Journal of
Drug Designing & Bioinformatics

JDDB, 1(2), 42-57, 2022
https://doi.org/10.58985/jddb.2023.v01i02.07
ISSN(Print):2959-3123

Review Article

Detection and diagnosis of pancreatic cancer: Computational biology approaches

Samvedna Singh , Shantanu Tomar, Madhu Goswami and **Shakti Sahi ***

School of Biotechnology, Gautaum Buddha University, Greater Noida, India

Article Information:
Received: 26 May 2023
Revised: 23 June 2023
Accepted: 23 June 2023

Corresponding Author:
Shakti Sahi
shaktis@gbu.ac.in

Academic Editor:
Prof. Dr. Samir Chtita

Keywords:
Pancreatic ductal Adenocarcinoma, Somatic mutation, KRAS, Tumor suppressor, Driver genes, Mutational analyses, Diagnosis, Computational tools, Databases.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the fatal cancers among all critical cancers. The progression of the disease is primarily due to the oncogene activation and inactivation of tumor suppressor genes causing genome instability and contributing to this malignancy in human cells. Somatic mutations drive cancer progression, and thus identification of such molecular alterations has the potential to deliver a deeper understanding of the nature of that tumor. Even though next-generation sequencing has discovered several functional mutations in *KRAS*, *TP53*, *CDNK2A*, *SMAD4*, and *BRCA1/2*, their clinical effects remain unclear. Pancreatic cancer remains unmanageable, with a 5-year survival rate of 5-10%. The biological significance of core driver genes, the importance of studying somatic mutations leading to the disease diagnosis their use in clinical practice and an account of computational tools and databases that assist in a detailed mutational analysis have been discussed in this review.

1. Introduction

1.1 Pancreatic cancer

Pancreatic cancer is the eighth leading cause of cancer-related deaths. In 2020 total of 495,773 cases was reported globally [1]. In 2022, new pancreatic cancer cases reported in the USA were 62,210 [2]. As per GLOBOCAN, pancreatic cancer is the 24th most common disease in India, approximately 10,860 new cases have been reported and ranked at 18th position in terms of the highest fatality rate [3]. People diagnosed with pancreatic cancer have a 5-year survival of 5-10%. The survival rate is affected by several factors. However, when diagnosed the specific cancer stage plays a crucial role [4]. Amongst pancreatic cancer, up to 93% are exocrine adenocarcinoma; the remaining 7% are pancreatic neuroendocrine tumors. The intraductal papillary mucinous and pancreatic intraepithelial neoplasia are significant precursors of PDAC [5].

1.2 Molecular Genetics of Pancreatic Cancer

Extensive research has established that pancreatic cancer is an inherited disease with various somatic mutations. Analyzing somatic mutations allows for differentiating pancreatic adenocarcinoma from other malignant neoplasms of the pancreas [6]. These mutations can be defined as alterations in the DNA sequence that may arise during replication be repaired incorrectly, or be left unrepaired. Several exogenous mutagens like chemicals, UVs, ionizing radiations, and endogenous mutagens

like reactive oxygen species, aldehydes, and repairing enzymes, can cause DNA damage. Different mutational processes have different unique patterns termed mutational signatures. Analyzing the signature patterns facilitates quantifying their effect on biological activity in a cancerous and non-cancerous genome [7]. A germinal mutation takes place in the germline. Germline mutations are also inherited, as the mutant cell participates in fertilization and passes the mutation to the next generation. Cancer due to the germline mutation is inherited or hereditary cancer. Next-generation analysis has proven promising in identifying germline mutations in genes, including CDKN21, TP53, BRCA2, ATM, MLH1, and BRCA1 responsible for pancreatic cancer progression in 5.5% of the cases. Spontaneous variations occur in somatic cells of a human body that include Single Nucleotide Variants (SNVs), chromosomal aberrations, Copy Number Variation (CNVs), insertion and deletions, which are known as "somatic mutations"[8]. Somatic mutations in the early-stage lead to developmental disorders, whereas intensifying accretion of these mutations for an extended period can lead to cancer progression.

1.3 Somatic Mutation

Somatic mutations cannot be passed down to the offspring except for the canine transmissible venereal tumor. Somatic mutations influence the antibodies, T cell, and B cell receptors. Some factors such as the environment, often trigger them, and they build up in any organism's DNA despite effective DNA repair mechanisms. Somatic mutations occur at a frequency of 2 to 6 mutations per million bases in healthy tissues [8]. As a result, somatic cells in the same organism may have different genotypes (somatic mosaicism) in healthy development and ageing. According to a study [9] point mutations ranging from 1000 to 20,000 and multiple insertions, deletions, and rearrangements contribute to cancer development and progression. These figures were derived from research involving millions of mutations in different cancer forms [10].

Somatic mutations include point mutations, repeats, deletions, insertions, multiplication, copy number loss, and other genomic variations. When somatic cells split, chromosomal somatic mutations occur. Chromosome breakages, inappropriate fixing, and unequal material exchangeduring chromosome separation cause structural aberrations during this period. These mutations disrupt genes and their pathways responsible for cell growth and proliferation, apoptosis, neovascularization, and other cancer hallmarks that lead to neoplasm development.

1.3.1 Somatic Mutation in Pancreatic Cancer

Somatic mutations are involved in the progression of cancers, which makes mutational profiling one of the foremost analyses of the other omics analysis to be considered in clinical practice. The majority of diagnosis at the clinical level is based on single-gene mutations. High throughput technologies have underlined that somatic alterations are a part of the process of growth and development. These somatic alterations may obstruct gene functions, such as the deactivation of tumor suppressor genes and oncogene activation and thus disrupt and deregulate crucial pathways that regulate normal cell growth [11]. Since almost no tumor can form without somatic mutations, they are essential to oncogenesis [12,13]. Since the existence or absence of particular mutations may dictate cancer therapy, determining a patient's mutational profile is essential in ensuring successful care. In colorectal, lung, pancreatic, and other cancer forms specific chemotherapeutics dependent on mutational status are already part of cancer therapies [14].

The accumulation of somatic point mutations, also known as single nucleotide variants (SNVs), in the genome can disrupt cell activity and lead to cancer initiation and progression. The entire repertoire of SNVs across a cancer genome (which can number in the thousands) can be used to infer clonal populations and research tumor evolution statistically. As a result, accurate identification of all somatic SNVs including those with low prevalence is critical since they can identify clones with desirable phenotypic characteristics. Biomedical investigators researching tumor progression also try to determine how particular clones are linked to properties like drug resistance, metastatic ability, and fitness under selective therapeutic pressures. Somatic mosaicism refers to the genetic heterogeneity caused by somatic mutations [15]. PDAC is known to

arise from PanINlesions (pancreatic intraepithelial neoplasia) by accumulating somatic changes in critical genes over time. (Fig.1) [16].

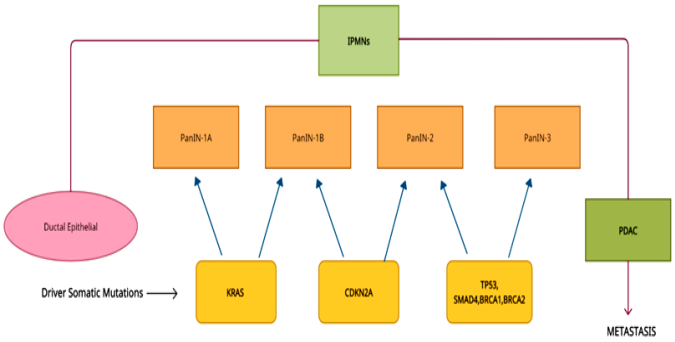

Figure 1: Driver gene mutations in pancreatic adenocarcinoma carcinogenesis, classification of pancreatic intraepithelial neoplasia (PanIN) precursor of pancreatic cancer, at its different stages (1A, 1B, 2, 3) due to the somatic mutations occurring in driver genes leading to cancer metastasis.

The occurrence of pancreatic cancer begins with precursor lesions such as intraductal papillary mucinous neoplasms (IPMN), pancreatic intraepithelial neoplasia (PanIN) and mucinous cystic neoplasms (MCN). One of the most common and well-described PDAC precursor lesions is PanIN. Gene mutations occurring at the Pancreatic intraepithelial neoplasia stage in due course advanced dysplastic condition. According to the dysplasia state precursor lesions exhibit varying levels of mucin, varied architectural patterns and different proliferation rates which eventually results in changes in gene functionality and cancer cell progression.

