Structural basis for multi-specific peptide recognition by the anti-IDH1/2 monoclonal antibody, MsMab-1

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Abstract

A point mutation in isocitrate dehydrogenase 1 (IDH1) and IDH2 is directly linked to the pathogenesis of certain types of tumors. To detect this mutation, several antibodies that can distinguish between mutant and wild-type enzymes have been established. One of which, MsMab-1, has a unique multi-specific character against several types of mutated IDH1/2. This promiscuous character is in remarkable contrast to the highly specific antigen recognition typically observed with a monoclonal antibody. We solved the crystal structure of MsMab-1 Fab fragment in complex with either IDH1 or IDH2-derived peptides. Based on the structure, it became clear that the peptide-binding pocket of the antibody is highly complementary to the core determinant shared between the IDH1 and IDH2, while leaving just enough space for the side chain of the pathogenic but not the wild-type amino acids located in the mutation position. Clarification of the molecular basis for the peculiar binding characteristics of MsMab-1 in atomic detail will help facilitating its diagnostic application, and may be used to develop better diagnostic reagents through structure-guided protein engineering.

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1. Introduction

Isocitrate dehydrogenase 1 (IDH1) and IDH2 catalyze oxidative carboxylation of isocitrate into α-ketoglutarate in the cytosol and the mitochondria, respectively [1,2]. Interestingly, several point mutations within the IDH1/2 genes have been identified in cancerous cells isolated from patients with glioma, acute myeloid leukemia, and cartilaginous tumors [3–6]. Majority of these mutations occur in the same amino acid position (Arg132 in IDH1 and Arg172 in IDH2, Fig. 1A), converting them to several types of other amino acids. Especially in glioma, Arg132/Arg172 is mutated to His, Cys, Ser, Gly, Met, and Lys [7]. It has been reported that the mutated IDH1/2 shows altered enzymatic property and produces D-2-hydroxyglutarate, which increases a risk of tumor malignancy [8,9].

Since there is a clear correlation between the mutation in the IDH1/2 and the pathogenesis, antibodies that can specifically recognize mutated but not wild-type enzymes are of great potential utility in the diagnostic or even the therapeutic applications. In fact, several monoclonal antibodies against the mutated IDH1/2 have been established and their application to the cancer diagnosis is being discussed [10].

We have isolated multiple murine antibodies that bind to the Arg-to-Gly mutant of human IDH1 (IDH1-R132G) but not to the wild type enzyme, from mice immunized with a synthetic peptide containing the mutation. One of the established antibodies, MsMab-1 (mouse IgG2a, κ), showed highly unique property in that it recognized several different IDH1 mutants without binding to the wild type version. Moreover, MsMab-1 could recognize several pathogenic IDH2 mutants while maintaining a strict distinction from the wild type IDH2 [11,12]. The broad specificity toward multiple pathogenic IDH1/2 mutants while maintaining a strict distinction from the wild-type enzyme suggests that this antibody may be used as a single diagnostic agent to detect various IDH mutations in a cancer specimen. We have already shown that the MsMab-1 can detect mutated IDH1/2 in glioma tissues by immunohistochemical staining, indicating its high potential in diagnostic applications [10,12]. Although the practical advantage of MsMab-1 in the cancer diagnosis is clear, it is difficult to reconcile that one antibody exhibits both highly selective and non-selective binding characters at the same time.

In this work, we performed structural analysis on the peptide binding by the MsMab-1 and clarified how the exclusive recognition of the mutated peptide was achieved while maintaining the
multi-specificity.

