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RAPID COMMUNICATION

Effect of lymph node micrometastases on prognosis of gastric carcinoma

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Abstract

AIM: To evaluate the relationship between lymph node micrometastases and prognosis of patients with gastric carcinoma and to evaluate the significance of the new assessment of nodal status in determining the pN categories in the 5th edition of the UICC TNM classification.

METHODS: A total of 850 lymph nodes from 30 patients with gastric carcinoma who underwent gastrectomy with lymphadenectomy were assessed by reverse transcription polymerase chain reaction assay in addition to histologic examination. Cytokeratin-20 gene marker was used in this assay.

RESULTS: Routine examination by HE staining confirmed metastasis in 233 lymph nodes from 20 patients. All these 233 lymph nodes were cytokeratin-20 positive. Moreover, lymph node micrometastases were detected in an additional 67 lymph nodes in 12 of these 20 patients. Lymph node micrometastases were also detected in 10 lymph nodes from 2 of 10 patients who had no obvious metastases identified by HE staining. Totally, lymph node micrometastases were identified by the reverse transcription polymerase chain reaction assay in 77 (12.5%) lymph nodes from 14 (46.7%) patients with gastric carcinoma. Of 27 patients who underwent curative resection, 7 (25.9%) were up-staged (from ⅠB stage to Ⅱ stage in 1 patient, from IB stage to ⅢA stage in 1 patient, from Ⅱ stage to ⅢA stage in 1 patient, from ⅢA stage to ⅢB stage in 1 patient, from ⅢA stage to Ⅳ stage in 1 patient, from ⅢB stage to Ⅳ stage in 2 patients). In a median follow-up of 32 (range 8-36) mo, Kaplan-Meier survival analysis showed significant improvements in median survival (22.86 ± 3.17 mo, 95% CI: 16.64-29.08 mo *vs* 18.00 ± 7.4 mo, 95% CI: 3.33-32.67 mo) of patients with negative lymph node micrometastases over patients with positive lymph node micrometastases (log-rank, $P < 0.05$).

CONCLUSION: Lymph node micrometastases have a significant impact on the current staging system of gastric carcinoma, and are significant risk factors for prognosis of patients with gastric carcinoma.

Key words: Gastric carcinoma; Lymph node micrometastases; Prognosis; Stage

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INTRODUCTION

Gastric carcinoma is one of the most common malignancies in China and lymph node metastasis is the most important prognostic factor for gastric carcinoma[1-3]. It was reported that some patients with gastric carcinoma despite undergoing curative resection of the tumor and regional lymph nodes still die of a recurrence[4-6]. To some degree, the fact may suggest the presence of lymph node micrometastases overlooked by the conventional histopathological examination. Recent advances in immunohistochemical and molecular biologic techniques have made it possible to detect lymph node micrometastases not evidenced by routine HE evaluation[7-12]. However, the effect of detection of lymph node micrometastases on determining the current staging system and prognosis of gastric carcinoma has not yet been extensively evaluated. Therefore, the aim of this study was to evaluate the significance of the new assessment of nodal status in determining the current staging system of gastric carcinoma. The relationship between lymph node micrometastases and prognosis of patients with gastric carcinoma was also evaluated. In this report, "micrometastasis" in regional lymph nodes was defined as metastasis that was detected only by RT-PCR assay rather than by ordinary HE staining.

MATERIALS AND METHODS

Patients and specimens

The presence of lymph node micrometastases was detected in the 850 dissected lymph nodes from 30 patients with gastric carcinoma who underwent gastrectomy at the Department of Gastrointestinal Pancreatic Surgery, Sun Yat-Sen University of Medical Sciences. There were 17 men and 13 women, ranging in age from 26 to 82 years, with a mean age of 56.8 years. None of these patients received preoperative chemotherapy or radiotherapy. Total gastrectomy was performed in 16 patients, distal subtotal gastrectomy in 13 patients, and proximal subtotal gastrectomy in 1 patient. One patient underwent D1 lymphadenectomy, 22 patients D2 lymphadenectomy, 4 patients D3 lymphadenectomy, and 3 patients palliative resection. According to the Lauren' criteria, 19 tumors were classified as diffusive type carcinomas and 11 tumors as intestinal type carcinomas. Depth of tumor invasion and extent of lymph node metastasis were classified according to UICC TNM classification.

Half of each resected lymph node was fixed in 10% formalin and embedded in paraffin for routine histopathological examination. The other half was stored in 1 mL RNA later (Sigma, USA) at 4℃ overnight, then transferred to a clean freezing tube and stored at 70℃ for RNA extraction.

