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Chemotherapeutic implications in microsatellite unstable colorectal cancer[1]

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Abstract

Chemotherapy for colorectal cancer is currently offered to patients based on the stage of their cancer, and there is evidence to show an overall survival benefit with 5-fluorouracil-based (5-FU) therapy for patients with lymph node metastasis who receive it. The pathogenesis of colorectal cancer involves genomic instability, with about 15% of tumors demonstrating a form of genomic instability called high-frequency microsatellite instability (MSI-H) and due to loss of DNA mismatch repair function, and the remainder of colorectal tumors lacking MSI-H with retained DNA mismatch repair function and called microsatellite stable (MSS), with a large proportion of these tumors demonstrating another form of genomic instability called chromosomal instability. There is now evidence to show that the form of genomic instability that is present in a patient's colorectal cancer may predict a survival benefit from 5-FU. In particular, patients whose colorectal tumors have MSI-H do not gain a survival benefit with 5-FU as compared to patients with MSS tumors. In vitro evidence supports these findings, as MSI-H colon cancer cell lines are more resistant to 5-FU compared to MSS cell lines. More specifically, components of the DNA mismatch repair system have been shown to recognize and bind to 5-FU that becomes incorporated into DNA and which could be a trigger to induce cell death. The binding and subsequent cell death events would be absent in colorectal tumors with MSI-H, which have lost intact DNA mismatch repair function. These findings suggest that: (a) tumor cytotoxicity of 5-FU is mediated by DNA mechanisms in addition to well-known RNA mechanisms, and (b) patients whose tumors demonstrate MSI-H may not benefit from 5-FU therapy. Future studies should include a better understanding of the cellular mechanisms of the DNA recognition of 5-FU, multi-centered prospective trials investigating the survival benefit of 5-FU based on genomic instability, and the investigation of alternative chemotherapeutic regimens for patients with MSI-H tumors to improve survival.

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Keywords

DNA mismatch repair; microsatellite instability; colorectal cancer; chemotherapy; treatment

1. Introduction

Approximately 147,000 cases will be diagnosed, and 57,000 individuals will die of colorectal cancer (CRC) in the US in 2005, ranking it second behind lung cancer as the deadliest cancer [1]. The most important prognostic indicator has traditionally been the stage of the tumor at diagnosis, with depth of invasion being the most critical factor [2]. However, there is considerable variation in the behavior of tumors even within the same pathologic stage, indicating that the molecular make-up of the tumor may be a more reliable predictor of its natural history and perhaps its biological response to chemotherapy. Among the most studied molecular markers correlated with survival in colorectal cancer is microsatellite instability (MSI). The finding of high frequency MSI (MSI-H) in CRC has been associated with a favorable patient prognosis when compared to patients with microsatellite stable (MSS) tumors [3,4], but paradoxically a poor response to 5-fluorouracil (5-FU)-based chemotherapy [5–7]. Here, we review the basic and clinical evidence for differences in chemotherapy responses for patients with MSI-H colorectal tumors, and the implications for future treatment of these patients.

2. Adjuvant chemotherapy with 5-fluorouracil

Treatment for advanced stage colorectal cancer includes surgical therapy and adjuvant chemotherapy. Most patients with advanced colorectal cancer that undergo surgical treatment alone will develop a recurrence of their disease. Adjuvant chemotherapy regimens have been tried with success to improve the outcome of patients with apparent residual disease after primary surgical resection [8,9]. Almost all adjuvant chemotherapy for advanced stage colorectal cancer involves the agent 5-fluorouracil (5-FU), typically in combination with levamisole or leukovorin [9]. Thus, 5-FU, introduced more than 40 years ago, remains the mainstay of chemotherapeutic treatment of colorectal cancer. In particular, 5-FU-based chemotherapy improves survival in patients with stage III colon cancer [10–12], and in patients with stage II and III rectal cancer [9]. Because colorectal cancer is so common, nearly every new chemotherapeutic agent has been tried. Other agents developed for colorectal cancer treatment, including irinotecan (camptothecin) and oxaliplatin, are not any more effective than 5-FU [13].

