Lung Cancer 82 (2013) 413–419

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Association of c-Met phosphorylation with micropapillary pattern and small cluster invasion in pT1-size lung adenocarcinoma

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ARTICLE INFO

Article history:
Received 26 October 2012
Received in revised form 23 August 2013
Accepted 10 September 2013

Keywords:
Micropapillary pattern
Small cluster invasion
c-Met
Lung adenocarcinoma
pT1
Prognosis

ABSTRACT

Lung adenocarcinomas with micropapillary pattern (MPP) are associated with frequent nodal metastasis. However, little is known about the mechanisms that underlie MPP-associated nodal metastasis. We have previously reported that pT1 lung adenocarcinomas with MPP are significantly associated with small cluster invasion (SCI) and lymphatic involvement. SCI is defined as markedly resolved acinar–papillary tumor structures with single or small clusters of carcinoma cells invading stroma within fibrotic foci. In this study, we hypothesized that c-Met activation may be involved in the MPP-SCI sequence, given that the c-Met tyrosine-kinase receptor and its ligand hepatocyte growth factor (HGF), play important roles in tumor cell motility and invasion. We analyzed 125 pT1-size lung adenocarcinomas for immunohistochemical expression of phosphorylated c-Met and its correlation with MPP, SCI, lymphatic involvement and prognosis. SCI was significantly more frequent in the MPP-positive group ($P < 0.0001$) and associated with lymphatic involvement ($P < 0.0001$) and nodal metastasis ($P = 0.021$). c-Met protein was detected in all tumors by immunohistochemistry as membranous and cytoplasmic staining. Phospho-c-Met (pc-Met) was positive in 119/125 tumors (95%) and expressed at high levels in 27 cases (22%). A high level of pc-Met expression was significantly associated with MPP ($P = 0.01$) and SCI ($P = 0.0059$). Moreover, in tumors with MPP or SCI, those expressing high levels of pc-Met were significantly more associated with lymphatic involvement. In p-Stage IA lung adenocarcinomas ($n = 99$), patients in the high pc-Met expression group showed significantly worse survival than patient in the low expression group ($P = 0.0313$). These results suggest that activation of c-Met through phosphorylation may be involved in MPP and SCI.

1. Introduction

Lung cancer is the leading cause of cancer deaths world-wide, and even with successful surgery, the prognosis is generally poor. The incidence of adenocarcinoma has been increasing in recent years [1]. Recent advances in diagnostic imaging have enhanced the capability for identifying lung cancer at early stage, and histopathological studies indicate the existence of good prognostic factors in lung adenocarcinomas. Although adenocarcinoma in situ (AIS)/pure bronchioloalveolar carcinoma (BAC) has been reported to be the only subtype without any invasive features and to have an excellent prognosis [2–4], other invasive subtypes of adenocarcinoma sometimes develop distant metastases soon after complete surgical resection in spite of their small size [3]. Even a significant fraction of the patients with stage IA lung adenocarcinoma experience recurrence and die after curative resection [5].

Micropapillary pattern (MPP) is characterized by the presence of papillary structures with tufts lacking a central fibrovascular core [6,7]. Patients with MPP-positive lung adenocarcinomas have poor prognosis and tend to present with extensive lymph node involvement and metastatic diseases [6,8–12]. However little is known about the mechanisms involved in MPP-associated lymph node metastasis. We previously reported that pT1 lung adenocarcinomas with MPP are significantly associated with small cluster invasion (SCI) and invade lymphatics [9,10]. SCI is defined as markedly resolved acinar–papillary tumor structures with single or small clusters of carcinoma cells invading stroma within fibrotic foci.

Given that c-Met tyrosine-kinase receptor and its ligand hepatocyte growth factor (HGF) play an important role in tumor cell motility and invasion, we hypothesized that c-Met activation may be involved in the MPP and SCI sequence. Several studies have documented that c-Met is highly expressed in non-small cell lung cancer

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0169-5002/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lungcan.2013.09.005

(NSCLC), and its overexpression is associated with an advanced cancer stage and shorter patient survival [13,14]. Furthermore, overexpression and activation of c-Met in lung adenocarcinoma have been found in a significant proportion of lung adenocarcinomas [15,16]. To test our hypothesis, we evaluated phosphorylated c-Met using immunohistochemistry in pT1 size lung adenocarcinomas, and analyzed the relationship between c-Met activation and MPP, SCI or prognosis.

2. Materials and methods

2.1. Patients

We reviewed 125 cases of pT1 size lung adenocarcinoma (≦3 cm) that had been surgically resected at the Department of Thoracic Surgery, Fukuoka University Hospital between April 1993 and December 2002. Anonymous use of redundant tissue is part of the standard treatment agreement with patients in our hospital when no objection is expressed. The study protocol was approved by the Ethics Committee of Fukuoka University. The pathological stage was determined according to the published standard for tumor, lymph node, and metastatic (TNM) classification of malignant tumor (International Union Against Cancer [UICC]) [17]. All patients underwent complete resection of the tumors. The mean follow-up period was 41 months (range 2–127 months).

