Tumor Biol. (2016) 37:3887–3895
DOI 10.1007/s13277-015-4255-8

ORIGINAL ARTICLE

Overexpression of Hexokinase 1 as a poor prognosticator in human colorectal cancer

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Received: 30 August 2015 / Accepted: 13 October 2015 / Published online: 17 October 2015

Abstract It has been suggested that hexokinase 1 (HK1) is involved in tumorigenesis. However, the expression dynamics of HK1 and its prognostic significance in human colorectal cancer (CRC) still remain unclear. The aim of the present study was to investigate the expression of HK1 and its prognostic significance in CRC. In this study, immunohistochemical analysis was used to examine the expression dynamics of HK1 in CRC tissues from two independent cohorts. Receiver operating characteristic curve analysis, Kaplan–Meier curves, and Cox regression analysis were utilized to investigate the prognostic significance. Results showed that a high expression of HK1 was observed in 106 of 393 (27.0 %) and 69 of 229 (30.1 %) of CRC in the training cohort and validation cohort, respectively. Further correlation analyses indicated that the increased HK1 expression was strongly correlated with the pN classification and TNM stage. Both cohorts showed a close association between the overexpression of HK1 and poorer overall survival. Importantly, multivariate analysis identified HK1 expression in CRC as an independent prognostic factor. Overexpression of HK1 may act as a significant biomarker of poor prognosis for patients with CRC.

Keywords Colorectal cancer · Hexokinase · Prognosis · Biomarker

Introduction

According to the global cancer statistics in 2011 [1], CRC was the second most common malignancy in females and the third in males. A 5-year survival rate for stage IV CRC patients was only 11.7 %. More than 1.2 million new CRC cancer cases and more than 600 thousand deaths occur each year worldwide. With the improvements in the diagnosis and treatment of CRC, the prognosis has improved over time. However, many CRC patients remain incurable [2, 3]. At present, predicting the prognosis of CRC patients mainly depends on the American Joint Committee on Cancer (AJCC) stage. However, this staging system is known to be imperfect due to the heterogeneous biological properties of CRC. Some reliable biomarkers, which can both guide treatment and help predict prognosis, have gradually come to the attention of clinician [4, 5]. It would be significant to identify suitable biomarkers for improving individualized treatment, even though previous studies exhibit a lack of appropriate qualities. In recent decades, more and more attention has been paid to the molecular metabolism, including glycolysis, of cancer cells.

The most important metabolic pathway for the growth and proliferation of cancer cells is glycolysis. The rapid cell growth and proliferation of cancer cells require high levels

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of energy consumption and result in an increased rate of glycolysis. Hexokinase is a kind of enzyme that catalyzes the conversion of glucose to glucose-6-phosphate at the first step in glycolysis. Four genes that encode Hexokinase (HK1, HK2, HK3, and HK4) have been found in vertebrates. The HK1 gene is localized in chromosome 10q22 and encodes a protein composed of 917 amino acids. HK1 is the first enzyme of glycolysis to be identified that couples cytosolic glycolysis to mitochondrial oxidative phosphorylation and produces ATP at mitochondrial outer membrane [6]. In addition, previous studies have suggested that HK1 also plays a role in regulating cell death, which may be associated with tumorigenesis and proliferation [7].

Recent studies showed that HK1 expression was upregulated in a variety of human cancers, such as breast, thyroid, kidney, and lung cancers, suggesting that HK1 may play an important role in tumorigenesis and progression [8–10]. Although the biological function of HK1 has been widely studied, the function and mechanism of HK1 in tumors remain unclear. In the present study, the expression dynamics of HK1 in the training and validation cohorts were examined using tissue microarray (TMA) and immunohistochemistry (IHC). The prognostic significance of HK1 expression in CRC was analyzed in these two cohorts.

Materials and methods

Patients and cohorts

In this study, to detect the association between the expression of HK1 and the prognosis of patients, paraffin-embedded pathological specimens from 622 patients with CRC who underwent initial resection were obtained from the Guangdong Institute of Gastroenterology, Sun Yat-sen University from January 1996 to December 2008 and the Sun Yat-sen University Cancer Center from January 2000 to November 2006 (Guangzhou, China). All CRC patients who underwent surgical resection were initially included. Three hundred and ninety-three patients were from the Guangdong Institute of Gastroenterology and 229 patients were from the Sun Yat-sen University Cancer Center. Patients who received neoadjuvant therapy were excluded from the study. MRI, CT, chest X-ray, abdominal ultrasonography, and/or bone scan were utilized to identify CRC-distant metastasis. The clinicopathological variables collected from the CRC database of the follow-up office included general information, surgical details, surgical techniques, degree of differentiation, depth of tumor invasion, nodal status, metastasis, and follow-up data. The pTNM status was assessed according to the criteria of the seventh edition of AJCC stage. All of the clinicopathological data of the enrolled patients were collected from the CRC database. The patients' follow-up was carried out according to an original plan. The patients were evaluated by MRI and/or CT at the hospital every 3 months for the first year, every 6 months for the second year, and annually thereafter. The primary endpoint was the overall survival (OS), which was defined as the time interval from surgery to death. The Guangdong Institute of Gastroenterology is attached to the Sixth Affiliated Hospital, Sun Yat-sen University. This study was approved by the Institutional Review Board (IRB) of the Sixth Affiliated Hospital and Cancer Center, Sun Yat-sen University.

