Biochemical and Biophysical Research Communications 448 (2014) 454–460

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Glutathione peroxidase-1 is required for self-renewal of murine embryonic stem cells

Qian-Yi Wang [1], Zhao-Shan Liu [1], Jie Wang, Hong-Xia Wang, Ang Li, Yang Yang, Xin-Zheng Wang, Yong-Qiang Zhao, Qiu-Ying Han, Hong Cai, Bing Liang, Nan Song, Wei-Hua Li, Tao Li *

State Key Laboratory of Proteomics, Institute of Basic Medical Sciences, National Center of Biomedical Analysis, 27 Tai-Ping Rd., Beijing 100850, China

ARTICLE INFO

Article history:
Received 23 April 2014
Available online 4 May 2014

Keywords:
Embryonic stem cells
Glutathione peroxidase-1
Self-renewal
Differentiation

ABSTRACT

Embryonic stem (ES) cells are pluripotent cells that are capable of giving rise to any type of cells in the body and possess unlimited self-renewal potential. However, the exact regulatory mechanisms that govern the self-renewal ability of ES cells remain elusive. To understand the immediate early events during ES cell differentiation, we performed a proteomics study and analyzed the proteomic difference in murine ES cells before and after a 6-h spontaneous differentiation. We found that the expression level of glutathione peroxidase-1 (GPx-1), an antioxidant enzyme, is dramatically decreased upon the differentiation. Both knockdown of GPx-1 expression with shRNA and inhibiting GPx-1 activity by inhibitor led to the differentiation of ES cells. Furthermore, we showed that during early differentiation, the quick degradation of GPx-1 was mediated by proteasome. Thus, our data indicated that GPx-1 is a key regulator of self-renewal of murine embryonic stem cells.

1. Introduction

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocysts [1]. The defining hallmarks of ES cells are the ability to give rise to all cell types within the body and the unlimited self-renewal potential [2]. This unlimited self-renewal capacity allows ES cells to maintain the undifferentiated state during dividing [2,3]. Defects in self-renewal mechanisms result in severe developmental problems. The elucidation of the self-renewal process and the underlying mechanisms provides the fundamental insights into development, aging and cancers [3]. It is believed that the pluripotency and the unlimited self-renewal potential of ES cells are under both cell-intrinsic and extrinsic control [4–7], however, the exact regulating mechanisms are not fully understood.

Recently, redox homeostasis has been recognized to play important roles in maintaining the self-renewal of ES cells [8]. ES cells have highly efficient machinery for antioxidant defense to maintain the redox homeostasis [9]. Reactive oxygen species (ROS), such as superoxide and hydrogen peroxide, are generated as a byproduct by mitochondria during aerobic metabolism [8–10], and these species can lead to DNA damage, senescence and cells death by causing oxidative damage to DNA and proteins [10]. GPx-1 is one of the antioxidant enzymes that modulate the overall redox homeostasis by reducing hydrogen peroxide to water, and therefore eliminating the toxic effects of ROS [10]. Recent studies have suggested the essential role of GPx-1 in many physiological and pathological procedures [11–13], but the role of GPx-1 in modulating ES cell self-renewal still remains elusive.

Much effort has been devoted to identifying potential regulators of ES cell self-renewal by comparing ES cells with their differentiated counterparts, however, the immediate early events upon ES cell differentiation are still largely unknown [14–17]. Here, by performing a differential proteomics study, we analyzed murine ES cells and the spontaneously differentiated cells, which were differentiated by withdrawing leukemia inhibitory factor (LIF), serum and feeder cells for 6 h, and we found that the expression level of GPx-1 was dramatically decreased upon the differentiation of ES cells. Both knockdown of GPx-1 expression and inhibition of GPx-1 activity resulted in the differentiation of ES cells. We further showed that during the early differentiation of ES cells, proteasome mediated the quick degradation of GPx-1. Thus, our data indicated that GPx-1 is required for self-renewal of murine embryonic stem cells.

* Corresponding author. Address: National Center of Biomedical Analysis, Bldg 11, Rm 226, 27 Tai-Ping Rd., Beijing 100850, China. Fax: +86 10 6824 6161.
E-mail address: tli@ncba.ac.cn (T. Li).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.04.139
0006-291X/

2. Materials and methods

2.1. Embryonic stem cell culture

Murine ES cells (SCRC-1002; American Type Culture Collection, Manassas, VA) were cultured in DMEM medium (Gibco), supplemented with 15% fetal bovine serum (Hyclon), 1000 U/ml LIF (Chemicon), 2 mM L-glutamine (Macgene), 1 mM sodium pyruvate (Gibco), 1% MEM nonessential amino acids (Gibco), 0.1 mM 2-mercaptoethanol (Sigma) and 1% penicillin–streptomycin (Macgene) on mouse embryonic fibroblast (MEF) feeder layers treated previously with mitomycin C (Sigma, M4287).

2.2. Differentiation of murine ES cells

Prior to differentiation, ES cells were disassociated by trypsin, the cell suspension was plated on a cultural dish for 30 min at 37 °C to remove the feeder cells. The purified cells were then cultured with ES cell culturing medium without LIF and serum in a new dish.

