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13. ABSTRACT *(Maximum 200 Words)*

Most research that requires the long-term propagation of prostate cancer (PCA) cells in culture is carried out with three cell lines: DU 145, PC-3, and LNCaP. All of these lines were derived from metastases; only one, LNCaP, makes prostate specific antigen (PSA) and/or androgen receptor. Neither DU 145 nor PC-3 exhibit any phenotypic markers specific for prostatic epithelial cells. Better models are needed. There is evidence that some prostatic fluids contain PCA cells. For this proposal, our goals are (1) to test the tumorigenicity of and to develop transplantable xenografts from PCA cells in prostatic fluid, (2) to develop methods for enhancing the tumorigenicity of small numbers of these PCA cells without deliberately altering their genes, (3) to test these methods for enhancement of tumorigenicity with prostatic fluid cells, and (4) to initiate clinical follow-up. To date, we have detected a marginal elevation of PSA in the blood of one mouse that received prostatic fluid cells 28 days after injection, unequivocal elevation of PSA in the blood of two mice 2-4 months after transplantation, and no elevation of PSA 6 months after transplantation. We have studied the coinjection of lethally irradiated, growth-factor-producing cells from several tumors with encouraging results in the case of some tumors.

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Introduction

The development of new approaches to the treatment of prostate cancer (PCA) would be greatly facilitated if there were a larger number of experimental models available. As we reviewed two years ago (Pretlow and Pretlow, 2000), (a) the rodent prostate seems almost irrelevant to us since it is biochemically, functionally, and embryologically quite different from the human prostate and (b) there are very limited numbers of human PCA epithelial cells that can be grown in the laboratory. Tissue culture lines derived from human PCAs are successful in less than 0.1% of human PCAs tested, and most such lines fail to make two molecules that are very important for most human PCAs: androgen receptor and prostate specific antigen (PSA). There are laboratories, including our own, that have reported the development of PCA xenografts from 5-10% of human PCAs; and most such xenografts make both PSA and androgen receptors, i.e., have functional properties in common with most human PCAs. If an investigator wants to develop a human PCA tissue culture line from a xenograft, there is an infinite supply of xenograft available, and tissue culture lines have been established from xenografts. One such line was developed from one of our xenografts (Sramkoski et al., 1999) and is available from the American Type Culture Collection (their catalogue number CRL-2505). Our goals in the proposed research are to assess the tumorigenicity of PCA cells in prostatic fluid, to develop methods to enhance the tumorigenicity of the small number of cells available there, to test those methods for the enhancement of tumorigenicity of small numbers of PCA cells, and to initiate clinical follow-up to find any correlations that may exist between the clinical courses of patients and whether or not their cells grow to form xenografts.

Body

Specific Aim 1 (also task 1). To test the tumorigenicity of cells obtained from the prostatic fluids (PF) of patients with PCA and to develop serially transplantable xenografts from these cells.

In months 1-18, we (statement of work, task 1 of the proposal) proposed to test the tumorigenicity of prostatic fluid cells obtained by prostatic massage from 30 patients. Our testing was delayed initially because our institution renovated some of the nude mouse facility and did not finish that task until approximately four months after this grant started. This restricted severely the number of cages available to our laboratory for several months; however, we are catching up rapidly. We have injected cells from the prostatic fluids of eleven patients into nude mice. Blood has been drawn from these mice 1, 2, 4, and 6 months (as the mice reached those intervals) after the injection of the cells. Elevated blood PSA has been observed in six mice injected with prostatic fluid cells from five patients; however, the elevated PSA has been of low magnitude (0.02-0.10 ng/ml). To date, we have not observed tumor histologically at the site of injection or in the lungs of mice injected with PF cells.

Specific Aim 2 (also task 2). To develop methods for enhancing the tumorigenicity of small numbers of PCA cells without deliberately altering their genes.

Specific Aim 2 is particularly important since the successful conduct of the proposed work for specific aim 3 is dependent on the identification of tumor cells that can be irradiated and coinjected with CWR22 with enhancement of the rate of growth of CWR22. Success in specific aim 2 might also have enormous significance for areas that are not directly related to this research proposal. For example, there are those who are interested in growing PCA cells from the blood of patients with PCA. We reported the growth as xenografts of PCA cells from the blood of two of eleven patients with metastatic PCA (Pretlow et al., 2000); however, if this is to provide a useful means of sampling patients' metastatic tumor for the purpose of predicting the appropriate drugs for specific

patients, any means that would permit the growth of PCA xenografts from a higher proportion of PCA patients would greatly facilitate this kind of research. We shall organize our progress report relevant to specific aim 2 with subheadings that name the coinjected, irradiated tumor cells.

