Protocol Detail Report

Printed By: CHEN, JINDONG
2/21/2013 11:44:01 AM

Protocol Information

Reference # 101477
Protocol # UCAR-2012-015
Protocol Type: Original
PI: CHEN, JINDONG
Approval Date: 5/30/2012
Submittal Date: 5/21/2012
Effective Date: 5/30/2012
Author: CHEN, JINDONG
Renewal Date: 5/30/2015
Status: Approved
Next Review Date: 5/30/2013
Inactive Date:
Expiration Date: 5/30/2015

Administrative Information 1

Reference Number 1.1

Reference Number (assigned by the system)
101477

Principal Investigator 1.2

CHEN, JINDONG | | jindong_chen@urmc.rochester.edu

Department 1.3

UROLOGY

Contact Information 1.4

Provide the following contact information for the PI and at least one other person who can make decisions about the animals in the PI's absence. UCAR or DLAM will attempt to contact the people on this list in the order given if there are questions about, or problems with your animals.

Name	UoR Mailing Address	UoR Telephone	Other Phone(s)	Email
Jindong Chen	Department of Urology mail box 656	585-273-1601	616-581-1639	jindong_chen@URMC.rochester.edu
Shuhui Si	Department of Urology mail box 656	585-273-1600	585-354-0810	shuhui_si@URMC.rochester.edu
Sue Scheon	Department of Urology mail box 656	585-275-1191	585-727-9225	sue_scheon@URMC.rochester.edu
Guan Wu	Department of Urology mail box 656		585-208-5433	guan_wu@URMC.rochester.edu

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Title

1.5

Provide a single title for the protocol.
Development of double knockout kidney cancer mouse models

Protocol Type

1.6

Protocol Type (assigned by the system)
Original

Author

1.7

The person who has responsibility for preparing the protocol should be named the author. It does not have to be the PI.

CHEN, JINDONG		jindong_chen@urmc.rochester.edu

Created By

1.8

Created By (assigned by the system)

UCAR, Protocol Admin		ucar@urmc.rochester.edu

Unrestricted View

1.9

The default for this question is NO. If you check YES, ALL users of the system will have access to this protocol.

☐ **Yes** ☑ **No**

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Protocol Associates

1.10

Each staff member who will be working with animals must be included on the protocol. In addition, you may wish to include administrative staff members as well. Each associate on the protocol may be assigned the role of Co-PI, Key Associate or Authorized to order animals. See Help for more information on these roles.
In addition to assigning special status to the associate, you must also list their responsibilities on the protocol (procedures that they will do, or other role if not working with animals) in the field labeled Responsibilities and describe their training to carry out these responsibilities in the field labeled Comments.

NOTE: Make sure to notify the UCAR office of all staff that you will be assigning the role of Co-PI. They will each need an account in the GRANITE system. Likewise, make sure to notify UCAR when someone leaves your laboratory so that they can be removed from the list, because all Associates listed on the protocol will have read-only access to your protocol after it is approved.

CHEN, JINDONG

Responsibilities colony management, administrative work

Comments Trained at Van Andel Research Institite in 2003, National Cancer Centre Singapore in 2011, Universiity of Rochester in 2012, with 9 years of experience in mouse modeling.

☑ **Co-Investigator** ☐ **Key Associate** ☑ **Authorized To Order Animals**

SCHOEN, SUSAN R.

Responsibilities administrative work

Comments

☑ **Co-Investigator** ☐ **Key Associate** ☐ **Authorized To Order Animals**

SI, SHUHUI

Responsibilities colony management including breeding, tagging, genotyping, monitoring, and euthanisia of animals

Comments Trained at Uiversity of Rochester in 2011, no experience in mouse work, will be further trained by Dr. Jindong Chen and vivarium staffs.

☑ **Co-Investigator** ☐ **Key Associate** ☐ **Authorized To Order Animals**

WU, GUAN

Responsibilities administrative work

Comments

☑ **Co-Investigator** ☐ **Key Associate** ☐ **Authorized To Order Animals**

Notes to UCAR

1.11

If you have any comments or questions for UCAR, provide them here.

General Protocol Information

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Scientific Abstract(s) 2.1

For each grant covered by this protocol provide theTitleAbstract

Please make sure statements relating to the animal model to be used in your studies are included. If this is a teaching protocol, provide the course description.

Development of double knockout kidney cancer mouse models
Malignant kidney tumors are subclassified into clear cell renal cell carcinoma (CCRCC), papillary renal cell carcinoma (PRCC), chromophobe renal cell carcinoma (CRCC), collecting duct carcinoma (CDC), and unclassified renal cell carcinoma. CCRCC is the most common one, accounting for approximate 75% of renal cell carcinoma cases. Studies of kidney cancer pathogenesis have been limited by the paucity of genetically defined animal models. VHL (von Hippel-Lindau) gene is the first important kidney cancer-related gene responsible for CCRCC and associated with VHL syndrome. VHL mutations have been identified in approximately 70% of CCRCC patients. However, knockout of VHL gene in the mouse kidney failed to cause kidney tumors except kidney cysts, implying that additional genetic alterations may be required besides VHL mutation. Recently, a new kidney cancer-related gene PBRM1 (Protein polybromo-1) was identified through exome sequencing in a series of primary CCRCC. Study showed that around 40% examined CCRCC cases carried PBRM1 gene mutations, indicating PBRM1 is the second most important kidney cancer-related gene following VHL. In addition, a PBRM1 partner gene called ARID1A is identified as mutated in around 30% of kidney cancers. PBRM1 and ARID1A can form a complex and synergetically suppress tumor formation. To determine the correlation of VHL, PBRM1, and ARID1A and their roles in tumorigenesis and better understand the pathogenesis of CCRCC, we decide to develop kidney-specific PBRM1, ARID1A knockout mouse models. Since tumorigenesis may require more than one genetic change to progress, knockout of PBRM1 or ARID1A individually may not guarantee formation of tumors as in the case of VHL knockout mouse model. Thus, we further want to double disrupt PBRM1 plus VHL and PBRM1 plus ARID1A to generate kidney-specific PBRM1-VHL and PBRM1-ARID1A double knockout mouse models. We expect that these mouse models will generate CCRCC tumors and are proper for pathogenesis study and act as powerful preclinical drug test tool that could lead to better treatment and care for patients with PBRM1/VHL mutations and other forms of cancer involving the same cancer-causing pathways.

