DOCTORAL THESIS

Mass spectrometry-based metabolomics study on KRAS-mutant colorectal cancer and rheumatoid arthritis

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Abstract

Ample studies have shown that perturbation of metabolic phenotype is correlated with gene mutation and pathogenesis of colorectal cancer (CRC) and rheumatoid arthritis (RA). Mass spectrometry (MS)-based metabolomics as a powerful and stable approach is widely applied to bridge the gap from genotype/metabolites to phenotype.

In CRC suffers, *KRAS* mutation accounts for 35%-45%. In previous study, SLC25A22 that encodes the mitochondrial glutamate transporter was found to be overexpressed in CRC tumor and thus to be essential for the proliferation of CRC cells harboring *KRAS* mutations. However, the role of SLC25A22 on metabolic regulation in *KRAS*-mutant CRC cells has not been comprehensively characterized. We performed non-targeted metabolomics, targeted metabolomics and isotope kinetic analysis of *KRAS*-mutant DLD1 cells with or without SLC25A22 knockdown using ultra-high performance liquid chromatography (UHPLC) coupled to Orbitrap MS and tandem MS (MS/MS). In global metabolomics analysis, 35 differentially regulated metabolites were identified, which were primarily involved in alanine, aspartate and glutamate metabolism, urea cycle and polyamine metabolism. Then targeted metabolomics analysis on intracellular metabolites, including tricarboxylic acid (TCA) cycle intermediates, amino acids and polyamines, was established by using LC-MS/MS coupled with an Amide BEH column. Targeted metabolomics analysis revealed that most TCA cycle intermediates, aspartate (Asp)-derived asparagine, alanine and ornithine (Orn)-derived polyamines were strongly down-regulated in

SLC25A22 knockdown cells. Moreover, the targeted kinetic isotope analysis using [U-$^{13}C_5$]-glutamine as isotope tracer showed that most of the ^{13}C-labeled TCA cycle intermediates were down-regulated in SLC25A22-silencing cells. Orn-derived polyamines were significantly decreased in SLC25A22 knockdown cells and culture medium. Meanwhile, accumulation of Asp in knockdown of GOT1 cells indicated that oxaloacetate (OAA) was majorly converted from Asp through GOT1. Exogenous addition of polyamines could significantly promote cell proliferation in DLD1 cells, highlighting their potential role as oncogenic metabolites that function downstream of SLC25A22-mediated glutamine metabolism. SLC25A22 acts as an essential metabolic regulator during CRC progression as promotes the synthesis of TCA cycle intermediates, Asp-derived amino acids and polyamines in *KRAS*-mutant CRC cells. Moreover, OAA and polyamine could promote KRAS-mutant CRC cell growth and survival.

Rheumatoid arthritis (RA) is a chronic, inflammatory and symmetric autoimmune disease and a major cause of disability. However, there is insufficient pathological evidence in term of metabolic signatures of rheumatoid arthritis, especially the metabolic perturbation associated with gut microbiota (GM). Based on consistent criteria without special diet and therapeutic intervention to GM, we enrolled 50 RA patients and 50 healthy controls. On basis of the platform of UHPLC-MS and GC-MS, were performed for the non-targeted metabolomics to investigate alterations of endogenous metabolites in response to RA inflammation and

interaction with GM. 32 and 34 significantly changed metabolites were identified in urine and serum of patients with RA, respectively. The altered metabolites were identified by HMDB, METLIN database or authentic standards, and mostly metabolites were attributed into tryptophan and phenylalanine metabolism, valine, leucine and isoleucine biosynthesis, aminoacyl-tRNA biosynthesis and citrate cycle. To obtain alterations of more components in tryptophan and phenylalanine metabolism, we developed and validated a targeted metabolomics method of 19 metabolites by using LC-QqQ MS. Combining the results of targeted metabolomics with global metabolomics, significantly up-regulated kynurenine (KYN), anthranilic acid (AA) and 5-hydroxylindoleacetic acid (HIAA) simultaneously in urine and serum was found to implicate the activation of tryptophan metabolism under the condition of RA, which acted pro-inflammatory roles in inflammation and was closely correlated with GM. IDO/TDO functioned as a pro-inflammation mediator was overexpressed in RA patients. Urinary kynurenic acid and serum serotonin that have impacts on anti-inflammation in immune system were down-regulated in RA patients. The levels of phenylacetic acid and phenyllactic acid serving as a pro-inflammatory and an anti-inflammatory agent, respectively, increased in serum of patients with RA. Moreover, certain essential amino acids (EAAs), and mostly conditional EAAs were decreased in RA patients, which have been reported to inhibit cell proliferation of immune cells. In particular, deficiency of branched chain amino acids (BCAAs, valine and isoleucine) was observed in serum of patients with RA, which may lead to muscle

loss and cartilage damage. The specificity of all altered metabolites resulted from RA was considerably contributed through the GM-derived metabolites. The findings revealed that GM-modulated RA inflammation was mainly resulted from tryptophan and phenylalanine metabolism, and amino acid biosynthesis, which may provide more information for better understanding the RA mechanism.

Key words: Metabolomics, mass spectrometry, colorectal cancer cell, rheumatoid arthritis, gut microbiome

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