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Effective Transgene Constructs to Enhance Gene Therapy with Trichostatin A

Hideki Hayashi et al.*
Nagasaki University
Japan

1. Introduction

Gene therapy has been proposed as a strategy for the treatment of intractable human diseases since the early 1990s. For the expression of a specific transgene in desired cells or tissues with the proper timing, many vectors carrying transgenes have been developed (Mátrai et al., 2010; Nayak & Herzog, 2010; Sliva & Schnierle, 2010). Retroviral, lentiviral, adenoviral, and adeno-associated viral vectors are used in various ways to achieve these goals. However, the introduced transgenes frequently become silenced in the host cells (Harbers et al., 1981; Jähner et al., 1982; Palmer et al., 1991). We searched for bioactive substances from Actinomycetes that enhance transgene expression, and found that trichostatin A (TSA), a histone deacetylase inhibitor (HDACi), enhanced several promoter activities remarkably (Y. Ma et al., 2009).

HDACis have been used as anti-cancer drugs, because they have various effects on tumor cells to arrest cell growth, induce apoptosis, inhibit metastasis, and enhance anti-tumor immunity, by regulating the expression of the relevant genes (Bolden et al., 2006; Haberland et al. 2009; X. Ma et al., 2009; Mai et al., 2005). Here, we developed effective TSA-inducible killer constructs to enhance the anti-cancer effects of TSA, by identifying the TSA-responsive element of the herpes simplex virus thymidine kinase (hsvTK) promoter, and TSA-dependently activating some cell-death-inducing genes. We determined the most relevant regions responsive to TSA, and constructed chimeric promoters with higher fold-increases and greater induced strengths with TSA, by replacing the weak TSA-responsible region (TSA2) of the CMV promoter with two or three copies of the TSA-responsible sites (TSA1) of the hsvTK promoter. In addition, the synthetic intron sequence (0.2kb) from the pRL-TK vector and the long 3'-untranslated region (1.0kb) from the pSV2-neo vector, including the SV40 late polyA site, were important for the basal expression of the transgene and the TSA-induction, respectively.

To create the TSA-inducible killer constructs, we placed the hsvTK gene for combination therapy with the prodrug Ganciclovir, and some strong death-inducing molecules (Bax, caspase8, and TRIF) under the control of the TSA-responsible chimeric promoters. They effectively killed the cells in which they were introduced, in a TSA-dependent manner. To evaluate the utility of the killer constructs for cancer gene therapy, the TSA-dependent death-inducing constructs were transferred to retroviral and adenoviral vectors.

*Yuhua Ma¹, Tomoko Kohno¹, Masayuki Igarashi², Kiyoshi Yasui¹, Koon Jiew Chua¹, Yoshinao Kubo¹, Motoki Ishibashi³, Ryuji Urae³, Shin Irie³ and Toshifumi Matsuyama¹.

¹ Nagasaki University, Nagasaki, Japan,

² Microbial Chemistry Research Center, Tokyo, Japan

³ MEDICAL CO. LTA, Fukuoka, Japan

2. TSA-induced transgene activation

2.1 Screening of actinomycetes products to enhance the transgene

Actinomycetes are the prime candidates in screens for new natural products as antitumor and antimicrobial bioactive agents, and the achievements have been well reviewed (Jensen et al., 2005; Park et al., 2008; Zheng et al., 2000). We screened the Actinomycetes products for substances that enhance the transgene promoter, using the luciferase reporter, pRL-TK vector (Promega). In the vector, the 0.75kb promoter region of hsvTK was placed upstream of the reporter Renilla luciferase gene. We found that a methanol extract of the MK616-mF5 strain increased the luciferase activity significantly, by about 41-fold, as compared to the control among 2,448 Actinomycetes products. The methanol extract from the Actinomycetes MK616-mF5 strain was purified by chromatography on a silica column and a C18 reverse-phase HPLC column, by monitoring the effects on the hsvTK-driven luciferase activities. Based on NMR and mass spectrometry analyses, we concluded that the natural product is TSA. TSA is a histone deacetylase inhibitor (HDACi), and some HDACis have recently been used in clinical trials for cancer treatment (Hoshino & Matsubara, 2010; X. Ma et al, 2009; Wagner et al., 2010). HDACi is thought to work by changing the acetylation status of core histones and thus modulating the gene expression patterns, including the genes associated with cell cycle arrest, apoptosis, and differentiation. Modifications of the N-terminal tails of core histones (the core histone is an octamer, consisting of an H3-H4 tetramer and two H2A-H2B dimers) play a crucial role in chromatin packing and gene expression. The acetylation of the core histone is illustrated in Fig. 1, although other modifications, including methylation, phosphorylation, and ubiquitination, also affect the chromatin remodeling (Gasper-Maia, et al., 2011; Mai et al., 2005). Generally, the positively-charged lysine residues in the N-terminal core histone tails, which protrude from the surface of the nucleosome, are tightly bound to the phosphate backbone of the DNA, thus maintaining the chromatin in a transcriptionally silent state (closed chromatin). The acetylation status of the core histone is controlled by the competitive activities of histone acetyl transferase (HAT) and histone deacetylase (HDAC). Acetylation neutralizes the positive charges on the core histone, and converts the chromatin to a more open state, enhancing the access of the transcriptional machinery, including RNA polymerase II, to the promoter region for gene expression. HDACis have been used as anti-cancer drugs, because they activate the expression of the TRAIL, Fas, Bid, and p53 genes to induce growth arrest and/or apoptosis of cancer cells (Bandyopadhyay et al., 2004; Nakata et al., 2004; Ogawa et al., 2004; Ruefli et al., 2001; Sonnemann et al., 2007) by inhibiting HDAC activities, and they also have anti-angiogenic and anti-metastatic effects (Bolden et al., 2006). In addition, HDACi can enhance anti-tumor immunity, either by directly affecting malignant cells to make them more attractive immune targets, or by altering immune cell activity and/or cytokine production. HDACi reportedly augments the immunogenicity of tumor cells by upregulating the expression of the major histocompatibility complex (MHC) class I and II proteins and co-stimulatory/adhesion molecules, such as CD40, CD80, CD86 and intercellular adhesion molecule-1 (ICAM-1) ((Maeda et al., 2000; Magner et al., 2000).

On the other hand, HDACis have been used to reactivate virally transduced genes and to amplify the expression of transgenes encoded by recombinant adenoviral or retroviral vectors (Chen et al., 1997; Dion et al., 1997; Khalighinejad et al., 2008; Kikuchi et al., 2007). However, the induction levels of the silenced transgenes were not sufficient to achieve satisfactory results.

