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Frontiers in Suicide Gene Therapy of Cancer

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Abstract

The National Cancer Institute (NCI) and the American Cancer Society (ACS) predict that 1,638,910 men and women will be diagnosed with cancer in the USA in 2012. Nearly 577,190 patients will die of cancer of all sites this year. Patients undergoing current systemic therapies will suffer multiple side effects from nausea to infertility. Potential parents, when diagnosed with cancer, will have to deposit oocytes or sperm prior to starting systemic radiation or chemo-therapy for the future genetic testing and in vitro fertilization, while trying to avoid risks of iatrogenic mutations in their germ cells. Otherwise, children of parents treated with systemic therapies, will be at high risk of developing genetic disorders. According to these predictions, this year will carry another, very poor therapeutic record again.

The ultimate goal of cancer therapy is the complete elimination of all cancer cells, while leaving all healthy cells unharmed. One of the most promising therapeutic strategies in this regard is cancer suicide gene therapy (CSGT), which is rapidly progressing into new frontiers.

The therapeutic success, in CSGT, is primarily contingent upon precision in delivery of the therapeutic transgenes to the cancer cells only. This is addressed by discovering and targeting unique or / and over-expressed biomarkers displayed on the cancer cells and cancer stem cells. Specificity of cancer therapeutic effects is further enhanced by designing the DNA constructs, which put the therapeutic genes under the control of the cancer cell specific promoters. The delivery of the suicidal genes to the cancer cells involves viral, as well as synthetic vectors, which are guided by cancer specific antibodies and ligands. The delivery options also include engineered stem cells with tropisms towards cancers. Main mechanisms inducing cancer cells' deaths include: transgenic expression of thymidine kinases, cytosine deaminases, intracellular antibodies, telomeraseses, caspases, DNases. Precautions are undertaken to eliminate the risks associated with transgenesis.

Progress in genomics and proteomics should help us in identifying the cancer specific biomarkers and metabolic pathways for developing new strategies towards clinical trials of targeted and personalized gene therapy of cancer.

Keywords

cancer suicide gene therapy; targeted therapy; personalized therapy; apoptosis; necrosis; brain cancer; ovarian cancer; pancreatic cancer; lung cancer; colon cancer; prostate cancer; breast cancer; testicular cancer; variable fragment antibodies; thymidine kinase; cytosine deaminase; intracellular antibodies; telomeraseses; caspases; DNases; viral vectors; stem cells

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Introduction

The NCI and the ACS predict that 1,638,910 men and women will be diagnosed with cancers of all sites in the USA in 2012 [1–2]. Nearly 577,190 patients will die of cancer this year. Patients undergoing current systemic therapies will suffer multiple side effects from nausea to infertility. Some cancers have particularly rapid pace of progression in the most vital organs, which results in the high rate of mortality, e.g., brain neoplasms. In other cancers, asymptomatic progression occurs to the advanced stages, which results in the high mortality e.g., ovarian or pancreatic cancers. Although deadly, but not immediately life threatening, other cancers impair dramatically the quality of various aspects of the patients' lives, e.g., colon, prostate, or breast cancers. Cancers, which disseminate by metastases into multiple vital organs and remain hidden within them, are beyond the capabilities of the local therapy and are extremely difficult to cure even with systemic therapies; thus are responsible for nearly 90% of cancer related deaths [1–2].

The serious problem for cancer therapy is the fact that patients undergoing current systemic therapies will suffer multiple side effects. Some of these side effects (e.g., nausea or vomiting) will cause the need for reduction of the dose of chemotherapeutics or radiation below the most effective levels, or withdrawal from a particular treatment altogether.

One of iatrogenic effects of systemic therapies may be patients' infertility. This prompts taking preventive measures. Potential parents, if diagnosed with cancer, may deposit oocytes or sperm prior to starting systemic radiation or chemo-therapies for the future genetic testing and in vitro fertilization [3]. Otherwise, children of parents, treated with systemic therapies, are at high risk of developing genetic disorders [4].

