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Review Article

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Role of *ADAMTS* and *BNC1* as Diagnostic, Prognostic, and Predictive Biomarkers in Pancreatic and Colorectal Cancer

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Abstract

Aggressive cancer such as pancreatic and colorectal cancer poses significant challenges for patient management due to late-stage diagnosis and therapeutic resistance. In lieu of this, several biomarkers, including those associated with aberrant DNA methylation are being investigated for diagnosis, prognoses, and therapeutic monitoring of these cancers. Several studies have reported that A Disintegrin And Metalloproteinase with ThromboSpondin motifs 1 (*ADAMTS1*) and zinc finger protein Basonuclin-1 (*BNC1*) gene methylation leading to gene expression/silencing has been associated with cancer progression, metastasis, and poor overall survival. Keeping this perspective in context, the potential utility of these methylation markers in pancreatic (PC) and colorectal cancers (CRC) is being investigated. However, limited review articles on the role of *ADAMTS* and *BNC1* gene methylation in PC and CRC have been published. Therefore, this review aims to comprehensively curate literature on *ADAMTS* and *BNC1* DNA methylation as a diagnostic, prognostic, and therapeutic marker in pancreatic and colorectal cancer. The review aims to provide understanding of the complex mechanisms underlying *ADAMTS1* and *BNC1* DNA methylation and their role as potential therapeutic targets for these hard-to-treat cancers.

Keywords: *ADAMTS*, *BNC1*, DNA methylation, Pancreatic cancer, Colorectal cancer

1. Introduction

Pancreatic cancer (PC) is a highly invasive malignant tumor that originates from pancreatic cells and results in the lowest five-year survival rates. Globally, pancreatic cancer is the third leading cause of cancer-related mortality, reflecting its aggressive nature due to challenges in early detection and treatment [1]. It is postulated that by the year 2030, pancreatic cancer is expected to become the second leading cause of cancer-related deaths [2]. Moreover, the Surveillance, Epidemiology, and End Results (SEER) database reports that only around 14% of PC cases are diagnosed during initial diagnosis leading to poor patient outcomes [3].

Colorectal Cancer (CRC) is a frequent major cause of cancer-related deaths globally which mostly begin as benign growths in the colon, such as adenomatous, which can gradually turn cancerous over time [4]. CRC is increasingly seen in younger adults. While most cases occur without a genetic link, approximately 10% arise from inherited genetic conditions such as Lynch syndrome or familial adenomatous polyposis [4]. Like PC, early detection of CRC remains a challenge despite the availability of screening tools [5]. Prognosis is poor in CRC, and several studies have reported on various biomarkers including those associated with DNA methylation [6–10].

Moreover, DNA methylation is a well-known phenomenon that manifests in various genomic loci, primarily at gene promoters and first exon, during the early stages of tumor formation, making DNA-methylated cancer genes potential candidates for diagnosis, prognosis and therapeutic targeting [11]. Due to its clinical significance, discoveries of abnormal DNA methylation in CRC have paved the way for FDA-approved screening tools like Cologuard and Epi proColon, which help detect the disease earlier and more effectively [12, 13]. Though these kits facilitate DNA methylation patterns, their utility as

prognostic tools is still under investigation especially in genetically diverse populations [12].

PC and CRC share aspects of their embryologic origin, which may help explain their overlapping molecular features and epigenetic alterations [14, 15]. Among these is the aberrant DNA methylation of genes such as *ADAMTS1* and *BNC1*, which have been detected in both tumor types. *ADAMTS1* (A Disintegrin And Metalloproteinase with Thrombospondin Motifs 1) encodes a matrix-degrading protease involved in modulating the tumor microenvironment and inhibiting angiogenesis. Hypermethylation of its promoter region leads to gene silencing, which may remove critical barriers to tumor invasion and metastasis. Similarly, *BNC1* (Basonuclin 1), a zinc finger transcription factor implicated in cellular proliferation and differentiation, is frequently silenced by promoter hypermethylation in various cancers, including pancreatic and colorectal, thereby contributing to uncontrolled cell growth and tumor progression [10, 15–18]. The detection of methylated *ADAMTS1* and *BNC1* in both PC and CRC suggests the presence of a shared epigenetic signature that may serve as a powerful biomarker for early detection. Therefore, establishing such a common methylation profile lays the groundwork for noninvasive screening tools with diagnostic, prognostic, and minimal residual disease monitoring potential, ultimately enabling more effective disease surveillance and improved patient outcomes [10, 15–19].

The primary aim of this review is to curate recent literature evidence on the role of methylated *ADAMTS1* and *BNC1* as emerging biomarkers in pancreatic and colorectal cancers. Given the increasing global burden of these aggressive malignancies, the lack of sensitive early detection methods, and the success of methylation-based diagnostics in colorectal cancer, this review is both timely and relevant. It highlights the translational potential of shared methylation signatures and underscores the importance of advancing epigenetic biomarker research to improve clinical outcomes in these hard-to-treat cancers.

2. Search strategy and selection criteria

A systematic literature review was performed to identify primary research examining *ADAMTS1*, *ADAMTS12*, and *BNC1* DNA methylation as diagnostic, prognostic, and predictive markers in PC and CRC. Peer-reviewed articles published from 2000 to 2025 were retrieved from PubMed, Scopus, Web of Science, and Google Scholar. The search employed combinations of targeted keywords, including *ADAMTS1* methylation, *ADAMTS12* methylation, *BNC1* methylation, pancreatic cancer, colorectal cancer, DNA methylation, epigenetics, and cell-free DNA.

Research articles qualified for inclusion if they constituted primary investigations of *ADAMTS1*, *ADAMTS12*, or *BNC1* methylation patterns in pancreatic or colorectal cancer. To capture the full scope of existing evidence, literature search was expanded to studies encompassing human tissue specimens, liquid biopsy materials, cancer cell lines, and other relevant biological models, contingent upon direct methylation evaluation. Included studies addressed the diagnostic, prognostic, predictive, or mechanistic significance of these methylation biomarkers.

