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Distinctive clinicopathological features of adenocarcinoma in situ and minimally invasive adenocarcinoma of the lung: A retrospective study

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ABSTRACT

Objectives: The aim of this study was to investigate distinguishing clinicopathological features, in addition to histological invasiveness, in adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) of the lung.

Materials and methods: Patients with lung adenocarcinoma who underwent surgery at our hospital between 2007 and 2014 were reviewed, focusing on computed tomography (CT) images, operative procedures and clinical outcomes, histopathology, Ki-67 immunostaining, and *EGFR*-mutation status. *EGFR* mutations were examined using a peptide nucleic acid–locked nucleic acid PCR clamp method. Group comparisons were investigated by Mann–Whitney *U* or Fisher's exact tests.

Results: Of 629 patients with lung adenocarcinoma who underwent surgery, 91 (14%) of 103 AIS (n = 34) or MIA (n = 69) tumors were reviewed. The ratio of male to female patients with MIA compared to AIS was significantly higher ($p < 0.02$). Of 103 tumors, 99 (96%) were non-mucinous. By CT, 74% of AIS appeared as pure ground-glass nodules and 75% of MIAs as part-solid ground-glass nodules. Pathological tumor diameters and Ki-67 labeling index (LI) values were significantly greater for MIAs compared to AIS ($p < 0.001$ for both). A Ki-67 LI of ≥2.8% indicated the presence of an MIA rather than an AIS. *EGFR* mutations were more frequently detected in MIAs (33/69, 48%) than AIS (9/34, 26%; $p = 0.055$). The ratio of exon 19 deletions to exon 21 missense mutations in MIAs tended to be higher than those in AIS ($p = 0.06$). Patients did not experience a local recurrence or metastasis after AIS and MIAs were removed by wedge resection, segmentectomy or lobectomy. Five-year recurrence-free survival rates were 100%.

Conclusion: Despite similar surgical outcomes for AIS and MIAs, we found differences in terms of gender, tumor diameters, CT findings, Ki-67 LI and a subset of *EGFR* mutations, highlighting the validity of classifying the two subtypes.

1. Introduction

In 2011, a new histologic classification was proposed for lung adenocarcinoma by the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society (IASLC/ATS/ERS) [1]. In 2015, the World Health Organization (WHO) defined two new subtypes of lung cancer: adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) [2]. AIS is a small (≤3 cm), localized adenocarcinoma with a pure lepidic growth that lacks stromal, vascular, alveolar space, or pleural invasion. MIA is a small (≤3 cm), solitary adenocarcinoma with a predominantly lepidic growth, showing ≤5 mm invasion along its greatest dimension, and lacking lymphatic, vascular, alveolar space, or pleural invasion. Both are usually subdivided into non-mucinous and, more rarely, mucinous variants. AIS and MIA are categorized as Tis and T1mi, respectively, in the eighth TNM classification of lung cancer [2,3].

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Generally, computed tomography (CT) findings of ground-glass versus solid opacities tend to correspond to lepidic versus invasive histologic patterns, respectively, as seen pathologically. Occasionally, however, contrary to predictions based on CT findings, several part-solid ground-glass nodules (GGNs) are revealed as AIS, while other pure GGNs are identified as MIAs in resected specimens [1,3].

It is hypothesized that morphologically, AIS and MIA represent a gradual malignant progression from the former to the latter; however, the biological activity and continuity involved in the transition remain unclear. Consequently, the clinical, biological and molecular characteristics of AIS and MIA need to be examined for a fuller comparison of both subtypes. The fraction of tumor cells positive for the nuclear antigen, Ki-67 (the Ki-67 labeling index or LI), is normally used to assess cancer cell proliferative activity [4]. Although mutations in the epidermal growth factor receptor (*EGFR*) gene are frequently detected in lepidic-type invasive adenocarcinomas, few studies have focused on the frequency or differences in *EGFR* mutations in AIS and MIA [5]. Regarding surgical outcomes, recent studies have revealed that patients with an AIS or MIA showed nearly 100% disease-free survival (DFS) with a complete resection [5–7].

