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Review

BORIS in human cancers – A review

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ABSTRACT

Brother of the regulator of the imprinted site (BORIS) or CTCFL is an 11 zinc finger (ZF) protein, which is considered to be a new oncogene. It is a paralogue of CCCTC-binding factor (CTCF), generated by a duplication event. BORIS is highly expressed in primary spermatocytes, although it is silenced at later stages of spermatogenesis. BORIS has either not been found in normal human tissues or cells or has been detected at very low levels. The expression of the BORIS gene is predominantly controlled by DNA-methylation, while its activation requires the demethylation of its promoter. Re-expression of BORIS in cancers is due to the hypomethylation of its promoter. High expression of BORIS protein and RNA correlates with the tumour size and grade in cancer patients. High percentages of BORIS transcripts were detected in breast, endometrial, prostatic and colon cancer patients. Lower percentages of BORIS were found in patients with melanoma and cancers of the head and neck. The expression of BORIS varied from low to high in lung, colon and ovarian cancer, melanoma and leukaemic cell lines. Lower expressions of BORIS were found in head and neck, breast, kidney, bladder, testicular and prostate carcinoma cell lines. An inhibitor of DNA methylation, 5-aza-2'-deoxy-cytidine (5-azadC), and histone deacetylase inhibitors induced or enhanced the expression of BORIS in various carcinoma cell lines. The silencing of BORIS induced apoptosis in tumorous cell lines. BORIS antitumor vaccines have been tested in mice with several cancers, based on the deletion of the DNA-binding ZF-region of the BORIS.

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1. About BORIS generally

Brother of the regulator of the imprinted site (BORIS) or CCCTC-binding factor-like protein (CTCFL) is an 11-zinc finger (ZF) protein, described as a transcriptional regulator. BORIS is a paralogue of CCCTC-binding factor (CTCF), a transcriptional repressor.^{1–3} BORIS acts as an antagonist to CTCF in normal and in cancer cells by binding to the same target sequences.^{2,4}

1.1. The BORIS gene

Human BORIS DNA consists of 27,931 bp, 11 exons and is located at chromosome 20q13.31 (Accession NC_000020). For

the human BORIS gene 566 single nucleotide polymorphisms (SNPs) have been reported, presently (<http://www.ncbi.nlm.nih.gov/snp>). The BORIS gene arose as a duplication event in early mammals.^{1,2} The similarity between human and other vertebrates' BORIS orthologues within the coding sequences for the 11-ZF domain is 80.4%, while for the N- and C-terminal domains it is less than 35%.⁵ BORIS human mRNA consists of 11 exons, 3493 bp, and the coding sequence is within exons 2–11, CDS 83–2074 bp (NCBI NM_080618, <http://www.ncbi.nlm.nih.gov>). BORIS transcripts have been detected in the testis of cattle, wallaby, platypus and the dragon, in the liver and the kidney of the platypus and in the brain, kidney and ovary of the bearded dragon.⁵ Transcription of BORIS is regulated by

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three alternative promoters A, B and C.⁶ Promoter A, located at –2071 to –1276 bp has high transcriptional activity.⁶ Promoter B is located at –1106 to –996 bp, while promoter C is at –821 to –622 bp upstream of the start site.⁶ All three promoters contain putative binding sites for Sp1, AP-2 transcription factors.⁶ Promoter A contains putative binding sites for CREB, promoter B for NF- κ B, N-myc, and promoter C for WT1 and EKLf transcription factors.⁶ The transcriptional start sites are at –1447, –899 and –658 bp upstream of the first ATG.⁶ Recently, 23 splice variants of the BORIS gene have been reported, with various numbers of ZF domains.⁷ The ZF domains of the BORIS represent specific DNA-binding sites, for example to the IGF2/H19-ICR region.^{7,8}

