ALK rearrangement: a high-frequency alteration in ovarian metastasis from lung adenocarcinoma

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Abstract

Background: Ovarian metastatic tumors from lung adenocarcinoma are rare, and a serial study of these tumors is lacking to date. Additionally, a better understanding of the clinicopathological and molecular characteristics of metastatic tumors is needed.

Methods: Seven cases of ovarian metastasis from lung adenocarcinoma from 2013 to 2017 at our institute were investigated. The results were combined with those found in literature review. A total of 16 cases were analyzed in the present study. We examined clinicopathological and immunohistochemical characteristics, further detected ALK rearrangement by FISH (fluorescence in situ hybridization), and assessed EGFR and KRAS mutations using Sanger sequencing or the amplification refractory mutation system (ARMS).

Results: The mean age of the patients was 44.6 years (range, 33–56 years). Eleven of sixteen patients developed ovarian tumors within a mean time of 18.5 months (range, 5–48 months) from the initial diagnosis of lung adenocarcinoma; 5 patients had lung tumors and ovarian masses simultaneously. Five tumors (5/16, 31%) occurred in the bilateral ovaries, and the others were unilateral ovarian tumors (11/16, 69%). All seven cases from our institute were positive for TTF-1 and Napsin A but negative for PAX8. In four cases, ALK (D5F3) was diffusely and strongly expressed, with ALK rearrangements (4/7, 57%). Overall, ALK rearrangement was found by FISH or immunohistochemistry in 11/16 (69%) cases. In two cases, EGFR mutations in exons 19 and 21, respectively, were found. One patient did not detected EGFR or ALK mutation in the metastatic tumor, but the primary lung adenocarcinoma did harbor an EGFR mutation. Two cases had no alterations in three genes above. Although the mean survival time of the patients with ALK rearrangement was longer than those without (mean survival time 25 m vs. 20 m), no statistical significance of the difference was found.

Conclusions: As the largest case series of ovarian metastasis from lung adenocarcinoma, our findings indicate that ALK rearrangement is the most common molecular alteration. Although patients with ALK rearrangement appear to have a better prognosis than do those without ALK rearrangement, more cases with longer follow-up and multivariant analysis are needed to clarify this point.

Keywords: Ovary, Metastatic lung adenocarcinoma, ALK rearrangement
Background
Lung cancer is the second most common cancer, and most cases are diagnosed at advanced stages. The 5-year survival rate of lung cancer is less than 20%. Lung cancer in non-smokers appears to be a distinct disease caused by driver mutations that are different from the genetic changes observed in lung cancer in smokers. In western countries, rearrangements in the gene (ALK) encoding anaplastic lymphoma receptor tyrosine kinase have been found in only 3–7% of lung adenocarcinomas; in contrast, EGFR mutation is approximately 10–15%, more frequent than ALK rearrangement [1, 2]. Nevertheless, these two alterations appear to be mutually exclusive in patients [3, 4], and only recent rare cases have shown concomitant alterations in ALK and EGFR [5, 6].

Ovarian metastatic tumors derive from many sites. The common primary sites include gastrointestinal tract, pancreas, and gynecologic tract (the cervix and uterus) [7]. However, lung carcinoma metastasis to the ovary is rarely reported. Reports of these metastatic tumors have included all major lung cancer histotypes, with small cell carcinomas comprising the largest proportion, followed by adenocarcinomas, large cell carcinomas and squamous cell carcinomas [8]. As stated above, lung adenocarcinoma metastases to the ovary are rare, and the incidence is not yet clear. Lung metastasis to uncommon sites occurs in less than 5% of cases, with metastatic adenocarcinoma to the ovary occurring in only 0.07%. So the frequency of total lung tumor metastasis to the ovary is estimated to be less than 0.01% [9]. More remarkably, although ovarian metastasis from lung carcinoma has an extremely low incidence, ALK rearrangement in these patients has occasionally been reported [10–17].

In the present study, we investigated 7 consecutive cases of lung adenocarcinoma metastasis to the ovary at our institute with cases-review in the literature. We obtained clinicopathological data regarding major molecular alterations for targeted therapy. To the best of our knowledge, this study is the largest panel of ovarian metastatic tumors from lung adenocarcinomas focusing on genetic alterations to date.

