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# Comparison of Chromosome 4 gene expression profile between lung telocytes and other local cell types

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## Abstract

Telocytes (TCs) are new cellular entities of mesenchymal origin described almost ubiquitously in human and mammalian organs (www.telocytes.com). Different subtypes of TCs were described, all forming networks in the interstitial space by homo- and heterocellular junctions. Previous studies analysed the gene expression profiles of chromosomes 1, 2, 3, 17 and 18 of murine pulmonary TCs. In this study, we analysed by bioinformatics tools the gene expression profiles of chromosome 4 for murine pulmonary TCs and compared it with mesenchymal stem cells (MSCs), fibroblasts (Fbs), alveolar type II cells (ATII), airway basal cells, proximal airway cells, CD8(+) T cells from bronchial lymph nodes (T-BL) and CD8(+) T cells from lungs (T-L). Key functional genes were identified with the aid of the reference library of the National Center for Biotechnology Information Gene Expression Omnibus database. Seventeen genes were up-regulated and 56 genes were down-regulated in chromosome 4 of TCs compared with other cells. Four genes (Akap2, Gpr153, Sdc3 and Tbc1d2) were up-regulated between one and fourfold and one gene, Svep1, was overexpressed over fourfold. The main functional networks were identified and analysed, pointing out to a TCs involvement in cellular signalling, regulation of tissue inflammation and cell expansion and movement.

**Keywords:** chromosome 4 • telocytes • mesenchymal stem cells • fibroblasts • alveolar type II cells • airway epithelial cells • lymphocytes

## Introduction

Telocytes (TCs) are newly described cells of the interstitial space [1, 2] which are ubiquitously distributed in mice and humans [3–17]. Telocytes are likely to have a mesenchymal origin [18] and are best characterized by very long extensions called telopodes (Tps) (for details see reviews [17, 19]. They were characterized in terms of ultrastructure [20, 21], immunophenotype [22], proteomic [23], gene profile [24–26] and miRNA imprint [27–29] and shown to be different from fibroblasts, mesenchymal cells or endothelial cells. Moreover, TCs display distinct electrophysiological properties [30–33]. The very long (tens to hundreds of micrometres) Tps classically described as an alternation of dilated regions—podoms and filamentous regions—podomers, were recently viewed by FIB-SEM tomography 3D reconstruction [2]. Therefore, the real aspect of Tps consists in regions with classical aspect of beads on a string appearance and 'ribbon-like' regions [34].

Telocytes were suggested to participate in intercellular information exchange and interactions by extracellular vesicle release [29, 35]. In addition, their secretome might have a modulatory role in

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**Table 1** Summary of up-regulated genes in TCs, as compared with others. (A) Genes up-regulated between zero and onefold in TCs as compared with others. (B) Genes up-regulated between one and fourfold in TCs as compared with others. (C) Genes up-regulated >fourfold in TCs as compared with others

| Compared pairs/fold up-regulated | >0 | >1 | >4 |
|---|---|---|---|
| TC5 *versus* others | 51 | 13 | 3 |
| TC10 *versus* others | 34 | 8 | 1 |
| TCs *versus* others | 17 | 5 | 1 |

