UPPSALA
UNIVERSITY

CRU Methodological Workshops 2000 and 2001

Cell culturing

Immunohistochemistry and in situ hybridisation

Radioimmunoassay, enzyme immunoassay and radioreceptor assay

Introduction to categorical data analysis, by examples

arranged by

Andrzej Madej and Xin Fu

CRU CENTRE FOR REPRODUCTIVE BIOLOGY IN UPPSALA

Foreword

This is a putting together of some abstracts and lists of speakers and participants for a set of CRU methodological workshops arranged by Drs Andrzej Madej, SLU and Xin Fu, Uppsala University during years 2000 and 2001. Despite the incompleteness we think it might be valuable for postgraduate students and others to have this information available on the internet for the sake of establishing scientific networks.

Uppsala December 2003,

Ulf Magnusson, Director CRU

Table of contents

Cell culturing 4

Immunohistochemistry and *in situ* hybridisation 12

Radioimmunoassay, enzyme immunoassay and radioreceptor assay 16

Introduction to categorical data analysis, by examples 19

Program for CRU – workshop on
CELL CULTURING

Tuesday May 30, 2000, 9.00-13.00
Fåhraeussalen, Patologen, UAS.

9.00 **Welcome**
9.05-9.20 **Basics on cell cultures.**
Michelle Watson, Dept. of Virology.
9.20-9.35 **Airway epithelial cells and cystic fibrosis.**
Charlotte Andersson, Dept. of Medical Cell Biology.
9.35-9.50 **3T3 mouse fibroblasts as indicator cells for screening of mycotoxin in feed.**
Torbjörn Lundh, Dept. of Animal Nutrition and Management.
9.50-10.00 **A human cell line panel representing defined types of drug resistance.**
Lena Lenhammar, Dept. of Clinical Pharmacology.
10.00-10.10 **Transformation of lymphocytes.**
Kristina Lagerstedt, Dept of Genetics and Phathology.
10.10-10.25 **In vitro and in vivo models of angiogenesis.**
Kerstin Eriksson, Dept. of Genetics and Pathology.
10.25-10.40 **Virusfabriker.**
Thomas Elfström, Dept. of Pediatrics.

10.40-11.00 **Coffee**

11.00-11.15 **Mammalian cell lines for transient transfections, stable and inducible expression and viral infections.**
Catharina Svensson, Dept. of Medical Biochemistry and Microbiology.
11.15-11.30 **Estrogen-dependent cell lines as indicators of exposure to natural and synthetic estrogens.**
Anders Johannisson, Dept. of Pathology, SLU.
11.30-11.45 **Pancreatic islets and islet cells.**
Eva Grapengiesser, Dept. of Medical Cell Biology.
11.45-12.00 **Human uterine cells.**
Xin Fu, Dept. of Obstetrics and Gynecology.
12.00-12.15 **Neuronal cells.**
Karl Åkerman, Dept. of Physiology.
12.15-12.30 **Practical aspects of cell culturing.**
Rajni Dyal, Dept. of Internal Medicine.

12.30-13.00 **Lunch and discussion.**

Coffee and lunch are free of charge.
Registration before 26 May: xin.fu@obstgyn.uu.se Fax: 559775
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Speakers

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Airway epithelial cells and Cystic Fibrosis

Charlotte Andersson
Dept. of Medical Cell Biology, Uppsala University

Cystic fibrosis (CF) is a lethal monogenetic disease caused by a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is a cAMP regulated chloride channel present in epithelial cells. It is possible to pharmacologically restore the function of CFTR. A good tool for these studies is to use airway epithelial cell lines with and without CFTR as well as primary cell cultures and freshly obtained nasal epithelial cells from patients and control subjects. Important factors for culture of freshly isolated airway epithelial cells is the growth medium. DMEM/Ham´s F12 has been shown to be a good medium. Serum contains TGF-β that inhibits airway epithelial cell proliferation and promotes differentiation.

The extra cellular matrix activates cell surface receptors and is important for phenotypic characteristics. Therefore, culturing on collagen or collagen-fibronectin-bovine serum albumin is beneficial. For removal of contaminating fibroblasts the cells is grown in serum free medium and selective trypsination can be performed. The disadvantages with freshly isolated cultures is the limited lifespan and that they can show an unstable Cl-transport phenotype.

