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*Review Article*

# Colon adenocarcinoma prognosis by BORIS a cancer-testis antigen

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**Abstract:** The brother of the regulator of imprinted sites (*BORIS*) is aberrantly expressed in many malignancies. The purpose of this article was to explore the relationship between prognosis and *BORIS* expression. The *BORIS* expression value with colorectal cancer samples and matched normal sample information was downloaded from the Oncomine database. For colorectal cancer patients, we selected the median *BORIS* expression as a threshold value and evaluated the prognosis. The expression rate of *BORIS* was higher in the cancer patients than the normal population ($P<0.001$). Similarly, *BORIS* expression was significant correlated with the N stage ($P<0.001$), M stage ($P<0.001$), and TNM stage ($P<0.001$). Results showed that patients with high expression of *BORIS* had poor 3-year overall survival (OS) rate in colon adenocarcinoma ($P=0.037$). Knockdown of *BORIS* by specific siRNAs caused suppressed cell viabilities in colon adenocarcinoma cell line. Our data indicated that *BORIS* may be a potential prognosis marker in colon adenocarcinoma.

**Keywords:** *BORIS*, prognosis, colon adenocarcinoma, colorectal cancer

## Introduction

Colorectal cancer (CRC) is the most common malignant cancer in the world [1-3] and has a high mortality [4]. It is essential to discover sensitive biomarkers for early screening [2, 5].

Brother of the regulator of imprinted sites (*BORIS*) is a transcription factor [6-11] and expresses in diverse human cancers [6, 12-19]. The previous research indicated that *BORIS* was aberrantly expression in colorectal cancer [8], while the correlation between *BORIS* expression and prognosis has not been documented.

In this study, for *BORIS* expression, 591 colorectal cancer patients and 581 normal samples were investigated. Subtypes of colorectal cancer include cecum adenocarcinoma, colon adenocarcinoma, colon mucinous adenocarcinoma, rectal adenocarcinoma, rectal adenocarcinoma, rectal mucinous adenocarcinoma, rectosigmoid adenocarcinoma, and rectosigmoid mucinous adenocarcinoma were applied for studying the correlation between *BORIS* and clinical-pathological characteristics. The patients were divided into low and high *BORIS* expression groups based on the median expression value [20-22]. Our analysis demonstrated that *BORIS* expression maybe a valuable indicator for predicting colon adenocarcinoma prognosis in 3-year overall survival rate.

## Materials and methods

### *Patients and tissue samples*

We collected 591 CRC patients and 581 normal samples to analyze the *BORIS* expression. The patient sample information include 88 cecum adenocarcinoma, 284 colon adenocarcinoma, 55 colon mucinous adenocarcinoma, 105 rectal adenocarcinoma, 8 rectal mucinous adenocarcinoma, 46 rectosigmoid adenocarcinoma, and 5 rectosigmoid mucinous adenocarcinoma. These sample data were obtained from Oncomine (Compendia Bioscience, ANN Arbor, MI) database [23, 24]. Clinicopathological information was documented in this study. Tissue

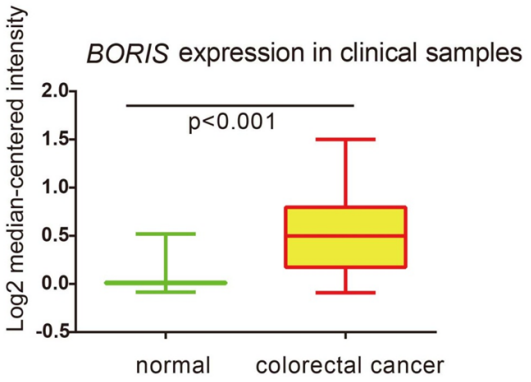


**Figure 1.** The expressions of *BORIS* in analyzed patient tissue were compared between cancer adjacent tissue and colorectal cancer tissue. Statistics were performed by student T test.

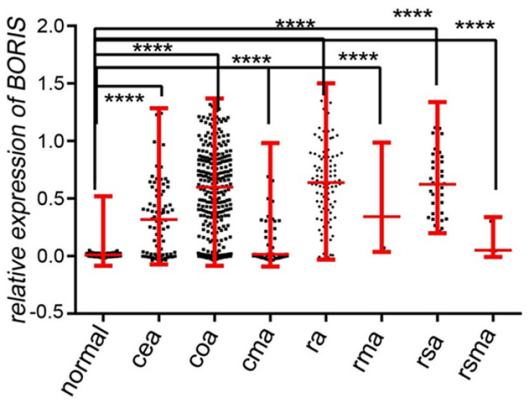


**Figure 2.** Subtypes of colorectal cancer were grouped for the analysis of BORIS expressions. BORIS expressions from 88 cecum adenocarcinomas (cea), 284 colon adenocarcinomas (coa), 55 colon mucinous adenocarcinomas (cma), 105 rectal adenocarcinomas (ra), 8 rectal mucinous adenocarcinomas (rma), 46 rectosigmoid adenocarcinomas (rsa), and 5 rectosigmoid mucinous (rsma) were compared with adjacent normal tissue. **** indicated the *P* value was lower than 0.001.

samples from 3 colorectal adenocarcinoma patients used in additional validation were obtained from Taizhou hospital of Zhejiang province.

