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Identification and validation of S100A7 associated with lung squamous cell carcinoma metastasis to brain☆

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Summary To identify potential markers associated with non-small cell lung cancer (NSCLC) metastasis to brain, comparative proteome analysis on two lung squamous cell carcinoma (SCC) cell lines, NCI-H226 and H226Br (the brain metastatic cell line of NCI-H226), was performed using two-dimensional electrophoresis (2-DE) followed by a tandem mass spectrometer with a matrix-assisted laser desorption/ionization (MALDI) source. Twenty differential proteins were identified, of which 6 proteins were up-regulated in H226Br cell compared with NCI-H226 cells, whereas 14 proteins were down-regulated. S100A7 and 14-3-3σ, two of candidate proteins significantly upregulated and downregulated in H226Br cell, were selected to verify the liability of the differential proteins by Western blot. The results were in accordance with 2-D data. To determine whether S100A7 overexpression is actually associated with SCC metastasis to brain, S100A7 protein was testified in 10 brain metastasis tissues from NSCLC, 38 primary NSCLC tissues including half matched local positive lymph nodes, 5 primary brain tumors and 2 non-cancer

Abbreviations: NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; Ad, adenocarcinoma; MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; PMF, peptide mass fingerprint; MS/MS, tandem MS; IHC, immunochistochemistry

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brain tissues by immunohistochemistry. Of particular interest to us was that the positive staining of S100A7 could be found in 3/5 (60%) brain metastases tissue from SCC and 8/21 (38%) the primary lung SCC tissues, while no positive staining was observed in the brain metastases tissue from Ad ($n = 5$), the primary adenocarcinoma (Ad) tissues ($n = 17$), the primary brain tumors ($n = 5$), all local positive lymph nodes from the primary NSCLC ($n = 19$) and non-cancer brain tissues ($n = 2$). These findings suggest that S100A7 expression is closely associated with SCC metastasis to brain and may be a potential biomarker for monitoring the development of SCC.

1. Introduction

Although development is continually made in diagnostic and therapeutic modalities, brain metastasis still frequently occurs in patients with NSCLC and overall survival is especially poor (3—6 months) [1]. Very little is known about how these metastases arise, and what characteristics the NSCLC cells with brain metastasizing potential possess. Answers to these questions are keys to bringing forward new strategies to prevent or control the growth of brain metastases. In fact, the molecular requirements for some metastatic processes may be tissue specific: the proclivity that lung cancer has for specific organs metastasis, such as brain, liver and bone, was noted many years ago. Some molecular factors, i.e. vascular endothelial growth factor, insulin-like growth factor-I (IGF-I), E-cadherin, ADAM9 and integrin $\alpha 3\beta 1$, have been reported to be related to the development of brain metastasis in NSCLC [2—6]. Recently, transcriptomic profiling of primary human carcinomas has identified gene expression patterns presented in the bulk primary cancer population predict a poor prognosis for patients. The existence of such signatures has been interpreted to mean that genetic lesions acquired early in carcinogenesis are sufficient for the metastatic process [7]. However, it is unclear whether these genes predicting metastasis are also based on the protein levels or functional mediators.

Proteomics provides an effective approach to study disease pathogenesis through analyzing alterations in protein expression and post-translational modifications due to malignant cell transformation in diseases. The proteomic approaches have been successfully applied to identify tumor-associated proteins in various types of cancer such as breast, prostate, and liver cancers [8—10]. With regard to lung cancer, proteome analysis has been carried out in tissues, sera, and cell lines from molecular to cellular level to study carcinogenesis and identify novel diagnostic markers and therapeutic targets [11—13]. 2-DE databases of NSCLC cell lines have also been established in these studies [14]. Nonetheless, only a few studies have been involved in proteomic analysis of lung cancer metastasis, perhaps due to lack of appropriate materials. Jiang et al. [13] performed the comparative study to differentiate the protein expression profiles of two human lung giant cell carcinoma cell lines with different metastatic potentials and found that there were 7 proteins up-regulated and 5 proteins down-regulated in the highly metastatic cell line PLA801D. However, so far there has been no systematic proteomics study associated with NSCLC brain metastasis.

In this study, we compared the proteome profilings of parental NCI-H226 cells and H226Br subline (an in vivo isolated brain metastatic variant) using 2-DE analysis. Previous study indicated that IGF-I mRNA level was higher in the brain metastatic H226Br cells compared to that of parental NCI-H226 cells and IGF-I was an autocrine growth regulator for human NSCLC cells metastatic to brain [3]. These results suggested that this pair of cell lines could be an ideal model for comparative proteome analysis of NSCLC brain metastasis-associated proteins. Twenty constantly differentially displayed proteins were identified. Overexpression of S100A7 could be found in brain metastasis H226 cell and 80% brain metastases tissues of SCC. These results provide the basis to look for potential markers for SCC metastasis to brain and provide some clues to understand the development of NSCLC brain metastasis.

2. Materials and methods

2.1. Chemicals and antibodies

DTT, urea, CHAPS, mineral oil, acrylamide, *N*′,*N*′-methylen-bis-acrylamide, Tris-base, glycine, SDS, ammonium persulphate, and TEMED were from Sigma (St. Louis, MO, USA). Immobilized pH-gradient (IPG) strips and IPG buffers were purchased from Amersham Pharmacia Biotechnology (Uppsala, Sweden). Other chemicals are commercially available (analytical grade). Monoclonal antibody to S100A7 was purchased from Imgenex (San Diego, CA, USA, catalog No.: IMG-409A) and this antibody was developed against recombinant psoriasin/HD-5 protein). Monoclonal mouse 14-3-3σ antibody (1.N.6) was purchased from Abcam (Cambridge, UK, catalog No.: ab14123).

2.2. Cell culture and sample preparation

Human SCC cell lines NCI-H226 and H226Br were kindly provided by Dr. Kang Fang (Department of Life Sciences, Taiwan Normal University, and Taipei). The cells were cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum at 37 °C and 5% (v/v) CO_2. The cell pellets were collected by centrifuge at $500 \times g$ for 5 min and washed three times with ice-cold PBS buffer. The cell pellets were then solubilized in lysis buffer containing 8 M urea, 4% (w/v) CHAPS, 40 mM Tris-base, 65 mM DTT, 1% IPG (v/v) buffer (pH 3—10 L), lysised for 1 h at 4 °C and centrifuged at $1{,}5000 \times g$ for 1 h at 4 °C. The supernatant was collected and dispensed, then stored at −80 °C. Protein concentrations were determined using the Bradford assay [15].