Modification Form for Permit BIO-UWO-0017

Permit Holder: Dale Laird

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

Jamie Simek

Cindy Pan

Ruchi Bhalla

Jared Churko

Silvia Peneula

~~Stephanie Langlois~~

Isabelle Plante

Xiang-Qun Gong

Cindy Shao

Jason Martin

Robert Lorentz

Jack Lee

Jennifer Siu

Additional Personnel
(Please list additional personnel here)

PASQUALE VECCHIO

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DH5 alpha, JM109	
Approved Cells	Human (primary), skin biopsies, Rodent (primary), transgenic alpha mutant mouse, Human (established), HELA, 293T, 293 Hek, Tumour cell lines, Rodent (established), Keratinocytes, NRK BiCR, MIRK, N2A,	C2 C12, L6, L10BIOBR - GFP L10BIOBR - HAPKK
Approved Use of Human Source Material	Human blood (whole), Human tissues (unpreserved), Human tissues (preserved), ODDD Patients and relatives	
Approved GMO	JM109, T-EASY, PcDNA3, pEGFP, retrovirus AP-2, SV 40 Large T antigen, HEK, E1A oncogenes, HeLa, pGFP-V-RS	
Approved use of Animals	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: [signature]

Classification: 2

Date of Last Biohazardous Agents Registry Form: Mar 27, 2009

Date of Last Modification (if applicable):

BioSafety Officer(s): J Stanley Dec 18/09

Chair, Biohazards Subcommittee: G.M. Kidder

See attached sheet

The CRL-2770 and CRL2771 are mouse melanoma cell lines. We are interested in investigating the role of pannexins and connexins as tumor suppressors in these cells. In the event the cells have sufficient pannexins and/or connexins we will knockdown their expression by RNAi technologies. If they are low in pannexins and/or connexins we will overexpress these molecules and examine the effect of these molecules on cell characteristics associated with cell migration and invasion. In the event that one or more pannexins/connexins affects their growth characteristics we will attempt to determine the mechanism involved.

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Cell Biology

ATCC® Number: CRL-2771™ Order this Item

Price: $338.00

Designations: L10BIOBR-MAPKK

Depositors: JL Arbiser

Biosafety Level: 2 [Cells containing SV40 viral DNA sequences]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: melanocyte

Source:
Cell Type: melanocyte;
Strain: B10.BR

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:** January 1, 2002

Applications: tumor model

Tumorigenic: Yes

Age: newborn

Comments: The L10BIOBR-MAPKK cell line (ATCC CRL-2771) was derived by infecting the immortalized murine melanocyte cell line, L10BIOBR, with pBABE which encodes a constitutively active MAPKK. The vector contains the SV40 viral DNA sequences and the puromycin resistance gene. The cells were selected in medium containing puromycin.The introduction of the MAPKK gene into melanocytes leads to tumorigenesis in nude mice, activation of the angiogenic switch and increased production of the proangiogenic factor, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs). Activation of MAP kinase signaling may be an important pathway involved in melanoma transformation. Inhibition of MAP kinase signaling may be useful in the prevention and treatment of melanoma. The L10BIOBR-MAPKK cell line and the corresponding negative control, L10BIOBR-GFP (CRL-2770), are a model for melanoma tumorigenesis and signal transduction [PubMed: 12514183].

Propagation: **ATCC complete growth medium:** Ham's F10 medium supplemented with 50 ng/ml TPA (Sigma Catalogue No. P-8139) and 7% horse serum
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
Temperature: 37.0°C

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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.
   An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/sq. cm. is recommended.
6. Incubate cultures at 37°C.

Interval: Subculture when cells reach a concentration of 4 X 10(4) cells/sq. cm.
Subcultivation Ratio: A subcultivation of 1:6 to 1:8 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: 24 hours

Related Products: recommended serum:ATCC 30-2040
derived from same cell line:ATCC CRL-2770

References:
89472: Govindarajan B, et al. Malignant transformation of melanocytes to melanoma by constitutive activation of mitogen-activated protein kinase kinase (MAPKK) signaling. J. Biol. Chem. 278: 9790-9795, 2003. PubMed: 12514183

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Cell Biology

ATCC® Number: CRL-1458™ Order this Item
Price: $264.00

Designations: L6
Depositors: D Schubert
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: Rattus norvegicus (rat)
Morphology: myoblast
PHOTO
Source: **Tissue:** skeletal muscle
Cell Type: myoblast myoblast;
Cellular Products: myosin

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)

Comments: The L6 myogenic line was isolated originally by Yaffe from primary cultures of rat thigh muscle maintained for the first two passages in the presence of methyl cholanthrene. [22581]
L6 cells fuse in culture to form multinucleated myotubes and striated fibers. The extent of cell fusion declines with passage and the cells should be frozen at low passage and periodically recloned with selection for fusion competent cells.
Tested and found negative for ectromelia virus (mousepox).

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. 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 (CO_2), 5%
Temperature: 37.0°C
Growth Conditions: The myoblastic component of this line will be depleted rapidly if the cells are allowed to become confluent.

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Subculturing:

Protocol: Subculture before the cells become confluent to retard the loss of differentiating ability that is observed as the cells are passaged.

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 1:20 to 1:40 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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

1064: Mandel JL, Pearson ML. Insulin stimulates myogenesis in a rat myoblast line. Nature 251: 618-620, 1974. PubMed: 4421831
22255: Richler C, Yaffe D. The in vitro cultivation and differentiation capacities of myogenic cell lines. Dev. Biol. 23: 1-22, 1970. PubMed: 5481965
22581: Yaffe D. Retention of differentiation potentialities during prolonged cultivation of myogenic cells. Proc. Natl. Acad. Sci. USA 61: 477-483, 1968. PubMed: 5245982
33164: Osawa H, et al. Identification and characterization of basal and cyclic AMP response elements in the promoter of the rat hexokinase II gene. J. Biol. Chem. 271: 17296-17303, 1996. PubMed: 8663388
33165: Osawa H, et al. Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. J. Biol. Chem. 271: 16690-16694, 1996. PubMed: 8663315

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Cell Biology

ATCC® Number: CRL-2770™ Order this Item
Designations: L10BIOBR-GFP
Depositors: JL Arbiser
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Mus musculus* (mouse)
Morphology: melanocyte
PHOTO
Source: **Cell Type:** melanocyte;
Strain: B10.BR
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:** January 1, 2002
Tumorigenic: No
Age: newborn
Comments: The L10BIOBR-GFP cell line was derived as a negative control for CRL-2771. The immortalized murine melanocyte cell line L10BIOBR was transduced with pDIVA-GFP and subjected to puromycin selection. Together, L10BIOBR-GFP (CRL-2770) and L10BIOBR-MAPKK (ATCC ® CRL -2771) are valuable cell models for oncogenic transformation and signal transduction studies for melanoma [PubMed: 12514183]. **Please note,** although the L10BIOBR-GFP cell line harbors the gfp gene, as verified by PCR analysis, the cell line does not express sufficient GFP protein for detection of GFP fluorescence by flow cytometry or fluorescence microscopy.
Propagation: **ATCC complete growth medium:** Ham's F10 medium supplemented with 50 ng/ml TPA (Sigma Catalogue No. P-8139) and 7% horse serum
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
Temperature: 37.0°C

Price: $338.00

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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.
   An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/sq. cm. is recommended.
6. Incubate cultures at 37°C.

Interval: Subculture when cells reach a concentration of 2 X 10(4) cells/sq. cm.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 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 hours

Related Products:
recommended serum:ATCC 30-2040
derived from same cell line:ATCC CRL-2771
Cell culture tested DMSO:ATCC 4-X
Erythrosin B vital stain solution:ATCC 30-2404
Trypan Blue vital stain solution:ATCC 30-2402

References:
89472: Govindarajan B, et al. Malignant transformation of melanocytes to melanoma by constitutive activation of mitogen-activated protein kinase kinase (MAPKK) signaling. J. Biol. Chem. 278: 9790-9795, 2003. PubMed: 12514183

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Cell Biology

ATCC® Number: CRL-1772™ Order this Item

Price: $256.00

Designations: C2C12

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: myoblast

PHOTO

Source:
Strain: C3H
Tissue: muscle
Cell Type: myoblast;

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)

Comments: This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903]
The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953]
Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23427]
Tested and found negative for ectromelia virus (mousepox).

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. 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

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Subculturing:

Protocol: IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT.
Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture.
Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

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 which 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.
   Inoculate at a cell concentration between 1.5 X 10 exp5 and 1.0 X 10 exp6 viable cells/75 cm2.
6. Incubate cultures at 37°C.

