NEDD9 overexpression predicts poor prognosis in solid cancers: a meta-analysis

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Background: The oncogenicity of neural precursor cell-expressed developmentally down-regulated 9 (NEDD9) has been demonstrated in multiple cancer types. However, the prognostic value of NEDD9 in some solid cancers remains controversial. Thus, this meta-analysis was conducted to evaluate the relationship between NEDD9 expression survival rates in solid tumors.

Method: Our meta-analysis included studies searched from various search engines with specific inclusion criteria and exclusion criteria. Combined HRs for overall survival (OS) and disease-free survival (DFS) or progression-free survival (PFS) or recurrence-free survival (RFS) or cancer-specific survival (CSS) were assessed using fixed-effects and random-effects models. The source of heterogeneity was identified by subgroup analysis. Additionally, publication bias was assessed using funnel plot and Egger’s regression asymmetry test.

Result: Eighteen studies with a total of 2,476 patients were retrieved for analysis. Pooled HRs and 95% CIs were calculated. Both OS (HR=1.82; 95% CI: 1.43–2.31) and DFS/PFS/RFS/CSS (HR=2.54; 95% CI: 1.93–3.33) indicated that NEDD9 overexpression is associated with poor OS in cancer patients with solid tumors.

Conclusion: NEDD9 overexpression might be a potential marker to predict prognosis in solid cancer patients.

Keywords: NEDD9, solid cancer, prognosis, meta-analysis

Introduction

Epidemiological data show that cancers have become one of the world’s leading causes of mortality.1 From the latest data, solid cancers accounted for over 90% of all types of cancers and the top five of new cases and deaths are lung cancer, breast cancer, prostate cancer, colon cancer and nonmelanoma skin cancer.1 Solid cancers are characterized by malignant tumors that form a discrete tumor mass. By contrast, lymphoproliferative malignancies diffusely infiltrate tissue without forming a mass. Although diagnosis of number of molecular markers and targeted therapies progressed with the efforts of many researchers and clinicians, the outcomes including death rates and overall survival (OS) of the majority of patients remain poor. Thus, more predictive molecular markers of solid cancers must be identified for prevention and individualized cancer treatment.

Neural precursor cell-expressed developmentally down-regulated 9 (NEDD9), also known as human enhancer of filamentation 1 (HEF1) and Crk-associated substrate lymphocyte type (Cas-L), belongs to the Crk-associated substrate family. NEDD9 coordinates the focal adhesion kinase and SRC signaling cascades that are involved in integrin-dependent adhesion and migration, invasion, cell apoptosis
and life cycle, and survival. NEDD9 overexpression contributes to solid cancer metastasis in lung, liver, breast, ovarian, colon, glioblastoma carcinoma, and cervical cancers. \textsuperscript{3,7–13} NEDD9 expression levels are a biomarker of cancer aggression and could be a prognostic factor of solid cancers. \textsuperscript{14} However, prognostic evidence is lacking due to studies with limited sample sizes or cancer types. This is why we evaluated the prognostic value of NEDD9 in solid tumors through meta-analysis to elucidate the clinical implications.

Methods
The meta-analysis was performed according to the PRISMA guideline and the statement for reporting systematic reviews and meta-analyses. \textsuperscript{15} Previously published studies were summarized and analyzed in this study (ethics board approval was not necessary).

Search strategy and study eligibility
We retrieved studies that measured NEDD9 expression and survival in solid cancer patients between 1995 and January 2019 from PubMed, EMBASE, Web of Science, and Google Scholar. The search terms included “NEDD9” or “neural precursor cell-expressed developmentally down-regulated 9” or “HEF1” or “human enhancer of filamentation 1” or “Cas-L” or “Crk-associated substrate lymphocyte type” and “neoplasms” or “cancer” or “tumor” or “malignancy” or “carcinoma” and “prognosis” or “survival”. Non-English language studies were excluded. Results were restricted to human studies of solid cancer and 402 entries were found.

