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Peptide-Mediated Transduction of Proteins and Nucleic Acids to Prevent and Treat Experimental Prostate Cancer

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14. ABSTRACT
The goal of this project is to prevent bone metastasis in early experimental prostate cancer using protein transduction: the ability of small peptides to facilitate the entry of large biologically active fusion protein cargos into cells. The hypothesis to be tested is that protein transduction can deliver therapeutic proteins to skeletal tissues and bone marrow in such a manner that they may facilitate the apoptotic, or programmed cell death of cancerous cells. Dr. Whalen and colleagues have previously shown that protein transduction can allow many proteins into organs previously unaccessible to other delivery methods (drugs, viral vectors etc). Her research team has constructed unique protein-transduction domains (PTD5 and Lys8) which facilitate the entry and activity of the tumor suppressive p53 or pro-apoptotic peptide Smac34 into prostate cancer cell lines. Unfortunately, these fusion peptides exhibited no or weak activities in prostate cancer cells. As an alternative, Dr. Whalen's team tried to explore the feasibility of adapting liposome mediated antisense gene therapy targeting EGFR for prostate cancer treatment, using murine models with xenografts of PC3 prostate cancer cells. Antisense gene therapy may prove to be useful for prostate cancer treatment.

15. SUBJECT TERMS
Drug delivery; protein therapy; prostate cancer; bone

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Introduction:

The goal in this project is to prevent the occurrence of bone metastasis in early experimental prostate cancer with systemic protein therapy via protein transduction. Protein transduction is based on the ability of small regions of proteins, called protein transduction domains (PTDs), to facilitate the entry of large biologically active fusion proteins into the cell lines in vitro, as well as cells and tissues of experimental animals (mice) in vivo. The hypothesis to be tested is that protein transduction can deliver therapeutic proteins to the bone marrow, periosteum, adjacent skeletal muscle and to the bone (osteocytes). Delivery of proteins in such a manner may facilitate the apoptotic, or programmed, cell death of cancerous cells and tissues of the bone. The first specific aim (Task #1 & #2; Statement of Work) of this project is to demonstrate the ability of protein transduction domain (PTD)-mediated delivery of p53 tumor suppressor to halt prostate cancer cell line proliferation in vitro, and to prevent and halt tumor development within and throughout the bone of immuno-incompetent mice in an established xenograft model of human prostate cancer in bone. The second specific aim (Task #3 & #4) was to develop an immuno-competent murine model of prostate cancer metastasis in order to ascertain the effects of PTD-based therapy on the development, of any, autoimmune responses.

Body

The first specific aim (Task #1 & #2; Statement of Work) of this project was to demonstrate the ability of protein transduction domain (PTD)-mediated protein delivery to prevent and halt tumor development within and throughout the bone of immuno-incompetent mice in an established xenograft model of human prostate cancer in bone.

Task #1 was to transduce the PC3 prostate cancer tumor cell line with p53 protein, a tumor suppressor protein, via protein transduction in vitro. This task was effort-intensive and would provide basis for other proposed Tasks. Dr. Whalen has accomplished Task #1.

The PC3 cell line was derived over several decades ago from the bone metastasis of a 56 year old man with prostate cancer. The PC3 cell line does not contain the tumor suppressor p53 protein (i.e. p53 null). Our hypothesis for Task #1 is that introduction of the p53 protein into PC3 cells will provide the cells with the missing tumor suppressor protein, thus halting cell growth and proliferation of the tumor cell line.

