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

Dongli Song a, Dragos Cretoiub, c, Minghuan Zheng a, Mengjia Qian a, Miaomiao Zhang a, Sanda M. Cretoiub, c, *, Luolan Chen d, Hao Fang e, *, Laurentiu M. Popescub b, c, †, Xiangdong Wanga, *
a Zhongshan Hospital, Fudan University Center for Clinical Bioinformatics, Shanghai Institute of Clinical Bioinformatics, Shanghai, China
b Division of Cellular and Molecular Biology and Histology, Department of Morphological Sciences, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
c Victor Babes /C223 National Institute of Pathology, Bucharest, Romania
d State Key Lab of Systems Biology, Chinese Academy of Science, Shanghai, China
e Department of Anesthesiology, Zhongshan Hospital and Jinhua Hospital of Fudan University, Shanghai, China

Received: September 24, 2015; Accepted: October 30, 2015

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 (Fs), 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...

†Deceased August 3, 2015.
*Correspondence to: Xiangdong WANG M.D., Ph.D.
E-mail: xiangdong.wang@clintransmed.org
Sanda M. CRETOIU, MD., Ph.D.
Email: sanda@cretoiu.ro
Hao FANG, M.D., Ph.D.
Email: fang.hao@az-hospital.sh.cn

© 2015 The Authors. Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
doi: 10.1111/jcmm.12746
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 | 00 | >1 | >4 |
|----------------------------------|----|----|----|
| TC5 versus others                | 51 | 13 | 3  |
| TC10 versus others               | 34 | 8  | 1  |
| TCs versus others                | 17 | 5  | 1  |

| Gene symbol | Folds (TC5 versus others/TC10 versus others) |
|-------------|---------------------------------------------|
|             | 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 CD6+ 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   |

| Gene symbol          | Folds (TC5 versus others/TC10 versus others) |
|----------------------|-----------------------------------------------|
|                      | 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 |
| 4930353I16Rik        | 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 |
| 5430416009Rik        | 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        | 0.98/0.38  | 0.12/0.35 | 7.93/6.85 | 34.35/29.18 | 26.31/22.64 | 20.37/17.21 | 12.22/10.32 |
| 9930414L06Rik        | 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 |
| Btf3I4               | 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 |
| Ccni2                | 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  | 5.8/15.02 | 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 |

© 2015 The Authors.
Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.
| Gene symbol | 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  |
| Ilgb3bp     | 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|
| Slt1        | 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|
| Smdd3b      | 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|13.91/8.67  | 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 |
| Txn2c12     | 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)

| Gene symbol | Fibroblast | Stem | ATII | CD8_T_BL | CD8_T_LL | Basal_cell | Duct_cell |
|-------------|------------|------|------|----------|----------|------------|-----------|
| 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  |

© 2015 The Authors.
Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.
stem cell proliferation and differentiation [36]. Other hypotheses, plead in favour of a role as progenitor cells during inflammatory/re-
pair 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 inducers/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 charac-
ters 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 air-
way 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 Biotech-
nology 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 × 10⁵ cells/cm² and harvested on days 5 (TC5) and
on days 10 (TC10), as previously described [28]. RNA isolation, prepara-
tion, labelling and hybridization for DNA microarray (The Mouse 4 × 44K
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 pro-
files 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 gener-
ated. Subsequent data processing was further analysed with Agilent Gene-
Spring GX software (version 11.5.1) software package and differentially
expressed genes were identified through fold change filtering. Hierarchi-
cally 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 expres-
sion 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, Fbno6, 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
doublenfold (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 air-
way 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 one-fold in TCs than in other cells (Table 2A) and 2 genes, Masp2 and Rngtt (Table 2B) were one to four-fold 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 one-fold 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 one-fold 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.

| 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 |
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 GTPases 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.

Acknowledgements

This work was partially supported (for DC) by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/141531. It was also funded by grants of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number 82/2012 and 194/2014. The work was supported by Zhongshan Distinguished Professor Grant (XDW), the National Nature Science Foundation of China (91230204, 81270099, 81320108001, 81270131, 81300010), the Shanghai Committee of Science and Technology (12JC1402200, 1243190207, 11410708600, 14431905100), Operation funding of Shanghai Institute of Clinical Bioinformatics, and Ministry of Education, Academic Special Science and Research Foundation for PhD Education (20130071110043).

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.

References

1. Popescu LM, Faussone-Pellegrini MS. TEOCYTES - a case of serendipity: the winding way from Interstitial Cells of Cajal (ICC), via Interstitial Cajal-Like Cells (ICLC) to TEOCYTES. J Cell Mol Med. 2010; 14: 729-40.
Telocytes have progenitor capacity and are a source of alphaSMA+ cells during repair. Histol Histopathol. 2015; 30: 615–27.