Lay Abstract 2.2

Provide a brief (100-200 word) overview of the proposed use of animals in terms comprehensible to the general lay public. Indicate how the animal studies proposed may ultimately benefit humans and/or animals.

Development of disease animal models allows researchers to investigate human diseases in ways which would be inaccessible in human patients. Mouse is the commonly selected animal for disease modeling due to their short generation time, prolific breeding, small size, and similar gene structure and function to human's. However, studies of kidney cancer pathogenesis have been limited by the paucity of genetically defined animal models. VHL is the first identified and important kidney cancer-related gene which is responsible for clear cell renal cell carcinoma (CCRCC). Of all kidney cancers, 80% belong to CCRCC. However, VHL-deficient mouse model failed to generate kidney tumors, implying that additional genetic changes in other kidney cancer-related genes may be required. Recently, a new kidney cancer-related gene PBRM1 was identified. PBRM1 mutations account for 40% kidney cancer cases, indicating PBRM1 is the second most important kidney cancer-related gene following VHL. The thord gene called ARID1A, which is a PBRM1 partner gene, is aiso associated with around 30% of kidney cancer cases. Thus, we decide to develop kidney-specific PBRM1 and ARID1A knockout mouse models respectively. In case of disruption of PBRM1/ARID1A alone may not guarantee the formation of tumors as in the case of VHL-deficent mouse model, we further plan to disrupt two genes simultaneously in a mouse model, namely, PBRM1 plus VHL and PBRM1 plus ARID1A, to generate kidney-specific PBRM1-VHL and PBRM1-ARID1A double knockout mouse models respectively. In this way, we expect that these mouse models will generate kidney tumors and are proper for pathogenesis study and can be used for drug screening tools in preclinical studies.

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Funding Source(s) 2.3

Provide a list of all sources of funding covered by this protocol. Note that the title is limited to 50 characters. If your title is longer, you may truncate it. The full title should be with the scientific abstract.

Funding Agency	Grant number	Title	PI
Department	Under review, see attachment below	Development of double knockout kidney cancer mouse models	Jindong Chen

Attach Grant 3

Attach grant 3.1

Please attach a copy of the following sections of your grant to this section in either MS Word or PDF format:AbstractSpecific AimsExperimental DesignVertebrate Animal Section

Please do not include Budget, Resources, Biosketches

The protocol and the grant must be reviewed to make sure all work on live animals described in the grant application is also described in the protocol.

Note: The grant will be removed from the protocol once the protocol is approved. If you do not want to attach you grant, email a copy to UCAR@urmc.rochester.edu.

5.0 Conversion Species Section 4

Species 4.1

4.1.1

MOUSE #1

Description 4.1.1.1

If you have more than one section for this species, provide a very brief description that will help distinguish this section from the others.

Sglt2-Cre mice carry Cre recombinase which only expresses in mouse kidney proximal tubules. PBRM1-flox mice were tagged by inserting two loxP sequences which can be recognized by Cre recombinase. When PBRM1-flox mice cross to Sglt2-Cre mice, PBRM1 gene will be deleted in the kidney proximal tubules only, not in other tissues. Similarily, when ARID1A-flox and VHL-flox mice cross to Sglt2-Cre mice, ARID1A and VHL will be disrupted in kidney proximal tubules only.

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Mouse strains

4.1.1.2

For each strain of mouse provideStrain nameAny known impairment, or special needs required to maintain this strain. If none, indicate NONE.Indicate (Y or N) whether you are willing to share the information that you have this strain with other investigators.

Strain	Impairment or special care	Share?
VHL-flox	none	Y
VHL knockout	Mice may develop kidney cysts. When mice are more than 6 months, kidneys may get enlarged due to cysts/yumors, close watch is needed. Once enlarged kidneys are palpable and observable, affected mice may become distressed and will be euthanized.	Y
PBRM1-flox	none	N
PBRM1 knockout	Mice may develop kidney cysts. When mice are more than 6 months, kidneys may get enlarged due to cysts/yumors, close watch is needed. Once enlarged kidneys are palpable and observable, affected mice may become distressed and will be euthanized.	N
ARID1A-flox	none	N
ARID1A knockout	Mice may develop kidney cysts. When mice are more than 6 months, kidneys may get enlarged due to cysts/yumors, close watch is needed. Once enlarged kidneys are palpable and observable, affected mice may become distressed and will be euthanized.	N
PBRM1-VHL knockout	Mice may develop kidney cysts. When mice are more than 6 months, kidneys may get enlarged due to	

