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Up-regulated Golgi phosphoprotein 2 (GOLPH2) expression in lung adenocarcinoma tissue

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ABSTRACT

Objectives: The aim of the study was to evaluate Golgi Phosphoprotein 2 (GOLPH2) expression in lung cancer.

Design and methods: GOLPH2 expression was analyzed from 178 lung cancer tumor tissue samples using immunohistochemistry. Levels of 133 lung cancer patients' serum GOLPH2 (sGOLPH2) were also quantitatively compared with 70 healthy individuals by a sandwich enzyme-linked immunosorbent assay (ELISA).

Results: All adenocarcinoma (ADC) tissue samples (100%) displayed strong staining, 83.6% of which displayed cytoplasmic diffused staining. However, for squamous cell carcinoma samples, only 16.3% displayed strong staining intensity. Statistically, GOLPH2 expression in tissues of ADC was significantly higher than in other types of lung cancer ($P<0.001$). Levels of sGOLPH2 were also detected. The mean value of sGOLPH2 concentration is 69 ng/ml in lung cancer patients and 53 ng/ml in healthy individuals.

Conclusions: Significantly elevated GOLPH2 expression was observed in ADC tumor tissues. The levels of sGOLPH2 were about 30% higher in lung cancer patients compared with healthy individuals.

Introduction

Golgi phosphoprotein 2 (GOLPH2, also known as GP73 and GOLM1) is a 73-kDa Golgi membrane glycoprotein coded by the *GOLM1* gene (Gene ID 51280) on homo-chromosome 9q21.33 with constitutive expression in cells of the epithelial lineage, especially in the prostate, gut, stomach, and within the central nervous system [1]. Although its biological function remains unknown, up-regulated GOLPH2 was observed in a number of diseases, including liver diseases [acute and chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC)] [2,3], Alzheimer's disease [4], Wilson's disease [5], prostate cancer [6–10], and renal cell cancer [11]. Recently, several studies reported that the levels of serum GOLPH2 (sGOLPH2) of patients with liver disease increased significantly [12–18], suggesting that GOLPH2 might serve as a potential serum marker for liver disease, especially for early HCC [13–15].

Lung cancer is the leading cause of cancer-related deaths in men and is second in women worldwide [19–21]. The 5-year survival rate for all stages combined is below 15% [22]. Lung cancer is grouped into small cell lung cancer (SCLC; 14%) and non-small cell lung cancer (NSCLC; 85%), and the clinical treatment strategies for different types of lung cancer are different. SCLC is often treated by chemotherapy, while NSCLC is often treated by surgical excision. Thus, accurate diagnosis is an important step toward efficacious treatment [22]. Being the most common form of lung cancer clinically, NSCLC could be further subclassified as adenocarcinoma (ADC; accounting for 50% of NSCLC, developed from gland epithelial cells), squamous cell carcinoma (SCC; accounting for 20% of NSCLC, developed from squamous epithelial cells), and large cell carcinoma (LCC; accounting for 10% of NSCLC), where ADC counts the most [23]. Most lung cancer patients have already become regionally advanced or metastatic at the time of diagnosis [24,25]. Since, there are no effective methods of early detection of lung cancer available currently, the determination of specific biomarkers in tissue and body fluid is of utmost importance in reducing the mortality of lung cancer.

Although the abnormal expression of GOLPH2 has been associated with several types of cancer, the association between lung cancer and GOLPH2 expression has not been studied previously. In this report, GOLPH2 expression was analyzed in lung cancer tissues. In particular,

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GOLPH2 expression was compared in ADC and SCC, two of the predominant types of NSCLC. The results indicated that GOLPH2 was significantly overexpressed in ADC tumor tissues. Furthermore, GOLPH2 expression in the cell lines of ADC and SCC origin was analyzed, and elevated levels of GOLPH2 expression was observed in ADC cell lines. Finally, a sandwich enzyme-linked immunosorbent assay (ELISA) was performed to detect the levels of sGOLPH2 in lung cancer patients and healthy individuals. The results showed that levels of GOLPH2 were increased in the sera of lung cancer patients. However, the differences between the mean values were not as significant as in liver-diseased patients.

Materials and methods

Generation of GOLPH2-specific mouse monoclonal antibodies (mAbs)

Mouse anti-GOLPH2 mAbs 5B12 and 5F10 were generated as described in Ref. [17]. In brief, a C-terminal His6-tagged recombinant GOLPH2 protein was expressed in BL21 bacteria (Invitrogen, USA) and purified by Ni-NTA affinity chromatography according to the manufacturer's protocols (Qiagen, USA). mAbs were prepared in BALB/c mice according to standard methods [26]. Ascitic fluids were collected, pooled, and isotyped with hybridoma subisotyping kit (Calbiochem, USA).

