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GENE THERAPY A PROMISING TREATMENT FOR BREAST CANCER: CURRENT SCENARIO IN PAKISTAN

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Abstract

Breast cancer is one of the most common cancers among women around the world. It accounts for 22.9% of all the cancers and 18% of all female cancers in the world. One million new cases of breast cancer are diagnosed every year. Pakistan has more alarming situation with 90,000 new cases and ending up into 40,000 deaths annually. The risk factor for a female to develop breast cancer as compared with male is 100 : 1. The traditional way of treatment is by surgery, chemotherapy or radiotherapy. Advanced breast cancer is very difficult to treat with any of the traditional treatment options. A new treatment option in the form of gene therapy can be a promising treatment for breast cancer. Gene therapy provides treatment option in the form of targeting mutated gene, expression of cancer markers on the surface of cells, blocking the metastasis and induction of apoptosis, etc. Gene therapy showed very promising results for treatment of various cancers. All this is being trialed, experimented and practiced outside of Pakistan. Therefore, there is an immense need that this kind of work should be started in Pakistan. There are many good research institutes as well as well-reputed hospitals in Pakistan. Presently, there is a need to develop collaboration between research institutes and hospitals, so that the basic work and clinical trials can be done to treat breast cancer patients in the country. This collaboration will prove to be very healthy and will not only strength research institute but also will be very beneficial for cancer patients.

Keywords: Breast cancer, Metastasis, Mutation, Apoptosis, Research Centers, Collaboration.

Introduction

Breast cancer has a very high ratio of occurrence in United States and probably all over the world among major cancers (Harris et al., 1993). Breast cancer is at third number in the list of most frequently occurring cancers after stomach and lung cancers. In women, it is the most common cancer and accounts for 21% of new cancer cases throughout the world. According to this ratio, 6.2% women in developed countries and 2.2% in developing countries on average are at life time risk of breast cancer (Parkin et al., 1999).

In Pakistan, breast cancer is the most frequently diagnosed cancer in women with a ratio of nearly 1 in 5 patients. Cumulative level of breast cancer is very low in Pakistan, in comparison with other countries. An average Pakistani woman would have estimated probability of 1-2% to be treated for breast cancer by the time she reaches her 65th birthday (Yousaf and Jaffery, 1985).

Surgery, hormonal therapy, radiotherapy and chemotherapy are the major treatments of breast cancer. These reduce the risk of breast cancer up to certain levels. These approaches can only suppress the tumor or cancer growth but they are unable to cure the disease permanently. As gene therapy is in use to treat several genetic disorders including cancer, so it can be a very promising treatment option for breast cancer patients as well. Till date many gene therapy approaches have been adopted in pre-clinical and clinical trials. Gene therapy can be broadly divided in six categories: (1) molecular chemotherapy, (2) anti-angiogenic gene therapy, (3) pro-apoptotic gene therapy, (4) mutation compensation, (5) genetic modulation of resistance/sensitivity, and (6) genetic immune-potentiation (Harris, 1998).

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Genetic Basis of Breast Cancer

Amplifications, deletions and gene mutations come under the somatic changes of genome of breast cancer. Oncogenes present on regions of several chromosomes get amplified in breast tumors. Tumor suppressor genes mutations are also cause of breast carcinomas. TP53 (Tumor Protein P53) which encodes the p53 transcription factor, and the CDH1 which encodes the cadherin cell adhesion molecule are the tumor suppressor genes (Ingvarsson, 1999).

The function of p53 is to suppress the cell proliferation through a multiprotein regulatory pathway and it focuses around the control of apoptosis and retinoblastoma gene (Malkin et al., 1990). Cellular-stresses activate p53 and these independent pathways of p53 depend upon distinct upstream regulatory kinases. These include a pathway dependent on the alternative product of the INK4 gene, $p14^{ARF}$, (which is activated by expression of oncogenes), an ataxia-telangectasia mutated (ATM)/human homologue of Rad53 (Chk2)-dependent pathway activated by DNA double-strand breaks and a third ATM, Chk2 and $p14^{ARF}$ independent pathway whose activity is increased by cytotoxic anti-tumour agents and ultraviolet light.

