The pancreatic cancer genome revisited

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Abstract | Pancreatic cancer is a genetic disease, and the recurrent genetic alterations characteristic of pancreatic cancer indicate the cellular processes that are targeted for malignant transformation. In addition to somatic alterations in the most common driver genes (*KRAS*, *CDKN2A*, *TP53* and *SMAD4*), large-scale studies have revealed major roles for genetic alterations of the SWI/SNF and COMPASS complexes, copy number alterations in *GATA6* and *MYC* that partially define phenotypes of pancreatic cancer, and the role(s) of polyploidy and chromothripsis as factors contributing to pancreatic cancer biology and progression. Germline variants that increase the risk of pancreatic cancer continue to be discovered along with a greater appreciation of the features of pancreatic cancers with mismatch repair deficiencies and homologous recombination deficiencies that confer sensitivity to therapeutic targeting. Wild-type *KRAS* pancreatic cancers, some of which are driven by alternative oncogenic events affecting *NRG1* or *NTRK1* — for which targeted therapies exist — further underscore that pancreatic cancer is formally entering the era of precision medicine. Given the vast developments within this field, here we review the wide-ranging and most current information related to pancreatic cancer genomics with the goal of integrating this information into a unifying description of the life history of pancreatic cancer.

Pancreatic neoplasms represent an important and rising public health burden worldwide. Epidemiological estimates for pancreatic cancer indicate that it is the seventh leading cause of global cancer deaths in industrialized countries and the third most common cause of death in the USA[1]. Pancreatic ductal adenocarcinoma (PDAC), the most common form of pancreatic cancer, was estimated to be responsible for 432,242 deaths worldwide in 2018 (REF.[1]). Key factors that contribute to poor PDAC outcomes include late clinical presentation related to insidious symptoms, lack of early detection strategies, complex biological features and limited therapeutic options[2]. The mainstay of PDAC treatment is cytotoxic therapy that, although resulting in a positive survival effect through developments in the past few years[3,4], leads to a modest, incremental improvement. Treatment is typically characterized by de novo and early development of acquired resistance[2]. Cachexia is a common presenting feature of PDAC, which further compounds treatment intolerance[5]. An alarming number of patients are diagnosed with metastatic PDAC, ~50% of all new diagnoses, and the average survival for this patient subset is <1 year[3].

PDAC is a disease with both genetic and epigenetic aspects to its formation, progression and development of resistance to therapy[6,7]. The PDAC genome, the focus of this Review, has been well described[8–14]. Initially, gene-focused studies identified the common driver genes of this disease and its major hereditary components[15]. These canonical PDAC genetic alterations are undruggable although major efforts are underway to develop novel therapeutic strategies[16,17]. Concurrent large-scale unbiased sequencing studies have revealed intertumoural and intratumoural heterogeneity at the transcriptional level with clear relationships to genetic features of the same tumour[18–22]. Thus, understanding of PDAC has largely advanced beyond its classic description, necessitating a reframing of the genetics of PDAC into a contemporary and more integrated understanding of this disease. In this Review, we summarize knowledge gleaned from studies of the PDAC genome in the context of its cellular origins, microenvironmental interactions and evolutionary growth dynamics during the life history of the neoplasm.

General features of the genome

Several large sequencing cohorts have been published in the past decade, each with unique sample sizes, quality assurances, sequencing methodologies and/or computational innovation[8–14]. The first, based on high-throughput Sanger sequencing of 20,661 protein-coding genes, reiterated the four main drivers of PDAC (*KRAS*, *CDKN2A*,

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Key Points

- The natural history of pancreatic cancer is characterized by both genetic and epigenetic alterations that contribute to its formation, progression and resistance to therapy.
- Most pancreatic cancers arise due to the accumulation of somatic alterations in a recurrent set of genes; however, some patients might develop pancreatic cancer owing to a genetic predisposition.
- Rare subsets of pancreatic cancers arise in association with a genetic alteration that is targetable.
- The pancreatic cancer stroma, inclusive of the immune system, acts as a dynamic selective pressure to which the neoplasm continuously adapts.
- Distinct genomic events are associated with pancreatic cancer phenotypes that are differentially sensitive to currently available therapies.

TP53 and *SMAD4*) and introduced the concept of core signalling pathways[8]. Sensitivity was high in this project owing to the use of xenograft enriched samples or low-passage cell lines, yet the sample size of 24 pancreaticobiliary cancers hampered identification of rare driver genes. Subsequent efforts relied on whole-exome sequencing with larger sample sizes, leading to identification of additional signalling pathways[9] and novel driver genes[9,10], and outlined the importance of *KRAS* wild-type PDAC for potential targeted therapies[10,11,23]. Whole-genome sequencing of yet even larger sample sizes revealed additional candidate driver genes and a range of genomic instability in PDAC, with the most unstable genomes highly correlated with inactivation of double-strand break repair genes and sensitivity to cisplatin[12]. When combined with laser capture microdissection, whole-genome sequencing has provided the highest sensitivity for identifying alterations in the PDAC genome and the roles of chromothripsis and whole-genome duplication in cancer progression[13,14,21]. Ultimately, with increased sample size and depth of coverage, the four main driver genes identified prior to the era of next-generation sequencing have remained at the forefront, although sufficiently greater detail with respect to mutational targets, genome structure and mechanisms of somatic alteration have been ascertained. The following summary represents a collective understanding of PDAC genetics based on these studies.

Single-nucleotide variants

The most prevalent form of genetic alteration in PDAC are single-nucleotide variants (SNVs)[8–14]. Although the absolute number of SNVs per PDAC reported amongst different studies probably varies according to the sequencing platform, computational pipelines and the purity of the DNA template used, estimates based on whole-genome sequencing of macro-dissected PDACs indicate ~2.64 mutations per megabase[12]. The number of SNVs per megabase might also vary by the region of the genome studied as non-coding regions have more SNVs per megabase than coding regions, possibly reflecting differences in selection pressures between these genomic regions[24]. The majority of SNVs are missense mutations, and many of these are passenger mutations that do not provide a fitness advantage to the cell[25,26].

Missense mutations that result both in a non-conservative change in the encoded amino acid and within a highly conserved region of the peptide are those most likely to change protein function[26–28]. Both deleterious and inconsequential SNVs occur in PDAC driver genes; thus this distinction is critical for annotation of germline and somatic variants and interpretation of the key genomic features of cancer[29–32]. In fact, the identification of recurrent deleterious mutations in previously understudied genes has supported identification of novel low-frequency PDAC driver genes such as in the *SLIT–ROBO* family or mediators of RNA splicing[8,9]. Nonsense mutations and small insertions and deletions that lead to frameshifts are deleterious by introducing a premature stop codon, which leads to degradation of the mutant transcript by nonsense-mediated decay or in some instances expression of a truncated protein[33]. Splice site mutations might have several consequences, including alternative splicing or intronic retention, that also lead to formation of a downstream premature stop codon within the aberrant transcript[34].

Although passenger mutations have little to no functional effect on protein function, they can nonetheless be relevant to cancer biology. Somatic missense mutations that develop in cancer cells can lead to the formation of novel peptides, whereas frameshift or splice site mutations might result in entirely novel stretches of amino acid sequences that, depending on their characteristics, can bind to MHC molecules as neoantigens that are recognized as non-self[35]. Furthermore, although most PDACs have a modest mutational burden[36] and hence a relatively low number of neoantigens[37], ~1% of PDACs arise in association with *MLH1*, *MSH2* or *MSH6* (either syndromic or sporadic) or somatic *POLE* mutations for which the tumour mutational burden is at least an order of magnitude higher[38,39] leading to an exceptionally high mutational burden and sensitivity to immune checkpoint inhibitor therapy[39,40].

