Modification Form for Permit BIO-RRI-0056

Permit Holder: Wei-Yang Lu

Approved Personnel
(Please stroke out any personnel to be removed)

Shuanglian Wang

Yun-Yan Xiang

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	[Estabslihed] (Human): A549. BEAS-2B, IB3-1, IB3-837	clone 9 (Rat) Hep G2 (Human)
Approved Use of Human Source Material		
Approved GMO		
Approved use of Animals	C57BL/6, BAL B/C Mice.	
Approved Toxin(s)		

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Nov 18, 2009

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____ Jan 25/10

Chair, Biohazards Subcommittee: _____

Brief Description of the use of Clone-9 and HepG2 cell lines

By Wei-yang Lu laboratory

Background information: Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mammalian brain. GABA is synthesized from glutamate by the activity of glutamic acid decarboxylase (GAD). GABA mediates signaling through activation of A- and B- type GABA receptors (GABA-A and GABA-B receptors). It has been long known that GABA exists in the circulating blood (plasma). Recent studies, including ours, demonstrated that GAD and GABA receptors are also expressed in non-neuronal cells. However, the roles of GABA-signaling in non-neuronal cells are not clear. The circulating GABA in the blood increases when liver is injured; and the increased GABA has been proposed to be associated with hepatic encephalitis. Our recent studies revealed that GAD and GABA-A and GABA-B receptor might be expressed in hepatocytes (primary liver cells). We intend to study the roles and intracellular signaling of GABA in the regulation function of hepatocytes. To this end we need to use cell lines of hepatocytes.

Why we need the cell lines: Clone-9 is line of rat hepatocytes and **HepG2** is a line of human hepatocytes (both lines of cells are at level-1). We need the two lines of hepatocytes because most of experiments are done in the rat and to explore whether GABA signaling also exists in human hepatocytes we request the two hepatocyte lines.

What will do with the cells lines: We will culture the cells and use these cells for RT-PCR and immunocytochemistry assays of GAD and GABA receptors. Also, we will make patch-clamp recordings in the cells, examining whether activation of GABA receptor induces transmembrane current in the cells. These studies will provide us novel results of the GABA signaling in hepatocytes, which bear important clinical significance.

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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Cell Biology

ATCC® Number: CRL-1439™ Order this Item **Price:** $329.00

Designations: clone 9

Depositors: ME Kalghn

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: Rattus norvegicus (rat)

Morphology: epithelial

Source:
Strain: Sprague-Dawley
Organ: liver
Disease: normal

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Isolation: **Isolation date:** 1968

Age: 4 weeks

Gender: male

Comments: Clone 9 (K-9) is an epithelial cell line isolated in 1968 from normal liver taken from a young male rat.
The line has been used for studies of in vitro carcinogenesis and is useful clonal assays for screening sera and other nutritional supplements.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

Related Links
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Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004 recommended serum:ATCC 30-2020
References:	21872: . Gene expression and carcinogenesis in cultured liver. New York: Academic Press; 1975. 22425: Weinstein IB, et al. Growth and structural properties of epithelial cell cultures established from normal rat liver and chemically induced hepatomas. Cancer Res. 35: 253-263, 1975. PubMed: 162864

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All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

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Cell Biology

ATCC® Number: HB-8065™ Order this Item **Price:** $272.00

Designations:	Hep G2
Depositors:	Wistar Institute
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial PHOTO
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma
Cellular Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; [3525] complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein) [3525]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (*Nucleofection technology from Lonza* Roche FuGENE® Transfection Reagents)
Receptors:	insulin; insulin-like growth factor II (IGF II) [22446]
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,12 D7S820: 10 F13A01: 5,7 F13B: 6,10 FESFPS: 11 LPL: 10,11 THO1: 9 TPOX: 8,9 vWA: 17
Cytogenetic Analysis:	modal number = 55 (range = 50 to 60); has a rearranged chromosome 1 [3525]
Age:	15 years adolescent
Gender:	male
Ethnicity:	Caucasian

