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REVIEW

Deoxyribonucleic acid methylation driven aberrations in pancreatic cancer-related pathways

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Abstract

Pancreatic cancer (PanCa) presents a catastrophic disease with poor overall survival at advanced stages, with immediate requirement of new and effective treatment options. Besides genetic mutations, epigenetic dysregulation of signaling pathway-associated enriched genes are considered as novel therapeutic target. Mechanisms beneath the deoxyribonucleic acid methylation and its utility in developing of epi-drugs in PanCa are under trails. Combinations of epigenetic medicines with conventional cytotoxic treatments or targeted therapy are promising options to improving the dismal response and survival rate of PanCa patients. Recent studies have identified potentially valid pathways that support the prediction that future PanCa clinical trials will include vigorous testing of epigenomic therapies. Epigenetics thus promises to generate a significant amount of new knowledge of biological and medical importance. Our review could identify various components of epigenetic mechanisms known to be involved in the initiation and development of pancreatic ductal adenocarcinoma and related precancerous lesions, and novel pharmacological strategies that target these components could potentially lead to breakthroughs. We aim to highlight the possibilities that exist and the potential therapeutic interventions.

Key Words: Methylation driven pathways; Pancreatic cancer methylation markers; Signaling pathway targeted therapy; PanCa enriched methylated pathway; Pre-cancer methylated pathways

Core Tip: Given the limited commercial availability of targeted epi-drugs and pathway-based biomarkers, it is important to generalize them for appropriate treatment of pancreatic cancer and related precancerous lesions. We also highlighted the clinical use of these therapeutic targets based on methylation driven pathways. This review will successfully help readers address current issues and support cutting-edge development of targeted therapies using epigenetically regulated pathways.

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INTRODUCTION

Pancreatic cancer (PanCa) is one of the fatal malicious carcinomas globally. Currently, PanCa is one of the foremost causes of death by cancer especially in the United States[1]. According to GLOBACON 2020, PanCa is the 12th most common cause of cancer with 495000 new cases worldwide, of which approximately 47% of new cases occurred in Asia and another significant proportion, 28%, in Europe[2]. By the end of 2022, the incidence could increase by 70%, equivalent to about 844000 new cases per year[3]. Recent studies elucidate deoxyribonucleic acid (DNA) methylation depiction from inflammatory diseases thus opening a new profile for the biomarker development in early prognosis. Cell-free DNA methylation, in particular, could be used to identify pre-neoplastic features in individuals with suspected pancreatic disorders. This is a clear non-invasive approach of PanCa pre-diagnosis[4]. It is observed through the years that PanCa consists of extremely fatal malignancies, having less than 5 year of survival rate. Early detection and treatment of this disease is hampered due to a lack of reliable diagnostic and prognostic markers[3]. It has been noted that there is epigenetic variance between populations which can be accounted for by a variety of racial, demographic, and vocational characteristics. Only a few research have examined the Pancreatic ductal Adenocarcinoma (PDAC) progression stage globally and the shifting epigenetic landscape in various ethnic groups[5]. Recent research has demonstrated the dynamic changes in the global DNA methylation and gene expression patterns play important roles in cancer development, including PanCa development. These findings offer important new information for understanding the onset and progression of this malignancy[5].

PanCa is clinically allied with an elevated rate of mortality. In terms of geographic features, Northern America and Europe show the maximum prevalence of PanCa where more males tend to get affected. There are an estimated 62210 (male) and 32970 (female) new cases in the United States alone in 2022, with an estimated 25970 male and 23860 PanCa-related deaths[2,3]. In South Eastern countries like India, the rate of incidence of PanCa are comparatively lower compared to the Western world. According to per year statistics in Eastern countries like in India, the rate of prevalence of PanCa seems to be 0.5 to 2.4 out of 100000 women and 0.2 to 1.8 out of 100000 men. Although, regardless of the prevalence of this deadly disease, patient survivability with PanCa is comparatively downcast with 1 to 5-year of relative survival rates for all stages[6]. The main reason for such miserable and prolonged consequences is perhaps because of the fact that this fatal disease is predominantly lacks any symptoms in the early stages. Meanwhile, the symptoms commence to expand largely and eventually tend to get metastatic in nature[7]. As a result, enucleating a metastatic tumor is frequently impossible. PanCa has a 1-year survival rate of 26%, and the 5-year survival rate is roughly 6% for advanced cancer and 22% for early stages when surgical removal of the tumor is still possible[8]. For this reason, few new therapeutic strategies like radiotherapy and chemotherapy are effective to mitigate the tumor size in selected PanCa patients[9]. Hypermethylation in DNA methylation can promote tumorigenesis. However, with histone and RNA methylation, both writers and erasers can be PanCa oncogenes such as *SMYD3, KDM1, MELLT3* and *FTO*[10].

The study of malignant genomic modifications has been ongoing over the past few decades, and it has become quite evident that epigenetics are crucial to carcinogenesis. DNA methylation, a key component of epigenetics, affects a variety of biological functions, including gene imprinting, genome stability, and cell differentiation. Hypermethylation and hypomethylation are the two categories of abnormal DNA methylation. DNA hypomethylation refers to less DNA methylation, which frequently causes disturbance of chromosome stability or increased aneuploidy. On the other hand, DNA hypermethylation refers to the buildup of methylation, which mostly results in transcriptional repression and reduced gene expression. Typically, abnormal DNA methylation can be seen in the promoter regions of transcription factors, which promotes the growth and metastasis of cancers[11,12]. DNA methylation plays a crucial role in the onset and progression of cancer. Early on in the tumorigenic process, DNA methylation alterations frequently take place. This phenomenon has been confirmed for the bladder, lung, breast, colorectal, and pancreatic pre-neoplastic lesions[13,14].

