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Review Article

Clinical characteristics and responses to chemotherapy and immune checkpoint inhibitor treatment for microsatellite instability gastric cancer

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Abstract: During the process of DNA replication, insertions or deletions of repeat sequences easily occur in microsatellites due to DNA polymerase slippage in instances of defective mismatch repair; this phenomenon is known as microsatellite instability. Based on genetic profiling, microsatellite instability gastric cancer is regarded as a separate subtype of gastric cancer that is associated with old age, the female sex, a distal gastric location, and a lower number of lymph node metastases. According to numerous retrospective studies, microsatellite instability is a favourable predictive marker for prognosis. However, during the perioperative period, gastric cancer patients with microsatellite instability after chemotherapy often exhibit a poor and unfavourable prognosis. This result still remains controversial. The efficacy of adjuvant chemotherapy in microsatellite instability-high tumours ranges from detrimental to beneficial effects. Due to the widespread expression of immune checkpoint molecules (such as programmed death-1 and programmed death-ligand 1) in tumours with microsatellite instability, immune checkpoint inhibitors have been utilized to treat microsatellite instability gastric cancer and tremendously improve the efficacy of treatment and survival of microsatellite instability patients. In this review, we attempt to outline the definitions of microsatellites and microsatellite instability, the methods used to screen for microsatellite instability, the clinical characteristics of microsatellite instability gastric cancer, and its responses to chemotherapy and immune checkpoint inhibitor treatment. Overall, determining the status of microsatellites is essential before developing a tailored treatment strategy for patients with microsatellite instability gastric cancer.

Keywords: Microsatellite instability, gastric cancer, immunotherapy, chemotherapy, mismatch repair

Introduction

Gastric cancer (GC) remains among the most common malignancies worldwide [1]. However, improved awareness of disease prevention, advanced detection methods, improved surgical techniques and effective chemotherapy drugs have all contributed to fight against this cancer. The incidence of GC has significantly decreased over the past 10 years, and the overall survival (OS) of GC patients has clearly improved. Unfortunately, GC remains to be the third leading cause of cancer-related deaths and has a poor prognosis [2-5]. According to related research, the prognosis of GC may not only depend on the stage of the disease, but may also be related to specific molecular biological characteristics [6, 7]. In fact, in 2014, the genetic characteristics of GC were described by The Cancer Genome Atlas (TCGA) Research Network, confirming that GC is a complex, heterogeneous disease. Based on the classification of TCGA Research Network, GC is divided into the following 4 subtypes (as shown in **Table 1**): (1) tumour-positive Epstein-Barr virus (EBV) infection; (2) microsatellite instability (MSI); (3) genome stable; and (4) chromosome unstable. MSI GC is identified as a separate GC entity. In Western countries, this type of GC accounts for 22% of the total number of GC cases [6]. MSI is

Table 1. Four gastric cancer subtypes described by TCGA, occurrence frequencies and main characteristics [6]

TCGA gastric cancer subgroups	Frequency (%)	Main characteristics
Epstein-Barr virus	9	EBV-CpG island methylator phenotype (CIMP)
		PD-L1/2 overexpression
		PIK3CA mutation
		CDKN2A silencing
		Immune cell signaling
Microsatellite instability	22	Hypermutation
		Gastric-CIMP
		MLH1 silencing
		Mitotic pathways
Genomically stable	20	Diffuse histology
		CDH1, RHOA mutation
		CLDN18-ARHGAP fusion
		Cell adhesion
Chromosomal instability	49	Intestinal histology
		TP53 mutation
		RTK-RAS activation

EBV, Epstein-Barr virus; MSI, Microsatellite instability; GS, Genomically stable; CIN, Chromosomal instability.

defined as a type of genomic instability in which functional deficiency of the mismatch repair (MMR) system causes insertions and deletions of bases within microsatellites during DNA replication [8]. In many malignancies, the results of MSI testing are considered an effective predictor of patient prognosis and responsiveness to chemotherapy and immunotherapy. In most hereditary nonpolyposis colorectal cancers (HNPCCs), the MSI phenotype is a distinctive feature that can help distinguish it from sporadic colorectal cancer [9]. In sporadic colorectal cancer, approximately 15% of patients exhibit MSI. Patients with MSI-high (MSI-H) colorectal cancer usually exhibit an improved prognosis and less aggressive biological behaviour but a poor response to 5-FU-based chemotherapy [10-12]. One proposed hypothesis suggested that, perhaps, chemotherapeutic drugs can attenuate inherent anti-tumor immunity of MSI-H patients. Another theory also proved that it is related to "injury-excision futile cycle" [13-16]. Due to the lack of MMR proteins in MSI-H patients, the DNA adducts that are formed by chemotherapeutic drugs could not be recognized by MMR proteins, which could not lead to chemotherapy-induced cytotoxic injury-excision futile cycle. Therefore, MSI-H patients have lower responsivity to chemotherapy strategy. However, recent studies revealed that the correlation between MSI-H status and the efficacy of adjuvant chemotherapy is still controversial. MSI-H GC patients who received adjuvant chemotherapy displayed significantly better prognosis than those suffering from microsatellite stability (MSS) [17-19]. Therefore, MSI-H status can be applied as a useful predictor, so as to predict the efficacy of adjuvant chemotherapy in colorectal cancer, but a large cohort of further studies still need to be done for the assessment of the correlation between MSI-H status and adjuvant chemotherapy efficacy in GC. Although the MSI-H phenotype has been identified as a predictive and prognostic factor of colorectal cancer, the relationship between the MSI phenotype and the clinical and pathological characteristics of GC has not been elucidated. Recently, studies have shown that patients with MSI-H tumours often also exhibit a considerable burden of mutation and that MSI in the coding sequences of genes is very likely to cause the production of aberrant proteins (such as truncated proteins) that are exogenous, novel antigens that can lead to specific immune responses favourable to prognosis [20]. Furthermore, studies have revealed that MMR deficiency may be a beneficial predictor of the clinical therapeutic effects of immune checkpoint inhibitors (ICIs) as the MSI-H phenotype is positively correlated with the expression of programmed death-ligand 1 (PD-L1) [21, 22]. Programmed death-1 (PD-1)

