Variations of chromosomes 2 and 3 gene expression profiles among pulmonary telocytes, pneumocytes, airway cells, mesenchymal stem cells and lymphocytes

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Abstract

Telocytes (TCs) were identified as a distinct cellular type of the interstitial tissue and defined as cells with extremely long telopodes (Tps). Our previous data demonstrated patterns of mouse TC-specific gene profiles on chromosome 1. The present study focuses on the identification of characters and patterns of TC-specific or TC-dominated gene expression profiles in chromosome 2 and 3, the network of principle genes and potential functional association. We compared gene expression profiles of pulmonary TCs, mesenchymal stem cells, fibroblasts, alveolar type II cells, airway basal cells, proximal airway cells, CD8+ T cells from bronchial lymph nodes (T-BL), and CD8+ T cells from lungs (T-LL). We identified that 26 or 80 genes of TCs in chromosome 2 and 13 or 59 genes of TCs up- or down-regulated in chromosome 3, as compared with other cells respectively. Obvious overexpression of Myl9 in chromosome 2 of TCs different from other cells, indicates that biological functions of TCs are mainly associated with tissue/organ injury and ageing, while down-expression of Pltp implies that TCs may be associated with inhibition or reduction of inflammation in the lung. Dominant overexpression of Sh3glb1, Tm4sf1 or Csf1 in chromosome 3 of TCs is mainly associated with tumour promotion in lung cancer, while most down-expression of Pde5 may be involved in the development of pulmonary fibrosis and other acute and chronic interstitial lung disease.

Keywords: TCs • mesenchymal stem cells • fibroblasts • chromosome 2 • chromosome 3 • lung

Introduction

Telocytes (TCs) were first described as a distinct cell type in 2010 [1, 2], followed by a significant growing of research globally [3, 4], as detailed in www.telocytes.com. TCs were found in multiple tissues and organs, such as heart [5–7], kidney [8] and urinary tract [9, 10], skin [11, 12] eye [13], mammary gland [14], digestive tract [15, 16], skeletal muscles [17] and neuromuscular spindles [18], uterus [19–22] and placenta [2], liver [23] and gall bladder [24, 25], pleura [26], trachea [27] and lungs [28]. TCs are mainly recognized and characterized by electron microscopy, the only technique able to highlight their characteristic extensions – the telopodes (Tps), consisting of thin segments – podomers, alternating with dilated regions – podoms [1]. Other characteristics of Tps include: (i) the unusual and varied
length, between tens and thousands of micrometres; (ii) the branch- 
ing network pattern, forming a labyrinthine system; (iii) the communi-
cations through homo- and heterocellular junctions exosome and
ectosome release [6, 20, 22]. TCS were found to link nerve fibres,
blood vessels, secretory acini and exocrine epithelial ducts [29–31],
and different cell types, e.g. macrophages, lymphocytes, mast cells,
stem cells [32–34]. TCS form 3-dimensional networks within organs/
tissues [20, 35]. Networks integrity may be affected in many patho-
logical conditions, such as systemic sclerosis [36], skin basal and
squamous cell carcinomas [37] and Crohn’s disease [38].
Telocytes differ from fibroblasts (Fs) and mesenchymal stem
(cells (MSCs) as demonstrated by miR signatures and genetic profiles
[15, 39, 40]. Proteomic signatures of the TCS are also supportive for
the uniqueness and helpful in understanding of the functions. The
data from omics studies demonstrated that elements within TCS are
involved in (i) intercellular signalling, (ii) mechanical sensing and
mechanochanical conversion task, (iii) tissue homeostasis and
remodelling/renewal, (iv) anti-oxidative stress and anti-ageing cellular
mechanisms, (v) cancer cell proliferation through the inhibition of
apoptosis [40, 41]. Our recent work explored patterns of mouse TC-
specific gene profiles on chromosome 1 and showed important roles
for TCS in the prevention of tissue inflammation and fibrogenesis,
development of lung inflammatory diseases or modulation of immune
cell responses [42]. However, dominant patterns and specificity of
gene and protein profiles of TCS which are different from other cells
existed in the lung is still not completed and unclear.
The present study undertakes an in-depth analysis to find out the
characters and patterns of TC-specific or TC-dominated gene expres-
sion profiles in chromosomes 2 and 3, investigate the network of
principle genes, and explore potential functional association.
Comparisons are made among pulmonary TCS, MSCs, Fs, alveolar
type II cells (ATII), airway basal cells (ABCs), proximal airway cells
(PACs), CD8+ T cells from bronchial lymph nodes (TBL) and CD8+ T
cells from lung (T-L), which may interact with TCS in the lung and
trachea. Furthermore, we applied the most complete reference library
of the National Center for Biotechnology Information (NCBI) Gene
Expression Omnibus database to identify key functional genes, and
characteristic networks by bioinformatics tools.

### Material and methods

#### Isolation and culture

Telocytes were isolated from the lung tissues of mice, primary
cultured in a concentration of $1 \times 10^6$ cells/cm², and harvested on
days 5 (TC D5) and on days 10 (TC D10), as described previously
[43]. RNA isolation, preparation, labelling, and hybridization were per-
formed for DNA microarray (The Mouse 4 × 44K Gene Expression
Array, Agilent, Shanghai, China). About 39,000+ mouse genes and
transcripts represented with public domain annotations were gained,
according to the protocol of One-Color Microarray-Based Gene
Expression Analysis. The hybridized arrays were washed, fixed and
scanned by the Agilent DNA Microarray Scanner (part number
G2505B).

#### Data collection and mining

We selectively collected gene expression profiles of pulmonary TCS on
days 5 (TC D5) and 10 (TC D10), Fs, MSCs, from our study [43], ATII,
ABCs, PACs, T-BL and T-L, from the NCBI Gene Expression Omnibus
database (GSE6846 [44], GSE27379 [45], GSE28651 [46]). The micro-
array was composed of 45,101 probes. We eliminated the probe sets
without corresponding official symbol, leaving 39,417 probes and
21,680 genes.
The gene expression profiles are from our earlier study, which are
composed of 23,861 probes, of pulmonary TCS on days 5 and 10, Fs
and MSCs are composed of 23,861 probes [43]. There were 13,236
probes and 11,545 genes after further eliminating the probes without
corresponding official symbol, which we focused on in the present
study. From the total of 11,545 genes, 917 genes of the chromosome 2
and 567 genes of the chromosome 3 were analysed.

#### Identification of differentially expressed genes

There are about 20,000–25,000 genes in mouse, of which about 85%
are similar with humans, and the propensity of functional changes was
reflected in different levels of the gene expression in particular cell
types. We used gene expression profiles between mouse lung cells to
seek for the specific regulated and identify genes specific to TCS and
their function. The fold change was utilized to identify differentially
expressed genes or simply differential genes. Up- or down-
regulated folds of TCS genes were calculated as compared with other cells
and subtracted its own multiple of TCS, after the average of gene expression
in each cell was obtained from the raw data of multi-databases, as
shown in Data S1.

#### Results

The final data analysis by bioinformatics tools showed that in
chromosome 2, 26 genes were overexpressed in TCS, as compared
with those in other cells (Table 1). Among them, 20 genes
(11110008F13Rik, 2310003F16Rik, 2900064A13Rik, Ab1, Ass1, Com-
md3, Commd7, Creb3l1, Dlgap4, Edf1, Id1, Manbal, Mocs3, Psmc3,
Sdcaq3, Sclc9a13, Sna1, Spc25, Tubb2c, Srxn1) were overexpres-
sed between 0 and 1 folds (Table 1A). Four genes, Dbd2d (Dysbin-
din domain-containing protein 2), Fbn1 (fibrillin 1), Tipi (tissue factor
pathway inhibitor) and Ak1 (adenylate kinase 1) genes, were over-
expressed one- to fourfold, in both TC D5 and TC D10, as compared
with other cells (Table 1B). Highest overexpressed gene, Myf9 (myo-
sin, light chain 9), was over fourfold up-regulated in both TC D5 and
TC D10 compared with other cells (Table 1C). 80 genes in TCS were
down-regulated, as compared with other cells (Table 2). Of them,
Gzfl, Ptp, Poir1b, Tasp1, Zbtb12 and Zbtb34 and Zfp120 were down-regulated
more than onefold in TCs compared with other cells.
A set of genes are specifically up- or down-regulated in pulmonary
TCS, as compared with other cells in chromosome 2 (Table 3), up-
or down-regulated genes more than 0-fold of TCS D5 were 576 or 341,
559 or 358, 228 or 689, 287 or 630, 277 or 640, 181 or 736, or 210
or 707, respectively, as compared with MSCs, Fs, ATII, T-BL, T-L,
ABCs, or PACs. Up- or down-regulated genes more than 0-fold of TCS
Table 1 Summary of genes expressed preferentially in TCs, as compared with others

| Compared pairs/fold up-regulated | >0 | >1 | >4 |
|----------------------------------|----|----|----|
| TC5 versus others                | 64 | 18 | 2  |
| TC10 versus others               | 56 | 13 | 4  |
| TCs versus others                | 26 | 6  | 2  |

