Clinicopathological Features, Immune Infiltration Landscape and Involved Signaling Pathways of the desmogleins family in Pancreatic Adenocarcinoma

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Research Article

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Abstract

Background

A growing body of evidence suggests that the DSG family plays a key role in tumorigenesis and progression; however, the function of DSG family members in PAAD remains unclear.

Methods

Comprehensive bioinformatics analysis was performed to investigate the clinicopathological characteristics, prognostic value, immunological features, and functional mechanisms of DSG family members in PAAD, using UALCAN, the HPA, Kaplan–Meier Plotter, cBioPortal, TISIDB, LinkedOmics, STRING and GSCALite Database.

Results

The expression of DSG family members was significantly higher in PAAD tissues compared with paraneoplastic or normal tissues, and their copy number variation was significantly associated with poorer clinicopathological characteristics and prognosis in PAAD patients. Furthermore, the roles of DSG family members in immune regulation are diverse and complex. Mechanistically, TP53 mutations are significantly associated with promoter methylation and the expression of DSG family members, and EGFR may be key to the role of DSG family members in PAAD. DSG family members activate several oncogenic pathways, including EMT, PI3K/AKT, and RAS/MAPK signaling pathway. In addition, we found that the expression of DSG family members was significantly correlated with sensitivity to multiple conventional chemotherapeutic agents and novel targeted drugs.

Conclusions

DSG family members play an oncogenic role in the development of PAAD and may serve as novel biomarkers or therapeutic targets.

1. Introduction

PAAD is a deadly gastrointestinal malignancy and unresectable or metastatic disease in approximately 80%-85% of patients, resulting in a five-year survival rate of less than 11% \[1\]. Global cancer statistics for 2020 show that PAAD ranked 7th in terms of tumor-caused deaths \[2\]. Despite great progress in medical diagnostic technology, the early diagnosis of PAAD still faces serious challenges. The sensitivity of CA19-9 for PAAD diagnosis is between 70% and 80%, but the specificity is only 50% \[3\]; furthermore, a sensitive early diagnostic index is still lacking. Surgical radical resection is still the main treatment; however, nearly
80% of PAAD patients are often not diagnosed until middle or terminal stages due to vague symptoms and have lost the opportunity for surgery \[4\]. Even if radical resection is performed, more than 50% of patients will experience recurrence within one year of surgery, and the five-year survival rate is only 25% \[1\]. In addition, PAAD is insensitive to both radiotherapy and chemotherapy, and despite recent advances in research on targeted therapy, antimetabolite therapy, and immunotherapy, they have not yet shown significant benefits in terms of overall patient survival. Therefore, it is urgent need to explore new diagnostic biomarkers and therapeutic targets for PAAD.

Desmogleins (DSGs), bridging granule core glycoproteins, are a group of transmembrane proteins that occupy an important position among the glycoproteins. Furthermore, they constitute intercellular bridging granules and are members of the cytocalciferin family, which is important in the adhesion process between epithelial cells. Adhesion junctions play a key role in the invasion and migration of cancer cells, and this suggests that the DSG family might be involved in tumorigenesis and development \[5\]. DSGs can occur as the following four isoforms: DSG1, DSG2, DSG3, and DSG4. Previous studies have pointed out that the expression of DSG1 is related to a poorer prognosis in anal canal squamous cell carcinoma \[6\], while DSG2, the most widely distributed isoform of the DSG family, leads to high levels of endocytosis. All human tissues show high levels of endogenous expression, and studies have shown that DSG2 can promote the development and progression of lung adenocarcinoma \[7-8\], ovarian cancer \[9\] and cervical cancer \[10\]. A recent study showed that DSG3 was overexpressed in squamous carcinoma cells, and that its overexpression was positively correlated with the clinical aggressiveness of tumors \[11\]. DSG4 has been less studied in humans, but some studies have reported its important part in the progression of wool traits in domestic animals \[12\]. However, the function of DSG family members in PAAD remains uncertain.

In this study, we used public databases and various bioinformatics analysis technologies to comprehensively study the possible functions and mechanisms of DSG family members in the development and progression of PAAD. First, we analyzed the differential expression of DSG family members in 33 human cancer samples (including PAAD) and paraneoplastic or normal samples using the cancer genome map (TCGA) and genotypic problem expression (GTEx) database. Then, we use HPA database to explore the protein level of DSG family members in PAAD tissue. In addition, according to the UALCAN database and Kaplan-Meier plotter database, we analyzed the correlation between the expression level of PAAD patients and their clinicopathological characteristics, overall survival rate and drug sensitivity. Through gene variation, immune infiltration, gene enrichment, PPI analysis and promoter methylation analysis, the potential molecular mechanism of DSG family members participating in the development and progress of PAAD was discussed. In conclusion, our research shows that DSG family members play a carcinogenic role in the development and progress of PAAD, and they are expected to become new molecular targets for patients with this deadly malignant tumor.