1.2. The BORIS protein

The human BORIS protein is 75.7 kDa, consists of 663 amino acids (a.a.). Its sequence status is complete and there is evidence at protein level (Q8NI51, www.uniprot.org). The N-terminal end is 256 a.a. long, the 11-ZF regions are located at 257–568 a.a. and the C-terminal end at 569–663 a.a. (www.uniprot.org). The ZF regions are 23 or 24 a.a. long (www.uniprot.org). The BORIS protein is homologous to the CTCF in the central 11-ZF DNA-binding domain, but not in the N- and C-terminal ends.^{1,2} The C-terminal end of the BORIS protein is predominantly unordered (lacks 3D structure), while computer analysis predicts that the N-terminal end may be disordered.⁹ The ZF-regions are strongly conserved between paralogues, while the N-terminal ends are significantly diverged.^{1,5} Both the N-terminal end and the full length BORIS protein may interact with PMRT7-DNA methylase and histones H1, H3, H2A,¹⁰ with the promoter of cerebroside sulfotransferase (CST).⁷ Furthermore, 16 proteins interacting with the N-terminal end of the BORIS have been classified as: (a) testis specific, (b) helicase associated, (c) transcription factors and (d) chromatin-associated proteins.⁸ The C-terminal end of the BORIS protein may interact with histones.¹⁰ The BORIS 23 splice variants encode 17 BORIS different proteins, isoforms, named A1 to A6, B0 to B7 and C1 to C9, according to promoter usage.⁷

The mouse BORIS protein or Ctcfl consists of 636 a.a. It is a 73.1 kDa protein, its sequence status is complete and there is evidence at protein level (Accession A2APF3 www.uniprot.org). Presently, two mouse isoforms are listed (www.uniprot.org).

1.3. BORIS in spermatogenesis, embryogenesis and normal tissues

BORIS appears to be restricted to the male germ cells. BORIS has been detected at early stages of spermatogenesis in spermatocytes and spermatogonia.^{1,2} At later stages of spermatogenesis, in spermatids and spermatozoa BORIS is silenced, while CTCF is re-activated.^{1,2} Transient expression of BORIS in male germ cells coincides with a decrease in CTCF expression and erasure of the global methylation pattern.^{1,2,11} In human testicular dissected tissue from the same male, the localisation of BORIS was nuclear in spermatocytes, while in spermatogonia it was cytoplasmic.¹² X-ray radiation induced microRNA, miR-709, which decreased the level of BORIS in mouse testis.¹³ In contrast, in testis of control animals, miR-

709 was low, while BORIS was high.¹³ miR-709 targets BORIS and represents a protective mechanism that prevents aberrant erasure of DNA methylation in mouse testis after radiation.¹³ Binding of BORIS to the promoters of SPANX-N and SPANX-A/D genes resulted in programmed displacement of CTCF and activation of these genes.¹⁴ SPANX-A/D genes encode nuclear envelope protein in early spermatids, while SPANX-N gene regulates the development of post meiotic spermatids in ejaculated spermatozoa.¹⁴ BORIS knock-out mice have defective spermatogenesis, small testes and increased cell death. This defect was evident at postnatal day 21, with a delayed production of haploid cells.¹⁵

Besides male germ cells, BORIS transcripts were detected in human oocytes and in 4 cells embryos at early stages of preimplantation development, while BORIS expression decreased in the early cleavage stage of the embryos.¹⁶

BORIS transcripts were not detected in normal peripheral blood lymphocytes, thymus,¹ skin,¹⁷ bone marrow,¹⁷ heart,¹⁷ epithelial bronchial cultures,¹⁸ adrenal,¹⁷ spleen,¹ intestine,¹ colon,¹ bladder,¹⁷ prostate,^{1,17} ovary,^{1,17} uterine¹⁷ and endometrial tissues.¹⁹ A very low level of BORIS transcripts was evident in normal human brain, lung, kidney, liver, spleen, stomach, colon, thymus and placenta.¹⁷ There was a very low level of BORIS protein in peripheral blood lymphocytes (7 of 36 donors),²⁰ and it was not present in primary breast cultures.²¹

1.4. Epigenetic control and BORIS functions

Epigenetic control includes conformational DNA modifications that do not change the sequence of the bases in DNA, methylation of the CpG islands in their promoters and histone modifications.²² Demethylation of BORIS promoter results in maximal activation of BORIS.¹² The BORIS promoter is hypomethylated in normal testis, while completely unmethylated in sperm.¹² By contrast, the BORIS promoter is strongly methylated in normal blood, prostate and bladder cells.¹²