Isolation might occur in copy number alteration in PDAC. These events are similar to structural alterations in which a chromosome substitutes for another. Chromothripsis is one of the standard techniques through which various structural alterations occur in one cataclysmic mitotic incident. Detection of chromothripsis through susceptible techniques shows that it can be located in 65% of PDACs, in many cases, before polyploidization. Chromothripsis can occur separately or with the additional complex genomic incident and involve multiple chromosomes resulting in gene amplification, deletions, or double minutes formation in either case. Alternatively, structural rearrangements could result from continuous genomic damage caused by a lack of DNA repair. Advance extensive studies of DNA copy-number changes (CNAs) led to the discovery of WGD in human tumors. WGD was more than twice as prevalent (13per cent each) as TERT promoter and oncogenic KRAS mutations.

In a comprehensive series of pancreatic cancers, whole-genome sequencing revealed 2.64 Mb of a mutational burden on an average per somatic mutation [17]. The four most frequently mutated tumor suppressor genes are The Kirsten rat sarcoma (KRAS) oncogene, the tumor suppressor protein 53 (TP53), SMAD family member 4 (SMAD4), and the cyclin-dependent kinase inhibitor 2A (CDKN2A). All these mutations dysregulate signaling pathways, thereby affecting the proliferation of tumor cells and crosstalk with the desmoplastic TNM (tumor, nodes, and metastasis) surrounding them [18].

Next-generation sequencing is an excellent technique for classifying and systematizing the full spectrum of somatic alterations and their characteristics. In sporadic pancreatic cancer studies whole-exome sequencing and whole-genome sequencing have led to identifying genes that, when mutated, can induce tumorigenesis [19]. KRAS, TP53, CDKN2A, and SMAD4 are the four main genetic alterations identified in PDAC and most mutations are point mutations [20]. The most prevalent KRAS and TP53 mutations are seen in early-stage intraepithelial neoplasia implyingthat they have a role in tumor initiation [12].

2. Mutations in pancreatic cancer

The classification of PDAC with the understanding of molecular, genetic, and morphological details will be beneficial in developing targeted and potent therapeutics in clinical practice. The detailed analysis of somatic variants will bring out essential findings. Other than top genes like *KRAS, TP53, SMAD4, CDKN2A*, and *BRCA1/2*studies have reported somatic mutations in various genes (Fig. 2) such as *ATM, TGFBR2, ARID2A, SF3B1, GNAS, EGFR, ERBB3, GAT6* that are involved in [20-23] crucial biological pathways causing PDAC. Driver genes in PDAC are listed below, and a few frequently reported mutations according to COSMIC and TCGA are mentioned in Table 1.

2.1 KRAS Mutation

In 90 per cent to 93 per cent of pancreatic tumors oncogenic KRAS mutations are detected. KRAS is a GTPase of size 21kDa, which gets activated on binding to GTP and deactivated upon binding with GDP. As KRAS gets activated, it further activates RAF family kinases RAF-1, BRAF, and ARAF. RAF family members thenget phosphorylated and activate MEK-1 and MEK-

2. These MEK-1 and MEK-2 further activate the extracellular regulatory kinases ERK-1 and ERK-2. These cause cell proliferations by bringing cytosolic and nuclear proteins like transcription factors ELK-1 and c-Jun [24]. The mutations cause constitutive activation of KRAS, resulting in various processes like uncontrolled proliferation, which causes cancer to develop and spread across the cells and tissues. KRAS is also responsible for regulating multiple signaling pathways which are reportedly involved in cancer progressions, such as PI3K-AKT, PLC- PKC, and RAL. Mutations of the codons G12, G13, or Q61, by and large, correspond to constitutively active KRAS, activated KRAS. The periodic mutations in K117 and A146 are also known to occur. Activating mutations in KRAS are reported in ninety-five per cent of pancreatic cancer cases. Of these ninety-nine per cent of all mutations occur in G12 (G12D- 50%) [25].

Mutations in KRAS highly contribute to the initiation and progression of pancreatic cancer. KRAS mutations alter RAS proteins. Practically every mutation in KRAS is SNVs in PDACs, appearing in codons 12(~91%), 13 (~2%), and 61(~7%). The mutations at codon 12 are reported to energize AKT/protein kinase B pathway providing resistance to apoptosis [26]. KRAS mutations in pancreatic cancer are due to neoplastic transformation. Most reports about its mutation have been on relatively small tumors, which lack the statistical justification to determine the appropriate association with the disease outcome [27]. KRAS oncogene has a mutational frequency of 20 to 100% and can be used for diagnostic purposes. A subset of tumors contains multiple

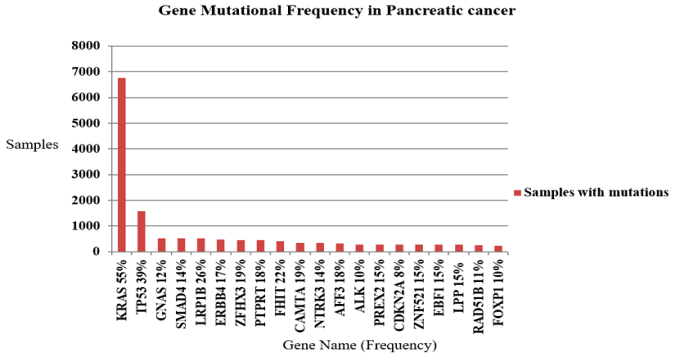

Figure 2: Gene mutation frequency of top 20 mutated genes in pancreatic cancer.

mutations in KRAS with some displaying evidence of biallelic mutations.

2.2 TP53 Mutation

TP53 also called antigen NY-CO-13 or p53 provides instructions for producing a protein called p53 which acts as a tumor suppressor. One of the functions of TP53 is the activation of target genes during DNA damage or oxidative stress and inducing apoptosis [28]. It enhances the expression level of CDKN1A due to which the cell cycle is arrested [29]. It is regarded as the Guardian of the Genome as it helps in cell division and DNA repair. In 70% of pancreatic cancer cases, TP53 is most frequently mutated resulting in its binding ability [24,30]. In a study of pancreatic adenocarcinoma patients, less mRNA expression of TP53 was associated with a poor disease prognosis. Clinical evidence suggests it can be a prognostic marker for diagnosis and therapy.

2.3 CDKN2A Mutation

The complex of the two cyclins CDK-4 and CDK-6 is involved in the cell cycle's phase transition from G1 to S. The tumor suppressor gene CDKN2A regulates the cell cycle progression by suppressing the CDK-4 and CDK-6 complex. The CDKN2A gene is located on chromosome 9p21 in the region that shows high-frequency loss of heterozygosity in various neoplasia. The tumor suppressor region of the CDKN2A gene encodes two distinctproteins, P16 and P14. P16 consists of three exons that arrest the cell cycle at the G1 phase thereby stopping cell growth [31]. The phosphorylation of retinoblastoma protein is obstructed. The retinoblastoma protein affects the E2F transcription factor and participates in the negative regulation of the cell cycle. Another protein, p14ARF, has a negative effect on cell growth as it stabilizes p53 activation and targets some CDKs at G1 and G2 phases thereby inducing apoptosis [32]. Mutations like promoter silencing, heterozygosity, or homozygous deletion disrupt the operation of CDKN2A. Some clinical studies on CDKN2A mutation reported these mutations as a prognostic and prophetical biomarker.

2.4 SMAD4 Mutation

SMAD4 acts as tumor suppressor gene. It is known to be deactivated in more than 50% of pancreatic

Table 1. List of genes and mutations predominantly involved in pancreatic cancer.

