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TITLE: Polyamine Analogues as Novel Anti-HER Family Agents in Human Breast Cancer

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14. ABSTRACT Elevated levels of all three naturally occurring polyamines, spermine, spermidine and putrescine, have been found in breast cancer tissues. Polyamine analogues have been shown to inhibit cell growth and in some cases induce apoptosis. My studies have demonstrated the ability of Progen (PG)-11144 and other oligoamines to inhibit cell growth in human breast cancer cell lines. These oligoamines can also suppress epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2) and estrogen receptor(ER)-alpha protein in multiple human breast cancer cell lines. Low dose long term exposure of T47D cells to PG-11144 decreases heat shock protein (HSP) 90 in addition to several HER family member proteins while not decreasing other receptors such as vitamin D receptor (VDR) and retinoic acid receptor (RAR)-beta. In addition to T47D cells, RAR-beta protein levels were maintained in MCF7 cells treated with PG-11144. Oligoamines PG-11144 and PG-11150 inhibit the growth of MCF7 and T47D breast cancer cell lines in a time and dose dependent manner. This project demonstrates that polyamine analogues are novel anti-HER family agents and contributes to their cytotoxicity in human breast cancer cells.					
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Introduction

The polyamines, spermine, spermidine and putrescine, are naturally occurring aliphatic cations that are essential for normal cell growth and differentiation (1,2). A number of studies have shown that polyamines play a key role in carcinogenesis and malignant transformation (3,4), thus making the polyamine pathway a therapeutic target of interest. Increased levels of all three naturally occurring polyamines have been found in many types of cancers, including breast cancer (5). Polyamine analogues have been developed to mimic the three natural polyamines and exploit the self-regulatory properties of polyamines. Treatment of human breast cancer cell lines with polyamine analogues has been shown to inhibit cell growth and in some cases induce apoptosis (6-8). One subset of polyamine analogues are conformationally restricted and long chain analogues named oligoamines (9). Our laboratory has focused on the oligoamine, Progen (PG)-11144, because of its effects in human breast cancer cells. Our studies have shown that oligoamines, especially PG-11144, inhibit growth of human breast cancer cell lines in culture and in mouse xenograft models. My studies have demonstrated the ability of PG-11144 to downregulate two members of the human epidermal growth factor receptor (HER) family: epidermal growth factor receptor (EGFR/HER1) and HER2. The overexpression of EGFR and HER2 is usually associated with more aggressive tumors and worse prognosis (10,11). Preliminary studies have also shown that PG-11144 inhibits cell growth in human breast cancer cell lines. Thus, the hypothesis underlying this proposal is that oligoamines are novel anti-HER family agents and oligoamine-induced down regulation of HER family members contributes to their cytotoxicity in human breast cancer cell lines. The studies proposed here are designed to elucidate the molecular mechanisms by which polyamine analogues inhibit the expression and activity of the HER family. The results of these experiments will lead to a better understanding of the cytotoxic action of polyamine analogues against human breast cancer and provide valuable information about the potential clinical application of oligoamines.

Body

Specific Aim1: To investigate the mechanisms by which oligoamines downregulate EGFR and HER2 expression.

It was previously shown that low dose long term exposure of T47D cells to PG-11144 decreased several HER family member proteins while not decreasing other receptors such as vitamin D receptor (VDR) and retinoic acid receptor (RAR)-beta. PG-11144 also has the ability to decrease heat shock protein (HSP) 90, which can stabilize HER2 protein.

