Molecular Characterization of Spontaneous Mesenchymal Stem Cell Transformation

Daniel Rubio1, Silvia Garcia1, Maria F. Paz1, Teresa De la Cueva1, Luis A. Lopez-Fernandez2, Alison C. Lloyd3, Javier Garcia-Castro1,4, Antonio Bernad5

1 Department of Immunology and Oncology, Centro Nacional de Biotecnologia, Consejo Superior de Investigaciones Científicas (CSIC), Universidad Autónoma de Madrid, Madrid, Spain, 2 Servicio de Farmacia, Hospital General Universitario Gregorio Marañón, Madrid, Spain, 3 Laboratory for Molecular Cell Biology, University College London, London, United Kingdom, 4 Andalusian Stem Cell Bank, Granada, Spain

Background. We previously reported the in vitro spontaneous transformation of human mesenchymal stem cells (MSC) generating a population with tumorigenic potential, that we termed transformed mesenchymal cells (TMC). Methodology / Principal Findings. Here we have characterized the molecular changes associated with TMC generation. Using microarrays techniques we identified a set of altered pathways and a greater number of downregulated than upregulated genes during MSC transformation, in part due to the expression of many untranslated RNAs in MSC. Microarray results were validated by qRT-PCR and protein detection. Conclusions / Significance. In our model, the transformation process takes place through two sequential steps; first MSC bypass senescence by upregulating c-myc and repressing p16 levels. The cells then bypass cell crisis with acquisition of telomerase activity, Ink4a/Arf locus deletion and Rb hyperphosphorylation. Other transformation-associated changes include modulation of mitochondrial metabolism, DNA damage-repair proteins and cell cycle regulators. In this work we have characterized the molecular mechanisms implicated in TMC generation and we propose a two-stage model by which a human MSC becomes a tumor cell.

INTRODUCTION

The development of a solid tumor is considered a multi-step process in which several molecular checkpoints must be altered to generate a tumor from a normal cell [1]. The acquired capabilities of tumor cells include their ability to proliferate continuously ignoring apoptosis or growth-inhibitory signals, generating their own mitogenic signals. In advanced phases of tumor development, a neoangiogenesis process takes place and finally tumor cells acquire the capacity of tissue invasion and metastasize to other organs. Generally, it is admitted that most tumors acquire these characteristics through genome instability, telomere stabilization and disruption of regulatory circuits [2].

A recent theory suggests the existence of cancer stem cells (CSC), a subpopulation of cells with tumorigenic potential that is lack in the rest of the cells within this tumor. CSC were reported for some tumor types including breast and lung cancer, leukemia and glioblastoma [3,4]. However, there is a great ignorance about how the “acquired capabilities” of tumor cells would take place; directly on adult stem cells, or on differentiated cells that suffer a dedifferentiation process. In this regard, CSC share several features with adult stem cells such as self-renewal ability, asymmetric division, and differentiation potential [5].

Adult human mesenchymal stem cell spontaneous immortalization and transformation were recently reported by our group [6], supporting the hypothesis of the stem cell origin of CSC. Independent laboratories have confirmed these data, reporting similar results using MSC derived from human or murine bone marrow [7–11]. In this regard, we have previously characterized the cellular sequence of steps necessary to transform a human MSC into a tumorigenic cell [6]. Following approximately 20 population doublings in vitro, mesenchymal stem cell cultures enter a senescence phase, but are able to bypass it at a high frequency. These cells then continue to divide until they reach a crisis phase. Only some samples are able to escape from this crisis phase spontaneously, but those that do have undergone tumorigenic transformation generating TMC.

However, until now genetic alterations implied in spontaneous MSC transformation are little known. Some groups have studied molecular pathways involved in the artificial transformation of MSC transduced with oncogenes [12,13]. In this study, we have characterized the molecular mechanisms implicated in TMC generation and we propose a two-stage model by which a human MSC becomes in vitro a tumor cell.

RESULTS

Comparative gene expression analysis of MSC transformation by microarray analysis

To analyze molecular differences associated with TMC generation, we performed microarray studies using mRNA from pre- and post-transformed MSC. Similar results using MSC derived from human or murine bone marrow were obtained.

Academic Editor: Joseph Najbauer, City of Hope Medical Center, United States of America

Received November 23, 2006; Accepted December 7, 2007; Published January 2, 2008

Copyright: © 2008 Rubio et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: DR and SG received predoctoral fellowships from the Spanish Ministry of Education and Science, SG-C and L LF received postdoctoral fellowships from the Ministry of Science and Technology and the Ministerio de Sanidad y Consumo (FIS; CP03/0031 and CP06/0267). This work was partially supported by Spanish Ministry of Science and Technology (CICYT) grants SAF2001-2262, SAF2005-0864 and GEN2001-1856-C13-02 to AB. The Department of Immunology and Oncology was founded and is supported by the Spanish National Research Council (CSIC) and by Pfizer.

Competing Interests: The authors have declared that no competing interests exist.

* To whom correspondence should be addressed. E-mail: abernad@cnic.es

1a Current address: Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain
1b Current address: Department of Regenerative Cardiology, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
1c Current address: Animalary facility, Centro Nacional de Servicio de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC), Universidad Autónoma de Madrid, Madrid, Spain
post-senescence MSC, and from TMC. From a general perspective, data analysis showed that the greatest changes were associated with TMC generation, as TMC functions were more different than post-senescence MSC, compared to pre-senescence MSC (Table 1). Although in a minor intensity, post-senescence MSC have the same altered functions that TMC. In both cases the principal category affected is “cancer” (Table 1). However, the main pathways deregulated in both, post-senescence MSC and TMC, are related to stress, toxic events and mitochondrial metabolism (Table 2). On the other hand, there was more down- than upregulated RNA transcripts associated with TMC generation (Figure S1). The main differences in mRNA expression profiles between pre- and post-senescence MSC are shown in Table 3. Table 4 shows differences between pre-senescence MSC and TMC.

Molecular differences in cell cycle-related proteins

We had reported differences in cell cycle progression in pre- and post-senescence MSC and TMC, including more rapid cell division in TMC [6]. Here we used microarray techniques to explore the cell cycle-related molecular differences in post-senescence MSC and TMC compared to pre-senescence MSC. Compared to pre-senescence MSC, post-senescence MSC showed few differences in cell cycle-related proteins. In contrast, TMC samples showed significant mRNA modulations; Cdk1 and Cdk4 as well as cyclins B1 and D2 were upregulated, whereas cyclin D1 was downregulated (Figure 1A).

We evaluated microarray experiments analyzed by qRT-PCR the expression of some of these genes. No expression difference was found between pre- and post-senescence MSC, while TMC overexpressed Cdk2 and Cdk6 (Figure 1B). In qRT-PCR experiments we also studied met-TMC, a cell line derived of lung metastases generated after s.c. inoculation of TMC in immuno-deficient mice (Rubio et al, unpublished results).

To determine whether the differences in mRNA levels gave rise to altered protein expression, we compared these samples by western blot. In TMC, cyclin B1, Cdk2 and Cdk6 were upregulated, whereas Cdk1 and Cdk4 remained constant. Cyclin D1 was downregulated from pre-senescence MSC to TMC (Figure 1C).

Upregulation of DNA repair pathways in transformed MSC

As DNA repair mechanisms are responsible for the bypass of senescence and crisis, as well as for tumor maintenance and progression [14], we analyzed the major proteins linked to these processes. We tested proteins involved in pathways including non-homologous end joining (NHEJ), base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and homologous recombination (HR).

Microarray analysis showed no mRNA significant differences between pre- and post-senescence MSC (Figure 2A). However protein analysis showed that DNA-PKcs was repressed in post-senescence MSC compared to pre-senescence MSC and a down-regulation of Rad51 after senescence bypass, although expression was restored and upregulated after crisis bypass in TMC (Figure 2C).

In contrast, mRNA levels were modulated differentially between TMC and pre-senescence MSC for several DNA repair-associated proteins. Specifically, DNA-PKcs and PCNA were upregulated in TMC, and XPA was downregulated compared to pre-senescence MSC. Although DNA polymerase μ and ERCC3 upregulation were statistically significant, differences in mRNA levels appeared to be minor, based on fold change and Z-score values (Figure 2A).

We performed qRT-PCR experiments to validate microarray results. No modulation of expression of DNA repair-related genes was detected between pre- and post-senescence MSC. In contrast, several of these genes were overexpressed in TMC, such as DNA-PKcs, DNA polymerase μ, RAD51 or ERCC4 (Figure 2B).

### Table 1. Comparative table of functions with a higher significance in selected genes for post-senescence MSC and TMC, obtained by Ingenuity Pathways Analysis software.

| Category                        | Process Annotation        | pre-sen/post-sen MSC | pre-sen MSC/TMC |
|---------------------------------|---------------------------|----------------------|-----------------|
|                                 |                           | Significance         | Molecules | Significance | Molecules |
| Cancer                          | Tumorigenesis             |                      |           |
| Cellular Movement               | Migration of eukaryotic cells | 4.38E-07              | 9         | 1.74E-18     | 25        |
| Tissue Development              | Developmental process of tissue | 1.35E-07              | 9         | 1.35E-17     | 23        |
| Gastrointestinal Disease        | Colorectal cancer         | 9.32E-12              | 9         | 1.75E-17     | 16        |
| Genetic Disorder                | Genetic disorder          | 8.00E-05              | 8         | 2.74E-17     | 26        |
| Cell Death                      | Cell death of eukaryotic cells | 3.06E-11              | 16        | 7.00E-20     | 34        |
| Organismal Survival             | Survival of mice          | 3.14E-08              | 7         | N/A          | N/A       |
| Organismal Survival             | Death of mammary          | 1.84E-04              | 6         | 3.62E-11     | 17        |
| Neurological Disease            | Cell death of neurons     | 4.71E-08              | 7         | 2.00E-05     | 7         |

In each category only the highest “Process Annotation” is represented.

