Biochemical and Biophysical Research Communications 424 (2012) 391–397

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

In vitro expanded bone marrow-derived murine (C57Bl/KaLwRij) mesenchymal stem cells can acquire CD34 expression and induce sarcoma formation *in vivo*

Song Xu [a,b,c,1], Ann De Becker [b,1], Hendrik De Raeve [d], Ben Van Camp [c], Karin Vanderkerken [c], Ivan Van Riet [b,c,*]

[a] Department of Lung Cancer Surgery, Lung Cancer Institute, Tianjin Medical University General Hospital, 300052 Tianjin, China
[b] Stem Cell Laboratory-Division Clinical Hematology, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium
[c] Department of Hematology and Immunology, Vrije Universiteit Brussel (VUB)-Myeloma Center, Laarbeeklaan 103, 1090 Brussels, Belgium
[d] Department of Anatomopathology, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium

ARTICLE INFO

Article history:
Received 11 June 2012
Available online 6 July 2012

Keywords:
Mesenchymal stem cells
Malignant transformation
Sarcoma

ABSTRACT

Mesenchymal stem cells (MSCs) have currently generated numerous interests in pre-clinical and clinical applications due to their multiple lineages differentiation potential and immunomodulary effects. However, accumulating evidence indicates that MSCs, especially murine MSCs (mMSCs), can undergo spontaneous transformation after long-term *in vitro* culturing, which might reduce the therapeutic application possibilities of these stem cells. In the present study, we observed that *in vitro* expanded bone marrow (BM) derived mMSCs from the C57Bl/KaLwRij mouse strain can lose their specific stem cells markers (CD90 and CD105) and acquire CD34 expression, accompanied with an altered morphology and an impaired tri-lineages differentiation capacity. Compared to normal mMSCs, these transformed mMSCs exhibited an increased proliferation rate, an enhanced colony formation and migration ability as well as a higher sensitivity to anti-tumor drugs. Transformed mMSCs were highly tumorigenic *in vivo*, resulting in aggressive sarcoma formation when transplanted in non-immunocompromised mice. Furthermore, we found that Notch signaling downstream genes (*hey1*, *hey2* and *heyL*) were significantly upregulated in transformed mMSCs, while Hedgehog signaling downstream genes *Gli1* and *Ptch1* and the Wnt signaling downstream gene *beta-catenin* were all decreased. Taken together, we observed that murine *in vitro* expanded BM-MSCs can transform into CD34 expressing cells that induce sarcoma formation *in vivo*. We assume that dysregulation of the Notch(+)/Hh(−)/Wnt(−) signaling pathway is associated with the malignant phenotype of the transformed mMSCs.

1. Introduction

A malignant neoplasm is a heterogeneous entity, containing both stroma and cancer cells. In the recent decades, much efforts were done to identify cancer-initiating cells or cancer stem cells (CSC) [1–5], although the existence of CSC is still in debate for several malignancies. On the other hand, some normal cell types, in particular mesenchymal stem cells (MSCs), have drawn great attention because they can transform into malignant cells spontaneously, or are induced by certain compounds and epigenetic modification [6–16].

MSCs, a population of multipotent non-haematopoietic progenitor cells, possess self-renewing, multi-lineage differentiation potential, immunomodulary properties, capacity for homing to injury/tumor sites and capacity for production of a variety of cytokines/chemokines. Therefore, these cells are considered to be useful for a number of therapeutic applications, including tissue regeneration, support of hematopoietic stem cells (HSC) engraftment, control of graft-versus-host disease (GVHD) and autoimmune diseases, prevention of rejection after solid organ transplantation, tissue engineering and also as gene therapy vehicles [17–20]. MSCs have been isolated not only from bone marrow but also from various other sources, including placenta, amniotic fluid, cord blood, fetal liver and adipose tissue [21–25]. Although the safety of MSC application has been emphasized in several reports [26,27], some groups demonstrated that MSCs can undergo spontaneous transformation *in vitro* and provided evidence that MSCs can behave as cancer stem cells in sarcomas [6–14]. Murine MSCs (mMSCs) seem to undergo a higher rate of transformation compared with human MSCs (hMSCs). This malignant transformation is associated with increased level of telomerase activity, p53 loss, oncogene activation, and/or accumulated chromosomal instability [8–13]. In addition, one recent study

* Corresponding author at: Stem Cell Laboratory-Division Clinical Hematology, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium. Fax: +32 02 477 6728.
E-mail address: ivan.vanriet@uzbrussel.be (I. Van Riet).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.06.118

demonstrated that not only long-term but also short-term cultured mMSCs could form malignant sarcoma *in vivo* [14].

Our initial study aimed to explore the role of MSCs in the tumor microenvironment. Unexpectedly, we observed during the primary culture of mMSCs, that *in vitro* expanded mMSCs underwent spontaneous transformation. The malignant transformed mMSCs (MT-mMSCs) showed a malignant phenotype, including an increased proliferation rate, altered morphology, less sensitivity to anti-tumor drugs and enhanced colony-formation ability and migration ability. Moreover, these cells could form sarcoma *in vivo*. However, compared to the observations of other groups, the type of the transformed cells in our study had different features. We found that MT-mMSCs had lost typical MSC markers CD90 and CD105 while acquiring CD34 expression. Furthermore, we found that MT-mMSCs exhibited dysregulated Notch, Hedgehog (Hh) and Wnt pathways compared to their normal counterparts, indicating a clinical relevance for sarcoma treatment by targeting Notch, Hh and Wnt pathways.

