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REVIEW

Different shades of pancreatic ductal adenocarcinoma, different paths towards precision therapeutic applications

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Background: Different histological and molecular subtypes of pancreatic ductal adenocarcinoma (PDAC), with different molecular composition and survival statistics, have recently been recognised.

Materials and methods: This review describes the currently available studies regarding molecular and histological subtypes in PDAC. Studies from major cohorts such as International Cancer Genome Consortium as well as smaller cohorts are reviewed. We discuss where the described subtypes overlap, where the discrepancies are and which paths forward could be taken regarding diagnosis, ontogeny and therapy.

Results: Four molecular subtypes with strong overlap among the different studies can be found, next to a list of mixed findings. Two of the four subtypes (epithelial classical and mesenchymal basal-like) were represented in every study and were often discriminated in other solid tumours as well. These two subtypes differ substantially in prognosis. One biomarker has been discovered, only discriminating these two subtypes, and insights into subtype-specific therapeutic vulnerabilities are scarce.

Conclusion: Subtypes can be reproducibly detected in cohorts of PDAC patients and two of them directly relate with prognosis. A consensus on the subtypes is warranted. Further discovery and validation studies are needed to identify strong biomarkers, to comprehend subtype ontogeny and to define strategies for precision medicine.

Key words: pancreatic ductal adenocarcinoma, subtyping, transcriptomics, cancer, histopathology

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent pancreatic carcinoma, with an overall 5-year survival (OS) of 8% [1–3]. It is the fourth leading cause of cancer death, and is expected to become the second leading cause of cancer death by 2030 [4–6]. Most PDACs are sporadic cancers, with no more than 10% accounted by familial/hereditary cancer [7]. In patients with a non-hereditary cancer, genomic analysis uncovered a prevalence of over 90% activating mutations in the *KRAS* gene, and common genetic alterations of *TP53*, *CDKN2A* and *SMAD4* [8–10]. No other mutations occur at a high frequency. Besides extensive heterogeneity in the mutational landscape among patients, there is also heterogeneity within a single patient [10–12].

Investigators have started deciphering molecular and histological subtypes in PDAC, which can lead to a more specific diagnosis and prognosis and opens perspectives for subtype-stratified treatment. This review documents the current knowledge on histological and transcriptomic subtypes in PDAC and suggests

ways forward to broaden our understanding and use of tumour subtyping for clinical perspectives.

Technology-driven subtyping in PDAC

Histological subtypes

At the histological level, PDAC is recognised as a heterogeneous disease consisting of different variants (e.g. medullary carcinoma, adenosquamous carcinoma [13–15]), which are described in the current WHO (World Health Organization) classification [16]. Schlitter et al. created a more comprehensive classification [17], in which 51% of tumours are classical. They also identified combined phenotypes, with a different histological feature present in over 30% of the tumour area, namely cribriform, gyriform, clear-cell, papillary, micropapillary or complex. Finally, they observed variants, which have no classical features, namely colloid, adenosquamous and papillary carcinoma. They also included medullary and tubular carcinomas as rare PDAC variants. Expert pancreatic pathologists should verify the reproducibility of this classification and confirm the most relevant subtypes. It should also be investigated whether and to what extent these histological subtypes and the molecular subtypes, as described below, overlap.

Transcriptomic driven subtyping

A seminal paper was published by Collisson et al. in 2011 who used gene expression microarray analysis on two sets of 27 and 36 primary resected microdissected samples, in addition to human and mouse cell lines [18]. They unveiled three subtypes: classical, quasi-mesenchymal (QM-PDA) and exocrine-like. The classical subtype showed high expression of epithelial and adhesion-associated genes, the QM-PDA subtype of mesenchymal genes and the exocrine-like subtype of digestive enzyme genes.

Several subsequent studies shared similar findings. Kim et al. described three molecular subtypes, based on microarray analysis of 96 resected non-microdissected samples [19]. The subtypes they reported were subtype 2, similar to the QM-PDA subtype, subtype 3 which resembles the exocrine-like subtype and subtype 1, which might resemble the classical subtype but was also enriched for immune pathways. The same team carried out miRNA expression profiles on 104 samples, again reporting three subtypes, of which it remains unclear how they match with their microarray-based subtypes [20].

The previous studies lack depth in their transcriptome analysis as they used microarray analysis, which is inferior to RNA sequencing (RNAseq) having a broader dynamic range and detecting low abundance transcripts; however, this technology came into practice after these papers were published.

Moffitt et al. analysed subtypes with gene expression microarrays by virtually microdissecting the tumour tissue from the stromal tissue, using 145 primary and 61 metastatic samples [21]. The findings were validated with RNAseq using a selection of these samples. They discerned two subtypes for the stromal tissue: normal and activated, in which the activated subtype was characterised by, amongst others, macrophage-related genes. They also described two subtypes for the tumour tissue: classical and basal-like. The basal-like subtype overlapped with basal-like subtypes in other cancers, and the classical subtype overlapped with the classical subtype from Collisson et al. [18]. Moffitt et al. found that the exocrine-like subtype mainly consisted of genes from normal exocrine pancreas. They did not investigate the combination of epithelial tumour cell and stromal cell signatures to define subtypes.

Janky et al. carried out gene expression microarrays on 118 resected samples [22]. They defined three clusters that overlapped for 92% with those of Collisson et al.

More recently, Bailey et al. and the International Cancer Genome Consortium (ICGC) published the first RNAseq-based subtyping using 96 samples, and integrating genomic analysis [23]. Four subtypes were identified: pancreatic progenitor (PP, similar to Collisson's classical subtype), squamous (similar to Collisson's QM-PDA subtype), aberrantly differentiated endocrine–exocrine (ADEX, similar to Collison's exocrine-like subtype) and a new subtype named immunogenic. The squamous subtype resembled squamous subtypes in other cancers and was associated with hypermethylation and suppression of genes that control the pancreatic endodermal cell-fate determination. The PP subtype was characterised by developmental transcriptional networks. The ADEX subtype was distinguished by transcriptional networks of later stages of pancreatic differentiation and development. The immunogenic subtype displayed signs of infiltrating B and T cells.

The Cancer Genome Atlas (TCGA) consortium, using 150 primary samples, applied the clustering techniques from Moffitt et al., Collisson et al. and Bailey et al., and reproduced the aforementioned classifications [10]. They noticed that in high-purity tumours, the basal-like and squamous subtypes overlapped strongly, as with the classical (Moffitt and Collisson) and PP subtypes. The other subtypes were attributed to lower purity of the samples, and may reflect gene expression from non-neoplastic tissues.

