Review Article

DNA METHYLATION SEQUENCING: A PROMISING TOOL FOR PANCREATIC CANCER DIAGNOSIS

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Abstract

Pancreatic cancer remains a deadly disease due to late diagnosis and limited treatment options. DNA methylation, a key epigenetic modification, plays a crucial role in cancer development and progression. Various research using DNA methylation patterns in pancreatic cancer tissues resulted in comparative evaluation of normal pancreas and cell lines resulted in the identification of potential biomarkers for diagnosis and therapy. During DNA methylation case studies led to identification 807 genes and 1505 CpG sites. Also, 289 differentially methylated CpG sites were also reported suggesting their vital contribution towards pancreatic cancer. In current review tried to explore the methylation approaches to identify important genes linked to gemcitabine, a common chemotherapy drug, identifying potential markers for patient response. This study sheds light on the link between DNA methylation and pancreatic cancer, paving the way for novel therapeutic targets and improved patient outcomes.

Keywords: Pancreatic Cancer, Pancreatic Ductal Adenocarcinoma (PDA), DNA Methylation, Biomarkers, Gemcitabine

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains a formidable foe, characterized by dismal survival rates. It holds the dubious distinction of being the third most lethal cancer in developed countries, with a mere 9% of five-year survival rate[1,2]. This aggressive malignancy originates in the pancreas ductal cells and often progresses silently in its early stages, facilitating its spread to other organs before detection.

PDAC can be categorized as either exocrine or neuroendocrine (endocrine tumors) based on the cell type from which it arises. Exocrine tumors, with PDAC being the most common variant, constitute roughly 93% of pancreatic cancers. The remaining 7% are neuroendocrine tumors (PNETs)[3]. The early diagnosis of PDAC presents a significant challenge. Additionally, the cancer exhibits a highly aggressive and fast-growing nature, portending a poor prognosis[2].

KRAS (Kirsten rat sarcoma viral oncogene homolog) mutations are recognized as crucial drivers of PDAC, present in over 90% of cases[4]. The current treatment

standard for PDAC patients revolves around chemotherapeutic cocktails, notorious for their high toxicity and limited targeting capabilities. Despite numerous clinical trials investigating optimized chemotherapeutic regimens for PDAC, overall survival rates have seen minimal improvement. The fact that most PDAC patients succumb to metastatic disease underscores the urgent need for novel therapeutic strategies that target not only the primary tumor but also the vulnerabilities specific to metastatic PDAC cells[3].

These compelling reasons fuel the pursuit of alternative blood-based biomarkers for pancreatic cancer. Such biomarkers hold immense promise for early-stage diagnosis. Liquid biopsies offer a non-invasive method for evaluating tumor characteristics and monitoring treatment response by detecting and analyzing genetic alterations in circulating cell-free DNA (cfDNA), circulating tumor cells (CTCs), and other biomarkers present in the blood[2].

Epigenetics, the study of heritable changes in gene expression that occur without alterations in the DNA sequence itself, offers a promising avenue for tackling PDAC. DNA methylation, a key epigenetic mechanism, involves the addition of methyl groups to DNA molecules, potentially silencing genes or altering their activity. Recent advancements in this field have spurred the exploration of DNA methylation as a potential source of epigenetic biomarkers for PDAC[5].

This review aims to synthesize current knowledge on epigenetic mechanisms in PDAC and highlight recent breakthroughs in the field of epigenetic biomarkers, particularly focusing on DNA methylation in exocrine pancreatic cancer. By leveraging existing knowledge, we hope to identify potential avenues for developing novel therapeutic interventions that target epigenetic dysregulation in PDAC.

2. Genetic Drivers of Pancreatic Cancer

Pancreatic cancer development is a complex process driven by the accumulation of genetic alterations in multiple genes. Mutations in specific genes can activate oncogenes (genes that promote cell growth) or inactivate tumor suppressor genes (genes that regulate cell division and prevent uncontrolled growth). These alterations disrupt normal cellular processes, leading to uncontrolled cell proliferation, invasion, and ultimately, tumor formation[8].

