Increased sensitivity and specificity of immunohistochemical (IHC) detection of HER2 expression is crucial as the role of Trastuzumab has expanded in the treatment of HER2-positive non breast cancer patients such as gastric cancer patients. Non-specific nuclear and cytoplasmic staining of the HER2 antibody clone 4B5 has been reported by other labs. In this study, we evaluated the specificity of HER2 clone 4B5 and a new HER2 antibody clone UMAB36 using a suite of methods including protein lysate arrays, western blots, FISH and IHC correlation screens on 129 breast and 158 colon cancer cases for HER2 expression. The protein lysate array and western results revealed that clone 4B5 recognizes three proteins: HER2, HER4, and ZSCAN18, a nuclear transcription protein. In comparison, clone UMAB36 recognized only HER2 protein in the same protein lysate array and western screen as clone 4B5. False negative results, based on correlation of IHC with HER2 FISH positives, were generated using clone 4B5 in 1 breast and 5 gastric of the total 287 cancer cases screened. Comparatively, no false negative results were observed using clone UMAB36 in which there was a 100% correlation between IHC and FISH screen. In this study, areas of normal gastric tissue often stained positive by HER2 clone 4B5, so we performed further analysis of 470 normal gastric cases using Ventana's BenchMark instrument. The results show that 278 normal gastric cases had positive stain with clone 4B5 compared to 3 cases with clone UMAB36. The high background by clone 4B5 may be due HER4 being upregulated in adjacent normal gastric tissue. Our results indicate clone UMAB36 has higher sensitivity and specificity than clone 4B5 in screening gastric tumors.

Introduction

The HER2 protein (ErbB-2) is a 185-kDa transmembrane tyrosine kinase (TK) receptor and a member of the epidermal growth factor receptor (EGFR) family. HER2 overexpression/amplification has been shown to be increased in 20% breast cancer and recent studies have found that tumors such as gastric, uterine and ovarian cancer have increased levels of HER2 expression. Increasing number of clinical trials have shown that gastric cancer with HER2 overexpression can benefit from the treatment of neutralizing antibody, Herceptin currently used to treat HER2 breast cancer patients. In clinical setting, HER2 immunohistochemistry (IHC) is the primary screening method to diagnose HER2 amplification in tumor tissues. However, studies have shown anti-HER clone 4B5 4B5 cross-reacted other proteins, IHC staining also indicated that it has strong non-specific cytoplasmic and nuclear staining in normal gastric mucosal cells and some gastric cancer samples. Using a protein lysate array which covers 85% of the human proteome, we have successfully identified and confirmed that the 4B5 bound to HER4 and a nuclear protein ZSCAN18 besides HER2. The non-specific binding accounts for the unexpected cytoplasmic and nuclear staining of 4B5 on normal gastric epithelium. Finally, we have developed a novel HER2 mouse monoclonal antibody UMAB36 with similar sensitivity to 4B5 but only reacted to HER2 across the 17,000 proteins on the protein chip. In breast cancer and 158 gastric cancer samples, UMAB36 showed 100% sensitivity and specificity comparing to the HER2 FISH results with no unspecific staining in the gastric mucosa layer. UMAB36 could provide an alternative high specific IHC reagent for HER2 amplification testing in gastric cancer population.

Anti-HER2 clone 4B5 Recognizes HER2, EGFR4 & ZSCAN18 Proteins by Protein Microarray, Western, & IHC

Anti-HER2 clone UMAB36 Recognizes Only HER2 Protein by Protein Microarray, Western, & IHC

Conclusions

1. We used a high density protein microarray chip technology for antibody specificity screening.
2. Frequently used rabbit monoclonal HER2 antibody 4B5 cross-reacts to other proteins (HER4, ZSCAN18).
3. A novel HER2 monoclonal antibody UMAB36 exhibits higher specificity and similar sensitivity compared with 4B5.
4. UMAB36 higher specificity for IHC screening may make it a better reagent for evaluation of HER2 amplification test in gastric cancer patients.

References