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Properties of a Telomerase-Specific Cre/Lox Switch for Transcriptionally Targeted Cancer Gene Therapy[1]

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Abstract

Telomerase expression represents a good target for cancer gene therapy. The promoters of the core telomerase catalytic [human telomerase reverse transcriptase (hTERT)] and RNA [human telomerase RNA (hTR)] subunits show selective activity in cancer cells but not in normal cells. This property can be harnessed to express therapeutic transgenes in a wide range of cancer cells. Unfortunately, weak hTR and hTERT promoter activities in some cancer cells could limit the target cell range. Therefore, strategies to enhance telomerase-specific gene therapy are of interest. We constructed a Cre/Lox reporter switch coupling telomerase promoter specificity with Cytomegalovirus (CMV) promoter activity, which is generally considered to be constitutively high. In this approach, a telomerase-specific vector expressing Cre recombinase directs excisive recombination on a second vector, removing a transcriptional blockade to CMV-dependent luciferase expression. We tested switch activation in cell lines over a wide range of telomerase promoter activities. However, Cre/Lox–dependent luciferase expression was not enhanced relative to expression using hTR or hTERT promoters directly. Cell-specific differences between telomerase and CMV promoter activities and incomplete sigmoid switch activation were limiting factors. Notably, CMV activity was not always significantly stronger than telomerase promoter activity. Our conclusions provide a general basis for a more rational design of novel recombinase switches in gene therapy.

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Introduction

Telomerase is a ribonucleoprotein reverse transcriptase that is minimally composed of RNA [human telomerase RNA (hTR)] and catalytic [human telomerase reverse transcriptase (hTERT)] subunits, which counteracts cell division–associated attrition of the telomeres of linear chromosomes by synthesizing new telomere DNA sequences from an internal template sequence in hTR [1–3]. Most normal cells do not express telomerase and are therefore subject to telomere-dependent senescence. However, telomerase activity is essential for immortalization of most cancer cells, and its inhibition results in delayed-onset apoptosis [4–8]. Thus, telomerase represents an exciting target for the development of novel anticancer therapeutics [9–12].

Differential expression of telomerase between normal and cancer cells is attributable to transcriptional regulation of hTR and hTERT. Both transcripts are readily detectable in most cancer cell lines and human malignancies, but are either absent or at very low levels in normal cells and tissues. Cloned hTR and hTERT promoter constructs also show selective activity in cancer cells and have been exploited for transcriptionally targeted gene therapy strategies, with the expectation that therapeutic transgenes can be expressed at high levels, specifically in cancer cells but not in normal cells [9,13–28].

Preclinical results of telomerase-specific gene therapy are encouraging. Therapeutic transgene expression has been demonstrated in multiple human cancer cell lines, whereas normal cells are generally not targeted by the hTR and hTERT promoters [9]. We previously reported that the telomerase promoters drive an efficient and selective expression of the bacterial nitroreductase (NTR) gene in several cancer cell lines and xenografts, but not in normal cells [15,16]. NTR catalyzes the rapid bioactivation of the relatively nontoxic prodrug CB1954, resulting in its conversion to a powerful alkylating agent. Activated CB1954 forms atypical DNA adducts that are poorly repaired, leading to efficient p53-independent apoptosis [29,30].

However, the telomerase promoters exhibit relatively weak activity in some target cells [15,16,24]. This could restrict therapeutic targeting potentials in applications such as enzyme/prodrug therapy, where there is good correlation between high transgene expression levels and significant therapeutic effects. In this respect, we have found the hTERT promoter to be particularly problematic. We have found that several cancer cell lines are inefficiently targeted by the NTR/CB1954 combination using the hTERT promoter. In our model, the stronger

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hTR promoter results in efficient therapeutic targeting of more cell lines than hTERT. However, the hTR promoter is also inefficient in some cases.

Therefore, strategies to improve the efficacy of telomerase gene therapy in cells with low promoter activity are of interest [21,24]. One approach to improving the expression levels of therapeutic transgenes in tissue-specific gene therapy makes use of the Cre/Lox switch. Phage P1–derived Cre recombinase catalyzes site-specific excision and circularization of stuffer DNA sequences flanked at the 5′ and 3′ ends by a specific 34-bp Cre-binding sequence (the LoxP site). Thus, Cre/Lox technology provides a valuable tool for studies of gene function [31,32].

Harnessed for gene therapy, a therapeutic transgene is separated from a strong constitutive promoter by a LoxP-flanked transcriptional termination signal. A weak tissue-specific promoter of interest drives the expression of Cre from a second vector, resulting in deletion of the stuffer and derepression of transgene expression. Thus, selectivity is neatly coupled with a constitutively high transcription rate. Several groups have applied this strategy in cancer gene therapy approaches [33–37]. Because the hTR and hTERT promoters show weak activity in some cancer cells, we reasoned that the Cre/Lox switch could be adapted to extend the effective target cell range for telomerase-specific gene therapy.

