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This project is a study of the potential role of retinoic acid receptor in the metastasis of prostate cancer. Initial studies identified the RXR-alpha expression to be highly correlated with retinoid sensitivity. In addition, clinical samples showed a trend towards a decreased expression in metastatic cells compared to the primary tumor cells. To determine the function of the retinoid receptor RXR-alpha, an adenovirus transducing RXR-alpha was created and tested in two ways. Prostate cancer cell lines were created to over express the gene. These RXR-alpha transduced cells were showed no change in the sensitivity to retinoids and the metastatic ability when used in a nude mouse model of experimental metastasis, a technique that was also newly developed in this study.

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FOREWORD

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N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

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INTRODUCTION:

The objective of this project is to determine the potential function of retinoic acid receptors in prostate cancer metastasis.

BODY:

1. Retinoic acid receptor expression

We have studied the expression of RXR-alpha and RAR-alpha protein in prostate cancer cell lines and in patients with prostate cancer. We used immunohistochemistry using antibody directed against the RXR-alpha protein and the RAR-alpha protein. Standard formalin fixed, paraffin embedded sections were studied. For cell lines, western blotting was also used. The current studies extend the results reported previously and have determined that as a general rule, RXR-alpha protein is expressed in retinoic acid sensitive cell lines but not in retinoic acid resistant cell lines. This raised an important possibility that (a) RXR-alpha protein expression may be a marker of retinoid sensitivity, and that (b) RXR-alpha protein expression may directly confer sensitivity to retinoids.

To extend these findings, we studied the expression of the RXR-alpha protein in the cancer cells of patients with prostate cancer by immunohistochemistry. Unlike the results seen with cell lines, the patients do not show such a clear-cut separation of the expression level. Rather, there is a wide range of expression, primarily centered at "low-moderate" level of expression, regardless of the type of cancer, such as the Gleason grade.

2. Construction of Adenovirus Vector Expressing RXR-alpha

In order to address the question of whether RXR-alpha receptor is directly involved in retinoid sensitivity, we constructed a human adenovirus vector able to transduce the RXR-alpha gene. This vector allows us to determine the effect of RXR-alpha expression in a wide range of cell lines and in clinical samples in vitro. A modified replication-deficient adenovirus vector was inserted with the human cDNA for RXR-alpha in the E1A region. After screening for the appropriate recombinants, a single clone was isolated which proved to be the correct clone.

The vector was initially characterized using test cell lines. The vector was grown in 293 cells engineered with E1A, and purified to a high titer. This vector was then tested in cell lines, and gave the expression of the protein of the correct size. By adjusting the multiplicity of infection, the protein expression could be increased to a level corresponding to 100 fold greater than cell lines with endogenous expression of RXR-alpha.

3. Effect of Transduction of RXR-alpha in Prostate Cancer Cell Lines

Transduction of the retinoid-resistant RXR-alpha negative cell lines with the engineered vector resulted in the expression of the RXR-alpha. Varying the multiplicity of infection allowed adjustment of the levels of protein expressed, so that it was possible to select the level of RXR-alpha protein expressed. These cells remained viable with normal growth characteristics.

These cells were then treated with retinoids. The retinoids tested were: 13-cis retinoic acid, 9 cis retinoic acid, all trans retinoic acid, and LGD1069 (Targretin). These were all used at the concentration of 1 μM. The cells remained viable and with normal growth characteristics.

4. Expression of A Gene Which May be Involved in Retinoid Resistance

In order to elucidate the mechanism of resistance to retinoids, despite the expression of appropriate receptors, we examined the expression of genes known to modify the retinoid sensitivity. Thus far, the data indicate that TGIF gene, which blocks the binding of RXR-alpha protein to its target and which interferes with SMAD activity, is expressed in the retinoid resistant prostate cancer cell lines. We are now determining its expression in clinical samples as well.

5. Establishment of a prostate cancer metastasis model.

Because no animal model for metastasis of prostate cancer cell lines was available, we developed an experimental model of prostate cancer metastasis. Nude mice were given an intracardiac injection of human prostate cancer cells. At 3-4 weeks after the injection, the animals were sacrificed, and organs assayed for the presence of human cells using the method described below.

We developed a highly sensitive method employing polymerase chain reaction directed against the human Alu repetitive sequence. This sequence is found only in humans and not in mice. Additionally, there are at least 100,000 copies of this repetitive sequence in the human genome. This means that the PCR target is already "pre-amplified," so that the assay is highly specific and sensitive for small numbers of human cells in mouse samples.

The sensitivity of this assay using titration curves was estimated to be 10 human cells in one million mouse cells. The PCR signal, quantitated using a digital image analysis method, was proportional to the input number of human cells in a mouse background.

Using the assay, injection of human prostate cancer cell lines resulted in a reproducible detection of human cells in the mouse femur. This may be considered a model for bone metastasis.

6. Metastatic ability of prostate cancer cells transduced with RXR-alpha

Using the prostate cancer cell lines transduced witht the RXR-alpha adenovirus vector, as described in section 3, we tested to see if the RXR-alpha transduction resulted in any difference in the metastatic ability. The metastatic ability of the cell lines was defined based on the animal assay described in item 5. There was no statistically significant difference in the number of human prostate cancer cells in the femur of nude mice at 4 weeks after intracardiac injection.

KEY RESEARCH ACCOMPLISHMENTS:

- Engineering of an RXR-alpha transducing adenovirus
- Demonstration of a correlation between RXR-alpha expression and retinoid sensitivity in cell lines, and lack of correlation in human patients
- Establishment of an experimental model of human prostate cancer metastasis in the nude mouse.
- Establishment of a method to detect small numbers of human cells in mouse tissues and cell samples.
- Demonstration that RXR-alpha expression does not affect retinoid sensitivity or metastatic ability.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes to include:

Manuscripts, abstracts, presentations; Publications, manuscripts

A manuscript is now in preparation detailing the results from items 1-3 listed above. It is likely that the results of the studies on the genes mediating retinoid resistance will be a separate manuscript. A manuscript is being prepared on the techniques of experimental model of metastasis and the assay for the detection of human cells.

CONCLUSIONS: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the annual and final reports.

REFERENCES:

None

APPENDICES:

None