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Bioengineered viral vectors for targeting and killing prostate cancer cells

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Enabling the transduction of therapeutic gene expression exclusively in diseased sites is the key to developing more effective treatments for advanced prostate cancer using viral-based therapy. While prostate cancers that express high levels of HER-2 are resistant to the killing effects of trastuzumab, they can be targeted for selective gene expression and destruction by lentiviruses with envelope proteins engineered to bind to this therapeutic antibody. More importantly, after intravenous injection, this trastuzumab-bound lentivirus is able to target castration-resistant prostate tumor xenografts, albeit with low efficiency. This proof of principle opens up multiple possibilities for the prevention and treatment of prostate cancer using a viral-based therapy. However, to be safe and more effective, the viral vectors must target prostate cancer cells more selectively and efficiently. A higher degree of specificity and efficiency of cancer cell targeting can be achieved by engineering viral vectors to bind to a specific cell surface marker and by controlling the expression of the therapeutic payload at transcriptional level, with a tissue-specific promoter, and at the translational level, with a regulatory sequences inserted into either the 5'UTR or 3'UTR regions of the therapeutic gene(s). The latter would be designed to ensure that translation of this mRNA occurs exclusively in malignant cells. Furthermore, in order to obtain a potent anti-tumor effect, viral vectors would be engineered to express pro-apoptotic genes, intracellar antibodies/nucleotide aptamers to block critical proteins, or siRNAs to knockdown essential cellular mRNAs. Alternatively, controlled expression of an essential viral gene would restore replication competence to the virus and enable selective oncolysis of tumor cells. Successful delivery of such bioengineered viruses may provide a more effective way to treat advanced prostate cancer.

Key words: prostate cancer, lentivirus, targeted viral delivery, anti-cancer therapy, HER-2

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Introduction

Prostate cancer is the most frequently diagnosed non-skin cancer in adult males in North America and is the second leading cause of cancer-related mortality.[1] While standard treatments such as radical prostatectomy, radiation therapy and androgen ablation improve the overall survival rates in prostate cancer patients, the emergence of recurrent, metastatic forms of castration-resistant/androgen-independent prostate cancer continues to be a major challenge, with no curative treatments presently available.[2,3]

Viral-based cancer therapy has emerged as a very promising approach to treat different types of cancers, including prostate cancers.[4-6] However, with most viral based therapies currently in clinical trials, viruses were applied locally by intra-tumoral injection and their ability for targeting metastatic cancer cells has been poorly addressed.[7-10] The following sections describe strategies to bioengineer therapeutic viral vectors to meet clinical needs for safely targeting and potentially eradicating advanced prostate malignancies.

Achievement of Selectivity for Targeting Prostate Cancers

Selective viral targeting of tissue-specific cell surface antigens. We and others observed that HER-2 is overexpressed

