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SEN LAB

Identifying an Activating Glycan Mutant in αX Subunit of αXβ2 Integrin

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Introduction

Integrins are α/β heterodimeric proteins that integrate extracellular and intracellular environments. Structural and functional studies have shown that integrin transition from a bent/closed conformation to an extended/open conformation. These conformational changes contain three major structural re-arrangements: the extension, headpiece opening, and leg-separation.[1]
Integrin αXβ2 has restricted expression in leukocytes. When activated, αXβ2 binds to its ligands—such as complement component iC3b—mediates cellular trafficking, leukocyte adhesion, phagocytosis, and T cell proliferation. αXβ2 dysfunction is associated with inflammatory diseases, such as atherosclerosis and hypersensitivity.[2] Both α and β integrin subunits are highly glycosylated. Studies show that complex N-linked glycosylations affect conformational dynamics on cell surfaces, and hence affect integrin functions, such as affinity and allosteric relay, as well.[3] **How glycans affect the activation of the integrin is not well understood.** At position N373 of the α subunit of αXβ2 is a high mannose glycan that is resistant to enzyme hydrolysis. To study the functional and structural roles of this glycan, we mutated an asparagine residue into serine (N373S variant). The effect of the mutation on the receptor-ligand affinity is assessed by erythrocyte rosetting, and the change in the conformation of the integrin is detected by monoclonal antibodies that identify structural epitopes. Molecular dynamics (MD) studies will measure the degree of freedom of the ligand binding domain and the αX I-domain. **The long term goal is to identify and characterize the N-glycosylation sites on integrin αXβ2 that are key regulators for integrin function.**