Medium Renewal: Every two to three days

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 30-2002
recommended serum:ATCC 30-2020

References:

22903: Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-727, 1977. PubMed: 563524
22953: Blau HM, et al. Plasticity of the differentiated state. Science 230: 758-766, 1985. PubMed: 2414846
23427: Katagiri T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage [published erratum appears in J Cell Biol 1995 Feb;128(4):following 713]. J. Cell Biol. 127: 1755-1766, 1994. PubMed: 7798324
28236: Chow YH, et al. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J. Virol. 71: 169-178, 1997. PubMed: 8985336
32828: Kessler PD, et al. Gene delivery to skeletal muscle results in sustanined expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. USA 93: 14082-14087, 1996. PubMed: 8943064
33069: Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

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

Jamie Simek
Cindy Pan
~~Katharine Toth~~
~~Steve Gelelli~~
Ruchi Bhalla
Jared Churko
~~Kyle Cowan~~
Silvia Peneula
Stephanie Langlois
Isabelle Plante
Xiang-Qun Gong
Cindy Shao
~~Jennifer Siu~~ Leave on list D.L.

Additional Personnel
(Please list additional personnel here)

JACK LEE
ROBERT LORENTZ
JASON MARTIN

* *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.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Mar 27, 2009

Signature of Permit Holder:

BioSafety Officer(s): Hawley Apr 21/09

Chair, Biohazards Subcommittee:

Modification Form for Permit BIO-UWO-0017

Permit Holder: Dale Laird

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DH5 alpha, JM109	
Approved Cells	Human (primary), skin biopsies, Rodent (primary), transgenic alpha mutant mouse, Human (established), HELA, 293T, 293 Hek, Tumour cell lines, Rodent (established), Keratinocytes, NRK BICR, MIRK, N2A,	B16 - F-0 B16 - F10
Approved Use of Human Source Material	Human blood (whole), Human tissues (unpreserved), Human tissues (preserved), ODOD Patients and relatives	
Approved GMO	JM109, T-EASY, PcDNA3, pEGFP, retrovirus AP-2, SV 40 Large T antigen, HEK, E1A oncogenes, HeLa	PGFP-N-RS VECTOR
Approved use of Animals	mice	
Approved Toxin(s)		

New cells to be used for connexin and pannexin studies. New media to be used to silence pannexin 1. Additional Biohazards are Level 1.

* *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.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Mar 27, 2009

Signature of Permit Holder:

BioSafety Officer(s): Wlantey Sept 21/09

Chair, Biohazards Subcommittee:

ORIGENE TECHNOLOGIES, INC.

HuSH shRNA Plasmid, pGFP-V-RS

pGFP-V-RS shRNA Cloning Plasmid
Catalog # TR30007

Product Description:

- Plasmid vector for cloning shRNA expression cassettes
- Designed for long term gene silencing studies
- Kanamycin (25ug/ml) and Puromycin resistance markers for easy selection of transformed or transfected cells
- U6 polymerase III promoter for shRNA expression
- MMLV LTR sequences for packaging into retroviral particles
- EcoRI and HindIII sites convenient for shuttling existing HuSH cassettes

Content: Each vial contains 5 ug of dried and purified plasmid DNA.

Storage and Stability: The plasmid is stable for at least 1 yr at -20°C from the date of shipment.

Guarantee: This product is guaranteed for the correct sequences and listed functions.

Related Products: Specific HuSH constructs are available at OriGene covering the full human, mouse and rat genomes.

Quality Control Assays

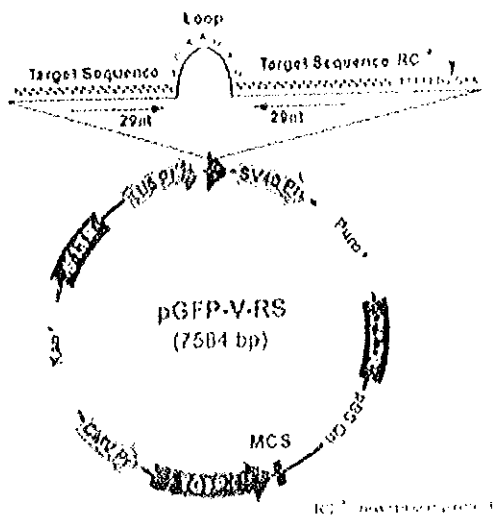
DNA Quantitation: The concentration of the purified plasmid was determined at OD_{260} by a UV spectrometer.

DNA Sequence Analysis: The final purified plasmid was sequenced to confirm its identity.

Functional Analysis:

1. Cloning: the pGFP-V-RS plasmid was digested with BamHI and HindIII and the digested fragment isolated. Multiple shRNA expression cassettes were cloned into this plasmid.
2. Inhibition of target gene: shRNA constructs cloned into pGFP-V-RS were verified for inhibition of target genes.
3. Stable cell lines: pGFP-V-RS was verified to generate stable cell lines using direct transfection.

Figure 1: Map of shRNA Cloning Vector pGFP-V-RS



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Subject: Re: Biohazard Modification Form: Laird lab
From: Dale Laird <Dale.Laird@schulich.uwo.ca>
Date: Mon, 21 Sep 2009 08:24:54 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer, These vectors are for transfections only. We do not anticipate making viral particles with them. Dale

Dale W. Laird, Ph.D.
Professor
Canada Research Chair in Gap Junctions and Disease
Department of Anatomy and Cell Biology
University of Western Ontario
Dental Science Building, Rm 00077
London, Ontario, Canada, N6A-5C1
Tel: (519) 661-2111 x86827
Fax: (519) 850-2562
Dale.Laird@schulich.uwo.ca
www.uwo.ca/anatomy/laird/index.htm

>>> Jennifer Stanley <jstanle2@uwo.ca> 18/09/2009 5:19 pm >>>
Hi Dr. Laird:

Thank you for your recent Biohazard Modification Form submission. Please clarify, as soon as possible, whether or not the retrovirus particles are being produced.

Thanks,
Jennifer

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Cell Biology

ATCC® Number: CRL-6475™ Order this Item

Price: $264.00

Designations: B16-F10

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: melanocyte

Source:
Organ: skin
Strain: C57BL/6J
Disease: melanoma

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 (technology from amaxa)

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. 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
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%

Subculturing:

Related Links
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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 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:
Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790

22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36: 3608-3615, 1976. PubMed: 953987

22192: Fidler IJ, Bucana C. Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. Cancer Res. 37: 3945-3956, 1977. PubMed: 908034

22243: Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197: 893-895, 1977. PubMed: 887927

23224: Briles EB, Kornfeld S. Isolation and metastatic properties of detachment variants of B16 melanoma cells. J. Natl. Cancer Inst. 60: 1217-1222, 1978. PubMed: 418183

23362: . . Nat. New Biol. 242: 148-149, 1973.

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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.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number:	CRL-6322™ Order this Item	**Price:**	$318.00
Designations:	B16-F0		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Mus musculus* (mouse)		
Morphology:	Spindle shaped		
Source:	**Organ:** skin **Strain:** C57Bl/6J **Disease:** melanoma		
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 technology from amaxa)		
Tumorigenic:	Yes		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. 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 (CO_2), 5% **Temperature:** 37.0°C		
Subculturing:			

Related Links

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

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 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:
Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790
22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36: 3608-3615, 1976. PubMed: 953987
22192: Fidler IJ, Bucana C. Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. Cancer Res. 37: 3945-3956, 1977. PubMed: 908034
22243: Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197: 893-895, 1977. PubMed: 887927
22424: Fidler IJ. Immune stimulation-inhibition of experimental cancer metastasis. Cancer Res. 34: 491-498, 1974. PubMed: 4812256
23224: Briles EB, Kornfeld S. Isolation and metastatic properties of detachment variants of B16 melanoma cells. J. Natl. Cancer Inst. 60: 1217-1222, 1978. PubMed: 418183
23362: . . Nat. New Biol. 242: 148-149, 1973.

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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: November 21, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

This form must also 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, Health Canada (HC) 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. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR: DALE W. LAIRD
SIGNATURE:
DEPARTMENT: ANATOMY & CELL BIOLOGY
ADDRESS: DSB 00072
PHONE NUMBER: x 86827
EMAIL: DALE.LAIRD@SCHULICH.UWO.CA

Location of experimental work to be carried out: Building(s) DSB Room(s) 00076 / 00070

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): CX43 MUTATIONS LINKED TO HUMAN DISEASE

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.

