Preoperative heat shock protein 47 levels identify colorectal cancer patients with lymph node metastasis and poor prognosis

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Abstract. Accumulating evidence suggests that overexpression of heat shock protein 47 (HSP47) increases cancer progression, and that HSP47 level in the tumor-associated stroma may serve as a diagnostic marker in various cancers. The present study aimed to evaluate whether HSP47 gene expression in colorectal cancer (CRC) tissues could be used to identify lymph node (LN) metastasis status preoperatively in patients with CRC. To do so, HSP47 gene expression was determined and its association with the clinicopathological characteristics of patients with CRC was analyzed. A total of 139 surgical specimens from patients with CRC and 36 patients with benign colonic disease undergoing surgery at Mie University Hospital were analyzed. HSP47 gene expression was determined by reverse transcription quantitative PCR using Power SYBR Green PCR methods. Expression level of HSP47 was significantly higher in CRC tissues compared with normal tissue from patients with benign colonic disease. Furthermore, high HSP47 expression was significantly associated with tumor progression, including high T stage, lymph node metastasis and venous invasion, and high TNM stage. High HSP47 expression may therefore serve as a novel predictive biomarker for determining patients with CRC and LN metastasis. According to Kaplan-Meier analysis, patients with high HSP47 expression level had significantly poorer overall survival than those with low HSP47 expression level. Furthermore, multivariate analyses identified HSP47 expression as an independent predictive marker for LN metastasis and poor overall survival in patients with CRC. In summary, the present study demonstrated that HSP47 expression may be considered as a novel biomarker for predicting LN metastasis status and prognosis in patients with CRC.

Introduction

Colorectal cancer (CRC) is the third most common non-cutaneous carcinoma and the third leading cause of cancer-associated mortality worldwide (1). The Tumor-Node-Metastasis (TNM) staging system established by the American Joint Committee on Cancer is widely used to predict prognosis and provide the most pertinent and effective treatment strategies in patients with CRC (2). According to the TNM staging system, the presence of positive lymph nodes (LN) determines the need for adjuvant chemotherapy in patients with colon cancer (3) and is associated with increased use of adjuvant radiation and chemotherapy in patients with rectal cancer (4). Although the 5-year-survival rate of patients with CRC and LN metastasis is only 69.2% (5), preoperative diagnosis of LN metastasis absence makes endoscopic mucosal resection or minimum bowel resection some possible options for patients with CRC. The development of tools for the accurate preoperative detection of LN metastasis might therefore serve an important role in optimizing the management of patients with CRC, such as minimizing surgery and evaluating the need for neoadjuvant chemotherapy and/or intensive adjuvant chemotherapy.

Non-invasive imaging modalities, including computed tomography (CT), magnetic resonance imaging (MRI), endorectal ultrasonography (EUS) and positron emission tomography/CT (PET/CT), are currently used for the preoperative diagnosis of LN metastasis in patients with CRC. However, these imaging modalities are unreliable, with relatively poor accuracy (22-73, 39-95, 62-83 and 63% for CT, MRI, EUS and PET/CT, respectively) (6,7).

The identification of predictive biomarkers of LN metastasis in CRC has mainly focused on the determination of the expression of several proteins by immunohistochemistry, of gene expression level (Carcinoembryonic antigen-related cell adhesion molecule 5 or Kallikrein-6 mRNAs and FER1 like member 4 non-coding RNAs) or DNA methylation in colorectal tissues (8-11). A previous study from our laboratory performed a comprehensive analysis using a proteomics approach and

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selected candidate proteins that might be used to identify LN metastasis in CRC. The expression level of HSP47 in CRC and the number of HSP47-positive spindle-shaped stroma cells in CRC stroma evaluated by immunohistochemistry were significantly associated with LN metastasis, and the number of HSP47-positive spindle-shaped stroma cells was identified as a novel predictive biomarker for LN metastasis and poor prognosis in patients with CRC (12). However, immunohistochemical analysis depends on tissue processing and staining conditions, properties of the immunohistochemical reagents, including antibodies, and visualization system. In addition, it is difficult to evaluate expression level objectively and/or quantitatively as required for clinical biomarkers. Furthermore, immunohistochemical analysis using formalin-fixed, paraffin-embedded samples from resected CRC tissues cannot be used to identify LN metastasis status preoperatively.