2. Materials and methods

2.1. mMSCs primary culture

Six-to-eight-week-old female C57Bl/KaLwRij mice (from Harlan CPB, the Netherlands) were sacrificed by cervical dislocation, and BM was collected by flushing out the content of femurs and tibias with RPMI 1640 (Lonza, Verviers, Belgium). Cells were then plated at 1×10^6 cells/cm^2 in McCoy's 5A media (Lonza, Verviers, Belgium) containing 10% FBS "Gold" (PAA Laboratories GmbH, Pasching, Austria), 1% L-glutamine (Lonza, Verviers, Belgium) and 1% penicillin/streptomycin (Lonza, Verviers, Belgium). After 24 h the culture was washed by PBS to remove non-adherent cells and replaced with fresh complete medium every 3 days. Once primary culture (passage 0) became 80–90% confluent, the cells were detached with 0.25% trypsin/1 mM EDTA (Lonza, Verviers, Belgium), and plated in complete medium at 1000 cells/cm^2 (Nunc, VWR International, Leuven, Belgium). Subsequent passages were performed similarly, but split ratios were 1:2. mMSCs were characterized by their fibroblast-like morphology, a distinctive panel of surface markers (CD90+, CD105+, CD34− and CD45−) and their differentiation potential towards adipocytes, osteoblasts and chondrocytes in special induction medium. FACS staining and tri-lineage differentiation induction were performed as described previously [28]. All the experiments were approved by the Ethical Committee for Animal Experiments, VUB (License No. LA1230281).

2.2. Thymidine incorporation assay

1×10^4 cells were plated in 96-well plates in McCoy's 5A with 10% FBS. Sixteen hours before harvest, cells were pulsed with 1 μCi [methyl-3H]thymidine (Amersham, Buckinghamshire, UK). Cells were harvested using a cell harvester (Inotech, Wohlen, Switzerland) onto fiberglass filters. Filters were dried for 1 h in a 60 °C oven, sealed in sample bags containing 4 mL of Optiscint Scintillation Liquid and radioactivity was counted using a 1450 Microbeta Liquid Scintillation Counter (all from Wallac, Turku, Finland). Results are expressed as the relative DNA synthesis.

2.3. Doubling time

Normal mMSCs or MT-mMSCs were plated at 2000 cells/well in 6-well culture plates, and the cells from each well were trypsinized and counted in duplicate with a hemocytometer every 2 days for a total 12 days. The growth curves were then plotted. The doubling time was calculated according to the equation: $TD = t \times \lg 2/\lg(Nt/N0)$, where $N0$ is the initial cell number, Nt is the end point cell number, and t is time interval.

2.4. 3D matrigel culture system

2×10^4 cells suspended in 100 μl of serum-free McCoy's 5A media were mixed with 400 μl Matrigel and overlaid on the surface of 24-well plate pre-coated with 500 μl of diluted (1:1) Matrigel layer. When the upper Matrigel became solidified, 200 μl McCoy's 5A with 10% FBS was added. After 15 days of incubation, the colony formations were compared.

2.5. Wound healing assay

1×10^6 cells/well were seeded in 6-well plates and cultured overnight at 37 °C in 5% CO_2. A straight scratch was gently made through the central axis of the plate using a micropipette tip. The plates were rinsed with PBS, and serum-free RPMI1640 was added. After 24 h of incubation, the number of cells migrating into the acellular area created by scratching was compared under an inverted microscope at a ×100 magnification. (Nikon ECLIPSE TS 100 with Nikonn Digital sight DS-Fi1).

2.6. Anti-tumor drugs treatment

Cells were cultured at a density of 2000 cells/well in a 96 well-plate at various concentrations of HDAC inhibitor-Vorinostat (0, 0.01, 0.1, 0.5, 1, 5, 10 and 50 μM) and proteasome inhibitor-Bortezomib (0, 0.1, 0.5, 1, 5, 10, 50 and 100 nM)). Three days later, cell viability was determined using CellTiter-Glo® (Promega, Madison, WI, USA) according to the manufacturer's protocol. The IC50 (concentration at which enzyme activity is inhibited by 50%) was calculated using Graphpad Prism (Graphpad Software, La Jolla, CA, USA).

2.7. In vivo transplantation of murine MSCs

To assess tumorigenesis *in vivo*, 2×10^6 transformed and non-transformed mMSCs were transplanted on the back of non-immunocompromised C57BL/KaLwRij mice (three mice per group). Two months later, when a visible mass was formed, the mass was removed and paraffin embedded sections were made and evaluated by an experienced pathologist.

2.8. Quantitative real time PCR

Total RNA was isolated from MSCs using Trizol (Invitrogen) and RNeasy Mini Kit (Qiagen), following the manufacturer's instructions. The concentration and purity of RNA were determined by the Quant-iT RNA BR Assay kit (Invitrogen) with Qubit fluorometer (Invitrogen). cDNA was synthesized using the Thermoscript RT-PCR system (Invitrogen) with random hexamers as primers. Quantitative real-time PCR analysis was done using the iCycler (Bio-Rad Laboratories) using the SYBR GreenER qPCR SuperMix for iCycler (Invitrogen) according to manufacturer's instructions. The primer sequences used are listed in Supplementary Table. Transcript levels were normalized to housekeeping gene beta-actin and analyzed by the relative quantification $2^{-\Delta\Delta Ct}$ method.

2.9. Statistical analysis

Statistical significance was assessed by comparing mean values (±SD) using the Mann Whitney test. $p < 0.05$ was considered as statistically significant. All experiments were repeated in triplicates at least.

