Original Article

Copy number gains of chromosome 17 identified by dual in situ hybridization in non-small cell lung cancer tissue correlate with overexpression of c-Myc

Patrapim Sunpaweravong[1,2]^, Patcharaporn Thongwatchara[1], Rassamee Chotipanvithayakul[3], Surasak Sangkhathat[2,4], Paramee Thongsuksai[2,5]

[1]Division of Medical Oncology, Department of Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand; [2]Department of Biomedical Science, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand; [3]Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand; [4]Department of Surgery, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand; [5]Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

Contributions: (I) Conception and design: P Sunpaweravong; (II) Administrative support: P Sunpaweravong, R Chotipanvithayakul, S Sangkhathat, P Thongsuksai; (III) Provision of study materials or patients: P Sunpaweravong, P Thongsuksai; (IV) Collection and assembly of data: P Sunpaweravong, P Thongwatchara; (V) Data analysis and interpretation: P Sunpaweravong, P Thongsuksai; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. Patrapim Sunpaweravong, MD. Division of Medical Oncology, Department of Medicine, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand. Email: spatrapi@medicine.psu.ac.th.

Background: c-Myc regulates multiple genes involved in cell proliferation in various cancer types including non-small cell lung cancer (NSCLC). Copy number gains of cytoband 17q25.3, along with chromosome 17, have been reported in NSCLC patients, emphasizing the clinical significance as a potential molecular target for therapy. The upregulation of c-Myc has been found to accelerate tumor development associated with duplication of the syntenic human cytoband 17q25.3. This study aimed to explore and compare the correlations of chromosome 17 copy number and c-Myc expression in NSCLC with the paired-normal respiratory epithelium and to examine their role as potential molecular targets for NSCLC therapy.

Methods: A total of 66 NSCLC tissue samples with paired-normal respiratory epithelium were examined. The copy number of chromosome 17 was determined by human epidermal growth factor receptor 2 (*HER2*)/centromeric enumeration probe of chromosome 17 (CEP17) dual in situ hybridization (DISH).

Results: Copy number gains of chromosome 17 were identified in 8 of 60 (13.3%) available NSCLC specimens. No copy number gains of chromosome 17 were demonstrated in the paired-normal respiratory epithelium. The mean *HER2* (1.2±0.3) and CEP17 (1.4±0.3) copy numbers of the normal respiratory epithelium were significantly lower than those of the NSCLC tissue [1.8±1.0 *vs.* 2.0±0.8, respectively (P<0.001)]. Twelve of 66 (18.2%) NSCLC patients had overexpression of c-Myc. Five (41.7%) of the patients whose tumors positive for c-Myc had *HER2* gene amplification [1] or copy number gain of chromosome 17 [4]. *HER2* gene amplification or copy number gain of chromosome 17 and high expression of c-Myc were associated with decreased overall survival.

Conclusions: Both biomarkers deserve further investigation to identify NSCLC patients with poorer survival outcomes requiring better therapeutic approaches.

Keywords: c-Myc; chromosome 17; copy number; respiratory epithelial tissue; non-small cell lung cancer (NSCLC)

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^ ORCID: 0000-0001-9787-270X.

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common cancers globally and the leading cause of cancer-related death (1), urging a better understanding in molecular tumorigenesis and multidisciplinary treatment options. c-Myc, as a transcription and replication factor, regulates multiple genes that control cell growth in varied cancer types including NSCLC. Copy number gains of cytoband 17q25.3, along with chromosome 17, have been reported in patients having NSCLC, emphasizing its clinical significance as a potential molecular target for therapy in this cancer (2). In pre-clinical studies, the upregulation of c-Myc has been found to lead to accelerated tumor development associated with duplication of the syntenic human cytoband 17q25.3, emphasizing the important role of this region in tumorigenesis (3,4).

Fluorescence in situ hybridization (FISH) has been accepted as a standard assay in the assessment of gene status or copy number of a chromosome. However, the FISH assay has limitations, mainly related to the requirement of dark-field fluorescence microscopy and lack of morphologic information (5). An alternative brightfield method including dual in situ hybridization (DISH) comprising chromogenic and silver in situ hybridization (CISH and SISH, respectively), has been developed and demonstrated the advantage of being able to analyse tissue heterogeneity more comprehensively with acceptable concordance (5). This study aimed to explore and compare the correlations of chromosome 17 copy number identified by DISH assay and c-Myc expression in NSCLC with the paired-normal respiratory epithelium and to examine their role as potential molecular targets for NSCLC therapy. We present the following article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2705/rc).

Methods

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Faculty of Medicine, Prince of Songkla University [protocol number REC 60-283-14-1]. Inform consent was waived.

Patient specimens and clinical information

This study included 66 cases of early-stage NSCLC (stages I–III) with paired-normal respiratory epithelial tissue. All patients had undergone radical surgery as their primary therapy. Tissue microarray slides were obtained from formalin-fixed, paraffin-embedded tissue sections, 3–4-μm in thickness, 3 mm in diameter (*Figure 1*). The copy number of the human epidermal growth factor receptor 2 (*HER2*) gene and chromosome 17 were evaluated by DISH. c-Myc protein expression was evaluated based upon immunohistochemistry (IHC) staining. Clinical information comprised the patient characteristics including demographic data, smoking status, NSCLC stage, histology, and survival outcome.

