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A Pilot Study of Antibody Drug Therapy to Regulate Cell Adhesion in Dental Implants

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Abstract

Dental implant therapy is a highly effective treatment for recovering occlusion after tooth loss. An important factor in the success of dental implants is establishing strong osseointegration. If more epithelial cells migrate to the implant-bone interface than mesenchymal stem cells, effective osseointegration may fail. Therefore, controlling epithelial cell adhesion and motility would be an effective strategy to increase the success rate of osseointegration. Laminin-332 is a major component of the basement membrane and is composed of three chains ($\alpha 3$, $\beta 3$ and $\gamma 2$). It is well-known that laminin-332 regulates cellular functions such as adhesion, proliferation, apoptosis and differentiation. These biological functions depend on changes in the structural arrangement of laminin-332 by proteolytic cleavage. It is well-known that cleavage of the $\alpha 3$ chain between its LG domains gives laminin-332 its biological function. In this study, we focused on LG domain cleavage and developed antibodies that target the LG domain cleavage site. We attempted to change the biological function of laminin-332 to control cell adhesion for the purpose of regulating dental implant therapy.

Keywords

Laminin-332, Monoclonal Antibody, Cleavage, Cell Adhesion

1. Introduction

Dental implant therapy is a key option for the recovery of occlusion following tooth loss [1] [2]. The success of dental implants is related to effective osseointegration [3] [4]. Strong osseointegration may be lost when epithelial cells invade the target sites earlier than mesenchymal stem cells [5] [6]. Therefore, the regulation of epithelial cells and mesenchymal stem cells could facilitate successful osseointegration in dental implant therapy.

Laminin-332 is an essential component of the basement membrane [7] [8]. Laminin-332 influences cell behavior by interacting with cell surface receptors and regulating cell adhesion, migration, cell-cell communication, proliferation and survival [7] [8]. These biological functions of laminin-332 depend on structural changes caused by cleavages [9] [10]. Laminin-332 is composed of three chains (α3, β3 and γ2) [11] [12]. Structural changes in α3 or γ2 by cleavage are known to play an important role in these biological functions [13] [14].

In the oral cavity, laminin-332 is also an important factor as it influences epithelial cell behavior in dental implant therapy and wound healing [15] [16] [17]. In dental implant therapy, oral epithelial cells play a critical role as they seal the peri-implant tissues when activated by laminin-332 [18]. Moreover, inadequate invasion of epithelial cells to the implant-bone interface induces failure of osseointegration [19].

In this study, we focused on the structural change in the α3 chain of laminin-332 by cleavage of LG domains. We attempted to suppress the biological function using monoclonal antibodies targeting the cleavage sites to influence epithelial cell adhesion. If the antibodies are able to control epithelial cell adhesion, we could develop antibody therapy to regulate successful osseointegration in dental implant therapy.

2. Materials and Methods

2.1. Antigen Production for Human Laminin-332

We focused on structural change in the α3 chain by cleavage of the LG3 and LG4 domains of laminin-332. When compared with mouse and chicken homolog (**Figure 1**), we synthesized a peptide containing cysteine for cleavage of the LG3 and LG4 domains of laminin-332 by targeting QLLQDTPVAS in the amino acid sequence connecting the LG3 and LG4 domains. To increase the immunogenicity, we synthesized the peptide with keyhole limpet hemocyanin (KLH) as the carrier protein.

2.2. Immunization, Hybridoma Generation and Selection for Monoclonal Antibody Production

The peptide antigen targeting the α3 chain was immunized to mice and hybridomas were generated. The animal experiments were conducted in the laboratory of Medical & Biological Laboratories Co., LTD (Nagoya, Japan). The animal experiments were followed the compliances of the animal experiment of Medical & Biological Laboratories Co., LTD. To select for monoclonal antibody production, we performed enzyme-linked immunosorbent assay (ELISA) for the peptide antigen for the α3 chain and KLH, and measured OD450 absorbance.

2.3. Cell Adhesion Assay

Human epithelial cell line Cas 9-22 was obtained from RIKEN Bio Resources, (Cell Engineering Division, Tsukuba, Japan). Each well of 96-well ELISA plates

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Laminin-5 alpha-3 LG3-4 human.prj    1 SLNKPPFLMLLKGSTRFNKTKTFRINQLLQDTFVASPRSVKVWQDA 46
Laminin-5 alpha-3 LG3-4 mouse.prj    1 SLNKPPFLMLFKSPKGFNKARSFNVNQLLQDAFQAA-RSIEAWQDG 45
Laminin-5 alpha-3 LG3-4 chicken.prj  1 HQYENLEPMLLK---EFKNPLRFKMLKDEKYFGHLTYSNVQHDQQN 43
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Figure 1. Production of antigen peptide. The antigen peptide targeted the *α*3 chain of human laminin-332 at the cleavage site between the LG3 and LG4 domains. Homology was assessed with mouse and chicken arraignment.

