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***Basic Study***

**Combined expression of CRM1 and CDK5 displayed higher prognostic accuracy for gastric cancer**

Sun YQ *et al*. Prognostic value of CRM1 and CDK5

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**Abstract**

***AIM***

To evaluate the predictive value of the expression of CRM1 and CDK5 in gastric cancer (GC) patients after gastrectomy.

***METHODS***

A total of 240 gastric cancer patients who received standard gastrectomy were enrolled in the study. The expression level of CRM1 and CDK5 was detected by immunohistochemistry. The correlations between CRM1 and CDK5 expression and clinicopathological factors were explored, univariate and multivariate survival analysis were used to identify prognostic factors for GC. Receiver operating characteristic analysis were used to compare the accuracy of the prediction of clinical outcome by the parameters.

***RESULTS***

The expression of CRM1 was significantly related to size of primary tumor (*P* = 0.005), Borrmann type (*P* = 0.006), degree of differentiation (*P* = 0.004), depth of invasion (*P* = 0.008), lymph node metastasis (*P* = 0.013), TNM stage(*P* = 0.002)and distant metastasis (*P* = 0.015). The expression of CDK5 was significantly related to sex (*P* = 0.048) and Lauren’s classification (*P* = 0.011). Multivariate Cox regression analysis identifies CRM1 and CDK5 co-expression status was an independent prognostic factor for overall survival (OS) of patients with GC. Integration of CRM1 and CDK5 expression could provide additional prognostic value for OS than CRM1 or CDK5 expression alone (*P* = 0.001).

***CONCLUSION***

The CRM1 and CDK5 co-expression status was an independent prognostic factors for patients with GC. Our results suggested that combined CRM1 and CDK5 expression could provide a better prognostic model for OS of GC patients.

**Keg words:** Gastric cancer; CRM1; CDK5; Prognosis

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**Core tip:** Our study shows that low expression of CRM1 and CDK5 was associated with poor prognosis of GC patients. The expression of CRM1 or CDK5 influenced the prognostic value of each other. Combined CRM1 and CDK5 expression provided a better prognostic power than their individual expression.

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**INTRODUCTION**

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide, although its incidence and mortality has decreased dramatically over the last 50 years[[1](#_ENREF_1)]. In 2011 there were about 420000 new cases diagnosed (70% were men and 30% were women) and 300000 death due to this disease in China[[2-4](#_ENREF_2)]. Clinically prognostic classification model for outcomes of GC patients is mainly UICC/AJCC TNM staging system based on the histopathological score[[5](#_ENREF_5)], whereas the underlying molecular and cellular processes during carcinogenesis of GC are ignored. Patients with the same TNM stage may have wide variations in survival owing to different genetic mutation status[[6](#_ENREF_6)]. Therefore, a better understanding of the molecular pathology might provide better prognostic biomarkers and guidance for a more precise treatment for the GC patients.

The human nuclear export protein chromosomal region maintenance/exportin1/Xpo1 (CRM1) has been reported to control multiple processes during cellular mitosis and is important in mediating nuclear export of cargo proteins that contain specific leucine-rich nuclear export signal (NES) consensus sequences[[7](#_ENREF_7),[8](#_ENREF_8)] . Previous studies have demonstrated that CRM1 is important for the functions of proteins such as EGFR, p53, p27, CDK5 and Akt1[[9-13](#_ENREF_9)]. The prognostic value of CRM1 expression have been reported in many types of cancer including ovarian cancer[[14](#_ENREF_14)], osteosarcoma[[15](#_ENREF_15)], glioma[[16](#_ENREF_16)], pancreatic cancer[[17](#_ENREF_17)] and oesophageal squamous cell carcinoma[[18](#_ENREF_18)]. However, whether CRM1 expression contributes to the development or progression of GC is not known.

CDK5 is a proline-directed serine/threonine kinase and participates in a variety of pathological and physiological functions[[19](#_ENREF_19),[20](#_ENREF_20)]. Increasing evidence suggested a role of CDK5 in cancer tumorigenesis and progression[[21](#_ENREF_21),[22](#_ENREF_22)]. Our previous work have demonstrated that in GC CDK5 down-regulation was an independent prognostic factor and the nuclear localization of CDK5 was critical for its tumor suppressor function[[23](#_ENREF_23)]. Given that CRM1 regulates CDK5 cytoplasm localization in neurons[[12](#_ENREF_12)], we hypothesized that the functional correlation between CRM1 and CDK5 may affect each other's prognostic power. In the present study, we examined the expression of CRM1 and CDK5 in 240 gastric tumor tissues and analyzed their correlation with patient clinicopathologcial features.

