Human Epidermal Growth-Factor Receptor 2 Overexpression in Gastric Carcinoma in Thai Patients

Pattana Sornmayura MD*, Budsaba Rerkamnuaychoke DMSc*,
Artit Jinawath MD, PhD*, Chakrapan Euanorasetr MD**

** Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
*** Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*

Objective: *To determine human epidermal growth-factor receptor 2 (HER2) protein-overexpression frequency and the concordance rate between immunohistochemistry and fluorescence in situ hybridization techniques in gastric carcinoma.*
Material and Method: *A retrospective analysis of gastric adenocarcinomas obtained from 224 adult patients between January 2000 and December 2008 were performed. The paraffin-embedded tissues were sliced into 4-μm-thick sections and analyzed for HER2 protein expression levels by immunohistochemistry (IHC) using an automated slide-staining IHC system. Breast carcinoma tissues were included in every staining batch as positive control. In order to detect and quantify amplification of the HER2, the authors performed fluorescence in situ hybridization (FISH) using PathVysion® HER2 DNA Probe Kit. The IHC results were independently recorded by two pathologists using the standard HER2 scoring system for gastric carcinoma. FISH results were interpreted using standard guideline as employed in breast carcinoma. The two-tailed-Fisher's exact test was used to assess the concordance between IHC and FISH results.*
Results: *HER2 protein overexpression level was identified in 9% (20 in 224 cases) of the gastric tumors; 80% of which were well or moderately differentiated and of the intestinal or mixed type. However, HER2-overexpressing tumors comprised only 16% of the intestinal/mixed-type or well/moderately differentiated tumors. There was no signal obtained from 29 specimens from FISH studies. Thus, the overall results of IHC and FISH methods were concordant in 88% (171 out of 195, $p < 0.001$).*
Conclusion: *There is a significant concordance rate between IHC and FISH in gastric carcinoma. The present study is the first HER2 study of such carcinoma in Thai patients.*

Keywords: *Gastric cancer, Gastric adenocarcinoma, HER2, Immunohistochemistry, FISH*

J Med Assoc Thai 2012; 95 (1): 88-95
Full text. e-Journal: *http://www.jmat.mat.or.th*

In 1999, the most recent year for which comprehensive data are available, stomach cancer was the eighth most common type of cancer diagnosed in men in Thailand, the estimated age-standardized incidence rate for males and females in Thailand was 4.1 and 2.4 per 100,000, respectively[1]. The average rate of approximately 3/100,000 per year[2] is much lower than that found in Japan, (46.8/100,000 per year), China (41.3/100,000 per year), or Korea (62.2/100,000 per year)[3]. Despite this relatively low rate, new strategies for the clinical management of gastrointestinal carcinoma in Thailand are needed. In its advanced stages, gastric cancer is not treatable through surgical resection, which is the standard treatment for early-stage disease, and has poor outcome despite the use of new treatment strategies, such as perioperative chemotherapy and adjuvant chemoradiation[4,5].

Human epidermal growth-factor receptor 2 (HER2) is a transmembrane receptor tyrosine kinase that regulates intracellular signals affecting cell growth, differentiation and survival through binding to various ligands[6]. Overexpression levels of HER2 resulting from amplification of HER2 is observed in 15 to 25% of breast cancers and is considered a predictor of aggressive tumor growth and poor patient outcomes[6]. Because treatment with an antibody that targets HER2 has been shown to improve clinical and survival outcomes for breast cancer[6,7], HER2 status has also been examined in gastric carcinoma. These studies have suggested that HER2 level is elevated in approximately 16 to 23% of gastric carcinoma cases[8-10] and that HER2 overexpression

Correspondence to:
Sornmayura P, Department of Pathology, Ramathibodi Hospital, Mahidol University, 270 Rama VI Rd, Bangkok 10400, Thailand.
Phone: 0-2201-1432, Fax: 0-2354-7266
E-mail: p2517s@yahoo.com

level correlates with poor prognosis and increased aggressiveness of the disease[11]. Furthermore, recently published data from a randomized controlled trial indicate that HER2-targeted treatment improves chemotherapeutic outcome in advanced HER2-positive gastric cancer[12]. However, many more studies are needed, including some that begin to address the possible differences in HER2 status in gastric carcinomas in the Eastern versus Western worlds or among individual countries.