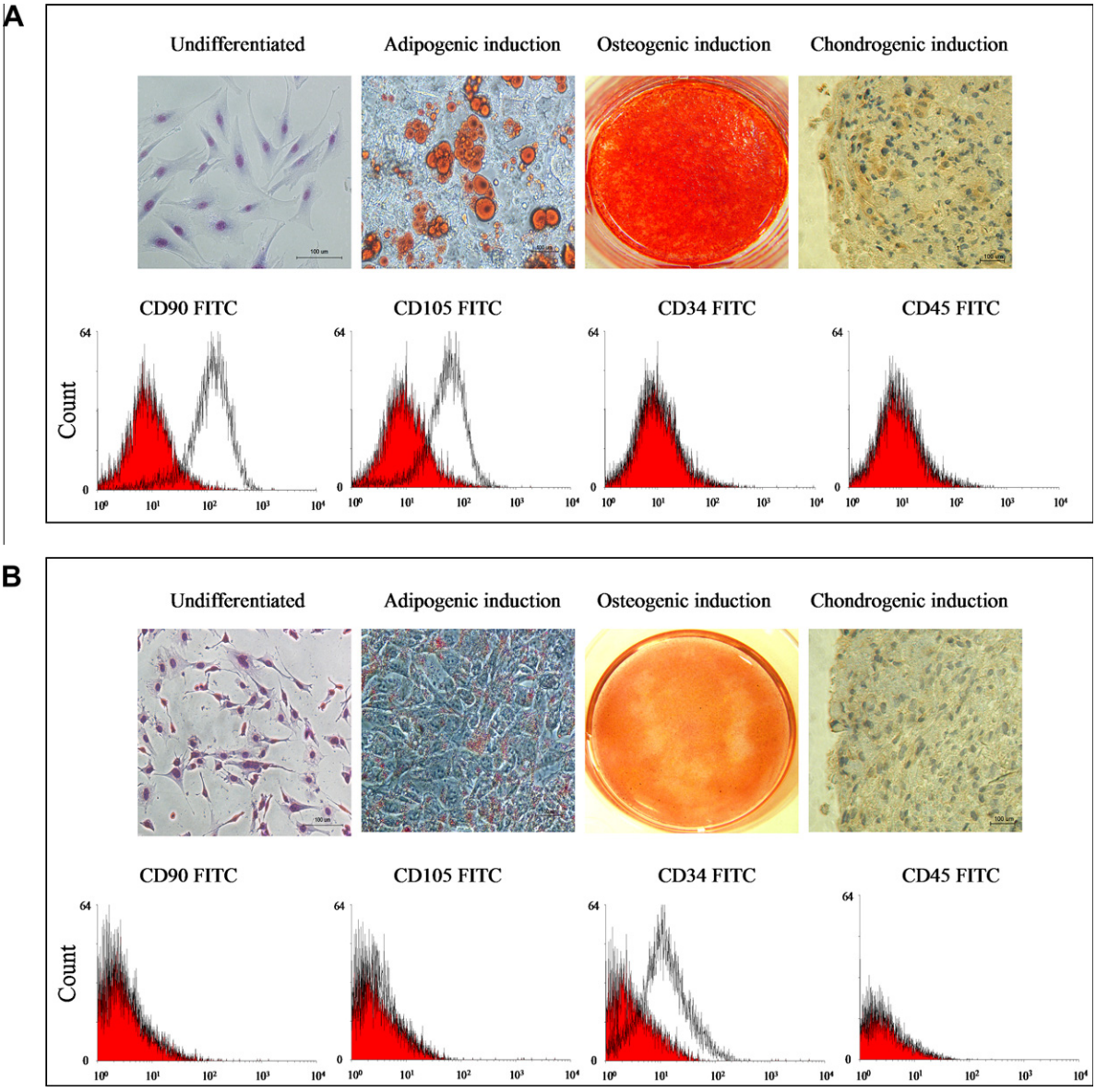


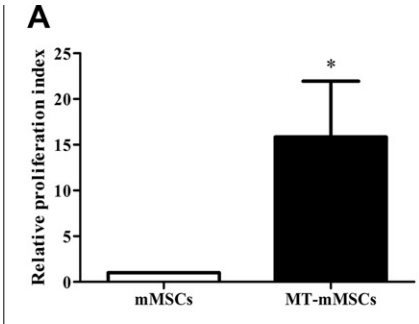
Fig. 1. Characteristics of normal and MT-mMSCs. (A) Characterization of normal murine MSCs (mMSCs). mMSCs show a large and spindle shape and exhibit robust tri-lineage differentiation. They are positive for CD90 and CD105, but negative to CD34 and CD45. (B) Characterization of malignant transformed murine MSCs (MT-mMSCs). MT-mMSCs have a small, round or irregular shape, and show a much lower tri-lineages differentiation capacity. MT-mMSCs are negative for CD90 and CD105 but positive for CD34. Differentiation into adipocytes, osteoblasts, and chondrocytes is shown by Oil Red O staining, alizarin red s staining and collagen type II staining. Scale bar = 100 μm.

