The non-coding RNA (ncRNA)-mediated high expression of polycomb group factor 1 (PCGF1) is a prognostic biomarker and is correlated with tumor immunity infiltration in liver hepatocellular carcinoma

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Background: Liver hepatocellular carcinoma (LIHC) has a poor prognosis worldwide. Polycomb group factor 1 (PCGF1) was recently reported to play a tumor suppressive role in cancers. However, the molecular mechanism and competitive endogenous ribonucleic acid (ceRNA) regulatory networks of PCGF1 in LIHC are still unclear.

Methods: We constructed a PCGF1 ceRNA regulatory network in LIHC and identified potential prognostic markers, especially for tumor immunity. We identified the gene expression profiles and conducted correlation and survival analyses of PCGF1 and the related RNAs. We also explored the clinicopathological features and diagnostic and prognostic values of PCGF1 and constructed a nomogram to predict 1-, 3-, and 5-year survival. Based on a variety of bioinformatics tools, we confirmed the PCGF1-related signaling pathways in LIHC. Finally, the role of PCGF1 in immune cell infiltration was also analyzed.

Results: We found that PCGF1 was overexpressed in LIHC (P<0.001) and was linked to a poor prognosis in terms of overall survival (OS, P=0.029), the progress-free interval (PFI, P=0.002), and disease-free survival (DFS, P=0.02). Hsa-miR-22-3p was highly negatively correlated with PCGF1. Further, 3 upstream long non-coding RNAs (lncRNAs) (i.e., AC016405.3, BX284668.6, and MIR4435-2HG) were confirmed to further research. PCGF1 was positively associated with pathologic tumor stages (P=0.001), histologic grade (P=0.030), alpha fetoprotein (AFP) level (P=0.030), and vascular invasion (P=0.022). The area under the curve of PCGF1 was 0.983 [confidence interval (CI): 0.972–0.994]. In the multivariate analyses, high PCGF1 expression remained an independent factor associated with OS [hazards ratio (HR): 1.696, P=0.027], DSS (HR: 2.139, P=0.024), and the PFI (HR: 1.512, P=0.034). We found that PCGF1 was involved in some malignancy-associated signaling pathways and plays a role in regulating the immune response.
Introduction

Liver hepatocellular carcinoma (LIHC) is common worldwide and has a poor prognosis, which places a huge burden on the global public health system, especially in China (1,2). In 2018, there were about 841,300 newly diagnosed liver cancer cases and 782,000 deaths, accounting for nearly 4.7% of all new cancer patients and 8.2% of all cancer deaths (3). LIHC accounts for more than 75% of new liver cancer cases and over 50% of those cases occur in China (4,5).

The leading risk factors for LIHC vary and include hepatitis B virus (HBV), hepatitis C virus (HCV), food contaminated with aflatoxin, long-term smoking, alcohol abuse, type 2 diabetes, and obesity (3,5). The influence of these major risk factors varies by region. In China and Eastern Africa, chronic HBV infection and frequent aflatoxin exposure are the main causes of LIHC, while in Japan and Egypt, HCV infection is the main cause of LIHC (1-3).

The curative treatment regimens for LIHC include surgery and radiofrequency ablation (5-7), but these are only applicable to patients with early-stage LIHC. Indeed, curative surgery is not suitable for advanced LIHC patients, for whom the standard of care includes transarterial chemoembolization, radiotherapy, and systemic therapy, such as chemotherapy, targeted therapy, and new immunotherapeutic agents (8-11). In recent decades, due to the implementation of a comprehensive multidisciplinary approach, the survival rate of LIHC patients has improved. However, the survival rate of LIHC is still lower than expected, as some patients show multicentric recurrence after curative treatment (5,11,12). Thus, novel therapeutic targets and promising prognostic biomarkers need to be identified for LIHC.

Polycomb group factor 1 (PCGF1), which is also known as nervous system polycomb1 (NSPc1), was first identified in 2001 (13,14). PCGF1 is a novel mammalian polycomb gene, belongs to the polycomb group (PcG) protein family, and plays a significant role in the development of the nervous system (15). The PcG protein family forms multiprotein complexes, which are classified as polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2) (16-18). These complexes function by suppressing gene transcription through epigenetic remodeling and were originally identified in drosophila melanogaster (16-18). Among the various PCGF proteins, PRC1 is typically involved in canonical polycomb repressive complex 1 (cPRC1) and non-canonical polycomb repressive complex 1 (ncPRC1) (16,19). As one of the members of the PRC1, PCGF1 is a protein coding gene and is overexpressed in the developing nervous system (15). The RING finger domain of PCGF1 is 93% homologous to Bmi1 (PCGF4) (13,18,20). Thus, PCGF1 may have similar functions to Bmi1, as it shares the same domain architecture (21).

Previous studies on PCGF1 have focused on the developing nervous system and stem cell function (22). The abnormal expression of PCGF1 has been implicated in cancer stem cell phenotypes and has been shown to promote tumorigenesis in various types of cancers (21,22). PCGF1 was shown to be overexpressed in colorectal cancer (CRC) and was linked to cancer progression and a poor prognosis (14). In CRC, PCGF1 contributes to stem cell enrichment and induces the activation of stem cell biomarkers (14). Research has shown that the downregulation of PCGF1 in gliomas leads to the inactivation of the c-myc signaling pathway and reduces cell proliferation (15). PCGF1 expression is increased in several cancer types (14) and regulates the ability of stem cell self-renewal by targeting retinol dehydrogenase 16 (RDH16) in glioma cells (21); however, to date, no studies have explored its prognostic and diagnostic value and the regulatory network of PCGF1 in LIHC. Additionally, the expression, prognosis, and mechanism of PCGF1, and the role of tumor immune infiltration in LIHC remain unclear.

In this research, we performed PCGF1 expression and survival analyses in human cancer. We also investigated the

Conclusions: We confirmed the upstream ceRNA regulatory network of PCGF1 in LIHC. PCGF1 has an oncogenic effect and correlates with tumor immunity.

