Int J Clin Exp Pathol 2014;7(9):6056-6063
www.ijcep.com /ISSN:1936-2625/IJCEP0001562

Original Article

Alpha B-crystallin correlates with poor survival in colorectal cancer

Chuanbing Shi[1*], Zhenyu He[2*], Ning Hou[3], Yijiang Ni[4], Lin Xiong[5], Pingsheng Chen[1]

[1]Department of Pathology and Pathophysiology, School of Medicine, Southeast University, Nanjing 210009, China; [2]Department of General Surgery, The Second Affiliated Hospital of Nanjing Medical University, Nanjing 210000, China; [3]Department of Pathology, Jiangsu Cancer Hospital, Nanjing 210000, China; [4]Department of Traumatic Surgery, Changzhou No. 2 People's Hospital Affiliated with Nanjing Medical University, Changzhou 213000, China; [5]Department of Pathology, The Second Affiliated Hospital of Nanjing Medical University, Nanjing 210000, China. [*]Equal contributors.

Received July 27, 2014; Accepted August 23, 2014; Epub August 15, 2014; Published September 1, 2014

Abstract: Alpha B-crystallin (CRYAB) is primarily found as major structural proteins of the ocular lens and is a principal member of the small heat shock protein (HSP) family. So far, CRYAB has been suggested to play critical roles in the development of several kinds of human cancers. However, the association between CRYAB expression and clinicopathological characteristics of colorectal cancer (CRC) has not been elucidated yet. In the present study, one-step quantitative PCR reverse transcription-polymerase chain reaction (qPCR) analysis of 18 samples of CRC and immunohistochemistry (IHC) analysis with 100 cases of CRC sample in tissue microarrays (TMA) were employed to evaluate the expression of CRYAB in CRC. The results suggested that CRYAB expression in the mRNA and protein levels was significantly higher in CRC tissues than in corresponding non-cancerous tissues ($P < 0.05$ and $P = 0.014$, respectively). The expression of CRYAB protein in CRC was significantly associated with distant metastasis ($P = 0.040$) and overall survival ($P = 0.003$). Kaplan-Meier method and multivariate survival analysis indicated that high expression of CRYAB ($P = 0.040$) and distant metastasis ($P = 0.005$) showed significant correlations with poor prognosis of CRC patients. The data imply that CRYAB expression is correlated with substantial clinical characteristics of CRC, and it may be identified as an unfavorable prognostic factor for CRC.

Keywords: CRYAB, CRC, qPCR, TMA, IHC

Introduction

Colorectal cancer (CRC) is third most prevalent cancer worldwide and the fourth of the leading causes of cancer mortality globally, accounting for almost 8% of all cancer deaths [1, 2]. Although the incidence of CRC in China is relatively lower than that in western countries, it has been increasing rapidly in recent years, particularly in developed regions [3, 4]. It is estimated that 25% of CRC patients experienced distant metastases and more than 50% of those with earlier-stage presentations developed metastatic disease [5, 6]. As a result, the CRC patients with metastasis encountered a dismal prognosis, with a 5-year survival rate of approximately 10% [7]. Over the last two decades, despite numerous therapeutic modalities for CRC treatment developed remarkably, including surgical resection, standard chemotherapy and adjuvant therapies, recurrence and metastasis remain the two most challenging problems in clinic and the overall survival rates of CRC patients stay disappointing [8]. Since accurate prognosis is essential for selecting the most effective treatment, considerable interests have been created in identifying factors that influence the prognosis of CRC patients. For now, the most widely studied prognostic factors are generally related to the pathological attributes of CRC, including tumor size, differentiation grade, TNM stage, and metastasis status [9]. However, these currently used predictors only provide broad categorization of risk and fail to exclusively identify biological characteristics of CRC patients. Hence, it is critical to screen novel and specific molecular biomarkers to stratify patients' outcomes, which would be

