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(54) Title: METHODS OF DERIVATION AND/OR PROPAGATION OF EPITHELIAL CELLS

(57) Abstract: The present invention relates to methods of derivation and/or propagation of epithelial cells from tissue comprising culturing said tissue in media containing an effective amount of a proprotein convertase inhibitor for a period of time allowing the derivation and/or propagation of epithelial cells.



Methods of derivation and/or propagation of epithelial cells

FIELD OF THE INVENTION

5 The present invention relates to methods of derivation and/or propagation of epithelial cells from tissue comprising culturing said tissue in media containing an effective amount of a proprotein convertase inhibitor for a period of time allowing the derivation and/or propagation of epithelial cells.

BACKGROUND OF THE INVENTION

10 Today, there is a need in regenerative medicine to improve the therapies for epithelial cell associated diseases and injuries. The use of these genes products or small molecules, independently or in combination, will improve the process of *ex-vivo* amplification for autologous and homologous cell based therapies. It will also be of interest for the development of therapeutic agents to enhance healing of epithelia such as the skin, cornea, oral and nasal mucosa etc.

20 There is also a need for the development of robust *in vitro* systems to study normal epithelial cells (ex: skin keratinocytes, corneal epithelial cells etc.). The current state of the art for the culture of human epithelial stem cells (skin, cornea and other stratified epithelia) involves the use of feeder cells (3T3 cells). Those cells were derived from mouse embryo in the laboratory of Howard Green (Todaro and Green, 1963). When cell cycle arrested (by irradiation or mitomycin c treatment), feeder cells can support the growth of human epithelial cells and allow to produce enough autologous material to treat large burn wounds (Gallico et al., 1984; Rheinwald and Green, 1975). However, the regulatory authorities constantly push towards the development of alternatives to get rid of the “xeno” component of the system (Feeder cells and serum).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 represents 3T3-J2 feeder cells (MEFs) and the Cultured Epidermal Autografts (CEA) procedure.

Figure 2 depicts the screening procedure.

Figure 3 shows the effects produced by inhibition of FURIN with a small molecule (Protease convertase inhibitor). FURIN inhibition reduces the signal of the rhodamine B fluorescence assay.

Figure 4 shows the results of the QPCR analysis of the expression of *DNp63*, *HOPX*, *LEKTI*, *IVL* and *KRT1*. It also shows the results of the quantification of KI67 positive cells by immunofluorescence analysis.

DESCRIPTION OF THE INVENTION

To identify the genes involved in the cross-talk between 3T3-J2 cells and human keratinocytes stem cells, the Inventors decided to screen the 3T3 cells with the mouse druggable genome siRNA library (QIAGEN). The library covered only 8'320 genes from the mouse genome. To validate the results of the primary screen, a secondary screen for the same 126 genes was performed. Other genes expected to have an effect in the culture system (Table 1) were also included. For the secondary screen, both individual ("deconvoluted"), and pools of siRNAs were tested in duplicates for each targeted gene. The secondary screen confirmed the effect for 70 genes (53 %) (Table 2).

Table 1

Gene Name	Gene ID GeneCards®
Agmo	319660
Arhgef25	52666
Bmf	171543
Bzap1	207777
Cacna1e	12290

Cdk2	12566
Ces3b	13909
Clec2d	93694
Csnk1a1	93687
Ctdsp2	52468
Dctn1	13191
Ddx39	68278
Ddx54	71990
Ddx58	230073
Dll3	13389
Dll4	54485
Dmbt1	12945
Dok1	13448
Dok2	13449
Dok5	76829
Efna1	13636
Egf	13645
Egln1	112405
Ehd4	98878
Eif3c	56347
Emd	13726
Epb4.2	13828
Ercc2	13871
Ewsr1	14030
Exosc8	69639
Eya3	14050
Ezh2	14056
F10	14058
Fbxo31	76454
Fbxo34	78938
Fbxw11	103583
Fcrl5	329693
Fgf7	14178
Fhl5	57756
Fkbp1a	14225
Fkbp8	14232
Flcn	216805
Ftmt	67634
Furin	18550
Fut8	53618
Fzd3	14365
Gal3st1	53897
Gcdh	270076
Gga1	106039

Gjb2	14619
Gm5867	545756
Gnb5	14697
Gne	50798
Gpd2	14571
Gpnmb	93695
Gpx7	67305
Gria1	14799
Gsn	227753
Hs3st1	15476
Htr4	15562
Htr6	15565
Il1f9	215257
Il20	58181
Il21r	60504
Il25	140806
Itgav	16410
Kcnj9	16524
Lifr	16880
Lmo2	16909
Mapk15	332110
Mapkapk2	17164
Mknk1	17346
Mllt6	246198
Mmp1b	83996
Necab2	117148
Nefh	380684
Nfkbie	18037
Nr1h2	22260
Nsmf	56876
Olfr1301	258889
Olfr32	18331
Olfr684	244187
Olfr731	258360
Olfr888	258416
Olfr922	258777
Olfr95	258506
Pabpn1	54196
Papln	170721
Pccb	66904
Pdlim7	67399
Pfn1	18643
Pgam2	56012
Pkia	18767

Pkn3	263803
Pla2g2d	18782
Pld3	18807
Pole	18973
Porcn	53627
Ppan	235036
Ppil2	66053
Prss22	70835
Prss48	368202
Prtn3	19152
Psma6	26443
Psmb4	19172
Psmc1	19179
Psmc2	19181
Psmc5	19184
Psmc2	21762
Ptk2	14083
Rpl35	66489
Rpl38	67671
Rpl4	67891
Rrm1	20133
Rrm2	20135
Sars	20226
Smc3	13006
Snrpd1	20641
Stat4	20849
Styxl1	76571
Tbck	271981
Tgfb1	21803
Tsen2	381802
Tssk2	22115
Vmn1r45	22297
Wdr92	103784
Wnk4	69847
Wnt3	22415
Xab2	67439
Xpo1	103573
Xpo7	65246