In the present study, the authors examined the HER2 status of 224 gastric carcinoma cases in Thailand using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) techniques to detect HER2 level and HER2 amplification respectively. Specifically, the authors' objectives were (1) to assess the incidence of HER2 status in gastric carcinoma; (2) to examine the relationship between the histologic characteristics (grade and stage) of the tumors and the IHC results; and (3) to determine the concordance between IHC and FISH results in analyzing HER2 status in gastric carcinoma.

Material and Method

The authors performed a retrospective analysis of gastric adenocarcinomas obtained from 224 adult patients (121 men and 103 women) in Ramathibodi Hospital (Bangkok, Thailand) between January 2000 and December 2008 (inclusive). The age range of the study population was 18 to 87 years (median, 58 years). The tissues had been obtained by resection, fixed in buffered 10% formalin and paraffin-embedded. For the present study, the paraffin-embedded tissues were sliced into 4-μm-thick sections and analyzed for HER2 level by IHC using a Ventana automated slide-staining IHC system (Benchmark XT; Ventana Medical Systems, Tucson, AZ, USA) and Pathway anti-HER-2/neu (4B5) rabbit monoclonal primary antibodies as the immunoreagent (Ventana Medical Systems). As a positive control, paraffin-embedded sections of invasive breast carcinoma tissue were included in every staining batch.

To detect and quantify amplification of HER-2, the authors performed FISH using a PathVysion HER2 DNA Probe Kit (Abbott Molecular, Abbott Park, IL, USA). This kit uses two labeled probes, a Spectrum Orange-labeled probe for the detection of HER-2 DNA and a Spectrum Green-labeled chromosome enumeration probe 17 (CEP17). This latter probe hybridizes to alpha satellite DNA located at the centromere of chromosome 17 and acts as an internal control that allows the determination of the relative number of HER2 gene copies. All procedures were carried out according to the manufacturer's instructions, and results were recorded as positive or negative for HER2 overexpression (HER2-positive or HER2-negative by FISH respectively). Cases failing to yield either a positive or a negative signal were excluded from the analysis.

The collected IHC data included patient age and sex, tumor site (gastric or gastroesophageal junction), width (longest dimension), histologic type (Lauren's classification, intermediate, diffuse, or mixed)[13], histologic grade (World Health Organization (WHO) classification, well, moderately, or poorly differentiated or undifferentiated)[14], depth of invasion, lymph node metastasis (TNM classification)[14], HER2 IHC staining pattern (membranous (partial, U-shaped, or complete) or cytoplasmic), HER2 IHC staining intensity (strong, weak, or faint) and percentage of cells exhibiting HER2 IHC immunoreactivity. The IHC findings were independently interpreted by two pathologists (blind to each other) applying a four-tiered scoring system recommended by a consensus panel on HER2 scoring for gastric cancer[15]. This system defines score 0 as undetectable reactivity or membranous reactivity in less than 10% of tumor cells; score 1+ as faint/barely perceptible membranous reactivity in more than 10% of tumor cells (partially membranous staining); score 2+ as weak-to-moderate complete or basolateral membranous reactivity in more than 10% of tumor cells and score 3+ as moderate-to-strong complete or basolateral membranous reactivity in more than 10% of tumor cells. Tumors scored as 2+ or 3+ by both pathologists were taken as IHC-positive for HER2 overexpression level (HER2-positive by IHC).

For statistical analysis, the two-tailed-Fisher's exact test was used to assess the concordance between the IHC and FISH results. Results are considered statistically significant at $p < 0.05$.

The present study was proved by ethical clearance committee on human rights related to researches involving human subjects, faculty of medicine, Ramathibodi hospital, Mahidol University (ID 04-52-44).

Results

The authors performed a retrospective study of 224 cases of gastric adenocarcinoma in adult men and women who had undergone tumor resection in Thailand between January 2000 and December 2008.

All but 15 of the cases were gastric tumors, and the remainder was esophagogastric junction tumors. Most of the tumors were diffuse and poorly differentiated, but a substantial fraction was of the intestinal or mixed type or exhibited high or moderate differentiation. The specific grades and stages are shown in Table 1 together with other tumor characteristics, including size, T-stage depth of invasion and N-stage regional lymph node metastasis.

