

Review

KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 Mutations in Pancreatic Cancer

Jonas Cicenas ^{1,2,3,*}, Kotryna Kvederaviciute ⁴, Ingrida Meskinyte ⁵,
Edita Meskinyte-Kausiliene ⁶, Aiste Skeberdyte ⁷ and Jonas Cicenas Jr. ⁸

¹ Vetsuisse Faculty, Institute of Animal Pathology, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland

² MAP Kinase Resource, Bioinformatics, Melchiorstrasse 9, 3027 Bern, Switzerland

³ Proteomics Centre, Institute of Biochemistry, Vilnius University, Sauletekio al. 7, LT-10257 Vilnius, Lithuania

⁴ Faculty of Mathematics and Informatics, Vilnius University, Naugarduko st. 24, LT-03225 Vilnius, Lithuania; k.kvederaviciute@mapkinases.eu

⁵ Ministry of Education and Science, A. Volano g. 2, LT-01516 Vilnius, Lithuania; ingrida.meskinyte@gmail.com

⁶ Energy and Biotechnology Engineering Institute, Aleksandro Stulginskio University, Studentų g. 11, LT-53361 Akademija, Lithuania; edita.meskinyte@gmail.com

⁷ Division of Human Genome Research Centre, Institute of Biosciences, Life Sciences Center, Vilnius University, Sauletekio al. 7, LT-10257 Vilnius, Lithuania; aiste.skeberdyte@gmail.com

⁸ International School of Law and Business, Laisvss pr. 58, LT-05120 Vilnius, Lithuania; jo.cicenas@gmail.com

* Correspondence: j.cicenas@mapkinases.eu; Tel.: +370-667-04267

Academic Editor: Samuel C. Mok

Received: 22 March 2017; Accepted: 25 April 2017; Published: 28 April 2017

Abstract: Pancreatic cancer is a disease that has a very high fatality rate and one of the highest mortality ratios among all major cancers, remaining the fourth leading cause of cancer-related deaths in developed countries. The major treatment of pancreatic cancer is surgery; however, only 15–20% of patients are candidates for it at the diagnosis of disease. On the other hand, survival in patients, who undergo surgery, is less than 30%. In most cancers, genome stability is disturbed and pancreatic cancer is not the exception. Approximately 97% of pancreatic cancers have gene derangements, defined by point mutations, amplifications, deletions, translocations, and inversions. This review describes the most frequent genetic alterations found in pancreatic cancer.

Keywords: KRAS; TP53; CDKN2A; SMAD4; BRCA1; BRCA2; mutation; pancreatic cancer; genetic variant

1. Introduction

As opposed to steady or diminishing rates of most solid tumors, pancreatic cancer remains one of the most deadly cancers. More than 43,000 patients are expected to die of pancreatic cancer in the USA and 91,500 in the European Union in 2017 [1,2]. There are also some alarming predictions that pancreatic cancer might soon become the second highest cause of cancer-related death in the USA and the third highest in the EU. In addition, virtually the only possible treatment of pancreatic cancer is surgery, but less than 20% of patients have operable tumors, and less than 30% of those patients survive surgery [3,4]. Moreover, diagnosis of pancreatic cancer is quite challenging because histological tests require invasive approaches, and high amounts of stromal cells in the tumor cause biopsies to be insufficient for correct diagnosis [4,5]. Given all of this, pancreatic cancer is one of the most investigated cancers both in basic and clinical science setups.

Most pancreatic cancers (97%) have gene alterations, such as amplifications, deletions, translocations, inversions, frameshifts, and substitutions. Disruptions of several genes in pancreatic

cancer are almost universal and are present in 70%–98% of patients [5–7]. Mutations of other genes are less frequent [8] and some of them are derived from germline inheritance [9,10] (Figure 1). The mutation of genes can either enhance the function of protein, sometimes making it even constitutively active or diminish the function, sometimes abolishing it completely. Subsequently, the changes in protein functions can cause uncontrolled proliferation, motility, and adhesion of cells, protection from apoptosis or autophagy, DNA repair problems, microsatellite instability, and other processes, which lead to the development, growth, and spread of cancer.

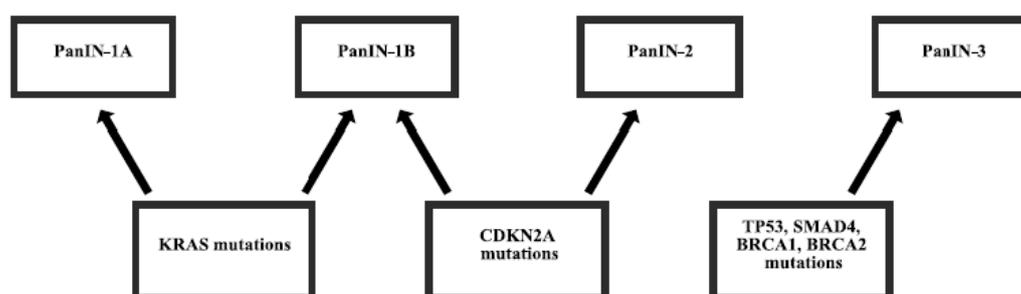


Figure 1. Mutations in the carcinogenesis of pancreatic adenocarcinoma. Pancreatic intraepithelial neoplasia (PanIN) grading.