This pathway may be mediated by other kinases such as the ATM relative ataxia-telangectasia and Rad3-related protein (ATR) (Vogelstein et al., 2001). The anti-proliferative action of p53 is exerted by induction of reversible or irreversible cell cycle arrest or apoptosis (Donehower et al., 1992). In solid tumors, p53 is the most commonly mutated tumor suppressor gene. Patients with rare hereditary disease, Li-Fraumeni syndrome also have a mutated p53 gene in their germline cells (Harris, 1998).

Mutated p53 gene is expected to present in 50% of all cancers. Mutant proteins are a defective target for binding of sequence specific DNA and in this way also for the transactivation of genes, upregulated by the wild-type proteins (Sigal and Rotter 2000). There are different mechanisms of p53 inactivation in breast cancer. In virus-associated cancers sequestration or enhanced degradation of p53 occurs through interaction with virally encoded proteins. Binding of MDM2 with p53 promotes the ubiquitination of the C-terminus of p53 and, hence, subsequent degradation. Association of p53 and MDM2 is prevented by interaction of $p14^{ARF}$ with MDM2 and thus stabilizing p53. Overexpression of MDM2 or deletion or epigenetic silencing of $p14^{ARF}$ can start inappropriate degradation of p53. There is another mechanism of inactivation which involves cytoplasmic sequestration of p53 protein and preventing nuclear localisation of the protein and thus inhibiting its activity (Vogelstein et al., 2001).

The other two very important caretaker genes are wild type BRCA1 and BRCA2 and their function is to repair the damaged DNA. BRCA1 is present on chromosome 17. Various studies indicate that mutations in BRCA1 are associated with 50 percent to 85 percent life-time risk of developing breast cancer. Carriers of BRCA1 gene mutation often develop breast cancer at a younger age as compared to the general population (Thompson and Easton, 2002). BRCA2 gene is located on chromosome 13. Women carrying BRCA2 mutations have similar chances of cancer development as are the BRCA1 carriers. Men carrying BRCA2 mutation have an increased risk of developing breast cancer (Campeau et al., 2008).

Chromosomal instability results due to deletions or defects in BRCA genes as cell loses the ability to repair the mistakes which occur during the normal process of replication, transcription and translation naturally (Patel et al., 1998).

Gene Therapy Approaches

The genetic alterations in tumor suppressor genes (TSGs) are the basis for the breast carcinomas. Strategies have been employed which target correction of these TSGs to cure breast carcinomas permanently. When introduced in breast cancer cells, many TSGs have shown induction of apoptosis or cell cycle arrest including p53 (Wang et al., 1993), p21 (Shibata et al., 2001), p27 (Craig et al., 1997), p16 (Bai et al., 2001; Campbell et al., 2000), Rb (Jiang and Zacksenhaus, 2002; Wang et al., 1993), BRCA1 (Holt et al., 1996), BRCA2 (Holt, 1997), Testin (Sarti et al., 2005), Maspin (Shi et al., 2002).

To date most trials target the most common altered TSGs in cancer which is p53. Human breast cancer cells when transduced with viral wild type p53 showed sufficient restoration of the

normal balance of cell proliferation and cell death of the breast cancer cell (Chen and Mixson, 1998; Zhang et al., 1994) demonstrated transfer of p53 into lung cancer by an adenoviral vector initially. Now, we will discuss some approaches to treat breast cancer by gene therapy.

1. Antisense Technology

Antisense technology involves the use of antisense molecules that can specifically inhibit the expression of pathogenic genes. Antisense oligodeoxynucleotides are short ssDNA molecules that modify gene expression by blocking the transfer of genetic information into protein. Many genes relevant to breast cancer such as αV integrin (Townsend et al., 2000), ribosomal protein P2 (Gardner-Thorpe et al., 2003), c-erbB-2 (Brysch et al., 1994), methylenetetrahydrofolate reductase (Sekhon et al., 2002), and protein kinase C-alpha (PKC-α) (Roychowdhury and Lahn, 2003; Lahn et al., 2004), have been successfully targeted by antisense oligonucleotides. LY900003 is currently in clinical development and it presents an antisense oligonucleotide to specifically block PKC-α (Roychowdhury and Lahn**,** 2003).

