Transdifferentiation Researches

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Abstract: Transdifferentiation is a non-stem cell transforming into a different type of cell, or a differentiated stem cell changing to another type of cells. Transdifferentiation is a type of metaplasia, which includes all cell fate switches, including the interconversion of stem cells. There are about 300 different types of cells in human and each specialized for a specific function. Most of our cells are matured cells (adult cells) and normally the adult cells are differentiated from than stem cells. The importance of the trandifferentiation is to transform the non-stem cell into a different type of cells that the old cells can transform to young cells. If we can transform the old cells to a young cell, we can keep the life living eternally and keep the life body in the younger stage forever. This is the really biological immortality – living eternal.
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Introduction

Transdifferentiation is a non-stem cell transforming into a different type of cell, a type of metaplasia, which includes all cell fate switches, including the interconversion of stem cells. It is a differentiated stem cell changing to another type of cells, the conversion of differentiated cells into another differentiated cell type (Slack and Tosh, 2001). Transdifferentiation is a lineage reprogramming. It is a real hope to Rejuvenate old cells, tissues or organs and even a whole living-body through cell transdifferentiation.

Turritopsis nutricula is a hydrozoan that can revert to the sexually immature (polyp stage) after becoming sexually mature. It is the only known metazoan capable of reverting completely to a sexually immature, colonial stage after having reached sexual maturity as a solitary stage. It does this through the cell development process of transdifferentiation. This cycle can repeat indefinitely that offers it biologically immortal (Ma and Yang, 2010).

There are many reports of transdifferentiation of adult stem cells that can form adult cell lineages (Brittan et al, 2002; Jiang et al, 2002; Korbling et al, 2002; Krause et al, 2001). Lagasse et al (2000) have studied mice lacking fumarylacetoacetate hydrolase, which develop a fatal metabolic liver disease and they have reported that purified hematopoietic stem cells can differentiate into hepatocytes in vivo and rescue the defect. They showed that bone marrow cells migrated to the liver and transform into fully functional hepatocytes that involved stem cells rather than fully differentiated cells (COPE, 2010). However, the conversion of mesenchymal cells into epithelial cells and the conversion of epithelial cells into mesenchymal cells may not be regarded as a transdifferentiation event as it involves at least one type of embryonic cells.

As the example of the transdifferentiation, in salamanders and chickens when the lens of the eye is removed, cells of the iris turn into lens cells. Although transdifferentiation is rare in vertebrates, it occurs in the fetal development of the oesophagus, when the tunica muscularis which is composed of smooth muscle transdifferentiates into skeletal muscle across. During this process, smooth muscle cells transform back into myoblasts, then line up and fuse to form myotubes which then become cylindrical skeletal muscle fibers.

Many contents and information of this article are collected from libraries and Internet to offer to the readers as the references to design potential projects.

Definition of Transdifferentiation

1. The change of a cell or tissue from one differentiated state to another. **2.** The differentiation of a tissue-specific stem cell into another type of cell as, for example, a bone marrow stem cell differentiating into a neuron (Medcinenet.com, 2010).

Experiments

When examining transdifferentiated cells, it is important to look for markers of the target cell type and the absence of donor cell markers which can be accomplished using green fluorescent protein or immunodetection. It is also important to examine the cell function, epigenome, transcriptome, and proteome profiles. Cells can also be evaluated based upon their ability to integrate into the corresponding tissue in vivo (Eda et al, 2010).

Experimental method example - TGF-β in mesenchymal transdifferentiation study

Cell culture

Mouse NMuMG breast epithelial tumor cells (can be obtained from American Type Culture Collection, Manassas, VA, USA) are routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 10 μg/ml insulin, 100 units/ml penicillin and 50 μg/ml streptomycin. Cells are grown in a 5% CO_2-atmosphere at 37°C.

Polyclonal and monoclonal antibodies

Preparation of antisera against ALK-1 (RRQ), ALK-2 (RRN), ALK-3 (KSI), ALK-4 (RVY), ALK-5 (VPN), ALK-6 (DET), ActR-II (ARC), ActR-IIB (RKP), BMPR-II (SMN and NRR), TβR-II (DRL), Smad2 (SED), and phosphorylated Smad2 (PS2). Mouse monoclonal anti-E-cadherin and anti-β-catenin antibodies can be purchased from Transduction Laboratories, Lexington, KY, USA. Two monoclonal antibodies (mAb), one which recognizes specifically Smad1 (mAb A-4, sc-7965) and one that recognizes primarily Smad3 but also Smad2 (mAb H-2, sc-7960) can be bought from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Monoclonal antibodies against the haemaglutinin and Flag epitopes are available by Boehringer Mannheim (Mannheim, Germany) and Sigma (St Louis, MO, USA).

