Van Andel Research Institute
Animal Study Protocol

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PLEASE TYPE

A. ADMINISTRATIVE DATA

Laboratory: Laboratory of Cancer Genetics

Principal Investigator: Bin Tean Teh, M.D., Ph.D.

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Telephone: 1-616-234-5296 Fax: 1-616-234-5297 Email: bin.teh@vai.org

Protocol Title: **Evaluation of the tumor-growth suppression role of LSAMP and HNT in mice**

Initial Submission ☒ Renewal ☐ or Modification ☐

List the names of all individuals authorized to conduct procedures involving animals under this protocol and identify key personnel (e.g., co-investigator(s)), providing their laboratory, telephone, fax, and email:

Name	**Dept/Affiliation**	**Phone**	**Fax**	**Email**
Jindong Chen	*Lab of Cancer Genetics*	*616.234.5578*	*616.234.5579*	*Jin-dong.chen@vai.org*
Bin Tean Teh	*Lab of Cancer Genetics*	*616.234.5296*	*616.234.5297*	*bin.teh@vai.org*
ChaoNan Qian	*Lab of Cancer Genetics*	*616.234.5538*	*616.234.5539*	*chaonan.qian@vai.org*

☒ Internally supported research

☐ Funding source: Not applicable.

Title of grant application: Not applicable.

Submission deadline or dates of funding: Not applicable.

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B. ANIMAL REQUIREMENTS

1. Species: *Mus musculus*
2. Strain or subspecies: *BALB/C nudes*
3. Approximate age, weight, or size: 4-6 weeks, 15-20 grams
4. Sex: female
5. Source (name of outside vendor or supplier):
   (NOTE: Animal order will not be processed without an approved protocol number on the order.)
   VARI Vivarium breeding stock
6. Average daily census: 50-80
7. Primary housing location(s):
   (NOTE: Facility manager must certify below that facility has the resource capability to support the study. If animals will be housed in a lab or anywhere else outside the central facility for more than 12 hours, provide building and room number.)
   VARI Vivarium SPF barrier facility
8. Location(s) where manipulation will be conducted:
   (NOTE: Animals cannot be removed from the animal facility and then returned. Animals cannot be taken to the 4th floor laboratory area and kept overnight without the Vivarium Director's approval.)
   VARI Vivarium
9. Number of animals to be used:
   Year 1: 750 Year 2: 750 Year 3: 300
   Total for the duration of entire study: 1800

C. TRANSPORTATION

Transportation of animals must conform to all institutional guidelines/policies and federal regulations. If animals will be transported on public roads or out of state, describe efforts to comply with USDA regulations. If animals will be transported between facilities, describe the methods and containment to be utilized. If animals will be transported within a facility, include the route and elevator(s) to be utilized.

It may be necessary, on a few occasions, to transfer live animals to the 4th or 5th floor for experimental purposes. These animals will be transported from the Vivarium in cages with filter tops on carts. The cages will be carried through the cage wash area, passed through the hall door and placed on a cart that has been placed outside the facility in the corridor. The mice will be transported to the 4th or 5th floor in the freight elevator. These mice will not be returned to the facility and will be euthanized within 12 hours of leaving the facility. The cages will be returned to the dirty cage wash area for cleaning and autoclaving.

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D. STUDY OBJECTIVES

Briefly explain in language **understandable to a layperson** the aim of the study and why the study is important to human or animal health, the advancement of knowledge, or the good of society.

Adult kidney cancer is one of the most common diseases (the eighth in men and tenth in women), which is known to have different histological types. Renal cell carcinoma (RCC) accounts for 80-85% of all kidney cancer in the United States and can be classified into clear cell renal cell carcinoma (CCRCC, 75%), papillary renal cell carcinoma (PRCC, 15%), chromophobe renal carcinoma (5%), and collecting duct RCC (1%) (1). Worldwide, approximately 150,000 people are diagnosed with renal cell carcinoma, resulting in 78,000 deaths annually. Recently, we identified two cancer-related genes *LSAMP* and *NORE1* in a previously reported Japanese hereditary kidney cancer family. Our preliminary results indicated that *LSAMP*, HNT (homolog of LSAMP) and *NORE1* inhibit tumor-cell growth in vitro, suggesting that they are good tumor suppressor gene candidates. We have established several LSAMP and HNT stable-transfected cell lines that constantly express LSAMP and HNT protein, separately. To further test their suppression feature of tumor growth in vivo and understand their functional role in carcinogenesis, we will inject these stable transfected cells into nude mice to see whether these genes also have the ability to inhibit in vivo tumor growth from these tumor cells.

