Modification Form for Permit BIO-RRI-0011

Permit Holder: Susan Meakin

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

Renee Phillips

Kayla Driver

Asghar Talebian

Alfonso Dietrich

Chunhui Li

James MacDonald

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Yeast, E.coli containing plasmids	
Approved Cells	Human (established), rodent (established, primary), NHP (established), Hek 293, 293T cells, SY5Y, IMR32, SK-N-AS, CHP-212, BE(2)-C, SK-N-DZ, SK-N-MC, SK-N-F1, Daoy, Daoy-TrkA/TrkB/TrkC (wt and	
Approved Use of Human Source Material		
Approved GMO	FRS2, FRS3, Shp2, FRS3 (shRNA), Nesca (shRNA), RasFrf1, SV 40 Large, T antigen, pCMX, pCDNA3.1, pEGFP, FRS2, FRS3, ShcB, ShcC, AKT, Fyn, NR2B, STEP isoforms, RasFrfl, Nesca, Axin, beta-catenin,	hTTP in pET30a AND hTTP AS HA TAG IN pCMV ARF6-GFP N98R AND N48I MUTANTS
Approved use of Animals		
Approved Toxin(s)		

hTTP WILL BE USED TO STUDY INTERACTIONS BETWEEN TTP AND NESCA (hTTP - human TRISTETRAPROLIN, ALSO CALLED ZFP36 ZINC FINGER PROTEIN - 36)
ARF6 WILL BE USED TO STUDY EFFECTS ON MACROPINOCYTOSIS IN MEDULLA-BLASTOMAS.

* *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: Susan Meah

Classification: 2

Date of Last Biohazardous Agents Registry Form: May 3, 2007

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

BioSafety Officer(s): Ronald Wozniak March 04, 2010

Chair, Biohazards Subcommittee:

THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 275, No. 23, Issue of June 9, pp. 17827–17837, 2000
Printed in U.S.A.

Interactions of CCCH Zinc Finger Proteins with mRNA

BINDING OF TRISTETRAPROLIN-RELATED ZINC FINGER PROTEINS TO AU-RICH ELEMENTS AND DESTABILIZATION OF mRNA*

Received for publication, February 25, 2000
Published, JBC Papers in Press, April 5, 2000, DOI 10.1074/jbc.M001696200

Wi S. Lai‡, Ester Carballo‡, Judith M. Thorn‡, Elizabeth A. Kennington‡, and Perry J. Blackshear‡§¶

From the ‡Office of Clinical Research and Laboratory of Signal Transduction, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709 and the §Departments of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Macrophages derived from tristetraprolin (TTP)-deficient mice exhibited increased tumor necrosis factor α (TNFα) release as a consequence of increased stability of TNFα mRNA. TTP was then shown to destabilize TNFα mRNA after binding directly to the AU-rich region (ARE) of the 3′-untranslated region of the TNFα mRNA. In mammals and in *Xenopus*, TTP is the prototype of a small family of three known zinc finger proteins containing two CCCH zinc fingers spaced 18 amino acids apart; a fourth more distantly related family member has been identified in *Xenopus* and fish. We show here that representatives of all four family members were able to bind to the TNFα ARE in a cell-free system and, in most cases, promote the breakdown of TNFα mRNA in intact cells. Because the primary sequences of these CCCH proteins are most closely related in their tandem zinc finger domains, we tested whether various fragments of TTP that contained both zinc fingers resembled the intact protein in these assays. We found that amino- and carboxyl-terminal truncated forms of TTP, as well as a 77 amino acid fragment that contained both zinc fingers, could bind to the TNFα ARE in cell-free cross-linking and gel shift assays. In addition, these truncated forms of TTP could also stimulate the apparent deadenylation and/or breakdown of TNFα mRNA in intact cells. Alignments of the tandem zinc finger domains from all four groups of homologous proteins have identified invariant residues as well as group-specific signature amino acids that presumably contribute to ARE binding and protein-specific activities, respectively.

Zinc finger domains within proteins can mediate interactions with DNA, RNA, other proteins, and small molecules such as diacylglycerols. One relatively uncommon class of zinc finger proteins contains fingers of the CCCH type, in which three cysteines and one histidine are thought to coordinate a single atom of zinc. Members of a subclass of the larger family of CCCH zinc finger proteins contain two tandem zinc fingers consisting of $CX_8CX_5CX_3H$, where X refers to variable amino acids, spaced exactly 18 amino acids apart. The prototype of proteins of this CCCH double zinc finger subclass is tristetraprolin (TTP),[1] also known as TIS11 and Nup475. It was first identified as the product of an immediate early response gene in fibroblasts and other cells stimulated with insulin, serum, or phorbol esters (1–4). The same stimuli that increase transcription of this gene also stimulate its rapid serine phosphorylation (5) and rapid nuclear to cytosol translocation (6) in fibroblasts.

