RESEARCH PAPER

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Abnormal expression of long non-coding RNA LINC01270 in glioma and its correlation with X-ray computed tomography signs

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This study focused on the association of LINC01270 and computed tomography (CT) signs with glioma development, to evaluate their potential in the early detection of glioma. Serum LINC01270 was evaluated in glioma patients and healthy individuals using PCR. The involvement of LINC01270 in glioma onset and development was evaluated by ROC and chi-square test. The association of LINC01270 with the CT signs and their combined effects in the diagnosis in glioma were also estimated. Serum LINC01270 was significantly elevated in glioma patients, which was closely associated with patients' advanced WHO grades and lower KPS. Both LINC01270 upregulation and CT findings showed significant potential in diagnosing glioma, and LINC01270 correlated significantly with the invasion risk and metastasis indicated on CT. The combination of LINC01270 expression and CT findings significantly improved the sensitivity and specificity of glioma diagnosis. Upregulated LINC01270 in glioma is associated with malignant and severe disease development and has significant diagnostic value. Combined detection of LINC01270 and CT findings could improve the diagnostic efficacy in glioma cases, thus optimizing clinical diagnosis.

Key words: glioma, lncRNA LINC01270, serum; combined diagnosis, CT examination

INTRODUCTION

Glioma is the most prevalent intracranial tumor and always occurs in the neuroectoderm (Cahill and Turcan, 2018). Glioma shows strong invasion, presenting a short survival time and poor prognosis. Currently, although various processes, such as mitochondrial metabolism (Chen et al., 2022c), have been reportedly associated with glioma onset, the direct molecular pathological mechanism of its onset remains unclear, thus increasing the difficulty in its diagnosis and therapy (Davis, 2018; Fu et al., 2020; Mudassar et al., 2020; Reifenberger et al., 2017). Surgical resection is one of the major treatments for glioma along with radiotherapy and chemotherapy, which could alleviate the mass occupying effect, relieve intracranial hypertension, and reduce the tumor cells (Norden and Wen, 2006; Wu et al., 2011). The higher the resection rates, the better the survival rate. Radiotherapy and chemotherapy are usually employed to assist postoperative surgical treatment, which could significantly benefit patients primarily receiving therapy and those with advanced glioma. However, due to the invasiveness of glioma, complete excision of the tumor tissues is difficult to achieve. Ensuring early detection of glioma could provide a reference for early intervention and benefit patient outcomes.

X-ray computed tomography (CT) is a commonly used diagnostic method for central nervous system neoplasms, which provides valuable information regarding tumor location, tumor size, and some tumor-induced

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secondary changes (Salazar et al., 1981). Surgical therapy and tumor recurrence could destroy the blood-brain barrier, disturbing the CT findings. Notably, especially hemorrhage can disturb the CT signals (Diaconis and Rao, 1980; Mathews et al., 2021). Additionally, CT is not effective in distinguishing recurrent tumor, and disease diagnosis is mainly based on imaging features, which lack quantitative auxiliary results and can cause misdiagnosis or missed diagnosis. Magnetic resonance imaging (MRI) is a more sensitive and specific examination for diagnosing malignant tumors. However, due to its high examination cost and long inspection time, MRI cannot be employed as a common examination method, and its usage in the early screening for patients in emergency or coma is limited. Recently, the identification of biomarkers has become a novel cancer research hot point, where non-coding RNAs (ncRNAs) have attracted special attention. In previous studies, long ncRNAs were considered reliable biomarker candidates, and several lncRNAs showed involvement in the occurrence and development of glioma, such as lncRNA DANCR, LINC01018, and MDHDH (Chen et al., 2022b; Xu et al., 2023; Yu et al., 2023). Some studies have also explored several candidate lncRNAs in glioma, which were related with the prognosis, ferroptosis, immune, and other critical pathological processes of glioma development (Chen et al., 2022a; Huang et al., 2022; Shi et al., 2022; S. Wu et al., 2023). Data from the online databases, starBase and GEPIA, showed that LINC01270 was significantly upregulated in head and neck squamous cell carcinoma and glioblastoma multiforme. Additionally, LINC01270 was revealed to regulate the progression of human cancers, such as lung adenocarcinoma, breast cancer, gastric cancer, and esophageal cancer (Jiang et al., 2022; Li et al., 2022a; Li et al., 2022b; Zhang et al., 2022). However, the available data was lacking to confirm the significance of LINC01270 in glioma. Moreover, if the dysregulation of LINC01270 is associated with glioma progression, the expression level of LINC01270 might complement the diagnostic efficiency of CT.