DISH

The copy number of chromosome 17 was determined by a *HER2*/centromeric enumeration probe of chromosome 17 (CEP17) bright-field DISH deoxyribonucleic acid (DNA) probe assay. DISH analysis was performed using a *HER2* DISH DNA probe cocktail (Ventana Medical Systems, Tucson, AZ, USA). *HER2* gene copy number was assessed using a SISH dinitrophenyl (DNP) detection kit, hapten labeling of the *HER2* gene and red ISH digoxigenin (DIG) labeling of the CEP17. Briefly, unstained tissue microarray sections were baked overnight, deparaffinised in xylene, dehydrated in a series of ethanol washes, and air dried. The slides then were incubated in ribonuclease (RNase) for 60 minutes followed by a 10-minute cycle of protease digestion and denaturation. DNP- and DIG-labeled probes were cohybridized to their respective specific DNA sequences. The ultraView SISH DNP detection kit, followed by the Red ISH DIG detection kit were used to detect the DNP-labeled *HER2* probe and the DIG-labeled chromosome 17 probe, respectively. The tissue microarray slides were then counterstained with hematoxylin II (Ventana Medical Systems). Image acquisition was performed using light microscopy. DISH counts were performed and the results were reported according to the established guideline. At least 100 non-overlapping intact interphase nuclei from each tissue sample were analyzed independently by 2 investigators (PS and Par T) to determine the average copy numbers and average ratios of *HER2* gene and chromosome 17. The mean copy numbers of *HER2* and chromosome 17 per cell were recorded, and the *HER2*:CEP17 ratio was calculated for each case. The following criteria adapted from the 2007 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) criteria for dual-color ISH to classify a gain of *HER2* gene or chromosome 17 was implemented.

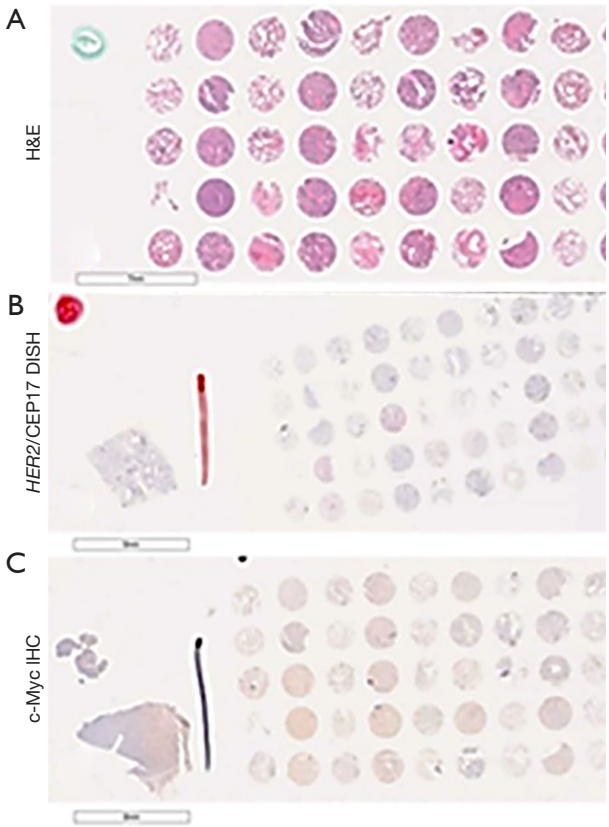

Figure 1 Tissue microarray slides stained for H&E, *HER2*/CEP17 DISH, and c-Myc IHC performed for tumor cells and paired-normal respiratory epithelium in NSCLC patients. Representative microscopic images (10×) of (A) H&E, (B) *HER2*/CEP17 DISH, and (C) c-Myc are shown. HER2, human epidermal growth factor receptor 2; CEP17, centromeric enumeration probe of chromosome 17; DISH, dual in situ hybridization; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer.

Copy gains of *HER2* gene: Gains were deemed in tissue specimens when the analysed nuclei presented an average ratio between the *HER2* gene and the reference CEP17 probe (i.e., *HER2*:CEP17) ≥2.2.

Clonal gains of *HER2* gene: Gains were defined when the analysed nuclei presented an average ratio between the *HER2* gene and the reference CEP17 probe (i.e., *HER2*:CEP17) <2.2 (unmet criterion of the copy gains of *HER2* gene), but ≥10% of the analysed nuclei demonstrated a *HER2*:CEP17 ratio ≥2.2.

Chromosome 17 copy gains or polysomy of chromosome 17: Gains of chromosome 17 were deemed when the analysed nuclei presented an average copy number ≥3 for both *HER2* gene and CEP17, and the *HER2*:CEP17 ratio was <2.2.