The present study aimed to evaluate whether HSP47 gene expression level could be used in CRC tissues to identify LN metastasis status preoperatively. To do so, HSP47 gene expression level was quantified by reverse transcription quantitative (RT-q)PCR in frozen CRC and control samples from surgical specimens and the association between HSP47 gene expression and clinicopathological characteristics of patients with CRC was analyzed.

Materials and methods

Patients and sample collection. Patients with primary CRC who underwent surgical resection at Mie University Medical Hospital, Japan between January 2000 and January 2005 were enrolled in the present study. Mean age of the patients was 69 years and mean tumor size was 40 mm. Patients with preoperative treatment, incomplete clinical data, inadequate follow-up or inadequate tissue samples were excluded. Patients with inflammatory bowel disease, familial adenomatous polyposis, hereditary non-polyposis colon cancer or other rare and complex types of tumors were also excluded from the present study. All tissue samples were collected from surgically resected specimens and were immediately frozen in liquid nitrogen and kept at -80°C until RNA extraction. A total of 175 surgical samples, comprising 36 normal mucosa samples from patients with benign colonic disease and 139 cancer tissue samples from patients with CRC were analyzed. The clinicopathological characteristics of patients with CRC were based on TNM classification using the American Joint Committee on Cancer (2). This cohort comprised 79 men and 60 women (average age, 67.7 years), of whom 33, 44, 33 and 29 patients had stages I, II, III and IV CRC, respectively. The median follow-up time was 32.9 months (range, 0.4-115 months). The 5-year survival rate was 80.1%. No patient received chemotherapy or radiotherapy before surgery and no perioperative mortality was observed. The research protocol was approved by Mie University Hospital Ethical Review Committee. All participants provided written informed consent and agreed to donate their clinical specimens for research purposes.

RNA extraction and RT-qPCR. Surgical specimens (200 μg) were homogenized using a Mixer Mill MM 300 homogenizer (Qiagen Sciences, Inc.) and total RNA was isolated using the RNeasy Mini Kit (Qiagen, Inc.) according to the manufacturers'

instructions. cDNA was synthesized using random hexamers and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' protocol.

HSP47 expression level was determined by RT-qPCR using Power SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and a Step One Plus Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The sequences of the primers were as follows: HSP47, forward 5'-ATGCAGAAGAAGGCTGTTGC-3', reverse 5'-GGCCTTGTTCTTGTCAATGG-3'; and β-actin, forward 5'-ACAGAGCCTCGCCTTTGC-3' and reverse 5'-GCGGCG ATATCATCATCC-3'. The amplification conditions were as follows: 95°C for 10 min, 40 cycles of 95°C for 15 sec, and 60°C for 1 min. After amplification, the products were subjected to an increasing temperature gradient from 60°C to 95°C at a rate of 0.3°C/sec with continuous fluorescence monitoring, to produce a melting curve. The HSP47 expression level in each sample was normalized to β-actin expression level using the $2^{-\Delta\Delta Ct}$ method (13).

Statistical analysis. All statistical analyses were performed using JMP version 10 (SAS Institute, Inc.). The results were expressed as the median values or as the mean ± standard deviation. Comparisons were made using non-parametric Mann-Whitney U-tests for continuous variables. Differences between groups were estimated using Pearson's χ^2 or Kruskal-Wallis tests. Dunn's tests were used as a method for multiple comparisons following Kruskal-Wallis test. Receiver operating characteristic (ROC) curves were established to determine cut-off expression values for predicting LN metastasis and prognosis using Youden's index. The Kaplan-Meier method was used to determine the cumulative probability of overall survival (OS) with Stage I-IV and disease-free survival (DFS) with Stage I-III patients with CRC, and differences were evaluated using log-rank tests. Prognostic factors were examined by univariate and multivariate analyses (Cox proportional hazards regression model). Logistic regression analysis was used to evaluate the factors influencing LN metastasis. P<0.05 was considered to indicate a statistically significant difference.