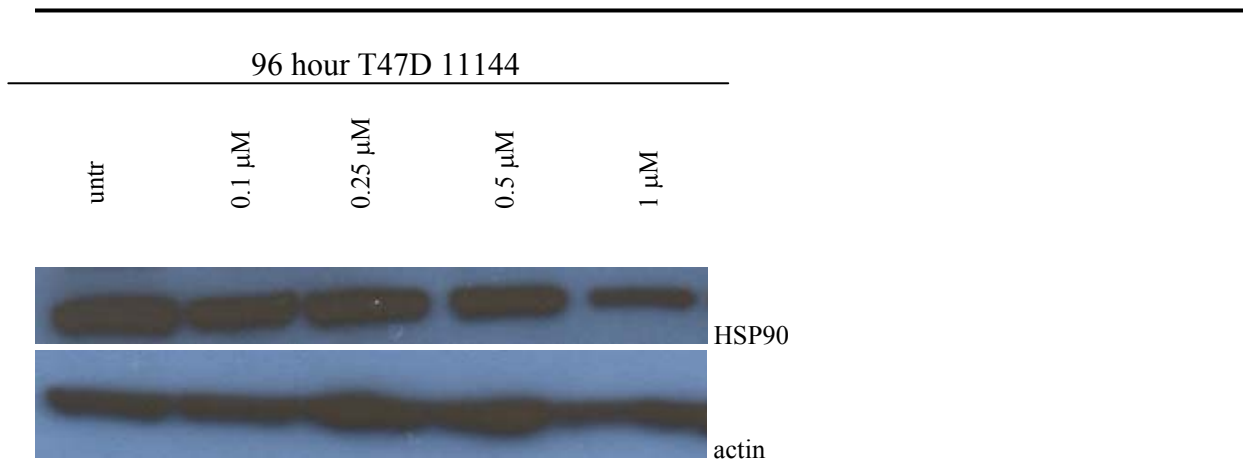


Figure 1. Western blot analysis of T47D breast cancer cell line treated with low doses of PG-11144 for 96 hours. An antibody for HSP90 was used. Actin was used as a control to ensure equal loading.

Previously no change in RAR-beta protein levels were observed in T47D cells treated with PG-11144. RAR-beta protein levels were also examined in MCF7 cells.

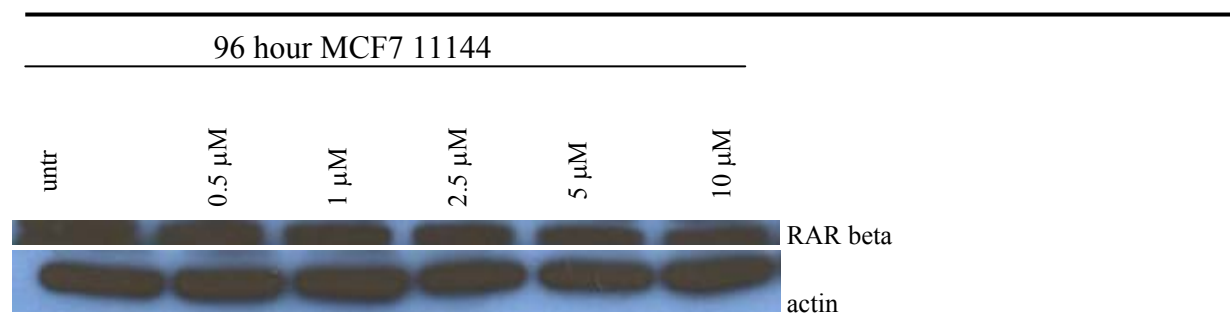


Figure 2. Western blot analysis of MCF7 breast cancer cell line treated with PG-11144 for 96 hours. An antibody for RAR-beta (51kDa) was used. Actin was used as a control to ensure equal loading.

In addition to T47D cells, RAR-beta protein levels were maintained upon PG-11144 treatment in MCF7 cells. This result further suggests that the decrease in HER family member proteins is not due to non-specific drug cytotoxicity.

Reverse transcriptase-polymerase chain reaction (RT-PCR) experiments were performed with PG-11144 treated T47D cells at 96 hours.



Figure 3: Reverse transcriptase-polymerase chain reaction (RT-PCR) results of T47D cells treated with various doses of PG-11144. RNA was isolated from oligoamine treated cells using the Trizol method. M-MLV reverse transcriptase was used to generate cDNA, followed by PCR to assess mRNA expression.