Table 2. Top tox list obtained by Ingenuity Pathways Analysis for post-senescence MSC and TMC

| MSC presen/MSCpost-sen | p-value | Ratio | Significance | Molecules |
|------------------------|---------|-------|--------------|-----------|
| Aryl Hydrocarbon Receptor Signaling | 1.97E-05 | 4/154 (0.026) | 2.73E-18 | 22 |
| Hepatic fibrosis       | 3.48E-03 | 2/85 (0.024) | 4.71E-27 | 41 |
| Xenobiotic Metabolism  | 7.83E-03 | 2/129 (0.016) | 2.30E-05 | 7 |
| Cytochrome P450 Panel  | 1.43E-02 | 1/14 (0.071) | 1.75E-17 | 16 |

| MSC presen/TMC | p-value | Ratio | Significance |
|----------------|---------|-------|--------------|
| Hepatic fibrosis | 2.11E-14 | 10/85 (0.118) | 2.73E-18 |
| FXF/RXR activation | 1.18E-03 | 2/20 (0.100) | 4.71E-27 |
| Mitochondrial dysfunction | 4.70E-03 | 3/125 (0.024) | 1.75E-17 |
| Hepatic cholestasis | 5.04E-03 | 2/20 (0.100) | 2.74E-17 |

LPS & IL-1 mediated inhibition of RXR function | 1.19E-02 | 3/185 (0.016) | 2.73E-18 |

LPS & IL-1 mediated inhibition of RXR activation | 1.19E-02 | 3/185 (0.016) | 2.73E-18 |

doi:10.1371/journal.pone.0001398.t002

doi:10.1371/journal.pone.0001398.t001

stable. In contrast, the principal category involved was “cancer” (Table 1). However, the main pathways deregulated in both, post-senescence MSC and TMC, are related to stress, toxic events and mitochondrial metabolism (Table 2). On the other hand, there was more down- than upregulated RNA transcripts associated with TMC generation (Figure S1). The main differences in mRNA expression profiles between pre- and post-senescence MSC are shown in Table 3. Table 4 shows differences between pre-senescence MSC and TMC:

### Table 2. Top tox list obtained by Ingenuity Pathways Analysis for post-senescence MSC and TMC

| MSC presen/MSCpost-sen | p-value | Ratio | Significance | Molecules |
|------------------------|---------|-------|--------------|-----------|
| Aryl Hydrocarbon Receptor Signaling | 1.97E-05 | 4/154 (0.026) | 2.73E-18 | 22 |
| Hepatic fibrosis       | 3.48E-03 | 2/85 (0.024) | 4.71E-27 | 41 |
| Xenobiotic Metabolism  | 7.83E-03 | 2/129 (0.016) | 2.30E-05 | 7 |
| Cytochrome P450 Panel  | 1.43E-02 | 1/14 (0.071) | 1.75E-17 | 16 |

| MSC presen/TMC | p-value | Ratio | Significance |
|----------------|---------|-------|--------------|
| Hepatic fibrosis | 2.11E-14 | 10/85 (0.118) | 2.73E-18 |
| FXF/RXR activation | 1.18E-03 | 2/20 (0.100) | 4.71E-27 |
| Mitochondrial dysfunction | 4.70E-03 | 3/125 (0.024) | 1.75E-17 |
| Hepatic cholestasis | 5.04E-03 | 2/20 (0.100) | 2.74E-17 |

LPS & IL-1 mediated inhibition of RXR function | 1.19E-02 | 3/185 (0.016) | 2.73E-18 |

LPS & IL-1 mediated inhibition of RXR activation | 1.19E-02 | 3/185 (0.016) | 2.73E-18 |
### Table 3. Main mRNA differences between pre- and post-senescence MSC.

| Genbank Acc No | Gene name                                                                 | x-fold change | p-value | z-score |
|---------------|---------------------------------------------------------------------------|---------------|---------|---------|
| NM_004932     | Cadherin 6, type 2, K-cadherin (fetal kidney)                              | -3.675        | <0.001  | -8.612  |
| AL049227      | Homo sapiens mRNA; cDNA DKFZp564N1116 (from clone DKFZp564N1116)           | -3.173        | <0.001  | -8.827  |
| NM_021197     | WAP four-disulfide core domain 1                                           | -3.151        | 0.005   | -8.772  |
| NM_005012     | Secreted frizzled-related protein 1                                        | -3.048        | 0.001   | -6.866  |
| AF070524      | Homo sapiens clone 24453 mRNA sequence                                     | -2.664        | 0.006   | -6.775  |
| AB046796      | KIAA1576 protein                                                          | -2.346        | 0.004   | -4.803  |
| NM_013381     | Thyrotrpin-releasing hormone degrading ectoenzyme                           | -2.272        | 0.004   | -5.253  |
| AK022997      | Homo sapiens cDNA FLJ12935 fls. clone NT2RP2004982                        | -2.265        | 0.003   | -5.048  |
| NM_006208     | Ectonucleotide pyrophosphatase/phosphodiesterase 1                         | -2.259        | 0.003   | -4.791  |
| NM_03211      | Hypothetical gene supported by AF038182; BC009203                         | -2.220        | 0.001   | -4.689  |
| AJ279081      | Chromosome 21 open reading frame 66                                        | -2.188        | <0.001  | -4.726  |
| NM_006389     | Oxygen regulated protein (150kD)                                           | -2.173        | 0.009   | -4.402  |
| NM_006267     | Latent transforming growth factor beta binding protein 1                   | -2.094        | <0.001  | -4.554  |
| NM_001271     | Chromodomain helicase DNA binding protein 2                                | -2.076        | 0.006   | -6.785  |
| NM_002937     | Ribonuclease. RNase A family. 4                                            | -2.016        | <0.001  | -3.940  |
| NM_000325     | Paired-like homeodomain transcription factor 2                             | -1.959        | <0.001  | -3.954  |
| NM_009910     | Plasminogen activator. tissue                                              | -1.903        | 0.001   | -4.655  |
| AK057782      | Homo sapiens cDNA FLJ25053 fls. clone CBL04266                            | -1.908        | 0.002   | -5.726  |
| NM_005382     | Neurofilament 3 (150kD medium)                                             | -1.884        | 0.001   | -3.872  |
| U67784        | G protein-coupled receptor                                                 | -1.855        | <0.001  | -3.732  |
| NM_012730     | Hypothetical protein PP1044                                                 | -1.833        | 0.002   | -3.514  |
| NM_002977     | Sodium channel. voltage-gated. type IX. alpha polypeptide                  | -1.823        | 0.000   | -3.512  |
| NM_032883     | Chromosome 20 open reading frame 100                                       | -1.804        | 0.005   | -3.561  |
| NM_004405     | Distal-less homeo box 2                                                    | -1.788        | 0.009   | -4.443  |
| AF263545      | Homo sapiens HUT11 protein mRNA. partial 3’ UTR                           | -1.776        | 0.007   | -5.334  |
| D13628        | Angiopoietin 1                                                             | -1.748        | <0.001  | -4.393  |
| NM_001957     | Keratin associated protein 1.5                                             | -1.738        | 0.001   | -4.048  |
| BC012486      | Keratin associated protein KRTAP2.1A                                       | -1.732        | <0.001  | -3.517  |
| NM_021226     | Hypothetical protein from clones 23549 and 23762                          | -1.694        | 0.007   | -3.864  |
| NM_000077     | Cyclin-dependent kinase inhibitor 2A (melanoma. p16. inhibits CDK4)        | -1.686        | 0.003   | -4.109  |
| AF070641      | Homo sapiens clone 24421 mRNA sequence                                     | -1.660        | 0.007   | -4.773  |
| NM_004288     | Pleckstrin homology. Sec7 and coiled/coil domains. binding protein         | -1.636        | 0.009   | -4.299  |
| NM_018476     | Brain expressed. X-linked 1                                                | -1.524        | <0.001  | -3.971  |
| AF161403      | Homo sapiens HSPC285 mRNA. partial cds                                      | 1.507         | 0.001   | 3.846   |
| NM_019111     | Major histocompatibility complex. class II. DR alpha                        | 1.598         | <0.001  | 3.582   |
| AP001660      | Homo sapiens genomic DNA. chromosome 21q. section 4/105                    | 1.654         | 0.003   | 4.720   |
| AK022034      | Homo sapiens cDNA FLJ11972 fls. clone HEMBB1001209                        | 1.667         | 0.008   | 3.744   |
| NM_005435     | Rho guanine nucleotide exchange factor (GEF) 5                             | 1.687         | <0.001  | 4.854   |
| NM_024081     | Transmembrane gamma-carboxyglutamic acid protein 4                         | 1.733         | <0.001  | 4.446   |
| NM_004938     | Death-associated protein kinase 1                                          | 1.776         | 0.008   | 5.389   |
| NM_004962     | Growth differentiation factor 10                                           | 1.817         | 0.004   | 5.410   |
| NM_009090     | Matrix Glb protein                                                         | 1.828         | 0.003   | 5.265   |
| NM_012068     | Activating transcription factor 5                                           | 1.832         | 0.002   | 3.701   |
| NM_014422     | Heat shock 27kD protein family. member 7 (cardiovascular)                  | 1.855         | 0.004   | 3.603   |
| NM_022719     | DiGeorge syndrome critical region gene DG5i; likely ortholog of mouse expressed sequence 2 embryonic | 1.879         | 0.003   | 3.887   |
| NM_004669     | Chloride intracellular channel 3                                           | 1.889         | 0.006   | 4.662   |
| NM_001673     | Asparagine synthetase                                                      | 1.896         | 0.006   | 3.594   |
| NM_000955     | Cartilage oligomeric matrix protein (pseudoachondroplasia. epiphyseal dysplasia 1. multiple) | 1.921         | <0.001  | 3.665   |
| NM_001353     | Aldo-keto reductase family 1. member C1 (dihydriol dehydrogenase 1; 20-alpha (3-alpha)-hydroxyster | 1.947         | <0.001  | 3.742   |
| NM_019105     | Tenascin XB                                                               | 2.036         | 0.001   | 4.471   |
We evaluated the correlation between RNA and protein levels by western blot analysis. Proteins were elevated in nearly all DNA repair pathways in TMC compared to pre-senescent MSC. These effects were protein- rather than pathway-specific. MMR was the only pathway that showed no differences between MSC and TMC, although we analyzed only PCNA in this pathway (Figure 2C).

