Review

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The Roles of CBX4 in Gastrointestinal Cancer

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Chromobox 4 (CBX4) is a polycomb group protein involved in epigenetic regulation via the polycomb repressive complex 1 (PRC1) and its small ubiquitin-like modifier (SUMO) ligase activity. It has been reported that CBX4 plays several oncogenic functions, contributing to tumor growth, metastasis, and therapeutic resistance. Elevated CBX4 expression is also correlated with poor patient prognosis and advanced tumor stages in multiple gastrointestinal cancers. Preclinical studies demonstrate that targeting CBX4 using small-molecule inhibitors, RNA interference, and monoclonal antibodies mitigates tumor progression and enhances treatment efficacy. Additionally, CBX4 exhibits potential as a diagnostic biomarker, with its high expression levels serving as an indicator of early-stage disease. Despite these advances, further research is needed to elucidate molecular mechanisms behind CBX4's involvement in carcinogenesis to accelerate the translation of tools and approaches targeting CBX4 to clinical settings. The aim of this paper is to review the role of CBX4 in gastrointestinal cancers, including gastric, liver, colorectal, and esophageal cancers, offering invaluable insights for developing novel diagnostic tools and targeted therapies to improve patient outcomes.

Keywords: gastrointestinal cancer; Chromobox 4; biomarker

Introduction

Digestive tract tumors, including gastric cancer, esophageal cancer, colorectal cancer, liver cancer, and pancreatic cancer, pose a significant global public health challenge due to their high incidence and mortality rates. According to the 2022 statistics on the Global Cancer Observatory, the incidence of digestive cancers shows an increasing trend worldwide, with notable differences in incidence and mortality rates across different regions, particularly in Asian countries such as Indonesia, Vietnam, and Myanmar [1]. In 2022, there were an estimated 4.91 million new cases of gastrointestinal cancers and 3.33 million deaths globally, with colorectal cancer cases claiming the majority, followed by gastric cancer, esophageal cancer, pancreatic cancer, liver cancer, and biliary tract cancer [2]. In China, digestive system tumors account for a substantial proportion of the cancer burden, with gastric cancer, colorectal cancer, and liver cancer being among the top five causes of cancer-related deaths [3]. The incidence and mortality rates of these cancers vary significantly by region, with particularly high rates in East Asia. Not only do digestive tract tumors cause great suffering to patients, but their high incidence and mortality rates also impose a heavy burden on society and families, highlighting the urgent need for strengthened prevention, early diagnosis, and treatment of these cancers.

The chromobox (CBX) family of proteins, which are crucial epigenetic regulators, has garnered significant attention due to their roles in various cancers. Studies have shown that CBX family members are differentially expressed in a variety of solid tumors, with the majority being upregulated in tumor tissues compared to normal tissues. For instance, CBX3 and CBX7 have been identified as key players in the development and progression of cancers such as liver, lung, pancreatic, and uterine tumors. CBX3 has been consistently associated with worse overall and relapse-free survival in patients with these cancers, whereas CBX7 has been found to have a tumor suppressive role in many cases [4]. A recent study has demonstrated that CBX3 is significantly upregulated in higher-grade tumors that exhibit stem cell-like traits, and its associated gene expression profiles are enriched with stemness markers and targets for the c-Myc transcription factor. This suggests that CBX3 may play a role in promoting cancer stemness, which is characterized by increased genomic alterations and a higher mutation burden [5]. On the other hand, CBX7 is downregulated in higher-grade tumors and is associated with a lower mutation burden, indicating its potential role in suppressing cancer stemness [6]. However, Chromobox 4 (CBX4), another important member of the CBX family, was recently reported to be closely related to tumorigenesis.

The *CBX4* gene, located on human chromosome 17, is 1689 bp in length and encodes 562 amino acids [7]. CBX4 is a member of the CBX protein family of the polycomb repressive complex 1 (PRC1). CBX4, along with CBX2, CBX6, CBX7, and CBX8, can act as component proteins of the PRC1 complex. It is involved in PRC1-mediated epigenetic modifications, thereby repressing gene transcrip-

tion. Unlike other CBX proteins, CBX4 has two small ubiquitin-like modifier-interacting motifs (SIMs) and thus differs from other members of the CBX family in that CBX4 is able to act as a ubiquitin-like modifier (SUMO) ligase that promotes SUMOylation of the proteins bound to it, regulating the biological functions of target proteins [8].

Accumulating evidence indicates that CBX4 is aberrantly expressed across multiple tumor types, underscoring its potential as an oncogene. For example, CBX4 is significantly overexpressed in osteosarcoma cell lines and tissues compared to normal tissues, where it drives tumor progression and metastasis by transcriptionally upregulating Runt-related transcription factor 2 (Runx2) [9]. Beyond osteosarcoma, CBX4 has been implicated in various cancers, including prostate cancer [10], leukemia [11], renal cell carcinoma [12], and lung adenocarcinoma [13]. In these contexts, CBX4 promotes tumor progression by enhancing angiogenesis and metastasis.

However, CBX4's role is particularly pronounced in gastrointestinal cancers, where it exhibits unique functions in promoting tumor growth, metastasis, and therapeutic resistance. In gastric cancer, CBX4 activates the Wingless/Integrated (Wnt)/β-catenin signaling pathway, accelerating tumor progression and correlating with poor patient prognosis [14,15]. In hepatocellular carcinoma (HCC), CBX4 facilitates angiogenesis and metastasis by SUMOylating hypoxia-inducible factor 1-alpha (HIF-1α), stabilizing it under hypoxic conditions, and activating downstream pro-angiogenic genes [16,17]. In colorectal cancer, CBX4 suppresses metastasis through epigenetic regulation involving histone deacetylase 3 (HDAC3), while also influencing circadian rhythm disruption and immune microenvironment changes [18,19]. In esophageal cancer, CBX4 enhances tumor invasion and radioresistance by regulating the epithelial-mesenchymal transition (EMT) and modulating the tumor immune microenvironment [20,21]. These findings highlight CBX4's multifaceted roles in gastrointestinal cancers and emphasize its potential as both a diagnostic biomarker and therapeutic target. Its overexpression is consistently associated with adverse patient outcomes, underscoring the need for further exploration of its molecular mechanisms and clinical applications.