The non-coding genome of PDAC has fewer cancer driver events than the coding genome, although some shared features of importance have been noted[24,41]. In one of the few studies of the non-coding PDAC genome, recurrent mutations in *cis*-regulatory promoter regions were substantially associated with alterations in gene expression related to transcriptional regulation[41]. Specific genes affected by promoter region mutations, including *RUNX3*, *ROBO1*, *SLIT2* and *CTNNA2*, have previously been implicated in PDAC[8,9,42]. Several members of the WNT signalling pathway are also affected by somatic mutations in associated regulatory elements[41]. Through integration of DNA methylation and mRNA expression analysis, 96 genes have been identified as silenced by methylation in PDAC including known genes *CDKN2A*, *CDKN2B*, *BRCA1* or *MGMT* and novel PDAC candidates *ZFP82* and *PARP6* (REF.[11]). In this same study, analysis of miRNAs revealed three clusters that contained several miRNAs previously implicated in cancer and/or PDAC specifically[11,43]. Moreover, *RNF43* mutations were correlated with miRNA cluster number 2. *RNF43* inactivation has been implicated in cystic precursors to PDAC[44], suggesting a special importance of this group of miRNAs for PDACs arising by this alternative mechanism[44] (BOX 1). Long non-coding (lnc)RNAs were also evaluated revealing two clusters

with potential importance for PDAC. For example, PDACs with basal-like features versus classic features showed differential expression of the lncRNAs UCA1, HNF1A-AS1 and NORAD, with EVADR identified as the most differentially expressed lncRNA associated with the classic subtype[11].

Mutational signatures

Somatic mutations arise during every cell division over the course of an individual's lifetime[45]. Mutations appear owing to one or more types of process that range from the intrinsic error rate of otherwise high-fidelity DNA replication, exogenous or endogenous mutagens, enzymatic modifications of DNA, or defective DNA repair. Many mutational processes lead to a specific type of somatic mutation, referred to as its mutational signature[46]. For example, by assessing the prevalence of the six possible base substitutions (C>A, C>G, C>T, T>A, T>C and T>G) in the context of the flanking 5′ and 3′ bases, 49 distinct single-base substitution (SBS) signatures have been found[46]. More than 80 mutational signatures are currently recognized that span SBS, double-base substitutions, indels and clustered bases, although the molecular basis for many remain unknown[46].

Generally speaking, the most prevalent mutational signatures in PDAC are related to ageing, smoking, defective DNA repair, dysregulated APOBEC (apolipoprotein B mRNA editing enzyme-catalytic polypeptide) and reactive oxygen species (ROS)[46–48]. Some signatures have also been found for which the aetiology remains unknown, such as SBS17 (REFS[14,48]). Mutational signatures, in general, are similar between matched primary and metastatic tumours[14,47,48], whereas mutational signatures seem to differ between coding and non-coding regions. Signatures SBS8 and SBS40, both of unknown aetiology, are more prevalent in non-coding non-enhancer regions, whereas the mutational signatures of enhancer regions are similar to those of coding regions[24]. The prevalence of ageing signature SBS1, largely characterized by deamination of 5-methylcytosine to thymine, is logical considering the median age of people with PDAC is in the seventh decade[1]. The ageing signature SBS5 is also frequent in PDAC although the mechanisms underlying this process are less clear as this signature is less well correlated with clock-like accumulation of mutations (which occurs at a constant rate and is therefore age-related)[46]. Signatures associated with smoking such as double base substitution 2 (DSB2) and small insertions/deletions 3 (ID3) are consistent with abundant epidemiological data indicating that smoking is a risk factor for pancreatic carcinogenesis[49,50]. DSB2 is characterized by doublet mutations most often affecting guanine pairs, a reflection of the strand bias of this mutational process, whereas ID3 is associated with 1-bp insertions or deletions occurring at short homopolymers[46].

Multiple forms of defective DNA damage repair also contribute to the PDAC genome landscape. SBS3 and ID6 are markers of defective homologous recombination repair of double-strand breaks; these signatures might be seen in association with germline and somatic inactivation of *BRCA1*, *BRCA2* or *PALB2* as well as in patients whose tumour exhibits favourable responses to platinum therapies (as platinum therapies are known to be effective in tumours with DNA repair gene aberrations)[12]. SBS3 is characterized by C to A, C to G, C to T, T to A, T to C, and T to G mutations in relatively equal abundance and ID6 is characterized by small deletions of >5 bp with extended stretches of overlapping microhomology at breakpoint junctions[46]. Defects in non-homologous end joining in PDAC are reflected by signature ID6 as well as ID8; this latter signature is not well characterized but shows features of clock-like accumulation[46]. Defective DNA mismatch repair consistently appears as signature SBS6 together with ID1 and ID2. This signature accounts for relatively fewer PDACs than the other DNA damage signatures, possibly reflecting the lower prevalence of PDACs with microsatellite instability phenotypes[51].

Mutations due to dysregulation of the cytidine deaminase family of APOBEC enzymes, reflected by SBS2 and SBS13, have been reported in several PDAC genome analyses[46–48]. APOBEC enzymes catalyse the deamination of cytosine to uracil with a preference for a TpC sequence context in experimental systems[52–54]. Mutations associated with SBS2 and SBS13 also show a high degree of strand coordination. They arise on the same parental allele and are on the same DNA strand, although SBS2 has a higher prevalence of transitions whereas SBS13 has a higher prevalence of transversion mutations[52].

Finally, a subset of PDACs also show mutations presumed to accumulate owing to ROS generation, detected as SBS18 (REF.[55]). Free radical species such as ROS or nitrogen oxide species are generated endogenously as by-products of normal cellular metabolism, including apoptosis and the inflammatory response, where they can cause >25 different forms of oxidative DNA lesions[56]. To date, the extent to which specific signatures are associated

Box 1 | Pancreatic cancer precursors

The most common precursor from which pancreatic ductal adenocarcinoma (PDAC) arises is pancreatic intraepithelial neoplasia (PanIN). PanINs are histologically classified into low-grade (LG PanIN) and high-grade (HG PanIN) according to the degree of dysplasia[178]. LG PanINs are more common than HG PanINs[163], probably because the latter rapidly transition to invasive cancer[83]. Evidence also indicates that HG PanINs are capable of migrating through the pancreatic ductal system, consistent with 3D visualizations of human pancreata that show discontinuity of HG PanINs[84,157]. Moreover, invasive carcinomas commonly colonize the ductal system and simulate HG PanINs, and hence true HG PanINs are exceedingly rare[83,84]. The histological features of LG PanINs, formerly referred to as PanIN1 and PanIN2, generally correlate with their genetic features[178,179]. *KRAS* mutations are associated with the transition of cuboidal ductal epithelium to columnar morphology with intracytoplasmic mucin[180]. The acquisition of *CDKN2A* alterations correlates with *KRAS* and is associated with nuclear enlargement, loss of polarity and mitotic figures akin to PanIN2 (REF.[179]). *TP53* alterations occur late during carcinogenesis and are associated with features of carcinoma in situ. They also probably herald the onset of invasion, as only 17% of patients with incidental HG PanIN in the absence of an invasive PDAC have *TP53* alterations[83].

Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cyst neoplasms (MCNs) are also recognized precursor lesions of invasive pancreatic cancer[181]. Whereas IPMNs are derived from the main pancreatic duct and/or its side branches, MCNs do not associate with the pancreatic duct system. Histologically, both IPMNs and MCNs are categorized into low-grade and high-grade on the basis of cytoarchitectural atypia, which is similar to PanIN classification[178]. *GNAS* mutations are exclusively found in IPMNs, whereas *RNF43*-inactivating mutations might be found in both IPMNs and MCNs[44]. Hence, the finding of a *GNAS* or *RNF43* mutation in an infiltrating PDAC suggests it arose from a cystic precursor.

with each form of DNA lesion is unknown, although SBS18 indicates general defective DNA base excision repair similar to that of *MUTYH* defects (*MUTYH* encodes a DNA glycosylase involved in oxidative DNA repair; germline mutations in *MUTYH* are associated with hereditable predisposition to colorectal polyposis and colon cancer, termed MUTYH-associated polyposis (MAP))[46,57,58].

Copy number and structural alterations

Next-generation sequencing methodologies have greatly enhanced understanding of alterations at the chromosome level[13,59]. One prevalent mechanism by which the PDAC genome is rendered complex is polyploidy, or whole-genome duplication[59]. Whole-genome duplication has been identified in ~20% of PDACs according to analysis of targeted sequencing data generated from bulk tissues[59], and in 45% of microdissected PDACs that underwent whole-genome sequencing[13]. In this latter example both the use of microdissected material and the development of computational methods for specific analysis of ploidy probably account for the higher prevalence reported. In both studies whole-genome duplication was associated with a higher number of copy number alterations and *TP53* somatic alterations compared with diploid tumours. Furthermore, copy number losses and gains become more prevalent and affect larger regions of DNA after whole-genome duplication, suggesting that both ongoing genomic instability and mis-segregation of chromosomes occur during the polyploidization event[13].

Although copy number alteration in PDAC might occur in isolation, these events are more commonly associated with other structural alterations in which part of one chromosome is translocated to another. One such common mechanism by which this occurs in PDAC is chromothripsis, a phenomenon in which multiple structural alterations occur in a single catastrophic mitotic event[13,60]. Highly sensitive methods of detection of chromothripsis indicate that it can be found in as many as 65% of PDACs, in many instances preceding polyploidization[13]. Chromothripsis might occur in isolation or in association with other complex genomic events and involving multiple chromosomes, in either instance leading to gene amplifications, formation of double minutes (that is, small fragments of extrachromosomal DNA) or deletions[13,61]. Alternatively, ongoing genomic damage due to DNA repair deficiency might also lead to structural rearrangements[12]. A survey of structural rearrangements in PDAC indicates that most (>80%) are intra-chromosomal. Of these intra-chromosomal events, the majority are rearrangements (58%), followed by fold-back or amplified inversions (25%) and deletions (13%). Inter-chromosomal translocations are less prevalent (<20%) and duplications of large genomic regions (including tandem duplications) are relatively uncommon, accounting for ~3% of events[12].

The genomic landscape

Cancer is a Darwinian process by which mutations occur randomly and provide the fuel upon which selection pressures act[62]. Thus, the genes somatically altered at high frequency in PDAC indicate the cellular pathways whose dysregulation is selected for a survival advantage during pancreatic carcinogenesis[7]. Although specific high-frequency targets are described separately below, these genetic events occur through a combination of stepwise accumulation and punctuated events in which two or more tumour suppressor gene alleles might be lost in a single event[13,63]. The genes most often somatically altered in PDAC are summarized in FIG. 1.

High-frequency somatic genetic alterations

KRAS mutations. *KRAS* activation is among the earliest genetic events known in PDAC, in which it signifies the transition from a normal centroacinar or ductal cell to an initiated cell[64] (FIG. 1a, FIG. 2). KRAS is a 21 kDa small GTPase that activates MAPK–ERK signalling, thus controlling cellular processes relating to proliferation, differentiation, migration and survival[65]. *KRAS* mutations are the most common oncogenic alteration in PDAC, occurring in ~90% of cases[8–14]. Given the overwhelming evidence supporting the role of chronic pancreatitis in pancreatic carcinogenesis[66,67], hyperactivity of MAPK–ERK signalling seems a requisite to maintain survival of a cell within the inflamed microenvironment. *KRAS* mutations have been shown to increase cellular fitness by protecting against inflammation-associated senescence and promoting autophagy, micropinocytosis and stress granule formation[17,68–70]. In human tissues *KRAS* mutations are found in low-grade pancreatic intraepithelial neoplasia (PanIN), and some PanINs might be associated with acinar to ductal metaplasia[71,72] (BOX 1).

Virtually all *KRAS* mutations in PDAC are SNVs occurring in codons 12 (~91%), 13 (~2%) and 61 (~7%)[8–14]. Subsequent to these activating mutations, allelic imbalance in association with whole-genome duplication and tumour progression might occur, further increasing the dosage of the mutant *KRAS* allele[48,73]. In some patients more than one mechanism of *KRAS* allelic imbalance is seen within different metastatic sites, indicating convergence on a net gain in MAPK–ERK signalling[48]. This finding has been independently observed in mouse models of PDAC[71]; furthermore, in the same study compelling evidence was shown for *Kras* allelic imbalance occurring in the setting of *Cdkn2a* homozygous deletion, whereas heterozygous loss of *Cdkn2a* was associated with alternative oncogenic events affecting *Kras*[74]. These findings indicate that PDACs undergo distinct evolutionary routes to increase *Kras* dosage depending on the mechanism of inactivation of *Cdkn2a*.

About 10% of PDACs do not have an activating mutation in *KRAS*[11]. These cases are notable for mutations or copy number alterations in alternative drivers such as activating mutations or amplifications of *BRAF*, *FGFR1* or *ERBB2*, inactivating mutations in *NF1*, *DUSP6* or *SPRED1* (REFS[10,11]) or fusions involving *NRG1* and *NTRK1* (REFS[23,75,76]), further underscoring that MAPK–ERK hyperactivity is ultimately the phenotype selected for. Wild-type *KRAS* PDACs also seem to be enriched in patients with germline mutations in known familial risk genes[11].

