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Original Article

G2M checkpoint pathway alone is associated with drug response and survival among cell proliferation-related pathways in pancreatic cancer

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Abstract: Given the severe side effects of the treatments and poor survival, prognostic and predictive biomarkers to guide management of pancreatic cancer are in critical need. We hypothesized that cell proliferation-related pathways are associated with drug response and survival in pancreatic cancer. Six Hallmark cell proliferation-related gene sets (G2M Checkpoint, E2F Targets, MYC Targets V1 and V2, Mitotic Spindle, p53 pathway) defined by MSigDB in gene set variant analysis were evaluated in 3 independent cohorts- TCGA-PAAD (n = 176), GSE57495 (n = 63), and GSE62452 (n = 69). G2M and E2F, as well as Mitotic and p53 pathway correlated highly with other gene sets. All pathways were significantly correlated with *MKI67* expression and its proliferation score, but none with cytolytic activity and the rate of pathologically complete resection (R0). All pathways were significantly associated with high alteration and expression of KRAS gene except for MYC v1. G2M, E2F, and p53 pathway were significantly associated with high alteration of TP53 gene. Interestingly, different pathways correlated with the AUC of different cancer therapeutics, such as Gemcitabine (Mitotic: r = 0.706 [P = 0.01]), Paclitaxel (MYC v2: r = -0.636 [P < 0.05]), Apatinib (Mitotic: r = -0.556 [P = 0.03]), Palbociclib (E2F: r = 0.675 [P < 0.01]), and Sorafenib (G2M: r = -0.593 [P = 0.03]). Among all six pathways, only G2M was consistently associated with worse patient survival in all three cohorts. In conclusion, each cell proliferation-related pathway was predictive of a unique agent, and the G2M score alone predicts survival in pancreatic cancer.

Keywords: Biomarker, cell cycle, gene sets, gene expression, GSVA, GSEA, tumor microenvironment, pathway analysis, pancreatic cancer, proliferation, Hallmark gene sets, survival, transcriptome

Introduction

Pancreatic cancer is one of the deadliest malignancies known, with a poor five-year survival rate of less than 5% in the United States [1]. Despite advances in imaging technologies and surgical techniques, the majority of patients present with metastatic disease burden, with only 20% of patients presenting with resectable disease. Surgical resection remains the mainstay of curative treatment for pancreatic cancer. Advancements in patient selection for surgery has allowed for improved median survival in patients [3]. For example, the identification of borderline resectability status has allowed surgeons to choose the appropriate candidates for an attempt at complete curative resection with microscopic tumor clearance (R0). In addition, pancreatic cancer resections that result in positive margins (R1 and R2 [R1/2]) are thought to be influenced by aggressive tumor biology and invasiveness of cancer.

These factors appear as influential, if not more, than surgical technique alone in the correlation to survival [2-4].

Understanding the aberrant cell proliferation of pancreas cancer has been heavily studied to identify targeted therapies to cease cancer progression and improve clinical outcomes. Gemcitabine, a cell cycle-specific inhibitor, has been the most common first-line chemotherapy regimen for patients with metastatic pancreatic cancer [5]. CDK inhibitors, such as Palbociclib and Dinaciclib, that target cell proliferation pathways have also been studied as targeted therapies [6, 7]. Treatment with combined CDK4/6 and MEK inhibitors has been used for their inhibitory effect on cell cycle progression while also modulating immune features of pancreatic cancer cells [8].

Unfortunately, the proliferation pathway in pancreatic cancer is hard to define. Adding to its complexity, the high amount of genomic heterogeneity of pancreas cancer and its tumor microenvironment have been associated with resistance to therapy [9, 10]. Thus far, no reliable molecular targets have been identified that can predict or influence the success of treatment. To this end, there is an urgent need to identify diagnostic, prognostic, and predictive biomarkers, as well as to develop more effective and less toxic therapeutic interventions.

The gene set variation analysis (GSVA) is a computational model that can be used to examine gene expression within a pathway rather than a single gene. Utilization of a pathway or gene-set approach more accurately considers gene coordination, increases model simplicity, and can increase the applicability of prediction models. We had previously reported the utility of a pathway score which was calculated by GSVA with Hallmark gene sets in several cancers [11-13], including pancreatic cancer. A high G2M pathway score in pancreatic cancer showed a link to *KRAS* or *TP53* alteration, which are known to be frequently altered, and are associated with poor prognosis [14]. Given this background, we hypothesized in this study that the other proliferation-related gene sets are also associated with worse survival and have unique characteristics in pancreatic cancer patients. We analyzed the pathways associated with characteristics in pancreatic cancer patients and determined which pathway score had the most prognostic value in patients with pancreatic cancer.

Materials and methods

Pancreatic cancer cohorts and their data

In the study, clinical and mRNA expression data of 176 patients who had a pathological diagnosis of pancreatic cancer were obtained from TCGA pancreatic adenocarcinoma (TCGA_PAAD) cohort through Genomic Data Commons Data Portal (GDC) (https://portal.gdc.cancer.gov) [15]. Mutation data were downloaded through cBioportal [16]. To survival analysis, other three cohorts, Chen et al. (GSE57495; n = 63) [17], and Hussain et al. (GSE62452; n = 69) [18] were obtained from the Gene Expression Omnibus (GEO) repository of the US National Institutes of Health (http://www.ncbi.nlm.ih.gov/geo/). In all analyses, the average gene value with multiple probes and gene expression data, which were transformed for $\log_2$ mRNA data for the effect of several drugs on the pancreatic cancer cell line was obtained from the publicly available dataset at CCLE.

Gene set expression analyses

Gene set variation analysis (GSVA) score [19] of the proliferation-related MSigDB Hallmark gene sets [5], including G2M target, E2F checkpoint, MYC v1 and v2, Mitotic, and p53 pathway, were defined as each pathway score using GSVA Bioconductor package (version 3.10), as we previously reported [20-23]. False discovery rate (FDR), as the adjusted p values, less than 0.25 for statistical significance, which was recommended by gene set enrichment analysis (GSEA) software (Lava version 4.0) [24], as we previously reported [25-32].

Statistical analysis

R software (version 4.0.1, R Project for Statistical Computing) and Microsoft Excel (version 16 for Windows) were used in all data analysis and data plotting. To divide the low and high pathway score groups, the median value of each score within cohorts were used. Two-tail Fisher's exact tests were used to analysis of comparisons between groups, as we described in each figure legends. Cox proportional hazard analyses were used to estimate hazard ratio (HR), 95% CI, and p-value. A p-value of less

than 0.05 was taken statistically significant. Tukey type boxplots show median and interquartile level values.

Results

G2M checkpoint and E2F targets scores demonstrated strong correlation and consistency among the 6 cell proliferation-related Hallmark gene sets in pancreatic cancer

Molecular Signatures Database (MSigDB) Hallmark defines 6 gene sets as cell proliferation-related in Gene Set Validation Analysis (GSVA): G2M checkpoint, E2F target, MYC targets v1, MYC targets v2, Mitotic spindle, and p53 pathway [33]. We utilized these cell proliferation-related gene sets as pathway scores and analyzed them in The Cancer Genome Atlas (TCGA) pancreatic adenocarcinoma cohort (TCGA-PAAD, n = 176). The genes included in each score are listed in Table S1. First, we studied how close these 6 scores relate to each other in pancreatic cancer. We found that the correlation of G2M checkpoint pathway score with E2F targets score was the strongest (**Figure 1A**; r = 0.976). On the other hand, the correlation of Mitotic spindle score with MYC targets v2 score was the weakest (r = 0.564). Next, we studied the pathways that constitute these scores in pancreatic cancer. We investigated the correlation by hallmark gene sets, and we used the categories defined by MSigDB Hallmark gene sets: Cell proliferation, Signaling, Pathway, Metabolic, DNA damage, Development, and Cellular component [33]. The gene sets that showed Spearman's rank correlation > 0.600 in each analysis were recognized as highly enriched. As expected, G2M checkpoint, E2F targets, and MYC targets v2 pathway scores were strongly correlated with cell proliferation-related gene sets (**Figure 1B**). On the other hand, Mitotic spindle and p53 pathway scores were highly correlated with signaling gene sets. Immune-related gene sets were not involved in any of the proliferation-related pathway scores. These results suggest that each of the cell proliferation-related pathway scores has unique characteristics.

All six scores were significantly correlated with cell proliferation, but not with immune activity or rate of pathological complete resection (R0)

In clinical practice, cell proliferation is commonly assessed with Ki67 expression, which is encoded by the *MKI67* gene. As expected, all six cell proliferation-related pathways were strongly correlated with *MKI67* expression (**Figure 2A**; all spearman rank correlation (r) > 0.500 and all P < 0.001). The proliferation score, calculated by Thorsson et al. [34], was also correlated with all six cell proliferation-related pathways score (**Figure 2A**). Based upon our previous observation that aggressive breast cancer is counterbalanced with the human immune response [12, 13, 35], it was of interest to investigate whether highly proliferative pancreatic cancer related with immune activity as estimated by cytolytic activity score (CYT) as established by Rooney et al. [36]. None of the cell proliferation scores were correlated with CYT (**Figure 2A**). Since pancreatic cancer with aggressive biology is unlikely to result in pathological complete resection (R0) [2, 4], we investigated whether any of the six scores correlated with less likelihood of achievement of an R0 resection. Surprisingly, none of the six scores were associated with a decreased R0 resection rate (**Figure 2B**).

