

# World Journal of *Clinical Cases*

*World J Clin Cases* 2019 December 6; 7(23): 3915-4171



**REVIEW**

- 3915** Overview of organic anion transporters and organic anion transporter polypeptides and their roles in the liver  
*Li TT, An JX, Xu JY, Tuo BG*

**ORIGINAL ARTICLE****Observational Study**

- 3934** Value of early diagnosis of sepsis complicated with acute kidney injury by renal contrast-enhanced ultrasound  
*Wang XY, Pang YP, Jiang T, Wang S, Li JT, Shi BM, Yu C*
- 3945** Value of elastography point quantification in improving the diagnostic accuracy of early diabetic kidney disease  
*Liu QY, Duan Q, Fu XH, Fu LQ, Xia HW, Wan YL*
- 3957** Resection of recurrent third branchial cleft fistulas assisted by flexible pharyngotomy  
*Ding XQ, Zhu X, Li L, Feng X, Huang ZC*
- 3964** Therapeutic efficacy of acupuncture combined with neuromuscular joint facilitation in treatment of hemiplegic shoulder pain  
*Wei YH, Du DC, Jiang K*
- 3971** Comparison of intra-articular injection of parecoxib *vs* oral administration of celecoxib for the clinical efficacy in the treatment of early knee osteoarthritis  
*Lu L, Xie Y, Gan K, Huang XW*

**Retrospective Study**

- 3980** Celiomesenteric trunk: New classification based on multidetector computed tomography angiographic findings and probable embryological mechanisms  
*Tang W, Shi J, Kuang LQ, Tang SY, Wang Y*

**Prospective Study**

- 3990** Interaction of arylsulfatases A and B with maspin: A possible explanation for dysregulation of tumor cell metabolism and invasive potential of colorectal cancer  
*Kovacs Z, Jung I, Szalman K, Baniás L, Bara TJ, Gurzu S*

**CASE REPORT**

- 4004** Recuperation of severe tumoral calcinosis in a dialysis patient: A case report  
*Westermann L, Isbell LK, Breitenfeldt MK, Arnold F, Röthele E, Schneider J, Widmeier E*

- 4011** Robotic wedge resection of a rare gastric perivascular epithelioid cell tumor: A case report  
*Marano A, Maione F, Woo Y, Pellegrino L, Geretto P, Sasia D, Fortunato M, Orcioni GF, Priotto R, Fasoli R, Borghi F*
- 4020** Primary parahiatal hernias: A case report and review of the literature  
*Preda SD, Pătrașcu Ș, Ungureanu BS, Cristian D, Bințișan V, Nica CM, Calu V, Strâmbu V, Sapalidis K, Șurlin VM*
- 4029** Diagnosis of Laron syndrome using monoplex-polymerase chain reaction technology with a whole-genome amplification template: A case report  
*Neumann A, Alcántara-Ortigoza MÁ, González-del Ángel A, Camargo-Diaz F, López-Bayghen E*
- 4036** *In-vitro* proliferation assay with recycled ascitic cancer cells in malignant pleural mesothelioma: A case report  
*Anayama T, Taguchi M, Tatenuma T, Okada H, Miyazaki R, Hirohashi K, Kume M, Matsusaki K, Orihashi K*
- 4044** Distant metastasis in choroidal melanoma with spontaneous corneal perforation and intratumoral calcification: A case report  
*Wang TW, Liu HW, Bee YS*
- 4052** Secondary Parkinson disease caused by breast cancer during pregnancy: A case report  
*Li L*
- 4057** Pulmonary embolism and deep vein thrombosis caused by nitrous oxide abuse: A case report  
*Sun W, Liao JP, Hu Y, Zhang W, Ma J, Wang GF*
- 4063** Micronodular thymic tumor with lymphoid stroma: A case report and review of the literature  
*Wang B, Li K, Song QK, Wang XH, Yang L, Zhang HL, Zhong DR*
- 4075** Diffuse large B cell lymphoma with bilateral adrenal and hypothalamic involvement: A case report and literature review  
*An P, Chen K, Yang GQ, Dou JT, Chen YL, Jin XY, Wang XL, Mu YM, Wang QS*
- 4084** Urethral pressure profilometry in artificial urinary sphincter implantation: A case report  
*Meng LF, Liu XD, Wang M, Zhang W, Zhang YG*
- 4091** Hydroxyurea-induced cutaneous squamous cell carcinoma: A case report  
*Xu Y, Liu J*
- 4098** Recurrent hypotension induced by sacubitril/valsartan in cardiomyopathy secondary to Duchenne muscular dystrophy: A case report  
*Li JM, Chen H*
- 4106** Complete duodenal obstruction induced by groove pancreatitis: A case report  
*Wang YL, Tong CH, Yu JH, Chen ZL, Fu H, Yang JH, Zhu X, Lu BC*

- 4111** Radiological aspects of giant hepatocellular adenoma of the left liver: A case report  
*Zheng LP, Hu CD, Wang J, Chen XJ, Shen YY*
- 4119** Mixed serous-neuroendocrine neoplasm of the pancreas: A case report and review of the literature  
*Xu YM, Li ZW, Wu HY, Fan XS, Sun Q*
- 4130** Rigid esophagoscopy combined with angle endoscopy for treatment of superior mediastinal foreign bodies penetrating into the esophagus caused by neck trauma: A case report  
*Wang D, Gao CB*
- 4137** Left armpit subcutaneous metastasis of gastric cancer: A case report  
*He FJ, Zhang P, Wang MJ, Chen Y, Zhuang W*
- 4144** Bouveret syndrome: A case report  
*Wang F, Du ZQ, Chen YL, Chen TM, Wang Y, Zhou XR*
- 4150** Fatal complications in a patient with severe multi-space infections in the oral and maxillofacial head and neck regions: A case report  
*Dai TG, Ran HB, Qiu YX, Xu B, Cheng JQ, Liu YK*
- 4157** Management of massive fistula bleeding after endoscopic ultrasound-guided pancreatic pseudocyst drainage using hemostatic forceps: A case report  
*Ge N, Sun SY*
- 4163** Pure squamous cell carcinoma of the gallbladder locally invading the liver and abdominal cavity: A case report and review of the literature  
*Jin S, Zhang L, Wei YF, Zhang HJ, Wang CY, Zou H, Hu JM, Jiang JF, Pang LJ*

**ABOUT COVER**

Editorial Board Member of *World Journal of Clinical Cases*, Consolato M Sergi, FRCP (C), MD, PhD, Professor, Department of Lab Medicine and Pathology, University of Alberta, Edmonton T6G 2B7, Canada

**AIMS AND SCOPE**

The primary aim of *World Journal of Clinical Cases* (WJCC, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

**INDEXING/ABSTRACTING**

The WJCC is now indexed in PubMed, PubMed Central, Science Citation Index Expanded (also known as SciSearch®), and Journal Citation Reports/Science Edition. The 2019 Edition of Journal Citation Reports cites the 2018 impact factor for WJCC as 1.153 (5-year impact factor: N/A), ranking WJCC as 99 among 160 journals in Medicine, General and Internal (quartile in category Q3).