Transdifferentiation of NMuMG cells and fluorescence microscopy of the actin cytoskeleton, E-cadherin, β-catenin and Smad proteins

NMuMG cells are seeded at a density of 2×10^4 cells/cm^2 on 22×22 mm glass coverslips coated with 0.1% gelatin. The next day, cells are stimulated with activin A, BMP-7, or TGF-β1 in the presence of 10% FBS. Inhibition of TGF-β1-mediated transdifferentiation of NMuMG cells is tested with the more general serine/threonine kinase and protein kinase C (PKC) inhibitor staurosporine (available by Sigma, St Louis, MO, USA), the PKC-α, -β1, -β2, -γ isoform-specific inhibitor bisindolyl-maleimide I (available by Calbiochem-Novabiochem Corp., La Jolla, CA, USA), the MEK inhibitor PD98059 (available by Biomol, Research Laboratories, Inc., Plymouth Meeting, PA, USA), the specific PI3-kinase inhibitor, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002; available by Biomol, Plymouth Meeting, PA, USA), the PI3-kinase inhibitor, wortmannin (available by Calbiochem-Novabiochem Corp. La Jolla, CA, USA) or the Src-specific inhibitor PP1 (available by Calbiochem-Novabiochem Corp., La Jolla, CA, USA). After 36 hours of stimulation, cells are processed for direct fluorescence of the actin cytoskeleton with 0.25 μM tetramethylrhodamine B isothiocyanate (TRITC)-conjugatedphalloidin (available by Sigma, St Louis, MO, USA) as described by Moustakas and Stournara (Moustakas and Stournaras 1999). For indirect immunofluorescence, specimens are incubated with a 200-fold dilution of mouse monoclonal anti-E-cadherin or anti-β-catenin antibody, 1,000-fold dilution of mouse monoclonal anti-Smad1 or anti-Smad2/3 antibodies, and 500-fold dilution of mouse monoclonal antihaemaglutinin (HA) or anti-Flag antibodies (for detection of adenovirally encoded proteins) followed by incubation with a 100-fold diluted TRITC-conjugated or fluorescein isothiocyanate (FITC)-E. Piek and others TGF-β-signalling in transdifferentiation 4559 conjugated goat anti-mouse IgG antibodies (available by Dako-PattsA/S, Glostrup, Denmark) as described by Moustakas and Stournara (Moustakas and Stournaras 1999). In double staining experiments, indirect immunofluorescence preceded the direct fluorescent phalloidin staining. All specimens are mounted on glass slides with fluoromount-G (available by Southern Biotechnology Associates, Birmingham, AL, USA) and are observed on an Olympus Vanox-T model AHBT microscope equipped with an AH2-RFL epifluorescent illumination unit. Photomicrographs are obtained with a 35 mm Olympus (C-35AD-4) camera on Kodak P400 black and white film.

Receptor affinity cross-linking studies

Activin A, BMP-7, and TGF-β1 are iodinated to comparable specific activity by the chloramine-T method. Affinity binding and cross-linking of iodinated growth factors to receptors on NMuMG cells, followed by immunoprecipitation with receptor-specific antisera.

Transient transfection studies

NMuMG cells are seeded at a density of 2×10^4 cells/cm^2 in 6-well tissue culture plates. The next day transient transfections are performed using the transfection reagent, following the manufacturer's protocol (Boehringer Mannheim, Mannheim, Germany). Cells are transfected with 1 μg 3TP-Lux reporter construct or 0.5 μg (SBE)4-Lux reporter construct. For ligand-induced reporter assays, 1 μg pcDNA3 is added, while for analysis of reporter activity induced by CA type I receptors, 1 μg plasmid carrying the respective receptor cDNAs is co-transfected. After 24 hours, cells are stimulated for 20 hours with activin A, BMP-7 and TGF-β1. In all transfections the β-galactosidase expression plasmid pCH110 (available by Pharmacia, Uppsala, Sweden)

served as an internal control to correct for transfection efficiency. β-Galactosidase activity is measured in 100 mM Na_2HPO_4/NaH_2PO_4 (pH 7.3), 1 mM $MgCl_2$, 100 mM β-mercaptoethanol and 0.67 mg/ml *o*-nitrophenyl-galactopyranoside (available by Sigma, St Louis, MO, USA).

Transient adenoviral infection studies

Adenoviral stocks are maintained and titred in 293T cells and their titre ranged between 2.5 and 9×10^8 plaque forming units (pfu) per ml. Establishment of optimal infection conditions of NMuMG cells is performed using a β-galactosidase-encoding virus and staining of fixed cell monolayers with 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal). Under optimal conditions, more than 90% of the cells are infected as determined by the blue, β-galactosidase-positive staining. Routine infections are performed at a multiplicity of infection (MOI) of 100 with single viruses. This corresponds to 1×10^2 pfu per cell. Titration experiments used MOIs from 5 to 500 and in multiple vector co-infections the described MOI of the individual virus is used but the total viral MOI is always kept at 150 maximum. NMuMG cells are seeded at a density of 5×10^4 cells/cm^2 in 24-well tissue culture plates (light microscopy and X-Gal staining assays) or at a density of 5×10^5 cells/cm^2 on glass coverslips positioned in 6-well plates (direct fluorescence and indirect immunofluorescence assays). The next day the culture medium is changed to DMEM containing 5% FBS 1 hour prior to the infection. Cells are infected at the appropriate MOI for 12 hours and then washed and fed fresh 5% FBS-DMEM. Cells are observed every 8 hours and finally assayed 48-60 hours post-infection.