S.No	Gene name	Mutation type	Position
1.	KRAS	MISSENSE	G12D, G12V, G12C, G13D, Q61H, G12R, G12A, G12S, A146T
2.	PIK3CA	MISSENSE	E545K, H1047R, E542K, R88Q, H1047L, N345K, E726K, G118D
3.	NRAS	MISSENSE	Q61R, Q61K, Q13R, G12D, Q61L
4.	FBXW7	MISSENSE	R465H, R505G, R465C
5.	BRAF	MISSENSE	V600E, V600M
6.	CDKN2A	STOP GAINED	R80*, R58*
7.	APC	STOP GAINED FRAMESHIFT	R1450*, R876*, R1114* T1556NFs3*
8.	3'UTR PTEN	MISSENSE FRAMESHIFT STOP GAINED	R130Q, R130G K267RFs*9, T319* R233*, R130*
9.	TP53	MISSENSE STOP GAINED SPLICE REGION	R175H, R248Q, R273C, R273H, R248W, R282W, Y220C, G245S, H179R, H193R, V157F, Y163C, R273L, C176F, I195T, R249S, E285K, C176Y R213*, R196*, R342*, R306*, Q192* T125T
10.	ARID1A	FRAMESHIFT STOP GAINED	D1850Tfs*33 R1989*
11.	IDH1	MISSENSE	R132H, R132C
12.	FGFR2	MISSENSE	S252W
13.	FGFR3	MISSENSE	S249C
14.	CTNN31	MISSENSE	S37F
15.	EGFR	MISSENSE	L858R
16.	GNAQ	MISSENSE	Q209F
17.	AKT1	MISSENSE	E17K
18.	ERBB2	MISSENSE	S310F
19.	GNA11	MISSENSE	Q209L
20.	PPP2R1A	MISSENSE	P179R
21.	BCOR	MISSENSE	N1459S
22.	HRAS	MISSENSE	Q61R
23.	POLE	MISSENSE	P286R
24.	SPECC1	FRAMESHIFT	N303TFs*63
25.	JAK1	FRAMESHIFT	K860NFs*16
26.	RPL22	FRAMESHIFT	K15RFs*5
27.	UBR5	FRAMESHIFT	E2121KFs*28
28.	CTCF	FRAMESHIFT	T204NFs*26
29.	KMT2D	FRAMESHIFT	F2354LFs*17
30.	AKAP9	FRAMESHIFT	K39RFs*17

adenocarcinomas, inactivation occurs due to homozygous deletion or intragenic mutation. SMAD-4 translocate itself in trimeric form into the nucleus activates gene expression and causes cell growth inhibition [33,34]. SMAD-4 proteins can transduce signals from the cell surface to the nucleus. SMAD-4 mediates TGF-β transduction and gene regulation. Transforming growth factors regulate proliferation, differentiation, motility, and necrobiosis [34].

2.5 BRCA1/2Mutation

BRCA1/2 gene is a tumor suppressor that plays a significant role in the recognition, transcription, regulation, and double-strand break repair of DNA to forestall cell types from developing mutations [35]. Somatic mutationsin BRCA1 and BRCA2 are reported in about 9% of PDAC patients. Somatic mutations of BRCA2 appear to be uncommon in tumors of the pancreas. The mechanism by which mutant BRCA2

contributes to pancreatic cancer development is unknown. Inactivation of several independent functions of BRCA2, such as remodeling of chromatin, transcriptional gene control, DNA damage repair, and cell development and also appears to provide a pathophysiological basis for the interrelation between BRCA2 mutations and pancreatic cancer [36].

3. Tools for mutational analysis

3.1 Tools used for somatic mutations

Several tools are available for detecting and analyzing somatic mutations (Table 2), users can choose the tool depending on the data type and user interface.

3.1.1 Mutalisk [37]

Mutalisk associates somatic mutations with genomic, transcriptional, and epigenomic features to understand better mutational processes that contribute to mutation generation. This web-based technology combines physical genome mapping with somatic mutation identification. The results are displayed using graphics and charts. Mutalisk only accepts VCF files as input.
http//mutalisk.org/analyze.php

3.1.2 VarMap [38]

VarMap is a web-based tool for mapping chromosomal coordinates to canonical UniProt sequences and associated protein 3D structures, including validation checks and structural annotation. It can consider patient variant information, environmental context, and spatial protein distribution of genetic variants.
https//bio.tools/VarMap

3.1.3 Somatic sniper [39]

Somatic sniper detects differences in single-nucleotide location between malignancy and normal samples. It uses the genotype likelihood model to compute the

Table 2. List of tools for somatic mutation detection and analysis.

S.No	Name of tools	Web-based/ language-based	Freely available	Feature	Input data	Links/source
1.	Broad GDAC firehouse [52]	Web-based	Yes	Performs various automated analyses. Mutational analyses, Correlation analyses, Differential expression analyses, and Pathway analyses across all types of cancers.	TCGA	http// gdac.broadinstitute. org/
2.	cBioportal [73]	Web-based	Yes	Allows correlation analyses for copy number alterations or methylation of genes. The portal also facilitates users to study gene(s) of interest with access of Onco Printer and Mutation Mapper	TCGA CCLE	http// cbioportal.org (Ceramiet *al.*, 2012)
3.	TCGA Clinical explorer [52]	Web-based	yes	Enables users to conclude relevant clinical information from TCGA data and allows them to translate the clinical data into the classification of drivers genes, miRNA and proteins	TCGA	http// genomeportal.stanford.edu/pan-tcga/ Weinstein *et al.*, 2013)
4.	TCGA4U [74]	Web-based	yes	Genomic alterations that occurred in the tumor can be understood using this tool to study the relationship of genomic alterations with clinical data.	TCGA	http// www.tcga4u.org, 8888
5.	UCSC Xena [75]	Web-based	yes	This tool performs the comparative analysis of tumor samples to normal samples to explore a gene expression whether it is up or down-regulated in one or more cancer types.	TCGA GDC ICGC GTEx TARGET TOIL	http// xena.ucsc.edu/ get ting-started/
6.	Vanno [76]	Web-based		Performs in-depth analysis of cancer-causing genome sequence alterations. Functional predictions and mutation landscapes of TCGA data can be derived.	TCGA	http// cgts.cgu.edu.tw/ vanno

Table 2 (*Continued*)

S.No	Name of tools	Web-based/ language-based	Freely available	Feature	Input data	Links/source
7.	MutEnricher [77]	Python-based software	yes	Investigate both coding and non-coding region for somatic mutation enrichment of the genome.	TCGA and other cancer databases.	https// github.com/ asoltis/ MutEnricher
8.	MutaLisk [37]	Web-based	yes	Perform the comparative analysis of somatic mutations along with physical mapping of the genome.	Data uploaded in file format (vcf format)	http// mutalisk.org/ analyze.php
9.	VarMap [38]	Web-based	Yes	Useful to map the genomic coordinates to protein.	The vcf file format is uploaded	https// www.ebi.ac.uk/ thornton-srv/ databases/cgi-bin/VarSite/ GetPage.pl? varmap=TRUE
10.	Somatic Sniper [39]	Software	Yes implemented in C	This tool identifies the single nucleotide positions to differentiate between normal and tumours genes in the form of a somatic scores.	dbSNP	http// gmt.genome.wustl.edu/packages/ somatic-sniper/
11.	MutaNet [40]	Software Codes in Python	yes	Perform the statistical analysis of mutations in the genome. This tool enables to identify the impactful mutations.	UniProt AureoWiki, PATRIC, Cytoscape, NCBI SRA, ReguIonDB, Regprecise	https// service.bioinformatik.uni-saarland.de/ mutanet/
12.	VarSim [41]	Software Code in Java and Python	yes	Simulates and validates the different types of variants such as large structural variants, SNV, insertions and deletions.	COSMIC dbSNP, DGV VCF file format	http// bioinform.github.io/ varsim/
13.	SomVarIUS [42]	Software written in Python 2.7.	yes	Using high-throughput sequencing can identify a somatic mutation in unpaired tissue samples	TCGA Takes sorted alignment files (.bam) as input and Output is in the variant call format (.vcf)	https// github.com/ kylessmith/ SomVarIUS
14.	MutaGene [43]	Web-based Python Package	yes	Web-based tool for identification of mutations and mutational processes to analyse genes and calculate the DNA and protein stability.	ICGC TCGA PCGP COSMIC(WGS)	https// www.ncbi.nlm.nih.gov/research/ mutagene/
15.	VarScan 2 [44]	Command-line software written in Java	yes	Detects copy number alterations and other somatic mutations from exome data of normal and tumor pairs.	NGS data SOLiD, Life/PGM, Roche/454	http// dkoboldt.github.io/varscan/using-varscan.html
16.	CHASM [46]	Language-based tool	yes	This tool discriminates somatic missense mutations as cancer drivers.	list of somatic missense mutations	http// wiki.chasmsoftware.org
17.	MutSig CV [47]	Language-based MATLAB2013a	yes	Examines the mutational changes found in DNA sequencing and identifies mutated genes.	MAF file	https// software.broadinstitute.org/cancer/cga/ mutsig

somatic score, the likelihood of genotype changes between tumor and normal samples.