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

DR. QING (CINDY) SHAO
DR. STEPHANIE LANGLOIS
DR. SILVIA PENUELA
DR. ISABELLE PLANT
DR. GREGORY GONG
JARED CHURKO
RUCHI BHALLA
STEVE CELETTI
KATHERINE TOTH
JENNIFER SIU
JAMIE SIMEK

1.0 Microorganisms

GRANT RENEWED APRIL, 2009

Laird, Dale W. CIHR Operating Grant

Cx43 mutations linked to human disease

Intercellular gap junction channels allowing the direct passage of small molecules and secondary messengers between most contacting cells are built from a library of 21 connexin (Cx) family members. At present, 8 distinct genetic diseases ranging from sensorineural deafness to developmental disorders have been linked to germ line mutations in the genes encoding connexins. To date, 42 mutations in the *GJA1* gene encoding the gap junction protein, Cx43 have been linked to the human developmental disorder known as oculodentodigital dysplasia (ODDD). This primarily autosomal dominant disease is typically characterized by syndactyly, camptodactyly, craniofacial abnormalities, enamel loss, incontinence and ophthalmic defects. Given that Cx43 is the predominant connexin expressed in over 35 distinct cell types, it is remarkable that patients have moderate to severe defects in some organs while other organs appear to remain free of developmental abnormalities and disease. Although many mutants are distributed on the cell surface, not unlike wild-type Cx43, all mutants tested to date exhibit complete or substantial loss-of-function and dominant-negative effects on co-expressed wild-type Cx43 with respect to gap junctional intercellular communication (GJIC). Mechanistically, at least some of these mutants appear to act as dominant-negatives by direct co-oligomerization with wild-type Cx43, although their effects in vitro and in vivo on other co-expressed connexins remain unknown. **Thus, we hypothesize that different ODDD-linked Cx43 mutants exhibit distinct cellular phenotypes and effects on co-expressed connexins manifesting in a loss of GJIC and perturbed cell differentiation, ultimately resulting in variable disease load.**

Aim 1: Examine the functional status, fate, dynamics and inter-connexin interactions of dominant and recessive ODDD-linked Cx43 mutants in reference cells and in cells obtained from ODDD patients. Dominant and recessive Cx43 mutants will be characterized with respect to (1) their ability to exert dominant and transdominant effects on GJIC, and (2) hemichannel function. These parameters will be assessed in defined reference cell models and primary cell cultures from ODDD-linked mutant mice that co-express Cx43 or other connexins typically co-expressed with Cx43 *in vivo*. FRET, co-immunoprecipitation and pulse-chase studies will be used to determine if the mutants establish direct interactions with co-expressed connexins and regulate their functional half-life. Finally, the expression, localization, phosphorylation status, and turnover of Cx43 will be examined in fibroblasts and/or tissue biopsies obtained from a cohort of patients harbouring Cx43 gene mutations.

Aim 2: *Characterize transgenic mouse models of ODDD that harbour missense or truncation mutations in distinct regions of the Gja1 gene, and analyze the consequences of the mutants on cell differentiation.* Interestingly, current mouse models harbouring different ODDD-linked mutants exhibit both similar and distinct phenotypes that echo the diversity of symptoms observed in ODDD patients. Thus, we will first finish the generation and characterization of a "knock-in" gene-targeted mouse in which the endogenous Cx43 coding sequence is replaced either by the human wild-type Cx43 coding sequence or the $Cx43^{fs260}$ mutant coding sequence. Together with the $Cx43^{G60S}$, $Cx43^{I130T}$, $Cx43^{G138R}$ and littermate control mice, we will compare the phenotypes of these 4 mutant mouse models of human ODDD by performing *in situ* analysis using combinations of microCT and microMRI analysis, in addition to immunohistochemistry for connexins and tissue specific differentiation markers. Cell differentiation and function will be further assessed in primary cell cultures and/or explants from mineralized tissue (osteoblasts and ameloblasts), where abnormalities are consistently prevalent in patients; smooth muscle cells and myofibroblasts from the bladder, as approximately 30-50% of the patient cohort report urinary problems; and the mammary gland (myoepithelial and luminal cells), where sub-clinical disease may be present. Lastly, BrdU incorporation and Ki67 immunolabeling as well as TUNEL and caspase 3 staining will be used to assess the effect of Cx43 mutants on cell proliferation and death, respectively.

These studies will combine reference cell models expressing ODDD-mutants, mouse models of ODDD and direct ODDD patient data to establish genotype/phenotype relationships and the role of Cx43 in cell differentiation and function.

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

Do you use microorganisms that require a permit from the CFIA? ○ 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	Health Canada or CFIA Containment Level
DH5 α E.coli	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500 ML	INVITROGEN	☒ 1 ○ 2 ○ 3
JM109	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500 ML	PROMEGA	☒ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ○ 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 in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒ Yes ○ No	SKIN BIOPSIES	Not applicable
Rodent	☒ Yes ○ No	TRANSGENIC & MUTANT MICE	2006-101
Non-human primate	○ Yes ☒ No		
Other (specify)	○ Yes ☒ No		

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	HELA, 293T, 293 HEK, TUMOR CELL LINES	ATCC
Rodent	☒ Yes ○ No	KERATINOCYTES, NAK D.ICR. MIR, N2A	CLONETIC, ATCC VENDOR, INVITROGEN
Non-human primate	○ Yes ☒ No		
Other (specify)	☒ Yes ○ No	MOCK	ATCC

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) THESE ARE ALL STANDARD WELL USED CELL LINES

2.4 For above named cell types(s) indicate HC or CFIA containment level required ○ 1 ☒ 2 ○ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☒ 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)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	ODDD PATIENTS AND RELATIVES	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)	ODDD PATIENTS AND RELATIVES	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Organs or Tissues (preserved)	ODDD PATIENTS AND RELATIVES	○ Yes ☒ No		☒ 1 ○ 2 ○ 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? ☒ YES ○ NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
SM109	T-EASY P. DNA3 (+)(-) PEGFP	PROMEGA INVITROGEN CLONETECH	CONNEXIN GENES PANNEXIN GENES	CELL LINES EXPRESSING PLASMIDS TEND TO GROW SLOWER AND FORM CELL-CELL OR CELL CHANNELS.

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

4.3 Will genetic modification(s) involving viral vectors be done? ☒ YES, complete table below ○ NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
RETROVIRUS	PP-2	DR. J. GARIPPO McGILL UNIVERSITY	CONNEXINS PANNEXINS	CELLS COMMUNICATE BETTER

* Please attach a Material Safety Data Sheet or equivalent. SEE B.U.A.-UNO-0017 AS INFORMATION IS CURRENTLY ON RECORD WITH SAFETY OFFICER

4.4 Will genetic sequences from the following be involved?

- HIV ○ YES, please specify _____ ☒ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ○ YES, specify _____ ☒ NO
- SV 40 Large T antigen ○ YES } H.E.K. cells ☒ NO
- E1A oncogene ○ YES } HeLa cells ☒ NO
- Known oncogenes ○ YES, please specify _____ ☒ NO
- Other human or animal pathogen and or their toxins ○ YES, please specify _____ ☒ NO

4.5 Will virus be replication defective? ☒ YES ○ NO

4.6 Will virus be infectious to humans or animals? ☒ YES ○ NO

4.7 Will this be expected to increase the containment level required? ○ YES ☒ NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? ○ YES ☒ NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? ○ YES ○ NO

5.3 How will the virus 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? ○ YES, number. _____ ○ NO ○ PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used MICE

6.3 AUS protocol # 2006-101

6.4 Will any of the agents listed be used in live animals ○ YES, specify. _____ ☒ NO

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

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 ☒ NO
- Pound source cats O YES ☒ NO
- Cattle, sheep or goats O YES ☒ NO
- Non-Human Primates O YES, please specify species _____ ☒ NO
- Wild caught animals O YES, please specify species & colony # _____ ☒ NO
- Birds O YES ☒ NO
- Others (wild or domestic) O YES, please specify _____ ☒ NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ☒ 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_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ☒ 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 lifestage of the insect? _____

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

_____
_____
_____

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊗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

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 10.0 ⊗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 plan 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 pathogenic agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
X YES, permit # if on-campus Bio - UWO - 0017
O NO
O NOT REQUIRED

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 [signature] Date: March 4, 09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: [signature]
Date: 31 March 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: [signature]
Date: March 27/09

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: Bio-UWO-0017 Expiry Date (3 years from Approval): March 26, 2012

Special Conditions of Approval:

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

..... Original Message .....
Subject:questions
Date:Tue, 03 May 2005 09:17:07 -0400
From:Dale Laird <Dale.Laird@fmd.uwo.ca>
To:jstanle2@uwo.ca
CC:Cindy Shao <Cindy.Shao@fmd.uwo.ca>

Dear Jennifer, I apologize for the delay in responding to your questions and comments on my last Biohazardous Agents Registry Form. Your email was sent to dwlaird@uwo.ca instead of dale.laird@fmd.uwo.ca and got held back for some reason. I found 650 emails on my UWO account of which many were not forwarded to my FMD account (as they should be). Anyway here is the additional information you requested.

