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New use of microsatellite instability analysis in endometrial cancer (Review)

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Abstract. The increasing incidence of obesity and diabetes due to changes in diet, earlier menarche, delayed menopause, late marriage, and declining birth rate have resulted in an increase in the number of endometrial cancer cases over the last few decades. Although surgical therapy is sufficient for early endometrial cancer, there is no effective therapy for patients with advanced and recurrent endometrial cancer. The oncogenic mechanism of endometrial cancer involves microsatellite instability (MSI) caused by dysfunction of DNA mismatch repair genes in 30% of patients. Immune checkpoint inhibitors, including anti-programmed death (PD)-1 and anti-PD-ligand 1 antibodies, are of interest as novel anticancer drugs; however, these drugs are currently expensive, and there is a need to select patients who will benefit from their use. The use of MSI analysis as a predictive biomarker for the therapeutic efficacy of these drugs may be useful for reducing the costs of drug therapy.

Contents

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1. Introduction

Microsatellites are repeat sequences of one to several DNA bases. These sequences are used for forensic identification and paternity testing because they are polymorphic, occurring widely in both coding and non-coding regions. Repeat errors during DNA replication are likely to occur in these regions and are usually repaired by DNA mismatch repair (MMR) genes. In neoplastic lesions that develop due to aberration of this mechanism, the microsatellite repeat number in tumor tissues differs from that in normal tissues (1). This phenomenon is called microsatellite instability (MSI) and is closely related to carcinogenicity of hereditary tumors, including Lynch syndrome and others (Fig. 1). MSI analysis is currently performed as secondary screening for patients suspected for Lynch syndrome.

MMR function is lost in 20-30% of patients with endometrial cancer (2,3). Lynch syndrome accounts for approximately 25% of these cases, and the majority involve hypermethylation of *MLH1* promoter or somatic mutations of MMR genes (4). A recent study showed that MSI analysis is effective as a predictive biomarker for the effect of immune checkpoint inhibitors, which are new anticancer drugs, including anti-PD-1 antibody and anti-PD-L1 antibody (5). This suggests that MSI analysis may be useful as a biomarker for the effect of immunotherapy for endometrial cancer. In this article, the utility of MSI analysis in patients with endometrial cancer and new testing procedures are discussed.

2. Classification of endometrial cancer by genetic alterations and MSI

Bokhman classified endometrial cancer into type 1 and 2 (6). Type 1 is characterized by relatively young onset, well-differentiated tumor with high expression of estrogen receptor (ER), and good prognosis. Type 2 is typically elderly-onset, ER-negative poorly differentiated cancer with a poor prognosis. Histologically, endometrioid adenocarcinoma has the highest incidence, followed by serous adenocarcinoma and clear cell adenocarcinoma. Type 1 cases are mostly well-differentiated endometroid adenocarcinoma, and Type 2 often involves other histological types (7,8). *PTEN*, *KRAS*, *CTNNB1* and *PI3KCA* mutations are frequently found in type 1 cases, whereas

HER2 and *TP53* mutations occur in type 2 (7-10). Although there are certain tendencies for mutated genes (11-13), the Bokhman classification is limited by its difficulty in classification of endometrial cancer associated with MSI and Lynch syndrome (2,3).

Using exome sequencing, The Cancer Genome Atlas Research Network categorized endometrial cancer into 4 types based on gene mutation pattern and frequency, copy number variation, and MSI status (13). These four types are referred to as POLE ultramutated, MSI hypermutated, copy-number low and copy-number high (Table I), and the incidences are 7.3, 28.0, 38.8 and 25.9%, respectively. All tumors categorized in the POLE ultramutated group carry mutations in the exonuclease domain of *POLE*, and possessed the highest incidence of other gene mutations such as *PTEN*, *PIK3R1* and *PIK3CA*. The copy-number low and high groups both have the lowest gene mutation rate and are categorized into two groups based on the existence of somatic copy number alterations. Distinct from these other types, the MSI type showed hypermethylation, which were mostly found in the *MLH1* promoter region, and has the second highest incidence of gene mutation following the POLE ultramutation type. MSI-type endometrial cancer is histologically characterized by lymphocyte invasion and immunogenicity (2). Because *MLH1* promoter methylation is a somatic event which leads to sporadic endometrial cancer (14), the effectiveness of immunotherapy should be determined not only in Lynch syndrome-related endometrial cancers, but also in sporadic cases classified in the MSI hypermethylated group.

