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ABOUT COVER

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REVIEW

Molecular alterations in pancreatic tumors

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Abstract

Genetic alterations in pancreatic tumors can usually be classified in: (1) Mutational activation of oncogenes; (2) Inactivation of tumor suppressor genes; and (3) Inactivation of genome maintenance genes controlling the repair of DNA damage. Endoscopic ultrasound-guided fine-needle aspiration has improved pre-operative diagnosis, but the management of patients with a pancreatic lesion is still challenging. Molecular testing could help mainly in solving these "inconclusive" specimens. The introduction of multi-gene analysis approaches, such as next-generation sequencing, has provided a lot of useful information on the molecular characterization of pancreatic tumors. Different types of pancreatic tumors (*e.g.*, pancreatic ductal adenocarcinomas, intraductal papillary mucinous neoplasms, solid pseudopapillary tumors) are characterized by specific molecular alterations. The aim of this review is to summarize the main molecular alterations found in pancreatic tumors.

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Core Tip: To date, in patients with pancreatic cancer there are no targetable molecules for personalized treatment in clinical practice. The introduction of multi-gene analysis approaches, such as massive parallel sequencing, has provided a lot of useful information regarding the molecular characterization of pancreatic tumors. However, a huge amount of data needs to be properly managed to determine the information that is useful and correct. A deeper knowledge of the molecular alterations characterizing pancreatic neoplasms may lead to new potential therapeutic targets for these tumors.

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INTRODUCTION

Genetic alterations in pancreatic tumors are usually classified in: (1) Tumors with activation of oncogenes (*e.g.*, *KRAS* mutation—more than 90% of pancreatic tumors); (2) Tumors harboring inactivation of tumor suppressor genes (*e.g.*, *p16/CDKN2A*, *TP53*, and *SMAD4*); and (3) Tumors with inactivation of genes controlling the repair of DNA damage (*e.g.*, *hMLH1* and *MSH2*)[1].

KRAS gene mutations inhibit the ability of KRAS protein to hydrolyze GTP (guanosine-5'-triphosphate), leaving the protein constitutively active, mediating cell survival and differentiation. *KRAS* is the most common marker used for single-gene testing. Its use is strongly limited by the identification of mutations also in low-grade pancreaticobiliary dysplasia or chronic pancreatitis (about 10%)[2-5]. To date, the "The Papanicolaou Society of Cytopathology guidelines" does not encourage testing of *KRAS* in bile duct strictures and solid pancreatic masses as a useful "single-gene" test.

P53 protein is involved in cell-cycle regulation, the apoptotic process, and plays a crucial role in the maintenance of genomic stability. Mutations in the *TP53* gene lead to inactivation of the normal protein function. In the presence of DNA damage, the functional loss of the p53 protein enhances cellular survival, facilitating the accumulation of further genetic mutations[6]. *TP53* is mostly inactivated by single-point mutations[7]. *TP53* gene inactivation is a very common event in pancreatic cancer (50% to 75% of pancreatic cancers harbor *TP53* mutations)[7-9].

CDKN2A/p16 maps on chromosome 9p and encodes the protein $p14^{ARF}$, an activator of p53 protein, and $p16^{INK4a}$ protein. This protein inhibits the progression of the cell cycle at the G1-S checkpoint binding of cyclin-dependent kinases (CDKs), as CDK4 and CDK6[10]. *CDKN2A/p16* was the first tumor suppressor gene that was shown to undergo silencing and promoter hypermethylation in pancreatic cancer[11]. The Rb/p16 pathway is down-regulated in most pancreatic cancers, almost all through p16 gene inactivation[11]. Mutations in the *CDKN2A* gene are associated with an increased risk of cancers. Moreover, *CDKN2A* alterations are also frequently observed in cancer cell lines.

SMAD4/DPC4 gene is located on chromosome 18q and the protein acts in the signal transduction cascade, involving transforming growth factor β (TGF-β). A loss of SMAD4 protein leads to unregulated cellular proliferation[12].

The aim of this review is to summarize the main molecular alterations found in pancreatic tumors, both in solid (*e.g.*, pancreatic ductal adenocarcinoma (PDAC), Table 1) and in cystic neoplasms (*e.g.*, intraductal papillary mucinous neoplasms, IPMN).