Inclusion criteria consisted of an evaluation of NEDD9 overexpression associated with OS (date of surgery to date of death as a result of any cause), disease-free survival (DFS) or progression-free survival (PFS) or recurrence-free survival (RFS) or cancer-specific survival (CSS), and immunohistochemistry (IHC) or ELISA analysis of NEDD9 expression. Tumors were classified by NEDD9 expression levels using the cutoffs defined in the studies (Table 1). All references of the included studies were scanned and studies of potential interest were reviewed for further analysis. Reviews, clinical endpoints other than OS or DFS/PFS/RFS/CSS, cutoff value for over/normal NEDD9 expression, histological type, and HR estimation. Some HR data were extracted from tables or Kaplan–Meier curves of NEDD9 overexpression and normal expression groups. We used the Newcastle–Ottawa Non-Randomized scale (NOS) as recommended by the Cochrane Non-Randomized Studies Methods Working Group for a quality assessment of the cohort studies. \textsuperscript{16,17} The NOS score ranged from 0 to 9 based on three board perspectives: study group selection, study group comparability, and ascertainment of the outcome of interest. A consensus NOS score for each study was obtained by a discussion between all investigators. Studies with a score ≥5 had a high-quality methodology.

Statistical analysis
Data were extracted from the primary publications and analyzed with Stata 12 (Stata Corporation, College Station, TX, USA). The HR and corresponding 95% CI estimates were calculated and pooled to determine the association of NEDD9 overexpression with OS and DFS/PFS/RFS/CSS. An HR>1 indicated a poor prognosis in patients with NEDD9 overexpression. The random-effects model and the fixed-effects model were conducted according to DerSimonian and Laird’s method and Mantel-Haenszel’s method, respectively. \textsuperscript{18,19} Statistical intrastudy heterogeneity was evaluated by the \( I^2 \) value to quantify the proportion of the total variation. The \( I^2 \) values of 25%, 50%, and 75% were the cutoff points of low, moderate, and high heterogeneity, respectively. \textsuperscript{20} We used the fixed-effects model to pool the results if relatively low or moderated heterogeneity existed (\( I^2 \leq 50\% \)). The random-effects model was used when the \( I^2 \) value was \( \geq 50\% \). If high or moderate heterogeneity existed, a subgroup analysis of the cancer characteristics was conducted to determine possible causes. \textsuperscript{21} Differences between the subgroups were calculated from the Cochrane Handbook for Systematic Reviews of Interventions. Outcome credibility was validated by sensitivity analysis.
| Study     | Country  | Tumor type | Case number | Age (years) | Gender (M/F) | TNM stage (I/II/III/IV) | Follow-up (months) | Detected method | Survival analysis | Cut-off value | Multivariate analysis | HR  |
|-----------|----------|------------|-------------|-------------|--------------|-----------------------|-------------------|----------------|-------------------|--------------|----------------------|-----|
| Afsar 2018 | Turkey   | Pancreatic cancer | 32          | Media 61    | 20/12        | NR                   | Max 27            | ELISA           | OS/PFS            | NR           | No                   | SC  |
| B-Babouly 2018 | Egypt | Bladder cancer | 105         | Mean 57     | 69/36        | 32/73 (I-II/III-IV)  | Mean 25           | IHC             | PFS              | Hs ≥4        | No                   | SC  |
| Erturk 2018 | Turkey   | Melanoma   | 112         | Mean 52     | NR           | Mean 20.8           | ELISA             | OS              | NR               | No Report    | SC                   |     |
| Harb 2017  | Egypt    | Bladder cancer | 50          | Mean 52     | 35/15        | 24/26 (T1-T2-T3)    | Max 35            | IHC             | OS               | Hs >4        | Yes Report           |     |
| Karabulut 2015 | Turkey | Gastric cancer | 68          | Media 60    | 49/19        | NR                   | Mean 8            | ELISA           | OS/PFS           | NR           | No                   | SC  |
| Kondo 2012  | Japan    | Lung cancer  | 60          | Mean 62.5   | 26/34        | 31/5/23/1 (I/II/III/IV) | 0–90             | IHC             | OS/RFS           | >30% of nuclear stained | Yes Report       |     |
| Li 2014    | China    | Colorectal cancer | 92          | Mean 62     | 52/40        | 15/30/37/10 (I/II/III/IV) (UICC stage) | 5–60            | IHC             | OS               | Hs >4        | Yes Report           |     |
| Li 2016    | China    | Breast cancer | 226         | Median 56   | 0/226        | 79/83/53/1 (I/II/III/IV) | Median 27       | IHC             | OS/DFS           | Hs ≥4        | Yes Report           |     |
| Liu 2014   | China    | Gastric cancer | 187         | NR          | 104/83       | 77/110 (I-II/III-IV) | 0–60            | IHC             | OS               | >25% of nuclear stained | No SC         |     |
| Lu 2015    | China    | Hepatocellular cancer | 164       | Median 49   | 133/31       | 32/73 (I-II/III-IV) (UICC stage) | 0–60            | IHC             | OS               | Hs ≥4        | Yes Report           |     |
| Mao 2013   | China    | Lung cancer  | 105         | Mean 60.4   | 63/42        | 46/59 (I-II/III-IV) (UICC stage) | 0–60            | IHC             | OS               | Positive cell score ≥3 | No SC         |     |
| Ostojc 2018 | Croatia | Lung cancer  | 71          | Median 492  | 112/53       | 55/110 (I-II/III-IV) (UICC stage) | 0–125           | IHC             | OS               | Positive cell score ≥3 | No SC         |     |
| Shi 2014   | China    | Gastric cancer | 125         | NR          | 61/64        | 73/52 (I-II/III-IV) | 0–60            | IHC             | OS               | Hs >4        | Yes Report           |     |
| Wang 2014  | China    | Ovarian cancer | 129         | NR          | 0/129        | 73/52 (I-II/III-IV) (FIGO stage) | 0–60            | IHC             | OS/PFS           | Nuclear staining | Yes Report       |     |
| Wang 2017  | China    | Renal cancer  | 68          | NR          | 69/39        | 53/15 (I-II/III-IV) | 0–60            | IHC             | CSS              | Hs ≥5        | No                   | SC  |
| Xue 2013   | China    | Pancreatic cancer | 106        | NR          | 65/41        | 54/52 (I-II/III-IV) | 0–60            | IHC             | OS               | Hs ≥4        | No                   | Report |