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cysts/yumors, close watch is needed. Once enlarged kidneys are palpable and observable, affected mice may become distressed and will be euthanized.	N	PBRM1-ARID1A knockout
Mice may develop kidney cysts. When mice are more than 6 months, kidneys may get enlarged due to cysts/yumors, close watch is needed. Once enlarged kidneys are palpable and observable, affected mice may become distressed and will be euthanized.	N	Sglt2-Cre
none	Y	

Authorized Amounts

4.1.1.3

Attachments: Help with Animal Use Categories

Provide the total number of NEW animals you plan to request for up to 3 years.
Add one field for each category (stress level C, D, or E) in which you are requesting animals. See the attached help section to determine the criteria for each category.
If you are breeding animals or buying pregnant females, all animals that you expect to be born must be included in your numbers, even if they cannot be used for experiments. Do not include animals used as embryos in your numbers.
Please include each animal in only one category -- the category that reflects the most invasive or potentially painful use (E>C).

Stress Category	Requested	OnOrder	Received	Available
CATEGORY C	1822	0	24	1798
Totals	**1822**	**0**	**24**	**1798**

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Outline of Animal Use and Justification of Numbers

4.1.1.4

Attachments: Help and Examples for Justification of Numbers

Provide an outline of the studies to be performed on live animals. For each study, indicate: the experimental groups and control groups to be tested, a simple listing of experimental procedures to be performed on each group, the number of animals in each group and the category of use (C, D, or E).

If more than one procedure (other than breeding, genotyping, ID, and other husbandry procedures and euthanasia) will be done on any animals, provide a flowchart or time line that shows what procedures will be done. Make sure to describe the maximum number of procedures that an individual animal might experience.

State how you determined group size. (Note: Group size may be justified by: a) the known or estimated variability in your control population(s) and the minimum statistically significant change you plan to determine experimentally (e.g. power analysis); and/or b) the amount of a component required for measurements, and the amount of that component that can be obtained from an individual animal.)

Explain how you determined the total number of animals that you have requested. Account for all animals, even those that will not be used experimentally, including breeders and pups. If you must breed animals that cannot be used experimentally, discuss why they cannot be used, and show the calculations you used to determine how many must be bred to obtain the number you need for experiments.

Please do not provide the entire Experimental Design section of your grant here. Provide a summary that describes what animals will experience, how many animals will experience it, and how you arrived at that number.

First, we will obtain the following mice for our study. These mice are floxed by insertion of LoxP sequences flanking the targeting genes and will grow normally because the knockout is not completed until the animal is exposed to Cre recombinase. The floxed gene will be disrupted once it is exposed to Cre recombinase which is supposed to cut the gene following the two special Cre sequences flanking it. Usually, Cre deletes a segment of gene in an experimental animal and has been used to generate animals with mutations limited to certain cell types.

Mice will be imported:

(1) Sglt2-Cre transgenic mouse from VARI (Van Andel Research Institute)
(2) VHL-flox/flox transgenic mice from VARI
(3) Kidney-specific VHL knockout mice (VHL-flox/flox; Sglt2-Cre) from VARI
(4) PBRM1-flox mice (PBRM1-flox/flox) from Massachusetts General Hospital, Harvard Medical School
(5) ARID1A-flox mice (ARID1A-flox/flox) from Massachusetts General Hospital, Harvard Medical School

We intend to import 10 mice (5 mice each sex) for each strain. In total, 50 transgenetic mice will be transferred to URMC vivarium.

Mice obtained from above sources will be used for i) generating PBRM1 knockout mice, ii) generating ARID1A knockout mice, iii) producing double PBRM1-VHL double knockout mice, iv) developing PBRM1-ARID1A double knockout mice. All the estimations listed below are calculated based on the average litter size is 10 mice.

EXPERIMENT #1 (year 1)

Number of mice needed in this experiment to generate kidney proximal tubule-specific PBRM1 knockout mice (PBRM1-flox/flox; Sglt2-Cre-tg/+). Below are the calculations:

Mice are needed at Step #1A: to create kidney proximal tubule-specific PBRM1 heterozygous knockout mice (PBRM1-flox/+; Sglt2-Cre-tg/+) in this step, two imported homozygous PBRM1-Flox mice (one each sex) will be crossed to two Sglt2 -Cre (tg/+) transgenic mice (one each sex) to produce knockout PBRM1 heterozygotes. The mice will breed at least twice. Of their offspring, 50% will be heterozygotes (PBRM1-flox/+; Sglt2-Cre-tg/+). Given an approximate litter of 10 pups, that should produce around 40 offspring and approximate 20 of them should be heterozygous knockout mice. Assuming the error rate is 10%, 44 mice are needed in this step to meet our requirement.

Total mice generated in Step #1A: 4x10+10%(4x10)=44

Mice are needed at Step #1B: to produce kidney proximal tubule-specific PBRM1 homozygous knockout mice (PBRM1-flox/flox; Sglt2-Cre-tg/+) two female and two male heterozygous PBRM1-flox mice generated in experiment #1A will be inbred to produce homozygous knockout PBRM1 mice. The mice will breed three times. Of their offspring, approximately 20% will be homozygotes. Given an approximate litter of 10 pups, that should produce 60 offspring and approximate 12 of them should be homozygous knockout mice. Assuming the error rate is 10%, 66 mice will be produced in this step.

