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Frequency of driver mutations in lung adenocarcinoma from female never-smokers varies with histological subtypes and age at diagnosis

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Abstract

Purpose—Our previous study revealed that 90% (47 of 52; 95% CI: 0.79–0.96) of Chinese never-smokers with lung adenocarcinoma harbor known oncogenic driver mutations in just four genes: *EGFR*, *ALK*, *HER2*, and *KRAS*. Here, we examined the status of known driver mutations specifically in female never-smokers with lung adenocarcinoma.

Experimental Design—Tumors were genotyped for mutations in *EGFR*, *KRAS*, *ALK*, *HER2*, and *BRAF*. Data on age, stage, tumor differentiation, histological subtypes, and molecular alterations were recorded from 349 resected lung adenocarcinomas from female never-smokers. We further compared the clinicopathological parameters according to mutational status of these genes.

Results—Two hundred and sixty-six (76.2%) tumors harbored *EGFR* mutations, 16 (4.6%) *HER2* mutations, 15 (4.3%) *EML4-ALK* fusions, seven (2.0%) *KRAS* mutations, and two (0.6%) *BRAF* mutations. In univariate analysis, patients harboring *EGFR* mutations were significantly older ($p<0.001$), whereas patients harboring *HER2* mutations were significantly younger ($p=0.036$). Higher prevalence of *KRAS* ($p=0.028$) and *HER2* ($p=0.021$) mutations was found in invasive mucinous adenocarcinoma (IMA). The frequency of *EGFR* mutations was positively correlated with acinar predominant tumors ($p=0.002$). Multivariate analysis revealed that older age at diagnosis ($p=0.013$) and acinar predominant subtype ($p=0.005$) were independent predictors of *EGFR* mutations. Independent predictors of *HER2* mutations included younger age ($p=0.030$) and IMA ($p=0.017$). IMA ($p=0.006$) and poor differentiation ($p=0.028$) were independently associated with *KRAS* mutations.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Conclusions—The frequency of driver mutations in never-smoking female lung adenocarcinoma varies with histological subtypes and age at diagnosis. These data have implications for both clinical trial design and therapeutic strategies.

Keywords

Lung adenocarcinoma; Female; Never smoker; EGFR mutation; HER2 mutation; Acinar; Mucinous; Age

Introduction

Lung cancer is the leading cause of death from malignant tumors in males, and the second leading cause of cancer death among females worldwide (1). Chinese females have a lung cancer incidence of 21.3 cases per 100,000 population (1). Histologically, lung cancer can be classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The latter mainly contains three subtypes: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Although tobacco smoking is the major risk factor for lung cancer (2–3), the disease also occurs in never-smokers, defined as those who smoked less than 100 cigarettes throughout their life (4). Never-smokers are more likely to be female and have tumors with adenocarcinoma histology (4–5). For example, up to 53% of lung cancers in women may not be caused by direct smoking (6).

Recently, adenocarcinomas in particular have been found to harbor recurrent driver mutations that can predict benefit from targeted therapies. The most prominent example involves epidermal growth factor receptor (*EGFR*) tyrosine kinase domain mutations that predict response to the *EGFR* tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib (7–9). Other commonly reported driver mutations in lung adenocarcinoma involved genes such as *KRAS*, *HER2*, *ALK*, and *BRAF*. Tumors harboring *HER2* mutations, *ALK* fusions, and *BRAF* mutations are sensitive to BIBW 2992 (10), crizotinib (11), and PLX4032 (12), respectively.

In 2011, a new multidisciplinary classification of lung adenocarcinoma was proposed by the International Association for the Study of Lung Cancer, American Thoracic Society, and European Respiratory Society (IASLC/ATS/ERS). In the new classification, the term bronchioloalveolar adenocarcinoma (BAC) was no longer used, and invasive adenocarcinomas were classified according to the predominant subtype (13). It is appealing to know the association between adenocarcinoma histological subtypes and prevalence of driver mutations.

Mutations in the tyrosine kinase domains of *EGFR* and *HER2* are both more frequent in adenocarcinomas, never-smokers, females, and Asian patients (14–16). We previously showed that up to 90% (47 of 52; 95% confidence interval: 0.79–0.96) of lung adenocarcinoma from Chinese never-smokers harbor known oncogenic driver mutations in just 4 genes: *EGFR*, *ALK*, *HER2*, and *KRAS* (17). Here, we extended our comprehensive mutational analyses of *EGFR*, *KRAS*, *ALK*, *HER2*, and *BRAF* to 349 never-smoking women with lung adenocarcinoma, and examined correlations between molecular alterations and clinicopathological features.