One of the most well-characterized genetic drivers of pancreatic cancer is the KRAS gene. Mutations in KRAS, particularly in codon 12, are found in over 90% of PDAC cases[8]. KRAS mutations activate a signaling pathway that promotes cell growth and survival. Other frequently mutated genes in PDAC include TP53 (tumor protein 53), a crucial tumor suppressor gene involved in cell cycle arrest and DNA repair, and CDKN2A (cyclin-dependent kinase inhibitor 2A), another tumor suppressor gene that inhibits cell cycle progression. Mutations in these genes contribute to uncontrolled cell proliferation and resistance to cell death pathways[9].

In addition to these core driver mutations, several other genes have been implicated in pancreatic cancer development. These include genes involved in DNA damage repair, cell adhesion, and signal transduction pathways. Identifying and understanding the specific genetic alterations in a patient's tumor can provide valuable insights for personalized treatment strategies. Mutations in oncogenes, such as KRAS, lead to their activation. Activated oncogenes promote uncontrolled cell growth and proliferation, ultimately resulting in tumor formation and pancreatic cancer [10] development as shown in Figure 1.
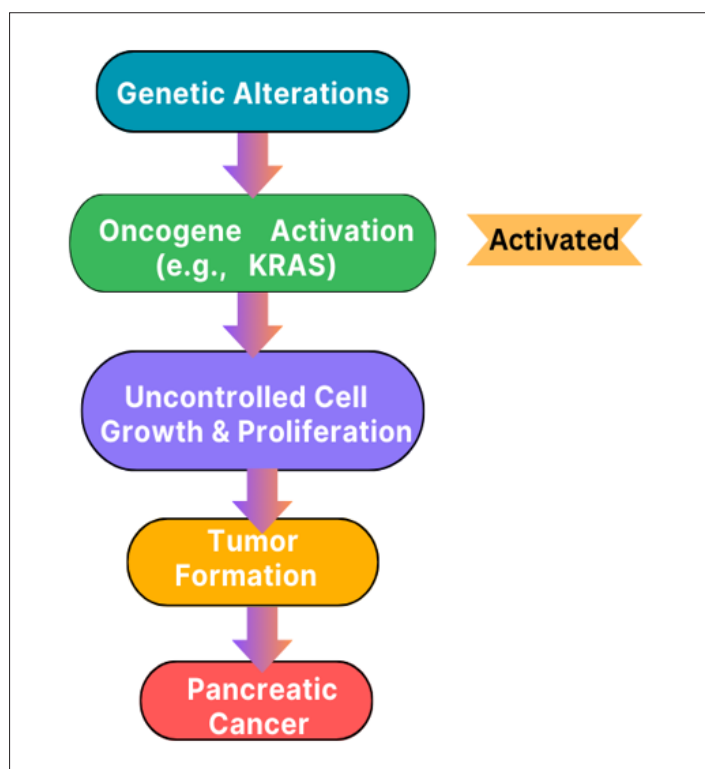
Figure 1: Development of Pancreatic Cancer through genetic alterations highlighting the key steps involved in how genetic alterations contribute to pancreatic cancer

3. DNA Methylation and Pancreatic Cancer

DNA methylation, a key epigenetic modification, plays a critical role in regulating gene expression. It involves the addition of a methyl group (CH3) to specific DNA molecules, primarily at cytosine-phosphate-guanine (CpG) dinucleotide sites[11]. This methylation process can influence gene activity without altering the underlying DNA sequence. In pancreatic cancer (PDAC), aberrant DNA methylation patterns have emerged as a significant hallmark of the disease.

Studies have shown that widespread changes in DNA methylation occur in PDAC compared to healthy pancreatic tissue[12,2]. These changes can involve both hypermethylation (increased methylation) and hypomethylation (decreased methylation) of specific CpG sites. Hypermethylation often leads to silencing of tumor suppressor genes, which normally act to control cell growth and division. Conversely, hypomethylation can activate oncogenes, genes that promote cancer development.

Research suggests that DNA methylation patterns in PDAC hold promise for several clinical applications. Firstly, they offer potential biomarkers for early detection of the disease. By analyzing methylation profiles in blood or pancreatic juice samples, it may be possible to identify individuals at risk for PDAC before symptoms develop[12]. Secondly, DNA methylation patterns may help predict patient prognosis and guide treatment decisions. For example, the methylation status of specific genes might indicate a patient's response to certain chemotherapeutic drugs[13].