As shown in Table 2, IHC analysis of HER2 level revealed 20 cases exhibiting complete/basolateral (U-shaped) membrane staining in at least 10% of the tumor cells; of these cases, staining was intense in 14 cases (IHC score 3+) and weak/moderate in 6 cases (IHC score 2+). There were five cases of partial membrane staining with faint density in at least 10% of tumor cells (IHC score 1+) and 199 cases with variable patterns and densities of staining in less than 10% of tumor cells (IHC score 0). IHC scores of 2+ and 3+ were considered positive for HER2 overexpression status, yielding a HER2-positive rate by IHC of 9% ($p = 0.002$).

Categorization of the IHC data according to histologic type (intestinal, diffuse, or mixed)[13] and histologic grade (well, moderately, or poorly differentiated)[14] revealed that 80% of the tumors identified HER2-positive (scores of 2+ or 3+) were well or moderately differentiated intestinal or mixed-type tumors. Of the intestinal and mixed-type tumors, 16% were HER2-positive and of the well and moderately differentiated tumors, 16% were IHC-positive.

FISH studies identified 34 of the 224 cases as positive for HER2 amplification and 161 cases as negative, but no signal was obtained for the remaining 29 samples (Table 3). Of the 20 cases scored as HER2-positive by IHC (scores of 2+ or 3+), 15 also scored positive by FISH, for a concordance rate of 15/20 (75%) ($p < 0.001$), and of 204 cases scored as HER2-negative by IHC (scores 0 or 1+), 156 also scored negative by FISH, for a concordance rate of 156/175 (89%) ($p < 0.001$). Overall, the results of the IHC and FISH methods were concordant in 88% (171) of the 195 cases that returned positive or negative FISH results ($p < 0.001$).

Discussion

Overexpression of HER2, as detected by IHC analysis of HER level or by FISH analysis of HER2 amplification, is a proven indicator of increased tumor aggressiveness and decreased overall survival in

Table 1. Characteristics of gastric tumors examined in the present study (n = 224)

Parameter	Type	Number
Width[a]	Median	4.35 cm
	Range	0.4-13 cm
Site	Esophagogastric junction	15
	Gastric	209
Type (histologic)[b]	Intestinal	49
	Diffuse	122
	Mixed	53
Grade[c]	Well differentiated	39
	Moderately differentiated	62
	Poorly differentiated	123
Invasion depth (TNM T-stage)[c]	T_1	14
	T_2	138
	T_3	59
	T_4	13
Regional lymph node metastasis (TNM N-stage)[c]	N_x	6
	N_1	41
	N_2	77
	N_3	65
	N_4	35

[a] Measured in the longest dimension
[b] Lauren's classification[13]
[c] WHO criteria[4]

Table 2. Rate of HER2 overexpression status in surgically resected gastric carcinomas, categorized by tumor type and grade

IHC score[a]		Type[b]			Grade[c]		
Grade	Total	Intestinal	Diffuse	Mixed	WD[c]	MD[c]	PD[c]
0	199	36	116	47	27	55	117
1+	5	2	2	1	2	1	2
2+	6	2	2	2	1	3	2
3+	14	9	2	3	9	3	2
Total	224	49	122	53	39	62	123
Total positive[d]	20	11	4	5	10	6	4
% positive[e]	8.9	22.4	3.3	9.4	25.6	9.7	3.3

[a] IHC scoring based on the recommendations of a consensus panel on HER2 scoring for gastric cancer[9]
[b] Histologic type (Lauren's classification)[13]
[c] Grade of differentiation (WHO criteria)[4] WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated
[d] Number of tumors of the type or grade shown that are HER2-positive (scored 2+ or 3+)
[e] Percentage of tumors of each type or grade that are HER2-positive

Table 3. Concordance between results of immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) analysis of HER2 overexpression status[a] in 224 surgically resected gastric tumors

IHC result		FISH result[b]			Concordance		
Score	n	NS[c]	Positive	Negative	n	Fraction	Rate (%)
0	199	28	19	152	199	152/171	89
1+	5	1	0	4	5	4/4	100
2+	6	0	3	3	6	3/6	50
3+	14	0	12	2	14	12/14	86
Total	224	29	34	161	224	171/195	88

[a] HER2 overexpression is defined as an IHC score of 2+ or 3+ or a positive FISH result
[b] Shading indicates concordant results (HER2-negative by both IHC and FISH or HER2-positive by both IHC and FISH)
[c] No signal obtained in FISH analysis

breast carcinoma[11]. HER2 overexpression is detected by IHC or FISH in 10 to 34% of invasive breast carcinoma cases[11] and the concordance rate between these two techniques is 73 to 98%[16]. A HER2-targeting antibody that blocks HER2-mediated signal transduction and thereby inhibits tumor cell proliferation and progression has been used successfully in the treatment of HER2-overexpressing (HER2-positive) breast carcinomas[17]. The success of HER2-targeted antibody therapy in breast carcinoma treatment has led to a variety of studies examining HER2 expression in carcinomas of the colon, bladder, ovary, endometrium, lung, cervix, head and neck, esophagus, and gastrointestinal tract[11]. In the present study, the authors examined 224 gastric carcinomas that had been surgically resected in Thailand and IHC analysis identified 20 (9%) of the cases as HER2-positive.

