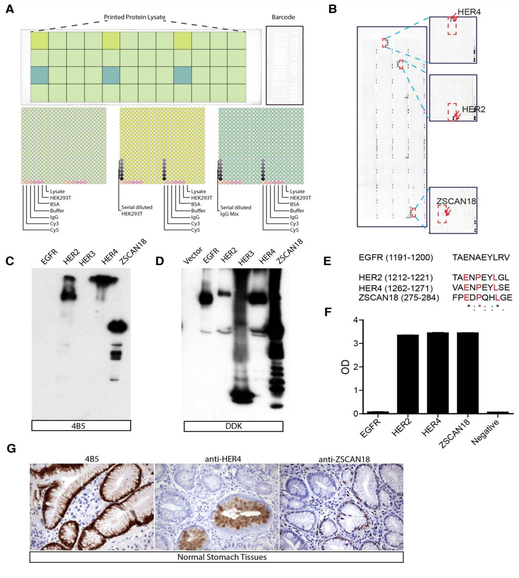
Develop a Highly Specific HER2 Antibody for IHC Screening in Breast and Gastric Cancers

Lixin Zhou[2], Kehu Yuan[1], Fangfang Ren[3], Lili Qi[1], Zhongwu Li[2], Guiyin Wu[1], Xiaozheng Huang[2], Yi Shen[1], Min Zhao[2], Wei Fu[1], Huibo Liu[1], Boyang Chu[1], Guangli Wang[1], Youmin Shu[1],Donghui Ma[1], Wei-wu He[1] & Jian Chen[4]
[1]OriGene technologies, 9620 Medical Center Dr., Suite 200, Rockville, MD 20850; [2]Department of pathology, Beijing Cancer Hospital, No.52 Fu-Cheng Road, Haidian District Beijing 100142, P.R.China; [3]Department of Human Anatomy and Histoembryology, Medical College of Soochow University, Suzhou 215123, P. R. China; [4]Institute of Functional Nano and Soft Materials (FUNSOM), Soochow University, Suzhou 215123, P. R. China.

Introduction

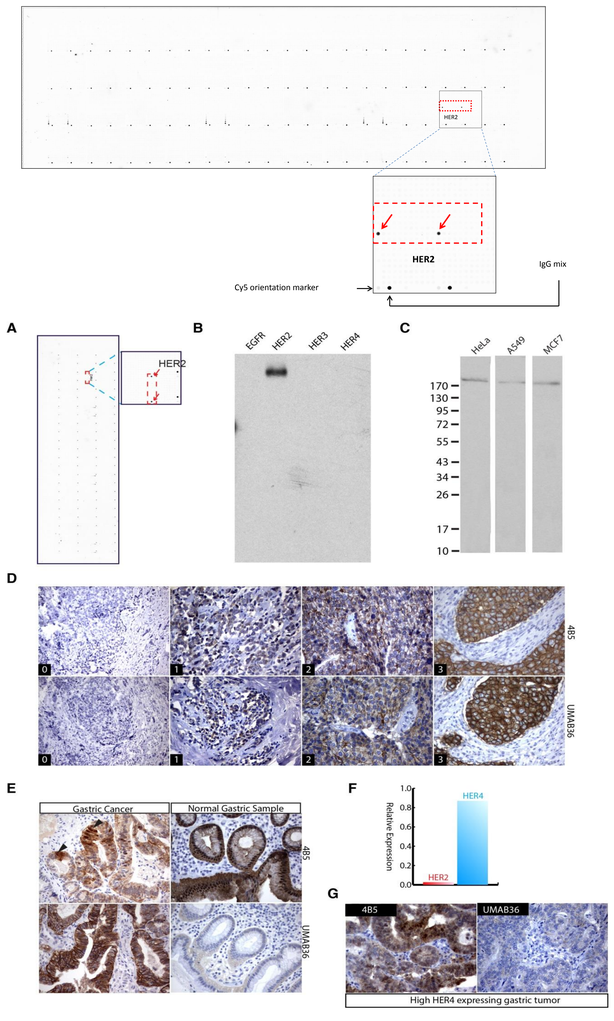
Human epidermal growth factor receptor 2 (HER2) is an orphan receptor tyrosine kinase member of the EGFR families and is found to be a key tumor driver gene. In breast cancer and gastric cancer, HER2 amplification can be effectively treated by its neutralizing antibody, Herceptin. In clinic, the HER2 immunohistochemistry (IHC) was used as the primary screening method to diagnose HER2 amplification. However, recent evidence suggested that the frequently used rabbit HER2 antibody 4B5 cross-reacted to another family member HER4. 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 unclear 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.

