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REVIEW ARTICLE

Microsatellite instability in cancer: a novel landscape for diagnostic and therapeutic approach

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Abstract

Defective DNA mismatch repair creates a strong mutator phenotype, recognized as microsatellite instability (MSI). Various next-generation sequencing-based methods for evaluating cancer MSI status have been established, and NGS-based studies have thoroughly described MSI-driven tumorigenesis. Accordingly, high-frequency MSI (MSI-H) has been detected in 81 tumor types, including those in which MSI was previously underrated. The findings have increased the use of immunotherapy, which is assumed to be efficient in tumors having a high mutation burden and/or neoantigen load. In MSI tumorigenesis, positively and negatively selected driver gene mutations have been characterized in colorectal cancers. Recent advancements in genome-wide studies of MSI-H cancers have developed novel diagnostic and therapeutic approaches, including CXCR2 inhibitor, a synthetic lethal therapy targeting the Werner gene and inhibition of nonsense-mediated mRNA decay. MSI is a predictive marker for chemotherapy as well as immunotherapy. Thus, analyses of MSI status and MSI-related alterations in cancers are clinically relevant. We present an update on MSI-driven tumorigenesis, focusing on a novel landscape of diagnostic and therapeutic approaches.

Keywords DNA mismatch repair · Microsatellite instability · Next-generation sequencing · Immunotherapy · Synthetic lethal therapy

Introduction

Microsatellite instability (MSI) is characterized by alterations in the genome-wide microsatellite repeats. MSI is observed in most cancers developed in patients with Lynch syndrome (Aaltonen et al. 1993; Ionov et al. 1993; Thibodeau et al. 1993), and in a subclass of sporadic cancers (Eso et al. 2020; Imai and Yamamoto 2008; Gelsomino et al. 2016; Yamamoto et al. 2012, 2014; Yamamoto and Imai 2015, 2019), in which it is primarily triggered by MLH1 methylation. DNA mismatch repair (MMR) genes are inactivated genetically or epigenetically, leading to increased frameshift mutations in various cancer-related genes, and resulting in tumor development. High frequency MSI (MSI-H) cancers have specific molecular, pathological, and clinical characteristics different from low-frequency MSI (MSI-L) or microsatellite stable (MSS) cancers, irrespective of tumor tissue origin.

The use of next-generation sequencing (NGS) to find genetic changes in many tumor types is a practical method for investigating MSI-H tumorigenesis, and for discovering novel diagnostic biomarkers and therapeutic targets (Yamamoto and Imai 2019). We present an update on MSI-driven tumorigenesis, focusing on a novel landscape for diagnostic and therapeutic approaches (Fig. 1).

MSI analyses in various tumor types by NGS

Evaluation of MSI status by polymerase chain reaction (PCR)-based assays and/or immunohistochemistry, has become a regular clinical practice for various types of advanced cancers (Ruiz-Bañobre and Goel 2019). From

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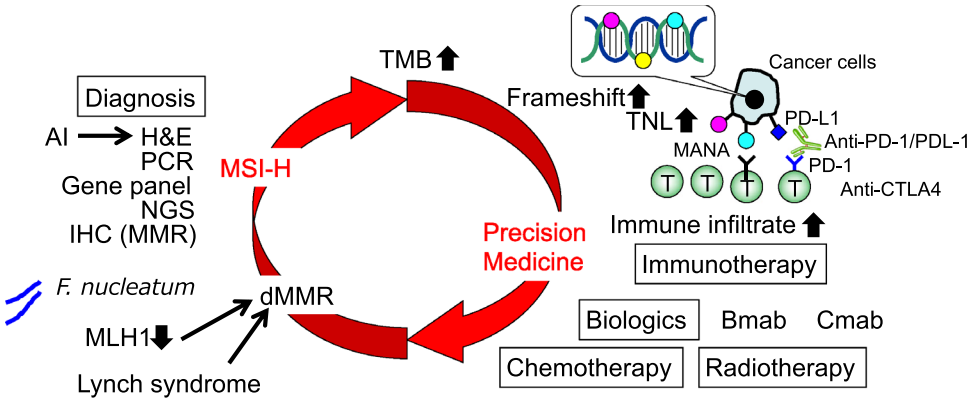

Fig. 1 Overview of MSI-H and precision medicine. MSI-driven tumorigenesis, focusing on a novel landscape for diagnostic and therapeutic approach, is shown