IHC

Expression of c-Myc was determined by IHC. In brief, sections from the tissue microarray blocks were deparaffinized in xylene, rehydrated in graded alcohol, and washed in wash-buffer solution. Positive controls as well as negative reagent controls as a reference were included in each staining run as a reference. An epitope retrieval process using Dako Target Retrieval Solution (1:10; Agilent Technologies, Santa Clara, CA, USA) at 105 ℃ for 20 min was performed. The slides were then loaded onto an autostainer and the following incubations were applied sequentially: 10-min peroxide blocking agent (3%), 10-min Power Block, 10-min proteinase K, and a rabbit monoclonal anti-c-Myc antibody (1/50, EP121; Cell Marque, Rocklin, CA, USA). A buffer rinse was performed following each step. The slides were counterstained with hematoxylin, then rinsed gently in reagent quality water, dehydrated in graded alcohol, cleared in xylene, and cover-slipped.

Analyses of the IHC-stained slides were performed using the 10× magnification objective of a light microscope. c-Myc protein expression was categorized as positive or negative based on an IHC semi-quantitative score. In detail, the percentage of positive tumor cells per slide (0–100%) was multiplied by the dominant intensity pattern of staining (0= negative or trace; 1+ = weak; 2+ = moderate; 3+ = intense), giving an overall score ranging from 0–300. Specimens with IHC 2/3+ ≥75% of the cells of interest or an overall score ≥150 were considered positive for c-Myc.

Statistical analysis

Differences in c-Myc protein expression and chromosome 17 copy number gains between NSCLC patient subgroups were compared using univariate statistics, including the comparison of 2 means (*t*-test) and the comparison of 2 proportions (Chi-squared test). Multivariate statistics and Cox's regression model to explore the relationship between c-Myc expression and the chromosome 17 copy number gains were also applied for the analysis. A P value of <0.05 was considered statistically significant. A Kaplan-Meier plotter and log-rank test were applied for survival analysis in each patient subgroup.

Results

The clinicopathological characteristics of the patients are shown in *Table 1*. The copy number gains of chromosome

Table 1 Clinical features of study patients (n=66)

Clinicopathological characteristics	n (%)
Age (years), Mean ± SD	61.8±10.9
Age	
≤65 years	40 (60.6)
>65 years	26 (39.4)
Gender	
Male	37 (56.1)
Female	29 (43.9)
Smoking status	
Smoker	31 (47.0)
Never-smoker	29 (43.9)
Unknown	6 (9.1)
Tumor histology	
Adenocarcinoma	46 (69.7)
Squamous carcinoma	20 (30.3)
Clinical stage	
I	43 (65.2)
II	20 (30.3)
III	3 (4.5)

SD, standard deviation.

17 were determined by *HER2*/CEP17 bright-field DISH DNA probe assay (*Figure 2*) and were found in 8 of 60 (13.3%) of the NSCLC specimens. No copy number gains of chromosome 17 were found in the paired-normal respiratory epithelium samples (*Table 2*). The mean *HER2* (1.2±0.3) and CEP17 (1.4±0.3) copy numbers of the normal respiratory epithelium were significantly lower than those of the NSCLC tissue [1.8±1.0 and 2.0±0.8, respectively (P<0.001)]. However, the mean *HER2*/CEP17 ratios between the normal respiratory epithelium and the NSCLC tissue were not statistically significantly different (0.8±0.1 and 0.9±0.5, respectively (P=0.16; *Table 3*).

Twelve of 66 (18.2%) NSCLC patients had overexpressed c-Myc protein detected by IHC. Five (41.7%) c-Myc positive patients also had *HER2* gene amplification (1) or copy number gains of chromosome 17 (4), identified by gains of both *HER2* and CEP17 (P=0.13; *Table 4* and *Figure 3*).

The median *HER2* and CEP17 copy numbers, as well as the median *HER2*/CEP17 ratios of the normal respiratory epithelium, were significantly lower than those of the NSCLC tissue, across all clinicopathological subgroups (*Table 5*). Multivariate analysis revealed that neither copy numbers of *HER2* and chromosome 17 or any clinicopathological characteristics were independent predictors of overall survival in the NSCLC patients (*Table 6*).

The prognostic significance of molecular features was evaluated by a Kaplan-Meier survival plotter and Cox proportional hazards model. NSCLC patients with *HER2* gene amplification or copy number gain (polysomy) of chromosome 17 had a greater tendency to be associated with poorer overall survival than those with negative *HER2* results, especially at 1 year [77.8 (95% CI: 36.5, 93.9) months *vs.* 96.1 (95% CI: 85.2, 99.0) months, respectively] and at 3 years [62.2 (95% CI: 21.3, 86.5) months *vs.* 71.1 (95% CI: 55.9, 81.9) months, respectively (P=0.954)] (*Figure 4* and *Table 7*).

To determine whether the combination of c-Myc expression and *HER2* copy number had a higher prognostic significance, 60 NSCLC patients whose tumors could be examined for both c-Myc and *HER2* copy number were categorized based on the status of both variables. Patients with positive expression of c-Myc and *HER2* amplification or gains of copy number of *HER2* (co-positive results) tended to have a more unfavorable prognosis than the other subgroups (*Figure 5* and *Table 7*).