Methods
Samples
Seven cases of lung carcinoma metastasis to the ovary, including 5 treated with surgery at Fudan Cancer Center and 2 consultation cases, were reviewed in the Department of Pathology of Fudan University Shanghai Cancer Center in 2013–2017. Clinical information and gross features were obtained from medical records. Formalin-fixed, paraffin-embedded tissue blocks or unstained slides were reprocessed for hematoxylin and eosin staining, immunohistochemistry and molecular analysis. All cases were reviewed by two senior pathologists who verified the diagnosis. The present study was approved by our institutional ethics committee. We also reviewed unselected reported cases of ovarian metastasis from lung adenocarcinoma in the English literature in PubMed, and 9 cases with molecular alterations were retrieved. In total, 16 cases were analyzed in the present study.

Immunohistochemistry
Immunohistochemistry analyses, including detection of PAX8, ER, PR, TTF-1, Napsin A, ALK (D5F3), CK7, CK20, CDX2 and HNF-1β, were performed for all 7 cases using a Ventana Benchmark XT autostainer (Ventana Medical Systems Inc., Tucson, AZ, USA). Appropriate positive and negative controls were included. A list of antibodies used in this study is shown in Table 1.

EGFR and KRAS mutational analysis
The mutational statuses of EGFR (exons 18, 19, 20 and 21) and KRAS (exon 2) were determined by polymerase chain reaction (PCR)-based direct gene sequencing, as previously described [18], or by the amplification refractory mutation system (ARMS). In brief, genomic DNA from the 7 tumors was extracted using a QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. The primers used for EGFR and KRAS are listed in Table 2. The PCR products were confirmed by agarose gel electrophoresis, purified using a DNA Clean/Extraction Kit (GeneMarkBio, A&D Technology, Beijing, China), and submitted for direct sequencing using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s protocol. The sequencing products were ethanol precipitated before being assessed using a 3500 Genetic Analyzer (Applied Biosystems), and the resulting sequence data were analyzed using Chromas software. Each mutation was verified in both the sense and antisense directions and was independently evaluated by two

| Antibody | Dilution | Clone | Manufacturer |
|----------|----------|-------|--------------|
| TTF-1    | Ready-use| SPT24 | Leica        |
| NapsinA  | 1:500    | KCG1.1| Abcam        |
| ALK      | Ready-use| D5F3  | Ventana      |
| PAX8     | 1:150    | MRQ3  | Cell Marque  |
| ER       | Ready-use| SP1   | Ventana      |
| PR       | Ready-use| 1E2   | Ventana      |
| CK7      | 1:100    | SP52  | Ventana      |
| CK20     | Ready-use| SP33  | Ventana      |
| CDX2     | 1:50     | EPR2764Y | Maxim    |
| HNF-1β   | 1:500    | polyclone | Sigma    |
| P63      | Ready-use| 4A4   | Roche        |
| P40      | Ready-use| ER8   | Maxim        |
served at 5–remaining 11 cases (11/16, 69%), ovarian tumors were metastatic tumors occurred simultaneously. For the cases (5/16, 31%), the lung adenocarcinoma and ovarian other patients had no smoking history (10/12, 83%). In 5 (2.5-pack years), 1 patient smoked 1-pack per day, and the smoking history was known, 1 patient was a light smoker years (mean 44.6 years). Among 12 patients for which

Among the seven cases in our cohort and the nine cases

### Table 2 The primers of EGFR and KRAS on hot spots

| EGFR    | Exon 18                      | Exon 19                      | Exon 20                      | Exon 21                      |
|---------|------------------------------|------------------------------|------------------------------|------------------------------|
|         | F: 5'-AGCATGCGAGGCAGGGCTGAC-3' | R: 5'-ATATACACCTTGCAAGGACTTG-3' | F: 5'-GATGCCATCTGGCCTTAC-3'  | F: 5'-TCAGAGCCTGGCAGACTG-3'  |
|         | R: 5'-CCAGATACCTGGGAGGGGTA-3' | R: 5'-AGCAGGGTCTAGACGACGG-3'  | R: 5'-TCGGTAACTGGTGCGGAC-3'  | R: 5'-AGTGGGTTGCTTACAGG-3'   |

in 3 cases (3/16, 19%) (Table 3). Serum CA125 was elevated with median values in the range of 100–500 U/ml in 5 recorded cases.

