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REVIEW

Noncoding RNAs as potential biomarkers to predict the outcome in pancreatic cancer

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Abstract: Pancreatic ductal adenocarcinoma (PDAC), a common digestive system cancer, is highly malignant and has a poor disease outcome. Currently, all available examination and detection methods cannot accurately predict the clinical outcome. Therefore, it is extremely important to identify novel molecular biomarkers for personalized medication and to significantly improve the overall outcome. The "noncoding RNAs" (ncRNAs) are a group of RNAs that do not code for proteins, and they are categorized as structural RNAs and regulatory RNAs. It has been shown that microRNAs and long ncRNAs function as regulatory RNAs to affect the progression of various diseases. Many studies have confirmed a role for ncRNAs in the progression of PDAC during the last few years. Because of the significant role of ncRNAs in PDAC, ncRNA profiling may be used to predict PDAC outcome with high accuracy. This review comprehensively analyzes the value of ncRNAs as potential biomarkers to predict the outcome in PDAC and the possible mechanisms thereof.

Keywords: pancreatic ductal adenocarcinoma, microRNA, long noncoding RNA, outcome prediction

Introduction

Pancreatic ductal adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC), a common digestive tumor, is highly malignant and has a poor patient outcome. The majority of patients have metastasis at diagnosis, and only approximately 15% of the tumors can be surgically removed.[1] Despite the refinement of surgical techniques for treatment and the constant modification of surgical approaches for improving the resection rate, the median survival time of PDAC is only 13.4 months after resection combined with gemcitabine.[2] For patients who are not candidates for curative surgery, the median survival time is only 7 months.[3]

Outcome prediction in pancreatic ductal adenocarcinoma

Pathological stage is the most important tool used to evaluate the outcome and predict the necessity and validity for adjuvant treatments in PDAC. Additionally, in the metastatic setting, only clinical prognostic factors, such as imaging examinations and serum tumor markers, are available to guide treatment decision making.[4–6]

Carbohydrate antigen 19-9 (CA19-9) has been extensively used to screen for PDAC in blood and predict the clinical cancer outcome.[7,8] However, several benign diseases and multiple types of digestive adenocarcinoma, particularly advanced gastrointestinal cancers, may cause elevated serum CA19-9 levels, which suggests that the specificity of CA19-9 requires improvement for PDAC outcome predication.[9] In the majority of patients with benign biliary obstruction, CA-19-9 levels are significantly elevated (>1,000 U/mL).[10] Therefore, it is difficult to distinguish whether high preoperative

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levels of CA19-9 are related to tumor recurrence or other diseases.

Furthermore, PDAC is heterogeneous both in biology and genetics. Promising tools for stratifying patients according to tumor biological behaviors and aberrant genetic alterations are required. Moreover, the majority of patients with PDAC remain asymptomatic until they present with locally advanced or distally metastatic and surgically inoperable disease.[11] Therefore, rather than a PDAC tissue biomarker, blood-based biomarkers are more critical to guide PDAC patient treatment.

Noncoding RNAs

"Noncoding RNAs" (ncRNAs) are a group of RNAs that do not code for proteins. They are categorized into two classes according to transcript size: "long noncoding RNAs" (lncRNAs) are greater than 200 nucleotides in length, and "small ncRNAs" are less than 200 nucleotides in length.[12–14] Among the small ncRNAs, microRNAs (miRNAs) consist of approximately 22 nucleotides and are the product of precursor miRNAs consisting of 60–110 nucleotides digested by the RNase III, Dicer.[15]

MicroRNAs

"miRNAs" are widely distributed small ncRNAs, primarily responsible for the negative regulation of gene expression.[16] Thus far, more than 2,500 miRNAs have been reported (miRBase, v 20.0).[17] The majority of miRNAs function via base-pairing with complementary sequences of the messenger RNA (mRNA) three prime untranslated region (3′UTR), degrading target gene mRNA, and inhibiting the protein translation of target genes.[18] miRNAs are involved in regulating various biological activities, including cell growth, differentiation, apoptosis, and proliferation,[15,19] indicating that miRNAs play an important role in the development and progression of various diseases.[20]

