Structural Analysis Sheds Light on the Heparan Sulfate-Binding Ability of Nonclassical MHC-I Molecule, MILL2

MILL2 is a nonclassical major histocompatibility complex class-I molecule associated with β_2-microglobulin. Previous reports suggested that MILL2 is involved in wound healing or nutrient metabolism; however, its molecular mechanisms remain unclear. Crystal structural analysis revealed that MILL2 has a unique basic patch located on the exposed surface of the α3 domain. Further functional study identified the patch as a binding site to heparan sulfate on the cell surface of fibroblast. Interestingly, two forms (open and closed) of MILL2 with different orientation onto β_2-microglobulin were observed. Binding study demonstrated that only MILL2 lacking β_2-microglobulin binds the heparin column. This structural plasticity supports the idea that MILL2 constitutively associates with β_2-microglobulin but releases it when bound to heparan sulfate.

Classical major histocompatibility complex class-I (MHC-I) molecules are type-I transmembrane proteins composed of extracellular α1, α2 and α3 domains associated with β_2-microglobulin (β_2m). They ubiquitously express on the surface of nucleated cells to present peptides derived from intracellular proteins. Cytotoxic T lymphocytes survey classical MHC-I molecules presenting abnormal peptides derived from viral or tumor proteins, and eliminate the cells expressing them. Thus, classical MHC-I molecules play a key role in adaptive immunity against viral infection and malignant transformation.

On the other hand, genes encoding proteins similar to classical MHC-I α chains are found in mammalian genomes. These MHC-I-like proteins are called nonclassical MHC-I molecules and play diverse roles such as in the regulation of natural killer cells or NKT cells, lipid metabolism, iron transfer, IgG transportation and so on. MHC class I-like located near the leukocyte receptor complex (*Mill*) gene family were identified in the genomes of rodents, marsupials and odd-toed ungulates [1–3]. The members of *Mill* encoding proteins, MILL1 and MILL2, are nonclassical MHC-I proteins associated with β_2m [4]. MILLs were reported to be involved in wound healing [5]; however, the molecular function remains largely unknown.

Recently, we successfully determined the crystal structure of MILL2 extracellular domain as a heterodimer with β_2m at 2.15 Å resolution [6]. The overall structure of MILL2 closely resembles that of other MHC-I molecules [**Fig. 1(A)**]. β_2m is located to the side of the α3 domain. The α1 and α2 domains have two α-helices lying on the β-sheet platform formed by seven β-strands. In ligand-presenting MHC-I molecules, antigen peptide or small molecules fit in the groove of these helices; however, the distance between the two helices of MILL2 is too narrow to permit ligand binding. Electrostatic analysis of MILL2 revealed a remarkable basic patch on the exposed surface [**Fig. 1(B)**]. Six basic amino acids forming this patch (Arg194, Arg200, Lys229, Arg232, Arg247 and Arg251) are located at the α3 domain near the β_2m interacting site [**Fig. 1(C)**]. Since this basic patch is unique to MILL2 among all classical and nonclassical MHC-I molecules, we hypothesized that it interacts with putative receptors or ligands by electrostatic interaction. MILL2 is known to bind to the cell surface of the fibroblast cell line NIH-3T3 [5]. We too confirmed that wild-type MILL2 tetramer bound to the surface of NIH-3T3 cells. In contrast, mutant tetramers in which some basic residues were replaced with alanine completely abolished binding to NIH-3T3 cells. In addition, treatment with trypsin, heparan sulfate (HS)-specific endoglycosidases or excess concentrations of heparin inhibited MILL2 tetramer-binding to NIH-3T3 cells. These results suggest that MILL2 binds to HS of glycoproteins on the cell surface of fibroblast.

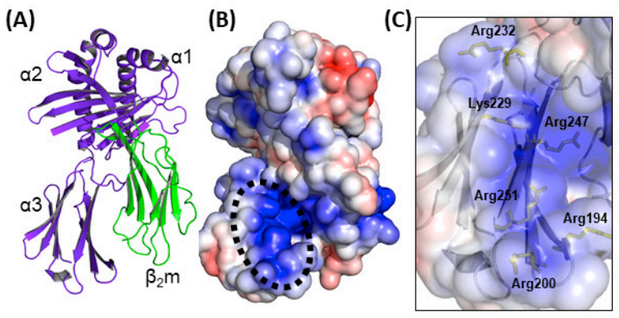

Figure 1: (A) Overall structure of MILL2 (closed form, green: β_2m, purple: MILL2 α chain). **(B)** Electrostatic surface potential of MILL2. Red and blue indicate negatively and positively charged areas, respectively. The black dotted circle indicates the MILL2-specific basic patch. **(C)** Magnified image of the basic patch area on the α3 domain.