NGS-based techniques, alternative computational methods using targeted or whole exome sequencing data have been developed to evaluate MSI (Foltz et al. 2017; Hirotsu et al. 2020; Ruiz-Bañobre and Goel 2019; Wang and Liang 2018) (Fig. 2). These methods are shown to precisely evaluate MSI status (Ganesh et al. 2019; Ruiz-Bañobre and Goel 2019). Recently, Fujimoto et al. characterized the whole genome mutational landscape of microsatellites in 21 cancer types (Fujimoto et al. 2020). Moreover, deep learning could reportedly predict MSI directly from tissues sections stained with hematoxylin and eosin, facilitating common MSI screening in the near future (Kather et al. 2019).

Noninvasive analyses of MSI and tumor mutation burden (TMB) using cell-free DNA (cfDNA)

Using a pan-cancer gene panel that included selected microsatellite sequences, Georgiadis et al. (2019) established a cfDNA-based analysis of MSI and TMB. The sensitivities for MSI-H ($n=23$) and TMB-High ($n=15$) were 78% and 67%, respectively, and specificity was > 99% ($n=163$). The findings showed the possibility of noninvasive pan-cancer detection and follow-up for cancer patients with MSI-H and/or TMB-High, presumed to have high sensitivity to immune checkpoint inhibitors (ICIs).

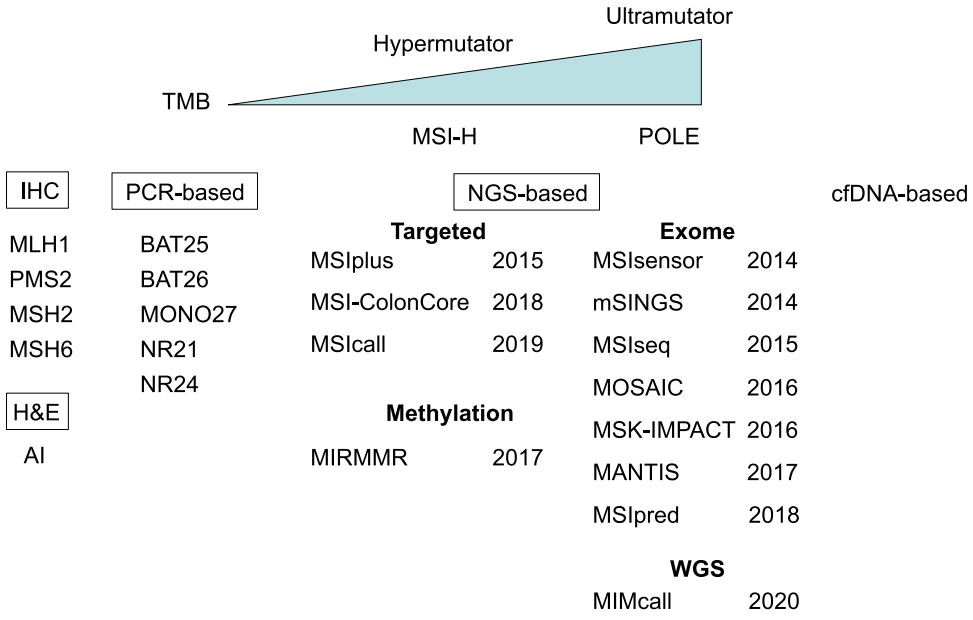

Fig. 2 TMB and evaluation of the MSI status. The MSI status is evaluated by immunohistochemistry, PCR-based assays, and NGS-based techniques

The origin of plasma circulating cfDNA variants determined by high-intensity sequencing

Using high-intensity sequencing, Razavi et al. (2019) determined the origin of cfDNA variants by analyzing cfDNA and corresponding leukocytes and tissue DNA in 124 metastatic cancer patients and 47 controls. The analyses showed high sensitivity and specificity, enabling detection of cancer-derived mutations and evaluation of TMB, MSI, mutation signatures and origins of somatic mutations detected in cfDNA. A large portion of cfDNA mutations (53% of cancer patients and 82% of controls) showed characteristics corresponding to clonal hematopoiesis. These findings suggest the importance of clonal hematopoiesis, and the need to further analyze corresponding leukocytes cfDNA sequencing for precise interpretation of variants.