In 2002, Calin et al identified a small genomic region in chromosome 13q14 that was commonly deleted in chronic lymphocytic leukemia and contained the *miR-15a* and *miR-16-1* genes, suggesting a link between these miRNAs and chronic lymphocytic leukemia.[21] That was the first time the significance of miRNA in human cancer was demonstrated. After this initial observation, additional miRNAs were found to be aberrantly expressed in various types of cancer,[22–25] which indicates that the expression of miRNAs may be a better indicator of tumor prognosis than conventional protein-coding gene arrays.[23]

Long noncoding RNAs

Unlike miRNAs, lncRNAs are longer in length and more diverse. They are not highly conserved, and have been considered the transcriptional "noise", by-product of RNA polymerase II and are not biologically functional for a long period of time.[26,27] However, recently, studies have revealed the significance of lncRNAs in the regulation of multiple biological processes at different levels, including chromosome modifications, transcription, and post-transcription processing.[28–31]

The increased knowledge of the noncoding transcriptome in humans has revealed that lncRNAs are also involved in the regulation of cancer progression. In multiple solid adenocarcinomas, evidence suggests the value of lncRNAs for clinical outcome evaluation, indicating that the lncRNA signature can also improve the outcome prediction of various cancers, including pancreatic cancer.[32–36]

Noncoding RNAs as circulating biomarkers in cancer

In 2008, Mitchell et al[37] reported remarkable insights into circulating miRNAs as biomarkers for cancer classification and prognostication. One of the most important advantages of using circulating miRNAs as biomarkers is their stability in plasma and serum, where they are most likely protected from RNase degradation by binding to Argonaute proteins.[37,38] Recent studies corroborated the potential of circulating miRNAs, which appears to extend well beyond the oncology field.[39,40] The specificity and simplicity of circulating miRNAs are particularly striking, suggesting that serum-based miRNAs could emerge as promising sources of biomarker information.

miRNAs are highly stable in plasma and have outcome-predictive potential in several malignancies thus far.[41,42] Therefore, it is also possible that other types of ncRNAs, such as lncRNAs, are also stable in plasma and serum. Recent studies have confirmed that lncRNAs are relatively stable in plasma samples.[43,44] However, the protective mechanism remains unclear.

Noncoding RNAs in pancreatic ductal adenocarcinoma outcome prediction

MicroRNAs as biomarkers for outcome prediction in pancreatic ductal adenocarcinoma

In 2005, the expression profiling of a large number of miRNAs demonstrated that 334 miRNAs are aberrantly expressed in PDAC.[23] For circulating miRNAs in the serum of PDAC patients, several groups of researchers have

demonstrated the presence and potential utility of circulating miRNAs (Table 1). Furthermore, the role of circulating miRNAs for PDAC outcome prediction has also been gradually revealed (Figure 1). Nevertheless, there are no direct correlations between circulating miRNA levels and CA19, which is not unexpected because these two factors are presumably regulated by different mechanisms.

MicroRNA-21

MicroRNA (miR)-21 is the most studied miRNA possibly related to the progression of pancreatic cancer, and this miRNA has several pancreatic cancer-related target genes, such as *PDCD4*, *PTEN*, and *TIMP3*.[45] Of these, *PDCD4*, a tumor-suppressor gene, is the direct target of miR-21.[46] Inhibiting the expression of miR-21 can upregulate the expression of *PDCD4*, reduce cell proliferation, and increase cell apoptosis simultaneously in the pancreatic cancer cell line MIA-Pa-Ca-2, indicating that miR-21 is a potential biomarker for oncological outcome and a target for treatment.[47] It has also been demonstrated that gemcitabine downregulates miR-21 expression and promotes the expression of its target gene, *FasL*. The combination of *FasL* and gemcitabine promotes the apoptosis of PDAC cells.[48]

The abnormal expression of seven miRNAs – miR-21, miR-20a, miR-24, miR-25, miR-99a, miR-185, and miR-191 – in plasma was detected through the examination of 197 cases of PDAC.[49] The positive rate of PDAC detection by the seven miRNA-based biomarkers was 96.2%, significantly higher than that of CA19-9 (46.2%) and carcinoembryonic antigen (30.8%) in the same sample set.[49] These miRNAs can differentiate PDAC from pancreatitis and act as markers for predicting outcome, particularly plasma miR-21, which can function as an independent indicator for patient outcome.[48,49]

MicroRNA-210

As a hypoxia marker in PDAC, miR-210 was detected with fourfold increased expression in pancreatic cancer patients compared with in normal tissues ($P<0.00004$).[50] Cancers with increased hypoxia have a poor outcome and greater resistance to chemotherapy and radiotherapy, and miR-210 likely mediates diverse epigenetic mechanisms, resulting in hypoxia.[51,52] Camps et al[53] showed that miR-210 in human breast cancer was not only a marker of tumor hypoxia in vivo but also an indicator of adverse outcomes clinically. Similarly, in PDAC patients, miR-210 may be a promising biomarker to predict disease outcome and sensitivity to chemotherapy.