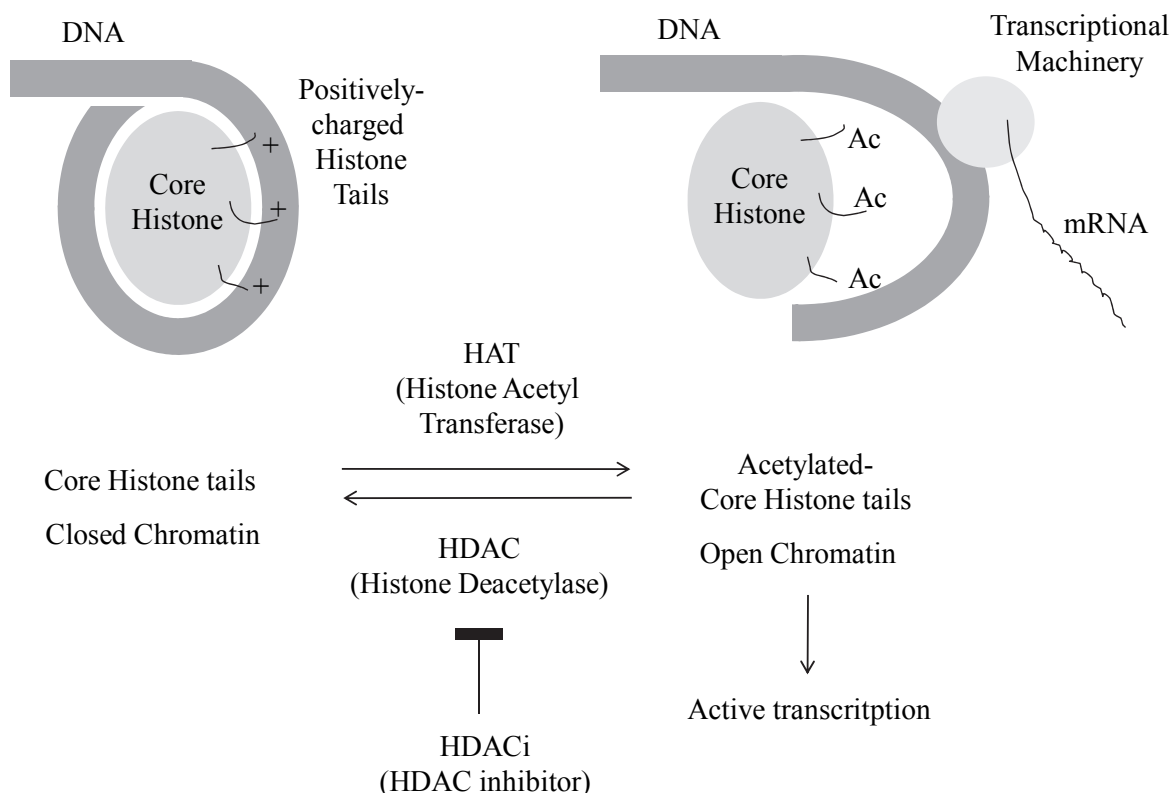


Fig. 1. Effects of HDAC inhibitors on chromatin structure and gene expression. In general, the acetylation of the positively-charged lysine residues within the N-terminal core histone tails, by histone acetyl transferase (HAT), converts the chromatin to a more open form for the access of the transcriptional machinery, including RNA polymerase II, to activate gene expression. In turn, when the acetylation is removed by histone deacetylase (HDAC), the chromatin returns to a closed form (transcriptionally silent). By inhibiting HDAC activities, the HDACi can alter the chromatin structure to an open form, to activate gene expression. —+: positively-charged lysine residues at N-terminal core histone, —Ac: acetylated lysine residues.

To develop effective vectors exhibiting high sensitivities to TSA and sustained strong activities in the presence of TSA, we examined the responsiveness of various promoters to TSA. HeLa cells were treated with 1.0 $\mu\text{g}/\text{ml}$ of TSA for 24 hours, after transfections with various Renilla luciferase constructs. The TSA treatment induced remarkable transgene expression with all of the tested promoters, as shown in Fig. 2. The hsvTK promoter exhibited the highest fold-increase, and the RSV and SV40 promoters also showed relatively high fold-increases. The responses of the CMV and β -actin promoters to TSA were poor. In contrast, the CMV promoter showed the greatest strength, but the hsvTK promoter activity was weak, even in the presence of TSA.

2.2 Analyses of the TSA-responsive elements in the hsvTK and CMV promoters

To assess which region of the hsvTK promoter is responsible for the TSA stimulation, we created a series of deletion mutants within the 0.7kb promoter region of the hsvTK gene.

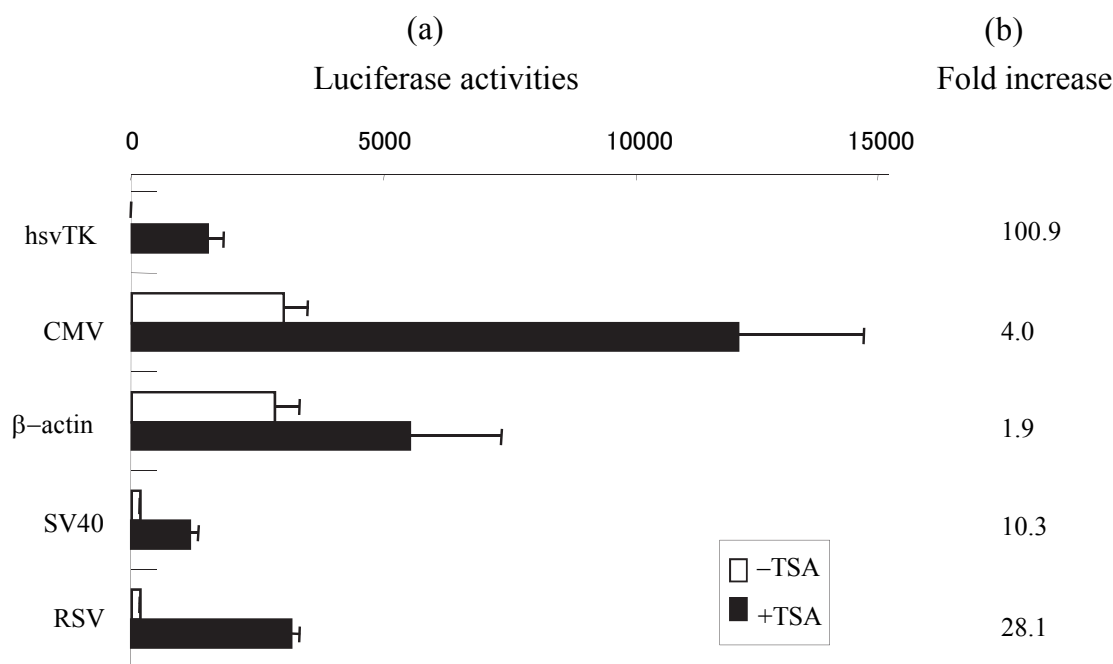


Fig. 2. Treatment with purified TSA leads to the induction of cellular and viral promoters. HeLa cells in a 96-well plate were transfected with 40ng of various constructs in FuGene 6 transfection mixtures. The transfected HeLa cells were treated with/without 1.0 μg/ml of TSA for 24hr, and the reporter assay was performed using a Renilla luciferase assay kit (Promega), according to the manufacturer’s instructions. (a) Luciferase activities. (b) Fold increase: the ratio of the luciferase activities in HeLa cells with or without TSA treatment. Promoters: hsvTK, herpes simplex virus thymidine kinase promoter; CMV, cytomegalovirus promoter; SV40, Simian Virus 40 early promoter; and RSV, Rous sarcoma virus LTR promoter. Values are means ± SD. This figure was reproduced from *Int. J. Integ. Biol.*, 5: 108-115, 2009.

