PROGRESS REPORT
Division of Molecular Regulation of Inflammatory and Immune Diseases

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Members (Matsushima's lab.)

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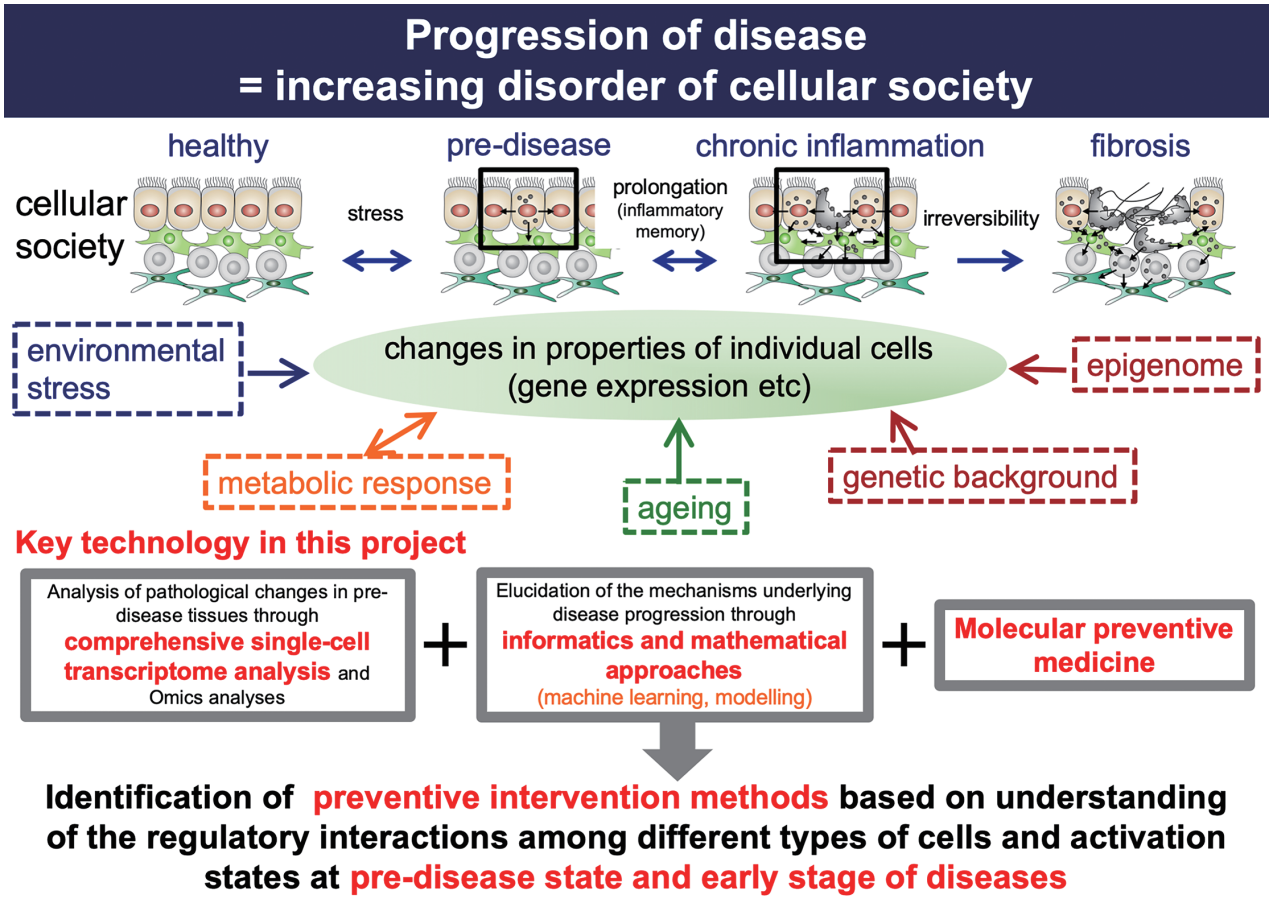
Division of Molecular Regulation of Inflammatory and Immune Diseases

