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RESEARCH

Development of Single-Vector Tet-on Inducible Systems with High Sensitivity to Doxycycline

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Abstract Single-vector Tet-on systems were developed to enable the tight regulation of transgenes in mammalian cells with a low dosage of doxycycline. Both the regulatory and the responsive units were integrated in a single vector and separated by a short DNA segment (214 bp). In the developed single-vector Tet-on systems, a high level of expression of the transgene can be induced by doxycycline at a concentration of as low as 1 ng/ml, which is 500–1,000 times lower than that usually utilized in other Tet-on systems. The single-vector Tet-on system developed here exhibited 3.5–10.8 times greater inducibility of the transgene in response to doxycycline than did a dual-vector system from a commercial source. Further studies indicate that the basal activity of Tet-on systems depends greatly on the strength of the promoter that controls the transactivator. The basal activity of Tet-on systems was high when the transactivator that was directed by the human cytomegalovirus promoter, and it was almost undetectable when the transactivator was placed under the control of a moderate strength mouse mammary tumor virus promoter. Moreover, the introduction of selectable markers allows the developed single-vector Tet-on systems to facilitate the generation of conditional transgenic cells and animals with high inducibility, low basal activity and detrimental effects of the long-term administration of doxycycline.

Keywords Tetracycline · Tet-on inducible system · Single-vector · Transgenic cell · CMV · MMTV

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Introduction

The expression of certain genes in specific cells or tissues is useful in elucidating their functions. However, the uncontrolled expression of transgenes may lead to the selection of cell lines with undesirable side effects, particularly when the gene products are either cytotoxic, inhibitory to cell growth or exhibiting multiple characteristics, which may lead to confounded conclusions. Controlling the expression of genes of interest in a temporally and spatially inducible manner may help to solve these problems. Among developed inducible systems the tetracycline-inducible expression system is widely adopted to express exogenous genes in various hosts, including embryonic stem cells [1], mammalian cells [2], transgenic mice [3], insects [4], and plants [5], either universally or in a tissue-specific manner [6–8].

The conventional dual-vector Tet-inducible system comprises two distinct plasmids, one of which contains the regulatory unit for the constitutive expression of the transactivator (tTA) (Tet-off system) or the reverse transactivator (rtTA) (Tet-on system) and the other consists of the Tet-controlled responsive unit for the expression of a gene of interest. The tTA in the Tet-off system binds to tet operator (TetO), which consists of tetracycline-responsive

elements (TREs) coupled minimal promoter, and induces the expression of transgenes in the absence of tetracycline. The persistent presence of tetracycline is required to maintain the low basal expression of the transgene before induction [9]. However, this system suffers from high basal expression and low efficiency of induction [10]. Additionally, the transgenic animals may experience adverse side effects because of the long-term administration of a high dosage of tetracycline or doxycycline [11–13]. The Tet-on system is established by replacing tTA with rtTA, which is inactive without Tet. The rtTA binds to TetO only when tetracycline or doxycycline (Dox), which is an analogue of tetracycline, is present, and allows the expression of a transgene to be induced in a dose-dependent manner.

Although a conventional Tet-on inducible system is a powerful tool for inducing the expression of a transgene in response to Tet in various hosts, it has been proven to be time-consuming, costly, labor-intensive and inefficient in generating a successful cell model or progeny [14, 15]. Several single vector-based Tet-on inducible systems, which contain both the rtTA expression cassette and the tetracycline-controlled responsive unit, were constructed in an attempt to overcome the disadvantages of conventional dual-vector inducible systems [16–18]. However, large plasmid size, high background expression and low inducibility were drawbacks of these single vector-based Tet-on inducible systems. Larger plasmids are less easily purified and more tightly restrict the size of the cloned gene. These problems limit their application in the generation of transgenic cells and animals [16, 17].

In this study, various simplified Tet-on inducible systems with both an rtTA gene and a tetracycline-controlled responsive unit carried in a single vector were constructed. The results indicate that the developed single-vector Tet-on system exhibited high inducibility of the transgenes. To eliminate the basal activity of Tet-on systems, the expression of rtTA gene was controlled by human cytomegalovirus (CMV) or mouse mammary tumor virus (MMTV) promoters. The orientation of the rtTA gene and the tetracycline-controlled responsive unit and the presence of repressor were also investigated to reduce the basal expression of the reporter gene. The results of this study showed that the basal activity of Tet-on systems is influenced by the promoter that controls the rtTA gene of the Tet-on inducible systems.