In a previous study of gastric carcinoma in Japan, Kameda et al[18] scored samples according to the number of HER2-immunoreactive cells and the intensity of the IHC staining reaction using a grading system ranging from minus (-) to 4+; if the 3+ and 4+ scores are assumed to be positive, then three (9%) of the 34 cases are positive, a rate similar to that found in the present study. In other Asian studies, Park et al[9] and Yano et al[10] reported that 16% of 182 cases and 23% of 200 cases, respectively are HER2-positive. The study reported by Yano et al[10] differed from the authors in its inclusion criteria; they only examined

invasive, intestinal-type gastric cancers, a histologic type that has been reported by several authors to have a high correlation with HER2 expression[19-22]. In previous studies of gastric carcinoma in Europe, Gravalos et al[23] Lemoine et al[19] and Allgayer et al[24] reported that 13% of 166 cases, 26% of 39 cases and 91% of 203 cases, respectively, are HER2-positive. The extremely high rate reported by Allgayer et al[24] may have been a consequence of their application of a high-sensitivity test and the scoring of IHC-positive cells using both membranous and cytoplasmic staining patterns.

Classification of the histologic findings in gastric carcinoma tends to yield the same histologic picture whether the Lauren[13] or WHO[14] criteria are used; the intestinal, mixed, and diffuse types of Lauren generally correspond to the well, moderately and poorly differentiated WHO grades, respectively. In our analysis, 80% of the HER2-positive cases were of the intestinal and mixed types or were well or moderately differentiated. However, the data have clearly shown that not all tumors of these particular types and grades have overexpressed HER2; only 16% of the intestinal/mixed-type tumors or 16% of the well/moderately differentiated tumors were HER2-positive. Several Asian and European reports have also noted a high correlation between HER2 overexpression level and well-differentiated/intestinal-type gastric tumors[23,19] and between HER2 amplification in intestinal-type gastric cancer[11,9], while E-cadherin mutations are most frequently found in diffuse-type tumors[11].

Methodological differences may be responsible for some of the variations in the reported data among Asian studies. Yano et al[10] studied only intestinal-type gastric carcinomas and Park et al[9] did not classify their IHC-positive tumors according to histologic type. In the study by Kameda et al[18], all three IHC-positive tumors are a papillary variant of the intestinal type. Why IHC-positive cases tend to be intestinal-type tumors and why some intestinal-type tumors do not overexpress HER2, is unknown, but the HER2-immunostaining staining pattern and heterogeneity characteristics in intestinal-type tumors are qualitatively different from those in breast carcinoma. Incomplete membrane staining in a U-shaped or basolateral pattern is frequently found in intestinal-type tumors and is usually interpreted as having the same meaning as complete membrane staining (Fig. 1a, 1b). Heterogeneous staining occurs at least three times as frequently in gastric tumors with moderate or strong HER2 overexpression level