Given its broad implications in tumorigenesis, CBX4 has also been implicated in other digestive tract tumors beyond esophageal squamous cell carcinoma [22]. To comprehensively address its role in gastrointestinal malignancies, we conducted a comprehensive review of the *CBX4* gene and its functions in gastrointestinal cancer. This review aims to consolidate existing knowledge and identify gaps for future research.

Survey Methodology

We undertook a systematic approach to gather relevant scientific literature and synthesize information related to the role of CBX4 in gastrointestinal cancers. Several major databases were selected for this purpose, including PubMed, Web of Science, Scopus, and Google Scholar, to ensure a comprehensive coverage of academic resources. A thorough search was conducted across these databases, and the search outputs were meticulously screened to identify studies that are directly relevant to the topic. Only the most pertinent and high-quality sources were compiled and utilized in the review.

The search strategy involved the use of carefully chosen keywords related to CBX4 and gastrointestinal cancers, such as "CBX4", "gastrointestinal cancer", "gastric cancer", "liver cancer", "colorectal cancer", "esophageal cancer", "oncogenic function", "diagnostic biomarker", "therapeutic target", and "epigenetic regulation". Boolean operators were employed to refine the search results and ensure that the most relevant studies were retrieved. To enhance the precision of the searches, medical subject headings (MeSH) terms were specifically utilized in PubMed.

Clear inclusion and exclusion criteria were established for the selection of literature. Studies were included if they employed well-defined experimental procedures, provided detailed data analysis methods, and had systematic and transparent methodologies. Studies were excluded if they had methodological ambiguities, small sample sizes (fewer than 10 participants or samples), or were not peer-reviewed. The publication timeframe was restricted to the most recent 5 to 10 years to ensure the inclusion of the latest research findings and to reflect the current state of knowledge on CBX4 in gastrointestinal cancers.

EndNote was used to organize and manage all relevant literature, which facilitated effective citation management and ensured a systematic approach to the review writing process. Throughout the preparation of this review, ongoing attention was paid to the latest research developments, with systematic updates to literature searches conducted to ensure the review's comprehensiveness and temporal validity. This rigorous and methodical approach ensured that the review provided a robust and up-to-date synthesis of the current understanding of CBX4's role in gastrointestinal cancers.

CBX4 Gene

CBX4 is a member of the polycomb group proteins and is involved in various biological processes, including cell proliferation, differentiation, apoptosis, and immune regulation. It plays a crucial role in chromatin modification and gene expression regulation as a component of PRC1. Here, we summarize the molecular mechanisms of *CBX4* in different contexts (Fig. 1).

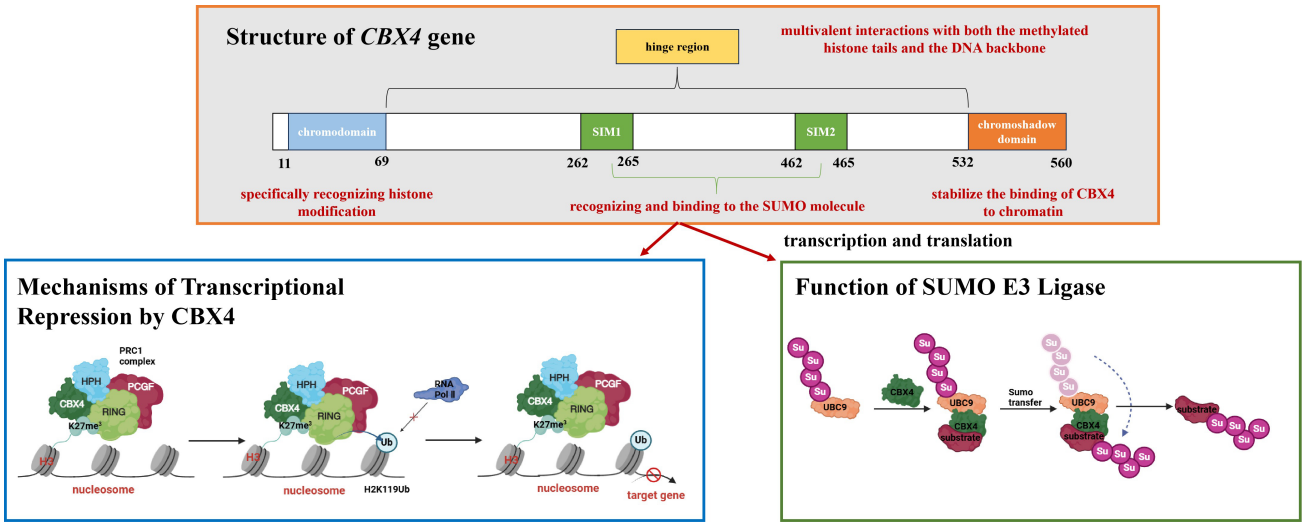

Fig. 1. The molecular mechanisms of *CBX4* in different contexts. *CBX4* binds H3K27me3 via the chromodomain, promotes oligomerization through the chromoshadow domain, enhances DNA binding with the hinge region, and regulates gene expression via SUMOylation, playing roles in both transcriptional and post-translational modifications. The figure was created using Microsoft PowerPoint 2021 (Microsoft Corporation, Redmond, WA, USA). *CBX4*, *Chromobox 4*; H3K27me3, Histone H3 lysine 27 trimethylation; SUMO, small ubiquitin-like modifier.

CBX4 Mediates Transcriptional Repression Through PRC1 Complex

Structure and Function of CBX4

As a member of the polycomb group (PcG) protein family, CBX4 contains a highly conserved chromatin-binding domain. The chromatin-binding domain of CBX4, also known as heterochromatin protein 1 gamma (HP1γ), is composed of several key structural elements that facilitate its interaction with chromatin and regulation of gene expression. The N-terminal chromodomain is responsible for recognizing and binding to the trimethylated lysine 9 mark on histone H3 (H3K9me3). This interaction is mediated by aromatic residues within the chromodomain that form stable contacts with the methylated lysine. The chromodomain consists of approximately 58 amino acids and features an antiparallel β-sheet structure packed against a C-terminal α-helix, allowing for specific recognition of the H3K9me3 peptide [23].

Between the chromodomain and the chromoshadow domain lies the hinge region, which is rich in basic amino acids and enables non-specific DNA binding, thereby enhancing the overall binding affinity of CBX4 to chromatin. This region is relatively flexible, allowing for multivalent interactions with both the methylated histone tails and the DNA backbone [24].

