Application Note

Engineered novel bioactive Nectin-4 cis-homodimer protein significantly enhances the binding of TIGIT

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Abstract

Nectin-4, also known as PVRL-4, is a type 1 integral membrane glycoprotein that can form a homo-cis dimer on the cell membrane. The extracellular region contains 3 domains, a distal immunoglobulin-like (Ig-like) V-type domain followed by two Ig-like C2-type domains. Nectin-4 is overexpressed on various tumor cells. It is involved in cell-cell junctions and plays a role in cancer progression by influencing cell adhesion, migration, and proliferation. Nectin-4 has been recently identified as a novel ligand of T cell immunoglobulin and ITIM domain (TIGIT) and interaction between Nectin-4 and TIGIT inhibits NK cell cytotoxicity thus suppressing immune responses, allowing cancer cells to evade immune surveillance. Nectin-4 and Nectin-1 can also interact in a heterophilic manner. As an emerging target of cancer therapeutics, a recombinant protein mimicking the Nectin-4 native dimer conformation can be crucial for therapeutic antibody and vaccine discovery.

The goals of this study are to: (a) create a novel soluble bioactive Nectin-4 homodimer molecule mimicking its native dimer quaternary structure on the cell surface and (b) evaluate the recombinant Nectin-4 dimer binding potency to its receptor vs the monomer form. The dimer can be used as an immunogen and an antigen to increase the potential of cancer therapeutic antibody and vaccine discovery when targeting quaternary structural epitopes.

To better mimic the native dimer conformation and quaternary structure, we designed a novel soluble Nectin-4 cis-dimer with 3 extracellular Ig-like domains fused to a dimer motif at the C-terminus. The Nectin-4 dimer protein was expressed and purified from HEK293 cells and confirmed by SDS-PAGE and Western blot analyses. We characterized the Nectin-4 dimer protein binding potency to TIGIT and Nectin-1 by ELISA. We also studied Nectin-4 dimer immunogenicity using a Nectin-4 dimer DNA immunization protocol to induce potent antibody responses in mice.

Very interestingly, the results demonstrated that the Nectin-4 dimer binding potency to TIGIT and Nectin-1 increased dramatically compared to the Nectin-4 monomer protein. The Nectin-4 dimer DNA immunization generated a high titer antibody response in mice. This novel Nectin-4 cis-dimer is not only bioactive but also has increased functional activities *in vitro*. It can be a very useful immunogen and antigen to advance Nectin-4 targeted drug discovery.

Method

Nectin-4 cis-dimer design and production:

The Nectin-4 cis-dimer was designed to express the extracellular 3 Ig-like domains with a dimer motif as a stabilization element followed by a poly-His tag at the C-terminus. HEK293T cells were transiently transfected with the Nectin-4 dimer expressing plasmid. The Nectin-4 dimer was purified through a Nickel column using the AKTA go™ protein purification system (Cytiva).

QC tests:

Multiple QC tests were performed to evaluate the quality and lot consistency of the purified Nectin-4 dimer protein. SDS-PAGE with reducing and non-reducing conditions were used to detect the dimer protein and its purity. Western blot and ELISA analyses were conducted to test the Nectin-4 specific antibody binding to the Nectin-4 dimer protein.

Nectin-4 and TIGIT or Nectin-1 binding assay:

The purpose of this study is to evaluate the Nectin-4 binding ability to its receptor TIGIT. The Nectin-4 dimer (Product Code: CSP-24016) and a commercial Nectin-4 monomer were coated on 96-well microtiter ELISA plates (2 µg/ml), then detected with TIGIT-Fc dimer (Product Code: CSP-24028) or Nectin-1-Fc with a serial dilution.

**Data presented as a poster at the 2024 AACR Special Conference in Cancer Research: Advances in Pancreatic Cancer Research*

Results

Nectin-4 cis-dimer design and expression

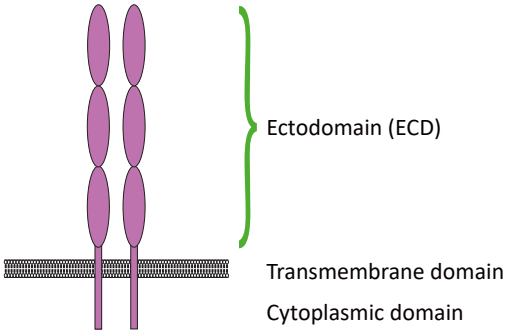

Figure 1. Schematic illustration of the Nectin-4 dimer on the cell surface. The extracellular, transmembrane and cytoplasmic domains are indicated.

