Academic Journal of Cancer Research 10 (2): 10-23, 2017
ISSN 1995-8943

DOI: 10.5829/idosi.ajcr.2017.10.23

Review on Gene Therapy of Prostate Cancer

Debela Abdeta

Wollega University, School of Veterinary Medicine, P.O. Box 395, Ethiopia

Abstract: Prostate cancer is the second most common cause of death from malignancy in men. Gene therapy comes as a powerful approach to specifically treat advanced prostate cancer. Cancer gene therapy may be defined as the transfer of recombinant DNA into human cells to achieve an antitumor effect. The prostate is ideal for gene therapy. It is an accessory organ, offers unique prostate-specific antigen and prostate-specific membrane antigen. Viral and non-viral means are being used to transfer the genetic material into tumor cells. Summarizing, viruses are obviously potential vectors yielding high transfection efficiencies with the risk of increased genetic instability of transfected host cells and induces strong immunological responses. Suicide gene therapy using genetically engineered mesenchymal stem cells or neural stem cells has the advantage of being safe, because prodrug administration not only eliminates tumor cells but consequently kills the more resistant therapeutic stem cells as well. The number of clinical trials utilizing gene therapy methods for PCA is increasing.The main limiting factors for the development of an effective gene therapy are efficiency of gene transfer, selectivity of tumor targeting and the immunogenic properties of the vectors as well as general safety considerations.

Key words: Prostate · Gene Therapy · Vectors · Cancer · Stem Cells

INTRODUCTION

Cancer is a disease characterized by a loss in the normal control mechanisms that govern cell survival, proliferation and differentiation. It is now well established that a small subpopulation of cells, referred to as tumor stem cells, reside within a tumor mass. They retain the ability to undergo repeated cycles of proliferation as well as migrate to distant sites in the body to colonize various organs in the process called metastasis. The genetic instability allows them to become resistant to chemotherapy and radiotherapy [1].

Prostate cancer has recently become the most commonly diagnosed male malignancy in industrialized countries and the second leading cause of cancer-related mortality in men. It is argued that disease confined to the prostate can be successfully treated by radiation or surgery, with adjuvant hormonal therapy. However, up to half of men with clinically localized disease are not cured by these approaches [2]. In the United Kingdom over 60% of men with prostate cancer have either locally advanced or metastatic disease at presentation and are incurable. Surgery is the first choice of treatment for patients with prostate cancer. However, surgical removal of the prostate is only successful for organ-confined disease. Tumors which already grow beyond the prostate capsule or which recur after surgery can be treated with androgen ablation. This is mostly successful, since the majority of untreated prostate tumors are androgen-dependent [3].

Although chemotherapy can have a role to play in patients with advanced prostate cancer, response rates are modest and the survival benefit marginal. There is thus clearly a need for novel therapies to improve current prospects for survival. Significant advances have been made in gene therapy over recent years as a result of developments in molecular and cell biology. These include the improvement of both viral and nonviral gene delivery systems, the discovery of new therapeutic genes, better understanding of mechanisms of disease progression and the emergence of better prodrug systems [4].

Prostate cancer displays several features that make it a good candidate for gene therapy. First, the primary tumor site is easy to access and image. Thus, treatments

Corresponding Author: Debela Abdeta, Wollega University, School of Veterinary Medicine, P. O. Box 395, Ethiopia.
E-mail: debela.abdeta@gmail.com.

can be readily and accurately injected into the tumor, assisted for example by transrectal ultrasonography. Second, although many prostate cancer–associated target molecules are also expressed on normal prostate tissue, because the prostate is not a vital organ, any damage to adjacent normal prostate tissue would not be a contraindication to initiating treatment [5].

Intensive research strongly improved knowledge about the molecular changes, which are thought to be associated with prostate cancer development and progression to therapy resistance. There are a large number of genetic alterations, which have been associated with prostate cancer and exploited to develop a gene therapeutic approach. Some of the most intensively studied molecules in prostate cancer are p53 and retinoblastoma gene (RB). A considerably important role in tumor progression and metastasis plays the extracellular matrix (ECM), which represents a natural barrier to tumor cells [6, 7].

An important key regulatory role in prostate cancer seems to play the androgen receptor (AR). This intracellular steroid receptor mediates the action of androgens and thereby regulates growth, function and differentiation of prostate cells [8]. It is expressed in primary prostate cancer as well as in advanced hormone-refractory tumors and in metastatic lesions [9, 10]. Furthermore, AR gene amplifications may occur in tumors, which recurred after androgen ablation therapy [11, 12].

Another important point in tumorigenesis and progression to therapy resistance is the ability of tumor cells to escape immune surveillance. Although the precise mechanisms underlying the failure of the natural immune system to prevent tumor formation are still unclear, there are several gene therapeutic approaches, which tend to re stimulate the patient's immune responses against cancer cells [13]. So this paper was written to review the gene therapy strategy in prostate cancer.

Molecular Pathogenesis: Prostate cancer has the highest heritability (42%) of all human cancers according to twin studies [14]. There are several susceptibility gene candidates, for example RNASEL and macrophage-scavenger receptor 1 (MSR1). RNASEL is suggested to be a tumor suppressor gene since it is involved in regulation of cell proliferation and apoptosis. Common alterations in this gene are base substitutions and a four-base deletion. The MSR1 gene encodes subunits of a macrophage-scavenger receptor that is involved in recognition of infectious agents. Base alterations in this gene lead to increased sensitivity to severe infections with both bacteria and viruses [15].

Most of the hereditary incidences seem to be due to polymorphism in genes that regulate prostate development and function [14]. The most studied polymorphism in prostate cancer is polyglutamine (CAG) repeats in the AR. Shorter repeats are associated with increased AR transcriptional activity and thus an increased risk for developing prostate cancer [15].

Like most sporadic cancers, somatic prostate cancer is a consequence of genetic alterations such as mutations, deletions, amplifications and chromosomal rearrangements as well as of epigenetic changes (Fig. 1). GSTP1 is a gene that encodes glutathione S-transferase (GSTP), which function is to defend prostate cells against genomic damage [5]. In more than 90 % of prostate cancer and 70 % of prostatic intraepithelial neoplasia this gene is inactivated because of hypermethylation of a CpG island in the regulatory region of the GSTP1 gene. The absence of GSTP leads to increased genomic instability that is a requirement for carcinogenesis. Since hypermethylation of GSTP1 is common in prostate cancer and neoplasia it could be used as a diagnostic marker [15, 16].

Gain or loss of specific alleles but also over expression of tumor growth-promoting genes, so-called oncogenes have often been described to stimulate proliferation and tumor progression, respectively [7]. On the other hand, tumor suppressor genes may be down regulated, lost or inactivated by mutations.The NKX3.1 gene is a homeobox gene that encodes a transcription factor that represses expression of the prostate specific antigen (PSA) gene. The loss of 8p21 where NKX3.1 is located is common and occurs at an early stage of the disease [15]. PTEN is a tumor suppressor gene encoding a phosphatase that regulates signal transduction pathways. The level of PTEN is decreased in prostate cancer due to allelic losses and mutations which promotes cell survival [15, 16].

P27 is a cyclin-dependent kinase inhibitor that is encoded by the CDKN1B gene. Reduced level of p27 is common in prostate cancer and is a result of allelic losses and loss of PTEN function. Alterations in CDKN1B are associated with poor prognosis [15]. The tumor suppressor gene p53 is mutated in several human cancers as well as in advanced prostate cancer. P53 regulates the cell cycle and upon DNA damage either induces cell cycle arrest for DNA repair or induces apoptosis. Thus, loss of p53 function increases cell survival [16].