Table 2

Gene Name	Gene ID GeneCards®	Reference to therapeutic use
Dll3	13389	-
Dll4	54485	-
Dmbt1	12945	(Müller et al., 2012)
Efna1	13636	(Gordon et al., 2013)
Furin	18550	(Gurtner et al., 2008)
Gpnmb	93695	(Rose et al., 2010)
Il20	58181	(Ouyang et al., 2011)
Itgav	16410	-
Lifr	16880	-
Mapk15	332110	-
Mmp1b	83996	(Rohani et al., 2014)
Papln	170721	-
Smc3	13006	(Wu and Couchman, 1997)

5

Then, a functional annotation analysis with the DAVID bioinformatics resources (Huang et al., 2009) on the list of putative hits has been performed. Only 3 pathways were enriched (Table 3). Most of genes identified in the secondary screen are involved in different cellular processes. To sort the putative hits in a comprehensive list, the subcellular annotations from GeneCards® (<http://www.genecards.org/>) were retrieved. This allowed to identify putative “feeder” genes (transmembrane receptors, growth factors, transmembrane ligands and other signaling molecules that influence epithelial stem cells growth *in vitro*). Several genes associated with epidermal homeostasis and wound repair are represented. For example, IL20 is a cytokine that is upregulated in psoriasis (a disease characterized by a hyper proliferative epidermis) (Ouyang et al., 2011). MMP1, a collagenase, is also upregulated in wounded skin and facilitates the migration of keratinocytes (Rohani et al., 2014). FURIN is also known to play a key role during wound repair (Gurtner et al., 2008).

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Table 3

Pathway (KEGG)	# genes	p-value
Proteasome	4	3.60E-03
Pyrimidine metabolism	4	2.50E-02
Glutathione metabolism	3	4.60E-02

5 In view of these findings, the present invention thus relates to methods of derivation and/or propagation of epithelial cells from tissue comprising culturing said tissue in media containing an effective amount of a modulator of i) one or more genes involved in epithelial cells propagation and/or differentiation, or of a ii) product of said genes for a period of time allowing the derivation and/or propagation of epithelial cells. Preferably, the one or more genes involved in epithelial cells propagation and/or differentiation will be selected from the group of gene listed in Table 1. Most preferably the one or more genes involved in epithelial cells propagation and/or differentiation will be selected from the group comprising Dll3, Dll4, Dmbt1, Efna1, Furin, Gpnmb, Il20, Itgav, Lifr, Mapk15, Mmp1b, Papln and Smc3, or from a combination of one or more of these genes.

Generally, the modulator will be selected from the group comprising a chemical agent, an antibody, an engineered protease, and enzymatically active RNA.

20 The modulator can either activate or silence the genes or inhibit or activate the product of said genes.

Most preferably, the enzymatically active RNA is selected from the group comprising a miRNA, a siRNA, a piRNA, a hnRNA, a snRNA, esiRNA, shRNA, decoys, RNA aptamers and an antisense oligonucleotide. One will appreciate that any compound with different formulations capable to inhibit or activate one or more physiological actions effected by a gene involved in epithelial cells propagation and/or differentiation is encompassed by the present invention.

Also provided is a cell culture media containing an effective amount of a modulator of i) one or more genes involved in epithelial cells propagation and/or differentiation, or of a ii) product of said genes for derivation and/or propagation of epithelial cells.

5

The present invention also provides a pharmaceutical composition comprising a modulator of i) one or more genes involved in epithelial cells propagation and/or differentiation, or of a ii) product of said genes for use in the treatment of epithelial cell associated diseases, disorders and injuries. Preferably, the pharmaceutical composition comprises a pharmaceutically effective amount of the active compound, i.e. a modulator of i) one or more genes involved in epithelial cells propagation and/or differentiation, or of a ii) product of said genes, optionally with one or more pharmaceutically acceptable carriers, diluents and adjuvants.

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The present invention also provides a pharmaceutical composition comprising a modulator of the invention for use in the treatment of epithelial cell associated diseases, disorders and injuries. Preferably, the pharmaceutical composition comprises a pharmaceutically effective amount of the active compound, i.e. a modulator of the invention, optionally with one or more pharmaceutically acceptable carriers, diluents and adjuvants.

20

Also provided herein is a kit comprising i) a pharmaceutical composition comprising a therapeutically effective amount of a modulator of the invention and/or a ii) a therapeutically effective amount of epithelial cells obtained according to the method of derivation and/or propagation of epithelial cells as described herein, pharmaceutically acceptable salts, solvates or esters thereof, for the treatment of epithelial cell associated diseases, disorders and injuries.

25

Among the hits identified as target genes that showed a significant impact on human keratinocyte's proliferation when silenced, the inventors identified the gene *Furin*. When an inhibitor of the *Furin* gene product was added to the culture medium, the inventors surprisingly observed an increase in keratinocyte proliferation and an inhibition of keratinocyte differentiation.

30

FURIN belongs to the proprotein convertase family that is a family of enzymes catalysing the proteolytic maturation of prohormones and proproteins (G. Thomas, 2002; Seidah and Prat, 2012). To date, the proprotein convertase family comprises FURIN, PC1/3, PC2, PC4, PACE4, PC5/PC6, PC7/LPC/PC8, SKI/S1P and PCSK9.