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|---------------------------------------------|
|             | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| (A) Genes up-regulated between 0- and 1-folds in TCs as compared with others |
| 1110008F13Rik | −0.41/−0.18 | −0.46/−0.25 | −0.73/−0.72 | −0.79/−0.80 | −0.85/−0.85 | −0.91/−0.91 | −0.91/−0.92 |
| 2310003F16Rik | −0.40/−0.04 | −0.49/−0.19 | −0.97/−0.97 | −0.52/−0.46 | −0.56/−0.50 | −0.82/−0.80 | −0.86/−0.84 |
| 2900064A13Rik | −0.13/−0.21 | −0.41/−0.46 | −0.37/−0.58 | −0.29/−0.54 | −0.30/−0.54 | −0.24/−0.51 | −0.19/−0.47 |
| Abi1         | −0.75/−0.67 | −0.43/−0.23 | −0.76/−0.76 | −0.29/−0.32 | −0.70/−0.71 | −0.82/−0.83 | −0.89/−0.90 |
| Ass1         | −0.44/−0.34 | −0.96/−0.95 | −0.82/−0.84 | −0.94/−0.95 | −0.80/−0.83 | −0.90/−0.92 | −0.80/−0.84 |
| Commd3       | −0.63/−0.13 | −0.63/−0.54 | −0.56/−0.62 | −0.78/−0.59 | −0.76/−0.60 | −0.13/−0.34 | −0.24/−0.65 |
| Commd4       | −0.29/−0.56 | −0.62/−0.91 | −0.58/−0.49 | −0.53/−0.99 | −0.54/−0.99 | −0.24/−0.87 | −0.60/−0.98 |
| Creb31       | −0.65/−0.66 | −0.93/−0.44 | −0.46/−0.35 | −0.99/−0.18 | −0.99/−0.52 | −0.86/−0.25 | −0.88/−0.29 |
| Dlgap4       | −0.79/−0.98 | −0.65/−0.96 | −0.44/−0.77 | −0.28/−1.00 | −0.58/−0.96 | −0.34/−0.37 | −0.38/−0.71 |
| Edf1         | −0.44/−0.17 | −0.40/−0.08 | −0.51/−0.73 | −0.73/−0.76 | −0.81/−0.61 | −0.26/−0.28 | −0.26/−0.66 |
| Id1          | −0.98/−0.70 | −0.97/−0.03 | −0.74/−0.20 | −0.99/−0.50 | −0.95/−0.45 | −0.27/−0.63 | −0.66/−0.59 |
| Manbal       | −0.49/−0.51 | −0.43/−0.43 | −0.77/−0.37 | −0.79/−0.19 | −0.66/−0.19 | −0.37/−0.20 | −0.71/−0.25 |
| Mocs3        | −0.81/−0.53 | −0.38/−0.02 | −0.30/−0.90 | −0.55/−0.95 | −0.51/−0.95 | −0.67/−0.55 | −0.63/−0.57 |
| Psmc3        | −0.64/−0.46 | −0.58/−0.20 | −0.34/−0.29 | −0.17/−0.58 | −0.18/−0.36 | −0.18/−0.67 | −0.23/−0.93 |
| Sdccag3      | −0.44/−0.46 | −0.48/−0.60 | −0.76/−0.37 | −0.77/−0.98 | −0.74/−0.94 | −0.73/−0.45 | −0.67/−0.18 |
| Slc39a13     | −0.51/−0.59 | −0.28/−0.77 | −0.13/−0.42 | −0.46/−0.73 | −0.20/−0.75 | −0.58/−0.72 | −0.91/−0.80 |
| Snai1        | −0.84/−0.26 | −0.37/−0.15 | −0.92/−0.67 | −0.93/−0.74 | −0.95/−0.58 | −0.95/−0.40 | −0.97/−0.59 |
| Spc25        | −0.41/−0.37 | −0.24/−0.40 | −0.79/−0.91 | −0.61/−0.91 | −0.41/−0.91 | −0.19/−0.58 | −0.07/−0.85 |
| Tubb2c       | −0.73/−0.59 | −0.84/−0.48 | −0.47/−0.40 | −0.74/−0.90 | −0.77/−0.89 | −0.73/−0.57 | −0.81/−0.74 |
| Srxn1        | −0.49/−0.68 | −0.71/−0.30 | −0.32/−0.86 | −0.96/−0.85 | −0.93/−0.83 | −0.69/−0.86 | −0.41/−0.93 |

(B) Genes up-regulated between 1- and 4-folds in TCs as compared with other

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|---------------------------------------------|
|             | DBMD2 | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| Dbndd2      | −0.61/−1.00 | −0.66/−0.98 | −0.90/−0.73 | −0.96/−0.96 | −0.95/−0.96 | −0.83/−0.87 | −0.81/−0.90 |
| Fbn1        | −0.95/−0.98 | −0.84/−0.53 | −0.89/−0.84 | −0.95/−0.82 | −0.95/−0.84 | −0.66/−0.87 | −0.89/−0.76 |
| Tp1p        | −0.60/−0.72 | −0.94/−0.58 | −0.85/−0.85 | −0.66/−0.95 | −0.73/−0.96 | −0.84/−0.78 | −0.82/−0.86 |
| Ak1         | −0.80/−0.74 | −0.84/−0.96 | −0.69/−0.93 | −1.00/−0.85 | −0.97/−0.87 | −0.94/−0.93 | −0.97/−0.92 |

(C) Genes up-regulated between >4-folds in TCs as compared with others

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|---------------------------------------------|
| Myl9        | −1.00/−0.96 | −0.92/−0.91 | −0.98/−0.92 | −1.00/−0.96 | −1.00/−0.98 | −0.98/−0.88 | −1.00/−0.94 |
Table 2: Summary of genes expressed preferentially in TCs, as compared with others

| Compared pairs/fold down-regulated | >0   | >1   | >4   |
|-----------------------------------|------|------|------|
| TC5 versus others                | 140  | 14   | 0    |
| TC10 versus others               | 236  | 38   | 0    |
| TCs versus others                | 80   | 6    | 0    |