Modifications of histones (H) may also affect BORIS. Methylation of lysine (K) residues of histone H4 created a permissive H3K4/dimethyl and H3K9/dimethyl chromatin status.²³ This enabled the binding of BORIS to the promoter of BAG-1 and an increase of BAG-1 expression in DNA methyltransferases (DNMT1 and DNMT3B) overexpressing cells.²³ BORIS stimulated the activity of protein methyltransferase 7 (PRMT7) which catalysed the methylation of the arginine, R, residues of histones H2A and H4.¹⁰ Coexpression of BORIS and PRMT7 was crucial for ICR methylation.¹⁰ BORIS binds preferentially to the paternal differentially methylated H19-ICR region, whereas CTCF binds to the unmethylated maternal allele and directs BORIS to the paternal allele.²⁴

Moreover, BORIS binds by its ZF domain to the upstream binding factor (UBF), a nuclear protein, a transactivator of RNA polymerase I, and plays a role in the maintenance of the chromatin structure.²⁵

2. BORIS in cancer

BORIS is considered to be a new oncogene, which is reactivated in cancer.² BORIS may derepress other oncogenes such

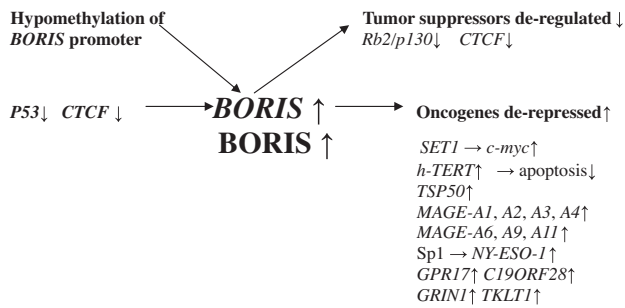


Fig. 1 – BORIS regulates tumor-suppressors and oncogenes in cancers.

as *c-myc* by inhibiting the activity of CTCF.^{2,4} CTCF, a transcriptional factor, suppressed expression of *c-myc* oncogene.^{2,26} BORIS also induced the expression of another oncogene TSP50.²⁷ Furthermore, BORIS may deregulate tumour suppressor genes such as CTCF^{2,4} and *Rb2/p130*.²⁸ *Rb2/p130* is induced by CTCF and regulates chromatin modifications in lung cancer²⁸ (Fig. 1).

BORIS is an inhibitor of apoptosis in cancer cells by activating *hTERT* transcription.²⁹ *hTERT* is essential for telomerase activity and is an endogenous inhibitor of apoptosis.³⁰ BORIS binds to the first exon of the *hTERT* gene and permits its transcription by counteracting the repressive effect of CTCF³⁰ (Fig. 1).

BORIS is decreased while tumour suppressor genes such as CTCF and *p53* are increased.^{2,6} Silencing of BORIS by siRNA, which targets the part of the gene for ZF region 10, decreased cell viability and induced apoptosis by activating caspase 3/7 in a breast cancer cell line.³¹

In 84% of the tested tumorous cell lines either promoters A and C or promoters B and C of the BORIS gene were used.⁶

2.1. Epigenetic control and BORIS functions in cancer

Overexpression of BORIS correlated with the hypomethylation of its promoter in primary testicular¹² prostate¹² and epithelial ovarian tissues of cancer patients.³² Partial demethylation of BORIS promoter was detected in ovarian,⁶ colon⁶ and lung cancer³⁴ and in leukaemic cell lines.⁶ Overexpression of BORIS correlated with the hypomethylation of *LINE-1*, a global DNA hypomethylation marker in epithelial ovarian³³ and prostate cancer patients.¹²

The hypomethylator 5-aza-2'-deoxy-cytidine (5-azadC) induced the expression of BORIS in breast,³⁵ lung,³⁴ bladder,¹² testicular,¹² prostate,¹² ovarian³² and melanoma¹⁷ cancer cell lines. BORIS was induced by 5-azadC in cancer cell lines with very low or undetectable level of BORIS. Furthermore, activation of BORIS by 5-azadC correlated with the derepression of the proto-oncogene *NY-ESO-1* in lung cancer.^{18,34} *NY-ESO-1* is a germ-line restricted gene, upregulated in cancer.^{18,34} BORIS enhanced the translocation of Sp1 to the nucleus, physically interacting with Sp1, which mediated the derepression of *NY-ESO-1*.¹⁸ Sp1 is a transcription factor that binds to GC-TC-rich promoter elements via ZF domains³⁶ and is overexpressed in malignancy³⁷ (Fig. 1 and Table 1).