Table 1 MicroRNAs (miRNAs) as predictive biomarkers in pancreatic ductal adenocarcinoma (PDAC) outcome

Study	Year	miRNA	N	Regulation	End point(s)
Bauer et al[73]	2012	87 miRNAs	45	Differentially expressed	Eighty-seven miRNAs have a value for the evaluation of cancer recurrence in patients and for people with a familial risk of PDAC
Ho et al[50]	2010	miR-210	11	↑	Serum miR-210 level is elevated in PDAC patients and may potentially serve as a useful biomarker for PDAC
Kawaguchi et al[71]	2013	miR-221	47	↑	Plasma miR-221 is a useful biomarker for predicting malignant outcomes in PDAC patients and may contribute to PDAC treatments
Kong et al[54]	2011	miR-21, miR-155, miR-196a	35	↑	Three miRNA-based biomarkers are able to differentiate PDAC from normal pancreas; circulating miR-196a level is valuable in predicting long-term survival of PDAC patients
LaConti et al[72]	2011	miR-100a, miR-10	6	↑	Circulating miR-100a and miR-10 serve as indicators of drug response in PDAC
Liu et al[49]	2012	miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, miR-191	197	↑	Seven miRNA-based biomarkers serve as a novel noninvasive approach for PDAC prognosis; serum miR-21 level is significantly correlated with PDAC overall survival
Morimura et al[42]	2011	miR-18a	19	↑	Plasma level of miR-18a was obviously lower in postoperative samples than in preoperative samples
Wang et al[67]	2009	miR-20, miR-210, miR-155, miR-196a	49	↑	Three miRNA-based biomarkers may serve as noninvasive early outcome prediction biomarker for PDAC
Wang et al[48]	2013	miR-21	177	↑	High-level serum miR-21 is significantly correlated with a lower overall survival of PDAC patients

Abbreviations: CA19-9, carbohydrate antigen 19-9; miR, microRNA.

