Comparison between deletion genes in BRCA1 and BRCA2 on tamoxifen.

Dr. Marjan Assefi *

University of North Carolina at Greensboro, Joint School of NanoScience and Nano Engineering, North Carolina, USA

Abstract

According to Doamekpor, BRCA1 and BRCA2 are human genes. They produce proteins that eliminate and suppress tumors. They repair the damages to our DNA. He stated that if a mutation happens in one of the genes and BRCA1 or BRCA2 do not work properly, DNA damages may not be repaired; this process leads to cancer. Tamoxifen is used to block the Estrogen hormone in breast cancer patients with Estrogen receptors. We are going to search from other papers and Raptor X to see which genes have mutations in BRCA1 and BRCA2 which have resistant to Tamoxifen. These studies connote the resistance in BRCA mutation and define the importance of domains with BRCA2 and BRCA1 (Stacey).

Keywords: BRCA1, BRCA2, Raptor X, Tamoxifen, Breast cancer, Estrogen Hormone, DNA, Tumor.

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Introduction

According to Mandal, almost from the beginning of life on earth, mankind was affected and suffered by breast cancer. Almost In every era in recorded history, it is mentioned and written about. Breast cancer's symptoms are visible. At late stages the lumps advance toward the tumors. This process has been recorded by physicians in ancient times. She stated that this is because; breast cancer is not like the other internal cancers. Breast lumps tend to show themselves. Galen's theories on breast cancer were accepted until the 17th century. She found that in 1680, Francoisdela Boe Sylvius a French scientist began to reject the funny theory of cancer. He hypothesized that cancer was not caused by the black bile. He said it cause from a chemical which transformed lymphatic fluids from acidic to acrid. In 1730s, a physician from Paris under the name of Daude Deshais Gendron rejected the theory of Galen. Mandal mentioned that he believed when glandular and nerve tissue get mix with lymph vessels, cancer begins to develop. In 1895, a Scottish surgeon George Beatson discovered when he removed ovaries from the breast cancer; the breast tumor began to shrink. From that time on, many surgeons began to remove both ovaries. They performed a radical mastectomy for breast cancer patients. The tumor began to shrink after removing the ovaries because of the estrogen.

Mandal found that Estrogen which is released from the ovaries helped the tumor to grow and to become larger. But removing the ovaries "Estrogen" helped the tumor to shrink meaning to get smaller. With the advent of modern medicine, from 1995, almost ten percent of breast cancer patients went under mastectomy. This period was followed by the new the rapies for breast cancer like: hormone treatments, surgeries and biological the rapies. Scientists isolated the genes that caused breast cancer, like the genes: ATM, BRCA2, and BRCA1. According to Doamekpor, BRCA1 and BRCA2 are human genes. They produce proteins that eliminate and suppress tumors. They repair the damages to our DNA. He stated that if a mutation happens in one of the genes and BRCA1 or BRCA2 do not work properly. DNA damages may not be repaired; this process leads to cancer. According to Web Med, the most commonly used hormone therapy for the treatment of cancer is Tamoxifen. Women who have breast cancer go under estrogen sensitivity test. This test shows if the patient has estrogen receptors(ER+) or not. Estrogen helps the growth of breast cancer cells. Tamoxifen blocks the effects of estrogen on these cells. This medicine is called (anti Estrogen). They stated that Tamoxifen slows or stops the growth of cancer cells which are already present in the body (Web Med). According to Lambert, Tamoxifen prevents the original breast cancer from coming back and also helps to prevent new cancer in the opposite breast. It reduces the risks of breast cancer in high risk woman for this disease. Tamoxifen blocks the action of Estrogen. Estrogen is a female hormone. Certain types of breast cancer need estrogen to grow (Lambert). Tamoxifen is used in breast cancer patients both men and women. Tamoxifen is used to lower woman's chance of developing breast cancer if she is in a high risk group (such as family history of breast cancer). Tamoxifen has been used for breast cancer patients who have estrogen receptor (Lambert). Advanced ER positive disease fail to respond to Tamoxifen once. In patients who are sensitive to Tamoxifen, the disease tends to a resistant phenotype (Lambert).