TTP-deficient mice appear normal at birth but rapidly develop a wasting syndrome accompanied by erosive arthritis, dermatitis, alopecia, autoantibodies, and myeloid hyperplasia. Essentially all of these abnormalities were prevented by the injection of monoclonal antibodies specific for mouse tumor necrosis factor (TNFα) (7). Macrophages derived from these mice exhibited enhanced TNFα release, accompanied by increases in TNFα mRNA levels (8). This was due to an increase in stability of TNFα mRNA in macrophages derived from TTP-deficient mice (9). These findings implicated TTP as an intracellular regulator of TNFα mRNA stability and thus of TNFα biosynthesis. More recently, we have shown that TTP deficiency has a similar effect on the stability of another mRNA containing a so-called class II AU-rich element (ARE), that encoding granulocyte-macrophage colony-stimulating factor (GM-CSF) (10). In this case, there was a marked stabilization of GM-CSF mRNA in bone marrow stromal cells derived from TTP-deficient mice compared with control cells, indicating that TTP is also a physiological regulator of GM-CSF mRNA stability and thus of GM-CSF secretion (10).

TTP can bind directly to the ARE of TNFα mRNA (9, 11). The integrity of both zinc fingers was required for this direct protein-RNA interaction, because a single C → R mutation within the CCCH motif from either finger abolished the ARE binding activity of TTP (11). The same mutations abrogated the ability of TTP to destabilize the TNFα mRNA in intact cells (11). These studies indicated that TTP could bind directly to the TNFα ARE and destabilize the TNFα mRNA in a zinc finger-dependent manner, apparently by initially stimulating its deadenylation (11).

Aside from TTP, two other members of this subclass have been identified to date in mammals: cMG1 (TIS11b, ERF1, and Berg-36) (12–15) and TIS11d (ERF2) (15, 16). All three of these proteins contain the two typical CCCH fingers, spaced 18 amino acids apart, with the sequence RYKTEL or a variant leading into each finger. Proteins with nearly identical double zinc fingers spaced 18 amino acids apart have been identified in

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¶ To whom correspondence should be addressed: A2-05 NIEHS, 111 Alexander Dr., Research Triangle Park, NC 27709. Tel.: 919-541-4899; Fax: 919-541-4571; E-mail: black009@niehs.nih.gov.

[1] The abbreviations used are: TTP, tristetraprolin; TNFα, tumor necrosis factor α; ARE, AU-rich element; GM-CSF, granulocyte-macrophage colony-stimulating factor; TZF, tandem zinc finger; PAGE, polyacrylamide gel electrophoresis; CMV, cytomegalovirus; PCR, polymerase chain reaction; bp, base pair(s).

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 284, NO. 43, pp. 29383–29390, October 23, 2009

RNA-destabilizing Factor Tristetraprolin Negatively Regulates NF-κB Signaling*

Received for publication, May 26, 2009, and in revised form, August 17, 2009 Published, JBC Papers in Press, September 8, 2009, DOI 10.1074/jbc.M109.024745

Jian Liang, Tianhua Lei, Yuting Song, Natalie Yanes, Yongfen Qi, and Mingui Fu[1]

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Tristetraprolin (TTP) is a CCCH zinc finger-containing protein that destabilizes mRNA by binding to an AU-rich element. Mice deficient in TTP develop a severe inflammatory syndrome mainly because of overproduction of tumor necrosis factor α. We report here that TTP also negatively regulates NF-κB signaling at the transcriptional corepressor level, by which it may repress inflammatory gene transcription. TTP expression inhibited NF-κB-dependent transcription. However, overexpression of TTP did not affect reporter mRNA stability. Instead, TTP functioned as a corepressor of p65/NF-κB. In support of this concept, we found that TTP physically interacted with the p65 subunit of NF-κB and was also associated with HDAC1, -3, and -7 *in vivo*. Treatment with histone deacetylase inhibitors or small interfering RNA induced HDAC1 or HDAC3 knockdown completely or partly abolished the inhibitory activity of TTP on NF-κB reporter activation. Consistently, chromatin immunoprecipitation showed decreased recruitment of HDAC1 and increased recruitment of CREB-binding protein on the *Mcp-1* promoter in $TTP^{-/-}$ cells compared with wild-type cells. Moreover, overexpression of TTP blocked CREB-binding protein-induced acetylation of p65/NF-κB. Taken together, these data suggest that TTP may also function *in vivo* as a modulator in suppressing the transcriptional activity of NF-κB.

The transcription factor NF-κB mediates the major inflammatory signal pathways and regulates the most inflammatory gene expression (1). Excessive and prolonged activation of NF-κB can cause massive damage to host tissue and can result in human inflammatory diseases such as atherosclerosis and arthritis (2). Thus, the activation of NF-κB must be terminated through multiple mechanisms, including recruitment of transcriptional corepressors (3–5).