| Gene symbol    | Folds (TC5 versus others/TC10 versus others) | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
|----------------|---------------------------------------------|------------|------|------|----------|----------|------------|-----------|
| 1500012F01Rik  | 1.00/2.24                                  | 2.51/4.68  | 0.09/0.29 | 0.83/1.11 | 1.30/1.68 | 2.50/3.01 | 4.17/4.95 |
| 160027N09Rik   | 0.02/0.26                                  | 0.24/0.53  | 3.26/2.85 | 5.59/4.77 | 6.52/5.68 | 6.23/5.32 | 6.80/5.85 |
| 1700058C13Rik  | 0.01/0.29                                  | 0.03/0.31  | 6.00/5.54 | 6.63/5.93 | 15.2/13.91 | 7.23/6.46 | 4.82/4.30 |
| 2010317E24Rik  | 0.71/2.34                                  | 1.58/4.04  | 0.03/0.47 | 0.23/0.70 | 1.38/2.34 | 10.45/14.81 | 10.14/14.46 |
| 2810408M09Rik  | 1.13/1.13                                  | 0.66/0.66  | 20.58/14.77 | 1.23/0.58 | 14.91/10.44 | 65.85/46.34 | 29.46/20.69 |
| 4921504E06Rik  | 0.16/0.37                                  | 0.31/0.55  | 6.25/5.27 | 0.87/0.57 | 8.86/7.40 | 11.69/5.56 | 9.10/7.51 |
| 6820408C15Rik  | 0.02/0.06                                  | 0.54/0.61  | 99.87/75.87 | 8.76/6.22 | 28.01/20.77 | 26.11/19.03 | 17.2/12.51 |
| Abca2          | 0.77/1.04                                  | 0.51/0.73  | 11.84/9.81 | 5.21/4.07 | 6.05/4.84 | 8.82/7.01 | 6.99/5.55 |
| Acvr2a         | 1.31/1.33                                  | 0.42/0.43  | 23.28/16.86 | 13.98/9.7 | 25.29/18.05 | 84.04/59.65 | 70.93/50.56 |
| Angpt2         | 0.52/0.45                                  | 1.97/0.31  | 16.78/0.65 | 9.96/1.22 | 5.33/2.03 | 14.94/3.34 | 20.00/1.38 |
| Api5           | 0.06/0.28                                  | 0.40/1.50  | 3.39/9.94 | 6.09/5.56 | 4.92/2.84 | 3.10/8.51 | 3.11/11.60 |
| Arhgap1        | 0.30/0.39                                  | 0.05/0.84  | 1.45/3.21 | 1.27/5.61 | 0.44/4.59 | 4.67/2.81 | 3.64/2.84 |
| Arpc5I         | 1.84/1.17                                  | 0.11/0.75  | 1.81/1.99 | 9.73/1.70 | 7.56/0.73 | 9.55/5.73 | 5.43/4.53 |
| Atr2           | 0.15/2.57                                  | 0.26/0.39  | 2.39/1.58 | 7.92/8.55 | 3.92/6.73 | 8.87/8.38 | 5.91/4.74 |
| B2m            | 1.13/0.44                                  | 0.57/1.06  | 2.50/1.63 | 4.18/3.06 | 4.50/2.19 | 2.32/1.70 | 1.22/1.32 |
| Catsper2       | 1.51/0.83                                  | 0.31/1.01  | 14.79/2.94 | 24.41/9.06 | 27.92/4.63 | 19.7/10.11 | 15.59/6.82 |
| Cbfa2T2        | 0.21/0.82                                  | 0.43/0.34  | 0.27/1.18 | 0.97/2.13 | 0.86/2.37 | 0.60/1.00 | 0.28/0.35 |
| Cdc7           | 1.89/1.49                                  | 6.28/0.30  | 1.05/10.46 | 1.2/16.91 | 0.10/19.66 | 2.18/13.55 | 2.49/10.73 |
| Cep110         | 1.61/0.62                                  | 0.28/0.92  | 0.84/0.24 | 7.14/0.87 | 4.90/0.79 | 5.53/0.51 | 2.91/0.22 |
| Chcd5          | 0.45/3.93                                  | 0.13/11.43 | 6.34/1.55 | 3.16/1.67 | 2.13/0.35 | 12.54/2.84 | 10.66/3.24 |
| Ciz1           | 0.30/2.69                                  | 0.21/0.81  | 1.53/0.90 | 0.84/7.16 | 0.68/4.99 | 7.8/5.53 | 4.74/2.93 |
| Cry2           | 0.11/1.18                                  | 0.15/0.69  | 13.77/0.05 | 4.83/3.43 | 2.42/2.38 | 10.9/13.88 | 11.47/11.46 |
| Ddx27          | 0.42/0.74                                  | 0.44/0.62  | 0.20/1.47 | 1.27/0.74 | 1.09/0.61 | 5.47/7.32 | 6.52/4.46 |
| Ddx31          | 0.31/0.36                                  | 0.93/0.91  | 0.51/2.65 | 1.79/0.87 | 0.75/9.21 | 5.42/17.28 | 5.09/13.41 |
| Depdc7         | 1.34/0.33                                  | 0.42/0.38  | 13.2/11.89 | 0.67/3.96 | 2.85/1.96 | 2.96/9.11 | 33.03/9.65 |

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| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|-------------------------------------------|
|             | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| Dsn1        | 0.61/0.26  | 1.33/0.31 | 6.39/6.62 | 39.29/12.48 | 47.16/10.54 | 12.99/6.54 | 23.88/5.26 |
| Elp4        | 0.76/1.09  | 0.25/1.12 | 5.62/0.30 | 1.29/1.37 | 1.03/1.21 | 14.19/5.74 | 10.08/6.88 |
| Ext2        | 0.44/0.46  | 0.68/1.16 | 23.82/0.23 | 31.23/1.21 | 21.21/0.41 | 20.92/4.07 | 11.20/3.83 |
| Fmn12       | 0.18/1.37  | 0.15/0.44 | 11.91/9.50 | 5.60/0.20 | 12.75/1.80 | 15.11/1.84 | 21.28/23.52 |
| Fupb3       | 0.04/0.99  | 0.42/1.88 | 14.72/5.69 | 34.28/34.4 | 32.79/41.90 | 93.41/11.27 | 73.83/20.93 |
| Gapvd1      | 0.04/0.18  | 0.09/3.47 | 3.56/5.04 | 5.04/13.37 | 3.84/8.91 | 3.82/4.01 | 3.24/2.43 |
| Gmeb2       | 0.30/1.39  | 0.70/0.70 | 15.73/5.58 | 17.11/1.21 | 7.65/0.98 | 2.14/13.63 | 2.63/9.73 |
| Hat1        | 0.56/0.99  | 0.09/1.32 | 12.93/24.06 | 49.03/30.61 | 46.29/21.08 | 26.19/20.45 | 29.7/11.00 |
| Il15ra      | 1.42/0.59  | 0.07/0.54 | 2.25/11.67 | 0.64/5.29 | 2.21/12.29 | 4.80/14.33 | 1.58/20.32 |
| Mapkb1      | 0.03/0.18  | 0.13/2.25 | 96.09/4.23 | 25.15/33.87 | 22.48/30.68 | 123.55/25.01 | 263.85/15.93 |
| Mga         | 0.68/0.24  | 0.34/0.68 | 4.24/12.63 | 4.57/28.72 | 5.14/27.86 | 8.89/78.35 | 7.84/62.23 |
| Mkks        | 0.26/0.64  | 0.25/0.71 | 1.66/4.26 | 3.04/5.77 | 2.84/4.51 | 6.40/4.39 | 6.51/3.77 |
| Mll10       | 0.11/1.18  | 0.06/0.54 | 5.21/2.72 | 32.27/10.63 | 27.76/16.87 | 15.03/12.57 | 12.67/9.62 |
| Mrps5       | 0.30/0.37  | 0.29/0.79 | 2.85/11.87 | 3.12/12.53 | 1.15/5.55 | 16.43/1.34 | 8.73/1.72 |
| Ncabi3      | 3.74/0.72  | 0.49/0.20 | 3.72/10.21 | 2.23/38.12 | 6.28/36.48 | 11.85/20.21 | 3.01/23.07 |
| Nr6a1       | 0.87/2.71  | 0.40/0.63 | 18.84/2.64 | 14.87/0.78 | 18.42/2.54 | 41.84/5.30 | 67.71/1.82 |
| Ntng2       | 0.19/0.21  | 0.25/0.33 | 0.4/82.62 | 10.52/20.87 | 12.47/18.91 | 4.3/102.97 | 1.66/221.24 |
| Nusap1      | 0.59/1.31  | 1.62/0.83 | 0.56/4.26 | 0.21/4.42 | 0.45/0.77 | 2.96/8.61 | 4.62/7.64 |
| Oftr73      | 0.10/0.04  | 0.27/0.82 | 1.12/0.60 | 4.73/1.36 | 1.03/1.30 | 20.44/3.31 | 5.83/3.40 |
| P2rx3       | 0.88/0.59  | 0.47/0.52 | 0.77/5.49 | 5.71/32.77 | 1.29/28.59 | 5.36/15.24 | 12.31/12.91 |
| Pdhx        | 1.09/0.82  | 0.10/0.80 | 11.01/2.93 | 7.06/0.82 | 5.76/1.16 | 5.97/16.23 | 6.61/8.67 |
| Phf20       | 0.77/3.35  | 0.87/0.37 | 0.88/2.17 | 5.00/1.11 | 3.20/3.81 | 1.03/7.36 | 0.67/1.62 |
| Polr3f      | 0.83/1.25  | 0.21/0.69 | 10.77/16.44 | 10.86/12.56 | 14.35/15.82 | 15.73/35.52 | 7.5/5.78 |
| Rae1        | 0.71/0.82  | 0.58/0.91 | 0.59/0.56 | 1.34/11.47 | 1.61/13.79 | 4.08/4.73 | 1.55/1.89 |
| Rbm38       | 1.52/0.58  | 0.11/0.70 | 18.93/2.24 | 5.43/1.14 | 12.22/1.26 | 10.19/8.33 | 8.49/7.36 |
| Scn1a       | 9.47/0.74  | 2.38/0.87 | 0.73/1.13 | 0.61/3.84 | 2.46/3.60 | 7.56/0.30 | 4.58/0.57 |
| Slc12a5     | 4.24/0.92  | 0.59/2.15 | 2.84/0.38 | 3.26/0.04 | 3.41/0.21 | 4.93/2.38 | 5.08/3.83 |
| Slc27a4     | 1.12/0.22  | 0.50/0.41 | 28.79/0.72 | 13.26/3.52 | 6.48/0.62 | 6.51/15.87 | 6.71/4.40 |
| Slc34a3     | 0.33/1.15  | 0.38/0.69 | 10.17/0.48 | 10.98/4.46 | 19.71/0.89 | 20.08/4.17 | 17.79/9.87 |
| Spata2      | 0.54/1.15  | 0.59/0.13 | 0.65/8.00 | 1.75/4.87 | 0.78/3.99 | 4.44/4.06 | 3.95/4.56 |
Table 2. Continued

