Actin gamma 1 is a critical regulator of pancreatic ductal adenocarcinoma

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Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) accounts for about 90% of pancreatic cancers, which represents one of the most lethal malignancies with a 5-year overall survival less than 10%. Identifying molecular biomarkers is invaluable in helping to predict clinical outcomes and developing targeted chemotherapies. Actin gamma 1 (ACTG1) is a kind of actin isoform that exists in almost all cell types as a component of the cytoskeleton, thus mediating cell viability. Although there have been studies revealing the prognostic significance of ACTG1 in several malignancies such as glioblastoma and hepatocellular carcinoma, its involvement and function in pancreatic cancer needs to be elucidated.

Methods: We retrospectively enrolled a cohort of PDAC patients after surgical resection \( (n = 149) \) and conducted immunohistochemistry experiments to explore the expression profile of ACTG1. Univariate and multivariate analyses were performed to investigate the clinical relevance of ACTG1. The functional role of ACTG1 in PDAC progression was further validated via both in vitro and in vivo studies.

Results: ACTG1 presented a higher expression in PDAC tissues than in nontumorous pancreatic tissues. ACTG1 level positively correlated with tumor stage, implying its potential role as a tumor promoter. Univariate and multivariate analyses identified that patients with lower ACTG1 showed a better overall survival compared to those with higher ACTG1 expression. Cellular and xenograft experiments confirmed the role of ACTG1 on facilitating tumor proliferation both in vitro and in vivo.

Conclusions: Our study revealed a pro-oncogenic role of ACTG1 in PDAC, which may help predict prognosis and serve as a novel therapeutic target.

Keywords: ACTG1, pancreatic adenocarcinoma, prognosis, proliferation
distinct prognosis, which is largely dependent on tumor heterogeneity. Therefore, identifying novel molecular prognostic biomarkers of PDAC is invaluable for prognosis prediction and personalized therapy.

Actins are highly conserved proteins that play critical roles in cell motility in almost all eukaryotic cells. There are three major isoforms of actin in eukaryotes, including alpha, beta, and gamma. Among them, actin gamma 1 (ACTG1) exists in most cell types as a cytoskeleton component, thus regulating cell motility. Mutations in ACTG1 had been reported to induce nonsyndromic hearing loss and Baraitser-Winter syndrome. Besides, ACTG1 interference can upregulate p-P65 level as well as suppress Akt phosphorylation in human nucleus pulposus cells, thus regulating the development of intervertebral disc degeneration. Of note, the correlation between ACTG1 and malignancies has been speculated due to its critical function in modulating cell proliferation, migration, and invasion. For example, ACTG1 exhibits significantly higher expression in skin cancer tissues than nontumorous skin tissues, and bioinformatic analyses suggested its role in regulating skin cell proliferation and migration through ROCK signaling pathway. ACTG1 is also overexpressed in hepatocellular carcinoma (HCC) tissues compared with adjacent normal tissues and can promote HCC proliferation by accelerating cell cycle, which is modulated by the upstream regulators, including Ras-related, associated with diabetes (RRAD) as well as microRNAs. Similarly, ACTG1 participates in the proliferation process of ovarian cancer cells and glioblastomas. However, the expression and function of ACTG1 in pancreatic cancer remain unknown.

Here in this study, we initially explored the protein expression profile of ACTG1 in human PDAC and identified its clinical significance on predicting patients’ overall survival. Furthermore, we conducted in vitro and in vivo assays to determine its involvement in PDAC proliferation.

**MATERIALS AND METHODS**

**Ethics**

This study had been approved by the Institutional Review Board of Chongqing Xinqiao Hospital. Each participant fully understood and signed an informed consent form. Animal studies were also approved by the Institutional Review Board of Chongqing Xinqiao Hospital and carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

**Patients’ enrollment and data collection**

We collected a retrospective cohort of patients to analyze protein expression of ACTG1 in PDAC. All cases underwent curative R0 surgical resection in our hospital. All patients were pathologically confirmed as PDAC without distant metastasis. Accordingly, we collected 149 cases to assess their clinicopathological characteristics and follow-up. Patients’ basic information and clinicopathological characteristics were obtained for all the cases [Table 1].