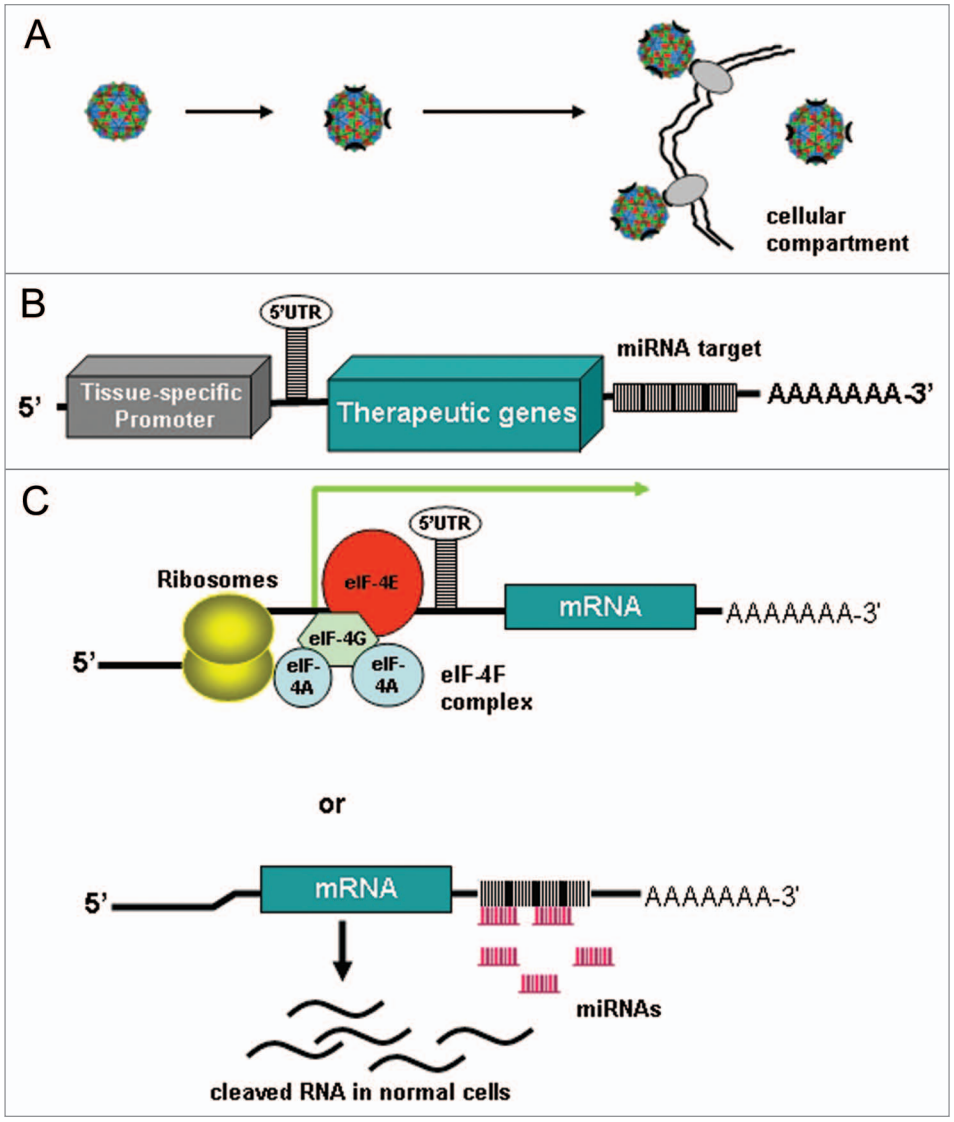

Figure 1. Strategies to achieve selective targeting of prostate cancers. (A) A tissue-specific cell surface antigen can be used to mediate selective viral targeting. (B) Tissue-specific expression of genes can be achieved by controlling gene expression at the transcriptional level with a tissue-specific promoter. (C) Cancer-specific expression of genes can be achieved by controlling gene expression at the translational level.

in the majority of prostate cancers.[11,12] Overexpression of HER-2, which causes activation of the PI3k/AKT pathways and promotion of cell proliferation, has been proposed to be a survival factor for prostate cancer cells in the absence of androgens.[11,13] While trastuzumab (Herceptin, Genentech, CA), a humanized monoclonal antibody, which neutralizes the HER-2 receptor, has been shown to be very effective in treating breast cancers, treatment of patients with prostate cancer showed poor efficacy.[14] A possible alternative therapeutic application of HER-2 overexpression in advanced prostate cancers could be to target viral gene therapies. As a proof of principle, we used a lentiviral vector which was pseudotyped with a modified Sindbis virus envelope protein such that it could bind to the Fc region of an IgG molecule (**Fig. 1A**). The Fab regions of the antibody determine the targeting specificity of the viral vector for any cell surface antigen.[15] Indeed, lentiviruses engineered to bind to trastuzumab selectively infected human prostate cancer cells such as LNCaP or C4-2 cells, which overexpress HER-2 on their surface. Tumor-specific targeting by trastuzumab-coated lentivirus was best demonstrated when these viruses, carrying a firefly luciferase expressing cassette, were injected intravenously (i.v.). In contrast to a non-specific diffused luminescent signal seen with the VSV-G packaged lentivirus, which has a broad cellular tropism, the trastuzumab-bound lenti virus gave rise to a tumor-enriched luminescence, indicating selective infection and expression of luciferase in prostate cancer cells.[12] Considering the significant dilution effect as a consequence of i.v. injections, it was encouraging that at least a small fraction of injected virus was able to infect prostate cancer tumors through this antibody-mediated targeting. By recognizing cell-specific surface antigens, viral vectors can infect and transduce expression of genes selectively. To ensure prostate-

specific targeting, other antibodies, such as those recognizing prostate membrane-specific antigen (PMSA)[16] and prostate stem cell antigen (PSCA),[17] can also be included in the targeted viral therapies.

