Table 4 Clinicopathological and molecular features of fifteen traditional serrated adenomas with high-grade dysplasia

No.	Age/sex	Location	Size (mm)	*KRAS* mutation	*BRAF* mutation	*PIK3CA* mutation	*MGMT* methylation	*MLH1* methylation	MSI status	LINE-1 methylation level	*IGF2* DMR0 methylation level	IGF2 expression
1	75/M	Rectum	8	c.35G>A (p.G12D)	Wild	Wild	(-)	(-)	MSS/MSI-low	58	70	Weak
2	54/F	Sigmoid colon	20	c.35G>A (p.G12D)	Wild	Wild	(+)	(-)	MSS/MSI-low	53.7	39.5	Strong
3	62/F	Transverse colon	15	c.38G>A (p.G13D)	Wild	Wild	(+)	(-)	MSS/MSI-low	53.3	72.0	No expression
4	84/M	Rectum	5	c.35G>A (p.G12D)	Wild	Wild	(-)	(-)	MSS/MSI-low	65.0	26.5	Moderate
5	85/M	Sigmoid colon	12	Wild	c.1799T>A (p.V600E)	Wild	(-)	(-)	MSS/MSI-low	58.0	45.5	Strong
6	48/M	Sigmoid colon	20	Wild	c.1799T>A (p.V600E)	Wild	(-)	(-)	MSS/MSI-low	53.7	40.5	Moderate
7	69/M	Sigmoid colon	10	Wild	c.1799T>A (p.V600E)	Wild	(-)	(-)	MSS/MSI-low	58.7	52.0	No expression
8	60/M	Descending colon	9	Wild	c.1799T>A (p.V600E)	Wild	(-)	(-)	MSS/MSI-low	59.0	41.5	Moderate
9	34/M	Sigmoid colon	18	Wild	c.1799T>A (p.V600E)	Wild	(+)	(-)	MSS/MSI-low	57.3	42.0	Strong
10	61/M	Rectum	10	Wild	c.1799T>A (p.V600E)	Wild	(-)	(-)	MSS/MSI-low	56.7	29.0	Strong
11	52/F	Ascending colon	15	Wild	c.1799T>A (p.V600E)	Wild	(+)	(+)	MSS/MSI-low	57.0	57.0	Moderate
12	70/F	Rectum	13	Wild	c.1799T>A (p.V600E)	Wild	(+)	(+)	MSS/MSI-low	63.0	84.5	Weak
13	66/F	Ascending colon	12	Wild	Wild	c.1624G>A (p.E542K)	(-)	(-)	MSS/MSI-low	49.7	28.0	Moderate
14	52/M	Sigmoid colon	12	Wild	Wild	Wild	(-)	(-)	MSS/MSI-low	48.0	44.5	Moderate
15	69/F	Rectum	13	Wild	Wild	Wild	(+)	(-)	MSS/MSI-low	44.3	80.0	Weak

HGD: High-grade dysplasia; MSI: Microsatellite instability; MSS: Microsatellite stable; TSA: Traditional serrated adenoma.

ples was small (n = 15), our findings require further confirmation from future independent studies.

Global DNA hypomethylation is associated with genomic instability, which leads to cancer[45-50]. As the LINE-1 or L1 retrotransposon constitutes a substantial portion (ca. 17%) of the human genome, the level of LINE-1 methylation is regarded as a surrogate marker of global DNA methylation[46,51]. We previously reported that LINE-1 methylation is highly variable but is strongly associated with a poor prognosis in CRC[45]. However, no previous study has reported the role of LINE-1 hypomethylation in serrated lesions. In serrated lesions, unlike the *IGF2* DMR0 methylation level, no significant difference was observed between the LINE-1 methylation level and histological type. We also found that the LINE-1 methylation levels in TSAs with HGD were significantly lower than those in TSAs. These results suggest that both *IGF2* DMR0 hypomethylation and LINE-1 hypomethylation are important epigenetic alterations in the progression of TSAs. Because the carcinogenic mechanism remains unclear, further analyses are needed to clarify the role in the TSA pathway of the hypomethylation of these locations.

