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Serum *ROCK1* mRNA is of great diagnostic value for glioma patients

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Abstract

The study aimed to measure the presence of rho-associated protein kinase 1 (*ROCK1*) mRNA in serum samples collected from glioma and investigate its diagnostic significance in glioma.

The presence of *ROCK1* mRNA was examined by quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between *ROCK1* mRNA and clinical characteristics was analyzed via Chi-square test. The criteria of diagnosis evaluation, including sensitivity, specificity, optimal cutoff point, and area under the curve (AUC) were determined through the receiver operating characteristic (ROC) curve analysis.

ROCK1 mRNA was significantly increased in serum samples collected from glioma patients compared to the controls ($P < .05$). Besides, high *ROCK1* mRNA expression was tightly related with Karnofsky Performance Status (KPS) score ($P = .024$) and World Health Organization (WHO) grade ($P = .029$). However, there was no association between *ROCK1* expression and gender, neurological disorders, family history and cigarette smoking (all, $P > .05$). In addition, the optimal cutoff point was 3.025, with the sensitivity and specificity of 88.89% and 79.25%, respectively. The AUC was 0.881, indicating that *ROCK1* was a diagnostic biomarker for glioma patients ($P < .0001$, 95% CI = 0.829–0.933).

Serum *ROCK1* mRNA is significantly up-regulated in glioma cases compared to healthy controls. *ROCK1* may be a potential diagnostic biomarker in glioma.

Abbreviations: AGE = agarose gel electrophoresis, AUC = area under the curve, GTP = guanosine triphosphate, KPS = Karnofsky Performance Status, miR = microRNA, qRT-PCR = quantitative real-time polymerase chain reaction, ROC = receiver operating characteristic, ROCK1 = rho-associated protein kinase 1, WHO = World Health Organization.

Keywords: biomarker, diagnostic, glioma, ROC, *ROCK1*

1. Introduction

Glioma is the most frequent type of primary malignant brain tumors and 70% cases are at advanced-grade when diagnosed, which is the most aggressive and common brain tumor.[1–3] Currently, gliomas were divided into 4 grades according to the World Health Organization (WHO) grading scale, which is based on the cytologic features and malignant degrees: pilocytic astrocytoma (I), diffuse astrocytoma (II), anaplastic astrocytoma (III), and glioblastoma (IV).[4–6] The precise grading of glioma is important for designing therapy strategies, evaluating prognosis, and monitoring the response to therapies.[7] Glioma mainly derives from neuroepithelial tissues and is of high mortality and morbidity, accounting for about 40% to 50% of intracranial cancers and approximately 1.5% of whole body tumors.[8,9] Because of ineffective therapies and the infiltrative development patterns, the survival rate of glioma patients is relatively low. In addition, the poor prognosis and high mortality are considered to be associated with late diagnosis of glioma.[10,11] Therefore, it is of great importance to find novel biomarkers for the early diagnosis of glioma.

Rho-associated coiled-coil containing protein kinase (ROCK) is the downstream regulator of RhoA and participates in regulating the effects of RhoA on cell adhesion, smooth muscle contraction, cell motility, and apoptosis.[12,13] ROCK is initially identified as a serine/threonine protein kinase that binds to guanosine triphosphate (GTP)-bound RhoA.[14,15] Rho-associated protein kinase 1 (*ROCK1*) is an isoform of ROCK and consists of a kinase domain, an N-terminal region, a PH domain, a coiled-coil domain, and a C-terminal domain.[16,17] *ROCK1* acts as an oncogene and is involved in a wide range of progressions, including cell migration, metastasis, and invasion.[18,19] Moreover, *ROCK1* was highly expressed in various tumor tissues and tumor cell lines, including osteosarcoma, gastric cancer, lung cancer, and prostate cancer.[19–22] However, no reports were directly studied the expression and diagnostic value of *ROCK1* in glioma.

