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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 PG-11144 and other oligoamines to inhibit cell growth in human breast cancer cell lines. These oligamines 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. This downregulation occurred with the 24 hour treatment of T47D cells. These studies were extended to evaluate the effects of long term, low dose exposure of PG-11144 in T47D cells; downregulation was observed in HER family protein and RNA levels. This project will demonstrate 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 completion of this project will also provide valuable information about the potential clinical application of oligoamines.					
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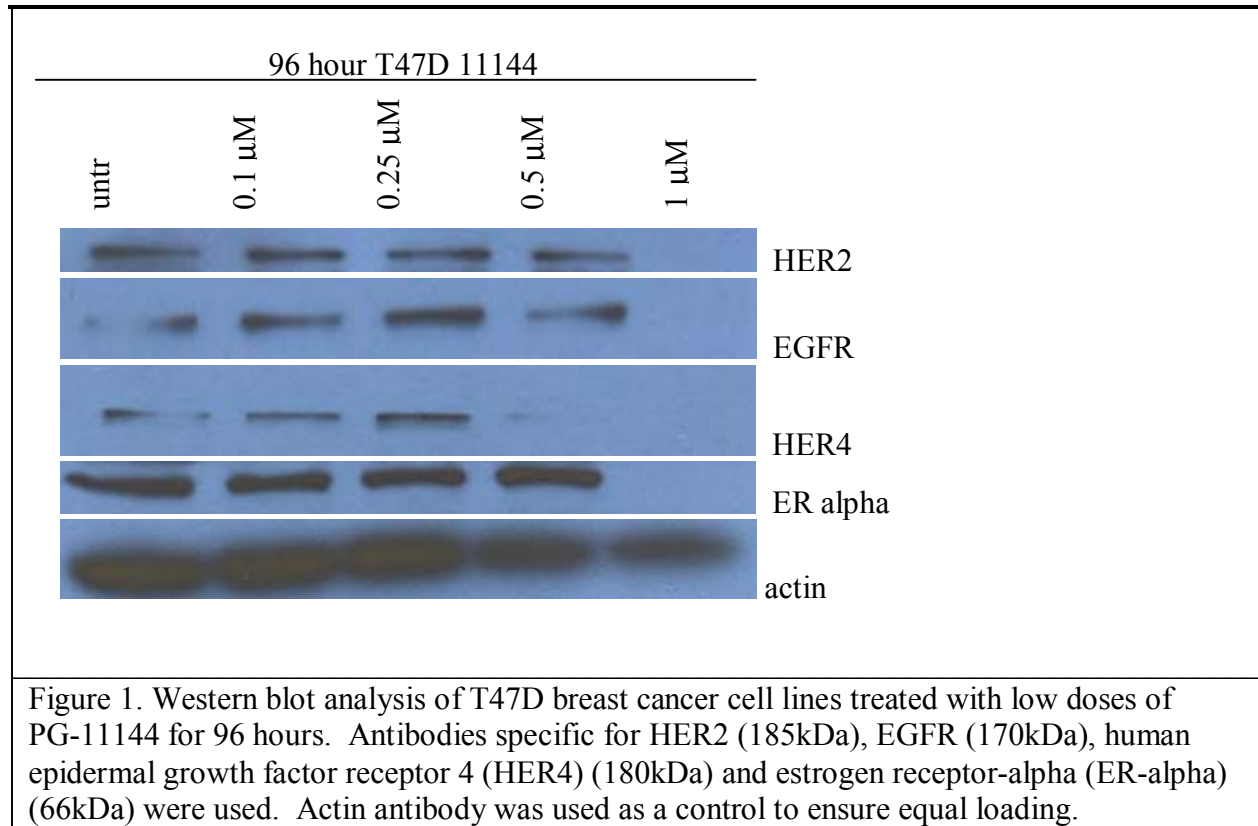
INTRODUCTION

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) and human epidermal growth factor receptor 2 (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.