1. Section 4.3 - please note that HEK 293 contains E1A oncogene
Response: Thank you this is noted.

2. Section 4.5
Response: -The AP-2 retroviral vector is the main one we have been using for several years. The documentation, vector source and description was placed on file with the Safey office several years ago. See manuscript reference Gallpeau et al., 1999, Cancer Research 59; 2384-2394. The 293GPG packaging cells which produce replication-defective virus are described in this same paper. The 293GPG packaging cells were orginally described in Ory et al., Proc Natl Acad Sci U S A. 1996 Oct 15;93(21):11400-6. Both the AP-2 vector and packaging cells are from Dr. Jacques Galipeau in Montreal. The MTA for these cells and vectors were done in collaboration with Dr. Chris Naus several years ago.
-More recently we have obtained thepH1.1-QCXIH retroviral vector (from GenScript) for shRNA studies. See (Barton and Medzhitov, 2002, PNAS 99; 14943-14945). The HEK293 derived packaging cells produce replication-incompetent viral particles. (See AmphoPack293 from BD Biosciences).
3: Section 1.2 - confirm E. coli DH5alpha,
Response: -Yes this is the E.coli we use,

4: Section 6.0 - do you have an animal protocol?
Response: -The animal studies being performed in this study are in conjunction with the Co-Principle Applicants, Drs. Kidder and Dr. Bernier. Their animal protocols have been modified to include this new grant. I do not have a separate animal protocol.

5: Section 8.3 - description of lindane use:
Response: -Lindane is disolved in DMSO at 50um and used as a gap junction channel inhibitor at a final concentration of 50nM.

I trust this answers all your questions. Again sorry for the delay.

Dale Laird

Dale W. Laird, Ph.D.
Professor
Canada Research Chair in Gap Junctions and Disease

S 2... 2005 1:43 PM

Modification Form for Permit BIO-UWO-0017

Permit Holder: Dale Laird

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

Jamie Simek

Cindy Pan

Ruchi Bhalla

Jared Churko

Silvia Peneula (Penuela)

Isabelle Plante

Xiang-Qun Gong

Cindy Shao

Jason Martin

Robert Lorentz

Jack Lee

~~Jennifer Siu~~

Additional Personnel
(Please list additional personnel here)

MICHAEL STEWART

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DH5 alpha, JM109	
Approved Primary and Established Cells	Human (primary), skin biopsies, Rodent (primary), transgenic alpha mutant mouse, Human (established), HELA, 293T, 293 Hek, Tumour cell lines, Rodent (established), Keratinocytes, NRK BiCR, MIRK, N2A,	Established cell lines: B16-F0, B16-F10, B16-BL6 (mouse skin melanoma)
Approved Use of Human Source Material	Human blood (whole), Human tissues (unpreserved), Human tissues (preserved), ODDD Patients and relatives	
Approved Genetic Modifications (Plasmids/Vectors)	JM109, T-EASY, PcDNA3, pEGFP, retrovirus AP-2, SV 40 Large T antigen, HEK, E1A oncogenes, HeLa, pGFP-V-RS	
Approved Use of Animals	mice	

Approved Biological 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 STORED, USED AND DISPOSED OF..*

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: 

Current Classification: 2 **Containment Level for Added Biohazards:** 1

Date of Last Biohazardous Agents Registry Form: Mar 27, 2009

Date of Last Modification (if applicable): Dec 21, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ **Date:** _____

Modification Form for Permit BIO-UWO-0017

Permit holder: Dale Laird

Additional Biohazards for approval:

Established cell lines:

B16-F0, B16-F10, B16-BL6 (mouse skin melanoma). The first two cell lines were obtained from ATCC (see data sheets enclosed) and are Biosafety level 1. The B16-BL6 cell line was originally derived from B16-F10 (see paper attached by Poste, et al.) and it was kindly provided to us by Dr. Moulay Alaoui-Jamali of the Lady Davis Institute at McGill University.

These cell lines will be used for cell culture in vitro, shRNA silencing of Pannexins, protein and RNA extractions, and other cell biology and biochemistry work currently performed with other cell lines in our lab. They will be handled and disposed according to the current biosafety regulations.

Cell Line Designation: B16-F0
ATCC® Catalog No. CRL-6322™

Table of Contents:

Cell Line Description

Organism: *Mus musculus* (mouse)
Strain: C57BL/6J
Tissue: melanoma; skin
Tumorigenic: yes, in syngeneic mice
Growth properties: adherent
Morphology: spindle shaped
Depositors: Naval Biosciences Laboratory
Comments: Confirmed as a murine cell line by ATCC. Tests for microbial contamination were negative.

DISCLAIMER: This cell line was deposited by the Naval Biosciences Laboratory. Researchers should be aware that one purpose of the NBL bank was to produce and distribute early passage cultures of cells from various clinical materials. Many of these lines, unlike most others available and published in the ATCC catalog, are primary and mixed. Those from tumors, for example, may consist of mixtures of stromal and cancer cells in which the former cell types predominate.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.

4. Resuspend the cell pellet with the recommended complete medium and dispense into a 25 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. *It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells, grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the

Product Information Sheet for CRL-6322

Lot number: 58254426

Designation: B16-F0 Description: Melanoma
Total Cells/mL: 8.5 x 10(5)
Expected Viability: 85.1% to 96.3%
Ampule Passage No.: Unknown
Population Doubling (PDL): N/A
Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)
Volume/Ampule: 1 mL
Date Frozen: 12/18/08

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 60% to 70% confluence in 2 days.

American Type Culture Collection
10801 University Boulevard
Manassas, VA 20110-2209

Ordering info: 800 638-6597 (USA and Canada)
703 365-2700 (elsewere) Fax: 7033652750
email: sales@atcc.org web site: www.atcc.org

2. **If the cells are still attached**, aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which 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.
   Subculture ratio: 1:6 to 1:10

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in **Culture Of Animal Cells: A Manual Of Basic Technique** by R. Ian Freshney, 5th edition, published by Wiley-Liss. N.Y., 2005.

Medium Renewal

Every 2 to 3 days

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO_2 in air atmosphere is then recommended).

ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) and ATCC® Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(Additional references may be available in the catalog description at www.atcc.org)

Fidler IJ. **Biological behavior of malignant melanoma cells correlated to their survival in vivo.** Cancer Res. 35: 218-224, 1975 PubMed: 75074100

Fidler IJ et al. **Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A.** Cancer Res. 36: 3608-3615, 1976 PubMed: 76255481

Fidler IJ and Bucana C. **Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes.** Cancer Res. 37: 3945-3956, 1977 PubMed: 78001804

Fidler IJ and Kripke ML. **Metastasis results from preexisting variant cells within a malignant tumor.** Science 197: 893-895, 1977 PubMed: 77236031

Briles EB and Kornfeld S. **Isolation and metastatic properties of detachment variants of B16 melanoma cells.** J. Natl. Cancer Inst. 60: 1217-1222, 1978 PubMed: 78173966

Nat. New Biol. 242: 148-149, 1973

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations

ATCC®

Product Information Sheet for CRL-6475

Lot number: 58078645

Designation: B16-F10 Description: Melanoma
Total Cells/mL: 7.3 x 10(5)
Expected Viability: 66.7% to 75.9%
Ampule Passage No.: Unknown
Population Doubling (PDL): N/A
Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)
Volume/Ampule: 1 mL
Date Frozen: 10/01/08

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 60% to 70% confluence in 2 days.

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B16-
first→ BL6 cells
report

[CANCER RESEARCH 40, 1636–1644, May 1980]

In Vitro Selection of Murine B16 Melanoma Variants with Enhanced Tissue-invasive Properties

G. Poste,[1, 2] J. Doll,[1] I. R. Hart,[3] and I. J. Fidler[3]

Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14263 [G. P., J. D.], and Cancer Biology Program, National Cancer Institute, Frederick Cancer Research Center, Frederick, Maryland 21701 [I. R. H., I. J. F.]

ABSTRACT

New assay methods have been devised to quantitate tumor cell invasion of tissues of differing histological complexity maintained as organ cultures *in vitro* (chorioallantoic membrane of chicken, mouse urinary bladder, and canine blood vessel). In addition to quantitating tumor cell invasion, these methods also allow recovery of invasive cells for comparison with noninvasive cells. These methods have been used to select variant sublines from murine B16-F1 and B16-F10 melanoma lines that display significantly greater tissue-invasive abilities than the parent lines. B16 variant sublines selected *in vitro* for increased invasiveness through the bladder wall or vein also show a significant increase in their ability to form spontaneous and experimental metastases *in vivo*. In contrast, cells from the same parent cell line selected for increased invasiveness through the chorioallantoic membrane do not show significant alterations in metastatic behavior. We conclude that invasive variants can be isolated from the parent B16 tumor by several *in vitro* methods and that the level of expression of the invasive phenotype *in vivo* may be determined by the severity of the selection procedure *in vitro*.

