

**Hot Topics Scholarship Advocate Reports from the  
2007 San Antonio Breast Cancer Symposium**

Topic: Diet and exercise as means of cancer prevention (WHEL study)

<b>Report Name</b>	<b>Pages</b>	<b>Author</b>
Diet and Prevention	2-3	Eleanore Anbinder
Opportunity for a novel therapeutic combination	4-5	Princess Nikky Onyeri
The protective effect of parity via p53 in breast cancer	6-9	Melanie Shouse
Exercise Lowers Insulin in Breast Cancer Survivors	10-11	Peggy Nicholson

## **Diet and Prevention**

Over the last ten or more years, a number of studies have tried to clarify the role of diet in the prevention of breast cancer. Results have been reported recently in several studies, including the Women's Intervention Nutrition Study (WINS), the Women's Healthy Eating and Living (WHEL) study, and a third study called Diet, Lifestyle and BRCA – Related Breast Cancer Among French Canadians.

The WHEL study looked at the results of a multi-institutional randomized controlled trial of dietary change in 3,008 women previously treated for early stage breast cancer, and were enrolled between 1995 and 2000. The data was presented at the 30<sup>th</sup> annual San Antonio Breast Cancer Symposium (SABCS) on Saturday 15, 2007. The basic premise of the WHEL study was to determine whether a diet high in fruits, vegetables, and fiber, plus a diet somewhat lower in fat would lower the risk of recurrence. The study was a large phase III clinical trial and was reported to the SABCS by Dr. John P. Pierce of the University of California, San Diego.

The study randomly assigned women to two dietary groups. About half of the women were asked to follow the government recommended 5 a day diet, consisting of five servings of fruit and/or vegetables and at least 20 grams of fiber and less than 30% of fat in daily food consumption. The rest of the women on the study were assigned to a group with a more strict diet of three servings of fruit, five of vegetables, 16 ounces of vegetable juice, 30 grams of fiber and only 15/20% of daily fat. All of the women on the study received support to help them stay with the diet. The goal was to find out whether fruits, vegetables, fiber and fat could reduce breast cancer recurrence as well as death from any cause, in early stage breast cancer survivors ages 18 to 70. The study was conducted over a 7 year period.

At the four year mark of the study, differences between the two groups was significant. Women in the group whose criteria for eating more fruits, vegetables and the limiting of fat intake were sticking with the increased diet. However, at 7.3 years of follow-up, there were no statistically significant differences in terms of the main proposition of the study and there was no indication

that a change in diet could affect the recurrence or new primary breast cancer, nor has the death rate changed.

The published conclusion in the report of the National Cancer Institute of September 26, 2007 is that the “WHEL authors say these results fairly demonstrate that early stage breast cancer survivors do not protect themselves from further breast cancer by adopting a diet very high in fruits, vegetables and fiber and somewhat lower in fat”.<sup>1</sup>

The previously published WINS Study focused more on the fat intake in a diet suggested that although observationally, rather than specifically, that low fat leads to less weight and may have some influence on early stage cancers. However, this is not yet proven.

The Canadian Study suggest that women with high energy intake who carry the BRCA mutations, regardless of physical activity and diet may still have the same risk as women with a more restricted energy intake. “Further research is warranted to confirm these associations in other study populations”.<sup>2</sup>

Review of the WHEL study presented at the SABCS and a look at these two studies teaches us that much more research is needed before a conclusion that more fruits, vegetables, fiber or a lower fat intake will definitely reduce the incidence of breast cancer or impact the recurrence of breast cancer.

