

43. Faria, D. et al. The calcium-activated chloride channel Anoctamin 1 contributes to the regulation of renal function. *Kidney Int* **85**, 1369-81 (2014).
44. Schroeder, B. C., Cheng, T., Jan, Y. N. & Jan, L. Y. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. *Cell* **134**, 1019-29 (2008).
45. Zhu, G., Zhang, Y., Xu, H. & Jiang, C. Identification of endogenous outward currents in the human embryonic kidney (HEK 293) cell line. *J Neurosci Methods* **81**, 73-83 (1998).
46. Neher, E. Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol* **207**, 123-31 (1992).
47. Ye, W. et al. The K⁺ channel KIR2.1 functions in tandem with proton influx to mediate sour taste transduction. *Proc Natl Acad Sci U S A* **113**, E229-38 (2016).
48. Perriere, G. & Gouy, M. WWW-query: an on-line retrieval system for biological sequence banks. *Biochimie* **78**, 364-9 (1996).
49. Wang, Y. Y., Chang, R. B., Waters, H. N., McKemy, D. D. & Liman, E. R. The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J Biol Chem* **283**, 32691-703 (2008).

Example 8 – Otopetrin Sequences

[0084] Human – OTO1 (SEQ ID NO:1):

MLEGLGSPASPRAAASASVAGSSGPAACSPSSAPRSPESPAPRRGGVRASVPQKLA
 EMLSSQYGLIVFVAGLLLLLAWAVHAAGVSKSDLLCFLTALMLLQLLWMLWYVGR
 SSAHRRLFRLKDTAGAGWLRGSITLFAVITVILGCLKIGYFIGFSECLSATEGVFPVT
 HSVHTLLQVYFLWGHAKDIIQSFKTLERFGVIHSVFTNLLWANGVLNESKHQLNEH
 KERLITLGFGNITTVLDDHTPQCNCPTLCTAISHGIYYLYPFNIEYQILASTMLYVL
 WKNIGRKVDSSHQHQKMQFKSDGVMVGAVLGLTVLAATIAVVVVYLIHIGRSKTKS
 ESALIMFYLYAITLLMLMGAAGLAGIRIYRIDEKSLDESKNPARKLDSDLLVGTASGS
 WLISWGSILAILCAEGHPRYTWYNLPYSILAIVEKYIQNLFIFESIHREPEKLSEDIQTLR
 VVTVCNGNTMPLASSCPKSGGVARDVAPQGKDMPPAANGNVCMRESHDKKEEEKQE
 ESSWGGSPSPVRLPRFLQGNARKKVLNRNIAAFLFLCNISLWIPPAFGCRPEYDNGLEEI
 VFGFEPWIIVVNLAMPFSIFYRMHAAASLFEVYCKI

CLAIMS

1. A method of identifying a modulator of otopetrin-mediated proton translocation activity comprising:
 - (a) contacting an otopetrin polypeptide, or functional portion thereof, or host cell expressing said polypeptide with a test compound; and
 - (b) determining a proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof;wherein an increase or decrease of at least 1% of the proton translocation activity, compared to an amount of proton translocation activity determined in an absence of the test compound, identifies the test compound as a modulator of the proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof.
2. The method of claim 1, wherein:
 - the otopetrin polypeptide comprises a mammalian otopetrin polypeptide, or functional portion thereof, wherein the mammalian otopetrin polypeptide comprises a polypeptide sequence selected from Otop1, Otop2, Otop3, and functional portions thereof;
 - the otopetrin polypeptide comprises an insect otopetrin polypeptide, or functional portion thereof;
 - the otopetrin polypeptide, or the functional portion thereof is associated with a cell membrane or synthetic membrane; and/or
 - the otopetrin polypeptide is: at least 80%; at least 85%; at least 90%; at least 95%; at least 96%; at least 97%; at least 98%; at least 99%; or 100%; identical to an otopetrin polypeptide sequence of any one of SEQ ID Nos:1-9.
3. The method of claim 1 or claim 2, wherein determining the proton translocation activity comprises: measuring conductivity, current, resistance, voltage potential across a membrane comprising the otopetrin polypeptide, or functional portion thereof; or intracellular pH or intracellular calcium within a host cell expressing an otopetrin polypeptide, or functional portion thereof.