Materials and Methods

Patients and samples

From October 2007 to July 2011, we consecutively procured lung tumors resected with curative intent at the Department of Thoracic Surgery, Fudan University Shanghai Cancer Center, Shanghai, China. Subjects eligible for this study had to meet the following criteria:

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pathologically confirmed lung adenocarcinoma; sufficient tissue for comprehensive mutational analyses. Patients who received neoajuvant chemotherapy were excluded. This research was approved by the Institutional Review Board of the Fudan University Shanghai Cancer Center, Shanghai, China. Written informed consent was obtained from all patients.

Mutational analyses

RNA was extracted as per standard protocol after frozen tumor specimens were dissected into TRIZOL (Invitrogen). Total RNA samples were reverse transcribed into complementary DNA (cDNA). *EGFR* (exons 18–22), *HER2* (exons 18 to 21), *KRAS* (exons 2 to 3), and *BRAF* (exons 11 to 15) were amplified by polymerase chain reaction (PCR) using cDNA. Amplified products were analyzed by direct dideoxynucleotide sequencing. To identify *EML4-ALK* fusions, multiple 5' primers were used along with a fixed 3' primer localizing to *ALK* exon 20 in order to detect all known *EML4* fusion variants as previously described (17). Note, in our previous study (17), we screened for more mutations; based upon results from the first 52 cases analyzed, subsequent tumors were analyzed for a more restricted set of mutations.

Clinicopathological variables

Clinicopathological data collected for analyses included age at diagnosis, pathological TNM stage, tumor differentiation, and histological subtypes of adenocarcinoma according to the new IASLC/ATS/ERS multidisciplinary classification of lung adenocarcinoma (13). TNM stages were evaluated in accordance with the seventh edition of the lung cancer staging classification system (18).

Statistical methods

Pearson's χ^2 test (when no cell of a contingency table has expected count less than five) or Fisher's exact test (when any cell of a contingency table has expected count less than five) was used to assess the association between two categorical variables. Independent sample t-test was applied to investigate correlation between a categorical variable and a continuous variable. For multivariate analyses, binary logistic regression model was employed. All the statistical analyses were performed in the SPSS for Windows (Version 16.0, Chicago, IL). *P*-values were two-tailed for all the tests. Statistical significance was set as $p<0.05$.

Results

Patient characteristics

A total of 349 never-smoking female lung adenocarcinoma cases met eligibility for this study. All patients were Chinese. A summary of patient characteristics was listed in Table 1. The median age at diagnosis was 58 years (range: 23–80). The number of patients in stages I–IV was 206, 33, 99, and 11 respectively. Seventy-seven (22.1%), 175 (50.1%), and 97 (27.8%) tumors were poorly, moderately, and well differentiated, respectively. The most common histological subtype was acinar predominant (52.4%), followed by papillary predominant (15.5%), solid predominant (13.2%), and lepidic predominant (9.7%). Other invasive types of adenocarcinoma included 14 (4.0%) invasive mucinous adenocarcinoma (IMA), six (1.7%) micropapillary predominant, and two (0.6%) enteric predominant. There were two (0.6%) adenocarcinoma in situ (AIS), and eight (2.3%) minimally invasive adenocarcinoma (MIA), all were nonmucinous. Detailed clinicopathological data for each patient was listed in Supplementary Table S1.

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Correlation between driver mutations and clinicopathological features

Two hundred and sixty-six (76.2%) tumors harbored *EGFR* mutations, 16 (4.6%) *HER2* mutations, 15 (4.3%) *EML4-ALK* fusions, seven (2.0%) *KRAS* mutations, and two (0.6%) *BRAF* mutations (Figure 1). All were mutually exclusive. Only 43 (12.3%) cases did not have any of these mutations.

Among *EGFR* tyrosine kinase domain mutations, 124 were exon 19 deletions, 111 were L858R, eight were G719X mutations in exon 18, and 10 were insertions in exon 20. Five T790M were detected in chemotherapy-naïve patients (four concurrent with L858R, one with G719S). Other aberrations were composed of 709ET=>D, E709K, F723I, L692V, and V689L in exon 18, K757M, 746 ELREAT ins L, 745–746 ins IPVAM, and 746–747 ins GVV in exon 19, S784Y, I768S, V774M, P776L, and S768I in exon 20, and L861Q, L833V, and V834L in exon 21.

All the 16 *HER2* mutations were exon 20 insertions. *EML-ALK* fusions involved six V1, four V2, two V3a/b, and three other fusion variants. *KRAS* mutations included three G12C, two G12D, one G12V, and one G12A. The two *BRAF* alterations were both V600E mutations.