Hence, this study confirmed the abnormal expression of LINC01270 in glioma and evaluated its diagnostic significance. Moreover, the combined effect of LINC01270 expression and CT findings in distinguishing glioma was estimated to improve the screening and monitoring of glioma.

METHODS

General patient information

This study was approved by the Ethics Committee of The First Affiliated Hospital of Jinzhou Medical University. A total of 113 glioma patients from 2018 to 2020 were enrolled as the glioma group. The control group included 62 healthy individuals, who showed no abnormalities in regular physical examinations of the brain and neural examinations. All study participants or their families provided written informed consent. The inclusion criteria for the glioma group were: 1) primarily diagnosis of glioma; 2) no anti-cancer therapies before enrollment; and 3) no history of other malignant tumors. Patients with recurrence were excluded. Among the enrolled glioma patients, 17 myxopapillary ependymomas patients and 6 pilocytic astrocytoma patients were classified as World Health Organization (WHO) Grade I, while 26 low-grade astrocytoma patients, 11 oligodendroglioma patients, and 12 mixed glioma patients were classified as WHO Grade II. WHO Grade III patients mainly included those diagnosed with anaplastic astrocytoma and anaplastic oligoastrocytoma. The WHO grading of the enrolled patients was according to previous studies (Louis et al., 2016; Wesseling and Capper, 2018). The glioma patients included 72 males and 41 females of average age 65.41 ± 7.26 years, while the control group comprised 39 males and 23 females with of average age 63.34 ± 4.59 years. The age and gender composition did not differ significantly between the two groups. The clinicopathological features of glioma patients are summarized in Table 1.

CT examination

CT examination was performed once after patients' diagnosis. CT was performed using LightSpeed VCT and AW4.4 workstation (GE Healthcare, USA), and the images were analyzed by at least two chief physicians and one CT specialist by the single-blind method. LightSpeed VCT provided stereoscopic imaging of the blood vessels and clarified the invasion boundary. The hemodynamic parameters obtained, such as cerebral blood volume and cerebral blood flow, also assisted in assessing the tumor extent, including invasion and metastasis (Harrer et al., 2008; Narang et al., 2011; Wilson et al., 2013). Patients underwent the CT in a supine position under calm breathing or holding their breath. The scan parameters were set as follows: tube voltage, 120 kV; current, 100 mA; layer thickness, 6.0 mm; collimation, 1.5 mm; scanning time, 6-8 s; and reconstructed thickness, 10 mm. The CT results were scored as 1-5 according to the degree of anomaly. The diameter, boundary, invasion, and morphology were of the tumor recorded. As extra-cranial metastasis of glioma is rare, only intracranial metastasis has been summarized in this study. CT can only screen the potential of metastasis, which

Table 1. Association of LINC01270 with patients' clinicopathological features.

	Cases	Low-LINC01270	High-LINC01270	*P*
Gender				0.683
Male	72	34	38	
Female	41	21	20	
Age (years)				0.729
≤ 55	45	21	24	
> 55	68	34	34	
Tumor size (cm)				0.227
≤ 4	53	29	24	
> 4	60	26	34	
Tumor location				0.315
Frontal	48	26	22	
Temporalis	65	29	36	
WHO grades				0.002
I-II	70	42	28	
III	43	13	30	
KPS				0.013
≤ 80	44	15	29	
> 80	69	40	29	

KPS: Karnofsky Performance Scale.

need further confirmation by MRI. Therefore, the metastasis was defined as "potential intracranial metastasis" in this study. The diameter changes were defined as the changes in the glioma 3 months after diagnosis. The other signs summarized in Table 2 were obtained during the first examination.

Data from online databases

The expression of LINC01270 in brain-related tumors, including head and neck squamous cell carcinoma and glioblastoma multiforme, was evaluated using the starBase (https://rnasysu.com/encori/index.php) and the GEPIA (http://gepia.cancer-pku.cn/index.html) databases, respectively.