Following this report, Mueller et al. analysed the transcriptomic data from Bailey et al., supplemented with undifferentiated tumours from the ICGC PACA-AU (Pancreatic Cancer Australia) cohort, and revealed five distinct clusters [24]. In cluster 1, squamous differentiation, TP63ΔN transcriptional targets and cell proliferation/cell cycle were overexpressed, which overlapped strongly with the squamous subtype. Cluster 4 was similar to the immunogenic subtype and was enriched for undifferentiated tumours. Undifferentiated tumours were also characterised by signatures of cluster 3, such as epithelial-to-mesenchymal transition (EMT) and MAPK signalling. Clusters 2 and 5 were associated with metabolism, epithelial cell differentiation and embryonic development.

The Collisson, Bailey and Moffitt studies were furthermore validated by Birnbaum et al., using fifteen public datasets, comprising a total of 846 primary tumours [25]. They concluded that the prognostic value derived from the Bailey and Moffitt signatures was still substantial in multivariate analysis, while that of Collisson was not. Multivariate analysis incorporating the Bailey classification, Collisson classification and Moffitt classification resulted in statistical significance for the Bailey classification and Moffitt stroma classification, indicating that these were complementary.

More recently, Puleo et al. integrated microenvironmental and epithelial components in their microarray RNA analysis derived

Table 1. Summary of studies that have subtyped pancreatic ductal adenocarcinoma (PDAC)

Study by	Defined subtypes (with potential overlap)				Other subtypes
Bailey et al. [23]	Squamous	PP	ADEX	Immunogenic	
Collisson et al. [18]	QM-PDA	Classical	Exocrine-like		
Moffitt et al. [21]	Basal-like	Classical			Activated stroma, Normal stroma
Puleo et al. [26]	Pure basal-like	Pure classical		Immune classic	Desmoplastic, Stroma activated[a]
Maurer et al. [28]	Basal-like	Classical			ECM-rich stroma, Immune-rich stroma
Kim et al. [19]	Subtype 2	Subtype 1?	Subtype 3	Subtype 1?	
Mueller et al. [24]	Cluster 1	Cluster 2	Cluster 5?	Cluster 4	Cluster 3
Janky et al. [22]	k3.cl3	k3.cl1	k3.cl2		
Noll et al. [30]	$KRT81^{+}HNF1A^{-}$	$KRT81^{-}HNF1A^{-}$	$KRT81^{-}HNF1A^{+}$		
Martinelli et al. [60]	$GATA6^{low}$		$GATA6^{high}$		
Sivakumar et al. [46]	Wnt^{-}/HH^{-}		Notch	Cell cycle	
Daemen et al. [41]	Glycolytic	Lipogenic			Slow proliferating
Seino et al. [39]	WRi		W^{-}		W^{+}

Each column corresponds to one overlapping subtype, except for the final column, containing other, non-overlapping subtypes. The first listed studies looked at transcriptomics to classify the tumours, while those below the bold line have looked at functional characteristics or potential markers.
Question marks are indicated when the study did not find a direct relation to a previously described subtype, but gene expression patterns indicate it likely belonging to this subtype.
Dark boxes are shown when the study did not find a subtype in this category.
[a]Desmoplastic and stroma activated contain mainly tumours belonging to the squamous/basal-like subtype.
PP, pancreatic progenitor; ADEX, aberrantly differentiated endocrine exocrine; QM-PDA, quasi-mesenchymal pancreatic adenocarcinoma; KRT81, keratin 81; HNF1A, hepatocyte nuclear factor 1-Alpha; HH, hedgehog; WRi, Wnt and R-spondin-independent organoids; W-, Wnt-non-secreting organoids; W+. Wnt-secreting organoid.

from 309 paraffin-embedded samples and distinguished five subtypes: pure classical, immune classical, pure basal-like, stroma activated and desmoplastic [26]. The pure classical subtype fits under classical/PP and contains mostly well-differentiated tumours. Furthermore, the immune classical subtype was also categorised under classical/PP. Pure basal-like tumours have acellular stroma, contain poorly differentiated tumours and are associated with metastatic spread. The stroma activated subtype is defined by a biologically active stroma, while the desmoplastic subtype is enriched for low tumour content and high expression of structural and vascularised stroma components (e.g. elastin). The immune classical and desmoplastic subtypes were characterised by an immune cell infiltrate, which resembles Moffitt's 'normal stroma' subtype, while the stromal infiltrate in the pure basal-like and stroma activated subtypes resembles Moffitt's 'activated stroma'. Activated stroma is defined by the presence of activated fibroblasts, resident fibroblasts undergoing a phenotypic shift by acquiring a myofibroblast-like phenotype. Tumours containing activated stroma are enriched in such myofibroblast-like cancer associated fibroblasts, expressing high levels of Alpha Smooth Muscle Actin (ACTA2) as well as Fibroblast Activation Protein alpha (FAP) and Osteonectin (SPARC) [27].

Another study subtyping epithelial and stromal content came from Maurer et al. who microdissected the epithelial and tumour compartments of 60 resected samples and carried out RNAseq [28]. They proposed two epithelial subtypes, basal-like and classical, and two stromal subtypes, Extra Cellular Matrix-rich stroma and immune-rich stroma. They did not find the exocrine-like subtype.

Finally, Connor et al. studied 224 primary and, importantly, 95 metastatic samples from 289 patients [29]. For the 19 paired samples, the primary tumour and metastases were molecularly conserved, and therefore detected as identical subtypes for both the Moffitt tumour, Collisson and Bailey classification. Interestingly, they also discovered that the basal-like tumours were enriched for hypoxia. Moreover, high hypoxia resulted in stable disease upon neoadjuvant therapy, whereas four patients with a partial response exhibited low hypoxia.

In summary, three distinct subtypes were identified by most groups and assigned to the epithelial content of the tumour (Table 1 and Figure 1). The first subtype is the basal-like, squamous or QM-PDA subtype, and secondly the classical or PP subtype. Interestingly, a basal-like and classical subtype can be found in many solid tumours, such as breast cancer and colorectal cancer (Supplementary Table S1, available at *Annals of Oncology* online). A third subtype found by five studies is the exocrine-like or ADEX subtype. The fourth subtype, the immunogenic subtype, displays a high expression of immunity genes that may come from the tumour epithelium or from immune cells. According to Moffitt et al., two subtypes of stroma exist: normal or activated. Puleo et al. integrated the epithelial and stromal transcriptomics and discovered that immune and stromal content differed between subtypes.

