Lung Cancer 86 (2014) 318–323

Contents lists available at ScienceDirect

Lung Cancer

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

Cytokeratin 19 expression in primary thoracic tumors and lymph node metastases

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ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form
18 September 2014
Accepted 22 September 2014

Keywords:
CK19
Lung
OSNA
Immunohistochemistry
TMA
LN

ABSTRACT

Background: The use of one-step nucleic acid amplification (OSNA), which allows for the rapid intraoperative detection of lymph node (LN) metastasis, is becoming more widely accepted in breast cancer. To provide basic data for the development of this method for lung tumors, we conducted a large-scale investigation of cytokeratin (CK) 19 expression in thoracic tumors.
Patients and methods: We examined CK19 expression in specimens from a total of 801 surgically resected samples of primary lung adenocarcinoma (ADC), squamous cell carcinoma (SQC), large-cell carcinoma (LCC), pleomorphic carcinoma (PC), large cell neuroendocrine carcinoma (LCNEC), small cell carcinoma (SCC), and carcinoid tumor (CT) as well as pleural malignant mesothelioma and lung metastatic deposits from breast cancer using tissue microarrays (TMAs) and whole sections. We also compared the CK19 expression status between primary sites and LN metastatic deposits.
Results: The overall rate of CK19 expression as observed on TMAs and whole sections in the 801 analyzed cases was 88.0%. CK19 expression was detected in 94.6% of ADCs, 93.6% of SQCs, 54.5% of LCCs, 54.8% of PCs, 77.4% of LCNECs, 31.8% of SCCs, 34.0% of CTs, and 92.9% of malignant mesotheliomas. Expression of CK19 was also detected in 90.9% of lung metastatic deposits from breast carcinomas. CK19 expression was maintained between CK19-positive primary sites and the corresponding LN metastatic deposits. Of note, a portion of CK19-negative primary tumors showed upregulation of CK19 protein expression in LN metastases.
Conclusions: Most thoracic tumors, except for PCs, CTs, and SCCs, were positive for CK19. We also found that CK19 expression was maintained between CK19-positive primary tumors and the corresponding LN metastatic deposits. These results may be useful in the development of the OSNA method for the intraoperative detection of LN metastasis in non-small cell lung cancer (NSCLC).

1. Introduction

In non-small cell lung cancer (NSCLC), lymph node (LN) metastatic status is one of the most predictable adverse prognostic factors [1–3]. In particular, in deciding on the most appropriate surgical procedure for patients with NSCLC, the presence or absence of intrathoracic metastatic LNs is one of the most important factors. Identification of LN metastasis in decision-making for surgical procedures is also of importance in other tumor types. For example, in breast cancer, sentinel lymph node (SLN) biopsy has recently become a standard surgical procedure in the decision of whether to undertake axillary LN dissection. However, intraoperative diagnosis of SLNs for metastasis using frozen sections has a sensitivity with immunohistochemistry (IHC) of only 50–70% compared with the permanent histologic sections of the same LN [4,5].

Cytokeratins (CKs) are keratin-containing intermediate filament proteins found in the intracytoplasmic cytoskeleton of epithelial tissue. CKs are of two types: acidic type I CKs and basic or neutral type II CKs. CKs are usually found in pairs, comprising a type I CK and a type II CK. Among the 20 epithelial CKs, CK19 is the lowest molecular weight (40 kDa) acidic keratin and is a specific cytoskeletal structure of simple epithelia; its expression is observed in normal and cancerous epithelial cells of organs such as the breast, colon, and lung [6,13].

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http://dx.doi.org/10.1016/j.lungcan.2014.09.018
0169-5002/

The one-step nucleic acid amplification (OSNA) assay is a novel technique that utilizes a loop-mediated isothermal amplification (RT-LAMP) method of gene amplification [7,8]. The assay is characterized by the quantitative measurement of a target mRNA, a brief reaction time, a high specificity for the target mRNA, and an absence of genomic DNA amplification. On the basis of these advantages, the OSNA assay has been developed as an alternative intraoperative method for the detection of tumor metastasis, and has been validated as diagnosis of LN metastases in breast, gastric, and colorectal cancers [9–12]. Also, the potential OSNA utility to non-small cell lung cancer patients has been reported [13]. To provide basic data for the development of the OSNA method for the evaluation of LN metastasis in lung tumors, we used immunohistochemistry to evaluate the CK19 expression rate in several different types of thoracic tumors. In addition, we evaluated the concordance of CK19 expression between primary tumors and their LN deposits.

2. Materials and methods

2.1. Case selection

The Institutional Review Board of our hospital approved the current study (2010-0077). The specimens used in this study were from previously constructed tissue microarray (TMA) blocks, which utilized a core sample measuring 2 mm in diameter, and they included samples from a total of 801 cases seen at the National Cancer Center Hospital (Tokyo, Japan) between 1997 and 2007. The cases consisted of 726 primary lung tumors, including 294 adenocarcinomas (ADCs), 157 squamous cell carcinomas (SQCs), 11 large cell carcinomas (LCCs), 42 pleomorphic carcinomas (PCs), 106 large-cell neuroendocrine carcinomas (LCNECs), 66 small cell carcinomas (SCCs), and 50 carcinoid tumors (CTs), as well as 42 malignant mesotheliomas and 33 metastatic lung deposits from breast carcinomas. Histological diagnoses were based on the latest World Health Organization classification with the aid of immunohistochemical panels [14]. Among all ADC cases, the predominant histological patterns were classified based on the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society (IASLC/ATS/ERS) classification [15].