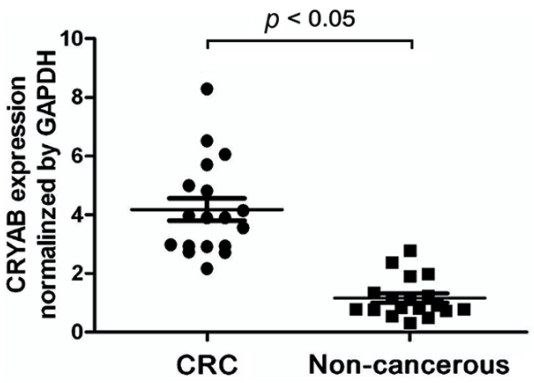

Figure 1. One-step quantitative real-time polymerase chain reaction (qPCR) was performed to evaluate the expression of CRYAB mRNA in colorectal cancer (CRC) tissues and matched non-cancerous tissues. Expression of CRYAB mRNA in CRC tissues (4.19 ± 0.351) was significantly higher than that of in matched tumor adjacent non-cancerous tissues (1.16 ± 0.160) ($P < 0.05$), when normalized to the GAPDH internal control.

helpful in the formulation of therapeutic strategies and further improvement of patients' survival in CRC.

Alpha B-crystallin (CRYAB) is primarily found as major structural proteins of the ocular lens and is a principal member of the small heat shock protein (HSP) family [10]. It is widely accepted that CRYAB acts as a molecular chaperone by preventing the aggregation of denatured proteins after the exposure to stresses including heat shock, radiation, and oxidative stress [11]. In addition to being a molecular chaperone, CRYAB is suggested to play important role in apoptosis inhibition, cellular protection, and proteasomal interactions [12]. Until recently, the importance of CRYAB in cancer development has drawn great attention and a growing number of studies indicate the intricate relationship between CRYAB expression and various human cancers. For instance, increased CRYAB expression was detected in mammary metaplastic carcinomas and CRYAB overexpression promoted brain metastasis in breast cancer, while silencing CRYAB inhibited distant metastasis [13, 14]. CRYAB regulates apoptosis by acting as an anti-apoptotic molecule and enhances tumorigenesis by modulating vascular endothelial growth factor (VEGF) [15]. Several studies reported the prognostic role of CRYAB in different human cancers and CRYAB appeared to be oncogenic [10, 16, 17]. However, the relationship between CRYAB expression and the clinicalpathological attributes of CRC is rarely evaluated. Whether CRYAB also acts oncogenically in CRC and whether CRYAB could be identified as a valuable biomarker for CRC prognosis or other important clinical items, related studies are of great importance.

In this present retrospective study, we detected the mRNA and protein expression of CRYAB in CRC, by using one step quantitative reverse transcription-polymerase chain reaction (qPCR) test in fresh tumor samples and immunohistochemistry (IHC) analysis in tissue microarray (TMA) respectively. The associations of CRYAB expression with the clinicopathological parameters of CRC, especially prognostic status, were further evaluated.

Materials and methods

CRC patient specimens

Formalin-fixed, paraffin-embedded tumor samples from 100 CRC cases and 94 matched peritumoral tissue specimens were collected in CRC patients from the Department of Pathology, the Affiliated Hospital of Nantong University from 2002 to 2007. All cases were reevaluated by two independent pathologists and the final decision was made by consensus if a conclusion differed. The original clinical data were collected from hospital medical records, including gender, age, tumor size, tumor location, histological type, tumor differentiation, serum CEA level, lymph node metastasis, distant metastasis, as well as TNM stage which was classified using the 7th American Joint Committee on Cancer (AJCC) staging system for CRC and overall survival status. None of the patients received radiotherapy, chemotherapy, or immunotherapy prior to enterectomy. A written informed consent and related pictures were also collected from each patient for publication of this present research. The study protocol was approved by the Human Research Ethics Committee of local hospital.