The C-terminal chromoshadow domain mediates protein-protein interactions, particularly through its pseudo-symmetric binding site, which recognizes specific peptide motifs. This domain is crucial for the oligomerization of CBX4 and the formation of higher-order chromatin structures [24]. The chromoshadow domain contains a β-sheet core and is capable of forming dimers or higher-order complexes, which further stabilize the binding of CBX4 to chromatin.

Beyond the chromoshadow domain, the C-terminal extension may be involved in additional protein-protein interactions that modulate the chromatin-binding activity and functional roles of CBX4. This region is less conserved but may contain motifs that interact with other chromatin-associated proteins or regulatory factors [25].

In summary, the chromatin-binding domain of CBX4, through its chromodomain and chromoshadow domain, specifically recognizes and binds to H3K9me3-marked chromatin, while the hinge region and C-terminal extension contribute to its multivalent binding and functional regulation. These structural features collectively enable CBX4 to have a significant impact on heterochromatin formation and gene expression control.

Mechanisms of Transcriptional Repression by CBX4

CBX4 mediates transcriptional repression through several key mechanisms. Initially, the chromodomain of CBX4 specifically recognizes and binds to Histone H3 lysine 27 trimethylation (H3K27me3), a critical step in targeting PRC1 to repressive chromatin regions. This interaction ensures the precise localization of PRC1, enabling it to exert its repressive functions on specific genes. Once bound to the methylation of the trimethylated lysine 27 on histone H3 (H3K27me3), CBX4 recruits other components of PRC1, such as really interesting new gene 1A/B (*Ring1A/B*), polycomb group RING Finger protein 1-6 (*PCGF1-6*), and polyhomeotic homolog 1-3 (*PHC1-*

3) [26]. These components collaborate to form a functional PRC1 complex, which is essential for the establishment and maintenance of repressive chromatin states. The Ring1A/B subunits of PRC1 possess ubiquitin ligase activity, catalyzing the monoubiquitination of histone H2A at lysine 119 (H2AK119ub) [27]. This modification further consolidates the repressive chromatin structure, leading to the silencing of target genes. By recruiting PRC1, CBX4 indirectly promotes the formation of H2AK119ub, thereby enhancing the stability of gene repression. Consequently, CBX4-mediated recruitment of PRC1 results in the repression of multiple genes involved in cell proliferation, differentiation, and metastasis. For instance, in clear cell renal cell carcinoma, CBX4 interacts with HDAC1 to suppress the transcription of the tumor suppressor gene Kruppel-like factor 6 (*KLF6*), thereby promoting tumor growth and metastasis [12]. Similarly, in lung adenocarcinoma, CBX4 represses the transcription of Zinc Finger E-box-binding homeobox 2 (ZEB2), inhibiting tumor metastasis while promoting proliferation through the upregulation of phosphoglycerate dehydrogenase (PHGDH) [13].

CBX4 has a significant impact on transcriptional repression through its dependency on the PRC1 complex. By recognizing H3K27me3 and recruiting PRC1, CBX4 facilitates the formation of H2AK119ub, leading to the silencing of target genes. This mechanism is evident in various cancers, where CBX4 influences tumor progression through interactions with other epigenetic factors such as HDAC1 [12] and ZEB2 [13]. Understanding the intricate functions of CBX4 in different cancer contexts provides valuable insights for developing targeted therapeutic strategies aimed at disrupting its oncogenic activities. Future research should further explore the specific mechanisms by which CBX4 interacts with PRC1 and other epigenetic regulators, as well as its role in other cancer types, to fully uncover its potential as a therapeutic target.

Catalytic Effect of Protein SUMOylation

While CBX4 is well-known for its role in transcriptional repression through PRC1-mediated mechanisms, it also functions as a SUMO ligase, highlighting its multifaceted role in chromatin regulation and post-translational modifications. This dual functionality underscores CBX4's importance in fine-tuning gene expression and cellular processes. Unlike other CBX family members, CBX4 stands out due to its two SIMs, which enable it to catalyze the SUMOylation of target proteins, thereby influencing their biological activity, stability, and cellular localization.

SUMOylation

Post-translational modification of proteins is an important mechanism that regulates cellular physiology. SUMO was discovered in 1996 as a homolog of ubiquitin [28], and since then, a variety of proteins have been found to undergo SUMOylation and participate in the regulation of cellular physiological functions. Humans possess at least four SUMO proteins, including SUMO-1, SUMO-2, SUMO-3, and SUMO-4; among them, only SUMO-1 is unable to polymerize, SUMO-2 is highly homologous to SUMO-3 [29], and SUMO-4 appears to be expressed only under stressful conditions [30]. Although the amino acid sequence homology between SUMO and ubiquitin is only 18%, the three-dimensional structures of SUMO and ubiquitin are indeed strikingly similar, and the enzymatic mechanism of SUMOylation is also relatively similar to that of ubiquitination, yet the enzymes involved in SUMOylation are completely different from those associated with ubiquitination [31]. SUMOylation proteins can modulate protein localization, function of target proteins, and regulation of their interactions [30]. A lot of protein substrates can be bound by SUMO molecules, whose specificity for substrate binding is reduced under stress; nevertheless, the principle of how SUMO molecules achieve specific binding of substrates through a limited number of binding and debinding enzymes is still unclear [32].

Catalyzed by SUMO-activating enzyme (E1), SUMO conjugation enzyme (E2), and SUMO ligase (E3), SUMOylation is regulated by the family of sentrin-specific proteases [33]. Similar to ubiquitination, SUMO molecules occur only on lysine residues of target proteins. The first step in the SUMO molecule coupling pathway is cleavage of its carboxyl terminus, exposing the bis-glycine residues required for coupling. Mature SUMO couples to SUMO E1 enzyme, which consists of two subunits, SUMO activating enzyme subunit 1 (SAE1) and SUMO activating enzyme subunit 2 (SAE2). Activation of the SUMO molecule requires adenosine triphosphate (ATP) hydrolysis for energy supply to generate the high-energy SUMO-E1 thioester bond [34]. Subsequently, the SUMO molecule undergoes transesterification, leading to transfer to ubiquitin-conjugating enzyme 9 (UBC9), the only known SUMO E2 enzyme [35]. Finally, with the help of SUMO E3 ligases, SUMO forms an isopeptide bond with a specific lysine residue on the substrate. Compared with E1 and E2 enzymes, SUMO E3 ligases are more diverse, demonstrating sequence or spatial environment specificity, and thus play a significant role in achieving substrate-specific recognition *in vivo* and facilitating SUMOylation of target proteins, greatly improving the efficiency of SUMO binding [36].

