In vivo multi-photon molecular imaging reveals inflammatory immune cell cross-talks in adult common diseases

Satoshi Nishimura

(1) Department of Cardiovascular Medicine, (2) Translational Systems Biology and Medicine Initiative, The University of Tokyo, Tokyo, Japan
snishi-tky@umin.ac.jp
Keywords: multi-photon, in vivo imaging, inflammation, metabolic syndrome, thrombus

Metabolic syndrome is a major risk factor of cardiovascular events, and adipose tissue remodeling based on chronic inflammation play a central role in obesity. To assess dynamic inflammatory cellular interplay between multiple cell-types in adult common diseases, including metabolic syndrome, obesity, and cardiovascular diseases, “in vivo molecular imaging technique” based on single- and multi-photon microscopy was developed. We first applied this technique to metabolic disease field. Our imaging visualized precise adipose tissue structures, and revealed close spatial and temporal interrelationships between angiogenesis and adipogenesis in obese adipose tissue (Figure A) [1]. In addition, increased leukocyte- platelet- endothelial cell interactions in the microcirculation of obese adipose were observed, a hallmark of inflammation [2]. Inflammatory cell kinetics, such as rolling, adhesion and extravasation was observed, clearly indicating the inflammatory status of obese adipose tissue. We also found that large numbers of CD8+ effector T cells infiltrated into obese adipose [3]. Infiltration by CD8+ T cells is essential for the initiation and development of adipose inflammation, showing that the CD8+ T cells are the key regulator of obese adipose tissue inflammation. These CD8+ T cells contribute to macrophage recruitment and adipose tissue inflammation associated with obesity. In addition, we recently found that B cells are major component of stromal vascular fraction of adipose tissue, and we clarified their function in vivo. These B cells secrete high amount of IL-10 in unstimulated conditions, and have new phenotype with anti-inflammatory properties against diet-induced-obesity inflammation. These B cells survive and secrete IL-10 under the stimulation of fatty acid, through TLR4/Myd88 pathways. Genetic and immunological B cell depletion ameliorated the adipose tissue inflammation and insulin resistance in obesity, indicating that these B cells can be the new therapeutic target against diabetic and obese conditions.

Our imaging also visualized multiple cell types specifically in real time and in vivo settings (Figure B), and single platelet kinetics can be visualized by this technique. We applied this technique to another field, cardiovascular diseases, to elucidate the multi-cellular process of developing thrombus in vivo. As for the thrombus formation, inflammations are recently considered to play a central role in pathogenesis of cardiovascular diseases, and contribute to the thrombotic response via cellular and humoral modulations. However, the direct contribution to thrombosis formation in vivo was not clear due to the lack of a real-time visualization technique of developing thrombus. Recently, we developed new animal models of developing thrombus by modifying in vivo imaging technique, and we visualized single platelet kinetics in developing thrombus (Figure C) [4].

In our models, discoid platelet aggregation on intact endothelium was triggered by ROS production within vessels, where such ROS signal was maintained with inflammatory

cytokines including TNF-alpha or IL-1. Pharmacological examination and kinetic analysis of thrombi revealed that initiation was regulated by the complex of GPIb-alpha with exposed von Willebrand factor through TNF-alpha/TNF-R1 axis in ECs. Moreover, integrin activation through and the actin cytoskeleton organization through Rac1 signaling is required for thrombus stability in late phase.

Using this technique, we found Lnk (an adapter protein) contribute to stabilization of developing thrombus in vivo via out-side-in integrin signaling [4]. In addition, we have succeeded in efficient production of human iPS-derived platelets by regulating the c-Myc reactivation process [5]. And to elucidate the clinical usefulness of these artificial platelets, we visualized that produced platelets have ability of thrombus formation in vivo [5].

Our imaging technique can be applied to bone marrows to visualize immune and stem cell functions during bone marrow reconstruction processes (Figure D). We have succeeded in visualizing stem cells, T cells, myeloid cells, megakaryocytes, platelets etc. in bone marrow with high time and spatial resolution using multi-photon in vivo molecular imaging technique. We found T cell proliferations have pivotal roles during re-constructive process of bone marrows after transplantations, closely related to haemtopoietic stem cell differentiation in niches.

Our results clearly demonstrated the power of our imaging technique to analyze complex cellular interplays in inflammatory diseases: metabolic syndrome, thrombus formation, and bone marrow reconstructive processes. Our imaging elucidate the parenchymal and stromal cell cross talks in inflammatory diseased conditions. This imaging might contribute to developing new therapeutic interventions against them.

References

1. Nishimura S, et al. Diabetes. (2007),56:1517.
2. Nishimura S, et al. J Clin Invest. (2008), 118: 710
3. Nishimura S, et al. Nature Medicine, (2009), 5;8:914.
4. Nishimura S, et al. J Clin Invest, (2010), 120(1): 179-190.
5. Takayama N & Nishimura S et al, J Exp Med. (2010), 207(13):2817-2830

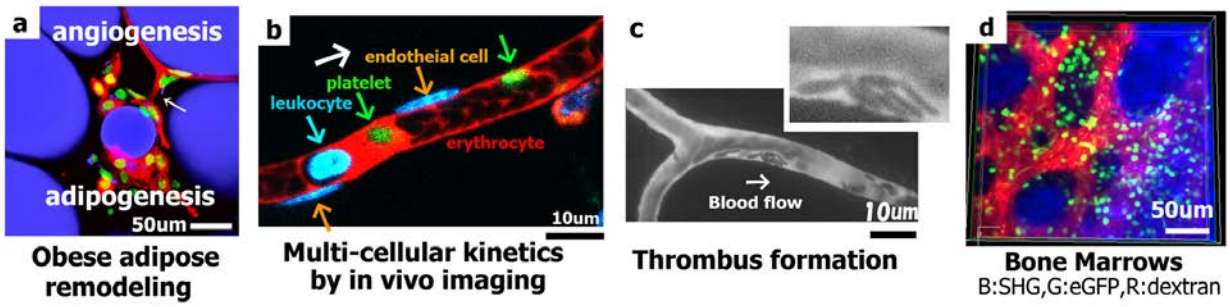

Figure. In vivo imaging visualizing inflammatory cell dynamics in diseased conditions. (a) Adipose tissue remodeling in obesity including adipogenesis and angiogenesis (b) Multi-cellular kinetics visualized by novel in vivo imaging technique (c) Thrombus formation and single platelet kinetics revealed by in vivo imaging (d) T cell proliferations in bone marrows after transplantation