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Responsible Electronic Editor: *Yan-Xia Xing*

Proofing Production Department Director: *Xiang Li*

**NAME OF JOURNAL**

*World Journal of Clinical Cases*

**ISSN**

ISSN 2307-8960 (online)

**LAUNCH DATE**

April 16, 2013

**FREQUENCY**

Semimonthly

**EDITORS-IN-CHIEF**

Dennis A Bloomfield, Bao-Gan Peng, Sandro Vento

**EDITORIAL BOARD MEMBERS**

<https://www.wjnet.com/2307-8960/editorialboard.htm>

**EDITORIAL OFFICE**

Jin-Lei Wang, Director

**PUBLICATION DATE**

December 6, 2019

**COPYRIGHT**

© 2019 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**

<https://www.wjnet.com/bpg/gerinfo/204>

**GUIDELINES FOR ETHICS DOCUMENTS**

<https://www.wjnet.com/bpg/GerInfo/287>

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**

<https://www.wjnet.com/bpg/gerinfo/240>

**PUBLICATION MISCONDUCT**

<https://www.wjnet.com/bpg/gerinfo/208>

**ARTICLE PROCESSING CHARGE**

<https://www.wjnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## Prospective Study

# Interaction of arylsulfatases A and B with maspin: A possible explanation for dysregulation of tumor cell metabolism and invasive potential of colorectal cancer

Zsolt Kovacs, Ioan Jung, Krisztina Szalman, Laura Bantias, Tivadar Jr Bara, Simona Gurzu

**ORCID number:** Zsolt Kovacs (0000-0002-1038-7769); Ioan Jung (0000-0001-6537-2807); Krisztina Szalman (0000-0002-7016-9923); Laura Bantias (0000-0002-2240-2540); Tivadar Jr Bara (0000-0002-8231-6310); Simona Gurzu (0000-0003-3968-5118).

**Author contributions:** Kovacs Z drafted the article and contributed to the gene expression study; Jung I and Bantias L contributed to the diagnosis and immunohistochemical assessment; Bara TJ contributed to the surgical interventions; Szalman K contributed to selection of patients for blood analysis; Gurzu S designed research and confer the final agreement for publication; Zsolt Kovacs and Krisztina Szalman have equally contribution to the paper.

**Supported by** the Romanian National Authority for Scientific Research, CNCSIS - UEFISCDI, No. 20 PCCF/2018.

**Institutional review board statement:** The Ethical Approval of Mures County Emergency Hospital and signed informed consent was obtained before surgery.

**Conflict-of-interest statement:** All authors have no conflicts of interest.

**CONSORT 2010 statement:** The guidelines of the CONSORT 2010 Statement have been adopted.

**Open-Access:** This article is an

**Zsolt Kovacs, Ioan Jung, Laura Bantias, Simona Gurzu,** Department of Pathology, University of Medicine, Pharmacy, Sciences and Technology "George Emil Palade", Targu Mures 530149, Romania

**Krisztina Szalman,** Department of Internal Medicine, University of Medicine, Pharmacy, Sciences and Technology "George Emil Palade", Targu Mures 530149, Romania

**Tivadar Jr Bara,** Department of Surgery, University of Medicine, Pharmacy, Science and Technology "George Emil Palade", Targu Mures 530149, Romania

**Simona Gurzu,** Research Center (CCAMF), University of Medicine, Pharmacy, Sciences and Technology, Targu Mures 540139, Romania

**Corresponding author:** Simona Gurzu, MD, PhD, Professor, Department of Pathology, University of Medicine, Pharmacy, Sciences and Technology, "George Emil Palade", 38 Gheorghe Marinescu Street, Targu Mures 540139, Romania. [simonagurzu@yahoo.com](mailto:simonagurzu@yahoo.com)  
**Telephone:** +40-745-673550  
**Fax:** +40-265-210407

## Abstract

### BACKGROUND

Although it has been shown that arylsulfatases are lost in colorectal cancer (CRC) cell lines, their exact role in the carcinogenesis and behavior of this cancer was not elucidated. No data about the correlation between serum and immunohistochemical (IHC) level of arylsulfatases (ARSA, ARSB) in patients with CRC were published yet.

### AIM

To evaluate the possible prognostic value of ARSA and/or ARSB in CRC, at circulating and protein levels.

### METHODS

The present study included 45 consecutive patients who were prospectively diagnosed with CRC. For IHC stains (protein expression) ARSA, ARSB and maspin expression were quantified. For these markers, cytoplasmic expression was taken into account. For gene expression study, circulating mRNA was isolated from all patients, before surgery. A group of 45 healthy patients without inflammatory or tumor pathologies was used as control group. Reverse transcription and Taqman Gene Expression Array were used for ARSB gene



open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Received:** September 27, 2019

**Peer-review started:** September 27, 2019

**First decision:** October 24, 2019

**Revised:** October 31, 2019

**Accepted:** November 15, 2019

**Article in press:** November 15, 2019

**Published online:** December 6, 2019

**P-Reviewer:** Fukushi K, Kanat O, Vynios D

**S-Editor:** Ma YJ

**L-Editor:** A

**E-Editor:** Qi LL



expression.

## RESULTS

The preoperative circulating RNA level of the ARSB gene was significantly decreased in patients with CRC ( $RQ < 1$ ), compared with the control group ( $RQ > 1$ ). A more significant decrease ( $RQ < 0.5$ ) occurred in ulceroinfiltrative maspin-positive adenocarcinomas, with a higher degree of tumor budding, diagnosed in locally advanced stages (pT3/4). ARSA/maspin immunopositivity indicated a higher risk for lymph node metastasis, while triple positivity for maspin/ARSA/ARSB and ARSB gene expression level  $< 0.5$  were indicators of CRC aggressive behavior, independent of lymph node status.

## CONCLUSION

The significant independent negative prognostic factors of CRC are the ulceroinfiltrative aspect, high budding degree, triple positivity for maspin, ARSA and ARSB, and low ARSB gene expression.

**Key words:** Arylsulfatase; Maspin; Colorectal cancer; ARSB gene; Blood; Tissue

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** In this paper we tried to emphasize the role of arylsulfatases (ARSA, ARSB) in colorectal cancer (CRC) behaviour and possible role of ARSB serum level in follow-up of patients. This is the first study in literature which proved that a low ARSB gene expression in serum ( $RQ < 1$ ) might be a non-invasive indicator of risk of CRC. Moreover, triple positivity for maspin/ARSA/ARSB and ARSB gene expression level  $< 0.5$  were proved to be indicators of CRC aggressive behavior, independent of lymph node status.

**Citation:** Kovacs Z, Jung I, Szalman K, Banias L, Bara TJ, Gurzu S. Interaction of arylsulfatases A and B with maspin: A possible explanation for dysregulation of tumor cell metabolism and invasive potential of colorectal cancer. *World J Clin Cases* 2019; 7(23): 3990-4003

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i23/3990.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i23.3990>

## INTRODUCTION

Colorectal cancer (CRC) usually affects people over 60 years of age and remains one of the leading causes of cancer mortality in Europe<sup>[1]</sup>. Although the involvement of several molecular factors in colorectal carcinogenesis and the invasiveness of CRC have been described, these processes are not yet fully understood<sup>[2]</sup>. In this paper, we sought to characterize the possible role of two arylsulfatases (ARSA and ARSB) in CRC behavior. As the correlation between the immunohistochemical (IHC) and gene expression of ARSB has not yet been examined in CRC, we aimed to formulate a new hypothesis about the molecular background of CRC.

Arylsulfatases are lysosomal enzymes that are able to catalyze the hydrolysis of sulfate esters. A total deficiency of ARSA is known to induce metabolic disorders such as metachromatic leukodystrophy, a lysosomal storage disorder characterized by the accumulation of cerebroside sulfate within lysosomes and the further destruction of the brain's white matter<sup>[3,4]</sup>.

Chondroitin sulfate and dermatan sulfate are the targets of ARSB, which are glycosaminoglycans. The total deficiency of ARSB leads to Maroteaux-Lamy syndrome, a genetic disorder with severe neurological dysfunction<sup>[4,5]</sup>. The pseudo-deficiency and/or extralysosomal localization of ARSA and/or ARSB is not known to cause serious health problems<sup>[4,6]</sup>.

