

Original Article

Pan-cancer analysis reveals a regulatory pattern of anoikis in human cancers

Xingjian Wang[#], Chao Yang[#], Xiaoyun Wang, Pengfei Duan^{*}

Department of Interventional Radiology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu 215006, China



Article Info

Abstract



Article history:

Received: October 24, 2023

Accepted: January 12, 2024

Published: February 29, 2024

Use your device to scan and read the article online



Anoikis emerges when a cell finds itself extricated from the appropriate extracellular matrix, leading to an interruption in integrin ligation and thus triggering programmed cellular demise. The cardinal role of Anoikis in the realms of tumor invasion and metastasis is undeniable, although our grasp on its precise influence within the convoluted landscape of cancer biology remains somewhat circumscribed. Notably, both the immune milieu of the tumor and its inherent aggression are correlated with the fluctuating variables of Anoikis. We conducted a thorough evaluation of the genes associated with anoikis and studied the regulatory patterns of these genes as well as the prognostic impact of anoikis in 33 different types of tumors. We provided functional annotations for the regulatory patterns linked to Anoikis. Additionally, we described the associations between immunological factors and genes associated with Anoikis. By applying gene set variation analysis (GSVA), we utilized the inherent abilities of 34 basic genes to calculate the Anoikis index. The Anoikis index is closely related to prognosis, immune microenvironment, immunotherapy, and other aspects. Our functional research revealed a correlation between immune cell infiltration, EMT, and a regulatory gene that is synonymous with adverse survival outcomes. In addition, our observations revealed a direct relationship between the expression of CEACAM5 and CEACAM6, the amplification of epithelial-mesenchymal transition (EMT) phenomenon, and a decrease in survival outcomes. The potential therapeutic utility of anoikis-related genes was highlighted by the possible links between TME, clinical samples, genetic mutations, drug resistance, and immunotherapy.

Keywords: Pan-cancer analysis, Regulatory pattern, Anoikis, Human cancers.

1. Introduction

Anoikis, a term derived from the Greek word for "homelessness," encapsulates a distinct form of apoptotic cell death arising from inadequate or improper cell adhesion [1]. At its core, Anoikis is a fundamental apoptotic process that plays a crucial role in the initiation and advancement of various malignancies [2-4]. This phenomenon is primarily instigated by the interplay of two apoptotic mechanisms: malfunctioning mitochondria or the activation of death receptors on the cell surface [5-7]. Originally pinpointed in epithelial and endothelial cells, Anoikis is indispensable for the orchestration of cancer invasion and metastasis [8].

The dysregulation of Anoikis has been implicated in the aggressive behavior of cancer cells, allowing them to evade the constraints of proper tissue architecture. Consequently, this process has garnered significant attention in cancer research, providing insights into the intricate interplay between cell adhesion, apoptosis, and the progression of malignant tumors. As our understanding of Anoikis deepens, it unveils promising avenues for targeted therapeutic interventions aimed at disrupting this pivotal process in cancer development and metastatic dissemination [3,7,8].

Numerous investigations have shown that the CPT1A imbalance in colorectal cancer is directly associated with

Anoikis resistance and ultimately results in a number of unfavorable outcomes, including cancer metastasis [3]. Furthermore, there is evidence that highlights the important contribution of CircCEMIP in prostate cancer cells. Exploiting the activity of CircCEMIP could potentially enhance protective autophagy, ultimately promoting resistance to anoikis [9]. At the same time, anoikis-mediated lipid signaling is the subject of increasing study. The prevention of anoikis in metastatic tumors has been linked to a number of molecular and pathway changes. Several partially acknowledged mechanisms that are associated with resistance to anoikis in various types of cancers involve alterations in carbohydrate and amino acid metabolism, along with specific modifications in the lipid metabolism process (such as a rise in fatty acid uptake and production) [10-12]. While the import of anoikis in comprehensive pan-cancer analyses has yet to be exhaustively evaluated, it is recognized that these anoikis-associated genes bear considerable influence in the orchestration of carcinogenesis, tumor invasion, and neoplastic infiltration.

Immunotherapy, a therapeutic approach that bolsters the patient's immune response to combat malignancies, has become an increasingly coveted avenue in the arena of cancer management. It has become widely accepted that, under normal physiological circumstances, immune cells residing in the tumor microenvironment (TME) possess

* Corresponding author.

E-mail address: duanpengfei621@163.com (P. Duan).

[#] These authors contributed equally

Doi: <http://dx.doi.org/10.14715/cmb/2024.70.2.8>

the capacity to discern and eradicate cancer cells—a phenomenon eloquently referred to as immunosurveillance [13,14]. However, cancer cells, in their cunning, possess the capability to alter the host's immune architecture to elude this immune scrutiny, achieved through the recruitment of immune-suppressive cell contingents and dampening the tumor's propensity to evoke an immune reprisal [15,16]. Undifferentiated primary tumors are more likely to migrate aggressively or develop distant metastases, which eventually cause tumor progression [17-19]. For immunotherapy, it is crucial to investigate how the tumor microenvironment is altered and immune cells are introduced [20,21].

The objective of this investigation was to ascertain the extent of Anoikis in disparate cancer varieties as cataloged in the TCGA. For this purpose, we employed gene set enrichment analysis (GSEA) indices derived from the MsigDB repository to scrutinize signatures pertinent to Anoikis-associated genes. Our results illuminated a potent correlation between hallmarks of cancer and the Anoikis score. This study imparts a comprehensive comprehension of the regulatory capabilities inherent to Anoikis infiltration, which may pave the path to novel therapeutic strategies and refined patient stratification.

2. Materials and Methods

2.1. Data collection

We accessed the Molecular Signatures Database (MsigDB) to procure the Anoikis gene set (designated as GOBP ANOIKIS). This particular resource, which can be located at <http://www.gsea-msigdb.org/gsea/msigdb/cards/GOBP-ANOIKIS>, encompasses an assembly of 34 genes that are intimately connected to the Anoikis biological process. Capitalizing on a dedicated R package, we computed the Anoikis score, thereby enabling an astute evaluation of the prevalence of Anoikis phenomena within tumorous entities [22]. Expression datasets are frequently analyzed using this approach to estimate the variance in the activity of biological pathways and processes within samples. It is based on an approach that is non-parametric and unsupervised. For categorization and further analysis, the Anoikis scores—which made use of an ideal cutoff—were split into two groups based on quantiles. The Anoikis profiles of tumor and adjacent non-tumor tissues were examined by comparing their differential gene expression (DEG) using the Limma program. DEG analysis was conducted for each specific cancer dataset and also for the PanCan dataset.

2.2. Analysis of anoikis profiles in cancers

The MsigDB database provided us with the Anoikis gene set file, known as GOBP ANOIKIS. This particular gene set consists of 34 genes that are related to Anoikis. We utilized the GSVA software to conduct gene set variation analysis (GSVA) and determine the extent of Anoikis enrichment in cancerous conditions. It is standard practice to evaluate the variance in pathway activity and biological processes within expression datasets using this non-parametric, unsupervised analytic technique. By setting optimal cutoffs, we calculated Anoikis scores, which were then categorized into two groups for further analysis. We utilized the Limma program to conduct differential gene expression (DEG) analysis in order to glance at the variations in Anoikis profiles between tumor and para-tumor

tissues. In this investigation, both a PanCaner dataset and individual cancer datasets were utilized.

2.3. Anoikis genes CNV and methylation profile in pan-cancer based on GSCA

The expansive TCGA database provides an entry point to the GSCA platform, an integrated web server dedicated to assimilating multiomics data, and can be accessed via <http://bioinfo.life.hust.edu.cn/web/GSCA/>. GSCA's investigative purview is broad, encompassing a multitude of research areas, such as copy number variation (CNV), methylation, pathway activity, and immunological infiltration. The GSCA platform probes the intricate relationship between mRNA expression of Anoikis genes and the incidence of CNV across various neoplastic categories. Furthermore, it scrutinizes the correlation between the abundance of mRNA expression and the degree of methylation associated with Anoikis genes within a variety of tumor types.

2.4. Anoikis genes SNV and immune infiltrates profile in pan-cancer based on GSCA

We examined the SNV data from 33 cancers [23] using the GSCA database. Seven kinds of harmful variants were included: missense mutations, nonsense mutations, frame shift ins, splice site mutations, frame shift deletions, in-frame mutations, and missense mutations. In order to examine the association between gene expression and immune cell infiltrations, a Spearman correlation analysis was conducted. The infiltration of immune cells was evaluated using the tool ImmuCellAI, which assessed 24 different types of immune cells.

