Pancreatic cancer driver mutations are targetable through distant alternative RNA splicing dependencies

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC), the most common histological subtype of pancreatic cancer, has one of the highest case fatality rates of all known solid malignancies. Over the past decade, several landmark studies have established mutations in KRAS and TP53 as the predominant drivers of PDAC pathogenesis and therapeutic resistance, though treatment options for PDACs and other tumors with these mutations remain extremely limited. Hampered by late tumor discovery and diagnosis, clinicians are often faced with using aggressive and non-specific chemotherapies to treat advanced disease. Clinically meaningful responses to targeted therapy are often limited to the minority of patients with susceptible PDACs, and immunotherapies have routinely encountered roadblocks in effective activation of tumor-infiltrating immune cells. Alternative RNA splicing (ARS) has recently gained traction in the PDAC literature as a field from which we may better understand and treat complex mechanisms of PDAC initiation, progression, and therapeutic resistance. Here, we review PDAC pathogenesis as it relates to fundamental ARS biology, with an extension to implications for PDAC patient clinical management.

INTRODUCTION

PDAC epidemiology and treatment

Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90% of all tumors of the pancreas, while the remaining 10% is comprised of predominantly pancreatic neuroendocrine tumors [1]. According to the most recent Surveillance, Epidemiology, and End Results (SEER) Program data, pancreatic cancer remains one of the deadliest solid malignancies in the United States, with a five-year survival of approximately 10% [2]. Routine screening is not practiced for early detection of pancreatic tumors, although high-risk patients with familial pancreatic cancer or known germline cancer-predisposing syndromes, accounting for 5–10% of all pancreatic cancer patients, may benefit from pancreatic screening and germline mutation testing [3–5].

Current therapy for PDAC patients includes surgical resection with adjuvant chemoradiation, increasing 5-year patient survival to approximately 20%, by some estimates [6, 7]. For over 80% of patients, however, PDAC is diagnosed as either borderline resectable, locally advanced, or metastatic disease, limiting eligibility for surgery [8–11]. Patients receiving systemic medical therapy either independent of surgery or in the adjuvant setting see significant yet minimal improvements in survival, though treatment options are often limited by patient tolerance [12–14]. While some PDACs harboring susceptibility-conferring mutations (e.g., BRCA1/2, ATM) are treatable via targeted medical approaches, such as poly (ADP-ribose) polymerase inhibition for PDACs with
DNA repair gene mutations, these constitute a minority of all PDACs [15–17]. Even the most successful systemic medical treatments, gemcitabine plus nab-paclitaxel or FOLFIRINOX (a combination of 5-fluorouracil, leucovorin, irinotecan, and oxaliplatin), have demonstrated only a modest improvement in median patient survival of about 2-4 months beyond the gemcitabine control arm median survival of about 6 months [18–20]. Nevertheless, there is strong evidence suggesting a role for neoadjuvant systemic therapy to improve resectability of borderline resectable lesions [9, 21, 22].

PDAC driver mutations and non-mutational driver-phenocopying mechanisms

Non-hereditary PDAC, accounting for about 90% of cases, is predominantly characterized by a well-established progression of mutational burden beginning with activating point mutations in the KRAS gene (about 90% of PDACs) [23–25]. Mutations in KRAS are often accompanied by secondary mutations, most commonly in tumor protein p53 (TP53, > 60% of cases), cyclin-dependent kinase inhibitor 2A (CDKN2A), and mothers against decapentaplegic homolog 4 (SMAD4) genes, conferring unique advantages to PDACs in therapy resistance and tumor aggression [26, 27]. Recent evidence via novel small molecule intervention and genetic ablation has shown that loss of oncogenic KRAS function, for example, is prone to initial tumor volume loss followed by tumor regrowth, either as a consequence of cancer cell heterogeneity in KRAS dependency or the presence of highly KRAS-dependent cells harboring the ability to undergo a stress-induced clonal escape mechanism mediated through advantageous functional alterations [28–32]. Epigenetic, metabolic, and immuno-modulatory processes have all been implicated in drug resistance and tumor maintenance in KRAS-mutant PDACs [33–37]. This suggests that even the most potent anti-KRAS targeted therapies are susceptible to mechanisms of therapy resistance.

Over the past decade, a wealth of information on PDAC RNA expression has contributed to a rapidly advancing understanding of the mechanisms by which these tumor cells may harbor treatable characteristics, either dependent or independent of tumor mutational status. Many studies of human tumor samples have led to a growing consensus on a two-subtype transcriptomic disease model described by the ‘Basal-like’ and ‘Classical’ gene signatures, which have been shown to correlate well with systemic therapy response, tumor aggression, and patient survival [38–46]. Work is ongoing to describe genetic characteristics of the PDAC stromal compartment, though early studies have shown a strong relationship between disease severity and stromal cell gene expression [39, 47, 48]. Even after subtyping PDACs by gene expression and mutational status, there still exists substantial variety in tumor therapy response and cellular characteristics in the preclinical setting [43, 44, 49], as recently reviewed in Du et al. [50]. Thus, there are likely other mechanisms, either epigenetic or otherwise hidden in summary gene expression data, by which tumors are initiated, maintained, and able to evade therapy that must be uncovered to effectively treat PDACs from several dependency-inspired angles.

Alternative RNA splicing in normal and cancer cells

Alternative RNA splicing biology/functions

As mentioned above, recent literature has focused on using gene expression data to characterize PDACs, such as for determination of tumor transcriptomic subtypes, evaluation of potential disease biomarkers, and discovery of novel targetable disease mechanisms. Concurrently, substantial work has been conducted to describe the epigenetic, proteomic, and broadly metabolic characteristics of the disease. Nevertheless, there exists a comparable lack of investigation into alternative RNA splicing (ARS), an extremely plastic genetic control mechanism by which cells monitor and respond to stress, regulate gene expression, and influence intra- and inter-cell communication [51–57].