Several studies question the existence of the ADEX or exocrine-like subtype, claiming that this subtype could be contaminated with exocrine cells. Although most studies included samples with a relatively high tumour cellularity [18, 22, 23], contamination could be possible. As Puleo et al. described, even a

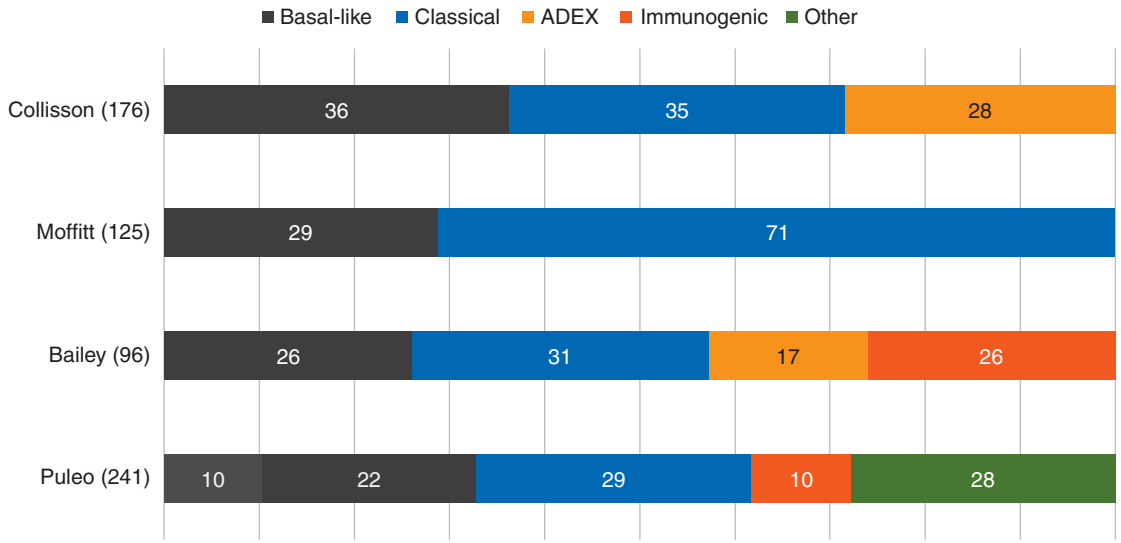

Figure 1. The distribution of subtypes in percentage for the main classification studies. Puleo's basal-like subtype is a combination of the pure basal-like (10%) and stroma activated (22%) subtypes, as genes belonging in the stroma activated group correspond to the basal-like subtype in other studies (*n* samples between brackets).

small amount of acinar cells can contaminate the transcriptomic results [26]. This theory is further supported by the fact that Collisson et al. were not able to find the exocrine-like subtype in cell lines of both humans ($n = 19$) and mice ($n = 15$) [18], although Noll et al. did find three exocrine-like cell lines [30]. Bailey et al. selected samples with at least 40% epithelial content, which should have removed impurity in their clustering analysis [23]. Additionally, Collisson et al. also detected the exocrine-like subtype in microdissected samples [18]. Another argument in favour of the existence of the exocrine-like subtype is that acinar enzymes stain positive in PDAC (see Human protein Atlas). This question remains an area for further investigation.

Subtypes based on metabolic and signal transduction pathway analysis

While the previous studies analysed human (resected) pancreatic tissue, other studies have used experimental models of PDAC, such as organoids and patient-derived xenografts (PDX), as well as cell lines. Although cell lines are easy to work with, they are limited as only the epithelial tumour cells are represented. Mouse models such as KPC mice have a *Kras* and *Tp53* mutation, replicating the tumour and its microenvironment; however, it is not fully clear if human PDAC heterogeneity is reflected well in this model [31]. Another experimental model is the PDX model, in which a piece of the tumour of a patient is implanted in an immunodeficient mouse. The model is efficient in studying human tumours and their response to therapy [32–34]; however, while this model reflects the original tumour, the implantation is not always successful and this model in immunodeficient mice may not fully reflect what happens in a patient. Humanised mouse models offer a valid alternative [35]. Organoids as experimental 3D model are becoming very popular because the architecture of the tumour is retained, and efforts are undertaken to co-culture with stromal cells [36, 37]. Therefore, this model has potential in performing (higher throughput) experiments on human tumours. Several studies have used organoids to study subtypes or therapeutic responses in PDAC [38–40].

Using 38 PDAC cell lines, Daemen et al. applied metabolite profiling using liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry and discovered that PDAC could be divided into three subtypes: slow proliferating, glycolytic and lipogenic [41]. The slow proliferating subtype displayed a high doubling time and was low in amino acids and carbohydrates. It is unclear if it corresponds to one of the transcriptomics driven subtypes. In contrast, the glycolytic subtype was driven by glycolytic and serine pathways and corresponded to the QM-PDA subtype. The lipogenic subtype was enriched for lipid and mitochondrial metabolites and correlated with the classical subtype [41]. It would be interesting to confirm the association between the glycolytic subtype and the QM-PDA subtype using a Ki67 staining, since only a positive correlation of Ki67 with recurrence has been described yet. The effect on survival remains conflicting between studies [42–44].

Global tyrosine patterns (phosphorylation of tyrosine) were investigated by Humphrey et al. using mass spectrometry on two series of PDAC cell lines (19 cell lines from ATCC, the American Type Culture Collection, and 17 cell lines from TKCC, The Kinghorn Cancer Centre), which could both be classified into three subtypes [45]. Cell–cell adhesion and EMT were characteristic for one subtype, perturbations in mRNA metabolism for the second subtype, and the third subtype was enriched for receptor tyrosine kinase signalling and showed enhanced sensitivity to erlotinib; however, the authors did not find a direct overlap with the other studies.

Sivakumar et al. applied master regulator analysis on co-expression networks on 7 existing datasets such as TCGA and ICGC for a total of 560 samples, and identified pathways associated with the Bailey subtypes [46]. The squamous subtype was characterised by repression of Hedgehog/Wnt signalling, while cell cycle signalling was involved in the immunogenic subtype, and ADEX and PP subtypes were enriched for Notch signalling.