2. Gene Mutations in Pancreatic Cancer

KRAS (also known as K-Ras 2, Ki-Ras, c-K-ras, or c-Ki-ras) is a small GTPase (21 kDa), which binds guanosine triphosphate and diphosphate nucleotides. It is activated when bound to GTP and deactivated when bound to GDP. Activated *KRAS* binds and activates RAF family kinases, RAF1, BRAF, and ARAF [11]. Activated RAFs phosphorylate and activate MEK1 and MEK2 kinases, which in turn phosphorylate and activate ERK1 and ERK2 kinases. ERKs phosphorylate various cytosolic and nuclear proteins, such as transcription factors ELK1 and c-JUN, leading to cell proliferation [12]. Due to this, mutations, which cause constitutive activation of *KRAS*, lead to uncontrolled proliferation and other processes causing cancer development and spreading. *KRAS* can also regulate other signaling pathways, such as PI3K-AKT, PLC-PKC, and RAL, which are also known to be involved in cancer progression [13]. *KRAS* is mutated in more than 20% of human cancers, mostly in pancreatic (more than 90%), colorectal and lung cancers [13] as well as leukemias [14] (Table 1). Mutations of the codons G12, G13, or Q61 are usually associated with constitutively active *KRAS*, and recurrent mutations in K117 and A146 seem to be additional hotspots (Figure 2). Ninety-five percent of pancreatic cancers carry activating mutations in *KRAS* and modifications in G12 account for 99% of all mutations (G12D—50%) [15]. On the other hand, G13 mutations are much rarer than in some other cancers (e.g., colorectal 17%). Clinical studies have shown that mutations of *KRAS* could be used as a significant prognostic biomarker, as well as a tool for therapy prediction. In a study that examined 136 pancreatic adenocarcinoma patients, 71 (52%) patients harbored point mutation in G12 (70) and Q61 (1) [16]. Patients with mutations showed a worse response to first-line gemcitabine-based chemotherapy (11.3%) than those with wild-type *KRAS* (26.2%) and poorer survival ($p = 0.001$). In addition, survival benefit was observed in the subgroup of patients treated with gemcitabine/erlotinib combination ($p = 0.002$), but no survival difference was observed in the subgroup of patients treated only with gemcitabine ($p = 0.121$). Another study in 173 patients, 121 (70%) of which had G12 mutations, showed no association with response to erlotinib alone ($p = 0.4$), but *KRAS* wildtype patients had an improved overall survival ($p = 0.005$) [17]. Phase I/II study using siRNA against G12D in combination with chemotherapy was performed in 15 locally advanced pancreatic cancer patients [18]. None of the 12 patients analyzed later showed tumor progression, 10 had stable disease, 2 displayed partial response, and median overall survival was 15.12 months. The study using a targeted deep sequencing assay detected *KRAS* mutations in 96/100 (96%) patients. The patients were then separated into two

groups: one having 0–2 mutations, and the other having 3. The presence of 3 mutations was prognostic for a better overall survival ($p = 0.004$) [19]. Interestingly, activating mutations in kinase downstream of KRAS signaling, BRAF (V599E), were found in 3/9 (33%) tumors, lacking KRAS mutations, but known to have microsatellite instability [20].