The following construction of the protein transduction domain coupled to the p53 protein was carried in collaboration with Paul D. Robbins, Ph.D (University of Pittsburgh).

i. An expression plasmid was constructed with the gene encoding the human p53 protein.

ii. The expression plasmid was cloned and expressed in insect cells and the presence of p53 protein was confirmed in cell lysates by Western immunoblot analysis. The resulting band appeared to be identical to wild-type, purified human p53 (positive control).

iii. The expressed p53 protein was chemically coupled to the PTD5 protein transduction domain (a novel PTD developed in this collaboration). The p53 protein was also separately coupled to the HIV Tat protein transduction domain (demonstrated by numerous investigators and reported in the literature to efficiently shuttle protein cargos into cells). An additional construct consisted of PTD5-coupled to a marker protein, beta-galactosidase. Evaluation of the ability of PTD5-p53 to transduce p53-null PC3 prostate cancer cell line and halt its -growth in vitro: PTD5-beta galactosidase marker protein efficiently entered PC3 (prostate-cancer derived) cells with almost 100 % efficiency (at the higher doses) and entry into the cells was dose dependent. PTD5-beta galactosidase efficiency of transduction into PC3 cells was almost identical to that of the positive control HIV-TAT protein transduction domain. These results demonstrated that the PTD5 protein transduction domain was capable of transducing the PC3 prostate cancer cell line in vitro. These data are consistent with experiments using PTD5-beta-galactosidase in other (non-prostate cancer) cell lines as well as primary cell cultures.

Additional constructs were also used, for example, PTD-4, 3, 2 and 1. These constructs were 8-12 amino acids long, and contained various numbers of lysines or arginines, amino acids hypothesized to be important in protein transduction efficiency. None of these alternative PTDs had the ability to transduce PC3 prostate cancer cell lines carrying the beta-galactosidase marker protein as well as PTD-5 or the positive control HIV- TaT protein transduction domains. The

PTD5-p53 and the TAT-p53 constructs were incubated with PC3 prostate cancer cells at various concentrations and the effect on cell proliferation was assessed colorimetrically with MTT (3-(45- dimethyl-2-thiazolyl)-2,5, diphinyltetrazolium bromide, Sigma).

Unfortunately, there was no effect on PC3 prostate cancer cell proliferation whatsoever, even at relatively high concentrations of PTD5 or HIV-TAT p53 protein transduction domains. These data suggested that the p53 protein biological activity may have been compromised during coupling to either of the two experimental transduction domains. Further attempts were made to resynthesize the p53 protein and couple it to PTD5 or HIV-TAT protein transduction domains. Additional attempts were made to couple active portions of the p53 protein (smaller bioactive peptides) to the PTDs. No construct was ever found to be capable of arresting the cell cycle or cause apoptosis of the PC3 cell line (or any other p53 null cell line).

Since PTD-p53 exhibited no effect on PC3 prostate cancer cell proliferation, it was not feasible to accomplish original Task #2, 3, and 4, which were to test the efficacy of PTD-p53 in xenograft tumors in immunodeficient mice and syngeneic mice. Other labs also experienced difficulty in obtaining functional activity of protein transduction domains carrying cargos of p53 or p53 peptides. Personal communications with Dr. Steven Dowdy (Swartze et al. Science 1999), reported inconsistencies in p53 activity following fusion of the p53 protein to the HIV-Tat related protein transduction domain. To test the hypothesis that PC3 cell growth could nonetheless be halted via alternative PTD-mediated cargos, Dr. Whalen tested the ability of a PTD5-smac construct created in our collaborator's laboratory (P.D. Robbins). SMAC is the abbreviation for 'secondmitochondria-derived activator of caspases. SMAC is considered to be a master regulator of apoptosis, programmed cell death in mammals. Specifically, smac induces the activation of procaspase-3 and promotes the enzymatic potential of mature caspase-3. Caspases are crucial regulators of apoptosis. PTD5-smac34 was constructed as outlined above, and consisted of the PTD5 protein construction domain and 34 amino acids from the amino-terminus of the smac protein. We found that the DU 145 cell line was the most susceptible to PTD5-smac34-induced cell death, as assayed by MTT. A dose dependent effect was demonstrated when PTD5-smac34 was added at various concentrations (25-200 uM). Furthermore, Dr. Whalen also found that the PTD5-smac34 construct did not require TRAIL (Apo-2L/tumor necrosis factor-related apoptosis inducing ligand) for its activity. Prostate cancer cell lines cultured with PTD5-smac34 viability was only slightly reduced when TRAIL was added to the cultures. Under normal conditions, TRAIL is necessary for the release of smac from mitochondria via translocation of BAX from the cytosol to the mitochondria. In her experiments, PTD5-smac34 did not need TRAIL to induce prostate cancer cell apoptosis.

