HspB5 correlates with poor prognosis in colorectal cancer and prompts epithelial-mesenchymal transition through ERK signaling

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Abstract

Alpha B-crystallin (HspB5) is abnormally expressed in tumor tissues and portends a poor prognosis in cancer patients. However, the role of HspB5 in colorectal cancer (CRC) is still unclear. Seventy CRC patients and 40 healthy volunteers were sampled from August 2012 to March 2015 in order to determine the clinical significance of HspB5. In vitro cellular studies were used to validate its molecular mechanisms in CRC. Our clinical data indicated that HspB5 was up-regulated, and had a positive association with TNM stage CRC patients. The expression level of HspB5 in CRC patients was closely correlated with MMP7 and E-cadherin, two core epithelial–mesenchymal transition (EMT) gene products. The in vitro studies revealed that high HspB5 expression could prompt tumor cell proliferation and invasion, as well as EMT. Gene-microarray analysis suggested three significant signaling pathways (PI3K, p38 and ERK) were involved in HspB5-induced EMT. Signal transduction pathway inhibitors and HspB5 gene knockdown models suggested that HspB5 promotes CRC tumorigenesis and EMT progression through ERK signaling pathways. In summary, HspB5 maybe trigger the EMT in CRC by activating the ERK signaling pathway. It is a potential tumor biomarker for CRC diagnosis and prognosis.

Introduction

Colorectal cancer (CRC) is the third most prevalent cancer in China and accounts for the major cause of cancer-related deaths[1]. As we know, the occurrence and progression of CRC maybe a multi-step, complex process associated with multiple oncogenes and tumor suppressors[2]. Although great efforts have been made to better understand the molecular mechanism relevant to CRC and to improve its therapy, these aspects of CRC are still largely unknown. The underlying pathogenesis associated with CRC requires further study for the development of novel diagnostic and therapeutic approaches.

Alpha B-crystallin (HspB5) is a cytoprotective molecular chaperone and small heat-shock protein[3]. It prevents the stress-induced, irreversible aggregation of denatured proteins, and
traps aggregation-prone proteins in reservoirs of nonnative, and refoldable intermediates, within soluble, large, and multimeric structures[4]. Thus, \textit{HspB5} is suggested to play a key role in cellular protection, apoptosis inhibition, and proteasomal interactions. Recent studies mainly focus on the presence of \textit{HspB5} in various types of solid tumors as a novel oncoprotein, as well as a prognostic marker[5, 6]. For example, \textit{HspB5} over-expression has been clinically detected in several different types of tumors including breast, renal, lung, and hepatic cancer, and is associated with poor prognosis in most of these patients[7–10]. However, little is known about the role and mechanism of \textit{HspB5} in CRC.

CRC is a malignant tumor that is characterized by a high potential for invasion and metastasis[11]. It is widely accepted that epithelial–mesenchymal transition (EMT) acts as a fundamental mechanism in tumor invasion and metastasis, especially in CRC[12]. EMT is a process by which epithelial cells lose epithelial properties to become mesenchymal stem cells. Carcinoma cells undergoing EMT in primary tumors lose their cell polarity and cell to cell adhesion capacity, gain invasive properties[13], enter the bloodstream, and then contribute to clonal outgrowth at these metastatic sites[14]. Related studies are of great importance in determining whether \textit{HspB5} expression facilitates tumor progression in CRC by inducing EMT.

In the present study, it was demonstrated that \textit{HspB5} could be identified as a valuable biomarker for clinicopathological parameters and poor prognosis in CRC patients. The potential molecular mechanisms by which \textit{HspB5}-induced EMT may be responsible for tumor invasion and metastasis was then explored. Taken together, this is the first report showing the clinical significance and molecular mechanism of \textit{HspB5} in CRC.

**Materials and methods**

**Cell lines**

Three CRC cell lines, including HCT116, SW480 and Lovo, were purchased from the American Type Culture Collection (Manassas, VA, USA). All cells were maintained in RPMI 1640 media supplemented with 10% fetal bovine serum under a 5% CO\textsubscript{2} incubator at 37˚C.