Materials and Methods

Reagents and Plasmids

Plasmids pTRE2hyg, which contained the minimal RNA polymerase II promoter with multiple TetO repeats, pTet-tTS plasmid, which contained a Tet-controlled transcriptional silencer tTS, pCMV-Tet3G plasmid, which contained a Tet-controlled transcriptional tTA, pTRE3G-IRES plasmid, which contains the minimal RNA polymerase II promoter with multiple tetO repeats, and pMAMneo were obtained from Clontech Laboratories (Palo Alto, CA). The pUHrT62-1, a plasmid with rtTA2s-M2 cDNA, was kindly provided by Dr. Hermann Bujard [19]. Restriction enzymes were purchased from New England Biolabs. All other reagents were reagent grade.

Construction of Plasmids

The plasmid pCRT$_{neo}$(-TRE2), which contained an expression cassette of rtTA2s-M2 under the control of CMV, was generated by inserting the rtTA2s-M2 gene from pUHrT62-1 into the *Hin*dIII–*Bam*HI sites of the pCDNA3.0 (Invitrogen). The plasmid pMRT$_{neo}$(-TRE2) was generated by replacing the CMV promoter (*Nru*I–*Hin*dIII sites) of pCRT$_{neo}$(-TRE2) plasmid with MMTV-LTR promoter from pMAMneo plasmid. The pTRE2-EGFP/hyg was constructed by inserting a green fluorescence protein (GFP) cDNA into the *Mlu*I–SalI sites of pTRE2hyg. The single plasmid carrying both rtTA2s-M2 and Tet-controlled GFP expression cassettes in tandem (ptCRT$_{neo}$/GFP) or opposite direction (poCRT$_{neo}$/GFP) was constructed by replacing the rtTA2s-M2 expression cassette (*Xho*I fragment) in pTRE-GFP/hyg plasmid with a 3.8 kb *Sal*I fragment containing the CMV-controlled rtTA2s-M2 as well as neomycin resistance gene (neo) expression cassettes from pCRT$_{neo}$(-TRE) (Fig. 1a). The Tet-on inducible systems with rtTA2s-M2 under the control of MMTV promoter, ptMRT$_{neo}$/GFP and poMRT$_{neo}$/GFP, were also constructed using similar strategy. The rtTA2s-M2 expression cassette (*Xho*I fragment) in pTRE-GFP/hyg plasmid was replaced with a 4.5 kb *Sal*I fragment from pMRT$_{neo}$(-TRE2), which contained a MMTV-controlled rtTA2s-M2 as well as a neomycin resistance gene (neo) expression cassette, to generate ptMRT$_{neo}$/GFP (tandem orientation) and poMRT$_{neo}$/GFP (opposite orientation) plasmids (Fig. 4a). The pTRE3G/GFP was constructed by inserting GFP cDNA (digested with *Bam*HI and *Eco*RV) from pTRE2-GFP/hyg into the *Bgl*II–*Eco*RV sites of pTRE3G-IRES.

The simplified Tet-on inducible plasmids with tTS, a Tet-controlled transcriptional silencer [20], were also constructed. The tTS expression cassette (2.1 kb) from the pTet-tTS plasmid [19] was amplified by PCR with the primer set RV-PME-5-TTS (5′-gatatcgtttaaacGAGCTTGGCCCATTG-3′) and BGLII-3-TTS (5′-agatctGGTCGAGCTGATACTTCCCGTCC-3′) and inserted into the *Ssp*I–*Bgl*II sites of pMRT*neo*(-TRE2) to generate ptTS/MRT$_{neo}$(-TRE2) vector. The 4.4 kb *Pme*I–*Not*I fragment from ptTS/MRT$_{neo}$(-TRE2)

