UNIVERSITI TEKNOLOGI MARA

MODULATION OF PURINE METABOLISM AND APOPTOTIC PATHWAY VIA ENT2 CRISPR/CAS9 KNOCKOUT IN COLORECTAL CANCER CELL LINES

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ABSTRACT

Colorectal cancer (CRC) is one of the most prevalent malignant cancers worldwide. Altered metabolic pathways are considered as a distinct property of cancer and in CRC the purine metabolism pathway has been shown to be one of the most affected pathway. Equilibrative nucleoside transporter 2 (ENT2) is a bidirectional transporter that mediates the uptake of purine and pyrimidine nucleosides and nucleobases, particularly; hypoxanthine. Although the purine metabolism pathway is the most impacted in CRC, not much is known on ENT2 role in CRC development and its association with altered purine metabolism pathway. Therefore, this study is aimed to determine the role of ENT2 in altered purine metabolism in the early and late stages of CRC by employing the CRISPR/Cas9 gene-editing tool to generate CRC cell lines with ENT2 knockout. The mRNA expression of ENT2 was determined by qRT-PCR in a panel of CRC cell lines (SW1116, SW480, HT29, DLD1, HCT15 and HCT116), representing the four stages of CRC and compared with the normal colon cell line; CCD-841CoN. *ENT2* expression was significantly higher ($p<0.05$) in all CRC stages cell lines. Two CRC cell lines (HT29 representing early stages and DLD1 representing late stages) with the high expression of *ENT2* were subjected to CRISPR/Cas9 knockout. Single cell-derived clones were screened using western blot and confirmed with Sanger sequencing. Two clones of the HT29 were expressed both partial and complete ENT2 knockout, whereas one clone of the DLD1 expressed almost complete ENT2 knockout. The ENT2 knockout (ENT2/KO) cells were tested on multiple functional assays, including cell proliferation, apoptosis pathway, substrates and enzyme levels related to the purine catabolism pathway and determination of ROS level. The results of this study indicated that CRC cell proliferation was lowered in both HT29/KO and DLD1/KO clones with a significant decrease in cell viability and colony formation in HT29/KO clones compared to the non-targeting control (NTC); $p=0.011$ and $p=0.001$, respectively. For the apoptosis pathway, mitochondrial membrane potential was significantly lower in HT29/KO clones than the NTC ($p=0.001$) however, there was no change in DLD1/KO clone compared to the NTC. Moreover, *P53*, *BAK* genes were upregulated in HT29/KO and DLD1/KO clones with significant upregulation of *Caspase-3* and *Caspase-9* while *Bcl-2* was downregulated in HT29/KO compared to the NTC, $p=0.001$. Hypoxanthine level and xanthine oxidase activity were significantly higher in HT29/KO; $p=0.001$, $p=0.022$ and DLD1/KO; $p=0.01$ and $p=0.026$, respectively, as compared to their respective NTC. The generation of ROS was higher in HT29/KO and DLD1/KO clones, with a significant increase in HT29/KO clones, $p=0.001$. Collectively, our findings demonstrated that ENT2/KO induced apoptosis as a consequence of ROS induction due to the increment of hypoxanthine and xanthine oxidase levels in both early and late stages of CRC with more effectiveness in the early stages. In this regard, targeting the ENT2 gene might be a novel CRC treatment strategy by increasing the ROS production via promoting the rate of purine catabolism pathway and key to predicting the possible success of improving the sensitivity to chemotherapies drugs to treat the CRC patients in personalized medicine.

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