The aim of this retrospective study was to compare AIS with MIA in terms of the clinical characteristics of patients, including CT findings, as well as tumor characteristics such as histological features, the Ki-67 labeling index and *EGFR* mutations.

2. Materials and methods

2.1. Patients

We retrospectively reviewed patients with lung adenocarcinoma who underwent a resection at our hospital between 2007 and 2014. This study was approved by the ethics committee at Saitama Medical University International Medical Center, which waived the requirement for written informed consent from individual patients.

2.2. CT images

We retrospectively reviewed the findings of preoperative high-resolution CT for 103 lesions in 91 patients. All CT images containing each nodule were acquired with 1-mm section thickness. Ground glass and solid components of lung nodules were observed on a liquid-crystal display with a window level of -500 to -700 Hounsfield units (HU), and a window width of 1500 to 2000 HU as a lung window setting.

A ground glass component was defined as a slight and homogenous increase in attenuation that did not obscure underlying vascular markings, whereas a solid component was defined as an area of increase in opacification that obscured underlying vascular markings in the lung window setting.

We measured total nodule size and the long axis of the largest solid component of each nodule on each axial image. We classified all nodules in CT findings into three types as follows: homogenous ground-glass nodule without a solid component (pure GGN); ground-glass nodule consisting of focal to multiple solid components (part-solid GGN); or a nodule that is a major solid (> 50%) with peripheral ground-glass opacity (solid-dominant nodule). The three types of CT images and all CT values were compared with histological subtypes.

2.3. Surgical procedures

The standard surgical treatment for a lung carcinoma at our institution has been a lobectomy with systemic lymph node dissection. A sublobar resection of a segmentectomy with hilar lymph node sampling or wedge resection, also known as a limited resection, was applied to small tumors representing pure-GGNs or part-solid GGNs on CT, or to synchronous multiple lung tumors.

2.4. Histopathology

Slides with tumor sections were reviewed according to the 2015 WHO classification for pulmonary adenocarcinoma. An AIS was defined as a small ($\leq$3 cm), localized adenocarcinoma with a pure lepidic growth that lacked stromal, vascular, alveolar space, or pleural invasion. An MIA was defined as a small ($\leq$3 cm), solitary adenocarcinoma with a predominantly lepidic growth, showing $\leq$5 mm invasion along the longest dimension, and that lacked lymphatic, vascular, alveolar space, or pleural invasion.

All sections were stained with a conventional hematoxylin and eosin stain for microscopic diagnosis, and subsequently with a van Gieson stain for elastic fibers to assess evidence of stromal, vascular, or pleural invasion. Specimens from all AIS and MIA cases were entirely sampled histologically and specifically evaluated for the presence or absence of invasion. In addition, all slides were immunostained for Ki-67 (clone MIB-1; Dako, Glostrup, Denmark). Immunoreactivity was detected using diaminobenzidine (DAB), and sections were counterstained with hematoxylin. The percentage of positive tumor cells, known as the Ki-67 LI, was calculated by counting more than 500 cells. All slides were reviewed by two pathologists.

2.5. Analysis of EGFR status

We analyzed the *EGFR* mutation status of freshly resected or formalin-fixed paraffin-embedded specimens. The mutation status of *EGFR* exons 18, 19, 20 and 21 was examined using a peptide nucleic acid–-locked nucleic acid PCR clamp method. This method can detect known mutations using PCR primers, each designed for a specific mutated sequence [8].

2.6. Statistical analysis

Continuous variables (expressed as a median) were compared using a Mann–Whitney *U* test. Frequencies were evaluated for categorical variables between the two histological subtypes using Fisher's exact test. Receiver operating characteristic (ROC) curves were plotted for the Ki-67 LI to confirm the optimal cut-off to predict histological subtypes. Overall survival (OS) was calculated from the date of surgery to the date of death from any cause. Recurrence-free survival (RFS) was calculated from the date of surgery to the date of disease recurrence. Survival curves were plotted by the Kaplan–Meier method. *P*-values of less than 0.05 were considered significant.