2.5. Gene set variation analysis

All TCGA samples underwent gene set variance analysis using the GSVA method. For this investigation, the HALLMARK pathway database from the Molecular Signatures Database (MSigDB) was utilized. Pathways that were highly relevant to the immune system were picked for more research. With the goal of establishing a bond between cancer hallmarks, the causal connection between each gene set's ssGSEA enrichment score and Anoikis score was calculated using Pearson's correlation coefficient.

2.6. Research of the correlation among anoikis and changes in the tumor microenvironment

The technique known as ESTIMATE, which utilizes data on genetic expression, is used to estimate the degree of stromal or immune cell infiltration in malignant tumor tissues. By employing the ESTIMATE algorithm, the immunological and stromal scores of each carcinoma tissue were calculated. The connection between Anoikis scores and these two scores was subsequently analyzed, taking into account the level of immune system cell infiltration, by utilizing the R software applications "estimate" and "limma." Furthermore, the linkage between tumor purity and the Anoikis score was concurrently investigated using the aforementioned techniques.

2.7. Investigation of the correlation of anoikis with tumor cell infiltration and immunomodulator genes in multiple cancers

The Anoikis levels and the prevalence of immune infil-

tration in multiple cancers were investigated by utilizing the TIMER2 database and ImmuCellAI. The TIMER2.0 database, which can be accessed at <http://timer.compgenomics.org/>, provides comprehensive data on tumor immunity [24]. Lymphocytes, macrophages, NK cells, neutrophils, and other immune infiltrate cells may accurately predict a patient's prognosis [25, 26]. We examined the correlation between the Anoikis score and the extent of immune infiltration across various types of tumors. We utilized the TIMER2 database to evaluate immunological infiltration with accuracy. The genes and Anoikis scores associated with immune cell infiltration, immune checkpoints, or other malignancies were shown on a Spearman correlation analysis heat map.

2.8. Survival analysis of numerous tumor types with various anoikis scores

Various kinds of cancer patients were divided into two groups, one with high Anoikis scores and the other with low scores, using a predetermined threshold determined by the survival package. The difference in prognosis between these two groups was examined using Kaplan-Meier curves, log-rank tests, and single-variable Cox estimating models. Furthermore, a single-variable Cox analysis was conducted to assess the overall impact of Anoikis on survival in all types of cancer.

2.9. Impact of anoikis on immunology therapy response

The impact of Anoikis on the outcomes of immune therapy was determined by considering three cohorts of PD1 inhibitor treatment [27–29]. Based on RECIST standards, the patients were categorized into groups of responders and non-responders. The mRNA expression data of these two groups was utilized to generate Anoikis scores, which were then compared to determine the association between Anoikis and the response to therapy.

2.10. Immunohistochemistry staining of CEACAM5, CEACAM6 in PAAD

To examine the protein expression levels of CEACAM5 and CEACAM6 in pancreatic tumor tissues compared to their matched normal tissues, we utilized the HPA database. Specifically, we obtained immunohistochemistry images of these tissues from HPA, which offers comprehensive information about the distribution of proteins within various human cells and tissues.

2.11. Statistical analysis

The log₂ transformation was applied to standardize all the gene expression data. Two sets of t-tests were employed to compare malignant tissue with healthy tissue, with a threshold of P 0.05 to determine statistical significance. For the survival analysis in this study, the Kaplan-Meier curve, log-rank test, and Cox proportional hazard regression model were utilized. Statistical significance was evaluated using a P value of <0.05. (*P value <0.05; **P value <0.01; ***P value <0.001). The necessary R packages, such as "ggplot2", "ggpubr", "survival", and "survminer", were obtained from either bioconductor packages or R packages. A statistical significance threshold of P<0.05 was established for each analysis. All statistical analyses were performed using the R software. (version 4.1.1)

3. Results

3.1. Expression of anoikis-related genes in pan-cancer

First, we obtained the clinical information and expression profiles for 31 malignancies from TCGA and GTEx. Then, we chose the GOBP_ANOIKIS gene set in the MsigDB database. Following the completion of these early steps, we compared the Anoikis pathway genes differential expression in TCGA tumor tissue to that in TCGA and GTEx normal tissue. We can observe that, whereas NTRK2 and PDK4 exhibit a declining trend in most malignancies, E2F1 and CEACAM6 in pancreatic cancer have a considerable rising trend. While BRCA and CESA showed the opposite tendency, anoikis pathway genes including CHOL, DLBC, PAAD, and others had typically elevated expression in the cancer types we discovered. (Figure 1A.) We next performed intragroup correlation analysis on the genes involved in the Anoikis pathway in both PAAD and pancreatic cancer (Figure 1B, 1C). At both the PAAD and pan-cancer levels, we discovered that CEACAM5 and CEACAM6 expression were positively connected with one another and negatively correlated with PDK4. Because the process of anoikis formation is regulated by multiple biological mechanisms and genomic interactions, we opted to analyze the gene co-interaction structure associated with anoikis. To accomplish it, our focus was on the protein-protein interaction network (PPI) of Anoikis genes available in the STING database. Our findings revealed an intricate framework of interactions, suggesting that Anoikis establishes a co-regulatory system (Figure 1D).

3.2. The analysis of survivability of anoikis gene in multiple carcinoma

By conducting single-factor Cox regression analysis and Kaplan-Meier survival analysis, we initially identified ITGA5 and ITGB1, among other factors, as posing a higher risk for pancreatic cancer (Figures 2A–B), while

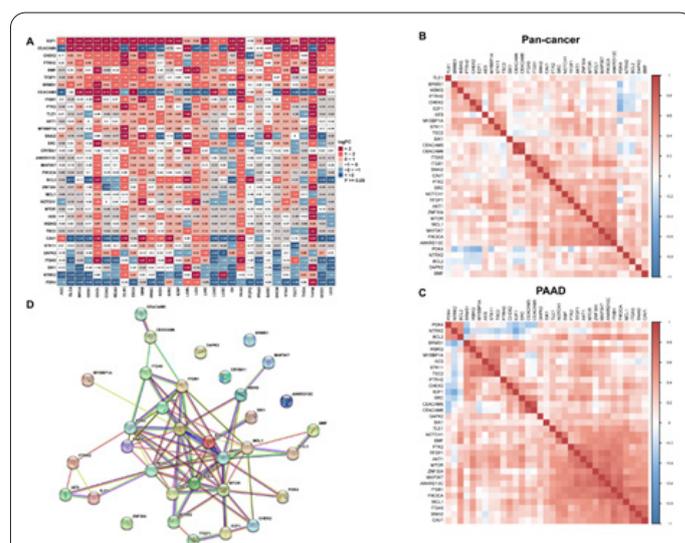


Fig. 1. The pan-cancer study examined the expression of genes associated with anoikis across multiple types of cancer. A: The genes related to Anoikis were compared between samples of tumors and normal tissues. B: The color becomes darker as the correlation between Anoikis genes in pan-cancer strengthens. C: The color intensity in PAAD is directly proportional to the strength of correlation between Anoikis genes. D: The STING database (<https://cn.string-db.org/>) provides information about protein-protein interactions involving Anoikis proteins.

Bcl-2, AES, etc. exhibit protective effects in the majority of malignancies. This information allowed us to further explore the influence of the Anoikis pathway gene on the survival prognosis of pancreatic cancer. Each gene in the anoikis pathway was given a risk score, and the significant number was determined using Cox analysis and Kaplan-Meier survival analysis (Figure 2C-D). This was done in consideration of the fact that some of the anoikis pathway genes are risk factors and some are protective factors in various cancers. The results mostly support our past analysis. ITGA5 and ITGB1 are pan-cancer risk factors, whereas BCL2 and AES are preventative factors.

3.3. In the context of pan-cancer, we examine the genetic alterations associated with genes related to anoikis

We initially computed the SNV mutation analysis of each of the 33 cancer types in order to explore the peculiarities of the Anoikis gene set in cancer (Figure 3A). The findings revealed that UCEC, SKCM, and COAD had the highest rates of the Anoikis pathway gene's total mutation. The top three mutation frequencies in pancreatic cancer, however, were PIK3CA, NOTCH1, and MTOR, whereas CAV1, IKBKG, and PTRH2 exhibited a very conservative trend. Then, we looked at the top 10 mutation frequencies in the Anoikis pathway genes across 29 different cancer types. PIK3CA ranked first with a 45% mutation frequency, followed by ITGA5 with a 4% mutation frequency. At least one SNV type alteration was present in more than 75% of cancer types (Figure 3B).