Quantitative real-time polymerase chain reaction (qPCR) analysis

Eighteen fresh CRC cancer tissue samples and 18 normal tumor-adjacent tissue samples were collected from the Department of Pathology, Affiliated Cancer Hospital of Nanjing Medical University, for qPCR analysis. Total RNA extrac-

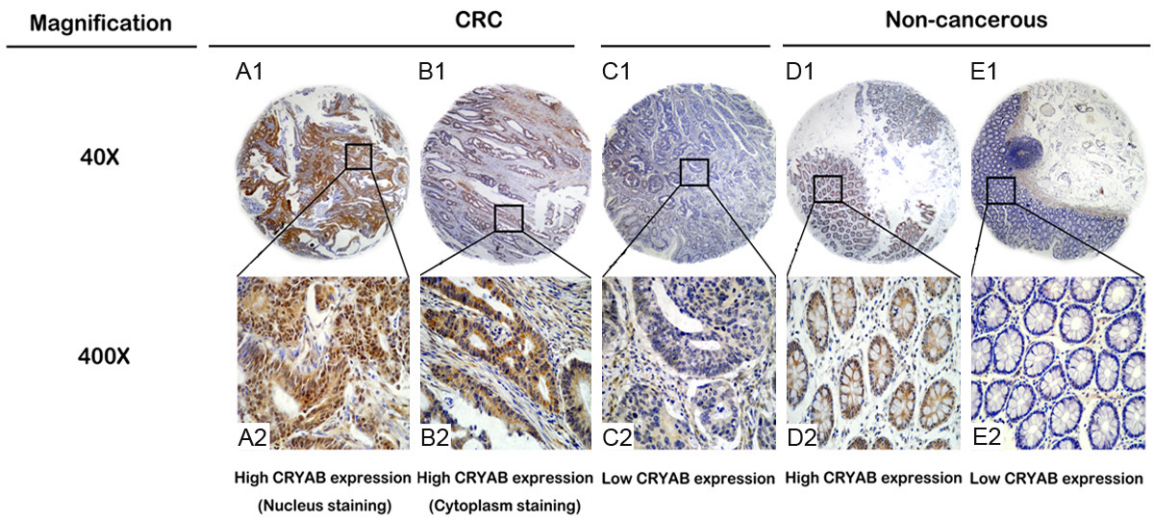

Figure 2. Representative figures of CRYAB protein expression in colorectal cancer (CRC) tissues and non-cancerous tissues by immunohistochemistry (IHC) analysis. A1 and A2. High expression of CRYAB in nucleus of CRC cells. B1 and B2. High expression of CRYAB in cytoplasm of CRC cells. C1 and C2. Low expression of CRYAB in CRC cells. D1 and D2. High expression of CRYAB in non-cancerous cells. E1 and E2. Low expression of CRYAB in non-cancerous cells. Magnification ×40 in A1, B1, C1, D1 and E1; ×400 in A2, B2, C2, D2 and E2.

tion, quality control, and one-step qRT-PCR analysis were performed as previously described [18]. The primers for CRYAB were as follows: forward primer 5'-CTT TGA CCA GTT CTT CGG AG-3' and reverse primer 5'-CCT CAA TCA CAT CTC CCA AC-3'. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA level was employed to standardize the measurements of the target gene and the primers for GAPDH were as follows: forward primer 5'-TGC ACC ACC AAC TGC TTA GC-3' and reverse primer 3'-GGC ATG GAC TGT GGT CAT GAG-5'. Amplification conditions consisted of 30 min at 42°C for reverse transcription and 2 min at 94°C for Taq activation, followed by 35 cycles at 95°C for 20 s, 56°C for 20 s, and elongation at 72°C for 30 s. Each measurement was performed in triplicate.

Tissue microarrays (TMA) construction and Immunohistochemistry (IHC) analysis

A total of 100 CRC and 94 normal tumor-adjacent tissues were enrolled in this present study. TMA was produced by Xinchao Biotech Co., Ltd. (Shanghai, China). Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded sections and arranged in the new recipient paraffin blocks. Tissue microarray was cut into 4-μm sections and placed on super frost charged glass microscope slides. IHC analysis was performed as previously described [18]. Deparaffinized sections (4 μm thick) from array blocks were separately stained on an Autostainer Universal Staining System (LabVision, Kalamazoo, MI, USA) using mouse anti-CRYAB antibody (1:400, Stressgen, Victoria, Canada). The secondary antibody used was horseradish peroxidase-conjugated antibody (Dako Cytomation, Carpinteria, CA, USA).