DNA methylation markers BAT3 (Bcl-associated gene) and SET1 coupled with BORIS modified H3K4 histone dimethylation in a colon carcinoma cell line.⁸ BAT3 binds to the BORIS promoter, recruits SET1A a direct BORIS interacting protein.⁸ SET1A is a H3K4 methyltransferase, while BAT3 is a protein recruitment factor.⁸

Table 1 – Known mechanisms of action of BORIS as a transcriptional regulator.

BORIS↑	BORIS – regulator of transcription	Type of cancer	References
Reactivation in cancer	Oncogene↑ MAGE-A10↑	HNSCC	[39]
Reactivation in cancer	PR promoter↑ PR↑ ER promoter↑ ER↑	Breast	[21]
Reactivation in cancer	BORIS binds to H19-DMR	Colon: HCT116	[24]
Reactivation in cancer	MAGE-A1↑ (not in all patients)	Melanoma	[17]
Induction by			
5aza-dC BORIS promoter hypomethylated	BORIS binding↑ to NY-ESO-1 promoter NY-ESO-1 gene↑	Lung: Calu-6↑↑ H460↑ A549↑↑↑ SKLC-6↑↑ H1184↑↑ H2087↑↑ H1299↑ H358↑↑ H1355↑ H228↑	[34]
5aza-dC BORIS promoter hypomethylated	BORIS recruits Sp1 BORIS binding↑ to NY-ESO-1 promoter NY-ESO-1 gene↑	Lung: Calu-6↑	[18]
Transfection	BORIS binding↑ to BAG-1 promoter BAG-1↑	Colon: HCT-116↑↑	[23]
Transfection and 5aza-dC	Oncogenes MAGEA3/6↑ MAGEA2↑ GPR17↑ MAGEA4↑ GRIN1↑ MAGEA11↑ H19↑ C19ORF28↑ TKL1↑	Oral keratinocytes: OKF6-Tert1	[38]
BORIS↓			
By siRNA OCM-8054↑	Caspase 3/7↑ apoptosis↑	Breast: MDA-MB-231	[31]
BORIS knock-down	BORIS binding↓ to BAG-1 promoter↓ BAG-1↓	Colon: HCT-116	[23]

5-azadC, 5-aza-2'-deoxy-cytidine, DNA hypomethylator.
PR, progesterone receptor; ER, oestrogen receptor.
DMR, differentially methylated region of the gene.
HNSCC, head and neck squamous cell carcinoma.

A histone deacetylase inhibitor, Depsipeptide FK228, induced the expression of BORIS in one lung carcinoma cell line.³⁴

2.2. BORIS in head and neck cancers

There are a few studies regarding the expression of BORIS in head and neck cancers. BORIS has been detected in primary head and neck squamous cell carcinoma (HNSCC)^{38,39} (Table 2). Moderate expression of BORIS was found in cancer cell lines originating from the central nervous system (CNS) such as glioblastomas⁶ and in neuroblastomas.⁴⁰ This was correlated with the methylation of the BORIS gene⁶ (Supplementary Table 3).

The expression of BORIS in these cancers was correlated to the upregulation of the proto-oncogenes MAGE-A3/6, MAGE-A4, MAGE-A11, GPR17 and C19ORF28.³⁸ No correlation was found between BORIS and the expression of other cancer-testis antigens (MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A10 and NY-ESO-1).³⁹

In these cancers promoters A and C of the BORIS gene were used.⁶

2.3. BORIS in breast cancer

There have been more studies regarding the expression of BORIS in breast cancers. BORIS protein has been detected in breast cancer patients in leukocytes isolated from their peripheral blood²⁰ and in tumorous tissues.²¹ BORIS protein level was correlated with the size of the tumours²¹ and with an increase of the BORIS mRNA level in primary tumours.^{20,21} By contrast, BORIS mRNA was not detected in eight breast cancer patients³⁵ (Table 2). In studies on patients, the expression of BORIS was not correlated to methylation.^{20,21,35} Most probably, the absence of BORIS in cancers, is due to high methylation. In breast cancer cell lines BORIS transcripts were detected at weak²¹ to moderate levels.^{6,40} Weak expression of BORIS was correlated to promoter methylation⁶ (Supplementary Table 3). The BORIS protein was found in the nucleus and cytoplasm of breast cancer cell lines.²¹ In breast cancer

cell lines, both promoters A and C of the BORIS gene were used.⁶

BORIS also activated promoters of the genes for progesterone and oestrogen receptors.²¹ A high level of BORIS was correlated with high levels of these receptors in breast cancer patients (Table 1).²¹ In breast cancer cell lines, both promoters A and C of the BORIS gene were used.⁶

