Cancer Medicine Journal

Research Article | Vol 3 Issue S1

ERBB2 FISH and Chromosome Microarray Testing of Gastroesophageal Adenocarcinomas at a Single Institution

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Received Date: October 13, 2020; **Accepted Date:** October 30, 2020; **Published Date:** November 7, 2020

ABSTRACT

Overexpression/amplification of erb-b2 receptor tyrosine kinase 2 (ERBB2) is a major prognostic factor in gastroesophageal cancers; it is currently the only biomarker established for the selection of targeted therapy for patients with advanced gastroesophageal adenocarcinoma (GEA). Current standard procedure for determining ERBB2 status in such patients is immunohistochemistry (IHC), followed by in situ hybridization (ISH), when IHC result is equivocal. Insufficient knowledge regarding the utilities of chromosomal microarray (CMA) has hindered its use as an adjunct tool in ERBB2 analysis. Here, we performed CMA on 7 formalin-fixed paraffin-embedded (FFPE) GEA specimens previously tested by ERBB2 fluorescence in situ hybridization (FISH) and evaluated the concordance and performance of CMA. CMA identified 4 (57.1%) samples with amplification of ERBB2, compared to 3 (42.9%) by FISH. CMA also detected several additional DNA copy number variants in these samples, which may have prognostic and therapeutic indications. Further case studies and clinical trials may provide evidence for the utility of CMA-based genomic studies in the management of patients with suspected ERBB2-positive gastroesophageal adenocarcinoma.

KEYWORDS

ERBB2; FISH; CMA; GEA

INTRODUCTION

Erb-b2 receptor kinase 2 (ERBB2), also known as human epidermal growth factor receptor 2 (HER2), is located on chromosome 17q12 and encodes a protein essential for cell proliferation and survival [1-4]. ERBB2 is overexpressed in multiple types of cancer, including between 7% to 38% of gastroesophageal adenocarcinomas (GEA), which is associated with a more aggressive course and worse prognosis [1,5]. GEA

Citation: Alexander Yu, ERBB2 FISH and Chromosome Microarray Testing of Gastroesophageal Adenocarcinomas at a Single Institution. Cancer Med J 3(S1): 39-46.

includes the fifth (stomach) and eighth (esophageal) most common cancers worldwide, and ERBB2 remains a primary biomarker for targeted therapy in these patients [1]. Currently, the standard method for establishing ERBB2 protein overexpression and DNA amplification is immunohistochemistry (IHC), followed by in situ hybridization (ISH) if IHC result is equivocal [2]. In 2016, College of American Pathologists (CAP), American Society for Clinical Pathology (ASCP), and American Society of Clinical Oncology (ASCO) developed a comprehensive guideline for ERBB2 testing and treatment for patients with GEA [1]. However, the guideline currently states "no recommendation" for or against genomic studies such as polymerase chain reaction (PCR) or chromosomal microarray (CMA) in GEA patients [1]. Interestingly, recent studies in breast cancers have shown upwards of 93% to 100% concordance rate of ERBB2 results between ISH and CMA, a 5% to 15% equivocal rate in IHC and ISH, respectively, and a more thorough detection of copy number variations (CNVs) of cancer-related genes like TP53 with CMA [6-8]. Thus, the determination of CNVs by high-density single nucleotide polymorphism (SNP) CMA could be a valuable adjuvant tool for analysis of ERBB2 amplification in GEA patients [3,9]. However, studies into ERBB2 concordance with respect to ISH and CMA in tissues derived from advanced GEA are lacking. Here, we performed CMA and fluorescence in situ hybridization (FISH) on 7 formalin-fixed paraffin-embedded (FFPE) specimens derived from patients with advanced GEA and evaluated the performance of CMA with respect to FISH results.