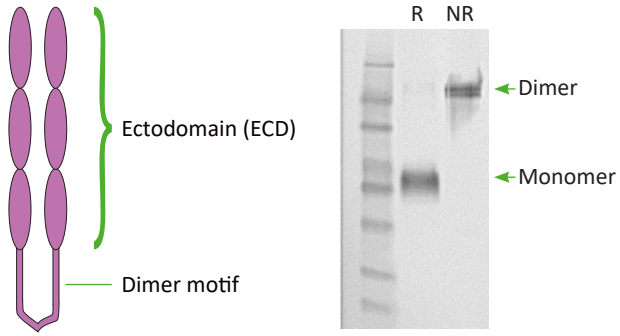

Figure 2. Schematic illustration of the Nectin-4 cis-dimer designed to express the extracellular domain fused with the dimeric motif (left). Purified Nectin-4 cis-dimer analysis using SDS-PAGE (right) under reduced (R) and non-reduced (NR) conditions

Nectin-4 cis-dimer bioactivities

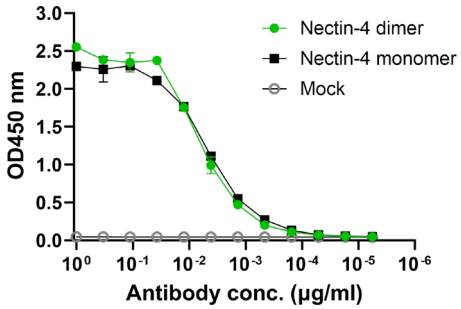

Figure 3. Nectin-4 specific monoclonal antibody has similar binding to monomer and dimer forms as measured by ELISA.

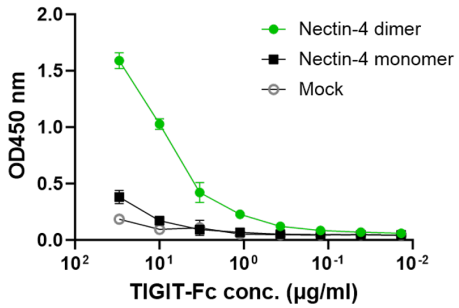

Figure 4. Nectin-4 cis-dimer significantly enhances binding to TIGIT-Fc dimer compared to Nectin-4 monomer. The binding assays were measured by ELISA.

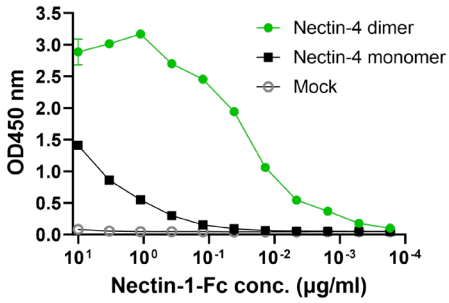

Figure 5. Nectin-4 cis-dimer significantly enhances binding to Nectin-1-Fc dimer compared to Nectin-4 monomer. The binding assays were measured by ELISA.

Results

Immunogenicity of Nectin-4 cis-dimer using DNA immunization

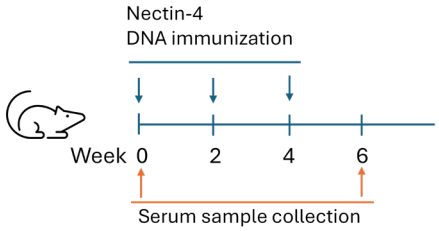

Figure 6. Nectin-4 cis-dimer using DNA immunization of mice to generate antibodies. DNA immunization was performed by intramuscular injection.
Group A – Nectin-4 dimer DNA immunization; mouse ID A1 – A4
Group B – Nectin-4 dimer DNA with immune engaging element; mouse ID B1 – B4