According to Thompson *et al*[15], 2015, out of around 250000 assessed CpG sites, 20000 hotspots were correlated with patient survival. The two categories that were survival (-) and survival (+) which represented the connection between higher methylation and survival. The survival (+) sites were more evenly dispersed intragenically, whereas the survival (-) sites tended to group close to the TSS (transcription start site), indicating hypermethylation of promoter regions. An increased methylation pattern, associated with shorter survival was observed in survival (-) groups, while reduced methylation led to longer survival times in survival (+) groups. Some of the important genes [within the top 10 in survival (-) groups] which were found to be hypermethylated were *FAM150A, ONECUT1, RASSF10, RNF207,* PanCa DH9. The tumor-suppressor role of these genes are well-established in other aggressive cancers. While the genes such as *PTPRN2, MAD1L1, CBFA2T3, COL5A1,* and *SHANK2* etc. made their way into the top ten differentially methylated genes in the survival (+) group[16]. Thus, this segmentation, together with the fact that promoter regions of genes are typically better defined and documented, led to the surviving sites producing a clearer recovery of functional annotation and genes overall[17]. There is growing evidence that DNA methylation can affect how genes are expressed, despite the fact that the majority of research on DNA methylation has focused on the methylation state of promoters and CpG islands. Importantly, a mutation of *KRAS* in acinar or ductal cells causes the development of pancreatic lesions, which is the causative genetic event in over 90% of PDAC cases (PanIN). Along with the *KRAS* mutation, subsequent deletion mutations or mutations of other types in tumor suppressor genes promote tumor growth and accelerate the course of the disease[10,18].

The Advantageous incidence of this systematic review is to summarize all the differential methylation pathways in several precancerous lesions of PanCa. The alterations in epigenetics occurring in PanCa also discussed in this review. This review also gives insight into landscapes of the early epigenetics in precursor lesions. In this review also highlighted the differentially methylated enriched signaling pathways and methylated modulators, and their therapeutic targets for precancerous as well as PanCa. In brief, we describe an overview of differentially methylated genes, highlighting their diagnostic or prognostic potential in PanCa related enriched pathways (Figure 1).

PRECANCEROUS LESIONS OF THE PANCREAS

PanCa shows a proclivity for almost about 5 to 7 years of retention rate. Over the years it has been observed that a significant number of patients execute an immensely impoverished prophecy. Recent clinical studies clearly depict that throughout a long period of time; over 10 to 11 years, cellular level observation shows a clear tendency to originate various invasive proficiency. These series of phenomena conciliate the detection factors as well as root out the precursor lesions in PanCa[19]. From recent clinical studies, it is observed that the prior detection of these precancerous lesions put forward the possibility to reduce the death rate. The studies also delineate the fact that several non-intrusive prototype lesions exhibit malignant PanCa. From the surgical history of PanCa, it has been observed that patients having a previous history of PDAC may have microscopic pancreatic intraepithelial neoplasms (PanINs). Furthermore, these multifocal PanINs is clinically allied with diagnosable lobulocentric atrophy. Moreover, Intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCN) are another couple of prominent pre-cancerous lesions which give rise to PanCa. These lesions are often considered to form cysts as well[20]. The surgical treatment and revelation of these MCN, IPMNs and PanINs can often create disturbance in the advancement to incursive PanCa. These eventually shows a high efficacy to save the lives of cancer patients[3,20].

PANCREATIC CANCER AND ITS EPIGENETICS LANDSCAPE

Due to epigenetic alterations, oncogenic signaling pathways specifically derived from transcriptional deregulation create a trademark to PanCa. 5-Hydroxymethylcytosine (5-hmC) is a chemical (epigenetic) modification of DNA at regulatory regions that result in the generation of 5-methylcytosine (5-mC) residue and has been thoroughly studied in PanCa. Genome analysis of 5-hmC occupied loci was done in the cell lines of short-passaged PanCa. As a result, surprising patterns of alteration were seen in neoplastic tissues in primary cancer patients[19,21]. It was observed that near the open chromatin regions, the 5-hmC was very much enhanced and thus tends to show upregulation of the allied transliteration [22]. The transcripts involve a few important oncogenic signaling pathways enmeshed in pancreatic neoplasia, such as *KRAS,* master regulator of cell cycle (*MYC), BRD4* and *VEGFA* where *BRD4* tends to be highly overexpressed in nature. In terms of functional approach, accession of 5-hmC at promoter *BRD4* was implicated along with the transcript expression elevation specifically in primary patient samples. It was also noticed that the in *in-vivo* experiments the growth of PanCa is highly inhibited by the *BRD4* blockage. Concisely, it can be said that in human PanCa and oncogenic enhancers, partisan enhancement and 5-hmC reallocation tend to be an important regulatory mechanism[22,23].

ROLE OF THE KEY PATHWAY MODULATORS IN PANCA AND ITS ASSOCIATED PRECANCEROUS LESSIONS

In PanCa, some important pathways are Raf/Ras/ERK. MEK interposes specific cellular responses to a few growth factor actions. In the past years, inhibitors emergence is highly noticed that directly target *KRAS*. This circumvents the long-harboured speculation that drugs cannot be produced by *KRAS*. In PDAC, several attempts have been made to target this

