Characterization of an Antiproliferative Factor from Interstitial Cystitis Patients

Thomas P. Conrads[1], Zoltan Szekely[2], Joseph J. Barchi[3], Chen-Ou Zhang[4], Kristopher R. Koch[4], and Christopher J. Michejda[2], Timothy D. Veenstra[1], Susan K. Keay[4]

[1]Laboratory of Proteomics and Analytical Technologies, SAIC-Frederick, Inc., [2]Structural Biophysics Laboratory; [3]Laboratory of Medicinal Chemistry, National Cancer Institute, Frederick, MD, 21702; and [4]Division of Infectious Diseases, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, 21201

Interstitial cystitis (IC) is a debilitating chronic painful bladder disorder from which approximately one million Americans suffer; its etiology is unknown. Abnormalities seen in the bladder of IC patients include petechial hemorrhages, ulcers that extend into the lamina propria, and thinning of the bladder epithelium to 1-2 cell layers. A novel antiproliferative factor (APF) has been described in the urine of 94% of symptomatic patients with IC that was determined to be uniquely produced by the bladder epithelial cells of these patients (1). The purified APF factor can induces several changes in normal bladder epithelial cells, including significantly decreased rates of proliferation (2).

Microcapillary reversed-phase liquid chromatography (μRPLC) coupled online to an ion-trap mass spectrometer (MS) operated in a data-dependent tandem MS mode was used to analyze urine preparations from IC patients. Microcapillary RPLC-MS/MS analysis of a biological isolate from the urine of patients suffering from IC and *de novo* sequencing of the active fraction resulted in the identification of a small O-linked glycopeptide (N-acetylneuramic acid/Galactose/N-acetylgalacosamine-TVPAAVVVA) with potent bladder epithelial cell antiproliferative acitivity. The amino acid sequence of this peptide shares 100% identity with a segment in the sixth trans-membrane domain of frizzled 8 (amino acids 541-549), a receptor for Wnt proteins. Northern blot analysis demonstrates that APF is expressed solely in the bladder epithelium of IC patients with no expression evident in normal human bladder epithelial cells in vitro.

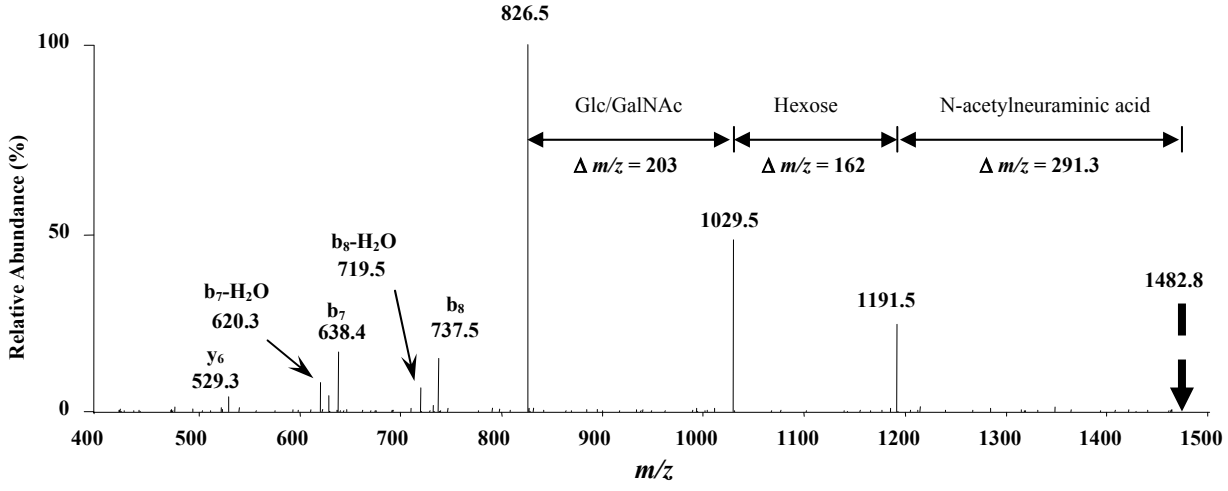

Figure 1. Tandem MS of the molecular ion at *m/z* = 1482.8. *De novo* sequence interpretation of this fragment ion spectrum indicates the presence of a small peptide glycosylated with N-acetylneuramic acid-hexose-N-acetyl(glucose or galactose)amine.