Previous studies have reported that SSAs with cytological dysplasia have accumulated genetic abnormalities and are at a high risk of progression to colorectal carcinoma[7,26,28]. Loss of staining for MLH1 leads to MSI and repeat tract mutation in genes such as *TGFβRII* is restricted to lesions with cytological dysplasia in SSAs[26,27,52,53]. In the current study, MSI-high was more frequently detected in SSAs with cytological dysplasia than in SSAs without. Our data indicate that in SSAs with cytological dysplasia, it is not hypomethylation of *IGF2* DMR0 or LINE-1 but rather MSI due to *MLH1* hypermethylation that plays an important role in the evolution to colorectal carcinoma.

The RAS-RAF-MEK-ERK signaling pathway is commonly altered in CRC and serrated lesions through oncogenic mutation of either *BRAF* or *KRAS*[15,21,25]. Moreover, CRCs with serrated morphology are particularly prone to mutations targeted by anti-epidermal growth factor receptor therapy. Therefore, as the variety of molecularly targeted agents for CRC increases, understanding of molecular alterations is becoming increasingly important[21,40]. *BRAF* and *KRAS* mutations are mutually exclusive and demonstrate a subtype specificity in serrated lesions[10,15,17-19,28]; they are most likely initiating events in the majority of HPs[54]. Previous studies have reported that *BRAF* is mutated with increasing frequency in SSAs (60%-100%)[3-5,9,11,16]. In the current study, *BRAF* mutations were detected in 49% of HPs and 87% of SSAs, respectively. Therefore, our data relating to the frequency of *BRAF* mutations in SSAs are consistent with previous reports. The activation of the RAS-RAF-MEK-ERK signaling pathway by *BRAF* or *KRAS* mutation is also common in TSAs. Previous studies have reported *BRAF* mutation rates in TSAs ranging from 27% to 55%[6,8,16,55], compared to *KRAS* mutation rates of 29%-46%[6,8]. In the cur-

rent study, *BRAF* and *KRAS* mutations were detected in 69% and 17% of TSAs, respectively. Thus, the wide variation in the relative proportion of *BRAF vs KRAS* mutations in different studies reflects differences in histological classification or small sample size.

In conclusion, we found that *IGF2* DMR0 hypomethylation can occur in the early stage of any histological types of serrated lesions; however, hypomethylation may be an infrequent epigenetic alteration in SSAs. These results imply that *IGF2* DMR0 hypomethylation may be a key epigenetic event that affects the progression of HPs. Our data also suggest that the hypomethylation of *IGF2* DMR0 and LINE-1 may play an important role in the progression of the TSA pathway.

COMMENTS

Background

The serrated pathway attracts considerable attention as an alternative colorectal cancer (CRC) pathway. Authors previously reported the association of *insulin-like growth factor 2* (*IGF2*) differentially methylated region (DMR)0 hypomethylation with poor prognosis and its link to global DNA hypomethylation [long interspersed nucleotide element-1 (LINE-1) hypomethylation] in CRC; however, to date, there have been no studies describing its role in the serrated pathway.

Research frontiers

Sessile serrated adenoma (SSA) and traditional serrated adenoma (TSA) are premalignant lesions, but SSA is the principal serrated precursor of CRC. In particular, there are many clinicopathological and molecular similarities between SSA and microsatellite instability (MSI)-high CRC, for example, right-sided predilection, *MLH1* hypermethylation, and frequent *BRAF* mutation. Therefore, SSAs are hypothesized to develop in some cases to MSI-high CRCs with *BRAF* mutation in the proximal colon. In contrast, a definite precursor of TSA has not been established. In addition, the key carcinogenic mechanism involved in this TSA pathway remains largely unknown. To investigate the role of *IGF2* DMR0 hypomethylation in serrated lesions they examined *IGF2* DMR0 methylation levels as well as other molecular alterations.

Innovations and breakthroughs

This is the first report of an association between histopathological findings and *IGF2* DMR0 hypomethylation in serrated lesions. *IGF2* DMR0 hypomethylation was less frequently detected in SSAs than in hyperplastic polyps (HPs), TSAs, and non-serrated adenomas. They also found that *IGF2* DMR0 and LINE-1 hypomethylations in TSAs and non-serrated adenomas with high-grade dysplasia were more frequently detected in TSAs and non-serrated adenomas, suggesting that such hypomethylation may play an important role in the progression of those tumors. Thus, their finding of differential patterns of *IGF2* DMR0 hypomethylation in serrated lesions may be a clue for elucidating the progression of serrated lesions.

