Prognostic Estimation Model for Oligodendroglioma: An mRNA-Sequence Data-Based Analysis

Qinghui Zhu
Beijing Tian Tan Hospital

Shaoping Shen
Beijing Tian Tan Hospital

Chuanwei Yang
Beijing Tian Tan Hospital

Mingxiao Li
Beijing Tian Tan Hospital

Xiaokang Zhang
Beijing Tian Tan Hospital

Haoyi Li
Beijing Tian Tan Hospital

Xuzhe Zhao
Beijing Tian Tan Hospital

Ming Li
Beijing Tian Tan Hospital

Cui Yong
Beijing Tian Tan Hospital

Xiaohui Ren
Beijing Tian Tan Hospital

Song Lin (linsong2005@126.com)
Beijing Tian Tan Hospital

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Abstract

**Object:** In contrast with the previous diagnostic strategy, which relied only on histopathologic evidence, the integrated diagnosis of oligodendroglioma based on the 5th edition of World Health Organization Classification of Tumors of the Central Nervous System (WHO CNS 5) criteria requires the codeletion of chromosome arms 1p and 19q and isocitrate dehydrogenase gene (IDH1 or IDH2) mutation (mt). The existing prognostic indicators may not be completely suitable for oligodendroglioma patients based on the new diagnostic criteria. We aimed to identify a prognostic prediction model for oligodendrogliomas based on the WHO CNS 5 classification.

**Methods:** We collected 175 glioma samples to investigate significant changes in mRNAs using the Chinese Glioma Genome Atlas (CGGA) database and to establish a prediction model for prognosis by Least Absolute Shrinkage and Selection Operator (LASSO) and Cox logistic analysis.

**Results:** Eighty-eight differentially expressed RNAs (DERNAs) were identified between the long survival group and the short survival group. Seven RNAs were selected to calculate risk scores. Risk level, age and Primary-or-Recurrence Status (PRS) type were used as factors for the prognostic model.

**Conclusion:** An individualized prognostic model for oligodendroglioma patients based on the WHO CNS 5 criteria was established. The predictive ability of this model was validated in a validation cohort, which demonstrated its predictive accuracy. In the future, more pathological evidence is needed to support our predictive model to further classify oligodendrogliomas.

Introduction

Oligodendroglioma is a subtype of glioma with a relatively favorable prognosis compared with those of others [1-3]. In contrast with the previous diagnostic strategy, which relied only on histopathologic evidence [4], the integrated diagnosis of oligodendroglioma based on the World Health Organization Classification of Tumours of the Central Nervous System (WHO CNS 5) criteria requires the codeletion of chromosome arms 1p and 19q and isocitrate dehydrogenase gene (IDH1 or IDH2) mutation (mt) [5]. The WHO CNS 5 proposes an integrated diagnosis based on the consideration of pathological features and molecular data, such as IDH and 1p/19q TERT molecular markers. Based on these new diagnostic criteria, the classification of oligoastrocytomas has been removed, along with the term “anaplastic” [5], which indicates that the consensus that the histopathological classification is not enough to predict the prognosis of the tumor has been established [6]. These revised diagnostic criteria for diffuse glioma are more reliable in guiding prognosis than classic histopathological methods [7].

According to prior reports, the 5-year survival rate of oligodendroglioma almost reaches 90% [8]. However, we also observed that some patients showed a shorter survival time, irrespective of the treatment they received or the WHO grade. Moreover, the disparity in survival also suggests that a more objective and optimal stratification of oligodendrogliomas that is not only dependent on pathological diagnosis is
urgently needed. Therefore, some studies regarding the molecular markers related to the survival of oligodendroglioma patients have aimed to stratify oligodendroglioma, with an attempt to identify possible therapeutic targets and improve the survival of oligodendroglioma patients. Several previous studies that aimed to identify prognostic indicators of oligodendroglioma proposed the use of CDKN2A/B, PTEN, NOTCH 1 and other biomarkers as classification criteria to reclassify the prognosis of oligodendroglioma, but there is no consistent conclusion [9−12]. Our previous work showed that oligodendroglioma patients with 1q19p copolysomy had a worse prognosis [13]. Most of these prognostic predictors that were assessed in previous studies were discussed based on the pathological diagnosis of oligodendrogliomas rather than an integrated diagnosis. In other words, the existing prognostic indicators may not be completely suitable for oligodendroglioma patients based on the latest diagnostic criteria.