In the present study, we attempted to determine the presence of *ROCK1* mRNA in glioma and assess its relationship with diagnosis of glioma patients.

2. Materials and methods

2.1. Patients and samples

Serum samples were taken from 126 glioma patients and 53 healthy blood donors in Tianjin Medical University General

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The authors declare that they have no conflict of interest.

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Table 1

Relationship between *ROCK1* expression and clinical factors.

Clinical characteristics	Case NO.	Expression High	Expression Low	χ^2	*P* value
Gender				1.530	.216
Male	67	53	14		
Female	59	41	18		
Neurological disorder				2.682	.102
Present	55	45	10		
Absent	71	49	22		
Family history				2.352	.125
Yes	62	50	12		
No	64	44	20		
Preoperative KPS score				5.088	.024
≦40	65	54	11		
>40	61	40	21		
Cigarette smoking				0.503	.478
Ever	54	42	12		
Never	72	52	20		
WHO grade				4.785	.029
I, II	78	53	25		
III, IV	48	41	7		

KPS = Karnofsky Performance Status, ROCK1 = rho-associated protein kinase 1, WHO = World Health Organization.

Hospital. Among the 126 glioma patients, there were 56 males and 70 females, who were grouped using the tumor-node-metastasis (TNM) stage. Their clinical parameters were recorded at diagnosis time and summarized in Table 1. None of the patients received any chemotherapy or radiotherapy before blood collection. The healthy controls were prospectively recruited from the medical examination center of the same hospital, and they were matched with cases in age and gender. Our present study was authorized by the Ethic Committee of Tianjin Medical University General Hospital. Informed consents were provided by all participants.

2.2. Serum specimens

After 12 hours overnight fasting, 5 mL peripheral blood samples were collected from all the participants using VP-AS109K Vacutainer tubes (Terumo Corporation, Tokyo, Japan). The blood samples were incubated for 30 minutes at room temperature then centrifuged at 1500 × g for 10 minutes to isolate serum samples. Subsequently, the serum specimens were centrifuged at 20,000 × g for 10 minutes under 4°C condition to remove cell debris, and then maintained at −80°C until use.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from 200 μL serum using Trizol reagent (Invitrogen, Carlsbad, CA). 2% agarose gel electrophoresis (AGE) was used to detect the integrity of the obtained RNA samples, and the purity of RNA sample was estimated using Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE). In AGE images, the complete RNA exhibited clear 28 s and clear 18 s bands, moreover, the brightness ratio of 28 s band to 18 s band was nearly 2:1. OD A260/A280 ratio of 1.8 to 2.0 suggested the high purity of the RNA sample, without the contamination of DNA or protein. The first stand of cDNA was synthesized by PrimerScript™ RT reagent kit (Takara, Kyoto, Japan). Then real-time PCR was conducted using Power SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA) in the ABI Prism 7500 Sequence Detector System Applied Biosystems (Foster City, CA). The used specific primer sequences were as follows: *ROCK1*: Forward 5'-AACATGCTGCTGGATAAA-TCTGG-3'; Reverse 5'-TGTATCACATCGTACCATGCCT-3'; *GAPDH*: Forward 5'-AAGACCTTGGGCTGGGACTG-3'; Reverse 5'-ACCAAATCCGTTGACTCCGA-3'. *GAPDH* was employed as an internal control. The amplification was carried out in a 20 μL volume containing 1 μL cDNA, 10 μL 2 × SYBR Green Supermix, 2 μL qRT-PCR primers, and 7 μL ddH2O. The reaction sets were as followed: 50°C 2 minutes and 95°C 10 minutes, followed by 40 cycles of 95°C for 30 s and finally 60°C for 1 minutes. The data were shown as cycle threshold (Ct). The levels of *ROCK1* mRNA were normalized to *GAPDH*, and calculated using the formula of $2^{-\Delta\Delta Ct}$. Each experiment was performed 3 times.