Overall, studying DNA methylation in PDAC is a rapidly evolving field with significant potential for improving pancreatic cancer diagnosis, prognosis, and treatment strategies. Selected genes reported increase and decrease in PDA like TFP12, RELN decrease expression and increase in progression while MET, ITAGA2 increased expression also leads into decline in patient survival rate as shown in detail in Table 1.

Table 1: Represents the DNA methylation genes that are related to alterations during PDA progression and metastasis

Category	Gene	Molecular function	Molecular phenotype in PDA	Functional phenotype in PDA	Reference
DNA methylation	TFP12	Serine proteinase inhibitor that evolved to inhibit the degradation of pro-metastasis extracellular matrix	Decrease expression by hypermethylation	Increases in progression, proliferation	6
	RELN	Act as a serine protease in the extracellular matrix that evolved during neuronal migration	Decrease expression by hypermethylation	Patient survival decreases but increases in migration, invasion	7
	MET	Acts as a receptor tyrosine kinase and is also involved in cell survival	Increases expression by hypermethylation	Patient survival decreases	8
	ITGA2	Involved in cell attachment in extracellular matrix	Increases expression by hypermethylation	Patient survival decreases	9

Table 2: cfDNA Methylation Analysis for Pancreatic Cancer Detection

Feature	Description
cfDNA	Fragmented DNA molecules circulating in the bloodstream, originating from various sources including dying cells.
Tumor-derived cfDNA	cfDNA released by tumor cells, potentially containing tumor-specific information.
cfDNA Methylation Analysis	Identifying and characterizing abnormal methylation patterns in cfDNA.
Pancreatic Cancer and DNA Methylation	Pancreatic cancer is associated with widespread alterations in DNA methylation (hypermethylation and hypomethylation).
Detection Method	Analyzing methylation status of cfDNA isolated from blood to potentially detect tumor-specific methylation changes.
Advantages over Traditional Biopsies	Non-invasive approach compared to invasive tissue biopsies.
Potential Applications	Development of non-invasive diagnostic tests for early pancreatic cancer detection.

4. Cell-Free DNA (cfDNA) Methylation: A Promising Route for early Pancreatic Cancer Detection

The diagnosis of pancreatic cancer remains a significant challenge due to the lack of specific symptoms in its early stages. Consequently, the disease often progresses to advanced stages before detection, hindering treatment options and significantly impacting patient outcomes[12]. In this context, the exploration of novel, non-invasive biomarkers for early pancreatic cancer detection holds immense promise. Cell-free DNA (cfDNA) methylation analysis has emerged as a promising approach for achieving this goal.

Circulating cfDNA consists of fragmented DNA molecules present in the bloodstream, originating from various sources including dying cells throughout the body. Tumor cells are known to release cfDNA into the bloodstream, offering a potential source of tumor-derived information[12]. cfDNA methylation analysis focuses on identifying and characterizing abnormal methylation patterns in cfDNA, potentially reflecting the methylation profile of the tumor itself.

Pancreatic cancer development is associated with

widespread alterations in DNA methylation patterns. These alterations can involve both hypermethylation (increased methylation) and hypomethylation (decreased methylation) of specific genes[12]. By analyzing the methylation status of cfDNA isolated from a patient's blood, researchers can potentially detect these tumor-specific methylation changes. This approach offers several advantages over traditional tissue biopsies, which can be invasive and often limited to accessible tumor sites.

Several studies have shown promising results for cfDNA methylation analysis in detecting pancreatic cancer[2]. Researchers have identified specific cfDNA methylation markers that are associated with the presence of pancreatic cancer. These markers hold promise for the development of non-invasive diagnostic tests that could facilitate earlier detection of the disease, potentially leading to improved patient outcomes[2]. Circulating cfDNA includes fragments released by tumor cells, offering a potential source of tumor-derived information. By analyzing variation in the methylation patterns of cfDNA, researchers can potentially mark methylation changes associated with pancreatic cancer[14]. Various categorial cfDNA features are reported highlighting crucial participation in pancreatic cancer as shown in Table 2. Several studies have shown promising results for cfDNA methylation analysis in detecting pancreatic cancer. Researchers are identifying specific cfDNA methylation markers associated with the presence of pancreatic cancer. These markers hold promise for the development of non-invasive diagnostic tests that could facilitate earlier detection, potentially leading to improved patient outcomes.

