PROGRESS REPORT
Division of Molecular Regulation of Inflammatory and Immune Diseases

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Members (Matsushima's lab.)

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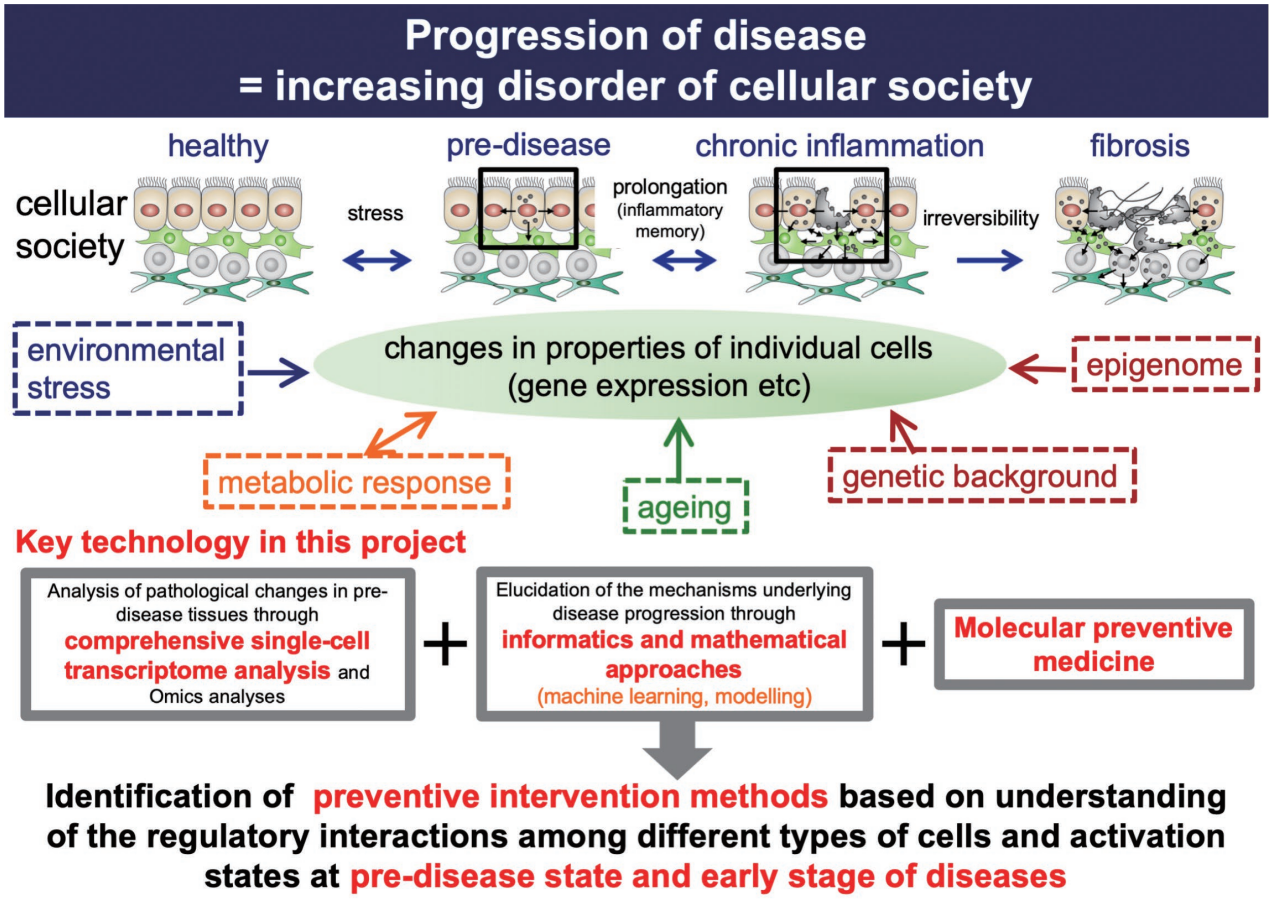
Division of Molecular Regulation of Inflammatory and Immune Diseases

Chairman: Kouji Matsushima, M.D., Ph.D.

Division of Molecular Regulation of Inflammatory and Immune Diseases aims to propose new preventive methods and treatment strategies against intractable inflammatory and immune diseases by investigating molecular mechanisms underlying inflammation and immune responses with particular interests in cytokines and chemokines.