http//gmt.genome.wustl.edu/packages/somatic-sniper/

3.1.4 MutaNet [40]

MutaNet was created to determine the impact of specific mutations on gene regulation and genome performance. MutaNet analyses antibiotic resistance gene alterations and their possible impact on antibiotic resistance in bacterial strains. MutaNeT analyses mutations in various genomic areas statistically. The program also includes

mutations in a given gene regulatory network to assess their global impact.
https//service.bioinformatik.uni-saarland.de/mutanet/

3.1.5 VarSim [41]

VarSim can simulate and validate various variants, including single nucleotide variants, minor indels, and significant structural variants. It is a comprehensive, automated computing framework that supports parallel computing and numerous read simulators. VarSim is the only program that can mimic SNVs, minor indels, and various types of SVs. VarSim's completeness makes it a near match to real-world sequencing investigations.
https//bioinform.github.io/varsim/

3.1.6 SomVarIUS [42]

A computational method for detecting somatic mutations in unpaired tissue samples using high-throughput sequencing data. SomVarIUS takes sorted alignment files (.bam) as input and produces predicted somatic mutations in the variant call format (.vcf) allowing it to be easily integrated into any conventional genome analysis pipeline. It also produces an extra output that includes all the information regarding the status of known cancer disease-associated mutations in samples.
https//github.com/kylessmith/SomVarIUS

3.1.7 MutaGene [43]

MutaGene can determine the context-dependent mutability of DNA locations and anticipated amino acid substitutions across the whole genome. Mutability can be used as a background model to identify probable driver mutations, relating cancer genetics to phenotype. It aids in decoupling the relative roles of mutagenesis and selection in carcinogenesis. Mutations from cancer samples can be submitted in VCF format, Mutagene can recognize them, break them down into individual mutational signatures, and determine the closely related cancer kind, primary location, and cluster of samples with similarmutational profiles.
https//www.ncbi.nlm.nih.gov/research/mutagene/

3.1.8 VarScan2 [44]

VarScan is a platform-independent mutation caller for targeted mutations. VarScan 2 detects somatic mutations and copy number changes (CNAs) in neoplasia–normal pairs of exome data. It may help discover germline mutations, multiplesample variants, somatic mutations, and somatic copy number modifications.
https//dkoboldt.github.io/varscan/using-varscan.html

3.1.9 MuTect [45]

MuTect was created by the Broad Institute for the accurate and reliable identification of somatic mutations in cancer genome next-generation sequencing data. It identifies somatic mutations using paired and normal and neoplasia cells as input. MuTect employs a variant detection statistic to determine whether a variation is more likely than a sequencing error. MuTect then searches for and removes six types of known sequencing artefacts MuTect has been frequently employed in cancer genomes research at the Broad Institute.
https//institute.org/cancer/cga/mutect

3.1.10 CHASM [46]

CHASM (Cancer-Specific High Throughput Annotation of Somatic Mutations) to distinguish and focus on missense mutations most likely to cause beneficial modifications that increase the normal cell's uncontrolled growth property. CHASM employs a random classifier forest technique to distinguish between synthetically manufactured passenger and driver missense mutations.
https//wiki.chasmsoftware.org

3.1.11 MutSigCV [47]

MutSig is an abbreviation for "mutational significance." MutSig analyses mutational changes discovered in DNA sequencing to identify genes that were changed more frequently than expected by chance, given the background mutation process. MutSigCV considers heterogeneity by employing patient-specific mutation frequencies and spectra and gene-specific mutation rates, expression, and replication times.
https//software.broadinstitute.org/cancer/cga/mutsig

3.2 Databases for mutation analyses

Various databases are available for studying somatic mutation in different aspects, some of the important databases and case studies are discussed below (Table 3).

3.2.1 Mutfunc [48]

Mutfunc is a mutational database that includes predictions based on a single nucleotide alteration in

three organisms (Humans, *E. coli*, Yeast). Protein stability, interaction interfaces, post-translational changes, and transcription factor binding sites are among the mechanisms investigated.
https//www.mutfunc.com/

3.2.2 Cancer 3D [49]

Cancer 3D is a free and open-source database that examines missense mutations in the context of protein structure in cancer. The Cancer3D database contains the findings of such investigations and data from The Cancer Genome Atlas (TCGA) and the Cancer Cell Line Encyclopedia (CCLE). The database also assists users in analyzing the distribution patterns of mutations and their association with changes in pharmacological activity using two algorithms e-Drug and e-Driver.
https//www.cancer3d.org/search

3.2.3 Intogene [50]

Intogene collects and analyses somatic mutations in hundreds of neoplasia genomes to identify cancer-driver genes. Intogene database employs seven distinct methods for identifying cancer driver genes and compiles the output data of driver genes and a library of mutational features that can be utilized to explain and comprehendthe mechanism of action.
https//www.intogen.org/search

3.2.4 TANRIC [51]

TANRIC is an open-source site that analyses long non-coding RNAs (lncRNA). These lncRNAs are crucial in cancer biology. TANRIC analyses lncRNAs in clinical and molecular data contexts using the expression patterns of cancer datasets from TCGA, CCLE, and other independent datasets. It is a useful tool for determining the function and clinical significance of

Table 3. List of databases dedicated to mutation analyses.

S.No	Name of databases	Description	Links
1.	Mutfunc [48]	The mutational analysis includes stability, interaction, modification and TF binding sites.	http//www.mutfunc.com/
2.	Cancer3D [49]	This database analyses the missense mutation regarding protein structure and helps the user to analyse the pattern of mutations.	http//www.cancer3d.org/search (TCGA CCLE)
3.	Intogene [50]	Intogene analyses the somatic mutations from tumor genomes for cancer driver genes identification. It uses different methods for driver genes identification and compiles the output file for better exploration and analysis.	https//www.intogen.org/search (TCGA ICGC)
4.	TANRIC [51]	TANRIC analysis includes the long non-coding RNAs in the context of clinical and molecular data.	https//www.tanric.org (TCGA, CCLE)
5	TCGA [52]	TCGA has complete data on cancer and is stored in the GDC portal. The generated information is from a cancer patient and can be used for clinical significance, mutational analysis and gene expression profiling.	https //www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga
6.	Cosmic [53]	Somatic mutation database. It has data from expert manual curation and genome-wide screen. Several browsing tools and datasets are present for comparative analysis of cancer.	https //cancer.sanger.ac.uk/cosmic
7.	TCPA [54]	Portal is used for the visualization and analysis of functional proteomics	https//tcpaportal.org/tcpa/ (TCGA)
8.	GEO database [55]	An NCBI database which has data from various high throughput methods, microarray experiments, next-generation sequencing etc. This database organised the data in a very informative form for easily accessible and better understanding.	https// www.ncbi.nlm.nih.gov/geo/ (NCBI)
9.	CMPD [78]	CMPD contains more than 2 million genetic alterations, two major components of CMPD are, a web interface for the database SOLite and another for retrieval of mutated protein sequences.	http//cgbc.cgu.edu.tw/cmpd
10.	ClinVar [56]	ClinVar provides all the information regarding the relationship between the human variation and phenotype.	https// www.ncbi.nlm.nih.gov/clinvar/

lncRNAs in cancer considerably facilitating lncRNA-related biological discoveries and clinical features.
https//www.tanric.org

3.2.5 TCGA [52]

The NCI and National Human Genome Research Institute collaborated on the Cancer Genome Atlas program. Over 12 years, the TCGA collected comprehensive cancer data from 11000 patients. The full cancer data set is processed and savedin the GDC portal. The information derived from the collected data includes clinical significance, molecular analysis, and gene expression profiling.
https//www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga

3.2.6 COSMIC [53]

Cancer somatic mutations catalogue. Cosmic is a repository for all somatic mutations associated with human cancer in a catalogue format with extensive analysis. There are two sorts of data in Cosmic expert manual curation and genome-wide screen data. Cosmic is organized by numerous discrete projects that present a variety of datasets and browsing tools for comparative research.
https//cancer.sanger.ac.uk/cosmic

3.2.7 TCPA [54]

The Cancer Proteome Atlas is a comprehensive resource for accessing, visualization and analyzing cancer functional proteomics. This resource provides an idiosyncratic opportunity to verify the findings from TCGA data and identify model celllines for functional investigation.
https//tcpaportal.org/tcpa/

3.2.8 GEO Database [55]

The GEO database is a freely accessible resource that distributes functional genomics microarray, next-generation sequencing, and other forms of high throughput data. Platform, sample, series, datasets, and profiles are several types of geo data. GEO search analysis can be done in numerous ways, including using GEO datasets to search for data relevant to their research and GEO profiles. A Gene expression can be investigated and retrieved at this gene-level base or further analysis.
https//www.ncbi.nlm.nih.gov/geo/