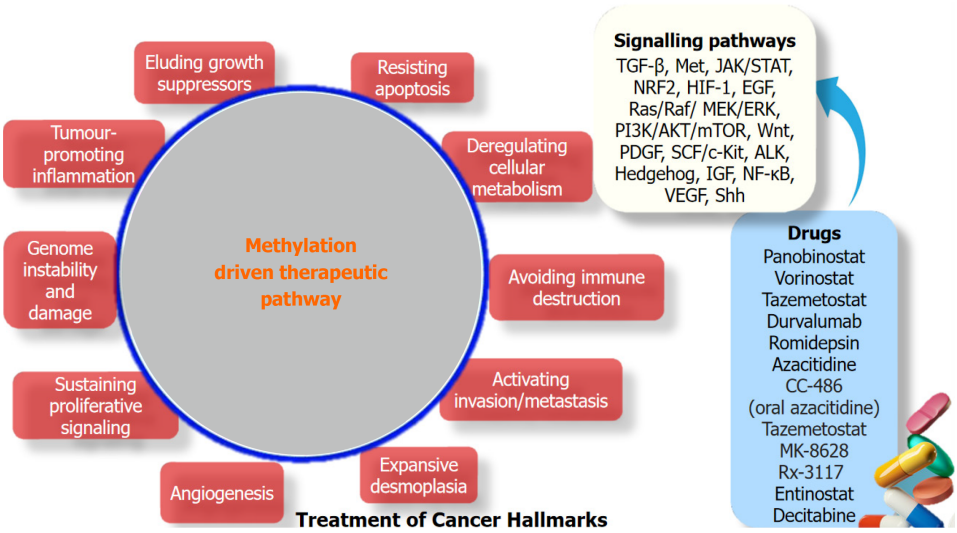

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Figure 1 Comprehensive visualization showcasing interaction between epigenetic pathways and probable drug treatments concerning pancreatic cancer. EGF: Epidermal growth factor; Ras/Raf/MEK/ERK: Rat sarcoma virus/Rapidly Accelerated Fibrosarcoma/Mitogen-activated protein kinase/extracellular-signal-regulated kinase; PI3K/AKT/mTOR: Phosphoinositide 3-kinases/Ak strain transforming/Mammalian target of rapamycin; Wnt: Wingless-related integration site; PDGF: Platelet-derived growth factor; SCF/c-Kit: Stem cell factor/receptor tyrosine kinase; ALK: Anaplastic lymphoma kinase; TGF-β: Transforming growth factor beta; HGF: Hepatocyte growth factor; JAK/STAT: Janus kinase/signal transducers and activators of transcription; BTK: Bruton tyrosine kinase; Src: Tyrosine-protein kinase (sarcoma); COX-2: Cyclooxygenase 2; NRF2: Nuclear factor erythroid 2–related factor 2; HIF-1: Hypoxia-inducible factor-1; PKCδ-PKD1: Protein Kinase Cδ-Polycystin 1, Transient Receptor Potential Channel Interacting; IGF: Insulin like growth factor; VEGF: Vascular endothelial growth factor.

important oncogenic pathway in various approaches. The downstream regulation of frequently mutated *KRAS* is eventually considered to be an esoteric drug target[24,25]. On the other hand, owing to the offsetting mechanism that involves the enzyme, geranylgeranyl transferase, the upstream regulation of *KRAS,* using inhibitors like farnesyltransferase has been completely nugatory[26]. It is also observed that the inhibitors like *MRTX8, AMG510* and others specifically target only the *KRAS* mutant variant such as G12C[27,28]. Over 1%–5% of PDACs portray this kind of mutation and the progress is really promising. In PDAC, to regulate the antitumor pursuit in *KRAS*, the genetic inhibition of some autophagy regulators reciprocally enhances the propensity of *ERK* inhibitors (Table 1)[29].

EPIDERMAL GROWTH FACTOR PATHWAY MODULATORS

Epidermal growth factor receptor (EGFR) functions in a significant way in PanCa specifically in terms of tumorigenesis. Epidermal growth factor (EGF) is one of the classic pathways that works in a dysregulated manner in PDAC and is thus often considered as a potent therapeutic target. EGF signaling pathway inhibitors are considered as one of the efficient and significant regulators for cellular viability. These pathway regulators often mediate a wide range of signaling activities, precisely Jak-STAT, Akt/PI3K, Ras/Raf/ and MEK/ERK[29,30]. In PanCa patients, it is often noticed that affirmative activation and regulation of *EGFR* works effectively for the activation of *KRAS* and *ERK* and this persuades the formation of tumor more profoundly[31]. It is found that in Phase III clinical trial, the add-on of erlotinib elevates more positive improvements in cell survival in PanCa patients. The presence of *KRAS* gene (wild type) in PDAC tumors with a tiny proportion also leads to a significant improvement in PDAC patient survival[27,31].

WNT PATHWAY MODULATORS

In case of tissue development and maintenance in both embryos and adults, The Wnt signaling pathway plays a critical role. Digressive activation of this Wnt pathway has been closely associated with cancers like PanCa, specifically to the severely affected digestive tract. It is observed that Cancer stem cells are strongly associated with the activation of this pathway[32]. Furthermore, a precise monoclonal antibody named Wnt inhibitor vantictumab, which eventually targets the decrepitate receptor. This depicts a huge responsive activity of tumors and is often found to be combined with gemcitabine[33].

Table 1 Targeting various characteristics of pancreatic carcinomas and their associated therapeutic strategies

Potent therapeutics	Cancer Hallmarks
TGF-β pathway inhibitors HGF; Met pathway inhibitors	Activating invasion/metastasis
JAK/STAT pathway inhibitors; BTK inhibitors; Src inhibitors; COX-2 inhibitors	Tumour-promoting inflammation
NRF2 pathway inhibitors; HIF-1 pathway inhibitors; PKCδ-PKD1 inhibitors; Amino acid transporter inhibitors; α-Glucosidase inhibitors	Deregulating cellular metabolism
EGF pathway inhibitors; Ras/Raf/ MEK/ERK pathway inhibitors; PI3K/AKT/mTOR pathway inhibitors; Wnt pathway inhibitors; PDGF pathway inhibitors; SCF/c-Kit pathway inhibitors; ALK pathway inhibitors; Hedgehog pathway inhibitors	Sustaining proliferative signalling
IGF pathway inhibitors; NF-κB pathway inhibitors	Resisting apoptosis
VEGF pathway inhibitors	Inducing angiogenesis
Shh pathway inhibitors; FAK inhibitors; Src inhibitors; EGFR inhibitors	Expansive desmoplasia
Aurora kinase inhibitors; Cyclin-dependent kinase inhibitors	Eluding growth suppressors
PD-L1 inhibitors; CTLA-4 inhibitors	Avoiding immune destruction
PARP inhibitors Photodynamic agents; Bromodomain inhibitors; HDAC inhibitors	Genome instability and damage

EGF: Epidermal growth factor; Ras/Raf/MEK/ERK: Rat sarcoma virus/Rapidly Accelerated Fibrosarcoma/Mitogen-activated protein kinase/extracellular-signal-regulated kinase; PI3K/AKT/mTOR: Phosphoinositide 3-kinases/Ak strain transforming/Mammalian target of rapamycin; Wnt: Wingless-related integration site; PDGF: Platelet-derived growth factor; SCF/c-Kit: Stem cell factor/receptor tyrosine kinase; ALK: Anaplastic lymphoma kinase; TGF-β: Transforming growth factor beta; HGF: Hepatocyte growth factor; JAK/STAT: Janus kinase/signal transducers and activators of transcription; BTK: Bruton tyrosine kinase; Src: Tyrosine-protein kinase (sarcoma); COX-2: Cyclooxygenase 2; NRF2: Nuclear factor erythroid 2–related factor 2; HIF-1: Hypoxia-inducible factor-1; PKCδ-PKD1: Protein Kinase Cδ-Polycystin 1, Transient Receptor Potential Channel Interacting; IGF: Insulin like growth factor; VEGF: Vascular endothelial growth factor.

