

Original Research

Prognostic value of protein tyrosine kinase 6 overexpression in cancers: a meta-analysis

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Abstract

Background: Protein tyrosine kinase 6 (PTK6) plays an important role in cell proliferation and differentiation. However, the functions of PTK6 appear highly context-dependent and differ depending on the cell type, as well as its intracellular localization. High PTK6 expression in tumor has been associated with poor pathological features and prognosis in some studies, but other studies have reported opposite results. Therefore, we performed this meta-analysis to derive more precise estimations of the association of PTK6 expression with prognosis and clinicopathological features in cancer patients. **Methods:** We conducted a literature search in PubMed, Ovid MEDLINE, and MEDLINE databases to cover all articles published until June 2021. All 1475 patients from the eight studies were included in the meta-analysis. Because of heterogeneity in PTK6 expression in non-tumor tissues, the included studies were divided into two subgroups according to PTK expression in non-tumor tissues: the low expression subgroup (LESG) or high expression subgroup (HESG). **Results:** Patients with high PTK expression showed significantly worse overall survival (OS) in LESG (Hazard Ratio (HR) = 2.53 [95% Confidence Interval (CI), 1.68–3.83], $p < 0.0001$), but significantly better OS in HESG (HR = 0.56 [95% CI, 0.40–0.78], $p = 0.0006$). PTK6 expression also showed different associations with clinicopathological features, such as advanced T classification, stage, and differentiation according to PTK6 expression in non-tumor tissues. **Conclusions:** PTK6 expression in tumor was a prognostic factor in patients with various cancers, but the direction of prognosis differs, depending on the degree of PTK6 expression in non-tumor tissues.

Keywords: PTK6; brk; prognosis; meta-analysis

1. Introduction

Protein tyrosine kinase 6 (PTK6), a member of a distinct family of non-receptor tyrosine kinases closely related to Src kinases, plays an important role in cell proliferation and differentiation by transferring signals from cell surface receptors to intracellular targets [1,2].

The PTK6 protein consists of a tyrosine kinase domain, along with SH2(Src-homology) and SH3 domains, of which SH3 seems more important for the regulation of catalytic activity [3]. Growing evidence suggests that PTK6 is involved in a variety of tissues and cancers and interestingly, the functions of PTK6 seem to depend on the cell types and its intracellular localization [4].

PTK6 expression has been detected in a variety of normal epithelial linings, which are generally well-differentiated cells. PTK6 is expressed in the gut, prostate, skin, and oral epithelium. Many *in vitro* and *in vivo* studies indicate that PTK6 promotes the differentiation of epithelial cells and modulates the survival of normal cells. PTK6 ex-

pression induces the differentiation of cultured human keratinocytes and the expression of epidermal differentiation markers, while ectopic overexpression of PTK6 in immortalized cell lines promote apoptosis [5].

PTK6 expression was first identified in metastatic breast cancer [6–9] and subsequently detected in several other cancers of the ovary [10], head and neck [11,12], lung [13,14], esophagus [15], cervix [16], bladder [17], pancreas [18] and stomach [19].

High PTK6 expression has been associated with poor pathological features and prognosis in some studies, but other studies have reported opposite results. Therefore, this meta-analysis, including all eligible studies, was performed to derive more precise estimations of the association of PTK6 expression with prognosis and pathological features in cancer patients.



