Expression of Mortalin Detected in Human Liver Cancer by Tissue Microarrays

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ABSTRACT

Mortalin is a highly conserved molecular chaperone in the heat shock protein (HSP) 70 family, which plays a role in carcinogenesis. The relationship between tumors and the expression of Mortalin is not fully elucidated. In this study, human tumor specimens from various organs of liver cancer at different stages and cultured liver cancer cells were used to study the expression pattern of Mortalin. Through immunohistochemistry we showed that Mortalin was significantly higher in tumors than in adjacent benign tissues. Using liver tissue microarrays tested on hepatocellular carcinomas, Mortalin expression was consecutively higher with advanced tumor stages. Mortalin expression on the cultured liver cancer cells were characterized with immunocytochemistry, Real-time PCR, and western blot. The results showed that the expression level is markedly higher in the SMMC 7721 (a liver-derived tumor cell line) than in the HL 7702 (a normal liver cell line) in the protein level only. Understanding the role of Mortalin in tumors may lead to development of a new therapeutic target in cancer treatment. Anat Rec, 294:1344–1351, 2011. © 2011 Wiley-Liss, Inc.

Key words: Mortalin; tumor; tissues microarrays; SMMC7721 cell line; HL 7702 cell line

Mortalin is a highly conserved molecular chaperone in the heat shock protein (HSP) 70 family, which is encoded by the nuclear gene HSPA9B (GeneID: 3313) localized on chromosome 5q31.1.1. It is translated in the cytoplasm and transported into mitochondria (Czarnecka et al., 2006). Mortalin is essential for translocating mitochondrial-targeted proteins in the inner matrix, which interacts with the wild-type tumor suppressor protein p53 (Wadhwa et al., 1998) and modulates the Ras-Raf-MAPK pathway (Mizukoshi et al., 2001; Wadhwa et al., 2003). There is some constitutive level of Mortalin in the normal tissues; however the findings of up-regulated Mortalin in tumor cells draw scientists' attention to its role in carcinogenesis. It has been reported that the Mortalin was

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elevated in human brain tumor, colon carcinoma, leukemia, and the immortalized cell lines derived from the tumors (Wadhwa et al., 2006). Yi et al. reported that Mortalin (HSPA9) is associated with positive venous infiltration and advanced tumor TNM stages in Hepatocellular carcinoma (HCC) and thus Mortalin suggested as a tumor marker for predicting early recurrence, which may have immediate clinical applications for cancer surveillance after curative surgery (Yi et al., 2008). At the same time, overexpression of Mortalin was sufficient to increase the malignancy of carcinoma cells. However, the mechanism of Mortalin in carcinogenesis is still elusive.

HCC is the most common liver malignancy, and normal liver tissue had very low constructive Mortalin expression (Massa et al., 1995). In this study, we investigated the Mortalin expression in various tumors and in liver cancer of different ages, genders, clinic stages, and types. At the same time, we observed change of cell viability after Mortalin overexpressed in Normal human hepatic cells, to approach the possibility that Mortalin as a candidate target for cancer therapy.

MATERIALS AND METHODS

Tissue Specimens

Thirty-one tumor samples of carcinoma were collected from different organs of the patients in Ruijin Hospital (Shanghai, China): three hepatic, four esophageal, ten gastric, six colonic, six rectal, and two breasts.

Tissue Microarrays

A liver tissue microarray (Shanxi Chaoying Biotechnology) was employed on the specimens of liver tumor or adjacent benign tissues from 200 donors: 139 cases hepatocellular carcinoma, 10 cases of cholangiocellular carcinoma, 26 cases of hepto-adenocarcinoma metastatic, 5 of clear-cell carcinoma, and 20 samples of paracarcinoma livers.

Cell Culture

The liver cell line HL 7702 and hepatocellular carcinoma cell line *SMMC 7721* were maintained in DMEM with high glucose (10%) and supplemented with 10% heat-inactivated fetal bovine serum. All cell lines were cultured at 37°C, in an atmosphere of 5% CO_2 and 95% air in a humidified incubator.

