Supporting Information

Experiments

Tissue glycoprotein extraction and lectin microarray analysis

Archival FFPE colon sections (2.0 mm diameter and 4 μm thickness) were purchased from Super BioChips Laboratories (Seoul, South Korea). Tissue specimens from colorectal cancer (CRC) patients with good prognosis (alive; n=34) and poor prognosis (deceased; n=11) were used in this study. The clinical characteristics of all subjects are provided in Suppl. Table S1.

Differential glycan profiling of tissue sections was performed as described previously.[1, 2] Briefly, FFPE tissue sections were deparaffinized, and each specimen was detached from the glass slide using a needle (21 G) under a microscope. Glycoproteins were extracted, followed by fluorescence-labeling with 10 μg of Cy3-succimidyl ester (SE; Buckinghamshire, UK). Free Cy3-SE was blocked with a probing buffer (0.5 M glycine in Tris-buffered saline containing 1% Triton X-100 (TBSTx)), the sample volume was adjusted to 200 μL, and aliquots (30 μL) of Cy3-labeled glycoprotein were applied to a lectin microarray LecChip (GlycoTechnica, Yokohama, Japan). Fluorescence signals were measured on a GlycoStation Reader 1200 (GlycoTechnica), and the data were processed with Array-Pro Analyzer software (version 4.5, Media Cybernetics, Silver Spring, MD). Note that it is necessary to adjust the concentrations of Cy3-labeled protein solution empirically to obtain appropriate signal intensities. The data were analyzed using a normalization procedure after a gain-merging process.[3]

Lectin affinity capturing

Streptavidin-coupled magnetic beads (10 μL, Dynabeads, Life Technologies, Carlsbad, CA) were incubated with 10 μL biotinylated *Aleuria aurantia lectin* (AAL) or *Agaricus bisporus agglutinin* (ABA) (100 ng/μL, J-oil Mills, Yokohama, JP) dissolved in PBS containing 1% Triton X-100 (PBSTx) at 4 °C for 30 min with shaking (×1400 rpm). As a control, the magnetic beads (10 μL) were incubated with 10 μL PBSTx at 4 °C for 30 min with shaking (×1400 rpm), which was referred to as "Input" for comparative analysis of the pre- and post-lectin-assisted fractionation during the following steps. The supernatants were then removed, and the beads were washed three times with 200 μL of PBSTx. After washing, the beads were incubated with 60 μL of the sample solution (30 μL Cy3-labeled glycoproteins and 30 μL probing buffer) overnight at 4 °C with shaking (×1400 rpm). The supernatants (*i.e.*, the pass fraction: lectin(–) and Input(–)) were collected, and then applied to a LecChip.

Statistical analysis

A Mann–Whitney U test was used to compare the lectin microarray data of the two CRC groups (good prognosis *vs.* poor prognosis; $p<0.05$).

References

1 A. Matsuda, A. Kuno, H. Ishida, T. Kawamoto, J.-i. Shoda and J. Hirabayashi, *Biochem. Biophys. Res. Commun.*, 2008, **370**, 259-263.

2 A. Kuno, A. Matsuda, Y. Ikehara, H. Narimatsu and J. Hirabayashi, in *Methods in Enzymology*, ed. F. Minoru, Academic Press, 2010, vol. 478, pp. 165-179.

3 A. Kuno, Y. Itakura, M. Toyoda, Y. Takahashi, M. Yamada, A. Umezawa and J. Hirabayashi, *J. Proteomics Bioinform*, 2008, **1**, 068-072.