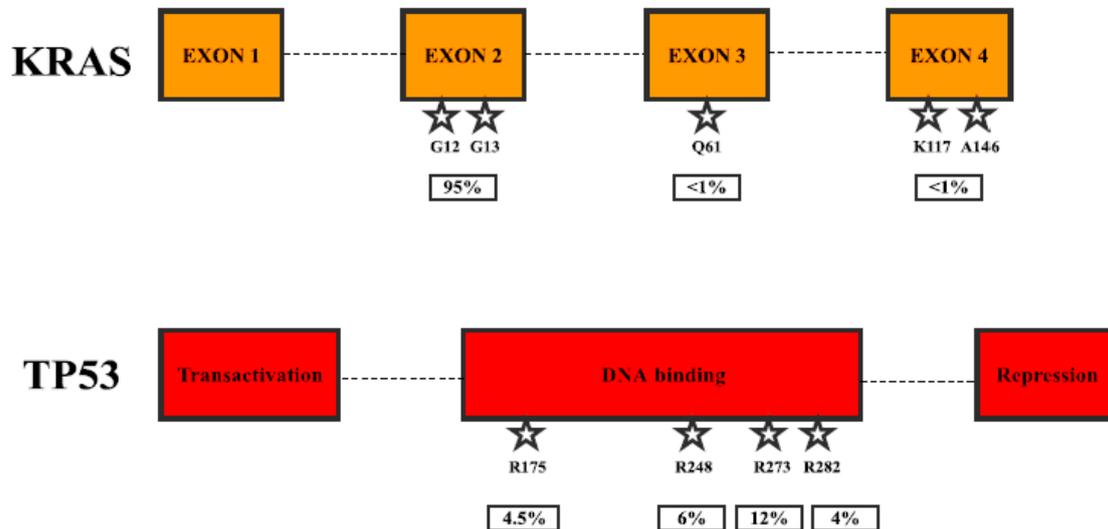


Figure 2. Most frequent mutations in pancreatic cancer. KRAS mutations by the exons and TP53 mutations by protein domain.

TP53 (also known as p53 or antigen NY-CO-13) is the tumor suppressor, which transcriptionally activates target genes in response to cellular stress such as oxidative stress or DNA damage and thus induces growth arrest or apoptosis [21]. It also increases cyclin-dependent kinase inhibitor CDKN1A expression, thus stopping cell cycle progression [22]. *TP53* is one of the most frequently mutated genes in all cancers and is mutated in 70% of pancreatic cancers [23], mostly resulting in the loss of DNA binding ability and thus subsequently gene transcription activation [24] (Figure 2, Table 1). Some clinical evidence suggests that *TP53* could be used as biomarker for prognosis and therapy prediction. A study in 57 pancreatic ductal adenocarcinoma patients assessed *TP53* mutations as well as mRNA expression [25]. It was observed that patients with low *TP53* mRNA expression were associated with worse prognosis ($p = 0.032$), and the results were more significant in patients with *TP53* wild-type genes ($p = 0.021$). One quite promising study was published recently, stating that mutated *TP53* caused poor prognosis to pancreatectomy through upregulation of PTRF (cavin-1) in patients with preoperative serum [26], but the publication was retracted due to the use of wrong antibodies in the study [27]. Nevertheless, the result seems to be interesting, and it is hopeful that the study will be repeated using suitable techniques. A study in pancreatic cancer patient tumors, of which 4/50 (8%) had a complete loss of *TP53* expression, 20/50 (40%) showed regular expression, and 26/50 (52%) patients showed overexpression, showed a significant improvement in progression-free survival ($p = 0.02$) for patients with regular expression compared to complete loss [28]. The study using a targeted deep sequencing assay detected *TP53* mutations in 13/100 (13%) patients, and the presence of 0–2 vs. 3 mutations was prognostic for better overall survival ($p = 0.004$) [19].

CDKN2A (also known as p16-INK4a, MTS-1, or CDK4I) is the tumor suppressor, which regulates cell cycle progression by inhibiting cyclinD-CDK4 and cyclinD-CDK6 complexes responsible for initiating the G1/S phase transition. This protein encoded by the *CDKN2A* gene, but is unrelated to another tumor suppressor ARF, encoded by the same gene. Inherited modifications in *CDKN2A* cause familial atypical multiple mole melanoma and an increased risk of pancreatic cancer [29,30]. It is one of the most frequently altered genes in cancer, and the incidence of mutations in sporadic pancreatic cancer is impressive, with inactivation occurring in 98% of cases [31] (Table 1). *CDKN2A* gene

disruption happens by different types of mutations, such as the loss of heterozygosity, homozygous deletion, or promoter silencing. There is some clinical evidence for the use of CDKN2A mutations as prognostic and predictive biomarker. The study performed in 88 pancreatic cancer patients, 69 of whom underwent pancreaticoduodenectomy and 19 did not undergo resection [32]. In 3'UTR C580T, the mutation rate was different between patients that had pancreaticoduodenectomy and patients that did not. In patients with surgery, 56 (81%) had the CC genotype, 13 (19%) had the CT genotype, and none had the TT genotype, and of the patients without surgery, 12 (63%) had the CC genotype, 5 (26%) had the CT genotype, and 2 (11%) had the TT genotype. There was significant disease-free survival between CC and CT/TT genotypes ($p = 0.039$). In another study, 5/120 (4%) of pancreatic cancer patients had CDKN2A mutations, namely G101W, E27X, and L65P [33]. Three of those patients had melanoma-pancreatic cancer kindred, suggesting that cancers were familial. Since only about 10% of pancreatic cancers are part of all familial cancers, the results showing substantially high mutation rates suggested that detected CDKN2A mutations are prevalent in the region where analysis was performed. Fluorescence in situ hybridization study showed CDKN2A deletion in 16/32 (50%) patients and overall survival was shorter in patients with gene deletion ($p = 0.002$) [34]. The study using a targeted deep sequencing assay detected CDKN2A mutations in 42/100 (42%) patients, and the presence of 0–2 vs. 3 mutations was prognostic for better overall survival ($p = 0.004$) [19].