While chemotherapy with 5-FU is the best treatment option and has become the standard of care in advanced stage colorectal cancer, individual patient tumor response rates are still overall poor. In a meta-analysis of randomized trials, continuous infusion of 5-FU had a tumor response rate of 22% [14]. In the seven randomized trials included in this meta-analysis, the tumor response rates ranged between 20 and 30%. There is no current method to determine which patient will have a tumor response to 5-FU. There are likely several reasons why some colorectal tumors do not respond to treatment with 5-FU. Importantly, the

loss of DNA mismatch repair within the patient's tumor is associated with no survival benefit from 5-FU.

3. Loss of DNA mismatch repair and microsatellite instability

Colorectal cancers develop as a consequence of genomic instability. That is, during the transformation process from normal to tumor, the DNA of a colonocyte is damaged in a particular pattern that drives tumorigenesis. Approximately 15% of sporadic colorectal tumors display MSI-H, mostly as a result of epigenetic silencing by hypermethylation of the *hMLH1* gene [15,16]. The remaining 85% of sporadic colorectal cancer can be termed microsatellite stable [MSS], with most tumors in this category following a molecular pathway characterized by chromosomal instability whereby sequential mutations and allelic loss of key regulatory genes culminate in the transformation of an adenoma into cancer [17,18].

The phenotype of MSI (particularly MSI-High, defined as ≥ 30% of DNA microsatellite markers mutated within a colorectal tumor) [19] is caused by a defective DNA MMR system. DNA MMR is an evolutionarily conserved system capable of repairing mispaired nucleotides and short mismatched insertion-deletion loops (IDLs) in DNA, presumably as a result of insertion mistakes made by DNA polymerase during replication. DNA MMR replaces the mispair on the newly synthesized daughter strand [20,21]. The mispair or IDL is recognized and bound by either hMutSα (a heterodimer of hMSH2 and hMSH6) or hMutSβ (a heterodimer of hMSH2 and hMSH3) in a highly orchestrated manner (Fig. 1). hMutSα is capable of binding to single base pair mismatches and single IDLs whereas hMutSα can only bind IDLs [22,23]. Recruitment of the hMutLα complex (heterodimer of hMLH1 and hPMS2) by hMutSα or hMutSβ subsequently targets the DNA to complete the repair process. Defects in the MMR genes *hMSH2, hMLH1*, and *hMSH6* in humans have been clearly linked to Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer, or HNPCC), an autosomal dominant condition in which one of the MMR genes is mutated in germline cells [24–30].

It has been noted that failure to correct post-replication mismatches results in a 10- to 100-fold increase in mutation rate [31,32]. Thus, absence of MMR results in a hypermutable environment with recognizable frameshift mutations in predominantly mono-, di-, and tri-nucleotide microsatellite tracts, as well as within genes that contain such sequences in their coding regions, such as *TGFBR2, ACVR2*, and *BAX* [33–38]. Mutation within these genes and the subsequent loss of protein function are thought to drive the pathogenesis of MSI-H colorectal tumors [39,40].

4. A more favorable prognosis for patients with MSI-H colorectal cancer (in the absence of adjuvant chemotherapy)

Clinically, tumors with MSI-H are correlated with the tumor's location in the *proximal colon*, have a *histological poor grade*, and are inversely related to allelic loss [41–43]. Microsatellite unstable tumors also tend to be diploid, mucinous with signet cell features,

and have a surrounding lymphoid reaction. This phenotype appears to be the result of rapid neoplastic progression in Lynch syndrome and sporadic tumors that exhibit MSI [43–45].