| | Folds (TC5 *versus* others/TC10 *versus* others) | | | | | | |
|---|---|---|---|---|---|---|---|
| Gene symbol | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| (A) | | | | | | | |
| 1700009N14Rik | −0.98/−0.99 | −0.97/−0.97 | −0.72/−0.8 | −0.41/−0.6 | −0.23/−0.47 | −0.47/−0.64 | −0.49/−0.65 |
| Aurkaip1 | −0.37/−0.09 | −0.46/−0.22 | −0.35/−0.32 | −0.43/−0.42 | −0.48/−0.47 | −0.61/−0.6 | −0.51/−0.5 |
| Fam176b | −0.73/−0.73 | −0.86/−0.86 | −0.09/−0.34 | −0.36/−0.55 | −0.56/−0.69 | −0.88/−0.92 | −0.94/−0.96 |
| Fbxo6 | −0.33/−0.17 | −0.56/−0.45 | −0.6/−0.64 | −0.83/−0.85 | −0.89/−0.9 | −0.77/−0.8 | −0.84/−0.86 |
| Hspg2 | −0.62/−0.7 | −0.21/−0.38 | −0.69/−0.82 | −0.7/−0.83 | −0.6/−0.77 | −0.76/−0.87 | −0.84/−0.91 |
| Macf1 | −0.74/−0.66 | −0.5/−0.35 | −0.64/−0.67 | −0.52/−0.56 | −0.47/−0.51 | −0.65/−0.68 | −0.46/−0.51 |
| Mast2 | −0.48/−0.15 | −0.62/−0.38 | −0.92/−0.9 | −0.96/−0.95 | −0.95/−0.94 | −0.81/−0.79 | −0.87/−0.85 |
| Otud3 | −0.61/−0.5 | −0.79/−0.73 | −0.4/−0.43 | −0.09/−0.16 | −0.18/−0.24 | −0.12/−0.2 | −0.11/−0.19 |
| Plekhm2 | −0.36/−0.6 | −0.17/−0.49 | −0.46/−0.76 | −0.51/−0.78 | −0.47/−0.77 | −0.77/−0.9 | −0.72/−0.87 |
| Tm2d1 | −0.32/−0.14 | −0.43/−0.27 | −0.39/−0.44 | −0.27/−0.35 | −0.36/−0.42 | −0.28/−0.35 | −0.23/−0.31 |
| Tmem59 | −0.51/−0.43 | −0.45/−0.36 | −0.99/−0.99 | −0.99/−0.99 | −0.99/−0.99 | −1/−1 | −1/−1 |
| Zcchc17 | −0.53/−0.45 | −0.67/−0.61 | −0.54/−0.61 | −0.08/−0.23 | −0.59/−0.65 | −0.62/−0.69 | −0.76/−0.8 |
| (B) | | | | | | | |
| Akap2 | −0.89/−0.81 | −0.73/−0.54 | −0.78/−0.73 | −0.78/−0.74 | −0.82/−0.78 | −0.79/−0.75 | −0.82/−0.78 |
| Gpr153 | −0.93/−0.92 | −0.66/−0.61 | −0.67/−0.72 | −0.98/−0.99 | −0.96/−0.97 | −0.98/−0.99 | −0.92/−0.93 |
| Sdc3 | −0.74/−0.62 | −0.88/−0.83 | −0.65/−0.62 | −0.84/−0.83 | −0.73/−0.71 | −0.79/−0.78 | −0.87/−0.87 |
| Tbc1d2 | −0.91/−0.78 | −0.99/−0.97 | −0.78/−0.6 | −0.99/−0.98 | −0.97/−0.94 | −0.8/−0.65 | −0.94/−0.9 |
| (C) | | | | | | | |
| Svep1 | −0.97/−0.97 | −0.84/−0.83 | −0.9/−0.92 | −0.95/−0.96 | −0.95/−0.97 | −0.95/−0.96 | −0.94/−0.95 |

**Fig. 1** Expression profiles of the selected genes as an active group of chromosome 4 of telocytes (TCs) isolated and cultured from mouse lungs on days 5 (D5) and 10 (D10), as compared with fibroblasts (Fbs), mesenchymal stem cells (MSCs), alveolar type II cells (ATII), airway basal cells (ABCs), proximal airway cells (PACs), $CD8^+$ T cells come from bronchial lymph nodes (T-BL), and $CD8^+$ T cells from lung (T-L) respectively (**A**). The profiles for entire genes are described in Supplementary Document 1. The selected core network and whole mouse network are linked by the documented functional interactions from various databases (see Materials and methods). Genes in each network are indicated in red and some of their nearest neighbours are indicated by dark grey nodes. A group of telocyte genes up-regulated and down-regulated more than zerofold as compared with all other cells and existed in telocytes on days 10 and 5 were selected as telocyte-specific or dominated genes in chromosome 4 (**A**). Top 50 up- or down-regulated genes of each cells were also evaluated and their distribution within chromosome 4 genes showed the difference between cells (**B**). Details of the selected network in each cell type are in Figures S1–S9.

