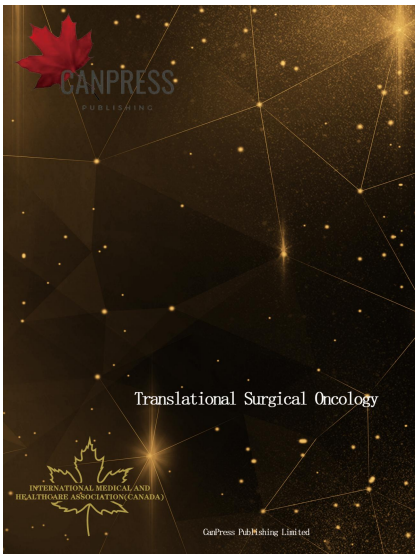
Update on transcription phenotypes in pancreatic cancer



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Abstract

Compelling evidence demonstrates that pancreatic cancer (PC) was characterized by inter-patient and intra-tumor heterogeneity, which prompted us to establish a clinically applicable molecular taxonomy. The established tumor subtypes based on transcription profiles in PC have the potential to indicate the prognosis of patients and new therapeutic vulnerability. However, the current tumor phenotyping remains nascent and does not inform clinical management. This review summarizes the current tumor subtypes of PC, their potential clinical relevance, and underlying driving force. We also clarify the relationship between tumor subtypes defined by different studies.

Keywords: Pancreatic cancer; Tumor phenotyping; Tumor subtypes; Molecular taxonomy

Introduction

Currently, there are many large-scale integrated bulk transcriptome and genome sequencing datasets, including the International Cancer Genome Consortium[1](ICGC) and The Cancer Genome Atlas[2](TCGA), which enabled the analysis of the molecular characteristics of cancer. Accumulating evidence[3,4] demonstrates that molecular differences could be identified in histopathologically indistinguishable tumors. Pancreatic cancer (PC) is a highly malignant and lethal tumor[5]. Although considered to the uniformly aggressive, there are dramatically different clinical outcomes and therapeutic responses to radiochemotherapy and immunotherapy among patients, highlighting the heterogeneity of PC[6]. Several tumor subtypes of PC have been identified using large-scale transcriptome sequencing. However, except clinical staging system, no established molecular classifiers can inform clinical management decisions for PC at this time. Compared with breast cancer[7], tumor phenotyping of PC is still in its infancy. Advances in single-cell and spatial sequencing technology permit capturing more comprehensive and precise molecular characteristics than previous bulk sequencing, which can identify the transcription profiles of tumor cells, stromal cells, and immune cells, respectively. Molecular taxonomy established by single-cell transcriptome sequencing that is based on the whole community of tumors, instead of tumor cells alone, might be the future exploration direction. In this review, we

will summarize the current status of tumor phenotyping of PC and the clinical relevance of these tumor subtypes. We attempt to clarify the relationship between tumor subtypes defined by different studies and the driving force behind them.

Identification of tumor subtypes based on transcriptome profiling

Accumulating single-cell RNA-seq studies[8-11] have found a remarkable heterogeneity of tumor cells in PC that tumor cells clustered by individual patients, while other cell types, including cancer-associated fibroblast (CAFs) and immune cells from all patients clustered together. The patients' responses to treatments are heterogeneous. This heterogeneity prompted us to define a molecular taxonomy to distinguish patients even if they have the same clinicopathological features. Molecular subtypes defined in lymphoma[12] and breast cancer7 have been successfully applied to guide treatment. Unfortunately, pancreatic cancer has been lagging behind. No robust molecular subtype in PC can guide preclinical therapeutic development, let alone clinical treatment. First, in 2011, Collisson et al.[13] generated gene expression microarray datasets from untreated resected primary PC samples and human and mouse PC cell lines. To overcome the difficulty of a paucity of tumor cells available in PC samples, the epithelium was microdissected away from the stroma for transcriptional profiling. Three molecular subtypes, including Classical, Quasi-mesenchymal (QM-PDA), and Exocrine-like, were identified using the non-negative matrix factorization (NMF) analysis. Each subtype has its specific gene expression. The Classical subtype highly expressed adhesion-associated and epithelial genes. The QM-PDA subtype was characterized by high expression of mesenchyme-associated genes. GATA6, a GATA-family transcription factor, was highly enriched in Classical subtype tumors and cell lines, while QM-PDA subtype tumors and cell lines had comparatively low expression of it. The Exocrine-like subtype had high expression of tumor cell-derived digestive enzyme genes. Classical and QM-PDA subtypes, but not Exocrine-like subtype, were identified in PC cell lines. However, the resected PC samples were microdissected to avoid the contamination of the normal pancreas adjacent to tumor. Therefore, the defined Exocrine-like subtype should be a bona fide subtype.