Sample collection

Peripheral blood was collected from the control group and the glioma group the next morning after their enrollment and stood for 1 h at room temperature. Serum samples were obtained by centrifugating twice at 4000 rpm for 10 min and stored in a refrigerator at -80°C for subsequent analyses.

Real-time qPCR

Serum samples were lysed with the TRIzol kit to isolate total RNA. After evaluating the purity of RNA, high-quality RNA (OD260/280=1.8-2.2) was used. Reverse transcription was conducted using HiScript II reverse Transcriptase to generate cDNA and stored at -20°C. Real-time qPCR was performed using the ABI Prism 7900 system (Shanghai Meixuan, China) with SYBR Green. The thermal cycle conditions were as follows: 95°C initial heating for 3 min followed by 40 cycles of 95°C for 30 s, 55°C for 60 s, and 72°C for 60 s. The relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method with GAPDH as the internal reference (Meng et al., 2018; Shaker et al., 2019). The sequences of the primers used were as follows:

LINC01270 forward: 5'-CTCACGAAAGCGCAGGAATG-3'; reverse: 5'-GCTCCAAAAGCAGACAAGCC-3'; GAPDH forward: 5'-GAGAACGGGAAGCTTGTCAT-3'; and reverse: 5'-CAGAGATGATGACCCTTTTGG-3'.

Statistical analyses

Statistical analyses were conducted using SPSS 26.0 software. Comparison between the groups was conducted using the student's t-test ($P<0.05$ indicating a significant difference). The diagnostic value of CT, LINC01270 expression, and their combined effects were estimated by ROC analysis. The significance of LINC01270 in glioma development and prognosis was assessed by the chi-square and Cox regression analyses.

RESULT

Dysregulation of LINC01270 and its correlation with the clinicopathological features

LINC01270 was upregulated in head and neck squamous cell carcinoma from the starBase database (Fig. 1A) and in glioblastoma multiforme from the GEPIA database (Fig. 1B). Glioma patients showed a significantly increased serum LINC01270 level compared with that of healthy individuals (Fig. 2A). The average serum level of LINC01270 was used as the cutoff to divide the glioma group patients into low-LINC01270 and high-LINC01270 groups. Patients with severe disease conditions, indicated by advanced WHO grades and low KPS scores, were mainly included in the high-LINC01270 group. Consistently, serum LINC01270 level showed a significant association with the advanced WHO grades ($P=0.002$) and low KPS scores ($P=0.013$) in the glioma group patients (Table 1).

Association between LINC01270 and CT signs

CT signs indicated changes in the tumor diameter, boundary, metastasis, morphology, and invasion risk in glioma patients. Based on the subgrouping of the glioma group patients, LINC01270 showed a close association with the potential intracranial metastasis ($P=0.003$) and invasion risk ($P=0.018$), where most of the patients with high invasion risk and metastasis showed high serum levels of LINC01270 (Table 2). Although the metastasis is relatively low in Grade I-II glioma patients, those with Graded III remain at a high risk of metastases. Among the 40 glioma patients with metastasis, 31 had Grade III glioma and 9 had Grade II glioma.

Table 2. Association of LINC01270 with CT signs of glioma patients.

	Cases	Low-LINC01270	High-LINC01270	*P*
Diameter changes				0.155
Thick or thin	77	41	36	
No changes	36	14	22	
Boundary				0.264
Clear	68	36	19	
Unclear	45	32	26	
Invasion risk				0.018
Low	74	42	32	
High	39	13	26	
Potential intracranial metastasis				0.003
Absent	73	43	3	
Present	40	12	28	
Morphology				0.148
Regular	62	34	28	
Irregular	51	21	30	

