A case of invasive mucinous adenocarcinoma of the lung showing stepwise progression at the primary site

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ABSTRACT

Objective: Invasive mucinous adenocarcinoma (IMA) is a variant of lung adenocarcinoma. We present one case of IMA with mixed mucinous and non-mucinous components, suggesting stepwise progression within the tumor.
Material and method: The two different components of IMA were separately examined by immunohistochemistry and performed amplicon sequencing (Ion Ampliseq Cancer Hotspot Panel v2, ilumina, San Diego, CA).
Result: Macroscopically, the IMA contained a small and well demarcated solid part. Tumor cells in the main part showed abundant intracytoplasmic mucin, whereas those in the solid part showed scant intracytoplasmic mucin and high-grade nuclear atypia. Both parts harbored the same *KRAS* p.G12 V mutation. The amplicon sequencing of the IMA showed oncogenic *TP53* p.P278 L mutation was detected only in the solid part.
Conclusion: Oncogenetic *TP53* mutation might promote stepwise progression of this case of IMA.

1. Introduction

Invasive mucinous adenocarcinoma (IMA) is a variant of lung adenocarcinoma [1]. The tumor cells in IMA are columnar and contain abundant intracytoplasmic mucin, mimicking goblet cells of the colon [1]. IMA is often positive for HNF4-alpha, but negative for TTF-1 [1]. IMA reveals genetic abnormalities similar to those of colorectal carcinoma and commonly shows *KRAS* mutation (in up to 90% of cases) [1,2].

Many pathological investigations have suggested that lung adenocarcinoma shows stepwise progression from atypical adenomatous hyperplasia, through adenocarcinoma *in situ* to minimally invasive adenocarcinoma. The majority of non-mucinous, TTF-1-positive and HNF4-alpha-negative adenocarcinomas follow this stepwise progression, but mucinous adenocarcinomas are relatively rare and the concept of stepwise progression in these tumors is still debatable [3,4]. On the other hand, some IMAs have non-mucinous component to some extent [6–8], and these have been described as mixed IMA [6] or mixed mucinous/non-mucinous adenocarcinoma [5,7]. However, those reports have not provided details about the morphological distributions of the mucinous and non-mucinous components. Recently, Kadota et al. reported that pure IMAs tended to harbor *KRAS* mutation more frequently than mixed IMAs [4], but the role of *KRAS* mutation in IMAs has not been fully elucidated.

We have experienced one case of IMA containing a small, well-demarcated solid part. Histologically, the main part of the tumor was pure mucinous-type IMA, but the solid part consisted of tumor cells with scant intracytoplasmic mucin and high-grade nuclear atypia. We hypothesized that the solid part represented progression from the main part, and examined the two parts using a molecular biological approach. This allowed us to prove that *TP53* gene alteration was associated with stepwise progression of this IMA.
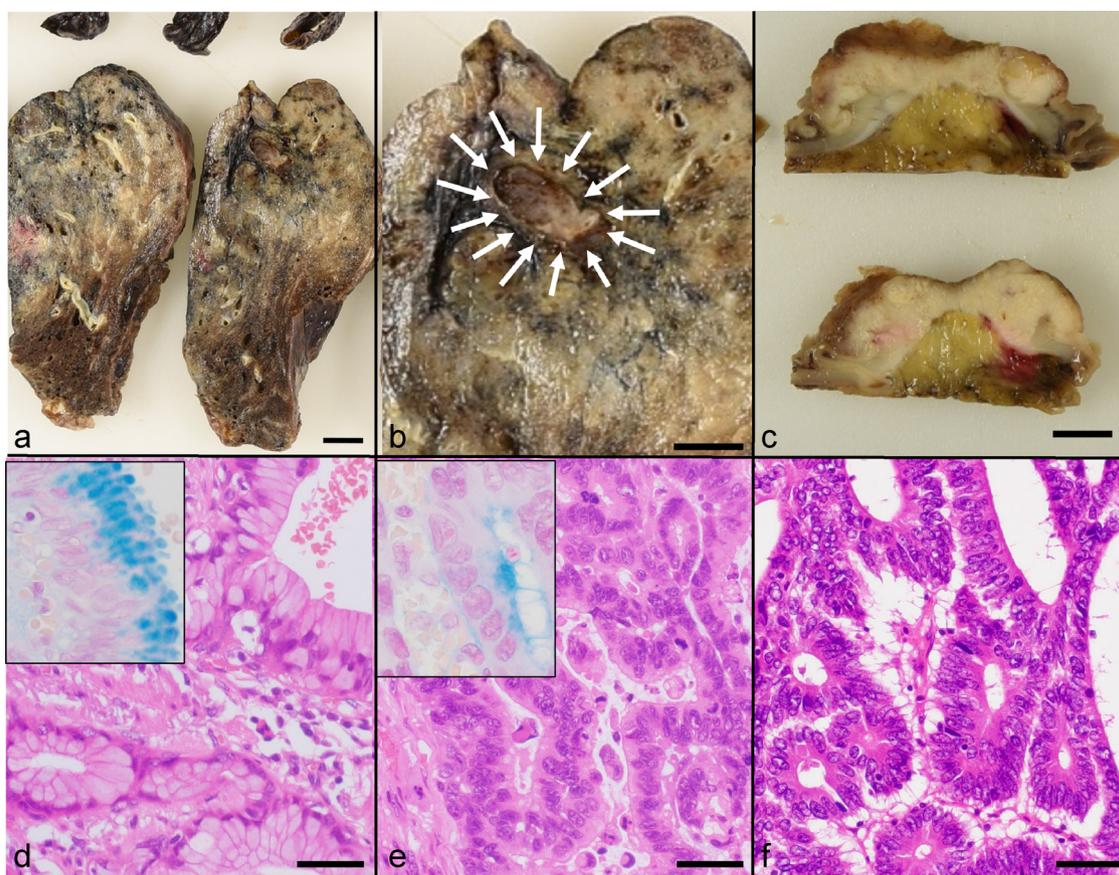
Fig. 1. Macroscopic and microscopic feature of the lung cancer and sigmoid colon cancer.

