論文の内容の要旨

論文題目　Transcriptional regulation of leukotriene B_4 type-1 receptor in leukocytes

和訳　ロイコトリエン B_4 第一受容体の白血球における転写調節

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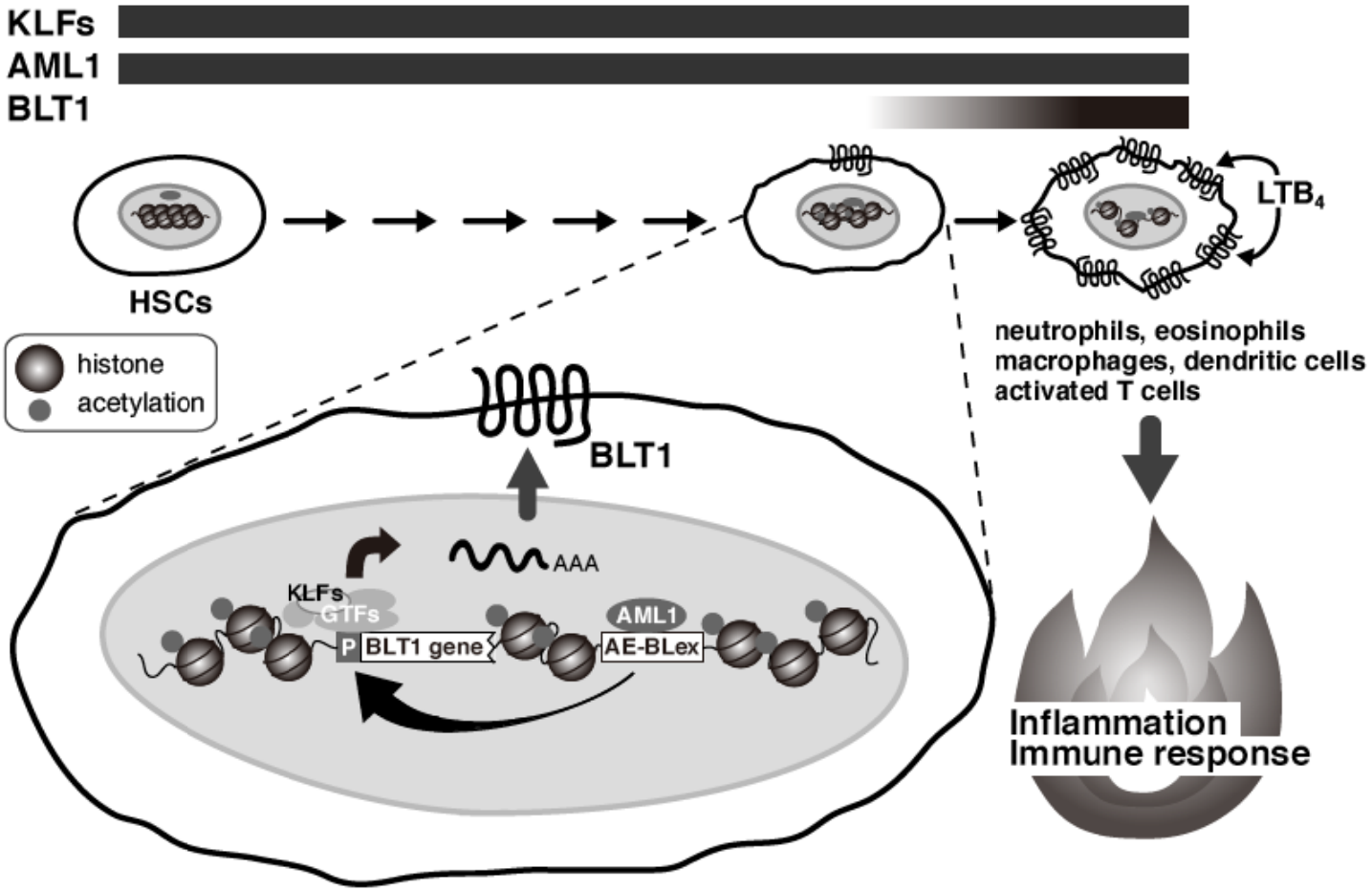
Leukotriene B_4 (LTB_4) is a lipid mediator with potent chemoattractant properties and rapidly generated from activated innate immune cells such as neutrophils, macrophages, and mast cells. Previously, our laboratory identified two types of LTB_4 receptors, which are LTB_4 type-1 receptor (BLT1) and type-2 receptor (BLT2). Several groups have certified the pivotal roles of BLT1 in peripheral leukocytes. BLT1-deficient mice have revealed that LTB_4-BLT1 pathway appears to play important roles in various inflammatory diseases, such as allergen-induced airway hyperresponsiveness, autoimmune arthritis and atherosclerosis. Although BLT1 is exclusively expressed in peripheral leukocytes, suggesting the stringent

