event in the early stage of hepatocarcinogenesis.[19,20] Moreover, some studies show that a *p16* deficiency does not fully restore the self-renewal capability of *Bmi-1*. In addition, reduced stem cell frequency occurs in *Bmi-1*-deficient neural stem cells, even when p16Ink4a and p19Arf are not expressed.[4,29,30] These studies indicate that there are additional downstream pathways that might mediate the effect of *Bmi-1* on self-renewal and cell proliferation.

From our gene expression analysis, we found that induction of *Bmi-1* in bone marrow cells resulted in an upregulation of *ABCB1*.[6] *ABCB1* appears to be a consistent feature of mammalian cells displaying resistance to multiple anticancer drugs, and has been postulated to mediate drug resistance.[31,32] Interestingly, recent findings also show expression of *ABCB1* in various stem cells,[33–35] which might make them less sensitive to cancer treatment. Increased expression of *ABCB1* was observed in HCC, particularly in early and well differentiated HCC, compared with the surrounding noncancerous region. *ABCB1* expression decreases with the progression of HCC, suggesting a reflection of tumor dedifferentiation.[36] We showed here that *ABCB1* expression was clearly altered in parallel with *Bmi-1* expression. High expression of both *Bmi-1* and *ABCB1* was observed in the early stage of hepatocarcinogenesis, which suggests their collaboration in maintaining the cell's ability for self-renewal, proliferation, and increased resistance from apoptosis. Although it is possible that *ABCB1* represents a novel downstream target for *Bmi-1*, further analysis is necessary to clarify the mechanism underlying the link between *Bmi-1* and *ABCB1* expression.

In summary, we evaluated the expression and involvement of the "stemness" gene, *Bmi-1*, in HCC, particularly in early stage hepatocarcinogenesis. The strong correlation observed between *Bmi-1* and *ABCB1* expression in HCC indicates a new regulatory pathway for *Bmi-1*, and reveals a potential novel target for enhancing future HCC treatment strategies.

Acknowledgments

This work was supported in part by a Keio University Grant-in-Aid for Encouragement of Young Medical Scientists. Grant-in-aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan to T.M.; Grants for the Health Labour Sciences Research and the Third Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare of Japan; Grant-in-aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan to M.S. Our sincere thanks to H. Suzuki, H. Abe, S. Kusakari, N Hashimoto, M. Konno, and T. Nagai for support throughout the work, M. Fujiwara and M. Iwata, for providing technical assistance, and also Dr. Toru Kiyono (Virology Division, National Cancer Center Research Institute of Japan).