SMAD4 (also known as DPC4 or MADH4) is tumor suppressor protein, which translocates to the nucleus as heterotrimeric SMAD2/SMAD3-SMAD4 complex after TGF β family receptors activation, where it activates the expression of genes and causes growth inhibition [35,36]. Mutations of SMAD4 occur in around 50% of pancreatic cancers, mostly leading to the loss of protein activated [37] (Table 1). Approximately 30% of mutations occur by homozygous deletion. Clinical research results show that SMAD4 inactivation by mutations could be used as a prognostic biomarker in pancreatic cancers. The study in 90 pancreatic cancer patients showed that SMAD4 mutations were present in 17/90 patients (19%) and gene mutation status was significantly associated with overall survival ($p = 0.006$) [38]. In another study, SMAD4 gene was inactivated in 8/25 (32%), 3/25 (12%) by homozygous deletion, and 5/25 (20%) by mutations in MH2 domain [39]. The study using a targeted deep sequencing assay detected SMAD4 mutations in 7/100 (7%) patients, and the presence of 0–2 vs. 3 mutations was prognostic for better overall survival ($p = 0.004$) [19].

BRCA1 (also known as RNF53) is a tumor suppressor, which acts as E3 ubiquitin-protein ligase that mediates the formation of Lys-6-linked polyubiquitin chains [40]. It is regulating cellular responses to DNA damage and thus is very important in DNA repair and G2/M cell cycle progression [41]. The gene is frequently mutated in quite a few familial cancers: it accounts for 45% of families with a high incidence of breast cancer and 80% of families with an elevated frequency of both breast and ovarian cancer [42]. Most of the BRCA1 mutations (88%) result in protein truncation and thus result in the loss of function of the protein and subsequently contribute to tumorigenesis. On the other hand, sporadic mutations of this gene is quite rare. Mutations of BRCA1 occur in approximately 6.6% of pancreatic patients and could be associated with familial pancreatic cancer risk [43] (Table 1). There is no direct clinical evidence that BRCA1 could be used as predictive biomarker, but a preclinical study using patient-derived xenografts showed that BRCA1 and BRCA2 mutations sensitize tumors to cisplatin chemotherapy [44].

Table 1. Gene mutation frequency in pancreatic cancer.

Gene	Frequency	Reference
KRAS	70%–95%	[15,16]
TP53	20%–76%	[23,24]
CDKN2A	49%–98%	[31,32]
SMAD4	19%–50%	[37,38]
BRCA1	6.6%–14%	[43,44]
BRCA2	3.6%–7.5%	[9,10,43,44]

BRCA2 (also known as FANCD1) is involved in double-strand break repair during the S phase of the cell cycle, by activating RAD51 recombinase. It is also involved in cytokinesis, centrosome duplication, and cell death [45]. Mutated and thus frequently inactivated BRCA2 cause an increased risk of developing breast, ovarian, prostate, stomach, and pancreatic familial cancers [46,47]. Mutations of BRCA2 are found in approximately 7.3% of familial pancreatic cancer patients and show the increased risk by up to 20-fold [9] (Table 1). However, in the case of sporadic cancer, very few cases of somatic mutations of BRCA2 are reported. A number of clinical studies were performed to estimate BRCA2 as potential prognostic and predictive biomarker for pancreatic cancer. The two-stage association study on pancreatic cancer that included 981 cases and 1991 controls in the first stage and 2603 cases and 2877 controls in a second stage showed that c.*532A>G variant located in the 3'-UTR is significantly associated with sporadic pancreatic cancer ($p < 0.0001$) [48]. A patient carrying rare BRCA2 1153insertionT mutation was unresponsive to gemcitabine therapy, but complete remission was reached, when cisplatin was added in combination with gemcitabine [49]. Another case of a pancreatic cancer patient with a 6174delT BRCA2 mutation showed prolonged survival after docetaxel, capecitabine, and gemcitabine combination followed by single agent irinotecan, despite prognostically unfavorable disease [50]. There was also a case of a patient with metastatic pancreatic cancer responding to a combination of mitomycin C and capecitabine [51]. All these cases combined show that BRCA2 mutations can be used as predictive biomarkers for increased sensitivity of pancreatic tumors to DNA-intercalating agents.

3. Conclusions

Most cancers, including pancreatic, are very genetically complex diseases. Many cancers develop because of gene mutations, which either render proteins in cells defective or totally delete them. Therefore, cancer research is striving towards the detection of as many genetic alterations in important genes as possible. New generation sequencing is an incredible technique, which helps to classify and systematize the entire range and characteristics of somatic and germline alterations [52]. It is now reaching a point where such a technique is going to be used by many laboratories for routine diagnostics, prognostics, and therapy prediction. There are also a growing number of companies that specialize in bioinformatics applications for clinical scientists to investigate data sets with hundreds of patient gene mutations and related clinical parameters. Up to now, gene mutation studies have discovered approximately 140 genes that can promote tumorigenesis when mutated. Normally, a tumor contains 2–8 of these “driver” mutations and the rest of them are passengers with no particular growth advantage for the tumor. It is important to reach a point at which our knowledge of the genes mentioned in this review will be sufficient to evaluate pancreatic cancer morbidity, mortality, and therapeutic approaches.