Total mice generated at Step #1B: 6x10+10% (6x10)=66

Mice are needed at Step #1C: to quickly obtain desired number of kidney proximal tubule-specific PBRM1 homozygous knockout mice (PBRM1-flox/flox; Sglt2-Cre-tg/+), two female and two male heterozygous PBRM1-Flox mice generated in experiment #1B will be inbred. The number of mice will meet the following phenotype analysis at 10 intervals. (for each interval, we will assess 3 genotypes:
1) PBRM1-floxhomozygous; Sglt2-Cre heterozygous, 2) double heterozygous, and 3) PBRM1 wild type; Sglt2-Cre heterozygous):

1) 2 months old
2) 4 months old
3) 6 months old
4) 8 months old
5) 10 months old
6) 12 months old
7) 14 months old
8) 16 months old
9) 18 months old
10) 20 months old

To calculate the mouse number of each group, a p value=0.05 and a power level between 80% and 90% were adopted (80%, needs 17 mice, 90% needs 22 mice). Thus, for each of these 10 groups, we will use 20 homozygous knockout mice per genotype group to assess the phenotype. In total, we need 200 homozygous knockout mice (10x20). In this experiment, three female and three male homozygous PBRM1-Flox knockout mice (PBRM1-flox/flox; Sglt2-Cre-tg/+) generated in experiment #1B will be inbred to produce homozygous knockout PBRM1 mice. Of their offspring, approximately 75% will be homozygotes. Thus, we need generate 267 pups to give rise to 200 homozygous knockout mice. Considering 10% error rate, we need to produce approximate 294 pups to meet our requirement. Given an approximate litter of 10 pups, the three pairs of mating mice need to breed 10 times.

Total mice will be produced in Step #1C: 300 (10x10x3)

Totals for Experiment #1 (year 2):
Breeding Step 1A: 44
Breeding Step 1B: 66
Breeding Step 1C: 300
Total: 410

EXPERIMENT #2 (year 1)

Number of mice needed in this experiment to generate kidney proximal tubule-specific ARID1A knockout mice (ARID1A-flox/flox; Sglt2-Cre-tg/+). Below are the calculations:

Mice are needed at Step #2A: to create kidney proximal tubule-specific PBRM1 heterozygous knockout mice (ARID1A -flox/+; Sglt2-Cre-tg/+) in this step, two imported homozygous ARID1A -Flox mice (one each sex) will be crossed to two Sglt2-Cre (tg/+) transgenic mice (one each sex) to produce knockout ARID1A heterozygotes. The mice will breed at least twice. Of their offspring, 50% will be heterozygotes (ARID1A -flox/+; Sglt2-Cre-tg/+). Given an approximate litter of 10 pups, that should produce around 40 offspring and approximate 20 of them should be heterozygous knockout mice. Assuming the

error rate is 10%, 44 mice are needed in this step to meet our requirement.

Total mice generated in Step #2A: 4x10+10%(4x10)=44

Mice are needed at Step #2B: to produce kidney proximal tubule-specific PBRM1 homozygous knockout mice (ARID1A -flox/flox; Sglt2-Cre-tg/+) two female and two male heterozygous ARID1A -flox mice generated in experiment #2A will be inbred to produce homozygous knockout PBRM1 mice. The mice will breed three times. Of their offspring, approximately 20% will be homozygotes. Given an approximate litter of 10 pups, that should produce 60 offspring and approximate 12 of them should be homozygous knockout mice. Assuming the error rate is 10%, 66 mice will be produced in this step.

Total mice generated at Step #2B: 6x10+10% (6x10)=66

1) Mice are needed at Step #2C: to quickly obtain desired number of kidney proximal tubule-specific ARID1A homozygous knockout mice (ARID1A -flox/flox; Sglt2-Cre-tg/+), two female and two male heterozygous ARID1A-Flox mice generated in experiment #2B will be inbred. The number of mice will meet the following phenotype analysis at 10 intervals (for each interval, we will assess 3 genotypes:
1) ARID1A-floxhomozygous; Sglt2-Cre heterozygous, 2) double heterozygous, and 3) ARID1A wild type; Sglt2-Cre heterozygous):

1) 2 months old
2) 4 months old
3) 6 months old
4) 8 months old
5) 10 months old
6) 12 months old
7) 14 months old
8) 16 months old
9) 18 months old
10) 20 months old

As calculated as at Step #1C (EXPERIMENT #1), total mice will be produced in Step #2C: 300 (10x10x3)

Totals for Experiment #2 (year 2):
Breeding Step 2A: 44
Breeding Step 2B: 66
Breeding Step 2C: 300
Total: 410

EXPERIMENT #3 (year 2)

Number of mice needed in this experiment to generate kidney proximal tubule-specific PBRM1-VHL double knockout mice (PBRM1-flox/flox; VHL-flox/flox; Sglt2-Cre-tg/+). Below are the calculations:

Mice are needed at Step #3A: to generate PBRM1-VHL double heterozygous mice (PBRM1-flox/+; VHL-flox/+; Sglt2-Cre-tg/+), two females (one PBRM1-flox/flox; Sglt2-Cre-tg/+ and one VHL-flox/flox; Sglt2-Cre-tg/+) and two males (one PBRM1-flox/flox; Sglt2-Cre-tg/+ and one VHL-flox/flox; Sglt2-Cre-tg/+) from above experiments will be used to set up two mating cages. Each mating pair will be inbred at least twice. Given an approximate litter of 10 pups, that should produce 40 (2x4x10) mice.