In 2015, Moffitt et al.[14] analyzed gene expression of microarray data from primary and metastatic PC tumors, PC cell lines, normal pancreas, and adjacent normal pancreas. To eliminate the interference of abundant stroma cells and intermixed normal endocrine and exocrine cells to primary tumors and the interference of cell types from host organ to metastatic tumors, virtual microdissection (please refer to their article for more details) of primary and metastatic PC samples was used. They identified two tumor-specific molecular subtypes, Classical and Basal-like, using NMF analysis. Interestingly, the Basal-like subtype identified in PC was molecularly similar to basal tumors in bladder[15,16] and breast[17] cancers. The gene enriched in the Classical subtype in this study overlapped with the Classical subtype in Collisson's study, so they retained the naming convention Classical. Moreover, GATA6 was also highly expressed in the Classical subtype, consistent with Collisson's study.

In 2016, Bailey et al.[18] performed a bulk RNA-seq from PC tumors with high epithelial content and defined four tumor-specific subtypes, Squamous, Pancreatic progenitor, Immunogenic, and aberrantly differentiated endocrine exocrine (ADEX), using an unsupervised analysis. An extended array-based mRNA expression dataset from PC tumors with the full range of tumor cellularity verified these four subtypes. Additionally, a comprehensively integrated genomic analysis was performed to determine the candidate genomic events important in each subtype. There was a correlation between these 4 tumor subtypes and specific histological characteristics: 1) Squamous subtype with adenosquamous carcinomas; 2) Pancreatic progenitor subtype and Immunogenic subtype with mucinous non-cystic (colloid) adenocarcinomas and carcinomas arising from IPMN, which are mucinous; 3) ADEX subtype with rare acinar cell carcinomas. Transcriptional network analysis showed that four core gene programs characterized the Squamous subtype, involving in inflammation, hypoxia response, metabolic reprogramming, TGF-β signaling, MYC pathway activation, and autophagy. Many of these genes were enriched in the C2-squamous-like class of tumors defined in the TCGA pan-cancer studies[19]. The Pancreatic progenitor was characterized by many pancreatic endoderm cell-fate determination-related transcription factors, such as PDX1, MNX1, HNF4G, HNF4A, HNF1B, HNF1A, FOXA2, FOXA3, and HES1, and gene programs regulating fatty acid oxidation, steroid hormone biosynthesis, drug metabolism, and O-linked glycosylation of mucins. ADEX subtype was defined by the transcriptional network involved in exocrine (NR5A2 and RBPJL) and endocrine differentiation (NEUROD1, NKX2-2, and MAFA). However, it remains controversial regarding whether the ADEX subtype exists oris the result of normal tissue contamination. Notably, some patient-derived PC cell lines (pure tumor cells) were enriched with gene programs associated with the ADEX subtype, supporting the former. Moreover, it is almost impossible to eliminate all normal and normal-like cells, especially those scattered cells in PC tissues using histopathological methods. The role of these cells in PC is still not fully understood as well. The Immunogenic subtype was associated with a significant immune infiltrate, whose gene programs included B cell signaling pathways, antigen-presenting, CD4/8+ T cell, and Toll-like receptor signaling pathways.