To form embryoid body (EB), murine ES cell aggregates were cultured in Ultra Low Attachment Plates (Corning Costar, 3471) with ES cell culturing medium lacking sodium pyruvate and LIF.

2.3. Protein identification by label-free LC–MS analysis

Murine ES cells were spontaneously differentiated. Proteins from undifferentiated and differentiated murine ES cells were extracted and digested as described previously [18]. The peptide mixture was acidified by formic acid for followed MS analysis. The label-free LC–MS analysis was performed as described by Shen et al. [19].

2.4. 2-dimensional electrophoresis (2-DE) and MALDI-TOF/TOF-MS analysis

Protein extracts of undifferentiated and differentiated murine ES cells were separated by 2-DE as described previously [20]. The 2-DE images were captured using ImageScanner (Amersham Pharmacia Biotech). Thirty-two spots representing significantly altered between undifferentiated and differentiated cells proteins were analyzed by ultraflex III MALDI-TOF/TOF-MS (Bruker). Peptide mass fingerprinting (PMF) data was searched on local Mascot v2.1 against the non-redundant protein database NCBInr and the MS/MS ion database search was conducted using Mascot (http://www.matrixscience.com).

2.5. RNA interference

Two RNA interference sequences for knockdown GPx-1, 5′-AGAAACCCTGCTGTCCCAG-3′ and 5′-GTTTGAGAAGTGCGAAGTG-3′ were sub-cloned into pLKO lentiviral vectors. 24 and 48 h after co-transfection of 293T cells with lentiviral packaging plasmids, the viral particles were harvested. The virus were concentrated by ultracentrifugation for 1.5 h at 25,000g and resuspended in D-PBS (Hyclon) before incubation with murine ES cells. At 36 h post-infection, ES cells were harvested for protein expression analysis and cell morphological observation.

2.6. Protein extraction and Western blotting

Cells were washed with PBS before being collected and lysed in lysis buffer (20 mM Tris–HCl pH 7.4, 150 mM NaCl, 0.5% Nonidet P-40 and protease inhibitor cocktail (Roche, 04693124001)). Cell debris was removed by centrifugation at 12,000g, for 10 min at 4 °C. Protein concentrations were determined using Bradford Dye (Bio-Rad, Hercules, CA). Total protein was separated by SDS–PAGE and transferred to Hybond-P PVDF membrane (GE Healthcare, Piscataway, NJ). Specific antibodies used for Western blotting are GPx-1 (Abcam, ab108427), Oct4 (Santa cruz, sc5279 and α-tubulin (Sigma, T5168).

2.7. Alkaline phosphatase staining

The differentiation phenotype of murine ES cells was evaluated by determining alkaline phosphatase (ALP) activity using ALP staining kit (Millipore, SCR004) according to the manufacturer's instruction. Briefly, cells in 24-well plates were fixed by 4% paraformaldehyde for 1–2 min and washed with Rinse Buffer. After wash, 0.5 ml staining solution was added to each well and incubated in dark at room temperature for 15 min. Aspirate staining solution and rinse the wells with Rinse Buffer again, the cells were then photographed.

2.8. Flow cytometry

The ES cells were disassociated by trypsin and then resuspended with 10% FBS in PBS. After 30 min blocking, cells were washed and incubated with SSEA-1 antibody (Santa cruz, sc21702-PE) or the control IgG for 30 min at room temperature. After wash with 1% FBS in PBS for 3 times, cells were resuspended in 0.5 ml PBS and analyzed on flow cytometer.

2.9. RT-PCR

Total RNA was extracted with TRIzol according to the manufacturer's guidelines (Invitrogen). CYP-26 mRNA was measured by RT-PCR with a Prime Script RT Master Mix kit (Takara) and the primers, 5′-TTCTGCAGATGAAGCGCAGG-3′ (forward) and 5′-TTTC GCTGCTTGTGCGAGGA-3′ (reverse). GAPDH was used as a control, the forward and reverse primers were 5′-CGACTTCAACAGCAA CTCCCACTCTTCC-3′ and 5′-TGGGTGGTCCAGGGTTTCTTACTCCTT-3′ respectively. The RT-PCR reactions (30 cycles) were migrated on a 1% agarose gel and stained with ethidium bromide.

3. Results

3.1. Proteomic identification of altered expression of proteins upon murine ES cell differentiation

In order to understand the immediate early events during ES cell differentiation, we sought to identify the proteomic difference before and after ES cell differentiation. Especially, the down-regulated proteins during differentiation may represent the potentially essential regulators for ES cells self-renewal. We first let the murine ES cells differentiate spontaneously by withdrawing LIF, serum and feeder cells. At 6 h post differentiation when the morphological change could be observed, the early differentiated cells together with their undifferentiated counterpart were collected and subjected to a label-free LC–MS analysis (Fig. 1A). Proteins that are uniquely expressed in undifferentiated cells and in differentiated cells were listed (Table 1). We then used 2-DE to analyze independent samples (Fig. 1A). The down-regulated proteins after differentiation were arrowed (Fig. 1B), and these protein spots were subjected to in-gel digested by trypsin, and the resulting peptides were analyzed by MALDI-TOF/TOF MS. GPx-1 was identified to be a down-regulated protein after differentiation, which is consistent with the data listed in Table 1 (Table 1 and Fig. 1C).