High scores in G2M checkpoint, E2F targets and p53 pathways are associated with KRAS and TP53 alteration, and the high score of any cell proliferation-related pathways is correlated with gene expression of KRAS and TP53 in pancreatic cancer

As KRAS and TP53 gene mutations are known to contribute to poor prognosis in pancreatic cancer [37], we hypothesized that the cell proliferation-related pathway scores are associated with alteration and expression of these genes. Indeed, all of the high cell proliferation pathway scores in pancreatic cancer, except for the MYC targets v1 score, were significantly associated with a higher percentage of alteration in the KRAS gene (**Figure 3**). A high score of the G2M checkpoint, E2F targets, and p53 pathway was significantly associated with a higher percentage of alteration in the TP53 gene (**Figure 3**; P = 0.026, 0.011, and 0.007, respectively). Furthermore, all six scores were moderately correlated with KRAS gene expression but not with TP53 gene expression (**Figure 3**).

G2M checkpoint pathway score was consistently and strongly associated with worse survival across all three independent cohorts

As we previously reported that enhanced cell proliferation was associated with worse survival in breast [12, 13] and pancreatic cancer [14],

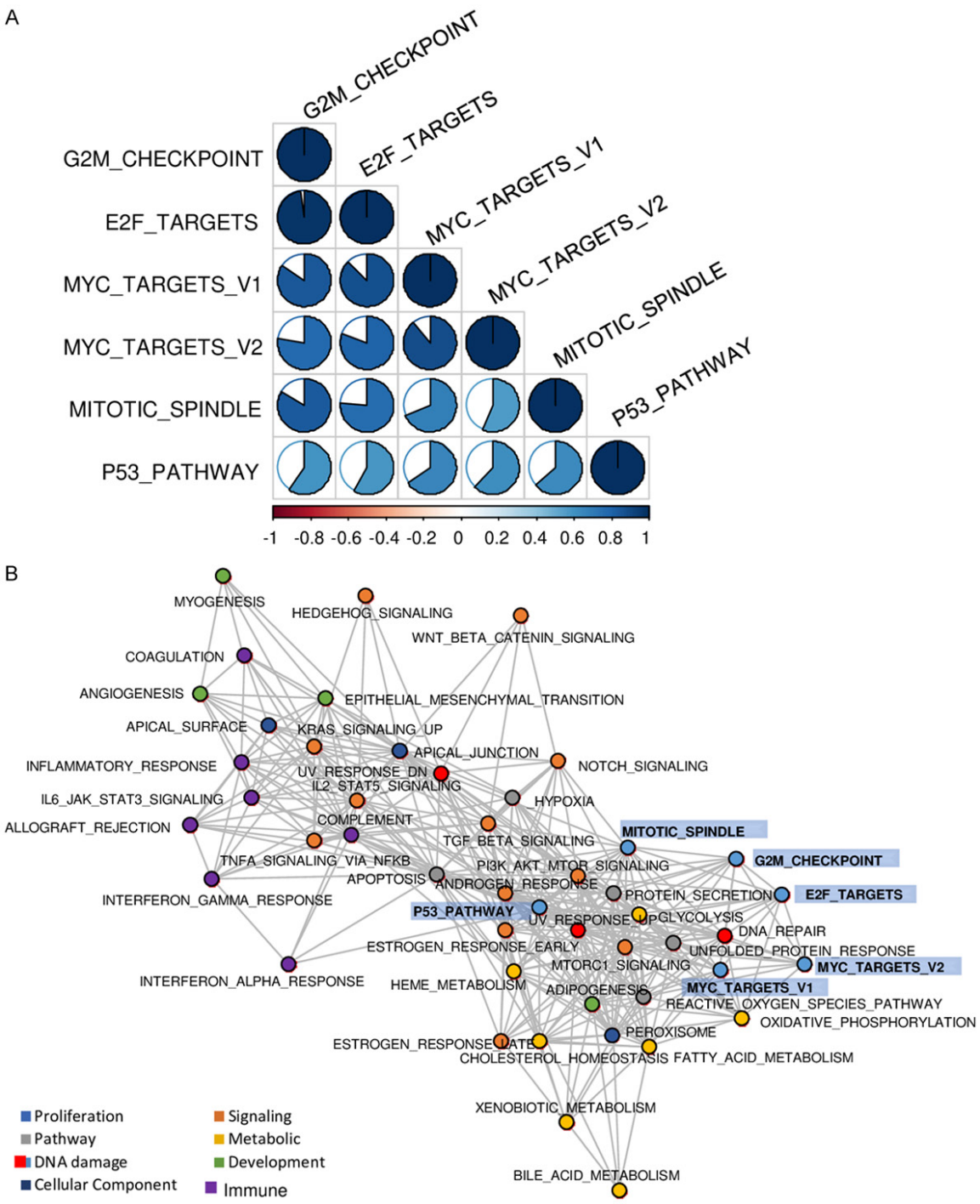


Figure 1. Characteristics of six cell proliferation-related pathway scores in the TCGA pancreatic adenocarcinoma cohort. A. Correlation matrix of six cell proliferation-related pathway scores. The correlation value was indicated by color (blue for positive correlation and red for negative correlation), while the magnitude of correlation was shown with circles. Spearman's rank correlation was used to the analysis. B. Network plot for highly correlations of hallmark gene sets. Spearman's rank correlation > 0.600 was defined as highly significantly correlated gene sets.

it was of interest to identify which cell proliferation-related score strongly related with survival in pancreatic cancer patients. We analyzed the hazard ratios (HR) of the six scores related to Overall survival (OS), Disease-free survival (DFS), and Disease-specific survival (DSS) in the TCGA (n = 176), as well as OS in GSE62452 (n = 69) and GSE57495 (n = 63) cohorts.

