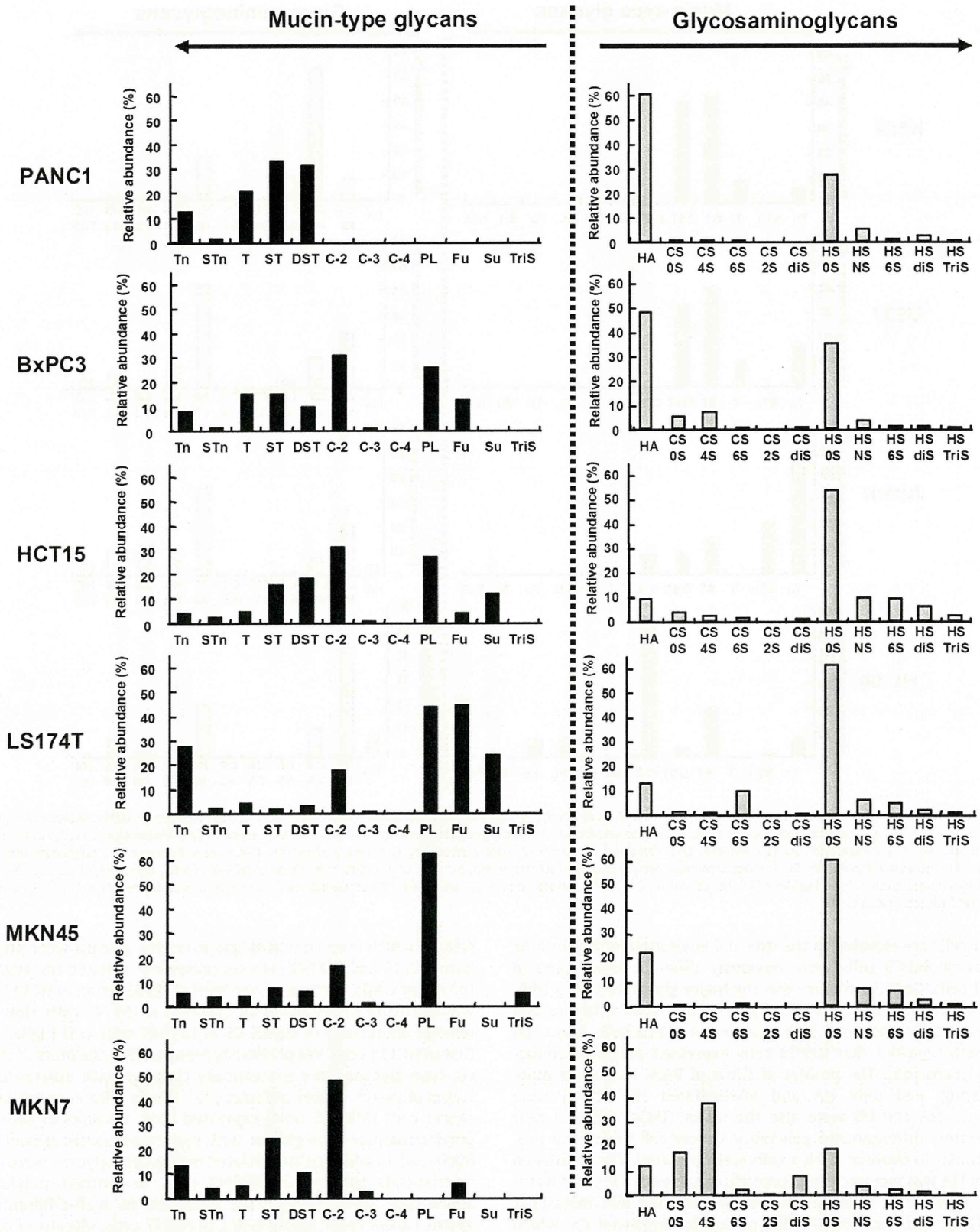

Fig.8. Characterization of six epithelial cancer cell lines. The contents of mucin-type glycans and GAGs are displayed as black bars and gray bars, respectively. Calculation of relative abundances and abbreviations are the same as in Fig. 7.

Although further studies are required, these results may indicate that GAGs play an important role in differentiation of gastric cancer cells.

As described above, it was revealed that our methods are useful to characterize the various cancer cell lines. In the future, we will apply these methods to compare *O*-glycan profiles between cancer

cells and normal cells and to reveal the tumor-specific alterations of *O*-glycan profiles.

Conclusion

We have developed an automatic system for releasing *O*-glycans from glycoproteins and applied the methods to the analysis of the released mucin-type glycans. In the current study, we developed methods for one-pot analysis of mucin-type glycans and GAGs. Serotonin affinity chromatography for group separation of mucin-type glycans based on the number of sialic acid residues [38] is also useful for collection of GAGs. As shown in Fig. 1, GAGs were strongly retained on the serotonin-immobilized column, and group separation of GAGs and mucin-type glycans was easily achieved. After collection of these glycans, mucin-type glycans were conveniently analyzed by MALDI–TOF MS and HPLC, and GAGs could be analyzed by CE as a mixture of unsaturated disaccharides after digestion with specific eliminases.

Two leukemia cancer cell lines (U937 and K562) showed similar profiles of mucin-type glycans and could not be discriminated only by comparing mucin-type glycans. However, GAG profiles showed obviously distinct characteristics (Fig. 7). In contrast, pancreatic cancer cell lines (PANC1 and BxPC3) showed similar GAG profiles but quite different mucin-type glycan profiles (Fig. 8). We also revealed that the profiles of GAGs as well as mucin-type glycans were dramatically altered at different differentiation stages of cancer cells, as determined by the analysis of MKN45 and MKN7 cells.

Based on these results, the current techniques will be useful to discover the novel biomarkers for diseases. However, the relationship between biological characteristics and *O*-glycan profiles observed in cancer cells might not be the same with that observed in actual physiological conditions. Therefore, to discover the practical glycan biomarkers for diagnosis of tumors, our method needs to be applied to the clinical samples such as serum or tissue samples. We are now applying the current methods to various kinds of biological samples. Furthermore, we are also developing methods for identification of proteins carrying specific glycans. These results will be shown in future publications.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ab.2011.12.017.

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