Blind CRYAB immunostaining observation and evaluation were simultaneously performed by two independent pathologists. IHC results were analyzed according to a previously described method [19]. Staining intensity was scored as follows: 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). The percentage of CRYAB-positive cells was also classified into 4 categories, where 1 was given for 0-10%, 2 for 11-50%, 3 for 51-80%, and 4 for 81-100%. The product of the intensity and percentage scores gave rise to the final CRYAB staining score [18, 19]. The degree of CRYAB staining was sorted by a two-level grading system, and staining scores were described as follows: a score below 3 (< 3) suggested low expression of CRYAB, while a score above 3 (≥ 3) suggested high expression of CRYAB.

Statistical analysis

The CRYAB mRNA expression normalized to GAPDH in CRC samples compared with match-

Table 1. Correlation of CRYAB expression with clinicopathological characteristics of 100 CRC patients

Groups	No.	CRYAB		χ^2	*P* value
		+	-		
Gender					
Male	61	36	25	0.066	0.797
Female	39	22	17		
Age (years)					
≥ 60	66	37	29	0.300	0.584
< 60	34	21	13		
Tumor size (cm)					
≥ 5	43	21	22	2.600	0.107
< 5	57	37	20		
Tumor location					
Colon	86	52	34	2.265	0.322
Rectum	10	5	5		
Ileocecal junction	4	1	3		
Histological type					
Adenocarcinoma	97	57	40	1.457	0.483
Squamous carcinoma	2	1	1		
Spindle cell carcinoma	1	0	1		
Tumor differentiation					
Well	4	4	0	2.913	0.233
Moderately	81	47	34		
Poorly	6	4	2		
Unknown	9	3	6		
Serum CEA level (ng/ml)					
≥ 15	11	7	4	0.332	0.565
< 15	59	32	27		
Unknown	30	19	11		
Lymph node metastasis					
Positive	34	19	15	0.095	0.758
Negative	66	39	27		
Distant metastasis					
Positive	19	15	4	4.225	0.040*
Negative	81	43	38		
TNM stage					
Stage I, II	61	31	30	3.310	0.069
Stage III, IV	39	27	12		
Overall survival					
Alive	59	27	32	8.846	0.003*
Dead	41	31	10		

*$P < 0.05$.

ing non-cancerous tissue samples was analyzed with the Wilcoxon non-parametric signed-rank test. The associations between CRYAB expression and clinicopathologic parameters of CRC were evaluated with χ^2 tests. Survival curves were produced using the Kaplan-Meier method. Univariate and multivariate Cox regression model were performed to detect the prognostic elements. For all tests, the significance level for statistical analysis was set at $P < 0.05$. All data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL) and STATA 12.0 (Stata Corporation, College Station, TX).

Results

Evaluation of CRYAB mRNA expression by qPCR

Total RNA was extracted from 18 CRC tissues and matched tumor adjacent tissues, and then subjected to one-step qPCR to determine CRYAB mRNA expression. When normalizing to GAPDH, the means of CRYAB mRNA in CRC tissues and that of the corresponding non-cancerous tissues were calculated as 4.19 ± 0.351 and 1.16 ± 0.160, respectively ($t = 7.857$, $P < 0.05$). CRYAB expression in the CRC samples was approximately 3.6-fold higher than that in matched non-cancerous tissues (**Figure 1**).

Evaluation of CRYAB protein expression by IHC

High CRYAB expression was observed in 58 (58.0%) of the 100 CRC samples compared with only 38 (40.4%) of 94 matched normal tumor-adjacent tissue samples. The difference was statistically significant ($\chi^2 = 5.987$, $P = 0.014$) according to the χ^2 test analysis and this result was consistent with the previous qPCR test in which elevated CRYAB mRNA level was witnessed. As is shown in **Figure 2**, positive IHC staining was mainly localized in the cytoplasm and nucleus of CRC cells.

Association between CRYAB expression and clinicopathological characteristics of CRC

The association of high CRYAB expression with the clinicopathological characteristics of CRC patients is shown in **Table 1**. High CRYAB expression was associated with distant metastasis ($P = 0.040$) and overall survival ($P = 0.003$). In comparison, other important clinical items, including gender, age, tumor size, tumor location, histological type, tumor differentiation, serum CEA level, lymph node metastasis and TNM stage, were barely associated with CRYAB expression (**Table 1**).