The ultimate goal of cancer therapy is the complete elimination of all cancer cells. Ideally, such a therapy would be leaving all healthy cells unharmed and would have no iatrogenic effects. Although not risks-free [5–6], one of the most promising therapeutic strategies in this regard is cancer suicide gene therapy (CSGT), which is rapidly progressing into new frontiers.

Cancers Treated with Cell Suicide Inducing Genes

Selective elimination of cancer cells is particularly critical in cases in which they are intermingled with the healthy cells. Therefore, the surgical resection unavoidably removes the functional, healthy cells together with the cancerous ones; thus impairs the patients' abilities to function normally. Radiation therapy affects all the exposed cells. While this therapy relies on the higher sensitivity of rapidly proliferating cells to the ionizing radiation, some of the healthy cells, including reproductive, immune, and hormonal systems' cells are most sensitive. Moreover, cancer initiating stem cells are shown to be resistant to radiation.

Chemotherapeutics penetrate into and affect all the cells. Most of them are relying upon the higher intake rate into rapidly proliferating cells and blocking mitosis, which lead to activating apoptotic cascades. Nevertheless, populations of the healthy cells, including those involved in regeneration and immunity, are also seriously affected. Moreover, side populations of ABCG2 expressing cancer stem cells are capable of expulsion of therapeutics, thereby developing resistance.

Efficacy of immunotherapy relies upon high specificity and sensitivity of the used antibodies, which attract the patient's immune response to the cells pointed with these antibodies. Any lack of specificity, or worse - cross reactivity with molecules on the surfaces of the healthy cells, directs killing power of immune systems towards the healthy cells. This misdirected immunological response may result in serious side effects, which the

patients will endure, worse than, if they would not be treated at all. All these cases qualify for suicide gene therapy trials, while the clinical diagnoses determine the choice of strategy.

Glioblastoma multiforme (GBM) is the most often and the most deadly brain cancer [7–13]. It is incurable. Nearly 13,700 patients, out of almost 22,910 newly diagnosed, will die this year in the USA [1–2]. The average survival time of patients with GBM, from the time of diagnosis to death, is approximately 14.6 months for the patients in the USA. However, even during those months, neurosurgical resection, followed by radiation and chemotherapy with Temozolomide, leads to serious impairment of the quality of life. This prompts attempts of delivery of suicidal genes into gliomas. It is often accomplished by targeted delivery by viral and non-viral vectors targeting over-expressed (e.g., EGFR) or uniquely mutated (e.g., EGFRvIII) receptors [14–31]. A spectrum of the targeted biomarkers is expanded with those being displayed on cancer initiating cells including CD133 and its variants [11–12]. Moreover, genetically engineered stem cells, with tropism toward the tumors, are used as the carriers delivering the suicidal genes [29–31].

Ovarian cancer is the most deadly neoplasm of the female reproductive system. Almost 15,500 women will die, out of nearly 22,280 newly diagnosed, this year in the USA [1–2]. Asymptomatic progression to the advanced stages, leads to the very high mortality rate, while more than 63% of women are diagnosed only at these advanced stages. Lifetime risk estimates for ovarian cancer among women in the general population indicate that 1.4 % (14 out of 1,000) will be diagnosed with ovarian cancer compared to up to 40 % of women (400 out of 1,000), who have harmful BRCA1 or BRCA2 mutations [32]. Radical therapy of the ovarian cancers involves oopherectomy and hysterectomy, which leave women infertile. Systemic therapy may lead to mutations in the oocytes' genomes. These iatrogenic effects of therapies prompt preventive collecting oocytes prior to therapy for the in vitro fertilization [4]. Moreover, SSEA-4, TRA-1-60, CD44, CD133 biomarkers defined recently on stem cells in germ cell tumors and epithelial carcinomas constitute a novel group of specific targets [33–40]. **Germ cell tumors** in male patients exhibit similar molecular profiles [34]. As in the ovaries, the same mutations are also responsible for **breast cancers**, which will be diagnosed in nearly 229,060 and will be cause of deaths of nearly 39,920 women this year in the USA [41–42]. Radical therapy of the advanced breast cancer - mastectomy leads to permanent disfiguring women's bodies. Although immunotherapy with Herceptin is very effective, it is restricted to women overexpressing Her2/neu. The cancer suicide gene therapy, which targets mutated receptors displayed on surfaces of ovarian and breast cancer cells e.g., EGFRvIII, offers a fertility saving and offspring protecting alternative [43–46].