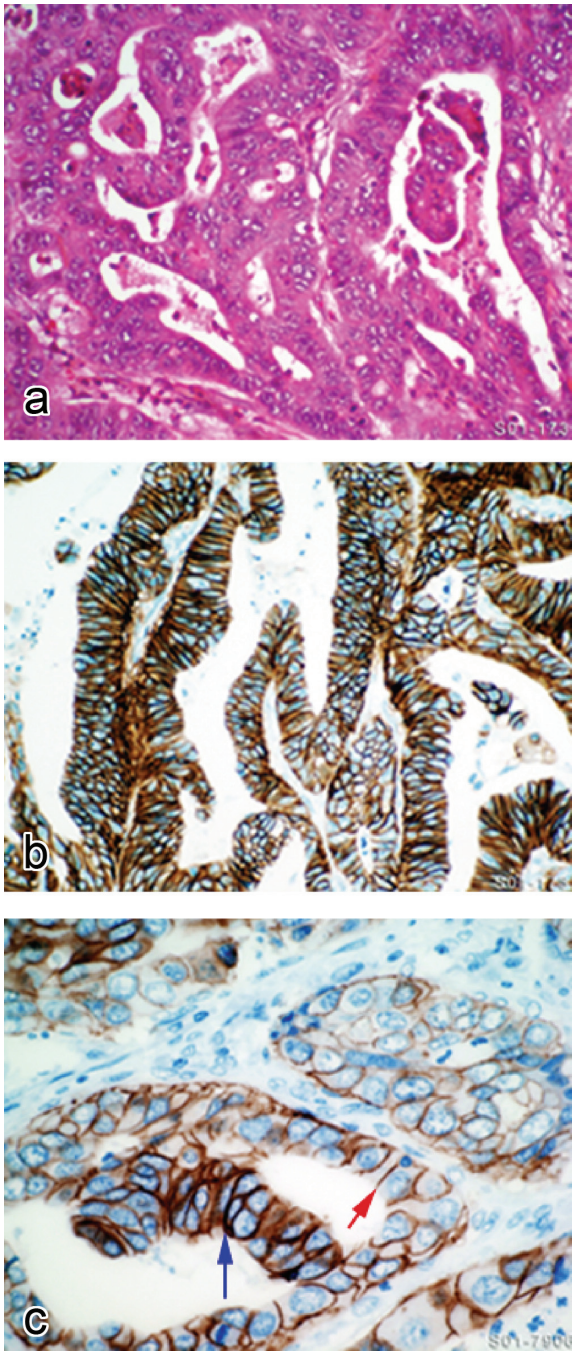

Fig. 1 The histology of intestinal type gastric carcinoma with pattern of HER2 IHC staining (a) gastric adenocarcinoma, intestinal type, Hematoxylin and eosin stain, x200; (B) HER2 expression level in intestinal type carcinoma, note the strong basolateral or "U"-shaped pattern; IHC analysis for HER2, x200; (C) IHC staining for HER2 yielding a heterogeneous staining pattern ranging from intense (large arrow) to faint (small arrow), x400

(Fig. 1c) as in breast carcinomas[15]. Future in-depth studies are needed to conclusively demonstrate a correlation between gastric cancer histology and HER2 overexpression status as detected by IHC.

Theoretically, HER2 amplification leading to HER2 overexpression status in the cellular membrane induces a molecular cascade promoting malignant transformation[25]. However, the discrepancies among the concordance results reported in the Asian studies suggest an unusual molecular mechanism. Noting that HER2 overexpression level can occur in the absence of HER2 amplification, Kameda et al[18] have suggested that gene amplification may not be the primary mechanism of HER2 overexpression status in gastric cancer. Indeed, HER2 overexpression might involve a different mechanism, such as upregulation of HER2 transcription or transcriptional activation by other genes or post-transcriptional events[17,26].

The authors' results demonstrate that the concordance rate between IHC and FISH results was high (88%) for gastric cancers in Thailand. Furthermore, intestinal and mixed-type tumors (well or moderately differentiated) were found to have a high incidence of HER2 overexpression level (correlation of 80% for IHC-positive cases). The 13% non-concordance rate observed in the present study has suggested that HER2 overexpression level (as determined by IHC) could occur without HER2 amplification (as determined by FISH) and vice versa. Additional studies are needed, which incorporate improved tissue sampling and handling and in which possible confounding factors, such as variation in the duration of formalin fixation, are eliminated, are needed. Because tumor samples containing cells scoring 2+ or 3+ in IHC immunoreactivity were scored as IHC-negative when these high-scoring cells comprise less than 10% of all cells, tissue sampling has a direct effect on IHC interpretation.

Despite the low overall incidence of HER2 overexpression status, the high incidence of gastric cancer in the Thai population means that a substantial number of patients stand to benefit from effective HER2-targeted treatment. The authors' basic data will require clinical correlation with survival rates, tumor stages, and management strategies to provide insight into the therapeutic potential of HER2 as a target for gastric cancer therapy.

Acknowledgments

The authors wish to thank Dr. Amnuay Thithapandha for his advice in preparing this paper. Professional editorial services were provided by L.B. Murata, PhD.

Potential conflicts of interest

Supported by Roche Thailand Limited and Roche Diagnostics (Thailand) Limited.