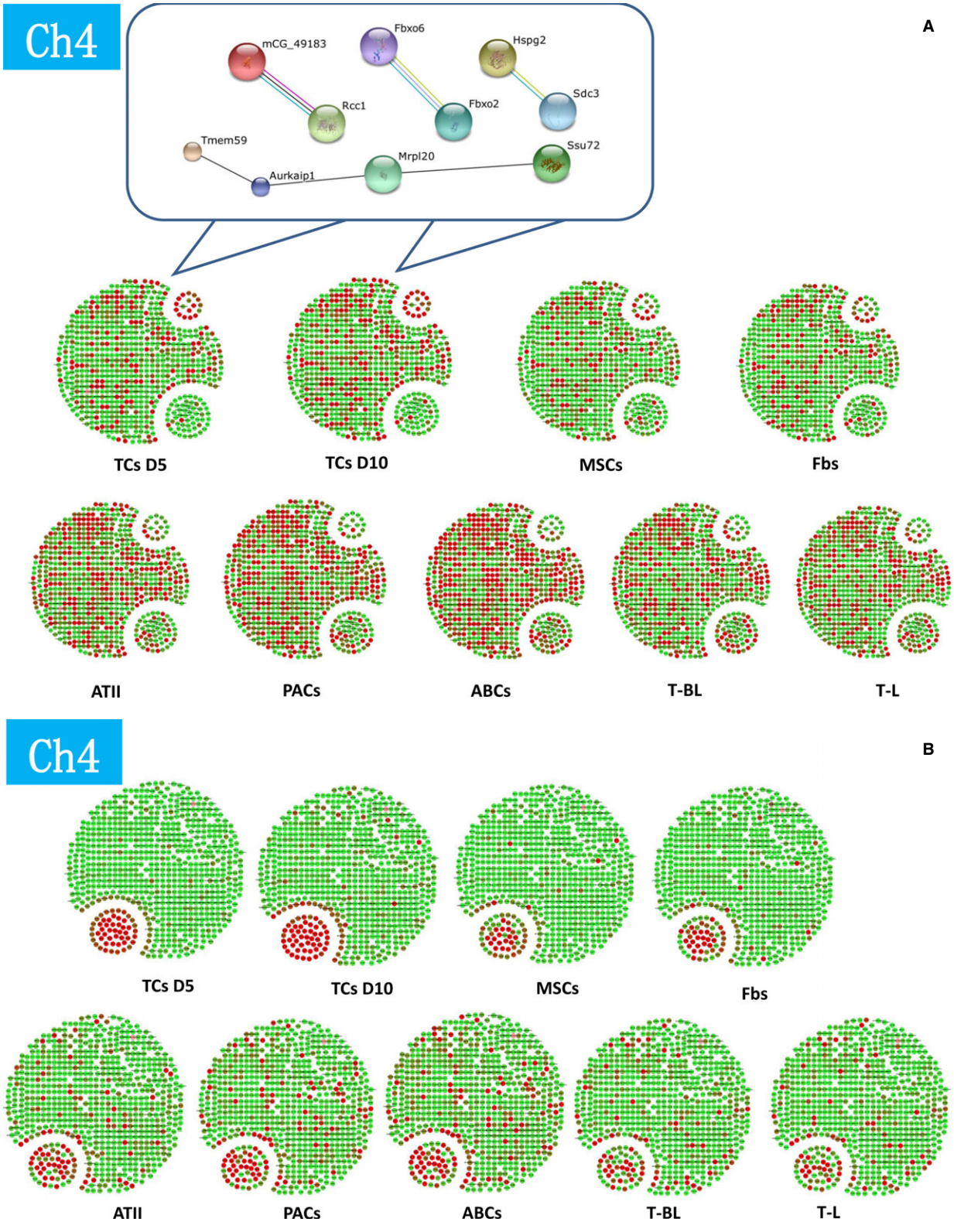

**Table 2** Summary of down-regulated genes in TCs, as compared with others. (A) Genes down-regulated between zero and onefold in TCs as compared with others. (B) Genes down-regulated between one and fourfold in TCs as compared with others

| Compared pairs/fold down-regulated | >0 | >1 | >4 |
|---|---|---|---|
| TC5 *versus* others | 70 | 3 | 0 |
| TC10 *versus* others | 142 | 10 | 0 |
| TCs *versus* others | 56 | 2 | 0 |