In one of the recently published reviews of the literature, we showed that fewer than 40 papers concerning the possible role of ARSA/ARSB in tumorigenesis were published in the English literature and indexed in the Medline database prior to 2018<sup>[4]</sup>. These studies were mainly based on cell lines and took into account malignant melanoma (about one paper), lung cancer (about two papers), urogenital cancer

(about 10 papers) and CRC (about 20 papers)<sup>[4]</sup>. Independently of carcinoma localization, a decreased expression pattern of ARSA and ARSB was described in the above-mentioned papers<sup>[7]</sup>.

In CRC, it seems that ARSB is expressed in normal colonic mucosa and shows a loss of intensity in tumor cells, with a role in carcinoma invasiveness and metastatic capacity<sup>[4,7,8]</sup>. To our knowledge, no data about the role of ARSA in CRC have yet been published<sup>[4]</sup>.

In this paper, we examined the IHC expression of ARSA and ARSB in 45 CRC specimens, together with circulating *ARSB* gene expression. All of the obtained data were correlated with clinicopathological parameters, including the degree of budding and overall survival rate<sup>[9]</sup>. For budding quantification, we used the IHC biomarker maspin, which is a serine protease that is known to be involved in the inhibition of tumor cell proliferation, angiogenesis and apoptosis promotion<sup>[9-12]</sup>. We chose Maspin for budding assessment, because it was proved to be a specific marker for budding quantification<sup>[11,12]</sup> and an reliable prognostic and possible predictive marker, for CRC<sup>[9-12]</sup>.

The aim of this study was to examine the unexplored pathological significance of ARSA and ARSB, and their possible interaction with maspin in patients with CRC. ARSA/ ARSB IHC expression level was correlated with maspin positivity and ARSB preoperative gene expression level, in order to see the possible positive correlation between ARSA/ ARSB and maspin protein level together with ARSB gene expression and tumor behavior and aggressivity.

## MATERIALS AND METHODS

### **Selection criteria and histological assessment**

The present study included 45 consecutive cases of patients who were prospectively diagnosed with CRC. The Ethical Approval of Mures County Emergency Hospital and signed informed consent was obtained before surgery.

In all of the patients, surgical resection was performed and blood was taken one day before surgery. Only those patients who survived for at least 20 d after surgery were included. No preoperative radiotherapy was performed before surgery. Cases from the upper rectum also involved the recto-sigmoid junction and no preoperative chemotherapy were administered. The patient follow-up period was between 20 and 509 d.

All of the cases comprised adenocarcinomas without distant metastases (M0). The eighth edition of the American Joint Committee on Cancer and the current World Health Organization classification were used for tumor staging. Tumor buds were quantified under light microscopy using the criteria proposed by Ueno *et al*<sup>[13]</sup> in 2012, adapted with the International Tumor Budding Consensus Conference criteria from 2016<sup>[14]</sup>. To ensure the objectivity of budding assessment, maspin quantification was also conducted, based on the criteria mentioned in the previously published papers<sup>[11,12]</sup>. Cases were divided into G1 (below five buds/HPF), G2 (five-nine buds) and G3 (over 10 isolated cells or clusters/HPF)<sup>[10-12]</sup>.

### **Immunohistochemical assessment of ARSA, ARSB and maspin**

ARSA, ARSB and maspin expression were quantified using the IHC markers presented in [Table 1](#). For ARSA, ARSB and maspin, cytoplasmic expression was taken into account. Although maspin can also show nuclear positivity, especially in tumor buds<sup>[12]</sup>, due to the low number of cases, the subcellular expression was not taken into account. Cases were classified as showing low or high expression, based on the percentage of positive cells and the intensity of immunostaining<sup>[10-12]</sup>.

### **ARSB gene expression**

Circulating mRNA was isolated from all 45 patients with CRC ([Table 2](#)), from which 2 mL of intravenous blood was taken one day before surgery. For the control group, we used blood from 45 healthy patients without inflammatory or tumor pathologies, in which colonoscopy was conducted for screening purposes.

Blood mRNA isolation was performed using a Roche High Pure RNA Isolation kit (Roche Diagnostics, Germany) in line with the user's guide. Reverse transcription was conducted using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, United States) following the recommended protocol. A Taqman Gene Expression Array was used for Arylsulfatase B gene expression, according to the user's guide (Applied Biosystems, United States), with the following PCR protocol: (1) UNG (uracil N-glycosylase) incubation 50°C, two minutes, one cycle (for preventing carryover contamination); (2) Enzyme activation 95°C, 20 s, one cycle; and (3)



**Table 1** Characteristics of immunohistochemical markers

Marker	Clone	Incubation	pH
Arylsulfatase A	Mouse monoclonal (Santa Cruz Biotechnology)	High pH (Dako)	9.0
Arylsulfatase B	Rabbit polyclonal (Abcam)	High pH (Dako)	9.0
Maspin	Mouse monoclonal (Santa Cruz Biotechnology)	0.01 mol/L citrate (Novocastra)	6.0

Denaturation, 95°C, one second with Anneal/Extend at 60°C, 20 s for 40 cycles.

### Statistical analysis

Statistical analysis was performed using GraphPad Prims8 software. A *P* value < 0.05 (with a 95% confidence interval) was considered statistically significant, calculated using the  $\chi^2$  (and Fisher's exact test and Yate's continuity correction) test and Kruskal-Wallis, while the Mann-Whitney test was used for *ARSB* gene expression. The possible correlation between the examined IHC markers and the *ARSB* gene was assessed using a Venn diagram. Overall survival was evaluated using Kaplan-Meier survival curves.

## RESULTS

### Immunohistochemical markers and clinicopathological factors

The immunoexpression of the three examined markers (*ARSA*, *ARSB* and maspin) was not correlated with the patients' gender or age (Tables 2-4). Although a slightly increased *ARSB* expression was seen in aging patients, it was not statistically significant when the age of 60 was taken into account (Table 3). The median age of patients was  $57 \pm 13.24$  years (range 26-86 years), most of whom ( $n = 26$ ; 57.8%) were diagnosed with CRC over the age of 60.

*ARSA* and *ARSB* expression was not influenced by tumor localization (Tables 2 and 3). Most of the maspin-negative cases ( $n = 19$ ; 42.22%) involved the distal colon, while the positive cases were located in the upper rectum (Table 4).

Most of the cases ( $n = 33$ ; 73.33%) showed an ulcero-infiltrative aspect, with high expression of all of the three examined markers. Independently of *ARSB* expression (Table 3), polypoid tumors were mostly maspin negative (Table 4) and showed low *ARSA* intensity (Table 2).

The *ARSA* and maspin intensity increased in parallel with tumor dedifferentiation, with only G1 cases associated with significantly low *ARSA* and negative maspin (Tables 2 and 4). *ARSB* expression was high in G1 and G2 cases and particularly decreased in G3 and G4 specimens (Table 3).

There was predominantly a loss of *ARSA* expression in cases diagnosed in locally advanced stages (pT3+4), without lymph node metastases, independently of the degree of tumor budding (Table 2). *ARSB* immune expression was not correlated with the pT stage, pN stage, lymph node ratio or the degree of tumor budding (Table 3). Most of the maspin-negative cases were diagnosed in the early stages (pT1+2) and did not show lymph node metastases or a high degree of budding (Table 4).

### *ARSB* gene expression and clinicopathological factors

In all of the patients included in the control group ( $n = 45$ ), without inflammatory or tumor disorders, the circulating *ARSB* gene expression level was higher than one (ranging from one-five), whereas blood levels higher than one were obtained from the blood of patients with CRC. After normalization with house-keeping, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene expression of *ARSB* was assessed using Kruskal-Wallis and Mann-Whitney tests<sup>[15]</sup>, with a significantly decreased *ARSB* gene expression ( $P < 0.0001$ ) found in patients with CRC (Figure 1).

As a low *ARSB* gene expression circulating level was found in all of the patients with CRC, after relative quantification ( $RQ < 1$ ) for statistical assessment (Table 5), the cases were divided into two groups ( $RQ < 0.5$  and  $RQ \geq 0.5$ ).