Keywords: Non-coding RNA (ncRNA); liver hepatocellular carcinoma (LIHC); polycomb group factor 1 (PCGF1); prognosis; immunity infiltration

Submitted Jun 10, 2022. Accepted for publication Aug 18, 2022.
doi: 10.21037/atm-22-3862
View this article at: https://dx.doi.org/10.21037/atm-22-3862
regulation of micro ribonucleic acids (miRNAs) and long non-coding RNAs (lncRNAs) related to PCGF1 in LIHC. Finally, we explored the value of PCGF1 in immune cells. In summary, we found links between non-coding RNA (ncRNA) and the upregulation of PCGF1, which may serve as a biomarker and regulate immune cell infiltration in LIHC. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3862).

Methods

RNA-seq data source availability and ethics statement

PCGF1 expression levels in 20 cancers were identified in The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). The RNA sequencing (RNA-seq) transcriptomic data [level 3 HTSeq—fragments per kilobase per Million (FPKM)] were converted to transcripts per million reads (TPM) format and log\(^2\) values for the study (23). Additionally, clinical information was retained, and duplicate samples were removed. Subsequently, we also extracted the PCGF1 data from the University of California Santa Cruz (UCSC) XEUC database (https://xenabrowser.net/datapages/), RNA-seq data in TPM format from TCGA, and genotype-tissue expression (GTEx) data uniformly processed by the Toil process (24). The RNA-seq data in the TPM format of TCGA cancer samples and the corresponding normal tissues of the GTEX were extracted, and the expression levels of the samples after log\(^2\) transformation were compared. The miRNA data for LIHC were also extracted from TCGA. According to the median expression level of PCGF1, all the data were divided into high and low expression groups. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Survival analysis of PCGF1 in cancers

A Kaplan-Meier (KM) analysis was conducted to determine the prognostic value of the PCGF1 characteristics and survival. We also extracted the survival data and clinical information of cancer patients from an article for subsequent analysis (25).

StarBase database analysis for candidate miRNA and lncRNA prediction

The StarBase database (http://starbase.sysu.edu.cn/) is widely used to determine target-miRNA interactions (26). We used it to analyze the correlations between ncRNA and gene expression in LIHC. We first predicted the upstream binding miRNAs of PCGF1 and confirmed the candidate miRNA-PCGF1 to predict the potential lncRNAs that might bind to miRNA-pcGF1. We also examined the correlations between lncRNA, miRNA, and PCGF1 expression in LIHC. Additionally, TCGA data were used to test the relationship between the expression levels and clinical outcomes using the R tool (version 3.6.3).

Correlations with PCGF1 expression and its clinical value in LIHC

Wilcoxon rank-sum tests and a logistic analysis were conducted to analyze the correlations between clinicopathological features and PCGF1 expression (27). The receiver operating characteristic (ROC) curve showed that PCGF1 had a good predictive value. Cox regression modeling was carried out to identify prognostic factors. Using the “rms” package of R (version 3.6.3) and a multivariate Cox model, a nomogram for predicting 1-, 3- and 5-year survival was established. The calibration curve was established, and the prediction probability of the nomogram was estimated. The consistency index (C-index) was calculated to evaluate the prediction accuracy of the nomogram.

Enrichment analysis and gene set enrichment analysis (GSEA)

Differentially expressed genes (DEGs) with a |log fold change (FC)| value >1.5 were identified using R language-related software DESeq2 (version 1.26.0) (27), and an adjusted P value less than 0.05 was set as the cutoff value for DEG identification. The “ClusterProfiler” software package was used to conduct the Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, and “ggplot2” software package was used to visualize the results (28). A GSEA (29) was conducted using R package ClusterProfiler (Version 3.14.3) (28) to confirm the significant functions and pathways (30). The terms with an adjusted P value less than 0.05 and a false discovery rate (FDR) value less than 0.25 were selected.

Immune infiltration analysis in LIHC

A single-sample GSEA (ssGSEA) was performed with the
GSVA package (version 1.34.0) (31), and the infiltration of 24 immune cells was estimated according to the inferred immune characteristics (32). The correlations between PCGF1 expression and these immune cells were evaluated by calculating the Pearson's correlation coefficients. The enrichment scores of the immune cells in the related samples were compared using the Wilcoxon rank sum test. A P value less than 0.05 was considered statistically significant.

Statistical analysis

R Statistics Software Package (version 3.6.3) was used for the statistical analysis. In the PCGF1 expression analysis, the Wilcoxon rank-sum test was used to compare the expression levels of PCGF1 in the tumor and normal tissues. The PCGF1 expression levels were defined as high or low based on the median value. The relationship between PCGF1 and clinicopathology was analyzed using the Wilcoxon rank-sum test and a logistic regression analysis. We conducted a Cox regression analysis to confirm the effects of the clinicopathology variables on survival time. A P value less than 0.05 was considered statistically significant.

Results

Expression levels of PCGF1 in cancers

We used TCGA data to investigate the expression levels of PCGF1 and its predictive value in different cancers. As Figure 1A shows, PCGF1 was more upregulated in tumor tissues than normal tissues, including LIHC, bladder urothelial carcinoma, kidney renal clear cell carcinoma (KIRC), cholangiocarcinoma, stomach adenocarcinoma (STAD), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma, breast invasive carcinoma (BRCA), kidney renal papillary cell carcinoma (KIRC), lung adenocarcinoma (LUAD), thyroid carcinoma (THCA), lung squamous cell carcinoma (LU_SC), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), and uterine corpus endometrial carcinoma (UCEC) (P<0.05; see Figure 1A). However, no significant difference in PCGF1 expression was observed in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), and pancreatic adenocarcinoma (PAAD) (P>0.05; see Figure 1A). Further, we extracted the PCGF1 data from the UCSC XENA database (http://xena.ucsc.edu/) and found that PCGF1 was also significantly more upregulated in LIHC, COAD READ, STAD, PAAD, UCEC, LUSC, PRAD, ESCA, KIRC, BRCA, CESC, and LUAD compared to TCGA and the GTEx corresponding normal controls (P<0.05; see Figure 1B-1M). To sum up, we found that PCGF1 was overexpressed in several cancers, and it may be a key driver of carcinogenesis in cancer.