| | Folds (TC5 *versus* others/TC10 *versus* others) | | | | | | |
|---|---|---|---|---|---|---|---|
| Gene symbol | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
| (A) | | | | | | | |
| 1700013G24Rik | 0.18/0.28 | 0.34/0.45 | 1.43/0.93 | 0.39/0.07 | 5.42/4.01 | 50.81/38.78 | 22.78/17.36 |
| 2210012G02Rik | 1.36/0.8 | 0.68/0.28 | 31.01/16.83 | 100.21/53.77 | 56/30.27 | 44.47/23.55 | 21.26/11.08 |
| 2610301B20Rik | 1.63/1.75 | 1.3/1.41 | 7.41/5.43 | 4.38/2.99 | 0.97/0.48 | 3.12/2.05 | 1.17/0.61 |
| 2610528B01Rik | 0.66/0.34 | 15.64/12.46 | 17.59/9.98 | 49.88/28.21 | 12.65/6.94 | 31.04/17.35 | 15.34/8.41 |
| 4930535I16Rik | 10.92/6.52 | 0.98/0.25 | 11.07/4.56 | 1.28/0.02 | 2/0.36 | 17.05/7.07 | 18.12/7.59 |
| 5430416O09Rik | 0.44/0.78 | 0.21/0.5 | 22.22/19.97 | 1.13/0.87 | 1.01/0.79 | 3.05/2.55 | 1.3/1.03 |
| 9430015G10Rik | 0.98/1.38 | 0.12/0.35 | 7.93/6.85 | 34.35/29.18 | 26.31/22.64 | 20.37/17.21 | 12.22/10.32 |
| 9930104L06Rik | 0.4/0.68 | 0.4/0.68 | 0.43/0.25 | 2.83/2.26 | 2.36/1.9 | 8.44/7.02 | 1.86/1.44 |
| AA415398 | 0.6/0.16 | 0.38/0.01 | 8.93/4.29 | 3.07/1.11 | 8.47/3.97 | 20.5/10.11 | 17.32/8.52 |
| Agmat | 0.35/0.57 | 0.09/0.27 | 3.74/3.04 | 4.14/3.26 | 7.97/6.53 | 19.18/15.67 | 13.9/11.38 |
| Anp32b | 0.43/0.8 | 1.35/1.96 | 1.59/1.39 | 2.42/2.07 | 1.88/1.62 | 14.09/12.48 | 14.37/12.8 |
| BC057079 | 0.14/0.46 | 0.33/0.7 | 3.03/2.77 | 7.53/6.75 | 6.72/6.1 | 3.87/3.41 | 6.1/5.47 |
| Btf3l4 | 0.49/1.07 | 0.11/0.53 | 2.78/2.84 | 2.63/2.58 | 1.29/1.29 | 4.31/4.22 | 3.69/3.63 |
| C430048L16Rik | 1.28/2.16 | 0.75/1.43 | 0.23/0.25 | 1.2/1.17 | 2.54/2.53 | 3.67/3.59 | 1.08/1.06 |
| Cap1 | 3.57/6.8 | 3.03/5.89 | 0.2/0.5 | 1.47/1.99 | 0.48/0.81 | 2.88/3.7 | 3.74/4.77 |
| Casp8ap2 | 0.1/0.45 | 0.29/0.7 | 3.9/3.71 | 38.04/35.47 | 51.64/48.86 | 4.79/4.4 | 1.56/1.4 |
| Ccnl2 | 0.52/1.57 | 0.07/0.8 | 1.09/1.57 | 16.55/20.03 | 15.86/19.48 | 4.59/5.69 | 3.58/4.51 |
| Chd5 | 0.87/0.28 | 7.33/4.71 | 21.45/10.24 | 40.34/19.1 | 36.74/17.61 | 69.19/33.06 | 27.74/13.02 |
| Clcnkb | 1.03/1.24 | 1.08/1.3 | 12.27/9.73 | 1.13/0.67 | 0.44/0.15 | 8.62/6.54 | 4.45/3.29 |
| Col16a1 | 0.43/0.3 | 3.97/3.52 | 2.13/1.08 | 1.25/0.45 | 2.43/1.25 | 4.61/2.62 | 2.81/1.47 |
| Cyp4a31 | 7.9/17.22 | 5.85/13.02 | 0.04/0.56 | 0.8/1.62 | 1.47/2.64 | 7.54/11.38 | 0.96/1.86 |
| Dennd4c | 0.44/0.82 | 0.06/0.34 | 167.81/154.43 | 223.55/199.84 | 388.03/351.76 | 177.75/158.54 | 132.18/118.48 |
| Dnajc11 | 0.04/0.35 | 0.16/0.51 | 7.39/6.99 | 11.28/10.37 | 4.03/3.71 | 5.72/5.21 | 4.66/4.25 |
| Eif2b3 | 0.31/0.56 | 0.72/1.05 | 6.17/5.24 | 4.11/3.32 | 2.29/1.82 | 7.03/5.77 | 7.08/5.85 |
| Gja10 | 0.28/1.02 | 0.2/0.9 | 0.91/1.21 | 1.69/2.01 | 7.08/8.17 | 7.59/8.6 | 2.81/3.28 |
| Gng10 | 0.15/0.05 | 0.94/0.77 | 1.94/0.97 | 17.25/10.86 | 10.63/6.66 | 3.4/1.85 | 2.47/1.26 |