Material and Methods

To identify genes responsible for Tamoxifen resistance in breast cancer have been applied by random transfection of CDNA libraries. In order to find novel genetics for dominant mechanisms in anti-estrogen resistance in human breast cancer, they have used cancer cell line ZR-75-1. This cell line is dependent on estrogen. Progression of these cells to anti estrogen resistance is low. Random regulation of gene expression in ZR-75-1 cells with 5 azacytidine produced anti- estrogen resistance (Lambert). Over 800 million ZR-75-1 cells have been infected with a murine retrovirus and subjected to culture with 1 Mmol/L of 4hydroxyTamoxifen for 4-5 weeks. 80 cell lines were generated to have resistance to 4 hydroxy Tamoxifen. Also they used PCR (Lambert, 2012). They used Fluorescence in situ hybridization for showing CAPANI and PIR12 cells had three copies of the BRCA2 gene (in Result section). I used RaptorX website to

show BRCA1 and BRCA2 domain site residues.

Results

According to Lambert, the pharmacology of Tamoxifen, in the structural and functional of the ER has a great role and sometimes causes resistance. Till now, no prominent mechanism has been found (Lambert). BRCA genes are responsible for Tamoxifen resistance in human breast cancer. Individual BRCA genes can change estrogen dependent breast cancer cells into estrogen independent Tamoxifen resistant cells in vitro (Lambert).

Expression of Urokinase type plasminogen activator (UPA) and its inhibitor PAI-1, HER2, epidermal growth factor receptor EGFR, Beta ER, TP53 over expression, and high expression of Thymidine Kinase were found to be associated with poor response to Tamoxifen(Lambert). Tamoxifen is metabolized as 4hydroxy Tamoxifen which binds to the ER. Alternative metabolizing could stimulate tumor growth. It is obvious from the all published studies that mutation of Alpha ER is quite rare. For ER Alpha, no regulation has been found between the specific variants and Tamoxifen resistance (Lambert). Lambert states that Genetic and epigenetic are important in disease treatment and Tamoxifen resistance. These genes include activated Ha-RAS, TGFBetha1, RAFi, IGFR, IRSI, CYR61, and activated AKT. In HER2 over expression is found in 25% of breast carcinomas and shows failure to Tamoxifen (Lambert). Three breast cancers anti estrogen resistance (BRCA) are found which are the cause of resistance in 15 cell lines and are distinct from hereditary Breast cancer genes (Lambert).

BRCA1: Lambert indicates that it is identified in 4 independent cell lines. The BRCA1 locus was mapped to chromosome 16q22-23. The scanning showed a transcript of 3.2 Kb highly expressed in the cell lines (Lambert). Transfectants displayed resistance to 4 hydroxy Tamoxifen and anti-estrogen fulvestrants. Human and rat homologue Crk- lead to substrate protein P130 CAS although BCRA1 is cable of by passing the dependence of ZR-75-1 cells (Lambert).

BRCA2: The second locus in anti-estrogen resistance was found in cell fusion- mediated gene transfer; PCR analysis on DNA from a radiation hybrid cell panel and in situ hybridization (Lambert). Map the BCRA2 locus on human chromosome 6p12.1-12.1 (Lambert). The candidate gene in BRCA2 locus is of 5 and 8 Kb.The complete codings are transformed into ZR-75-1 cells to confirm the role of protein in anti-estrogen-resistant cell proliferation. According to Stacey, they sequenced BRCA2 C.6174delT mutations; they cancerous were resistant to carboplatin treatment (Figure 2).Patient UK1999 they found out a 137bp deletion inside exon11 of the gene, 7bp3 to the c.6174delT mutation (Stacey). Stacey states that sequence of BRCA2 from patient UK2223 led to another result, they found out an 8bp deletion in exon 11, 5bp, 3 to the c.6174delT mutation. The result was restoration of the BRCA2 ORF, with the loss of 3 amino acids. A 6bp direct repeat was in relation with the deletion in one of the tumors UK1999 (Stacey).

