Overcoming tamoxifen resistance: A primer for "Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen"

Jennifer Thomalla

Purpose

To discuss and explain Hurtado *et al.* (2008) "Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen" which offers a description of the regulation of the *ERBB2* gene at the transcriptional level. Tamoxifen resistance can occur via crosstalk between ER/ERBB2. Understanding the regulation of *ERBB2* enables researchers to develop methods to knockdown expression of ERBB2, thus decreasing the amount of ER/ERBB2 crosstalk ultimately restoring tamoxifen sensitivity.

*Biology Department, University of Minnesota Duluth,

Introduction

Approximately one in eight women will develop breast cancer in their lifetime (cancer.gov). Several histologic varieties of the disease exist, each characterized by unique biomarkers. Estrogen receptor positive (ER+) breast cancer, which expresses estrogen receptors, relies on estrogen to grow and accounts for approximately 70% of all breast cancer cases (cancer.gov). The choice of therapy administered to the patient depends on the ER status. If the patient is ER+, hormone therapy can be an effective treatment. If the patient does not have ERs, hormone therapy is ineffective.

Estrogen is a hormone necessary for several normal physiological processes in female organs such as development, reproduction, lactation, cell growth, and cell differentiation (Jordan 2002). Estrogen is produced when regulative hormones signal to the ovaries that estrogen production is appropriate for normal body function (Jordan 2002). Estrogen travels through the body via the bloodstream affecting certain female organs (Samavat and Kurzer 2015). These target tissues recognize estrogen by using ERs, which have a high binding affinity for estrogen (Zhu and Conney 1998).

Estrogen acts on two nuclear receptors that act as transcription factors: estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ). While the role of ERβ is less clear, the role of ERα in gene transcription has been more extensively studied (Speirs et al 1999). Classical estrogen signaling by ERα commences when estrogen binds to the receptor inducing **dimerization**. The dimerized receptor proteins then bind to short sequences of DNA, estrogen response elements (EREs), in the promoter region of target genes (Osborne and Schiff 2005). **Coactivator proteins** are recruited to the protein complex which induces the activation of gene transcription. ERs can also downregulate gene expression by recruiting **corepressors** to the protein complex (Smith et al 1997).

Dimerization
A reaction that joins two molecular subunits together forming a single dimer.

Coactivators
Proteins that increase gene expression by binding to a transcription factor. Coactivators cannot induce transcription alone.

Corepressors
Proteins that decrease gene expression by binding and inhibiting a transcription factor.

Corresponding Author:
Jennufer Thomalla
thom4878@d.umn.edu

Estrogen receptor positive breast cancer

Cancer cells can develop via several mechanisms. Normally, cells undergo cell division for maintenance of body tissues. To divide into daughter cells, cells must first replicate their DNA before distributing it to the new daughter cells. ERs regulate many genes including ones that drive cell division (Osborne and Schiff 2005). Over time, cells continually divide, increasing the chance of a mutation during DNA replication. When cells that have damaged DNA divide, they pass on the mutation to all daughter cells. Some mutations occur in DNA segments that code for proteins significant to the progression of the cell cycle. If these proteins are changed in such a way that they alter the cell cycle, there can be an overgrowth of cells. These cells continue to divide and pass on the mutated DNA, becoming cancerous and eventually forming a tumor.

Endocrine Therapy and Tamoxifen

Antiestrogen
A substance that inhibits the effects of estrogen.

Endocrine therapy, or hormone therapy, uses synthetic molecules that interfere with the body's natural hormones in order to prevent or treat cancer. It has been employed for over a hundred years as a treatment for breast cancer (Jordan 2002). Endocrine therapy is currently used as a first-line treatment for women with ER+ breast cancer as well as for women who exhibit an elevated risk of developing the disease. The efficacy and general tolerability of endocrine therapy make it a good alternative to chemotherapy.

Several endocrine therapies regulate ERs. One type of these are the selective estrogen receptor modulators (SERMs), including the **antiestrogen** tamoxifen. Tamoxifen works as an antagonist to competitively inhibit estrogen receptors in selective tissues, thereby blocking cancer cell growth. Tamoxifen is currently being used for treatment of ER+ breast cancer and as an adjuvant therapy for prevention of breast cancer in women at high risk of developing the disease (Chang et al 2011).