Author Contributions: Jonas Cicenas has written most of the article with some help of Kotryna Kvederaviciute and Edita Meskinyte-Kiausiliene. Ingrida Meskinyte has written most of the introduction with some help of Jonas Cicenas. Jonas Cicenas Jr. was responsible for IT and computer graphics. Aiste Skeberdyte mostly contributed to the improvement of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. American Cancer Society. Key Statistics for Pancreatic Cancer. How Common is Pancreatic Cancer? Available online: <https://www.cancer.org/cancer/pancreatic-cancer/about/key-statistics.html> (accessed on 16 February 2017).
2. United European Gastroenterology. Pancreatic Cancer Set to Become Third Biggest Cancer Killer in EU Next Year. Available online: <https://www.ueg.eu/press/releases/ueg-press-release/article/pancreatic-cancer-set-to-become-third-biggest-cancer-killer-in-eu-next-year/> (accessed on 16 February 2017).
3. Carrato, A.; Falcone, A.; Ducreux, M.; Valle, J.W.; Parnaby, A.; Djazouli, K.; Alnwick-Allu, K.; Hutchings, A.; Palaska, C.; Parthenaki, I. A systematic review of the burden of pancreatic cancer in Europe: Real-world impact on survival, quality of life and costs. *J. Gastrointest. Cancer* **2015**, *46*, 201–211. [CrossRef] [PubMed]