Although, the number of **prostate cancers** is declining, still nearly 28,170 men will die of the disease this year [1–2]. However, nearly 241,740 newly diagnosed and 2 million cancer surviving men will suffer daily problems associated with this cancer's progression. Due to the anatomical passage of the urinary and reproductive tracks through the prostate, surgery on the prostate cancer in many cases leads to iatrogenic complications: erectile dysfunction and urinary incontinence. Several biomarkers have been identified for the prostate cancer including PSMA, androgen, CXCR4 or EpCAM [47–52]. Those serious side effects propel trials of suicide gene therapy of the prostate cancers [53–63].

Nearly 43,920 Americans will be newly diagnosed with **pancreatic cancer** this year. Almost 37,930 of them will die [1–2]. This translates into an average 4% one year survival rate (4 patients surviving a year out of 100 diagnosed). Surgery, including pancreatic transplantation, is effective in the early stages. However, the anatomical location results in asymptomatic progression of the neoplasm to the advanced stages. Unbearable pain develops with this cancer's progression. Often, non-specific symptoms are associated with impairment of the numerous functions of pancreas within the digestive and hormonal

systems. Those functions result from diversity of specialized cells with variety of lineage specific display profiles. Discoveries of new biomarkers make pancreatic cancer another good candidate for targeted cancer suicide gene therapy [64–67].

Cancers of the digestive system include **gastric and colon cancers**. An estimated 103,170 people will be newly diagnosed with colon in 2012 [1–2]. Almost 51,960 of the patients will die during the year, while suffering from increasing malnutrition. Several unique biomarkers are present on the cancer cells including well established carcinoembryonic antigen (CEA) [68]. To that, there are added newly emerging biomarkers, including those on the colon cancer stem cells CD44 and CD133 [68–72]. Not only they facilitate early diagnosis due to shedding of these biomarkers into the patients' blood, but also they are targets for diagnostic molecular imaging and targeted therapies. The targeted therapies include cancer suicide gene therapies [73–77].

Almost 160,340 people will die of the **lung cancer** this year [1–2]. More than 226,180 people will be newly diagnosed. This cancer will take more lives than any other cancer. The overall 5 year survival rate is 15% (15 out of 100 diagnosed patients will survive 5 years). Progression of cancer not only is reducing the active area of oxygen supply, but also is often associated with pleural effusion, which rapidly fills up large volume of pleural cavity and suppresses the lungs' volumes. Both lead to deaths by asphyxia. Contributing factors include clones of cancer stem cells resistant to therapies, which are identified with the recently discovered biomarkers [78–81]. They propel targeted cancer suicide gene therapy trials [82–85].

Receptors Displayed on Living Cells - Targets for Therapeutic Transgenes' Vectors

The most essential element of attaining high therapeutic efficacy, while avoiding iatrogenic effects, is the precise delivery of the therapeutics to the treated cells only. This is also the case for cancer suicide gene therapy. However, only a few unique, qualitative biomarkers, which are present exclusively on cancer cells, have been identified. They offer the ultimate targeting precision for delivering the suicidal genes' carrying vectors.

Epidermal growth factor receptors constitute a family of the receptors ErbB 1–4. First member of this family - an epidermal growth factor receptor (EGFR) or ErbB1 is present on most healthy cells and their cancerous derivatives. However, the number of receptors on cells may differ. While a normal healthy glial cell displays ~ 3 × 10 ∧ 4 receptors, the malignant glioma cell may display ~ 2–3 × 10 ∧ 6 receptors. It is the result of increased levels of gene expression or / and multiple copies of genes in cancer cells, what is leading to an increased number of the gene expression products - cell surface receptors. Supplying the same concentrations of vectors to both, glial and glioma cells in patients with brain tumors, would result in two orders of magnitude higher therapeutics' saturation of glial cells than normal cells. Nevertheless, the apoptosis inducing transgenes would cause harm in healthy cells, if no other protective measures would be involved. However, the EGFR variant III – the truncated product of deletion mutation of the gene, is present uniquely on the cancer cells including brain, lung, ovarian, and many others. As such, it is an excellent immunogen for cancer vaccines [86]. This receptor is a target for recombinant adenoviral vector [87]. The EGFRvIII is the target for genetically engineered variable fragment antibodies, which guide delivery of the suicidal genes into ovarian and breast cancers [88]. Another member of this receptors family is Her2 or ErbB2. Its over-expression in breast and other cancers broadcasts poor prognosis. It is a target for immunotherapy. Engineering multivalent adapters refine precision of delivery to this receptor by viral vectors in gene therapy [89].