Predicted upstream miRNAs of PCGF1 in LIHC

There is increasing evidence that ncRNAs are actively involved in gene expression regulation (33). Thus, we sought to determine whether PCGF1 was also regulated by ncRNAs. First, we used the StarBase database to predict the upstream miRNAs of PCGF1. A total of 16 possible upstream miRNAs were predicted to potentially target PCGF1. For better visualization, a miRNA-PCGF1 regulatory network was created using Cytoscape software (see Figure 3A). As a negative regulator of gene expression, miRNA plays an important role in many biological processes. As Figure 3B shows, among all the upstream miRNAs, hsa-miR-22-3p was negatively correlated with PCGF1, and hsa-miR-23c was positively correlated with PCGF1 in LIHC. Subsequently, we evaluated the expression and prognostic value of hsa-miR-22-3p in LIHC using TCGA data, which was more overexpressed in normal tissues than LIHC tissues (P<0.05; see Figure 3C) and linked...
Figure 1 PCGF1 expression levels in cancers. (A) PCGF1 expression levels in LIHC and other types of human cancers based on TCGA database. ns, P≥0.05; **, P<0.01; ***, P<0.001. (B-M) PCGF1 expression levels in in PRAD, STAD, COAD READ, UCEC, LIHC, LUSC, KIRC, CESC, ESCA, PAAD, LUAD, and BRCA based on the UCSC XENA database. *, P<0.05; ***, P<0.001. PCGF1, polycomb group factor 1; TPM, transcripts per million; TCGA, The Cancer Genome Atlas; ns, no significance; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, esophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma; UCSC XENA database (http://xena.ucsc.edu/).
Figure 2 Clinical outcomes of PCGF1 expression in LIHC and COAD READ. (A-C) OS, PFI, and DSS in LIHC; (D-F) OS, PFI, and DSS in COAD READ; (G) OS in COAD; and (H) OS in READ. HR, hazards ratio; LIHC, liver hepatocellular carcinoma; PCGF1, polycomb group factor 1; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; OS, overall survival; PFI, progress-free interval; DSS, disease-specific survival.

Figure 2 Clinical outcomes of PCGF1 expression in LIHC and COAD READ. (A-C) OS, PFI, and DSS in LIHC; (D-F) OS, PFI, and DSS in COAD READ; (G) OS in COAD; and (H) OS in READ. HR, hazards ratio; LIHC, liver hepatocellular carcinoma; PCGF1, polycomb group factor 1; COAD, colon adenocarcinoma; READ, rectum adenocarcinoma; OS, overall survival; PFI, progress-free interval; DSS, disease-specific survival.

to unfavorable DSS (P=0.002; see Figure 3D), the PFI (P=0.001; see Figure 3E), and OS (P<0.001; see Figure 3F). Thus, upstream hsa-miR-22-3p appears to act as tumor suppressive miRNA, and the hsa-miR-22-3p-PCGF1 axis may be the potential pathway in LIHC.

Upstream lncRNAs of hsa-miR-22-3p in LIHC

LncRNAs act as competitive endogenous RNAs (ceRNAs) and interact with messenger RNAs (mRNAs) via the miRNA binding sites. Next, to ascertain whether hsa-miR-22-3p was modulated by some lncRNAs, we used the
Figure 3 Hsa-miR-22-3p was identified as a potential upstream miRNA of PCGF1 in LIHC. (A) The miRNA-PCGF1 network was established by Cytoscape. (B) The expression correlation analysis for miRNA-PCGF1 in LIHC using the StarBase database. (C) The expression of hsa-miR-22-3p in TCGA-LIHC. The DSS (D), PFI (E) and OS (F) of hsa-miR-22-3p in TCGA-LIHC. ***, P<0.001. miRNA, micro ribonucleic acid; TPM, transcripts per million; LIHC, liver hepatocellular carcinoma; PCGF1, polycomb group factor 1; TCGA, The Cancer Genome Atlas; DSS, disease-specific survival; PFI, progress-free interval; OS, overall survival.

StarBase database to examine the upstream lncRNAs of hsa-miR-22-3p. The regulatory network of lncRNA and hsa-miR-22-3p is shown in Figure 4. We used TCGA data sets to examine the expression levels and predictive values of selected lncRNAs. Only 4 lncRNAs (i.e., LINC01184, AC016405.3, BX284668., and MIR4435-2HG) were
significantly higher in LIHC (P<0.001; see Figure 5A) and were correlated with a poorer OS (P<0.05; see Figure 5B-5E). Additionally, overexpressed MIR4435-2HG indicated poor DSS (P=0.011; see Figure 5F), and high levels of LINC01184 (P=0.02; see Figure 5G) and AC016405.3 (P=0.035; see Figure 5H) were linked to a poor PFI in LIHC. Taken together, based on the classical sponge theory/competitive ceRNA hypothesis, these results indicate that lncRNA levels are inversely linked to miRNA levels and positively linked to mRNA levels. We used the StarBase database to examine the correlation between 4 lncRNAs and hsa-miR-22-3p or PCGF1 expression in LIHC (see Table 1). We found that AC016405.3, BX284668.6 and MIR4435-2HG downregulate hsa-miR-22-3p, and increase the expression of PCGF1 in LIHC.

**Correlation between PCGF1 expression and clinical characteristics in LIHC patients**

To investigate the relationship between PCGF1 expression and the clinical features of LIHC, we compared the clinical features of 371 patients with LIHC and divided them into high and low PCGF1 expression groups (see Table 2). As Table 2 and Figure 6A-6E show, the high expression of PCGF1 is closely related to tumor (T) stage (T1 vs. T2, T3, & T4, P<0.001), pathologic stage (Stage I vs. Stages II, III, & IV, P=0.001), vascular invasion (no vs. yes, P=0.012), histologic grade (G1 & G2 vs. G3 & G4, P=0.002), and alpha fetoprotein (AFP) level (≤400 vs. >400 ng/mL, P=0.045). Additionally, a statistically significant correlation was found with node (N) stage, metastasis (M) stage, Child-Pugh grade, age, gender, residual tumor, and race in PCGF1-high (P<0.05, see Figure S2A-2G). To further confirm that the expression of PCGF1 was associated with the poor prognosis of LIHC patients, we performed a logistic regression analysis (see Table 3). High PCGF1 expression levels were positively correlated with pathologic stage [Stages I, II, & IV vs. Stage I, OR: 2.239, 95% confidence interval (CI): 1.461–3.451, P=0.001], histologic grade (G3 & G4 vs. G1 & G2, OR: 1.606, 95% CI: 1.047–2.473, P=0.030), AFP level (>400 vs. ≤400 ng/mL, OR: 1.878, 95% CI: 1.070–3.345, P=0.030), and vascular invasion (yes vs. no, OR: 1.731, 95% CI: 1.085–2.777, P=0.022).