Macroscopically, the lung tumor was broadly extended from the hilum to the peripheral lung (S8 and 9 segments), showing a mucinous cut surface (a). In the tumor, a small, well demarcated, small solid part (arrows) was detected (b). The patient had also undergone sigmoidectomy one month before (c). In the main part of lung cancer, tumor cells resembling goblet cells of the colon showed lepidic, papillary and acinar growth (d). In the solid part, tumor cells with scant intracytoplasmic mucin and high-grade nuclear atypia showed a cribriform structure (e). Alcian-blue stain showed less intracytoplasmic mucin than those in main part (b and d, inlet). The histology of the sigmoid colon cancer (f) was very similar to that of the solid part of the lung cancer (e).

2. Clinical summary

An 82-year-old Japanese man visited the University of Tsukuba Hospital because of increased sputum production. A nodule with ground glass opacity was detected in the lower lobe of the right lung by computed tomography (CT). He had been diagnosed as having mucinous adenocarcinoma of the lung by CT-guided biopsy at the previous hospital. He underwent lobectomy of the right lower lobe four months later (Fig. 1a and b).

At the time of admission, sigmoid colon cancer had also been detected, and he underwent sigmoidectomy one month before the lobectomy (pT3N0, pStage II, UICC 8th edition) (Fig. 1c).

No recurrence or distant metastasis of either tumor was detected during postoperative follow-up (six months).

3. Pathological findings

Gross examination demonstrated an ill-demarcated and glossy tumor extending from the hilum to the peripheral lung (S8 and 9 segments) (Fig. 1a). The tumor measured 8.5 × 7.5 x 3.5 cm and included a small, well demarcated solid part measuring 1.5 x 1.0 cm (Fig. 1b). On microscopic examination, the tumor cells showed lepidic, papillary and acinar growth. They were columnar with small nuclei and abundant intracytoplasmic mucin (goblet cell type) (Fig. 1d). In the solid part, tumor cells showed a cribriform structure and had large nucleus (Fig. 1e). They showed less intracytoplasmic mucin than those in main part (Alcian-blue stain, Fig. 1b and d, inlet). In the small area

surrounding the solid part, tumor cells intermediate in form between the main and solid parts were evident, showing a slight amount of intracytoplasmic mucin and mild nuclear atypia.

