The molecular mechanisms of cholesteryl ester transfer protein (*CETP*) as a drugresistance in breast cancer (BC)

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Introduction: Cancer cells exhibit increased levels of intracellular cholesterol that has been implicated in cancer aggressiveness and resistance [1]. *CETP* maintains cellularhomeostasis and it has been previously reported to be a cell survival gene [2]. In this study,we aimed to knock down *CETP* in BC cells to reduce resistance towards current therapies, and to investigate the molecular pathways involved.

Methodology: *CETP* expression in MCF-7 and MDA-MB 231 breast cancer cells were determined using Western blotting, RT-qPCR and immunofluorescent staining. Intracellular cholesterol levels were measured using cholesterol assay and various staining techniques. Cellular proliferation and apoptosis were measured using MTT and Phosphatidylserine exposure assay post Tamoxifen (TAM) and acetyl-plumbagin treatments, pre and post *CETP* knock-down. Thereafter, RT² Profiler™ PCR arrays were used to determine the gene expression profiles of human lipoprotein signalling and cholesterol metabolism pathway andthe human cancer resistance pathways that were affected post *CETP* knock-down. Kaplan- Meier plots were also generated and survival outcomes were compared between high and low *CETP* expression in different types of cancers. A mice xenograft study was performed to measure tumour growth rate between untreated and *CETP* knocked-down MDA-MB 231cells.

Results: The preliminary results showed that knocking-down *CETP* resulted in an increase in apoptosis in MCF-7 cells when treated with TAM (by 10-40%). Furthermore, *CETP* knock-down with the addition of a cholesterol-depleting agent increased apoptosis by 10 fold, possibly due to a decrease in cholesteryl ester (CE) content. Similar results were observed in MDA-MB-231 cells. In MCF-7 cells, *CETP* knock-down decreased cancer resistance through a decrease in cholesterol synthesis genes and in MDA-MB 231 cells; cholesterol efflux increased with increased estrogen receptors thus ppossibly sensitised these cells to targeted hormone therapeutic drugs. Lastly, high levels of *CETP* in estrogen positive BC cells reduces survival rate up to 100-150 days by 40% and 50% in triple negative BC cells. The mice xenograft study showed that there was an 86.45% reduction in tumour growth when *CETP* was knocked down compared to the non-transfected cell.

Conclusion: *CETP* could thus serve as a potential drug-resistance marker in cancer cells,more specifically BC. Further validation in BC patient samples is required in future.

References: [1] Li YC, Park MJ, Ye S-K, Kim C-W, Kim Y-N: Elevated levels of cholesterol-rich lipid rafts in cancer cells are correlated with apoptosis sensitivity induced by cholesterol-depleting agents. The American journal of pathology 2006, 168(4):1107-1118. [2] Esau, L.,Sagar, S., Bangarusamy, D., and Kaur, M. (2016). Identification of *CETP* as a molecular target for estrogen positive breast cancer cell death by cholesterol depleting agents. Genes& cancer 7, 309-322.

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