5

In one instance, the present invention also concerns a method of derivation and/or propagation of epithelial cells from tissue comprising culturing said tissue in media containing an effective amount of a proprotein convertase inhibitor for a period of time allowing the derivation and/or propagation of epithelial cells.

10

The tissue can be cultured in the presence of fibroblast feeder cells. Feeder cells support the growth of epithelial and epithelial stem cells by producing growth factors and providing adhesion molecules and ECM components for cell attachment. Preferably, the fibroblast feeder cells are selected from the group comprising mouse embryonic and human fibroblasts, either primary cells or immortalized cells (such as 3T3 cells), or a combination thereof. Human fibroblast feeder cells are usually derived from several human cell types, such as human foreskin fibroblasts (hFFs) or adult Fallopian tube epithelial cells. Not wishing to be bound to any theory, it is believed that the use of such feeder cells, or conditioned media derived from such feeder cells, provides one or more substances necessary to promote the growth of the epithelial and epithelial stem cells and/or prevent or decrease the rate of differentiation of such cells. Such substances are believed to include membrane-bound and/or soluble cell products that are secreted into the surrounding medium by the cells. In addition, those of skill will also recognize that one or more substances produced by the feeder cells, or contained in the conditioned media, can be identified and added to the cell culture media of the invention to obviate the need for such feeder cells and/or such conditioned media.

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Usually, the epithelial cells comprise epithelial stem cells. Preferably, the method of the invention improves the number of proliferative epithelial stem cells, preferably by inhibiting their terminal differentiation.

The proprotein convertase inhibitor is selected from the group comprising a FURIN inhibitor, a PC1/3 inhibitor, a PC2 inhibitor, a PC4 inhibitor, a PACE4 inhibitor, a

PC5/PC6 inhibitor, a PC7/LPC/PC8 inhibitor, an SKI/S1P inhibitor and a PCSK9 inhibitor, or a combination of one or more of these inhibitors. Preferably, the proprotein convertase inhibitor is selected from the group comprising a FURIN inhibitor, a PC4 inhibitor, a PACE 4 inhibitor, a PC5/6 inhibitor, and a PC1/3 inhibitor.

- 5 More preferably, the proprotein convertase inhibitor is a FURIN inhibitor or a combination of one or more of these inhibitors.

When the proprotein convertase inhibitor is a FURIN inhibitor, the effective amount of the FURIN inhibitor is about 1 to about 100 μ M, or about 10 to about 75 μ M, or
10 about 50 μ M.

PCT applications No. WO 2009/023306, WO2013029182 and WO2013138665 A1, as well Becker et al., 2012 in Journal of Biological Chemistry, which are hereby incorporated by reference in their entirety, disclose FURIN inhibitors.

15

The proprotein convertase inhibitor can also be selected among the group comprising Hexa-D-arginine (potent inhibitor of FURIN, PACE4 and PC1) and Decanoyl-Arg-Val-Lys-Arg-CMK (potent inhibitor of FURIN, SPC3/PC1/PC3, PACE4, SPC6/PC5/PC6 and SPC7/LPC/PC7/PC8).

20

The tissue is usually selected among the group comprising a stratified epithelium comprising keratinocytes and a pseudostratified epithelium (simple epithelium) comprising keratinocytes. Preferably, the stratified epithelium is selected from the group comprising cornea, skin, esophagus, oral mucosa, conjunctiva, vagina, and
25 cervix (ectocervix) whereas the pseudo-stratified or simple epithelium is selected from the group comprising trachea, lung stomach, intestine, pancreas, thymus and bladder.

The keratinocytes can be any type of preferably primary keratinocyte. In some
30 examples, the primary keratinocyte is a foreskin keratinocyte, vaginal keratinocyte or cervical keratinocyte.

In some embodiments, the primary keratinocytes are obtained by a tissue biopsy. In some examples, the tissue biopsy is taken from the skin (e.g., the cutaneous and/or mucosal squamous epithelium).

- 5 The stratified or pseudostratified epithelium comprising keratinocytes are cultured in the presence of a proprotein convertase inhibitor for any suitable period of time to allow for derivation and/or propagation of epithelial cells. In some aspects, the keratinocytes are cultured in the presence of a proprotein convertase inhibitor for at least 15 days, at least 20 days, at least 40 days, at least 60 days, at least 100 days, at least 150 days, at least 200 days, at least 250 days, at least 300 days, at least 350 days, at least 400 days, at least 450 days, or at least 500 days.

Usually, the epithelial cells that have been derived and/or propagated from any of the above-mentioned tissue are recovered using techniques known in the art.

- 15 Also provided is a cell culture media containing an effective amount of a proprotein convertase inhibitor for derivation and/or propagation of epithelial cells. Any culture media suitable for derivation and/or propagation of epithelial cells from tissue in the presence of fibroblast feeder cells or not, envisioned by a person skilled in the art is encompassed.

20

- In case of derivation and/or propagation of epithelial cells from Human keratinocytes, the cells were usually propagated on top of irradiated 3T3-J2 cells with cFAD culture medium, a 3:1 ratio of DMEM and Ham's F12 culture medium, supplemented with insulin (5 µg/mL), Triiodothyronine (2×10^{-9} M, Sigma), hydrocortisone (0.4 µg/mL, Calbiochem) and cholera toxin (10^{-10} M, ICN). Cells were incubated in 10% CO₂ humid atmosphere at 37°C.

- The present invention also provides a pharmaceutical composition comprising a proprotein convertase inhibitor for use in the treatment of epithelial cell associated diseases, disorders and injuries. Preferably, the pharmaceutical composition comprises a pharmaceutically effective amount of the active compound, i.e. proprotein convertase inhibitor, optionally with one or more pharmaceutically acceptable carriers, diluents and adjuvants.