As shown in Table 2, patients with *EGFR* mutations were significantly older than *EGFR* wild-type patients (mean age, 58.9 versus 54.5 years; $p<0.001$), whereas patients harboring *HER2* mutations were significantly younger than those who did not (mean age, 52.6 versus 58.1 years; $p=0.036$). There were no significant differences in the distributions of disease stage, or tumor differentiation. Clinicopathological characteristics among *EGFR* mutation subtypes were not statistically different (Supplementary Table S2). Since the number of cases with *KRAS* or *BRAF* mutations was low, we only listed individual patient characteristics for these patients in Supplementary Table S1.

Based on the findings of correlation between age and the presence of driver mutations, we further equally stratified patients into three categories according to age at diagnosis: ≤54, >54 and ≤61, and >61 years old. The three age groups contained 121 (34.7%), 111 (31.8%), and 117 (33.5%) patients, respectively. Figure 2 showed the distribution of molecular drivers among various age groups. Although more than half of the patients harbored *EGFR* mutations in each category, the frequency of *EGFR* mutation increased significantly from 66.9% in patients ≤54 years to 76.6% in patients >54 and ≤61 years, and 85.5% in those >61 years ($p=0.004$). The mutation rate of *HER2* was 9.1% in patients ≤54 years, whereas only 3.6% of patients >54 and ≤61 years, and one patient (0.9%) in the oldest category harbored *HER2* mutations ($p=0.008$). The incidences of *KRAS* mutation, *EML4-ALK* fusion, and *BRAF* mutation, respectively, were low and comparable among the age categories.

The distribution of driver mutations according to adenocarcinoma histological subtypes was shown in Figure 3. We compared frequency of driver mutations in each individual subtype to all other subtypes (Supplementary Table S3). All the AIS and MIA harbored *EGFR* mutations. The frequency of *EGFR* mutations was positively correlated with acinar predominant tumors (83.1% versus 68.7%; $p=0.002$), and negatively with invasive mucinous adenocarcinoma (28.6% versus 78.2%; $p<0.001$) and solid predominant tumors (60.9% versus 78.5%; $p=0.009$). Significantly higher prevalence of *KRAS* (14.3% versus 1.5%; $p=0.028$) and *HER2* (21.4% versus 3.9%; $p=0.021$) mutations was found in IMA.

Multivariate analyses of predictors of driver mutations

Correlations among *EGFR*, *HER2*, and *KRAS* mutations with clinicopathological features were further evaluated by logistic regression analysis incorporating patient age, TNM stage, tumor differentiation, and histological subtypes. We used median age (58 years) as cutoff

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value. Odds ratios (OR), 95% confidence intervals (CI), and *P*-values were listed in Table 3. Older age at diagnosis (OR=1.93, 95% CI: 1.15–3.24; p=0.013) and acinar predominant subtype (OR=2.10, 95% CI: 1.25–3.52; p=0.005) were independent predictors of *EGFR* mutations. Independent predictors of *HER2* mutations included younger age at diagnosis (OR=4.17, 95% CI: 1.15–15.11; p=0.030) and IMA subtype (OR=5.81, 95% CI: 1.37–24.72; p=0.017). IMA subtype (OR=14.30, 95% CI: 2.15–95.10; p=0.006) and poor differentiation (OR=6.91, 95% CI: 1.24–38.60; p=0.028) were independently associated with *KRAS* mutations.

Discussion

While the incidence of lung cancer in men seems to have reached a plateau, lung cancer rates in women are still rising (1). In parallel, translational research has progressed to the point where we can define the disease at a clinically relevant molecular level. Previous studies have demonstrated distinct molecular features in Asian female never-smokers with lung adenocarcinoma (14–16). However, our study is the first to clarify the spectrum of well-identified oncogenic driver mutations specifically in a large set of lung adenocarcinoma from East Asian never-smoking women, and to analyze correlations between driver mutations and clinicopathological characteristics, especially histological subtypes of adenocarcinoma in line with the new IASLC/ATS/ERS classification of lung adenocarcinoma.