Results

Differential gene expression patterns of HSP47 in normal colonic mucosa and CRC tissues. HSP47 expression level was significantly higher in CRC tissues (n=139) compared with normal colonic mucosa (n=36; P=0.0201; Fig. 1A), and significantly higher in CRC with LN metastasis compared with CRC without LN metastasis (P=0.0470; Fig. 1A). In addition, HSP47 expression level was significantly higher in stage IV CRC tissues compared with stage I CRC tissues (P=0.0186; Fig. 1B).

Association between HSP47 expression level and clinicopathological characteristics in CRC. The associations between HSP47 expression level and various clinicopathological characteristics in patients with CRC were evaluated after dividing the 139 CRC cases into two groups, based on HSP47 expression level (high vs. low; Table I). The high/low cut-off values were determined by ROC analysis for LN metastasis (cut-off 0.0969762). Of the 139 CRC samples, 49.6% (69/139) cases had high HSP47 expression (>0.0969762) and 50.4%
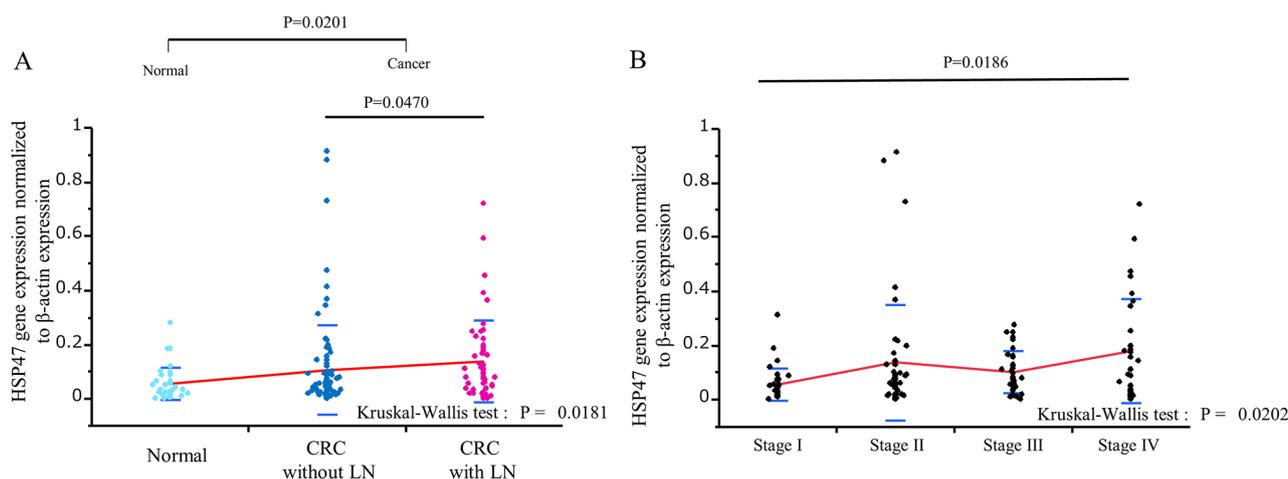
Figure 1. Reverse transcription quantitative PCR analysis of HSP47 gene expression in normal colonic and CRC samples. Data were normalized by β-actin expression level. Bars represent standard deviations. Statistical analysis was performed using Kruskal-Wallis test and Dunn's test. (A) HSP47 expression level was significantly higher in CRC samples compared with normal samples, and in CRC with LN metastasis compared with CRC without LN metastasis. (B) HSP47 gene expression level in 139 CRC tissues subdivided according to Tumor-Node-Metastasis staging. HSP47, heat shock protein 47; LN, lymph node metastasis; CRC, colorectal cancer.

(70/139) had low expression. Furthermore, high HSP47 expression level was significantly associated with advanced T stage (P=0.0163), LN metastasis (P=0.0186), venous invasion (P=0.0328), and advanced TNM stage (P=0.0115; Table I).

Differential gene expression patterns of HSP47 in normal colonic mucosa and CRC tissues. A comparison of HSP47 expression between colon cancer and rectum cancer samples revealed that there was no significant difference in expression between the two cancer types (Fig. S1).