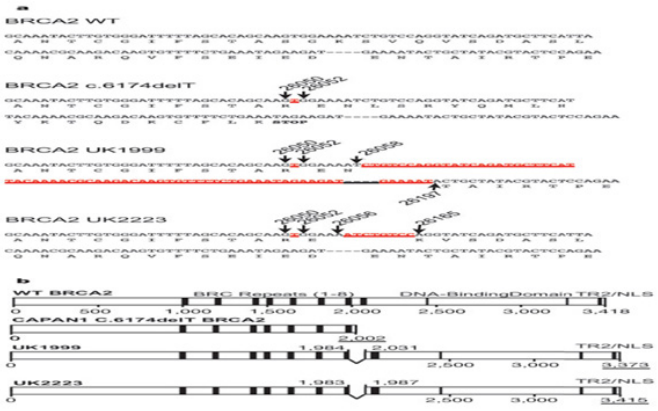

Figure 2. *Identification of BRCA2 ORF restoring mutations in carboplatin resistant tumors in BRCA2c.6174delT mutation carriers (Stacey).*

BRCA3: On the southern blots, the third BCRA locus was identified. PCR analysis shows that this integration locus is located on chromosome 1p21. This BRCA3 locus often causes anti-estrogen resistance. Transfectant show resistance to 4 hydroxy Tamoxifen (Lambert). Transformation of BRCA3 gene into another human breast cancer cell line (MCF7) also showed resistance to pure anti-estrogen to these cells (Lambert). Lambert states that sequence analysis of the BCRA3 CDNA revealed a novel gene product containing a Src homology 2 (SH2) domains and partial homology to cell division cycle protein 48 of bacteria, yeast, and mammalian species and a GDP exchange factor (GEF) domain.

We used sequences of BRCA1 and BRCA2 to find binding residues of them in RaptorX. As you see in this Figure1, we wanted to show domain region of BRCA1 and BRCA2 with RaptorX. In the left image, in BRCA1, you see binding residues which are C39, H41, C61, and C64 for ligand Zn. But in right image, you can see those binding residues which are N237, G238, G239 A240, and S241 for ligand DT (Lambert).

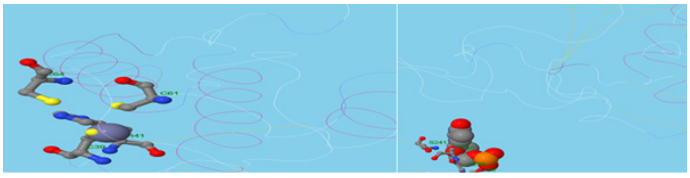

Figure 1. *The left image is for BRCA1, and the right image is for BRCA2 from RaptorX. We wanted to identify BRCA1 and 2 domain region.*

According to Stacey states that comparative genomic hybridization in combination with fluorescence in situ hybridization showed that CAPANI and PIR12 cells had three copies of the BRCA2 gene. Five BRCA2 copies in PIR1-6 were in contrast with complementary DNA and genomic DNA from PIRI-12. It showed that all PIR clones also carried the c.6174delT BRCA2 allele (Stacey). PIR1-11 also showed that BRCA2 alleles carrying deletions of up to 58kb, of the c.6174delT mutations. The new BRCA2 species did not have the three repetitions of BRC and DBD. When they sequenced BRCA2 in PIR12; it showed the presence of a 458bp deletion surrounding the c.6174delT mutation (Stacey, 2007). It lacked 153 amino acids residues; two BRC repeats but produced an almost full length BRCA2 protein. In almost all cases, examination of nucleotide sequences surrounding BRCA2 deletions in PIR1-

12revealed, short regions of sequence (Stacey, 2007). They were associated with the end of the deleted region (Table 1).