**Patients and follow-up**

Fresh CRC tissue and adjacent nontumor tissue was collected from 70 consecutive patients who underwent colorectal surgery between August 2012 and March 2015 at Songjiang Hospital Affiliated Shanghai First People’s Hospital. CRC differentiation was defined according to Dukes’ grading system described by the World Health Organization. None of the patients received chemotherapy, radiotherapy or other special treatment before surgery. Forty normal colonic samples were obtained via colonoscopy from healthy people who underwent a health check in Songjiang Hospital Affiliated Shanghai First People’s Hospital. Follow-ups were terminated on May 15, 2016, following the procedures described in this paper[15, 16].

**Gene expression profile chip**

Six human colonic tumors (N = 6) and their adjacent nontumor tissue (N = 6), as well as four normal intestinal mucosa tissue samples (N = 4) were included and subjected to gene expression microarray in the study. Subsequently, human gene microarray hybridization was performed according to the manufacturer’s instructions (Agilent Technologies, Santa Clara, CA, USA). Briefly, hybridization run conditions were as follows: speed (10 rpm), run time (up to 17 h), temperature (65˚C). After the reaction was complete, the hybridization chip was washed with Gene Expression Wash Buffer Kit in wash tank staining dishes (Thermo Shandon, Wal-tham, MA, USA). After generating the microarray scan images through the Agilent Microarray...
Scanner (Agilent Technologies), the data was extracted using Feature Extraction software 10.7 (Agilent Technologies). The data were normalized using the Quantile algorithm, Gene Spring Software 11.0 (Agilent Technologies). Finally, the obtained data was exported directly into the SAM software for SAM analysis. Genes were considered to be differentially expressed based on the criteria of P<0.005, fc2, and mean7.

**RNA extraction and qRT-PCR**

Seventy human colonic tumors, their adjacent nontumor tissue, and 40 normal intestinal mucosa samples were analyzed via qRT-PCR (Table 1). Total RNA of pancreatic tissue was isolated with a Trizol reagent (Invitrogen, CA, USA) according to the manufacturer’s instructions. The total RNA was treated with RNase-free DNase to remove the residual genomic DNA. 1 μg of total RNA was reverse-transcribed to the first-strand cDNA that was used as template for PCR amplification. The thermal amplification step was conducted in the following conditions: 95˚C denaturation for 30s followed by 40 cycles of 95˚C denaturation for 5s, and 60˚C for 30s. In addition, HCT116, SW480 and Lovo cell lines were also analyzed via qRT-PCR as described previously.

**Immunofluorescence**

CRC patient samples were subjected to immunofluorescence analysis. A monoclonal, anti-human HspB5 antibody (Santa Cruz, CA, USA) was used to detect the expression of HspB5 in colonic tissue samples. Paraffin-embedded tissue sections of colorectal cancers were stained as follows: the samples were incubated with a polyclonal anti-HspB5 antibody overnight at 4˚C, and then with a secondary antibody for 60 min at 37˚C.

**Cell transfection**

Small interfering RNAs (siRNA) were constructed and synthesized by Shanghai GenePharma Co, Ltd (Shanghai, China). Lipofectamine 2000 was purchased from Invitrogen Life Technologies (Carlsbad,CA, USA). Control siRNA and siRNA-HspB5 were transfected into Lovo cells using Lipofectamine, according to the manufacturer’s instructions. Briefly, 20pmol HspB5 siRNA or control siRNA was incubated with Lovo cells for 6h. The interference efficiency of the HspB5 gene was defined using qRT-PCR and western blotting. The siRNA target sequences are listed below.

siRNA sequences: 5’-CCAUCACCCGUAGAAGAGAATT 3’;  
Antisense: 5’-UUCUCUUCACGGGUGAGTT 3’;  
Control RNA sequences: 5’-UUCUCGGAACGUGACGUATT 3’;  
Antisense: 5’-ACGUGACACAGUUCCGGAGATT 3’.