Research on inflammation and fibrosis by comprehensive single-cell transcriptome analysis

Conventional immunological and pathological approaches to inflammation aimed to examine overall or averaged qualitative and quantitative changes in whole organs or tissues consisting of thousands of cells. As a result, it has been difficult to understand the complex processes occurring in the pre-disease state, in which changes occur in small, localized groups of cells that then influence surrounding cells, eventually



resulting in changes to the cellular society. To overcome this limitation, in this project we are analyzing inflamed tissues at the single-cell level by using BD Rhapsody-based our novel single-cell RNA-seq (scRNA-seq) method—TAS-Seq. (Shichino et al. 2022, *Commun. Biol*.1:602). By using this technology, we analyzed silica-induced pulmonary fibrosis model and we found that C1q as a specific marker of lung interstitial macrophages, and C1q acts as a profibrotic mediator in silica-induced lung fibrosis (Ogawa et al. 2022, *Biochem. Biophys. Res. Commun*. 599:113-119). In addition, we continuously developed an updated version of TAS-Seq, TAS-Seq3 of which gene-detection sensitivity was enhanced. TAS-Seq3 could detect up to 4000 genes in single nuclei from adult mouse brain and could be combined with a spatial transcriptomics Curio Trekker platform. We are now developing TAS-Seq3 for high-resolution spatial transcriptomics Stereo-seq, for the analysis of fixed cells, and increasing its cell throughput up to 0.2-1M cells. We also established temporal network analysis of cell-cell communications in time-course scRNA-seq data of bleomycin-induced pulmonary fibrosis model, and identify hub cells and associated extracellular molecules, and now verifying the roles of the molecules by using genetically-modified mice. In addition, we also analyzed BLM-injured murine lung organoid model and identified the induction of aberrant basaloid cells (ABCs) and possible important genes that contribute to ABCs induction (Bin et al. 2025, *Inflamm. Regeneration*. in press). Furthermore, we are collecting scRNA-seq data of the lung samples of human interstitial lung disease patients, fibrotic rat lungs, and murine lungs of various lung fibrosis models to establish species-wide atlas of lung fibrosis. Through the analysis, we identified PF-ILD-specific epithelial cells (progressive disease-specific) subsets and gene signature of lung macrophages of non-PF-ILD samples (associated with good prognosis), and further investigated the roles of the cells and gene signatures in lung fibrosis. In addition, we collaborated with various research groups through our TAS-Seq family technologies and accelerates the research of various inflammatory diseases.

Collaborators:

Shigeyuki Shichino, Satoshi Ueha

Research on combination cancer immunotherapy

Recent advances in immune-checkpoint inhibitors, such as CTLA-4 and PD-1 antibodies, have revolutionized cancer therapy. We found that depletion of $CD4^+$ cells, including regulatory T cells, using anti-CD4 antibodies induced strong tumor-specific $CD8^+$ T-cell responses and anti-tumor effects against subcutaneous tumors, surpassing those achieved by checkpoint inhibitors. Moreover, combining anti-CD4 with anti-PD-1/PD-L1 antibodies produced robust synergistic effects, achieving complete tumor regression with immune memory in several cancer models (Cancer Immunol Res. 2015). TCR repertoire analysis of tumor tissues and draining lymph nodes (dLNs) revealed that anti-CD4 and anti-PD-L1 treatments induced expansion of diverse tumor-reactive clones with a progenitor-exhausted phenotype (“clonal spreading”) in an $Fscn1^+$ mature regulatory DC–dependent manner (Front Immunol. 2019; Cancer Immunol Res. 2023). Based on these findings, we are now conducting clinical development of the humanized anti-CD4 antibody IT1208 (J Immunother Cancer 2019; Cancer Immunol Res. 2021).

To better characterize tumor-reactive T cells induced during clonal spreading, we sought to identify their defining surface markers. Using Fucci transgenic mice and multi-site tumor models, we tracked $CD8^+$ T-cell clones across dLNs and tumors by integrating bulk and single-cell TCR/RNA/CITE-seq analyses. We found that clones proliferating in dLNs subsequently expanded in tumors and mediated potent anti-

tumor immunity. CLEC12A and CD200 were identified as novel surface markers that efficiently enrich tumor-reactive clones in dLNs, outperforming conventional CD44^{hi} or PD-1^{+}Ly108^{+} Tpex markers. Importantly, these markers also enriched tumor-reactive T cells in human oral cancer–draining lymph nodes, highlighting their translational relevance. These findings provide valuable tools for monitoring immune responses and identifying tumor-reactive clones in both preclinical and clinical settings (in submission).