Table 1. *BRCA2 genomic deletions observed in CAPAN1and PIR clones (Stacey).*

Clone name	Name of the allele	Sequence of genomic deletion (5'→3')*	No of bases deleted	No of clones
CAPAN1	c.6174delTǂ	(Exon 11) 26052 26052 (Exon 11) TTGTGGGATTTTTAGCAAG(T)GGAAAATCTGTCCAGGTATCAGAT	1	-
PIR1, 3-6	BRCA2ΔA‡	(Exon11) 25451 83997 (Exon 27) AATGTTGAAG**ATCAAA**(AAAACACT...TTATCAAA)GTCCTTTATCACT	58,545	5
PIR2	BRCA2ΔB‡	(Exon 11) 2537583990 (Exon 27) TTCTGATGAGGTATAT(AATGATTC...AGA**TATAT**)TATCAAAGTCCTTT	58,614	1
PIR7-11	BRCA2ΔC‡	(Exon 11) 25312 65211 (Exon 22) TCTCTCCGAAA**AACAA**(GATACTTA…GG**AACAA**)GGTTTATCAAGGGA	39,898	5
PIR12	BRCA2ΔD‡	(Exon 11) 25857 26316 (Exon 11) TAGCACGCATTCACA**T**(AAGGTTTT... TAGAAAG**T**)TCCTTACACAAAG	458	1

Nucleotide sequences at the BRCA2 deletion sites in the clones are shown.

*Sequence numbers flanking the deletions are shown and are based on GenBank accession no. AY436640.

ǂ Reference 10.

‡ New deletions. Microhomologies are indictaed with overlapping sequences in bold and underlined with deleted sequences shown in parentheses.

Discussion

Expression of BCRA1p130Cas protein has been analyzed. The data showed that both in univariate and in multivariate analysis, a high expression level of BRCA1p130 Cas in 8% of the primary tumors was associated with an increased rate of relapse, and accordingly a high expression level of BRCA1p130cas was associated with a poor response to first line Tamoxifen therapy (Lambert). Proteins with tumors with high BCRA1p130cas levels showed reduced response compared with low intermediate levels of BCRA130cas.

Lambert states that the response of Breast Cancer to Tamoxifen treatment is determined by the different properties the tumor cells have. Sex steroid hormone receptor negative tumors are unlikely to respond the antiestrogen. High levels of BRCA1p130cas protein were found in malignant tumors. These variable levels were also found in nonmalignant breasts (Lambert). All breast tumors are derived from the estrogen dependent epithelial precursor cell population, one third of the carcinomas are ER Alpha negative, but do not express EGF receptor (Lambert). The majority of breast cancers are ER Alpha positive growing tumors. According to (Stacey), cells with loss of BRCAs function are highly sensitive to (ADP-ribose) polymerase and are defective in homologous recombination. The resistance to PARP inhibition can be acquired by a mutation in BRCA2. PIR clones from the human CAPA/ni pancreatic cancer cell line were derived. They carry c.6174delT frame shift mutation. PIR clones can cause DNA damage induced RAD5 nucleau foci. New BRCA2 isoforms were expressed as a result of intragenic deletion of c.6174delT mutation causing the ORF to be restored. When BRCA2 deficient cells reconstituted with revertant BRCA2 alleles; the PARP inhibitor sensitivity and HR deficiency were rescued (Stacey).

Conclusion

According to (Stacey), most of the deletions in BRCA2 were related to small tracts of homology. It is possible that they arose from error prone repair which was caused by BRCA2 deficiency. ORF restoring mutations were existed in ovarian tumors from c.614delT mutation. These studies connote the resistance in BRCA mutation and define the importance of domains with BRCA2 and BRCA1.

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*Correspondence to

Dr. Marjan Assefi
Joint School of NanoScience and Nano engineering
University of North Carolina at Greensboro
North Carolina, USA
Email: m_assefi@uncg.edu