All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, this is a modified version of R commander that includes statistical functions that are frequently used in biostatistics [9].

3. Results

3.1. Patients investigated

We reviewed 91 patients (14%) with AIS or MIA, enrolled from among 629 patients with lung adenocarcinoma who underwent surgery between 2007 and 2014. Of these, synchronous multiple primary lung carcinomas were documented in 15 (16%) patients who had undergone resections for between two to four tumors. Of the 15 patients, eight had combined lesions of AIS and MIA, and the remaining seven had a lepidic-type invasive adenocarcinoma combined with AIS or MIA. All seven patients with invasive adenocarcinoma underwent a lobectomy with a hilar and mediastinal lymph node dissection. Sixty-five patients underwent a segmentectomy or lobectomy, and 38 patients underwent a wedge resection (Table 1). Adjuvant chemotherapy and radiotherapy, before or after surgery, was not undertaken by any patient.

Table 1
Characteristics of 103 tumors according to histological subtype.

Characteristics		AIS (n = 34)	MIA (n = 69)	*P* value
Age	Median (Range)	66 (40–79)	67 (40–82)	0.65
Gender (male)	n (%)	8 (24)	33 (48)	0.02
Total tumor size on CT (cm)	Median (Range)	1.0 (0.4–2.4)	1.5 (0.5–2.6)	< 0.001
Solid component size on CT (cm)	Median (Range)	0 (0–0.7)	0.3 (0–0.9)	< 0.001
CT image				
Pure ground-glass nodule		25	12	< 0.001
Part-solid ground-glass nodule		6	52	
Solid-dominant nodule		3	5	
Total tumor diameter (cm)	Median (Range)	0.8 (0.4–2.0)	1.1 (0.4–2.2)	< 0.001
Invasive component (cm)	Median (Range)	0	0.2 (0.1–0.5)	
Variant				
Non-mucinous type		32	67	0.59
Mucinous type		2	2	
EGFR mutation				
Present		9	33	0.055
Absent		25	36	
Location				
Right upper lobe		8	20	0.41
Right middle lobe		3	10	
Right lower lobe		7	17	
Left upper lobe		9	17	
Left lower lobe		7	5	
Surgical procedure				
Wedge resection		16	22	0.31
Segmentectomy		7	15	
Lobectomy		11	32	

CT, computed tomography; AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; EGFR, epidermal growth factor receptor.

3.2. Clinicopathological features and surgical procedures

The Table 1 shows comparisons of clinicopathological features according to the histological subtypes of 103 tumors from 91 patients (40 males). The median time interval between preoperative CT scans for image review and surgery was 13 days (range, 1–103 days). Based on the 2015 WHO classification of pulmonary adenocarcinoma, we selected 103 tumors (34 AIS and 69 MIAs) from 91 patients (Fig. 1). Both AIS and MIAs were mostly non-mucinous. Although an overlap of age and the numbers of each gender with tumors was noted, a significant difference in age between patients with AIS or MIA was not observed. Both subtypes were more common in females than in males; however, the ratio of males to females was significantly higher among patients with MIA than those with AIS ($p < 0.02$).

As for histological variants, 32 tumors (31%) were identified as being non-mucinous AIS, 67 (65%) as non-mucinous MIA, and two each (2%) as mucinous AIS and MIA, respectively. Regarding histological subtypes of invasive components in 69 MIAs, an acinar pattern was detected in 50 (72%) and a papillary pattern in 19 (28%) adenocarcinomas. Micropapillary, solid, colloid, fetal or invasive mucinous patterns were not found in invasive components.