2. Materials and methods

2.1. IgG production and cloning

MsMab-1 was produced using mouse medial iliac lymph node methods, and its heavy and light chain genes were cloned as described previously [12]. Institutional animal committee of Tohoku University approved our study. The HA mutant of MsMab-1 was produced using a QuikChange Lightning site-directed mutagenesis kit (Agilent Technologies Inc., Santa Clara, CA). Chinese hamster ovary (CHO)-K1 cells (American Type Culture Collection, Manassas, VA) were transfected with the plasmids using a Gene Pulser Xcell electroporation system (Bio-Rad Laboratories Inc., Waltham, MA) at 1 mC. MsMab-1 IgG was digested by papain (Papain-agarose: Thermo Fisher Scientific Inc.) in PE buffer (20 mM potassium sodium phosphate buffer pH 7.0, 10 mM EDTA) containing 25 mM Cys-HCl pH 7.0. After dialysis against TBS buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl), Fc fragment was removed from the digested mixture by Protein A-Sepharose (GE Healthcare, Billerica, MA) before crystalization. For crystallization of the complex with a ligand peptide, 1/10 volume of 10 mM peptide dissolved in water was added to the purified Fab sample just before the crystalization. Synthetic peptides IDH1(Ser) (KPIIIGSHAYGDQYRA) and IDH2(Ser) (TKPITIGSCHAHGQYKA) were purchased from Sigma-Aldrich Corp.

The crystallization screening of the IDH1(Ser)-Fab complex was performed using the sitting drop vapor diffusion method with the Index (Hampton Research, Aliso Viejo, CA) and Classics Neo (Qiagen, Hilden, Germany) kits. The initial condition was optimized, and diffraction-quality crystals were obtained under the condition of 0.15 M ammonium sulfate, 0.2 M potassium sodium tartrate with the hanging drop vapor diffusion method. The crystal for the IDH2(Ser)-Fab complex was obtained under the same condition. For the diffraction data collection, the crystals were soaked in the same solution containing 10% (v/v) ethylene glycol, and was rapidly frozen with liquid nitrogen.

2.4. X-ray data collection and processing

The diffraction data sets were collected at BL44XU beamline, SPring-8 (Hyogo, Japan), and the diffraction images were processed with XDS [13]. For the Fab-IDH1(Ser) complex, the initial phases were calculated by molecular replacement method with PHASER [14] using a Fab structure of mouse IgG2a (PDID: 4EBQ) as a search model. The initial phases were then improved by density modification with DM [15], and an initial model was automatically built with ARP/wARP [16]. The model was refined with phenix.refine [17] and iterative manual editing using COOT [18]. For IDH2(Ser)-Fab complex, the initial phases were calculated by molecular replacement method using the structure of IDH1(Ser)-Fab as a search model, and the resultant model was refined with manual editing on COOT and REFMAC5 [19]. The model validation was carried out with MOLPROBITY [20], and all figures were prepared with PyMOL (http://www.pymol.org/). Diffraction data statistics and final refinement statistics are shown in Supplement Table 1.

3. Results and discussion

3.1. MsMab-1 Fab crystal structure with a mutant IDH1-derived peptide

We evaluated the binding of MsMab-1 toward various IDH1-derived 19-mer peptides by ELISA. As shown in Fig. 1B, MsMab-1 showed no binding toward wild-type peptide containing Arg132, but recognized peptides containing His, Ser, Leu and Gly at the same site (black bars). As the binding signal was the highest for the peptide with Ser substitution, we used a 12-mer peptide containing Ser mutation (designated as IDH1(Ser) hereafter) for the co-crystallization with the MsMab-1 Fab. Crystals grew under multiple conditions, and we successfully obtained diffraction quality crystals after optimization. From the diffraction dataset collected using the BL44XU at SPring-8, initial phases were calculated by
molecular replacement method, and the final structure was refined at the resolution of 1.93 Å. The crystal contained two copies of peptide-Fab complexes in the asymmetric unit. Electron densities were clearly visible for all 12 residues of IDH1(Ser) peptide bound to one Fab (molecule 1) in the asymmetric unit, while the C-terminal 2 residues of the peptide bound to the other Fab (molecule 2) were disordered. Nevertheless, the visible 10 residues had the same conformation in the two molecules. As the variable region of the antibody molecules were also virtually identical, we will only discuss the structure of the molecule 1 hereafter. We will also use the peptide residue numbering based on their original IDH sequence positions throughout the paper.