and PD-L1 are negative immunoregulators. The combination of PD-1 on the surface of cytotoxic T lymphocytes (CTLs) and PD-L1 on the surface of tumor cells can inhibit the specific anti-tumor immune response, thus achieving the purpose of tumor cells immune escape. Therefore, the blockade of PD-1/PD-L1 pathway may trigger the activation of CTLs-mediated specific anti-tumor immune response again pharmacologically. Pembrolizumab is considered as anti-PD-1 monoclonal antibody, so, its efficacy in patients with non-colorectal MSI-H/deficient-MMR (dMMR) cancer was endorsed in Phase II KEYNOTE-158 trail, including 233 patients with 27 tumour types [23]. A total of 80 patients with non-colorectal MSI-H/dMMR cancer obtained clinical benefits from anti-PD-1 therapy with pembrolizumab. In this review, the evidence regarding the association between the MSI-H phenotype and GC is summarized, and special emphasis is placed on the molecular biological characteristics, clinical manifestations, prognosis, and effects of chemotherapy and ICIs observed in MSI GC.

Microsatellites and MSI

Microsatellites are also known as simple repeats or short tandem repeats, and their repetitive units are composed of 1-6-nucleotide DNA motifs [24]. Microsatellites are widely distributed in the human genome and account for approximately 3% of the total genome.

Since microsatellites are composed of repetitive DNA sequences, insertions or deletions of repeated units easily occur within microsatellites due to DNA polymerase slippage during DNA replication. In this case, if the MMR system is deficient, these aberrations are not identified and repaired, leading to the changes of microsatellites length called MSI phenotype [25].

Diagnostics of MSI

The polymerase chain reaction (PCR) amplification of microsatellite markers with specific primers has been widely employed as a mainstream method to evaluate MSI. In 1997, the American National Cancer Institute (NCI) recommended a panel of 5 microsatellite markers (BAT-25, BAT-26, D2S123, D5S346 and D17S250) to check MSI status [26]. By comparing the variation size in microsatellites between tumour and normal tissues, MSI can be evaluated as a “shift” with only one microsatellite or more microsatellites. However, in subsequent studies, researchers observed that dinucleotide markers (D5S346, D2S123 and D17S250) have been shown to be less sensitive and specific than mononucleotide markers in the detection of MMR deficiency [27]. Therefore, in 2002, the HNPCC seminar suggested that dinucleotide markers should be replaced by mononucleotide markers [28] and an ideal panel of 5 mononucleotide markers (BAT-25, BAT-26, NR-21, NR-24 and NR-27) was created for MSI detection [25]. Among these 5 MSI markers, if two or more microsatellite markers show instability, the mutations are defined as MSI-H; if only one microsatellite site shows a mutation, it is considered MSI-low (MSI-L); and MSS indicates no mutation site [26].

Immunohistochemistry (IHC) is widely used to detect the lack of MMR proteins (MLH1, MSH2, MSH6 and PMS2). These proteins are usually positively expressed in normal nuclei on IHC. The loss of protein expression indicates the occurrence of MMR deficiency, providing indirect evidence of MSI. In MSI detection, IHC and PCR show a high degree of consistency (≥90%) [29], and these methods are complementary.

Clinicopathological characteristics of MSI gastric cancer

In 2014, the genetic characteristics of GC were described in detail. According to genotype profiling, GC was classified into 4 subtypes, and the MSI type was an independent subtype [6]. MSI GC has been described as having a high rate of mutation in the gene coding region, involving signalling pathways associated with tumorigenesis, such as *PIK3CA*, *ERBB3* and *EGFR* genes, and *MLH1* gene promoter methylation leading to *MLH1* gene silencing [6, 30]. The MSI phenomenon is usually detected in colonic and endometrial cancer. This phenomenon has also been detected in GC, and the clinicopathological characteristics of MSI GC have been reported [31-34]. A recent meta-analysis of MSI GC was reported. This meta-analysis systematically investigated the relationship between MSI and the clinicopathological characteristics and OS of GC patients [34]. This meta-analysis included 48 studies, involving a total of 18612 gastric cancer patients. Of all patients, 9.2% showed MSI (1718 of 18612).