“A pharmaceutically effective amount” refers to a chemical material or compound which, when administered to a human or animal organism induces a detectable pharmacologic and/or physiologic effect.

- 5 The respective pharmaceutically effect amount can depend on the specific patient to be treated, on the disease, disorder or injury to be treated and on the method of administration. Further, the pharmaceutically effective amount depends on the specific peptide used. The treatment usually comprises a multiple administration of the pharmaceutical composition, usually in intervals of several hours, days or weeks.
- 10 The pharmaceutically effective amount of a dosage unit of the peptide of the invention usually is in the range of 0.1 ng to 100 mg per kg of body weight of the patient to be treated. However, it is understood that the suitable dosage of the pharmaceutical composition of the present invention will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any and the
- 15 nature of the effect desired.

Acceptable carriers, diluents and adjuvants which facilitates processing of the active compound into preparation which can be used pharmaceutically are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as

20 phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl orbenzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than

25 about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose,

30 mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN®, PLURONICS® or polyethylene glycol (PEG). The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. Remington's Pharmaceutical Sciences, by E.W. Martin, Mack Publishing Co., Easton, Pa., 15th

Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compounds or molecules.

The form of administration of the pharmaceutical composition may be systemic or topical. For example, administration of such a composition may be various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, buccal routes or via an implanted device, and may also be delivered by peristaltic means.

The pharmaceutical composition comprising a pharmaceutically effective amount of the proprotein convertase inhibitor, as described herein, as an active compound may also be incorporated or impregnated into a bioabsorbable matrix, with the matrix being administered in the form of a suspension of matrix, a gel or a solid support. In addition the matrix may be comprised of a biopolymer.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the proprotein convertase inhibitor, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and [gamma] ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT(TM) (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration must be sterile. This is readily accomplished for example by filtration through sterile filtration membranes.

One aspect of the present invention also concerns a method of treating an epithelial cell associated disease, disorder or injury in a patient in need thereof characterized in that it comprises

(i) obtaining a tissue from said patient in need thereof, or from an individual,

- (ii) subjecting the tissue to the method of derivation and/or propagation of epithelial cells as described herein,
- (iii) recovering the epithelial cells derived and/or propagated from the tissue, and
- (iv) contacting said epithelial cells with the patient in need thereof.

5

Alternatively or additionally, the method of treating an epithelial cell associated disease, disorder or injury may comprise a step of administering a therapeutically effective amount of proprotein convertase inhibitor, after or concomitantly to contacting the epithelial cells with the patient in need thereof.

10

Preferably, the epithelial cell associated disease, disorder or injury is selected among the non-limiting group comprising a wound such a burn or ulcer, bed sores, a skin disease, chronic wound, lacerations, abrasions, contusions, skin cuts, surgical wounds, chemical and/or thermal wounds, bites, stings and corneal wound.

15

In some aspects, the method comprises treating the patient in need thereof with an organotypic tissue equivalent prepared according to the methods disclosed herein. The primary keratinocytes are then obtained by a tissue biopsy of the patient to be treated with the organotypic tissue equivalent (thus, the organotypic tissue equivalent is an autograft).

20

Further provided is the use of a pharmaceutical composition of the invention in the preparation of a medicament for the treatment of an epithelial cell associated disease, disorder or injury in a patient in need thereof.

25

Also provided herein is a kit comprising i) a pharmaceutical composition comprising a therapeutically effective amount of proprotein convertase inhibitor and/or a ii) a therapeutically effective amount of epithelial cells obtained according to the method of derivation and/or propagation of epithelial cells as described herein, pharmaceutically acceptable salts, solvates or esters thereof, for the treatment of epithelial cell associated diseases, disorders and injuries.

30

EXAMPLES

Material & Methods**5 Culture of 3T3-J2 (Feeder cells)**

3T3-J2 cells were propagated in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 8% Bovine Serum (BS, Thermo Scientific) and incubated in 10% CO₂ atmosphere at 37°C. For serial amplification, 3T3 cells were inoculated every 7 days at low density (1 to 5 x 10⁵ cells per 162 cm² flasks. The culture medium was replaced every 3 to 4 days. For keratinocytes propagation, 3T3-J2 cells were irradiated with a dose of 60 Gy and then plated at a density of 2.5 x 10⁴ cells/cm².

15 Culture of Human keratinocytes (Identical protocol for all epithelial cells derived from stratified, pseudostratified or simple epithelia, ex: cornea, skin, esophagus, oral mucosa, conjunctiva, prostate, bladder, vagina, thymus)

Human keratinocytes were propagated on top of the irradiated 3T3-J2 cells with cFAD culture medium, a 3:1 ratio of DMEM and Ham's F12 culture medium (Amimed), supplemented with insulin (5 µg/mL, Sigma), Triiodothyronine (2 x 10⁻⁹ M, Sigma), hydrocortisone (0.4 µg/mL, Calbiochem) and cholera toxin (10⁻¹⁰ M, ICN). Cells were incubated in 10% CO₂ humid atmosphere at 37°C. For serial amplification, pre-confluent keratinocytes were trypsinized (0.05% trypsin and 0.1% EDTA) and seeded at appropriate density once a week. The culture medium was changed every 2 to 3 days for mass culture or every 4 days for colony forming efficiency. EGF was added at each feeding (10 ng/mL, Upstate Biotechnology Inc.). For FURIN inhibition experiments, the Proprotein Convertase Inhibitor (50 µM, Calbiochem) was added daily in cFAD medium.