Table 2. Univariate and multivariate analysis of prognostic factors in 100 CRC patients for overall survival

	Univariate analysis			Multivariate analysis		
	HR	*P* value	95% CI	HR	*P* value	95% CI
CRYAB expression						
High versus Low	2.52	0.011*	1.23-5.14	2.20	0.040*	1.04-4.70
Gender						
Male versus Female	1.51	0.219	0.78-2.92			
Age (years)						
≥ 60 versus < 60	0.87	0.658	0.46-1.64			
Tumor size (cm)						
≥ 5 versus < 5	1.30	0.391	0.71-2.41			
Tumor location						
Colon versus Rectum versus Ileocecal junction	0.72	0.418	0.33-1.59			
Histological type						
Adenocarcinoma versus Non-adenocarcinoma	1.09	0.934	0.15-7.92			
Tumor differentiation						
Well versus Moderately versus Poorly	1.89	0.222	0.68-5.27			
Serum CEA level (ng/ml)						
≥ 15 versus < 15	2.33	0.057	0.98-5.57			
Lymph node metastasis						
Positive versus Negative	2.80	0.001*	1.51-5.19	1.67	0.340	0.58-4.80
Distant metastasis						
Positive versus Negative	6.85	0.001*	3.58-13.11	3.77	0.005*	1.48-9.61
TNM stage						
Stage I-II versus Stage III-IV	0.25	0.001*	0.13-0.49	0.73	0.641	0.19-2.80

*$P < 0.05$.

Survival analysis

Based on univariate Cox regression analyses concerning potential predictive factors, high CRYAB expression ($P = 0.011$), lymph node metastasis ($P = 0.001$), distant metastasis ($P = 0.001$) and tumor TNM stage ($P = 0.001$) were closely involved in the overall survival of 100 CRC patients. For further confirmation, we completed multivariate Cox regression and the data showed that CRYAB expression ($P = 0.040$) and distant metastasis ($P = 0.005$) were two independent predictors of overall survival (**Table 2**). Kaplan-Meier survival curves in **Figure 3** indicated that CRC patients with high CRYAB expression and positive distant metastasis encountered significantly poor survival time.

Discussion

Crystallins, which are the major structural proteins in lens, are initially categorized into four major families: α, β, γ and δ. It is subsequently reported that α-, β- and γ-crystallins are found in almost all vertebrate lenses [17]. CRYAB is one member of α-crystallins which belong to HSP family and functions as a molecular chaperone to protect proteins from aggregation, thereby promoting cell survival [20]. Lately, CRYAB has been linked to many biologic characteristics in human cancers and a large number of studies report various relationships between CRYAB expression and different kinds of tumors. CRYAB assists apoptosis resistance by inhibiting caspase-3 activation and promots oncogenic transformation [21]. CRYAB supports cell migration and invasion and localizes to the infiltrative edge of malignant glioblastomas [22]. CRYAB enhances tumorigenesis by modulating vascular endothelial growth factor (VEGF) and confers anti-VEGF resistance in breast cancer [23, 24]. Moreover, several studies indicates that high expressions of CRYAB associates with poor prognosis of cancer patients [10, 16, 22, 25-27]. All the above infor-

