GASTRIC CANCER AND PERSPECTIVE VIEW OF HER2 BIOMARKER

Dr. Ganesh Adhikari

ABSTRSCT: *The advent of human epidermal growth factor receptor-2 (HER2) Status and biomarker testing has modified the treatment and prognosis of gastric, breast and colorectal cancer. Epidermal growth factor receptor and HER-2 family maneuvers a key role in the exploitation of gastric cancer by detecting the epidermis express in gastric cancer tissue and to investigate serum and tissue HER2. Targeted drugs i.e. antineoplastic therapies such as EGFR tyrosine kinase inhibitors have application in the treatment of gastric cancer.*

OBJECTIVES: To scrutinize the manifestation of HER2 in gastric cancer and assess their association with clinic-pathological hallmarks: the tumorigenesis and the metastasis of gastric tumor.

METHODS: 1. Analysis of a prospective database with 68 patients for gastric cancer in the first affiliated hospital of Anhui Medical University range from 2013.4-2014.3 Immunohistochemistry staining(IHC) was used to detect the expression of tissue HER2 in 68 cases of gastric cancer and adjacent non-cancer tissue and 40 cases of adjacent normal EGFR and HER2. Preoperative serum samples were extracted from 68 gastric tumor patients and 40 healthy volunteers. Serum and tissue of HER2 were determined using an enzyme-linked immunosorbent assay (ELISA).
2. To analysis the relationship of clinic pathological features with the expressions HER2 and the correlation of diagnosis and prognosis with gastric cancer.
3.All data were processed by SPPSS.13.0

Results: There was significant difference between the overexpression of Her2 in gastric cancer and the low expression in adjacent non-cancer tissue and adjacent normal epithelial tissue（$P<0.01$. Serum Her2 were significantly higher in patients with gastric cancer than those in normal control group（$P<0.01$）. The Her2 levels have no relationship to sex, age, tumor location and histological grades（$P>0.05$）, but had significantly statistic difference between lymph node metastasis, Histological differentiation and Dukes stage（$P<0.01$）.

CONCLUSION: The overexpression of Her2 in gastric cancer apprise that the HER2 may maneuver an important role in progression of gastric cancer; serum HER2 can be used as a new tumor marker to esteem the procession of gastric cancer.

KEYWORD: Gastric Cancer, GF (growth factor), CEA (carcino-embriyogenic antigen) Human Epidermal Growth Receptor2(HER2); Immunohistochemistry(IHC); Tumor node metastasis(TNM); ELISA (Enzyme linked immunosorbent assay)

1.INTRODUCTION

In gastric cancer, is a malignant (cancer) cells are formed in the lining of the stomach that are located in the upper part of the abdomen. Universally, gastric cancer is the 3rd most common cause of mortality and 4th most commonly diagnose cancer [1-2]. Gastric cancer is usually diagnosing with metastasis in advanced stage. There are reports that depending on the diagnosis, overall survival ragging from 5% to 90%. [3] Gastric cancer (adenocarcinoma or less commonly lymphoma) can occur in any part of the stomach. The main two tumor sites of gastric adenocarcinoma are proximal to (cardia) and distal (non-cardia). despite a decline in distal gastric cancers, proximal tumors have been increasing in incidence since 1970s, especially male in western [4-5]. As the cancer grows it can spread through its wall and nearby organs like esophagus, liver, pancreas, duodenum etc. Early diagnosis and surgical removal of all parts of stomach is often delayed because there are few distinct symptoms before the cancer spreads like epigastric pain, nausea, vomiting, heart burn weight loss and GERD. Therefore, it is very important to improve the rates of early diagnosis and treatment to improve its rate. Since 2005, gene expression microarray analysis was founded as pre-warning system for

advanced gastric cancer. [6] Lacking of biomarker for the diagnosis of cancer is still the primary obstacle for early diagnosis. Both environment and familial were among the risk factor. Serologic markers and histological precursor lesions of gastric cancer and early perception of gastric cancer using these markers are reviewed.