Alternative RNA splicing is a choreography by which RNA is processed to expand the protein diversity of eukaryotic organisms, via activity of the spliceosome, an RNA-protein complex. Exons, introns, and other noncoding RNA segments are recognized by RNA binding proteins (RBPs, including SR proteins and hnRNPs, among others) at conserved cis-regulatory RNA sequences to promote or suppress – in a summative fashion – RNA segment retention in the final mRNA product [58–60]. Estimates suggest that most genes with multiple exons undergo ARS to produce multiple distinct protein isoforms [61], and significant variation exists across tissue types as to the predominance of a given isoform, likely due to tissue-specific RBP expression and conditional RBP activity [62].

Several studies have posited that most alternative splicing events seen in next generation sequencing studies result from noisy aberrant splicing, leading to non-functional protein products [63–65]. Nevertheless, there is a growing basis of evidence for this noise and also for regulated diversification of isoform expression specifically contributing to organism and tissue development, normal cellular physiology, and pathology that might provide insight into cancer pathophysiology and therapy development [66, 67]. An active field of study is centered on the development of novel programmatic methods for assessing expression of annotated or novel protein isoforms using RNA sequencing platforms, though many investigators have also found success in home-brewed
pipelines for analysis of this data [68–72]. This, along with the development of reliable long-read RNA sequencing technologies to enable precise quantification of transcripts at the whole mRNA level as well as continually advancing paired-end RNA sequencing at the single cell level, positions studies of ARS at a uniquely opportune time to uncover meaningful biochemical knowledge necessary for biomedical advancement.

**ARS mechanisms in cancer pathogenesis**

ARS has been implicated in the initiation and maintenance of solid and non-solid malignancies [73–75]. There are several ways that ARS may be modified in cancer, including mutations in cis-regulatory RNA sequences, splicing protein post-translational modifications, and alterations in splicing protein primary sequences as well as expression levels, as reviewed in Escobar-Hoyos et al. [76]. While recent studies have demonstrated that RBPs known to interact with the spliceosome and globally alter ARS are mutated at a low rate in PDACs and two of the most common PDAC precursor lesions, pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasm [77, 78], mechanistic understanding of their role in pathogenesis of PDACs and other solid tumors is extremely limited. Furthermore, there is a notable dearth of knowledge on the splicing-regulatory roles of the most commonly mutated genes in PDAC, TP53 and KRAS.

To address this lack of knowledge, we recently uncovered a novel mechanism by which PDACs with KRAS and TP53 mutations (a combination found in most PDACs) promote cancer pathogenesis via modified splicing of GTPase-activating protein (GAP) mRNAs and subsequent amplification of KRAS signaling [32]. Increased hnRNPK expression downstream from mutant p53 leads to increased retention of cytosine-rich exons in GAP mRNAs, thus leading to dysfunctional GAPs that are limited in their ability to transition KRAS from its active GTP-bound state to its inactive GDP-bound state. In the study, we show that KRAS- and TP53-mutant PDACs are selectively susceptible to spliceosome inhibition using H3B-8800 (an inhibitor of the SF3B complex, critical in spliceosome function and currently in phase I clinical trials) [79, 80], induction of hnRNPK functional loss, and correction of the cytosine-rich GAP splicing alterations using targeted oligonucleotide delivery. Thus, through this oncogenic dependency, it is likely that PDAC cells with both KRAS and TP53 mutations could be targeted in the clinic, though more work must be conducted to evaluate whether or not these findings of efficacy will translate to studies on primary human pancreatic tumors. Importantly, TP53 and KRAS mutations commonly co-occur in several solid malignancies, including lung adenocarcinoma and colorectal cancer, opening the possibility that tumors harboring this combination of mutations might also carry the same ARS oncogenic mechanisms and therapeutic susceptibilities as KRAS/TP53-mutant PDACs [81, 82].

Others have also demonstrated that aberrant mRNA splicing in PDACs may generate meaningful tumor biomarkers while also contributing to tumor progression and drug resistance [83–91]. For example, CD44, a cell-surface glycoprotein that undergoes extensive splicing of its 20 exons, is differentially spliced between PDAC and normal pancreas tissue [88]. Furthermore, expression of the ‘standard’ CD44 isoform (CD44s) as opposed to ‘variant’ forms (CD44v) is strongly associated with an epithelial-to-mesenchymal transition process in PDAC cells, and inclusion of variant exons v3 and v6 in the CD44 mRNA product is uniquely associated with cancer metastasis [89, 90]. Other studies have shown that ARS alterations in PDACs strongly target extracellular matrix components [86, 91]. In another instance, the pyruvate kinase (PKM) gene was shown to predominantly produce the PKM2 isoform in gemcitabine-resistant PDAC cells, for which metabolic gemcitabine sensitivity could be restored following targeted antisense oligonucleotide delivery to promote production of the alternative PKM1 isoform [85]. Some studies have recently presented large-scale analyses of PDAC splice variant expression, though there is yet substantial work needed to harmonize these findings with the growing biomedical understanding of PDAC as well as routine clinical practice [84, 86].

**Treating alternative RNA splicing defects**

**ARS dependencies are targetable in PDACs, other malignancies, and non-malignant diseases**

Both targeted oligonucleotide delivery and small molecule inhibition of the spliceosome have been shown to be effective at treating several carcinomas in preclinical models, in addition to other splicing-focused therapies [32, 73]. Targeted oligonucleotides can be quickly generated to correct mutated, improperly spliced, or otherwise defective mRNA products with high specificity and efficacy when properly delivered to target cells [92]. Perhaps the most famous example of dysfunctional mRNA gene product correction is that of nusinersen (Spinraza), approved by the FDA in 2016 as the first medical treatment for spinal muscular atrophy, offering strong proof-of-concept for clinical efficacy of this treatment modality [93, 94]. New oligonucleotide delivery methods have improved clinicians’ abilities to deliver therapeutic doses into difficult-to-reach tumors, like PDACs, taking advantage of tumor microenvironmental factors such as acidic pH, for example [75, 95, 96]. As an alternative method for targeting ARS pathogenic mechanisms, small molecule spliceosome modulators have demonstrated efficacy in treating cancers preferentially susceptible to spliceosome dysfunction [80], such as those harboring heterozygous mutations in SF3B1, a critical component
of the spliceosome for which complete functional loss is synthetic lethal [97].

