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ANTISENSE TECHNIQUE TO TREAT BREAST CANCER – A REVIEW

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ABSTRACT
There are many genes which are responsible for developing breast cancer especially, BRCA2 (Breast Cancer 2) and HER2 are extensively involved in developing breast cancer and hence it is the centre of attractions for all the researchers. Nano-particles conjugated with the anti-HER2 monoclonal antibodies are called as "Trastazumab" which directly target the HER2 gene. The major advantage of this technology is that the cells can be prevented before they evolve in to mature stages i.e. metastases production. The BRCA2 gene belongs to the family of tumor suppressor genes and its protein product is responsible for the error free repair mechanisms of DNA. This BRCA2 gene interacts with RAD51 gene to fix the DNA breaks. Mutation in BRCA2 gene such as insertion and deletion leads to breast cancer. More than 800 mutations are found in this gene that lead to increased risk of the breast cancer. Furthermore, BRCA2 gene is also associated with various cancers like prostate, ovarian, fallopian, male breast cancer. Researchers believe that altered products produced due to defects in this gene are unable to interact with the gene RAD51 and cannot repair the DNA. Antisense RNA is the tool which can used to block any RNA or DNA to synthesize its product. In this review we focus in using Antisense RNA against the sense RNA of an altered BRCA2 gene to block the altered affectivity of that gene on the DNA repair mechanism. However, Antisense RNA technique may not help in treating breast cancer, it can better manage the breast cancer to occur.

Keywords: Breast cancer, Genes, Mutation, Antisense RNA

INTRODUCTION

A germline mutation in BRCA1 or BRCA2 predisposes to the development of breast and ovarian cancer. The risk of developing breast cancer is associated with the mutation in BRCA1 or BRCA2 genes, which is derived from families with affected individuals and from population based studies appears to be variable within the families. Many molecular diagnosis techniques have been developed to detect mutations in these two genes for the persons who are susceptible to breast cancer. Treatment of breast cancer with BRCA1 or BRCA2 related tumors is similar to sporadic forms of these cancers. However, new classes of drugs that specifically target the BRCA1/2 signaling pathways are being studied. Furthermore the hormone estrogen is the potent stuff for the proliferation of onco-cells. So treatments are targeted to the genes which respond to estrogen production like estrogen-receptor genes (ER genes) like HER2. Many nano-particles are used to treat breast cancer which targets directly the genes which are estrogen responsive like HER2 gene. Thus nowadays various techniques and treatments are available to cure breast cancer but no treatment is specific which can target a particular gene[1].

Antisense can be a useful technique to atleast manage the genes which are diverging from the natural product of any gene. Antisense technique is the one which can direct against a specific gene. When the genetic sequence of a particular gene is known to be causative of a particular disease, it is possible to synthesize a strand of nucleic acid (DNA, RNA or a chemical analogue) that will bind to the messenger RNA (mRNA) produced by that gene and inactivate it, effectively turning that gene "off". The mRNAs are used to translate into some proteins. When antisense RNA is used against a specific gene which generates a defective protein product, it makes a double stranded molecule i.e. SENSE Strand (actual mRNA) and Antisense Strand which is exactly complementary to that of the sense strand. As a result the mRNA cannot be translated into the protein because double stranded strands are not supposed to be translated. So according to this concept we are making an antisense strand against a specific sequence of BRCA2 gene to at least manage the breast cancer[2].

Breast Cancer

Hereditary breast and ovarian cancer (HBOC) resulting from mutations in BRCA1 and BRCA2 is the most common form of both hereditary breast and ovarian cancers and occurs in all ethnic and racial populations. The overall prevalence of BRCA1/2 mutations is estimated to be from 1:400 to 1:800. 187delAG mutation occurs with a frequency of about 1.1% in individuals of Ashkenazi Jewishin. 5385insC mutation has an estimated prevalence of 0.1% - 0.15%[3]. 6174delT occurs with a frequency of about 1.5%.