2. Methods and Materials
Ethics Statement: This study was approved by the Ethics Committee of the Cancer Hospital Shenzhen, Chinese Academy of Medical Sciences and was conducted in strict accordance with the principles of the Declaration of Helsinki. All data in this study were retrieved from online databases. No human or animal testing was involved.

2.1. Expression Analysis

In this study, R (version 4.0.3) was used to analyze the expression of DSG family member genes in cancerous and paracancerous tissues in the PAAD project from the TCGA database and normal pancreatic data from the GTEx database (https://commonfund.nih.gov/GTEx) (accessed October 8, 2022). The statistical significance of differences in expression were assessed by the Wilcoxon test. Then, the expression of DSG family member genes in PAAD patients with different clinicopathological features were obtained from the UALCAN database (http://ualcan.path.uab.edu) (accessed October 9, 2022) and statistically analyzed by Welch's t test. \( p < 0.05 \) was considered to be statistically significant\(^\text{13–14}\).

2.2. HPA Analysis

Immunohistochemical staining of DSG2 in PAAD tissues was analyzed using the HPA database (https://www.proteinatlas.org/) (accessed on October 15, 2022). The HPA contains immunohistochemical staining of tissue sections and cell lines that allows for the analysis of differential protein expression in tumor tissues. The protein expression levels were classified into the following four categories based on the intensity and quantification of staining: negative, weak, moderate and strong\(^\text{15}\).

2.3. Survival Analysis

The Kaplan–Meier Plotter database (http://www.kmplot.com/) (accessed on October 16, 2022) was used to analyze the correlation between the expression of DSG family members and overall survival (OS) and relapse-free survival (RFS) in PAAD patients\(^\text{16}\). Log-rank \( P \) values were calculated using the "Survival" R package (version 2.38), and \( P < 0.05 \) was considered statistically significant\(^\text{17}\).

2.4. Gene Variation Analysis

The cBioPortal database (http://www.cbioportal.org/) (accessed on October 18, 2022) was used to analyze the genetic variants of DSG family members in PAAD and to further determine the correlation between the variants and clinicopathological features\(^\text{18}\). Statistical significance was assessed by the chi-square test, and \( P < 0.05 \) was considered statistically significant. In addition, statistical significance of genetic mutations in DSG family members with OS and disease-free survival (DFS) in patients with PAAD was assessed by the log-rank test, and \( P < 0.05 \) was considered statistically significant\(^\text{19}\).

2.5. Methylation Analysis

To assess the association between the expression of DSG family members and promoter methylation level, the level of PAAD promoter methylation under different conditions were analyzed using the UALCAN
database. The statistical significance of the differences was assessed by Welch's t test, and \( P < 0.05 \) was considered statistically significant\(^{20-21}\).

### 2.6. Immune Infiltration Analysis

Correlations of the expression of DSG family members with the infiltration levels of immune cells and the expression of immune molecules in PAAD were analyzed using the TISIDB database (http://cis.hku.hk/TISIDB/) (accessed on October 22, 2022). Statistical significance was assessed by Spearman's test, and \( P < 0.05 \) was considered statistically significant\(^{22}\). This database was then used to analyze the differences in the expression of DSG family members in different immune subtypes of PAAD. The statistical significance of the differences in expression levels was assessed using the Kruskal–Wallis test, and \( P < 0.05 \) was considered statistically significant\(^{22}\). In addition, the TIMER database (http://timer.cistrome.org/) (accessed on October 23, 2022) was used to analyze the correlation between copy number alterations of DSG family members and the level of infiltration of the six classes of immune cells in PAAD. The statistical significance of the differences was assessed by the Wilcoxon rank sum test, and \( P < 0.05 \) was considered statistically significant\(^{23-24}\).

### 2.7. Gene Enrichment Analysis

The LinkedOmics database (http://www.linkedomics.org/) (accessed on October 18, 2022) was used to select the 800 genes most closely associated with DSG family members that were also coexpressed\(^{25}\). The Metascape database (https://metascape.org) (accessed November 3, 2022) was used to visualize the biological processes (BP), cellular components (CC), molecular functions (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) of DSG family members and their 800 coexpressed genes\(^{26}\). In addition, pathway enrichment of DSG family members was performed using the GSCALite database (http://bioinfo.life.hust.edu.cn/web/GSCALite/) (accessed on November 16, 2022)\(^{27}\).