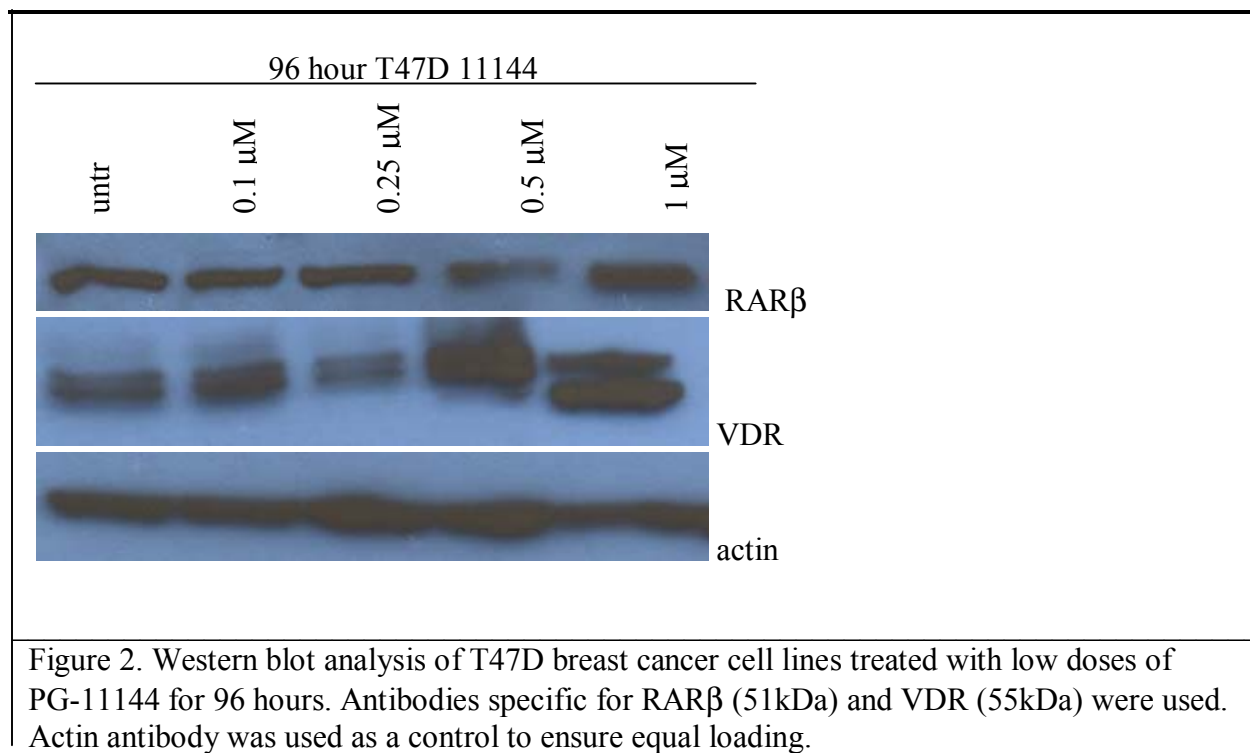
BODY

Specific Aim 1: To investigate the mechanisms by which oligoamines downregulate EGFR and HER2 expression.