High HSP47 expression level predicts LN metastasis in patients with CRC. The present study investigated various factors associated with LN involvement by univariate and multivariate logistic analyses (Table II). Advanced T stage (P=0.0007), lymphatic invasion (P=0.0043), venous invasion (P=0.0002) and high HSP47 expression (P=0.0042) in CRC were significantly associated with LN metastasis following univariate analysis. Furthermore, multivariate analysis identified high HSP47 expression [odds ratio (OR): 2.3946, 95% confidence interval (CI): 1.116397-5.209946; P=0.0249] as the only independent predictor of LN metastasis in patients with CRC (Table II).

High HSP47 gene expression affects prognosis in patients with CRC. Patients with CRC were divided into two groups according to HSP47 expression level determined by ROC analysis for prognosis (cut-off 0.0924641). Kaplan-Meier analysis indicated that disease-free survival tended to be poorer in the high-expression group, although the difference was not significant (Fig. 2A); however, OS was also significantly poorer in the high-expression group compared with the low-expression group (P=0.0003, log-rank test; Fig. 2B). Cox's univariate and multivariate analyses of clinicopathological factors related to OS in patients with CRC are presented in Table III. The results from univariate analysis indicated that poor OS was significantly associated with advanced T stage (P<0.0001), LN metastasis (P=0.0040), hepatic metastasis

(P<0.0001), distant metastasis (P<0.0001), lymphatic invasion (P=0.0056), venous invasion (P=0.0051) and high HSP47 expression in CRC (P=0.0021). In addition, multivariate analysis identified hepatic metastasis [hazard ratio (HR): 9.1250, 95% CI: 3.7270458-22.288004; P<0.0001] and high HSP47 expression (HR: 2.7407, 95% CI: 1.1623509-6.1627421; P=0.0224) as independent prognostic factors in patients with CRC (Table III).

Association between HSP47 expression and LN counts in patients with CRC. The association between LN metastasis status and HSP47 expression level in patients with CRC was evaluated. Patients were classified according to the stage of LN metastasis based on the Japanese Society for Cancer of the Colon and Rectum 7th edition (N0, no metastasis; N1, 1-3 pericolic/perirectal; intermediate LN metastases; N2, ≥4 pericolic/perirectal, intermediate LN metastases; N3, main LN metastasis including lateral LN metastasis) (14). Kaplan-Meier analysis demonstrated that patients with N3 had significantly poorer OS than the other groups (P<0.0001; Fig. 3A). HSP47 expression level in N3 patients was significantly higher compared with N0 patients (P=0.0253; Fig. 3B). ROC curves were also generated to assess the potential significance of HSP47 expression level for the detection of LN metastasis status in patients with CRC. The results demonstrated that HSP47 expression level could discriminate between N1-3 and N0 CRC patients, with an AUC value of 0.60138 (Fig. 3C). In addition, HSP47 level could also discriminate between N2-3 and N0-1 CRC patients, with an AUC of 0.84392 (Fig. 3D).

Discussion

The present study investigated the gene expression pattern of HSP47 in CRC tissues by RT-qPCR. HSP47 expression level was significantly higher in CRC tissues compared with normal tissue from patients with benign colonic disease and was significantly associated with tumor progression indicated by high T stage, LN metastasis and venous invasion and high

Table I. Association between HSP47 expression level and the clinicopathological characteristics in patients with colorectal cancer.

| Variable | n | HSP47 expression | | P-value |
		High (n=69)	Low (n=70)	
Age, years				0.1072
<69	70	30	40	
≥69	69	39	30	
Sex				0.4479
Male	79	37	42	
Female	60	32	28	
Histology				0.2908
Undifferentiated	9	6	3	
Differentiated	130	63	67	
Tumor size, mm				0.1247
<40	49	20	29	
≥40	90	49	41	
T classification				0.0163[a]
T1-T2	39	13	26	
T3-T4	100	56	44	
Lymph node metastasis				0.0186[a]
Present	88	37	51	
Absent	51	32	19	
Lymphatic invasion				0.1811
Present	124	64	60	
Absent	15	5	10	
Venous invasion				0.0328[a]
Present	113	61	52	
Absent	26	8	18	
Hepatic metastasis				0.1376
Present	20	13	7	
Absent	119	56	63	
Peritoneal metastasis				0.9918
Present	2	1	1	
Absent	137	68	69	
Distant metastasis				0.1041
Present	16	11	5	
Absent	123	58	65	
Recurrence				0.1041
Present	24	15	9	
Absent	84	38	46	
Stage				
I	33	9	24	0.0115[a]
II	44	21	23	
III	33	21	12	
IV	29	18	11	

(Pearson's χ^2); [a]$P<0.05$. The cut-off value for high/low HSP47 expression was 0.0969762. HSP47, heat shock protein 47.