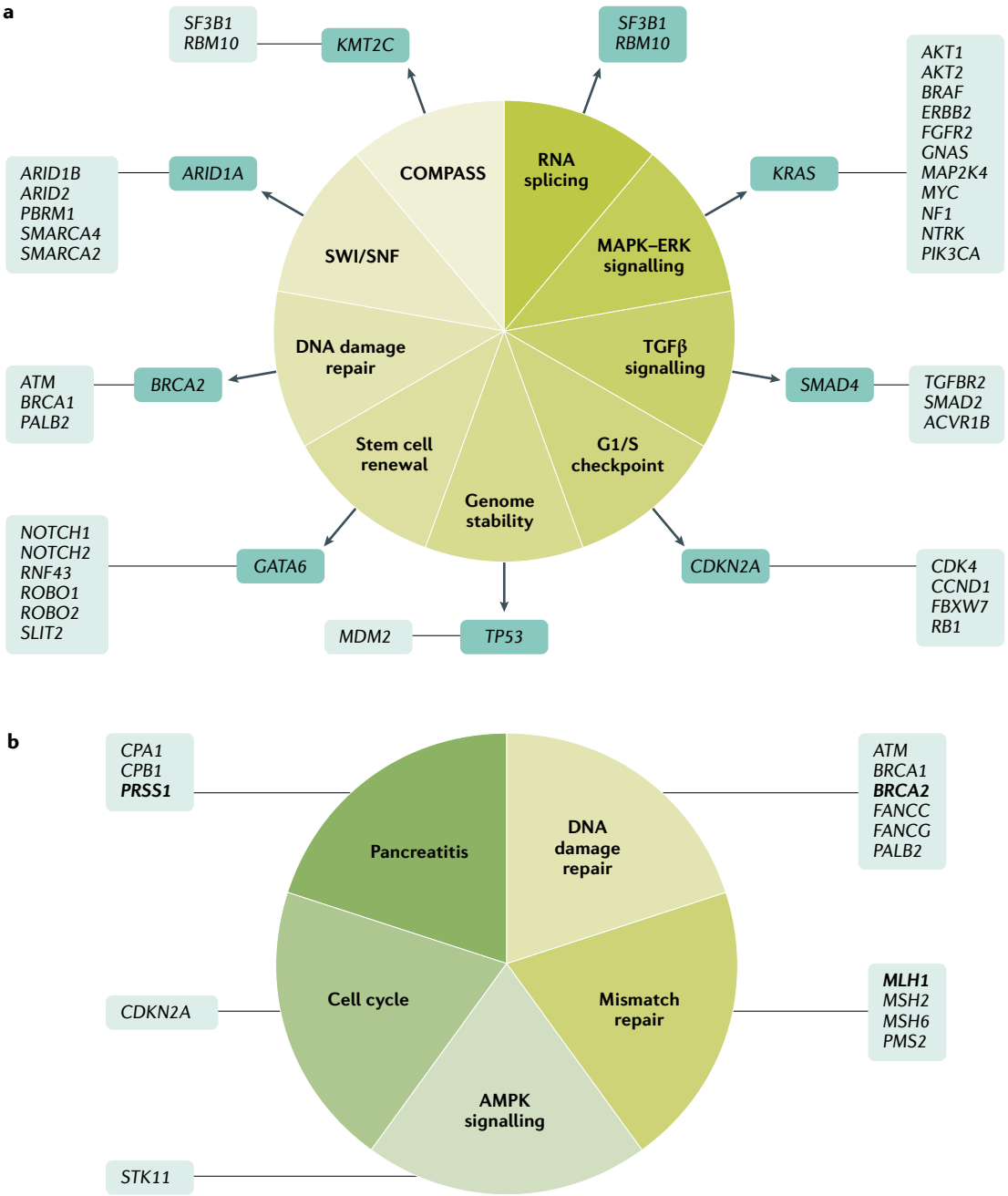

Fig. 1 | **The most commonly altered driver genes in pancreatic cancer organized by molecular function. a** | Somatic alterations. The genes most often targeted for each pathway are in the darker green boxes. Less-common targets are shown in the accompanying boxes in alphabetical order. **b** | Germline alterations. Categories with multiple genes are listed alphabetically with the most commonly affected genes in bold.

CDKN2A *inactivation.* *CDKN2A* is a tumour suppressor gene whose protein product controls the G1/S checkpoint. *CDKN2A* encodes two proteins: the INK4 family member p16 (or p16INK4a) and p14ARF[77]. Whereas p16 arrests the cell cycle in the G1 phase by inhibiting binding of CDK4 or CDK6 with cyclin D1, p14ARF initiates p53-dependent cell cycle arrest. The mutational profile of *CDKN2A* or *CDKN2B* indicates that p16 is the most likely target, as a substantial proportion of mutations do not affect the p14ARF coding region[78]. Inactivation of *CDKN2A* is found in 90% of PDACs by multiple mechanisms, each occurring in approximately equal proportions: homozygous deletion, mutation coupled with loss of the wild-type allele, or hypermethylation[11,12,72]. In PDACs in which *CDKN2A* is not inactivated, alternative mechanisms of inhibiting the G1/S checkpoint — including *RB1* inactivation by somatic mutation or hypermethylation, CDK4 amplification or CCND1 amplification — have been identified, indicating convergence on and selection for loss of the G1/S checkpoint[10,79]. *CDKN2A* inactivation is most often found in association with *KRAS* mutations[72,80];

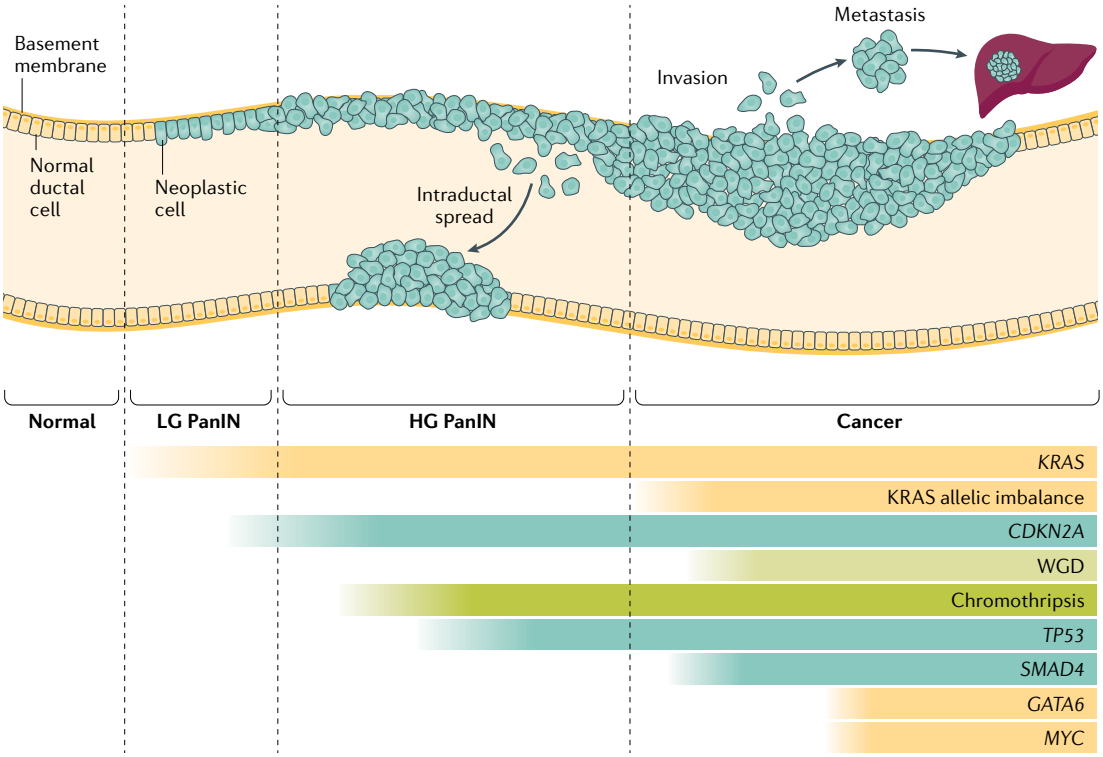

Fig. 2 | **Revised genetic progression model of pancreatic cancer of the most commonly altered genes.** *KRAS* and *CDKN2A* mutations are characteristic of low-grade pancreatic intraepithelial neoplasia (LG PanIN), whereas *TP53* alterations typify high-grade precursors (HG PanIN) and the presence of a lethal neoplasm. *SMAD4* alterations are selected for at the moment of invasion, or shortly thereafter. Metastasis is a relatively late event in the evolutionary history of pancreatic cancer and commonly contains the same driver gene alterations present in the primary tumour. The timing of *GATA6* and *MYC* amplification is less well understood but probably occurs in association with chromothripsis or with whole-genome duplication (WGD) that is correlated with *TP53* alterations.

as a consequence, cellular fitness and tumour cell proliferation increases fivefold or more[81,82].

