Poster Presentation

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Structural and biochemical characterization of the 4-1BB/4-1BBL/Galectin 9 signaling axis

Aruna Bitra[1], Dirk M Zajonc[1]
[1]*La Jolla Institute For Allergy And Immunology, San Diego, United States*
E-mail: aruna@lji.org

4-1BB (CD137) is a tumor necrosis factor receptor super family (TNFRSF) member that undergoes receptor trimerization upon interacting with its trimeric ligand (4-1BBL) and form 4-1BB/4-1BBL hetero – hexameric complexes to promote antiviral immunity. 4-1BB signaling is greatly reduced in Galectin-9 (Gal-9) deficient mice, suggesting a pivotal role of Gal-9 in 4-1BB activation. Gal-9 is a tandem-repeat carbohydrate-binding protein that engages 4-1BB receptors to either facilitate the clustering of individual 4-1BB/4-1BBL hetero-hexameric complexes or induce formation of a "closed state" in 4-1BB, to facilitate the stable recruitment of cytoplasmic signaling components.
In an attempt to elucidate the 4-1BBL – 4-1BB – Gal-9 interaction, we have first determined 4-1BB binding to both 4-1BBL and Gal-9 using Surface Plasmon Resonance (SPR). Secondly, we have attempted to structurally characterize the formation of 4-1BB/4-1BBL hetero – hexameric complex and 4-1BB – Gal-9 interaction.
To achieve our objectives, we have determined the crystal structure of mouse 4-1BB in 3 different space groups (P21, P21212 and P43) to a maximum resolution of 2.2 Å using sulfur-SAD phasing. The overall architecture of 4-1BB is similar to other TNFRSFs with four cysteine rich domains (CRD) that contain 10 disulfide linkages. The CRD1 of 4-1BB is smaller and lack the conserved β-β′ strands in comparison to other TNFRSF members. Additionally, the orientation of CRD3 with respect to CRD2 also differs from others, hence making the structure of 4-1BB slightly distinctive to other TNFRSFs. In the crystal structure, we have mapped two Asn residues within CRD4 that are N-linked glycosylated and mediate 4-1BB binding to Gal-9. Mutation of either of this N-linked glycosylation sites did not affect the binding affinity towards Gal-9. SPR studies also revealed that Gal-9 could only bind to mammalian expressed N-linked glycans that have terminal galactose on 4-1BB, since Gal-9 is specific for terminal galactose moieties that are not found in insect cell expressed glycans. Structural studies of a 4-1BB/Gal-9 complex have been unsuccessful due to the flexible interaction between both proteins via N-linked glycans.
4-1BB binds with high affinity to 4-1BBL via CRD1 and CRD2. SPR studies further revealed that, unlike other TNF receptors, no cross-species interaction between human 4-1BB and mouse 4-1BBL was observed, suggesting structural adaptations of the binding sites found within 4-1BB and 4-1BBL in both mice and humans. Biochemical data also suggests that mouse 4-1BBL can covalently form dimers or timers via 2 unique cysteine residues that are absent in human 4-1BBL. To fine tune the structural differences between mouse and human 4-1BBL, we have collected the native dataset for m4-1BBL to a resolution of 2.3 Å in P21 space group, however phasing via MR has been unsuccessful so far, suggesting gross structural differences between the previously solved human 4-1BBL and mouse 4-1BBL structure. Attempts to crystallize the stable complex of 4-1BB/4-1BBL yield weak diffracting crystals and hence further trails to improve the diffraction quality for structure solution are currently underway.
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