The short, rare minisatellites BORIS-MS2 in the 5' upstream region of the gene, were associated with a high risk for breast cancer in nearly 800 young patients compared to over 400 benign cancers as controls.⁴¹ The BORIS-MS2 were not analysed in other cancers.

2.4. BORIS in lung cancer

The expression of BORIS was not tested in patients with lung cancers. BORIS transcripts were expressed in lung carcinoma cell lines at high^{6,18} to moderate levels.^{6,34} This was correlated to BORIS promoter methylation⁶ (Supplementary Table 3). Induction of BORIS by a hypomethylator was found in cell lines with low levels of BORIS^{18,34} (Supplementary Table 3). BORIS protein was detected both in the nucleus and in the cytoplasm of an untreated lung carcinoma cell line.³⁴

In these cell lines both promoters A and C of the BORIS gene were used.⁶

2.5. BORIS in kidney, bladder, prostatic and testicular cancer

BORIS was detected in prostatic patients in high percentage⁴⁰ (Table 2). The BORIS protein was found in the cytoplasm of primary prostatic tumours.¹² The expression of BORIS was not tested in kidney, bladder and testicular cancer patients. BORIS was weakly to moderately expressed in prostatic,¹² testicular,^{6,12} bladder¹² and kidney⁶ carcinoma cell lines (Supplementary Table 3). Weak expression of BORIS was correlated to the methylation of the BORIS promoter in kidney cancer^{6,12} (Supplementary Table 3). In a prostatic cancer cell line BORIS was weakly expressed in the nucleus^{6,12} however, after 5-aza-dC treatment it was detected around the nucleus.¹²

Table 2 – BORIS in cancer patients.

Cancer type	BORIS+ patients/total patients	Detection level (techniques)	References
HNSCC	9/52 (17%)	RNA (RT-PCR)	[39]
Breast	L 77/87; 41/58 12/19; 5/6 11/19; 6/6; 11/12	Protein (IHC) Protein (Western) RNA (RT-PCR)	[20,21] [20,21] [20,21,40]
Prostate	9/10	RNA (RT-PCR)	[40]
Endometrial	73/95	RNA (rt-RT-PCR)	[19]
Mixed mesodermal	24/31	RNA (rt-RT-PCR)	[19]
Colon	8/10	RNA (RT-PCR)	[40]
Melanoma			
Primary	4/25 (16%)	RNA (RT-PCR)	[17]
Metastatic	13/38 (34%)	RNA (RT-PCR)	[17]
HNSCC – head and neck squamous cell carcinoma. L – leukocytes. IHC – immunohistochemistry; rt – real time.			

The testicular teratocarcinoma cell line used mostly promoter A and less promoters B and C of the BORIS gene. The kidney cancer lines used both promoters A and C.⁶

2.6. BORIS in ovarian, uterine and cervical cancers

BORIS was studied by several investigators in patients with gynaecological cancers. BORIS was expressed in higher percentage in endometrial and lower in uterine mixed mesodermal cancer patients¹⁹ (Table 2). BORIS was highly expressed in tumours with a high grade.¹⁹ A high level of BORIS correlated with the expression of other CG genes such as MAGE-A9 (24 of 122 patients) and DSCR8 (16 of 122 patients) at the lower level.¹⁹

BORIS was moderately³² to highly expressed in ovarian cancer cell lines.⁶ It was not detected in a cervical adenocarcinoma cell line.²⁹ A high expression of BORIS was correlated with the unmethylation of the promoter.⁶ BORIS transduction did not alter global methylation in ovarian cancer cell lines and was not sufficient for other CG genes (MAGE-A1, NY-ESO-1, XAGE-1) expression.⁴² When overexpressed in ovarian cancer cell lines, BORIS protein was detected in the nucleus.⁴²

In ovarian cell lines, either promoters A and C or promoters B and C of the BORIS gene were used.⁶

2.7. BORIS in colon carcinomas

There are a few studies regarding BORIS in colon carcinomas. High percentage of BORIS transcripts were detected in colon cancer patients⁴⁰ (Table 2).