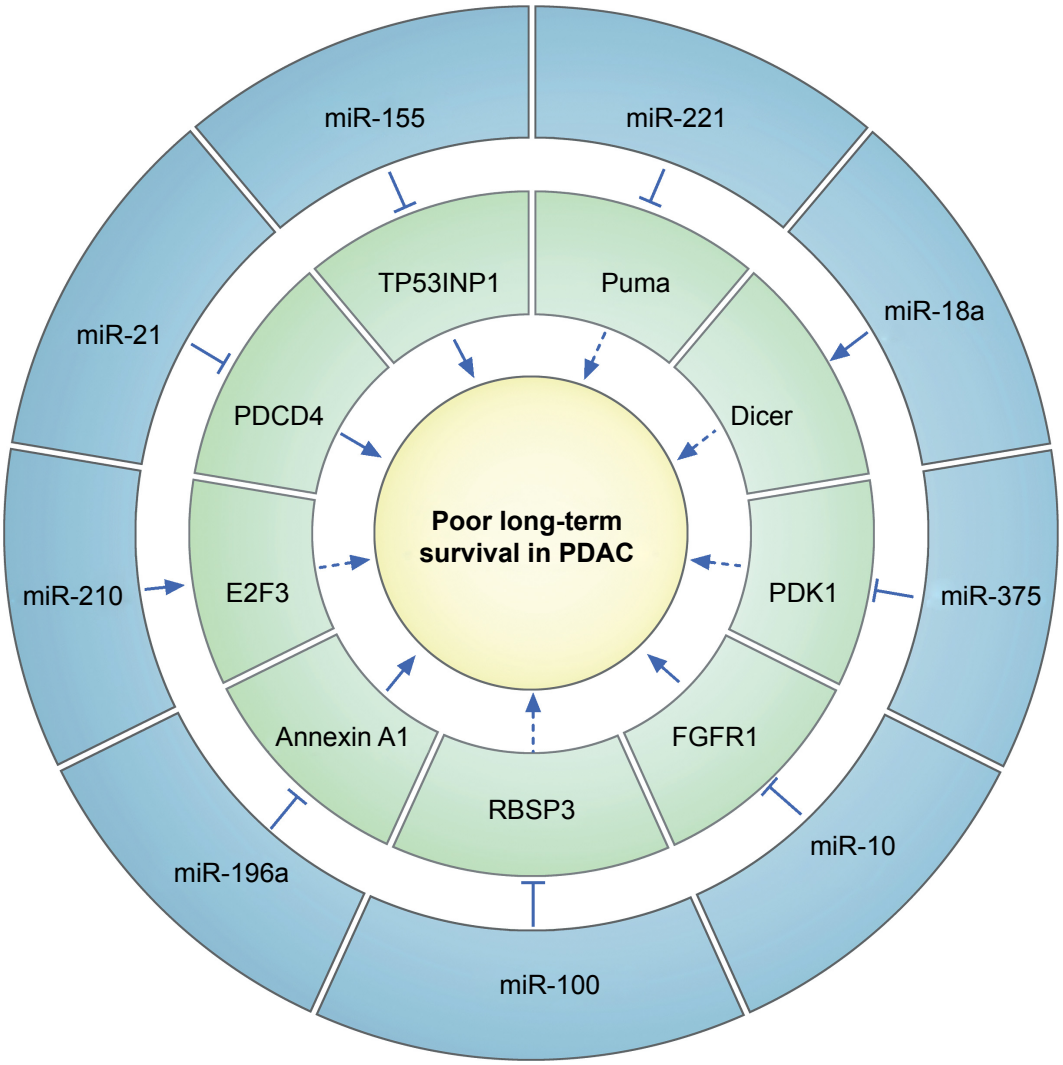

Figure 1 The regulation of long-term survival of pancreatic ductal adenocarcinoma (PDAC) by microRNAs (miRs).
Abbreviations: E2F3, E2F transcription factor 3; FGFR1, fibroblast growth factor receptor 1; PDCD4, programmed cell death 4; PDK1, 3-phosphoinositide-dependent protein kinase-1; TP53INP1, tumor protein 53-induced nuclear protein 1 gene; PUMA, p53 upregulated modulator of apoptosis; RBSP3, RB1 serine phosphates from human chromosome 3.

MicroRNA-196a

The serum miR-196a level was found to have a potential value in predicting the median survival time of PDAC patients.[54] Although the mechanism by which miR-196a promotes PDAC progression has not been completely elucidated, high expression of miR-196a is also associated with a poor outcome in pancreatic adenocarcinoma patients.[55]

Further functional analysis showed that the expression level of miR-196a in solid tumors promotes cell proliferation and suppresses cell apoptosis through downregulation of annexin 1.[56] Overexpressed annexin 1 is a frequent biological marker and correlates with the differentiation of PDAC during tumorigenesis.[57] These results suggest that miR-196a contributes to cancer progression and may be a prospective biomarker in PDAC.

MicroRNA-155

miR-155 is a typical multifunctional miRNA and was one of the first miRNAs found to be elevated in human cancer.[58] An approximately twelvefold upregulation of the expression of miR-155 has been verified in PDAC tissues compared with in normal pancreatic tissues. miR-155 is overexpressed in PDAC cells and represses the expression of the tumor protein 53-induced nuclear protein 1 gene (*TP53INP1*), which is a proapoptotic stress-induced p53 target gene.[59] *TP53INP1* induces autophagy-dependent cell death and plays a tumor-suppressor role in multiple cancers, such as gastric cancer and pancreatic cancer.[60,61] In serum, miR-155 can be used to differentiate the sera from PDAC patients from that of healthy controls.[54] The ability of miR-155 to differentiate PDAC sufferers from healthy people demonstrates its utility as a first-line serum biomarker for PDAC outcome prediction.

