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Elevated Mortalin correlates with poor outcome in hepatocellular carcinoma

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ABSTRACT

Although several lines of evidence existed suggesting that Mortalin was linked with survival in malignant tumors; it has been barely described regarding the prognostic involvement of its expression in hepatocellular carcinoma (HCC). Herein, to understand the prognostic meaning of Mortalin expression, Immunohistochemistry was undertaken to observe the immunohistochemical characteristics of Mortalin on HCC tissue microarray consisting of 90 cases of HCC and its paired normal control dots, followed by detailed statistical analysis with the accompanying clinicopathological variables available, including gender, age, tumor size, differentiation, cirrhosis, vascular invasion, clinical stage, T classification and intrahepatic metastases. Meanwhile, Kaplan-Meier survival curve was plotted to analyze the prognostic difference for patients with high and low expression of Mortalin. It was exhibited that Mortalin was over-expressed in HCC tissues relative to paired normal control and elevated Mortalin significantly correlated with vascular invasion, clinical stage and intrahepatic metastasis. Kaplan-Meier survival analysis revealed that Mortalin was remarkably associated with overall survival and disease-free survival. Multivariate Cox regression analysis showed that expression of Mortalin was an independent prognostic factor in HCC. Collectively, the data we provided here support the prognostic prediction value of Mortalin in HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death in China, accounting for 70–90% of all primary liver cancers [1]. Due to the lack of typical symptoms and earlier routine screening of HCC, the patients with HCC at the time of diagnosis was usually at advanced stage, especially at stages III and IV, few patients therefore will have an opportunity to receive curative treatment, either primary resection or liver transplantation [2]. Consequently, it is pivotal to identify the key biomarkers, allowing for earlier prediction for diagnosis and prognosis in the development and progression of HCC.

Mortalin, aliased HSPA9B, GRP75, PBP74 or MOT, is a member of the heat shock protein 70 gene family [3]. Thus, this protein is a heat-shock cognate protein, acting as a highly conserved molecular chaperone [4]. Several lines of evidence [5] existed indicating that Mortalin was heavily involved in the cell proliferation, stress response and maintenance of the mitochondria. A recent study by Yun et al. [6] suggested that human Mortalin had the ability for malignant transformation, over-expression of which could therefore contribute to the carcinogenesis. Moreover, several mechanistic studies that follow showed that up-regulation of Mortalin was found to be able to induce the epithelial-mesenchymal transition (EMT) of cancer cells [7-9], which is strongly suggestive of its heavy implication in the development of cancers.

In the present study, to understand the prognostic implication of Mortalin in HCC, immunohistochemistry was performed on HCC tissue microarray comprised 90 cases of HCC and its paired dots. Subsequently, detailed statistical analysis ensued with the accompanying clinicopathological variables available. At the same time, prognostic significance of Mortalin expression was evaluated using Kaplan-Meier survival curve.

2. Patients and methods

2.1. Clinical tissues

The present study was approved by the Medical Ethics Committee at the Center Hospital of Karamay. The tissue microarray used for the immunostaining analysis of Mortalin was commercially purchased from Shanghai Outdo Biotech. Co. Ltd. (Catalog number: HLivH180Su07,

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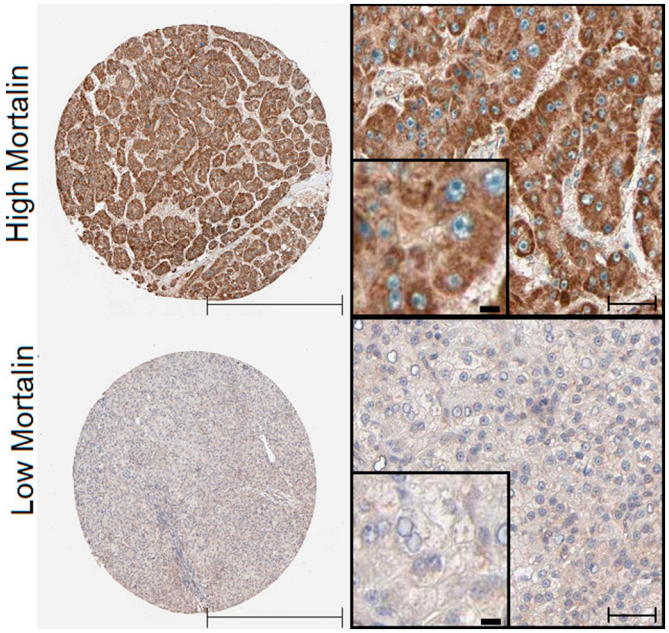