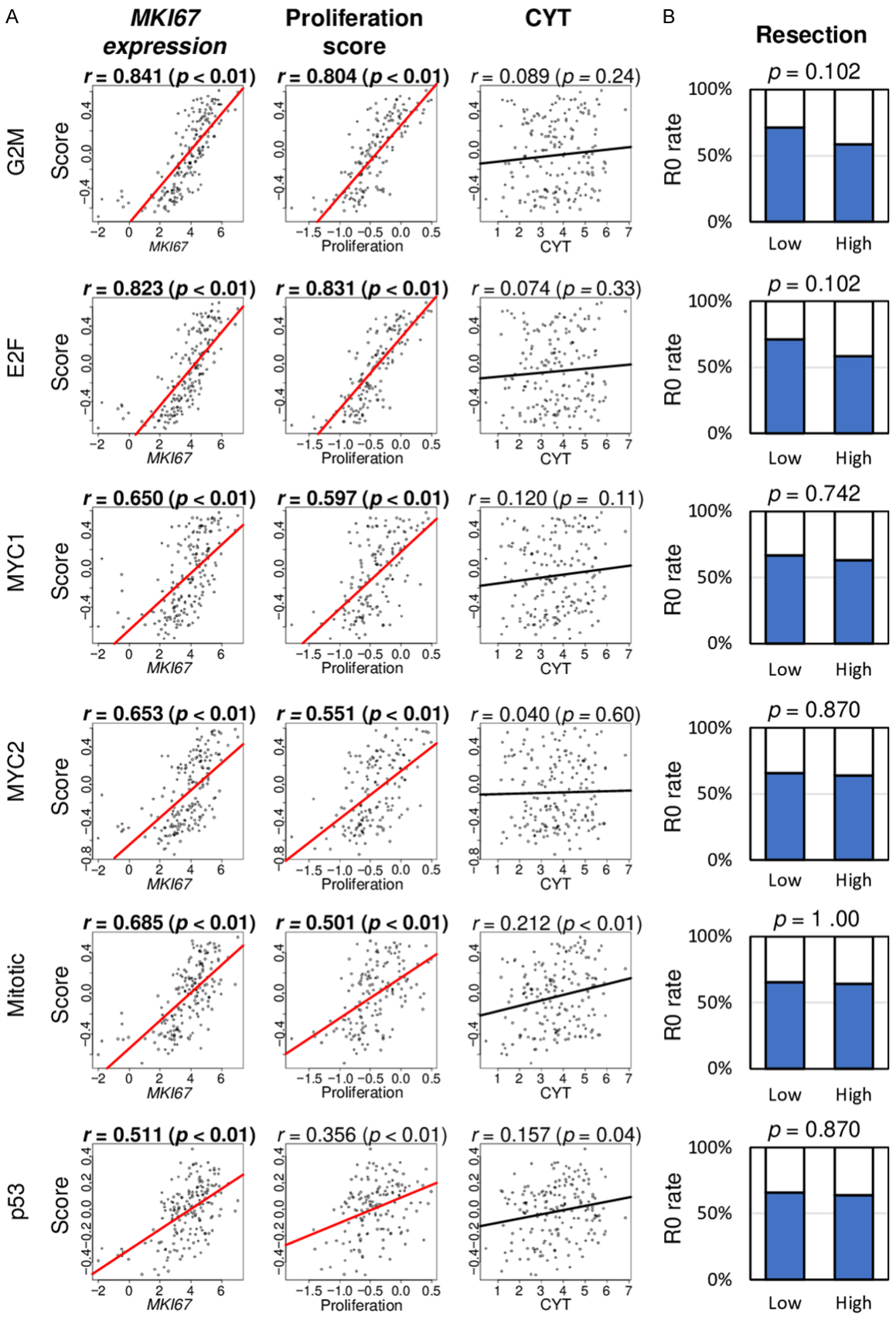
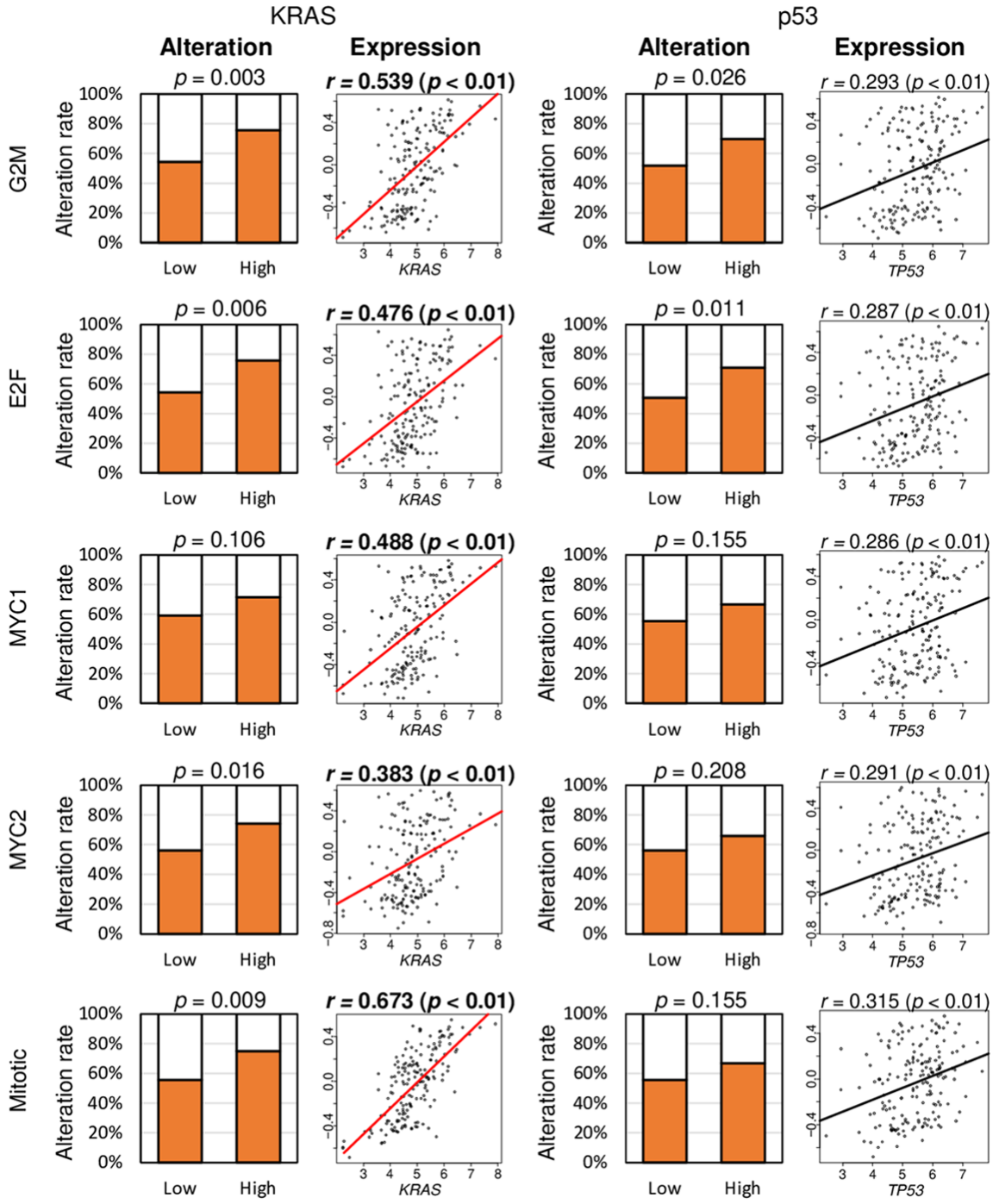

A
MKI67 expression
Proliferation score
CYT
B
Resection
G2M
E2F
MYC1
MYC2
Mitotic
p53
Score
MKI67
Proliferation
CYT
r = 0.841 (p < 0.01)
r = 0.804 (p < 0.01)
r = 0.089 (p = 0.24)
r = 0.823 (p < 0.01)
r = 0.831 (p < 0.01)
r = 0.074 (p = 0.33)
r = 0.650 (p < 0.01)
r = 0.597 (p < 0.01)
r = 0.120 (p = 0.11)
r = 0.653 (p < 0.01)
r = 0.551 (p < 0.01)
r = 0.040 (p = 0.60)
r = 0.685 (p < 0.01)
r = 0.501 (p < 0.01)
r = 0.212 (p < 0.01)
r = 0.511 (p < 0.01)
r = 0.356 (p < 0.01)
r = 0.157 (p = 0.04)
p = 0.102
p = 0.102
p = 0.742
p = 0.870
p = 1 .00
p = 0.870
R0 rate
100%
50%
0%
Low
High

Figure 2. Association of six cell proliferation-related pathway scores with cell proliferation parameters, immune cytolytic activity, and pathological complete resection in the TCGA cohort. A. Correlation plots of cell proliferation-related pathway scores with *MKI67* expression, Proliferation score, and Cytolytic Activity score (CYT). Spearman's rank correlation was used for the analysis. B. Bar plots of the comparison of the low and high score group with R0 resection ratio. Fisher's test was use for the analysis. The median value was used as a cut-off to divide low and high groups for each pathway. G2M, G2M Checkpoint; E2F, E2F Targets; MYC1, MYC Targets V1; MYC2, MYC Targets V2; Mitotic, Mitotic Spindle; p53, p53 pathway.



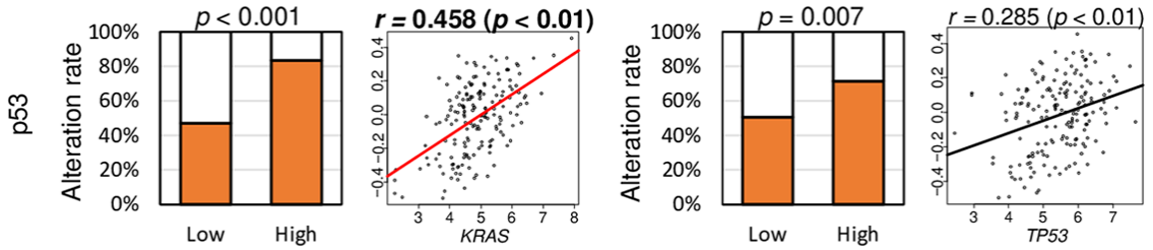


Figure 3. The association of six cell proliferation-related pathway scores with alteration and expression of *KRAS* and *TP53* genes in the TCGA cohort. Bar plots of the percentage of patients with alteration of *KRAS* and *TP53* genes between low and high gene sets. The median value was used to divide low and high score groups as a cut-off. The Fisher's test was used for the analysis. Correlation plots of cell proliferation-related pathway scores with gene expression of *KRAS* and *TP53*. Spearman's rank correlation was used for the analysis. G2M, G2M Checkpoint; E2F, E2F Targets; MYC1, MYC Targets V1; MYC2, MYC Targets V2; Mitotic, Mitotic Spindle; p53, p53 pathway.

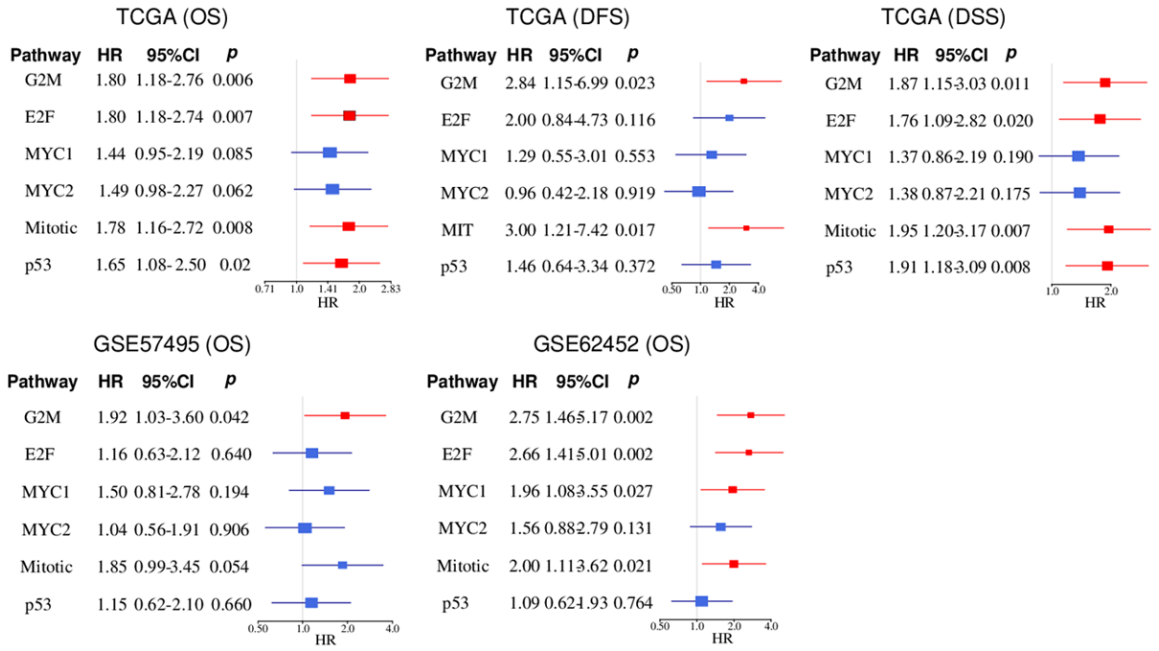


Figure 4. Association of six cell proliferation-related scores and patient survival in pancreatic cancer. Forest plots of hazard ratio (HR) for high cell proliferation-related pathway score in the TCGA (n = 176), GSE57495 (n = 63), and GSE62452 (n = 69). The median value was used as a cut-off to divide low and high groups within the cohort. The blue line indicates non-significance and the red line indicated significance. G2M, G2M Checkpoint; E2F, E2F Targets; MYC1, MYC Targets V1; MYC2, MYC Targets V2; Mitotic, Mitotic Spindle; p53, p53 pathway.