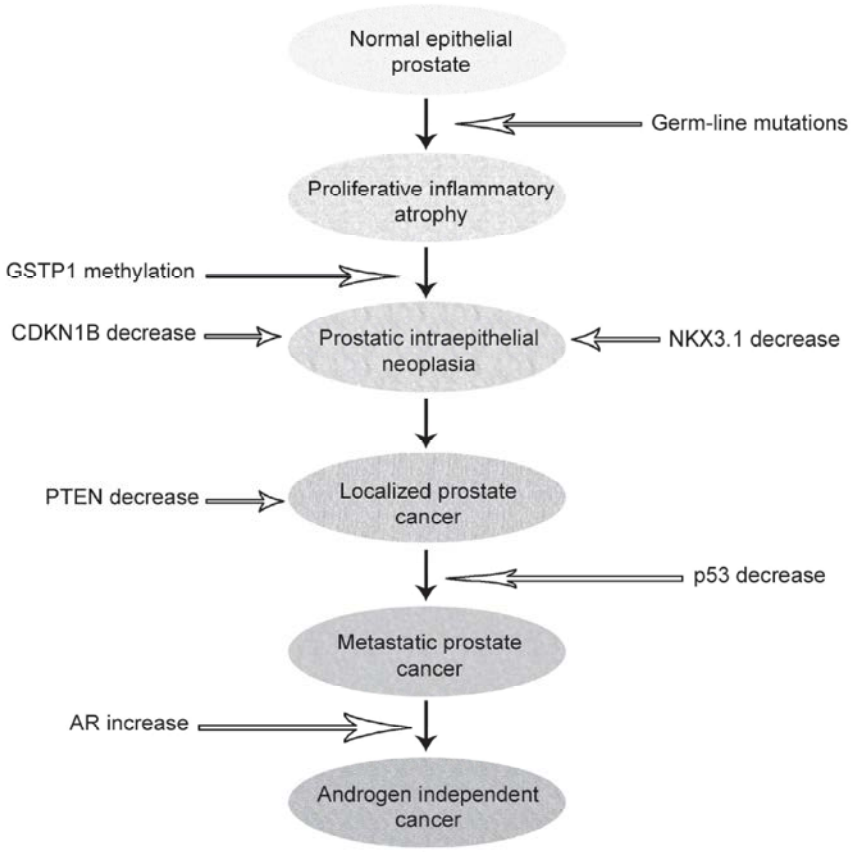

Fig. 1: Molecular pathogenesis of prostate cancer

At a late stage of disease when the cancer has become metastatic patients are usually treated with androgen withdrawal. However, the cancer will eventually emerge to androgen-independent because of amplification and mutations in the androgen receptor [15, 16].

Androgen receptor represses expression of multiple genes mediating androgen synthesis, DNA synthesis and proliferation while stimulating genes mediating lipid and protein biosynthesis. Androgen levels in CRPC appear adequate to stimulate AR activity on enhancer elements, but not suppressor elements, resulting in increased expression of AR and AR repressed genes that contribute to cellular proliferation [17].

The standard treatment for metastatic prostate cancer (PCa) is surgical or medical castration to reduce circulating androgens (androgen deprivation therapy (ADT) and suppress activity of the AR, but patients invariably relapse with more aggressive castration-resistant prostate cancer (CRPC) [18].

Mechanisms that may contribute to restoring AR activity in CRPC include AR mutations or alternative splicing, increased intratumoral androgen synthesis, increased co activator expression and activation of several kinases that may directly or indirectly sensitize AR to low levels of androgens[19]. Mechanisms contributing to the increased AR mRNA in CRPC include AR gene amplification in about one-third of patients with CRPC and increased E2F activity in RB-deficient tumors [20].

Gene Delivery Methods: One major question in gene therapy is the efficient and safe transfer of the genetic material into the target tissue. Gene therapy for cancer currently necessitates the transfer of recombinant DNA into human cells in order to achieve an antitumor effect and efficient gene transfer requires the use of a vector. All vectors contain at a minimum the transgene of interest linked to a promoter to drive its expression. The ideal vector should be specific to the target cell and deliver DNA efficiently into cells. It should be nontoxic to the patient and environment, nonimmunogenic, nonmutagenic and ideally produced cheaply at high concentrations. There are an increasing number of vectors and delivery methods available for gene transfer. Factors that may determine which vector is most ideal for a particular study

include maximal transgene size permissible, tendency to provoke inflammatory/immune responses, persistence of gene transfer and the ability to deliver the transgene to non dividing cells, target cell specificity and transduction efficiency [4].

Viral Vectors: Viral vectors represent potential gene delivery vehicles. Especially with respect to a use in patients, highly attenuated or non-replicative viruses represent good candidates for gene delivery. Among the broad range of viruses, retro-, adeno-associated and Herpes simplex viruses are the most frequently used in prostate cancer. According to the differences concerning infection strategies, the different viral vectors may have advantages as well as disadvantages [21].

Retroviruses: Because of their stable integration into the target cell genome and transmission to the progeny of the transduced parent cell, retroviral vectors can potentially lead to sustained transgene expression. However, retroviral entry into the cell nucleus is cell-division–dependent, which may be a significant problem when considering the treatment of cancers with low mitotic rates, such as prostate cancer. Other limitations include relatively low transduction rates in vivo, the rapid inactivation of retroviruses by human complement, as well as the potential to induce insertional mutagenesis and secondary malignancies [21].

Retroviral vectors induce the insertion of the therapeutic gene into the host genome, resulting in stable and efficient transfection. This makes their use in humans critical due to high risk of genetic instability and mutagenesis which limits their use in the clinic though can be overcome by lentiviruses [22].

Lentiviruses, such as the human immunodeficiency virus, are a subfamily of retroviruses that are able to integrate into nondividing cells. Lentivirus vectors are also able to sustain prolonged transgene expression. Safety concerns, however, including the risk of insertional mutagenesis, have limited the use of these vectors. Although lentiviral vectors obviously represent potential gene delivery tools, their pathogenicity is a major critical point with respect to their use in patients [23].

Adenoviruses and Adeno-associated Vectors: Adenovirus vectors are the most common viral vehicles used for prostate cancer gene therapy in human clinical trials. This is because of the advantages of efficient transduction and easy manipulation in vitro. It is also relatively straightforward to produce high titers of purified virus. Moreover, adenoviruses infect both dividing and nondividing cells and their DNA is not incorporated into the host genome, minimizing concerns about insertional mutagenesis. The major disadvantages are the transient expression of its DNA insert and the immune responses generated in response to the vector [24]. They are most frequently used in prostate cancer clinical trials and intensive research has resulted in improvement of safety, specificity and cytotoxicity. Nevertheless, the use of adenoviral vectors in the clinic may be limited due to their strong immunogenicity [22].

A major consequence of the anti-adenovirus immune response is a marked reduction of transgene expression following multiple dosing, which appears to result mainly from stimulation of neutralizing antibody responses, although adenovirus-specific cytotoxic T lymphocytes (CTLs) have been detected. In addition to specific anti-adenovirus transgene–directed immune responses, nonspecific inflammation can also significantly reduce transgene expression. It has been possible to increase the duration of adenovirus gene expression through the use of CTL blocking agents or immunosuppressive drugs [25, 26].

Safety, however, is still an issue with systemic use of adenovirus vectors because this has been associated with acute liver injury, resulting from the release of cytokines, in several mouse models. However, low doses of modified E1- deleted viruses can be used with minimal toxicity even in animals with damaged livers, although in some cases a lower level of transgene expression was seen [27].

New adenoviral vectors have been developed that have no adenoviral genes within their genome but retain sequences essential for replication and packaging of the genome. These “gutless” vectors can carry very large DNA inserts of up to 35 Kb, compared with 8kb in the former adenoviral vectors and do not express viral proteins, which has the additional advantage of limiting the host immune response. The adeno-associated virus vector is replication- deficient and is unique in that it requires co-infection with another adenovirus for productive infection in cell cultures. Though adeno associated viruses may infect nondividing cells and elicit little immune response, they have lost their ability to integrate specifically into the target cell genome, raising concerns of potential insertional mutagenesis [22].