TNM stage. High HSP47 expression level also served as a novel predictive biomarker for patients with CRC and LN metastasis, and as an independent prognostic factor in patients with CRC.

Table II. Univariate and multivariate analyses of associations with lymph node metastasis in patients with colorectal cancer.

	Univariate analysis			Multivariate analysis		
Variable	OR	95% CI	P-value	OR	95% CI	P-value
Age (<69 vs. ≥69 years)	1.2321	0.617647-2.4697243	0.5534	-	-	-
Sex (female vs. male)	1.2840	0.6395638-2.5783842	0.4810	-	-	-
Tumor size (≥40 vs. <40 mm)	2.0237	0.9624956-4.4365109	0.0632	-	-	-
T classification (T3-4 vs. T1-2)	5.0000	1.8374783-12.775448	0.0007[a]	2.1400	0.7456599-6.9057309	0.1606
Pathology (poor or mucinous vs. moderate/well differentiated)	1.4128	0.335628-5.5889128	0.6218	-	-	-
Lymphatic invasion (present vs. absent)	9.4595	1.8123151-174.04766	0.0043[a]	1.0124	0.0343703-29.441451	0.9935
Venous invasion (present vs. absent)	9.1875	2.5583968-58.877309	0.0002[a]	5.2985	0.8617945-102.43786	0.0752
HSP47 gene expression (≥0.0969762 vs. <0.0969762)	2.8846	1.3963896-6.0536216	0.0042[a]	2.3946	1.116397-5.209946	0.0249[a]

Logistical regression model; [a]P<0.05. The cut-off value for high/low HSP47 gene expression was 0.0969762. OR, odds ratio; CI, confidence interval; HSP47, heat shock protein 47.
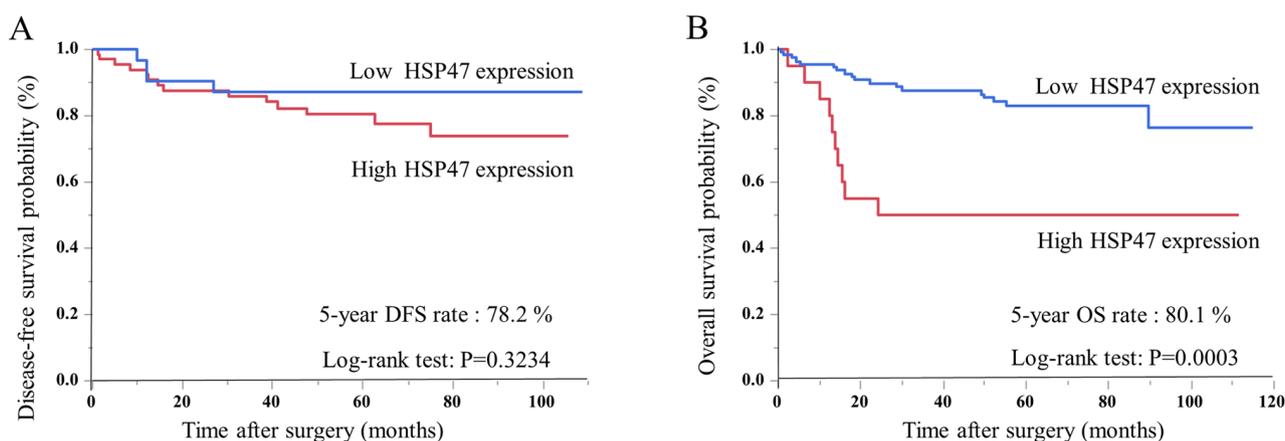
Figure 2. Survival curves of patients with CRC after curative surgery according to HSP47 gene expression status. Kaplan-Meier method was used to determine the cumulative probability of overall survival with Stage I-IV and disease-free survival with Stage I-III patients with CRC, and differences were evaluated using log-rank tests. (A) Disease-free survival. Patients with high HSP47 gene expression levels tended to have poorer disease-free survival than those with low expression levels, although the difference was not significant (P=0.3234, log-rank test). (B) Overall survival. Patients with high HSP47 gene expression levels had significantly poorer overall survival than those with low expression levels. (P=0.0003, log-rank test). CRC, colorectal cancer; HSP47, heat shock protein 47.

