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An Androgen-Inducible Proximal Tubule-Specific Cre-Recombinase Transgenic Model

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Abstract

To facilitate the study of renal proximal tubules, we generated a transgenic mouse strain expressing an improved Cre-recombinase (iCre) under the control of the kidney androgen regulated protein (KAP) promoter. The transgene was expressed in the kidney of male mice but not in female mice. Treatment of female transgenic mice with androgen induced robust expression of the transgene in the kidney. We confirmed the presence of Cre-recombinase activity and the cell-specificity by breeding the KAP2-iCRE mice with ROSA26 reporter mice. X-gal staining of kidney sections from male double transgenic mice showed robust staining in the epithelial cells of renal proximal tubules. β -galactosidase staining in female mice became evident in proximal tubules after administration of androgen. This model of inducible Cre-recombinase in the renal proximal tubule should provide a novel useful tool for studying the physiological significance of genes expressed in the renal proximal tubule. This has advantages of other current models where cre-recombinase expression is constitutive, not inducible.

Keywords

Cre recombinase; proximal tubule; kidney; testosterone

Introduction

The kidney plays an important role in the maintenance of homeostasis by regulating the components and volume of body fluids. The kidney is also one of the most complex organs both in terms of its function and cellular diversity. In addition to an extensive vasculature which serves to filter blood and retrieve critical nutrients, the kidney contains an array of

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tubules and collecting ducts lined by epithelial cells exhibiting vastly differing transport mechanisms depending on their locations in the nephron. This complexity has made the genetic analysis of renal function problematic because there remains a paucity of proven cell-specific promoters to direct expression of proteins to specific nephron segments.

Conventional gene knockouts have some serious limitations, not the least of which is embryonic or postnatal lethality. For example, deletion of Pax-2, a transcriptional regulator of paired-box family, results in the failure of the mesonephros and metanephros to form (19). It can also be difficult to interpret the phenotype if the deleted gene is expressed in multiple cell types and tissues. Angiotensinogen, the substrate for angiotensin-II, is expressed in the renal proximal tubule but also in the liver, brain, heart and adipose tissue (9). Angiotensinogen-deficient mice die before weaning, and exhibit severe renal abnormalities (3; 10). However, it remains unclear if the loss of renal or extra-renal angiotensinogen is the cause of death.

In order to circumvent these limitations, Cre-recombinase strategies have been utilized to control the temporal and spatial deletion of a target gene (17). The bacteriophage P1 derived Cre-recombinase can mediate the deletion, insertion, translocation or inversion of a DNA segment flanked by loxP sites. Spatial specificity is obtained by placing expression of Cre-recombinase under the regulation of a cell-specific promoter. Temporal control over the deletion is obtained by either using a promoter with the desired temporal characteristics or the use of an inducible Cre-recombinase. Several recent reviews highlight how the Cre-LoxP system can be used to effectively target diverse cell types in the kidney (7; 8; 20). Unfortunately, few promoters provide the exquisite kidney-specificity that would be desired in these experiments.

We previously used the kidney androgen induced protein (KAP) promoter to specifically target angiotensinogen expression to the proximal tubules of the kidney (4). This promoter is androgen-inducible thus providing a tool for regulated or inducible expression (2; 5). Unpublished studies suggest that the robust kidney-specific expression we obtained in these mice was due to the unique juxtaposition of regulatory elements not only in the KAP promoter but also within the angiotensinogen gene itself. Based on this, we designed a new vector (pKAP2) which retains the most attractive features of spatial and temporal expression, lacks expression of angiotensinogen, and incorporates a site for the insertion of any coding cDNA (1). We used this to generate mice exhibiting proximal tubule and androgen-specific expression of human renin (11). Herein, we report a new model expressing Cre-recombinase under the control of the KAP-angiotensinogen chimeric construct. Like other models, it is expressed in the kidney, has low level mRNA in some extra-renal tissues (although without evidence of cre-recombinase activity), and within the kidney it is restricted to proximal tubules and is strongly inducible by androgen. Importantly, the transgene is silent in untreated females.