In this cohort of patients, more women showed MSI than men. A clear relationship exists between the status of MSI and an age of 65 years or older. The risk of MSI in the intestinal-type is greater than that in fuse/mixed-type GC. A significant relationship exists between the status of MSI and the position of the middle/low stomach. A significant relationship exists between MSI and the absence of lymph node metastasis. A clear relationship exists between MSI and TNM stage I and/or II at diagnosis. According to this meta-analysis, the pooled hazard ratio (HR) of the OS of patients with MSI versus those with non-MSI GC was 0.69 ($P < 0.001$). MSI GC patients are associated with better OS. *KRAS* mutations are important for determine the efficiency of EGFR-targeted antibodies in metastatic colorectal cancer [35]. *KRAS* mutations have important effects on cell proliferation and inhibition of apoptosis due to dysregulation of the MAPK signalling pathway. Therefore, Karol Pollom et al. analysed the role of *KRAS* mutations in MSI GC based on 595 GC patients. These researchers found a total of 24 patients with *KRAS* mutations, including 18 patients with MSI and 6 patients with MSS. The MSI patients with *KRAS* mutations were older, most of these patients were female, and these patients had a better prognosis. In contrast, the MSS patients with *KRAS* mutations showed a more advanced TNM stage, and these patients had a worse prognosis and results following treatment [36]. The effect of the status of MSI on OS was also assessed. The median OS of the MSS was 10 months, while the median OS of the MSI patients was 108 months ($P < 0.001$) [36]. Many studies have shown that a positive correlation exists between the histological intestinal type and the MSI phenotype, while diffuse and mixed histology GC are rarely associated with MSI GC, further demonstrating that the MSI phenotype is usually associated with a better prognosis [34]. In sporadic MSI colorectal cancer, the *BRAF* V600E mutation due to MSI is frequently reported, but the *BRAF* mutation has never been reported in MSI GC [30]. Furthermore, it is well-known that the hypermethylation of the *MLH1* gene promoter region caused by H. *pylori* infection is closely related to MMR deficiency in GC, resulting in the status of MSI-H [37]. MSI-H tumours often have a high burden of mutation. Compared with MSS tumours, MSI-H tumours have the potential to encode novel, non-self antigens, subsequently attracting more lymphocytes to accumulate in the tumour, thereby inducing a strong immune response (**Figure 1**).

However, interferon γ released by CTLs can further induce tumour cells and immune cells to express PD-L1 [38]; therefore, the combination of PD-1 and PD-L1 can inhibit the immune response mediated by CTLs (**Figure 2**).

Tadayoshi Hashimoto et al. detected the expression of MLH1 and PD-L1 in 285 patients. These researchers observed that 85.7% of the 28 patients with MLH1-negative GC showed an MSI-H phenotype, but these patients showed a higher expression of PD-L1 than the MLH1-positive tumour patients [39], which can achieve immune escape. The immune evaluation of the microenvironment in dMMR tumours showed the intensive aggregation of tumour infiltrating lymphocytes and the extensive expression of immune checkpoint molecules, such as PD-L1, LAG-3, IDO, and CTLA4 [40, 41].

Response of MSI GC to chemotherapy

Numerous studies have been conducted to define the prognostic role of MSI-H. Tadayoshi Hashimoto et al. found that 85.7% of MLH1 negative patients showed an MSI-H phenotype and that these patients were significantly less likely to respond to pre-operative chemotherapy than patients with MLH1-positive tumours. These researchers hypothesized that ICIs might be more suitable for patients with MLH1-negative tumours [39]. JY et al. analysed 1990 patients with GC and found that in MSS/MSI-L patients with stage II or III GC, adjuvant chemotherapy based on 5-FU showed better disease-free survival. However, this study did not show any benefit among patients with MSI-H GC. The status of MSI in GC appears to be a possible guideline for the 5-FU-based chemotherapy drugs in stage II or III GC [42]. Similarly, in 2015, Kim et al. [43] observed a similar result as follows: the prognosis of stage II MSI-H GC patients was significantly worse after they received chemotherapy. Therefore, these researchers believed that the MSI-H phenotype is related to good prognosis, but the prognostic value of MSI-H can be attenuated by chemotherapy. To determine the correlation between MSI and survival among patients receiving chemotherapy, a Medical Research Council Adjuvant Gastric Infusional Chemo-