There have been few studies examining the relationship between age at diagnosis and the status of multiple driver mutations concurrently in lung cancer. In those that studied associations with just *EGFR* mutations, the data are conflicting (19–21). Two studies retrospectively genotyped lung cancer specimens for *EGFR* from large clinical trials, and revealed that *EGFR* mutations were associated with younger age of onset (20–21). However, these trials mainly enrolled Caucasian patients. Moreover, a considerable percentage of patients in both studies were males, smokers, or diagnosed with non-adenocarcinoma histology (20–21). This age discrepancy could be partly due to the fact that female lung cancer patients, who are enriched for *EGFR* mutations, tend to be younger at diagnosis than male patients (22). More recently, a study on 98 Korean females with NSCLC stratified patients into 5-year age groups and showed that *EGFR* mutation rate significantly increased with age (19), which is consistent with our results. Never-smokers and adenocarcinomas comprised, respectively, 84% and 83% of this population (19). Collectively, these data along with ours highlight again that never-smoking women with lung adenocarcinoma represent a unique subset of patients with the disease.

HER2 mutations were found to be more prevalent in East Asian ethnicity, adenocarcinoma histology, females, and never-smokers (14–15). According to a meta-analysis of published studies, *HER2* mutations were present in a small proportion of NSCLC patients, namely 2.1% and, 1.5% of Asian and, Caucasian cases, respectively (23). Consistent with our data, this study also showed that *HER2* mutated patients were significantly younger at diagnosis (23). Here, we report a mutation rate of 4.6% in Chinese never-smoking females with adenocarcinoma. When limited to the youngest one-third of patients, the frequency of *HER2* mutation was 9.1% in all the patients, and 27.5% in *EGFR* wild-type patients. These data have implications for clinical trials seeking to enrich for patients with *HER2* mutant tumors.

To our knowledge, however, a mechanistic basis to explain an association between the occurrence of driver mutations and age at diagnosis is unclear. As this relationship was revealed in female never-smokers, female hormones might play a role. Estrogens potentially played a role in promoting lung cancer (24). Increased estrogen levels were significantly associated with poorer survival among lung cancer patients (25). Immunohistochemical

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studies showed that *EGFR* mutations correlated with higher expression of estrogen receptors (26–27). In a cohort study, women experiencing a longer fertile life (measured as period from age of menarche to age of menopause or age at diagnosis) were found to have a higher proportion of *EGFR* mutations (28). Although these data are not sufficient to support our findings, future studies on female hormones might help to elucidate the mechanisms underlying the relationship between somatic mutations and age at diagnosis in lung adenocarcinoma from female never-smokers.

With regard to histologic molecular correlations, previous studies revealed a high prevalence of *EGFR* mutations in adenocarcinomas formerly classified as nonmucinous BAC or with nonmucinous BAC patterns (29–30) which probably fell into AIS, MIA, and lepidic predominant subtype in the new classification (13). Our study showed that all the AIS and MIA (all were nonmucinous in this study), and 82.4% (28 out of 34) of lepidic predominant adenocarcinoma harbored *EGFR* mutations, while only one *HER2* mutation and no other driver mutations were detected in lepidic predominant adenocarcinoma, although statistical significance was not reached, probably due to the limited numbers of these subtypes. A negative correlation between *EGFR* mutations and solid subtype had also been reported (31–32). Motoi and colleagues (32) showed that *EGFR* mutations were present in 9.1% (3 out of 33) of the acinar subtype, without statistical significance. However, we could not find a related study enrolling East Asian patients. To our knowledge, our study is the first to report a positive association between *EGFR* mutations and the acinar predominant subtype.

It has been well demonstrated that invasive mucinous adenocarcinoma (formerly mucinous BAC) is correlated with an absence of *EGFR* mutations and the presence of *KRAS* mutations (29, 33–35). The present study confirmed these molecular histological correlations. Casali and colleagues (35) reported that HER2/neu immunohistochemical expression significantly characterized mucinous BAC. However, they did not analyze *HER2* mutation in their study (35). Our study is the first to show that IMA subtype was significantly correlated with the presence of *HER2* mutations. A possible explanation is the high frequency of *HER2* mutations in our series of nearly 350 East Asian female never-smoking lung adenocarcinoma patients which contributed to achieving statistical significance. Together with the findings that *EGFR* mutations were frequently detected in AIS, MIA, and lepidic predominant adenocarcinomas, our study confirmed the distinctive molecular nature between formerly mucinous BAC and adenocarcinoma formerly classified as nonmucinous BAC or with a nonmucinous BAC component, thus supporting the removal of the term BAC in the new classification (13).

In addition to our previous study (17), we also detected two V600E *BRAF* mutations in lung adenocarcinoma from never-smoking women, accounting for a mutation rate of 0.6%. Paik and colleagues (36) screened 697 patients with lung adenocarcinoma for *BRAF* mutations, and found that this molecular alteration was only present in current or former smokers. However, Marchetti and colleagues (37) also detected V600E *BRAF* mutations in never-smokers, and revealed that this mutation subtype was more prevalent in females than in males.