RNA extraction

Lymph node samples were homogenized in 1 mL of Trizol reagent (Invitrogen) per 50-100 mg of tissue using a power homogenizer. RNA extraction was carried out according to the protocol recommended by the manufacturer. Total RNA was dissolved in diethylpyrocarbonate-treated water and the volume and quality of RNA were then assessed with an ultraviolet spectrophotometer.

Access RT-PCR

Complementary DNA (cDNA) was synthesized and amplified from total RNA using the Access RT-PCR system (Promega). The primer sequences used for CK-20 detection are 5'-ggtcgcgactacagtgcatattaca-3'(sense) and 5'-cctcagcagccagtttagcattatc-3' (anti-sense)[13,14]. cDNA synthesis was monitored by beta-actin RT-PCR using the following primers: 5'-caaatgcttctaggcggact -3'(sense) and 5'-atgctatcacctccccctgtg -3'(anti-sense). RT-PCR was performed in a 25 μL reaction mixture containing 11 μL nuclease-free water, 5 μL 5 × reaction buffer, 0.5 μL dNTP (10 mmol/L), 0.5 μL each of beta-actin primers (20 μmol/), 1.25 μL each of CK-20 primers (10 μmol/), 1 μL $MgSO_4$ (25 mmol/), 0.5 μL AMV reverse transcriptase (5 u/μL), 0.5 μL Tfl DNA polymerase (5 u/μL), and 3 μL RNA sample. The Access RT-PCR condition was set up as follows: 1 cycle at 48℃ for 45 min (reverse transcription), 1 cycle at 94℃ for 2 min (AMV RT inactivation), followed by 40 cycles at 94℃ for 30 s (denaturation, at 62℃ for 1 min (annealing) and at 68℃ for 1.5 min (extension), then a final extension at 68℃ for 7 min. The resultant cDNA products of CK-20 and beta-actin were 121 base pairs and 381 base pairs, respectively. The RT-PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide.

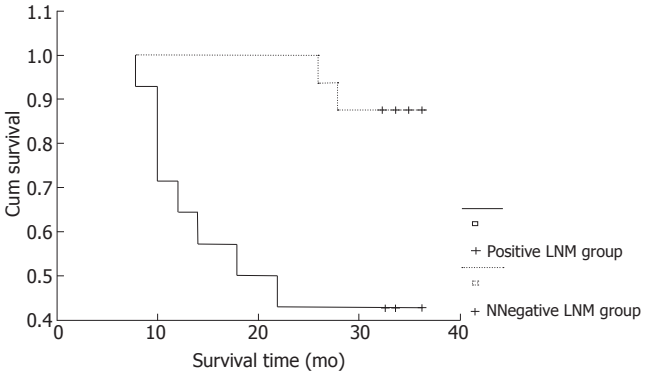

Figure 1 Correlation between lymph node micrometastases and survival of patients with gastric carcinoma (Kaplan-Meier survival analysis).

Statistical analysis

The relationship between lymph node micrometastases and survival of patients with gastric carcinoma was evaluated by Kaplan-Meier survival analysis and log-rank test. $P < 0.05$ was considered statistically significant.

RESULTS

Routine examination by HE staining confirmed metastasis in 233 lymph nodes from 20 patients. All these 233 lymph nodes were cytokeratin-20 positive. Moreover, lymph node micrometastases were detected in an additional 67 lymph nodes in 12 of these 20 patients. Lymph node micrometastases were also detected in 10 lymph nodes from 2 of 10 patients who had no obvious metastases identified by HE staining. Totally, lymph node micrometastases were identified by reverse transcription polymerase chain reaction assay in 77 (12.5%) lymph nodes from 14 (46.7%) patients with gastric carcinoma. Of 27 patients who underwent curative resection, 7 (25.9%) were up-staged (from ⅠB stage to Ⅱ stage in 1 patient, from ⅠB stage to ⅢA stage in 1 patient, from Ⅱ stage to ⅢA stage in 1 patient, from ⅢA stage to ⅢB stage in 1 patient, from ⅢA stage to Ⅳ stage in 1 patient, from ⅢB stage to Ⅳ stage in 2 patients). In a median follow-up of 32 (range 8-36) mo, Kaplan-Meier survival analysis showed significant improvements in median survival (22.86 ± 3.17 mo, 95% CI: 16.64-29.08 mo *vs* 18.00 ± 7.48 mo, 95% CI: 3.33-32.67 mo) of patients with negative lymph node micrometastases over patients with positive lymph node micrometastases. The difference between these two groups was statistically significant (log-rank, $P < 0.05$) (Figure 1).

