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(54) **Title: CULTURE MEDIUM**

(57) **Abrégé/Abstract:**
The invention relates to improved culture methods for expanding epithelial stem cells and obtaining organoids, to culture media involved in said methods,and to uses of said organoids.

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(54) **Title**: CULTURE MEDIUM

(57) **Abstract**: The invention relates to improved culture methods for expanding epithelial stem cells and obtaining organoids, to culture media involved in said methods,and to uses of said organoids.

## CLAIMS

1. A method for expanding epithelial stem cells comprising:

providing a population of epithelial stem cells;

providing a culture medium comprising an ErbB3/4 ligand, a receptor tyrosine kinase ligand and a BMP inhibitor;

contacting the stem cells with the culture medium; and

culturing the cells under appropriate conditions.

2. The method of claim 1, wherein the culture medium further comprises a Wnt agonist.

3. The method of claim 1 or claim 2, wherein the ErbB3/4 ligand is a neuregulin polypeptide.

4. A culture medium comprising a receptor tyrosine kinase ligand and a BMP inhibitor, characterised in that the culture medium further comprises an ErbB3/4 ligand.

5. The culture medium of claim 4, wherein the culture medium further comprises a Wnt agonist.

6. The culture medium of claim 4 or claim 5, wherein the ErbB3/4 ligand is a neuregulin polypeptide.

7. The culture medium of claim 6, wherein the neuregulin polypeptide comprises or consists of the amino acid sequence recited in SEQ ID NO: 27.

8. The culture medium of any one of claims 4-7, wherein the receptor tyrosine kinase ligand is selected from EGF, HGF, PDGF and FGF (e.g. FGF7 and/or FGF10).

9. The culture medium of any one of claims 5-8, wherein the Wnt agonist is an Lgr5 agonist.

10. The culture medium of claim 9, wherein the Lgr5 agonist is Rspondin.

11. The culture medium of any one of claims 4-10, wherein the BMP inhibitor is Noggin.

12. The culture medium of any one of claims 4-11, wherein the culture medium further comprises a TGF-beta inhibitor.

13. The culture medium of any one of claims 4-12, wherein the culture medium further comprises: (i) a Notch inhibitor and/or a prostaglandin pathway activator, and/or (ii) a

cAMP pathway activator and/or a BMP pathway activator, and/or (iii) a p38 inhibitor, gastrin and/or nicotinamide, and/or (iv) a ROCK inhibitor, and/or (v) testosterone.

14. The culture medium of any one of claims 4-13, further comprises a p53 stabilising agent.

15. The culture medium of claim 4, wherein the culture medium comprises:

(i) an ErbB3/4 ligand (e.g. human neuregulin β-1), EGF, FGF (e.g. FGF10), HGF, a TGF-β inhibitor (e.g. A83-01), nicotinamide, one or more Wnt agonists (e.g. an Lgr5 agonist), a cAMP pathway activator (e.g. forskolin), gastrin, a BMP inhibitor (e.g. Noggin), a Wnt agonist (e.g. Wnt conditioned medium) and a Rock inhibitor (e.g. Y27632), or

(ii) an ErbB3/4 ligand (e.g. human neuregulin β-1), one or more receptor tyrosine kinase ligands (e.g. EGF), a BMP inhibitor (e.g. Noggin) and one or more Wnt agonists (e.g. an Lgr5 agonist) and testosterone.

16. The method according to any one of claims 1-3, wherein the culture medium is a culture medium as described in any one of claims 4-15.

17. An organoid obtainable or obtained by a method of any one of claims 1-3 or 16.

18. The organoid of claim 17, wherein the organoid is a cancer organoid.

19. The organoid of any one of claims 17, wherein the organoid is obtained from normal tissue.

20. An organoid according to any one of claims 17-19 in a culture medium according to any one of claims 4-15.

21. Use of an organoid as defined in any one of claims 17-19, or a cell derived from said organoid, in a drug discovery screen; toxicity assay; research of tissue embryology, cell lineages, or differentiation pathways; gene expression studies including recombinant gene expression; research of mechanisms involved in tissue injury or repair; research of inflammatory and infectious diseases; studies of pathogenetic mechanisms; or studies of mechanisms of cell transformation or aetiology of cancer.