References

1. Attasara P, Srivatanakul P, Sriplung H. Cancer incidence in Thailand: stomach. In: Khuhaprema T, Srivatanakul P, Attasara P, Sriplung H, Wiangnon S, Sumitsawan Y, editors. Cancer in Thailand (2001-2003). Vol. V. Bangkok: Bangkok Medical Publisher; 2010:25-7.
2. Vilaichone RK, Mahachai V, Tumwasorn S, Wu JY, Graham DY, Yamaoka Y. Molecular epidemiology and outcome of Helicobacter pylori infection in Thailand: a cultural cross roads. Helicobacter 2004; 9: 453-9.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010 Jun 17. [Epub ahead of print; PMID: 20560135].
4. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 2006; 355: 11-20.
5. Macdonald JS, Smalley SR, Benedetti J, Hundahl SA, Estes NC, Stemmermann GN, et al. Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 2001; 345: 725-30.
6. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353: 1659-72.
7. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344: 783-92.
8. Kim MA, Jung EJ, Lee HS, Lee HE, Jeon YK, Yang HK, et al. Evaluation of HER-2 gene status in gastric carcinoma using immunohistochemistry, fluorescence in situ hybridization, and real-time quantitative polymerase chain reaction. Hum Pathol 2007; 38: 1386-93.
9. Park DI, Yun JW, Park JH, Oh SJ, Kim HJ, Cho YK, et al. HER-2/neu amplification is an independent

prognostic factor in gastric cancer. Dig Dis Sci 2006; 51: 1371-9.
10. Yano T, Doi T, Ohtsu A, Boku N, Hashizume K, Nakanishi M, et al. Comparison of HER2 gene amplification assessed by fluorescence in situ hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer. Oncol Rep 2006; 15: 65-71.
11. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. Ann Oncol 2008; 19: 1523-9.
12. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376: 687-97.
13. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965; 64: 31-49.
14. Fenoglio-Preiser CM, Carneiro F, Correa O, Guilford P, Lambert R, Megraud F, et al. Tumors of the stomach. In: Hamilton SR, Aaltonen LA, editors. World Health Organization Classification of Tumors. Pathology and genetics of tumors of the digestive system. Lyon: IARC Press; 2000: 37-52.
15. Hofmann M, Stoss O, Shi D, Buttner R, van de Vijver M, Kim W, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology 2008; 52: 797-805.
16. Schnitt SJ. Breast cancer in the 21st century: neu opportunities and neu challenges. Mod Pathol 2001; 14: 213-8.
17. Garcia I, Vizoso F, Martin A, Sanz L, Abdel-Lah O, Raigoso P, et al. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. Ann Surg Oncol 2003; 10: 234-41.
18. Kameda T, Yasui W, Yoshida K, Tsujino T, Nakayama H, Ito M, et al. Expression of ERBB2 in human gastric carcinomas: relationship between p185ERBB2 expression and the gene amplification. Cancer Res 1990; 50: 8002-9.
19. Lemoine NR, Jain S, Silvestre F, Lopes C, Hughes CM, McLelland E, et al. Amplification and overexpression of the EGF receptor and c-erbB-2 proto-oncogenes in human stomach cancer. Br J Cancer 1991; 64: 79-83.
20. Lin JT, Wu MS, Shun CT, Lee WJ, Sheu JC, Wang TH. Occurrence of microsatellite instability in gastric carcinoma is associated with enhanced expression of erbB-2 oncoprotein. Cancer Res 1995; 55: 1428-30.
21. Polkowski W, van Sandick JW, Offerhaus GJ, ten Kate FJ, Mulder J, Obertop H, et al. Prognostic value of Lauren classification and c-erbB-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction. Ann Surg Oncol 1999; 6: 290-7.
22. Wu MS, Shun CT, Wang HP, Sheu JC, Lee WJ, Wang TH, et al. Genetic alterations in gastric cancer: relation to histological subtypes, tumor stage, and Helicobacter pylori infection. Gastroenterology 1997; 112: 1457-65.
23. Gravalos C, Marquez A, Garcia-Carbonero R, Rivera F, Colomer R, Sastre J, et al. Correlation between HER2/neu overexpression/amplification and clinicopathological parameters in advanced gastric cancer patients: a prospective study. Proceedings of the 2007 ASCO Gastrointestinal Cancers Symposium (Abstract No 89). January 19-21, 2007. Orlando, Florida.
24. Allgayer H, Babic R, Gruetzner KU, Tarabichi A, Schildberg FW, Heiss MM. c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. J Clin Oncol 2000; 18: 2201-9.
25. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244: 707-12.
26. Hollywood DP, Hurst HC. A novel transcription factor, OB2-1, is required for overexpression of the proto-oncogene c-erbB-2 in mammary tumour lines. EMBO J 1993; 12: 2369-75.