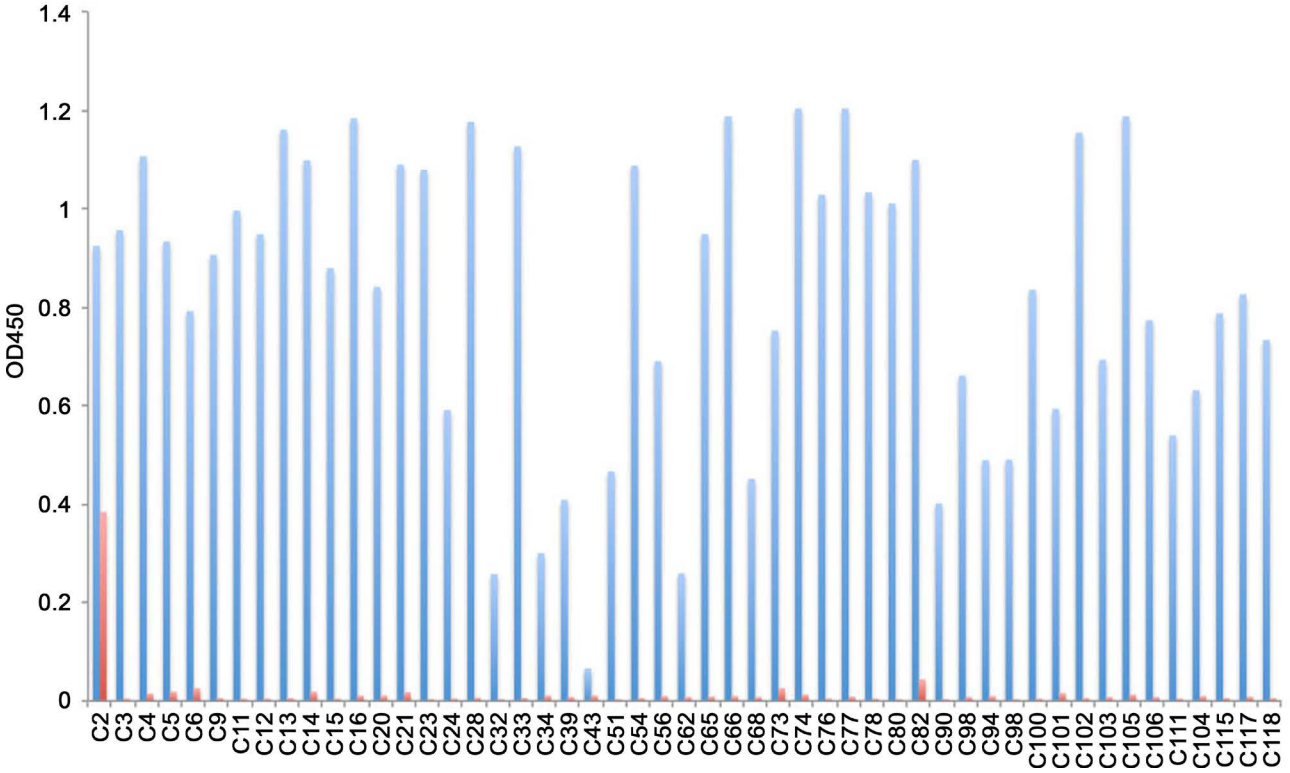

Figure 2. Selection of hybridoma clones. Hybridoma clones were selected based on their affinity to the antigen peptide and the carrier protein, KLH. Red column was represented the affinity to the antigen peptide and blue column was represented to the affinity to the carrier protein, KLH.

(Costar, Cambridge, MA, USA) was coated with recombinant human laminin-332 (10 μg/mL) with hybridoma clones or culture medium and then blocked with 1.2% bovine serum albumin. The wells were washed with phosphate-buffered saline (PBS) twice. Cells (2×10^5) were placed into each well and incubated at 37˚C in the CO_2 incubator for 30 min. After the non-adherent cells were removed, the adherent cells were fixed with 4% paraformaldehyde and washed with PBS twice. The wells were stained with 0.5% (w/v) crystal violet in 20% (v/v) methanol for 10 min. After washing with water, the wells were measured with OD550 nm. The results were analyzed using Student's t-test.