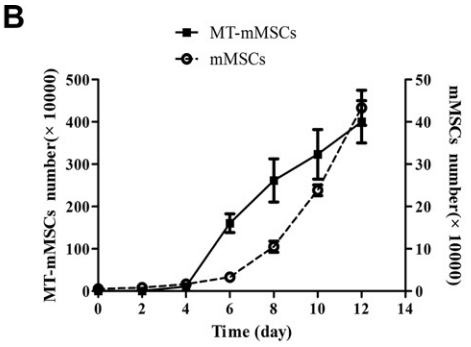
3. Results and discussion

3.1. Characteristic alteration of MT-mMSCs

Since MSCs received a lot of interest for various clinical applications, the safety problems of MSC therapy have also been raised. In long term *in vitro* cultures, mMSCs have been reported to gradually lose their stem cell properties and transform into malignant cells due to genetic instability, p53 depletion, and/or oncogene activation (9–12). For hMSCs, the reports so far were controversial. Some said hMSCs would not transform even though undergoing a long term *in vitro* culture, while others observed that a subgroup of hMSCs were able to transform into sarcoma cells. In our experience, we never observed any malignant transformation of hMSCs in all cultures up to passage 15, but the proliferation of some hMSCs went down after passage 10. However, we did observe that mMSCs were more prone to accelerate their growth in the late passages (more than passages 8–10). However, we only observed very occasional incidence (less than 10%) of mMSCs spontaneous transformation into sarcoma cells, which is not consistent with the previous studies mentioning that mMSCs frequently underwent malignant transformation.

In our cultures, mMSCs at passages 4 and 5 exhibited a typical fibroblast-like morphology, were positive for CD90 and CD105 but negative for CD34 and CD45. They have the capacity to differentiate into chondrocytes, osteoblasts, and adipocytes (Fig. 1A), which meet to criteria to define MSCs proposed by Mesenchymal and Tissue Stem Cell committee of the International Society for Cellular Therapy (ISCT) [29]. However, in one primary culture following the same expansion condition, we surprisingly observed that mMSCs at passage 8–9, suddenly started to grow faster, accompanied with that the morphology changed from large and spindle to small, round and irregular shape (Fig. 1B). Moreover, MT-mMSCs, in contrast to the normal MSCs, lost both stem cells





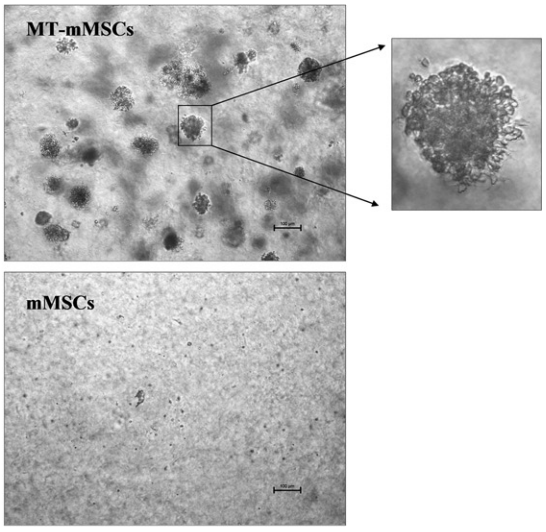
Doubling Time:

MT-mMSCs *vs* mMSCs = 21.05±1.02 *vs* 33.69±3.11 hours

C

	Vorinostat	
	mMSCs	MT-mMSCs
IC50	5.45 μM	1.24 μM

	Bortezomib	
	mMSCs	MT-mMSC
IC50	13.58 nM	4.93 nM



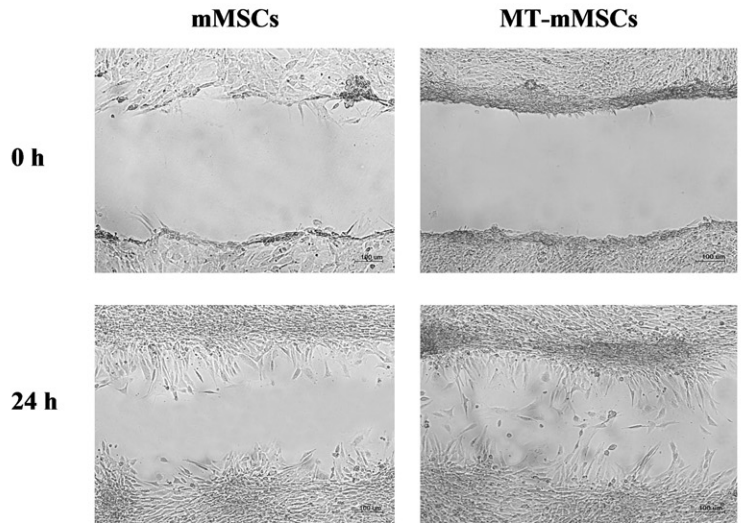


Fig. 2. MT-mMSCs have a malignant phenotype. (A) The proliferation rate of MT-mMSCs is much higher than normal mMSCs, as examined by the thymidine incorporation assay. (B) Doubling time of MT-mMSCs is much shorter than normal mMSCs. (C) MT-mMSCs are more sensitive to HDAC inhibitor Vorinostat and proteasome inhibitor Bortezomib. (D) MT-mMSCs show an enhanced colony formation *in vitro*. One of three independent experiments is shown. (E) MT-mMSCs have higher migration capacity in the wound healing assay. One representative picture of five experiments is shown. $^{*}p < 0.05$.