In 2020, Chan-Seng-Yue et al[20]. performed RNA-seq of laser capture microdissection (LCM) purified tumors from 248 patients with PC, including many stage IV patients. Thus, it can capture a wider spectrum of disease. The NMF was applied to extract tumor-specific expression signatures. The

cohort was divided into five tumor-specific subtypes according to the gene expression continuum of NMF signatures, which they termed Basal-like-A, Basal-like-B, Hybrid, Classical-A, and Classical-B. The Hybrid subtype presented multiple expression signatures. Single-cell RNA-seq was performed for 13 resectable and 2 metastatic PC tumors to further explore intra-tumor subtypes. Normal human pancreas-specific signatures from previous single-cell RNA-seq study[21] were used to discriminate normal from malignant epithelial cells. Seven tumor clusters were identified and scored for Basal-like/Classical signatures. Interestingly, Classical and Basal-like clusters coexisted in the same tumors (13/15 PC patients). Both Bulk-seq and single-cell RNA-seq suggested that there was a positive correlation between Basal-like signatures and epithelial-mesenchymal transition (EMT) program. Juiz et al.[22] performed single-cell RNA-seq for biopsy-derived PC organoids and found that Classical and Basal-like clusters coexisted in the organoids from the same patients. Recently, Williams et al.[23] also demonstrated tumor cells co-expressing Basal-like and Classical signatures were present in most patients (> 90%). There existed a Classical-Basal-like polarization continuum and co-expressor tumor cells may represent an intermediate state between subtype poles. These results indicated that a greater complexity than expected regarding tumor subtypes, intra-tumor heterogeneity, and the plasticity of tumor subtypes need to be further studied. Classical-A/B subtypes highly expressed a set of transcription factors related to pancreatic lineage differentiation, including HNF1A, HNF4G, GATA6, GATA4, ONECUT2, and NKX2-2. The copy number gains in GATA6 were more frequent in Classical-A/B subtypes than in Basal-like-A/B subtypes.

In 2022, Hwang et al.[24] optimized single-nucleus RNA-seq for frozen PC specimens, which included 18 treatment-naïve and 25 chemotherapy treated tumors. Unsupervised clustering was used and 14 malignant cell programs were identified that reflected either subtype, including Classical, Squamoid, Basaloid, Mesenchymal, Acinar-like, Neuroendocrine-like, and neural-like progenitor (NRP), or cell state. The Classical subtype strongly overlapped with the previous defined Classical subtype[13,18]. Squamoid subtype highly expressed genes related to epidermis development/proliferation, keratinocyte differentiation, and cornification. Basaloid subtype was enriched for pathways involved in stemness, ribosomal proteins, ribosomal RNA processing, cell migration/invasion, cell-cell and cell-extracellular matrix junctions, EMT, and metallothioneins-related genes. The Mesenchymal subtype was characterized by EMT, matrisome, extracellular matrix production, and stemness. Acinar-like subtype had high copy number alterations and was considered as an aberrantly differentiated exocrine subtype. Neuroendocrine-like subtype was termed because of the gene expression of CNTN4, CTNND2, NRXN3, RELN, SEMA5A, NRCAM, and AUTS2, which involved in neuronal development/migration/adhesion. NRP subtype was significantly enriched in ‘brain tissue enhanced’ genes (e.g., NRXN3). To decipher how these tumor subtypes are spatially organized in the tumor microenvironment (TME), the NanoString GeoMx human whole transcriptome atlas was performed. Three multicellular communities were identified using unsupervised clustering. Different tumor subtypes preferred coexisting with specific cancer-associated fibroblasts (CAFs) and immune cell types. For example, in community 1, NRP and Neuroendocrine-like subtypes matched with neurotropic CAFs and CD8+ T cells, which were all enriched in TME of patients after treatment. This study reminds us that besides the transcription feature of tumor cells, the transcription feature of other cell types (e.g. CAFs and immune cells) that play a vital role in TME, should also be considered to predict patients’ prognosis and conduct patient selection for specific therapy.

