INHIBITION OF INTERLEUKIN-8 BY RNA-INTERFERENCE CAUSES REDUCED SURVIVAL AND INCREASED DRUG-SENSITIVITY IN PROSTATE CANCER CELLS.

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Background: Intereukin-8 (IL-8) is a multifunctional chemokine absent in normal epithelial and in primary prostate cancer cells but expressed in hormone-refractory (androgen-independent, AI) prostate cancer cells (e.g., PC-3). IL8 level is increased in circulation of prostate cancer patients with advanced disease. As a correlative of IL-8 expression, AI cells also exhibit constitutively activated nuclear factor kappa B (NF-kB) and protein kinase B (AKT) which are known positive regulators of cell survival, and inhibitors of chemotherapy drug-induced cell death. How NF-Kb and Akt are activated in AI cells and how they are connected to chemo-resistance is not well understood. We hypothesized that suppression of IL-8 synthesis should reduce NF-kB and AKT activation and decrease cell survival, and thus increase cytotoxicity of anti cancer drugs. We further hypothesized that IL-8 suppression by RNA interference is an effective method to reduce the activities of NF-kB and AKT in AI prostate cancer cells.

Experimental design: PC-3 cells were transiently transfected with IL-8siRNA (Dharmacon) using Dharmafect transfection protocol. Non-target scrambled siRNA served as control siRNA (C-Si RNA). RNA interference was determined by quantitative real-real time RT-PCR and ELISA. Protein expressions were identified by SDS-PAGE followed by immunoblotting with the appropriate antibodies. NF-kB activity was determined by assaying luciferase activity of transiently transfected cultures, with TK renilla as control. The cell proliferation activity was determined by colorimetric MTT assay and flow cytometry.

Results and discussion: IL8-siRNA decreased both IL8 expression (>90%) and of its two cognate receptors, CXCR-1 and CXCR-2 mRNA by 51 and 32 %, respectively when compared to control-siRNA transfectants. IL-8siRNA transfected PC-3 cells showed 20-40% decrease in cell proliferation activity and caused an inhibition of cell-cycle progression; cells were arrested in G1/S interface (25.3 % increase in G1 and 73% decrease in S-phase, Fig 1). Further,
suppression of IL-8 synthesis decreased cyclin D1 and CDK4 complex as well as phospho-Rb and cyclin B1 levels (Fig 2A). A 27% decrease in the NF-kB activity and a significant decrease in phospho-AKT were observed in IL8–siRNA transfected cells. Our investigation showed a significant increase in apoptosis of IL8siRNA transfectants, with and without drug treatment. Biochemical analysis showed a significant decrease in anti-apoptotic proteins, Bcl-2 and Bcl-xL but a large increase in pro-apoptotic protein BAD and BAX and activated caspase-9 were observed in IL-8siRNA transfectants (Fig 2B) suggesting a higher sensitivity for cytotoxic chemotherapeutic drugs when IL-8 is suppressed. As shown in Fig 3, a 40% increase in staurosporine cytotoxicity in IL-8siRNA-PC-3 cells shows increased drug sensitivity in IL-8 siRNA transfectants.

Conclusions: IL-8 is a growth promoting and survival factor in AI prostate cancer and inhibition of IL-8 by genetic silencing (siRNA) may significantly improve therapeutic response by promoting pro-apoptotic pathway and inhibiting survival pathway; thus proving an advantage for combination therapy in advanced prostate cancer. (Grant Support NIH_R01CA061038 (BLL))