They were transfected into HeLa cells, and the Renilla luciferase activities were measured after a 24 hr treatment with 1.0 μg/ml of TSA. The responses to TSA were reduced by the deletion of the region between -231 and -111. Therefore, this region is important for the activation of the HSV-tk promoter by TSA, and we named this region TSA1. The TSA1 region contained an octamer motif (ATTTGCAT), an NF-Y binding site (CCAAT), and a GC box. These elements are also reportedly important for the TSA-activation of other promoters (Hirose et al., 2003), consistent with our results. The TSA treatment also increased the gene expression by the CMV promoter, although the fold-increase was low. The reporter construct deletion mutant -77 almost lost its basal and TSA-induced activities. The region -281 ~ -77 is important for weak activation by TSA, and thus we named it TSA2. The weak TSA-stimulation may be related to the degenerate octamer motif (ATTTGCgT), the partial NF-Y binding site (CCAAg), and the GC box in the TSA2 region.

2.3 Development of chimeric promoters that significantly enhance transgene expression

A strong, drug-inducible promoter is urgently needed for cancer treatments, such as suicide gene therapy. The TSA1 site within the hsvTK promoter was important for the highest fold-increase. On the other hand, the CMV promoter showed the greatest induced strength, and the TSA2 site was relevant to the weak responsiveness to TSA. We succeeded in constructing chimeric promoters with a higher fold-increase and greater induced strength to TSA, by replacing the weak TSA2 site of the CMV promoter with two or three copies of the TSA1 sites of the hsvTK promoter. A schematic representation of the chimeric promoters is shown in Fig. 3a. The reporter assay revealed that the chimeric promoter HC1, containing two copies of TSA1, and the chimeric promoter HC3, containing three copies of TSA1, amplified transgene expression in response to TSA by 220.7-fold and 94.2-fold, respectively (Fig. 3b,c). The two chimeric promoters retained induced strength comparable to that of the CMV promoter.

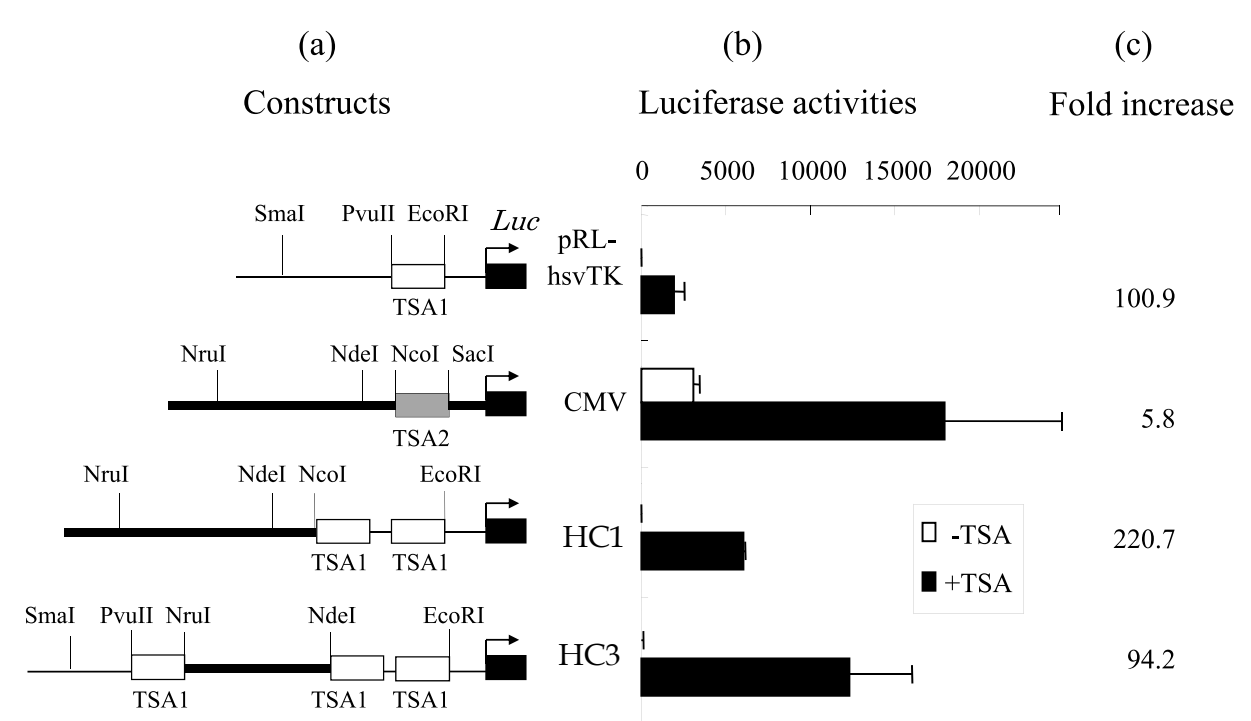


Fig. 3. The chimeric promoter of hsvTK and CMV enhanced transgene expression. (a) Schematic representations of the chimeric promoters. The open horizontal box represents the regulatory region in the hsvTK promoter (shown as a thin line) responsive to TSA (TSA1); the stippled horizontal box represents the regulatory region in the CMV promoter (shown as a thick line) responsive to TSA (TSA2); and the HC1 and HC3 chimeric promoters containing TSA1 and fragments of the CMV promoter are shown as a thick line. The restriction enzyme sites used to make the mutants are indicated. (b) Reporter assay of chimeric promoters. HeLa cells in a 96-well plate were transfected with 40ng of the various constructs in FuGene 6 transfection mixtures. The transfected HeLa cells were treated with/without 1.0 µg/ml of TSA for 24hr, and the Renilla luciferase activities were measured. Experiments were performed in triplicate. Values are means ± SD. (c) Fold increase: the ratio of luciferase activities in HeLa cells with or without TSA treatment. This figure was reproduced from *Int. J. Integ. Biol.*, 5: 108-115, 2009.

As shown in Fig. 4, we also found that a synthetic intron (0.2kb) from the pRL-TK vector and the long 3'-untranslated region (1.0kb) from the pSV2-neo vector are important for the basal expression of the transgene and the TSA-induction, respectively. The long 3'-untranslated region lowered the basal transgene activities in the HC3-L construct, and enhanced the TSA-stimulated transgene activities in all of the constructs (CMV-L, HC1-L, and HC3-L).