Immunohistochemistry shows strong membranous staining of human epidermal growth factor receptor-2 (HER2) in gastric cancer (A) and negative in adjacent normal epithelial tissues.
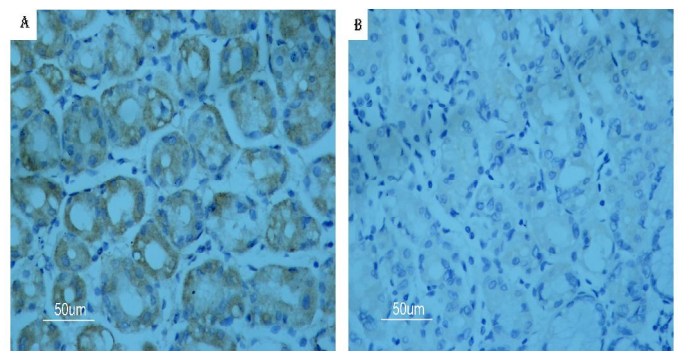
Examples of human epidermal growth factor receptor-2 immunohistochemical expression and amplification in primary and metastatic gastric cancer.

Therefore, the aims and objective of my thesis are to provide or to update our knowledge of HER2 in the context of gastric cancer and to describe the clinical trials. Our main objectives that are more focus here are: To inspect the manifestation of HER2 in gastric cancer, to determine the significance of serum HER2 and tissue HER2 in gastric cancer patients, to study the relationship between tissue HER2 and serum HER2 in gastric cancer patients and finally to access the association of HER2 with tumor genesis and metastasis of gastric cancer.

2. MATERIAL AND METHODS

2.1 Sample collection

From January 2013 to January 2014, a total of 68 patients with newly diagnosed gastric cancer received elective surgery at the Department of General Surgery in the First Affiliated Hospital of Anhui Medical University. An additional panel of 40 serum samples were taken from healthy people who had healthy physical examinations in the same session whose fasting sample were receive before surgery. while collecting fasting venous blood 4ml of EDTA in anticoagulant tube Fasting venous blood,3000r/min centrifugation for 10 minute. The age of the subjects in both the healthy and cancer groups was 36-81 years. Tissue samples were liquidated and stirred at -20°C until analyzed. The base line of these characteristics were located surrounding about 5cm of specimens. No patients received chemotherapy or other intervention prior to surgery.

2.2 General Information

68 patients with gastric cancer, male/female:45/23 cases: aged 36 to 81years Among patients 46 were high grade, poorly differentiated:73/3 cases: Duke stage: a/b of 46 cases, C/D of 30 cases: not all patients receiving any chemo-oradiotherapy.40 cases of gastric adenomas:25males and 15 females: aged 30-52yrs mean 40years: mixed/fluffy/tubular:9/11/20.

2.3 Reagents

Rabbit anti-human VEGF monoclonal antibody, Rabbit anti-human HER2 monoclonal antibody, ELISA KITS, EDTA Fluid, PBS buffer reagent and DAB or more agents from Anhui Anke Biotechnology Co. Ltd, immuno-histochemical detection kit (Universal): from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. Distilled water, xylene. hydrogen peroxide, alcohol, hematoxylin etc. (laboratory of Anhui Medical university)

2.4 Instruments

While performing ELISA test following instruments were used Slicer, LEICA RM2135 type: Shanghai Leica Instruments Electric oven, DHG-9202-os type: Science Instrument Co., Ltd. Shanghai three rounds (Medical) microwave experiments: The Shanghai Institute of Medicine Heated water tank, DK-8D type; PYX-DHS-4X5V type Water-incubator:from Shanghai Yuejin Medical Instrument. Fume Hood: Yixing modern experimental plant Tissue spread roast machine: Matt Hubei Medical Electronics -20 ℃ ultra-low temperature freezer: Japan's Sanyo Microplate reader: The US company BIOTEK Fridge: Meiling Optical Microscope: Olympus Japan Timer Slides and cover slips: Anhui Anke Biotechnology Co., Ltd. A measuring cylinder (500ml) and two volumetric flasks (2500ml)
Wet box: two

2.5 Elisa Assay

After the collection of the venous blood sample from patients (68) and control (40), the samples were centrifuged at 3000 rpm for 3 minutes and then stored at -20°C until the completion of assay. Various samples were drawn from each of the patient at the time of endoscopy or before surgery. Sera were assayed for anti HER2 antibody by using a commercial ELISA Kit (Beijing Zhongshan Jingqioa Company). The assay was performed according to the recommendation of the manufacturer.