### Changes in tumor suppressors and oncogenes expression related with MSC transformation

To explore the role of tumor suppressor gene inactivation in MSC senescence and crisis bypass, we analyzed p16, p21 and p53. In a previous publication we have shown that c-myc protein overexpression is linked to senescence bypass and is maintained in TMC; expression is linked to senescence bypass and is maintained in TMC;

| Genbank Acc No | Gene name | x-fold change | p-value | z-score |
|----------------|-----------|---------------|---------|---------|
| NM_000095      | Keratin 7 | 3.027         | <0.001 | 6.389   |
| NM_000095      | Nuclear factor I/B | 2.844 | <0.001 | 6.389 |
| NM_004750      | Cytokine receptor-like factor 1 | 3.027 | <0.001 | 6.610 |
| AK055188       | Homo sapiens cDNA FLJ30626 | 3.340 | 0.002 | 7.033 |
| AF311912       | Secreted frizzled-related protein 2 | 3.365 | 0.001 | 7.630 |
| AL00218        | Homo sapiens mRNA; cDNA DFFZp586N1323 | 3.422 | 0.002 | 7.428 |
| U14383         | Mucin 8. tracheobronchial | 3.791 | 0.004 | 7.836 |
| NM_000095      | Cartilage oligomeric matrix protein (pseudoachondroplasia. epiphyseal dysplasia 1, multiple) | 3.796 | 0.003 | 7.483 |
| AK000819       | Homo sapiens cDNA FLJ20812 | 3.953 | <0.001 | 8.800 |
| NM_015863      | Surfactant protein B | 4.343 | <0.001 | 13.305 |
| NM_005332      | Interferon. alpha-inducible protein 27 | 5.236 | <0.001 | 12.417 |

Array data were filtered according to Z-score (>3.5 and < -3.5) and p-value (<0.01).

We compared mRNA regulation differences in the oncogenes c-myc and telomerase in post-senescent MSC and TMC with pre-senescent MSC. We found no differences in oncogene mRNA transcript levels in these populations (Figure 3A). Nonetheless, in a previous publication we have shown that c-myc protein overexpression is linked to senescence bypass and is maintained in TMC;

Table 3. cont.

| Genbank Acc No | Gene name | x-fold change | p-value | z-score |
|----------------|-----------|---------------|---------|---------|
| AB046843       | KIAA1623 protein | 2.040 | 0.001 | 5.225 |
| AF111170       | Homo sapiens 14q32 Jagged2 gene. complete cds; and unknown gene | 2.051 | <0.001 | 4.030 |
| NM_007223      | Putative G protein coupled receptor | 2.126 | 0.006 | 6.832 |
| AK057721       | Homo sapiens cDNA FLJ33159; clone UTERU2000465 | 2.134 | 0.002 | 5.097 |
| NM_003480      | Microfibril-associated glycoprotein-2 | 2.307 | 0.002 | 4.891 |
| AK024396       | Acetyl-Coenzyme A synthetase 2 (AMP forming)-like | 2.330 | 0.002 | 6.343 |
| BC015794       | Hypothetical protein FLJ10097 | 2.367 | <0.001 | 5.142 |
| AK024428       | Pleckstrin homology. Sec7 and coiled/coil domains 4 | 2.415 | 0.007 | 6.613 |
| AK024240       | Homo sapiens cDNA FLJ14178; clone NT2RP2003339 | 2.451 | 0.001 | 6.199 |
| NM_005264      | GDNF family receptor alpha 1 | 2.465 | <0.001 | 6.065 |
| L48728         | Homo sapiens T cell receptor beta (TCRBV1051) gene. complete cds | 2.626 | 0.004 | 7.592 |
| NM_005556      | Keratin 7 | 2.795 | 0.007 | 5.917 |
| NM_007281      | Scrapie responsive protein 1 | 2.828 | <0.001 | 6.276 |
| NM_005596      | Nuclear factor I/B | 2.844 | <0.001 | 6.389 |
| NM_004750      | Cytokine receptor-like factor 1 | 3.027 | <0.001 | 6.610 |
| AK055188       | Homo sapiens cDNA FLJ30626; clone CTONG2001911. weakly similar to UBIQUITIN CARBOXYL-TERMINAL | 3.340 | 0.002 | 7.033 |
| AF311912       | Secreted frizzled-related protein 2 | 3.365 | 0.001 | 7.630 |
| AL00218        | Homo sapiens mRNA; cDNA DFFZp586N1323 (from clone DFFZp586N1323) | 3.422 | 0.002 | 7.428 |
| U14383         | Mucin 8. tracheobronchial | 3.791 | 0.004 | 7.836 |
| NM_000095      | Cartilage oligomeric matrix protein (pseudoachondroplasia. epiphyseal dysplasia 1, multiple) | 3.796 | 0.003 | 7.483 |
| AK000819       | Homo sapiens cDNA FLJ20812; clone AD5E01316 | 3.953 | <0.001 | 8.800 |
| NM_015863      | Surfactant protein B | 4.343 | <0.001 | 13.305 |
| NM_005332      | Interferon. alpha-inducible protein 27 | 5.236 | <0.001 | 12.417 |

Array data were filtered according to Z-score (>3.5 and < -3.5) and p-value (<0.01).
### Table 4. Main mRNA differences between pre-senescence MSC and TMC.