Breast cancer usually starts in the tissues of breast. Breast cancers are of two types such as ductal carcinoma, a common type of breast cancer, which starts within the tubes of nipple which transmits the milk and lobular carcinoma begins in lobules a part of breast that produce milk. Breast cancer may either be invasive or non-invasive. Invasive are those tumor cells which have the ability to invade into their surrounding tissues and non-invasive cells will not invade. Non-invasive breast cancers are also called as "in-situ".

Symptoms of breast cancer

General symptoms are breast lumps or lump in the armpit which is hard possess uneven edges and usually does not hurt. Change in the size, shape or feel of the breast or nipple. Secretion of fluid from the nipple will be bloody, clear to yellow or green color and look like pus. Symptoms of advanced breast cancer may includes bone pain, breast pain or discomfort, skin ulcers, swelling of one arm (next to the breast with cancer) and Weight loss.

Treatment for breast cancer

Treatment is based on many factors, including type and stage of the cancer, sensitive of cancer to certain hormones, over expression of gene HER2/neu. General cancer treatments are chemotherapy medicines to kill cancer cells, radiation therapy to destroy cancerous tissue, surgery to remove cancerous tissue, lumpectomy to remove breast lumps and mastectomy to remove all or part of the breast and possible nearby structures. Hormonal therapy is prescribed to women with ER-positive breast cancer to block certain hormones that fuel cancer growth. An example of hormonal therapy is the drug tamoxifen which blocks the effects of estrogen that helps breast cancer cells to survive and grow. Women with estrogen-sensitive breast cancer are benefited by this drug. Targeted therapy, also called biologic therapy, is a newer type of cancer treatment where special anticancer drugs are used that target certain changes in a cell that can lead to cancer. One such drug is trastuzumab (Herceptin) which will be useful for women with HER2 positive breast cancer. Most women receive a combination of treatments. For women with stage I, II, or III breast cancer, the main goal is to treat the cancer and prevent it from returning (curing). For women with stage IV cancer, the goal is to

improve symptoms and help them live longer. In most cases, stage IV breast cancer cannot be cured.

- Stage 0 and DCIS -- Lumpectomy plus radiation or mastectomy is the standard treatment. There is some controversy on how best to treat DCIS.
- Stage I and II -- Lumpectomy plus radiation or mastectomy with some sort of lymph node removal is the standard treatment. Hormone therapy, chemotherapy, and biologic therapy may also be recommended following surgery.
- Stage III -- Treatment involves surgery, possibly followed by chemotherapy, hormone therapy, and biologic therapy.
- Stage IV -- Treatment may involve surgery, radiation, chemotherapy, hormonal therapy, or a combination of these treatments.

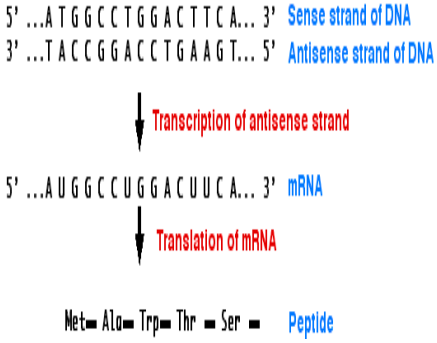
BRCA genes

Mutations in a number of genes are now known to cause susceptibility to breast and/or ovarian cancer. In the context of high-risk families, the most important genes are BRCA1 and BRCA2. BRCA1 was localized in chromosome 17q by genetic linkage in 1990[4] and subsequently was cloned in 1994[5]. Studies to date suggest that BRCA1 accounts for the majority of families containing multiple cases of breast and ovarian cancer[6,7]. The BRCA2 gene was reported in 1994 by Professor Michael Stratton and Dr Richard Wooster (Institute of Cancer Research, UK)[8]. This gene is located in the long arm of chromosome 13 at position 13q12.3 spanning the DNA from base pairs 31,787,616 to 31,871,804. BRCA2 belongs to the tumor suppressor gene family and the protein encoded by this gene is involved in the repair of chromosomal damage with an important role in the error free repair of DNA double strand breaks[9]. Though the structures of both BRCA1 and BRCA2 are very different, some functions are interrelated. Both the genes interact with the gene RAD51 gene to fix breaks in DNA. Certain variations and mutations in BRCA2 gene cause an increased risk of breast cancer. Many researchers have identified more than 100 mutations in this gene. These mutations are generally deletions or insertions of a small number of DNA base pairs. Abnormal gene product cannot interact with the gene RAD51 and thus DNA repairs cannot be satisfied. As a result mutations build up and form a tumor.