2.6 Elisa Methods:

The kit will be placed at room temperature.various sample of the configured 100ul sample were placed in microliter plate. close the plate covering with microliter and incubated for 2 hrs. at a temperature of 37 ℃.Antibody reagent (biotin-labeled)100ul solution was added to each incubated tube at 37 ℃, for 1 hour.After incubation, the reaction plates were washed and well dried 3 times for 3 minutes.Microliter were subsequently added to the reaction liquid 100μl, 37 ℃, and culture for 1 hr.The plate was discarded in the presence of liquid, and washed repeatedly for 5 times in the interval of 3 minutes and thought dry the plate.100ul color solution were placed in each solution let for reaction for 10 seconds than placed for 37 ℃, incubated for 20 min (protected from light). 50ul solution were subsequently added to each reaction. It was measured at 450 nm wavelength while placing in a microplate reader.

2.7 Statistical Analysis

SPSS for window version 13(SPSS, USA) was used to analyzed results. In serum HER2 level the difference was analyzing in between serum and normal tissue by using T-test. Statistical significance was set at $P < 0.05$. The experimental group was obtained control group HER2 mean value to the absorbance of the standard. The resulting value of each sample drawn into the equation corresponding to tissue HER2 concentration, serum HER2 concentration, serum EGFR≥6.5 mg / L (control group ± 2s) [25] is positive, EGFR <6.5 μ g / L was negative; serum HER2≥5.9 mg / L as positive, HER2 <5.9 mg / L was negative.

In each cases according to immunohistochemistry kit demanding PBS as a negative control with color intensity and color ranged. To show high magnification five positive sells were selected. The number in each field of vision to take 100 gastric cancer cells taking 500 gastric cancers were semi-quantitative scoring method of low determination. Serum her2 cells were occasional expression of membrane, cytoplasm, positive cells stained brown, vision positive cells positive cells, ≤5% regarded as (-), ≥25 regarded% (+), 25-50% treated as (++), ≥50% deemed (+++); HER2-positive mainly in the cell membrane, can also be a small part in the cytoplasm, the cells appear yellow granules as positive. Analyzing Results: Ratings are based on positive staining extent (points), 0: no coloring; 1: pale yellow; 2: brown; 3: tan. Ratings are based on percentages of positive cells (min), 0: <5%, 1: 5% to 10%, 2: 11% to 50%, 3: 51-80% 4:> 80%, based on the final results of the two-phase take determination: negative (-) 0; weakly positive (+) 1 to 4 points; moderately positive (+) 5 to 8 minutes, strongly positive (+++) 9 to 12 points. Low expression: negative and weak positive expression: positive and strongly positive.

2.8 Data processing

SPSS 13.0, Statistical analysis, comparison between groups application test, showing results of measurement data with ± s; significance level α = 0.05.

2.9 Principles and Methods

IHC used for detecting cells of tissue section. The working principle of IHC is antibody binding to antigen in sample tissue. In 1941 ALBERT COON applied this concept and implement this procedure.

The most commonly used to make tissue samples, the most basic method is paraffin, paraffin sections can be better preserved tissue morphology, and can be serially sectioned, have helped comparative study of various dyed; but also long-term retention for retrospective experimental studies; paraffin sections in the production process, within the organization will inevitably have some impact antigen exposure, but these effects can be reduced by antigen retrieval performed. Therefore, the production method in IHC tissue samples in the preferred paraffin. Antibody immunohistochemistry experiments in general applications, including monoclonal and polyclonal antibodies, monoclonal antibody is a clone of B lymphocytes and antibodies secretion is produced. Obtained mainly fused hybridism cells by the immunized animal. Polyclonal antibodies are immune serum antigen animals through direct immunization of purified after refining in the blood of animals are obtained by a number of B lymphocyte clone mixture secreted more antibodies

2.10 IHC Experimental Procedure

Rinse sections in PBS-Tween 20 for 2x2min

Serum Blocking: incubate sections with normal serum block – species same as secondary antibody, for 30 minutes to block non-specific binding of immunoglobulin. Note: since this protocol uses avid in-biotin detection system, avid in/biotin block may be needed based on tissue type. If you do, the avid in/biotin block should be done after normal serum block. Primary antibody with related solution incubated for I hour at room temperature or placed overnight at 4°c. wash in PBS for 20.Secondary Antibody: incubate sections with biotinylated secondary antibody at appropriate dilution in PBS for 30 minutes at room temperature.