HSP47 is a 47-kDa member of a group of heat shock proteins with unique collagen-specific binding characteristics (15), which was first characterized by Kurkinen et al (16) in murine parietal endoderm cells. Heat shock proteins play an important role as molecular chaperones in protein folding, by assembling newly synthesized proteins or reassembling misfolded proteins (17). HSP47 binds to nascent single polypeptide chains of procollagen immediately after entry into the endoplasmic reticulum and dissociates from procollagen chains in the Golgi apparatus (18). When aberrant procollagen accumulates in the endoplasmic reticulum due to heat shock, depletion of ascorbic acid or after treatment with α, α'-dipyridyl, which inhibits procollagen secretion and causes accumulation of procollagen in the endoplasmic reticulum (19). Some studies have suggested that procollagen gene expression level and rates of procollagen synthesis were regulated with HSP47 (20), and that HSP47 expression was increased in fibrotic human lung, kidney and skin (21-24).

HSP47 has been mapped to human chromosome 11q13.5, which is a known 'hot spot' in a number of human cancers (25). Interestingly, altered HSP47 expression has been identified in several cancers, including sarcoma, esophageal squamous cell carcinoma and ulcerative colitis-associated carcinoma (26-28). Maitra et al (29) reported HSP47 immunolabeling in the tumor-associated stroma and suggested that it may act as a novel diagnostic and therapeutic marker in pancreatic carcinoma. Furthermore, we previously reported that the number of HSP47-positive spindle-shaped stroma cells in the cancer stroma of patients with CRC could serve as a novel predictive biomarker of LN metastasis, early recurrence and poor prognosis (8). In addition, HSP47 silencing has been shown

Table III. Univariate and multivariate analyses of associations with overall survival in patients with colorectal cancer.

(Cohort 1: Protein analysis)	Univariate analysis			Multivariate analysis		
Variables	HR	95% CI	P-value	HR	95% CI	P-value
Age (<69 vs. ≥69 years)	1.7043	0.8012998-3.8397503	0.1682	-	-	-
Sex (male vs. female)	1.3229	0.6220246-2.9802979	0.4731	-	-	-
Tumor size (≥40 vs. <40 mm)	1.8287	0.8144726-4.6473787	0.1486	-	-	-
T classification (T3-4 vs. T1-2)	13.3454	2.8403789-238.09155	<0.0001	3.8129	0.733633-70.061812	0.1260
Lymph node metastasis	3.0116	1.4279703-6.5249787	0.0040[a]	1.3758	0.5677664-3.3313589	0.4761
Peritoneal metastasis	6.5208	0.3625655-31.578247	0.1555	-	-	-
Hepatic metastasis	16.5158	7.466573-36.758521	<0.0001	9.1250	3.7270458-22.288004	<0.0001
Distant metastasis	7.2862	3.2929176-15.473565	<0.0001	2.0538	0.7842089-5.1884429	0.1403
Pathology (poor or mucinous vs. moderate/well differentiated)	1.6081	0.3825837-4.5888813	0.4652	-	-	-
Lymphatic invasion) (present vs. absent	1.6488	0.4826371-2.07195	0.0056[a]	2.1415	0.0606119-16.498407	0.4061
Venous invasion (present vs. absent)	7.6486	1.628946-136.4308	0.0051[a]	1.3460	0.2462182-25.080995	0.7719
HSP47 gene expression (≥0.0924641 vs. <0.0924641)	3.7958	1.6753552-8.1191931	0.0021[a]	2.7407	1.1623509-6.1627421	0.0224[a]