DU 145

Four coinjection experiments have been carried out with DU 145. In all cases, 250 CWR22 cells were used as the viable (not irradiated) target cells the growth of which we wished to accelerate by coinjection of lethally irradiated tumor cells that might produce growth factors that would accelerate the growth of CWR22. The first of these experiments was a pilot experiment to see if there might be an optimal range of doses for the coinjection of DU 145 cells. In the first experiment, we injected one animal with each combination of cells. The injection mixtures included 375,000, 750,000, 1,500,000, 3,000,000, 6,000,000, and 12,000,000 DU 145 cells. For comparison, two animals received 250 CWR22 cells in the absence of coinjected, lethally irradiated cells; and two animals received 12,000,000 lethally irradiated DU 145 cells but no CWR22 cells. We were encouraged by the fact that two animals that received similar doses of DU 145 grew tumors most rapidly, i.e., animals that received 750,000 and 1,500,000 lethally irradiated DU 145 cells grew tumors such that the minimum dimensions of the tumors were 6 mm 47 days after injection. With greater precision than we expected, data from the other animals in this experiment supported the preliminary interpretations that (a) there was an optimal dose of DU 145 cells and (b) coinjected DU 145 cells accelerated the growth of the very small number (250) of injected, viable CWR22 cells. As the doses increased above the 0.75 and 1.5 million-cell doses, tumors grew progressively more slowly as shown in the table entitled "First DU 145 Coinjection Experiment." The data were consistent with the data from our testing of growth factors in soft agar in that increases or decreases from the optimal dose resulted in decreased growth stimulation. The one deviant and unexpected data point resulted from the fact that one of the two animals that received only the 250 CWR22 cells with no coinjected, irradiated cells also developed a tumor during the six-month duration of the experiment. Worse than that, that animal's tumor grew to 6 mm in 61 days.

First DU 145 Coinjection Experiment

CWR22 (cells injected)	DU 145, irradiated (cells injected)	Days to 6 mm tumor
250	12,000,000	no tumor at 6 months
250	6,000,000	124
250	3,000,000	82
250	1,500,000	47
250	750,000	47
250	375,000	91
250	0	61
250	0	no tumor at 6 months
0	12,000,000	no tumor at 6 months
0	12,000,000	no tumor at 6 months

In the next experiment, conditions were altered only by increasing the number of mice to 3 animals/group that were injected with a narrower range of doses of irradiated DU 145 cells: 3 million, 1.5 million, 0.75 million, and 0.375 million cells. While the results of this experiment were consistent with the previous experiment in that the successful doses of irradiated CWR22 cells were similar (not the same) to those observed previously, the experiment was a failure in the sense that only two animals showed signs of developing tumor at 104 days, and the experiment was terminated after 104 days. The two animals that developed tumor included one of the three animals that was coinjected with 0.75 million cells and one of the three animals that received 0.375 million

cells. Neither of the animals that received 250 CWR22 cells without coinjected DU 145 cells developed tumor.

In reviewing the previous two experiments, it occurred to me that our original design was flawed. If the "control" group of mice always included the same number of mice as each group that received graded doses of irradiated tumor cells, ineffective coinjections might always be more encouraging than merited. For example, in the second experiment with coinjected, irradiated DU 145 cells described above, a total of 4 groups (3 animals/group or 12 animals in 4 groups) received coinjected DU 145 cells, while there were only 3 animals that received 250 CWR22 cells without coinjected cells. If the appearance of tumors were entirely random and unrelated to coinjected cells, the results would always be encouraging, i.e., there would be a four-fold probability that "experimental" animals would develop tumors before "control" animals. There are two other DU 145 coinjection experiments in progress. One of these experiments is sufficiently advanced to lead us to believe that the effect of coinjecting DU 145 is small. While many animals that received coinjected DU 145 have developed and are developing tumors, some animals that received only 250 CWR22 cells in the absence of DU 145 cells appear to be developing tumors. If the fourth DU 145 experiment fails to show outstanding enhancement of CWR22 cell growth from the coinjection of DU 145 cells, we can conclude that DU 145 cells should not be pursued in depth before other kinds of cells are investigated.

