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ORIGINAL ARTICLE

Investigation of the Relationship Between DNA Mismatch Repair Genes and Microsatellite Instability in Solid Tumors

Solid Tümörlerde DNA Yanlış Eşleşme Onarım Genleri ile Mikrosatellit İnstabilite Arasındaki İlişkinin Araştırılması

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ABSTRACT

Aim: In this study, we aimed to detect the MSI status and somatic mutations in MMR genes (MSH2, MSH6, MLH1, PMS2) of a total of 55 solid tumors diagnosed with colorectal, endometrium and ovarian cancer by NGS method and to reveal the relationship between them.
Material and Method: DNA isolation was performed by taking 10-micron sections from paraffin-embedded tissue samples of 55 patients diagnosed with kolorektal, endometrium ve ovarian solid tümörlerinin and Kapa NGS DNA extraction kit was used for sequence analysis. The purity and concentration of the DNA obtained was measured by Qubit fluoremeter, and NadPrep DNA Universal Library Preparation Kit was used for high quality library preparation. Bioinformatics analyses were performed on the Genomize Seq platform. The MSI value was analysed by Roche Navify Mutation Caller software and percentage MSI values were determined using the MSIsensor2 pipeline for secondary analysis of NGS.
Results: All ovarian tumors were in the MSI-Stable category. The average MSI value was 2.01. One sample had an MSI of zero. In addition, no mutations in the MSH2 and MLH1 genes were detected in any of the ovarian tumors. 3 of 4 endometrial tumors were in the MSI-Stable category, and 1 tumor was in the MSI-low (MSI value: 13.2) category. No variants were detected in the MSH2 and PMS2 genes in endometrial tumors. 2 of the 41 colorectal tumors (Case4, Case16) were in the MSI-High category. No variants were detected in the MMR genes in 19 tumors.
Conclusion: Although frame-shift and stop-gain mutations were detected in 23 tumors that would cause protein deficiency in MMR genes, MSI-H was not detected, as expected, except for two colorectal tumors. Therefore, the results of our study emphasise the need to define new predictive biomarkers clinically within the framework of algorithms to predict response to immunotherapy and determine prognosis.

Keywords: Colorectal cancer, Endometrium cancer, Ovarian cancer, MMR, MSI

ÖZ

Amaç: Bu çalışmada kolorektal, endometrium ve over kanseri tanısı almış toplam 55 solid tümörün MSI durumunu ve MMR genlerindeki (MSH2, MSH6, MLH1, PMS2) somatik mutasyonları NGS yöntemi ile tespit etmeyi ve aralarındaki ilişkiyi ortaya koymayı amaçladık.
Gereç ve Yöntem: Kolorektal, endometrium ve yumurtalık kanseri tanısı almış 55 solid tümör örneklerinden 10 mikronluk kesitler alınarak DNA izolasyonu gerçekleştirilmiş ve dizi analizi için Kapa NGS DNA ekstraksiyon kiti kullanılmıştır. Elde edilen DNA'nın saflığı ve konsantrasyonu Qubit floremetre ile ölçülmüş, yüksek kalitede kütüphane hazırlanması için NadPrep DNA Universal Library Preparation Kit kullanılmıştır. Biyoinformatik analizler Genomize Seq platformunda gerçekleştirilmiştir. MSI değeri Roche Navify Mutation Caller yazılımı ile analiz edilmiş ve yüzde MSI değerleri NGS'nin ikincil analizi için MSIsensor2 boru hattı kullanılarak belirlenmiştir.
Bulgular: Tüm yumurtalık tümörleri MSI-Stabil kategorisindeydi. Ortalama MSI değeri 2,01 idi. Bir örneğin MSI değeri sıfırdı. Ayrıca, yumurtalık tümörlerinin hiçbirinde MSH2 ve MLH1 genlerinde mutasyon tespit edilmemiştir. 4 endometriyal tümörün 3'ü MSI-Stabil kategorisinde ve 1 tümör MSI-düşük (MSI değeri: 13,2) kategorisindeydi. Endometriyal tümörlerde MSH2 ve PMS2 genlerinde herhangi bir varyant tespit edilmemiştir. 41 kolorektal tümörün 2'si (Vaka4, Vaka16) MSI-Yüksek kategorisindeydi. MMR genlerinde 19 tümörde herhangi bir varyant tespit edilmemiştir.
Sonuç: MMR genlerinde protein eksikliğine neden olabilecek çerçeve kayması ve stop-gain mutasyonları 23 tümörde tespit edilmesine rağmen, iki kolorektal tümör dışında beklendiği gibi MSI-H tespit edilmemiştir. Bu nedenle, çalışmamızın sonuçları, immünoterapiye yanıtı öngörmek ve prognozu belirlemek için algoritmalar çerçevesinde klinik olarak yeni öngörücü biyobelirteçlerin tanımlanması gerektiğini vurgulamaktadır.