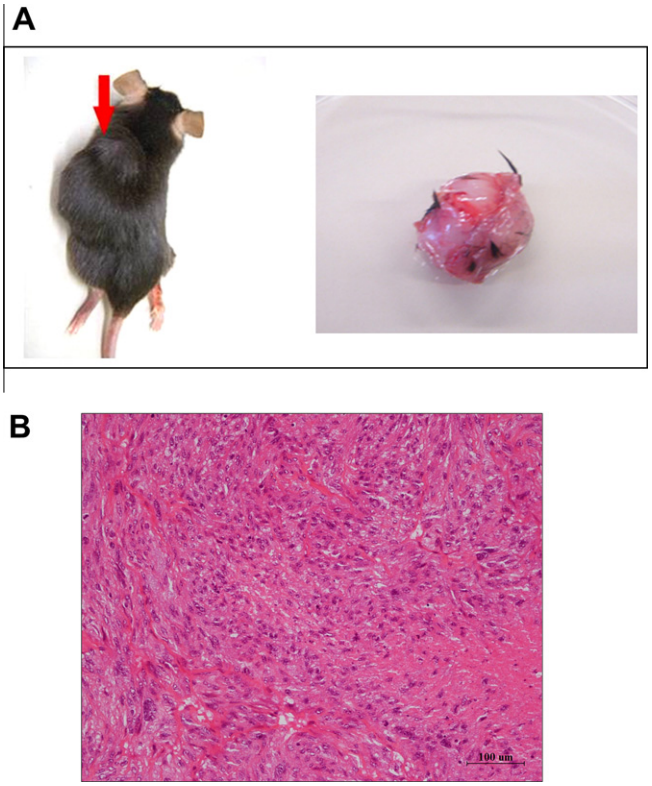


Fig. 3. MT-mMSCs form sarcoma *in vivo*. (A) 2×10^6 MT-mMSCs were injected s.c. into naive mice. Two months later, a visible tumor mass formed in situ. $n = 3$. (B) Histological analysis showed undifferentiated spindle cells with elongated or pleomorphic nuclei and scant acidophilic cytoplasm with mitotic activity representing a highly aggressive sarcoma. One representative picture is shown. Scale bar, 100 μm.

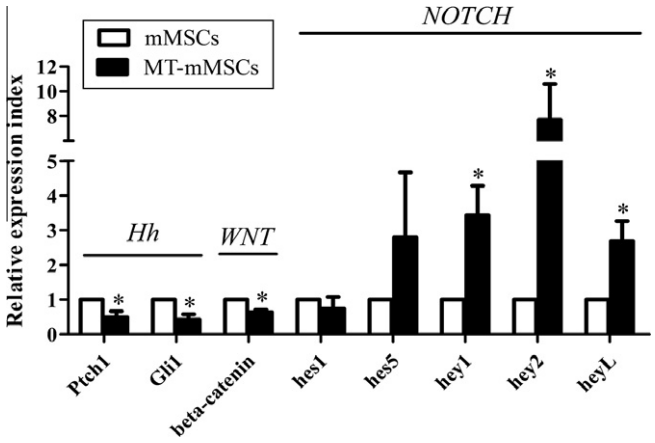


Fig. 4. MT-mMSCs exhibit over-activated Notch and de-activated Hh and Wnt pathway activity. By real time PCR, MT-mMSCs show significant lower Hh pathway (Ptch1 and Gli1) and Wnt pathway (beta-catenin) but higher Notch pathway (hey1, hey2 and heyL) activity compared to normal mMSCs (passage 5). $^*p < 0.05$.

markers CD90 and CD105, whereas they acquired CD34 expression compared to normal mMSCs (Fig. 1B). Meanwhile, the potential of tri-lineage differentiation of MT-mMSCs was dramatically abolished (Fig. 1B).

3.2. Malignant phenotype of MT-mMSCs

We observed that MT-mMSCs exhibited a 15-fold higher proliferation rate compared to normal mMSCs, (Fig. 2A). The doubling time of MT-mMSCs was much shorter than normal mMSCs (MT-mMSCs *vs* mMSCs = 21.05 ± 1.02 *vs* 33.69 ± 3.11 h) (Fig. 2B). HDAC inhibitors and proteasome inhibitors represent two types of novel potent anti-tumor agents which are increasingly used for the treatment of hematological and solid malignancies, including sarcoma [30–36]. We observed that MT-mMSCs were more sensitive to the HDAC inhibitor-Vorinostat and the proteasome inhibitor-Bortezomib, showing a decreased IC50 compared to normal mMSCs (Vorinostat: MT-mMSCs *vs* mMSCs = 1.24 μM *vs* 5.45 μM; Bortezomib: MT-mMSCs *vs* mMSCs = 4.93 nM *vs* 13.58 nM) (Fig. 2C). Besides the increased proliferation and reduced sensitivity to anti-tumor drugs, the transformed cells lost contact inhibition which was another growth feature of "malignant" cells. In 3D matrigel culture, MT-mMSCs acquired anchorage independent growth ability (Fig. 2D). The *in vitro* wound healing assay revealed that MT-mMSCs have a more robust migratory ability than normal mMSCs (Fig. 2E). All these observations indicate that these mMSCs went through a "malignant" transformation process.

3.3. MT-mMSCs generate sarcoma in vivo

It is widely known that CD34 is the marker for hematopoietic stem cells but CD34 is also a marker of Kaposi Sarcoma regardless of its clinical subtype or tumor stage [37]. Since there were no reports that MSCs could transdifferentiate into hematopoietic cells, we therefore estimated that these mMSCs transformed into CD34 positive sarcoma cells. We transplanted MT-mMSCs s.c. in non-immunocompromised mice to test if transformed mMSCs could form tumor *in vivo*. After 2 months visible tumors were formed *in situ* on the back where MT-mMSCs were injected (Fig. 3A). However, there was no tumor formation in the mice injected with non-transformed mMSCs. The tumor was removed and by histological analysis it could be classified as an "undifferentiated high-grade" sarcoma (Fig. 3B).