The total tumor size ($p < 0.001$), pathological tumor diameter ($p < 0.001$) and solid component size ($p < 0.001$) on CT were significantly greater in MIAs compared to AIS. Significant relationships between histological subtypes, and tumor location or surgical procedures were not found. With regard to surgery, 60 tumors (58%) were removed by sublobar resection (wedge resection or segmentectomy) and 43 tumors (42%) were removed by lobectomy.

3.3. Pathological findings and CT image review

We retrospectively reviewed the findings of preoperative high-resolution CT for 103 tumors from 91 patients. Of 34 AIS, 25 (74%) tumors appeared as a pure GGN on CT, while 52 (75%) of 69 MIAs appeared as a part-solid GGN (Table 1). Most tumors showed a general correlation between pathological diagnosis and CT image pattern (Fig. 1).

In contrast, nine AIS (26%) showed solid components on CT, and subsequently had the appearance of part-solid GGNs or solid nodules. Twelve MIAs (17%) had no solid components and subsequently appeared as pure-GGNs. With regard to reasons for this discrepancy, we demonstrated that a subset of AIS, having the appearance of a part-solid GGN on CT imaging, was due to (1) aggregates of histiocytes within alveolar spaces or lymphocytes of alveolar walls, (2) collapsed alveolar spaces, or (3) a benign scar. These areas were recognized as solid components. Meanwhile, a subset of MIAs with a pure GGN appearance was caused by (4) small invasive foci in the wall of pre-existing vessels within the tumor, or (5) sparsely proliferated areas consisting of papillary or acinar structures. The former (4) led to MIAs being labeled as pure GGN because the margins of normal vessel structures were judged to remain outlined on a CT image. The latter (5) was aerated histologically and thus appeared as a non-solid component [10].

All mucinous variants of two AIS and two MIAs appeared as solid-dominant nodules on CT imaging due to consisting of mucin-rich tumor cells and alveolar spaces filled with mucin, as determined histologically.

3.4. Differentiation using Ki-67 LI

The Ki-67 LI is used as a marker of cellular proliferation and was significantly higher in MIAs (median, 5.23%; range, 1.64–13.3) than in AIS (median, 1.75%; range, 0.54–4.32; $p < 0.001$; Fig. 2A). A ROC curve revealed that the optimal cutoff value for predicting a MIA was a Ki-67 LI of 2.76% (area under the curve, 0.93; 95% confidence interval [CI], 0.885–0.977; sensitivity, 92.4%; specificity, 76.5%; Fig. 2B).

3.5. Distribution of EGFR mutations in histological subtypes

EGFR mutations were detected in 9 of 34 AIS (26%) and in 33 of 69 MIAs (48%); these tended to be more frequent in MIAs than in AIS ($p = 0.055$; Table 1), but did not reach significance ($p = 0.053$) after excluding four mucinous tumors (two AIS and two MIAs). Of all tumors, 17 (17%) showed deletion mutations in exon 19 (E746–A750del, L747–A750del, L747–A750del + T751S, L747–S752del, L747–S752del + P753S, L747–S752del + E746V, and L747–E749del + A750P), and 25 (24%) had missense mutations in exon 21 (L858R and L861Q). With regard to tumors harboring exons 19 and 21 mutations, the ratio of exon 19 deletions (17 tumors) to exon 21 missense mutations (16 tumors) in MIAs tended to be higher than those in AIS (one to nine tumors; $p = 0.06$; Fig. 3). The existence of both exon 19 and 21 mutations was not noted in any tumor. An *EGFR* mutation was not found in any mucinous type AIS or MIA.

3.6. Clinical outcomes after surgery

Regarding surgical outcomes, 23 (68%) of 34 AIS, and 37 (54%) of 69 MIAs were removed by wedge resection or segmentectomy, and the remaining 11 (32%) and 32 (46%) by lobectomy, respectively (Table 1). For the four mucinous variants (4%) of the 103 tumors, one was removed by wedge resection and three by lobectomy.