Transfection and Mortalin Overexpression in HL-7702 Cells

The pcDNA3/Mortalin containing Mortalin full-length cDNA was transfected into HL-7702 cells using Lipofectamine 2000 (Invitrogen, Carlsbad). Two micrograms of plasmid DNA per 5×10^6 cells were used for transfection. Neomycin-resistant colonies were isolated in the medium supplemented with neomycin analog G418-sulfate (1 mg/mL; Amresco, Solon). The expression level of Mortalin protein was identified using Western blotting. Briefly, the cell pellets were lysed in SDS loading buffer (50 mM Tris, pH 6.7, 2% SDS, 10% glycerol, 0.06% bromophenol blue, 100 mM dithiothreitol), separated by 10% SDS-PAGE and subsequently transferred to nitrocellulose membrane. Immunoblots were blocked in PBS (pH 7.4) containing 5% skim milk, 0.5% Tween-20, and incubated with Mortalin antibody (Santa Crutz, diluted 1:500) for 2 hr at room temperature. After washing, the membrane was then incubated with HRP conjugated rabbit anti-goat IgG for 1 hr at room temperature. Immunoreactive bands were visualized using the ECL western blotting detection system kit (Amersham Pharmacia Biotech) according to the manufacturer's recommended protocol. The membranes used for Mortalin detection were reprobed with actin polyantibody and the corresponding secondary antibody to normalize the signal strength of Mortalin. The transfected cell line over-expressed with Mortalin was provided by Hongyan Li in our institute (Liu et al., 2005).

Immunohistochemistry

Immunohistochemistry was performed as follows: The sections were deparaffinized in xylene and dehydrated in a graded alcohol series, and endogenous peroxidase was blocked with 30% hydrogen peroxide. Heat-induced epitope retrieval was performed for 20 min by microwaves at about 97 to 100°C with a 0.01 mol/L concentration of citrate buffer (pH 6.0). After the slides cooled at room temperature for 30 min, they were rinsed with PBS and with BSA for 30 min, and then incubated overnight with a primary antibody (anti-Mortalin Ab13529 1:200, Abcam) in 4°C. The slides then were washed three times with PBS and then incubated with a secondary Biotin-labeled Goat Anti-Mouse IgG at 20°C for 30 min. Afterward, they were rinsed with PBS three times and then reacted with DAB for 10 min. The sections were counterstained with hematoxylin for 10 min, and then washed by PBS for three times. The level of the Mortalin expression on the slides was reviewed and rated by two individual scorers.

Immunocytochemistry

HL 7702 and SMMC 7721 Cells were seeded onto glass coverslips in six wells with the concentration of about 5×10^4/mL each well, and were washed with cold PBS and fixed with a pre-chilled methanol-acetone (1:1, v/v) mixture for 5 min on ice. Fixed cells were washed with PBS, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and blocked with 2% BSA in PBS for 20 min. Signal detection was obtained by treatments of consecutive primary antibodies (anti-Mortalin Ab13529 1:200,Abcam Cambridge, UK) and secondary antibody (goat anti-mouse IgG 1:1000, Boshide, China). Hochest 33258 was used to stain the nucleus. Cells were imaged using a Leica DM2500 confocal microscope with an argon laser (488 nm). Images were pictured and processed using Leica DFC300FX and Adobe Photoshop (Adobe Systems, Mountain View, CA).

Immunofluorescence

HL 7702 and SMMC 7721 Cells were seeded onto glass coverslips in six wells with the concentration of about 5×10^4/mL each well, and were washed with cold PBS and fixed with a pre-chilled methanol-acetone (1:1, v/v) mixture for 5 min on ice. Fixed cells were washed with PBS, permeabilized with 0.2% Triton X-100 in PBS for 10 min, and blocked with 2% BSA in PBS for 20 min. Signal detection was obtained by a mixture of primary antibodies (1:100 dilutions for Mortalin) and goat anti-