4. Rahib, L.; Smith, B.D.; Aizenberg, R.; Rosenzweig, A.B.; Fleshman, J.M.; Matrisian, L.M. Projecting cancer incidence and deaths to 2030: The unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res.* **2014**, *74*, 2913–2921. [[CrossRef](#)] [[PubMed](#)]
5. Smit, V.T.; Boot, A.J.; Smits, A.M.; Fleuren, G.J.; Cornelisse, C.J.; Bos, J.L. KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. *Nucleic Acids Res.* **1988**, *16*, 7773–7782. [[CrossRef](#)] [[PubMed](#)]
6. Caldas, C.; Hahn, S.A.; da Costa, L.T.; Redston, M.S.; Schutte, M.; Seymour, A.B.; Weinstein, C.L.; Hruban, R.H.; Yeo, C.J.; Kern, S.E. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat. Genet.* **1994**, *8*, 27–32. [[CrossRef](#)] [[PubMed](#)]
7. Redston, M.S.; Caldas, C.; Seymour, A.B.; Hruban, R.H.; da Costa, L.; Yeo, C.J.; Kern, S.E. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. *Cancer Res.* **1994**, *54*, 3025–3033. [[PubMed](#)]
8. Maurice, D.; Pierreux, C.E.; Howell, M.; Wilentz, R.E.; Owen, M.J.; Hill, C.S. Loss of SMAD4 function in pancreatic tumors: C-terminal truncation leads to decreased stability. *J. Biol. Chem.* **2001**, *276*, 43175–43181. [[CrossRef](#)] [[PubMed](#)]
9. Goggins, M.; Schutte, M.; Lu, J.; Moskaluk, C.A.; Weinstein, C.L.; Petersen, G.M.; Yeo, C.J.; Jackson, C.E.; Lynch, H.T.; Hruban, R.H.; et al. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res.* **1996**, *56*, 5360–5364. [[PubMed](#)]
10. Carnevale, J.; Ashworth, A. Assessing the significance of BRCA1 and BRCA2 mutations in pancreatic cancer. *J. Clin. Oncol.* **2015**, *33*, 3080–3081. [[CrossRef](#)] [[PubMed](#)]
11. Marais, R.; Light, Y.; Paterson, H.F.; Marshall, C.J. Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. *EMBO J.* **1995**, *14*, 3136–3145. [[PubMed](#)]
12. Gallo, A.; Cuzzo, C.; Esposito, I.; Maggiolini, M.; Bonofiglio, D.; Vivacqua, A.; Garramone, M.; Weiss, C.; Bohmann, D.; Musti, A.M. Menin uncouples Elk-1, JunD and c-Jun phosphorylation from MAP kinase activation. *Oncogene* **2002**, *21*, 6434–6445. [[CrossRef](#)] [[PubMed](#)]
13. Cicenys, J.; Tamosaitis, L.; Kvederaviciute, K.; Tarvydas, R.; Staniute, G.; Kalyan, K.; Meskinyte-Kausiliene, E.; Stankevicius, V.; Valius, M. KRAS, NRAS and BRAF mutations in colorectal cancer and melanoma. *Med. Oncol.* **2017**, *34*, 26. [[CrossRef](#)] [[PubMed](#)]
14. Al-Kzayer, L.F.; Sakashita, K.; Al-Jadiry, M.F.; Al-Hadad, S.A.; Ghali, H.H.; Uyen, L.T.N.; Liu, T.; Matsuda, K.; Abdulkadhim, J.M.; Al-Shujairi, T.A.; et al. Analysis of KRAS and NRAS Gene mutations in Arab Asian Children with acute leukemia: High frequency of RAS mutations in acute lymphoblastic leukemia. *Pediatr. Blood Cancer* **2015**, *62*, 2157–2161. [[CrossRef](#)] [[PubMed](#)]
15. Bamford, S.; Dawson, E.; Forbes, S.; Clements, J.; Pettett, R.; Dogan, A.; Flanagan, A.; Teague, J.; Futreal, P.A.; Stratton, M.R.; et al. The cosmic (catalogue of somatic mutations in cancer) database and website. *Br. J. Cancer* **2004**, *91*, 355–358. [[CrossRef](#)] [[PubMed](#)]
16. Kim, S.T.; Lim, D.H.; Jang, K.T.; Lim, T.; Lee, J.; Choi, Y.L.; Jang, H.L.; Yi, J.H.; Baek, K.K.; Park, S.H.; et al. Impact of KRAS mutations on clinical outcomes in pancreatic cancer patients treated with first-line gemcitabine-based chemotherapy. *Mol. Cancer Ther.* **2011**, *10*, 1993–1999. [[CrossRef](#)] [[PubMed](#)]
17. Boeck, S.; Jung, A.; Laubender, R.P.; Neumann, J.; Egg, R.; Goritschan, C.; Ormanns, S.; Haas, M.; Modest, D.P.; Kirchner, T.; et al. KRAS mutation status is not predictive for objective response to anti-EGFR treatment with erlotinib in patients with advanced pancreatic cancer. *J. Gastroenterol.* **2013**, *48*, 544–548. [[CrossRef](#)] [[PubMed](#)]
18. Golan, T.; Khvalevsky, E.Z.; Hubert, A.; Gabai, R.M.; Hen, N.; Segal, A.; Domb, A.; Harari, G.; David, E.B.; Raskin, S.; et al. Rnai therapy targeting kras in combination with chemotherapy for locally advanced pancreatic cancer patients. *Oncotarget* **2015**, *6*, 24560–24570. [[CrossRef](#)] [[PubMed](#)]
19. Hayashi, H.; Kohno, T.; Ueno, H.; Hiraoka, N.; Kondo, S.; Saito, M.; Shimada, Y.; Ichikawa, H.; Kato, M.; Shibata, T.; et al. Utility of assessing the number of mutated KRAS, CDKN2A, Tp53, and SMAD4 genes using a targeted deep sequencing assay as a prognostic biomarker for pancreatic cancer. *Pancreas* **2017**, *46*, 335–340. [[CrossRef](#)] [[PubMed](#)]
20. Calhoun, E.S.; Jones, J.B.; Ashfaq, R.; Adsay, V.; Baker, S.J.; Valentine, V.; Hempen, P.M.; Hilgers, W.; Yeo, C.J.; Hruban, R.H.; et al. BRAF and FBXW7 (CDC4, FBW7, AGO, SEL10) mutations in distinct subsets of pancreatic cancer: Potential therapeutic targets. *Am. J. Pathol.* **2003**, *163*, 1255–1260. [[CrossRef](#)]

