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Summary of Doctoral Dissertation

Degree requested Doctor of Pharmaceutical Science Applicant's name Nabila Mutassim Murad Abdu

Title of Doctoral Dissertation

Molecular basis for ANGPTL2 recognition of LILRB2 immune checkpoint receptor

(免疫チェックポイント受容体 LILRB2 に対する ANGPTL2 認識の分子基盤**)**

Background

Up-to-date, an indispensable number of auto-immune diseases and cancers are difficult to be cured. Despite the extraordinary success of the known immune checkpoints; the programmed cell death 1 (PD1) and the CTLA-4 inhibitors, the treatment acquired resistance is still in an increase (1). Therefore, developing therapies that target new immune checkpoints is needed.

Leukocyte immunoglobulin-like receptor subtype B2 (LILRB2) is a member of immune checkpoint receptors, with four highly homologous extracellular immunoglobulin (Ig)-like domains and intracellular ITIM (Immunoreceptor tyrosine-based inhibitory motif) expressed on the surface of the myeloid cells. LILRB1, which is highly homologous to LILRB2, is expressed in broad immune cells. These receptors induce immune tolerance against healthy self-cells through the recognition of major histocompatibility complex class 1 (MHCI) molecules and especially have a crucial role in cancer development through the interactions with the human leukocyte antigen-G (HLA-G) expressed by the tumor cells (2).

Angiopoietin-like receptor 2 (ANGPTL2), has been recently reported to interact with LILRB2 to inhibit the differentiation of the hematopoietic stem cells, hence leading to a poor prognosis in leukemia (3). The overexpression of LILRB2 and ANGPTL2 is related to numerous solid tumors, including, non-small cell lung cancer (4), pancreatic cancer (5), breast cancer (6) and colorectal cancer (7). The detailed information on the interaction of ANGPTL2 and LILRB2 is poorly understood and yet to be elaborated on.

This research aims to study the molecular recognition of ANGPTL2 to LILRB2, to pave the way for the development of an alternative effectively durable antitumor treatment. In this study, through the mutagenesis and the surface plasmon resonance (SPR) analysis, the crucial recognition sites of ANGPTL2 to LILRB2 were determined.

Results and discussion:

1: ANGPTL2 specifically binds to LILRB2D1D2

First, to clarify the specificity of ANGPTL2 to LILRB molecules, the binding activity of the full-length ANGPTL2 expressed in the mammalian cells immobilized on the sensor chip to soluble LILRB2 proteins was studied by the SPR analysis. ANGPTL2 was found to be specifically binding to the first two N-terminal Ig-like domains of LILRB2 (LILRB2D1D2) but not to LILRB1D1D2.

ANGPTL2 is known to form multimers in the human body, therefore, the avidity effect of ANGPTL2 binding to LILRB2 was studied in the opposite direction. As expected, ANGPTL2 showed binding to LILRB2D1D2 with slow

association/dissociation kinetics. This suggests that the multimerized ANGPTL2 physiologically acquires a strong binding affinity to LILRB2 on the cell surface. However, the recombinant ANGPTL2 proteins tend to form heterogeneous multimers and aggregation and are also heavily digested during the storage.

2: ANGPTL2 FLD could bind to LILRB2 D1D2

The fibrinogen-like domain (FLD) is expected to be the putative functional receptor-binding domain of ANGPTL2. Therefore, to avoid the multimerization through the coiled-coil domain of ANGPTL2, I established the new expression and purification system of ANGPTL2 FLD. ANGPTL2 FLD expressed in *E.coli* was purified by size exclusion chromatography and showed binding activity to LILRB2.

3: ANGPTL2 FLD recognition domain in LILRB2

To determine the responsible LILRB2 domain for ANGPTL2 binding, LILRB2D1 and LILRB2D2 proteins were prepared, furthermore, to specifically determine the ANGPTL2FLD binding region on LILRB2, the mutagenesis SPR assay was performed using LILRB1 as a negative control.

4: ANGPTL2 FLD recognition site in LILRB2

There are 36 different amino acid residues between LILRB1D1D2 and LILRB2D1D2 in total, through studying the effect of these mutations on the binding of LILRB2 to ANGPTL2, the most crucial binding amino acid residues to ANGPTL2FLD could be determined.

In summary, this study could show the most important recognition site of LILRB2 for ANGPTL2 binding. This region was found to be far from the HLA class I binding region on LILRB2. The binding analysis of this study revealed a weak-binding fast association and fast dissociation type of kinetics between ANGPTL2FLD and LILRB2 interaction by the monovalent binding, whereas, in fact, ANGPTL2 in the physiological conditions tend to form multimers which might lead to stronger binding with more exaggerated signaling by the clustering of LILRB2 on the cell surface. The results of this study will pave the way to developing new specific immunotherapy targeting the multifunctional LILRB2 that is capable to treat the current uncurable related diseases.

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