Ribonucleic interference (RNAi) technology is another closely related area (Scherr et al., 2003). RNAi showed specific downregulation of c-myc which was sufficient to inhibit the growth of breast cancer MCF-7 cells ***in vitro*** and ***in vivo*** (Wang et al., 2005). Ribozymes are another approach in ablation of oncogenes. They are RNA molecules capable of acting as enzymes (Rossi, 1992). Ribozymes were used for the treatment of human immunodeficiency virus (HIV) initially (Rossi et al., 1992). Hammerhead ribozyme is the simplest and is approximately 30 nucleotides long. Possibility to produce ***trans***-acting ribozymes directed against RNA sequences of interest highlighted the importance of hammerhead ribozymes. Since then, genetically modified ribozymes have been designed, produced and given to cells to 'knock down' the expression of specific genes. Keeping this in view, adenovirus-mediated ribozyme targeting of HER-2/neu showed inhibition of in vivo growth of breast cancer cells in a mouse model (Suzuki et al., 2000).

Another strategy targets disruption of normal cellular localization of growth receptors. The proto-oncogene ***erbB-2*** is of particular interest. Downregulation of cell surface erbB-2 levels and induction of apoptosis in erbB-2-overexpressing breast cancer cells resulted on delivery of a gene encoding an anti-erbB-2 intracellular single-chain antibody (sFv) (Wright et al., 1997; Grim et al., 1998).

2. Suicide Genes

In late 1980s, it was suggested that there is a possibility that introduction of so-called suicide genes renders cancer cells more sensitive to chemotherapeutics or toxins. Suicide gene therapy has two categories: toxin gene therapy and enzyme-activating pro-drug therapy. Toxin gene therapy includes the transfection of genes that express toxic molecules. On the other hand, enzyme-activating pro-drug therapy explains the transfection of genes able to express enzymes that can selectively activate specific pro-drugs. The latter one has been variously called gene-directed enzyme pro-drug therapy (GDEPT), gene pro-drug activation therapy (GPAT), suicide gene therapy or virally directed enzyme pro-drug therapy (VDEPT) (Niculescu-Duvaz and Springer, 2005). Gene-directed enzyme pro-drug therapy is a two phase treatment of cancer. The gene for the enzyme is delivered to the breast cancer cell in the first phase while in the second a nontoxic pro-drug is administered, which is then converted into a toxic metabolite by the foreign enzyme expressed in the tumor. The enzymes recommended for GDEPT for breast cancer are divided into two categories. The first one contains 'foreign' enzymes which are nonmammalian in origin, such as carboxypeptidase G2 (CPG2), bacterial cytosine deaminase (Friedlos et al., 2002; Stribbling et al., 2000; Niculescu-Duvaz and Springer, 2005) and viral TK (Gadal et al., 2004; Grignet-Debrus et al., 2000). The second category contains enzymes which are of human origin, such as cytochrome ***P***450 isophorms (Niculescu-Duvaz and Springer, 2005; Kammertoens et al., 2000). Most probably, the two best examples of this strategy are cytosine deaminase (CD) and thymidine kinase (TK), which convert 5-fluorocytosine and ganciclovir (GCV), respectively, into their toxic drug forms.

Many experimental models have shown that combination therapy with cytokines and suicidal genes can be used (Chen et al., 1995; O'Malley et al., 1996). An adenoviral vector carrying the interleukin-2 (IL-2), granulocyte-macrophage

colony-stimulating factor (GM-CSF) or HSV-1 thymidine kinase (HSV-TK) was injected in breast cancer cells grown as xenografts in BALB/c mice. Both cytokine genes combined with HSV-TK showed a sufficient reduction in tumor growth in comparison with HSV-TK alone (Majumdar et al., 2000). Efficacy of GDEPT system is enhanced by a double transfer of suicide genes. Two distinct types of pro-drugs are activated by transfecting cancerous cells with two different suicide genes. Results reveal that, the antitumoral effect exerted by two different suicide gene systems (CD and cytochrome *P*450 2B1) is more efficient to each single system alone for murine mammary tumors (Kammertoens et al., 2000).

3. Gene Therapy to Induce Apoptosis

Impaired apoptotic signaling plays an important role in tumor initiation and progression in different types of cancers. Apoptotic resistance of cancerous cells has hazardous effects because not only it increases the growth of the tumor but also provides resistance to host defense mechanism and different types of therapeutics (Bold et al., 1997; Nylandsted et al., 2000). Identification of genes involved in apoptotic induction offers a good target for breast cancer gene therapy. Strategies have been employed which deliver those genes that are responsible for apoptotic death and also transmit death signals to the adjacent tumor cells. These proapoptotic strategies in breast cancer include functional replacement of TSGs, suicide gene therapy, death receptor and ligand systems and pathways, BCL-2 family proteins.