3. Results

3.1. Selection of Hybridoma Clones

The hybridoma clones were bound with antigen peptides and the OD450 measurements ranged from 0.066 to 1.203 (Figure 2, blue bars). The carrier protein, KLH, was also strongly bound to some clones (Figure 2, red bars). In the criteria

of the cut off value in the OD450 was less than 1.0 with the affinity of the antigen peptides, and which was over 0.1 of OD450 with the affinity of the carrier protein, KLH was cut off. Therefore, we selected clones that could bind strongly with the antigen peptide but not with KLH, such as C77.

3.2. Cell Adhesion Activity

To investigate the influence of the monoclonal antibody on the cell adhesion function of epithelial cells for the *α*3 chain of laminin-332, we compared it with the cell adhesion function of human epithelial cells from the Cas9-22 cell line. The monoclonal antibody significantly decreased cell adhesion for the laminin-332 *α*3 chain when compared with no monoclonal antibody (**Figure 3**) in both laminin-332 doses, 1 and 10 μg/mL.

4. Discussion

In the present study, we developed monoclonal antibodies targeting the *α*3 chain of human laminin-332, which is altered by cleavage between the LG3 and LG4 domains. We succeeded in generating hybridoma clones by immunization of the mice using antigen peptide with the carrier protein, KLH. From the hybridoma clones, we selected the clone with a high affinity for the antigen peptide and a low affinity for the carrier protein, KLH. Finally, we generated a monoclonal antibody targeting the cleavage site of the LG domains in the *α*3 chain of human laminin-332. Moreover, we investigated whether our monoclonal antibody could regulate the adhesion function of epithelial cells.

We revealed that the monoclonal antibody targeting the *α*3 chain of human laminin-332 significantly decreased the adhesion function of epithelial cells. Laminin-332 is well-known to play an important role in cell adhesion via structural changes in the *α*3 or *γ*2 chains [13] [14]. The change in the structure of the *α*3 chain occurs by cleavage between the LG domains from LG1 to LG5 [20]. It is known that cleavage between LG3 and LG4 is one of the most important events

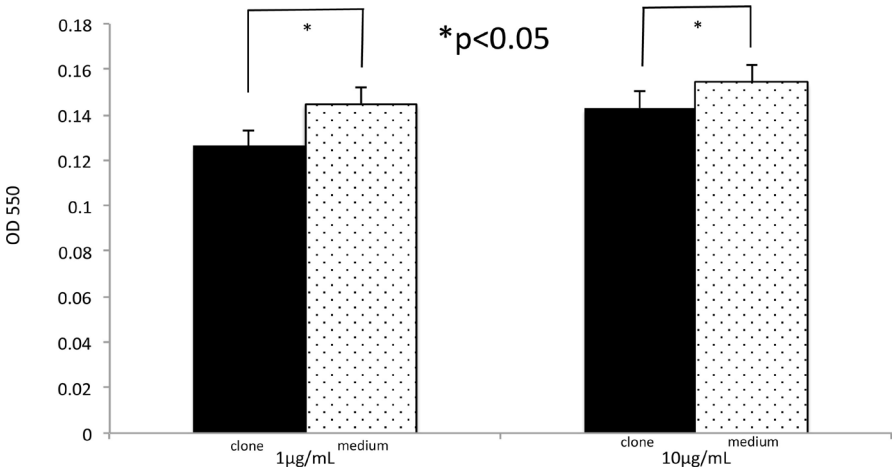

Figure 3. Adhesion assay of human epithelial cells. We revealed that the monoclonal antibody decreased the human epithelial cell adhesion function.

for activating the biological function of laminin-332 [13] [14].

Strong osseointegration is crucial for successful dental implant therapy [3] [4]. Invasion of primarily epithelial cells, rather than mesenchymal stem cells, to the implant-bone interface may cause failure of osseointegration [19]. Therefore, regulating epithelial cell function would be an effective strategy to obtain successful osseointegration in dental implant therapy.

In a future study, we are planning to investigate whether mesenchymal stem cells or osteoblasts would be influenced by the monoclonal antibody for laminin-332. Laminin-332 is also detected in periosteum, not only in the basement membrane [21]. Moreover, laminin-332 affects osteoblast differentiation and calcification [22] [23] [24]. Therefore, laminin-332 plays important roles not only in epithelial cell function but also in the function of mesenchymal cells.

5. Conclusion

We concluded that our generated monoclonal antibody, which activates structural changes in laminin-332, has the potential to regulate epithelial cells in dental implant therapy.

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