**Pathological features**

On gross examination, the ovarian tumors exhibited smooth outer surface. Macroscopic omental cake was present in one case (LTO_2). The mean size of 13 ovarian tumors with available data was 11 cm (range, 4–21.5 cm). The cut surface was predominantly solid in 7 cases, whereas it was cystic in two cases; the other 4 cases included mixed solid and cystic surfaces. The solid area of the tumor had a medium firmness level (LTO_1, Fig. 1). Large cavities in two of the tumors (LTO_3 and 7) were filled with dark brown viscous liquid and showed partial papillary architecture. The clinical and macroscopic features of all cases are summarized in Table 3.

Upon microscopic observation, all 16 cases were found to be adenocarcinomas, including 5 cases with solid or prominent solid features, 8 cases with prominent acinar features (Fig. 2a-d) and 2 cases with a mixed solid and acinar pattern. One case did not represent the histological subtype in the article. LTO_1 was characterized by solid features, and the tumor cells had distinct cell membrane and little mucin, similar to squamous cell carcinoma, but were negative for p63 and p40. Prominent solid patterns and sheets of tumor cells were also observed in other four cases, presenting polymorphous epithelioid cells with eosinophilic or clear cytoplasm and unclear cell borders. Remarkably, signet ring cells were observed in 3 cases. Tumor cells with an eosinophilic cytoplasm were attached to the cystic wall and exhibited hemorrhage and inflammatory infiltration, with a mixed acinar and solid growth pattern in LTO_3. The acinar features included large tubules but with little mucus in the lumen in 8 cases. Signet ring cells were observed in LTO_16. Additionally, acinar features with micropapillaries in acinar cells were found in LTO_5, and eosinophilic, mucinous fluid was observed in some acinar cells. In LTO_7, prominent mucin were present in acinar cells, which showed a cribriform pattern. In the present case series, the acinar pattern was slightly more common than solid pattern. However, 4/5 cases with solid features harbored ALK rearrangement.

**Immunohistochemistry**

ER, PR, HNF-1β and PAX8 expression was negative in our 7 cases, while TTF-1, Napsin A and CK7 were expressed in all 7 cases. No cases were positive for CK20 or CDX2. LTO_1 was negative for p63 and p40. Four of 7 cases showed diffuse and strong positivity for ALK (D5F3) (Fig. 2e). Interestingly, the 2 cases with solid or prominent solid features were both ALK positive. The immunohistochemistry results are presented in Table 3.

**Genetic alterations in EGFR and KRAS and ALK rearrangement**

In seven cases, sequencing was successful and revealed mutations in EGFR exons 18, 19, 20 and 21.

**ALK rearrangement based on FISH**

Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular) was used for ALK testing, as described previously [19]. Fifty or more non-overlapping nuclei were counted by two professional molecular pathologists according to standard criteria in our laboratory. The positive cut-off for ALK rearrangement was at least 15% tumor cells with a split pattern and/or single orange signal without a corresponding green signal [20]. An increased number of nuclei or alternative nuclei were counted if the results were close to the cut-off values.