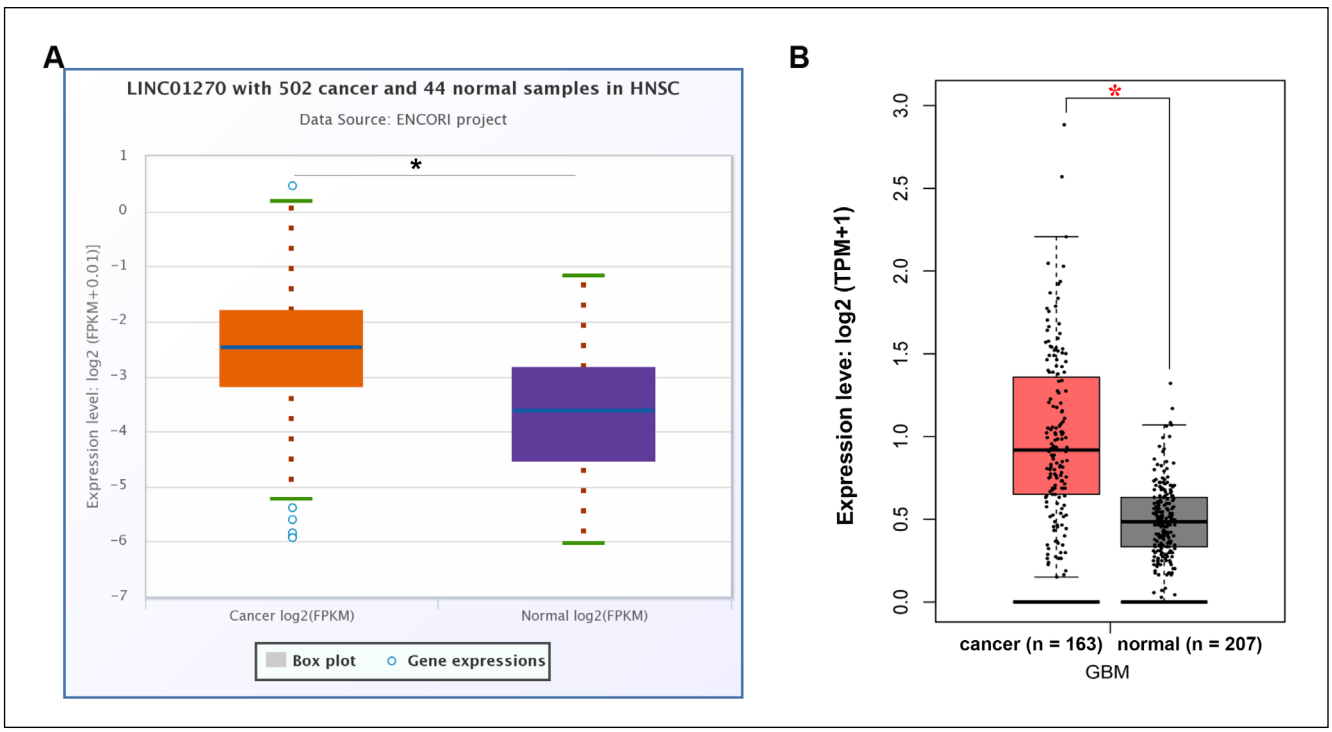

Fig. 1. Data of LINC01270 expression in head and neck squamous cell carcinoma (A) and glioblastoma multiforme (B) from the online database, starBase (A) and GEPIA (B). The data from starBase database were plotted with the values of $\log_2$ (FPKM+0.01) and the presented as mean ± SD (A). The data from the GEPIA database were plotted with the values of $\log_2$ (TPM+1) and presented as median and 95% CI (B). All samples from the two databases were tissues. *P<0.05.

