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研究課題名（和文）Effects of Runx1-associated lncRNA on asthma pathogenesis

研究課題名（英文）Effects of Runx1-associated lncRNA on asthma pathogenesis

研究代表者

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研究成果の概要（和文）：Runx1欠損マウスはヒト喘息疾患様の気道炎症が自然発症するが、その機序は分かっていない。遺伝子座の分析結果、Runx1遺伝子上流に存在するロングノンコーディングRNA（long non-coding RNA（lncRNA））を発見した。Runx1-lncRNAは発現量が非常に少ないが、CD4T細胞特異的な発現を示し、遺伝子ノックダウンにより既知のRunx1標的遺伝子の発現異常が認められた。Runx1-lncRNA欠損マウスはRunx1欠損マウス様な喘息様疾患が発症することが明らかになった。この研究で「Runx1-lncRNAがRunx1の作用に影響を与えると考えられる」と結論される。

研究成果の概要（英文）：Runx1 knockout mice spontaneously develop lung inflammation resembling human asthma; however, its mechanism is not known yet. Examination of Runx1 locus shows a long non-coding RNA (lncRNA) located near Runx1 gene. This novel lncRNA shows a very low expression, but it is restricted to helper T lymphocytes. Knockdown of Runx1-lncRNA shows differential expressions of Runx1 target genes. Also, Runx1-lncRNA knockout mouse develops lung pathologies similar to Runx1 knockout mice. Therefore, we conclude that Runx1-lncRNA is functionally related to Runx1 protein.

研究分野：　医歯薬学

キーワード：　CD4T細胞　Runx1転写因子　long non-coding RNA　ヒト喘息疾患発症

１．研究開始当初の背景

It has been well documented that Runx1 transcription factor plays crucial roles in many developmental processes including hematopoiesis. Interestingly, Runx1 knockout mice also suffer from numerous autoimmune diseases such as asthma-like lung inflammation in addition to well-known defects in hematopoiesis. Indeed, our laboratory found an evidence that Runx1 protein might be directly related to immune responses in addition to blood cell differentiation since our data showed that helper T cells of Runx1-deficient mice produced more IL-4 (one of the critical cytokines for inflammatory responses) compared to wild-type control mice indicating that Runx1 regulates IL-4 expression and that the absence of Runx1 results in the abnormal de-repression of IL-4.

To further investigate how Runx1 might directly regulate immune responses, I took a bioinformatics approach. To this end, I examined Runx1 locus using public databases of mouse genomics, and found a novel long non-coding RNA (lncRNA) encoded near Runx1 gene. Therefore, it was of interest to examine whether this Runx1-lncRNA help and/or cooperate with Runx1 protein for proper function of Runx1 in the regulation of immune responses.

２．研究の目的

The main purpose of this study was to test the hypothesis that Runx1-lncRNA is functionally associated with Runx1 protein. This was based on the fact that many lncRNAs have been shown to interact with the proteins encoded by nearby genes and the fact that Runx1 protein and Runx1-lncRNA are indeed located side by side.

To prove such a functional relationship, first I had to characterize Runx1-lncRNA biochemically in terms of expression patterns and genomic structures. Obtained expression and structural information would be used to further examine its potential function by using knockdown systems in vitro and by generating knockout mice. The phenotypes observed by these functional experiments would be compared to Runx1 knockout mice to confirm the hypothesis.

３．研究の方法

The very first objective of this study was the biochemical characterization of Runx1-lncRNA. I planned to biochemically analyze Runx1-lncRNA by real-time PCRs from various tissues of mice including lymphocytes such as T and B cells. Secondly I planned to examine the potential functions of Runx1-lncRNA by performing siRNA-mediated knockdown experiments. Specifically, I could knockdown Runx1-lncRNA from activated helper T cells and monitor changes in cytokine levels such as IL-4. Basically I would survey whether an absence of Runx1-lncRNA has similar effects to phenotypes of Runx1-deficient cells.

If above approaches led to positive outcomes, I would generate a knockout mouse line by using ES (embryonic stem) cell technologies already established in our laboratory in which Runx1-lcnRNA is genetically removed from mouse. This would help us to study function of Runx1-lncRNA in more physiological settings.

Knockout mice for Runx1-lncRNA would be analyzed by many ways. First the mice would be examined for developmental defects since Runx1-deficient mice show numerous developmental defects such as proper differentiation of CD4+ helper and CD8+ killer T cells. Also, activated helper T cells will be examined for expression of IL-4 to confirm the results from in vitro knockdown assays. Then the mouse would be kept for 3-5 months and examined for diseases since Runx1-deficient mice develop asthma-like symptoms from 3-5 month old.