We have performed CNV and methylation analysis on the gene set in this article in order to further investigate the properties of the Anoikis pathway gene set. First, we performed CNV analysis on 33 malignancies, and the findings

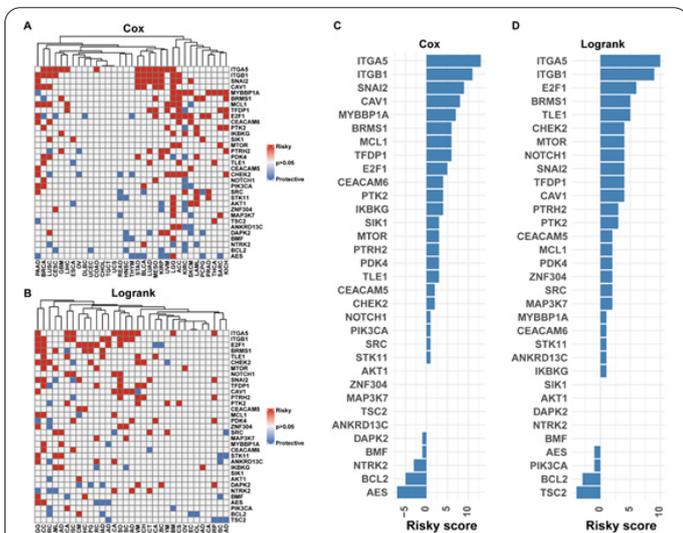


Fig. 2. The analysis of the survival of Anoikis genes in various types of cancer. (A) Through a univariate Cox regression analysis, we identified regulatory genes associated with Anoikis that carry both a pan-cancer risk prognosis and a favorable prognosis. Notably, outcomes with $P > 0.05$ are indicated in gray. Conversely, those with $P < 0.05$ are color-coded based on hazard ratio (HR): instances where $HR > 1$ are highlighted in red, and instances where $HR < 1$ are displayed in blue. (B) A survival analysis using the Kaplan-Meier method was conducted for pan-cancer, focusing on genes associated with Anoikis. In this analysis, genes categorized as red indicate a higher risk, while genes categorized as blue indicate a more favorable outcome. (C, D) The risky score of univariate Cox regression analysis and Kaplan-Meier survival analysis.

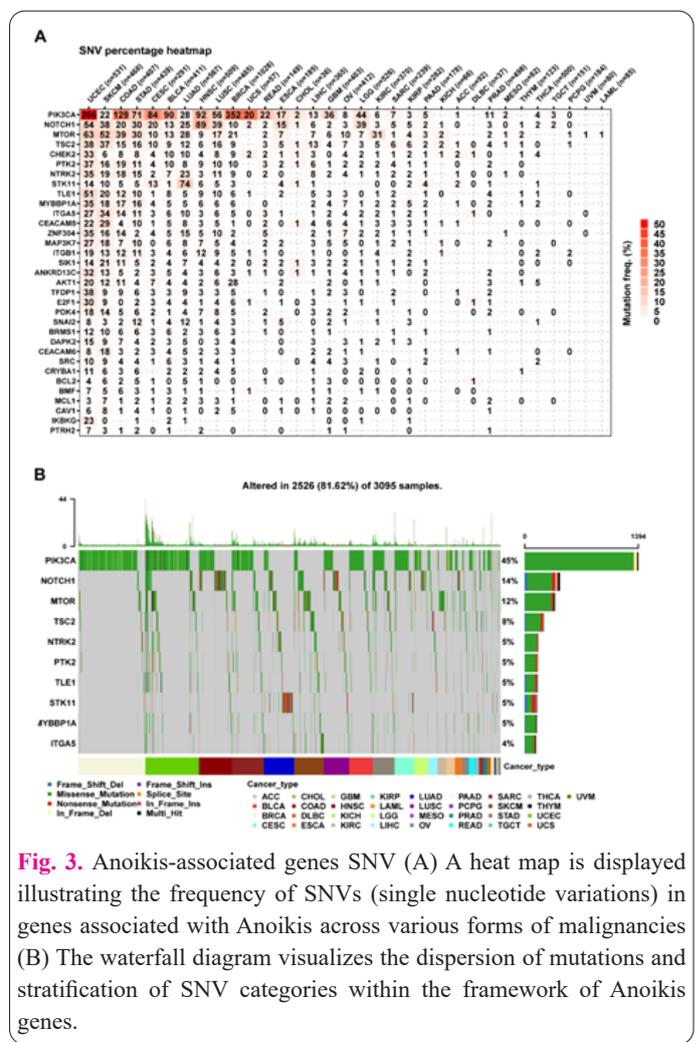


Fig. 3. Anoikis-associated genes SNV (A) A heat map is displayed illustrating the frequency of SNVs (single nucleotide variations) in genes associated with Anoikis across various forms of malignancies (B) The waterfall diagram visualizes the dispersion of mutations and stratification of SNV categories within the framework of Anoikis genes.

revealed that PTK2, E2F1, and SRC had significant mutation frequencies, but MYBB1A, BCL2, and MTOR had a more moderate trend (Figure 4A). Subsequently, we explored the relationship between CNV and the expression levels of mRNA. The CNV of the Anoikis pathway gene was essentially positively linked with each gene's mRNA expression in BRCA, LUCS, and OV. At the pan-cancer level, PTK2, AKT1, and TFDP1 mRNA expression were typically consistent with the incidence of CNV mutations, but PDK4 and CNV were essentially not statistically significant or negatively linked. (Figure 4B.) In 14 malignancies, we examined the methylation levels in tumor tissues and healthy tissues. PDK4, Notch1, and ITGB1 methylation levels in BRCA were noticeably greater than those in healthy tissues, but BMF exhibited a negative trend. CAV1 has the least methylation in KIRC. ITGA5, NOTCH1, and SNAI2 all displayed a reversal trend with regard to their respective mRNA expression when we conducted an analysis of the relationship between methylation levels and mRNA expression in various types of cancer. We found that there is a strong negative correlation between the Anoikis pathway gene and mRNA expression in PAAD. (Figure 4C-D.)

By utilizing Spearman correlation analysis, we performed calculations to determine the association between the penetration of immune cells and the expression of gene mRNA (Figure 5). The study showed that BCL-2 was adversely associated with neutrophils, nTreg, and other immune cells, but that it was positively connected with a considerable number of immune cells. At the same time, BMF, NTRK2, NOTCH1, and other anoikis genes showed

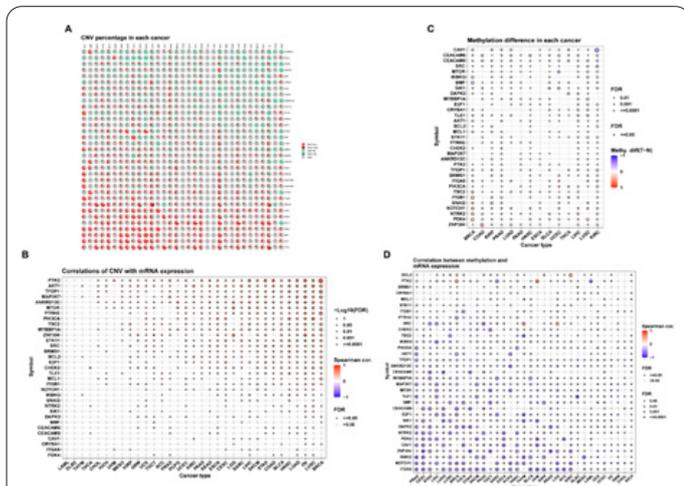


Fig. 4. Anoikis genes CNV and Methylation Profile (A) Pie plot summarizes the CNV of inputted genes within the chosen types of malignancies (B) Figure provides the correlation of CNV with mRNA expression (C) The summary provides a visual representation of the variation in methylation levels between neoplastic and healthy specimens concerning the particular genes chosen within the defined cancer categories (D) The connection between methylation and mRNA expression is summarized in the figure.

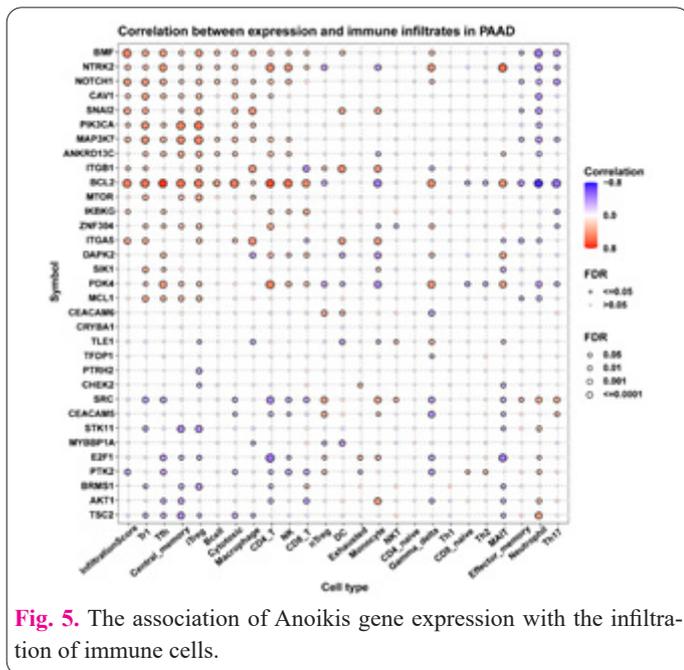


Fig. 5. The association of Anoikis gene expression with the infiltration of immune cells.

positive changes in iTreg, CD4+T, and NK cells.