Recently, Seino et al. carried out transcriptome analysis on a tumour organoid library from 39 patients to identify three subtypes based on Wnt signalling pathway association:

Table 2. Clinicopathological characteristics of the two main subtypes: basal-like and classical

	Basal-like	Classical
Overall survival	[10–19.2] months	[19–43.1] months
Disease-free survival	[4.6–10.9] months	[13.5–20.6] months
Well differentiated (grade 1)	18%	50%
Moderately differentiated (grade 2)	37%	39%
Poorly differentiated (grade 3)	45%	11%

Overall survival and disease-free survival are shown in months, while grades 1–3 are shown in percentages. Percentages amount to 100% per subtype [18–23, 25, 26].

Wnt-secreting organoids (W^+), Wnt-non-secreting organoids (W^-) and Wnt and R-spondin-independent organoids (WRi) [39]. The W^- subtype needs exogenous Wnt and R-spondin ligand, W^+ subtype depends on R-spondin, but is independent of exogenous Wnt ligand, and the WRi subtype does not require Wnt signal activation. In light of the current testing of Wnt inhibitors in clinical trials [47, 48], the latter two studies may be of significance (see further).

Other subtyping strategies

Researchers have also used other techniques to classify PDAC, such as Waddell et al., who classified PDAC into four subtypes by investigating patterns of genomic structural variation [49]. They defined four subtypes based on distribution and frequency of structural rearrangements, called stable, unstable, locally rearranged and scattered.

Using multiomic profiling on patient-derived tumour xenografts (PDTX), Nicolle et al. found a basal and a classical subtype [33]. The basal subtype was highly glycolytic (as seen by Daemen et al. [41]), less differentiated and showed characteristics of EMT. A subgroup of the classical subtype had extensive immune infiltration, likely corresponding to the immunogenic subtype, although they note that this subtype is based on the stromal content. Further results with PDTX were described by Lomberk et al. showing that the subtypes are characterised by distinct chromatin states (epigenetics), which are correlated with differential methylation patterns and a corresponding change in transcription levels of nearby genes [34].

Koay et al. used CT imaging and discovered that high delta tumours, with a distinct border between the tumour and the surrounding parenchyma, were more aggressive and more likely to develop distant metastases [50]. These tumours had more mesenchymal features and contained less stromal cells, similar to the pure basal-like subtype [26].

Some studies have based their strategy on the immune environment. Wartenberg et al. discovered a small population of patients with PD-L1 expression that grouped in the immune-exhausted subtype with poor prognosis [51]. Knudsen et al. noticed that PD-L1 expression was associated with a glycolytic metabolic preference, which is associated with the basal-like subtype [41, 52]. Furthermore, Wartenberg et al. found a subgroup with high Foxp3 and a poor prognosis, which seemed to overlap with the basal-like subtype [51]. This was corroborated by Knudsen et al. although they reported a correlation between M2 macrophages ($CD68^+CD163^+$) and poor prognosis [51, 52]. Koay et al. found more T-regulatory cells in the high delta tumours [50]. More research on the immune system is needed to unravel its role in PDAC.

Clinicopathological characteristics of the subtypes

The difference in survival between the basal-like subtype and other subtypes is substantial in all studies (Table 2): a median OS of 10–19.2 months was determined in the basal-like subtype compared with 19–43.1 months for the classical subtype. Disease-free survival was 4.6–10.9 for the basal-like subtype and 13.5–20.6 for the classical subtype. The differentiation grade also differed (Table 2): basal-like tumours were more often poorly differentiated, while classical tumours were more often well differentiated [18–23, 25, 26].

Various articles have reported that PDACs originating from the body or tail are more aggressive compared with those originating in the head of the organ [53, 54]. Dreyer et al. compared survival in 94 patients who were classified as head non-squamous, head squamous, body/tail non-squamous and body/tail squamous. The squamous subtype was overrepresented in tumours of the body/tail region, with substantially worse survival [55].

Path forward: surrogate markers for pragmatic subtyping

A pragmatic classifier is essential to subtype a patient, even with a small biopsy sample from either the primary or metastatic tumour. This classifier could be built into the routine diagnostics, giving faster results than RNAseq analysis. A study by Noll et al. aimed at identifying such markers through array-based differential expression analysis of subtyped PDAC cell lines [30]. Interesting biomarkers were checked for heterogeneous expression in the Protein Atlas. Two showed strong and subtype-specific staining: KRT81 for QM-PDA and HNF1A for the exocrine-like subtype. Both were negative in the classical subtype. Applying the same markers, Muckenhuber et al. investigated patient outcome and treatment response in a recent study [56]. They used two independent cohorts ($n = 262$ and 130) and included a cohort of advanced-stage PDAC ($n = 125$); however, 1% of the first cohort, 14% of the second cohort and 4% of the advanced-stage cohort showed expression of both markers. Later, Kuhlmann et al. published that cadherin-17 (CDH17) and galectin-4 (LGALS4) were co-localised on tumour cells of the exocrine-like subtype [57]. Altogether, this would give three different biomarkers (CDH17, LGALS4, HNF1A), in addition to CYP3A5, also reported by Noll et al., to differentiate the exocrine-like subtype from the classical and QM-PDA subtype; however, according to Bailey et al., HNF1A, CYP3A5 and CDH17 are expressed more in the PP than the ADEX subtype, and LGALS4 is expressed more in the PP and immunogenic subtypes [23]. Puleo et al. found CYP3A5 and LGALS4 to be more

expressed in the classical subtype [26]. Together, this questions the use of these markers and calls for a selection of surrogate markers driven by the largest sequencing efforts.

Using RNAscope, an *in situ* hybridisation technique, Aung et al. identified GATA6 as a biomarker of the classical subtype in their molecular profile-driven prospective clinical trial (COMPASS) [58]. They confirmed that immunohistochemistry for GATA6 strongly correlated with the RNAscope results [59]. Collisson already reported that GATA6 was low in QM-PDA compared with the other subtypes [18]. Bailey et al. confirmed epigenetic silencing of GATA6 in their squamous subtype [23] while Martinelli et al. noted that low GATA6 basal-like tumours had a poor prognosis [60]. Seino et al. discovered that GATA6 regulates the extent of Wnt niche, thus the low GATA6 subtype would be Wnt independent [39].