The preoperative *ARSB* gene circulating level did not prove to be correlated with patients' age or gender, either in the presence or absence of lymph node metastases (Table 5). *ARSB* was found to have a significantly lower gene expression profile in ulcero-infiltrative tumors, especially those from the upper rectum, diagnosed in locally advanced stages (pT3+4), which showed a high degree of budding (over 10 buds/HPF) and a high grade of dedifferentiation (Table 5).

### Correlation of immunohistochemical markers with *ARSB* gene expression

**Table 2** Correlation between arylsulfatase A expression and pathological aspects of colorectal cancer

Characteristics	Number	ARSA expression		<i>P</i> value
		Low	High	
Age, yr				
≤ 60	19	8	11	0.76 <sup>1</sup>
> 60	26	13	13	
Gender				
Male	26	12	14	> 0.99 <sup>1</sup>
Female	19	9	10	
Macroscopic aspect				
Polypoid	12	9	3	0.04 <sup>1</sup>
Ulceroinfiltrative	33	12	21	
Microscopic aspect				
G1	14	11	3	0.02 <sup>2</sup>
G2	24	9	15	
G3+4	7	2	5	
Localization				
Proximal	8	5	3	0.50 <sup>2</sup>
Distal	21	10	11	
Upper rectum	16	6	10	
Lymph node ratio				
< 0.1	34	19	15	0.04 <sup>1</sup>
≥ 0.1	11	2	9	
Lymph node metastasis				
Absent	29	17	12	0.03 <sup>1</sup>
Present	16	4	12	
pT stage				
≤ T2	15	3	12	0.01 <sup>1</sup>
≥ T3	30	18	12	
Budding grade				
G1	15	8	7	0.81 <sup>2</sup>
G2	9	4	5	
G3	21	9	12	
Maspin expression				
Positive	22	3	19	< 0.0001 <sup>1</sup>
Negative	23	18	5	
ARSB expression				
Low	11	5	6	0.92 <sup>1</sup>
High	34	16	18	

<sup>1</sup>Fisher's exact test.<sup>2</sup> $\chi^2$  test. Significant differences are shown in bold. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated, 4 mucinous.

No correlation was found between ARSA and the tissue protein level of ARSB (Table 2) or its RNA circulating level (Table 5). The ARSB protein level in surgical specimens was inversely correlated with the preoperative ARSB gene circulating level (Table 5).

A significant direct correlation was observed between maspin and the tissue protein level of both ARSA (Table 2) and ARSB (Table 3). The maspin protein tissue level was inversely correlated with the preoperative ARSB gene circulating level (Table 5).

A Venn diagram illustrates that from the 45 cases, 14 (31.11%) showed positivity for maspin, high ARSA and ARSB protein levels and low ARSB gene expression (Figure 2). All of the 14 cases were ulceroinfiltrative carcinomas of the upper rectum, showing a high degree of budding.

**Table 3 Correlation between arylsulfatase B expression and pathological aspects of colorectal cancer**

Characteristics	Number	ARSB expression		<i>P</i> value
		Low	High	
Age, yr				
≤ 60	19	7	12	0.09 <sup>1</sup>
> 60	26	4	22	
Gender				
Male	26	5	21	0.34 <sup>1</sup>
Female	19	6	13	
Macroscopic aspect				
Polypoid	12	7	5	0.003 <sup>1</sup>
Ultero-infiltrative	33	4	29	
Microscopic aspect				
G1	14	2	12	0.007 <sup>2</sup>
G2	24	4	20	
G3+4	7	5	2	
Localization				
Proximal	8	2	6	0.99 <sup>2</sup>
Distal	21	5	16	
Upper rectum	16	4	12	
Lymph node ratio				
< 0.1	34	9	25	0.70 <sup>1</sup>
≥ 0.1	11	2	9	
Lymph node metastasis				
Absent	29	9	20	0.27 <sup>1</sup>
Present	16	2	14	
pT stage				
≤ T2	15	5	10	0.46 <sup>1</sup>
≥ T3	30	6	23	
Budding grade				
G1	15	3	12	0.82 <sup>2</sup>
G2	9	2	7	
G3	21	6	15	
Maspin expression				
Positive	22	2	20	0.03
Negative	23	9	14	

<sup>1</sup>Fisher's exact test.<sup>2</sup> $\chi^2$  test. Significant differences are shown in bold. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated, 4 mucinous.

### Overall survival

Examination of the independent prognostic value of the examined prognostic factors showed no correlation with overall survival for patients' age or gender (Figure 3), or with the tumor grade of differentiation (Figure 4). A longer but not significant overall survival was associated with lymph node status (Figure 4). Ultero-infiltrative tumors (Figure 3) with a high degree of budding and diagnosed in the T3-4 stage, especially those of lower rectum, presented a shorter overall survival rate (Figure 4). Triple high protein expression of ARSA, ARSB and maspin, correlated with low ARSB gene expression, were indicators of short overall survival (Figure 2).

Multivariate correlation indicates that negative prognostic value was associated with ultero-infiltrative carcinomas of the lower rectum, with a high degree of budding, triple positivity for ARSA/ARSB/maspin and low ARSB gene expression.

**Table 4** Correlation between maspin expression and pathological aspects of colorectal cancer

Characteristics	Number	Maspin expression		P value
		Negative	Positive	
Age, yr				
≤ 60	19	9	10	0.66 <sup>1</sup>
> 60	26	14	12	
Gender				
Male	26	13	13	0.86 <sup>1</sup>
Female	19	10	9	
Macroscopic aspect				
Polypoid	12	10	2	0.01 <sup>1</sup>
Ulceroinfiltrative	33	13	20	
Microscopic aspect				
G1	14	11	3	0.03 <sup>2</sup>
G2	24	10	14	
G3+4	7	2	5	
Localization				
Proximal	8	1	7	< 0.0001 <sup>2</sup>
Distal	21	19	2	
Upper rectum	16	3	13	
Lymph node ratio				
< 0.1	34	19	15	0.26 <sup>1</sup>
≥ 0.1	11	4	7	
Lymph node metastasis				
Absent	29	19	10	0.01 <sup>1</sup>
Present	16	4	12	
pT stage				
≤ T2	15	13	2	0.001 <sup>1</sup>
≥ T3	30	10	20	
Budding grade				
G1	15	14	1	<0.001 <sup>1</sup>
G2	9	5	4	
G3	21	4	17	

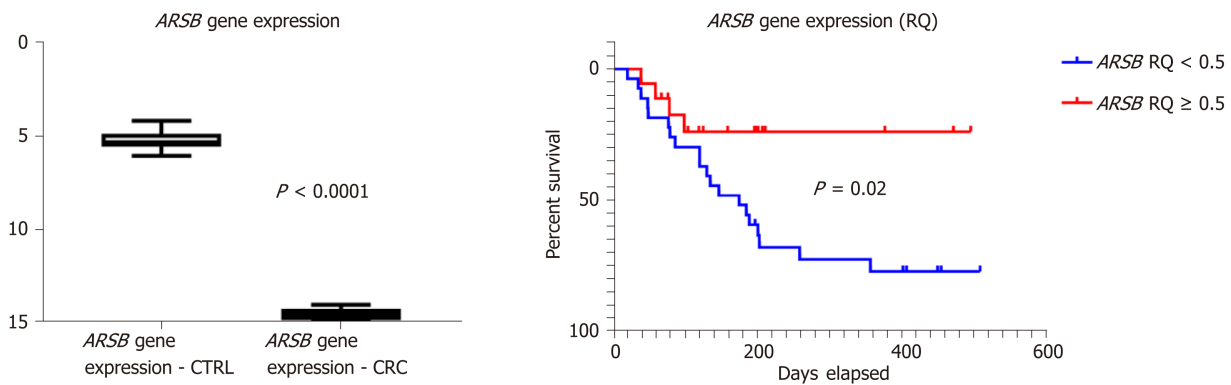
<sup>1</sup>Fisher's exact test.<sup>2</sup> $\chi^2$  test. Significant differences are shown in bold. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated, 4 mucinous.

