457c Engineering Antibodies against the Epidermal Growth Factor Receptor to Block Dimerization

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Epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase involved in the regulation of cellular proliferation and differentiation. It is overexpressed and/or mutated in a wide variety of carcinomas, and thus EGFR has emerged as an important target for cancer therapy. This has been validated by antibodies such as the FDA-approved Cetuximab (ERBITUX, ImClone Systems Incorporated), which blocks EGFR signaling by interfering with ligand binding. We have identified single-chain antibodies (scFvs) specifically directed at epitopes associated with receptor dimerization but not associated with ligand binding. These epitopes were targeted by using disulfide-bonded peptides that mimic loops in EGFR as an antigen. A nonimmune human scFv library was screened for binding to the peptides using yeast surface display. The selected scFvs were then engineered for improved binding to the full EGFR ectodomain using random mutagenesis and screening. We have shown that rabbit polyclonal antibodies against these peptide epitopes reduce EGFR tyrosine phosphorylation in human mammary epithelial cells (HMECs). Additionally, EGFR on the HMEC surface can simultaneously bind these antibodies and the ligand epidermal growth factor (EGF). Thus, we have employed an alternative strategy for using antibodies to block EGFR signaling by preventing receptor dimerization. These antibodies could potentially be used as therapeutics in synergy with ligand-blocking antibodies such as Cetuximab or against the mutant EGFR vIII, which can signal in the absence of ligand.