Vaccinia and Herpes Viruses: The Vaccinia virus, a member of the pox virus family, is naturally cytopathic but can be engineered to a non-cytopathic form that retains its infectious activity. It has several characteristics that

offer a potential advantage for gene therapy. Vaccinia has a large genome of 186kb, allowing the incorporation of a large transgene insert and it replicates DNA and transcribes RNA in the cytoplasm without being transported to the cell nucleus, thus avoiding any potential insertional mutagenesis. Disadvantages include systemic toxicity, high immunogenicity and only transient transgene expression. Vaccinia virus–based vectors have been used mainly for the delivery of antigens to tumors to elicit host cell immune responses as a means of targeting the cancer cells for immune-based destruction. Other viruses, which were shown to be useful vector constructs in prostate cancer patients, are Vaccinia viruses. These DNA poxviruses have a large genome and are therefore able to express large foreign proteins. Moreover, they are able to transfect dividing as well as non-dividing cells [21, 28].

Herpes simplex viral vectors are also frequently used in prostate cancer gene therapy. These viruses contain double stranded DNA and replicate in the nucleus of the host cell. They are commonly used vectors to induce the expression of prodrug activation genes [29, 30]. The main advantages of herpes viruses are their large insert size of 35kb and their ability to infect dividing and non dividing cells. However, they are limited by their potential pathogenicity, poor transduction efficiency, transient gene expression and strong immunological responses [31].

Non -viral Delivery Systems: Nonviral gene transfer systems include chemical methods, such as the use of liposomes and physical methods, such as microinjection electroporation. Liposomes are relatively cheap, nontoxic, nonimmunogenic lipids and can be used for DNA coating to protect DNA from degradation until it reaches the target cell. The lipid envelope fuses with the target cell membrane and the gene is delivered directly to the cytoplasm [21].

However, the low efficiency of transgene delivery is the main limiting step. On the other hand, the safety of this technique has been verified in a phase I study in which liposomes containing the interleukin-2 (IL-2) gene were injected into the prostates of patients with advanced prostate cancer [32].Hybrid vectors, combining viral and synthetic approaches, have been devised to overcome their respective limitations. Adenovirus liposome complexes have resulted in a 1000- fold increase in gene transfer efficiency relative to naked plasmid. Transgenes of up to 48kb have been successfully transferred using this technique [33].

The most basic form of delivery strategy is to deliver the plasmid directly to the desired site, i.e., the tumor. Although this method is cheap and can be simple to perform, the cellular uptake of the plasmid DNA/RNA and expression of the transgene occur with low efficiency. Furthermore, DNA that is internalized into the cell is susceptible to endonuclease activity, thus limiting the duration of transgene expression. However, because no immune responses are generated with this system, research is ongoing to try and bypass the present limitations [21].

Use of Tissue-specific Promoters in Prostate Cancer Gene Therapy: The use of specific promoters allows restricting gene activation to the target site and this in turn may contribute to reduced toxic side effects. Unfortunately, only a limited number of prostate-specific promoters such as PSA or rat probasin (rPb) have been identified so far [34, 35]. PSA and rPb promoters are strong and efficient promoters; however, their activation is strongly regulated through androgens. In patients with hormone refractory prostate cancer, who, in general, already underwent androgen ablation androgen-regulated promoters may therefore have only limited efficacy. In order to overcome this problem androgen-independent prostate specific promoters like osteocalcin or the prostate-specific membrane antigen (PSMA) may be more useful [36]. Another possibility is to modify androgen-dependent promoters like rPb in order to render them active also in the absence of androgens by introducing a retinoic acid-response element [37].

Another interesting strategy is the use of an osteocalcin promoter [29]. Since osteocalcin is a major non-collagenous bone matrix protein, which is highly expressed in prostate cancer cells but also in osteoblasts, this osteocalcin-driven promoter allows a prostate tumor cell specific activation of the therapeutic gene not only in the primary tumor but also in metastatic lesions, which are frequently found in lymph nodes and bone [38]. This treatment was shown to be well tolerated in patients with locally recurrent prostate cancer and in patients with lymph node and bone metastasis of hormone-refractory prostate cancer [29]. Osteocalcin promoter driven adenoviral mediated gene delivery was also shown to be useful to co-target prostate tumor epithelial cells and bone stromal cells. This strategy allows inhibiting the intercellular communication between the epithelium and the stroma, which is thought to have a strong influence on prostate tumor growth and progression [38].

Recently, conditionally replication-competent vectors have been developed where the adenoviral replication is driven by a specific promoter such as PSA, vitamin D3, or osteocalcin to further increase cytotoxicity and acquire tissue specificity [39]. Pramudji *et al*. [40] investigated the advantages to use a caveolin-1 promoter, which should not only be more active than PSA promoters but also additionally direct tumor associated endothelial cells, thus producing a bystander effect. Their results in fact supported the concept of targeting prostate tumor cells and tumor-associated endothelial cells simultaneously.

Similarly, the promoter of the DD3PCA3 gene was described to be useful in gene therapy to specifically deliver therapeutic agents to prostate cancer. DD3PCA3 expression is restricted to prostate tissue and was found to be highly up regulated in prostate cancer. Preclinical experiments demonstrated that DD3PCA3-based adenoviral constructs are attractive candidates to be used in prostate cancer gene therapy [41].

Stem Cell Based Gene Therapy in Prostate Cancer: The introduction of stem cells (SCs) in cancer gene therapy is attributed mainly to the powerful advantage of it being a vector or cellular vehicle. Stem cell based cancer gene therapy is based on its tumor tropic property. The tumor homing ability of SCs holds therapeutic advantages compared to other vehicles such as proteins, antibodies, nanoparticles and viruses. Viruses or non migratory vector-producing cells have been utilized but demonstrated many shortcomings in effective delivery of the therapeutic agents. Virus-mediated gene therapies have limitation because of the difficulty in tracking cancer cells [42].

Another uprising evidence for using stem cells in cancer therapy is that it is nowadays broadly accepted that cancer is a stem cell disease. Compared with conventional chemotherapy, suicide gene therapy using SCs had no significant adverse effect on the weight gain of the animals. Such safety and efficacy imply a great potential of SC cell line expressing transgenes of interest for biologic study or clinical application [43, 44].

Stem Cell Delivery as a Vector: Gene-directed enzyme prodrug therapy known as well as virus-directed enzyme prodrug therapy or suicide gene therapy did not demonstrate a satisfactory final result in clinical trials in the past. The primary cause for this failure was the missing tumor specificity of this approach. However, SC based prodrug cancer therapy is quite different from the classical prodrug gene cancer therapies. The main differences from earlier versions of cancer gene therapies are tumor homing able of MSCs, *in vitro* preparation of therapeutic SCs by plastic ability and having prodrug converting genes integrated as DNA provirus in therapeutic SCs. Stable and effective production of prodrug converting enzymes under strong retroviral promoter is a great advantage of stem cell delivery as a vector [45].

Mechanisms of Stem Cell Based Gene Therapy: Considering the issue that MSCs play dual roles in tumor genesis through potentiating tumor growth, enhancement of neovascularization, or differentiating into tumor stromal fibroblasts, suicide genes are of particular interest because they facilitate the development of MSCs in antitumor therapy [46].

The novel strategy of stem cell based gene therapy in prostate cancer includes silencing gene expression, expression of intracellular antibodies, blocking cells' vital pathways and transgenic expression of caspases and DNases. SC based gene therapy consists of two phases of treatment. In the first step, the gene for a foreign enzyme (bacterial, yeast or viral) is delivered and targeted to the tumor by transduction of SCs. In the second step, the enzymatic activity of the gene product converts less toxic prodrug to cytotoxic substance at the tumor lesion. Therefore, the active cytotoxic substances produced by enzymatic process within transduced MSCs effectively demolish the neighboring or surrounding tumor cells, which are called bystander effect. In addition, the process of killing tumor and therapeutic stem cells can induce host immune responses mediated by natural killer (NK) cells and T-cells, which is called distant bystander effect [47].