Submitted by:  
Eleanor F. Anbinder  
Art beCAUSE Foundation  
Framingham, MA

References:

1. National Cancer Institute Report: 9/26/2007
2. Poster #2027, San Antonio Breast Cancer Symposium: Diet, Lifestyle and BRCA related breast cancer among French Canadians

## **OPPORTUNITY FOR A NOVEL THERAPEUTIC COMBINATION**

ErbB-2 (also known as HER2) is associated with approximately 20-30% of all breast cancer and is amplified or overexpressed in aggressive breast tumors, associated with the poorest overall survival. Notch-1 is a potent cell fate determinant and oncogene that is also overexpressed with its ligand, Jagged-1 in aggressive breast cancer. However, inflammatory breast cancer (IBC) is a type of breast cancer with high metastasis due to the presence of florid lymphovascular tumor emboli. Therefore, Notch signaling is very important for the survival and proliferation of breast tumors, of which ErbB-2 positive tumors are treated with trastuzumab plus chemotherapy. Unfortunately, trastuzumab resistance is common and recurrence is also inevitable. However, more experiments were carried out to investigate whether a novel crosstalk between ErbB-2 and Notch-1 contributes to the resistance.

The results showed that trastuzumab increased the amount of nuclear accumulation of active intracellular Notch-1 and Notch target proteins. Trastuzumab also polarized the localization of both Notch-1 and ligand Jagged-1 near the cell surface. In addition, trastuzumab increased the interaction between Notch-1 and presenilin-1 and increased the proteolytic activity of a g-secretase, which was inhibited by an inhibitor (GSI).

Therefore, inhibition of ErbB-2 by trastuzumab reactivates Notch-1 which is a potent breast oncogene by two mechanisms shown below:

1. Increasing the availability of Notch-1 and Jagged-1 at the cell surface through a Dynamin 2- dependent process.
2. Increasing g-secretase activity.

The above two results suggest that trastuzumab combinations may be effective in ErbB-2 positive tumors and may delay or prevent the onset of resistance.

However, for targeting upregulated notch signaling in the CD133+ stem cells, the results equally suggest the following:-

1. That activation of Notch signaling pathways are essential for maintenance of viable tumor emboli and CD 133+ stem cells.
2. That inhibiting Notch pathways with gamma- secretase inhibitors may achieve selective targeting of stem cells within the lymphovascular emboli and ultimately a novel, effective therapy for inflammatory breast cancer.

**SUBMITTED BY:**

Princess Nikky Onyeri  
Princess Nikky Breast Cancer Foundation  
Abuja, FCT, Nigeria

**Resources:**

Lecture Number 75

Authors: Clodia Osipo, Parul Patel, Lu Hao, Loren Whitehouse, Peter Strack, Todd Golde, Kathy Albain and Lucio Miele

Poster Number: 3094

Sanford H Barsky, Yi Xio, Yin Ye

## **The protective effect of parity via p53 in breast cancer**

A woman's reproductive history is well-known to be her strongest non-genetic risk factor for breast cancer. An early age at first pregnancy (before age 24) confers a 50% lifetime reduction in breast cancer risk compared with nulliparous women (those who have never carried a pregnancy to full term). Scientists have been working to determine the molecular mechanism that confers this added protection, in order to develop preventative therapies and a means to increase the effectiveness of chemo based on an individual's tumor biology. One theory to explain this protective effect is that parity (pregnancy) causes "maturing" of the mammary gland through epithelial cell differentiation that may result in cells with a less proliferative character which are less susceptible to carcinogenic transformation. While it has been demonstrated that parity induces a high level of mammary differentiation, its protective value has been disproved via experiments that induced mammary cell differentiation in the absence of estrogen and progesterone; this offered no protective effect. Another possible explanation is known as the "cell-fate" hypothesis, that parity-induced hormone exposure during this critical period of development leads to permanent changes in gene expression (turning specific genes on and off) and signal transduction (the ability of cells to communicate with one another). These changes then determine the response of the mammary gland to carcinogen-induced cellular stress later in life. This hypothesis has received backing from recent studies which have identified differences in key gene expression patterns between parous and nulliparous mouse models, with genes that promote cell growth and proliferation being down-regulated by parity and genes that inhibit growth being up-regulated by parity.