The mRNA expression level of ACTG1 (presented as transcripts per million, TPM) was extracted from GEPIA (http://gepia.cancer-pku.cn) website according to the RNAseq data from TCGA database and GTEx database, including 179 pancreatic cancer tissues and 171 normal pancreatic tissues.

**Immunohistochemistry staining**

The tumor samples from all the 149 cases were obtained and formalin-fixed and paraffin-embedded for immunohistochemistry (IHC) staining. Briefly, specimen sections were dewaxed and rehydrated, incubated in 3% H₂O₂ for blocking endogenous peroxidase activity. Next, the sections were incubated in citrate buffer (pH 6.0) overnight at 4°C, followed by sequential incubation with secondary antibodies and diaminobenzidine. After being counterstained with hematoxylin, stained sections were evaluated by two independent pathologists. The staining

| Table 1: Expression level of ACTG1 protein in PDAC patients |
|----------------|-----------------|-----------------|----------|
| Variables      | Cases (n=149)   | ACTG1 level     | P        |
| Age (years)    |                 |                 |          |
| ≤60            | 62              | 32              | 30       | 0.792 |
| >60            | 87              | 43              | 44       |       |
| Sex            |                 |                 |          |
| Female         | 51              | 25              | 26       | 0.817 |
| Male           | 98              | 50              | 48       |       |
| Location       |                 |                 |          |
| Head           | 92              | 42              | 50       | 0.146 |
| Body or tail   | 57              | 33              | 24       |       |
| Pathological grade |           |                 |          |
| Grade 1-2      | 95              | 53              | 42       | 0.077 |
| Grade 3        | 54              | 22              | 32       |       |
| T stage        |                 |                 |          |
| T1             | 38              | 26              | 12       | 0.032*|
| T2             | 66              | 28              | 38       |       |
| T3             | 45              | 21              | 24       |       |
| Vessel invasion|                 |                 |          |
| Negative       | 99              | 50              | 49       | 0.954 |
| Positive       | 50              | 25              | 25       |       |
| Lymph node metastasis |           |                 |          |
| Negative       | 87              | 40              | 47       | 0.207 |
| Positive       | 62              | 35              | 27       |       |

Note: * Statistically significant by Chi-square test.
intensity was scored as 1 (no), 2 (mild), 3 (moderate), and 4 (strong). The percentage of positively stained cells was scored as 1 (0–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The total score was obtained by multiplying the two scores above, ranging 1–16. According to the receiver operating characteristic (ROC) curve, all patients were sub-grouped into low-ACTG1 group (IHC score ≤8.5, n = 75) or high-ACTG1 group (IHC score >8.5, n = 74).

Cell culture and shRNA transduction

Human PDAC cell lines PANC-1 and BxPC-3 were obtained from the American Type Culture Collection (ATCC). BxPC-3 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin. PANC-1 and HEK293 cells were cultured in DMEM medium supplemented with 10% FBS and penicillin-streptomycin. All cells were incubated in an incubator with a humidified atmosphere of 5% CO2 at 37°C.

ACTG1-specific shRNA lentiviruses (ACTG1-KD#1 and ACTG1-KD#2) and scrambled control lentiviruses containing nonspecific shRNA were synthesized by Sigma-Aldrich, as reported previously. The shRNA transduction was conducted according to the manufacture’s standard procedure and validated by Western blotting.

Western blotting

Cells were lysed by NP-40 lysis buffer and centrifuged at 12,000 rpm for 30 min to obtain the supernatants. Protein lysates were subjected to SDS-PAGE and transferred onto the PVDF membrane. Blotting membranes were then blocked with 5% BSA and incubated with primary antibodies (ACTG1, #ab200046, Abcam; GAPDH, #2118, Cell Signaling Technology) at 1:1000 dilution overnight at 4°C. The horseradish peroxidase-conjugated secondary antibodies were then added to incubate for another 45 min at 25°C. The signal detection was achieved using super-sensitive enhanced luminol-based chemiluminescent solution.

