Genetic comparison of mouse lung telocytes with mesenchymal stem cells and fibroblasts

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Abstract

Telocytes (TCs) are interstitial cells with telopodes – very long prolongations that establish intercellular contacts with various types of cells. Telocytes have been found in many organs and various species and have been characterized ultrastructurally, immunophenotypically and electrophysiologically (www.telocytes.com). Telocytes are distributed through organ stroma forming a three-dimensional network in close contacts with blood vessels, nerve bundles and cells of the local immune system. Moreover, it has been shown that TCs express a broad range of microRNAs, such as pro-angiogenic and stromal-specific miRs. In this study, the gene expression profile of murine lung TCs is compared with other differentiated interstitial cells (fibroblasts) and with stromal stem/progenitor cells. More than 2000 and 4000 genes were found up- or down-regulated, respectively, in TCs as compared with either MSCs or fibroblasts. Several components or regulators of the vascular basement membrane are highly expressed in TCs, such as Nidogen, Collagen type IV and Tissue Inhibitor of Metalloproteinase 3 (TIMP3). Given that TCs locate in close vicinity of small vessels and capillaries, the data suggest the implication of TCs in vascular branching. Telocytes express also matrix metalloproteases Mmp3 and Mmp10, and thus could regulate extracellular matrix during vascular branching and de novo vessel formation. In conclusion, our data show that TCs are not fibroblasts, as the ultrastructure, immunocytochemistry and microRNA assay previously indicated. Gene expression profile demonstrates that TCs are functionally distinct interstitial cells with specific roles in cell signalling, tissue remodelling and angiogenesis.

Keywords: telocytes • mesenchymal stem cells • fibroblasts • gene expression profile • interstitial cells • stroma
• connective tissue • lung

Introduction

Recent electron microscopic studies have identified telocytes (TCs), a distinct type of interstitial cells, in many cavity and non-cavity organs [1–20]. Telocytes are defined by their very long prolongations – called telopodes (Tps; generally, 2–3/cell; length of up to hundreds of μm) – which emerge from a relatively small cellular body. It has been shown that TCs form a 3D network through the organ interstitium surrounding organ-specific structures, blood capillaries, immune cells and nerve endings. As a specific functional property, TCs are key players in intercellular signalling, at both short and long distance. Thus, the long Tps establish direct contacts (junctions) with neighbouring cells and contribute to the (directional) transport of long-range signals driven by TCs [21]. Local (paracrine) signalling of TCs is achieved by shedding vesicles [8, 20, 22].

The ultrastructural portrait of TCs was recently complemented with the immunophenotypical and electrophysiological characterization and the specific microRNA expression signature [20, 22, 23]. However, the gene expression profile for this type of cells has not been reported yet. Prompted by these studies, we sought to compare

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| Compared pairs/fold up-regulated | >2 | >10 | >30 | >100 |
|----------------------------------|----|-----|-----|------|
| TCs vs. MSCs                     | 2921 | 500 | 174 | 44 |
| TCs vs. Fbs                      | 3173 | 661 | 295 | 85 |

(A) Genes up-regulated more than 100-folds in telocytes (TCs) as compared with mesenchymal stem cells (MSCs) and fibroblasts (Fbs)