Here we report the development of a telomerase-specific Cre/Lox switch regulating the expression of the *luciferase* gene. In this system, luciferase expression is controlled by the Cytomegalovirus (CMV) promoter and by a LoxP-flanked stuffer fragment harboring the SV40 late polyadenylation signal upstream of the *luciferase* gene. Expression of Cre, mediated by hTR, hTERT, or SV40 promoters, excises the stuffer facilitating the luciferase expression (Figure 1). This model allowed us to test quantitatively whether a telomerase-specific Cre/Lox switch could potentially enhance therapeutic transgene expression levels relative to direct expression through the hTR or hTERT promoters.

We selected a panel of four cancer cell lines with a wide range of hTR and hTERT promoter activities in order to examine switch activation over a range of low to high Cre expression levels. Cre was expressed in a promoter- and cell-specific manner, effectively derepressing luciferase expression over a wide range of concentrations. Unexpectedly, however, the switch did not confer significantly enhanced luciferase expression in any of the cells tested here.

These results were explained by cell-specific differences between telomerase and CMV promoter activities, and by a sigmoid relationship between Cre expression levels and switch activation efficiency. Switch activation saturated at very low Cre doses and increased Cre expression did not confer increased luciferase expression. Because of these attributes of Cre-mediated excision in cells, a very large differential in selective and constitutive promoter activities is required to confidently predict enhancement of gene expression by the Cre/Lox switch. In our model, the CMV promoter was not always significantly stronger than hTR or hTERT. Importantly, because viral promoters have no biomarkers of activity in human tissues, we conclude that Cre/Lox switches using viral promoters cannot be applied in a hypothesis-led fashion as experimental clinical therapeutics. These factors limit the application of the Cre/Lox switch in its current configuration for telomerase-specific gene therapy and are also relevant for successful development of Cre/Lox switches regulated by other promoters. Based on these findings, we highlight possible modifications to significantly improve the performance and predictability of other novel Cre/Lox switches for gene therapy.

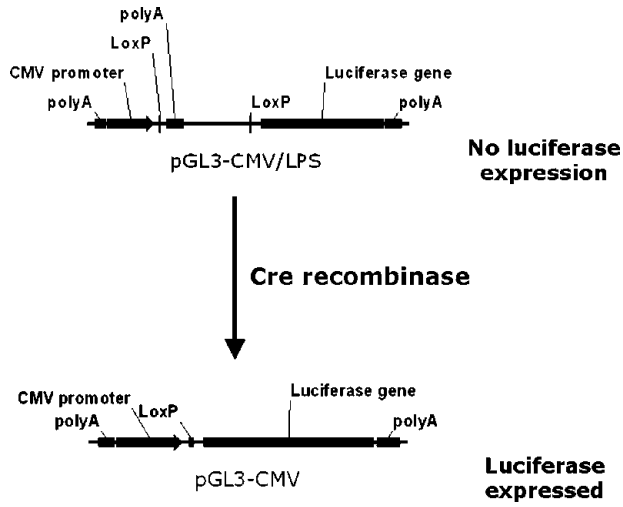

Figure 1. *Regulation of luciferase expression by the Cre/Lox switch. Transcription of the luciferase gene in vector LPS is repressed by the presence of a LoxP-flanked stuffer fragment harboring the SV40 late polyadenylation signal upstream of the luciferase gene. In the presence of Cre protein expression, in this case directed by the hTR and hTERT promoters, the stuffer and polyadenylation signal are excised, leading to the derepression of CMV-dependent luciferase expression.*

Materials and Methods

Cell Lines and Plasmids

In this study, we used the human cancer cell lines 5637 (bladder carcinoma), C33A (cervical carcinoma), A2780 (ovarian adenocarcinoma), and A549 (lung adenocarcinoma). The relative targeting efficiency of these cell lines by hTR- or hTERT-directed enzyme–prodrug therapy has previously been reported [15,16]. All vectors reported in this study are based on the pGL3 reporter vector backbone (Promega, Madison, WI). The hTR- and hTERT-luciferase reporter vectors (pGL3-hTR and pGL3-hTERT) contain 867- and 572-bp fragments of the hTR and hTERT promoters, respectively, which have previously been shown to direct the selective expression of transgenes in tumor cells [15,16]. The control vectors pGL3-SV40 and pGL3-Basic were obtained from Promega. To construct the hTR-, hTERT-, SV40-, and promoterless-Cre expression vectors, the *luciferase* gene was deleted from the pGL3 series of luciferase reporters by an *Nco*I/*Xba*I digest. The ends were made blunt with Klenow fragment, and the vectors were religated. Cre was inserted downstream of each promoter as a *Hin*dIII fragment