Tissue microarray construction and immunohistochemical analysis

Tissue microarrays consist of paraffin blocks in which up to 1000 separate tissue cores are assembled in array fashion to allow multiplex histological analysis [11]. In the present study, TMA was constructed by two trained researchers. In the tissue microarray construction, tumor areas of each primary tumor was identified by two senior pathologists, then two cores (1 mm diameter) were punched from these representative tumor areas and deposited into a recipient block at defined positions with the tissue array instrument (Beecher Instruments, Alphelys, France).The recipient blocks were subsequently cut into 5-μm sections for IHC staining. TMA slides were deparaffinized and rehydrated through graded alcohols before being exposed to the antigen retrieval system (10 mM sodium citrate, 0.05 % Tween-20, pH 6.0 for 25 min). Endogenous peroxidase was blocked with 0.3 % hydrogen peroxide for 10 min at room temperature. After incubation with the primary rabbit polyclonal HK1 antibody (1:200, Cell Signaling Technology) overnight at 4 °C, the sections were stained with diaminobenzidine in an Envision system (DakoCytomation, Glostrup, Denmark). The slides were finally counterstained with hematoxylin (Zymed Laboratories Inc, San Francisco, CA, USA). A negative control was obtained by replacing the primary antibody with a normal rabbit IgG. Known immunostaining-positive slides were used as positive controls.

Evaluation of immunohistochemical analysis

Each TMA spot was assigned an intensity score from 0 to 3 (0, 1, 2, or 3) by two trained researchers. The number of positive tumor cells divided to the total number of tumor cells was assigned using 5 % increments (0 %, 5 %, 10 % . . . 100 %). *H* score (range of 0–300) was achieved by adding the sum

Table 1 Difference of the clinicopathological characteristics of two Hexokinase 1 expression levels in colorectal cancers

Variable	HK1 expression							
	Training cohort				Validation cohort			
	Cases	Low	High	*P* value*	Cases	Low	High	*P* value*
Age (years)				0.024*				0.363
≤60.0	200	156 (78.0 %)	44 (22.0 %)		129	87 (67.4 %)	42 (32.6 %)	
>60.0	193	131 (67.9 %)	62 (32.1 %)		100	73 (73.0 %)	27 (27.0 %)	
Gender				0.127				0.949
Female	173	133 (76.9 %)	40 (23.1 %)		87	61 (70.1 %)	26 (29.9 %)	
Male	220	154 (70.0 %)	66 (30.0 %)		142	99 (69.7 %)	43 (30.3 %)	
Differentiation				0.759				0.128
Well/moderate	330	240 (72.7 %)	90 (27.3 %)		193	131 (67.9 %)	62 (32.1 %)	
Poor	63	47 (74.6 %)	16 (25.4 %)		36	29 (80.6 %)	7 (19.4 %)	
pT classification				0.081				0.093
T1/T2	70	57 (81.4 %)	13 (18.6 %)		92	70 (76.1 %)	22 (23.9 %)	
T3/T4	323	230 (71.2 %)	93 (28.8 %)		137	90 (65.7 %)	47 (34.3 %)	
pN classification				0.024*				0.023*
N0	229	177 (77.3 %)	52 (22.7 %)		169	125 (74.0 %)	44 (26.0 %)	
N1	164	110 (67.1 %)	54 (32.9 %)		60	35 (58.3 %)	25 (41.7 %)	
pM classification				0.049*				0.141
M0	365	271 (74.2 %)	94 (25.8 %)		206	147 (71.4 %)	59 (28.6 %)	
M1	28	16 (57.1 %)	12 (42.9 %)		23	13 (56.5 %)	10 (43.5 %)	
TNM stage				0.038*				0.005*
I/II	219	169 (77.2 %)	50 (22.8 %)		150	114 (76.0 %)	36 (24.0 %)	
III/IV	174	118 (67.8 %)	56 (32.2 %)		79	46 (58.2 %)	33 (41.8 %)	

HK1 Hexokinase 1

*Significant at $P<0.05$

obtained from multiplying the intensity score by the proportion of area stained (*H* score=I1XP1+I2XP2+I3XP3). And a final *H* score was the average of two cores from the same representative tumor areas.

