UNIVERSITI TEKNOLOGI MARA

METABOLOMICS PROFILING OF SERUM SAMPLES OF MALAYSIAN PATIENTS WITH COLORECTAL CANCER

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PhD

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Colorectal cancer (CRC) is the second most common cancer in the world and in Malaysia. The gold standard for diagnosis is the use of colonoscopy and sigmoidoscopy. The invasive nature of diagnostic tools and asymptomatic nature of the disease may contribute to most Malaysian patients being diagnosed at late stage with poor prognosis. Thus, there is a need for non-invasive biomarkers for CRC. Alterations in metabolite profile reflect changes in metabolism and therefore metabolomics has been used to identify the differential metabolites between CRC and healthy individuals. The aim of the study is to identify potential biomarkers and affected pathways by performing serum untargeted metabolic profiling of CRC using LCMS-QTOF. Serum samples from 50 CRC and 50 age matched healthy individuals were used. The raw data generated were analysed using software Mass profiler professional (MPP). Partial least squares discriminant analysis (PLS-DA) models generated based on profiled data were capable of discriminating most of the normal sera from colorectal sera. The pathways analysis was done using Metaboanalyst. The biomarkers were then validated using a new set of sera from 20 CRC and 20 normal subjects. The involvement of purine metabolism and fatty acid oxidation pathways as altered pathways in CRC were validated using normal and colon cancer cell lines of different stages. Hypoxanthine/xanthine, uric acid and HPRT1 levels and xanthine oxidase activity were determined. The levels of acetylcarnitine and the rate of fatty acid oxidation were determined with and without the presence of etomoxir, an inhibitor of CPT1. *In silico* data analysis was done using public data GSE21510 and GSE32323 from Gene Expression Omnibus database (GEO) series, web-based GEO2R with a p-value smaller than 0.01, an absolute log fold-change greater than 1 as filters. The results showed 11 metabolites were differentially expressed between CRC patients and controls ($p < 0.05$). The differential metabolites were hypoxanthine, tyrosine, acetyl carnitine, xanthine, uric acid, methionine, pipecolic acid, 5-oxoproline, lysophosphatidylcholine, lysophosphatidylethanolamine and citric acid. The use of these 11 metabolites was able to discriminate CRC and normal samples in the new samples set with 80% accuracy using random forest algorithm. These metabolites were then linked to metabolic pathways using KEGG database, and the data showed that the major pathways affected were purine metabolism, fatty acid oxidation, citric cycle, phospholipid catabolism, glutathione metabolism and lysine degradation. *In-silico* data mining showed alterations in gene expression in purine metabolism, fatty acid oxidation, methionine metabolism, glutathione metabolism, histone methylation and histone acetylation. Xanthine oxidase activity were low in CRC compared to normal cells (0.02 mU versus 0.09 mU respectively). The levels of hypoxanthine/xanthine and HPRT were higher in cancer than normal cells while uric acid levels were low in CRC cells. For fatty acid oxidation, the levels of acetylcarnitine were higher in CRC compared to normal cells. The levels of acetylcarnitine were reduced by 40% when cells were inhibited with etomoxir. Oxygen consumption rates showed that fatty acid oxidation was not significantly affected in CRC. In conclusion, the 11 metabolites have the potential to be used as biomarkers to detect CRC in serum of patients with high accuracy. Purine metabolism was among several pathways that were affected in CRC patients.

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