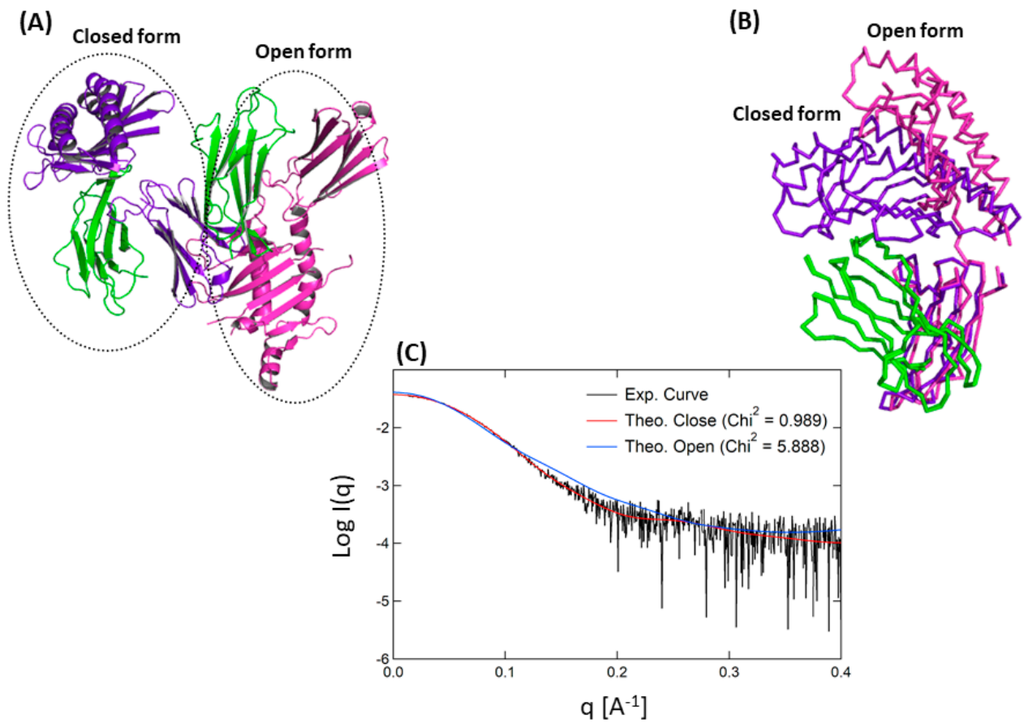

Figure 2: (A) Crystal packing of MILL2 (open and closed forms, green: β_2m, purple and magenta: MILL2 α chain). **(B)** Superimposition of the two conformations based on the position of β_2m. The coloring is the same as **(A)**. **(C)** SAXS profiles of MILL2 (black line: experimental data, red and blue lines: theoretical model of closed and open forms, respectively).

Interestingly, two conformations of MILL2 are observed in the crystals [**Fig. 2(A)**]. In one form, the α1-α2 domains are anchored with β_2m (closed form), similar to typical MHC-I molecules. On the other hand, in another form, the α1-α2 domains are not associated with β_2m and are located far from the α3-β_2m domains (open form) [**Fig. 2(B)**]. Small-angle X-ray scattering (SAXS) profiles demonstrated that the majority of MILL2 in solution exists in the closed conformation [**Fig. 2(C)**]. On the other hand, heparin affinity chromatography revealed that β_2m dissociates from MILL2 when MILL2 binds to heparin. MILL2 should exist in the open conformation since the fixing of the α1-α2 domain by β_2m is completely lost. These observations suggested that MILL2 constitutively associates with β_2m but releases it when bound to HS on the surface of fibroblast. Since β_2m generally supports the structural stability of MHC-I molecules, β_2m likely contributes to the proper folding and stability of MILL2. On the other hand, upon binding to HS, the dissociation of β_2m from MILL2 might contribute to increasing the binding surface toward HS of heavily glycosylated cell surface proteins.

Taken together, structural analysis revealed that the "orphan molecule" MILL2 is the first MHC-I-like molecule having HS-binding ability. MILL2 presumably associates with HS-proteoglycans (HSPG) on the surface of fibroblast, which include the syndecan family important in the progression of wound healing. Further investigations for ligand evaluation, especially syndecans, will clarify MILL2-mediated wound healing.

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