**The diagnostic and prognostic value of PCGF1 expression in LIHC**

To better understand the clinical benefits of PCGF1 evaluations, a ROC curve analysis was performed to determine its prognostic value. The area under the curve (AUC) of PCGF1 for identifying tumors from normal

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**Figure 4** The network of lncRNA-hsa-miR-22-3p established by Cytoscape. lncRNA, long non-coding RNA.
tissues was 0.983 (95% CI: 0.972–0.994). Thus, PCGF1 has a high value in terms of its diagnostic sensitivity and specificity (see Figure 6F). Additionally, the univariate Cox analysis showed that the high expression of PCGF1 was related to a decrease in OS [hazards ratio (HR): 1.692, P=0.006], DSS (HR: 1.756, P=0.022), and the PFI (HR: 1.683, P=0.001) (see Table 4).

To further examine the survival-related factors, we used a Cox proportional-risk regression model for the multivariate analysis. High PCGF1 expression remained an independent factor associated with poor OS (HR: 1.696, P=0.024), DSS (HR: 2.139, P=0.024), and the PFI (HR: 1.512, P=0.034).
Table 1 The correlations between lncRNA and miRNA or lncRNA and PCGF1 in LIHC was analyzed using the StarBase database

| miRNA/PCGF1 | LncRNA         | R value | P value     |
|-------------|----------------|---------|-------------|
| hsa-miR-22-3p | LINC01184       | 0.003   | 9.55E–01    |
| hsa-miR-22-3p | AC016405.3      | −0.323  | 1.89E–10    |
| hsa-miR-22-3p | BX284668.6      | −0.186  | 3.26E–04    |
| hsa-miR-22-3p | MIR4435-2HG     | −0.168  | 1.16E–03    |

Gene

| miRNA/PCGF1 | LncRNA         | R value | P value     |
|-------------|----------------|---------|-------------|
| PCGF1       | LINC01184      | 0.073   | 1.57E–01    |
| PCGF1       | AC016405.3     | 0.291   | 9.59E–09    |
| PCGF1       | BX284668.6     | 0.224   | 1.20E–05    |
| PCGF1       | MIR4435-2HG    | 0.3     | 3.08E–09    |

lncRNA, long non-coding RNA; miRNA, micro ribonucleic acid; PCGF1, polycomb group factor 1; LIHC, liver hepatocellular carcinoma.

Table 2 Relationship between PCGF1 expression and clinicopathological features in patients with LIHC

| Characteristics                | Low expression of PCGF1 (N=185) | High expression of PCGF1 (N=186) | P value |
|--------------------------------|---------------------------------|---------------------------------|---------|
| Age, median (IQR)              | 61.5 (51, 69)                   | 61 (52, 68)                     | 0.759   |
| Age, n (%)                     |                                 |                                 | 0.914   |
| ≤60                            | 87 (23.5)                       | 90 (24.3)                       |         |
| >60                            | 97 (26.2)                       | 96 (25.9)                       |         |
| Gender, n (%)                  |                                 |                                 | 0.130   |
| Female                         | 53 (14.3)                       | 68 (18.3)                       |         |
| Male                           | 132 (35.6)                      | 118 (31.8)                      |         |
| Race, n (%)                    |                                 |                                 | 0.204   |
| Asian                          | 73 (20.3)                       | 85 (23.7)                       |         |
| Black or African American      | 7 (1.9)                         | 10 (2.8)                        |         |
| White                          | 101 (28.1)                      | 83 (23.1)                       |         |
| T stage, n (%)                 |                                 |                                 | 0.001   |
| T1                             | 109 (29.6)                      | 72 (19.6)                       |         |
| T2                             | 35 (9.5)                        | 59 (16.0)                       |         |
| T3                             | 34 (9.2)                        | 46 (12.5)                       |         |
| T4                             | 5 (1.4)                         | 8 (2.2)                         |         |
| N stage, n (%)                 |                                 |                                 | 0.622   |
| N0                             | 125 (48.8)                      | 127 (49.6)                      |         |
| N1                             | 1 (0.4)                         | 3 (1.2)                         |         |

Table 2 (continued)
Table 2 (continued)

| Characteristics                  | Low expression of PCGF1 | High expression of PCGF1 | P value |
|----------------------------------|-------------------------|--------------------------|---------|
| M stage, n (%)                   |                         |                          | 1.000   |
| M0                               | 133 (49.3)              | 133 (49.3)               |         |
| M1                               | 2 (0.7)                 | 2 (0.7)                  |         |
| Pathologic stage, n (%)          |                         |                          | 0.001   |
| Stage I                          | 102 (29.4)              | 69 (19.9)                |         |
| Stage II                         | 34 (9.8)                | 52 (15)                  |         |
| Stage III                        | 33 (9.5)                | 52 (15.0)                |         |
| Stage IV                         | 3 (0.9)                 | 2 (0.6)                  |         |
| Residual tumor, n (%)            |                         |                          | 0.044   |
| R0                               | 166 (48.5)              | 158 (46.2)               |         |
| R1                               | 4 (1.2)                 | 13 (3.8)                 |         |
| R2                               | 1 (0.3)                 | 0 (0)                    |         |
| Histologic grade, n (%)          |                         |                          | 0.057   |
| G1                               | 35 (9.6)                | 20 (5.5)                 |         |
| G2                               | 91 (24.9)               | 86 (23.5)                |         |
| G3                               | 51 (13.9)               | 71 (19.4)                |         |
| G4                               | 6 (1.6)                 | 6 (1.6)                  |         |
| AFP (ng/mL), n (%)               |                         |                          | 0.040   |
| ≤400                             | 115 (41.4)              | 98 (35.3)                |         |
| >400                             | 25 (9.0)                | 40 (14.4)                |         |
| Child-Pugh grade, n (%)          |                         |                          | 0.497   |
| A                                | 112 (46.9)              | 105 (43.9)               |         |
| B                                | 9 (3.8)                 | 12 (5.0)                 |         |
| C                                | 1 (0.4)                 | 0 (0)                    |         |
| Vascular invasion, n (%)         |                         |                          | 0.029   |
| No                               | 115 (36.5)              | 91 (28.9)                |         |
| Yes                              | 46 (14.6)               | 63 (20.0)                |         |