Positively and negatively selected driver gene mutations in MSI-H CRCs

In MSI tumorigenesis, excessive genome instability causes not only positively selected mutations that contribute to tumor development, but also negatively selected mutations which have detrimental effects on cancer cells (Jonchere et al. 2018) (Fig. 3). The positively and negatively selected driver gene mutations identified, indicate that genome instability has a dual role in MSI-H CRC development.

Except for a small number of detrimental mutations detected in coding regions, negatively selected mutations were found mainly in long noncoding repeat sequences of the 5′ or 3′ UTR. Although around 10% of mutations were shown to change RNA expression, their functional effects need further analysis. Intriguingly, several negatively selected mutations were shown to upregulate tumor suppressive roles in MSI tumorigenesis, while others were shown to downregulate oncogenic roles. These results are in keeping with the contradictory activation or inactivation, respectively, in tumorigenesis. Results also emphasize that MSI in noncoding UTRs have an essential tumor suppressive effect in MSI tumorigenesis.

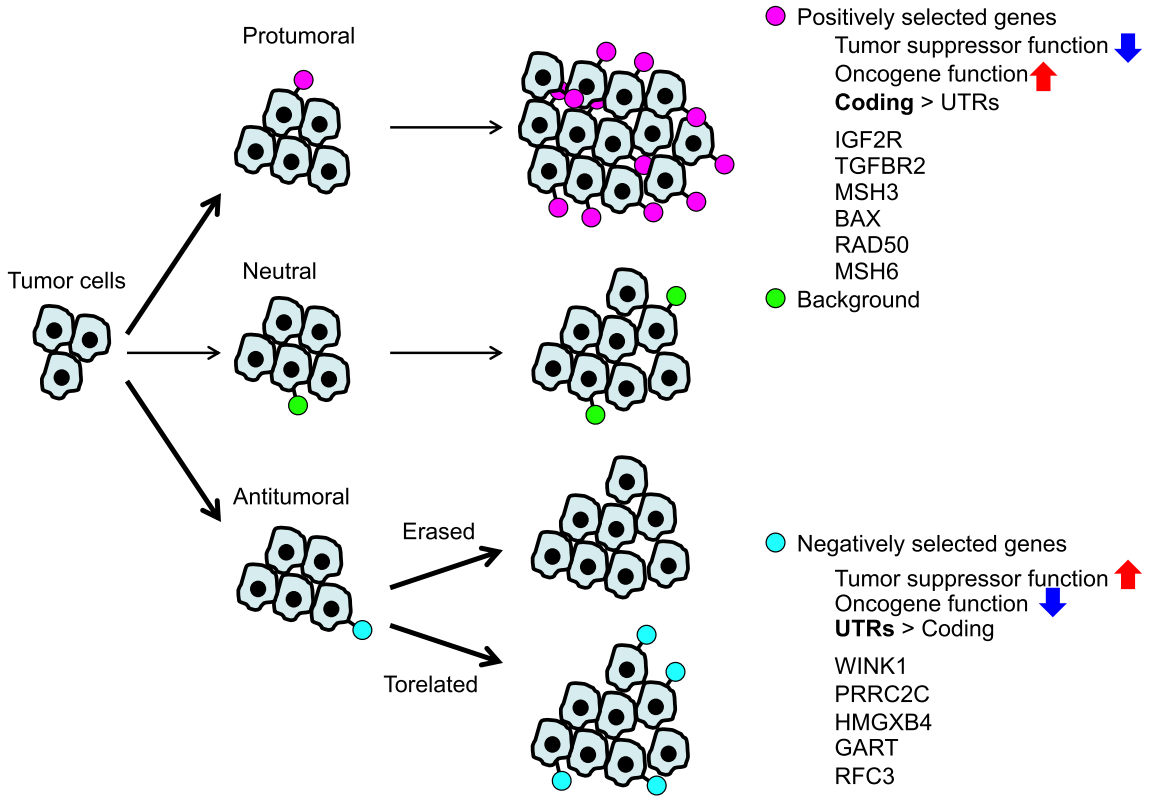

Fig. 3 Positively and negatively selected driver gene mutations in MSI-H CRCs. In MSI tumorigenesis, excessive genome instability causes not only positively selected mutations that contribute to tumor development but also negatively selected mutations which have detrimental effects on cancer cells

Ultra-hypermutated phenotype caused by DNA polymerase epsilon inactivation

For DNA replication fidelity, replicative DNA polymerases, exonucleolytic proofreading, and DNA MMR are necessary. Inactivating mutations of the polymerase epsilon (POLE) or delta (POLD1) result in an ultra-hypermutated phenotype that is represented by an excessive number of mutations (Gargiulo et al. 2016; Mittica et al. 2017; Nebot-Bral et al. 2017) (Figs. 1, 2). Owing to the high immunogenicity, patients with POLE-mutant tumors may also be sensitive to immunotherapy (Domingo et al. 2016; Ganesh et al. 2019) (Fig. 2).