INTRODUCTION

Invasion of lymphatics and blood vessels by malignant cells provides a major pathway for the dissemination of neoplastic cells within the body (for reviews, see Refs. 7, 8, and 27). In addition, following their arrest in the capillary beds of different organs, circulating tumor cells invade the wall of these vessels and escape into the extravascular tissue(s) where they establish metastases. Despite its obvious importance in the metastatic process, relatively little is known about the mechanism(s) of tumor invasion. This deficiency reflects the formidable technical problems encountered in studying invasion *in vivo* and the lack of quantitative methods for studying invasion *in vitro* and for recovering invasive cells once invasion has taken place.

A substantial body of evidence has been assembled in the last few years which indicates that primary malignant neoplasms are not homogeneous entities of cells with uniform properties but instead contain subpopulations of tumor cells with widely differing metastatic abilities (for reviews, see Refs. 7, 8, and 27). This phenotypic heterogeneity dictates that examination of heterogeneous unselected tumor cell populations may offer little insight into the cellular properties responsible for invasion and/or metastasis if only very few cells within the population express these behavioral traits. A more productive approach to the experimental analysis of the cellular properties needed for successful invasion is to isolate invasive tumor cell subpopulations and compare them with poorly invasive or noninvasive tumor cells derived from the same parent cell population. In this report, we describe methods for the *in vitro* isolation of a series of murine B16 melanoma cell variants with enhanced invasive properties and an initial characterization of their behavior *in vivo*.

MATERIALS AND METHODS

Cells. The origin and properties of the B16-F1 (low potential for lung colonization) and B16-F10 (high potential for lung colonization) sublines of the B16 melanoma have been described in detail previously (5, 12). Cultures of mouse embryo fibroblasts were prepared by trypsinization of 14- to 16-day-old C57BL/6 mouse fetuses as described elsewhere (29). Homogeneous peritoneal macrophage cultures were obtained from C57BL/6 mice inoculated i.p. with sodium thioglycollate (Baltimore Biological Laboratories, Cockeysville, Md.) as described previously (29). All cells were incubated in plastic flasks or Petri dishes in CMEM[4] (12, 29). The components of CMEM were obtained from Flow Laboratories, Inc., Rockville, Md., and the Grand Island Biological Co., Grand Island, N. Y. Cell cultures were incubated at 37° in a humidified atmosphere containing 5% CO_2 in air. All cultures were free of *Mycoplasma* species and pathogenic murine viruses (12), and they remained so throughout the experiments. In certain experiments, cell cultures (except macrophages) were labeled with [^{125}I]IdUrd as detailed in Ref. 5. Briefly, actively growing nonconfluent monolayer cell cultures were incubated for 24 hr in CMEM containing [^{125}I]IdUrd (0.2 μCi/ml; specific activity, 200 μCi/mmol; New England Nuclear, Boston, Mass.). This method labels more than 95% of the cells and does not alter their metastatic behavior *in vivo* (5).

Assay of Tumor Cell Invasion in CAM and Selection of Invasive Cell Variants. Newly fertilized eggs were incubated at 38° in a humidified atmosphere (relative humidity, approximately 75%) in an egg incubator (Favorite Incubator; Leahy Mfg. Co., Higginsville, Mo.) until Day 8 after fertilization, when the CAM was dropped using the false air sac method as described elsewhere (28). Two days later, the CAM was harvested and dissected by a sterile technique into pieces approximately 0.625 inch square for eventual transfer to the

[1] Recipient of USPHS Research Grant CA 18260 and NIH Core Support Grant 17609 to Roswell Park Memorial Institute Cancer Cell Center.

[2] To whom requests for reprints should be addressed.

[3] Recipient of National Cancer Institute Contract NO1-CO-75380 with Litton Bionetics Inc.

Received November 1, 1979; accepted February 7, 1980.

[4] The abbreviations used are: CMEM, Eagle's minimal essential medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and 2-fold concentrated vitamin solution; [^{125}I]IdUrd, [^{125}I]iododeoxyuridine; CAM, chorioallantoic membrane; o.d., outside diameter; i.d., internal diameter; MEM, Eagle's CMEM (see above) without serum; HBSS, Hanks' balanced salt solution.

penetrating the new CAM's were, in turn, harvested and passaged again through fresh CAM's up to 14 times. These cells will be described by the prefix CP (CAM-passage) followed by the number of times the cells had been passaged through CAM (*e.g.*, B16-F1-CP10 = B16-F1 cells passaged 10 times through the CAM).

In most selection experiments, nutrient agar was used in the lower chamber. Normal CAM cells were unable to grow in agar, but B16 tumor cells could form colonies in agar because of their loss of anchorage dependence. In certain experiments, fluid culture medium (CMEM) or factors which are chemotactic for macrophages or leukocytes and/or certain tumor cells were placed in the bottom compartment to determine if penetration of radiolabeled tumor cells was affected by such chemotactic stimuli. Bacterial culture filtrates containing leukotactic factors were produced from cultures of *Escherichia coli* (WHO reference strain 16) as described by Ward *et al.* (41). Serum fractions containing leukotactic factors were produced by zymosan activation of human serum in the presence of 1 M ϵ-aminocaproic acid (40). Zymosan-activated sera were incubated with trypsin using the method described by Orr *et al.* (25) to generate material containing factors from the third and fifth components of complement which are chemotactic for some tumor cells (25). All of the above materials were added to MEM at a final concentration of 75 μl/ml. In other experiments, the bottom chamber was filled with cell-free culture supernatant fluid harvested from the same tumor cell population added to the upper chamber.

Assay of Tumor Cell Invasion in Mouse Urinary Bladder and Selection of Invasive Cell Variants. Adult male C57BL/6 mice were killed by ether inhalation. The mice were submerged in a 5% Wescodyne solution (West Chemical Products, New York, N. Y.), immersed in 70% ethanol for 2 min, and placed into a laminar flow hood. The abdominal cavity was opened in a sterile fashion, and the urogenital system was fully exposed (Fig. 3). Urine within the urinary bladder was expressed by gentle finger pressure (sterile surgical gloves were worn during these procedures), after which the urethra was occluded by application of a hemostat. One testis was displaced from the scrotal sac, and the ductus deferens was identified and spread across an open pair of forceps, with care being taken not to rupture the duct. The ductus deferens was penetrated (Fig. 3) using a 27-gauge needle (Becton, Dickinson, and Company, Rutherford, N. J.) attached to a tuberculin syringe, and 0.3 to 0.4 ml of tumor cell suspension (0.9 to 1.2 $\times$ 10^6 cells) was injected slowly. The injected cells reached the urethra via the emergence of the ductus deferens in the prostrate, and retrograde urethral flow carried suspended tumor cells to the bladder which gradually became filled with cells (Fig. 4). A ligature of 2-0 chromic catgut (Detnatel, Queens Village, New York, N. Y.) was placed around the neck of the bladder distal to the entrance of the ureters and tied tightly (Fig. 4). The bladder was then excised from the abdominal cavity using hot scissors (flamed) in order to cauterize the cut tissue. The ligated isolated bladder was then vigorously rinsed in 4 changes of HBSS containing gentamicin sulfate (0.1 mg/ml; Schering Corporation, Kenilworth, N. J.). The excised bladders were then placed in sterile 60-mm plastic Petri dishes containing a preformed base of semisolid agar produced by pouring 5 ml of agar (see below) into dishes followed by chilling at 4° for 10 min to solidify the agar. The semisolid agar medium was prepared by

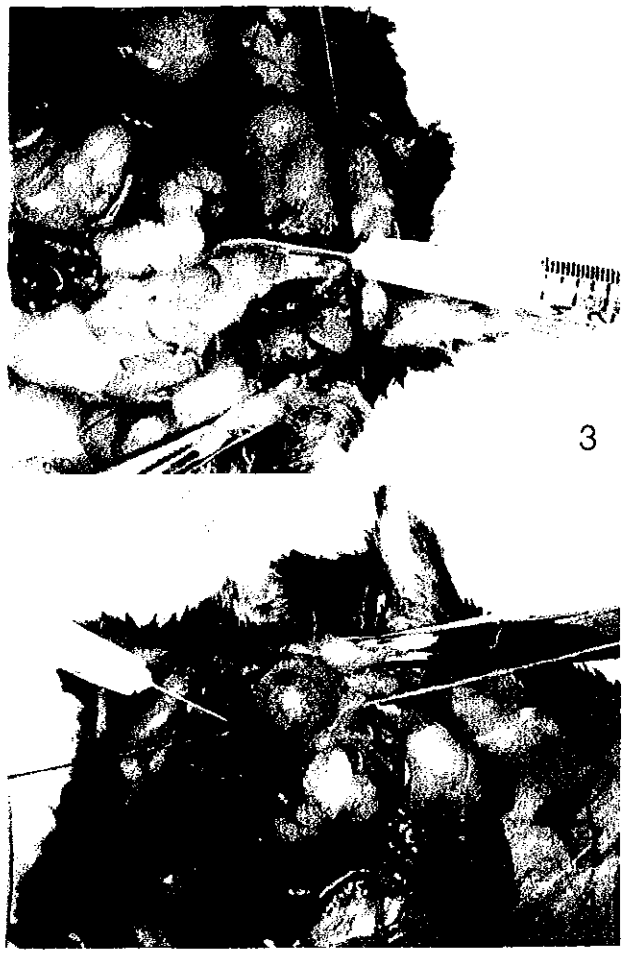

Fig. 3. Retrograde filling of murine urinary bladder with tumor cells via the vas deferens. The bladder has been emptied mechanically, and the ductus deferens is cannulated by a 27-gauge needle.