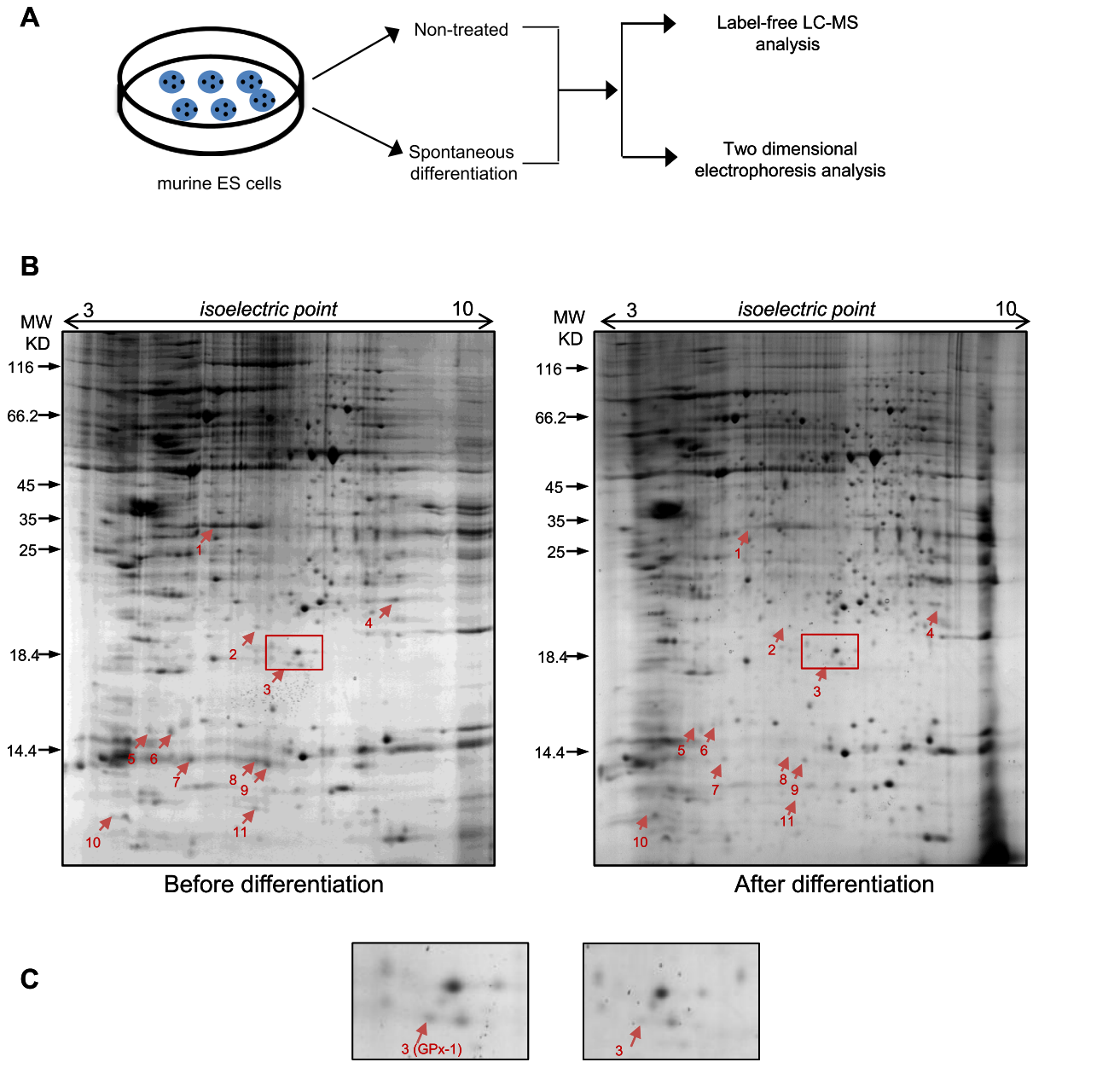

Fig. 1. 2-DE analysis of undifferentiated and differentiated murine ES cells. (A) Scheme for identifying the proteomic difference before and after ES cell differentiation. (B) Proteins from the extracts of undifferentiated (left panel) and differentiated murine ES cells (right panel) were separated on 24-cm non-linear gradient IPG strips and 12.5% SDS–PAGE, followed by coomassie brilliant blue staining. Eleven down-regulated proteins after differentiation are arrowed. (C) The rectangular areas in (B) are magnified, respectively.