### 2.8. Construction of the Functional PPI Network

The STRING database (https://string-db.org/) (accessed on November 18, 2022) was used to connect the genes with the strongest PPI with DSG family members in order to create a functional PPI network of DSG family members\(^{28}\). The impact of different genes in the PPI network was scored using Cytoscape (version 3.7.2)\(^{29-30}\).

### 2.9. Drug Sensitivity Analysis

The GSCALite database was used to analyze the correlation between the expression of DSG family members and sensitivity to various chemotherapeutic or targeted therapeutic agents for PAAD. The statistical significance of differences was assessed by Spearman's test. \( P < 0.05 \) was considered statistically significant\(^{27}\).

### 3. Result
3.1. Aberrant Expression of DSG Family Members across Cancers

To explore the transcription levels of DSG family members in different tissues, including various cancer, adjacent paraneoplastic and normal one, we analyzed data from TCGA and GTEx database. It was showed that DSG4 had low transcription levels in almost all cancer and paraneoplastic tissues (Fig. 1D). DSG1/3 had high transcriptional activity in only a few cancer and paraneoplastic tissues, including skin cutaneous melanoma (SKCM) tissues, breast cancer (BRCA), esophageal cancer (ESCA), and lung squamous cell carcinoma (LUSC). Compared to normal and paraneoplastic tissues, the transcription levels of DSG1/3 family members were significantly higher in LUSC and PAAD tissues but significantly decreased in BRCA, ESCA and SKCM tissues (Fig. 1A, C). Meanwhile, DSG2 showed a higher transcription level in the majority of tumors and was significantly increased in 25 cancer tissues compared to paraneoplastic and normal tissues, including cholangiocarcinoma (CHOL), PAAD and liver hepatocellular carcinoma (LIHC), but significantly decreased in 4 cancer tissues, including acute myeloid leukemia (LAML), low-grade glioma of the brain (LGG), pheochromocytic and paraganglioma (PCPG), and SKCM tissues. These findings suggest that the expression of DSG family members varies significantly among different cancers; however, their transcription levels were all significantly elevated in PAAD, highlighting the latent part of this family in PAAD.

3.2. Abnormal Expression of DSG2 Proteins in Tissues from PAAD

To observe the expression of DSG family proteins in PAAD tissues, we analyzed immunohistochemical staining of DSG2 proteins using data from the PAAD project in the HPA database (DSG1/3/4 data were missing). Cancer tissues from 12 PAAD patients were stained with HPA004896 antibody, and the results showed that all 12 tissue samples were positive for DSG2 indicators and all were moderately or highly stained. The ratio of moderate staining - high staining was 1:2 (Fig. 1E). In addition, cancer tissues from nine PAAD patients were stained with CAB025122 antibody, and these results pointed out that all nine tissue samples were positive for the DSG2 index. The ratio of highly stained to moderately stained cells was 1:2 (Supplementary Figure S1). Based on these data, DSG2 protein stained positively in all pancreatic cancer samples. Thus, DSG2 proteins were moderately and highly expressed in PAAD tissues, which is similar to the expression at the transcription level.

3.3. The Relationship between the Expression of DSG Family Members and the Clinicopathological Characteristics and Prognosis of PAAD Patients

We analyzed the relationship between the expression of DSG family members and the clinicopathological characteristics of PAAD patients using R (version 4.0.3) based on the PAAD project data in the UALCAN database. The results showed that high expression of DSG1/4 was significantly correlated with race, with DSG1 expression being significantly higher in Caucasians than in Asians ($p = 7.564 \times 10^{-3}$) (Fig. 2A). Conversely, DSG4 expression was lower in Caucasians than in Asians ($p = 4.134 \times 10^{-2}$) (Fig. 2B).
Interestingly, the expression level of DSG2 was significantly higher in TP53-mutated PAAD tissues than in TP53 wild-type tumors \((p = 1.716 \times 10^{-2})\) (Fig. 2C), and the high expression of DSG2 was significantly correlated with poor initial treatment outcome \((p < 0.05)\) (Fig. 2D). The expression level of DSG3 was significantly correlated with age, cancer stage and tumor grade (Fig. 2E), and high expression of DSG3 was significantly correlated with poor initial treatment outcome \((p < 0.001)\) (Fig. 2F). However, there was no significant correlation between the expression levels of DSG family members and other clinicopathological characteristics, such as history of chronic pancreatitis, sex, N stage, history of alcohol consumption or history of diabetes mellitus (Supplementary Figure S2).

