EXPERIMENTAL CELL RESEARCH 314 (2008) 1202–1216

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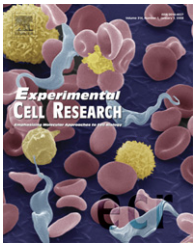

Research Article

Inducible endothelial cell-specific gene expression in transgenic mouse embryos and adult mice

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ARTICLE INFORMATION

Article Chronology:
Received 3 June 2007
Revised version received
23 December 2007
Accepted 24 December 2007
Available online 15 January 2008

Keywords:
TET-ON
TET-OFF
Activator
Responder
Tie-2 promoter
Endothelium
Tetracycline regulated
gene expression
MAdCAM-1

ABSTRACT

Utilizing both the TET-OFF and TET-ON systems in combination with transcriptional control elements of the Tie-2 gene, we have established a series of transgenic activator and responder mice for TET-regulated endothelial cell-specific transgene expression in double transgenic mouse embryos and in adult mice. TET-regulated expression of LacZ reporter genes could be achieved in virtually all endothelia in mid gestation stage mouse embryos. In contrast in adult mice, using the very same Tie-2 tTA activator mouse strain, we observed striking differences of TET-induced gene expression from various inducible expression constructs in different vascular beds. Non-endothelial expression was never detected. The prominent differences in completeness of TET-induced endothelial expression highlight the still underestimated critical role of the responder mouse lines for uniform TET-induced gene expression in heterogeneous cell populations such as endothelial cells. Interestingly, in double transgenic mice inducibly expressing several different adhesion molecules, no adverse effects were observed even though these proteins were robustly expressed on endothelial cells in adult tissues. These transgenic model systems provide versatile tools for the TET-regulated manipulation of endothelial cell-specific gene expression in the entire embryonic vasculature and distinct vascular beds in adult mice.

Introduction

Functional analysis of gene products in the adult vasculature by genetic approaches such as constitutive expression of dominant-negative acting transgenes or deletion of genes has often been hampered by embryonic lethal phenotypes of conventional transgenic and knock-out mice. Lethal null mutations in genes relevant for vascular development and function include VEGF-A and its receptors, Angiopoietins and Tie receptors, members of the TGFβ family and their receptors as well as signalling molecules such as rasGAP and transcription factors such as Fli-1 and numerous other genes [1–3]. With few

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[5] This work is dedicated to the memory of Werner Risau (1953–1998), in whose department this project was initiated.

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doi:10.1016/j.yexcr.2007.12.026

exceptions, early embryonic lethality has severely limited the functional analysis of these genes during adulthood.

Despite the advances made by the development of cell lineage-specific recombination technology [4–7], functional analysis of genes expressed in endothelial cells (ECs) during late gestation and in adult tissues remains difficult. To facilitate cell and tissue-specific as well as temporal regulation of gene expression, inducible systems are required that allow for the reversible manipulation of gene expression. A small number of inducible gene expression systems have been established and are suitable for use in transgenic animals. To avoid unwanted induction of endogenous gene products, these systems utilize chimeric transcription factors whose ligand binding- and DNA-interaction domains are derived from bacterial or invertebrate transcriptional regulator genes. Target gene activation is achieved by fusion of these domains to highly potent mammalian or artificial transcriptional activation domains. Such systems include the ecdysone-inducible system [8], the lac-repressor based system [9] and the most commonly used tetracycline-inducible system [10–12].

To allow for endothelial cell selectivity of TET-system transgenes, we contemplated the potential applicability of promoter elements from various EC-specifically expressed genes that had previously been investigated by reporter gene analysis in transgenic mice. The control elements considered included those of the Flk-1, ICAM-2, PECAM-1, Tie-1, Tie-2 and VE-cadherin genes. Some EC-specific genes are downregulated in adult vessels (Tie-1 [13] and Flk-1 [14]), the promoters of some lacked specificity (ICAM-2 and PECAM-1 [15,16]) or were incompletely characterized (VE-cadherin [17]). Therefore, we used the Tie-2 promoter/enhancer elements, which we have previously shown to reliably direct LacZ reporter gene expression to ECs both during embryogenesis and adulthood [18].

Consequently, we established different types of activator mouse lines expressing tTA (TET-OFF) or rtTA (TET-ON) under the transcriptional control elements of the mouse Tie-2 receptor tyrosine kinase gene. Additionally, we raised various types of responder mouse lines. Several combinations of responder and activator mouse crosses were identified, in which a Tie-2 tTA transgene product leads to doxycycline repressible expression of a LacZ reporter gene exclusively in endothelial cells of embryonic and adult mice. In an effort to investigate the consequences of inducible ectopic expression of adhesion molecules on leukocyte recruitment across endothelium, responder mice for inducible expression of select adhesion molecules were also created.