3.4. Elucidating anoikis scores and its varied influences on prognostic outcomes across an array of cancer types

In order to measure Anoikis, we evaluated the GSVA enrichment score for the Anoikis signature (referred to as the Anoikis score) in each type of cancer. There is significant variation in Anoikis scores across different types of tumors (Figure 6A). At the same time, we divided the cancer into two groups according to the above Anoikis score and the best cut-off value. It's clear that the group with lower expression demonstrates a notably superior prognosis relative to the group with elevated expression in various cancers such as PAAD, BLCA, and KIRP. It should be noted that in HNSC, the group characterized by higher expression demonstrably exhibits a superior prognosis when contrasted with the group with lower expression. (Figure 6B) At the same time, HNSC is also the cancer with the

highest Anoikis enrichment score. We explored the association between the scores derived from anoikis-associated genes and the prognostic outcomes from the pan-cancer dataset. We assessed the survival of patients employing measures such as overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI). The results of Cox regression analysis on 33 different types of cancer indicated a significant correlation between the anoikis score and overall survival (OS) in 7 specific types of cancer, namely: MESO, LGG, ACC, KIRP, PAAD, BLC, and SKCM (Figure 7A). In our study, we investigated the correlation between the anoikis score and DSS in individuals with cancer. The impact of the anoikis score on DSS was observed in a total of seven cancer types, namely KIRP, LGG, ACC, PAAD, MESO, HNSC, and BLCA (Figure 7B). The Cox regression analysis revealed that having a higher anoikis score posed a risk factor for PAAD, BRCA, KIRP, and ACC (Figure 7C). Furthermore, we inspected the correlation between the anoikis score and disease-free interval (DFI), noting the influence of the anoikis score on the DFI in patients afflicted with PAAD, KIRP, LGG, BLCA, ACC, and BRCA (Figure 7D). To sum up, we found that in PAAD, anoikis score is positively correlated with OS, DSS, DFI, and PFI, which may be a good indicator to predict the prognosis of patients with PAAD.

3.5. Anoikis-associated biological pathways

To investigate the pathways related to anoikis, we star-

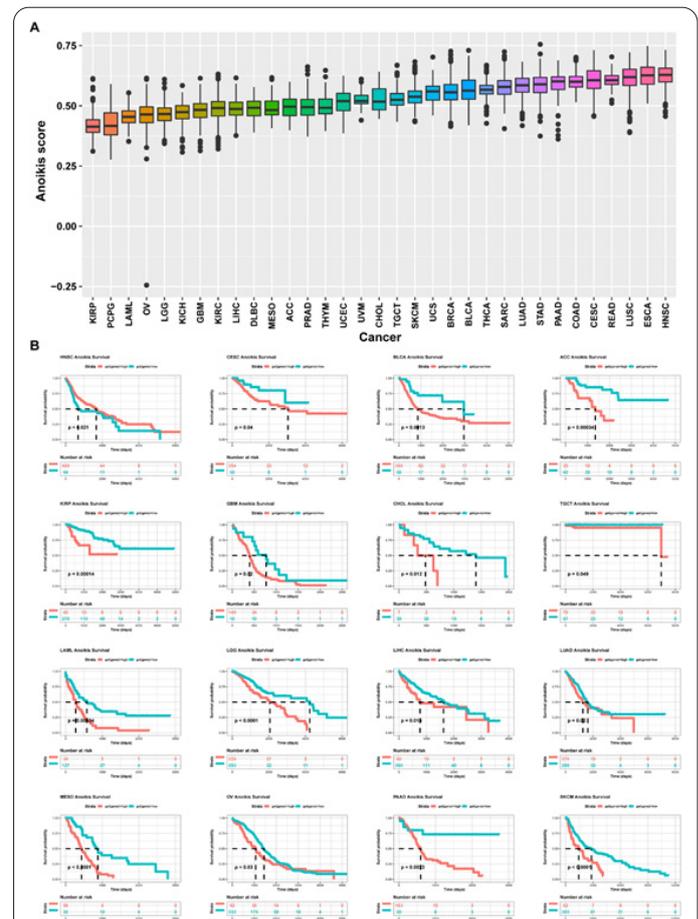


Fig. 6. The diverse prognostic impact of Anokis scores across multiple cancer forms is defined. (A) Anoikis is characterized by a profile of pan-cancer GSVA enrichment scores. (B) A set of Kaplan-Meier curves displaying the correlation between stratified Anoikis scores and cancer survival in multiple cases.

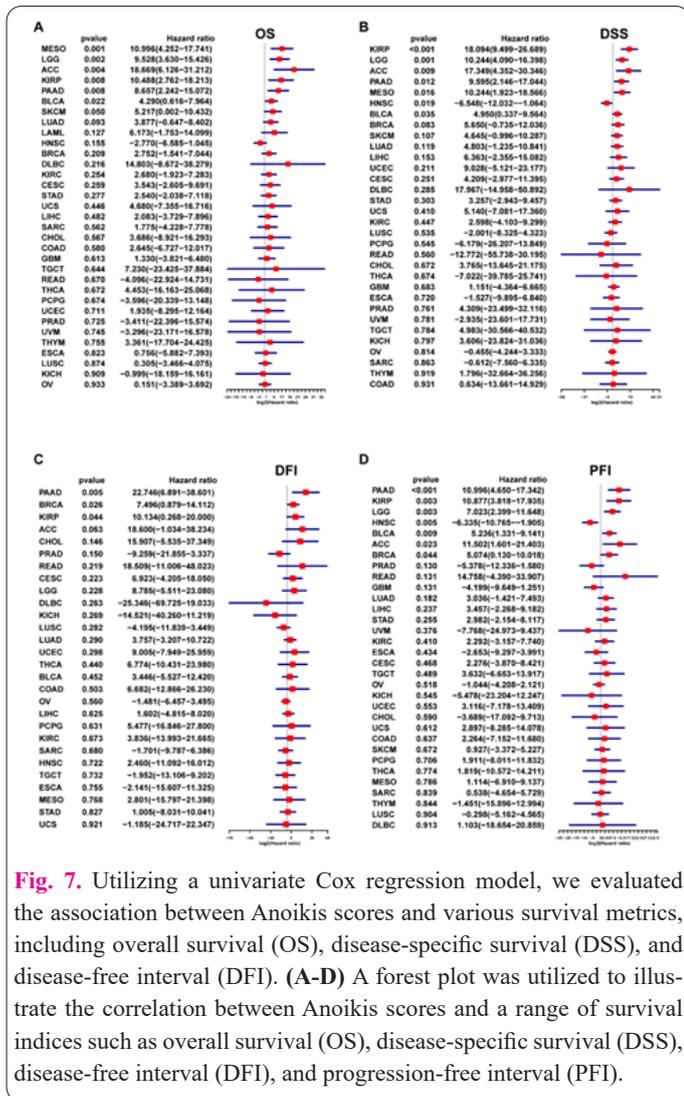


Fig. 7. Utilizing a univariate Cox regression model, we evaluated the association between Anoikis scores and various survival metrics, including overall survival (OS), disease-specific survival (DSS), and disease-free interval (DFI). (A-D) A forest plot was utilized to illustrate the correlation between Anoikis scores and a range of survival indices such as overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI).

ted by generating an anoikis score. This involved assessing the mRNA expression patterns of 34 genes associated with anoikis in a variety of cancer types using the ssGSEA method. This score provides an approximate indication of the overall expression level of anoikis. Secondly, we used the HALLMARK path dataset of the MsigDB database to perform the GSEA analysis and completed the correlation analysis of the Anoikis score and biological pathways with the pheatmap package. Confirming the fact that anoikis is a crucial regulator of the immune system, more than 30 types of cancer exhibited a positive association with immunological mechanisms, including TGF-beta signaling and TNFA signaling via NFKB. At the same time, our research shows that in most cancers, Anoikis scores are negatively correlated with FATTY and BILE ACID METABOLISM, PEROXISOME, and other pathways. (Figure 8)