Next, we analyzed the correlation between the expression of DSG family members and the prognosis of PAAD patients using the Kaplan–Meier Plotter database. The results showed that increased expression of DSG2 and DSG3 was significantly associated with shorter OS and RFS \((P < 0.05)\), and increased expression of DSG4 was associated with shorter OS \((P < 0.05)\) (Fig. 2F, G). In conclusion, the results of this study showed that high expression of DSG family members in PAAD patients was associated with detrimental clinicopathological features and poor prognosis. This suggests that DSG family members may play an oncogenic role, and they could serve as novel biomarkers for PAAD.

### 3.4. Mutations in the DSG Family Member Genes Were Associated with Worse Clinicopathological Characteristics and a Worse Prognosis for PAAD Patients

To further explore the mechanisms associated with the differential expression of DSG family members in PAAD, we analyzed the genetic variants of DSG family members using the cBioportal online tool. The data were collected from four databases, including TCGA, and out of a total of 807 PAAD samples, 23 samples \((2.6\%)\) had mutations in DSG family member genes. Among them, the gene with the highest mutation frequency was DSG2 \((2.4\%)\), and the main types of mutations were amplification and deep deletion (Fig. 3A); furthermore, there was a significant co-occurrence of mutations in DSG family members in the same sample (Supplementary Material S1). On this basis, we analyzed the clinicopathological characteristics and prognosis of PAAD patients with and without mutations in the DSG family genes. The results showed that mutations in DSG family genes were significantly correlated \((P < 0.05)\) with clinicopathological features of PAAD, such as race, tumor location, tumor focality and tumor mutational burden (TMB) (Fig. 3B, C, D, E). In terms of prognosis, PAAD patients with mutations in DSG family genes had significantly shorter OS \((P < 0.05)\) (Fig. 3F, G).

### 3.5. Correlation between the Methylation Levels of Promoters of the DSG Family Members and the Clinicopathological Characteristics of PAAD Patients

We next explored the relationship between epigenetic modifications of DSG family members and the clinicopathological characteristics of PAAD patients. Based on data from the PAAD project in the UALCAN database, we analyzed the relationship between the level of promoter methylation of DSG family members and the clinicopathological characteristics of PAAD patients. As shown in Fig. 3H, the level of promoter methylation of DSG1/2 was significantly higher in tumor tissues of patients than in
paracancerous tissues \( (p = 3.204 \times 10^{-3} / p = 9.975 \times 10^{-4}) \). In contrast, the level of promoter methylation of DSG3/4 in tumor tissues was significantly lower than that in paraneoplastic tissues \( (p = 4.764 \times 10^{-4} / p = 2.939 \times 10^{-3}) \). However, the levels of promoter methylation of DSG family members did not significantly correlate with clinicopathological features such as cancer stage, tumor grade or N stage (Supplementary Figure S3). In addition, we found that the level of promoter methylation of DSG2 was significantly increased in patients with TP53 mutation \( (p = 3.237 \times 10^{-3}) \) (Fig. 3I), whereas the level of promoter methylation of DSG3/4 was significantly decreased \( (p = 4.102 \times 10^{-3} / p = 3.644 \times 10^{-3}) \) (Fig. 3I). Thus, TP53 mutation have different effects on the methylation of different members of the DSG family, which is consistent with previous studies\[^{31}\].

### 3.6. The Immune Landscape of the Expression and Variations of DSG Family Members in PAAD Patients

Next, using data from the PAAD project in the TISIDB database, we explored the relationship between the expression of DSG family members and the level of infiltration of tumor immune cells and multiple immunomodulators based on various immunological markers (Supplementary Material S2). Our results showed that the role of DSG family members in tumor immunomodulation was inconsistent. First, DSG2 was negatively correlated with the level of infiltration of multiple immune cells, including Act CD8, Tfh cells and eosinophils (Table 1, Supplementary Figure S4A), and significantly negatively correlated with 16 immune-promoting factors, including ADORA2A, CD160 and IL10 (Fig. 4A). Thus, high expression of DSG2 may be mainly associated with suppression of the immune response to PAAD. In contrast, DSG3 was positively correlated with the level of infiltration of several immune cells with high killing capacity, including Act CD4, Tcm CD4 and CD56dim (Table 1, Supplementary Figure S4B); furthermore, DSG3 was positively correlated with most immunomodulators (immune promoters, MHC molecules, chemokines and chemokine receptors) in PAAD (Fig. 4B-E). These findings suggest that high DSG2 expression in PAAD associated with its immunosuppressive tumor microenvironment may promote immune escape from PAAD; meanwhile, DSG3 may play a role in immune activation.