21. Levy, N.; Yonish-Rouach, E.; Oren, M.; Kimchi, A. Complementation by wild-type p53 of interleukin-6 effects on m1 cells: Induction of cell cycle exit and cooperativity with c-Myc suppression. *Mol. Cell. Biol.* **1993**, *13*, 7942–7952. [[CrossRef](#)]
22. Bates, S.; Ryan, K.M.; Phillips, A.C.; Vousden, K.H. Cell cycle arrest and DNA endoreduplication following p21WAF1/CIP1 expression. *Oncogene* **1998**, *17*, 1691–1703. [[CrossRef](#)] [[PubMed](#)]
23. Hwang, R.F.; Gordon, E.M.; Anderson, W.F.; Parekh, D. Gene therapy for primary and metastatic pancreatic cancer with intraperitoneal retroviral vector bearing the wild-type p53 gene. *Surgery* **1998**, *124*, 143–150. [[CrossRef](#)]
24. Kern, S.E.; Pietenpol, J.A.; Thiagalingam, S.; Seymour, A.; Kinzler, K.W.; Vogelstein, B. Oncogenic forms of p53 inhibit p53-regulated gene expression. *Science* **1992**, *256*, 827–830. [[CrossRef](#)] [[PubMed](#)]
25. Grochola, L.F.; Taubert, H.; Greither, T.; Bhanot, U.; Udelnow, A.; Wurl, P. Elevated transcript levels from the MDM2 p1 promoter and low p53 transcript levels are associated with poor prognosis in human pancreatic ductal adenocarcinoma. *Pancreas* **2011**, *40*, 265–270. [[CrossRef](#)] [[PubMed](#)]
26. Xiang, J.F.; Wang, W.Q.; Liu, L.; Xu, H.X.; Wu, C.T.; Yang, J.X.; Qi, Z.H.; Wang, Y.Q.; Xu, J.; Liu, C.; et al. Mutant p53 determines pancreatic cancer poor prognosis to pancreatectomy through upregulation of cavin-1 in patients with preoperative serum CA19-9 ≥ 1000 u/mL. *Sci. Rep.* **2016**, *6*, 19222. [[CrossRef](#)] [[PubMed](#)]
27. Xiang, J.F.; Wang, W.Q.; Liu, L.; Xu, H.X.; Wu, C.T.; Yang, J.X.; Qi, Z.H.; Wang, Y.Q.; Xu, J.; Liu, C.; et al. Retraction: Mutant p53 determines pancreatic cancer poor prognosis to pancreatectomy through upregulation of cavin-1 in patients with preoperative serum CA19-9 ≥ 1000 u/mL. *Sci. Rep.* **2016**, *6*, 25115. [[CrossRef](#)] [[PubMed](#)]
28. Ormanns, S.; Siveke, J.T.; Heinemann, V.; Haas, M.; Sipos, B.; Schlitter, A.M.; Esposito, I.; Jung, A.; Laubender, R.P.; Kruger, S.; et al. Perk, PAKT and p53 as tissue biomarkers in erlotinib-treated patients with advanced pancreatic cancer: A translational subgroup analysis from AIO-PK0104. *BMC Cancer* **2014**, *14*, 624. [[CrossRef](#)] [[PubMed](#)]
29. Hussussian, C.J.; Struewing, J.P.; Goldstein, A.M.; Higgins, P.A.; Ally, D.S.; Sheahan, M.D.; Clark, W.H., Jr.; Tucker, M.A.; Dracopoli, N.C. Germline p16 mutations in familial melanoma. *Nat. Genet.* **1994**, *8*, 15–21. [[CrossRef](#)] [[PubMed](#)]
30. Vasen, H.F.; Gruis, N.A.; Frants, R.R.; van Der Velden, P.A.; Hille, E.T.; Bergman, W. Risk of developing pancreatic cancer in families with familial atypical multiple mole melanoma associated with a specific 19 deletion of p16 (p16-leiden). *Int. J. Cancer* **2000**, *87*, 809–811. [[CrossRef](#)]
31. Schutte, M.; Hruban, R.H.; Geradts, J.; Maynard, R.; Hilgers, W.; Rabindran, S.K.; Moskaluk, C.A.; Hahn, S.A.; Schwarte-Waldhoff, I.; Schmiegel, W.; et al. Abrogation of the RB/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res.* **1997**, *57*, 3126–3130. [[PubMed](#)]
32. Chen, J.; Li, D.; Killary, A.M.; Sen, S.; Amos, C.I.; Evans, D.B.; Abbruzzese, J.L.; Frazier, M.L. Polymorphisms of p16, p27, p73, and MDM2 modulate response and survival of pancreatic cancer patients treated with preoperative chemoradiation. *Ann. Surg. Oncol.* **2009**, *16*, 431–439. [[CrossRef](#)] [[PubMed](#)]
33. Ghiorzo, P.; Gargiulo, S.; Nasti, S.; Pastorino, L.; Battistuzzi, L.; Bruno, W.; Bonelli, L.; Taveggia, P.; Pugliese, V.; Borgonovo, G.; et al. Predicting the risk of pancreatic cancer: On CDKN2A mutations in the melanoma-pancreatic cancer syndrome in Italy. *J. Clin. Oncol.* **2007**, *25*, 5336–5337. [[CrossRef](#)] [[PubMed](#)]
34. Luo, Y.; Tian, L.; Feng, Y.; Yi, M.; Chen, X.; Huang, Q. The predictive role of p16 deletion, p53 deletion, and polysomy 9 and 17 in pancreatic ductal adenocarcinoma. *Pathol. Oncol. Res.* **2013**, *19*, 35–40. [[CrossRef](#)] [[PubMed](#)]
35. Dai, J.L.; Turnacioglu, K.K.; Schutte, M.; Sugar, A.Y.; Kern, S.E. DPC4 transcriptional activation and dysfunction in cancer cells. *Cancer Res.* **1998**, *58*, 4592–4597. [[PubMed](#)]
36. Cao, D.; Ashfaq, R.; Goggins, M.G.; Hruban, R.H.; Kern, S.E.; Iacobuzio-Donahue, C.A. Differential expression of multiple genes in association with MADH4/DPC4/SMAD4 inactivation in pancreatic cancer. *Int. J. Clin. Exp. Pathol.* **2008**, *1*, 510–517. [[PubMed](#)]
37. De Bosscher, K.; Hill, C.S.; Nicolas, F.J. Molecular and functional consequences of SMAD4 c-terminal missense mutations in colorectal tumour cells. *Biochem. J.* **2004**, *379*, 209–216. [[CrossRef](#)] [[PubMed](#)]
38. Blackford, A.; Serrano, O.K.; Wolfgang, C.L.; Parmigiani, G.; Jones, S.; Zhang, X.; Parsons, D.W.; Lin, J.C.; Leary, R.J.; Eshleman, J.R.; et al. SMAD4 gene mutations are associated with poor prognosis in pancreatic cancer. *Clin. Cancer Res.* **2009**, *15*, 4674–4679. [[CrossRef](#)] [[PubMed](#)]