Immune checkpoint immunotherapy

Frameshift mutation-enriched MSI-H tumors have been characterized by high Immunoscores, marked infiltration of immune cells (particularly mutation-specific CD8 + and CD4 + tumor-infiltrating lymphocytes (TILs) and macrophages), inhibitory PD-1/PD-L1 cells, and type I interferons-enriched microenvironment (Ganesh et al. 2019; Mlecnik et al. 2016). Accordingly, the outcomes of clinical trials that evaluated ICIs in patients with MSI-H tumors are encouraging (Ganesh et al. 2019; Le et al. 2015, 2017; O'Neil et al. 2017; Overman et al. 2017, 2018). The efficacy of the anti-PD-1 antibody was shown in 12 types of MSI-H cancers (Le et al. 2017). These results further suggest that a substantial portion of mutant-derived neoantigens render MSI-H tumors sensitive to ICIs, irrespective of tissue origin. Many clinical trials are presently underway to evaluate the efficiency of various ICIs in MSI-H metastatic CRC (mCRC) and other tumors (Ganesh et al. 2019).

KEYNOTE-164 (NCT02460198) study assessed the efficacy of pembrolizumab in previously treated MSI-H/deficient MMR (dMMR) mCRC. Registered patients with MSI-H/dMMR mCRC were 124 (61 for cohort A and 63 for cohort B). Overall response rate (ORR) was 33% for both cohorts. Thus, pembrolizumab was effective in patients with MSI-H/dMMR mCRC, and had manageable side effects (Le et al. 2020).

KEYNOTE-158 (NCT02628067) phase II study of pembrolizumab, was conducted in patients with formerly treated, unresectable or metastatic MSI-H/dMMR non-colorectal cancer. In total, 233 patients with 27 tumor types were registered. High-ranking tumor types were endometrial (21.0%), gastric (10.3%), cholangiocarcinoma (9.4%), and pancreatic (9.4%) cancers. Median follow-up period was 13.4 months and ORR was 34%. These results suggest that anti-PD-1 therapy using pembrolizumab is effective in patients with formerly treated advanced MSI-H/dMMR non-colorectal cancer (Marabelle et al. 2020).

In addition to PD-1 blocking, monospecific and bispecific antibodies, cellular therapies, cytokines, and vaccines that target various immune checkpoint components, macrophages and other molecules involved in innate immunity are actively investigated (Fig. 4).

Biomarkers for immune checkpoint immunotherapy

Various biomarkers predicting response to ICIs are presently being investigated. MSI-H accumulates frameshift mutations in cancer cells, leading to specific molecular traits, including higher tumor mutational burden/load (TMB/TML) (Innocenti et al. 2019), increased tumor neoantigen load (TNL), and marked TILs (Fig. 5). These alterations are correlated with increased sensitivity to ICIs (Chang et al. 2018), and hence high TMB and/or TNL is associated with a better response to ICIs (Panda et al. 2018). Moreover, compared with subclonal neoantigens, clonal neoantigens reportedly play more important roles in producing efficient anti-tumor immunity (McGranahan et al. 2016). The immunoscore is determined as the lymphocyte distribution in invasion margins and the center of tumors (Galon et al. 2013). Estimation of immune status by means of Immunoscore is reportedly helpful in predicting response to ICIs and tumor recurrence beyond MSI (Mlecnik et al. 2016).

Immunopathologic stratification of MSI-H CRC for ICIs

To classify MSI-H CRC patients, Llosa et al. integrated PD-L1 expression at the invasive front and histopathologic characteristics (percentage of extracellular mucin) to create a combined PD-L1 and mucin (CPM) score. This score distinguished which patients respond to ICIs. If confirmed in larger studies, MSI testing with CPM score may optimize immunotherapeutic interventions for MSI-H CRC patients (Llosa et al. 2019).