Selection of cut-point value

Receiver operating characteristic (ROC) curve analysis was applied to determine the cutoff score using the 0, 1-criterion [12]. At the final *H* score, the sensitivity and specificity for each outcome under study was plotted to generate various ROC curves. The score closest to the point with both maximum sensitivity and specificity was selected as the cutoff score. Tumors designated as "low expression" were those with scores below or equal to the cutoff value, whereas those designated as "high expression" were the tumors with scores above the cutoff value. The following clinicopathological features were dichotomized for ROC curve analysis: differentiation (well/moderate or poor), pT status (T1–T2 or T3–T4), pN status (N0 or N1), pM status (PM0 or pM1), TNM stage (I + II or III + IV), and survival (death due to CRC or censored [lost to follow-up, alive, or dead from other causes]).

Statistical analysis

Difference of the clinicopathological characteristics of two HK1 expression levels were analyzed with the chi-square test for categorical variables. Differences between quantitative data that meet normality and homogeneity were analyzed using Student's *t* test. The statistical significance of the survival analysis between the HK1 expression and patient survival was estimated by the Kaplan–Meier curves with a log-rank test. Multivariate Cox proportional hazard regression model was constructed using the forward stepwise method with an entry criterion of $P<0.05$ and a removal criterion of $P>0.10$. All of the statistical analyses were performed with the SPSS software version 16 (SPSS, Chicago, IL, USA). *P* values< 0.05 were considered to indicate statistical significance.

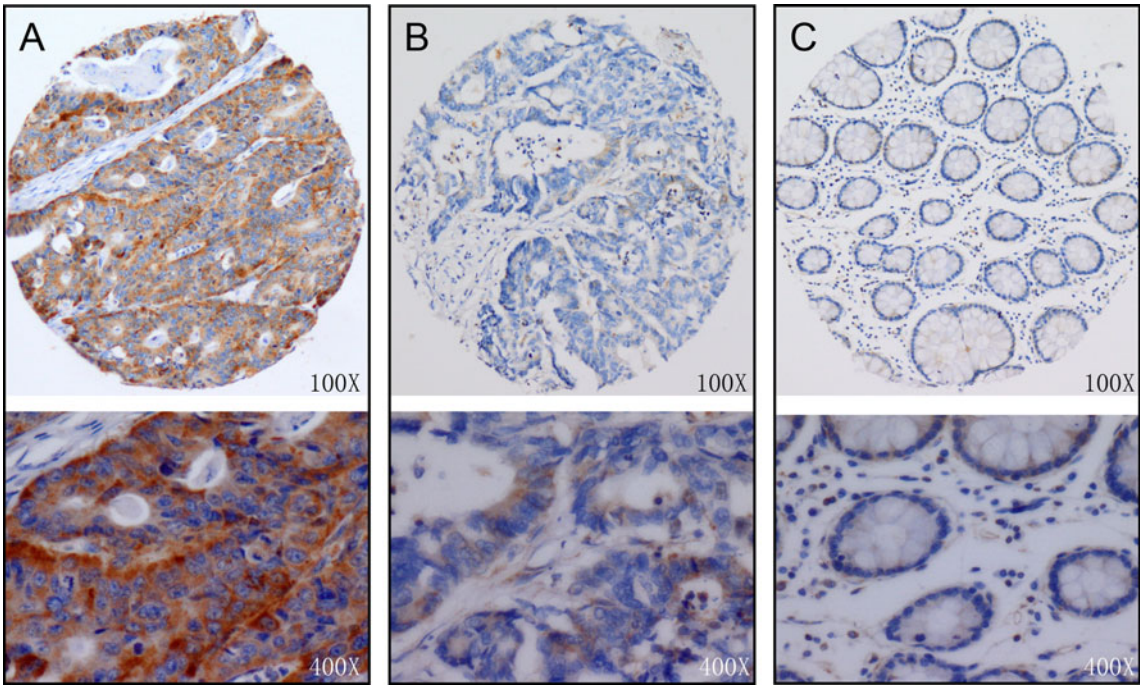

Fig. 1 IHC staining of representative high- and low-HK1-expressing samples of CRC and adjacent normal colorectal mucosa. **a** High expression of HK1 was observed in a CRC tissue, in which more than 90 % tumor cells revealed positive immunostaining of HK1. The intensity was assigned a score of 3. **b** A CRC case demonstrated a low expression of HK1, in which less than 60 % tumor cells showed immunoreactivity to HK1. The intensity was assigned a score of 1. **c** The corresponding adjacent mucosal tissue showed negative expression of HK1

Results

Patient characteristics and expression dynamics of HK1

A total of 622 CRC patients (393 patients in the training cohort and 229 patients in the validation cohort) were enrolled for TMA analysis in this study. Of these, 362 patients were male, and 260 were female. There were 369 stage I or II patients and 253 stage III or IV patients. The follow-up time for all of the patients ranged from 0.5 to 123.5 months (mean 58.4 months). During follow-up, a total of 179 patients died. In the training cohort, 220 patients were men (56.0 %) and 173 (44.0 %) were women, with a mean age of 58.7 years. The average follow-up time was 60.1 months. In the validation cohort, 142 (62.0 %) patients were men, and 87 (38.0 %) were women. The mean age was 57.3 years. The average follow-up time was 55.42 months. The detailed information of clinicopathological characteristics was showed in Table 1.