A major limitation of this study was the lack of survival data. Since the cases were collected from 2007 to 2011, survival data of these patients were far from maturity. However, it would be of interest to evaluate the prognostic impact of clinical variables, histological patterns, and mutation types, and further research in this aspect is warranted.

In summary, through analyses of 349 Chinese female never-smokers with lung adenocarcinoma, we found that the frequency of driver mutations varies with histological

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subtypes and age at diagnosis. Given the potential effectiveness of TKIs, our findings have implications for translational research as well as clinical therapeutic strategies.

Translational Relevance

We carried out comprehensive mutational analyses of *EGFR*, *KRAS*, *ALK*, *HER2*, and *BRAF* on lung adenocarcinomas from 349 Chinese never-smoking women, and examined correlations between molecular alterations and clinicopathological features. In logistic regression analyses, older age at diagnosis (p=0.013) and acinar predominant subtype (p=0.005) were independent predictors of *EGFR* mutations. Independent predictors of *HER2* mutations consisted of younger age at diagnosis (p=0.030) and invasive mucinous adenocarcinoma (IMA) subtype (p=0.017). IMA subtype (p=0.006) and poor differentiation (p=0.028) were independently associated with *KRAS* mutations. *HER2* mutations are present in a small proportion of NSCLC patients. However, in our study, when limited to the youngest one-third of patients, the frequency of *HER2* mutations was 9.1% in all the patients, and 27.5% in *EGFR* wild-type patients. These data may help inform both clinical trial design and therapeutic strategies for the treatment of never smoking women with lung adenocarcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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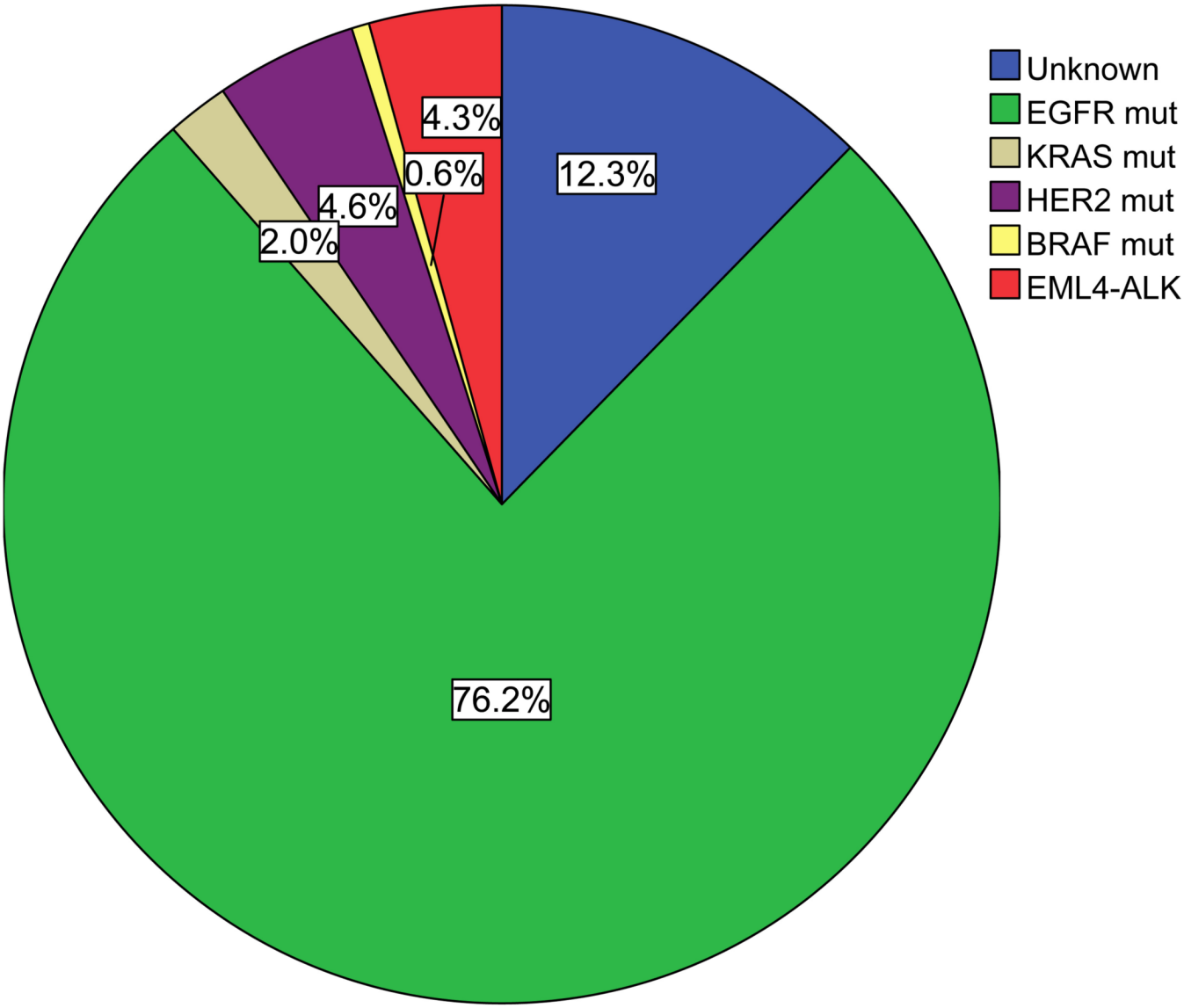