The success of SC based gene therapy depends on several factors, which are the catalytic activity of the enzyme encoded by suicide gene, a suitable combination of prodrug enzyme, the migration ability of the SC vector to target tumor cells, sufficient transgene expression and the extent of bystander effect [43].

Recently, it was reported indeed that expression of the bifunctional suicide gene *CD: UPRT* increases radio sensitization and bystander effect of 5-FC in PC cells. Using bifunctional suicide gene, researchers have previously shown, in *vitro* and *in vivo* studies, that the bystander effect mediated by *CD: UPRT* gene directed enzyme prodrug therapy without a direct cell to cell contactor functional gap junctions [48].

Gene Therapy Strategies in Prostate Cancer: There are currently four main approaches to prostate cancer gene therapy: the replacement of deficient tumor-suppressor genes with genes that enhance apoptosis, the introduction of an effector gene that can stimulate the host's immune response by activating tumor-specific CTLs, suicide gene therapies involving transfection of tumor cells with a gene that produces an enzyme that converts a prodrug into a toxic agent and the use of oncolytic viruses [4].

Enhancing Apoptosis: Mutations in the tumor-suppressor gene *p53* are observed in 25 to 75% of prostate cancers, more commonly in advanced tumors although it is thought to function in the G1 checkpoint activated in response to DNA damage and radiation dependent apoptosis. The ability of over expressed *p53* to inhibit the growth of primary cultures derived from radical prostatectomy specimens has been demonstrated, even when the *p53* status is normal [49]. The further potential therapeutic efficacy of *p53* gene delivery has been suggested by the observation that prostate cancer cell lines infected with wild type *p53* adenovirus were not tumorigenic [50]. P53-dependent cell cycle arrest may function through the action of the CKI3 p21 [51]. P21 expression is induced by p53, either by over expression of p53 or after DNA damage. Cells over expressing p21 accumulate in G1 and mice lacking p21 have defects in the G1 checkpoint induced by DNA damage. Together, these data support the idea that p21 participates in the G1 checkpoint mediated by p53 [52].The therapeutic effects of *p53* gene delivery are likely to be due to enhancement of apoptosis as well as other "bystander" mechanisms such as anti- angiogenesis. Administration of adeno virus p21 can significantly extend mouse survival and decrease tumor volume. In addition, p21 was more effective than p53 in suppressing tumor cell growth, suggesting that CKIs may prove beneficial in the treatment of prostate and perhaps other cancers [53].

P53 encodes a transcription factor, the targets of which include genes that regulate cellular responses to DNA damage, cell cycle progression and genomic stability [54]. Loss of p53 results in an inability of some cell types to undergo apoptosis and this may be a primary mechanism that gives rise to tumors in cells lacking p53 [55]. P53-dependent cell cycle arrest can function through the action of the Cdk inhibitor p21 [52]. Both of these genes can arrest the cell cycle in G1 when over expressed. Viral-mediated gene therapy approaches have been used to deliver p53 to a number of malignancies with the goal of suppressing tumor growth in vivo [56]. Injection of Ad5CMV-p53 into subcutaneous squamous cell carcinoma nodules significantly reduced further tumor development [53].

P21 may be a more potent growth inhibitor of some cell types than p53. Whereas it is clear that p21 transcription is induced in p53-infected cells, the level of p21 produced via p53-mediated transcription may not be as high as that obtainable by adenovirus-mediated transduction because of differences in promoter strength or translational efficiencies of endogenous and exogenous p21 mRNAs [57]. The presence of p53 may allow for appropriate induction of apoptosis or G1 arrest in response to DNA damage in certain circumstances.

Clinical trials using replication deficient adenoviral vectors encoding *p53* directly injected into the prostate gland under ultrasonic or magnetic resonance imaging are underway. A study has also demonstrated growth inhibition of prostate cancer by an adenovirus expressing a "novel" tumor-suppressor gene, *pHyde* [58]. *In vitro* introduction of this recombinant vector led to a decrease in growth of the human prostate cancer cell lines DU145 and LNCaP in culture.

In vivo injection of the virus reduced DU145 tumors in nude mice compared with untreated control or viral control–treated DU145 tumors. Introduction of the *pHyde* gene resulted in apoptosis and stimulated *p53* expression. Oncogenes that may be activated in prostate cancer include c-*myc, bcl-2,* c-*met* and *ras*. Disruption of c-*myc* over expression using antisense c-*myc* transduced by a replication deficient retrovirus led to a 95% reduction in the tumor volume of DU145 prostate cancer cell xenografts [58]. The *bcl-2* gene is over expressed in androgen-independent prostate cancer.

It has been shown that proteolytic activation of caspase-7 is a common event in LNCaP cells undergoing apoptosis [59]. The over expression of caspase-7 induced by transfection of LNCaP cells with an adenoviral vector expressing the gene resulted in apoptosis of these cells after 72 hours. It was possible to induce apoptosis despite the over expression of the apoptosis suppressor gene *bcl-2.*

In the study by Godbey and Atala on Directed apoptosis in Cox-2-overexpressing cancer cells through expression-targeted gene delivery [60] the polycation poly (ethylenimine) was used to none virally introduce the genes into the target cells. The plasmid introduced was under the control of the cyclooxygenase-2 (COX-2)

promoter because constitutive COX-2 over expression has been implicated in tumorigenesis. Thus, coculture of normal cells and COX-2 over expressing prostate cancer cells (PC3) revealed a higher reporter expression in the cancer cells. This targeting method was then used to direct the expression of inducible forms of caspases 3 and 9 following which the cells underwent apoptosis. Thus, in this particular study, the heightened COX-2 expression levels of the cancer cells were used for guidance of gene expression in transfected cells.

Enhancing Immunological Responses: Prostate cancer has several factors that make it a good candidate for adoptive immunotherapy. Although prostate cancer is a visceral tumor, the primary tumor site is relatively easy to access and image. Effector immune cells can be readily and accurately injected directly into the tumor assisted by transrectal ultrasonography. In addition, prostate cancer expresses a number of unique tumor and tissue markers including prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA) and members of the *ErbB* gene family. These markers not only can serve for screening and monitoring of prostate cancer, but also those markers expressed on the cell surface may provide useful targets for active and passive immunotherapy. Finally, although many of these prostate cancer–associated antigens are also expressed on normal prostate tissue, the prostate is not a vital organ and thus any damage to neighboring cells can be accepted as a consequence of treatment [4].

However, active immunization against prostate cancer may be of limited efficacy because in many cases the tumor cells are of low immunogenicity. Defects in major histocompatibility complex (MHC) class I expression are observed in 85% of primary and 100% of metastatic tumors [61] suggesting that evasion of MHC class I tumor-associated antigens is important in tumor development. One method of generating an immune response despite down regulation of MHC class I is the use of lymphocytes possessing chimeric receptors. In their research Immuno-Gene Therapy of Established Prostate Tumors Using Chimeric Receptor-redirected Human Lymphocytes Pinthus *et al* [62] have used "chimeric immune receptors" that possess antibody-like specificity linked to T cell triggering domains in order to redirect immune effector cells toward tumors. This approach combines the effector functions of T cells with the ability of antibodies to recognize a presented antigen with high specificity and without MHC restriction. Using this approach, anti-*erbB2* chimeric receptors were introduced into human lymphocytes and tested against human prostate cancer xenografts in a severe combined immunodeficiency disease (SCID) mouse model. Local delivery of *erbB2*- specific chimeric receptor-bearing lymphocytes, together with systemic IL-2 administration, resulted in retardation of both tumor growth and PSA secretion, prolongation of survival and complete tumor elimination in a significant number of mice.

Antitumor responses can also be enhanced by presenting tumor antigens in the context of high levels of transduced cytokines. Granulocyte macrophage colony-stimulating factor (GMCSF) has emerged as a cytokine with significant efficacy in the induction of an antitumor immune response [63]. GM-CSF may be transduced into autologous or allogeneic tumor cells ex vivo using a viral vector. The transduced tumor cells are then irradiated both to minimize malignant potential and to improve immunogenicity. The cells are then reintroduced by vaccination into the patient. Tumor cell vaccine-expressed GM-CSF may activate quiescent antigen-presenting cells, which then present processed antigen to both CD4 (helper) and CD8 (cytotoxic) T cells, activating a systemic antitumor immune response.