Cell proliferation

Cell counting kit-8 (CCK-8, Dojindo, Osaka, Japan) was introduced to measure the proliferative capacity of PDAC cells. Briefly, transduced cells were seeded in 96-well plates and cultured for different time points (day 1, 2, 3, and 4). Each well was then treated with 10 μl of CCK-8 reagent, followed by incubation at 37°C for another 1 h before the detection of absorbance at 450 nm by using a microplate reader.

In vivo xenografts

BALB/c nude mice at 4 weeks old were purchased for in vivo xenograft assay. Briefly, 5 × 10^6 stable-transduced PANC-1 and BxPC-3 cells were subcutaneously injected into the mice. After 5 days, the tumor diameter was measured by a vernier caliper every 5 days for 1 month, and the tumor volume was calculated according to the following formula: Volume = (π × length × width^2)/6. After 1 month, all mice were sacrificed, and the subcutaneous xenografts were isolated and pictured.

Statistics

Statistical analyses were conducted with the SPSS 20.0 Software. Associations between ACTG1 expression and clinicopathological characteristics were tested by Chi-square test. Kaplan–Meier analyses and log-rank test were used to evaluate the overall survival of enrolled PDAC cases. The multivariate Cox regression model was used to identify independent prognostic factors. Student’s t-test was selected to validate the statistical difference of cellular experiments. All cellular experiments were conducted in triplicate and repeated three times. P < 0.05 was considered as statistically significant.

RESULTS

Patients’ information

Our retrospective cohort includes 149 PDAC patients. Briefly, the median age of all cases was 68 years, ranging from 39–87 years. Among them, 51 cases were females, and 98 cases were males. Nighty-two cases possessed tumors on the head of the pancreas, while the other 57 cases were on the pancreatic body or tail location. As for the pathological differentiation grade, 23 cases were identified as well-differentiated (grade I), 72 cases as moderately-differentiated (grade II), and the other 54 cases as poorly differentiated (grade III). The tumor size information was also retrieved and classified according to the TNM staging system. Accordingly, 38 cases were detected with stage T1, 66 cases with stage T2, and the other 45 cases with stage T3. Among all the 149 cases, 87 cases showed negative lymph node metastasis, while the other 62 cases exhibited positive lymph node metastases. Although 99 cases showed no vascular invasion according to the surgical record, up to 50 cases were identified with positive vessel invasion, and superior mesenteric vessel as the most frequently involved vessel.

ACTG1 expression in PDAC tissues

The mRNA levels of ACTG1 in PDAC tissues and normal pancreatic tissues were extracted from GEPIA online server (http://gepia.cancer-pku.cn). As a result, ACTG1-mRNA level was significantly higher in PDAC tissues than that in nontumorous pancreatic normal tissues were extracted from GEPIA (Figure 1a, P < 0.001). Moreover, patients...
with higher ACTG1-mRNA level exhibited poorer disease-free survival according to the Kaplan-Meier survival curves [Figure 1b, \( P = 0.021 \)]. The results implied that ACTG1 may be upregulated in PDAC and played oncogenic roles, which encouraged us to explore its protein expression pattern. As revealed by IHC experiments,
ACTG1 protein is widely expressed in the cytoplasm of both normal pancreas and PDAC tissues. However, ACTG1 exhibited distinct expression levels in different PDAC tissues [Figure 1c, d]. To better illustrate the clinical relevance of ACTG1 in PDAC, we divided the cohort into high- and low-ACTG1 expression groups according to the ROC curve [Figure 1e, P < 0.001]. Thus, the high-ACTG1 group contained 74 patients, while the low-ACTG1 group contained 75 cases.