Pragmatically defining subtypes that will describe the tumour optimally for each patient will be challenging due to inter- and intrapatient heterogeneity, especially when subtyping in a biopsy specimen. Assessing this heterogeneity, for example by comparing different tumour regions of one patient, is crucial and becomes increasingly important when looking at metastases that might have a different genetic makeup. The current knowledge of molecular subtypes has been mostly focussed on resected tumours, which represent <20% of the whole patient population [2]. Only the study by Connor et al. has investigated metastatic samples, which they found are molecularly conserved compared with their primary tumour; however, this study mainly focussed on liver metastases, which could bias their findings. An ongoing clinical trial will give more insights in metastatic samples, as they focus exclusively on characterising subtypes in biopsies of metastatic patients [61].

In conclusion, GATA6 is a potential biomarker to distinguish classical from basal-like tumours; however, if there are more than two subtypes, as suggested by the leading studies, GATA6 cannot be used as a single classifier and consensus signatures should be elaborated while considering tumour heterogeneity.

Path forward: understanding subtype ontogeny

An area that remains largely unexplored is the role of the tumour ontogeny and at what stage of tumourigenesis the subtype divergence comes up. PDAC precursor lesions comprise PanIN (Pancreatic Intraepithelial Neoplasia) and IPMNs (Intraductal Papillary Mucinous Neoplasm). Data suggest that PDAC might have different cells of origin with most evidence for acinar and duct cells [62, 63]. Transitions from one cell type to the other (acinar to ductal metaplasia) and the possibility of one cell type developing one type of precursor lesion (acinar-PanIN and duct-IPMN) have been reported [64, 65]. Data from Lee et al. recently suggested that acinar cell derived PDAC was closer to the classical subtype than duct derived tumours, based solely on cytokeratin 20 expression [66]. The gene signatures of the PP and ADEX subtype also suggest, respectively, a resemblance with a progenitor ductal state or an aberrant differentiation state of the adult acinar (and endocrine) cell lineage. In other solid tumours, more research has already gone into this aspect. In breast cancer, the same mutational profile can generate different subtypes depending on whether luminal or basal cells were targeted [67]. In colorectal cancer, different precursor lesions (tubular versus serrated adenomas) give rise to different tumour subtypes [68]. In squamous cell carcinoma, tumours from the interfollicular epidermis give rise to well-differentiated tumours, while those from the hair follicle showed EMT and increased metastatic potential [69].

Not only the cell of origin or its differentiation stage could dictate the subtype, studies also show transition of one subtype into another. This has been reported for tumours gaining TP63, which reprograms the tumour to the basal-like subtype [70]. In mouse models, BET inhibitors can shift the tumour from squamous to classical [71]. Extrinsic factors such as inflammation can also drive a specific subtype, as reported in colorectal cancer [72]. Inflammation in chronic pancreatitis is an important risk factor for development of PDAC [73], but no data exist correlating this with molecular subtypes.

In summary, understanding the ontogeny of the molecular subtypes which is intrinsically associated with the cell of origin, the sequence of mutations and the rewiring of signalling pathways will provide information that can contribute to the consensus classification and holds promise for subtype-tailored therapeutic targeting.

Path forward: subtype-driven therapies

As common mutations in PDAC are not targetable, research has been focussed on precision medicine to target specific, less common mutations such as *BRCA1/2* [74]. In patients with a *BRCA* mutation, platinum therapy and PARP inhibitors can be given to substantially improve OS in advanced disease [75]. In *KRAS* wild-type patients, alternative drivers such as *BRAF* can be targeted [10]. While microsatellite instability can be used for targeted therapy in some solid tumours, this only occurs in 0.5% of patients with PDAC. Several studies have tried using genomic characterisation to refine treatment, with varying success rates [76–78]; however, based on a recent large-scale study of 3600 PDAC patients, only 17% harbour a potentially targetable genomic alteration [79].

A new development in cancer therapy is determining the genome with circulating tumour DNA (ctDNA) to refine therapy. Research on this topic is emerging, with only a few promising studies published so far in the field [80]. In PDAC, no study has been carried out to guide therapy with ctDNA. They were only focussed on diagnosis and prognosis [81]. Studies that explored whether the ctDNA can be used as molecular snapshots of the pancreatic tumour subtypes are likely desirable.

In an attempt to advance the use of precision medicine in PDAC, stratified therapeutic regimens for the subtypes described in Table 1 have been investigated. These data are scarce, speculative and should be interpreted with caution because of lack of clinical evidence. Most studies focussed on the basal-like subtype. Mixed observations are reported on response to standard drugs used in the adjuvant and palliative setting: Collisson et al. discovered that human cell lines of the QM-PDA subtype *in vitro* were more sensitive to gemcitabine than the classical subtype [18]. Moffitt et al. stated that the basal-like subtype would respond better to unspecified adjuvant therapy, according to retrospective

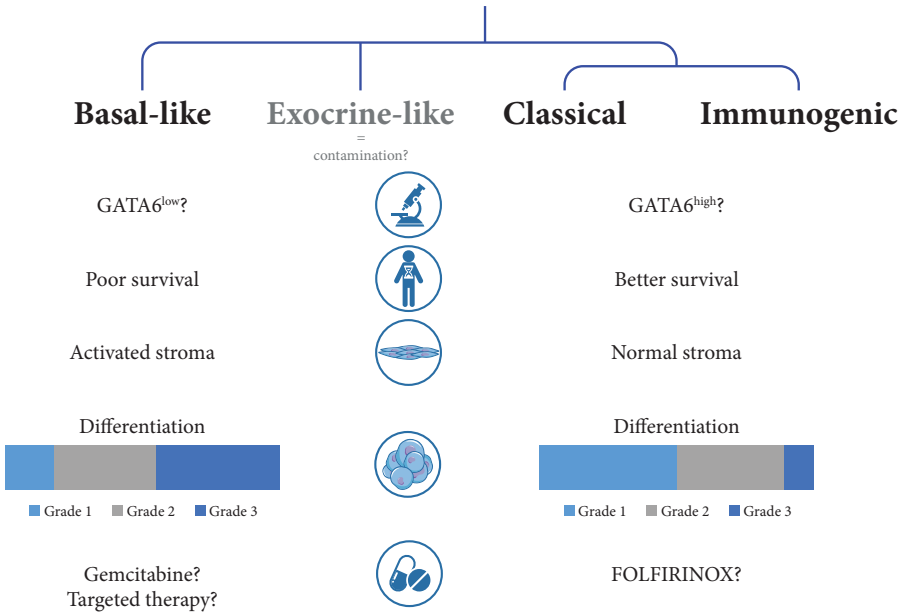

Figure 2. Summarising figure of the different subtypes in pancreatic ductal adenocarcinoma (PDAC). We show a hypothetical and simplified phylogenetic tree for the proposed different subtypes, and list potential biomarkers, clinicopathological differences and potential therapeutics for the basal-like subtype and the classical (and immunogenic) subtype. Question marks indicate possibilities that need to be further explored.