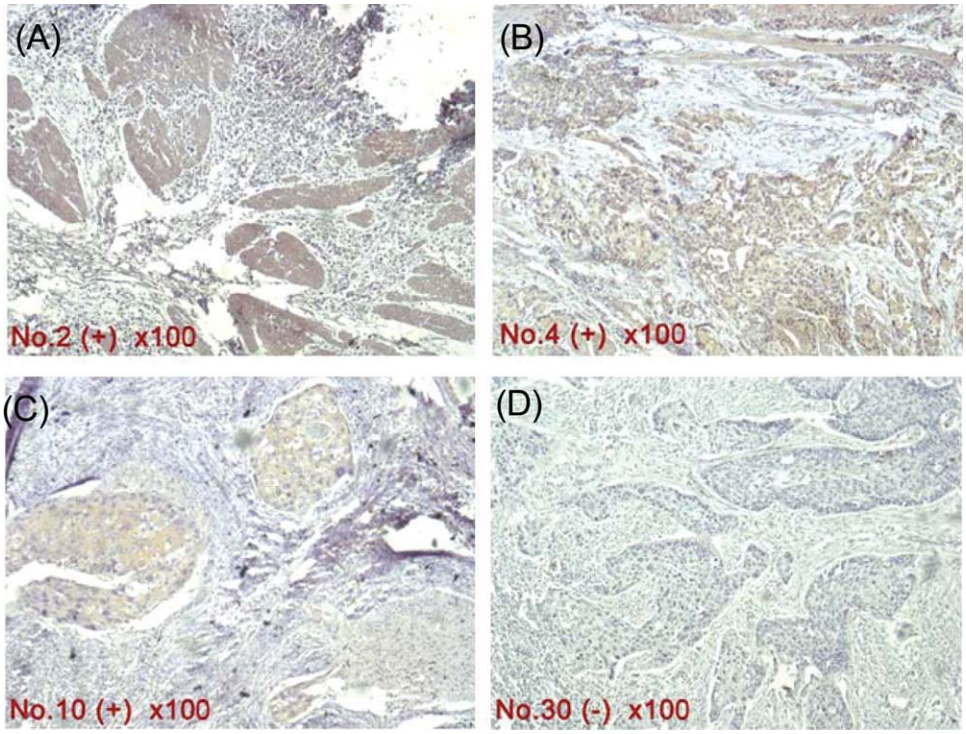

Fig. 1. Images listed above are part of the 30 various tumor specimens processed with immunohistochemical staining. The red numbers refer to the code of the sample, and the visual field is under a low power magnifying lens (×100). Under the microscope, the typical Mortalin-positive-staining image shows pancytoplasmic disseminated refractive particles with colors varying from light brown to brown. **A:** Sample No. 2: gastric adenocarcinoma, Stage II; **B:** Sample No. 4: squamous cell carcinoma of esophagus, Stage I; **C:** Sample No. 10: squamous cell carcinoma of esophagus, Stage II; **D:** Sample No. 30: squamous cell carcinoma of esophagus, Stage II, negative (processing without the anti-Mortalin antibodies staining).

mouse IgG with fluorochromes was treated as secondary antibody. Cells were imaged using a Leica DM2500 confocal microscope with an argon laser (488 nm). Images were pictured and processed using Leica DFC300FX and Adobe Photoshop (Adobe Systems, Mountain View, CA).

Western Blot Analysis

Cells were lysed in RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% Doc, 0.1% sodium dodecyl sulfate, and 50 mM Tris/HCl (pH 8.0)] supplemented with a 1 g/mL aprotinin and 100 g/mL phenylmethylsulfonyl fluoride. The cell suspension was incubated on ice for 30 min then centrifuged at 20,000*g* for 15 min at 4°C. The supernatants were collected for further analysis. The protein concentration of the samples was determined by Bradford assay. A total of 20 g of proteins were separated by 10% or 15% sodium dodecyl sulfate-polycrylamide gels and transferred onto nitrocellulose membranes (Amersham Pharmacia Biotech, Little Chalfont, UK). Membranes were blocked with 5% (W/V) fat-free dry milk in TBS-T buffer (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.05% Tween-20) and incubated overnight at 4°C with relevant primary antibodies, followed by washing and incubation with appropriate horseradish peroxidase-conjugated secondary antibodies.

Immunocomplexes were visualized using the enhanced chemiluminescence. Western blotting detection system (Cell Signaling Technology) with exposure of the membranes to X-ray film (Eastman Kodak, Rochester). GAPDH and Lamin B1 were used to ensure equivalent loading of whole and nuclear cell protein respectively. The signal intensity of the respective bands was quantified by a scanning densitometer using an image analysis system with Scion Image version 4.03 software.

The Glucose Deprivation Procedure and the d3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) Reduction Assay

The exponentially growing cells in the wells were gently washed twice with glucose-free DMEM medium for 6, 12, 24, 36, and 48 hr. The MTT reduction assay was involved in estimating cell viability. MTT (Pufei, Shanghai, China) was added to the culture medium at the end of incubation. After further incubation at 37°C for 4 hr, media was removed. Cells were lysed and formazan was dissolved with 150 μL dimethyl sulfoxide. Absorbance at 492 nm was measured with a microplate reader (Thermo Scientific, Anaheim) and results were expressed as the percentage of MTT reduction, assuming the absorbance of control cells as 100%.

Statistical Analysis

The staining score data is reported in means plus 95% confidence intervals. One-way analysis of variance (ANOVA), independent t test and X^2 test and Fisher's

exact test were used to analyze the scores of both tumor and normal cell groups. The P value was <0.05 which is considered significant.

RESULTS

The Expressions of Mortalin were Up-regulated in Various Tumors

The levels of the Mortalin expression were classified according to the staining intensity of the immunostaining. Compared to adjacent benign tissues, the expression levels of Mortalin were always significantly higher in the cancer tissue rather than paracarcinoma tissue from vicinities (Fig. 1).

TABLE 1. TMA analysis result: distribution classified by age (above or below 55 years old), gender, and TNM stages

	All patients		
Characteristics	No.	Percent	P value
Age			
<55	110	57.9%	>0.05
≥55	80	42.1%	
Gender			
Female	51	26.8%	<0.05
Male	139	73.2%	
TNM Stages			
T0	6	3.7%	
T1	12	7.3%	<0.05
T2	59	36.0%	<0.05
T3-4	87	53.0%	>0.05

The Expressions Patterns of Mortalin in Liver Cancers

The patterns of Mortalin expression in liver cancers were characterized according to age, gender, different TNM stages, and pathological diagnosis (Table 1). There was no difference in Mortalin expression between male and female liver caner patients ($P > 0.05$). Significantly higher expression was noticed in patients 55 years old or above (1.68 ± 1.39) versus the younger group (1.48 ± 1.24, $P < 0.05$).