The first gene which was identified with a function of inhibiting apoptosis was BCL-2 and it plays an important role in breast cancer (Vaux et al., 1988; Lin et al., 2001). Experiments on nude mice have showed that BCL-xs gene which is a dominant negative repressor of Bcl-2 and Bcl-xL induced apoptosis in human mammary tumors (Ealovega et al., 1996). The down regulation of proapoptotic Bik has been linked to the development of breast cancers. Significant induction of apoptosis was observed in orthotopic tumor tissues in nude mice and in four breast cancer cell lines in vitro (Zou et al., 2002).

In breast cancer cells, there are number of death receptor and ligand systems that function in regulation of apoptosis. These include tumor necrosis factor-related apoptosis-inducing ligands (TRAIL), Fas ligand (FasL) and tumor necrosis factor-α. Experimental data showed that death of breast tumor cells occurred due to the rapid production and expression of TRAIL protein on introduction of *TRAIL* gene into subcutaneous human breast cancer xenografts in nude mice and breast cancer cells using an adenoviral vector (Griffith et al., 2000; Lin et al., 2002).

Several proteins have been identified which help in promoting tumorigenesis by inhibiting apoptosis (Jaattela, 1999; Nylandsted et al., 2000). Major stress-inducible Hsp70 (also known as Hsp72 or Hsp70i) may be categorized as cancer-relevant antiapoptotic protein. Tumor cell lines and human breast tumors express Hsp70. Classical transfection of antisense Hsp70 cDNA (asHsp70) or inhibition of its synthesis by adenoviral transfer causes significant breast cancer cells death. Bcl-2 and Bcl-X_L could not save death induction of breast tumor cells by as Hsp70. There is a good possibility to treat therapies resistant cancers by neutralizing Hsp70 (Nylandsted et al., 2000).

The adenovirus type 5 E1A protein has demonstrated antitumor effects through the induction of apoptosis, inhibition of cell cycle progression and sensitization to chemotherapeutic agents and radiation (Hung et al., 2000; Ueno et al., 2000). Regression of tumors in nude mice and induction of apoptosis in breast cancer cells is demonstrated by adenovirus-directed expression of dominant-negative ER receptor (Lee et al., 2001).

4. Anti-Angiogenic Gene Therapy

Angiogenesis plays an important role in establishment and spread of tumor. In this regard, antiangiogenic gene therapy can prove a good approach for treating breast carcinomas (Davidoff and Nathwani, 2004). Plasmids encoding angiostatin and endostatin and complexed with liposomes inhibited breast cancer in nude mice (Chen et al., 1999; Oga et al., 2003). Antiangiogenic therapy alone can not control the promulgated breast cancer in humans. Combining antiangiogenic therapy with other strategies, both conventional and gene transfer-based can produce good results. Antiangiogenic therapy can be complemented by both chemotherapic and

hormonal approaches due to their different mechanisms of antitumoral action.

In a recent experiment, it was observed that the association of angiostatin with tamoxifen produced better results than either approach used alone in a transgenic mouse model of breast cancer (Sacco et al., 2002). Further, the potential efficacy of delivery of endostatin gene can be used to treat metastatic breast cancer to brain (Oga et al., 2003). Recombinant proteins that inhibit angiogenesis have shown regression in tumors of mouse models when administered systemically (Wu et al., 1997; Sim et al., 2002).

5. Immunotherapy

An immune response can be harnessed and used for eradicating out the cancerous cells. Immunotherapy can be divided into two functional types: active immunotherapy and passive immunotherapy. The former approach employs tumor vaccines and immunostimulatory cytokines stimulates the patient's immune response to generate an antitumor immunity; while the later approach explains how to administer pre-formed elements of the immune system, such as antitumor cytokines, tumor antibodies, or tumoricidal effector cells to patients, with a plan of directly kill the cancer cell.

An immune response is triggered on alarm signals induced by pathogens (Strong, 2000). Activation of a number of immune effector cells can be triggered by direct introduction of immunostimulatory molecules encoding genes into breast cancer cells. Systemic injection of cytokines such as IL-2 was focus of initial studies (Siegel and Puri, 1991). Biologically active cytokine can be delivered at a tumor site without manipulating with tumor cells ex vivo (Bubenik et al., 1998). Usage of autologous or allogenic genetically modified normal cells for local secretion of immunostimulatory molecules was recommended for this purpose.