*Cell lines and culture*

The Caco-2 colorectal cancer cell line was used and cultured as in previous methods [8].

*Immunohistochemistry assay*

Colorectal carcinoma cancer tissue and the paired adjacent normal tissue were collected from Taizhou hospital and then were applied in immunohistochemistry assays which were performed by SHANGHAI OUTDO BIOTECH CO., LTD, China. Primary BORIS antibody bought from Santa Cruz (sc-377085) was used in these experiments. Brown signal indicated BORIS expression in the slides.

*Cell proliferation analysis*

The cell line Caco-2 was applied to investigate *BORIS* function in colorectal carcinoma cancer cells. The Caco-2 cells were seeded in 96-well plates, plated for 1000 cells per well the day before transfection. Five replicates were performed to avoid variation. MTT assay was applied to assess cell viability three days after transfection of siRNA [8].

*Statistical analysis*

Based on the median *BORIS* expression as a threshold, the patients were classified into low and high expression groups [20-22]. Chi-square test was used to compare the *BORIS* expression with clinicopathological features [25]. The "survival" package in R (R Foundation for Statistical Computing) was used for survival curve analysis. A difference was considered significant if $P<0.05$. All Statistical analyses were performed using GraphPad Prism 6 software (GraphPad Softward Inc., La Jolla, CA, USA).

## Results

For the explicit description of whether *BORIS* expression was associated with colorectal cancer in humans, we analyzed *BORIS* using the publicly available Oncomine database. As shown in **Figure 1**, *BORIS* expression levels were dramatically elevated in colorectal cancer samples. Interestingly, quantitative comparisons showed that expression levels of *BORIS* in normal samples were significantly lower than in cecum adenocarcinoma, colon adenocarcinoma, colon mucinous adenocarcinoma, rectal adenocarcinoma, rectal mucinous adenocarcinoma, rectosigmoid adenocarcinoma and rectosigmoid mucinous adenocarcinoma (**Figure 2**).

Next, the expression levels of *BORIS* were categorized into high and low expression groups in CRC patients (**Table 1**). As shown in **Table 2**, we found that the expression of *BORIS* significantly correlated with different cancer sub-

**Table 1.** The expression of *BORIS* in different types of colorectal cancer

| Group | Expression of *BORIS* | | N |
|---|---|---|---|
| | Low | High | |
| Cecum Adenocarcinoma | 34 (38.6%) | 54 (61.4%) | 88 |
| Colon Adenocarcinoma | 56 (19.7%) | 228 (80.3%) | 284 |
| Colon Mucinous Adenocarcinoma | 34 (61.8%) | 21 (38.2%) | 55 |
| Rectal Adenocarcinoma | 10 (9.5%) | 95 (90.5%) | 105 |
| Rectal Mucinous Adenocarcinoma | 3 (37.5%) | 5 (62.5%) | 8 |
| Rectosigmoid Adenocarcinoma | 0 (0.0%) | 46 (100.0%) | 46 |
| Rectosigmoid Mucinous Adenocarcinoma | 3 (60.0%) | 2 (40.0%) | 5 |

*BORIS* = brother of regulator of imprinted sites.

**Table 2.** Correlation between *BORIS* expression and clinicopathological features in colorectal cancer

| Features (n=591) | *BORIS* expression | | P |
|---|---|---|---|
| | Low (n=140) | High (451) | |
| Gender | | | 0.62 |
| Male (n=314) | 71 | 243 | |
| Female (n=271) | 66 | 205 | |
| Unknown (n=6) | | | |
| Age, years | | | 0.989 |
| <60 (n=162) | 38 | 124 | |
| ≥60 (n=423) | 99 | 324 | |
| Unknown (n=6) | | | |
| T stage | | | 0.208 |
| T0, T1 (n=20) | 6 | 14 | |
| T2 (n=103) | 21 | 82 | |
| T3 (n=398) | 89 | 309 | |
| T4 (n=60) | 20 | 40 | |
| Unknown (n=10) | | | |
| N stage | | | 0.000 |
| N0 (n=329) | 96 | 233 | |
| N+ (n=251) | 41 | 210 | |
| Unknown (n=11) | | | |
| M stage | | | 0.000 |
| M0 (n=440) | 114 | 326 | |
| M1 (n=82) | 7 | 75 | |
| Unknown (n=69) | | | |
| Clinical TNM stage | | | 0.000 |
| I-II (n=311) | 94 | 217 | |
| III-IV (n=258) | 40 | 218 | |
| Unknown (n=22) | | | |

*BORIS* = brother of regulator of imprinted sites. Unknown = not provided data.

types ($P$<0.001), N stage ($P$<0.001), M stage ($P$<0.001) and clinical TNM stage ($P$<0.001). To further investigate the significance of the prognostic role in colorectal cancer, 3-year overall survival rate were estimated (**Figure 3**). The high *BORIS* expression did not correlate with shorter 3-year overall survival rate ($P$=0.28), reversely high *BORIS* expression correlated with even better survival.