While tamoxifen is mostly effective and tolerable to patients, there are associated risks. Risks of taking tamoxifen include the development of endometrial cancer, stroke, pulmonary embolism, and deep vein thrombosis (Gail et al. 1999). Premenopausal women are subject to additional risks like premature menopause, bone loss, and loss of fertility. The degree of risk is dependent on individual factors such as race, age, and the duration of treatment (Gail et al. 1999). Tamoxifen can be safely taken for up to ten years, but the risk of developing endometrial cancer increases with time. In addition to these risks, *de novo* and acquired resistance occurs with tamoxifen treatment. Understanding the mechanisms of tamoxifen resistance could bring insight into the development of new treatments to overcome this resistance (Osborne and Schiff 2005).

Tamoxifen resistance via crosstalk between ER and ERBB2

Tamoxifen resistance can occur from crosstalk between the ER and ERBB2 pathways. ERBB2 is a tyrosine kinase receptor that activates downstream kinases and can contribute to increased cell division. The downstream kinases activated by ERBB2 can also phosphorylate ERs. The phosphorylation of ERs and associated coactivators by intracellular kinases switches the ability of tamoxifen to act as an agonist in the tamoxifen-ER complex instead of the normal antagonist role. Tamoxifen normally works as an ER antagonist and competitively inhibits ERs to repress the transcription of genes regulated by the ER. However, when downstream kinases of the ERBB2 receptor activate the ER, tamox-

ifen can work as an agonist and increase transcription of ER regulated genes. The activation of ERs, even in the presence of tamoxifen, triggers gene expression and can cause tumor growth (Arpino et al. 2008). Understanding the regulation of the *ERBB2* gene can inform our understanding of the contribution of ERBB2 to tamoxifen resistance.

Crosstalk is when one signaling pathway affects another pathway. The crosstalk between the ER and ERBB2 pathways is an established mechanism of tamoxifen resistance. Elevated levels of the ERBB2 receptor contribute to tamoxifen resistance. The regulation of *ERBB2* on a transcriptional level was largely undetermined before the Hurtado et al. (2008) study. Hurtado et al. (2008) showed that two proteins, the paired box 2 gene product (PAX2) and amplified in breast cancer 1 (AIB1), competitively regulate *ERBB2*. When PAX2 binds to the ER preventing AIB1 from binding, transcription is repressed. When the opposite occurs and AIB1 binds to the ER, PAX2 is unable to bind and AIB1 activates transcription. Clinically, patients who have more AIB1 and less PAX2 tend to have a worse prognosis with an increased risk of relapse after tamoxifen treatment than patients who have more PAX2 than AIB1 (Hurtado et al. Figure 4 2008). A more complete understanding of the regulatory mechanism of *ERBB2* allows researchers to evaluate strategies that decrease the expression of *ERBB2* which would decrease the crosstalk between ER and ERBB2 and ultimately combat tamoxifen resistance.

Motif
Short, recurring patterns in DNA that provide some kind of biological function.

Transfection
The introduction of nucleic acids into a cell.

Determining the regulation of *ERBB2*: Advancements made by Hurtado et al

Identification of ER binding sites in the ERBB2 gene

Hurtado et al. (2008) studied the regulation of the *ERBB2* gene and ERBB2 protein overexpression to better understand tamoxifen resistance. Understanding *ERBB2* regulation could lead to better breast cancer treatment by preventing *ERBB2* expression to limit ER/ERBB2 crosstalk. The ultimate goal was to understand how decreasing the amount of crosstalk could restore tamoxifen sensitivity in tamoxifen resistant tumors.

Because ERs play an integral role in breast cancer and are key players involved in tamoxifen therapy, the researchers began their study by performing chromatin immunoprecipitation (ChIP) analysis to assess the locations of ER binding sites within the genome (See Figure 1). Via this process, they identified 8,525 ER binding sites. A new ER binding site was identified within an intron of the *ERBB2* gene (Hurtado et al. Figure 1a 2008). They further investigated this particular binding site because of the well-known mechanism of tamoxifen resistance induced by crosstalk between ER and ERBB2.