**MATERIALS AND METHODS**

***Patients and specimens***

The study cohort was composed of samples from 240 patients (178 men and 62 women, mean age: 59.5 years) with gastric adenocarcinoma, who had undergone gastrectomy at the Department of Gastric Surgery, Fujian Medical University Union Hospital, between January 2009 and December 2009. Following surgery, routine chemotherapy was given to patients with advanced disease and no radiation treatment was administered to any of the patients. Eligibility criteria for patients included in this study are: (1) histologically proven adenocarcinoma; (2) not with other gastric tumors such as gastric stromal tumor; (3) no history of gastrectomy or other malignancy; (4) not received the neoadjuvant chemotherapy; and (5) availability of complete clinicopathological and survival data (Figure 1). The study was performed with the approval of the ethics committee of Fujian medical union Hospital. Written consent was given by the patients for their information and specimens to be stored in the hospital database and used for research.

***Clinicopathological and survival data***

The clinical and pathological data were recorded prospectively for the retrospective analysis. The clinicopathological data for the 240 GC patients included age, sex, size of primary tumor, location of primary tumor, degree of differentiation, histological type, Lauren’s classification, Borrmann type, depth of invasion, lymph node metastasis, TNM stage, vessel invasion and distant metastasis. The pathologic stage of the tumor was re-assessed according to the 2010 International Union Against Cancer (UICC) on gastric cancer TNM classification (seventh edition)[[5](#_ENREF_5)]. Overall survival (OS) was defined as the time from curative surgery to death or the last clinical follow-up. After surgery, all patients were followed by outpatient visits, telephone calls and letters every 3 months in the first 2 years, every 6 mo in the next 3 years and every year afterwards or until death. The deadline for follow-up was October 2015. All patients had follow-up records for more than 5 years.

***Immunohistochemistry***

Paraffin blocks that contained sufficient formalin-fixed tumor specimens were serial sectioned at 4 μm and mounted on silane-coated slides for Immunohistochemistry (IHC) analysis. The sections were deparaffinized with dimethylbenzene and rehydrated through 100, 100, 95, 85, and 75% ethanol. Antigen retrieval treatment was done in 0.01 mol/L sodium citrate buffer (autoclaved at 121°C for 2 min, pH 6.0) and endogenous peroxidase was blocked by incubation in 3% H2O2 for 10 min at room temperature. The sections were then washed in phosphate buffer saline (PBS) and blocked with 10% goat serum (ZhongShan Biotechnology, China) for 30 min and incubated with rabbit anti-human CRM1 (ab24189, 1:200 dilution, Abcam) or CDK5 (sc-173, 1:150 dilution; Santa Cruz Biotechnology) antibody in a humidified chamber at 4 °C overnight. Following three additional washes in PBS, the sections were incubated with HRP-conjugated secondary antibody for 30 min at room temperature. The visualization signal was developed with diaminobenzidine (DAB) solution and all slides were counterstained with 20% hematoxylin. Finally all slides were dehydrated and mounted on cover slips. For negative controls, the primary antibody diluent was used to replace primary antibody.

***Evaluation of immunostaining intensity***

The IHC-stained tissue sections were reviewed under microscope by 2 pathologists who were blinded to the clinical parameters, and scored independently according to the intensity of cellular staining and the proportion of stained tumor cells[[6](#_ENREF_6)]. The CRM1 and CDK5 proteins were immunohistochemically stained yellowish to brown in the cytoplasm and/or nuclei of cancer cells. The expression pattern of CRM1 and CDK5 was all or none in tumor tissues, suggesting the score for the proportion of stained tumor cells was unavailable. The staining intensity was scored as 0 (no staining), 1(weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown) (Figure 2). The CRM1 and CDK5 protein expression was considered low if the score was 1 or less and high if it was 2 or more.

