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Large Cell Lung Carcinoma with Unusual Imaging Feature, Immunophenotype and Genetic Finding

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Abstract We present a case of large cell lung carcinoma in sixty-one year old male with typical lung cancer symptoms but unusual radiological presentation and immunophenotype. Tumor morphological finding related to its radiological finding was suggestive for large cell lymphoma or carcinoma, but its immunophenotype made confusion for pathological diagnosis. No p53 mutations were detected in genetic investigation. Multidisciplinar diagnostic approach to some tumors is useful for their final diagnosis.

Keywords Large cell lung carcinoma · Immunophenotype · Imaging · Tumor genetic · Diagnosis

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Introduction

Imaging feature of large cell lung carcinoma (LCLC) is usually similar to peripheral lung tumors [1]. These tumor cells express Epithelial Membrane Antigen (EMA), AE1/AE3 (pancytokeratin) and Thyroid-Transcriptive-Factor-1 (TTF-1). Neuroendocrine antigens are expressed in majority of LCLCs [2–4].

Mutations in p53 tumor suppressor gene play important role in the genesis of many human tumors, including LCLC [5]. Inactivating p53 mutations are detected in up to 50% of LCLC, they are well characterized and associated closely with smoking [6]. These mutations occur in many codons, mostly in the regions between exons 5 and 8 with recognized hotspots located at codons 157–158, 175, 245–249, 273, and 282 [7, 8].

We point out difficulties in diagnosis of LCLC with unusual imaging feature and immunohistochemical finding. Lung carcinoma propagated mostly in mediastinum with a little involving of lung parenchyma according to the radiological and CT-scan findings. Cancer cells expressed EMA, TTF-1 and vimentin without expression of AE1/AE3 and any of neuroendocrine antigens.

Case Outline

Clinical Finding

Sixty-one-year old man, Caucasian, smoker habitant, 40 years/20 cigarettes per a day, suffered from short breath, dry cough and intermittent temperature up to 38°. Intermittent hemoptisis appeared a month after the first symptoms.

Blood count report detected anemia (red blood cells–3.34×10^6 and hemoglobin–107 g/L). Biochemical finding was within regular range.

Chest X-ray revealed mediastinal widening of tumor. CT scan indicated mediastinal lymphadenopathy located in anterior mediastinum (55×45mm), bilateral paratracheal, retrocaval, in aorticopulmonary window (70×50mm), descending to the bifurcation and right hilum (up to 37 mm) (Fig. 1, 2). A small irregular solid mass of higher density comparing to lymph nodes was present lateral to the vena cava superior with extension into the anterior segment of right upper lobe (Fig. 2).

Pathological Finding

Two percutaneous needle biopsies and one transbronchial biopsy were performed, but all of them were insufficient for pathological diagnosis. Diagnostic thoracotomy was done. Tumor was mostly located in mediastinal lymph node package confirming CT finding. Three pieces of tumor were extirpated for pathological diagnosis. Microscopically, tumor was composed of predominantly ovoid and round, closely packed cells, with rich pale, basophilic or eosinophilic cytoplasm (Fig. 3a, b) and zones of necrosis (Fig. 3b). Large, hyperchromatic or pale and vesicular nuclei were centrally located in tumor cells with basophilic cytoplasm, otherwise they were peripherally located in eosinophilic tumor cells. One or more nucleoli were prominent in tumor cells. Bi- or multinucleated tumor cells were found. Scattered lymphocytes were between tumor cells.

Immunohistochemistry

A broad spectrum of monoclonal antibodies was applied to exclude possible primary sites of the tumor according to its morphology. Immunostaining was performed by incubating tissue sections with appropriate sera for 60 min at room temperature in humidity chamber, using the streptavidin-biotin technique (LSAB+ Kit, Peroxidase Labeling, K0690, DAKO Cytomation, Denmark). Antigen-antibody complexes were visualized with 3-amino-9-ethylcarbasole (AEC, No. K3469, DAKO Cytomation, Denmark) or diaminobenzidine hydrochloride (DAB, No. K3468, DAKO Cytomation, Denmark) substrate solution. The cell nuclei were contrastained with Mayer's haematoxylin. The control stainings included omission of the primary.

