

Cholesterol promotes prostate cancer progression and metastasis

Gina C.Y. Chu, Ruoxiang Wang, Haiyen E. Zhau, Michael Freeman, Stephen Freedland, Edwin Posadas, and Leland W. K. Chung

Uro-Oncology Research Program, Departments of Medicine and Surgery, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA. 90048

Background: Cholesterol is an essential lipid component required for the assembly and maintenance of cell membranes and functions in cell adhesion, signal transduction, and cellular homeostasis. High levels of systemic cholesterol are associated with prostate cancer (PC) development and progression and were identified by metabolomics in PC bone metastases. Recently hypercholesterolemia was also suggested to be associated with the development of castration-resistant PC (CRPC) after androgen deprivation therapy (ADT) in patients with bone metastasis.

Methods: Indolent and aggressive human PC cells, including RWPE-1, LNCaP, C4-2, C4-2B, and ARCaP_M were subjected to qRT-PCR analysis of cholesterol synthesizing genes and measurement of total cholesterol levels in the PC cells. PC3 cells were intracardially inoculated into nude mice primed with either normal or high cholesterol diet for three weeks prior to inoculation, and the mice were subjected to weekly bioluminescence imaging to monitor the development of metastasis, and mouse bone marrow was aspirated to isolate disseminated tumor cells (DTCs) for downstream biochemical analysis at the end of the experiment. PC cells were treated with 20 μ g/ml of cholesterol for 48h or 6 μ M of DZ-SIM for 16h followed by western blot analysis or total cholesterol measurement. Mice bearing 22Rv1 subcutaneous tumors were treated with vehicle, DZ, SIM, and DZ-SIM at 5mg/kg followed by weekly tumor and body weight measurement to determine the DZ-SIM therapeutic efficacy *in vivo*.

Results: Our preclinical data demonstrated that a high cholesterol diet promoted and enhanced metastasis of PC cells in mice and increased the number of DTCs in mouse bone marrow where these DTCs expressed elevated levels of malignant markers. We also showed that aggressive PC cells have higher intracellular cholesterol levels and express increased levels of cholesterol biosynthesis genes compared to normal or indolent PC cells. Moreover, exogenous cholesterol directly binds smoothelin (SMO) and activates Gli1 and downstream FOXM1, which is a master regulator governing the reprogramming of indolent PC cells to express increased levels of EMT markers, RANKL, Shh and Shh targets in LNCaP cells. Targeting intratumoral cholesterol by our recently developed DZ-SIM, a conjugate between a tumor targeting heptamethine carbocyanine dye, DZ, and cholesterol-lowering simvastatin (SIM), has been shown to effectively lower cholesterol levels, attenuated the cholesterol-mediated Gli1-FOXM1 signaling, and inhibit the cell and tumor growth of 22Rv1 cells *in vitro* and *in vivo* compared to SIM by a mechanism involving mitochondria and lysosomes targeting.

Conclusions: Cholesterol has been implicated with PC pathogenesis, and our results strongly demonstrated that cholesterol induced reprogramming and malignancy of PC cells by activating Gli1 and FOXM1 signaling and promoted PC growth and metastasis in mice. Targeting intratumoral rather than systemic cholesterol by DZ-SIM blocks cholesterol-induced Gli1-FOXM1 signaling and effectively inhibit PC cell and tumor growth *in vivo* and in mice.

Supported by DoD Idea Development Award and Phileover Foundation Award