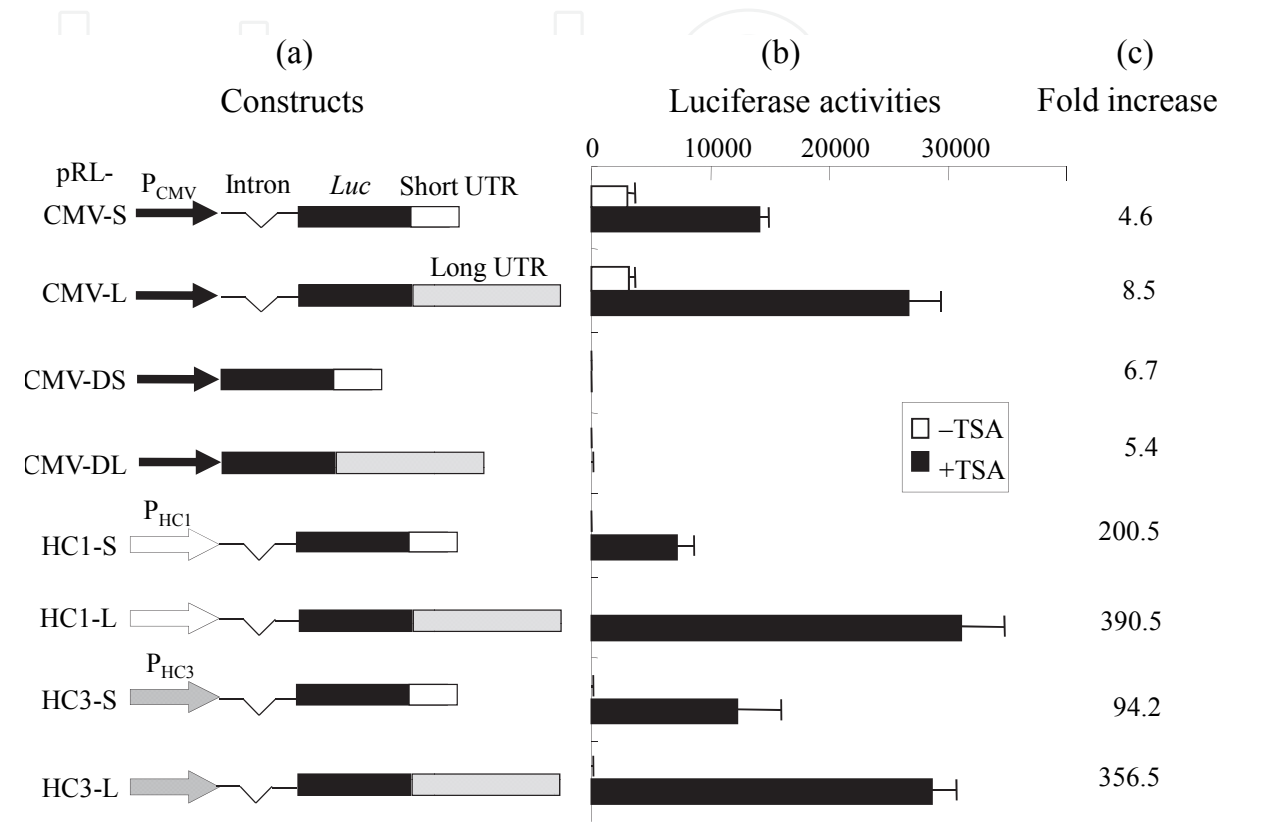


Fig. 4. Development of a modified CMV construct with intron and polyA sequences. (a) Transgene constructs modified with a synthetic intron and short and long 3'-untranslated regions. The closed solid horizontal box (■) represents Renilla luciferase. The open horizontal box (□) and the striped horizontal box (▨) represent the short and long 3'-untranslated regions (UTR), respectively. The broken line (---) represents the synthetic intron. The solid arrow (→), open arrow (⇨), and striped arrow (⇨) represent the CMV, HC1, and HC3 chimeric promoters, respectively. (b) Reporter assay of the constructs. HeLa cells in a 96-well plate were transfected with 40ng of the various constructs in FuGene 6 transfection mixtures. The transfected HeLa cells were treated with/without 1.0 µg/ml of TSA for 24 hr, and the Renilla luciferase activities were measured. Experiments were performed in triplicate. Values are means ± SD. (c) Fold increase: the ratio of luciferase activities in HeLa cells with or without TSA treatment. This figure was reproduced from *Int. J. Integ. Biol.*, 5: 108-115, 2009.

2.4 TSA-dependent induction of the stably integrated transgene under the chimeric promoters to kill target cells by different mechanisms.

Considering the various escape mechanisms employed by cancer cells, we introduced the molecules that can activate different cell death-inducing mechanisms. We placed the hsvTK

gene for combination therapy with the prodrug Ganciclovir, and some strong death-inducing molecules (Bax, caspase8, and TRIF) under the TSA-responsible chimeric promoters. Suicide gene therapy has been proposed as a strategy for the treatment of cancer for many years (Culver et al., 1992; Fillat et al., 2003; Mooleten et al., 1990). One of the most widely used prodrug-activating gene therapies is the thymidine kinase gene of the herpes simplex virus (hsvTK), in combination with the non-toxic prodrug Ganciclovir (GCV) (Fig. 5). The principal effect of the hsvTK/GCV system is to generate GCV triphosphate (GCV-PPP) from the non-toxic prodrug GCV, by the introduced hsvTK and several cellular kinases, including guanylate kinase. The produced GCV-PPP inhibits the mammalian DNA polymerase due to its analogy with deoxyguanosine triphosphate, and leads to cell death. The hsvTK is required for the formation of GCV-PPP; however, the GCV treatment eradicated the majority of the population, even though only 10% of the cell population was expressing hsvTK (Freeman et al., 1993). The killing of hsvTK-negative cells by adjacent hsvTK positive cells is called “bystander killing effects”, which depend mainly upon gap