The therapeutic utility of a targeted anti-cancer drug relies heavily on rapid and accurate tumor profiling, often in practice requiring immunohistochemical staining of fixed tissue to inform clinical decision-making. Currently, gene expression and subtyping methods for PDACs, though holding high potential for clinical translation, rely on a days-to-weeks-long approach involving RNA quantification and subsequent analysis. Evaluation of splicing changes, however, may be feasibly conducted through simple RT-PCR methods, enabling highly specific and rapid identification of actionable ARS dependencies. Our recent work, together with the corpus of evidence supporting clinical translation of ARS events for cancer therapy, provides a compelling vision of future oncology practice involving targeted ARS tumor profiling through scalable RNA amplification and visualization methods [98].

**FUTURE PROSPECTS**

Advancements in high-throughput RNA sequencing technologies over the past decade have led to substantial growth in the understanding of RNA splicing in cancer, and specifically PDAC. While ours and other studies have established strong connections between well-studied molecular alterations and splicing changes, several fundamental questions remain unanswered about the role of ARS in PDAC and, more broadly, cancer pathogenesis as whole. Evidence is limited on the capacity of ARS alterations to phenocopy mutational signatures as well as the role of ARS in cellular transformation downstream from bona fide cancer-initiating genomic mutations. The recent expanse of data on non-malignant pancreatic tumor co-conspirator cells – including cancer-associated fibroblasts and tumor-infiltrating immune cells, for example – further opens an exciting opportunity to understand how ARS might contribute to tumor cell immune evasion as well as the drastic desmoplastic collagen deposition in PDACs. Further investigation of these mechanisms may likely translate to clinically effective therapeutics, in addition to enabling a well-rounded understanding of cancer pathogenesis, maintenance, drug resistance, and immune evasion.

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**CONFLICTS OF INTEREST**

LEH is a consultant for KDx Diagnostics Inc. and OncoGenesis Inc. The comments described here are not related to the interests of either company. SDL sits on the Scientific Advisory Boards of Episteme Prognostics and NYBO Therapeutics.

**REFERENCES**

1. Luo G, Fan Z, Gong Y, Jin K, Yang C, Cheng H, Huang D, Ni Q, Liu C, Yu X. Characteristics and Outcomes of Pancreatic Cancer by Histological Subtypes. Pancreas. 2019; 48:817–22. https://doi.org/10.1097/MPA.0000000000001338. [PubMed]
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019; 69:7–34. https://doi.org/10.3322/ caac.21551. [PubMed]
3. Hruban RH, Canto MI, Goggins M, Schluick R, Klein AP. Update on familial pancreatic cancer. Adv Surg. 2010; 44:293–311. https://doi.org/10.1055/j. gvasu.2010.05.011. [PubMed]
4. Canto MI, Hruban RH, Fishman EK, Kamel IR, Schluick R, Zhang Z, Topazian M, Takahashi N, Fletcher J, Petersen G, Klein AP, Axilbund J, Griffin C, et al. Frequent detection of pancreatic lesions in asymptomatic high-risk individuals. Gastroenterology. 2012; 142:796–804. https://doi.org/10.1053/j. gastro.2012.01.005. [PubMed]
5. Canto MI, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, Fishman EK, Brune K, Axilbund J, Griffin C, Ali S, Richman J, Jagannath S, et al. Screening for Early Pancreatic Neoplasia in High-Risk Individuals: A Prospective Controlled Study. Clin Gastroenterol Hepatol. 2006; 4:766–81. https://doi.org/10.1016/j.cgh.2006.02.005. [PubMed]
6. He J, Ahuja N, Makary MA, Cameron JL, Eckhauser FE, Choti MA, Hruban RH, Pawlik TM, Wolfgang CL. 2564 resected periampullary adenocarcinomas at a single institution: Trends over three decades. HPB (Oxford). 2014; 16:83–90. https://doi.org/10.1111/hpb.12078. [PubMed]
7. Cleary SP, Gryfe R, Guindí M, Greig P, Smith L, MacKenzie R, Strasberg S, Hanna S, Taylor B, Langer B, Gallinger S. Prognostic factors in resected pancreatic adenocarcinoma: Analysis of actual 5-year survivors. J Am Coll Surg. 2004; 198:722–31. https://doi.org/10.1016/j. jamcollsurg.2004.01.008. [PubMed]
8. Chu LC, Goggins MG, Fishman EK. Diagnosis and Detection of Pancreatic Cancer. Cancer J. 2017; 23:333–42. https://doi.org/10.1097/PPO.0000000000000290. [PubMed]
9. Gillen S, Schuster T, Meyer zum Büschenfelde C, Fries H, Kleeff J. Preoperative/Neoadjuvant Therapy in Pancreatic Cancer: A Systematic Review and Meta-analysis of Response and Resection Percentages. PLoS Med. 2010; 7:e1000267. https://doi.org/10.1371/journal.pmed.1000267. [PubMed]
10. Matsuno S, Kato S, Nakamura R, Kobari M, Sato T. [Hematogenous metastasis in pancreatic cancer]. [Article in Japanese]. Gan No Rinsho. 1985; 31:537–43. [PubMed]

www.oncotarget.com 528 Oncotarget
11. Kleeff J, Korec M, Apte M, La Vecchia C, Johnson CD, Biankin AV, Neale RE, Tempéro M, Tuveson DA, Hruban RH, Neoptolemos JP. Pancreatic cancer. Nat Rev Dis Primers. 2016; 2:16022. https://doi.org/10.1038/nrdp.2016.22. [PubMed]

12. Ducreux M, Seufferlein T, Van Laethem JL, Laurent-Puig P, Smolenicky C, Malka D, Boige V, Hollebecque A, Conroy T. Systemic treatment of pancreatic cancer revisited. Semin Oncol. 2019; 46:28–38. https://doi.org/10.1053/j.seminoncol.2018.12.003. [PubMed]

