Expression of CRM1 and CDK5 shows high prognostic accuracy for gastric cancer

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AIM
To evaluate the predictive value of the expression of chromosomal maintenance (CRM)1 and cyclin-dependent kinase (CDK)5 in gastric cancer (GC) patients after gastrectomy.

METHODS
A total of 240 GC 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 analyses were used to identify prognostic factors for GC. Receiver operating characteristic analysis was 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.
CONCLUSION
CRM1 and CDK5 co-expression was an independent prognostic factors for GC. Combined CRM1 and CDK5 expression could provide a prognostic model for OS of GC.

Key words: Gastric cancer; CRM1; CDK5; Prognosis

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Core tip: Our study shows that low expression of chromosomal maintenance (CRM)1 and cyclin-dependent kinase (CDK)5 was associated with poor prognosis of gastric cancer patients. The expression of CRM1 or CDK5 influenced the prognostic value of each other. Combined CRM1 and CDK5 expression had better prognostic power than their individual expression had.
slides were dehydrated and mounted on coverslips. For were counterstained with 20% hematoxylin. Finally, all peroxidase-conjugated secondary antibody for 30 min in PBS, the sections were incubated with horseradish-
at 4 °C. 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-buffered 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, Cambridge, MA, United States) or CDK5 (sc-173, 1:150 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, United States) antibody in a humidified chamber at 4 °C overnight. Following three additional washes in PBS, the sections were incubated with horseradish-peroxidase-conjugated secondary antibody for 30 min at room temperature. The visualization signal was developed with diaminobenzidine solution and all slides were counterstained with 20% hematoxylin. Finally, all slides were dehydrated and mounted on coverslips. For negative controls, the primary antibody diluent was used to replace primary antibody.

Evaluation of immunostaining intensity
The stained tissue sections were reviewed under a microscope by two 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 (Figure 2). 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 and high if it was ≥ 2.

Statistical analysis
IBM SPSS version 19.0 (SPSS, Chicago, IL, United States) was used for all statistical analyses. χ2 and Fisher’s exact tests 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 have been significant prognostic factors according to the univariate analysis. Receiver operating characteristic (ROC) analysis was used to compare the accuracy of the prediction of clinical outcome by the parameters. All P values were two-sided and statistical significance was determined at P < 0.05.

RESULTS
Expression status of CRM1 and CDK5 in GC
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
The correlation between 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
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 OS for GC patients with low expression of CDK5 ($P < 0.05$) (Figure 4B).

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, respectively. The mean survival time was 41.5 mo for patients with CRM1 and CDK5 low expression; 46.9 mo for those with CRM1 or CDK5 low expression; and 61.1 mo for those with CRM1 and CDK5 high expression (Table 3).

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

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

In our previous work, we demonstrated that down-regulation of CDK5 in GC was an independent prognostic factor. To improve the prognostic accuracy of OS in GC patients, we combined CRM1 and CDK5 expression to generate a predictive model. ROC analysis was applied to compare the prognostic accuracy between
combined CRM1 and CDK5 expression and CRM1 or CDK5 expression alone. Combination of CRM1 and CDK5 expression showed significantly higher prognostic accuracy [area under the curve (AUC): 0.622, 95%CI: 0.551-0.694, \( P = 0.001 \)] 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 has demonstrated that the karyoplasm localization of CDK5 is important for its multiple pathological and physiological functions, including neuronal migration during brain development, neuronal cell survival and tumor development and differentiation. The role of CRM1 in cell migration and proliferation is well-established, and its association with CDK5 has been reported in various studies. The combined expression of CRM1 and CDK5 in gastric cancer tissues may indicate a more aggressive phenotype, influencing patient prognosis.
progression\(^{[23-27]}\). CDK5 has no intrinsic nuclear localization signal and its nuclear localization relies on p27\(^{[12]}\). In the absence of p27, two weak NESs on CDK5 bind to CRM1, leading to the cytoplasmic shuttle of CDK5\(^{[12]}\). 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\(^{[23]}\). 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\(^{[28-30]}\). Forgues et al\(^{[31]}\) 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 that CRM1 exerts a tumor suppressive role in GC. Considering the oncogenic role of CDK5 in many other types of cancer such as hepatocellular carcinoma\(^{[24]}\), breast cancer\(^{[32]}\), we explored the correlation between CDK5 or CRM1 expression and clinicopathological variables in GC patients.