Discussion

NSCLC represents the leading cause of cancer-related deaths worldwide. Treatment outcome for NSCLC remains poor, indicating the need for better understanding of tumorigenesis and therapeutic development. c-Myc oncogene, the cellular homologue of the avian myelocytomatosis virus transforming sequence, encodes a nuclear transcription and replication factor. Alterations of c-Myc protein levels due to gene amplification, enhanced transcription, increased RNA or protein stability, chromosomal translocation, or viral insertion are involved in the initiation and progression of various cancer types (6). c-Myc stimulates the transcription of various target genes involved in cell growth and tumorigenesis and has been demonstrated to promote DNA replication via non-transcriptional mechanisms (7). Moreover, c-Myc has been reported to be associated with chemotherapy resistance and poor survival outcomes in various cancer types including NSCLC (8). In NSCLC, c-Myc has been recognized as an important oncogene in the initiation and progression of the

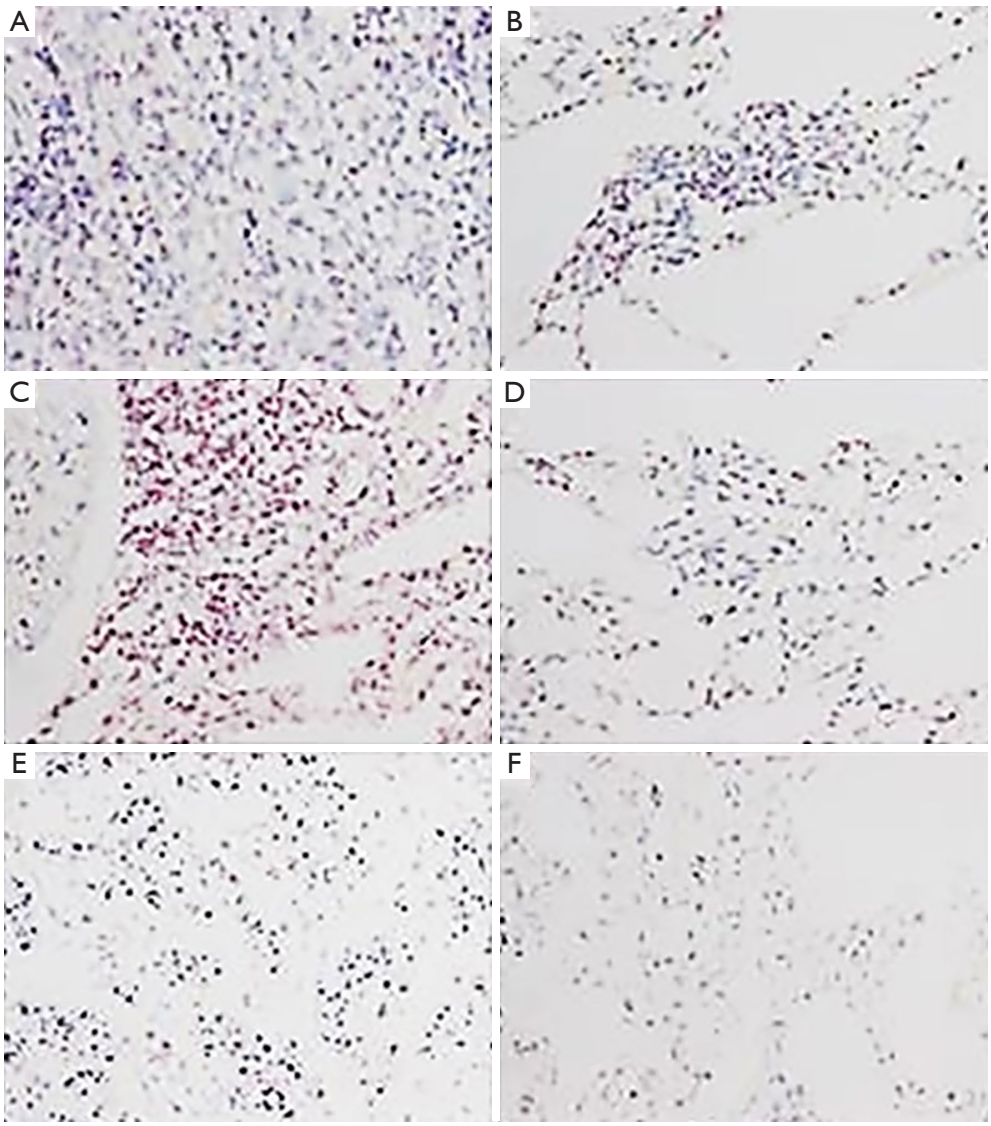

Figure 2 *HER2*/CEP17 DISH in the study NSCLC patients. Representative microscopy images (10×) of NSCLC (A,C,E) and paired-normal respiratory epithelium tissue (B,D,F) showing normal *HER2*/CEP17 (A,B,D,F), copy number gains of *HER2* and chromosome 17 or polysomy (C), and *HER2* amplification (E). HER2, human epidermal growth factor receptor 2; CEP17, centromeric enumeration probe of chromosome 17; DISH, dual in situ hybridization; IHC, immunohistochemistry; NSCLC, non-small cell lung cancer.

tumor (9). Gains in copy numbers of c-Myc were found to be associated with lung adenocarcinoma in never-smokers (10). Increased activation of c-Myc has been observed in lung squamous cell carcinomas, compared to premalignant lesions in the same patients (11).