Although strict exclusion parameters were not applied, specific publication formats received lower priority unless they offered critical contextual information. Such formats included conference abstracts, editorials, case reports, non-English language articles, exclusively animal-based investigations, and research limited to gene expression without methylation data. The central criterion guiding study inclusion was whether the findings substantively advanced knowledge of the methylation-dependent functions of *ADAMTS1*, *ADAMTS12*, or *BNC1* in PC and CRC.

The selection procedure commenced with preliminary screening of titles and abstracts, subsequently followed by thorough full-text assessment of candidate articles. To guarantee thorough coverage, reference lists from all selected studies underwent manual examination to locate further relevant publications.

3. DNA methylation

DNA methylation, considered as an epigenetic mechanism, is crucial in gene expression regulation. This process involves CpG sites, short DNA sequences of cytosine and guanine nucleotides linked by a phosphate group, within the DNA molecule that serve as primary targets of DNA methylation. Notably, CpG sites that undergo methylation tend to form clusters of approximately 500 base pairs with high guanine and cytosine nucleotides. These clusters are designated as CpG islands. The DNA methylation process involves a family of enzymes known as DNA methyltransferases (DNMTs) that recognize and bind to specific CpG sites, catalyzing the transfer of a methyl group (-CH3) to the fifth carbon of the cytosine residue. This transfer is accomplished using S-adenosyl methionine (SAM) as a donor, creating a modified form of the DNA molecule called 5-methylcytosine (5Mc) [20, 21]. The attached methyl groups recruit Methyl-CpG-Binding Domain (MBD) proteins, that silence gene expression by recognizing and forming stable complexes with methylated CpG dinucleotides. Such complexes recruit chromatin-modifying enzymes, leading to chromatin compaction and transcriptional repression, effectively inhibiting gene expression [20].

In mammals, the establishment and maintenance of these patterns is performed by DNMT3, and DNMT1. In normal conditions, proper DNA methylation patterns ensure precise gene expression regulation and stable gene silencing. Therefore, aberrant DNA methylation patterns are considered a major epigenetic hallmark in various types of cancer, including PC and CRC [9, 14].

3.1. DNA methylation in pancreatic and colorectal cancer

Epigenetic modifications can dysregulate the cell cycle, which is crucial to cancer progression. In PC, these epigenetic changes affect cell cycle control mechanisms. Hypermethylation-induced silencing of tumor suppressor genes disrupts the cell-cycle checkpoints leading to impaired DNA replication and repair process. For instance, the *CDKN2A* (p16) tumor suppressor gene, which inhibits cyclin-dependent kinases (CDKs) as a cell cycle regulation process, is frequently silenced through hypermethylation. This allows CDKs to remain active consistently, resulting in dysregulation of the G1 to S transition and compromising genomic integrity [21, 22]. On the other hand, hypomethylation promotes uncontrolled cell proliferation via overexpression of oncogenes and amplification of signaling pathways involved in cell cycle progression [21, 23]. This dual mechanism, involving hypermethylation-mediated silencing of tumor suppressor genes and hypomethylation-induced oncogene activation, creates an environment supporting proliferation of pancreatic cancer cells as shown in Figure 1 [9, 24]. Together, these dual epigenetic mechanisms result in dysregulated cell cycle progression, genomic instability, and ultimately cancer development [16]. Therefore, it is essential to unravel these underlying mechanisms and identify potential biomarkers, such as *ADAMTS1* and *BNC1*, to develop targeted therapeutic strategies and advance early detection methods in both PC and CRC [10, 25].