TTP[2] is an RNA-binding protein required for the rapid degradation of mRNAs containing AU-rich elements (6). Targets regulated by TTP include the mRNAs encoding TNFα (7), granulocyte-macrophage colony-stimulating factor (8), and interleukin-2 (9), etc. Mice deficient in TTP develop an inflammatory syndrome characterized by cachexia, spontaneous arthritis, dermatitis, and neutrophilia (10). The inflammatory syndrome in $TTP^{-/-}$ mice is caused mainly by overproduction of TNFα, as neutralizing antibodies reactive with TNFα prevent most of the inflammatory symptoms in $TTP^{-/-}$ mice (10). Overexpression of TNFα in $TTP^{-/-}$ mice may be explained by its prolonged mRNA half-life, but other mechanisms may also exist. Accumulating evidence indicates that TTP may have additional functions besides influencing cytokine mRNA stability. In *Schizosaccharomyces pombe*, a TTP-related protein is required for effective transmission of a pheromone-induced Ras/mitogen-activated protein kinase (MAPK) signal (11). In addition, a *nim/cdc25* mutant can be complemented by either the Cdc2 kinase or a *TTP/TIS11* gene, suggesting a cell cycle effect (12). A TTP/TIS11-related protein in *Saccharomyces cerevisiae* is required for normal metabolism and retards cell growth when overexpressed (13). TTP is induced during apoptosis in response to the breast cancer susceptibility protein BRCA1 (14). Furthermore, continuous expression of TTP at physiological levels causes apoptotic cell death (15, 16). These observations indicate that TTP protein might influence regulatory pathways that regulate survival, differentiation, or proliferation. In a genome-wide analysis of TTP-affected glucocorticoid targets, the half-lives of many TTP target mRNAs were not increased in $TTP^{-/-}$ cells, suggesting a regulatory role for TTP not limited to mRNA turnover (17). In addition, TTP is shuttled between the cytoplasm and nucleus (18). It promotes mRNA decay in the cytoplasm. However, what it does in the nucleus is unknown.

We report here that TTP also negatively regulates NF-κB signaling at the transcriptional corepressor level. It suppresses the transcriptional activity of p65/NF-κB by recruiting HDACs on the NF-κB target gene promoters. These results suggest that TTP may control the inflammatory response through multiple mechanisms, including inhibition of transcription in the nucleus and promotion of mRNA decay in the cytoplasm.

MATERIALS AND METHODS

Cells—Littermate wild-type and $TTP^{-/-}$ day 14.5 embryos were used to generate MEF cell lines $67^{+/+}$ and $66^{-/-}$, respectively (provided by Dr. Perry J. Blackshear, NIEHS, NIH, Research Triangle Park, NC). Cells were grown as a monolayer in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% fetal bovine serum, 2 mM L-glutamine, and 100 units/ml each penicillin and streptomycin. The mouse macrophage cell

* This work was supported by a James and Esther King New Investigator research grant and an American Heart Association beginning grant-in-aid (to M. F.).

[1] To whom correspondence should be addressed: Burnett School of Biomedical Sciences, College of Medicine, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816. Tel.: 407-823-1306; Fax: 407-823-0956; E-mail: mfu@mail.ucf.edu.

[2] The abbreviations used are: TTP, tristetraprolin; TNFα, tumor necrosis factor α; HDAC, histone deacetylase; MEF, mouse embryonic fibroblast; HA, hemagglutinin; CBP, cAMP-responsive element-binding protein-binding protein; IKKβ, IκB kinase β; siRNA, small interfering RNA; LPS, lipopolysaccharide; IL-1β, interleukin-1β; GST, glutathione *S*-transferase; ChIP, chromatin immunoprecipitation; STAT, signal transducer and activator of transcription; TAD, transcriptional activation domain.