| Genbank Acc No. | Gene name                                                                 | x-fold change | p-value   | z-score |
|-----------------|----------------------------------------------------------------------------|---------------|-----------|---------|
| NM_000165       | Gap junction protein, alpha 1, 43KD (connexin 43)                          | -52.904       | <0.001    | -6.640  |
| BC014245        | Homo sapiens, Similar to RIKEN cDNA 1110014B07 gene, clone MGC:20766 IMAGE:4586039, mRNA, complete c | -45.496       | <0.001    | -6.311  |
| NM_000089       | Collagen, type I, alpha 2                                                  | -44.113       | <0.001    | -6.041  |
| NM_002421       | Matrix metalloproteinase 1 (interstitial collagenase)                     | -31.671       | <0.001    | -5.512  |
| NM_006475       | Osteoblast specific factor 2 (fasciclin I-like)                           | -31.206       | <0.001    | -5.513  |
| NM_052947       | Heart alpha-kinase                                                        | -30.941       | <0.001    | -5.499  |
| NM_000089       | Collagen, type I, alpha 2                                                  | -30.183       | <0.001    | -5.443  |
| NM_002937       | Ribonuclease, RNase A family, 4                                           | -30.113       | <0.001    | -5.911  |
| NM_013372       | Cysteine knot superfamily 1, BMP antagonist 1                              | -27.678       | <0.001    | -5.301  |
| NM_006475       | Osteoblast specific factor 2 (fasciclin I-like)                           | -25.377       | <0.001    | -5.225  |
| NM_006063       | Sarcomeric muscle protein                                                 | -25.291       | <0.001    | -5.502  |
| M96843          | Striated muscle contraction regulatory protein                            | -24.615       | <0.001    | -5.414  |
| NM_002526       | 5’ nucleotidase (CD73)                                                    | -24.069       | <0.001    | -5.258  |
| NM_021242       | Hypothetical protein STRAIT11499                                          | -22.708       | <0.001    | -6.307  |
| AK027274        | Homo sapiens cDNA FLJ14368 fis, clone HEMBA100112                         | -21.750       | <0.001    | -7.398  |
| AB033025        | KIAA1199 protein                                                          | -21.257       | <0.001    | -4.880  |
| NM_032348       | Hypothetical protein MGC3047                                               | -20.763       | <0.001    | -4.840  |
| NM_009963       | Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase) | -20.328       | <0.001    | -5.091  |
| AB058761        | KIAA1858 protein                                                          | -20.016       | <0.001    | -5.644  |
| NM_006211       | Proenkephalin                                                             | -19.361       | <0.001    | -4.731  |
| AK055725        | Maternally expressed 3                                                     | -19.350       | <0.001    | -4.747  |
| NM_001038       | Fibrillin 1 (Marfan syndrome)                                             | -18.750       | <0.001    | -4.679  |
| AB011145        | KIAA0573 protein                                                          | -18.677       | <0.001    | -5.912  |
| NM_006988       | A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1 | -18.388       | <0.001    | -4.649  |
| AB014511        | ATPase, Class II, type 9A                                                 | -17.675       | <0.001    | -4.701  |
| NM_000393       | Collagen, type V, alpha 2                                                 | -17.533       | <0.001    | -4.628  |
| NM_0049419      | Integrin, alpha 2b (platelet glycoprotein llb/lllla complex, antigen CD41B) | -17.439       | <0.001    | -5.061  |
| NM_007268       | Ig superfamily protein                                                    | -17.320       | <0.001    | -4.951  |
| NM_015966       | Weakly similar to glutathione peroxidase 2                                | -16.943       | <0.001    | -4.525  |
| NM_016551       | Heptacellulare carcinoma novel gene-3 protein                             | -16.524       | <0.001    | -4.966  |
| NM_002593       | Procollagen C-endopeptidase enhancer                                     | -16.332       | <0.001    | -4.476  |
| NM_000358       | Transforming growth factor, beta-induced, 68kD                           | -16.120       | <0.001    | -4.438  |
| NM_031442       | Brain cell membrane protein 1                                             | -15.709       | <0.001    | -4.508  |
| NM_015364       | MD-2 protein                                                              | -15.455       | <0.001    | -4.753  |
| NM_002487       | Necedin homolog (mouse)                                                   | -15.122       | <0.001    | -5.116  |
| AF109681        | Integrin, alpha 11                                                        | -15.057       | <0.001    | -4.708  |
| NM_000916       | Oxytocin receptor                                                         | -14.278       | <0.001    | -4.260  |
| AL136693        | Duodenal cytochrome b                                                     | -14.265       | <0.001    | -4.350  |
| AK055976        | Thymosin, beta 4, X chromosome                                            | -14.159       | <0.001    | -4.233  |
| AK025931        | Homo sapiens cDNA: FLJ22278 fis, clone HRC03745                          | -13.834       | <0.001    | -4.949  |
| BC002025        | Homo sapiens, clone IMAGE:335762, mRNA, partial cds                       | -13.804       | <0.001    | -4.193  |
| NM_007085       | Follistatin-like 1                                                         | -13.508       | <0.001    | -4.164  |
| NM_022726       | Elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 4 | -12.892       | <0.001    | -4.526  |
| NM_005847       | Solute carrier family 23 (nucleobase transporters), member 2             | -12.779       | <0.001    | -4.511  |
| NM_030781       | Collectin sub-family member 12                                            | -12.550       | <0.001    | -5.284  |
| NM_000993       | Collagen, type V, alpha 1                                                 | -12.506       | <0.001    | -4.033  |
| NM_001353       | Aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysterol reductase) | -12.332       | <0.001    | -4.011  |
| NM_002064       | Glutaredoxin (thioltransferase)                                           | -12.314       | <0.001    | -4.005  |
| NM_020404       | Tumor endothelial marker 1 precursor                                      | -12.138       | <0.001    | -4.086  |
| NM_000962       | Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase) | -11.886       | <0.001    | -4.382  |
| Genbank Acc No. | Gene name | x-fold change | p-value | z-score |
|----------------|-----------|---------------|---------|---------|
| NM_022360      | Human epididymis-specific 3 beta | −11.816 | <0.001 | −4.766 |
| BC009078       | Homo sapiens, clone MGC:17624 IMAGE:3855543, mRNA, complete cds | −11.792 | <0.001 | −4.284 |
| AB051443       | KIAA1656 protein | −11.742 | <0.001 | −7.230 |
| NM_031440      | Transmembrane protein 7 | −11.686 | <0.001 | −5.635 |
| NM_002421      | Matrix metalloproteinase 1 (interstitial collagenase) | −11.641 | <0.001 | −3.916 |
| NM_024031      | Hypothetical protein MGC3121 | −11.606 | <0.001 | −3.916 |
| AF200348       | Melanoma associated gene | −11.516 | <0.001 | −3.901 |
| NM_031426      | Hypothetical protein FLJ12783 | −11.244 | <0.001 | −3.923 |
| AK055903       | Homo sapiens cDNA: FLJ12952 fis, clone COL07036 | −11.134 | <0.001 | −3.847 |
| NM_012104      | Beta-site APP-cleaving enzyme | −10.628 | <0.001 | −3.819 |
| AL133640       | Homo sapiens mRNA; cDNA DKFZp586C1021 | −10.618 | <0.001 | −4.345 |
| U17077         | BENE protein | −10.516 | <0.001 | −4.085 |
| NM_024563      | Hypothetical protein FLJ14054 | −10.297 | <0.001 | −4.710 |
| NM_004098      | Empty spiracles homolog 2 (Drosophila) | −10.242 | <0.001 | −3.715 |
| AK023413       | Homo sapiens cDNA FLJ13351 fis, clone OVARC1002156, weakly similar to Danio rerio uridine kinase mRNA | −10.209 | <0.001 | −4.692 |
| NM_000090      | Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant) | −10.049 | <0.001 | −3.684 |
| NM_003247      | Thrombospondin 2 | −9.958 | <0.001 | −3.667 |
| NM_005086      | Sarcospan (Kras oncogene-associated gene) | −9.955 | <0.001 | −4.800 |
| NM_004265      | Fatty acid desaturase 2 | −9.904 | <0.001 | −3.660 |
| NM_014244      | A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 2 | −9.728 | <0.001 | −4.028 |
| AF131817       | Homo sapiens clone 25023 mRNA sequence | −9.691 | <0.001 | −4.916 |
| AK054816       | Ferritin, heavy polypeptide 1 | −9.653 | <0.001 | −3.629 |
| AL050370       | Homo sapiens mRNA; cDNA DKFZp566C0546 (from clone DKFZp566C0546) | −9.494 | 0.001 | −4.871 |
| NM_003246      | Thrombospondin 1 | −9.217 | <0.001 | −3.635 |
| NM_004660      | DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide, Y chromosome | −9.185 | <0.001 | −3.666 |
| NM_014678      | KIAA0685 gene product | −9.118 | <0.001 | −3.584 |
| NM_016588      | Neuritin | −8.963 | <0.001 | −4.233 |
| NM_001235      | Serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 2 | −8.923 | <0.001 | −3.503 |
| AK057865       | Thy-1 cell surface antigen | −8.798 | <0.001 | −4.707 |
| NM_023927      | Hypothetical protein FLJ21313 | −8.689 | <0.001 | −3.574 |
| NM_013253      | Dickkopf homolog 3 (Xenopus laevis) | −8.612 | <0.001 | −4.497 |
| AF111170       | Homo sapiens 14q32 Jagged2 gene, complete cds; and unknown gene | −8.553 | <0.001 | −3.800 |
| AF334710       | Homo sapiens pyruvate dehydrogenase kinase 4 mRNA, 3' untranslated region, partial sequence | −8.534 | 0.001 | −4.138 |
| NM_022143      | NAG14 protein | −8.469 | <0.001 | −3.575 |
| NM_002422      | Matrix metalloproteinase 3 (stromelysin 1, progelatinase) | −8.442 | 0.001 | −3.777 |
| AK055249       | Homo sapiens cDNA FLJ30687 fis, clone FC88F2000379 | −8.415 | <0.001 | −3.917 |
| AJ279081       | Chromosome 21 open reading frame 66 | −8.329 | <0.001 | −4.091 |
| NM_001541      | Heat shock 27kD protein 2 | −8.279 | <0.001 | −3.537 |
| NM_017680      | Asporin (LRR class 1) | −8.279 | <0.001 | −5.816 |
| NM_004148      | Dual specificity phosphatase 2 | −8.249 | <0.001 | −4.407 |
| AL049227       | Homo sapiens mRNA; cDNA DKFZp564N1116 (from clone DKFZp564N1116) | −8.230 | <0.001 | −4.831 |
| NM_018977      | Calcium channel, voltage-dependent, gamma subunit 6 | −8.127 | <0.001 | −5.033 |
| AY040094       | Serine protease HTRA3 | −7.690 | <0.001 | −4.676 |
| NM_002761      | Protamine 1 | −7.636 | <0.001 | −3.924 |
| NM_001850      | Collagen, type VIII, alpha 1 | −7.617 | <0.001 | −3.919 |
| AB011538       | Slit homolog 3 (Drosophila) | −7.569 | <0.001 | −3.813 |
| AB033073       | Similar to glucosamine-6-sulfatases | −7.473 | 0.001 | −3.627 |
| NM_003885      | Cyclin-dependent kinase 5, regulatory subunit 1 (p35) | −7.415 | <0.001 | −4.046 |
| NM_030786      | Intermediate filament protein syncoilin | −7.325 | <0.001 | −3.751 |
| NM_001864      | Cytochrome c oxidase subunit Vlla polypeptide 1 (muscle) | −7.295 | <0.001 | −3.654 |
### Table 4. cont.