PCGF1, polycomb group factor 1; LIHC, liver hepatocellular carcinoma; IQR, interquartile range; AFP, alpha fetoprotein.

The analysis also showed that pathologic stage (Stage I vs. Stages II, III, & IV) had predictive advantages for OS (HR: 2.154, P=0.002), and the PFI (HR: 1.775, P=0.002) in LIHC patients. T stage (T1, T2, & T3 vs. T4) (HR: 3.656, P=0.038) and pathologic stage (Stage I vs. Stages II, III, & IV) (HR: 2.705, P=0.003) also showed the similar trend in DSS.

Next, as Figure 7A-7F show, a nomogram was constructed to predict the 1-, 3-, and 5-year OS, PFI, and DSS in TCGA-LIHC. Pathological stage and PCGF1 were incorporated into the nomogram to predict OS (C-index: 0.624) and the PFI (C-index: 0.656). A DSS predictive nomogram was established by T stage, pathological stage and PCGF1, and the C-index was 0.714. A calibration curve was drawn to verify the effectiveness of the nomogram.
Figure 6 Correlation of PCGF1 expression with clinical pathological characteristics in LIHC patients and ROC curve of PCGF1. (A) T stage; (B) pathologic stage; (C) vascular invasion; (D) histologic grade; and (E) AFP (ng/mL). (F) The ROC curve of PCGF1 had an AUC value of 0.983 between LIHC tissues and normal tissues. *, P<0.05; **, P<0.01; ***, P<0.001. PCGF1, polycomb group factor 1; TPM, transcripts per million; AFP, alpha fetoprotein; FPR, false positive rate; TPR, true positive rate; AUC, area under the curve; CI, confidence interval; LIHC, liver hepatocellular carcinoma; ROC, receiver operating characteristic.

Table 3 Logistic regression models estimated the association between PCGF1 and clinical pathologic characteristics

| Characteristics                              | Total (N) | OR       | P value |
|----------------------------------------------|-----------|----------|---------|
| T stage (T3 & T4 vs. T1 & T2)                | 368       | 1.522 (0.949–2.459) | 0.083   |
| N stage (N1 vs. N0)                          | 256       | 2.953 (0.372–60.132) | 0.351   |
| M stage (M1 vs. M0)                          | 270       | 1.000 (0.119–8.434) | 1.000   |
| Pathologic stage (Stages II, III, & IV vs. Stage I) | 347   | 2.239 (1.461–3.451) | <0.001  |
| Gender (male vs. female)                     | 371       | 0.697 (0.449–1.077) | 0.105   |
| Race (Black or African American & White vs. Asian) | 359 | 0.740 (0.486–1.122) | 0.157   |
| Age (>60 vs. ≤60)                            | 370       | 0.957 (0.636–1.439) | 0.832   |
| Residual tumor (R1 & R2 vs. R0)              | 342       | 2.732 (1.005–8.675) | 0.062   |
| Histologic grade (G3 & G4 vs. G1 & G2)       | 366       | 1.606 (1.047–2.473) | 0.030   |
| AFP (ng/mL) (>400 vs. ≤400)                  | 278       | 1.878 (1.070–3.345) | 0.030   |
| Child-Pugh grade (B & C vs. A)               | 239       | 1.280 (0.530–3.152) | 0.583   |
| Vascular invasion (yes vs. no)               | 315       | 1.731 (1.085–2.777) | 0.022   |

PCGF1, polycomb group factor 1; OR, odds ratio; AFP, alpha fetoprotein.
### Table 4 Cox regression analysis for OS, DSS, PFI in LIHC patients