| Gene symbol | Folds (TC5 versus others/TC10 versus others) | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
|-------------|---------------------------------------------|------------|------|------|----------|----------|------------|----------|
| Sptlc3      |                                             | 0.42/0.74  | 0.31/1.10 | 26.19/5.08 | 2.31/7.18 | 13.32/8.05 | 179.36/10.23 | 250.13/8.40 |
| Ss18l1      |                                             | 0.33/1.71  | 1.15/1.85 | 24.07/1.10 | 70.3.5/5.51 | 106.73/3.61 | 142.18/1.19 | 87.02/0.81 |
| Surf6       |                                             | 0.14/2.78  | 0.03/0.71 | 0.36/0.10 | 1.56/1.44 | 0.98/0.59 | 4.86/3.33 | 5.65/2.12 |
| Timm10      |                                             | 1.11/0.80  | 0.16/0.19 | 0.79/7.50 | 0.44/7.32 | 0.44/9.91 | 3.52/10.71 | 2.82/4.98 |
| Trub2       |                                             | 0.41/1.52  | 0.58/1.33 | 0.34/0.72 | 1.71/1.45 | 0.55/1.77 | 4.38/4.31 | 1.61/1.68 |
| Tltl9       |                                             | 0.06/2.02  | 0.03/0.34 | 13.59/16.46 | 4.75/4.47 | 8.44/10.41 | 63.39/8.51 | 39.11/7.1 |
| Yme111      |                                             | 1.17/14.07 | 0.17/3.87 | 4.23/0.82 | 10.71/0.64 | 12.14/2.59 | 21.28/7.73 | 18.6/4.72 |
| Arl6ip6     |                                             | 0.33/5.28  | 0.27/0.91 | 0.09/2.37 | 1.77/2.62 | 1.30/2.61 | 3.37/4.03 | 2.27/4.19 |
| Cep152      |                                             | 0.58/2.53  | 1.26/7.61 | 1.95/0.32 | 28.84/1.17 | 18.63/0.56 | 5.03/3.14 | 2.63/3.27 |
| Chd6        |                                             | 0.54/0.90  | 1.20/0.97 | 11.69/10.67 | 70.65/11.16 | 61.55/20.31 | 58.35/20.35 | 34.4/18.12 |
| Ddb2        |                                             | 3.10/1.00  | 0.72/1.06 | 10.74/0.56 | 81.94/1.53 | 91.16/0.66 | 88.59/3.99 | 61.48/3.57 |
| Dnmt3b      |                                             | 1.85/0.69  | 0.05/0.56 | 1.45/2.66 | 16.44/1.80 | 14.99/11.27 | 3.61/151.12 | 1.68/211.91 |
| Dut         |                                             | 0.04/2.30  | 0.36/0.50 | 0.80/1.54 | 2.32/5.41 | 1.35/3.55 | 14.44/1.97 | 9.05/1.33 |
| Emilin3     |                                             | 0.83/0.44  | 0.86/0.29 | 26.92/0.25 | 42.07/1.28 | 59.95/0.79 | 7.44/4.22 | 12.92/4.95 |
| Entpd6      |                                             | 1.61/1.68  | 0.30/0.48 | 41.36/0.66 | 38.04/0.30 | 17.21/0.32 | 17.79/3.07 | 12.23/2.46 |
| Mett5       |                                             | 0.61/0.10  | 0.37/0.06 | 2.72/10.00 | 6.83/2.21 | 4.9/6.00 | 6.01/46.04 | 5.83/28.46 |
| Myef2       |                                             | 0.58/0.69  | 0.11/0.33 | 0.74/1.38 | 0.91/3.04 | 2.12/3.57 | 4.87/9.78 | 3.94/8.22 |
| Rif1        |                                             | 0.94/1.19  | 1.76/3.64 | 0.98/14.02 | 5.85/10.48 | 7.01/7.97 | 3.27/6.11 | 2.11/0.65 |
| Sfmtb2      |                                             | 2.56/2.25  | 3.08/1.98 | 1.09/1.49 | 7.35/0.88 | 9.00/1.40 | 2.16/7.25 | 0.94/5.39 |

(B) Genes down-regulated between 1- and 4-folds in TCs as compared with others

| Gene symbol | Folds (TC5 versus others/TC10 versus others) | Gzf1 | 1/3.12 | 3.24/1.05 | 17.78/47.88 | 13.78/42.76 | 10.39/19.69 | 8.17/20.02 | 1.12/13.88 |
|-------------|---------------------------------------------|------|--------|----------|-------------|-------------|-------------|-----------|-----------|
| Pltp        |                                             | 9.94/3.83 | 2.17/3.01 | 189.9/16.60 | 7.67/31.99 | 8.30/21.33 | 110.19/13.79 | 96.66/22.44 |
| Polr1b      |                                             | 1.83/1.59 | 1.33/1.47 | 7.01/5.03 | 6.45/10.25 | 3.06/18.41 | 10.71/37.36 | 8.50/46.25 |
| Tasp1       |                                             | 2.19/7.78 | 1.12/8.13 | 1.96/2.59 | 15.91/2.34 | 12.47/4.49 | 20.97/8.59 | 8.31/9.50 |
| Zbb34       |                                             | 2.82/4.42 | 1.78/2.60 | 58.06/2.67 | 131.6/19.40 | 78.44/15.48 | 69.44/25.45 | 58.72/10.27 |
| Zfp120      |                                             | 1.92/1.70 | 1.84/1.62 | 17.79/11.67 | 21.29/13.60 | 18.08/11.68 | 11.75/7.33 | 8.68/5.36 |

D10 were 431 or 486, 408 or 509, 238 or 679, 294 or 623, 288 or 629, 182 or 735, or 222 or 695, as compared with MSCs, Fbs, ATII, T-BL, T-L, ABCs or PACs respectively. Up- and down-regulated genes more than 0-fold of TCs were 406 or 316, 388 or 338, 204 or 655, 262 or 598, 251 or 603, 158 or 712, or 180 or 665, as compared with MSCs, Fbs, ATII, T-BL, T-L, ABCs or PACs respectively.

In chromosome 3, 13 genes were higher than 0-fold in TCs, as compared with those in other cells (Table 4), of which 10 genes (Ag1, Ecm1, Golm4, Kcnab1, Lce1a2, Nnx1, Pde4dip, Plekho1, Psrc1, Rhoc, Rit1, Scamp3, Sec22b) were overexpressed 0- to 1-fold (Table 4A). Three genes Sh3gb1 (SH3-domain GRB2-like B1 – endophilin), Tm4sf1 (transmembrane 4 superfamily member 1) and Csfl (colony stimulating factor 1) were overexpressed more than one fold, in both TC D5 and TC D10, as compared with other cells (Table 4B). 59 genes in TCs were down-regulated, as compared with other cells (Table 5). Of them, 1700013F07Rik, Amy1, Anp32e,
Dnase2b, Fmo5, Pde5a, Phf17, Rwdd3 and Trim33 were down-regulated more than onefold, in both TC D5 and TC D10, as compared with other cells.