| Genbank Acc No. | Gene name | x-fold change | p-value | z-score |
|-----------------|-----------|---------------|---------|---------|
| AK001058        | Homo sapiens cDNA FLJ10196 fs, clone HEMBA1004776 | −7.238 | <0.001 | −3.569 |
| NM_001375       | Deoxyribonuclease II, lysosomal | −7.202 | <0.001 | −3.719 |
| NM_002414       | Antigen identified by monoclonal antibodies 12E7, F21 and O13 | −7.199 | <0.001 | −3.630 |
| AL080135        | Hypothetical protein DKFZp434I143 | −7.174 | <0.001 | −4.265 |
| BF680501        | Putative membrane protein | −7.108 | <0.001 | −6.487 |
| NM_017980       | Hypothetical protein FLJ10044 | −6.999 | <0.001 | −3.665 |
| M68874          | Phospholipase A2, group IV (cytosolic, calcium-dependent) | −6.795 | <0.001 | −5.273 |
| NM_006552       | Lipophilin A (uroglobin family member) | −6.550 | <0.001 | −3.540 |
| AK054724        | Homo sapiens cDNA FLJ30162 fs, clone BRACE2000565 | −6.401 | <0.001 | −3.877 |
| AK025015        | Homo sapiens cDNA: FLJ21362 fs, clone COL02886 | −6.389 | <0.001 | −3.580 |
| NM_02776        | Kallikrein 10 | −6.375 | <0.001 | −3.741 |
| AF380356        | Homo sapiens PBX1 mRNA, complete cds | −6.263 | <0.001 | −4.673 |
| NM_004529       | Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3 | −6.221 | 0.003 | −4.190 |
| NM_025258       | NG37 protein | −6.045 | 0.002 | −4.951 |
| BC016964        | Homo sapiens, clone MGC:21621 IMAGE:418577, mRNA, complete cds | −5.945 | <0.001 | −4.086 |
| AK022198        | Homo sapiens cDNA FLJ12136 fs, clone MAMMA1000312 | −5.889 | 0.008 | −4.879 |
| AK056857        | Homo sapiens cDNA FLJ32295 fs, clone PROST2001823, weakly similar to TRANSCRIPTION FACTOR SP1 | −5.879 | 0.001 | −4.255 |
| NM_032514       | Microtubule-associated protein 1 light chain 3 alpha | −5.830 | <0.001 | −4.491 |
| AK024734        | Homo sapiens cDNA: FLJ21081 fs, clone CAS02995 | −5.816 | <0.001 | −4.229 |
| BC017981        | Homo sapiens, Similar to Riken cDNA 2700038C09 gene, clone MGC:24600 IMAGE:4245342, mRNA, complete c | −5.685 | 0.001 | −3.761 |
| AF220030        | Tripartite motif-containing 6 | −5.666 | <0.001 | −4.166 |
| NM_001458       | Filamin C, gamma (actin binding protein 280) | −5.649 | <0.001 | −3.748 |
| AK025786        | Homo sapiens cDNA: FLJ22133 fs, clone HEP200529 | −5.544 | <0.001 | −3.707 |
| NM_001935       | Dipeptidylpeptidase IV (CD26, adenosine deaminase complexing protein 2) | −5.426 | <0.001 | −4.964 |
| AF131851        | Hypothetical protein | −5.282 | <0.001 | −5.808 |
| NM_031957       | Keratin associated protein 1.5 | −5.278 | <0.001 | −3.813 |
| AK057853        | Homo sapiens cDNA FLJ25124 fs, clone CBR06414 | −5.132 | <0.001 | −5.054 |
| BC040224        | Homo sapiens, clone MGC:4762 IMAGE:3537945, mRNA, complete cds | −5.044 | <0.001 | −4.122 |
| NM_000451       | Short stature homeobox | −4.956 | <0.001 | −3.845 |
| U12767          | Nuclear receptor subfamily 4, group A, member 3 | −4.840 | <0.001 | −4.340 |
| NM_006517       | Solute carrier family 16 (monocarboxylic acid transporters), member 2 (putative transporter) | −4.815 | <0.001 | −4.004 |
| NM_018692       | Chromosome 20 open reading frame 17 | −4.722 | <0.001 | −5.134 |
| NM_005130       | Heparin-binding growth factor binding protein | −4.683 | 0.004 | −6.093 |
| AK026141        | Homo sapiens cDNA: FLJ22488 fs, clone HRC10948, highly similar to HSU72928 Human clone 23803 mRNA | −4.639 | <0.001 | −3.517 |
| AK055391        | Homo sapiens cDNA FLJ30829 fs, clone FEBRA2001790, highly similar to Xenopus laevis RRM-containing | −4.535 | <0.001 | −5.706 |
| AL359052        | Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 1968422 | −4.486 | <0.001 | −3.606 |
| NM_003012       | Secreted frizzled-related protein 1 | −4.471 | 0.002 | −3.815 |
| BC015134        | Homo sapiens, clone IMAGE:3934391, mRNA | −4.455 | 0.005 | −4.111 |
| AK055501        | Homo sapiens cDNA FLJ30939 fs, clone FEBRA2007414 | −4.436 | <0.001 | −3.795 |
| AF000994        | Ubiquitously transcribed tetranic peptide repeat gene, Y chromosome | −4.392 | <0.001 | −4.574 |
| NM_002089       | GRO2 oncogene | −4.319 | <0.001 | −4.839 |
| NM_018271       | Hypothetical protein FLJ10916 | −4.274 | <0.001 | −5.289 |
| NM_006030       | Calcium channel, voltage-dependent, alpha 2/delta subunit 2 | −4.269 | 0.005 | −5.657 |
| NM_052969       | Ribosomal protein L39-like | −4.156 | <0.001 | −3.920 |
| NM_022773       | Hypothetical protein FLJ12681 | −4.146 | <0.001 | −4.175 |
| NM_004753       | Short-chain dehydrogenase/reductase 1 | −4.134 | <0.001 | −4.386 |
| D13628          | Angiopoietin 1 | −4.075 | 0.003 | −5.116 |
| NM_004675       | Ras homolog gene family, member I | −4.027 | 0.001 | −3.549 |
| NM_001532       | Solute carrier family 29 (nucleoside transporters), member 2 | −4.009 | <0.001 | −3.337 |
| U16306          | Chondroitin sulfate proteoglycan 2 (versican) | −3.959 | <0.001 | −3.505 |
| Genbank Acc No. | Gene name | x-fold change | p-value | z-score |
|----------------|-----------|---------------|---------|---------|
| NM_024806      | Hypothetical protein FLJ23554 | 2 | <0.001 | 3.678 |
| AF152529       | Protocadherin gamma subfamily B, 8 pseudogene | 3.770 | 0.003 | 4.390 |
| NM_006383      | DNA-dependent protein kinase catalytic subunit-interacting protein 2 | 3.676 | 0.001 | 4.543 |
| NM_020169      | Latexin protein | 3.670 | <0.001 | 3.817 |
| AK055969       | Homo sapiens cDNA FLJ31407 fs, clone NT2NE2000137 | 3.494 | 0.006 | 4.646 |
| BC011406       | Homo sapiens, clone MGC:9758 IMAGE:3855620, mRNA, complete cds | 3.281 | <0.001 | 3.672 |
| NM_014553      | LBP protein; likely ortholog of mouse CRTR-1 | 3.676 | 0.001 | 4.543 |
| NM_006821      | Peroxisomal long-chain acyl-coA thioesterase | 3.221 | <0.001 | 3.869 |
| NM_005098      | Musculin (activated B-cell factor-1) | 3.198 | 0.002 | 4.057 |
| AK022355       | Homo sapiens cDNA FLJ12293 fs, clone MAMMA1001815 | 3.178 | <0.001 | 3.925 |
| NM_003178      | Synapsin II | 3.130 | 0.004 | 4.693 |
| U14383         | Mucin 8, tracheobronchial | 3.043 | 0.009 | 4.336 |
| NM_004257      | TGF beta receptor associated protein -1 | 3.028 | 0.006 | 4.330 |
| AK021632       | Homo sapiens cDNA FLJ11570 fs, clone HEMBA1003309 | 2.987 | 0.009 | 3.984 |
| NM_002108      | Histidine ammonia-lyase | 2.987 | <0.001 | 3.812 |
| NM_032880      | Hypothetical protein MGC15730 | 2.956 | 0.001 | 3.585 |
| BC015160       | Homo sapiens, clone IMAGE:3885940, mRNA, partial cds | 2.873 | 0.004 | 3.919 |
| AK055509       | Homo sapiens cDNA FLJ30947 fs, clone FEBC2007714 | 2.829 | <0.001 | 3.925 |
| D86964         | Dedicator of cyto-kinesis 2 | 2.801 | 0.002 | 3.595 |
| NM_006359      | Tumor necrosis factor (ligand) superfamily, member 6 | 2.731 | 0.005 | 3.872 |
| NM_006494      | Solute carrier family 16 (monocarboxylic acid transporters), member 6 | 2.681 | 0.005 | 3.662 |
| AB007964       | KIAA0495 | 2.677 | 0.001 | 3.586 |
| X68994         | H.sapiens CREB gene, exon Y | 2.654 | 0.004 | 3.554 |
| NM_013227      | Aggrecan 1 (chondroitin sulfate proteoglycan 1, large aggregating proteoglycan, antigen identified b | 2.628 | 0.007 | 3.646 |
| AF019226       | Glioblastoma overexpressed | 2.411 | 0.006 | 3.507 |
| AK054905       | Homo sapiens cDNA FLJ30343 fs, clone BRACE2007502 | 1.931 | 0.006 | 4.281 |
| AF339768       | Homo sapiens clone IMAGE:119716, mRNA sequence | 2.544 | 0.004 | 3.639 |
| NM_003631      | Thrombomodulin | 2.620 | 0.001 | 3.636 |
| NM_006187      | 2’-5’-oligoadenylate synthetase 3 (100 kD) | 2.628 | 0.007 | 3.724 |
| AK025390       | Homo sapiens cDNA: FLJ121737 fs, clone CDLF3396 | 2.772 | 0.001 | 3.713 |
| AF130074       | Homo sapiens clone FLB9348 PRO2523 mRNA, complete cds | 2.805 | 0.001 | 4.057 |
| AB032962       | KIAA1136 protein | 2.838 | 0.001 | 4.111 |
| AJ420570       | Homo sapiens cDNA FLJ14752 fs, clone NT2RP3003071 | 2.870 | <0.001 | 3.839 |
| NM_014811      | KIAA0649 gene product | 2.873 | 0.007 | 4.113 |
| NM_004171      | Solute carrier family 1 (glial high affinity glutamate transporter), member 2 | 2.950 | 0.008 | 4.227 |
| NM_013982      | Neuregulin 2 | 2.985 | 0.001 | 3.982 |
| NM_032808      | Hypothetical protein FLJ14594 | 3.020 | <0.001 | 4.172 |
| AB002366       | KIAA0368 protein | 3.085 | 0.009 | 4.403 |
| NM_018700      | Tripartite motif-containing 36 | 3.129 | 0.001 | 4.306 |
| BC003376       | ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R) | 3.172 | <0.001 | 3.568 |
| AK023283       | Homo sapiens cDNA FLJ13221 fs, clone NT2RP4002075 | 3.221 | <0.001 | 3.869 |
| AK054766       | Homo sapiens cDNA FLJ30204 fs, clone BRACE2001496 | 3.329 | 0.001 | 3.717 |
| NM_002829      | Protein tyrosine phosphatase, non-receptor type 3 | 3.429 | <0.001 | 3.617 |
| NM_001618      | ADP-ribosyltransferase (NAD+; poly [ADP-ribos] polymerase) | 3.444 | 0.001 | 4.504 |
| NM_001445      | Fatty acid binding protein 6, ileal (gastrotropin) | 3.450 | 0.001 | 4.096 |
| NM_024565      | Hypothetical protein FLJ14166 | 3.528 | 0.002 | 3.896 |
| L05148         | Zeta-chain (TCR) associated protein kinase (70 kD) | 3.599 | <0.001 | 3.759 |
| NM_005525      | Hydroxysteroid (11-beta) dehydrogenase 1 | 3.601 | <0.001 | 3.525 |
| NM_016931      | NADPH oxidase 4 | 3.657 | 0.004 | 4.289 |
| AK057339       | Actin like protein | 3.693 | <0.001 | 4.038 |
Moreover, TMC also have telomerase activity which is not found in pre- or post-senescence MSC [6].