Tissue-specific expression of genes can be achieved by controlling gene expression at the transcriptional level with a tissue-specific promoter. We demonstrated that, through recognizing HER-2, trastuzumab-bound lentivirus can selectively infect prostate cancer cells in vitro. However, after i.v. injection, we observed that some lentiviral mediated luminescent signals appeared outside the tumor sites, although they were predominantly enriched in the tumors.[12] Given that safety is a primary concern of any viral based gene therapy, the ability to more stringently limit viral directed gene expression to tumor cells is a very important consideration. The probasin-derived ARR_2PB promoter has been shown to be capable of directing tightly controlled prostate-specific gene expression.[18] It was only after this promoter was placed in front of the firefly luciferase gene that the luminescent signal was entirely restricted to the prostate tumors. By using a prostate-specific promoter to control gene expression at the transcriptional level, viral-directed gene expression can be restricted to prostate cancer cells (**Fig. 1B**). This approach can thus be used in the design of viral vectors to further address virus safety issues.

Cancer-selective expression of genes can be achieved by controlling gene expression at the translational level. While viral-directed gene expression can largely be restricted in a tissue-specific manner by transcriptional control with a tissue-specific promoter, basal expression of a therapeutic gene in normal or non-targeted tissues can result in toxic effects. Consequently, it may limit the use of such viral vectors for therapeutic purposes. It has been demonstrated that when a complex 5'UTR fragment of FGF-2 is placed in front of a reporter or therapeutic genes, it could efficiently restrict the expression of these genes to cancer cells.[19,20] This is because efficient translation of mRNA with a bulky 5'UTR is largely dependent on the cellular level of elF-4E, which is highly expressed in prostate cancer cells.[19] ElF-4F forms a complex with elF-4G and elF-4A and functions as a helicase to unwind the bulky 5'UTR and consequently facilitate translation of downstream mRNAs[21,22] (**Fig. 1C**). In addition, it has also been shown that sequences complementary to specific microRNAs (miRNAs) inserted at the 3'UTR of some essential viral genes (**Fig. 1B**) can result in selective amplification of viruses in cancer cells, if these miRNAs are absent or low in cancer cells.[4,23,24] These reports indicate that control of gene expression at the mRNA translational level can provide further stringency to limit viral-directed gene expression to cancer cells.

Selection of Anti-Cancer Therapeutic Genes for Viral Expression

Gene-directed enzyme pro-drug systems. Gene-directed enzyme pro-drug systems such as herpes thymidine kinase (HTK), in combination with the pro-drug ganciclovir (GCV), are perhaps the most commonly used anti-cancer gene therapy system both in experimental models and clinical trials.[4,25] HTK, whose substrate specificity is distinct from cellular thymidine kinases, can convert GCV to a toxic phosphorylated form allowing killing of cells that express HTK as well as regional cell killing through a bystander effect.[26] Indeed, we found that when trastuzumab was bound to lentiviruses carrying an HTK expression cassette, C4-2 and LNCaP prostate cancer cells could be selectively infected by this virus and shown to express HTK. Addition of GCV consequently resulted in substantial cell killing as compared to cells infected with trastuzumab-bound control lentivirus carrying a GFP expression cassette.[12] Besides HTK in combination with pro-drug GCV, there are other successful gene-directed enzyme pro-drug systems, such as cytosine deaminase (CD) from bacteria or yeast with 5-fluorocytodine (5-FC) and bacterial nitroreductase (NfsB) with 5-(azaridin-1-yl)-2,4-dinitrobenzamide (CB1954).[25]

Viral mediated expression of pro-apoptotic genes. One of the important hallmarks of cancer cells is their ability to evade apoptotic programmed cell death.[27] Induction of apoptosis in malignant cells is a major goal of cancer therapy in general. Induced or exogenous expression of numerous apoptosis-regulating genes has been evaluated for this purpose and has been shown to elicit profound anti-tumor effects.[28-30] Exogenous expression of pro-apoptotic genes using viral vectors may result in more efficient cell killing, particularly when used in combination with cytotoxic drugs.[31,32]

Viral expression of siRNAs, intra cellular antibodies, peptides and nucleotide aptamers for specific gene knockdowns. RNA interference (RNAi) has emerged as a very promising approach to specifically knockdown critical growth promoting proteins and thereby treat cancer.[33] For example, it has been shown that knocking down the androgen receptor with an RNAi strategy suppresses the growth of androgen-dependent and castration-resistant prostate tumors in animal models.[34,35] However, currently a severe limitation on using siRNAs therapeutically is the problem of in vivo delivery. We anticipate that viral-mediated expression of siRNAs to target specific mRNAs, which encode proteins essential for cell growth or survival in prostate cancer cells, may solve this problem and eventually provide a means to efficiently deliver siRNAs to tumors in vivo.