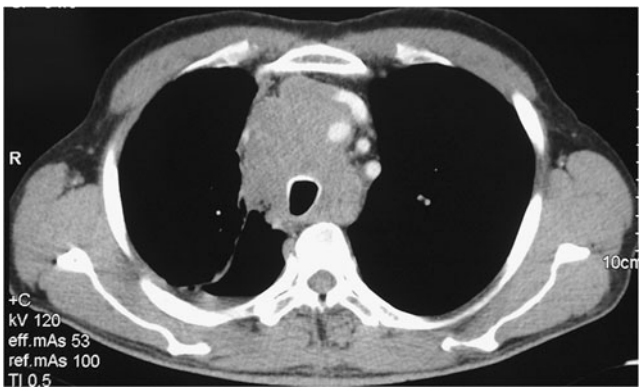

Fig. 1 Contrast enchanced CT. Mediastinal lymphadenopathy

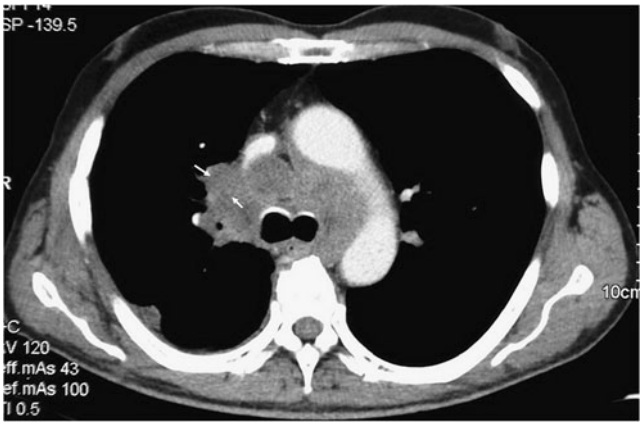

Fig. 2 Contrast enchanced CT. Mediastinal lymphadenopathy and tumor mass lateral to vena cava superior (*arrows*)

Tumor cells exhibited vimentin (Fig. 4a), Epithelial Membrane Antigen (EMA) (Fig. 4b) and TTF-1 (Fig. 4c). Ki-67 (Fig. 4d) and p53 (Fig. 4e) were expressed in almost 100% of tumor cells.

Large cell lymphomas were excluded, as well as various carcinomas with clear cell morphology (adrenal cortex, kidney, liver) as well as different mesenchimal (malignant melanoma, paraganglioma, Schwanoma and liposarcoma) and pleural tumors (malignant mesothelioma, solitary fibrous tumor of pleura). AE1/AE3 was not exhibit in tumor cells. Thyreoglobulin absence excluded thyroid gland origin of tumor. Neuroendocrine antigens were not expressed—CD56 (NCAM), CD57 (Leu-7), Neuron-Specific-Enolase (NSE), synaptophisin, chromograninA and Protein Gene Product 9.5 (PGP9.5).

LCLC without neuroendocrine differentiation was diagnosed. This conclusion was confirmed by the second opinion of the foreign pathologist.

Genetic Analysis

We have investigated p53 gene mutations by polymerase chain reaction and DNA sequence analysis of exons 5, 6, 7, 8 and 9. DNA was extracted from the paraffin-embedded tumor tissue sample, using xylene extraction of the paraffin followed with the phenol/chloroform DNA extraction method. Each polymerase chain reaction (PCR) was performed using 100 ng of genomic DNA. Primers sequences flanking exons 5–9 of the TP53 gene were chosen according to