**Results**

**Clinicopathological data**

Among the seven cases in our cohort and the nine cases previously reported, patient ages ranged from 33 to 56 years (mean 44.6 years). Among 12 patients for which smoking history was known, 1 patient was a light smoker (2.5-pack years), 1 patient smoked 1-pack per day, and the other patients had no smoking history (10/12, 83%). In 5 cases (5/16, 31%), the lung adenocarcinoma and ovarian metastatic tumors occurred simultaneously. For the remaining 11 cases (11/16, 69%), ovarian tumors were observed at 5–48 months (mean 18.5 months) following the diagnosis of the lung adenocarcinoma. Computed tomography revealed bilateral ovarian tumors in only five patients (5/16, 31%); the others presented unilateral tumors (11/16, 69%). Concurrent metastatic sites were present in most cases (13/16, 81%), including the bone (6 cases), brain (5 cases), supraclavicular lymph node (3 cases), liver (2 cases), and pleural lymph node (1 case) but were absent in 3 cases (3/16, 19%) (Table 3). Serum CA125 was elevated with median values in the range of 100–500 U/ml in 5 recorded cases.
| Case     | Age (years) | Smoking history | Side | Tumor size (cm) | Surgery                      | Gross appearance                                                                 | Morphological features                                                                 | Interval time between primary lung cancer and metastasis to ovary and concurrent metastatic sites | ALK (D5F3) | ALK-FISH | EGFR status | KRAS status | Targeted treatment | Follow-up |
|----------|-------------|-----------------|------|-----------------|------------------------------|----------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|------------|----------|-------------|-------------|-------------------|-----------|
| LTO_1    | 43          | N/A             | U    | L,11*7*3        | TAH-BSO                      | solid, with minor honeycomb cut-section                                             | solid                                                                                 | 30 m, no concurrent sites                                                       | Positive   | Rearrangement | Neg.        | Neg.        | No                | 10 m, DOD   |
| LTO_2    | 39          | no              | B    | L,44*3,4*3*2    | TAH-BSO and omentectomy       | solid                                                                             | acinar                                                                                | 5 m, no concurrent sites                                                        | negative   | No        | exon 19 mutation | Neg.        | Gefitinib         | 13 m, DOD  |
| LTO_3    | 52          | no              | U    | L, 21.5*18*8    | TAH-BSO                      | Cystic prominent, focal solid area                                                  | Acinar+solid                                                                          | Synchronous, bone, left supraclavicular lymph node                                 | negative   | No        | Neg.        | Neg.        | No                | 36 m, alive |
| LTO_4    | 50          | N/A             | U    | L,16*15*6       | USO                          | solid and cystic, focal papillary architected                                       | acinar                                                                                | 6 m, brain and bone                                                            | Positive   | Rearrangement | Neg.        | Neg.        | No                | 19 m, alive |
| LTO_5    | 56          | no              | B    | R, 8.5*5*4.5(L), 3*2*1 | BSO + appendectomy         | solid, solid predominant                                                             | acinar                                                                                | 19 m, bone                                                                     | Positive   | Rearrangement | Neg.        | Neg.        | No                | 22 m, alive |
| LTO_6    | 35          | no              | U    | /               | TAH-BSO                      | /                                                                                  | solid predominant                                                                     | Synchronous, brain and bone                                                      | Positive   | Rearrangement | Neg.        | Neg.        | Cislotinib        | 24 m, alive |
| LTO_7    | 55          | no              | U    | R, 14*10*9      | /                            | Cystic with old dark brown, viscus liquid                                           | acinar                                                                                | 7 m, liver                                                                     | negative   | No        | Neg.        | Neg.        | No                | 8 m, DOD   |
| LTO_8(10)| 54          | 2.5-pack year   | B    | R, 2–4                   | BSO                          | /                                                                                  | Thick trabeculae or solid nests                                                     | 1.2 m, brain                                                                   | Positive   | Rearrangement | Neg.        | Neg.        | No                | /          |
| LTO_9(11)| 39          | 1-pack per day  | B    | 15 by 10               | /                            | /                                                                                  | /                                                                                     | /                                                                      | /          | /        | /          | /           | /                 | /         |
| LTO_10(12)| 50        | /               | B    | 12.8*12.2*11.7     | TAH-BSO                      | Mixed cystic and solid adnexal mass with intracytoplasmic mucinous and signet-ring cells | Solid with intracytoplasmic mucinous and signet-ring cells                          | 39 m, liver and bone                                                           | Rearrangement | Neg.       | /            | Cislotinib   | /                  | /          |
| LTO_11(13)| 38        | No              | U    | L                     | /                            | /                                                                                  | /                                                                                  | Synchronous, bone                                                              | Neg. exon 21 | mutation | Erlotinib       | 24 m, DOD   | /                  | /          |
| LTO_12(13)| 47        | /               | U    | R                     | BSO                          | solid                                                                               | solid predominant                                                                    | 20 m, right supraclavicular lymph node                                           | /          | /        | Rearrangement | Neg.        | Cislotinib        | 12 m, alive |
| LTO_13(14)| 33        | No              | U    | L,79*5.9              | Adnexal mass biopsy           | solid                                                                               | acinar                                                                              | Synchronous, bilateral supraclavicular lymph node                               | Positive    | /         | Neg.        | /           | Cislotinib        | 4 m, alive |