Then, we further explored the relationship between copy number alterations of DSG family members and the infiltration level of tumor-infiltrating cells based on data from the PAAD project in the TIMER database. The results showed that copy number alteration of DSG may affect the infiltration level of six dominant immune cells, including dendritic cells, CD4 + T cells, CD8 + T cells and B cells, especially those with arm-level gain and high amplification (Fig. 4F, G, H, I) (Supplementary Material S3).

Finally, we investigated the relationship between the expression levels of DSG family members and immune subtypes. As shown in Supplementary Figure S4, the expression of DSG3 differed significantly among the five immune subtypes. Among them, the highest expression of DSG3 was found in PAAD tissues of the C2 subtype, while the lowest expression of DSG3 was found in PAAD tissues of the C3 subtype.
In conclusion, the roles of DSG family members in the immune regulation of the PAAD tumor microenvironment are complex and diverse. Immune escape was associated with high DSG2 expression, which indicates that DSG2 may be a potential target for the treatment of PAAD.

3.7. Enrichment Analysis of the DSG Family Members and 800 Coexpressed Genes

To further explore the functional mechanisms of DSG family members in PAAD development, we performed GO and KEGG enrichment analyses (Supplementary Material S5) of DSG family members and 800 coexpressed genes based on PAAD project data from the LinkedOmics and Metascape databases (Supplementary Material S4). GO analysis indicated that DSG family members and 800 coexpressed genes were mainly enriched in the BP terms "cell division", "cell junction organization" and "cell–cell adhesion" (Fig. 5A), the CC terms "cell–cell junction", "cell-substrate junction" and "actin cytoskeleton" (Supplementary Figure S6A), and the MF terms "cell adhesion molecule binding", "actin binding" and "phospholipid binding" (Supplementary Figure S6B). KEGG analysis indicated that DSG family members and 800 coexpressed genes may play important roles in various pathways related to cancer development and progression, such as "adherens junction", "cell cycle", "pancreatic cancer" and "p53 signaling pathway" (Fig. 5B). Based on this, we further analyzed the mechanism of action of DSG family members in various oncogenic pathways using data from the PAAD project in the GSCA database. As shown in Fig. 5C, the DSG family plays an active role in the EMT signaling pathway, and DSG1/2/3 are important activators of the "PI3K/AKT signaling pathway", the "RTK signaling pathway", the "RAS/MAPK signaling pathway" and the "TSC/mTOR signaling pathway". In addition, the DSG family is involved mainly in "apoptosis" and the "cell cycle". These findings reveal the mechanism by which DSG family members may promote the development of PAAD by activating multiple oncogenic pathways and enhancing the survival and motility of PAAD cells.

3.8. Construction and Analysis of the PPI Network associated with DSG Family Members

To construct and analyze the PPI network of DSG family members in PAAD patients, we used the STRING database to identify the 24 genes that have the strongest PPI with DSG family members (Fig. 5D) (Supplementary Material S6), and then Cytoscape software was used to map the associated PPI network. The larger the circle and the darker the color, the higher the number of PPIs associated with that gene. The results showed that EGFR showed the highest score in the PPI network of DSG family members (Fig. 5E) (Supplementary Material S7), indicating that EGFR plays an important role in the PPI network and is closely related to DSG family members. In addition, an interesting finding was the direct PPI interaction between DSG1/2 and EGFR (Fig. 5D), suggesting that the biological function of the DSG family in PAAD may be related to EGFR.

3.9. The Expression of DSG Family Members Affects the Sensitivity to Treatment with Multiple Drugs
Finally, we analyzed the association between the expression of DSG family members and therapeutic sensitivity to various chemotherapeutic and targeted agents using the GSCALite database. The results showed that the expression of DSG3 was positively correlated with sensitivity to various targeted drugs, including neratinib, fluorouracil and erlotinib (Fig. 5F). In contrast, the expression of DSG2 was negatively correlated with the sensitivity of PAAD patients to various targeted or chemotherapeutic drugs, including gemcitabine, docetaxel and paclitaxel; however, it was positively correlated with the sensitivity to trametinib, erlotinib and gefitinib. We searched the ClinicalTrials.gov database (https://clinicaltrials.gov/) (accessed December 3, 2022) for relevant clinical studies of the three drugs mentioned above and found that clinical trials of trametinib for PAAD are ongoing, while erlotinib has been used in unresectable locally advanced PAAD. As a promising biomarker for predicting the sensitivity of multiple drugs to PAAD, DSG2 expression in particular correlates significantly with the sensitivity of both currently used chemotherapeutic and targeted drugs; therefore, DSG2 warrants further clinical studies and basic research for validation.