## Correlations between ACTG1 expression and patients’ characteristics

To further probe the possible role of ACTG1 in PDAC progression, we firstly assessed ACTG1 expression. As shown in Table 1, patients with more advanced T stage exhibited significantly higher ACTG1 protein level (P = 0.032), indicating its possible involvement in tumor growth. In contrast, no significant correlation was found between ACTG1 with age, gender, location, pathological grade, vessel invasion, or lymph node metastasis (all P > 0.05).

Since online database indicated an unfavorable role of ACTG1 on predicating PDAC’s clinical outcomes [Figure 1b], using our retrospective cohort, we assessed the clinical significance of ACTG1 as well as other clinicopathological parameters [Table 2]. Accordingly, neither patients’ age [Figure 2a, P = 0.338] nor patients’ gender [Figure 2b, P = 0.586] exhibited significant effect on the overall survival time after surgical resection. On the other hand, tumors located at the head of pancreas resulted in poorer overall survival compared to those located on the body or tail of pancreas [Figure 2c, P = 0.012]. As expected, a poor differentiation grade also correlated with shorter overall survival time [Figure 2d, P = 0.004]. The tumors’ T stage was another prognostic factor as more advanced T stage indicated poorer overall survival [Figure 2e, P = 0.002]. Besides, patients with positive vascular invasion [Figure 2f, P < 0.001] or lymph node metastasis [Figure 2g, P = 0.042] also exhibited poorer overall survival. Of note, higher ACTG1 was correlated with a significantly poorer overall survival (mean survival time 16.9 ± 1.9 months) compared to those with lower ACTG1 (mean survival time 28.0 ± 1.8 months) [Figure 2h, P < 0.001].

Multivariate analysis [Table 3] revealed ACTG1 as a novel independent prognostic biomarker of PDAC (HR = 2.433, 95% CI 1.629–3.634, P < 0.001), highlighting its potential role in predicting overall survival. Other independent prognostic factors included tumor location (HR = 0.653, 95% CI 0.430–0.992, P = 0.046), pathological grade (HR = 1.562, 95% CI 1.041–2.344, P = 0.031), T stage (HR = 1.954, 95% CI 1.214–3.148, P = 0.006), vessel invasion (HR = 2.242, 95% CI 1.458–3.449, P < 0.001), as well as lymph node metastasis (HR = 1.895, 95% CI 1.234–2.909, P = 0.003).

## ACTG1 enhances PDAC growth both in vitro and in vivo

To further validate the tumor-related role of ACTG1 in PDAC, we conducted knockdown assays in both PANC-1 and BxPC-3 human PDAC cell lines [Figure 3a]. According to the immunoblotting data, the expression level of ACTG1 decreased to about 30% in knockdown groups (ACTG1-KD#1 and ACTG1-KD#2) compared to the scrambled group (control) in BxPC-3 cells. Similarly, the knockdown efficiency of ACTG1-shRNAs reached to about 50% in PANC-1 cells. We next subjected the transfected cells to proliferation assay by CCK-8 method. As shown in Figure 3b, silencing ACTG1 significantly inhibited the proliferation capacities of both PANC-1 and BxPC-3 cells.

We next constructed the xenograft models by subcutaneously injecting control cells (transfected with scrambled shRNA) and experimental cells (transfected with ACTG1-KD#1-shRNA), respectively. By monitoring the in vivo tumor growth curve, we found that the xenografts of ACTG1-KD#1 group showed a significantly slower growth rate in both PANC-1 and BxPC-3 xenografts [Figure 3c]. Finally, we isolated the subcutaneous xenografts and

