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Type	Poster
Session	Protein structure and function
Title	BNCT: development of a novel Boron Delivery Antibody (BDA) by means of specific residues replacement
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Motivation

Boron Neutron Capture Therapy (BNCT) is a tumor cell-selective radiotherapy based on a nuclear reaction that occurs when the isotope boron-10 (10B) is radiated by low-energy thermal neutrons or epithermal neutrons, triggering a nuclear fission response to produce an alpha particle (4He) and lithium-7 (7Li). BNCT differs from modern approaches, since the probability of thermal neutrons being captured by a 10B is much higher than that of being captured by atoms in human cells, thus allowing a selective administration of irradiation to cells. Hence the need to create novel delivery agents containing 10B with high tumor selectivity, but also exhibiting low intrinsic toxicity, fast clearance from normal tissue and blood, and with no pharmaceutical effects. Synthetic chemistry has led to the development of specific tumor-targeting 10B delivery agents, such as boron-containing small molecules, boron compound conjugates and boron delivery nanoparticles [1]. Recently, Boronophenylalanine (BPA) was approved by the Ministry of Health, Labour and Welfare of Japan as a boron delivery agent for BNCT of advanced or recurrent head and neck cancers last year [2]. Monoclonal antibodies (mAbs) have shown a great potential as boron delivery agents due to their ability to recognize tumor-associated epitopes, making them a topic that has been extensively investigated. Antibodytherapeutic molecules conjugates, exhibiting higher boron concentration in tumors, have been synthesized, but their size could be a problem in crossing the blood-brain barrier. This work is aimed at improving the potential of monoclonal antibodies applied to BNCT therapy identifying the best residues within them to accept a boron atom, with a focus on head and neck cancer. For this purpose, Cetuximab, an epidermal growth factor receptor (EGFR) inhibitor used for the treatment of metastatic colorectal cancer, metastatic non-small cell lung cancer and head and neck cancer, was chosen [3]. Cetuximab is a chimeric monoclonal antibody capable of inhibiting EGFR and decelerating tumor growth. Since EGFR activation induces macropinocytosis, the efficient cellular uptake of Boron atoms inserted into the antibody is guaranteed [4].

Methods

From a subset of boron containing ligands, we selected BPA and boronic acid derivatives, based on their scaffold similarity with amino acids and their use in BNCT radiotherapy. Firstly, a mutagenesis pipeline has been developed to investigate candidates: 1) Side chains containing boron derived above have been used as ligands in docking studies for the replacement of residues; 2) Cetuximab residues able to mimic the ligand both as structural and chemical-physical features were chosen (Tyr, Ser, Trp, His, Phe); 3) Each of them was "deprived" of the side chain mutating to Ala and subsequently to Gly. The reason of this mutation was to check whether the candidate ligand side chain was able to reposition itself exactly in the regions previously occupied by the native residues. Any mutation around the binding site of EGFR membrane protein has been excluded from the analysis, as it is essential for this study and for possible uses in BNCT therapy that such interaction is preserved to maintain the desired selectivity. Docking simulations were carried out for each of the proposed mutations. Additional docking calculations were run on the native antibody to get an initial assessment of any ligand pocket and of the best affinity scores. The results were analysed by visual inspection, selecting the best residues to be mutated, taking into account not only affinity score levels but also orientation, overlapping degree and ligand distance from the respective side chains of mutated residues. The same results were then validated by a python script.

Results

Results have therefore led to the identification of Tyr as the best residue for mutation. The similarity of Tyr with Boronophenylalanine relies on similar steric and polar features. In fact, the only hydrophobic feature is not sufficient by itself to perform the best pocket occupancy and on the other side, the polar component should integrate it. This is why Ser and Thr side chains, endowed of an OH group, were not able to perform

so well as Tyr. Finally, to evaluate if the mutated residues are able to maintain the correct antibody folding, Molecular Dynamic simulations are ongoing. Overall, this work has led to the development of a BDA Identification Pipeline that can be customized for other case studies: starting from a ligands library, a sub-set with steric and polar characteristics similar to the 21 amino acids is selected. These molecules are then used as probe in docking simulations to identify residues to mutate. Subsequently antibody folding validation by Molecular Dynamics leads to the identification of a novel modified residue which constitutes the BDA.

Info

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[4] Nakase I, Aoki A, Sakai Y, Hirase S, Ishimura M, Takatani-Nakase T, Hattori Y, Kirihata M. Antibody-Based Receptor Targeting Using an Fc-Binding Peptide-Dodecaborate Conjugate and Macropinocytosis Induction for Boron Neutron Capture Therapy. ACS Omega. 2020 Sep 2;5(36):22731-22738. doi: 10.1021/acsomega.0c01377.

Figure

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Availability	-

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