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| Case      | Age (years) | Smoking history | Side | Tumor size (cm) | Surgery                        | Gross appearance                                                                 | Morphological features                      | Interval time between primary lung cancer and metastasis to ovary and concurrent metastatic sites | ALK (D5F3) | ALK-FISH | EGFR status | KRAS status | Targeted treatment | Follow-up |
|----------|-------------|-----------------|------|-----------------|-------------------------------|---------------------------------------------------------------------------------|---------------------------------------------|---------------------------------------------------------------------------------|------------|-----------|--------------|-------------|------------------|-----------|
| LTO_14   | 41          | No              | U    | L: 10           | Left salpingo-oophorectomy    | solid                                                                          | acinar                                                                      | 8 m, pleural                                                                      | Rearrangement | Neg.     | Neg.         | Crizotinib | 10 m, alive     |           |
| LTO_15   | 45          | No              | U    | R:11            | TAH-BSO                       | Solid                                                                          | Solid nests with abundant granular and extensive signet ring cell change     | 48 m, no concurrent sites                                                                | /           | /         | Mutation in lung cancer | /           | 1 m, DOD       |           |
| LTO_16   | 37          | No              | U    | L: 9*5.8        | No resection                  | Mixed solid and cysts                                                          | Acinar and several signet-ring cells                                          | Synchronous, brain                                                                   | Pos in lung cancer | Rearrangement in lung cancer | /           | Alectinib | 20 m, alive |

Abbreviation: TAH-BSO total abdominal hysterectomy and bilateral salpingo-oophorectomy, m month, N/A not applicable, U unilateral, B bilateral, DOD dead of disease, L left, R Right
and KRAS exon 2; a frameshift mutation of EGFR exon 19 was detected in one of seven cases (Fig. 3, Case LTO_2). Wild-type EGFR and KRAS genes were found in the remaining 6 cases, though ALK rearrangement was present in 4. Among all 16 cases, FISH or immunohistochemistry revealed an ALK rearrangement in 10. For one patient (LTO_16), molecular examination of the ovarian tumor was not performed, but the lung cancer harbored an ALK rearrangement. Based on the high concordance of ALK status between the primary and metastatic tumors [20], we presumed ALK rearrangement in 11/16 (68.8%) cases in the present study. Two cases showed EGFR mutation in exon 19 and 21, respectively. One patient did not exhibit an EGFR mutation or ALK rearrangement in the metastatic tumor, but the primary lung adenocarcinoma did carry an EGFR mutation. The remaining 2 cases had no EGFR or KRAS mutation or ALK rearrangement.

Fig. 1 LTO_1 showed a solid tumor with a grey-yellow cut surface and focal hemorrhage.

Fig. 2 Histological subtypes of ovarian metastatic lung adenocarcinomas. a and b, LTO_1 and LTO_6, solid subtype; c and d, LTO_4 and LTO_5, acinar subtype; e, ALK (D5F3) diffuse expression in LTO_1; f, ALK rearrangement with split orange and green signals by FISH.
Follow-up
The 7 patients in our cohort were followed up until June 7, 2019. The follow-up time of the 9 patients from the English literature were obtained from the original articles. All 16 patients underwent chemotherapy, and 8 received targeted drugs: gefitinib and Tarceva for EGFR mutations and crizotinib and alectinib for ALK rearrangements. Five patients died in 10 months (m), 13 m, 8 m, 24 m and 1 m, after the diagnosis of ovarian metastasis from lung adenocarcinoma, including a patient harboring an ALK rearrangement (LTO_1) who did not undergo targeted therapy and had hypothyroidism for 3 years. Two patients, both carrying an EGFR mutation (LTO_2 and LTO_11), were treated with gefitinib or Tarceva. One patient (LTO_15) exhibited an EGFR mutation in the lung tumor but the mutation status of the ovarian metastatic tumor was unknown. The fifth patient who died (LTO_7) exhibited no ALK/EGFR alterations and did not undergo targeted therapy. Of the 9 surviving patients, 8 had ALK rearrangements. Hence, two groups were formed based on the molecular alterations present. The first group included 11 patients with ALK rearrangements. The mean survival of this group was 25 m, with only 1 death from the disease. The other group of 5 patients did not have ALK rearrangements; the mean survival was 20 m, but only 1 patient survived. Although survival was not significantly different between the two groups \((P = 0.110)\), longer follow-up with additional samples may clarify the difference in survival between these two groups (Fig. 4). Additional details are listed in Table 3.