Patients with MSI-H tumors have been associated with a more favorable prognosis compared to patients with MSI-L or MSS tumors [3,4]. One study which examined the clinical outcome in 607 young patients (< 50 years of age) with colorectal cancer found that MSI-H tumors had a significant survival advantage (hazard ratio of 0.42, $p < 0.0001$) over MSS tumors [3]. In addition, MSI-H tumors had a significantly lower likelihood of metastasizing to regional lymph nodes (odds ratio 0.33, $P < 0.001$) and distant organs (odds ratio 0.49, $P = 0.02$). Indeed, the favorable prognosis of MSI-H CRC have been documented by several other studies [46,47], and a recent systematic review of pooled studies confirmed the relationship between tumor MSI-H and patient survival, with a combined hazard ratio for overall survival associated with MSI-H at 0.65 (95% CI 0.59 to 0.71) [4]. The biologic basis for the tumor MSI-H and patient survival association has not been established. It has been postulated that the lymphocyte infiltration seen in MSI-H tumors is a reflection of an enhanced host immune response invoked by the presence of numerous mutated products [44,48]. Several studies have correlated a lymphocytic infiltration in CRC with increased survival [49,50]. Others have also suggested that the enormous mutational burden resulting from loss of MMR activity may be self-limiting in that essential cell functions may be hindered [51]. However, these explanations remain speculative at this time, and the molecular mechanism underlying the relatively indolent behavior of MSI-H tumors remains elusive. The fact that patients with MSI-H tumors have a better prognosis over patients with MSS tumors originally confounded some studies examining 5-FU chemotherapy, with most lacking the appropriate control group for comparative purposes.

5. Response to 5-fluorouraci-based chemotherapy in patients with MSI-H tumors

Patients with tumors that exhibit high-frequency MSI (MSI-H) are typically treated the same as those without the MSI-H phenotype, with the stage of the tumor being the major determinant of which patients will benefit from adjuvant chemotherapy. However, recent evidence indicates that the MSI status of the tumor may also be important.

Paradoxically, the favorable natural history of MSI-H CRC does not parallel its response to chemotherapy. Early reports appeared to indicate that 5-FU adjuvant chemotherapy appeared to be beneficial for patients with MSI-H CRC, but these studies were limited by small or non-randomized study population [52–55]. Recent studies powered by larger sample sizes and with appropriate control groups have demonstrated that patients with MSI-H tumors do not appear to derive any benefit from 5-FU-based adjuvant chemotherapy (Table 1), and there is evidence to suggest that chemotherapy may even be detrimental to patients with MSI-H CRC. In one study of 204 patients with sporadic stage II and III CRC, retrospective survival analysis failed to show a difference in survival among patients with MSI-H tumors irrespective of whether 5-FU was administered ($p = 0.52$) [5]. In contrast, there was a significant survival advantage with 5-FU chemotherapy among patients with MSI-L and MSS tumors ($p = 0.0478$). Similarly, another study of 505 stage II and III colorectal cancer

patients failed to show a survival benefit with 5-FU in patients with MSI-H colorectal cancers ($p = 0.4$), while patients with MMR-competent MSI-L and MSS tumors benefited with improved survival with 5-FU ($p = 0.0001$) [7]. Another study examined 570 tissue specimens from patients with stage II and III CRC who had been enrolled in randomized trials of 5-FU-based chemotherapy [6]. Among 287 patients not receiving chemotherapy, the 5-year survival rate was higher among patients with MSI-H tumors compared to those with MSH-L or MSS tumors (88.0% vs. 68.4%, $p = 0.004$), a finding consistent with the more favorable prognosis of MSI-H tumors. However, among patients who received chemotherapy, those with MSI-H tumors were associated with a slightly lower 5-year survival rate compared to the MSH-L or MSS tumors (70.7% vs. 75.5%, $p = 0.66$). Furthermore, among patients with MSI-H tumors, chemotherapy with 5-FU was associated with a worse outcome (hazard ratio for death, 2.14, $p = 0.11$). In addition to the lack of 5-FU survival benefit in patients with sporadic MSI-H tumors, stage III patients with HNPCC do not demonstrate a 5-year survival benefit with 5-FU over untreated patients [56] (Table 1). Collectively, these results suggest that 5-FU-based chemotherapy does not prolong survival in patients with MSI-H CRC and may even be detrimental. On a molecular level, these findings suggest that 5-FU-mediated cytotoxicity may be dependent on intact DNA MMR gene function.