To investigate the pattern of HK1 expression in CRC tissues, 622 CRC specimens and corresponding normal colorectal mucosa were subjected to IHC analysis in this study. HK1 staining was mainly found on the cytoplasm (Fig. 1a–c) of CRC cells. The IHC staining of representative high- and low-HK1-expressing samples of CRC and adjacent normal colorectal mucosa is shown in Fig. 1.

Selection of optimal cut-point value for HK1 expression

ROC curve analysis was applied to determine a single optimal cutoff score for the expression dynamics of HK1 in various patterns. The *H* score of HK1 expression mentioned above ranged from 0 to 300. The ROC curves for each clinicopathologic characteristics clearly showed the point on the curve closest to (0.0, 1.0) that maximized both sensitivity and specificity of the outcome. According to the ROC curve analysis, the *H* score for HK1 expression above the cutoff value of 185 was defined as “high expression” and tumors with scores below or equal to the obtained cutoff value were considered “low expression”. According to the *H* score, 106 tumors were defined as “high expression” and 287 tumors were defined as “low expression” in the training cohort. And in the validation cohort, 69 tumors were considered as “high expression” and 160 tumors were considered as “low expression”. The corresponding AUCs are described in Fig. 2.

Association between HK1 expression and clinicopathological variables

In the training cohort, further analyses using chi-squared test showed that HK1 expression was significantly associated with age (P=0.024), pN classification (P=0.024), pM classification (P=0.049), and TNM stage (P=

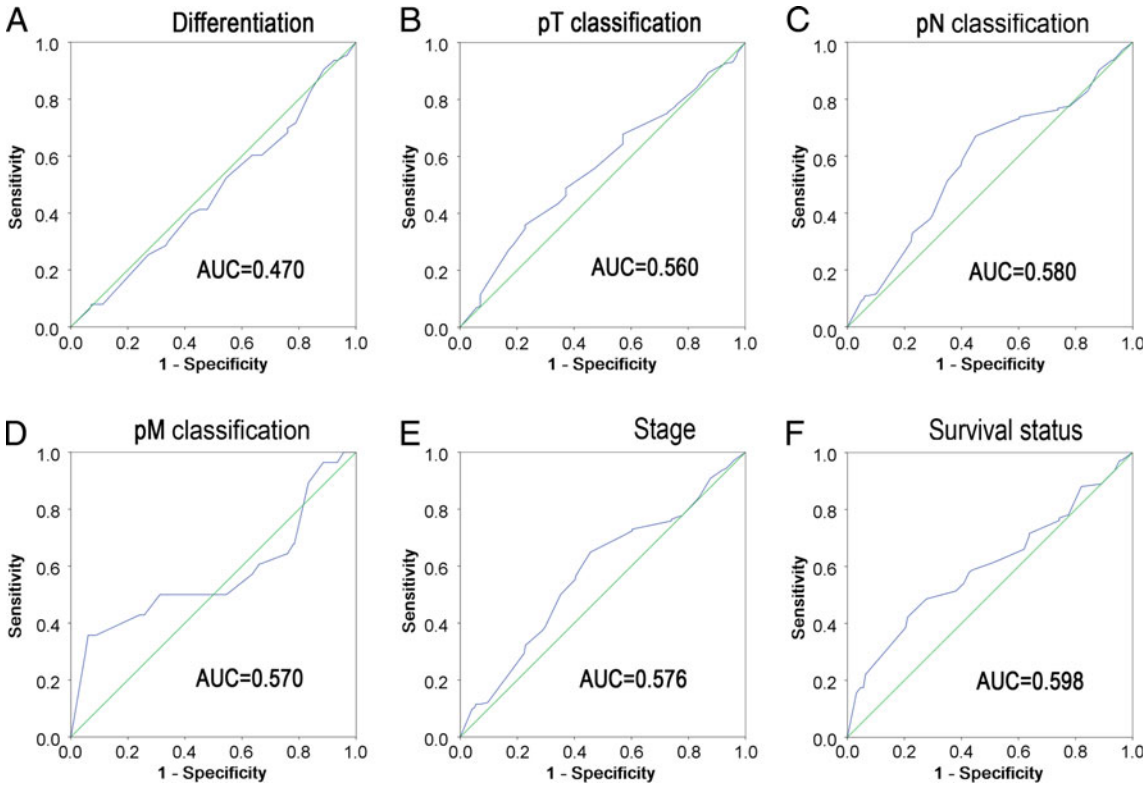

Fig. 2 ROC curve analysis was applied to determine the cutoff value for the expression dynamics of HK1 in colorectal carcinoma. The sensitivity and specificity for each outcome were plotted: **a** differentiation (AUC=0.470), **b** pT classification (AUC=0.560), **c** pN classification (AUC=0.580), **d** pM classification (AUC=0.570), **e** TNM stage (AUC=0.576), and **f** survival status (AUC=0.598), *AUC* area under the curve

0.038). In the validation cohort, significant differences were found in pN classification (P=0.023) and TNM stage (P=0.005) (Table 1). High HK1 expression was more common in patients with higher pN, pT (ns), TNM stage, or distant metastases (ns in validation cohort). Higher expression was also more common in older patients in the training cohort.

