cells in day 7 (Figure. 3B and 3C). Cells on day 3 were classified into two subpopulations based on their expression level of proteasomal degradation. Because major population on days 7 and 13 exhibited high proteasomal degradation expression (Figure. 3B), the pathway may serve as a crucial signaling mechanism for the survival of the cells under the proteotoxic stress, and cells with downregulated proteasomal degradation may undergo cell deaths, forming the bottleneck-like projection observed by a trajectory analysis (Figure. 3D).

The aforementioned findings led to the

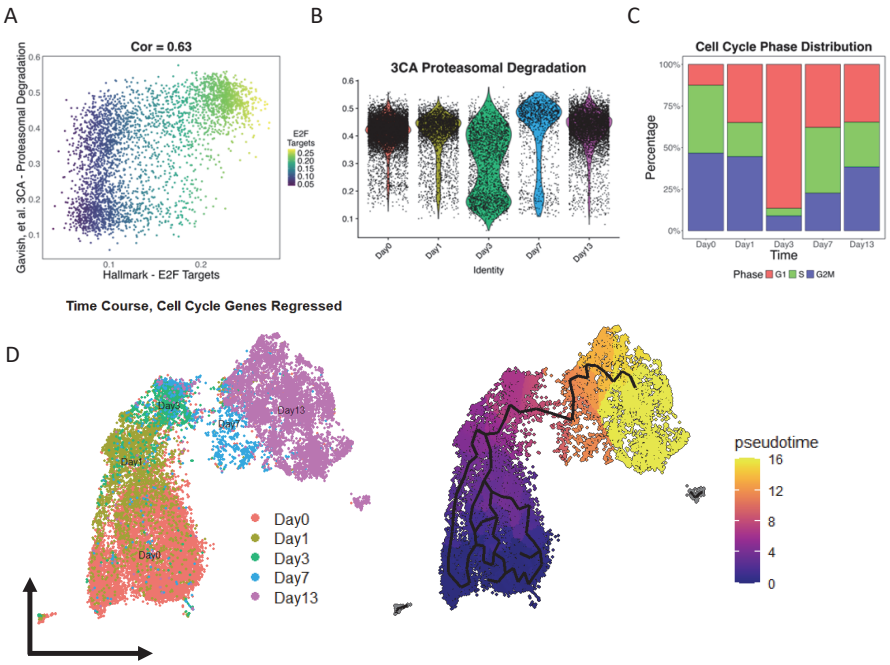

Figure 3. Multi-timepoints single cell analysis during adaptation to aneuploid cell.
(A) Positive correlation between proteasomal degradation activity and cell cycle arrest.
(B) Appearance of cells with low proteasomal degradation activity at day 3.
(C) Large proportion of cell cycle arrest was observed at day 3.
(D) Pseudotime analysis of cell adaptation to aneuploidy.

conclusion that cell survival during aneuploid establishment is significantly influenced by proteotoxic stress. This conclusion was further substantiated through a series of experiments. Our approach may facilitate the identification of tumor-intrinsic stress indicators, such as proteotoxicity stress, which could inform the development of novel therapeutic regimens and pan-tumor research.

Major Collaborators:

Shicheng Zhang (the University of Tokyo), Tomoko Yamamori-Morita and Akihiro Ohashi (National Cancer Center East)

Publication (# correspoinding author) (fiscal year 2023-2024)

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.)
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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.)

Division of Integrated Research

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. We have 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. In this year, we continued to improve the compounds to exhibit similar effects at lower concentrations than previous compounds,

and actually identified several compounds with stronger effects. 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), Nakamura, H. (Institute of SCIENCE TOKYO)

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)

Developmental Engineering Research Support Program at Tokyo University of Science

Since 2015, we have been conducting a developmental engineering research support program at Center for Animal Disease Models, established under the Ministry of Education,

Culture, Sports, Science and Technology's (MEXT) Strategic Research Foundation Grant-aided Project for Private Universities. This research support project has been taken over as a project of RIBS from 2021. In this support project, we support cleaning mice, freezing mice embryos, recovery of mice from frozen embryos, and generation of genetically modified mice.

In this year, we generated 6 strains of genetically modified mice. Additionally, we conducted cleaning of 11 strains, embryo freezing for 18 strains, and restoration from frozen embryos for 9 strains. In recent years, requests for the generation of Knock-in mice, such as reporter mice or floxed mice have been subject to increasing demand compared to the generation of simple Knock-out mice.

Since August 2021, we have employed one technician with support from the Tokyo University of Science to assist in improving our technical capabilities. Moving forward, we aim to further enhance both our technical expertise and reliability to strongly support not only our institute but also the broader research conducted at our university at a higher level.

Collaborators:

Watanabe, S.

Publications

Tai Y, Sakaida Y, Kawasaki R, Kanemaru K, Akimoto K, Brombacher F, **Ogawa S**, Nakamura Y, Harada Y. Foxp3 and Bcl6 deficiency synergistically induces spontaneous development of atopic dermatitis-like skin disease. *Int Immunol*. 2023 Sep 5;35(9):423-435. doi: 10.1093/intimm/dxad018.

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