４．研究成果

The first aim of this study was the characterization of Runx1-lncRNA. To examine expression patterns of Runx1-lncRNA throughout various tissues, I performed quantitative real-time PCR using numerous cell types including T cell, B cells and myeloid cells. And it was found that Runx1-lncRNA is expressed at a very low levels in all cell types examined which is not uncommon for lncRNAs. However, Runx1-lncRNA also lacks introns; so that it was very difficult to differentiate real Runx1-lncRNA transcripts from genomic DNA contamination or transcriptional noises. After numerous

attempts to define expression patterns of Runx1-lncRNA, it was concluded that Runx1-lncRNA is indeed expressed by helper T lymphocytes, but the expression levels were very low and unstable.

The second aim of the study was to find out potential functions of Runx1-lncRNA. Since Runx1-lncRNA is the closest neighboring gene to Runx1, we hypothesized that Runx1-lncRNA might function similarly to Runx1 proteins. To this end, we knock-downed Runx1-lncRNA from activated helper T cells by siRNA transduction in vitro. Since Runx1-lncRNA is not expressed abundantly, it was relatively simple to remove most of Runx1-lncRNA from activated helper T cells. After knockdown of Runx1-lncRNA, the cells were examined for potential phenotypes by real-time PCR and flow cytometry. Then it was found that knockdown of Runx1-lncRNA resulted in increased levels of IL-4 (3-4 folds) which is one of the most prominent phenotypes of Runx1-deficient helper T cells. Therefore, it seemed that Runx1-lncRNA is indeed necessary for Runx1 to regulate IL-4 as we hypothesized.

These promising preliminary data prompted us to generate a mouse knockout (KO) line in which Runx1-lncRNA is genetically removed to gain detailed insights into the physiological functions of Runx1-lncRNA. The generated Runx1-lncRNA knockout mice were successfully born, and they showed normal development and remained healthy. To confirm the results of in vitro studies, I harvested helper T cells and examined IL-4 levels after activation, and found that Runx1-lncRNA-deficient helper T cells indeed showed around 2-fold increases in IL-4 expression compared to wild-type control mice. However, the degrees of the increase was smaller than in vitro knockdown studies.

It was of great interest to find out whether Runx1-lncRNA KO mouse eventually develop asthma-like symptoms due to increased levels of secreted IL-4 similarly to Runx1-deficient mouse. Therefore, I examined Runx1-lncRNA KO mouse at the later stages (age of 3-5 months) by histologies because Runx1-deficient mice develop asthma-like symptoms from the age of 3 months. Indeed I found that these Runx1-lncRNA KO mice develop spontaneous infiltration of leukocytes in lungs and airways which exactly resemble the pathologies of both Runx1 KO mouse and human asthma.

Combined together, it was concluded that Runx1-lncRNA might function together with Runx1 protein to regulate expression of IL-4 as we hypothesized and that the absence of either Runx1 or Runx1-lncRNA results in spontaneous inflammation in lungs.

Recent development of next-generation sequencing has been generating tremendous amounts of genomic data. Therefore, I revisited mouse genomic databases for recently uploaded information and found that Runx1-lncRNA locus might also contain a potential enhancer element near Runx1-lncRNA. Therefore, it was possible that Runx1-lncRNA KO mouse might have lost both Runx1-lncRNA gene and a potential transcriptional enhancer. In other words, it is not possible to exclude a possibility that phenotypes observed in Runx1-lncRNA KO mouse resulted from the knockout of the potential enhancer. To resolve this important issue, further studies are necessary to dissect the contributions by Runx1-lncRNA and the observed enhancer sequence in the regulation of Runx1-mediated IL-4 regulation.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　0　件）

〔学会発表〕（計　5　件）

1. Fine-tuning of Runx1 function by a lncRNA, The 43th Annual Japanese Society of Immunology Meeting, Kyoto, Japan, 2014-12-13

2. Modulation of Runx complex function by a lncRNA, Kyoto T cell conference, Kyoto, Japan, 2014-05-17

3. Runx-mediated regulation of CC chemokine expression, The 42th Annual Japanese Society of Immunology Meeting, Chiba, Japan, 2013-12-12

4. Runx-mediated immunoregulation, The 3rd CSI-JSI-KAI Joint Symposium on Immunology, Po-hang City, Korea, 2013-12-02

5. Rediscovering Runx transcription factors as modulators of immune homeostasis, Cytokines 2013, San Francisco, USA, 2013-10-03

〔図書〕（計　0　件）

〔産業財産権〕
○出願状況（計　0　件）
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名称：
発明者：
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〔その他〕
ホームページ等

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