One gene whose expression is postulated to convey this protection is the well-studied tumor suppressor gene p53, known as the "guardian of the genome". P53 is the most commonly altered gene in human breast cancers, and its absence can cause spontaneous development of mammary tumors in animal models. P53 mediates the cellular response to DNA damage in a wide variety of organisms, and can evoke DNA repair, cell-cycle arrest, or apoptosis (cell death) depending on the type of cellular insult endured. P53 is activated in the nucleus of the cell and is shuttled to the cytoplasm for degradation. Interestingly, it has been found that parity leads to the induction, expression, activation, and localization of p53 in the cell nucleus, whereas the "nulliparous" gland typically displays inactive p53 sequestered in the cytoplasm. This parity-induced

activation of p53 has been validated through gene expression studies that have also shown higher levels of p21 and MDM2, two of the key gene targets that are upregulated by active p53 and work to prevent tumor development. Therefore, upon carcinogen invasion, the parous mammary gland would have ample resources to combat DNA-damaging effects via p53 activation, leading to DNA repair or cell-cycle arrest before carcinogenic transformation and tumor development can take place. The central role of p53 in parity-induced protection was demonstrated by Medina et al., who found that mice lacking p53 were unable to mount a protective response to carcinogens even after pregnancy.

Parity and its effect on P53 activity has been studied extensively in mouse and rat models, but human tissue studies have been uncommon. A new IRB-approved study by Dr. Sallie Smith-Schneider et al. at Baystate Medical Center explored the regulation of p53 expression by parity in human breast tissue. Smith-Schneider hypothesized that radiation-induced DNA damage would result in increased p53 activation in parous women versus nulliparous women. They used a 5-Gy dose of Gamma irradiation to induce p53 activation in mammary tissue extracted from 42 parous and nulliparous women after breast reduction surgery. The subjects were divided into three groups: (1) nulliparous (NP): 11 women; (2) early parity (EP) before age 23: 24 women; and (3) late parity (LP) after age 23: 7 women. These groupings were based on their responses to pre-operative questionnaires which also accounted for onset of menses, last menses, and family history of breast cancer. Two portions of tissue were cultured for 24 hours at 37 degrees, and then exposed to 5Gy of ionizing radiation to induce DNA damage. The tissue was incubated again for 6 hours, while control tissue remained non-irradiated. A polyclonal antibody to p53 was used to perform immunohistochemical (IHC) analysis, and p53 staining was scored by a pathologist as follows: 0 = none; 1+ = weak; 2+ = moderate; 3+ = strong. Differences in p53 scores were then compared between the irradiated and non-irradiated tissue.

Results indicated that parity does indeed confer raised activation levels of p53 after DNA damage in human breast tissue. Pregnancy in general conferred a p53 score of 3.46 (p-value .0024) versus p53 score of 2.57 for nulliparous women. Early pregnancy specifically conferred a raised p53 score of 3.73 with a p-value of .001, while later pregnancy after 23 years reduced the p53 score to 3.14. Interestingly, the p53 score also rose with an increasing number

of full-term pregnancies, with the strongest level of p53 activation shown after 2 or 3+ pregnancies (3.54 and 3.72, respectively, p-value .001).

Multivariate model estimates examining effects of parity via change in p53 levels were calculated with potential confounding factors taken into account that have the potential to bias the results: current age, age at menarche, family history, oral contraceptive use, and menopausal status. While the first two were determined to be potential confounders, the last three exerted no significant effect on outcomes. The adjusted difference between the p53 score in breast tissue from nulliparous women versus women who undergo early parity was 1.44 (p-value .002). This indicates that early pregnancy significantly increased the number of luminal epithelial cells in the mammary gland which could respond to DNA damage through the upregulation of the tumor suppressor gene p53.

In conclusion, this study demonstrated for the first time in human mammary tissue the results which have been seen in rat and mouse models, namely that a full-term pregnancy (particularly at an early age) increases the responsiveness of the tumor suppressor gene p53 to DNA damage induced by carcinogens. The protection conferred by early parity has thus been shown to be mediated by the activity of p53. These results may pose profound implications for the development of targeted therapies for breast cancer. A particularly interesting corollary to this finding is the discovery by Sivaraman et al. that treatment of adolescent-age mice with pregnancy levels of estrogen and progesterone for approximately a month in order to mimic early parity conveys the same protective effect as actual pregnancy, with equal induction of p53 and other gene expression signatures which are upregulated during parity. If confirmed in human studies, this could provide a simple protective public-health solution to the breast cancer pandemic which could avoid 20,000 of the 40,000 breast cancer deaths suffered in the United States each year by offering an achievable means of protection to the group most highly at risk, those who choose not to undergo early pregnancy.