In this study, expression of c-Myc was significantly more demonstrable as positive IHC staining in tumorous tissue than normal respiratory tissue, confirming a role of c-Myc in NSCLC development. The expression of c-Myc was significantly less frequent in the adenocarcinoma subtype. In addition, NSCLC patients who had overexpressed c-Myc tended to have poorer overall survival than those who did not, highlighting the significance of this protein during stepwise lung carcinogenesis.

In a pre-clinical study, the upregulation of c-Myc lead to multiple changes in nuclear organization and selective chromosome repositioning including mouse chromosome 11 in PreB v-abl/myc cells, which were hypothesized to possibly

Table 2 Copy numbers of *HER2* and chromosome 17 and c-Myc status between respiratory epithelium and non-small cell lung cancer tissue samples (n=66)

	Normal respiratory epithelium, n (%)	NSCLC tissue, n (%)	P value*	Adenocarcinoma n=46, n (%)	Squamous carcinoma n=20, n (%)	P value*
HER2 (DISH of *HER2* and CEP17)			0.001			0.013
Positive (*HER2*/CEP17 ratio ≥2.2)	0	1 (1.7)		1 (2.5)	0	
Gains of *HER2* and CEP17 (Copy number ≥3 and *HER2*/CEP17 ratio <2.2)	0	8 (12.3)		2 (5.0)	6 (30.0)	
Negative	66 (100.0)	51 (85.0)		37 (92.5)	14 (70.0)	
Not available	0	6		6	0	
cMyc expression (IHC 2/3+ ≥75% or score ≥150)			<0.001			0.005
Positive	0	12 (18.2)		4 (8.7)	8 (40.0)	
Negative	66 (100.0)	54 (81.8)		42 (91.3)	12 (60.0)	
Not available	0	0		0	0	

*, Chi-square or Fisher exact test. HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer; DISH, dual-color in situ hybridization; CEP17, centromeric enumeration probe of chromosome 17; IHC, immunohistochemistry.

Table 3 Mean copy numbers of *HER2* and chromosome 17 between respiratory epithelium and non-small cell lung cancer tissue samples (n=66)

	Normal respiratory epithelium, mean ± SD	NSCLC tissue, mean ± SD	P value*
Mean *HER2* copy number	1.2±0.3	1.8±1.0	<0.001
Mean CEP17 copy number	1.4±0.3	2.0±0.8	<0.001
Mean *HER2*/CEP17 ratio	0.8±0.1	0.9±0.5	0.156

*, *t*-test. HER2, human epidermal growth factor receptor 2; SD, standard deviation; NSCLC, non-small cell lung cancer; CEP17, centromeric enumeration probe of chromosome 17.

Table 4 Correlation of copy numbers of *HER2* and chromosome 17 and c-Myc status in non-small cell lung cancer patients (n=66)

	c-Myc Negative, n (%)	c-Myc Positive, n (%)	P value*
HER2 positive or gains of *HER2* and CEP17 (polysomy)	10 (18.5)	5 (41.7)	0.125
HER2 negative	44 (81.5)	7 (58.3)	–

*, Chi-square or Fisher exact test. HER2, human epidermal growth factor receptor 2; CEP17, centromeric enumeration probe of chromosome 17; CEP, centromeric enumeration probe.

promote neoplastic transformation including the mouse plasmacytoma (PCT). Accelerated tumor development has usually been associated with duplication of the mouse subcytoband 11E2 (syntenic to human chromosome 17, cytoband 17q25.3), supporting the importance of this chromosomal region in tumorigenesis (12). The 17q25 chromosomal region has been reported to be involved in a variety of human cancers including ovary (13,14), prostate (15), leukemia (16), neuroblastoma (17,18), esophagus (19-21), sarcoma (22,23), and breast (24). The frequent observations of allelic gain or loss in this chromosomal region in various types of cancers suggest that oncogenes or tumor suppressor genes, respectively, are located there. Frequent copy number gains of chromosome 17 have been proposed as a new phenomenon in NSCLC patients. Sunpaweravong *et al.* reported that gains of chromosome 17 were observed in approximately one third (38.9%) of NSCLC patients while a majority (61%) harbored