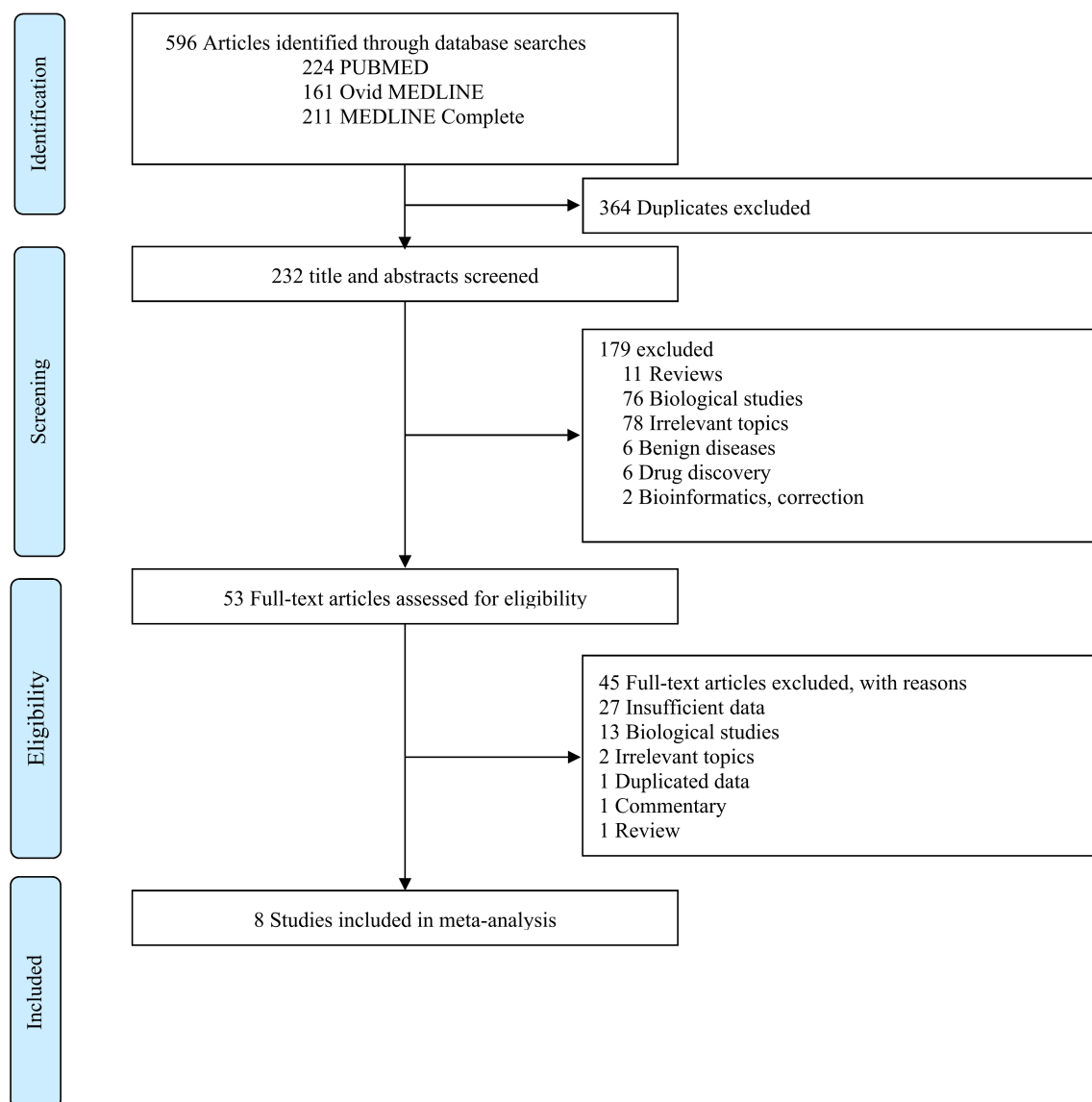


Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram showing the search process of studies included in this meta-analysis.

2. Materials and methods

2.1 Literature search and inclusion criteria

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [20]. We conducted a literature search in PubMed, Ovid MEDLINE, and MEDLINE databases to cover all articles published until June 2021, with the following search terms in their titles, abstracts or keyword lists: ‘PTK6 or Protein tyrosine kinase 6 or Brk or Breast tumor kinase’ and ‘cancer or carcinoma or malignancy or tumor or neoplasm’. Furthermore, we inspected the reference lists of the relevant articles to identify additional articles. We evaluated all potentially relevant articles by examining their titles and abstracts. Subsequently, the full text of eligible articles was screened to retrieve all articles that met the inclusion criteria.

Studies that discussed the following points were included in this meta-analysis: (i) evaluation of PTK6 expression by immunohistochemical (IHC) staining; (ii) relationships between PTK6 expression level and clinicopathological features of the patients or sufficient published data to estimate the hazard ratio (HR) for survival; and (iii) articles written in English.