HK1 expression as a significant prognostic factor

In the training cohort, the assessment of survival using Kaplan–Meier analysis and a univariate Cox proportional hazard regression model revealed that a high expression of HK1 was correlated with worse overall survival (log-rank, P<0.0001 and P<0.001, Fig. 3 and Table 2). Moreover, other

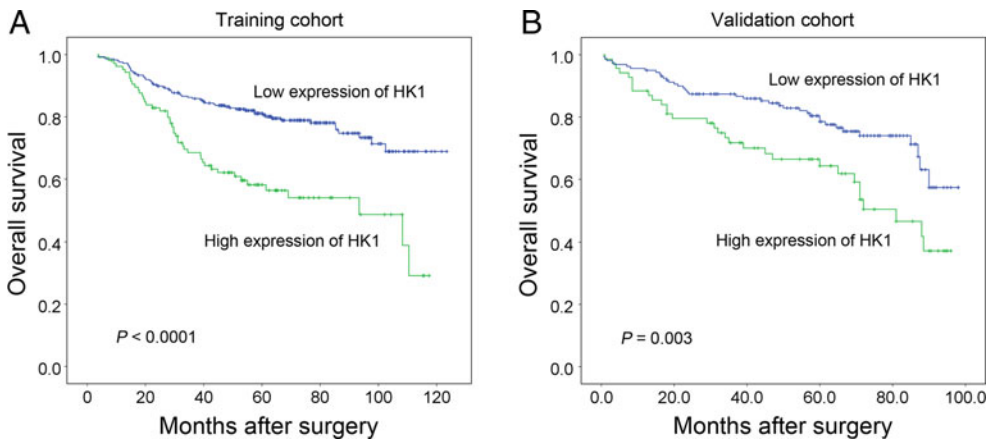

Fig. 3 Survival curves for the 393 CRC patients in the training cohorts and 229 patients in the validation cohorts according to the expression dynamics of HK1 (log-rank test). **a** Probability of survival of 393 CRC patients in the training cohorts: low expression of HK1, n=287; high expression of HK1, n=106 (P<0.0001). **b** Probability of survival of the 229 CRC patients in the validation cohort: low expression of HK1, n = 160; high expression of HK1, n=69 (P=0.003)

Table 2 Univariate analysis of Hexokinase 1 expression and clinicopathologic variables in patients with colorectal cancers (Cox proportional hazards regression)

Variable	Training cohort			Validation cohort		
	All cases	Hazard ratio (95 % CI)	*P* value*	All cases	Hazard ratio (95 % CI)	*P* value*
Age (years)			0.039*			0.341
≤60.0	200	1.0		129	1.0	
>60.0	193	1.496 (1.021–2.191)		100	1.257 (0.785–2.012)	
Gender			0.956			0.676
Female	173	1.0		87	1.0	
Male	220	0.989 (0.677–1.445)		142	1.110 (0.680–1.814)	
Differentiation			<0.001*			0.134
Well/moderate	330	1.0		193	1.0	
Poor	63	2.370 (1.537–3.653)		36	1.587 (0.867–2.902)	
pT classification			0.016*			0.001*
T1/T2	70	1.0		92	1.0	
T3/T4	323	2.098 (1.150–3.826)		137	2.758 (1.531–4.965)	
pN classification			<0.001*			<0.001*
N0	229	1.0		169	1.0	
N1	164	2.305 (1.574–3.376)		60	2.419 (1.505–3.888)	
pM classification			<0.001*			<0.001*
M0	365	1.0		206	1.0	
M1	28	9.500 (5.977–15.099)		23	8.501 (4.787–15.097)	
HK1 expression			<0.001*			0.004*
Low	287	1.0		160	1.0	
High	106	2.462 (1.680–3.607)		69	1.997 (1.244–3.205)	

CI confidence interval, *HK1* Hexokinase 1

**P* values<0.05

clinicopathological variables, including age (P=0.039), tumor differentiation (P<0.001), pT classification (P=0.016), pN classification (P<0.001), and pM classification (P<0.001), were also found to be prognostic factors (Table 2). In the validation cohort, a similar result that showed the prognostic significance of a high expression of HK1 was obtained (log-