To identify potential prognostic factors and establish a prognostic estimation model for oligodendrogliomas based on the WHO CNS 5 classification, we analysed the data of the China Glioma Genome Atlas (CGGA) database and the information of patients in the Department of Neurosurgical Oncology, Beijing Tiantan Hospital, Capital Medical University.

**Material And Methods**

**CGGA Data**

RNA sequencing and corresponding clinical data were retrieved from the CGGA database (DataSet ID: mRNAseq_693 and mRNAseq_325) [14−18]. Oligodendroglioma patient data were selected and divided into 2 cohorts (training cohort and validation cohort). The corresponding clinical data of the oligodendroglioma patients are summarized in Table 1. We collected 165 oligodendroglioma samples (109 in the training cohort and 56 in the validation cohort) to investigate significant changes in mRNAs and to establish a prognostic model.
<table>
<thead>
<tr>
<th></th>
<th>training cohort</th>
<th>validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td>109</td>
<td>56</td>
</tr>
<tr>
<td>OS(days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg.</td>
<td>1860.39</td>
<td>3207.93</td>
</tr>
<tr>
<td>Mid.</td>
<td>1696</td>
<td>3673</td>
</tr>
<tr>
<td>SD</td>
<td>892.29</td>
<td>1302.947</td>
</tr>
<tr>
<td>min</td>
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<td>284</td>
</tr>
<tr>
<td>max</td>
<td>4725</td>
<td>4793</td>
</tr>
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</tr>
<tr>
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<td>77</td>
<td>40</td>
</tr>
<tr>
<td>Dead</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
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<td>46</td>
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<tr>
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<td></td>
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<td>103</td>
<td>55</td>
</tr>
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<td>&gt;60</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Radio treatment</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>51</td>
</tr>
<tr>
<td>No</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Chemo treatment</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>29</td>
</tr>
<tr>
<td>No</td>
<td>33</td>
<td>27</td>
</tr>
</tbody>
</table>

**Table 1**
Corresponding clinical data of the patients in two cohorts

**Statistical Analysis**
Statistical analysis was conducted with R software (version 4.1.3. http://www.r-project.org). The packages in R that were used in this study are reported in the Additional file 1. The reported statistical significance levels were all obtained from two-sided tests.

**Screening of Differentially Expressed (DE) mRNAs and Calculating Risk Score**

Since many low-grade glioma studies have used 5-year survival as an indicator of long-term survival [19,20], we used 1825 days ($\approx 5$ years) as the standard to classify the length of patient survival time. The DESeq2 package was used to filter the DE mRNAs between the long survival patients (> 1825 days) and short survival patients (survival time $\leq$ 1825 days) in the testing cohort with the criteria of an $|\log_2$-fold-change$| \geq 1.0$ and a false discovery rate p value of $< 0.001$. The expression levels of the mRNAs are presented in a heatmap (Fig. 1). The least absolute shrinkage and selection operator (LASSO) regression analysis was used to select the most useful predictive mRNAs from the mRNA-seq and survival data [21]. The risk score was calculated for each patient and was used to divide patients into two different groups (high-risk and low-risk). Survival analysis of target mRNAs was performed using the survival package. The ROCR, glmnet and caret packages were used to assess the predictive accuracy.

**Establishment of a Prognostic Model**

The multivariable Cox regression analysis began with the following factors: sex, age (patients who were younger than 60 years were classified into the Low-Age group; otherwise, they were classified into the High-Age group), Primary-or-Recurrence Status (PRS) type, and risk. A prognostic model was generated to predict the survival time of patients suffering from oligodendroglioma. To provide the clinician with an intuitive method for assessing the possibility of survival at a specific time, we established a prognostic nomogram model based on Cox logistic analysis in the testing cohort.