A standard version of the **cluster of differentiation 44** (CD44s) is present on cells from variety of tissues, including those of epithelial origin as prostate, ductal epithelium of breast, mucosa, and many others, as well as on cells in neoplasms. However, alternative splicing patterns (CD44v) are present on various cancers and their metastases including cancers of the lungs, bladder, breast. In particular CD44v6, specific for epithelial cancers, is an antigen for monoclonal antibodies in immuno- and gene therapies [90–91]. The recombinant antibodies are manufactured to guide suicide gene therapy vectors against ovarian cancers [88].

Tumorigenic cancer cells, with stem cell profiles, display **cluster of differentiation 133** (CD133) / prominin [11–12, 92], often in association with **CXCR4**. They are resistant to cisplatin treatment. Therefore, CD133 and CXCR4 became the points for delivery of the lentivirus driven suicidal genes [92].

Carcino-embryonic antigen (CEA) is present on the luminal surfaces of the mucosal cell, but amplified and diffused on all surfaces of cancer cells [68]. It has become the cancer biomarker, which after coupling antibodies with radionuclides, is detected by diagnostic imaging. It is shed by cancerous cells into blood of cancer patients, so it is routinely detected in lab tests. It is also the target for the vectors delivering therapeutic genes [93–94].

Folic acid aka vitamin B9 is imported into the cells with the aid of the **folate receptor** (FR). Folic acid is used in synthesis and repair of the genomic DNA. Therefore, highly mitotic cancer cells, which have high demands for the folates, are characterized by over-expression of the folate receptors on their surfaces. As such, they become guides for delivery of therapeutics. Folate linked nanoparticles transfer HSV TK into prostate and nasopharyngeal cancer cells [95].

Also **transferrin receptor** (TfR) aka cluster of differentiation 71 (CD71) is overexpressed on cancer cells to meet their high demands for iron [96]. Iron chelating enzymes, ribonucleotide reductase and cytochrome-c reductase, heavily influence cancer cell metabolism. Depletion of iron is one of the therapeutic strategies of cancer therapy. Radioactive isotopes of iron are used in nuclear medicine. Iron is imported into cells by transferrin receptor. The same mechanism is used to deliver therapeutic suicidal genes [88, 97–99].

Mucins are displayed on cell surfaces as glycosylated proteins. Although present on normal cells, their expression onto the cancer cells is greatly upregulated. Moreoverer, the carbohydrates present on breast, pancreatic, and ovarian cancer cells are fewer and simpler. This translates in variation in antibody cross reactivity between MUC1 labeling normal versus cancer cells. Moreover the MUC-1/Y is often replaced by the MUC-1/Z form. These features are exploited for making specific antibodies. These antibodies serve as the guides for the vectors with the therapeutic cargo to the cancer cells [88, 100–101].

Cancer stem cells or cancer intiating cells have recently been suggested as responsible for propeling growth of tumors. Resistance to radiation and chemotherapy has been attributed to the clones of cancer stem cells. Therefore, the biomarkers of stem cells become potential, novel guides for targeted therapies. Among them, **stage specific embryonic antigen** 4 (SSEA-4) and **tumor resistance antigen** 1–60 (TRA-1-60) have been identied on the pluripotent stem cells of the embryonal carcinomas of the testes and ovaries [33–34]. It is worth noting, that these biomarkers of pluripotency are being expressed only on undifferentiated pluripotent stem cells, while ceasing to express immediately upon the cells' differentiation. Therefore, they are unique biomarkers for delivery of the therapeutic transgenes to the pluripotent stem cells only.