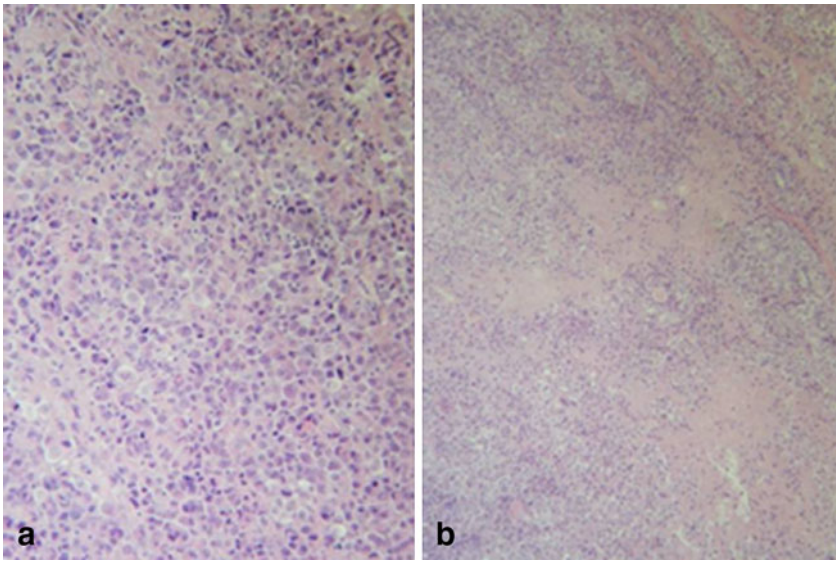

Fig. 3 Morphology of LCLC: **a** mixture of large ovoid tumorous cells with pale and eosinophilic cytoplasm and large pale or hyperchromatic nuclei with prominent nucleoli H&Ex20; **b** large areas of tumor necrosis, H&Ex10