3.4. Notch(+)/Hh(−)/Wnt(−) in MT-mMSCs

Notch, Hh, and WNT singling pathways are known to play fundamental roles in cell fate decisions and differentiation. It is, therefore, not surprising that the downstream proteins of Notch, Hh, and Wnt have been implicated in tumorigenesis, functioning as either oncogenes or tumor suppressor proteins depending on the cellular context. In our study, we examined whether and at which level Notch, Hh, and Wnt pathways downstream genes are changed in the transformed mMSCs. We demonstrated that MT-mMSCs, compared to normal mMSCs, exhibited significantly elevated levels of Notch downstream genes *hey1*, *hey2* and *heyL*, but downregulated Hh downstream Gli1 and Ptch1 as well as Wnt downstream beta-catenin expression (Fig. 4). These data indicate that the Notch pathway is over-activated, while the Hh and Wnt pathways are both de-activated in transformed mMSCs. The over-activated Notch pathway and de-activated Wnt pathway have already been demonstrated in sarcoma, and treatment strategies based on targeting both pathways have been proposed in previous studies [38–42]. Although our data do not allow to conclude that dysregulation of Notch and Wnt pathways triggers the malignant transformation of MSCs, they show at least that these pathways are differentially expressed in transformed mMSCs. Targeting these pathways for treatment of sarcoma is clinically relevant. However, for the Hh pathway, our observation that MT-mMSCs have a decreased *Gli* and *Ptch1* expression is not in accordance with previous observations that inhibition of the Hh pathway decreases sarcoma cell growth [43–45]. This indicates that the Hh pathway might play a different and complex role in the sarcoma initiation and expansion.

Taken together, our data provide evidence that MSCs are progenitor cells for sarcoma and that CD34 acquisition is associated with the MSCs transformation process. Transformed mMSCs show over-activation of Notch and de-activation of Hh and Wnt pathways. Targeting these pathways might have therapeutic potential for sarcoma treatment.

Acknowledgments

We would like to thank Nicole Arras, Wim Renmans, Angelo Willems, Carine Seynaeve, for their expert technical assistance, and Prof. Zhou Qinghua for his administrative support. We thank Merck & Co for providing Vorinostat. Our research work is supported by Grants from the FWO-Vlaanderen, Vlaamse Liga tegen Kanker (Stichting Emmanuel Van der Schueren), the Vrije Universiteit Brussel (HOA) and the Scientific Foundation Willy Gepts (WFWG) UZ Brussel. Xu S is supported by CSC-VUB scholarship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.06.118.

References

[1] B. Choijamts, S. Jimi, T. Kondo, Y. Naganuma, T. Matsumoto, M. Kuroki, H. Iwasaki, M. Emoto, CD133+ cancer stem cell-like cells derived from uterine carcinosarcoma (malignant mixed *Müllerian tumor*), Stem Cells 29 (2011) 1485–1495.

[2] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. USA 100 (2003) 3983–3988.

[3] D.B. Gürsel, B.J. Shin, J.K. Burkhardt, K. Kesavabhotla, C.D. Schlaff, J.A. Boockvar, Glioblastoma stem-like cells-biology and therapeutic implications, Cancers (Basel) 3 (2011) 2655–2666.

[4] D.G. Tang, L. Patrawala, T. Calhoun, B. Bhatia, G. Choy, R. Schneider-Broussard, C. Jeter, Prostate cancer stem/progenitor cells: identification, characterization, and implications, Mol. Carcinogr. 46 (2007) 1–14.

[5] H. Chiba, G. Ishii, T.K. Ito, K. Aoyagi, H. Sasaki, K. Nagai, A. Ochiai, CD105-positive cells in pulmonary arterial blood of adult human lung cancer patients include mesenchymal progenitors, Stem Cells 26 (2008) 2523–2530.

[6] R. Tasso, A. Augello, M. Carida', F. Postiglione, M.G. Tibiletti, B. Bernasconi, S. Astigiano, F. Fais, M. Truini, R. Cancedda, G. Pennesi, Development of sarcomas in mice implanted with mesenchymal stem cells seeded onto bioscaffolds, Carcinogenesis 30 (2009) 150–157.

[7] J. Tolar, A.J. Nauta, M.J. Osborn, A. Panoskaltsis Mortari, R.T. McElmurry, S. Bell, L. Xia, N. Zhou, M. Riddle, T.M. Schroeder, J.J. Westendorf, R.S. McIvor, P.C. Hogendoorn, K. Szuhai, L. Oseth, B. Hirsch, S.R. Yant, M.A. Kay, A. Peister, D.J. Prockop, W.E. Fibbe, B.R. Blazar, Sarcoma derived from cultured mesenchymal stem cells, Stem Cells 25 (2007) 371–379.

[8] M.L. Suvà, N. Riggi, J.C. Stehle, K. Baumer, S. Tercier, J.M. Joseph, D. Suvà, V. Clément, P. Provero, L. Cironi, M.C. Osterheld, L. Guillou, I. Stamenkovic, Identification of cancer stem cells in Ewing's sarcoma, Cancer Res. 69 (2009) 1776–1781.

[9] F. Tirode, K. Laud-Duval, A. Prieur, B. Delorme, P. Charbord, O. Delattre, Spontaneous expression of embryonic factors and p53 point mutations in aged mesenchymal stem cells: a model of age-related tumorigenesis in mice. Mesenchymal stem cell features of Ewing tumors, Cancer Cell 11 (2007) 421–429.

