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Mass spectrometry-based N-linked glycomic profiling as a means for tracking pancreatic cancer metastasis

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ABSTRACT

The aberrant glycosylation profile on the surface of cancer cells has been recognized for its potential diagnostic value towards assessing tumor progression. In this study, we initially investigate N-glycan profiles on the surface of normal (HPDE) and cancerous (Capan-1, Panc-1, and MIA PaCa-2) pancreatic cell lines, which are from different sites of pancreatic tumor. The enzymatically deglycosylated total N-glycans are permethylated via a quantitative solid-phase method and then analyzed by using MALDI-TOF MS and MALDI-QIT-TOF MS. We demonstrate that the level of high-mannose type glycans is higher among Capan-1 cells—pancreatic cancer cells that have metastasized to the liver—than that observed among Panc-1 and MIA PaCa-2 cells—pancreatic cancer cells from the pancreas duct head and tail regions, respectively. Furthermore, the relative abundance of highly-branched sialyted N-glycans is significantly up-regulated on Panc-1 and MIA PaCa-2 pancreatic cancer cells compared to that of normal HPDE pancreas cells. Taken together, these results indicate that specific N-glycosylation profile changes in pancreatic cancer cells can be used to not only distinguish between normal and cancerous cells but also provide more information on their location and metastatic potential.

1. Introduction

The abnormalities in glycosylation profile manifested among cancer cells—including an increase in sialyation, fucosylation, high-branched glycans, and Lewis type antigens—and the integral roles of glycoconjugates in various cell-cell and cell-protein interactions underscore the importance of illuminating light on pancreatic cancer cell glycosylation.[1] Furthermore, given differences in the biological and physiological characteristics of cancerous cells from normal cells, as well as the metastasis of cancer cells and their resistance to hormonal/chemo therapy, the role of glycoconjugates is not only necessary but also practical. From this perspective, a close examination of the molecular glycoconjugate abnormalities associated among different types of pancreatic cancer cells could provide the basis for the selection of novel pancreatic cancer biomarkers and potential patient-specific targeted therapies.

Currently, pancreatic cancer is most often examined via conventional imaging methods.[2] Such methods, however, have proven difficult during diagnosis due to the location—central with respect to both sagittal and coronal planes—of the pancreas, its small dimensions, and the limited spatial resolution of the imaging modality. Consequently, while a qualitative approach to the examination of pancreatic cancer biomarkers is valuable, concurrent quantitative methods must be employed. In particular, due to the abnormally high levels of sialylation observed among cancer cells, the quantitative analysis of sialylated glycans is a central point of emerging studies. While conventional high performance liquid chromatography (HPLC) analysis confers the ability to qualitatively and quantitatively distinguish glycoconjugates isomers, it is mired

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by a relatively low peak resolution and sensitivity.[3] Mass spectrometry (MS), on the other hand, offers an alternative with its high sensitivity and peak resolution for carbohydrate identification;[4] MALDI-TOF MS, in particular, is being developed as a platform for the qualitative and quantitative analysis of glycoconjugates.[5]

Herein, we propose to utilize a MS-based analytical method to investigate the surface-bound-N-glycan profiles for three pancreatic cancer cell lines—representative of different pancreatic cancer locations—and a normal pancreatic cell line. The membrane fractionations of pancreatic cancer and normal pancreatic cells are enzymatically deglycosylated and subsequently permethylated via a quantitative solid-phase method to obtain corresponding N-glycans. The total permethylated N-glycans, including acidic and neutral glycans, are then analyzed via MALDI-TOF MS. Additional detailed information with regards to the sialylated glycans highly expressed on the surface of normal and cancerous pancreatic cells are obtained via MALDI-QIT-TOF MS. We envision that such glycoconjugate profiles obtained from our study will contribute to the discovery of novel glycoconjugate-based biomarkers and to the elucidation of the mechanism of pancreatic cancer metastasis.

2. Results and discussion

2.1. MS profiling of total N-glycans from pancreatic cancer cells

In the present study, we carry out a comprehensive analysis of N-glycan profiles of the membrane glycoproteins isolated from normal (HPDE) and cancerous (Capan-1, Panc-1, and MIA PaCa-2) pancreatic cells as shown in Fig. 1. Briefly, cell membrane proteins were fractionated from pancreatic cell lysate by ultracentrifugation and then N-linked glycans were released from the cell membrane proteins by peptide N-glycosidase F (PNGase F). Next, the released N-linked glycans were purified using porous graphitic carbon (PGC) cartridges via solid-phase extraction. Finally, the purified N-linked glycans were analyzed by MALDI-TOF MS combined with solid-phase permethylation and tandem MS. Representative MALDI-TOF mass spectra of the N-glycans from the pancreatic cells are obtained (Supplementary data Fig. 1), and all N-linked glycan structures detected as $[M+Na]^+$ in positive mode are listed in Table 1. Our results show the presence of various 41 N-glycan structures, including that of high-mannose type glycans and complex bi-, tri-, and tetra-antennary glycans with sialic acid and/or fucose residues. The composition of the N-glycans are predicted from experimental mass values using GlycoMod tool (ExPASy: SIB bioinformatics resource portal),[6] and further corroborated for detailed structure via tandem mass spectrometric analysis.