Related Links
- NCBI Entrez Search
- Cell Micrograph
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Comments:
The cells express 3-hydroxy-3-methylglutaryl-CoA reductase and hepatic triglyceride lipase activities. [23557]
The cells demonstrate decreased expression of apoA-I mRNA and increased expression of catalase mRNA in response to gramoxone (oxidative stress). [26594]
There is no evidence of a Hepatitis B virus genome in this cell line. [1205] [22909]

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C

Subculturing:
Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: Twice per week

Preservation:
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CRL-10741
derivative:ATCC CRL-11997
purified DNA:ATCC *HB-8065D*

References:

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3525: Knowles BB, Aden DP. Human hepatoma derived cell line, process for preparation thereof, and uses therefor. US Patent 4,393,133 dated Jul 12 1983
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26594: Cuthbert C, et al. Regulation of human apolipoprotein A-I gene expression by gramoxone. J. Biol. Chem. 272: 14954-14960, 1997. PubMed: 9169468
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32752: Dubuisson J, Rice CM. Hepatitis C virus glycoprotein folding: disulfide bond formation and association with calnexin. J. Virol. 70: 778-786, 1996. PubMed: 8551615
32830: Yamaguchi Y, et al. Biochemical characterization and intracellular localization of the Menkes disease protein. Proc. Natl. Acad. Sci. USA 93: 14030-14035, 1996. PubMed: 8943055
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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

,nust be completed by each Principal Investigator holding a grant administered by the University of , Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is ıbed in the laboratory or animal work proposed. The form must also be completed if any work is proposed ɔlving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require ublic Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR: Wei-Yang Lu
SIGNATURE: Weiyang Lu
DEPARTMENT: Physiology and Pharmacology
ADDRESS: Roberts Research Institute
PHONE NUMBER: (519) 663-5777 ext. 24282
EMERGENCY PHONE NUMBER(S):
EMAIL: wlu33@uwo.ca

Location of experimental work to be carried out: Building(s) Roberts R.I. Room(s) 2233A1, 2250, 2234

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: Canadian Institutes of Health Research, Canadian Cystic Fibrosis Foundation
GRANT TITLE(S): GABAergic regulations of airway epithelium in asthma
Understand the role of chloride channel GABA-A-receptor in CF lung disease and its relationship to CFTR protein

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:

Yun-Yan Xiang
Shuanglian Wang

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓ YES O NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)?
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ✓ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
A549	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	50 mL	ATCC	✓ 1 O 2 O 3
BEAS-2B	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	50 mL	ATCC	O 1 ✓ 2 O 3
IB3-1	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	50 mL	ATCC	O 1 ✓ 2 O 3
IB3-B37	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	50 mL	ATCC	O 1 ✓ 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

see table 2.3 pl

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ✓ YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes ✓ No		Not applicable
Rodent	O Yes ✓ No		
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes ✓ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	✓ Yes O No	A549 IB3-1 BEAS-2B IB3-B37	ATCC
Rodent	O Yes ✓ No		
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes ✓ No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 ✓ 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES ✓ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES ✓ NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below ✓NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ✓NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ✓NO
- SV 40 Large T antigen ✓YES BEAS-2B O NO
- E1A oncogene O YES IB3-1 ✓NO
- Known oncogenes O YES, please specify _____ ✓NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ✓NO

4.5 Will virus be replication defective? O YES ✓NO

4.6 Will virus be infectious to humans or animals? O YES ✓NO

4.7 Will this be expected to increase the containment level required? O YES ✓NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ✓NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ✓YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used C57BL/6, BALB/c mice

6.3 AUS protocol # to be submitted

6.4 Will any of the agents listed be used in live animals O YES, specify: _____ ✓NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES √Ø NO
- Pound source cats O YES √Ø NO
- Cattle, sheep or goats O YES √Ø NO
- Non-human primates O YES, please specify species _____ √Ø NO
- Wild caught animals O YES, please specify species & colony # _____ √Ø NO
- Birds O YES √Ø NO
- Others (wild or domestic) O YES, please specify _____ √Ø NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES √Ø NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES √Ø NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES √Ø NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 √Ø NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

SIGNATURE Wenjang Lu

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 Ø 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____

Ø NO, please certify

O NOT REQUIRED for Level 1 containment

→ Level 2 Inspection completed Nov 17/09 JL.