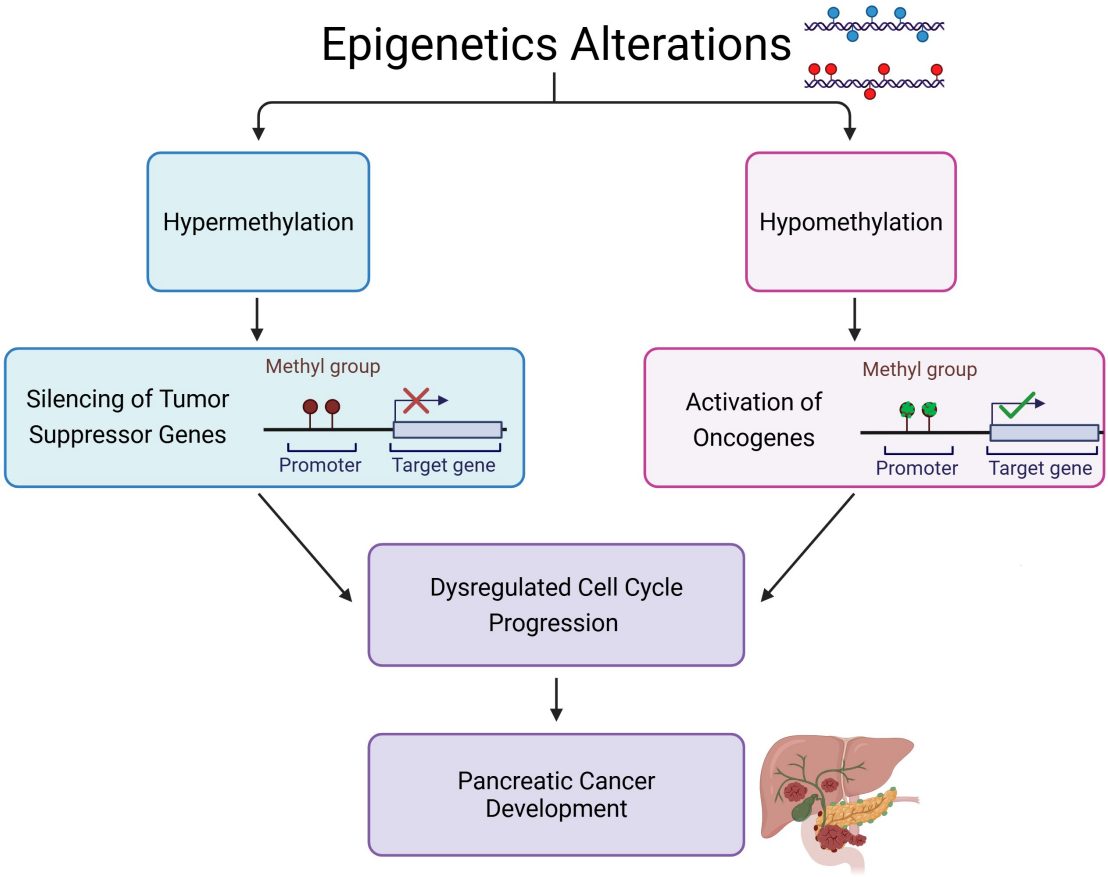

Figure 1: Epigenetic Regulation of Cell Cycle Dysregulation in Pancreatic Cancer. The diagram shows how epigenetic alterations contribute to pancreatic cancer. Hypermethylation silences tumour suppressor genes, while hypomethylation activates oncogenes. Both lead to dysregulated cell cycle progression, ultimately resulting in pancreatic cancer development.

3.2. DNA Methylation of *ADAMTS-1* and *BNC1* in Pancreatic and Colorectal Cancer

ADAMTS-1, a member of the *ADAMTS* family, is a protease enzyme that has a tissue-specific expression pattern associated with functional roles such as extracellular matrix (ECM) remodeling, angiogenesis regulation, tissue development and homeostasis [26, 27]. Dysregulation of the ECM turnover can lead to tissue dysfunction [26]. The importance of *ADAMTS-1* in regulating angiogenesis is attributed to its anticancer and anti-inflammatory properties. It acts as a putative anti-angiogenic factor, modulating blood vessel formation vital for normal development [27]. Furthermore, *ADAMTS-1* contributes to skeletal, follicular, and lung development, ensuring proper tissue structure and function [26]. Therefore, hypermethylation of *ADAMTS1*'s promoter regions leads to reduced expression or silencing of the gene, critically affecting its function. Studies

have demonstrated that this dysregulation could facilitate tumor cell invasion, metastasis, and survival, thereby promoting tumor development and progression [8, 10, 15, 28].

An analysis of DNA methylation biomarkers in PC revealed that *ADAMTS1* showed cancer-specific methylation patterns in primary pancreatic tumors and precursor lesions known as Pancreatic Intraepithelial Neoplasms (PanINs) [26]. The methylation status of *ADAMTS1* was found to be significantly correlated with the loss of gene expression in PC cell lines and primary tumor samples [26]. Moreover, treatment with DNA demethylating agents resulted in a significant re-expression of *ADAMTS1*, which indicates that its methylation status directly influences gene expression on PC. Heavy methylation patterns have been reported in *ADAMTS1* promoter regions both in PC cell lines and primary tumors. On the other hand, minimal or no methylation was observed in normal pancreatic tissues [26].

Similarly in CRC, *ADAMTS1* promoter hypermethylation has been reported in more than 80% of CRC cell lines and in around 70% of biopsies while lower hypermethylation has been reported in adenomas and normal tissues. Such hypermethylation in CRC is linked to reduced or silenced *ADAMTS1* expression, altering its tumour-suppressive role [29]. The increased frequency of *ADAMTS1* silencing from adenomas to high-grade carcinomas suggests its role during the adenoma–carcinoma transition, with potential implications as an early biomarker for CRC dynamics [29].

In addition to *ADAMTS-1*, *BNC1* also shows notable methylation changes in these cancers. BNC is a highly conserved zinc finger protein expressed in specific cell types such as skin keratinocytes and testicular and ovarian germ cells. Mechanistically, *BNC1* serves as a transcription factor and plays a significant role in cellular differentiation, proliferation and maintenance of ribosomes [30, 31]. Aberrant expression of *BNC1* promoter methylation has been attributed to tumor progression in lungs, pancreatic, renal, lymphocytic leukemia, and brain cancers.

In PC, silencing of *BNC1* due to promoter hypermethylation is a notable phenomenon. This silence disrupts *BNC1*'s function as a transcription factor, affecting regulation of genes involved in cellular differentiation and proliferation. On the other hand, studies have demonstrated that over-expressing *BNC1* in PC cell lines leads to inhibition of colony formation and cell proliferation in vitro. These findings suggest that restoring *BNC1* expression or its tumor-suppressive functions could potentially affect PC progression, leading to better patient outcomes [31].