[10] Y. Shima, T. Okamoto, T. Aoyama, K. Yasura, T. Ishibe, K. Nishijo, K.R. Shibata, Y. Kohno, K. Fukiage, S. Otsuka, D. Uejima, T. Nakayama, T. Nakamura, T. Kiyono, J. Toguchida, In vitro transformation of mesenchymal stem cells by oncogenic H-rasVal12, Biochem. Biophys. Res. Commun. 353 (2007) 60–66.

[11] M. Miura, Y. Miura, H.M. Padilla-Nash, A.A. Molinolo, B. Fu, V. Patel, B.M. Seo, W. Sonoyama, J.J. Zheng, C.C. Baker, W. Chen, T. Ried, S. Shi, Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation, Stem Cells 24 (2006) 1095–1103.

[12] C. Josse, R. Schoemans, N.A. Niessen, M. Delgaudine, A.C. Hellin, C. Herens, P. Delvenne, V. Bours, Systematic chromosomal aberrations found in murine bone marrow-derived mesenchymal stem cells, Stem Cells Dev. 19 (2010) 1167–1173.

[13] D. Rubio, S. Garcia, M.F. Paz, T. De la Cueva, L.A. Lopez-Fernandez, A.C. Lloyd, J. Garcia-Castro, A. Bernad, Molecular characterization of spontaneous mesenchymal stem cell transformation, PLoS ONE 3 (2008) e1398.

[14] J.O. Jeong, J.W. Han, J.M. Kim, H.J. Cho, C. Park, N. Lee, D.W. Kim, Y.S. Yoon, Malignant tumor formation after transplantation of short-term cultured bone marrow mesenchymal stem cells in experimental myocardial infarction and diabetic neuropathy, Circ. Res. 108 (2011) 1340–1347.

[15] S. Zhang, Z. Han, Q. Kong, J. Wang, B. Sun, G. Wang, L. Mu, D. Wang, Y. Liu, H. Li, Malignant transformation of rat bone marrow-derived mesenchymal stem cells treated with 4-nitroquinoline 1-oxide, Chem. Biol. Interact. 188 (2010) 119–126.

[16] I.W. Teng, P.C. Hou, K.D. Lee, P.Y. Chu, K.T. Yeh, V.X. Jin, M.J. Tseng, S.J. Tsai, Y.S. Chang, C.S. Wu, H.S. Sun, K.D. Tsai, L.B. Jeng, K.P. Nephew, T.H. Huang, S.H. Hsiao, Y.W. Leu, Targeted methylation of two tumor suppressor genes is sufficient to transform mesenchymal stem cells into cancer stem/initiating cells, Cancer Res. 71 (2011) 4653–4663.

[17] Y.L. Tang, Autologous mesenchymal stem cells for post-ischemic myocardial repair, Methods Mol. Med. 112 (2005) 183–192.

[18] K. Le Blanc, I. Rasmusson, B. Sundberg, C. Götherström, M. Hassan, M. Uzunel, O. Ringdén, Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells, Lancet 363 (2004) 1439–1441.

[19] M. Kassem, B.M. Abdallah, Human bone-marrow-derived mesenchymal stem cells: biological characteristics and potential role in therapy of degenerative diseases, Cell Tissue Res. 331 (2008) 157–163.

[20] A. Nakamizo, F. Marini, T. Amano, A. Khan, M. Studeny, J. Gumin, J. Chen, S. Hentschel, G. Vecil, J. Dembinski, M. Andreeff, F.F. Lang, Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas, Cancer Res. 65 (2005) 330.

[21] P.S. In 't Anker, S.A. Scherjon, C. Kleijburg-van der Keur, G.M. de Groot-Swings, F.H. Claas, W.E. Fibbe, H.H. Kanhai, Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta, Stem Cells 22 (2004) 1338–1345.

[22] P.S. In 't Anker, S.A. Scherjon, C. Kleijburg-van der Keur, W.A. Noort, F.H. Claas, R. Willemze, W.E. Fibbe, H.H. Kanhai, Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation, Blood 102 (2003) 1548–1549.

[23] A. Erices, P. Conget, J.J. Minguell, Mesenchymal progenitor cells in human umbilical cord blood, Br. J. Haematol. 109 (2000) 235–242.

[24] P.A. Zuk, M. Zhu, P. Ashjian, D.A. De Ugarte, J.I. Huang, H. Mizuno, Z.C. Alfonso, J.K. Fraser, P. Benhaim, M.H. Hedrick, Human adipose tissue is a source of multipotent stem cells, Mol. Biol. Cell. 13 (2002) 4279–4295.

[25] C. Campagnoli, I.A. Roberts, S. Kumar, P.R. Bennett, I. Bellantuono, N.M. Fisk, Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow, Blood 98 (2001) 2396–2402.

[26] M. Vilalta, I.R. Dégano, J. Bagó, D. Gould, M. Santos, M. García-Arranz, R. Ayats, C. Fuster, Y. Chernajovsky, D. García-Olmo, N. Rubio, J. Blanco, Biodistribution, long-term survival, and safety of human adipose tissue-derived mesenchymal stem cells transplanted in nude mice by high sensitivity non-invasive bioluminescence imaging, Stem Cells Dev. 17 (2008) 993–1003.