Table 3 Cox multivariate analyses of prognostic factors on overall survival

Variables	Hazards ratio	95 % CI	*P* value*
Training cohort			
Differentiation (poor vs well/moderate)	1.469	0.907–2.377	0.118
pT classification (T3/ T4 vs T1/T2)	1.248	0.666–2.337	0.489
pN classification (N1 vs N0)	1.871	0.780–4.484	0.160
pM classification (M1 vs M0)	8.011	4.368–14.692	<0.001*
HK1 expression (high vs low)	2.493	1.664–3.733	<0.001*
Validation cohort			
Differentiation (poor vs well/moderate)	1.587	0.837–3.006	0.157
pT classification (T3/ T4vs T1/T2)	1.936	1.065–3.519	0.030*
pN classification (N1 vs N0)	0.973	0.275–3.443	0.966
pM classification (M1 vs M0)	3.935	1.155–13.402	0.028*
HK1 expression (high vs low)	1.755	1.072–2.875	0.025*

CI confidence interval, *HK1* Hexokinase 1

*Significant at P<0.05

rank, $P=0.003$ and $P=0.004$, Fig. 3 and Table 2). Of the other clinicopathological variables, the univariate analysis demonstrated that pT classification ($P=0.001$), pN classification ($P<0.001$), and pM classification ($P<0.001$) affected the patients' overall survival (Table 2).

Given that the features that were observed to have a prognostic influence in univariate analysis may be covariates, HK1 expression and those clinicopathologic variables that were found to be significant in the univariate analysis were further examined through multivariate analysis. In the training cohort, multivariate analysis showed that high expression of HK1 was a significant independent prognostic factor for poor overall survival [hazard ratio (HR), 2.493; 95 % confidence interval (CI), 1.664–3.733; $P<0.001$; Table 3]. The HK1 expression in the validation cohort showed similar results (HR, 1.755; 95 % CI, 1.072–2.875; $P=0.025$; Table 3).

Discussion

It has been shown in previous studies that biomarkers were useful for predicting prognosis in many tumors. Furthermore, biomarkers were found to be even superior to the AJCC stage in some studies [4, 5, 13–18]. However, because of the inhomogeneity and lack of reproducibility, some of the results from these studies remained conflicting [14, 15]. A large-scale research is needed to discover the novel and specific biomarkers. Recently, some investigators have documented that HK1 expression was significantly higher in tumor tissues, such as breast, thyroid, kidney, and lung cancers [8–10], indicating an important role of HK1 in the progression of carcinogenesis, but few studies have investigated the role of HK1 in colorectal cancer.

HK1 is known to play an important role in glycolytic activity via phosphorylation of glucose. Several studies have proved that malignant tumor cells increase the glycolytic activity in vivo and in vitro [19, 20], thus suggesting the fact that the glycolytic pathway and glycolysis-related genes HK1 may participate in tumorigenesis. Bryson et al. showed that HK1 overexpression in an established epithelial cell line had leaded to protection against oxidant-induced cell death [21]. This protection was glucose-dependent, which suggested that the increase in glucose phosphorylation may be the main mechanism for cellular protection by HK1 and that overexpression of HK1 protected cells against damage from oxidative stress. In addition, the specificity of HK1 binding to the mitochondria may also make a contribution to cell protection, in which the special porin, a 30-kDa protein in the outer membrane of the mitochondria called voltage-dependent anion channel (VDAC) is involved [22, 23]. The binding of HKs to the mitochondria may be dependent on the phosphorylation state of VDAC, which allowed the transport of many solutes and metabolites in and out of the intermembrane space [24–27], and thus protected cells from death. These results suggested that HK1 might play an important role in the process of tumorigenesis and tumor growth.

HK1 is overexpressed in a large variety of tumors, including colorectal cancer, breast cancer, and thyroid cancer (listed at http://www.proteinatlas.org/ENSG00000156515), suggesting that overexpression of HK1 may be a general mechanism in tumoral development. In our study, HK1 expression in CRC was associated closely with the pN classification and TNM stage in both cohorts, which indicated that HK1 may function as a key biomarker in the processes of tumor development and thus has the potential to predict prognosis in CRC patients. Rathmell JC et al. [28] revealed a similar result in their study. Twenty-five patients who had undergone resection of CRC were selected randomly and 76 % of them show a high expression of HK1. The multivariate analyses of our cohorts confirmed that HK1 expression was an independent prognostic factor of CRC. Although the mechanisms of the predictive value of HK1 for CRC patients were not further investigated in the present study, our findings provided the evidence that the overexpression of HK1 was significantly correlated with poor prognosis in CRC. HK1 could be a useful biomarker additive to the AJCC TNM staging system for patients with CRC and may be used to identify patients who are more likely to have a short cancer-specific survival. As a result, a more aggressive treatment could be considered for those patients who exhibit a high expression of HK1.

