BORIS, Brother of the Regulator of Imprinted Sites, Is Aberrantly Expressed in Hepatocellular Carcinoma

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Background: The brother of the regulator of imprinted sites (*BORIS*) is a novel member of the cancer testis antigen gene family, which are normally expressed only in spermatocytes, but abnormally activated in different malignancies. **Aim:** The aim of this study was to explore the expression of *BORIS* in hepatocellular carcinoma (HCC) and its correlation with the clinicopathologic features and prognosis of HCC. **Methods:** We investigated *BORIS* expression in HCC cell lines and 105 primary HCC clinical surgical specimens using real-time polymerase chain reaction and Western blot analysis. We further examined the correlation of *BORIS* with a liver stem cell marker (CD90) in HCC tissues by histochemical double staining. The correlation of *BORIS* with clinicopathologic features and prognosis of HCC was analyzed using patient data. **Results:** The expression of *BORIS* was found in SMMC-7721, BEL-7402, and Huh-7, but not in hep-G2 cells. The expression rate of *BORIS* was significantly higher in the HCC tissues than in the adjacent noncancerous tissues ($p=0.000$). *BORIS* expression was correlated with the tumor size ($p=0.000$), CD90 expression ($p=0.000$), and satellite nodule ($p=0.000$). Kaplan–Meier survival curves showed that patients with positive expression of *BORIS* had lower overall survival rate ($p=0.003$). **Conclusions:** Our data indicate that *BORIS* may be an auxiliary diagnosis index and a novel favorable prognostic indicator of HCC.

Background

HEPATOCELLULAR CARCINOMA (HCC) is a common malignant tumor especially in East Asia, resulting in more than 250,000 deaths each year in China (Kensler *et al.*, 2003; Farazi and DePinho, 2006; Jemal *et al.*, 2008). In spite of enormous efforts to improve clinical treatment, the overall survival of patients with HCC remains unsatisfactory because of high incidence of reoccurrence and metastasis. Recent research efforts on stem cells have shed light on new therapeutic approaches for the eradication of cancer stem cells (CSCs) in HCC (Yang *et al.*, 2008; Zhang *et al.*, 2008; Mishra *et al.*, 2009). There is a crucial early role for epigenetic alterations in cancer in addition to their ability to substitute for genetic variation later in tumor progression (Feinberg *et al.*, 2006). Numerous recent findings in the molecular biology of cancer suggest that the neoplastic phenotype arises and is maintained through genome-wide modifications associated with epigenetic changes (Lyko and Brown, 2005; El-Osta, 2006). Brother of the regulator of imprinted sites (*BORIS*) is a unique epigenetically acting, tumor-promoting, transcription factor expressed in different types of human and mouse cancer cells that have been recently described (Klenova *et al.*, 2002).

The *BORIS* gene was first described as a DNA-binding protein that shares 11 zinc-finger (ZF) domains with CCCTC-binding factor (CTCF) (Filippova *et al.*, 1996; Qi *et al.*, 2003), but differs from this molecule at the N- and C-termini (Loukinov *et al.*, 2002). Lobanekov *et al.* originally identified the CTCF as a transcription factor regulating *cmyc* expression (Lobanekov *et al.*, 1990; Filippova *et al.*, 1996), which was subsequently found to act as a tumor suppressor (Filippova *et al.*, 1998; Rasko *et al.*, 2001; Filippova *et al.*, 2002). *BORIS* belongs to the same protein family as CTCF and has gained increasing attention due to its decreasing methylation of DNA. *BORIS* protein is absent in nonmalignant male tissues with the exception of testis, and is totally absent in females. However, *BORIS* transcripts were detected in more than half of the cancer cell lines (Klenova *et al.*, 2002). Several collaborating laboratories have initiated studies of *BORIS* expression in a variety of primary cancers (Hong *et al.*, 2005; Vatolin *et al.*, 2005). As yet, there have been no reports about the expression of *BORIS* in HCC.

Based on the above discoveries, in the present study, we investigated *BORIS* expression in a total of 105 tissue samples of HCC and several liver cancer cell lines by immunohistochemical, polymerase chain reaction (PCR), and Western blot

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analysis, and demonstrated that BORIS expression was a reliable indicator for disease diagnosis and its poor prognosis. The expression of CSC marker CD90 is related to the expression of BORIS in HCC tissue.