2.2. Immunohistochemistry and evaluation

Heat-induced epitope retrieval with a target retrieval solution 9 (Dako, Carpinteria, CA, USA) was performed. Incubation with primary antibody to CK19 (RCK108, 1:50, Dako) was conducted using an automated stainer (Dako) following the vendor's protocol. ChemMate EnVision™ (Dako) detection kits were used. Immunohistochemical staining was scored independently by two observers (K.M. and K.T.). Discrepancies in judgment were resolved by means of a joint viewing of the slides under a multiheaded microscope. We defined immune-positive cases as those with a mean positive area $\geq$10%.

First, we performed CK19 staining on TMA sections. Then, the after mentioned 96 CK19-negative cases were subjected to CK19 immunostaining using the whole section from the largest area of the tumor blocks.

2.3. CK19 expression in LN metastasis

To elucidate the differences in CK19 expression between primary sites and their corresponding LN metastases, we selected the CK19-positive cases based on TMA data and the CK19-negative cases based on whole sections at primary tumor sites from patients with LN tumor deposits. CK19-positive cases were defined as those in which more than 5% of the tumor cells in the LN were positive.

2.4. Statistical analyses

Statistical analysis of CK19 expression among the different histological subtypes of ADCs was performed using Fisher's exact test with the SPSS Statistics 22 program (IBM Corporation, Somers, NY, USA).

3. Results

3.1. CK19 expression in TMA sections

CK19 expression was detected in 643 (80.3%) of all 801 analyzed cases. In primary lung and pleural tumors, CK19 expression was detected in 278 (94.6%) of 294 ADCs, 147 (93.6%) of 157 SQCs, 6 (54.5%) of 11 LCCs, 23 (54.8%) of 42 PCs, 82 (77.4%) of 106 LCNECs, 21 (31.8%) of 66 SCCs, 17 (34.0%) of 50 CTs, and 39 (92.9%) of 42 malignant mesotheliomas (Fig. 1). CK19 expression was also detected in 30 (90.9%) of 33 lung metastatic deposits from breast carcinomas (Figs. 1 and 2).

3.2. CK19 expression rates together with whole section data

Additional whole section analysis increased the CK19 expression from 80.3% to 88.0% of all cases. The expression rate in metastatic deposits from breast carcinoma remained unchanged (90.9%). Among primary lung and thoracic tumors, 62 (40%) of 155 negative cases were found to be changed to positive for CK19-expression using whole section analysis: 14 (87.5%) of 16 ADCs, 4 (40%) of 10 SQCs, 3 (60%) of 5 LCCs, 7 (36.8%) of 19 PCs, 11 (45.8%) of 24 LCNECs, 18 (40%) of 45 SCCs, 4 (12.1%) of 33 CTs, and 1 (33.3%) of 3 malignant mesothelioma (Fig. 3).

Final CK19 expression rates conjunction with TMA and whole sections was 99.3% ADC, 96.2% SQC, 81.8% LCCs, 71.4% PC, 87.7% LCNEC, 59.1% SCC, 42.0% CT, and 95.2% malignant mesotheliomas (Fig. 1).

3.3. Correlation between CK19 expression and IASLC/ATS/ERS classification of lung ADCs

Among the 294 ADCs, the most common histological subtype was papillary-predominant, with 79 cases (26.9%), followed by 66 solid-predominant cases (22.4%), 54 acinar-predominant cases (18.4%), 46 invasive mucinous ADC cases (15.6%), 27 micropapillary-predominant cases (9.2%), and 22 lepidic-predominant cases (7.5%). There were no cases of mucinous adenocarcinoma in situ (AIS) or minimally invasive adenocarcinoma (MIA). When subdividing ADCs based on the IASLC/ATS/ERS classification, there were no statistical differences in CK19 expression ($P = 0.31$). Complete positivity was observed in papillary-, acinar-, micropapillary-, and lepidic-predominant ADCs. The other subtypes also demonstrated highly positive CK19 expression, with rates of 98.5% in solid-predominant and 97.8% in invasive mucinous ADCs.

3.4. CK19 expression in LN metastases

We also examined the CK19 expression in LN metastases from 38 lung tumors: 22 cases that were CK19-positive at the primary sites (10 ADCs, 7 SQCs, 1 LCC, 2 LCNECs, 1 SCC, and 1 CT) and 16 cases that were CK19-negative at the primary sites (1 ADC, 2 SQCs, 1 LCC, 3PCs, 4 LCNECs, 2 SCCs, and 3 CTs).

All LN deposits from cases that were CK19-positive at the primary sites were positive for CK19. In addition, 7 (43.8%) of 16 LN