TARGETED ENZYME PRODRUG THERAPY FOR METASTATIC PROSTATE CANCER – A COMPARATIVE STUDY OF THREE FUSION PROTEINS

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Abstract: Prostate cancer (PC) is the most common non-skin malignancy and the second leading cause of cancer-related death in American men, yet remains essentially incurable. Our objective is to develop a targeted, non-viral, enzyme/prodrug therapy to treat metastatic PC with minimal side effects. To achieve this, we have designed three novel fusion proteins (FP) each consisting of human annexin V (AV) and an enzyme without human homologs, thereby avoiding activation in healthy tissues. AV serves as the targeting arm via a high affinity to phosphatidylserine (PS), which is tightly segregated to the cytoplasmic leaflet in healthy cells but robustly and consistently expressed on the outer leaflet of tumor cells, their metastases, and tumor vasculature. PS expression was increased prior to FP treatment via low dose (50 pM) docetaxel treatment. The FP enzymes are: (i) purine nucleoside phosphorylase (PNP) – converts fludarabine (FD) into 2-fluoroadenine (2-FA), (ii) L-methioninease (MT) – converts methionine (Met) to methanethiol and selenomethionine (SeMet) to methylselenol, and (iii) cytosine deaminase (CD) – converts 5-fluorocytosine (5-FC) to 5-fluorouracil. Binding strength of FPs to PS and cytotoxicity of all three systems were evaluated for human PC-3 prostate cancer cells.

Binding of all FPs to PC-3 cells was found to be relatively strong with Kd ranging from ~0.06-0.6 nM. PNP-AV/FD produced 80% cell death at concentrations as low as 5 µM and was as effective as 2-FA, whereas FD treatment alone caused minimal cytotoxicity at concentrations up to 10 µM. MT-AV/SeMet treatment produced 90% cell death at SeMet concentrations of 500 µM after 3 days, whereas SeMet alone was not cytotoxic until 1000 µM concentrations. CD-AV/5-FC treatment produced only 60% cytotoxicity at concentrations of 5000 µM 5-FC in 9 days. Docetaxel treatment showed a significant increase in killing velocity and efficacy for the PNP-AV/FD and CD-AV/5-FC systems. The three novel FP/prodrug systems show promise for the targeted treatment of PC with minimal side effects. Future directions will consist of in vivo studies and studies with PEGylated FPs.