The ligands and antibodies for the aforementioned receptors serve as the guides for the delivery of the vectors to these receptors. Nevertheless, it is necessary to keep in mind, that therapeutics guided by these ligands and antibodies will deliver therapeutic genes to cancer cells only, if they are uniquely specific. However, if for a particular antibody, there is any cross reactivity between cancer and healthy cells' receptors, then obviously the therapeutic genes will affect healthy cells, what will manifest as the side effects, on the same way as with the non-specific systemic therapies. Therefore, continued effort towards defining the molecular display profiles on the spectra of heterogenous populations of clones of single, living cancer cells, should be the primary goal for designing targeted, personalized therapy [102–103].

Cancer Specific Promoters

For therapy involving expression of genes leading to the cells' deaths, it is essential that these genes are targeted to and expressed in all and only targeted cancer cells, but not in healthy cells. Targeting to the cancer cells can be accomplished with the aid of ligands or / and antibodies specific for the molecules displayed on the surfaces of the living cells as discussed above. Expressing in the cancer cells can be restricted by selection of promoters of the genes, which are upregulated in the investigated cancers as determined by genomics and proteomics, i.e., promoters, which are lineage, oncogene, biomarker, or induction specific. The promoters of these genes are engineered into the DNA constructs driving effectively expression of the therapeutic cancer suicide genes. Therefore, the cancer suicide genes are expressed only in cancer cells. It is an important safety measure, so that if a suicidal gene is erroneously delivered into the healthy cells, it is not expressed – it remains inactive.

Two orders of magnitude higher expression of **epidermal growth factor receptors** (EGFR) in cancer cells over normal cells provides a rationale for using their promoters, while engineering the constructs driving expression of the cancer suicide inducing transgenes. This approach is further enhancement of the strategy, which also involves delivering trangenes through the receptors mutated only on cancer cells or present on pluripotent cancer stem cells [33].

Similarly to EGFR, **transferrin receptors** (TfR) are more heavily displayed on rapidly proliferating cancer cells, than on quiescent, normal, healthy cells. Therefore, the TfR promoters are efficiently used for expressing suicidal genes [88].

Carcinoembryonic antigen (CEA) is the result of the high CEA gene expression in various cancers including gastric and colorectal carcinomas. Therefore, the transgenes under control of its promoter are expressed only in those cancers. Further improvement of the expression occurs, when the transgene, e.g., cytosine deaminase, is set under the control of the Cre/LoxP regulation system [[104].

Telomerase is an RNA polymerase, which lengthens telomeres. Majority of cancers greatly over-express the hTERT subunit, while immortalizing the cells. Therefore, it is used as a promoter of suicidal genes in cancer cells. An example of such a strategy involves transduction of the ovarian cancers with HSV TK under the telomerase promoter [105–109].

P**rostate specific antigen** (PSA) and p**rostate specific membrane antigen** (PSMA) are uniquely over-expressed by prostate cancer cells. Therefore, their promoters are incorporated into the constructs for cancer suicide genes, which are expressed into prostate cancers [59, 63].

Cytokeratins, uniquely present in epithelial cells, are histopathological biomarkers of the neoplasms of the epithelial lineage. They are also used to determine EM and ME transitions,

which occur during cancerogenesis and metastasis. For the human embryonic stem cells, they are also biomarkers of differentiation into one of three germ layers. **Cytokeratin 18 and 19** (CK19) are among these biomarkers. The promoter for CK19 is used to drive expression of the transgenes within epithelial cells [110].

Physiologically, prostaglandin-endoperoxide synthase (PTGS) aka **cyclooxygenase** (Cox) catalyzes formation of prostaglandins, prostacyclin and thromboxane. Aspirin is best known inhibitor of Cox providing relief from inflammation and pain. The Cox gene has a very high transcriptional activity in colorectal cancers. This prompts its' promoter use, after delivering the vectors through the coxsackievirus and adenovirus receptors (CAR), for using it in cancer suicide gene therapy of gastrointestinal cancers [111].