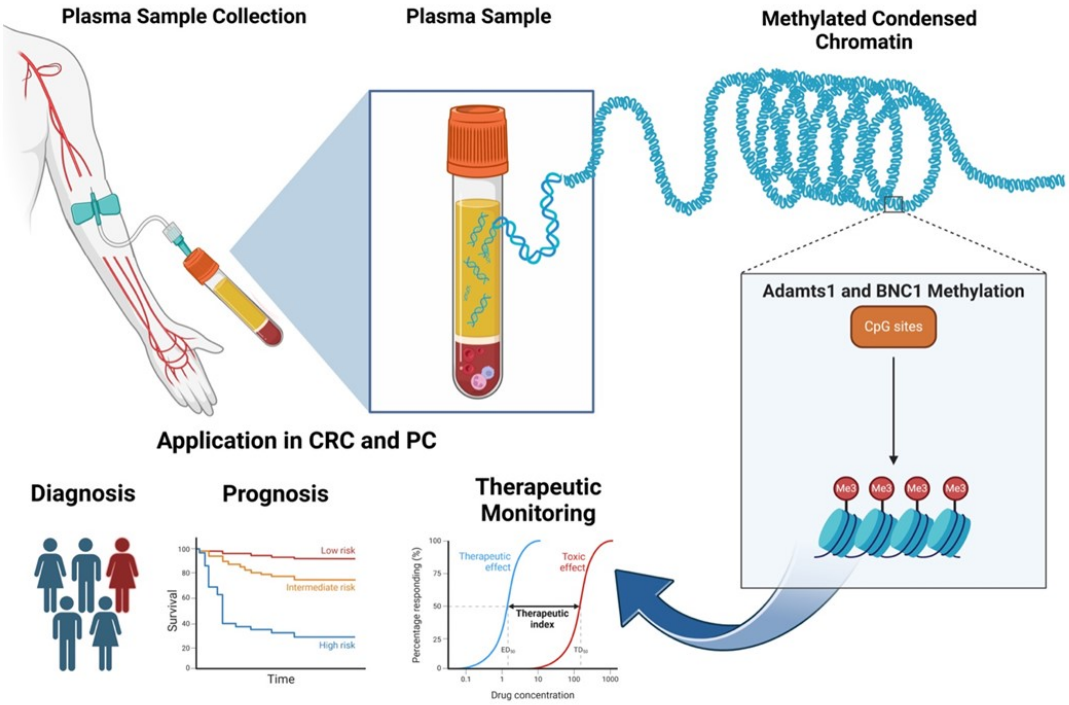

Figure 2: Plasma-based Detection and Functional Applications of *ADAMTS1* and *BNC1* Methylation in Pancreatic and Colorectal Cancer. This schematic illustrates the process of plasma sample collection and downstream analysis of cell-free DNA (cfDNA) containing methylated chromatin fragments. Specific CpG site hypermethylation in genes such as *ADAMTS1* and *BNC1* can be detected and used as epigenetic biomarkers. These methylation changes have demonstrated clinical utility in diagnosis, prognosis, and therapeutic monitoring of colorectal cancer (CRC) and pancreatic cancer (PC). Applications include early detection, patient risk stratification, and treatment response assessment, supporting the development of personalized therapeutic strategies.

A comparable pattern is observed in CRC, aberrant methylation of the *BNC1* gene via epigenetic modification has been reported in early precursor lesions/adenomas with increased aberrant modification observed in late-stage CRC. This observation

indicates involvement of aberrant methylation patterns in *BNC1* early in the tumor development stages [32]. *BNC1* methylation has been associated with reduced gene expression, particularly in moderately differentiated CRCs, suggesting its potential role as a tumor suppressor [32]. These findings suggest that *BNC1* methylation is a potential biomarker for early detection and risk assessment in CRC and may be a target for novel therapeutic interventions [32].

Several studies have documented that *ADAMTS1* hypermethylation is associated with inhibition of its proteolytic activity for ECM components thus facilitating desmoplastic stroma formation and angiogenesis in the pancreatic tumour micro-environment [15, 33, 34]. Furthermore, promoter methylation–mediated silencing of *BNC1* gene accelerates genomic instability by affecting p53-dependent transcription and telomere maintenance leading to poor patient survival [35]. Nano-enabled "Methylation-On-Beads" (MOB) assays that couple silica superparamagnetic beads with bisulphite-converted cfDNA have boosted analytical sensitivity for these loci, enabling single-molecule detection in as little as 200 µL of serum [36]. Combining *ADAMTS1* and *BNC1* methylation readouts with circulating mutant KRAS or CA19-9 increases the composite area-under-the-curve to >0.90, underscoring their translational potential for blood-based screening of high-risk cohorts [15]. This means that the combined markers can distinguish between healthy individuals and those with PC, particularly in early stages, making them promise for blood-based screening in high-risk populations. A graphic summary of the clinical relevance and workflow of plasma-based detection of *ADAMTS1* and *BNC1* methylation in pancreatic and colorectal cancer is illustrated in Figure 2.

4. Role of *ADAMTS* 1, 5, and 12 as diagnostic, prognostic, and predictive biomarkers in pancreatic cancer

4.1. *ADAMTS1* DNA Methylation in Diagnosing Pancreatic Cancer

The potential utility of aberrant *ADAMTS-1* DNA methylation as an early diagnostic marker for PC has been an area of interest for some researchers globally [10, 15, 18]. This topic was prompted by a study that discovered a lower *ADAMTS1* mRNA expression in PC tissue than in non-cancerous pancreas (p value:0.002). While *ADAMTS1* was initially believed to have antiangiogenic effects on PC, it was later found to promote local invasion and lymph node metastasis. These findings have stimulated further research on *ADAMTS1* altered expression in PC [37]. A hallmarks study by Yi Mi et al., reported on high frequency of *ADAMTS1*(67%) and *BNC1* (91%) DNA methylation in several PC cell lines. After correlating the abnormal methylation status with the expression patterns for these genes, both genes showed a lack of endogenous gene expression and significant re-expression after demethylating agent 5-aza-2-deoxycytidine (DAC) treatment in these cell lines. Bisulfite sequencing analysis also confirmed the promoter associated CpG island methylation in *BNC1* and *ADAMTS1* promoters. Using methylation-specific polymerase chain reaction (MSP) analysis, methylation of *BNC1* and *ADAMTS1* was compared between different conditions (normal, pancreatitis, PanIN, and invasive cancers). Increased methylation patterns were observed both in invasive cancers compared to normal pancreatic tissues ($p<0.001$) and chronic pancreatitis ($p<0.001$) [10]. A low frequency of *ADAMTS1* methylation was observed in non-cancerous diseases such as pancreatitis [10]. Including pancreatitis samples in this comparison aids in elucidating the distinction between cancer-associated methylation and inflammatory-associated methylation. Interestingly, a significant quantitative difference between PanINs and invasive cancers in *ADAMTS1* and *BNC1* ($p<0.001$) was observed. The study reported that *BNC1* methylation can be detected in the early stages of pancreatic carcinogenesis, such as PanIN while *ADAMTS1* methylation was only found in invasive stages [10]. These findings support the potential use of *ADAMTS1* and *BNC1* genes as diagnostic markers [10]. Similarly, a pilot study to determine whether *BNC1* and *ADAMTS1* promotes DNA methylation in pancreatic cancer patients' serum using MOB reported that for all stages of PC, *ADAMTS1* and *BNC1* demonstrated reliable sensitivity and specificity. However, *ADAMTS1* or *BNC1* individually demonstrate lower specificity and sensitivity indicating their limitation as to individual diagnostic markers [10].

