

## Accepted Manuscript

Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells

S. Garcia, M.C. Martín, R. de la Fuente, J.C. Cigudosa, J. Garcia-Castro, A. Bernad

PII: S0014-4827(10)00065-0  
DOI: doi:[10.1016/j.yexcr.2010.02.016](https://doi.org/10.1016/j.yexcr.2010.02.016)  
Reference: YEXCR 8378

To appear in: *Experimental Cell Research*

Received date: 5 February 2010  
Accepted date: 12 February 2010



Please cite this article as: S. Garcia, M.C. Martín, R. de la Fuente, J.C. Cigudosa, J. Garcia-Castro, A. Bernad, Pitfalls in spontaneous in vitro transformation of human mesenchymal stem cells, *Experimental Cell Research* (2010), doi:[10.1016/j.yexcr.2010.02.016](https://doi.org/10.1016/j.yexcr.2010.02.016)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

**PITFALLS IN SPONTANEOUS IN VITRO TRANSFORMATION OF HUMAN MESENCHYMAL STEM CELLS**

García S<sup>1</sup>, Martín MC<sup>2</sup>, de la Fuente R<sup>3</sup>, Cigudosa JC<sup>2</sup>, García-Castro J<sup>3,4,5</sup> and Bernad A<sup>1,3,5</sup>

<sup>1</sup> Regenerative Cardiology Department. Centro Nacional de Investigaciones Cardiovasculares Carlos III. Melchor Fernández Almagro, 3. 28029 Madrid, Spain

<sup>2</sup> Cytogenetic Unit. Centro Nacional de Investigaciones Oncológicas. Melchor Fernández Almagro, 3. 28029 Madrid, Spain

<sup>3</sup> Department of Immunology and Oncology. Centro Nacional de Biotecnología. UAM. Campus de Cantoblanco. 28049 Madrid, Spain

<sup>4</sup> Área de Biología Celular y del Desarrollo. Instituto de Salud Carlos III. Ctra Majadahonda-Pozuelo Km 2,200. 28220 – Majadahonda. Madrid, Spain

<sup>5</sup> **Corresponding authors**

Antonio Bernad. Regenerative Cardiology Department. Centro Nacional de Investigaciones Cardiovasculares Carlos III. Melchor Fernández Almagro, 3. 28029 Madrid, Spain  
Tel.: 00 34 91 453 1274/ 12 77; Fax: 00 34 91 453 12 40; [abernad@cnic.es](mailto:abernad@cnic.es)

Javier García-Castro. Área de Biología Celular y del Desarrollo. Instituto de Salud Carlos III. Ctra Majadahonda-Pozuelo Km 2,200. 28220 – Majadahonda. Madrid, Spain  
Tel: 00 34 918223288; Fax: 00 34 918223269; E-mail: [jgcastro@isciii.es](mailto:jgcastro@isciii.es)

Spontaneous *in vitro* transformation of human primary cells was, and continues to be, a scarcely described phenomenon. Only the description of the generation of the HaCAT cell line [1] is a canonical example, worldwide accepted. More recent examples included the emergence of tumorigenic populations upon *in vitro* culture of fetal human mesenchymal stem cells (hMSC), induced by GM-CSF and IL-4, [2] and bone marrow hMSC [3]. Other examples have also been reported after very long-term *in vitro* culture of telomerized hMSC-TerT [4]. In this scenario, our previous results [5-7] were only a new observation to be added to this list. Only very recently similar results have been published [8], reporting spontaneous malignant transformation in 46 % of bone marrow-derived hMSC long-term cultures (5–106 weeks). However, other authors reported opposite results [9].

Since our original publication, we continued to work on the definition and characterization of the hMSC spontaneous transformation phenomenon. Unexpectedly, however, we have been unable to obtain a single new human MSC transformation event (about 15 independent new adipose tissue samples –both adult and pediatric-, and 25 attempts of independent set of cultures). What we can, however, clearly state is that the *ex vivo* culture of human MSC under the conventional conditions promotes a variable but significant level of genomic instability, concurring with an important level of aneuploidy (Estrada et al., submitted). In any case, in all experiments, after a clear senescent phase the culture was exhausted. When we began to observe this central discrepancy we tried to evaluate several factors that could influence it, but finally, we carried out a STR (short tandem repeats) analysis to control the possibility of cross-contamination between the different TMC (for transformed mesenchymal stem cells) samples or between TMC and MSC cultures.