**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 |
| Gpr3 | 0.71/1.32 | 0.61/1.18 | 0.55/0.54 | 0.9/0.83 | 2.68/2.59 | 6.6/6.3 | 2.08/1.97 |
| Guca2b | 0.49/1.03 | 0.39/0.89 | 6.42/6.38 | 20.5/19.76 | 0.41/0.38 | 1.58/1.49 | 1.93/1.83 |
| Htr6 | 0.05/0.32 | 0.11/0.39 | 2.89/2.55 | 6.02/5.23 | 3.42/2.98 | 17.29/15.2 | 7.1/6.21 |
| Itgb3bp | 1.69/3.05 | 0.19/0.8 | 0.32/0.46 | 1.37/1.54 | 1.09/1.27 | 2.05/2.26 | 2.07/2.29 |
| Lrp8 | 1.39/1.05 | 0.33/0.14 | 1.94/0.85 | 3.72/1.88 | 8.57/4.92 | 8.29/4.66 | 6.82/3.79 |
| Mdn1 | 0.05/0.2 | 4.47/5.26 | 2.63/2.04 | 22.03/17.71 | 17.2/13.99 | 3.22/2.42 | 3.95/3.03 |
| Mrpl50 | 1.02/1.86 | 0.62/1.3 | 1.23/1.32 | 1.47/1.49 | 0.87/0.91 | 5.43/5.46 | 3.48/3.53 |
| Mysm1 | 0.25/0.63 | 0.1/0.43 | 1.13/1.03 | 5.56/5.09 | 9.49/8.87 | 1.59/1.4 | 0.57/0.46 |
| Nfx1 | 0.43/0.7 | 0.47/0.75 | 6.99/5.95 | 12.45/10.37 | 7.26/6.08 | 16.28/13.58 | 11.53/9.63 |
| Padi1 | 2.1/2.28 | 0.69/0.79 | 1.6/1.01 | 3.92/2.7 | 1.26/0.72 | 53.37/39.8 | 39.89/29.84 |
| Pnrc2 | 0.51/0.96 | 0.52/0.96 | 0.52/0.44 | 2.07/1.82 | 1.01/0.87 | 4.13/3.69 | 2.29/2.02 |
| Ppie | 0.2/0.42 | 0.02/0.21 | 8.3/7.04 | 25.22/21.02 | 12.55/10.53 | 12.31/10.15 | 9.68/7.99 |
| Ppp1r8 | 0.03/0.39 | 0.59/1.14 | 14.59/14.36 | 16.61/15.85 | 8.84/8.55 | 9.57/9.09 | 12.5/11.96 |
| Prpf4 | 0.29/0.99 | 1.69/3.16 | 2.13/2.53 | 2.49/2.83 | 2.09/2.44 | 2.16/2.46 | 2.69/3.06 |
| Psip1 | 0.7/1.66 | 1.75/3.31 | 0.03/0.18 | 6.21/7.02 | 4.28/4.95 | 0.41/0.56 | 0.73/0.93 |
| Rbm12b | 0.85/1.48 | 0.01/0.36 | 0.77/0.74 | 3.16/2.96 | 1.96/1.86 | 42.69/40.57 | 21.65/20.66 |
| Rere | 0.04/0.53 | 0.03/0.53 | 114.9/123.97 | 372.04/389.75 | 288.69/306.63 | 246.05/257.22 | 213.01/223.85 |
| Sit1 | 0.93/1.91 | 0.02/0.55 | 18.38/20.41 | 58.46/62.81 | 54.15/59.01 | 8.62/9.3 | 3.99/4.38 |
| Slc1a7 | 0.2/0.6 | 1.06/1.75 | 5.81/5.62 | 1.34/1.21 | 6.5/6.18 | 6.15/5.74 | 2.64/2.45 |
| Slc24a2 | 0.97/0.64 | 0.33/0.11 | 9.22/5.23 | 3.07/1.41 | 8.6/4.76 | 12.23/6.81 | 18.21/10.41 |
| Smpdl3b | 0.11/0.34 | 1.56/2.08 | 8.18/7.07 | 2.69/2.16 | 7.17/6.08 | 2.72/2.17 | 3.6/2.94 |
| Snip1 | 0.2/0.24 | 0.14/0.17 | 23.93/17.75 | 54.13/39.28 | 68.21/50.28 | 13.91/9.87 | 7.79/5.45 |
| Tle1 | 0.01/0.37 | 0.21/0.65 | 4.58/4.54 | 1.34/1.26 | 1.77/1.71 | 0.43/0.37 | 0.85/0.79 |
| Trim14 | 0.45/0.52 | 0.26/0.32 | 19.25/14.53 | 112.18/83.33 | 51.76/38.86 | 21.63/15.83 | 3.67/2.49 |
| Txndc12 | 2.21/2.33 | 0.22/0.26 | 10.57/7.78 | 4.87/3.33 | 1.62/0.96 | 7.28/5.08 | 6.21/4.33 |
| Ubxn11 | 4.39/4.71 | 0.2/0.27 | 12.07/9.12 | 21.95/16.25 | 12.9/9.6 | 9.9/7.17 | 4.79/3.37 |
| Usp1 | 0.72/1.35 | 0.1/0.51 | 0.77/0.78 | 5.14/4.97 | 3.1/3.04 | 1.59/1.51 | 1.81/1.74 |
| Wwp1 | 0.01/0.12 | 0.27/0.41 | 43.21/34.92 | 46.36/36.37 | 61.11/48.69 | 81.23/63.75 | 83.25/65.68 |
| (B) | | | | | | | |
| Masp2 | 13.13/11.07 | 3.8/3.1 | 9.4/5.49 | 23.91/14.1 | 38.75/23.43 | 27.31/16.13 | 20.68/12.18 |
| Rngtt | 1.03/1.48 | 1.25/1.75 | 1.26/1.02 | 7.58/6.44 | 4.49/3.83 | 2.87/2.35 | 2.3/1.87 |