Fig. 1. High and low expression of Mortalin, detected by IHC on HCC tissue microarray. The magnification of dots was 5 fold, and the corresponding scale bar represents 50 μm; the magnification of high power field in response to where boxed on dots was 20 fold, the scale bar stands for 10 μm; as for the insets, the magnification was 40 fold, and the scale bar is 5 μm. Immunostaining of Mortalin was chiefly membranous and cytoplasmic, shown were representative figures picked out of 90 dots captured.

Shanghai, China). The array consisted of 90 paired HCC tissues and its corresponding normal controls. Staging and grading of the samples was assessed in accordance with the World Health Organization classification and grading system. None of the samples were collected from patients undergoing chemoradiotherapy prior to resection. Informed consent was obtained from each participants involved. The corresponding clinicopathological information, including age, clinical stage, tumor grade, lymph node metastasis and overall survival, was available for each hepatocarcinoma tissue dot arrayed on tissue microarray.

2.2. Immunohistochemistry (IHC)

In brief, the hepatocarcinoma TMA was deparaffinized and rehydrated. Heat-induced epitope retrieval was performed using citrate buffer (pH = 6.0) and a microwave histoprocessor (Haier, Qingdao, Shandong, China), after which the tissue sections were incubated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. Tissue sections were then incubated with a primary antibody to Mortalin (dilution at 1:200, ab2799, Abcam, Cambridge, UK) overnight in a humidified chamber at 4 °C. Immunostaining was visualized using a labeled horseradish peroxidase (HRP)-conjugated AffiniPure mouse Anti-rabbit IgG antibody with 3,3′-diaminobenzidine as a chromogen (Dako Canada Inc., Mississauga, Ontario, Canada), and the tissue was counterstained with hematoxylin.

2.3. Immunoscoring

The section was evaluated under a light microscope, and cellular localization of the protein and the immunostaining intensity of each section were assessed by two pathologists. The staining patterns were scored based on the signal intensity as follows: negative (no positive staining), denoted as —; weak (< 15% of cells with positive staining), represented as +; medium (> 15% but < 30%), ++; and strong (> 30% of cells with positive staining), +++. For the clinicopathological analysis, the negative and weak samples were classified as low expression, whereas medium and strong samples were classified as high expression. Additionally, the use of a general rabbit anti-human IgG in place of the primary antibody served as a negative control, breast cancer tissue as positive control.

2.4. Statistical analysis

Statistical analysis was conducted using SPSS 17.0 version (SPSS, Chicago, USA) and GraphPad Prism 5.0. Data are expressed as the mean ± standard deviation (SD) and were analyzed using the Chi-square test or Fisher's Exact test (when the frequency count was < 5 in contingency table). Kaplan-Meier survival curves were plotted, and log-rank test was carried out. P value < 0.05 was defined as statistically significant versus the control.

3. Results

3.1. Mortalin was over-expressed in HCC tissues

The original study regarding Mortalin in HCC by Yi X and colleagues [10] reported that Mortalin was remarkably up-regulated in liver cancer tissues using two-dimensional gel electrophoresis approach. Suggested by this study, attempt was made to replicate the observation reported by Yi X et al. surrounding Mortalin in HCC and its paired normal control tissues of our own. IHC method was undertaken to verify the expression of Mortalin in our own HCC cases, totaling 90. Results from IHC exhibited that immunostaining of Mortalin was appreciably membranous and cytoplasmic; while, nuclear staining was hardly observable in HCC (Fig. 1). In spite of its highly heterogeneous expression in both HCC and normal control tissues, with the intensity of immunostaining varying from case to case from being negative, weak, moderate and to strong positive (Fig. 2); Taken as a whole, Mortalin was displayed to be pronouncedly up-regulated in HCC as compared with paired normal controls (Table 1). To facilitate the clinicopathological significance analysis of its expression, the Mortalin expression was broadly stratified into two layers, which is to say, high and low expression according to the staining intensity (Fig. 1). Cross-Table analysis was performed between high and low expression of Mortalin versus clinicopathological variables available, including gender, age, tumor size, differentiation, cirrhosis, vascular invasion, clinical stage, T classification and intrahepatic metastases. Detailed statistical analysis showed that high expression of Mortalin significantly correlated with vascular invasion, clinical stage, and intrahepatic metastasis; whereas no significant correlation can be identified between Mortalin expression versus gender, age, tumor size, differentiation, cirrhosis, and T classification (Table 1), explicitly suggesting that Mortalin was associated with metastasis of HCC.