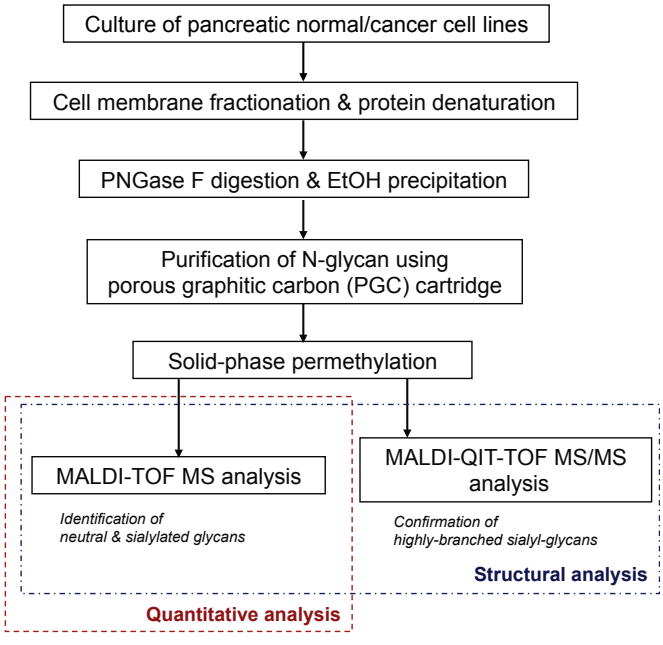

Fig. 1. Analytical scheme for the analysis of N-linked glycans isolated from pancreatic normal/cancer cells.

2.2. Quantitative analysis of N-glycans via solid phase-permethylation

Relative quantities of N-glycans among normal and cancerous pancreatic cells were determined by MALDI-TOF MS analysis coupled with solid-phase permethylation, which has proven to be a reliable and reproducible method in the quantification of total oligosaccharides such as sialic acid-containing glycans.[7] As shown in Fig. 2, a different N-glycosylation pattern is observed on Capan-1 pancreatic cancer cells than that on other pancreatic cells—both normal and cancerous. While the amount of high-mannose type glycans is higher in Capan-1 cells (71.3%) than in HPDE, Panc-1, and MIA PaCa-2 cells (29.4, 29.5, and 24.9%, respectively), the amount of complex type glycans is lower in Capan-1 cells than in HPDE, Panc-1, and MIA PaCa-2 cells. Some previous studies have reported that the elevation of high mannose type glycans correlates with progression of cancer.[8–11] For example, Johns et al. showed that the high-mannose glycans are more prevalent on the surface of cancer cells, relative to normal cells.[11] Maria et al. also revealed that the level of high-mannose oligosaccharides derived from serum are increased during breast cancer progression, suggesting that cancer cells may display the high-mannose oligoscaccharides because of an incompeletion of the glycosylation proecess in the Golgi.[9] More specifically, a detailed analysis reveals that the fucosylated form of glycans observed in Capan-1 cells represents 16.9% of the total N-glycan pool; in comparison, 65.1, 54.3, and 59.0% are fucosylated in HPDE, Panc-1, and MIA PaCa-2 cells, respectively. Similarly, the sialylated form of glycans observed in Capan-1 cells represents 22.4% of the total N-glycan pool while 44.5, 47.5 and 59.7% are sialylated in HPDE, Panc-1, and MIA PaCa-2 cells, respectively. Given that Capan-1 cells are pancreatic cancer cells that have metastasized to the liver—Panc-1 and MIA PaCa-2 cells are pancreatic cancer cells present in the pancreatic duct head and tail region, respectively—Most recently, Powers et al. reported on spatial profiling of the location and distribution of N-glycans in formalin-fixed paraffin embedded (FFPE) pancreas tissues. They showed that a high-mannose type glycan, Hex8HexNAc2, was predominantly found in the tumor region of the tissue,[12] and this is correlated to our result in Capan-1. On the other hand, Zhao et al. revealed that the level of both highly-branched and sialylated N-glycans increased in the pancratic cancer serum.[13] The elevation of these highly-brached and sialylated glycans is consistent with our result in Panc-1 and MIA PaCa-2. However, these stuides on pancreatic cancer have not been in part targeted toward specific type of cells and tissues. Therefore, our results indicate that the N-glycan surface profile of pancreatic cancer cells could be used to predict the site of pancreatic tumor metastasis. It should be noted that while recent studies have reported on the glycosylation of pancreatic cancer cell lines derived from a singular source of human pancreatic adenocarcinoma,[14,15] no previous studies on the N-glycosylation profile of pancreatic cancer cells derived from different sites exist to the best of our knowledge.

Additionally, MS analysis is used to take a closer look into sialylated N-glycan levels for both normal and cancerous pancreatic cells. Given that an increased level of sialylation on the cell surface is a representative characteristic for most tumoral cells,[16,17] our results that show significantly higher levels of highly-branched (tri-