Vectors of Suicidal Genes

Tropisms of natural or engineered viruses towards specific receptors are the foundations for constructing viral vectors for suicide cancer gene therapy. The attachment of these vectors to the targeted cells is contingent upon recognition of specific receptors on the cells' surfaces by the ligands on the vectors. In other words, only the viruses with the very specific ligands on their surfaces will anchor onto the specific receptors on the cells and vice versa - targeting the specific cells will require engineering viruses displaying ligands matching exactly those receptors, which are displayed on the targeted cells. Those interactions, between cell receptors and viral ligands are in vivo modulated by the immune system involving toll like receptors. Identical principles rule designing of the nonviral vectors. Similarly, tropism of the cells, which are bioengineered to deliver therapeutic cargo to cancers, is driven by selective interactions between the ligands and receptors. The entry of vectors, through receptor mediated endocytoses or membrane fusions, also requires specific set of domains. These domains promote vectors' escape from endosomal and / or lysosomal pathways. The other domains facilitate entries into nuclei. Replication, assembly, and egress or latency, all determine dynamics of interactions between the vector and the cell. All these elements have decisive effect upon the choice of the vectors, as well as engineering therapeutic cargo carrying cells, in designing cancer suicide gene therapies.

Herpes simplex virus (HSV) belongs to a family of herpesviridae - enveloped DNA viruses. They bind to the receptors through orthologs of their three main ligand glycoproteins: gB, gH, and gL, while sometimes employing accessory proteins. These ligands play decisive roles in the primary routes of viruses' entries in oral, ocular, and genital forms of the disease. The HSVs possess high tropism towards the cell receptors of the nervous system [112]. This tropism is utilized for engineering recombinant viruses delivering the suicide inducing genes into cancer cells [113]. The therapeutic bystander effects are enhanced by inclusion of connexin coding sequences into the constructs [114–115].

Lentivirus belongs to a family of retroviridae – enveloped, single stranded RNA retroviruses. The most known member of this group is Human immunodeficiency virus (HIV). It is a lentivirus that causes acquired immunodeficiency syndrome (AIDS). The viruses' ligands have affinity towards CD4, which is present on the cells of the human immune system such as CD4+ T cells, macrophages, and dendritic cells [116]. To exert its activity after the entry into the cell, the viral RNA genome has to be reverse transcribed into double-stranded DNA, which is imported into the cell nucleus and integrated into the cellular DNA. This virus is used to deliver the therapeutic genes to leukemia cells [117–121]. The recombinant lentivirus is used to deliver deoxycytidine kinase. Recombinant lentivirus is effective in delivering suicide genes through the mucin receptor into pancreatic cancer cells, while sparing healthy cells. It also demonstrates affinity towards the epithelial

ovarian carcinoma expressing mucin. The recombinant lentivirus is also used to deliver suicidal genes into gliomas.

Adenovirus is a non-enveloped virus consisting of a double-stranded, linear DNA genome and a capsid. Naturally, it resides in adenoids and may be a cause of upper respiratory tract infections. The viruses utilize cells' coxsackievirus and adenovirus receptor (CAR) for the adenoviral fiber protein for entry into nasal, tracheal, and pulmonary epithelia [122]. The main problems to overcome are low levels of the CARs on the cancer cells and chromatization by histone deacetylases. The recombinant virus is capable for delivering thymidine kinase and cytosine deaminase achieving therapeutic effects [123]. The adenovirus engineered with the H19 enhancer / DMD-H19 promoter complex induces apoptosis only in the cancer cells with loss of imprinting of the insulin-like growth factor 2 gene (IGF2). Artificial “death switches” are introduced into cancer cells by adenoviruses to initiate apoptosis [124–130]. Replication-competent adenovirus-mediated suicide gene therapy (ReCAP) is in the clinical trials for newly-diagnosed prostate cancer.