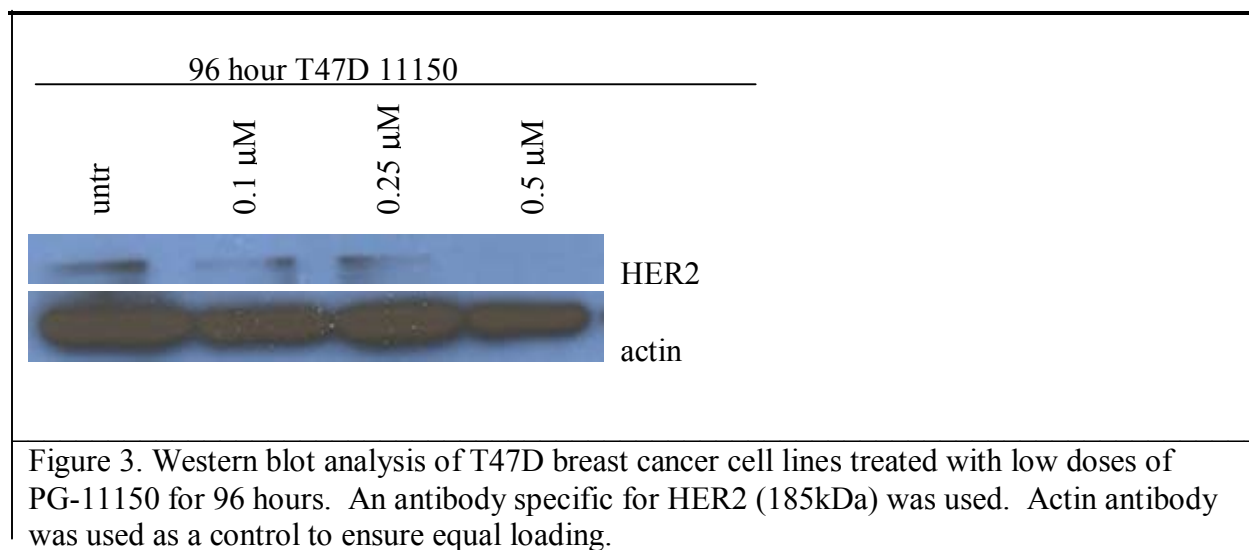
My studies have shown that the oligoamine, PG-11144, downregulates the expression of EGFR and HER2 protein in several human breast cancer cell lines as documented by Western blot analysis. In particular, this downregulation occurred with the 24 hour treatment of T47D cells. These studies were extended to evaluate the effects of long term, low dose exposure of PG-11144 in T47D cells. (Figure 1 and Figure 2).



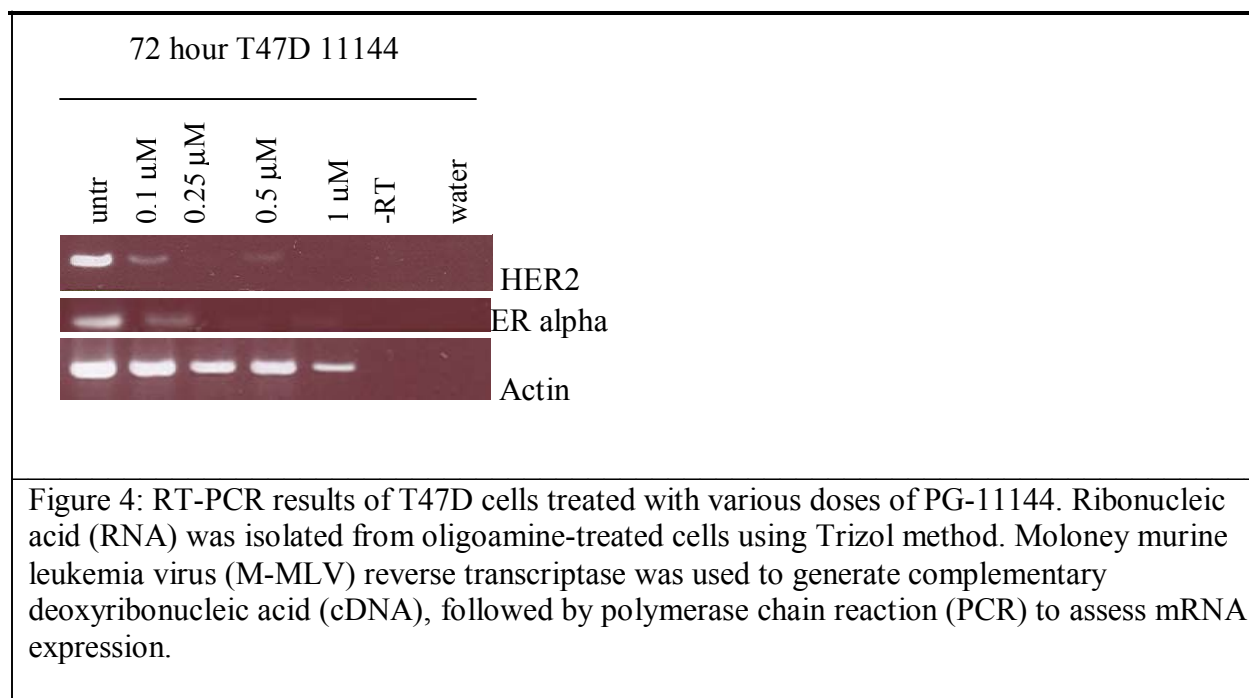
96 hour 1 μ M treatment of T47D cells decreased HER2, EGFR, HER4 and ER-alpha expression. The effects of PG-11144 on other receptors, retinoic acid receptor beta (RAR β) and vitamin D receptor (VDR) were also evaluated.



