Meditopia: Meditope-Enabled Bonds and their Affinity with Cetuximab

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Abstract

A meditope is a peptide found in the fragment antigen-binding (Fab) region of an antibody, and can be used to bind substances, such as monoclonal antibodies (mAbs) and pharmaceuticals. We centered on four mutations that can increase the affinity between the meditope and the mAb, allowing for a stronger bond. Our focus was on the anti-epidermal growth factor receptor mAb cetuximab, which tracks the overexpression of the epidermal growth factor receptor (EGFR) by cancer cells. Utilizing a meditope, cetuximab can be linked to chemotherapy, creating a more effective treatment that only attacks cancer cells, significantly reducing possible long-term side effects. In this study, yeast display and deep mutational scanning tracked, enhanced, and joined four mutations within the meditope, increasing affinity, or bond strength, for cetuximab 10-fold, from Kd 130 nm to 15 nm. Those mutations led to heightened cetuximab recruitment to EGFR-overexpressing cancer cells, and therefore more efficient targeting of cancer. The methods used in this study can develop other effective meditope-linked monoclonal antibodies and chemotherapy treatments. The Mahtomedi MSOE Center for BioMolecular Modeling MAPS Team will use 3-D modeling and printing technology to examine structure-function relationships of meditope-mAb binding within cetuximab. The visual model will be a valuable tool in developing our story.

Antibody-Drug Conjugates

An Antibody Drug Conjugate (ADC) is the chemically linked combination of a monoclonal antibody (mAb) and a chemotherapeutic drug. ADCs allow for targeted cancer-fighting, increasing the tolerability of the drug by searching for and targeting overexpressed cells without harming healthy cells (Ponziani, et al.). We focused on the monoclonal antibody cetuximab, which tracks the expression of Epidermal Growth Factor Receptor (EGFR) hormones which are overexpressed in cancer cells.

Meditopes

A meditope is a type of cyclic peptide located in the fragment-antigen binding (Fab) region of the antibody. Meditopes are 12 amino acids that bind between the heavy and light chains of the monoclonal antibody cetuximab, a site not found on human antibodies (Figure 3) (Bzymek, et al.). Although not naturally occurring, meditopes have been grafted to human mAbs such as trastuzumab (King & Williams). The meditope links the monoclonal antibody and the drug with a cleavable chemical bond, allowing specific chemical signals to cause the meditope-antibody conjugation to separate from the drug. Cetuximab in conjunction with a drug is an internalizing treatment. It utilizes Receptor-Mediated Endocytosis (RME), the process of binding to an antigen receptor site to enter the cancerous cell where the drug is released and apoptosis occurs (Ponziani, et al.). Meditopes are useful for linking ADCs because they only bind to specific sites—meaning no unintentional bonds—and can increase the utility of mAbs by allowing for additional interactions between proteins. But there is one major issue with the meditope for cetuximab: “many of [its] potential applications…are limited by its moderate affinity” (Van Rosmalen, et al.).

Figure 3

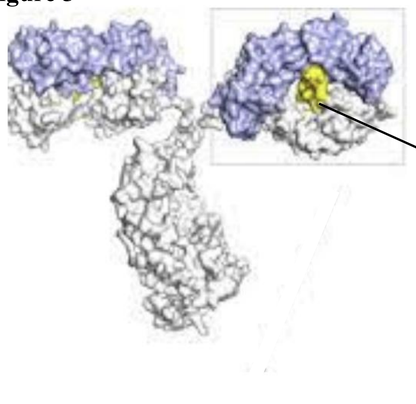
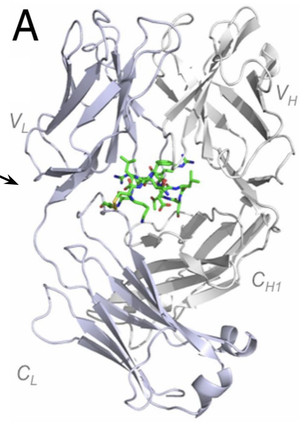


Figure 2



The Mutation

Binding affinity describes the bond strength between two things--like a meditope and a mAb. It originates in amino acid interactions and the non-covalent chemical bonds they form. The way amino acids interact also inherently relies on peptides, like a meditope, because binding affinity also depends on interactions between side-chains and main-chains (Du, et al.). Increasing bond affinity leads to higher cellular toxicity, but also decreased penetration of tumors, meaning the goal is a balance between the two. It can also cause more successful and accurate binding. Van Rosmalen, et al., used deep mutational scanning and yeast display to screen all single amino acid substitutions for the previously un-mutated meditope CQFDLSTRRLKC, and a select set of double substitutions (Figure 2). They then proceeded to identify mutations that, when combined, would enhance each others’ strength in order to find the mutation that would increase the bond affinity between the meditope and cetuximab the most. Simulations and experimental data both showed that “positions 1, 3, 5, and 6” on the meditope are most tolerable to mutation (Martijn, et al.). The end result was the tetra mutant Q1V/D3N/S5G/K10R, a combination of four mutations whose distinct locations can be seen on Figure 1, and are color-coded as green, red, brown, and gray respectively on Figure 4. Each mutation represents a substitution made to an amino acid on the meditope--an attempt to increase affinity piece by piece in search of higher overall bond affinity. Q1V denotes the change of amino acid one from glutamine to valine, D3N the shift from aspartic acid to asparagine, S5G a swap of serine and glycine, and K10R is the replacement of amino acid 10, lysine, by arginine. These substitutions are shifts from hydrophilic and polar to hydrophobic and nonpolar, negative charge to uncharged polar, uncharged polar to uncharged polar, and from 6 hydrogens to 8, respectively.