PAI-1 assays

NMuMG cells, grown to 90% confluence, are rinsed in PBS and medium is changed to methionine- and cysteine-free MCDB 10^4 (available by SVA, Uppsala, Sweden) containing 0.1% bovine serum albumin (BSA). Subsequently, activin A, BMP-7 or TGF-β1 are added for two hours, followed by labelling for two hours with 1 μCi/ml [35S]methionine/[35S]cysteine (available by Amersham, Buckinghamshire, UK). Thereafter, PAI-1 production is assayed.

Endogenous E-cadherin, β-catenin and Smad2/3 and adenovirally encoded receptor and Smad detection by western blotting

Normal NMuMG cells or adenovirus-infected cells are grown to 90% confluence. Cells are rinsed in PBS and serum-starved for three hours in DMEM containing 0.1% BSA, followed by addition of 2 nM activin A, 5.7 nM BMP-7, or 400 pM TGF-β1. After stimulation for 0, 60 minutes or 36 hours cells are put on ice, rinsed with PBS and lysed in solubilisation buffer (125 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM PMSF, 1.5% Trasylol, and 1% Triton X-100) for 40 minutes. Cell lysates are quantified by Bradford analysis for protein content and extracts representing equal total protein amounts are separated by sodium dodecyl sulfate (SDS)-gel electrophoresis using an 8.5% (Smad2 phosphorylation analysis) or a 7% (all other experiments) polyacrylamide gel, followed by wettransfer of the proteins to Hybond-C extra nitrocellulose membranes (available by Amersham, Buckinghamshire, UK). Non-specific binding of proteins to the membranes is blocked in TBS-T buffer (0.01 M Tris-HCl, pH 7.4, 0.15 M NaCl, 0.1% Tween 20) containing 3% BSA. The primary Smad antibodies are diluted 1,000-fold, the E-cadherin antibody is diluted 500-fold, the β-catenin antibody is diluted 1,000-fold, the anti-HA antibody 300-fold and the anti-Flag antibody 500-fold in TBS-T and secondary horseradish peroxidase-conjugated goat antirabbit IgG antibody (available by Amersham, Buckinghamshire, UK) is used at a 10,000-fold dilution in TBS-T. Detection is performed by enhanced chemiluminescence (ECL) (Piek et al. 1999).

Discussion

Life is a physical and chemical process. From ontology aspect, the world is timeless and the life exists forever as any other body in the nature. The nature of life is that life is a process of negative entropy, evolution, autopoiesis (auto-organizing), adaptation, emergence and living hierarchy. Up to now, there is no scientific evidence to show that life body and non-life body obey the same natural laws. But, all the researches are made by the methods of biology, biochemistry and molecular biology, etc. It is very possible that the life and non-life are essential different in the biophysics, i.e. the quantum level. In the future, it is possible to make artificial life by either biological method or electronic technique (Hongbao Ma 2005b).

Human bodies are composed of a huge array of cells. There are about 300 different types of cells, each specialized for a specific function. For example the nerve cells conduct signals around the body, muscle cells contract to produce movement and intestinal cells absorb food from the gut. Many common diseases such as cancer, heart disease or stroke, cause damage to specific structures and organs within the body. To repair the organs requires being able to regenerate the correct types of cell in the damaged areas. One possible method is to graft in stem cells, which may be able to replace any type of cell. Another

possibility is to replace the missing function by causing transdifferentiation (or conversion) of existing cells. This strategy is likely to work best in the case of endocrine organs whose function is to secrete hormones into the blood. This is because the exact location of an endocrine gland within the body does not matter, and, so long as it has access to the bloodstream, a replacement organ could be successfully created by transdifferentiation in an unusual position. Understanding how trandifferentiation happens and creating useful transdifferentiation (e.g. reprogramming cells from the liver to become pancreatic beta cells or using mesenchymal stem cells from bone marrow to repair bone and other tissues) enable us to use it in the therapy applications.

Related to human activities, there are two aspects of the world: One is the observed world (epistemology) and the other is the existed world (ontology). From the epistemology angle, time and space are relative (observed) (Ma 2003). From the ontology angle, time and space are absolute (existed) and the universe is a timeless world, which means that all the past, the present and the future exist eternally. All the life is a kind of existence in the universe, and from this aspect the life exist eternally (Ma, 2003). The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Embryonic stem cells are derived from the inner cell mass of blastocyst stage embryos. Somatic stem cells are generally believed to differentiate only into cells characteristic of the tissue wherein they reside. Stem Cell is the original of life. All cells come from stem cells (Hongbao Ma 2005a). Most of our cells are matured cells, i.e. adult cells, rather than stem. The importance of the trandifferentiation is to transform the non-stem cell into a different type of cells. If we can transform the old cells to a young cell, we can keep the life living eternally and keep the life body in the younger stage forever. This is the really biological immortality – living eternal – we will not die.

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