The high score of the G2M checkpoint and Mitotic spindle pathways were associated with poor OS, DFS, and DSS in all cohorts of TCGA and GSE62452; whereas E2F targets and p53 pathway scores were associated with only OS and DSS of TCGA cohort (**Figure 4**). The MYC targets v1 score was associated with OS in GSE62452 alone. The G2M checkpoint score consistently demonstrated the highest HR in all the survival analyses across all the cohorts (HR; 1.80, 2.84, 1.87, 1.92, 2.75, respectively). None of the other gene sets within the Hallmark gene sets showed significant differences across all cohorts (Tables S2 and S3). These results suggest that the G2M checkpoint pathway score has the highest potential to be used as a prognostic biomarker in pancreatic cancer among the six cell proliferation-related pathway scores.

Cell proliferation-related pathway scores predict response for different therapeutic agents used in pancreatic cancer treatment

As the cell proliferation-related pathway scores are associated with survival, we hypothe-

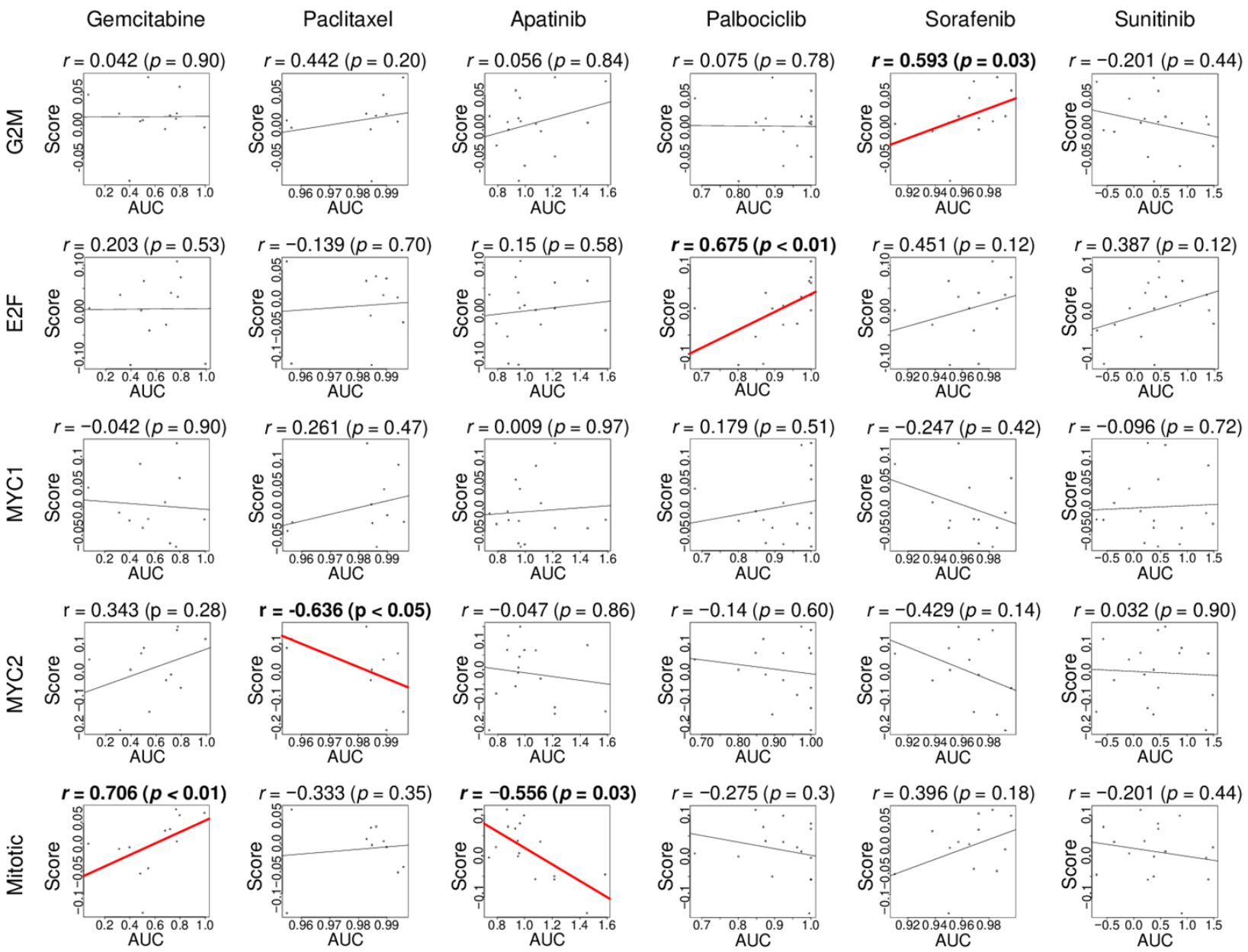
sized that the score also correlates with response to treatment. Since we do not have access to pancreatic cancer patient cohorts with transcriptome and drug response data, we examined the drug sensitivity of pancreatic cancer cell lines, including Gemcitabine, Paclitaxel, Apatinib, Palbociclib, Sorafenib, and Sunitinib, utilizing the Cancer Cell Line Encyclopedia [38]. We found that the G2M checkpoint score correlated moderately with the area under the curve (AUC) of Sorafenib sensitivity (**Figure 5**; $r = 0.593$, $P = 0.03$). The E2F targets score correlated strongly with AUC for Palbociclib (**Figure 5**; $r = 0.675$, $P < 0.01$). The MYC targets v2 score negatively correlated with AUC for Paclitaxel (**Figure 5**; $r = -0.636$, $P < 0.05$). The Mitotic spindle score correlated strongly with AUC for Gemcitabine (**Figure 5**; $r = 0.706$, $P = 0.01$), and negatively with AUC for Apatinib (**Figure 5**; $r = -0.556$, $P = 0.03$). These results suggest that the cell proliferation-related pathway scores may have the potential to predict drug treatment response for pancreatic cancer.

Discussion

In this study, we evaluated six cell proliferation-related pathways (G2M checkpoints, E2F targets, MYC target v1, MYC target v2, Mitotic spindle, and p53 pathway) in pancreatic cancer using the GSVA scoring method with the MSigDB Hallmark gene sets collection. The pathways strongly correlated with each other, especially the G2M checkpoint with E2F target scores. The pathways also highly correlated with gene sets classified into other categories, such as metabolic, DNA damage, cellular component, but the distributions of these components were different in six cell proliferation-related gene sets. All six cell proliferation-related gene sets was strongly correlated with *MKI67* gene expression, and the high score of all six cell proliferation-related gene sets was significantly associated with a high proliferation score. Among them, high mitotic spindle score alone was associated with high cytolytic activity. None were associated with the R0 resection rate. A high score of all cell proliferation-related gene sets was significantly associated with a high rate of *KRAS* gene alteration except for MYC targets v1 score. A high score of G2M checkpoint, E2F targets, and p53 pathway was significantly associated with high rate of *TP53* gene alteration. All six gene sets were correlated with KRAS gene expression but not with TP53 gene expression. Interestingly, cell proliferation-related pathway scores predicted response to different therapeutic agents used in pancreatic cancer treatment, such as Gemcitabine, Paclitaxel, Apatinib, Palbociclib, and Sorafenib. A high score of all six gene sets was associated with worse prognosis except for MYC target v2. Finally, only the G2M checkpoint score showed significant association with worse patient survival across all cohorts.

To date, many studies have reported the expression of various genes and signal transduction pathways in cancer. These act in an intricate manner to promote cancer growth and/or treatment resistance. Therefore, it can be difficult to define the complex signaling in cancer by identifying a single gene. GSVA is a useful tool to illustrate a wide perspective of the signaling pathways in cancer. We have previously reported several gene set pathway scores were associated with clinical outcomes in cancer using the GSVA method. *KRAS* signaling was significantly associated with anti-cancer immune microenvironment as well as improved survival in breast cancer [11]. In pancreatic cancer, a high G2M checkpoint score was significantly associated with worse patient survival, particularly after margin-positive resection [14]. In the majority of cases, pathological margin-negative R0 resection has better survival compared with margin-positive resection, thought to be reflective of the aggressive biology of the cancer in the subset of margin positive patients [2-4]. However, none of the 6 cell proliferation-related scores predicted the R0 resection rate in this study. Proliferation-related gene sets score showed association with tumor aggressiveness, such as pathological grade, AJCC stage, and *MKI67* expression. The score of MYC targets v1 and v2 in breast cancer [39] and the G2M checkpoint score in pancreatic cancer strongly correlated with *MKI67* gene expression and with worse patient survival [14].