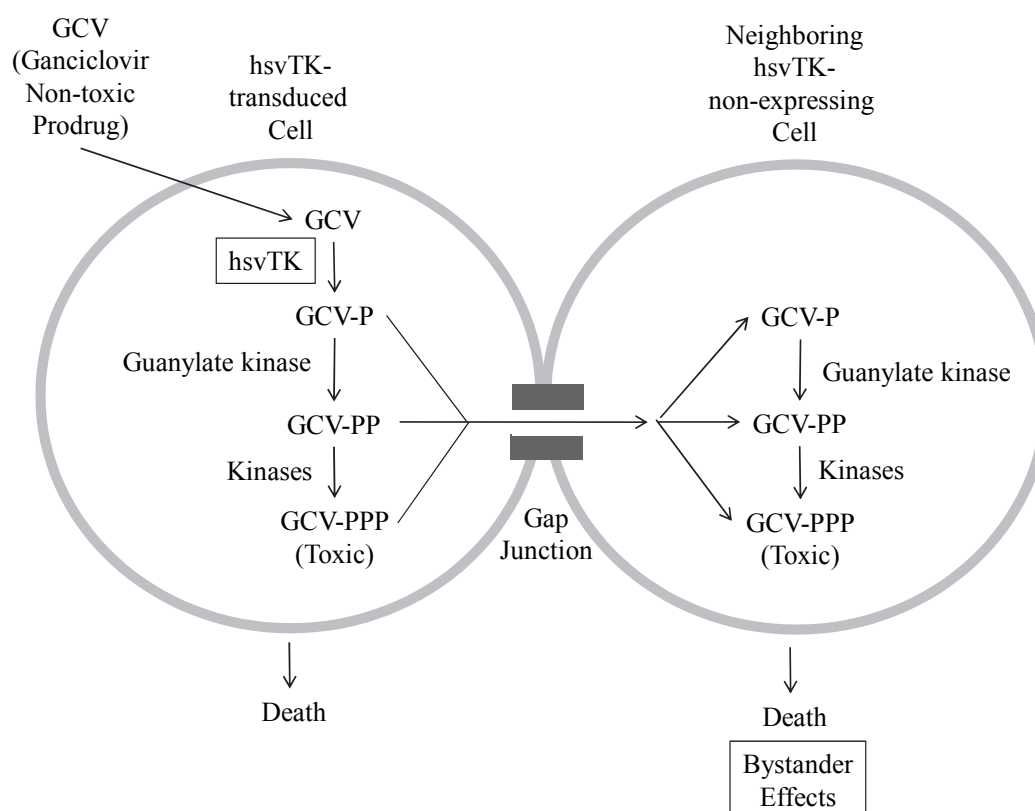


Fig. 5. Principle of suicide gene therapy with bystander effects. The non-toxic prodrug Ganciclovir (GCV) is assimilated into the target hsvTK-transduced cell, and is converted into the toxic triphosphorylated GCV (GCV-PPP) by hsvTK and several cellular kinases. GCV-PPP inhibits the mammalian DNA polymerase due to its analogy with deoxyguanosine triphosphate, and leads to cell death. In addition, GCV-PPP is transferred to the neighboring hsvTK-non-expressing cell via the gap junction, and the neighboring cell is also killed by the toxic GCV-PPP (Bystander killing effects).

junction formation, and has been established as a potentially powerful tool in cancer gene therapy (Fick et al., 1995; Denning & Pitts, 1997; Mesnil et al., 1996). To enhance the killing effects on cancer cells, the hsvTK suicide gene therapy is employed with other anti-cancer treatments, such as chemotherapy, radiotherapy, and immunotherapy. Especially, the combination therapy of hsvTK/GCV and HDAC inhibitors (HDACi) including TSA is promising for cancer therapy (Ammerpohl et al., 2004; Yamamoto et al., 2003), because HDACis have anti-cancer effects by inducing tumor cells to undergo differentiation, growth arrest and/or apoptosis, and by enhancing anti-tumor immunity, by altering gene expression through chromatin structure remodeling.

Other molecules (Bax, Caspase8, and TRIF) that induce apoptosis by activating different pathways should be beneficial for the treatment of various tumors, because each cancer employs various strategies to escape from the host immune surveillance and to gain growth advantages (Liu et al., 2011; Luo et al., 2009; Pavet et al., 2011). As shown in Fig. 6, two core pathways for inducing apoptosis exist: the extrinsic and intrinsic pathways. In the intrinsic pathway, the mitochondrion plays a central role. The mitochondrial membrane integrity is controlled by pro-apoptotic molecules (Bax, Bak, Bid, etc.) and anti-apoptotic molecules (Bcl-2, Bcl-x_L, etc.). The disruption of the membrane integrity by various stimuli, including DNA-damaging agents, increases the permeability and allows the release of cytochrome *c* and second mitochondria-derived activator of caspase (Smac)/direct IAP binding protein with low pH (DIABLO). The cytochrome *c* forms an apoptosome with apoptotic protease-activating factor-1 (Apaf-1) and caspase9 in the cytosol, and the Smac/DIABLO antagonizes the inhibitor of apoptosis proteins (IAPs) to enhance the activation of caspase9 and caspase3. As a result, the executioner caspase3 is effectively activated, to induce cell death. Actually, Bax overexpression has been reported to cause cell death in various cell types (Kitanaka et al., 1997; Tamm et al., 1998; Xiang et al., 1996). Caspase8 is the key enzyme in the extrinsic pathway. When the death receptors (Fas, DR3, DR4, etc.) are activated by their ligands, they recruit caspase8 via an adaptor molecule, Fas-associated death domain (FADD), and form the death-inducing signaling complex (DISC) to activate caspase8. The activated caspase8 not only activates caspase3 directly, but also activates the intrinsic pathway by removing the N-terminal region of Bid, thus producing its active, truncated form (tBid). Both signals work together to induce cell death by activating the executioner caspase3. TIR domain-containing adaptor protein (TRIF) is a key molecule that mediates the signals from pathogens via Toll-like receptor3 (TLR3) and TLR4, to trigger type I interferon production and inflammatory responses via interferon regulatory factor3 (IRF3) and Nuclear factor κ B (NF κ B), respectively (Kawai & Akira, 2010). Among these molecules related to the innate immune system, TRIF is a strong death-inducing molecule that activates caspase8 via receptor interacting proteins (RIPs) and FADD (Kaiser & Offermann et al., 2005).

To evaluate the utility of these TSA-responsive killer constructs for cancer gene therapy, we introduced the TSA-inducible hsvTK gene constructs to a well-characterized retroviral vector. Retroviral vectors are useful tools for the long-term expression of stably integrated transgenes in target cells, although there are risks due to insertional mutagenesis and the generation of replication competent retroviruses. We used a retroviral vector, pQC (Clontech) that self-inactivates the 5' LTR to avoid promoter interference, and to reduce the risk of transactivation of genome sequences around the integration site. The hsvTK, Bax, Caspase8, and TRIF genes were placed downstream of the chimeric promoters (P_{HCL} and P_{HC3}), with the P_{PGK}-driven puromycin selection marker (Fig. 7).