Fig. 4. Tumor cell suspension (0.3 to 0.4 ml) has been injected into the ductus deferens, and the urinary bladder is filled by retrograde flow. Hemostat occludes the penile urethra and ligature is 2-0 catgut.

mixing 20 ml 2 $\times$ MEM, 5 ml fetal calf serum, 5 ml tryptose phosphate broth (all reagents from Grand Island Biological Co.), and 20 ml 1.25% agar (Difco Laboratories, Detroit, Mich.). The excised bladders were placed on the agar base, neck vertically up, and semisolid supporting agar, composed of 2 volumes of agar base to 1 volume of CMEM, was added to a point just below the ligature. Each bladder was restrained in the vertical position by stretching the free ends of the ligating catgut at 180° to one another and placing the Petri dish lid on the free ends. Thus, solidification of the agar maintained the bladders in the correct upright position. One bladder was placed in a single Petri dish, and between 5 and 10 bladders were used to obtain cells at each passage level (see below). Individual bladders were removed from the agar at daily intervals after incubation at 37° in a humidified atmosphere containing 5% CO_2 for up to 5 days. The agar situated around the site where the base of the bladder had rested was then pipetted up and added to 1 ml CMEM in a single well of a Costar 16-mm multiwell dish (Costar, Cambridge, Mass.). These dishes were incubated under the same conditions as described above, and the wells were examined daily for tumor colonies. When tumor

hollow fiber capillary bundles have been replaced by a 15-cm length of ultrahigh-molecular-weight polyethylene tube. The vein is fitted onto this tube before being placed into the chamber. Segments of dog femoral vein were perfused *in situ* with ice-cold 0.9% NaCl solution before removal from the animal. Collateral vessels were tied off or occluded by a diathermy needle before removing the main portion of vein. Veins were fitted onto the tube by inserting the tube into the lumen of the vessel for a sufficient distance to completely cover the tube. Two encircling catgut ties were then made at each end of the tube, and the excess vein was trimmed with a scalpel. The tube and vessel were then placed into the polycarbonate outer chamber and sealed with screw-fit end pieces and silicon rubber sealing O-rings. Both ends of the tube were then connected to 0.132 inch i.d. x 0.183 inch o.d. gas-permeable medical grade silicone tubing (Dow Corning Corp., Midland, Mich.) to create a closed perfusion circuit with an intervening reservoir of culture medium. The tubing between the reservoir and the input side of the chamber was passed through a 500-ml conical flask containing a mixture of 5% CO_2 in air to ensure correct gaseous exchange. Tumor cells (1×10^6) in CMEM (1.5 ml) were then injected into the outer chamber, and the entire apparatus was incubated at 37° in a walk-in hot room. For invasion assays, ^{125}I-IdUrd-labeled cells were injected into the outer chamber, and the number of cells penetrating the wall of the vessel was determined by measuring the radioactivity recovered in the inner perfusion circuit. For selection experiments, nonradiolabeled cells were introduced, and invasive cells harvested from the internal circuit were reinjected into chambers containing fresh vessels. The invasive cell populations recovered by serial passage through veins will be referred to by the prefix BV (blood vessel) followed by the number of times the cells have been passaged through veins.

Animals. Specific-pathogen-free C57BL/6 mice were obtained from the Animal Production Area, Frederick Cancer Research Center, and the West Seneca Laboratories, Roswell Park Memorial Institute.

Metastasis Formation. The ability of cells to form experimental metastases after i.v. injection was measured by injecting 5×10^4 to 5×10^5 viable cells as a single-cell suspension in 0.2 ml HBSS into the tail vein of unanesthetized adult C57BL/6 mice matched for age, weight, and sex. Mice were killed 18 or 21 days later, and the number of lung metastases was determined with a dissecting microscope as described previously (6, 9).

The following methods were used to assay the ability of cells to form spontaneous metastases: (*a*) C57BL/6 mice were given s.c. injections in the external ear of 5×10^4 cells in 0.1 ml HBSS, and the number of lung metastases was determined 4 weeks later; (*b*) C57BL/6 mice were given s.c. injections in the ear as above. The ear plus the growing tumor were then amputated 10 days later (7), and formation of regional lymph node and lung metastases was determined 4 to 6 weeks later; or (*c*) C57BL/6 mice were given i.m. injections of 2.5×10^4 viable cells in 0.5 ml HBSS in the footpad of the hind leg. In some animals, the "primary" tumor was amputated after 4 weeks, and metastasis formation was determined 4 weeks thereafter. In other animals, the "primary" tumor was not amputated, and metastasis formation was measured 6 weeks after initial tumor cell injection. In all of the protocols, mice were matched for age, weight, and sex.

RESULTS

***In Vitro* Selection of B16 Melanoma Cell Variants with Increased Ability to Invade CAM.** The ability of B16-F1 melanoma cells to invade and penetrate CAM maintained *in vitro* and the invasive behavior of variants selected from B16-F1 cells by serial passage of cells that successfully penetrate the CAM are shown in Table 1. The results indicate that the proportion of cells in the parent cell population that penetrate through the CAM to reach the lower chamber (Fig. 2) is very low. Serial passage of the invasive cell fractions recovered from the lower chamber produced a significant increase in the proportion of cells penetrating the CAM. Maximum efficiency of invasion was achieved after 10 passages through the CAM (Table 1).

Confirmation that invasion in this system involves selection (enrichment) of subpopulations with increasing invasive properties was provided by the finding that the "nonpenetrating" cell fractions recovered from CAM's inoculated with early-passage populations contained significantly fewer invasive cells than did the starting inoculum (Table 1). With increasing CAM passage, however, the proportion of invasive cells recovered in the nonpenetrating fraction increased. After 10 passages, the efficiency of invasion by the reinoculated nonpenetrating fraction was similar to that of the original inoculum, suggesting that the cell population was now composed almost exclusively of invasive cells. Thus, in early passages, invasion was a nonrandom process which selects for invasive cell subpopulations. With subsequent passaging, the proportion of invasive cells in the population was enriched; by 10 passages, invasion had become a random process and the population consisted of cells with invasive properties.

The invasive behavior of variants at any given passage level is highly reproducible, and significant variation has not been detected using different CAM preparations. In addition, assays

Table 1

Invasiveness of B16-F1 mouse melanoma cells during serial passage in chick CAM in vitro

Aliquots of 1×10^6 viable B16-F1 melanoma cells in CMEM were added to the ectodermal surface of portions of CAM in invasion chambers. Cells penetrating the CAM were recovered from the bottom chamber of the invasion apparatus 7 days later and reinoculated onto fresh CAM preparations. This was repeated up to 14 times. At each passage level, [^{125}I]IdUrd-labeled cells (penetration assay) and nonradiolabeled cells (serial passaging) derived from a common parent culture were used.

Passage no.	% of inoculated cells penetrating the CAM[a]	% of "nonpenetrating" cell fraction penetrating new CAM[b]
1	2.4 ± 0.6[c]	Not detectable
2	4.1 ± 1.1	1.3 ± 0.4
4	11.6 ± 2.3	3.9 ± 0.8
6	17.4 ± 3.7	13.7 ± 2.1
8	23.7 ± 4.4	18.4 ± 2.7
10	31.6 ± 4.9	27.6 ± 4.3
14	30.6 ± 4.2	33.4 ± 4.6

[a] Cell-associated [^{125}I]IdUrd radioactivity recovered in the bottom chamber as a percentage of total radioactivity in the original cell inoculum. Inocula contained between 3.5 and 6×10^5 cpm/10^6 cells.

[b] Nonradiolabeled cells (1×10^6) were added to CAM invasion chambers and incubated for 7 days at 37° after which "nonpenetrating" cells which failed to reach the bottom chamber were recovered from the culture medium in the upper chamber and from the CAM (see "Materials and Methods"). These were cultured *in vitro* to generate sufficient numbers of cells, labeled with [^{125}I]IdUrd, and reinoculated onto fresh CAM to assay cell penetration as described in Footnote *a*.