13. Arslan C, Yalcin S. Current and future systemic treatment options in metastatic pancreatic cancer. J Gastrointest Oncol. 2014; 5:280–95. https://doi.org/10.3978/j.issn.2078-6891.2014.030. [PubMed]

14. Maréchal R, Demols A, Gay F, de Maertelaer V, Arvanitaki AE, Mikhail S, Chung V, Picozzi VJ, Sohal D, Blais EM, Holter S, Aderka D, Paluch-Shimon S, Kaufman B, Gershoni-Baruch R, Hedley D, Moore MJ, Friedman E, et al. Overall survival and clinical characteristics of pancreatic cancer in BRCA mutation carriers. Br J Cancer. 2014; 111:1132–8. https://doi.org/10.1038/bjc.2014.418. [PubMed]

15. Golan T, Kanji ZS, Epelbaum R, Devaud N, Dagan E, Holter S, Aderka D, Paluch-Shimon S, Kaufman B, Gershoni-Baruch R, Hedley D, Moore MJ, Friedman E, et al. Molecular profiling of patients with pancreatic cancer: Initial results from the know your tumor initiative. Clin Cancer Res. 2018; 24:5018–27. https://doi.org/10.1158/1078-0432.CCR-18-0531. [PubMed]

16. Hahn SA, Greenhalf B, Ellis I, Sina-Frey M, Rieder H, Korte B, Gerdes B, Kress R, Ziegler A, Raeburn JA, Campra D, Grutzmann R, Rehder H, et al. BRCA2 Germline Mutations in Familial Pancreatic Carcinoma. J Natl Cancer Inst. 2003; 95:214–21. https://doi.org/10.1093/jnci/95.3.214. [PubMed]

17. Fishvaian MJ, Bender RJ, Halverson D, Rahib L, Hendifiar AE, Mikhail S, Chung V, Picozzi VJ, Sohal D, Blais EM, Mason K, Lyons EE, Matrisian LM, et al. Molecular profiling of patients with pancreatic cancer: Initial results from the know your tumor initiative. Clin Cancer Res. 2018; 24:5018–27. https://doi.org/10.1158/1078-0432.CCR-18-0531. [PubMed]

18. Chiaravalli M, Reni M, O’Reilly EM. Pancreatic ductal adenocarcinoma: State-of-the-art 2017 and new therapeutic strategies. Cancer Treat Rev. 2017; 60:32–43. https://doi.org/10.1016/j.ctrv.2017.08.007. [PubMed]

19. Conroy T, Desseigne F, Ychou M, Bouché O, Gourgou-Bourgade S, de la Borderie Resectable Pancreatic Cancer. Ann Surg. 2015; 261:12–7. https://doi.org/10.1097/SLA.0000000000000867. [PubMed]

20. Ferrone CR, Marchegiani G, Hong TS, Ryan DP, Deshpande V, McDonnell EI, Sabbatino F, Santos DD, Allen JN, Blaszkowsky LS, Clark JW, Faris JE, Goyal L, et al. Radiological and Surgical Implications of Neoadjuvant Treatment With FOLFIRINOX for Locally Advanced and Borderline Resectable Pancreatic Cancer. Ann Surg. 2015; 261:12–7. https://doi.org/10.1097/SLA.0000000000000867. [PubMed]

21. Kanda M, Matthaei H, Wu J, Hong SM, Yu J, Borges M, Hruban RH, Maitra A, Kinzler K, Vogelstein B, Goggins M. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology. 2012; 142:730–733.e9. https://doi.org/10.1053/j.gastro.2011.12.042. [PubMed]

22. Caldas C, Kern SE. K-ras mutation and pancreatic adenocarcinoma. Int J Pancreatol. 1995; 18:1–6. https://doi.org/10.1007/BF02825415. [PubMed]

23. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perou CH. Most human carcinomas of the exocrine pancreas contain mutant K-Ras genes. Cell. 1988; 53:549–54. https://doi.org/10.1016/0092-8674(88)90571-S. [PubMed]

24. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek D, Quinn MC, Robertson AJ, Fadlullah MZ, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015; 518:495–501. https://doi.org/10.1038/nature14169. [PubMed]

25. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek D, Quinn MC, Robertson AJ, Fadlullah MZ, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015; 518:495–501. https://doi.org/10.1038/nature14169. [PubMed]

26. Huijbers M, Voskuilen M, Schuurman E, van der Burg ME, van der Valk P, Schmitz-Pepping H, et al. Tumor Microenvironment Remodeling Enables Bypass of Oncogenic KRAS Dependency in Pancreatic Cancer. Cancer Discov. 2020; 10:1058–77. https://doi.org/10.1158/2159-8290.CD-19-0597. [PubMed]

27. Tenzer S, Muley T, Böttcher C, Horlings HM, Haupts A, Tjan-Heijnen VC, et al. KRAS-driven pancreatic ductal adenocarcinoma: The role of microenvironmental TGFβ signaling. Oncotarget. 2018; 9:6203–16. https://doi.org/10.18632/oncotarget.25144. [PubMed]

28. Tenzer S, Muley T, Böttcher C, Horlings HM, Haupts A, Tjan-Heijnen VC, et al. KRAS-driven pancreatic ductal adenocarcinoma: The role of microenvironmental TGFβ signaling. Oncotarget. 2018; 9:6203–16. https://doi.org/10.18632/oncotarget.25144. [PubMed]

29. Tachez M, Gebauer F, Petersen C, Arnold D, Trepel M, Wiegsscheider K, Schafhausen P, Bockhorn M, Izbic J, Yekebas E. Sequential neoadjuvant chemoradiotherapy (CRT) followed by curative surgery vs. primary surgery alone for resectable, non-metastasized pancreatic adenocarcinoma: NEOPA- a randomized multicenter phase III study (NCT01900327, DRKS00003893, ISRCTN82191749). BMC Cancer. 2014; 14:411. https://doi.org/10.1186/1471-2407-14-411. [PubMed]