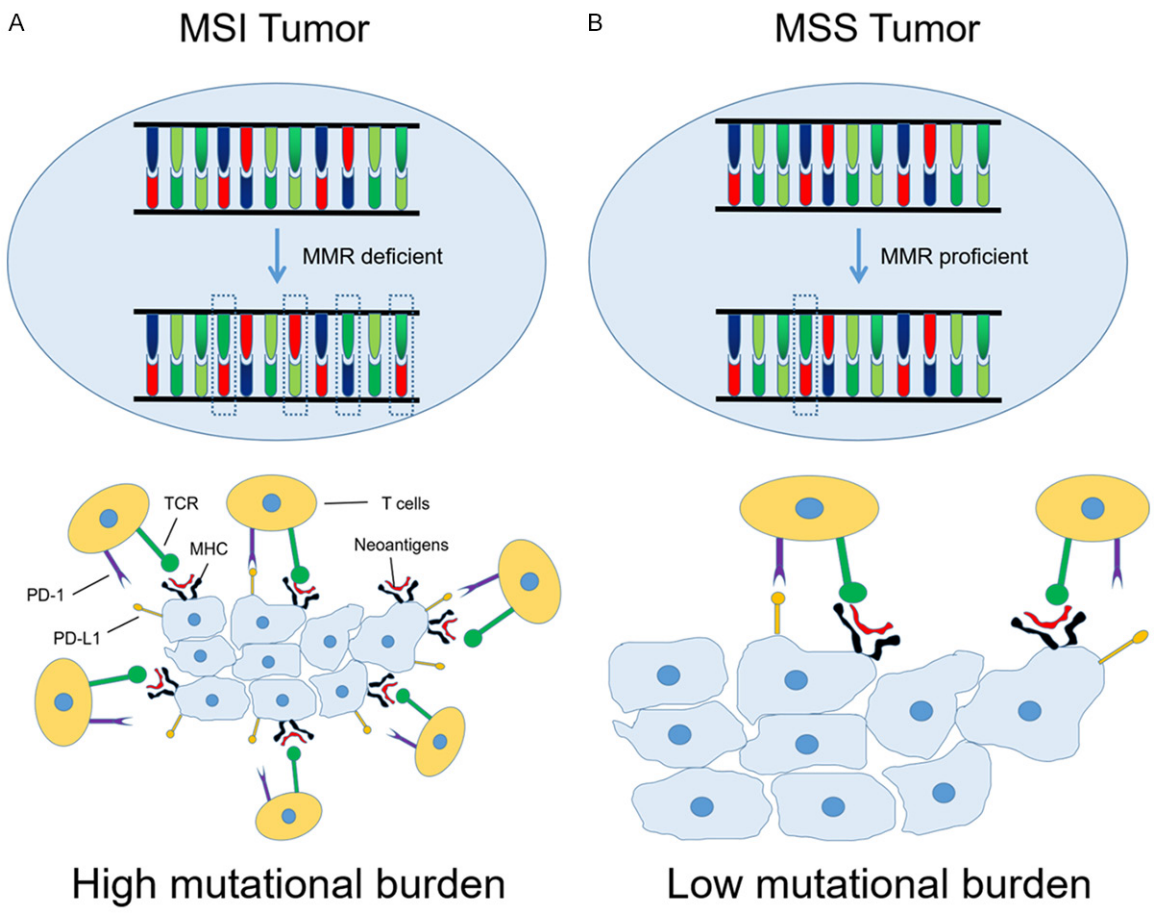

Figure 1. Immune microenvironment of MSI and MSS tumours. A. MSI tumours have a high mutation burden with a large number of T cells infiltrating the tumour tissue; B. MSS tumours often show a low mutation burden rarely with tumour infiltrating lymphocytes in the tumour tissue.

therapy (MAGIC) trial was conducted [44]. In this study, the total survival time of MSI-H or dMMR patients who only underwent surgery was dramatically longer than that of MSS or MSI-L patients. However, the overall median survival time of MSI-H or dMMR patients receiving surgery plus chemotherapy was obviously lower than that of MSS or MSI-L patients (9.6 *vs* 19.5 months). These researchers believed that the MSI-H status was related to a positive prognostic outcome in patients undergoing surgical operation alone and that the determination of MSI-H or dMMR could be applied to screen patients for perioperative chemotherapy. Besides MSI-H status, the prognostic role of MSI-L status was also explored in GC. In 2020, Dan Jiang et al. analyzed the prognosis of 96 GC patients with MSI status, including 12 MSI-L patients. The result showed that 83.3% MSI-L GC patients were assessed as poor response to neoadjuvant chemotherapy. Compared with MSS tumour patients, MSI-L patients presented poor disease-free survival (DFS) (P=0.018) with a HR of 2.839 [45]. In a cell experiment, the ability of MMR proteins to induce cell death after binding to 5-FU was indicated to be 30 times higher than that of MMR deficiency [46].

One proposed hypothesis suggests that MSI-H GC shows a negative therapeutic response to chemotherapy due to the negative effect of chemotherapy on the immune system, which can reduce the inherent positive effect of the MSI-H phenotype on prognosis. The detailed molecular mechanism of this effect has also been explored. Due to the formation of DNA adducts induced by chemotherapeutic drugs, various type of DNA damage, such as base mismatch, intra-strand cross-linking or inter-strand cross-linking, can be induced. Then, MMR protein can recognize such DNA damage and lead to iterative rounds of MMR known as futile