(Continued)
and the publication bias was assessed by visual inspection of the funnel plots. Funnel plot asymmetry was determined by Egger’s linear regression test. A two-sided \( P < 0.05 \) was considered statistically significant.

## Results

### Search results and study characteristics

Eighteen studies were eligible for the pooled meta-analysis. Eligible studies were published between 2012 and February 2019 with a total of 2476 patients. A flowchart depicting the selection of literature is shown in Figure 1.

All included studies were retrospectively analyzed. The sample size ranged from 32 to 601 (median 137). NEDD9 overexpression was reported in 1309 (52.9%) of the 2476 included patients. Amongst all study cohorts, 3 evaluated lung cancer, \(^{23,27,33} \) 4 assessed gastric cancer, \(^{28,30,32,39} \) 3 undertook urinary bladder carcinoma, \(^{31,34,36} \) 2 illustrated pancreatic carcinoma, \(^{29,38} \) and single studies focused on breast cancer, \(^{25} \) colorectal cancer, \(^{34} \) hepatocellular carcinoma, \(^{26} \) renal carcinoma, \(^{37} \) melanoma, \(^{35} \) and ovarian cancer. \(^{3} \) Studies were conducted in China (11), Japan (1), Turkey (3), Croatia (1), and Egypt (2). The Newcastle–Ottawa Quality Assessment Scale indicated that the quality scores ranged from 6 to 8 (median 7) with a relatively high study quality. The main characteristics of the 18 eligible publications are reported in Table 1. Seven studies \(^{8,23,25,32,36,38} \) reported that the HR of DFS/PFS/RFS/CSS associated with NEDD9 expression. Sixteen studies \(^{8,23,33,38} \) included OS and a follow-up time of 8–125 months. The HR and 95% CI were directly recorded in 11 studies. \(^{8,23,26,28,31,34,35} \) HR was inferred from the remaining studies using the Tierney method to estimate HR from Kaplan–Meier survival curves.