Cox proportion hazard regression model; [a]P<0.05. CI, confidence interval. The cut-off value for high/low HSP47 gene expression was 0.0924641; HSP47, heat shock protein 47.
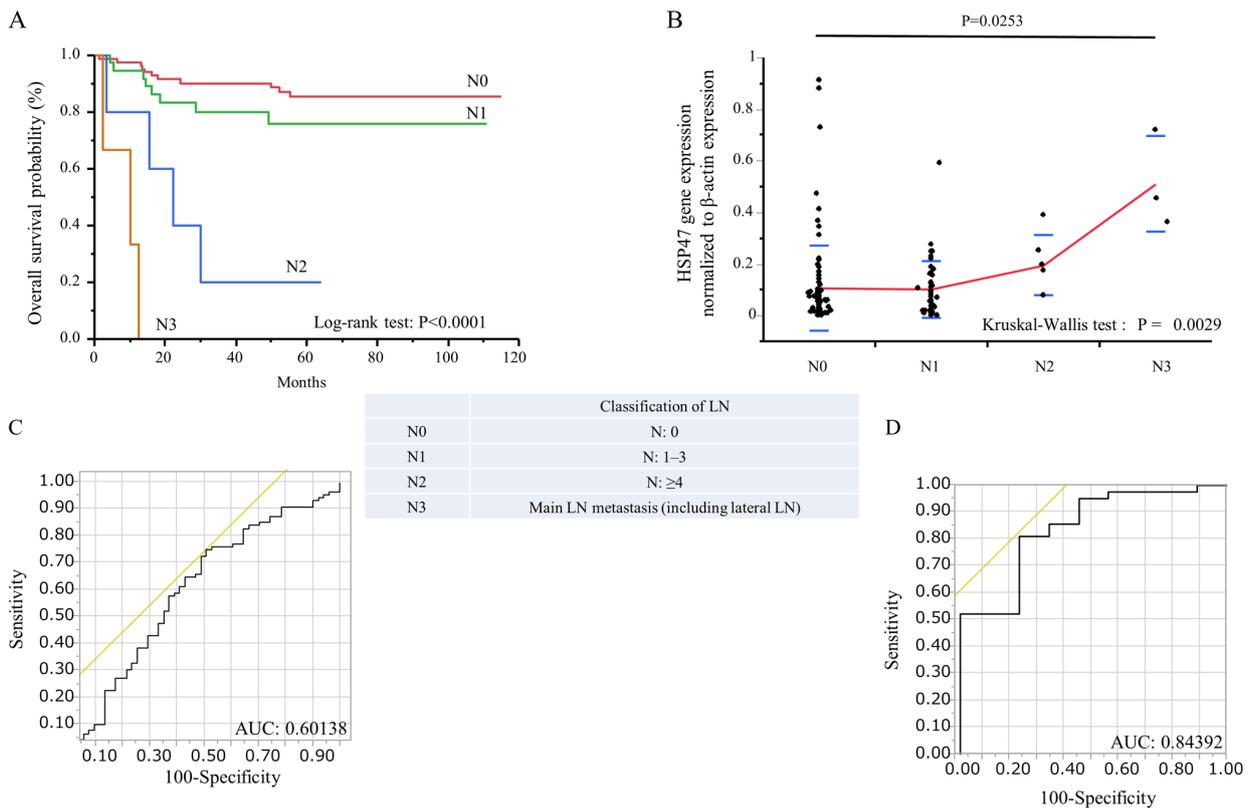
Figure 3. Association between HSP47 expression level and N stage. (A) Survival curves for patients with CRC after curative surgery according to N stage. Patients with high N stage had poorer OS than those with low N stage (P<0.0001, log-rank test). (B) HSP47 gene expression levels in 139 patients with CRC subdivided by N stage. HSP47 gene expression level was significantly higher in N3 compared with N0 patients with CRC. (C) ROC curve analysis using HSP47 expression level to distinguish between CRC with and without LN metastasis (N0 vs. N1-3). HSP47 expression level yielded an AUC value of 0.60138 for detecting CRC with LN metastasis. (D) ROC curve analysis using HSP47 expression level to detect CRC with severe LN metastasis (N0-1 vs. N2-3). HSP47 expression level yielded an AUC value of 0.84392 for discriminating between N2-3 CRC and N0-1 CRC. AUC, area under the curve; CRC, colorectal cancer; HSP47, heat shock protein 47; ROC, receiver operating characteristic.

to inhibit cell proliferation, migration and invasion in cervical squamous cell carcinoma (30).