The survival of mice with intracerebral breast cancer metastasis was prolonged by intratumoral injection of IL-2-secreting syngeneic/allogeneic fibroblasts transfected with DNA from breast cancer cells (Lichtor et al., 2005). T-cell subsets ($CD8^+$ and natural killer (NK)/LAK cells) mediated the antitumor response predominantly (Lichtor et al., 2005; Slos et al., 2001). A variety of other cytokines such as the GM-CSF, IL-4, ILl-12, IL-18, interferon-alpha and IL-23 have been used (Yu et al., 1993; Lo et al., 2003).

6. Genetic Vaccines

Genetic vaccines are another approach. In the last three decades, many tumors associated with antigens have been identified and cloned (Knuth et al., 1991; Knuth et al., 1989). These antigens have been used to produce vaccines for immunotherapy of cancer. Most tumor antigens are utilized as immunotherapy targets because they are aberrantly expressed on tumor cells. In this regard, a growth advantage for breast cancer cells has been provided by aberrant expression of Her-2/neu/erbB-2 overexpression (Yip and Ward, 2002). Currently, under investigation genes for the vaccine production in breast carcinomas are carcino-embryonic antigen (Kawashima et al., 1999), Fos-related antigen 1 (Fra-1) (Kustikova et al., 1998; Luo et al., 2005), tumor cell-associated extracellular matrix metalloproteinase inducer (EMMPRIN) (Tang et al., 2005), MAGE-1 (Toso et al., 1996), MUC-1 (Ioannides et al., 1993), hTERT (Vonderheide et al., 1999), and B7-H4 (Salceda et al., 2005; Tringler et al., 2005).

A recent approach is a combination of different vaccines. By utilizing various cytokines and co-stimulatory molecules as molecular adjuvants combination vaccine strategies tend to enhance the effect of genetic vaccines in cancers (Chang et al., 2004). In this way, these approaches combine the 'genetic' and 'conventional' immunotherapeutic approaches. Studies suggest that GM-CSF enhances antigen processing and presentation by dendritic cells so it has been used as vaccine adjuvant for breast cancer (Von Mehren et al., 2001). In another study, it was observed that injecting mice with Her-2/neu encoding plasmids and augmentation with cytokines enhances the efficacy against breast cancer (Chang et al., 2004).

7. Dendratic Cells (DCs) Applications

DCs are powerful antigen-presenting cells and have central role in generation of immune responses. They process the antigens and present epitopes to the surface MHC molecules to have interaction with T cells (Sakai et al., 2004). Many costimulatory molecules and cytokines that are required to sustain and direct the immune response are expressed by DCs (Banchereau and

Steinman, 1998; Thery and Amigorena, 2001). Many studies for producing anticancer vaccines have focused on using DCs ability to present tumor antigens in a proper way.

Gene transfer approaches including DCs rely on direct modification of DCs by gene modification *in vitro* and then administration of modified APCs or DCs as a vaccine. For modification purposes, genes encoding breast cancer antigens, cytokines and molecules involved in antigen presentation were used. Suppression of breast cancer in BALB-*neu*T mice was observed with a vaccine having modified DCs by adenoviruses encoding nonfunctional tumor antigens, such as nonsignaling HER-2/*neu (*Sakai et al., 2004). $CD8^+$ cytotoxic T lymphocytes induced by DCs transduced with a Tat fusion protein (from the HIV) having the extracellular domain of Her2/neu killed Her2/neu-expressing breast cancer cells specifically *(*Sakai et al., 2004). For breast cancer therapy both $CD4^+$ and $CD8^+$ mammoglobin-specific T cells can be induced by dendritic cells transduced with Tat mammaglobilin (Viehl et al., 2005).

8. Monoclonal Antibodies

Another approach is transferring genes encoding antibodies to known tumor antigens in vivo. Genetically modified antibodies molecules contain a constant region of human antibody molecule and a variable region of monoclonal antibody directed against antigen of interest. This antibody has long half-life in human body due to the absence of neutralizing immune response and maintains all the functions of natural antibodies at the same time (Larin et al., 2004).