Anahtar Kelimeler: Kolorektal kanser, Endometrium kanseri, Yumurtalık kanseri, MMR, MSI

Introduction

Approximately 20,000 mutations occur in each cell every day and these are regularly repaired by DNA repair mechanisms (1,2). In cases where cells are threatened by the failure of repair mechanisms, apoptosis or programmed cell death occurs to prevent cell proliferation. Cancer cells are genomically unstable cells that have gained the ability to proliferate (3,4). This neoplastic transformation of cancer cells is mainly due to the accumulation of somatic mutations in the DNA of the cells. In humans, the acquired immune system plays an important role in the immune response against cancer cells due to its ability to specifically target nonspecific antigens (2-5). Based on this understanding, various immunotherapies have been developed, including cancer vaccines, monoclonal antibodies and immune checkpoint inhibitors. In recent years,

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immunotherapy has become an important pillar of cancer treatment along with chemotherapy, surgery, radiation and targeted therapies. In this context, in 2013, Science magazine declared immunotherapy for cancer treatment as the "Breakthrough of the Year" (6).

A recent US National Comprehensive Cancer Network (NCCN) guideline recommends that tumors should have microsatellite instability (MSI), mismatch repair genes (MMR) and tumor mutation burden (TMB) detected for the use of the FDA-approved drugs pembrolizumab, nivolumab and ipilimumab in the treatment of many types of cancer. Microsatellite instability is a state of hypermutability caused by genetic or epigenetic inactivation of DNA mismatch repair genes. The presence of MSI therefore represents phenotypic evidence that MMR genes are not functioning normally. Cells with abnormally functioning MMR are unable to correct errors that occur during DNA replication and consequently accumulate errors. The US National Cancer Institute (NCI) has identified 5 microsatellite regions as markers for the presence and categorization of MSI. These regions are BAT25, BAT26 containing two mononucleotide repeats and D2S123, D5S346 and D17S250 containing 3 dinucleotide repeats. The tumor is classified as MSI-High (MSI-H) if 2 or more of these regions are unstable, MSI-Low (MSI-L) if less than 2, and MSI-Stable (MSS) if none of the repeat regions are unstable (7). Variations in MMR genes (MLH1, MSH2, MSH6 and PMS2), which play an important role in the integrity and protection of DNA, contribute to the development of carcinoma in individuals. Immunohistochemical staining of MMR genes on tumor samples reveals the risk status according to the presence or absence of deficiency at the protein level. Variations in MMR genes can be inherited or somatic as in Lynch Syndrome (8). When tumor-specific variations in MMR genes are considered, MLH1 variants are more associated with high colorectal cancers, while MSH2 variants are more associated with non-colon neoplasms. However, the carcinogenesis process in individuals with MSH6 variants progresses more slowly than in MSH2 and MLH1 variant carriers. The tumor association of variants in the PMS2 gene is weaker than the others (9,10).

Laboratory analyses have shown that tumors characterized by MSI-H undergo hypermutation as a result of accumulation of somatic mutations and exhibit a number of molecular and biological alterations, with high numbers of tumor-infiltrating lymphocytes (tumor infiltrative) abundantly expressing peptides that act as neoantigens to elicit an immune response (11-22). At the same time, researchers have shown that tumors with the MSI-H profile also tend to express high levels of checkpoint proteins, including PD-1 and PD-L1, and reported that tumors with this profile respond better to PD-L1/PD-1 blocking drugs (21,22). These studies have led to the hypothesis that MSI-H may be a pan-cancer biomarker that predicts response to immunotherapy independent of tumor histology and entirely dependent on the genetic composition of the tumor (22,23).

In a study on colorectal cancers, it was shown that despite poor histological differentiation, tumors with MSI-H profile have less aggressive biological behavior than CNS tumors (24). MSI is a predictive biomarker for chemotherapy and immunotherapy in cancers. Therefore, identification of the MSI profile and MSI-related molecular and biological changes in tumors is of clinical importance (25). The MSI-IVD kit was the first tumor agnostic diagnostic method to detect MSI status using a PCR-based method of 5 microsatellite regions recommended by the NCI to determine the MSI profile. However, in recent years, next-generation sequencing (NGS) methods have been used to detect the MSI-H profile, which increase sensitivity by analyzing many more microsatellite regions (26). Researchers have also emphasized that NGS is a suitable method for determining the MSI profile as well as discovering new diagnostic biomarkers and therapeutic targets and for risk assessment (26-28).

In gastrointestinal and gynecologic cancers such as colorectal carcinoma, gastric adenocarcinoma, duodenal adenocarcinoma, small bowel adenocarcinoma, carcinoma of the uterus and ovary, and endometrial carcinoma, MSI-H and high TMB almost always coexisted, whereas in melanoma, squamous cell carcinoma, and lung carcinoma, high TMB was observed quite frequently, whereas MSI-H was very rare. These data suggest that the MSI-H pathway appears to be a common phenomenon in gastrointestinal and gynecologic carcinogenesis, but somehow less involved in other cancers (18).

In this study, we investigated the MSI status and somatic mutations in MMR genes of colorectal, endometrial and ovarian solid tumors by NGS method and investigated the relationship between them.

