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Genes involved in leukotriene synthesis pathway are dynamically regulated during lung development in Rhesus monkeys

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ABSTRACT

Background: Leukotrienes play critical roles in many inflammatory lung diseases and several antagonists of their receptors have been used in the clinical settings. However, the physiological functions of leukotrienes in lung development are still unclear.

Method: The expression levels of 34 genes involved in leukotriene synthesis and function pathway in the lungs of Rhesus monkey during different developmental time points were determined on a MiSeq platform and analyzed by the reads per kilobase of transcript per million mapped reads (RPKM) method.

Results: The results showed that the expression levels of *PLA2G1B, PLA2G10, PLA2G2D, ALOX5*, and *ALOX5AP* increased dramatically in the lung of Rhesus monkey, reflecting the changes in the pulmonary environment after delivery. Additionally, the different expression patterns between molecules related to LTB4 and LTC4 synthesis suggested distinct roles of LTB4 and LTC4 in lung development. Finally, the constant expression of CysLT1 during the development process provided new information to the pharmaceutical basis of the use of leukotriene receptor antagonists in the clinical setting.

Conclusion: The expression levels of several key genes involved in leukotriene synthesis changed dramatically during lung development in Rhesus monkeys, suggesting the potential roles of leukotrienes in lung development in this animal model.

1. Introduction

The lung is an important organ for gas exchange in terrestrial organisms, including humans. Lung development is a finely orchestrated and complicated process regulated by the interactions of many signal pathways [1]. Many studies have shown that the same genetic networks function in both lung organogenesis and pulmonary disease pathogenesis [2], highlighting the importance of elucidating functional signaling

Abbreviations: RPKM, reads per kilobase of transcript per million mapped reads; FGF, fibroblast growth factor; ETS, E26 transformation-specific or E-twenty-six; LTs, leukotrienes; ALOX5, arachidonate 5-lipoxygenase; LTA4, leukotriene A4; LTB4, leukotriene B4; LTC4, leukotriene C4; LTD4, leukotriene D4; LTE4, leukotriene E4; CysLTs, cysteinyl leukotrienes; PLA2, phospholipase A2; ALOX5AP, arachidonate 5-lipoxygenase activating protein; LTA4H, leukotriene A4 hydrolase; LTC4S, leukotriene C4 synthase; GGT, γ-glutamyl transpeptidase; DPEP, dipeptidase

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pathways. These signaling pathways participate in almost all metabolic processes in organisms and are related to disease states. The main functions of in vivo signaling pathways include the regulation of physiological processes and reaction with stimuli in the external environment in order to maintain homeostasis. However, the extent of signaling pathway activity is also important. Both hyperactivation and insufficient activation of different pathways can cause various diseases. Various transcription factors, such as fibroblast growth factor (FGF) [3], thyroid transcription factor-1 [4], β-catenin, forkhead box, SRY-related HMG-box, and E26 transformation-specific or E-twenty-six (ETS) [5], play key roles in lung development. However, identification of novel signaling pathways involved in lung development is critical.

Leukotrienes (LTs), a type of lipidic biological signal molecules, are synthesized from arachidonic acid by arachidonate 5-lipoxygenase (ALOX5) and multiple enzymes. LTs are expressed at high levels in response to inflammatory stimuli [6]. LTs, particularly the arachidonic acid/5-hydroperoxyeicosatetraenoic acid/ leukotriene A4 (LTA4)/ leukotriene C4(LTC4)/ leukotriene D4(LTD4)/ leukotriene E4(LTE4) signaling pathway, participate in various allergic lung diseases, such as asthma, chronic obstructive pulmonary disease, obliterative bronchiolitis after lung transplantation, and interstitial lung diseases [7]. The inhibition of LT pathways by antagonists of the membrane receptor of cysteinyl leukotrienes (CysLTR1) could alleviate clinical symptoms [8]. Most previous studies have focused on changes in the LT pathway and the mechanisms of CysLTR1 antagonists in the context of inflammation.

Notably, however, the use of CysLTR1 antagonists is restricted to patients who are older than 2 years of age in China. The reason for this restriction is still not clear, and the expression patterns of LT receptors and other key molecules in these pathways have not been elucidated. Thus, additional studies are needed to improve rational drug use and elucidate the physiological functions of LTs. Therefore, in this study, we evaluated the expression patterns of key molecules in the LT synthesis pathway in the lungs of Rhesus monkeys during lung development.