30. Tachez M, Gebauer F, Petersen C, Arnold D, Trepel M, Wiegsscheider K, Schafhausen P, Bockhorn M, Izbic J, Yekebas E. Sequential neoadjuvant chemoradiotherapy (CRT) followed by curative surgery vs. primary surgery alone for resectable, non-metastasized pancreatic adenocarcinoma: NEOPA- a randomized multicenter phase III study (NCT01900327, DRKS00003893, ISRCTN82191749). BMC Cancer. 2014; 14:411. https://doi.org/10.1186/1471-2407-14-411. [PubMed]

31. Tachez M, Gebauer F, Petersen C, Arnold D, Trepel M, Wiegsscheider K, Schafhausen P, Bockhorn M, Izbic J, Yekebas E. Sequential neoadjuvant chemoradiotherapy (CRT) followed by curative surgery vs. primary surgery alone for resectable, non-metastasized pancreatic adenocarcinoma: NEOPA- a randomized multicenter phase III study (NCT01900327, DRKS00003893, ISRCTN82191749). BMC Cancer. 2014; 14:411. https://doi.org/10.1186/1471-2407-14-411. [PubMed]
30. Waters AM, Der CJ. KRAS: The critical driver and therapeutic target for pancreatic cancer. Cold Spring Harb Perspect Med. 2018; 8:a031435. https://doi.org/10.1101/ cshperspect.a031435. [PubMed]

31. Zeitouni D, Pylayeva-Gupta Y, Der CJ, Bryant KL. KRAS mutant pancreatic cancer: No lone path to an effective treatment. Cancers (Basel). 2016; 8:45. https://doi.org/10.3390/cancers8040045. [PubMed]

32. Escobar-Hoyos LF, Penson A, Kannan R, Cho H, Pan CH, Singh RK, Apken LH, Hobbs GA, Luo R, Lecomte N, Babu S, Pan FC, Alonso-Curbelo D, et al. Altered RNA Splicing by Mutant p53 Activates Oncogenic RAS Signaling in Pancreatic Cancer. Cancer Cell. 2020; 38:198–211.e8. https://doi.org/10.1016/j.ccell.2020.05.010. [PubMed]

33. Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, Perera RM, Ferrone CR, Mullarky E, Shyh-Chang N, Kang Y, Fleming JB, Bardeesy N, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature. 2013; 496:101–5. https://doi.org/10.1038/nature12040. [PubMed]

34. Azizan N, Suter MA, Liu Y, Logsdon CD. RAGE maintains high levels of NFkB and oncogenic Kras activity in pancreatic cancer. Biochem Biophys Res Commun. 2017; 493:592–7. https://doi.org/10.1016/j.bbrc.2017.08.147. [PubMed]

35. Kitajima S, Asahina H, Chen T, Guo S, Quiceno LG, Cavanaugh JD, Merlino AA, Tange S, Terai H, Kim JW, Wang X, Zhou S, Xu M, et al. Overcoming Resistance to Dual Innate Immune and MEK Inhibition Downstream of KRAS. Cancer Cell. 2018; 34:439–452.e6. https://doi.org/10.1016/j.ccell.2018.08.009. [PubMed]

36. Hamarsheh S, Groß O, Brummer T, Zeiser R. Immune modulatory effects of oncogenic KRAS in cancer. Nat Commun. 2020; 11:5439. https://doi.org/10.1038/s41467-020-19288-6. [PubMed]

37. Eser S, Schnieke A, Schneider G, Saur D. Oncogenic KRAS signalling in pancreatic cancer. Br J Cancer. 2014; 111:817–22. https://doi.org/10.1038/bjc.2014.215. [PubMed]

38. Colisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, Cooc J, Weinkle J, Kim GE, Jakkula L, Feiler HS, Ko AH, Olshen AB, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med. 2011; 17:500–3. https://doi.org/10.1038/nm.2344. [PubMed]

39. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SGH, Headley KA, Rashid NU, Williams LA, Eaton SC, Chung AH, Smyla JK, Anderson JM, Kim HJ, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. 2015; 47:1168–78. https://doi.org/10.1038/ng.3398. [PubMed]

40. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, Miller DK, Christ AN, Bruxner TJ, Quinn MC, Nourse C, Murtaugh LC, Harliwong I, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016; 531:47–52. https://doi.org/10.1038/nature16965. [PubMed]

41. Aguirre AJ, Nowak JA, Camarda ND, Moffitt RA, Ghazani AA, Hazar-Rethinam M, Raghavan S, Kim J, Brais LK, Ragon D, Welch MW, Reilly E, McCabe D, et al. Real-time genomic characterization of advanced pancreatic cancer to enable precision medicine. Cancer Discov. 2018; 8:1096–111. https://doi.org/10.1158/2159-8290.CD-18-0275. [PubMed]

42. Tiriac H, Belleau P, Engle DD, Plenker D, Deschênes A, Somerville TDD, Froeling FEM, Burkhart RA, Denrence RE, Jang GH, Miyabayashi K, Young CM, Patel H, et al. Organoid profiling identifies common responders to chemotherapy in pancreatic cancer. Cancer Discov. 2018; 8:1112–29. https://doi.org/10.1158/2159-8290.CD-18-0349. [PubMed]

43. Aung KL, Fischer SE, Denrence RE, Jang GH, Dodd A, Creighton S, Southwood B, Liang SB, Chadwick D, Zhang A, O’Kane GM, Alhaba H, Moura S, et al. Genomics-driven precision medicine for advanced pancreatic cancer: Early results from the COMPASS trial. Clin Cancer Res. 2018; 24:1344–54. https://doi.org/10.1158/1078-0432.CCR-17-2994. [PubMed]

44. Rashid NU, Peng XL, Jin C, Moffitt RA, Volmar KE, Belt BA, Panni RZ, Nywening TM, Herrera SG, Moore KJ, Hennessey SG, Morrison AB, Kawalerski R, et al. Purity Independent Subtyping of Tumors (PurlIST), A Clinically Robust, Single-sample Classifier for Tumor Subtyping in Pancreatic Cancer. Clin Cancer Res. 2020; 26:82–92. https://doi.org/10.1158/1078-0432.CCR-19-1467. [PubMed]