Table 2 Relationships between different CRM1 and CDK5 protein expression 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 | \(\chi^2\) | \(P\) 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    | 0.026\(^1\) |
| \(> 5\)                                        | 141   | 41                            | 72                          | 28                            |          |           |
| Location of primary tumor                      |       |                               |                             |                               |          |           |
| Lower 1/3                                      | 56    | 18                            | 19                          | 19                            | 10.848   | 0.009     |
| 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                             | 0.035    | 0.197     |
| I + II type                                    | 89    | 20                            | 38                          | 31                            |          |           |
| II + IV type                                   | 141   | 41                            | 71                          | 29                            |          |           |
| Degree of differentiation                     |       |                               |                             |                               |          |           |
| Well/moderate                                  | 96    | 18                            | 43                          | 35                            | 11.278   | 0.850     |
| Poor and not                                   | 144   | 45                            | 71                          | 28                            |          |           |
| Lauren's classification                        |       |                               |                             |                               |          |           |
| Intestinal type                                | 46    | 17                            | 24                          | 5                             | 11.278   | 0.019\(^1\) |
| Diffuse type                                   | 194   | 46                            | 90                          | 58                            |          |           |
| Histological type                              |       |                               |                             |                               |          |           |
| Papillary                                      | 7     | 2                             | 3                           | 2                             | 11.278   | 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                                             | 40    | 4                             | 13                          | 10                            |          |           |
| T3                                             | 62    | 16                            | 27                          | 19                            |          |           |
| T4                                             | 111   | 35                            | 57                          | 19                            |          |           |
| Lymph node metastasis                          |       |                               |                             |                               |          |           |
| N0                                             | 63    | 15                            | 22                          | 26                            | 10.996   | 0.088     |
| N1                                             | 40    | 9                             | 22                          | 9                             |          |           |
| N2                                             | 43    | 7                             | 26                          | 10                            |          |           |
| N3                                             | 94    | 32                            | 44                          | 18                            |          |           |
| TNM stage                                      |       |                               |                             |                               |          |           |
| I                                              | 44    | 8                             | 17                          | 19                            | 10.996   | 0.088     |
| II                                             | 55    | 14                            | 24                          | 17                            |          |           |
| III                                            | 123   | 33                            | 65                          | 25                            |          |           |
| IV                                              | 18    | 8                             | 8                           | 2                             |          |           |
| Vessel invasion                                |       |                               |                             |                               |          |           |
| Negative                                       | 230   | 62                            | 105                         | 63                            |          |           |
| Positive                                       | 10    | 1                             | 9                           | 0                             |          |           |
| Distant metastasis                             |       |                               |                             |                               |          |           |
| Negative                                       | 222   | 55                            | 106                         | 61                            |          |           |
| Positive                                       | 18    | 8                             | 8                           | 2                             |          |           |

\(^1\)P < 0.05, statistical significance. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.
### 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  | 0.000 |
| > 5                            | 41.1                          | 30.4                    | 37.516        |          |       |
| Location of primary tumor      |                               |                         |               |         |
| Upper 1/3                       | 51.8                          | 38.7                    | 44.354        | 28.888  | 0.000 |
| 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  | 0.000 |
| I + II type                     | 81.9                          | 71.5                    | 64.835        |          |       |
| III + IV type                   | 42.6                          | 30.4                    | 38.102        |          |       |
| Degree of differentiation       |                               |                         |               |         |
| Well/moderate                   | 57.1                          | 57.1                    | 50.857        | 1.026   | 0.752 |
| 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                       | 57.2                          | 57.2                    | 48.339        |          |       |
| Tubular                         | 75.0                          | 53.6                    | 53.850        |          |       |
| Mucinous                        | 60.2                          | 48.2                    | 51.110        |          |       |
| Signet-ring-cell                |                               |                         |               |         |
| Depth of invasion               |                               |                         |               |         |
| T1                             | 97.5                          | 94.9                    | 78.311        | 64.970  | 0.000 |
| 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  | 0.000 |
| 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  | 0.000 |
| II                             | 76.1                          | 61.3                    | 60.241        |          |       |
| III                            | 40.7                          | 29.2                    | 38.186        |          |       |
| N/                             | 27.8                          | 16.7                    | 22.518        |          |       |
| Vessel invasion                 |                               |                         |               |         |
| Negative                        | 60.8                          | 49.3                    | 50.492        | 8.264   | 0.004 |
| Positive                        | 20.0                          | 20.0                    | 23.400        |          |       |
| Distant metastasis              |                               |                         |               |         |
| Negative                        | 60.7                          | 50.6                    | 51.544        | 20.223  | 0.000 |
| Positive                        | 16.7                          | 16.7                    | 22.518        |          |       |
| CRM1 expression                 |                               |                         |               |         |
| Low                            | 54.1                          | 39.7                    | 44.590        | 7.707   | 0.005 |
| High                           | 67.0                          | 61.5                    | 56.540        |          |       |
| CDK5 expression                 |                               |                         |               |         |
| Low                            | 49.5                          | 39.3                    | 53.058        | 6.234   | 0.013 |
| High                           | 63.6                          | 53.4                    | 43.438        |          |       |
| CRM1/CDK5 expression            |                               |                         |               |         |
| CRM1 and CDK5 Low              | 47.6                          | 34.3                    | 41.487        | 13.683  | 0.001 |
| 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. CRM: Chromosomal maintenance; CDK: Cyclin-dependent kinase.
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.