STEM CELL FACTOR/C-KIT PATHWAY MODULATORS

In several cell lines of PanCa, the occupancy of c-Kit has been clearly mentioned. The stem cell factor tends to reinforce the differentiation as well as the proliferation of cells and also seems to be expressing towards its ligands. Masitinib tends to strongly inhibit both the platelet derived growth factor and stem cell factor signaling pathways, thus delivering such extremely promising outcomes. This affirmative feedback is often found to be combined with gemcitabine[34]. Moreover, this c-Kit pathway inhibitors effluxes the overexpressed *ACOX1* marker which elucidates the efficiency in cancer patients [35].

PI3K/AKT/MTOR PATHWAY MODULATORS

The inhibitors of some specific signaling pathways like PI3K/mTOR and Akt bring into play some indispensable control over multifarious processes that are closely related to the growth and survival of cells in case of disease as well as health [36]. The mTOR/Akt and PI3 pathways also play distinctive key roles in several important cellular mechanisms like cell invasion, adhesion, and migration[37].

ROLE OF EPIGENETIC MODULATED PATHWAYS IN PANCA

Whole genome and exome sequencing has shown that a considerable portion of PDAC patients also carries non-germline mutations in chromatin remodelling complexes and epigenetic regulators, such as *ARID1A/B, MLL2/3/4, PBRM1, SMARCA2/4,* and *KDM6A* in addition to germline mutations. Moreover, the inactivation of *KDM6A, MLL3,* and *MLL5* (histone modification enzymes) and non-germline mutations in *ARID1A* occurred simultaneously with oncogenic *KRAS* in insertional mutagenesis screening of sleeping beauty transposon[38]. Vincent *et al*[39] discovered that the histone-modifying enzyme-coding genes were mutated in all of the malignancies in our screen. These mutations helped oncogenic *KRAS* accelerate the progression of PDAC, indicating that changes to the epigenome are crucial for accelerating PDAC. These results demonstrate the importance of epigenetic regulation in the progression of PanCa[40].

Transcriptional silencing is linked to abberant CpG island methylation of multiple tumor suppressor genes, including p16, in pancreatic and other carcinomas. In 15% of PanCa, the p16 gene is reported to be inactive due to hypermethylation of the CpG island. With higher PanIN grades, there is a greater tendency for the loss of p16 protein production, The *ppENK* gene exhibits anomalous methylations in pancreatic carcinomas, as was recently established using representational difference analysis and methylation CpG island amplification (MCA)[41].

PENK DNA methylation has been widely observed in precancerous lesions of varying severity, including extra- and intraluminal PTs and CPs, PanINs, IPMNs, and mucinous cystic neoplasms. Changes in PENK methylation increased with increasing coverage of tumor tissue, but were absent in autoimmune pancreatitis (AIP) and adjacent normal pancreatic tissue[3].

The m6A demethylase *ALKBH5* was found to be downregulated in a gemcitabine-treated patient-derived xenograft model, and its overexpression made PDAC cells more sensitive to treatment. Reduced *ALKBH5* levels predict poor clinical outcome in PDAC and other malignancies. Furthermore, both *in vitro* and *in vivo,* downregulation of *ALKBH5* greatly promotes PDAC cell proliferation, migration, and invasion, whereas overexpression has the opposite impact. The m6A global profile indicated changes in the expression of certain *ALKBH5* target genes, such as Wnt inhibitor 1 (WIF-1), which associated with Wnt signaling pathway mediation and WIF-1 transactivation[42].

Met-enkephalin, a tonically active inhibitory factor that interacts with the opioid growth factor receptor, is encoded by the *ppENK* gene. Met-enkephalin was found to slow the growth of various human cancers, including PanCa, according to Zagon and colleagues. Comb and associates claim that the CpG island methylation of *ppENK* directly prevented a positively active transcription factor from binding, which in turn suppressed the production of the gene. Given this, it is a possible outcome that cell growth and carcinogenesis of the pancreas are promoted because of the methylation of the ppEK gene[41]. Moreover, α-catenin, angiogenesis inhibitor *BAI3, CTNNA2, DPP6* (dipeptidyl-peptidase), *GUCY1A2* (guanylate cyclase), heterotrimeric G-protein-coupled receptor, protein kinases like *PRKCG,* and *Q9H5F0*- these genes were often altered at significantly lower frequencies[43]. According to the reports of Li *et al*[44] a total of 16420 genes having methylation information were found to be differently methylated, including 40 and 831 significantly hypomethylated and hypermethylated genes, respectively. *SARM1, IRX4, IRF4, FOXC2, EN2, ZSCAN23, PTPN5, HOXB4, CACNA1,* and *IGF2BP1* were the 10 genes with the most significantly different methylation patterns. The 10 genes with the most different methylation patterns were *REG4, C11orf34, BRD9, S100A16, HIST1H2BK, STATH, LRRC31, UBD, MIR548A1,* and *PSMG3*[45].

Processes like the differentiation of neurons in the CNS, neuropeptide signaling pathway and organ development at the embryonic stage, were the most observed enrichment functions. According to the study, these genes were primarily engaged in signaling pathways for neuroactive ligand-receptor interaction, cAMP, salivary secretions, glutamatergic synapses, calcium, morphine addiction, circadian rhythm, nicotine addiction, and pancreatic secretions. Genes that significantly affect survival were included as taxonomic features in order to define molecular subtypes of PDAC in relation to prognosis[46]. An important finding from the univariate Cox proportional hazards regression model developed for clinical factors indicated that age should be considered as a significant parameter related to patient survival. These 135 significantly differentially methylation genes were included in the above-mentioned multivariate regression model together with age as a covariate to find variables that independently influence prognosis. Using multivariate Cox regression models, 78 differentially methylation genes substantially linked with prognosis were discovered[46,47].