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Wenjang Lu Date: Sept. 16, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kidder
Date: 18 Nov. 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: Ronald Noseworthy
Pending Level 2 Inspection Date: Sept. 22, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: J Stanley
Date: Nov. 17/09

Approval Number: B10-RA-0096 Expiry Date (3 years from Approval): NOV 17, 2012

Special Conditions of Approval:

* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED*

Funding Agency: Canadian Institutes of Health Research

Grant Title: **GABAergic regulations of airway epithelium in asthma**

During the development of asthma, airways in the lung undergo structural remodeling and functional alterations, which are characterized by goblet cell (GC) hyperplasia and airway hyper-responsiveness (AHR). The mechanism of such asthmatic reactions remains unclear. Novel data from our recent studies have shown that airway epithelial cells (AECs) in the lung express glutamic acid decarboxylase (GAD), the key enzyme for the synthesis of γ-aminobutyric acid (GABA), the major inhibitory transmitter in the brain. Furthermore, AECs also express subunits for A-type GABA receptors ($GABA_AR$s), and these subunits are known to form chloride channels in neurons. Our preliminary results strongly suggest that $GABA_AR$s provide an unexpected excitatory and autocine/paracrine function in AECs. Remarkably, the levels of GAD and $GABA_AR$ subunits expressed in AECs increased dramatically in BALB/c mice that were sensitized and challenged with ovalbumin (OVA), a widely used animal model of asthma. The present project intends to study the role of GABA and $GABA_AR$s in asthmatic reaction, with particular regard to the underlying mechanisms for AEC transdifferentiation.

To demonstrate the role of the AEC GABAergic system in asthmatic reactions, the allergen-sensitive BLAB/c mice and allergen-insensitive C57BL/6 mice will be sensitized with OVA, and then challenged with OVA. The expression levels of GAD and $GABA_AR$s in AECs of allergen-challenged mice will be examined and correlated to the changes of airway resistance. Allergen-challenged BALB/c mice will be treated intranasally with $GABA_AR$ inhibitor; to examine whether blocking GABA signaling improves pulmonary functions.

Our preliminary data showed that T_{H2} cytokine interleukine-13 (IL-13) increased in the lung of OVA-treated BALB/c mice. Intranasal administration (i.n.) of IL-13 enhanced GAD and $GABA_AR$s in AECs. Conversely, i.n. $GABA_AR$ inhibitor suppressed the extent of CG hyperplasia and mucus production, but did not affect the level of IL-13 in the lung of the OVA-challenged mice. These results suggest that in asthma the GABAergic activation in AECs is downstream of the IL-13-initiated signaling. In neurons, phosphorylation of the $GABA_AR$ β_2-subunit by Akt initiates translocation of the receptor to the plasma membrane. In addition, $GABA_AR$ activation depolarizes the neural progenitors, consequently triggering neural proliferation and differentiation. To explore the pathway by which T_{H2} cytokines enhances $GABA_AR$ expression in AECs, we will determine whether similar mechanisms exist in the AECs. IL-13 will be applied to BALB/c mice to determine whether it activates PI3K/Akt and phosphorylates $GABA_AR$ in AECs. PI3K or Akt inhibitor will be administrated before application of cytokines to determine whether PI3K/Akt signaling regulates the T_{H2} cytokine-increased expression of $GABA_AR$s in AECs. AECs, namely A549 and BEAS-2B cells, grown in air-liquid surface will be treated with IL-13 to show that IL-13 increases $GABA_AR$s in the apical membrane of AECs.

