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# Evaluation of laser microdissection as a tool in cancer glycomic studies

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**Abstract**

Laser microdissection (LMD) is a recent development that enables the isolation of specific cell populations from tissue sections. This study focuses on the potential of LMD as a tool in cancer glycomics using colon cancer as a model. LMD was performed on hematoxylin and eosin stained frozen tissue sections. Tumor cells and normal epithelial cells were selectively microdissected. *N*-Glycans from the LMD- and the bulk tissue-derived samples were liberated by hydrazinolysis and then labeled with 2-aminopyridine. After sialidase digestion, the resulting asialo-*N*-glycans were analyzed by normal and reversed phase HPLC combined with mass spectrometry. Comparison of the various *N*-glycan profiles with the aid of LMD identified seven characteristic *N*-glycans with significantly different expression profiles between normal and cancerous cells that could not be detected by conventional analysis. Thus, LMD is a potent and useful tool for analyzing variations in the expression of *N*-glycans by overcoming the problem of tissue sample heterogeneity.


*Keywords:* Colon cancer; Glycomics; Microdissection; *N*-Glycans; Pyridylamination

It is well known that glycans on the cell surface or in the extracellular space play important roles in cellular differentiation, adhesion, and proliferation [1,2]. The biosynthesis of glycans is tissue-specific and is regulated not only by physiological conditions, but also by pathological conditions such as tumorgenesis [3–5]. Aberrant glycosylation of membrane components occurs in essentially all types of human cancers, and many glycosyl epitopes constitute tumor-associated carbohydrate antigens (TACAs) [6–8]. Many lines of evidence suggest that the TACAs function mainly as adhesion molecules and contribute to cancer metastasis [7,9–11]. Alteration of the expression profile of TACAs in certain types of cancer has prompted researchers to evaluate their potential use as diagnostic and/or prognostic tools.

The application of glycomics to cancer research can highlight changes in the expression profile of the glycans occurring during tumor development and progression, leading to the identification of new molecular markers or potential therapeutic targets. However, because cancer tissue is composed of multiple subpopulations of cells, including normal epithelial cells, stromal cells, inflammatory cells, and angiogenic elements, accurate molecular analysis requires isolation of the tumor cells. Laser microdissection (LMD) is a recently developed technique that permits the reliable procurement of specific cell populations from tissue sections under direct microscopic observation. The laser-assisted microdissection technique has already been extensively used to isolate specific types of cells for the molecular analysis of DNA, RNA, and protein. However, in the field of glycan research, only one application of this technique for the analysis of glycosaminoglycans in postmortem human LASIK corneas has been reported [12]. Because LMD is a highly time consuming technique, feasibility

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and usefulness of this procedure must be thoroughly evaluated prior to its application in the analysis of glycans in cancer specimens.

In this study, we have investigated the potential of LMD as a tool in cancer glycomic studies using colon cancer as a model. Asialo-PA-*N*-glycans were prepared from bulk colon cancer tissue, bulk normal colon tissue, and from both normal colonic epithelial cells and cancerous colonic cells isolated from the bulk tissue using LMD. The *N*-glycans were then analyzed by normal and reversed phase HPLC in combination with mass spectrometry. LMD enabled us to identify seven characteristic *N*-glycans which displayed remarkable differences in the expression profile between normal and cancerous colon cells that could not be detected by conventional techniques. Our results demonstrate the usefulness of LMD for the accurate analysis of *N*-glycans in cancer glycomic studies.

## Materials and methods

*Standard PA-oligosaccharides*. The structures and abbreviations of the authentic PA-oligosaccharides used in this study are listed in Table 1. Authentic PA-sugars were obtained from the following suppliers: 224F from Takara (Shiga, Japan); 22bis, ag22bisF, $G_1$22bisF, and 22bisF from Seikagaku Co. (Tokyo, Japan). Ag22bis was prepared by digestion of 22bis with Jack bean β-galactosidase (Seikagaku Co.). The structure of ag22bis was verified by normal and reversed phase HPLC analyses, combined with successive exoglycosidase digestions and by mass spectrometric analysis.