| Genes vs. Fbs | Genes | Folds | Genes | Folds | Genes | Folds |
|---------------|-------|-------|-------|-------|-------|-------|
| Ctgf          | 6151  | Tm4sf1| 217   | Sprr1a| 2971  |
| Sprr1a        | 2593  | Sulf1 | 212   | Cck   | 1242  |
| Myl9          | 1668  | Chi3l3| 204   | Wtcd2 | 551   |
| Tagln         | 1545  | Vopp1 | 198   | Serinc2| 527  |
| Cck           | 1206  | Mfas1 | 198   | Chi3l3| 369   |
| Nid1          | 1143  | Myh14 | 194   | Gipr1 | 355   |
| Sdpr          | 1004  | Gpn   | 185   | Gppk1 | 284   |
| Crlf1         | 942   | Dsp   | 182   | Trf   | 259   |
| Anxa8         | 799   | Mmp10 | 177   | Myh14 | 246   |
| Cdc9          | 718   | Khdrbs3| 175  | Gsta3 | 244   |
| Wtcd2         | 660   | Atp1b1| 174   | Gpr56 | 222   |
| Sox4          | 501   | Papss2| 171   | Cyp61 | 210   |
| Dhcr24        | 496   | Gprc5c | 168   | Gprc5c| 204   |
| Timp3         | 445   | Prt2c1| 165   | Tjp2  | 202   |
| Trim44        | 410   | Gas6  | 165   | Atp1b1| 194   |
| Serpine1      | 376   | Rbp1  | 161   | Lz1   | 181   |
| Marcksl1      | 356   | Foxq1 | 156   | Aldh1a2| 167   |
| Hs6st2        | 335   | Cbic  | 149   | Gpx2  | 152   |
| Gpr56         | 331   | Aldh1a2| 149  | Dsp   | 150   |
| Nrg1          | 327   | Cdh2  | 136   | Khdrbs3| 146   |
| Trf           | 306   | Crc1  | 133   | Acp5  | 143   |
| Bmp4          | 298   | Mmp3  | 131   | Rbp1  | 141   |
| Cyba          | 293   | Gpx2  | 126   | Gprc5c| 137   |
| Thy1          | 280   | Gprc5c| 125   | Clu   | 131   |
| Lnc32         | 278   | Fstl1 | 125   | Tmc4  | 128   |
| Rab34         | 269   | Lama2 | 120   | Acp5  | 114   |
| Dpysl3        | 263   | Tjp2  | 117   | Epb4.114b| 114   |
| Decr1         | 256   | Igfl9 | 116   | Mfsd6 | 109   |
Table 1. Continued

TCs vs. Fbs

| Gene   | Folds | Gene   | Folds | Gene   | Folds |
|--------|-------|--------|-------|--------|-------|
| Gsta3  | 240   | Bcr    | 110   | Cblc   | 107   |
| Evl    | 237   | Lce1i  | 108   | Acta1  | 105   |
| Tmem45a| 233   | Rnf128 | 107   | F11r   | 101   |
| Aldh1a1| 225   | Klh13  | 106   |        |       |
| Fzd1   | 223   | Echdc2 | 103   |        |       |
| Cryab  | 219   | Trim16 | 101   |        |       |
| Lyz1   | 217   |        |       |        |       |

(B) Genes up-regulated between 30- and 100-folds in telocytes (TCs) as compared with mesenchymal stem cells (MSCs) and fibroblasts (Fbs)

TCs vs. Fbs

| Gene   | Folds | Gene   | Folds | Gene   | Folds | Gene   | Folds |
|--------|-------|--------|-------|--------|-------|--------|-------|
| Wnt11  | 100   | Letmd1 | 47    | Pdgfb  | 97    | Pcgf5  | 36    |
| F3     | 98    | Rpgrpi1| 46    | Aldh1a1| 93    | Fxyd3  | 36    |
| Pdgfb  | 97    | Trp53i11| 46   | Itpa    | 90    | Ctsk   | 35    |
| Fxyd6  | 94    | Hebp2  | 46    | Fxyd6  | 87    | Ctgf   | 35    |
| Fhl2   | 94    | Dkk3   | 45    | Tns1   | 79    | Ctb    | 35    |
| Nox4   | 93    | Cryab  | 45    | St14   | 78    | Lama5  | 35    |
| Ptprf  | 93    | Pvr13  | 44    | Lce1i  | 78    | Evpl   | 34    |
| Tgfbi11| 93    | P2rx2  | 44    | Grip1  | 77    | Col4a6 | 34    |
| Ddah1  | 92    | A2bp1  | 43    | S100a16| 76    | Chst4  | 34    |
| Cd99   | 92    | Cyba   | 43    | Klh13  | 74    | Apoe   | 33    |
| Irx1   | 87    | Cyrb1  | 42    | Tnk1   | 74    | Pik3r6 | 33    |
| Pdlim1 | 86    | Cobl   | 42    | Mmrn2  | 74    | Panx1  | 33    |
| Epb4.113| 86    | Pdlim3 | 41    | Rpgrpi1| 72    | Rnu1b6 | 33    |
| Tuft1  | 86    | Map3k9 | 41    | Gsta3  | 71    | Nppb   | 33    |
| Msln   | 83    | Tlr13  | 41    | Endod1 | 71    | Sema6a | 33    |
| Panx1  | 83    | Tjp3   | 41    | Scrm1a | 69    | Serpinb6b| 33   |
| Clic5  | 83    | Grh12  | 41    | Tcstd2 | 69    | Apoc2  | 32    |
| Ggh    | 83    | Sdcbp2 | 41    | Mboat1 | 68    | Villa  | 32    |
| Bst1   | 79    | Cd14   | 41    | Gas6   | 67    | Irx1   | 31    |
| Mansc1 | 79    | Krt17  | 41    | Dapk2  | 66    | Isyna1 | 30    |
| Sico3a1| 78    | Loxl2  | 40    | Gpsf3l | 65    | Map3k9 | 30    |
| Gene       | Folds |
|------------|-------|
| Tnfsf15    | 78    |
| Il6        | 78    |
| Saa3       | 77    |
| Fgd3       | 77    |
| Ecndc2     | 77    |
| Mapk13     | 75    |
| Tnfrsf11b  | 75    |
| Basp1      | 70    |
| Slc4a11    | 70    |
| Bst1       | 69    |
| F3         | 69    |
| Ubqin2     | 69    |
| Adam8      | 68    |
| Parp8      | 67    |
| Sox4       | 67    |
| Egfl7      | 66    |
| Gsta3      | 64    |
| Tnk1       | 64    |
| Fzd2       | 64    |
| Gpm6b      | 63    |
| Cgn        | 62    |
| Unc13b     | 61    |
| Celsr1     | 61    |
| Mmrm2      | 61    |
| Dok2       | 61    |
| Tpm2       | 60    |
| Ppfibp2    | 60    |
| Npr3       | 60    |
| Cpsf3I     | 59    |
| Peg13      | 59    |
| Arhgef16   | 59    |
| Lass3      | 58    |