Surprisingly, none of the TMC populations obtained presented an STR profile compatible with the original human MSC samples and were quite similar among them. In addition, when we compared the TMC STR profile with a panel of human tumor cell lines, we found that is quite similar to the one corresponding for the HT1080 cell line. HLA genotype analyses confirmed these data. Finally, we repeated the karyotype analysis of old and new samples. We observed that although there was a high heterogeneity among the clones and samples selected after de co-culture, the most characteristic genomic alterations [der(5) and t(3;11)] present in TMC [5] are also common to the cell line HT1080 that we have available in our lab. This result again, supports the proposed working hypothesis (Figure 1, summarizes the most relevant results).

Currently, the most plausible explanation for the MSC spontaneous transformation phenomenon we described is thus an artifact mediated by an unnoticed minimal cross-contamination of some of the original samples with HT1080 cells.

## Functional interaction between human MSC and HT1080 cells during long-term *in vitro* culture

The previously described data imply that there must be an effect of the human MSC on the tumor cells to control their expansion during the long-term cultures. TMC appeared in the last phases of the long-term cultures of human MSC. It is difficult to understand why the potential contaminating tumoral cells did not start to overgrow the cultures in the first weeks, but only 3-4 months later.

Prior to our initial communication, there were few and conflictive publications focused on this topic. Nakamura et al. reported an antitumor effect of rat MSC on glioblastoma [10]. On the contrary, Fierro et al. reported positive effects of MSC co-cultures of breast cancer cell lines [11]. Since then, the scientific literature continues to show similar results, strongly suggesting that probably there must be tumor cell-type specificity in the effect. In addition, it was recently demonstrated, both *in vitro* and *in vivo*, that MSC have the potential to migrate and intercalate in tumor capillary network, form Cx43-based gap junctions and increase the production of ROS, promoting capillary apoptosis and network degeneration [12].

Because the scientific literature was non-conclusive on the potential effect, we next designed a set of experiments in which we mixed HT1080-EGFP cells with hMSC obtained from different donors (in 3 dilutions: 1/10,000, 1/100,000 and 1/500,000) and maintained the mixed cultures until the end of the culture or the evident appearance of HT1080-like cells. Twice per week, presence of EGFP in the cultures was analyzed by flow cytometry, PCR and fluorescent microscopy. In most of the co-cultures, we detected a delay in the emergence of the HT1080-EGFP cells with respect to the expected kinetics in the case there were no functional interaction with MSC. A summary of the results obtained is included in Figure 2.

In conclusion, our results strongly suggest that the significative emergence of HT1080-EGFP cells was delayed respect the theoretical expected time and, in some cases, HT1080 were undetectable for weeks before they began to overgrow the culture. These data are compatible with our hypothesis that a minimal HT1080 contamination could have been present in some of our hMSC cell cultures since first weeks, and remained undetectable for months until hMSC advanced senescence manifests and further development of the crisis phase; then, the hMSC deterioration allowed HT1080 cells to invade the flask and growth with their normal duplication rates.

## References

1. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *Cell Biol* 106 (1988) 761-771.
2. Xu W, Qian H, Zhu W, Chen Y, Shao Q, Sun X, Hu J, Han C, Zhang X. A novel tumor cell line cloned from mutated human embryonic bone marrow mesenchymal stem cells. *Oncol Rep* 12 (2004) 501-508.
3. Wang Y, Huso DL, Harrington J, Kellner J, Jeong DK, Turney J, McNiece IK. Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. *Cytotherapy* 7 (2005) 509-519.
4. Serakinci N, Guldborg P, Burns JS, Abdallah B, Schrødder H, Jensen T, Kassem M. Adult human mesenchymal stem cell as a target for neoplastic transformation. *Oncogene* 23 (2004) 5092-5094.
5. Rubio D, Garcia-Castro J, Martín MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. *Cancer Res* 65 (2005) 3035-3039.
6. Rubio D, Garcia S, De la Cueva T, Paz MF, Lloyd AC, Bernad A, Garcia-Castro J. Human mesenchymal stem cell transformation is associated with a mesenchymal-epithelial transition. *Exp Cell Res* 314 (2008) 691-698.
7. Rubio D, Garcia S, Paz MF, De la Cueva T, Lopez-Fernandez LA, Lloyd AC, Garcia-Castro J, Bernad A. Molecular characterization of spontaneous mesenchymal stem cell transformation. *PLoS ONE* 3(1):e1398.
8. Røslund GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, Mysliwicz J, Tonn JC, Goldbrunner R, Lønning PE, Bjerkvig R, Schichor C. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Res* 69 (2009) 5331-5339.
9. Bernardo ME, Zaffaroni N, Novara F, Cometa AM, Avanzini MA, Moretta A, Montagna D, Maccario R, Villa R, Daidone MG, Zuffardi O, Locatelli F. Human Bone Marrow-Derived Mesenchymal Stem Cells Do Not Undergo Transformation after Long-term In vitro Culture and Do Not Exhibit Telomere Maintenance Mechanisms. *Cancer Research* 67 (2007) 9142-9.
10. Nakamura K, Ito Y, Kawano Y, Kurozumi K, Kobune M, Tsuda H, Bizen A, Honmou O, Niitsu Y, Hamada H. Antitumor effect of genetically engineered mesenchymal stem cells in a rat glioma model. *Gene Ther.* 11 (2004):1155-1164.
11. Fierro FA, Sierralta WD, Epuñan MJ, Minguell JJ. Marrow-derived mesenchymal stem cells: role in epithelial tumor cell determination. *Clin Exp Metastasis* 2 (2004) 313-319.
12. Otsu K, Das S, Houser SD, Quadri SK, Bhattacharya S, Bhattacharya J. Concentration dependent inhibition of angiogenesis by mesenchymal stem cells. *Blood* 113 (2009) 4197-4205.