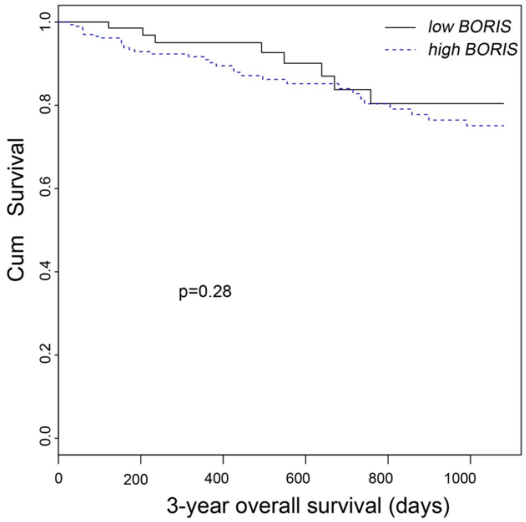


**Figure 3.** Kaplan-Meier survival cures for colorectal cancer patients according to *BORIS* expression.

Due to *BORIS* being expressed differently in colorectal cancer subtypes (**Figure 2**), it is worth to investigate the correlation between *BORIS* and subtypes of colorectal cancer. We selected four subtypes to perform survival curve analysis (**Figure 4**). Interestingly, the results showed that high *BORIS* expression had worse outcomes in colon adenocarcinoma ($P$=0.037). There were no significant differences in cecum adenocarcinoma ($P$=0.361), colon mucinous adenocarcinoma ($P$=0.987), or rectal adenocarcinoma ($P$=0.36), respectively. To further validate the expression of *BORIS* in colon adenocarcinoma, we examined the *BORIS* expression in colorectal cancer tissues

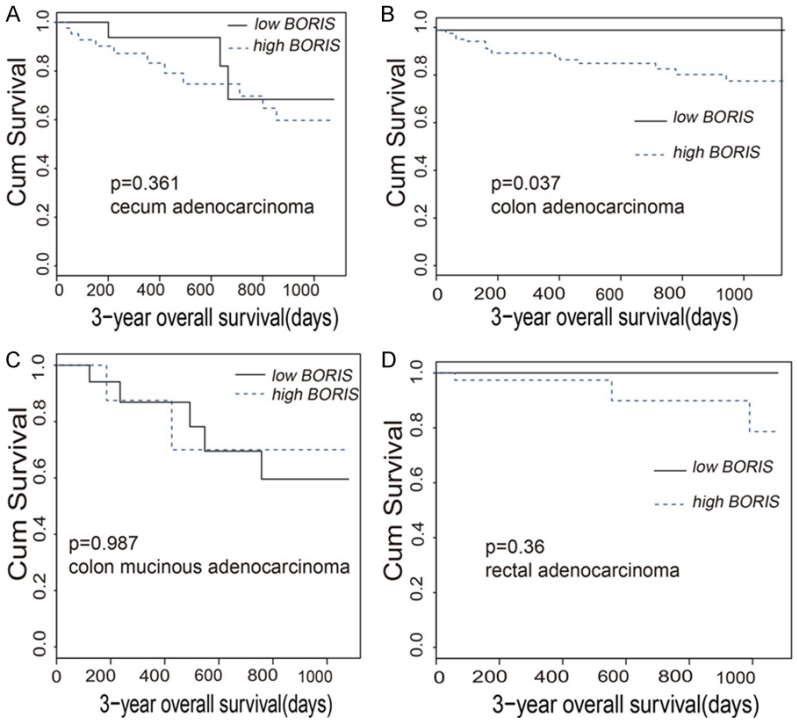


**Figure 4.** Kaplan-Meier survival curves for different colorectal cancer subtypes patients according to *BORIS* expression.