The Mortalin expression in a hepatocellular carcinoma was significantly higher than the normal ones ($P < 0.05$; Fig. 2). Because the NM stages in our samples are all N0M0, so the classification was based on the advance of TNM stages from T0, T1, T2, T3, to T4. The Mortalin expression at T1 ($1.33 + 1.70$) was higher than T0 ($1.17 + 0.17$, $P < 0.05$). At T2, T3, and T4 stages ($1.76 + 2.08$) the expression of Mortalin was higher than at T1 ($P < 0.05$). However, there was no significant difference among the groups T2, T3, and T4 (Fig. 3).

The immunocytochemistry staining (Fig. 4) of Mortalin in HL-7702 and SMMC-7721 cell lines and the tumor line of SMMC-7721 was positive, which was verified by the western blotting in which the Mortalin expression of SMMC-7721 line was higher than the HL-7702 line (Fig. 5).

On the basis of the classification of liver cancer regarding pathological diagnosis, Mortalin was expressed the most highly in HCC, followed by cholangiocellular carcinoma and finally the liver clear cell carcinoma ($P < 0.05$).

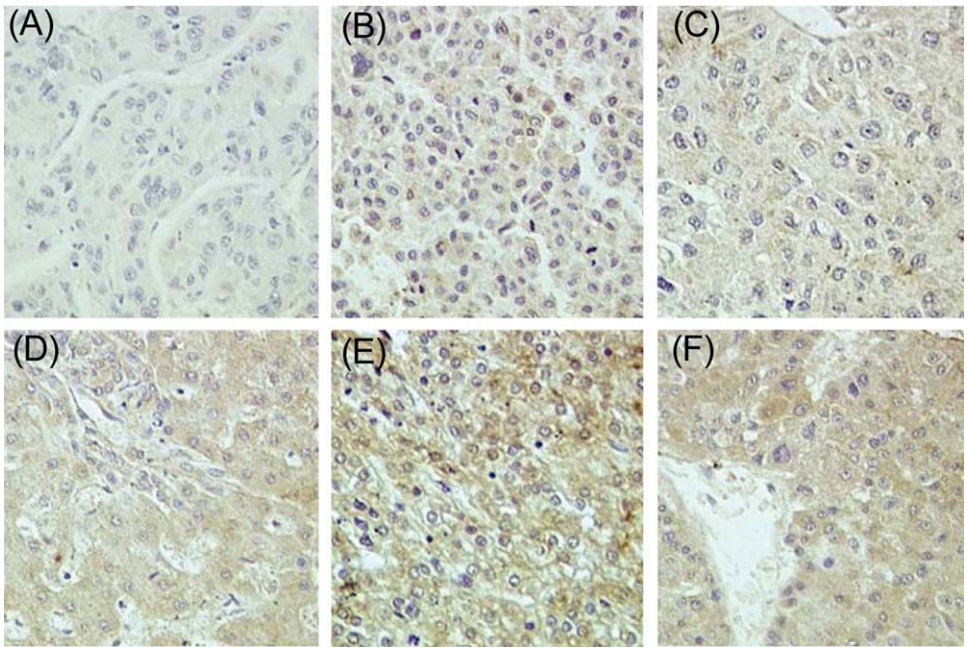

Fig. 2. Mortalin immunostaining in HCC showing a staining score (×200).Under a 20× high power lens (Leica DM2500), the sections were classified according to staining intensity as follows: **A:** Staining intensity, 0 (total absence of staining). **B:** staning intensity, 1+ (very weak staining). **C:** staning intensity, 2+ (mild staining). **D:** staning intensity, 3+ (moderate staining). **E:** staning intensity, 4+ (strong staining). **F:** staning intensity, 5+ (very heavy staining).