Transformed cell lines undergo a crisis 15-20 subcultures after transformation. Cells that escape from crisis acquire unlimited growth capacity and are immortal. Transformed cells often become less differentiated and lose cell-type specific features such as tight junctions. There are a few cell lines available that keep the characteristics of the native epithelium, e. g., the CF bronchial epithelial cell line (CFBE41o-), the human bronchial epithelial cell line (16HBEo-) and CF submucosal epithelial cells line CFSME. NHBE (normal human bronchial epithelial cell line) is a non-immortalized cell line representative for basal cells in the airways. The disadvantage is that they grow very slowly and change in phenotype over time.

Also cells that are transfected with CFTR and express large amounts the protein can be used. C127 (a mouse mammary cell line) and BHK (baby hamster kidney cell line) are examples of cell lines that have been transfected with WT-CFTR, ΔF508CFTR and mock (transfecter vector only). Culture is performed in a selective medium in order to keep a high CFTR-expression level.

Nasal epithelial cells are easily obtained with a small brush from the inferior nasal concha. Experiments can be directly performed on these cells. The cells are ciliated cells from the respiratory epithelium. The advantage of these cells is that the efficiency of treatment can be determined for each individual.

3T3 Mouse Fibroblasts as Indicator Cells for Screening of Mycotoxin in Feed

Torbjörn Lundh and Johan Widestrand
Swedish University of Agricultural Sciences, Department of Animal nutrition and Management, P.O. Box 7024, 750 07 Uppsala, Sweden

Background

Mycotoxins are substances produced by different fungal species. Our main interest are focused on the group of trichothecene mycotoxins, which are frequently found in Sweden. The extremely toxic HT-2 toxin and T-2 toxin belongs to the type A-group while the more common deoxynivalenol and nivalenol trichothecenes in the B-group. The analysis of trichothecenes in feed and food is mainly performed by chemical analysis as gas chromatographic techniques. Since this type of analysis are very time consuming and expensive, a more simple and cost efficient bioassay has been developed.

Methods

Cellculturing and freezing: Mouse fribroblasts (3T3-Swiss albino CCL-92) was cultured in DMEM supplemented with 10% FCS, L-glutamine (4 mM) and antibiotics. The cells reach a saturation density at 3.9 x 10^4 cells/cm^2. The cells were subcultivated twice weekly with 0.05% trypsin and 0.02% EDTA in PBS before they reached the saturation density. The cells were seeded at a concentration of 3 x 10^5 cells /T25 flask.
The cells were cultivated to near confluence, trypsinated, placed in a freezing solution consisted of DMEM 80%, DMSO 10% and FCS 10% and kept at –135 ^oC.

Bioassays: Feed extracts in acetonitrile/ water (84:16) was cleaned- up by using a MycoSep™ column. The cells were seeded in micro titre plates and exposed to the toxic extracts. The toxic effect on the cells was determined using three different colorimetric bioassays. The BrdU (5-bromo-2´-deoxyuridine) bioassay was used to measure the incorporation of BrdU in the DNA proliferating cells. The metabolic activity was measured using the MTT (3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide) bioassay. The release of lactate dehydrogenase (LDH) from cells with damaged plasma membranes was determined using the LDH-bioassay.

Results

With the BrdU method it is possible to detect trichothecene toxins at very low levels in feeding stuffs. For T-2 and HT-2 toxins the detection limit are lower than for the GC-method (IC_{50} 5-13 ng toxin/ml cell culture medium). The IC_{50} value (ng toxin/ ml cell culture medium) for deoxynivalenol and nivalenol are 263 and 365 respectively.
No significant differences was found between spiked wheat samples and pure toxins.

Conclusion

By measuring the BrdU incorporation in 3T3-cells exposed to feed extracts provides a useful method for screening of trichothecenes at concentrations frequently found in feeding stuffs.

Literature

Johan Widestrand 2001. Assessment of trichothecene contamination. Doctoral thesis, Swedish University of Agricultural Sciences, Agria 274.