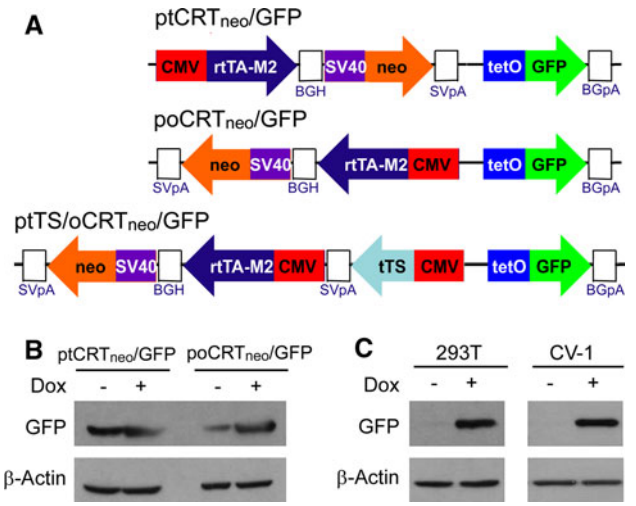

Fig. 1 Characterization of CMV-based, simplified Tet-on inducible systems. **a** Structure of single plasmid vectors. *Arrows* indicate directions of transcription for GFP, rtTS-M2, and neo genes, which are determined by tetO inducible promoter, CMV and SV40 promoters, respectively. BGpA contains rabbit β globin poly(A), SVpA is bi-directional SV40 viral poly(A), and BGH is bovine growth hormone poly(A) signal. CMV-based Tet-on inducible systems contain a tTA rtTA2s-M2 gene, a responsive element that comprises a GFP reporter gene under the control of tetO flanking with a TetCMVmin promoter and a neomycin-resistant gene. Regulatory and responsive units are either tandem (ptCRT$_{neo}$/GFP) or oppositely orientated (poCRT$_{neo}$/GFP). Tet-controlled transcriptional silencer, tTS, under the control of a CMV promoter was used to construct ptTS/oCRT$_{neo}$/GFP plasmid. **b** ptCRT$_{neo}$/GFP- and poCRT$_{neo}$/GFP-transfected HEK 293T cells (1×10^6 cells) were treated with buffer only or buffer that contained 100 ng/ml Dox at 37°C for 24 h. Following incubation, cell lysate was prepared and subjected to western blot analysis. **c** ptTS/oCRT$_{neo}$/GFP-transfected HEK 293T and CV-1 cells (1×10^6 cells) were treated without or with 100 ng/ml Dox at 37°C for 24 h before being subjected to western blot analysis. β-Actin was used as an internal control

was then inserted into the *Nru*I–*Not*I sites of poMRT$_{neo}$/GFP to generate the ptTS/oMRT$_{neo}$/GFP vector. The ptTS/oCRT$_{neo}$/GFP vector, a CMV-based Tet-on inducible vector, was constructed by replacing a 2.2 kb *Nru*I–*Not*I fragment of ptTS/oMRT$_{neo}$/GFP with the 1.5 kb *Nru*I–*Not*I fragment of ptTS/CRT$_{neo}$(-TRE2).

Cell Culture and Plasmid Transfection

Human embryonic kidney cell line (HEK 293T), monkey kidney cell line (CV-1), human cervical cancer cell line (HeLa), and mouse normal mammary gland epithelial cell line (NMuMG) were grown in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS, Biological Industries), 100 units/ml penicillin (Invitrogen), and 100 μg/ml streptomycin (Invitrogen) in a 37°C humidified culturing chamber containing 95% air and 5% CO_2.

The developed simplified Tet-on inducible systems (1 μg) and the dual-vector inducible systems (1 μg each of the responsive and regulatory plasmids) were transfected into cells using the TurboFect (Fermentas) transfection kit, following the manufacturer's instructions. Briefly, DNA mixed with 4 μl TurboFect reagent in 200 μl Opti-MEM® I Reduced Serum Media (Invitrogen) and incubated under room temperature for 20 min. After incubation, the DNA/TurboFect mixture (200 μl) was added into 60 mm cultural dish with cell density of around 60% confluency at 37°C for 3 h. After transfection, cells were washed by PBS buffer (10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) and replaced with the normal culture medium with mock or Dox for additional 24 h. GFP expression in the transfected cells was monitored by western blot analysis.

Western Blotting

Cell lysate was prepared as described elsewhere [21]. Briefly, cells were harvested and lysed in RIPA buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na_3VO_4, and 1 μg/ml leupeptin). Cell lysate (50 μg) was separated on a 10% SDS-PAGE. Protein bands were then transferred to the PVDF membrane (millipore) by electroblotting, followed by probing with primary antibodies specifically against β-actin and GFP (Santa Cruz Biotechnology). The signals were detected using an enhanced chemiluminescence system.

