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Differential N-glycan patterns identified in lung adenocarcinoma by N-glycan profiling of formalin-fixed paraffin-embedded (FFPE) tissue sections

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ABSTRACT

N-glycan profiling is a powerful approach for analyzing the functional relationship between N-glycosylation and cancer. Current methods rely on either serum or fresh tissue samples; however, N-glycan patterns may differ between serum and tissue, as the proteins of serum originate from a variety of tissues. Furthermore, fresh tissue samples are difficult to ship and store. Here, we used a profiling method based on formalin-fixed paraffin-embedded (FFPE) tissue sections from lung adenocarcinoma patients. We found that our method was highly reproducible. We identified 58 N-glycan compositions from lung adenocarcinoma FFPE samples, 51 of which were further used for MS^n-based structure prediction. We show that high mannose type N-glycans are upregulated, while sialylated N-glycans are downregulated in our FFPE lung adenocarcinoma samples, compared to the control samples. Our receiver operating characteristic (ROC) curve analysis shows that high mannose type and sialylated N-glycans are useful discriminators to distinguish between lung adenocarcinoma and control tissue. Together, our results indicate that expression levels of specific N-glycans correlate well with lung adenocarcinoma, and strongly suggest that our FFPE-based method will be useful for N-glycan profiling of cancer tissues. *Significance:* Glycosylation is one of the most important post-translational protein modifications, and is associated with several physiopathological processes, including carcinogenesis. In this study, we tested the feasibility of using formalin-fixed paraffin-embedded (FFPE) tissue sections to identify changes in N-glycan patterns and identified the differentially expressed N-glycans of lung adenocarcinoma. Our study shows that the FFPE-based N-glycan profiling method is useful for clinical diagnosis as well as identification of potential biomarkers, and our data expand current knowledge of differential N-glycan patterns of lung adenocarcinoma.

1. Introduction

Lung cancer is the leading cause of cancer-related mortalities in the world, and is characterized by poor prognosis [1]. Lung adenocarcinoma, which accounts for 40% of all diagnosed lung cancer cases, is a histological subgroup of non-small cell lung cancer, and while the most frequent form, it remains difficult to detect at an early stage. If detected during the early stages, the 5-year survival rate increases from 15% to over 60% [2]. To date, the molecular mechanisms responsible for lung adenocarcinoma carcinogenesis and progression have remained unclear. Therefore, the identification of biomarkers to detect early stage lung adenocarcinoma, together with the discovery of suitable molecular targets, will be critical for successful treatment.

Glycosylation is one of the most common post-translational protein modifications, and is associated with several physiopathological processes. Protein glycosylation patterns appear to be altered in various types of cancers, and are believed to impact the degree of malignancy [3]. Several recent studies focused on the correlation between N-glycan pattern or changes and lung cancer prognosis or progress, including the identification of biomarkers for early detection [2,4–6], the aberrant glycosylation of glycoprotein [7–10] and the function of glycosyltransferase [11–13]. The understanding of the role of glycosylation in cancer not only provides novel candidate biomarkers, but also allows for identifying therapeutic targets. Sialyl Lewis X (SLe^X), which consists of a sialic acid α2,3-linked to galactose with fucose α1,3-linked to GlcNAc, is assumed a suitable marker of lung adenocarcinoma [14,15].

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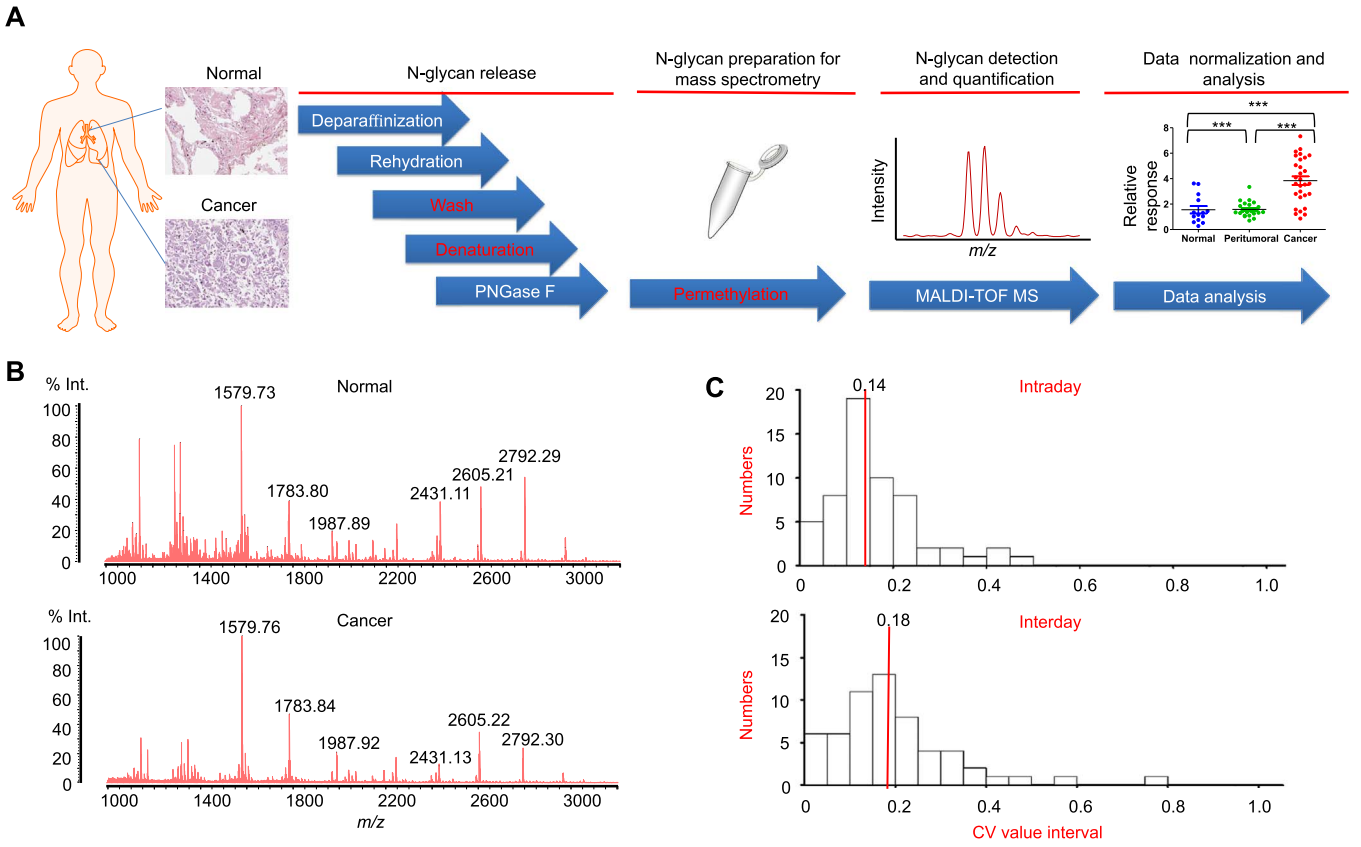