**Apparent Performance of the Prognostic Model in the Training Cohort**

The Hosmer–Lemeshow test was used to verify the accuracy of the prognostic model. A calibration curve was plotted to visualize the results. The model was subjected to bootstrapping validation (1,000 bootstrap resamples) to plot the calibration curves. Harrell's concordance index (C-index) was used to calculate the degree of discrimination between the predicted value and the observed value.

**Independent Validation of the Prognostic Model**

The performance of the prognostic model was tested in a validation cohort. The Cox regression formula established in the training cohort was applied to all patients in the validation cohort. Risk scores were calculated and used in the Cox regression prognostic model. The C-index and calibration curve were derived based on the model.
Quantitative Reverse Transcription Polymerase Chain Reaction using Clinical Samples

Our patients’ tumour tissues were used for qRT–PCR. We chose patients with oligodendroglioma, IDH mut, and 1p/19q codeletion and obtained their cryopreserved tumour samples along with the corresponding clinical data. Total RNA was isolated from cells and brain tissues. M-MLV reverse transcriptase (TaKaRa Bio, Japan) was used to synthesize cDNA. qRT–PCR was performed using a Roche LightCycler® 480II system (Roche, Switzerland). GAPDH was used as an internal reference to quantify the mRNAs. qRT–PCR assays were performed in triplicate. The primer and probe sequences of the genes used for qRT–PCR analysis are listed in Additional file 2. Using patients with a survival time of more than 5 years as the control group, CT values were converted to relative expression to validate the expression differences.

Results

Identification of Differentially Expressed RNAs (DERNAs)

A total of 165 oligodendroglioma samples from CGGA mRNA sequencing data in two cohorts were selected based on IDHmut,1p/19q codeletion status. After normalization, Eighty-eight mRNAs were identified to be significantly DE (|log2-fold-change| ≥ 1.0 and false discovery rate p < 0.001) between the long survival and short survival groups. The heatmap of the DERNAs is shown in Fig. 1.

LASSO Gene Selection and Risk Score Calculation

Eighty-eight DERNAs were reduced to 7 mRNAs in the LASSO regression model by using the minimum criteria for risk factors (Fig. 2A and B). These mRNAs were features with nonzero coefficients in the LASSO logistic regression model based on 109 patients in the training cohort. These genes were used to calculate the patients’ risk scores. The distribution of risk scores is shown in Fig. 2C and D. As the risk score increased, the survival time of patients decreased, and the mortality rate increased. Survival analysis was conducted with the DERNAs combined with the corresponding clinical data. Receiver operating characteristic (ROC) curves were generated to validate the accuracy of the risk score for predicting survival time. The area under the ROC curve (AUC = 0.679) indicated that the risk score had an acceptable prediction accuracy for survival.

Establishment of the Individualized Prognostic Model

Using univariate Cox regression, the indicators with p values of < 0.05 (age, PRS status, risk level) were selected as components of the established prognostic model. Age > 60 years, the presence of recurrence rather than primary tumour, and high-risk level led to worse prognosis in patients with oligodendroglioma. The ROC curve indicated that the predictive ability of the model was improved after age and PRS status were included (Fig. 3A). A nomogram was generated to present the results. At the same time, based on
the prognostic model, the DynNom package and the shinyPredict package were used to generate scripts to individually predict the prognosis of oligodendroglioma patients (Fig. 3C and D).

Evaluation of the Prognostic Model in the Training Cohort

The calibration curves of the prognostic nomogram for the probability of survival at 3 years, 5 years and 8 years showed similarities in the observed and predicted values in the training cohort (Fig. 3E). The Hosmer–Lemeshow test suggested that there was no departure from perfect fit (p = 0.9997) (Table 2). The prediction nomogram yielded a C-index of 0.912 (95% CI, 0.679 to 0.981) in the training cohort.