BORIS transcripts were moderately⁴⁰ to highly⁶ expressed in colon cancer cell lines (Supplementary Table 3). The cell lines weakly expressing BORIS were methylated⁶ (Supplementary Table 3). Colon carcinoma cell lines mostly used promoters A and C.⁶ However, in one colon carcinoma line promoter B and less frequently promoters A and C were used.⁶

2.8. BORIS in melanoma

Studies on BORIS in melanoma patients are not numerous.¹⁷ BORIS was expressed in a low percentage (16%) in primary melanomas and in higher percentage (34%) in metastatic melanoma patients¹⁷ (Table 2). There was a lack of strict association between the expression of BORIS and other CT genes (MAGE-A1, MAGE-A2, MAGE-A4, MAGE-B1 and NY-ESO-1) in melanomas.¹⁷ BORIS expression was moderate^{6,17} to high in melanoma cell lines.⁴⁰ However, BORIS was not expressed in all the melanoma cell lines.¹⁷ Methylation was not tested in most of the melanomas, most probably it was high in the cell lines with undetectable BORIS (Supplementary Table 3).

In one melanoma cell line, both promoters A and C of the BORIS gene were used.⁶

2.9. BORIS in leukaemia

There are no data about BORIS in leukaemic patients. However, BORIS was weakly to highly expressed in leukaemic cell lines⁶ (Supplementary Table 3). High expression of BORIS was

detected in unmethylated cell lines.⁶ In a leukaemic cell line A5, A6, B4, B5 and C6 isoforms were detected.⁷

In leukaemic cell lines, mostly promoters A and C, less frequently promoter B and infrequently promoters A and C of the BORIS gene were used.⁶

2.10. BORIS in experimental therapy

There are *in vivo* experimental therapeutical studies in mice having various carcinomas, based on modifications of BORIS. An antitumour vaccine based on deleting the BORIS DNA-binding ZF-domain, has been constructed.⁴³ The protein-based BORIS vaccine induced a significant level of antibodies, while the DNA-based vaccine generated CD8⁺ cytotoxic T-lymphocytes and also contained IL-12 and IL-18.⁴³ The cytotoxicity inducing vaccine was efficient against mammary cancer,^{43,44} glioma, leukaemia and mastocytoma in mice.⁴³ The most recent therapeutic approach is based on vaccination with dendritic cells loaded with a truncated form of BORIS.⁴⁵ This vaccine induced strong anti-cancer immunity, inhibited the growth of mammary cancer and reduced metastasis in experimental mice.⁴⁵

There are no reports about methylating agents as anticancer therapeutics, which may decrease the high expression of BORIS in cancers.

2.11. Future studies

Future studies may yield new data about BORIS in patients with lung, kidney, bladder or testicular cancer as well as leukaemia. The regulatory roles of BORIS in the activation of new oncogenes and the de-regulation of new tumour suppressors genes should be studied, further. The methylation status of BORIS promoter in cell lines with low expression or undetectable level of BORIS has not been well documented. High methylation status would explain controversial data about absence of BORIS in some cancers. Furthermore, histone modifications and the involvement of microRNAs in epigenetic control of BORIS should be studied in the future.

The BORIS antitumour vaccines have not been tested in human trials. To our knowledge, there are no data about methylating agents which may decrease the high level of BORIS in cancers. Methylating agents would be efficient in cancers with hypomethylated or unmethylated BORIS promoter.

In conclusion, BORIS is transiently expressed in spermatogenesis. It is undetectable or very weakly detectable in normal human tissues. BORIS is reactivated in cancers due to the hypomethylation of its promoter. A high expression of BORIS correlated with the size and grade of cancers. A high percentage of BORIS was detected in breast, endometrial, prostatic and colon carcinoma patients, while a lower percentage was present in patients with melanoma and cancer of the head and neck. BORIS expression was weak to strong in various tumorous cell lines.

Conflict of interest statement

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ejca.2011.09.009](https://doi.org/10.1016/j.ejca.2011.09.009).

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