The tumor cells of the sigmoid colon cancer were very similar to those in the solid part of the lung cancer (Fig. 1f), so the latter were thought to represent intratumoral metastasis from the sigmoid colon cancer or partial progression of the main part. We analyzed tumor tissues from the three areas (sigmoid colon cancer and the two components of the lung cancer) immunohistochemically. Tumor cells in the main part were TTF-1(-), HNF4-alpha(+), CDX2(-), p53(-). Although tumor cells in the solid part were TTF-1(-) and HNF4-alpha(+), similar to the main part, they were CDX2(+) and p53(+). On the other hand, tumor cells in the sigmoid colon cancer were TTF-1(-), HNF4-alpha(+), p53(+) and CDX2(+) (Fig. 2a–f). We then examined *KRAS* mutation at codon 12 and 13 using PCR (SRL, Tokyo, Japan). *KRAS* p.G12 V mutation was detected in both components of the lung cancer, whereas *KRAS* p.G12D mutation was detected in the sigmoid colon cancer. On the basis of these results, the lung cancer was diagnosed as invasive mucinous adenocarcinoma (IMA), pT4aN0, pStage IIIA (UICC: 8th edition).

In order to examine in detail the molecular biological features of the two different IMA components, we performed amplicon sequencing using Ion Ampliseq Cancer Hotspot Panel v2 (ilumina, San Diego, CA). Each DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples from the two IMA parts separately after macrodissection. We collected mutations whose allele frequency (AF) was over 10%. *KRAS* p.G12 V and *TP53* p.P72A mutation was commonly
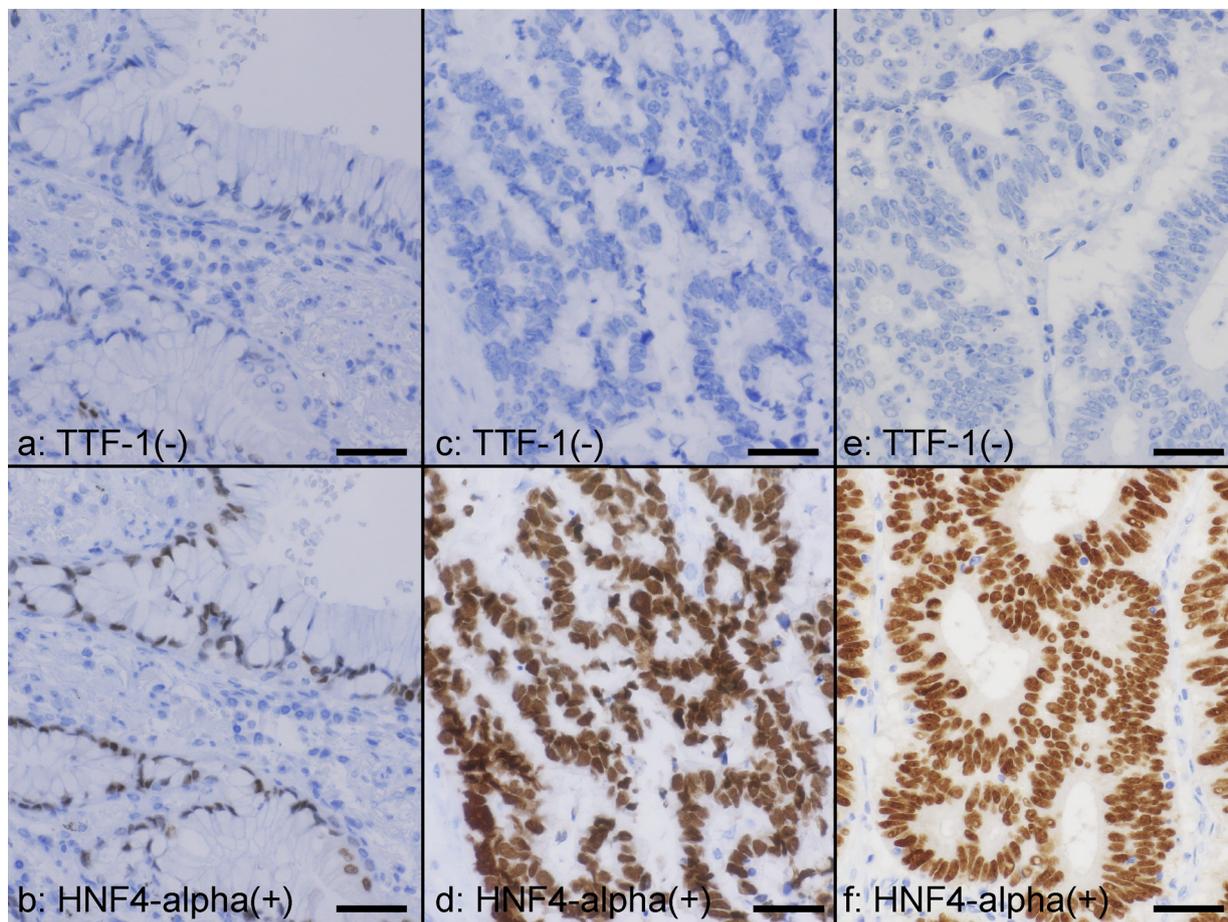
Fig. 2. Immunohistochemical feature of lung cancer and sigmoid colon cancer.
In the main part of the IMA, the tumor cells were TTF-1(-) (a), HNF4-alpha(+) (b), CDX2(-) and p53(-). Tumor cells in the solid part were also TTF-1(-) (c) and HNF4-alpha(+) (d), but CDX2(+) and p53(+). Tumor cells in the sigmoid colon cancer showed the same immunohistochemical pattern as that of the solid part of the IMA: TTF-1(-) (e), HNF4-alpha(+) (f) CDX2(+) and p53(+).