However, Bobby Bhatia et al. [29]. found that HK1 was frequently upregulated in the olomoucine-treated medulloblastomas, which indicated a better overall survival of medulloblastomas patients. This result was opposite to the previous studies. The authors assumed that HK1 had played a role distinct from glycolysis in differentiated (e.g., normal cerebellum) or non-proliferating tissue (e.g., olomoucine-treated medulloblastoma) which needed to be further confirmed. Several studies have also reached a similar conclusion in human brain tumors [30, 31]. This contradiction may be due to the special role HK1 played in different tissues. In the study of Li W et al. [32], HK1-shRNA was used to knock down HK1 expression in primary cultured esophageal squamous cell carcinoma (ESCC) cells. They showed that silence of HK1 not only inhibited cell proliferation in vitro but also induced a reduction of phospho-S6 kinase, an important kinase in the mammalian target of rapamycin (mTOR) pathway [33], indicating that in addition to metabolic function, HK1 might be involved in the signaling transduction and has an oncogenic effect which was associated with the mTOR pathway. The phosphatidylinositol 3-kinase (PI3K)/Akt/ mTOR pathway is a central hub for the regulation of cell proliferation. Several studies have recently suggested that the PI3K/Akt/mTOR signaling pathway was implicated in the pathogenesis and progression of invasive

cancers [34–36]. We may come to a conclusion that HK1 plays a crucial role in CRCs, in which not only metabolic activity but also signaling transduction is involved.

In this study, we found that a high expression of HK1 remained a significant independent prognostic factor indicating poor overall survival in multivariate analysis. Further analysis using chi-squared test for the training cohort demonstrated that HK1 expression exhibited a statistically significant correlation with the pN classification and TNM stage. This finding was consistent with the results from previous studies [8–10]. Further studies are needed to clarify the potential mechanism of HK1 in cancer progression in CRC. Our study is the first and largest study to investigate the prognostic significance of HK1 expression in CRC patients by IHC analysis in two large cohorts. High expression of HK1 was an independent predictor of poor prognosis, as evidenced by the Kaplan–Meier curves and multivariate Cox proportional hazards regression analysis. Therefore, the examination of HK1 overexpression could be used as an additional effective tool in identifying those CRC patients at increased risk of tumor progression, thus predicting poor survival outcomes and guiding a more aggressive treatment regimen. In conclusion, in the present study with two large cohorts of CRC patients, high expression of HK1 was recognized to be a strong and independent predictor of poor survival. The expression of HK1 in patients with CRC could be used as an additional parameter for identifying patients with a higher risk of tumor progression and optimizing the individualized treatment strategies for CRC patients.

Compliance with ethical standards This study was approved by the IRB of the Sixth Affiliated Hospital and Cancer Center, Sun Yat-sen University.

Conflicts of interest None

Declaration of funding interests This work is supported by the National Natural Science Foundation of China (81400604).

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
2. Mitry E, Rachet B, Quinn MJ, Cooper N, Coleman MP. Survival from cancer of the rectum in England and Wales up to 2001. Br J Cancer. 2008;99 Suppl 1:S30–2.
3. Brenner H, Bouvier AM, Foschi R, Hackl M, Larsen IK, Lemmens V, et al. Progress in colorectal cancer survival in Europe from the late 1980s to the early 21st century: the EUROCARE study. Int J Cancer. 2012;131:1649–58.
4. Wu XR, He XS, Chen YF, Yuan RX, Zeng Y, Lian L, et al. High expression of CD73 as a poor prognostic biomarker in human colorectal cancer. J Surg Oncol. 2012;106:130–7.
5. Zou Y, Chen Y, Wu X, Yuan R, Cai Z, He X, et al. CCL21 as an independent favorable prognostic factor for stage III/IV colorectal cancer. Oncol Rep. 2013;30:659–66.
6. Smith TA. Mammalian hexokinases and their abnormal expression in cancer. Br J Biomed Sci. 2000;57:170–8.
7. Pastorino JG, Hoek JB. Hexokinase II: the integration of energy metabolism and control of apoptosis. Curr Med Chem. 2003;10:1535–51.
8. Hooft L, van der Veldt AA, van Diest PJ, Hoekstra OS, Berkhof J, Teule GJ, et al. [18F]fluorodeoxyglucose uptake in recurrent thyroid cancer is related to hexokinase i expression in the primary tumor. J Clin Endocrinol Metab. 2005;90:328–34.
9. Millon SR, Ostrander JH, Brown JQ, Raheja A, Seewaldt VL, Ramanujam N. Uptake of 2-NBDG as a method to monitor therapy response in breast cancer cell lines. Breast Cancer Res Treat. 2011;126:55–62.
10. Oparina NY, Snezhkina AV, Sadritdinova AF, Veselovskii VA, Dmitriev AA, Senchenko VN, et al. Differential expression of genes that encode glycolysis enzymes in kidney and lung cancer in humans. Genetika. 2013;49:814–23.
11. Battifora H. The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. Lab Investig. 1986;55:244–8.
12. Zlobec I, Steele R, Terracciano L, Jass JR, Lugli A. Selecting immunohistochemical cut-off scores for novel biomarkers of progression and survival in colorectal cancer. J Clin Pathol. 2007;60:1112–6.
13. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313:1960–4.
14. Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol. 2009;27:186–92.
15. Nosho K, Baba Y, Tanaka N, Shima K, Hayashi M, Meyerhardt JA, et al. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. J Pathol. 2010;222:350–66.
16. Huang Y, Li W, Chu D, Zheng J, Ji G, Li M, et al. Overexpression of matrix metalloproteinase-21 is associated with poor overall survival of patients with colorectal cancer. J Gastrointest Surg. 2011;15:1188–94.
17. Lin KY, Tai C, Hsu JC, Li CF, Fang CL, Lai HC, et al. Overexpression of nuclear protein kinase CK2 alpha catalytic subunit (CK2alpha) as a poor prognosticator in human colorectal cancer. PLoS One. 2011;6, e17193.
18. Lin AY, Chua MS, Choi YL, Yeh W, Kim YH, Azzi R, et al. Comparative profiling of primary colorectal carcinomas and liver metastases identifies LEF1 as a prognostic biomarker. PLoS One. 2011;6, e16636.
19. Dang CV, Semenza GL. Oncogenic alterations of metabolism. Trends Biochem Sci. 1999;24:68–72.
20. Oronsky BT, Oronsky N, Fanger GR, Parker CW, Caroen SZ, Lybeck M, et al. Follow the ATP: tumor energy production. A perspective. Anticancer Agents Med Chem. 2014.
21. Bryson JM, Coy PE, Gottlob K, Hay N, Robey RB. Increased hexokinase activity, of either ectopic or endogenous origin, protects renal epithelial cells against acute oxidant-induced cell death. J Biol Chem. 2002;277:11392–400.
22. Azoulay-Zohar H, Israelson A, Abu-Hamad S, Shoshan-Barmatz V. In self-defence: hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death. Biochem J. 2004;377:347–55.
23. Sun L, Shukair S, Naik TJ, Moazed F, Ardehali H. Glucose phosphorylation and mitochondrial binding are required for the protective effects of hexokinases I and II. Mol Cell Biol. 2008;28:1007–17.