Role of PAX2

The sequence analysis of the ER binding sites found via ChIP analysis revealed that the ER binding sites have a PAX transcription factor **motif** (Hurtado et al. Figure 1b 2008). This was of interest because PAX2 is expressed in some breast cancers (Muratovska 2003). PAX2 can also mediate tamoxifen stimulated endometrial cancer development (Wu et al. 2005). Hurtado et al. (2008) wanted to identify the role of PAX2 in *ERBB2* regulation.

ChIP analysis was performed to determine the binding of PAX2 to ER binding sites after pretreatment with either tamoxifen or estrogen. PAX2 was recruited to the ER binding site within the *ERBB2* gene after being treated with either tamoxifen or estrogen (Hurtado et al. Figure 1c 2008). However, PAX2 binds to ER binding sites only after treatment with tamoxifen.
The new observations that cells treated

with estrogen recruit PAX2 led the group of researchers to develop the hypothesis that PAX2 is a transcriptional repressor of *ERBB2* gene expression.

A previous study done by Bates and Hurst (1997) showed that the ER downregulates *ERBB2*. Hurtado et al. (2008) hypothesized that the ER binding site within the *ERBB2* gene was a cis-regulatory element (CRE), or region of noncoding DNA that regulates transcription of the nearby gene. They proposed that ER binding to the CRE would repress *ERBB2* expression. To test if the ER binding site is used to repress *ERBB2,* MCF-7 cells were treated with a control, tamoxifen, or estrogen. Reverse transcription polymerase chain reaction (RT-PCR) showed that estrogen treatment as well as the tamoxifen treatment decreased the amount of *ERBB2* mRNA (Hurtado et al.

Antibody
A protein produced in response to and counteracting any specific molecule, called an antigen.