Decreased expression of HER2 mRNA in conjunction with decreased protein expression would imply that polyamine analogues regulate HER2 at the transcriptional level. However, at 96 hours mRNA levels were maintained suggesting another level of regulation is involved.

Previous studies using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays confirm that PG-11144 inhibits the growth of several breast cancer cell lines. Further studies included multiple time points and an additional oligoamine, PG-11150.

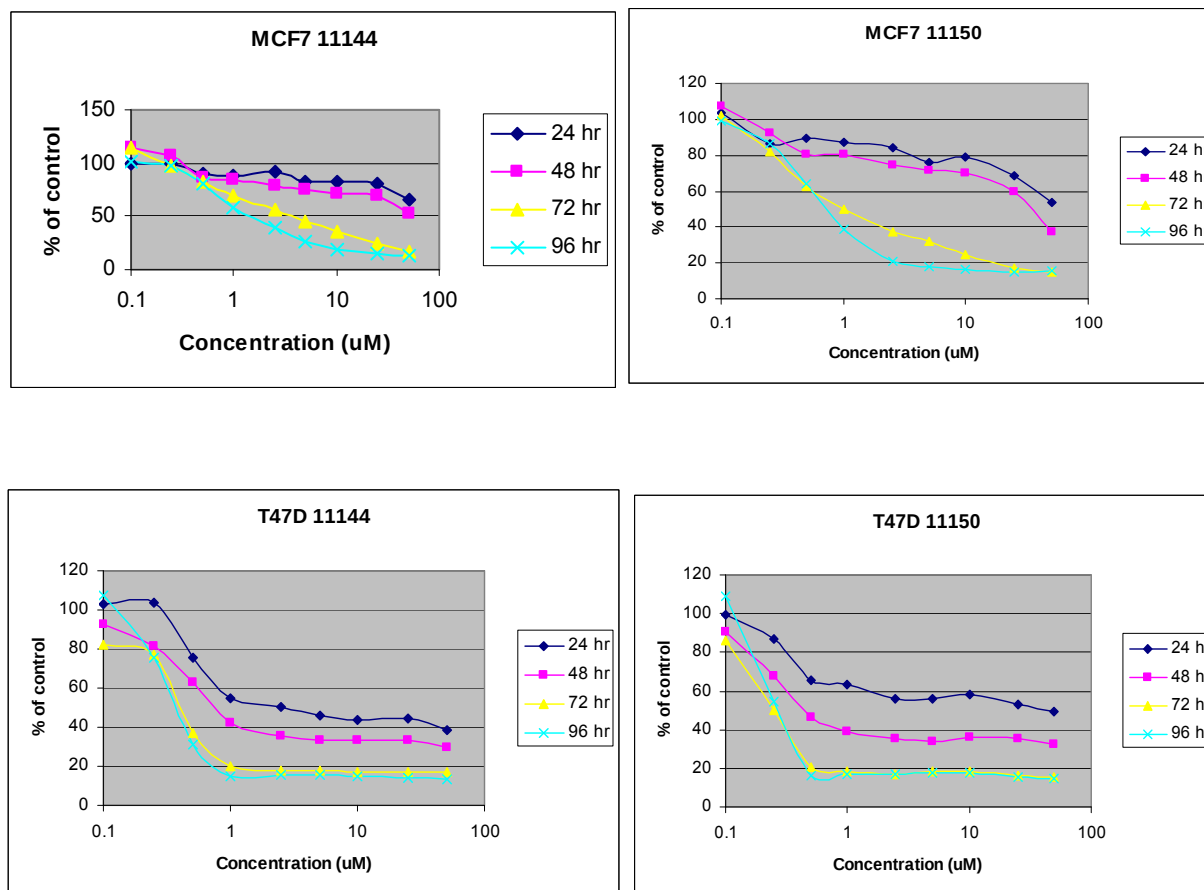


Figure 4: MTT Assays were performed on MCF7 and T47D breast cancer cell lines using oligoamines PG-11144 and PG-11150 at 24, 48, 72 and 96 hours. The range of oligoamine concentrations were 0.1 to 50 μ M.