TMB is a predictive marker for response to ICIs in MSI-H mCRC

MSI is a marker for good response to ICIs. Patients with MSI-H mCRC treated with PD-1 had good disease control as well as promising progression-free survival (PFS). Nevertheless, ORR to pembrolizumab and nivolumab is variable and frequently less than half, indicating that further predictive markers are necessary. All 13 TMB-high patients and 3 of 9 TMB-low patients reportedly responded to ICIs, suggesting that TMB is a significant independent marker to

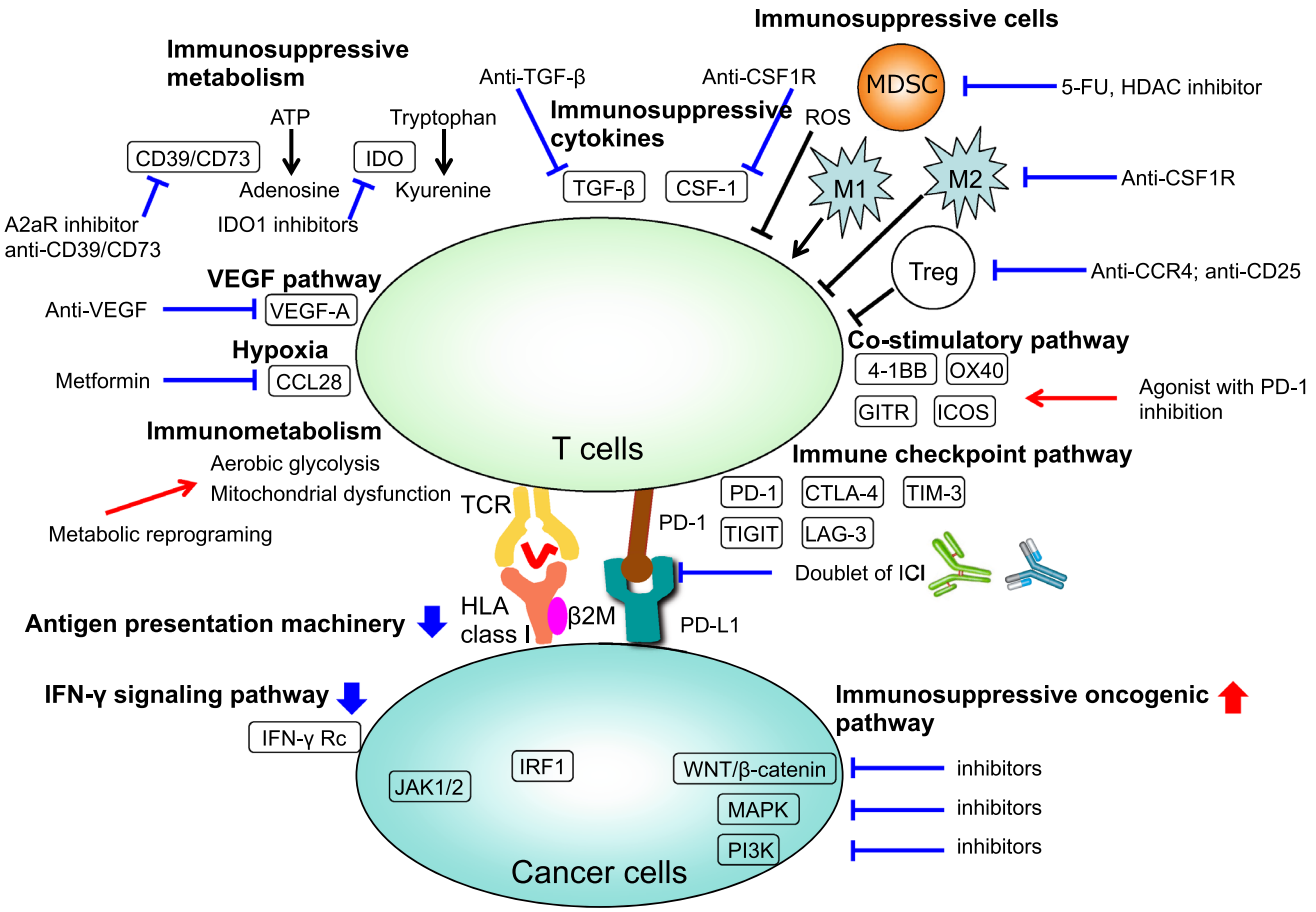

Fig. 4 Immune checkpoint immunotherapy. Monospecific and bispecific antibodies, cellular therapies, cytokines, and vaccines that target various immune checkpoint components, macrophages, and other molecules involved in innate immunity are actively investigated