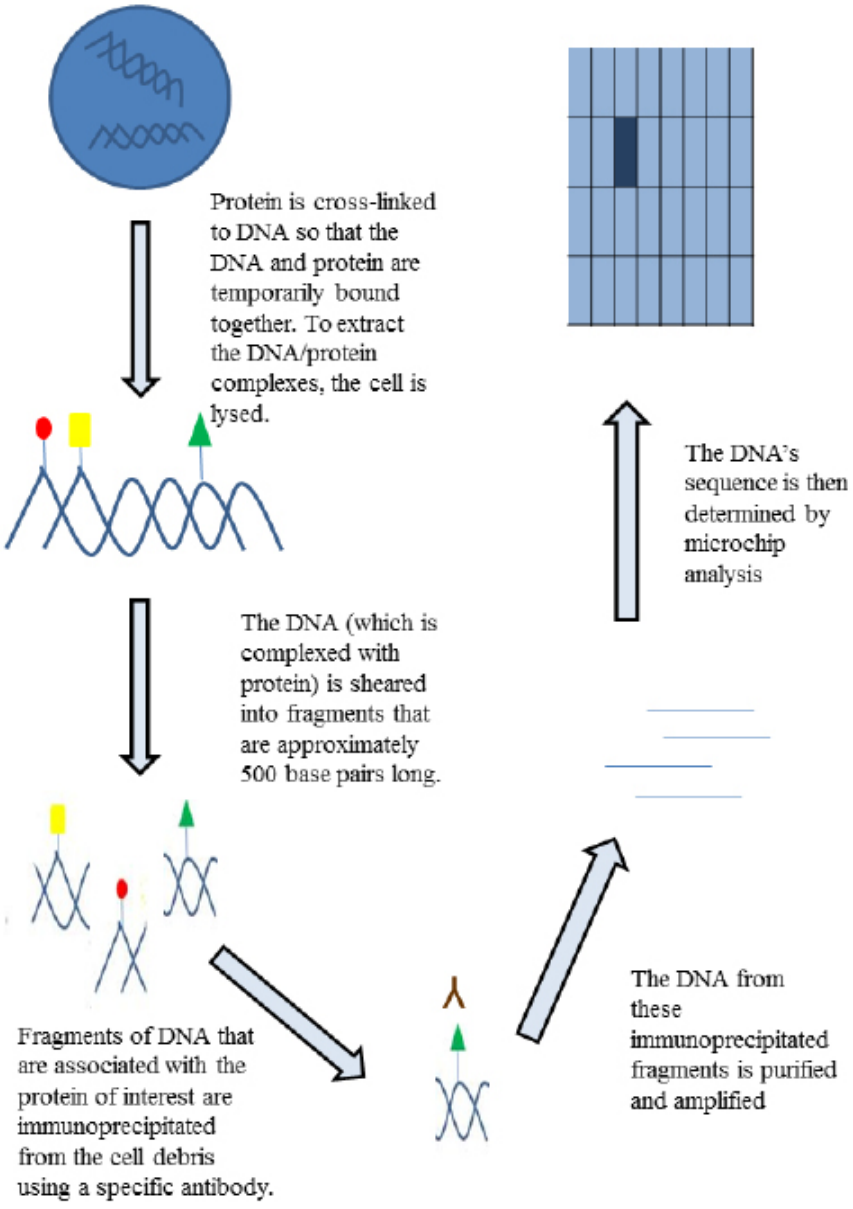

Figure 1 Chromatin Immunoprecipitation. Chromatin immunoprecipitation is a molecular biology technique that allows researchers to analyze interactions between DNA and proteins (Collas 2004).