The most commonly used HER2 diagnostic monoclonal antibody (4B5) is not specific



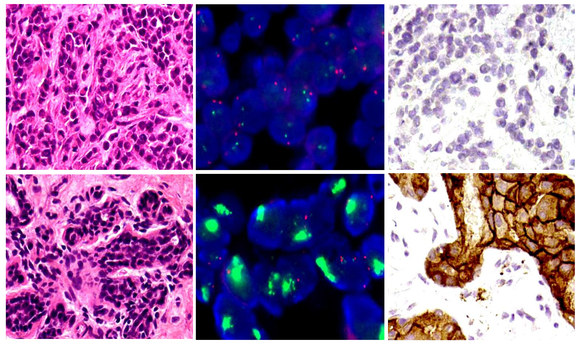
By using OriGene's 10K protein microarray chip, we discovered that Roche's PATHWAY HER2 (4B5 clone) cross-reacts with ZSCAN18 and Her4. The cross-reactivity was confirmed by WB, ELISA and IHC tests.

Ultra-specific HER2 antibody (UMAB36) for anatomic pathology application



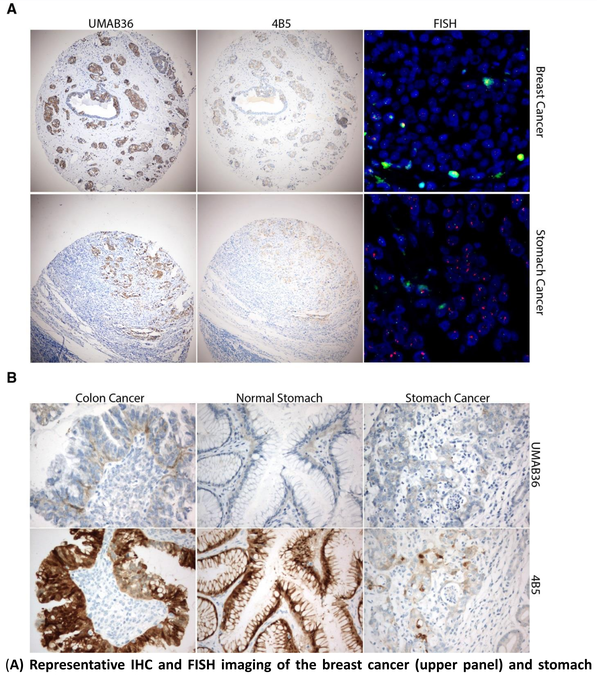
(A) UMAB36 binding results on the 10K protein lysate chip. The block with HER2 positive signals were enlarged and the positive signals were pointed by red arrows. (B) Western blot analysis of HEK293T cell lysates expressing different DKK tagged EGFR family members with UMAB36. (C) Western blot analysis 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.

UMAB36 IHC performance matches to the FISH test



IHC staining on FISH identified HER2 negative or positive breast cancer tissue with an ultra-specific anti-HER2 mAb (UMAB36).

4B5 and UMAB36 IHC staining and FISH tests on large collections of tumor tissues



(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) IHC results of 4B5 and UMAB36 in normal colon (left), normal stomach (middle) and stomach cancer (right).

Both 4B5 and UMAB36 IHC scores have high correlation with FISH results

Table 1. Summary of HER2 scores diagnosed by UMAB36, 4B5 and FISH in breast cancer and stomach cancer cases.

Tissue Type	FISH Result	IHC Score / Cases	UMAB36 0	UMAB36 1	UMAB36 2	UMAB36 3	4B5 0	4B5 1	4B5 2	4B5 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. Summary of HER2 scores by UMAB36, 4B5 and FISH in pancreas, thyroid, colon and ovarian cancer samples.

Tissue Type	FISH Result	IHC Score / Cases	UMAB36 0	UMAB36 1	UMAB36 2	UMAB36 3	4B5 0	4B5 1	4B5 2	4B5 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 developed 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 could be a better IHC screening reagent for 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.