**Table 1.** Continued

| Gene       | Folds |
|------------|-------|
| Cald1      | 40    |
| Brsk1      | 40    |
| Ppp1r9a    | 40    |
| Stxbp2     | 39    |
| Rab25      | 39    |
| Stfa3      | 39    |
| Cald1      | 39    |
| Brsk1      | 39    |
| Lmo7       | 38    |
| Timp1      | 38    |
| Id1        | 38    |
| Rnf130     | 37    |
| Serping1   | 37    |
| Csf2rb     | 37    |
| Olfr1383   | 37    |
| Sulf2      | 37    |
| Nhs1l      | 37    |
| Itm2a      | 37    |
| Slamf9     | 37    |
| Caznb3     | 36    |
| Spint1     | 36    |
| Tubal1a    | 36    |
| Rgs17      | 36    |
| Col4a6     | 36    |
| Tpm1       | 36    |
| Scmn1a     | 35    |
| Sirpb1a    | 35    |
| Col4a6     | 36    |
| Tpm1       | 36    |
| Scmn1a     | 35    |
| Sirpb1a    | 35    |
| Tja3       | 35    |
| Clic3      | 35    |
| Klf13      | 35    |
| Lrc33      | 35    |
| Gprc5a     | 35    |
| Abcc3      | 35    |