High throughput screening of 3T3-J2 (Discovery strategy)

30 The mouse druggable genome siRNA library was first pooled (4 siRNAs per gene) by the Biomolecular Screening Facility (BSF) and then spotted on 96 well plates (BD Falcon) in duplicates. Briefly, 10 µL of pooled siRNAs (QIAGEN) were spotted (40 nM

final concentration) with 0.4 μ L of RiboCellIn transfection reagent (BioCellChallenge) and 10 μ L of OptiMem (Life Technologies) with the Caliper Sciclone platform (PerkinElmer). The first and last columns were spotted with scramble siRNA (negative control) and mouse specific cell death cocktail siRNAs (positive control) (QIAGEN) respectively. After 15 min incubation at RT, 10'000 irradiated 3T3-J2 cells (in 80 μ L of DME/BS) were seeded in each well using a Multiflow liquid dispenser (Biotek). Assay plates were then incubated overnight in 10% CO₂ atmosphere at 37°C. The next day, the medium was removed using an ELx405 (Biotek) and 600 normal human epidermal cells (strain YF29) in 100 μ L of cFAD were dispensed in each well with the Multiflow. The plates were then incubated for one week in 10% CO₂ atmosphere at 37°C with one medium change (cFAD without EGF) on the third day after seeding using the ELx405 and the Multiflow (Biotek). After 7 days of culture, the plates were fixed for 15 min with 3.4% formaldehyde (Sigma-Aldrich) and stained for 10 min with 3% rhodamine B (Sigma). Next, the plates were washed 9 times with PBS using the ELx406 (Biotek) and the remaining rhodamine B was then re-suspended in 100 μ L of PBS by 20 min incubation at RT on an orbital shaker at 90 rpm. Finally, the total fluorescence of the rhodamine B was measured using an Infinite F500 plate reader (Tecan). A Z' was computed to assess the assay's quality both during the assay development and the screening campaign (Zhang et al., 1999).

Immunocytochemistry

Cell samples were fixed with 4% paraformaldehyde (PFA) for 15 min. The samples were washed three times with PBS and permeabilized with 0.3% Triton X-100 (Sigma) in PBS for 10min. After another three consecutives washes with PBS, the samples were blocked with 2% bovine serum albumin (BSA) in PBS for 45 min and then incubated overnight at 4°C with primary antibody diluted in blocking solution. After three washes with PBS, the samples were incubated with corresponding conjugated secondary antibodies diluted in PBS for 45 min at RT. DNA was counterstained with DAPI for 10 min. Coverslips were washed twice with PBS and mounted on microscope slides.

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CLAIMS

1. A method of derivation and/or propagation of epithelial cells from tissue
5 comprising culturing said tissue in media containing an effective amount of a
proprotein convertase inhibitor for a period of time allowing the derivation and/or
propagation of epithelial cells.
2. The method of claim 1 characterized in that the tissue is culture in the
10 presence of fibroblast feeder cells.
3. The method of claim 1 or 2 characterized in that the epithelial cells comprise
epithelial stem cells.
- 15 4. The method of anyone of the preceding claims characterized in that the
number of proliferative epithelial cells is improved.
5. The method of anyone of the preceding claims characterized in that the
terminal differentiation of the epithelial cells is inhibited.
20
6. The method of anyone of the preceding claims characterized in that the
proprotein convertase inhibitor is selected from the group comprising a FURIN
inhibitor, a PC4 inhibitor, a PACE 4 inhibitor, a PC5/6 inhibitor, and a PC1/3 inhibitor.
- 25 7. The method of anyone of the preceding claims characterized in that the tissue
is a stratified epithelium comprising keratinocytes, a pseudostratified epithelium or a
simple epithelium.
8. The method of claim 7 characterized in that the stratified epithelium is
30 selected from the group comprising cornea, skin, esophagus, oral mucosa,
conjunctiva, vagina, and cervix (ectocervix).
9. The method of anyone of the preceding claims characterized in that the
epithelial cells are recovered.

10. A cell culture media containing an effective amount of a proprotein convertase inhibitor for derivation and/or propagation of epithelial cells.

5 11. A proprotein convertase inhibitor for use in the treatment of epithelial cell associated diseases, disorders and injuries.

12. A method of treating an epithelial cell associated disease, disorder or injury in a patient in need thereof characterized in that it comprises

- 10 (i) obtaining a tissue from said patient in need thereof, or from an individual,
(ii) subjecting the tissue to the method of derivation and/or propagation of epithelial cells according to anyone of claims 1 to 9,
(iii) recovering the epithelial cells derived and/or propagated from the tissue, and
(iv) contacting said epithelial cells with the patient in need thereof.

15

13. The method of claim 12 characterized in that it further comprises administering a therapeutically effective amount of proprotein convertase inhibitor, after or concomitantly to contacting the epithelial cells with the patient in need thereof.

20

14. A kit comprising

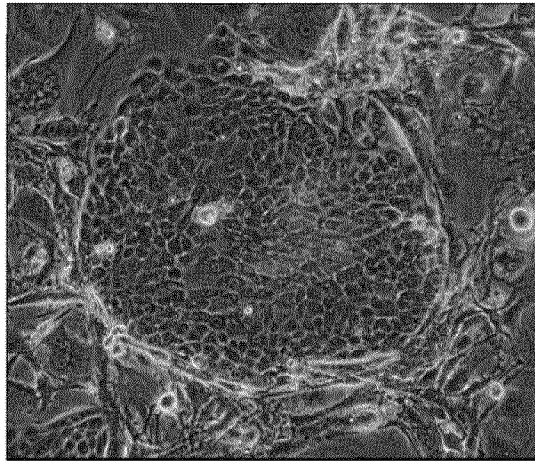
- i) a pharmaceutical composition comprising a therapeutically effective amount of proprotein convertase inhibitor and/or a
ii) a therapeutically effective amount of epithelial cells obtained according to anyone

25

of claims 1 to 9,
pharmaceutically acceptable salts, solvates or esters thereof, for the treatment of epithelial cell associated diseases, disorders and injuries.