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   | 0.026*  |
| Tumor size (≥ 5 vs < 5 cm)                      | 0.723       | 0.232          | 2.060| 1.309-3.243   | 0.002*  |
| 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   | 0.0002* |
| CDK5 and CRM1 expression (low/high vs high/high)| 0.568       | 0.254          | 1.765| 1.074-2.903   | 0.025*  |
| (low/low vs high/high)                          | 0.769       | 0.269          | 2.158| 1.274-3.657   | 0.004*  |
| Borrmann type (type early, I vs II and III)     | NA          | NA             | NA   | NA            | NA      |

*P < 0.05, statistical significance. NA: Not available.
and neuroendocrine thyroid cancer[25], it is possible that the shift of CDK5 function in GC affects 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 glycogen synthase kinase-3β phosphorylation and acts as a tumor suppressor in GC[33]. The differential expression or activities of other CDK5-binding partners such as CDK5RAP3 may also affect the functions of CDK5 and CRM1 among different cancer types.

The fact that either CDK5 or CRM1 expression could influence the prognostic power of the other (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 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 effective markers for predicting therapeutic outcomes, but the profound molecular roles of CRM1 and CDK5 in GC remain 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, although 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-up is probably needed for these patients after surgery.

**COMMENTS**

**Background**

To evaluate the prognostic value of the expression of chromosomal maintenance (CRM1) and cyclin-dependent kinase (CDK5) for gastric cancer (GC) patients after gastrectomy.

**Research frontiers**

CDK5 downregulation was an independent prognostic factor and the nuclear localization of CDK5 was critical for its tumor suppressor function in GC. Given that CRM1 regulates CDK5 karyoplasm localization in neurons, we hypothesized that the functional correlation between CRM1 and CDK5 may affect the prognostic power of each molecule. In the present study, we examined the expression of CRMI and CDK5 in 240 gastric tumor tissues and analyzed their correlation with patient clinicopathological features.

**Innovations and breakthroughs**

CRM1 and CDK5 coexpression was an independent prognostic factor for patients with GC. The present results suggested that combined CRM1 and CDK5 expression could provide a better prognostic model for overall survival (OS) of GC patients.

**Applications**

The present results supported 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 prognostic of the CRM1 and/or CDK5 low patients, more frequent follow-up is probably needed for these patients after surgery.