Results and Discussion

Construction and Characterization of Simplified CMV-Based Tet-on Expression Vectors

The reverse transactivator rtTA2s-M2, an rtTA derivative that has been suggested to exhibit a considerably low background activity and a wide range of induction [19], was utilized herein to construct simplified Tet-on inducible systems. To investigate the efficacy of the constructed simplified Tet-on inducible systems GFP was used as a reporter. The ptCRT$_{neo}$/GFP vector was constructed by placing rtTA2s-M2 under the control of the CMV promoter (Fig. 1a). CMV promoter is widely utilized to induce high-level expression of genes of interest in various mammalian cells [20]. The regulatory (rtTA2s-M2 expression cassette) and responsive elements (Tet-controlled transgene expression unit) were separated using a short non-coding DNA sequence of 214 bp to reduce the size of the simplified Tet-on inducible vector (around 6.43 kb). The reduction of the size of the simplified Tet-on inducible expression vector may increase the transfection efficiency of the simplified

Tet-on inducible system. Moreover, the presence of two single restriction sites, *Ase*I and *Drd*I, can be exploited to linearize the plasmids.

Characterization of Simplified CMV-Based Simplified Tet-on Expression Vectors

The constructed ptCRT$_{neo}$/GFP plasmid could be readily transfected into HEK293T and CV-1 cell lines using a conventional liposome-based transfection reagent (data not shown). However, the expression of GFP was detected 1 day following transfection even without induction (Fig. 1b). The high basal expression of GFP may have been caused by mutual interference between regulatory and responsive elements [17], which were arranged in a tandem orientation and separated by a 214 bp-long DNA segment in the ptCRT$_{neo}$/GFP plasmid. As shown in the previous study, a long intervening DNA sequence between the regulatory and responsive elements is probably required to eliminate mutual interference between regulatory and responsive elements [17]. However, the size of the simplified Tet-on inducible vector may be greatly increased by using a long intervening DNA segment. To solve this problem, the regulatory and responsive elements were oppositely oriented to generate the plasmid poCRT$_{neo}$/GFP. Interestingly, the background expression of GFP in the HEK293T cells that were transfected with poCRT$_{neo}$/GFP was markedly reduced, although not completely eliminated (Fig. 1b). This result suggests that the short spacing between the rtTA-M2 expression cassette and the tetracycline-controlled responsive unit may be responsible for the mutual interference between them and can be reduced, at least partially, by arranging them in opposite orientations in the plasmid [17].

Although the developed CMV-based Tet-on inducible systems, poCRT$_{neo}$/GFP and ptCRT$_{neo}$/GFP, exhibited moderate to high basal expression of GFP in HEK293T cells, they were responsive to Dox (Fig. 1b). The induced GFP level in poCRT$_{neo}$/GFP-transfected HEK293T cells upon treatment with 100 μg/l Dox was clearly increased compared to the uninduced GFP level, whereas the inducibility of the GFP expression in ptCRT$_{neo}$/GFP-transfected cells was low, probably owing to the high background expression of GFP before treatment (Fig. 1b).

Effect of tTS on Basal Expression of Transgene

Previously, tTS, a Tet-controlled transcriptional silencer, was demonstrated to block the expression of Tet-regulated transgenes in the absence of tetracycline [20, 22, 23]. The binding of tetracycline releases tTS from the tetO promoter and enables transcription [16, 22, 24]. Therefore, tTS was used to determine whether the basal activity of the developed simplified Tet-on inducible system could be further reduced. The tTS-bearing simplified Tet-on inducible system ptTS/oCRT$_{neo}$/GFP (~8.53 kb) (Fig. 1a) was constructed. Recombination may have occurred in the single-vector Tet-on system, ptTS/oCRT$_{neo}$/GFP, in *E. coli* because of the presence of two CMV promoters, leading to the deletion of a 2.1 kb fragment that contains both SVpA and tTS (Fig. 1a). Therefore, the integrity and the size of the plasmid were verified by the retraction mapping following amplification and isolation from *E. coli* (data not shown). Interestingly, no rearrangement or shortage of the cloned ptTS/oCRT$_{neo}$/GFP plasmid was observed in this investigation, probably because the rearrangement occurred in only a very small fraction of DNA.