3.2. Reduced expression of GPx-1 upon murine ES cell differentiation

To confirm the above finding, murine ES cells were differentiated for different time course and the expression of GPx-1 was determined by Western blot. Consistently, an obvious decrease of GPx-1 expression was observed at 6 h post differentiation, and GPx-1 was almost undetectable at 24 h after differentiation. Interestingly, Oct4, the well accepted ES cell marker, remained constant at this stage of differentiation (Fig. 2A). To rule out the possibility that the down-regulation of GPx-1 was caused by the serum starvation, we then cultured the ES cells under the condition for EB formation [21], similar results were obtained (Fig. 2B). Together, these data suggested that GPx-1 is a key regulator for ES cell self-renewal.

3.3. Knockdown of GPx-1 led to the differentiation of ES cells

To determine the role of GPx-1 in ES cell self-renewal, we knocked down GPx-1 using two different short hairpin RNAs (shRNAs) sequences (Fig. 3A). The knockdown of GPx-1 resulted in differentiation of ES cells, as shown by cell morphology (Fig. 3B) and alkaline phosphatase (ALP) staining (Fig. 3C). We then counted the numbers of densely packed and alkaline phosphatase-positive ES cell colonies before and after GPx-1 knockdown, the number of ES colonies were significantly reduced in GPx-1-knockdown cells (Fig. 3D). To further confirm the differentiation of ES cells caused by GPx-1 knockdown, we chose to examine the expression of SSEA-1, the ES cell marker[22], and CYP-26, the marker of differentiated cells [23,24], in ES cells before and after

Table 1
List of uniquely expressed proteins that identified by label-free LC-MS in undifferentiated or differentiated ES cells.

Number	Accession	Protein name	Score	Unique expression	
				Before differentiation	After differentiation
1	gi|51810	Heterogeneous nuclear ribonucleoprotein U	391.1		✓
2	gi|15384	Heterogeneous nuclear ribonucleoprotein A/B isoform 1	246.76		✓
3	gi|27041	Ras-GTPase-activating protein SH3-domain binding protein	237.05		✓
4	gi|20867	Stress-induced-phosphoprotein 1	229.54		✓
5	gi|67025	60S ribosomal protein L11	182.29		✓
6	gi|13205	ATP-dependent RNA helicase DDX3X	181.09		✓
7	gi|15122	Hemoglobin subunit alpha	176.13	✓	
8	**gi|1**10957	Putative ATP-dependent RNA helicase PI10	166.88		✓
9	gi|18663	Phosphoglycerate kinase 2	155.22		✓
10	gi|18102	Nucleoside diphosphate kinase A	141.47		✓
11	gi|20042	40S ribosomal protein S12	140.03	✓	
12	gi|271564	Hypothetical protein	139.81	✓	
13	gi|17420	CDK-activating kinase assembly factor MAT1	134.72	✓	
14	gi|114873	Down syndrome cell adhesion molecule-like protein	129.06		✓
15	gi|108147	Bifunctional purine biosynthesis protein PURH	126.34		✓
16	gi|14775	GPx1 Glutathione peroxidase 1	117.99	✓	
17	gi|16796	LIM and SH3 protein 1	116.09	✓	
18	gi|22608	Nuclease sensitive element binding protein 1	115.06		✓
19	gi|53605	Nucleosome assembly protein 1 -like 1	112.72		✓
20	gi|100504508	CDNA sequence BC085271	105.79		✓
21	gi|56086	SET nuclear oncogene	105.12		✓

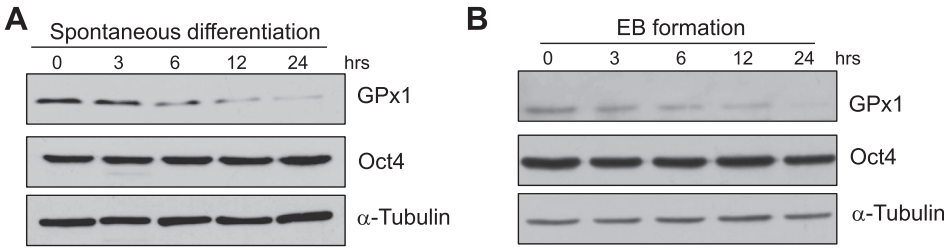

Fig. 2. GPx-1 expression is decreased upon differentiation of murine ES cells. Immune blot analysis of GPx-1 and Oct4 expression in ES cells during the spontaneous differentiation (A) or EB formation (B). α-Tubulin blots indicate loading of lanes.

GPx-1 knockdown. The inhibition of GPx-1 expression in ES cells with shRNAs led to the appearance of CYP-26 expression, as a control, the CYP-26 expression could also be detected in ES cells cultured under EB formation condition for 3 days (Fig. 3E). Consistently, the expression of SSEA-1 in ES cells after GPx-1 knockdown was dramatically decreased (Fig. 3F). Together, these data indicated that GPx-1 expression is critical for maintaining the undifferentiated state of ES cells.

3.4. Inhibition of GPx-1 activity caused the differentiation of ES cells

Further, we tested if GPx-1 modulates ES cell self-renewal through its enzymatic activity. We treated ES cells with mercaptosuccinic acid, the specific inhibitor of GPx-1 activity. The inhibition of GPx-1 activity led to the significant ES cell differentiation, as shown with CYP-26 and SSEA-1 expression (Fig. 4A and B), as well as ALP staining (Fig. 4C). The ES cell colonies numbers were significantly reduced in cells treated with GPx-1inhibitor (Fig. 4D). Thus, GPx-1 is likely to regulate ES cell self-renewal via its enzymatic activity.