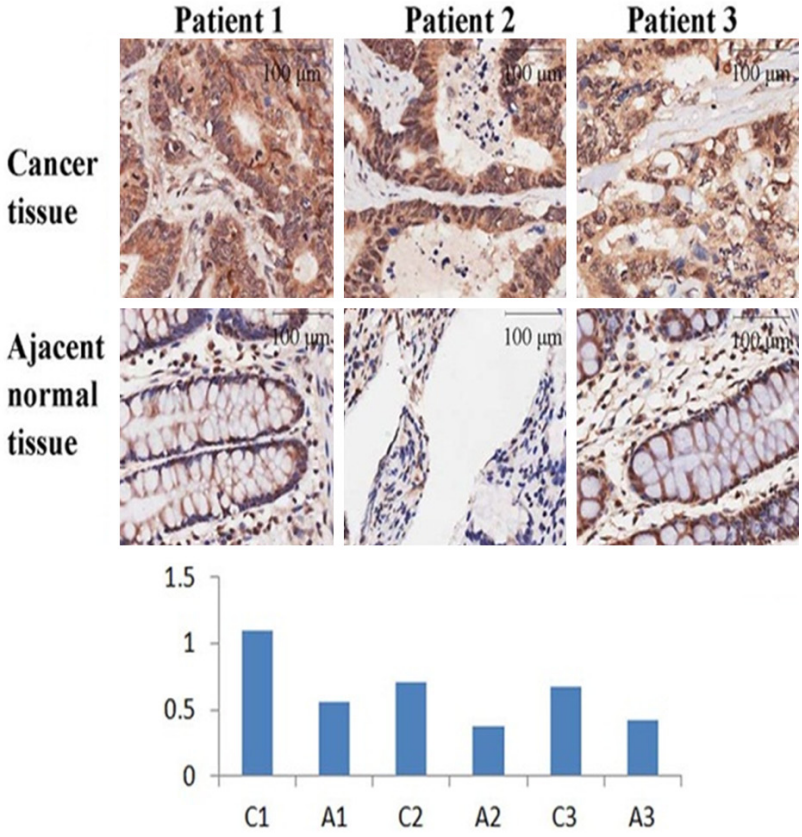


**Figure 5.** Immunohistochemistry (IHC) was performed to test the expression of *BORIS* between paired colorectal adenocarcinoma tissue and adjacent normal tissue from 3 patients. Brown signals indicated the expression of BORIS. Gray values from each IHC picture were plotted to show the BORIS expression. Cancer tissue from patient 1 (C1), adjacent normal tissue from patient 1 (A1), cancer tissue from patient 2 (C2), adjacent normal tissue from patient 2 (A2), cancer tissue from patient 3 (C3), adjacent normal tissue from patient 3 (A3).

and the paired adjacent normal tissue by immunohistochemistry (IHC) method. As shown in **Figure 5**, we found abundant *BORIS* that was abnormal in colon adenocarcinoma tissue but not in the adjacent normal tissue. At the same time, we evaluated the cell proliferation ability of Caco2 (Colon Adenocarcinoma Cell Line) by siRNA knockdown of *BORIS*. In line with our expectation, *BORIS* expression supports colorectal adenocarcinoma cell proliferation (see **Figure 6**).

## Discussion

*BORIS*, is a member of the cancer-testis antigen family [26] and considered to be an oncogene [8, 25, 27-31]. In this study, the decrease in colon adenocarcinoma cell proliferation ability after knockdown of *BORIS* by siRNA suggests that *BORIS* may play a crucial role in colon adenocarcinoma (see **Figure 6**).

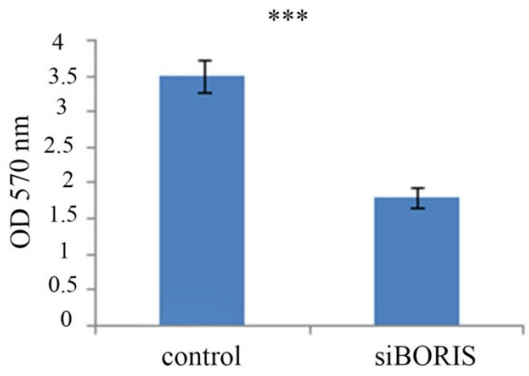


**Figure 6.** To test the impact of *BORIS* on colorectal adenocarcinoma, *BORIS* knockdown was performed on a colorectal adenocarcinoma cell line Caco-2. *BORIS* siRNA was transfected into Caco-2 cells and the cell viability was tested three days after the transfection. Three stars indicated significant suppression of the cell viability under the treatment of *BORIS* knockdown.

It is worth mentioning that this is the first time investigating the prognosis of *BORIS* expression in colorectal cancer. In addition, for 3-year overall survival rate, we also found that high *BORIS* expression showed the correlation with poor prognosis in colon adenocarcinoma. Future studies may yield new data about *BORIS* in patients with colorectal cancer. More and more studies should focus on the function of *BORIS* in the process of carcinogenesis and molecular mechanisms in colon adenocarcinoma. Although the underlying mechanism is still unclear, the reliable prognostic value explains that *BORIS* may be a useful marker and therapeutic target for colon adenocarcinoma.

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## Disclosure of conflict of interest

None.

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