3.2. Over-expressed Mortalin correlated with unfavorable outcome in HCC

Having seen that Mortalin was indeed markedly up-regulated in HCC relative to paired normal controls, next we attempted to explore the prognostic significance of its expression. As stated in the preceding, the expression of Mortalin was broadly stratified into high and low expression based on the immunostaining intensity, based on which, Kaplan-Meier survival analysis was performed between high and low expression of Mortalin versus the overall survival and disease-free

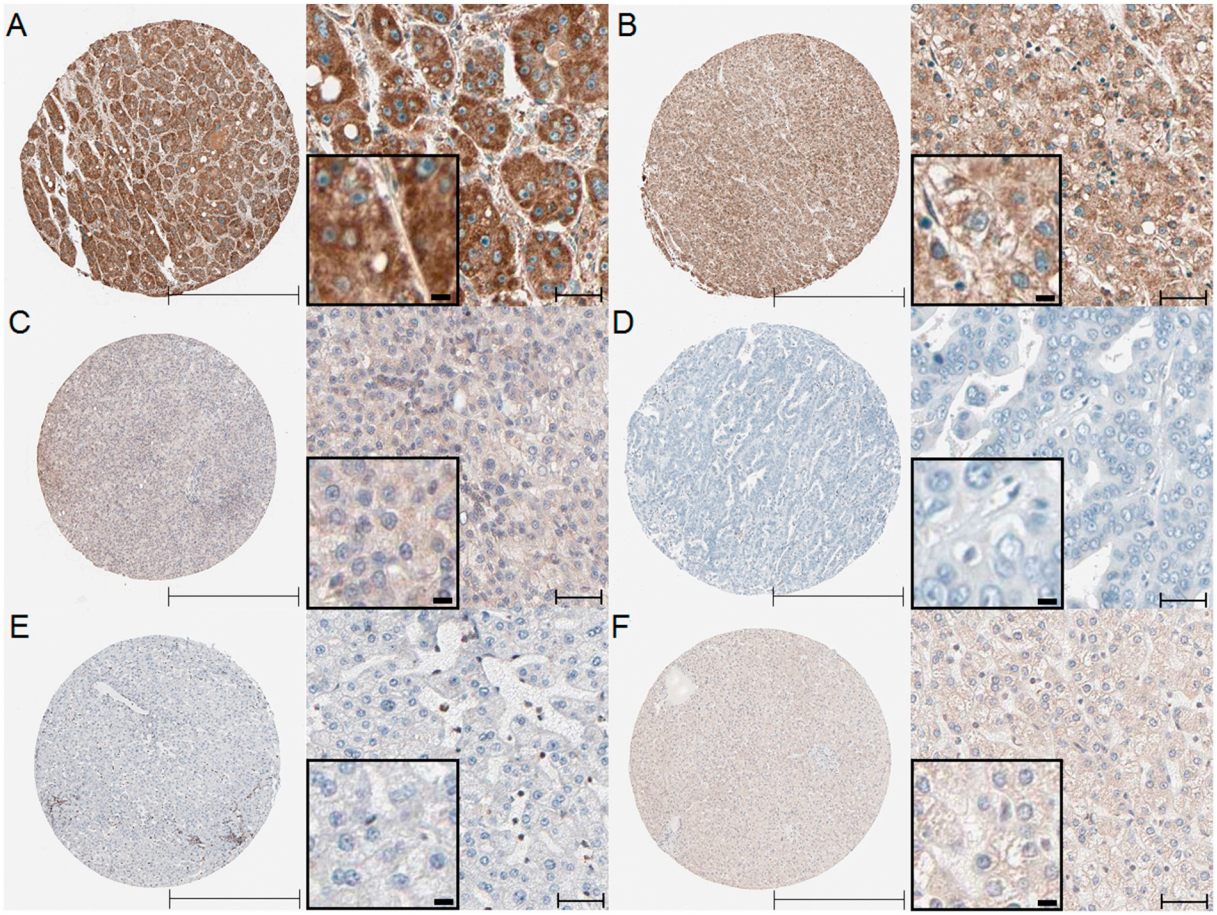

Fig. 2. Heterogeneous expression of Mortalin in HCC and normal liver control tissues. A: Strong immunostaining; B: Moderate staining; C: weak staining; D: negative staining of Mortalin in HCC tissues; E: negative staining of Mortalin in normal liver tissue; F: weak staining of Mortalin in normal liver tissue. Scale bar labeled on the overview of dots stands for 50 μm, scale bar shown on the high power field represents 100 μm, insets whose scale bar were 200 μm.

survival time. Analysis from Kaplan-Meier survival plots displayed that over-expression of Mortalin was significantly associated with overall survival (OS) and lower disease free survival (DFS) rates of patients with HCC (Fig. 3). In order to further analyze the prognostic significance of Mortalin expression, multivariate Cox regression analysis was carried out. It revealed that Mortalin expression was an independent prognostic factor for HCC in addition to clinical stage and intrahepatic metastases (Table 2), indicative of independent prognostic value of Mortalin expression.

4. Discussion

In this report, Mortalin was exhibited to be dramatically over-expressed in HCC tissues relative to paired normal controls; and elevated Mortalin was found to significantly correlate with vascular invasion, clinical stage, intrahepatic metastasis and overall prognosis. Results from multivariate Cox regression analysis showed that Mortalin expression was an independent prognostic factor in HCC. All the data we obtained here were in support of the prognostic prediction value of Mortalin in HCC.