4.2. *ADAMTS1* and *ADAMTS12* methylation in pancreatic cancer prognosis and therapeutic response

Evidence suggests that treatment can cause reversible changes in DNA promoter methylation [38]. Nonetheless, there is a deficiency in prospective research that tracks the methylation patterns in PC patients based on their prognosis, treatment outcomes, and recurrence rates [39]. Throughout research, the DNA methylation status of *ADAMTS1* and *ADAMTS12* holds promise as a prognostic marker for PC. For example, *ADAMTS1* DNA methylation, reported more frequently in stage II (85%) than in PanIN or stage I PDAC (25%) may indicate PC progression over time, evidencing its utility as a biomarker of response [40]. Subsequently, another study validated a biomarker panel in cell-free tumor DNA of 39 patients with different stages of pancreatic cancer. Results revealed that the cell-free DNA methylation status of *ADAMTS1* and *BNC1* is sensitive and specific for early detection of pancreatic cancer when curative tumor resection is still feasible [15]. The methylation of *ADAMTS1* was positive in 87.5% of patients with stage I cancer, 77.8% of patients with stage IIA, 90% with stage IIB, and 100% with stage III/IV PC indicating its utility as a prognostic marker [15].

In addition, studies have also investigated the potential role of *ADAMTS12* as a prognostic and chemotherapy response marker. A study analyzing serum samples of 58 resected patients and 87 patients with either metastatic or locally advanced

PC, reported higher expression of *ADAMTS12* in PC patients compared to normal controls ($p < 0.0001$). It was also reported that in resected patients, high levels of *ADAMTS12* were associated with poor outcomes (HR: 2.07, p-value:0.04). Also, in the phase III Metastatic Pancreatic Adenocarcinoma Clinical Trial (MPACT) trial, researchers assessed initial and follow-up levels in another 372 patients with metastatic PC who were chemotherapy treated with nab-paclitaxel with gemcitabine (n = 184) or gemcitabine monotherapy (n = 188). Results revealed that nab-paclitaxel patients showed undetectable *ADAMTS12* levels before treatment and had prolonged survival compared to those who did not receive the treatment (p value: 0.0046). On the other hand, patients with consistently undetectable or reduced *ADAMTS12* levels during treatment showed better survival patterns (p value:0.0054). The study concluded that *ADAMTS12* can serve as a prognostic blood marker for stromal activation, and its levels can facilitate prediction of prognosis and therapeutic response [41]. These findings collectively highlight the clinical potential of *ADAMTS1* and *ADAMTS12* as non-invasive biomarkers for early pancreatic cancer diagnosis and prognosis (Table 1).

Table 1: Summary of studies on *ADAMTS1* and *ADAMTS12* as diagnostic and prognostic biomarkers in pancreatic cancer

Biomarker	Purpose	Methods	Findings	Significance	Limitations	Reference
ADAMTS1 (METH-1)	Prognostic	RT-PCR of tumour and adjacent tissue	*ADAMTS1* mRNA downregulated in PC vs. normal Higher expression within tumours correlated with lymph node metastasis and worse prognosis	Indicates prognostic utility of *ADAMTS1* expression (higher levels = worse outcome)	Small sample (n=18), and no protein data. Further validation in high-risk populations needed	[37]
ADAMTS1	Diagnostic	MSP, qPCR, bisulphite sequencing in cell lines and serum	Promoter methylation of *ADAMTS1* detected in 67% of PC cell lines and early-stage tumours Re-expression after demethylation was detectable in serum using MOB assay	Supports *ADAMTS1* as a potential early diagnostic biomarker when used with *BNC1*	Cell-line based data Limited serum sample size *ADAMTS1* alone has moderate sensitivity	[10]
ADAMTS1	Diagnostic	cfDNA methylation in plasma (MOB assay)	*ADAMTS1* methylation detectable in cfDNA with 87.2% sensitivity and 95.8% specificity; combined with *BNC1*, detection reached 97.3% sensitivity	Validates non-invasive early detection using a two-gene methylation panel	Small cohort Low sensitivity for *ADAMTS1* alone	[15]

ADAMTS1: A Disintegrin And Metalloproteinase with ThromboSpondin motifs 1; METH-1: human ortholog of the protein ADAMTS1; ADAMTS12: A Disintegrin And Metalloproteinase with ThromboSpondin motifs 12; RT-PCR: Real-Time polymerase chain reaction; MSP: Methylation-specific PCR; qPCR: quantitative polymerase chain reaction; cfDNA: circulating cell-free DNA; MOB: Methylation-on-Beads protocol; mRNA: messenger RNA; PC: Pancreatic Cancer; BNC1: Basonuclin-1.

4.3. *ADAMTS1* DNA Methylation in diagnosing Colorectal Cancer

ADAMTS1 DNA methylation analysis emerges as a candidate for enhancing the early detection of CRC, highlighting its potential as a valuable noninvasive diagnostic marker. A study on 20 colon cancer cell lines reported increased hypermethylation patterns in *ADAMTS1* (85%) genes. In addition, in 116 adenoma and carcinoma samples, *ADAMTS1* hypermethylation was observed to be 37% and 71% respectively, indicating early hypermethylation as a marker of disease progression [18].