***Statistical analysis***

The IBM SPSS 19.0 (SPSS, Chicago, IL) was used for all statistical analyses. Χ2 and Fisher’s exact test were used to analyze categorical data. Univariate survival analysis was performed using the Kaplan-Meier method, and the significance of difference between groups was analyzed using the log-rank test. The stepwise Cox proportional hazards regression model was used for multivariate survival analysis with adjustments for variables that may be significant prognostic factors according to the univariate analysis. Receiver operating characteristic (ROC) analysis were used to compare the accuracy of the prediction of clinical outcome by the parameters. All *P* values were 2-sided and statistical significance was determined at *P* < 0.05.

**RESULT**

## *Expression status of CRM1 and CDK5 in GC*

First we examined CRM1 and CDK5 protein expression in tumor tissues from 240 GC patients using immunohistochemistry. The expression of CRM1 and CDK5 proteins were scored as low in 149 (62.08%) and 91(37.92%) samples, and high in 91 (37.92%) and 149 (62.08%) samples, respectively. Based on the combined expression of CRM1 and CDK5, we classified the patients into three subtypes: CRM1 and CDK5 high (n = 63), CRM1 or CDK5 low (n = 114) and CRM1 and CDK5 low (n = 63).

***Correlation between CRM1 and CDK5 expression and Clinicopathological parameters in GC patients***

Second, the correlation between the expression of CRM1 and CDK5 and the clinicopathological features were analyzed (Table 1). CRM1 expression was significantly related to size of primary tumor (*P* = 0.005), Borrmann type (*P* = 0.006), degree of differentiation (*P* = 0.004), depth of invasion (*P* = 0.008), lymph node metastasis (*P* = 0.013), TNM stage(*P* = 0.002)and distant metastasis (*P* = 0.015). The expression of CDK5 was significantly related to sex (*P* = 0.048) and Lauren’s classification (*P* = 0.011). The correlation between combined CRM1 and CDK5 expression and the clinicopathological features was also analyzed. The combined CRM1 and CDK5 expression was significantly related to size of primary tumor (*P* = 0.026), degree of differentiation (*P* = 0.007), Lauren’s classification (*P* = 0.019), lymph node metastasis (*P* = 0.015), TNM stage(*P* = 0.035)and vessel invasion (*P* = 0.021) (Table 2).

***Prognostic value of CRM1 and CDK5 expression***

To elucidate the prognostic value of CRM1 and CDK5 expression, univariate Kaplan–Meier and multivariate Cox regression analyses were employed. Univariate analysis revealed that the patient OS was significantly associated with size and location of primary tumor, Borrmonn type, degree of differentiation, depth of invasion, lymph node metastasis, TNM stage, vessel invasion, distant metastasis, CRM1 expression and CDK5 expression, but not with sex, age at surgery, histological type, and Lauren’s classification (Table 3). The hazard ratio and 95% confidence interval for patient OS were compared among the subgroups. The OS were poorer in patients with low expression of CRM1 or CDK5 in comparison to the corresponding patients with high CRM1 or CDK5 expression (Figure 3).

The 3- and 5-year cumulative survival rates were 54.1% and 39.7% for patients with low CRM1 expression, and 67.0% and 61.5% for those with high CRM1 expression. The mean survival time for patients with low and high expression of CRM1 was 44.6 and 56.5 months respectively. Clearly, GC patients with low expression of CRM1 had a poorer prognosis than those with high CRM1 expression (P < 0.05) (Figure 4A). The 3- and 5-year cumulative survival rates were 49.5% and 39.3% for GC patients with low expression of CDK5, and 63.6% and 53.4% for those with high CDK5 expression. The mean survival time for GC patients with low and high expression of CDK5 was 43.4 and 53.1 mo respectively, suggesting a shorter overall survival for GC patients with low expression of CDK5 (P < 0.05) (Figure 4B).

Next, we evaluated the prognostic value of the combined CRM1 and CDK5 expression. The patients with simultaneous high expression of CRM1 and CDK5 displayed better survival in comparison with the rest of the patients in Kaplan–Meier analysis (Figure 4C). The 3- and 5-year cumulative survival rates were 47.6% and 34.3% for the simultaneous low CRM1 and CDK5 expression patient group, 55.9% and 45.2% for the CRM1 or CDK5 low expression patient group, and 73.0% and 66.7% for the simultaneous high CRM1 and CDK5 expression patient group. The mean survival time was 41.5 months for patients with CRM1 and CDK5 low expression, 46.9 months for those with CRM1 or CDK5 low expression,and 61.1 months for those with CRM1 and CDK5 high expression (Table 3).

