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**Development of HCT116-Folfox Resistant Colon Cancer Cells and Characterisation of Exosomes derived from HCT116-Folfox Resistant Cells**

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**ABSTRACT**

Exosomes are extracellular nanovesicles released by almost all living cells, including colorectal cancer (CRC) cells, into the biological fluids. Exosomes are natural carriers of proteins and nucleic acids that reflects the parent cells. Therefore, exosomes have been investigated extensively as potential biomarkers and therapeutic targets for various diseases. However, the potential of exosomes as biomarkers to detect Folfox-resistant CRC has not been fully explored and validated. This research aims to characterise exosomes derived from Folfox-resistant HCT116 CRC cell line as potential targets for further development of biomarkers among CRC patients with chemo-resistance. HCT116 cell line was selected to develop drug resistance due to its initially high sensitivity towards drug treatment. The normal CRC cell line HCT116 (HCT116-C) was induced with 5 cycles of drug treatment (48 hours in culture medium containing 25µM of 5-fluorouracil (5-FU) and 0.625µM of oxaliplatin followed by 12 days in drug-free culture medium) to develop the corresponding Folfox-resistant derivative clones (HCT116-R). HCT116-C and HCT116-R cell lines were compared for cell viability in 5-FU and oxaliplatin as well as the rate of migration in wound healing assay. Exosomes derived from the HCT116-R cell line were characterised by transmission electron microscopy, and subjected to particle size measurement and zeta potential analysis. As compared to the normal HCT116-C cell line, HCT116-R cell line had higher IC50 values with 2.5 to 6-fold change when treated with 5-FU and oxaliplatin. The migration rate of HCT116-R cell line was about 19 percent higher than that of HCT116-C cell line in wound healing assay. HCT116-R exosomes are morphologically cup-shaped nanovesicles that range from 100 to 170nm in size. In conclusion, we have successfully developed a stable HCT116-Folfox resistant colon cancer cells, and the characteristics of exosomes derived from this cell line are in concordance with the literature.