39. Singh, P.; Srinivasan, R.; Wig, J.D. SMAD4 genetic alterations predict a worse prognosis in patients with pancreatic ductal adenocarcinoma. *Pancreas* **2012**, *41*, 541–546. [[CrossRef](#)] [[PubMed](#)]
40. Boulton, S.J. Brca1-mediated ubiquitylation. *Cell Cycle* **2006**, *5*, 1481–1486. [[CrossRef](#)] [[PubMed](#)]
41. Deng, C.X.; Wang, R.H. Roles of BRCA1 in DNA damage repair: A link between development and cancer. *Hum. Mol. Genet.* **2003**, R113–R123. [[CrossRef](#)]
42. Xu, C.F.; Solomon, E. Mutations of the BRCA1 gene in human cancer. *Semin. Cancer Biol.* **1996**, *7*, 33–40. [[CrossRef](#)] [[PubMed](#)]
43. Stadler, Z.K.; Salo-Mullen, E.; Patil, S.M.; Pietanza, M.C.; Vijai, J.; Saloustros, E.; Hansen, N.A.; Kauff, N.D.; Kurtz, R.C.; Kelsen, D.P.; et al. Prevalence of brca1 and BRCA2 mutations in ashkenazi jewish families with breast and pancreatic cancer. *Cancer* **2012**, *118*, 493–499. [[CrossRef](#)] [[PubMed](#)]
44. Lohse, I.; Borgida, A.; Cao, P.; Cheung, M.; Pintilie, M.; Bianco, T.; Holter, S.; Ibrahimov, E.; Kumareswaran, R.; Bristow, R.G.; et al. BRCA1 and BRCA2 mutations sensitize to chemotherapy in patient-derived pancreatic cancer xenografts. *Br. J. Cancer* **2015**, *113*, 425–432. [[CrossRef](#)] [[PubMed](#)]
45. Rudkin, T.M.; Foulkes, W.D. BRCA2: Breaks, mistakes and failed separations. *Trends Mol. Med.* **2005**, *11*, 145–148. [[CrossRef](#)] [[PubMed](#)]
46. Friedenson, B. BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. *MedGenMed* **2005**, *7*, 60. [[PubMed](#)]
47. Welch, P.L.; King, M.C. BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. *Hum. Mol. Genet.* **2001**, *10*, 705–713. [[CrossRef](#)] [[PubMed](#)]
48. Huang, L.; Wu, C.; Yu, D.; Wang, C.; Che, X.; Miao, X.; Zhai, K.; Chang, J.; Jiang, G.; Yang, X.; et al. Identification of common variants in BRCA2 and MAP2K4 for susceptibility to sporadic pancreatic cancer. *Carcinogenesis* **2013**, *34*, 1001–1005. [[CrossRef](#)] [[PubMed](#)]
49. Sonnenblick, A.; Kadouri, L.; Appelbaum, L.; Peretz, T.; Sagi, M.; Goldberg, Y.; Hubert, A. Complete remission, in BRCA2 mutation carrier with metastatic pancreatic adenocarcinoma, treated with cisplatin based therapy. *Cancer Boil. Ther.* **2011**, *12*, 165–168. [[CrossRef](#)]
50. James, E.; Waldron-Lynch, M.G.; Saif, M.W. Prolonged survival in a patient with BRCA2 associated metastatic pancreatic cancer after exposure to camptothecin: A case report and review of literature. *Anticancer Drugs* **2009**, *20*, 634–638. [[CrossRef](#)] [[PubMed](#)]
51. Chalasani, P.; Kurtin, S.; Dragovich, T. Response to a third-line mitomycin C (MMC)-based chemotherapy in a patient with metastatic pancreatic adenocarcinoma carrying germline BRCA2 mutation. *JOP* **2008**, *9*, 305–308. [[PubMed](#)]
52. Meldrum, C.; Doyle, M.A.; Tohill, R.W. Next-generation sequencing for cancer diagnostics: A practical perspective. *Clin. Biochem. Rev.* **2011**, *32*, 177–195. [[PubMed](#)]