Last, the clinicopathological parameters that were correlated with the patient survival in the univariate analysis were included in the multivariate analysis. The CRM1 and CDK5 co-expression status, tumor size, tumor location, and TNM stage were independent prognostic factors for patients with GC, whereas vessel invasion and Borrmonn type were not (Table 4).

***Improvement of the CDK5 prognostic model with CRM1 expression***

In our previous work, we have demonstrated that down-regulation of CDK5 in GC was an independent prognostic factor. To improve the prognostic accuracy of GC patient OS, we combined CRM1 and CDK5 expression to generate a predictive model. Receiver operating characteristic analysis was applied to compare the prognostic accuracy between combined CRM1 and CDK5 expression and CRM1 or CDK5 expression alone. We found that the combination of CRM1 and CDK5 expression showed significantly higher prognostic accuracy (AUC 0.622, 95%CI: 0.551 to 0.694, *P =* 0.001) than CRM1 expression alone (AUC 0.585, 95%CI: 0.512-0.657, *P =* 0.024) or CDK5 expression alone (AUC 0.575, 95%CI: 0.503-0.648, *P =* 0.045) (Figure 5). All these results indicated that the combined CRM1 and CDK5 expression provided better prognostic power for GC patient OS.

**DISCUSSION**

Increasing evidence demonstrated that the karyoplasm localization of CDK5 was important for its multiple pathological and physiological functions including neuronal migration during brain development, neuronal cell survival and tumor development and progression[[23-27](#_ENREF_23)]. CDK5 has no intrinsic nuclear localization signal (NLS) and its nuclear localization relies on p27[[12](#_ENREF_12)]. In the absence of p27, two weak nuclear export signals (NES) on CDK5 bind to CRM1, leading to the cytoplasmic shuttle of CDK5[[12](#_ENREF_12)]. In this study, low CDK5 expression was associated with poorer prognosis (Figure 4B), which was consistent with our previous discovery that CDK5 acted as a tumor suppressor in GC[[23](#_ENREF_23)]. However, CRM1 is usually considered as an oncogene and involved in the nuclear export of a number of proteins including p53, p21, c-ABL and FOXOs[[28-30](#_ENREF_28)]. Forgues *et al*[[31](#_ENREF_31)] found that cytoplasmic sequestration of CRM1 is frequently associated with hepatocellular carcinoma. In this work, high CRM1 expression was associated with longer GC patient survival (Figure 4A), suggesting CRM1 may exert a tumor suppressive role in GC. Considering the oncogenic role of CDK5 in many other types of cancer such as hepatocellular carcinoma[[24](#_ENREF_24)], breast cancer[[32](#_ENREF_32)] and neuroendocrine thyroid cancer[[25](#_ENREF_25)], it is possible that the shift of CDK5 function in GC affect the function of CRM1. In addition, we recently found that CDK5RAP3, a binding protein of the CDK5 activator p35, negatively regulates the β-catenin signaling pathway by repressing GSK-3β phosphorylation and acts as a tumor suppressor in gastric cancer[[33](#_ENREF_33)]. The differential expression or activities of other CDK5 binding partners such as CDK5RAP3 may also impact on the functions of CDK5 and CRM1 across different cancer types.

The fact that either CDK5 or CRM1 expression could influence each other’s prognostic power (Figure 4C) seemed to support this hypothesis. Further analysis with ROC revealed that combination of CRM1 and CDK5 expression showed significantly higher prognostic accuracy than CRM1 expression alone or CDK5 expression alone (*P =* 0.001) (Figure 5), indicating that combined CRM1 and CDK5 expression show more prognostic power for OS of patients with GC. Taken together, our present study suggested that CRM1 and CDK5 should receive considerable attention as an effective marker for predicting therapeutic outcomes, but the profound molecular roles of CRM1 and CDK5 in GC remains far from being fully elucidated and need further research.

In addition, we found that low CRM1 expression was associated with lymph node metastasis in GC (Table 1). This suggested that the identification of CRM1 expression in preoperative mucosal biopsies from GC patients may indicate the necessity for a more aggressive lymphadenectomy, albeit further studies in a larger cohort of patients are needed.

