Investigating the effect of cholesterol depletion on the epithelial to mesenchymaltransition of breast cancer cells

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BACKGROUND & HYPOTHESIS: Breast cancer is the most prevalent cancer among women globally. Metastasis, the spread of cancer cells from the primary tumour to a secondary site, accounts for approximately 90% of cancer-related mortality [1]. The epithelial to mesenchymal transition (EMT) is a critical cellular program underlying the metastatic spread of cancer cells. The role of cholesterol in EMT and metastasis has not yetbeen established; however, there is a link between cholesterol and cancer cell growth and proliferation. This study serves to determine the role of cholesterol in the induction of EMT and metastasis, and delineate molecular pathways affected during EMT and cholesterol depletion.

METHODS: An *in vitro* model of EMT was established using the NMuMG cell line treated with TGFβ. The induction of EMT was confirmed by immunocytochemistry and qPCR. Cholesterol levels were measured using a cholesterol quantitation assay andimmunocytochemical lipid stains using cholesterol depleting agents MβCD, KS-01 and simvastatin. Changes in cell viability after EMT induction and treatment with cholesterol depleting agents were measured using viability assays and immunocytochemistry. Changes in gene expression in EMT and cholesterol pathways were observed using immunocytochemistry and qPCR.

RESULTS: The induction of EMT in NMuMG cells resulted in a change from epithelial to mesenchymal morphology. Expression of E-cadherin was significantly reduced and expression of Vimentin was significantly increased after EMT induction. After induction of EMT, the cell proliferation marker Ki67 was reduced and viabilityassays show a decrease in cell proliferation as cells transition from a proliferative to an invasive state. Cellular cholesterol content decreased significantly after EMT induction and after treatment with cholesterol depleting agents. Cholesterol pathway alterations after EMT induction suggest a significant role of cholesterol in EMT. Immunocytochemistry and qPCRshow a change in EMT-related pathways after cholesterol depletion.

CONCLUSION: Cholesterol plays a complex role in the induction of EMT, which differs based on cancer type. Cholesterol depletion significantly changes the expression of EMT-related genes and cholesterol pathways are altered upon EMT induction. Cholesterol depletion may serve as a therapeutic target for metastatic cancer in combination with current treatment strategies.

REFERENCES: [1] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis.science. 2011 Mar 25;331(6024):1559-64.

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