Targeted Serum Metabolite Profiling for Colorectal Cancer Progression Monitoring

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Colorectal cancer (CRC) is one of the most prevalent cancers worldwide, and a major cause of human morbidity and mortality. A number of current efforts are focused on earlier detect of colon cancer using a variety of technologies including genomics, proteomics and metabolomics. Research focused on CRC disease status surveillance using metabolomics or other approaches has not been reported; Close monitoring of disease progression (DP) in CRC can be critical for patients' prognosis management and treatment decisions. In this study we investigate a targeted LC-MS/MS approach for serum metabolic profiling to monitor CRC patient disease progression, using a panel of significantly altered metabolites as potential biomarkers. The targeted platform allows the detection of 162 metabolites, representing more than 20 different classes (such as amino acids, carboxylic acids, pyridines, etc.) from 25 important metabolic pathways (e.g., TCA cycle, amino acid metabolism, purine and pyrimidine metabolism, glycolysis, etc.). In this study, 131 metabolites were reproducibly detected in the 49 samples, with an average coefficient of variation (CV) of 7.1%. Both univariate and multivariate statistical methods were used for metabolite biomarker selection. After applying the univariate Mann-Whitney U-test, 19 metabolites from different classes, such as monosaccharides, amino acids, carboxylic acids, and nucleosides, showed a significant statistical difference (p<0.05) between CRC DP and other CRC disease status [complete remission(CR) + stable disease (SD)]. Furthermore, highly significant changes (defined as p<0.01) between CRC DP and other CRC disease status

(CR+SD) were found for six metabolites, namely succinate, N2, N2-dimethylguanosine, adenine, citraconic acid methylmalonate, and 1-methylguanosine. We established the individual ROCs for each of these six metabolites for monitoring the CRC disease progression. Some of these metabolites had good AUROCs, such as 0.83 for succinate and 0.82 for N2, N2 dimethylguanosine, which have better performance than carcinoembryonic antigen (CEA) (or its sequential sample ratio) alone.

Furthermore, PLS-DA was utilized to identify the performance of multiple metabolite biomarkers in combination for monitoring CRC DP. Variable importance in projection (VIP) scores from the PLS-DA of all metabolites were calculated to evaluate those metabolites that contributed most to the differentiation of CRC DP from CR and SD. Interestingly, when the VIP threshold was set to 2, five out of the six metabolites (succinate, N2, N2-dimethylguanosine, adenine, citraconic acid and 1-methylguanosine) that had $p<0.01$ were again selected as important biomarkers for CRC DP monitoring.

A PLS-DA model using only these five core metabolite biomarkers was then applied to evaluate the performance of this approach for CRC DP monitoring, and the ROC curve generated for this metabolite model is shown in Figure 1A. The AUROC for this five metabolite model demonstrated excellent performance with an AUROC of 0.91, a sensitivity of 0.83 and a specificity of 0.94. To further test the robustness of this model, MCCV was applied with three different specificities. The true classification models clearly outperformed the random permutation models (Figure 1B), suggesting that the five core metabolite biomarker model is reliable for the CRC DP monitoring.

To the best of our knowledge, this is the first study using an LC-MS/MS targeted serum metabolic profiling approach for CRC disease progression surveillance. Our results suggest the potential usefulness of metabolic profiling for CRC disease monitoring.

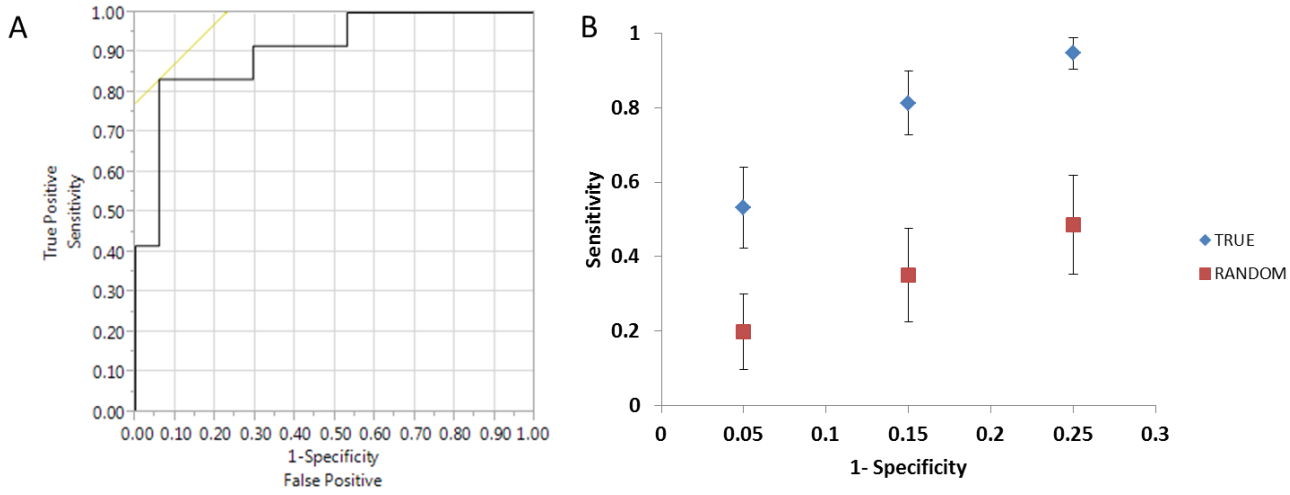

Figure 1. (A) ROC curve of PLS-DA model using five metabolites (with VIP>2) for DP vs. CR + SD: AUROC= 0.91; sensitivity= 0.83; specificity= 0.94. (B) Monte Carlo cross validation (MCCV) PLS-DA results from the same 5 metabolites: True, true class models; Random, random permutation model. The testing group specificities were 0.95, 0.85, and 0.75.