What qualifies *ADAMTS1* as a suitable early diagnostic marker is the clear distinction between its methylation status in normal mucosa/healthy patients (0%) and in malignant lesions (71%). Surprisingly, two out of three CRC cell lines lacked expression of *ADAMTS1* indicating that other unknown mechanisms may be at play [18]. However, further investigation is required to determine if the methylation of *ADAMTS1*, Cellular Retinoic Acid-Binding Protein 1 (*CRABP1*), and nuclear receptor subfamily 3, group C, member 1(*NR3C1*) genes is exclusive to CRC or if it is also present in other types of tumors within the hereditary nonpolyposis colon cancer (HNPCC) spectrum. It was concluded that with refined microarray screening, *ADAMTS1* was detected and *CRABP1* and *NR3C1*, encountered cancer-specific hypermethylation in CRC [18]. Subsequently, another supporting research showed that gene-specific promoter hypermethylation is an early event in colorectal tumorigenesis, demonstrated by hypermethylation of *ADAMTS1* in polyps irrespective of size. This study also found that O-6-methylguanine-DNA methyltransferase (MGMT) and Mucosa Associated Lymphoid Tissue Translocation 1 (MALT) are

also hypermethylated in CRC patients indicating these genes are appropriate for the early detection of colorectal lesions in an unstable colon as part of a panel [42]. Another study revealed that *ADAMTS-1* was down-regulated in CRC samples compared to normal colons, with the most significant decrease of approximately 20% in early-stage CRC [43].

Several commercial assays and diagnostic kits have incorporated methylated MGMT for clinical applications, especially in glioblastoma, but its relevance in colorectal cancer (CRC) has also been supported by recent studies. For example, the therascreen® MGMT Pyro® Kit (QIAGEN) is CE-marked for detecting MGMT promoter methylation using pyrosequencing, and studies [44] have suggested its applicability in CRC. In addition, the Epi proColon® test, which targets methylated SEPT9, demonstrates its clinical potential in circulating tumor DNA for early CRC detection, paving the way for similar assays to include MGMT and *ADAMTS1* [45]. Although *ADAMTS1* is not yet commercially targeted, its early hypermethylation in polyps suggests strong potential as a candidate biomarker in future multiplex methylation panels [46].

4.4. *ADAMTS* 1, 5, and 12 methylation in assessing colorectal cancer prognosis and therapeutic response

Assessing the methylation and expression of *ADAMTS1*, -5, -12 genes emerge also as promising prognostic markers and therapeutic targets in CRC. Analysing DNA methylation profile of these genes alongside pathological diagnosis can assist in predicting the prognosis of CRC tumours. A study demonstrated that *ADAMTS1* was one of twelve genes identified as hub genes because they had high connectivity within a clinically significant module using Metascape for gene analysis. Levels of *ADAMTS1* and the other genes significantly correlate with a risk score, indicating their potential importance in predicting outcomes for CRC [47]. In another research, the function of *ADAMTS12* in the progression of CRC was examined. The study found that *ADAMTS12* expression was predominantly located in the fibroblasts bordering tumour cells or macrophages at the invasive cancer margins. Also, the levels of *ADAMTS12* expression were significantly associated with the histological grade of the tumour, depth of invasion, presence of lymph node metastasis, and Dukes' stage. Furthermore, low or absent *ADAMTS12* expression within the tumour stroma was associated with poor overall/disease-free survival. Meanwhile, patients expressing *ADAMTS12* had a favourable prognosis. It was concluded that the expression of *ADAMTS12* in the stroma of CRC samples is essential in preventing tumour growth. As a result, *ADAMTS12* expression can serve as a reliable prognostic marker for CRC [48]. Furthermore, another member of the *ADAMTS* family, *ADAMTS5*, is potentially an interesting therapeutic target in CRC. A study found that *ADAMTS5* was hypermethylated and downregulated in tumour tissues compared with non-tumour tissues ($p < 0.001$). The findings showed that increasing *ADAMTS5* levels hindered CRC cells' migration and invasion abilities suggesting its potential as a valuable biomarker in CRC disease-free survival [49]. These findings position *ADAMTS1*, *ADAMTS5*, and *ADAMTS12* as emerging markers for CRC prognosis and treatment response, pending further clinical validation (Table 2).

Table 2: Summary of Studies on *ADAMTS* family as diagnostic and prognostic biomarkers in Colorectal Cancer (CRC)

Biomarker	Purpose	Methods	Findings	Significance	Limitations	Reference
ADAMTS1	Diagnostic	DNA methylation profiling in CRC tissues	*ADAMTS1* hypermethylated in 71% of colorectal carcinomas and 37% of adenomas; 0% methylation in normal mucosa	Suggests cancer-specific and early-stage diagnostic utility for CRC	Moderate sample size. Lacks protein or functional data	[18]
ADAMTS1	Diagnostic	Methylation microarray on CRC tissue samples	*ADAMTS1* included in a panel of genes frequently methylated in benign and malignant CRC lesions	Supports field effect and early-stage epigenetic involvement in CRC	Sample size moderate. Needs further validation	[42]
ADAMTS1	Prognostic	Methylation-based survival model using TCGA	*ADAMTS1* part of a 9-gene methylation signature predicting poor overall survival in CRC	Suggests potential prognostic utility via methylation status	Model-based; lacks prospective clinical validation	[22]
ADAMTS1	Expression analysis	RT-PCR of CRC and normal colon tissues	*ADAMTS1* significantly downregulated in CRC, especially in early-stage (Stage A) tumours	Indicates possible tumor suppressor role via transcriptional silencing	No methylation analysis; no survival correlation	[21]