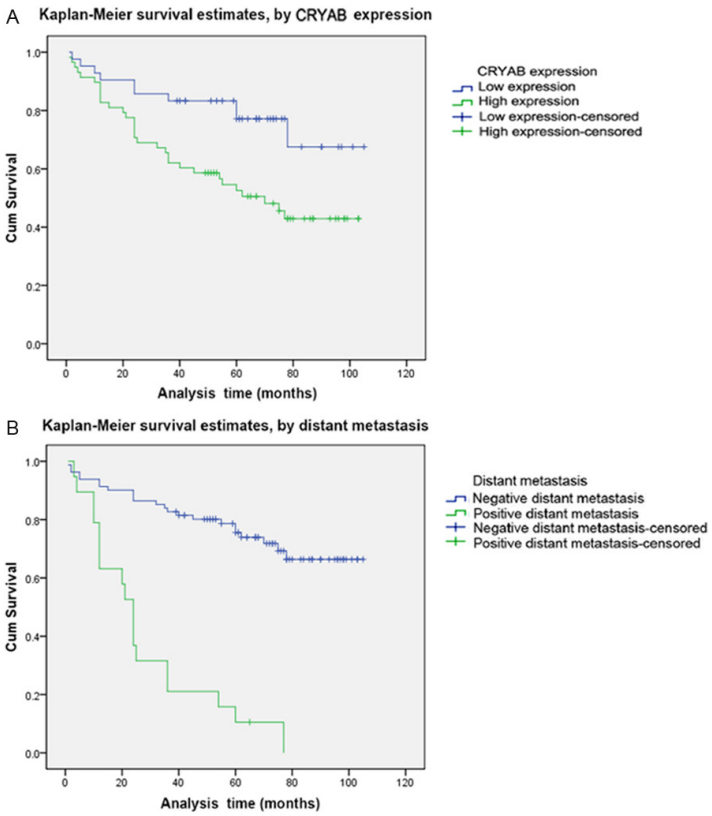

Figure 3. Survival analysis of 100 colorectal cancer (CRC) patients by Kaplan-Meier method. A. Overall survival rate in patients with high CRYAB expression (green line) was significantly lower than that in patients with low CRYAB expression (blue line). B. Overall survival rate in patients with positive distant metastasis (green line) was significantly lower than that in patients with negative distant metastasis (blue line).

mation signify a positive oncogenic characteristic of CRYAB. What is the performance of CRYAB in CRC and whether CRYAB could be used as a novel biomarker for diagnosis and prognosis in CRC, this present study was carried out.

We conducted qPCR test and IHC analysis to detect the expression CRYAB with CRC samples, in mRNA and protein level respectively. The results of qPCR test exhibited a statistically elevated level of mRNA expression of CRYAB in 18 fresh CRC tissue samples than that in matched non-cancerous tissues. Similarly, the data of IHC analysis in TMA with 102 CRC samples also indicated a higher level of CRYAB protein expression in CRC comparing to that in related non-cancerous tissues. It is clear that the results of qPCR and IHC analysis were consistent and hence suggested that CRYAB expression played an important role in CRC tumorigenesis. In addition, we found that high CRYAB protein expression was correlated with certain clinicopathological parameters, including distant metastasis and overall survival. Our results are in line with previous studies which reported the expression and characteristic in various types of human cancers [10, 16, 25].

For survival research, in univariate analysis mode, we identified that high CRYAB expression, lymph node metastasis, distant metastasis and tumor TNM stage were all significantly associated with the overall survival CRC patients. Furthermore, multivariate analysis was executed and the result confirmed that CRYAB protein expression and distant metastasis could be recognized as two independent prognostic factors for CRC prognosis. Kaplan-Meier curve also showed that CRC patients with high CRYAB expression and positive distant metastasis encountered a significantly poorer overall survival. The above data agree with several latest studies, which also elucidated the predictive role of CRYAB in certain cancers [16, 26, 27].

However, a number of studies stated the opposite function of CRYAB in which CRYAB showed inhibitory or at least irrelevant role in tumor development, such as in head and neck squamous cell carcinoma, nasopharyngeal carcinoma and lung cancer [28-30]. Our present data conflict with these studies. In my view, the exact role of CRYAB in human cancer is far from known and the characteristic of CRYAB in certain type of tumor could be affected by many elements, including the differences in the tumor types, antibody origins, or experiment protocols.

In conclusion, this study was the first to report the expression of CRYAB in CRC and investigate the relationship between CRYAB and clinical attributes of CRC patients, especially prognosis status. Our results indicated that CRYAB may be identified as a new prognostic biomarker for CRC. Further researches that enroll larger samples and elucidate the mechanisms of CRYAB action are necessary.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Pingsheng Chen, Department of Pathology and Pathophysiology, School of Medicine, Southeast University, Nanjing, China. Tel: +86-25-8327-2507; Fax: +86-25-8327-2507; E-mail: chenps@seu.edu.cn

References

[1] Huang F, Zhang Q, Ma H, Lv Q, Zhang T. Expression of glutaminase is upregulated in colorectal cancer and of clinical significance. Int J Clin Exp Pathol 2014; 7: 1093-100.

