Proteomic analysis identifies S100A8 and S100A9 as serological markers for colorectal cancer

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Introduction
Colorectal cancer (CRC) is the third most common malignancy in the world, and represents the main cause for cancer deaths in Europe and the USA. In Korea, CRC occupies the fourth position in the mortalities caused by cancer, and its incidence still continues to increase. The risk of recurrence and subsequent death due to CRC is closely related to the stage of the disease at the time of primary diagnosis. Various serum markers for CRC are available among which carcinoembryonic antigen (CEA) is the most commonly used. However, this marker lacks sensitivity as well as specificity for screening an average risk population (1). Therefore, new cancer biomarkers are needed that will further enhance detection of the disease and trigger a follow-up colonoscopy.

Methods
We applied 2D-DIGE together with Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) tandem mass spectrometry to detect differentially expressed proteins in CRC tissues compared to matched normal tissues (n=6). Four proteins that were markedly over-expressed in cancer tissues were selected and confirmed by Semi-quantitative RT-PCR, Western blot and immunohistochemistry. In addition, a total of 10 plasma samples from CRC patients are included in the Western blot analysis. The top six abundant proteins (serum albumin, immunoglobulin G, immunoglobulin A, transferrin, haptoglobin, and antitrypsin) were depleted by MARS (Agilent technology) column prior to Western blot analysis.

Results
We identified 34 spots that were significantly up-regulated and 17 spots down-regulated with intensity changes greater than two-fold (Student’s t-test, p<0.05). Expression of both mRNA and protein in CRC for four proteins, adenosylhomocysteinase, Nm23-H1, S100A8 and S100A9, was further evaluated by semi-quantitative RT-PCR and Western blot. Immunohistochemistry analysis showed that adenosylhomocysteinase and Nm23-H1 were overexpressed in tumor cell cytoplasm and S100A8 and S100A9 proteins were strongly expressed in tumor infiltrating immune cells. Western blot analysis with fractionated plasma samples, we found that S100A8 and S100A9 were detected as significantly increased in plasmas of CRC patients (n=10) compared to healthy controls (n=10). The AUC curve was 0.79 for S100A8 and 0.83 for S100A9. Therefore, we suggest S100A8 and S100A9 as candidates for serological biomarkers that aid CRC diagnosis.

Innovative aspects
- We showed that the serum levels of S100A8 and S100A9 were significantly higher in CRC patients than in healthy controls, suggesting that both proteins or only S100A8 or S100A9 might represent a new serum marker for CRC.

References