An integrative analysis of spatial and single-cell transcriptomic data was utilized to examine intra- and inter-tumor heterogeneity. Kim et al.[25] delineated an integrative map of tumor subtypes and cancer-associated fibroblasts in PC. They identified a novel tumor subtype, Ep_VGLL, which exhibited intermediate characteristics between the Classical and Basal-like subtypes and was associated with a worse prognosis. Ep_VGLL1 was spatially correlated with two major populations representing the Classical and Basal-like subtypes in PC, indicating that Ep_VGLL1 might serve as a bridging population between these two subtypes.

Clinical relevance of tumor subtypes

Tumor phenotyping based on transcriptional similarities and differences has the potential to optimize current imaging and pathological classification. Patients with the same clinical stages and tumor grades could be subdivided into different subtypes. Multiple studies have shown that tumor subtypes were related to patients’ prognosis[26]. Those patients with presumed poor prognosis might benefit from neoadjuvant therapy and clinical trial recruitment, instead of standard of care. Tumor phenotyping can also help guide therapeutic development. The response of patients with PC to adjuvant chemotherapy (e.g. Gemcitabine and FOLFIRINOX) and immunotherapy (e.g. anti-PD-1 antibody) is heterogeneous[27]. The current challenge is to screen patients who are sensitive to these treatment regimens. Porter et al.[28] found that FOLFIRINOX combination chemotherapy induced a shift of both Classical and QM-PDA toward a more QM-PDA state in PC tumors samples and cell lines. The plasticity of Classical/QM-PDA states in PC influenced response to therapy. This study highlights the relationship between tumor subtypes and therapeutic vulnerabilities and resistances. However, it still needs to be further investigated.

In Collisson’s study[13], the stratification by transcription phenotypes provides a useful prognostication in PC patients. Specifically, the Classical subtype was associated with longer overall survival (OS), whereas the QM-PDA subtype was

related to high tumor grade and shorter OS. Multivariate Cox regression analysis indicated that transcription phenotype in this study was an independent predictor of OS of PC (P = 0.024). Moreover, QM-PDA subtype cell lines were more sensitive to gemcitabine than the classical subtype, whereas erlotinib was more effective in Classical subtype cell lines, suggesting there is a subtype-specific treatment response.

In Moffitt's study[14], there was a significant association between tumor-specific subtypes and the prognosis of PC patients. Patients identified as the Classical subtype had a longer median survival time (19 v.s. 11 months) and higher 1-year survival rate (70% v.s. 40%) compared with those identified as the Basal-like subtype. Multivariate Cox regression analysis showed that tumor-specific subtype classification was independently associated with survival (P = 0.003). In addition, patients with Basal-like tumors showed a strong trend toward better response to adjuvant therapy (hazard ratio (HR): 0.38; 95% confidence interval (CI): 0.14–1.09), but patients with Classical tumors didn't. Thus, patients with Basal-like tumors appeared to benefit from adjuvant therapy, whereas patients with Classical tumors might more benefit from other therapies although they had a better prognosis. However, it needs to be studied further in the future.

In Bailey's study[18], a total of 93 PC patients were divided into 4 molecular subtypes based on their transcriptional profiles. The Kaplan–Meier (KM) analysis suggested that patients with the Squamous subtype had a significantly worse prognosis in comparison to other subtypes (P = 0.0302). Multivariate analysis indicated that the Squamous subtype was an independent prognostic factor (Squamous v.s. Others; P = 0.0086). In this study, a new Immunogenic subtype of PC was identified, which may provide decision support in the patient selection for drugs and targeted therapies.

In Chan-Seng-Yue's study[20], the correlation analysis between tumor subtypes and clinicopathological features was conducted. The results indicated that patients with early stages (Stage I/II) had a higher proportion of Classical-A/B subtypes compared to those with Stage IV (P = 0.03). Basal-like-A subtype was rare in resectable tumors, absent in locally advanced tumors, and more frequently in Stage IV (P = 0.00003). Basal-like-A, instead of Basal-like-B, was related to chemoresistance and showed a trend towards a worse prognosis. Taken together, different clinical stages had different tumor-specific subtypes. Tumor-specific subtypes might change along with the tumor progress. Basal-like-A/B was related to the advanced stages and worse survival, which was consistent with previous understanding.