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stem cell proliferation and differentiation [36]. Other hypotheses, plead in favour of a role as progenitor cells during inflammatory/repair processes [37]. Telocytes have recently been shown to act as progenitor cells in adulthood, being able to differentiate in cells like interstitial cells of Cajal, myofibroblasts and even in fibroblasts [38]. Also, during morphogenesis, it might be possible to behave like inductors/regulators of differentiation for parenchymal cells [38, 39].

Our previous studies identified characters and patterns of TCs-specific or TCs-dominated gene profiles in chromosome 1, 2, 3, 17 and 18 using global comparison between TCs and other cell types found in the mouse lung tissue [24–26]. To further study the characters and patterns of TC-specific or TC-dominated gene expression profiles, we currently performed a detailed analysis for chromosome 4, and investigated the characteristic gene networks and potential functional association using bioinformatics tools. Pulmonary TCs in cell culture, harvested on day 5 (TC5) and on day 10 (TC10) were compared with mesenchymal stem cells (MSCs), fibroblasts (Fbs), alveolar type II cells (ATII), airway basal cells (ABCs), proximal airway cells (PACs), $CD8^+$ T cells from bronchial lymph nodes (T-BL) and $CD8^+$ T cells from lung (T-L). Key functional genes were identified with the aid of the reference library of the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus database.

# Material and methods

## Isolation and culture

Telocytes were isolated from the lung tissues of mice, primary cultured in a concentration of $1 \times 10^5$ cells/cm$^2$, and harvested on days 5 (TC5) and on days 10 (TC10), as previously described [28]. RNA isolation, preparation, labelling and hybridization for DNA microarray (The Mouse $4 \times 44$K Gene Expression Array; Agilent, Shanghai, China), we gained about 39,000+ mouse genes and transcripts represented with public domain annotations, 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

The gene expression profiles of pulmonary TC5 and TC10, Fbs and MSCs were collected from a previous study [28]. Gene expression profiles for ATII, ABCs, PACs, T-BL and T-L were obtained from the NCBI Gene Expression Omnibus database (GSE6846 [40], GSE27379 [41], GSE28651 [42]). The microarray was composed of 45,101 probes. First, we eliminated the probe sets without corresponding official symbol, leaving 39,417 probes and 21,680 genes.

## Identification of differentially expressed genes

The identification of differentially expressed genes was done as the method described in our previous study [24]. Briefly, after the acquired data normalized with quantile normalization, the probe level (*_norm_RMA.pair) files and gene level (*_RMA.calls) files were generated. Subsequent data processing was further analysed with Agilent GeneSpring GX software (version 11.5.1) software package and differentially expressed genes were identified through fold change filtering. Hierarchically clustered was performed with the Agilent GeneSpring GX software (version 11.5.1). Gene Ontology analysis and String Network analyses were performed with the standard enrichment computation method to uncover the relevance among variant proteins expressed by variant genes.

Eight-five per cent of mouse genes (approx. 20,000–25,000 genes) is very similar with the human genes. This study investigates gene expression profiles of chromosome 4 in different lung cell populations to search for TC-specific regulated genes. Up- or down-regulated folds of TC-genes were calculated by comparison with other cells and subtracted its own multiple of TC, after the average of gene expression in each cells.