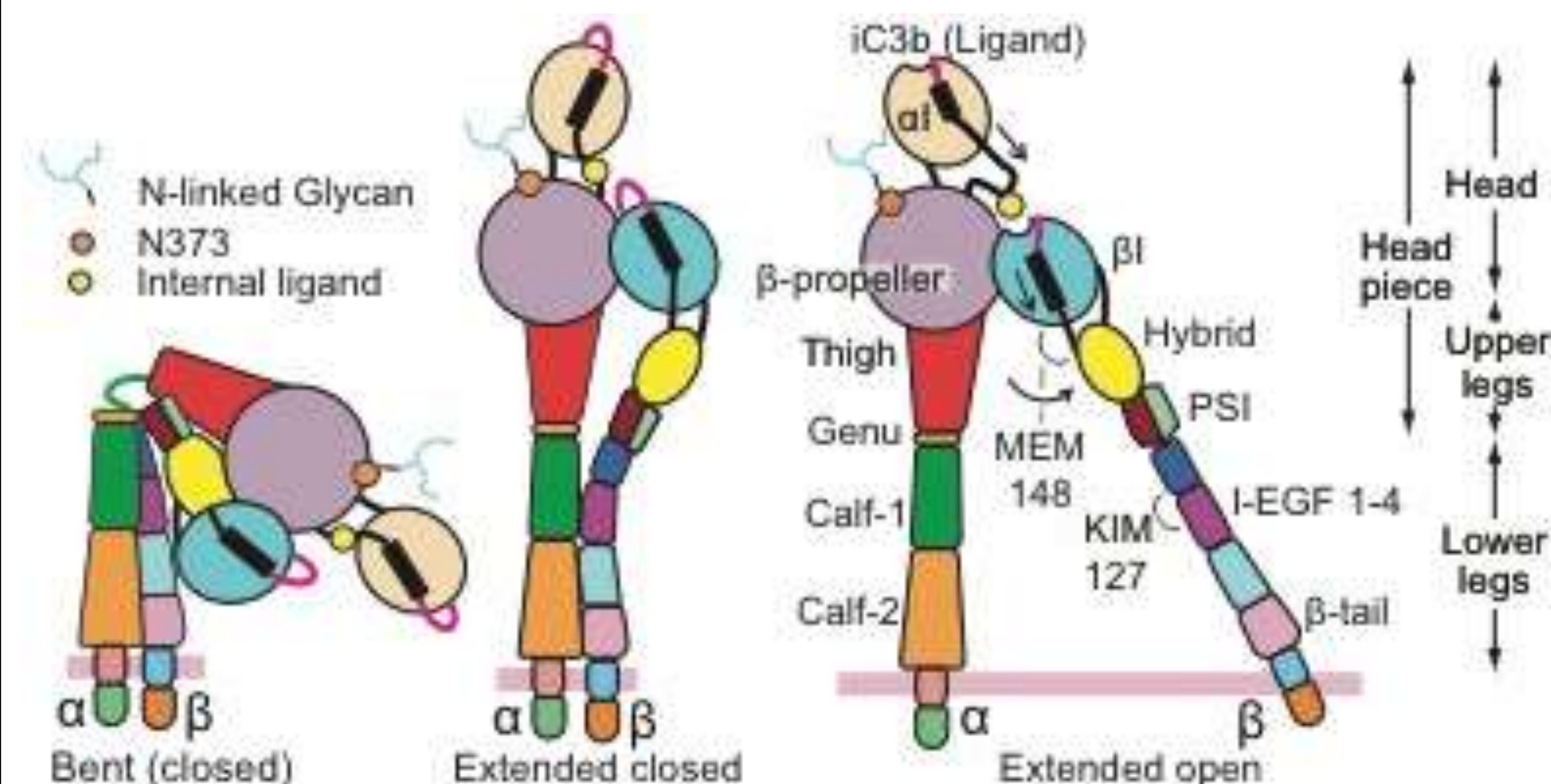

Fig1. Integrin domain organization and conformational states.

Methods

- **Rosetting assay:** Sheep erythrocytes are sensitized with IgM anti-Forssman and human C5-deficient serum. E-IgM-iC3b or E-IgM in PBS are incubated for 1 hour at 37 °C in the presence of 1 mM Mg^{2+}/Ca^{2+}. After 3 washes, the percentage of cells with rosette formation (at least 5 erythrocytes/HEK293T cells; over 100 HEK293T cells have been examined) is assayed by microscopy.
- **Epitope exposure:** For immunofluorescence flow cytometry, HEK 293T transfectants are stained with FITC-labelled CBR LFA 1/7 and 647-labelled KIM127 in the presence of 1 mM Mg^{2+}/Ca^{2+} to recognize the activation-dependent epitopes.
- **MD simulation:** Pyrosetta utilization will generate slow structures to confirm N373 glycan effect on αI-domains' rotational degree of freedom.