Although there is no homology between SUMO E3 ligases, almost all SUMO E3 ligases contain the V/I-X-V/I-V/I motif [37] and may also contain hydrophobic amino acids such as leucine [38]. The IVIV motif (SIM1) at positions 262 to 265 and the VILL motif (SIM2) at positions 462 to 465 of CBX4 have therefore been recognized as E3 ligase motifs. The hydrophobic core of SIM2 is surrounded by negatively charged aspartic acid and glutamic acid, which increase the SUMO affinity [39]. SUMO modification usually occurs on the lysine residue of substrate protein, and

these lysine residues are usually located on the ψ-K-X-D/E sequence, in which ψ represents a hydrophobic amino acid residue. However, under different conditions, such as cell stress, the specificity of SUMO binding may change, and the substrates of SUMO tend to increase, thus allowing for differential responses to cellular states under stress [40].

Function of SUMO E3 Ligase

In 2003, the first CBX SUMO-modifying substrate was identified: C-terminal Binding Protein (CtBP). It was revealed that CBX4 could bind to CtBP, specifically targeting lysine 428 for SUMO modification [41]. Subsequently, a series of CBX4 substrates have been identified, including smad interacting protein 1 (SIP1), where CBX4 mediates SUMO modification, thereby regulating the transcriptional activity of the SIP1 at positions 391 and 866, which are dependent on helicic acid [42]. Another substrate is homeodomain-interacting protein kinase 2 (HIPK2). Upon DNA damage, HIPK2 promotes the phosphorylation of CBX4, after which the phosphorylated CBX4 facilitates the SUMO modification of HIPK2, establishing a self-regulatory feedback mechanism between the substrate and the E3 ligase to address DNA damage and suppress gene transcription [43]. Further studies have identified additional substrates for CBX4-mediated SUMOylation, including DNA methyltransferase 3 alpha (*Dnmt3a*) [44], CCCTC-binding factor (*CTCF*) [45], and B lymphoma Mo-MLV insertion region 1 (*BMI-1*) [46], among others. These findings highlight the diverse roles of CBX4 in post-translational modifications and its implications in various cellular processes.

Given the characteristics of SUMO E3 ligases, the amino acid sequences of SIMs in CBX4, specifically residues 262–265 and 462–465, can be identified as SIMs and are considered conserved sequences [16]. The absence of these SIMs leads to the loss of CBX4's ability to promote the SUMOylation of its substrates, thereby preventing its localization within the polycomb protein complex. SUMOylation enhances the binding of CBX4 to H3K27me3 and can be regulated by Sentrin/SUMO-specific protease 2 (SENP2), thereby enabling CBX4 to exert its transcriptional repression function mediated by the PcG protein complex [47].

The Roles of CBX4 in Gastrointestinal Cancer

CBX4 has been reported to be associated with the growth and metastasis of a variety of tumors, and genomic analysis has also yielded a significant increase in the expression of CBX4 in tumors [48]. The mechanisms by which CBX4 promotes the growth of tumor cells can be divided into two main types: one relying on PcG-based transcriptional repressive activity, and another based on the SUMO E3 ligase activity of CBX4. In this review, we summarize the association of CBX4 with the four main types of gastrointestinal cancers (Fig. 2 and Table 1, Ref. [15–19,21,22,49–62]).

CBX4 and Gastric Cancer

Gastric cancer (GC) stands as one of the most prevalent malignant tumors globally, with notably high incidence rates in East Asia, including China, Japan, and South Korea [63]. Extensive research has revealed that the overexpression of CBX4 in GC is intrinsically linked to tumor progression, drug resistance, and adverse prognoses. In GC, elevated CBX4 expression is significantly associated with tumor size, pathological differentiation, and reduced patient survival. A recent study reported that CBX4 expression in GC tissues was markedly higher than in normal tissues, with high CBX4 levels correlating with poor prognosis. Furthermore, CBX4 expression is closely related to tumor stage and lymph node metastasis, highlighting its crucial role in GC development and progression [14].

CBX4 orchestrates the proliferation and invasion of GC cells through multiple signaling pathways. For instance, CBX4 activates the Wnt/β-catenin pathway, enhancing GC cell proliferation and invasiveness. Fang and Pan [15] found that CBX4 promotes Wnt/β-catenin activation, thereby increasing GC cell malignancy. CBX4 also influences GC cell behavior through the Notch pathway and N-glycosylation. Guo and Gao [49] revealed that CBX4 upregulates actin-binding Rho activating protein (*ABRACL*), promoting GC cell proliferation and migration while inhibiting apoptosis. CBX4 binds to the *ABRACL* promoter, transcriptionally regulating its expression and facilitating GC progression. CBX4 can also activate cell division cycle 20 (*CDC20*), promoting tumor progression and stem cell-like properties in GC. CBX4 inhibition significantly suppresses GC cell proliferation, migration, and metastasis. Mechanistically, CBX4 interacts with H3K4me3 to upregulate *CDC20* mRNA, maintaining GC cell stemness and promoting malignancy [50].

The dynamic and heterogeneous expression of CBX4 in GC is vital for mediating chemotherapy resistance. Ma *et al.* [51] indicated that high expression of BMI-1, which shares regulatory mechanisms with CBX4, is associated with drug tolerance in GC. BMI-1 influences GC stem cell properties, enhancing doxorubicin resistance by regulating EMT and drug resistance-related proteins. A further study revealed that CBX4 reduces chemotherapy sensitivity by inhibiting cellular senescence. CBX4 promotes yes-associated protein 1 (YAP1) SUMOylation, inhibiting the Hippo pathway and enhancing GC cell drug resistance and malignancy. Specifically, CBX4 modifies YAP1 at K97 and K280 with SUMO-1, competing with YAP1-S127 phosphorylation and enhancing YAP1 stability and nuclear translocation [52].