<table>
<thead>
<tr>
<th>Interval</th>
<th>Alive</th>
<th>Dead</th>
<th>Predicted Alive</th>
<th>Predicted Dead</th>
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<tr>
<td>[0.118,0.125]</td>
<td>38</td>
<td>6</td>
<td>38.829471</td>
<td>5.170529</td>
</tr>
<tr>
<td>(0.125,0.153)</td>
<td>15</td>
<td>2</td>
<td>14.392965</td>
<td>2.607035</td>
</tr>
<tr>
<td>(0.153,0.422)</td>
<td>6</td>
<td>3</td>
<td>5.205934</td>
<td>3.794066</td>
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<tr>
<td>(0.422,0.498)</td>
<td>16</td>
<td>17</td>
<td>16.571630</td>
<td>16.428370</td>
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<tr>
<td>(0.498,0.889)</td>
<td>2</td>
<td>4</td>
<td>2.000000</td>
<td>4.000000</td>
</tr>
</tbody>
</table>

Independent Validation of the Prognostic Model

The calibration curves in the validation cohort showed good agreement between the predictions and observations (Fig. 3F). The Hosmer–Lemeshow test yielded a nonsignificant p value (P = .8921), and the C-index of the nomogram for the prediction of survival was 0.778 (95% CI, 0.769 to 0.787).

Validation of RNA Expression Differences between Tumour Tissues of Patients

The cryopreserved tumour samples of oligodendroglioma patients in the Department of Neurosurgical Oncology, Beijing Tiantan Hospital, Capital Medical University were obtained. Samples from the long survival group and short survival group were selected for qRT–PCR to calculate the relative expression levels. We sought to validate the differential expression of the seven target genes (LOXL2, APLN, SLC12A5, TROAP, NUF2, ANGPT2, CTHRC1) among these samples. Unfortunately, however, although the differences in the mean relative gene expression between groups were consistent with expectations, except for APLN and ANGPT2, there were no statistically significant differences in gene expression between the two groups (Fig. 4B). Due to the limitations of preservative methods and the time span of the study, a suitable number of samples was difficult to obtain, resulting in insufficient sample size. The small sample size may be the reason the results of qRT–PCR analysis did not reflect the results of LASSO regression. The results will be more credible if RNA sequencing can be performed on different groups of patients in the future.
Discussion

Using the clinical and RNA-seq information contained in the CGGA database, we identified 88 genes with differential expression between patients with a survival time of more than 5 years and less than 5 years. Then, we used LASSO regression to further refine the number of genes that could be used to predict prognosis. This approach can be used not only to select mRNAs based on the strength of their univariate association with survival but also to calculate a risk score and perform risk stratification that is sufficient for predicting prognosis. We tried to identify pathological evidence to validate the gene expression differences we observed, but the qRT–PCR analysis was ineffective. More studies on the molecular mechanisms associated with oligodendroglioma prognosis are needed in the future.

Accurate prognosis prediction is an important component of the individualized treatment of tumours [22]. Based on the new diagnostic criteria of the WHO CNS 5, the widely recognized markers with prognostic significance for oligodendroglioma possess limitations. Cao L. et al. analysed the information of 4568 patients with oligodendroglioma; established a prognostic model based on the Surveillance, Epidemiology, and End Results (SEER) database; and proposed that radiotherapy, age, tumour location, grade and surgical resection are independent prognostic factors of oligodendroglioma [23]. However, they only focused on oligodendrogliomas diagnosed by histopathological criteria, and molecular factors were not considered. The survival analysis of high-grade oligodendroglioma by Liu S. et al was comprehensive and more detailed in the collection of clinical information [24]. Family circumstances were also included in the survival analysis. This approach was consistent with the International Classification of Functioning, Disability and Health guidelines, which were based on a comprehensive bio-psycho-social view and were adopted by the World Health Organization in 2001. In addition to assessments of pathology or physiological changes, assessments of the treatments of cancer patients should consider individual patient needs in daily life [25]. The disadvantage of this study was that it lacked molecular pathological information for a more accurate diagnosis of patients and prediction of prognosis. Unlike previous studies, our study is a rare prognostic study of oligodendroglioma combined with clinical and molecular data. In our study, the AUC value of the ROC curve of the prognostic model that integrated clinical and molecular data was higher than that of the prognostic model that contained only molecular data, and the C-index value of our nomogram was higher than that of the Cao L study, which contained only clinical information; these results demonstrate that the integration of clinical and molecular data can predict prognosis more effectively. In the future, more clinical information can be collected from patients for larger and more detailed prognostic analysis to establish a more accurate individualized prognostic prediction model.