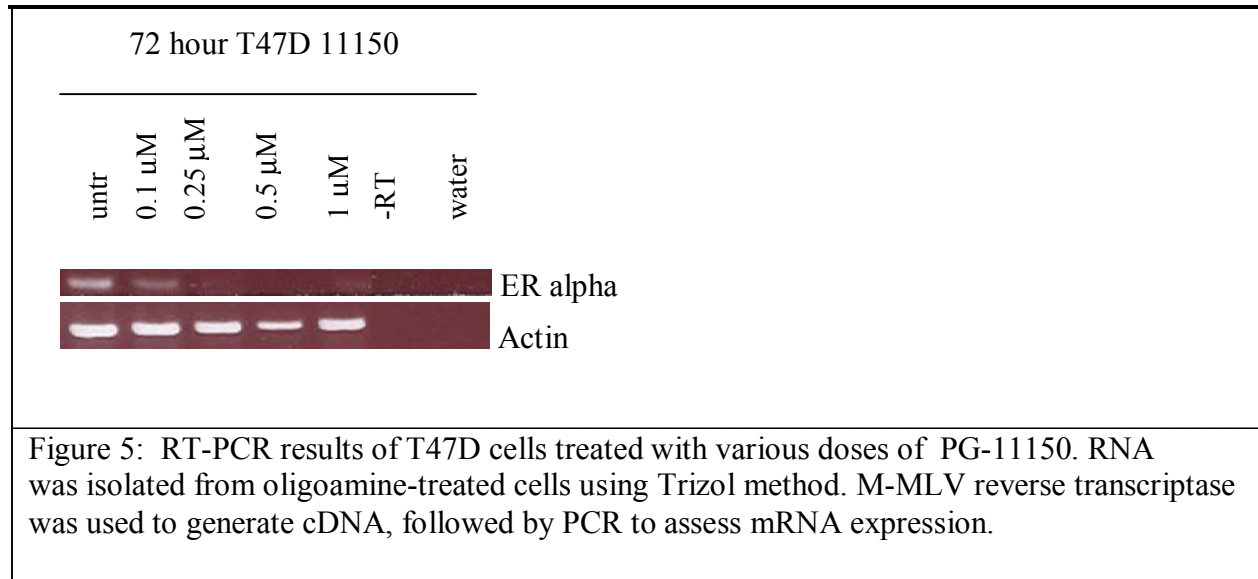
The 96 hour low dosage treatment of T47D cells with PG-11144 does not decrease RAR β or VDR suggesting that the results in Figure 1 are not due non specific drug toxicity. The effect of the oligoamine, PG-11150, on 96 hour T47D cells on HER2 protein was examined.