Results

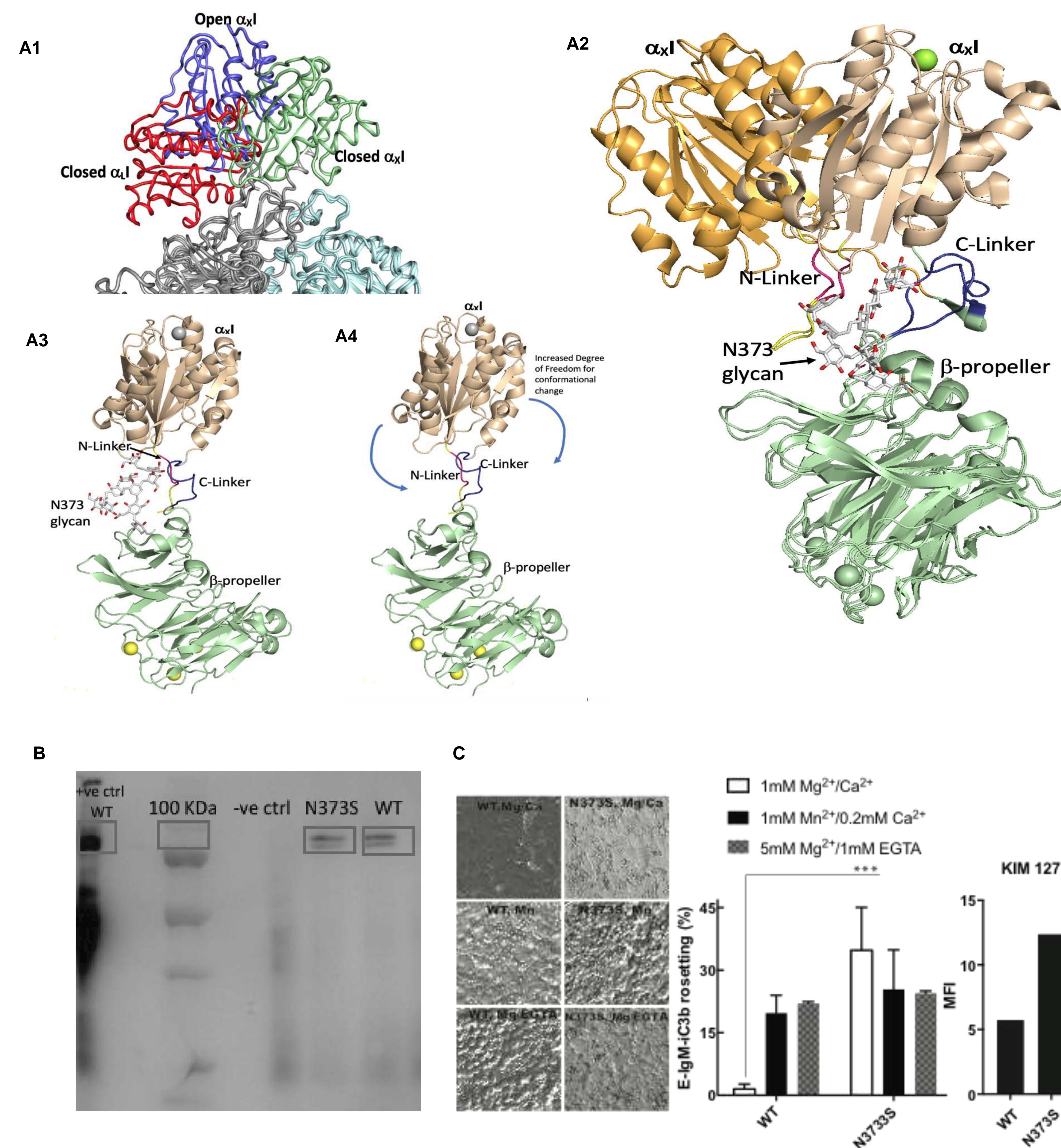

Fig2. A1-4. The Pymol model on the top left indicates the overlap of αI-domains of LFA-1 (red) closed, αXβ2 open (deep blue), and αXβ2 closed (red) conformations (PDB ID codes 5e6u, 4neh, and 5es4 respectively). The Pymol model on the left shows the αXβ2 closed αX I-domain (wheat) with the β-propeller domain (pale green). The N373 glycan (grey) is positioned close to the C-linker (deep blue) and is attached to the αX I-domain. The N-linker is shown in yellow with a segment colored hot pink. The Pymol models A3 and A4 show αXβ2 closed αX I-domain without the N373 glycan. The arrow represents the increase in degree of freedom for conformational change that the αX I-domain acquires upon glycan removal. **A4.** Superimposed αXβ2 open (bright orange) and αXβ2 closed (wheat) conformations. **B.** Western blot analysis of HEK293T cells transfected with WT and N373S variant. **C.** Rosetting assay of transfected 293T cell and KIM127 epitope measurements by FACS to the right.

Discussion

- The mutation did not affect the expression of the integrin.
- Removal of the N-glycosylation at residue 373 of the αX subunit increases the active form of the αXβ2 integrin on the cell surface.
- N373S mutation shifts the structural dynamics towards the extended state as probed by KIM127.
- N373S mutation is probably regulating the integrin activation by influencing the conformational rearrangement.
- **Hypothesis:** On the constitute activation by N373S, we predict that the glycan at the N373 residue limits the freedom of the αI-domain.
- **Future directions:**

1. Molecular dynamics studies to understand how the N373 glycan is modulating and affecting the structural rearrangements of the integrin.
2. The headpiece opening of the mutant will be measured by the binding of the monoclonal antibody, MEM148.
3. The function of the mutant N373S variant will be detected by phagocytosis assay of transfected K562 cells.
4. Transfecting the possible N-glycosylation mutants of both subunits of the αXβ2 integrin to nail down the ones modulating and affecting the receptor conformational rearrangements and function.

References

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