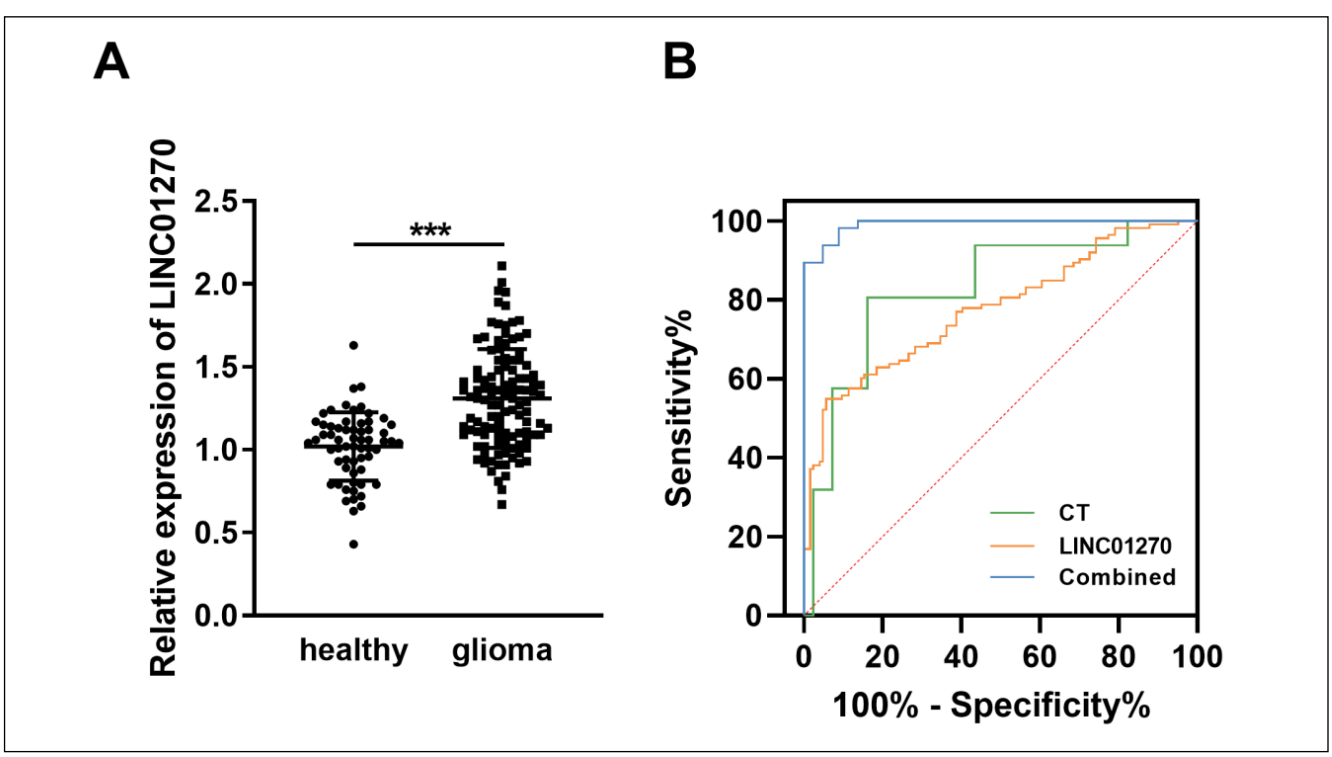

Fig. 2. Serum expression of LINC01270 in glioma (A) and the diagnostic significance in glioma (B). LINC01270 and CT examination could discriminate glioma patients from healthy individuals, and their combination could improve the sensitivity and specificity of glioma. The relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method normalized to GAPDH. ***P<0.001.

Diagnostic significance of CT, LINC01270, and their combination diagnosis

The CT signs and upregulation of serum LINC01270 showed significant diagnostic value for glioma, which distinguishes glioma patients from healthy individuals (Fig. 2B). CT showed a higher sensitivity than LINC01270 expression in diagnosing glioma, while the specificity of the latter was relatively higher than the former (Table 3). The combination of CT and LINC01270 improved the sensitivity and specificity of glioma diagnosis compared with the diagnostic efficacy of CT and LINC01270 individually (Table 3).

DISCUSSION

Glioma shows invasive growth, and the imaging features and the early stage of glioma lack typical symptoms, increasing the difficulty in early detection and delaying the optimal therapeutic time. Although great progress has been made in the therapeutic strategies for glioma, the therapeutic efficacy and patient outcomes remain unsatisfactory due to the high malignancy and rapid progression to advanced stages. Differently expressed lncRNAs are considered novel biomarkers of human cancers, which predict tumor progression and patient outcomes. Previously, LINC01270 was identified as a prognosis-related lncRNA in triple-negative breast cancer, which was associated with the patients' overall survival and regulated cell viability and motility (Ping et al., 2021). Several lncRNA expression profiles in different human cancers also showed the dysregulation of LINC01270, and its functional role was revealed based on these data. For example, a constructed co-expression network revealed the upregulation of LINC01270 in lung adenocarcinoma, and a recent study confirmed that LINC01270 could promote the progression of lung adenocarcinoma via regulation of miR-326 (Wang et al., 2019; Zhang et al., 2022). The abnormal expression of LINC01270 in glioma was reported previously, and it mediated drug resistance and immunosuppressive microenvironment in head and neck cancers (Chen et al., 2022c). Similarly, online databases confirmed the upregulation of LINC01270 in head and neck squamous cell carcinoma and glioblastoma multiforme. Moreover, the collected clinical tissue samples validated the upregulation of LINC01270 in the serum samples of 113 glioma patients. The upregulation of LINC01270 was significantly associated with the WHO grades and KPS scores of the patients, which are critical factors in evaluating disease severity and development, suggesting the potential involvement of LINC01270 in glioma development (Bai et al., 2020; Gunawan et al., 2020).