### Meta-analysis results

Moderate intrastudy heterogeneity was observed \((I^2=58.4\%)\) in 16 studies where OS was determined by univariate analysis or multivariate analysis. The random-effects model was used to pool the results. The combined HR estimate of OS was 1.82 (95% CI: 1.43–2.31; \( P < 0.01 \)). NEDD9 overexpression was associated with poor OS (Figure 2).

Studies were divided into five groups by cancer location for subgroup analysis: (1) digestive system cancer \((n=8);^{24,26,28,30,32,38,39} \) (2) respiratory system cancer \((n=3);^{23,27,33} \) (3) breast and ovarian cancer \((n=2);^{8,25} \) (4) urinary system cancer \((n=2);^{31,34} \) and (5) melanoma \((n=1);^{35} \) The

### Table 1 (Continued).

![Table showing study characteristics](link)
combined HR estimate for OS in the digestive system cancer group was 1.61 (95% CI: 1.32–1.95; $I^2=8.4\%$). The HR estimate for OS in the respiratory system cancer group was 1.96 (95% CI: 0.68–5.70; $I^2=85\%$), 3.96 (95% CI: 2.26–6.94; $I^2=0\%$) for breast and ovarian cancer groups, and 2.44 (95% CI: 1.54–3.86; $I^2=0\%$) for urinary system groups.

Figure 1 Flow diagram of the study selection process.

Figure 2 Forest plot of NEDD9 overexpression with overall survival.
There was no apparent intrastudy heterogeneity ($I^2=0\%$) among 7 studies where DFS/PFS/RFS/CSS was determined by univariate analysis. The fixed-effects model was used to pool the results. The combined HR estimate was 2.54 (95% CI: 1.93–3.33; $P=0.142$), which indicated that NEDD9 overexpression was associated with poor DFS/PFS/RFS/CSS (Figure 3). The studies were subdivided by cancer location: digestive system (n=2),$^{23}$ respiratory system (n=1),$^{23}$ breast and ovarian cancer (n=2),$^{8,25}$ and urinary system cancer (n=2).$^{36,37}$ The combined HR estimate for DFS/PFS/RFS/CSS were 1.71 (95% CI: 1.11–2.64; $I^2=0\%$) in the group digestive system cancer and 2.59 (95% CI: 1.56–4.28; $I^2=0\%$) in the group breast and ovarian cancers. The HR estimate for DFS/PFS/RFS/CSS in urinary cancer was 4.03 (95% CI: 2.15–7.55; $I^2=45.9\%$).

**Publication bias**

Funnel plot and Egger’s test were used to evaluate the publication bias. The shape of the funnel plots did not show obvious asymmetry for OS determined by univariate analysis or multivariate analysis (Figure 4). Egger’s test also failed to provide evidence of significant publication bias. Publication bias was also investigated for DFS/PFS/RFS/CSS obtained by univariate analysis and the funnel plots showed basic symmetry (Figure 5). Our results suggested that publication bias was not evident.

**Discussion**

Regarding prognostic value of level of NEDD9 and survival rates of solid tumors patients, we use the tool of meta-analysis to demonstrate that the high expression of NEDD9 acts as a prediction of the poor outcome for patients with solid tumors.

NEDD9 contains multiple functional modules for protein interaction, yet lacks any known enzymatic function. Adult lung and kidney tissues, which are rich in immature lymphoid cells, as well as the fetal brain, contain the highest levels of NEDD9.$^{40–42}$ NEDD9 is crucial in
integrin-dependent signaling cascades to activate cell migration and is involved in lateral communication to Ras signaling cascades. NEDD9 can drive mesenchymal-type movement by activating RAC2 GTPase and WAVE in complex with GEF DOCK3 to inhibit GTPase Rho and amoeboid movement. Invasion is accompanied by ECM proteolysis through activation of MMP14, MMP2, and MMP9 metalloproteinases. 

