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Predicting the pathological response to neoadjuvant chemoradiation using untargeted metabolomics in locally advanced rectal cancer

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ABSTRACT

Purpose: The present study aimed to identify a panel of potential metabolite biomarkers to predict tumor response to neoadjuvant chemo-radiation therapy (NCRT) in locally advanced rectal cancer (LARC).
Experimental design: Liquid chromatography–mass spectrometry (LC–MS)-based untargeted metabolomics was used to profile human serum samples (n = 106) from LARC patients treated with NCRT. The samples were collected from Fudan University Shanghai Cancer Center (FUSCC) from July 2014 to January 2016. Statistical methods, such as partial least squares (PLS) and Wilcoxon rank-sum test, were used to identify discriminative metabolites between NCRT-sensitive and NCRT-resistant patients according to their tumor regression grade (TRG). This trial is registered with Clinical Trials.gov, number NCT03149978.
Results: A panel of metabolites was selected as potential predictive biomarkers of pathological response to NCRT. A total of 4810 metabolic peaks were detected, and 57 significantly dysregulated peaks were identified. These 57 metabolic peaks were used to differentiate patients using PLS in a dataset containing NCRT-sensitive (n = 56) and NCRT-resistant (n = 49) patients. The combination of 57 metabolic peaks had AUC values of 0.88, 0.81 and 0.84 in the prediction models using PLS, random forest, and support vector machine, respectively, suggesting that metabolomics has the potential ability to predict responses to NCRT. Furthermore, 15 metabolite biomarkers were identified and used to construct a logistic regression model and explore dysregulated metabolic pathways using untargeted metabolic profiling and data mining approaches.
Conclusions: A panel of metabolites has been identified to facilitate the prediction of tumor response to NCRT in LARC, which is promising for the generation of personalized treatment strategies for LARC patients.

For locally advanced rectal cancer (LARC) patients, preoperative neoadjuvant chemo-radiation therapy (NCRT) followed by total mesorectal excision (TME) is the standard treatment [1–3]. Previous studies have shown that NCRT has better local control and lower toxicity than adjuvant chemoradiotherapy [4–6]. In clinical practice, however, pathological responses to NCRT demonstrated obvious heterogeneity. Approximately 10–30% of patients show pathologic complete response, 40–45% show variant tumor regression, and the remaining 20–30% have no response to NCRT [7–9]. Therefore, more cost-effective, predictive biomarkers of NCRT for LARC patients are needed to maximize patient benefits and minimize adverse effects.

To date, the pathological tumor response to NCRT has been evaluated using imaging of tumor morphology. Monguzzi L et al. reported that the mean value of the apparent diffusion coefficient from MRI could be used to predict the pathological response to NCRT [10], which was confirmed by Genovesi D et al. in a single-site prospective study [11]. Similarly, Zhang et al. reported the use of the standardized uptake value from positron emission tomography (PET) scans to assess the pathological response of LARC patients to NCRT [12]. However, the changes in tumor morphology typically occur later than the changes at the biological

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and molecular levels. Therefore, image diagnosis is not an ideal method to evaluate the NCRT sensitivity at early stages and cannot accurately predict therapeutic responses prior to NCRT.

With recent advancements in molecular and systems biology, molecular biomarkers have been discovered to predict the response of rectal cancer patients to NCRT. Huh et al. reported that high levels of CD44 mRNA in pretreatment biopsies are associated with a poor tumor regression [13]. Yan et al. reported that patients with high Smac levels, low Ki-67 expression and negative vascular endothelial growth factor (VEGF) expression are more sensitive to NCRT [14]. Kim et al. reported that the candidate markers CORO2A rs1985859 and the putative marker FAM101A rs7955740 are valuable to predict sensitivity to NCRT [15]. These three studies focused on investigating the association between the specific molecular markers or single-nucleotide polymorphisms (SNPs) of genes and the pathological response to NCRT. However, the generalization of these studies is limited by a wide confidence interval of the prediction, a retrospective study design or a small sample size. In addition, these results explored only the association rather than the predictive capability of these biomarkers toward the therapeutic response of NCRT in LARC patients.