The *KRAS* and *TP53* genes are associated with high rates of alteration in pancreatic cancer [37] and are linked to progression of disease. Among the six cells proliferation-related pathway scores, the E2F checkpoint, G2M targets, and p53 pathway scores were correlated with expression of *KRAS* and *TP53* gene mutations in pancreatic cancer.


Gemcitabine
Paclitaxel
Apatinib
Palbociclib
Sorafenib
Sunitinib
G2M
E2F
MYC1
MYC2
Mitotic
Score
AUC
r = 0.042 (p = 0.90)
r = 0.442 (p = 0.20)
r = 0.056 (p = 0.84)
r = 0.075 (p = 0.78)
r = 0.593 (p = 0.03)
r = −0.201 (p = 0.44)
r = 0.203 (p = 0.53)
r = −0.139 (p = 0.70)
r = 0.15 (p = 0.58)
r = 0.675 (p < 0.01)
r = 0.451 (p = 0.12)
r = 0.387 (p = 0.12)
r = −0.042 (p = 0.90)
r = 0.261 (p = 0.47)
r = 0.009 (p = 0.97)
r = 0.179 (p = 0.51)
r = −0.247 (p = 0.42)
r = −0.096 (p = 0.72)
r = 0.343 (p = 0.28)
r = -0.636 (p < 0.05)
r = −0.047 (p = 0.86)
r = −0.14 (p = 0.60)
r = −0.429 (p = 0.14)
r = 0.032 (p = 0.90)
r = 0.706 (p < 0.01)
r = −0.333 (p = 0.35)
r = −0.556 (p = 0.03)
r = −0.275 (p = 0.3)
r = 0.396 (p = 0.18)
r = −0.201 (p = 0.44)

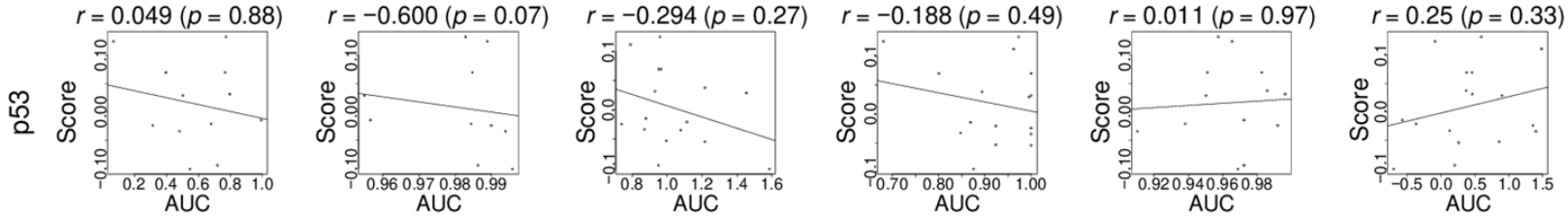


Figure 5. Correlation between the cell proliferation-related scores and drug response. Correlation plots of the association in human pancreatic cancer cell lines between six proliferation-related gene set score and sensitivity to pancreatic cancer drugs, including Gemcitabine, Paclitaxel, Apatinib, Palbociclib, Sorafenib, and Sunitinib. *P*-value and rho (*r*) were analyzed with spearman's rank correlation coefficient. G2M, G2M Checkpoint; E2F, E2F Targets; MYC1, MYC Targets V1; MYC2, MYC Targets V2; Mitotic, Mitotic Spindle; p53, p53 pathway.

Surgical resection remains the mainstay of curative treatment for pancreatic cancer [40]. However, the majority of patients present with metastatic disease at the time of diagnosis, and therefore systemic chemotherapy remains the primary treatment option for most patients. As rates of drug resistance have made chemotherapy less effective, a bigger problem arises. Regimens of FOLFIRINOX (FFX) and Gemcitabine/Nab-paclitaxel (GNP) are used in metastatic patients, and large centers have transitioned these regimens as neoadjuvant treatment for patients with locally advanced pancreatic cancer [41, 42]. With this, an increasing number of patients are treated with multiple chemotherapeutic regimens with the intent of downstaging the cancer and enabling complete microscopically negative resection [43], or sparing major surgery altogether. Despite the widespread use of neoadjuvant therapy, it is unclear which regimen is associated with the best possible survival. The recent notion that adjuvant FFX may improve survival in patients already treated with neoadjuvant therapy and tumor resection has been under investigation [44].

Several other pathway-targeted chemotherapies have been studied. Palbociclib, a CDK4 inhibitor, leads to inhibitory effects at different stages of the tumor cell and within the tumor microenvironment, which collectively drives down cancer cell proliferation and invasion [45]. Sorafenib, on the other hand, inhibits signaling pathways such as RAF/MEK/ERK cascades resulting in decreased cell proliferation [46], and target receptors such as VEGFR PDGFR-β for anti-angiogenic effects. Sorafenib also inhibits DNA synthesis and induces tumor cell death in various cancers [47]. Combination therapies have shown potent anti-proliferative and pro-apoptotic efficacy in pancreatic cancer cells [48, 49]. Preclinical studies and on-going phase I clinical trials have demonstrated the safety and efficacy of combinatorial radio-chemotherapy plus surgery in pancreatic cancer, including the combination of sorafenib and Vorinostat [50]. Additionally, Sunitinib inhibits endothelial cell proliferation angiogenic proliferation as a broad-spectrum receptor tyrosine kinase (RTK) inhibitor [51]. Though Sunitinib treatment has impressive results in treating neuroendocrine pancreatic tumors, phase II clinical trials in advanced or metastatic pancreatic cancer have shown its consistent failure [52, 53].

In our study, proliferation-related gene sets did not show correlation to a sensitivity for Sunitinib or Apatinib, which is a multiple kinase inhibitor with bioactivity against VEGFR-2, PDGFR-β, c-Kit, and c-src [54]. These agents were proven to have a survival benefit in several cancers such as gastric, colon, and breast cancer [55]. Apatinib has demonstrated substantial potential as a new therapeutic option due to its ease of administration, compliance rate, low toxicity, and improved outcomes [56]. Cheng-Ming et al. reported the potential use of Apatinib in the treatment of pancreatic cancer [57]. The ability to target even a minority of pancreatic cancer patients is significant due to the current limitations in treatment options. A biomarker that can predict survival and response to chemotherapy, such as the proliferation-related gene set score, may be able to improve treatment efficacy, reduce toxicity, and improve patients' quality of life. Based on our study, we speculate that proliferation-related scores can have utility as a predictive biomarker of response to chemotherapies in pancreatic cancer patients.

Our study has its limitations. This study is a retrospective study. In order to establish the utility of cell proliferation-related gene sets as a biomarker in predicting the effectiveness of chemotherapies, a prospective study will be required.

In conclusion, we have demonstrated that the G2M pathway score can serve as a tool for identifying patients who are likely to have poor survival in pancreatic cancer. Our findings support the need for clinical trials to evaluate these gene set scores as a predictive biomarker for response to chemotherapy.

Acknowledgements

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Disclosure of conflict of interest

None.

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Table S1. Member genes of the Hallmark proliferation-related pathway gene sets