3.5. Proteasome mediated the GPx-1 degradation

The quick decrease of GPx-1 expression indicated that this was unlikely regulated at transcriptional level, we then tested whether GPx-1 undergoes degradation upon ES cell differentiation. We treated ES cells with MG132, the proteasome inhibitor, and NH_4Cl, the lysosome inhibitor, respectively. Upon differentiation, GPx-1 down-regulation was significantly blocked by MG132, while NH_4Cl did not show any effect (Fig. 4E). Thus, the decreased expression of GPx-1 was mainly through proteasome-mediated degradation.

4. Discussion

In this study, we sought to understand the biological events happened in the early stage of murine ES cell differentiation. To do so, we let ES cells differentiate spontaneously by withdrawing LIF, serum and feeder cells. At the time point of 6-h post-differentiation, we started to observe the morphological change of the cells, the differentiated cells and the undifferentiated counterparts were subjected to proteomic analysis. We have found that GPx-1 is required for maintaining the undifferentiated state of murine embryonic stem cell.

To eliminate the toxic effect of ROS, ES cells are highly proficient in antioxidant defense [9,25,26]. Various antioxidant mechanisms of stem cells have been characterized [8,27,28]. A transcriptomic study has showed that several oxidative stress resistance genes, including members of *GPx* family, underwent considerable down-regulation when ES cells differentiated. In line with these reports, we have showed that the protein expression level of GPx-1 was markedly reduced in hours post spontaneous differentiation. ROS has also been shown to be essential for stem cell differentiation [29,30], therefore, it is reasonable to speculate that the decreased