Herceptin is a well-studied example of it. This humanized monoclonal antibody binds directly to Her-2-positive tumor cells and displays efficient inhibition of tumor growth. Antibody-dependent cellular toxicity is the basis of tumor rejection strategy. In this case, killing mechanisms are activated directly when Fc surface receptor on opsonized breast cancer cells binds with natural killer cells (Larin et al., 2004).

9. Miscellaneous Gene Therapy Approaches

Evidences suggest that downregulation of Major Histocompatibility Complex (MHC) molecules, costimulatory molecules and some other ligands can help escaping breast cancer cells from immune control (Hui et al., 1984; Wallich et al., 1985). $CD4^+$ activation takes place due to presentation of MHC II and this results in secretion of cytokines. These secreted cytokines along with presented MHC I $CD8^+$ cytotoxic T cells and result in cell necrosis (Ruppert et al., 1997). Host immune system recognition and antitumor activity can be enhanced by introducing such deficient receptors and ligands using gene therapy approach (Tanaka et al., 1985).

Conventional therapies fail in many cases of breast cancers due to the resistance to DNA damaging agents such as chemotherapy and radiotherapy. Different mechanisms responsible for breast cancer multi drug resistance have been elucidated. The main mechanisms include modulation in apoptotic function, multidrug resistance-associated protein 1 (MRP1, ABCC1), deletion or amplification of topoisomerase II, breast cancer resistance protein (ABCP, BCRP, MXR, ABCG2) and cellular overproduction of P-Glycoprotein (Staud and Pavek, 2005; Pavek et al., 2005).

Down regulation of apoptosis and over-expression of antiapoptotic genes play a vital role in failure of radiotherapy and chemotherapy at the present stage of breast cancer treatment. It is stated that p53 plays no part in chemotherapy and radiotherapy resistant tumors so this can be the basis for TSG combination therapies. Combination with other anticancer treatments can be beneficial due to the low toxicity of p53 in initial trials and linkage of p53 with apoptosis. Keeping this in view, adenovirus p53 can demonstrate tumor regression and sensitize local and metastatic breast cancer by doxorubicin therapy (Lebedeva et al., 2001).

After both chemo and radio therapies, the cellular response to DNA damage is mediated by p53. p53 results in cell cycle arrest and finally induce apoptosis (Pruschy et al., 2001; Dasika et al., 1999). When DNA is damaged by radiotherapy, there is a cell cycle arrest at the G1 checkpoint as well as p53 dependent transcription activation of p21 (Macleod et al., 1995). The loss of this checkpoint is linked with decrease in tumor cell apoptosis (Szumiel, 1994).

ErbB-2 a proto-oncogene is widely studied in breast cancer. A high correlation is reported

between resistance to different therapies and overexpression of epidermal growth factor receptor (Sartor, 2000). Human breast cancer cells were radiosensitized by overexpression of dominant negative epidermal growth factor receptor CD533 mediated by adenoviruses (Lammering et al., 2001; Lammering et al., 2001).

Human telomerase reverse transcriptase (***TERT***) is a potential target for active-specific immunotherapy. However, it has been proved difficult to induce effective specific tumor antigen-specific immunity consistently by various TERT vaccine formulations. To overcome this difficulty, new adjuvant strategies such as utilizing chemokines to attract T-cells and antigen-presenting cells have been employed (Yamano et al., 2007).

In a study, Brade et al. (2003), tested the hypothesis that thermosensitivity can be enhanced by heat-directed suicide gene therapy in locally recurrent breast cancer (LRBC). Given the relapse rate in the following thermoradiotherapy for LRBC and the fact that cells can be intrinsically resistant to heat (Kampinga et al, 1995; Henle et al, 1997).

An adenoviral vector (Ad.70b.CDTK) was constructed in which the hsp70b promoter controls the expression of dual prodrug-activating *E.coli* cytosine deaminase/herpes simplex virus thymidine kinase (CDTK) fusion gene. When expressed in the presence of the prodrugs 5-fluorocytosine (5-FC) and ganciclovir (GCV), the CDTK fusion protein has been shown previously to be highly cytotoxic in a variety of tumor cell types both in vitro and in vivo (Kim et al., 1998; Rogulski et al., 1997; Xie et al., 1999). Importantly, this therapeutic strategy was shown to act synergistically with heat treatment in a human prostate cancer model (Blackburn et al., 1998). Here it was shown for the first time that adenovirus-mediated, heat-directed expression of a CDTK fusion gene can significantly decrease the survival of thermoresistant human breast cancer cell lines treated with clinically relevant doses of pro-drugs and heat.