Figure 1.
Frequency of driver mutations in lung adenocarcinoma from female never-smokers. The majority (87.7%) of patients harbored a known mutation in *EGFR*, *HER2*, *ALK*, *KRAS* or *BRAF*.

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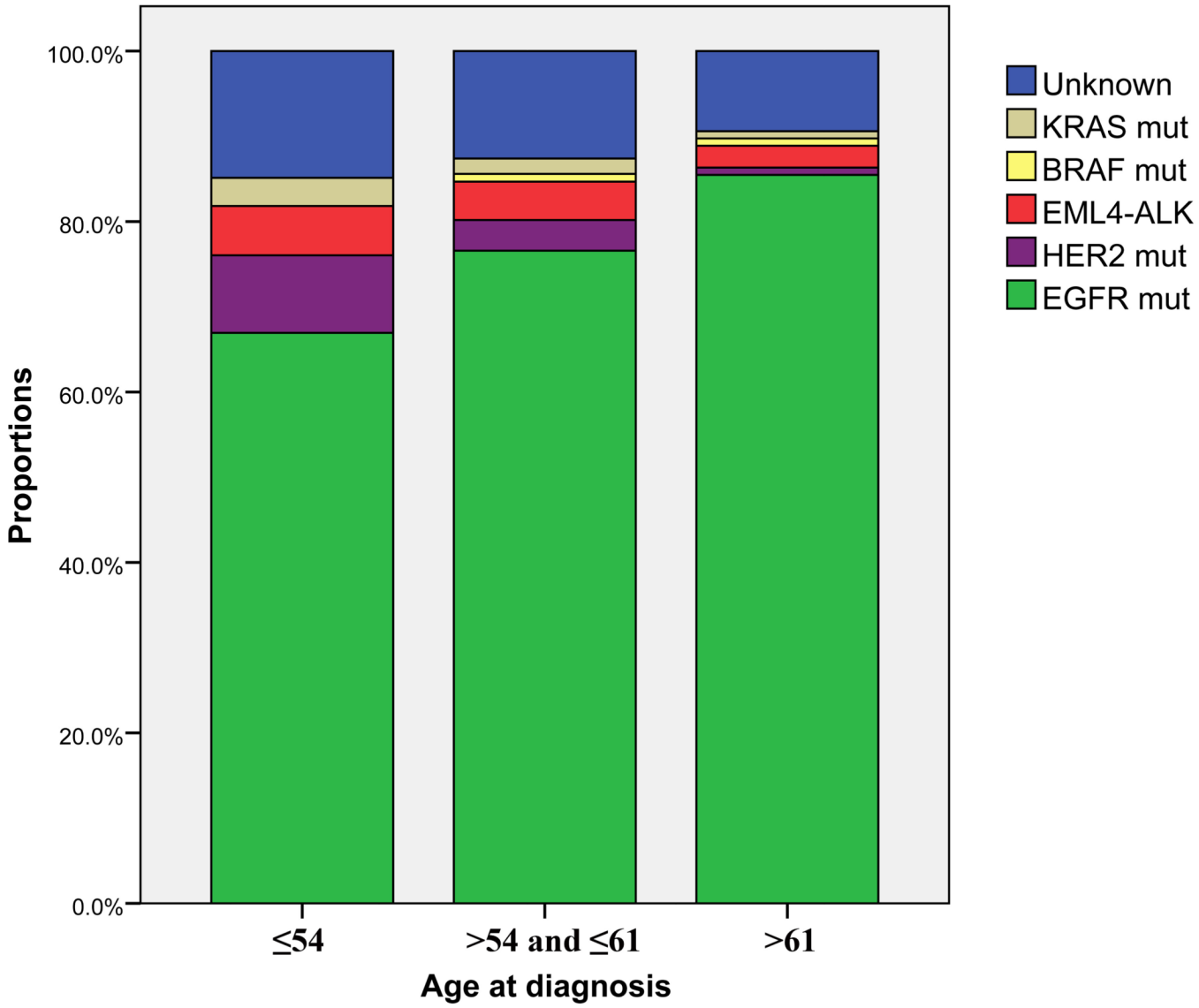

Figure 2.

