Specificity Evaluation of Two HER2 Antibody Clones UMAB36 and 4B5 on Gastric and Breast Cancer Tissues. (UMAB36 Is Mono-Specific on HER2 While 4B5 Cross -reacts With Multiple Proteins In IHC Assays)

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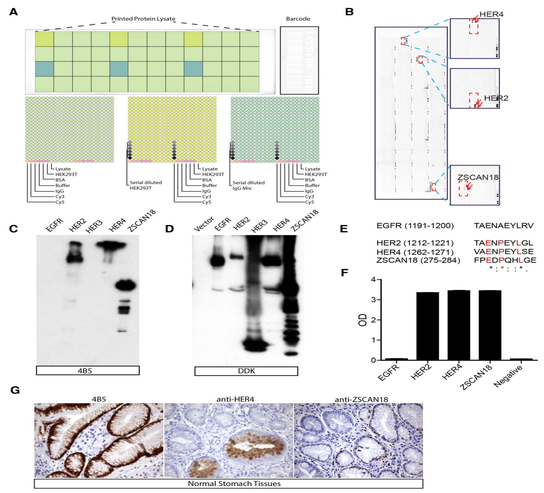
Abstract

Increased sensitivity and specificity of immunohistochemical (IHC) detection of HER2 expression is crucial as the role of Trastuzumab have expanded in the treatment of HER2 positive non breast cancer patients such as gastric cancer patients. Nonspecific 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 FISH HER2 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 specificity and sensitivity than clone 4B5 in screening gastric tumors.

Introduction

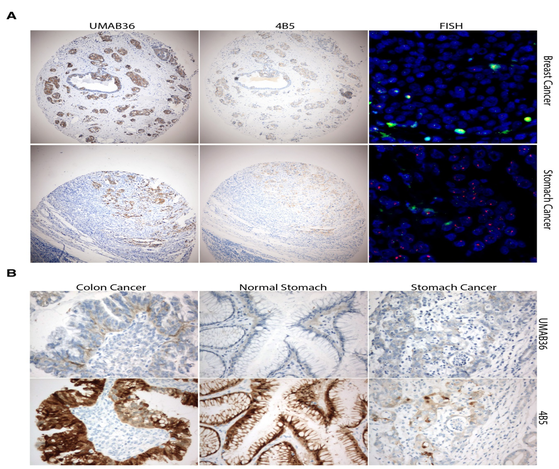
The HER2 protein (ErbB-2) is a 185-kDa transmembrane tyrosine kinase (TK) receptor and a member of the epidermal growth factor receptor (EGFR) family. HER2 overexpression/amplification has been shown to be increased in 20% breast cancer and recent studies have found that tumors such as gastric, uterine and ovarian cancer have increase 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 HER+ 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-HER2 clone 4B5 cross-reacted other proteins. IHC staining also indicated that it has strong non-specific cytoplasmic and nucleus 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 nunclear 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 129 breast cancer and 158 gastric cancer samples, UMAB36 showed 100% sensitivity and specificity comparing to the HER2 FISH reference 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



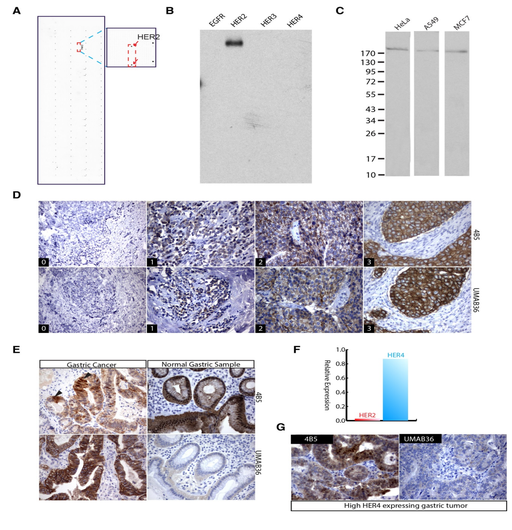
(A) Cartoon of 10K protein array chip. (B) HER2 clone 4B5 10k chip binding result showing HER2, HER4, and ZSCAN18 detected in screen. (C) Western blot analysis of HEK293T cell lysates expressing different DKK tagged EGFR family members and ZSCAN18 screened with HER2 clone 4B5 (D) Duplicate blot of HEK293T in panel C screened with anti-DDK (E) Overlapping sequences where clone 4B5 cross reacts with multiple (F) Relative HER4 and HER2 expression level of a representative gastric cancer tissue from OriGene's biorepository with high levels of HER4 but low levels of HER2 using qPCR analysis. (G) IHC staining by 4B5, HER4 and ZSCAN18 of the representative normal gastric tissues.