MTT assays confirm both PG-11144 and PG-11150 inhibits the growth of MCF7 and T47D breast cancer cell lines in a time and dose dependent manner: the longer the cell line's exposure to oligoamines and the greater the oligoamine concentration the greater the effect on growth inhibition.

Specific Aim 2: To determine the role of EGFR and HER2 in oligoamine induced cytotoxicity.

I have recently obtained a HER2 overexpressing MCF7 cell line and will be completing the following experiments. These cells will be treated with oligoamines and compared using a series of assays, including MTT assays. Fluorescence activated cell sorting (FACS) analysis will be used to examine cell cycle distribution. Apoptosis will be examined via DNA fragmentation and Western blots of apoptotic proteins such as cytochrome c, fas, and caspases 3 and 9.

Specific Aim 3: To determine if oligoamines can overcome endocrine resistance.

Experiments using tamoxifen-resistant and sensitive human breast cancer cell lines will address the goals set forth in this aim. Experiments similar to those aforementioned with the MCF7 HER2 overexpressing cell line will also be completed in these cell lines.

As a member of the Cellular and Molecular Medicine Graduate Program (CMM), which integrates the fields of medicine and basic science, I have participated in CMM sponsored events such as the annual CMM Fall Retreat and Distinguished Lecture Series. My formal training consists of weekly lab meetings to present results and discuss future directions, a weekly breast cancer translational research conference, and annual courses addressing the fundamentals of cancer biology and novel approaches to cancer prevention and therapeutics. Moreover, there have been multiple opportunities to present my research at numerous events including the Breast Cancer Research Program Seminar and the Breast Cancer Research Program Retreat. Most recently, I presented my research at the Polyamines Gordon Research Conference.

Key Research Accomplishments

- Low dose long term exposure of T47D cells to PG-11144 decreases heat shock protein (HSP) 90 in addition to several HER family member proteins while not decreasing other receptors such as vitamin D receptor (VDR) and retinoic acid receptor (RAR)-beta.
- In addition to T47D cells, exposure of MCF7 cells to PG-11144 does not decrease RAR-beta protein.
- At 96 hours T47D HER2 mRNA levels were maintained suggesting the decrease in HER2 protein is not primarily due to changes at the transcriptional level.
- MTT assays confirm both PG-11144 and PG-11150 inhibits the growth of MCF7 and T47D breast cancer cell lines in a time and dose dependent manner.

Reportable Outcomes

Posters and Presentations:

Polyamine Analogues as Novel Anti-HER Family Agents in Human Breast Cancer.

Richards T. Breast Cancer Program Seminar, Johns Hopkins University, January 2009.

Polyamine Analogues as Novel Anti-HER Family Agents in Human Breast Cancer.

Richards T. Breast Cancer Program Retreat, Johns Hopkins University, June 2009.

Polyamine Analogues as Novel Anti-HER Family Agents in Human Breast Cancer.

Richards T. Polyamines Gordon Research Conference, June 2009.

Conclusions

Low dose long term exposure of T47D cells to PG-11144 decreases heat shock protein (HSP) 90 in addition to several HER family member proteins while not decreasing other receptors such as vitamin D receptor (VDR) and retinoic acid receptor (RAR)-beta. Exposure of MCF7 and T47D cells to PG-11144 does not decrease RAR-beta protein further suggesting that the decrease in HER family member proteins is not a result of non-specific drug cytotoxicity. At 96 hours T47D HER2 mRNA levels were maintained suggesting the decrease in HER2 protein is not primarily due to changes at the transcriptional level. MTT assays confirm both PG-11144 and PG-11150 inhibits the growth of MCF7 and T47D breast cancer cell lines in a time and dose dependent manner. Further experiments with a HER2 overexpressing MCF7 cell line and MCF7 tamoxifen resistant cell line will explore the potential clinical application of oligoamines.

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