Applications

In the current study, authors found that the *IGF2* DMR0 methylation levels of SSAs were significantly higher compared with those of HPs (microvesicular HPs), TSAs, and non-serrated adenomas. They also showed that *IGF2* DMR0 hypomethylation was less frequently detected in SSAs compared with HPs, TSAs, and non-serrated adenomas. Therefore, their data challenge the common conception of discrete molecular features of SSAs *vs* other serrated lesions (TSAs and HPs) and may have a substantial impact on clinical and translational research, which has typically been performed with the dichotomous classification of SSAs.

Terminology

IGF2 DMR: IGF2 expression is controlled by CpG-rich regions known as *IGF2* DMRs in CRC. In particular, *IGF2* DMR0 hypomethylation has been suggested as a surrogate-biomarker for *IGF2* loss of imprinting. LINE-1: Global DNA hypomethylation is associated with genomic instability, which leads to cancer. As the long interspersed nucleotide element-1 or L1 retrotransposon constitutes a substantial portion of the human genome, the level of LINE-1 methylation is regarded as a surrogate marker of global DNA methylation. Serrated pathway: The serrated neoplasia pathway has attracted considerable attention as an alternative pathway of CRC development, and serrated lesions exhibit unique clinicopathological or molecular features. Of the serrated lesions, SSAs are hypothesized to develop in some cases to MSI-high CRCs with *BRAF* mutation in the proximal colon.

Peer review

The authors investigated the hypomethylations of *IGF2* DMR0 and LINE-1; MSI; and mutations of *KRAS*, *BRAF*, and *PIK3CA* in patients with serrated lesions and non-serrated adenomas. The results demonstrated that *IGF2* DMR0 hypomethylation can occur in the early stage of any histological types of serrated lesions; however, hypomethylation may be an infrequent epigenetic alteration in SSAs. The authors also revealed that the hypomethylation of *IGF2* DMR0 and LINE-1 may play an important role in the progression of the TSA pathway. This article may have a substantial impact on clinical and translational research in the progression of serrated lesions related to malignant transformation.

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RAPID COMMUNICATION

CCAAT/enhancer binding protein α (C/EBPα)$^+$ M2 macrophages contribute to fibrosis in IgG4-related disease?

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Abstract

IgG4-related disease (IgG4-RD) is a new disease entity characterized by type 2 helper T (Th2)-dominant inflammation and progressive fibrosis. We found the infiltration of strange cell populations in the fibrotic lesions of submandibular gland specimens obtained from 15 patients with IgG4-RD. These cells expressed CCAAT/enhancer binding protein a (C/EBPα). Many of the cell populations were identified with M2 macrophages. The degrees of infiltration of C/EBPα$^+$M2 macrophages and the ratio of fibrotic lesions in the specimens were correlated ($r^2 = 0.83$, $p < 0.01$). We also analyzed the expression of C/EBPα in other chronic inflammatory disorders: synovium in rheumatoid arthritis (RA), liver tissue in chronic viral hepatitis, and mucosa in ulcerative colitis. The specimens from RA and chronic viral hepatitis showed infiltration of C/EBPα$^+$ cells, but there were few C/EBPα-positive cells in ulcerative colitis. Fibrosis is not a major issue in ulcerative colitis. In conclusion, we found the remarkable infiltration of C/EBPα$^+$M2 macrophages in cases of chronic inflammation with fibrosis, including IgG4-RD. This primitive study also disclosed that most of C/EBPα$^+$M2 macrophages localized in fibrotic lesions, and the degree of the infiltration and the ratio of fibrotic area were correlated.

Keywords

CCAAT/enhancer binding protein α, Fibrosis, IgG4-related disease, Macrophage

History

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Organ fibrosis occurs and proceeds irreversibly with many chronic inflammatory disorders. It often becomes clinically problematic in pulmonary fibrosis, liver cirrhosis, and diabetic nephropathy. Fibrosis also occurs in inflammatory bowel diseases and rheumatic disorders. Extracellular matrix is deposited by activated fibroblasts in the fibrosis, but the mechanisms of the fibrosis remain the critical issue to be elucidated.