Materials and Methods

Construction of KAP2-iCre transgene and CMV-iCre plasmid

The transgene was generated using the pKAP2 plasmid previous described (1; 11). The codon optimized iCre was amplified from plasmid pBlue-iCre (a gift from Dr. Rolf Sprengel, Department of Molecular Neuroscience, Max-planck Institute for Medical Research, Heidelberg, Germany) using the primers GTGCGGCCGCGCGCGCAATTA and GTGCGGCCGCGCTTTTCCAGTCA. This plasmid encodes an improved Cre-recombinase designed to lower the CG content, improve mammalian codon usage, and exhibit higher level expression in mammalian cells (14). PCR amplified iCre was cloned

into the pcDNA3.1/V5-hisTOPO vector. After sequencing the iCre fragment was then inserted into the *NotI* site of the pKAP2-hAGT construct for generation of transgenic mice.

Cell culture and flow cytometry

Immortalized mouse convoluted proximal tubule cells PKSV-PCT (PCT3) (generously provided by Dr. Anna Meseguer, Centre d'investigacions en Bioquímica i Biologia Molecular (CIBBIM), Barcelona, Spain) were cultured in a modified medium as described (15). The cells were transfected with the Cre Stoplight plasmid (22) (a generous gift from Dr. Thomas E. Hughes, Yale University, New Haven, USA) alone or co-transfected with CMV-iCre plasmid using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). The cells were further cultured for 48 hours, fixed with 4% paraformaldehyde, filtered through 70 μ m nylon meshes before subjecting to flow cytometry (Becton Dickinson FACS DiVA Flow Cytometry and Cell Sorter, BD Bioscience).

Generation of KAP2-iCre transgenic mouse

The transgene was released by digesting with *Ata* II and *Nde* I and purified by agarose gel electrophoresis and recovered using Qia-quick purification kit (Qiagen, Valencia, CA). The purified DNA was resuspended in microinjection buffer (10 mM Tris-Cl, pH7.5, 0.1 mM EDTA, 2 μ g/ml) and microinjected into the pronuclei of fertilized oocytes from C57BL/6J X SJL/J mice using standard procedures. The transgenic founders were backcrossed to C57BL/6J for a minimum of 5 generations. Transgenic progeny were identified by PCR analysis of tail DNA using the primers GGCCTTTGAACGCACTGAC and AGGGGCAGCCACACCAT. To induce expression of the transgene, female mice (6-8 months of age) were treated for 5 days with a subcutaneously implanted testosterone pellet (5 mg) designed to continuously release testosterone for 21 days (Innovative Research of America, Sarasota, FL) (5). In some experiments designed to assess if the transgene is super-induced, adult male mice were implanted with the same dose of testosterone. All experimental protocols were approved by the University of Iowa Animal Care and Use committee.

RNA isolation and RNase protection assay

Total RNA was isolated from organs of mice using Trizol reagent (MRC, Cincinnati, Ohio). A fragment was amplified from the iCre plasmid using primers: TGGCCTTTGAACGCACTGAC and AGGGGCAGCCACACCAT and subcloned to pCRII-TOPO plasmid (Invitrogen, Carlsbad, CA). The plasmid was linearized with *Hind* III and the antisense probe was labeled using T7 RNA polymerase (Stratagene, La Jolla, CA). An internal control, mouse 28S rRNA was also labeled using pTRI-28S as template (Ambion, Austin, TX). The sizes of protected fragments were 350 and 105 nucleotides, respectively. RNase protection assay was performed using the RPA III kit (Ambion, Austin, TX) on 50 μ g of total RNA.