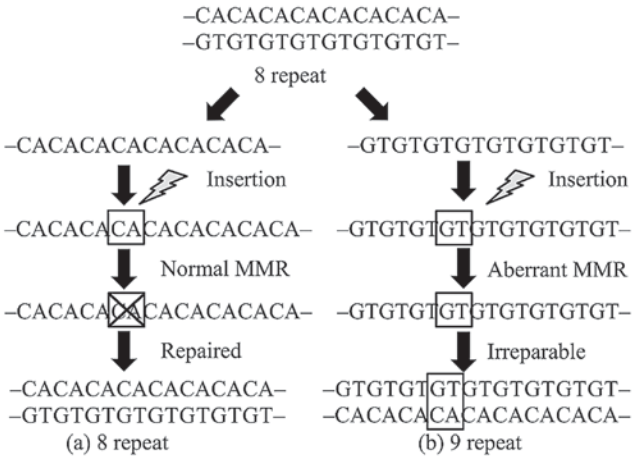

Figure 1. MSI is caused by aberrant MMR genes. (a) Even if insertion occurs during DNA replication, the microsatellite repeat number is repaired by the MMR mechanism and generally returns to the original value. (b) If the MMR mechanism is dysfunctional due to MMR gene aberrations, the insertion is not repaired and the repeat number increases. These variations of microsatellite repeat numbers that are not repaired due to replication errors caused by MMR gene aberrations are referred to as MSI. MSI, microsatellite instability; MMR, mismatch repair.

3. MSI analysis as a predictive biomarker for the efficacy of immune checkpoint inhibitors

Cancer cells have two mechanisms to avoid the host immune response; the first involving the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathway, and the second linked with programmed cell death-1 (PD-1) and PD ligand (PD-L1) (15). Activated T cells express PD-1, and its interaction with PD-L1 decreases T cell activity (16,17). Physiologically, PD-L1 is expressed in organs related to immune tolerance, including the tonsils, lungs and placental syncytiotrophoblasts (18,19). Expression of PD-L1 on the surface of tumor cells causes the tumor to avoid host T-cell activity (20). Therefore, blocking of the PD-1 interaction with PD-L1 in such cancers is likely to enhance the host immune response and have an antitumor effect (Fig. 2). This has been shown in malignant melanoma and non-small-cell cancer, and an effect on ovarian cancer has been found in gynecological diseases (20,21).

Le *et al* conducted a phase 2 study using an anti-PD-1 antibody, pembrolizumab, given every two weeks at 10 mg/kg in 11 patients with colon cancer associated with MMR deficiency (group A), 21 patients with colon cancer without MMR aberration (group B), and 9 non-colorectal cancer patients with MMR deficiency (group C) (5). The objective response rate (ORR) and 20-week progression-free survival (PFS) were 40 and 78% in group A, 0 and 11% in group B, and 71 and 67% in group C. Median PFS and overall survival (OS) could not be examined in group A, but were 2.2 and 5.0 months, respectively, in group B. Compared to group B, the patients in group A had significantly lower hazard ratios of 0.10 (P<0.001) for disease progression and 0.22 (P=0.05) for death. Exome sequencing showed that group B (wild-type MMR function) had significantly fewer somatic mutations than groups A and C (MMR deficient) (73 vs. 1778, P=0.007).

Although confirmation by phase 3 trials needs to be awaited, the results above suggest that anti-PD-1 antibody may be a new therapeutic candidate in cancer patients with aberrant MMR genes. Howitt *et al* found that MSI-type endometrial cancer had 7-fold higher neoepitope levels in comparison with microsatellite-stable (MSS) cancer (22). In POLE- and MSI-type cancers, the number of CD3- and CD8-positive cells invading cancer tissues was significantly higher than that in MSS-type cancer (P=0.001 and P<0.001, respectively), with no significant differences between POLE- and MSI-type cancers (P=0.86, P=0.29) (22). Since the incidence of somatic mutation is high in tumors associated with MSI, it is suggested that proteins with new immunogenicity are produced in these tumors, leading to excessive T cell infiltration (23-25).

Expression of PD-L1 in tumors is not necessarily a precise marker to estimate the therapeutic effect of PD-1/PD-L1 checkpoint blockade (26), and the creation of a new strategy is imperative. MSI analysis may be a candidate predictive biomarker for the effect of immunotherapy, including immune checkpoint inhibitors.

4. New modalities of MSI analysis and perspectives

The Bethesda panel is the conventional approach for MSI analysis, which is optimized for the secondary screening of Lynch syndrome (27). This method uses a PCR assay at 5 microsatellites in total, consisting 3 dinucleotide repeats (D2S123, D5S346, D17S250) and 2 mononucleotide repeats (BAT26, BAT25), and determines differences in repeat number between tumor and non-tumor regions. Cases with ≥2, 1 and 0 positive markers are classified as MSI-high (MSI-H), MSI-low (MSI-L), and microsatellite stable (MSS), respectively. In the Bethesda panel, dinucleotide repeats

Table I. Classification and characteristics of endometrial cancer [modified from (13)].

