Prognostic and clinicopathological significance of Gankyrin overexpression in cancers: evidence from a meta-analysis

Xiaotong Zhao1,2,*
Fangzhou Liu2,3,*
Yuan Zhang3
Peihua Li1
1Department of Otolaryngology, Affiliated Hospital of Xuzhou Medical College, Xuzhou, 2Department of Otolaryngology, The Affiliated Cancer Hospital, Nanjing Medical University, Nanjing, Jiangsu, People’s Republic of China
3These authors contributed equally to this work

Abstract: Many studies have indicated that Gankyrin is a promising and novel prognostic tumor biomarker. However, the results of different studies remained controversial. Hence, a meta-analysis was undertaken to investigate the association between Gankyrin expression and cancer prognosis. Eligible studies were identified by searching the electronic databases PubMed, Embase, and Cochrane Library up to November 2015. Prognostic value of Gankyrin expression was evaluated by hazard ratio with 95% confidence interval (CI). Meanwhile, relative risk (RR) with 95% CI was used to assess the effects of Gankyrin expression on clinicopathological parameters. In total, ten studies with 1,326 patients were included for final analysis. A significant association was found between Gankyrin overexpression and poorer overall survival in patients with cancer (hazard ratio = 1.73, 95% CI: 1.29–2.31, P = 0.000). In the subgroup analysis, the association was also detected in Chinese patients and patients with cancers of the digestive system. The pooled RR indicated that Gankyrin overexpression was related to advanced tumor–node–metastasis stage (RR = 0.72, 95% CI: 0.60–0.86, P = 0.000), positive lymph node metastasis (RR = 1.66, 95% CI: 1.41–1.94, P = 0.000), and distant metastasis (RR = 1.43, 95% CI: 1.20–1.70, P = 0.000). The meta-analysis demonstrated that Gankyrin is a novel biomarker for predicting cancers, especially digestive system cancers, and is more suitable for predicting cancer prognoses in Asians.

Keywords: Gankyrin, cancer, prognosis, meta-analysis

Introduction

Gankyrin protein (also named as PSMD10, p28[Gank], or p28) is a small protein (25 kDa). It was initially identified and characterized as the p28 component of a regulatory subunit of the 26S proteasome in 1998.1 “Gann” means cancer in Japanese, and ankyrin is functional domain that is involved in protein–protein interactions. Gankyrin protein contains two typical domains including seven ankyrin repeats and Rb-recognition motif (LXCXE), and the name of Gankyrin was derived from these factors.2,3 Previous studies showed that Gankyrin enhances cancer cell proliferation through interaction with p53 and Rb,4,5 and the aberrant expression of Gankyrin is associated with cancer progression and poor prognoses. Although many clinical studies suggested that the overexpression of Gankyrin was a predictor for worse outcome in cancers,6,7 some other conflicting conclusions were arrived.8,9 Therefore, the prognostic value of Gankyrin overexpression remains controversial. To overcome the limitation of the single study, this meta-analysis was conducted to investigate the relationship between Gankyrin expression and its prognostic value in cancers.
Materials and methods

Search strategy
A systematic literature search of the databases PubMed, Embase, and Cochrane Library was performed with the following strategy: “Gankyrin” OR “p28” OR “p28Gank” OR “PSMD10” AND “prognosis” OR “prognostic” OR “survival” OR “outcome” AND “cancer” OR “carcinoma” OR “neoplasm” OR “tumor” OR “malignant” up to November 2015. To explore additional studies, we also inspected the references of the included studies. The eligible reports were identified independently by two investigators (Zhao and Liu).

Inclusion criteria
Eligible studies have to meet the following inclusion criteria: 1) had to evaluate the Gankyrin expression in human cancer tissues; 2) had to evaluate the association between Gankyrin expression and cancer prognosis; and 3) had to have sufficient information to estimate the hazard ratios (HRs) with 95% confidence intervals (CIs). The exclusion criteria were as follows: 1) reviews, case reports, letters, and conference abstracts and 2) overlapping data. The flow diagram of study selection is presented in Figure 1.

Data extraction
Data from eligible studies were extracted independently by two investigators (Zhao and Liu) according to the inclusion and exclusion criteria mentioned earlier.