The aim of this research is to further prove that LSAMP and HNT can suppress tumor growth in vivo and is a tumor suppressor gene.

E. RATIONALE FOR ANIMAL USE

(Use additional sheets if necessary.)

1. Explain your rationale for animal use.
   (NOTE: The rationale should include reasons why non-animal models cannot be used.)

   No in vitro system can really replicate the complex processes of cancer cell growth in living animals. Cell-growth suppression role of a gene in vitro does not means that the gene is a tumor suppressor. This in vivo experiment will provide an excellent way to examine the effect of LSAMP/HNT on tumor growth. Thus results based on this experiment will give us much reliable information for understanding the role of LSAMP and HNT genes.

2. Justify the appropriateness of the species selected.
   (NOTE: The species selected should be the lowest possible on the phylogenetic scale.)

   The use of non-mammals has significant limitations in research. Although many different non-mammalian species (such as frogs, squid, zebrafish, and birds) can model a specific component of a system, there is not one single non-mammalian species that models a complete mammalian system accurately and reliably.

   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 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 bearing human tumor with high metastatic ability can mimics the process of tumor cell spreading in human.

3. Justify the number of animals to be used.
   (NOTE: The number of animals should be the minimum number required to obtain statistically valid results.)

   Experiment :

Ten tumor cell lines A-498, ACHN, 786-O, 769-P, SW-156 (RCC); COLO-205, HCT-116, SW-620 (Colorectal cancer); MCF-7 (Breact cancer), and SKOV-3 (Ovarian cancer) will be transfected with EGFP-LSAMP, EGFP-HNT, and EGFP-vector control plasmids. These cell lines exhibit LSAMP and/or HNT promoter methylations with downregulated expression in these genes. Successfully stable transfected cell lines will be selected for tumor growth experiment in nude mice. At least six stable transfected cell lines for each gene (LSAMP, HNT) will be selected for this experiment. For each selected cell line, two stable EGFP-LSAMP or EGFP-HNT transfected cell clones and two EGFP-vector transfected clones from one cell line will be used in the study. The stable transfected cell lines will be injected subcutaneously into the nude mice. There are 10 mice per group and the experiment will be repeated three times. Please refer to table below:

Number Breakdown for Experiment #l:
EGFP-LSAMP or -HNT Expression vector- 10 mice x 2 clones x 3 times x 6 cell lines.....… 360 mice
Empty EGFP vector - 10 mice x 2 clones x 3 times x 6 cell lines.....360 mice
Untransfected original cell line controls 10 mice x 3 times x 6 cell lines…..……..180 mice

Total mice for one gene experiment……..900 mice

Grand Total for Protocol:

900 mice x 2 gene experiments= 1800 mice

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F. DESCRIPTION OF EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES (Use additional sheets if necessary)

Briefly explain the experimental design and specify all animal procedures. This description should allow the IACUC to understand the experimental course of an animal from its entry into the experiment to the endpoint of the study.

This project is designed to investigate the tumor growth inhibition of LSAMP and HNT genes.

Tumour studies: The LSAMP/HNT stable transfected cell clones used for the soft agar assays will be used to analyze tumour growth in nude mice in this study. The work will be done in accordance with institutional animal care guidelines. Cells will be collected, re-suspended in culture medium DMEM. We will inject 300 μl (7 $X10^6$ cells) subcutaneously into the right flank of female 5–6-week-old athymic BALB/c nude mice (Charles Rivers Laboratories). The tumours will be measured with calipers weekly for six weeks. Tumour volume will be calculated as length × height × width × 0.5. After six weeks, the mice will be killed and tumours be dissected and weighed. Tumour weights and volumes is suposed to give comparable results. We will confirm the expression of *LSAMP/HNT* for several tumours by RT–PCR

Specifically address the following:

1. **Experimental injections or inoculations**
   (substances, e.g., infectious agents, adjuvants, etc.; dose, sites, volume, route, and schedules)

   We will inject 300 μl (7 $X10^6$ cells) subcutaneously into the right flank of female 5–6-week-old athymic BALB/c nude mice.