The inducibility and background expression of ptTS/oCRT$_{neo}$/GFP were studied by transfecting into HEK293T and CV-1 cells. As shown in Fig. 1c, the basal expression of GFP was almost absent from the ptTS/oCRT$_{neo}$/GFP-transfected HEK293T or CV-1 cells without Dox. Treatment with Dox (100 μg/l) induced marked expression of GFP. This result suggests that tTS in the CMV-based Tet-on inducible system can effectively suppress the basal expression of the transgene.

Dose-Dependent Expression of Transgene in Simplified Tet-on Inducible System

The dose-dependent expression of GFP in response to Dox was demonstrated in HeLa cells that were transfected with ptTS/oCRT$_{neo}$/GFP (Fig. 2). Interestingly, the expression of GFP could be induced by a Dox concentration of as low as 0.1 ng/ml. The induction of GFP was much higher at a concentration of 1 ng/ml, which is 500–1,000 times lower than that (0.5–1 μg/ml) used in the conventional dual-vector [9, 10] and previously developed single-vector Tet-on systems [16–18]. Adverse side effects may occur owing to the long-term administration of a high dosage of tetracycline or doxycycline [11–13]. The results of the present

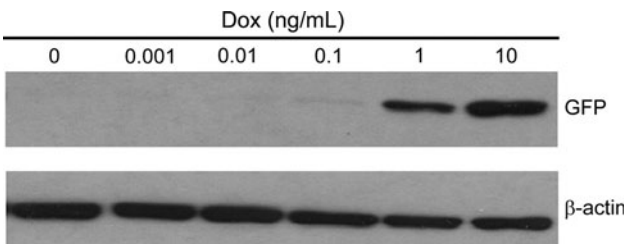

Fig. 2 Dox dose-dependent expression of GFP. Plasmid ptTS/oCRT$_{neo}$/GFP-transfected HeLa cells (1×10^6 cells) were treated with various concentrations of Dox (0.001, 0.01, 0.1, 1, and 10 ng/ml) at 37°C for 24 h before cell lysate was prepared. Western blot analysis was performed using the antibody specifically against GFP. *β*-Actin was used as an internal control

study suggest that the functional tetracycline-controlled transgene can be effectively delivered into mammalian cells by the developed single-vector Tet-on system with high inducibility and possibly low adverse effects.

Comparison with Conventional Dual-Vector Tet-on Inducible Systems

The basal expression and the inducibility of the GFP reporter in the simplified Tet-on system, ptTS/oCRT$_{neo}$/GFP, and the conventional dual-vector Tet-on inducible systems were compared (Fig. 3a). All except for the dual-vector system pTRE2-GFP/hyg and pCRT$_{neo}$(-TRE2) pair exhibited extremely low basal expression of GFP (Fig. 3a) without doxycycline. Among the Tet-on inducible systems tested, the dual-vector Tet-on system pTRE2-GFP/hyg and pTet-tTS pair [19] exhibited the lowest inducibility of GFP expression. Among three Tet-on systems with high inducibility, the developed single-vector system ptTS/oCRT$_{neo}$GFP exhibited a slightly higher Dox-inducible expression of GFP than the other two dual-vector systems, which were the pTRE2-EGFP/hyg and pCRT$_{neo}$(-TRE2) and the pTRE2-EGFP/hyg and ptTS/oCRT$_{neo}$(-TRE2) pairs (Fig. 3a). The lower panel in Fig. 3a presents the quantitation of the western blot result.

The inducibility of the GFP reporter in ptTS/oCRT$_{neo}$/GFP and the Clontech Tet-on 3G system, a commercialized dual-vector system, was also compared. Interestingly, the developed single-vector system exhibited a higher inducibility of GFP expression than did the Clontech Tet-on 3G system. Upon treatment with 1 ng/ml Dox, expression of the GFP reporter was observed in ptTS/oCRT$_{neo}$/GFP-transfected HEK293 cells, but not in HEK293 cells that were transfected with Clontech Tet-on 3G system (Fig. 3b). The expression levels of GFP in the ptTS/oCRT$_{neo}$/GFP-transfected cells were 3.5–10.8 times higher than those in the Clontech Tet-on 3G system (Fig. 3b, lower). The GFP inducibility difference between two inducible system is high at low concentration of Dox (1 μg/ml), whereas the inducibility difference was reduced at high concentration of Dox. These studies suggest that the developed single-vector system exhibits low basal expression and high transgenic inducibility. The unequal efficiencies of delivery of different plasmids during co-transfection may be responsible for the low inducibility of the transgene in the conventional dual-vector inducible systems, especially the pTRE2-GFP/hyg and pTet-tTS pair and the Clontech Tet-on 3G system. Additionally, the above results suggest that the designed regulatory element in plasmids pCRT$_{neo}$(-TRE2), ptTS/oCRT$_{neo}$(-TRE2) and ptTS/oCRT$_{neo}$/GFP efficiently support the high expression of transgenes in response to Dox.