In chromosome 3 (Table 6), up- or down-regulated genes more than 0-fold of TCs D5 were 345 or 222, 352 or 215, 377 or 210, 386 or 214, 353 or 201, 366 or 190, 130 or 437, or 137 or 430, as compared with Fbs, MSCs, ATII, T-BL, T-L, ABCs or PACs respectively. Up- or down-regulated genes more than 0-fold of TCs D5 were 265 or 302, 263 or 304, 138 or 429, 188 or 437, 189 or 427, or 190 or 427, as compared with Fbs, MSCs, ATII, T-BL, T-L, ABCs or PACs respectively. Up- and down-regulated genes more than 0-fold of chromosome 2 and 3, including the number and names of up- or down-regulated genes more than 0-fold among different cells, were listed in Data S2.

Table 3 The number of genes specifically up- or down-regulated in pulmonary telocytes, as compared with other cells respectively

| Compared pairs          | Up>0 | Up>1 | Up>4 | Down>0 | Down>1 | Down>4 |
|-------------------------|------|------|------|--------|--------|--------|
| TC5 versus stem         | 576  | 194  | 50   | 341    | 80     | 14     |
| TC10 versus stem        | 431  | 136  | 41   | 486    | 152    | 27     |
| TCs versus stem         | 406  | 116  | 30   | 316    | 73     | 14     |
| TC5 versus fibroblast   | 559  | 201  | 79   | 358    | 107    | 17     |
| TC10 versus fibroblast  | 408  | 166  | 61   | 509    | 178    | 33     |
| TCs versus fibroblast   | 388  | 140  | 56   | 338    | 93     | 14     |
| TC5 versus ATII         | 228  | 102  | 36   | 689    | 504    | 289    |
| TC10 versus ATII        | 238  | 104  | 35   | 679    | 516    | 296    |
| TCs versus ATII         | 204  | 86   | 30   | 655    | 476    | 268    |
| TC5 versus CD8BL        | 287  | 174  | 89   | 630    | 689    | 303    |
| TC10 versus CD8BL       | 294  | 196  | 97   | 623    | 485    | 284    |
| TCs versus CD8BL        | 262  | 160  | 81   | 598    | 461    | 262    |
| TC5 versus CD8LL        | 277  | 178  | 92   | 640    | 487    | 306    |
| TC10 versus CD8LL       | 288  | 187  | 97   | 629    | 482    | 289    |
| TCs versus CD8LL        | 251  | 162  | 84   | 603    | 458    | 263    |
| TC5 versus basal cell   | 181  | 89   | 42   | 736    | 612    | 414    |
| TC10 versus basal cell  | 182  | 101  | 41   | 735    | 601    | 406    |
| TCs versus basal cell   | 158  | 79   | 36   | 712    | 573    | 375    |
| TC5 versus duct cell    | 210  | 118  | 50   | 707    | 552    | 358    |
| TC10 versus duct cell   | 222  | 117  | 51   | 695    | 548    | 345    |
| TCs versus duct cell    | 180  | 103  | 42   | 665    | 522    | 320    |

The relationships of the more than 0-fold up-regulated genes of chromosome 2 and 3 in TC D5 and/or TC D10 were analysed by String Network analysis (www.string-db.org), as compared with other cells, to identify direct (physical) and indirect (functional) associations between selected genes of TCs. TC-specific or dominating genes in TC D5 and TC D10 were selected by up- or down-expression more than 0-fold, as compared with other cells. Figure 1A and C demonstrated the distribution of such active gene group in chromosome 2 and 3 of all cells, and interactions or potential functional links of those genes of TCs.

In chromosome 2, about 30-50% of TCs genes showed similar patterns of gene expression in MSCs, Fbs or ATII, while 5-15% of TCs genes showed similarities with ABCs, PACs, T-BL or T-L. Top 50 up- or down-regulated genes of each cell were also evaluated and their distribution within chromosome 2 genes showed the difference between them, as shown in Figure 1B. High expressed genes of each cell within chromosome 2 were evaluated and distributed as red col-

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Table 4 Summary of genes expressed preferentially in TCs, as compared with others

| Compared pairs/fold up-regulated | >0 | >1 | >4 |
|----------------------------------|----|----|----|
| TC5 versus others                | 42 | 10 | 0  |
| TC10 versus others               | 30 | 7  | 2  |
| TCs versus others                | 13 | 3  | 0  |

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|---------------------------------------------|
|             | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| Agl         | –0.31/–0.25 | –0.42/–0.37 | –0.64/-0.6 | –0.54/-0.5 | –0.81/-0.79 | –0.63/-0.6 | –0.79/-0.77 |
| Ecm1        | –0.96/–0.96 | –0.9/-0.9 | –0.91/-0.91 | –0.96/-0.96 | –0.85/-0.85 | –0.48/-0.47 | –0.24/-0.23 |
| Golim4      | –0.65/–0.63 | –0.24/-0.2 | –0.86/-0.85 | –0.8/-0.79 | –0.86/-0.86 | –0.65/-0.63 | –0.48/-0.45 |
| Kcna1       | –0.94/–0.95 | –0.39/-0.54 | –0.73/-0.8 | –0.62/-0.71 | –0.9/-0.93 | –0.24/-0.42 | –0.65/-0.73 |
| Lce1a2      | –0.16/–0.3 | –0.41/-0.52 | –0.01/-0.18 | –0.03/-0.2 | –0.24/-0.37 | –0.83/-0.86 | –0.93/-0.94 |
| Nexn        | –0.59/-0.47 | –0.3/-0.08 | –0.82/-0.76 | –0.9/-0.87 | –0.96/-0.95 | –0.64/-0.52 | –0.78/-0.71 |
| Pde4dip     | –0.65/-0.69 | –0.52/-0.58 | –0.19/-0.29 | –0.11/-0.22 | –0.4/-0.47 | –0.71/-0.75 | –0.68/-0.72 |
| Plekho1     | –0.07/-0.3 | –0.8/-0.85 | –0.8/-0.85 | –0.85/-0.89 | –0.97/-0.98 | –0.95/-0.97 | –0.98/-0.99 |
| Prsc1       | –0.8/-0.7 | –0.78/-0.67 | –0.81/-0.73 | –0.72/-0.59 | –0.62/-0.45 | –0.32/0 | –0.38/-0.1 |
| Rhoc        | –0.57/-0.52 | –0.5/-0.43 | –0.85/-0.83 | –1/-1 | –0.92/-0.91 | –0.6/-0.55 | –0.73/-0.69 |
| Rit1        | –0.54/-0.53 | –0.66/-0.65 | –0.58/-0.57 | –0.69/-0.68 | –0.66/-0.65 | –0.25/-0.22 | –0.57/-0.55 |
| Scamp3      | –0.34/-0.34 | –0.55/-0.55 | –0.79/-0.79 | –0.61/-0.61 | –0.72/-0.72 | –0.82/-0.82 | –0.83/-0.83 |
| Sec22b      | –0.41/-0.08 | –0.5/-0.23 | –0.49/-0.21 | –0.55/-0.3 | –0.63/-0.43 | –0.56/-0.32 | –0.65/-0.46 |

| (B) Genes up-regulated between 1- and 4-folds in TCs as compared with other |
|---------------------------------------------|
| Sh3glb1 | –0.73/-0.68 | –0.62/-0.54 | –0.81/-0.77 | –0.79/-0.75 | –0.8/-0.76 | –0.69/-0.62 | –0.7/-0.63 |
| Tm4sf1  | –1/-1 | –0.6/-0.67 | –0.85/-0.88 | –1/-1 | –1/-1 | –0.99/-0.99 | –0.99/-0.99 |
| Csf1    | –0.71/-0.65 | –0.73/-0.68 | –0.93/-0.92 | –0.98/-0.98 | –0.98/-0.97 | –0.91/-0.9 | –0.92/-0.91 |

Our (Fig. 1B). The distribution of the high expressed genes and low expressed genes both in TC D5 and TC D10 indicates that they are in the centre of the small cluster and different from the other cells. Among the 26 co-up-expressed genes (Table 1A-C), 7 genes were found to have certain interactions (Fig. 1A).