patient data [21]. Martinelli et al. assessed data from ESPAC-3 and observed that patients with a low GATA6 expression responded poorly to the adjuvant therapy 5-fluorouracil (5-FU)/leucovorin when compared with GATA6 expressors [60]. According to Muckenhuber et al., the ADEX subtype could benefit from FOLFIRINOX, based on their data of subtyped patients treated with FOLFIRINOX or gemcitabine [56].

More recently, Aung et al. carried out the first prospective trial where patients with both locally advanced or metastatic PDAC were included [58]. A biopsy was used for RNAseq to classify the patients. One hundred eighteen patients with sequencing data received treatment with modified FOLFIRINOX (m-FOLFIRINOX) or gemcitabine with nab-paclitaxel. The best progression-free survival (PFS) and OS were reached in patients who had a tumour belonging to the classical subtype, treated with m-FOLFIRINOX. Those belonging to the basal-like subtype and treated with m-FOLFIRINOX showed the worst PFS. Targetable genetic alterations were found in 30% of the patients [59].

Novel treatment strategies in a subtype-specific manner were also explored. In the TCGA data, Sivakumar et al. noticed that the basal-like subtype might benefit from unspecified targeted therapy [46]. Daemen et al. suggested that ENO2 could be targeted in the basal-like subtype [41]. According to Noll et al., who used cell lines, the exocrine-like subtype might be resistant to tyrosine kinase inhibitors (TKis) through CYP3A5 [30]. Bailey et al. suggested that the immunogenic subtype, with PD1 signalling upregulation, might be a potential target for immune modulators [23].

It should be emphasised that the above publications have not been replicated and use different approaches, thus it is uncertain which therapies would be more effective in which subtypes. The difference in progression and survival between the two main subtypes also makes evaluating a therapeutic effect difficult. The pathways that differ among the subtypes should be investigated for clinical utility. In the era of precision medicine and immunotherapy, we should promptly design prospective studies evaluating gene-driven therapies.

Discussion

Conclusion and perspectives

For PDAC, four to five subtypes have been described, of which the basal-like and classical subtypes can be found in each of the reported studies. The basal-like subtype, with a poor prognosis, is characterised by EMT and TP63 expression. These tumours are often poorly differentiated and seem to respond better to gemcitabine. The classical subtype, with a better prognosis, is associated with epithelial genes, pancreatic transcription factors and high expression of GATA6. These well-differentiated tumours appear to respond better to FOLFIRINOX. The exocrine-like subtype, characterised by exocrine genes, might be caused by acinar cell contamination, an issue that deserves further investigation. The immunogenic subtype is characterised by immunity genes, which may originate from an immune infiltrate. Furthermore, several studies have defined separate subtypes for the stroma, where activated stroma has a poor prognosis.

As shown in Figure 2, major progress has been made in the area of subtyping for PDAC, although no consensus is reached. The biology of PDAC, both ontogeny and stroma, should be further explored, and more potential biomarkers should be investigated. Above all, more translational trials are needed to study which therapies are more effective in which subtype.

