論 文 の 内 容 の 要 旨
Abstract

論文題目

Biochemical analysis for cell adhesion mechanism by LI-cadherin

（LI-cadherinによる細胞接着機構に関する生化学的解析）

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Chapter 1. Introduction

Liver Intestine-cadherin (LI-cadherin) is a member of the cadherin superfamily. Cadherins are a family of glycoproteins responsible for calcium ion-dependent cell adhesion [1]. In the human body, expression of LI-cadherin is observed in normal small intestine and colon cells [2], as well as in various cancer cells, such as gastric adenocarcinoma, colorectal cancer, and pancreatic cancer [2–5]. Previous studies indicated that LI-cadherin may have both positive and negative effects on cancer progression [5,6]. In the case of gastric cancer, LI-cadherin is considered promising for imaging because its expression is not observed in normal gastric cells but is expressed on gastric cancer cells at a higher rate than other gastric cancer markers, such as CDX2 and CK20 [2,7]. For these reasons, LI-cadherin has been focused as a target for cancer therapy and diagnosis.

Extracellular cadherin (EC) repeats of LI-cadherin have sequence homology with those of classical cadherins [8]. However, LI-cadherin exhibits distinct structural features compared to classical cadherins such as the number of EC repeats and the short cytoplasmic domain which does not require interaction with other proteins to achieve cell adhesion [9,10]. Although LI-cadherin is a promising target for treatment and diagnosis of cancer, a poor understanding of the molecular basis of LI-cadherin function has been an obstacle to designing molecules that exhibit the desired properties.

Therefore, in this study, I aimed to elucidate the mechanisms of LI-cadherin-dependent cell adhesion at the molecular level. In addition, I investigated the effect of anti-LI-cadherin antibodies on LI-cadherin-dependent cell adhesion, to evaluate the possibility of using such antibodies for the imaging and treatment of cancers expressing LI-cadherin. In particular, the knowledge obtained from the analysis of the cell adhesion mechanism influenced my investigations of the antagonistic or agonistic activity of each antibody in LI-cadherin-dependent cell adhesion. This information was also applied to elucidate the roles of LI-cadherin in cancer cells at the molecular level.

Chapter 2. Dimerization mechanism and structural features of human LI-cadherin

First, LI-cadherin was expressed as recombinant protein. Measurement of the dissociation constant (K_D) using analytical ultracentrifugation (AUC) revealed that LI-cadherin forms two types of homodimer: EC1-4 homodimer and EC1-2 homodimer. Crystal structure of LI-cadherin homodimer revealed the novel architecture of LI-cadherin EC1-4 homodimer, which is different from other cadherins' homodimer. Biochemical and computational analysis were performed to elucidate the detailed characteristics of the EC1-4 homodimer. Molecular dynamics simulation indicated that noncanonical Ca^{2+}-free linker between EC2 and EC3 is highly flexible when homodimer is not formed. Phe224 was identified as a critical residue for the homodimerization of EC1-4 through the analysis using size exclusion chromatography-multi angle light scattering (SEC-MALS) and differential scanning calorimetry (DSC). Cell-based assay has proved that Phe224 is also indispensable for LI-cadherin-dependent cell adhesion. Cell-based assays using truncated LI-cadherin suggested that formation of EC1-2 homodimer is not sufficient to maintain LI-cadherin-dependent cell adhesion.

These results indicated the unique molecular characteristics of LI-cadherin and suggested that LI-cadherin plays unique roles in human body.

Chapter 3. Analysis of how anti-LI-cadherin antibodies affect LI-cadherin-dependent cell adhesion

Epitope of each antibody was analyzed using hydrogen deuterium exchange mass spectrometry (HDX-MS) and isothermal titration calorimetry (ITC). Some types of IgG and Fab exhibited inhibitory effect on LI-cadherin-dependent cell adhesion whereas some others exhibited enhancement effect. The underlying mechanisms of inhibitory or enhancement activity of each antibody against LI-cadherin-dependent cell adhesion was described based on the knowledge obtained from the analysis on cell adhesion mechanism.

Although further investigation using clinical samples and *in vivo* experiments are necessary to validate the potency of each antibody, my study demonstrates the possibility of

regulating LI-cadherin function and performing stable imaging of cancer cells using anti-LI-cadherin antibodies.

Chapter 4. Molecular mechanism underlying the increased risk of colorectal cancer metastasis due to LI-cadherin gene single nucleotide polymorphisms (SNPs)

Two single nucleotide polymorphisms (SNPs) were found in the LI-cadherin gene [11]. The SNPs are responsible for the amino acid changes of Lys115 to Glu and Glu739 to Ala [11].

Homodimerization tendency of both EC1-4 and EC1-2 homodimer were decreased by the mutation of Lys115 to Glu. MD simulation revealed the partial conformation change of the molecule by the mutation of Lys115 to Glu. Cell-based assay showed the decrease of cell adhesion ability by the amino acid changes caused by the SNPs.

The results indicated that the amino acid change by SNPs decrease the homodimerization tendency of LI-cadherin, which is necessary for LI-cadherin-dependent cell adhesion. The risk of cancer metastasis seems to be increased by the weakened cell adhesion ability which enhances the migration of cancer cells from primary tumor. This study is the first to consider the role of LI-cadherin in cancer cells at the molecular level.

Chapter 5. Summary

As little was known regarding LI-cadherin at the molecular level prior to this study, I believe that the knowledge obtained from my research will advance our understanding of the roles of LI-cadherin in both normal and cancer cells, and will aid in the development of therapeutic and diagnostic molecules targeting LI-cadherin.

As future perspectives, I expect that analysis using additional anti-LI-cadherin antibodies would contribute not only to increasing the number of candidates with potential for clinical application, but would also deepen our understanding of the association state of LI-cadherin on the cell membrane.

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