Approximately 75% of the total 40 mice will be PBRM1-VHL double heterozygous mice (PBRM1-flox/+; VHL-flox/+; Sglt2-Cre-tg/+). Thus, roughly 30 mice will be PBRM1-VHL double heterozygous. Considering 10% error rate, 44 mice should be produced in this step.

In total, mice needed at Step 3A: 44

Mice are needed at Step #3B: to produce PBRM1-VHL double knockout mice, PBRM1-VHL double heterozygous (PBRM1-flox/+; VHL-flox/+; Sglt2-Cre-tg/+) mice will be inbred (3 mating cages). We will expect to obtain approximate six PBRM1-VHL double knockout mice at this step. From this cross, around 5% of the offspring will be PBRM1-VHL double knockout mice. Given an approximate litter of 10 pups, we need 120 pups (6/5%) (12 litters) to give rise to 6 PBRM1-VHL double knockout mice.

Considering 10% error rate, 132 pups are needed at this step #3B.

In total, mice needed in Step 3B: 132 (120+120/10%)

Mice are needed at Step #3C: to produce enough number of PBRM1-VHL double knockout mice (PBRM1-flox/flox; VHL-flox/flox; Sglt2-Cre-tg/+) for phenotype analysis, PBRM1-VHL double knockout mice from Step #3B will be further inbred. The phenotype will be also assessed at 10 intervals:

1) 2 months old
2) 4 months old
3) 6 months old
4) 8 months old
5) 10 months old
6) 12 months old
7) 14 months old
8) 16 months old
9) 18 months old
10) 20 months old

For each of these 10 groups, we will use 20 homozygous knockout mice per genotype group to assess the phenotype as above. In total, we need 200 homozygous knockout mice (10x20). In this experiment, three female and three male homozygous PBRM1-VHL-Flox knockout mice (PBRM1-flox/flox; VHL-flox/flox; Sglt2-Cre-tg/+) generated in experiment #3B will be inbred to produce required homozygous double knockout PBRM1-VHL mice. Of their offspring, approximately 75% will be homozygotes. Thus, we need generate 267 pups to give rise to 200 homozygous knockout mice. Considering 10% error rate, we need to produce approximate 294 pups to meet our requirement. Given an approximate litter of 10 pups, the three pairs of mating mice need to breed 10 times.

Total mice will be produced in Step #3C: 300 (10x10x3)

Totals for Experiment #3 (year 2):
Breeding Step 3A: 44
Breeding Step 3B: 132
Breeding Step 3C: 300
Total: 476

EXPERIMENT #4 (year 3)

Number of mice needed in this experiment to generate kidney proximal tubule-specific PBRM1-ARID1A double knockout mice (PBRM1-flox/flox; ARID1A-flox/flox; Sglt2-Cre-tg/+). Below are the calculations:

Mice are needed at Step #4A: to generate PBRM1-ARID1A double heterozygous mice (PBRM1-flox/+; ARID1A-flox/+; Sglt2-Cre-tg/+), two females (one PBRM1-flox/flox; Sglt2-Cre-tg/+ and one ARID1A-flox/flox; Sglt2-Cre-tg/+) and two males (one PBRM1-flox/flox; Sglt2-Cre-tg/+ and one ARID1A-flox/flox; Sglt2-Cre-tg/+) from above experiments will be used to set up two mating cages. Each mating pair will be inbred at least twice. Given an approximate litter of 10 pups, that should produce 40 (2x4x10) mice.

Approximately 75% of the total 40 mice will be PBRM1-ARID1A double heterozygous mice (PBRM1-flox/+; ARID1A-flox/+; Sglt2-Cre-tg/+). Thus, roughly 30 mice will be PBRM1-ARID1A double heterozygous. Considering 10% error rate, 44 mice

should be produced in this step.

In total, mice needed at Step 4A: 44

Mice are needed at Step #4B: to produce PBRM1-ARID1A double knockout mice, PBRM1-VHL double heterozygous (PBRM1-flox/+; ARID1A-flox/+; Sglt2-Cre-tg/+) mice will be inbred (3 mating cages). We will expect to obtain approximate six PBRM1-VHL double knockout mice at this step. From this cross, around 5% of the offspring will be PBRM1-VHL double knockout mice. Given an approximate litter of 10 pups, we need 120 pups (6/5%) (12 litters) to give rise to 6 PBRM1-ARID1A double knockout mice. Considering 10% error rate, 132 pups are needed at this step #4B.

In total, mice needed in Step 4B: 132 (120+120/10%)

Mice are needed at Step #4C: to produce enough number of PBRM1-ARID1A double knockout mice (PBRM1-flox/flox; ARID1A-flox/flox; Cre-tg/+) for phenotype analysis, PBRM1-ARID1A double knockout mice from Step #4B will be further inbred. The phenotype will be also assessed at 10 intervals, as Step #3C (EXPERIMENT #3), the same number of mice (300) will be used at this step.