6. DNA mismatch repair: A mediator of chemotoxicity

In addition to its role in recognizing and directing repair of polymerase mistakes in DNA, the human MMR system is also capable of recognizing certain DNA adducts caused by exogenous alkylation damage [57–59]. The SN1 methylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) creates O^6-methylguanine as its principal adduct. This adduct is recognized by the MMR system, and results in a G_2/M cell cycle arrest and cell death [57]. Similarly, when 6-thioguanine (6-TG) is incorporated into the DNA of MMR-proficient cells, a G_2/M cell cycle arrest is induced [58]. Both the MNNG adduct and 6-TG are thought to be recognized by the MMR system because of mispairing with T (or C) with the altered nucleotide on the newly synthesized DNA strand. Cell cycle arrest at the G_2/M checkpoint prevents mutations (namely G to A transitions) in daughter cells. Adducts formed by cisplatin and carboplatin intercalate and distort DNA, which are also recognized by the MMR proteins [60–62] (Table 2). However, substituted amine analogues of cisplatin, namely oxaliplatin, tetraplatin, transplatin, JM335, and JM216, form different types of adducts and are not apparently recognized by the MMR system [60]. Similarly, 8-azaguanine (8-AG) is not recognized in MMR-intact cells [58]. Irinotecan, the topoisomerase I inhibitor, seems to induce its toxicity independent of the DNA MMR system [63–65]. Indeed, several compounds that induce toxicity upon colorectal cancer cells may work independently of DNA MMR, and some of these drugs will need further evaluation to exploit as independent treatments for patients with MSI-H colorectal cancer (Table 2).

7. DNA mismatch repair and 5-FU recognition

5-FU is a fluoropyrimidine that is incorporated into RNA (mRNA, rRNA, and tRNA), is an inhibitor of thymidylate synthetase (which catalyzes the conversion of dUMP to dTMP), and has some incorporation into DNA [66–68] (Fig. 2). The cytotoxic effects of 5-FU have

traditionally been attributed to its ability to inhibit thymidylate synthetase and its interference with RNA processing [66]. Under normal conditions as shown in Fig. 2, dUTPase prevents the incorporation of dUTP and FdUTP into DNA by dephosphorylating the nucleotides to dUMP and FdUMP, respectively [69,70] (enzyme #13, Fig. 2). However, since 5-FU can inhibit thymidylate synthetase, accumulation of dUMP and FdUMP occurs, which exhausts the ability of dUTPase to metabolize dUTP and FdUTP. As dUTP and FdUTP levels rise and those of TTP fall, dUTP and FdUTP replace TTP as substrates for DNA polymerases, and are incorporated into DNA [66] (enzyme #11, Fig. 2). Nonetheless, uracyl-*N*-glycosylase, an enzyme that removes uracil bases from DNA after the spontaneous deamination of deoxycytidine, will typically remove the incorporated uracil bases (enzyme #14, Fig. 2). In spite of this, TTP is not available and the DNA strand will be repaired using dUTP or FdUTP as a substrate. 5-FU incorporation into DNA had been previously observed [67,71,72], but the consequences of this phenomenon was not known until recently as there was no reported correlation between 5-FU incorporation into DNA and cytotoxicity [71–73]. The first demonstration of MMR-mediated 5-FU toxicity came from in vitro studies demonstrating that cell lines with intact MMR function were selectively killed with 5-FU treatment, while MSI-H cells were resistant to 5-FU [68]. These results were corroborated by a subsequent study demonstrating that colon cancer cells with biallelic hypermethylation of hMLH1 lost their resistance to 5-FU in the presence of a demethylating agent [74]. More recent studies have shown that hMutSα can recognize and bind DNA containing 5-FU [75,76], indicating that resistance to 5-FU in MMR-deficient cells may be attributable to the direct interaction between 5-FU and MMR proteins. Indeed, hMutSα, the heterodimer of hMSH2 and hMSH6, shows greater affinity for 5-FU incorporated into DNA than its natural substrate, a typical base mispair [75].