A study[8] estimated that disease was linked to BRCA2 in 74% of families, providing preliminary evidence that BRCA1 and BRCA2 together might account for most high risk breast cancer families. There is particularly clear evidence that BRCA1 and BRCA2 together are likely to account for the majority of high-risk breast-ovarian cancer families. In a study of 145 breast-ovarian cancer families, there were 10 families with strong evidence against linkage to BRCA, of these 3 have been identified due to BRCA1 mutation and 7 have been identified by BRCA2 mutation[11]. The aims of this collaborative study were twofold, first to estimate the respective proportions of different types of high risk cancer families in which the disease is due to BRCA1 and BRCA2 and to determine on what proportion was due to unidentified genes and second to estimate the penetrance of BRCA2 in a large data set.

Gene products of abnormal and abnormal BRCA2 gene

Normal gene product of BRCA2 codes for 380 kd protein with 3418 amino acids. Eight 30-40 residue motifs were found in exon 11 mediates binding of BRCA2 to RAD51. BRCA2 is normally located in the nucleus and contains phosphorylated residues[12]. Like BRCA1, BRCA2 is expressed in most of the tissues and cell types, indicating that the gene expression does not account for the tissue restricted phenotypes of breast and ovarian cancers. BRCA2 transcriptions are induced in late G_1 phase of the cell cycle and remain elevated during the S phase, indicating its role in DNA synthesis[13]. The BRCA2 susceptibility protein interacts with the RAD51 protein, a key component in homologous recombination and double-strand break repair[14,15]. Through this interaction, BRCA2 regulates the availability and activity of RAD51, which coats single-strand DNA to form a nucleoprotein filament that invades and pairs with a homologous DNA duplex to initiate strand exchange[16]. When this function is lost, it probably allows for the accumulation of other genetic defects that are themselves directly responsible for cancer formation.



Abnormal gene product of BRCA2 is reported due to frameshift deletions, insertions or nonsense mutations that predict premature truncation of protein transcription, consistent with the loss of function that is expected with clinically significant mutations in tumor suppresser genes.

Variation in breast cancer risk by position in BRCA2 mutation carriers

Cancer occurrence in 164 families with breast and ovarian cancer and germline mutations in BRCA2 gene were studied previously. Mutations in the central portion of the gene were associated with the higher ratio of breast cancer. The mutations were between nucleotides 3059-4075 and 6503-6629. Generally the mutations were deletions and insertions of some few nucleotides in this region of the gene taking to the new defective gene product which was not able to interact with the gene RAD51 to repair the DNA breaks. In a study[17] based on 25 families with germline BRCA2 mutations reported that families with high proportion of breast cancer tended to have mutations located within a 3.3 kb region in exon 11 of BRCA2 gene. They called this region of BRCA2 as OCCR (Ovarian Cancer Cluster Region) bounded by nucleotides 3035 and 6629.