## DISCUSSION

The present study confirms the possible role of ARSA and ARSB in colorectal carcinogenesis and tumor invasiveness. As no similar studies have previously been published in the Medline database, it is difficult to interpret the obtained results. Accordingly, we took into account the well-known clinicopathological prognostic parameters and maspin, one of the serine proteases intensely examined by our team and used in daily diagnosis for tumor budding quantification<sup>[9-12]</sup>.

As the previous studies proved the unquestionably prognostic role of tumor stages, the present findings show that this parameter remains an independent prognostic factor, but the most strongly statistically independent prognostic value was found for the degree of tumor budding, especially for ulceroinfiltrating tumors, in accordance with other published papers<sup>[12-14]</sup>.

In the early stages (pT1/2N0 cases with low grade budding), maspin was mostly lost in tumor cells compared with normal mucosa. Maspin was found to be positive in advanced stages (pT3/4N1-2 with high grade budding), whereas ARSA was firstly high, then lost in patients with pT3/4N0 carcinomas, and re-expressed in cases with lymph node metastases. These aspects confirm that ARSA is positively maintained in the early stages, as in normal colonic mucosa, functioning as a protector of local invasiveness but not lymph vessel invasion. The ARSA protein level then decreases in



**Figure 1** *ARSB* gene expression level is decreased in blood of patients with colorectal cancer, versus control group (left), the low level being a negative prognostic factor (right). CRC: Colorectal cancer.

parallel with local tumor invasiveness but is re-expressed when tumor cells invade the lymph vessel inva, independently of the degree of tumor budding.

Regarding ARSB, the present study confirms the previously supposed loss of ARSB expression in patients with CRCs, compared with healthy patients<sup>[7,8]</sup>. For the first time in the literature, we have aimed to prove the possible mechanism of CRC cell ARSB-related aggressiveness. Firstly, we showed that the RNA circulating level of the *ARSB* gene ( $RQ < 1$ ) can be used as a screening indicator of CRC, especially in patients with  $RQ < 0.5$ . On the one hand, the indicators of local aggressiveness, such as the ulcero-infiltrative aspect, advanced stage (T3,4), a high degree of budding and maspin positivity, were correlated with an *ARSB* gene circulating level of  $< 0.5$ , independently of the presence or absence of lymph node metastases. On the other hand, independently of the tumor stage or other clinicopathological parameters, the ARSB protein level expressed in normal colonic mucosa was maintained in CRC cells in over 75% of cases, especially in ulcero-infiltrative, well-differentiated (G1) adenocarcinomas. An inverse correlation between the circulating and protein level of ARSB was statistically shown. These aspects confirm that in early stages of colorectal carcinogenesis, maspin and ARSB are lost and then re-expressed in tumor cells, as an indicator of aggressiveness.

Although based on a small number of cases, the present study demonstrates that in early stages of carcinogenesis, both ARSA and ARSB protein expression is maintained in tumor cells. Then, the evolution depends on the arylsulfatase that is expressed the most, in correlation with other genetic or environmental factors. A loss of ARSA might indicate a high capacity of tumor cells for local invasiveness and a high risk of local recurrence after surgery. Independently of the depth of infiltration, in cases with high ARSA intensity, there is a higher risk of lymph vessel invasion and lymph node metastases, independently of ARSB expression. Although ARSB is maintained in tumor cells, its circulating level decreases in parallel with the depth of tumor infiltration. Maspin positivity remains an indicator of a high degree of tumor budding, especially for locally advanced carcinomas.

It is also worth mentioning that a triple positivity for ARSA/ARSB/maspin, correlated with an *ARSB* gene circulating level of  $< 0.5$ , is an indicator of a lower survival rate, independently of the other clinicopathological parameters. This association was found in one third of the cases, with a high degree of budding in each case.

In the literature, the ARSB-related potential aggressive behavior of G2/3 ulcero-infiltrative CRC is thought to be induced by either the epithelial-mesenchymal transition of CRC cells or ARSB-mediated hypoxia in colonic epithelial cells, underlining the importance of this enzyme in human CRC<sup>[7,8,16-19]</sup>.

Based on the obtained data and data from literature, it is tempting to emit a hypothesis regarding the possible role of arylsulfatases in carcinogenesis and evolution of CRC, and their possible interaction with maspin. As we have mentioned, till now, it was proved that loss of ARSA/ARSB induce metabolic disorders<sup>[3-6]</sup>. It was also proved and confirmed in our material that ARSA/ARSB protein level decreases in carcinomas<sup>[4]</sup>. Based on these facts and our result, we can suppose that, in CRC, the arylsulfatases are firstly lost, possible as result of metabolism imbalance, which is specific for malignant tumors<sup>[20]</sup>. On the other hand, in parallel with decreased serum expression of *ARSB* gene, decreased protein levels are seen in tumor cells, in early stages.

In parallel with lymph vessels invasion, when the tumor cells metabolism is

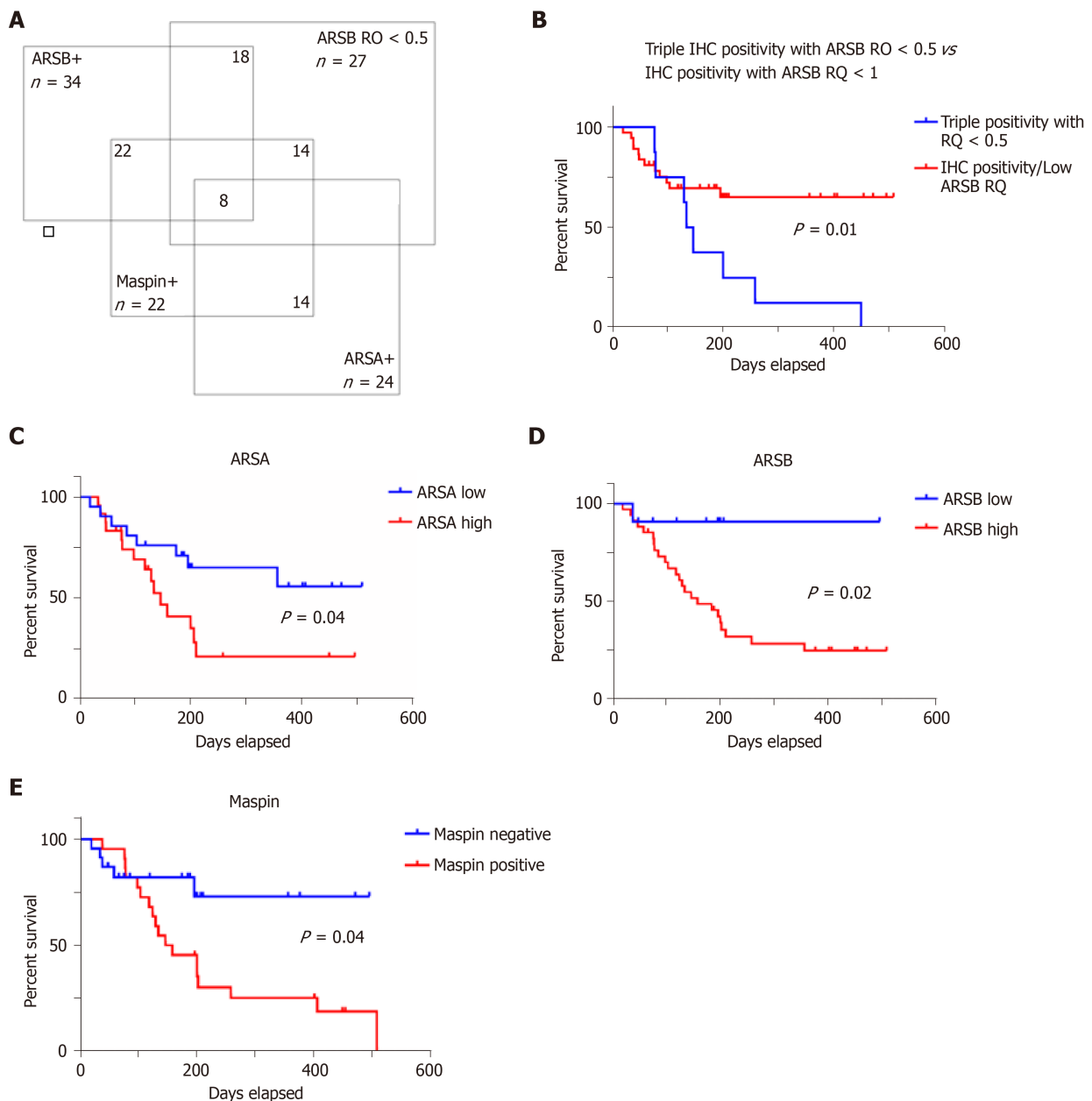
**Table 5 Correlation between arylsulfatase A gene expression and pathological aspects of colorectal cancer**