MATERIALS AND METHODS

This study included seven 7 FFPE specimens derived from patients with advanced GEA collected during the period of July 2012 to May 2017. The study was approved by the UTMB's Institutional Review Board (IRB #02-089). FISH assay was conducted by using PathVysion HER-2 DNA Probe Kit (Abbott Laboratories, Abbott Park, IL, USA) according to the manufacturer's instructions. Section of patient's FFPE tissue sample was first heat fixed onto a glass slide. Sample and PathVysion probes (ERBB2 and chromosome enumeration probe 17, CEP17) were then denatured and probes hybridized to the patient sample (Abbott Laboratories, Abbott Park, IL, USA). After washing to remove excess probes, ERBB2 and CEP17 signals were observed under a fluorescent microscope (CytoVision, Leica Biosystems Inc., Buffalo Grove, IL, USA). ERBB2 status was evaluated by using the 2016 ASCO/CAP guidelines for GEA [10].

Chromosomal microarray (CMA) was conducted by using the OncoScan CNV Assay Kit (Affymetrix, Santa Clara, CA, USA), which utilizes 335,000 probes for copy number variations (CNVs) [11]. Patient's genomic DNA (gDNA) was extracted and purified from FFPE tissue samples by using QIAamp DNA FFPE Tissue Kit (Qiagen Inc, Valencia, CA, USA). Patient gDNA and probe were hybridized with a gap between them, which was then filled with A/T or G/C nucleotides (Affymetrix, Santa Clara, CA, USA). DNA was cleaved, amplified via PCR, and hybridized to a chip containing the SNP and oligonucleotide probes, which was then scanned to a computer (Affymetrix, Santa Clara, CA, USA). A CNV profile was generated and analyzed by using the Chromosomal Analysis Suite (ChAS, Affymetrix, Santa Clara, CA, USA). CNVs including ERBB2 status were evaluated considering Mikhail et al. (2019) recommendations for CMA [12].

RESULTS

Table 1 summarizes the clinical features of the seven patients whose GEA tissues were used in this study. Four of the seven patients with advanced GEA presented with metastasis to brain or regional lymph nodes. Three patients demonstrated a related esophageal comorbidity. Six patients reported a significant smoking history. Figure

1 depicts the copy number variants (CNVs) apparent in each patient's tissues. Table 2 summarizes the concordance results with respect to ERBB2 amplification between CMA and FISH analyses. Six cases demonstrated concordance between CMA and FISH regarding ERBB2 amplification (3 amplified, 3 not amplified) between microarray and FISH assays (Table 2). There was one case (patient 6) with discordant results on ERBB2 status between FISH and CMA with respect to the primary tumor (Table 2, Case 6, ERBB2 amplified by CMA but not amplified by FISH). Table 3 reports several CNVs detected in the GEA samples. Of the CNVs, the most frequent changes included gains in chromosomes 8q/20 (6/7 cases), 2/12p/17q (5/7 cases), 13q (4/7 cases), but losses in chromosomes 18q (6/7 cases) and 9p/16p (4/7 cases). Below is highlighted information for each patient's clinical course.

	Patient #1	Patient #2	Patient #3	Patient #4	Patient #5	Patient #6	Patient #7
Age	69	71*	56*	37	51	70	52
Gender	M	M	F	M	M	M	M
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Hispanic	Caucasian	Caucasian
Cancer Type	EAC	EAC	EAC	Gastric	Gastric	EAC	EAC
Primary Tumor Site	Esophagus	Esophagus	GE Junction	Stomach	Stomach	Esophagus	GE Junction
Metastasis Site	R frontall Lobe	None	None	None	8/16 perigastric lymph nodes	R cerebellum	1/9 regional lymph nodes, brain, paraspinal muscle
Related Comorbiditie s	Barrett's esophagus	Esophageal reflux	NA	NA	NA	NA	GERD
Smoking History	1 ppd, 20 pack-yrs	1 ppd, 58 pack-yrs	1.5 ppd, 61.5 pack-yrs	Former smoker	None	1.5 ppd, 37.5 pack-yrs	Former smoker

Table 1: Clinical features of seven patients with GEA.
Note: *Patient deceased. EAC: Esophageal Adenocarcinoma, GERD: Gastroesophageal Reflux Disease, PPD: Pack Per Day.