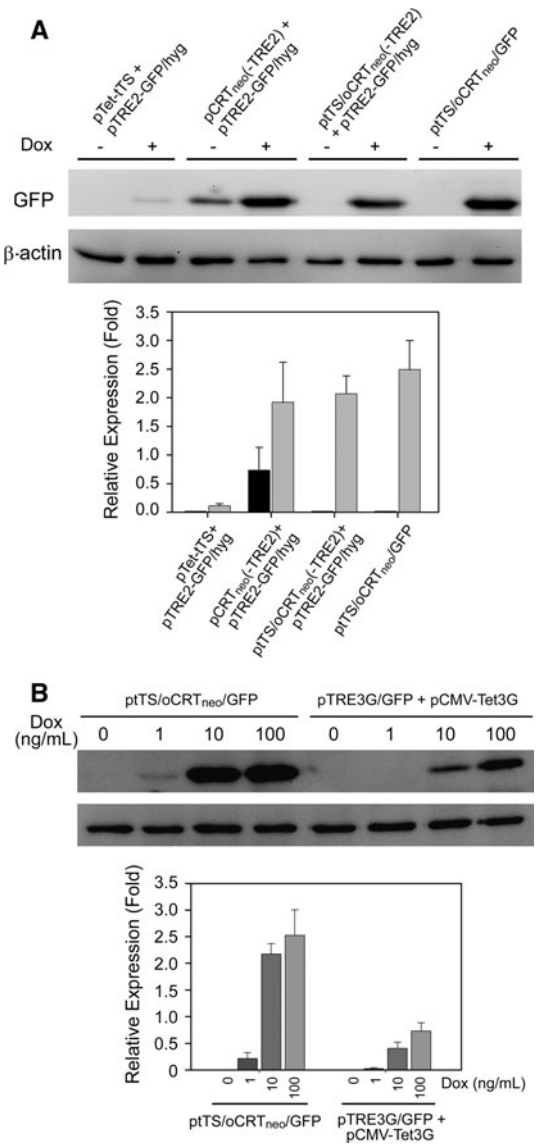

Fig. 3 Expression of GFP reporter in HEK 293T cells with different Tet-on inducible systems. **a** (*Top*) HeLa cells were transfected with dual-vector Tet-on inducible systems, pTRE2-GFP/hyg + pTet-tTS, pTRE2-GFP/hyg + pCRT$_{neo}$(-TRE2), and pTRE2-GFP/hyg + ptTS/oCRT$_{neo}$(-TRE2) plasmid pairs, or single-vector Tet-on system ptTS/oCRT$_{neo}$/GFP, as described in "Materials and Methods" section. After transfection, cells were treated with buffer only or 100 ng/ml Dox at 37°C for 24 h before western blot analysis. *β*-Actin was used as an internal control. *Bottom* Quantitation of the GFP expression relative to that of *β*-actin was shown on the *bar graph*. Each *data* is presented as the mean ± SD from three independent measurements. **b** (*Top*) HEK 293T cells were transfected with single-vector Tet-on system ptTS/oCRT*neo*/GFP, or Clontech 3G systems, pTRE3G/GFP and pCMV-Tet3G pair, as described in "Materials and Methods" section. Following transfection, cells were treated with buffer only or various concentrations of Dox (1, 10, and 100 ng/ml) at 37°C for 24 h before western blot analysis. *β*-Actin was used as an internal control. *Bottom* Quantitation of the GFP expression relative to that of *β*-actin was shown in the *bar graph*. Each *data* is presented as the mean ± SD from three independent measurements