Imaging examination has been considered one of the most routine screening methods for glioma, showing the details and characteristics of lesions in high resolution. CT examination, a common imaging examination method, can elicit changes in the tumor size, surrounding tissue conditions, metastasis, and other information related to tumor progression (Salazar et al., 1981). In this study, CT results of glioma patients showed a significant diagnostic potential with relatively high sensitivity, though the specificity was unsatisfying. Currently, studies have focused on the combination of CT with other technologies, such as positron emission tomography/CT, to improve diagnostic efficacy (Bogsrud et al., 2019; Kim et al., 2022; Li et al., 2021). Routine blood routine examination is a common test in the clinic with the advantage of being non-invasive. Serum LINC01270 showed diagnostic potential for glioma in the present study, and its specificity was higher than that of CT examination, but the sensitivity was unsatisfactory. Moreover, in the CT results, LINC01270 levels were correlated with the invasion risk and metastasis in glioma patients, which is consistent with its association with the clinicopathological features of the glioma patients. In addition, the diagnostic significance of LINC01270 and its correlation with the CT findings suggested that LINC01270 could complement the diagnostic efficacy of CT. The ROC analysis revealed that, the combination of LINC01270 and CT improved the diagnostic sensitivity and specificity in glioma case. Therefore, the serum expression

Table 3. Diagnostic efficacy of CT, LINC01270, and their combination.

	Sensitivity	Specificity	AUC
CT	80.53%	77.42%	0.828
LINC01270	54.87%	93.55%	0.777
Combined diagnosis	93.81%	95.16%	0.992

AUC: area under the curve.

of LINC01270 would be a novel addition in the diagnosis of glioma with CT. Moreover, the noninvasive characteristics of serum biomarkers could improve the convenience of clinical diagnosis. The identification of diagnostic biomarkers mainly directs to the early screening of glioma. Patients enrolled in this study were in relatively early stages of glioma (WHO grade I-III). The significance of LINC01270 combined with CT in distinguishing glioma patients could benefit the early detection of low-grade glioma. Additionally, the etiology of malignant tumors could affect the expression of lncRNAs. Therefore, the significance of LINC01270 might change in other subtypes of glioma, such as isocitrate dehydrogenase (IDH)-mutant type and IDH-wide type glioma, which needs further exploration.

However, this study has some limitations that warrant further investigations. Current studies have noticed the prognostic value of lncRNAs or other ncRNAs in glioma and other malignant tumors (Chandra Gupta et al., 2017; Chi et al., 2019; Goyal et al., 2021). The prognosis prediction investigation requires a long-term follow-up, and the tumor tissue expression could be more suitable. Due to the limitation of the study period and sample type, the function of LINC01270 in predicting patients outcomes in glioma had not been confirmed. Moreover, the increased expression of LINC01270 was observed in head and neck squamous cell carcinoma, as indicated by data from the online databases. Therefore, the dysregulation of LINC01270 might also occur in other brain disorders. Future studies should focus on the significance of LINC01270 in distinguishing malignant brain tumors from benign brain disorders. This is a single-center study with a relatively small sample size, and a selection bias might exist; hence, future studies should expand the sample size and research scope. On the other hand, whether the LINC01270 expression changes after surgery was not elicited in this study. The changing expression of LINC01270 in the patients' serum or tumor tissues might be associated with the recovery and prognosis, which is also a potential indicator in glioma cases. Therefore, other than following up patients' disease conditions and development, future research should also focus on the changes in LINC01270 expression.

CONCLUSION

According to the above findings, the upregulation of serum LINC01270 in glioma cases is closely associated with malignant and severe disease development and can distinguish glioma patients from healthy individuals. CT examination can be used for diagnosing glioma, and the results should be correlated with serum LINC01270 expression. The combination of CT examination and serum LINC01270 levels could improve their diagnostic efficacy in glioma cases.

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