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| Gene     | Folds | Gene     | Folds | Gene     | Folds | Gene     | Folds |
|----------|-------|----------|-------|----------|-------|----------|-------|
| Dapk2    | 58    | Sgk1     | 35    | Psmg2    | 47    |          |       |
| Plac9    | 58    | Ankrd1   | 34    | Col4a4   | 46    |          |       |
| Msrb2    | 58    | Mid1ip1  | 34    | Csf2rb2  | 45    |          |       |
| Ckb      | 57    | Coro1a   | 34    | Tmem88   | 45    |          |       |
| Fam83h   | 57    | Cd248    | 34    | Cd97     | 45    |          |       |
| Vcan     | 56    | Acta1    | 34    | Ppl      | 45    |          |       |
| Acp5     | 56    | Inadl    | 33    | P2rx2    | 44    |          |       |
| Csf1r    | 56    | Sesn3    | 33    | A2bp1    | 43    |          |       |
| Ap1s3    | 56    | Evpl     | 33    | Akr1c13  | 43    |          |       |
| Ptx3     | 56    | C3       | 33    | St6gal1  | 42    |          |       |
| Trmc4    | 56    | Tpm2     | 33    | Efnb1    | 41    |          |       |
| Rpgrip1  | 55    | Pila     | 33    | Dok2     | 41    |          |       |
| Ctsw     | 55    | H19      | 33    | Adam8    | 41    |          |       |
| Wwec1    | 54    | Ptkb3    | 32    | Clic5    | 41    |          |       |
| Glip1    | 54    | Zfhx3    | 32    | Sh3bgr   | 40    |          |       |
| Hes6     | 54    | Fcer1g   | 32    | Fgd3     | 39    |          |       |
| Tacstd2  | 54    | Stab1    | 32    | Csf2rb   | 39    |          |       |
| Nsd1     | 54    | Col1a2   | 32    | Olfr1383 | 39    |          |       |
| Cyb561   | 53    | Igfbp2   | 31    | H19      | 39    |          |       |
| Fcgr2b   | 53    | Vcam1    | 31    | Sirp1b1a | 39    |          |       |
| Cdc42ep5 | 53    | Chpfl2   | 31    | Fcer1g   | 38    |          |       |
| Mdfi     | 52    | Npnb     | 31    | Sdc39a4  | 38    |          |       |
| Gaintl4  | 52    | Ccl27a   | 31    | Fcgr4    | 38    |          |       |
| Anxa8    | 52    | Ccl2     | 31    | Sh3bgr   | 38    |          |       |
| Plcg2    | 52    | Tnafip3  | 31    | Sdc22a18 | 38    |          |       |
| Col4a4   | 51    | Fnbp11   | 31    | Alcam    | 38    |          |       |
| Acp5     | 50    | Marveld3 | 31    | Stfa3    | 38    |          |       |
| Btg3     | 49    | Spin2    | 30    | Pfibbp2  | 37    |          |       |
| Ltbp2    | 48    | Sh3bgr   | 30    | Clic3    | 37    |          |       |
| Cd93     | 47    | Adams9   | 30    | Csf1r    | 37    |          |       |
| Gadd45b  | 47    | Abcc3    | 30    | Spin2    | 36    |          |       |
| Afap112  | 47    | Lcp1     | 30    | Lamc2    | 36    |          |       |
murine lung TCs with mesenchymal stem cells (MSCs) and fibroblasts (Fbs) to identify the genes which are specifically regulated in TCs. We choose lung TCs as these are well-characterized ultrastructurally and immunohistochemically in situ and in vitro [4, 5, 11, 16, 17].

**Method and Materials**

**Cell lines and tissue sampling**

Mouse colonies were maintained in Animal Research Center of Fudan University, Shanghai, China. Lung samples were obtained from 20 to 25 g male BABL/c mice, 4–6 weeks of age. The mice were killed with an overdose of anaesthetic and the lungs were harvested for the isolation of TCs. The animal study was approved by the Ethic Committee for Animal Care and Use, Fudan University. Mesenchymal stem cells and fibroblast cell lines were obtained from Sciencell Research Laboratories (Cat. no. M7500-57, Carlsbad, CA, USA) and from Chinese Academy of Science (Cat. no. GNM28, Shanghai, China) respectively.

**Isolation and primary culture of telocytes from lung tissues**

Lung tissues were cut into small pieces and harvested under sterile conditions and collected into sterile tubes containing Dulbecco’s Modified

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**Table 2. Summary of genes less expressed in TCs, as compared with mesenchymal stem cells (MSCs) and fibroblasts (Fbs)**

| Compared pairs/fold down-regulated | >2 | >10 | >30 | >100 |
|-----------------------------------|----|-----|-----|------|
| TCs vs. MSCs                      | 4365 | 175 | 32  | 5    |
| TCs vs. Fbs                       | 5451 | 326 | 63  | 16   |

(A) Genes down-regulated more than 100-folds in telocytes (TCs) as compared with mesenchymal stem cells (MSCs) and fibroblasts (Fbs)

**Table 2. Continued**

| Gene | Folds | Gene | Folds | Gene | Folds |
|------|-------|------|-------|------|-------|
| Acacb | 68    | Slx   | 37    | Odz4 | 58    |
| Angpt1 | 67    | Gchfr | 35    | Elf2s1 | 58  |
| Csprs | 67    | Hc    | 35    | Pde8b  | 54   |
| Gm4951 | 67    | Ptgir | 33    | Ebf3  | 46   |
| Mtapl1b | 65    | Accn2 | 32    | Angpt1 | 46  |
| Serpin9b | 59    | Masp2 | 32    | Rsf2d  | 45   |
| Cx6a2 | 59    | Cbr2  | 31    | Ifi202b | 45  |
| Matn2 | 57    | Col5a3 | 30    | Fbln1  | 37   |
| Pla2g2e | 54    | Ifi204 | 35    |
| Nrxn3 | 49    | Thbs2 | 35    |
| Cbr2 | 49    | Mx2   | 34    |
| Ebf3 | 48    | Ndufa4l2 | 34    |
| Olm15 | 47    | Tgfbr3 | 31    |
| Pparc1a | 45    | Car6  | 31    |