Non-viral vectors are designed and synthesized *de novo* by biotechnologies of biomolecular engineering. They are engineered at the various levels of complexity. In general, they primarily provide the structural framework for condensation of the transgenic DNA. The vectors based poly(oligoD)arginine are engineered to condense TK gene into small nanoparticles or to assemble into dendrimers. These nanoparticles are used to transfect and kill ovarian, breast, and prostate cancer cells [95, 131–134]. Their targeting selectivity towards cancer cells is enhanced by adding ligands or antibodies as the guides towards the cell receptors [88]. Delivery of the therapeutic transgenes can be further enhanced by adding superparamagnetic nanoparticles or rendering the vectors superparamagnetic and driving the vectors into the neoplasms by electromagnetic pulses [88]. The liposomes offer an option for encapsulation and enhanced penetration through all cell membranes [95]. Selectivity of these vectors towards specific cells is enhanced by intercalating the lipid layer with the ligands or antibodies to create immnuno-liposomes. Nanobodies against MUC-1 linked with polyethylene glycol (PEG) - polyethylenimine (PEI) are the bases to induce apoptosis in the MUC-1 over-expressing breast cancer cells. The synthetic antibodies anchoring dsDNA constitute the founding framework for the complex biotag vectors, which incorporate signaling domains for cell entry, lysosomal escape, and nuclear entry of the therapeutic transgenes [88].

A major problem for gene therapy is low efficacy in delivery and expression of therapeutic genes. **Bioengineered stem cells** are being tested for their potential of resolving this problem for two reasons: precise targeting and efficient expression. The human stem cells can be delivered directly into the tumor. The human embryonic stem cells, mesenchymal stem cells, as well as the induced stem cells are bioengineered to deliver therapeutics. Some of them they have affinity for targeting gliomas, while the others towards breast cancer metastasis to the brain; all after intravenous injection [135–148]. This feature makes them perfect vectors for carrying therapeutic genes. The recombinant version of thymidine kinase shows enhanced over the wild type activity after being secreted, while effective in inflicting bystander effects [140–141]. Adding the kappa chain leader and endoplasmic reticulum export signal improves secretion; thus therapeutic effects [142]. Adding valproic acid significantly enhances activity of thymidine kinases [142]. The stem cells are being tested for their potential for carrying the suicidal genes also into variety of other tumors [135–148].

Mechanisms of Inducing Cancer Cells `Death

Induction of cancer cells' suicide can be accomplished on several ways. The ultimate goal is to eliminate all cancer cells and their nucleic acids carrying genetic information. The goal is also to spare all healthy cells including those of the reproductive system.

Thymidine kinase (TK) is an ATP-thymidine 5'-phosphotransferase present in all living cells. It is also present in viruses including herpes simplex virus (HSV), varicella zoster virus (VZV], and Epstein-Barr virus [EBV]. Physiologically, this enzyme converts deoxythymidine into deoxythymidine 5'- monophosphate (TMP), which is further phosphorylated to deoxythymidine diphosphate and thereafter to deoxythymidine triphosphate by thymidylate kinase and nucleoside diphosphate kinase respectively. As the triphosphate, it is incorporated into the synthesized DNA molecule by DNA polymerases or viral reverse transcriptases. Some dNTP analogs have the ability to terminate the DNA synthesis upon their incorporation into synthesized DNA. Ganciclovir is a synthetic analogue of 2'-deoxy-guanosine with such synthesis termination capability. Termination of synthesis triggers the apoptotic signaling cascades. This route of cancer suicide gene therapy involves two stages. First, HSV-TK gene is delivered and expressed in cancer cells. In most cases, it is delivered by viral vectors. Second, the suicidal gene delivery is followed by provision of Ganciclovir. HSV-TK is effectively used in cancer suicide gene therapy by expression in targeted cells to exert intracellular effects outlined above [15–19, 22–24]. Alternatively, TK is also secreted into the extracellular fluids by genetically engineered cells [135–148]. Thereafter, it is internalized by surrounding cancer cells to cause their death. A new recombinant version – 007 is shown to be more effective than the wild type [140]. Using valproic acid, as an inhibitor of deacetylases, enhances its efficacy [141].