Fig. 1. N-glycan profiling from tissue sections. (A) General workflow. N-glycans were released from tissue sections, permethylated and analyzed by MALDI-TOF-MS; data were then normalized within Markview using the total area for subsequent analysis. (B) Mass spectra of N-glycan peaks of one normal and one cancer tissue sections. (C) The distribution of coefficient of variation (CV) of N-glycan peaks for intraday (top) and interday (bottom) from three repeated experiments, the data were normalized using total area, with median of 0.14 and 0.18, respectively.

Core fucose is the α1,6-fucose in the innermost GlcNAc residue of N-glycans, which is catalyzed by the glycosyltransferase FUT8. Increase in FUT8 expression or core fucosylation was reported for various cancers, such as hepatomas [16], ovarian cancer [17], pancreatic cancer [18], colon cancer [19] and non-small cell lung cancer [12]. Principally, β1,6-GlcNAc side chain branching (synthesized by GnT-V) appears to be upregulated in cancer, whereas β1,4-GlcNAc (bisecting GlcNAc, synthesized by GnT-III) appears to be downregulated [20]. In contrast, an increase in GnT-V expression appears to impair malignancy of lung cancer [13]. These earlier studies suggested that specific changes in N-glycan pattern provide a promising starting point for early cancer diagnosis.

The sample types used in N-glycan analysis have varied from cell line [21], serum [4], tissue [22] and formalin-fixed paraffin-embedded (FFPE) tissue sections [23]. However, N-glycan pattern of samples originating from cell line may not reflect the real N-glycan information in cancer tissue since the environment of cell line differ from the environment of cancer cells *in vivo* [22,24]. Furthermore, blood may present a source of contamination, even after thorough washing. This represents a major problem especially for lung tissue samples, which are well-perfused and thus difficult to purify [22]. In addition, fresh or frozen tissue samples are often inconvenient to use, as they are difficult to store or ship. In comparison, FFPE tissue sections are easy to handle, are clinically well-described, possess histological scale information and are readily available in sufficient numbers [23,25].

Several recent studies focusing on N-glycan analysis used FFPE tissue sections in combination with MALDI-TOF mass spectrometry. Mass spectrometry imaging (MSI) is an emerging technique applied to visualize the spatial distribution of N-glycan on FFPE tissue sections [26–28]. A recent study evaluated the feasibility that analyzing N-glycans using FFPE tissue sections replacing fresh tissues [29]. However, there are few reports that utilize FFPE tissue sections to analyze N-glycan patterns of clinical settings.

Here, we used a MALDI-MS-based N-glycan profiling method to analyze differential N-glycans in lung adenocarcinoma specifically from FFPE tissue sections. We show that our method is convenient, fast, and reliable, making it a suitable approach for marker identification in a clinical setting. Furthermore, we identified upregulated high mannose type and downregulated sialylated N-glycans in lung adenocarcinoma compared to the controls using FFPE samples.

2. Materials and methods

2.1. Materials

Ethanol, acetone, xylene and chloroform were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). DL-Dithiothreitol (DTT), urea, thiourea, ammonium bicarbonate, sodium hydroxide (NaOH), 2,5-dihydroxybenzoic acid (DHB), standard peptides, trifluoroacetic acid (TFA), acetic acid and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich. Water, 1-propanol, methanol and acetonitrile were purchased from J.T. Baker. Peptide Nglycosidase F (PNGase F) was purchased from Promega Corporation (WI, USA). 96-Well plate C18-Sep-Pak cartridges were obtained from Waters Corporation (MA, USA). Tissue section samples of discovery set and validation set were purchased from Beijing Raisedragon'S Co., Ltd. and Fanpu Biotech, Inc. Co., Ltd., respectively. The tissue sections are 5 μm thick and their area are > 0.5 cm^2. The overview of sample information was shown in Table 2, the details of sample information was shown in Supplementary Tables and Supplementary Fig. 1. Silicon slide