We hypothesize that, during the development of asthma, T_H2 cytokines, including Il-13, enhance the expression of GAD and $GABA_AR$s in AECs. Consequently, the activated-GABAergic signaling induces AEC transdifferentiation, and hence alterations of airway function. This study may lead to novel treatments of asthma.

Funding Agency: Canadian Cystic Fibrosis Foundation

Grant Title: **Understand the role of chloride channel GABA-A-receptor in CF lung disease and its relationship to CFTR protein**

Cystic fibrosis (CF), a common genetic disorder, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) which is a membrane chloride channel located at airway epithelial cells. There are other chloride channels existing in airway epithelial cells and may be able to reverse the effects of CF. Recent studies in our laboratory indicated that a specific chloride channel – GABA-A-receptor is located on the epical membrane of airway cells and an autocrine/paracrine GABA signaling system do existent in bronchial epithelial cells. Deficit in CFTR function increase the expression of GABA-signaling molecules. In this study, GABA-signaling in bronchial epithelial cells (BECs) will be investigated both in $CFTR^{-/-}$ mice and matched wild-type C57BL/6 mice, and in several cultured lines of human BECs, namely BEAS-2B, IB3-1 (BEC line isolated from a patient with cystic fibrosis) and IB3-837 (IB3-1 expressing wild type CFTR, a cell line from other research laboratory at the University of Toronto) cells by Western blot, immunohistochemistry/ immunocytochemistry and patch-clamp recordings. This proposal initiates studies of the interactions between CFTR and GABA signaling in the cells. Understanding the crucial role of GABA signaling in airway epithelial cells in the pathological course of CF lungs will provide a new target for therapies of CF.

Cell Biology

ATCC® Number:	CCL-185™	Order this Item	Price:	$256.00

Designations:	A549
Depositors:	M Lieber
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** lung **Disease:** carcinoma
Cellular Products:	keratin
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1972
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 11 D16S539: 11,12 D5S818: 11 D7S820: 8,11 THO1: 8,9.3 TPOX: 8,11 vWA: 14
Cytogenetic Analysis:	This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6) (q11;q27); ?del(6) (p23); del (11) (q21), del(2) (q11), M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell; and N12 and N17 usually had 4 copies.
Isoenzymes:	G6PD, B

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Age:	58 years
Gender:	male
Ethnicity:	Caucasian
Comments:	This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. [23218] Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway. [58030] The cells are positive for keratin by immunoperoxidase staining.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 2 X 10(3) and 1 X 10(4) viable cells/cm2. Do not exceed 7 X 10(4) cels/cm2. 6. Incubate cultures at 37°C. **Interval:** Maintain cultures at a cell concentration between 6 X 10(3) and 6 X 10(4) cell/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	about 22 hours

recommended serum:ATCC 30-2020

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2004

References:

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transcription factor as a mechanism producing airway mucosal inflammation. J. Virol. 70: 8773-8781, 1996. PubMed: 8971006
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58030: Lieber M, et al. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer 17: 62-70, 1976. PubMed: 175022

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ATCC® Number: CRL-9609™ Order this Item **Price:** $256.00

Designations: BEAS-2B

Depositors: The United States of America

Biosafety Level: 2 [CELLS CONTAIN PAPOVAVIRUS]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Organ: lung
Tissue: bronchus
Disease: normal
Cell Type: epithelialvirus transformed

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Applications: transfection host (Roche FuGENE® Transfection Reagents)

Tumorigenic: No

Comments: Epithelial cells were isolated from normal human bronchial epithelium obtained from autopsy of non-cancerous individuals. [21937]
The cells were infected with an adenovirus 12-SV40 virus hybrid (Ad12SV40) and cloned. [21937]
The cells retain the ability to undergo squamous differentiation in response to serum, and can be used to screen chemical and biological agents for ability to induce or affect differentiation and/or carcinogenesis. [21937]
The cells stain positively for keratins and SV40 T antigen.