Melanie Shouse  
Komen St. Louis Research Advocate Committee  
St. Louis, MO

## **Bibliography**

Britt K., Ashworth A., Smalley M. “Pregnancy and the risk of breast cancer.” *Endocrine-Related Cancer*, 14: 907 – 933, 2007.

D’Cruz, CM, Moody SE, Master SR, Hartman JL, Keiper EA, Imielinski MB, Cox JD, Wang JY, Ha SI, Keister BA, and Chodosh LA. “Persistent Parity-Induced Changes in growth factors TGF-B3, and differentiation in the rodent mammary gland.” *Mol. Endocrinol.*, 16 (9): 2034-2051, 2002.

Kuperwasser C., Pinkas J., Hurlbut GD, Naber SP, and Jerry DJ, “ Cytoplasmic Sequestration and Functional Repression of p53 in the Mammary Epithelium is Reversed by Hormone Treatment.” *Cancer Research* 2000 May 15; 60: 2723-2729.

Medina D., “Breast Cancer: The Protective Effect of Pregnancy.” *Clinical Cancer Research*, 10: 380s – 384s, Jan 1, 2004 (suppl.).

Medina, D., and Kittrell, FS. “p53 Function is Required for Hormone-Mediated Protection of Mouse Mammary Tumorigenesis.” *Cancer Research* 2003 Oct. 1; 63: 6140-3.

Sivaraman L., Conneely O.M., Medina D., O’Malley B.W. “p53 is a potential mediator of pregnancy and hormone-induced resistance to mammary carcinogenesis.” 98 (22): 12379 – 12384, Oct. 23, 2001.

## Exercise Lowers Insulin in Breast Cancer Survivors

It is known that breast cancer survivors use exercise programs and proper diet to reduce levels of insulin in their blood cells. There are many studies that provide a high body mass index (BMI) or high caloric intake on breast cancer prognosis in research that underlies mechanisms and complications for breast cancer prevention as well as treatment.

While there is evidence that BMI influences breast cancer risk, the relationship is modest and varies with menopausal status and other factors. BMI is more consistently identified as an important adverse prognostic factor. Women with higher BMI have higher circulating insulin levels associated with cancer cells of a patient with insulin resistance and hyperinsulinism are sensitive to insulin stimulation is an important unanswered question. Insulin signaling is therefore a potential mediator of the effects of BMI on breast cancer prognosis. It has been recognized for decades that restriction of energy intake reduces carcinogenesis and reduces aggressive cancer behavior in laboratory models. More recent experimental data provide early evidence that the growth of some cancers is increased when caloric intake of the host or patient is increased. Michael Pollak, MD provided various studies of women in the United Kingdom who have associated increased body mass index with increase risk of breast cancer. The research includes insulin and insulin-related growth factors. It has been shown that higher fasting insulin levels or c-peptide levels (a marker of insulin production) at time of diagnosis are associated with poorer outcome. Since obesity and hyperinsulinemia are common (and increasing) in affluent societies, the public health impact of the adverse impact of these factors on breast cancer prognosis may be considerable.

Obesity is linked to hyperinsulinemia (above baseline level insulin). Exercise and proper diet has proven to reduce insulin. Exercise has proven beneficial for a cancer patient through treatment as well as afterwards. This practice has proven to provide a long-term survivorship, depending on the initial diagnose.

The medical field has viewed that insulin is a product of pancreatic B-cells that regulates systemic conserving energy. Insulin and/or Insulin Growth Factor (IGF) receptor may have unrecognized functions in higher organs.

In closing, the findings suggest that a breast cancer patient invest in their health through continual exercise and proper diet for long-term survivorship and fewer complications.

Submitted by:  
Peggy Nicholson  
Sisters Network Baltimore Chapter  
Gwynn Oak, MD 21207