45. Colisson EA, Bailey P, Chang DK, Biankin AV. Molecular subtypes of pancreatic cancer. Nat Rev Gastroenterol Hepatol. 2019; 16:207–20. https://doi.org/10.1038/s41575-019-0109-y. [PubMed]

46. Maurer C, Holstrom SR, He J, Laise P, Su T, Ahmed A, Hibshoosh H, Chabot JA, Oberstein PE, Sepulveda AR, Genkinger JM, Zhang J, Iuga AC, et al. Experimental microdissection enables functional harmonisation of pancreatic cancer subtypes. Gut. 2019; 68:1034–43. https://doi.org/10.1136/gutjnl-2018-317706. [PubMed]

47. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffi G, Lucito MS, Sivajothi S, Armstrong TD, Engle DD, et al. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting cancer-associated fibroblasts. Cancer Discov. 2019; 9:1102–23. https://doi.org/10.1158/2159-8290.CD-18-0349. [PubMed]

48. Ogawa Y, Masugi Y, Abe T, Yamazaki K, Ueno A, Fujii KM, Shiers Y, Kimura H, Sumida C, Kohashi T, et al. Organoid profiling to evaluate single-sample classifier for tumor subtyping in pancreatic cancer. Cancer Discov. 2020; 10:1112–29. https://doi.org/10.1158/2326-6066.CD-19-1323. [PubMed]
49. Lomberk G, Blum Y, Nicolle R, Nair A, Gaonkar KS, Marisa L, Mathison A, Sun Z, Yan H, Elarouci N, Armenoult L, Ayadi M, Ordog T, et al. Distinct epigenetic landscapes underlie the pathobiology of pancreatic cancer subtypes. Nat Commun. 2018; 9:1978. https://doi.org/10.1038/s41467-018-04383-6. [PubMed]

50. Du Y, Zhao B, Liu Z, Ren X, Zhao W, Li Z, You L, Zhao Y. Molecular subtyping of pancreatic cancer: Translating genomics and transcriptomics into the clinic. J Cancer. 2017; 8:513–22. https://doi.org/10.7150/jca.17622.

51. Cammas A, Lewis SM, Vagner S, Holeik M. Post-transcriptional control of gene expression through subcellular relocation of mRNA binding proteins. Biochem Pharmacol. 2006; 76:1395–403. https://doi.org/10.1016/j.bcp.2008.05.022. [PubMed]

52. Solier S, Barb J, Zeeberg BR, Varma S, Ryan MC, Kohn KW, Weinstein JN, Munson PJ, Pommier Y. Genome-wide analysis of novel splice variants induced by topoisomerase I poisoning shows preferential occurrence in genes encoding splicing factors. Cancer Res. 2010; 70:8055–65. https://doi.org/10.1158/0008-5472.CAN-10-2491. [PubMed]

53. Bianchi M, Crinelli R, Giacomini E, Carloni E, Radici L, Scarpa ES, Tasiini F, Magnani M. A negative feedback mechanism links UBC gene expression to ubiquitin levels affecting RNA splicing rather than transcription. Sci Rep. 2019; 9:1–19. https://doi.org/10.1038/s41598-019-54973-7. [PubMed]

54. Kishore S, Stamm S. The snoRNA HBII-52 regulates alternative splicing of the serotonin receptor 2C. Science. 2006; 313:230–2. https://doi.org/10.1126/science.1118265. [PubMed]

55. Mu Y, Otsuka T, Horton AC, Scott DB, Ehlers MD. Activity-dependent mRNA splicing controls ER export and synaptic delivery of NMDA receptors. Neuron. 2003; 40:581–94. https://doi.org/10.1016/S0896-6273(03)00676-7. [PubMed]

56. Xu Z, Weiss A. Negative regulation of CD45 by differential homodimerization of the alternatively spliced isoforms. Nat Immunol. 2002; 3:764–71. https://doi.org/10.1038/nri822. [PubMed]

57. Cheng C, Yaffe MB, Sharp PA. A positive feedback loop couples Ras activation and CD44 alternative splicing. Genes Dev. 2006; 20:1715–20. https://doi.org/10.1101/gad.1430906. [PubMed]

58. Black DL. Mechanisms of Alternative Pre-Messenger RNA Splicing. Annu Rev Biochem. 2003; 72:291–336. https://doi.org/10.1146/annurev.biochem.72.121801.161720. [PubMed]

59. Quinones-Valdez G, Tran SS, Jun HI, Bahn JH, Yang EW, Zhan L, Brümmer A, Wei X, Van Nostrand EL, Pratt GA, Yeo GW, Graveley BR, Xiao X. Regulation of RNA editing by RNA-binding proteins in human cells. Commun Biol. 2019; 2:19. https://doi.org/10.1038/s42003-018-0271-8. [PubMed]

60. Van Nostrand EL, Pratt GA, Yee BA, Wheeler EC, Blue SM, Mueller J, Park SS, Garcia KE, Gelboin-Burkhart C, Nguyen TB, Rabano I, Stanton R, Sundararaman B, et al. Principles of RNA processing from analysis of enhanced CLIP maps for 150 RNA binding proteins. Genome Biol. 2020; 21:90. https://doi.org/10.1186/s13059-020-01982-9. [PubMed]

61. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. Alternative isoform regulation in human tissue transcriptomes. Nature. 2008; 456:470–6. https://doi.org/10.1038/nature07509. [PubMed]

62. Van Nostrand EL, Freese P, Pratt GA, Wang X, Wei X, Xiao R, Blue SM, Chen JY, Cody NAL, Dominguez D, Olson S, Sundararaman B, Zhan L, et al. A large-scale binding and functional map of human RNA-binding proteins. Nature. 2020; 583:711–9. https://doi.org/10.1038/s41586-020-2077-2. [PubMed]

63. Pickrell JK, Pai AA, Gilad Y, Pritchard JK. Noisy Splicing Drives mRNA Isoform Diversity in Human Cells. PLoS Genet. 2010; 6:e1001236. https://doi.org/10.1371/journal.pgen.1001236. [PubMed]