The collected characteristics of the included studies were as follows: first author, year of publication, number of patients, clinicopathological features (lymph node [LN] involvement, stage, T classification, and pathological grade), and survival outcomes (overall survival [OS] and disease-free survival [DFS]) along with their HRs with 95% confidence intervals (CIs). When HR values of both univariate and multivariate analyses were available, the data from the multivariate analysis were selected preferentially.

Two investigators independently extracted data from each eligible study using a standard data collection form and assessed the risk of bias. Discrepancies were resolved through discussion.

2.2 Statistical analysis

Pooled odds ratios (ORs) with 95% CIs were calculated to assess the association between PTK6 expression level and clinicopathological features such as LN metastasis, advanced stage, T classification, and pathological grade. HRs with 95% CIs were estimated using statistical values directly extracted from the original articles. If HRs with their 95% CIs were not provided, they were estimated from the Kaplan-Meier curves using the plotdigitizer software. The significance of the OR and HR was determined using the Z-test. Statistical significance was set at $p < 0.05$.

The traditional Q -test and I^2 statistic were used to evaluate heterogeneity. A p value ≥ 0.10 for the Q -test or $I^2 \leq 50\%$ indicates that there was no significant heterogeneity among the studies, and the fixed-effect model (Mantel-Haenszel method) was used. However, the random-effects model (DerSimonian-Laird method) was adopted when significant heterogeneity was observed ($p < 0.10$, $I^2 > 50\%$).

Publication bias was assessed using Begg's test and Egger's linear regression test [21,22]. Statistical significance was determined by t -test, and a p value of <0.05 , which was representative of significant publication bias.

3. Results

3.1 Results of the literature search

Fig. 1 shows a flow diagram illustrating the literature search process. Altogether, 596 potentially relevant articles were initially identified, of which 364 duplicates were removed. Of the remaining articles, 179 were excluded by screening the titles and abstracts. The full text of the remaining 53 potentially eligible studies was reviewed, and 45 articles were further excluded: 27 articles did not present appropriate data for meta-analysis; 13 articles were biological studies and the data of one study [8] was duplicated in another article by the same authors [9]. Finally, eight studies were included in the meta-analysis [9,11–17].

3.2 Characteristics of the included studies

The main characteristics and clinicopathological findings of the selected studies are presented in Table 1 (Ref. [9,11–17]). Most of the studies were retrospective. All 1475 patients from the eight studies were included in the meta-analysis. All patients were surgically treated, except for nasopharyngeal cancer patients [12] and none of the patients received neoadjuvant treatment. All studies used immunohistochemistry (IHC) to assess PTK6 expression status and provided the criteria for PTK6 expression status, although slightly different cutoff values were adopted to define PTK6 expression. IHC staining was performed on non-tumor tissues as a reference in all the studies. Breast cancer

patients received adjuvant hormonal therapy, radiotherapy, and/or chemotherapy when indicated [9] and curative radiotherapy with or without chemotherapy was administered to patients with nasopharyngeal cancer [12].

3.3 PTK6 expression in tumor tissues and non-tumor tissues

The rates of high PTK6 expression varied, ranging from 29.1% to 68.8%. Non-tumor tissues from the larynx and esophagus showed high expression of cytoplasmic PTK6 [11,15], while other non-tumor tissues were stained less than tumor tissues.

3.4 Impact of PTK6 expression on clinicopathological features

As mentioned above, non-tumor tissues showed heterogeneity in PTK6 expression. Therefore, the included studies were divided into two subgroups according to PTK expression in non-tumor tissues: the low expression subgroup (LESG) or high expression subgroup (HESG), and subgroup analysis was thus performed.

From seven studies [11–17], 1049 patients were included in the meta-analysis of ORs with 95% CIs for LN metastasis and T classification. There was no significant association between PTK6 expression and LN metastasis (OR = 1.10 [95% CI, 0.59–2.06], $p = 0.76$) (Supplementary Fig. 1), but a positive correlation between high PTK expression and advanced T classification was observed in the whole group (OR = 1.85 [95% CI, 1.23–2.77], $p = 0.003$) and LESG (OR = 2.35 [95% CI, 1.69–3.28], $p < 0.00001$) (Supplementary Fig. 2).