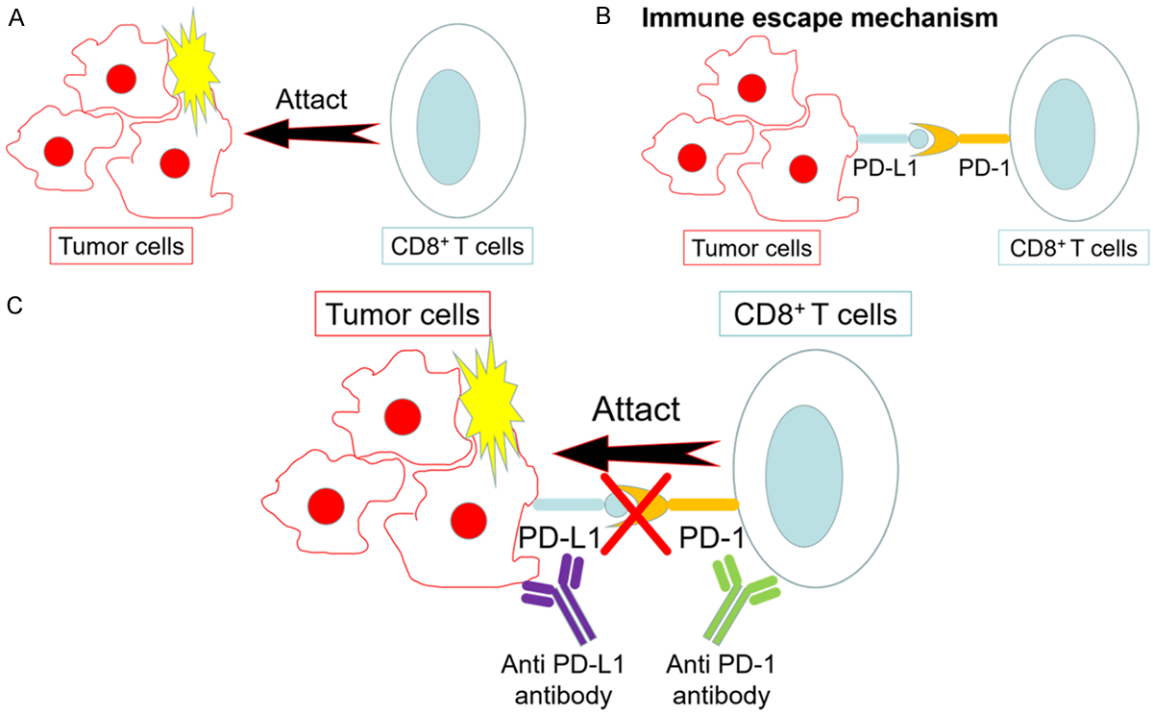

Figure 2. Mechanism of tumour immune escape and targeted therapy with immune checkpoint inhibitors against MSI. A. CD8-positive T cells attack tumour cells; B. When PD-L1 on the surface of tumour cells combines with PD-1 on the surface of CD8-positive T cells, the immune escape mechanism is activated; C. Immune checkpoint inhibitor (the anti-PD-1/L1 antibody) blocks the binding of PD-1 and PD-L1, thus activating the specific immune response mediated by CTLs.

cycling, apoptosis, cell cycle arrest, and autophagy through different signalling pathways [13-16]. Therefore, in the clinic, the use of chemotherapy for GC should receive sufficient attention. Before the formulation of chemotherapy strategies, the clinical characteristics and molecular phenotypes of patients should be evaluated in detail, and individualized treatment strategies should be more widely applied in clinical practice.

However, recently, with the increase of depth and breadth of MSI GC researches, it was found that using MSI status to predict the response of MSI GC to chemotherapy is disputable. In 2019, a large-scale meta-analysis, including 1174 GC patients (84 MSI-H and 1090 MSS/MSI-L patients) was performed [17]. No correlation exists between MSI status and the efficacy of adjuvant chemotherapy for GC. Similarly, the predictive value of MSI-H for adjuvant chemotherapy in large groups of GC patients was assessed by Jin won Kim in 2020 [18]. In an analysis consisting of 162 MSI-H patients, adjuvant chemotherapy exhibited a significant improvement on DFS and OS (P=0.047 and P=0.043, respectively). In 2019, Georg Martin Haag et al. obtained a similar result that MSI-H GC patients undergoing adjuvant chemotherapy illustrated a significantly better OS compared with MSS tumors (P=0.014), indicating that the MSI-H status is a favorable prognostic indicator in GC patients experiencing neoadjuvant treatment [19].

Therefore, MSI status can effectively predict the efficacy of chemotherapy in colorectal cancer patients, but the correlation between them in GC is still debatable. More prospective studies are necessary to investigate the predictive role of MSI status for chemotherapy response in GC.

Response of MSI GC to immunotherapy

ICIs, such as anti-PD-1 monoclonal antibodies (e.g., nivolumab and pembrolizumab) and anti-CTLA-4 monoclonal antibodies (ipilimumab), have been widely used in the targeted treatment of cancer. As previously mentioned,

because of the high mutation burden in MSI-H tumours, the mismatch, deletion and insertion of bases easily occur in the coding regions of genes, which may result in the expression of abnormal proteins. These abnormal endogenous proteins (peptides) are presented on the cell surface by major histocompatibility complex (MHC) I, and CTLs are recruited and infiltrate tumour tissue to recognize and kill tumour cells. However, interferon γ released by CTLs can induce tumour cells to express PD-L1. PD-1 and PD-L1 are negative immunoregulation factors. The combination of PD-L1 on the surface of tumour cells and PD-1 on the surface of CTLs can inhibit the specific antitumor immune response of CTLs to achieve immune escape of tumour cells.

With the help of ICIs, the immune response can be activated again, and the tumour can subsequently be treated based on the fact that the PD-L1/PD-1 pathway can be blocked pharmacologically. ICIs have achieved exciting clinical outcomes in advanced melanoma and colorectal cancer [21]. Relevant studies have also been performed in GC. In 2016, the KEYNOTE-012 trial was established to assess the safety and activity of the PD-1 antibody pembrolizumab against recurrent or metastatic gastric or gastroesophageal junction adenocarcinoma positive for PD-L1. Among 39 patients, 8 patients (22%) were determined to have an overall response [22]. In the Keynote-059 trial, 295 patients with GC or advanced gastroesophageal junction adenocarcinoma were treated with pembrolizumab. Among these patients, 57% were determined to be positive for PD-L1. The objective response rate and median (range) response duration were both superior to those of patients with PD-L1-negative tumours. Regarding MSI-H, the objective response rate (57%) is more obvious. Therefore, pembrolizumab was authorized by the FDA for the therapy of GC positive for PD-L1 with pre-treatment metastasis in 2017 [47]. Nivolumab, which is another PD-1 fully human IgG4 monoclonal antibody inhibitor of PD-1, has also been evaluated in patients with advanced GC or gastro-oesophageal junction cancer [48]. Compared with the placebo group, the median OS period and 12-month OS rates were both increased in the nivolumab group. Moreover, nivolumab was helpful in prolonging the median OS of the patients regardless of the positive or negative expression of PD-L1 [48]. Likewise, the efficacy and safety of ICIs were also investigated in advanced GC or gastroesophageal junction cancer by Cong Chen [49]. Briefly, MSI-H patients exhibited higher objective response rate and disease control rate than MSS patients. Meanwhile, anti-PD-1/PD-L1 therapeutic strategy was more effective for patients with EBV+, MSI-H, PD-L1+ or high tumor mutational burden. In metastatic GC patients, comprehensive molecular characteristics of clinical responses to PD-1 inhibitors were depicted [50]. Remarkable response to pembrolizumab was observed in MSI-H patients, (overall response rate 85.7%), which was consistent with above outcomes. The combination of nivolumab and ipilimumab (anti-CTLA-4 antibody) has also been applied in the checkmate 032 study, and this combination showed the increased benefits for patients positive for PD-L1 [51]. However, the efficacy of ICIs in MSI-H GC may not be as good as that reported in colorectal cancer due to the small sample size. In the future, research investigating the efficacy of ICIs in MSI-H GC is warranted.