Patients and tissue samples

The study protocol was approved by the Guangzhou Medical College institutional review board with informed consent. Paraffin-embedded tumor tissues from 178 patients were obtained from the Affiliated Hospital of Guangzhou Medical College. The age of the patients ranged from 16 to 84 years, with a median of 61 years. There were 115 males and 63 females. 65 patients had a smoking history. Cases were selected according to tissue availability and were not considered for any known preoperative or pathologic prognostic factors. Histologic diagnosis was established according to the guidelines of the World Health Organization [27]. Among these patients, 116 (55.5%) were adenocarcinoma (ADC), 49 (23.4%) were squamous cell carcinoma (SCC), 8 (3.8%) were other NSCLC (containing large cell carcinoma, pleiomorphic carcinoma, and sarcomatoid carcinoma of the lung), and 5 (2.4%) were SCLC. Lung cancer was staged using the standard classification system (pTNM: pathologic TNM stage [28]. The stage grouping of the pTNM subsets was T = primary tumor, N = regional lymph nodes, M = distant metastasis). Among these patients, 41 (23.0%) had pT1 status, 104 (58.4%) had pT2, and 14 (7.9%) had pT3/4. For pN status, 76 (42.7%) had pN0 status, 25 (14.0%) had pN1, 47 (26.4%) had pN2, and 11 (6.2%) had pNx. For pM status, 149 (93.7%) had pM0 status and 10 (6.3%) had pM1/x.

Paraffin-embedded tumor tissues were cut at 5 μm thickness and placed on microscopic slides. These Slides were dried in a 60 °C oven for 6 h before storage at 4 °C.

Cell culture

Huh7 is a hepatocarcinoma cell line obtained from the American Type Culture Collection. A549, SPC-A-1, LTEP-a2, GLC-82, and 95-D are ADC-derived cell lines [29–33]. L-78 (LTEP-78) and H157 (NCI-H157) are SCC-derived cell lines [34,35]. These lung cancer cells were generously provided by Dr. Duanqing Pei. Huh7 and H157 were cultured in Dulbecco's modified Eagle's medium (Gibco, USA). The others were in RPMI 1640 (Hyclone, USA). Both media were supplemented with 10% fetal bovine serum (Hyclone, USA) and 100 IU/ml penicillin-streptomycin (Gibco, USA). 1015, 1010 and 0818 are cell lines we generated from lung cancer tissue. 1015 and 1010 are ADC-derived cell lines and 0818 is a SCC-derived cell lines.

Cell immunofluorescence staining and confocal microscopy

Huh7 was cultured on the cover slips in six-well plates. Following removal of the culture medium, the cells were fixed in 4% pre-cooled paraformaldehyde for 15 min and permeabilized with 0.5% Triton X-100 for 5 min on ice. Then the cells were incubated for 1 h with a mAb (5B12 or 5F10; 1:100 diluted in PBS) at room temperature. Tetramethylrhodamine isothiocyanate-labeled goat anti-mouse IgG secondary antibody (Chemicon, USA) was applied to the cells for 1 h at room temperature. The slides were then incubated with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI; KPL, USA) for 5 min. Confocal images were taken using the Leica confocal microscopy imaging system (TCS SP2 AOBS; Leica, Switzerland). The two-color images were exported as TIFF images.

Immunohistochemistry

Deparaffinization was done as follows. The slides were soaked twice in xylene for 10 min each. After the slides were rehydrated, antigen retrieval was achieved by heating the slides in citrate buffer (pH 6.0; Zhongshan Golden Bridge, Beijing, China) for 2 min in boiled water in a pressure cooker. They were then blocked for 15 min in 3% H_2O_2 in phosphate-buffered saline (PBS; 0.01 M, pH 7.4). To detect GOLPH2, 5B12 (1:100 diluted in PBS) was used and detected by horseradish peroxidase (HRP)-labeled goat anti-mouse IgG secondary antibody (Zhongshan Golden Bridge, China), followed by 3, 3′-diaminobenzidine staining (Zhongshan Golden Bridge, China) according to the manufacturer's suggestion.

Evaluation of the immunohistochemical (IHC) staining

Images of the IHC slides were captured with a high-resolution digital microscope camera (Olympus, Japan). The staining intensity was scored by three independent investigators without prior knowledge of the clinical details of the specimens. A five-tier grading system (0 to 4) was used for GOLPH2 staining intensity and pattern: negative staining (±, score 0), less than 10% positive staining cells or complete negative staining [a 10% threshold (calculated the percentage of positive cells in one field of vision) was used to determine negativity versus positivity, which did not consider the staining intensity grade]; weak staining (1+, score 1), more than 10% but less than 100% positively stained cells, with minute granules outpocketing to the Golgi apparatus; moderate staining (2+, score 2), a thick and more intense staining for almost all cells; and strong positive staining (3+, score 3), chunky, dark crude staining; for some very high expression slides, the staining was not like the typical Golgi apparatus localization but showed cytoplasmic diffused staining. These slides were scored as four grades (4+, score 4).

Serologic tests

Serum samples were collected from 133 patients (85 ADC, 33 squamous, 10 other NSCLC, and 5 SCLC) and 70 healthy individuals who all gave prior consent. A sandwich ELISA was performed to quantify the sGOLPH2 of patients and healthy individuals. ELISA was performed as follows. 96-well ELISA plates (Costar, USA) were pre-coated with 10 μg/ml 5B12 and blocked using 2% (w/v) bovine serum albumin. Purified recombinant GOLPH2 protein was used as a standard with a series of concentration gradients in duplicates. Serum samples were diluted 1:10 in PBS and added in duplicates to the wells for 1 h at 37 °C. Subsequently, 8 μg/ml HRP-conjugated 5F10 was applied into the plates for 1 h at 37 °C. The colorimetric reaction was performed with tetramethylbenzidine (Sangon, Shanghai, China) for 10 to 30 min before it was stopped by adding 50 μl of 2 M sulfuric acid to each well. Absorbance was measured at 450 nm by a UV star