Figure 1

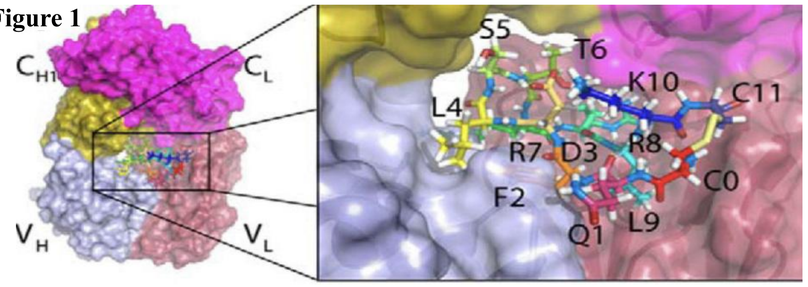
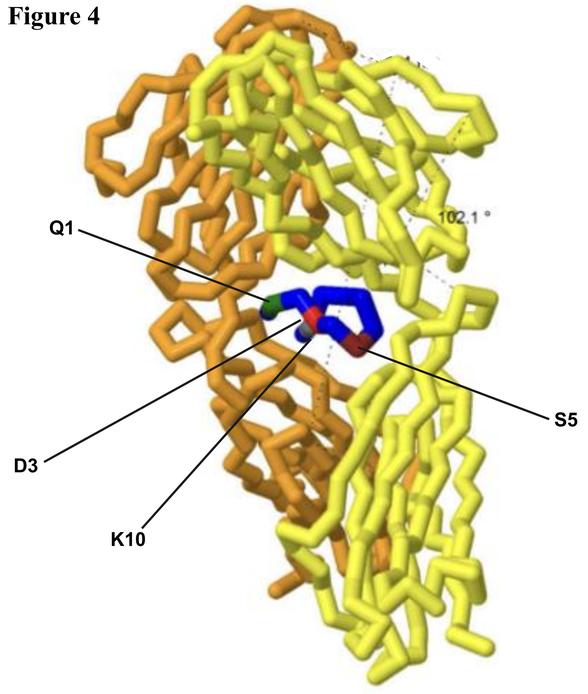


Figure 4



Increase in Affinity

This mutation increased bond affinity nearly 10-fold, meaning the dissociation of cetuximab from the meditope was originally 130 nanometers, but shrunk to 15 nanometers. According to Erijman, et al, there are two key factors that can increase bond affinity--geometric complementarity and hydrogen bonds. Hydrogen bonds increase the amount of free energy to be used in binding, leading to stronger bonds, and stabilize the interaction, reducing the chance of negative outcomes. Geometric complementarity simply means that two things connect well--like a key in a lock--increasing stability and bond strength. Therefore, this increase in affinity was most likely caused by higher geometric complementarity between the substituted amino acids and the binding sites. We infer that the binding site for Q1 was hydrophobic, leading it to bond better with hydrophobic amino acid V. The binding site for D3 bonded better with the polar N, and S5’s binding site better connected with G. Additionally, the swap to uncharged polar protein N increased the chance of hydrogen bonding, as did the substitution of R in K10’s binding site--this increased the hydrogen count, which was higher in the meditope overall after the mutation than before, and therefore the likelihood of hydrogen bonding. Due to higher affinity, the meditope and cetuximab have a higher chance of successful bonding and subsequently staying together while traveling throughout the body to antigen receptors found on the outer membrane of cancer cells--all due to higher bond strength and stability. This mutation also allows the ADC to maintain the proper balance between cellular toxicity and tumor penetration ability, meaning more effective cancer-fighting. Initially, with the non-mutated meditope, high levels of bonding only occurred at a “peptide concentration of 500 nm”, but the mutated meditope had high binding levels at 50 nm. This means that significantly less meditope is needed for the same amount of cancer killed. Therefore, the higher affinity between the tetramutant meditope and cetuximab also led to “more efficient…targeting of EGFR-overexpressing tumor cells” (Van Rosmalen, et al).

Conclusion

Most chemotherapeutic drugs are invasive and incredibly damaging to their hosts. This leads to long-term health effects that heavily impact patients’ quality of life--all because of damage done to healthy cells. By using antibody drug conjugates with high binding affinity, patients’ long-term effects are reduced significantly, as the ADC is able to target and kill cancer cells with little or no harm done to healthy cells. The use of peptide bonds in ADCs is not limited to this meditope for cetuximab--the development of other ADCs in the hopes of limiting damage to patients is ongoing. Additionally, there is a high chance that the strategy utilized by Rosmalen, et al. in this research can be more widely applied to the “affinity mutation of protein-protein or protein-peptide interactions.” This would mean other highly effective cancer treatments with low risk to patients, and, as explained earlier, increasing affinity often means that less drug is required--implying the possibility of lower drug costs. In sum, this research, both alone and if successfully applied to other meditopes, has the power to save lives, increase quality of life for patients, and even reduce the amount of money required for treatment.

References

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