DISCUSSION

Despite improved surgical treatment, the prognosis of gastric cancer remains poor currently[15-19]. pN category is one of the most important prognostic factors for gastric carcinoma. The pN classification of gastric carcinoma, based on the number of metastatic lymph nodes, has been adopted by the current American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) TNM staging system. According to the AJCC/UICC pN categories, Hundahl *et al*[20] reported that stage-stratified

5 and 10-year survival rates of patients with gastric carcinoma are as follows: stage I A, 78%/65%; stage I B, 58%/42%; stage Ⅱ, 34%/26%; stage ⅢA, 20%/14%; stage ⅢB, 8%/3%; and stage Ⅳ, 7%/5%. Kodera *et al*[21] also reported that the number of metastatic nodes after D2 lymphadenectomy reflects prognosis well and is a strong independent prognostic factor for gastric carcinoma as shown by multivariate analysis. Histopathological examination of resected lymph nodes using HE staining has been the gold standard for diagnosis of lymph node metastasis. However, micrometastases consisting of one to a few cells in lymph nodes resected during gastrectomy are often overlooked by the conventional histopathological method. Therefore, the purpose of this study was to evaluate the significance of detection of lymph node micrometastases in determining the current staging system of gastric carcinoma. The relationship between lymph node micrometastases and prognosis of patients with gastric carcinoma was also evaluated.

Recent advances in immunohistochemical and molecular biologic techniques have made it possible to detect lymph node micrometastases not evidenced by routine HE staining. It was reported that micrometastases are identified in regional lymph nodes from 28%-68.1% of patients[22-26]. In the current study, we applied the reverse transcription polymerase chain reaction assay to detect micrometastases in the lymph nodes resected from 30 cases of stage I -Ⅳ gastric carcinomas. Totally, lymph node micrometastases were identified in 77 (12.5%) lymph nodes from 14 (46.7%) patients with gastric carcinoma. The tumor stage was upgraded in 25.9% (7/27) of patients who underwent curative resection. Similar to our results, Okada *et al*[13] assessed 435 lymph nodes from 28 patients with gastric carcinoma who underwent gastrectomy with lymphadenectomy using the multiple-marker RT-PCR assay in addition to histologic examination. Of 28 patients who underwent curative resection, the disease stage was upgraded in 10 patients by genetic diagnosis (from Stage I A to Stage I B in 5 patients, from Stage I B to Stage ⅢA in 2 patients, from Stage I B to Stage Ⅳ in 1 patient, from Stage I B to Stage Ⅱ in 1 patient, and from Stage Ⅱ to Stage ⅢB in 1 patient). Lee *et al*[8] applied AE1/3 immunohistochemical staining to detect micrometastases in 3625 regional lymph nodes that were dissected in gastrectomy specimens from 153 patients with early-stage gastric carcinoma (46 patients) and advanced gastric carcinoma (107 patients). Micrometastases were identified in 191 lymph nodes from 75 patients. Twenty-eight of those patients were up-staged. These results indicate that much careful assessment of the lymph node status must be followed in the histopathological examination of resected specimens. Lymph node micrometastases may improve the current staging system of gastric carcinoma and should be validated in future trials as an alternative clinical index.

The prognostic value of lymph node micrometastases for patients with gastric carcinoma is still controversial. Ishida *et al*[23] reported that gastric carcinomas with micrometastases have significantly worse prognoses at stage Ⅱ. Lee *et al*[8] also reported that patients with lymph node micrometastases have a decreased 5-year survival rate (49%) compared with patients without lymph node micrometastases (76%) for both early and advanced gastric carcinoma. However, Fukagawa *et al*[27] reported that the presence of immunohistochemically-detected micrometastases in regional lymph nodes does not affect the survival of Japanese patients with pT2N0M0 gastric carcinoma who have undergone gastrectomy with D2 lymph node dissection. The 5-year and 10-year survival rates of patients with and without micrometastases were 94%/79% and 89%/74%, respectively. The differences were not statistically significant ($P > 0.05$). Our results showed significant improvements in median survival (22.86 ± 3.17 mo, 95% CI: 16.64-29.08 mo *vs* 18.00 ± 7.48 mo, 95% CI: 3.33-32.67 mo) of patients with negative lymph node micrometastases over patients with positive lymph node micrometastases. The difference between these two groups was statistically significant (log-rank, $P < 0.05$). These conflicting observations may be explained by the small sample size and selection bias. To draw a further conclusion, larger sample and multi-center investigations on gastric carcinoma are needed.

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