Statistical analysis
HRs with their 95% CIs were calculated to evaluate the association between Gankyrin expression levels and the cancer prognosis (OS). If available, we obtained data from article directly. If not, we calculated data from Kaplan–Meier curves. Meanwhile, the impact of Gankyrin expression on clinicopathological parameters was performed by relative risks (RRs) with their 95% CIs. The heterogeneity among the included studies was checked by the chi-squared Q test. If the heterogeneity exists ($P<0.10$ or $I^2>50\%$), the random-effects model was used; otherwise, the fixed-effects model was used. Subgroup analyses were conducted to explore the source of heterogeneity. Moreover, a sensitivity analysis was carried out to evaluate whether any single study could have effects on the pooled HRs by sequentially discarding individual studies. Egger’s test and Begg’s funnel plots were applied to assess publication biases. All statistical analyses were performed using Stata 12.0 (StataCorp LP, College Station, TX, USA). $P$-value $<0.05$ was considered statistically significant.

Results

Study characteristics
According to the search strategy, a total of 51 studies were selected after the initial search. All the studies were identified by the inclusion and exclusion criteria mentioned earlier. Finally, ten eligible studies, published from 2008 to 2015, were included in this meta-analysis. The main characteristics of the eligible studies are summarized in Table 1. A total of 1,326 patients from the People’s Republic of China, Japan, and Korea were involved. Of all the eligible studies, nine were in English language, whereas one was in Chinese (seven were...
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about digestive system cancers [hepatocellular cancer, colorectal cancer, esophageal cancer, gastric cancer, and cholangiocarcinoma cancer] and three were about other cancer types [non-small-cell lung cancer, glioma cancer, and liposarcoma]. IHC was the main method used to detect the Gankyrin expression in these studies, but in one study, it was detected by real-time polymerase chain reaction.

Meta-analysis results

Gankyrin expression and OS

As shown in Figure 2, Gankyrin overexpression was significantly associated with poor OS in cancers (HR = 1.73, 95% CI: 1.29–2.31, P = 0.000). Owing to the heterogeneity detected in these studies (P = 0.019, I^2 = 54.6%), the random-effects model was applied.

To explore the source of heterogeneity, subgroup analyses were performed by nationality, cancer type, detecting method, and cutoff value. In our subgroup analysis, a significant association between Gankyrin overexpression and poor OS was observed in the “People’s Republic of China” subgroup (HR = 1.72, 95% CI: 1.40–2.11, P = 0.000), “digestive system cancers” subgroup (HR = 1.62, 95% CI: 1.05–2.49, P = 0.028), “IHC” subgroup (HR = 1.66, 95% CI: 1.20–2.29, P = 0.002), and “cutoff value median” subgroup (HR = 1.71, 95% CI: 1.29–2.27, P = 0.000; Table 2).

Table 1 Main characteristics of eligible studies in this meta-analysis

| First author | Year | Case nationality | Case number | Cancer type | Method | Cutoff | HR (95% CI) for OS | NOS score |
|-------------|------|-----------------|-------------|-------------|--------|--------|--------------------|-----------|
| Wang et al  | 2015 | People’s Republic of China | 166 | Non-small-cell lung cancer | IHC | Score ≥ 4 | 1.51 (1.02–2.24) | 8 |
| Wu et al    | 2015 | People’s Republic of China | 100 | Colorectal cancer | IHC | 5% | 3.21 (1.01–10.17) | 7 |
| Hwang et al | 2014 | Korea | 204 | Liposarcoma | IHC | Score ≥ 2 | 2.21 (1.22–4.00) | 6 |
| Jing et al  | 2014 | People’s Republic of China | 193 | Hepatocellular carcinoma | IHC | Median | 1.24 (0.85–1.82) | 8 |
| Zheng et al | 2014 | People’s Republic of China | 85 | Cholangiocarcinoma | IHC | Median | 2.11 (1.13–3.93) | 8 |
| Zheng et al | 2014 | People’s Republic of China | 124 | Gastric cancer | RT-PCR | Median | 2.26 (1.40–3.66) | 7 |
| Yang et al  | 2012 | People’s Republic of China | 138 | Glioma cancer | IHC | Score ≥ 3 | 2.50 (1.07–5.84) | 8 |
| Fu et al    | 2011 | People’s Republic of China | 201 | Hepatocellular carcinoma | IHC | Median | 1.74 (1.15–2.66) | 7 |
| Ortiz et al | 2008 | Japan | 72 | Esophageal cell carcinoma | IHC | 50% | 2.89 (0.70–11.91) | 7 |
| Umemura et al | 2008 | Japan | 43 | Hepatocellular carcinoma | IHC | 50% | 0.15 (0.04–0.64) | 7 |

Abbreviations: HR, hazard ratio; CI, confidence interval; OS, overall survival; NOS, Newcastle–Ottawa scale; IHC, immunohistochemistry; RT-PCR, real-time polymerase chain reaction.