Table 2. Continued

(B) Genes down-regulated between 30- and 100-folds in telocytes (TCs) as compared with mesenchymal stem cells (MSCs) and fibroblasts (Fbs)

**Table 2.**

| Gene | Folds | Gene | Folds | Gene | Folds |
|------|-------|------|-------|------|-------|
| Car6 | 323   | Ccl5 | 282   |
| Odz4 | 275   | Hoxc6 | 146  |
| Tenn4 | 269   | Cdsn | 159   |
| Pla2g2e | 253   | Ifi203 | 63    |
| Cdsn | 229   | Gdpd2 | 85    |
| Gld5 | 209   |       |       |
| Rarres2 | 180   |       |       |
| Hoxc6 | 152   |       |       |
| Ndufa4l2 | 150   |       |       |
| Hoxc10 | 133   |       |       |
| Rhd | 122   |       |       |
| Plin4 | 113   |       |       |
| Gm2022 | 105   |       |       |
| Car9 | 102   |       |       |

**Table 2.** Continued

| Gene | Folds | Gene | Folds | Gene | Folds |
|------|-------|------|-------|------|-------|
| Serpinb9f | 95    | Tbx15 | 44    | Tbx15 | 93   |
| Foxg1 | 94    | Dmrc1c2 | 42    | Hoxc10 | 92   |
| Mst1 | 88    | Igt2bp3 | 41    | Nrk2-5 | 84   |
| Ifi203 | 82    | Ifk | 41    | Gbp3  | 72   |
| Avil | 75    | Paip1 | 38    | Lpar4 | 67   |
| Hsd17b14 | 69    | Rps3a | 38    | Hoxb9 | 66   |

murine lung TCs with mesenchymal stem cells (MSCs) and fibroblasts to identify the genes which are specifically regulated in TCs. We choose lung TCs as these are well-characterized ultrastructurally and immunohistochemically in situ and in vitro [4, 5, 11, 16, 17].
Eagle’s Medium (DMEM, Gibco, NY, USA), supplemented with 100 UI/ml penicillin and 0.1 mg/ml streptomycin (Sigma Chemical, St. Louis, MO, USA), and the samples were brought to the cell culture room immediately. Samples were further rinsed with sterile DMEM and minced into fragments about 1 mm³, which were then incubated at 37°C for 4 hrs on an orbital shaker, with 1 mg/ml type II collagenase (Sigma-Aldrich, St. Louis, MO, USA) in PBS without Ca²⁺ and Mg²⁺. Dispersed cells were separated from non-digested tissue by the filtration through a 40-µm diameter cell strainer (BD Falcon, Franklin, NJ, USA), harvested by centrifugation, and resuspended in DMEM supplemented with 10% foetal calf serum (Gibco, NY, USA), 100 UI/ml penicillin and 0.1 mg/ml streptomycin. Cell density was counted in a haemocytometer and viability was assessed using the Trypan blue. Cells were distributed in 25 cm² culture flasks at a density of $1 \times 10^5$ cells/cm² and maintained at 37°C in a humidified atmosphere (5% CO₂) until becoming semiconfluent (usually 4 days after plating). Culture medium was changed every 48 hrs. Cultured cells were examined by phase contrast microscope, under an inverted Olympus phase contrast microscope (1×51).

**RNA isolation and preparation**

Mouse lung telocytes were isolated after 5 days of culture. Mouse MSCs and fibroblasts were cultured and collected on days 5 and 10 respectively. RNA preparation was performed using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions, including a DNase digestion treatment. The amount and quality of RNA were measured by NanoDrop-1000 spectrophotometer and with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

**Fig. 1** Hierarchical cluster analysis of the differentially expressed genes among telocytes (TCs), mesenchymal stem cells (MSCs) and fibroblasts (Fbs).