Biomarker	Purpose	Methods	Findings	Significance	Limitations	Reference
ADAMTS12	Prognostic	Immunohisto-chemistry and clinical correlation in CRC tissues	Low stromal *ADAMTS12* expression associated with deeper invasion, higher grade, lymph node metastasis, and poorer survival	Stromal *ADAMTS12* may serve as a favorable prognostic marker	Only stromal-specific, no methylation or therapeutic data	[48]
ADAMTS5	Prognostic and Therapeutic	Methylation analysis and functional assays	*ADAMTS5* hypermethylated and downregulated in CRC; re-expression reduced cell invasion without affecting proliferation or apoptosis	Supports tumor suppressor role and potential as therapeutic target	Preclinical in vitro data; lacks patient survival data	[24]

ADAMTS1: A Disintegrin And Metalloproteinase with ThromboSpondin motifs 1; ADAMTS5: A Disintegrin And Metalloproteinase with ThromboSpondin motifs 5; ADAMTS12: A Disintegrin And Metalloproteinase with ThromboSpondin motifs 12; CRC: Colorectal Cancer; TCGA: The Cancer Genome Atlas Program; RT-PCR: Real-Time polymerase chain reaction.

5. Role of *BNC1* as diagnostic, prognostic, and predictive biomarker in pancreatic cancer and colorectal cancer

5.1. Role of *BNC1* as a potential diagnostic marker in pancreatic cancer

Recent research underscores the potential of *BNC1* promoter methylation as an effective epigenetic biomarker for PC, particularly PDAC [15]. A study reported *BNC1* methylation in 65.1% of PDAC patients (25/39) compared to 6.3% in healthy individuals (6/95 controls) indicating a strong association between *BNC1* methylation and malignant changes in the pancreas. In the same study, diagnostic accuracy improved considerably when *BNC1* was evaluated alongside *ADAMTS1*. Using this two-gene combination, cancer patients were distinguished from non-cancer controls based on methylation analysis [15]. This notable detection rate illustrates the potential of a combined methylation panel to improve diagnostic accuracy. Moreover, the study also investigated methylation patterns in cfDNA and observed that early-stage pancreatic cancer, particularly in stages I and II, could be reliably detected, indicting its utility in treatment dynamics and patient management early on [15]. These results suggest that assessing *ADAMTS1* and *BNC1* methylation in cfDNA may be a valuable strategy for early detection, thereby enhancing patient outcomes through prompt treatment. Overall, the findings support the utility of *BNC1*, either independently or in conjunction with *ADAMTS1*, as a meaningful diagnostic marker for PC, especially when identified through blood-based cfDNA analysis [15](Table 3).

Table 3: Summary of Studies on *BNC1* as a diagnostic and prognostic biomarker in pancreatic cancer

Purpose	Methods	Findings	Significance	Limitations	Reference
Diagnostic	Methylation-specific PCR, bisulphite sequencing, qPCR in PC cell lines and tissues	*BNC1* promoter methylated in 91% of PC cell lines and PanINs Gene re-expressed after 5-Aza treatment	Suggests strong early-stage tissue-based diagnostic value	Data limited to in vitro and tumour tissue	[10]
Prognostic	cfDNA methylation biomarkers in PC	Proposes *BNC1* as a possible prognostic marker based on its methylation pattern, but no survival data provided	Theorized prognostic relevance that needs a clinical validation	No original data or outcome analysis presented	[40]
Diagnostic	Methylation analysis of plasma cfDNA using MOB assay	*BNC1* methylation detected in cfDNA of PC patients with 64.1% sensitivity. The sensitivity improved to 97.3% when combined with *ADAMTS1*	Demonstrates non-invasive early detection potential in combination panels	*BNC1* alone has modest sensitivity Cohort size was small	[15]

Purpose	Methods	Findings	Significance	Limitations	Reference
Diagnostic	Review of cfDNA methylation studies in PC	Summarizes that *BNC1* methylation is detectable in plasma during early-stage PC	Reinforces potential value of cfDNA-based *BNC1* detection	No original clinical or experimental data	[9]

BNC1: Basonuclin-1; qPCR: quantitative polymerase chain reaction; PC: Pancreatic Cancer; cfDNA: circulating cell-free DNA; MOB: Methylation-on-Beads protocol; PanINs: Pancreatic Intraepithelial Neoplasms; 5-Aza: 5-azacytidine; ADAMTS1: A Disintegrin And Metalloproteinase with ThromboSpondin motifs 1.

5.2. Role of *BNC1* as potential diagnostic marker in Colorectal Cancer

DNA methylation in *BNC1* genes has been reported as one of the top 50 hypermethylated Differentially Methylated Regions (DMRs) in CRC [50]. Hypermethylation in *BNC1* genes has been reported in 85% of tumor samples, clearly distinguishing precancerous and cancerous lesions versus inflamed/healthy tissue. It is possible that demethylation treatment could help reverse some of the mRNA alterations that may be caused by systematic methylation. Furthermore, systematic changes in methylation patterns were detected early on in CRC carcinogenesis, occurring in precursor lesions and CRC indicating that DNA hypermethylation is an early event that can serve as a tool in colorectal cancer diagnosis [50].

6. Limitations

Studies have shown the promising findings regarding *ADAMTS* and *BNC1* as emerging biomarkers in PC and CRC, but several limitations limit the strength and generalizability of current conclusions [15, 17, 18, 23, 37, 43, 48, 49].

Most studies of *ADAMTS1* and *BNC1*-related methylation-based biomarkers in PC and CRC rely on small, single-center, and often retrospective cohorts, and they exhibit considerable methodological heterogeneity, limiting standardization and comparability [15, 18, 32, 42, 50]. Having small sample sizes can limit the statistical power of the study and raise concerns about reproducibility. To accurately assess the efficiency of *ADAMTS1* and *BNC1* as biomarkers for PC and CRC, larger and demographically diverse cohorts are needed to refine effect estimates, validate assay cut-offs, and establish the clinical utility of these biomarkers [51].