Therapeutic antibodies are most often designed to recognize and neutralize growth factor receptors on the cell surface. Modern technology and novel knowledge gained in the current post-genomic era have allowed the discovery of many novel molecular targets involved in cancer progression possible. However, many anti-cancer target molecules are intracellular, which make them difficult for antibodies to access. Viruses may be one of the best tools to direct specific interaction of antibodies with critical antigens in the intracellular compartments of living cells. The most commonly used format of such intracellular antibodies, "intrabodies," are single chain antibodies (scFv). It has been shown that intrabody targeting of hTERT attenuates the immortality of cancer cells.[36] An alternative approach is to have a virus express a small peptide or aptamer that binds to an intracellular protein with high affinity to inhibit the growth or survival of cancer cells.[37]

A further refinement of this strategy is to use a virus that expresses a nucleotide aptamer, a fragment of nucleotide that can specifically bind to and inhibit the function of proteins.[38]

Future Prospective for Developing Effective Viral Therapies for Prostate Cancer

Lentivirus, a retrovirus derived from HIV-1 virus,[39,40] has been widely used to deliver genes in basic and applied research.[39] It has been used for genome-wide functional studies of gene expression, to re-program stem cells, to create transgenic animals, and for clinical applications.[39,40] Several studies have demonstrated that there is no enrichment for lentiviral integration sites in close proximity to proto-oncogenes or within tumor suppressor genes.[41-43] Therefore, overall lentiviral vectors can be considered relatively safe for use in the clinical trial protocols. The VSV-G pseudotyped lentivirus has a broad spectrum of cell tropisms and can efficiently infect both mitotic and post-mitotic cells.

Lentiviruses with antibodies recognizing a prostate cell-specific surface antigen bound to their envelope can specifically infect prostate cells (**Fig. 1A**). If a prostate-specific promoter is used to drive expression of a factor with anti-cancer properties, expression of this factor will be restricted to prostate cells (**Fig. 1B**). When a complex 5'UTR, such as that cloned from rat FGF-2, is inserted in front of an mRNA, transcript expression can only occur under conditions in which sufficient elF-4E is available, such as is the case in the majority of cancer cells (**Fig. 1C**). Preferential expression in cancer cells can be further enhanced by inclusion of target sequences in the 3'UTR for specific miRNAs, which are highly expressed in normal tissues but which have low or no expression in cancer cells (**Fig. 1B**). Hence by combining a viral envelope with a tissue-specific antibody attached, a tissue-specific promoter, a complex 5'UTR requiring abundant elF-4E, and a 3'UTR with specific miRNA target sequences, one can create a safe and highly specific virus for targeting cancer cells for expression of a therapeutic gene.

Lentivirus has been engineered to be non-replicative for bio-safety reasons. Though the virus can be targeted to the tumor sites after systematic administration, its ability to transduce only a small number of cells is a significant hurdle to its use as therapeutic tool. To overcome this limitation, a conditional replication competent virus should be considered. Some naturally mutated or bioengineered recombinant viruses are replication-competent within some cancer cells because they are capable of exploiting molecular defects of cancer cells to avoid replication suppression.[4,5,44] Replication of such viruses can eventually cause oncolysis and death of cancer cells with a subsequent amplification of virus titre.[4,45,46] In a bladder cancer mouse-human xenograft model, we demonstrated that intra vesical instillation of the replication competent Vesicular Stomatitis virus (VSV) can eradicate established bladder tumors.[47-49] In vitro, we observed that a single plaque forming unit of VSV can kill at least 3,250 prostate cancer cells within three days (unpublished observation). If some of the tissue targeting principles described above for lentiviral vectors can be incorporated into an oncolytic virus such as VSV, then one can envision a very effective virus therapy to treat prostate and other cancers. However, while clinical trials with oncolytic viruses show some very promising results, to demonstrate efficacy, their application in cancer treatment requires that they be combined with traditional chemotherapy or radiotherapy.[4,5,44]

Conclusion

Bioengineered viral vectors have considerable potential as valuable therapeutic tools to safely treat cancer. By restricting their ability to only infect prostate cancer cells through the use of tissue-specific promoters, antibodies that target specific cell surface antigens, and cancer-specific translational controls, we can safely deliver their therapeutic payload. However, to be truly effective in controlling advanced metastatic cancers, where delivery of a sufficient therapeutic gene payload may be the limiting factor, it may be necessary to build these tissue selection components into an oncolytic, replication competent virus.

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