Regarding HSP47 gene expression, Morino *et al* (31) demonstrated that tumor cell lines derived from metastatic carcinomas that remained metastatic in animals can synthesize higher levels of HSP47. Naitoh *et al* (24) also reported that HSP47 expression level was upregulated by 8-fold in keloid lesions. However, the association between HSP47 expression level and tumor progression, prognosis and metastasis in cancer patients remains unclear. To the best of our knowledge, the present study was the first to suggest that high HSP47 expression level in CRC primary tumors may be considered as an independent marker for the prediction of LN metastasis and poor prognosis in patients with CRC.

Neoadjuvant chemotherapy or chemoradiotherapy are currently used as treatments for patients with advanced rectal cancer and have been shown to decrease local recurrence and increase survival (32). Correct preoperative staging plays therefore a critical role in determining whether patients with rectal cancer should undergo neoadjuvant therapy. Similarly, preoperative neoadjuvant treatments and decisions regarding the range of LN dissection depend on accurate LN staging in patients with colon cancer (33). In the present study, HSP47 expression value in the group with LN was significantly higher than in the group without LN (0.137848±0.149551 vs. 0.106957±0.166488). In addition, patients with N2 or N3 tumors had significantly poorer OS than other patients, and HSP47 gene expression level was also significantly higher in N2 and N3 patients compared with N0 and N1 patients. This was supported by markedly higher AUC values for comparisons between N2-3 and N0-1 CRC patients (AUC=0.84392). Furthermore, HSP47 expression was identified as the only independent predictive factor for LN metastasis in patients with CRC. Evaluation of HSP47 expression level may therefore help deciding the appropriate neoadjuvant chemotherapy to be used, and determining the extent of LN dissection (local or wide excision). HSP47 expression level may therefore help decreasing the size of surgery and evaluate the requirement of neoadjuvant chemotherapy in patients with CRC before surgery.

Previously, we demonstrated that the number of HSP47-positive spindle cells in the stroma of CRC may serve as a novel predictive biomarker of LN metastasis, early recurrence and poor prognosis via immunostaining (12). In the present study, we used mRNA to examine LN metastasis and prognostic factors of CRC patients. Although it is easy to evaluate the prognostic factor of CRC using protein staining, the objectivity can be poor and the evaluation can be dependent of the observer. Evaluating mRNA expression may be a more useful method because it allows quantification, reducing human bias and leading therefore to a more accurate evaluation of LN metastasis and prognosis in patients with CRC. Although HSP47 expression level was quantified in frozen surgical specimens rather than in preoperative biopsy samples, the results from the present study suggested that evaluation of HSP47 expression level in biopsy samples may represent a promising clinical tool for the preoperative prediction of LN metastasis, allowing therefore the determination of appropriate treatment strategies in patients with CRC. It may be worth mentioning that the current study did not actually use preoperative biopsies, although it aimed to determine if it

could be possible to determine the LN metastasis in patients with CRC.

In summary, the results from the present study indicated that HSP47 expression level in CRC tissues may be considered as a potential biomarker for predicting LN metastasis status and prognosis in patients with CRC. These results suggested that assessing LN metastasis may lead to a reduction in surgery in CRC and may help deciding about preoperative chemotherapy in rectal cancer. By examining the OS via evaluation of HSP47 expression, it may be possible to predict before surgery that postoperative chemotherapy may be required for patients with both colon and rectal cancers.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

KM, YT, MaK and YO designed the study. TI, YN, SO, TS, HF, JH, MiK, TA, YI and YM performed the experiments. KM, YT and YO acquired, analyzed and interpreted data. KM and YT drafted the manuscript. MaK supervised and coordinated the study. All the authors were involved in writing the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The research protocol was approved by Mie University Hospital (Mie, Japan) Ethical Review Committee (approval no. 2632). All participants provided written informed consent and agreed to donate their clinical specimens for research purposes.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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