In Hwang' study[24], they investigated the prognostic relevance of tumor subtypes by scoring them in TCGA and ICGC datasets. Multivariable Cox regression analysis showed NRP (P = 0.02) and Squamoid (P = 0.03) subtypes were associated with shorter time to progression (TTP). The Classical subtype was associated with longer TTP (P < 0.001) and OS (P = 0.02), indicating that the Classical subtype was beneficial to prognosis as in previous studies[13,14,20].

Correlation between tumor subtypes defined in different studies

Tumor transcription phenotyping is influenced by multiple variables, such as tumor purity, clustering method, sequencing platform, and sample size. Each study has established a molecular taxonomy according to its own understanding of transcription profiles of tumor subtypes. There is an overlapping tumor subtype relationship between different studies[13,14,18,20,24]. Nevertheless, the same patient could be defined as different tumor subtypes in different studies. These inconsistent molecular taxonomies are confusing. Here, we will clarify the relationship among these tumor subtypes (Table 1). Moffitt et al.[14] compared the defined tumor subtype with the counterpart of Collisson et al.[13] Their finding supported that the previous Classical subtype is a bona fide group, and the Classical_Moffitt subtype strongly overlapped with the Classical_Collisson subtype. QM-PDA_ Collisson subtype is partially driven by stromal composition and overlapped with Basal-like subtype in Moffitt classification. Exocrine-like subtype (might correspond to normal pancreas) from Collisson et al was not found by Moffitt et al. Bailey et al.[18] also compared their transcription classification with previous tumor subtypes defined in Collisson et al.[13] and Moffitt et al.[14] Three of the tumor subtypes in this study directly overlapped with Collisson classification. QM-PDA_Collisson subtype, Classical_Collisson/Moffitt subtype, and Exocrine-like_Collisson subtype were renamed Squamous_Bailey subtype, Pancreatic progenitor_Bailey subtype, and ADEX_Bailey subtype, respectively. Around half of the Squamous_Bailey subtype fell within the Basal-like_Moffitt subtype. In addition to previously defined tumor subtypes, a new subtype, termed Immunogenic, was identified in Bailey et al. Chan-Seng-Yue et al.[20] divided previously defined Classical and Basal-like subtypes into two subtypes, Classical A/B and Basal-like A/B, respectively. A Hybrid subtype was termed due to multiple expression signatures and could not correspond to any previous classification. Hwang et al.[24] refined malignant classification using the scRNA-seq method and found that the Classical_Hwang subtype strongly overlapped with the previously defined Classical subtype. Both the Basaloid and Squamoid_Hwang subtypes overlapped significantly with the Basal-like_Moffitt subtype. However, Squamoid and Mesenchymal_Hwang subtypes did not exhibit significant overlap with bulk RNA-seq defined Squamous_Bailey subtype and QM-PDA_Collisson subtype.

The potential driving force behind tumor subtypes

Although several molecular subtypes of PC based on transcription characteristics have been established, little is known about the driving force underlying these tumor-specific