2.4. Statistical analysis

All data analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL), and the figures were plotted by GraphPad Prism version 5.0 (GraphPad, San Diego, CA). It was considered as statistical significance if *P* was less than 0.05. The expression levels of *ROCK1* were shown as mean ±standard deviation (SD), and their comparison between glioma patients and healthy controls was carried out using student *t* test. Chi-square test was adopted to compare the relationship of *ROCK1* expression and clinical factors of glioma patients. Receiver operating characteristics (ROC) analysis was performed to evaluate the diagnostic value of *ROCK1* through calculating the sensitivity, specificity and area under the curve (AUC) in glioma.

3. Results

3.1. Up-regulation of ROCK1 mRNA in serum samples collected from glioma

Quantitative Real-time PCR was performed to measure the presence of *ROCK1* mRNA in serum collected from glioma and healthy controls. The serum level of *ROCK1* mRNA in glioma was 4.11 ± 0.96, while that in the controls was only 2.41 ± 1.00. It could be concluded that *ROCK1* mRNA was obviously increased in serum collected from glioma compared to the controls (Fig. 1, $P < .05$).

3.2. Relationship between ROCK1 expression and clinical factors

The included glioma patients were divided into high expression group (n = 84) and low expression group (n = 32) according to their median serum *ROCK*1 mRNA levels. Chi-square test was used to illustrate the association between *ROCK1* and clinical parameters of glioma patients. The result showed that high *ROCK1* mRNA level was significantly correlated with preoperative Karnofsky Performance Status (KPS) score ($P = .024$) and WHO grade ($P = .029$). However, no obvious relationship was found between *ROCK1* expression and gender ($P = .216$), neurological disorders ($P = .102$), family history ($P = .125$) or cigarette smoking ($P = .478$) (Table 1).

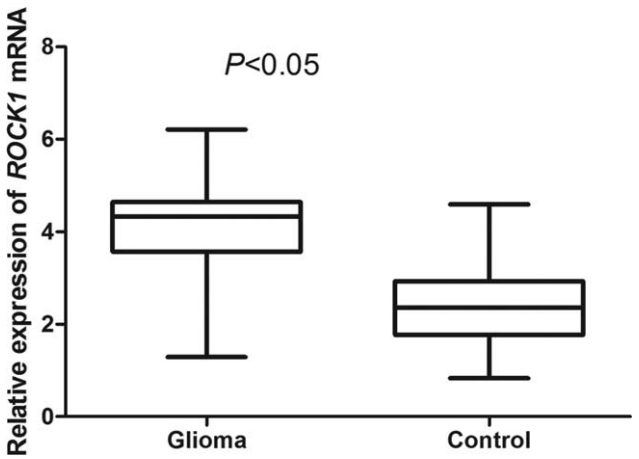

Figure 1. The presence of *ROCK1* mRNA in serum samples collected from glioma and healthy controls was determined using real-time PCR. Serum *ROCK1* mRNA level was increased in glioma compared to the controls ($P < .05$). PCR = polymerase chain reaction, ROC = receiver operating characteristic, ROCK1 = rho-associated protein kinase 1.

3.3. Diagnostic accuracy of serum ROCK1 in glioma

The ROC curve was profiled to assess the potential significance of serum *ROCK1* mRNA levels in diagnosis of glioma. As shown in Figure 2, the sensitivity and specificity were 88.89% and 79.25%, respectively, with an optimal cutoff point of 3.025. Besides, the AUC was 0.881, indicating that *ROCK1* was a diagnostic marker for glioma ($P < .0001$, 95% CI = 0.829–0.933).