Homo sapiens zinc finger protein 36, C3H type, homolog (mouse) (ZFP36), mRNA

Comment Features Sequence

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VERSION     NM_003407.2  GI:141802261
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REFERENCE   1  (bases 1 to 981)
  AUTHORS   Schichl,Y.M., Resch,U., Hofer-Warbinek,R. and de Martin,R.
  TITLE     Tristetraprolin impairs NF-kappaB/p65 nuclear translocation
  JOURNAL   J. Biol. Chem. 284 (43), 29571-29581 (2009)
   PUBMED   19654331
  REMARK    GeneRIF: attenuation of NF-kappaB activity is at least in part due
            to an interference of TTP with the nuclear import of the p65
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REFERENCE   2  (bases 1 to 981)
  AUTHORS   Balakathiresan,N.S., Bhattacharyya,S., Gutti,U., Long,R.P.,
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  TITLE     Tristetraprolin regulates IL-8 mRNA stability in cystic fibrosis
            lung epithelial cells
  JOURNAL   Am. J. Physiol. Lung Cell Mol. Physiol. 296 (6), L1012-L1018 (2009)
   PUBMED   19363120
  REMARK    GeneRIF: enhanced stability of IL-8 mRNA in TTP-deficient cystic
            fibrosis lung epithelial cells serve to drive the proinflammatory
            cellular phenotype in the lung
REFERENCE   3  (bases 1 to 981)
  AUTHORS   Ogilvie,R.L., Sternjohn,J.R., Rattenbacher,B., Vlasova,I.A.,
            Williams,D.A., Hau,H.H., Blackshear,P.J. and Bohjanen,P.R.
  TITLE     Tristetraprolin mediates interferon-gamma mRNA decay
  JOURNAL   J. Biol. Chem. 284 (17), 11216-11223 (2009)
   PUBMED   19258311
  REMARK    GeneRIF: Tristetraprolin plays an important role in turning off
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REFERENCE   4  (bases 1 to 981)
  AUTHORS   Takahashi,N., Sato,N., Takahashi,S. and Tojo,A.
  TITLE     Gene-expression profiles of peripheral blood mononuclear cell
            subpopulations in acute graft-vs-host disease following cord blood
            transplantation
  JOURNAL   Exp. Hematol. 36 (12), 1760-1770 (2008)
   PUBMED   18814951
  REMARK    GeneRIF: downregulation of antiinflammatory factors, such as
            TNFAIP3, KLF2, ZFP36, and BTG1, seems to be involved in
            acceleration of immune response, thus exacerbation of acute GVHD.
REFERENCE   5  (bases 1 to 981)
  AUTHORS   Suzuki,T., Tsutsumi,A., Suzuki,H., Suzuki,E., Sugihara,M.,
            Muraki,Y., Hayashi,T., Chino,Y., Goto,D., Matsumoto,I., Ito,S.,
            Miyazawa,K. and Sumida,T.
  TITLE     Tristetraprolin (TTP) gene polymorphisms in patients with
            rheumatoid arthritis and healthy individuals
  JOURNAL   Mod Rheumatol 18 (5), 472-479 (2008)
   PUBMED   18536977
  REMARK    GeneRIF: the disease duration in Rheuamtoid arthritis patients with
            Tristetraprolin (TTP) genotype GG was shorter than that of patients
            with genotypes AA/AG
REFERENCE   6  (bases 1 to 981)
  AUTHORS   Taylor,G.A., Thompson,M.J., Lai,W.S. and Blackshear,P.J.
  TITLE     Phosphorylation of tristetraprolin, a potential zinc finger
            transcription factor, by mitogen stimulation in intact cells and by
            mitogen-activated protein kinase in vitro
  JOURNAL   J. Biol. Chem. 270 (22), 13341-13347 (1995)
   PUBMED   7768935
REFERENCE   7  (bases 1 to 981)
  AUTHORS   Heximer,S.P. and Forsdyke,D.R.
  TITLE     A human putative lymphocyte G0/G1 switch gene homologous to a
            rodent gene encoding a zinc-binding potential transcription factor
  JOURNAL   DNA Cell Biol. 12 (1), 73-88 (1993)
   PUBMED   8422274
REFERENCE   8  (bases 1 to 981)
  AUTHORS   Taylor,G.A., Lai,W.S., Oakey,R.J., Seldin,M.F., Shows,T.B.,
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            Eddy,R.L. Jr. and Blackshear,P.J.
  TITLE     The human TTP protein: sequence, alignment with related proteins,
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  JOURNAL   Nucleic Acids Res. 19 (12), 3454 (1991)
   PUBMED   2062660
REFERENCE   9  (bases 1 to 981)
  AUTHORS   DuBois,R.N., McLane,M.W., Ryder,K., Lau,L.F. and Nathans,D.
  TITLE     A growth factor-inducible nuclear protein with a novel
            cysteine/histidine repetitive sequence
  JOURNAL   J. Biol. Chem. 265 (31), 19185-19191 (1990)
   PUBMED   1699942
REFERENCE   10 (bases 1 to 981)
  AUTHORS   Lai,W.S., Stumpo,D.J. and Blackshear,P.J.
  TITLE     Rapid insulin-stimulated accumulation of an mRNA encoding a
            proline-rich protein
  JOURNAL   J. Biol. Chem. 265 (27), 16556-16563 (1990)
   PUBMED   2204625
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from M92843.1.
            On Apr 5, 2007 this sequence version replaced gi:4507960.

            Sequence Note: removed 1 base from the 5' end that did not align to
            the reference genome assembly.

