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The goal of this project is to test and to develop a new class of drugs, polyamide, which can selectively and effectively inhibit the function of transcription factors whose aberrant regulation of gene expression is associated with human breast cancer. The Her2/neu oncogene promoter is selected as a model for this study. The key approach is to prevent the binding of two transcription factors, ESX and AP-2, to the consensus DNA binding sites contained within the Her2/neu promoter resulting in inhibition of transcription factor function. If successful, the drugs will be able to prevent the targeted transcription factors from binding to DNA and thus they cannot enhance the activity of the proteins associated with the cancer cell. These new types of drugs have potential to be designed specifically to block the cellular proteins that are implicated in human breast cancers. Not only will these drugs have the potential to become a new type of anticancer agent they may also be used as tools to identify other proteins involved in breast cancer.

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FOREWORD

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NA 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).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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NA In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Lory Vogley 9/18/99

PI - Signature Date

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INTRODUCTION

Transcription factors are potential targets for antitumor agents because abnormal regulation of gene expression plays an important role in cancer. The ability to preferentially block gene expression by interfering with transcription factor-DNA complexes could be a powerful tool for explaining how aberrant gene expression contributes to neoplastic phenotypes. The strategy for developing gene-specific transcription inhibitors is to target a DNA binding by antitumor drugs. Several drugs were involved in this study. We will investigate a new class of DNA minor groov binding ligands, pyrrole-imidazole polyamides (PA), as potential transcription factor-specific inhibitors of gene expression. Recently, polyamides designed to interfere with TFIIIA binding to its promoter response element were shown to be potent and specific inhibitors of 5S RNA gene transcription. Such compounds have also been shown to specifically inhibit the replication of endogenously integrated virus within the genome of human blood cells. Polyamides specifically targeted to the Ets and the AP-2 binding sites within the promoter of the Her2/neu oncogene were specially focused. Deregulation of Her2/neu expression is associated with 20-30% of primary human breast cancers. As comparison, some other DNA binding drugs, such as distamycin and chromomycin A3, are also tested at the same condition. In this project we will also test the combination of two drugs each targeting the different transcription factor binding site within the Her2/neu promoter. Progress toward creating effective inhibitors will be evaluated with a prioritized sequence of cell-free and cellular assays. The initial assessment will employ a cell-free gel mobility shift assay to determine the ability of drugs to inhibit transcription factor-DNA complex formation. Agents found to be effective and selective in reducing complex formation will then be tested for their ability to specifically inhibit promoter function in cell-free and cellular assays. This information will contribute to the right direction to manipulate the chemistry of compounds and further enhance their potency and specificity.

PROGRESS REPORT:

Pyrrole and imidazole containing polyamides (PA) represent a new class of synthetic DNA binding ligands with remarkably high affinity and sequence-specificity. The code for their sequence-specific DNA recognition is based on a side-by-side pairing of the heterocyclic amino acid units within the minor groove of DNA. In this project, the activities of two polyamides, PA-2 and PA-10, were first tested both in cell-free and in whole cell system. PA-2 was designed to bind immediately downstream of the ESX core binding site while PA-10 was designed to bind the AP-2 binding site of the Her2/neu promoter.

The quantitative footprint titration analysis showed that PA-2 and PA-10 bind to their target sites with equilibrium association constant of $1.4x10^{10}$ M^{-1} and $8.7x10^{10}$ M^{-1} respectively. In this study, electrophoretic mobility shift assay was used to test the ability of each drug to inhibit its target binding site within the Her2/neu promoter. Incubation of PA-2 or PA-10 with a DNA oligonucleotide containing the binding site from Her2/neu promoter followed by the addition of ESX or AP-2 protein resulted in a concentration-dependent inhibition of transcription factor-DNA complex formation. Ten nM of PA-2 inhibited complex formation up to 95 % while 1 nM resulted in a detectable decrease in complex formation. Eight nM of PA-10 inhibited complex formation 95% while as little as 0.5 nM resulted in a detectable decrease in complex formation. The result showed that 2.2 nM of PA-2 and 1.2 nM of PA-10 are required to inhibit complex formation by 50%.

To determine whether the effects of drugs on transcription factor-DNA complex formation resulted in an ability to influence biological function, *in vitro* transcription assays were performed. The plasmid DNA containing the Her2/neu promoter was used as a template and SKBR-3 nuclear extracts as transcription machinery, resulting in a 760 base transcript. Drugs were incubated with DNA template prior to the addition of nuclear extracts and nucleotides. The result demonstrates the ability of drugs to block synthesis of the 760 base transcript in a concentration-dependent manner. For example, 0.5 μM PA-10 inhibited transcript synthesis by 95% while 0.1 μM blocked the transcript less than 30% compared with the untreated control. Drug concentrations of 2 μM for distamycin, 0.5 μM for PA-2, and 0.2 μM for PA-10 were required to inhibit transcription by 50%. There was some evidence of the production of partial transcripts when higher drug concentrations were used. Comparison of the abilities of the polyamides with that of distamycin to inhibit transcript synthesis reveals that the potency of former compounds are about one order of magnitude greater. The very high affinity constant of polyamides likely contributes to the difference in activity. Studied are now underway to detect the effect of combination of drugs targeting both transcription binding sites within the Her2/neu promoter in the *in vitro* transcription assays.

To evaluate the effectiveness of polyamides as Her2/neu transcription inhibitions in the whole cell system, Northern analysis and RNA synthesis assays were performed. AP-2, AP-10 along with several other drugs were tested in the Northern analysis. Treatment with 50 μM of distamycin for 48 hours decreases the Her2/neu mRNA of SKBR-3 cells

by 50% while the mRNA of GAPDH is probed at the same time as comparison. However, no significant change of Her2/neu mRNA was detected after up to 20 μM polyamide treatment. The result in our lab also strongly indicated that majority of the polyamide was located in the nucleus. It is unclear why the polyamides are all over the nucleus but do not show any functional activity.

In the process of the RNA synthesis assays, we have developed a more sensitive condition to detect the drug effects. When the traditional method for this assay was applied, the result showed that some drugs including distamycin and polyamides not only inhibit uridine incorporation, but also decrease uridine uptake at a comparable level. This observation makes it difficult to study the effect of RNA synthesis by using the uridine incorporation assay. The separation of the effects for uridine uptake from incorporation was greatly improved by adding 25 μM of cold uridine in the medium. The high uridine concentration at a saturated level for the uridine uptake helps to eliminate the drug effect on the uridine uptake.

The Her2/neu mRNA was reported having a relative long half-life, at about 7.5 hours. After 7 hours, the protein protection of the Her2/neu mRNA was also reported, which make its half-life even longer. To detect the drug activity effectively, establishment of stable cell line containing the Her2/neu promoter with the luciferase report is in process. If successful, it will allow us to detect the drug effects in a more convenient and sensitive way.