Materials and Methods

Ethics

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved by the medical ethics committee of the West China hospital, Sichuan University. All patients ($n=141$) involved in the study voluntarily offered written informed consent when approached by research personnel.

Tissue samples

HCC tissue samples ($n=105$) were obtained from patients who underwent surgical treatment without prior radiotherapy or chemotherapy treatment at the West China hospital between 2005 and 2006 (Chengdu, China). Normal liver samples ($n=20$) and cirrhosis samples ($n=16$) were collected from individuals without cancer and were used as controls. Clinical information on gender, age, serum hepatitis B surface antigen (HBsAg), serum alpha fetoprotein (AFP), cirrhosis, satellite nodule, and tumor capsule of patients were received from the West China Hospital. All samples were obtained with previous written consent. The histological cell types were assigned according to the criteria of the WHO classification.

Cell lines and culture

Different human liver cancer cell lines, Hep-G2, and Huh-7 were obtained from the American Type Culture Collection (ATCC). BEL-7402 and SMMC-7721 cell lines were obtained from the Committee of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in the DMEM or RPMI-1640 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C with 5% CO₂.

Immunohistochemical and histochemical double staining

The embedded tissues were cut into 4- μ m-thick sections for histological studies and stained using hematoxylin-eosin. BORIS protein was detected with a specific rabbit anti-human polyclonal antibody (Abcam). Paraffin-embedded tumor sections were boiled in a cooker for 4 min in citrate buffer. Then, slides were treated with 3% hydrogen peroxide 20–30 min before blocking with 3% bovine serum albumin. The tumor sections were incubated with the primary antibody (dilution, 1:50) overnight at 4°C. For negative control, the primary antibody incubation was replaced with 3% blocking solution. Sections were washed in PBS three times and incubated with goat-anti-rabbit IgG (Jackson ImmunoResearch) for 1 h at room temperature. After further washing, the samples were covered with a Vectashield mounting medium (Vector Laboratories) and stored at -20°C. Negative control slides were probed with normal goat serum under the same experimental conditions.

To determine the relationship between the expression of BORIS and CSC marker CD90, we performed histochemical double staining for BORIS (Abcam) and CD90 (Dako) on the same sections using Envision Doublestain System (Dako).

Deparaffinization and antigen retrieval were performed as described above. After quenching the endogenous peroxidase activity with a peroxidase-blocking reagent (Dako), tissue sections were incubated with mouse monoclonal anti-CD90 antibodies for 1 h at room temperature, followed by rinsing with a washing buffer. Then, the tissue sections were subjected to secondary staining for BORIS with the sequential steps of quenching, incubation with anti-BORIS antibodies, incubation with anti-goat IgG antibodies, and detection of the phosphatase activity in accordance with the manufacturer's instructions (Envision Doublestain System; Dako).

Immunofluorescence detection of BORIS and CD90 derived from frozen liver cancer tissue sections were performed using Alexa fluorescent dye-conjugated antibodies (Invitrogen). Fluorescent images were acquired at 2-mm Z-axis intervals using a confocal microscope (Leica TCS SP5 II).

Immunohistochemical staining and assessment of BORIS levels in HCC and paracancer tissues

Immunohistochemical analysis was done by staining normal ($n=20$), cirrhosis ($n=16$), paracancer tissues ($n=34$), and HCC ($n=58$) using the Vecta stain Elite ABC standard kit (Vector Laboratories) as suggested by the manufacturer. Immunohistochemical staining was evaluated by using the immunoreactivity score (IRS) as previously described (Gion *et al.*, 2001). In brief, the percentage of BORIS-positive cells was divided into four categories (<10%, 11%–50%, 51%–80%, and >80%, with corresponding scores of 1, 2, 3, and 4, respectively), whereas the staining intensity was given a value between 0 (no detectable immunostaining) and 3 (strong immunostaining). The IRS (0–12) was then calculated by multiplying the score values. Scoring was done in a blinded fashion by two independent scorers, with each slide read twice.

Reverse transcription-PCR

To determine the BORIS transcription in human HCC cell lines, total RNA was extracted from cells with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA was reverse-transcribed with 25 units of MMLV reverse transcriptase (Promega) and oligo-dT as primer. The resulting cDNAs were amplified with the following oligonucleotide sequences: BORIS, 5-CCCATTGTGCCACCATCA-3 (forward), BORIS, 5-AGCATGCAAGTTGCGCA-3 (reverse). Actin transcription was used as an invariant endogenous control. The primer sequences were as follows: actin F: 5-TCATCACCATTGGCAATGAG-3 (forward), actin R: 5-CACTGTGTTGGCGTACAGGT-3 (reverse).