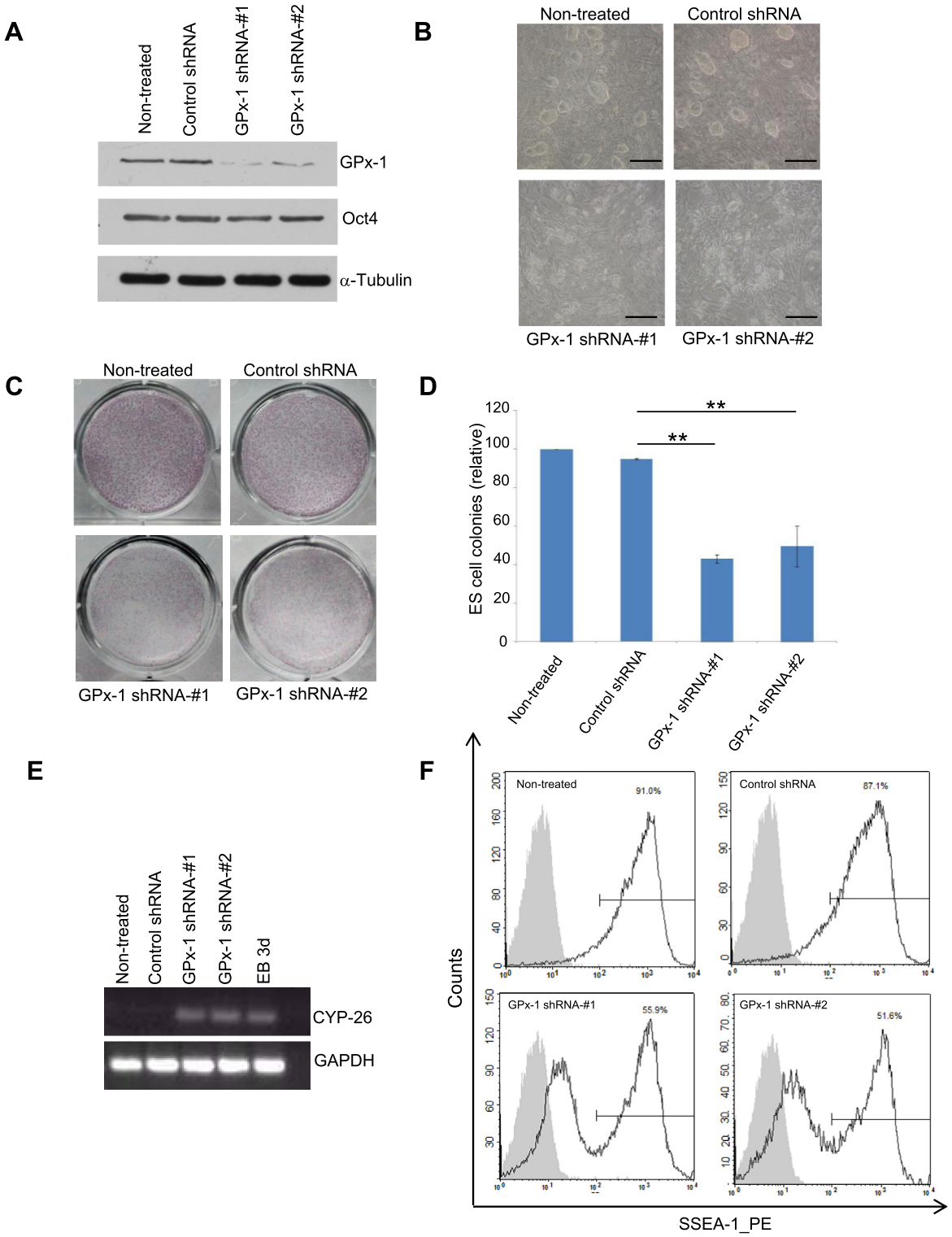

Fig. 3. Knockdown of GPx-1 resulted in the differentiation of murine ES cells. ES cells were infected with lentivirus that expressing the control shRNA and GPx-1 shRNAs. (A) Immune blot analysis of GPx-1 expression, and α-tubulin blots indicate loading of lanes. (B) Cell morphologies were shown in phase contrast images, scale bars, 100 μm. (C) Representative images for ALP stained ES cells. (D) Quantification of densely packed and alkaline phosphatase-positive ES cell colonies. Data represent three independent experiments. For each experiment, colony numbers were normalized to that of non-treated group. One-way ANOVA was used for multiple-group comparisons, error bars, mean ± SEM, $^{**}p < 0.01$. (E) RT-PCR analysis for CYP-26 expression in ES cells 36 h after shRNA transfection. The ES cells cultured under EB formation condition for 3 days as positive control. (F) Flow cytometric analysis of SSEA-1 expression in ES cells after GPx-1 knockdown.

GPx-1 expression leads to the increase of ROS and thus promotes ES cell differentiation.

The focus on the damaging effects of ROS has now been changed to the homeostatic ROS level, since increasing evidence suggested that low levels of ROS are necessary for maintaining the capacity of self-renew of stem cells [31–33]. With the treatment of GPx-1 inhibitor, we observed a more significant effect than the treatment of specific knockdown of GPx-1 with shRNAs. It could be due to the