4. The method of any one of claims 1 to 3, wherein determining the proton translocation activity comprises expressing the otopetrin polypeptide, or functional portion thereof in a cell, wherein:

the cell is a mammalian cell;

the cell is an oocyte; and/or

the cell is a HEK-293 cell or a CHO cell.

5. The method of any one of claims 1 to 4, wherein the test compound is selected from the group consisting of a small organic molecule, small inorganic molecule, electrophile, polysaccharide, peptide, protein, antibody, nucleic acid, and an extract, wherein the extract is from a biological material is selected from a bacteria, plant, fungi, animal cell, and animal tissue.

6. A method of identifying a modulator of otopetrin-mediated proton translocation activity comprising:

(a) measuring a proton translocation activity mediated by an otopetrin polypeptide, or a functional portion thereof, (i) in the presence of a test compound, and (ii) in the absence of the test compound; and

(b) determining a difference between the proton translocation activity measured in (a)(i) compared to the proton translocation activity measured in (a)(ii), thereby identifying the test compound as a modulator of the proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof.

7. The method of claim 6, wherein:

the otopetrin polypeptide comprises a mammalian otopetrin polypeptide, or functional portion thereof;

the mammalian otopetrin polypeptide comprises a polypeptide sequence selected from a murine or human Otop1, Otop2, Otop3, and functional portions thereof;

the otopetrin polypeptide comprises an insect otopetrin polypeptide, or functional portion thereof;

the otopetrin polypeptide, or the functional portion thereof is associated with, or integrated in a cell membrane or synthetic membrane; and/or

the otopetrin polypeptide is: at least 80%; at least 85%; at least 90%; at least 95%; at least 96%; at least 97%; at least 98%; at least 99%; or 100%; identical to an otopetrin polypeptide sequence of any one of SEQ ID Nos:1-9.

8. The method of claim 6 or claim 7, wherein:

measuring the proton translocation activity comprises measuring conductivity, current, voltage potential or resistance across a membrane comprising the otopetrin polypeptide, or functional portion thereof; and/or

measuring the proton translocation activity comprises measuring conductivity, current, intracellular pH, intracellular calcium, or voltage potential or resistance across a membrane comprising the otopetrin polypeptide, or functional portion thereof in response to a change in pH.

9. The method of any one of claims 6 to 8, the method comprising inducing a change in pH on one side of the otopetrin polypeptide, wherein:

the change in pH is an extracellular change in pH;

the change in pH is an increase in pH;

the change in pH is a decrease in pH; and/or

the change in pH is at least 0.1 pH units.

10. The method of any one of claims 6 to 9, wherein:

the heterologous or homologous cell is a mammalian cell; and/or

the heterologous or homologous cell is an oocyte.

11. The method of any one of claims 6 to 10, wherein the test compound is selected from the group consisting of a small organic molecule, small inorganic molecule, polysaccharide, peptide, protein, antibody, nucleic acid, and an extract, wherein the extract is from a biological material is selected from a bacteria, plant, fungi, animal cell, and animal tissue.

12. A system for sensing proton translocation activity through a membrane channel, the system comprising:

one or more heterologous otopetrin polypeptides integrated into a membrane of a cell, wherein the cell comprises one or more nucleic acids that direct the expression of the one or more heterologous otopetrin polypeptides and the cell comprises a fluorescent pH indicator or a fluorescent calcium indicator, ~~optionally~~ wherein:

the cell is an immortalized cell;

the cell is HEK-293 or Cos-7;

the one or more heterologous otopetrin polypeptides are selected from Otop1, Otop2 and Otop3 or functional portions thereof;

the otopetrin polypeptide is: at least 80%; at least 85%; at least 90%; at least 95%; at least 96%; at least 97%; at least 98%; at least 99%; or 100%; identical to an otopetrin polypeptide sequence of any one of SEQ ID Nos:1-9;

the cell is transfected with a fluorescent reporter;

the cell is loaded with a fluorescent reporter;

the fluorescent reporter is pHluorin;

the fluorescent pH indicator is selected from pHrodo RED, DFFDA, and BCECF, and/or the fluorescent calcium indicator is Fura 2 AM or Fluo-4 or Fluo-3.