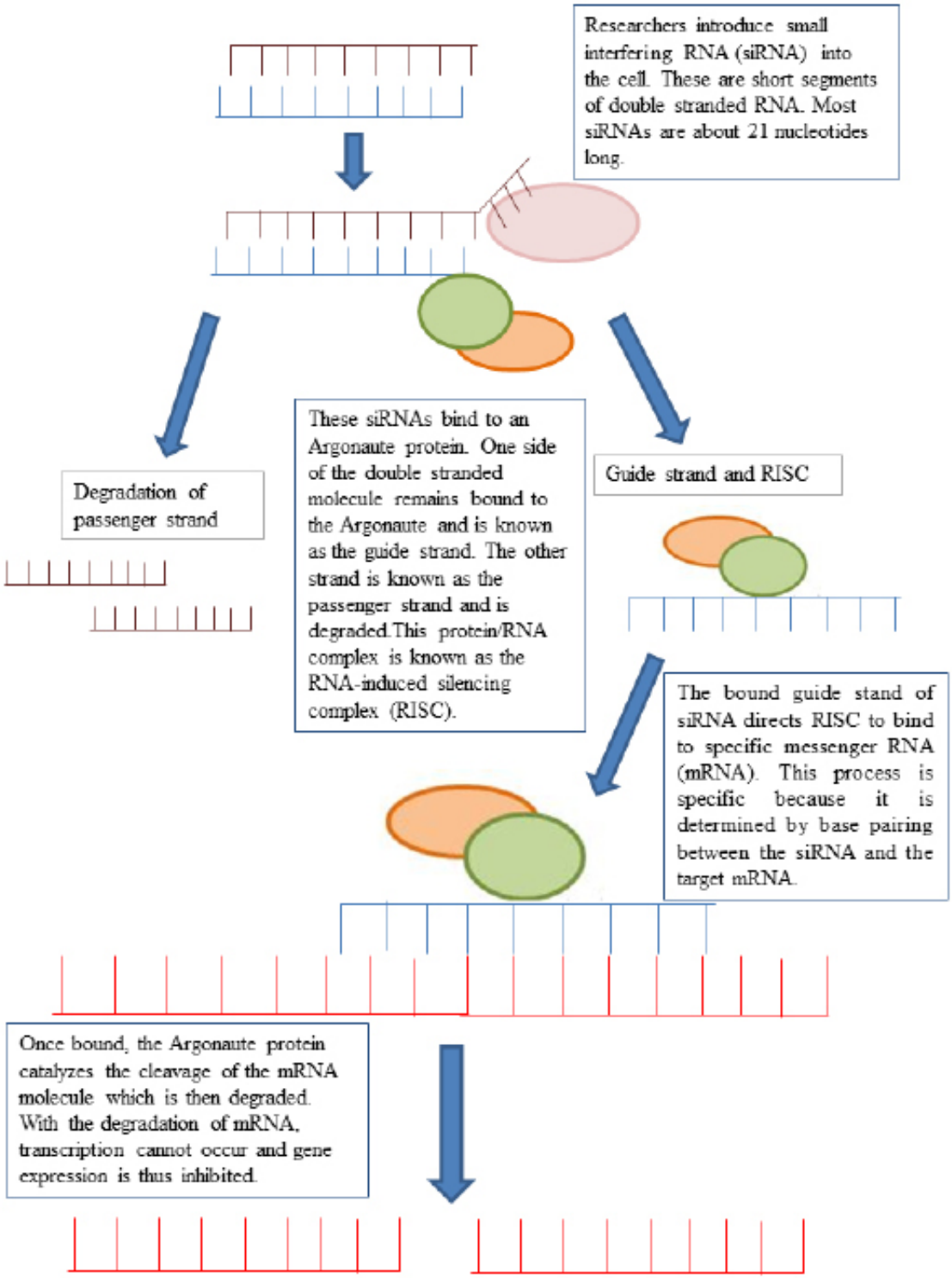

Figure 2 RNA interference (RNAi). RNAi is a biological process that inhibits gene expression by RNA molecules called short interfering RNA (siRNA (Bagasra 2004).

Figure 1d 2008). This information supports the hypothesis that the ER binding site is a CRE that plays a role in repressing *ERBB2*. Of all the prognoses of ER+ breast cancer, the worst are the cases with elevated ERBB2 levels (Kun et al. 2003). Therefore, tamoxifen is most effective when *ERBB2* is properly repressed and not amplified.

Enhancer
Short DNA sequence that affects transcription regulation. When bound with activator proteins, the enhancer increases transcription.

Clinical translation
A research discipline with the goal to translate lab-based research into applied clinical applications. It is commonly known as the "bench to bedside" discipline.

Because PAX2 was recruited to ER binding sites when cells were treated with estrogen, Hurtado et al. (2008) developed the hypothesis that PAX2 is a transcriptional repressor of *ERBB2* gene expression. Scientists can determine a molecule's role by knocking down or silencing the genes that transcribe for the molecule. In this study, PAX2 was silenced using short interfering RNA (siRNA) (See Figure 2). Cells were treated with either a control siRNA or the *PAX2* siRNA. The samples of each **transfection** group were subsequently treated with either estrogen, tamoxifen, or a control.

PAX2 is required for the repression of *ERBB2* gene expression. Cells treated with the control siRNA and then treated with either estrogen or tamoxifen, knocked down *ERBB2* levels of mRNA relative to the vehicle (Hurtado et al. Figure 2b 2008). *PAX2* siRNA cells that were treated with estrogen or tamoxifen had no reduction of *ERBB2* mRNA, indicating that PAX2 is required for the estrogen or tamoxifen dependent reduction in *ERBB2* mRNA levels. Reduction in PAX2 also leads to an increase in cell number after estrogen or tamoxifen treatment indicating cell division rates were increased (Hurtado et al. Figure 2c 2008). Blocking ERBB2 signaling with Herceptin, an anti-ERBB2 **antibody**, reduced the cell division back to background levels demonstrating that the enhanced cell division was due to ERBB2. This indicated that with the knockdown of PAX2, tamoxifen actually increases cell number, supporting the hypothesis that PAX2 is a transcription repressor of the *ERBB2* gene.

Involvement of AIB1

Tamoxifen resistant breast cancers typically have elevated levels of AIB1 in addition to increased ERBB2 levels. AIB1 acts as a coactivator to ERs (List et al. 2001, Osborne et al. 2003). Past studies have shown that AIB1 is a key player in oncogenesis in mice and have established effects in tumorigenesis in humans by AIB1 acting as an ER coactivator (Anzick et al. 1997, Torres-Arzayus et al. 2004). Therefore Hurtado et al. (2008) hypothesized that AIB1 and PAX2 competitively interact to regulate *ERBB2* transcription, with AIB1 as an activator and PAX2 as a repressor.