Table 1 Main genetic alterations detectable in pancreatic ductal adenocarcinoma

Type of pancreatic lesion	Genetic alteration	Reported frequency (%)	Type of alterations	Role in clinical practice
PDAC	*KRAS*	70-90	Point mutations	Diagnostic/prognostic
	TP53	50-75	Point mutations/LOH	Prognostic
	CDKN2A/p16	90-98	Point mutations/LOH	Prognostic/genetic surveillance
	SMAD4	40-60	Point mutations/LOH	Prognostic
	BRCA1/2	5-10	Point mutations	Predictive/genetic surveillance
	NTRK1-3	< 1	Gene fusions	Predictive
	MSI	< 2	LOF	Predictive

The percentages quoted are estimated from the literature cited in the paper. Diagnostic role appears mainly in preoperative material. In bold those markers recommended for clinical practice by National Comprehensive Cancer Network 2019 guidelines. PDAC: Pancreatic ductal adenocarcinoma; LOH: Loss of heterozygosity.

PDAC

Wide genome analysis

According to data obtained by whole-exome sequencing analysis, PDAC harbors an average of about 60 genetic alterations, and most of them are point mutations[1,8,13]. Based on these alterations, 12 cellular pathways genetically altered in pancreatic neoplasia have been identified[8]. Massive sequencing studies carried out on PDACs revealed that alterations may be found in some well-known genes (*e.g.*, *KRAS*, *CDKN2A*, *TP53*, *ARID1A*, *SMAD4*) or in novel genes, that may be involved in DNA damage repair (*e.g.*, *ATM*), chromatin modification (*e.g.*, *EPC1* and *ARID2*), or in neoplastic carcinogenesis (*e.g.*, *KDM6A* and *PREX2*)[14]. Whole-exome sequencing analysis has defined some putative therapeutic targets (*e.g.*, RBM10) associated with longer survival in patients with pancreatic cancers, others associated with improved survival (*e.g.*, KRAS p.Q61H mutation), and others defining sensitivity to target therapies in PDAC models (*e.g.*, BRAF mutations as sensitivity markers for treatment with vemurafenib)[15].

An expression analysis study led to the cluster of PDAC in 4 different subtypes: (1) Squamous; (2) Pancreatic progenitor; (3) Immunogenic; and (4) Aberrantly differentiated endocrine exocrine[16]. Also analysis of the structural genomic alterations in PDACs have been classified into four different subtypes: (1) "Stable", when PDACs contain less than 50 structural variations; (2) "Locally rearranged", when PDACs exhibit a focal event on one or two chromosomes; (3) "Scattered subtype", when PDACs show a fewer number of chromosomal damages and less than 200 structural variations; and (4) "Unstable", when PDACs exhibit more than 200 structural events[17].

KRAS

KRAS is the most frequently mutated oncogene in pancreatic cancers (> 95%) and the most frequent gene mutated in PDAC (from 70% to 95%)[8,15,18,19]. The acquisition of a *KRAS* mutation represents an early and initiating event in PDACs. However, the low frequency of progression of precursor lesions to PDAC suggests that additional alterations are needed for neoplastic progression[20]. In PDAC, the mutations in *KRAS* are not located only in exon 2, but they have also been found in other exons[14,15,21]. However, the mutations harbored in exon 2 exhibited a similar association with survival, while cases mutated in exon 3 seem to have a remarkably favorable prognosis[15]. Coexistent *KRAS* mutations were detected in the same pancreatic neoplastic mass more frequently than in other tumors[21-23].

KRAS analysis may be of particular interest in the case of doubtful or inconclusive diagnoses (*e.g.*, specimens with cytological atypia or acellular specimens). It is well-established that cytopathology together with *KRAS* analysis allows improved diagnosis of PDAC in endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) specimens[24-31]. Finding a *KRAS* mutation in EUS-FNA material may: (1) Indicate that a re-evaluation of the cytopathology report is needed (mainly if doubtful); (2) Indicate a second FNA or surgery[21]; and (3) Lead to a reduction in

false-negative diagnoses[32].

A worse prognosis has been observed in patients with tumors harboring coexistent *KRAS* mutations together with TP53 alterations and/or loss of SMAD4 protein[33,34]. KRAS p.G12C variant is a mutation that could be targeted by KRAS-G12C-specific inhibitors[35]. PDAC which also harbors the p.G12C alteration may benefit from this treatment. KRAS-wild type PDACs represent a distinct molecular subtype of pancreatic cancer that could benefit from tailored treatments, including BRAF antagonists and MAPK inhibitors[36].