To this end, we first raised transgenic mice carrying bidirectional constructs coding for a reporter gene and the adhesion molecule E-selectin. In these lines, inducible expression of both the reporter LacZ and E-selectin, could be observed in endothelial cells in several tissues with the highest number of positive vessels in the brain [19]. Finally, several mouse lines, transgenic for a unidirectional MAdCAM-1 construct, have been established and found to allow for very robust TET-inducible endothelial cell-specific expression.

The endothelial cell-selective transactivator mice described here will be valuable tools to investigate the role of mutated or ectopically expressed gene products in the vasculature of mouse embryos and of adult mice.

Materials and methods

Cloning strategies for transgene constructs and DNA purification

The tet operator-multiple cloning site plasmid pUHD 10-3, the bidirectional tet operator-plasmid pBI-4, the tet operator-LacZ plasmid pUHG 16-3, the human CMV promoter/enhancer rtTA and rtTA2^s-M2 expression vectors pUHG 17-1 and pUHrT 61-1, the human CMV promoter/enhancer tTA expression vector pUHD 15-1, were all provided by H. Bujard (ZMBH, Heidelberg). (http://www.zmbh.uni-heidelberg.de/bujard/homepage.html).

For construction of pHHtTANS (Tie-2 tTA plasmid) the tTA open reading frame was amplified with Vent DNA-polymerase (New England Biolabs, Frankfurt, Germany) using the primers PKrtTA (5′-AAACTGCAGACCATGTCTAGATTAGATAAAAGT-3′) and KrtTAV (5′-GGATATCCTCGCGCCCCCTACCCA-3′), digested with PstI and EcoRV and ligated into MluI digested, blunt-ended and Sse 8387I recut pHHNS [18]. For construction of pHHrtTA2^s-M2NS (Tie-2 rtTA2^s-M2 plasmid) the open reading frame of the rtTA2^s-M2 transactivator was amplified with Vent DNA-polymerase using the primers M2-N (5′-AGGCCTG-CAGGAATTCACCATGTCTAGACTGGAC-3′) and M2-C (5′-CGT-ACGCGTCTGGATCCTTACTTAGTTACCCG-3′), digested with PstI and MluI and ligated into Sse8387I/MluI digested pHHNS.

pTRE BH LacZ was created by ligation of a 317 bp XhoI/BamHI fragment from pUHD 10-3 and a 475 bp BamHI/HindIII fragment harboring the minimal Tie-2 promoter into XhoI and HindIII digested pHH [18].

A plasmid containing four copies of the chicken beta-globin insulator [20] flanking a neo-expression cassette (pJC 13-1 ΔLCR) was derived from pJC 13-1 (a gift from Gary Felsenfeld, Bethesda, USA) by cutting with EcoRI, releasing the LCR fragment and religating the plasmid. The neo-cassette was then replaced by insertion of a partially (TC) filled in SalI/XhoI fragment from pSL1190 (Pharmacia) into the partially (GA) filled in BamHI site of pJC 13-1 ΔLCR resulting in the plasmid pGEM4Z-4X INS. This construct was cut with BamHI (present in the fragment from pSL1190), partially filled in with GA and recut with NotI, then two fragments, a partially (TC) filled in XhoI/Bsu36I fragment from pUHG 16-3 (containing the tet-operators 5′ of the CMV immediate early promoter) and a Bsu36I/NotI fragment (harboring part of the LacZ open reading frame and the poly(A) signal from the bovine growth hormone gene) were inserted to give rise to p4X INS TRE LacZ. For microinjection this construct was linearized at a unique SalI site.

For construction of the E-sel-TRE-LacZ construct, a XbaI/HincII fragment harboring the open reading frame of mouse E-selectin was ligated into NheI/EcoRV digested pBI-4 to create pBI-4 E-sel. A StuI/XbaI fragment from pBI-4 E-sel, containing part of the TET-response element (TRE) in addition to the E-selectin open reading frame was ligated into StuI/NheI cut pBI-4 LacZ (a pBluscript derivative of pBI-4).

pBI-4 MAdCAM was created by ligating a MluI/XbaI blunt fragment from pBSII-MAdCAM (derived from pCDM8 MAdCAM, a gift from M. Briskin; Millenium Pharmaceuticals, Cambridge) into MluI/EcoRV cut pBI-4.

The TRE-MAdCAM-1 construct was created by ligating a 442 bp XhoI/SacII TET-response element (TRE) containing