In chromosome 3, about 50–60% of TCs genes showed similar patterns of gene expression in Fbs, MSCs, PACs or ABCs, while 0–20% of TCs genes showed similarities with ABCs, PACs, T-BL or T-L. Top 50 up- or down-regulated genes of each cell were also evaluated and their distribution within chromosome 3 genes showed the difference between them, as shown in Figure 1D. High expressed genes of each cell within chromosome 3 were evaluated and distributed as red colour (Fig. 1D). The distribution of up-expressed genes and down-expressed genes in TC D5 and TC D10 indicates that they are in the centre of the small cluster and different from the other cells. Among the 16 co-up-expressed genes (Table 4A and B), no clear or certain interactions (Fig. 1C) were found. The hierarchical cluster analysis of the differentially expressed genes (Fig. 2) clearly shows that TCs are poorly related to the other cell lines.

**Discussion**

Mouse chromosomes are the best studied mammalian chromosomes and are considered as gold standard of human comparative map, although genomic rearrangements occur during evolution. Certain
Table 5 Summary of genes expressed preferentially in TCs, as compared with others

| Compared pairs/fold down-regulated | >0  | >1  | >4  |
|-----------------------------------|-----|-----|-----|
| TC5 versus others                 | 79  | 12  | 0   |
| TC10 versus others                | 137 | 22  | 1   |
| TCs versus others                 | 59  | 9   | 0   |

| Gene symbol | Folds (TC5 versus others/TC10 versus others) | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
|-------------|---------------------------------------------|------------|------|------|----------|----------|------------|-----------|
| 1700027A23Rik | 0.9/1.57 | 0.08/0.46 | 162.04/220.27 | 4.95/7.07 | 0.43/0.94 | 28.1/38.49 | 8.2/11.48 |
| 2810403A07Rik | 0.81/1.11 | 0.7/0.99 | 0.47/0.72 | 3.33/4.07 | 4.16/5.04 | 1.31/1.7 | 0.96/1.3 |
| 4932438A13Rik | 1.12/1.11 | 0.9/0.9 | 4.4/4.39 | 76.24/75.99 | 78.16/77.9 | 9.48/9.44 | 8.12/8.09 |
| 4933421E11Rik | 1.61/2.74 | 0.22/0.74 | 0.3/0.87 | 5.03/7.64 | 4.6/7.02 | 2.41/3.89 | 0.14/0.64 |
| A530020G20Rik | 0.13/0.92 | 0.02/0.73 | 6.3/11.39 | 1.91/3.94 | 2.91/5.64 | 2.4/4.77 | 1.14/2.63 |
| Acadm | 5.66/5.92 | 0.22/0.27 | 4.5/4.72 | 6.8/7.1 | 4.87/5.09 | 6.25/6.53 | 5.1/5.33 |
| Adh6a | 0.46/0.67 | 0.44/0.65 | 5.68/6.63 | 8.32/9.65 | 11.57/13.37 | 50.97/58.39 | 25.27/29.03 |
| Ahcyl1 | 0.62/0.88 | 0.14/0.32 | 1.5/1.91 | 1.14/1.49 | 0.51/0.76 | 2.4/2.95 | 1.9/2.38 |
| Abl3 | 1.08/1.53 | 1.35/1.87 | 0.45/0.77 | 0.95/1.38 | 5.61/7.05 | 0.82/1.22 | 1.64/2.21 |
| Atpl1b | 0.44/0.93 | 0.99/1.66 | 2.2/3.29 | 28.57/38.63 | 22.24/30.15 | 10.08/13.85 | 15.11/20.59 |
| Car3 | 0.25/0.29 | 0.04/0.07 | 3.72/3.87 | 4.07/4.23 | 6.87/7.12 | 40.74/42.05 | 22.72/23.47 |
| Clcc1 | 1.62/2.56 | 0.03/0.39 | 0.08/0.47 | 0.81/1.45 | 0.7/1.31 | 3.29/4.83 | 3.25/4.77 |
| Cryz | 3.65/6.13 | 3.67/6.15 | 0.91/1.92 | 0.17/0.79 | 0.0/0.53 | 0.51/1.32 | 2.14/3.8 |
| Cts1 | 0.22/0.15 | 1.18/1.05 | 151.36/142.4 | 336.91/317.03 | 271.25/255.24 | 245.94/231.42 | 92.66/87.15 |
| Gnat2 | 1.35/1.57 | 0.19/0.3 | 1.09/1.29 | 2.4/2.72 | 4.24/4.73 | 8.71/9.63 | 1.89/2.17 |
| Gpsm2 | 1.89/2.66 | 1.39/2.03 | 0.36/0.72 | 3.42/4.59 | 5.15/6.78 | 14.01/17.99 | 18.95/24.24 |
| Hax1 | 0.16/0.6 | 0.66/1.3 | 1.25/2.1 | 0.9/1.62 | 0.94/1.68 | 0.6/1.21 | 0.84/1.54 |
| Hltf | 0.31/0.45 | 0.45/0.61 | 3.28/3.75 | 18.55/20.78 | 18.24/20.44 | 7.82/8.83 | 3.57/4.09 |
| Hps3 | 1.39/1.42 | 0.56/0.8 | 0.8/0.82 | 8.25/8.37 | 6.09/6.18 | 2.97/3.02 | 0.26/0.27 |
| Ints3 | 0.83/0.94 | 0.23/0.3 | 27.92/29.65 | 13.8/14.68 | 6.12/6.54 | 23.06/24.5 | 26.27/27.9 |
| Isg2012 | 0.09/0.62 | 0.06/0.57 | 2.61/4.35 | 4.77/7.55 | 3.23/5.27 | 5.9/9.22 | 6.17/9.62 |
| Lass2 | 0.06/0.12 | 0.01/0.07 | 5.54/5.9 | 2.13/2.3 | 1.64/1.79 | 1.12/1.23 | 0.41/0.49 |
| Lrrc40 | 0.4/0.16 | 0.42/0.18 | 102.03/84.89 | 256.01/213.26 | 257.71/214.68 | 537.73/448.12 | 349.78/291.43 |
| Lrrcc1 | 0.1/0.63 | 0.19/0.77 | 0.6/1.37 | 2.23/3.8 | 1.26/2.35 | 5.2/8.21 | 2.45/4.13 |
| Mtosd8 | 0.71/0.3 | 0.45/0.11 | 11.71/8.67 | 24.91/18.72 | 19.66/14.72 | 32.59/24.56 | 33.42/25.19 |
| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|--------------------------------------------|
|             | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| Mrpl24      | 0.64/1.57  | 0.06/0.66 | 1.1/2.27 | 3.1/5.41 | 1.9/3.56 | 1.85/3.45 | 1.82/3.41 |
| Mrpl9       | 0.29/0.47  | 0.44/0.64 | 3.16/3.73 | 7.18/8.3 | 5.33/6.19 | 8.23/9.48 | 7.87/9.08 |
| Ndufb5      | 1/1.86     | 0.01/0.44 | 2.17/3.55 | 2.34/3.79 | 0.88/1.69 | 2.78/4.42 | 2.95/4.66 |
| Odf2l       | 1.26/1.48  | 0.17/0.29 | 5.61/6.26 | 11.82/13.08 | 7.95/8.83 | 44.13/48.56 | 37.95/41.78 |
| Papss1      | 1.13/1.25  | 0.44/0.52 | 2.44/2.62 | 1.53/1.66 | 0.28/0.34 | 1.73/1.88 | 1.64/1.78 |
| Pgrmc2      | 1.11/1.55  | 0.08/0.31 | 4.75/5.96 | 1.45/1.97 | 2/2.62 | 29.73/36.17 | 15.37/18.8 |
| Plk4        | 0.46/0.88  | 0.71/1.2 | 2.33/3.29 | 11.64/15.29 | 12.8/16.79 | 7.3/9.69 | 12.53/16.43 |
| Prpf38b     | 1.18/0.93  | 0.68/0.49 | 11.25/9.81 | 50/44 | 66.2/58.3 | 44.75/39.37 | 34.78/30.58 |
| Rabggtb     | 0.81/0.52  | 1.09/0.75 | 8.12/6.64 | 53.93/45.01 | 57.59/48.07 | 37.82/31.52 | 48.92/40.81 |
| Rapgef2     | 0.29/0.68  | 0.63/1.11 | 2.8/3.92 | 10.47/13.85 | 8.15/10.86 | 0.24/0.6 | 0.61/1.09 |
| Rps4x       | 0.11/0.67  | 0.62/1.45 | 0.39/1.1 | 0.85/1.79 | 1.99/3.51 | 0.67/1.53 | 0.54/1.32 |
| Sars        | 3.16/4.85  | 1.32/2.27 | 1.03/1.86 | 0.4/0.98 | 0.09/0.54 | 0.41/0.99 | 0.3/0.83 |
| Setdb1      | 0.25/0.87  | 0.6/1.39 | 0.03/0.55 | 1.29/2.42 | 1.07/2.1 | 2.73/4.58 | 1.8/3.19 |
| Slah2       | 1.29/1.68  | 1.79/2.27 | 3.92/4.77 | 0.21/0.42 | 0.87/1.19 | 3.28/4.02 | 3.03/3.72 |
| Slt33a1     | 0.75/0.58  | 0.72/0.55 | 5.56/4.91 | 2.19/1.87 | 1.75/1.48 | 3.02/2.62 | 0.75/0.58 |
| Snc4        | 0.07/0.85  | 0.89/2.26 | 0.12/0.94 | 14.46/25.7 | 14.77/26.25 | 2.22/4.56 | 6.29/11.58 |
| Sohlh2      | 0.02/0.11  | 0.08/0.17 | 1.69/1.93 | 0.91/1.07 | 1.86/2.11 | 1.58/1.8 | 1.85/2.1 |
| Spata5      | 0.06/0.88  | 0.21/1.16 | 0.19/1.12 | 0.32/1.36 | 0.4/1.49 | 1.15/2.83 | 1.51/3.47 |
| Syt6        | 0.23/0.6   | 0.21/0.57 | 0.15/0.5 | 0.55/1.02 | 0.43/0.87 | 1.27/1.96 | 0.29/0.69 |
| Tbl1xr1     | 2.36/1.6   | 0.89/0.47 | 2.59/1.79 | 4.02/2.9 | 3.66/2.61 | 16.91/12.89 | 13.56/10.29 |
| Txnip       | 0.77/0.21  | 1.35/0.6 | 54.04/36.58 | 107.86/73.33 | 65.79/44.6 | 36.39/24.53 | 26.15/17.53 |
| Ubln4       | 0.18/0.58  | 0.21/0.63 | 11.94/16.36 | 5.82/8.15 | 2.66/3.91 | 28.73/38.87 | 40.6/54.79 |
| Wdr77       | 0.14/0.7   | 0.97/1.92 | 3.67/5.93 | 1.52/2.74 | 0.79/1.66 | 2.28/3.88 | 2.96/4.88 |
| Ythdf3      | 0.22/0.54  | 0.64/1.07 | 3.08/4.17 | 6.8/8.87 | 6.44/8.41 | 3.68/4.92 | 3/4/06 |
| Zzz3        | 0.1/0.64   | 0.73/1.58 | 0.1/0.64 | 3.91/6.33 | 3.67/5.96 | 1.29/2.42 | 0.6/1.39 |