MicroRNA-18a

MiR-18a is located in the miR-17-92 cluster and is highly expressed not only in pancreatic cancer tissues but also in patient serum.[42,62] This miRNA was reported as a potential oncogene in various types of cancer, with its elevation caused by genome amplification or transcriptional activation by *c-myc*.[63] *c-myc* alters the sensitivity of chemotherapeutic drugs by affecting the expression of the cell-cyclin protein cyclin D1.[64] Transcriptional activation of cyclin D1 and c-myc induces the Gap 1- to Synthesis-phase transition in the cell cycle and results in DNA replication and ultimately mitosis, which are responsible for cell proliferation.[65]

A recent study showed the prognostic value of miR-18a in PDAC. MiR-18a was significantly lower in postoperative samples than preoperative samples (P=0.0077).[42] Therefore, the relationships between miR-18a, *c-myc*, and cyclin D1 suggest that miR-18a is a potential outcome-predictive miRNA in PDAC.

Combination of microRNAs

miRNAs as a whole can significantly increase the reliability of outcome prediction. The expression of miR-21, miR-210, miR-155, and miR-196a in plasma has been shown to be increased in PDAC patients.[66] Receiver operating characteristic curve analysis has shown that the specificity and sensitivity were significantly higher through miRNA combination, with values of 89% and 64%, respectively.[67]

A single gene or protein may not accurately predict patient outcome and guide treatment. The reliability is significantly increased if several miRNAs can predict the outcome. Other studies have also demonstrated that the putative target genes for these predictive miRNAs are involved in complex signaling networks that can affect pancreatic cancer tumor progression and predict disease outcome.[68–70]

Other microRNAs

Other circulating miRNAs, such as miR-10, miR-34b, miR-100a, miR-221, and miR-375, have also been confirmed as valuable biomarkers for predicting the clinical outcome in PDAC patients via the regulation of their target genes.[71–73]

Long noncoding RNAs as biomarkers for outcome prediction in pancreatic ductal adenocarcinoma

As yet, there is no direct evidence that circulating lncRNAs are molecular markers for PDAC outcome prediction; however, this has been reported in other digestive tumors, such as hepatocellular carcinoma and gastric cancer.[43,74] Moreover, several lncRNAs have significant correlations with PDAC outcome and are involved in cancer progression. Therefore, these types of lncRNAs should also be investigated (Table 2).

Metastasis-associated lung adenocarcinoma transcript 1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is the first lncRNA discovered to be related to the prognosis of PDAC. MALAT1 is located on chromosome 11q13 and was initially discovered by Ji et al[75] when observing the metastatic process of non-small-cell lung cancer in 2003. Then Lin et al[35] identified the negative expression of MALAT1 in the majority of adjacent normal tissues and more positive expression in PDAC tissues using in situ hybridization. In the next study, the expression of MALAT1 was also shown to be elevated in PDAC tissues, and its expression level was significantly related to the long-term survival of PDAC patients, which was considered an independent predictor of outcome.[76]

HOX transcript antisense RNA

HOX transcript antisense RNA (HOTAIR) is the first lncRNA found to have regulatory functions of reverse transcription.[77] HOTAIR was also confirmed as related to the invasion and metastasis of solid tumors and is considered an important biomarker for disease outcome prediction.[78–81] In PDAC, the higher expression of HOTAIR is related to cell invasion and tumor prognosis. Furthermore, in pancreatic cancer cell lines, HOTAIR knockout can reduce the proliferation and invasion of the tumor, and induce apoptosis, which further suggests that HOTAIR functions as both an oncogene and a negative prognostic factor.[81]

PVT1

Among lncRNAs, PVT1 functions as a MYC protein and oncogene, and these roles have been confirmed in various solid tumors, including Burkitt's lymphoma, breast cancer, and lung cancer.[82] In studies of PDAC cells, You et al[83] used whole genome and transposon genetic screening to demonstrate that the functional inactivation of PVT1 enhanced the sensitivity of gemcitabine to the human pancreatic tumor cell line AsPDAC-1. In addition to drug sensitivity, MYC and PVT1 co-amplification is related to the rapid progression of and poor outcome in breast cancer.[84] Therefore, the predictive role of PVT1 is quite promising in PDAC patients.