A phase I trial of eight immunocompetent patients treated with autologous GM-CSF vaccine prepared from ex vivo retroviral transduction of surgically harvested cells showed that the technique was safe and that antitumor immune responses were inducible [64]. Phase II studies have commenced with GMCSF generated allogeneic vaccines. Preliminary analysis of the initial trial approved to use direct transrectal prostatic gene therapy injection has suggested that this approach is safe [58].

Studies employing other cytokine genes such as *IL-2* are also underway. In a phase II trial intratumoral injection of a plasmid coding for *IL-2* formulated in a liposomal, cationic lipid mixture vehicle led to decreases in serum PSA levels at 2 weeks post injection in 80% of the patients, with no grade 3 or 4 toxicity reported [65].

Another vaccine strategy is the DNA vaccine. In this approach, an expression cassette containing the transgene against which an immune response is desired is injected directly into host cells. The expression of the transfected gene in vivo then promotes immune responses. DNA vaccines are easier to prepare than peptide- or viral-based vaccines and are safe, as they are none replicating. Efficacy can be improved by fusion of the desired transgene with the sequence for a pathogen-derived gene to elicit a stronger immune

response. The preclinical testing of a PSA-based DNA vaccine in mice resulted in a strong humoral immune response against PSA-positive tumors [66].

The major drawback with the strategy of cytoreductive immunotherapy is the limited tumor burden the immune system can eliminate and thus applicability may be limited to low bulk disease. Also, the harvesting and culture of autologous or allogeneic tumor or immune cells for ex vivo gene therapy is costly and challenging technically [4].

Suicide Gene Therapy: Suicide gene therapy involves the conversion of an inactive prodrug into toxic metabolites that can lead to cell cycle arrest and death. Active drug is limited spatially to the transduced cells and adjacent surrounding cells, facilitating higher drug concentrations without increased normal tissue toxicity. Bystander mechanisms markedly enhance efficacy of tumor destruction such that tumors can be eradicated following transduction of only 10% of neoplastic cells with suicide genes. Activation of ganciclovir by herpes simplex virus thymidine kinase (HSV-tk) has been the most widely investigated system [4].

The HSV-tk system is characterized by the highly effective phosphorylation of ganciclovir. The toxic product of this process cannot cross cell membranes, allowing it to accumulate within the cell. Incorporation into newly synthesized DNA causes termination of synthesis and cell death. Marked tumor growth inhibition and suppression of spontaneous and induced metastases have been demonstrated after injection of the vector containing the *HSV-tk* gene directly into prostate tumors with subsequent treatment with ganciclovir in the orthotopic mouse model [66].

A phase I clinical trial has been carried out in patients with recurrent prostate cancer using a replication-deficient adenovirus vector containing the thymidine kinase gene injected directly into the prostate followed by intravenous ganciclovir [67]. A statistically significant prolongation of the PSA doubling time from a mean of 9.8 months to 13.3 months was achieved after the first cycle of gene therapy. Grade 4 toxicity was encountered after the vector injection in only one of 18 patients. Even after repeated injections, side effects were generally mild and self -limiting.

An additive response was observed in patients receiving a second cycle of gene therapy with further prolongation of the mean PSA doubling time. The cytosine deaminase (*CD*) gene isolated from *E. coli* encodes for an enzyme that is not normally present in mammalian cells that transforms cytosine to uracil. This enzyme converts 5-fluorocystine to 5-fluorouracil, which inhibits RNA and DNA synthesis. Preclinical studies have shown greater efficacy in killing tumor cells compared with the herpes simplex thymidine kinase/ganciclovir system [68]. Clinical studies are now being initiated. The *E. coli* nitroreductase /CB1954 combination is also an attractive candidate for clinical evaluation because it generates a potent DNA cross-linking agent that can kill both dividing and non dividing cells by induction of apoptosis via a p53-independent mechanism.

Furthermore, the efficacy that has been demonstrated in xenograft models required only three cycles of prodrug administration. Djeha *et al*. [69] injected a replication-deficient adenovirus expressing high levels of nitroreductase intratumoral and combined this with systemic CB1954 treatment. They found that a single injection of the virus (7.5 \ 109 to 2 \ 1010 particles) followed by CB1954 resulted in a decrease in tumor growth of human prostate cancer xenografts (PC3 cell line) in nude mice. Several trials are currently taking place looking at the potential of combining radiotherapy with gene therapy treatments. Chhikara *et al*. [70] treated subcutaneous murine prostate tumors with an intratumoral injection of an adenovirus expressing the *HSV-tk* gene followed by systemic ganciclovir or local radiation therapy or the combination of gene and radiotherapy.

Both single-therapy modalities resulted in a 38% decrease in tumor growth compared to untreated controls, but the combined treatment resulted in a decrease of 61%. Preliminary data suggest that this approach is also safe in humans. Teh *et al*. [71] have treated men with newly diagnosed prostate cancer with a combination of radiotherapy, in situ gene therapy consisting of an adenovirus expressing the thymidine kinase gene, together with valacyclovir and in high-risk patients' hormonal therapy. At a median follow up of 5 months, the acute toxicities were limited, with no patient experiencing a grade 3 or greater acute toxicity. More recently at a median follow-up of 22.3 months, no grade 3 or greater late toxicity was seen [71].

To further increase the effectiveness of suicide gene therapy, several groups have developed approaches to deliver both the cytosine deaminase and *HSV-tk* genes and treat with both 5- fluorocystine and ganciclovir. Freytag *et al*. [72] have developed a lytic replication-competent adenoviral vector encoding an HSV-tk–CD

fusion protein and used it for the treatment of patients with a local recurrence of prostate cancer at least 1 year after the completion of definitive radiotherapy treatment. The virus was injected intratumoral into 16 patients, followed by systemic treatment with ganciclovir and 5- fluorocystine. The treatment was well tolerated with a reduction in PSA in nearly 50% of patients. Furthermore, two patients showed a lack of detectable carcinoma at their 1 year follow-up biopsies. Freytag *et al*. [72] have also used the same gene therapy in combination with radiotherapy for the treatment of patients with newly diagnosed intermediate- to high risk prostate cancer. The authors reported no significant side effects and acute urinary and gastrointestinal toxicities were similar to those expected with the radiotherapy treatment.

Oncolytic Viruses: Viruses alone can infect and kill tumor cells without the insertion of a cytotoxic transgene. Certain viruses, including adenoviruses and HSV, have as part of their normal life cycle a lytic phase that is lethal to the host cell [4].

The use of oncolytic HSV for the treatment of cancer has progressed to phase I clinical trials, although this is for the treatment of gliomas. G207 is a replication-competent HSV that is mutated so that viral propagation is confined to tumor cells and to limit neurovirulence. Although G207 is mainly being assessed clinically for the treatment of gliomas, with regard to prostate cancer G207 was capable of conferring cytotoxicity to several prostate tumor cell lines and either growth retardation or tumor eradication of mouse xenografts [73].

This construct showed potent PSA-selective cytotoxic activity in preclinical testing [74] and a phase I trial has been carried out [75]. A total of 20 patients with locally recurrent prostate cancer following radiotherapy were treated. The study revealed that intratumoral injection of the construct was safe and it led to a decrease in PSA in a dose-responsive manner. Additional studies have demonstrated that combination treatment of CG7060 and radiation leads to synergistic prostate tumor cytotoxicity [76].