Characteristics	Number	ARSB gene expression (RQ)	P value	ARSB gene expression (RQ)		P value
				RQ < 0.5	RQ ≥ 0.5	
Age, yr						
≤ 60	19	0.4801 ± 0.2711	0.669 <sup>1</sup>	10	9	0.53 <sup>2</sup>
> 60	26	0.5125 ± 0.3261		17	9	
Gender						
Male	26	0.4938 ± 0.2892	0.959 <sup>1</sup>	14	12	0.37 <sup>2</sup>
Female	19	0.5058 ± 0.3250		13	6	
Macroscopic aspect						
Polypoid	12	0.4339 ± 0.2530	0.395 <sup>1</sup>	4	8	0.04 <sup>2</sup>
Ultero-infiltrative	33	0.5225 ± 0.3172		23	10	
Microscopic aspect						
G1	14	0.4155 ± 0.3034	0.295 <sup>3</sup>	4	10	0.01 <sup>4</sup>
G2	24	0.5496 ± 0.2793		17	7	
G3+4	7	0.4915 ± 0.3747		6	1	
Localization						
Proximal	8	0.3246 ± 0.2332	0.115 <sup>3</sup>	5	3	0.06 <sup>4</sup>
Distal	21	0.4911 ± 0.3179		9	12	
Upper rectum	16	0.5962 ± 0.2824		13	3	
Lymph node ratio						
< 0.1	34	0.5057 ± 0.3020	0.926 <sup>1</sup>	20	14	> 0.99 <sup>2</sup>
≥ 0.1	11	0.4799 ± 0.3293		7	4	
Lymph node metastasis						
Absent	29	0.4749 ± 0.2838	0.545 <sup>1</sup>	17	12	> 0.99 <sup>2</sup>
Present	16	0.5422 ± 0.3358		10	6	
pT stage						
≤ T2	15	0.4987 ± 0.3296	0.9652 <sup>1</sup>	3	12	0.0002 <sup>2</sup>
≥ T3	30	0.4989 ± 0.2997		24	6	
Budding grade						
G1	15	0.5193 ± 0.2963	0.9774 <sup>3</sup>	4	11	0.001 <sup>4</sup>
G2	9	0.4835 ± 0.3272		5	4	
G3	21	0.4894 ± 0.3086		18	3	
ARSA IHC expression						
Low	21	0.5164 ± 0.2751	0.7561 <sup>3</sup>	13	8	> 0.99 <sup>2</sup>
High	24	0.4835 ± 0.3275		14	10	
ARSB IHC expression						
Low	11	0.4849 ± 0.3034	0.9613 <sup>1</sup>	4	7	0.08 <sup>2</sup>
High	34	0.5034 ± 0.3050		23	11	
Maspin IHC expression						
Positive	22	0.5066 ± 0.2981	0.9878 <sup>1</sup>	18	4	0.005 <sup>2</sup>
Negative	23	0.4979 ± 0.3175		9	14	

<sup>1</sup>Mann-Whitney test.<sup>2</sup>Fisher's exact test.<sup>3</sup>Kruskal-Wallis test.<sup>4</sup> $\chi^2$  test. Significant differences are shown in bold. G1: Well differentiated; G2: Moderately differentiated; G3: Poorly differentiated, 4 mucinous.

accelerated (metabolic reprogramming), compared with normal metabolism, as it was previously described in literature<sup>[20]</sup>, arylsulfatases might be upregulated and induce tumor cells aggressiveness. It was previously proved that metabolic adaptation of tumor cells is realized through aerobic glycolysis, also known as Warburg effect<sup>[20]</sup>, which is mediated by arylsulfatases and is dysregulated in metabolic disorders<sup>[4]</sup>. As regarding maspin, it was previously proved that its expression is correlated with hypoxic-induced angiogenesis, via VEGF-A (Vascular Endothelial Growth Factor), in