3.2.9 ClinVar [56]

ClinVar is a freely accessible database that contains all information about the relationships between human variants and phenotypes. ClinVar reviews submissions identifying variations detected in patient samples, claims about their clinical significance, submitter information, and supporting data. ClinVar enables us to comprehend the relationship between human variants and observed health states and the history of that interpretation.
https//www.ncbi.nlm.nih.gov/clinvar/

3.3 Analysis of Mutations using COSMIC

The Catalogue of Somatic Mutation (COSMIC) is a comprehensive and systematic database for studying the role of somatic mutations in human cancers. It lists various mutations, including gene fusions, copy number variations, non-coding, drug resistance, and coding mutations. It contains the library of cancer-causing genes, Cancer Gene Census (CGC) assembled by specialists from various medical reporting, pharmaceutical development, and laboratory research [53] and tools for analysis (Fig. 3). The most recent release contains around 6 million coding mutations from 1.4 million samples from over 26,000 studies, this approach uses hidden Markov models to predict protein missense variations' functional, genetic, and phenotypic implications. Cosmic uses TCGA gene expression level 3 data and methylation data from the ICGC portal for TCGA investigations. COSMIC provides for discovering new cancer treatment targets and biomarkers by providing detailed information on mutation distributions, mutational signature analyses and effects. Improve the collection of clinical trial cohorts. Identify driver mutations and associated genes to aid in patient diagnosis.

For example, researchers in a study [57] used the COSMIC database, which contains somatic mutations from The Cancer Genome Atlas (TCGA) and several smaller-scale investigations. Researchers used multi-label classification algorithms and the Disease Ontology hierarchy to find cancer subtype-specific biomarkers.

Saha *et al.* [21] used databases such as TCGA and COSMIC to perform mutation annotation and harmful property prediction analysis. They expected that TP53 would be the most frequently altered gene (41 per cent) among the 114 reported somatic mutations, followed by KRAS, SMAD4, CTNNB1, and ERBB3. We uncovered a new TP53 hotspot mutation (p.A138V, in 17 per cent of all patients).

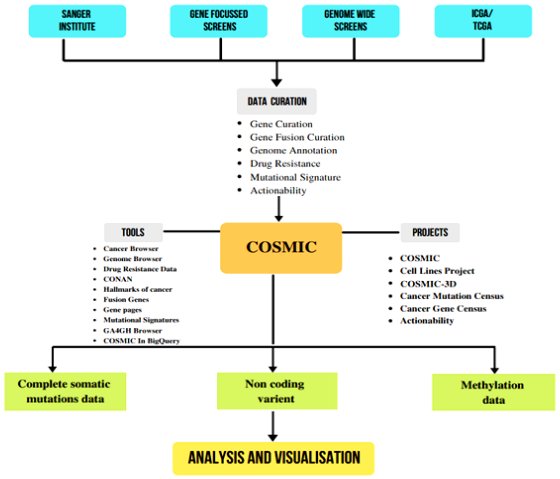

Figure 3: Overview of tools and projects available for data analysis and their applications.

3.4 Analysis of TCGA Data for Somatic Mutation

TCGA, The Cancer Genome Atlas (https// cancergenome.nih.gov/) has genome-wide data from over 30 cancer types and thousands of somatic mutations that advance the understanding of tumorigenesis. To identify somatic mutations, exome sequencing data is used that allows the detection of SNVs, Single amino acid substitutions. In addition to Mutational Analysis, TCGA is used for Survival Analysis, Correlation Analysis, Methylation Analysis, Exploration of cancer drivers, Differential Analysis, and Pathway Analysis. In a study, Baek and Lee [58] analyzed whole-exome sequencing data of 134 PDAC patients. They discovered five genes, KRAS, CDKN2A, TTN, TP53, and KCNJ18, mutated in the beginning stages of tumorigenesis. In another latest study, Hwang *et al.* [59] used TCGA gene expression data for unsupervised clustering and identified three distinct molecular subtypes belonging to three different pathways and were also able to validate them in another cohort using each subtype-specific gene (200 were chosen). Various powerful yet easy-to-use tools (Fig. 4) are also provided to analyze and visualize TCGA data, such as The Broad GDAC portal, TCGA Clinical Explorer, Cancer3D, TCGA4U, and UCSC Xena and Vanno, which allow for performing mutation analysis.

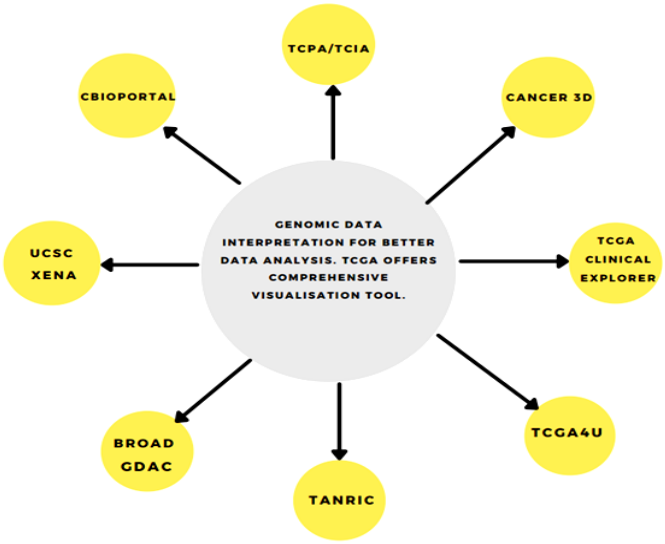

Figure 4: Tools for better visualization and interpretation of multidimensional data are available from TCGA.

4. Current treatment and novel opportunities

4.1 Anti-RAS therapy

Surgery followed by adjuvant chemotherapy is the only possible treatment option for PDAC, but only 15–20 per cent of patients are suitable for surgery [60]. The only targeted treatment for PDAC is a combination of gemcitabine and an epidermal growth factor receptor (EGFR) inhibitor, which can improve life by a statistically significant but clinically unsatisfactory twelve days compared to gemcitabine alone. Patients with advanced pancreatic cancer are treated with multiagent combination chemotherapy, such as irinotecan/oxaliplatin/5- fluorouracil or nab-paclitaxel/ gemcitabine, although their median overall survival is > a year. In PDAC, therapeutic methods have been mainly ineffective, with no treatment prolonging lifebeyond one year following diagnosis. In 93 per cent of pancreatic cancers, KRAS mutations arefound. There are additional opportunities for therapeutics targeting individual mutant KRAS isoforms, particularly with small molecule inhibitors of KRAS G12C. KRAS testing will be required to determine the particular KRAS mutation present [61].

4.2 KRAS inhibitors

The development of KRAS inhibitors has proven difficult due to various reasons. Competitive inhibitors have a very high affinity for GTP. After binding to the GTP binding site that cannotbe overtaken, inhibitors of allosteric groups have been challenging to create due to the lack ofpockets for drug binding on the KRAS surface [62].

4.3 G12C inhibitors

KRAS G12C inhibitors are present in only 1% of pancreatic cancer cases, which is very uncommon [63,64]. In the 12th position, glycine-to-cysteine mutation triggered the KRAS oncoprotein, increasing tumor cell cycle progression. The mutated cysteine is located near a switch II pocket (P2). A small molecule known as Sotorasib (AMG 510) inhibits KRAS G12C in a reversible and specific manner via a unique interaction with the P2 pocket [65]. A study shows that G12C inhibitors can bind with a recently discovered P2 surface pocket on KRAS and covalently bind to the mutant G12C protein's reactive cysteine residue, according to a study. Another inhibitor of KRAS G12C, adagrasib (MRTX849), had a confirmed response in one patient with pancreatic cancer [66].

4.4 G12D inhibitors

RAS-selective inhibitor RMC-6236 binds to cyclophilin A, a chaperone protein, and constructs a tri-complex with the specific RAS protein. Multiple RAS mutants, notably KRAS G12V and KRAS G12D, have their signaling inhibited in their GTP-bound conformations [67]. KRAS G12D inhibitors are in preclinical development. One direct inhibitor, which is MRTX1133, is currently undergoing research trials.