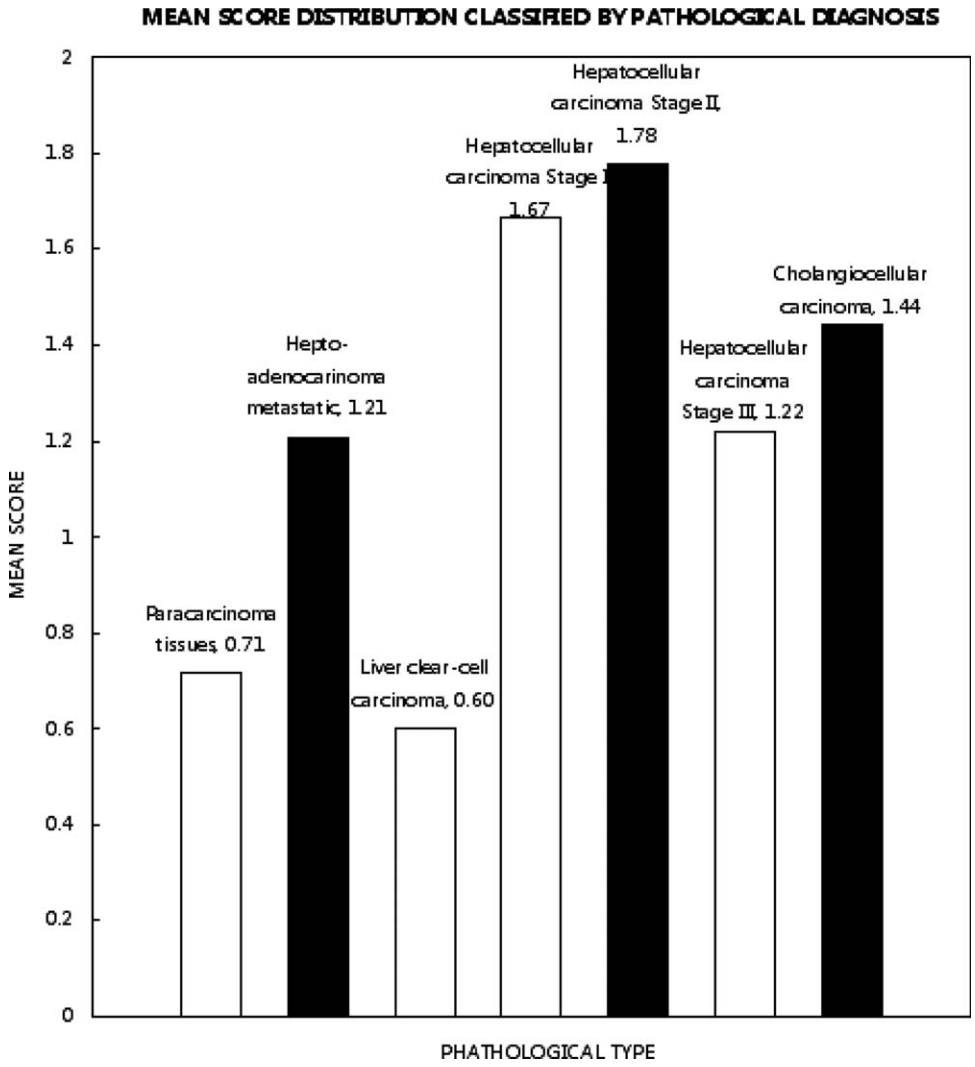

Fig. 3. TMA analysis result distribution classified by pathological diagnosis by Bar chart.

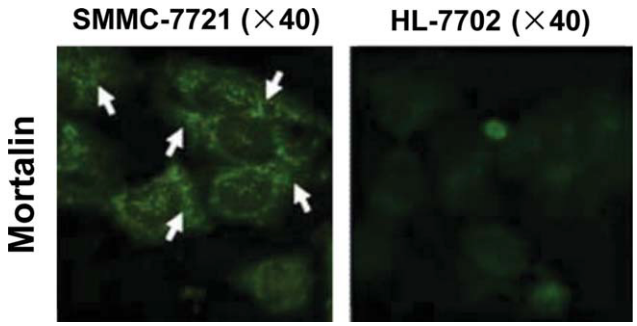

Fig. 4. Immunolocalization and immunofluorescence difference of Mortalin between SMMC-7721 and HL-7702 cells. In (**A**), Mortalin showed pancytoplasmic disseminated green fluorescent dots (arrows) in the SMMC-7721 cells, whereas in (**B**) the HL-7702 was totally negative.

Mortalin Protects the Liver Cells from GD Injury

HL-7702 cells were transfected with pcDNA3/Mortalin, and selected for stable clones. Western blot analysis revealed a higher expression level of Mortalin in transfected cells compared with HL-7702 cells (Fig. 6A). MTT assays were applied to investigate the role of Mortalin on HL-7702 under GD stress (Fig. 6B). After exposure to a glucose-free medium for 12 hr, 33.37% of the HL-7702 cell died. However, the viability in the Mortalin-overexpressing cells transfected with pcDNA3/Grp75 significantly improved over the control at all times ($P < 0.05$).