   Two hours before termination, each mouse will receive an intraperitoneal injection of 0.2 ml BrDU labeling reagent (Amersham Biosciences, RPN 20). This reagent is used to indicate proliferative cells following sacrifice. The MSDS for BrDU is enclosed.

2. **Blood withdrawals**
   (volume, frequency, withdrawal sites, and methodology)

   Not applicable.

3. **Surgical procedures**
   (provide details of survival and non-survival surgical procedures in Section G.)

   Not applicable.

4. **Radiation**
   (dosage and schedule)

   Not applicable.

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5. **Methods of restraint**
   (e.g., restraint chairs, collars, vests, harnesses, slings, etc.)
   Include how animals are restrained for routine procedures like blood withdrawals. Prolonged restraint must be justified with appropriate oversight to ensure it is minimally distressing. Describe any sedation, acclimation, or training to be utilized.

   Not applicable.

6. **Animal identification methods**
   (e.g., ear tags, tattoos, collar, cage card, implant, etc.)

   Cage cards and ear notches.

7. **Other procedures**
   (e.g., survival studies, tail biopsies, etc.)

   None.

8. **Resultant effects, if any, that the animals are expected to experience**
   (e.g., pain or distress, ascites production, etc.)

   Animals will be observed daily. Animals will be monitored for weight loss, lethargy, loss of appetite, and euthanized when necessary. The animals will specifically be watched to see if the tumor development impedes eating or drinking. While every effort will be made to avoid causing pain/distress to mice, some mice may experience adverse effects associated with tumor. Tumor burden is limited with 1 cm^3. A loss of > 20% body weight is indicative for euthanasia.

9. **Other potential stressors**
   (e.g., food or water deprivation, noxious stimuli, environmental stress)
   In addition, specify procedures to monitor and minimize distress. If a study is USDA Classification E, indicate any non-pharmaceutical methods to minimize pain and distress.

   No other potential stressor is expected to occur.

10. **Experimental endpoint criteria**
    (e.g., tumor size, percentage body weight gain or loss, inability to eat or drink, behavioral abnormalities, clinical symptomatology, or signs of toxicity)
    Experimental endpoint criteria must be specified when the administration of tumor cells, biologics, infectious agents, radiation, or toxic chemicals are expected to cause significant symptomatology or are potentially lethal. List the criteria to be used to determine when euthanasia is to be performed. Death as an endpoint must <u>always</u> be scientifically justified.

    The expected endpoint of the experiment is day 42 (six weeks).

If any of the animals in this protocol meet the following criteria for morbidity or mortality, they will be sacrificed at that time. The normal endpoint for the subcutaneous tumor model is the growth of the tumor to the limit of 1 cm^3. A loss of > 20% body weight is indicative of euthanasia at any time during the experiment. When mice appear distressed, e.g., lethargy, ulcerations, loss of appetite for more than one day, or when the tumor development impedes eating or drinking, the mice will be euthanized. This is not considered an endpoint of the experiment, unless they appear to cause distress to the animal.

11. **Veterinary care**
(indicate desired plan of action in case of animal illness, e.g., initiate treatment, call investigator prior to initiating treatment, euthanize)

Daily veterinary care will be provided to all animals by the Vivarium staff. The Vivarium staff will consult with the attending veterinarian Dr. Joan Koelzer (616) 437-6415 or the alternate attending veterinarian Dr. Diane Egedy (616) 827-2950 when necessary. In the case animals are found sick or dead the PI will be notified via email and phone. PI will be notified with symptomology, disposition and animal identifier. In the event the PI cannot be reached, associates in the PI's lab will be contacted. In the event PI and his/her associates cannot be contacted any sick mice will be treated at the discretion of the Vivarium staff or attending veterinarian. Any animals found dead will be placed in a -20 refrigerator.