Current and Future Directions of Prostate Cancer Gene Therapy: The promise of gene therapy lies in its potential for selective potency. To achieve this aim, cancer gene therapy strategies attempt to exploit the biological uniqueness of each particular tumor. The human prostate is an accessory organ and is not required for potency or urinary continence. Thus unique targets showed by PCA and the normal prostate are candidates for new treatment. The Prostate Expression Database is an online resource designed to access and analyzes gene expression information derived from the human prostate [15]. It has revealed more than 55 000 expressed sequence tags (ESTs) from 43 cDNA libraries; of these approximately 500 are prostate-unique [15]. The presence of this large number of prostate-unique promoters and candidate antigens promises to facilitate the design of prostate-specific gene therapy. Ideally, both the clinical and genetic features of PCA should be taken into account in the design of effective prostate gene therapeutic strategies [77].

Gene therapy comes as an important approach for therapeutic intervention in PCa. Clinical trials for PCa have been demonstrating that gene therapy is relatively safe and well tolerated, although some improvements are yet to be developed and Current biotechnological methodologies, especially genomic studies, are adding important aspects to this area[78]. Gene transfer technology allows an incredible diversity of treatment possibilities. This diversity can be used to complement traditional therapies, as well as provide radically new frontiers for treatment. Gene transfer therapy can rely on the current information known about the genetics of cancer formation, bringing a more sophisticated and personalized approach to therapy.

CONCLUSIONS

In conclusion the expanding field of genomics provides an exciting new resource for the design of prostate specific gene therapy strategies. The obstacles to the development of gene-based human therapeutics are significant but the rewards are promising. In the future, the wide use of patients and tumor genomic analysis as well as the assessment of host humoral and cellular immunity will facilitate a better selection of the most appropriate gene therapy per patient. Safety testing and also technical improvements are inevitable and researchers should always keep "an open eye" when using viral vectors. Then they undoubtfully represent potential delivery vehicles for gene transfer.

ACKNOWLEDGEMENTS

First of all I would like to express my heartfelt thanks to almighty God for His mercifulness, saving my personal life and keeping my life in all my ways. My thanks also go

to Addis Ababa University, school of medicine, department of pharmacology for giving me the chance to write this paper. I finally, express my thanks to my close friends and class mates those shared me love and experience.

REFERENCES

1. Edward, C. and C. Alan, 2015. Cancer Chemotherapy, In Clinical Pharmacology: 9th ed. Ed., Katzung, B.G.
2. Brawley, O., E. Giovannucci and B. Kramer, 2000. Epidemiology of Prostate Cancer. Philadelphia: Lippincott Williams & Wilkins.
3. Brinkmann, A.O., L.J. Blok, P.E. de Ruiter, P. Doesburg, K. Steketee, C.A. Berrevoets and J. Trapman, 1999. Mechanisms of androgen receptor activation and function. J. Steroid Biochem Mol. Biol., 69: 307-313.
4. Iris, E.E., H. Petra, B. Georg and K. Helmut, 2005. Gene Therapy Strategies in Prostate Cancer, Department of Urology, University of Innsbruck, Austria, Current Gene Therapy, 5: 1-10.
5. Angelika, D., 2005. Prostate cancer gene therapy based on an adenoviral vector with tissue specific expression, Uppsala University School of Engineering, ISSN 1401-2138, 1-23
6. Shi, X., N. Nesslinger, A. Deitch, P. Gumerlock and R. White, 2002. Complex functions of mutant pp: 53 alleles from human prostate cancer. *Prostate*, 51: 59-72.
7. Nupponen, N., L. Kakkola, P. Koivisto and V. Tapio, 1998. Genetic alterations in hormone-refractory recurrent prostate carcinomas. Am. J. Pathol., 153: 141-148.
8. Wilson, J., F. George and M. Renfree, 1995. *T*he endocrine role in mammalian sexual differentiation. Recent Prog. Horm. Res., 50: 349-364.
9. Culig, Z., A. Hobisch, M.V. Cronauer, C. Radmayr, A. Hittmair, J. Zhang, M. Thurnher, G. Bartsch and H. Klocker, 1996. Androgen receptor status of lymph node metastases from prostate cancer. Prostate, 28: 129-135.
10. Ruizeveld de Winter, J.A., P.J. Janssen, H.M. Sleddens, M.C. Verleun-Mooijman, J. Trapman, A.O. Brinkmann, A.B. Santerse, F.H. Schröder and T.H. van der Kwast, 1994. Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer. Am. J. Pathol., 144: 735-746.
11. Yang, L., L. Wang, H.K. Lin, P.Y. Kan, S. Xie, M.Y. Tsai, P.H. Wang, Y.T. Chen and C. Chang, 2003. Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3 and MAPK, three distinct signal pathways in prostate cancer cells. Biochem. Biophys. Res. Commun., 305: 462-469.
12. Zhao, X.Y., P.J. Malloy, A.V. Krishnan, S. Swami, N.M. Navone, D.M. Peehl and D. Feldman, 2000. Glucocorticoids can promote androgen independent growth of prostate cancer cells through a mutated androgen receptor. Nature Medicine, 6: 703-706.
13. Metzger, E., J.M. Müller, S. Ferrari, R. Buettner and R. Schüle, 2003. A novel inducible transactivation domain in the androgen receptor: implications for PRK in prostate cancer. EMBO J., 22: 270-280.
14. Grönberg, H., 2003. Prostate cancer epidemiology. Lancet; 361: 859-864.
15. Nelson, W., A. De Marzo and W. Isaacs, 2003. Prostate cancer. New England Journal of Medicine, 349: 366-381.
16. Porkka, K. and T. Visakorpi, 2004. Molecular mechanisms of prostate cancer. European Urology; 45: 683-691.
17. Cai, C., H.H. He, S. Chen, I. Coleman I.H. Wang, Z. Fang, P.S. Nelson, X.S. Liu, M. Brown and S.P. Balk, 2011. Androgen Receptor Gene Expression in Prostate Cancer Is Directly Suppressed by the Androgen Receptor Through Recruitment of Lysine-Specific Demethylase, Cancer Cell AR Suppresses Its Gene Transcription, pp: 457-471.
18. Stanbrough, M.J., G. Bubley, K. Ross, R.G. Todd, A. R.Mark, M.P. Trevor, G.F. Phillip and P.B. Steven, 2006. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res, 66: 2815-2825.
19. Yuan, X. and S. Balk, 2009. Mechanisms mediating androgen receptor reactivation after castration. Urol. Oncol., 27: 36-41.
20. Sharma, A., W.S. Yeow, A. Ertel, I. Coleman, N. Clegg, C. Thangavel, C. Morrissey, X. Zhang X.C.E. Comstock, A.K. Witkiewicz, L. Gomella, E.S. Knudsen, P.S. Nelson and K.E. Knudsen, 2010. The retinoblastoma tumor suppressor controls androgen signaling and human prostate cancer progression. J. Clin. Invest., 120: 4478-4492.