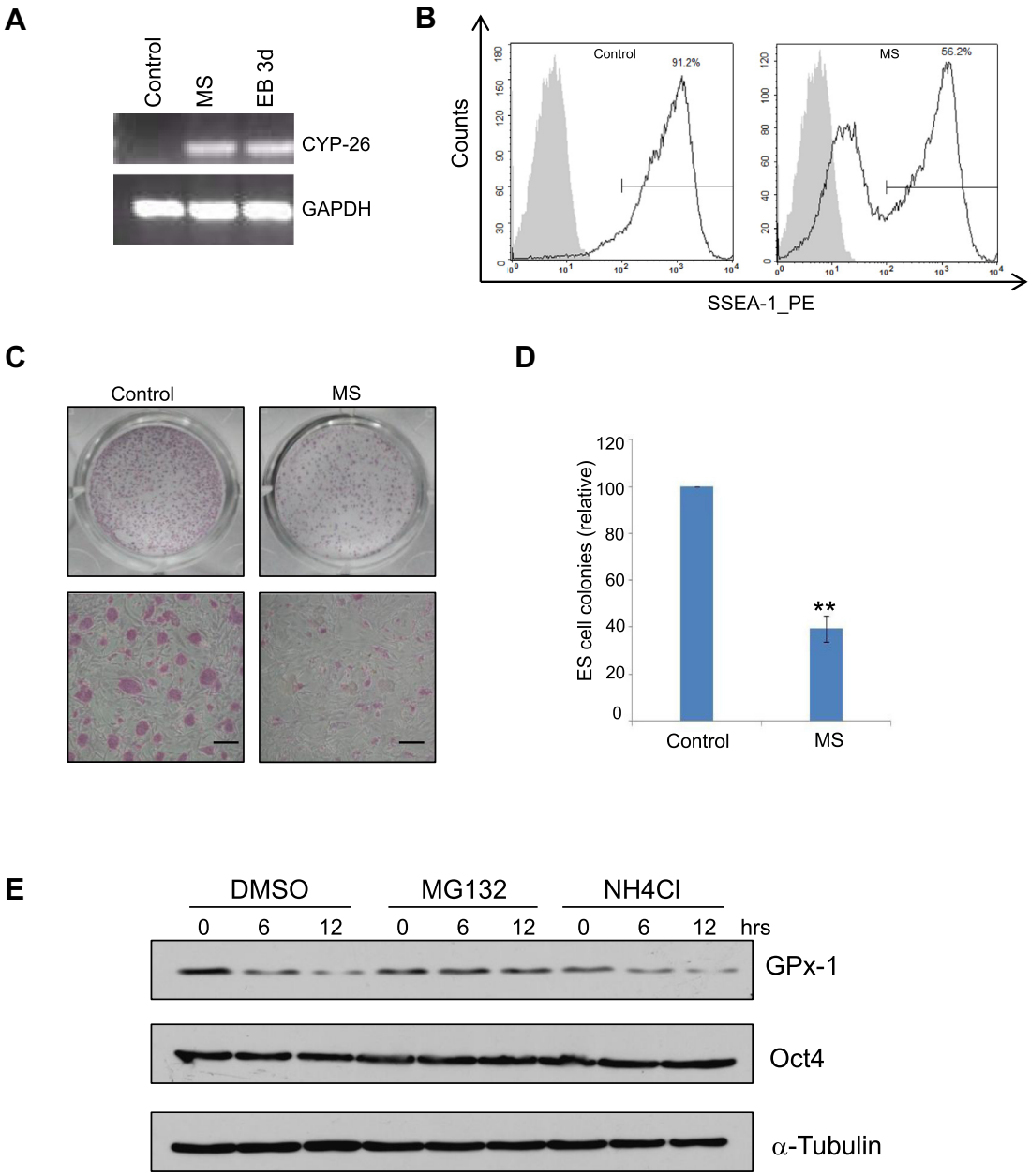

Fig. 4. Inhibition of GPx-1 activity led to ES cell differentiation and proteasome mediates the degradation of GPx-1 upon differentiation. Murine ES cells treated with or without GPx-1 activity inhibitor, mercaptosuccinic acid (MS, 40 mM), for 24 h. RT-PCR analysis for CYP-26 appearance (A), flow cytometric analysis of SSEA-1 expression (B) and ALP staining of these cells, scale bars, 100 μm (C). The number of ES colonies in (C) was quantified, data represent three independent experiments, error bars, mean ± SEM, $^{**}p < 0.01$ (D). (E) Murine ES cells were pre-treated with MG132 (10 μM) or NH_4Cl (10 mM) and then subjected to the spontaneous differentiation process, DMSO treatment was used as control. Immune blot analysis of GPx-1 and Oct4, α-tubulin blots indicate loading of lanes.

inhibition effect of mercaptosuccinate on other members of GPx family [10,34]. The balanced activity of GPx-1 as well as other GPx proteins is most likely to contribute to the subtly orchestrated balance of the overall intracellular ROS levels, which play a pivotal role in modulating ES cell self-renewal and the differentiation. As such, it is not surprising to observe the re-expression of GPx-1 when ES cells continue to differentiate for weeks [35].

Proteasome-based quick degradation of GPx-1 at the early stage of ES cell differentiation suggests that this protein is a sensitive regulator of ES cell self-renewal. Further study regarding the regulation of expression, modification and activity of GPx-1 in ES cells will certainly provide important insights into stem cell biology.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by National High-Tech R&D Program of China (2014AA020501), National Basic Research Program of China (2013CB530803), China National Natural Science Foundation (Nos. 81372250, 31270934, 81302595 and 81301745), Beijing Municipal Science and Technology Commission (2011103), SKLP-K201102 and AMMS-2012CXJJ030.