IgG4-related disease (IgG4-RD) is a new disease entity characterized by elevated levels of serum IgG4 and remarkable infiltration of IgG4-bearing plasmacytes and fibrosis in the involved organs. We have investigated the peculiar pathogenesis of type 2 helper T (Th2)-dominant inflammation and progressive fibrosis [1]. During our research, we found the infiltration of strange cell populations in the fibrotic lesions of submandibular gland specimens obtained from 15 patients with IgG4-RD. Clinical and histopathological data was shown in Table 1. These cells expressed CCAAT/enhancer binding protein α (C/EBPα) (anti-CEBPA antibody, Fitzgerald Industries International, Inc. North Acton, MA, USA). Many of the cell populations were identified with M2 macrophages as they expressed CD163 (anti-CD163 antibody, Epitomics. Burlingame, CA, USA) (Figure 1a, b), but all M2 macrophages did not express C/EBPα. Microscopic images of all cases were digitalized (DP Controller version 2.3.1.231; Olympus, Tokyo, Japan) and then Scion Image version 4.0.3.2 software (Scion Corporation, Frederick, MD, USA) was used to assess the fibrotic lesions. The degrees of infiltration of C/EBPα$^+$M2 macrophages and the ratio of fibrotic lesions in the specimens were correlated ($r^2 = 0.83$, $p < 0.01$). We also analyzed the expression of C/EBPα in other chronic inflammatory disorders: synovium in rheumatoid arthritis (RA), liver tissue in chronic viral hepatitis, and mucosa in ulcerative colitis. We examined five cases for each disease. C/EBPα-positive cells were observed in synovium of RA (30–50/HPF) and in the liver tissue of chronic hepatitis (20–40/HPF), which presented with chronic inflammation with fibrosis. On the other hand, there were few C/EBPα-positive cells in ulcerative colitis. Fibrosis is not a major issue in ulcerative colitis (Figure 2a–c).

C/EBPα is expressed on the terminally differentiated cells of various tissues, including the hematopoietic system, liver, adipocytes, and lungs, and plays an important role in cellular differentiation. C/EBPα is expressed on myeloid progenitor cells in the hematopoietic system. Blood cell differentiation tends to be inversely correlated with the degree of C/EBPα expression. C/EBPα is a crucial transcription factor

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Table 1. Clinical and pathological findings of 15 cases with IgG4-related disease.

Case No.	Age	Sex	Analyzed organ	Serum IgG4 (mg/dL)	CD163 (cells/HPF)	C/EBPα (cells/HPF)	CD163+ C/EBP α + (cells/HPF)	Distribution of CD163+C/EBPα +	Ratio of fibrosis in specimen (%)
1	69	M	SM	173	78	42	26	IF	53.4
2	67	M	SM	626	26	28	12	IF	27.2
3	64	M	SM	1410	58	20	9	IF	19.8
4	57	M	SM	402	55	12	7	IF	17.5
5	64	M	SM	257	28	8	7	IF	14.6
6	51	F	SM	768	62	23	5	IF	9.5
7	65	M	SM	548	46	36	13	IF	16.7
8	74	M	SM	374	38	16	14	IF	17.9
9	64	M	SM	2210	61	12	11	IF	20.4
10	69	M	SM	1420	36	13	13	IF	15.6
11	55	F	SM	870	39	8	6	IF	8.9
12	74	F	SM	963	21	3	3	IF	6.0
13	62	M	SM	796	26	11	10	IF	18.2
14	71	M	SM	457	39	24	18	IF	25.4
15	57	M	SM	329	33	26	18	IF	36.2

HPF high power field, *C/EBPα* CCAAT/enhancer binding protein α, M male, F female, SM submandibular gland, IF interfollicular.

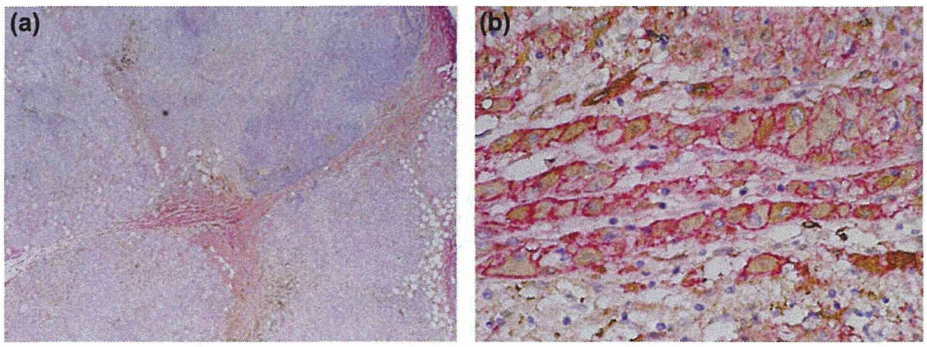

Figure 1. Identification of cells expressing C/EBPα. Double immunostaining with CD163 (*red*) and CCAAT/enhancer binding protein α (C/EBPα) (*brown*) in the submandibular glands of IgG4-related disease. C/EBPα-positive cells are observed along the fibrosis. These cells express CD163 (**a.** magnification ×40, **b.** magnification ×400).