3.6. Pan-cancer analysis of the correlation between the Anoikis Scores and Tumor microenvironment

Increasing research evidence points towards the pivotal function of the tumor immune microenvironment in the initiation and advancement of tumors (22, 23). Hence, delving deeper into the connection between the tumor microenvironment (TME) and Anoikis expression becomes essential. We employed the ESTIMATE algorithm to gauge stromal scores, immune scores, the ESTIMATE score, and tumor purity across 33 distinct cancer types. We then examined the relationship between Anoikis expres-

sion levels and these four scores. Our findings indicate a significant positive correlation between the expression of Anoikis-related genes and the stromal scores, immune scores, and ESTIMATE Score in pan-cancer analysis for PAAD, PCPG, LAML, PRAD, LGG, COAD, KIRP, and OV. Nevertheless, this correlation was not observed in CHOL, DLBC, MESO, and LAML. At the same time, the above cancers were negatively correlated with tumor purity. (Figure 9A) Then we analyzed the tumor microenvironment from the aspects of the immune-related pathway, the matrix/metastasis-related pathway, and the DNA loss

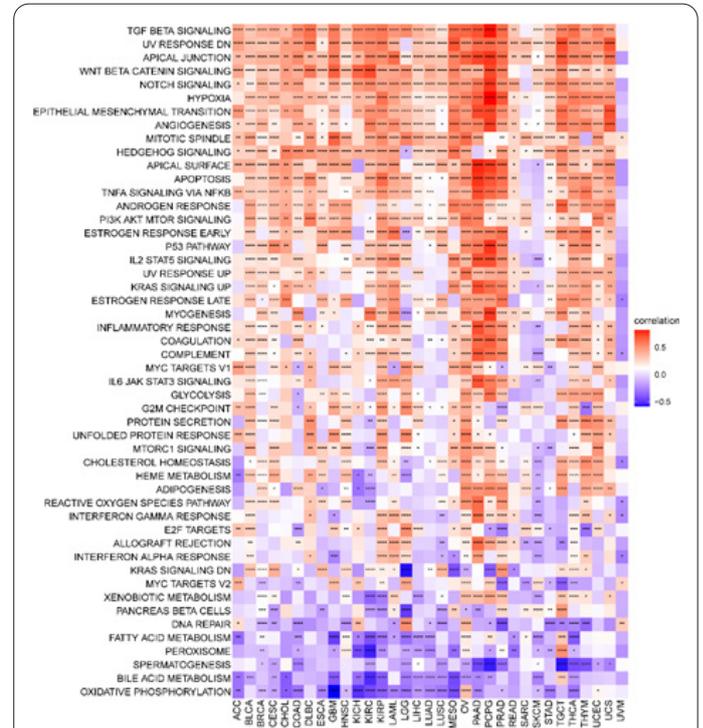


Fig. 8. The Anoikis score is strongly linked to the key pathways in cancer, particularly the immune pathway. Positive correlation is represented by the color red, negative correlation by blue, and the intensity of the color indicates the strength of the correlation.

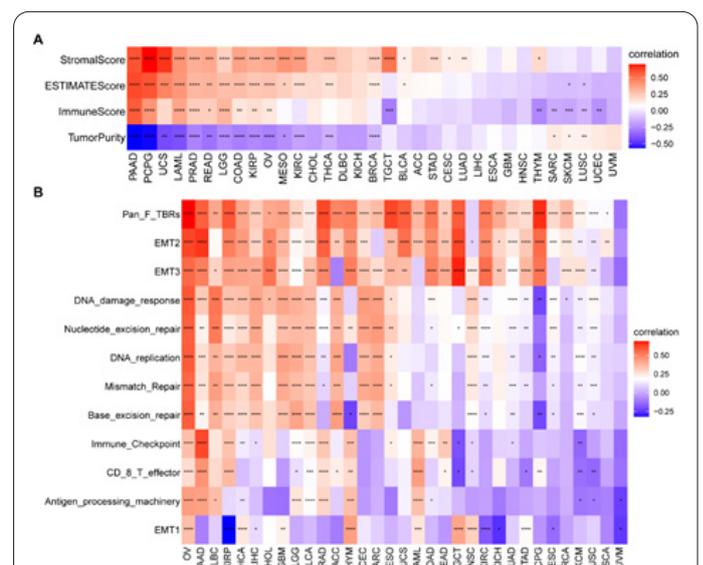


Fig. 9. The correlation between the Anoikis Scores and Tumor microenvironment (A) Tumor purity, ESTIMATES score, immune score, and the stromal score of 33 types of tumors. (B) The relationship between Anoikis Scores and Tumor microenvironment was distinguished by different signatures (immune-relevant signature, mismatch-relevant signature and stromal-relevant signature).

repair-related pathway, respectively. We found that in OV and PAAD, the Anoikis score basically showed a positive correlation trend with the above three related pathways. From the perspective of pancreatic cancer, the Anoikis score was mainly concentrated in EMT2, EMT3, and Pan_F_TBRs and other matrix/transfer-related pathways, which coincides with the definition of Anoikis(Figure 9B).

3.7. Pan-cancer analysis of the anoikis score and immune cell infiltration

To investigate the correlation between the immune response and the anoikis score, we conducted a comprehensive analysis across various types of cancer. Utilizing the TIMER2 and ImmuCellAI databases, we examined the relationship between the anoikis score and the level of immune infiltration. Figure 10A clearly demonstrates that there are significant associations between the anoikis score and the abundance of infiltrating immune cells. In particular, we noticed a relationship between the anoikis score and the incidence of B cells across 12 cancer types, CD4+ T cells in 10 cancer varieties, CD8+ T cells spanning 14 cancer types, macrophages in 13 different cancers, neu-

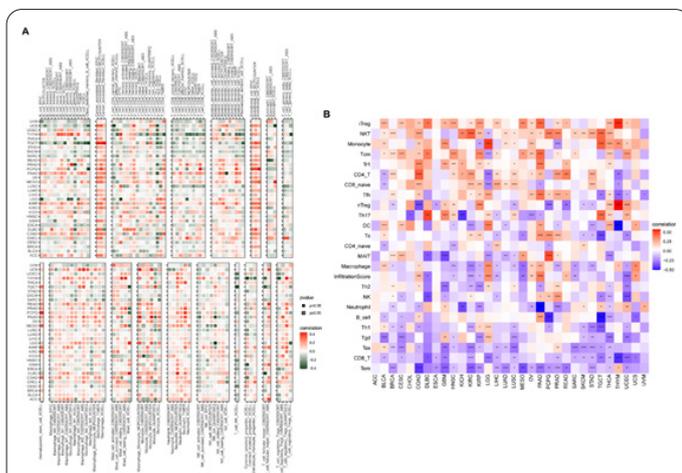


Fig. 10. The analysis of Anoikis score and immune cell infiltration (A) Relationship between Anoikis score and immune cell infiltration in different cancers by TIMER2 database (<http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/>). (B) The TME-infiltrating cell of 32 types of tumors by ImmuCellAI(<http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/>).

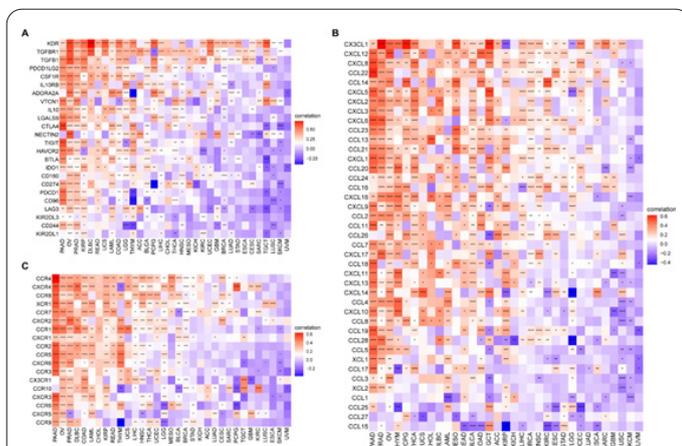


Fig. 11. Co-expression of the Anoikis score and genes related to the immune system. (A) There exists a connection between the Anoikis score and genes related to immunosuppression as well as immune checkpoints. (B-C) The relevance between the Anoikis score and proteins related to chemokines and their receptors. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

trophils present in 13 cancer forms, and DCs noted in 15 types of cancer (Figure 10B).

Furthermore, in our analysis of 33 tumors, we discovered connections between the Anoikis score and immune-related genes. These genes were responsible for encoding proteins involved in immunosuppression, immunoregulation, chemotaxis, and chemokine receptors. The heatmap that resulted from our analysis demonstrated a strong positive correlation between Anoikis score and nearly all immune-related genes in cases of pancreatic adenocarcinoma (PAAD), ovarian cancer (OV), and prostate adenocarcinoma (PRAD) (Figure 11). However, in UVM, SKCM, and LUSC, the Anoikis score showed a significant negative correlation trend with immune-related genes.