30

Figure 1



3T3-J2 feeder cells (MEFs)
Fetal bovine serum

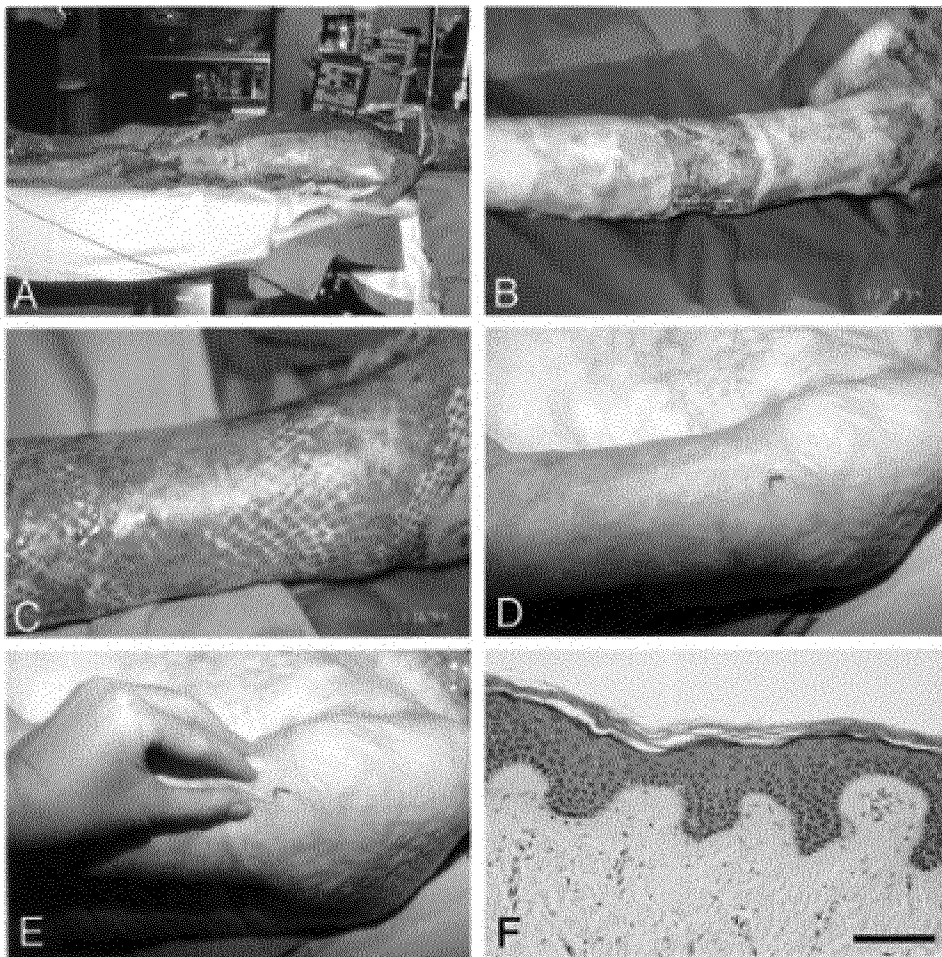


Figure 2

