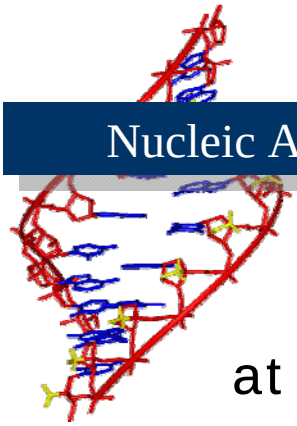
University of Southern Denmark

Nucleic Acid Center **Seminar** no. 67



Friday 22 August 2014
at 14:15 in the FKF Colloquium-room

Prof. Jagat Kanwar

Nanomedicine-Laboratory of Immunology and Molecular Biomedical Research (NLIMBR), Deakin University, Geelong, Australia

" EpCAM aptamer-siRNA targeted chimera and LNA modified Nucleolin aptamer inhibits tumor growth through downregulation of cancer stem cell markers"

Web: http://www.deakin.edu.au/research/stories/2012/06/25/the-fight-against-cancer

Abstract*:* Epithelial cell adhesion molecule (EpCAM), marker of cancer initiating cells and pluripotency marker is overexpressed in various cancers and regards as potential target for cancer therapeutics and drug discovery. Targeting EpCAM using larger molecule such as antibody, affibody, ankyrins were approached earlier, yet chemical antibody, aptamer regards one of the best methods to target EpCAM positive cancers. In the current study, we chimerized the EpCAM aptamer, 19ntd RNA aptamer with siRNA targeting EpCAM (EpApt-siEp), thereby we achieve knockdown of EpCAM in EpCAM expressing cells. This double selection ensures the target specificity and sensitivity by weeding off the off-target effects. EpApt-siEp was processed invitro by dicer enzyme, hence in the cells; similar mechanism for the generation of siRNA would be adopted. The EpApt-siEp construct significantly silenced EpCAM in Weri-Rb1 and MCF7 cells, evaluated by qPCR, northern and Western blotting ($P<0.005$). The expression of EpICD in RB primary tumor samples revealed out the cancer stem cell (CSC) property of EpCAM and its regulated stem cell marker expression. Pluripotency markers, SOX2, OCT4, NANOG, and CD133 were studied in RB cell line, Weri-Rb1 upon silencing EpCAM using siRNA and EpApt-siEp construct. The knockdown of EpCAM downregulated stem cell markers, inhibited cell proliferation and induced cellular cytotoxicity ($P<0.01$). The invivo studies using epithelial cancer model, MCF7 xenograft, as a proof of concept showed partial tumor regression with complete tumor growth inhibition without any toxicity in the animals ($P<0.0001$). The tumor tissues showed downregulation of EpCAM, MRP1, ABCG2, BCL-2, stathmin and survivin ($P<0.05$), upregulation of BAX and ATM upon EpApt-siEp treatment ($P<0.001$ and 0.05). Our results collectively revealed that EpApt-siEp construct can be potentially used for eradicating EpCAM positive cancer cells as well as CSCs, sparing normal EpCAM negative surrounding cells.