Although clonal spreading mobilizes diverse tumor-reactive clones, it remained unclear whether these clones follow uniform or heterogeneous dynamics of expansion and contraction within tumors. To address this, we developed a multi-site tumor model that enables temporal tracking of hundreds of CD8^{+} T-cell clones within individual mice. By integrating clonal expansion kinetics with single-cell transcriptomic data, we identified a specific gene module—termed the "expansion signature"—that predicts the proliferative potential of individual clones. This signature was enriched in PD-1^{+}Ly108^{+} progenitor-exhausted cells and strongly correlated with intratumoral clonal expansion and favorable responses to PD-1 blockade therapy. Expression of the signature during treatment was associated with durable anti-tumor immunity, whereas its decline preceded clonal contraction. Thus, this "pan-immunotherapy" signature provides a powerful framework for monitoring clonal dynamics and therapeutic efficacy across diverse immunotherapy modalities (Nat Commun 2025).

Collaborators:

Satoshi Ueha

Research on chemokine receptors and signaling molecules

Chemotaxis of immune cells is the movement of immune cells infiltrating into the tissue and accumulating in the inflamed region upon stimulation by chemotactic factors such as chemokines and cytokines that are secreted during inflammation. The chemotaxis is an essential immune reaction that protects our body, on the other hand, deeply involved in the pathogenesis of cancer, viral infection, choroidal neovascularization and inflammatory diseases, including atopic dermatitis and graft-versus-host disease (J Clin Invest.1999, Nature Immunology. 2003, Proc Nat Acad Sci USA. 2007, PLoS ONE. 2016). Through research on the molecular mechanisms of chemokine signals, we identified a novel chemokine receptor-associated molecule "FROUNT" (Terashima et al. Nature Immunology. 2005). We are investigating new drug agents to treat cancer and inflammatory diseases, by focusing on the molecular mechanisms of chemokine signals by the FROUNT (Terashima et al. J Immunology. 2009, Biochem. J. 2014, Nature Communications. 2020). Recently, we have demonstrated that FROUNT is also involved in inflammatory cytokine production and activation other than chemotaxis and have reported its involvement in the pathogenesis of crescentic glomerulonephritis and anxiety disorders (Kidney International. 2022, Frontiers in Pharmacology. 2022, Int J Mol Sci. 2023, Sci Rep. 2024, Pharmacol Pharm Sci. 2025). We have developed a novel FROUNT inhibitor, FN-01, and have initiated research aimed at its practical application in the treatment of intractable diseases.

Collaborators:

Yuya Terashima

Publications

Kouji Matsushima, M.D., Ph.D.

1. Kurokawa M, Goya T, Kohjima M, Tanaka M, Iwabuchi S, Shichino S, Ueha S, Hioki T, Aoyagi T, Takahashi M, Imoto K, Tashiro S, Suzuki H, Kato M, Hashimoto S, Matsuda H, Matsushima K, Ogawa Y. Microcirculatory

disturbance in acute liver injury is triggered by IFNγ-CD40 axis. J Inflamm (Lond). 221(1):23, 2024.
2. Taketomi Y, Higashi T, Kano K, Miki Y, Mochizuki C, Toyoshima S, Okayama Y, Nishito Y, Nakae S, Tanaka S, Tokuoka SM, Oda Y, Shichino S, Ueha S, Matsushima K, Akahoshi N, Ishii S, Chun J, Aoki J, Murakami M. Lipid-orchestrated paracrine circuit coordinates mast cell maturation and anaphylaxis through functional interaction with fibroblasts. Immunity. 57(8):1828-1847, 2024.
3. Okabe Y, Toda E, Urushiyama H, Terashima Y, Kunugi S, Kajimoto Y, Terasaki M, Matsushima K, Saito A, Yamauchi Y, Nagase T, Shimizu A, Terasaki Y. Antifibrotic effect of disulfiram on bleomycin-induced lung fibrosis in mice and its impact on macrophage infiltration. Sci Rep. 14(1):23653, 2024.