From four studies [11–15], 748 patients were included in the meta-analysis of ORs with 95% CIs for tumor stage (I, II vs. III, IV). Compared with low PTK6 expression, tumors with high PTK6 expression showed significantly lower rates of advanced stages in HESG (OR = 0.60 [95% CI, 0.38–0.95], $p = 0.03$), while no significant association was observed in LESG (OR = 2.23 [95% CI, 0.85–5.85], $p = 0.10$) (Supplementary Fig. 3).

For pathological grade, tumors with high PTK6 expression showed a significantly lower rate of advanced pathological grade in HESG (OR = 0.13 [95% CI, 0.04–0.44], $p = 0.001$) (Supplementary Fig. 4). The results are summarized in Table 2.

3.5 Impact of PTK6 expression on survival

From five studies [11,12,15–17], 823 patients were included in the meta-analysis of HRs with 95% CIs for OS. There was no correlation between PTK expression and overall survival (HR = 1.41 [95% CI, 0.61–3.25], $p = 0.42$); however, it became apparent when the analysis was restricted to subgroup: patients with high PTK expression showed significantly worse OS in LESG (HR = 2.53 [95% CI, 1.68–3.83], $p < 0.0001$) (Fig. 2A), but significantly better OS in HESG (HR = 0.56 [95% CI, 0.40–0.78], $p = 0.0006$) (Fig. 2B).

Table 1. Characteristics of the included studies.

Study	Primary cancer	Criteria for PTK6 overexpression	N (%) of PTK6 over-expression	LN metastasis (high vs. low)	Stage 3/4 (high vs. low)	T classification (high vs. low)	Histologic grade (high vs. low)	HR for OS	HR for DFS	PTK6 expression in non-tumor tissues
Aubele (2008) [9]	Breast	0 (no staining); 1+ (light); 2+ (moderate); 3+ (strong) - High expression: 2+/3+ - Low expression: 0/1+	293/426 (68.8%)	NA	NA	NA	NA	NA	0.681 (0.468–0.992) $p = 0.045$	Negative
Fan (2011) [14]	Lung	IHC scoring (0–12): proportion of immunopositive cells (0–4) \times staining intensity (0–3) - PTK-positive : 2–12 - PTK-negative: 0–1	65/122 (53.3%)	38/65 vs 16/57	25/65 vs 11/57	46/65 vs 27/57	55/64 vs 42/56	NA	NA	Less than cancer cells
Zhao (2013) [13]	Lung	Staining index (0–9): Staining intensity (0–3) \times percentage of positive cells (0–3) - High expression: >median staining index value - Low expression: <median staining index value	51/104 (49.0%)	23/51 vs 25/53	15/51 vs 17/53	8/51 vs 6/53	19/51 vs 20/53	NA	NA	Negative
Liu (2013a) [11]	Larynx	Expression score (0–7): Staining intensity (0–3) + Proportion of immunopositive cells (0–4) - High expression: 6–7 - Medium expression: 3–5 - Low expression: 1–2	39/134 (29.1%)	10/98 vs 12/36	42/98 vs 20/36	40/98 vs 13/36	40/98 vs 33/36	0.48 (0.22–1.03) $p = 0.06$	NA	Positive
Liu (2013b) [12]	Nasopharynx	Expression score (0–7): Staining intensity (0–3) \times Proportion of immunopositive cells (0–3) - High expression: IRS > 5.0 - Low expression: IRS \leq 5.0	113/178 (63.5%)	55/113 vs 31/65	102/113 vs 43/65	88/113 vs 32/65	NA	2.038 (1.051–3.951) $p = 0.035$	NA	Negative
Chen (2014) [15]	Esophagus	Immunoreactivity score (IRS): Staining intensity (0–3) \times percentage of positive cells (0–4) - PTK-positive: IRS 5–12 - PTK-negative: IRS 0–4	104/210 (49.5%)	40/104 vs 53/106	32/104 vs 45/106	72/104 vs 72/106	11/104 vs 37/106	0.579 (0.402–0.835) $p = 0.003$	0.557 (0.391–0.795) $p = 0.001$	Positive
Wang (2016) [16]	Cervix	Immunoreactivity score (0–12): Staining intensity (0–3) \times percentage of positive cells (0–4) - High expression: Immunoreactivity score: 7–12 - Low expression: Immunoreactivity score: 0–6	69/150 (46.0%)	20/69 vs 21/81	NA	20/69 vs 21/81	45/69 vs 61/81	5.999 (1.622–22.191) $p = 0.0073$	NA	Negative
Xu (2017) [17]	Bladder	Expression score (0–7): Staining intensity (1–4) \times Proportion of immunopositive cells (0–4) - High expression: Immunoreactivity score: >8.5 - Low expression: Immunoreactivity score: 1–8.5	56/151 (37.1%)	18/56 vs 13/95	NA	39/56 vs 42/95	40/56 vs 59/95	2.527 (1.416–4.510) $p = 0.0017$	NA	Negative