Discussion
The most common non-small cell lung cancer (NSCLC) metastatic sites are the brain [21], followed by the bone and liver [22]. All other organ metastases comprise less than 5% of cases, and thus, they may be defined as uncommon metastases. The anatomical sites in decreasing order of frequency are the soft tissue, kidney, pancreas, spleen, peritoneum, intestine, bone marrow, eye, ovary, thyroid, heart, breast, tonsil nasal cavity [9], and gastric region [23]. The total frequency of lung metastasis to the ovary is estimated to be less than 0.01% [9]. Due to the paucity of reported cases, the clinicopathological features of these tumors remain unknown. To the best of our knowledge, this is the largest number of cases of ovarian metastasis from lung adenocarcinoma examined to date. Although the ovary is an uncommon metastatic site in the female reproductive tract, uterine cervix metastasis from lung adenocarcinoma harboring ALK rearrangement has been reported [24], as have concurrent cervix and breast metastases [25]. In our cohort, the minority (19%) of ovarian metastatic tumors involved only...
one metastatic site, whereas the majority of ovarian metastatic tumors were concurrent with other metastatic sites (81%), especially the bone and brain.

Ovarian mucinous carcinoma accounts for only 3–4% of all primary tumors [26], and most cases constitute metastatic tumors from diverse sites. As previously noted by Lee et al. [27], metastatic tumors of the ovary tend to show bilateral masses. However, unilateral masses were common in our cohort of metastatic lung adenocarcinomas (11/16, 68.8%), in contrast to Krukenberg tumors with bilateral masses. This is consistent with unilateral ovarian metastasis in twelve of nineteen cases in Irving’s report [8]. Furthermore, tumors greater than 10 cm in size tended to be primary ovarian tumors, but the mean size in the present study was 11 cm. These findings indicate that ovarian metastatic tumors from lung adenocarcinomas have distinct clinicopathological features compared with those from other organs, and some characteristics may result in misdiagnosis of primary ovarian tumors.

Metastatic mucinous carcinomas mainly include those of the gastrointestinal tract, pancreatic, and gynecologic organs [27, 28]. Lung adenocarcinoma metastases to the ovary are extremely rare and comprise less than 0.01% of cases [9]. Although metastatic ovarian tumors prior to the primary lung adenocarcinomas were not found in our cohort, 5 cases (16%) of ovarian tumors were reported prior to the lung carcinoma, including 1 adenocarcinoma [8]. Hence, differentiating primary ovarian tumors from metastatic tumors is a major challenge, especially for mucinous ovarian carcinomas. A panel of immunohistochemical markers should be used for differential diagnosis. PAX8 is recognized as a sensitive marker of the gynecologic tract [29], but it was absent in all 7 cases in our cohort. TTF-1 and Napsin A, which are commonly-used pulmonary-origin markers, are helpful in distinguishing these cases from cases of primary ovarian mucinous carcinoma [30]. TTF-1 and Napsin A were expressed in all 7 cases, and metastatic lung adenocarcinoma was further supported with the negative results for PAX8, HNF-1β, ER and PR, and history of lung adenocarcinoma.

Dominant oncogenes in NSCLC are associated with different biological behaviors manifesting as distinct patterns of metastasis, and ALK rearrangement predisposes to rare sites of pericardial and pleural disease [31, 32]. Interestingly, in the present study, 11/16 (69%) cases harbored ALK rearrangements in an unselected rare ovarian metastasis from a lung adenocarcinoma as the most frequent molecular alteration. EML4-ALK fusion genes are predominantly observed in younger, nonsmoking/light smoking female populations, especially in East Asia. Histopathologically, this ALK rearrangement in lung adenocarcinoma appears to promote the unique features of solid and/or signet ring cells [33]. Other features have been found in cases with ALK rearrangements, whereby mixed subtype adenocarcinomas were the most common subtype, followed by the acinar predominant subtype, papillary predominant subtype [34], micropapillary and cribriform growth [35, 36], bronchioloalveolar carcinoma (BAC) and low-grade endobronchial mucocoeplidmoid carcinoma. Rare casesof adenosquamous carcinoma and mucocoeplidmoid carcinoma has been reported [35]. Although there is one reported case of ovarian metastatic lung adenocarcinoma with comprehensive solid and signet ring cells [16], regrettably, there was no further genetic analysis of this case. Our results also indicated that the solid phenotype is highly suggestive of ALK alteration, and 4/5 cases with solid features harbored ALK rearrangement. However, the acinar pattern was slightly more common than was the solid pattern in patients with ALK rearrangement (6 acinar vs. 5 solid), which is consistent with previous reports in which lung adenocarcinoma with ALK rearrangement was likely to be related to acinar components [37].