Total mice will be produced in Step #4C: 300 (10x10x3)

Totals for Experiment #4 (year 3):
Breeding Step 4A: 44
Breeding Step 4B: 132
Breeding Step 4C: 300
Total: 476

TOTALS FOR PROTOCOL:
Mice imported 50
Experiment #1 410
Experiment #2 410
Experiment #3 476
Experiment #4 476
Total: 1822

Grand total number for this study is 1822 mice

Tissue harvest only

4.1.1.5

The items below refer to animals that will be used for tissue harvest ONLY. No procedures other than colony management (breeding, genotyping, etc.) and euthanasia are done on them.

- ☐ All Animals on this protocol will be used for tissue harvest only.
- ☑ Some animals on this protocol will be used for tissue harvest only.

4.1.1.5.1

All Animals on this protocol will be used for tissue harvest only.

4.1.1.5.2

Some animals on this protocol will be used for tissue harvest only.

Groups for tissue harvest only

4.1.1.5.2.1

Identify the groups of animals that will be used for tissue harvest only.
VHL-flox, PBRM1-flox, ARID1A-flox, and Sglt2-Cre mice.

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Procedures I

4.1.1.6

Check each procedure that will be done on the protocol.

- [x] Breeding
- [x] Genotyping
- [x] Animal Identification
- [ ] Non-standard housing (including individual housing)
- [ ] Special diet or water

4.1.1.6.1

Breeding

Breeding

4.1.1.6.1.1

Check all that apply to your protocol.

- [x] Standard breeding: 1-3 females:1 male, animals separated before birth of pups
- [ ] Continuous breeding: animals not separated before birth of pups
- [x] Standard weaning (Mice - 21-28 days, Rats - by 23 days)
- [ ] Delayed weaning (Mice - after 28 days, Rats - after 23 days)
- [ ] Animals will be euthanized before weaning.

4.1.1.6.1.1.1

Standard breeding: 1-3 females:1 male, animals separated before birth of pups

Colony manager(s) for standard breeding

4.1.1.6.1.1.1.1

Please list the staff members who will be responsible for colony management.
Shuhui Si, Jindong Chen

4.1.1.6.1.1.2

Continuous breeding: animals not separated before birth of pups

4.1.1.6.1.1.3

Standard weaning (Mice - 21-28 days, Rats - by 23 days)

4.1.1.6.1.1.4

Delayed weaning (Mice - after 28 days, Rats - after 23 days)

4.1.1.6.1.1.5

Animals will be euthanized before weaning.

4.1.1.6.2

Genotyping

Genotyping

4.1.1.6.2.1

Check all statements that apply.

- [x] Genotyping using DLAM tail biopsy SOP (see link)
- [ ] Other method

4.1.1.6.2.1.1

Genotyping using DLAM tail biopsy SOP (see link)

4.1.1.6.2.1.2

Other method

4.1.1.6.3

Animal Identification

Animal Identification

4.1.1.6.3.1

Check all the methods you plan to use.

- ☐ Marker or dye
- ☐ Tattooing
- ☑ Ear punch or notch
- ☐ Tag/Band
- ☐ Transponder implantation (non-surgical)
- ☐ Toe clipping

Animals must be less than 7 days of age

4.1.1.6.3.1.1

Marker or dye

4.1.1.6.3.1.2

Tattooing

4.1.1.6.3.1.3

Ear punch or notch

4.1.1.6.3.1.4

Tag/Band

4.1.1.6.3.1.5

Transponder implantation (non-surgical)

4.1.1.6.3.1.6

Toe clipping

4.1.1.6.4

Non-standard housing (including individual housing)

4.1.1.6.5

Special diet or water

Procedures II

4.1.1.7

Check all procedures that will be done on this protocol.

- ☐ Administration of experimental substances
- ☐ Controlled substances
- ☐ Paralytic agents
- ☐ Animal pathogens
- ☑ Tumor studies
- ☐ Bone marrow transplant/reconstitution
- ☐ Antibody production - Monoclonal
- ☐ Antibody production - Polyclonal
- ☐ Cells or cell lines

4.1.1.7.1

Administration of experimental substances

4.1.1.7.2

Controlled substances

4.1.1.7.3

Paralytic agents

4.1.1.7.4

Animal pathogens

4.1.1.7.5

Tumor studies

Tumor studies

4.1.1.7.5.1

If tumors are being induced using injection of cells, cell lines, or chemical substances, make sure you have checked Administration of Experimental Substances and provided the requested information in the table.

Describe your procedure for inducing tumors.

Tumors are spontaneous and not experimentally induced.

Tumor endpoints

4.1.1.7.5.2

Describe tumor monitoring and endpoints, include frequency of monitoringstaff responsible for monitoringtumor size, and clinical conditions that would cause you to euthanize your animals before experimental endpoints are met.

For mice of VHL knockout, PBRM1 knockout, ARID1A knockout, PBRM1-VHL double knockout, PBRM1-ARID1A double knockout, monitoring for possible spontaneous tumors/cysts will be done once a week. Dr. Shuhui Si will be responsible for monitoring. In Dr. Si's absence, Dr. Jindong Chen will monitor tumors. Once affected mice appear with palpable/observable tumors/cysts or distress for any reasons, they will be euthanized.

Tumor endpoints - UCAR guidelines

4.1.1.7.5.3

Will UCAR Policy on Tumor Endpoints be followed?

- [x] Yes
- [ ] No

4.1.1.7.5.3.1

Yes

4.1.1.7.5.3.2

No

4.1.1.7.6

Bone marrow transplant/reconstitution

4.1.1.7.7

Antibody production - Monoclonal

4.1.1.7.8

Antibody production - Polyclonal

4.1.1.7.9

Cells or cell lines

Procedures III

4.1.1.8

Check all procedures that will be done on this protocol.