Expression of foreign genes in mammalian cells using transient transfections, stable or inducible expression and viral infections

Catharina Svensson, Dept of Medical Biochemistry and Microbiology, Uppsala University

Viruses require the metabolic function of a living - and mostly dividing - host cell to produce progeny virus. To ensure optimal conditions for virus production, several viral activities are involved in reprogramming host cell activities. The co-evolution of viruses and their hosts therefore gives a strong hint on essential cellular processes. Different strategies have been applied to study the effect of both single genes and cascades of viral regulatory events.

Transient transfections usually demonstrate a limited efficiency (approx 5-30 %). Although this method has proven very informative when dissecting the function of viral regulators on known targets (reporter), the low efficiency prevents analyses of host cell response.

Stable transfections are extensions of the transient transfections, including an additional step involving selection of cells where the transfected DNA has been incorporated into the host cell genome. This selection is usually based on the co-integration of a selectable marker – often a gene conferring drug resistance. Although the frequency of integration is low (approx 10^{-5}), the expansion of individual cell clones give rise to new cell lines with continuous expression of the gene of interest (GOI) in 100 % of the cells.

A general problem with stable expression of viral regulatory proteins is their toxicity. We have solved this problem by creating an integrated GOI (in this case the adenovirus E1A gene) which is **inducible** upon addition of a tetracyclin derivative (using the TetON system from Clontech). This approach allows a dose-dependent expression of the GOI, which can also be followed in a time course experiment. The effect of the GOI can be measured on individual host cell genes or globally, using micro array technology.

Viral infections allow for a complete analysis of host cell reprogramming due to their capacity to infect 100 % of the target cells. With the aid of the multitude of viral mutants available, it is possible to ascribe different regulatory functions to individual viral genes. However, viruses may also be reconstructed to serve as carriers of foreign genes, thereby allowing gene expression, but not virus production. With a combination of recombinant viruses and the TetON system it has become possible to express foreign, toxic genes to well defined levels.

Pancreatic islets and islet cells

Eva Grapengiesser

The islets of Langerhans are multihormonal endocrine microorgans representing 1-2% of the pancreas. They appear as clusters of 3003-5000 cells embedded within the exocrine tissue. In the mouse, 50-80% of the cells are beta-cells secreting insulin. Additional hormones are secreted by alfa$_1$-cells (somatostatin;10-20%), alfa$_2$-cells (glucagon;10-20 %) and PP-cells (pancreatic polypeptide; < 5%). In previous studies it has been assumed that the reaction of the whole islet represents the beta-cells. To study the Ca^{2+}-signals for hormone secretion in individual beta-cells and other types of cells, the islets were isolated from the pancreas and dispersed into single cells and small clusters. The cells were allowed to attach to coverslips used as the bottom of a perifusion chamber placed on the stage of a microscope. Cytoplasmic Ca^{2+} was measured in single cells using microfluorometric technique. Preliminary identification of the living cells was based on differences in cell size and nucleus/cytoplasmic ratio. After the experiments the identification was performed with immunohistological staining for insulin, glucagon and somatostatin. Using this protocol it has been possible to demonstrate that each of the four types of islet cells has an intrinsic ability to generate oscillations of cytoplasmic Ca^{2+}.

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Program for CRU – workshop on
IMMUNOHISTOCHEMISTRY AND
***IN SITU* HYBRIDISATION**

Monday December 4, 2000, 9.00-16.30
Skoogsalen, Ing. 78/79, UAS.

9.00 Welcome
Andrzej Madej, Dept. of Animal Physiology, SLU.
9.05-10.05 **Immunocytochemistry in ultrastructural research**
Agneta Lukinius, Dept. of Pathology, Rudbecklab.
10.05-10.20 **Some hints for immunostaining**
Anders Ahlander, Dept. of Anatomy, BMC.
10.20-10.45 **Immunohistochemical analysis of xenograft rejection**
Anna Björkland, Dept. of Clin. Immunology, Rudbecklab.
10.45-11.10 **Colocalization and quantification of immunostaining by confocal microscope**
Zhanchun Li, Dept. of Internal Medicine, UAS.
11.10-11.35 **Immunocytochemistry in mammary tumour biology**
Eva Hellmen, Dept. of Anatomy and Histology, SLU.
11.35-12.00 **Carbonic anhydrases - histochemistry and immunohistochemistry**
Yvonne Ridderstråle, Dept. of Animal Physiology, SLU.