[c] Mean ± S.E. derived from measurements on 8 replicate chambers at each passage.

Invasion of Different Tissues by Variants Selected in CAM, Bladder, and Vein. Selection of cells for invasiveness in one tissue may not necessarily enable them to invade other tissues. This was demonstrated by showing that CAM-passaged invasive variants did not differ from unselected parent F1 cells in their ability to invade bladder or vein (Table 4). In contrast, bladder- and vein-passaged variants were highly invasive in the CAM, urinary bladder, and vein preparations (Table 4).

Formation of Spontaneous and Experimental Metastases by Invasive Variants. Assay of the ability of CAM-passaged invasive cell variants to form experimental metastases after i.v. injection or spontaneous metastases after s.c. injection into the external ear revealed that their metastatic activity did not differ significantly from parental B16-F1 cells (Table 5). In contrast, cells from invasive variants selected by passaging in bladder organ culture (BP series) or perfused vein (BV series) produced significantly more pulmonary metastases than did parent B16-F1 cells following both i.v. (experimental metastasis) and s.c. (spontaneous metastasis) injection (Table 5). Indeed, the metastatic activity of these invasive variants is comparable to that of the highly metastatic B16-F10 line which was selected from the B16-F1 line for its ability to produce lung metastases following i.v. injection (5).

Table 3

Invasiveness of B16-F1 mouse melanoma cells during serial passage in segments of dog femoral vein maintained in a perfusion apparatus in vitro

Segments of dog femoral vein in a perfusion culture apparatus were injected with 1 × 10^6 B16-F1 cells and maintained for 2 weeks at 37°. Cells that penetrated the blood vessel wall were recovered from the inner perfusion circuit (see "Materials and Methods") and reinjected onto fresh vein preparations. This process was repeated up to 10 times. Replicate populations of [^{125}I]dUrd-labeled cells (penetration assay) and nonlabeled cells (serial passaging) from the same parent culture were used at each passage.

Passage no.	% of inoculated cells penetrating vessel wall[a]	% of "nonpenetrating" cell fraction penetrating new vessel[b]
1	1.6 ± 0.4[c]	0
2	19.3 ± 4.2	11.3 ± 2.9
3	32.4 ± 4.9	20.7 ± 3.6
4	36.9 ± 5.6	30.7 ± 4.8
6	35.8 ± 3.7	31.3 ± 4.2
8	31.6 ± 4.4	32.7 ± 5.0

[a] Cell-associated [^{125}I]dUrd radioactivity recovered in the inner perfusion circuit as a percentage of the total radioactivity in the original cell inoculum.
[b] Cells were injected into the outer chamber as in Footnote *a*, and the chamber was incubated for 4 days at 37°. Cells in the outer chamber which failed to attach to the vessel were aspirated, cultivated *in vitro* for a short period to provide sufficient cells, labeled with [^{125}I]dUrd, and reinjected onto fresh vessels, and their penetration was assayed as in Footnote *b*.
[c] Mean ± S.E. derived from measurements on 2 chambers at each passage.

Table 4

Invasiveness of B16 melanoma cell variants in different tissues in vitro

Cell[a]	% of cell penetration[b]		
	CAM	Bladder	Vein
Parent B16-F1	2.3 ± 0.5[c]	1.3 ± 0.4	1.6 ± 0.4
B16-F1-CP10	29.8 ± 4.3	1.9 ± 0.6	2.3 ± 0.5
B16-F1-BP8	38.2 ± 5.1	30.7 ± 4.3	24.7 ± 3.9
B16-F1-CP10, BP4	30.9 ± 4.0	22.6 ± 4.6	14.3 ± 3.1
B16-F1-BV6	47.2 ± 6.8	26.5 ± 4.1	33.2 ± 4.5

[a] The suffix designations for B16-F1 cells are: CP10, 10 passages in CAM; BP8, 8 passages in bladder organ cultures; CP10, BP4, 10 passages in CAM followed by 4 passages in bladder organ cultures; BV6, 6 passages through vein preparations.
[b] Preparations of the indicated cell types labeled with [^{125}I]dUrd were assayed for their ability to penetrate CAM, mouse bladder organ cultures, and perfused dog femoral vein as described in Tables 1, 2, and 3, respectively.
[c] Mean ± S.E. derived from measurements on 4 replicate assay preparations for CAM and bladder and 2 for vein.

The BL6 variant selected from B16-F10 cells by passaging in intact bladder is also more efficient than the parental B16-F10 cells in producing spontaneous metastases. However, unlike the BP variants passaged in bladder organ culture, the BL6 variant is less efficient than the parent B16-F10 line in producing experimental metastases after i.v. injection (Table 6).

DISCUSSION

A variety of *in vitro* systems have been used to study invasion of normal tissues by malignant tumor cells. Attempts to study invasion by allowing normal and tumor cell populations to interact in monolayer culture (1–3, 13, 15) are probably oversimplified since tumor cells are not required to invade an organized tissue matrix. Invasion of tumor cells into aggregates of normal cells (10, 16) suffers from similar disadvantages. In addition, doubts must be expressed about the functional significance of tumor cell infiltration into aggregates in view of

Table 5

Metastasis formation by cell variants selected from B16-F1 melanoma cells

Cell[a]	Metastasis formation	
	Spontaneous[b]	Median no. of pulmonary colonies[c]
B16-F1	2/20 (10)[d]	9 (2–27)[e]
B16-F10	12/20 (60)	111 (23–196)
B16-F1-CP10	2/19 (11)	7 (0–31)
B16-F1-BP8	14/20 (70)	92 (18–210)
B16-F1-CP10, BP4	10/19 (53)	47 (12–129)
B16-F1-BV4	13/18 (72)	104 (31–224)
B16-F1-BV8	17/20 (85)	123 (27–185)

[a] The suffix designations for B16-F1 cells are as in Table 4, Footnote *a*.
[b] Number of animals with lung metastases and total number of animals given injections. C57BL/6 mice were given injections of 5 × 10^4 viable cells in the external ear (s.c.), the injected ear was amputated 10 days later, and metastasis formation was assayed 4 weeks after amputation.
[c] C57BL/6 mice given i. v. injections of 5 × 10^4 cells, and metastasis formation was assayed 18 days later. Results derived from measurements on 10 animals/group.
[d] Numbers in parentheses, percentage.
[e] Numbers in parentheses, range.

Table 6

Metastasis formation by an invasive cell variant (BL6) derived from B16-F10 melanoma cells

Route of injection	Cell	Experiment 1	Experiment 2
		No. of mice bearing lung tumor nodules	
s.c.[a] (external ear)	B16-F10	3/9 (33)[b]	1/12 (17)[b]
	B16-BL6	6/8 (75)[b]	7/10 (70)[b]
i.m. (footpad)	B16-F10	2/7 (29)[b,c]	1/9 (22)[b,d]
	B16-BL6	7/9 (78)[b,c]	4/5 (80)[b,d]
		Median no. of lung colonies	
i.v.	B16-F10	441 (170–>500)[e,f]	231 (66–481)[e,g]
	B16-BL6	148 (43–274)[e,f]	83 (16–258)[e,g]

[a] Mice were given injections of 5 × 10^4 viable cells in the external ear s.c. and autopsied 4 weeks after injection.
[b] Numbers in parentheses, percentage.
[c] Mice given injections of 2.5 × 10^4 viable cells in the footpad; primary lesion amputated after 4 weeks and mice killed 4 weeks later.
[d] Autopsied 6 weeks after injection without amputation.
[e] Number of lung nodules formed 3 weeks after i.v. injection of 5 × 10^5 cells median. Numbers in parentheses, range.
[f] Experiment 1, 8 animals/group.
[g] Experiment 2, 15 animals/group.

or i.m., where invasiveness is a prerequisite for metastasis, it is able to exploit its advantage over the F10 cells and metastasize more effectively.

The importance of the selection procedure in determining the invasive phenotype is also illustrated by the present results in the properties of CAM-passaged cells. These variants do not differ from parental B16-F1 cells in their ability to invade other tissue (*i.e.*, bladder or vein). In addition, invasive variants selected in the CAM do not differ from the B16-F1 parent cells in their metastatic behavior *in vivo*. Comparison of CAM-selected variants, in which invasiveness and metastatic ability are functionally dissociated, with variant sublines selected in bladder and vein in which these traits are coupled could provide insight into the contribution of specific cellular alterations to these particular behavior properties.

As emphasized elsewhere (8, 27), the search for properties uniform to all invasive cells may well be unproductive. We consider, however, that the likelihood of identifying properties which are expressed with a relatively high degree of consistency in invasive tumor cells will be substantially increased by studying variant cell populations selected specifically for this phenotype.