**FIGURE LEGENDS.**

**Figure 1. A)** STR analyses of short-term MSC culture (MSC16), pre- and postsenescent (MSC-16 S, PS) and transformed (TMC-14 1-3, TMC-16 1-2) cell cultures. Several control cell lines (A549, Caco, H441, Hela, Saos, T98G and U87MG) were analyzed in parallel, including STR patterns of HT1080 and HT1080-eGFP cell lines cultured in our laboratory. **B)** HLA haplotypes of TMC-14, TMC-16 and HT1080. **C)** Karyotype of our HT1080 and HT1080-eGFP cell lines showing the same specific translocations of TMCs previously published.

**Figure 2.** Co-culture of the hMSC and HT1080 transduced with eGFP. Graphs show the eGFP percentage detected by flow cytometry in mixed cultures. Dilution showed are 1 HT1080 cell per 10,000 (red), 100,000 (blue) or 500,000 (green) hMSC generated from 3 different donors (PN17, PN32, PN33). Lower right panel show the mRNA relative expression of Cx43, quantificated by qRT-PCR, in short-term cultures of hMSC, postsenescent hMSC (MSC-PS) and TMC/HT1080 cultures.

**A**

	D3S1358	Vwa	D16S539	D2S1338	D8S1179	D2S11	D18S51	D19S433	TH01	FGA	XY
MSC-16	14 15	17	12 13	23 25	13 15	31.2	12 13	14 15	7	21 23	X Y
MSC-16 S	14 15	17	12 13	23 25	13 15	31.2	12 13	14 15	7	21 23	X Y
MSC-16 PS	14 15	17	12 13	23 25	13 15	31.2	12 13	14 15	7	21 23	X Y
TMC-14 1	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X
TMC-14 2	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X
TMC-14 3	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X Y
TMC-16 1	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X
TMC-16 2	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X
HT1080	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X Y
HT1080 eGFP	16	14 19	9 12	25 26	13 14	28 30	12 18	13.2 15	6	22 25	X Y
A549	16	14	11 12	24	13 14	29	14 17	13	8 9.3	23	X Y
Caco	13 17	16 18	12 13	17 25	12 14	30 32	12	14.2	6 7	19	
H441	17 18	17	9 13	17 19	8 14	32.2	18 19	14	7 9.3	24 25	
Hela	14 18	16 18	9 10	17	12 13	27 28	16	12.2 13.2	7	21	X X
Saos	14 18	18	12 13	18	10 12	28 30	15	13	6 9	22 25	
T98G	16	17 20	23	19 24	13 14	28 32.2	13 16	12	7 9.3	21	X Y
U87MG	16 17	15 17	12	20 23	10 11	28 32.2	13 14	14.2 15	9.3	18 28	

**B**

TMC-14	A*31DHJF,A*68EBCE	DRB1*03DRNV,DRB1*04DYMY	DRB3*01ANEX,DRB4*01ARYD
TMC-16	A*31DHJF,A*68EBCE	DRB1*03DRNV,DRB1*04DYMY	DRB3*01ANEX,DRB4*01ARYD
HT1080	A*31DHJF,A*68EBCE	DRB1*03DRNV,DRB1*04DYMY	DRB3*01ANEX,DRB4*01ARYD

**C**

HT1080	49-119, XY, der(5)t(5;5)(p15;?), der(11)t(3;11)(?:q25)
HT1080-eGFP	60-95, XY, der(5)t(5;5)(p15;?), der(11)t(3;11)(?:q25)