X-gal staining and Immunohistochemistry

ROSA26 mice (B6.129S4-Gt(*ROSA*)26^{*tm1Sor*}/J) expressing LacZ after Cre-mediated recombination were obtained from the Jackson Laboratory (stock # 003474). ROSA26 mice were bred with KAP2-iCre transgenic mice to establish double transgenic heterozygote mice for study. Mice were sacrificed by CO₂ asphyxiation and perfused transcardially with cold PBS with 2 mM MgCl₂ and 4% paraformaldehyde (PFA) in PBS (pH 7.4). Organs were dissected and post fixed in 4% PFA for 2 hours and then in 30% sucrose overnight. 400 μ m vibratome sections were washed in permeabilization solutions (0.01% sodium deoxycholate, 0.02% NP-40 in PBS) for 1 hour and stained in X-gal solution (1 mg/ml X-gal, 5 mM potassium ferricyanide and 5 mM potassium ferrocyanide in PBS) overnight at 37°C. For

immunohistochemistry, sections were embedded in paraffin after X-gal staining and cut into 5 μ m sections and counter-stained with hematoxylin, AQP1, or AQP3 antibody (Alpha Diagnosis, San Antonio, TX) following the standard immunostaining procedures. Images were visualized under standard (X-gal appears blue) and under indirect fiber optic illumination (X-Gal appears Red, Dolan Jenner, Boxborough, MA) using a Nikon Eclipse E600 microscope. The indirect illumination was particularly helpful when low level β -Gal activity was observed as red staining on a yellow/brown background.

Results

Generation and Tissue-Specific Expression of KAP2-iCre Transgenic Mice

At the inception of this project we generated three different constructs utilizing the KAP2-angiotensinogen chimeric promoter to express several different variants of Cre-recombinase (cytoplasmic, nuclear and codon optimized) (Figure 1A). To confirm the activity of Cre-recombinase, we first placed each Cre-recombinase variant under the control of a human cytomegalovirus immediate-early gene (CMV) promoter, and performed co-transfections with the Cre-Stoplight plasmid in PCT3 cells. The Cre-Stoplight expresses DsRed in the absence of Cre-recombinase and eGFP in the presence of Cre-recombinase (22)(Figure 1B). PCT3 cells transfected with Cre Stoplight alone produced only DsRed, whereas cells transfected with both Cre Stoplight and CMV-iCre, produced eGFP (Figure 1C). The other Cre variants also showed evidence of recombinase activity but at a lower level than iCre.

A total of 26 transgenic founders for the three constructs were obtained. Each was bred to establish lines and was examined for evidence of Cre-recombinase expression. Twenty five of 26 lines either did not express the transgene, expressed it very weakly or exhibited ubiquitous weak expression. Only one line (line 29066/2) encoding the “improved” codon optimized Cre-recombinase showed evidence of kidney-specific expression. Strong expression of Cre-recombinase mRNA was detected in the kidney, with weaker expression evident in brain, heart and liver (Figure 2). The level of expression in extra-renal tissues was generally lower than in kidney in replicate assays from other mice (data not shown). We next crossed KAP2-iCre mice with ROSA26 reporter mice to assay for evidence of Cre-recombinase activity in renal and extrarenal tissues where we saw evidence of transgene mRNA. Expression of LacZ is induced in this strain of ROSA26 mice in response to Cre-recombinase (16).

In the liver, very few robustly stained hepatocytes (much less than 1% of cells) were evident in KAP2-iCre-positive ROSA-positive mice (Figure S1A). β -Gal activity was occasionally also observed in liver sections from KAP2-iCre-negative ROSA-positive (Figure S1B). In the brain, the only robust staining was observed in the choroid plexus, and this was observed in both KAP2-iCre-positive (Figure S2A-B) and -negative (Figure S2C) ROSA-positive mice suggesting endogenous β -Gal activity. No detectable X-gal staining was found in submandibular gland and spleen and only light staining was evident in testes and ovary (data not shown).

Androgen Induction of KAP2-iCre Expression

Under baseline conditions, there was little or no expression of the transgene in any tissue in female mice (data not shown). In male transgenic mice, exogenous testosterone did not affect the level of iCre mRNA in the kidney (Figure 3A). Importantly however, there was a robust induction of iCre expression in the kidney in female mice in response to exogenous testosterone (Figure 3B).

