学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Molecular recognition mechanism of novel KIR2DS1 specific monoclonal antibodies

（新規ＫＩＲ２ＤＳ１特異的モノクローナル抗体の分子認識機構の解析）

Background

Natural killer (NK) cells play critical roles in host defense against types of tumors, microbial infections, hematopoietic and solid organ transplantations and autoimmune diseases. The activation of NK-cell effector functions is regulated by multiple types of activating and inhibitory receptors, as designated as paired receptors, that recognize the ligands expressed on potential target cells. Killer cell immunoglobulin-like receptor (KIR) family consists of polymorphic but highly homologous receptors regulates human NK cell functions. Especially, two paired receptors against human leukocyte antigen (HLA)-C allotypes with Lys80, activating KIR2DS1 and inhibitory KIR2DL1, have only six different amino acid residues, and hard to be discriminated by monoclonal antibodies (mAbs). In our laboratory, three novel rat anti-KIR2DS1 specific mAbs were successfully generated. These mAbs showed high specificity to KIR2DS1 and were clarified to recognize the Asn163 residue of KIR2DS1 by the mutation assay using surface plasmon resonance (SPR). Interestingly, only 1C7 mAb additionally recognized Thr154 residue. These specific mAbs against KIR2DS1 are expected to be used for the basic research to clarify the expression properties of KIR2DS1 in the NK cell-related immune disorders, and to be developed as candidates for therapeutic antibody drugs as well. In this study, I studied the understanding of the molecular recognition mechanism of these original anti-KIR2DS1 mAbs for future clinical applications in the effective regulation of NK cell functions.

Results and discussion

1. Crystal structure of KIR2DS1

The recombinant protein of the KIR2DS1 ectodomain (residues 1-200) was expressed in Escherichia coli as inclusion bodies, refolded, and purified by size exclusion chromatography and anion exchange chromatography. To understand the molecular recognition of KIR2DS1 by the novel antibodies, 7.8 mg/ml of the purified KIR2DS1 was crystallized in 0.2 M Ammonium sulfate, 0.1 M HEPES pH 7.5, 25% (w/v) PEG 3350 at 20℃ by the sitting-drop method. The crystal structure of KIR2DS1 demonstrated a well-conserved KIR2D structure including the orientation of side residues compared with KIR2DL1 (PDB ID: 1IM9), which is consistent with the difficulty of discrimination by mAbs so far.

2. Binding properties of KIR2DS1 by three mAbs and Thermodynamic analysis of Fabs-KIR2DS1 interactions

To verify the binding affinity of KIR2DS1-specific mAbs (1C7, 5B12, 3E11) toward KIR2DS1, kinetic analysis was performed by using SPR analysis at 25℃. The mAbs were injected onto the immobilized C-terminal biotinylated KIR2DS1 molecules. All mAbs were reasonably well-fitted to the bivalent analyte model with slow dissociation. Among them, 1C7 mAb showed the highest affinity to KIR2DS1 with the apparent dissociation constants (K_D) fitted by 1:1 Langmuir binding model, apparent K_D = 1.6-3.3 nM, 15-44 nM and 22-234 nM for 1C7 mAb, 5B12 mAb and 3E11 mAb, respectively. The Fab fragment of 1C7 mAb also showed the highest affinity to KIR2DS1 (1C7 Fab: K_D = 0.45 μM, 5B12 Fab: K_D = 11 μM, 3E11 Fab: K_D = 8.3 μM). To understand the binding properties of mAbs to KIR2DS1, thermodynamic analysis of the Fabs-KIR2DS1 binding was performed by SPR analysis. The K_d values were determined at the different temperatures (12℃, 15℃, 20℃, 25℃ and 30℃) by equilibrium analysis. Binding enthalpy (ΔH), entropy (ΔS) and heat capacity (ΔCp) were obtained by van't Hoff analysis. The 1C7 Fab-KIR2DS1 and 5B12 Fab-KIR2DS1 interaction showed the enthalpy-driven recognition. However, the 3E11 Fab-KIR2DS1 interaction showed the entropy-driven recognition. Based on the previous mutation analysis, 1C7 mAb was suggested to have a wider interaction surface (conclude Asn163 and Thr154) than the others (only conclude Asn163), which could result in the difference of the binding affinities with KIR2DS1. Therefore, I focused on the 1C7 mAb for further binding, structural and functional analyses and optimization for clinical application.

3. Crystal structure of 1C7 Fab fragment

For further optimization of the CDR sequences and understanding the molecular recognition of KIR2DS1 at the atomic level, I established the preparation method of high purity of 1C7 Fab protein by papain digestion. The purified 1C7 Fab fragment was successfully crystallized under the condition of 0.1 M Tris pH 8.5, 30% (w/v) PEG100 at 20℃ by the sitting-drop method. Parts of CDR regions were not visible in the electron density map possibly due to the flexible structure. It was suggested that the flexible 1C7 VH_CDR3 and VL_CDR2 structures play an important role in highly specific binding to KIR2DS1 using these flexible regions similar to typical antigen-antibody interaction.

Conclusion

In this study, I clarified the crystal structure of KIR2DS1, which showed the highly conserved structure with KIR2DL1, and there was no big difference with each other. The kinetic and thermodynamic analysis for three anti-KIR2DS1 specific mAbs-KIR2DS1 binding showed that these three mAbs would have similar but different binding characteristics, and 1C7 mAb showed the highest affinity to KIR2DS1 with slow dissociation rate. The crystal structure of 1C7 Fab fragment was consistent with the entropically-driven binding property in SPR analysis. The flexible loops in the VH_CDR3 and VL_CDR2 regions of 1C7 Fab might play an important role in highly specific binding for KIR2DS1.