In conclusion, our results suggested that combined CRM1 and CDK5 expression was an independent prognostic factor for OS and showed more prognostic power in GC patients. Considering the inferior prognosis of the CRM1 and/or CDK5 low patients, more frequent follow-ups are probably needed for these patients after surgery.

**COMMENTS**

***Background***

To evaluate the prognostic value of the expression of CRM1 and CDK5 for gastric cancer (GC) patients after gastrectomy.

***Research frontiers***

CDK5 down-regulation was an independent prognostic factor and the nuclear localization of CDK5 was critical for its tumor suppressor function in gastric cancer. Given that CRM1 regulates CDK5 karyoplasm localization in neurons, we hypothesized that the functional correlation between CRM1 and CDK5 may affect each other's prognostic power. In the present study, the authors examined the expression of CRM1 and CDK5 in 240 gastric tumor tissues and analyzed their correlation with patient clinicopathologcial features.

***Innovations and breakthroughs***

The CRM1 and CDK5 co-expression status was an independent prognostic factors for patients with GC. Our results suggested that combined CRM1 and CDK5 expression could provide a better prognostic model for OS of GC patients.

***Applications***

The presented results suggested that combined CRM1 and CDK5 expression was an independent prognostic factor for OS of GC patients and showed more prognostic power than individual factors alone. Considering the inferior prognosis of the CRM1 and/or CDK5 low patients, more frequent follow-ups are probably needed for these patients after surgery.

***Peer-review***

The authors investigate whether combined expression of CDK5 and CRM1 correlates with clinic-pathological parameters in gastric cancer. The manuscript is sound and the experiments/correlations are well-performed.

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**E:\10月内投出去\CRM-1数据\WJG\figure 1.tif**

**Figure 1 Eligibility criteria for patient inclusion.**

**E:\10月内投出去\CRM-1数据\WJG\figure 2.tif**

**Figure 2 Immunohistochemical staining of CRM1 and CDK5 expression in gastric cancerous tissue and the criteria for immunohistochemistry scoring.** Score 0: no staining, Score 1: weak staining, Score 2: moderate staining, Score 3: strong staining. The protein expression was considered low if the score was 1 or less and high if it was 2 or more. Scale bar = 100μm.

**E:\10月内投出去\CRM-1数据\WJG\figure 3.tif**

**Figure 3 Forest plot showing hazard ratios (oblongs) and 95%CI (bars) for overall survival of subgroups from the 240 gastric cancer patients with different CRM1 (left) and CDK5 (right) expression status.** HR: Hazard ratio; OS: Overall survival.

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**Figure 4 Kaplan–Meier analysis of the correlation between the expression of CRM1 (A), CDK5 (B) and combined CRM1 and CDK5 expression (C) and the overall survival of gastric cancer patients.**

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**Figure 5 Receiver operating characteristic analysis of the sensitivity and specificity of the predictive value of the combined CRM1 and CDK5 expression model, CRM1 expression model and CDK5 expression model.** AUC: Area under the curve.