Chatterjee *et al*[5], identified "regulation of ion transport", "alpha/beta interferon signaling", "morphogenesis and development" and "transcriptional dysregulation" as the four most statistically significant extended terms. Voltage-gated ion channels are membrane proteins that selectively transport ions and are activated by changes in membrane potential. The activation of channels permits potassium ions to move along the electrochemical gradient. Hypermethylation of the *KCNA3* gene promoter may explain the poor expression of Kv1.3 in PDAC. The modulation of ion channels has been demonstrated to play a significant role in the regulation of cell death, evasion, and survival in the context of PDAC invasion and development.

In a study by Nones *et al*[48] 25 pathways were reported to be significantly affected by DNA methylation in PDACs. Axon guidance was one of the most significant (adjusted *P* value 5 1.91E-05) and was supported by MetaCore pathway analysis. This pathway was recently implicated in PDAC. Other pathways identified here as enriched for genes aberrantly methylated including cell adhesion, hedgehog signaling, TGF-b, integrin signaling and WNT/NOTCH signaling are well-known key cancer signaling previously described to be genetically altered in PDAC. WNT signaling has been reported to be aberrant methylated in PDAC cell lines. Our results confirm that this pathway is aberrantly methylated in this large cohort of PDAC. Stellate cell activation (adjusted *P* value3.26E-05) another interesting pathway identified here as significantly affected by DNA methylation deserves further investigation due its importance in PDAC. Pancreatic stellate cells are the main fibroblastic cells in PDAC and are known to interact with PanCa cells creating the fibrotic microenvironment of PDAC. It is hypothesized that the fibrous microenvironment of PDAC creates a barrier that impairs the delivery of chemotherapeutic drugs and promotes aggressive behaviour of tumor cells. Known genes are involved in astrocyte activation [cyclooxygenase-2, transforming growth factor-beta receptor 1, EGFR, tumor necrosis factor-alpha and MET were hypomethylated in PDAC and confirmed by bisulfite amplicon deep sequencing[48].

miRNAs are frequently suppressed in cancer cells and have the potential to act as tumor suppressors. Several miRNAs have been implicated in the development and spread of cancer in the pancreas, and it may one day be possible to stop the disease's progression by increasing the activity of a particular miRNA within a cell. The Food and Drug Administration (FDA) -approved drug Miravirsen, which employs miRNA to treat hepatitis C, has sparked interest in miRNA-based medicines for the treatment of PanCa. Regrettably, no treatments employing miRNAs or siRNAs that are comparable to them have been tried in clinical trials to treat PanCa, therefore miRNA will not be explored in great detail in this review. Nevertheless, we recommend individual study into the state of the art in miRNA studies[49].

In addition to confirming the mutations in the tumor suppressor and classical PDAC-associated oncogenes listed above, sequencing efforts have also revealed mutations in a variety of chromatin-modifying enzymes and complexes. The chromatin remodelers like, SWI/SNF family which alters nucleosome structure using ATP and accessibility of DNA in order to control gene transcription, includes the *ARID1A* component as one of their most often altered genes. 6% of the *ARID1A* mutations in human PDAC were found using multiplatform sequencing analysis. Although the role of *ARID1A*

as a PDAC-associated tumor suppressor gene is well documented, lymph node or distant metastases do not coincide with its expression levels. Instead, they are related to tumor stage and differentiation. When *ARID1A* is knocked out, acinar to ductal metaplasia and PanIN lesions develop as a result[41]. It's interesting to note that a recent study using genetically altered PanCa mice models demonstrated the importance of the survival gene Arid1a, whose absence inhibits cell development and causes cell death. In Ras-driven animal models, the deletion of *ARID1A* also prevents cell growth, leading to the emergence of inactive and low-quality cystic precursor lesions known as IPMNs[50]. The progression of Arid1a-deficient progenitor cells to adenocarcinomas, however, occurs during carcinogenesis *via* routes involving *Tp53* loss or Myc overexpression. PDAC has also been linked to mutations in the SWI/SNF subunits *SMARCB1, ARID1B, BRG1, PBRM1, SMARCA2,* and *SMARCA4.* In PanCa cell lines, *BRG1* inactivating mutations and deletions have been discovered. *BRG1,* a crucial entity of the SWI/SNF chromatin remodelling complex, is an ATP-dependent helicase. Neoplastic lesions that mirror human intraductal papillary mucinous neoplasms are produced as a result of *BRG1* deletion and *KRAS* mutation, which aids in the progression of PDAC[41,51].

Methylation at particular genomic locations may put patients at risk for tumor recurrence following total surgical removal and may be a sign of local and/or systemic metastasis. The surgical resection margin methylation profile may be used as a biologic marker in the absence of histologic disease to detect remaining pancreatic tissue that is susceptible to tumor recurrence or that harbours multi-focal disease throughout the gland. The link between methylation abnormalities and Auto immuno pancreatitis (AIP), a representative Immunoglobulin G4 (IgG4)-related illness, has yet to be determined. Through methylation array research using the Methylation 450K BeadChip array, the scientists discovered that sphingosine kinase inhibitor (SKI) may have a major methylation anomaly in AIP and explored the connection of SKI with AIP clinicopathological characteristics. AIP had a considerably lower SKI methylation ratio than PDAC and nurse practitioner (NP). Furthermore, the immunohistochemical staining-index (SI) score for SKI in AIP was substantially greater than in NP, despite no significant difference between AIP and PDAC[52]. Both the serum IgG4 concentration and the SKI methylation ratio showed a strong negative connection between the SI score and the methylation ratio. SI and the serum IgG4 concentration were shown to be somewhat positively correlated. Givien that SKI is regarded as an oncogene, hypomethylation of SKI and carcinogenesis may be connected to AIP[53]. Additionally, the association between serum IgG4 levels and SKI methylation raises the possibility that SKI plays the role in the aetiology of AIP. *NPTX2*, along with Cyclin D2, *FOXE1, TFPI2, ppENK,* and p16 all had hypermethylation events (10%) according to a research by Kinugawa *et al*[54]. However, compared to NCA and NP, AIP had a considerably greater*TFPI2* methylation ratio.

