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

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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

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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...
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) 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.

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 (ID04-52-44).

**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 esophago gastric 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>
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<tr>
<th>Parameter</th>
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<tr>
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</tr>
<tr>
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<tr>
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<td>T3</td>
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<tr>
<td>N4</td>
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*a Measured in the longest dimension
b Lauren’s classification(13)
c WHO criteria(4)
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...
This page includes a discussion on the correlation between HER2 expression and various histologic types of gastric cancer. The text highlights the intestinal-type gastric cancers, which have been reported to have a high correlation with HER2 expression. Previous studies in Europe and Asia have reported varying rates of HER2-positive cases, with some attributed to methodological differences in testing.

Classification of histologic findings in gastric carcinoma tends to yield the same histologic picture regardless of whether the Lauren or WHO criteria are used. The intestinal, mixed, and diffuse types of Lauren generally correspond to well, moderately, and poorly differentiated WHO grades, respectively. Analysis of HER2-positive cases revealed that while 80% were intestinal or mixed types or well or moderately differentiated, only 16% of intestinal/mixed-type tumors or 16% of well/moderately differentiated tumors were HER2-positive.

Methodological differences may explain variations in the reported data among Asian studies. Yano et al. studied only intestinal-type gastric carcinomas and Park et al. did not classify their IHC-positive tumors according to histologic type. In the study by Kameda et al., all three IHC-positive tumors were a papillary variant of the intestinal type.

The text also mentions that 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. Heterogeneous staining occurs at least three times as frequently in gastric tumors with moderate or strong HER2 overexpression level.

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.

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Potential conflicts of interest

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9. Park DI, Yun JW, Park JH, Oh SJ, Kim HJ, Cho YK, et al. HER-2/neu amplification is an independent


การตรวจโปรตีน HER2 ของมะเร็งกระเพาะอาหารในภูมิไทย

พัฒนา ศรุงยา, บุญมา ถุกฤทธิ์ชัย, อาทิศ จินาวัฒน์, จักรพันธ์ เอื้อนรเศรษฐ์

วัตถุประสงค์: เพื่อค้นหาอุปกรณ์การแสดงออกของโปรตีน human epidermal growth-factor receptor 2 (HER2) และวิเคราะห์ความสอดคล้องระหว่างเทคนิคทาง immunohistochemistry (IHC) กับเทคนิค fluorescence in situ hybridization (FISH) ซึ่งใช้วิเคราะห์โปรตีน HER2 ของมะเร็งกระเพาะอาหาร จำนวน 224 ราย ระหว่างปี พ.ศ. 2543 ถึง พ.ศ. 2551 โดยใช้ชิ้นเนื้อที่มีอาการพินิจคัดแยกหน้าประมาณ 4 ในคร่อมและนำกลับมานำวิเคราะห์ย่อยนต์โปรตีน HER2 ด้วยเทคนิคทาง IHC โดยใช้ระบบย้อมสีอัตโนมัติ Ventana หัวโค้ชชิ้นเนื้อที่เป็นมะเร็งตามเป็น positive control สำหรับชิ้นเนื้อที่ทำการศึกษาทุก ๆ ชุด และยังวิเคราะห์HER2 gene amplification ซึ่งใช้เทคนิค FISH ด้วย Path Vysion HER2 DNA Probe Kit ชิ้นเนื้อที่มีผลการย่อยสีอย่างน้อย 2 ชุดของการศึกษาด้วย IHC โดยแปลผลและบันทึกโดยพยายามที่จะลดการใช้เทคนิค FISH นั้นใช้การแปลผลโดยอาศัยเกณฑ์มาตรฐานเดียวกันกับมะเร็งเต้านม วิเคราะห์ความสอดคล้องระหว่างผลของ IHC และ FISH นั้นได้ประเมินโดยใช้ two-tailed-Fisher’s exact test

ผลการศึกษา: พบโปรตีน HER2 overexpression 8.9 % ของผู้ป่วยทั้งหมด (20 ใน 224 ราย) ซึ่งพบว่า 80% ของมะเร็งกลุ่มเป็นกลุ่ม well และ moderately differentiated type หรือ intestinal และ mixed type อย่างไรก็ตามพบในมะเร็งชนิดเป็น 16% ของมะเร็งชนิด intestinal/mixed type หรือ well/moderately differentiated ที่จะมีโปรตีนHER2 overexpression อัตราความสอดคล้องระหว่างผลของ IHC และ FISH คือ 87.7% หรือ 171 ใน 195 ราย เนื่องจากมีจำนวน 29 ราย ที่ไม่มีผลการแปลผลโดยการศึกษาด้วยเทคนิค FISH

สรุป: อุปกรณ์การตรวจสอบโปรตีน HER2 overexpression ได้ใช้เป็นการศึกษาในผู้ป่วยมะเร็งกระเพาะอาหารในภูมิไทย แต่พบว่ามีอัตราความสอดคล้องสูงระหว่างการศึกษาด้วยเทคนิค IHC และ FISH เชนเทียบกับที่พบในมะเร็งเต้านม การศึกษาเป็นการศึกษาแรกสำหรับHER2 ของมะเร็งกระเพาะอาหารในภูมิไทย