**Table 1 Relationships between CRM1 and CDK5 protein expressions (immunohistochemical staining) in gastric cancer tissues and various clinicopathological variables**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **variables** | **Total** | **CRM1 expression** | | | |  | **CDK5 expression** | | | |
| **Low (*n =* 149)** | **High (*n =* 91)** | ***χ*2** | ***P* value** |  | **Low (*n =* 91)** | **High (*n =* 149)** | ***χ*2** | ***P* value** |
|  |
| Gender |  |  |  |  |  |  |  |  |  |  |
| Male | 178 | 110 | 68 | 0.024 | 0.877 |  | 61 | 117 | 3.893 | 0.0481 |
| Female | 62 | 39 | 23 |  | 30 | 32 |
| Age at surgery(yeas) |  |  |  |  |  |  |  |  |  |  |
| ≤ 60 | 120 | 78 | 42 | 0.867 | 0.352 |  | 46 | 74 | 0.018 | 0.894 |
| > 60 | 120 | 71 | 49 |  | 45 | 75 |
| Size of primary tumor (cm) |  |  |  |  |  |  |  |  |  |  |
| ≤ 5 | 99 | 51 | 48 | 7.995 | 0.0051 |  | 35 | 64 | 0.470 | 0.493 |
| > 5 | 141 | 98 | 43 |  | 56 | 85 |
| Location of primary tumor |  |  |  |  |  |  |  |  |  |  |
| Upper 1/3 | 56 | 33 | 23 | 5.290 | 0.152 |  | 22 | 34 | 1.718 | 0.633 |
| Middle 1/3 | 59 | 39 | 20 |  | 21 | 38 |
| Lower1/3 | 103 | 59 | 44 |  | 37 | 66 |
| More than 1/3 | 22 | 18 | 4 |  | 11 | 11 |
| Borrmann type |  |  |  |  |  |  |  |  |  |  |
| Early stage | 10 | 4 | 6 | 10.118 | 0.0061 |  | 5 | 5 | 0.774 | 0.679 |
| I + II type | 89 | 46 | 43 |  | 32 | 57 |
| III + IV type | 141 | 99 | 42 |  | 54 | 87 |
| Degree of differentiation |  |  |  |  |  |  |  |  |  |  |
| Well/moderate | 96 | 49 | 47 | 8.287 | 0.0041 |  | 30 | 66 | 3.021 | 0.082 |
| Poor and not | 144 | 100 | 44 |  | 61 | 83 |
| Lauren’s classification |  |  |  |  |  |  |  |  |  |  |
| Intestinal type | 46 | 33 | 13 | 2.254 | 0.176 |  | 25 | 21 | 6.527 | 0.0111 |
| Diffuse type | 294 | 116 | 78 |  | 66 | 128 |
| Histological type |  |  |  |  |  |  |  |  |  |  |
| Papillary | 7 | 4 | 3 | 2.958 | 0.398 |  | 3 | 4 | 7.052 | 0.070 |
| Tubular | 187 | 112 | 75 |  | 63 | 124 |
| Mucinous | 20 | 13 | 7 |  | 10 | 10 |
| Signet-ring cell | 26 | 20 | 6 |  | 15 | 11 |
| Depth of invasion |  |  |  |  |  |  |  |  |  |  |
| T1 | 40 | 18 | 22 | 11.908 | 0.0081 |  | 15 | 25 | 2.145 | 0.543 |
| T2 | 27 | 13 | 14 |  | 8 | 19 |
| T3 | 62 | 38 | 24 |  | 21 | 41 |
| T4 | 111 | 80 | 31 |  | 47 | 64 |
| Lymph node metastasis |  |  |  |  |  |  |  |  |  |  |
| N0 | 63 | 29 | 34 | 10.781 | 0.0131 |  | 23 | 40 | 4.868 | 0.182 |
| N1 | 40 | 29 | 11 |  | 11 | 29 |
| N2 | 43 | 26 | 17 |  | 14 | 29 |
| N3 | 94 | 65 | 29 |  | 43 | 51 |
| TNM stage |  |  |  |  |  |  |  |  |  |  |
| I | 44 | 18 | 26 | 15.074 | 0.0021 |  | 15 | 29 | 1.058 | 0.787 |
| II | 55 | 33 | 22 |  | 19 | 36 |
| III | 123 | 82 | 41 |  | 49 | 74 |
| IV | 18 | 16 | 2 |  | 8 | 10 |
| Vessel invasion |  |  |  |  |  |  |  |  |  |  |
| Negative | 230 | 141 | 89 | 1.423 | 0.233 |  | 88 | 142 | 0.278 | 0.598 |
| Positive | 10 | 8 | 2 |  | 3 | 7 |
| Distant metastasis |  |  |  |  |  |  |  |  |  |  |
| Negative | 222 | 133 | 89 | 5.94 | 0.0151 |  | 83 | 139 | 0.352 | 0.553 |
| Positive | 18 | 16 | 2 |  | 8 | 10 |

1*P* < 0.05, statistical significance.