	POLE (ultramutated)	MSI (hypermutated)	Copy number low	Copy number high
Frequency	7.3%	28.0%	38.8%	25.9%
Copy number aberrations	Low	Low	Low	High
MSI status	Mixed	High	Stable	Stable
Mutation rate	Very high 232x10^6 mutations/Mb	High 18x10^6 mutations/Mb	Low 2.9x10^6 mutations/Mb	Low 2.3x10^6 mutations/Mb
Genes commonly mutated	*POLE* (100%) *PTEN* (94%) *PIK3CA* (71%) *PIK3R1* (65%) *FBXW7* (82%) *ARID1A* (76%) *KRAS* (53%) *ARID5B* (47%)	*PTEN* (88%) *RPL22* (37%) *KRAS* (35%) *PI3CA* (54%) *PIK3R1* (40%) *ARID1A* (37%)	*PTEN* (77%) *CTNNB1* (52%) *PIK3CA* (53%) *PIK3R1* (33%) *ARID1A* (42%)	*TP53* (92%) *PPP2R1A* (22%) *PIK3CA* (47%)
Histological type	Endometrioid	Endometrioid	Endometrioid	Endometrioid, Serous, mixed
Tumor grade	Mixed (grade 1-3)	Mixed (grade 1-3)	Grade 1 and 2	Grade 3
Progression-free survival	Good	Intermediate	Intermediate	Poor

Mb, megabase.

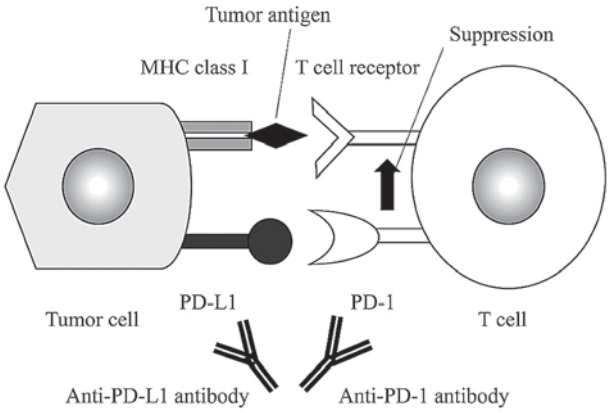

Figure 2. Action of antibodies against PD-L1 and PD-1 in tumor cells. Binding of PD-L1 expressed in tumor cells and PD-1 on the surface of T cells induces immune tolerance. Anti-PD-L1 antibody and anti-PD-1 antibody bind to PD-L1 and PD-1, respectively, to block immune tolerance, resulting in an enhanced antitumor effect of T cells.

have been shown to have less sensitivity and specificity than mononucleotide repeats (28), with particularly low sensitivity in patients with non-colorectal cancer, or tumors related to *MSH6* mutation (29-33). Consequently, the pentaplex panel was developed as a procedure with higher sensitivity and specificity, and has been proposed as a replacement for the Bethesda panel (28,33-38). This panel uses 5 mononucleotide repeats (NR-21, NR-22, NR-24, BAT-25, BAT-26) as markers. A modified pentaplex panel with replacement of NR-22 with NR-27 is also used (39). Pagin *et al* developed a hexaplex panel method using 6 mononucleotide repeats (NR-21, NR-22, NR-27, BAT-25, BAT-26, BAT-40) as markers and showed that this approach had higher sensitivity and specificity than the pentaplex panel in patients with *MSH6* mutation and those with non-colorectal cancer (40).

The type of microsatellite marker that is most appropriate for MSI analysis remains uncertain. Upon consideration of the use of MSI analysis as a predictive biomarker for the effect of anticancer drugs in endometrial cancer, the development of an optimal method for MSI detection in endometrial cancer, due to both somatic mutation and Lynch syndrome, is required. Hause *et al* developed the MOSAIC method for cross-sectional MSI analysis in 18 cancer types using the cancer exomes from the Cancer Genome Atlas (TCGA) database (41). In this model, a total of 223,082 microsatellites from exome sequencing were investigated to estimate mean mutation numbers in tumor and normal tissues obtained from cancer cases in the database. 17,564 microsatellites were identified as loci especially unstable in MSI-H tumors, which were located frequently in known oncogenes, suggesting that the other loci may also be located in so far unknown oncogenes. Characteristic microsatellite regions were involved among specific types of cancer, which distinguished four cancer-specific signatures based on MSI patterns. The MOSAIC method had a high sensitivity and specificity in identifying MSI-H tumors, with a possibly higher diagnostic accuracy in endometrial cancer compared to conventional MSI panels. The incidence of MSI-H tumor was highest in endometrial cancer among 18 types of tumors.

There is an ongoing debate about the methods for MSI analysis that can include results for unknown MMR genes in endometrial cancer. Therefore, the method proposed by Hause *et al* (41) may be an effective new approach with wider application compared to current MSI analysis optimized for Lynch syndrome.

5. Conclusion

MSI is found in approximately 30% of cases of endometrial cancer. Immunotherapy is a promising therapeutic strategy for MSI-type endometrial cancer; however, this therapy is very expensive and there is a need to select patients who will benefit from the therapy. The current MSI assay is optimized for Lynch syndrome, whereas many cases of MSI-type endometrial cancer are caused by *MLH1* promoter methylation or somatic mutation, and a new method of MSI analysis focused on these cancers is needed. MSI analysis for advanced endometrial cancer may contribute to establishment of new therapeutic strategies, including neoadjuvant therapy, for patients with this cancer.

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