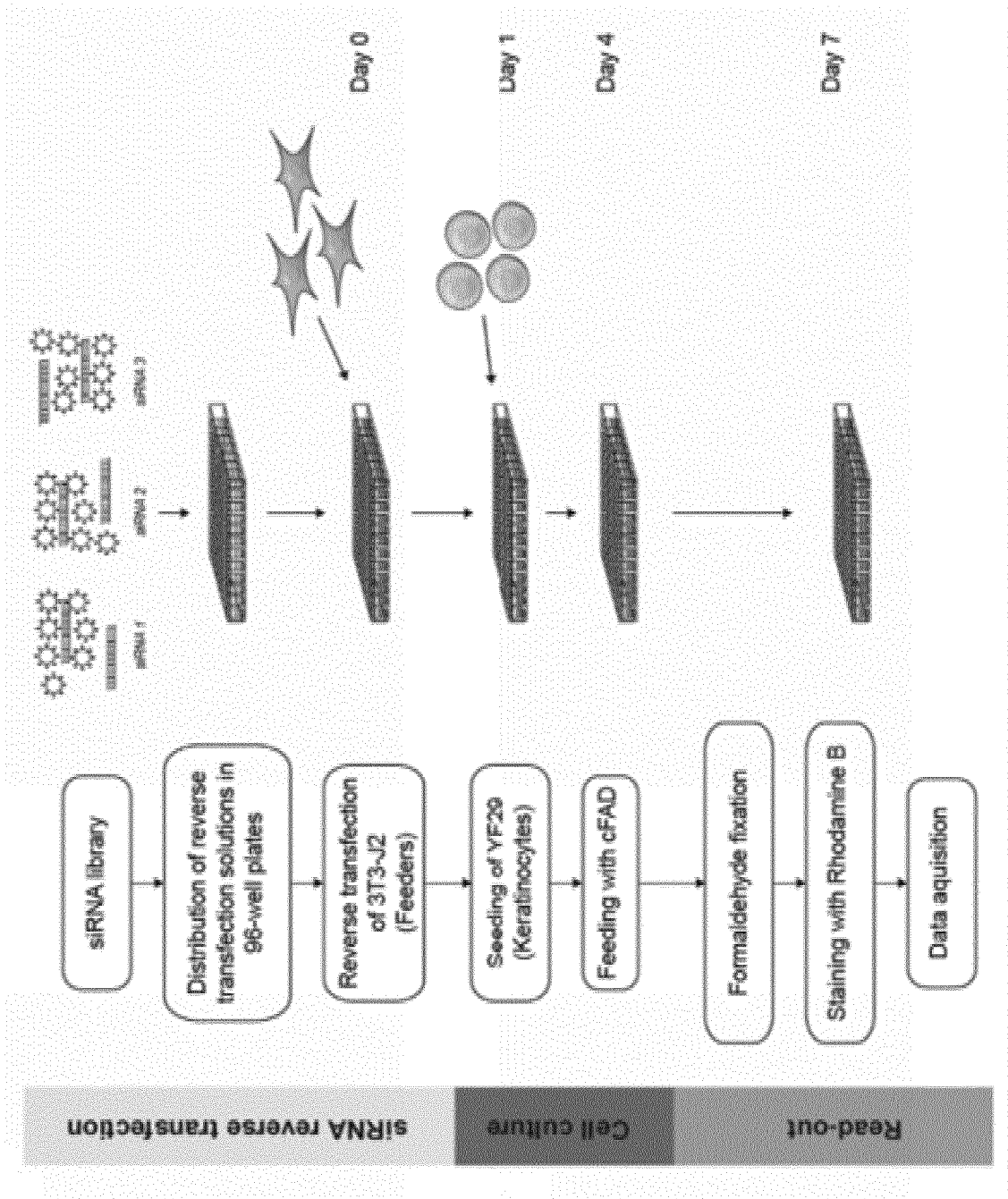


Figure 3

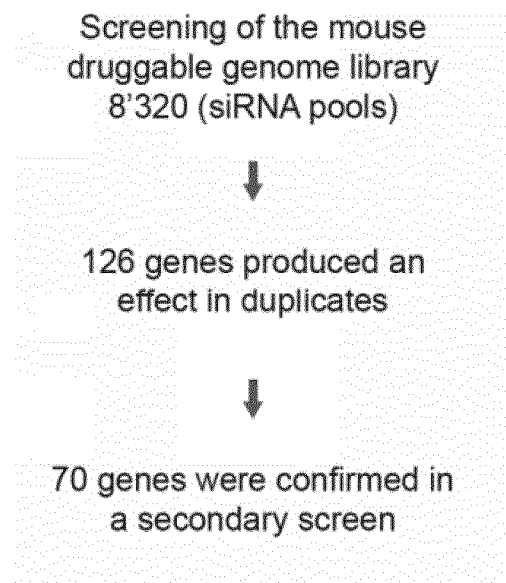
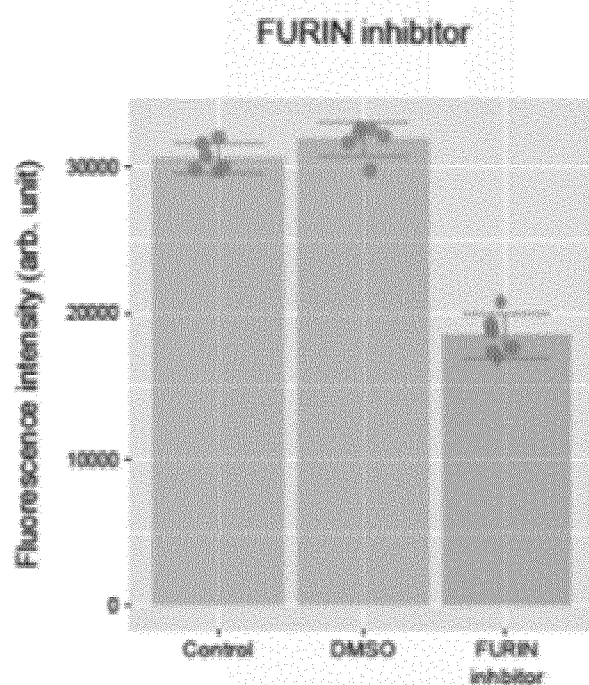