**DISCUSSION**

Mesenchymal stem cells can be easily isolated and expanded in culture to generate large numbers of cells for cellular therapies. Human MSC in early passage are safe although stressful conditions (as they are cultured for a long time) can turn them in immortal and occasionally they became tumorogenic [6]. Further research is necessary to understand this process in order to develop better protocols for culture adult stem cells, as it has been showed better protocols for culture adult stem cells, as it has been demonstrated recently [19]. Here, we describe several molecular alterations in our spontaneous human MSC transformation model that affect cell cycle regulation, oncogene expression, mitochondrial metabolism, DNA repair mechanisms and inactivation of tumor suppressor genes.

TMC versus pre-senescence MSC analysis showed that functions with a higher significance are related, with expected, with transformation, genetic disorders and cell death (Table 1). We described previously that post-senescence MSC are non-tumorigenic and their cellular behaviour in culture was very similar to pre-senescence MSC [6]. Interestingly, post-senescence MSC versus pre-senescence MSC array analysis also showed the same functions altered than TMC, although in smaller grade (Table 1), suggesting a pre-tumoral state of pre-senescence MSC.

We observed expression of many untranslated RNAs in MSC concurring with reports which show a large and “silent” mRNA pool in stem cells [20], this could be the reason why, following MSC transformation, we identified more downregulated than upregulated genes in arrays experiments (Figure S1). Comparison of mRNA and protein expression in pre- and post-senescence MSC and in TMC showed variation in RNA and protein regulation. Cyclin D2, Cdk1 and PCNA mRNA were upregulated in TMC compared to MSC, although their protein levels did not change; whereas c-Myc, Cdk2, Cdk6, DNA ligase IV and DNA polymerases mRNA levels remained stable but their protein levels were upregulated. Translational control could thus be important for adult stem cells, and retention of large numbers of silenced transcripts might allow rapid stem cell differentiation to other lineages in response to appropriate stimuli. These data also indicates the limitations of results based on RNA-exclusive analysis of MSC.

Telomerase activity has been found in almost all human tumors but not in adjacent normal cells [21] and maintenance of telomere stability is required for the long-term proliferation of tumor cells [22]. The escape from cellular senescence and thus becoming immortal by activating telomerase is required by most tumor cells for their ongoing proliferation [23]. In our model, during TMC generation these cells acquire a detectable telomerase activity [6]. Telomerase promotes MSC immortalization and, in conjunction with additional events, produces cell transformation [12,13,24]. These additional events usually implied an oncogene deregulation.

One of the most important oncogenes involved in MSC transformation is c-myc. In our spontaneous model, senescent and post-senescence MSC, as well as TMC, overexpress c-myc [6]. Consistent with our previous results, data from other groups have shown that c-myc seems to be essential to spontaneously transform MSC [7,9,10]. In this regard, Funes et al. used retroviral vectors to introduce human telomerase (TERT), HPV-16 E6 and E7, H-Ras and SV40 small T antigen (ST), individually or in combination, in human MSC. The combination of TERT, E6, E7 and H-Ras did not induce MSC transformation. Only MSC transduced with ST becomes transformed cells [10]. ST inactivates phosphatase 2A, hence, TMC can be an appropriate model to study MSC transformation and understand the genetic and epigenetic changes involved in this process.

**Table 4. cont.**

| Genbank Acc No. | Gene name | x-fold change | p-value | z-score |
|-----------------|-----------|---------------|---------|---------|
| NM_024771       | Hypothetical protein FLJ13848 | 3.881 | 0.001 | 5.345 |
| NM_032047       | UDP-GalNAc:betaGal beta-1,3-N-acetylgalosaminyltransferase 5 | 4.172 | 0.002 | 4.415 |
| NM_014962       | BTB (POZ) domain containing 3 | 4.237 | 0.002 | 5.362 |
| NM_017780       | KIAA1416 protein | 4.243 | <0.001 | 3.977 |
| AB018295        | KIAA0752 protein | 4.556 | <0.001 | 5.016 |
| NM_006622       | Serum-inducible kinase | 4.639 | 0.001 | 4.742 |
| NM_003651       | Cold shock domain protein A | 4.961 | <0.001 | 4.080 |
| AF268419        | Homo sapiens chondrosarcoma CSAG1c mRNA sequence | 5.423 | <0.001 | 3.659 |
| AK055111        | Homo sapiens cDNA FLJ30549 fis, clone BRAWH2001484, weakly similar to POLYPEPTIDE N-ACETYLGLALACTOSAM | 5.503 | <0.001 | 3.909 |
| NM_006597       | Heat shock 70kD protein 8 | 5.664 | 0.001 | 3.622 |
| NM_019844       | Solute carrier family 21 (organic anion transporter), member 8 | 5.919 | 0.000 | 4.271 |
| AB037727        | Cask-interacting protein 1 | 7.351 | <0.001 | 3.597 |
| AL137311        | Homo sapiens mRNA; cDNA DKFZp761G02121 (from clone DKFZp761G02121); partial cds | 8.238 | <0.001 | 3.661 |
| BC014584        | Homo sapiens, clone IMAGE:4074062, mRNA | 8.280 | 0.001 | 3.982 |
| NM_003785       | G antigen, family B, 1 (prostate associated) | 8.326 | <0.001 | 3.582 |
| NM_005010       | Neuronal cell adhesion molecule | 8.533 | <0.001 | 4.478 |
| NM_033642       | Fibroblast growth factor 13 | 9.666 | <0.001 | 4.016 |
| NM_018476       | Brain expressed, X-linked 1 | 11.140 | <0.001 | 3.946 |
| NM_002364       | Melanoma antigen, family B, 2 | 12.281 | <0.001 | 4.005 |

* Array data were filtered according to Z-score (>3.5 and < -3.5) and p-value (<0.01). doi:10.1371/journal.pone.0001398.t004

TMC Molecular Characterization
resulting in c-myc stabilization [25], suggesting that c-myc might be necessary to transform MSC.

We explored DNA repair mechanisms to elucidate their role in MSC transformation. Post-senescent MSC showed downregulation of DNA-PKcs, ERCC3 and Rad51 proteins, each of which is associated to a distinct DNA repair pathway. Extremely restricted clonal selection takes place during cell crisis, and only cells with functional DNA repair mechanisms would continue to grow. TMC have a higher metabolic rate and divide more rapidly than pre- or post-senescence MSC, with a consequent increase in DNA damage. Proteins that participate in DNA repair are upregulated in TMC compared to MSC; this, together with telomere length maintenance, could permit cell survival, despite oxidative damage to DNA and be responsible for TMC karyotype stabilization. Recently it has been published the dependency on oxidative phosphorylation during MSC transformation [11]. We have not detected statistically significant changes of these genes in our microarray experiments (Table S1), although potential pathways leading to changes in post-senescence MSC and TMC revealed changes in stress, toxic events and mitochondrial metabolism pathways (Table 2). The definitive role of mitochondrial respiration on spontaneous MSC transformation remains to be investigated.

A chromosome 5 alteration and a (3;11) translocation are recurrent, stable features of in vitro cultured TMC [6]. The telomerase gene map to human chromosome 5, suggests that it is activated by internal amplification of this chromosome in TMC. Chromosome 11 alterations are recurrent in tumors [26]. Although we did not detect a target gene in the 3;11 translocation in our model, genes involved in cell transformation are likely to be located in this region [27–29].

As tumor suppressor genes are major targets in neoplastic transformation, we analyzed their expression in these cells. The tumor suppressor Rb is implicated in several cancer types [18]. In our model of MSC transformation, Rb protein levels are upregulated progressively, and Rb is inactivated by a phosphorylation mechanism in TMC, as described [30]. In addition of Rb, loss of p53 function is common in many tumor types [18], but this pathway appeared to be functional in our model, as p53 was upregulated and phosphorylated in UV-irradiated cells. We observed higher basal p53 levels in TMC than in MSC, even when they had not been exposed to UV irradiation. In TMC, p16 mRNA and protein were entirely absent, and the Ink4a/Arf locus had been deleted. The increase in basal p53 may thus be due to stabilization by the ubiquitin protein ligase MDM2, due to the lack of p16 [31]. Identical results, p16 locus deletion and normal p53 activity, was detected in telomerase-immortalized human MSC [32]. The results suggest that p16 inhibition is essential for TMC generation, as is the case for human malignancies including glioblastoma, melanoma, pancreatic adenocarcinoma, non-small cell lung cancer, bladder carcinoma and oropharyngeal cancer, where this tumor suppressor is frequently lost [33].

Figure 1. Cell cycle regulation. (A) x-fold change, p-value and Z-score of cell cycle regulators expression measured by microarray analysis between pre- and post-senescence MSC and post-senescence MSC and TMC. (B) Relative mRNA expression of Cyclin D1 (CCND1), and cyclin-dependent kinases 2 (CDK2) and 6 (CDK6) in pre- and post-senescence MSC, TMC and met-TMC analyzed by qRT-PCR. (C) Western blot analysis of cell cycle regulator protein expression in pre- and post-senescence MSC and two TMC samples. α-tubulin was used as loading control.

doi:10.1371/journal.pone.0001398.g001
Finally, we propose a two-stage model in which a mesenchymal stem cell becomes a tumor cell (Figure 4). The first step, the senescence bypass or M1 phase, is associated with c-myc overexpression and p16 repression; many DNA repair proteins are subsequently downregulated. Telomere shortening provokes the cell crisis phase, or M2, in which cells undergo stringent selection. TMC then upregulate many DNA repair proteins, which may be necessary for crisis bypass. Finally, escape from crisis is associated with telomere stabilization, Rb hyperphosphorylation and p16 deletion that seems to be essential to promote transformation [33,34]. TMC also upregulate many DNA repair proteins, which may be necessary for crisis bypass. These levels are maintained in TMC and could permit cell survival, despite oxidative damage to DNA.