Western blot analysis

For Western blot analysis, lysates from cells were prepared according to Klenova *et al.* (1993) with modifications. Lysates from testis tissues were prepared as follows; tissues were homogenized in the lysis buffer at the ratio 50 mm³ tissue/100 mL of buffer. The homogenate was kept on ice for 30 min, filtered through gauze, and centrifuged for 15 min, at 4°C and 13,000 rpm. Samples containing high concentration of lipids were additionally precipitated with acetone. The supernatant was discarded, the pellet dried at room temperature, and resuspended in sodium dodecyl sulfate loading/lysis buffer. Western blot assay was conducted as described previously,

and membranes were probed with anti-BORIS antibody (Abcam; dilution 1:100) or anti- α -tubulin antibody (Sigma; dilution 1:200). Detection was performed using enhanced chemiluminescence reagent (Amersham Biosciences, now GE Healthcare) according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation or median with range. SPSS program for Windows (version 15.0; SPSS, Inc.) was used for statistical analysis. Comparisons of BORIS tumor expression with clinical and pathologic features were evaluated by using chi-square tests or two-tailed Fisher's exact test. Overall survival analyses were estimated by using the Kaplan-Meier method. The Student's *t*-test was used to detect differences between groups. All tests performed were two-tailed. A difference was considered significant if $p < 0.05$.

Results

BORIS expression in HCC cell lines

BORIS expression was investigated in different human liver cell lines by means of reverse transcription-polymerase chain reaction (RT-PCR) and Western blot method. As shown in (Fig. 1), we found that normal testis (positive control), BEL-7402, SMMC-7721, and Huh-7 cells constitutively express BORIS mRNA and protein. However, BORIS could not be detected in Hep-G2 cells or normal liver tissue.

BORIS expression in HCC clinical samples

To screen the state of BORIS expression *in vivo*, we investigated BORIS protein expression by immunohistochemistry, in a total of 105 primary HCC, 20 normal liver, and 16 cirrhosis tissues. Fifty-eight cases of BORIS expression (55.2%) localized in the cytoplasm and the nucleolus were found in HCC surgical resection samples, whereas the remaining 47 cases (44.8%) displayed almost undetectable BORIS expression. BORIS expression was not detected in cirrhosis or

TABLE 1. CORRELATION BETWEEN BORIS PROTEIN EXPRESSION AND CLINICOPATHOLOGICAL FEATURES OF HEPATOCELLULAR CARCINOMA

Features n = 105	BORIS negative n = 47	BORIS positive n = 58	p
Age (years)			0.646
< 50 n = 51	24	27	
≥ 50 n = 54	23	31	
Gender			0.976
Female, n = 18	8	10	
Male, n = 87	39	48	
Serum HBsAg			0.689
Negative, n = 15	6	9	
Positive, n = 90	41	49	
CD90			0.000
Negative, n = 35	26	9	
Positive, n = 70	21	49	
Serum AFP			0.059
Negative, n = 34	20	14	
Positive, n = 71	27	44	
Cirrhosis			0.203
No, n = 17	10	7	
Yes, n = 88	37	51	
Tumor size (cm)			0.000
< 5 n = 42	26	5	
≥ 5 n = 63	21	53	
Satellite nodule			0.000
No, n = 61	37	24	
Yes, n = 44	10	34	
Tumor capsule			0.070
Complete n = 50	27	23	
No/incomplete n = 55	20	35	

p-Value < 0.05 is bolded.

BORIS, brother of the regulator of imprinted sites; HBsAg, hepatitis B surface antigen; AFP, alpha fetoprotein.

FIG. 1. Detection of the brother of the regulator of imprinted sites (BORIS) mRNA and protein in four human liver cancer cell lines by reverse transcription-polymerase chain reaction (A) and Western blot (B). Expression of BORIS mRNA and protein in SMMC-7721 (lane 2), BEL-7402 (lane 3), and Huh-7 (lane 4), but not in hep-G2 (lane 5). Human testicular tissues (lane 1) were used as positive control; normal liver tissue (lane 6) was used as nonmalignant negative control.