Previous studies have shown that LOXL2, a member of the lysyl oxidase (LOX) family, not only promotes glioma cell proliferation, migration, and invasion and induces the epithelial-to-mesenchymal transition (EMT) process but also reduces the sensitivity of glioma cells to temozolomide (TMZ) [26]. APLN is activated by VEGF signalling and hypoxia-responsive elements in the APLN promoter, stimulates angiogenic sprouting, and plays a necessary and sufficient role in tumour angiogenesis [27]. TROAP
activates the Wnt/β-Catenin pathway and upregulates the expression of its downstream targets to play a tumour-promoting role [28]. ANGPT2 activates angiogenesis through VEGFA, normalizes tumour blood vessels, and promotes the malignant transformation of glioblastoma [29,30]. NUF2 has a potential role in glioma growth and TMZ resistance [31]. The CTHRC1 gene contributes to tissue repair in vascular remodelling in response to injury by limiting collagen matrix deposition and promoting cell migration [32]. Among these differentially expressed genes, in addition to the increased expression of the genes mentioned above, which are related to cell differentiation and proliferation, the decreased expression of SLC12A5 aroused our interest. SLC12A5 (encoding the KCC2 protein) acts to stabilize nerve cell potential, and its reduced expression correlates with the development of epilepsy [33–38]. Consistent with our findings, Yang and Gao et al. found that the expression of SLC12A5 in patients with shorter survival time was significantly lower than that in patients with longer survival time [39]. Paradoxically, epilepsy has been reported in some studies as an independent beneficial factor for glioma prognosis [40]. On the one hand, this finding may be due to the fact that epilepsy is thought to be associated with IDH1 mutations in low-grade gliomas [41], and IDH mutations are associated with better glioma prognosis. On the other hand, the seizures may cause some patients to seek treatment more actively, which may result in a better prognosis [42,43]. The genes involved in risk score calculation can be used not only to predict the prognosis of oligodendroglioma patients and explain the occurrence and progression of oligodendroglioma but also to provide directions for potential therapeutic targets of oligodendroglioma. ISL2, a nuclear and chromatin-associated transcription factor [44], regulates the transcription of ANGPT2 by binding to the ANGPT2 promoter. When ISL2 expression was found to be reduced, oligodendroglioma cell proliferation was reduced. TMZ combined with anti-ISL2 therapy may be effective in oligodendroglioma in vitro and in tumour-bearing animal models [29]. For this possible treatment, more clinical studies are required to study its safety and efficiency.

Another noteworthy point in the present study is that no prognostic benefit of radiotherapy or chemotherapy was observed in the CGGA oligodendroglioma cohort. Long-term follow-up of radiotherapy or chemotherapy in oligodendroglioma is lacking. The effect of various treatment modalities on the prognosis of oligodendroglioma is still inconclusive. Cao L. et al. observed no survival benefit of radiotherapy in the treatment of oligodendrogliomas, while chemotherapy was an independent favourable prognostic factor in WHO grade 3 oligodendrogliomas [23]. The survival benefit of radiotherapy assisted by TMZ therapy was observed in the EORTC study 26053 – 22054 [45]. Weller et al. found that the Procarbazine, Lomustine, and Vincristine (PCV) protocol alone proved effective for the treatment of WHO grade 2 oligodendroglioma [46]. Several studies have also indicated that radiotherapy concurrent with PCV therapy can prolong the overall survival of WHO grade 3 oligodendroglioma patients [47]. In our study, treatments showed no significant effect on the prognosis of patients with oligodendroglioma in the CGGA database, and we considered that this result may be due to a bias in the treatment of patients. This bias in therapy was due to the suspected nature of the tumour based on intraoperative findings or histopathology. For instance, patients with better intraoperative findings or histopathological properties may be treated conservatively, while those with worse characteristics may be referred for
radio/chemotherapy. A prospective study committed to disclosing the prognostic impact of postoperative radiotherapy and/or chemotherapy in WHO CNS5 patients may be warranted in the future.