64. Melamud E, Moult J. Stochastic noise in splicing machinery. Nucleic Acids Res. 2009; 37:4873–86. https://doi.org/10.1093/nar/gkp471. [PubMed]

65. Sorek R, Shamir R, Ast G. How prevalent is functional alternative splicing in the human genome? Trends Genet. 2004; 20:68–71. https://doi.org/10.1016/j.tig.2003.12.004. [PubMed]

66. Wan Y, Larson DR. Splicing heterogeneity: Separating signal from noise. Genome Biol. 2018; 19:1–10. https://doi.org/10.1186/s13059-018-1467-4. [PubMed]

67. Skotheim RJ, Nees M. Alternative splicing in cancer: Noise, functional, or systematic? Int J Biochem Cell Biol. 2007; 39:1432–49. https://doi.org/10.1016/j.biocel.2007.02.016. [PubMed]

68. Trincado JL, Entizne JC, Hysenaj G, Singh B, Skalic M, Elliott DJ, Eyras E. SUPPA2: Fast, accurate, and uncertainty-aware differential splicing analysis across multiple conditions. Genome Biol. 2018; 19:40. https://doi.org/10.1186/s13059-018-1417-1. [PubMed]

69. Anders S, Reyes A, Huber W. Detecting differential usage of exons from RNA-seq data. Genome Res. 2012; 22:2008–17. https://doi.org/10.1101/gr.133744.111. [PubMed]

70. Vaquero-Garcia J, Barrera A, Gazzara MR, Gonzalez-Vallinas J, Lahens NF, Hogenesch JB, Lynch KW, Barash Y. A new view of transcriptome complexity and regulation through the lens of local splicing variations. Elife. 2016; 5:e11752. https://doi.org/10.7554/eLife.11752. [PubMed]

71. Li Y, Knowles DA, Humphrey J, Singh SK, Skalic M, Elliott DJ, Eyras E. SUPPA2: Fast, accurate, and uncertainty-aware differential splicing analysis across multiple conditions. Genome Biol. 2018; 19:40. https://doi.org/10.1186/s13059-018-1417-1. [PubMed]

72. Trincado JL, Entizne JC, Hysenaj G, Singh B, Skalic M, Elliott DJ, Eyras E. SUPPA2: Fast, accurate, and uncertainty-aware differential splicing analysis across multiple conditions. Genome Biol. 2018; 19:40. https://doi.org/10.1186/s13059-018-1417-1. [PubMed]