Patient	OncoScan ERBB2 Copy #	OncoScan CEP17 Copy #	ERBB2 FISH Result (ERBB2 count:CEP17 count; ERBB2/CEP17 Ratio)
1	18.67	2.33	Amplified (200:20;10)
2	2.33	2	Amplified (123:41; 3)
3	2.33	2	Amplified (131:96;1.26)
4	2	2	Not Amplified (44:34;1.29)
5	2	2	Not Amplified (70:56;1.25)
6	3	2	Not Amplified (75:65;1.15)
7	2	2	Not Amplified (77:54;1.43)

Table 2: Comparison of ERBB2 results from chromosomal microarray and FISH analysis.
Note: Six of seven cases showed the same results regarding ERBB2 amplification (3 amplified, 3 not-amplified) between microarray and FISH assays. There was one case (#6) with discordant results on ERBB2 status.

Patient	Chromosome Losses	Chromosome Gains
1	1p,5,7q,9p,11q,12,17p,18,19,22q	1q,2,3q,7,8,9,10p,12q,13q,15q,16q,17,20,22q,X,Y
2	1p,3p,4,7q,8p,9,10,11,12q,14q,16,17p,18q,19,21q,22q,X,Y	1q,2,3q,5p,6,7p,8q,10p,12p,13q,17q,18q,19,20
3	1p,3,4,6p,9,10q,12,14q,16,17p,18q,19,21q,22,X	2,5,6,8q,12p,13q,15q,17q,18,19,20,Xq
4	4q	8,21q
5	4p,18	2,4,12,19q,20
6	5q,6,8p,9p,10p,14q,16p,18q,21	1q,2,3q,4,5q,6,7,8,9q,10,12,13q,14q,15q,16,17q,18p,19,20
7	3p,5,7q,10q,13q,14q,15q,16,17p,18,19,21q,22q,Y	1q,3,6,8q,11p,12,16q,17q,19,20,21,X

Table 3: Summary of microarray analysis in seven GEA specimens.
Note: OncoScan microarray analysis reveals several common DNA copy number variations in these cases, including gains in chromosomes 8q/20 (6/7 cases), 2/12p/17q (5/7 cases), 13q (4/7 cases), and losses in chromosomes 18q (6/7 cases) and 9p/16p (4/7 cases). CEP17 used as control.

Patient 1 was a 69-years old Caucasian male with a past medical history of distal esophageal intramucosal adenocarcinoma with high grade dysplasia diagnosed in 2010 by esophagogastroduodenoscopy (EGD) and biopsy. The patient declined surgery and treatment at the time. Later in the year, he was admitted to neurology for work up of left upper extremity weakness, involuntary jerking movement, paresthesia, and ipsilateral facial asymmetry. MRI of the brain on 2012 showed a hyper vascular lesion (3.4 cm × 3.2 cm × 3.3 cm), likely metastasis, in the posterior R frontal lobe. Chest CT also demonstrated increased size of the original esophageal mass. The patient elected craniotomy with resection of the brain lesion. Pathology reported metastatic adenocarcinoma, histologically consistent with previously diagnosed esophageal primary. ERBB2 was positive by FISH study. He was prescribed Carboplatin + Taxol (carbo/taxol) chemotherapy with concurrent radiotherapy for 5 weeks in addition to decadron.

