# 論文の内容の要旨

## 論文題目：Humanization and characterization of anti-epiregulin monoclonal antibody

（抗エピレギュリンモノクロナール抗体のヒト型化と機能評価）

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The epidermal growth factor (EGF) signaling system consists of at least seven ligands, namely, EGF, amphiregulin, transforming growth factor α, heparin-binding EGF, betacellulin, epiregulin (EREG), and epigen (Yarden and Sliwkowski, 2001). These ligands bind to the extracellular region of the epidermal growth factor receptor (EGFR) and induce a conformational change, leading to receptor dimerization and activation. Activated EGFR stimulates a variety of intracellular signaling pathways, including the mitogen-activated protein kinase pathway and the phosphoinositide 3-kinase/Akt pathway, leading to cell proliferation, cell survival, and angiogenesis (Yarden and Sliwkowski, 2001; Fischer et al., 2003). In tumors, the EGFR ligands are produced by either the malignant cells themselves or the

surrounding stromal cells, causing constitutive activation of EGFR (Salomon et al., 1995). Of the EGFR ligands, EREG is produced as a transmembrane precursor that exerts a mitogenic effect on various cell types, including hepatocytes and human cancer cells, in particular, on epithelial tumor cells (Toyoda et al., 1995). EREG is expressed at relatively low levels in most adult tissues and its expression has been found to be high in many human cancers, including those of the colon, breast, prostate, and ovary (Baba et al., 2000; Freimann et al., 2004; Tørring et al., 2005; Revillion et al., 2007). A number of studies have examined the involvement of EREG in tumorigenesis and showed the oncogenic effects of its overexpression. Because of its involvement in the oncogenesis, EREG has been considered as a therapeutic target. To this end, several anti-EREG murine monoclonal antibodies (mAbs) have been generated to successfully detect tumor cells in transplanted mouse models with high sensitivity (unpublished data). However, one of the primary problems in developing monoclonal antibodies as clinical drugs is the human anti-mouse antibody response (HAMA), which limits the administration of murine antibodies to humans (Carter, 2001). When humanizing the therapeutic murine anti-EREG antibody, the key issue is to preserve their affinity without losing the biological activity. The purpose of this study was to reduce the immunogenicity of the murine 9E5 monoclonal antibody through antibody humanization, while maintaining acceptable binding activity.

In this report, I describe the design, construction, and expression of humanized anti-EREG antibody accomplished by variable domain resurfacing studies performed on the basis of the computer-aided three-dimensional (3D) structure of the variable domain fragment (Fv) of a murine anti-EREG monoclonal antibody. Initially, human consensus sequence was used as a template to design the amino acid sequence of the variable region of humanized antibody. However, the initial version of humanized antibody (HM0) was shown to have significantly lower antigen-binding affinity. Grafting the

CDRs of murine antibodies onto appropriate human frameworks has often resulted in lower affinity or specificity for the target antigen. There are some general strategies for the recovering the binding affinity of humanized antibodies prepared by the CDR grafting method. These strategies often require several trial-and-error approaches (Gram et al., 1992). In this study, a reliable 3D model of the variable regions of the 9E5 antibody was built by computer-aided homology modeling. This molecular model suggested that the residue 49 in the light chain variable region (VL) underlying CDR was critical for antigen binding. The back mutation L49Y (tyrosine to histidine) generated the humanized version HM1, which produced an antibody that had binding ability similar to that of its murine counterpart. Importantly, mutation of one amino acid in the framework was sufficient to recover the binding capacity of a humanized antibody. Additionally, the heavy and light chain frameworks of HM1 showed a humanness score (Abhinandan and Martin, 2007) of 0.270 (heavy) and 0.092 (light), respectively, suggesting that the immunogenicity of the humanized anti-EREG antibodies was much lower than that of the original murine antibody. The reduced immunogenicity and enhanced capacity to bind to cancer cells may aid the application of the humanized anti-EREG antibody HM1 in clinical settings.

This study also supports the idea that HM1 contains a human IgG1 constant region that is effective in recruiting human immune system effector function, including complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). All these results show that the humanized anti-EREG antibody is successfully expressed as an IgG1 complete antibody and that this antibody is able to bind specifically to human colorectal carcinoma cells. The humanized anti-EREG antibody has the potential for further development as a candidate therapeutic antibody.