            Publication Note:  This RefSeq record includes a subset of the
            publications that are available for this gene. Please see the
            Entrez Gene record to access additional publications.
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      961 cgcatctctg tttctgagtg a
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Modification Form for Permit BIO-RRI-0011

Permit Holder: Steve Meakin

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

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James MacDonald

~~Ian Grant~~

Additional Personnel
(Please list additional personnel here)

Kayla Driver
Renee' Phillips

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Approved Cells	Human (established), rodent (established, primary), NHP (established), Hek 293, 293T cells, SY5Y, IMR32, SK-N-AS, CHP-212, BE(2)-C, SK-N-DZ, SK-N-MC, SK-N-F1, Daoy, Daoy-TrkA/TrkB/TrkC (wt and	
Approved Use of Human Source Material		
Approved GMO	~~plasmid,~~ FRS2, FRS3, Shp2, FRS3 (shRNA), Nesca (shRNA), RasFrf1, SV 40 Large, T antigen, pCMX, pCDNA3.1, pEGFP, FRS2, FRS3, ShcB, ShcC, AKT, Fyn, NR2B, STEP isoforms, RasFrfl, Nesca, Axin, beta-catenin,	
Approved use of Animals		
Approved Toxin(s)		

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** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

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Signature of Permit Holder: Susan Mark

Classification: 2

Date of Last Biohazardous Agents Registry Form: May 3, 2007

Date of Last Modification (if applicable): Aug 4, 2009

BioSafety Officer(s): Ronald Noseworthy JPlunkey Dec 21/09

Chair, Biohazards Subcommittee: G.M. Kidder

Makin Lab. BIO-RF1-0011

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

~~Jupinder Bains~~
~~Andrew Lu~~
Jennifer Forsyth
~~Jennifer Gerasimoff~~
Chunhui Li
~~Kim Brookes~~
~~Todd Hryciw~~
~~Sara LeMay~~
James MacDonald

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~~Pat Rutka~~

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Signature of Permit Holder: Susan Mak

BioSafety Officer(s): Nancy July 27/09

Chair,Biohazards Subcommittee: G.M. Kilder Aug 4/09

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Yeast, E.coli containing plasmids	
Approved Cells	Human (established), rodent (established, primary), NHP (established), Hek 293, 293T cells, SY5Y, IMR32, SK-N-AS, CHP-212, BE(2)-C, SK-N-DZ, SK-N-MC, SK-N-F1, Daoy, Daoy-TrkA/TrkB/TrkC (wt and	U87MG U W426 U341 U373
Approved Use of Human Source Material		
Approved GMO	SV 40 Large, T antigen, pCMX, pCDNA3.1, pEGFP, FRS2, FRS3, ShcB, ShcC, AKT, Fyn, NR2B, STEP isoforms, RasFrll, Nesca, Axin, beta-catenin, FGFRs, GST fusion vectors, pAS, pACT, pGad, pAd-Easy,	
Approved use of Animals		
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.*

Classification: 2

Date of last Biohazardous Agents Registry Form: May 3, 2007

Signature of Permit Holder: Duncan Mak

BioSafety Officer(s): Jtunley July 27/09

Chair,Biohazards Subcommittee: O.L. Kilder

The research in my lab addresses the mechanisms activated by the receptor tyrosine kinases termed Trk in the developing and mature nervous systems. The Trk family includes 3 members, TrkA (activated by nerve growth factor), TrkB (activated by brain-derived neurotrophic factor) and TrkC (activated by neurotrophin 3). My research addresses (1) mechanisms of how TrkA can activate cell death in tumors of the nervous system such as medulloblastomas and glioblastomas, (2) mechanisms where by TrkB facilitates long-term potentiation and memory, via ShcC, RasGrf1 and the NMDA receptors, (3) mechanisms of neuronal survival and neuronal process formation, via Nesca, RasGrf1 and FRS3 and (4) mechanisms of how FRS3 facilitates cortical neuron development and migration during cortical neurogenesis. We address these questions using a variety of molecular and cell biology approaches including transgenic mice, primary neuron cultures, knock down studies using knock-out mice or loss of expression via siRNA or shRNA approaches as well as transfection and over-expression approaches using plasmids or adenoviral expression in both established and primary neuronal cell culture.

Subject: RE: Meakin protocol
From: Ron Noseworthy <rnoseworthy@robarts.ca>
Date: Mon, 27 Jul 2009 13:02:16 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: jmacdon@robarts.ca

[illegible] with plasmids [illegible]

[illegible]

[illegible] the Meakin [illegible]

[illegible] several notes need to be made. First she has plasmids that express the following oncogenes: myc although not clear if in some cases it is just a myc tag and not the whole gene, fos, src, ras, fyn and SV40 large T antigen. Also need to do a search to see if [illegible] of them [illegible] not sure which. She also has plasmids that can be used to generate infectious replication defective Adenovirus, retrovirus and lentiviral vectors. [illegible]

[illegible]

Subject: [illegible]
Date: Mon, [illegible] May 2009 [illegible]
From: Kabani [illegible]
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: [illegible] Kidney [illegible]
References: [illegible]

Hi Jennifer,

have no issues with the Kidney, Radiation and [illegible] modification. [illegible] the Canada modification leaves me uncertain as [illegible] has [illegible] as level [illegible] while [illegible] has them as level [illegible]. [illegible] guess we go with [illegible] as [illegible] our governing body, [illegible] then how will [illegible] the work?