Figure 4

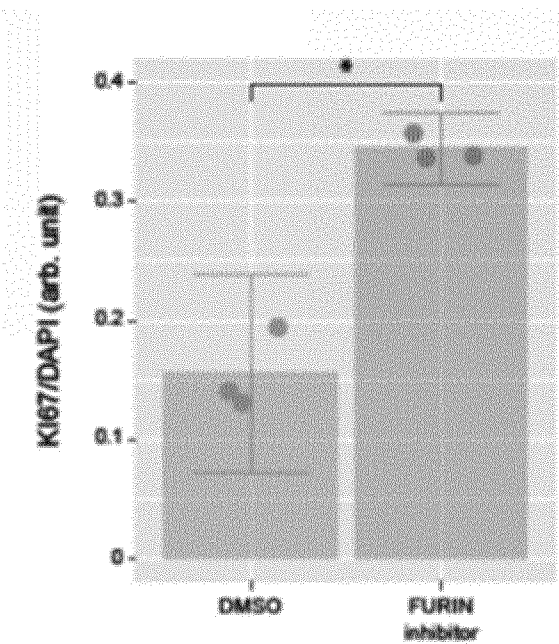
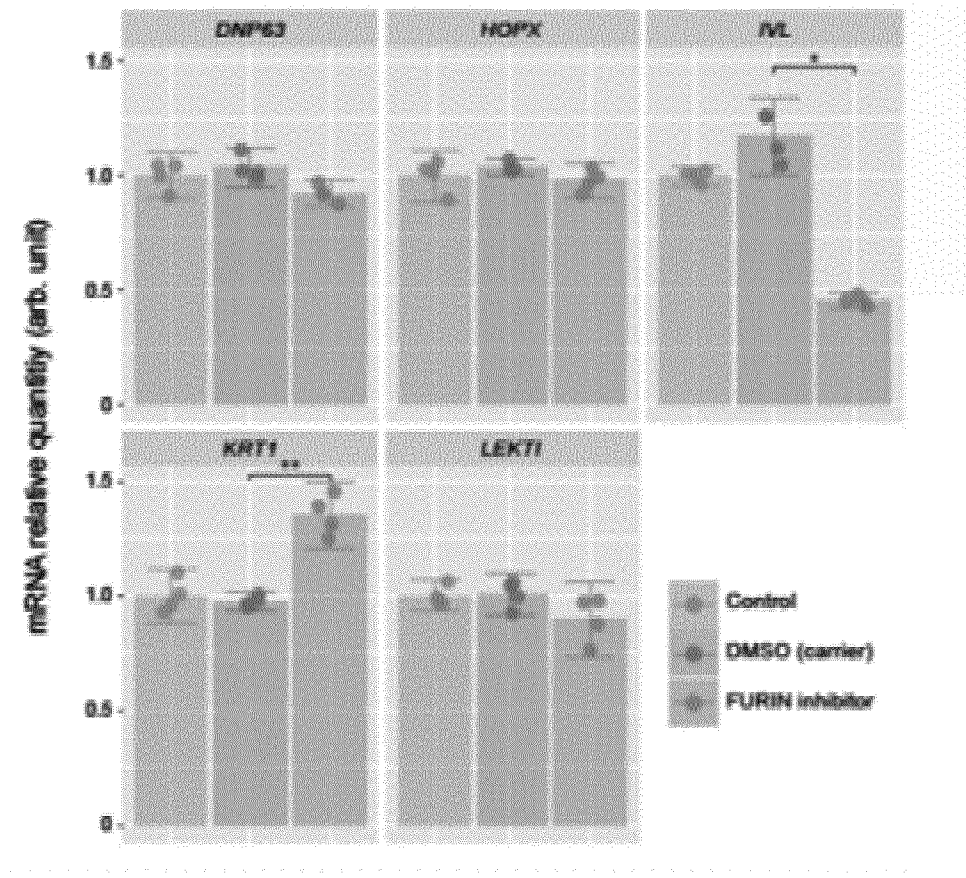


**Reduced assay signal
(confirmed the result of the screen)**

**Reduced expression of late
differentiation markers (IVL)**

Increased proliferation (KI67)

Figure 4 (cont')



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/077479

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N5/071 A61K38/08 A61P17/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BECKER G L ET AL: "Highly Potent Inhibitors of Proprotein Convertase Furin as Potential Drugs for Treatment of Infectious Diseases", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 287, no. 26, 22 June 2012 (2012-06-22), pages 21992-22003, XP007921976, ISSN: 0021-9258, DOI: 10.1074/JBC.M111.332643 [retrieved on 2012-04-26] cited in the application page 21995 ----- -/--	10,11



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 February 2018

Date of mailing of the international search report

13/02/2018

Name and mailing address of the ISA/

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Fax: (+31-70) 340-3016

Authorized officer

Teyssier, Bertrand

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/077479

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAN C D ET AL: "Cleavage of endogenous Î3ENaC and elevated abundance of Î ENaC are associated with increased Natransport in response to apical fluid volume expansion in human H441 airway epithelial cells", PFLÜGERS ARCHIV - EUROPEAN JOURNAL OF PHYSIOLOGY, vol. 462, no. 3, 12 June 2011 (2011-06-12), pages 431-441, XP019938306, ISSN: 1432-2013, DOI: 10.1007/S00424-011-0982-X page 432; figures 2,4 -----	10
X	EDIN N F J: "The role of interleukin-13 in the removal of hyper-radiosensitivity by priming irradiation", JOURNAL OF RADIATION RESEARCH, vol. 55, no. 6, 25 June 2014 (2014-06-25), pages 1066-1074, XP055447891, JP ISSN: 0449-3060, DOI: 10.1093/jrr/rru053 page 1069; figure 4 -----	10
X	HO H ET AL: "Small Molecule Proprotein Convertase Inhibitors for Inhibition of Embryo Implantation", PLOS ONE, vol. 8, no. 12, E81380, 4 December 2013 (2013-12-04), XP055447895, DOI: 10.1371/journal.pone.0081380 page 3 -----	10
X	SEIDAH N G & PRAT A: "The biology and therapeutic targeting of the proprotein convertases", NATURE REVIEWS. DRUG DISCOVERY, vol. 11, no. 5, 20 April 2012 (2012-04-20), pages 367-383, XP055259923, GB ISSN: 1474-1776, DOI: 10.1038/nrd3699 cited in the application the whole document -----	11
X	GARY T: "Furin at the cutting edge: from protein traffic to embryogenesis and disease", NATURE REVIEWS MOLECULAR CELL BIOLOGY, vol. 3, no. 10, October 2002 (2002-10), pages 753-766, XP009131959, ISSN: 1471-0072 cited in the application the whole document ----- -/--	11

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/077479

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BANERJEE Y ET AL: "Targeting PCSK9 for therapeutic gains: Have we addressed all the concerns?", ATHEROSCLEROSIS, vol. 248, 9 March 2016 (2016-03-09), pages 62-75, XP029511000, ISSN: 0021-9150, DOI: 10.1016/J.ATHEROSCLEROSIS.2016.02.018 the whole document -----	1-14