TP53 *and* SMAD4 *inactivation.* Unlike *KRAS* and *CDKN2A*, which are virtually always targeted during carcinogenesis and before invasion into the pancreatic parenchyma, *TP53* and *SMAD4* inactivation are relatively late events that, when identified, signify the presence of a neoplasm with lethal potential[83,84]. *TP53* is a tumour suppressor gene whose protein product serves as a major guardian of genome integrity by modulating transcription, DNA repair, genomic stability, cell cycle control and apoptosis[85]. Alterations of *TP53* in cancer occur in 80% of PDACs, the majority of which are missense mutations in association with allelic loss that confer gain of function via altered DNA binding and interactions with other transcription factors[80,85]. Consequences of these gain-of-function mutations include cell cycle activation, loss of apoptosis regulation and metabolic changes[85]; these phenotypes are most likely selected for to maintain survival in association with an increasingly unstable genome during clonal expansion within the pancreatic ducts[7]. A subset of PDACs exhibit *TP53* loss-of-function mutations via truncating mutations or homozygous deletion[8–12,80]. Although these mutations also confer a lethal phenotype, the specific mechanisms by which they promote lethal PDAC have been relatively less explored[86,87]. *TP53* alterations in PDAC are also highly correlated with polyploidization[13,59] and entosis (a specialized form of epithelial cell death in which a viable cell is engulfed by another)[88,89]. *TP53* alterations do not seem to specifically cause whole-genome duplication, but might allow cell survival in the presence of a genome doubling event[90].

SMAD4, also a tumour suppressor, is a mediator of the canonical TGFβ signalling pathway that controls tissue homeostasis within the pancreatic epithelium and other tissue types[91]. Inactivation of *SMAD4* occurs in just over 50% of resected PDACs by homozygous deletion or somatic alteration with loss of the wild-type allele[92]. About 10% of PDACs have inactivating *TGFBR2* mutations or deletions that are mutually exclusive with SMAD4 loss[8–12]. Inactivating mutations of *ACVR1B* also occur in a very small number of PDACs[10,93–95]; a comparison of the transcriptomes of TGFBR2-activated and ACVR1B-activated cells has shown that they are similar and induce CDKN1A/p21 expression[96]. Loss of SMAD4 promotes growth by loss of intracellular canonical TGFβ pathway signalling leading to increased migratory behaviour, immune evasion and autocrine activation[97]. *SMAD4* inactivation specifically coincides with the moment of invasion, indicating that it is selected for to maintain cell survival and fitness upon exposure of the neoplastic cells to the stromal microenvironment[98].

Considering that the presence or absence of SMAD4 expression also correlates with the mode of invasion — that is collective or mesenchymal, respectively[98] — we posit that specific features of the stromal microenvironment that are yet uncharacterized act as a potent selective pressure that favours neoplastic cells with SMAD4 loss. The occasional observation of subclonal loss of SMAD4 expression also favours this interpretation[80].

Low-frequency somatic genetic alterations

Several genes are recurrently altered in PDAC but at frequencies that do not exceed 10% of analysed cases in large-scale studies. Although many of these genetic targets have been known for some time, emerging data indicate some might serve as biomarkers of PDAC subtypes with differing therapeutic susceptibilities[13,22,99,100].

SWI/SNF and COMPASS complexes. Inactivating nonsense, frameshift or splice site mutations in chromatin modifier genes are present in up to 10% of PDACs[8–11], indicating that epigenetic dysregulation is selected for by mutational processes. Two chromatin remodelling complexes are affected by inactivating mutations in PDAC, the Switch/Sucrose-Nonfermentable (SWI/SNF) complex and the COMPASS complex[101,102]. Genes targeted within the SWI/SNF complex include *ARID1A* and *ARID1B*, both of which are components of the BAF subunit, or *ARID2* and *PBRM1*, both of which are components of the PBAF subunit. *SMARCA2* and *SMARCA4*, which are present within both the BAF and PBAF subunits, might also be affected in a minority of PDACs[8–11]. *ARID1A* is genetically inactivated at a slightly higher rate (~6% of PDACs) than all other genes in the SWI/SNF complex, indicating a more general importance for disruption of the BAF subunit[11]. Furthermore, mutations in any one SWI/SNF gene are mutually exclusive of each other, indicating convergence for loss of function of this complex[103]. In normal cells the SWI/SNF complex contains DNA-stimulated ATPase activity and utilizes the energy provided by ATP hydrolysis to remodel chromatin through nucleosome sliding and removal[104]. These distinct SWI/SNF family complexes bind to a multitude of genomic loci, including distal enhancers, promoters and CCCTC-binding factor binding sites at which they facilitate and maintain DNA accessibility to regulate gene transcription[105]. Although SWI/SNF is often associated with promoting gene activation it can also target to, and position, nucleosomes to enable the binding of repressive transcription factors or to establish a repressive chromatin state[105].

Genes targeted within the COMPASS complex include the histone H3 lysine 4 (H3K4) methyltransferases *KMT2C* and *KMT2D* and the complex-related gene *KDM6A*, which acts as a H3 lysine 27 (H3K27) lysine demethylase[102,106]. *KMT2C* and *KMT2D* are the only lysine methyltransferases within the COMPASS complex that associate with *KDM6A*; as described for SWI/SNF, mutations in any one of these three genes are mutually exclusive of each other[12,22]. These proteins serve as major regulators of monomethylation at enhancer regions, indicating convergence for loss of function of the COMPASS complex.

Genetic inactivation of genes in the SWI/SNF or COMPASS complexes is correlated with the development of basal-like transcriptional features, particularly if this event occurs early in the evolutionary life history of the neoplasm[22]. Collectively, mutually exclusive inactivation of genes involved in either the SWI/SNF or COMPASS complexes implicates attenuation of enhancer activity as an important factor in the development of basal-like transcriptional features and worse outcome[22,102,107,108]. TP63-mediated enhancer reprogramming has been shown to drive the development of basal-like features[107–110], and indeed TP63 expression is highly sensitive and specific for basal-like features in PDAC tissues[22].

GNAS. *GNAS* encodes the α-subunit portion of a G-signalling complex found in multiple tissue types, including bone, skin and endocrine tissues[111]. Spontaneous missense mutations in codons 201 or 227 of *GNAS* form the genetic basis of McCune–Albright syndrome, which is characterized by one or more endocrinopathies[112]. At the cellular level, these mutations lead to a constitutively active α-subunit, increased levels of intracellular cAMP, and promotion of the actions of downstream hormones[112]. With respect to pancreatic neoplasms, *GNAS* mutations were originally identified in intraductal papillary mucinous neoplasms (IPMNs), a variant precursor of invasive PDAC (BOX 1), in which they seem to be among the earliest events in cystogenesis[72,113]. In large-scale sequencing studies of PDAC, somatic mutations in *GNAS* at codon 201 are identified in up to 10% of cases[11]. Inactivating mutations in *RNF43* are also found at low frequency in invasive PDACs (<10%)[11]. *RNF43* is an E3 ubiquitin ligase with intrinsic activity that has a role in modulating WNT signalling by promoting the degradation of WNT receptors[114,115]. Because of the high specificity of *GNAS* or *RNF43* mutations for cystic neoplasms, the presence of *GNAS* and/or *RNF43* mutations in an invasive cancer arising in the pancreas highly suggests that it arose from this alternative pathway of carcinogenesis[116,117].

