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13. ABSTRACT (Maximum 200 Words) Purpose: The purpose of this project was to investigate the utility of inducible siRNA vectors for analyzing signal transduction pathways in breast cancer cell lines. Scope: This was a one year project designed to test the feasibility of a novel approach, which, if it showed promise, would lead to more extensive research. Progress: Studies were initiated to test two strategies for construction of inducible siRNA vectors. These strategies made use of vectors described in publications that appeared subsequent to submission of the grant application. Four sites within the HER2 cDNA sequence - positions 672, 2190, 2796, and 4287 - were identified as favorable targets for siRNA inhibition based on known properties of such sites. Vectors were constructed targeting each of these sites with the two different inducible siRNA vectors. SK-BR3 cells were obtained from ATCC and transfections were initiated to generate stable derivative lines expressing the TET repressor. After accomplishing these initial objectives, further progress was delayed because of serious illness in the P.I.'s family requiring a prolonged absence, together with the almost simultaneous resignation of the individual primarily assigned to the project.				
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INTRODUCTION

A short interfering RNA (siRNA) is a double stranded RNA, usually 19-21 base pairs in length, with one strand complementary to the mRNA of a target gene (1). Ideally, the presence of such an RNA molecule leads to specific and nearly complete shut down of expression from the target gene. Plasmid vectors are available that allow stable expression of an siRNA in transfected cells (2, 3). This technology provides a powerful new tool for studying the numerous genetic and epigenetic events implicated in the pathogenesis of breast cancer. In particular, this method provides a method to approach a type of question not addressed by other methods, namely: For any given cell line, which pathways, and which specific components of those pathways, are *necessary* for the malignant behavior of the cell? Current vectors for stable expression of an siRNA in mammalian cell lines depend on specific RNA polymerase III (pol III) dependent promoters, which have properties compatible with synthesizing an siRNA (2-4). This proposal aimed to evaluate modifications of such vectors which would render siRNA expression inducible. The study focused on methods for introducing the tetracycline responsive element (TRE) into the pol III dependent histone H1 or U6 promoters as a means of generating an inducible siRNA expression vector. The purpose of this project was to investigate the utility of inducible siRNA vectors for analyzing signal transduction pathways in breast cancer cell lines. This was a one year project of limited scope designed to test the feasibility of a novel approach, which, if it showed promise, would lead to more extensive investigation.

BODY

Studies were initiated to test two strategies for construction of inducible siRNA vectors. Rather than constructing vectors *de novo* as originally proposed, we chose to begin with vectors reported to have the desired characteristics described in publications that appeared subsequent to submission of the grant application (6, 7). Four sites within the HER2 cDNA sequence – positions 672, 2190, 2796, and 4287 – were identified as favorable targets for siRNA inhibition based on known properties of such sites. Vectors were constructed targeting each of these sites with the two different inducible siRNA vectors. SK-BR3 cells were obtained from ATCC and transfections were initiated to generate stable derivative lines expressing the TET repressor.

After accomplishing these initial objectives, further progress was delayed because of serious illness in the P.I.'s family requiring a prolonged absence, together with the almost simultaneous resignation of the research biologist primarily assigned to the project. The current plan is to continue to evaluate the plasmids already constructed following the plan outlined in Tasks 3 and 4 of the Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS

1. Inducible siRNA vectors were obtained from external sources: pU6B (ref. 6) was obtained by request from C. Z. Song of the University of Washington, Seattle. pSuperior was purchased from Oligoengine (ref. 7).
2. The HER2 sequence was analyzed for sequences likely to provide effective targets for siRNA inhibition.

3. The four different target sequences were cloned into two candidate inducible siRNA vectors.

REPORTABLE OUTCOMES

1. A panel of plasmids have been constructed to express siRNA targeted against four different sites in the HER2 cDNA sequence under the control of a tetracycline inducible RNA polymerase III promoter.

CONCLUSIONS

The use of inducible siRNA vectors has the potential to provide new insights into to the roles of the various signaling pathways and their individual components in the pathogenesis of breast cancer. The utility of the plasmids constructed in the initial phase of this project remains to be determined by transfection experiments.

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APPENDICES: None