4. Discussion

In recent years, there has been increasing interest in the functions of DSG family members in tumors. Relevant studies have found that DSG family members have potential tumor suppressor or promoter roles in various malignancies, such as lung cancer\textsuperscript{[7–8]}, cervical cancer\textsuperscript{[10]}, head and neck squamous carcinoma\textsuperscript{[11]}, and thyroid cancer\textsuperscript{[32]}. However, there is a lack of functional studies on DSG family members in PAAD, and no systematic bioinformatics analysis has been performed. In this study, the first bioinformatics analysis of the functions of DSG family members in PAAD was performed to comprehensively identify the functions and potential mechanisms of DSG family members in PAAD development and progression. This was evaluated in terms of gene expression, gene variants, promoter methylation, immune cell infiltration, gene pathway enrichment, PPI and drug sensitivity.

DSG family members have different roles in different cancer tissues or even in the same cancer tissue. Myklebust\textsuperscript{[6]} showed that high expression of DSG1 was associated with tumor size, lymph node metastasis and poorer prognosis in squamous cell carcinoma of the anal canal. Similarly, another study\textsuperscript{[33]} showed that high DSG3 expression was associated with invasive growth in urothelium carcinoma and colorectal cancers. All of these studies demonstrated the pro-cancer function of DSG3, which is consistent with the findings in this study which showed that DSG3 expression was elevated in PAAD patients and significantly associated with poor patient prognosis. However, for DSG2, previous studies\textsuperscript{[34]} found that inhibition of DSG2 expression promoted the migration and invasion of PAAD cells, whereas in the present study, DSG was found to be highly expressed in PAAD tissues and significantly correlated with clinicopathological characteristics such as race, age, cancer stage, and tumor grade; furthermore, PAAD patients with high DSG expression had a worse prognosis. Similarly, Kamekura\textsuperscript{[35]} found that DSG2 expression was increased in human colon cancer tissue specimens and that DSG2 deletion resulted in reduced proliferation of mouse colonic epithelial cells, and deletion inhibited the growth of transplanted tumors. However, Yang\textsuperscript{[36]} found that DSG2 expression was lower in colon cancer than in paracancerous tissues and that patients with low DSG2 expression had a poorer prognosis. These
studies reflect the complexity and diversity of DSG2 functions and mechanisms of action in cancer tissues, and the reason for this may be related to the fact that DSG2 acts on different downstream signaling pathways in specific cellular environments. This further highlights the need for an in-depth study of the downstream pathways in which DSG2 acts.

In this study, we found that EGFR plays an important role in the PPI network of DSG family members, whose expression products interact with a variety of proteins. Furthermore, we found that there is a direct PPI interaction between DSG1/2 and EGFR, suggesting that the biological function of the DSG family in PAAD may be related to EGFR. It has been reported that in lung cancer,[7] there is a direct interaction between DSG2 and EGFR at the cell membrane, and DSG2 is required for EGFR binding to Src; in addition, DSG2 silencing suppresses the malignant phenotype of tumor cells by inhibiting the EGFR-Src-Rac1-PAK1 signaling pathway. Ungewiịs[37] further used atomic force microscopy in live intestinal cells and in cell-free cell devices to demonstrate that DSG2 and EGFR interact directly through their extracellular compartments and that DSG2 binds to ligands to inhibit EGFR tyrosine kinase activity. This study also reported that EGFR requires DSG2 to localize to the cell boundary when bound to Src. Furthermore, it was found that Src-mediated phosphorylation of the EGFR Y845 site enhanced the anti-apoptotic and pro-proliferative functions of cells, whereas inhibition of DSG2 expression led to a decrease in the phosphorylation level of the EGFR Y845 site, thus diminishing the proliferative capacity of cells[38]. In summary, EGFR may be one of the key signaling molecules mediating DSG family functions, and the DSG family is involved in tumorigenesis and development by regulating the EGFR pathway.

Another finding of interest is that TP53 mutations may affect the level of promoter methylation of most members of the DSG family, and whether this effect can promote PAAD development and progression deserves further study. In addition, CpG methylation in the promoter region is traditionally thought to be associated with inactivation of gene expression, so it is generally considered to be a repressive marker; however, recent studies have found that DNA methylation has diverse functions in different tissues and different gene regions[39–41]. Our study found that the level of promoter methylation and expression of DSG2 were significantly higher in TP53 mutant PAAD tissues than in TP53 wild-type PAAD tissues, indicating that DSG2 may be a potential downstream target of mutant TP53. By increasing DSG2 expression in PAAD, it regulates the methylation level of its promoter, which in turn promotes the development and progression of PAAD. In-depth study of this potential mechanism will identify new targets to prevent the development and progression of PAAD driven by TP53 mutations.