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Disclosure

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References

1. Kumar V, Abbas AK, Aster JC, Perkins JA. Robbins Basic Pathology. 10th edition. Amsterdam, the Netherlands: Elsevier 2018; 935 p.
2. NIH. Cancer stat facts: pancreatic cancer [Internet] 2015; https://seer.cancer.gov/statfacts/html/pancreas.html (12 March 2019, date last accessed).
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017; 67(1): 7–30.
4. Ferlay J, Colombet M, Soerjomataram I et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries and 25 major cancers in 2018. Eur J Cancer 2018; 103: 356–387.
5. Rahib L, Smith BD, Aizenberg R et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014; 74(11): 2913–2921.
6. Ferlay J, Partensky C, Bray F. More deaths from pancreatic cancer than breast cancer in the EU by 2017. Acta Oncol 2016; 55(9–10): 1158–1160.
7. Bartsch DK, Gress TM, Langer P. Familial pancreatic cancer: current knowledge. Nat Rev Gastroenterol Hepatol 2012; 9(8): 445–453.
8. Knudsen ES, O'Reilly EM, Brody JR, Witkiewicz AK. Genetic diversity of pancreatic ductal adenocarcinoma and opportunities for precision medicine. Gastroenterology 2016; 150(1): 48–63.
9. Biankin AV, Waddell N, Kassahn KS et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012; 491(7424): 399–405.
10. Raphael BJ, Hruban RH, Aguirre AJ et al. Integrated genomic characterization of pancreatic ductal adenocarcinoma. Cancer Cell 2017; 32(2): 185–203.
11. Witkiewicz AK, McMillan EA, Balaji U et al. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun 2015; 6: 6744.
12. Adamo P, Cowley CM, Neal CP et al. Profiling tumour heterogeneity through circulating tumour DNA in patients with pancreatic cancer. Oncotarget 2017; 8(50): 87221–87233.
13. Wilentz RE, Goggins M, Redston M et al. Genetic, immunohistochemical, and clinical features of medullary carcinoma of the pancreas: a newly described and characterized entity. Am J Pathol 2000; 156(5): 1641–1651.
14. Herxheimer G. Über heterologe cancroide. Beitr Pathol Anat 1907; 41: 348–412.
15. Madura JA, Jarman BT, Doherty MG et al. Adenosquamous carcinoma of the pancreas. Arch Surg 1999; 134(6): 599–603.
16. Chen W. Pancreas—WHO classification of tumors [Internet]. PathologyOutlines 2018; http://www.pathologyoutlines.com/topic/pancreaswho.html (19 March 2019, date last accessed).
17. Schlitter AM, Segler A, Steiger K et al. Molecular, morphological and survival analysis of 177 resected pancreatic ductal adenocarcinomas (PDACs): identification of prognostic subtypes. Sci Rep 2017; 7(1): 41064.
18. Collisson EA, Sadanandam A, Olson P et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med 2011; 17(4): 500–503.
19. Kim S, Kang MJ, Lee S et al. Identifying molecular subtypes related to clinicopathologic factors in pancreatic cancer. Biomed Eng Online 2014; 13(Suppl 2): S5.
20. Namkung J, Kwon W, Choi Y et al. Molecular subtypes of pancreatic cancer based on miRNA expression profiles have independent prognostic value. J Gastroenterol Hepatol 2016; 31(6): 1160–1167.
21. Moffitt RA, Marayati R, Flate EL et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet 2015; 47(10): 1168–1178.
22. Janky R, Binda MM, Allemeersch J et al. Prognostic relevance of molecular subtypes and master regulators in pancreatic ductal adenocarcinoma. BMC Cancer 2016; 16(1): 1–15.
23. Bailey P, Chang DK, Nones K et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016; 531(7592): 47–52.
24. Mueller S, Engleitner T, Maresch R et al. Evolutionary routes and KRAS dosage define pancreatic cancer phenotypes. Nature 2018; 554(7690): 62–68.
25. Birnbaum DJ, Finetti P, Birnbaum D et al. Validation and comparison of the molecular classifications of pancreatic carcinomas. Mol Cancer 2017; 16: 168.
26. Puleo F, Nicolle R, Blum Y et al. Stratification of pancreatic ductal adenocarcinomas based on tumor and microenvironment features. Gastroenterology 2018; 155(6): 1999–2013.
27. Kota J, Hancock J, Kwon J, Korc M. Pancreatic cancer: stroma and its current and emerging targeted therapies. Cancer Lett 2017; 391: 38–49.
28. Maurer HC, Holmstrom SR, He J et al. Experimental microdissection enables functional harmonisation of pancreatic cancer subtypes. Gut 2019; 68: 1034–1043.
29. Connor AA, Denroche RE, Jang GH et al. Integration of genomic and transcriptional features in pancreatic cancer reveals increased cell cycle progression in metastases. Cancer Cell 2019; 35(2): 267–282.
30. Noll EM, Eisen C, Stenzinger A et al. CYP3A5 mediates basal and acquired therapy resistance in different subtypes of pancreatic ductal adenocarcinoma. Nat Med 2016; 22(3): 278–287.
31. Hingorani SR, Wang L, Multani AS et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell 2005; 7(5): 469–483.
32. Hiroshima Y, Zhang Y, Murakami T et al. Efficacy of tumor-targeting Salmonella typhimurium A1-R in combination with anti-angiogenesis therapy on a pancreatic cancer patient-derived orthotopic xenograft (PDOX) and cell line mouse models. Oncotarget 2014; 5(23): 12346–12357.
33. Nicolle R, Blum Y, Marisa L et al. Pancreatic adenocarcinoma therapeutic targets revealed by tumor-stroma cross-talk analyses in patient-derived xenografts. Cell Rep 2017; 21(9): 2458–2470.
34. Lomberk G, Blum Y, Nicolle R et al. Distinct epigenetic landscapes underlie the pathobiology of pancreatic cancer subtypes. Nat Commun 2018; 9(1): 1978.
35. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nat Rev Immunol 2007; 7(2): 118–130.
36. Boj SF, Hwang C-I, Baker LA et al. Organoid models of human and mouse ductal pancreatic cancer. Cell 2015; 160(1–2): 324–338.
37. Tsai S, McOlash L, Palen K et al. Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models. BMC Cancer 2018; 18(1): 335.
38. Tiriac H, Belleau P, Engle DD et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. Cancer Discov 2018; 8(9): 1112–1129.
39. Seino T, Kawasaki S, Shimokawa M et al. Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. Cell Stem Cell 2018; 22(3): 454–467.
40. Huang L, Holtzinger A, Jagan I et al. Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell- and patient-derived tumor organoids. Nat Med 2015; 21(11): 1364–1371.
41. Daemen A, Peterson D, Sahu N et al. Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors. Proc Natl Acad Sci U S A 2015; 112(32): E4410–E4417.
42. Kim H, Park CY, Lee JH et al. Ki-67 and p53 expression as a predictive marker for early postoperative recurrence in pancreatic head cancer. Ann Surg Treat Res 2015; 88(4): 200–207.