The Meakin one is ok but several notes need to be made. First she had plasmids that express the following oncogenes: myc although not clear if in some cases it is just a myc tag and not the whole gene, fos, src, ras, fyn and SV40 large T antigen. Also need to do a search to see if the FRE? or [illegible] has oncogenic properties associated with [illegible] of them [illegible] not sure which. She also has plasmids that can be used to generate infectious replication defective Adenovirus, retrovirus and lentiviral vectors. In addition she has the vector plasmids to construct Semliki Forest virus vectors. [illegible] with the notes what the containment level is but we need comments from her [illegible] the vectors and oncogenes are in current use or not. Need [illegible] diligence on the Semliki virus vector [illegible] used.
Greg

Meakin

Meakin

------ Original Message --------
Subject: Re: semliki forest virus vectors - containment level request
Date: Fri, 26 Jun 2009 08:56:28 -0400
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Semliki virus and vectors are classified as risk group 2 human pathogens,
therefore you should follow the guidelines for containment level 2 work. I
also suggest that you do a short risk assessment on the work intent of your researcher to determine if additional requirements are necessary.

Have a good day

Genevieve Lacroix, M.Sc.
A/Head, Importation and Biosafety Programs
Chef intérimaire/Importation et servuce de biosécurité
Office of Laboratory Security / Bureau de la sécurité des laboratoires
Public Health Agency of Canada / Agence de la santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
Tel: (613) 941-8810
Fax: (613)941-0596
genevieve_lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

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Cell Biology

ATCC® Number:	HTB-14™ Order this Item	**Price:**	$256.00
Designations:	U-87 MG	**Depositors:**	J Ponten
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
			Photo

Source: **Organ:** brain
Tumor Stage: classified as grade IV as of 2007
Disease: glioblastoma; astrocytoma

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.

Related Cell Culture Products

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Tumorigenic: Yes

Antigen Expression: Blood Type A, Rh+

Cytogenetic Analysis: This is a hypodiploid human cell line with the modal chromosome number of 44 occurring in 48% of cells. The rate of higher ploidy was 5.9%., Twelve markers were common to all cells, including der(1)t(1;3) (p22;q21), der(16)t(1;16) (p22;p12), del(9) (p13) and nine others. The marker der(1) had two copies in most cells. There was only one copy of normal X. N1, N6 and N9 were not found.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 1
Me-2, 1
PGM1, 2
PGM3, 1

Age: 44 years

Gender: female

Ethnicity: Caucasian

Comments: This is one of a number of cell lines derived from malignant gliomas (see also ATCC HTB-15 and ATCC HTB-16) by J. Ponten and associates from 1966 to 1969.
Mycoplasma contamination was eliminated in September 1975.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%
Atmosphere: 5% CO2 in air recommended
Temperature: 37.0°C

Meakin list.

Adenovirus

pMd- TRKA

- TRKB
- TrK C - See description of
- Sh Pinder

~~[redacted]~~

- DN Pinchen
- WT Pinchen
- DN cdc 42
- CA cdc 42
- WT cdc 42
- PKC alpha
- PKC zeta
- JNK 1
- JNK 2
- DN JNK1
- DN JNK2
- GFP NESCA
- GFP
- Mono RED

2007-06

BIO-RRI-0011

robarts RESEARCH

BIOHAZARDOUS AGENTS REGISTRY FORM

Reviewed by Biosafety Subcommittee: February 2006

This form must be completed by each Principal Investigator when completing a grant application or grant renewal to be administered by the Robarts Research Institute, if the use of biohazardous and/or infectious agents is proposed. For any proposed animal work involving the use of biohazardous agents or animals carrying zoonotic agents infectious to humans, this form must also be completed.

COMPLETED FORMS ARE TO BE RETURNED TO BIOSAFETY SUBCOMMITTEE CHAIR, ROOM 3-34.1.

*If there are any changes to the information on these forms (excluding grant title and funding agencies) a new form must be completed and sent to the Biosafety Subcommittee Chair **BEFORE** implementation of these changes can occur.*

If multi-team grants are being applied for, each individual investigator of the team must submit a Biohazardous Agents Registry Form to the Biosafety Subcommittee Chair.