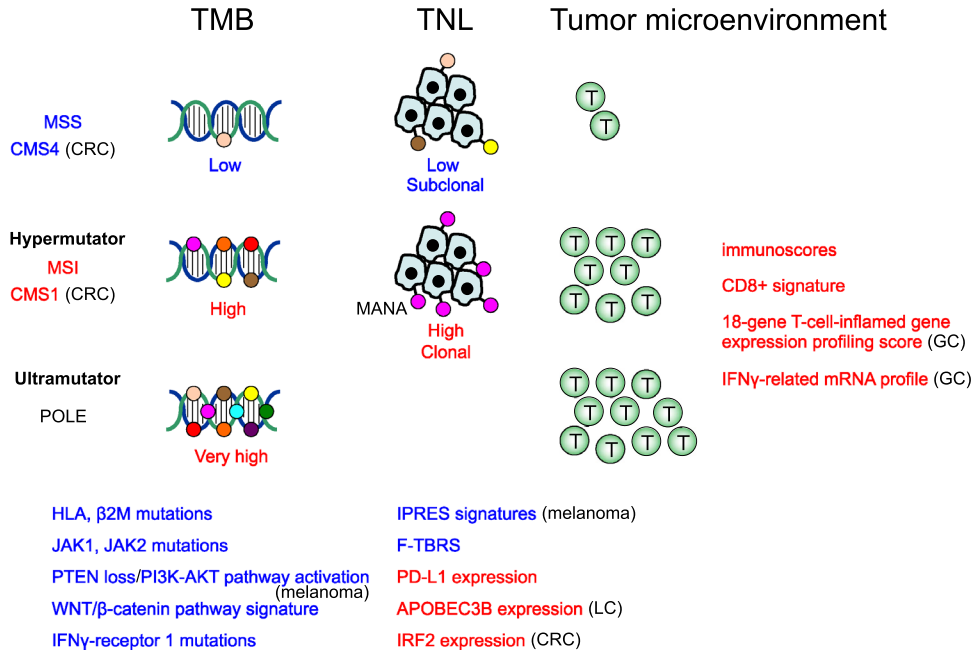

Fig. 5 Biomarkers for immune checkpoint immunotherapy. Various biomarkers predicting response to ICIs are presently being investigated. Markers predicting resistance (in blue) or high sensitivity (in red) to ICI therapy are shown

predict response to ICIs in MSI-H mCRC patients. If confirmed in prospective studies, TMB may guide the order and/or combinations of ICIs in MSI-H mCRC (Schrock et al. 2019).

Genetic diversity of MSI-H tumors influences anti-PD-1 immunotherapy response

Despite tumor immunogenicity, patients with MSI-H tumors show variable responses to ICIs, and about half are resistant to treatment. It is reported that the degree of MSI and subsequent TMB partially affects variable responses to PD-1 blocking in MSI-H cancers. The accumulation of insertion–deletion (indel) mutational load was especially correlated with response. The genome-wide evaluation of MSI degree and TMB may be necessary to predict responses to anti-PD-1 blocking across MSI-H tumors (Mandal et al. 2019).

Immune escape in MSI-H cancer

Immune evasion mechanisms help MSI-H tumor cells evade the immune response despite high TMB/TNL and prominent TILs. This mechanism explains, at least in part, why not all MSI-H tumors show good response to ICIs. Since 30–50% of MSI-H tumors do not respond to ICIs, a predictive biomarker is clinically needed.

Genetic and/or epigenetic inactivation of the human leukocyte antigen (HLA) class I, β2-microglobulin (B2M), and antigen-processing genes are often found in MSI-H tumors (Fig. 4). Frameshift mutations are frequently detected in target coding microsatellites in these genes (Hirata et al. 2007). Markers predicting resistance to ICI therapy, including acquired mutations in the *JAK1, JAK2* and *B2M* genes, were found in melanoma patients, but the role in other MSI-H tumors is not well defined (Ganesh et al. 2019) (Fig. 5). Ongoing clinical trials focusing on ICIs in MSI-H tumors will investigate immune evasion mechanisms to identify predictive biomarkers for primary and acquired resistance (Ganesh et al. 2019; Havel et al. 2019; Llosa et al. 2015; Ozcan et al. 2018).