The expression level of CBX4 is closely associated with poor prognosis in GC. Kang Lin *et al.* [53] ana-

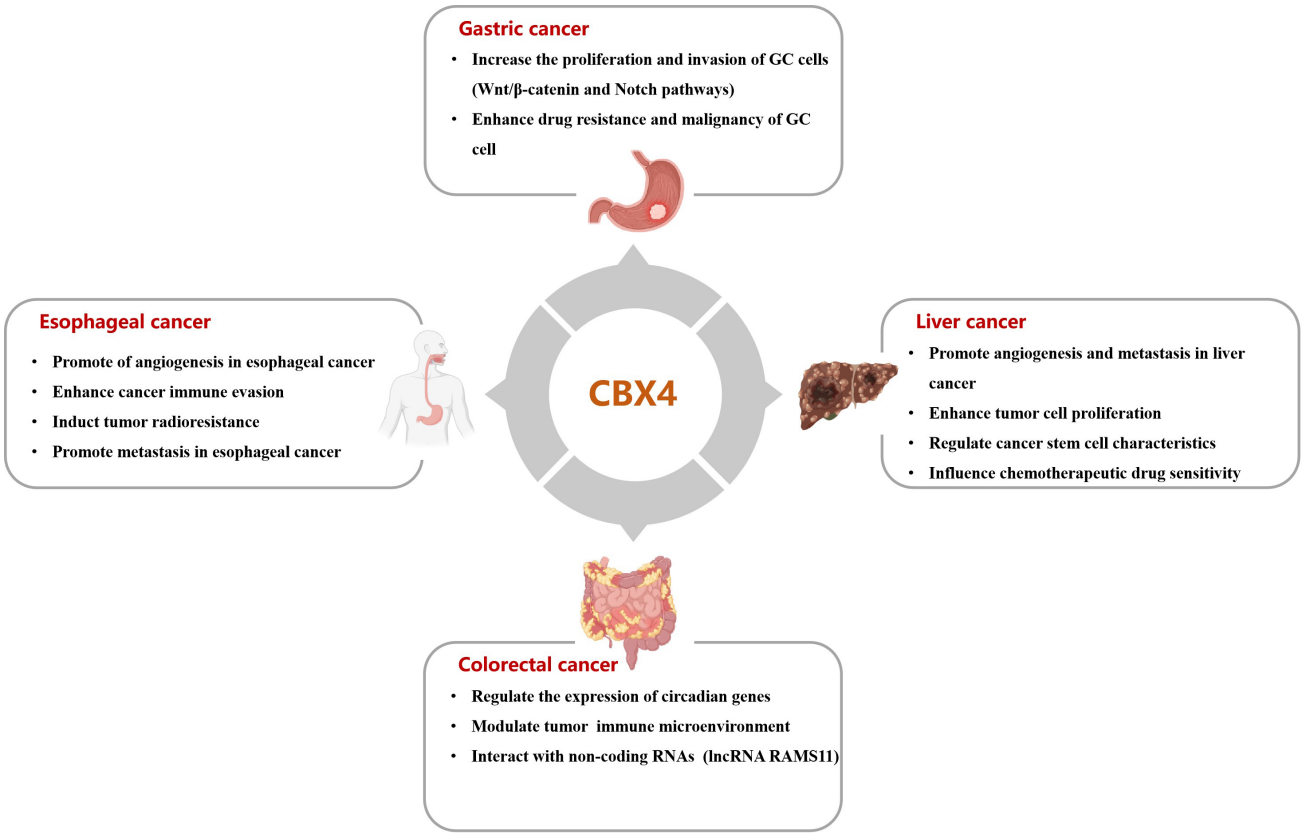

Fig. 2. The association of CBX4 with the four main types of gastrointestinal cancers. The figure was created using Microsoft PowerPoint 2021 (Microsoft Corporation, Redmond, WA, USA). CBX4, Chromobox 4; GC cells, gastric cancer cells; Wnt, Wingless/Integrated; RAMS11, RNAs associated with metastasis 11.

lyzed CBX4 expression in GC using databases, finding that high CBX4 levels correlate with poor overall survival, progression-free survival, and post-progression survival. Thus, their Kaplan–Meier analysis revealed that high CBX4 expression significantly reduces overall survival in GC patients, reinforcing its role as a poor prognostic biomarker. Additionally, CBX4 expression predicts recurrence risk, with patients having high expression more likely to experience postoperative recurrence and metastasis [54]. These findings corroborate CBX4's potential as a diagnostic and prognostic marker.

In summary, CBX4 is essential for GC oncogenesis and progression, with its overexpression linked to adverse patient outcomes. By virtue of its ability to regulate cell proliferation, invasion, and apoptosis through various signaling pathways, CBX4 emerges as a promising diagnostic marker and therapeutic target. Inhibiting CBX4 significantly impairs GC cell proliferation and migration, suggesting its utility in treatment strategies. Additionally, combining low-dose cisplatin with CBX4 inhibitors enhances chemotherapy sensitivity, thereby improving therapeutic efficacy. Future research should delve deeper into the mechanisms underlying the roles of CBX4 in GC to generate insights for developing more effective diagnostic and therapeutic approaches.

CBX4 and Liver Cancer

Liver cancer poses a significant threat to patient health and life due to its aggressive nature. Liver cancer, a type of malignant tumor, is primarily categorized into two types: primary liver cancer and secondary liver cancer [64]. Primary liver cancer, specifically HCC, arises from liver parenchymal cells or bile duct cells. In contrast, secondary liver cancer, also known as metastatic liver cancer, results from the metastasis of malignant tumors from other organs or tissues to the liver via the bloodstream or lymphatic system. Common primary tumors include colorectal cancer, gastric cancer, lung cancer, and pancreatic cancer [55].

A case-control study involving 334 liver cancer patients and 321 controls revealed a significant association between specific single-nucleotide polymorphisms (SNPs) in CBX4 and the risk of liver cancer. Notably, the *CBX4* polymorphism rs2289728 was found to be associated with a significant reduction of liver cancer risk. These SNPs mitigate the risk of tumor occurrence by inhibiting CBX4 expression [65].

CBX4 has been shown to promote angiogenesis and metastasis in liver cancer. Chen *et al.* [16] demonstrated that CBX4 enhances the expression of vascular endothelial growth factor (*VEGF*) in liver cancer cells by promoting

Table 1. The roles of CBX4 in different types of gastrointestinal cancer.