24. Maldonado EN, Lemasters JJ. Warburg revisited: regulation of mitochondrial metabolism by voltage-dependent anion channels in cancer cells. J Pharmacol Exp Ther. 2012;342:637–41.
25. Colombini M. VDAC structure, selectivity, and dynamics. Biochim Biophys Acta. 1818;2012:1457–65.
26. Takahashi Y, Tateda C. The functions of voltage-dependent anion channels in plants. Apoptosis. 2013;18:917–24.
27. Martel C, Wang Z, Brenner C. VDAC phosphorylation, a lipid sensor influencing the cell fate. Mitochondrion. 2014.
28. Rathmell JC, Fox CJ, Plas DR, Hammerman PS, Cinalli RM, Thompson CB. Akt-directed glucose metabolism can prevent Bax conformation change and promote growth factor-independent survival. Mol Cell Biol. 2003;23:7315–28.
29. Bhatia B, Potts CR, Guldal C, Choi S, Korshunov A, Pfister S, et al. Hedgehog-mediated regulation of PPARgamma controls metabolic patterns in neural precursors and shh-driven medulloblastoma. Acta Neuropathol. 2012;123:587–600.
30. Wolf A, Agnihotri S, Munoz D, Guha A. Developmental profile and regulation of the glycolytic enzyme hexokinase 2 in normal brain and glioblastoma multiforme. Neurobiol Dis. 2011;44:84–91.
31. Clatot F, Gouerant S, Mareschal S, Cornic M, Berghian A, Choussy O, et al. The gene expression profile of inflammatory, hypoxic and metabolic genes predicts the metastatic spread of human head and neck squamous cell carcinoma. Oral Oncol. 2014;50:200–7.
32. Li W, Xu Z, Hong J, Xu Y. Expression patterns of three regulation enzymes in glycolysis in esophageal squamous cell carcinoma: association with survival. Med Oncol. 2014;31:118.
33. Tsouko E, Khan AS, White MA, Han JJ, Shi Y, Merchant FA, et al. Regulation of the pentose phosphate pathway by an androgen receptor-mTOR-mediated mechanism and its role in prostate cancer cell growth. Oncogenesis. 2014;3, e103.
34. Paplomata E, O'Regan R. The PI3K/AKT/mTOR pathway in breast cancer: targets, trials and biomarkers. Ther Adv Med Oncol. 2014;6:154–66.
35. Li H, Zeng J, Shen K. PI3K/AKT/mTOR signaling pathway as a therapeutic target for ovarian cancer. Arch Gynecol Obstet. 2014.
36. Beck JT, Ismail A, Tolomeo C. Targeting the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway: an emerging treatment strategy for squamous cell lung carcinoma. Cancer Treat Rev. 2014;40:980–9.