Finally, we analyzed the role of the DSG family in PAAD immunity and explored the relationship between the expression of DSG family members and the sensitivity to targeted or chemotherapeutic agents in PAAD patients. The final analysis showed that the DSG family is closely associated with the clinical outcomes of PAAD. First, the immune system is complex. In addition to serving as the first line of defense against various pathogens, immune cells can provide surveillance functions by identifying and destroying latent cancer cells. Immunocompetent tumor-infiltrating cells are unique immune cells that infiltrate the tumor microenvironment by detecting tumor antigens and releasing proinflammatory and
immune molecules that regulate immune function\textsuperscript{[42]}. Furthermore, although cancer immunotherapy has been shown to improve survival in various cancers, remission rates in PAAD patients remain low\textsuperscript{[43]}. Therefore, a comprehensive study of the interaction between tumor and immune cells would help elucidate the pathogenesis of cancer and develop new immunotherapeutic strategies. We investigated the relationship between the level of immune cell infiltration and the expression of DSG family members in PAAD tissues. The results showed that DSG2 and DSG3 have completely different roles in PAAD immune regulation, which highlights the diversity and complexity of the roles of DSG family members in PAAD tumor immune regulation. Similarly, another study\textsuperscript{[44]} found that B7 family members may play different roles in tumor immune regulation. This finding indicates members of the same family may have different roles in tumor immune regulation, and this may be important for maintaining the balance between immune effectiveness and autoimmune suppression. However, regardless of their consistent regulatory functions, DSG2/3 is functionally active in tumor immune regulation of PAAD. For example, DSG2 was negatively correlated with the infiltration level of several killer immune cells, including Act CD8, Tfh cells and eosinophils, and significantly negatively correlated with 16 immune-promoting factors, including ADORA2A, CD160 and IL10. Therefore, high DSG2 expression may suppress the body's immune response to PAAD, which in turn promotes PAAD immune escape. In contrast, DSG3 was positively correlated with the infiltration level of multiple killer immune cells (including Act CD4, Tcm CD4 and CD56dim) and most immunomodulators (immune promoters, MHC molecules, chemokines and chemokine receptors) in PAAD, suggesting that DSG3 may be an immune activating molecule in PAAD. These studies suggest that the DSG family is a potential target for immunotherapy in PAAD and a molecular indicator that can be used to predict the efficacy of immunotherapy. In addition, our study found that the expression of DSG2 was significantly correlated with the therapeutic resistance of PAAD to a variety of targeted or chemotherapeutic agents, including gemcitabine, docetaxel and paclitaxel; however, it was positively correlated with the sensitivity of trametinib, erlotinib and gefitinib. Clinical trials of trametinib for PAAD are currently ongoing, but there are also clinical trials confirming that trametinib suppresses PAAD tumors\textsuperscript{[45]}. In addition, erlotinib has been used in unresectable locally advanced pancreatic cancers. Therefore, research and development of PAAD-targeted drugs that target DSG family members will have good prospects for application.

This study is the first bioinformatic analysis of the function of DSG family members in PAAD. We found significant differences in the expression of DSG family members in different cancers, and these proteins may play different roles in different cancers. The expression level and variants of DSG family members were significantly correlated with poorer clinicopathological features and poorer prognosis in PAAD patients. This finding may be related to the involvement of DSG family members in the activation of multiple oncogenic pathways and regulation of the immune response in PAAD patients. Another finding was that TP53 mutations have a significant effect on the level of promoter methylation of most DSG family members, and DSG2/3 may be a potential downstream target driving the onset and development of PAAD with TP53 mutations. In addition, we found that DSG family members were associated with sensitivity to treatment with multiple PAAD-targeting or chemotherapeutic agents. Therefore, DSG family members may be candidate targets for PAAD-targeted therapy or molecular markers for predicting drug
sensitivity; however, the present study has some limitations. For example, the number of databases included in this study may be insufficient due to the limited availability of data. In addition, this study provided only a bioinformatic analysis of the functions and mechanisms of DSG family involvement in the development and progression of PAAD, and more experimental data are still needed to further confirm the tumor-promoting role of DSG family members in PAAD.