TP53

EUS-FNA sensitivity in the diagnosis of pancreatic malignant lesions can be improved by also implementing the evaluation of the *TP53* gene[37-39]. P53 protein overexpression has been detected in specimens with pancreatic cancer but not in those with chronic pancreatitis. TP53 alterations have been detected in 50%-75% of PDACs. Combining the evaluation of p53 protein expression with histological examination improves the sensitivity of diagnosis of pancreatic cancers, with a high specificity [37,38]. The sensitivity of EUS-FNA in the diagnosis of PDAC is further improved by combining p53 and Ki67 staining[38].

The combination of p53 and CA19.9 increases the sensitivity of cytology but it can negatively affect the specificity[39]. A worse patient prognosis has been correlated with loss of p53 protein, mainly if p53 loss is combined with KRAS alterations and loss of SMAD4 protein expression[33,34]. In a recent study based on next-generation sequencing (NGS) results from the CONKO-001 phase III trial, it has been demonstrated that patients with *TP53* mutated PDAC tumors benefit from adjuvant gemcitabine treatment[40].

SMAD4

Approximately 50% of pancreatic cancers show SMAD4 protein inactivation, due to homozygous deletion and intragenic mutations[10,41-43]. Loss of SMAD4 generally occurs late in pancreatic carcinogenesis, and it has been frequently observed in pancreatic adenocarcinomas, but not in extra-pancreatic lesions[44]. In reactive and inflammatory diseases of the pancreas (*e.g.*, chronic pancreatitis) SMAD4 activity is usually preserved. Loss of SMAD4 protein has also been linked with an increased risk of developing metastases and worse prognosis[33,34,43,45,46]. In PDAC, *SMAD4* mutations lead to the prevention of the normal transduction of TGF-β signals; TGF-β inhibitor has shown efficacy in a preclinical investigation[47].

A study by Hsieh *et al*[48] demonstrated that SMAD4 deficiency in PDAC cells led to higher sensitivity to gemcitabine. On the contrary, *SMAD4* mutated cells were sensitive to gemcitabine similar to that in cells with wild-type SMAD4[48]. These data suggest that the *SMAD4* copy number may be a therapeutic marker for PDAC treatment with gemcitabine[49]. In this study, it was observed that SMAD4 deficiency led to upregulation of cell cycle-related genes, such as CDK1, with consequent higher sensitivity to other agents modulating the cell cycle such as clofarabine, cytarabine, darinaparsin, and olaparib[48,49].

CDKN2A

More than 95% of sporadic pancreatic carcinomas harbor a *CDKN2A* gene inactivation due to intragenic mutation, usually coupled with the loss of the other allele, promoter hypermethylation, or homozygous deletion of both alleles[50-52]. *CDKN2A* is involved in familial pancreatic cancer, although *CDKN2A* germline mutations in patients with pancreatic cancer occur rarely (0.6%)[53,54]. Patients harboring *CDKN2A* mutations are more likely to report a family history of pancreatic cancer than those without *CDKN2A* alterations[54]. Surveillance protocols from age 40 are recommended for *CDKN2A* mutation carriers[55].

Decreased expression of p16 protein has been associated with a tendency for the tumor to be larger than in those with normal p16 expression levels. Pancreatic neoplasms with loss of p16 expression, due to mutations and/or promoter hypermethylation, are significantly larger. Moreover, the survival period in these patients was significantly shorter when compared to those with a pancreatic tumor characterized by intact p16 functions[33,56,57]. CDK4/6 is a potential target in CDKN2A-deficient tumors and the efficacy of CDK4 inhibitors has been confirmed in PDAC preclinical models[58].