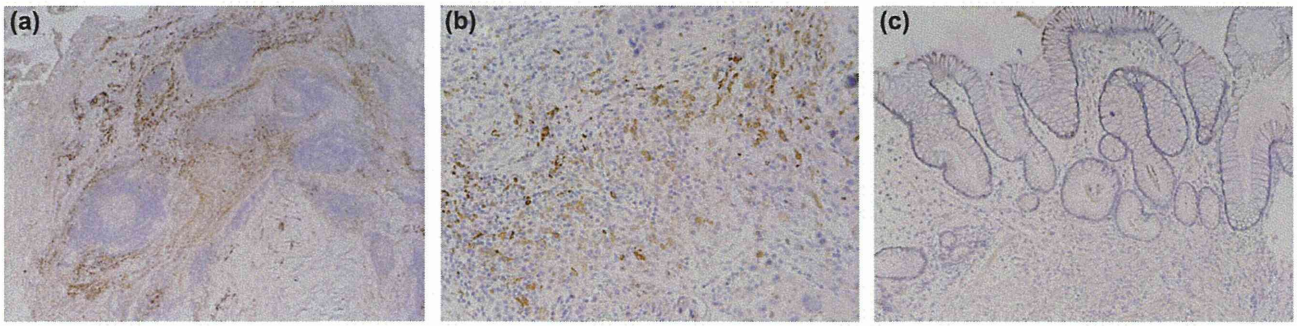

Figure 2. Distribution of C/EBPα-positive cells in other diseases. (a) Rheumatoid arthritis (magnification x40). (b) Liver cirrhosis due to hepatitis B virus (magnification ×200). (c) Ulcerative colitis (magnification x100). C/EBPα-positive cells are observed in the synovium of rheumatoid arthritis and cirrhotic liver tissues presenting fibrosis. In ulcerative colitis, for which fibrosis is not really a problem, there is little C/EBPα expression.

in the differentiation from common myeloid progenitor (CMP) to granulocyte/macrophage progenitor (GMP) and is no longer expressed in mature granulocytes and macrophages. It is not usually important in lymphoid differentiation [2].

The origin and the role of C/EBPα$^+$M2 macrophages are unknown. It was recently reported that C/EBPα could mediate the transdifferentiation of pre-B cells to macrophages [3,4]. If this transdifferentiation occurs in chronic inflammatory disorders with fibrosis, the origin of C/EBPα$^+$M2 macrophages could be pre-B cells in the bone marrow. Rituximab is an effective treatment for IgG4-RD [5]; but, the target of anti-CD20 antibodies in IgG4-RD is unclear because IgG4-RD is characterized by Th2-dominant inflammation and fibrosis. Rituximab can be transferred to the bone marrow. If our hypothesis is correct, one of the mechanisms by which rituximab regulates the pathogenesis of IgG4-RD is by depletion of pre-B cells in the bone marrow, which would prevent the transdifferentiation of pre-B cells into macrophages by C/EBPα. We must prove this hypothesis with experimental animals and further analyze other diseases characterized by fibrosis in our next studies.

With respect to the role of C/EBPα$^+$M2 macrophages, it is known that macrophages contribute to fibrosis [6]. This primitive study disclosed that most of C/EBPα$^+$M2 macrophages localized in fibrotic lesions, and the degree of the infiltration and the ratio of fibrotic area were correlated. Macrophages are classified as M1 macrophages, which contribute to

inflammation, and M2 macrophages, which play a role in tissue repair. M2 macrophages are mainly involved in the fibrosis. It is also unknown that C/EBPα$^+$M2 macrophages are involved in RA. There are many synovial fibroblasts, but few fibrosis in the synovium of RA. Over production of matrix metalloproteinase (MMP) leads to collagen degradation in RA [7]. It is considered that cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and transforming growth factor (TGF)-β regulate the expression of mRNA of MMP [8]. It is estimated that in those cytokines environments fibrosis is difficult to occur. C/EBPα$^+$ cells were not detected in the specimens with ulcerative colitis. It was considered that the factors other than the polarity of Th1-Th2 are also present. Research about the relationship between macrophages and fibrosis in cirrhosis is in progress. The inhibition of macrophage migration inhibitory factor (MIF) can lead to suppression of liver fibrosis [9]. It is important to analyze how fibrosis is affected by the presence or absence of C/EBPα expression.