### Table 1. qRT-PCR using the following primers.

| Gene | Forward (5’to3’) | Reverse (5’to3’) |
|------|------------------|-----------------|
| E-cadherin | CACCCACGTACAAGGGTGCTAGG | AACAGCTGTGGAGATGCGAG |
| MMP7 | CTACAGTGAGGACAGGCTCA | CACTCCACATCTGGGCTTCT |
| HspB5 | TTCCCAGGCCTACTTCCCT | TCCTGGGGCTCTTCAAGTTT |

https://doi.org/10.1371/journal.pone.0182588.t001
Western blot

Western blotting was used to investigate HspB5, MMP-7 and E-cadherin protein expression in Lovo cells. Briefly, the samples were incubated overnight at 4°C with a polyclonal anti-HspB5 antibody (Santa Cruz, CA, USA), an anti-MMP7 antibody (Santa Cruz, CA, USA), and an anti-E-cadherin antibody (Abcam, Cambridge, MA, USA). This was followed by incubation with a secondary antibody for 60 min at 37°C. Finally, western blot bands were captured through a gel imaging system (Bio-Rad, Hercules, CA, USA).

Invasion and metastasis assays

Transwell migration assays (pore size, 8 μM) were performed to analyze tumor invasion and metastasis. Lovo cells were selected and respectively co-cultured with siRNAs, Ly294002 (25μM final concentration, PI3K inhibitor, Calbiochem, Billerica, MA, USA), SB202190 (5μM final concentration, p38 inhibitor, Calbiochem, Billerica, MA, USA), and PD98039 (20μM final concentration, ERK inhibitor, Calbiochem, Billerica, MA, USA). Briefly, cells were loaded on the upper compartment of the six-well Transwell chamber (pore size 8 μm; Dow Corning Corp, Burlington, NJ, USA), and allowed to migrate across an 8.0μm pore size polycarbonate membrane for 24 h. As a result of the chemo-attractant properties, cells migrated to the bottom surface of the membrane and were then counted to evaluate their invasion and metastasis abilities.

Cell proliferation assay

Lovo cells were added to a 96-well plate at 1×10⁴/100μl/well concentration. At four different time points (0, 24, 48, and 72h), Cell Counting Kit-8 (CCK-8) solution was added into each well and incubated for 2hrs. The plate was read at 450 nm for determining the number of viable cells.

Colony formation assays

Lovo cells were seeded into 12-well plates and allowed to grow for about 4 to 5 days at 37°C until small colonies could be detected. Cell culture plates containing colonies were gently washed with phosphate-buffered saline solution and fixed with 3.7% formaldehyde for 10 min. Finally, the number of colonies was recorded.

Statistical analysis

All data were analyzed with SPSS 19.0 software (Shanghai, China) through the $X^2$ and Student t-test. Values were expressed as the mean±standard deviation (SD). P < 0.05 was considered to be statistically significant.

Ethical statement

The study protocol was approved by the committee for the Ethical Issue in First People’s Hospital, Shanghai Jiaotong University (No.201304), and written informed consent was obtained from each patient. All methods were conducted in accordance with the Guidelines for Experiments of Shanghai Jiaotong University.

Results

HspB5 over-expression in CRC patients

There was a significant difference in gene expression among CRC patients. When compared to healthy people, CRC patients were associated with the up-regulation of 1871 genes and down-regulation of 1478 genes. In addition, colonic tumor specimens and adjacent nontumor tissue
from each CRC patient was collected to further detect the differentially expressed genes. Between these two groups, 1823 genes were differentially expressed in tumor tissue. Of these, 1018 genes were up-regulated and 805 were down-regulated. Hierarchical clustering of gene expressions is shown in Fig 1A.

The microarray gene expression data analysis of the three significant tumor genes is summarized in Table 2. HspB5 expression in colonic tumor tissue was significantly lower than that of normal tissues.