BRCA1-2

Alterations in the BRCA pathway and defects in DNA maintenance (such as genomic

instability and the BRCA mutational signature) may have implications for the therapeutic selection of patients with pancreatic tumors[17]. *BRCA1/2* mutation frequencies range from 5% to 10%, and BRCA alterations have been detected both in sporadic and familial PDAC[1,59]. Patients with PDAC harboring germline *BRCA1* or *BRCA2* mutations showed a longer progression-free survival if treated with a PARP inhibitor[60,61]. According to the recommendation from the International Cancer of the Pancreas Screening Consortium, for *BRCA1* a consensus of 69.9% was reached for recommending that *BRCA1* mutation carriers undergo surveillance, whereas no consensus was reached on family history criteria for *BRCA1* mutation carriers[55]. For carriers of *BRCA2* mutations, the consensus (agreement of 93%) was to recommend surveillance for mutation carriers who have a blood relative with pancreatic cancer[55]. For *BRCA2* mutation carriers with a germline variant (deleterious), the recommended age to initiate surveillance is generally 50 years[55]. With regard to the surveillance protocols for high-risk individuals, the consensus is that pancreatic imaging with magnetic resonance imaging (MRI)/magnetic retrograde cholangiopancreatography and/or EUS should be the first-line test for pancreatic surveillance[55]. Pancreatic computed tomography is reserved for individuals unable to undergo MRI or EUS[55].

Other alterations

Even if uncommon, MSI/dMMR (Microsatellite Instability/defective DNA mismatch repair) has been described in about 1%-2% of PDAC[62,63]. PDACs with MSI are usually associated with medullary histology and are rarely mutated in *KRAS* or *TP53* genes[62,64]. National Comprehensive Cancer Network (NCCN) guidelines recommend the MSI and MMR tests in locally advanced and metastatic pancreatic carcinomas[65]. NCCN recommends the treatment of PDAC with the PD-1 (programm-ed cell death-1) inhibitor, Pembrolizumab, only in those patients with MSI-H (high-MSI) or dMMR advanced/metastatic pancreatic cancer[65].

BRAF mutations are an uncommon event in PDAC[1]. Some evidence suggests that patients with pancreas tumors harboring *BRAFV600E* mutations may benefit from treatment with RAF-MEK-targeted therapy[66].

The *MGMT* promoter can be hypermethylated in PDAC[34,67]. In 1998, treatment with temozolomide in advanced pancreatic cancer has been tried in a phase II study, but no relevant clinical response was observed[68]. *ARID1A* oncosuppressor protein deficiency was significantly associated with poor outcome in PDAC patients[15]. Amplifications and copy-number gains of oncogenes such as *ERBB2*, *MET*, and *FGFR1* may be detected in pancreatic tumors[17]. The inactivation of several genes (*e.g.*, *ROBO1*, *ROBO2*, *SLIT2*, and *RNF43*) leads to an aberrant WNT (Wingless-related integration site) signaling[15,17]. Unresectable non-metastatic pancreatic carcinomas may also harbor mutations in *GRM8* and *TRIM33* genes[69], while only a small fraction (approximately 5%) of pancreatic adenocarcinomas showed Cyclin E overexpression[70]. Different to solid pseudopapillary neoplasia (SPN), *CTNNB1* mutations are uncommon in PDAC[15,71]. In PDAC, the frequency of *NTRK* (neurotrophic tropomyosin receptor kinase) fusion is very low (about 0.3%), but clinical trials revealed that the selective TRK (tropomyosin receptor kinase) inhibitors are also effective in PDAC harboring *NTRK* rearrangement[58]. NCCN guidelines suggest the use of a TRK inhibitor in *NTRK* gene fusion-positive advanced/metastatic pancreatic cancer[61,65].

Organoids are a preclinical model that is becoming increasingly important for studying tumor behavior because they can simulate metastases, microtumors, and the tumor microenvironment better than "classical" monolayer culture systems[72]. An interesting preclinical model for the study of PDAC is the set-up of three-dimensional (3D)-tumoroids *in vitro* culture systems[72-78]. These organoids can also be generated from a resected PDAC and are amenable to therapeutic screening as well as genetic and biochemical perturbation[77]. In a study by Boj *et al*[76], organoids derived from murine and human PDAC generated lesions similar to pancreatic intraepithelial neoplasia (PanIN) and progressed to invasive PDAC. Intriguingly, the expression of mutated KRAS protein (KRASG12D) in PDAC organoids was sufficient to induce a preinvasive neoplasm[76]. PDAC tumoroid cultures retain the capacity to maintain tumor stroma and characteristics of the primary tumor including the long-term (> 44 d) production of CEA (carcinoembryonic antigen) and CA19-9 (carbohydrate antigen 19-9)[74]. This 3D cell culture model of PDAC would help the diagnostics, investigation of genetic drivers, and identification of novel therapeutic targets[72]. Moreover, this culture could also allow clarification on how the immunosuppressive mechanism affects the growth and stasis of tumors[74]. Moreover, another important aspect is that organoids are suitable for storage in biobanks and used for further

research, ensuring access to relevant sample numbers[78].