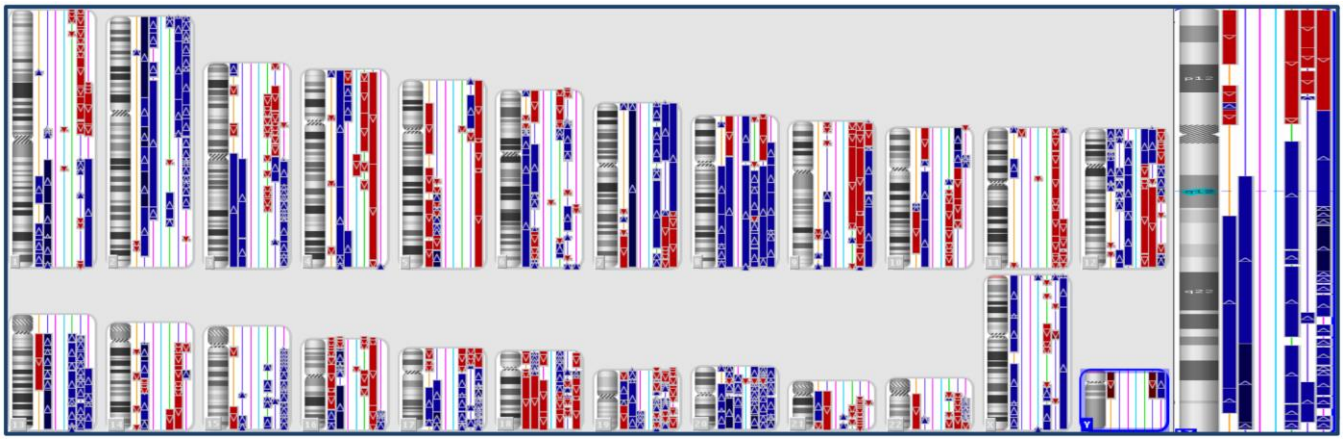

Figure 1: Microarray exhibits similar genetic profile for patients with GEA. OncoScan microarray analysis generated a CNV profile for 7 patients with GEA. Profile displays chromosome deletions (red bars, copy number <2) and additions (blue bars, copy number >2). Right to left, patients 1-7. Larger figure at right shows chromosome 17 with indicated q12 ERBB2 region (light blue line).

Patient 2 was a 71-years old Caucasian male admitted to the hospital in 2017 with a chief complaint of dysphagia and was found to have a lower esophageal mass. Biopsy revealed adenocarcinoma 38 cm - 44 cm from the incisors, confirmed by pathology. Abdominal/chest CT ruled out metastases. Esophageal ultrasonography (EUS) findings demonstrated esophageal mass between 38 cm - 44 cm per ultrasound. The tumor was seen to be invading the muscularis propria, but the adventitia was intact. ERBB2 by IHC was equivocal (score 2+) and ERBB2 was amplified by FISH analysis. The patient denied therapy and was lost to follow-up.

Patient 3 was a 56-year-old Caucasian female diagnosed with gastroesophageal junction adenocarcinoma in 2015 by EGD and EUS. ERBB2 biopsy sample was equivocal (score 2+) by IHC and ERBB2 DNA was amplified by FISH analysis. Chest/abdominal CT and PET scan ruled out evidence of metastasis. She elected chemoradiation (carboplatin, paclitaxel, and radiotherapy) combined with surgical excision. However, prior to surgical excision, she succumbed to sepsis with renal failure and died two months after diagnosis.

Patient 4 was a 37-years old Caucasian male diagnosed with stage IIIb gastric adenocarcinoma in 2015. EGD and pathology confirmed poorly differentiated gastric

adenocarcinoma without metastasis. ERBB2 was not amplified by FISH. The patient elected neo-adjuvant chemotherapy with epirubicin, cisplatin and continuous 5-fluorouracil (ECF) and then has surgical re-evaluation.

Patient 5 was a 51-years old Hispanic male diagnosed with an antral ulcer that was positive for adenocarcinoma in 2012. ERBB2 was not amplified by FISH. He underwent exploratory laparotomy and biopsy of portal lymph node and sub-total gastrectomy with Roux-en-Y reconstruction. Lymph node biopsies demonstrated metastasis to 8/16 periportal lymph nodes. Following surgery, the patient elected for 5-fluorouracil (5-FU) with leucovorin and concurrent chemo radiation with 5-FU. Post chemotherapy, he reported doing well with no complaints aside from constipation.