4. Discussion

Glioma is a tumor that occurs in neural ectoderm. It is difficult to completely remove by surgical resection because of high invasion and metastasis, and glioma is not sensitive to radiotherapy or chemotherapy. The cause of glioma is complex, which may be related to the chemical carcinogens, ionizing radiation, and heredity.[23] Besides, changes of these factors drive abnormal expression of cancer-related genes, including activation of oncogenes, and inactivation of anti-oncogenes. So far, various biomarkers and signaling pathways involved in cell progression and tumorigenesis have been studied in glioma. Wang et al showed that microRNA *(miR)-132* enhanced the activation of transforming growth factor-β (TGF-β) signaling via suppressing *SMAD7* in glioma cells.[24] Cheng et al. revealed that *miR-218* was an efficient and novel marker for prognosis of glioma patients.[25] Wang et al[26] reported that *RAB34* was related with progression and prognosis of glioma. What is more, Zhang et al[27] investigated the function of *ROCK1* on the proliferation and metastasis grade of glioma, and the results revealed that up-regulation of *ROCK1* played important roles in the carcinogenesis, progression, and invasion of glioma, Thus it attracted great interest to value the clinical role of *ROCK1* in glioma patients.

ROCK1, a key downstream effector of the small GTPase RhoA, is a serine/threonine kinase and mediates various cellular responses, including cell proliferation, growth, and apoptosis via microtubule network organization and effects on the cytoskeleton.[28,29] Aberrant expression of *ROCK1* has been observed in several cancers and proved to be related to the tumor development and progression. Zhang et al demonstrated that over-expression of *ROCK1* was found in laryngeal squamous cell carcinoma.[30] Chen et al explained that the expression of *ROCK1* in the myolytic left atrial myocytes of mitral regurgitation (MR) patients was significantly higher than the controls.[31] In addition, *ROCK1* has been studied as a biological marker in various diseases. Smit et al claimed that *ROCK1* was a potential drug target for BRAF mutant melanoma.[32] Akagi et al showed that *ROCK1* was a novel prognostic biomarker for vulvar cancer.[33] In the present study, the attention was paid on the expression of *ROCK1* in glioma and its relationship with diagnosis of glioma.

In our study, the underlying role of *ROCK1* in glioma diagnosis was assessed through a series of determinations. First, we determined the presence of *ROCK1* mRNA in serum samples collected from glioma and healthy controls using the quantitative real-time PCR and the results revealed that serum level of *ROCK1* mRNA was increased notably in glioma compared to normal controls, which was in accordance with the previous studies. Besides, high *ROCK1* mRNA level was significantly related with preoperative KPS score and WHO grade, indicating *ROCK1* might be involved in the progression of glioma. Furthermore, we established the ROC curve to detect the diagnostic value of serum *ROCK1* for glioma patients. The results showed that AUC exhibiting a global summary of the diagnostic performance of *ROCK1*, and the AUC value showed that *ROCK1* could discriminate glioma patients from the healthy individuals.

Though the diagnostic performance of *ROCK1* in glioma has been investigated in the study, its mechanism on glioma is still unclear. There was a report suggesting that the RhoA/ROCK pathway might be related to abnormal myometrial contractility in obese pregnant women.[34] Hallgren et al demonstrated hat the rho/ROCK signaling pathway was regarded as a sensor of tissue compliance.[35] These may provide us with research interests.

In summary, we explored the diagnostic value of *ROCK1* in glioma. Serum *ROCK1* mRNA level was significantly higher in glioma than that in the controls and *ROCK1* was of great