### Table 2: Overall survival of PDAC patients

| Variables                        | Cases (n=149) | Overall survival months | P     |
|----------------------------------|--------------|-------------------------|-------|
| Age (years)                      |              |                         |       |
| ≤60                              | 62           | 23.5%                   | 22.2±2.5 | 0.338 |
| >60                              | 87           | 31.0%                   | 23.4±1.7 |       |
| Sex                              |              |                         |       |
| Female                           | 51           | 27.7%                   | 21.3±2.2 | 0.586 |
| Male                             | 98           | 28.1%                   | 24.1±2.0 |       |
| Location                         |              |                         |       |
| Head                             | 92           | 21.8%                   | 19.6±1.6 | 0.012* |
| Body or tail                     | 57           | 37.7%                   | 27.9±2.6 |       |
| Pathological grade               |              |                         |       |
| Grade 1-2                        | 95           | 33.4%                   | 26.5±2.1 | 0.004* |
| Grade 3                          | 54           | 19.5%                   | 17.7±1.9 |       |
| T stage                          |              |                         |       |
| T1                                | 38           | 45.4%                   | 28.2±2.5 | 0.002* |
| T2                                | 66           | 23.2%                   | 23.5±2.4 |       |
| T3                                | 45           | 18.6%                   | 16.6±2.3 |       |
| Vessel invasion                  |              |                         |       |
| Negative                         | 99           | 37.8%                   | 27.6±2.0 | <0.001*|
| Positive                         | 50           | 7.1%                    | 14.3±1.7 |       |
| Lymph node metastasis            |              |                         |       |
| Negative                         | 87           | 35.5%                   | 25.9±2.1 | 0.042* |
| Positive                         | 62           | 17.5%                   | 17.9±1.5 |       |
| ACTG1 level                      |              |                         |       |
| Low                               | 75           | 42.5%                   | 28.0±1.8 | <0.001*|
| High                             | 74           | 12.6%                   | 16.9±1.9 |       |

Note: * Statistically significant by log-rank test.
confirmed that silencing ACTG1 can indeed suppress PDAC growth [Figure 3d].

**DISCUSSION**

Actins are highly conserved proteins that play roles in maintaining cytoskeleton and cell motility. Although the beta isoform, β-actin, is commonly recognized as a housekeeping gene in various cell types, the gamma actin ACTG1 seems distinctly expressed in different tissues. Dysregulated expression of ACTG1 has been reported in various diseases such as rheumatoid arthritis,[22] osteoarthritis,[23] and pulmonary hypertension.[24] Of note, ACTG1 showed diverse expressions and functions in different malignancies. While both the mRNA and protein levels of ACTG1 were down-regulated in ovarian cancer tissues compared to the nontumorous ovarian tissues, its higher mRNA level indicated improved clinical outcomes.[11] The function of ACTG1 in ovarian cancers seems similar to that in substantia nigra cells. As reported by Liu et al.[25] in a Parkinson’s disease model, capsaicin treatment induced upregulation of ACTG1. Moreover, overexpression of ACTG1 increased the cell apoptosis in their Parkinson’s disease cell model.

On the other hand, upregulated ACTG1 expression was identified in hepatocellular carcinoma, which was correlated with poorer overall survival by enhancing cell proliferation and inhibiting cell apoptosis.[9,26] Similarly, uterine cancers harbored 5%–20% of ACTG1 gene amplification or overexpression, whose higher expression was correlated with unfavorable prognosis.[27] Intriguingly, bioinformatic analyses revealed that high expression of ACTG1 may promote colon adenocarcinoma cell growth, but was relative to higher survival rate.[28] Therefore, detailed and specific roles of ACTG1 in distinct cancer types needs further investigation. According to our data, ACTG1 was significantly upregulated in PDAC tissues compared with nontumorous pancreatic tissues on both mRNA and protein levels. Moreover, PDAC patients with higher ACTG1 protein level exhibited poorer overall survival compared to those with lower ACTG1 expression. Multivariate analysis also validated the independent effect of ACTG1 on negatively affecting PDAC patients’ overall survival.