### Table 2. Gene microarray results for differentially expressed tumor genes in CRC patients.

| Gene name                         | P     | Fold |
|----------------------------------|-------|------|
| HspB5(tumor: adjacent nontumor)  | 0.033409 | 0.031731 |
| HspB5(tumor: normal tissues)     | 0.006775 | 0.632249 |
| MMP7(tumor: adjacent nontumor)   | 0.000114 | 243.7458 |
| MMP7(tumor: normal tissues)      | 0.000275 | 260.6385 |
| E-cadherin (tumor: adjacent nontumor) | 0.377876 | 1.169679 |
| E-cadherin (tumor: normal tissues) | 0.432123 | 0.897454 |

https://doi.org/10.1371/journal.pone.0182588.t002
in both adjacent nontumor tissue and normal intestinal mucosa samples (P<0.05). High MMP7 expression was found in tumor tissue while E-cadherin expression was not altered (P>0.05). This result showed that there was a significant difference in HspB5 and MMP7 expression among CRC patients, but not in E-cadherin.

To validate the microarray results, 70 CRC patients and 40 healthy people were chosen and sampled material subjected to the further study using qRT-PCR. As seen in Table 3, HspB5, MMP7 and E-cadherin were validated to be differentially expressed when comparing colonic tumor tissue and adjacent nontumor samples from CRC patients. Contrary to the down-regulation of HspB5 found in gene microarray results, HspB5 in colonic tumor tissue was significantly higher at mRNA level (colonic tumor tissue vs. adjacent nontumor tissue, P<0.05). The same result was found in CRC patients vs. healthy controls. E-cadherin in qRT-PCR analysis was also validated to be significantly down-regulated among these CRC tissue samples (P<0.05) while it did not show an obvious change when measured using gene microarray. MMP7 was over-expression in colonic tumor tissue (P<0.05), showing the same results from both qRT-PCR and gene microarray analysis. A comparison between qRT-PCR and gene microarray is described in Fig 1B. In summary, qRT-PCR analysis confirmed three differently expressed genes in CRC patients which included up-regulation of HspB5 and MMP7, and down-regulation of E-cadherin.

HspB5-labeled fluorescence experiments demonstrated that tumor tissue from CRC patients exhibited a stronger green fluorescence than their normal controls, and HspB5 labeled green fluorescence was found near the nucleus of the cell (Fig 1C). The experiments clearly revealed that HspB5 levels had markedly increased in CRC patients at the protein level (stage 4>stage 3>stage 2>stage 1>normal intestinal mucosa tissue), which is consistent with TNM descriptors.

HspB5 over-expression correlates with clinicopathological parameters and poor prognosis in CRC patients

The relationship between HspB5 over-expression and clinicopathological characteristics in CRC patients is shown in Table 4. The groups with high HspB5 expression levels accounted for 61.4% (43/70) of the total number of patients. High HspB5 expression was associated with TNM staging in 70 CRC patients (P<0.05). However, other important clinical characteristics, including age, sex, tumor size, tumor differentiation, lymph node metastasis, and tumor location were not directly related to HspB5 expression.

Patient follow-up was terminated on May 15, 2016, with the follow-up period for each of the patients ranging from 12 to 48 months. In order to analyze the overall survival rate, data from three year post-operative follow-up visits of 35 patients was used. Fig 1D reveals that the patients with high levels of HspB5 had a shorter overall survival rate than those with low expression. More patient samples will be collected to analyze the survival rates in future studies.

HspB5 promotes cell proliferation and invasion

Three different CRC cell lines, HCT116, SW480 and Lovo cells, were considered as the in vitro cellular model in this study. Western blot analysis showed that HspB5 protein levels in Lovo

Table 3. qRT-PCR confirmations of 3 differentially expressed genes among 70 CRC patients.

| Gene name | tumor tissue | adjacent nontumor tissue | normal tissue | Regulation |
|-----------|--------------|--------------------------|---------------|------------|
| HspB5     | 24.9±16.6    | 12.6±6.6                 | 7.9±3.5       | Up         |
| MMP       | 29.7±78.1    | 1                        | -             | Up         |
| E-Cadherin| 0.7±0.5      | 1                        | -             | Down       |

https://doi.org/10.1371/journal.pone.0182588.t003
cells were significantly higher than those in HCT117 and SW480 cells (Fig 2A). HspB5 mRNA in Lovo cells was also highly expressed compared to the other two cell lines. Thus, Lovo cells were chosen to determine the molecular mechanism of HspB5 in CRC.