Immune suppression and resistance to ICIs by KRAS-IRF2 axis in CRC

Oncogenic KRAS reportedly suppresses the immune system in CRC by inhibiting IRF2 expression, resulting in downregulation of interferon responsive genes, increased expression of CXCL3, enrollment of myeloid-derived suppressor cells, and consequent resistance to ICI therapy (Hänggi and Ruffell 2019; Liao et al. 2019).

Although MSI-H is a potent predictive marker of good response to ICIs, a subset of MSI-H CRC patients does not respond to anti-PD-1 therapy (O'Neil et al. 2017; Overman et al. 2017). IRF2 expression levels were positively correlated with response to anti-PD-1 therapy, and could be a novel predictive biomarker of response to therapy in MSI-H CRC. Accordingly, IRF2 reduction in MC38 cells, an MSI-H cell line that responds to ICIs, leads to resistance to ICIs. *KRAS* status is reportedly not related to anti-PD-1 therapy efficacy (Overman et al. 2018). Therefore, while *KRAS/BRAF* status is not a predictive marker of response to ICIs in MSI-H CRC, *KRAS* mutation may be a predictive marker of primary resistance to ICIs by IRF2 reduction in MSS CRC. Accordingly, the combination of ICIs with CXCR2 inhibitor may be promising in *KRAS*-mutated CRC patients (Katoh et al. 2013; Steele et al. 2016).

Identification of WRN as a synthetic lethal therapeutic target in MSI-H tumors

Inhibition of poly (ADP-ribose) polymerase (PARP) in patients with deficient homologous recombination DNA repair pathway is the first successful synthetic lethal cancer therapy. Other targets for synthetic lethal therapy have been studied (Chan et al. 2019; Kategaya et al. 2019; Lieb et al. 2019). Based on large-scale functional genomic screening of cancer cell lines, MSI-H cancer cells have been reported to be selectively susceptible to inactivation of WRN (Behan et al. 2019) (Fig. 6). WRN is a RecQ family DNA helicase, which is possibly inhibited using small molecules. Preclinical study of such small molecules in patients with MSI-H tumors is expected in the near future (Kawasaki et al. 2008).

Patient-derived xenografts (PDXs) and matched cell lines identify pharmacogenomic susceptibilities in CRC

Lazzari et al. (Lazzari et al. 2019) developed a 2D cell line (xeno-cell lines, XL) platform, which originated from PDXs of CRC with matched germline genomic DNA. Dependency on the *WRN* gene was evaluated in MSS, MSI-H, and MSI-like XLs using a functional approach with reverse genetics. MSI-H XLs were dependent on *WRN* gene expression, while MSS XLs were not. Interestingly, one MSS XL showing transcriptionally MSI-like characters was vulnerable to *WRN* loss. The XL platform is a useful method for validation of genes and studies to find new drug targets in CRC.