To determine whether this was the case, the research team implemented experiments to assess the binding of each respective transcription factor to the *ERBB2* CRE. To determine if AIB1 and PAX2 compete for binding, the researchers used *PAX2* siRNA to knockdown *PAX2* and then measured AIB1 binding to the *ERBB2* CRE. The experiment showed increased AIB1 binding to the ERBB2 **enhancers** in cells treated with both estrogen and tamoxifen when *PAX2* levels were reduced by siRNA (Hurtado et al. Figure 2d 2008). This indicates that the presence of PAX2 can inhibit binding of AIB-1 to the ERBB2 CRE.

Comparing two cell lines with different levels of PAX2 and AIB1 validated the role of AIB1 in tamoxifen resistance. MCF-7 cells already have amplified levels of AIB1 and high levels of PAX2. These cells are sensitive to tamoxifen due to the high levels of PAX2. A tamoxifen resistant (TamR) cell line was derived from the MCF-7 line by growing cells in the presence of tamoxifen until they developed resistance. Tamoxifen resistance was confirmed in the TamR cells by the quantification of mRNA levels. Tamoxifen reduced *ERBB2* mRNA levels in the MCF-7 cells, but not in the TamR cells (Hurtado et al. Figure 2b 2008). Both cell lines have similar levels of ER. AIB1 levels were similar between the cells but PAX2 levels were lower in the TamR cells. TamR cells had elevated ERRB2 levels without the amplification of the ERBB2 locus and low levels of PAX2 (Hurtado et al. Figure 3a 2008). Hurtado et al. (2008) recognized that low levels of PAX2 are a

potential explanation of why there is an increased amount of ERBB2 in TamR cells (Hurtado et al. Figure 3a 2008).

If AIB1 did compete with PAX2, the activation rather than the repression of *ERBB2* would occur and could contribute to higher levels of ERBB2 in TamR cells. ChIP experiments showed lower AIB1 binding at *ERBB2* CRE after tamoxifen and estrogen treatments (Hurtado et al. Figure 3b 2008). This result indicated that PAX2 interferes with AIB1 binding to the *ERBB2* CRE. Higher levels of AIB1 contributed to tamoxifen resistance by competing with PAX2 and acting as a transcription activator, resulting in elevated levels of ERBB2. AIB1 recruitment to the ER binding site was elevated in TamR cells compared to MCF-7 cells when each were treated with tamoxifen (Hurtado et al. Figure 3b 2008). To test the hypothesis that this phenomenon was due to PAX2 competition, an experiment was performed that introduced PAX2 to the TamR cells. With the addition of PAX2 into the TamR cells, the number of viable cells and protein decreased after treatment with tamoxifen indicating the restoration of tamoxifen sensitivity in the TamR cells (Hurtado et al. Figure 3c 2008). This indicated that PAX2 repressed the transcription of *ERBB2*.

Clinical implications

These findings are clinically important for all breast cancer patients. To link these laboratory findings to real patients Hurtado et al used immunohistochemistry techniques to determine the presence of AIB1 and PAX2 in 109 patients with ER+ breast cancer. These tumors were characterized as PAX2 positive or negative and AIB1 positive or negative. Following these characterizations, the patients' clinical outcome was determined and compared by their histological subtype. Tumors that were PAX2 positive had a significantly higher recurrence-free clinical outcome than patients who were PAX2 negative. Tumors with positive AIB1 expression usually had a worse clinical outcome than those tumors that were AIB1 negative. Tumors that were both PAX2 positive and AIB1 negative had the lowest recurrence rate out of all the subtypes (Hurtado et al. Figure 4 2008). These tumors also had lowest ERBB2 levels (Hurtado et al. 2008).