siRNAs were used to reduce the levels of HspB5 expression in Lovo cells. As seen in Fig 2B, western blot analysis showed that there was a 90% reduction of HspB5 protein levels after siRNA-mediated gene knockdown. Fig 2B showed that HspB5 mRNA levels were significantly lower after the introduction of HspB5 siRNA (0.07±0.02 vs.1, P<0.01). The control siRNA group failed to reveal any differences in HspB5 expression before and after siRNA interference. These data clearly revealed that gene silencing with siRNA successfully knocked out the HspB5 expression in Lovo cells, which could serve as the in vitro model to investigate the tumor invasion and metastasis in CRC.

There were obvious signs of tumor cell proliferation and invasion in Lovo cells, as seen in Fig 3. After siRNA-HspB5 treatment for 6 h, the number of invasive cells decreased markedly along with the inhibition of HspB5 expression (Fig 3A). The colony forming assay (Fig 3B) and CCK-8 assay (Fig 3C) revealed that Lovo cell proliferation was significantly suppressed by HspB5 siRNA. In addition, the control siRNA group failed to reveal any differences compared to Lovo cells. Based on this data, this study clearly shows that HspB5 over-expression could prompt the ability of tumor cell proliferation and invasion in CRC.

Table 4. Correlation between HspB5 and clinicopathological characteristics in 70 CRC patients.

| Variables                  | N  | HspB5 |   | P  |
|----------------------------|----|-------|---|----|
|                            |    | High  | Low|    |
| Patient                    | 70 | 43    | 27|    |
| Sex                        |    |       |   | 0.776|
| Male                       | 44 | 34    | 10|    |
| Female                     | 26 | 19    | 7 |    |
| Tumor diameter (cm)        |    |       |   | 0.144|
| ≥5cm                       | 25 | 16    | 9 |    |
| <5cm                       | 45 | 37    | 8 |    |
| Location                   |    |       |   | 0.248|
| Colon                      | 27 | 18    | 9 |    |
| Rectum                     | 43 | 34    | 9 |    |
| TNM stage                  |    |       |   | 0.042|
| I                          | 3  | 3     | 0 |    |
| II                         | 14 | 7     | 7 |    |
| III                        | 20 | 18    | 2 |    |
| IV                         | 33 | 25    | 8 |    |
| Lymphatic metastasis       |    |       |   | 0.584|
| Yes                        | 32 | 24    | 8 |    |
| No                         | 38 | 29    | 9 |    |
| Distant metastasis         |    |       |   | 0.622|
| Yes                        | 9  | 7     | 2 |    |
| No                         | 61 | 46    | 15|    |
| Tumor differentiation      |    |       |   | 0.084|
| Low                        | 18 | 13    | 5 |    |
| Middle                     | 49 | 38    | 11|    |
| High                       | 3  | 2     | 1 |    |

https://doi.org/10.1371/journal.pone.0182588.t004
HspB5 induces CRC EMT \textit{in vivo} and \textit{in vitro}

To investigate the effect of HspB5 on inducing EMT, the expression of two EMT hallmarks, E-cadherin and MMP7, were assessed \textit{in vivo} and \textit{in vitro}. Our clinical data indicated that HspB5 was up-regulated in CRC patients. Accompanied by HspB5 over-expression, up-regulation of MMP7 and down-regulation of E-cadherin were found in CRC patients (Fig 1B). This implies that elevated HspB5 expression induces EMT in CRC. After HspB5 gene silencing with siRNA, up-regulation of E-cadherin expression and down-regulation of MMP7 expression were founded in Lovo cells (Fig 2B). The \textit{in vitro} study further established that the increased expression of HspB5 induces EMT in colorectal cancer.