(B) Genes down-regulated between 1- and 4-folds in TCs as compared with others

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|--------------------------------------------|
|             | 1700013F07Rik | 6.5/7.08 | 1.64/1.84 | 34.73/37.52 | 2.1/2.34 | 7.8/8.48 | 29.96/32.38 | 47.84/51.65 |
|             | Amy1         | 2.81/3.86 | 8.06/10.55 | 9.44/12.32 | 7.37/9.67 | 14.79/19.13 | 31.07/39.88 | 24.64/31.68 |
|             | Anp32e       | 8.69/11.5 | 2.79/3.89 | 121.76/157.32 | 245.2/316.52 | 243.57/314.42 | 356.1/459.55 | 464.63/599.52 |
|             | Dnase2b      | 2.08/2.96 | 1.85/2.67 | 1.08/1.68 | 1.08/1.67 | 9.48/12.48 | 10.22/13.44 | 6.68/8.87 |
Table 5. Continued

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|---------------------------------------------|
|             | Fibroblast         | Stem            | ATII           | CD8_T_BL         | CD8_T_LL         | Basal_cell       | Duct_cell       |
| Fmo5        | 3.8/6              | 3.36/5.35       | 3.52/5.58      | 4.08/6.4         | 1.75/3.01        | 21.33/31.55      | 5.4/8.33        |
| Pde5a       | 4.09/2.99          | 3.94/2.87       | 4.58/3.37      | 39.25/30.54      | 12.26/9.39       | 44.99/35.04      | 15.96/12.29     |
| Phf17       | 1.24/1.76          | 3.48/4.52       | 1.04/1.52      | 3.4/4.42         | 3.67/4.75        | 11.3/14.14       | 4.48/5.75       |
| Rwdd3       | 2.98/4.43          | 2.52/3.8        | 2.98/4.43      | 14.07/19.53      | 10.16/14.21      | 28.88/39.73      | 8.77/12.31      |
| Trim33      | 1.93/5.56          | 1.06/3.62       | 4.2/10.64      | 26.9/61.48       | 17.78/41.07      | 3.18/1.17        | 2.63/7.13       |

Table 6 The number of genes specifically up- or down-regulated in pulmonary telocytes, as compared with other cells respectively

| Compared pairs | Up>0 | Up>1 | Up>4 | Down>0 | Down>1 | Down>4 |
|----------------|------|------|------|--------|--------|--------|
| TC10 versus fibroblast | 265  | 116  | 40   | 302    | 126    | 28     |
| TC5 versus fibroblast   | 345  | 161  | 51   | 222    | 87     | 17     |
| TCs versus fibroblast   | 255  | 100  | 33   | 212    | 78     | 12     |
| TC10 versus stem        | 263  | 109  | 35   | 304    | 115    | 23     |
| TC5 versus stem         | 352  | 134  | 41   | 215    | 63     | 12     |
| TCs versus stem         | 247  | 85   | 27   | 199    | 58     | 12     |
| TC10 versus ATII        | 138  | 67   | 22   | 429    | 306    | 177    |
| TC5 versus ATII         | 377  | 278  | 137  | 190    | 93     | 32     |
| TCs versus ATII         | 367  | 268  | 124  | 128    | 62     | 20     |
| TC10 versus CD8BL       | 188  | 117  | 59   | 379    | 302    | 186    |
| TC5 versus CD8BL        | 214  | 143  | 81   | 353    | 265    | 162    |
| TCs versus CD8BL        | 181  | 113  | 57   | 346    | 254    | 153    |
| TC10 versus CD8LL       | 168  | 99   | 51   | 399    | 307    | 197    |
| TC5 versus CD8LL        | 201  | 138  | 66   | 366    | 278    | 163    |
| TCs versus CD8LL        | 164  | 96   | 47   | 362    | 269    | 154    |
| TC10 versus basal cell  | 95   | 55   | 19   | 472    | 388    | 261    |
| TC5 versus basal cell   | 130  | 65   | 26   | 437    | 345    | 222    |
| TCs versus basal cell   | 87   | 45   | 16   | 429    | 339    | 218    |
| TC10 versus duct cell   | 120  | 56   | 26   | 447    | 373    | 234    |
| TC5 versus duct cell    | 137  | 81   | 29   | 430    | 321    | 199    |
| TCs versus duct cell    | 110  | 51   | 21   | 420    | 312    | 187    |

Human disease genes were discovered by comparative genomics using the information derived from mapped mouse mutations, although they are not the simplest model for human comparison. In humans, chromosome 2 has the largest sequenced base pairs (237, 712, 649) [47], working with all of the autosomes in humans, spanning the second largest amount of total base pairs (242, 751, 149).
and representing 16.2% of the total DNA [48–51]. Over 31 exactly known diseases were proposed to be associated with genes on chromosome 2. In mouse, chromosome 2 is entirely sequenced and has 3146 genes encoding 1780 proteins [47], of which 917 genes were measured by bioinformatics tools in the present study. Our data demonstrated that there were 26 or 80 up- or down-regulated genes of chromosome 2 in TCs, as compared with MSCs, Fbs, ATII, T-BL, T-L, ABCs or PACs.