Distribution of driver mutations in three age categories. The mutation rate of *EGFR* increased progressively with age, from 66.9% in patients ≤54 years to 85.5% in cases >61 years (p=0.004). The frequency of *HER2* mutations reached 9.1% in the youngest one-third of patients, and declined significantly with increasing age (p=0.008).

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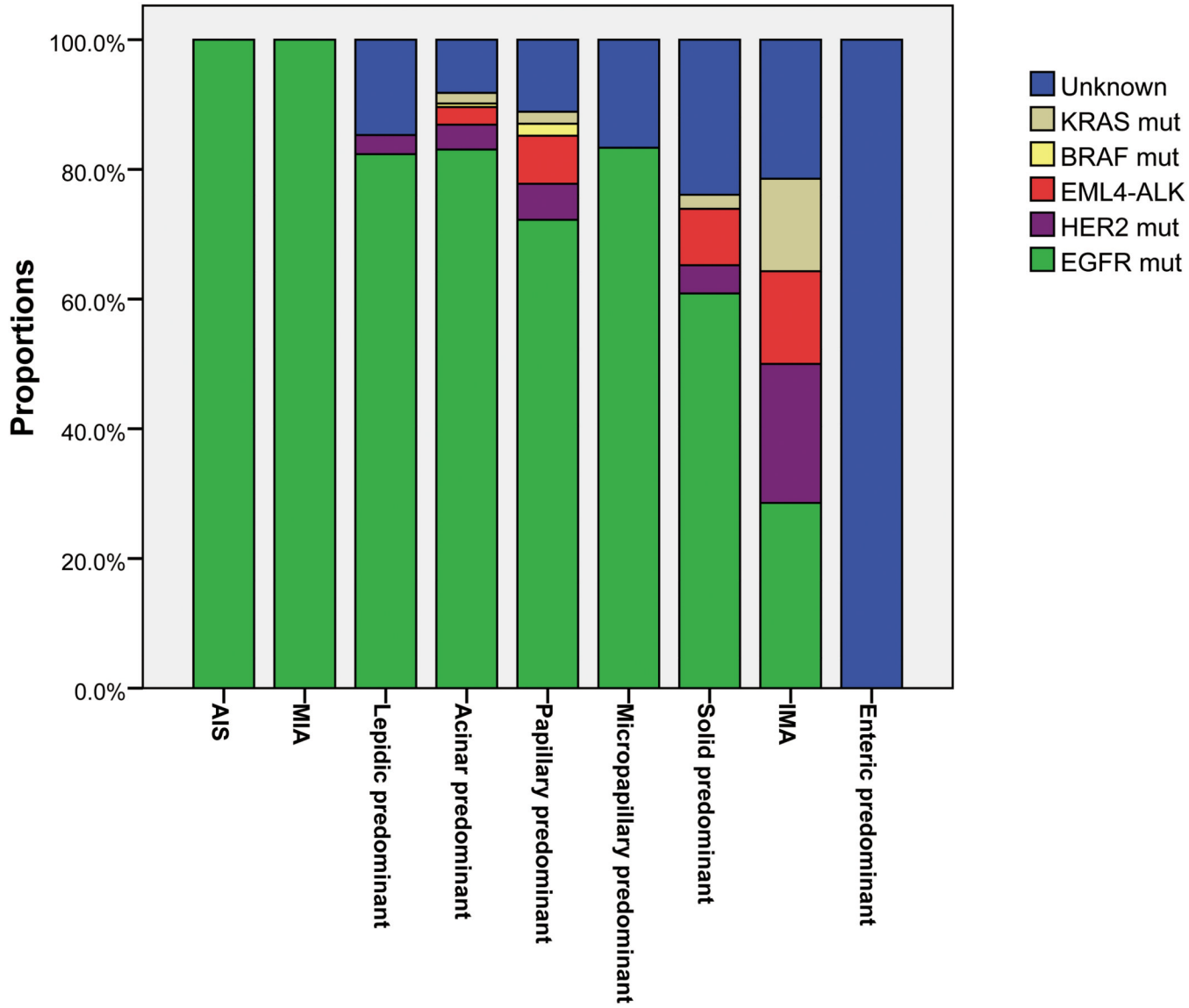

Figure 3.

