NOVEL ENZYME PRODRUG THERAPY OF BREAST CANCER DESIGNED FOR EFFECTIVE DELIVERY TO THE TUMOR

Presenter: Roger Harrison¹, PhD

Collaborators: Carla Kurkjian², MD & Vassilios Sikavitsas¹, PhD
¹School of Chemical, Biological and Materials Engineering, University of Oklahoma & ²Hematology-Oncology Section, University of Oklahoma Health Sciences Center

The purpose of this project is to develop a novel enzyme prodrug therapy in which methioninase (MT) is targeted by the human protein annexin V (ANX) to the breast tumor vasculature, using selenomethionine (SeMet) as the prodrug. Annexin V is known to bind with high affinity to phosphatidylserine (PS) in phospholipid bilayers. PS has recently been shown to be expressed on the external surface of endothelial cells that line the blood vessels in tumors but is not expressed on the external surface of the vascular endothelium in normal organs. MT catalyzes the conversion of methionine to methanethiol, α-ketobutyrate, and ammonia. It also catalyzes the conversion of SeMet to toxic methylselenol, α-ketobutyrate, and ammonia.

The MT-ANX fusion protein was produced by recombinant E. coli and then purified by chromatography. The binding of the biotinylated fusion protein (FP) to the surface of human endothelial cells and MCF-7 and MDA-MB-231 human breast cancer cells was measured in vitro by determining the dissociation constant $K_d$. The stability of binding of the FP was measured over 3 days for all the cell lines. The in vitro cytotoxicity of MT-ANX was determined for endothelial cells and the two breast cancer cell lines using the Alamar Blue assay for measuring cell viability. The in vivo cytotoxicity of the MT-ANX/SeMet enzyme prodrug system for MDA-MB-231 cancer cells implanted in nude mice was determined.

The yield of purified MT-ANX fusion protein was 30 mg per liter of E. coli culture broth, and the protein purity was >97%. The $K_d$ for each cell line tested was obtained from the specific binding data to give the following results: 0.5 ± 0.2 nM for endothelial cells, 6.2 ± 1.6 nM for MCF-7 breast cancer cells, and 4.9 ± 0.9 nM for MDA-MB-231 breast cancer cells. These results indicate that the binding of MT-ANX to these cells is relatively strong. Data for the binding stability of MT-ANX showed that the binding declined for all cell lines over 3 days; however, the FP was still present at day 3. Enzyme prodrug treatment of the endothelial cells with MT-ANX and SeMet gave significant cell killing for 500 and 1000 µM SeMet at days 1, 2, and 3 (p < 0.001). At 1000 µM SeMet, the cell viability was only 3% and 2% of the control at days 2 and 3, respectively. With no MT-ANX present, significant cell cytotoxicity was not observed at the levels of SeMet tested. For the two cancer cell lines, killing occurred at lower SeMet concentrations than for the endothelial cells. In the in vivo test of the cytotoxicity of the enzyme prodrug treatment in nude mice, the treatment was 10 mg/kg of MT-ANX injected i.p. on day 0 and 10 mg/kg of SeMet injected i.p. on days 1, 2, and 3, with this treatment sequence repeated two more times. The result was that the tumor size in the treatment group of mice steadily declined after treatment, while tumor size in the three control groups (untreated, treated with SeMet only, and treated with MT-ANX only) steadily increased over the same time period, with the tumor size in the treatment group becoming significantly lower than in the control groups after 12 days since the start of treatment (p < 0.001). After 47 days since the start of treatment, the tumor size in the treated group was nearly zero and was 4% of that in the untreated group.

Based on the data obtained to date, there is evidence that therapy with the MT-ANX/SeMet enzyme prodrug system has the potential to be highly effective in causing cytotoxicity to tumors wherever they occur in the body with minimal side effects. This new enzyme prodrug therapy system to treat breast cancer appears promising.

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