Future prospects

MSI-H/dMMR status may not be the only indicator of the application of ICIs or may not be an accurate indicator. Compared with MSI-H/dMMR or the level of PD-1/PD-L1 expression, tumor mutation burden (TMB) is emerging as a more accurate, comprehensive, powerful and potential biomarker for the prediction of the efficacy of ICIs, because there is an inconsistency between MSI-H/dMMR status and TMB. Most patients with MSI-H/dMMR had high TMB levels. However, not all patients with high TMB levels presented MSI-H/dMMR or high PD-1/PD-L1 expression [52]. Therefore, the detection of TMB levels would be more required indicator for immunotherapy application. In term of MSI detection methodology, PCR is still a mainstream technology. But recently, next generation sequencing (NGS)-based comprehensive detection technology appeared in many laboratories for the diagnosis of cancers and the establishment of therapeutic regimens. As a potential detection type, NGS can not only evaluate MSI status, but also determine TMB, so, it is thought to be a larger advantage. Otherwise, PD-1/PD-L1 expression, the number of tumour

infiltrating lymphocytes, and other immune checkpoint molecules expression such as PD-L2 and CTLA-4 also need to be paid enough attention. The combination of different types of ICIs may be more effective than a single ICI. The combination of immunotherapy plus chemotherapy as the first-line treatment may provide promising treatment results for advanced GC with PD-L1 positive and MSI-H [53]. In the future, comprehensive and thorough analysis of MSI-H/dMMR status, TMB, PD-1/PD-L1/PD-L2/CTLA4 expression and the number of tumour infiltrating lymphocytes would be more helpful, important and accurate for the prediction of response to immunotherapy for patients. All these lay the foundation for the development of individualized precise treatment strategy.

Conclusions

Microsatellites, also known as short tandem repeats, are widely distributed in the genome. In the process of DNA replication, insertions and deletions of bases are common. In the presence of MMR protein, these replication errors can be recognized and corrected. However, the MMR protein loses its function of recognizing and correcting replication errors if it is defective, which leads to MSI. Moreover, MSI occuring in regions for gene coding regions often leads to the production of abnormal novel antigens. GC is among the most aggressive malignant tumours with high metastatic potential. According to the Cancer Genome Atlas research network, GC is divided into four subtypes, including MSI GC. MSI GC has a unique immune microenvironment and therapeutic response that is mainly related to old age, the female sex, a distal gastric location and the histological intestinal type. However, for MSI GC, chemotherapy often leads to adverse actions, which may be observed because chemotherapy drugs can attenuate the inherent antitumor immune response of MSI GC. Because MSI tumours often have more immune checkpoint molecules (e.g., PD-1, PD-L1 and PD-L2), these results suggest that immunotherapy based on ICIs may be an effective treatment strategy for MSI GC. Therefore, determining the status of MSI may help the development of individualized and tailored treatment strategies for GC patients.

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Disclosure of conflict of interest

None.

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References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136: E359-86.

[2] Ratti M, Lampis A, Hahne JC, Passalacqua R and Valeri N. Microsatellite instability in gastric cancer: molecular bases, clinical perspectives, and new treatment approaches. Cell Mol Life Sci 2018; 75: 4151-4162.

[3] Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J and Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376: 687-697.

[4] Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, Dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD and Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet 2014; 383: 31-39.

[5] Wilke H, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov

O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD and Ohtsu A. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. Lancet Oncol 2014; 15: 1224-1235.

[6] Cancer Genome Atlas Research Network. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014; 513: 202-209.

[7] Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S and Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. Nat Med 2015; 21: 449-456.

[8] Yamamoto H, Adachi Y, Taniguchi H, Kunimoto H, Nosho K, Suzuki H and Shinomura Y. Interrelationship between microsatellite instability and microRNA in gastrointestinal cancer. World J Gastroenterol 2012; 18: 2745-2755.

[9] Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, Seruca R, Iacopetta B and Hamelin R. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology 2002; 123: 1804-1811.

[10] Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M and Gallinger S. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003; 349: 247-257.

[11] Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat B, Foster NR, Torri V, Ribic C, Grothey A, Moore M, Zaniboni A, Seitz JF, Sinicrope F and Gallinger S. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 2010; 28: 3219-3226.

[12] Yang G, Zheng RY and Jin ZS. Correlations between microsatellite instability and the biological behaviour of tumours. J Cancer Res Clin Oncol 2019; 145: 2891-2899.

[13] Zeng X and Kinsella TJ. A novel role for DNA mismatch repair and the autophagic processing of chemotherapy drugs in human tumor cells. Autophagy 2007; 3: 368-370.

[14] Gupta D, Lin B, Cowan A and Heinen CD. ATR-Chk1 activation mitigates replication stress caused by mismatch repair-dependent processing of DNA damage. Proc Natl Acad Sci U S A 2018; 115: 1523-1528.

