UNIVERSITI TEKNOLOGI MARA

CHARACTERISATION OF METABOLIC CHANGES IN COLORECTAL CANCER CELLS OF DIFFERENT STAGES

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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

Accurate diagnosis and staging of colorectal cancer (CRC) lead to better prognosis. Identification of molecular biomarkers may prove useful, but there is a lack of data on the molecular pathophysiology of CRC progression. Characterisation of metabolic changes in CRC cells may provide useful tools for study of potential therapeutic drug actions. This study was aimed to characterise the metabolites profiles in CRC cells of different stages and the metabolic pathways affected. In this study, normal colon cell lines; CCD 840 CoN and CRC cells lines of different stages; SW 1116 (stage A), HT 29 and SW 480 (stage B), HCT 15 and DLD-1 (stage C), and HCT 116 (stage D) were used. The global metabolomics profiling was performed using liquid chromatography-mass spectrometry (LC/MS). Mass Profiler Professional and Metaboanalyst software were used for statistical and pathway analysis. METLIN database was used for identification of metabolites. MS/MS was used for validation of detected metabolites. Validation of the affected metabolic pathways in CRC was performed in which expression of SLC6A14 (amino acid transporter), riboflavin kinase and FAD synthetase (enzymes in riboflavin metabolism pathway) were determined using Western blot. L-methionine and FAD levels, cells viability and rate of apoptosis activity were determined with and without the presence of alpha-methyltryptophan, and lumichrome which are inhibitors of SLC6A14, and riboflavin uptake, respectively. The results showed that there were differential metabolites identified in CRC cells of different stages, which could be used as potential metabolite biomarkers for staging. (R)-1-O-[b-D-glucopyranosyl-(1->6)-b-D-glucopyranoside]-1,3-octanediol, lauroyl diethanolamide, lysoPE(0:0/18:1(11Z)), lysoPE(0:0/22:5(4Z,7Z,10Z,13Z,16Z)), phosphocholine, S-(formylmethyl)glutathione, and N-acetyl-DL-methionine were the most important differential metabolites identified in stage A. In stage B, 1,2,4-nonadecanetriol, dodecanoylcarnitine, hericene B, l-hexanoylcarnitine, pyroglutamic acid and S-furanopetasitin were the differential metabolites while in stage C, the differential metabolites were bis-γ-glutamylcystine, hippuric acid, lumichrome, lysoPE(16:1(9Z)/0:0), PE(22:5(7Z,10Z,13Z,16Z,19Z)/15:0), and purine. FAD, methyhiomethyl butyrate, muzanzagenim, 3b-hydroxy-5-cholenoic acid, ATP, L-glutamate and lysoPE(0:0/20:0) were the differential metabolites identified in CRC cells stage D. The results showed that L-methionine and riboflavin metabolisms were the most prominent pathways affected in CRC cells as the cancer advances. The results also showed that increased level of methionine observed in metabolomics study is probably due to increased expression of SLC6A14. However, expressions of riboflavin kinase and FAD synthetase in CRC were similar to normal cells and hence could not account for the increased level of FMN and FAD as observed in the metabolomics study. The results also showed that metabolites profiles identified in intracellular and secreted CRC cells were different. In conclusion, this study identified several novel differential metabolites which can be used as potential biomarkers for staging. In this study, for the first time, profiles of the intracellular as well as the secreted metabolites were determined which provides a more complete picture of the metabolic derangement that occurs in the development and progression of CRC. The affected metabolic pathways identified in this study may lead to further understanding of the pathophysiology of CRC and hence, its application for treatment strategies.

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