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Normal p53 protein is a transcription factor and a suppressor regulating the expression of a wide range of genes involved in apoptosis, growth control, and inhibiting the proliferation of tumor cells in animal models. Mutations that inactivate the p53 gene are important steps in tumor progression. The purpose of this project is to use combinatorial methods to select RNA oligomers that will bind to the critical portion of mutant p53 protein to restore its ability to bind to specific DNA targets that allow it to act as a transcription factor. Such an RNA species could mimic the ability of antibodies directed against the same portion of p53 protein to restore wild-type function to mutants. Such RNAs could be expressed from viral vectors that could infect tumor cells and restore normal p53 functions, resulting in apoptosis. A critical region of the p53 peptide sequence has been chosen as the target for the selection of binding RNA oligomers. Five rounds of selection have been completed.

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Christopher J. Green JUNE 10, 1999

PI - Signature Date

Table of Contents

Introduction

The purpose of this research is to produce a nucleic acid sequence that will bind to mutant human p53 protein to enhance its tumor suppressor ability. Normal p53 protein is a transcription factor and a suppressor regulating the expression of a wide range of genes involved in apoptosis, growth control, and inhibiting the proliferation of tumor cells in animal models. Mutations that inactivate the p53 gene are important steps in tumor progression and often affect the protein's DNA binding ability. These mutant p53 proteins have significant changes in their structure (for a review, see Erlanson and Verdine, 1994). If a method can be found to return the structure of a cancer cell's mutant p53 to more wild type form, the cell's normal apoptotic mechanisms may be activated, resulting in the cells self-destruction. The use of specific nucleic acid sequences to bind to p53 protein to accomplish this task has some significant advantages. Combinatorial methods can be used to screen a large number of possible RNA sequences. Once effective RNA oligomers are found, it is conceivable that viral vectors can be constructed that can infect cells and express the desired RNA to restore normal function to mutant p53 protein.

During the past year the principal investigator on this project was replaced due to the original investigator leaving employment at SRI International. The use of combinatorial chemistry to find an RNA sequence that can bind to the p53 protein was continued but focused on one critical part of the target instead of the total p53.

The carboxyl terminus of the p53 protein has been demonstrated to negatively regulate its specific DNA binding ability. A synthetic 22-mer peptide from the carboxyl terminus (residues 361-382) of p53 protein can restore the DNA binding and transcriptional activity of some mutant p53 proteins (Selinova *et al.*, 1997). Antibodies directed at the carboxyl terminus of p53 protein have been shown to enhance the specific DNA binding ability of wild-type p53 protein and to restore the ability of mutant p53 protein to bind DNA(Hupp *et al.*, 1992, Halazonetis and Kandil, 1993, Niewolik *et al.*, 1995, and Abarzua *et al.*, 1996). When single chain Fv fragments derived from monoclonal antibodies directed against p53 is expressed in human tumor cells, their mutant p53 proteins's transcriptional activity was restored *in vivo* (Fromentel, *et al.*, 1999).

Rather than using the entire p53 protein molecule as a target for the selection of RNA oligomers, a synthetic 22-mer peptide was made and purchased from Bachem Bioscience Inc. This peptide corresponds to residues 361-382 of the human p53 protein (See Figure 1). Since this peptide is positively charged it makes a good candidate for the use of combinatorial methods to select a RNA oligomer. The template used to produce the random RNA oligomer is shown in Figure 2. The T7 RNA polymerase template containing a 40-base-long random sequence is used to generate a ^{32}P-labeled set of RNA oligomers. This RNA is then incubated with the 22–mer p53 peptide and electrophoresed through a non denaturing acrylamide gel.

Any RNA that binds to the peptide is retarded by the net reduction in charge and an increase in the size of the complex. The positions of the radio labeled RNA are detected by phosphor imaging. See Figure 3 for an example. The RNA that is retarded by binding to the peptide is excised from the gel and eluted. This RNA that is enriched for species that bind to the p53 peptide is then converted back into a T7 RNA polymerase template by reverse transcriptase-PCR using the primers in Figure 2. This new template is then used again to generate more RNA for another round of selection. This procedure can be repeated any number of cycles.