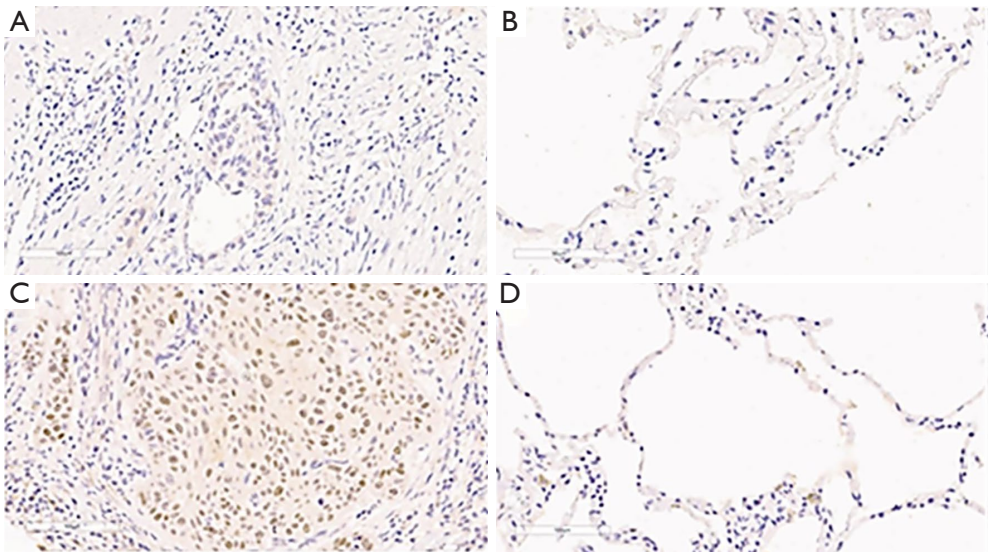

Figure 3 c-Myc expression in NSCLC tissue and paired-normal respiratory epithelium. Representative microscopy images (10×) of NSCLC (A,C) and paired-normal respiratory epithelium (B,D) show c-Myc negative staining (A,B,D) and positive staining (C). NSCLC, non-small cell lung cancer.

Table 5 Median copy numbers of *HER2* and chromosome 17 between respiratory epithelium and non-small cell lung cancer tissue samples (n=66)

	n (%)	*HER2* copy number			CEP17 copy number			*HER2*/CEP17 ratio		
		Normal respiratory epithelium	NSCLC tissue	P value*	Normal respiratory epithelium	NSCLC tissue	P value*	Normal respiratory epithelium	NSCLC tissue	P value*
Age										
≤65 years	40 (60.6)	1.1 (1.0, 1.2)	1.5 (1.2, 2.0)	<0.001	1.4 (1.2, 1.5)	1.7 (1.4, 2.2)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.01
>65 years	26 (39.4)	1.1 (1.0, 1.1)	1.5 (1.3, 1.9)	<0.001	1.3 (1.2, 1.4)	1.9 (1.6, 2.2)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.06
Gender										
Male	37 (56.1)	1.1 (1.0, 1.2)	1.5 (1.2, 2.0)	<0.001	1.3 (1.2, 1.4)	1.8 (1.5, 2.3)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.006
Female	29 (43.9)	1.1 (1.0, 1.2)	1.6 (1.3, 1.9)	<0.001	1.3 (1.3, 1.4)	1.8 (1.4, 2.0)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.12
Smoking status										
Smoker	31 (47.0)	1.1 (1.0, 1.2)	1.5 (1.2, 2.0)	<0.001	1.3 (1.2, 1.5)	1.8 (1.5, 2.1)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.01
Never-smoker	29 (43.9)	1.1 (1.1, 1.2)	1.6 (1.2, 1.9)	<0.001	1.3 (1.2, 1.4)	1.7 (1.4, 2.0)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.17
Unknown	6 (9.1)	1 (1.1, 1.1)	1.7 (1.3, 2.0)	0.02	1.3 (1.3, 1.4)	1.9 (1.5, 2.3)	0.02	0.8 (0.8, 0.8)	0.9 (0.8, 0.9)	0.11
Tumor histology										
Adenocarcinoma	46 (69.7)	1.1 (1.0, 1.2)	1.4 (1.2, 1.6)	<0.001	1.3 (1.2, 1.4)	1.6 (1.4, 1.9)	<0.001	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.09
Squamous carcinoma	20 (30.3)	1.1 (1.0, 1.5)	2.0 (1.5, 3.0)	<0.001	1.3 (1.3, 1.7)	2.3 (1.9, 3.2)	<0.001	0.8 (0.8, 0.8)	0.9 (0.8, 0.9)	0.003
Clinical stage										
I	43 (65.2)	1.1 (1.0, 1.2)	1.5 (1.2, 1.9)	<0.001	1.3 (1.2, 1.4)	1.7 (1.5, 2.1)	<0.001	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.07
II	20 (30.3)	1.1 (1.1, 1.3)	1.5 (1.3, 2.1)	0.003	1.4 (1.3, 1.6)	1.8 (1.5, 2.4)	0.01	0.8 (0.8, 0.9)	0.9 (0.8, 0.9)	0.01
III	3 (4.5)	1.0 (1.0, 1.0)	1.5 (1.3, 1.8)	0.35	1.3 (1.2, 1.4)	1.9 (1.6, 2.1)	0.4	0.8 (0.8, 0.8)	0.8 (0.8, 0.9)	0.27

Data are presented as median (IQR). *, *t*-test. HER2, human epidermal growth factor receptor 2; CEP17, centromeric enumeration probe of chromosome 17; IQR, interquartile range; NSCLC, non-small cell lung cancer.