In conclusion, we found the remarkable infiltration of C/EBPα$^+$M2 macrophages in cases of chronic inflammation with fibrosis, including IgG4-RD. This is the first report of C/EBPα$^+$M2 macrophages being present in fibrosis. It is necessary to examine the function and regulation of this cell group in fibrosis.

Conflict of interest

None.

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EXPERT OPINION

DNA synthesis inhibitors for the treatment of gastrointestinal cancer

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Introduction: Intensive laboratory, preclinical and clinical studies have identified and validated molecular targets in cancers, leading to a shift toward the development of novel, rationally designed and specific therapeutic agents. However, gastrointestinal cancers continue to have a poor prognosis, largely due to drug resistance.

Areas covered: Here, we discuss the current understanding of DNA synthesis inhibitors and their mechanisms of action for the treatment of gastrointestinal malignancies.

Expert opinion: Conventional agents, including DNA synthesis inhibitors such as fluoropyrimidines and platinum analogs, remain the most effective therapeutics and are the standards against which new drugs are compared. Novel DNA synthesis inhibitors for the treatment of gastrointestinal malignancies include a combination of the antimetabolite TAS-102, which consists of trifluorothymidine with a thymidine phosphorylase inhibitor, and a novel micellar formulation of cisplatin NC-6004 that uses a nanotechnology-based drug delivery system. The challenges of translational cancer research using DNA synthesis inhibitors include the identification of drugs that are specific to tumor cells to reduce toxicity and increase antitumor efficacy, biomarkers to predict pharmacological responses to chemotherapeutic drugs, identification of ways to overcome drug resistance and development of novel combination therapies with DNA synthesis inhibitors and other cancer therapies, such as targeted molecular therapeutics. Here, we discuss the current understanding of DNA synthesis inhibitors and their mechanisms of action for the treatment of gastrointestinal malignancies.

Keywords: antimetabolite, DNA synthesis inhibitor, drug delivery system, drug resistance, platinum analogs, translational cancer research

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1. Introduction

Cancer is a major public health problem in the US and other developed countries. DeSantis *et al.* reported that 1,665,540 new cancer cases are expected in the US in 2014 [1].Gastrointestinal cancer refers to malignancy of the gastrointestinal tract and accessory organs involved in digestion, including the esophagus, stomach, biliary system, pancreas, small intestine, colon, rectum and anus. An estimated 18,170 new cases of esophageal cancer, 22,220 new cases of stomach cancer, 136,830 new cases of colon and rectal cancer, 46,420 new cases of pancreatic cancer, 9,160 new cases of small intestine cancer and 33,190 new cases of liver and intrahepatic bile duct cancer will be diagnosed in 2014. Despite advances in surgery, radiation therapy, systemic chemotherapy and supportive therapies, the 5-year relative survival rates for all cancer in the US is ~ 66% for patients diagnosed between

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Article highlights.

- TAS-102 is a novel combination antimetabolite which consists of trifluorothymidine with a thymidine phosphorylase inhibitor.
- NC-6004 is a novel micellar formulation of cisplatin which uses a nanotechnology-based drug delivery system.
- The challenges of translational cancer research using DNA synthesis inhibitors include the identification of drugs that are specific to tumor cells, biomarkers to predict pharmacologic responses, identification of ways to overcome drug resistance, and development of novel combination therapies.

This box summarizes key points contained in the article.

2003 and 2009, and followed through 2010. Thus, the development of novel cancer therapeutics is urgently needed to improve cancer prognosis.