Distribution of driver mutations according to adenocarcinoma histological subtypes. All the adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) harbored *EGFR* mutations. Significantly higher frequency of *KRAS* (14.3% versus 1.5%; p=0.028) and *HER2* (21.4% versus 3.9%; p=0.021) mutations was found in invasive mucinous adenocarcinoma (IMA). Frequency of *EGFR* mutations was positively associated with acinar predominant tumors (83.1% versus 68.7%; p=0.002), and negatively with IMA (28.6% versus 78.2%; p<0.001) and solid predominant tumors (60.9% versus 78.5%; p=0.009).

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Table 1

Demographics and clinicopathological features of 349 never-smoking female lung adenocarcinoma patients

Variables	No. of patients	%
Age (years)		
≤30	3	0.9
31~40	13	3.7
41~50	60	17.2
51~60	147	42.1
61~70	82	23.5
71~80	44	12.6
Mean ± SD (range)	57.9 ± 10.3 (23–80)	
Stage		
IA	125	35.8
IB	81	23.2
IIA	26	7.4
IIB	7	2.0
IIIA	91	26.1
IIIB	8	2.3
IV	11	3.2
Differentiation		
Poor	77	22.1
Moderate	175	50.1
Well	97	27.8
Histological subtype		
AIS[a]	2	0.6
MIA[b]	8	2.3
Lepidic predominant	34	9.7
Acinar predominant	183	52.4
Papillary predominant	54	15.5
Micropapillary predominant	6	1.7
Solid predominant	46	13.2
IMA[c]	14	4.0
Enteric predominant	2	0.6
Total	349	

[a] AIS, adenocarcinoma in situ.

[b] MIA, minimally invasive adenocarcinoma.

[c] IMA, invasive mucinous adenocarcinoma.

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Table 2

The association between clinicopathological characteristics and mutational status of *EGFR*, *HER2*, and *EML4-ALK* in 349 never-smoking women with lung adenocarcinoma

Clinicopathological variables	*EGFR*			*HER2*			*EML4-ALK*		
	Mut[a]	Wild[b]	*P*	Mut	Wild	*P*	Mut	Wild	*P*
Age (years)									
Mean	58.9	54.5		52.6	58.1		54.5	58.0	
SD	9.7	11.4	<0.001	6.6	10.4	0.036	8.8	10.3	0.190
Differentiation									
Poor	51	26		5	72		4	73	
Moderate	138	37		7	168		8	167	
Well	77	20	0.066	4	93	0.634	3	94	0.786
Stage									
I	166	40		8	198		6	200	
II	24	9		1	32		3	30	
III	68	31		7	92		5	94	
IV	8	3	0.116	0	11	0.653	1	10	0.150
Stage I									
IA	99	26		5	120		5	120	
IB	67	14	0.533	3	78	1.000	1	80	0.407

[a]Mut, mutant type.

[b]Wild, wild type.

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Table 3

Multivariate analyses of factors that might affect the presence of *EGFR*, *HER2* or *KRAS* mutations

***EGFR* mutation**				
Variable	Category	OR	95% CI	*P*
Age	≥58 years/<58 years	1.93	1.15–3.24	0.013
Stage	I+II/III+IV	1.46	0.84–2.54	0.180
Differentiation	Poor/Moderate+Well	0.64	0.35–1.16	0.144
Histological subtype	Acinar predominant/Others[a]	2.10	1.25–3.52	0.005
HER2 mutation				
Variable	Category	OR	95% CI	*P*
Age	<58 years/≥58 years	4.17	1.15–15.11	0.030
Stage	I+II/III+IV	0.72	0.24–2.15	0.551
Differentiation	Poor/Moderate+Well	1.65	0.50–5.42	0.408
Histological subtype	IMA/Others[b]	5.81	1.37–24.72	0.017
KRAS mutation				
Variable	Category	OR	95% CI	*P*
Age	<58 years/≥58 years	2.32	0.42–12.98	0.337
Stage	I+II/III+IV	1.16	0.22–6.10	0.858
Differentiation	Poor/Moderate+Well	6.91	1.24–38.60	0.028
Histological subtype	IMA/Others[b]	14.30	2.15–95.10	0.006

[a] Others, subtypes other than acinar predominant.

[b] Others, subtypes other than invasive mucinous adenocarcinoma.