FIG. 2. The immunoreactivity score (IRS) values for 34 BORIS-positive samples (2.43 ± 0.51) in paracancer tissues were lower than 58 BORIS-positive samples (5.22 ± 0.53) in hepatocellular carcinoma (HCC) tissues ($p = 0.01$).

normal liver tissues. Analyses of the relationship between BORIS expression and various clinicopathological parameters are listed in Table 1. Expression of BORIS was significantly correlated with tumor size ($p=0.000$) and satellite nodule ($p=0.000$), but not with age, gender, serum HBSAg, cirrhosis, or tumor capsule (Table 1). On the other hand, we calculated that the IRS values for 34 BORIS-positive samples (2.43 ± 0.51) in adjacent noncancerous tissues were lower than 58 BORIS-positive samples (5.22 ± 0.53) in HCC tissues ($p=0.01$) (Fig. 2).

BORIS present in HCC associated with CD90 expression

We performed histochemical double staining for BORIS and CD90 in 105 primary HCC specimens. CD90 expression was observed in 70 cases (66.7%), localized in the plasma

membrane and the cytoplasm, and BORIS expression was observed in 58 cases (55.2%) localized in the nucleolus and the cytoplasm (Fig. 3). Forty-nine of the 105 cases (46.7%) showed double staining, and the expression of BORIS was correlated with CD90 ($p=0.000$, Table 1). We also found BORIS expression in HCC tissue associated with the expression of CD90 using multiple fluorescence staining technique (Fig. 3).

Prognostic significance of BORIS expression

To elucidate the prognostic role of BORIS in HCC, overall survival rates were estimated by Kaplan-Meier survival curves (Fig. 4). The expression of BORIS was associated with poor prognosis of patients with HCC ($p=0.003$, log-rank test). Overall survival rate and 5-year survival rate of patients with BORIS expression were lower than those that did not express

FIG. 3. Detection of BORIS and CD90 protein in normal human liver (A, B), HCC (D–J), and normal testicular tissues (C) by immunohistochemistry (IHC) and immunofluorescence. Different residual fluorescence is observed in the same hepatoma carcinoma cell (arrow).

FIG. 4. Kaplan-Meier survival curves for BORIS-positive group versus BORIS-negative group in 105 patients with HCC showed a highly significant separation ($p=0.003$, log-rank test).

BORIS (46.8% vs. 20.7% [overall survival rate] and 38.3% vs. 15.6% [5-year survival rate], respectively, $p<0.05$, Fig. 4). On the other hand, the presence of satellite nodule tends to be higher in the BORIS-positive group (34/58 cases, 58.6%) when compared to the BORIS-negative group (10/47 cases, 21.3%, $p=0.000$).

Discussion

A number of recent studies have found that epigenetic changes play an important role in the progression of cancer. In epithelial ovarian cancer, the BORIS/CTCF expression ratio is also associated with a higher stage of tumor and decreased rate of survival (Woloszynska-Read *et al.*, 2011). It has been suggested that the expression of BORIS displaces CTCF in the genome and leads to proliferation of cancer cells. Ghochikyan *et al.* (2007) demonstrated that BORIS protein was always expressed in pancreatic carcinoma, but there was no significant expression in normal tissues. D'Arcy *et al.* (2008) showed that normal glands of the breast were negative for BORIS, whereas all types of breast carcinoma expressed this protein with very high incidence. BORIS has also been shown to be expressed in many other cancers, such as head and neck squamous cell carcinoma, prostate cancer, endometrial cancer, colon carcinoma, and melanoma (Vatolin *et al.*, 2005; Risinger *et al.*, 2007; Kholmanskikh *et al.*, 2008; Cuffel *et al.*, 2011; Makovski *et al.*, 2012). The results of these studies, including ours, are similar in that they show BORIS expression to be more intense in the carcinoma nest than in normal or benign lesions. However, BORIS, as a regulatory protein of DNA demethylation, has not been reported to play a role in liver tumors. This is the first study showing the expression of BORIS in HCC and its role in disease prognosis. We studied protein levels of BORIS in a large number of cases of HCC and compared them to noncancerous tissues and observed that BORIS was expressed in 55.2% of the HCC cases. Our study also showed that the expression of BORIS in HCC is significantly related to the CSC marker CD90 in HCC tissues.