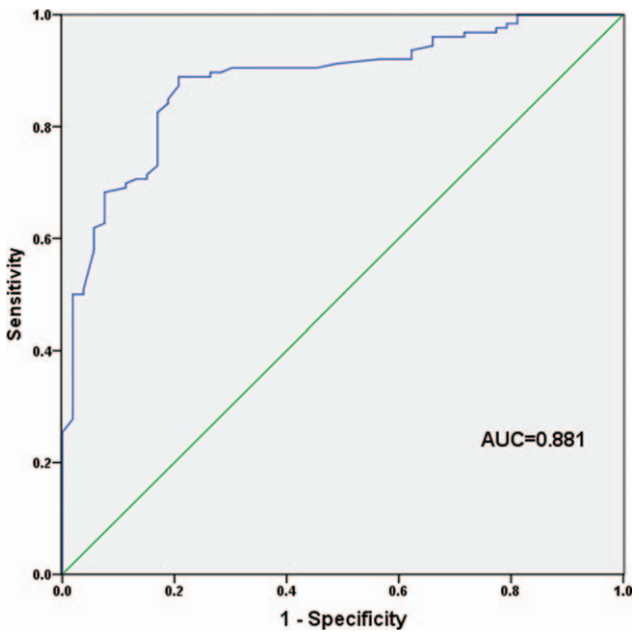

Figure 2. ROC curve was plotted to evaluate the diagnostic performance of *ROCK1* in glioma. The AUC was 0.881, suggesting that *ROCK1* was a diagnostic marker for glioma patients ($P < .0001$). ROC = receiver operating characteristic, ROCK1 = rho-associated protein kinase 1.

diagnostic significance in glioma. What is more, the precise mechanism of *ROCK1* in glioma is still dismal and needs more investigations and efforts in the future work.

