Cellular Cholesterol Lowering Action of Metformin Leads to Prevent Proliferation and Epithelial to Mesenchymal Transition in Breast Cancer Cells

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Earlier literature suggetsed that metformin, a well-known anti-diabetic drug, showed anticancer activity in various cancer types. Few clinical studies showed the low serum cholesterol and low TAG level in metformin treated patients. Earlier literature also indicated an existence of positive association between high cholesterol and cancer. However, the mechanism of metformin in relation with cellular cholesterol has not yet been studied. This study aimed to find out a molecular mechanism involved in metformin-inhibited cell growth and metastasis in breast cancer cells. Tumor sample based clinical study found the higher expressions of cellular cholesterol regulatory genes (e.g. HMGCoR, LDLR) in malignant breast cancer tissues as compared to benign tissues. Our cell culture study found that treatment of breast cancer MDA-MB-231 cells with metformin decreased cellular cholesterol level with concomitant inhibition of various genes (e.g. SREBF1 and LDLR) which maintain the homeostasis of cellular cholesterol. Cell cultue based experimental study documented that Metformin inhibited cell proliferation, migration, colony and spheroid formation of metatstatic breast cancer MDA-MB-231 cells. As a mechanism it was identified by RT-PCR that metformin treatment inhibited anti-apoptotic markers (Bcl2, BCLxl) and mesenchymal marker genes (Vimentin, N-cadherin) transcript levels with simultaneous enhancement of apoptotic markers (Caspase3, Bax) and epithelial marker genes E-cadherin and Keratin19, indicated an inhibitory effect of Metformin in proliferation and EMT of breast cancer cells. Less number of colony and spheroid formation has been observed in Metformin treated breast cancer cells. RT-PCR analysis also found that Metformin treatment inhibited stemness marker CD44 and BMI-1 in metastatic breast cancer MDA-MB-231 cells. Moreover, Metformin-inhibited cancer cell proliferation and migration was reversed by the exogenous treatment of cholesterol. Similarly, cholesterol treatment reversed the metformin-inhibited Bcl2, Vimentin, BMI-1 expression. Moreover, zymography data documented that cholesterol treatment upregulated metformin-inihibited MMP activity. These findings unravel a significant contribution of cellular cholesterol on EMT and stemness of breast cancer cells since cholesterol depleting MBCD inhibited cancer cell proloferation and EMT. Moreover, high level of cholesterol and cholesterol regulatory gene expressions was noted in cancer tissues. This study suggests a new molecular mechanism where metformin inhibits proliferation, EMT and stemness of breast cancer cells presumably by lowering cellular cholesterol level.

Keywords: Breast Cancer, Epithelial to mesenchymal transition, Stemness, Metformin, Cholesterol