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). TAS-Seq outperformed than other major scRNA-seq technologies in terms of gene sensitivity, accuracy of the detection of cell population abundancy, and sensitivity of cell-cell interaction. 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 developed an updated version of TAS-Seq, TAS-Seq2 of which gene-detection sensitivity and its utility were enhanced. TAS-Seq2 not only could apply to nanowell-based system (e.g. BD Rhapsody), but also to droplet-based system (e.g. 10X Chromium) and plate-based system (e.g. Smart-seq2). TAS-Seq2 could detect 1.5-2 times more genes than original 10X Chroimum v3 (10X TAS-Seq2), than TAS-Seq (BD Rhapsody) in mouse spleen and human frozen PBMC samples. We are now developing TAS-Seq2 for high-resolution spatial transcriptomics Stereo-seq, for the analysis of fixed cells, and increasing its cell throughput by 10-100 times. 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. We also established ex vivo organoid system that recapitulate the responses of bleomycin-induced lung injury, and found that the ex vivo system could also induce human IPF-related cell subsets that could not be observed in murine models. 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-Seq2 analysis and accelerates the research of various inflammatory diseases.

Collaborators:

Shigeyuki Shichino, Satoshi Ueha

Research on Hepatitis B virus infection

Infection by hepatitis B virus (HBV) causes chronic hepatitis. WHO estimates that 240 million people suffer from chronic HBV hepatitis, and 680 thousand people die from cirrhosis and hepatic cancer induced by chronic HBV infection. Currently, HBV vaccine can be used to prevent HBV infection, and anti-viral agents such as Interferon and Entecavir are used for the prevention of progression of chronic HBV hepatitis to HBV cirrhosis and hepatic cancer. However, elimination and eradication of HBV in patients with chronic HBV hepatitis have not been achieved. Moreover, in patients who achieved successful suppression of HBV by using anti-viral agents, cessation of the anti-viral agents causes viral replication and relapse in most cases. HBV cccDNA, which cannot easily be eradicated with anti-viral agents, are thought to be responsible for these difficulties underlying the treatment of HBV infection. Therefore, discoveries of new drug agents targeting cccDNA are warranted. In this research project, we aim to investigate low molecular compounds that eradicate HBV cccDNA by multi-step drug screening.

Collaborators:
Chandrasekar Balachandran

Research on combination cancer immunotherapy

Recent advances in the use of immune-checkpoint inhibitory antibodies, such as CTLA-4 and PD-1 antibodies, have brought a huge impact on cancer treatment. We found that the depletion of CD4+ cells, including regulatory T cells, by using anti-CD4 antibodies could induce tumor-specific CD8+ T cell response and anti-tumor effects against subcutaneous tumors, even better than immune-checkpoint inhibitory antibodies. Moreover, the combination of anti-CD4 antibody and anti-PD-1/PDL-1 antibody showed robust synergistic effects on anti-tumor responses, achieving complete cancer regression with immune memory in several cancer models (Ueha et al. Cancer Immunol Res. 2015). TCR repertoire analysis of the tumor tissue and draining lymph node (dLN) revealed that anti-CD4 and anti-PD-L1 treatment induced the expansion of diverse tumor-reactive clones with progenitor-exhausted phenotype ("clonal spreading") in an Fscn1^{+} mature regulatory DC-dependent manner. (Front Immunol. 2019, Cancer Immunol Research 2023). Based on these findings, we are now in clinical development of humanized anti-CD4 antibody IT1208 (Shitara et al. J Immunother Cancer. 2019, Cancer Immunology Research 2021). Using a bilateral tumor model that allows tracking of CD8^{+} T cell clones in an individual mouse, we demonstrated that a metabolically active subpopulation of progenitor-exhausted T cells maintained clonal longevity and mediated intratumoral T cell expansion rates. TCR repertoire analysis of the cell-cycle reporter mice revealed the contribution of T cell clones proliferating in the dLN to anti-tumor responses. We found that the anti-tumor effects of alarmin HMGN1, which enhances CD8^{+} T-cell responses through activation of DCs, is strongly associated with the DNA-binding capacity of HMGN1, while being independent of TLRs and STING which have been implicated in previous studies.

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).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.

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8. Motomura K, Matsuzaka T, Shichino S, Ogawa T, Pan H, Nakajima T, Asano Y, Okayama T, Takeuchi T, Ohno H, Han SI, Miyamoto T, Takeuchi Y, Sekiya M, Sone H, Yahagi N, Nakagawa Y, Oda T, Ueha S, Ikeo K, Ogura A, Matsushima K, Shimano H. Single-cell transcriptome profiling of pancreatic islets from early diabetic mice identifies Anxa10 for Ca2+ allostasis toward β-cell failure. Diabetes. 73(1):75-92, 2024.
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