[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-917.

[3] Xu AG, Yu ZJ, Jiang B, Wang XY, Zhong XH, Liu JH, Lou QY, Gan AH. Colorectal cancer in Guangdong Province of China: a demographic and anatomic survey. World J Gastroenterol 2010; 16: 960-5.

[4] Li Y, Wei J, Xu C, Zhao Z, You T. Prognostic significance of cyclin d1 expression in colorectal cancer: a meta-analysis of observational studies. PLoS One 2014; 9: e94508.

[5] Bartlett DL, Chu E. Can metastatic colorectal cancer be cured? Oncology (Williston Park) 2012; 26: 266-75.

[6] Lundberg IV, Lofgren Burstrom A, Edin S, Eklof V, Oberg A, Stenling R, Palmqvist R, Wikberg ML. SOX2 Expression Is Regulated by BRAF and Contributes to Poor Patient Prognosis in Colorectal Cancer. PLoS One 2014; 9: e101957.

[7] Patel A, Sun W. Ziv-aflibercept in metastatic colorectal cancer. Biologics 2014; 8: 13-25.

[8] Ji D, Chen Z, Li M, Zhan T, Yao Y, Zhang Z, Xi J, Yan L, Gu J. MicroRNA-181a promotes tumor growth and liver metastasis in colorectal cancer by targeting the tumor suppressor WIF-1. Mol Cancer 2014; 13: 86.

[9] Chen C, Wang L, Liao Q, Huang Y, Ye H, Chen F, Xu L, Ye M, Duan S. Hypermethylation of EDNRB promoter contributes to the risk of colorectal cancer. Diagn Pathol 2013; 8: 199.

[10] Annertz K, Enoksson J, Williams R, Jacobsson H, Coman WB, Wennerberg J. Alpha B-crystallin - a validated prognostic factor for poor prognosis in squamous cell carcinoma of the oral cavity. Acta Otolaryngol 2014; 134: 543-50.

[11] den Engelsman J, van de Schootbrugge C, Yong J, Pruijn GJ, Boelens WC. Pseudophosphorylated alphaB-crystallin is a nuclear chaperone imported into the nucleus with help of the SMN complex. PLoS One 2013; 8: e73489.

[12] Ousman SS, Tomooka BH, van Noort JM, Wawrousek EF, O'Connor KC, Hafler DA, Sobel RA, Robinson WH, Steinman L. Protective and therapeutic role for alphaB-crystallin in autoimmune demyelination. Nature 2007; 448: 474-9.

[13] Chan SK, Lui PC, Tan PH, Yamaguchi R, Moriya T, Yu AM, Shao MM, Hliang T, Wong SI, Tse GM. Increased alpha-B-crystallin expression in mammary metaplastic carcinomas. Histopathology 2011; 59: 247-55.

[14] Malin D, Strekalova E, Petrovic V, Deal AM, Al Ahmad A, Adamo B, Miller CR, Ugolkov A, Livasy C, Fritchie K, Hamilton E, Blackwell K, Geradts J, Ewend M, Carey L, Shusta EV, Anders CK, Cryns VL. alphaB-crystallin: a novel regulator of breast cancer metastasis to the brain. Clin Cancer Res 2014; 20: 56-67.

[15] Nahomi RB, Wang B, Raghavan CT, Voss O, Doseff AI, Santhoshkumar P, Nagaraj RH. Chaperone peptides of alpha-crystallin inhibit epithelial cell apoptosis, protein insolubilization, and opacification in experimental cataracts. J Biol Chem 2013; 288: 13022-35.

[16] Tang Q, Liu YF, Zhu XJ, Li YH, Zhu J, Zhang JP, Feng ZQ, Guan XH. Expression and prognostic significance of the alpha B-crystallin gene in human hepatocellular carcinoma. Hum Pathol 2009; 40: 300-5.

[17] Chen P, Ji W, Liu FY, Tang HZ, Fu S, Zhang X, Liu M, Gong L, Deng M, Hu WF, Hu XH, Chen XW, Li ZL, Li X, Liu J, Li DW. Alpha-crystallins and tumorigenesis. Curr Mol Med 2012; 12: 1164-73.