2.2. Pathologic evaluation

Surgically resected specimens were routinely fixed in 10% formalin, and the entire tumor nodules were processed into paraffin blocks for histopathological examination. Tissue sections were cut 4-μm thick, including the largest cut surface of the tumor, and stained with hematoxylin and eosin (H&E) and elastica-van Gieson stain. Histological subtyping was performed according to the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS)

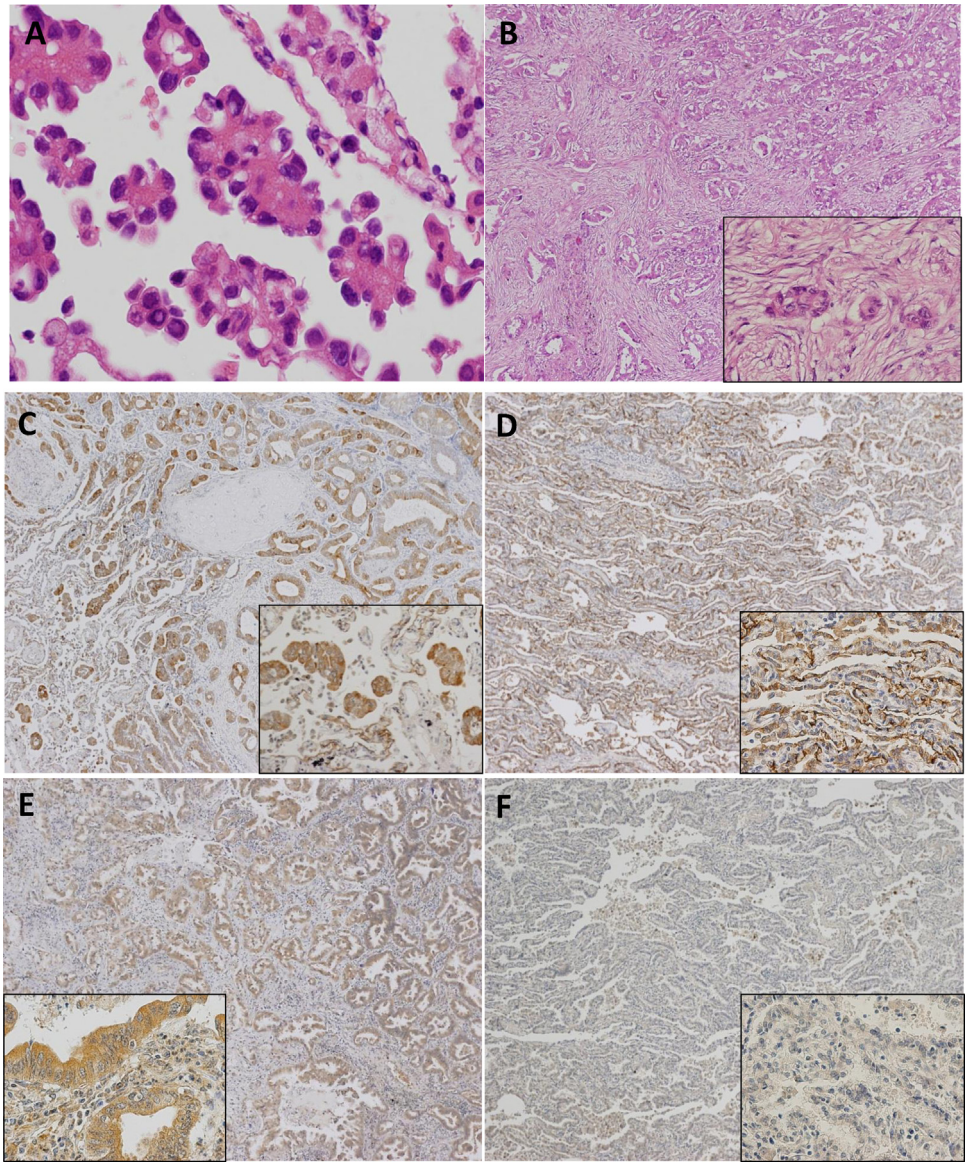

Fig. 1. Micopapillary component observed in invasive lung adenocarcinoma. (A) Micropapillary tufts lacking a central fibrovascular core (40×). (B) Small cluster invasion observed in invasive lung adenocarcinoma. Inset shows isolated small clusters of invading carcinoma cells in the fibrotic focus (100×). (C, D) Immunohistochemical staining of c-Met expression in lung adenocarcinoma. c-Met was detected as membranous and cytoplasmic staining not only in MPP and SCI positive cases (C) (100×), but also in negative cases (D) (100×). (E, F) Immunohistochemical staining of pc-Met expression in lung adenocarcinoma. pc-Met was detected as membranous and cytoplasmic staining in MPP and SCI positive cases (E) (100×), but not detected for MPP and SCI negative cases (F) (100×).