5. Epigenetic Regulators and DNA Methylation in PDAC

DNA methylation, as discussed previously, plays a critical role in regulating gene expression in pancreatic ductal adenocarcinoma (PDAC). However, the establishment and maintenance of these methylation patterns are orchestrated by a complex machinery involving various epigenetic regulators. There is strong correlational links between these regulators and DNA methylation in PDAC development are reported till date[15].

5.1 DNA Methyltransferases (DNMTs)

These enzymes are responsible for adding methyl groups to DNA, leading to gene silencing. DNMT1, the primary maintenance methyltransferase, ensures the faithful inheritance of methylation patterns during cell division [16]. Aberrant upregulation of DNMT1 activity has been observed in PDAC, contributing to the silencing of tumor suppressor genes that would otherwise regulate cell growth and proliferation[16]. Studies have shown that DNMT1 overexpression correlates with poor prognosis in PDAC patients[20]. This highlights the potential of targeting DNMT1 activity as a therapeutic strategy.

5.2 DNA Methyltransferase Inhibitors (DNMTis)

A new class of drugs has emerged as a promising therapeutic strategy by inhibiting DNMT activity and reactivating silenced tumor suppressor genes. Pre-clinical researches reported that DNMTis can inhibit PDAC cell growth and induce apoptosis (programmed cell death)[16]. However, further research is needed to optimize their efficacy and clinical application in PDAC treatment. Combination therapies with other chemotherapeutic agents are also being explored to improve treatment outcomes[21].

5.3 Ten-Eleven Translocation (TET) Enzymes

These enzymes function antagonistically to DNMTs by catalyzing the removal of methyl groups from DNA, promoting gene activation. Mutations or silencing of TET genes have been implicated in various cancers, including PDAC[18]. Restoring TET activity represents a potential therapeutic strategy for PDAC by promoting the re-expression of silenced tumor suppressor genes that can inhibit cancer cell growth and survival [21].

5.4 Histone Modifications

DNA methylation often co-occurs with alterations in histone modifications, another layer of epigenetic regulation. Chromatin remodelers and histone-modifying enzymes play a crucial role in regulating

DNA accessibility and gene expression. Disruptions in these processes can contribute to the aberrant methylation patterns observed in PDAC[19].

Understanding the interplay between these epigenetic regulators and DNA methylation holds immense promise for the development of novel therapeutic strategies for PDAC. By targeting these regulators, researchers aim to reverse the silencing of tumor suppressor genes and disrupt the growth and survival of cancer cells[20]. The development of drugs targeting epigenetic regulators is a promising route for PDAC treatment. However, further research is needed to optimize their efficacy and overcome challenges associated with drug delivery and specificity. Each regulators has a specific function, and alterations in their activity can contribute to PDAC development. DNMTs and histone modifications often jointly work together towards silencing of tumor suppressor genes, while TET enzymes and DNMTis can counteract this silencing. Understanding these mechanistic interactions opens a new promising approaches for the development of novel therapeutic strategies combating PDAC. Some detail functional and active role ın PDAc by epigenetic regulators are shown in Table 3.

6. Challenges in Developing and Utilizing cfDNA Methylation Biomarkers for PDAC Detection

While cfDNA methylation analysis holds immense promise for the early detection of pancreatic ductal adenocarcinoma (PDAC), significant challenges emerges and are needed to be addressed before it can be widely adopted in clinical practice. This section explores some of the key hurdles researchers are working to overcome[23].

6.1 Technical Challenges

A. Limited cfDNA Yield: PDAC tumors often shed relatively low amounts of cfDNA into the bloodstream compared to other cancers. This limited amount can make it challenging to obtain sufficient cfDNA for robust methylation analysis[12]. Optimizing isolation techniques and developing more sensitive detection methods are crucial for overcoming this hurdle.