**Fig. 2** Gene ontology of the genes with at least twofolds difference among telocytes (TCs), mesenchymal stem cells (MSCs) and fibroblasts (Fbs), analysed under following categories: Biological Processes (A), Cellular Components (B) and Molecular Function (C). ($P \leq 0.01$).
RNA labelling, array hybridization and DNA microarray

The Mouse 4 × 44K Gene Expression Array (Agilent, Shanghai, China) with about 39,000 mouse genes and transcripts represented with public domain annotations was applied for the analysis of gene profiles of mouse lung telocytes, MSCs and fibroblasts. Sample labelling and array hybridization were performed according to the protocol of One-Color Microarray-Based Gene Expression Analysis (Agilent Technology). Briefly, 1 μg of total RNA from each sample was linearly amplified and labelled with Cy3-dCTP. The labelled cRNAs were purified by RNAeasy Mini Kit (Qiagen). The concentration and specific activity of the labelled cRNAs (pmol Cy3/μg cRNA) were measured by NanoDrop ND-1000. One microgram of each labelled cRNA was fragmented by adding 11 μl 10 × Blocking Agent and 2.2 μl of 25 × Fragmentation Buffer, and heated at 60°C for 30 min. 55 μl 2 × GE Hybridization buffer was added to dilute the labelled cRNA. Hundred microlitre of hybridization solution was dispensed into the gasket slide and assembled to the gene expression microarray slide. The slides were incubated for 17 hrs at 65°C in an Agilent Hybridization Oven. The hybridized arrays were washed, fixed and scanned with the Agilent DNA Microarray Scanner (part number G2505B).

Fig. 3 String Network of the proteins that are differentially expressed among telocytes (TCs), mesenchymal stem cells (MSCs) and fibroblast (Fbs). A group of 46 genes are found connected functionally. Strong associations are represented by thick lines.
Data analysis

The acquired array images were analysed with Agilent Feature Extraction software (version 10.7.3.1). Quality normalization and subsequent data processing were performed with the GeneSpring GX v11.5.1 software package. The genes detected in all samples were chosen for further data analysis. Differentially expressed genes were identified through Fold Change filtering and hierarchically clustered by the Agilent GeneSpring GX software (version 11.5.1). Gene ontology and String Network analyses were performed with the standard enrichment computation method to study the relation among variant proteins expressed by variant genes. Fisher’s exact test was used to find more overlaps between the descriptive list and the GO annotation list than would be expected by chance. The P-value denoted the significance of GO terms enrichment in the descriptive genes.

Results and discussions

The quality of gene data after filtering and the distribution of data sets were assessed and visualized by Box-Plot. There was no significant difference in distributions of log2 ratios among TCs, MSCs and fibroblasts (Figure S1).

Gene expression analysis

Gene expression array data show that more than 500 genes are at least 10 times higher expressed in TCs comparing with either MSCs or fibroblasts (Table 1). Several genes are found 100 times up-regulated in TCs versus fibroblasts (Cdh2, Cyba, Rnf128, Dyps3, Fst1, Rbp1, Gm12892, Cdh2, Aldh1a1, Gm5864) or MSCs (Rbp1 and Glipr1; Table 1A). Additional genes are significantly overexpressed in TCs comparing with MSCs or fibroblasts (Table 1B). Table 2 is a summary of genes found to be down-regulated in TCs. Although many genes are less expressed in TCs comparing with MSCs or fibroblasts, very few are found at least 100 times down-regulated in TCs. Table 2A and B show the genes with known functions that are found at least 30 times down-regulated specifically in TCs comparing with MSCs and fibroblasts.

Hierarchical cluster and gene ontology analyses

The hierarchical cluster of the genes with more than twofold changes among telocytes, MSCs and fibroblasts is shown in Figure 1. Remarkably, the MCSs and fibroblast gene expression profiles relate each other to higher extent than to TCs supporting the view that TCs have a distinct gene expression pattern. In fact this is an important additional proof that TCs and fibroblasts are different cells. The GO analysis indicates that the genes differentially expressed in TCs are mainly involved in development, in tissue and organ morphogenesis and in transport and maintenance of a biological compound to a specific location (Fig. 2A). In addition, many of the differentially expressed genes likely function in extracellular compartments (Fig. 2B) and may play roles in cell survival, growth and differentiation through autocrine and paracrine activity (Fig. 2C). The relationships, including direct (physical) and indirect (functional) associations, of those genes were analysed by String Network analysis (www.string-db.org). Among the 156 co-expressed genes, 46 genes were found to have certain interactions (Fig. 3).