In this study, we also investigated BORIS mRNA and protein expression in HCC cell lines or primary HCC tissues, and evaluated the correlation of BORIS expression to clinical outcome of HCC patients, and we found that BORIS expression in HCC tissue was significantly higher than that in normal tissue, which suggests that BORIS may be involved in the genesis and development of liver cancer, and it could be used as an indicator of liver cancer. These results combine with the findings that BORIS was expressed in different human liver carcinoma cell lines suggest that BORIS is likely to be associated with malignant liver cells.

Our results demonstrated that BORIS was very frequently expressed in HCC when compared to noncancerous tissue. We obtained more informative results probably because of the larger number of cases examined by immunohistochemistry analysis. We found that the expression of BORIS is also correlated with high proliferation activity and poor overall survival rate. Our study also revealed that the expression of BORIS in HCC is significantly related to the CSC marker CD90 in HCC tissue. The research efforts on BORIS and CD90 have shed light on new directions for the eradication of CSCs. The expression of BORIS in CSCs provides evidence that BORIS could be a therapeutic target in treating liver cancer. Kaplan-Meier survival analysis showed that the expression of BORIS significantly correlated with a shorter survival time of HCC patients ($p=0.003$). Moreover, the expression of BORIS also showed a tendency to correlate with high tumor metastasis rate in the liver (Table 1). These results have revealed that the expression of BORIS strongly reflects the biological aggressiveness of HCC and plays an important role in its progression. These results also suggest that HCC patients with BORIS expression could probably have a relapse with poor prognosis. BORIS could be used as a prognostic indicator for the HCC, and inhibiting BORIS expression could prove to be an adjuvant therapeutic approach in treating HCC.

In summary, our study has shown for the first time that BORIS is expressed in HCC tissues, and its expression significantly correlates with poor prognosis. Furthermore, we demonstrated that the expression of CSC marker CD90 is related to the expression of BORIS in HCC tissue and HCC cell lines. The expression of BORIS in CSCs could provide experimental basis for relevant studies.

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Authors' Contributions

B.L., Y.Q., and K.F.C. designed and directed the study. Y.Q., B.L., K.F.C., W.Q.H., B.H., and Y.G.W. performed the majority of the experiments and composition of the manuscript. Y.G., W.Q.H., and Y.G.W. were responsible for data collection and analysis, and reviewing and scoring the degree of immunostaining of sections. All authors have read and approved the final manuscript.

Author Disclosure Statement

The authors declare that they have no competing interests.