Another major limitation is the scarcity of prospective biomarker trials. Most supporting evidence for *ADAMTS1* and *BNC1* comes from retrospective analyses of resected tissues or case-control studies [10, 15, 32]. There is a need for prospective studies to be designed with predefined biomarker hypotheses, standardized assays, or adequately powered clinical endpoints. For early detection, methylation assays are typically evaluated using samples obtained after diagnosis, which can inflate sensitivity estimates [14, 39, 46].

Furthermore, methodological variability further weakens the current evidence. Most available data rely on bisulfite-based DNA methylation methods, such as MSP, qMSP, pyrosequencing, or array platforms [8, 10, 11, 15, 44]. Bisulfite conversion poses challenges, including fragmenting DNA, introducing conversion-related bias, and reducing effective template quality [52, 53].

Emerging bisulfite-free methylation technologies, including Illumina's enzymatic methylation workflows and PacBio single-molecule real-time (SMRT) sequencing, allow direct detection of methylated bases and can minimize technical artifacts associated with bisulfite treatment [53]. Although these approaches improve CpG-resolution and reproducibility, *ADAMTS1* and *BNC1* methylation signatures have not yet been extensively evaluated with these platforms.

Notably, the prognostic significance of *BNC1* in colorectal cancer remains unknown, although the *BNC1* promoter has been studied for methylation in pancreatic cancer and other tumor types [10, 15, 31]. Existing colorectal cancer methylation research, including large multi-gene discovery efforts, rarely evaluates *BNC1* in a focused manner or assesses its association with survival outcomes [32, 42, 50]. Thus, any statement regarding *BNC1* as a prognostic biomarker in colorectal cancer must remain preliminary. Well-powered CRC cohorts with long-term follow-up and independent validation are required to determine whether *BNC1* adds value beyond established clinicopathologic and molecular prognostic indicators.

7. Future implications

Research on *ADAMTS1* and *BNC1* in the context of pancreatic and colorectal cancer has been reported. However, the function and impact of methylation in PC and CRC patients is still limited. While previous studies have shed light on these genes, a more comprehensive understanding is needed to guide the development of targeted therapies and improve outcomes for those affected by these complex malignancies. This underscores the importance of further investigation to elucidate their mechanisms and advance our knowledge of treatment strategies. Furthermore, large prospective clinical trials evaluating the methylation status of *ADAMTS1* and *BNC1* as biomarkers need to be done to understand their utility in PC and CRC. While both genes have shown promise in aiding diagnosis, rigorous testing is necessary to establish their effectiveness and reliability in clinical settings. In addition, even though *ADAMTS1* has already demonstrated its utility as a diagnostic marker, further

research is required to establish *BNC1* as a prognostic indicator specifically for colorectal cancer. Prognostic markers are essential for predicting disease progression and outcomes and can significantly impact patient management and treatment decisions. Therefore, further pre-clinical and clinical studies are required to explore the prognostic significance of *BNC1* in colorectal cancer to help provide valuable insights into disease prognosis and personalized treatment strategies.

Moreover, multi-omics techniques remain an important direction for future research in colorectal and pancreatic cancer. As it integrates genomes, epigenomics, transcriptomics, proteomics, metabolomics, and microbiome data which might aid in the detection of early molecular changes that promote tumor development and progression. Additionally, combining clinical and quantitative analyses of multi-omics data together will help us to understand mutations at the molecular level and gain a deeper understanding of multiple biological pathways. Therefore, with this technological advancement, both cancers can be diagnosed, prognosed, treated, and prevented with greater accuracy in the future, highlighting the potential of integrated datasets to improve early detection [54].

8. Conclusion

This review highlights the potential of DNA methylation biomarkers, specifically *ADAMTS1*, *ADAMTS12*, and *BNC1*, in diagnosing, predicting outcomes, and monitoring treatment for pancreatic and colorectal cancers. Research indicates that abnormal methylation of *ADAMTS1* and *BNC1* occurs early in cancer development, is linked to gene silencing, tumor growth, and worse clinical results. Notably, these changes can be detected not just in tumor tissues but also in circulating cell-free DNA, making them promising candidates for non-invasive early detection and ongoing disease monitoring.

From a clinical perspective, *ADAMTS1* and *BNC1* might offer significant benefits compared to traditional biomarkers. Established markers like CA19-9 for pancreatic cancer and SEPT9 for colorectal cancer often suffer from inconsistent sensitivity and specificity, particularly in the early stages of disease. In contrast, the methylation of *ADAMTS1* and *BNC1* shows high specificity for tumors, appears early in precursor lesions, and is reliably detectable in liquid biopsy samples. Additionally, analyzing the methylation of both *ADAMTS1* and *BNC1* together consistently provides better diagnostic accuracy than focusing on one marker alone, making them suitable candidates for inclusion in multi-marker screening tests, especially for high-risk individuals.

Looking ahead, the landscape of cancer diagnostics is likely to evolve with advancements in liquid biopsy technologies, multi-omics approaches, and detailed methylation profiling. Incorporating *ADAMTS1* and *BNC1* methylation into future diagnostic practices could enhance early cancer detection, allow for ongoing monitoring of treatment responses, and improve recurrence predictions. However, before these methods can be widely adopted in clinical settings, extensive large-scale studies, standardized testing procedures, and validation across different populations will be essential. Overall, the methylation of *ADAMTS1* and *BNC1* shows great promise as valuable biomarkers that could greatly improve precision diagnostics and personalized treatment strategies for pancreatic and colorectal cancers.

9. Declarations

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Author Contributions

AK, LS and HN conceptualized and wrote the manuscript with Illustrations. AN and AR edited the final version of the manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Conflicts of Interest

No conflict of interest to declare.

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