THERAPEUTIC ASPECTS OF EPIGENETIC MODULATED PATHWAYS IN PDAC AND ITS ASSOCIATED PRECANCEROUS LESIONS

PDAC is a deadly illness with few therapy options. According to new research, PDAC includes numerous layers of epigenetic alterations. Because the change is possibly reversible, it is a possible therapeutic target. Epigenetic changes can potentially affect the tumor microenvironment, modulating and enhancing treatment. Because epigenetic changes occur early in the disease, epigenetic markers can also be employed as diagnostic screening tools. Immunotherapy is being used more frequently to treat solid organ tumors, however there is no benefit for PDAC because most patients do not respond to these new treatments[55,56]. Because epigenetic processes regulate underlying immune cell activities, resulting in an anti-tumor response, combining immunotherapy and epigenetic therapy may improve patient outcomes even more. PDACs are currently classified as three to five subtypes according on the system used[57,58]. Using transcriptomic profiling, two primary molecular subtypes of PDAC were discovered: classical and basal[59]. The traditional kind has a better prognosis and clinical significance. Basal subtypes have altered the methylation of effectors and inhibitors of the Wnt signaling pathway by analyzing the epigenomic landscape. Classical tumors are hypomethylated, resulting in upregulation of the cholesterol transporter *NCP1L1*[60]. Furthermore, basal tumors were discovered to contain dysregulation of multiple genes related with established oncogenic signaling networks, including the *MYC*, erythroblastic oncogene B/*EGFR*, and transforming growth factor *(TGF)* signaling pathways. Chronic pancreatitis is a well-known risk factor for PDAC, which is consistent with the previously documented general link between tumor and inflammation[61, 62]. Early stage PanCa caused by inflammation is linked to epigenetic alterations. Damage to the pancreatic epithelium during a pancreatitis episode results in long-lasting transcriptional and epigenetic remodelling that creates epithelial memory that protects against strokes in the future[63].

Reader proteins, have lately been identified as prospective therapeutic targets, in particular the chromatin adaptors of the bromo and extra C-terminal (BET) family, after directly engaging with the histone tails with acetylated lysine residues, these proteins with bromodomains can bind transcription factors to the DNA, boosting the acetylation-induced transcriptional activation. BET proteins use the epigenetic landscape in this way to support the growth of PDAC cells. Given the wide variety of abnormal epigenetic marks that are possible targets for the advancement of anticancer therapy, the study of and application of epigenetic enzyme inhibitors for the anti-cancer therapy show promise[49,64].

Cell interactions and released substances can cause epigenetic alternations. It has been demonstrated that PDAC cells induced DNA methylation of the *SOCS1* gene, acytokine supressor and cancer promoting growth factor, to boost tumor cell proliferation *in vitro*[63,65]. Clinical evidences demonstrating a higher 3-mo overall survival in patients missing *SOCS1* methylationl end credence to this. In PDAC, lysine demethylase 3A (*KDM3A*) is an effective epigenetic regulator of immunotherapy responses. This enzyme controls the *EGFR* expressions[66]. Tumors produced by cancer cells deficient in *KDM3A* have infiltrating immune cells that are responsive to immunotherapy. To distinguish between PDAC and cancer precursor phase, methylation -specific electrophoresis was used to determine the methylation status of the *MUC1, MUC2,* and *MUC4* genes in pancreatic fluids[67]. Additionally, the methylation status of the mucin genes was examined

using machine learning, and it was discovered that *MUC1* and *MUC4* hypomethylation levels were significantly correlated with poor prognosis[68].

Through the suppression of Hedgehog (Hh) signaling, improved gemcitabine delivery was shown in preclinical investigations. Clinical studies were conducted for a number of cancers, but they were unsuccessful and did not progress to phase III trials[69]. However, preclinical research using epigenetic targeting of the proteins known as BET bromodomains, which controls the transcriptional output of Hh signaling, demonstrated positive results *in vitro,* suggesting possible synergistic therapeutic approaches[70]. BET bromodomain proteins are thought to be crucial contribution to PDAC development and are a topic of active investigations[71]. Based on the transcription factor GATA-binding factor 6 (*GATA6*)'s function as a regulator of the traditional PDAC subtype identity, the method to induce subtype switching in PDAC has been further investigated. A basal state is provided in PDAC by *GATA6* depletion[72]. As a regulator of *GATA6* transcription in PDAC, the histone methyltransferase zeste homologue 2 (*EZH2*) enhancer prevents the decreased *EZH2-GATA6* and induced gene signatures present in traditional PDAC subtypes. Therefore, a potential target for PDAC treatment in the future is the *EZH2-GATA6* axis[73]. Tazemetostat, an *EZH2* inhibitor, has been FDA-approved for use in the treatment of advanced epithelioid sarcoma and is currently being investigated in a phase II research in conjunction with ICI in the treatment of other solid tumors, including PDAC (NCT04705818)[74]. A hostile squamous cell subtype is promoted to differentiate in PDAC by epigenetic silencing of *GATA6*. Using genome-wide epigenetic mapping of the alterations 5-methylcytosine and 5-hydroxymethylcytosine (5hmC), this epigenetic dysregulation was demonstrated[75]. Due to decreased production of the enzyme 5-methylcytosine hydroxylase *TET2*, these transcriptional subtypes exhibit a higher loss of 5hmC. In addition, reduction of *SMAD4* expression revealed decreased 5hmC and *GATA6,* resulting in a more squamous-like tumor. Blocking DNA methylation by utilizing the DNA methyltransferase (DNMT) inhibitor 5-azacytidine slows the growth of typical PDAC tumor. In contrast, utilising the same medication or DNMT knockdown *via* small interfering RNA boosted hyaluronic acid synthesis, ultimately increasing the advancement of PDACI[76]. Epithelial cells from normal pancreata and PDAC underwent transcriptomic and DNA methylomic analysis, which identified a subpopulation characterised by hypomethylation of repetitive regions, which in turn triggers an interferon-linked transcriptional programme[77]. The relationship between cell-of-origin and epigenetics and tumor heterogeneity can be seen in the fact that tumors with low methylation were more aggressive than tumors with high methylation, which kept more of their cell-of-origin characteristics[78].