As for the functional mechanism, ACTG1 can affect both cell proliferation and apoptosis. For example, downregulation of hsa-miR-497-5p, the upstream regulator of ACTG1, was reported to be correlated with unfavorable prognosis of hepatocellular carcinoma by promoting cell proliferation.[29] Furthermore, COX10-AS1 (COX10 antisense RNA 1) can promote glioblastoma cell proliferation and inhibited glioblastoma apoptosis through upregulating ACTG1 at a miR-361-5p dependent manner, further highlighting the therapeutic value of targeting ACTG1 in malignancies.[12] Consistently, in this study, we proved that ACTG1 can promote PDAC progression via
enhancing cell growth both in vitro and in vivo. Silencing ACTG1 resulted in impaired cell viability and decreased tumor growth capacity. Besides proliferation and apoptosis, ACTG1 may also participate in regulating cell migration and invasion. For example, single-cell analysis indicated that ACTG1 might be involved in the regulation of renal...
cancer metastasis.\(^\text{29}\) Although our clinical data did not find any significant correlation between ACTG1 expression with vascular invasion or lymph node metastasis, whether ACTG1 participates in the metastasis of PDAC remains to be further investigated.

In conclusion, ACTG1 is remarkably associated with the postoperative prognosis of PDAC, in that higher ACTG1 can serve as an independent unfavorable prognostic biomarker. \textit{In vitro} and \textit{in vivo} data validated that ACTG1 can promote PDAC progression at least partially by enhancing tumor growth.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30.
2. Luo G, Fan Z, Gong Y, Jin K, Yang C, Cheng H, et al. Characteristics and outcomes of pancreatic cancer by histological subtypes. Pancreas 2019;48:817-22.
3. Kamisawa T, Wood LD, Itoi T, Takaori K. Pancreatic cancer. Lancet (London, England) 2016;388:73-85.
4. Ideno N, Mori Y, Nakamura M, Ohtsuka T. Early detection of pancreatic cancer: Role of biomarkers in pancreatic fluid samples. Diagnostics (Basel, Switzerland) 2020;10:1056.
5. van Wijk E, Krieger E, Kemperman MH, De Leenheer EM, Huygen PL, Rivière JB, van Bon BW, Hoischen A, Kholmanskikh SS, O’Roak BJ, Cremers CW, et al. A mutation in the gamma actin 1 (ACTG1) gene causes autosomal dominant hearing loss (DFNA20/26). J Med Genet 2003;40:879‑84.
6. Rivière JB, van Bon BW, Hoischen A, Kholmanskikh SS, O’Roak BJ, Gilissen C, et al. De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. Nat Genet 2012;44:440‑4, S1-2.
7. Wu T, Jia X, Feng H, Wu D. ACTG1 regulates intervertebral disc degeneration via the NF-κB-p65 and Akt pathways. Biochem Biophys Res Commun 2021;545:54-61.
8. Dong X, Han Y, Sun Z, Xue J. Actin Gamma 1, a new skin cancer pathogenic gene, identified by the biological feature-based classification. J Cell Biochem 2018;119:1406‑19.
9. Yan Y, Xu H, Zhang L, Zhou X, Qian X, Zhou J, et al. RRAD suppresses the Warburg effect by downregulating ACTG1 in hepatocellular carcinoma. Onco Targets Ther 2019;12:1691‑703.
10. Gao B, Li S, Tan Z, Ma I, Liu J. ACTG1 and TLR3 are biomarkers for alcohol-associated hepatocellular carcinoma. Oncol Lett 2019;17:1714‑22.
11. Chen S, Wang X, Yuan J, Deng C, Xie X, Kang J. Reduced levels of actin gamma 1 predict poor prognosis in ovarian cancer patients. J Obstet Gynaecol Res 2020;46:1827‑34.
