Mutation Compensation in Breast Cancer Gene Therapy: a New Promising Approach

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Abstract

Despite development and efforts being made worldwide in order to prevent deaths from breast cancer, it remains one of the leading causes of death for women. Available treatment modalities appear limited success and consequently new and complementary strategies have arisen. The last decade molecular basis of breast cancer has been extensively studied and delineated making gene therapy a potential new therapeutic approach. Gene therapies approaches can be generally categorized as follows: mutation compensation, molecular chemotherapy, proapoptotic gene therapy, antiangiogenic gene therapy, genetic immunopotentiation and genetic modulation of resistance/sensitivity. Clinical trials in which a multidisciplinary approach was followed combining gene therapy with chemotherapy or radiation therapy have shown promising results. Purpose of this review paper is to analyze mutation compensation as a promising approach in treating breast cancer.

Keywords: *breast cancer, gene therapy, mutation compensation*

Introduction

Despite the development of early detection methods, breast cancer remains the most common female malignancy. Chromosomal alterations or loss of tumor suppressor function and oncogenes is believed to influence cell growth and development. The loss of heterozygosity (LOH) is recognized on several chromosomes: 1, 3, 4 – 11, 13, 16 – 18, 22, X[1-3]. Probably it is a result of loss or inactivation of TSG (tumor suppressor gene)[3]. This tumor suppressor gene regulates the cell growth as well as the cell adhesion or protease activity. In addition, abnormalities of other proteins might interact with the gene product resulting cancer, even if there is not any TSG-gene mutation.

Familiar breast cancer has been investigated in the last years, especially concerning the BRCA-1 and BRCA-2 genes. Defect DNA repair function is associated with these genes resulting in carcinogenesis. On the other hand the expression of BRCA-1 gene seems to be reduced in most sporadic cases[4]. As a matter of fact other factors and mechanisms might be involved in the malignant process, such as the nuclear phosphoprotein p53 which repairs DNA damage as well as it inhibits the growth of abnormal cells[5]. According to the current data, mutations of the p53 gene increase the relative risk of relapse about 33%[6].

Furthermore, the growth factor receptor c-erbB-2 / HER-2 (neu) and the nuclear transcription factor C-myc are involved in the carcinogenesis of the breast[7]. The HER-2 gene encodes a tyrosine kinase receptor protein, a component of cell growth regulation. Worst prognosis is associated with the existence of the C-myc gene, which encodes a nuclear phosphoprotein[8].

Efforts have been made in order to achieve a better therapeutic result by using new gene mutation methods, like TSG replacement, application of RNA and

ribozymes, molecular chemotherapies, antisense mechanisms. In addition, antiangiogenic, immunological and proapoptotic genetic therapeutical approaches have been investigated giving promising results. However, the reported clinical response of these new methods remains low.

In the present review, data from the current literature has been reviewed in order to investigate whether the new gene therapies of the breast could present an alternative to the existing therapies.

TSG gene therapy

The already-existing new therapeutic methods consist of genetic tumor growth suppression with tumor genes replacement therapies or ablation of oncogenes.

According to current evidence, the replacement of the p53 gene remains the most investigated topic. Antiangiogenetic factors, proapoptotic proteins and immune upregulation might be involved in the final therapeutical result after viral p53 introduction in human breast cancer cells[9]. It is important to point out that as far as the existing trials are concerned, not only the p53 transduced tumor cells are killed, but also their neighboring cells[10]. Last, but not least, other TSGs like mda7[11], BRCA-1[12], BRCA-2[13], Rb[14], p27[15] might present as therapeutic target.

Apart from the viral induction antisense oligodeoxynucleotides also block the transfer of genetic information. These nucleotides are short ssDNA molecules[16]. Other suspected mechanisms that lead to mRNA inhibition are translation arrest, inhibition of transcription or splicing. Concerning the current literature, efforts have been made to suppress oncogenetic genes which are protein kinase C-alpha (PKC-a)[17], Bcl-2[18], insulin like growth factor receptor (IGF-IR)[19], plasma membrane calcium ATPase[20]. PKC-a running studies, in addition to chemotherapy, seem to have promising results[17].

Application of ribonucleic interference technology (RNAi) provides a possible specific down-regulation mechanism of the c-myc gene[21]. Furthermore, RNA molecules, the ribozymes are involved in the formation of covalent bonds in RNA strands. According to some studies ribozymes might affect not only mRNAs in cancer cells but also those in normal cells. That could suggest an important problem concerning the therapeutic targets. So, dimer minimized ribozymes (minizymes) have been investigated concerning their ability to suppress cyclin AD1 and hst-1 oncogenes of the breast[22].

Last but not least, molecular chemotherapy by using the so called 'suicide genes' provides another method of breast cancer gene therapy[23]. Via viruses genes that express toxic molecules or activate enzymes of specific pro-drugs are introduced in the cancer cells. Due to the so called bystander effect (effect also on nontransduced cancer cells) this method of the enzyme-activation is not limited only on the cells which are genetically modified to express an activating enzyme[23,24]. Induction of anti-tumoral immunity and transfer of phosphorylated ganciclovir between tumor cells might explain this effect. The enzyme-activating pro-drug therapy consists of genes of both human and non-human origin, eg. cytochrome p450 isophorms, thymidine kinase[23].

More efficient might be the transfection of cancer cells with two different suicide genes, due to the activation of two pro-

drugs. In addition combination of suicide and cytokines genes, such as IL-2 gene is supposed to achieve better results according to relative experiments[25]. Promising results are published concerning the development of genetic prodrug activation which is affecting the same cancer transcription[26,27]. On the contrary the tumor regression is reported to be not significant. According to other trials the use of MetXia-p450, a recombinant retroviral vector which is encoding the human cytochrome p450 type 2B6 gene, might present a safe and promising approach[26]. The cytochrome p450 enzymes are mainly located in the liver and convert the cyclophosphamide to an active phosphoramide and acrolein[26].

Lastly, it has been postulated that intracellular single-chain antibodies could down-regulate the cell surface erb-2 levels. Erb-2 is proto-oncogene which is overexpressed in breast cancer cells[28].

Conclusions

Mutation compensation of genes involved in breast cancer has been an issue of extensive investigation. However the majority of the trials report a low clinical response as well as toxicity. Adenoviral vectors are reported to have a high transgene expression but not a satisfactory tumor regression.

In addition transfer of genetic information might be blocked with antisense oligodeoxynucleotides, which are short ssDNA molecules. Few studies exist concerning this method. The use of PKC-a antisense oligonucleotide additionally to chemotherapy might have important results.

Furthermore, application of ribonucleic interference technology (RNAi) could be used for the specific down-regulation of the c-myc gene. This homologous to the gene RNAs affect the posttranscriptional mechanisms. In addition RNA molecules the ribozymes might suppress the cyclin D1 and hst-1 oncogenes.

Lastly, the "suicide genes" are another method of molecular chemotherapy of breast cancer. Induction of antitumoral immunity and transfer of phosphorylated ganciclovir between tumor cells might explain the observed "bystander effect", which is characterized by enzyme activation not exclusively of the transduced cancer cells. More efficient might be the tranfection of cancer cells with two different suicide genes or the combination of suicide genes and cytokine genes (IL-2 gene).

As a conclusion, it may be supported that the mutation compensation breast cancer gene therapy is very challenging. Future research should focus on the development of new gene transfer vectors in order to increase the existing low efficacy. ■

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