subtypes. The current challenge is to find the factors that determine and maintain tumor subtypes. Chan-Seng-Yue et al.[20] collected biopsies from six PC patients at different time points and found that there was no change in tumor subtypes with tumor progression in four patients (4/6). Their genomes were nearly identical with tumor progression. However, a pronounced change in tumor phenotype was observed in two patients (2/6) after surgery and therapy. Genomic events were observed in these two patients as well. This data supported the premise that the plasticity of tumor subtype might be driven by genomic instability. Chan-Seng-Yue et al.[20] found that TP53 mutations, complete loss of CDKN2A, and TGF-β signaling were enriched in Basal-like-A/B subtypes, and an intact allele of SMAD4, a key gene in TGF-β signaling, was observed in most of patients defined as Basal-like-A subtype. In contrast, SMAD4 mutations were enriched in Classical-A subtype. However, no single genomic event could determine tumor subtypes. It is possible that the constellation of genomic aberrations in the tumor gives rise to tumor subtypes which could change due to ongoing random genomic aberrations during progression or after chemotherapy. The intra-tumor heterogeneity might be explained by random genomic instability. Moffitt et al.[14] correlated tumor subtypes with gene mutation and found that KRAS mutation (p.Gly12Asp) was significantly enriched in the Basal-like subtype, while KRAS mutation (p.Gly12Val) was only found in the Classical subtype. Furthermore, the Basal-like subtype had a lower SMAD4 expression compared to the Classical subtype, supporting SMAD4 loss confers a more aggressive phenotype. Similarly, the single genomic event could not explain the RNA-defined tumor subtypes of all patients. Bailey et al.[18] found that the Squamous subtype (termed as Basal-like in other studies) was associated with mutations in TP53 and KDM6A and upregulated TP63 expression. A genetically engineered mouse model (KrasG12D/+; Trp53fl/+; TAp63fl/fl KPC mice) was used to investigate the functional consequence of this genomic event in defining the Squamous subtype. The data showed that mice with the mutation had more aggressive disease with increased metastatic potential than TP53-null mice. RNA-seq data revealed that mutant TP53 regulated the expression of Squamous subtype-associated genes. Here, three studies[14,18,20] supported that the Basal-like/Squamous subtype was associated with TP53 mutation.

Given that TFs play a crucial role in determining cell fate, many studies have focused on how TFs contribute to tumor subtype formation[29]. Collisson et al.[13] revealed that GATA6 expression was enriched in the Classical subtype. Furthermore, RNAi knockdown of GATA6 impaired tumor cell growth in Classical subtype PC cell lines, but not in QM-PDA subtype cell lines, supporting the subtype-specific role of GATA6 in the Classical subtype. Moffitt et al.14 also found that the Classical subtype was enriched for genes related to GATA6 overexpression. Bailey et al.[18] found Squamous/Basal-like subtype was associated with hypermethylation and concordant downregulation of genes involved in pancreatic endodermal cell-fate determination (e.g., GATA6), therefore leading to a loss of endodermal identity. Pancreatic progenitor/Classical subtype was characterized by TFs network containing PDX1, MNX1, HNF4G, and HNF1A/B. Moreover, Chan-Seng-Yue et al.[20] identified the upregulated TFs in the Classical-A/B subtype, which in particular included GATA6. GATA6 expression was subsequently used to distinguish Classical and Basal-like subtypes in PC[30,31]. Taken together, GATA6 might be an important driving factor in the Pancreatic progenitor/Classical subtype. However, our understanding of key TFs in other tumor subtypes is still largely limited.

Conclusion

Significant progress has been made in tumor phenotyping for pancreatic cancer. In summary, two reproducible tumor subtypes in PC can be identified in different cohorts: 1) Classical_Moffitt subtype/Classical_Collisson subtype/Pancreatic progenitor_Bailey subtype/Classical A/B_Chan-Seng-Yue subtype/Classical_Hwang subtype; 2) QM-PDA_Collisson subtype/Basal-like_Moffitt subtype/Squamous_Bailey subtype/Basal-like A/B_Chan-Seng-Yue subtype/Basaloid_Hwang subtype. The first subtype is a pancreatic lineage precursor and enriched for GATA6 expression. The second subtype, a worse phenotype, exhibits loss of endodermal identity and is associated with a worse prognosis and TP53 mutation. However, any kind of tumor classification could not currently inform clinical management as in other cancers. Clinically applicable molecular taxonomy for PC remains to be explored. Importantly, advances in single-cell and spatial technologies will help further investigate the intra-tumor subtype heterogeneity and plasticity of tumor subtypes after radiochemotherapy and immunotherapy. Refined tumor phenotyping will eventually improve the prognosis of patients with PC in the future.

Conflict of Interest Statement

The authors declare no competing interests.

Authors' contributions

Conceptualization, KC, RZ, PFW; Literature Search, KC, RZ, PFW; Writing – original draft, KC, RZ; Writing – review & editing, XDT, YMY, and JH; Supervision, JH and YMY. All authors read and approved the final version of the manuscript.