Mortalin, also named as HSPA9B, GRP75, PBP74 [11], or MOT-2 [12], is a member of the heat shock protein 70 gene family. Mortalin is primarily localized to the mitochondria but is also found to reside in the endoplasmic reticulum, plasma membrane and cytoplasmic vesicles [3]. Being a heat-shock cognate protein, Mortalin was expected to play multifaceted roles related to cell survival, control of proliferation, stress response and maintenance of the mitochondria, as extensively reviewed by Kaul SC and colleagues [3,4]. In cancer, Mortalin seemed to have received much attention during past decades. The original cancer study mentioning the term Mortalin actually came from an investigation of MKT-077 [12], a cationic rhodacyanine dye analogue, which binds to Mortalin, thereby abrogating its interactions with p53 in terms of pharmacological toxicity. Since then, different studies surrounding Mortalin in cancer has been successively emerged, including exploration of its imaging [13], suppression [14,15], interactive protein [16], structure [5,17], inhibitor [18] and clinicopathological significance of its expression [19,20]. The first report regarding clinicopathological meaning of Mortalin expression was from colorectal cancer [19] where Mortalin was found to be over-expressed in colorectal adenocarcinoma and correlated with poor survival. Later, this observation was extended to other types of tumor, including liver [10], gastric [21], breast [22], pancreatic [23], cholangio [7,24], and lung cancer [25]. These independent observations seem to bear out the prognostic value of Mortalin that was remarkably associated with adverse outcome.

Despite Mortalin was reported to be over-expressed in most human cancers, regardless of types; it has been scarcely described resolving around the prognostic implication of Mortalin expression

Table 1
Clinicopathological significance of Mortalin expression in HCC.

Clinicopathological variables	Total	Mortalin expression		χ^2	*P* value
		High	Low		
HCC	90	72	18	60.119	0.000
Paired normal control	90	20	70		
Gender					
Male	58	47	11	0.109	0.787
Female	32	25	7		
Age (years)					
> 50	56	46	10	0.425	0.590
≤50	34	26	8		
Tumor size (cm)					
< 5	41	32	9	0.179	0.793
≥5	49	40	9		
Differentiation					
Well	68	57	11	2.542	0.131
Poor	22	15	7		
Cirrhosis					
Yes	61	49	12	0.013	1.000
No	29	23	6		
Vascular invasion					
Presence	63	54	9	4.286	0.048
Absence	27	18	9		
Clinical stage					
I–II	28	17	11	9.448	0.004
III–IV	62	55	7		
T classification					
$T_{1–2}$	16	10	6	3.725	0.081
$T_{3–4}$	74	62	12		
Intrahepatic metastases					
Absence	33	20	13	12.249	0.001
Presence	57	52	5		

Table 2
Univariate and multivariate Cox regression analyses of the prognostic parameters in HCC.