19. Roatesi I, Radu BM, Cretoiu D, et al. Uterine telocytes: a review of current knowledge. Biol Reprod. 2015; 93: 10.

20. Ullah S, Yang P, Zhang L, et al. Identification and characterization of telocytes in the uterus of the oviduct in the Chinese soft-shelled turtle, Pelodiscus sinensis: TEM evidence. J Cell Mol Med. 2014; 18: 2385–92.

21. Cantarero I, Luesma MJ, Alvarez-Dotu JM, et al. Transmission electron microscopy as key technique for the characterization of telocytes. Curr Stem Cell Res Ther. 2015; doi: 10.2174/1574888X106661501131134.

22. Zhou Q, Wei L, Zhong C, et al. Cardiac telocytes are double positive for CD34/PDGFR-alpha. J Cell Mol Med. 2015; 19: 2036–42.

23. Zheng Y, Cretoiu D, Yan G, et al. Protein profiling of human lung telocytes and microvascular endothelial cells using iTRAQ quantitative proteomics. J Cell Mol Med. 2014; 18: 1035–59.

24. Sun X, Zheng M, Zhang M, et al. Differences in the expression of chromosome 1 genes between lung telocytes and other cells: Mesenchymal stem cells, fibroblasts, alveolar type II cells, airway epithelial cells and lymphocytes. J Cell Mol Med. 2014; 18: 801–10.

25. Zheng M, Sun X, Zhang M, et al. Variations of chromosomes 2 and 3 gene expression profiles among pulmonary telocytes, pneumocytes, airway cells, mesenchymal stem cells and lymphocytes. J Cell Mol Med. 2014; 18: 2044–60.

26. Wang J, Ye L, Jin M, et al. Global analyses of Chromosome 17 and 18 genes of lung telocytes compared with mesenchymal stem cells, fibroblasts, alveolar type II cells, airway epithelial cells, and lymphocytes. Biol Direct. 2015; 10: 9.

27. Cismasu VB, Radu E, Popescu LM. miR-193 expression differentiates telocytes from other stromal cells. J Cell Mol Med. 2011; 15: 1071–4.

28. Zheng Y, Zhang M, Qian M, et al. Genetic comparison of mouse lung telocytes with mesenchymal stem cells and fibroblasts. J Cell Mol Med. 2013; 17: 567–77.

29. Cismasu VB, Popescu LM. Telocytes transfer extracellular vesicles loaded with microRNAs to stem cells. J Cell Mol Med. 2015; 19: 351–8.

30. Sheng J, Shim W, Lu J, et al. Electrophysiology of human cardiac atrial and ventricular telocytes. J Cell Mol Med. 2014; 18: 355–62.

31. Cretoiu SM, Radu BM, Banciu A, et al. Isolated human uterine telocytes: immunocytochemistry and electrophysiology of T-type calcium channels. Histocem Cell Biol. 2015; 143: 83–94.
epithelial transition by PARAXIS during somitogenesis. *Dev Dyn.* 2013; 242: 1332–44.

45. Papin J, Subramaniam S. Bioinformatics and cellular signaling. *Curr Opin Biotechnol.* 2004; 15: 78–81.

46. Sreedharan S, Almen MS, Carlini VP, et al. The G protein coupled receptor Gpr153 shares common evolutionary origin with Gpr162 and is highly expressed in central regions including the thalamus, cerebellum and the arcuate nucleus. *FEBS J.* 2011; 278: 4881–94.

47. Kehoe O, Kalia N, King S, et al. Syndecan-3 is selectively pro-inflammatory in the joint and contributes to antigen-induced arthritis in mice. *Arthritis Res Ther.* 2014; 16: R148.

48. Toyofuku T, Morimoto K, Sasawatari S, et al. Leucine-rich repeat kinase 1 regulates autophagy through turning on TBC1D2-dependent Rab7 inactivation. *Mol Cell Biol.* 2015; 35: 3044–58.

49. Gilges D, Vinit MA, Callebaut I, et al. Polydom: a secreted protein with pentraxin, complement control protein, epidermal growth factor and von Willebrand factor A domains. *Biochem J.* 2000; 352: 49–59.

50. Longatti A, Lamb CA, Razi M, et al. TBC1D14 regulates autophagosome formation via Rab11- and ULK1-positive recycling endosomes. *J Cell Biol.* 2012; 197: 659–75.

51. Mack NA, Whalley HJ, Castillo-Lluva S, et al. The diverse roles of Rac signaling in tumorigenesis. *Cell Cycle.* 2011; 10: 1571–81.

52. Carroll B, Mohd-Naim N, Maximiano F, et al. The TBC/RabGAP Armus coordinates Rac1 and Rab7 functions during autophagy. *Dev Cell.* 2013; 25: 15–28.