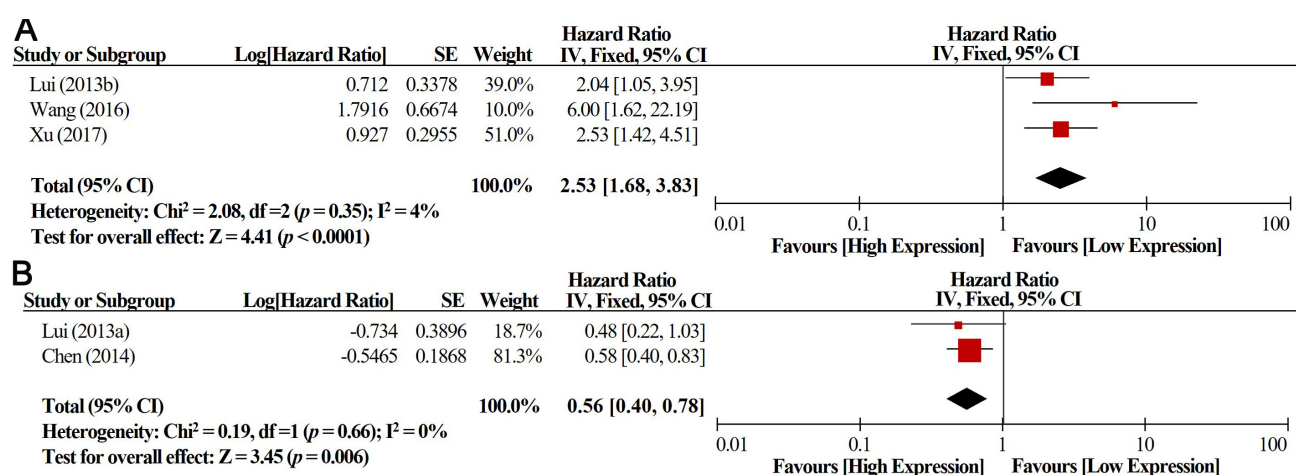


Fig. 2. Forest plots of hazard ratio for overall survival. (A) Low expression subgroup; (B) High expression subgroup.

Table 2. Odds ratios for clinicopathological features.

	Low expression subgroup (LESG)	High expression subgroup (HESG)	All included
LN metastasis	1.64 (0.93–2.90) $p = 0.09$	0.41 (0.15–1.09) $p = 0.07$	1.10 (0.59–2.06) $p = 0.76$
Advanced stages	2.23 (0.85–5.85) $p = 0.10$	0.60 (0.38–0.95) $p = 0.03$	1.29 (0.57–2.92) $p = 0.54$
T classifications	2.35 (1.69–3.28) $p = 0.00001$	1.12 (0.70–1.78) $p = 0.65$	1.85 (1.23–2.77) $p = 0.003$
Pathologic grades	1.11 (0.76–1.62) $p = 0.61$	0.13 (0.04–0.44) $p = 0.001$	0.57 (0.24–1.39) $p = 0.22$

Meta-analysis of HRs with 95% CIs for DFS was performed in only two studies of 636 patients. Compared to low PTK6 expression, high PTK6 expression in cancer tissues was associated with improved DFS (HR = 0.61 [95% CI, 0.47–0.79], $p = 0.0002$) (Fig. 3).

3.6 Publication bias

Begg's test and Egger's test were conducted for all ORs and HRs, and none of the p values of either test were less than 0.05, indicating that there were no substantial publication biases.