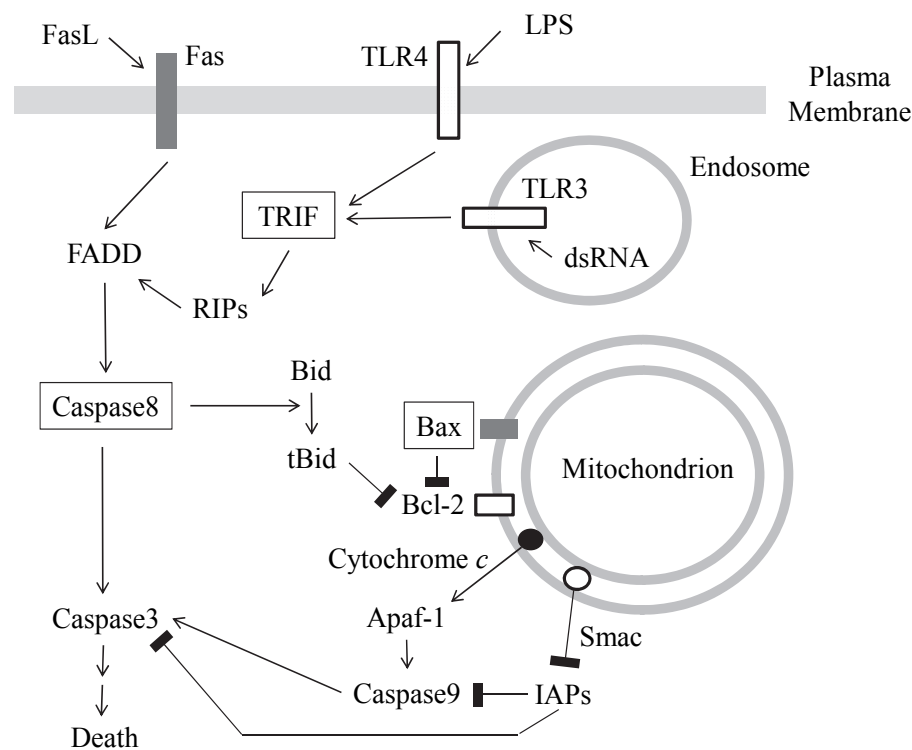


Fig. 6. Apoptosis pathways activated by Bax, Caspase8, and TRIF. Bax activates the intrinsic pathway of apoptosis by antagonizing the anti-apoptotic molecules (Bcl-2, Bcl-x_L, etc.) and increasing the permeability of the mitochondrion. The cytochrome *c* released from the intermembrane space of the mitochondrion forms an apoptosome with Apaf-1 and caspase9 in the cytosol, and leads to cell death by activating the executioner caspase3. Caspase8 is the key enzyme in the extrinsic pathway, where death receptors (Fas, DR4, DR5, etc.) activated by their ligands form the death-inducing signaling complex (DISC) with FADD and caspase8. The activated caspase8 leads to cell death by activating the executioner caspase3. TRIF is not only a key molecule that mediates the signal from pathogens via TLR3 and TLR4 to trigger type I interferon production and an inflammatory response, but also a strong death-inducing molecule by activating caspse8 via RIPs and FADD. Apaf-1, apoptotic protease-activating factor-1; DR4, Death receptor 4; FADD, Fas-associated death domain; TRIF, TIR domain-containing adaptor protein; TLR3, Toll-like receptor 3; RIP, receptor interacting proteins. —▶ stimulatory signal. —| inhibitory signal.

To verify the activities of the constructs, we examined whether the cells in which these constructs were transiently introduced are killed by TSA treatment. The 293T cells with the P_{HC1}- or P_{HC3}-hsvTK gene showed TSA- and GCV-dependent cell death. The cells with the P_{HC1}- or P_{HC3}-driven Bax, Caspase8, or TRIF genes were also killed with 0.1~0.3 µg/ml TSA treatment. Among them, TRIF triggered the most effective cell death. To examine the effects of TSA on the stably-introduced transgene expression, we obtained stable clones to express the death-inducing molecules under the control of the HC1 and HC3 promoters, by selection with puromycin for several weeks after the retroviral infection. The 293T cells containing the P_{HC1}-TRIF gene showed drastic cell death (apoptosis) upon a treatment with 0.1µg/ml TSA for 24h, as compared to the control 293T cells containing P_{CMV} only (Fig. 8). The chimeric promoter P_{HC1} maintained the TSA-responsiveness after becoming stably-integrated into the genome DNA.

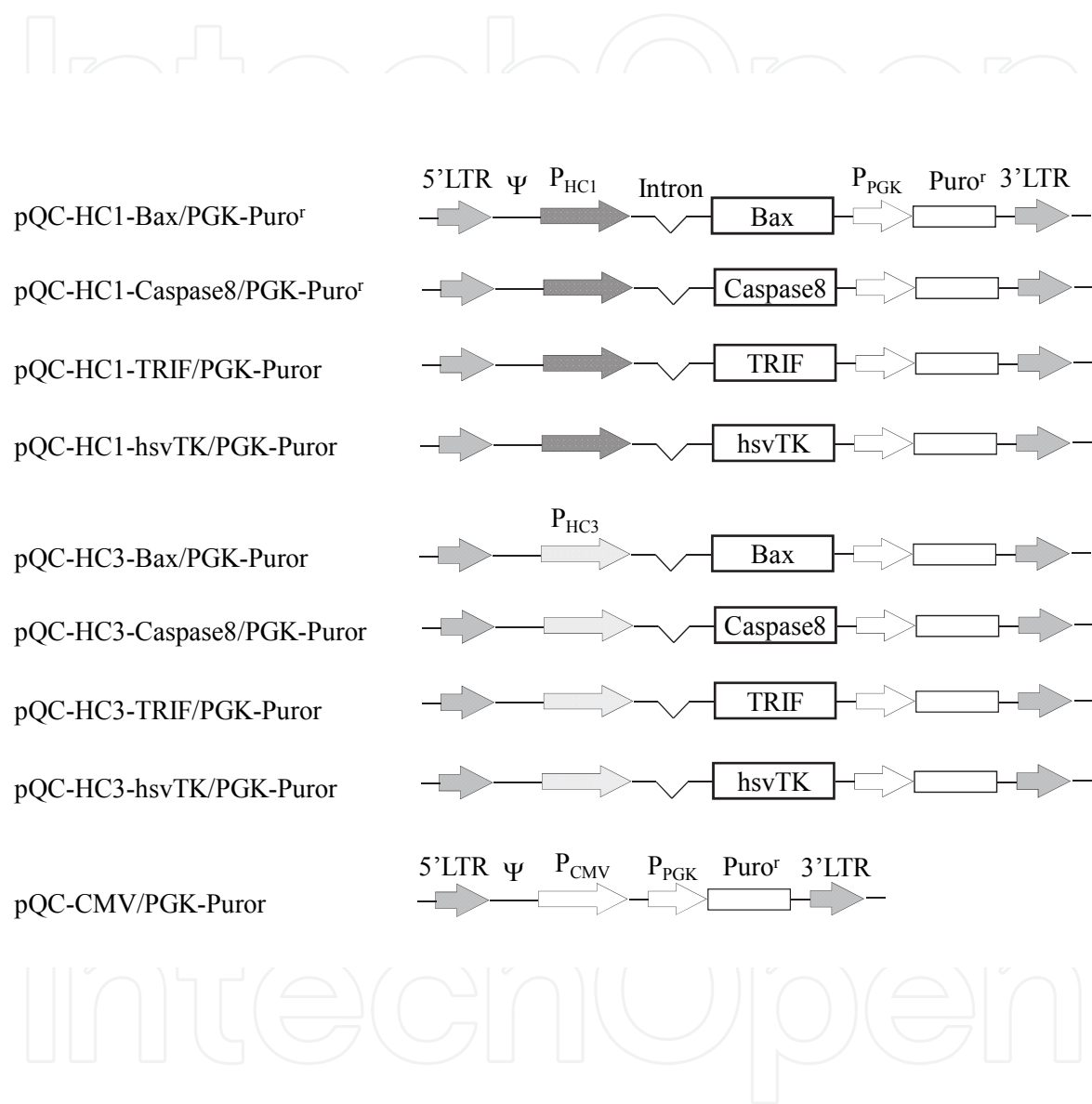


Fig. 7. Retroviral constructs. The retroviral vector, pQC (Clontech), that self-inactivates the 5' LTR to avoid promoter interference, was used to construct the expression plasmids. 5' LTR, the hybrid long terminal repeat, consists of the CMV type I enhancer and the mouse sarcoma virus promoter; Ψ, packaging signal; P_{HC1}, P_{HC3}, and P_{CMV}: the CMV, HC1, and HC3 chimeric promoters, respectively; Intron, the synthetic intron; P_{PGK}-Puro^r, PGK (murine phosphoglycerate kinase) promoter-driven puromycin resistance gene; 3' LTR, the Moloney murine leukemia virus LTR deleted in the U3. The death inducing genes (Bax, Caspase8, TRIF, and hsvTK) are boxed.