Cytosine deaminase (CD) leads the hydrolysis reaction of cytosine to uracil with release of ammonia. If the modified site is recognized by endonucleases, then the phosphodiester bond in the DNA is broken, while initiating repair by incorporation of a new cytosine. However, upon provision of non-toxic prodrug - 5-fluorocytosine (5-FC), cytosine deaminase converts it into 5-fluorouracil (5-FU), which can inhibit cancer cell growth. Transgenic expression of CD in cancer cells leads to their deaths [31, 73, 94]. Cytosine deaminase in tandem with thymidine kinase under the carcinoembryonic antigen promoter is tested on lung cancers. Transfection by the engineered adenovirus and expression under the cytomegalovirus promoter, the double suicide gene constructs, are tested for inducing suicide of breast cancer cells. Alternatively, stem cells are engineered to express and secrete cytosine deaminase to kill neighbouring cells. Transduced mesenchymal stem cells with lentivirus driven cytosine deaminase are injected into gliomas. The engineered cells are injected directly into the cancerous tumors or delivered through intravenous injecting to reach cancers based upon their tropism [123, 148].

Reactive oxygen species (ROS) are by-products of cellular metabolism, while being primarily generated in mitochondria. Moderate levels of ROS may promote the cell divisions and differentiation. Increased metabolism, which occurs in cancer cells, may lead to significantly accelerated reactions of ROS with the genomic DNA causing its damage, with membrane lipids affecting their permeability, and with proteins causing reduced enzymatic activity and increased proteolysis susceptibility. These reactions lead to apoptosis and / or necrosis. In healthy cells, the balance between production and neutralization of ROS is retained by the antioxidative enzymes (AOEs). However, either increasing levels of ROS, or blocking AOEs, lead to shifting this balance towards unquenched ROS, thus to oxidative stress and death. The first mechanism is used in various modalities of radiation therapy, which cause generation of free radicals. The main problem with this approach is iatrogenic effect of ionizing radiation onto the healthy cells. This limits the effective therapeutic dose.

Alternatively, the AOEs are blocked by the intracellular antibodies expressed from the DNA constructs delivered via EGFRvIII, CEA, or TfR mediated endocytosis [33, 88]. The combination of both routes, blocking of the AOEs, which makes them more sensitive to ROS, followed by low doses of radiation, which increases ROS, are currently in progress.

Telomerase is a ribonucleoprotein responsible for maintaining functions of telomeres. Levels of its both components: RNA (hTR) and protein (hTERT) are increased in cancer cells. Putting bacterial nitroreductase gene under the telomerase promoter facilitates expression of the enzyme, which in turn converts a non-toxic prodrug into the cytotoxic alkylating agent [108].

Triggering of apoptotic cascades involves activation of **caspases.** Under two systems regulating transcription, muristerone and TetOn, the human, constitutively active caspases expressed from the viral vectors, effectively induce apoptosis of the human embryonic kidney and breast cancer cell lines [128]. Transgenic expression of `death switches', bax and caspase 9, triggers apoptotic cascades and kills the cancer cells [126–129]. The final stage of many different routes leading to cancer cells' suicides is executed by **DNase**s, what is manifested by hallmarks of apoptosis: collapse of chromatin and disintegration of the genomic DNA. The DNA constructs for the constitutively active DNases are first led by the multifunctional biotags to the EGFRvIII on the ovarian cancer cells, and after escaping from the endosomal / lysosmal pathway into the cell nuclei. Thereafter, targeting transgenically expressed DNases, through nuclear pore complexes into the nuclei, leads to the destruction of the genomic DNA and cancer cells' deaths [88].

Conclusions

Progress in genomics and proteomics should help us in identifying the specific targets for developing new strategies for clinical trials of the targeted and personalized gene therapy of cancer.

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Abbreviations

CSGT	cancer suicide gene therapy
ROS	reactive oxygen species
HSVTK	Herpes simplex virus thymidine kinase
ROS	reactive oxygen species
Fv	variable antibody fragment
CD	cytosine deaminase
SSEA-4	stage specific embryonic antigen
TRA-1-60	tumor resistance antigen 1–60
CD44	cluster of differentiation 44
CD133	cluster of differentiation 133
EGFR	epidermal growth factor receptor

PSA	prostate specific antigen
PSMA	prostate specific membrane antigen
CEA	carcino-embryonic antigen
TfR	transferrin receptor
MUC	mucin receptor
FR	folate receptor

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