**Table 2 Relationships between different CRM1 and CDK5 protein expressions status in gastric cancer tissues and various clinicopathological variables**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Total** | **CRM1 and CDK5 High expression** | **CRM1 or CDK5 Low expression** | **CRM1 and CDK5 Low expression** | ***χ*2** | ***P* value** |
|
| Gender |  |  |  |  |  |  |
| Male | 178 | 42 | 87 | 49 | 2.553 | 0.279 |
| Female | 62 | 21 | 27 | 14 |
| Age at surgery(yr) |  |  |  |  |  |  |
| ≤ 60 | 120 | 35 | 54 | 31 | 1.109 | 0.574 |
| > 60 | 120 | 28 | 60 | 32 |
| Size of primary  tumor (cm) | |  |  |  |  |  |
| ≤ 5 | 99 | 22 | 42 | 35 | 7.275 | 0.0261 |
| > 5 | 141 | 41 | 72 | 28 |
| Location of primary tumor |  |  |  |  |  |  |
| Lower 1/3 | 56 | 18 | 19 | 19 | 10.848 | 0.093 |
| Middle 1/3 | 59 | 14 | 32 | 13 |
| Upper 1/3 | 103 | 22 | 52 | 29 |
| More than 1/3 | 22 | 9 | 11 | 2 |
| Borrmann type |  |  |  |  |  |  |
| Early stage | 10 | 2 | 5 | 3 | 6.035 | 0.197 |
| I + II type | 89 | 20 | 38 | 31 |
| III + IV type | 141 | 41 | 71 | 29 |
| Degree of differentiation |  |  |  |  |  |  |
| Well/moderate | 96 | 18 | 43 | 35 | 10.027 | 0.0071 |
| Poor and not | 144 | 45 | 71 | 28 |
| Lauren’s classification |  |  |  |  |  |  |
| Intestinal type | 46 | 17 | 24 | 5 | 7.875 | 0.0191 |
| Diffuse type | 194 | 46 | 90 | 58 |
| Histological type |  |  |  |  |  |  |
| Papillary | 7 | 2 | 3 | 2 | 11.127 | 0.850 |
| Tubular | 187 | 44 | 87 | 56 |
| Mucinous | 20 | 5 | 13 | 2 |
| Signet-ring cell | 26 | 12 | 11 | 3 |
| Depth of invasion |  |  |  |  |  |  |
| T1 | 40 | 8 | 17 | 15 | 10.996 | 0.088 |
| T2 | 27 | 4 | 13 | 10 |
| T3 | 62 | 16 | 27 | 19 |
| T4 | 111 | 35 | 57 | 19 |
| Lymph node metastasis |  |  |  |  |  |  |
| N0 | 63 | 15 | 22 | 26 | 15.845 | 0.0151 |
| N1 | 40 | 9 | 22 | 9 |
| N2 | 43 | 7 | 26 | 10 |
| N3 | 94 | 32 | 44 | 18 |
| TNM stage |  |  |  |  |  |  |
| I | 44 | 8 | 17 | 19 | 13.543 | 0.0351 |
| II | 55 | 14 | 24 | 17 |
| III | 123 | 33 | 65 | 25 |
| IV | 18 | 8 | 8 | 2 |
| Vessel invasion |  |  |  |  |  |  |
| Negative | 230 | 62 | 105 | 63 | 7.757 | 0.0211 |
| Positive | 10 | 1 | 9 | 0 |
| Distant metastasis |  |  |  |  |  |  |
| Negative | 222 | 55 | 106 | 61 | 4.191 | 0.123 |
| Positive | 18 | 8 | 8 | 2 |

1*P* < 0.05, statistical significance.