12. Zhou C, Jiang X, Liang A, Zhu R, Yang Y, Zhong I, et al. COX10-AS1 facilitates cell proliferation and inhibits cell apoptosis in glioblastoma cells at post-transcription level. Neurochem Res 2020;45:2196‑203.
13. Sun S, Xu L, Zou Q, Wang G. BPARNAseq: A babysitter package for retrospective and newly generated RNA-seq data analyses using both alignment-based and alignment-free quantification method. Bioinformatics 2021;37:1319‑21.
14. Niu M, Lin Y, Zou Q. sgRNAACNN: Identifying sgRNA on-target activity in four crops using ensembles of convolutional neural networks. Plant Mol Biol 2021;105:483‑95.
15. Zou Q, Xing P, Wei L, Liu B. Gene2vec: Gene subsequence embedding for prediction of mammalian N6-methyladenosine sites from mRNA. RNA 2019;25:205‑18.
16. Liu H, Xu Y, Zhang Q, Yang H, Shi W, Liu Z, et al. Prognostic significance of TBL1XR1 in predicting liver metastasis for early stage colorectal cancer. Surg Oncol 2017;26:13‑20.
17. Liu H, Gong Z, Li K, Zhang Q, Xu Z, Xu Y. SRPK1/2 and PI3Kα exert opposite functions by modulating SRSF1-guided MKRN2 alternative splicing in colon adenocarcinoma. J Exp Clin Cancer Res 2021;40:1‑16.
18. Pang X, Gong K, Zhang X, Wu S, Cui Y, Qian B-Z. Osteopontin as a multifaceted driver of bone metastasis and drug resistance. Pharmacol Res 2019;144:235‑44.
19. Pan D, Xia X-X, Zhou H, Jin S-Q, Lu Y-Y, Liu H, et al. COCO enhances the efficiency of photoreceptor precursor differentiation in early human embryonic stem cell-derived retinal organoids. Stem Cell Res Ther 2020;11:1‑12.
20. Chen T, Liu H, Liu Z, Li K, Qin R, Wang Y, et al. FGF19 and FGF4 promotes the progression of gallbladder carcinoma in an autocrine pathway dependent on GPRB1-cAMP-EGR1 axis. Oncogene 2021;40:4941‑53.
21. Van Roessel S, Kasumova GG, Verheij J, Najarian RM, Maggino I, De Pastena M, et al. International validation of the eighth edition of the American Joint Committee on Cancer (AJCC) TNM staging system in patients with resected pancreatic cancer. JAMA Surg 2018;153:e183617.
22. Liu J, Chen N. A 9 mRNAs-based diagnostic signature for rheumatoid arthritis by integrating bioinformatic analysis and machine-learning. J Orthop Surg Res 2021;16:44.
23. Li C, Luo J, Xu X, Zhou Z, Ying S, Liao X, et al. Single cell sequencing revealed the underlying pathogenesis of the development of osteoarthritis. Gene 2020;757:144939.
24. Wu M, Wu Y, Huang J, Wu Y, Wu H, Ji Q, et al. Protein expression profile changes of lung tissue in patients with pulmonary hypertension. PLoS One 2020;15:e021853.
25. Liu J, Liu H, Zhao Z, Wang J, Guo D, Liu Y. Regulation of Actg1 and Gsta2 is possible mechanism by which capsaicin alleviates apoptosis in cell model of 6-OHDA-induced Parkinson’s disease. Biosci Rep 2020;40:Ibr20191796. doi: 10.1042/bsr20191796.
26. Tian L-I, Qian B, Jiang X-H, Liu Y-S, Chen T, Jia C-Y, et al. MicroRNA-497-5p is downregulated in hepatocellular carcinoma and associated with tumorigenesis and poor prognosis in patients. Int J Genomics 2021;2021:6670390.
27. Richter C, Mayhew D, Rennhack JP, So J, Stover EH, Hwang JH, et al. Identification of differently expressed genes associated with prognosis and growth in colon adenocarcinoma based on integrated bioinformatics analysis. Front Genet 2019;10:1245.
28. Liu K, Gao R, Wu H, Wang Z, Han G. Single-cell analysis reveals metastatic cell heterogeneity in clear cell renal cell carcinoma. J Cell Mol Med 2021;25:4260‑74.