4.5 SOS inhibitors

SOS1 is a Guanine exchange factor that converts GDP to GTP to activate KRAS and GTPase- activating proteins. KRAS signaling is controlled by enzymes that catalyze the intrinsic hydrolysis of GTP back to GDP to inactivate KRAS. The guanine exchange factor SOS1 catalyzes the conversion of GDP to GTP to trigger KRAS and degrade the interaction of the SOS1-KRAS complex, preventing KRAS from storing GTP. Treatment with a MEK inhibitor reduces SOS1 phosphorylation by ERK and relieves negative response to SOS1, allowing SOS1-mediated feedback loops to restore RAS-mediated signaling. New small molecule SOS1 inhibitors impair SOS1-KRAS binding in various KRAS mutations. The SOS1 inhibitor BI-3406 reduced GTP-bound RAS and reduced proliferation in practically all KRAS codon 12 and 13 mutants examined. It worked in tandem with MEK inhibitors to prevent feedback reactivation [65].

4.6 Immunotherapy-based treatment strategies for sporadic PDAC

The combination of immunotherapy and chemotherapy has a considerable impact on PDAC. Several positive prediction markers for immune checkpoint inhibitors (ICIs) have been reported in recent studies, including an increased level of MSI-H, overexpression of PD-L1, increased TMB, and gene mutations. SMAD4 and TSC2 mutations were reported in stage 4 pancreatic cancer treated with immunotherapy. The patient responded partially to treatment, with the lesions diminishing and gradually decreasing. Responses of this magnitude are extremely rare in metastatic pancreatic cancer [68].

After surgical resection recurrence of pancreatic cancer still occurs in a high percentage of patients within the first two years. Using immunotherapy in conjunction with other treatments like chemotherapy and/or radiation in both neoadjuvant and adjuvant settings has improved the survival rate of the patients [69]. In the adjuvant trial, a phase II multi-institutional study that examined the use of algenpantucel-L immunotherapy in conjunction with chemotherapy and chemoradiotherapy produced 62% disease-free survival and 86% overall survival after 12 months. Although the survival of patients did not improve at the time of phase III IMPRESS clinical trial [70, 71]. In a recent trial, 30 patients in Japan received the OCV-C01 multi-peptide vaccine from the KIF20A protein, which contains peptides from the VEGFR1, VEGFR2, and the vascular endothelial growth factor receptor (VEGFR)1. Results demonstrated that 58.6% of patients had cytotoxic lymphocyte responses against KIF20A. In the realm of pancreatic cancer immunotherapy-based treatment, encouraging outcomes have been seen. However, the success of the therapy will depend on the prediction of

further combinatorial trials aimed at various mutations [72].

5. Discussion

PDAC is one the most lethal cancer with a terrible prognosis. Currently, no screening measures can detect cancer in its early stages which is why its poor overall survival. Individual characteristics lifestyle diabetes and other diseases are some risk factors that provide some indication for screening and etiological prevention. Surgical removal of pancreatic cancer is often difficult due to the organ's location, therefore, studying mutations and targeting them with combination drug therapies becomes crucial. The four significant most significant factors to consider when researching the disease are the four significant mutant driver genes (KRAS, TP53, CDKN2A, and SMAD4) and their biochemical pathways pathway, PI3K/AKT signaling pathway, Janus kinase and activator of transcription (JAK/STAT), and MAPK pathways are crucial pathways involved in pancreatic cancer. Current treatment includes chemo-drugs such as gemcitabine, Folfirinox, and 5-Fluorouracil (5-FU). These drugs are used in combination with other anticancer drugs. The advancement of sequencing technologies and tumor genetic profiling have reported various genes, pathways, potential prognostic markers, and mutations involved in pancreatic cancer that have helped in providing detailed insights into the mechanism of onset of the disease. However, despite these efforts, pancreatic cancer remains unmanageable. Novel screening and diagnostic methods for detecting resectable PDAC early on, neoadjuvant therapy to increase the number of patients eligible for curative resection. Somatic mutation detection and adjuvant therapy to improve postoperative survival in curative resections and palliative disease patients will overcome the challenges in PDAC management. Somatic mutations play a significant role in the development and progression of cancer disease; therefore, mutational profiling is a crucial step in therapeutic decision making.

6. Conclusions

In this review, a detailed account of somatic mutation and its different types, along with top mutations in PDAC and the characterization of driver genes has been studied in the present study. Numerous tools, variant analysis pipelines, and databases for analyzing mutation treatment options and new possibilities for PDAC are also discussed. Studying somatic mutations in pancreatic cancer can not only help strengthen the disease mechanism but will also help in dictating the treatment possibilities.

Authors' contributions

Conceptualized and drafted the review, S.S. and S.S.; Data mined and analyzed the information, S.S.; S.T. and M.G.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021, 71 (3), 209–249.
2. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2022. CA Cancer J Clin. 2022, 72 (1), 7–33.
3. Gaidhani, R.H.; Balasubramaniam, G. An epidemiological review of pancreatic cancer with special reference to India. Indian J Med Sci. 2021, 73, 99.
4. Ryan, D.P.; Hong, T.S.; Bardeesy, N. Pancreatic Adenocarcinoma. New England Journal of Medicine. 2014, 371 (11), 1039–1049.
5. Maitra, A.; Hruban, R.H. Pancreatic cancer. Annual Review of Pathology: Mechanisms of Disease. 2008, 3 (1), 157–188.
6. Alexandrov, L.B.; Nik-Zainal, S.; Wedge, D.C.; Aparicio, S. A. J. R.; Behjati, S.; Biankin, A.V.; Bignell, G.R.; Bolli, N.; Borg, A.; Børresen-Dale, A.L.; et al. Signatures of mutational processes in human cancer. Nature. 2013, 500 (7463), 415–421.
7. Omichessan, H.; Severi, G.; Perduca, V. Computational tools to detect signatures of mutational processes in DNA from tumours: A review and empirical comparison of performance. PLoS One. 2019, 14 (9), e0221235.
8. Martincorena, I.; Campbell, P.J. Somatic mutation in cancer and normal cells. Science. (1979). 2015, 349 (6255), 1483–1489.
9. Cibulskis, K.; Lawrence, M.S.; Carter, S.L.; Sivachenko, A.; Jaffe, D.; Sougnez, C.; Gabriel, S.; Meyerson, M.; Lander, E. S.; Getz, G. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples.