73. Inoue D, Chew GL, Liu B, Michel BC, Pangallo J, D’Avino AR, Hitchman T, North K, Lee SC, Bitner L, Block A, Moore
AR, Yoshimi A, et al. Spliceosomal disruption of the non-canonical BAF complex in cancer. Nature. 2019; 574:432–6. https://doi.org/10.1038/s41586-019-1646-9. [PubMed]
74. Wang Z, Lo HS, Yang H, Gere S, Hu Y, Buetow KH, Lee MP. Computational analysis and experimental validation of tumor-associated alternative RNA splicing in human cancer. Cancer Res. 2003; 63:655–7. [PubMed]
75. Kahles A, Lehmann KV, Toussaint NC, Hüser M, Stark SG, Sachsenberg T, Stegle O, Kohlbacher O, Sander C, Caesar-Johnson SJ, Demchok JA, Felau I, Kasapi M, et al. Comprehensive Analysis of Alternative Splicing Across Tumors from 8,705 Patients. Cancer Cell. 2018; 34:211–224.e6. https://doi.org/10.1016/j.ccell.2018.07.001. [PubMed]
76. Escobar-Hoyos L, Knorr K, Abdel-Wahab O. Aberrant RNA splicing in cancer. Annu Rev Cancer Biol. 2019; 3:167–85. https://doi.org/10.1146/annurev-cancerbio-030617-050407.
77. Fujikura K, Hosoda W, Felsenstein M, Song Q, Reiter JG, Zheng L, Beleva Guthrie V, Rincon N, Dal Molin M, Dudley J, Cohen JD, Wang P, Fischer CG, et al. Multiregion whole-exome sequencing of intraductal papillary mucinous neoplasms reveals frequent somatic KLF4 mutations predominantly in low-grade regions. Gut. 2020 Oct 7. https://doi.org/10.1136/gutjnl-2020-321217. [Epub ahead of print]. [PubMed]
78. Noë M, Niknafs N, Fischer CG, Hackeng WM, Beleva Guthrie V, Hosoda W, Debjyotik M, Papp E, Adleff V, White JR, Luchini C, Pea A, ScarpA A, et al. Genomic characterization of malignant progression in neoplastic pancreatic cysts. Nat Commun. 2020; 11:4085. https://doi.org/10.1038/s41467-020-17917-8. [PubMed]
79. Buonamici S, Yoshimi A, Thomas M, Seiler M, Chan B, Caleb B, Darman R, Fekkes P, Karr C, Keaney GF, Klimke VM, Kuni K, Lee L, et al. H3B-8800, an Orally Bioavailable Modulator of the SF3b Complex, Shows Efficacy in Spliceosome-Mutant Myeloid Malignancies. Blood. 2016; 128:966–966. https://doi.org/10.1182/blood.\v128.22.966.966.
80. Seiler M, Yoshimi A, Darman R, Chan B, Keaney G, Thomas M, Agrawal AA, Caleb B, Csibi A, Sean E, Fekkes P, Karr C, Klimke V, et al. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. Nat Med. 2018; 24:497–504. https://doi.org/10.1038\_nm\_4493. [PubMed]
81. Ye J, Lin M, Zhang C, Zhu X, Li S, Liu H, Yin J, Yu H, Zhu K. Tissue gene mutation profiles in patients with colorectal cancer and their clinical implications. Biomed Rep. 2020; 13:43–8. https://doi.org/10.3892\_br\_2020\_1303. [PubMed]
82. Ding L, Getz G, Wheeler DA, Mardis ER, McLellan MD, Cibulskis K, Sougnez C, Greulich H, Muzny DM, Morgan MB, Fulton L, Fulton RS, Zhang Q, et al. Somatic mutations affect key pathways in lung adenocarcinoma. Nature. 2008; 455:1069–75. https://doi.org/10.1038\_nature07423. [PubMed]
83. Hayes GM, Carrigan PE, Beck AM, Miller LJ. Targeting the RNA splicing machinery as a novel treatment strategy for pancreatic carcinoma. Cancer Res. 2006; 66:3819–27. https://doi.org/10.1158/0008-5472.CAN-05-4065. [PubMed]
84. Yu M, Hong W, Ruan S, Guan R, Tu L, Huang B, Hou B, Jian Z, Ma L, Jin H. Genome-Wide Profiling of Prognostic Alternative Splicing Pattern in Pancreatic Cancer. Front Oncol. 2019; 9:773. https://doi.org/10.3389/fonc.2019.00773. [PubMed]
85. Calabretta S, Bielli P, Passacantilli I, Pilozzi E, Vendri C, Capurso G, Balestra A, Sette C. Modulation of PKM alternative splicing by PTB1 promotes gemcitabine resistance in pancreatic cancer cells. Oncogene. 2016; 35:2031–9. https://doi.org/10.1038\_onc.2015.270. [PubMed]
86. Wang J, Dumartin L, Mafficini A, Ulug P, Sangaralingam A, Alamary NA, Radon TP, Salvia R, Lawlor RT, Lemoine NR, ScarpA C, Chelala C, Cmogr-parl-urcevic T. Splice variants as novel targets in pancreatic ductal adenocarcinoma. Sci Rep. 2017; 7:2980. https://doi.org/10.1038\_s41598-017-03354-z. [PubMed]
87. Adesso L, Calabretta S, Barbagallo F, Capurso G, Pilozzi E, Geremia R, Delle Fave G, Sette C. Gemcitabine triggers a pro-survival response in pancreatic cancer cells through activation of the MNK2/eIF4E pathway. Oncogene. 2013; 32:2848–57. https://doi.org/10.1038\_onc.2012.306. [PubMed]
88. Gansauge F, Gansauge S, Zobywalski A, Scharnweber C, Link KH, Nussler AK, Beger HG. Differential Expression of CD44 Splice Variants in Human Pancreatic Adenocarcinoma and in Normal Pancreas. Cancer Res. 1995; 55:4599–503. [PubMed]
89. Zhao S, Chen C, Chang K, Kannad A, Jagirdar J, Kumar AP, Freeman JW. CD44 expression level and isoform contributes to pancreatic cancer cell plasticity, invasiveness, and response to therapy. Clin Cancer Res. 2016; 22:5592–604. https://doi.org/10.1158\_1078-0432.CCR-15-3115. [PubMed]
90. Rall CJ, Rustgi AK. CD44 Isoform Expression in Primary and Metastatic Pancreatic Adenocarcinoma. Cancer Res. 1995; 55:1831–5. [PubMed]
91. Arafat H, Lazar M, Salem K, Chipitsyna G, Gong Q, Pan TC, Zhang RZ, Yeo CJ, Chu ML. Expression of CD44 Splice Variants in Human Pancreatic Adenocarcinoma and in Normal Pancreas. Cancer Res. 1995; 55:5499–503. [PubMed]
92. Yu M, Hong W, Ruan S, Guan R, Tu L, Huang B, Hou B, Jian Z, Ma L, Jin H. Genome-Wide Profiling of Prognostic Alternative Splicing Pattern in Pancreatic Cancer. Front Oncol. 2019; 9:773. https://doi.org/10.3389/fonc.2019.00773. [PubMed]
93. Passini MA, Bu J, Richards AM, Kinnecon C, Sardi SP, Staneck LM, Hua Y, Rigo F, Matson J, Hung G, Kaye EM, Shihabuddin LS, Krainer AR, Yoshimi A, et al. Spliceosomal disruption of the non-canonical BAF complex in cancer. Nature. 2019; 574:432–6. https://doi.org/10.1038/s41586-019-1646-9. [PubMed]
94. Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E, Norris DA, Xia S, Bennett CF, et al. Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study. Lancet. 2016; 388:3017–26. https://doi.org/10.1016/S0140-6736(16)31408-8. [PubMed]

95. Teo J, McCarroll JA, Boyer C, Youkhana J, Sagnella SM, Duong HT, Liu J, Sharbeen G, Goldstein D, Davis TP, Kavallaris M, Phillips PA. A Rationally Optimized Nanoparticle System for the Delivery of RNA Interference Therapeutics into Pancreatic Tumors in Vivo. Biomacromolecules. 2016; 17:2337–51. https://doi.org/10.1021/acs.biomac.6b00185. [PubMed]

96. Ämmälä C, Drury WJ, Knerr L, Ahlstedt I, Stillemark-Billton P, Wennberg-Huldt C, Andersson EM, Valeur E, Jansson-Löfmark R, Janzén D, Sundström L, Meuller J, Claesson J, et al. Targeted delivery of antisense oligonucleotides to pancreatic β-cells. Sci Adv. 2018; 4:3386–403. https://doi.org/10.1126/sciadv.aat3386. [PubMed]

97. Lee SCW, North K, Kim E, Jang E, Obeng E, Lu SX, Liu B, Inoue D, Yoshimi A, Ki M, Yeo M, Zhang XJ, Kim MK, et al. Synthetic Lethal and Convergent Biological Effects of Cancer-Associated Spliceosomal Gene Mutations. Cancer Cell. 2018; 34:225–241.e8. https://doi.org/10.1016/j.ccell.2018.07.003. [PubMed]

98. Credle JJ, Itoh CY, Yuan T, Sharma R, Scott ER, Workman RE, Fan Y, Housseau F, Llosa NJ, Bell WR, Miller H, Zhang SX, Timp W, et al. Multiplexed analysis of fixed tissue RNA using Ligation in situ Hybridization. Nucleic Acids Res. 2017; 45:e128. https://doi.org/10.1093/nar/gkx471. [PubMed]