Figure 4 Funnel plots for OS.
Abbreviation: OS, overall survival.

Figure 5 Funnel plots for DFS/PFS/RFS/CSS.
Abbreviations: DFS, disease-free survival; PFS, progression-free survival; RFS, recurrence-free survival; CSS, cancer-specific survival.
transition (EMT) is a transdifferentiation strategy where carcinoma cells acquire the ability to execute the steps of invasion-metastasis cascade.\textsuperscript{45} Epithelial cells express mesenchymal markers and cytoskeletal changes that increase motility and invasiveness during EMT. NEDD9 also interacts with key proteins to coordinate the signaling cascades controlling cell cycle transitions, apoptosis, migration, and invasion.\textsuperscript{43,46,47} NEDD9 expression has been linked to tumorigenesis of various malignancies, including cancers of the breast,\textsuperscript{25} colon,\textsuperscript{25,48,49} pancreas,\textsuperscript{29} head and neck,\textsuperscript{50} ovaries,\textsuperscript{8} gastric,\textsuperscript{24,30,32} lung,\textsuperscript{23,27,51} liver,\textsuperscript{52} kidney,\textsuperscript{53} glioblastoma,\textsuperscript{54} and neuroblastoma.\textsuperscript{55} However, some studies have the opposite point of view. One example is a study from Ostojić et al (2018) that is also included in our meta-analysis.\textsuperscript{33} It is interesting that they concluded the relationship between NEDD9 expression and survival did not reach statistically significant difference and there was a trend toward the correlation of higher NEDD9 to longer survival. The explanation is that expression of NEDD9 over certain level inevitably leads to apoptosis.\textsuperscript{56} Furthermore, it is also possible that elevated expression of NEDD9 may indicate better responses to therapy.\textsuperscript{23} Although there are some different opinions, our findings through meta-analysis suggested that elevated NEDD9 expression was correlated with poor survival in most solid cancers. Further studies are needed to elucidate the mechanisms of NEDD9 in cancer pathogenesis.

The prognostic value of NEDD9 in solid cancer is controversial despite previous investigations of this marker in multiple cancers. This is the first meta-analysis to analyze NEDD9 overexpression in relation to OS or DFS/PFS/RFS/CSS of solid cancer patients. We systematically evaluated the survival data of 2393 solid cancer patients from 18 eligible studies. We demonstrated that NEDD9 overexpression was associated with poor survival in solid cancer patients. The detrimental effect of NEDD9 overexpression on survival was statistically significant in OS or DFS/PFS/RFS/CSS. NEDD9 could be a prognostic biomarker and novel therapeutic target of solid cancer.

Several limitations should be considered when interpreting the findings of our meta-analysis. Differences among dilution solubility, antibodies, and cutoff values influenced the assessment of NEDD9 overexpression. A large multicenter clinical study using consistent antibodies and cutoff values is needed to validate our results. Language bias may exist in our meta-analysis because the search strategy was limited to English. Most eligible studies included Asian populations, especially Chinese, leading to population homogeneity. Some studies did not report HR and 95% CI directly. Data extracted by Tierney’s methods may introduce bias to the original data.

Conclusion

Here, we provide evidence that NEDD9 overexpression is associated with poor OS or DFS/PFS/RFS/CSS in solid cancer patients. Further investigation is required to elucidate the role of NEDD9 overexpression and provide new insights into tumor metastasis and progression for the development of target-specific therapies.

Author contributions

Conception/Design: Fei Zheng. Provision of study materials: Yang Gu, Jingjing Lu, Chen Chen. Collection and extract data: Yang Gu, Jingjing Lu. Data analysis and statistical guidance: Yang Gu. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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