| Characteristics                          | HR (95% CI) for OS | P value | HR (95% CI) for DSS | P value | HR (95% CI) for PFI | P value |
|------------------------------------------|--------------------|---------|---------------------|---------|---------------------|---------|
|                                          | Univariate analysis|         | Multivariate analysis|         | Univariate analysis|         | Multivariate analysis|         | Multivariate analysis|         | P value |
| PCGF1                                    | 1.692              | 0.006   | 1.696               | 0.027   | 1.756              | 0.022   | 2.139               | 0.024   | 1.683              | 0.001   | 1.512   | 0.034   |
|                                          | (1.166–2.455)      |         | (1.063–2.704)       |         | (1.085–2.842)      |         | (1.107–4.131)       |         | (1.228–2.307)      |         | (1.031–2.219)     |
| T stage (T1 & T2 & T3 vs. T4)            | 3.681              | <0.001  | 2.489               | 0.073   | 6.012              | <0.001  | 3.656               | 0.038   | 2.747              | 0.002   | 1.921   | 0.151   |
|                                          | (1.912–7.085)      |         | (0.920–6.735)       |         | (2.839–12.732)     |         | (1.077–12.414)      |         | (1.443–5.230)      |         | (0.788–4.684)     |
| N stage (N0 vs. N1)                      | 2.004              | 0.333   |                     |         | 3.562              | 0.080   | 1.896               | 0.389   | 1.385              | 0.648   |         |         |
|                                          | (0.491–8.181)      |         |                     |         | (0.858–14.785)     |         | (0.442–8.135)       |         | (0.342–5.611)      |         |         |
| M stage (M0 vs. M1)                      | 4.032              | 0.018   | 1.331               | 0.700   | 5.102              | 0.025   | 0.905               | 0.913   | 3.442              | 0.037   | 1.776   | 0.394   |
|                                          | (1.267–12.831)     |         | (0.311–5.697)       |         | (1.230–21.161)     |         | (0.148–5.503)       |         | (1.080–10.967)     |         | (0.474–6.653)     |
| Pathologic stage (Stage I vs. Stages II, III, & IV) | 2.074              | <0.001  | 2.154               | 0.002   | 2.887              | <0.001  | 2.705               | 0.003   | 2.310              | <0.001  | 1.775   | 0.002   |
|                                          | (1.418–3.032)      |         | (1.340–3.462)       |         | (1.705–4.888)      |         | (1.390–5.253)       |         | (1.683–3.170)      |         | (1.225–2.574)     |
| Histologic grade (G1 vs. G2, G3, & G4)   | 1.216              | 0.443   |                     |         | 1.231              | 0.524   | 1.211               | 0.369   |                     |         |         |         |
|                                          | (0.738–2.004)      |         |                     |         | (0.649–2.335)      |         | (0.798–1.838)       |         |                     |         |         |         |
| Residual tumor (R0 vs. R1 & R2)          | 1.571              | 0.194   | 1.640               | 0.246   | 1.515              | 0.167   |                     |         |                     |         |         |         |
|                                          | (0.795–3.104)      |         | (0.711–3.782)       |         | (0.841–2.730)      |         |                     |         |                     |         |         |         |
| AFP (ng/mL) (≤400 vs. >400)              | 1.056              | 0.827   | 0.849               | 0.625   | 1.056              | 0.793   |                     |         |                     |         |         |         |
|                                          | (0.646–1.727)      |         | (0.442–1.634)       |         | (0.705–1.581)      |         |                     |         |                     |         |         |         |
| Age (≤60 vs. >60)                        | 1.248              | 0.214   | 0.880               | 0.573   | 0.952              | 0.740   |                     |         |                     |         |         |         |
|                                          | (0.880–1.768)      |         | (0.566–1.370)       |         | (0.710–1.275)      |         |                     |         |                     |         |         |         |
| Gender (female vs. male)                 | 0.816              | 0.260   | 0.840               | 0.452   | 0.973              | 0.861   |                     |         |                     |         |         |         |
|                                          | (0.573–1.163)      |         | (0.533–1.323)       |         | (0.713–1.327)      |         |                     |         |                     |         |         |         |
| Race (Asian vs. Black or African American & White) | 1.316              | 0.146   | 1.469               | 0.117   | 1.225              | 0.188   |                     |         |                     |         |         |         |
|                                          | (0.909–1.906)      |         | (0.908–2.377)       |         | (0.905–1.658)      |         |                     |         |                     |         |         |         |

OS, overall survival; DSS, disease-specific survival; PFI, progress-free interval; LIHC, liver hepatocellular carcinoma; HR, hazards ratio; CI, confidence interval; PCGF1, polycomb group factor 1; AFP, alpha fetoprotein.
Figure 7 Construction and validation of nomograms based on PCGF1 expression and other clinical factors. A nomogram for predicting the probability of 1-, 3- and 5-year OS (A), DSS (C) and PFI (E). Calibration plots of the nomogram for predicting the OS (B), DSS (D) and PFI (F) likelihood. PCGF1, polycomb group factor 1; LIHC, liver hepatocellular carcinoma; OS, overall survival; PFI, progress-free interval; DSS, disease-specific survival.
Identification of the high and low PCGF1-related DEGs in LIHC

To study the differential expression of PCGF1 between the high and low expression groups, we first identified 532 DEGs based on a $|\log_2 FC| > 1.5$ and an adjusted $P$ value <0.05. Of the 532 DEGs, 422 were upregulated and 120 were downregulated (see Figure 8A). The top 20 genes that were positively or negatively correlated with PCGF1 are presented in a heatmap (see Figure 8B).

Functional annotation and predicted signaling pathways of PCGF1-associated DEGs

To predict the functional enrichment implications of PCGF1 in LIHC, the ClusterProfile software package was used to analyze the enrichment of GO and KEGG pathways. GO analysis was performed based on an adjusted $P$ value less than 0.05 and $Q$ value less than 0.2 to divide them into a biological process (BP) group (229 items), cellular composition (CC) group (44 items), and a molecular function (MF) group (49 items) (see Figure 8C). The KEGG pathways were mainly involved in some oncogenic signaling pathways, such as the Ras and PI3K-AKT signaling pathways (see Figure 8C).

Related signaling pathways of PXGF1 based on GSEA

To further quantify the role of PCGF1 in tumorigenesis and progression, a GSEA was performed based on a $p$ adjusted value <0.05 and a FDR <0.25 in the Molecular Signatures Database (MSigDB) [c2.all.v7.0.symbols.gmt (Curated)]. The GSEA analysis showed that the PCGF1-associated DEGs were significantly enriched in cancer pathways (see Figure 9A-9F), such as the PI3K-AKT signaling pathway, the Wingless/Integrated (WNT) signaling pathway,
PCGF1-related signaling pathways were analyzed by GSEA. NES, normalized enrichment score; FDR, false discovery rate; PCGF1, polycomb group factor 1; GSEA, gene set enrichment analysis.

**Role of PCGF1 in immune cells based on the ssGSEA**

Tumor-infiltrating lymphocytes (TILs) are considered independent predictors of adverse OS in cancer patients (34,35). The relationship between PCGF1 expression and infiltration levels in 24 immune cell subtypes in the LIHC microenvironment was studied by a Wilcoxon rank-sum test. The enrichment score of the T cells, B cells, a cluster of differentiation 8 (CD8) T cells, cytotoxic cells, dendritic cells (DCs), iDCs, macrophages, mast cells, neutrophils, natural killer (NK) CD56dim cells, NK cells, pDCs, T central memory (Tcm), T effector memory (Tem), T gamma delta (Tgd), T helper cell 1 (Th1) cells, and regulatory T cells (Tregs) were enriched in the low PCGF1 group (P<0.05; see Figure 10A). Next, we conducted a Spearman analysis to explore the relationship between PCGF1 and the infiltration levels of TILs. We observed that PCGF1 expression was negatively associated with mast cells (r=-0.276; P<0.001), DCs (r=-0.273; P<0.001), B cells (r=-0.277; P<0.001), neutrophils (r=-0.282; P<0.001), and cytotoxic cells (r=-0.289; P<0.001) (see Figure 10B-10G).