2. Materials and methods

2.1. Theory

LTs play an important role in various allergic diseases. However, the physiological functions of LTs are still unclear. The objective of this study was to elucidate the expression patterns of LT receptors and other key molecules in the LT synthesis pathway during fetal, neonatal, and youth periods in Rhesus monkeys. Knowledge of the expression pattern of these genes will shed light on the physiological functions of LTs and the use of LT-related drugs in the clinical setting.

2.2. Design

The evolutionary distance between Rhesus monkeys and humans is approximately 25 million years, which is closer than that between mice and humans (about 95-70 million years). Therefore, Rhesus monkeys were chosen as the experimental animals in this study. Rhesus monkeys have a life span of about 30 years and a gestation period of about 165 days. Based on the last menstruation after estrus, samples were collected during different developmental stages, including the pregnant period (45 days, n = 1; 70 days, n = 1; 100 days, n = 3; 137 days, n = 1; 157 days, n = 1; and 163 days, n = 1), neonatal period (4 days after birth, n = 1; 5 days after birth, n = 1; and 7 days after birth, n = 1), and youth period (5 years after birth, n = 2; and 7 years after birth, n = 1). All animal experiments complied with ARRIVE guidelines and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The study was approved by the Ethics Committee of Sichuan University.

Animals were anesthetized by pentobarbital sodium at the indicated times. Fetuses were removed by standard surgical methods. The lungs were isolated, washed with phosphate-buffered saline (PBS), and stored in liquid nitrogen immediately. For newborn and adult monkeys, the lung samples were obtained by biopsies in the same region. The obtained samples were washed with PBS and treated with TRIzol immediately.

2.3. Sequencing

Total RNA was isolated using TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The quality of total RNA was checked using a spectrophotometer and gel electrophoresis. cDNA was synthesized using a commercial kit (Takara, Dalian, China) with total RNA.

cDNA was sequenced on a MiSeq platform (Illumina, San Diego, CA, USA). The data were subjected to a BLAST search against the Rhesus monkey genome in the Ensembl database (http://www.ensembl.org/). The expression levels of target genes were normalized and calculated by the reads per kilobase of transcript per million mapped reads (RPKM) method.

2.4. Expression analysis

Expression levels of 34 key molecules in the LT synthesis pathway, including enzymes catalyzing the production of LTs and LT receptors, e.g., phospholipase A2 (PLA2), ALOX5, arachidonate 5-lipoxygenase activating protein (ALOX5AP), leukotriene A4 hydrolase (LTA4H), leukotriene C4 synthase (LTC4S), γ-glutamyl transpeptidase (GGT), dipeptidase (DPEP), LTB4R1, LTB4R2, CysLT1, and CysLT2, were evaluated. Their expression levels during different developmental periods were analyzed by unsupervised clustering and heatmap analysis.

2.5. Statistics

The heat map of gene expression profiles was drawn with the R package “pheatmap”. The differences in gene expression between the two groups were estimated by two-tailed *t*-tests, and results with p values less than 0.05 were considered significant.

3. Results

3.1. Molecules related to LTA4 synthesis

Molecules involved in LTA4 synthesis included members of the PLA2 family, *ALOX5*, and *ALOX5AP*. A total of 20 PLA2 family members were evaluated in this study (Fig. 1); of these, only *PLA2G1B*, *PLA2G15*, *PLA2G12A*, and *PLA2G4B* mRNAs were expressed constantly at a relatively high level (RPKM > 10) at all time points. The expression levels of *PLA2G6*, *PLA2G4C*, and *PLA2G16* were increased at late time points, whereas *PLA2G4A*, *PLA2G7*, and *PLA2G3* were expressed constantly (RPKM > 1) at all time points. *PLA2G10* and *PLA2G2D* were not expressed during gestation, but were observed during the newborn period. *PLA2G5*, *PLA2G2E*, *PLA2G2F*, *PLA2G12B*, *PLA2G4F*, *PLA2G4D*, and *PLA2G2C* were not expressed at any time point. The expression pattern of *PLA2G2A* was irregular. Additionally, the expression levels of *PLA2G1B*, *PLA2G10*, and *PLA2G2D* increased dramatically after delivery. Notably, all three of these lipases belong to the secretory PLA2 family, which liberate arachidonic acid. These results suggested that during pregnancy, arachidonic acid could be generated, and after delivery, more PLA2 may be needed for the liberation of arachidonic acid.

ALOX5 and *ALOX5AP* mRNAs were expressed at very low levels in the early and late fetal periods. Intriguingly, in newborns, the expression levels of both genes increased dramatically (Fig. 1). This elevation, together with the above results, indicated that the synthesis of LTA4 could change dramatically after birth.