Hyperactivation of ERK signaling is responsible for HspB5-induced EMT

Using the Kyoto Encyclopedia of Genes and Genomes and Pathway Profiler databases, the three significant signaling pathways, phosphatidylinositol 3-kinase (PI3K), p38 mitogen activated protein kinase (p38MAPK) and extracellular regulated protein kinases (ERK), were detected in this study. These pathways have previously been reported in metastasis and
carcinogenesis [13]. To further explore the role of the signaling pathways in HspB5-induced EMT, Ly294002 (PI3K inhibitor), SB202190 (p38 inhibitor) and PD98039 (ERK inhibitor) were used to treat Lovo cells. As shown in Fig 4A, down-regulation of MMP7 and up-regulation of E-cadherin at protein level in ERK inhibitor-treated groups was observed, but not in PI3K and p38 inhibitor groups. The latter two groups indicated a typical EMT phenotype due to the hyperactivation of ERK. Furthermore, cellular invasion and proliferation assays were used to investigate the cellular characteristics. After blocking ERK, PI3K and p38 signaling, cellular invasion and proliferation assays were evidently inhibited by the ERK signaling pathway (Fig 4B–4D). The aforementioned data clearly revealed that the ERK signaling pathway may be crucially involved in invasion, proliferation and EMT. Finally, siRNA-HspB5 was used to treat Lovo cells at 3 and 6h. This result indicated that the ERK signaling pathway was significantly blocked after HspB5 knockdown, thereby showing that HspB5 could regulate ERK (Fig 4A). Taken together, these results suggest a possible mechanism by which HspB5 induces EMT by activating ERK signaling in CRC cells.

**Fig 3.** HspB5 promotes cell proliferation, invasion and colon formation. A: Transwell Matrigel invasion assays. The number of invasive cells in the HspB5 siRNA-treated group decreased obviously compared with those in the control group. B: In colony forming assay, cell proliferation was significantly suppressed by HspB5 siRNA and ERK inhibitor (N = 3). Lovo cells vs. HspB5 siRNA (5.0±0.5% vs. 4.3±0.3%). C: CCK-8 assay. The cell proliferation was significantly suppressed 48 hours after HspB5 siRNA transfection. HspB5 over-expression could prompt tumor cell proliferation in CRC.

https://doi.org/10.1371/journal.pone.0182588.g003
Discussion

In this study, we explored the clinical significance and underlying mechanism of HspB5 in CRC pathogenesis. First, expression of HspB5 was significantly increased in CRC tissue compared with that in adjacent non-tumor or normal intestinal mucosa tissues. Second, HspB5 over-expression was closely correlated with the TNM stage and poor prognosis in clinical practice. This is the first report on the molecular mechanism of HspB5 and its involvement in tumor invasion and metastasis in CRC. This study clearly indicates that HspB5 is a candidate tumor suppressor which was markedly up-regulated in CRC, and potently induces the EMT process by activating the ERK signaling pathway.

The gene expression profiles chip is the latest DNA analysis technology using a whole-genome expression design[17]. It has been widely applied to screen the differentially expressed genes as potential biomarkers in cancer patients. With the great genetic scale, high throughput and objectivity, it allows the monitoring of tens of thousands of genes from each patient. As reported previously, HspB5 aggravated the oncogenic transformation[18]. This is achieved by involving extensive regulatory functions in cell apoptosis, proliferation, migration, invasion, drug resistance, and cell cycle regulation through inhibiting caspase-3 activation[19, 20], and anti-VEGF (vascular endothelial growth factor) resistance[5].

In this study, we found that HspB5 was obviously up-regulated in qRT-PCR while HspB5 was down-regulated according to gene microarray results. In order to explain the contradiction, the five samples analyzed with the gene chip were validated using qRT-PCR. The over-regulation of HspB5 mRNA of three samples and down-regulation of HspB5 mRNA of two samples was confirmed. We further investigated the level of HspB5 mRNA expression of 70
CRC patients, in which 43 HspB5 mRNA cases were up-regulated and 27 HspB5 mRNA cases were down-regulated. In addition, E-Cadherin in qRT-PCR was down-regulated while it had not an obvious difference in gene microarray. To the best of our knowledge, it was impossible that gene expression in tumor patients was up to 100% positive rate due to the individual differences[21]. Thus, the discrepancies in the results of qRT-PCR and gene microarray analysis could be attributed to the individual differences in clinical patients. It is necessary that qRT-PCR is performed to validate the microarray results.