The MsMab-1 Fab fragment assumes a typical Fab structure consisting of four immunoglobulin-like β-sandwich domains, and the ligand peptide is packed into a pocket formed by CDR loops L2, L3, H1, and H3 (Fig. 2A). The peptide sits on a bent H3 (Fig. 2B), which is exceptionally short (4 residues) compared to typical H3 loops in the database [21,22]. This feature made the antigen binding groove narrow and deep, burying most of the surface of the antigen peptide. The N-terminal half of the peptide has extended conformation and lies along the narrow cleft between heavy and light chains. At Gly131 in the middle, the peptide backbone bends abruptly and forms single turn helix ascending from the bottom of the cleft (Fig. 2B), and projects the C-terminal two residues that are disordered in the molecule 2 out of the pocket (Fig. 2C, asterisk). The side chains of peptide residues Ile128, Ile130, His133, and Tyr135 all made intimate VDW contacts with the surface of Fab (Fig. 2C, 2D), indicating their fundamental contribution to the interaction. The presence of Gly at the position 131 also seems essential, since its Cz atom is in a direct contact with the floor of the cleft and presence of any side chain would not be tolerated (Fig. 2D). The side chain of the pathogenic mutation residue, Ser132, is pointing toward a cavity formed by the Fab and the Ile129 of the peptide itself, with its hydroxyl oxygen making a direct hydrogen bond with His95 (based on the Chothia’s numbering scheme, [23]) of antibody heavy chain (Fig. 2C).

In order to simulate the binding of various IDH1 peptides, we modeled different side chains for the residue 132 based on the crystal structure of IDH1(Ser)-Fab complex (Fig. 3). When the mutations shown to be permissive for the MsMab-1 binding were introduced, the side chains were well accommodated in the cavity. However, the wild-type residue Arg is too large and expected to cause steric clash with either the antibody or the peptide itself. Therefore, the multi-specific property of MsMab-1 as well as its inability to recognize wild-type IDH1 is warranted by creating a right size of cavity through a unique cooperation between antigen binding pocket and the bound peptide itself.
3.2. Ala substitution for His95 of heavy chain changes ligand recognition property

On the Fab side, the H3 loop residue His95 constitute the wall of the cavity described above, and defines its volume (Fig. 3). We speculated that changing the side chain of His95 to a smaller one may enlarge the cavity, enabling a design of mutant antibody with a broader specificity. To test this hypothesis, we prepared a recombinant mutant MsMab-1 containing Ala in place of His95 (HA mutant). The HA mutant version of MsMab-1 IgG was stably expressed using CHO-K1 cells, and purified from the culture supernatant (with a typical yield of 0.5 mg from 2 L media). In a separate experiment, we also confirmed that the original wild-type MsMab-1 can be recombinantly produced at similar level (data now shown), indicating that the mutation does not affect the stability of the antibody. The purified HA MsMab-1 was evaluated for the binding toward different IDH1 peptides by using the same ELISA format as the original MsMab-1 (Fig. 1B, white bars). Contrary to our expectation, the mutant antibody did not show broader specificity than the original antibody; rather, the specificity became narrower because the HA MsMab-1 could only bind peptides carrying Gly and Ser at the position 132. We note that Tyr32 in the H1 loop sits right next to the His95, with its side chain phenolic ring pushed back by the His95 imidazole (Fig. 3). Retrospectively, the vacancy created by the removal of the His95 side chain would be readily filled by that of Tyr32, making the cavity even smaller. Although our simple subtraction strategy of structure-guided re-engineering to obtain an antibody with a desired property did not work, the result confirmed the essential role of His95 in discriminating amino acid types present in the residue 132 of IDH1, validating our crystal structure.