Rinse in PBS-Tween 20 for 3x2min.

Detection: incubate sections in FITC-Avid in D in PBS for 30 minutes at room temperature. The slide is protected from light and covered with aluminum foil or black box from 1st step till end.

Rinsing in PBS -20 for 3times up to 2min Counterstain is done with PI or DAPI if required.

Rinse sections in PBS-Tween 20 for 2x2min. 95% ethanol is used to dehydrate for 2 min or 100% ethanol for 2 times up to 3 minutes.Mounting medium used to coverslip with anti-fade.

3.RESULTS

3.1 Serum level of HER2 in gastric cancer patients and control group.

Serum HER2 level was significantly more (8.5 ± 2.3ng/ml) in the expressed group than in other patients (3.5 ± 1.2ng/ml) HER2 level ($t=12.74$, $p<0.05$). Cut-off level of ELISA above or below which the tested samples were considered positive or negative was calculated as the mean concentrations of 40 serum samples from healthy individuals +2SD, we take cut-off 5.9ng/ml.

3.2 Tissue level of HER2 in gastric cancer patients and control group.

Subject were 68 gastric cancer patients who underwent surgical resection. Tissue expression of HER2 was positive in 52 cases (76% of examined gastric cancer patients) and HER2 positive were highly differentiated. As shown in table no.1 there was a significant difference between the overexpression of her2 in gastric cancer and the low expression in adjacent normal epithelial tissue($p<0.01$).

Table 1. Relation between adjacent non-cancer tissue, adjacent normal epithelia tissue and gastric cancer.

group	n	her2（+）	χ^2	P p
adjacent noncaner tissues	68	15	40.273✳	＜0.01
adjacent normal epithelial tissues	40	13	20.321△	<0.01
Gastric cancer	68	52		

Note:comparison between adjacent noncancer tissue and gastric cancer，△comparison between adjacent normal epithelial tissues and gastric cancer

3.4 The relationship between tissue HER2 and serum HER2.

Common positive 38 case, common negative 7 case, and they have a positive correlation by Pearson Chi-Square test ($x^2=0.948$, $r=0.335$, $p=0.023$).

3.5 correlation between serum/tissue HER2 level and clinico-pathological parameters.

Table 2 EGFR and Her2 expression and CRC relationship clinico-pathological parameters (n)

parameters	n	Her2（+）	P vALUE	SERUM Her2（+）	P VALUE
gender					
male	45	36	0.337	29	0.328
female	23	16		12	
age(years),					
<60	22	14	0.084	10	0.084
≥60	46	38		31	
Size					
<5cm	44	31	0.216	25	0.428
≥5cm	24	21		16	
Histological differentiation					
Well/moderate	29	15	<0.01	4	<0.01
poor	39	37		37	
location					
cardia	32	25	0.762	21	0.397
body/antrum	36	27		20	
pathological stage(TNM stage)					
stages1,2	27	15	0.001	9	<0.01
stages 3,4	41	37		32	

lymph node metastasis					
yes	27	14	<0.01	9	<0.01
no	41	38		32	

The relationship between tissue Her2 and serum HER2:
Common positive 38 case, common negative 7 case, and they have a positive correlation by perarson Chi-Square test (x^2=0.948, r=0.335, p=0.023).

*According to age, sex and tumor location: As we can see in table no.2, no significant relationship was found (p > 0.05).

*According to histological grade: As shown in table no.2 and both the serum HER2 and tissue HER2 was significantly higher in patients with poor histological differentiation than to those with well/moderate HER2 level (p < 0.05).