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G. SURGERY

If proposed, complete the following:
(NOTE: Use additional sheets if necessary.)

1. Identify and describe the surgical procedure(s) to be performed. Include preoperative procedures (e.g., fasting, analgesic loading), and monitoring and supportive care during surgery. Include the aseptic methods to be utilized.

   Not applicable.

2. Who will perform surgery and what are their qualifications and/or experience?

   Not applicable.

3. Where will surgery be performed and postoperative care provided (building and rooms)?

   Not applicable.

4. If survival surgery, describe postoperative care required, frequency of observation, and identify the responsible individual(s). Include detection and management of postoperative complications during work hours, after hours, weekends, and holidays.

   Not applicable.

5. If non-survival surgery, describe how humane euthanasia is enacted and how death is determined.

   Not applicable.

6. Are paralytic agents used during surgery? If yes, please describe how ventilation will be maintained and how pain will be assessed.

   Not applicable.

7. Has major survival surgery been performed on any animal prior to being placed on this study? [Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, or limb amputation).]
   If yes, please explain:

   No

8. Will more than one major survival surgery be performed on an animal while on this study?
   If yes, please justify:

   Not performed

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H. PAIN OR DISTRESS CATEGORY:

Check the appropriate category and indicate the approximate number of animals in each.
NOTE: The sum of all three categories should equal the total cited in both Section B, question #9 (total number of animals used for the duration of the entire study) and Section E, question #3 (provide a justification for the total number of animals used in this protocol).

		Number of Animals
☒	Category 1 – Minimal, Transient, or No Pain or Distress.	1800
☐	Category 2 – Pain or Distress Relieved by Appropriate Measures	
☐	Category 3 – Unrelieved Pain or Distress***	

******NOTE: If animals are indicated in Category Three, a written scientific justification is required to explain why the appropriate use of anesthetics, analgesics, sedatives, or tranquilizers during and/or following painful or distressful procedures are contraindicated in this study.***

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I. ANESTHESIA, ANALGESIA, TRANQUILIZATION, OTHER AGENTS

For animals indicated in Section H, category 2, specify the anesthetics, analgesics, sedatives, or tranquilizers that are to be used. Include the name of the agent(s), the dosage, route, and schedule of administration.

Anesthesia will be used in this protocol in conjunction with the surgery and the terminal cardiac puncture. The anesthesia will be administered according to *SOP #6.005 – Isoflurane Anesthesia in Mice*, which is attached.

Post-surgerical analgesia is not expected to be necessary. However, the staff is alerted to watch for the following behavioral or the attitudinal changes which may be indicative of pain.

Behavioural Changes Potentially Indicative of Pain

- reluctance to move
- abnormal posturing
- teeth grinding
- decreased appetite
- vocalization

Attidunal Changes Potentially Indicative of Pain

- anxiety
- apprehension
- hypersensitivity
- depression
- uncommon, overt aggression

If these symptoms persist for more than one day after the surgery the administration of analgesic and/or tranquilizing agents may be required. Please consult the PI if such symptoms are noted.

J. METHOD OF EUTHANASIA OR DISPOSITION OF ANIMALS AT END OF STUDY

Indicate the proposed method of euthanasia. If a chemical agent is used specify the dosage and route of administration. If the method(s) of euthanasia include those **not** recommended by the AVMA Panel Report on Euthanasia (e.g., decapitation or cervical dislocation without anesthesia), provide scientific justification why such methods must be used. Indicate the method of carcass disposal if not described in Section K below.

K. HAZARDOUS AGENTS

Use of hazardous agents requires the approval of the institutional Biosafety Office/Committee. Attach documentation of approval for the use of recombinant DNA or potential human pathogens.