Further, we conducted a GSEA to quantify the immune-related-associated signaling pathways of PCGF1. PCGF1-high expression was significantly enriched in the interleukin (IL)-12, IL-4 and IL-13, and IL-10 signaling pathways, CD22 mediated B cell receptor (BCR) regulation, neutrophil pathways, and interferon-gamma pathways (see Figure 11A-11F). In summary, PCGF1 appears to play a role in the regulation of the tumor immune response.

**Discussion**

LIHC is a major aggressive cancer of the digestive system worldwide, which is common in patients with chronic liver disease and cirrhosis (36). There have been some marked improvements in the management of LIHC since
Figure 10  The relationship of immune cell infiltration between PCGF1 expression in LICHC. (A) Enrichment scores of 24 immune cell in high- and low-PCGF1 expression groups based on the Wilcoxon rank-sum test. ns, P ≥ 0.05; *, P<0.05; **, P<0.01; ***, P<0.001. (B) The correlations between PCGF1 expression and the infiltration levels of 24 immune cells using the Spearman analysis. (C-G) The negatively expressed correlations between PCGF1 and mast cells, DC, B cells, neutrophils, and cytotoxic cells. aDC, activated dendritic cell; CD8, cluster of differentiation 8; iDC, immature dendritic cell; NK, natural killer; pDC, Plasmacytoid dendritic cells; Tcm, T central memory; Tem, T effector memory; TFH, T follicular helper; Tgd, T gamma delta; Th1, T helper cell 1; Th17, T helper cell 17; Th2, T helper cell 2; Treg, regulatory T cell; PCGF1, polycomb group factor 1; TPM, transcripts per million; LIHC, liver hepatocellular carcinoma; DC, dendritic cell.
Figure 11 (A-F) Immune-related signaling pathways mediated by PCGF1 expression using a GSEA. PCGF1, polycomb group factor 1; GSEA, gene set enrichment analysis; NES, normalized enrichment score; FDR, false discovery rate.

2015, especially in the rapidly evolving field of systemic therapies, such as radiofrequency ablation, microwave ablation, cryoablation, and immunotherapy (37-40). With the development of a comprehensive treatment mode, the quality of life and OS of LIHC patients have been significantly improved. However, a useful predictive or prognostic LIHC biomarker has yet to be identified for use in daily practice, especially in immunotherapy (41).

PCGF1 is a member of the PCG protein family and plays an important role in the development of the mammalian nervous system. Studies have confirmed that PCGF1 plays an important role in the self-renewal of embryonic stem cells and is expressed in many tumors (13,21). The molecular mechanisms of PCGF1 in LIHC progression remain speculative and require further investigation. In our previous research, we found that PCGF1 was overexpressed in glioma, and is a prognostic biomarker related to tumor immunity (42). In this study, we conducted a comprehensive expression and survival analysis based on TCGA data and found that PCGF1 is highly expressed in several cancers.

The KM (OS, DSS, and the PFI) analyses showed that high PCGF1 expression was associated with poor survival in LIHC. Ji et al. found that PCGF1 is upregulated in CRC and is associated with decreased OS rates (14). We also described the upregulation of PCGF1 in CRC tissues. In summary, our observations suggest that PCGF1 functions as a candidate oncogene and is unlikely to be limited to LIHC and CRC.

There is accumulating evidence that various ncRNAs play key roles in the pathogenesis of human cancer (43). It is increasingly recognized that ncRNAs interact with each other via a ceRNA network and crosstalk to regulate gene expression (44). MiRNAs are widely expressed in human cells and can regulate gene expression as negative regulators (45). Thus, we used the online StarBase tool to explore the upstream regulatory miRNAs of PCGF1. Ultimately, a total of 16 miRNAs were identified as upstream regulatory oncogenic miRNAs of PCGF1 in LIHC. These miRNAs appear to work as tumor suppressor miRNAs.

We also performed expression validation and survival
analyses and found a high negative correlation between hsa-miR-22-3p and PCGF1 in LIHC. We found ample evidence that hsa-miR-22-3p plays a key role in the development of various tumor cells (46,47). Chen et al. (48) showed that hsa-miR-22-3p acts as a tumor suppressor that inhibits cell proliferation and induces cell cycle arrest and apoptosis by targeting specificity protein 1 (SP1) in LIHC. Further, catalpol negatively regulates MTA3 through hsa-miR-22-3p in LIHC cells to achieve an antigrowth effect (49).

We also used the StarBase database to examine the upstream lncRNAs of the hsa-miR-22-3p/PCGF1 axis in LIHC. Under the ceRNA network and crosstalk hypothesis, upstream lncRNAs should be overexpressed in LIHC and act as oncogenic lncRNAs. Based on the expression, survival, and correlation analyses, only AC016405.3, BX284668.6, and MIR4435-2HG were identified as potential regulators of the hsa-miR-22-3p-PCGF1 axis and as functioning as oncogenes in LIHC.

In BRCA, AC016405.3 acts as an oncogenic lncRNA by targeting hsa-miR-22-3p to regulate ERBB3 (50). However, Ren et al. (51) reported that AC016405.3 is downregulated in glioblastoma and targets miR-19a-5p to regulate the expression of Ten-Seven Translocation 2 (TET2) and inhibit cell proliferation and metastasis. BX284668.6 is highly expressed in TCGA-LIHC and is associated with a poor prognosis, but the function and mechanism of BX284668.6 in cancers are unknown. This needs to be explored further.

MIR4435-2HG enhances the development of LIHC (52,53). For example, Shen et al. (53) reported that MIR4435-2HG is involved in tumor proliferation and metastasis through the miR-22-3P/YWHAZ axis, which is related to a poor prognosis and overexpression. Based on these results, AC016405.3, BX284668.6, and MIR4435-2HG/hsa-miR-22-3p/PCGF1 axis were used to construct a ceRNA network axis in LIHC.