Seven patients had a lepidic-type invasive adenocarcinoma accompanied by ipsilateral or contralateral AIS, or MIAs. These seven patients had undergone a lobectomy with lymph node dissection for the dominant tumor and an additional resection of the wedge resection or a lobectomy (middle lobe) for multifocal GGNs. Consequently, the

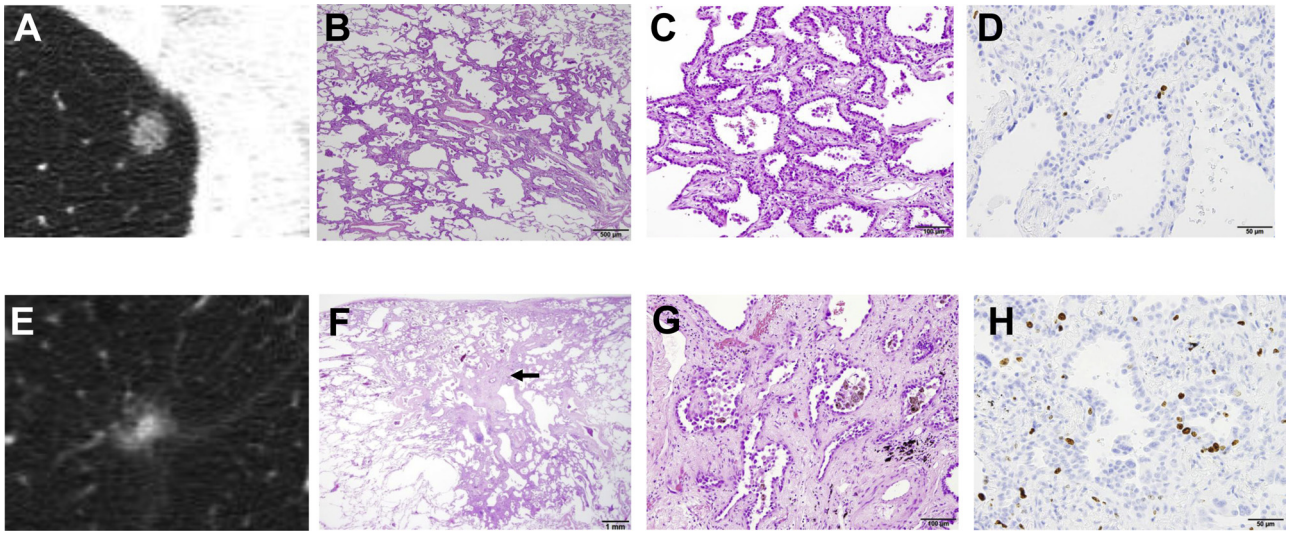

Fig. 1. Non-mucinous adenocarcinoma in situ without an epidermal growth factor receptor (*EGFR*) mutation (A–D). (A) A computed tomography scan shows a pure ground-glass nodule consisting of a circumscribed ground-glass nodule lacking any solid component. (B) The circumscribed non-mucinous tumor grew purely with a lepidic pattern; invasion or scarring foci were not seen (hematoxylin and eosin stain). (C) Atypical pneumocytes are crowded and have slightly hyperchromatic nuclei (hematoxylin and eosin stain). (D) Staining with an antibody to Ki-67 can be observed in only a few tumor cells.
Non-mucinous minimally invasive adenocarcinoma with an *EGFR* mutation of exon 19 (E746–A750del Type 2) (E–H). (E) A computed tomography scan shows a part-solid nodule consisting mostly of a ground-glass nodule with a small solid component. (F) This subpleural adenocarcinoma tumor consisted primarily of lepidic growth with a small area of invasion of less than 0.5 cm (arrow; hematoxylin and eosin stain). (G) Acinar-like glands invading the fibrous stroma (hematoxylin and eosin stain). (H) Staining with antibody to Ki-67 can be observed in a few more tumor cells compared with non-mucinous adenocarcinoma in situ (Fig. D).

patients did not show lymph node metastasis pathologically and all were diagnosed as p-Stage IA.