Figure 2 A forest plot of HR for the relationship between high Gankyrin expression and OS.

Note: Weights are from random-effects analysis.

Abbreviations: HR, hazard ratio; OS, overall survival; ES, effect size; CI, confidence interval.
Gankyrin expression and clinicopathological parameters

As shown in Figure 3 and Table 3, increased Gankyrin was significantly associated with poorer tumor–node–metastasis (TNM) stage (RR =0.72, 95% CI: 0.60–0.86, P=0.000), positive lymph node metastasis (RR =1.66, 95% CI: 1.41–1.94, P=0.000), and positive distant metastasis (RR =1.43, 95% CI: 1.20–1.70, P=0.000), but not with sex (P=0.271) and histological grade (P=0.070). Owing to insufficient data, we did not detect the relationship between Gankyrin overexpression and other clinicopathological parameters.

Sensitivity analysis

Sensitivity analysis showed no significant changes in HRs when any individual study was discarded (Figure 4).

Publication bias

Egger’s test and Begg’s funnel plots were applied to assess publication biases. The P-value of Egger’s test for

### Table 2 Main results of subgroup analyses

| Categories     | Studies (n) | Number of patients | HR (95% CI) for OS | Heterogeneity |
|----------------|-------------|--------------------|--------------------|---------------|
| OS             | 10          | 1,326              | 1.73 (1.29–2.31)   | 54.60         |
| Nationality    |             |                    |                    |               |
| People’s Republic of China | 7           | 1,007              | 1.72 (1.40–2.11)   | 11.30         |
| Japan          | 2           | 115                | 0.66 (0.04–11.91)  | 88.30         |
| Cancer type    |             |                    |                    |               |
| Digestive system cancers | 7           | 818                | 1.62 (1.05–2.49)   | 66.30         |
| Methods        |             |                    |                    |               |
| IHC            | 9           | 1,202              | 1.66 (1.20–2.29)   | 56.00         |
| Cutoff value   | 4           | 603                | 1.71 (1.29–2.27)   | 32.40         |

Abbreviations: HR, hazard ratio; CI, confidence interval; OS, overall survival; P< sub h, P heterogeneity; IHC, immunohistochemistry.

Figure 3 (Continued)
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Table 3

| Categories                     | Studies (n) | Number of patients | RR (95% CI) | Heterogeneity |
|--------------------------------|-------------|--------------------|-------------|---------------|
|                                |             |                    |             | P (%)  | P_h  | P-value | Model       |
| Sex                            |             |                    |             |        |       |         |             |
| Male vs female                 | 8           | 960                | 0.94 (0.83–1.05) | 0.00   | 0.497 | 0.271   | Fixed effects |
| Histological grade             |             |                    |             |        |       |         |             |
| Well, moderate vs poor         | 5           | 570                | 0.77 (0.57–1.02) | 74.60  | 0.003 | 0.070   | Random effects |
| TNM stage                      |             |                    |             |        |       |         |             |
| I, II vs III, IV               | 8           | 960                | 0.72 (0.60–0.86) | 58.70  | 0.018 | 0.000   | Random effects |
| Lymph node metastasis          |             |                    |             |        |       |         |             |
| + vs –                         | 5           | 578                | 1.66 (1.41–1.94) | 0.00   | 0.965 | 0.000   | Fixed effects |
| Distant metastasis             |             |                    |             |        |       |         |             |
| + vs –                         | 6           | 815                | 1.43 (1.20–1.70) | 65.30  | 0.013 | 0.000   | Random effects |

Abbreviations: RR, relative risk; CI, confidence interval; P, P_h, P-value; TNM, tumor–node–metastasis.
OS ($P=0.946$) indicated no publication bias among these studies. The symmetric funnel plots also suggested no evidence of publication bias in this meta-analysis (Figure 5). Because of the limited number of included studies ($n<10$), the publication bias for clinicopathological parameters was not assessed.