	Univariate analysis		Multivariate analysis		
	P	Regression coefficient (SE)	*P*	Relative risk	95.0% *CI*
Mortalin expression	0.011	0.652 (0.264)	0.025	1.833	1.023–3.387
Gender	0.065	0.762(0.365)	0.201	1.637	0.769–3.483
Age (years)	0.567	0.167(0.291)	0.276	1.404	0.762–2.587
Tumor size (cm)	0.876	0.026(0.168)	0.534	0.890	0.615–1.287
T classification	0.073	0.103(0.183)	0.560	1.147	0.723–1.820
Clinical stage	0.004	0.409(0.140)	0.049	1.356	0.977–1.882
Cirrhosis	0.578	0.404(0.226)	0.890	1.030	0.674–1.576
Vascular invasion	0.051	0.428(0.166)	0.065	1.556	0.979–1.932
Intrahepatic metastasis	0.014	−0.472(0.179)	0.032	0.605	0.406–0.900

in HCC in spite of one recent study by Yi X and colleagues [10] where they used liver cancer stead of the term HCC. In line with the observation made by Yi et al. [10], Mortalin was shown to be markedly up-regulated in HCC in comparison with paired normal controls in our setting. In addition, up-regulation of Mortalin was exhibited to pronouncedly correlate with vascular invasion, clinical stage and intrahepatic metastasis, which is fully concordant with findings described by Yi et al. [10], suggesting that Mortalin was closely related to metastasis of cancer. As supporting evidence, lines of evidence [7-9] existed displaying that up-regulation of Mortalin was capable of inducing the epithelial-mesenchymal transition, which could account for why elevated Mortalin was associated with metastasis of cancer. In terms of prognostic meaning, over-expressed Mortalin was found to be not only associated with unfavorable overall prognosis but also with disease-free survival in our own cases. Unfortunately, there has been a shortage of prognostic analysis of Mortalin expression in Yi X and colleagues' investigation [10]. In breast [22], pancreatic [23] and lung [25] cancers where Mortalin was concertedly reported to be an independent prognostic factor that closely correlated with disease free survival and overall survival, which is in total agreement with our own finding in HCC, strongly suggestive of its independent prognostic value in cancer, irrespective of the types. Despite this, there is lack of mechanistic investigation on biological roles mediated by Mortalin in HCC cells, which will be out of the scope of this study that deserves to be investigated in the experiment that follows.

In conclusion, Mortalin was displayed to be remarkably elevated in HCC relative to paired normal control tissues; and over-expressed Mortalin significantly correlated with vascular invasion, clinical stage, and intrahepatic metastasis of HCC. Prognostically, up-regulated Mortalin can independently predict the poor overall survival in HCC, suggestive of its remarkable prognostic value in patients with HCC.

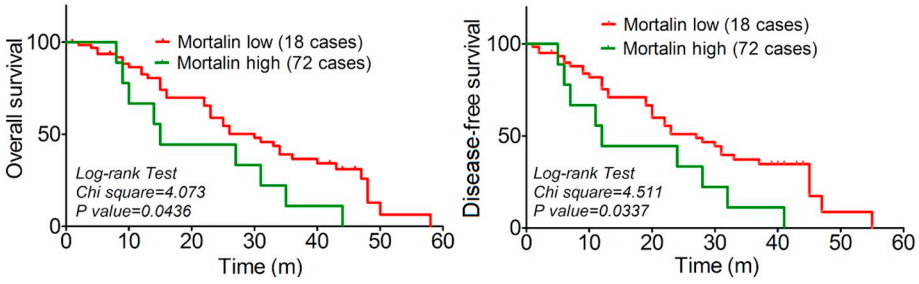

Fig. 3. Over-expressed Mortalin was significantly associated with unfavorable Overall prognosis (OS) and disease-free survival (DFS). The number of Mortalin high and low expression was 72 and 18 cases, respectively. Log-rank test was employed to analyze the difference between HCC patients with high and low Mortalin expression.

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Declaration of competing interest

The authors declare that they have no conflict of interests.

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