

# N-glycosylation status of $\beta$ -haptoglobin in sera of patients with colon cancer, chronic inflammatory diseases and normal subjects

Seung-Yeol Park<sup>1,2,3</sup>, Seon-Joo Yoon<sup>2,3</sup>, Yeon-Tae Jeong<sup>1</sup>, Jin-Man Kim<sup>4</sup>, Ji-Yeon Kim<sup>5</sup>, Bradford Bernert<sup>6</sup>, Thomas Ullman<sup>6</sup>, Steven H. Itzkowitz<sup>6</sup>, Jung-Hoe Kim<sup>1</sup>, Sen-itiroh Hakomori<sup>2,3</sup>

<sup>1</sup>Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

<sup>2</sup>Division of Biomembrane Research, Pacific Northwest Research Institute and Departments of Pathobiology and Global Health, University of Washington, Seattle, WA

<sup>3</sup>Departments of Pathobiology and Global Health, University of Washington, Seattle, WA

<sup>4</sup>Department of Pathology and Cancer Research Institute, Chungnam National University College of Medicine, Daejeon, Republic of Korea

<sup>5</sup>Department of Surgery, Chungnam National University College of Medicine, Daejeon, Republic of Korea

<sup>6</sup>Division of Gastroenterology, Department of Medicine, Mount Sinai School of Medicine, One Gustave Levy Place, New York, NY

N-glycosylation status of purified  $\beta$ -haptoglobin from sera of 17 patients, and from sera of 14 healthy volunteer subjects, was compared by blotting with various lectins and antibodies. Patients in this study were diagnosed as having colon cancer through histological examination of each tumor tissue by biopsy. Blotting index of serum  $\beta$ -haptoglobin with *Aleuria aurantia* lectin (AAL) was clearly higher for cancer patients than for healthy subjects. No such distinction was observed for blotting with three other lectins and two monoclonal antibodies. To determine tumor-associated reactivity of AAL binding as compared to inflammatory processes in colonic tissues,  $\beta$ -haptoglobin separated from sera of 5 patients with Crohn's disease (CD), and 4 patients with ulcerative colitis (UC), was studied. All these cases, except one case of UC, showed AAL index lower than that in cancer cases, similarly to healthy subjects. The higher AAL binding of  $\beta$ -haptoglobin in colon cancer patients than in healthy subjects appeared to be due to  $\alpha$ -L-fucosyl residue, since it was eliminated by bovine kidney  $\alpha$ -fucosidase treatment. N-linked glycans of serum haptoglobin from colon cancer patients vs. healthy subjects were released by N-glycanase, fluorescence-labeled, and subjected to normal-phase high performance liquid chromatography (NP-HPLC). Glycan structures were determined based on glucose unit (GU) values and their changes upon sequential treatment with various exoglycosidases. Glycosyl sequences and their branching status of glycans from 14 cases of serum  $\beta$ -haptoglobin were characterized. The identified glycans were sialylated or nonsialylated, bi-antennary or tri-antennary structures, with or without terminal fucosylation.

Some tumor-associated carbohydrate antigens are released into the bloodstream, and their enhanced levels defined by specific monoclonal antibodies (mAbs) have been utilized for tumor diagnosis [for review see Refs. 1 and 2]. More recently, plant lectins have been utilized to detect altered glycosyl epitopes associated with haptoglobins, the serum "acute phase" glycoproteins whose glycosylation status changes under vari-

ous pathological and physiological conditions.<sup>3-5</sup> In these studies, lectins are often used rather than antibodies, since labeled lectins are widely available, less expensive than antibodies, and display consistent binding specificity and reactivity. The level of serum haptoglobin is enhanced in various types of cancer and inflammation, but its glycosylation status is different from one type of cancer to another. However, our

**Key words:** haptoglobin, colon cancer, lectin blotting, N-linked glycan, HPLC

**Abbreviations:** 2-AB: 2-aminobenzamide; AAL: *Aleuria aurantia* lectin; BSA: bovine serum albumin; CD: Crohn's disease; ELISA: enzyme linked immunosorbent assay; GU value: glucose unit value; HPLC: high performance liquid chromatography; HRP: horseradish peroxidase; IBD: inflammatory bowel disease; PBS: phosphate-buffered saline (10 mM phosphate buffer/2.7 mM potassium chloride/137 mM sodium chloride; pH 7.4); PHA-E: *Phaseolus vulgaris*-E lectin; PHA-L: *Phaseolus vulgaris*-L lectin; PNGase F: Peptide: N-glycosidase F; PVDF: polyvinylidene difluoride; RT-PCR: reverse transcriptase-polymerase chain reaction; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; SNA: *Sambucus nigra* lectin; TBS: Tris-buffered saline; UC: ulcerative colitis.

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**Correspondence to:** Sen-itiroh Hakomori, PNRI, 720 Broadway, Seattle, WA 98122-4302, USA. Fax: 1-206-726-1212. E-mail: hakomori@u.washington.edu or Jung-Hoe Kim, Department of Biological Sciences, Korea Advanced Institute of Science and Technology, 373-1, Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea. Fax: +82-42-350-5614. E-mail: kimjh@kaist.ac.kr