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

For questions regarding this form, please contact Biosafety Subcommittee Chair at ext. 34125.

1.0 Contact Information

PRINCIPAL INVESTIGATOR: Dr. Susan Meakin

SIGNATURE: Susan Meakin

DATE: May 3rd, 2007

DEPARTMENT: Cell Biology and Stem Cell Biology, Dept of Biochemistry

ADDRESS: Robarts Research Institute Office 3-16.1

TELEPHONE: 663-5777 ext. 34304

EMAIL: smeakin@robarts.ca

Location of experimental work to be carried out

Building(s): Robarts Research Institute

Room(s): 3-20.1, 3-17.1 Tissue Culture Room

**For work being performed at Institutions affiliated with the Robarts Research Institute, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Robarts Research Institute Biosafety Subcommittee Chair. See Section 13.0, Approvals*

GRANT TITLE(S): 1. TrkA Activation of Autophagy in Human Neural Tumors
2. The Role of the FRS3 Adapter in Regulating β-catenin Signaling and Cellular Proliferation
3. The Roles of the FRS adapters in Stem Cell Survival and Proliferation
4. TrkB, ShcC and RasGrf1 Regulate NMDA Receptor Activity
5. Nesca, a Novel Signaling Adapter that Regulates Neuronal Growth and Function
6. Autophagy Induced Cell Death in Human Brain Tumors

ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) EXPLAINING THE BIOHAZARD(S) USED.

FUNDING AGENCY/AGENCIES: Cancer Research Society: Grants #1 and #2

Krembil/ORDCF: Grant #3, NSERC: Grant #4 CIHR: Grant #5 (Applied), McDonnell Foundation: Grant #6 (Applied)

Anticipated Grant End Date: Aug 31, 2008 (both CRS grants), Sept, 2012 (CIHR), March 2012 (NSERC)

Names of all personnel working under Principal Investigator's supervision in this location:

Dr. James MacDonald (Res. Assoc.)
Mrs. Sara LeMay (Res. Tech)
Dr. Todd Hryciw (PDF)
Dr. Sandy Vascotto (PDF)
Mrs. Kim Brookes (Ph.D. Student)
Mr. Chunhui Li (Res. Tech)
Ms. Jennifer Gerasimoff (M.Sc. Student)
Ms. Jennifer Forsyth (M.Sc. Student)
Mr. Andrew Lu (Summer Student)
Mr. Jupinder Bains (Summer Student)

Note : A list of human pathogens categorized according to Risk Group can be obtained by calling the Office of Laboratory Security directly at (613) 957-1779 or accessing their Web site : http://www.phac-aspc.gc.ca/ols-bsl/index.html

2.0 Microorganisms

2.1 Does your work involve the use of microorganisms? YES X NO O
If **NO**, please proceed to Section 3.0

2.2 Please complete the table below

Name of Microorganism	Is microorganism a known human pathogen? YES/NO	Is microorganism a known animal pathogen? YES/NO	Is microorganism a known zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?	Health Canada or CFIA Containment Level (select one)
Yeast	No	No	No	500 ml	1O 2X 3O
Bacteria (E.coli) containing plasmids	No	No	No	1000 ml	1O 2X 3O
					1O 2O 3O

BIOHAZARDOUS AGENTS REGISTRY
Please read this Form, complete and submit to Risk Management, Rm. 3-54

3.0 Cell Culture

3.1 Does your work involve the use of cell cultures? YES X NO O
If NO, please proceed to Section 4.0.

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

Cell Type	Is this cell type used in your work? YES / NO	Established or Primary *	Supplier of Primary Cell Culture Tissue
Human	Yes	Established (see below)	
Rodent	Yes	Both (See Below)	E17, P1, P3, P7, P30 Mice
Non-human primate	Yes	Cos 7 cells (established)	
Other (specify)	Sf21 Insect Cells, HEK 293T, cortical, neurospheres, hippocampal, motoneurons, nnr5, Medulloblastoma, gliomas, neuroblastomas, nnr5, PC12.		

* i.e. derived from fresh tissue

Complete the following table.

Specific Cell Line	Source / Supplier	HC or CFIA Containment Level (select one)
nnr5, PC12	In house	1 O 2 X 3 O
Medulloblastoma, Gliomas	In house, ATCC	1 O 2 X 3 O
~~HEK, Cos 7~~	~~In house, ATCC~~	1 O 2 X 3 O

4.0 Use of Human Source Materials

4.1 Does your work involve the use of human source materials? YES O NO X
If NO, please proceed to Section 5.0

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

Human Source Material	Specify Source, or Not Applicable (NA)	Is Human Source Material known to be infected with an infectious agent? YES/NO	Name of Infectious Agent	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid				1O 2O 3O
Human Blood (fraction) or other Body Fluid				1O 2O 3O
Human Organs (unpreserved)				1O 2O 3O
Human Tissues (unpreserved)				1O 2O 3O