References

[1] M.J. Evans, M.H. Kaufman, Establishment in culture of pluripotential cells from mouse embryos, Nature 292 (1981) 154–156.
[2] A. Mohyeldin, T. Garzon-Muvdi, A. Quinones-Hinojosa, Oxygen in stem cell biology: a critical component of the stem cell niche, Cell Stem Cell 7 (2010) 150–161.
[3] S. He, D. Nakada, S.J. Morrison, Mechanisms of stem cell self-renewal, Annu. Rev. Cell Dev. Biol. 25 (2009) 377–406.
[4] R. Jaenisch, R. Young, Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming, Cell 132 (2008) 567–582.
[5] H. Niwa, J. Miyazaki, A.G. Smith, Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells, Nat. Genet. 24 (2000) 372–376.
[6] H. Niwa, How is pluripotency determined and maintained?, Development 134 (2007) 635–646
[7] S.J. Morrison, A.C. Spradling, Stem cells and niches: mechanisms that promote stem cell maintenance throughout life, Cell 132 (2008) 598–611.
[8] K. Wang, T. Zhang, Q. Dong, E.C. Nice, C. Huang, Y. Wei, Redox homeostasis: the linchpin in stem cell self-renewal and differentiation, Cell Death Dis. 4 (2013) e537.
[9] G. Saretzki, L. Armstrong, A. Leake, M. Lako, T. von Zglinicki, Stress defense in murine embryonic stem cells is superior to that of various differentiated murine cells, Stem Cells 22 (2004) 962–971.
[10] E. Lubos, J. Loscalzo, D.E. Handy, Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities, Antioxid. Redox Signal. 15 (2011) 1957–1997.
[11] F. Antunes, D. Han, E. Cadenas, Relative contributions of heart mitochondria glutathione peroxidase and catalase to H(2)O(2) detoxification in in vivo conditions, Free Radic. Biol. Med. 33 (2002) 1260–1267.
[12] G. Cohen, P. Hochstein, Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes, Biochemistry 2 (1963) 1420–1428.
[13] Y. Fu, H. Sies, X.G. Lei, Opposite roles of selenium-dependent glutathione peroxidase-1 in superoxide generator diquat- and peroxynitrite-induced apoptosis and signaling, J. Biol. Chem. 276 (2001) 43004–43009.
[14] J. Nichols, B. Zevnik, K. Anastassiadis, H. Niwa, D. Klewe-Nebenius, I. Chambers, H. Scholer, A. Smith, Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4, Cell 95 (1998) 379–391.
[15] A.A. Avilion, Multipotent cell lineages in early mouse development depend on SOX2 function, Genes Dev. 17 (2003) 126–140.
[16] K. Mitsui, Y. Tokuzawa, H. Itoh, K. Segawa, M. Murakami, K. Takahashi, M. Maruyama, M. Maeda, S. Yamanaka, The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells, Cell 113 (2003) 631–642.
[17] M. Dejosez, J.S. Krumenacker, L.J. Zitur, M. Passeri, L.F. Chu, Z. Songyang, J.A. Thomson, T.P. Zwaka, Ronin is essential for embryogenesis and the pluripotency of mouse embryonic stem cells, Cell 133 (2008) 1162–1174.
[18] M.P. Washburn, D. Wolters, J.R. Yates 3rd, Large-scale analysis of the yeast proteome by multidimensional protein identification technology, Nat. Biotechnol. 19 (2001) 242–247.
[19] Z. Shen, P. Li, R.J. Ni, M. Ritchie, C.P. Yang, G.F. Liu, W. Ma, G.J. Liu, L. Ma, S.J. Li, Z.G. Wei, H.X. Wang, B.C. Wang, Label-free quantitative proteomics analysis of etiolated maize seedling leaves during greening, Mol. Cell. Proteomics 8 (2009) 2443–2460.
[20] B.F. Jin, K. He, H.X. Wang, B. Bai, T. Zhou, H.Y. Li, J.H. Man, B.Y. Liu, W.L. Gong, J. Wang, A.L. Li, X.M. Zhang, Proteomics analysis reveals insight into the mechanism of H-Ras-mediated transformation, J. Proteome Res. 5 (2006) 2815–2823.
[21] E.Y. Fok, P.W. Zandstra, Shear-controlled single-step mouse embryonic stem cell expansion and embryoid body-based differentiation, Stem Cells 23 (2005) 1333–1342.
[22] M. Evans, Discovering pluripotency: 30 years of mouse embryonic stem cells, Nat. Rev. Mol. Cell Biol. 12 (2011) 680–686.
[23] M.A. Lane, A.C. Chen, S.D. Roman, F. Derguini, L.J. Gudas, Removal of LIF (leukemia inhibitory factor) results in increased vitamin A (retinol) metabolism to 4-oxoretinol in embryonic stem cells, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13524–13529.
[24] D. Duval, M. Malaise, B. Reinhardt, C. Kedinger, H. Boeuf, A p38 inhibitor allows to dissociate differentiation and apoptotic processes triggered upon LIF withdrawal in mouse embryonic stem cells, Cell Death Differ. 11 (2004) 331–341.
[25] S. Parrinello, E. Samper, A. Krtolica, J. Goldstein, S. Melov, J. Campisi, Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts, Nat. Cell Biol. 5 (2003) 741–747.
[26] R.A. Busuttil, M. Rubio, M.E. Dolle, J. Campisi, J. Vijg, Oxygen accelerates the accumulation of mutations during the senescence and immortalization of murine cells in culture, Aging Cell 2 (2003) 287–294.
[27] S. Ghaffari, Oxidative stress in the regulation of normal and neoplastic hematopoiesis, Antioxid. Redox Signal. 10 (2008) 1923–1940.
[28] J. Case, D.A. Ingram, L.S. Haneline, Oxidative stress impairs endothelial progenitor cell function, Antioxid. Redox Signal. 10 (2008) 1895–1907.
[29] S. Pervaiz, R. Taneja, S. Ghaffari, Oxidative stress regulation of stem and progenitor cells, Antioxid. Redox Signal. 11 (2009) 2777–2789.
[30] X. Shi, Y. Zhang, J. Zheng, J. Pan, Reactive oxygen species in cancer stem cells, Antioxid. Redox Signal. 16 (2012) 1215–1228.
[31] Y.Y. Jang, S.J. Sharkis, A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche, Blood 110 (2007) 3056–3063.
[32] M. Schmelter, B. Ateghang, S. Helmig, M. Wartenberg, H. Sauer, Embryonic stem cells utilize reactive oxygen species as transducers of mechanical strain-induced cardiovascular differentiation, FASEB J. 20 (2006) 1182–1184.
[33] C.I. Kobayashi, T. Suda, Regulation of reactive oxygen species in stem cells and cancer stem cells, J. Cell. Physiol. 227 (2012) 421–430.
[34] J. Chaudiere, E.C. Wilhelmsen, A.L. Tappel, Mechanism of selenium-glutathione peroxidase and its inhibition by mercaptocarboxylic acids and other mercaptans, J. Biol. Chem. 259 (1984) 1043–1050.
[35] Y.M. Cho, S. Kwon, Y.K. Pak, H.W. Seol, Y.M. Choi, D.J. Park, K.S. Park, H.K. Lee, Dynamic changes in mitochondrial biogenesis and antioxidant enzymes during the spontaneous differentiation of human embryonic stem cells, Biochem. Biophy. Res. Commun. 348 (2006) 1472–1478.