DISCUSSION

The mammalian Mortalin was first cloned by Wadhwa (Wadhwa et al., 1993). As a member of the HSP 70 family, this highly conserved protein plays a vital role in multiple processes of cell life ranging from stress response, intracellular trafficking, cell proliferation,

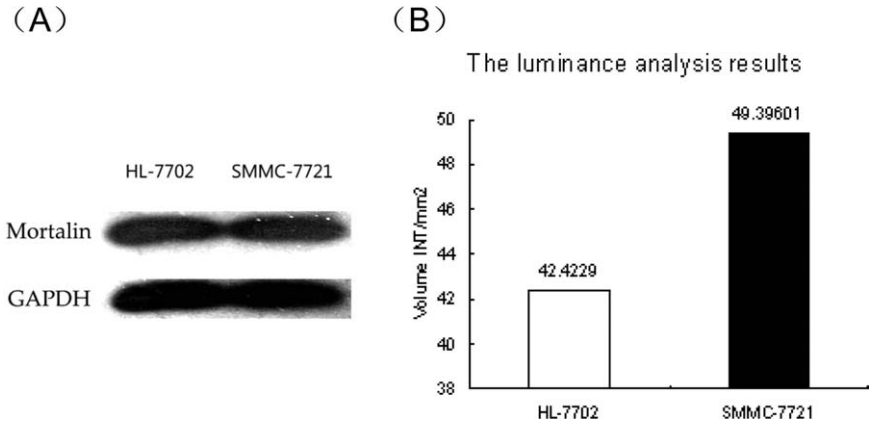

Fig. 5. **A:** The Western blot result of Mortalin in HL-7702 and SMMC-7721. Both of the cell lines contain certain amounts of Mortalin. **B:** The luminance analysis results by Quantity One. The Volume INT per mm^2 numbers indicates the expression level of SMMC-7721 over HL-7702., that is, the malignant cells had more Mortalin proteins.

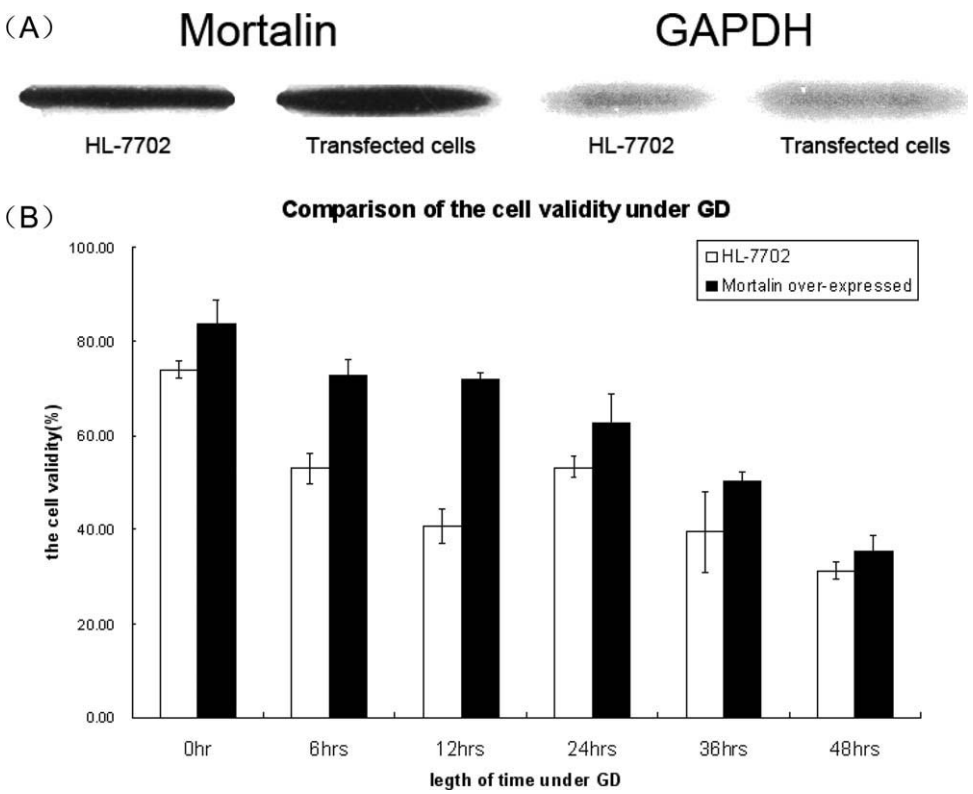

Fig. 6. **A:** The Western blot result of the transfected Mortalin-over-expressing cells and the normal liver cell line HL-7702. This picture shows an up-regulation of Mortalin in those transfected cells, that is, the transfection procedure has successfully been done. **B:** Mortalin-suppressed the decreased cell viability induced by glucose deprivation injury. This bar chart compared the cell viability of the normal liver cells and the Mortalin-over-expressing cells under GD in different time groups. The values presented are the mean ± SEM (N = 3) of representative experiments. *$P = 0.0157$, $P < 0.05$ compared with the controls.

differentiation, and carcinogenesis. It was constitutively expressed in almost every organ, and the levels of Mortalin were varied from various tissues. The role of Mortalin in inner mitochondrial matrix was complex (Kaul et al., 2007).