Other long noncoding RNAs

The mitogen-activated protein kinase (MAPK) signaling pathway is closely related to PDAC metastasis.[85,86] Tahira et al[87] evaluated 38 PDAC samples to examine the expression of

Table 2 Long noncoding RNAs (lncRNAs) as predictive biomarkers in pancreatic ductal adenocarcinoma (PDAC) outcome

Study	Year	lncRNA	Size (bp)	Cytoband	Regulation	Potential function and mechanism
Kim et al[81]	2013	HOTAIR	2,158	12q13.13	↑	HOTAIR expression is increased in PDAC and is associated with more aggressive tumors
Lin et al[35]	2007	MALAT1	8,708	11q13.1	↑	MALAT1 is a potential marker for PDAC cells and potential participant in the molecular cell biology of PDAC
Liu et al[75]	2014	MALAT1	8,708	11q13.1	↑	Overexpression of MALAT1 is an independent predictor of disease-specific survival of PDAC
Tahira et al[87]	2011	MAP3K14	906–1,260	17q21.31	↑	nc
	2011	PPP3Cb	3,165	10q22.2	↑	nc
	2011	DAPK1	5,942	9q21.33	↑	nc
You et al[83]	2011	PVT1	1,716	8q24	↑	PVT1 is a potential biomarker for the rational design of chemotherapies for PDAC

Abbreviations: DAPK1, death-associated protein kinase 1; HOTAIR, HOX transcript antisense RNA; MALA1, metastasis-associated lung adenocarcinoma transcript 1; MAP3K14, mitogen-activated protein kinase 14; nc, not clear; PPP3Cb, protein phosphatase 3, catalytic subunit, beta isozyme; PVT1, plasmacytoma variant translocation 1.

intergenic and intronic lncRNAs in PDAC and metastatic tissues using gene microarray analysis. Their results revealed that the expression levels of nine MAPK signaling pathway-related intronic lncRNAs were significantly increased, which indicates that lncRNAs are involved in the regulation of PDAC.

Perspectives

ncRNAs are becoming potential prognostic markers in PDAC, with support from several studies (Tables 1 and 2). Several serum miRNAs, such as miR-21 and miR-196a, are negatively correlated between expression and overall survival. Additionally, several lncRNAs have also been found to be elevated in PDAC tissues, and their expression levels were significantly related to the outcome of PDAC patients. These findings suggest the potential value of ncRNAs in predicting the prognostic outcome of PDAC.

Moreover, increasing evidence suggests a role for ncRNAs in PDAC biology and progression.[88] Recent studies have demonstrated that a variety of ncRNAs are frequently deregulated in PDAC and crosstalk with various biological processes, including epithelial-mesenchymal transition, cancer apoptosis regulation, and cancer-related signaling pathways, which may be crucial in tumor progression and affect patient outcome.[89–93]

However, as far as we are aware, no clinical trials are currently ongoing with ncRNA profiles as biomarkers in PDAC. The several studies described herein examined the predictive potential of ncRNAs in PDAC. Although they highlighted the promising roles of these molecules, four challenges remain to their clinical application: (1) The use of ncRNA for prognostic purposes requires assurance that the measured concentration represents the actual amount in the samples – however, commonly used approaches lack this assurance;[94] (2) lncRNA is not conserved evolutionarily, and it is difficult to study these molecules using animal models prior to clinical applications; (3) some ncRNAs are abnormally expressed during the development and progression of various diseases, including tumors, cardiovascular diseases, and immune-system diseases, and the specificity of detection in a particular tumor requires further study;[95–97] (4) PDAC is less common than other tumor types, such as lung cancer and colon cancer[98] and, further, a relatively smaller proportion of patients with PDAC are candidates for resection, further diminishing the potential pool of patients with easily accessible specimens.

Conclusion

The increased understanding of the roles of ncRNAs as tumor suppressors or oncogenes will help design novel

ncRNA-based approaches for outcome prediction and targeted therapy that counteract the aberrant expression of ncRNAs responsible for malignant progression. ncRNAs combined with other prognostic methods are a new direction for tumor biomarkers in the future that will allow clinicians to identify patients with the worst outcomes and then prolong survival.

Author contributions

Kaizhou Jin, Guopei Luo and Xianjun Yu designed study. Kaizhou Jin and Guopei Luo collected data. Zhiwen Xiao and Zuqiang Liu analyzed data. Chen Liu, Shunrong Ji, Jin Xu, Liang Liu, Jiang Long, Quanxing Ni and Xianjun Yu interpreted data. Kaizhou Jin, Zhiwen Xiao and Zuqiang Liu wrote the draft. Guopei Luo, Chen Liu, Shunrong Ji, Jin Xu, Liang Liu, Jiang Long, Quanxing Ni and Xianjun Yu revised it critically. All the authors approved the accuracy and integrity of the work and approved the version to be published.

Disclosure

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