PTC Cell-Specific Expression of KAP2-iCre

In males, strong β -Gal staining was evident in the renal cortex of a KAP2-iCre-positive ROSA26-positive double transgenic (Figure 4A-B). Very weak staining was observed in outer stripe of the outer medulla in kidney from KAP2-iCre-negative ROSA26-positive mouse (Figure 4E-F). High magnification revealed the staining to be in proximal tubule cells (Figure 4C-D), which was confirmed by immunostaining with a proximal tubule-specific marker (Figure 5). β -Gal staining co-localized with the proximal tubule marker AQP1 but not with the collecting duct marker AQP3 in KAP2-iCre-positive/ROSA26-positive double transgenic mice (Figure 5).

Detecting β -Gal staining in females was more problematic due to background staining in ROSA-positive KAP2-iCre-negative mice. Non-transgenic and transgenic female mice without testosterone treatment exhibited X-gal staining in the outer stripe of the medulla, where S3 segments of the proximal tubule are localized (Figure 6 A-F). After testosterone treatment, β -Gal staining was clearly evident throughout the renal cortex in the S1 and S2 segments of the proximal tubule (Figure 6G-I). This was more evident under high magnification using indirect fiber optic illumination (compare Figure 6I to panels C and F). Due to the endogenous β -Gal activity, it was impossible to determine if specific cre-mediated recombination was occurring in the S3 segment.

Discussion

Here we describe the development and characterization of transgenic mice expressing iCre under control of the KAP promoter. Expression of the transgene was most robustly observed in the kidney, although Cre-recombinase mRNA could be detected at low levels in several other tissues including the brain and liver. Previous studies have shown that KAP is abundantly, but not exclusively expressed in the kidney. KAP has also been reported to be expressed in number of extra-renal tissues albeit at very low levels (18). That angiotensinogen is also expressed in liver and brain, and is part of the transgenic backbone used here may account for transgene expression in these tissues (21). Of note, we did not observe evidence for specific Cre-recombinase enzyme activity in these tissues.

Crossing Cre transgenic mice with ROSA26 reporter mice allowed us to confirm proximal tubule-specific Cre-recombinase activity in these mice. In male transgenic mice, iCre activity was specifically found in the renal proximal tubules, confirmed by co-localization with AQP1, but not AQP3. In female mice, iCre was only expressed after the mice were treated with testosterone. Based on the staining we observed in non-transgenic female mice, we conclude that the staining in the outer stripe of the outer medulla was due to activity of the endogenous β -galactosidase and not ectopic Cre-recombinase activity. The apparent endogenous β -Gal staining in the outer medulla of the female non-transgenic mouse will not limit the use of female mice for future studies because this is an artifact of β -Gal system and not the promoter driving cre-recombinase. As in any cell-specific knockout or knockdown experiment, the investigator will have to demonstrate the effectiveness of the deletion on their target gene in the cells of interest.

Several transgenic mouse strains that express Cre-recombinase in renal proximal tubules have been reported under the control of the gamma-glutamyl transpeptidase (γ GT) (6), phosphoenolpyruvate carboxykinase (PEPCK) (12), and the sodium-glucose co-transporter type 2 (SGLT2) promoters (13). The KAP2-iCre mouse has the advantage of being much more tissue restricted than the PEPCK promoter which is highly active in the liver. Moreover, expression of the KAP2-iCre transgene is androgen inducible. We previously demonstrated in KAP-human angiotensinogen transgenic mice that testosterone regulated expression of the transgene was dose and time dependent, would decay back to baseline