Table 6 Multivariate Cox regression analyses of copy numbers of *HER2* and chromosome 17 and clinicopathological characteristics for overall survival in study non-small cell lung cancer patients (n=66)

	Crude HR (95% CI)	Adjusted HR (95% CI)	P value (LR-test)
Age: >65 *vs.* ≤65 years	0.63 (0.28, 1.41)	0.82 (0.32, 2.1)	0.683
Gender: female *vs.* male	0.78 (0.36, 1.7)	0.68 (0.16, 2.91)	0.611
Smoking status: Reference = Smoker			0.769
Never-smoker	0.84 (0.38, 1.88)	1.57 (0.34, 7.18)	
Unknown	1.2 (0.34, 4.23)	1.62 (0.36, 7.21)	
Tumor histology: squamous carcinoma *vs.* adenocarcinoma	1.88 (0.87, 4.06)	2.3 (0.8, 6.58)	0.126
Clinical Stage: Reference = Stage I			0.112
Stage II	2.67 (1.23, 5.81)	2.6 (1.06, 6.35)	
Stage III	1.56 (0.2, 11.89)	1.16 (0.13, 10.53)	
c-Myc tumor status: Positive *vs.* Negative	1.46 (0.55, 3.89)	1.21 (0.36, 4.14)	0.76
HER2 tumor status: Reference = Negative			0.399
Positive or polysomy	1.06 (0.31, 3.57)	0.56 (0.13, 2.37)	
Not available	1.39 (0.47, 4.08)	2.03 (0.6, 6.86)	

HER2, human epidermal growth factor receptor 2; HR, hazard ratio; CI, confidence interval; LR, likelihood ratio.

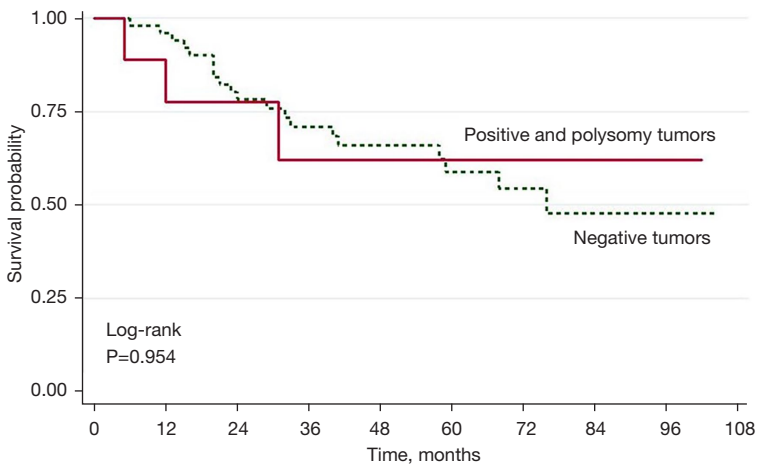

Figure 4 Overall survival in NSCLC patients, stratified by copy numbers of *HER2* and chromosome 17. HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer.

clonal gains of cytoband 17q25.3. These patients also had a tendency towards poorer survival outcome (2).

HER2 has been well established as a driver and therapeutic target in several cancers including NSCLC (25). However, the term "*HER2*-positive NSCLC" represents a distinct *HER2* aberration, not only at the molecular level but also in clinical entities and potential therapeutic agents. *HER2* amplification has been reported in 10–20% of NSCLC patients while HER2 protein overexpression has been observed in 2.4-38% and *HER2* mutations, such as in-frame insertions, have been detected in 2–4% (26-29). The Cancer Genome Atlas (TCGA) Research Network reports that tissue specimens from NSCLC patients harboring *HER2* amplification have not been found to consistently

Table 7 Survival of non-small cell lung cancer patients stratified by *HER2* copy number and c-Myc status

Variable	n	1-year survival, % (95% CI)	3-year survival, % (95% CI)	5-year survival, % (95% CI)
HER2 (n=60)				
Negative	51	96.1 (85.2, 99.0)	71.1 (55.9, 81.9)	58.9 (41.9, 72.4)
Positive and polysomy	9	77.8 (36.5, 93.9)	62.2 (21.3, 86.5)	62.2 (21.3, 86.5)
c-Myc (n=66)				
Negative	54	96.3 (86.0, 99.1)	72.4 (57.7, 82.7)	56.3 (40.0, 69.8)
Positive	12	83.3 (48.2, 95.6)	53.3 (19.9, 78.3)	53.3 (19.9, 78.3)
HER2 and c-Myc (n=60)				
c-Myc and *HER2* negative	44	95.5 (83.0, 98.8)	71.6 (55.3, 82.8)	58.1 (39.9, 72.5)
c-Myc negative and *HER2* positive	4	100.0 (–, –)	100.0 (–, –)	100.0 (–, –)
c-Myc positive and *HER2* negative	7	100.0 (–, –)	71.4 (25.8, 92.0)	71.4 (25.8, 92.0)
c-Myc positive and *HER2* positive	5	60.0 (12.6, 88.2)	40.0 (5.2, 75.3)	40.0 (5.2, 75.3)

HER2, human epidermal growth factor receptor 2; CI, confidence interval.