A clinical trial examining the medication in solid tumor types, including PDAC, and the recent FDA approval of the *EZH2* inhibitor tazemetostat for the treatment of advanced epithelioid sarcoma show a potential clinical relevance of the found *EZH2-GATA6* axis in PDAC tumor[79]. Numerous researches have examined how DNA methylation mechanisms control the expression of genes in various TME components[80]. For instance, 5-azacytidine, a DNA methyltransferase (DNMT) inhibitor, inhibited global DNA methylation in epithelial PDAC cells and cancer-associated fibroblasts (CAFs), which slowed the evolution of PDAC[75]. In immunocompetent PDAC models, DNMT inhibition increased CD4 and CD8 T-cell infiltration and significantly reduced tumor size. Espinet *et al*[77] have discovered a link between low DNA methylation levels and subpar PDAC patient outcomes. They show that tumors with low levels of overall DNA methylation in the epithelial cells exhibit increased expression of endogenous retroviral transcripts, robust double-stranded RNA sensing machinery engagement, activation of an interferon signature, and stromal cell reprogramming that is pro-tumourigenic in the PDAC TME. Clinical trials for a sequential strategy based on HDAC/DNMT inhibition, chemotherapy, and then PD-L1 blocking are now being conducted in PDAC, and the findings are highly anticipated[81].

Specifically, nucleoside-like inhibitors induce cytotoxicity through DNA damage brought on by the creation of DNMT-DNA abducts, disrupt DNA methylation, and encourage the re-expression of dormant genes. Both outcomes support anticancer action[82]. Additionally, RNA modification of N6-methyladenosine (m6A) is a unique strategy for dynamic and reversible epigenetic control that has been discovered by researchers. By triggering the Wnt signaling cascade and changing Wnt I[82].

Inhibitory factor 1 (*WIF-1*), m6A accelerates the course of PanCa. Demethylase, m6A rubber, and the alkylation repair protein 5 (*ALKBH5*) homolog are increased in gemcitabine-treated sensitized PDAC cells. By demethylating m6A and consequently reducing *WIF-1* and deactivating Wnt signaling, it slows the growth of tumors. *In vitro* and *in vivo* development and invasiveness are accelerated when PanCa cells lack *ALKBH5*[42,83]. As a result, *ALKBH5* might be a brand-new target for PanCa treatment. Numerous studies have shown how DNMT inhibitors affect PanCa cell lines *in vitro* by inhibiting them, radiosensitizing them, and immunological sensitizing them. PanCa DNA repair regulation is mediated by H3K36 methylation. H3K36 is a SETD2-dependent protein that is essential for HR repair. Demethylating H3K36 by demethylase *KDM4A* alters heart rate. A transcription factor for *MHCII, RFXAP* has been linked to the inhibition of tumor growth. PDAC survival was favourably linked with *RFXAP* deficiency[84]. Ding *et al*[85] found that the natural flavonoid fisetin regulates H3K36 methylation to promote *RFXAP* and *KDM4A* expression and interferes with HR, leading to DNA damage and PDAC S-phase arrest[85]. Therefore, this tactic may constitute a cutting-edge therapeutic method for treating PanCa. DNMT inhibitors (DNMTis) are undertaking Phase I/II clinical trials in patients with PanCa and have been shown to sensitize PDAC cells to immune checkpoint blockade treatment and chemotherapy [86]. Decitabine, alongside 5-aza, and guadecitabine are DNMTs used for PDAC. Haematological malignancies are also accepted to be treated with HDAC inhibitors (HDACis)[87]. Another therapeutic epigenetic approach for PanCa patients is HDAC inhibition. In PanCa cells, HDAC is, which includes *SAHA* and *CUDC-101*, can downregulate apoptotic inhibitory proteins including survivin and XIAP. Additionally, these HDACs can make PanCa more radiosensitive and make it cytotoxic[88]. AR-42, which is another potent HDACi against PanCa cells, can inhibit cell proliferation *via* inducing cell cycle arrest at G2 phase. Additionally, it can induce DNA damage, apoptosis, and p53 expression, suggesting that it may have therapeutic promise for the treatment of PanCa[89]. In addition to that, reader proteins with different bromodomains that attract proteins implicated in tumor initiation and elongation are blocked by the BET inhibitor JQ1 from binding to the BET domain. In the framework of personalized medicine, Bian *et al*[90] defined a novel

technique for PDAC classification and management based on sensitivity to JQ1 treatment. In order to select PanCa patients with unregulated c-MYC signaling pathways and demonstrate that these selected tumors exhibited greater susceptibility to BET inhibitor JQ1 treatment, the technique involved molecularly characterizing patient xenografts. According to the study, administering BET inhibitors in conjunction with conventional anticancer regimens may constitute an efficient therapy option for individuals who have been carefully chosen and categorized (Table 2)[83,91].

Mechanisms of faulty negative control of cell proliferation, in particular immune evasion, can also produce abnormal proliferation in the development of gastrointestinal malignancies, in addition to unchecked proliferation brought on by cell cycle dysregulation[92]. For instance, it was discovered by researchers that during PanCa, H3K4me3 of the *BCL2L1, CFLAR,* and *MCL-1* gene promoters upregulates the production of the anti-apoptotic proteins *Bcl-x, FLIP*, and *MCL-1,* as well as the *BAK1, BAX,* and *BCL2L11* gene promoters of Bak and Bax. Proapoptotic proteins like the Bim protein, for example, have their expression downregulated[93]. These six apoptosis-controlling genes are all essential for PanCa growth and development[94].