regulation of its transcription, the precise mechanism of the BLT1 expression is not fully understood. In this thesis, I present two findings of the transcriptional regulation in human promyelocytic leukemia cell line (HL-60 cells), in which the BLT1 expression is upregulated by stimulation with retinoic acid (RA).

First, I analyzed a core-promoter contributing to the BLT1 expression. The core-promoter of BLT1 possesses a crucial site (GC box) for the recognition of specificity protein 1 (Sp1). Interestingly, western blot analyses and electrophoretic mobility shift assays revealed that Sp1 is not produced in HL-60 cells, and that other proteins which belong to Kruppel-like factor (KLF) family could bind to this site. It has been reported that KLF1, KLF2 and KLF3 are specifically expressed in hematopoietic cells. I confirmed the expressions of these factors in HL-60 cells by PCR and western blot analyses, and examined the abilities of these factors for the activation of the BLT1 promoter by reporter assays. Thus, some KLFs, *e.g*., KLF1, can work as regulators for the BLT1 expression in HL-60 cells.

The core-promoter activity is not enhanced directly by the stimulation with RA in HL-60 cells, indicating the possible existence of enhancer region(s) at other loci. Therefore, I searched for the region involving in the enhanced expression of BLT1 in my second theme. DNase I-hypersensitivity analyses revealed an activated region in the RA-stimulated cells,

termed AE-BLex, at the intron-I:exon-II boundary of the BLT1 gene. By the reporter assays, I elucidated that AE-BLex acts as an enhancer for the BLT1 promoter. AE-BLex possesses two acute myeloid leukemia 1 (AML1/Runx1) recognition sites. These sites are pivotal for the enhancer function because the BLT1 expression was impaired by the knockdown of AML1 in HL-60 cells. The enhancement of the BLT1 expression during the RA-induced differentiation of HL-60 cells is due to a loosening of the chromatin structure around AE-BLex, which leads to the incremental binding of AML1. The AML1/AE-BLex complex was further confirmed in other BLT1-expressing leukemia cell lines and human peripheral leukocytes. Thus, AML1 enhances the BLT1 expression through binding to AE-BLex, which is accessible in leukocytes. This thesis shows a part of the regulation of the BLT1 expression in HL-60 cells, and may be helpful to understand the transcriptional mechanisms in leukocytes.



Schematic model of BLT1 expression in leukocytes

The gradation of each bar indicates the expression levels of KLFs, AML1 and BLT1 during hematopoiesis. In HSCs (hematopoietic stem cells), the BLT1 gene is silenced due to inactivation of the BLT1 promoter and AE-BLex. The chromatin structures of the BLT1 promoter and AE-BLex are altered by histone acetylation during the terminal differentiation. GTFs containing KLFs are recruited to the BLT1 promoter. Following recruitment to AE-BLex, AML1 facilitates the activity of the BLT1 promoter. Upregulated BLT1 expression is crucial for inflammation and the immune response in leukocytes (e.g., neutrophils, eosinophils, activated T-cells, dendritic cells and macrophages). GTFs; general transcription factors, P; BLT1 promoter.