detected in both the main and the solid part, but *TP53* p.P278 L mutation was detected only in the solid part. *TP53* p.P72A mutation was also detected in non-tumorous tissue of this patient.

4. Discussion

This 82-year-old Japanese man had two synchronous malignancies, sigmoid colon adenocarcinoma and lung IMA, for which he had undergone two consecutive operations. In the IMA, we detected one small well demarcated lesion showing histological and immunohistochemical characteristics similar to those of sigmoid colon cancer. We suspected that this solid part represented tumor-to-tumor metastasis from the sigmoid colon cancer, or stepwise progression of the IMA.

In order to clarify this issue, we examined *KRAS* mutation in the sigmoid colon cancer and the two components of the IMA, since colon cancer and IMA frequently contain *KRAS* mutation and its status was thought to be informative for clarifying the nature of the tumor parts. Interestingly, the same *KRAS* p.G12 V mutation was detected in both parts of the IMA, but a different mutation (p.G12D) was detected in the sigmoid colon cancer. Therefore, we concluded that the solid part of the IMA was not metastasis from colon adenocarcinoma, but rather represented focal progression of the IMA.

In order to analyze the tumor mutation characteristics, we analyzed both two components of the IMA by amplicon sequencing. The result indicated that tumor cells in both parts harbored the similar *KRAS* p.G12 V mutation and *TP53* p.P72A mutation. The clinical significance of *TP53* (p.P72A) mutation is not known in the Clinvar databases (ID:

12351). This mutation was also detected in non-tumorous tissue, so it could be SNIP and not related to stepwise progression. Interestingly, only tumor cells in the solid part had *TP53* p.P278 L mutation, which is known to be oncogenic in the Clinvar databases (ID: 232497) and in previous reports [9,10]. Morphologically, tumor cells in the solid part showed reduced mucin and high grade nuclear atypia, indicating dedifferentiation. These results indicated that the tumor cells acquired *TP53* p.P278 L mutation in the main part of the IMA as it progressed and formed the solid part.

When discussing the pathological definition of IMA, the proportion of goblet-type tumor cells is still a debatable issue: some cases show a pure mucinous pattern, whereas others have a non-mucinous component [3–7]. The ratio of mucinous/non-mucinous components has differed among reports, and the relationship between the two components was not been well investigated. Genetically, the proportion of *TP53* mutation in IMA has also different among reports. Boland et al. reported that two of 42 IMAs (5%) had *TP53* mutation, and that *KRAS* and *TP53* mutation were exclusive [5]. Shim et al. reported that one of 27 *KRAS* wild-type IMAs (5%) showed *TP53* p.R273C muration [10]. However, neither of these reports referred to the non-mucinous component. Rhigi et al. and Hwang et al. reported that 24 of 52 (46%) and five of 21 (24%) cases of mucinous adenocarcinoma of the lung showed *TP53* mutation by next-generation sequencing [6,7]. These two reports examined not only pure IMA but also mixed IMA (containing a non-mucinous component) and adenocarcinoma with mucinous features.

The present case appears to be very informative when considering the definition and progression of IMA, suggesting that pure goblet cell-

type IMA can progress to scant-mucinous adenocarcinoma and that *TP53* mutation might be a factor involved in such progression. Further investigations are required using more cases in order to confirm the biological significance of *TP53* mutation in IMA.

5. Conclusion

We have presented a case of IMA in which stepwise progression in the primary site appeared to have occurred. The tumor cells showing stepwise progression had scant intracytoplasmic mucin and high-grade nuclear atypia, and contained oncogenic*TP53* mutation.

Compliance with ethical standards

The study followed the ethical declaration of Helsinki.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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