5. Conclusions

In summary, this study provides a comprehensive investigation of the functions of DSG family members, and the findings indicate that they may play an oncogenic role in the development and progression of PAAD. The DSG family members could be novel molecular targets for PAAD therapy, although further functional validation and mechanistic studies should be carried out in a timely manner.

Abbreviations

PAAD
pancreatic adenocarcinoma
DSG
desmogleins
EGFR
epidermal growth factor receptor
EMT
epithelial-mesenchymal transition
PPI
protein–protein interaction
TCGA
The Cancer Genome Atlas
GTEx
Genotype-Tissue Expression
OS
overall survival
RFS
recurrence-free survival
DFS
disease-free survival
BP
biological process
CC
cellular components
MF
molecular function
KEGG
Kyoto Encyclopedia of Genes and Genomes.

Declarations

Author Contributions: Z.L. (Zhenyu Lin), Y.D. (Yongxing Du) and Y.D. (YunJie Duan) conceived and designed the study. Z.L. (Zhenyu Lin), X.Y.(Xin Yin), T.M.(Teng Ma), Y.X.(Yunliang Xie) collected the data. Z.L. (Zhenyu Lin), W.Z. (Wei Zhang) and Z.H.(Zhangkan Huang) performed the analyses. Z.L. (Zhenyu Lin) and Y.D. (Yongxing Du) wrote the manuscript. X.C.(Xu Che) supervised the study. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

References


Tables
Table 1
Relationship between DSG family expression and the abundance of tumor-infiltrating immune cells in PAAD.

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Notes: * p < 0.05; ** p < 0.01; *** p < 0.001.
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Notes: * p < 0.05; ** p < 0.01; *** p < 0.001.
Figure 1

Expression analysis of the DSG family in pan-cancer and PAAD, and HPA analysis of DSG2 in PAAD. (A) The expression level of DSG1; (B) the expression level of DSG2; (C) the expression level of DSG3; (D) the expression level of DSG4. ns, \( p \geq 0.05 \); * \( p < 0.05 \); ** \( p < 0.01 \); *** \( p < 0.001 \). (E) The protein level of DSG2 (40×).
The relationship between the expression levels of DSG family members and clinicopathologic features, TP53 mutation status, and prognosis of PAAD patients. (A) The relationship between expression levels of DSG1 and race; (B) the relationship between expression levels of DSG4 and race; (C) the expression levels of DSG2 in the TP53 mutant PAAD tissues were significantly higher than those in the TP53 wild-type PAAD tissues; (D, F) high expression of DSG2/3 was significantly correlated with a worse initial treatment.
effect; (E) the relationship between expression levels of DSG3 and patient’s age, cancer stages and tumor grade; (G, H) the increased expression levels of DSG2, DSG3 and DSG4 were significantly correlated with shorter OS and RFS. * p < 0.05; ** p < 0.01; *** p < 0.001.

**Figure 3**
Genetic variation analysis and promoter methylation analysis of the DSG family members in patients with PAAD. (A) Gene variation characteristics of the DSG family in PAAD patients; (B, C, D, E) the variation in the DSG family was significantly correlated with race, tumor location, tumor focality and TMB of PAAD patients; (E, F) PAAD patients with the genetic variation in the DSG family genes had shorter OS; (H) the promoter methylation levels of DSG1/2 in the tumor tissues of patients were significantly higher than those in the paracancerous tissues, but the levels of DSG3/4 were the opposite; (I) the promoter methylation level of DSG2 was significantly higher in TP53 mutant PAAD tissues than in TP53 wild-type PAAD tissues, but the level of DSG3/4 was the opposite.
Figure 4

Analysis of the immune infiltration of the DSG family in PAAD patients. (A–E) DSG2 was negatively correlated with various types of immune promoters, including IL10, CD160, and ADORA2A, while DSG3 was positively correlated with most immunomodulators (immune promoters, molecules, chemokines, and chemokine receptors). (F–I) The copy number alteration of DSG may affect the infiltration levels of six types of dominant immune cells. ns, p \geq 0.05; * p < 0.05; ** p < 0.01; *** p < 0.001.
Gene enrichment analysis, PPI network construction, and drug sensitivity analysis of the DSG family in PAAD patients. (A) The GO enrichment of the BP terms of the DSG family and 800 coexpressed genes; (B) the KEGG enrichment of the DSG family and 800 coexpressed genes; (C) the DSG family played an activating role in a variety of oncogenic pathways; (D, E) EGFR played an important role in the PPI network, and it was closely related to the DSG family; (F) the expression of the DSG family was negatively...
correlated with sensitivity to various PAAD-targeting or chemotherapeutic drugs, including gemcitabine, docetaxel, and paclitaxel.

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