Although excellent predictive models were identified, some problems still existed in our study. Constrained by conditions, the study only contained data from CGGA and lacked verification performed using other databases. Most of the cases in the CGGA database are patients treated in our hospital, so there may have been some bias in the selection of patient data included in the analysis. At the same time, our study is a retrospective study, and animal models and molecular mechanism studies are needed to support and explain our conclusions in the future.

Conclusion

We established an individualized prognostic model for oligodendroglioma patients based on the WHO CNS5 criteria using risk level, age, and PRS status. The predictive ability of this model was validated in a validation cohort and demonstrated predictive accuracy. In the future, more pathological evidence is needed to support our predictive model to further classify oligodendrogliomas into different survival subgroups.

Declarations

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors

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Author Contributions

Conception and design: Qinghui Zhu, Song Lin

Collection and assembly of data: Qinghui Zhu, Mingxiao Li, Xiaokang Zhang, Xuzhe Zhao, Ming Li

Data analysis and interpretation: Qinghui Zhu, Haoyi Li, Shaoping Shen, Chuanwei Yang

Cell biological experiments: Qinghui Zhu

Manuscript writing: Qinghui Zhu

Final approval of manuscript: All authors

Competing interests

The authors declare that they have no competing interests.

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25. U. Tschiesner, E. Linseisen, M. Coenen, S. Rogers, U. Harreus, A. Berghaus, A. Cieza, Evaluating sequelae after head and neck cancer from the patient perspective with the help of the international


Figures

Figure 1

Heatmap of DERNAs. The expression differences of DERNAs are shown in the heatmap. In the two groups of patients with different survival times, 88 differentially expressed genes were identified.

Figure 2

Gene selection using the LASSO binary logistic regression model. (A) LASSO coefficient profiles of the 88 DERNAs. A coefficient profile plot was produced against the log (lambda). (B) The area under the receiver operating characteristic (AUC) curve was plotted versus log(l). Dotted vertical lines were drawn at the optimal values by using the minimum criteria and the 1 standard error of the minimum criteria (the 1-SE criteria). 7 mRNAs were selected in the LASSO regression model by using the minimum criteria for risk factors. Patients’ risk scores were calculated based on these mRNAs. Distribution of patients in
ascending order of risk score. (C) Distribution curve showing the distribution of risk score. (D) Distribution of survival time showing that survival time decreases with increasing risk score and the incidence of time to death increases.

**Figure 3**

(A) ROC curves demonstrate the predictive ability of risk scores (AUC=0.679) versus the use of risk level and age and PRS-type (AUC=0.738). The predictive power of the predictive model is shown after age, and the PRS type were included has a better predictability of prognosis. (B) Dynamic nomogram plots were generated to visualize the prognostic model, (C and D) and scripts were generated to predict the probability of survival for a patient at a specific timepoint. (E) The calibration curves in the training cohort show that the predicted values at 3 years, 5 years and 8 years are close to the observed values. (F) The predicted values in the validation cohort at 3 years, 5 years and 8 years were compared with the observed values, and the fitted curves showed that the prediction accuracy was acceptable. External validation suggested that the model had the highest accuracy in predicting 8-year survival.

**Figure 4**

(A) Electropherogram of PCR products (M: DL20001GAPDH2GAPDH negative control3LOXL24LOXL2 negative control5APLN6APLN negative control7SLC12A58SLC12A5 negative control9TROAP10TROAP negative control11ANGPT212negative control13NUF214NUF2 negative control15CTHRC116CTHRC1 negative control) (B) The mean differences in gene expression between the two groups, except for APLN, were consistent with our risk model, but no significant differences were observed in gene expression between the two groups.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile.xlsx