- ☐ Blood sampling (not at time of euthanasia)
- ☐ Other fluid sampling
- ☐ Radiation
- ☐ Imaging
- ☐ Trauma (including burns)
- ☐ Seizures (experimentally induced)
- ☐ Death as an endpoint

4.1.1.8.1

Blood sampling (not at time of euthanasia)

4.1.1.8.2

Other fluid sampling

4.1.1.8.3

Radiation

4.1.1.8.4

Imaging

4.1.1.8.5

Trauma (including burns)

4.1.1.8.6

Seizures (experimentally induced)

4.1.1.8.7

Death as an endpoint

Procedures IV

4.1.1.9

Check each of the following that will be done on this protocol:

- ☐ Behavioral studies
- ☐ Water scheduling
- ☐ Food scheduling
- ☐ Stress (experimentally produced)
- ☐ Prolonged restraint

4.1.1.9.1

Behavioral studies

4.1.1.9.2

Water scheduling

4.1.1.9.3

Food scheduling

4.1.1.9.4

Stress (experimentally produced)

4.1.1.9.5

Prolonged restraint

Procedures - Surgical

4.1.1.10

Please check all that apply.

- ☐ Non-recovery surgery (NOT including standard euthanasia procedures)
- ☐ Recovery surgery

4.1.1.10.1

Non-recovery surgery (NOT including standard euthanasia procedures)

4.1.1.10.2

Recovery surgery

Procedures - Other

4.1.1.11

List and describe any additional procedures that will be performed on live animals.

Euthanasia - rodent

4.1.1.12

Check all methods of euthanasia that will be used on animals in this section.

Note that appropriate secondary physical methods includeCervical dislocationDecapitationRemoval of a major organOpening the chestPerfusion with a fixative

- ☑ Carbon dioxide (including secondary physical method) See link below.
- ☐ Anesthetic overdose following UCAR guidelines (including secondary physical method for verification)
- ☐ Anesthesia followed by perfusion with fixative
- ☐ Euthanasia of embryos, fetuses or neonates using UCAR guidelines (see link below).
- ☐ Cervical dislocation without sedation
- ☐ Decapitation without sedation
- ☐ Euthanasia following non-recovery surgical procedure
- ☐ Other euthanasia procedure

4.1.1.12.1

Carbon dioxide (including secondary physical method) See link below.

4.1.1.12.2

Anesthetic overdose following UCAR guidelines (including secondary physical method for verification)

4.1.1.12.3

Anesthesia followed by perfusion with fixative

4.1.1.12.4

Euthanasia of embryos, fetuses or neonates using UCAR guidelines (see link below).

4.1.1.12.5

Cervical dislocation without sedation

4.1.1.12.6

Decapitation without sedation

4.1.1.12.7

Euthanasia following non-recovery surgical procedure

4.1.1.12.8

Other euthanasia procedure

Animal Pain, Distress or Impairment

4.1.1.13

Are any of the experimental procedures, genetic manipulations (i.e., transgenic or knockout animals, localized delivery of a transgene) or other techniques described in this protocol expected to result in animal pain, distress or impairment of normal physiological function (i.e., ambulation, maintenance of hydration and caloric intake, normal elimination)?

Do any transgenic/knockout/mutant animals being used have characteristics that would cause pain, distress, impairment or require special care?

Note: Do not discuss monitoring and endpoints for procedures described under any of the following:

Tumor studies
Bone marrow transplant/reconstitution
Radiation
Trauma
Seizures
Stress
Recovery Surgery

Pain, distress and impairment for each of these is discussed along with the procedure, elsewhere in the protocol.

- [x] Yes, addressed elsewhere in the protocol
- [ ] Yes, not addressed elsewhere in the protocol
- [ ] No

4.1.1.13.1

Yes, addressed elsewhere in the protocol

4.1.1.13.2

Yes, not addressed elsewhere in the protocol

4.1.1.13.3

No

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Consideration of Alternatives 1

4.1.1.14

Attachments: Animal Testing Alternatives

Explain why you must use live animals for your studies, and briefly justify your choice of species, strain(s) and animal models(s) described in this protocol.

In this study, mice will be used for kidney cancer knockout models due to the following reasons. Since no in vitro experiments or nonmammalian model can replicate the complex processes of tumorigenesis in humans. Live animals usually meet a determined taxonomic equivalency to humans, so as to react to disease or its treatment in a way that resembles human physiology as needed. Many drugs, treatments and cures for human diseases have been developed with the use of animal models. Of the animals chosen, mice have significant advantages over other mammals as research subjects. Due to their short generation time and prolific breeding, investigators are able to perform studies in a cost-effective, time-efficient manner while using minimal numbers of animals. Due to their small size, they can be easily handled and have relatively minimal housing and care needs. Due to their common use as research subjects, a voluminous body of literature is available encompassing their breeding, housing, development & care. In addition, this complete mammalian widespread use has led to the intensive investigation of all aspects of mice in research (behavioral, genetic, biochemical, etc.) and the development of highly useful cell lines, biochemical products and more. Mice bear their young in utero which mimics human gestation and facilitates studies on early embryonic development. Finally, although mice are low on the phylogenetic scale, the structure and function of genes is very similar between mice and humans.