References

- Cuffel C, Rivals J-P, Zaugg Y, *et al.* (2011) Pattern and clinical significance of cancer-testis gene expression in head and neck squamous cell carcinoma. *Int J Cancer* 128:2625–2634.
- D'Arcy V, Pore N, Docquier F, *et al.* (2008) BORIS, a paralogue of the transcription factor, CTCF, is aberrantly expressed in breast tumours. *Br J Cancer* 98:571–579.
- El-Osta A (2006) Mechanisms of abnormal gene expression in tumor cells. *EXS* No 96:351–361.
- Farazi PA, DePinho RA (2006) Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer* 6:674–687.
- Feinberg AP, Ohlsson R, Henikoff S (2006) The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 7:21–33.
- Filippova GN, Fagerlie S, Klenova EM, *et al.* (1996) An exceptionally conserved transcriptional repressor, CTCF, employs different combinations of zinc fingers to bind diverged promoter sequences of avian and mammalian c-myc oncogenes. *Mol Cell Biol* 16:2802–2813.
- Filippova GN, Lindblom A, Meincke LJ, *et al.* (1998) A widely expressed transcription factor with multiple DNA sequence specificity, CTCF, is localized at chromosome segment 16q22.1 within one of the smallest regions of overlap for common deletions in breast and prostate cancers. *Genes Chromosomes Cancer* 22:26–36.
- Filippova GN, Qi CF, Ulmer JE, *et al.* (2002) Tumor-associated zinc finger mutations in the CTCF transcription factor selectively alter its DNA-binding specificity. *Cancer Res* 62:48–52.
- Ghochikyan A, Mkrtichyan M, Loukinov D, *et al.* (2007) Elicitation of T cell responses to histologically unrelated tumors by immunization with the novel cancer-testis antigen, brother of the regulator of imprinted sites. *J Immunol* 178:566–573.
- Gion M, Mione R, Leon AE, *et al.* (2001) A valuable marker for breast cancer management. A confirmatory multicentric study on 603 cases. *Eur J Cancer* 37:355–363.
- Hong JA, Kang Y, Abdullaev Z, *et al.* (2005) Reciprocal binding of CTCF and BORIS to the NY-ESO-1 promoter coincides with derepression of this cancer-testis gene in lung cancer cells. *Cancer Res* 65:7763–7774.
- Jemal A, Siegel R, Ward E, *et al.* (2008) Cancer statistics. *CA Cancer J Clin* 58:71–96.
- Kensler TW, Qian GS, Chen JG, *et al.* (2003) Translational strategies for cancer prevention in liver. *Nat Rev Cancer* 3:321–329.
- Kholmanskikh O, Loriot A, Brasseur F, *et al.* (2008) Expression of BORIS in melanoma: lack of association with MAGE-A1 activation. *Int J Cancer* 122:777–784.
- Klenova EM, Morse HC 3rd, Ohlsson R, *et al.* (2002) The novel BORIS+CTCF gene family is uniquely involved in the epigenetics of normal biology and cancer. *Semin Cancer Biol* 12:399–414.
- Klenova EM, Nicolas RH, Paterson HF, *et al.* (1993) CTCF, a conserved nuclear factor required for optimal transcriptional activity of the chicken c-myc gene, is an 11-Zn-finger protein differentially expressed in multiple forms. *Mol Cell Biol* 13:7612–7624.
- Lobanenkov VV, Nicolas RH, Adler VV, *et al.* (1990) A novel sequence-specific DNA binding protein which interacts with three regularly spaced direct repeats of the CCCTC-motif in the 5'-flanking sequence of the chicken c-myc gene. *Oncogene* 5:1743–1753.
- Loukinov DI, Pugacheva E, Vatolin S, *et al.* (2002) BORIS, a novel male germ-line-specific protein associated with epigenetic reprogramming events, shares the same 11-zinc-finger domain with CTCF, the insulator protein involved in reading imprinting marks in the soma. *Proc Natl Acad Sci U S A* 99:6806–6811.
- Lyko F, Brown R (2005) DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. *Natl Cancer Inst* 97:1498–1506.
- Makovski A, Yaffe E, Shpungin S, *et al.* (2012) Intronic promoter drives the BORIS-regulated expression of FerT in colon carcinoma cells. *J Biol Chem* 287:6100–6112.
- Mishra L, Banker T, Murray J, *et al.* (2009) Liver stem cells and hepatocellular carcinoma. *Hepatology* 49:318–329.
- Qi CF, Martensson A, Mattioli R, *et al.* (2003) CTCF functions as a critical regulator of cell-cycle arrest and death after ligation of the B cell receptor on immature B cells. *Proc Natl Acad Sci U S A* 100:633–638.
- Rasko JE, Klenova EM, Leon J, *et al.* (2001) Cell growth inhibition by the multifunctional multivalent zinc-finger factor CTCF. *Cancer Res* 61:6002–6007.
- Risinger JL, Chandramouli GVR, Maxwell GL, *et al.* (2007) Global expression analysis of cancer/testis genes in uterine cancers reveals a high incidence of BORIS expression. *Clin Cancer Res* 13:1713–1719.
- Vatolin S, Abdullaev Z, Pack SD, *et al.* (2005) Conditional expression of the CTCF-paralogous transcriptional factor BORIS in normal cells results in demethylation and derepression of MAGE-A1 and reactivation of other cancer-testis genes. *Cancer Res* 65:7751–7762.
- Woloszynska-Read A, Zhang W, Yu J, *et al.* (2011) Coordinated cancer germline antigen promoter and global DNA hypomethylation in ovarian cancer: association with BORIS/CTCF expression ratio and advanced stage. *Clin Cancer Res* 17:2170–2180.
- Yang ZF, Ho DW, Ng MN, *et al.* (2008) Significance of CD90+ cancer stem cells in human liver cancer. *Cancer Cell* 13:153–166.
- Zhang F, Chen XP, Zhang W, *et al.* (2008) Combined hepatocellular cholangiocarcinoma originating from hepatic progenitor cells: immunohistochemical and double-fluorescence immunostaining evidence. *Histopathology* 52:224–232.

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