G2M checkpoint: ABL1, AC027237.1, AC091021.1, AMD1, ARID4A, ATF5, ATRX, AURKA, AURKB, BARD1, BCL3, BIRC5, BRCA2, BUB1, BUB3, CASP8AP2, CBX1, CCNA2, CCNB2, CCND1, CCNF, CCNT1, CDC20, CDC25A, CDC25B, CDC27, CDC45, CDC6, CDC7, CDK1, CDK4, CDKN1B, CDKN2C, CDKN3, CENPA, CENPE, CENPF, CHAF1A, CHEK1, CHMP1A, CKS1B, CKS2, CTCF, CUL1, CUL3, CUL4A, CUL5, DBF4, DDX39A, DKC1, DMD, DR1, DTYMK, E2F1, E2F2, E2F3, E2F4, EFNA5, EGF, ESPL1, EWSR1, EX01, EZH2, FANCC, FBX05, FOXN3, G3BP1, GINS2, GSPT1, H2AFV, H2AFX, H2AFZ, HIF1A, HIRA, HIST1H2BK, HMGA1, HMGB3, HMGN2, HMMR, HNRNPD, HNRNPU, HOXC10, HSPA8, HUS1, ILF3, INCENP, JPT1, KATNA1, KIF11, KIF15, KIF20B, KIF22, KIF2C, KIF4A, KIF5B, KMT5A, KNL1, KPNA2, KPNB1, LBR, LIG3, LMNB1, MAD2L1, MAP3K20, MAPK14, MARCKS, MCM2, MCM3, MCM5, MCM6, MEIS1, MEIS2, MKI67, MNAT1, MT2A, MTF2, MYBL2, MYC, NASP, NCL, NDC80, NEK2, NOLC1, NOTCH2, NSD2, NUMA1, NUP50, NUP98, NUSAP1, ODC1, ODF2, ORC5, ORC6, PAFAH1B1, PBK, PDS5B, PLK1, PLK4, PML, POLA2, POLE, POLQ, PRC1, PRIM2, PRMT5, PRPF4B, PTTG1, PTTG3P, PURA, RACGAP1, RAD21, RAD23B, RAD54L, RASAL2, RBL1, RBM14, RPA2, RPS6KA5, SAP30, SFPQ, SLC12A2, SLC38A1, SLC7A1, SLC7A5, SMAD3, SMARCC1, SMC1A, SMC2, SMC4, SNRPD1, SQLE, SRSF1, SRSF10, SRSF2, STAG1, STIL, STMN1, SUV39H1, SYNCRIP, TACC3, TENT4A, TFDP1, TGFB1, TLE3, TMPO, TNPO2, TOP1, TOP2A, TPX2, TRA2B, TRAIP, TROAP, TTK, UBE2C, UBE2S, UCK2, UPF1, WRN, XPO1, YTHDC1
E2F Targets: AK2, ANP32E, ASF1A, ASF1B, ATAD2, AURKA, AURKB, BARD1, BIRC5, BRCA1, BRCA2, BRMS1L, BUB1B, CBX5, CCNB2, CCNE1, CCP110, CDC20, CDC25A, CDC25B, CDCA3, CDCA8, CDK1, CDK4, CDKN1A, CDKN1B, CDKN2A, CDKN2C, CDKN3, CENPE, CENPM, CHEK1, CHEK2, CIT, CKS1B, CKS2, CNOT9, CSE1L, CTCF, CTPS1, DCK, DCLRE1B, DCTPP1, DDX39A, DEK, DEPDC1, DIAPH3, DLGAP5, DNMT1, DONSON, DSCC1, DUT, E2F8, EED, EIF2S1, ESPL1, EXOSC8, EZH2, GINS1, GINS3, GINS4, GSPT1, H2AFX, H2AFZ, HELLS, HMGA1, HMGB2, HMGB3, HMMR, HNRNPD, HUS1, ILF3, ING3, IPO7, JPT1, KIF18B, KIF22, KIF2C, KIF4A, KPNA2, LBR, LIG1, LMNB1, LUC7L3, LYAR, MAD2L1, MCM2, MCM3, MCM4, MCM5, MCM6, MCM7, MELK, MKI67, MLH1, MMS22L, MRE11, MSH2, MTHFD2, MXD3, MYBL2, MYC, NAA38, NAP1L1, NASP, NBN, NCAPD2, NME1, NOLC1, NOP56, NUDT21, NUP107, NUP153, NUP205, ORC2, ORC6, PA2G4, PAICS, PAN2, PCNA, PDS5B, PHF5A, PLK1, PLK4, PMS2, PNN, POLA2, POLD1, POLD2, POLD3, POLE, POLE4, POP7, PPM1D, PPP1R8, PRDX4, PRIM2, PRKDC, PRPS1, PSIP1, PSMC3IP, PTTG1, RACGAP1, RAD1, RAD21, RAD50, RAD51AP1, RAD51C, RAN, RANBP1, RBBP7, RFC1, RFC2, RFC3, RNASEH2A, RPA1, RPA2, RPA3, RRM2, SHMT1, SLBP, SMC1A, SMC3, SMC4, SMC6, SNRPB, SPAG5, SPC24, SPC25, SRSF1, SRSF2, SSRP1, STAG1, STMN1, SUV39H1, SYNCRIP, TACC3, TBRG4, TCF19, TFRC, TIMELESS, TIPIN, TK1, TMPO, TOP2A, TP53, TRA2B, TRIP13, TUBB, TUBG1, UBE2S, UBE2T, UBR7, UNG, USP1, WDR90, WEE1, XPO1, XRCC6, ZW10
MYC Targets V1: ABCE1, AC004086.1, ACP1, AIMP2, AP3S1, APEX1, BUB3, C1QBP, CAD, CANX, CBX3, CCNA2, CCT2, CCT3, CCT4, CCT5, CCT7, CDC20, CDC45, CDK2, CDK4, CLNS1A, CNBP, COPS5, COX5A, CSTF2, CTPS1, CUL1, CYC1, DDX18, DDX21, DEK, DHX15, DUT, EEF1B2, EIF1AX, EIF2S1, EIF2S2, EIF3B, EIF3D, EIF3J, EIF4A1, EIF4E, EIF4G2, EIF4H, EPRS, ERH, ETF1, EXOSC7, FAM120A, FBL, G3BP1, GLO1, GNL3, GOT2, GSPT1, H2AFZ, HDAC2, HDDC2, HDGF, HNRNPA1, HNRNPA2B1, HNRNPA3, HNRNPC, HNRNPD, HNRNPR, HNRNPU, HPRT1, HSP90AB1, HSPD1, HSPE1, IARS, IFRD1, ILF2, IMPDH2, KARS, KPNA2, KPNB1, LDHA, LSM2, LSM7, MAD2L1, MCM2, MCM4, MCM5, MCM6, MCM7, MRPL23, MRPL9, MRPS18B, MYC, NAP1L1, NCBP1, NCBP2, NDUFAB1, NHP2, NME1, NOLC1, NOP16, NOP56, NPM1, ODC1, ORC2, PA2G4, PABPC1, PABPC4, PCBP1, PCNA, PGK1, PHB, PHB2, POLD2, POLE3, PPIA, PPM1G, PRDX3, PRDX4, PRPF31, PRPS2, PSMA1, PSMA2, PSMA4, PSMA6, PSMA7, PSMB2, PSMB3, PSMC4, PSMC6, PSMD1, PSMD14, PSMD3, PSMD7, PSMD8, PTGES3, PWP1, RACK1, RAD23B, RAN, RANBP1, RFC4, RNPS1, RPL14, RPL18, RPL22, RPL34, RPLP0, RPS10, RPS2, RPS3, RPS5, RPS6, RRM1, RRP9, RSL1D1, RUVBL2, SERBP1, SET, SF3A1, SF3B3, SLC25A3, SMARCC1, SNRPA, SNRPA1, SNRPB2, SNRPD1, SNRPD2, SNRPD3, SNRPG, SRM, SRPK1, SRSF1, SRSF2, SRSF3, SRSF7, SSB, SSBP1, STARD7, SYNCRIP, TARDBP, TCP1, TFDP1, TOMM70, TRA2B, TRIM28, TUFM, TXNL4A, TYMS, U2AF1, UBA2, UBE2E1, UBE2L3, USP1, VBP1, VDAC1, VDAC3, XPO1, XPOT, XRCC6, YWHAE, YWHAQ
MYC Targets V2: AIMP2, BYSL, CBX3, CDK4, DCTPP1, DDX18, DUSP2, EXOSC5, FARSA, GNL3, GRWD1, HK2, HSPD1, HSPE1, IMP4, IPO4, LAS1L, MAP3K6, MCM4, MCM5, MPHOSPH10, MRTO4, MYBBP1A, MYC, NDUFAF4, NIP7, NOC4L, NOLC1, NOP16, NOP2, NOP56, NPM1, PA2G4, PES1, PHB, PLK1, PLK4, PPAN, PPRC1, PRMT3, PUS1, RABEPK, RCL1, RRP12, RRP9, SLC19A1, SLC29A2, SORD, SRM, SUPV3L1, TBRG4, TCOF1, TFB2M, TMEM97, UNG, UTP20, WDR43, WDR74
Mitotic spindle: ABI1, ABL1, ABR, AC027237.1, ACTN4, AKAP13, ALMS1, ALS2, ANLN, APC, ARAP3, ARF6, ARFGEF1, ARFIP2, ARHGAP10, ARHGAP27, ARHGAP29, ARHGAP4, ARHGAP5, ARHGDIA, ARHGEF11, ARHGEF12, ARHGEF2, ARHGEF3, ARHGEF7, ARL8A, ATG4B, AURKA, BCAR1, BCL2L11, BCR, BIN1, BIRC5, BRCA2, BUB1, CAPZB, CCDC88A, CCNB2, CD2AP, CDC27, CDC42, CDC42BPA, CDC42EP1, CDC42EP2, CDC42EP4, CDK1, CDK5RAP2, CENPE, CENPF, CENPJ, CEP131, CEP192, CEP250, CEP57, CEP72, CKAP5, CLASP1, CLIP1, CLIP2, CNTRL, CNTROB, CSNK1D, CTTN, CYTH2, DLG1, DLGAP5, DOCK2, DOCK4, DST, DYNC1H1, DYNLL2, ECT2, EPB41, EPB41L2, ESPL1, EZR, FARP1, FBXO5, FGD4, FGD6, FLNA, FLNB, FSCN1, GEMIN4, GSN, HDAC6, HOOK3, INCENP, ITSN1, KATNA1, KATNB1, KIF11, KIF15, KIF1B, KIF20B, KIF22, KIF2C, KIF3B, KIF3C, KIF4A, KIF5B, KIFAP3, KLC1, KNTC1, KPTN, LATS1, LLGL1, LMNB1, LRPPRC, MAP1S, MAP3K11, MAPRE1, MARCKS, MARK4, MID1, MID1IP1, MYH10, MYH9, MYO1E, MYO9B, NCK1, NCK2, NDC80, NEDD9, NEK2, NET1, NF1, NIN, NOTCH2, NUMA1, NUSAP1, OPHN1, PAFAH1B1, PALLD, PCGF5, PCM1, PCNT, PDLIM5, PIF1, PKD2, PLEKHG2, PLK1, PPP4R2, PRC1, PREX1, PXN, RAB3GAP1, RABGAP1, RACGAP1, RALBP1, RANBP9, RAPGEF5, RAPGEF6, RASA1, RASA2, RASAL2, RFC1, RHOF, RHOT2, RICTOR, ROCK1, SAC3D1, SASS6, SEPT9, SHROOM1, SHROOM2, SMC1A, SMC3, SMC4, SORBS2, SOS1, SPTAN1, SPTBN1, SSH2, STAU1, STK38L, SUN2, SYNPO, TAOK2, TBCD, TIAM1, TLK1, TOP2A, TPX2, TRIO, TSC1, TTK, TUBA4A, TUBD1, TUBGCP2, TUBGCP3, TUBGCP5, TUBGCP6, UXT, VCL, WASF1, WASF2, WASL, YWHAE
p53 pathway: ABAT, ABCC5, ABHD4, ACVR1B, ADA, AEN, AK1, ALOX15B, ANKRA2, APAF1, APP, ATF3, BAIAP2, BAK1, BAX, BLCAP, BMP2, BTG1, BTG2, CASP1, CCND2, CCND3, CCNG1, CCNK, CCP110, CD81, CD82, CDH13, CDK5R1, CDKN1A, CDKN2A, CDKN2AIP, CDKN2B, CEBPA, CGRRF1, CLCA2, COQ8A, CSRNP2, CTSD, CTSF, CYFIP2, DCXR, DDB2, DDIT3, DDIT4, DEF6, DGKA, DNTTIP2, DRAM1, EI24, ELP1, EPHA2, EPHX1, EPS8L2, ERCC5, F2R, FAM162A, FAS, FBXW7, FDXR, FGF13, FOS, FOXO3, FUCA1, GADD45A, GLS2, GM2A, GPX2, H2AFJ, HBEGF, HDAC3, HEXIM1, HINT1, HIST1H1C, HIST3H2A, HMOX1, HRAS, HSPA4L, IER3, IER5, IFI30, IL1A, INHBB, IP6K2, IRAK1, ISCU, ITGB4, JAG2, JUN, KIF13B, KLF4, KLK8, KRT17, LDHB, LIF, LRMP, MAPKAPK3, MDM2, MKNK2, MXD1, MXD4, NDRG1, NHLH2, NINJ1, NOL8, NOTCH1, NUDT15, NUPR1, OSGIN1, PCNA, PDGFA, PERP, PHLDA3, PIDD1, PITPNC1, PLK2, PLK3, PLXNB2, PMM1, POLH, POM121, PPM1D, PPP1R15A, PRKAB1, PRMT2, PROCR, PTPN14, PTPRE, PVT1, RAB40C, RACK1, RAD51C, RAD9A, RALGDS, RAP2B, RB1, RCHY1, RETSAT, RGS16, RHBDF2, RNF19B, RPL18, RPL36, RPS12, RPS27L, RRAD, RRP8, RXRA, S100A10, S100A4, SAT1, SDC1, SEC61A1, SERPINB5, SERTAD3, SESN1, SFN, SLC19A2, SLC35D1, SLC3A2, SLC7A11, SOCS1, SP1, SPHK1, ST14, STEAP3, STOM, TAP1, TAX1BP3, TCHH, TCN2, TGFA, TGFB1, TM4SF1, TM7SF3, TNFSF9, TNNI1, TOB1, TP53, TP63, TPD52L1, TPRKB, TRAF4, TRAFD1, TRIAP1, TRIB3, TSC22D1, TSPYL2, TXNIP, UPP1, VAMP8, VDR, VWA5A, WRAP73, WWP1, XPC, ZBTB16, ZFP36L1, ZMAT3, ZNF365