# Results

Table 1 presents the global analysis of chromosome 4 genes in lung TCs. We found that 17 genes were up-regulated and 56 genes were down-regulated in chromosome 4 of TCs. Among the up-regulated genes, 12 genes (1700009N14Rik, Aurkaip1, Fam176b, Fbxo6, Hspg2, Macf1, Mast2, Otud3, Plekhm2, Tm2d1, Tmem59, Zcchc17) were overexpressed between zero and onefold (Table 1A), 4 genes (Akap2, Gpr153, Sdc3, Tbc1d2) were up-regulated between one and fourfold (Table 1B) and one gene, Svep1, was overexpressed over fourfold, in both TC D5 and TC D10, as compared with other cells (Table 1C). The genes highly expressed in TC5 were similar with

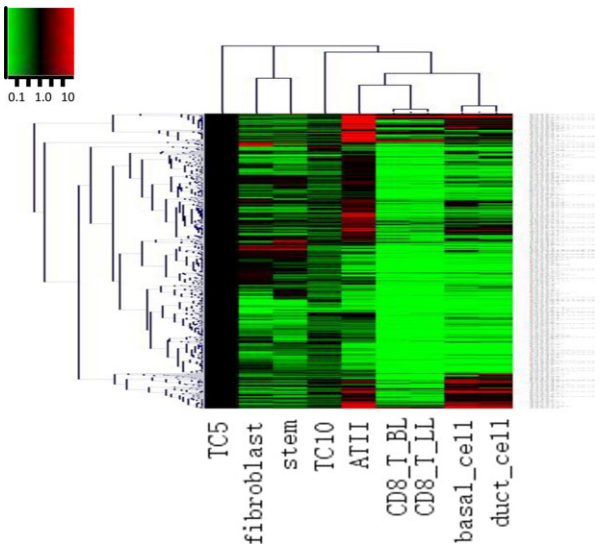


**Fig. 2** Hierarchical cluster analysis of the differentially expressed genes on chromosomes 4 among telocytes (TCs), mesenchymal stem cells (MSCs), fibroblasts (Fbs), lymphocytes from lungs (T-LL) and from bronchial lymph nodes (T-BL), alveolar type II cells (ATII), proximal airway cells (PAC) and airway basal cells (ABC). The differences are described by fold changes and the expression value of genes in TC5 are controls.

those in TC10 and different from MSCs, Fbs, ATII, ABCs, PACs, T-BL or T-L. The direct (physical) and indirect (functional) relationships, including associations, of these genes were analysed by String Network analysis and the interactions and potential functional links between these genes are displayed in Figure 1.

Among the down-regulated genes, 54 genes were expressed zero and onefold in TCs than in other cells (Table 2A) and 2 genes, Masp2 and Rngtt (Table 2B) were one to fourfold lower in TCs than in other cells.

Details of up- or down gene variations of chromosome 4 were listed in Table S1. The hierarchical cluster plot of the differentially expressed genes illustrated as coded colours (Fig. 2) clearly shows that TCs are less related with the other cells.

Table 3 presents a set of genes were found specifically up- or down-regulated in pulmonary TCs, as compared with Fbs, MSCs, ATII, ABCs, PACs, T-BL or T-L respectively. A set of genes up- or down-regulated more than onefold in TC5 were 233 or 49, 249 or 46, 78 or 408, 123 or 378, 125 or 375, whereas the genes up- or down-regulated more than onefold in TC10 were 163 or 92, 164 or 94, 71 or 410, 133 or 372 and 123 or 368.

## Discussion

Mouse genome is extremely valuable for research since the human and mouse genomes are remarkably similar not only in the structure of their chromosomes but also at the level of DNA sequence. Chromosome 4 represents more than 6 per cent of the total DNA in cells and likely contains 1000–1100 genes [43]. In humans, many genetic disorders stemming from chromosome 4 genes are described, *e.g.* achondroplasia, facioscapulohumeral muscular dystrophy, Huntington's disease, to name but a few. Mouse chromosome 4 has a total number of genes of 2430 which encode a number of 1270 proteins.

This study was dedicated to the global analysis of chromosome 4 genes of lung TCs compared with Fbs, MSCs, ATII, ABCs, PACs, T-BL and T-L of which 720 genes were measured by bioinformatics tools.