CWR91

In our original research proposal, we stated: "We wish to test the possibility that rapidly growing xenografts, CWR22Rv1 and CWR91, from two different patients may make autocrine growth factors or other environmental alterations that may explain their rapid growth and may stimulate other PCA cells to grow more rapidly." We received misinformation from the Army by telephone relevant to the date when our grant was to start, i.e., we were told by telephone that it would likely start at least two months before it started. Not to waste time and because the time required for the first generation of a tumor out of our nitrogen freezer is quite variable, we got CWR91 out of the freezer and injected it into nude mice immediately after receiving the letter from the Army announcing their intention to make an award and after being told on the telephone by a representative of the Army that the award would probably be made in less than one month.

The CWR91 from the freezer grew much more rapidly than usual, and we then had to decide what to do with it. We decided to expand the tumor into other mice so that we could start that experiment soon after the grant was activated. The expansion of the tumor went as expected; however, the army activation of the grant did not. Having expanded the tumor, despite the absence of support, we decided to go ahead and do a preliminary experiment with CWR91. Unequivocally, animals that were coinjected with CWR91 cells in addition to viable CWR22 were much less likely to develop tumors than animals that received CWR22 cells alone. We had known from data from another laboratory, not yet reported, that CWR91 makes TGF-beta. TGF-beta may inhibit the growth of CWR22 (we do not have data that are relevant to TGF-beta and CWR22 but do know that TGF-beta inhibits the growth of some other kinds of cells). Despite our knowledge of the production of TGF-beta by CWR91, the very rapid growth rate of CWR91 suggested that it was a PCA cell that might produce useful autocrine or paracrine growth factors and should be tested coinjected with CWR22. We hoped that CWR91's rapid growth might indicate the production of growth factors that would stimulate other PCAs as xenografts.

New Choice of Cells

BT-549, HS-578, K-562, MDA-MB-2, LOX IMVI, NCI-H23, SF-295, and SNB-75

We have recently donated CWR22 and a post-castration, post-regression, relapsed, serially transplantable derivative of CWR22, designated CWR22R, to the Biological Testing Branch of the National Cancer Institute. They want to distribute these tumors to other research laboratories. In the process of talking with the leaders of that part of the National Cancer Institute, we learned that they have RNA profiles of some of the tumors that they distribute. While these RNA profiles are not yet published and are confidential, the profiles indicate which tumors make large amounts of specific growth factors. We selected tumors from them that make different growth factors that are known to be heavily expressed by some human PCAs and/or known to stimulate some PCA cells in vitro. The ability to select tumors based on knowledge of the RNA profile should enhance the efficiency of our selection process. We obtained the following tumors from the Biological Testing Branch of the National Cancer Institute recently: BT-549, HS-578, K-562, MDA-MB-2, LOX IMVI, NCI-H23, SF-295, and SNB-75. We have injected them into two mice each. One of these tumors, LOX IMVI, an amelanotic melanoma, grows extraordinarily rapidly (two weeks from the injection of frozen cells until sacrifice of animals with tumors at least six mm in least dimension) and is being tested as described above.

KG 1A

A single experiment has been started with KG1A; however, that experiment has progressed for only slightly over one month, and it is too early to predict the results.

LOX IMVI

A single experiment has been started with LOX IMVI; however, that experiment has progressed for only one week, and it is too early to predict the results.

Specific aim 3 is dependent upon success in specific aim 2. Progress on specific aim 2 is proceeding rapidly, and we shall start specific aim 3 after tumor lines are identified that markedly enhance the growth of CWR22 when irradiated and coinjected.

Specific aim 4 is being pursued with the collection of the clinical data described in that aim for use in future correlations between clinical data and the extent of our success in growing patients' prostatic fluid PCA cells as xenografts.

Key Research Accomplishments

Prostatic fluid cells from eleven patients have been injected into nude mice. Only three of these patients' cells have been in mice as long as six months, i.e., have final results with the sacrifice of the mice. The others are continuing.

Testing of DU 145 as an irradiated feeder cell to be coinjected with PCA cells in completed.

Testing of KG 1A and LOX IMVI as irradiated feeder cells to be coinjected with PCA cells has been started.

Cells known to make large amounts of RNA for relevant growth factors have been obtained and injected into mice for expansion and testing after sufficient expansion. These cells include BT-549, HS-578, K-562, MDA-MB-2, NCI-H23, SF-295, and SNB-75 in addition to those currently being tested.

Reportable Outcomes

There are no reportable outcomes.

Conclusions

The only conclusion that is final is that coinjection of DU 145 cells enhances the growth of CWR22 cells only slightly and with considerable variation from experiment to experiment.

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Appendices

No appendices are included.