**Peer-review**

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

**REFERENCES**

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20072007]
2. Yang L. Incidence and mortality of gastric cancer in China. World J Gastroenterol 2006; 12: 17-20 [PMID: 16440411 DOI: 10.3748/wjg.v12.i1.17]
3. Chen W, Zheng R, Zeng H, Zhang S. The updated incidences and mortalities of major cancers in China, 2011. Chin J Cancer 2015; 34: 502-507 [PMID: 26370301 DOI: 10.1186/s40880-015-0042-6]
4. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu QX, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132 [PMID: 26080342 DOI: 10.3322/caac.21338]
5. Washington K. 7th edition of the AJCC cancer staging manual: stomach. Ann Surg Oncol 2010; 17: 3077-3079 [PMID: 20882416 DOI: 10.1245/s10434-010-1362-z]
6. Shou ZX, Jin X, Zhao ZS. Upregulated expression of ADAM17 is a prognostic marker for patients with gastric cancer. Ann Surg Oncol 2012; 256: 1014-1022 [PMID: 22668812 DOI: 10.1097/SLA.s0013182592565]
7. Fukuda M, Asano S, Nakamura T, Adachi M, Yoshida M, Yanagida M, Nishida E. CRM1 is responsible for intracellular transport mediated by the nuclear export signal. Nature 1997; 390: 308-311 [PMID: 9384386 DOI: 10.1038/36904]
8. Ossareh-Nazari B, Belcheire F, Dargemont C. Evidence for a role of CRM1 in signal-mediated nuclear protein export. Science 1997; 278: 141-144 [PMID: 9311922 DOI: 10.1126/science.278.5335.141]
9. Stommel JM, Marchenko ND, Jimenez GS, Moll UM, Hope TJ, Wahl GM. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. EMBO J 1999; 18: 1660-1672 [PMID: 10075936 DOI: 10.1093/emboj/18.6.1660]
10. Saji M, Vasko V, Kada F, Allbritton EH, Burman KD, Ringel MD. Akt1 contains a functional leucine-rich nuclear export sequence. Biochem Biophys Res Commun 2005; 332: 167-173 [PMID: 15896313 DOI: 10.1016/j.bbrc.2005.04.169]
11. He W, Wang X, Chen L, Guan X. A crosstalk imbalance between p27(Kip1) and its interacting molecules enhances breast carcinogenesis. Cancer Biol Ther 2012; 27: 399-402 [PMID: 22690887 DOI: 10.1089/cbt.2010.0802]
12. Zhang J, Li H, Herrup K. Cdk5 nuclear localization is p27-dependent in nerve cells: implications for cell cycle suppression and caspase-3 activation. J Biol Chem 2010; 285: 14052-14061 [PMID: 20189989 DOI: 10.1074/jbc.M109.068262]
13. Lo HW, Ali-Seyed M, Wu Y, Bartholomeusz G, Hsu SC, Hung MC. Nuclear-cyttoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1. J Cell Biochem 2006; 98: 1570-1583 [PMID: 16552725 DOI: 10.1002/jcb.20876]
14. Noske A, Weichert W, Niesporek S, Röske A, Buckendahl AC, Koch I, Schouli J, Dietel M, Denkert C. Expression of the nuclear export protein chromosomal region maintenance/exportin 1/Xpo1 is a prognostic factor in human ovarian cancer. Cancer 2008; 112: 1733-1743 [PMID: 18306389 DOI: 10.1002/cncr.23354]
15. Yao Y, Dong Y, Lin F, Zhao H, Shen Z, Chen P, Sun YJ, Tang LN, Zheng SE. The expression of CRM1 is associated with prognosis in human osteosarcoma. Oncol Rep 2009; 21: 229-235 [PMID: 19082467]
16. Shen A, Wang Y, Zhao Y, Zou L, Sun L, Cheng C. Expression of CRM1 in human gliomas and its significance in p27 expression and clinical prognosis. Neurosurgery 2009; 65: 153-159; discussion 159-160 [PMID: 19574837 DOI: 10.1227/01.NEU.0000348550.47441.4B]
17. Huang WY, Yue L, Qiu WS, Wang LW, Zhou XH, Sun YJ. Prognostic value of CRM1 in pancreas cancer. Clin Invest Med 2009; 32: E315 [PMID: 20003838]
18. van der Watt PJ, Zemanay W, Govender D, Hendricks DT, Parker MJ, Leander VD. Elevated expression of the nuclear export protein, CRM1 (exportin 1), associates with human oesophageal squamous cell carcinoma. Oncol Rep 2014; 32: 730-738 [PMID: 24898882]
19. Choi JH, Banks AS, Estall JL, Kajimura S, Boström P, Laznik D, Ruas JL, Chalmers MJ, Kamenecka TM, Blüher M, Griffin PR, Spiegelman BM. Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPARGamma by CKD5. Nature 2010; 466: 451-456 [PMID: 20651683 DOI: 10.1038/nature09291]
20. Hisanaga S, Endo R. Regulation and role of cyclin-dependent kinase activity in neuronal survival and death. J Neurochem 2010; 115: 1309-1321 [PMID: 21040475 DOI: 10.1111/j.1471-4159.2010.07050.x]
21. Lindqvist TJ, Wahl GM. A leucine-rich nuclear export signal in the p53 tetramerization domain: regulation of subcellular localization and p53 activity by NES masking. J Biol Chem 2010; 285: 14052-14061 [PMID: 10.1016/j.jbc.2010.04.169]
22. Tripathi BK, Qian X, Mertins P, Wang D, Papageorge A, Carr S, Lowsy DR. CDK5 negatively regulates Rho by phosphorylating and activating the Rho-GAP and tumor suppressor functions of DCL1. Cancer Res 2014; 74: 1574-1574 [DOI: 10.1158/1538-7445.AM2014-1574]
23. Cao L, Zhou J, Zhang J, Wu S, Yang X, Zhao X, Li H, Luo M, Yu Q, Lin G, Lin H, Xie J, Li P, Hu X, Zheng C, Bu G, Zhang YW, Xu H, Yang Y, Huang C, Zhang J. Cyclin-dependent kinase 5 decreases in gastric cancer and its nuclear accumulation suppresses gastric tumorigenesis. Clin Cancer Res 2015; 21: 1419-1428 [PMID: 25690906 DOI: 10.1158/1078-0432.CCR-14-1950]
24. Ehrlich SM, Liebl J, Ardelt MA, Lehr T, De Toni EN, Mayr D,
Sun YQ et al. Prognostic value of CRM1 and CDK5