As of the end of the May 14th, 1999 reporting period five cycles have been completed. After several more cycles have been completed, the double stranded DNA product from the RT-PCR reaction will be ligated into a pBluescript plasmid vector and the resulting clones sequenced using an Applied Biosystems 310 automated capillary electrophoresis sequencer. The sequences will be examined for common features and individual clones will be selected for RNA transcription and the determination of their relative binding abilities to the p53 protein. The human p53 gene cloned into a baculovirus expression vector that was described in the last report will be used as a source for this protein.

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Halazonetis, T. D., and Kandil, A. N. (1993) *EMBO J.,* **12**, 5057-5064.

Hupp, T. R. *et al.* (1992) *Cell* ,**71**, 875-886.

Niewolik, D. *et al.* (1995) *Oncogene,* **10**, 881-890.

Selinova, G. *et al.* (1997) *Nature Medicine,* **3**, 632-638.

Figure 1. Target peptide corresponds to residues 361-382 of the human p53 protein.

H-Gly-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-Ser-Lys-Lys-Gly-Gln-Ser-Thr-Ser-Arg-His-Lys-Lys-OH

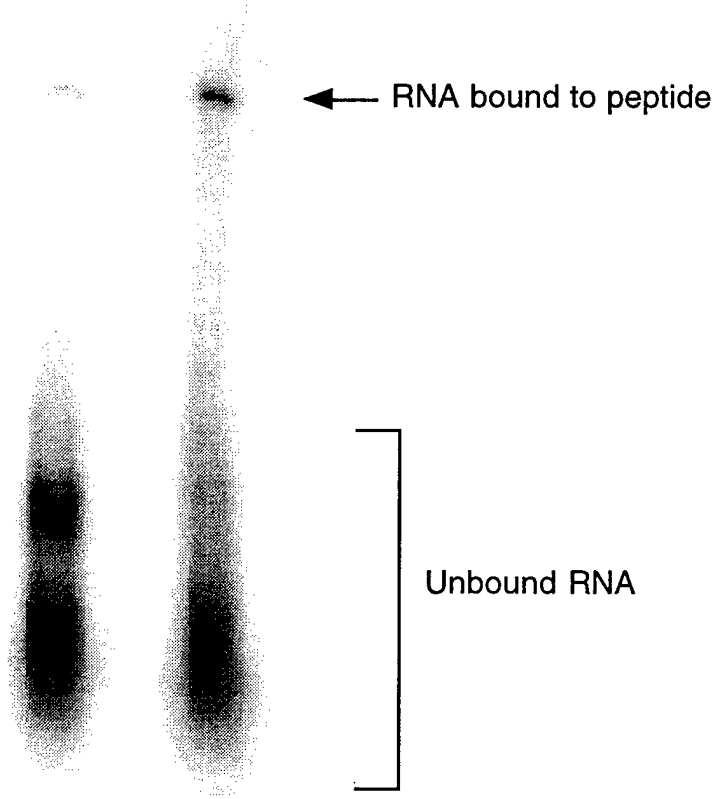
Figure 2. T7 transcription template for RNA oligomer and PCR primers. The T7 promoter is underlined.

5'<u>TAATACGACTCACTATAGGG</u>CGAATTCGGGTT$(N)_{40}$CCCTTTAGTGAGGGTTAATT
3'ATTATGCTGAGTGATATCCCGCTTAAGCCCAA$(N)_{40}$GGGAAATCACTCCCAATTAA

5'-primer 5'TAATACGACTCACTATAGGGCGAATTCGGGTT

3'-primer 5'AATTAACCCTCACTAAAGGG

Figure 3. Non denaturing gel electrophoresis of the fifth round of selection of RNA sequences that bind to the p53 22-mer peptide. The left lane contained the RNA without any peptide. The right lane contained 100 ng of the peptide.



Key Research Accomplishments

- Refocused project on selecting RNAs that bind specifically to a 22-mer peptide rather than the entire molecule. This peptide contains the sequences critical for the DNA binding functions of the p53 protein.

- Performed 5 rounds of selection of RNA oligomers that bind the p53 peptide.

- Wild-type p53 protein produced from a baculovirus expression vector. This will be used to test whether RNA species that bind to the 22-mer peptide will also bind to the intact protein.

- No manuscripts have yet been prepared on this research. After sequences have been found that have significant binding to the peptide and to the whole p53 protein a paper will be submitted.