21. Eder, J.P., P.W. Kantoff, K. Roper, G.X. Xu, G.J. Bubley, J. Boyden, L. Gritz, G. Mazzara, W.K. Oh, P. Arlen, K.Y. Tsang, D. Panicali, J. Schlom and D.W. Kufe, 2000. A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. Clin. Cancer Res., 6: 1632-1638.
22. Bonnet, M., J. Tartaglia, F. Verdier, P. Kourilsky, A. Lindberg, M. Klein and P. Moingeon, 2000. Recombinant viruses as a tool for therapeutic vaccination against human cancers. Immunol. Lett., 74: 11-25.
23. Buchschacher, G. and F. Wong-Staal, 2000. Development of lentiviral vectors for gene therapy for human diseases. *Blood*, 95: 2499-2504.
24. Ali, M., N. Lemoine and C. Ring, 1994. The use of DNA viruses as vectors for gene therapy. Gene Ther., 1: 367-384.
25. Dai, Y., E.M. Schwarz, D. Gu, W.W. Zhang, N. Sarvetnick and I.M. Verma, 1995. Cellular and humoral immune responses to adenoviral vectors containing factor IX gene: tolerization of factor IX and vector antigens allows for long-term expression. Proc Natl Acad Sci, USA; 92(5): 1401-1405.
26. Kay, M.A., A.X. Holterman, L. Meuse, A. Gown, H.D. Ochs, P.S. Linsley and C.B. Wilson, 1995. Long-term hepatic adenovirus-mediated gene expression in mice following CTLA4Ig administration. Nat Genet; 11(2): 191-197.
27. Nakatani, T., S. Kuriyama, K. Tominaga, T. Tsujimoto, A. Mitoro, M. Yamazaki, H. Tsujinoue, H. Yoshiji, S. Nagao and H. Fukui, 2000. Assessment of efficiency and safety of adenovirus mediated gene transfer into normal and damaged murine livers. Gut., 47(4): 563-570.
28. Gulley, J., A.P. Chen, W. Dahut, P.M. Arlen, A. Bastian, S.M. Steinberg, K. Tsang, D. Panicali, D. Poole, J. Schlom and J. Michael Hamilton, 2002. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen independent prostate cancer. Prostate, 53: 109-117.
29. Kubo, H., T.A. Gardner, Y. Wada, K.S. Koeneman, A. Gotoh, L. Yang, C. Kao, S.D. Lim, M.B. Amin, H. Yang, M.E. Black, S. Matsubara, S. Nakagawa and M. Gillenwater, 2003. Phase I dose escalation clinical trial of adenovirus vector carrying osteocalcin promoter-driven herpes simplex virus thymidine kinase in localized and metastatic hormone-refractory prostate cancer. Hum. Gene Ther., 14: 227-241.
30. Park, H.S., J. Cheon, H.Y. Cho, Y.H. Ko, J.H. Bae, D.G. Moon and J.J .Kim, 2003. In vivo characterization of a prostate-specific antigen promoter-based suicide gene therapy for the treatment of benign prostatic hyperplasia. Gene Ther., 10: 1129-1134.
31. Kaminski, J.M., J.B. Summers, M.B. Ward, M.R. Huber and Boris Minev, 2003. Immunotherapy and prostate cancer. Cancer Treat. Rev., 29: 199-209.
32. Belldegrun, A., C.L. Tso, A. Zisman, J. Naitoh, J. Said, A.J. Pantuck, A. Hinkel J.deKernion J and R. Figlin, 2001. Interleukin 2 gene therapy for prostate cancer: phase I clinical trial and basic biology. Hum Gene Ther., 12(8): 883-892.
33. Cotton, M., E. Wagner, K. Zatloukal, S. Phillips, D.T. Curiel and M.L. Birnstiel, 1992. High efficiency receptor-mediated delivery of small and large 48 kilobase gene constructs using the endosome-disruption activity of defective or chemically inactivated adenovirus particles. Proc Natl Acad Sci. USA; 89(13): 6094-6098.
34. Latham, J.P., P. Searle, V. Mautner and N. James, 2000. Prostate specific antigen promoter/enhancer driven gene therapy for prostate cancer: construction and testing of a tissue-specific adenovirus vector. Cancer Res., 60: 334-341.
35. Pang, S., 2000. Targeting and eradicating cancer cells by a prostate-specific vector carrying the diphtheria toxin A gene. Cancer Gene Ther., 7: 991-996.
36. Ikegami, S., T. Tadakuma, S. Suzuki, I. Yoshimura, T. Asano and M. Hayakawa, 2002. Development of gene therapy using prostatespecific membrane antigen promoter/enhancer with Cre Recombinase/ LoxP system for prostate cancer cells under androgen ablation condition. Jpn. J. Cancer Res., 93: 1154-1163.
37. Furuhata, S., H. Ide, Y. Miura, T. Yoshida and K. Aoki, 2003. Development of a prostate-specific promoter for gene therapy against androgen-independent prostate cancer. Mol. Ther., 7: 366-374
38. Matsubara, S. Wada, Y. Gardner, Egawa, M. Park, M.C. Hsieh, H.E. Zhau, C. Kao, S. Kamidono, J.Y. Gillenwater and L.W.K. Chung, 2001. A conditional replication competent adenoviral vector, Ad-OC-E1a, to cotarget prostate cancer and bone stroma in an experimental model of androgen-independent prostate cancer bone metastasis. Cancer Res., 61: 6012-6019.

39. Hsieh, C., L. Yang, L. Miao, F. Yeung, C. Kao, H. Yang, H.E. Zhau and L. Chung, 2002. A novel targeting modality to enhance adenoviral replication by vitamin D(3) in androgen-independent human prostate cancer cells and tumors. Cancer Res., 62: 3084-3092.
40. Pramudji, C., S. Shimura, S. Ebara, G. Yang, J. Wang, C. Ren, Y. Yuan, S.A. Tahir, T.L. Timme and T.C. Thompson, 2001. In situ prostate cancer gene therapy using a novel adenoviral vector regulated by the caveolin-1 promoter. Clin. Cancer Res., 7: 4272-4279.
41. Schalken, J., D. Hessels and G. Verhaegh, 2003. New targets for therapy in prostate cancer: differential display code 3 (DD3 (PCA3), a highly prostate cancer-specific gene. Urology, 62: 34-43.
42. Pulkkanen, K. and S. Yla-Herttuala, 2005. Gene therapy for malignant glioma: current clinical status, Molecular Therapy, 12(4): 585-598.
43. Cihova, M., V. Altanerova and C. Altaner, 2011. Stem cell based cancer gene therapy," Molecular Pharmaceutics, 8(5): 1480-1487.
44. Zarogoulidis, P., K. Darwiche, A. Sakkas, L. Yarmus, H. Huang, Q. Li, L. Freitag, K. Zarogoulidis and M. Malecki, 2013. Suicide gene therapy for cancer—current strategies, Journal of Genetic Syndrome & Gene Therapy, 9: 4.
45. Jae, H., J. Hong and S. Yun, 2014. Stem Cell Based Gene Therapy in Prostate Cancer, Hindawi Publishing Corporation, BioMed Research International, pp: 1-8.
46. Suzuki, K., R. Sun, M. Origuchi, M. Kanehira, T. Takahata, J. Itoh, A. Umezawa, H. Kijima, S. Fukuda and Y. Saijo, 2011. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization, Molecular Medicine, 17(7-8): 579-587.
47. Guido, M., M. Pacenti, C. Parolin, F. Farinati and G. Palù, 2005. Systemic efficacy of combined suicide/cytokine gene therapy in a murine model of hepatocellular carcinoma, Journal of Hepatology, 42(5): 728-735.
48. Cavarretta, I.T., V. Altanerova, M. Matuskova, L. Kucerova, Z. Culig and C. Altaner, 2010. Adipose tissue-derived mesenchymal stem cells expressing prodrug-converting enzyme inhibit human prostate tumor growth, Molecular Therapy, 18(1): 223-231.
49. Asgari K., I.A. Sesterhenn, D.G. McLeod, K. Cowan, J.W. Moul, P. Seth and S. Srivastava, 1997. Inhibition of the growth of pre-established subcutaneous tumor nodules of human prostate cancer cells by single injection of the recombinant adenovirus p53 expression vector. Int. J. Cancer., 71(3): 377-382.
50. Ko, S., A. Gotoh, G.N. Thalmann, H.E. Zhau, D.A. Johnston, W.W. Zhang, C. Kao and L.W.K. Chung, 2008. Molecular therapy with recombinant p53 adenovirus in an androgen-independent, metastatic human prostate cancer model. Hum Gene Ther., 7(14): 1683-1691.
51. Sherr, C. and J. Roberts, 1995.Inhibitors of mammalian G, cyclin-dependent kinases. Genes & Dcv., 9: 149-163.
52. Deng, C., P. Zhang, J. Harper, S. Elledge and P. Leder, 1995. Mice deficient in pp: 21 develop normally but have defects in G1 checkpoint function. Cell, 82: 675-684.
53. Clayman, G.L., A.K. El-Naggar, J.A. Roth, W.W. Zhang, H. Goepfert, D. Taylor and T.J. Liu, 1995. In vivo molecular therapy with p53 adenovirus for microscopic residual head and neck squamous carcinoma. Cancer Res., 55: 1-6.
54. Hinds, P. and R. Weinberg, 1994. Tumor suppressor genes. Cure. Opin. Genet. Dcv., 4: 135-1411.
55. Fujiwara T., E.A. Grimm, T. Mukhopadhyay, D.W. Cai, L.B. Owen-Schaub and J.A. Roth, 1993. A retroviralwild-typep53 expression vector penetrates human lung cancer Spheroids and inhibits growth by inducing apoptosis. Cancer Research., 53: 4129-4133.
56. Harper, J.W., S.J. Elledge, K. Keyomarsi, B. Dynlacht, L. HTsai, P. Zhang, S. Dobrowolski, C. Bai, L. Connel-Crowley, E. Swindell, M.P. Fox and N. Wei, 1995. Inhibition of cyclin-dependent kinases by pp: 21. Mol. Biol. Cell., 6: 387-400.
57. Steiner, M. and J. Gingrich, 2000. Gene therapy for prostate cancer: J. Urol., 164(4): 1121-1136.
58. Marcelli, M., G.R. Cunningham, M. Walkup, Z. He, L. Sturgis, C. Kagan, R. Mannucci, I. Nicoletti, B. Teng and L. Denner, 1999. Signaling pathway activated during apoptosis of the prostate cancer cell line LNCaP: over expression of caspase-7 as a new gene therapy strategy for prostate cancer. Cancer Res., 59(2): 382-390.
59. Godbey, W. and A. Atala, 2003. Directed apoptosis in Cox-2-overexpressing cancer cells through expression-targeted gene delivery. Gene Ther., 10(17): 1519-27.
60. Blades, R., P. Keating, L. McWilliam, N. George and P. Stern, 1995. Loss of HLA class I expression in prostate cancer: implications for immunotherapy. Urology., 46(5): 681-686.
61. Pinthus J.H., T. Waks, K. Kaufman-Francis, D.G. Schindler, A. Harmelin, H. Kanety, J. Ramon and Z. Eshhar, 2003.Immuno-gene therapy of established prostate tumors using chimeric receptor-redirected human lymphocytes. Cancer Res., 63(10): 2470-2476.