Literature Search

4.1.1.15

Attachments: Search for Alternatives information from Miner Library

In the table below, list the databases that were used to determine that Appropriate alternative models (Reduction, Refinement, Replacement) do not existThis work is not an unnecessary duplication of research

Note that the keyword field has a 50 character limit. If you need more space, simply add one or more lines (using the +) to the table.

Database	Years Covered	Keywords	Date of search
PubMed	1980-2012	PBRM1, ARID1A, VHL, Sglt2-Cre, kidney cancer, knockout mouse models, mice, clear cell renal cell carcinoma, kidney proximal tubule.	March 22, 2012

Consideration of Alternatives 2

4.1.1.16

List sources other than a search of the literature, such as, conference attendance, committee membership, journal reviewing, professional expertise and training.

Discuss any potential alternative models considered and your reasons for excluding them.

To our knowledge, we are the first group to develop kidney proximal tubule-specific knockout mouse models through Sglt2-Cre and loxP system. Up todate, no other groups in the world has tried to disrupt these genes in the kidney proximal tubules which is the most important tubules in the kidney. VHL gene has been disrupted in the mouse kidney distal tubules, which failed to produce tumors. We speculate that CCRCC originates from proximal tubules, not distal tubules. PBRM1 was recently identified to be associated with kidney cancer. No report has tried to develop ARID1A kidney cancer knockout mouse model.

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Location of Animal Work

4.1.1.17

Will animals be removed from the vivarium?

- [x] No
- [ ] Yes

4.1.1.17.1

No

4.1.1.17.2

Yes

Procedures done in vivarium

4.1.1.18

Will procedures (including breeding and colony management) be performed on animals in the vivarium?

- [ ] No
- [x] Yes, in the housing room(s)
- [x] Yes, in a vivarium procedure room

4.1.1.18.1

No

4.1.1.18.2

Yes, in the housing room(s)

Housing Rooms Procedures

4.1.1.18.2.1

List each type of procedure (including breeding and other colony management) that will be done in housing rooms:

Procedures
Breeding
Weaning
Ear punch/notch

4.1.1.18.3

Yes, in a vivarium procedure room

Procedure Room Procedures

4.1.1.18.3.1

List all procedures that will be done in vivarium procedure rooms:

Room #	Procedure(s)
TBD	Tail biopsy
TBD	Euthanasia

Certifications and PI Agreement

5

Protocol Detail Report

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Certification Statement 1

5.1

Animals that I request for this study will be used in accordance with regulations and standards as promulgated by the New York State Health Department, the United States Department of Agriculture, the Public Health Service and the University of Rochester.

- [x] Yes
- [ ] No

This protocol cannot be approved unless you can answer yes to this statement.

5.1.1

Yes

5.1.2

No

Certification Statement 2

5.2

I have designed procedures to be performed on animals so that pain or discomfort to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research.

- [x] Yes
- [ ] No

This protocol cannot be approved unless you can answer yes to this statement.

5.2.1

Yes

5.2.2

No

Certification Statement 3

5.3

To the best of my knowledge, the studies proposed do not unnecessarily duplicate any others in the published literature.

- [x] Yes
- [ ] No

This protocol cannot be approved unless you can answer yes to this statement.

5.3.1

Yes

5.3.2

No

Certification Statement 4

5.4

I have given serious consideration to the use of in vitro, less invasive, less painful, or other alternative techniques to the use of animals, as described in the application.

- [x] Yes
- [ ] No

This protocol cannot be approved unless you can answer yes to this statement.

5.4.1

Yes

5.4.2

No

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Certification Statement 5

5.5

I have concluded that the species, animal numbers, and procedures to be used are the most appropriate for the proposed investigation.

- [x] Yes
- [ ] No

This protocol cannot be approved unless you can answer yes to this statement.

5.5.1

Yes

5.5.2

No

Certification Statement 6

5.6

All hazardous agents, as identified by Environmental Health & Safety review, will be used according to the guidelines stipulated by Risk Management and Environmental Safety, Radiation Safety, and/or the Institutional Biosafety Committee.

- [ ] Not applicable
- [x] Yes
- [ ] No

This protocol cannot be approved unless you can answer yes or NA to this statement.

5.6.1

Not applicable

5.6.2

Yes

5.6.3

No

Principal Investigator Responsibility

5.7

By providing my electronic signature on this question, I certify that Ihave read this protocol andtake responsibility for following all its provisions.

Provide your name below.

Note that this protocol cannot be submitted until you have answered this question and provided an electronic signature. You must be logged into your account to submit an e-signature. It cannot be submitted from another account.
Jindong Chen

Use of Hazards - UCAR Use Only

6

Hazardous Agents

6.1

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Hazards not in Master List

6.2

List all hazardous substances not currently included on the Hazard Master List that are proposed for use on this protocol. Types include:Chemical (HC)Radionuclides (RN)Non-recombinant human pathogen (NP)Infectious agent (IA) Recombinant DNA (or RNA) molecules (RM)Human materials (HM)

Name	Hazard Type

UCAR Use Only

7

Protocol Number

7.1

Key Words

7.2

CO2 EUTHANASIA

TRANSGENIC/KNOCKOUT

TUMOR

BREEDING

7.2.1

CO2 EUTHANASIA

7.2.2

TRANSGENIC/KNOCKOUT

7.2.3

TUMOR

7.2.4

BREEDING

Notes

7.3

Append notes here.