12.00-12.45 Lunch and Coffee

12.45-13.10 **mRNA detection in tissues vs immunohistochemistry**
Kristofer Rubin, Dept. of Med. Biochemistry and Microbiology, BMC.
13.10-13.35 **Icke-radioaktiv in situ hybridisering**
Marie Torstensson, Dept. of Plastic and Handsurgery, UAS.
13.35-14.20 **Hybridisation histochemistry applied to embryonic and neuronal tissue**

Finn Halböök, Dept. of Neuroscience, BMC.
14.20-15.25 **PCR in situ hybridisation for HPV virus detection**
Jan Sällström, Dept. of Pathology, Rudbecklab.
15.25-15.45 **Somatostatin receptors: from basic research to clinical practise**
Eva Tiensuu Janson, Dept. of Internal Medicine, UAS.
15.45-16.10 **The oestrogen receptor - how to explain the cytoplasmic staining**
Elisabeth (Lisa) Persson, Dept. of Anatomy and Histology, SLU.
16.10-16.30 **Discussion** Moderated by *Andrzej Madej, Dept. of Animal Physiology, SLU.*

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Program for CRU – workshop on RADIOIMMUNOASSAY, ENZYME IMMUNOASSAY AND RADIORECEPTOR ASSAY

Wednesday May 30, 2001, 8.30-14.00
Lecture Hall FU 26 in HVC, entrance from Undervisningsplan 6F or from Ulls väg 27D, Ultuna

8.30 - 9.00 Coffe
9.00 Welcome
Andrzej Madej, Dept. of Animal Physiology, SLU.
Xin Fu, Dept. of Obstetrics and Gyneacology, UU.

9.05-9.40 **Introduction to immunoassay design**
Mats Stridsberg, Dept of Clinical Chemistry ,UU.

9.40-10.15 **Neuropeptides**
Fred Nyberg, Dept. of Pharmaceutical Biosciences, UU.

10.15-10.50 **Prostaglandins**
Hans Kindahl, Dept. of Obstetrics and Gynaecology, SLU.

10.50 -11.00 **Break**

11.00-11.45 **Radioligand binding assays for receptors**
Ingrid Lundell, Dept. of Pharmacology , BMC.

11.45-12.20 **Non-species specific hormones - methodological aspects**
Mats Forberg, Dept. of Clinical Chemistry, SLU.

12.20-12.45 **Quality control system for assays**
Andrzej Madej, Dept. of Animal Physiology, SLU.

12.45-13.00 **Discussion** Moderated by *Andrzej Madej, Dept. of Animal Physiology, SLU.*
Xin Fu, Dept. of Obstetrics and Gyneacology, UU.

13.00-13.45 Lunch

Coffee and lunch are free of charge.
Registration before 30 May is depending on the availability of places.

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Speakers

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Program for CRU – workshop on
INTRODUCTION TO CATEGORICAL DATA ANALYSIS, BY EXAMPLES

Monday November 5, 2001, 13.00-16.45
Lecture Hall "Hedstrandsalen", Ing. 70
Akademiska Sjukhuset

13.00 - 13.10 Coffee

13.10 - 13.15 **Welcome**
Lotta Rydhmer, Dept. Animal Breeding and Genetics, SLU

The lectures are given by Patrik Öhagen, Dept. Ruminant Med. and Vet. Epidemiology, SLU,

13.15-14.00 **What's so special about categorical data?**

14.00-14.15 Break

14.15-15.00 **An example on how the use of standard methods can go wrong**

15.00-15.30 Coffee break

15.30-16.15 **Do you need more sophisticated statistical methods?**

16.15-16.45 **Questions and discussion**
moderated by Nils Lundeheim, Dept. Animal Breeding and Genetics, SLU

This workshop in statistics will start from the very basics about methods for analysing Categorical Data. We will try to indicate how to get more knowledge from a data set by using more advanced (but still comprehensible) methods.

Contact Patrik Öhagen for more information about the workshop: Patrik.Ohagen@epid.slu.se

Workshop and coffee are free of charge.

Registration before November 2nd to: xin.fu@kbh.uu.se fax: 018 559775

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