This study demonstrates that PAX2 acts as a transcription repressor at the *ERBB2* promoter. It also shows that AIB1 and PAX2 compete for binding which subsequently determines whether *ERBB2* is expressed. This new information on regulation of *ERBB2* will allow researchers to potentially develop methods to limit its expression in patients who are resistant to tamoxifen because of previous knowledge of ERBB2 crosstalk with ERs. Significantly, this study showed that tamoxifen resistant cells have low levels of PAX2 and that reintroducing PAX2 to these cells restores tamoxifen sensitivity. **Clinical translation** of this phenomenon could positively impact a large population of women whose breast cancer tumors are resistant to tamoxifen.

PAX genes have established roles in embryogenesis and have been linked to oncogenesis in other tissues (Muratovska et al. 2003, Wang et al. 2008). One of its main roles is stimulating the development of the kidneys, eyes, and mammary glands. Studies have linked PAX2 to the mediation of tamoxifen acting like an estrogen ligand to the ER in tissues like the endometrium. PAX2 can also influence the development of other cancers like ovarian cancer (Muratovska et al. 2003). The Hurtado et al. (2008) study's findings were surprising because PAX2 usually acts as a transcription activator at other genomic locations. However, in Hurtado et al. (2008) it is shown to be repressing transcription of *ERBB2*.

A follow-up study to Hurtado et al. (2008)

was done to evaluate PAX2 and AIB1 as potential prognostic factors in breast cancer. This study found that high levels of AIB1 were indeed a negative factor for disease free survival in women who were not taking tamoxifen (Alkner et al. 2013). AIB1 was no longer a negative factor in patients who had the same AIB1 levels after tamoxifen treatment. This showed that AIB1 is a potential target for anti-cancer therapies. In women that were not treated with tamoxifen, PAX2 was not a prognostic factor. In treated women, however, PAX2 was a factor. In premenopausal women it was a positive prognostic factor for disease-free survival. In postmenopausal women it was a negative prognostic factor. While it is clear that AIB1 is a negative clinical prognostic factor and can be used to predict tamoxifen effectiveness, PAX2 has not been conclusively identified as a prognostic factor (Alkner et al. 2013). More clinical studies need to be done to determine this.

Endocrine therapy resistance is a significant problem because tamoxifen is the most common treatment of patients with ER+ breast cancer. There are only a few known clinical markers of tamoxifen resistant breast cancer, including elevated levels of AIB1 and ERBB2. Hurtado et al. (2008) showed that PAX2 is a tamoxifen recruited transcriptional repressor of *ERBB2* giving it the potential to be a predictive biomarker. This study also showed that AIB1 binding competes with PAX2 binding at the ER binding site causing increased *ERBB2* expression. Tamoxifen effectiveness is determined by the balance of AIB1 and PAX2 levels.

The discovery of PAX2 as a transcription repressor was somewhat surprising to researchers because the only previously known role of the protein was as an activator in the endometrium, contributing to endometrial cancer (Wu et al. 2005). In parallel, tamoxifen usually works as an antagonist in breast but has agonist effects in the endometrium. It is possible that PAX2 displays specific tropism and may be what determines the action of tamoxifen in different tissues.

Acknowledgements

I would like to thank Dr. Shannon Stevenson, Dr. Tim Craig, and Dr. Elizabethada Wright for their continual assistance in the writing process. I would also like to thank Dr. Andrew Skildum for his expertise in breast cancer biology that made this publication possible.

Author Biography

Jennifer Thomalla is a junior at the University of Minnesota Duluth where she is pursuing a double major in biology and political science. She works as an undergraduate research assistant for Dr. Andrew Skildum. Dr. Skildum studies antiestrogen resistance in human breast cancer. In her free time, Jennifer enjoys hunting, fishing, playing softball, and volunteering at the Solvay Hospice House in Duluth.

References

Alkner S, Bendahl PO, Grabau D, Malmstrom P, Ferno M, Ryden L, South Swedish Breast Canc Grp. 2013. The role of AIB1 and PAX2 in primary breast cancer: Validation of AIB1 as a negative prognostic factor. Ann Oncol 24(5):1244-52.

Anzick S, Kononen J, Walker R, Azorsa D, Tanner M, Guan X, Sauter G, Kallioniemi O, Trent J, Meltzer P. 1997. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277(5328):965-8.