several days after withdrawal of the steroid, and could be modulated in males by either castration or flutamide, an anti-androgen (5). High concentrations of estrogen could also induce the transgene in female mice. Consequently, the level of Cre-recombinase expression is likely to be controllable in both males and females.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bianco RA, Keen HL, Lavoie JL, Sigmund CD. Untraditional methods for targeting the kidney in transgenic mice. *Am J Physiol Renal Physiol.* 2003; 285:F1027–F1033. [PubMed: 14600026]
2. Davisson RL, Ding Y, Stec DE, Catterall JF, Sigmund CD. Novel mechanism of hypertension revealed by cell-specific targeting of human angiotensinogen in transgenic mice. *Physiol Genomics.* 1999; 1:3–9. [PubMed: 11015555]
3. Davisson RL, Kim HS, Kregge JH, Lager DJ, Smithies O, Sigmund CD. Complementation of reduced survival, hypotension and renal abnormalities in angiotensinogen deficient mice by the human renin and human angiotensinogen genes. *J Clin Invest.* 1997; 99:1258–1264. [PubMed: 9077534]
4. Ding Y, Davisson RL, Hardy DO, Zhu L-J, Merrill DC, Catterall JF, Sigmund CD. The kidney androgen-regulated protein (KAP) promoter confers renal proximal tubule cell- specific and highly androgen-responsive expression on the human angiotensinogen gene in transgenic mice. *J Biol Chem.* 1997; 272:28142–28148. [PubMed: 9346970]
5. Ding Y, Sigmund CD. Androgen-Dependent Regulation of Human Angiotensinogen Expression in KAP-hAGT Transgenic Mice. *Am J Physiol Renal Physiol.* 2001; 280:F54–F60. [PubMed: 11133514]
6. Dworniczak B, Skryabin B, Tchinda J, Heuck S, Seesing FJ, Metzger D, Chambon P, Horst J, Pennekamp P. Inducible Cre/loxP recombination in the mouse proximal tubule. *Nephron Exp Nephrol.* 2007; 106:e11–e20. [PubMed: 17356303]
7. Gawlik A, Quaggin SE. Deciphering the renal code: advances in conditional gene targeting. *Physiology (Bethesda).* 2004; 19:245–252. [PubMed: 15381752]
8. Igarashi P. Kidney-specific gene targeting. *J Am Soc Nephrol.* 2004; 15:2237–2239. [PubMed: 15284310]
9. Ingelfinger JR, Zuo WM, Fon EA, Ellison KE, Dzau VJ. In situ hybridization evidence for angiotensinogen messenger RNA in the rat proximal tubule. An hypothesis for the intrarenal renin angiotensin system. *J Clin Invest.* 1990; 85:417–423. [PubMed: 2298917]
10. Kim HS, Kregge JH, Kluckman KD, Hagaman JR, Hodgins JB, Best CF, Jennette JC, Coffman TM, Maeda N, Smithies O. Genetic control of blood pressure and the angiotensinogen locus. *Proc Natl Acad Sci USA.* 1995; 92:2735–2739. [PubMed: 7708716]
11. Lavoie JL, Lake-Bruse KD, Sigmund CD. Increased Blood Pressure in Transgenic Mice Expressing Both Human Renin and Angiotensinogen In The Renal Proximal Tubule. *Am J Physiol Renal Physiol.* 2004; 286:F965–F971. [PubMed: 15075192]
12. Rankin EB, Tomaszewski JE, Haase VH. Renal cyst development in mice with conditional inactivation of the von Hippel-Lindau tumor suppressor. *Cancer Res.* 2006; 66:2576–2583. [PubMed: 16510575]