Anti- HER2 clone UMAB36 IHC Stain Correlated to FISH Test Better Than Clone 4B5



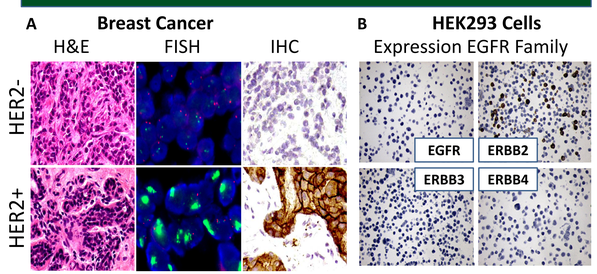
(A) Representative IHC and FISH imaging of the breast cancer (upper panel) and stomach cancer (lower panel) tissues where 4B5 (1+) and UMAB36 (2+) showed discrepant results. (B) Known negatives tissues of HER2 protein stained with HER2 clone UMAB36 upper panels and clone 4B5 lower panels.

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



(A) UMAB36 binding results on the 10K protein lysate chip. The block with HER2 positive signals were enlarged and the positive signals were pointed by red arrows. (B) Western blot analysis of HEK293T cell lysates expressing different DKK tagged EGFR family members with UMAB36. (C) Western blot analysis of endogenous HER2 with UMAB36 in different cancer cell lines. (D) IHC staining of breast cancer tissues with different HER2 scores using 4B5 (upper panel) or UMAB36 (bottom panel). (E) IHC staining of gastric cancer tissues (left) or normal gastric tissues (right) with 4B5 (upper) or UMAB36 (bottom). Cytoplasmic and nuclear 4B5 staining were pointed by arrowheads. (F) Relative HER4 and HER2 expression level of a representative gastric cancer tissue from OriGene's biorepository with high levels of HER4 but low levels of HER2 using qPCR analysis. (G) IHC staining by 4B5 (left) or UMAB36 (right) of the representative gastric cancer tissues in F which expressed high levels of HER4 but low level of HER2.

anti-HER2 clone UMAB36 IHC Specificity



(A) IHC staining with anti-HER2 mAb (UMAB36) on FISH identified HER2 negative upper panel or positive lower panel breast cancer tissue. (B) HEK293 cells overexpressing individual members of the EGFR family and stained with anti-HER2 clone UMAB36. Only ERBB2 positive HEK293 results in positive stain with clone UMAB36.

HER2 clone UMAB36 IHC Has High Correlation With HER2 FISH Test

Table 1. Clinical study of 129 breast cancer and 158 colon cancer cases of HER2 by evaluating IHC of anti-HER2 clone UMAB36 & 4B5 with HER2 FISH test.

			UMAB36				4B5			
Tissue Type	FISH Result	IHC Score / Cases	0	1	2	3	0	1	2	3
Breast	FISH NEG	93	29	38	26	0	51	29	13	0
	FISH POS	36	0	0	4	32	0	1	4	31
Stomach	FISH NEG	103	73	21	9	0	95	5	3	0
	FISH POS	55	0	0	10	45	0	5	5	45

Table 2. Clinical study of HER2 expression/amplification on pancreas, thyroid, colon, and ovarian cancer cases screen by IHC anti-HER2 (clone UMAB36 and 4B5) and FISH.

			UMAB36				4B5			
Tissue Type	FISH Result	IHC Score / Cases	0	1	2	3	0	1	2	3
Pancreas	FISH NEG	12	11	1	0	0	10	2	0	0
	FISH POS	0	0	0	0	0	0	0	0	0
Thyroid	FISH NEG	12	12	0	0	0	11	1	0	0
	FISH POS	0	0	0	0	0	0	0	0	0
Colon	FISH NEG	12	12	0	0	0	11	1	0	0
	FISH POS	0	0	0	0	0	0	0	0	0
Ovary	FISH NEG	11	6	4	1	0	9	2	0	0
	FISH POS	1	0	0	1	0	0	0	1	0

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

1. Ross JS. Update on HER2 testing for breast and upper gastrointestinal tract cancers. Biomark Med 2011;5(3):307-18.
2. Schrohl AS, et al. Epidermal growth factor receptor 2 (HER2) immunoreactivity: specificity of three pharmacodiagnostic antibodies. Histopathology 2011;59(5):975-83.
3. Arteaga CL, et al. Treatment of HER2-positive breast cancer: current status and future perspectives. Nat Rev Clin Oncol 2012;9(1):16-32.
4. Ma D, et al. Using protein microarray technology to screen anti-ERCC1 monoclonal antibodies for specificity and applications in pathology. BMC Biotechnology, 2012, 12: 88
5. Hechtman JF and Polydorides AD. HER2/neu gene amplification and protein overexpression in gastric and gastroesophageal junction adenocarcinoma: a review of histopathology, diagnostic testing, and clinical implications. Arch Pathol Lab Med 2012;136(6):691-7.
6. Fornaro L, et al. Anti-HER agents in gastric cancer: from bench to bedside. Nat Rev Gastroenterol Hepatol 2011;8(7):369-83.