*Tissue*. Paired samples of normal and cancerous colon were obtained from the same patient by a standard colectomy procedure. Areas of tissues examined were selected by an experienced gastrointestinal pathologist. The tissue was cut into blocks, embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan), snap frozen in liquid nitrogen, and stored at −80 °C until use. This study was approved by Local Ethics Committee of Osaka Medical Center for Cancer and Cardiovascular Diseases. Informed consent was obtained from the patient.

*Laser microdissection*. Frozen tissue sections (8 μm thick) of either cancerous colon or normal colonic mucosa were cut on a cryostat, CM 1900 microtome (Leica, Milton Keynes, UK). Tissue sections were thaw mounted on to a film-coated glass slide (90FOIL-SL25, Leica), briefly air dried, and then fixed at room temperature in 95% ethanol for 1 min. Staining was performed by the following procedure. Sections were immersed in Mayer's hematoxylin solution (Muto Pure Chemicals, Tokyo, Japan) for 30 s at room temperature, washed with phosphate-buffered saline (PBS) until a vivid blue color appeared, and then immersed in pure eosin solution (Muto Pure Chemicals) for 2 s. The sections were then dehydrated in 100% ethanol for 30 s and air dried. Laser microdissection (LMD) was performed using a Leica AS LMD system.

*Preparation of protein extracts*. After microdissection, the microdissected cells were carefully transferred from a PCR tube to a glass centrifuge tube using water and a micropipette. The collected cells were lyophilized and then solubilized in a 1:1 mixture of hexafluoroisopropanol (HFIP) [13,14] and 0.2% acetic acid. Control samples, which had not been subjected to LMD, were prepared from the frozen tissue sections cut directly into the solubilization mixture. The protein concentration was determined with a BCA protein assay kit (Pierce, Rockford, IL) using bovine serum albumin as a standard. A protein sample of 300 μg was collected, concentrated, and used for the preparation of PA-*N*-glycans.

*Preparation of PA-N-glycans*. *N*-Glycans were liberated from the glycoproteins by hydrazinolysis at 100 °C for 10 h and then re-*N*-acetylated with acetic anhydride in a saturated sodium bicarbonate solution as previously described [15]. The reducing ends of the liberated *N*-glycans were labeled with a fluorophore, 2-aminopyridine, by reductive amination [16]. The excess reagents were removed by phenol–chloroform extraction and cation-exchange chromatography [17]. The resulting PA-*N*-glycans were further purified by normal phase HPLC according to the method of Nakakita et al. [18] with minor modifications. Briefly, the lyophilized PA-*N*-glycans were dissolved in water and then injected into a TSKgel Amide-80 column (4.6 × 75 mm, Tosoh, Tokyo, Japan). The solvents used were

Table 1
Structures and elution positions in HPLC of standard PA-oligosaccharides

| Abbreviation | Structure | Elution position in HPLC | |
|---|---|---|---|
| | | RP (GU) | NP (GU) |
| ag22bis | GlcNAcβ1-2Manα1(6)<br>GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc-PA<br>GlcNAcβ1-2Manα1(3) | 11.45 | 5.37 |
| 22bis | Galβ1-4GlcNAcβ1-2Manα1(6)<br>GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc-PA<br>Galβ1-4GlcNAcβ1-2Manα1(3) | 12.72 | 6.78 |
| ag22bisF | GlcNAcβ1-2Manα1(6) Fucα1(6)<br>GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc-PA<br>GlcNAcβ1-2Manα1(3) | 14.90 | 5.66 |
| $G_1$22bisF | Galβ1-4GlcNAcβ1-2Manα1(6) Fucα1(6)<br>GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc-PA<br>GlcNAcβ1-2Manα1(3) | 15.86 | 6.29 |
| 22bisF | Galβ1-4GlcNAcβ1-2Manα1(6) Fucα1(6)<br>GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc-PA<br>Galβ1-4GlcNAcβ1-2Manα1(3) | 16.80 | 7.02 |
| 224F | Galβ1-4GlcNAcβ1-2Manα1(6) Fucα1(6)<br>Galβ1-4GlcNAcβ1(4)Manβ1-4GlcNAcβ1-4GlcNAc-PA<br>Manα1(3)<br>Galβ1-4GlcNAcβ1(2) | 13.91 | 7.96 |