Development and characterization of high-affinity monoclonal antibodies targeting ErbB3

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Epidermal growth factor receptors (EGFRs), also called ErbBreceptors, are considered the canonical receptor tyrosine kinases (RTKs). Notably, the dimerization of ErbB3 with ErbB1 or ErbB2 is known to induce the activation of the kinase signaling. ErbB1/2 are known to be involved in numerous forms of cancers and it has been shown that upon targeting ErbB1/2 by inhibitors-based therapies, ErbB3 is up-regulated and seems to participate in tumor progression and resistance. Furthermore, although ErbB3 is endowed with a tyrosine kinase domain, it lacks a proper kinase activity, making tyrosine kinase inhibitors (TKIs) ineffective. To provide an alternative anti-cancer therapy, a possible strategy is preventing ErbB3 heterodimerization with monoclonal antibodies. In this context, using the hybridoma technique, we developed two monoclonal antibodies targeting ErbB3, called hAb1 and hAb2. The aim of this work is to characterize the binding modalities of these monoclonal antibodies. We demonstrate the high affinity of hAb1 and hAb2 antibodies for the ErbB3 receptor through ELISA and BLI assays, and their effective suppression of ligand-dependent stimulation by in vitro experiments. Using X-ray crystallography we identified the epitope residues involved in the binding to the hAb1 antibody, while by means of single particle cryo-electron microscopy we determined the location of the binding site of hAb1 and hAb2 in the presence of the ligand NRG1β. In conclusion, we have identified the structural determinants that regulate the interaction of hAb1 and hAb2 with the ErbB3 receptor using a combined approach of structural biology techniques, functional and binding studies.