Patient 6 was 67-years old Caucasian male with a past medical history of hypertension and hyperlipidemia who was evaluated for a growing chest wall mass in 2014. Chest CT demonstrated masses invading into the pectoralis muscle. Biopsy of the mass demonstrated intermediate grade spindle cell neoplasm with a myxoid background suggestive of dermatofibrosarcoma protuberans (DFSP). The patient also reported a 4 months history of worsening dysphagia and hoarseness. The CT scan later revealed a distal esophageal mass causing stenosis with air-fluid level. An EGD was performed with biopsies showing invasive well-differentiated adenocarcinoma. PET scan revealed no enlarged lymph node or metastatic disease. He elected to start neoadjuvant chemoradiation for the esophageal cancer and began carboplatin/paclitaxel and radiotherapy completed within the year. He underwent an Ivor Lewis esophagectomy, feeding jejunostomy, pyloroplasty with resection of the chest wall dermatofibrosarcoma protuberans in 2016. In 2017, he presented with neurological symptoms and CT scan demonstrated brain metastasis of the initial esophageal adenocarcinoma. ERBB2 by IHC was equivocal (score 2+) and ERBB2 DNA was not amplified by FISH study. He underwent resection and completed radiation of the brain metastasis and currently has no evidence of disease.

Patient 7 was a 50-years old Caucasian male diagnosed in 2015 with Stage IIIA esophageal adenocarcinoma with metastasis to 1/9 regional lymph nodes. ERBB2 by IHC was equivocal (score 2+) and ERBB2 DNA was not amplified by FISH. He elected video-assisted thoracic surgery which was complicated by distal esophageal perforation and laparoscopic esophagectomy with percutaneous endoscopic gastrostomy placement. He also continued adjuvant chemotherapy with epirubicin, cisplatin, and 5-FU (ECF). In 2016, following neurological symptoms, CT imaging demonstrated paraspinal muscle and brain metastasis with biopsy and pathology confirming metastatic adenocarcinoma. Chest X-Ray later in the year also demonstrated lung mass (origin unknown). Radiotherapy and systemic treatment were recommended for the paraspinal muscle metastasis.

DISCUSSION

The current practice guidelines in GEA designate ERBB2 testing for all tumor specimens (primary and metastasis), as the addition of trastuzumab can prolong both progression-free and overall survival per the 2010 Trastuzumab for Gastric Cancer (ToGA) clinical trial in patients with good performance status, low cardiac risk, and candidates for systemic therapy [1]. In these patients, the current algorithm utilizes first line IHC followed by ISH testing if IHC result is equivocal (2+) [1]. Patients with 3+ IHC, ERBB2:CEP17 ratios >2 with equivocal 2+ IHC status, or more than six ERBB2 signals, on average via ISH, with equivocal 2+ IHC status are considered trastuzumab responders and should receive anti-ERBB2 therapy [1]. However, the guidelines acquiesce in a subset of 2+ IHC equivocal patients, there exists around 4.1% of gastric cancers from the ToGA trial that presented with

chromosome 17 polysomy, which manifests as an increase in CEP17 copy number above 3.0, caused primarily by intrachromosomal segmental duplication near the centromeric region, typically involving the ERBB2 gene [1]. As a result, the ERBB2 copy number in these patients' present challenges and ambiguity in the interpretation of ERBB2 ISH results. CMA profiling may be able to overcome these challenges as CMA provides a more complete coverage of multiple chromosomal regions and can accurately detect the length of copy number variants (Figure 1). Furthermore, CMA has been shown in studies to have above 90%, and as high as 100% concordance rate with FISH in breast and other ERBB2-positive cancer tissue samples, suggesting low compromise in detection sensitivity.6, 8 In our study, CMA detected patients 1, 2, 3, and 6 with gains of the ERBB2 region (copy numbers >2), while FISH detected duplications amplifications in patients 1, 2, 3 (Table 2). These results suggest not only high concordance, but also the possibility of CMA having higher sensitivity than FISH, although confirmation with regards to trastuzumab responders and CMA profiling is needed to validate this hypothesis. One potential cause for ERBB2 amplification variability in tumor samples that has been explored includes intratumor heterogeneity. GEA intratumor variation has been reported in gastric cancers, defined by focal IHC or ISH positivity1. OncoScan CMA utilizes the Tumor Scan™ (TuScan™) algorithm which can interpret large populations of intratumoral heterogeneity based on an initial estimation of tumor percentage in the sample, allowing for detection of ERBB2 heterogeneity [7]. This further suggests that CMA may aid in determining extent of clonal divergence and tumor progression in cases of ERBB2 uncertainty.