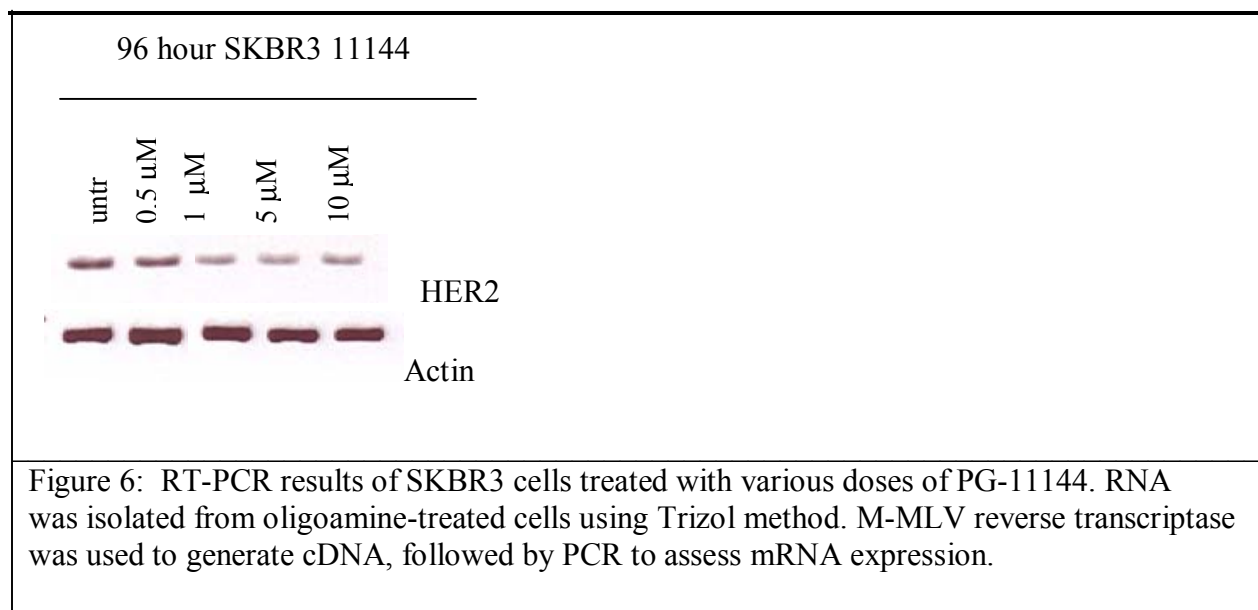
Like PG-11144, PG-11150 can decrease HER2 protein expression. However the downregulation is observed at a lower dosage, 0.5 μ M. Reverse transcriptase polymerase chain reaction (RT-PCR) experiments were performed to determine if messenger ribonucleic acid (mRNA) levels correlated with protein levels.



A decrease in both T47D HER2 and ER alpha mRNA was observed in long term, low dose PG-11144 exposure. A similar experiment was done with another oligoamine, PG-11150.



Low doses of PG-11150 also has the ability to downregulate ER alpha mRNA with long exposure times. RT-PCR was performed on HER2 overexpressing SKBR3 cells treated with PG-11144.



In SKBR3 cells high dosage PG-11144 downregulates HER2 mRNA less effectively than low dosage PG-11144 in T47D cells. This may be due to the HER2 overexpression in SKBR3 cells. Real time polymerase chain reaction studies will be completed to quantify these results and examine the effects of oligoamines on other cell lines.

Specific Aim 2: To determine the role of EGFR and HER2 in oligoamine-induced cytotoxicity. Future experiments will address the goals set forth in this aim.

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

Future experiments will address the goals set forth in this aim.

As a member of the Cellular and Molecular Graduate Program (CMM) I participate in CMM sponsored events including the CMM Fall Retreat and Distinguished Lecture Series. My formal training consists of courses addressing the fundamentals of cancer biology and novel approaches to cancer prevention and therapeutics, bi-weekly lab meetings to present results and discuss future directions and a weekly breast cancer translational research conference. I have also had the opportunity to present my research at Johns Hopkins events such as the CMM Retreat, Breast Cancer Program Seminar and the Breast Cancer Program Retreat.