13. The system of claim 13, further comprising a fluorescent-imaging plate reader (FLIPR); and/or

a test compound, wherein the test compound is in contact with the one or more otopetrin polypeptides.

14. A cell comprising a heterologous otopetrin polypeptide, wherein:

the otopetrin polypeptide is selected from Otop1, Otop2 and Otop3;

the otopetrin polypeptide is: at least 80%; at least 85%; at least 90%; at least 95%; at least 96%; at least 97%; at least 98%; at least 99%; or 100%; identical to an otopetrin polypeptide sequence of any one of SEQ ID Nos:1-9;

the cell is transfected with nucleic acid directing the expression of the heterologous otopetrin polypeptide;

the cell is an insect cell or a reptilian cell and the heterologous otopetrin polypeptides are mammalian otopetrin polypeptides; and/or

the cell is a non-human cell and the heterologous otopetrin polypeptides are human otopetrin polypeptides.

15. A synthetic membrane or synthetic lipid bilayer comprising an otopetrin polypeptide.

16. The method of claim 1, wherein:

the method comprises comparing the proton translocation activity determined in (b) to a proton translocation activity determined for the otopetrin polypeptide, or functional portion thereof, in the absence of the test compound;

an increase of at least 1% of the proton translocation activity indicates the test compound is an agonist of the proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof; or

a decrease of at least 1% of the proton translocation activity indicates the test compound is an antagonist of the proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof.

17. The method of claim 3, wherein:

the conductivity, voltage potential, current, resistance, intracellular pH, intracellular calcium, or the is measured in response to a change in pH;

determining the proton translocation activity comprises a patch clamp technique, voltage clamp technique, measurement of whole cell current, or use of a pH sensitive indicator or calcium-sensitive indicator; or

the pH sensitive indicator comprises pHluorin or pHrodo Red, DFFDA, and BCECF

the calcium-sensitive indicator comprises Fura-2, Flou-4 or Flou-3

the change in pH is an extracellular change in pH;

the change in pH is a change in intracellular pH

the change in pH is an increase in pH;

the change in pH is a decrease in pH; and/or

the change in pH is at least 0.1 pH units.

18. The method of claim 5, wherein:

the test compound has a molecular weight in a range of 50 to 500,000 Daltons;

the test compound is at a concentration in a range of: 1 pM to 100 mM; 1 pM to 10 mM; 1 pM to 1 mM; 10 pM to 100 mM; 10 pM to 10 mM; 10 pM to 1 mM; 100 pM to 100 mM; 100 pM to 10 mM; or 100 pM to 1 mM; or

the test compound is at a concentration of: at least 1 pM; at least 10 pM; at least 100 pM; at least 1 nM; at least 10 nM; at least 100 nM; at least 1 μ M; at least 10 μ M; at least 100 μ M; or at least 1 mM.

19. The method of claim 6, wherein:

the difference is at least a difference of 1%;

the proton translocation activity measured in (a)(i) is at least 1% greater than the proton translocation activity measured in (a)(ii) and the test compound is identified in (b) as an agonist of the proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof;

the proton translocation activity measured in (a)(i) is at least 1% less than the proton translocation activity measured in (a)(ii) and the test compound is identified in (b) as an antagonist of the proton translocation activity mediated by the otopetrin polypeptide, or the functional portion thereof;

measuring the proton translocation activity comprises a patch clamp technique, measurement of whole cell currents, two-electrode voltage clamping, or a fluorescence assay using a voltage-sensitive dye; or

measuring the proton translocation activity comprises expressing the otopetrin polypeptide, or functional portion thereof in a heterologous or homologous cell.

20. The method of claim 11, wherein:

the test compound has a molecular weight in a range of 50 to 500,000 Daltons;

the test compound is at a concentration in a range of: 1 pM to 100 mM; 1 pM to 10 mM; 1 pM to 1 mM; 10 pM to 100 mM; 10 pM to 10 mM; 10 pM to 1 mM; 100 pM to 100 mM; 100 pM to 10 mM; or 100 pM to 1 mM; or

the test compound is at a concentration of: at least 1 pM; at least 10 pM; at least 100 pM; at least 1 nM; at least 10 nM; at least 100 nM; at least 1 μ M; at least 10 μ M; at least 100 μ M; or at least 1 mM.