Keratan sulfate disaccharide: specific targeting to langerin and possible applications to COPD

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(1) Introduction

In chronic obstructive pulmonary disease (COPD), chronic obstructive bronchitis leads to bronchial obstruction and respiratory failure that are not fully reversible. A variety of immune cells such as macrophages, neutrophils, T-lymphocytes, B-lymphocytes and dendritic cells are activated and accumulated in the bronchi. Here, we propose that the keratan sulfate (KS) disaccharide L4 and oligomeric derivatives thereof are potential mitigators of COPD. They suppress the expression of inflammatory cytokines through langerin in murine dendritic cells, which is one of the molecular mechanisms to modulate the COPD disease features in model mice.

There are still some unsolved questions, although we show the possibility to develop the novel L4-based therapy for the patients suffering from COPD.

(2) Keratan sulfate (KS) proteoglycan

Keratan sulfate (KS) is composed of repeating units comprised of LactoNac disaccharides, namely 2-O-galactosyl- and 4-O-acetyl glucosamine (GlcNAc). KS is now classified into three types, conical KS-I, skeletal KS-II and brain KS-III.

(3) COPD: chronic obstructive pulmonary disease

COPD is the third commonest cause of death in the world in 2019. Majority causes of COPD are tobacco smoke, secondhand smoke, air pollution and asthma.

Genetic predisposition: Loss of α1-antitrypsin

Chronic inflammation

Oxidative stress

Secretion of mucin

Activation of neutrophil and macrophage

Proteases

Airway obstruction

Emphysema

Bronchitis

(4) Reduced KS expression in COPD and smoked mouse lung

Immunohistochemical analysis reveals the reduction of KS expression in the lung of COPD patients and 3 month-smoked mouse lung. Red indicates KS.

(5) Langerin: a binding partner of L4

Langerin was identified as a C-type lectin receptor in 2000. Langerin expression is selective and highly enriched in Langerhans cells (LCs), a subset of dendritic cells (DCs). Among various candidates for sugar ligands of langerin, we identified L4.

Primary structure of mouse langerin

Type-II membrane protein

Cytoplasmic

Transmembrane

L4

KS

L4 polymer

ELISA

IC50: 3.5 mM

2.7 μM

2.1 nM

Langerin

L4

Triangle L4

L4 polymer

PPE

PBS

(6) L4 and L4 derivatives

Four types of sulfation pattern in KS disaccharide. L4 and L3 bind to langerin, indicating 6-O-sulfation group in galactose is essential to binding. Expecting to strengthen the binding, triangle L4 and L4 polymer are generated by chemical synthesis.

Sulfation types in KS disaccharide

L4

L3

L4 polymer

L4

Triangle L4

L4 polymer

(7) Binding affinity of L4 and L4 derivatives to recombiant langerin

Competitive enzyme-linked immunosorbent assay (ELISA) reveals that L4 binds to recombinant langerin with an IC50 value of 3.5 mM. L4 derivatives such as triangle L4 and L4 polymer shows much stronger binding than L4 monomer. The IC50 values indicate the concentration for 50% inhibition of L4 binding.

(8) L4 and L4 derivatives suppress inflammatory cytokine expression in BMDCs

qPCR analysis reveals the modulation of the mRNA expression of inflammatory cytokine (IL-6 and TNFα) and anti-inflammatory cytokine (IL-10) in mouse BMDCs (bone-marrow derived dendritic cells). BMDCs expressing langerin were incubated with L4 (100μg/mL) or L4 derivatives (100μg/mL) for 2 days.

(9) L4 prevents progression of emphysema in COPD mouse model

Experimental design for monitoring emphysema by micro-computed tomography (micro-CT). PPE: porcine pancreatic elastase.

(10) Histological analysis of the lung of COPD mouse model

Representative images of the hematoxylin and eosin-stained lung sections of COPD mouse model (day 21 after intratracheal spray of L4 and PPE). Scale bar, 200 μm. The mean linear intercept is reduced by L4 treatment.

(11) Summary and perspective

Oligomeric L4 binds to langerin stronger than L4. Langerin functions to suppress COPD symptoms by modulating cytokine expression. Structural analysis indicates that KS3 in CRD is important in recognition of langerin. Rab11 dependent endosomal recycling of langerin is also reported.

(12) Future plans

To develop novel L4-based therapy for COPD, more detailed molecular functions of langerin should be demonstrated.

- Clarify the downstream signaling of langerin
  - Identify the binding partners
  - Is langerin phosphorylated?
  - What is the function of proline-rich motif?
- Evaluate the effect in human COPD patients etc.

COI Disclosure Information

We have no financial relationships to disclose.