Tumors in the immortal state for continuous proliferation compete for basic cellular needs (space, nutrient, and oxygen) in hostility of the environments. Heat shock proteins, such as Mortalin, may serve as safeguards to maintain homeostasis and integrity of protein

interactions. The observation that tumor cells often have elevated levels of HSPs associates with tumorigenesis (Kaul et al., 2007). The cellular expression of Mortalin may raise the threshold of stress-induced apoptosis (Yang et al., 2008). Notably here, we confirmed that the level of Mortalin was elevated in various tumor tissues, which supports the hypothesis of up-regulation of Mortalin in promoting carcinogenesis. By virtue of its role in mitochondrial biogenesis, the up-regulation of Mortalin may help the metabolism of the tumor cells and may modulate the proteins such as p53 in the malignant cell elimination (Wang et al., 2002). Mortalin causes cytoplasmic sequestration of p53 by binding to its carboxyl terminus amino acid residues 312–352. Previous studies have shown that the cationic inhibitor of Mortalin, MKT-077, competes with Mortalin for p53 binding and results in translocation of p53 to the nucleus followed by rapid apoptosis (Wadhwa et al., 2000). Some data also suggested that Mortalin-based cytoplasmic sequestration of p53 in leukemic cell model can be reversed using MKT-077 and will result either in transcription-based apoptosis (following nuclear translocation of p53) or non-transcription-based apoptosis (following mitochondrial translocation of p53; Walker et al., 2008). These evidences explored that the interaction of Mortalin and p53 is one of the key point in the process of cell apoptosis. In our present study we reported the up-regulated Mortalin in tumor tissues, and we hypothesized the up-regulated Mortalin bind with p53 in cytoplasm, and its sequestration of p53 can inhibit both transcription-based apoptosis and non-transcription-based apoptosis. Since apoptosis plays a critical role that it maintains a balance with cell division, we supported the hypothesis that the interaction of Mortalin and p53 can result in the disorders of cell division and it could be one of the mechanisms of tumorigenesis.

In this study, we depicted the up-regulated Mortalin in tumor tissues from various organs and confirmed that Mortalin protein levels were up-regulated in tumor tissues and tumor cell lines in dishes, and the protein up-regulation was isolated from the transcription. This may suggest that either the Mortalin translation was increased or Mortalin degradation was decreased. In TMA analysis from Group T0 to T4, we found that Mortalin expression was especially high in malignant ones. On the basis of the tissue classifications, hepatocellular carcinoma had the highest in contrast with hepato-adenocarcinoma, or matastatic ones. MTT assays demonstrated that the cells with a function gain of Mortalin reduced the decreased cell viability by glucose deprivation. The data from our experiments primitively confirm that Mortalin may serve as a molecular tumor marker, and that understanding of Mortalin may provide insights into the mechanisms of the malignancy of the tumor and provide us a new target for the treatment in future.

LITERATURE CITED

Beere HM. 2004. The stress of dying: the role of heat shock proteins in the regulation of apoptosis. J Cell Sin 117:2641–2651.

Camp RL, Charette LA, Rimm DL. 2000. Validation of tissue microarray technology in breast carcinoma. Lab Invest 80:1943.

Czarnecka AM, Campanella C, Zummo G, Cappello F. 2006. Mortalin: when a close friend becomes a close enemy. Cancer Biol Ther 5:714–720.

Deocaris CC, Kaul SC, Wadhwa R. 2007. Heat shock chaperone Mortalin and carcinogenesis. Heat Shock Prot Cancer 21:141–157.

Deocaris CC, Widodo N, Ishii T, Kaul SC, Wadhwa R. 2007. Functional significance of minor structural and expression changes in stress chaperone mortalin. Ann N Y Acad Sci 1119:165–175.

Hinterberger M, Reineke T, Storz M, Weder W, Vogt P, Moch H. 2007. D2–40 and calretinin—a tissue microarray analysis of 341 malignant mesotheliomas with emphasis on sarcomatoid differentiation. Mod Pathol 20:248–255.

Hsu WM, Lee H, Juan HF, Shih YY, Wang BJ, Pan CY, Jeng YM, Chang HH, Lu MY, Lin KH, Lai HS, Chen WJ, Tsay YG, Liao YF, Hsieh FJ. 2008. Identification of GRP75 as an independent favorable prognostic marker of neuroblastoma by a proteomics analysis. Clin Cancer Res 14:6237–6245.

Kaul SC, Deocaris CC, Wadhwa R. 2007. Three faces of Mortalin: a housekeeper, guardian and killer. Exp Gerontol 42:263–274.

Kaul SC, Taira K, Pereira-Smith OM, Wadhwa R. 2002. Mortalin: present and prospective. Exp Gerontol 37:1157–1164.