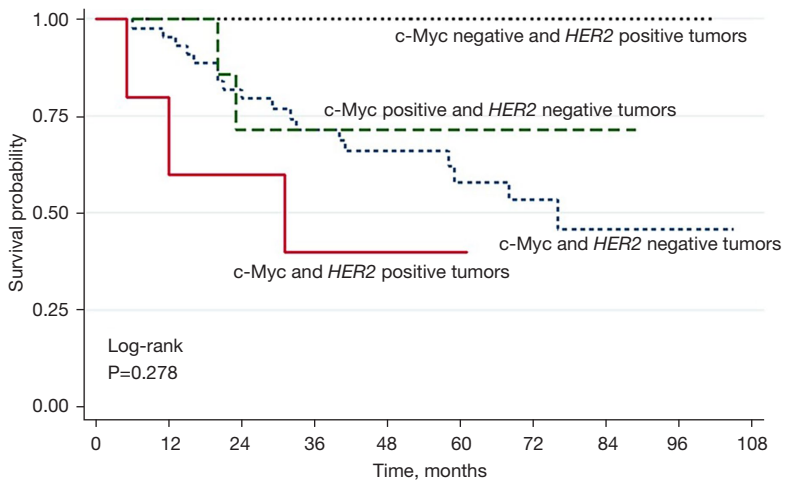

Figure 5 Overall survival in NSCLC patients, stratified by c-Myc expression and *HER2* status. HER2, human epidermal growth factor receptor 2; NSCLC, non-small cell lung cancer.

associate with those carrying the *HER2* mutation (27). Arcila *et al.* reported a group of 11 *HER2*-amplified lung cancer patients who had no co-existing *HER2* mutations (30). However, the concurrence of *HER2* amplifications and *HER2* mutations have been reported in other studies, ranging from 3-50% of NSCLC patients (28,31,32).

Different *HER2* detection methods and assessment criteria can impact *HER2* results and interpretation. To detect *HER2* amplification, in situ hybridization (ISH) remains the most used method. Both CISH and SISH have been accepted as a valid alternative to FISH and provide similar results in evaluating *HER2* and CEP17 copy numbers (33). DISH and FISH are quantitatively-based techniques which have been used for the quantification of intracellular nucleic acids, however, FISH is more costly and time-consuming, requiring complex interpretation of fluorescency features. The major advantage of DISH images acquired from bright-field microscopy is higher degree in providing tissue morphology, especially for assessing tumor heterogeneity (5). We used the bright-field ISH assay to assess the copy numbers of *HER2* and chromosome 17 in early-stage NSCLC patients. In this

study, 13.3% of the available NSCLC specimens were found to have copy number gains of chromosome 17 while no copy number gains of chromosome 17 were observed in the paired-normal respiratory epithelium specimens. Additionally, the mean copy numbers of *HER2* and CEP17 were also higher than those of the paired-normal respiratory epithelium specimens, emphasizing the important role of cancer-associated genes located on this chromosome which contribute to NSCLC tumorigenesis, including *HER2*/neu (17q12) in the EGFR family.

NSCLC has been characterized by extensive genomic instability at various levels, ranging from mutations in nucleotides to aneuploidy resulting from gains or losses of DNA copy numbers or entire chromosomes (8). Similar to our study, polysomy of chromosome 17 has been previously reported as a frequent phenomenon in NSCLC (25,31). However, apart from this study, the prognostic significance of polysomy of chromosome 17 has not been well established before. We, therefore, encourage further investigations to evaluate and confirm the potential prognostic and/or predictive value of chromosome 17 polysomy in NSCLC development and treatment outcomes.

We found that 41.7% of c-Myc positive NSCLC patients also had *HER2* gene amplification or copy number gains of the entire chromosome 17, identified by gains of both *HER2* and CEP17. Additionally, we found that NSCLC patients who harbored the *HER2* gene amplification or had copy number gains of chromosome 17 and positive expression of c-Myc tended to be associated with poor overall survival. The results from this study suggest that copy number gains of chromosome 17 and c-Myc overexpression could be promising prognostic biomarkers for NSCLC. However, this study is a preliminary report of a limited number of tumor samples, therefore further investigations are required to confirm our findings.

Conclusions

The *HER2* DISH DNA probe provides a reliable way to detect the chromosome 17 copy number in NSCLC patients and could be considered as an alternative assay to FISH. We found that copy number gains of chromosome 17 correlated with overexpression of c-Myc, emphasizing an important genetic aberration on this chromosome in NSCLC tumorigenesis. Both biomarkers may be used as prognostic factors to identify NSCLC patients with poorer survival outcomes and are potential targets for therapeutic development.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2705/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2705/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2705/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Faculty of Medicine, Prince of Songkla University (protocol number REC 60-283-14-1). Inform consent was waived.

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