Arpino G, Wiechmann L, Osborne CK, Schiff R. 2008. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: Molecular mechanism and clinical implications for endocrine therapy resistance. Endocr Rev 29(2):217-33.

Bagasra O and Prilliman KR. 2004. RNA interference: The molecular immune system. J Mol Histol 35(6):545-53.

Bates NP and Hurst HC. 1997. An intron 1 enhancer element mediates oestrogen-induced suppression of ERBB2 expression. Oncogene 15(4):473-81.

Breast Cancer. Cancer.gov. Retrieved February 19, 2015, from http://www.cancer.gov.

Chang BY, Kim SA, Malla B, Kim SY. 2011. The effect of selective estrogen receptor modulators (SERMs) on the tamoxifen resistant breast cancer cells. Toxicol Res 27(2):85-93.

Collas P. 2010. The current state of chromatin immunoprecipitation. Mol Biotechnol 45(1):87-100.

Gail M, Costantino J, Bryant J, Croyle R, Freedman L, Helzlsouer K, Vogel V. 1999. Weighing the risks and benefits of tamoxifen treatment for preventing breast cancer. J Natl Cancer Inst 91(21):1829-46.

Hurtado A, Holmes KA, Geistlinger TR, Hutcheson IR, Nicholson RI, Brown M, Jiang J, Howat WJ, Ali S, Carroll JS. 2008. Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. Nature 456(7222):663-6.

Jordan C. 2002. Historical perspective on hormonal therapy of advanced breast cancer (vol 24,pg A3, 2002). Clin Ther 24(4):717.

Kun Y, How LC, Hoon TP, Bajic VB, Lam TS, Aggarwal A, Sze HG, Bok WS, Yin WC, Tan P. 2003. Classifying the estrogen receptor status of breast cancers by expression profiles reveals a poor prognosis subpopulation exhibiting high expression of the ERBB2 receptor. Hum Mol Genet 12(24):3245-58.

List H, Reiter R, Singh B, Wellstein A, Riegel A. 2001. Expression of the nuclear coactivator AIB1 in normal and malignant breast tissue. Breast Cancer Res Treat 68(1):21-8.

Muratovska A, Zhou C, He S, Goodyer P, Eccles M. 2003. Paired-box genes are frequently expressed in cancer and often required for cancer cell survival. Oncogene 22(39):7989-97.

Osborne CK and Schiff R. 2005. Estrogen-receptor biology: Continuing progress and therapeutic implications. J Clin Oncol 23(8):1616-22.

Osborne CK, Bardou V, Hopp TA, Chamness GC, Hilsenbeck SG, Fuqua SA, Wong J, Allred DC, Clark GM, Schiff R. 2003. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. J Natl Cancer Inst 95(5):353-61.

Samavat H and Kurzer MS. 2015. Estrogen metabolism and breast cancer. Cancer Lett 356(2):231-43.

Smith CL, Nawaz Z, O'Malley BW. 1997. Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. Mol Endocrinol 11(6):657-66.

Speirs V, Parkes AT, Kerin MJ, Walton

DS, Carleton PJ, Fox JN, Atkin SL. 1999. Coexpression of estrogen receptor alpha and beta: Poor prognostic factors in human breast cancer? Cancer Res 59(3):525-8.

Torres-Arzayus M, de Mora J, Yuan J, Vazquez F, Branson R, Rue M, Sellers W, Brown M. 2004. High tumor incidence and activation of the PI3K/AKT pathway in transgenic mice define AIB1 as an oncogene. Cancer Cell 6(3):263-74.

Wang Q, Fang W, Krupinski J, Kumar S, Slevin M, Kumar P. 2008. Pax genes in embryogenesis and oncogenesis. J Cell Mol Med 12(6A):2281-94.

Wu H, Chen Y, Liang J, Shi B, Wu G, Zhang Y, Wang D, Li R, Yi X, Zhang H, et al. 2005. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. Nature 438(7070):981-7.

Zhu B and Conney A. 1998. Functional role of estrogen metabolism in target cells: Review and perspectives. Carcinogenesis 19(1):1-27.