It is not clear how the MMR system recognizes 5-FU incorporated into DNA. Unlike bulky intercalating adducts such as cisplatin, or O^6-methylguanine adducts formed by treatment with MNNG, 5-FU incorporation may not physically distort the DNA double helix because it does not interfere with interstrand hydrogen bonding (Fig. 3). How 5-FU might be recognized by DNA mismatch repair is speculation, but it may involve the highly charged fluorine atom that may deform the DNA double strand enough to be recognized by MMR proteins.

The downstream signaling pathways triggered by MMR recognition of modified DNA have been partially elucidated for some chemotherapeutic agents but remains undefined for 5-FU. For example, introduction of O^6-MeG into DNA results in a G_2/M cell cycle arrest that is dependent on an intact MMR system and involves the ATM and Rad3-related (ATR) and CHK1 kinases [77]. p53, another downstream target of ATR [78], has also been shown to become phosphorylated during MMR-mediated repair of DNA damaged by O^6-MeG [79]. Apoptosis induced by hMutSα recognition of O^6-MeG lesions appears to involve mitochondrial signaling that activate both caspase-dependent and caspase-independent pathways [80]. The cellular response invoked by MMR in response to cisplatin involves activation of c-ABL, which promotes apoptosis through regulation of P73, a P53-related protein [81,82]. Much less is known about the signaling cascade induced by MMR recognition of 5-FU. Restoration of MMR function in MMR-deficient colon cancer cells results in restoration of G_2/M cell cycle arrest after treatment with 5-FU [76,83], but it is not

known whether the same pathways delineated for other agents are involved. Interestingly, apoptosis was noted to occur at low levels in both MMR-proficient and MMR-deficient cells after exposure to 5-FU and irradiation [83], which likely may represent the non-MMR-mediated toxicities of 5-FU.

8. Conclusion

In summary, MSI represents a promising disease marker for CRC because of the favorable prognosis associated with MSI-H CRC. However, recent data suggests that it may also serve as a reliable marker for response to chemotherapy. An intact DNA MMR system appears to be necessary to mediate the cytotoxicity of several chemotherapeutic agents, including 5-FU. Both cell cycle arrest and cell death following exposure to 5-FU have been shown to be dependent on MMR proteins, and recognition of 5-FU incorporation into DNA by the MMR proteins appears to be a critical step in this process. Although the cellular mechanisms for the DNA recognition of 5-FU by MMR need to be elucidated, the discrimination of MMR recognition of incorporated 5-FU has important clinical implications in the treatment of CRC, since several studies have now shown that patients with MSI-H tumors do not derive any benefit from and may even be harmed by 5-FU-based adjuvant chemotherapy. There is currently an impetus for tailoring chemotherapeutic regimens based on the molecular profile of the tumor, and it is conceivable that MSI status may be a contraindication to 5-FU treatment in the future. However, prospective, randomized, and well-controlled studies are needed for absolute confirmation before any recommendations can be implemented. Furthermore, additional agents that might be beneficial towards patient survival regardless of the MSI status of the tumor need to be furthered explored.