Table S2. Association of other hallmark gene sets scores and patient survival in pancreatic cancer in the TCGA cohort. Cox proportional hazard analyses were used to estimate hazard ratio (HR), 95% CI, and *p*-value. The median value was used as a cut-off to divide low and high groups within cohorts

Category	Pathway	TCGA (OS)					TCGA (DFS)					TCGA (DSS)				
		HR	95% CI		*P*		HR	95% CI		*p*		HR	95% CI		*P*	
Cellular Component	APJ	1.28	0.85	1.94	0.24		1.86	0.79	4.35	0.15		**1.68**	**1.04**	**2.71**	**0.03**	*
	APS	**1.70**	**1.11**	**2.61**	**0.01**	*	2.04	0.85	4.88	0.11		**2.04**	**1.26**	**3.31**	**0.00**	*
	PER	1.12	0.74	1.69	0.61		0.94	0.41	2.18	0.89		1.21	0.76	1.93	0.43	
Development	ADI	1.37	0.90	2.08	0.14		1.18	0.51	2.73	0.70		1.48	0.92	2.38	0.10	
	ANG	1.44	0.95	2.18	0.09		**2.67**	**1.11**	**6.46**	**0.03**	*	**1.76**	**1.10**	**2.83**	**0.02**	*
	EMT	1.51	0.99	2.29	0.06		**4.34**	**1.65**	**11.39**	**0.00**	*	**1.73**	**1.08**	**2.78**	**0.02**	*
	MYO	0.88	0.58	1.34	0.56		1.07	0.47	2.46	0.87		1.13	0.71	1.79	0.62	
	PAN	0.83	0.55	1.26	0.38		**0.40**	**0.17**	**0.95**	**0.04**	*	0.98	0.62	1.56	0.94	
	SPE	0.93	0.62	1.41	0.74		0.67	0.29	1.52	0.34		1.01	0.64	1.62	0.95	
DNA damage	DNA	1.37	0.90	2.08	0.14		0.60	0.26	1.38	0.23		1.37	0.86	2.19	0.19	
	UVD	1.43	0.94	2.16	0.09		2.46	1.03	5.91	0.04		1.49	0.94	2.39	0.09	
	UVU	**1.53**	**1.00**	**2.32**	**0.05**	*	1.13	0.49	2.62	0.77		**1.69**	**1.05**	**2.73**	**0.03**	*
Immune	ALL	1.21	0.80	1.83	0.37		1.16	0.49	2.76	0.73		1.27	0.80	2.02	0.31	
	COA	1.47	0.97	2.24	0.07		1.27	0.55	2.90	0.58		**1.87**	**1.15**	**3.02**	**0.01**	*
	COM	**1.82**	**1.20**	**2.77**	**0.01**	*	1.40	0.59	3.34	0.45		**1.97**	**1.22**	**3.17**	**0.01**	*
	IFA	**1.68**	**1.11**	**2.55**	**0.01**	*	1.34	0.58	3.09	0.49		**1.60**	**1.00**	**2.56**	**0.05**	*
	IFG	1.31	0.86	1.98	0.21		0.95	0.41	2.22	0.91		1.26	0.79	2.01	0.32	
	IL6	1.40	0.92	2.12	0.12		1.35	0.56	3.23	0.51		1.57	0.98	2.52	0.06	
	INF	1.44	0.95	2.19	0.09		1.12	0.47	2.67	0.79		**1.74**	**1.08**	**2.80**	**0.02**	*
Metabolic	BIL	0.86	0.57	1.30	0.46		0.82	0.36	1.91	0.65		0.87	0.54	1.38	0.55	
	CHO	1.68	1.10	2.56	0.02	*	1.17	0.52	2.66	0.71		**1.62**	**1.01**	**2.60**	**0.04**	*
	FAT	1.39	0.92	2.12	0.12		0.89	0.39	2.03	0.78		1.37	0.86	2.19	0.19	
	GLY	1.76	1.15	2.69	0.01	*	1.90	0.82	4.40	0.13		**2.08**	**1.27**	**3.39**	**0.00**	*
	HEM	1.29	0.85	1.95	0.23		0.72	0.32	1.64	0.43		1.40	0.88	2.25	0.16	
	OXI	0.95	0.63	1.44	0.81		0.73	0.31	1.72	0.47		0.87	0.54	1.38	0.54	
	XEN	1.67	1.10	2.54	0.02	*	1.35	0.59	3.08	0.48		**1.72**	**1.07**	**2.75**	**0.02**	*
Pathway	APO	1.88	1.22	2.88	0.00	*	2.18	0.92	5.19	0.08		**2.17**	**1.33**	**3.53**	**0.00**	*
	HYP	1.93	1.26	2.97	0.00	*	2.23	0.97	5.10	0.06		**2.22**	**1.36**	**3.63**	**0.00**	*
	PRO	1.49	0.98	2.27	0.06		1.15	0.50	2.66	0.75		1.56	0.97	2.51	0.07	
	REA	1.10	0.73	1.66	0.66		0.78	0.34	1.77	0.55		1.05	0.66	1.67	0.83	
	UNF	1.14	0.75	1.74	0.53		0.74	0.32	1.70	0.48		1.17	0.73	1.88	0.52	
Signaling	AND	**1.86**	**1.22**	**2.84**	**0.00**	*	**2.43**	**1.02**	**5.83**	**0.05**	*	**1.97**	**1.22**	**3.18**	**0.01**	*
	ERE	**1.95**	**1.28**	**2.99**	**0.00**	*	2.15	0.93	4.94	0.07		**2.18**	**1.34**	**3.55**	**0.00**	*
	ERL	**2.22**	**1.45**	**3.41**	**0.00**	*	1.09	0.48	2.49	0.84		**2.43**	**1.49**	**3.96**	**0.00**	*
	HED	0.79	0.52	1.20	0.27		1.25	0.54	2.91	0.60		1.00	0.63	1.60	0.99	
	IL2	1.35	0.89	2.04	0.16		1.49	0.62	3.55	0.37		1.44	0.90	2.30	0.12	
	KRD	0.98	0.65	1.49	0.94		1.51	0.65	3.50	0.34		1.07	0.67	1.70	0.78	
	KRU	1.47	0.97	2.23	0.07		1.85	0.76	4.49	0.17		**1.70**	**1.06**	**2.73**	**0.03**	*
	MTO	**1.56**	**1.02**	**2.38**	**0.04**	*	1.79	0.76	4.23	0.18		**1.70**	**1.05**	**2.76**	**0.03**	*
	NOT	1.51	1.00	2.29	0.05		2.25	0.97	5.23	0.06		**1.79**	**1.11**	**2.89**	**0.02**	*
	PI3	**1.56**	**1.02**	**2.37**	**0.04**	*	1.16	0.51	2.64	0.73		**1.68**	**1.04**	**2.70**	**0.03**	*
	TGF	**1.67**	**1.10**	**2.54**	**0.02**	*	**3.21**	**1.32**	**7.85**	**0.01**	*	**2.15**	**1.31**	**3.51**	**0.00**	*
	TNF	**1.56**	**1.03**	**2.37**	**0.04**	*	2.01	0.86	4.69	0.11		**1.85**	**1.15**	**2.97**	**0.01**	*
	WNT	1.29	0.85	1.95	0.23		1.87	0.80	4.34	0.15		1.43	0.90	2.29	0.13	