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

| Compared pairs | Up >0 | Up >1 | Up >4 | Down >0 | Down >1 | Down >4 |
|---|---|---|---|---|---|---|
| TC10 *versus* fibroblast | 367 | 163 | 53 | 353 | 92 | 23 |
| TC5 *versus* fibroblast | 510 | 233 | 69 | 210 | 49 | 12 |
| TCs *versus* fibroblast | 354 | 149 | 45 | 197 | 42 | 12 |
| TC10 *versus* stem | 425 | 164 | 43 | 295 | 94 | 19 |
| TC5 *versus* stem | 551 | 249 | 59 | 169 | 46 | 11 |
| TCs *versus* stem | 419 | 144 | 33 | 163 | 45 | 11 |
| TC10 *versus* ATII | 171 | 71 | 17 | 549 | 410 | 229 |
| TC5 *versus* ATII | 174 | 78 | 20 | 546 | 408 | 225 |
| TCs *versus* ATII | 147 | 61 | 12 | 522 | 383 | 201 |
| TC10 *versus* CD8BL | 225 | 133 | 60 | 495 | 372 | 201 |
| TC5 *versus* CD8BL | 229 | 123 | 65 | 491 | 378 | 205 |
| TCs *versus* CD8BL | 204 | 110 | 52 | 470 | 346 | 181 |
| TC10 *versus* CD8LL | 208 | 123 | 56 | 512 | 368 | 194 |
| TC5 *versus* CD8LL | 217 | 125 | 59 | 503 | 375 | 208 |
| TCs *versus* CD8LL | 185 | 107 | 50 | 480 | 342 | 178 |
| TC10 *versus* basal cell | 128 | 57 | 16 | 592 | 497 | 308 |
| TC5 *versus* basal cell | 131 | 57 | 20 | 589 | 499 | 316 |
| TCs *versus* basal cell | 111 | 44 | 13 | 572 | 472 | 287 |
| TC10 *versus* duct cell | 156 | 85 | 32 | 564 | 464 | 267 |
| TC5 *versus* duct cell | 155 | 82 | 33 | 565 | 461 | 271 |
| TCs *versus* duct cell | 144 | 69 | 27 | 553 | 436 | 239 |



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We found that 17 genes were up-regulated and 56 genes were down-regulated in chromosome 4 of TCs as compared with other cell types.

Four genes, Akap2, Gpr153, Sdc3, Tbc1d2, were found to be more than onefold up-regulated in TCs as compared with other cell types. Akap2 (A-kinase (PRKA) anchor protein 2) gene encodes a protein involved in signalling pathways (G Protein signalling pathways and signal transduction PKA) and in modulation of actin filament dynamics [44, 45]. Gpr153 (G protein-coupled receptor 153) gene encodes an orphan receptor with elusive functions [46]. Sdc3 (syndecan 3) gene encodes a cell surface proteoglycan (heparan sulphate) involved in the organization of cell shape by affecting the actin cytoskeleton, possibly by transferring signals from the cell surface which seems to have a selectively pro-inflammatory function [47]. Tbc1d2 (TBC1 domain family member 2A) gene encodes a protein found in cell junctions and cytoplasmic vesicles and is apparently involved in positive regulation of GTPase activity and vesicle trafficking [48]. Svep1 (sushi, von Willebrand factor type A, EGF and pentraxin domain containing 1) gene encodes a protein involved in cell adhesion [49]. Small GTP ases regulate intracellular trafficking (budding, transport and fusion of vesicles) [50] and also intervene in cytoskeletal remodelling, migration and adhesion events [51]. Therefore, all these up-regulated genes encode proteins involved in cell signalling pathways and cytoskeleton organization and imply that TCs could integrate signals and auto-regulate its own fate, integrating autophagy with endocytic trafficking [52]. Moreover, since there are no data regarding the involvement of these four genes in any pulmonary pathology, the precise significance of those up-regulated genes in TCs still remains unclear.

Among the down-expressed genes in TCs, Masp2 (mannan-binding lectin serine peptidase 2) and Rngtt (RNA guanylyltransferase and 5′-phosphatase) genes were one to fourfold lower comparative with other cells.

## Conclusion

Our data showed, by global analyses, that 73 TCs-specific or dominant genes in chromosome 4 are different from other lung tissue resident cells or immune migrated cells. Current findings are supportive for our previous studies of TC-specific gene profiles and potential functional correlations, pointing out the same suggested roles for TCs [24–26]. Thus, TCs appear once more to have a significant role in cellular signalling, regulation of tissue inflammation, and cell expansion and movement.

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## Conflicts of interest

The authors declare that they have no competing interests.

## Supporting information

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

**Figure S1** Details of the selected core network genes in telocytes isolated from the mouse lung and cultured for 10 days in chromosome 4.

**Figure S2** Details of the selected core network genes in telocytes isolated from the mouse lung and cultured for 5 days in chromosome 4.

**Figure S3** Details of the selected core network genes in mouse mesenchymal stem cells in chromosome 4.

**Figure S4** Details of the selected core network genes in mouse fibroblasts in chromosome 4.

**Figure S5** Details of the selected core network genes in mouse alveolar type II cells in chromosome 4.

**Figure S6** Details of the selected core network genes in mouse proximal airway cells in chromosome 4.

**Figure S7** Details of the selected core network genes in mouse airway basal cells in chromosome 4.

**Figure S8** Details of the selected core network genes in mouse $CD8^+$ T cells come from bronchial lymph nodes in chromosome 4.

**Figure S9** Details of the selected core network genes in mouse $CD8^+$ T cells from lung in chromosome 4.

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