For all 91 patients, the median follow-up duration from the date of surgery was 60 months (20 to 112 months). Three patients, two with MIA and one with AIS, died 30, 43, and 85 months, respectively, after surgery due to other malignancies. The 5-year overall survival (OS) rate was 97.5% (Fig. 4A). Seven patients with lepidic adenocarcinoma displayed from 28 to 101 months' (median 53) survival without a local recurrence or distant metastasis. None of the patients experienced a recurrence, with the 5-year recurrence-free survival (RFS) rate being 100% (Fig. 4B).

4. Discussion

In 2015, the WHO defined two new subtypes of lung cancer: AIS and MIA [2]. It is hypothesized that morphologically, AIS and MIA represent a gradual malignant progression from the former to the latter. However, how such subtypes transition remains unclear [2,3]. Our results demonstrated several key differences between AIS and MIAs in terms of the ratio of males to females, preoperative CT images, Ki-67 labeling index and *EGFR* mutations in addition to microscopic histological invasion, although similar favorable outcomes were achieved after resection of the two subtypes.

For example, with regard to demographics, the female predominance seen among patients with AIS or MIAs has also been

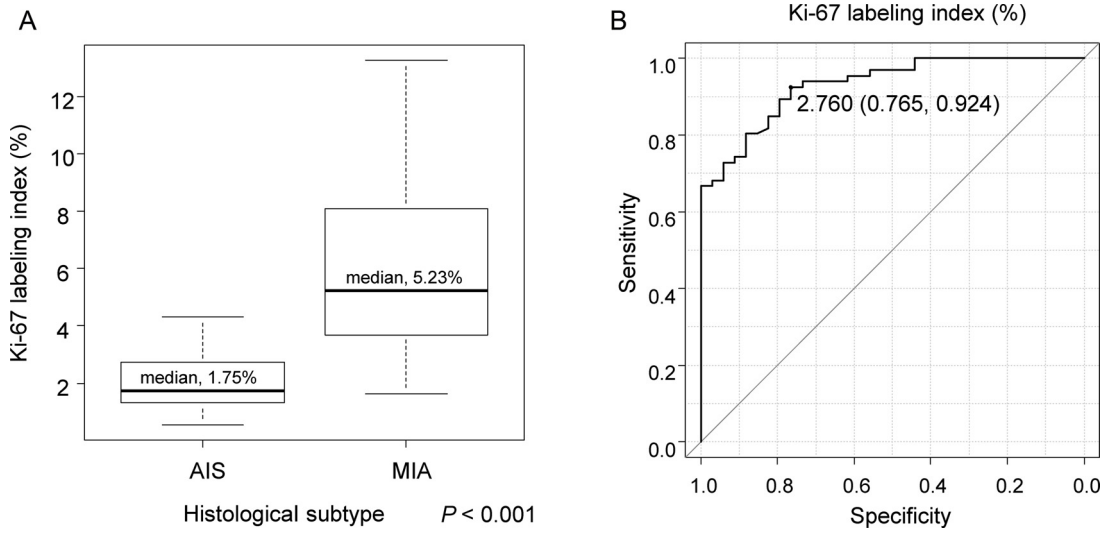

Fig. 2. (A) Comparison of Ki-67 labeling index (LI) between adenocarcinomas in situ (AIS) and minimally invasive adenocarcinomas (MIAs). The Ki-67 LI was significantly higher for MIAs (median, 5.23%) than for AIS (median, 1.75%). The boxes extend from the 25th to 75th percentiles of the data and the line in the middle represents the median. The upper and lower bars represent the distance from the 10th to 90th percentiles from the median. (B) A receiver operating characteristic curve showing a Ki-67 LI of 2.76% as the optimal cutoff value for predicting an MIA.