[15] Li Z, Pearlman AH and Hsieh P. DNA mismatch repair and the DNA damage response. DNA Repair (Amst) 2016; 38: 94-101.

[16] Fu D, Calvo JA and Samson LD. Balancing repair and tolerance of DNA damage caused by alkylating agents. Nat Rev Cancer 2012; 12: 104-120.

[17] Zhao F, Yuan X, Ren D, Shen G, Wang Z, Zheng F, Ahmad R, Ma Z and Zhao J. Predicting the efficacy of 5-fluorouracil-based adjuvant chemotherapy in gastric cancer by microsatellite instability: a meta-analysis. J Environ Pathol Toxicol Oncol 2019; 38: 21-28.

[18] Kim JW, Cho SY, Chae J, Kim JW, Kim TY, Lee KW, Oh DY, Bang YJ and Im SA. Adjuvant chemotherapy in microsatellite instability-high gastric cancer. Cancer Res Treat 2020; 52: 1178-1187.

[19] Haag GM, Czink E, Ahadova A, Schmidt T, Sisic L, Blank S, Heger U, Apostolidis L, Berger AK, Springfeld C, Lasitschka F, Jäger D, von Knebel Doeberitz M and Kloor M. Prognostic significance of microsatellite-instability in gastric and gastroesophageal junction cancer patients undergoing neoadjuvant chemotherapy. Int J Cancer 2019; 144: 1697-1703.

[20] Kloor M and von Knebel Doeberitz M. The immune biology of microsatellite-unstable cancer. Trends Cancer 2016; 2: 121-133.

[21] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B and Diaz LA Jr. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015; 372: 2509-2520.

[22] Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, McRee AJ, Lin CC, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M and Bang YJ. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. Lancet Oncol 2016; 17: 717-726.

[23] Marabelle A, Le DT, Ascierto PA, Di Giacomo AM, De Jesus-Acosta A, Delord JP, Geva R, Gottfried M, Penel N, Hansen AR, Piha-Paul SA, Doi T, Gao B, Chung HC, Lopez-Martin J, Bang YJ, Frommer RS, Shah M, Ghori R, Joe AK,

Pruitt SK and Diaz LA Jr. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol 2020; 38: 1-10.

[24] Srivastava S, Avvaru AK, Sowpati DT and Mishra RK. Patterns of microsatellite distribution across eukaryotic genomes. BMC Genomics 2019; 20: 153.

[25] Campanella NC, Berardinelli GN, Scapulatempo-Neto C, Viana D, Palmero EI, Pereira R and Reis RM. Optimization of a pentaplex panel for MSI analysis without control DNA in a Brazilian population: correlation with ancestry markers. Eur J Hum Genet 2014; 22: 875-880.

[26] Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN and Srivastava S. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998; 58: 5248-5257.

[27] Deschoolmeester V, Baay M, Wuyts W, Van Marck E, Van Damme N, Vermeulen P, Lukaszuk K, Lardon F and Vermorken JB. Detection of microsatellite instability in colorectal cancer using an alternative multiplex assay of quasi-monomorphic mononucleotide markers. J Mol Diagn 2008; 10: 154-159.

[28] Murphy KM, Zhang S, Geiger T, Hafez MJ, Bacher J, Berg KD and Eshleman JR. Comparison of the microsatellite instability analysis system and the Bethesda panel for the determination of microsatellite instability in colorectal cancers. J Mol Diagn 2006; 8: 305-311.

[29] Funkhouser WK Jr, Lubin IM, Monzon FA, Zehnbauer BA, Evans JP, Ogino S and Nowak JA. Relevance, pathogenesis, and testing algorithm for mismatch repair-defective colorectal carcinomas: a report of the association for molecular pathology. J Mol Diagn 2012; 14: 91-103.

[30] Normanno N, Rachiglio AM, Lambiase M, Martinelli E, Fenizia F, Esposito C, Roma C, Troiani T, Rizzi D, Tatangelo F, Botti G, Maiello E, Colucci G and Ciardiello F. Heterogeneity of KRAS, NRAS, BRAF and PIK3CA mutations in metastatic colorectal cancer and potential effects on therapy in the CAPRI GOIM trial. Ann Oncol 2015; 26: 1710-1714.

[31] Beghelli S, de Manzoni G, Barbi S, Tomezzoli A, Roviello F, Di Gregorio C, Vindigni C, Bortesi L, Parisi A, Saragoni L, Scarpa A and Moore PS. Microsatellite instability in gastric cancer is associated with better prognosis in only stage II cancers. Surgery 2006; 139: 347-356.

[32] Corso G, Pedrazzani C, Marrelli D, Pascale V, Pinto E and Roviello F. Correlation of microsatellite instability at multiple loci with long-term survival in advanced gastric carcinoma. Arch Surg 2009; 144: 722-727.

[33] Marrelli D, Polom K, Pascale V, Vindigni C, Piagnerelli R, De Franco L, Ferrara F, Roviello G, Garosi L, Petrioli R and Roviello F. Strong prognostic value of microsatellite instability in intestinal type non-cardia gastric cancer. Ann Surg Oncol 2016; 23: 943-950.

[34] Polom K, Marano L, Marrelli D, De Luca R, Roviello G, Savelli V, Tan P and Roviello F. Meta-analysis of microsatellite instability in relation to clinicopathological characteristics and overall survival in gastric cancer. Br J Surg 2018; 105: 159-167.