Initial investigations with human pancreatic cell lines showed that silencing *KMT2D* lowered the number and proportion of cells in G0/G1, which was accompanied by a drop in H3K4me1/2/3, indicating that histone methylation is actually involved in cells cycle management[95]. Further research has primarily focused on CKI control. *P15* and *P21* genes, which encode two often reported CKIs, show higher levels of H3K27me3 and H3K9me3 and lower levels of H3K4me2/3 in gastrointestinal malignancies such as GC, CRC, HCC, and PanCa[96]. Upstream lncRNAs such as *BLACAT1, SNHG17,* and *CASC15* can decrease P15 and P21 expression and cause G0/G1 checkpoint deficit. DZNep (3-deazaneplanocin A), a powerful pharmacologic inhibitor of S-adenosylhomocysteine hydrolase, modifies chromatin accessibility *via* inhibiting histone methyltransferases such as *EZH2*[97,98]. It results in a large decrease in H3K27me3 (a primary repressive histone mark) levels, as well as a significant decrease in cell proliferation and migration in CRC. Similar effects can be seen in PanCa, with decreased global H3K27me3 levels leading to re-expression of miR-218, limiting cell growth, encouraging apoptosis, and finally triggering cell cycle arrest in PanCa cells[99]. Another study found that DZNep significantly modulates miR-663a and miR-4787-5p expression and consecutively suppresses TGFb1-induced EMT signaling in PanCa[98,100]. UNC1999, an EZH2-specific inhibitor, not only lowers the abnormal H3K27 methylation that characterizes PanCa cells, but it also slows cancer cell proliferation in three model systems[101]. Furthermore, chaetospirolactone has been shown to suppress the activity of the epigenetic regulator *EZH2* and consistently decrease H3K27me3 to allow for the transcription of *DR4*, which then binds to TRAIL and culminates in the activation of initiator caspase-8 and the formation of the death-inducing signaling complex[102]. As a result, diosgenin, garcinol, *FBW7* and curcumin analogue CDF have also been identified as potential agents targeting *EZH2* to prevent the development of PanCa[97,103]. Amalgamation treatment with the HMT inhibitor panH3K9me chaetocin and an aurorakinase A (*AURKA*) inhibitor reduces H3K9 methylation at the centrosome, generating mitotic abnormalities that eventually drive aberrant mitotic checkpoint responses and eventually mitotic catastrophe in PanCa[104].

CONCLUSION

Since PanCa patients have a dismal prognosis, understanding the molecular events that drive this terrible tumor disease is critical for developing alternative and more effective treatment regimens and determining trustworthy diagnostic indicators. The role of epigenetics in the initiation, development, and evolution of PDAC has been demonstrated by advances in high throughput sequencing and genome-wide association studies. This review covers the major epigenetic signaling pathways as well as how the epigenetic machinery is altered or 'hijacked' in PanCa. Recent epigenetic research has considerably expanded our understanding of the regulatory characteristics involved in PanCa initiation, and progression, along with metastasis tumor. As discussed in this article, DNA-based epigenetic processes have been shown to play a role in PanCa and may serve as potential therapeutic targets aimed at rectifying epigenetic dysregulation of cellular machinery. Initial clinical trials with DNMT inhibitors at stages I-III are presently underway, paving the path for the creation of innovative, and hopefully more successful, 'epidrugs' for patients with PanCa. As a result, we believe that targeting epigenetic regulators and modulators with successful pharmaceutical or even immunotherapeutic techniques would be a game changer in the fight against this aggressive cancer. One significant restriction of using such epigenetic reprogramming of PDAC tumors is the danger of pleiotropic effects, which occur when certain components of the epigenetic machinery have opposite effects in different cellular compartments. Recent improvements in single-cell sequencing technologies that provide multi-omics information from the genome and transcriptome may be useful in determining the specific involvement of the several players in the epigenetic regulation of PDAC tumors. Overall, manipulating the epigenetic machinery, either alone or as part of a combination treatment plan, has the potential to reprogram the aggressive PDAC tumor profile to a less aggressive or easily identifiable and curable state, thereby benefiting patients in the future. In conclusion, we conclude that when cancer-associated signaling pathways are evaluated as a combined shift in "genomic-epigenomic-and-nuclear" structure, an even more realistic picture of PanCa will be obtained. Early preneoplastic lesions in this organ appear to require only a few mutations to initiate a process of aberrant organogenesis *via* self-reinforcing pathological loops. During metastatic progression, epigenomic landscapes defined by the differential acquisition of enhancers and super-enhancers appear to be required to maintain inheritable, cancer-associated gene expression patterns that support the heterogeneous differentiation of human PanCa tumors. This has given unique insights into an arsenal of novel, potentially actionable signaling pathways that were not previously achieved through genomic analyses, supporting the notion that effective future PDAC therapeutic regimens will require precision medicine approaches that include epigenomic targets.

Table 2 Current ongoing trails targeting epigenetic therapy (combination drugs) in Pancreatic Cancer

Drug names	Combination agents	Trail phase	NCT number
Panobinostat vorinostat	Various antineoplastic drugs	Phase 1	NCT03878524
Vorinostat	Capecitabine + radiation	Phase 1/2	NCT00948688
Tazemetostat	Durvalumab/gemcitabine	Phase 2 recruiting	NCT04705818
Durvalumab	Tazemetostat	Phase 2	NCT04705818
Romidepsin, azacitidine	Durvalumab, lenalidomide, nab-paclitaxel	Phase 1/2 recruiting	NCT04257448
Azacitidine	Chemotherapy after progression	Phase 2 active	NCT01845805
Vorinostat	Gemcitabine, sorafenib +/−, radiation	Phase 1 active	NCT02349867
CC-486 (oral azacitidine)	–	Phase 2 active	NCT01845805
Azacitidine, not recruiting	Pembrolizumab	Phase 2 active	NCT03264404
Tazemetostat	Durvalumab	Phase 2	NCT04705818
MK-8628	-	Phase 1 completed	NCT02259114
Rx-3117	Nab-paclitaxel	1,2	NCT03189914
Entinostat	Nivolumab	Phase 2 completed	NCT03250273
Decitabine	Tetrahydrouridine	Phase 1 completed	NCT02847000
Vorinostat	Capecitabine	Phase 1 completed	NCT00983268
Azacitidine	nab-Paclitaxel, carboplatin	Phase 1 completed	NCT01478685
Vorinostat	NPI-0052 (marizomib)	Phase 1 completed	NCT00667082
Azacitidine	Pembrolizumab	Phase 2 recruiting	NCT03264404
Azacitidine	Abraxane, gemcitabine	Phase 2 active	NCT01845805
Entinostat	Nivolumab	Phase 2 active	NCT03250273

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