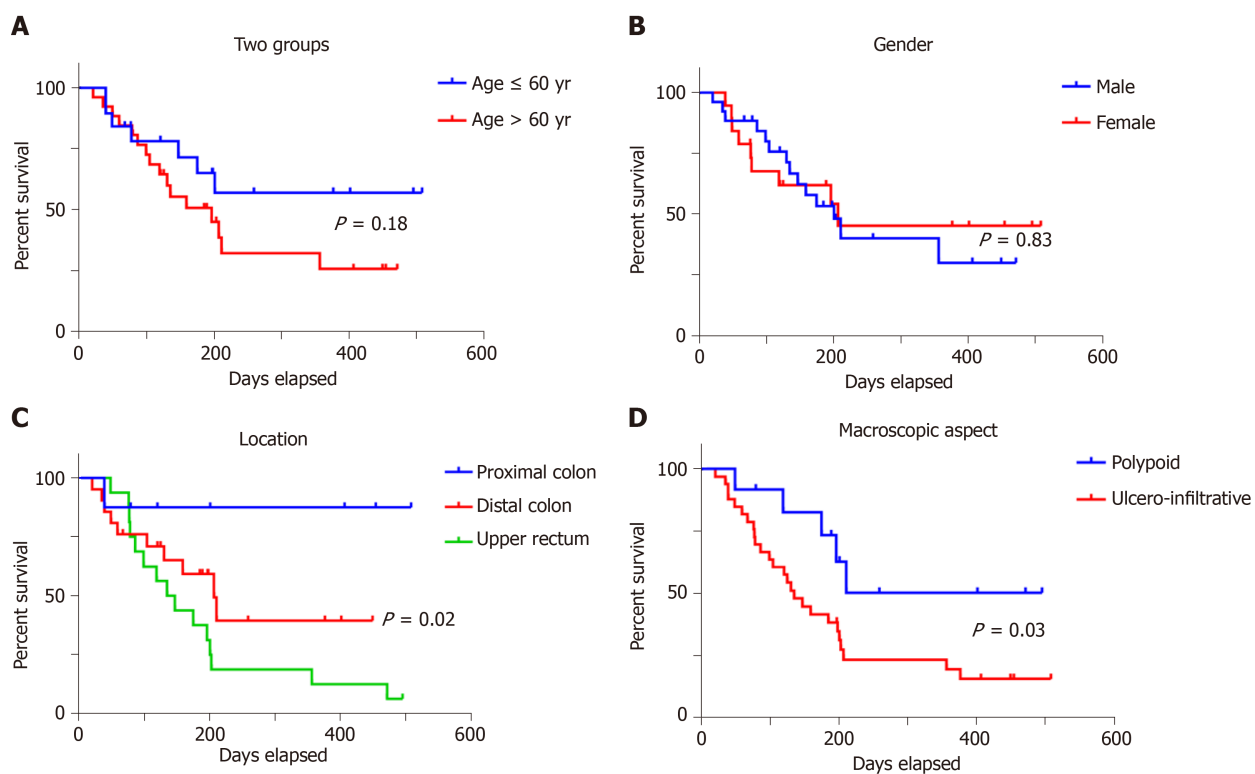




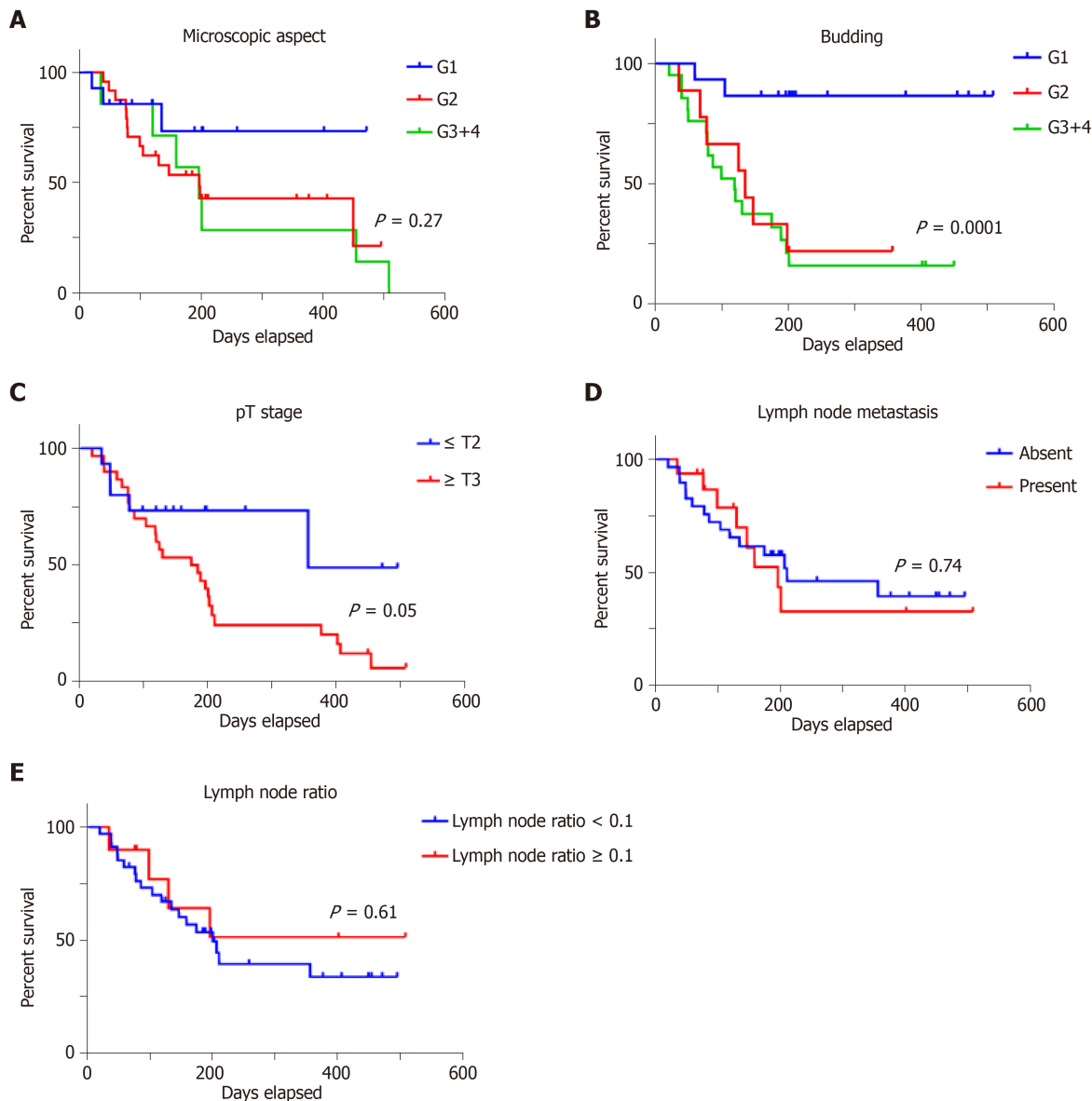
**Figure 2** One third of colorectal cancer specimens (14/45 cases) show triple positivity for ARSA/ARSB/maspin and low circulating *ARSB* gene expression level (A), as independent negative prognostic factor (B); the overall survival also depends on independently evaluation of protein tissue level of ARSA (C), ARSB (D) and maspin (E).

several malignant tumors such as gastric carcinoma<sup>[21]</sup>, squamous cell carcinoma<sup>[22]</sup>, liposarcoma<sup>[23]</sup> and CRC<sup>[11]</sup>. As this showed that, a maspin-arylsulfatases interaction might exist, we can suppose that this interaction evokes the genetically-induced dysregulation of tumor cell metabolism.

Further studies are needed to elucidate the molecular mechanism associated with arylsulfatases in colorectal carcinogenesis and the behavior of this tumor.



**Figure 3** In colorectal cancer, overall survival rate is not influenced by patients' age (A) or gender (B) but depends on tumor localization (C) and macroscopic aspect (D).



**Figure 4** In colorectal adenocarcinomas, overall survival rate does not depend on grade of differentiation (A) but is strongly influenced by tumor budding degree (B); a slightly correlation is proved with depth of infiltration (C) but lymph node status is not an independent prognostic factor (D, E).

## ARTICLE HIGHLIGHTS

### Research background

Arylsulfatase A and B (ARSA, ARSB) are lysosomal enzymes playing an important role in cellular metabolism. It is described in the literature the genetic condition characterized by a total deficiency of these two enzymes but few aspects are known about their role in carcinogenesis and evolution of colorectal cancer (CRC).

### Research motivation

As no data about the correlation of ARSA, ARSB and maspin were published yet, this study is original and represents first step in understanding the arylsulfatases influence upon malignant cells.

### Research objectives

This paper aimed to compare the immunohistochemical (IHC) stain and gene expression profile of ARSA and ARSB in CRC. Correlation with maspin and classical clinicopathological parameters was also done.

### Research methods

For IHC study, the expression of ARSA, ARSB and maspin were quantified in the cytoplasm of CRC cells. For gene expression study circulating mRNA, using Roche HighPure RNA kit, was isolated from all patients before surgery. A group of 45 healthy patients without inflammatory or

tumor pathologies was used as a control group. Reverse transcription and Taqman Gene Expression Array were used for Arylsulfatase B gene expression. Statistical analysis was performed using GraphPad Prims8 software using the  $\chi^2$  and Kruskal-Wallis test, while the Mann-Whitney test was used for ARSB gene expression, considering a *P*-value < 0.05 (with a 95% confidence interval) statistically significant.

### Research results

The preoperative gene expression level of ARSB was significantly decreased in patients with CRC (RQ < 1), compared with the control group (RQ > 1). A more significant decrease (RQ < 0.5) occurred in ulcero-infiltrative maspin-positive adenocarcinomas, with a higher degree of tumor budding, diagnosed in locally advanced stages (T3/4). The ARSB protein level in surgical specimens was inversely correlated with the preoperative ARSB gene circulating level.

### Research conclusions

High IHC expression of ARSA and ARSB, correlated with maspin positivity can be used as indicators of prognosis of CRC. Triple positivity of ARSA/ARSB/maspin correlated with an ARSB gene circulating level of <0.5, is an indicator of a lower survival rate, independently of the other clinicopathological parameters.

### Research perspectives

Based on the high IHC and low gene expression of ARSB, further investigations should be done, to elucidate the precise mechanism of this contradictory protein-gene expression profile.