KEY RESEARCH ACCOMPLISHMENTS

- Low dose, long term exposure of T47D cells to PG-11144 decreases ER-alpha protein along with multiple members of the HER family.
- Low dose, long term exposure of T47D cells to PG-11144 does not decrease the protein levels of other receptors such as RAR β and VDR.
- In T47D cells HER2 and ER alpha mRNA levels are downregulated with low dose, long term PG-11144 exposure.
- PG-11144 decreases HER2 mRNA levels in SKBR3 cells.

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, June 2008.

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

Richards T. Breast Cancer Program Retreat, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, June 2008.

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

Richards T. Department of Defense Breast Cancer Research Program Era of Hope Meeting, July 2008.

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

Richards T. Cellular and Molecular Medicine Program Fall Retreat, Johns Hopkins University, September 2008.

CONCLUSIONS

Low dose, long term exposure of T47D cells to PG-11144 decreases ER-alpha protein along with multiple members of the HER family. However, this decrease is not observed in protein levels of other receptors such as levels of other receptors such as RAR β and VDR. This result suggests that the effects seen on HER family members and ER-alpha is not a result of non-specific toxicity. In T47D cells, HER2 and ER alpha mRNA levels are also downregulated with low dose, long term PG-11144 exposure. PG-11144 also decreases HER2 mRNA levels in HER2 overexpressing SKBR3 cells. Further experiments will be completed to further assess the effects of oligoamines on human breast cancer cell lines and compare the effects of PG compounds on wild type MCF-7 and MCF-7 tamoxifen resistant lines. The completion of this project 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.

REFERENCES

1. Davidson NE, Hahm HA, McCloskey DE, Woster PM & Casero RA Jr. Clinical aspects of cell death in breast cancer: the polyamine pathway as a new target for treatment. *Endocrine-Related Cancer*. 6:69-73, 1999.
2. Marton LJ & Pegg AE. Polyamines as therapeutic targets. *Annual Review of Pharmacology and Toxicology*. 35:55-91. 1995.
3. Thomas T & Thomas TJ. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. *Cellular and Molecular Life Sciences*. 58:244-58, 2001.
4. Tabib A & Bachrach U. Polyamines induce malignant transformation in cultured NIH 3T3 fibroblasts. *The International Journal of Biochemistry & Cell Biology*. 30:135-46, 1998.
5. Wallace HM, Duthie J, Evans DM, Lamond S, Nicoll KM & Heys SD. Alterations in polyamine catabolic enzymes in human breast cancer tissue. *Clinical Cancer Research*. 6: 3657-3661, 2000.
6. Pledgie A, Huang Y, Hacker A, Zhang Z, Woster PM, Davidson NE, & Casero RA Jr. Spermine oxidase SMO(PAOh1), not N1-acetylpolyamine oxidase PAO, is the primary source of cytotoxic H₂O₂ in polyamine analogue-treated human breast cancer cell lines. *Journal of Biological Chemistry*. 280:39843-51. 2005.
7. McCloskey DE, Casero RA Jr, Woster PM & Davidson NE. Induction of programmed cell death in human breast cancer cells by an unsymmetrically alkylated polyamine analogue. *Cancer Research*. 55: 3233-6, 1995.
8. Casero RA Jr. & Woster PM. Terminally alkylated polyamine analogues as chemotherapeutic agents. *Journal of Medicinal Chemistry*. 44: 1-26, 2001.
9. Huang Y, Pledgie A, Casero RA Jr. & Davidson NE. Molecular mechanisms of polyamine analogs in cancer cells. *Anti-cancer Drugs*. 16:229-41, 2005.
10. Isola JJ, Holli K, Oksa H, Teramoto Y & Kallioniemi OP. Elevated erbB-2 oncoprotein levels in preoperative and follow-up serum samples define an aggressive disease course in patients with breast cancer. *Cancer*. 73:652-658, 1994.
11. Nicholson RI, Gee JM & Harper ME. EGFR and cancer prognosis. *European Journal of Cancer*. 37: S9-S15, 2001.