GATA6 *and* MYC. *GATA6* and *MYC* are both targets of gene amplification in PDAC in association with whole-genome duplication or chromothripsis[13,21,22]. GATA6 is a transcription factor that contributes to the normal development of mesodermal and endodermal tissues including the pancreas[118,119]. In human PDAC cells and tissues *GATA6* amplification, or its transcriptional upregulation, occurs late during carcinogenesis and activates canonical WNT signalling[120]. *GATA6* amplification or overexpression correlates with the classic transcriptional phenotype as well as with improved overall survival[20,21,99,100]. *GATA6* amplification is also correlated with *SMAD4* deletion, in part because they are in close proximity to each other on chromosome 18, suggesting they are dually targeted during chromothripsis events[21]. In some PDACs *GATA6* might instead undergo hypermethylation-mediated silencing[11]. Loss of GATA6 expression is correlated with basal-like features of PDAC and with poor outcome[20,21].

MYC is part of a family of transcription factors that form a network to regulate metabolism and cell

proliferation and induce expression of genes required for these processes[121]. Unlike GATA6, which is subject to either gain or loss of expression, MYC is usually only upregulated by gene amplification[8–14]. *MYC* amplification promotes cancer progression though altered metabolic pathways, survival signals in the setting of hypoxia, and promotion of cell competition[121,122]. *MYC* amplification is inversely correlated with *GATA6* amplification, basal-like features of PDAC and poor outcome[22,99,100].

ROBO/SLIT *family.* Mutations in the *ROBO/SLIT* family of genes[9], or their regulatory elements[41], have been described in a minority of PDACs. Although the frequency of PDACs with mutations in this gene family are low, they are consistently identified in large-scale unbiased sequencing studies[8–11]. To date, the functional consequence of these mutations for this tumour type remains unknown. In breast and intestinal cancers these genes have a role in regulation of WNT signalling and self-renewal[123,124], thus these mechanisms might be similarly affected in PDAC.

SF3B1 *and* RBM10. Mutations in genes whose protein products have a role in mRNA splicing are also recurrently identified in PDAC. The most notable genes identified include *SF3B1* and *RBM10* (REFS[9,10,12,48]). *SF3B1* mutations are prototypically heterozygous missense mutations; the presence of hotspots and the absence of nonsense or frameshift mutations in *SF3B1* suggest that these mutations are likely to result in a gain or change-of-function of the protein product. *SF3B1* mutations have been shown to disrupt interactions of SF3B1 with SUGP1, leading to aberrant use of cryptic splice sites during RNA splicing[125]. By contrast, inactivating nonsense or frameshift mutations are common for *RBM10*, indicating that loss of function is selected for. *RBM10* is a member of the RNA-binding motif gene family and is involved in pre-mRNA splicing and posttranscriptional regulation, including of *TP53* (REF.[126]).

Germline alterations

Several excellent reviews have described the genetic basis of familial PDAC[127,128]; thus we only briefly review germline alterations here. Alterations in PDAC risk genes might contribute to disease incidence by increasing the rate of PDAC initiation, but might also increase the rate of clonal expansion once initiation has occurred[129,130]. The most commonly affected genes are discussed next and summarized in FIG. 1b.

Whole-genome sequencing of >500 PDAC kindreds indicated that the genetic basis of familial PDAC is polygenic; that is, although each kindred had one or more risk alleles, the frequency of any one variant never exceeded 3% of the population studied[130]. In unselected populations of patients with apparently sporadic PDAC, germline testing has shown that ~5% of patients have a germline mutation[131,132]; this number approaches 20% of patients in regions enriched for individuals with Ashkenazi Jewish ancestry[133]. Thus, as most newly diagnosed patients with germline mutations do not have a family history of PDAC, current recommendations are that all newly diagnosed patients should undergo germline testing, particularly as a subset of these mutations are therapeutically actionable[133].

The best-characterized germline variants linked to PDAC are *BRCA1*, *BRCA2* and *PALB2*, the Fanconi anaemia genes *FANCC* and *FANCG*, and *ATM*. Mutations in *BRCA1* and *BRCA2* are the most commonly identified germline variant, accounting for up to half of all germline mutations found[130,133]. All of these gene products are components of the DNA double-strand break repair machinery[134]. Mutations in *BRCA1*, *BRCA2*, *PALB2*, *FANCC* or *FANCG* increase genomic instability by faulty homologous recombination at stalled replication forks and hence increase the rate at which somatic mutations occur, as seen by the signature SBS3 and ID6 (REF.[46]). Additional genes that increase PDAC risk when present in the germline include *CDKN2A* (FAMMM syndrome), *TP53* (Li–Fraumeni syndrome), the mismatch repair genes *MLH1*, *MSH2*, *MSH6* or *PMS2* (Lynch syndrome) and *STK11* (also known as *LKB1*) (Peutz–Jeghers syndrome)[128]. Patients with familial forms of chronic pancreatitis due to germline mutations in *PRSS1* are at extremely high risk of PDAC, probably because of the chronic duration of inflammation over the lifetime of the individual[135,136]. Germline mutations in *CPA1* and *CPB1* have also been identified, implicating endoplasmic reticulum stress in PDAC development[137].

The genome in context

More than half of all SNVs present in a cancerous epithelial cell have occurred prior to the initiating event as a result of random DNA errors that accumulate within each cell's lineage[138] (FIG. 3). Activating *KRAS* mutations, the most common initiating genetic alteration, probably also occur both randomly and commonly given that high-sensitivity methods consistently identify mutant *KRAS* in phenotypically normal pancreatic cells or in samples of chronic pancreatitis from otherwise healthy individuals[139,140]. This finding is entirely consistent with studies of other histologically normal tissues that exhibit mutations in known driver genes[141,142]. Thus, *KRAS* mutations alone are insufficient for development of PDAC. Some PDACs have no demonstrable *KRAS* mutations and activate aspects of MAPK–ERK signalling by alternative genetic events, for example *BRAF*, *PIK3CA* or *NF1* alterations (as discussed earlier)[11].