References

1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74–108.
2 Sakamoto M, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. *Hum Pathol* 1991; **22**: 172–8.
3 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105–11.
4 Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer* 2003; **3**: 895–902.
5 van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H, Berns A. Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. *Cell* 1991; **65**: 737–52.
6 Mori T, Kiyono T, Imabayashi H *et al.* Combination of hTERT and bmi-1, E6, or E7 induces prolongation of the life span of bone marrow stromal cells from an elderly donor without affecting their neurogenic potential. *Mol Cell Biol* 2005; **25**: 5183–95.
7 Haga K, Ohno S, Yugawa T *et al.* Efficient immortalization of primary human cells by p16INK4a-specific short hairpin RNA or Bmi-1, combined with introduction of hTERT. *Cancer Sci* 2007; **98**: 147–54.
8 Dimri GP, Martinez JL, Jacobs JJ *et al.* The Bmi-1 oncogene induces telomerase activity and immortalizes human mammary epithelial cells. *Cancer Res* 2002; **62**: 4736–45.
9 Song LB, Zeng MS, Liao WT *et al.* Bmi-1 is a novel molecular marker of nasopharyngeal carcinoma progression and immortalizes primary human nasopharyngeal epithelial cells. *Cancer Res* 2006; **66**: 6225–32.
10 Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 1999; **397**: 164–8.
11 Jacobs JJ, Scheijen B, Voncken JW, Kieboom K, Berns A, van Lohuizen M. Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes Dev* 1999; **13**: 2678–90.
12 van Kemenade FJ, Raaphorst FM, Blokzijl T *et al.* Coexpression of BMI-1 and EZH2 polycomb-group proteins is associated with cycling cells and degree of malignancy in B-cell non-Hodgkin lymphoma. *Blood* 2001; **97**: 3896–3901.
13 Bea S, Tort F, Pinyol M *et al.* BMI-1 gene amplification and overexpression in hematological malignancies occur mainly in mantle cell lymphomas. *Cancer Res* 2001; **61**: 2409–12.
14 Vonlanthen S, Heighway J, Altermatt HJ *et al.* The bmi-1 oncoprotein is differentially expressed in non-small cell lung cancer and correlates with INK4A-ARF locus expression. *Br J Cancer* 2001; **84**: 1372–6.
15 Kim JH, Yoon SY, Kim CN *et al.* The Bmi-1 oncoprotein is overexpressed in human colorectal cancer and correlates with the reduced p16INK4a/p14ARF proteins. *Cancer Lett* 2004; **203**: 217–24.
16 Qin ZK, Yang JA, Ye YL *et al.* Expression of Bmi-1 is a prognostic marker in bladder cancer. *BMC Cancer* 2009; **9**: 61.
17 Wang H, Pan K, Zhang HK *et al.* Increased polycomb-group oncogene Bmi-1 expression correlates with poor prognosis in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2008; **134**: 535–41.
18 Sasaki M, Ikeda H, Itatsu K *et al.* The overexpression of polycomb group proteins Bmi1 and EZH2 is associated with the progression and aggressive biological behavior of hepatocellular carcinoma. *Lab Invest* 2008; **88**: 873–82.
19 Hui AM, Sakamoto M, Kanai Y *et al.* Inactivation of p16INK4 in hepatocellular carcinoma. *Hepatology* 1996; **24**: 575–9.
20 Kaneto H, Sasaki S, Yamamoto H *et al.* Detection of hypermethylation of the p16(INK4A) gene promoter in chronic hepatitis and cirrhosis associated with hepatitis B or C virus. *Gut* 2001; **48**: 372–7.
21 Genda T, Sakamoto M, Ichida T *et al.* Cell motility mediated by rho and Rho-associated protein kinase plays a critical role in intrahepatic metastasis of human hepatocellular carcinoma. *Hepatology* 1999; **30**: 1027–36.
22 Hirohashi S, Ishak K, Kojiro M *et al.* Tumours of the liver and intrahepatic bile ducts. In: Hamilton SR, Aaltonen LA, eds. *Pathology and Genetics of Tumours of the Digestive System*. Lyon: IARC Press, 2000; 157–202.
23 Neo SY, Leow CK, Vega VB *et al.* Identification of discriminators of hepatoma by gene expression profiling using a minimal dataset approach. *Hepatology* 2004; **39**: 944–53.
24 Francis NJ, Kingston RE. Mechanisms of transcriptional memory. *Nat Rev Mol Cell Biol* 2001; **2**: 409–21.
25 Buchenau P, Hodgson J, Strutt H, Arndt-Jovin DJ. The distribution of polycomb-group proteins during cell division and development in Drosophila embryos: impact on models for silencing. *J Cell Biol* 1998; **141**: 469–81.
26 Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M. Stem cells and cancer: the polycomb connection. *Cell* 2004; **118**: 409–18.
27 Nagao K, Tomimatsu M, Endo H, Hisatomi H, Hikiji K. Telomerase reverse transcriptase mRNA expression and telomerase activity in hepatocellular carcinoma. *J Gastroenterol* 1999; **34**: 83–7.
28 Oh BK, Jo Chae K, Park C *et al.* Telomere shortening and telomerase reactivation in dysplastic nodules of human hepatocarcinogenesis. *J Hepatol* 2003; **39**: 786–92.
29 Molofsky AV, He S, Bydon M, Morrison SJ, Pardal R. Bmi-1 promotes neural stem cell self-renewal and neural development but not mouse growth and survival by repressing the p16Ink4a and p19Arf senescence pathways. *Genes Dev* 2005; **19**: 1432–7.
30 Molofsky AV, Pardal R, Iwashita T, Park IK, Clarke MF, Morrison SJ. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. *Nature* 2003; **425**: 962–7.
31 Kartner N, Evernden-Porelle D, Bradley G, Ling V. Detection of P-glycoprotein in multidrug-resistant cell lines by monoclonal antibodies. *Nature* 1985; **316**: 820–3.

32 Riordan JR, Deuchars K, Kartner N, Alon N, Trent J, Ling V. Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. *Nature* 1985; **316**: 817–9.
33 Bunting KD, Galipeau J, Topham D, Benaim E, Sorrentino BP. Effects of retroviral-mediated MDR1 expression on hematopoietic stem cell self-renewal and differentiation in culture. *Ann NY Acad Sci* 1999; **872**: 125–40, discussion 40–1.
34 Bunting KD. ABC transporters as phenotypic markers and functional regulators of stem cells. *Stem Cells* 2002; **20**: 11–20.
35 Keshet GI, Goldstein I, Itzhaki O *et al.* MDR1 expression identifies human melanoma stem cells. *Biochem Biophys Res Commun* 2008; **368**: 930–6.
36 Vander Borght S, Komuta M, Libbrecht L *et al.* Expression of multidrug resistance-associated protein 1 in hepatocellular carcinoma is associated with a more aggressive tumour phenotype and may reflect a progenitor cell origin. *Liver Int* 2008; **28**: 1370–80.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. ATP-binding cassette transporter B1 (*ABCB1*) expression in hepatocellular carcinoma (HCC) cell lines and clinical samples. (a) Quantitative real-time PCR and Western blot of *ABCB1* in HCC cell lines. Expression of *ABCB1* was significantly higher in KIM-1 cells compared with the other cell lines. (b) *ABCB1* mRNA expression levels in HCC clinical cases. The relative mRNA expression levels in tumor tissues (black column, T) and corresponding non-cancerous, background liver tissues (gray column, N) (left panel). High levels of *ABCB1* expression were observed in well differentiated HCC. The average expression levels of *ABCB1* were higher in tumor tissues than in the non-cancerous background liver tissues (2.30 *vs* 1.23, $P = 0.21$) (right panel). (c) Immunostaining of ABCB1 in well differentiated HCC. An irregular and thicker form of canalicular pattern with cytoplasmic staining was observed in the tumor region compared with the non-cancerous background region (magnification, ×100). Black arrows outline the border between the non-cancerous background region (N) and the tumor region (T). (d) ABCB1 expression in moderately differentiated HCC (magnification, ×200). Only an irregular canalicular pattern was observed (a, H&E stain; b, corresponding ABCB1 staining).

doi: 10.1111/j.1349-7006.2009.01431.x