ADI, Adipogenesis; ALL, Allograft rejection; AND, Androgen response; ANG, Angiogenesis; APO, Apoptosis; APS, Apical surface; APJ, Apical junction; BIL, Bile acid metabolism; CHO, Cholesterol homeostasis; COA, Coagulation; COM, Complement; DNA, DNA repair; EMT, Epithelial mesenchymal transition; ERE, Estrogen response early; ERL, Estrogen response late; FAT, Fatty acid metabolism; GLY, Glycolysis; HED, Hedgehog signaling; HEM, Heme metabolism; HYP, Hypoxia; IFA, Interferon alpha response; IFG, Interferon gamma response; IL2, IL2/JAK/STAT5 signaling; IL6, IL6/JAK/STAT3 signaling; ; INF, Inflammatory response; KRD, KRAS signaling down; KRU, KRAS signaling UP; MTO, Mtorc1 signaling; MYO, Myogenesis; NOT, Notch signaling; OXI, Oxidative phosphorylation; PAN, Pancreas beta cell; PER, Peroxisome; PI3, PI3K/AKT/MTOR signaling; PRO, Protein secretion; REA, Reactive oxygen species pathway; SPE, Spermatogenesis; TGF, TGF beta signaling; TNF, TNFa signaling via NFkB; UNF, Unfolded protein response; UVD, UV response down; UVU, UV response up; XEN, Xenobiotic metabolism; WNT, WNT beta catenin signaling.

Table S3. Association of other hallmark gene sets scores and patient survival in pancreatic cancer in the GSE57495 and GSE62452 cohorts. Cox proportional hazard analyses were used to estimate hazard ratio (HR), 95% CI, and *p*-value. The median value was used as a cut-off to divide low and high groups within cohorts

Category	Pathway	GSE57495 (OS)					GSE62452 (OS)				
		HR	95% CI		*P*		HR	95% CI		*P*	
Cellular Component	APJ	**2.44**	**1.29**	**4.61**	**0.01**	*	1.14	0.65	2.00	0.64	
	APS	1.39	0.75	2.58	0.29		1.61	0.91	2.84	0.10	
	PER	1.12	0.61	2.05	0.72		0.98	0.55	1.74	0.94	
Development	ADI	0.99	0.54	1.82	0.98		1.03	0.58	1.81	0.92	
	ANG	0.90	0.49	1.65	0.74		0.86	0.49	1.51	0.60	
	EMT	1.25	0.68	2.29	0.48		1.04	0.59	1.83	0.89	
	MYO	0.59	0.32	1.10	0.10		0.96	0.54	1.70	0.88	
	PAN	0.76	0.41	1.39	0.37		0.61	0.35	1.08	0.09	
	SPE	1.14	0.62	2.10	0.66		1.06	0.60	1.86	0.85	
DNA damage	DNA	0.72	0.39	1.32	0.29		1.49	0.84	2.64	0.17	
	UVD	1.44	0.78	2.66	0.24		1.07	0.61	1.90	0.81	
	UVU	0.96	0.52	1.76	0.89		1.05	0.60	1.84	0.87	
Immune	ALL	0.94	0.52	1.73	0.85		1.16	0.65	2.04	0.62	
	COA	1.06	0.58	1.94	0.85		0.77	0.44	1.37	0.37	
	COM	0.98	0.53	1.79	0.94		1.07	0.61	1.88	0.82	
	IFA	1.39	0.75	2.56	0.30		**1.94**	**1.05**	**3.58**	**0.03**	*
	IFG	0.82	0.45	1.51	0.53		1.23	0.69	2.18	0.48	
	IL6	0.87	0.47	1.59	0.64		1.21	0.68	2.14	0.52	
	INF	0.98	0.54	1.80	0.95		1.15	0.65	2.04	0.62	
Metabolic	BIL	0.77	0.42	1.42	0.40		**0.45**	**0.25**	**0.80**	**0.01**	*
	CHO	1.53	0.83	2.84	0.18		0.99	0.56	1.73	0.96	
	FAT	1.39	0.76	2.55	0.29		0.83	0.47	1.46	0.53	
	GLY	1.72	0.93	3.20	0.08		**1.88**	**1.05**	**3.34**	**0.03**	*
	HEM	1.09	0.59	1.99	0.79		1.41	0.80	2.48	0.24	
	OXI	1.01	0.55	1.86	0.96		0.98	0.54	1.75	0.94	
	XEN	0.94	0.51	1.73	0.85		0.85	0.48	1.49	0.57	
Pathway	APO	**2.41**	**1.29**	**4.50**	**0.01**	*	1.03	0.59	1.82	0.92	
	HYP	1.43	0.77	2.62	0.26		1.42	0.81	2.49	0.22	
	PRO	1.87	1.00	3.48	0.05		1.28	0.72	2.25	0.40	
	REA	1.25	0.68	2.29	0.47		1.01	0.58	1.79	0.96	
	UNF	0.97	0.53	1.78	0.92		**1.91**	**1.07**	**3.42**	**0.03**	*
Signaling	AND	1.54	0.83	2.85	0.17		1.28	0.72	2.28	0.40	
	ERE	1.01	0.55	1.85	0.98		1.05	0.59	1.84	0.88	
	ERL	1.13	0.62	2.08	0.69		0.95	0.54	1.68	0.87	
	HED	1.20	0.65	2.21	0.56		0.63	0.36	1.10	0.11	
	IL2	0.85	0.47	1.57	0.61		1.00	0.57	1.77	0.99	
	KRD	0.67	0.36	1.23	0.19		0.74	0.42	1.29	0.28	
	KRU	0.88	0.48	1.62	0.69		0.98	0.56	1.73	0.95	
	MTO	**2.01**	**1.08**	**3.76**	**0.03**	*	**1.94**	**1.08**	**3.51**	**0.03**	*
	NOT	1.05	0.57	1.92	0.89		0.90	0.51	1.59	0.72	
	PI3	1.63	0.88	3.00	0.12		1.55	0.88	2.75	0.13	
	TGF	1.26	0.69	2.31	0.45		0.86	0.49	1.51	0.60	
	TNF	0.89	0.48	1.63	0.70		0.84	0.48	1.47	0.54	
	WNT	0.93	0.50	1.70	0.80		1.05	0.60	1.85	0.86	

ADI, Adipogenesis; ALL, Allograft rejection; AND, Androgen response; ANG, Angiogenesis; APO, Apoptosis; APS, Apical surface; APJ, Apical junction; BIL, Bile acid metabolism; CHO, Cholesterol homeostasis; COA, Coagulation; COM, Complement; DNA, DNA repair; EMT, Epithelial mesenchymal transition; ERE, Estrogen response early; ERL, Estrogen response late; FAT, Fatty acid metabolism; GLY, Glycolysis; HED, Hedgehog signaling; HEM, Heme metabolism; HYP, Hypoxia; IFA, Interferon alpha response; IFG, Interferon gamma response; IL2, IL2/JAK/STAT5 signaling; IL6, IL6/JAK/STAT3 signaling; ; INF, Inflammatory response; KRD, KRAS signaling down; KRU, KRAS signaling UP; MTO, Mtorc1 signaling; MYO, Myogenesis; NOT, Notch signaling; OXI, Oxidative phosphorylation; PAN, Pancreas beta cell; PER, Peroxisome; PI3, PI3K/AKT/MTOR signaling; PRO, Protein secretion; REA, Reactive oxygen species pathway; SPE, Spermatogenesis; TGF, TGF beta signaling; TNF, TNFa signaling via NFkB; UNF, Unfolded protein response; UVD, UV response down; UVU, UV response up; XEN, Xenobiotic metabolism; WNT, WNT beta catenin signaling.