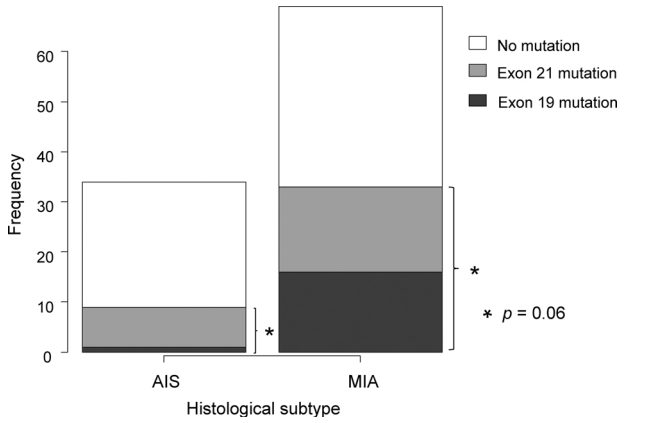

Fig. 3. Comparison of epidermal growth factor receptor (*EGFR*) exon mutations between two adenocarcinoma subtypes. Minimally invasive adenocarcinomas (MIAs) tended to harbor more exon 19 mutations than adenocarcinomas in situ (AIS; $p = 0.06$).

described by others [11,12]. Interestingly, the proportion of males was significantly greater among patients with MIAs compared to AIS in this study.

In addition, CT finding of a pure GGN or a part-solid GGN has been generally predictive of a diagnosis of AIS or MIA, respectively, and has subsequently been useful for the determination of appropriate surgical procedures. In the eighth edition TNM classification of lung cancer, a pure GGN measuring ≤3 cm and a GG predominantly part-solid nodule (≤3 cm) with solid size ≤5 mm represents cTis and cT1mi, respectively [3]. It is well recognized that cTis and cT1mi do not always correspond to AIS and MIA, respectively, and that the pT category may change after full histological evaluation. We have demonstrated reasons for discordance between cT and pT for approximately 20% of all nodules according to a detailed comparison of CT images and histology. Our data suggest that the vast majority of AIS and MIAs may be accurately predicted based on the careful evaluation of CT image. However, further studies of more cases of AIS and MIAs are required to verify this observation.

Another key difference between AIS and MIAs related to cell proliferation, which is a pivotal feature in the progression of lung cancer. The immunohistochemical assessment of the nuclear antigen, Ki-67, is used to assess cell proliferation and is associated with tumor growth. Several reports have described how a high Ki-67 LI (21–25%) significantly correlated with a poor prognosis in patients with lung cancer [4,13–15]. We found that the Ki-67 LI was significantly higher in MIA compared to AIS in an early phase of lung adenocarcinoma. Furthermore, the optimal cutoff value of the Ki-67 LI between the two subtypes was 2.76%. These results verified a malignant progression from AIS to MIA. In addition, a Ki-67 LI of 2.8% may be used as an indicator to differentiate between the two subtypes. Nonetheless, it is noteworthy that the Ki-67 LI value (5.27%) for MIAs we determined was far lower than the values (21–23%) that had been previously reported as poor prognostic factors for lung cancers.

Differences between AIS and MIAs also related to the *EGFR* gene, in which driver mutation is a representative genetic aberration found in lung adenocarcinomas, particularly in the East Asian population. In our study, *EGFR* mutation rates were 26% for AIS and 48% for MIA. Such mutation rates are similar to those of previous reports of 27.3% for AIS and 42.9% for MIA, or 39.0% for both AIS and MIA [5,12]. In general, a lung adenocarcinoma is thought to follow a linear multistep progression, whereby atypical adenomatous hyperplasia progresses to AIS and MIA, and is then followed by development into a lepidic invasive adenocarcinoma [12,16]. Interestingly, in our study, the mutation ratio of exon 19 to 21 was 13% and 94% in AIS and MIAs, respectively; tumors with exon 19 mutations tended to be more numerous among MIAs compared to AIS. These results allude to the initial development of some MIAs, except for the chronological transformation of AIS to MIAs.