4. Discussion

PTK6 expression in normal tissues is detected in the gut, prostate, skin, and oral epithelium. However, PTK6 is not expressed in the normal mammary glands. Among the studies included in this meta-analysis, non-tumor tissues of the larynx and esophagus showed high expression of PTK6, while it was not detected in non-tumor tissues of the breast, lung, nasopharynx, cervix, and bladder.

PTK6 is implicated in the regulation of various signaling pathways controlling the differentiation and maintenance of normal tissues, as well as tumor growth [3]. PTK6 also appears to modulate the survival of normal epithelial cells. However, its functions in tumorigenesis and metastasis are not fully understood; recent findings suggest that PTK6 has opposing functions in normal and cancer tissues [3]. Moreover, the functions of PTK6 appear highly context-dependent and differ depending on the cell type, as well as its intracellular localization.

In this meta-analysis, the included studies were divided into two subgroups according to PTK6 expression in non-tumor tissues: LESG (low) and HESG (high). Although PTK expression in cancer tissues was not prognostic in the meta-analysis including all studies, it became a significant prognostic factor in subgroup analysis: high PTK6 expression was associated with better prognosis in HESG and worse prognosis in LESG.

This discrepancy may originate from the unique mechanism of PTK6 function. It could be postulated that the overexpression of PTK6 could promote tumor differentiation and play an inhibitory role in tumor progression in HESG [11,23]. In addition, the loss of PTK6 in non-tumor tissues may be a critical event in cancer transformation [11]. On the other hand, PTK6 seems to function as a proto-oncogene in tumor tissues and is directly involved in proliferation, migration, and invasion in cancer cells of LESG [24]. It has been suggested that PTK6 interacts with ErbB family members, especially HER3 and HER4, and enhances EGF-induced proliferation and ERK1/2 activation in breast cancer tissues [8,9,13].

In the meta-analysis of ORs for pathological features, high PTK6 expression was associated with a lower incidence of advanced stage and high histologic grade in HESG, whereas there was an increased risk of T3/T4 classification in LESG. These findings may also support the hypothesis that PTK6 plays an inhibitory role in normal tissues but promotes the progression of cancer transformation in tumor tissues.

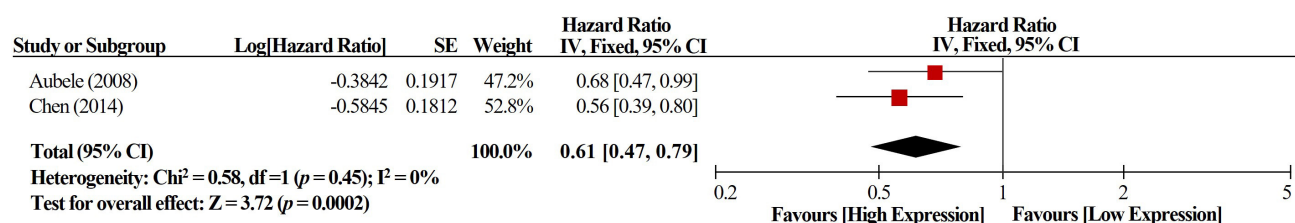


Fig. 3. Forest plot of hazard ratio for disease-free survival.

This meta-analysis had several limitations. This meta-analysis included only a small number of studies currently available, even though a thorough systemic review was performed. There was significant heterogeneity among the studies in the meta-analysis of ORs for pathological features. A random-effects model was used to minimize its effect on the results; however, the pooled ORs may nevertheless be affected.

5. Conclusions

To the best of our knowledge, this is the first meta-analysis to evaluate the prognostic significance of PTK6 expression in cancer tissue. The results suggest that PTK6 expression is a prognostic factor in patients with various cancers, but the direction of prognosis differs, depending on the degree of PTK6 expression in non-tumor tissues. There are still unanswered questions about how PTK6 expression is regulated and the functions of PTK6 in cancer, therefore more translational and clinical studies are needed to understand the role of PTK6 in the progression of malignant tumors.

Author contributions

JHK conceived the original idea. STP and SYJ participated in literature searching, data extraction and original draft preparation. HSK carried out statistical analysis and data interpretation and JJL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at <https://www.imrpress.com/journal/FBL/27/2/10.31083/j.fbl2702060>.

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