CYSTIC PANCREATIC TUMORS

IPMN

KRAS mutations are harbored by over 90% of low-grade PanIN[79], and mutant *KRAS* is sufficient to initiate the development of PanINs and IPMNs[20,80-83] (Table 2).

The distinction between IPMNs and mucinous cystic neoplasms (MCNs) from non-neoplastic pancreatic cysts may be helped by analysis of pancreatic cyst fluid. In fact, a *KRAS* mutation is highly specific for mucinous differentiation, but not for identifying MCNs[84]. *KRAS* alterations have a very high specificity but low sensitivity for MCNs and IPMNs (approximately 15% and 70%, respectively). If *KRAS* analysis is combined with that of *GNAS*, the sensitivity increases[85,86]. The differential diagnosis of cystic mucinous lesions (IPMN and MCN), mainly when the pre-operative cytology is non-diagnostic or when the CEA cyst fluid levels are indeterminate, may be helped by the analysis of *KRAS* and Loss-of-Heterozygosity (LOH)[87].

IPMNs rarely harbor mutations in the *TP53* gene (approximately 10%). The overexpression of *TP53* was more commonly observed in IPMNs of the pancreatobiliary type with invasion[88]. In a cohort of IPMN patients, the overexpression of TP53, together with loss of function of SMAD4, was strongly associated with patient survival[88]. In IPMNs, SMAD4 loss of function was rarely detected; SMAD4 loss is more common in IPMNs of the pancreatobiliary type with invasion[88].

The *GNAS* gene encodes the α-subunit of the stimulatory G-protein (Gαs). This subunit regulates the adenylate cyclase activity through Gαs-coupled receptors. Alterations in GNAS may determine the characteristic IPMN phenotype[89]. *GNAS* activating mutations are reported prevalently in IPMN (approximately 40%-60%) [85,88,90-92] and invasive pancreatic cancers, only if arising in association with an IPMN[79,93]. In the majority of IPMNs (approximately 90%), at least one of the *KRAS* or *GNAS* genes harbor mutations[94], and in about half of IPMN (approximately 40%), a *GNAS* alteration coexists with a *KRAS* mutation[90]. The combination of *KRAS* and *GNAS* mutations helps in distinguishing between a serous cystic neoplasm (SCN) and an IPMN with high sensitivity and specificity. In fact, if most IPMNs have a *GNAS* and/or a *KRAS* alteration, no SCNs harbor either mutation. Besides, detecting a mutation in the *GNAS* gene in cyst fluid may help to distinguish IPMNs from MCNs[95].

RNF43 mutation frequency in IPMN is about 25%, ranging from 10% to 75%[93,96]. These mutations are often inactivating alterations and are found in association with LOH.

CDKN2A/p16 inactivating mutations have also been found in IPMNs with high-grade dysplasia, other than in pancreatic adenocarcinoma[97].

Germline mutations of *STK11/LKB1* genes have been associated with IPMNs and invasive pancreatic cancer[98,99]. Besides, somatic mutations of *STK11/LKB1* are observed in about 5% of patients with sporadic IPMNs and pancreatic cancers[98,99].

A high amount of DNA and high-amplitude mutations in the pancreatic cyst fluid may be indicators of malignancy, helping to identifying malignant cystic lesions[86].

The use of NGS in pancreatic cyst fluid allows high sensitivity and specificity in classifying pancreatic cancers, mainly for the diagnosis of IPMN with advanced neoplasia[100].