## REFERENCES

- Rosso T, Malvezzi M, Bosetti C, Bertuccio P, Negri E, La Vecchia C. Cancer mortality in Europe, 1970-2009: an age, period, and cohort analysis. *Eur J Cancer Prev* 2018; **27**: 88-102 [PMID: 27472086 DOI: 10.1097/CEJ.0000000000000282]
- Obuch JC, Ahnen DJ. Colorectal Cancer: Genetics is Changing Everything. *Gastroenterol Clin North Am* 2016; **45**: 459-476 [PMID: 27546843 DOI: 10.1016/j.gtc.2016.04.005]
- Echeverri Olga Y, Salazar Diego A, Rodríguez-Lopez A, Janneth G, Almeciga-Díaz Carlos J, Barrera Luis A. Understanding the Metabolic Consequences of Human Arylsulfatase A Deficiency through a Computational Systems Biology Study. *Cent Nerv Syst Agents Med Chem* 2017; **17**: 72-76 [PMID: 27160716 DOI: 10.2174/1871524915666160510124150]
- Kovacs Z, Jung I, Gurzu S. Arylsulfatases A and B: From normal tissues to malignant tumors. *Pathol Res Pract* 2019; **215**: 152516 [PMID: 31262576 DOI: 10.1016/j.prp.2019.152516]
- Ittiwut C, Boonbuamas S, Srichomthong C, Ittiwut R, Suphapeetiporn K, Shotelersuk V. Novel Mutations, Including a Large Deletion in the ARSB Gene, Causing Mucopolysaccharidosis Type VI. *Genet Test Mol Biomarkers* 2017; **21**: 58-62 [PMID: 27797586 DOI: 10.1089/gtmb.2016.0221]
- Prabhu SV, Bhattacharyya S, Guzman-Hartman G, Macias V, Kajdacsy-Balla A, Tobacman JK. Extra-lysosomal localization of arylsulfatase B in human colonic epithelium. *J Histochem Cytochem* 2011; **59**: 328-335 [PMID: 21378286 DOI: 10.1369/0022155410395511]
- Bhattacharyya S, Tobacman JK. Arylsulfatase B regulates colonic epithelial cell migration by effects on MMP9 expression and RhoA activation. *Clin Exp Metastasis* 2009; **26**: 535-545 [PMID: 19306108 DOI: 10.1007/s10585-009-9253-z]
- Bhattacharyya S, Feferman L, Tobacman JK. Increased expression of colonic Wnt9A through Sp1-mediated transcriptional effects involving arylsulfatase B, chondroitin 4-sulfate, and galectin-3. *J Biol Chem* 2014; **289**: 17564-17575 [PMID: 24778176 DOI: 10.1074/jbc.M114.561589]
- Gurzu S, Szentirmay Z, Jung I. Molecular classification of colorectal cancer: a dream that can become a reality. *Rom J Morphol Embryol* 2013; **54**: 241-245 [PMID: 23771065 DOI: 10.1159/000350687]
- Gurzu S, Szentirmay Z, Toth E, Jung I. Possible predictive value of maspin expression in colorectal cancer. *Recent Pat Anticancer Drug Discov* 2013; **8**: 183-190 [PMID: 22963136 DOI: 10.2174/157489213805290619]
- Gurzu S, Szentirmay Z, Popa D, Jung I. Practical value of the new system for Maspin assessment, in colorectal cancer. *Neoplasma* 2013; **60**: 373-383 [PMID: 23581409 DOI: 10.4149/neo\_2013\_049]
- Banias L, Gurzu S, Kovacs Z, Bara T, Bara T, Jung I. Nuclear maspin expression: A biomarker for budding assessment in colorectal cancer specimens. *Pathol Res Pract* 2017; **213**: 1227-1230 [PMID: 28780084 DOI: 10.1016/j.prp.2017.07.025]
- Ueno H, Kajiwaru Y, Shimazaki H, Shinto E, Hashiguchi Y, Nakanishi K, Maekawa K, Katsurada Y, Nakamura T, Mochizuki H, Yamamoto J, Hase K. New criteria for histologic grading of colorectal cancer. *Am J Surg Pathol* 2012; **36**: 193-201 [PMID: 22251938 DOI: 10.1097/PAS.0b013e318235edee]
- Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Fléjou JF, Hansen TP, Hartmann A, Kakar S, Langner C, Nagtegaal I, Puppa G, Riddell R, Ristimäki A, Sheahan K, Smyrk T, Sugihara K, Terris B, Ueno H, Vieth M, Zlobec I, Quirke P. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. *Mod Pathol* 2017; **30**: 1299-1311 [PMID: 28548122 DOI: 10.1038/modpathol.2017.46]
- Marusteri M, Bacarea V. Comparing groups for statistical differences: how to choose the right statistical test? *Biochem Med* 2010; **20**: 15-32 [DOI: 10.11613/BM.2010.004]
- Vicente CM, Lima MA, Yates EA, Nader HB, Toma L. Enhanced tumorigenic potential of colorectal cancer cells by extracellular sulfatases. *Mol Cancer Res* 2015; **13**: 510-523 [PMID: 25477293 DOI: 10.1158/1541-7786.MCR-14-0372]
- Bhattacharyya S, Feferman L, Han X, Ouyang Y, Zhang F, Linhardt RJ, Tobacman JK. Decline in arylsulfatase B expression increases EGFR expression by inhibiting the protein-tyrosine phosphatase SHP2 and activating JNK in prostate cells. *J Biol Chem* 2018; **293**: 11076-11087 [PMID: 29794138 DOI: 10.1074/jbc.RA117.001244]
- Bhattacharyya S, Tobacman JK. Hypoxia reduces arylsulfatase B activity and silencing arylsulfatase B replicates and mediates the effects of hypoxia. *PLoS One* 2012; **7**: e33250 [PMID: 22428001 DOI: 10.1371/journal.pone.0033250]

- 10.1371/journal.pone.0033250]
- 19 **Bhattacharyya S**, Feferman L, Tobacman JK. Arylsulfatase B regulates versican expression by galectin-3 and AP-1 mediated transcriptional effects. *Oncogene* 2014; **33**: 5467-5476 [PMID: [24240681](#) DOI: [10.1038/onc.2013.483](#)]
  - 20 **Romero-Garcia S**, Lopez-Gonzalez JS, Báez-Viveros JL, Aguilar-Cazares D, Prado-Garcia H. Tumor cell metabolism: an integral view. *Cancer Biol Ther* 2011; **12**: 939-948 [PMID: [22057267](#) DOI: [10.4161/cbt.12.11.18140](#)]
  - 21 **Gurzu S**, Kadar Z, Sugimura H, Orlowska J, Bara T, Bara T, Szederjesi J, Jung I. Maspin-related Orchestration of Aggressiveness of Gastric Cancer. *Appl Immunohistochem Mol Morphol* 2016; **24**: 326-336 [PMID: [26067133](#) DOI: [10.1097/PAI.0000000000000189](#)]
  - 22 **Ciortea CD**, Jung I, Gurzu S, Kóvecsi A, Turdean SG, Bara T. Correlation of angiogenesis with other immunohistochemical markers in cutaneous basal and squamous cell carcinomas. *Rom J Morphol Embryol* 2015; **56**: 665-670 [PMID: [26429157](#)]
  - 23 **Jung I**, Gurzu S, Turdean S, Ciortea D, Sahlean DI, Golea M, Bara T. Relationship of endothelial area with VEGF-A, COX-2, maspin, c-KIT, and DOG-1 immunoreactivity in liposarcomas versus non-lipomatous soft tissue tumors. *Int J Clin Exp Pathol* 2015; **8**: 1776-1782 [PMID: [25973067](#)]



Published By Baishideng Publishing Group Inc  
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA  
Telephone: +1-925-2238242  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <https://www.f6publishing.com/helpdesk>  
<https://www.wjgnet.com>