43. Striefler JK, Sinn M, Pelzer U et al. P53 overexpression and Ki67-index are associated with outcome in ductal pancreatic adenocarcinoma with adjuvant gemcitabine treatment. Pathol Res Pract 2016; 212(8): 726–734.
44. Karamitopoulou E, Zlobec I, Tornillo L et al. Differential cell cycle and proliferation marker expression in ductal pancreatic adenocarcinoma and pancreatic intraepithelial neoplasia (PanIN). Pathology 2010; 42(3): 229–234.
45. Humphrey ES, Su S-P, Nagrial AM et al. Resolution of novel pancreatic ductal adenocarcinoma subtypes by global phosphotyrosine profiling. Mol Cell Proteomics 2016; 15(8): 2671–2685.
46. Sivakumar S, de Santiago I, Chlon L, Markowetz F. Master regulators of oncogenic KRAS response in pancreatic cancer: an integrative network biology analysis. PLoS Med 2017; 14(1): e1002223.
47. El-Khoueiry A. Safety and efficacy study of PRI-724 in subjects with advanced solid tumors [Internet] 2017; https://clinicaltrials.gov/ct2/show/NCT01302405 (12 March 2019, date last accessed).
48. Pharmaceuticals N. A study of LGK974 in patients with malignancies dependent on Wnt ligands [Internet] 2018; https://clinicaltrials.gov/ct2/show/NCT01351103 (12 March 2019, date last accessed).
49. Waddell N, Pajic M, Patch AM et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015; 518(7540): 495–501.
50. Koay EJ, Lee Y, Cristini V et al. A visually apparent and quantifiable CT imaging feature identifies biophysical subtypes of pancreatic ductal adenocarcinoma. Clin Cancer Res 2018; 24(23): 5883–5894.
51. Wartenberg M, Cibin S, Zlobec I et al. Integrated genomic and immunophenotypic classification of pancreatic cancer reveals three distinct subtypes with prognostic/predictive significance. Clin Cancer Res 2018; 24(18): 4444–4454.
52. Knudsen ES, Vail P, Balaji U et al. Stratification of pancreatic ductal adenocarcinoma: combinatorial genetic, stromal, and immunologic markers. Clin Cancer Res 2017; 23(15): 4429–4440.
53. Artinyan A, Soriano PA, Prendergast C et al. The anatomic location of pancreatic cancer is a prognostic factor for survival. HPB (Oxford) 2008; 10(5): 371–376.
54. Koskela HM, Syrjanen KJ, Korkeila EA. Disease outcome of patients with pancreatic cancer in a cohort treated outside clinical trials. Anticancer Res 2013; 33(12): 5491–5494.
55. Dreyer SB, Jamieson NB, Upstill-Goddard R et al. Defining the molecular pathology of pancreatic body and tail adenocarcinoma. Br J Surg 2018; 105(2): e183–e191.
56. Muckenhuber A, Berger AK, Schlitter AM et al. Pancreatic ductal adenocarcinoma subtyping using the biomarkers hepatocyte nuclear factor-1A and cytokeratin-81 correlates with outcome and treatment response. Clin Cancer Res 2018; 24(2): 351–359.
57. Kuhlmann L, Nadler WM, Kerner A et al. Identification and validation of novel subtype-specific protein biomarkers in pancreatic ductal adenocarcinoma. Pancreas 2017; 46(3): 311–322.
58. Aung KL, Fischer SE, Denroche RE et al. Genomics-driven precision medicine for advanced pancreatic cancer—early results from the COMPASS trial. Clin Cancer Res 2018; 24(6): 1344–1354.
59. O'Kane GM, Fischer S, Denroche R et al. Integrative molecular profiling and response to chemotherapy on the COMPASS trial. J Clin Oncol 2019; 37(Suppl 4): 188.
60. Martinelli P, Carrillo-De Santa Pau E, Cox T et al. GATA6 regulates EMT and tumour dissemination, and is a marker of response to adjuvant chemotherapy in pancreatic cancer. Gut 2017; 66(9): 1665–1676.
61. Renouf DJ. Prospectively defining metastatic pancreatic ductal adenocarcinoma subtypes by comprehensive genomic analysis (PanGen) [Internet] 2018; https://clinicaltrials.gov/ct2/show/NCT02869802 (11 April 2019, date last accessed).
62. Rooman I, Real FX. Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development? Gut 2012; 61(3): 449–458.
63. Krah NM, Murtaugh LC. Differentiation and inflammation: 'best enemies' in gastrointestinal carcinogenesis. Trends Cancer 2016; 2(12): 723–735.
64. Ferreira RMM, Sancho R, Messal HA et al. Duct- and acinar-derived pancreatic ductal adenocarcinomas show distinct tumor progression and marker expression. Cell Rep 2017; 21(4): 966–978.
65. Kopp JL, von Figura G, Mayes E et al. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. Cancer Cell 2012; 22(6): 737–750.
66. Lee AYL, Dubois CL, Sarai K et al. Cell of origin affects tumour development and phenotype in pancreatic ductal adenocarcinoma. Gut 2018; 68: 487–498.
67. Van Keymeulen A, Lee MY, Ousset M et al. Reactivation of multipotency by oncogenic PIK3CA induces breast tumour heterogeneity. Nature 2015; 525(7567): 119–123.
68. Fessler E, Drost J, van Hooff SR et al. TGFβ signaling directs serrated adenomas to the mesenchymal colorectal cancer subtype. EMBO Mol Med 2016; 8(7): 745–760.
69. Latil M, Nassar D, Beck B et al. Cell-type-specific chromatin states differentially prime squamous cell carcinoma tumor-initiating cells for epithelial to mesenchymal transition. Cell Stem Cell 2017; 20(2): 191–204.
70. Somerville TDD, Xu Y, Miyabayashi K et al. TP63-mediated enhancer reprogramming drives the squamous subtype of pancreatic ductal adenocarcinoma. Cell Rep 2018; 25(7): 1741–1755.
71. Andricovich J, Perkail S, Kai Y et al. Loss of KDM6A activates super-enhancers to induce gender-specific squamous-like pancreatic cancer and confers sensitivity to BET inhibitors. Cancer Cell 2018; 33(3): 512–526.
72. Fessler E, Medema JP. Colorectal cancer subtypes: developmental origin and microenvironmental regulation. Trends Cancer 2016; 2(9): 505–518.
73. Pinho AV, Chantrill L, Rooman I. Chronic pancreatitis: a path to pancreatic cancer. Cancer Lett 2014; 345(2): 203–209.
74. Garrido-Laguna I, Hidalgo M. Pancreatic cancer: from state-of-the-art treatments to promising novel therapies. Nat Rev Clin Oncol 2015; 12(6): 319–334.
75. Golan T, Kanji ZS, Epelbaum R et al. Overall survival and clinical characteristics of pancreatic cancer in BRCA mutation carriers. Br J Cancer 2014; 111(6): 1132–1138.
76. Aguirre AJ, Nowak JA, Camarda ND et al. Real-time genomic characterization of advanced pancreatic cancer to enable precision medicine. Cancer Discov 2018; 8(9): 1096–1111.
77. Pishvaian MJ, Bender RJ, Halverson D et al. Molecular profiling of patients with pancreatic cancer: initial results from the know your tumor initiative. Clin Cancer Res 2018; 24(20): 5018–5027.
78. Lowery MA, Jordan EJ, Basturk O et al. Real-time genomic profiling of pancreatic ductal adenocarcinoma: potential actionability and correlation with clinical phenotype. Clin Cancer Res 2017; 23(20): 6094–6100.
79. Singhi AD, George B, Greenbowe JR et al. Real-time targeted genome profile analysis of pancreatic ductal adenocarcinomas identifies genetic alterations that might be targeted with existing drugs or used as biomarkers. Gastroenterology 2019; 156(8): 2242–2253.
80. Sorber L, Zwaenepoel K, Deschoolmeester V et al. Circulating cell-free nucleic acids and platelets as a liquid biopsy in the provision of personalized therapy for lung cancer patients. Lung Cancer 2017; 107: 100–107.
81. Berger AW, Schwerdel D, Reinacher-Schick A et al. A blood-based multi marker assay supports the differential diagnosis of early-stage pancreatic cancer. Theranostics 2019; 9(5): 1280–1287.