Metabolomics provides the global and quantitative measurement of endogenous small molecular metabolites within a biological system [16–18] and is well acknowledged in cancer research [19–21]. Metabolomics simultaneously measures thousands of metabolites, and describes a holistic and dynamic profile of disease progression [22]. Currently, metabolomics has been successfully utilized in biomarker discovery for the early diagnosis of cancer, targeted therapy and response prediction [23–27]. Studies confirmed the feasibility of metabolic markers in early diagnosis of colorectal cancer. Wei J et al. analyzed the serum samples of colorectal cancer patients and non-cancer subjects. Oleamine, pyruvic acid, three carboxylic acid and ornithine cycle related metabolites, which are closely associated with the occurrence of colorectal cancer, were identified as differentially expressed markers between colorectal cancer patients and non-intestine cancer patients [28]. In their subsequent study with a larger sample size, urine samples were analyzed to make early diagnosis of colorectal cancer and differentially expressed metabolic markers, including cresol and aminobutyric acid, etc. were screened out. The area under the receiver operating characteristic (ROC) curve was 0.998 in the test sample, while the diagnostic sensitivity and specificity of serum CEA in colorectal cancer is only about 50–70% [29]. Uchiyama et al. successfully distinguished patients between colorectal cancer and intestinal adenoma by the screened serum metabolic markers [30]. However, to the best of our knowledge, no studies have reported the use of metabolite biomarkers for predicting response to NCRT at a personalized level.

In the present study, we designed a prospective cohort study of locally advanced rectal cancer to identify potential metabolite biomarkers for the prediction of tumor response to NCRT. The cohort recruitment began in July of 2014, with a target enrollment of 300 patients. For each patient, serum and urine samples before and during preoperative chemo-radiation therapy were obtained for metabolomics. Here, we report the results from the first 106 patients who were prospectively recruited in the study.

Materials and methods

Eligibility criteria

Between July 2014 and January 2016, a total of 106 patients with clinical T3-4 and/or N+ rectal cancer without distant metastasis were enrolled at Fudan University Shanghai Cancer Center (FUSCC). The following inclusion criteria were considered: (1) patients scheduled to receive CRT followed by TME surgery; (2) without metabolic diseases, such as diabetes mellitus or hyperthyroidism; and (3) informed consent was signed and obtained before the treatment. The CRT included intensity-modulated radiation therapy (IMRT) of 50 Gy in 25 fractions concurrently with capecitabine-based chemotherapy. Two weeks after the completion of CRT, one additional cycle of chemotherapy was administered according to the guidelines of the center. Surgery was scheduled at 8 weeks after the completion of CRT. TME was mandatory, whereas the form of surgery (anterior resection or abdominal-perineal resection) and whether a temporary colostomy should be performed were decided by the surgeon. The study was approved by the institutional review board of FUSCC.

Pathological evaluation of tumor response

Pathological tumor response was evaluated according to the 2010 American Joint Committee on Cancer (AJCC) tumor regression grade (TRG) system, which recorded the degree and the volume of residual primary tumor cells. Details of AJCC TRG system are defined as follows: Grade 0, defined as no viable cancer cells; Grade 1, characterized by single or small groups of tumor cells; Grade 2, involves residual cancer outgrown by fibrosis, but fibrosis still predominates; and Grade 3, defined as the minimal or no tumor cells killed. The NCRT-sensitive patients were defined as those with TRG Grades 0–1, while the NCRT-resistant patients were defined as those with TRG Grades 2–3.

Collection of serum samples

According to a published protocol [31], biological samples of the enrolled patients were collected in five consecutive time-points: baseline (within two weeks before beginning of CRT); early-phase CRT (5 fractions after beginning); middle-phase CRT (15 fractions after beginning); late-phase CRT (25 fractions after beginning); and surgery (within 2 days before surgery). All participants were in an overnight fasting state, and 5 mL of peripheral venous blood was drawn in the morning. The blood was allowed to clot for 30 min, followed by centrifugation at 3000 rpm for 15 min. Then the serum supernatant was collected, separated into 5 aliquots (200 μL of each aliquot) and immediately frozen in liquid nitrogen. The serum samples were then stored at −80 °C until further analyses. However, only baseline serum samples were analyzed in the current study.

Reagents and materials

LC–MS-grade water (H_2O), acetonitrile (ACN), methanol (MeOH), 0.1% formic acid (FA) in water and 0.1% FA in ACN were purchased from Honeywell (Muskegon, MI, USA). Ammonium fluoride (NH_4F) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in LC–MS-grade water prior to use.

Serum sample preparation

The details of serum sample preparation, LC–MS analysis, data processing and preparation have been described in a previous publication [31] and are provided in the Supporting Information.

LC–MS analysis

All serum samples were randomly injected during data acquisition. During data acquisition, blank samples (75% methanol in water) and QC samples (prepared by pooling aliquots of all subject samples) were injected every 8 samples, and the test mixture

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