[27] M.E. Bernardo, N. Zaffaroni, F. Novara, A.M. Cometa, M.A. Avanzini, A. Moretta, D. Montagna, R. Maccario, R. Villa, M.G. Daidone, O. Zuffardi, F. Locatelli, 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 Res. 67 (2007) 9142–9149.

[28] A. De Becker, P. Van Hummelen, M. Bakkus, I. Vande Broek, J. De Wever, M. De Waele, I. Van Riet, Migration of culture-expanded human mesenchymal stem cells through bone marrow endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3, Haematologica 92 (2007) 440–449.

[29] M. Dominici, K. Le Blanc, I. Mueller, I. Slaper-Cortenbach, F. Marini, D. Krause, R. Deans, A. Keating, Dj. Prockop, E. Horwitz, Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement, Cytotherapy 8 (2006) 315–317.

[30] A. Hrzenjak, F. Moinfar, M.L. Kremser, B. Strohmeier, E. Petru, K. Zatloukal, H. Denk, Histone deacetylase inhibitor vorinostat suppresses the growth of uterine sarcomas in vitro and in vivo, Mol. Cancer 9 (2010) 49.

[31] J. Sonnemann, L. Dreyer, M. Hartwig, C.D. Palani, T.T. Hong le, U. Klier, B. Bröker, U. Völker, J.F. Beck, Histone deacetylase inhibitors induce cell death and enhance the apoptosis-inducing activity of TRAIL in Ewing's sarcoma cells, J Cancer Res Clin Oncol. 133 (2007) 847–858.

[32] S. Yamamoto, K. Tanaka, R. Sakimura, T. Okada, T. Nakamura, Y. Li, M. Takasaki, Y. Nakabeppu, Y. Iwamoto, Suberoylanilide hydroxamic acid (SAHA) induces apoptosis or autophagy-associated cell death in chondrosarcoma cell lines, Anticancer Res. 28 (2008) 1585–1591.

[33] A. Hrzenjak, M.L. Kremser, B. Strohmeier, F. Moinfar, K. Zatloukal, H. Denk, SAHA induces caspase-independent, autophagic cell death of endometrial stromal sarcoma cells by influencing the mTOR pathway, J. Pathol. 216 (2008) 495–504.

[34] Y. Shapovalov, D. Benavidez, D. Zuch, R.A. Eliseev, Proteasome inhibition with bortezomib suppresses growth and induces apoptosis in osteosarcoma, Int. J. Cancer 127 (2010) 67–76.

[35] F. Bersani, R. Taulli, P. Accornero, A. Morotti, S. Miretti, T. Crepaldi, C. Ponzetto, Bortezomib-mediated proteasome inhibition as a potential strategy for the treatment of rhabdomyosarcoma, Eur. J. Cancer 44 (2008) 876–884.

[36] G. Lu, V. Punj, P.M. Chaudhary, Proteasome inhibitor Bortezomib induces cell cycle arrest and apoptosis in cell lines derived from Ewing's sarcoma family of tumors and synergizes with TRAIL, Cancer Biol. Ther. 7 (2008) 603–608.

[37] R. Russell Jones, G. Orchard, B. Zelger, E. Wilson Jones, Immunostaining for CD31 and CD34 in Kaposi sarcoma, J. Clin. Pathol. 48 (1995) 1011–1016.

[38] J. Roma, A. Masià, J. Reventós, J. Sánchez de Toledo, S. Gallego, Notch pathway inhibition significantly reduces rhabdomyosarcoma invasiveness and mobility in vitro, Clin. Cancer Res. 17 (2011) 505–513.

[39] P. Zhang, Y. Yang, P.A. Zweidler-McKay, D.P. Hughes, Critical role of notch signaling in osteosarcoma invasion and metastasis, Clin. Cancer Res. 14 (2008) 2962–2969.

[40] F. Engin, T. Bertin, O. Ma, M.M. Jiang, L. Wang, R.E. Sutton, L.A. Donehower, B. Lee, Notch signaling contributes to the pathogenesis of human osteosarcomas, Hum. Mol. Genet. 18 (2009) 1464–1470.

[41] Y. Cai, A.B. Mohseny, M. Karperien, P.C. Hogendoorn, G. Zhou, A.M. Cleton-Jansen, Inactive Wnt/beta-catenin pathway in conventional high-grade osteosarcoma, J. Pathol. 220 (2010) 24–33.

[42] I. Matushansky, E. Hernando, N.D. Socci, J.E. Mills, T.A. Matos, M.A. Edgar, S. Singer, R.G. Maki, C. Cordon-Cardo, Derivation of sarcomas from mesenchymal stem cells via inactivation of the Wnt pathway, J. Clin. Invest. 117 (2007) 3248–3257.

[43] U. Tostar, R. Toftgård, P.G. Zaphiropoulos, T. Shimokawa, Reduction of human embryonal rhabdomyosarcoma tumor growth by inhibition of the hedgehog signaling pathway, Genes Cancer 1 (2010) 941–951.

[44] N. Kawabata, K. Ijiri, Y. Ishidou, T. Yamamoto, H. Nagao, S. Nagano, S. Maeda, S. Komiya, T. Setoguchi, Pharmacological inhibition of the Hedgehog pathway prevents human rhabdomyosarcoma cell growth, Int. J. Oncol. 39 (2011) 899–906.

[45] H. Yamanaka, T. Oue, S. Uehara, M. Fukuzawa, Hedgehog signal inhibitor forskolin suppresses cell proliferation and tumor growth of human rhabdomyosarcoma xenograft, J. Pediatr. Surg. 46 (2011) 320–325.