3.3. Crystal structure of MsMab-1 Fab in complex with mutated IDH2-derived peptide

The remaining puzzle about the promiscuous binding properties of MsMab-1 is its ability to recognize pathogenic IDH2 peptide whose sequence is similar but distinct from that of IDH1. Within the 10-residue region of IDH1 peptide making direct contact with the MsMab-1, sequence variations exist at two positions: the Ile129 and Tyr135 in IDH1 are replaced by Thr169 and His175 in IDH2, respectively (Fig. 1A). The first position (Ile129) exposes its side chain group to the solvent, suggesting that the Thr residue of IDH2 is allowed in this position (Fig. 2D). However, the side chain of the second position (Tyr135) contributes significantly to the interaction, by a hydrophobic packing with the side chain of Ile55 from the L2 loop as well as a water-mediated hydrogen bonding network involving main chain carbonyls (Fig. 4A). To understand the mechanism underlining the dual-specificity, we crystalized MsMab-1 Fab in the presence of a 16-mer IDH2-derived peptide containing a pathogenic Arg-to-Ser mutation at the residue 172 (i.e., IDH2(Ser)). From the crystal structure solved at a resolution of 1.93 Å, it became clear that the antibody indeed recognized IDH2-derived peptide in a manner indistinguishable from that of IDH1 peptide (Fig. 4B). First, the Fv part of the antibody superimposes with that of the IDH1(Ser)-complex with an RMSD value of 0.39 Å for a total of 215 residues, and virtually no difference is observed for the conformation of the side chains of all residues making direct contacts with the ligand peptides (Fig. 4C). To understand the mechanism underlining the dual-specificity, we crystalized MsMab-1 Fab in the presence of a 16-mer IDH2-derived peptide containing a pathogenic Arg-to-Ser mutation at the residue 172 (i.e., IDH2(Ser)). From the crystal structure solved at a resolution of 1.93 Å, it became clear that the antibody indeed recognized IDH2-derived peptide in a manner indistinguishable from that of IDH1 peptide (Fig. 4B). First, the Fv part of the antibody superimposes with that of the IDH1(Ser)-complex with an RMSD value of 0.39 Å for a total of 215 residues, and virtually no difference is observed for the conformation of the side chains of all residues making direct contacts with the ligand peptides (Fig. 4C). This indicates that the antibody does not adjust its structure for the recognition of two different peptides. At the peptide side, the central 10 residues (Pro167-Gly176) are visible in the electron density map. When superposed onto the Fab-IDH1(Ser) complex structure using the Fv
portion, the two peptides have essentially identical conformation, showing an RMSD value of 0.20 Å for 10 matched Cα atoms. As expected, Thr169 is pointing away from the antibody, consistent with the lack of peptide discrimination at this position (Fig. 4D). For the second subtype-specific residue, the His175 perfectly emulates conformation of the Tyr135 and keep the same ring stacking with the Ile55L (Fig. 4A,B,D). Furthermore, acquisition of an additional water restored the hydrogen bonding network, making the interface virtually identical despite the Tyr to His replacement (Fig. 4B). Thus, we conclude that MsMab-1 accomplishes the dual specificity by recognizing common structural feature shared between IDH1 and IDH2. Furthermore, the structural environment of the pathogenic mutation residue is conserved between IDH1 and IDH2, explaining why MsMab-1 exhibits promiscuity (i.e., the dual recognition and the large choice of amino acid at the mutation position) without recognizing the wild-type IDH1/2 proteins.

The point mutation at Arg132 of IDH1 and Arg172 of IDH2 is commonly observed in 50–80% of gliomas [7]. A rapid detection of this mutation in a patient is necessary to enable accurate diagnosis required for designing appropriate therapeutic strategies. Particularly, the diagnosis by the immunohistochemical staining of a pathological tissue sample is considered superior to the genetic analysis of the patient, because it can be implemented more easily in a wide variety of clinical situations. Although the potential of using the immunohistochemistry in the diagnosis of gliomas has been postulated [24], there is one drawback to this method. So far, specific antibodies for several pathogenic mutated IDHs have been developed and commercially available. However, since over ten types of pathogenic mutation at the key Arg residue have been reported on both IDH1 and IDH2, one must search for many different antigens in a tissue specimen. So far, antibodies specific for several pathogenic mutated, but not wild-type, sequences of IDH have been developed and commercially available. Each antibody was raised against individual mutant sequence, and must be used in combination or as a mixture to detect unknown mutation. In contrast, the MsMab-1 can be used alone to detect many disease-causing mutations in both IDH1 and IDH2, simplifying the diagnostic procedure. Although this property was counter-intuitive due to the well-established antibody’s role as a highly selective binder, our structural analyses unraveled the mechanism underlying this seemingly puzzling phenomenon. It is anticipated that the structural information obtained in this study can be used to engineer better antibodies with improved affinity and/or modified specificity, contributing to the development of easy and accurate diagnostic tools for human glioma.

Accession codes

Coordinates and structure factors have been deposited in the Protein Data Bank under accession codes 5GIR for MsMab-1 Fab with IDH1(Ser), and 5GIS for MsMab-1 Fab with IDH2(Ser).
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Transparency document

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2016.08.110.

References