Hazardous Agent	Yes	No	Agent	Biosafety Approval Date	Tracking Number
Radionuclides	☐	☒			

Biological agents	☐	☒			
Hazardous chemicals or drugs	☐	☒			
Recombinant DNA	☐	☒			

Additional safety considerations:

1. Study Conducted at Animal Biosafety Level: 1 ☐ 2 ☒ 3 ☐ 4 ☐

2. Practices and procedures required for the safe handling and disposal of contaminated animals and material associated with this study to include methods for the removal of radioactive waste and, if applicable, the monitoring of radioactivity:

   Not applicable.

3. Other:

   Not applicable.

L. BIOLOGICAL MATERIAL/ANIMAL PRODUCTS FOR USE IN ANIMALS

(e.g., cell lines, antiserum, etc.)

1. Specify Material:

   Not applicable.

2. Source:

   Material Sterile or Attenuated: Yes ☒ No ☐

   If derived from rodents, has the material been MAP/RAP/HAP tested*?*
   (MAP - Mouse Antibody Production;
   RAP - Rat Antibody Production;
   HAP - Hamster Antibody Production)

   NOTE: If Yes, attach copy of results: Yes ☒ No ☐

3. I certify that the MAP/RAP/HAP-tested materials to be used have not been passed through rodent species outside of the animal facility in question and/or the material is derived from the original MAP/RAP/HAP-tested sample. To the best of my knowledge the material remains uncontaminated with rodent pathogens.

   Initials of Principal Investigator: Bin Tean Teh, M.D., Ph.D.

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M. TRANSGENIC AND KNOCKOUT ANIMALS

Describe any phenotypic consequences of the genetic manipulations to the animals. Describe any special care or monitoring that the animals will require.

Not applicable

N. SPECIAL CONCERNS OR REQUIREMENTS OF THE STUDY

List any special housing, equipment, animal care (e.g., special caging, water, feed, or waste disposal, environmental enhancement, etc.).

Not applicable.

O. PRINCIPAL INVESTIGATOR CERTIFICATIONS

1. I certify that I have attended the institutionally required investigator training course.

   Year of course attendance: **2000** Location: **VARI**
2. I certify that I have determined that the research proposed herein is not unnecessarily duplicative of previously reported research.
3. I certify that all individuals working on this proposal who are at risk are participating in the Institution's Occupational Health and Safety Program.
4. I certify that the individuals listed in Section A are authorized to conduct procedures involving animals under this proposal, have attended the institutionally required investigator training course, and received training in the biology, handling, and care of this species; aseptic surgical methods and techniques (if necessary); the concept, availability, and use of research or testing methods that limit the use of animals or minimize distress; the proper use of anesthetics, analgesics, and tranquilizers (if necessary); and procedures for reporting animal welfare concerns.
5. For all Category 3 Proposals *(see Section H)*:
   I certify that I have reviewed the pertinent scientific literature and the sources and/or databases noted below, and have found no valid alternative to any procedures described herein which may cause more than momentary pain or distress, whether it is relieved or not.
6. I certify that I will obtain approval from the IACUC before initiating any significant changes in this study.
7. I certify that I will notify the IACUC regarding any unexpected study results that impact the animals. Any unanticipated pain or distress, morbidity, or mortality will be reported to the attending Veterinarian and the IACUC.
8. I certify that I am familiar with and will comply with all pertinent institutional, state, and federal rules and policies.

Principal Investigator:

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Name: Bin Tean Teh, M.D., Ph.D. Signature: Date: 9/2/2003

P. CONCURRENCES

Supervisory concurrence as applicable:

Name: Signature: Date:

Safety Office/Committee Certification of Review and Concurrence:
(Required of all studies utilizing hazardous agents.)

Name: Signature: Date:

Facility manager/Veterinarian certification of resource capability in the indicated facility to support the proposed study:

Facility:

Name: Signature: Date:

Facility:

Name: Signature: Date:

Comments:

Attending Veterinarian certification of review and consultation on proper use of anesthetics and pain relieving medications for any painful procedures:

Name: Signature: Date:

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Q. FINAL APPROVAL

Certification of review and approval by the Institutional Animal Care and Use Committee:

Name: Signature: Date:

List any attachments here:

Not applicable.