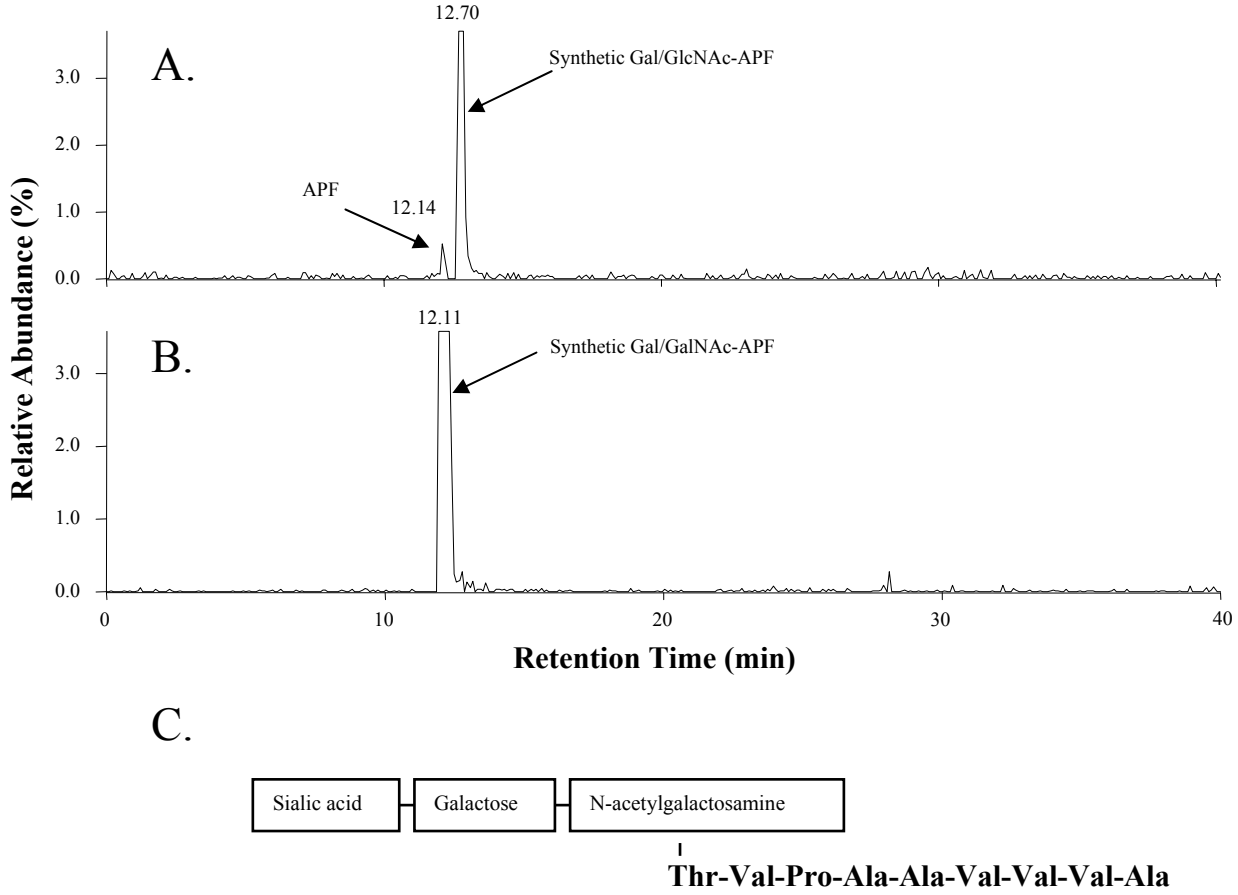

Figure 2. Total synthesis was conducted to generate both putative glycosylated forms of APF: Gal-GlcNAc-TVPAAVVVA (A.) and Gal-GalNAcTVPAAVVVA (B.). Each of these glycoforms was used to spike an "as isolated" APF preparation that was desialated. The two spiked APF samples were analyzed by μRPLC-MS/MS. The ability to resolve both glycoforms allowed the determination of native APF to be (Sialic acid)-Gal-GalNAc-TVPAAVVVA (C.).

Acknowledgements
This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12400. The content of this abstract does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

References
1. Keay SK, Zhang CO, Shoenfelt J, Erickson DR, Whitmore K, Warren JW, Marvel R, Chai T. (2001) *Urology* **57**, 9-14.
2. Keay S, Zhang CO, Shoenfelt JL, Chai TC. (2003) *Urology* **61,** 1278-1284.