[35] Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, Valtorta E, Schiavo R, Buscarino M, Siravegna G, Bencardino K, Cercek A, Chen CT, Veronese S, Zanon C, Sartore-Bianchi A, Gambacorta M, Gallicchio M, Vakiani E, Boscaro V, Medico E, Weiser M, Siena S, Di Nicolantonio F, Solit D and Bardelli A. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012; 486: 532-536.

[36] Polom K, Das K, Marrelli D, Roviello G, Pascale V, Voglino C, Rho H, Tan P and Roviello F. KRAS mutation in gastric cancer and prognostication associated with microsatellite instability status. Pathol Oncol Res 2019; 25: 333-340.

[37] Leung SY, Yuen ST, Chung LP, Chu KM, Chan AS and Ho JC. hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. Cancer Res 1999; 59: 159-164.

[38] Cho J, Lee J, Bang H, Kim ST, Park SH, An JY, Choi MG, Lee JH, Sohn TS, Bae JM, Kang WK, Kim S and Kim KM. Programmed cell death-ligand 1 expression predicts survival in patients with gastric carcinoma with microsatellite instability. Oncotarget 2017; 8: 13320-13328.

[39] Hashimoto T, Kurokawa Y, Takahashi T, Miyazaki Y, Tanaka K, Makino T, Yamasaki M, Nakajima K, Ikeda JI, Mori M and Doki Y. Predictive value of MLH1 and PD-L1 expression for prognosis and response to preoperative chemotherapy in gastric cancer. Gastric Cancer 2019; 22: 785-792.

[40] Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM and Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015; 5: 43-51.

[41] Ma C, Patel K, Singhi AD, Ren B, Zhu B, Shaikh F and Sun W. Programmed death-ligand 1 expression is common in gastric cancer associated with epstein-barr virus or microsatellite instability. Am J Surg Pathol 2016; 40: 1496-1506.

[42] An JY, Kim H, Cheong JH, Hyung WJ, Kim H and Noh SH. Microsatellite instability in sporadic gastric cancer: its prognostic role and guidance for 5-FU based chemotherapy after R0 resection. Int J Cancer 2012; 131: 505-511.

[43] Kim SY, Choi YY, An JY, Shin HB, Jo A, Choi H, Seo SH, Bang HJ, Cheong JH, Hyung WJ and Noh SH. The benefit of microsatellite instability is attenuated by chemotherapy in stage II and stage III gastric cancer: results from a large cohort with subgroup analyses. Int J Cancer 2015; 137: 819-825.

[44] Smyth EC, Wotherspoon A, Peckitt C, Gonzalez D, Hulkki-Wilson S, Eltahir Z, Fassan M, Rugge M, Valeri N, Okines A, Hewish M, Allum W, Stenning S, Nankivell M, Langley R and Cunningham D. Mismatch repair deficiency, microsatellite instability, and survival: an exploratory analysis of the medical research council adjuvant gastric infusional chemotherapy (MAGIC) trial. JAMA Oncol 2017; 3: 1197-1203.

[45] Jiang D, Shu C, Zhang W, Sun L, Zhang M, He Y, Owen G, Jin W, He D, Deng X and Liu X. Low level of microsatellite instability correlates with short disease-free survival of gastric cancer patients undergoing neoadjuvant chemotherapy. Virchows Arch 2020; [Epub ahead of print].

[46] Carethers JM, Chauhan DP, Fink D, Nebel S, Bresalier RS, Howell SB and Boland CR. Mismatch repair proficiency and in vitro response to 5-fluorouracil. Gastroenterology 1999; 117: 123-131.

[47] Fashoyin-Aje L, Donoghue M, Chen H, He K, Veeraraghavan J, Goldberg KB, Keegan P, McKee AE and Pazdur R. FDA approval summary: pembrolizumab for recurrent locally advanced or metastatic gastric or gastroesophageal junction adenocarcinoma expressing PD-L1. Oncologist 2019; 24: 103-109.

[48] Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK, Yeh KH, Yoshikawa T, Oh SC, Bai LY, Tamura T, Lee KW, Hamamoto Y, Kim JG, Chin K, Oh DY, Minashi K, Cho JY, Tsuda M and Chen LT. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer re fractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017; 390: 2461-2471.

[49] Chen C, Zhang F, Zhou N, Gu YM, Zhang YT, He YD, Wang L, Yang LX, Zhao Y and Li YM. Efficacy and safety of immune checkpoint inhibitors in advanced gastric or gastroesophageal junction cancer: a systematic review and meta-analysis. Oncoimmunology 2019; 8: e1581547.

[50] Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, Liu XQ, Sher X, Jung H, Lee M, Lee S, Park SH, Park JO, Park YS, Lim HY, Lee H, Choi M, Talasaz A, Kang PS, Cheng J, Loboda A, Lee J and Kang WK. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. Nat Med 2018; 24: 1449-1458.

[51] Janjigian YY, Bendell JC, Calvo E, Kim JW, Ascierto PA, Sharma P, Ott PA, Bono P, Jaeger D, Evans TRJ. CheckMate-032: phase I/II, open-label study of safety and activity of nivolumab (nivo) alone or with ipilimumab (ipi) in advanced and metastatic (A/M) gastric cancer (GC). J Clin Oncol 2016; 34: 4010.

[52] Zhao P, Li L, Jiang X and Li Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. J Hematol Oncol 2019; 12: 54.

[53] Jin H, Li P, Mao C, Zhu K, Chen H, Gao Y and Yu J. Pathological complete response after a single dose of anti-PD-1 therapy in combination with chemotherapy as a first-line setting in an unresectable locally advanced gastric cancer with PD-L1 positive and microsatellite instability. Onco Targets Ther 2020; 13: 1751-1756.