In a novel approach, the survivin which is a member of inhibitor of apoptosis (IAP) gene family has been used for treating breast cancer. Survivin is localized to components of the mitotic apparatus and is expressed in mitosis in a cell cycle–dependent fashion (Li et al., 1998). Survivin is present in very low amounts in normal adult tissues but its expression is upregulated in most human cancers cells (Ambrosini et al., 1997; Velculescu et al., 1999). Survivin pathway in cancer was targeted with a replication-deficient adenovirus encoding a survivin Thr^{34}→Ala mutant, which abolishes a phosphorylation site for $p34^{cdc2}$-cyclin B1 (O'Connor et al., 2000). Here, it was shown that initiation of the mitochondrial apoptotic pathway was caused in various tumor cell types, exhibited no toxicity for normal human cells, and suppression of tumor growth in three different xenograft breast cancer models *in vivo* occurred. A Model of different GT approaches to treat breast cancer is given in Fig. 1.

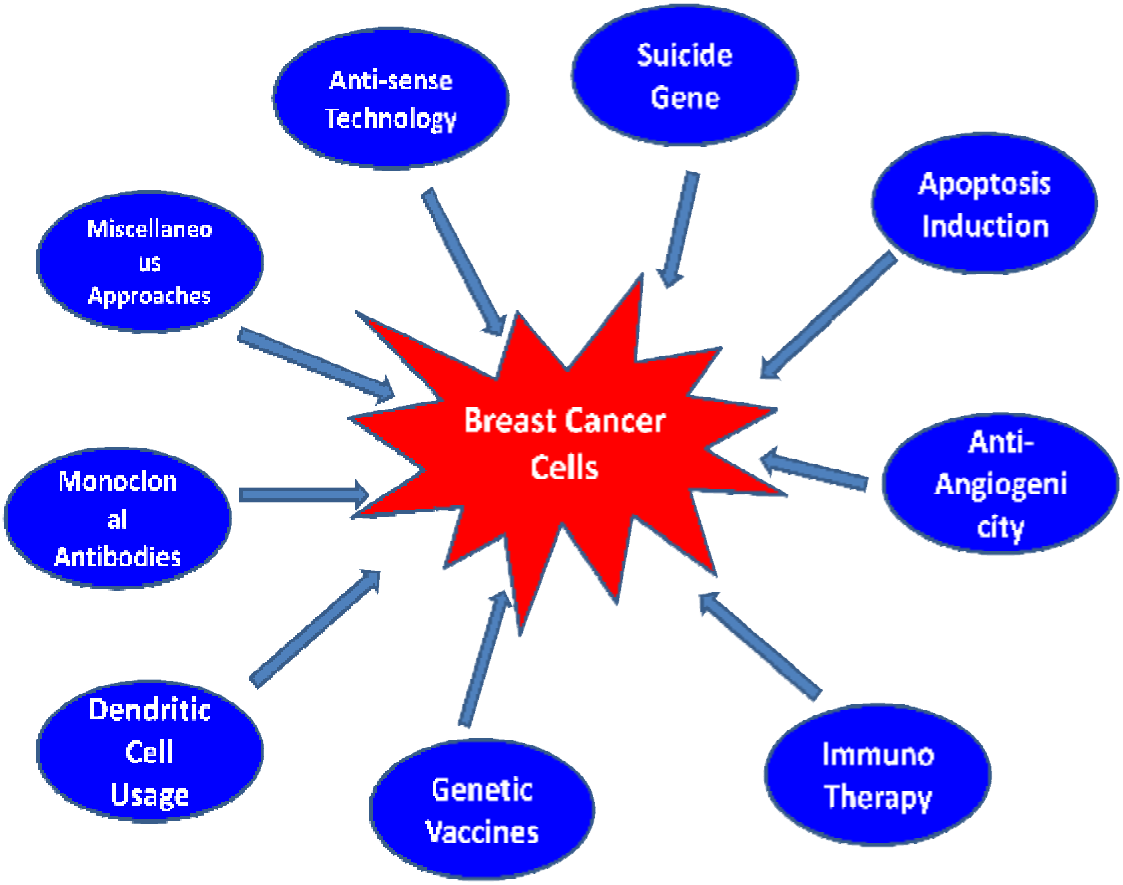

Fig. 1. Model of different gene therapy approaches to treat breast cancer.