Cancer type	Roles of CBX4	Mechanism
Gastric cancer	Proliferation and invasion	Activates the Wnt/β-catenin pathway, enhancing gastric cancer cell proliferation and invasiveness [15].
		Upregulates *ABRACL*, promoting gastric cancer cell proliferation and migration while inhibiting apoptosis [49].
		Activates *CDC20*, promoting tumor progression and stem cell-like properties [50].
	Chemotherapy resistance	Inhibits the Hippo pathway and enhances drug resistance and malignancy of gastric cancer cells under the regulation of BMI-1 [51].
		Modifies YAP1 at K97 and K280 with SUMO1, competing with YAP1-S127 phosphorylation and enhancing YAP1 stability and nuclear translocation [52].
	Cancer prognosis	Significantly correlates with poor overall survival, progression-free survival, and post-progression survival [53,54].
Liver cancer	Angiogenesis and metastasis	Stabilizes HIF-1α under hypoxic conditions by promoting its SUMOylation, thereby activating downstream genes (e.g., *VEGF*, *SDF-1*, *ANG2*) involved in angiogenesis and metastasis [16].
		Promotes the migration and invasion of liver cancer cells by upregulating *VEGF* expression [17].
	Cell proliferation and cycle progression	Promotes the G1-to-S phase transition by regulating cell cycle-related genes (e.g., *PCNA*, *CCNE2*), thereby enhancing cell proliferation [56].
	Cancer stemness regulation	Maintains cancer stem cell properties by regulating the nuclear translocation of YAP1, enhancing its transcriptional activity, and sustaining self-renewal and tumorigenicity [57].
	Promotion of viral hepatocellular carcinoma	Promotes EMT via specific signaling pathways, enhancing cancer cell invasiveness and migration [58].
	Cancer prognosis	Significantly associated with poor prognosis, high tumor differentiation, high microvascular density, and distant and hematogenous metastasis [56].
Colorectal cancer	Carcinogenesis	Interacts with HDAC3 to inhibit *Runx2* promoter activity and expression [18].
	Circadian rhythm disruption and immune microenvironment regulation	Interacts with core circadian genes (*CLOCK*, *PER1*, *PER3*, and *CRY2*) and influences immune cell infiltration in the tumor microenvironment [19].
	Invasion	Binds to lncRNA RAMS11 to promote TOP2α transcriptional activation [59].
	Cancer prognosis	Significantly associated with poor prognosis, low survival rates, and high recurrence rates [60,61].
Esophageal cancer	Metastasis	Enhances cell migration and invasion by regulating EMT [21].
	Immune evasion	Modulates tumor immune microenvironment by promoting infiltration of immunosuppressive cells (e.g., tumor-associated macrophages, regulatory T cells) [22].
	Radioresistance	Regulates autophagic activity to repair radiation-induced DNA damage and promote cell survival [55].
	Cancer prognosis	Significantly associated with poor prognosis, promoting tumorigenesis, immune evasion, and radioresistance [62].

ABRACL, actin-binding Rho activating protein; *CDC20*, cell division cycle 20; BMI-1, B lymphoma Mo-MLV insertion region 1; YAP1, yes-associated protein 1; HIF-1α, hypoxia-inducible factor 1-alpha; *VEGF*, vascular endothelial growth factor; *SDF-1*, stromal cell-derived factor 1; *ANG2*, Angiopoietin-2; *PCNA*, proliferating cell nuclear antigen; *CCNE2*, cyclin E2; EMT, epithelial-mesenchymal transition; HDAC3, histone deacetylase 3; *Runx2*, Runt-related transcription factor 2; *CLOCK*, circadian locomotor output cycles kaput; *PER1*, period circadian regulator 1; *PER3*, period circadian regulator 3; *CRY2*, cryptochrome circadian regulator 2; TOP2α, Topoisomerase II alpha.

the SUMOylation of hypoxia inducible factor 1 subunit alpha (HIF-1α). This interaction stabilizes HIF-1α under hypoxic conditions, leading to its accumulation in the cell nucleus and the subsequent activation of downstream genes, including *VEGF*, stromal cell-derived factor 1 (*SDF-1*), and Angiopoietin-2 (*ANG2*), which play crucial roles in tumor angiogenesis and metastasis. Furthermore, CBX4 overexpression significantly increases the *in vitro* angiogenesis and cell migration ability of the liver cancer cell line MHCC97L, an effect that is dependent on its SIMs. CBX4 also promotes the migration and invasion of liver cancer cells by upregulating VEGF expression [17].

High levels of CBX4 are closely associated with the proliferation and cell cycle progression of liver cancer cells.

Wang *et al*. [56] reported that high expression of CBX4 in liver cancer cells is closely related to tumor cell proliferation and cycle progression. CBX4 promotes the cell cycle transition from the G1 phase to the S phase by regulating the expression of cell cycle-related genes, such as proliferating cell nuclear antigen (*PCNA*) and cyclin E2 (*CCNE2*), thereby enhancing cell proliferation. Additionally, CBX4 further promotes cell cycle progression by inhibiting the expression of p16. Moreover, CBX4 can regulate the characteristics of cancer stem cells, evident in Zhao *et al*.'s research [57], which found that CBX4 maintains cancer stem cells in liver cancer cells by regulating the nuclear translocation of YAP1. Specifically, CBX4 overexpression promotes the accumulation of YAP1 in the cell nucleus, enhancing its transcriptional activity and maintaining the self-renewal and tumor-forming ability of cancer stem cells. CBX4 also further enhances the characteristics of cancer stem cells by inhibiting the expression of microRNA-424 (*miR-424*).

The expression level of CBX4 in patients is highly correlated with chemotherapeutic drug sensitivity. MiR-195 in patient plasma exerts a tumor-suppressive effect by directly targeting CBX4 and inhibiting its expression. MiR-195 overexpression significantly inhibits the proliferation, invasion, and migration of liver cancer cells, an effect that can be partially reversed by CBX4 overexpression. Moreover, high expression of CBX4 is significantly associated with poor prognosis in liver cancer patients, indicating its potential as a therapeutic target. A retrospective analysis of 727 liver cancer patients found that high expression of CBX4 was significantly associated with poor prognosis. Specifically, high expression of CBX4 was significantly correlated with high tumor differentiation, high microvascular density, distant metastasis, and hematogenous metastasis. Additionally, high expression of CBX4 was significantly associated with shorter overall survival and disease-free survival in patients, confirming CBX4 as an independent prognostic marker. A recent study also reported that CBX4 expression was significantly higher in liver cancer tissues than in non-tumor tissues, and its high expression was significantly associated with tumor size, differentiation degree, and tumor, node, metastasis (TNM) staging. Furthermore, high expression of CBX4 was significantly correlated with serum alpha fetoprotein (AFP) levels in patients, suggesting that CBX4 may be a potential diagnostic marker [56].