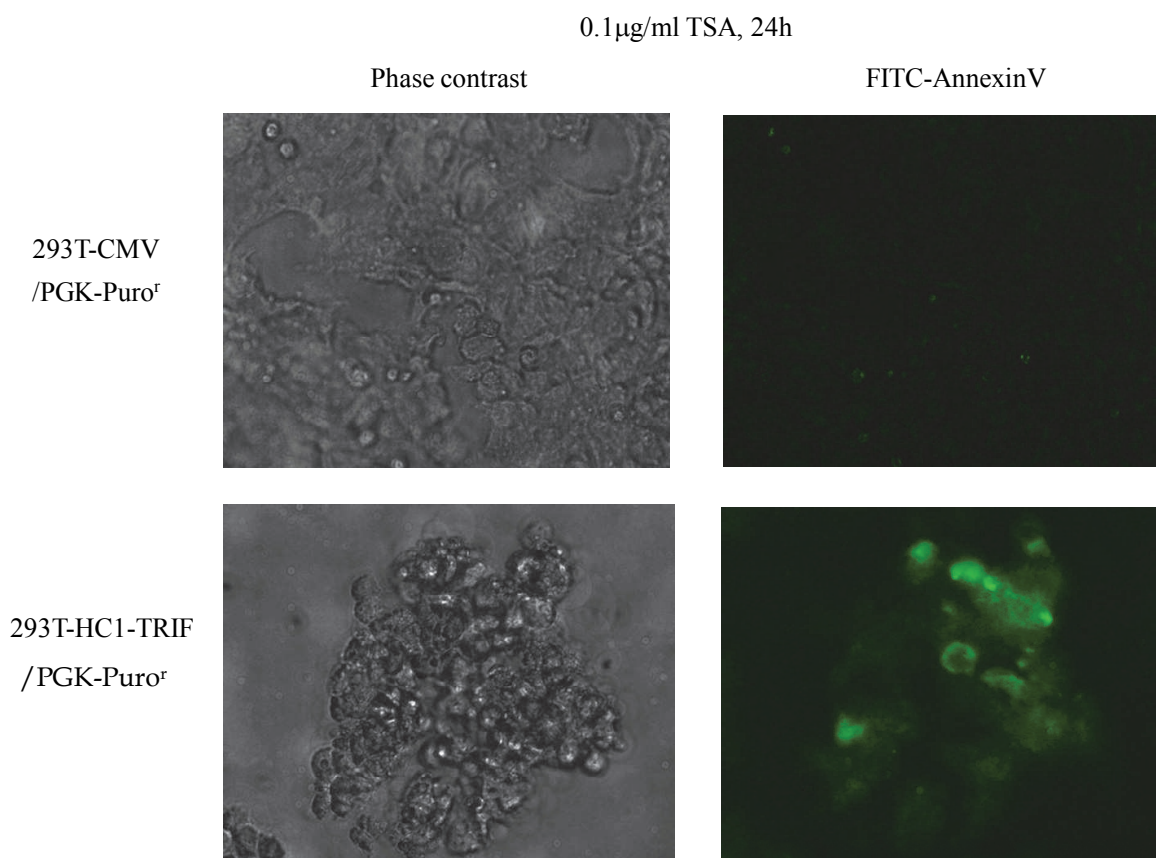


Fig. 8. TSA-induced cell death in the cells stably containing the P_{HC1} -TRIF transgene. The HEK293T cells introduced with the P_{HC1} -TRIF gene, or the control P_{CMV} promoter only, were selected in medium containing 1 μ g/ml puromycin for more than two weeks to obtain stable clones. The cells were then treated with 0.1 μ g/ml TSA for 24h to activate the P_{HC1} promoter. To detect the apoptotic cells, FITC-conjugated Annexin V was used according to the manufacturer's instructions. The HEK293T cells containing the P_{HC1} -TRIF gene showed drastic cell death (apoptosis), as compared to the control 293T cells containing P_{CMV} only.

On the other hand, adenoviral or adeno-associated viral vectors are preferable to retroviral or lentiviral vectors, to avoid insertional mutagenesis by the transgene in target cells. In addition, the vectors can be inserted with the long 3'-untranslated region (UTR), to enhance the TSA-responsiveness between the inverted terminal repeats (ITRs). We constructed several adenoviral vectors containing the death-inducing genes (Bax, Caspase8, TRIF, and hsvTK) under the chimeric promoters (P_{HC1} and P_{HC3}) with the long 3' UTR sequence (Fig. 9). To monitor the transfection and infection efficiencies easily, we added the EF1 α promoter-driven GFP. The recombinant adenovirus constructs were transferred from a shuttle vector (Clontech) with the specific homing endonucleases I-CeuI and PI-SceI, and showed GFP expression in the HEK293 packaging cells.