Notably, lung cancer with ALK rearrangement represents only a small subset of NSCLC, but metastatic ovarian tumors were found to occur in most ALK fusion-positive cases. Why the ovary is the favored site of these lung cancers is not known. In our cohort, the ALK protein was expressed in 4 cases (57%), and 100% concordance with the FISH results was observed. Most evidence demonstrates good correlation between the immunohistochemical staining of ALK (clone D5F3) and FISH [38]. To reduce the economic burden, patients should be screened by ALK immunohistochemistry to ensure whether they are suitable for targeted therapy. Notably, pleural metastatic tumors weakly express ALK (clone D5F3), but 75% were positive for ALK rearrangement by FISH; therefore, the results of immunohistochemical and FISH may be inconsistent for distant metastases [38].

Recently, Gupta R et al. demonstrated a decreased median survival rate for ALK-positive tumors with uncommon sites of metastasis compared with common sites [31]. However, only one patient with ovarian metastatic ALK-positive tumors was reported in their paper (this was not reanalyzed in the current study due to lack of detail information). According to our data, slightly increased survival compared with triple-negative (no EGFR/KRAS/ALK alterations) and EGFR/KRAS mutation cases may be associated with uncommon metastatic ovary tumors with ALK rearrangement, though this difference was not statistically significant. As mentioned, patients with ALK rearrangement have a better prognosis than do those with wild-type EGFR [39]. Hence, patients with ALK rearrangement and uncommon sites of metastasis to the ovary may achieve better outcomes and longer survival times. Because of the paucity of ovarian metastasis from lung adenocarcinoma, longer follow-up times and more samples are needed for further research.
The *EGFR* mutation is approximately 10–15%, more frequent than *ALK* rearrangement [1, 2]. Histologically, patients with *EGFR* mutation more frequently display BAC or papillary components, but the incidence of solid features is lower than that in other patients [40, 41]. One patient in our group with an *EGFR* mutation in exon 19 (p.746-750del) showed the acinar subtype, which conformed to the morphological features, and the mutation was in concordance with that of the primary lung adenocarcinoma. According to previous reports, there is an approximate discordance rate of 16.2–27% between primary lung adenocarcinomas and corresponding metastases. One case showed L858R in a primary lung cancer but 2235-2249del in ovarian metastatic tumors [42]. Because the responsiveness to *EGFR* tyrosine kinase inhibitors tends to correlate with the *EGFR* mutation status in metastatic lesions compared with primary tumors [42, 43], metastatic tumors should be retested. Patients with rare *EGFR* mutations in metastatic ovarian carcinomas are likely to have a worse prognosis than are those with tumors with *ALK* rearrangement.

**Conclusions**

In summary, our findings indicate that *ALK* rearrangement is the most common molecular alteration in lung tumor metastasis to the ovary, followed by *EGFR* mutation. Although patients with *ALK* rearrangement appear to have a better prognosis than do those without *ALK* rearrangement, more cases with longer follow-up and multivariate analysis are needed to clarify this point.

**Abbreviations**

ALK: anaplastic lymphoma receptor tyrosine kinase; ARMS: amplification refractory mutation system; BAC: bronchioloalveolar carcinoma; NSCLC: Non-small-cell lung cancer

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**Authors’ contributions**

RB and WTY designed the study and interpreted data. RB wrote the manuscript and WTY modified it. QMB, XLZ and XYZ analyzed FISH and Sanger Sequence results. XC and WHJ performed the immunohistochemical staining. RB, XLX, SXT, YFC, HJG, XYT, BC, HLG diagnosed these rare cases. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Ethics approval and consent to participate**

The present study was approved by Fudan university cancer center ethics committee and consented to participate.

**Consent for publication**

We confirm that the person’s data in any form in this paper were approved by the person according to our consent form of Fudan university cancer center.

**Competing interests**

The authors declare that they have no competing interests.

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