3.8. Anoikis score with immunotherapy response and drug sensitivity

According to our previous analysis, when the score of the Anoikis pathway is high, the immune microenvironment is in a relatively immunosuppressive state. We speculate that patients with a high score on the Anoikis pathway have worse immunotherapeutic efficacy. We discussed the relationship between Anoikis score and immunotherapy in SKCM, advanced non-small cell lung cancer, and urothelial cancer. Using the GSE78220 database, we discovered that SKCM patients with immune tolerance had a higher Anoikis score. Furthermore, we conducted survival analysis based on the optimal cutoff value and observed that patients with high scores had a worse prognosis. Lastly, based on these findings, we evaluated the proportion of immunotherapy effectiveness in both the high and low Anoikis score groups. In the high-score group, 62% of patients exhibited immune tolerance, while the low-score group had 80% of patients who were responsive to immunotherapy. (Figure 12A-C) Therefore, we have reason to believe that immunotherapy is less effective in patients with high expression of the Anoikis score. Furthermore, we found that in advanced non-small cell lung cancer patients receiving anti-PD-1/PD-L1 treatment, the prognosis of the high-rating group was poor. At the same time, it was shocking that the proportion of disease progression in the high-rating group was 100% (Figure 12D-F). Finally, we conducted the same analysis on urothelial carcinoma according to the above process, and the results showed that the immunotherapy effect of the high-rating group was poor. Among them, the proportion of immune tolerance in high-rating groups also reached an amazing 91% (Figure 12G-I). In a word, patients with a high Anoikis score are in an immunosuppressive microenvironment, and the immunotherapy effect is poor, which may provide new ideas for clinical immunotherapy.

Additionally, we have explored other research areas unrelated to immunotherapy, where we investigated the connection between the mRNA expression of genes associated with Anoikis and the IC50 values of drugs obtained from the GDSC. It was observed that AKT1, TLE1, SRC, SIK1, and PTK2 exhibited a consistent positive correlation trend with nearly all of the drugs that were selected for analysis. (the higher the gene expression, the worse the non-immunotherapy effect), except 17-AGG. MAP3K7, BCL2, STK11, and BMF showed a significant negative correlation trend with them (Figure 13A). Later, we selected CTRP drugs for the same analysis. The results showed that CAV1, TLE1, and SRC showed a positive correlation

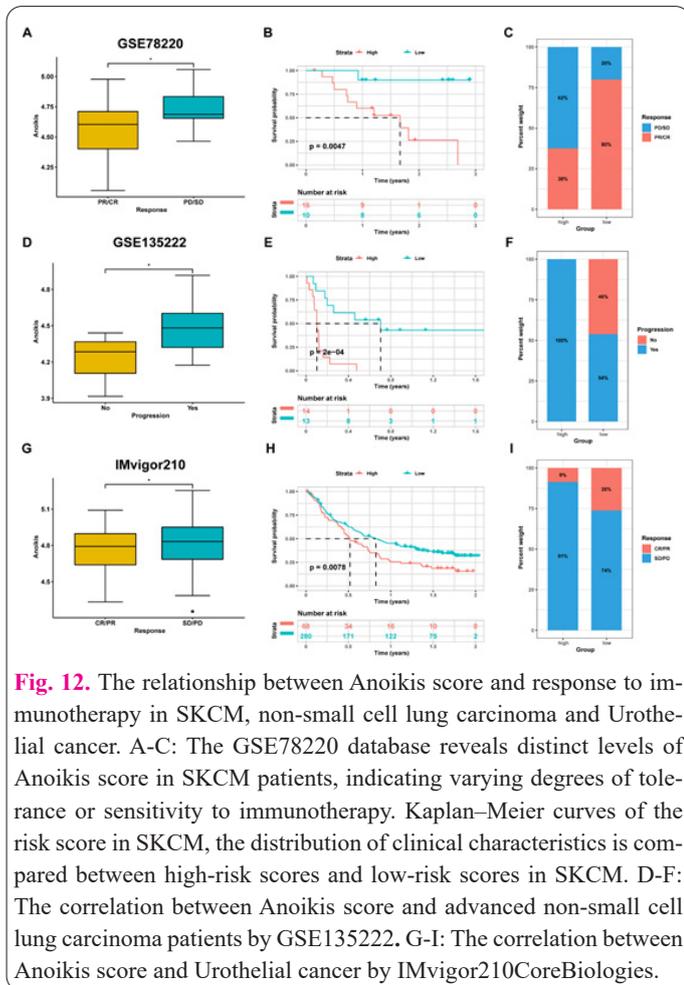


Fig. 12. The relationship between Anoikis score and response to immunotherapy in SKCM, non-small cell lung carcinoma and Urothelial cancer. A-C: The GSE78220 database reveals distinct levels of Anoikis score in SKCM patients, indicating varying degrees of tolerance or sensitivity to immunotherapy. Kaplan–Meier curves of the risk score in SKCM, the distribution of clinical characteristics is compared between high-risk scores and low-risk scores in SKCM. D-F: The correlation between Anoikis score and advanced non-small cell lung carcinoma patients by GSE135222. G-I: The correlation between Anoikis score and Urothelial cancer by IMvigor210CoreBiologies.

trend with more than ordinary drugs, while AKT1, BCL2, and STK11 showed a negative correlation (Figure 13B).

3.9. Identification of CEACAM5,6 as a key Anoikis-related gene in PAAD

After we identified CEACAM5 and CEACAM6 as risk factors for PAAD patients' prognosis using univariate Cox regression analysis, we utilized the clinical data from the TCGA and GSE62452 databases to conduct KM survival analysis (Fig. 14A, B). The patients who exhibited high levels of CEACAM5 and CEACAM6 expression experienced a less favorable prognosis. Furthermore, to evaluate the protein expression of CEACAM5 and CEACAM6, we analyzed the immunohistochemistry (IHC) data obtained from the HPA database, as shown in Figures 14C-F. Meanwhile, we performed a preliminary analysis of the differential expression of CEACAM5 and CEACAM6 in both normal pancreatic tissues and tumor tissues. (Figure. 1A). The findings from both of these databases strongly correlate with each other. Normal pancreatic tissue demonstrated moderate CEACAM5 and CEACAM6 IHC staining, while tumor tissues presented with intense staining.

4. Discussion

Anoikis plays a crucial role in protecting the organism by preventing shed cells from reattaching to an incorrect location on a new substrate and inhibiting their growth [9]. Drawing on a range of research, it's been shown that the initiation of anoikis apoptosis hinges on both internal and external pathways [5,30]. Various intracellular signals, such as DNA damage and endoplasmic reticulum stress,

can trigger the activation of Anoikis, whereas the regulation of apoptosis resides primarily with mitochondria [31]. The inability to regulate anoikis is a possible feature of cancer cells, which aids in the invasion and migration of tumors, the creation of distant organ metastases, and the acquisition of drug resistance [32–34]. Nevertheless, there is a scarcity of research examining the influence of genes associated with anoikis on pancreatic cancers.

We used a widely-used technique called gene set enrichment analysis (GSEA) to assess the features of Anoikis in 10422 samples across 34 types of cancer [35,36]. We demonstrated that the Anoikis score is associated with several cancer markers, namely EMT and immune [37–39]. The anti-tumor impact of anoikis is undeniable, and prior research has shown that it is still fundamentally a sort of apoptosis. Anoikis resistance is one of the critical elements that cannot be disregarded in the evolution of cancer, according to an increasing number of studies [40–46]. According to our GSEA research, oxidative stress, EMT, and energy metabolism make up the majority of anoikis resistance. At the same time, we discovered that the prognosis for most cancers was poorer with a higher Anoikis score. Therefore, it is trustworthy that the Anoikis score

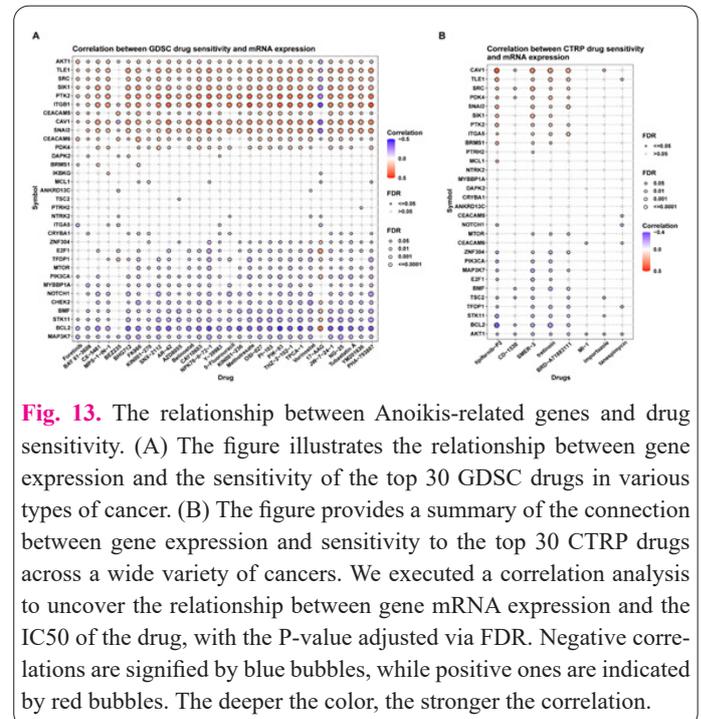


Fig. 13. The relationship between Anoikis-related genes and drug sensitivity. (A) The figure illustrates the relationship between gene expression and the sensitivity of the top 30 GDSC drugs in various types of cancer. (B) The figure provides a summary of the connection between gene expression and sensitivity to the top 30 CTRP drugs across a wide variety of cancers. We executed a correlation analysis to uncover the relationship between gene mRNA expression and the IC50 of the drug, with the P-value adjusted via FDR. Negative correlations are signified by blue bubbles, while positive ones are indicated by red bubbles. The deeper the color, the stronger the correlation.