**Discussion**

The Gankyrin gene is located on human chromosome Xq22.3 and encodes a protein with seven ankyrin repeats, which is required for protein–protein interactions.\(^1,2,5\) Gankyrin was considered to enhance tumor onset and progression by modulating the phosphorylation of Rb and the ubiquitylation of p53.\(^3\) In 2000, Higashitsuji et al identified the Gankyrin as a gene that was consistently overexpressed in hepatocellular cancer and then opened the investigative prolog in Gankyrin expression in malignant tumor. In the following years, Gankyrin have attracted much attention, especially in Asia. It was reported that Gankyrin was negatively or weakly expressed in normal tissues, but was highly expressed in various cancers.\(^10–19,26–30\) Although the focus area of the research is in digestive system cancers, the role of Gankyrin in other malignant tumors has received increasing attention in recent years. Several studies have found that Gankyrin might be an important prognostic marker for malignant cancers.\(^10–17\) However, some conflicting conclusions have been reported. Umemura et al\(^18\) came to the opposite conclusion that positive expression of Gankyrin might predict a better prognosis in hepatocellular cancer. Moreover, an insignificant result was obtained by Jing et al.\(^19\) Since the prognostic value of Gankyrin expression remained inconclusive, a meta-analysis was carried out to address the controversial issue.

To the best of our knowledge, the present analysis is the first meta-analysis to evaluate the role of Gankyrin as a cancer prognostic. Here, we identified ten studies including 1,326 cases. We observed that Gankyrin overexpression was associated with a poor prognosis in patients with cancer ($HR=1.73, 95\% CI: 1.29–2.31, P=0.000$). Additionally, the results revealed that elevated Gankyrin expression was related to TNM stage, lymph node metastasis, and distant metastasis. Based on the results, Gankyrin might act as...
a reliable biomarker for cancers and could help us to predict clinical outcomes of patients with cancer.

To explain the heterogeneity in OS, subgroup analyses were performed. It revealed that a high Gankyrin expression was related to poor prognosis in Chinese patients (HR =1.72, 95% CI: 1.40–2.11, P=0.000), but was not statistically significant in Japanese patients (HR =0.66, 95% CI: 0.04–11.91, P=0.776). Because most of patients enrolled in this meta-analysis were Chinese, it demonstrated that Gankyrin may play a greater role in Chinese patients. We deduced that the discrepancy might be caused by geno-type difference and the small sample size (only two studies with 115 Japanese patients were included). The results might not be representative, and more large size studies are necessary to enhance the reliability of the conclusion. The results also indicated that elevated Gankyrin expression was significantly associated with poor OS in digestive system cancers (HR =1.62, 95% CI: 1.05–2.49, P=0.028). In terms of methods for detection, we found that IHC was widely applied and considered as an effective method (HR =1.66, 95% CI: 1.20–2.29, P=0.002). In addition, we found that the heterogeneity of OS reduced in the “median” subgroup. This may be because different standards of cutoff values caused the heterogeneity. Significant heterogeneity was also found for the association between evaluated Gankyrin with clinicopathologic features and distant metastasis. The random-effects model was applied. In view of the limited number of studies (n=5, 8, 6, respectively), the subgroup analyses were not performed. The heterogeneity could be explained by the differences in characteristics of patients, detecting methods, cutoff values, antibodies, and so on. Therefore, to increase the reliability of the conclusions, more homogeneous samples are needed.

Although efforts were made to conduct this meta-analysis, some limitations should be acknowledged. First, most of the included studies focused on the digestive system cancers, and the small number of the studies focusing on each type of cancer might weaken our conclusions; thus, more efforts are needed in the future. Second, all the studies included were from Asian countries; hence, our conclusion might be more applicable to the Asian populations. It is difficult to make a reliable conclusion for other regions. Third, if the study did not provide HRs with 95% CIs, we had to extract data from Kaplan–Meier curves, and the calculated data might be less reliable. Fourth, although most of the studies detected the Gankyrin expression by IHC, the different antibody concentrations and the variable cutoff value might influence the results.

Conclusion

In conclusion, the meta-analysis indicated that Gankyrin overexpression was significantly associated with poor prognosis and might be a promising biomarker in predicting clinical outcomes for cancer patients, especially patients with cancers of the digestive system. Further clinical researches are needed to strengthen our conclusions and confirm the precise prognostic value of Gankyrin in cancers.

Disclosure

The authors report no conflicts of interest in this work.

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