Currently, few studies exist exploring the differences in treatment outcome for ERBB2 FISH-negative, CMA-positive GEA patients; consequently, studies from other primary cancers of ERBB2 status may provide insight into CMA utility in ERBB2 status discernment. Haskell et al. [7] utilized both CMA and FISH on breast cancer cases to develop an integrated approach to ERBB2 DNA amplification and interpretation. They found that while ISH analysis reported essential ERBB2 data, CMA had the potential to employ multiple control regions based off an estimation of clonal heterogeneity, as previously described using the TuScanTM algorithm [7]. As a result, CMA provided the potential for not only covering a larger scope of genomic changes, but also the potential for higher accuracy in ERBB2 gene analysis. While the current ERBB2 GEA interpretation guidelines report difficulty in selecting control regions for segmental amplifications of chromosome 17 in addition to heterogeneity between malignant and benign cells [7], recent advances with validated CMA algorithms such as TuScanTM in addition to utilization of other control regions besides chromosome 17 may ultimately resolve these issues. Currently, there exists no recommendation of using CMA due to insufficient evidence [7]. However, advances in CMA utility may address equivocal cases and provide a third tier of interpretation for patients with uncertain ERBB2 GEA status.

Since CMA offers a genomic wide view of tissue changes, it can detect more CNVs than in a single region alone (e.g., chromosome 17 ERBB2 gene locus), resulting in possible prognostic and therapeutic implications [6]. Davison et al. [9] conducted SNP CMA on superficial GEA specimens and noted that although copy number gains for ERBB2, EGFR, and MET receptor tyrosine kinases were generally mutually exclusive, they found a few cases of co-amplification of ERBB2 with EGFR, MET, or KRAS. Overall, over 40% of the GEA cases had a copy gain of at least one of ERBB2, EGFR, MET, or KRAS genes capable of activating downstream MAP kinase signal transduction pathways [9]. Interestingly, point mutations in TP53 tend to be the most common abnormalities in GEA and breast cancers, none of the 41

cases in this study involved copy number abnormalities in TP53 [6,9]. Other studies have also explored deletions in CDKN2A and FHIT, mutations in PIK3CA, DNA methylation defects, and other genomic profiles as possible biomarkers for predicting prognosis and treatment response in GEA patients [9,13]. As shown in this report, CMA utilization may serve as a valuable tool in categorizing and stratifying cancer patients by therapy resistance and gene target therapy for optimal care [6].

Ultimately, this study helps illuminate the value of CMA in GEA evaluation. Our study calls for the necessitates of further research regarding ERBB2 concordance status of CMA with FISH, CMA with IHC, and CMA with respect to anti-ERBB2 therapy response in patients to fully elucidate the utility of CMA in ERBB2 status determination. In closing, the utilization of CMA may result in more accurate ERBB2 interpretation in patients diagnosed with advanced GEA, ultimately improving targeted treatments and patient outcomes.

ACKNOWLEDGMENT

This work was supported partially by the National Institutes of Health (NIH) fund (5TL1TR001440-02), for which AY and SL are predoctoral trainees, as well as the NIH/NIAID T35 Infectious Diseases and Inflammatory Disorders Training Program (AI78878-10), for which SL is the predoctoral trainee.

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