13. Rubera I, Poujeol C, Bertin G, Hasseine L, Counillon L, Poujeol P, Tauc M. Specific Cre/Lox recombination in the mouse proximal tubule. *J Am Soc Nephrol*. 2004; 15:2050–2056. [PubMed: 15284291]
14. Shimshek DR, Kim J, Hubner MR, Spergel DJ, Buchholz F, Casanova E, Stewart AF, Seeburg PH, Sprengel R. Codon-improved Cre recombinase (iCre) expression in the mouse. *Genesis*. 2002; 32:19–26. [PubMed: 11835670]
15. Soler M, Tornavaca O, Sole E, Menoyo A, Hardy D, Catterall JF, Vandewalle A, Meseguer A. Hormone-specific regulation of the kidney androgen-regulated gene promoter in cultured mouse renal proximal-tubule cells. *Biochem J*. 2002; 366:757–766. [PubMed: 12030848]
16. Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet*. 1999; 21:70–71. [PubMed: 9916792]
17. Stec DE, Sigmund CD. Modifiable gene expression in kidney: kidney-specific deletion of a target gene via the cre-loxP system. *Exp Nephrol*. 1998; 6:568–575. [PubMed: 9807030]
18. Toole JJ, Hastie ND, Held WA. An abundant androgen-regulated mRNA in the mouse kidney. *Cell*. 1979; 17:441–448. [PubMed: 455472]
19. Torres M, Gomez-Pardo E, Dressler GR, Gruss P. Pax-2 controls multiple steps of urogenital development. *Development*. 1995; 121:4057–4065. [PubMed: 8575306]
20. Wu F. Conditional targeting in the kidney. *Nephron Physiol*. 2007; 107:10–16.
21. Yang G, Merrill DC, Thompson MW, Robillard JE, Sigmund CD. Functional expression of the human angiotensinogen gene in transgenic mice. *J Biol Chem*. 1994; 269:32497–32502. [PubMed: 7798251]
22. Yang YS, Hughes TE. Cre stoplight: a red/green fluorescent reporter of Cre recombinase expression in living cells. *Biotechniques*. 2001; 31:1036, 1038, 1040–1036, 1038, 1041. [PubMed: 11730010]

Figure 1. Map of KAP2-iCre transgene and confirmation of Cre activity in PCT3 cells

A. A schematic map of the transgene is shown. B. A schematic representation of the Cre Stoplight plasmid is shown. After cre-mediated recombination, the DsRed is “floxed” out and eGFP is brought into close proximity to the CMV promoter. C. FACS sorting of PCT3 cells transfected with Stoplight alone (left) or co-transfected with Stoplight and CMV-iCre.

Figure 2. Tissue-specific expression of KAP2-iCre

RNase protection assay was performed on whole tissue RNAs from a male non-transgenic (Tg-) and male transgenic (Tg+) mice. The position of the Cre-recombinase and 28S RNAs is shown. Br, brain; Lg, lung; Ht, heart; Sg, submandibular gland; Lv, liver; Sp, spleen; Ts, testes; K, kidney. Representative of at least 6 different mice.

Figure 3. Androgen-induction of iCre transcription in the kidney

Total RNA isolated from kidneys of testosterone treated or untreated male (A, n=4 each $\pm$ androgen) and female (B, n=4 each $\pm$ androgen) mice was analyzed using RPA. The genotype and treatment with androgen are indicated by the + and -.

Figure 4. Expression of lacZ in kidneys of male KAP2-iCre/ROSA26 double transgenic mice
 X-gal staining of kidney section from male KAP2-iCre+/ROSA26+ double transgenic mice (A, B, E and F), and male KAP2-iCre-/ROSA26+ transgenic mice (C and D). Images in A, C, D, and E were taken with direct illumination, whereas images in B and F were taken under indirect fiber optic illumination. C is a higher magnification of the indicated area in A and D is a higher magnification of the indicated area in C. Representative of at least 4 different mice. Bar=100 μ m.

Figure 5. Localization of LacZ in renal proximal tubules in male KAP2-iCre/ROSA26 double transgenic mice

A. X-gal staining followed by hematoxylin counter-staining. B. X-gal staining followed by AQP3 labeling. C-D. AQP1 staining showed the co-localization of lacZ and AQP1 in the epithelial cells of proximal tubules. D. Higher power magnification of the indicated area of panel C. Bar=100 μ m.

Figure 6. Expression of LacZ in kidneys of female KAP2-iCre/ROSA26 double transgenic mice
Endogenous galactosidase activity was found in the outer strip of medulla of KAP2-iCre-/ROSA26+ (A-C) and untreated female KAP2-iCre+/ROSA26+ mice (D-F). Blue staining in the cortex in testosterone-treated female KAP2-iCre+/ROSA26+ mice (G-I). B, E and H are images taken under indirect fiber optic illumination. C, F and I are higher magnification of the indicated frames in B, E and H. The arrow denotes the boundary of the renal capsule. Bar=100 μ m. Representative of at least 3 different mice. Glomeruli are indicated by g.