Effect of Promoter that Controls tTA in Basal Activity of Inducible System

The CMV promoter has been demonstrated to have much higher strength than many other promoters, including SV40 and MMTV promoters [25]. Therefore, the high expression level of the rtTA2s-M2 gene in cells may be partially responsible for the high basal expression of the transgene in cells with ptCRT$_{neo}$/GFP. At a low level of rtTA2s-M2 expression, the background expression of transgene may be reduced, as revealed by the effect of transactivation silencer tTS on the basal activity of the ptTS/oCRT$_{neo}$/GFP inducible system (Fig. 1c). To test this hypothesis, the MMTV, a hormone-regulated promoter that contains several hormone response elements [26, 27], was used to control the expression of tTA rtTA2s-M2 in the simplified Tet-on inducible systems, ptMRT$_{neo}$/GFP, poMRT$_{neo}$/GFP and ptTS/oMRT$_{neo}$/GFP (Fig. 4a). As shown in Fig. 4b, all three MMTV-based, simplified Tet-on inducible systems exhibited extremely low basal activity in the absence of Dox. The inducibility of GFP expression in all three MMTV-based, Tet-on inducible systems in response to treatment with Dox (100 ng/ml) was high, suggesting that replacing the CMV promoter with the MMTV promoter had no effect on the inducibility of transgene. These results suggest that the strength of the promoter that controls the expression of rtTA2s-M2 is essential in reducing the basal activity of the simplified Tet-on inducible system.

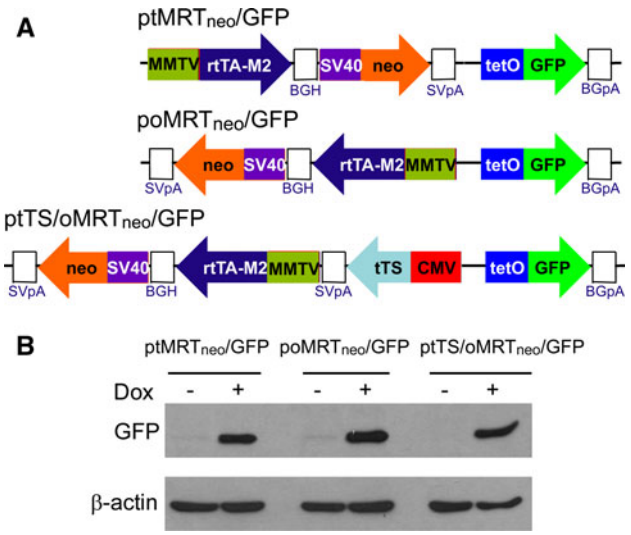

Fig. 4 Expression of GFP in NMuMG cells that were transfected with MMTV-based Tet-on single vector inducible systems. **a** Structure of MMTV-based Tet-on systems. *Arrows* indicate directions of transcription for GFP, rtTS-M2, and neo genes, which are controlled by tetO inducible promoter, MMTV, and SV40 promoters, respectively. **b** ptMRT$_{neo}$/GFP, poMRT$_{neo}$/GFP, and ptTS/oMRT$_{neo}$/GFP transiently transfected NMuMG cells were treated with 100 ng/ml Dox at 37°C for 24 h. Expression of GFP was determined by western blot analysis. *β*-Actin was used as an internal control

Conclusion

In this work, simplified Tet-on inducible systems were constructed in a single vector by integrating regulatory and responsive elements, which were separated using a short intervening DNA segment (214 bp). The small size of the developed single-vector Tet-on systems (ranging from 6.5 to 8.5 kb) allows a large transgene to be cloned and delivered into various mammalian systems. The developed single-vector Tet-on system exhibited high inducibility and low basal activity for the expression of transgenes. The developed single-vector Tet-on system exhibited higher inducibility (3.5–10.8 folds) of the expression of GFP reporter than did a commercialized Clontech 3G system. Interestingly, Dox at a low concentration (1 ng/ml) markedly induced the transgene on the developed single-vector Tet-on systems compared to 1 ng/ml Dox used in previously described systems, potentially reducing the adverse effects of the long-term administration of tetracycline or doxycycline on transgenic animals. The reduction of basal activity is also important to prevent the unwanted side-reactions in the transgenic models. The strength of the promoter that controls the tTA was critical to control the basal activity of Tet-on systems. The basal activity of the single-vector Tet-on system with the high-strength promoter CMV is high and can only be moderately reduced by oppositely orienting both regulatory and responsive units. The basal activity of the Tet-on system is almost undetectable when the tTA is placed under the control of the moderate-strength promoter MMTV or integrating a Tet-controlled transcription silencer, rtTS. In summary, the single-vector Tet-on inducible systems that exhibit low basal activity and high inducibility in response to low dosage of Dox were developed.

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Conflict of interest None.

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