62. Dranoff, G., E. Jaffee, A. Lazenby, P. Golumbek, H. Levitsky, K. Brose, V. Jackson, H. Hamada, D. Pardoll and R.C. Mulligan, 1993. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony stimulating factor stimulates potent, specific and long-lasting anti-tumor immunity. Proc Natl Acad Sci, USA, 90(8): 3539-3543.
63. Simons, J.W., B. Mikhak, J. Chang, A.M. DeMarzo, M.A. Carducci, M. Lim, C.E. Weber, A.A. Baccala, M.A. Goemann, S.M. Clift, D.G. Ando, H.I. Levitsky, L.K. Cohen, M.G. Sanda, R.C. Mulligan, A.W. Partin, H.B. Carter and S. Piantadosi, 1999. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony stimulating factor using ex vivo gene transfer. Cancer Res., 59(20): 5160-5168.
64. Pantuck, A., A. Zisman and A. Belldegrun, 2000. Gene therapy for prostate cancer at the University of California, Los Angeles: preliminary results and future directions. World J. Urol., 18(2): 143-147.
65. Kim, J.J., N.N. Trivedi, D.M. Wilson, S. Mahalingam, L. Morrison, A. Tsai, M.A. Chattergoon, K. Dang, M. Patel, L. Ahn, J.D. Boyer, A.A. Chalian, H. Schoemaker, T. Kieber-Emmons, M.A. Agadjanyan and D.B. Weiner, 1998. Molecular and immunological analysis of genetic prostate specific antigen (PSA) vaccine. Oncogene; 17(24): 3125-3135.
66. Thompson, T., 1999. In situ gene therapy for prostate cancer. Oncol Res., 11(1): 1-8.
67. Herman, J.R., H.L. Adler, E. Aguilar-Cordova, A. Rojas-Martinez, S. Woo, T.L. Timme, T.M. Wheeler, T.C. Thompson and P.T. Scardino, 1999. In situ gene therapy for adeno carcinoma of the prostate: a phase I clinical trial. Hum Gene Ther., 10(7): 1239-1249.
68. Singhal, S. and L. Kaiser, 1998. Cancer chemotherapy using suicide genes. Surg Oncol Clin North Am., 7(3): 505-536.
69. Djeha, A.H., T.A. Thomson, H. Leung, P.F. Searle, L.S. Young, D.J. Kerr, P.A. Harris, A. Mountain and C.J. Wrighton, 2001. Combined adenovirus-mediated nitro reductase gene delivery and CB1954 treatment: a well-tolerated therapy for established solid tumors. Mol Ther., 3(2): 233-240.
70. Chhikara, M., H. Huang, M.T. Vlachaki, X. Zhu, B. Teh, K.J. Chiu, S. Woo, B. Berner, E.O'. Smith, K.C. Oberg L.K. Aguilar, T.C. Thompson, E.B. Butler and E.A. Cordova, 2001. Enhanced therapeutic effect of HSV-tk+GCV gene therapy and ionizing radiation for prostate cancer. Mol Ther., 3(4): 536-542.
71. The, B.S., E. Aguilar-Cordova, K. Kernen, C.C. Chou, M. Shalev, M.T. Vlachaki, B. Miles, D. Kadmon, W.Y. Mai, J. Caillouet, M. Davis, G. Ayala, T. Wheeler, J. Brady, L.S. Carpenter, H.H. Lu, J.K. Chiu, S.Y. Woo, T. Thompson and E.B. Butler, 2003. PhaseI/II trial evaluating combined radiotherapy and in situ gene therapy with or without hormonal therapy in the treatment of prostate cancer a preliminary report. Int J. Radiat Oncol Biol. Phys., 51(3): 605-613.
72. Freytag, S.O., H. Stricker, J. Pegg, D. Paielli, D.G. Pradhan, J. Peabody, M. DePeralta- Venturina, X. Xia, S. Brown, M. Lu and J.H. Kim, 2003. Phase I study of replication-competent adenovirus-mediated double-suicide gene therapy in combination with conventional-dose three-dimensional conformal radiation therapy for the treatment of newly diagnosed, intermediate- to high-risk prostate cancer. Cancer Res., 63(21): 7497-7506.
73. Oyama, M., T. Ohigashi, M. Hoshi, M. Murai and K. Uyemura, 2000. Oncolytic viral therapy for human prostate cancer by conditionally replicating herpes simplex virus 1 vector G207. J. Cancer Res., 91(12): 1339-1344.
74. Rodriguez, R., E.R. Schuur, H.Y. Lim, G.A. Henderson, J.W. Simons and D.R. Henderson, 1997. Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate specific antigen-positive prostate cancer cells. Cancer Res., 57(13): 2559-2563.
75. Chen, Y., T. DeWeese, J. Dilley, Y. Zhang, Y. Li, N. Ramesh, J. Lee, R. Pennathur-Das, J. Radzyminski, J. Wypych, D. Brignetti, S. Scott, J. Stephens, D.B. Karpf, D.R. Henderson and D.C. Yu, 2001. A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. Cancer Res., 61(20): 7464- 7472.
76. Chen, Y., T. DeWeese, J. Dilley, Y. Zhang, Y. Li, N. Ramesh, J. Lee, R. Pennathur-Das, J. Radzyminski, J. Wypych, D. Brignetti, S. Scott, J. Stephens, D.B. Karpf, D.R. Henderson and D.C. Yu, 2001. CV706, a prostate cancer-specific adenovirus variant, in combination with radiotherapy produces synergistic antitumor efficacy without increasing toxicity. Cancer Res., 61(14): 5453-5460.
77. Mabjeesh, N., H. Zhong and J. Simons, 2002. Gene therapy of prostate cancer: current and future directions, endocrine-related cancer, 9: 115-139.
78. Etel, R. and A. Marcello, 2003. Molecular aspects of prostate cancer: implications for Future directions, International Braz J. Urol., 29(5): 401-411.