Previous studies have shown that high PCGF1 expression is related to clinicopathological features and a poor prognosis (14,42). Additionally, similar results were observed in relation to LIHC, including in relation to the pathologic stage, histologic grade, AFP level, and vascular invasion. PCGF1 may be more predictive in early and late periods of cancer. In addition, for a biomarker to be successful, it must have appropriate detection accuracy. In recent years, while we have been keen to study novel biomarkers in serum or tissue biomarkers, their utility in clinical practice has been limited, except for AFP (41). Thus, we need to constantly search for biomarkers that can be used in the clinical diagnosis and prediction of LIHC. PCGF1 showed a promising diagnostic value for LIHC and had an AUC of 0.983 in the ROC analyses. We also constructed a predictive nomogram that included PCGF1 expression, T stage, and pathologic stage to determine the OS, DSS, and PFI of LIHC patients. Physicians can use this nomogram to identify high-risk patients. Thus, PCGF1 has the potential as a diagnostic and prognostic indicator of LIHC.

PcG complexes are involved in diverse BPs, including cell stages, cellular signaling and cancer, and positively regulate gene transcription, and modify non-histone substrates (54). PCGF4 (BMI1) is an important oncogene in CRC, which can downregulate the expression of cyclin-dependent kinase inhibitor 2A (CDKN2A) (55). Additionally, the gene product of PCGF2 (Mel-18) is very similar to that of PCGF4 in structure and is also a member of the PCG family (42). In gastric cancer, the expression of PCGF4 and PCGF2 were negatively correlated. Unlike PCGF4, which is an oncogene, PCGF2 acts as a tumor suppressor (56-57). Conversely, PCGF6 acts as a master regulator to Nanog homeobox (Nanog), octamer-binding transcription factor 4 (Oct4), and SRY-related HMG box transcription factor 2 (Sox2) expression in embryonic stem cells (58). Yan et al. reported that PCGF1 function is strongly and mechanistically associated with tumorigenesis and progression, and the c-Myc signaling pathway takes part in regulating cell proliferation in glioblastoma (15). We speculated that the abnormal expression of PCGF1 interferes with the transmission of various signal pathways related to tumorigenesis and progression. However, the role of PCGF1 in the occurrence and development of LIHC requires further study.

We then conducted a bioinformatics analysis to confirm the biological function and mechanisms of PCGF1 in LIHC. We found that PCGF1 was mainly involved in ion channel activity, gated channel activity, the immunoglobulin complex, the extracellular matrix, and neuron-to-neuron synapses. The KEGG analysis and the additional GSEA showed that PCGF1 expression was associated with the Ras, PI3K-AKT, FOXO, and WNT signaling pathways, autophagy, and the cell cycle. The literature demonstrated that these pathways play a pivotal role in tumorigenesis and progression, including proliferation, stemness, neovascularization, apoptosis, and epithelial-mesenchymal transition (59-63). Based on our findings, we hypothesized that PCGF1 functions as a proto-oncogene by promoting invasiveness and metastasis in LIHC; however, the detailed
mechanisms should be tested in other experiments.

The tumor microenvironment (TME) is a complex ecology in which immune cells are critical components. In the TME, immune cells can have divergent effects on tumorigenesis (e.g., anti-tumorigenic and pro-tumorigenic effects) according to the environment and tumor type (64). During cancer development and progression, the variation of TME components controlled by the host immune system can affect the immunophenotype and disease progression (64,65). In our previous study, we found that the expression of PCGF1 in glioma was positively correlated with Th2 cells, and negatively correlated with T follicular helper (TFH) enrichment, traditional Chinese medicine, and Tgd (42). However, our LIHC data demonstrated that PCGF1 expression was negatively correlated with mast cells, DCs, B cells, neutrophils, and cytotoxic cells. Further, several immune-related pathways were confirmed by GSEA, such as the IL-12, IL-4, IL-13, and IL-10 signaling pathways, CD22-mediated BCR regulation, neutrophil pathways, and interferon-gamma pathways. The function of B cells in the TME can predict the poor prognosis of cancer, and the production of cytokines promotes immune suppressive phenotypes in tumor aggression (64). Neutrophils can release VEGF to promote tumor angiogenesis in the peritumoral stroma of LIHC (66). DCs harbor the potential to recognize, capture, and present antigens to T cells, which serve as immune system sentinels. However, the TME can combine with DCs to block its function and promote tumor progression (64,65).

Above all, we speculate that PCGF1 is a biomarker for predicting immune cell infiltration and a potential therapeutic target in LIHC. We identified the main mechanism of PCGF1 in LIHC; however, this study still had some limitations. First, it only used TCGA data, which may indicate bias. Second, to confirm the credibility of the results, our hypothesis needs to be further validated by experiments.

**Conclusions**

In summary, our research showed that PCGF1 is upregulated in multiple cancers and is a diagnostic and prognostic biomarker in LIHC. We further identified the upstream regulatory network of PCGF1, and used lncRNAs (i.e., AC016405.3, BX284668.6, and MIR4435-2HG) to construct a hsa-miR-22-3p/PCGF1 network. Further, our research suggests that PCGF1 may play a crucial role in immune infiltration and act as an oncogene in LIHC.

**Acknowledgments**

The authors appreciate the academic support from the AME Hepatocellular Carcinoma Collaborative Group.

**Funding:** The study was supported by 2021 Shandong Medical Association Clinical Research Fund—Qilu Special Project (No. YXH2022ZX02206).

**Footnote**

**Reporting Checklist:** The authors have completed the TRIPOD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3862/rc

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3862/coif). The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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(English Language Editor: L. Huleatt)

Cite this article as: Liu J, Xu Y, Sun C, Yang S, Xie J, Samant H, Xin X. The non-coding RNA (ncRNA)-mediated high expression of polycomb group factor 1 (PCGF1) is a prognostic biomarker and is correlated with tumor immunity infiltration in liver hepatocellular carcinoma. Ann Transl Med 2022;10(16):898. doi: 10.21037/atm-22-3862