One gene Myl9 (myosin, light polypeptide 9) was overexpressed most in TCs, different from other cells. Myl9 regulatory gene encodes the regulatory light chains of myosin II molecule, known to play a central role in cell adhesion, migration and division. Recent results showed Myl9 as the only gene differentially expressed in the aged versus young injured arteries [52] implying that it may be related to tissue/organ injury and ageing. Therefore, it is possible that the over-expression of Myl9 in pulmonary TCs may play an important role in lung injury and ageing. There were six genes, e.g. Pltp, Gzf1, Polr1b, Tasp1, Zbtb34 and Zfp120, down-expressed most in TCs, different from other cells. The Pltp (phospholipid transfer protein) gene is widely expressed in the body, and plays an important role in lipid metabolism, immune modulation, lipopolysaccharide binding or neurodegenerative disease [53]. Pltp is highly expressed within the lung epithelium, in chronic obstructive pulmonary disease or pulmonary inflammation [54]. TCs may play an important role of inhibiting inflammation in the lung. Roles of Gzf1 (GDNF-inducible zinc finger protein 1), Polr1b (polymerase (RNA) I polypeptide B), Tasp1...
(taspase threonine aspartase 1), Zbtb34 (zinc finger and BTB domain containing 34) or Zfp120 (zinc finger protein 120) genes or proteins in the lung remain unclear. Thus, there is a further need to clarify the exact mechanisms and functions of these genes in TCs.

Mouse chromosome 3 has a total number of genes of 993 which encode a total of 669 proteins [47]. Human chromosome 3 has ~7% of the human genome probably related with, at least, 121 diseases that are associated with 105 genes [55] and also spanning the third largest amount of total base pairs (199,446,827) and represented about 6.5% of the total DNA in cells [56–59]. The chromosome 3 has 1550 genes, of which 567 genes of chromosome 3 were measured by bioinformatics tools in the present study. We showed that there were 13 or 59 up- or down-regulated genes of chromosome 3 in TCs, as compared with Fbs, MSCs, ATII, T-BL, T-L, ABCs or PACs. There were three genes, e.g. Sh3glb1, Tm4sf1 and Csf1, overexpressed in TCs.

Sh3glb1 gene encodes SH3-domain GRB2-like B1 or endophilin, known to have an extremely close relationship with Bax-interacting factor-1 (bif-1) [60, 61], involved in cell survival and proliferation under metabolic stress and evasion of apoptosis. SH3glb1 is a membrane curvature-inducing protein interact with BECN1 though UVRAG and regulates the post-Golgi trafficking of membrane-integrated ATG9A for autophagy. At the premalignant stage, allelic loss of Sh3gb1 could enhance Myc-induced chromosomal instability and
result in the up-regulation of anti-apoptotic proteins, including MCL1 and BCL2L1 [61], being responsible for enabling cells to survive and proliferate under metabolic stress and evasion of apoptosis. Endophillin is a membrane curvature-inducing protein that interacts with autophagy related beclin 1, although UV radiation resistance associated gene (Uvrag) and regulates the post-Golgi trafficking of membrane-integrated autophagy related 9A (Atg9A) protein. At the premalignant stage, allelic loss of Sh3glb1 enhances Myc-induced chromosomal instability and results in the up-regulation of anti-apoptotic proteins, including MCL1 and BCL2L1 [61]. So far, there is no reported association with any lung disease, however, we cannot exclude a role for TCs as having pro-proliferative effects through inhibition of apoptosis as showed in a previous study [41].

Tm4sf1 (transmembrane 4 superfamily member 1) is a distant member of the transmembrane 4 superfamily of cell-surface proteins characterized by the presence of four hydrophobic domains [62]. It is highly expressed in different carcinomas, e.g. in lung cancer [62], and lowly expressed in normal tissues [63]. Colony stimulating factor 1 (macrophage) (Csf1) plays an important role in cancer metastasis and invasion. It is highly expressed in different carcinomas and expressed at relatively low levels (if at all) in many normal tissues [63]. High expression of Csf1 can increase metastasis and invasion of pulmonary adenocarcinomas [64]. For example, Tm4sf1 it was up-regulated in human adenocarcinoma A549 cell line, suggesting a poor prognosis for anticancer therapy [65]. Overexpression of TM4SF1 and Csf1 in lung TCs may have a role in the development of lung cancer. Among down-expressed genes in TCs, Pde5a (cGMP-specific phosphodiesterase 5A) has an obvious association with acute and chronic interstitial lung disease. Overexpression of Pde5a may accelerate the formation of pulmonary fibrosis, while down-expression of Pde5a has important roles and effects in pulmonary fibrosis-associated pulmonary hypertension [66, 67]. Therefore, we

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**Figure 2** Hierarchical cluster analysis of the differentially expressed genes on chromosomes 2 and 3 among telocytes (TCs), mesenchymal stem cells (MSCs), fibroblasts (Fbs), lymphocytes from lungs (T-LL) and from bronchial lymph nodes (T-BL), alveolar type II cells (ATII), proximal airway cells (PAC) and airway basal cells (ABC).
concluded that Tm4sf1 and Csf1 found to be overexpressed in lung TCs may have a role in tumor promotion. There were nine genes, e.g. 1700013F07Rik, Amy1, Ang32e, Dnase2b, Fmo5, Pde5a, Phf17, Rwdd3 and Trim33, down-expressed most in TCs, different from other cells.

Among them, only phosphodiesterase 5a (Pde5a) cGMP-specific gene is obviously associated with acute and chronic interstitial lung disease. Its high expression promotes the pulmonary fibrosis, while the inhibition of Pde5a expression ameliorates right ventricular failure and pulmonary, when is associated with bleomycin, through a reduction in reactive oxygen species [68]. Therefore, Pde5a low expression in lung TCs may have therapeutic effect on pulmonary fibrosis and other acute and chronic interstitial lung disease, probably by modulation of oxidative stress levels, as previously shown [41].

In conclusion, the present study compared genetic variations of chromosome 2 and 3 of pulmonary TCs with other related cells, e.g. Fbs, MSCs, ATII, T-BL, T-L, ABCs or PACs. Our data showed a number of TCs-specific or dominant genes in chromosomes 2 and 3, different from other lung tissue resident cells or infiltrated cells. The TCs signatures of chromosome 2 and 3 genes indicate TCs may be mainly associated with anti-inflammatory responses, the prevention of lung cancer formation and development or protective effects on pulmonary fibrosis or acute and chronic interstitial lung diseases.

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Conflicts of interest

The authors confirm that there are no conflicts of interest.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Data profiles for all genes.

Data S2 Details of up- or down regulated gene expression variations of chromosome 2 and 3.

Figure S1 Details of the selected core network genes in TCs isolated from the mouse lung and cultured for 5 days in chromosome 2.

Figure S2 Details of the selected core network genes in TCs isolated from the mouse lung and cultured for 10 days in chromosome 2.

Figure S3 Details of the selected core network genes in mouse mesenchymal stem cells in chromosome 2.

Figure S4 Details of the selected core network genes in mouse fibroblasts in chromosome 2.

Figure S5 Details of the selected core network genes in mouse alveolar type II cells in chromosome 2.

Figure S6 Details of the selected core network genes in mouse airway basal cells in chromosome 2.

Figure S7 Details of the selected core network genes in mouse proximal airway cells in chromosome 2.

Figure S8 Details of the selected core network genes in mouse CD8+ T cells come from bronchial lymph nodes in chromosome 2.

Figure S9 Details of the selected core network genes in mouse CD8+ T cells from lung in chromosome 2.

Figure S10 Details of the selected core network genes in TCs isolated from the mouse lung and cultured for 10 days in chromosome 3.

Figure S11 Details of the selected core network genes in TCs isolated from the mouse lung and cultured for 5 days in chromosome 3.

Figure S12 Details of the selected core network genes in mouse mesenchymal stem cells in chromosome 3.

Figure S13 Details of the selected core network genes in mouse fibroblasts in chromosome 3.

Figure S14 Details of the selected core network genes in mouse alveolar type II cells in chromosome 3.

Figure S15 Details of the selected core network genes in mouse airway basal cells in chromosome 3.

Figure S16 Details of the selected core network genes in mouse proximal airway cells in chromosome 3.

Figure S17 Details of the selected core network genes in mouse CD8+ T cells come from bronchial lymph nodes in chromosome 3.

Figure S18 Details of the selected core network genes in mouse CD8+ T cells from lung in chromosome 3.
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