With regard to survival analysis, patients with AIS or MIA have been shown by others to have a 100% 5-year RFS or 100% 5-year DFS after surgery [5,7,11,17–24]. Our data are consistent with these previous studies. In the present study, 68% of all patients with AIS and 54% with MIAs underwent sublobar resections (wedge resection or segmentectomy); such patients subsequently showed a 100% 5-year RFS. Several recent reports have also described a lack of tumor recurrence after sublobar resections for AIS or MIA [11,20,24].

Finally, several limitations of this study should be acknowledged. First, this was a retrospective study in which potential selection biases cannot be ruled out. Our study was conducted at a single institution in an Asian country. The lung adenocarcinomas of East Asian patients are known to have different genetic features to those of Western patient populations, such as more common *EGFR* mutations. Second, regarding the choice of surgical procedures, we intentionally performed a limited resection of tumors appearing as a pure GGN or part-solid GGN on CT imaging; however, in some cases, the attending surgeons made the shift to a lobectomy with a lymph node dissection because of the

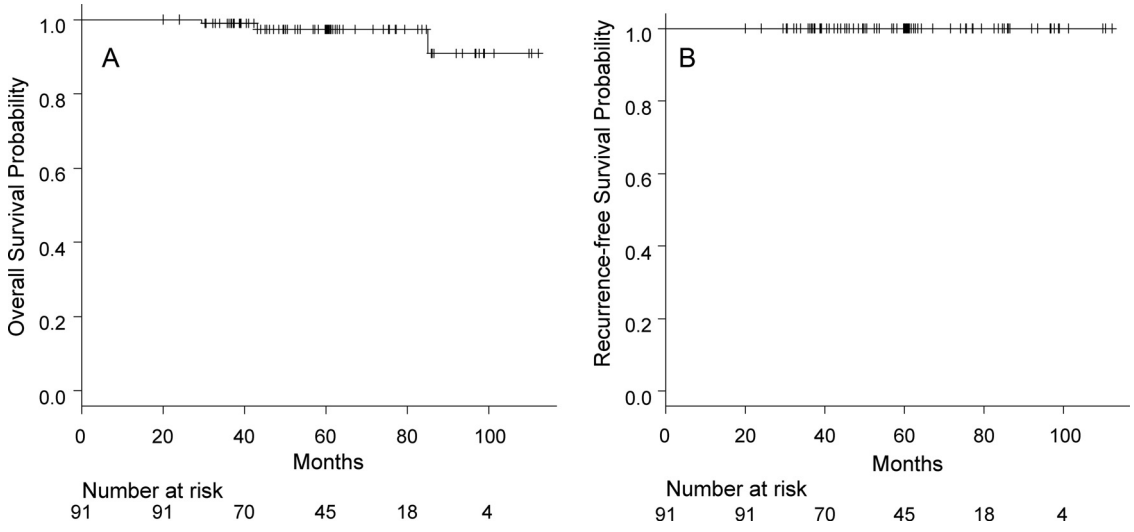

Fig. 4. Kaplan–Meier survival curves. Patients with adenocarcinomas in situ (AIS) or minimally invasive adenocarcinomas (MIAs) had a 5-overall survival rate of 97.5% (A) and a 5-year recurrence-free survival of 100% (B).

intraoperative suspicion of an invasive tumor. Third, AIS or MIAs were diagnosed with the agreement of two more senior pathologists when these were assessed differently after a diagnosis of histological invasiveness.

In conclusion, although patients with AIS and MIA shared the same favorable outcome after complete resection, we have demonstrated histological and genetic characteristics that can be used to distinguish between these subtypes in lung adenocarcinoma: MIA cases were more likely to be male, show higher mutation rates of *EGFR* and have a significantly higher Ki-67 LI in an early phase of lung adenocarcinoma.

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Conflict of interest

The authors declare no conflict of interest.

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