Propagation: **ATCC complete growth medium:** The base medium for this cell line (BEBM) along with all the additives can be obtained from Lonza/Clonetics Corporation as a kit: BEGM, Kit Catalog No. CC-3170. ATCC does not use

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the GA-1000 (gentamycin-amphotericin B mix) provided with the BEGM kit. Note: Do not filter complete medium.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The flasks used should be precoated with with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin dissolved in BEBM medium .

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Add 2.0 to 3.0 ml of 0.25% Trypsin - 0.53mM EDTA solution containing 0.5% polyvinylpyrrolidone (PVP) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 5 to 10 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
3. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
4. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
5. Discard supernatant and resuspend cells in fresh growth medium. Inoculate new flasks at 1500 to 3000 cells per sq. cm. The culture flasks used should be pre-coated with a mixture of 0.01mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01mg/ml bovine serum albumin dissolved in BEBM medium (see reference below).
6. Place culture flasks in incubators at 37C.

Interval: Subcultured before reaching confluence.
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 1% PVP and 7.5% DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:

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ATCC® Number:	**CRL-2777™** Order this Item	**Price:**	**$338.00**
Designations:	IB3-1 [JHU-52]		
Depositors:	PL Zeitlin		
Biosafety Level:	2 [Cells contain SV40 and Adenovirus 12 DNA viral sequences]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
	PHOTO		
Source:	**Organ:** bronchus **Disease:** cystic fibrosis **Cell Type:** epithelial immortalized with Ad12-SV40 hybridimmortalized with adenovirus 12 - SV40 virus hybrid (Ad12-SV40)		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Restrictions:	Part of the Johns Hopkins Special Collection		
Isolation:	**Isolation date:** 1992		
Cytogenetic Analysis:	modal chromosome number = 80 to 90. There are an average of four chromosome 7 per cell. The phenylalanine 508 deletion in the gene coding for the cystic fibrosis transmembrane regulator is present on at least one chromosome. [70685]		
Age:	7 years		
Gender:	male		
Ethnicity:	White		
Comments:	IB3-1 (ATCC CRL-2777) is an immortalized cell line created in 1992 from a primary culture of		

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bronchial epithelia cells isolated from a patient with cystic fibrosis. The culture was transformed with a hybrid virus, adeno-12-SV40 [PubMed: 1849726]. The IB3-1 are deficient in cyclic AMP-medicated protein kinase A activation of chloride conductance, which is diagnostic of Cystic Fibrosis [PubMed: 7679117]. Genotypically, the cell line is a compound heterozygote containing the delta F508 mutation and a nonsense mutation, W1282X, with a premature termination signal [PubMed: 10518596].The cells stain positively for SV40 T antigen [PubMed: 1849726].They can be used for studies of the mutant cystic fibrosis transmembrane regulatory protein and its interaction with the chloride channel.The S9 cell line (ATCC CRL-2778) and the C38 cell line (ATCC CRL-2779) were derived from the IB3-1 cell line.The CF phenotype present in the IB3-1 cells was corrected in the S9 and C38 cell line by transfection with wild-type adeno-associated viral cystic fibrosis transmembrane conductance regulator (AAVCFTR).

Propagation:

ATCC complete growth medium: LHC-8 Basal Medium (Invitrogen catalog #12679-015), 95%; fetal bovine serum, 5%
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The flasks used should be precoated with a mixture of 0.01 mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/ml bovine serum albumin dissolved in culture medium.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37° C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new precoated culture vessels.
   An inoculum of 3 X 10(3) to 8 X 10(3) viable cells/cm2 is recommended. Do not exceed 1 X 10(5) cells/cm2.
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 4 X 10(3) and 4 X 10(4) cells/cm2.
Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended
Medium Renewal: Two to three times weekly

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time:

29 hrs

Related Products:

source culture:ATCC JHU-52
derivative:ATCC CRL-2778
recommended serum:ATCC 30-2020
derivative:ATCC CRL-2779

References:

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