[18] Fu M, Gu X, Ni H, Zhang W, Chang F, Gong L, Chen X, Li J, Qiu L, Shi C, Bao J. High expression of inositol polyphosphate phosphatase-like 1 associates with unfavorable survival in hepatocellular carcinoma. Int J Clin Exp Pathol 2013; 6: 2515-22.

[19] Ni S, Xu L, Huang J, Feng J, Zhu H, Wang G, Wang X. Increased ZO-1 expression predicts valuable prognosis in non-small cell lung cancer. Int J Clin Exp Pathol 2013; 6: 2887-95.

[20] Sanbe A. Molecular mechanisms of alpha-crystallinopathy and its therapeutic strategy. Biol Pharm Bull 2011; 34: 1653-8.

[21] Petrovic V, Malin D, Cryns VL. alphaB-crystallin promotes oncogenic transformation and inhibits caspase activation in cells primed for apoptosis by Rb inactivation. Breast Cancer Res Treat 2013; 138: 415-25.

[22] Ho PY, Chueh SC, Chiou SH, Wang SM, Lin WC, Lee IL, Yang HY, Peng HC, Lai MK. AlphaB-crystallin in clear cell renal cell carcinoma: tumor progression and prognostic significance. Urol Oncol 2013; 31: 1367-77.

[23] Kase S, He S, Sonoda S, Kitamura M, Spee C, Wawrousek E, Ryan SJ, Kannan R, Hinton DR. alphaB-crystallin regulation of angiogenesis by modulation of VEGF. Blood 2010; 115: 3398-406.

[24] Ruan Q, Han S, Jiang WG, Boulton ME, Chen ZJ, Law BK, Cai J. alphaB-crystallin, an effector of unfolded protein response, confers anti-VEGF resistance to breast cancer via maintenance of intracrine VEGF in endothelial cells. Mol Cancer Res 2011; 9: 1632-43.

[25] Mao Y, Zhang DW, Lin H, Xiong L, Liu Y, Li QD, Ma J, Cao Q, Chen RJ, Zhu J, Feng ZQ. Alpha B-crystallin is a new prognostic marker for laryngeal squamous cell carcinoma. J Exp Clin Cancer Res 2012; 31: 101.

[26] Moyano JV, Evans JR, Chen F, Lu M, Werner ME, Yehiely F, Diaz LK, Turbin D, Karaca G, Wiley E, Nielsen TO, Perou CM, Cryns VL. AlphaB-crystallin is a novel oncoprotein that predicts poor clinical outcome in breast cancer. J Clin Invest 2006; 116: 261-70.

[27] Volkmann J, Reuning U, Rudelius M, Hafner N, Schuster T, Becker V Ros A, Weimer J, Hilpert F, Kiechle M, Durst M, Arnold N, Schmalfeldt B, Meindl A, Ramser J. High expression of crystallin alphaB represents an independent molecular marker for unfavourable ovarian cancer patient outcome and impairs TRAIL- and cisplatin-induced apoptosis in human ovarian cancer cells. Int J Cancer 2013; 132: 2820-32.

[28] Boslooper K, King-Yin Lam A, Gao J, Weinstein S, Johnson N. The clinicopathological roles of alpha-B-crystallin and p53 expression in patients with head and neck squamous cell carcinoma. Pathology 2008; 40: 500-4.

[29] Lung HL, Lo CC, Wong CC, Cheung AK, Cheong KF, Wong N, Kwong FM, Chan KC, Law EW, Tsao SW, Chua D, Sham JS, Cheng Y, Stanbridge EJ, Robertson GP, Lung ML. Identification of tumor suppressive activity by irradiation microcell-mediated chromosome transfer and involvement of alpha B-crystallin in nasopharyngeal carcinoma. Int J Cancer 2008; 122: 1288-96.

[30] Campbell-Lloyd AJ, Mundy J, Deva R, Lampe G, Hawley C, Boyle G, Griffin R, Thompson C, Shah P. Is alpha-B crystallin an independent marker for prognosis in lung cancer? Heart Lung Circ 2013; 22: 759-66.