Nat. Biotechnol. 2013, 31 (3), 213–219.
10. Park, S.; Kim, S.J.; Yu, D.; Peña-Llopis, S.; Gao, J.; Park, J.S.; Chen, B.; Norris, J.; Wang, X.; Chen, M.; et al. An integrative somatic mutation analysis to identify pathways linked with survival outcomes across 19 cancer types. Bioinformatics. 2016, 32 (11), 1643–1651.
11. Esteller, M. Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. Br. J. Cancer. 2006, 94 (2), 179–183.
12. Jones, S.; Zhang, X.; Parsons, D.W.; Lin, J.C.H.; Leary, R.J.; Angenendt, P.; Mankoo, P.; Carter, H.; Kamiyama, H.; Jimeno, A.; et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science (1979). 2008, 321 (5897), 1801–1806.
13. Martini, M.; Vecchione, L.; Siena, S.; Tejpar, S.; Bardelli, A. Targeted therapies: how personal should we go? Nat. Rev. Clin. Oncol. 2012, 9 (2), 87–97.
14. Van Cutsem, E.; Köhne, C.H.; Láng, I.; Folprecht, G.; Nowacki, M.P.; Cascinu, S.; Shchepotin, I.; Maurel, J.; Cunningham, D.; Tejpar, S.; et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: Updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J. Clin. Oncol. 2011, 29 (15), 2011–2019.
15. Dou, Y.; Gold, H.D.; Luquette, L.J.; Park, P.J. Detecting somatic mutations in normal cells. Trend. Gen. 2018, 34 (7), 545–557.
16. Waddell, N.; Pajic, M.; Patch, A.M.; Chang, D.K.; Kassahn, K.S.; Bailey, P.; Johns, A.L.; Miller, D.; Nones, K.; Quek, K.; et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015, 518 (7540), 495–501.
17. Kamisawa, T.; Wood, L.D.; Itoi, T.; Takaori, K. Pancreatic Cancer. The Lancet. 2016, 388 (10039), 73–85.
18. Collisson, E.A.; Bailey, P.; Chang, D.K.; Biankin, A.V. Molecular subtypes of pancreatic cancer. Nat. Rev. Gastroenterol Hepatol. 2019, 16 (4), 207–220.
19. Casolino, R.; Paiella, S.; Azzolina, D.; Beer, P.A.; Corbo, V.; Lorenzoni, G.; Gregori, D.; Golan, T.; Braconi, C.; Froeling, F. E. M.; et al. Homologous recombination deficiency in pancreatic cancer: A systematic review and prevalence meta-analysis. J. Clin. Oncol. 2021, 39 (23), 2617–2631.
20. Hayashi, A.; Hong, J.; Iacobuzio-Donahue, C.A. The pancreatic cancer genome revisited. Nat. Rev. Gastroenterol Hepatol. 2021, 18 (7), 469–481.
21. Saha, G.; Singh, R.; Mandal, A.; Das, S.; Chattopadhyay, E.; Panja, P.; Roy, P.; DeSarkar, N.; Gulati, S.; Ghatak, S.; et al. A novel hotspot and rare somatic mutation p.A138V, at TP53 is associated with poor survival of pancreatic ductal and periampullary adenocarcinoma patients. Mol. Med. 2020, 26 (1), 59.
22. Crowley, F.; Park, W.; O'Reilly, E.M. Targeting DNA damage repair pathways in pancreas cancer. Cancer Met. Rev. 2021, 40 (3), 891–908.
23. Lowery, M.A.; Jordan, E.J.; Basturk, O.; Ptashkin, R.N.; Zehir, A.; Berger, M.F.; Leach, T.; Herbst, B.; Askan, G.; Maynard, H.; et al. Real-time genomic profiling of pancreatic ductal adenocarcinoma: potential actionability and correlation with clinical phenotype. Clin. Cancer Res. 2017, 23 (20), 6094–6100.
24. Cicenas, J.; Kvederaviciute, K.; Meskinyte, I.; Meskinyte-Kausiliene, E.; Skeberdyte, A.; Cicenas, J. KRAS, TP53, CDKN2A, SMAD4, BRCA1, and BRCA2 mutations in pancreatic cancer. Cancers (Basel) 2017, 9 (12), 42. https://doi.org/10.3390/cancers9050042.
25. 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 (2), 355–358.
26. Vizan, P.; Boros, L.G.; Figueras, A.; Capella, G.; Mangues, R.; Bassilian, S.; Lim, S.; Lee, W.N. P.; Cascante, M.K. Ras codon-specific mutations produce distinctive metabolic phenotypes in human fibroblasts. Cancer Res. 2005, 65 (13), 5512–5515.
27. Rachakonda, P.S.; Bauer, A.S.; Xie, H.; Campa, D.; Rizzato, C.; Canzian, F.; Beghelli, S.; Greenhalf, W.; Costello, E.; Schanne, M.; et al. Somatic mutations in exocrine pancreatic tumors: Association with patient survival. PLoS One. 2013, 8 (4), e60870.
28. 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 (12), 7942–7952.
29. Bates, S.; Ryan, K.M.; Phillips, A.C.; Vousden, K.H. Cell cycle arrest and DNA endoreduplication following P21Waf1/Cip1 expression. oncogene 1998, 17 (13), 1691–1703.
30. Kern, S.; Pietenpol, J.; Thiagalingam, S.; Seymour, A.; Kinzler, K.; Vogelstein, B. Oncogenic forms of P53 inhibit P53-regulated gene expression. Science (1979). 1992, 256 (5058), 827–830.
31. McWilliams, R.R.; Wieben, E.D.; Rabe, K.G.; Pedersen, K.S.; Wu, Y.; Sicotte, H.; Petersen, G.M. Prevalence of CDKN2A mutations in pancreatic cancer patients: implications for genetic counseling. Eur. J. Hum.Gen. 2011, 19 (4), 472–478.
32. Cairns, P.; Mao, L.; Merlo, A.; Lee, D. J.; Schwab, D.; Eby, Y.; Tokino, K.; van der Riet, P.; Blaugrund, J.E.; Sidransky,

D. Rates of P16 (MTS1) Mutations in primary tumors with 9p loss. Science (1979). 1994, 265 (5170), 415–417.
33. Zhao, M.; Mishra, L.; Deng, C.X. The role of TGF-β/ SMAD4 signaling in cancer. Int. J. Biol. Sci. 2018, 14 (2), 111–123.
34. Liang, C.; Xu, J.; Meng, Q.; Zhang, B.; Liu, J.; Hua, J.; Zhang, Y.; Shi, S.; Yu, X. TGFB1-induced autophagy affects the pattern of pancreatic cancer progression in distinct ways depending on SMAD4 status. Autophagy. 2020, 16 (3), 486–500.
35. Rosen, M.N.; Goodwin, R. A.; Vickers, M.M. BRCA Mutated pancreatic cancer: A change is coming. World J. Gastroenterol. 2021, 27 (17), 1943–1958.
36. Vietri, M.T.; D'Elia, G.; Caliendo, G.; Albanese, L.; Signoriello, G.; Napoli, C.; Molinari, A.M. Pancreatic cancer with mutation in BRCA1/2, MLH1, and APC Genes: phenotype correlation and detection of a novel germline BRCA2 mutation. genes (Basel). 2022, 13 (2), 321.
37. Lee, J.; Lee, A. J.; Lee, J.K.; Park, J.; Kwon, Y.; Park, S.; Chun, H.; Ju, Y.S.; Hong, D. Mutalisk: A web-based somatic mutation analysis toolkit for genomic, transcriptional and epigenomic signatures. Nucleic Acids Res. 2018, 46 (W1), W102–W108.
38. Stephenson, J.D.; Laskowski, R.A.; Nightingale, A.; Hurles, M.E.; Thornton, J.M. VarMap: A web tool for mapping genomic coordinates to protein sequence and structure and retrieving protein structural annotations. Bioinfor. 2019, 35 (22), 4854–4856.
39. Larson, D.E.; Harris, C.C.; Chen, K.; Koboldt, D.C.; Abbott, T.E.; Dooling, D.J.; Ley, T.J.; Mardis, E.R.; Wilson, R. K.; Ding, L. Somatic sniper: Identification of somatic point mutations in whole genome sequencing data. Bioinfor. 2012, 28 (3), 311–317.
40. Hollander, M.; Hamed, M.; Helms, V.; Neininger, K. MutaNET: A tool for automated analysis of genomic mutations in gene regulatory networks. Bioinform. 2018, 34 (5), 864–866.
41. Mu, J.C.; Mohiyuddin, M.; Li, J.; Bani Asadi, N.; Gerstein, M.B.; Abyzov, A.; Wong, W.H.; Lam, H.Y.K. VarSim: A high-fidelity simulation and validation framework for high-throughput genome sequencing with cancer applications. Bioinform. 2015, 31 (9), 1469–1471.
42. Smith, K.S.; Yadav, V.K.; Pei, S.; Pollyea, D.A.; Jordan, C.T.; De, S. SomVarIUS: somatic variant identification from unpaired tissue samples. Bioinform. 2016, 32 (6), 808 –813.
43. Goncearenco, A.; Rager, S.L.; Li, M.; Sang, Q.X.; Rogozin, I.B.; Panchenko, A.R. Exploring background mutational processes to decipher cancer genetic heterogeneity. Nucleic Acids Res. 2017, 45 (W1), W514–W522.
44. Koboldt, D.C.; Zhang, Q.; Larson, D.E.; Shen, D.; McLellan, M.D.; Lin, L.; Miller, C.A.; Mardis, E.R.; Ding, L.; Wilson, R.K. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. 2012, 22 (3), 568–576.
45. Do Valle, Í.F.; Giampieri, E.; Simonetti, G.; Padella, A.; Manfrini, M.; Ferrari, A.; Papayannidis, C.; Zironi, I.; Garonzi, M.; Bernardi, S.; et al. Optimized pipeline of MuTect and GATK tools to improve the detection of somatic single nucleotide polymorphisms in whole-exome sequencing data. BMC Bioinform. 2016, 17 (S12), 341.
46. Carter, H.; Samayoa, J.; Hruban, R. H.; Karchin, R. Prioritization of driver mutations in pancreatic cancer using cancer-specific high-throughput annotation of somatic mutations (CHASM). Cancer Biol. Ther. 2010, 10 (6), 582–587.
47. Lawrence, M.S.; Stojanov, P.; Polak, P.; Kryukov, G.V.; Cibulskis, K.; Sivachenko, A.; Carter, S.L.; Stewart, C.; Mermel, C.H.; Roberts, S.A.; et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013, 499 (7457), 214–218.
48. Wagih, O.; Galardini, M.; Busby, B.P.; Memon, D.; Typas, A.; Beltrao, P. A resource of variant effect predictions of single nucleotide variants in model organisms. Mol. Syst. Biol. 2018, 14 (12).
49. Porta-Pardo, E.; Hrabe, T.; Godzik, A. Cancer3D: Understanding cancer mutations through protein structures. Nucleic Acids Res. 2015, 43 (D1), D968–D973.
50. Gonzalez-Perez, A.; Perez-Llamas, C.; Deu-Pons, J.; Tamborero, D.; Schroeder, M.P.; Jene-Sanz, A.; Santos, A.; Lopez-Bigas, N. IntOGen-mutations identifies cancer drivers across tumor types. Natz. Methods. 2013, 10 (11), 1081–1082.
51. Li, J.; Han, L.; Roebuck, P.; Diao, L.; Liu, L.; Yuan, Y.; Weinstein, J. N.; Liang, H. TANRIC: An interactive open platform to explore the function of LncRNAs in cancer. Cancer Res. 2015, 75 (18), 3728–3737.
52. Weinstein, J.N.; Collisson, E.A.; Mills, G.B.; Shaw, K.R.M.; Ozenberger, B.A.; Ellrott, K.; Shmulevich, I.; Sander, C.; Stuart, J.M. The cancer genome atlas pan-cancer analysis project. Nat. Genet. 2013, 45 (10), 1113–1120.
53. Tate, J.G.; Bamford, S.; Jubb, H.C.; Sondka, Z.; Beare, D.M.; Bindal, N.; Boutselakis, H.; Cole, C.G.; Creatore, C.; Dawson, E.; et al. COSMIC: The catalogue of somatic mutations in cancer. Nucleic Acids Res. 2019, 47 (D1), D941–D947.
54. Li, J.; Lu, Y.; Akbani, R.; Ju, Z.; Roebuck, P.L.; Liu, W.; Yang, J.Y.; Broom, B.M.; Verhaak, R.G. W.; Kane, D.W.; et al. TCPA: A resource for cancer functional proteomics data. Nat. Methods. 2013, 10 (11), 1046–1047.
55. Barrett, T.; Wilhite, S.E.; Ledoux, P.; Evangelista, C.; Kim, I.F.; Tomashevsky, M.; Marshall, K.A.; Phillippy, K.H.;