Table 1. Tumor subtypes of pancreatic cancer

Study	Year	Specimens	Sequencing platform and methodology	Tumor purity	Subtypes	Correlation	Potential driving force	Clinical relevance
Collisson et al.	2011 (first study)	Untreated resected primary PC tumors and human and mouse PC cell lines	Microarray; NMF	Microdissected; High	1) Classical; 2) Quasi-mesenchymal (QM-PDA); 3) Exocrine-like	/	GATA6 and KRAS addiction in Classical subtype	Longer OS for Classical subtype; High tumor grade and shorter OS for QM-PDA subtype; More sensitive to Gemcitabine and erlotinib in QM-PDA subtype and Classical subtype respectively;
Moffitt et al.	2015	Primary and metastatic PC tumors, PC cell lines, normal pancreas, and adjacent normal pancreas	Microarray; NMF	Virtual microdissection; Median	1) Classical; 2) Basal-like	Classical_Moffitt subtype overlapped with Classical_Collisson subtype; Basal-like_Moffitt subtype partially overlapped with QM-PDA_Collisson subtype	KRAS mutation (p.Gly12Asp) in Basal-like subtype; KRAS mutation (p.Gly12Val) in Classical subtype	Longer median survival time and higher 1-year survival rate in Classical subtype; Better response to adjuvant therapy in

								Basal-like subtype
Bailey et al.	2016	Untreated primary PC tumors	Bulk RNA-seq and Microarray; NMF	Bulk RNA-seq (> 40%); Microarray (full range of tumor cellularity)	1) Squamous; 2) Pancreatic progenitor; 3) Immunogenic; 4) Aberrantly differentiated endocrine exocrine (ADEX)	QM-PDA_Collisson subtype was renamed Squamous_Bailey subtype; Classical_Collisson/Moffitt subtype was termed Pancreatic progenitor_Bailey subtype; Exocrine-like_Collisson subtype was renamed ADEX_Bailey subtype	TP53 and KDM6A mutations and upregulated TP63 expression in Squamous subtype; Hypermethylation and concordant downregulation of GATA6 in Squamous subtype; Upregulation of PDX1, MNX1, HNF4G, HNF1A/B in Pancreatic progenitor	Worse prognosis in Squamous subtype
Chan-Seng-Yue et al.	2020	PC tumors containing Stage IV patients	Bulk RNA-seq; NMF	LCM; High	1) Basal-like-A; 2) Basal-like-B; 3) Hybrid; 4) Classical-A; 5) Classical-B	Previous Classical subtype was separate into Classical A and B_Chan-Seng-Yue subtype; Previous Basal-like subtype was separate into	TP53 mutations, complete loss of CDKN2A, and TGF-β signaling in Basal-like-A/B subtypes;	Higher proportion of Classical-A/B subtypes in early stages (Stage I/II);

						Basal-like A and B_Chan-Seng-Yue subtype	SMAD4 mutations in Classical-A subtype	More frequently in Stage IV for Basal-like-A subtype; Chemoresistance and worse prognosis in Basal-like-A subtype
Hwang et al.	2022	Treatment-naïve and treated PC tumors	SnRNA-seq; unsupervised clustering-consensus NMF	Epithelial compartment was separated using cell markers and CAN	1) Classical; 2) Squamoid; 3) Basaloid; 4) Mesenchymal; 5) Acinar-like; 6) Neuroendocrine-like; 7) neural-like progenitor (NRP)	Classical_Hwang subtype overlapped with previous defined Classical subtype; Basaloid, Squamoid_Hwang subtype overlapped with Basal-like_Moffitt subtype	NRXN3 in NRP subtype; GATA6 in Classical subtype	Shorter TTP in NRP and Squamoid subtypes; Longer TTP and OS in Classical

NMF: non-negative matrix factorization; LCM: laser capture microdissection; snRNA-seq: single-nucleus RNA sequencing; CNA: copy-number alterations; TTP: time to progression; OS: overall survival

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