According to the American Cancer Society, cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. It is caused by the accumulation of genetic mutations and epigenetic alterations in oncogenes and tumor suppressor genes [2,3]. Cancer chemotherapy has changed since curative treatments were identified for previously fatal malignancies with rapid cell growth, such as acute leukemia [4]. As many chemotherapies affect mitosis, tumors with high growth rates are more sensitive to chemotherapy because a larger proportion of the targeted cells are undergoing cell division at any time. However, conventional chemotherapy is less effective against slow growing cancers, including gastrointestinal cancers. Additionally, intratumoral heterogeneity may contribute to the varying sensitivity of cancer cells to chemotherapy, as well as to drug resistance [5].

There are a number of strategies in the administration of chemotherapeutic drugs, including combination chemotherapy, combined modality chemotherapy, postoperative (adjuvant) chemotherapy, preoperative (neoadjuvant) chemotherapy and salvage chemotherapy. Chemotherapy is also employed as part of the multimodal treatment of cancer, such as esophageal cancer, thereby allowing for more limited surgery. Adjuvant and neoadjuvant chemotherapy can extend life and prevent disease recurrence following surgical resection of gastrointestinal cancers, including esophageal, gastric, colorectal and pancreatic cancer [6].

Recently, there has been a shift toward developing novel, rationally designed and specific agents for cancer therapy [2,7,8] Among gastrointestinal cancers, there are novel molecularly targeted therapeutics, including the tyrosine kinase inhibitors imatinib and sunitinib for gastrointestinal stromal tumors [9,10], regorafenib for metastatic colorectal cancer [11] and gastrointestinal stromal tumors [12], sunitinib and everolimus for pancreatic neuroendocrine tumors and erlotinib in combination with gemcitabine for advanced pancreatic carcinoma [13]. Additionally, therapeutic monoclonal antibodies have been developed, including a humanized anti-VEGF monoclonal antibody, bevacizumab, for metastatic colorectal cancer [14], a chimeric anti-EGFR monoclonal antibody, cetuximab, for metastatic colorectal cancer [15], a human monoclonal antibody to EGFR, panitumumab, for metastatic colorectal cancer, a humanized anti-Her2 receptor monoclonal antibody, trastuzumab, for metastatic gastric or gastroesophageal junction adenocarcinoma [16,17] and a human monoclonal antibody to the Her2 receptor, ramucirumab, for metastatic gastric or gastroesophageal junction adenocarcinoma. Moreover, recombinant fusion proteins have been developed, such as ziv-aflibercept, consisting of the binding portions of VEGF from VEGF receptors 1 and 2 fused to the Fc portion of immunoglobulin G1, for metastatic colorectal cancer [18]. However, despite the remarkable successes of the molecularly targeted agents discussed above, the prognosis of gastrointestinal cancer remains poor due to drug resistance.

New therapies for gastrointestinal cancers are not likely to replace cytotoxic agents, many of which act by damaging DNA. Rather, cytotoxic agents combined with molecularly targeted drugs will continue to be used in chemotherapy for gastrointestinal cancers. Here, we discuss the current understanding of DNA synthesis inhibitors and their mechanisms of action for the treatment of gastrointestinal cancers in order to improve patient prognosis.

2. DNA synthesis inhibitors

Traditionally, cancer drugs have been discovered through large-scale testing of synthetic chemicals and natural products in proliferating animal tumor systems, including mouse allograft preclinical cancer models using murine leukemia cells, human xenograft models using immunodeficient mice and *in vitro* human cancer cell line models, such as the anticancer drug screen conducted in 60 human tumor cell lines by the United States National Cancer Institute (NCI) [4,19]. Over time, this system has evolved into one that combines both *in vitro* human cancer cell lines with human xenograft models. Most of the agents discovered in these drug screens interact with DNA or its precursors, inhibiting the synthesis of new genetic material and causing damage to DNA in both normal and malignant cells. Unfortunately, none of the screening systems have successfully predicted outcome of clinical trials [20,21].

The drugs used in cancer chemotherapy are varied in structure and mechanism of action. Most chemotherapeutic drugs work by impairing mitosis, effectively targeting fast-dividing cells. These drugs prevent mitosis through a number of mechanisms, including damaging DNA and inhibiting the cellular machinery involved in cell division. Interestingly, many of these drugs inhibit DNA synthesis.

DNA synthesis is the creation of new DNA molecules through the process of DNA replication, wherein a replication initiator protein splits the existing cellular DNA and makes a copy of each split strand. The copied strands are then joined together with their template strand to form a new DNA molecule. DNA replication proceeds in three enzymatically