| Table 3 | Genes up- or down-regulated in telocytes (TCs) relative to both mesenchymal stem cells (MSCs) and fibroblasts (Fbs) |
|---------|---------------------------------------------------------------------------------------------------|
| Gene name | TCS vs. Fbs | TCS vs. MSCs |
|          | Fold change | Reg | Fold change | Reg |
| Ctgf     | 6150        | Up  | 35         | Up  |
| Mmp10    | 177         | Up  | 56         | Up  |
| Mmp3     | 131         | Up  | 25         | Up  |
| Col4a4   | 46          | Up  | 51         | Up  |
| Col4a6   | 34          | Up  | 36         | Up  |
| Col4a5   | 8           | Up  | 32         | Up  |
| Unc13b   | 61          | Up  | 7          | Up  |
| Mapk13   | 75          | Up  | 13         | Up  |
| Igsf9    | 115         | Up  | 3          | Up  |
| Glipr1   | 54          | Up  | 355        | Up  |
| Clic5    | 83          | Up  | 41         | Up  |
| Myh14    | 194         | Up  | 245        | Up  |
| Aldh1a1  | 225         | Up  | 92         | Up  |
| Aldh1a2  | 148         | Up  | 167        | Up  |
| Rbp1     | 161         | Up  | 141        | Up  |
| Gpro5c   | 125         | Up  | 136        | Up  |
| Gsta3    | 64          | Up  | 70         | Up  |
| Plac9    | 57          | Up  | 63         | Up  |
| Fgd3     | 77          | Up  | 39         | Up  |
| Dok2     | 60          | Up  | 41         | Up  |
| Scnn1a   | 35          | Up  | 68         | Up  |
| Car6     | 323         | Down| 31         | Down|
| Osd4     | 275         | Down| 59         | Down|
| Oz/ten-m | 269         | Down| 56         | Down|
| Cdsn     | 229         | Down| 153        | Down|
| Hoxc6    | 152         | Down| 207        | Down|
| Ili203   | 82          | Down| 150        | Down|
**TCs are potentially involved in tissue remodelling and basement membrane homeostasis**

A set of genes are specifically up- or down-regulated in TCs comparing with both fibroblasts and MSC (Table 3). As last two cell types are developmentally and functionally quite different, one being progenitors and the other differentiated, specialized cells, this set of genes should connect to the specific biological activities of TCs among the other stromal cells. Thus, we have found that several genes with roles in tissue remodelling and repair are significantly up-regulated in TCs (Tables 1A and 3): connective tissue growth factor (CTGF) [24, 25], Transgelin (Tagln) [26], Nidogen 1 (Nid1) [27, 28], tissue inhibitor of metalloproteinase 3 (TIMP3) [29], collagen type IV, alpha 1 (Col4a1), alpha 2 (Col4a2), alpha 3 (Col4a3), alpha 4 (Col4a4), alpha 5 (Col4a5) [28, 30], Matrix Metalloproteinase 10 (Mmp10) [31–33], Matrix Metalloproteinase 3 (Mmp3) [31–33] and Retinol-binding protein 1 (RBP1). RBP1 (also known as CRABP-I, CRBP, CRBP1, CRBP1, RBPP, RBPC) is required in tissue remodelling [34]. Regarding the molecular mechanisms, RBP1 delivers vitamin A to other cells through the plasma membrane protein STRA6 involved in JAK/STAT signalling and the intracellular metabolism of the vitamin [35]. Remarkably, two main components of basement membrane, Collagen type IV and Nidogen 1 are up-regulated in the cultured TCs comparing with both MSCs and fibroblasts. Moreover, TIMP3 is an extracellular matrix-anchored metalloproteinase inhibitor that acts specifically to increase vascular (endothelial) basement membrane stability [36, 37]. As TCs express Matrix Metalloproteinases Mmp3 and Mmp10 also, it is likely that TCs are involved in both basement membrane assembly (stability) and surrounding extracellular matrix remodelling.

**Concluding remarks**

Overall, the data indicate that TCs are clearly distinct from both MSCs and fibroblasts, and the gene signature of TCs suggests specific biological functions in (a) development and tissue morphogenesis, (b) biological compound transport and (c) extracellular matrix remodelling. It has been proposed that TCs play essential roles in angiogenesis given that TCs are frequently found in close vicinity of small vessels and express angiogenesis-related factors (VEGF, NO) and pro-angiogenic microRNAs [22]. The data presented here bring additional support to this view suggesting that TCs may also regulate vascular basement membrane remodelling as key step in vascular branching and de novo vessel formation.

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**Conflict 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:

**Figure S1 Box-Plot of Quality assessment of gene data after filtering.** After normalization, the distributions of log2 ratios among all samples are nearly the same.

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