Gene Therapy in Pakistan

In Pakistan, conventional treatment such as surgery, chemotherapy, radiotherapy and hormonal therapy is being practiced for breast cancer. These treatments are beneficial in many cases as they suppress tumor growth sufficiently. The beneficence from these treatments also depends on stage of cancer. Patients with detected cancer with early stages are benefited to greater extent as compared to those with last stages. Despite of these treatments, cancer relapse has been observed in many cases. This relapse often becomes deadly for the patients (Iqbal et al., 2010).

Conventional therapies are used most frequently to cure breast cancer but usually fail to permanently treat patient. With very encouraging results on animals and clinical trials on human, gene therapy shows very promising results. In addition, surgery, chemotherapy and radiotherapy are very painful procedures for patients and side effects of these therapies often become unbearable. Many approaches of gene therapy for treating breast cancer are in trials but all this is being done in developed countries outside Pakistan. There is a great need of gene therapy treatment of breast cancer in Pakistan. Many good research institutes like Center of Excellence in Molecular Biology (CEMB) (www.cemb.edu.pk), School of Biological Sciences (SBS) (http://pu.edu.pk/home/department/59/School-of-Biological-Sciences), Atta-ur-Rehman School of Applied Biosciences (ASAB-NUST) (www.asab.edu.nust.pk), HEJ Research Institute, Karachi University (http://www.iccs.edu/hej/index.php,National Institute of Biotechnology and Genetic Engineering (NIBGE) (www.nibge.org), alongwith well-reputed hospitals, such as Shoukat Khanum Cancer Hospital (www.shaukatkhanum.org.pk), Agha Khan Hospital (www.hospitals.aku.edu), etc., Nuclear

Medicine, Oncology And Radiotherapy Institute (NORI) Hospital, Atomic Energy Commission Nuclear medicine/Cancer Treatment (http://www.paec.gov.pk/nori/patient.htm), Institute of nuclear medicine and oncology (INMOL) can plan and work together for such treatment in Pakistan.

Gene therapy can be promoted in Pakistan by linking hospitals like Shoukat Khanum Cancer Hospital, Agha Khan Hospital, NORI, INMOL etc. with research institutes such as CEMB, SBS, HEJ, NIBGE, ASAB-NUST, etc. All these institutes have the cell culture facilities and also tools for gene manipulations. In addition to develop linkage at national level, collaborations should be developed between institutes in Pakistan and researcher centers and hospitals which are working in area of breast cancer gene therapy abroad, so that this technology could be immediately transferred in Pakistan. There is no good linking among research centers and hospitals in Pakistan, which is a need of the hour. Initially, experts from hospitals and research centers should make a working group so that they could plan that what can be done in our setup and then the work should be started on it on priority basis. In addition, some researchers can go abroad to get training in advanced lab practicing gene therapy. All the basic facilities are available at both hospitals and research centers and there is only a need to develop good relationship. It will not only benefit research students and researchers but also thousands of cancer patients in Pakistan.

Conclusion

Till-date many strategies have been used for treating breast cancer and majority of them have focused on p53. For gene therapy intratumoral route is a best choice of administration. Although adenoviral vectors present higher transgene expression but a small minority of patients provide clinical evidence of tumor regression.

Antiangiogenic gene therapy is also a good approach but antiangiogenis therapies can not alone treat breast cancer. They need augmentation by other therapies like conventional therapy. Intramuscular delivery route of antiangiogenic factor gene can be helpful in treating metastatic breast cancer to the brain.

Immunotherapy for breast cancer is another approach. New genetic vaccines can be developed by better understanding the nature of antigens which provoke better antitumor response and by identification of novel tumor antigens.

Breast cancer gene therapy is a difficult task to perform. Till-date, transgene expression has not been achieved up to the desire. For this, there is a need to device new gene therapy strategies and new transgenes should be searched for. Also there is a huge need to focus on new gene therapy vectors because presently used vectors have not showed great success. A better understanding of molecular pathways which lead to breast cancer is also required.

Pakistan has very high ratio of deaths due to breast cancer so pre clinical and clinical trials should be immediately started here for better treatment of breast cancer in the country. Gene therapy can only be made more beneficent if more and more countries put their efforts in it and an effective linkage could be developed among hospitals and research centers locally.

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