Both acute and chronic pancreatitis greatly increase the risk of developing PDAC. In mice, PDAC progression is accelerated in the presence of associated chronic pancreatitis[143], and in humans, many of the known risks factors for PDAC are commonly characterized by the induction of chronic inflammation[135,136]. The mechanisms by which pancreatitis promotes the development of PDAC are complex[144–146] but are influenced by the cell of origin[147,148], the context by which inflammation occurs[149,150] and the extent that KRAS signalling is co-opted for survival in the setting of inflammation[151,152]. Activated *KRAS* itself might then induce a feedforward mechanism that sustains survival in part by inducing a pro-neoplastic immunoenvironment or by downregulating other cellular pathways that limit formation of acinar to ductal metaplasia[146,153–155]. Thus, sustained activation of MAPK–ERK signalling increases the fitness

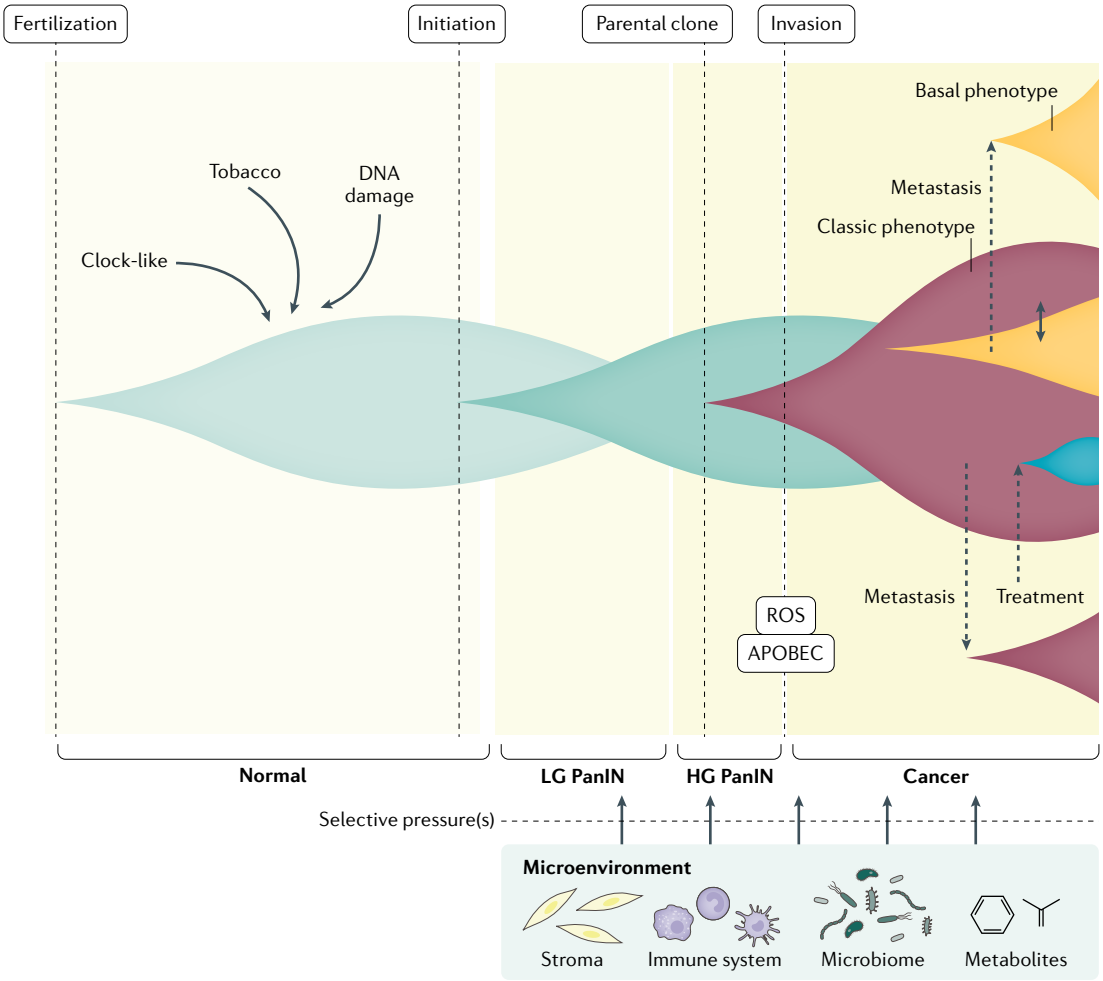

Fig. 3 | **Schematic of the clonal dynamics associated with pancreatic cancer formation and progression.** Following fertilization, a clonal lineage develops that occupies the normal pancreas (light blue). Mutational processes that contribute to accumulation of mutations in this lineage are largely clock-like but might include smoking signatures or evidence of ongoing DNA damage. Initiation of a normal cell occurs, most often by mechanisms that sustain MAPK–ERK signalling. The intraductal neoplasm, depicted in turquoise, acquires additional mutations with subsequent increases in cellular fitness and the formation of the malignant clone with lethal potential (red). Invasive cancer develops that undergoes subclonal evolution leading to intratumoural heterogeneity for genetic alterations (bright blue). Additional mutational processes further contribute to accumulation of mutations, for example, due to reactive oxygen species (ROS) or dysregulated APOBEC. Invasive cancers might have classic (red) or basal (yellow) transcriptional features with the potential to transform to the other type (indicated by the bold double arrow), leading to cellular heterogeneity for these phenotypes. Both classic and basal phenotypes have the potential to metastasize. Selective pressures that sculpt the extent of intratumoural heterogeneity before and after invasion include the desmoplastic stroma, the immune system, the microbiome and nutrient availability. Treatment further acts as a selective pressure that selects for resistance clones. Dissemination of cells to distant organs may similarly be influenced by these selective pressures, as well as pressures specific to the novel organ microenvironment. HG PanIN, high-grade pancreatic intraepithelial neoplasia; LG PanIN, low-grade pancreatic intraepithelial neoplasia.

of the cellular population to withstand the inflamed environment[68,156]. Continued accumulation of genetic damage by ageing in association with one or more superimposed mutational processes (such as smoking) increases the chance of accumulation of additional alterations, such as in *CDKN2A* leading to loss of the G1/S checkpoint and further increasing the fitness of this mutant clone[45]. Mutant clones containing both *KRAS* and *CDKN2A* alterations are histologically compatible with PanIN2, yet PanIN2 is not an obligate PDAC precursor because an autopsy series demonstrated the presence of these lesions in 28% of individuals who succumbed to unrelated causes[157]. Loss of the G1/S checkpoint probably increases the rate of DNA damage to the cells by promoting catastrophic events such as chromothripsis[13,74] and by providing the selection pressure for *TP53* alterations to limit apoptosis in the context of genotoxic stress[85]. This idea is consistent with observations of allelic loss of *TP53* and *SMAD4* in a single event in some PDACs[13]. *TP53* alterations are highly correlated with tumours with high-grade cytological features (PanIN3), increased proliferation and the ability to migrate throughout the pancreatic ductal system[84]. *TP53* alterations might also actually signify the presence of

Box 2 | **Transcriptional phenotypes of pancreatic cancer**

An important facet of pancreatic ductal adenocarcinoma (PDAC) biology that is intimately linked to its genomic features is that of molecular subtypes[182]. As mentioned for genomic analyses, the number of proposed molecular subtypes of PDAC varies according to sample sets and genomic and computational approaches[18–20]. Currently the field accepts two major subtypes: classic and basal-like[11]. Classic-type PDAC is characterized by the histological features typically attributed to this tumour type, such as poorly formed infiltrating glands, single-cell invasion, nuclear pleomorphism, and a high nuclear to cytoplasmic ratio. A hallmark of this subtype is also upregulation of the pancreatic lineage marker GATA6 by amplification or transcriptional upregulation[21,22]. By contrast, basal-like PDACs are characterized by solid or nesting patterns of growth, abundant pink cytoplasm, and a relatively low nuclear to cytoplasmic ratio[22]. In some tumours frank squamous features are seen. Genomic features associated with the development of this subtype are inactivation of *ARID1A*, *KMT2C* or *KMT2D*, allelic imbalance of *KRAS* and amplification of *MYC*[21]. This subtype can also be recognized by immunohistochemical labelling for cytokeratins 5 and 6 and nuclear expression of TP63. Phylogenetic studies suggest that histologically evident basal-like features in human PDAC tissues represent an outgrowth of a subclonal population, thus classic features might be the originating molecular subtype of this disease. By both multiregion sampling and single-cell analysis, both classic and basal-like features have been demonstrated to coexist in the same primary tumour, and classic and basal-like features can be seen in different metastases in the same patient[21,22,183]. However, an intraductal xenotransplantation model with patient-derived organoids has shown that even pure basal-like organoids can transition to a classic phenotype, underscoring the importance of cellular plasticity in general in PDAC[184]. In clinical trials, basal-like features are associated with a worse response to mFOLFIRINOX and decreased overall survival[99,185].