MCN

MCNs harbor alterations also commonly found in PDAC. MCNs frequently have *KRAS* gene mutations, mainly in MCNs with high-grade dysplasia[101]. The diagnosis of mucinous cysts may be helped by the presence of a *KRAS* mutation in cyst fluid[86]. p16/CDKN2A expression is altered in MCN, even if in a lower percentage (about 15%) if compared to that of PDAC[102]. P53 has been reported with aberrant expression in MCN with high-grade dysplasia[103]. *TP53* alterations are often associated with aggressiveness and seem to be involved in progression to PDAC[92]. Mutations of *RNF43* have been reported in MCN[92]. *PIK3CA* alterations were described with very low frequency in MCN and in association with high-grade dysplasia and invasive adenocarcinoma[104]. As in IPMN, no *VHL* mutations were detected in MCN[92], but, different to IPMN, the *GNAS* gene is not mutated in MCN[96]. The LOH of *Dpc4/Smad4* contributes to MCN progression in mice with *KRAS-G12D* mutation[105], confirming that the *SMAD4* gene acts as a PDAC tumor suppressor[106]. Whole-exome sequencing performed on a cohort of MCN revealed

Table 2 Main genetic alterations detectable in cystic pancreatic lesions

Type of pancreatic lesion	Genetic alteration	Reported frequency (%)	Type of alterations	Role in clinical practice
IPMN	*KRAS*	90	Point mutations	Diagnostic
	TP53	10	Point mutations/LOH	Prognostic
	GNAS	40-60	Point mutations	Diagnostic
	RNF43	25	Point mutations/LOH	Diagnostic
MCN	*KRAS*	0-25% (LG); 50-90% (HG)	Point mutations	Diagnostic
	CDKN2A/p16	0-10 (LG); 50 (HG)	Point mutations/LOH	Prognostic
	TP53	0 (LG); 20-50 (HG)	Point mutations/LOH	Prognostic
SCN	*VHL*	40-60	Point mutations/LOH	Diagnostic

The percentages quoted are estimated from the literature cited in the paper. Diagnostic role appears mainly in preoperative material. IPMN: Intraductal papillary mucinous neoplasms; MCN: Mucinous cystic neoplasia; LG: Low-grade mucinous cystic neoplasia; HG: High-grade mucinous cystic neoplasia; SCN: Serous cystic neoplasm; LOH: Loss of heterozygosity.

that the tumors harbored about 16 somatic mutations per tumor, lower than the number of mutations observed in IPMN (about 27 per tumor)[92].

SCN

VHL mutations are frequently reported in SCN (from 40% up to 60% of cases)[100,107]. SCNs usually do not harbor alterations in genes frequently mutated in IPMN, or MCN (*i.e.*, *KRAS*, *GNAS*, *TP53*), helping to distinguish SCNs from the other mucinous neoplasia[100,108]. The lack of *CTNNB1* mutations allows the differentiation of SCN from SPN. Moreover, SCNs do not harbor mutations in genes frequently altered in neuroendocrine pancreatic tumors[109]. Whole exome sequencing analysis performed on a cohort of eight SCNs revealed that almost all tumors harbored a LOH on chromosome 3p[92]. An average of only 10 non-synonymous somatic mutations was detected in SCNs[92], far less than the average observed in PDAC.

OTHER PANCREATIC TUMORS

SPN

The *CTNNB1* gene (codons from 32 to 37) encodes for a region that plays a crucial role in the regulation of the β-catenin protein[110,111]. Alterations within this *CTNNB1* region usually block β-catenin phosphorylation, inhibiting degradation of the protein[112]. *CTNNB1* mutations are characteristic of pancreatic SPN[71,113,114]. Different to PDAC, SPNs are not mutated in *KRAS*, *TP53*, or *SMAD4* genes, and *CTNNB1* mutations are the main molecular alteration detected[92]. DNA array CGH (comparative genomic hybridization) performed on a pediatric case of SPN revealed a loss in chromosome band 11p15.5, a chromosomal region encoding for the *HRAS* gene[115]. As suggested by Selenica *et al*[116], even if inhibition of the Wnt pathway may be an intuitive therapeutic option for this disease, the evidence that clinically advanced Wnt pathway inhibitors target components upstreaming β-catenin activity is a clear limitation. For this reason, future drugs should be designed to target the β-catenin protein directly[116] (Table 3).

