BIO_04 - Conformational dynamics behind the inhibition of Notch1 NRR region by the antineoplastic antibody Brontictuzumab

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Introduction: Notch is an evolutionarily conserved signaling pathway comprising four human receptors (Notch1-4) involved in critical cellular processes, like cell differentiation. Pathogenic alterations of the receptors are related to several cancer types, making the Notch pathway a promising target for drug design. Monoclonal antibodies (mAbs) against Notch proteins emerge as a target-specific alternative to nonspecific gamma-secretase inhibitor drugs. There are five reported mAbs targeting the Notch pathway, but only Brontictuzumab (BRON) targets Notch1. Although it is established that BRON binds to the Notch1 NRR region, no structural information is available to elucidate how this mAb prevents Notch1 activation.

Objective: Propose a binding mode between Brontictuzumab and NRR and elucidate the conformational dynamics involved in their interaction.

Methodology: BRON Fv (Fragment variable) was modeled with RoseTTAFold and assessed with MolProbity and QMEAN. Validation of the docking procedure using ClusPro 2.0 was based on the redocking of a synthetic Fab/NRR complex (PDB: 3L95). The comparison of the results with the crystal structure used DockQ. Then, BRON was docked with the NRR from 3L95. Molecular Dynamics (MD) of Fab-NRR, BRON-NRR, and NRR apo (PDB: 3I08) used AmberTools19. Trajectories were analyzed with cpptraj and pyPcazip.

Results: We obtained an Fv model for BRON that successfully satisfied stereochemical requirements, with no Ramachandran outliers detected and a QMEANDisCo global score of 0.8. Redocking of 3L95 showed that the docking pose with the lowest energy score was closest to the experimental structure (DockQ score: 0.91). Thus, the lowest energy model from the BRON- NRR docking was selected for MD. Simulations showed that the complex with BRON was stable (average RMSD: 2.49 Å). BRON restricted NRR conformation and allowed it to explore new regions of the conformational space. Moreover, BRON formed a hydrogen bond with L1710, thus obstructing the S2 cleavage site.

Conclusion: We introduced a possible binding mode between BRON and the NRR region from Notch1 that obstructs the S2 cleavage site and affects the conformations explored by the receptor, possibly leading to the inhibition of Notch1 activation that is related to its anti-cancer activity.

Keywords: Notch; Cancer; Antibody