การตรวจโปรตีน HER2 ของมะเร็งกระเพาะอาหารในผู้ป่วยไทย

พัฒนา ศรมยุรา, บุษบา ฤกษ์อำนวยโชค, อาทิตย์ จินาวัฒน์, จักรพันธ์ เอื้อนรเศรษฐ์

วัตถุประสงค์: *เพื่อค้นหาอุบัติการณ์การแสดงออกของโปรตีน human epidermal growth-factor receptor 2 (HER2) และอัตราความสอดคล้องระหว่างเทคนิคทาง immunohistochemistry (IHC) กับเทคนิค fluorescence in situ hybridization (FISH) ซึ่งใช้ตรวจหาโปรตีนและ HER2 gene amplification ในมะเร็งกระเพาะอาหาร*

วัสดุและวิธีการ: *ศึกษาข้อมูลย้อนหลังในผู้ป่วยทุกรายที่เป็นโรคมะเร็งในกระเพาะอาหารและได้รับการผ่าตัดกระเพาะจำนวน 224 ราย ระหว่างปี พ.ศ. 2543 ถึง พ.ศ. 2551 โดยใช้ชิ้นเนื้อที่ฝังในพาราฟินนำมาตัดให้หนาประมาณ 4 ไมครอน แล้วนำมาวิเคราะห์หาระดับโปรตีน HER2 ด้วยเทคนิคทาง IHC โดยใช้ระบบย้อมสีอัตโนมัติของเครื่อง Ventana ทั้งนี้ได้ใช้ชิ้นเนื้อที่เป็นมะเร็งเต้านมเป็น positive control สำหรับชิ้นเนื้อที่ทำการศึกษาทุก ๆ ชุด และยังวิเคราะห์หา HER2 gene amplification ซึ่งใช้เทคนิค FISH ด้วย Path Vysion HER2 DNA Probe Kit ขั้นตอนทั้งหมดดำเนินการตามวิธีการใช้งานของผู้ผลิตเครื่องมือ ผลของการศึกษาด้วย IHC ได้แปลผลและบันทึกโดยพยาธิแพทย์สองคน โดยใช้เกณฑ์มาตรฐานสำหรับระดับ HER2 ในมะเร็งกระเพาะอาหารส่วนผลของ FISH นั้นใช้การแปลผล โดยอาศัยเกณฑ์มาตรฐานเดียวกันกับมะเร็งเต้านม อัตราความสอดคล้องระหว่างผลของ IHC และ FISH นั้นได้ประเมินโดยใช้ two-tailed-Fisher's exact test*

ผลการศึกษา: *พบโปรตีน HER2 overexpression 8.9 % ของผู้ป่วยทั้งหมด (20 ใน 224 ราย) ซึ่งพบว่า 80% ของมะเร็งเหล่านี้เป็นกลุ่ม well และ moderately differentiated หรือ intestinal และ mixed type อย่างไรก็ดีในทางกลับกันพบว่าเพียง 16% ของมะเร็งชนิด intestinal/mixed type หรือ well/moderately differentiated ที่จะมีโปรตีน HER2 overexpression อัตราความสอดคล้องระหว่างผลของ IHC และ FISH ที่ได้คือ 87.7% หรือ 171 ใน 195 ราย เนื่องจากมีจำนวน 29 ราย ที่ไม่มีสัญญาณให้ตรวจวิเคราะห์ได้จากการศึกษาด้วยเทคนิค FISH*

สรุป: *อุบัติการณ์ของโปรตีน HER2 overexpression ในผู้ป่วยไทยค่อนข้างต่ำเมื่อเทียบกับการศึกษาอื่น ๆ แต่พบว่ามีอัตราความสอดคล้องสูงระหว่างการศึกษาด้วยเทคนิค IHC และ FISH เช่นเดียวกับที่พบในมะเร็งเต้านมการศึกษานี้เป็นการศึกษาแรกสำหรับ HER2 ของมะเร็งกระเพาะอาหารในผู้ป่วยไทย*