Abbreviations

5-FU	5-fluorouracil
MMR	DNA mismatch repair
MSI-H	high-frequency microsatellite instability
MSI-L	low frequency microsatellite instability
MSS	microsatellite stable
CRC	colorectal cancer
TS	thymidylate synthase
O^6-MeG	O^6-methylguanine
6-TG	6-thioguanine
8-AG	8-azoguanine

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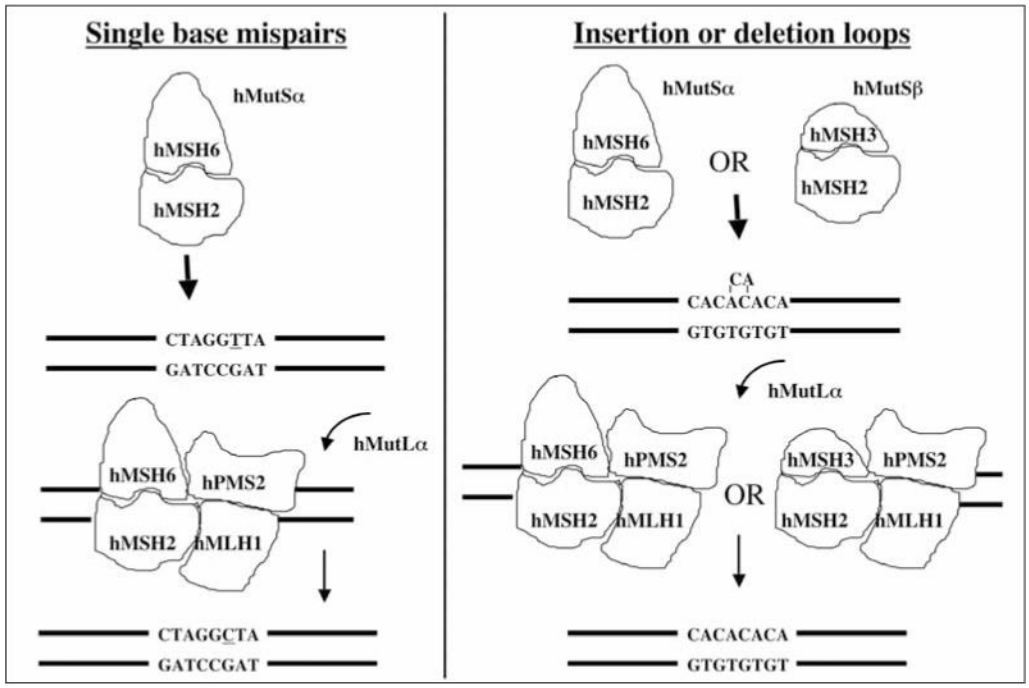

Fig. 1.

Schematic for DNA mismatch repair. The heterodimer hMutSα recognizes and binds single mispairs and small insertion-deletion loops (IDL), whereas hMutSβ only binds IDLs. The subsequent events to effect excision and re-synthesis of the DNA are identical between the two heterodimers. The heterodimer hMutSα has been shown to recognize 5-FU incorporated into DNA.

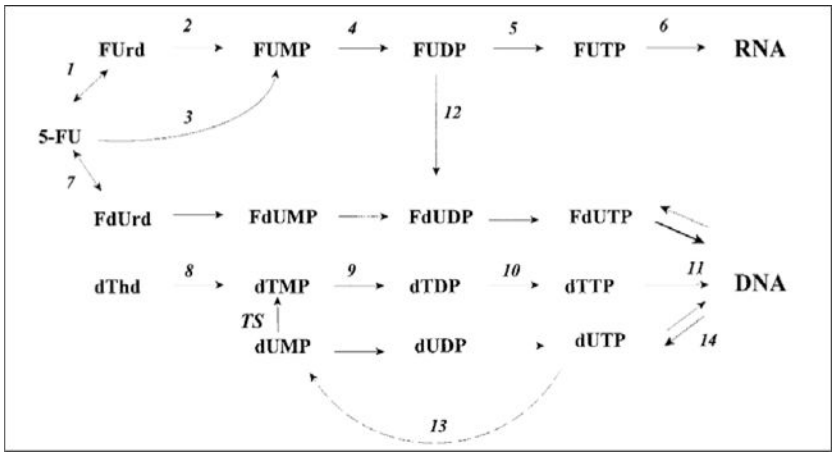

Fig. 2.