Kaul Z, Yaguchi T, Kaul SC, Hirano T, Wadhwa R, Taira K. 2003. Mortalin imaging in normal and cancer cells with quantum dot immunoconjugates. Cell Res 13:503–507.

Lanneau D, Brunet M, Frisan E, Solary E, Fontenay M, Garrido C. 2008. Heat shock proteins:essential proteins for apoptosis regulation. J Cell Mol Med 12:743–761.

Lanneau D, Thonel A, Maurel S, Didelot C, Garrido C. 2007. Apoptosis versus cell differentiation role of heat shock proteins HSP90, HSP70, and HSP27. Prion 1:53–60.

Liu Y, Liu W, Song XD, Zuo J. 2005. Effect of GRP75/mthsp70/PBP74/mortalin overexpression on intracellular ATP level, mitochondrial membrane potential and ROS accumulation following glucose deprivation in PC12 cells. Mol Cell Biochem 268:45–51.

Massa SM, Zuo J, Longo FR, Wang J, Sharp FR. 1995. Cloning of rat grp75,an hsp70 family member, and its expression in normal and ischemic brain. J Neurosci Res 40:807–819.

Mosser DD, Morimoto RI. 2004. Molecular chaperones and the stress of oncogenesis. Oncogene 23:2907–2918.

Mizukoshi E, Suzuki M, Misono T, Loupatov A, Munekata E, Kaul SC, Wadhwa R, Imamura T. 2001. Cell-cycle dependent tyrosine phosphorylation on Mortalin regulates its interaction with fibroblast growth factor-1. Biochem Biophys Res Commun 280: 1203–1209.

Rupert L, Marcus F, Joerg RS, Hans-Juergen W, Heinz H. 2008. Expression and clinical significance of Glucose Regulated Proteins GRP78 (BiP) and GRP94 (GP96) in human adenocarcinomas of the esophagus. BMC Cancer 8:70.

Takano S, Wadhwa R, Mitsui Y, Kaul SC. 2001. Identification and characterization of molecular interactions between glucose-regulated proteins (GRPs) Mortalin/GRP75/peptide- binding protein 74 (PBP74) and GRP94. Biochem J 357:393–398.

Wadhwa R, Kaul SC, Ikawa Y, Sugimoto Y. 1993. Identification of a novel member of mouse hsp 70 family: its association with cellular mortal phenotype. J Biol Chem 268:6615–6621.

Wadhwa R, Kaul SC, Mitsui Y. 1994. Cellular mortality to immortalization: mortalin. Cell Struct Funct 19:1–10.

Wadhwa R, Sugihara T, Yoshida A, Nomura H, Reddel RR, Simpson R, Maruta H, Kaul SC. 2000. Selective toxicity of MKT-077 to cancer cells is mediated by its binding to the hsp70 family protein mot-2 and reactivation of p53 function. Cancer Res 60:6818–6821.

Wadhwa R, Taira K, Kaul SC. 2002. An Hsp70 family chaperone, Mortalin/mthsp70/PBP74/Grp75: what, when, and where? Cell Stress Chaperones 7:309–316.

Wadhwa R, Takano S, Kaur K, Deocaris CC, Pereira-Smith OM, Reddel RR, Kaul SC. 2006. Upregulation of mortalin/mthsp70/Grp75 contributes to human carcinogenesis. Int J Cancer 118: 2973–2980.

Wadhwa R, Takano S, Rober M, Yoshida A, Nomura H, Reddeli RR, Mitsui Y, Kaul SC. 1998. Inactivation of tumor suppressor p53 by Mot-2, a hsp70 family member. J Biol Chem 273:29586–29591.

Wadhwa R, Yaguchi T, Hasan MK, Taira K, Kaul SC. 2003. Mortalin-MPD (mevalonate pyrophosphate decarboxylase) interactions and their role in control of cellular proliferation. Biochem Biophys Res Commun 302:735.

Walker CW, Böttger SA. 2008. A naturally occurring cancer with molecular connectivity to human diseases. Cell Cycle 7:2286–2289.

Wang S, Fusaro G, Padmanabhan J, Chellappan SP. 2002. Prohibitin colocalizes with Rb in thenucleus and recruits N-CoR and HDAC1 for transcriptional repression. Oncogene 21:8388–8396.

Yang L, Liu X, Hao J, Yang Y, Zhao M, Zuo J, Liu W. 2008. Glucose-regulated protein 75 suppresses apoptosis induced by glucose deprivation in PC12 cells through inhibition of Bax conformational change. Acta Biochim Biophys Sin 339–348.

Yi X, Luk JM, Lee NP, Peng J, Leng X, Guan XY, Lau GK, Beretta L, Fan ST. 2008. Association of mortalin (HSPA9) with liver cancer metastasis and prediction for early tumor recurrence. Mol Cell Proteomics 7:315–325.