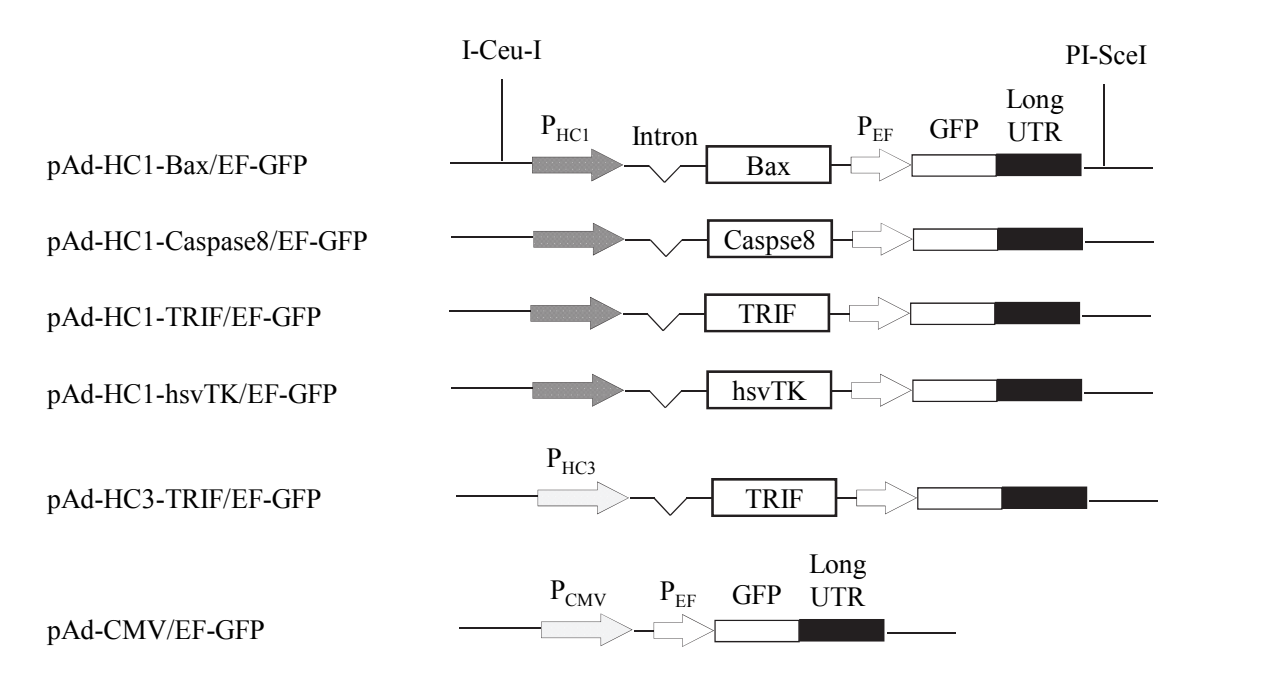


Fig. 9. Adenoviral constructs. The recombinant adenovirus constructs were created by transferring the fragments from a shuttle vector, pTRE-Shuttle2 (Clontech), with the specific homing endonucleases I-CeuI and PI-SceI to an adenoviral vector deleted in the E1 and E3 regions, pAdeno-X (Clontech). P_{HC1} , P_{HC3} , and P_{CMV} : the CMV, HC1, and HC3 chimeric promoters, respectively; Intron, the synthetic intron; $P_{PGK-Puro^r}$, PGK (murine phosphoglycerate kinase) promoter driving the puromycin resistance gene; Long UTR, long 3'-untranslated region. The death inducing genes (Bax, Caspase8, TRIF, and hsvTK) are boxed.

3. Specific delivery of transgenes to target cells

Another challenge is to deliver the transgene to the desired cells or tissues. Some specific delivery systems have been developed by engineering the viral envelope proteins of Lentivirus or Retrovirus, in a procedure referred to as pseudotyping, and incorporating specific antibodies and ligands (Mátrai et al., 2010). The pseudotyping with vesicular stomatitis virus glycoprotein (VSV-G) as a viral envelope protein has broadened the natural tropism of the virus to infect basically all human and murine cell types (Burns et al., 1993). Using various viral envelopes, the recombinant viruses can be introduced into different target cells, including lymphocytes and nerve cells (Colin et al., 2009; Funke et al., 2009; Miletic et al., 2004). Some viruses have two envelope proteins with distinct functions, to bind to the host receptor and to fuse with the membrane for entry (Buchholz et al., 2009). The E1 and E2 proteins of Sindbis virus (Morizono et al., 2005; Yang et al., 2006), and the H and F proteins of measles virus (Frecha et al. 2008; Funke et al., 2008) have been well

characterized for the recombinant virus to infect a desired target cell (retargeting) with high transduction efficiency. The principle of retargeting using the measles H and F proteins is illustrated in Fig. 10. The natural receptor recognition of the measles H protein is ablated, and a ligand is attached that recognizes the cognate receptor on the target cell. Subsequent virus entry is mediated by the F protein pH-independently at the plasma membrane, and the transgene is delivered into the target cell. Using specific markers on target cells (specific epitope and antibody binding, or ligand and receptor binding), the transgene will be retargeted to the desired cell type. By combining this specific delivery system with a suicide gene, selective cell death of the target cells was achieved (Funke et al., 2008). Our TSA-responsible killer gene constructs can be applied to this retargeting system. Finally, these TSA-responsible killer gene constructs can be used to eliminate the undesirable cells generated by inadvertent insertional mutagenesis in the process of gene therapy, as a biosafety measure.

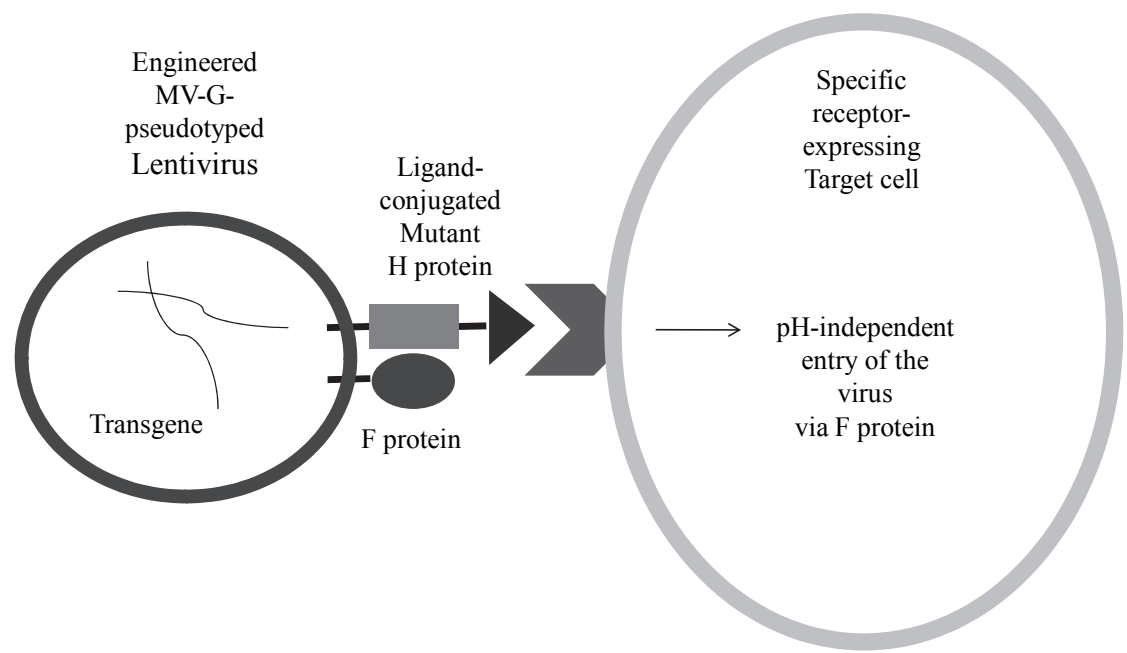


Fig. 10. Specific delivery of a transgene. The natural receptor recognition of the measles H protein is ablated, and a ligand is attached that recognizes the cognate receptor on the target cell. The virus entry is mediated by the F protein pH-independently at the plasma membrane, and the transgene is delivered into the target cell. Using specific epitope and antibody binding, or ligand and receptor binding, the transgene is delivered to the desired target cells.

4. Conclusions

Considering the various anti-cancer effects of TSA, an HDACi with the ability to reactivate silenced transgenes by altering the chromatin structure, we have developed TSA-inducible cell death systems to enhance the anti-cancer effects of TSA. The chimeric promoters P_{HC1} and P_{HC3} showed high TSA-responsiveness to express the death-inducing genes (hsvTK,

Bax, Capase8, and TRIF), and the cells in which the killer constructs were introduced showed TSA-dependent cell death. Combined with specific delivery systems to target cancer cells, these killer constructs will be beneficial for cancer gene therapy. We are planning to investigate the killer efficacies of other HDACis besides TSA, because various HDACis are now available for cancer therapy. The *in vivo* trials of these constructs with HDACis will be the crucial next step.

5. Acknowledgments

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This book aims at providing an up-to-date report to cover key aspects of existing problems in the emerging field of targets in gene therapy. With the contributions in various disciplines of gene therapy, the book brings together major approaches: Target Strategy in Gene Therapy, Gene Therapy of Cancer and Gene Therapy of Other Diseases. This source enables clinicians and researchers to select and effectively utilize new translational approaches in gene therapy and analyze the developments in target strategy in gene therapy.

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Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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