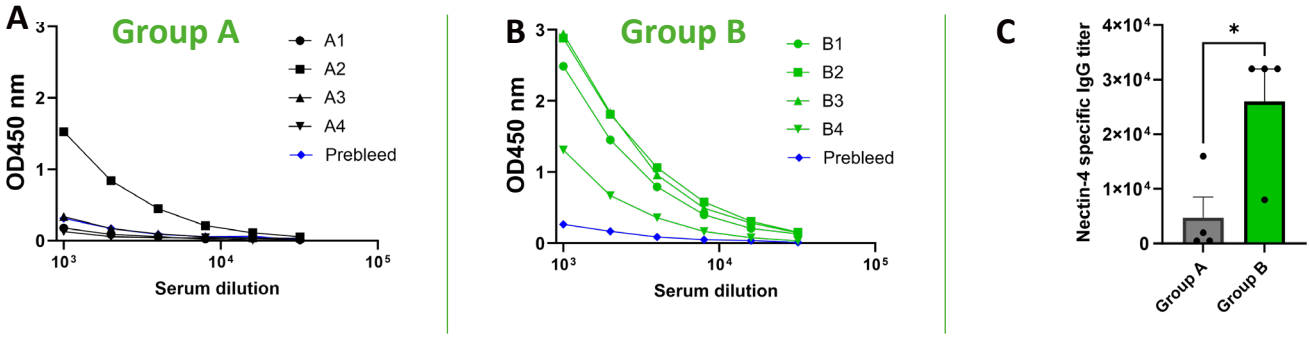

Figure 7. Nectin-4 dimer-specific antibody responses after the 3rd DNA immunization in mice from (A) Group A , (B) Group B, and (C) a comparison of antibody titers in both groups. The immune engaging element significantly enhanced the antibody responses in Group B compared to Group A.

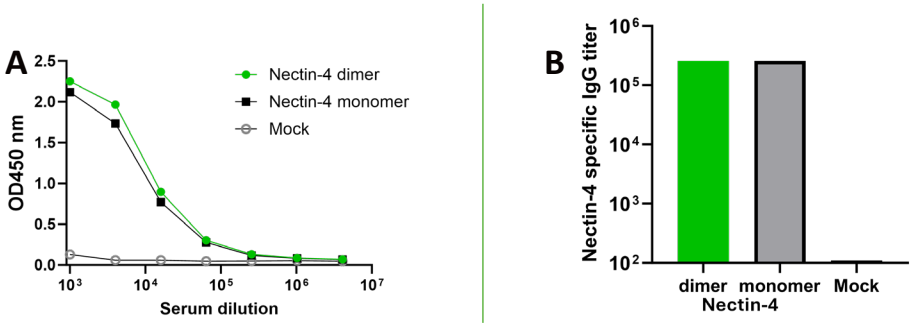

Figure 8. Nectin-4-specific polyclonal antibodies in sera from immunized mice can bind to the Nectin-4 dimer and monomer similarly. Group B mouse serum titration (A) and antibody titers (B) against the Nectin-4 dimer, Nectin-4 monomer or mock antigen.

Conclusion

- This novel Nectin-4 cis-dimer protein is bioactive and in the desirable conformation: (a) resulting in an increased binding potency to its receptor TIGIT and to Nectin-1, and (b) eliciting strong antibody responses in mice when using DNA immunization.
- This novel Nectin-4 dimer protein can be a very useful molecule for cancer research, therapeutic antibody and vaccine discovery. The Nectin-4 dimer protein can be used to:
  (i) elicit antibody responses against the dimer quaternary epitopes,
  (ii) screen antibodies targeting more conformational epitopes, and
  (iii) study the Nectin-4 and TIGIT ligand/receptor interactions.

Product codes

Nectin-4 dimer product code: CSP-24016, TIGIT-Fc dimer product code: CSP-24028

References

1. Reches A, et al. Nectin4 is a novel TIGIT ligand which combines checkpoint inhibition and tumor specificity. J Immunotherapy Cancer. 2020; 8(1): e000266
2. Harrison O, et al. Nectin ectodomain structures reveal a canonical adhesive interface. Nature Structural & Molecular Biology (2012); 19: 906