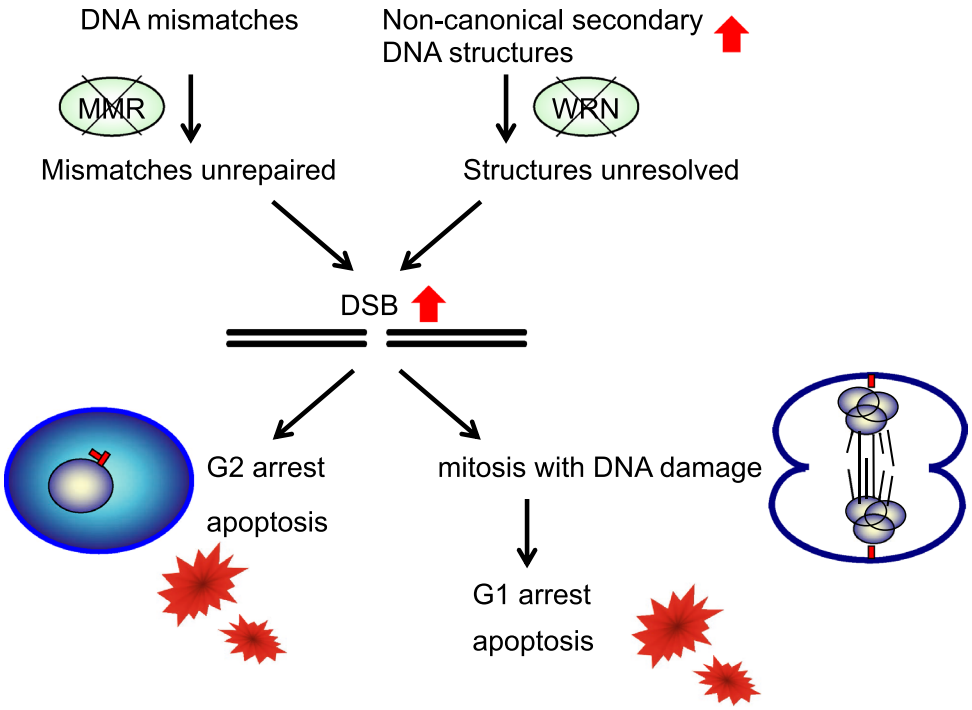

Fig. 6 Identification of WRN as a synthetic lethal therapeutic target in MSI-H tumors. MSI-H cells arrest in G2 or progress into mitosis with DNA damage, resulting in G1 arrest or apoptosis

A comprehensive gastric cancer (GC) PDX collection captures cancer cell-intrinsic transcriptional MSI characters

Corso et al. (2019) developed a comprehensive GC PDX platform that could find and confirm novel drug targets and optimize therapeutic strategies. Moreover, transcriptomic analysis of GC PDXs identified a cancer cell intrinsic MSI signature that recognized a subset of MSS patients with MSI transcriptional characters and better prognosis. Thus, a multilevel GC PDX platform could identify an MSI gastric signature, contributing to precision medicine progress in GC.

Targeting nonsense-mediated mRNA decay (NMD) in MSI-H CRCs

mRNAs with a premature termination codon (PTC) are usually degraded by NMD system. NMD increases deficiencies caused by partially inactivated function of tumor suppressor genes with frameshift mutations (Bhuvanagiri et al. 2010; Popp and Maquat 2018; Usuki et al. 2004). Owing to the large number of mRNAs with PTC in MSI-H cancers, NMD can significantly inhibit the complete expression of tumor suppressor genes (Chan et al. 2009; El-Bchiri et al. 2005, 2008).

Compared with MSS CRC, MSI-H CRC reportedly expresses high levels of SMG1/6/7 and UPF1/2, which are crucial NMD activators (Bokhari et al. 2018). Many mRNAs with PTC are re-expressed by inhibition of the NMD activity. There are some mRNAs encoding mutated proteins with detrimental action against MSI carcinogenesis. Suppression of NMD by its inhibitor amlexanox, reportedly decreased MSI-H cancer growth in vivo, but not in MSS cancers. Amlexanox is useful for patients with recurring aphthous stomatitis (Ballal 2014). Thus, suppression of the oncogenic action of NMD appears to be an efficient approach for tailoring treatment of MSI-H CRC.

From the viewpoint of immunotherapy, NMD inhibitors receive the attention. NMD inhibitors are estimated to increase anti-tumor T cell immunity in MSI-H CRC by the cell surface presentation of abundant MSI-derived neoantigens. Whether NMD inhibitors synergistically function with ICIs in patients with MSI-H tumor needs further clarification.

Adaptive mutability of CRCs in response to targeted therapies

The occurrence of drug resistance restricts the efficacy of targeted therapies in human cancers. It is reported that homologous recombination repair and MMR genes were downregulated, and error-prone polymerases were upregulated by inhibition of epidermal growth factor receptor (EGFR)/BRAF in drug-resistant cells (Russo et al. 2019). Expression of MMR proteins was decreased in PDXs and cancer specimens through therapy. Thus, DNA damage, MSI, and increased mutability were induced by inhibition

of EGFR/BRAF, suggesting that cancer cells manipulate adaptive mutability to escape therapeutic pressures.

Concluding remarks

MSI-H is observed in various tumor types, including those for which MSI-H was previously underrated. The recent advancements in genome-wide investigation of MSI-H cancers has led to the discovery of new diagnostic and therapeutic strategies, including CXCR2 inhibitor, a synthetic lethal therapy targeting WRN, and NMD inhibition. Combining genome and phenotype-based information with clinical data will facilitate the development of innovative diagnostic and therapeutic approaches in the era of NGS and precision medicine.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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