Intracellular metabolism of 5-FU. The numbers denote the following enzymes: (1) uridine phosphorylase, (2) uridine kinase, (3) orotate phsophoribosyltransferase, (4) and (9) pyrimidine kinase, (5) and (10) pyrimidine diphosphate kinase, (6) RNA polymerase, (7) thymidine phosphorylase, (8) thymidine kinase, (11) DNA polymerase, 12 ribonucleotide reductase, (13) deoxyuridine triphosphate pyrophosphatase, (14) uracil-DNA-glycosylase. TS = thymidylate synthase. 5-FU can be incorporated into RNA, and DNA due to its ability to block TS and exhaust the availability of dTTP, leaving only dUTP or FdUTP available for new DNA synthesis.

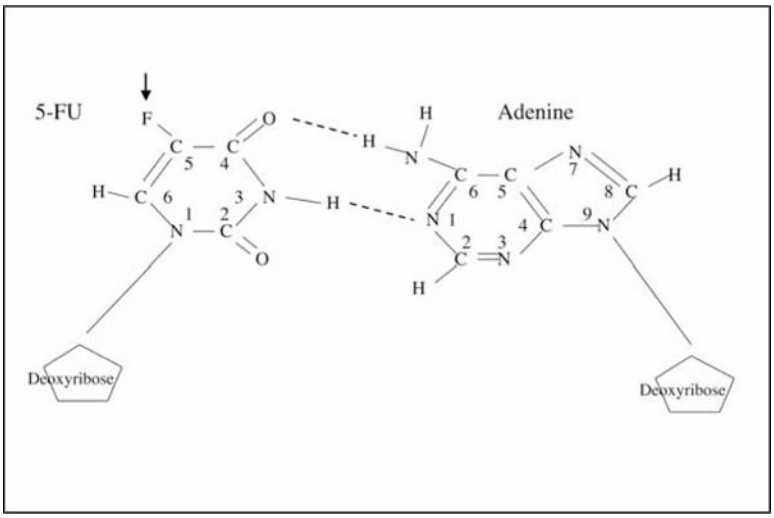

Fig. 3.

5-Fluorodeoxyuracil pairing with adenine in DNA. The fluorine molecule is at the 5 position (arrow), and does not interfere with hydrogen binding with adenine, but is recognized by DNA mismatch repair proteins.

Table 1

Studies of 5-FU treatment, survival, and MSI status

Published Study (year)	Total Number of Patients	Number of 5-FU treated patients	Stage assessed for survival	MSI-H survival benefit with 5-FU?	MSS/MSI-L survival benefit with 5-FU?
Ribic et al. (2003)	570	283	II–III	NO	YES
Carethers et al. (2004)	204	66	II–III	NO	YES
deVos et al. (2004) *	92	28	III	NO	N/A
Jover et al. (2006) **	754	260	II–III	NO	YES

* HNPCC patients only. The other studies contained mostly sporadic patients.

** Only 505 patients were stage II–III. Seven patients had a germline MMR gene mutation.

Table 2

Some chemotherapeutic compounds and relationship with MMR substrate recognition

Compound	Does MMR recognition mediate toxicity?	References
N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)	YES	57, 84
6-thioguanine	YES	58
cisplatin, carboplatin	YES	60–62
oxaliplatin, tetraplatin, transplatin, JM335, and JM216	NO	60
8-azaguanine	NO	58
5-fluorouracil	YES	68
doxorubicin, epirubicin, mitoxantrone	YES	64
bleomycin	NO	85
dacarbazine	YES	86
irinotecan/camptothecin, topotecan	NO	63–65
docetaxel, paclitaxel	NO	64
mitomycin C	NO	87