*According to Duke's stage (TNM stage): from table no.2 and we can see that both the serum HER2 and tissue HER2 was also significantly higher in patients with TNM stage 3,4 compared to those with TNM stage 1,2 (p < 0.05).

*According to lymph node metastasis: as shown in table no. 2, comparing those with lymph node metastasis, serum HER2 and tissue HER2 was highly significant in patients who did not have lymph node metastasis (p < 0.05).

4. DISCUSSION

Through investigation and application of the testing methodologies commonly used to detect HER2 in gastric cancer specimens, other studies have shown HER2 IHC scoring system would be suitable to score gastric cancer samples accurately and more reproducibly. These findings emphasize the need to validate the methods for determining HER2 status before designing any clinical trial involving a new tumor type. [15] Our investigation of the validity of using IHC and ELISA methodologies to detect HER2 positivity in gastric cancer has also shown a high level of concordance (93.5%) between the two tests. In another recently published study using these same assays in gastric cancer specimens, concordance between the results was 86.9% [16] The concordance between the IHC findings in surgically resected tumors and biopsy specimens is very important in gastric cancer clinically. If a satisfactory concordance rate is obtained, HER2 status can be evaluated by IHC in un-resectable cases as well as cases of recurrence after gastrectomy. Early 4,00000 cases were diagnosed which leads around 300000 death accounts 23.2% deaths. [17] In spite of different methodological finding, chemotherapy and radiation therapy have less likely improvement within the last decade by improving quality of life and the prognosis. Several clinical and pathological staging with molecular markers would enable a more precise identification of patient with the highest or lowest risk of relapse following gastric cancer surgery.

4.1 Serum level of HER2 in gastric cancer patients and control group.

As we can see from the table no.1, serum HER2 was significantly higher in patients with gastric cancer compared to those in control group. Serum HER2 has high sensitivity and is moderately specific (60% sensitive and 44% specific, at cut off value of 5.9ng/ml). similar findings were seen in some other studies done in breast cancer. Their results also revealed the fact that serum HER2 levels in cancer group was significantly higher than that in healthy control group. [18]

4.2 Tissue HER2 level in gastric cancer patients and control group.

Tissue expression of HER2 was positive in 76% of examined gastric cancer patients, whereas it was positive in only 32.5% of examined control group. Significant difference was found between overexpression of HER2 in gastric cancer and the low expression in adjacent normal epithelial tissue.

4.3 The relationship between tissue HER2 and serum HER2.

A positive correlation has been found between tissue HER2 and serum HER2 by using Pearson chi-square test (p < 0.05). in another study about HER2 in breast cancer by J. Cancer et al. [19] they also found high statistical correlation between serum HER2 level and tissue HER2 level, which was also in accordance with another previous study by Molina et al. 2003

4.4 Correlation between serum/tissue HER2 level and clinico-pathological parameters.

In our study, no significant relationship was found between serum/tissue HER2 level with age, sex and tumor location (p > 0.05). whereas, both serum HER2 and tissue HER2 was significantly higher in patients with poor histological differentiation, same was found is patients with TNM stage 3,4. Both serum HER2 and tissue HER2 was more significant in patients in Duke's stage 3,4 comparing to those in stage 1,2. Similar result was seen in another study performed in patients with breast cancer. (reference) except in lymph node involvement. In their study elevated HER2 level was also found among patients with lymph node involvement, while in our study, both serum HER2 and tissue HER2 was found highly significant in those who did not have lymph node metastasis. [20]

5.CONCLUSSION

5.1 Over expression of Serum her2 in gastric cancer patients is comparatively higher. Hence we can provide this theory for the diagnosis, prognosis and targeted therapy of the patient.

5.2 Expression of tissue her2 was higher in adenoma tissue

5.3 Tissue HER2 and serum HER2 have no relation with age sex and location.

5.4 Both serum and tissue her2 are significant in those without lymph node metastasis it was also significant in patients with poor histological differentiation and those in duke stage (3 and 4 stage). since the overexpression of her2 was closely association with on patient's poor histological differentiation and duke stage, we can use HER2 as a new tumor marker to monitor the progression of gastric cancer.

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