The essential steps in TMC generation described here are basically in agreement with results of other authors working in MSC transformation [7–11,32] and these alterations are very similar to molecular changes associated with transformation of other cell types. In epithelial cells, spontaneous immortalization of human keratinocytes exhibited a small number of chromosomal aberrations, reduced p16INK4a mRNA, elevated telomerase activity and functionally normal p53 [35]. Immortalization of human prostate cells by c-myc revealed overexpression of the c-myc and Bmi-1 oncogenes, as well as loss of p14ARF expression [36,37], while overexpression of c-myc immortalizes freshly isolated human foreskin fibroblasts displayed a marked decrease in expression of p14ARF [38,39].

In sum, all these evidences strongly suggest that cells with a mesodermal origin could require a common sequence of oncogenic events to become a tumor cells. How these processes are coordinated or associated with the critical cell evolution/selection revealed in the culture [6] remains to be studied in deep. The cause/consequence relationship of this molecular signature with the recently characterized mesenchymal to epithelial transition (Rubio, D. et al, in press) or other potentially involved mechanisms remains also to be determined.

**MATERIALS AND METHODS**

**Isolation of MSC and cell culture**

MSC were isolated as described [6]. Briefly, adipose tissue from non-oncogenic patients were digested with collagenase P and

---

### Table: DNA Repair-Related Gene Expression

| Gene        | MSC pre-sen/MSC post-sen | MSC pre-sen/TMC |
|-------------|--------------------------|-----------------|
|             | x-fold change | p-value | Z-score | x-fold change | p-value | Z-score |
| DNA-PKc     | -1.121        | 0.23    | -0.438  | 1.572        | <0.05   | 0.7225   |
| Ku70        | 1.125         | 0.27    | 0.5895  | 1.86         | 0.45    | 0.8165   |
| XRCC4       | 1.128         | 0.14    | 0.693   | 1.789        | 0.46    | 0.5065   |
| ligase IV   | -1.045        | 0.65    | -0.309  | 1.063        | 0.32    | 0.0185   |
| PolU        | 1.126         | 0.05    | 0.889   | 1.263        | <0.01   | 0.505    |
| Polε        | -1.122        | 0.32    | -0.4145 | 1.1258       | 0.39    | -0.1305  |
| PCNA        | 1.081         | 0.78    | 0.4415  | 3.676        | <0.01   | 2.029    |
| ERCC3       | -1.097        | 0.25    | -0.546  | 1.386        | <0.05   | 0.5215   |
| XPA         | 1.056         | 0.38    | -0.3205 | -1.932       | <0.01   | -1.113   |
| XPF         | nd            | nd      | nd      | nd           | nd      | nd       |
| XPG         | nd            | nd      | nd      | nd           | nd      | nd       |
| Rad51       | -1.113        | 0.52    | -0.64   | -1.118       | 0.26    | -0.2075  |

---

**Figure 2. DNA repair regulation.** (A) x-fold change, p-value and Z-score of DNA repair-related gene expression measured by microarray analysis between pre- and post-senescence MSC and post-senescence MSC and TMC. (B) Relative mRNA expression of ERCC3, DNA ligase IV (LIG IV), DNA polymerase b (POLb) and µ (POLµ), RAD51, XPA and XRCC4 in pre- and post-senescence MSC, TMC and met-TMC analyzed by qRT-PCR. (C) Western blot analysis of DNA repair-related protein expression in pre- and post-senescence MSC and two TMC samples. α-tubulin was used as loading control. doi:10.1371/journal.pone.0001398.g002

---

**Figure 3. TMC Molecular Characterization.**
Figure 3. Regulation of oncogenes and tumor suppressor genes.

(A) x-fold change, p-value and Z-score of oncogenes and tumor suppressor genes measured by microarray analysis between pre- and post-senescence MSC and post-senescence MSC and TMC. (B) Relative mRNA expression of p16 analyzed by qRT-PCR in different samples of pre-senescence MSC (n = 4), post-senescence MSC (n = 3) and TMC (n = 3). (C) Homozygous deletion analysis of p14, p15 (D) and p16 (E) genes. β-actin was used as internal PCR control. Control cell lines were normal lymphocytes (NL), HCT116 and MDA-MB231. (F) Western blot analysis of p21 expression in pre- and post-senescence MSC, TMC and met-TMC. α-tubulin was used as loading control. (G) Analysis of p53 activation following UV irradiation of cells. p53 levels and phosphorylation were tested in pre- and post-senescence MSC, in two TMC samples and a sample of met-TMC. α-tubulin was used as loading control. (H) Rb protein levels and phosphorylation tested in pre- and post-senescence MSC, two samples of TMC and a met-TMC sample. α-tubulin was used as loading control.

doi:10.1371/journal.pone.0001398.g003
cultured (37°C, 5% CO₂) in MSC medium (DMEM plus 10% FCS, 2 mM glutamine, 50 μg/ml gentamycin) and passaged when they reached 85% confluence. TMC and met-TMC were cultured under the same conditions.

**Microarray labeling**

Total RNA was isolated from four biological replicates of pre- and post-senescence MSC and from TMC using TriReagent Solution (Sigma) following manufacturer’s instructions. RNAs were purified with MegaClear (Ambion) and integrity confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies). Total RNA (1.5 μg each) was amplified using the Amino Allyl MessageAmp aRNA kit (Ambion); we obtained 15–60 μg of amino-allyl amplified RNA (aRNA). Mean aRNA size was 1500 nucleotides, as measured using the Agilent 2100 Bioanalyzer. For each sample, 2.5 μg of aRNA was labeled with one aliquot of Cy3 or Cy5 Mon NHS Ester (CyDye Post-labeling Reactive Dye, Amersham) and purified with the Amino Allyl MessageAmp aRNA kit. Cy3 and Cy5 incorporation were measured using 1 μl probe in a Nanodrop spectrophotometer (Nanodrop Technologies). For each hybridizations, 80–100 pmol of Cy3 and Cy5 probes were mixed, dried by speed-vacuum, and resuspended in 9 μl RNase-free water. Labeled aRNA was fragmented by adding 1 μl of 10× fragmentation buffer (Ambion) and incubating (70°C, 15 min). The reaction was terminated with 1 μl stop solution (Ambion).

**Slide treatment and hybridization**

Slides containing 22,102 annotated genes corresponding to the Human 70-mer oligo library (V2.2) (Qiagen-Operon) were obtained from the Genomics and Microarray Laboratory (Cincinnati University). Information on printing and the oligo set can be found at http://microarray.uc.edu. Slides were prehybridized (42°C, 45–60 min) in 6× SSC, 0.5% SDS and 1% BSA, then rinsed 10 times with distilled water. Fragmented Cy5 and Cy3 aRNA probes were mixed (80–100 pmol of each label) with 10 μg PolyA (Sigma) and 5 μg Human Cot-DNA (Invitrogen) and dried in a speed-vacuum. Each probe mix was resuspended in 60 μl of hybridization buffer (50% formamide, 6× SSC, 0.5% SDS, 5× Denhardt’s solution). Probes were denatured (95°C, 5 min) and applied to the slide using a LifterSlip (Erie Scientific). Slides were incubated (48°C, 16 h) in hybridization chambers (Array-It; Telechem International) in a water bath. After incubation, slides...
were washed twice with 0.5× SSC, 0.1% SDS (5 min each), three times with 0.5× SSC (5 min) and finally in 0.05× SSC (5 min), then dried by centrifugation (563 g, 1 min). Images from Cy3 and Cy5 channels were equilibrated and captured with an Axon 4000B scanner and spots quantified using GenePix 5.1 software.

Four independent biological replicates were “dye swapped” and studied (6 hybridizations). Data were analyzed using Almazen software. Each replicate was lowess-normalized and the log ratios were calculated with the corresponding standard deviation and z-score. We obtained adjusted p-values using limma by FDR [40]. Differentially expressed genes were selected by filtering signal intensity (>64), z-score (>3.5 or < −3.5) and p-value (<0.01).

Pathways analysis
By using Ingenuity Pathways Analysis (IPA), potential pathways leading to changes in MSC-post-senescent and TMC were created. This web-delivered application reveals relevant networks by comparing gene expression data with known pathways and interactions between genes (http://www.ingenuity.com). The filtered expression data set for MSC-post-senescent and TMC regulated genes were uploaded as tab-delimited text into IPA for generating biological networks. Each gene identifier was mapped to its corresponding object in the Ingenuity Pathways Knowledge Base. This software assigned a score for all networks that were ranked on the probability that a collection of genes equal to or greater than the number in a network could be achieved by chance alone (a score of 2 represents a 99% confidence level, and 3 a 99.9%). Biological functions are then calculated an assigned to each network.

Quantitative real-time PCR (qRT-PCR)
cDNA was generated from 100 ng of total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems) in a 10 µl final reaction volume. Real-time PCR reactions were performed in triplicate using two dilutions (1/50, 1/500; 3 µl/µl) of each cDNA, 1× TaqMan Assay-On-Demand (Hs00233365_m1, Cdkn2a; Hs00195591_m1, snail; Hs00161904_m1, slug) or primers described in Table S2, 1× SYBR Green PCR Master Mix or 1× TaqMan Universal PCR Master Mix (Applied Biosystems) in a volume of 8 µl in 384-well optical plates, or using Universal ProbeLibrary (Roche). PCR reactions were run on an ABI Prism 7900HT (Applied Biosystems) and SDS v2.2 software was used to analyze the results with the Comparative Ct Method (ΔΔCt).