**Table 3 Univariate analysis of the correlation between clinicopathological parameters and survival of patients with gastric cancer**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Clinicopathological parameters** | **Cumulative survival rates (%)** | | **Mean survival time (mo)** | **Log-Rank test** | ***P* value** |
| **3-yr** | **5-yr** |
| Gender |  |  |  |  |  |
| Male | 66.1 | 48.3 | 49.022 | 0.092 | 0.762 |
| Female | 56.6 | 48.0 | 49.324 |
| Age at surgery (yr) |  |  |  |  |  |
| ≤ 60 | 60.8 | 48.1 | 49.510 | 0.022 | 0.882 |
| > 60 | 57.2 | 47.9 | 49.285 |
| Size of primary tumor (cm) |  |  |  |  |  |
| ≤ 5 | 84.8 | 73.4 | 66.451 | 44.251 | 0.0001 |
| > 5 | 41.1 | 30.4 | 37.516 |
| Location of primary tumor |  |  |  |  |  |
| Upper 1/3 | 51.8 | 38.7 | 44.354 | 28.888 | 0.0001 |
| Middle 1/3 | 42.4 | 33.9 | 39.508 |
| Lower 1/3 | 76.5 | 66.7 | 61.597 |
| More than 1/3 | 31.8 | 22.7 | 30.500 |
| Borrmann type |  |  |  |  |  |
| Early stage | 90.0 | 90.0 | 72.186 | 41.770 | 0.0001 |
| I + II type | 81.9 | 71.5 | 64.835 |
| III+IV type | 42.6 | 30.4 | 38.102 |
| Degree of differentiation |  |  |  |  |  |
| Well/moderate | 70.8 | 60.3 | 57.397 | 8.644 | 0.0031 |
| Poor and not | 49.8 | 39.9 | 44.056 |
| Lauren’s classification |  |  |  |  |  |
| Intestinal type | 66.8 | 50.7 | 53.287 | 0.649 | 0.420 |
| Diffuse type | 56.2 | 47.4 | 48.471 |
| Histological type |  |  |  |  |  |
| Papillary | 57.1 | 57.1 | 50.857 | 1.026 | 0.752 |
| Tubular | 57.2 | 47.0 | 48.339 |
| Mucinous | 75.0 | 53.6 | 53.850 |
| Signet-ring cell | 60.2 | 48.2 | 51.110 |
| Depth of invasion |  |  |  |  |  |
| T1 | 97.5 | 94.9 | 78.311 | 64.970 | 0.0001 |
| T2 | 88.9 | 74.1 | 67.889 |
| T3 | 59.2 | 46.0 | 48.764 |
| T4 | 37.8 | 25.2 | 34.461 |
| Lymph node metastasis |  |  |  |  |  |
| N0 | 88.9 | 80.8 | 70.120 | 59.862 | 0.0001 |
| N1 | 69.5 | 69.5 | 61.079 |
| N2 | 58.1 | 34.9 | 43.674 |
| N3 | 33.0 | 23.3 | 32.911 |
| TNM stage |  |  |  |  |  |
| I | 97.7 | 95.4 | 78.211 | 71.616 | 0.0001 |
| II | 76.1 | 61.3 | 60.241 |
| III | 40.7 | 29.2 | 38.186 |
| IV | 27.8 | 16.7 | 22.518 |
| Vessel invasion |  |  |  |  |  |
| Negative | 60.8 | 49.3 | 50.492 | 8.264 | 0.0041 |
| Positive | 20.0 | 20.0 | 23.400 |
| Distant metastasis |  |  |  |  |  |
| Negative | 60.7 | 50.6 | 51.544 | 20.223 | 0.0001 |
| Positive | 16.7 | 16.7 | 22.518 |
| CRM1 expression |  |  |  |  |  |
| Low | 54.1 | 39.7 | 44.590 | 7.707 | 0.0051 |
| High | 67.0 | 61.5 | 56.540 |
| CDK5 expression |  |  |  |  |  |
| Low | 49.5 | 39.3 | 53.058 | 6.234 | 0.0131 |
| High | 63.6 | 53.4 | 43.438 |
| CRM1/CDK5 expression |  |  |  |  |  |
| CRM1 and CDK5 Low | 47.6 | 34.3 | 41.487 | 13.683 | 0.0011 |
| CRM1 or CDK5 Low | 55.9 | 45.2 | 46.873 |
| CRM1 and CDK5 High | 73.0 | 66.7 | 61.069 |

1*P* < 0.05, statistical significance.

**Table 4 Multivariate analysis of the correlation between clinicopathological parameters and survival time of patients with gastric cancer**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Covariates** | **Coefficient** | **Standard error** | **HR** | **95%CI for HR** | ***P* value** |
| Tumor location (cardia *vs* others) | 0.451 | 0.202 | 1.570 | 1.057-2.333 | 0.0261 |
| Tumor size (≥ 5 *vs* *<* 5 cm) | 0.723 | 0.232 | 2.060 | 1.309-3.243 | 0.0021 |
| Vessel invasion (positive *vs* negative) | NA | NA | NA | NA | NA |
| TNM stage (stage III and IV *vs* I and II) | 1.086 | 0.243 | 1.961 | 1.839-4.768 | 0.0001 |
| CDK5 and CRM1 expression | |  |  |  |  |
| (low/high *vs* high/high) | 0.568 | 0.254 | 1.765 | 1.074-2.903 | 0.0251 |
| (low/low *vs* high/high) | 0.769 | 0.269 | 2.158 | 1.274-3.657 | 0.0041 |
| Borrmann type (type early, I, II *vs* III, IV) | NA | NA | NA | NA | NA |

1*P* < 0.05, statistical significance. NA: Not available.