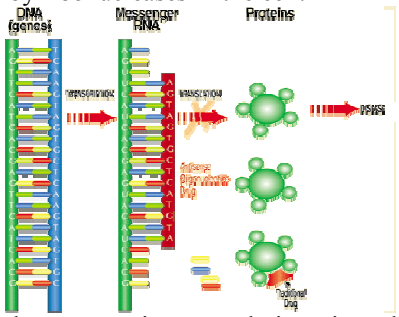
Heterogeneity and penetrance of BRCA2 & BRCA1 genes in breast cancer

Many researches were done on the heterogeneity and penetrance of the genes BRCA1 and BRCA2 in development of breast cancer. The contribution of both the genes to inherited breast cancer was assessed by linkage and mutation analysis in 237 families, each with at least four cases of breast cancer collected by the Breast Cancer Linkage Consortium. Families were included without involvement of other types of breast cancer. Ultimately, the breast cancer disease was linked to mutation in BRCA1 gene was estimated about 52% of families, to BRCA2 in 32% and to neither gene in 16% of families, suggesting other predisposition of the genes.

Antisense Technique

The antisense concept was first conceived in 1978 and has been through various technical refinements. Antisense compounds are designed to have the right nucleotide sequence to bind specifically and interfere with associated mRNA for the production of particular protein. To create antisense drugs, special chemically stabilized nucleotides are synthetically linked together in short chains of about 12-30 nucleotides (called oligonucleotides). Each antisense drug is designed with the right complementary genetic code to bind to a specific sequence of nucleotides in its mRNA target to form a short area of double strands. However, RNA can form duplexes just as DNA does. All that is needed is a second strand of RNA whose sequence of bases is complementary to the first strand; e.g., 5′ C A

U G 3′ mRNA and 3′ G U A C 5′ Antisense RNA. The second strand is called the antisense strand because its sequence of nucleotides is the complement of message sense. When mRNA forms a duplex with a complementary antisense RNA sequence, translation is blocked. This may occur because the ribosome cannot gain access to the nucleotides in the mRNA or duplex RNA is quickly degraded by ribonucleases in the cell.



So, our main goal to use antisense technique is to block the gene BRCA2 or BRCA1 which lead to production of defective gene products. As indicated earlier, the specific frameshift mutations in nucleotides of exon 11 in BRCA2 leads to defective gene. Molecular genetic testing and many more techniques help us to identify these mutations. Once identified, the antisense strand can be made which is exactly complementary to the sense strand of BRCA2 gene which is defective.

PRIMER DESIGNING TOOL

As indicated previously, the frameshift mutations occur in the region of BRCA2 between nucleotides 3059-4075 and 6503-6629. The primer is made for the synthesis of the antisense strand. Nowadays various types of software are available to design the primers like "PRIMER3". The whole BRCA2 gene sequence was copied in the primer3 and the primers according to the above regions of nucleotides were selected. So, for the synthesis of antisense following primers could be selected

3051 CTCCTGGGTT CAAGTGATTC TCCTGTGGCA GCCTCCGGAG TAGCTGGGAC

6501 TAAAATGGAT GTGTTATTAT TAGCTGAACT CCTTAGTTTA CTTTAGAGTT

The selection of the primers is the most crucial thing. By using Primer3 software, the primers of few oligonucleotides and the whole antisense RNA are synthesized using PCR. The novel antisense is now directed against the defective frameshift mutated gene and then the gene will be blocked due to the formation of double stranded RNA.

CONCLUSION

We can conclude that antisense technique can be used to treat any type of defective gene which can be lead to altered gene product. As antisense binds to a sense strand of a defective gene, it forms a double stranded molecule which cannot be translated as double strands cannot be detected by ribosomes. Antisense strands make double stranded molecule by making hydrogen bonds with the sense strands. Thus, the antisense strands complementary to the nucleotides from 3059-4075 and 6503-6629 by synthesizing primers of few oligonucleotides of region 3051 and 6501 respectively in BRCA2 gene. Once the primers are designed, whole antisense strand can be synthesized and directed against the defective gene which blocks the BRCA2 gene, thus inhibiting the altered gene product formation. As a result, ribosomes cannot synthesize the gene and thus decreasing the risk of breast cancer. It is not sure that, antisense technique is able to cure breast cancer completely, but however it can manage extensively the disease by decreasing the defective gene product.

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