To date, HspB5 has been reported to be over-expressed in the multiple tumors, and is associated with poor prognosis and recurrence of human cancer. In this study, HspB5 over-expression was founded in CRC patients. This is consistent with other types of cancers, such as breast, thyroid, renal papillary cell, gastric, oral, and hepatocellular carcinoma[8, 22, 23]. Results from previous studies suggested that HspB5 over-expression may play an important role in CRC. Subsequently, we studied the relationship between HspB5 over-expression and poor prognosis in CRC patients. Histopathological characteristics, such as the TNM stage, larger tumor size, presence of vascular invasion, intrahepatic spreading, and lymph node metastasis, were regarded as the significant hallmarks of poor prognosis in CRC patients[24]. HspB5 expression was found to be up-regulated with TNM stage cancer (P = 0.042), indicating that HspB5 could be regarded as a novel biomarker for CRC prognosis in such patients.

Since CRC cells undergoing EMT is a critical initiation event for tumor progression, their detection and identification will aid in the development of new therapies that specifically target EMT, improve patient prognosis, and reduce therapy resistance[25]. Playing a key role in cellular proliferation, invasion and metastasis, HspB5 was responsible for the poor prognosis in HspB5-positive cancer patients[26]. HspB5 was validated as a potent inducer of EMT, an important mechanism during cancer invasion transformation and metastasis. However, HspB5-induced EMT in CRC was unknown. We firstly demonstrated that HspB5 expression could be inhibited by siRNAs in Lovo cells. After blocking HspB5 expression, the ability of tumor cell proliferation, invasion and metastasis in CRC was dramatically reduced. E-cadherin and MMP7 [27] are thought to promote tumor metastasis and progression in a variety of human cancers by inducing EMT. Changes in E-cadherin levels were related to the adhesion of epithelial cells, allowing cancer cells to cross the basement membrane and invade surrounding tissue. MMP7 plays an important role in tumor metastasis and regulation of cell migration through the breakdown of the extracellular matrix. Next, our study illustrated the down-regulation of E-cadherin and up-regulation of MMP7 in vivo and in vitro, especially in CRC patients. Furthermore, a recent paper reported that HspB5 promoted tumor migration and invasion capability through EMT signaling in CRC SW480 cell and nude mice model[28]. Thus, these data revealed that CRC cells had undergone an EMT process that was identified as a result of the increase in HspB5 proteins.

Out of further interest, we investigated the possible pathway by which HspB5 participates in cell metastasis of colorectal carcinoma. We found that the inhibitor of ERK, but not PI3K/Akt or p38, attenuated the EMT progression in CRC. We demonstrated that PD98039, which serves as an ERK inhibitor, successfully up-regulated the level of E-cadherin, and down-regulated the level of MMP7 in vitro, leading directly to decreased migration and invasion in tumor cells. A study had indicated that the PI3K/Akt, ERK and Raf/MAPK signaling pathways played an important role in the process of EMT in human cancers[29]. In theory, the up-regulation of HspB5 expression would participate in PI3K, p38 and ERK signaling in CRC cells[30–32]. Bewilderingly, the ERK, but not PI3K and p38 signaling pathways may be crucially involved in the invasion, proliferation and EMT induced by HspB5 over-expression in Lovo Cells. One plausible explanation is that the functions of PI3K/Akt, ERK and Raf/MAPK in EMT might depend on the different cell types[33].
Conclusion
In a word, this study provides a better understanding on both the functional role and molecular mechanism of HspB5 in human CRC. Our current work indicates that HspB5 is a novel marker for the unfavorable prognosis in CRC patients after surgery due to its capacity to facilitate cancer cell migration and invasion. Notably, HspB5 may induce EMT via the ERK signaling pathway in CRC.

Acknowledgments
The authors are grateful to the National Natural Science Foundation of China (No 81370569), Science & Technology Commission of Shanghai Songjiang (No QK1102) and Songjiang District Commission of Health and Family Planning (No 2012-III-03).

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