Unfortunately, the efficacy of PTD5-smac, particularly at low dosage, was marginal (Appendix 1), which prevented further studies using in vivo animal models.

As another alternative, Dr. Whalen and the team have tried to explore the feasibility of adapting liposome mediated antisense gene therapy targeting epidermal growth factor receptor (EGFR) for treatment of prostate cancer. While it has previously been shown that antisense gene therapy can be beneficial in slowing or stopping the growth of various types of cancer, no study has yet

confirmed the efficiency of this technique for treatment of prostate cancer patients. The experimental design was to determine the half-life of the antisense RNA in various tissues after injection and the potential toxicity of the antisense RNA. The efficacy of antisense RNA will be assessed based on tumor size changes in murine models with xenografts of PC3 prostate cancer cells. After confirmed tumor growth liposome mediated antisense gene therapy would be administered to one group and no liposomes administered to the control group. At the conclusion of treatments, mice were to be sacrificed, tumor + margin tissues harvested/measured, and Dr. Whalen intended to compare groups to determine if there would be any statistically significant and/or clinically relevant outcomes. Unfortunately, there was not enough time for Dr. Whalen to test rigorously of the proposed studies due to the time and budget limit of the proposed project. Although Dr. Whalen was not able to extensively explore the antisense technology for prostate cancer treatment, other labs, e.g. Dr. Martin Gleave's lab at the Vancouver Prostate Center, have generated strong clinical and pre-clinical data demonstrating the potential of the antisense approach for prostate cancer treatment (Gleave ME, et al, 2001; Lamoureux F, et al, 2011; Miyake H, et al, 2000). These studies took a long time (>10 years) and required huge amount of funds and resources. It was beyond the scope of this project to move antisense RNA approach into pre-clinical and clinical settings.

Key Research Accomplishments:

1. Generated sufficient PTD-p53 fusion peptide for functional tests.

2. Transduced the PC3 prostate cancer tumor cell line with p53 protein, a tumor suppressor protein, via protein transduction in vitro. Unfortunately, the PTD-p53 exhibited no activity in prostate cancer cells.

3. As an alternative, generated sufficient PTD5-smac34 fusion peptide for functional tests.

4. Transduced the PC3 prostate cancer tumor cell line with smac protein, a pro-apoptotic protein, via protein transduction in vitro. The PTD-smac fusion protein at high dose induced apoptosis in prostate cancer cell lines in vitro.

5. As another alternative, Dr. Whalen's team developed a strategy to target EGFR using antisense RNA technology to inhibit prostate cancer cell growth.

Reportable Outcomes:

1. Abstract of this work was presented at the 9th Annual Scientific Retreat on September 20 - 22nd in Washington, DC. The title of the Abstract: Treatment of Experimental Prostate Cancer via Protein Transduction of a PTD-5/smac-trail fusion peptide.

Conclusions:

The results from this project showed that the tumor suppressor p53 or pro-apoptotic protein smac delivered into prostate cancer cells via Protein Transduction domains (PTD) exhibited weak or no activities. Thus, delivery of proteins via protein transduction into prostate cancer cells as a potential therapy may not be feasible. As an alternative, antisense RNA targeting key signaling proteins such as EGFR may be promising. These findings will help guide future prostate cancer research directions.

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Appendices: 1.

Appendix: Graph 1
PTD5-smac34 Alone Reduces PCa Cell Proliferation in a Dose Dependant Manner