Additionally, recent studies have highlighted the role of CBX4 in the pathogenesis and progression of viral HCC, particularly in the context of chronic hepatitis C virus (HCV) infection. HCC is the most common type of primary liver cancer and a leading cause of cancer-related deaths worldwide. Chronic viral infections, such as hepatitis B virus (HBV) and HCV, are major risk factors for HCC, accounting for a significant proportion of cases globally. A recent study has demonstrated that CBX4 promotes EMT by activating specific signaling pathways, thereby enhancing the invasive and migratory capabilities of HCC cells. CBX4 has been shown to interact with long noncoding RNAs (lncRNAs) RNAs associated with metastasis 11 (RAMS11), to regulate gene expression and further drive tumor progression [58].

Taken together, the role of CBX4 in liver cancer is complex and multifaceted, involving the promotion of angiogenesis, influence on cell proliferation and cycle, regulation of cancer stem cell characteristics, and modulation of chemotherapeutic drug sensitivity. With high expression significantly associated with poor prognosis in liver cancer patients, CBX4 expression level holds important clinical value for the diagnosis, prognosis prediction, and treatment guidance of liver cancer. The specific molecular mechanisms of CBX4 in liver cancer should be explored further to aid in the development of novel therapeutic strategies targeting CBX4.

CBX4 and Colorectal Cancer

CBX4 has been implicated in the development and progression of colorectal cancer (CRC). Recent research has not only elucidated the multifaceted roles of CBX4 in CRC but also highlighted its potential clinical significance, particularly in the regulation of tumor biological behavior and the development of therapeutic targets.

One of the key mechanisms by which CBX4 exerts its effects in CRC is through epigenetic regulation. Specifically, CBX4 has been shown to suppress the metastasis of CRC by HDAC3 binding to the *Runx2* promoter, thereby inhibiting the expression of Runx2. This process underscores the importance of the CBX4-HDAC3 interaction in the suppression of oncogene transcription, particularly for Runx2, a transcription factor that is essential for promoting metastasis. Notably, the metastasis-suppressing function of CBX4 is independent of its SUMO E3 ligase activity, chromodomain, and PRC1, suggesting potential non-canonical functions of CBX4 in CRC [18].

In addition to its role in epigenetic regulation, CBX4 has been implicated in the disruption of circadian rhythms and immune infiltration in CRC. Research has shown that the upregulation of CBX4 in CRC is closely associated with circadian rhythm disruption. CBX4 interacts with core circadian genes such as circadian locomotor output cycles kaput (*CLOCK*), period circadian regulator 1 (*PER1*), period circadian regulator 3 (*PER3*), and cryptochrome circadian regulator 2 (*CRY2*), and affects various cell types in the tumor immune microenvironment, including B cells, $CD4^+$ T cells, myeloid-derived suppressor cells, and cancer-associated fibroblasts [19]. These findings reveal the potential role of CBX4 in immune evasion and provide new insights into its function within the tumor microenvironment.

Furthermore, CBX4 has been shown to regulate the expression of oncogenes through interactions with lncRNAs. Silva-Fisher *et al*. [59] identified the lncRNA

RNAs associated with metastasis 11 (RAMS11) as vital for the progression of metastatic CRC. The study found that lncRNA RAMS11 promotes the transcriptional activation of Topoisomerase II alpha (TOP2α) by binding to CBX4, thereby enhancing the invasiveness of tumors and their resistance to topoisomerase inhibitors. This mechanism reveals a new role for CBX4 in lncRNA-mediated oncogene regulation, further enriching its functional spectrum in CRC.

From a clinical perspective, numerous studies have indicated that high expression of CBX4 is associated with poor prognosis in CRC patients [60]. Specifically, high expression of CBX4 is related to low survival rates and high recurrence rates, suggesting that CBX4 can serve as an important biomarker for the prognosis of CRC. Moreover, analysis of clinical data from 136 patients revealed that the expression level of CBX4 is closely associated with the risk of tumor recurrence, with an area under the receiver operating characteristic (ROC) curve of 0.794, indicating that CBX4 has high predictive value for early recurrence of CRC [61].

The regulatory mechanisms of CBX4 in CRC are complex and diverse, involving the regulation of circadian genes, changes in the immune microenvironment, and interactions with lncRNAs. Its high expression is associated with poor prognosis and high recurrence rates, making it an important biomarker for the diagnosis and prognosis prediction of CRC. Additionally, the role of CBX4 in inhibiting tumor metastasis and regulating drug sensitivity provides important clues for the development of new therapeutic strategies. Future research should further explore the multiple regulatory mechanisms of CBX4 in an attempt to provide more comprehensive solutions for the diagnosis and treatment of CRC.

CBX4 and Esophageal Cancer

Recent research has highlighted the significant role of CBX4 in the development and progression of esophageal cancer, particularly in esophageal squamous cell carcinoma (ESCC). CBX4, a member of the CBX family, has been identified as a key oncogene that influences multiple aspects of tumor biology, including proliferation, migration, invasion, and response to therapy. CBX4 overexpression is a common feature in ESCC tissues, and its expression level correlates with tumor aggressiveness [20]. Functionally, CBX4 promotes ESCC cell proliferation and colony formation *in vitro* and tumorigenicity *in vivo*. It enhances cell migration and invasion by regulating the EMT process, thereby facilitating tumor metastasis. Mechanistically, CBX4 interacts with various signaling pathways, such as the Phosphoinositide 3-kinase (PI3K) - protein kinase B (AKT) -mechanistic target of Rapamycin (mTOR) pathway, to drive tumorigenesis [21]. Additionally, CBX4 modulates the tumor immune microenvironment by promoting the infiltration of immunosuppressive cells, such as tumor-associated macrophages and regulatory T cells, thus contributing to immune evasion [22].

One of the notable functions of CBX4 is its role in conferring radioresistance to ESCC cells. By regulating autophagic activity, CBX4 enhances the repair of radiation-induced DNA damage and promotes cell survival. Specifically, CBX4 suppresses the expression of beclin 1, a key autophagy regulator, thereby inhibiting autophagy and reducing the effectiveness of radiotherapy. This mechanistic insight suggests that targeting CBX4 could potentially sensitize ESCC cells to radiation treatment [66].