Pancreatic neuroendocrine tumors

Pancreatic neuroendocrine tumors (PanNETs) show a distinct landscape of molecular alterations if compared to the other pancreatic tumors. The mutation frequency in PanNET was lower than that observed in poorly differentiated neuroendocrine carcinomas (45% and 83%, respectively)[117], and the incidence of mutations was higher in PanNET with a high risk of progression than those with low risk[117]. *MEN1* alterations (both mutations and LOH) have been found in up to 70% of PanNETs and have been associated with a better prognosis[118,119]. PanNETs harbor alterations in those genes involved in the chromatin remodeling complex, such as loss of ATRX and DAXX proteins expression[118]. Mutations in the mTOR (mammalian target of rapamycin) pathway genes (*e.g.*, *PIK3CA*, *PTEN*, and *TSC2*) have been detected in

Table 3 Main genetic alterations detectable in other pancreatic tumors

Type of pancreatic lesion	Genetic alteration	Reported frequency (%)	Type of alterations	Role in clinical practice
SPN	*CTNNB1*	90-100	Point mutations	Diagnostic
PanNET	*MEN1*	70	Point mutations/LOH	Diagnostic/prognostic
	VHL	25	Point mutations/LOH	Diagnostic
AAC	*CTNNB1*	5-25	Point mutationsLOF	Diagnostic
	MSI	5-15	LOF	Predictive?

The percentages quoted are estimated from the literature cited in the paper. Diagnostic role appears mainly in preoperative material. SPN: Solid pseudopapillary neoplasm; PanNET: Pancreatic neuroendocrine tumor; AAC: Acinic adenocarcinoma; LOH: Loss of heterozygosity; LOF: Loss of function.

PanNET[118]. For example, *PTEN* and *TSC2* genes have been observed as inactivating in primary PanNET and their low protein levels were associated with shorter overall and disease-free survival[120]. *VHL* inactivation, due to deletion or methylation, was also observed in up to 25% of PanNETs[121,122].

Acinar cell carcinomas

Activating mutations in *CTNNB1* and inactivating mutations in *APC* genes have been observed in up to 25% of acinar cell carcinomas (ACCs). *KRAS* mutations are an uncommon event in this type of neoplasia[123]. Nevertheless, *TP53* mutations were also found only in a low fraction of ACCs[123-125], deletion of the *TP53* region (chromosome band 17p13.1) was detected by FISH in about half of a cohort of 54 ACCs[125]. Alterations found in IPMN (*e.g.*, *GNAS* and *RNF43*) or in PanNET (*e.g.*, *MEN1*) were rarely found in ACC[126] A genome-wide analysis performed on ACCs revealed a median number of 137 point mutations per tumor and *COL12A1*, *FRY*, *FRYL*, and *PLB1* were the most frequently mutated genes[124]. Intriguingly, a genome-wide analysis identified no recurrent point mutations in ACCs[124]. Microsatellite instability was detected in a fraction of ACC ranging from 7% to 14% of cases[127]. ACC with MSI did not exhibit distinct morphological or clinical features[128].

CONCLUSION

To date, there are no targetable molecules available for personalized patient treatment of pancreatic tumors in clinical practice, as stated by the current European Society for Medical Oncology (ESMO) guidelines[129]. EUS-FNA has improved pre-operative diagnosis[130-132], but the management of patients with a pancreatic lesion is still challenging. In fact, in a subset of cases, such as lesions with atypical/suspicious cytopathologic features, the pre-operative diagnosis remains inconclusive[133]. Performing a molecular characterization could help mainly in solving these "inconclusive" specimens. The introduction of multi-gene analysis approaches, such as NGS, has provided a lot of useful information regarding the molecular characterization of pancreatic tumors[134]. EUS-FNAC (FNA cytology) is a useful diagnostic tool for pancreatic lesions[135] and molecular analysis can be successfully performed on cytological smears[21]. However, it has becoming increasingly crucial to have sufficient material for histological, immunohistochemical, and molecular characterization. Pancreatic FNAB (FNA biopsy) provides enough material to allow proper histological assessment, immunostaining, and molecular techniques [*e.g.*, NGS, digital polymerase chain reaction (PCR)][136-138].

A huge amount of data needs to be properly managed to determine the information that is useful and correct[139]. The recent ESMO guidelines have outlined their indications for the use of NGS in the characterization of metastatic cancers. As regards PDAC, it is not currently recommended to perform multigene NGS in daily practice[140]. However, ESMO encourages multigene sequencing in order to get access to innovative drugs. Moreover, NGS can be an alternative technique to PCR-based assays if it is not associated with extra cost for the public health care system if the patient is informed about the putative benefits of this analysis[140]. In conclusion, a deeper knowledge of the molecular alterations characterizing pancreatic neoplasms may lead to new potential therapeutic targets for these tumors.

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