invasive PDAC because incidental PanIN3 without associated PDAC rarely have *TP53* mutations[83], *TP53* alterations are highly correlated with polyploidization[13,59], and polyploidization correlates with environmental stress such as when invading novel microenvironments[158,159]. In this instance, the novel microenvironment referred to is the pancreatic parenchyma beyond the basement membrane.

The pancreatic stromal response to an invasive carcinoma is an expansive topic in itself[160]. The pancreatic stroma undoubtedly contributes to pancreatic carcinogenesis via clinically evident or subclinical pancreatitis[144], and this process becomes exacerbated once invasion occurs[7]. The stromal response to epithelial injury in the normal pancreas includes fibroblast activation, immune suppression, remodelling of the extracellular matrix and trophic signals to promote re-epithelialization, all features coordinated in large part by TGFβ[161,162]. In cancer, this radically altered microenvironment forms yet another potent selection pressure that we posit is the reason for loss of SMAD4 in ~55% of PDACs[92]. Evidence in favour of this notion is that once cancerization of the ductal system is ruled out, *SMAD4* alterations have not been found in PanIN3 (REF.[83]), SMAD4 is a central mediator of TGFβ signalling[97], and *SMAD4* loss itself has been shown to be a subclonal event in some PDACs[80,163]. Thus, *TP53* inactivation might promote whole-genome duplication to increase the adaptive ability of the neoplasm in general, whereas loss of canonical TGFβ signalling, most often by SMAD4, is the mechanism by which the newly invasive PDAC adapts to the stromal microenvironment specifically.

Mutational processes continue within the infiltrating neoplasm. In addition to clock-like processes, mutations might accumulate owing to defective DNA double-strand or mismatch repair systems, DNA damage in association with ROS and dysregulation of APOBEC. Mutations due to defective double-strand break repair or DNA mismatch repair might occur in the context of inactivated DNA repair genes[12], or in association with inflammatory responses to neoantigens[47]. Whole-genome duplication, with or independent of chromothripsis probably further adds to intratumoural heterogeneity by increasing the diversity of the genome[13,59]. This diversity manifests as variable gene dosages that are adjusted for a net survival advantage in the context of the immediate microenvironment and available nutrients[74]. As a result, by the time a PDAC is clinically evident it contains demonstrable intratumoural heterogeneity characterized by subclones that differ with respect to somatic alterations, allelic gains or losses and gene deletions or amplifications[48] (BOX 2). In some patients with resectable PDAC these subclones contain mutations or amplifications of known driver genes that are associated with disease recurrence after adjuvant therapy[48]. By contrast, sequencing studies of treatment-naive stage IV PDAC failed to identify any genetic heterogeneity, indicating these two patient cohorts differ by at least one major clonal expansion[164]. Ultimately, despite the complexity of a primary PDAC genome, focused studies of the genomic alterations associated with metastasis indicate that metastatic efficiency is largely established by the driver genes that accumulate during carcinogenesis[14,82,165], after which epigenomic and metabolic perturbations have a greater role[166,167].

Therapeutic vulnerabilities of the genome

PDAC has traditionally been considered an undruggable neoplasm[16]. However, large-scale studies of the PDAC genome have increasingly revealed features that render PDAC therapeutically susceptible. Genotyping of a patient's tumour for prospective clinical management has become routine clinical practice owing to the finding that 5–20% of unselected patients have a germline alteration in a PDAC predisposition gene[133]. Moreover, the Know Your Tumour retrospective study found that patients who received a therapy that matched their genetic profile had improved outcomes compared with those who did not[168], although this finding largely reflects patients with tumours with *BRCA2* mutations and microsatellite instability.

Up to 25% of PDACs have actionable molecular alterations, defined as a molecular alteration for which there is clinical or strong preclinical evidence of a predictive benefit from a specific therapy[168]. The most prevalent genetic alterations that are considered actionable include those with a germline or somatic mutation in a DNA double-strand break repair gene, such as *BRCA1*, *BRCA2* or *PALB2*; these PDACs are more responsive to cisplatin than PDACs that are wild-type for these genes[169]. Moreover, patients with metastatic PDAC with germline *BRCA* mutations who received the PARP inhibitor olaparib in the maintenance setting exhibited longer progression-free survival than patients who received placebo[170]. Inactivating mutations in mismatch repair genes, seen in ~1% of PDACs[39], lead to a dramatically increased mutational burden that renders these

neoplasms sensitive to immune checkpoint inhibitors such as pembrolizumab[40]. Wild-type *KRAS* tumours, which account for 5–10% of PDACs, also represent opportunities for immediate intervention, particularly in those patients whose tumours have *NTRK* fusions that confer susceptibility to larotrectinib[23] or *NRG1* fusions that show responses to afatinib[75,76]. Although *KRAS* mutations are undruggable, the rare *KRAS* variant G12C shows promise for therapeutic targeting[171]. This mutation results in a predominantly GTP-bound KRAS oncoprotein containing the aberrant cysteine next to the P2 pocket of the switch II region. The P2 pocket is present only in the inactive GDP-bound conformation of KRAS and has been exploited to establish covalent inhibitors of $KRAS^{G12C}$ (REFS[172,173]); early data from patients with PDAC with this rare mutation demonstrate responsiveness[171].

Examples of genetic alterations with potential but yet unproven benefit include *ERBB2–HER2* amplification in 2% of PDACs[174], suggesting a potential role for the many targeted therapies to this protein. However, at this time no defined guidelines exist for management of PDAC with *HER2* amplification, and the lack of benefit in $HER2^{+}$ gastric cancer indicates that tumour type-specific factors might need to be taken into consideration[175]. Methylthioadenosine phosphorylase co-deletion with *CDKN2A* or *CDKN2B* on chromosome 9p represents another potential therapeutic strategy by virtue of its potential synthetic lethality with methionine adenosyltransferase IIα (MAT2A) and the arginine methyltransferase, PRMT5 (REFS[176,177]).

Conclusions

Laboratory and clinical research over the past decade has contributed substantially to our understanding of the molecular pathways altered in pancreatic malignancies. Next-generation sequencing modalities have revealed the genomic features of this disease, some of which are actionable and hence indicative of therapeutic vulnerabilities. Thus, the field has entered the era of personalized approaches for the management of PDAC, with promise for halting the rising rate of deaths from PDAC.

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Author contributions

C.A.I.-D. and A.H. contributed to researching data for the article, made a substantial contribution to discussion of content, and wrote and reviewed/edited the manuscript before submission. J.H. wrote and reviewed/edited the manuscript before submission.

Competing interests

The authors declare no competing interests.

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