457b Targeting L-Methioninase to Human Cancer Cells

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Results obtained over the past 40 years have demonstrated that tumor cells of all types tested have an elevated growth requirement for methionine compared to normal cells. Numerous lines of cancer cells are unable to survive and grow when the amino acid methionine is replaced in the medium with homocystine. However, normal adult cell lines survive and grow well with this substitution. Therefore, the enzyme methioninase was targeted to the surface of cancer cells in order to develop a treatment for cancer that is more selective than current therapies. All exogenous methionine in the vicinity of the cancer cells will potentially be depleted with methioninase bound to the cell surface.

The present study developed a novel fusion protein targeted specifically to the urokinase receptor on tumor cells. The fusion protein contains two components: the amino terminal fragment of human urokinase (amino acids 1-49) and methioninase (containing 398 amino acids), an enzyme that catalyzes the breakdown of methionine to alpha-ketobutyrate, methanethiol, and ammonia. The urokinase receptor is overexpressed on many different types of cancer cells.

A process was developed to produce the fusion protein in Escherichia coli and then purify it. The gene for the amino terminal fragment (ATF) of urokinase was joined to the gene for methioninase, and the fusion gene was ligated to the pET 30 EK/LIC vector and transformed into E. coli strain BL21(DE3). This vector adds with an amino-terminal 6xHis tag to the overexpressed protein. In the cloning, the gene for the HRV 3C protease was added just ahead of the ATF gene. The fusion protein was overexpressed and then was purified by immobilized metal affinity chromatography. The 6xHis tag was cleaved off using HRV 3C protease.

The specific binding of the fusion protein to urokinase receptors was demonstrated by measuring the displacement of the fusion protein from MCF-7 human breast cancer cells by urokinase. MCF-7 breast cancer cells are methionine dependent and express the cell surface urokinase receptor. The urokinase concentration necessary to produce a 50% displacement of fusion protein was determined to be approximately 10 nM. It was also determined that human epidermal growth factor, over the same concentration range, did not produce any significant displacement of the fusion protein.

The effect of the fusion protein on the migration and proliferation of MCF-7 breast cancer cells, SK-LU-1 lung cancer cells, and PC-3 prostate cancer cells was determined using a culture wounding assay, which measures cell proliferation and migration. SK-LU-1 and PC-3 cancer cells also are methionine dependent and express the urokinase receptor. For a concentration range of 10-1000 nM of fusion protein, there was a dose-related inhibition of both the migration and proliferation of the three types of cancer cells on days 2 and 3 following fusion protein treatment. For the breast cancer cells, the mean cell number in the proliferation test after 3 days was 5 times less for the cells treated with 1000 nM fusion protein compared to vehicle treated controls. One of the most important findings of this study is that treatment with the fusion protein was significantly more effective than free L-methioninase in inhibiting cell migration and proliferation at 1000 nM, which supports our rationale for targeting L-methioninase to the surface of the cancer cells.

The nude mouse xenograft model was used in a preliminary study to determine the influence of the fusion protein upon the growth and metastasis of tumor breast tumor cells in vivo. In this study, MCF-7 human breast cells, suspended in Matrigel were injected into the flank of nude mice. These cells are stably transfected with the beta-galactosidase reporter gene so that metastasis of the cancer cells can be determined and quantified. The development of tumor masses was monitored over a period of 30 days. Treatment groups received either the fusion protein (three mice each treated with 12 microliters/day at a

concentration of 5000 nM, equal to 12 micrograms/day assuming a molecular weight of 196,000 Da for the homotetrameric fusion protein) or vehicle in the control group (two mice) administered by continuous infusion s.c. to the tumor site over a period of 14 days using an Alzet osmotic infusion pump. The results of this study demonstrated that the fusion protein was not cytotoxic to the nude mice since none of the treated animals died or showed signs of whole animal or organ cytotoxicity during the 14-day treatment period. The total number of cancer cells/gram of tissue was reduced by an average of 50% in the fusion protein treatment group compared to the control group. Further, lung metastases were found in all of the control animals, while none were found in the fusion protein treated mice.

In conclusion, the results of this study indicate that targeting methioninase to cell surface receptors of cancer cells is potentially an effective approach to improve the treatment of cancer with fewer adverse side effects than conventional chemotherapy.