22. An organoid according to any one of claims 17-19, or a cell derived from said organoid, for use in medicine.

23. A culture medium comprising a p53 stabilising agent.

24. The method according to claim 16, wherein the epithelial stem cells are lung epithelial stem cells, and wherein the receptor tyrosine kinase ligand is one or more FGFR2b ligands.

25. The method of claim 24, wherein the one or more FGFR2b ligands is FGF7 and/or FGF10.

26. The method of claim 24 or claim 25, wherein the culture medium further comprises one or more components selected from the group consisting of: B27, N-acetylcysteine, Nicotinamide, a ROCK inhibitor, a TGF-beta inhibitor and a p38 inhibitor.

27. The method of claim 24 or claim 25, wherein the culture medium further comprises: B27, N-acetylcysteine, Nicotinamide, a ROCK inhibitor, a TGF-beta inhibitor and a p38 inhibitor.

28. The method of any one of claims 24-27, wherein the culture medium further comprises one or more further receptor tyrosine kinase ligands.

29. The method of claim 28, wherein the one or more further receptor tyrosine kinase ligands are EGF and/or amphiregulin.

30. The method of any one of claims 1-3, 16 or 24-29, wherein the method further comprises a step of replacing the culture medium with a culture medium that does not comprise an ErbB3/4 ligand.

31. A lung organoid which comprises a population of lung epithelial stem cells, wherein the lung organoid is obtainable by or obtained by a method of any one of claims 24-30.

32. A lung organoid which comprises a population of lung epithelial stem cells, wherein the lung organoid further comprises ciliated cells.

33. The lung organoid of claim 31, wherein the lung organoid comprises ciliated cells.

34. The lung organoid of claim 32 or claim 33, wherein the ciliated cells are moving synchronously.

35. The lung organoid of any one of claims 31-34, wherein the lung organoid is a human lung organoid.

36. The lung organoid of any one of claims 31-35 obtained from normal lung tissue, primary lung cancer or metastatic lung cancer which has been passaged for at least 4 passages.

37. Use of a lung organoid as defined in any one of claims 31-36, or a cell derived from said organoid, in a drug discovery screen; drug screening; personalized medicine; a toxicity assay; research of tissue embryology, cell lineages, or differentiation pathways; gene expression studies including recombinant gene expression; research of mechanisms involved in tissue injury or repair; research of inflammatory and infectious diseases; studies of pathogenetic mechanisms; or studies of mechanisms of cell transformation or aetiology of cancer.

38. A lung organoid according to any one of claims 31-36, or a cell derived from said organoid, for use in therapy.

39. A lung organoid according to any one of claims 31-36, or a cell derived from said organoid, for use in diagnosis.

40. A method for studying the effectiveness of one or more drugs for treating a pulmonary viral infection, wherein the method comprises:

stimulating one or more pulmonary virus-infected organoids with the one or more drugs and

measuring the change in motility of the one or more lung organoids.

41. The method of claim 40, wherein the method further comprises measuring the change in incidence of fused organoids and/or the change in incidence of organoids with a mesenchymal-like phenotype.

42. A method for studying the effectiveness of one or more drugs for treating a disease, wherein the method comprises:

stimulation of one or more disease organoids with said one or more drugs, and measuring the change in motility of epithelial cells in the organoids by measuring (a) the change in incidence of fused organoids, (b) the change in rotation of organoids, (c) the change in motility of organoids and/or (d) the change in incidence of cells with a mesenchymal-like phenotype,

and correlating a change in motility of epithelial cells in the organoids with drug efficacy.

43. The method of any one of claims 40-42, wherein the change in motility of organoids, the change in incidence of fused organoids, change in rotation of organoids and/or the change in incidence of organoids with a mesenchymal-like phenotype is compared to: (i) a healthy control organoid and/or (ii) a an organoid that has not been stimulated with the one or more drugs.

44. The method of any one of claims 40-43, wherein the pulmonary viral infection is an RSV infection.

45. The method of any one of claims 40-44, wherein the one or more lung organoids are lung organoids according to any one of claims 31-36.