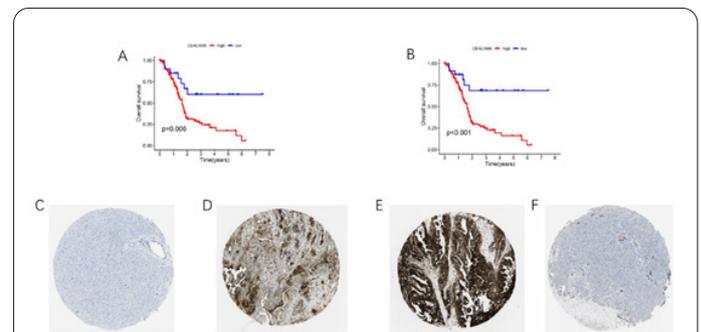


Fig. 14. CEACAM5,6 as a Key Anoikis-Related Gene in PAAD. (A) Survival analysis of CEACAM5. (B) Survival analysis of CEACAM6. (C-D) Differential expression of CEACAM5 in tumor and cancer tissues through IHC. (E-F) Differential expression of CEACAM6 in tumor and cancer tissues through IHC.

may, to some degree, indicate the resistance of Anoikis.

Furthermore, we examined the correlation between the Anoikis score and the efficacy of anti-PD1 treatment in individuals diagnosed with SKCM, advanced non-small cell lung cancer, and urothelial carcinoma. In patients who responded to PD1 blocking, we discovered a slight but substantial reduction in the Anoikis score (Figure 12). Our research also found that patients with high Anoikis scores had positive correlations with many immune cells, including tumor-associated macrophages, CAF, and Treg cells, indicating that these patients were in an immunosuppressive milieu. Together, these results imply that Anoikis score inhibition boosts PD1 treatment sensitivity.

To summarize, we have conducted a comprehensive analysis and offer a fresh perspective on the role of Anoikis in cancer, highlighting its dysregulatory impact. At the same time, we observed a direct connection between the Anoikis score and both the ImmuneScore and Stromalcore within the tumor microenvironment. Conversely, we noticed an opposite relationship with tumor purity. These results indicate that genes related to Anoikis could play a significant role in the immune microenvironment. Additionally, through pathway enrichment analysis, we discovered a strong association with immune checkpoints, EMT, and other factors. These findings potentially offer new insights into the molecular mechanisms and pathways involved in treating cancer immune evasion and metastasis. In addition, we have also offered fresh perspectives on the correlation between Anoikis and other characteristic features of cancer, its prognosis, and various sets of genes. Among these novel associations, it is particularly crucial to explore the Anoikis-triggered Epithelial-Mesenchymal Transition (EMT), as well as the impact of Anoikis-related genes on the movement of immune cells and the regulation of chemotaxis factors.

Declarations

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The original contributions presented in the study are included in the article/Supplementary Material Further inquiries can be directed to the corresponding author.

Authors' contributions

XJ.W. wrote the article; XY.W., C.Y. processed the data analysis; C.Y. and PF.D. revised the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding

This study was supported by the Suzhou Science and Technology Plan Project (No. SYS2020124).

Acknowledgments

With sincere thanks to the teams of authors of the open source software involved in this study, including but not limited to R studio, we also thank the distributors of the R packages and the online databases referenced in this research.

Availability of data and materials

The expression profiles and clinical data of TCGA and GTEx are sourced from the UCSC XENA website: <https://xenabrowser.net/datapages/>

R language version 4.1.1

The Anoikis gene set is sourced from the MsigDB database

GOBP_ANOIKIS

http://www.gseamsigdb.org/gsea/msigdb/cards/GOBP_ANOIKIS

References

- Fang W, Chen Z (2023) Identification of anoikis related subtypes and construction of prognostic model in hepatocellular carcinoma. *Cell Mol Biol* 69:219-228. doi: 10.14715/cmb/2023.69.9.34
- Jin L, Chun J, Pan C, Kumar A, Zhang G, Ha Y et al (2018) The PLAG1-GDH1 Axis Promotes Anoikis Resistance and Tumor Metastasis through CamKK2-AMPK Signaling in LKB1-Deficient Lung Cancer. *Mol Cell* 69:87-99. doi: 10.1016/j.molcel.2017.11.025
- Wang YN, Zeng ZL, Lu J, Wang Y, Liu ZX, He MM et al (2018) CPT1A-mediated fatty acid oxidation promotes colorectal cancer cell metastasis by inhibiting anoikis. *Oncogene* 37:6025-6040. doi: 10.1038/s41388-018-0384-z
- Ye G, Yang Q, Lei X, Zhu X, Li F, He J et al (2020) Nuclear MYH9-induced CTNBN1 transcription, targeted by staurosporin, promotes gastric cancer cell anoikis resistance and metastasis. *Theranostics* 10:7545-7560. doi: 10.7150/thno.46001
- Li S, Chen Y, Zhang Y, Jiang X, Jiang Y, Qin X et al (2019) Shear stress promotes anoikis resistance of cancer cells via caveolin-1-dependent extrinsic and intrinsic apoptotic pathways. *J Cell Physiol* 234:3730-3743. doi: 10.1002/jcp.27149
- Simpson CD, Anyiwe K, Schimmer AD (2008) Anoikis resistance and tumor metastasis. *Cancer Lett* 272:177-185. doi: 10.1016/j.canlet.2008.05.029
- Zhong X, Rescorla FJ (2012) Cell surface adhesion molecules and adhesion-initiated signaling: understanding of anoikis resistance mechanisms and therapeutic opportunities. *Cell Signal* 24:393-401. doi: 10.1016/j.cellsig.2011.10.005
- Li AE, Ito H, Rovira II, Kim KS, Takeda K, Yu ZY et al (1999) A role for reactive oxygen species in endothelial cell anoikis. *Circ Res* 85:304-310. doi: 10.1161/01.res.85.4.304
- Yu Y, Song Y, Cheng L, Chen L, Liu B, Lu D et al (2022) CircCE-MIP promotes anoikis-resistance by enhancing protective autophagy in prostate cancer cells. *J Exp Clin Canc Res* 41:188. doi: 10.1186/s13046-022-02381-7
- Corbet C, Bastien E, Santiago DJJ, Dierge E, Martherus R, Vander LC et al (2020) TGFbeta2-induced formation of lipid droplets supports acidosis-driven EMT and the metastatic spreading of cancer cells. *Nat Commun* 11:454. doi: 10.1038/s41467-019-14262-3
- Andre S, Sanchez-Ruderisch H, Nakagawa H, Buchholz M, Kopitz J, Forberich P et al (2007) Tumor suppressor p16INK4a--modulator of glycomic profile and galectin-1 expression to increase susceptibility to carbohydrate-dependent induction of anoikis in pancreatic carcinoma cells. *Febs J* 274:3233-3256. doi:

- 10.1111/j.1742-4658.2007.05851.x
12. Cieply B, Koontz C, Frisch SM (2015) CD44S-hyaluronan interactions protect cells resulting from EMT against anoikis. *Matrix Biol* 48:55-65. doi: 10.1016/j.matbio.2015.04.010
 13. Joyce JA (2005) Therapeutic targeting of the tumor microenvironment. *Cancer Cell* 7:513-520. doi: 10.1016/j.ccr.2005.05.024
 14. Belli C, Trapani D, Viale G, D'Amico P, Duso BA, Della VP et al (2018) Targeting the microenvironment in solid tumors. *Cancer Treat Rev* 65:22-32. doi: 10.1016/j.ctrv.2018.02.004
 15. Malmberg KJ, Ljunggren HG (2006) Escape from immune- and nonimmune-mediated tumor surveillance. *Semin Cancer Biol* 16:16-31. doi: 10.1016/j.semcancer.2005.07.007
 16. Walsh SR, Simovic B, Chen L, Bastin D, Nguyen A, Stephenson K et al (2019) Endogenous T cells prevent tumor immune escape following adoptive T cell therapy. *J Clin Invest* 129:5400-5410. doi: 10.1172/JCI126199
 17. Albin A, Mirisola V, Pfeffer U (2008) Metastasis signatures: genes regulating tumor-microenvironment interactions predict metastatic behavior. *Cancer Metast Rev* 27:75-83. doi: 10.1007/s10555-007-9111-x
 18. Veschi V, Verona F, Lo IM, D'Accardo C, Porcelli G, Turdo A et al (2020) Cancer Stem Cells in Thyroid Tumors: From the Origin to Metastasis. *Front Endocrinol* 11:566. doi: 10.3389/fendo.2020.00566
 19. Celia-Terrassa T, Jolly MK (2020) Cancer Stem Cells and Epithelial-to-Mesenchymal Transition in Cancer Metastasis. *Csh Perspect Med* 10doi: 10.1101/cshperspect.a036905
 20. Reck M, Remon J, Hellmann MD (2022) First-Line Immunotherapy for Non-Small-Cell Lung Cancer. *J Clin Oncol* 40:586-597. doi: 10.1200/JCO.21.01497
 21. Ott PA, Hu-Lieskovan S, Chmielowski B, Govindan R, Naing A, Bhardwaj N et al (2020) A Phase Ib Trial of Personalized Neoantigen Therapy Plus Anti-PD-1 in Patients with Advanced Melanoma, Non-small Cell Lung Cancer, or Bladder Cancer. *Cell* 183:347-362. doi: 10.1016/j.cell.2020.08.053
 22. Hanzelmann S, Castelo R, Guinney J (2013) GSEA: gene set variation analysis for microarray and RNA-seq data. *Bmc Bioinformatics* 14:7. doi: 10.1186/1471-2105-14-7
 23. Liu CJ, Hu FF, Xie GY, Miao YR, Li XW, Zeng Y et al (2023) GSCA: an integrated platform for gene set cancer analysis at genomic, pharmacogenomic and immunogenomic levels. *Brief Bioinform* 24:bbac558. doi: 10.1093/bib/bbac558
 24. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q et al (2020) TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res* 48:W509-W514. doi: 10.1093/nar/gkaa407
 25. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A et al (2013) CD4(+) follicular helper T cell infiltration predicts breast cancer survival. *J Clin Invest* 123:2873-2892. doi: 10.1172/JCI67428
 26. Ni J, Wang X, Stojanovic A, Zhang Q, Wincher M, Buhler L et al (2020) Single-Cell RNA Sequencing of Tumor-Infiltrating NK Cells Reveals that Inhibition of Transcription Factor HIF-1 α Unleashes NK Cell Activity. *Immunity* 52:1075-1087. doi: 10.1016/j.immuni.2020.05.001
 27. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S et al (2016) Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* 165:35-44. doi: 10.1016/j.cell.2016.02.065
 28. Jung H, Kim HS, Kim JY, Sun JM, Ahn JS, Ahn MJ et al (2019) DNA methylation loss promotes immune evasion of tumours with high mutation and copy number load. *Nat Commun* 10:4278. doi: 10.1038/s41467-019-12159-9
 29. Snyder A, Nathanson T, Funt SA, Ahuja A, Buross NJ, Hellmann MD et al (2017) Contribution of systemic and somatic factors to clinical response and resistance to PD-L1 blockade in urothelial cancer: An exploratory multi-omic analysis. *Plos Med* 14:e1002309. doi: 10.1371/journal.pmed.1002309
 30. Laguinde LM, Samara RN, Wang W, El-Deiry WS, Corner G, Augenlicht L et al (2008) DR5 receptor mediates anoikis in human colorectal carcinoma cell lines. *Cancer Res* 68:909-917. doi: 10.1158/0008-5472.CAN-06-1806
 31. Rath E, Moschetta A, Haller D (2018) Mitochondrial function - gatekeeper of intestinal epithelial cell homeostasis. *Nat Rev Gastro Hepat* 15:497-516. doi: 10.1038/s41575-018-0021-x
 32. Pierce CJ, Simmons JL, Broit N, Karunarathne D, Ng MF, Boyle GM (2020) BRN2 expression increases anoikis resistance in melanoma. *Oncogenesis* 9:64. doi: 10.1038/s41389-020-00247-1
 33. Rutherford TR, Elder AM, Lyons TR (2021) Anoikis resistance in mammary epithelial cells is mediated by semaphorin 7a. *Cell Death Dis* 12:872. doi: 10.1038/s41419-021-04133-5
 34. Wang L, Li C, Wang J, Yang G, Lv Y, Fu B et al (2022) Transformable ECM Deprivation System Effectively Suppresses Renal Cell Carcinoma by Reversing Anoikis Resistance and Increasing Chemotherapy Sensitivity. *Adv Mater* 34:e2203518. doi: 10.1002/adma.202203518
 35. Shen XT, Xie SZ, Xu J, Yang LY, Qin LX (2022) Pan-Cancer Analysis Reveals a Distinct Neutrophil Extracellular Trap-Associated Regulatory Pattern. *Front Immunol* 13:798022. doi: 10.3389/fimmu.2022.798022
 36. Sun Z, Zhao Y, Wei Y, Ding X, Tan C, Wang C (2022) Identification and validation of an anoikis-associated gene signature to predict clinical character, stemness, IDH mutation, and immune infiltration in glioblastoma. *Front Immunol* 13:939523. doi: 10.3389/fimmu.2022.939523
 37. Wang LN, Zhang ZT, Wang L, Wei HX, Zhang T, Zhang LM et al (2022) TGF- β 1/SH2B3 axis regulates anoikis resistance and EMT of lung cancer cells by modulating JAK2/STAT3 and SHP2/Grb2 signaling pathways. *Cell Death Dis* 13:472. doi: 10.1038/s41419-022-04890-x
 38. Cheng X, Shen T, Liu P, Fang S, Yang Z, Li Y et al (2022) mir-145-5p is a suppressor of colorectal cancer at early stage, while promotes colorectal cancer metastasis at late stage through regulating AKT signaling evoked EMT-mediated anoikis. *Bmc Cancer* 22:1151. doi: 10.1186/s12885-022-10182-6
 39. Chen Z, Liu X, Zhu Z, Chen J, Wang C, Chen X et al (2022) A novel anoikis-related prognostic signature associated with prognosis and immune infiltration landscape in clear cell renal cell carcinoma. *Front Genet* 13:1039465. doi: 10.3389/fgene.2022.1039465
 40. Yang Y, Jiao Y, Mohammadi MR. (2023). Post-translational modifications of proteins in tumor immunotherapy and their potential as immunotherapeutic targets. *Cell Mol Biomed Rep* 3(4):172-84. doi: 10.55705/cmbr.2023.378480.1091.
 41. Ali Salman R. Prevalence of women breast cancer. (2023). *Cell Mol Biomed Rep* 3(4):185-96. doi: 10.55705/cmbr.2023.384467.1095.
 42. Kanwal N, Al Samarrai OR, Al-Zaidi HM, Mirzaei AR, Heidari MJ. (2023). Comprehensive analysis of microRNA (miRNA) in cancer cells. *Cell Mol Biomed Rep* 3(2):89-97. doi: 10.55705/cmbr.2022.364591.1070
 43. Li X, Mohammadi MR. (2023). Combined Diagnostic Efficacy of Red Blood Cell Distribution Width (RDW), Prealbumin (PA), Platelet-to-Lymphocyte Ratio (PLR), and Carcinoembryonic Antigen (CEA) as Biomarkers in the Diagnosis of Colorectal Cancer. *Cell Mol Biomed Rep* 3(2):98-106. doi: 10.55705/cmbr.2023.374804.1088.
 44. Alsaedy HK, Mirzaei AR, Alhashimi RA. (2022). Investigating the structure and function of Long Non-Coding RNA (LncRNA) and its role in cancer. *Cell Mol Biomed Rep* 2(4):245-53. doi: 10.55705/cmbr.2022.360799.1062.

45. Alavi M, Rai M, Martinez F, Kahrizi D, Khan H, Rose Alencar De Menezes I, Douglas Melo Coutinho H, Costa JG (2022). The efficiency of metal, metal oxide, and metalloids nanoparticles against cancer cells and bacterial pathogens: different mechanisms of action. *Cell Mol Biomed Rep* 2022 2(1):10-21. doi: 10.55705/cnbr.2022.147090.1023.
46. Zhang T, Wang B, Su F, Gu B, Xiang L, Gao L et al (2022) TC-F7L2 promotes anoikis resistance and metastasis of gastric cancer by transcriptionally activating PLAUR. *Int J Biol Sci* 18:4560-4577. doi: 10.7150/ijbs.69933