5.0 **Genetically Modified Organisms and Cell lines**

5.1 Will genetic modifications be made to the organism, virus or cell line? YES X NO O
If NO, please proceed to Section 6.0

5.2 Will genetic sequences from any of the following be involved?

- HIV YES O NO X

If YES, specify: _____

- HTLV 1 or 2 YES O NO X

If YES, specify: _____

- Other human or animal pathogen and/or their toxins YES X NO O

If YES, specify: Lentiviral vectors_____

5.2 Will intact genetic sequences be used from:

- SV 40 Large T antigen YES X NO O
- Adeno E1A YES O NO X
- Known or suspected oncogenes YES O NO O X

If YES, specify: _In all mammalian expression vectors,_ _____

5.4 Will a live vector(s) (viral or bacterial) be used for gene transduction? YES X NO O

If YES, name vector: _pCMX, pEGFP, pGex, pTapTag, pCAL, pAdEasy, _____

5.5 List specific vector(s) to be used:_Adenoviral, Lentiviral (Invitrogen), pBPX (insect)_____

5.6 Will vector be replication defective? YES X NO O

5.7 Will vector be infectious to humans or animals? YES X NO O

5.8 Will this be expected to increase the Containment Level required? YES X NO O

6.0 **Human Gene Therapy Trials**

6.1 Will human clinical trials using the vector(s) in 5.5 be conducted? YES O NO X
If NO, please proceed to Section 7.0
If YES, attach a full description of the make-up of the virus.

6.2 Will vector be able to replicate in the host? YES O NO O

6.3 How will the vector be administered? _____

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

_____

6.5 Has human ethics approval been obtained? YES O NO O

Approval # _____

7.0 Animal Experiments

7.1 Will any of the agents listed be used in live animals? YES ○ NO X
If NO, please proceed to section 8.0

7.2 Name of animal species to be used. _____

7.3 AUS protocol # _____

7.4 If using murine cell lines have they been tested for murine pathogens? YES ○ NO ○

8.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs YES ○ NO X
- Pound source cats YES ○ NO X
- Sheep or goats YES ○ NO X
- Non- Human Primates YES ○ NO X

If YES specify species _____

- Wild caught animals YES ○ NO X

If YES specify species _____

9.0 Biological Toxins

9.1 Will toxins of biological origin be used? YES ○ NO X
If NO, please proceed to Section 10.0
If YES, please name the toxin _____

9.2 What is the LD_{50} (specify species) of the toxin? _____

10.0 Import Requirements

10.1 Will the agent be imported? YES X NO
If NO, please proceed to Section 11.0
If YES, country of origin _____

10.2 Has an Import Permit been obtained from HC for human pathogens? YES X NO ○

10.3 Has an import permit been obtained from CFIA for animal pathogens? YES X NO ○

10.4 Has the import permit been sent to Biosafety Subcommittee Chair? YES X NO ○

If YES, Permit # "P-1385" for transformed 293 cells _____

11.0 Training Requirements for Personnel Named on Form

All personnel named in section 1.0 of this form who will be using any of the above named agents are required to attend the following training courses given by OH&S

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 2.0 to 10.0 have been trained as required

SIGNATURE Susan Meakin

12.0 Containment Levels

12.1 For the work described in sections **2.0 to 10.0** select the highest HC or CFIA Containment Level required. 1○ 2 X 3○

With Lentiviral work operationally handled with level 3 practices

12.2 Has the facility been certified by Biosafety Subcommittee Chair for this level of containment?

YES X NO ○

If YES, give date: ~~August 2003~~ February 28, 2007 and permit number: 2007-02(3-17)

13.0 Approvals

Robarts Research Institute

Signature [signature] Date May 03, 2007

Biosafety Officer for the Institution where experiments will take place

Signature____________________ Date____________________

Biosafety Officer of Robarts Research Institute (if different than above)

Signature____________________ Date____________________

Note: This permit will be in effect from May 04, 2007 to May 2010 subject to annual facility re-certification.

Hi Jennifer,
>>>
>>>
>>>
>>> Could you please let me know if the attached material has OHS approval?
>>>
>>>
>>>
>>> Material: recombinant adenovirus vector-mediated neurotrophin-3 receptor (Ad-TrkC)
>>>
>>> Researcher: Dr. Susan Meakin
>>>

Nancy McCreery wrote:

Hi Jennifer - Dr. Meakin is planning on sending the below materials off campus.

pACT2-ShcB : DNA # 1042
pGEX4T2-ShcB : DNA # 1293
pGEX4T2-ShcB SH2 : DNA # 1427
pGEX4T2-ShcB PTB : DNA # 1425
pRK5-ShcB : DNA # 1268

Nancy