Brandl L, Kirchner T, Zahler S, Gerbes AL, Vollmar AM. Targeting cyclin dependent kinase 5 in hepatocellular carcinoma--A novel therapeutic approach. J Hepatol 2015; 63: 102-113 [PMID: 25660209 DOI: 10.1016/j.jhep.2015.01.031]

25 Pozo K, Castro-Rivera E, Tan C, Plattner F, Schwach G, Siegl V, Meyer D, Guo A, Gundara J, Mettlach G, Richer E, Guevara JA, Ning L, Gupta A, Hao G, Tsai LH, Sun X, Antich P, Sidhu S, Robinson BG, Chen H, Nwariaku FE, Pfragner R, Richardson JA, Bibb JA. The role of Cdk5 in neuroendocrine thyroid cancer. Cancer Cell 2013; 24: 499-511 [PMID: 24135281 DOI: 10.1016/j.ccr.2013.08.027]

26 Merk H, Zhang S, Lehr T, Müller C, Ulrich M, Bibb JA, Adams RH, Bracher F, Zahler S, Vollmar AM, Liebl J. Inhibition of endothelial Cdk5 reduces tumor growth by promoting non-productive angiogenesis. Oncotarget 2016; 7: 6088-6104 [PMID: 26755662]

27 Zhang J, Li H, Yabut O, Fitzpatrick H, D’Arcangelo G, Herrup K. Cdk5 suppresses the neuronal cell cycle by disrupting the E2F1-DP1 complex. J Neurosci 2010; 30: 5219-5228 [PMID: 20392944 DOI: 10.1523/JNEUROSCI.5628-09.2010]

28 Connor MK, Kotchertkov R, Cariou S, Resch A, Lupetti R, Beniston RG, Melchior F, Hengst L, Slingerland JM. CRM1/Ran-mediated nuclear export of p27(Kip1) involves a nuclear export signal and links p27 export and proteolysis. Mol Biol Cell 2003; 14: 201-213 [PMID: 12529437 DOI: 10.1091/mbc.E02-06-0319]

29 Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat Med 2001; 7: 228-234 [PMID: 11175855 DOI: 10.1038/84683]

30 Vogt PK, Jiang H, Aoki M. Triple layer control: phosphorylation, acetylation and ubiquitination of FOXO proteins. Cell Cycle 2005; 4: 908-913 [PMID: 15917664 DOI: 10.4161/cc.4.7.1796]

31 Forges M, Difilippantonio MJ, Linke SP, Ried T, Nagashima K, Feden J, Valerie K, Fukasawa K, Wang XW. Involvement of Crm1 in hepatitis B virus X protein-induced aberrant centriole replication and abnormal mitotic spindles. Mol Cell Biol 2003; 23: 5282-5292 [PMID: 12861014 DOI: 10.1128/MCB.23.15.5282-5292.2003]

32 Chiker S, Pennaneach V, Loew D, Dingli F, Biard D, Cordelieres FP, Gemble S, Vacher S, Bieche I, Hall J, Fernet M. Cdk5 promotes DNA replication stress checkpoint activation through RPA-32 phosphorylation, and impacts on metastasis free survival in breast cancer patients. Cell Cycle 2015; 14: 3066-3078 [PMID: 26237679 DOI: 10.1080/15384101.2015.1078020]

33 Wang JB, Wang ZW, Li Y, Huang CQ, Zheng CH, Li P, Xie JW, Lin JX, Lu J, Chen QY, Cao LL, Lin M, Tu RH, Lin Y, Huang CM. CDK5RAP3 acts as a tumor suppressor in gastric cancer through inhibition of β-catenin signaling. Cancer Lett 2017; 385: 188-197 [PMID: 27793695 DOI: 10.1016/j.canlet.2016.10.024]

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