Clinically, elevated CBX4 expression is associated with advanced tumor stages, larger tumor sizes, and poor patient outcomes in adenocarcinoma of the esophagogastric junction [67]. The expression level of CBX4 shows a strong correlation with tumor angiogenesis and immune cell infiltration, indicating its potential as a biomarker for assessing tumor malignancy and predicting patient prognosis. Furthermore, CBX4 expression is linked to the response to immune checkpoint inhibitors, suggesting its utility in guiding immunotherapy strategies [68,69].

Given its oncogenic functions and clinical relevance, CBX4 represents an attractive therapeutic target for the treatment of esophageal cancer. Preclinical studies have demonstrated that CBX4 targeting, either through genetic knockdown or pharmacological inhibition, can effectively suppress tumor growth and enhance the efficacy of conventional therapies [62]. These findings underscore the therapeutic potential of CBX4-targeting approaches in improving treatment outcomes of esophageal cancer patients. In summary, CBX4 plays a multifaceted role in esophageal cancer by promoting tumorigenesis, facilitating immune evasion, and conferring radioresistance. Its expression level serves as a valuable biomarker for diagnosis, prognosis prediction, and treatment guidance in digestive tract tumors. Future research should focus on further elucidating the molecular mechanisms underlying CBX4's functions and exploring the clinical application of CBX4-targeting therapies.

CBX4 in Tumor Immune Evasion

In addition to its regulatory role in gastrointestinal malignancies, CBX4 has emerged as a key player in tumor immune evasion. Accumulating evidence indicates that CBX4 modulates immune checkpoint molecules and T cell function through dual mechanisms of epigenetic regulation and metabolic reprogramming, thereby exerting immunosuppressive effects within the tumor microenvironment. Recent studies have uncovered a dual role for CBX4 in immune checkpoint control: On the one hand, CBX4 directly suppresses programmed cell death protein 1 (PD-1) expression by promoting PRC1-mediated H2AK119ub1 and PRC2-mediated H3K27me3 modifications, which accumulate repressive histone marks at the programmed cell death 1 (*Pdcd1*) promoter and enhance T cell anti-tumor

activity [22]. On the other hand, CBX4 indirectly impairs T cell effector function by stabilizing transcription factors Kruppel-like factor 3 (KLF3) and Specificity protein 1 (SP1) through SUMOylation, leading to upregulation of the metabolic molecule Aldob [70]. This, in turn, disrupts glycolysis and ATP synthesis. These findings highlight CBX4 as a critical regulator that both directly modulates immune checkpoints via epigenetic mechanisms and indirectly suppresses T cell function through metabolic reprogramming, thereby promoting tumor immune evasion.

Moreover, CBX4 exhibits functional heterogeneity across different tumor types. For instance, high CBX4 expression correlates with prolonged survival in breast cancer, whereas elevated CBX3 and CBX5 expression are associated with poor prognosis. This divergence suggests that CBX family members engage in distinct immune regulatory mechanisms within the tumor microenvironment [70]. In the context of Kras mutations, *CBX4* deletion induces genomic instability, which promotes tumorigenesis through Hippo pathway inactivation. Although this study did not directly address immune evasion mechanisms, genomic instability may indirectly alter immune cell function by modifying signaling pathways within the tumor microenvironment, offering a novel perspective on CBX4's role in immune evasion [71].

Collectively, CBX4 directly regulates PD-1 expression via epigenetic modifications and indirectly suppresses T cell function through metabolic reprogramming, positioning it as a central player in tumor immune evasion. The functional diversity of CBX family members in the tumor microenvironment underscores their potential as targets for immunotherapy. Future research should investigate the dynamic regulatory mechanisms of CBX4 within the immune microenvironment and its interactions with other signaling pathways, such as the Hippo pathway, to fully elucidate its role in immune evasion. These insights will provide a critical foundation for the development of targeted immunotherapies.

Future Perspectives

A growing understanding of CBX4's dual role in epigenetic regulation and post-translational modifications opens new avenues for therapeutic intervention in gastrointestinal cancers. Future research should focus on dissecting the molecular mechanisms through which CBX4 interacts with other epigenetic factors, such as HDAC1 and general control non-derepressible 5 (GCN5), to modulate gene expression and tumor progression. Elucidating these interactions could reveal novel therapeutic targets and spur on combination of therapeutic strategies for enhancing treatment efficacy. Additionally, the development of specific inhibitors targeting CBX4's SUMO E3 ligase activity holds promise for overcoming drug resistance, a major challenge in cancer therapy. Thus, preclinical studies should prioritize the evaluation of these inhibitors in relevant models to assess their safety and efficacy.

Furthermore, exploring CBX4's role in the tumor microenvironment and its impact on immune evasion could provide insights into immune-oncology approaches. The integration of CBX4-targeting therapies with existing treatment modalities, such as chemotherapy and immunotherapy, may offer synergistic benefits. Notably, the role of CBX4 in microsatellite instability-high (MSI-high) CRC remains underexplored. MSI-high CRC is characterized by a unique genetic and immune landscape, and understanding how CBX4 influences tumor progression and immune responses in this context could reveal new therapeutic strategies. Future studies should investigate the interaction of CBX4 with MSI-related genes and its impact on immune checkpoint inhibitors, which are commonly used in MSI-high CRC. The translation of these findings into clinical applications will be crucial to validate CBX4's potential as a next-generation therapeutic target in gastrointestinal cancers.

Conclusion

This comprehensive review elucidates the multifaceted roles of CBX4 in gastrointestinal cancers, particularly in CRC. CBX4, as a member of the PcG protein family, plays a pivotal role in epigenetic regulation through its interactions with histone modifications and other chromatin-associated factors. CBX4 not only functions as a transcriptional repressor via PRC1-mediated mechanisms but also acts as a SUMO E3 ligase, influencing post-translational modifications of key proteins involved in tumor biology. Elevated CBX4 expression has been consistently linked to poor prognosis in various gastrointestinal cancers, including GC and CRC, further solidifying its role as a biomarker for disease aggressiveness. Additionally, CBX4 has been shown to impact tumor immune infiltration and correlate with immune cell subsets such as B cells, $CD4^+$ T cells, myeloid-derived suppressor cells, and cancer-associated fibroblasts, suggesting its potential involvement in immune escape within the tumor microenvironment. These findings highlight CBX4's complexity and potential as a therapeutic target in gastrointestinal cancers.

Availability of Data and Materials

Not applicable.

Author Contributions

XJZ, JD, YPC, and ZML contributed to the study design. XJZ and JD conducted the literature search. All authors were involved in the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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