Author contributions

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References

[1] Kiang KM, Zhang XQ, Leung GK. Long non-coding rnas: the key players in glioma pathogenesis. Cancers 2015;7:1406–24.
[2] Wen PY, Kesari S. Malignant gliomas in adults. New Engl J Med 2008;359:492–507.
[3] Du W, Pang C, Wang D, et al. Decreased FOXD3 expression is associated with poor prognosis in patients with high-grade gliomas. PLoS One 2015;10:e0127976.
[4] Hueng DY, Tsai WC, Chiou HY, et al. DDX3X biomarker correlates with poor survival in human gliomas. Int J Mol Sci 2015;16:15578–91.
[5] Vigneswaran K, Neill S, Hadjipanayis CG. Beyond the World Health Organization grading of infiltrating gliomas: advances in the molecular genetics of glioma classification. Ann Transl Med 2015;3:95.
[6] Sun J, Liao K, Wu X, et al. Serum microRNA-128 as a biomarker for diagnosis of glioma. Int J Clin Exp Med 2015;8:456–63.
[7] Ryu YJ, Choi SH, Park SJ, et al. Glioma: application of whole-tumor texture analysis of diffusion-weighted imaging for the evaluation of tumor heterogeneity. PLoS One 2014;9:e108335.
[8] Birner P, Gatterbauer B, Oberhuber G, et al. Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. Cancer 2001;92:165–71.
[9] Diao J, Xia T, Zhao H, et al. Overexpression of HLA-DR is associated with prognosis of glioma patients. Int J Clin Exp Pathol 2015;8:5485–90.
[10] Turner JD, Williamson R, Almefty KK, et al. The many roles of microRNAs in brain tumor biology. Neurosurg Focus 2010;28:E3.
[11] Wei X, Chen D, Lv T, et al. Serum MicroRNA-125b as a potential biomarker for glioma diagnosis. Mol Neurobiol 2014.
[12] Shi J, Zhang YW, Yang Y, et al. ROCK1 plays an essential role in the transition from cardiac hypertrophy to failure in mice. J Mol Cell Cardiol 2010;49:819–28.
[13] Loirand G, Guerin P, Pacaud P. Rho kinases in cardiovascular physiology and pathophysiology. Circ Res 2006;98:322–34.
[14] Ongusaha PP, Qi HH, Raj L, et al. Identification of ROCK1 as an upstream activator of the JIP-3 to JNK signaling axis in response to UVB damage. Sci Signal 2008;1:ra14.
[15] Leung T, Chen XQ, Manser E, et al. The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. Mol Cell Biol 1996;16:5313–27.
[16] Lock FE, Hotchin NA. Distinct roles for ROCK1 and ROCK2 in the regulation of keratinocyte differentiation. PLoS One 2009;4:e8190.
[17] Jacobs M, Hayakawa K, Swenson L, et al. The structure of dimeric ROCK I reveals the mechanism for ligand selectivity. J Biol Chem 2006;281:260–8.
[18] Xi ZW, Xin SY, Zhou LQ, et al. Downregulation of rho-associated protein kinase 1 by miR-124 in colorectal cancer. World J Gastroenterol WJG 2015;21:5454–64.
[19] Shin JY, Kim YI, Cho SJ, et al. MicroRNA 135a suppresses lymph node metastasis through down-regulation of ROCK1 in early gastric cancer. PLoS One 2014;9:e85205.
[20] Cui G, Cui M, Li Y, et al. MiR-186 targets ROCK1 to suppress the growth and metastasis of NSCLC cells. Tumour Biol J Int Soc Oncodevelop Biol Med 2014;35:8933–7.
[21] Liu X, Choy E, Hornicek FJ, et al. ROCK1 as a potential therapeutic target in osteosarcoma. J Orthop Res Off Publ Orthop Res Soc 2011;29:1259–66.
[22] Brown M, Roulson JA, Hart CA, et al. Arachidonic acid induction of Rho-mediated transendothelial migration in prostate cancer. Br J Cancer 2014;110:2099–108.
[23] Luo Q, Huang H, Deng Y, et al. Lentivirus-mediated shRNA targeting ZNF217 suppresses cell growth, migration, and invasion of glioma cells in vitro. Nan Fang yi Xue Xue Bao J South Med Univ 2015;35:1024–7.
[24] Wang ZH, Zhang QS, Duan YL, et al. TGF-beta induced miR-132 enhances the activation of TGF-beta signaling through inhibiting SMAD7 expression in glioma cells. Biochem Biophys Res Commun 2015;463:187–92.
[25] Cheng MW, Wang LL, Hu GY. Expression of microRNA-218 and its clinicopathological and prognostic significance in human glioma cases. Asian Pacific J Cancer Prevent APJCP 2015;16:1839–43.
[26] Wang HJ, Gao Y, Chen L, et al. RAB34 was a progression- and prognosis-associated biomarker in gliomas. Tumour Biol J Int Soc Oncodevelop Biol Med 2015;36:1573–8.
[27] Zhang P, Lu Y, Liu XY, et al. Knockdown of Rho-associated protein kinase 1 suppresses proliferation and invasion of glioma cells. Tumour Biol J Int Soc Oncodevelop Biol Med 2015;36:421–8.
[28] Montefusco MC, Merlo K, Bryan CD, et al. Little ROCK is a ROCK1 pseudogene expressed in human smooth muscle cells. BMC Genet 2010;11:22.
[29] Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. Nature reviews. Mol Cell Biol 2003;4:446–56.
[30] Zhang J, He X, Ma Y, et al. Overexpression of ROCK1 and ROCK2 inhibits human laryngeal squamous cell carcinoma. Int J Clin Exp Pathol 2015;8:244–51.
[31] Chen HC, Chang JP, Chang TH, et al. Enhanced expression of ROCK in left atrial myocytes of mitral regurgitation: a potential mechanism of myolysis. BMC Cardiovasc Dis 2015;15:33.
[32] Smit MA, Maddalo G, Greig K, et al. ROCK1 is a potential combinatorial drug target for BRAF mutant melanoma. Mol Syst Biol 2014;10:772.
[33] Akagi EM, Lavorato-Rocha AM, Maia Bde M, et al. ROCK1 as a novel prognostic marker in vulvar cancer. BMC Cancer 2014;14:822.
[34] O'Brien M, Carbin S, Morrison JJ, et al. Decreased myometrial p160 ROCK-1 expression in obese women at term pregnancy. Reprod Biol Endocrinol RB&E 2013;11:79.
[35] Hallgren O, Rolandsson S, Andersson-Sjoland A, et al. Enhanced ROCK1 dependent contractility in fibroblast from chronic obstructive pulmonary disease patients. J Transl Med 2012;10:171.