Western blot
Cell extracts were fractionated in 6%–15% SDS-PAGE, followed by transfer to PVDF membranes. We used antibodies to cyclins A clone E23 (1/200), D1 DCS-11 (1/1000), and D2 DCS-3.1 (1/1000) from Labvision; cyclin B1 sc-595 (1/200), cyclin D3 sc-182 (1/200), cdc2 sc-747 (1/200), cdk2 sc-163 (1/200), cdk4 sc-260 (1/200) and cdk6 sc-177 (1/200) were from Santa Cruz Biotechnology, DNA ligase IV sc-11748 (1/200) from Santa Cruz Biotechnology. We also used anti-DNA polymerase μ [23], -PCNA Ab-1 (Calbiochem, 1/100), and -DNA polymerase β Ab-1 (1/500), -ERCC1 Ab-2 (1/200), -XPA Ab-1 (1/200), -XPF Ab-1 (1/200), and -XPG Ab-1 (1/200) from Labvision, -Rad-31 (Pharmping, 1/3000), -p21 sc-397 (Santa Cruz Biotechnology 1/1000), -p53 DO-1 (Merck, 1/1000), and -Rb (1/2000, overnight, 4°C), -phospho Ser 780-Rb (1/1000, overnight, 4°C), -phospho Ser 795-Rb (1/1000, overnight, 4°C), -phospho Ser 807/811-Rb (1/1000, overnight, 4°C) from Cell Signalling. We used anti-tubulin 9026 (Sigma, 1/5000). Incubation was 1 h at room temperature unless otherwise specified, followed by peroxidase-labelled goat anti-mouse, goat anti-rabbit or rabbit anti-goat antibody (Dako, 1/2000, 1 h, RT). Blots were developed using ECL (Amersham).

p53 activation assay
We induced p53 upregulation and activation in UV-irradiated pre- and post-senescent MSC, TMC, and met-TMC (15 JU/m²). Extracts were collected 18 h after irradiation and used in western blot with anti-p53 or -phospho Ser15-p53 antibodies. β-tubulin was used as control.

Analysis of p16ink4a, p15ink4b and p14ARF CpG island methylation status
We determined DNA methylation patterns in the CpG islands of p16ink4a, p15ink4b and p14ARF tumor suppressor genes by chemical conversion of unmethylated, but not methylated, cytosine to uracil, followed by methyl-specific PCR (MSP). Amplification using primers specific for methylated or modified unmethylated DNA [41,42]. Placental DNA treated in vitro with Sss I methyltransferase was used as positive control, and DNA from normal lymphocytes as negative control for methylated alleles. Each PCR sample (12 µl) was separated in non-denaturing 6% polyacrylamide gels, ethidium bromide-stained, and visualized with UV illumination. Promoter methylation status of these genes was verified by bisulfite genomic sequencing of CpG islands. Both strands were sequenced. Primers for bisulfite genomic sequencing and methylation-specific PCR were designed according to genomic sequences around presumed transcription start sites of the genes studied. Primer sequences and PCR conditions for methylation analysis are available on request.

Bisulfite treatment
Genomic DNA was EcoRI-digested to shear DNA and achieve complete chemical conversion after bisulfite treatment. Sodium bisulfite conversion of genomic DNA (1 µg) was performed as described [41,42], with modifications. Briefly, NaOH was added to denature DNA (0.3 M final concentration) and incubated (15 min, 37°C). Fresh bisulfite solution (2.5 M sodium metabisulfite and 125 mM hydroquinone, pH 5.0) was added to each sample, and incubation continued (16 h, 50°C, in the dark). Modified DNA was purified using Wizard DNA purification resin (Promega) and eluted in water at 60°C. After desulfonation with NaOH (0.3 M final concentration; 10 min, 37°C), isolation was continued with 0.3 volume of 10.5 M ammonium acetate, followed by incubation (5 min, RT). Modified DNA was precipitated using 2.5 volumes of 100% ethanol and glycogen (5 mg/ml) as a carrier. The pellet was washed with 70% ethanol, dried, and eluted in distilled water.

Homoygous deletion analysis
We analyzed fragments of the p16INK4a-E1z, E2, p14ARF-E1β, and p15INK4b genes as described [16] to detect homoygous deletion in TMC. Comparative multiplex PCR was performed as described [43] to analyze each gene locus, using the β-actin fragment as internal control. Normal lymphocytes (NL) were used as negative control of tumor suppressor gene methylation, HCT116 (colorectal cancer line) as positive control of Ink4a/Arf locus methylation and MDA-MB231 (mammary adenocarcinoma) as control of Ink4a/Arf locus deletion.

SUPPORTING INFORMATION
Figure S1 Comparison of mRNA differences between pre- and post-senescent MSC and in TMC. Microarray analysis pattern of overall mRNA differences between pre- and post-senescent MSC (A), and pre-senescent MSC and TMC (B). MA plots are shown,
being A: log-ratio of two expression intensities vs. M: the mean log-expression of the two.

Found at: doi:10.1371/journal.pone.0001398.s001 (4.09 MB TIF)

Table S1 Main mRNA differences between pre-senescent MSC and TMC focused in genes implicated in bioenergetic pathways.

Found at: doi:10.1371/journal.pone.0001398.s002 (0.11 MB DOC)

Table S2 Primers used for q-RT-PCR analysis with Universal ProbeLibrary protocol.

Found at: doi:10.1371/journal.pone.0001398.s003 (0.04 MB DOC)

REFERENCES

1. Hahn WC, Weinberg RA (2002) Rules for making human tumor cells. N Engl J Med 347: 1593–1603.
2. Hahn WC, Weinberg RA (2002) Modelling the molecular circuitry of cancer. Nat Rev Cancer 2: 311–341.
3. Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414: 105–111.
4. Dalerba P, Cho RW, Clarke MF (2007) Cancer stem cells: models and concepts. Annu Rev Med 58: 267–284.
5. Pardal R, Clarke MF, Morrison SJ (2003) Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 3: 985–992.
6. Rubio D, García-Castro J, Martín MC, de la Fuente R, Cigudosa JC, et al. (2005) Spontaneous human adult stem cell transformation. Cancer Res 65: 3033–3039.
7. Miura M, Miura Y, Padilla-Nash HM, Molinolo AA, Fu B, et al. (2006) Accumulated Chromosomal Instability in Murine Bone Marrow Mesenchymal Stem Cells Leads to Malignant Transformation. Stem Cells 24: 1095–1103.
8. Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, et al. (2006) Sarcoma Derived from Cultured Mesenchymal Stem Cells. Stem Cells 25: 371–379.
9. Wang Y, Huo DL, Harrington K, Jelkner J, Jeong DK, et al. (2005) Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. Cytoteraphy 7: 509-519.
10. Zhou YZ, Bosch-Marce M, Okuyama H, Krishnamachary B, Kimura H, et al. (2006) Spontaneous Transformation of Cultured Mouse Bone Marrow-Derived Stromal Cells: A Model of Age-Related Tumorigenesis In Mouse. Cancer Res 66: 10849–10854.
11. Li H, Fan X, Kowit RS, Jo Y, Mosquín B, et al. (2007) Spontaneous Expression of Embryonic Factors and p53 Point Mutations in Aged Mesenchymal Stem Cells: A Model of Age-Related Tumorigenesis. Cancer Res 67: 10889-10898.
12. Funes JM, Quintiero M, Hernandez S, Martinez D, Quintero M, et al. (2007) Transformation of human mesenchymal stem cells increases their dependency on oxidative phosphorylation for energy production. Proc Natl Acad Sci U S A 104: 6223–6228.
13. Serakinci N, Goldburg P, Burns JS, Abdullah B, Schroeder H, et al. (2004) Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 23: 5993–5998.
14. Kastan MB, Bartek J (2004) Cell-cycle checkpoints and cancer. Nature 432: 375–382.
15. Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M (1999) The p16INK4a and deletion of p15INK4b are frequent events in human esophageal carcinogenesis. Cancer Res 59: 3126–3135.
16. Xing EP, Nie Y, Wang LD, Yang GY, Yang CS (1999) Aberrant methylation of the p16INK4A promoter in colon carcinoma cell lines. Nucleic Acids Res 33: e73.
17. Zongaro S, de Stanchina E, Colombo T, D’Incalci M, Giulotto E, et al. (2005) Association of p16(INK4a) and pRb inactivation with immortalization of human lymphoma cell lines. Blood 105: 2149–2156.
18. Milyavsky M, Shats I, Erez N, Tang X, Senderovich S, et al. (2003) Prolonged culture of telomerase-immortalized human fibroblasts leads to a premalignant phenotype. Cancer Res 63: 7147–7157.
19. Benanti JA, Wang ML, Myers HE, Robinson KL, Grandori C, et al. (2007) Epigenetic Down-Regulation of ARF Expression Is a Selection Step in Immortalization of Human Fibroblasts by c-Myc. Mol Cancer Res 5: 2169–2185.
20. Pegram LD, Megensil MD, Lange BJ, Nowell PC, Rowley JD, et al. (2000) t(3;11) translocation in treatment-related acute myeloid leukemia fuses MLL with the GMS (guanidine 3’ monophosphate synthetase) gene. Blood 96: 4360–4362.
21. Labana M, Nemo T, Komatsu N, Machida H, Miyoshi I, et al. (2000) Constitutional t(3;11)(p21;q23) in a family, including one member with lymphoma establishment of novel cell lines with this translocation. Cancer Genet Cytoenet 117: 28–31.
22. Chau BN, Wang JY (2003) Coordinated regulation of life and death by RB. Nat Rev Cancer 3: 130–138.
23. Serakinci N, Goldburg P, Burns JS, Abdullah B, Schroeder H, et al. (2004) Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 23: 5093–5098.
24. Serakinci N, Goldburg P, Burns JS, Abdullah B, Schroeder H, et al. (2004) Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene 23: 5093–5098.
25. TMC Molecular Characterization

ACKNOWLEDGMENTS

We thank L. Almonacid for qRT-PCR analysis, C. Mark and C. Pantol for editorial support.

Author Contributions

Conceived and designed the experiments: DR, AB, JG. Performed the experiments: DR, SG, TD, MP. Analyzed the data: DR, SG, AL, JG, LL. Contributed reagents/materials/analysis tools: AL. Wrote the paper: DR, AB, AL, JG.

January 2008 | Issue 1 | e1398

TMC Molecular Characterization

PLoS ONE | www.plosone.org

15

PLOS ONE