REFERENCES

1. Abercrombie, M., and Heaysman, J. E. M. Invasive behavior between sarcoma and fibroblast populations in cell culture. J. Natl. Cancer Inst., *56:* 561–569, 1976.
2. Armstrong, P. B., and Lackie, J. M. Studies on intercellular invasion *in vitro* using rabbit peritoneal neutrophil granulocytes (PMNS). I. Role of contact inhibition of locomotion. J. Cell Biol., *65:* 439–462, 1975.
3. Barski, G., Leon, B., Le Francois, D., and Belehradek, J., Jr. Cell movement as related to oncogenesis. Blood Cells, *2:* 453–466, 1976.
4. Easty, D. M., and Easty, G. C. Measurement of the ability of cells to infiltrate normal tissues *in vitro*. Br. J. Cancer, *29:* 36–49, 1974.
5. Fidler, I. J. Metastasis: quantitative analysis of distribution and fate of tumor emboli labeled with ^{125}I-5-iodo-2′-deoxyuridine. J. Natl. Cancer Inst., *45:* 775–782, 1970.
6. Fidler, I. J. Selection of successive tumor lines for metastasis. Nat. New Biol., *242:* 148–149, 1973.
7. Fidler, I. J. Tumor heterogeneity and the biology of cancer invasion and metastasis. Cancer Res., *38:* 2651–2660, 1978.
8. Fidler, I. J., Gersten, D. M., and Hart, I. R. The biology of cancer invasion and metastasis. Adv. Cancer Res., *28:* 149–250, 1978.
9. Fidler, I. J., and Kripke, M. L. Metastasis results from preexisting variant cells within a malignant tumor. Science (Wash. D.C.), *197:* 893–895, 1977.
10. Gershman, H., and Rosen, J. J. Cell adhesion and cell surface topography in aggregates of 3T3 and SV40-virus-transformed 3T3 cells: visualization of interior cells by scanning electron microscopy. J. Cell Biol., *76:* 639–651, 1978.
11. Hart, I. R. The selection and characterization of an invasive variant of the B16 melanoma. Am. J. Pathol., *97:* 587–600, 1979.
12. Hart, I. R., and Fidler, I. J. An *in vitro* quantitative assay for tumor cell invasion. Cancer Res., *38:* 3218–3224, 1978.
13. Knyrim, K., and Paweletz, N. Cell interactions in a "bilayer" of tumor cells: a scanning electron microscope study. Virchows Arch. B Cell Pathol., *25:* 309–325, 1977.
14. Leighton, J. Invasive growth and metastasis in tissue culture systems. Methods Cancer Res., *4:* 85–124, 1968.
15. Leung, T.-K., and Babai, F. Etude ultrastructurale de l'invasion des cultures primaires du foie par des cellules cancéreuses. Virchows Arch. B Cell Pathol., *29:* 267–280, 1979.
16. Liebrich, W., and Paweletz, N. Ultrastructure of aggregates of tumor and normal cells and of mixed aggregates. Virchows Arch. B Cell Pathol., *22:* 39–53, 1976.
17. Liotta, L. A., Abe, S., Robey, P. G., and Martin, G. R. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. Proc. Natl. Acad. Sci. U. S. A., *76:* 2268–2272, 1979.
18. Liotta, L. A., Kleinerman, J., Catanzaso, P., and Rynbrandt, D. Degradation of basement membrane by murine tumor cells. J. Natl. Cancer Inst., *58:* 1420–1431, 1977.
19. Liotta, L. A., Tryggrason, K., Garbisa, S., Hart, I., and Foltz, C. M. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. Nature (Lond.), *284:* 67–68, 1980.
20. Mareel, M., de Bruyne, G., and de Ridder, L. Invasion of malignant cells into ^{51}Cr-labeled host tissues in organotypical culture. Oncology (Basel), *34:* 6–9, 1977.
21. Mareel, M., de Ridder, L., de Brabander, M., and Vakaet, L. Characterization of spontaneous, chemical and viral transformants of a C3H/3T3 type mouse cell line by transplantation into young chick blastoderms. J. Natl. Cancer Inst., *54:* 923–929, 1975.
22. Mareel, M., Kint, J., and Meyvisch, C. Methods of study of the invasion of malignant C3H-mouse fibroblasts into embryonic chick heart *in vitro*. Virchows Arch. B Cell Pathol., *30:* 95–111, 1979.
23. Nicolson, G. L., Winkelhake, J. L., and Nussey, A. C. An approach to studying the cellular properties associated with metastasis. *In:* L. Weiss (ed.), Fundamental Aspects of Metastasis, pp. 291–305. Amsterdam: North Holland Publishing Co., 1976.
24. Noguchi, P. D., Johnson, J. B., O'Donnell, R., and Petricciani, J. C. Chick embryonic skin as a rapid organ culture assay for cellular neoplasia. Science (Wash. D.C.), *199:* 980–983, 1978.
25. Orr, W., Varani, J., and Ward, P. A. Characteristics of the chemotactic response of neoplastic cells to a factor derived from the fifth component of complement. Am. J. Pathol., *93:* 405–420, 1978.
26. Poole, A. R. Tumor lysosomal enzymes and invasive growth. *In:* J. T. Dingle (ed.), Lysosomes in Biology and Pathology, Vol. 3, pp. 303–337. Amsterdam: North Holland Publishing Co., 1973.
27. Poste, G., and Fidler, I. J. The pathogenesis of cancer metastasis. Nature (Lond.), *283:* 139–146, 1980.
28. Poste, G., and Flood, M. K. Cells transformed by temperature-sensitive mutants of avian sarcoma virus cause tumors *in vivo* at permissive and nonpermissive temperatures. Cell, *17:* 789–800, 1979.
29. Poste, G., and Kirsh, R. L. Rapid decay of tumoricidal activity and loss of responsiveness to lymphokines in inflammatory macrophages. Cancer Res., *39:* 2582–2590, 1979.
30. Pourreau-Schneider, N., Felix, H., Haemmerli, G., and Sträuli, P. The role of cellular locomotion in leukemic infiltration: an organ culture study on penetration of L5222 rat leukemia cells into the chick embryo mesonephros. Virchows Arch. B Cell Pathol., *23:* 257–264, 1977.
31. Reese, D. H., Friedman, R. D., Smith, J. M., and Sporn, M. B. Organ culture of normal and carcinogen-treated rat bladder. Cancer Res., *36:* 2525–2527, 1976.
32. Reich, E. Tumor-associated fibrinolysis. Fed. Proc. *32:* 2174–2175, 1973.
33. Roblin, R., Chou, I. N., and Black, P. H. Proteolytic enzymes, cell surface charge and viral transformation. Adv. Cancer Res., *22:* 203–260, 1975.
34. Scher, C. D., Haudenschild, C., and Klagsbrun, M. The chick chorioallantoic membrane as a model system for the study of tissue invasion by viral transformed cells. Cell, *8:* 373–382, 1976.
35. Schirrmacher, V., Shantz, G., Clauer, K., Komitowski, D., Zimmermann, H.-P., and Lohmann-Matthes, M.-L. Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice. I. Tumor invasiveness *in vitro* and metastasis formation *in Vivo*. Int. J. Cancer, *23:* 233–244, 1979.
36. Schleich, A. B., Frick, M., and Mayer, A. Patterns of invasive growth *in vitro*. Human decidua graviditatis confronted with established human cell lines and primary human explants. J. Natl. Cancer Inst., *56:* 221–237, 1976.
37. Sherman, M. I., Strickland, S., and Reich, E. Differentiation of early mouse embryonic and teratocarcinoma cells *in vitro*: Plasminogen Activator Production. Cancer Res., *36:* 4208–4216, 1976.
38. Sylvén, B. Biochemical and enzymatic factors involved in cellular detachment. *In:* S. Garattini and G. Franchi (eds.), Chemotherapy of Cancer Dissemination and Metastasis, pp. 129–138. New York: Raven Press, 1973.
39. Tickle, C., Crawley, A., and Goodman, M. Cell movement and the mechanism of invasiveness: a survey of the behaviour of some normal and malignant cells implanted into the developing chick wing bud. J. Cell Sci., *31:* 293–322, 1978.
40. Vallota, E. H., and Müller-Eberhard, H. J. Formation of C3a and C5a anaphylatoxins in whole human serum after inhibition of the anaphylatoxin inactivator. J. Exp. Med., *137:* 1109–1123, 1973.
41. Ward, P. A., Lepow, I. H., and Newman, L. J. Bacterial factors chemotactic for polymorphonuclear leukocytes. Am. J. Pathol., *52:* 725–736, 1968.
42. Wiseman, L. L. Contact inhibition and the movement of metal, glass and plastic beads within solid tissues. Experientia (Basel), *34:* 26–28, 1976.