Sherman, P.M.; Holko, M.; et al. NCBI GEO: Archive for functional genomics data sets—update. Nucleic Acids Res. 2012, 41 (D1), D991–D995.
56. Landrum, M.J.; Lee, J.M.; Riley, G.R.; Jang, W.; Rubinstein, W.S.; Church, D.M.; Maglott, D.R. ClinVar: Public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014, 42 (D1), D980–D985.
57. Amar, D.; Izraeli, S.; Shamir, R. Utilizing somatic mutation data from numerous studies for cancer research: proof of concept and applications. Oncogene. 2017, 36 (24), 3375–3383.
58. Baek, B.; Lee, H. Prediction of survival and recurrence in patients with pancreatic cancer by integrating multi-omics data. Sci. Rep. 2020, 10 (1), 18951.
59. Hwang, J.W.; Jang, S.K.; Lee, D.J. Genomic analysis of pancreatic cancer reveals 3 molecular subtypes with different clinical outcomes. Medicine. 2021, 100 (14), e24969.
60. Qian, Y.; Gong, Y.; Fan, Z.; Luo, G.; Huang, Q.; Deng, S.; Cheng, H.; Jin, K.; Ni, Q.; Yu, X.; Liu, C. Molecular alterations and targeted therapy in pancreatic ductal adenocarcinoma. J. Hematol. Oncol. 2020, 13 (1), 130.
61. Lambert, A.; Schwarz, L.; Borbath, I.; Henry, A.; Van Laethem, J.L.; Malka, D.; Ducreux, M.; Conroy, T. An update on treatment options for pancreatic adenocarcinoma. Ther. Adv. Med. Oncol. 2019, 11, 175883591987556.
62. Lee, M.S.; Pant, S. Personalizing medicine with germline and somatic sequencing in advanced pancreatic cancer: current treatments and novel opportunities. American Society of Clinical Oncology Educational Book. 2021, No. 41, e153–e165.
63. Spencer-Smith, R.; O'Bryan, J.P. direct inhibition of RAS: quest for the holy Grail? Semin. Cancer Biol. 2019, 54, 138–148.
64. Bailey, P.; Chang, D.K.; Nones, K.; Johns, A.L.; Patch, A.M.; Gingras, M.C.; Miller, D.K.; Christ, A.N.; Bruxner, T. J.C.; Quinn, M.C.; et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016, 531 (7592), 47–52.
65. Simanshu, D.K.; Nissley, D.V.; McCormick, F. RAS Proteins and their regulators in human disease. Cell. 2017, 170 (1), 17–33.
66. Hayashi, A.; Hong, J.; Iacobuzio-Donahue, C.A. The Pancreatic Cancer Genome Revisited. Nat Rev Gastroenterol Hepatol. 2021, 18 (7), 469–481.
67. Sakamoto, K.; Masutani, T.; Hirokawa, T. Generation of KS-58 as the First K-Ras(G12D)-inhibitory peptide presenting anti-cancer activity in vivo. Sci. Rep. 2020, 10 (1), 21671.
68. Ye, Y.; Zheng, S. Successful immunotherapy for pancreatic cancer in a patient with TSC2 and SMAD4 mutations: a case report. Front. Immunol. 2021, 12.
69. Kole, C.; Charalampakis, N.; Tsakatikas, S.; Frountzas, M.; Apostolou, K.; Schizas, D. Immunotherapy in combination with well-established treatment strategies in pancreatic cancer: current insights. Cancer Manag Res. 2022, 14, 1043–1061.
70. Hardacre, J.M.; Mulcahy, M.; Small, W.; Talamonti, M.; Obel, J.; Krishnamurthi, S.; Rocha-Lima, C.S.; Safran, H.; Lenz, H.J.; Chiorean, E.G. Addition of algenpantucel-L immunotherapy to standard adjuvant therapy for pancreatic cancer: a phase 2 study. J. Gastro. Surg. 2013, 17 (1), 94–101.
71. Hewitt, D.B.; Nissen, N.; Hatoum, H.; Musher, B.; Seng, J.; Coveler, A.L.; Al-Rajabi, R.; Yeo, C.J.; Leiby, B.; Banks, J.; et al. A phase 3 randomized clinical trial of chemotherapy with or without algenpantucel-L (hyperacute-pancreas) immunotherapy in subjects with borderline resectable or locally advanced unresectable pancreatic cancer. Ann. Surg. 2022, 275 (1), 45–53.
72. Torphy, R.J.; Zhu, Y.; Schulick, R.D. Immunotherapy for pancreatic cancer: barriers and breakthroughs. Ann. Gastro. Surg. 2018, 2 (4), 274–281.
73. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The CBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012, 2 (5), 401–404.
74. Huang, Z.; Duan, H.; Li, H. Identification of gene expression pattern related to breast cancer survival using integrated TCGA datasets and genomic tools. Biomed Res. Int. 2015, 2015, 1–10.
75. Goldman, M.J.; Craft, B.; Hastie, M.; Repečka, K.; McDade, F.; Kamath, A.; Banerjee, A.; Luo, Y.; Rogers, D.; Brooks, A.N.; et al. Visualizing and interpreting cancer genomics data via the xena platform. Nat. Biotechnol. 2020, 38 (6), 675–678.
76. Huang, P.J.; Lee, C.C.; Tan, B. C.M.; Yeh, Y.M.; Huang, K.Y.; Gan, R.C.; Chen, T.W.; Lee, C.Y.; Yang, S.T.; Liao, C.S.; et al. Vanno: a visualization-aided variant annotation tool. Hum Mutat. 2015, 36 (2), 167–174.
77. Soltis, A.R.; Dalgard, C.L.; Pollard, H.B.; Wilkerson, M.D. MutEnricher: A flexible toolset for somatic mutation enrichment analysis of tumor whole genomes. BMC Bioinform. 2020, 21 (1), 338.
78. Huang, P.J.; Lee, C.C.; Tan, B. C.M.; Yeh, Y.M.; Julie Chu, L.; Chen, T.W.; Chang, K.P.; Lee, C.Y.; Gan, R.C.; Liu, H.; et al. CMPD: Cancer mutant proteome database. Nucleic Acids Res. 2015, 43 (D1), D849–D855.