B. Fragmentation of cfDNA: Circulating cfDNA molecules are fragmented, making it difficult to identify the tissue of origin and distinguish tumor-derived methylation patterns from those originating from healthy cells[24]. Novel approaches are needed

Table 3: Epigenetic Regulators and their Roles in PDAC

Epigenetic Regulator	Function	Role in PDAC
DNA Methyltransferases (DNMTs)	Add methyl groups to DNA, leading to gene silencing.	Aberrant up-regulation can silence tumor suppressor genes, promoting PDAC development. DNMT1 over expression correlates with poor prognosis[15,19].
DNA Methyltransferase Inhibitors (DNMTis)	Inhibit DNMT activity, reactivating silenced tumor suppressor genes.	Pre-clinical studies show promise in inhibiting PDAC cell growth and inducing apoptosis. Further research needed for clinical application[16,21].
Ten-Eleven Translocation (TET) Enzymes	Remove methyl groups from DNA, promoting gene activation.	Mutations or silencing of TET genes are linked to PDAC. Restoring TET activity is a potential therapeutic strategy to re-express tumor suppressor genes[17,21].
Histone Modifications	Modifications on histone proteins regulate DNA accessibility and gene expression.	Disruptions in histone modifications, like mutations in histone demethylases, can contribute to aberrant methylation patterns in PDAC[18,22].

to differentiate between these sources and ensure the specificity of cfDNA methylation markers for PDAC detection.

C. Heterogeneity of PDAC: PDAC tumors exhibit significant intra-tumor heterogeneity, meaning methylation patterns can vary within the same tumor. This can lead to challenges in identifying reliable methylation markers that are representative of the entire tumor. Furthermore, inter-tumor heterogeneity exists between different patients, requiring the development of panels of methylation markers to improve diagnostic accuracy across diverse populations[25].

6.2 Analytical Challenges

A. Standardization of Methods: Currently, there is a lack of standardization in cfDNA isolation protocols, bisulfite conversion techniques, and methylation detection methods. This variability can lead to inconsistencies in results and make it difficult to compare data from different studies[15]. Establishing standardized protocols and reference materials is essential for reliable and reproducible cfDNA methylation analysis in PDAC diagnostics.

B. Data Analysis and Interpretation: Analyzing the vast amount of data generated by cfDNA methylation profiling presents a significant challenge. Developing robust bioinformatic tools and algorithms is crucial for identifying and interpreting methylation patterns that are specifically associated with PDAC[26]. These tools should account for tumor heterogeneity and potential confounding factors such as age, lifestyle, and co-existing diseases.

6.3 Clinical Challenges

A. Validation of Biomarkers: Rigorous clinical validation studies are needed to establish the accuracy, sensitivity, and specificity of cfDNA methylation markers for PDAC detection. These studies should involve large patient cohorts and compare the performance of cfDNA methylation analysis with existing diagnostic methods[14]. Demonstrating the cost-effectiveness of cfDNA methylation testing compared to traditional

diagnostic procedures will also be crucial for its wider adoption in clinical practice.

B. Integration with Clinical Workflow: Developing workflows for seamlessly integrating cfDNA methylation analysis into routine clinical practice requires further exploration. This includes establishing appropriate cut-off values for methylation markers and determining the optimal timing for cfDNA methylation testing in the diagnostic pathway for PDAC.

Despite these challenges, ongoing research is actively addressing these limitations. Advancements in cfDNA isolation techniques, bisulfite conversion methods, and next-generation sequencing technologies are continuously improving the sensitivity and accuracy of cfDNA methylation analysis. Furthermore, large-scale collaborative efforts are underway to identify and validate robust cfDNA methylation markers for PDAC detection. By overcoming these challenges, cfDNA methylation analysis has the potential to revolutionize the early detection of PDAC, leading to improved patient outcomes.

6. Conclusion

Pancreatic ductal adenocarcinoma (PDAC) remains a highly aggressive malignancy with a dismal prognosis. Early diagnosis and the development of more effective therapeutic strategies are critical for improving patient outcomes. DNA methylation, a key epigenetic modification, has emerged as a promising route for understanding PDAC pathogenesis. This review has explored the current understanding of DNA methylation in PDAC, highlighting its role in gene regulation and its potential as a source of biomarkers. We discussed the identification of differentially methylated CpG sites and candidate genes associated with PDAC, offering insights into the underlying molecular mechanisms of the disease. Furthermore, the potential of cfDNA methylation analysis for early detection and its clinical applications in risk stratification, treatment monitoring, and minimal residual disease detection were explored. Despite the immense potential

of DNA methylation sequencing, significant challenges need to be addressed for its wider adoption in clinical practice. In conclusion, DNA methylation sequencing holds immense promise for revolutionizing the diagnosis and management of PDAC. By overcoming current challenges and pursuing future directions, researchers can work on the way for the development of novel epigenetic therapies and improve patient outcomes against devastating PDAC disease.

Conflict of Interest: None

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