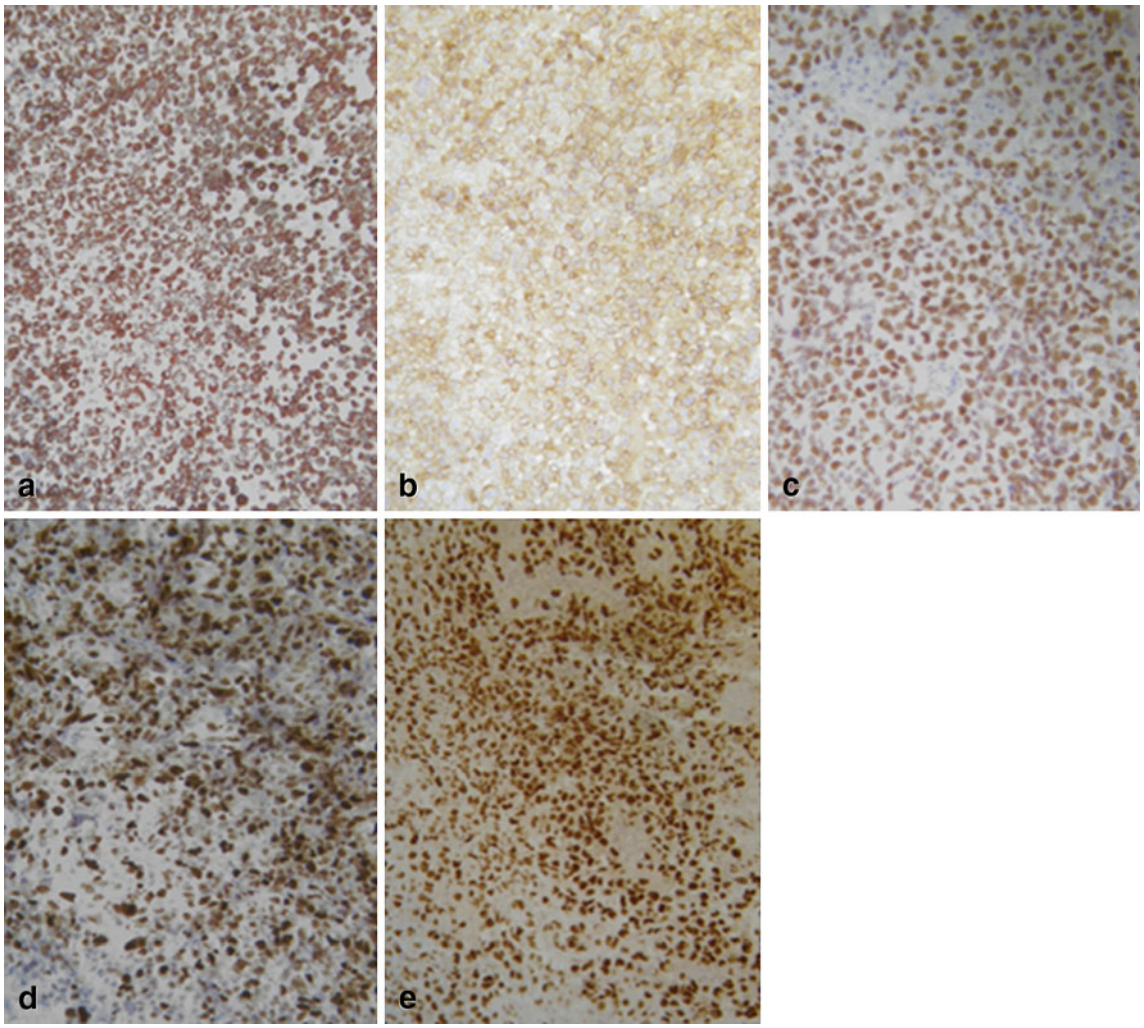

Fig. 4 Immunophenotype of LCLC: **a** intracytoplasmatic, perinuclear reactivity, vimentinx20; **b** membrane, linear immunoreactivity suggested epithelial origin of tumor cells, EMAx20; **c** nuclear positivity in tumor cells suggested lung cell origin of carcinoma, TTF-1×20; **d** high proliferation of tumor cells, Ki67×20; and **e** their high mitotic activity, p53×20

previously reported sequences [9]. PCR product for exons 5, 6, 7, and 8/9 were 325, 230, 139, and 330 bp long, respectively. Obtained PCR products were subsequently sequenced using ABI Prism BigDye 3.1 sequencing system according to manufacturer protocol. No p53 mutations were detected in our patient.

Discussion

CT finding of most lung large cell carcinomas is frequently as single, peripheral mass or nodule with irregular shape and margins and signs of lobulatin without bulky lymphadenopathy or cavity formation. In particular, it is necessary to distinguish LCLC from adenocarcinoma because it may be difficult to distinguish them by biopsy specimens [1, 10, 11]. The prominent CT feature of our case was bulky lymphadenopathy with small irregular tumor unclearly bordering with lymph nodes. This finding was suggestive for lymphoma. This imaging feature is different from the literature data. Explanation might be the fact that the most of literature data regarded to large cell neuroendocrine carcinoma and the diagnosis in our case was LCLC without neuroendocrine differentiation.

According to the morphological finding in differential diagnosis diffuse large cell non Hodgkin lymphoma, mesenchimal tumors, metastasis and LCLC were considered. Large spectrum of monoclonal antibodies were applied to exclude lymphoma, mesenchimal tumors and metastasis. Only TTF-1, EMA, vimentin, Ki67 and p53 were expressed. TTF-1 tissue-specific expression in lung and thyroid tumor cells has recently been used as a marker for the differential diagnosis primary from metastatic lung carcinoma. Its expression could be found in small- and non-small lung carcinoma and in some benign lung tumors of alveolar epithelia origin [2, 3, 12, 13].

Epithelial origin of tumor was conclusive by EMA positivity while AE1/AE3 was not expressed as usual in the other epithelial lung tumors. Vimentin paranuclear high intracytoplasmatic expression in tumor cells, without AE1/AE3 expression, made confusion for pathological diagnosis. Lung and pleural tumors exhibit vimentin in spindle cell carcinoma and mesothelioma, but in 38% lung adenocarcinoma. Vimentin expression in LCLC was not noted to our best knowledge [1, 2, 14, 15].

High Ki-67 and p53 expression in tumor cells was suggestive for poor prognosis of large cell carcinoma exhibited in WHO lung classification blue book as well as in the other papers [2, 3, 16]. Overall expression of proliferative markers suggested LCLC. Detection of increased levels of p53 protein in tumor cells by immunohistochemical analysis usually indicates the presence of p53 gene mutations, because missense mutations stabilize p53 protein and prolonge its half-life. However, p53 may be temporarily stabilized by some mechanism other than mutation [17, 18]. This fact was a reason why all of the overexpressed p53 proteins do not show genetic abnormalities in this case.

Conclusion

Multidisciplinary diagnostic approach to some tumors is useful for their final diagnosis.

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