Material and Methods

DNA isolation, Sequencing analyses and MSI value

DNA isolation from 10-micron sections of paraffin-embedded tissues whose diagnosis was confirmed by pathological examination was performed using the Kapa NGS DNA extraction kit (Roche molecular systems, Inc., Germany). The purity and concentration of the DNA obtained were measured using a Qubit fluoremeter (ThermoFisher Scientific, USA). To generate a high-quality library from double stranded DNA (dsDNA), we used the NadPrep DNA Universal Library Preparation Kit (Nanodigmbio (Nanjing) Biotechnology Co., Ltd, China), which includes the Library Prep Module and Adapter Primer Module. NAD panels within 5' biotinylated probes, optimised for targeted capture applications in NGS, were used for libraries prepared using the NadPrep DNA Universal Library Preparation Kit (for MGI). In this study, 500 ng of DNA from each library was used for hybrid capture. After this step, the KAPA HyperPETE Pan Cancer Panel (Roche, USA, 2021) kit was used. With this process, 190 MSI loci related to somatic variation of MMR genes and somatic oncology applications were analysed. The MSI value was analysed by Roche Navify Mutation Caller software (Roche, USA, 2021) and percentage MSI values were determined using the MSIsensor2 pipeline for secondary analysis of NGS data. The algorithm categorises MSI values between 0-10 as MSI-stable, between 10-20 as MSI-Low and between 20-100 as MSI-High.

After these procedures, a single-stranded circular DNA library was prepared using the MGIEasy Circularisation Kit (MGI Tech Co., Ltd, China). Single-stranded circular DNAs were converted into nanoballs (DNBs) by rolling circle amplification using the DNB SEQ-G50RS high-throughput sequencing kit (MGI Tech Co., Ltd, China). The sequencing cartridge was then prepared, and the DNBs were placed in the DNB tube and inserted into the instrument. Samples were passed through the flow cell in the instrument and sequencing was performed on the DNBSEQ-G50RS instrument (MGI Tech Co., Ltd, China). Bioinformatic analysis of the data obtained from the study was performed on the Genomize Seq (v8.0.4) platform. The bioinformatic analysis of the data obtained from the study was carried out on the Genomize Seq platform.

Results

Of the 55 solid tumors that constituted the sample group in the study, 41 were colorectal, 10 ovarian and 4 endometrial. All of the ovarian tumors were in the MSI-Stable category. The mean MSI value was 2.01. One sample had an MSI value of zero. In addition, none of the ovarian tumors had mutations in the MSH2 and MLH1 genes. The highest number of variants was detected in MSH6 gene (7 unique) and 4

Table 1. MSI status of endometrial and ovarian tumors and mutations detected in MMR genes

Case number	Tumor type	MSI Value	MSI Category	MLH1	MSH6	**PMS2**
Case1	Ovarian	2,80	MSS		p.L1167fs[b]	
Case2	Ovarian	2,90	MSS			
Case3	Ovarian	1,90	MSS			
Case4	Ovarian	1,00	MSS		p.T86fs[c] , p.F1088fs[c]	p.F788fs[b]
Case5	Ovarian	1,00	MSS			p.R20Q[a]
Case6	Ovarian	0,00	MSS			p.R20Q[a]
Case7	Ovarian	1,90	MSS			
Case8	Ovarian	3,80	MSS		p.D284fs[b], p.W414*[c], p.R1005*[c], p.F1088fs[c],p.C1269*[c]	p.Q342*[c], p.F173fs[b]
Case9	Ovarian	2,90	MSS			
Case10	Ovarian	1,90	MSS			p.R20Q[a]
Case11	Endometrium	4,50	MSS	p.Q409fs[b],p. S467fs[c]	p.V509E[b], p.L1167fs[b],	
Case12	Endometrium	2,8	MSS	p.V267fs[b],p.E324*[c]		
Case13	Endometrium	2,10	MSS	p.Q328*[c]	p.E1120*[c]	
Case14	Endometrium	13,2	MSI-Low	p.R265C[c]	p.Q160fs[b],p.F1088fs[c],p.Q1155fs[b]	

[a] Benign variant according to ACMG Classification

[b] Likely Pathogenic variant according to ACMG classification

[c] Pathogenic variant according to ACMG classification

* stop gained mutation

fs: frame shift mutation

different variants were detected in PMS2 gene. Of the four endometrial tumors, three were in the MSI-stable category and one tumor was in the MSI-low category (MSI value:13.2). The mean MSI of endometrial tumors was 5.65. As in ovarian tumors, none of the endometrial tumors had variants in the MSH2 gene.

Table 2. MSI status of colorectal tumors and mutations detected in MMR genes

Case number	MSI Value	MSI Category	MLH1	MSH2	MSH6	PMS2
Case1	3,20	MSS				
Case2	0,00	MSS				p.R810fs[b], p.P440fs[b]
Case3	1,90	MSS				
Case4	45,40	MSI-High			p.F1088fs[c]	
Case5	0,00	MSS			p.F1088fs[c]	c.1145-1G>T[b]
Case6	3,00	MSS				
Case7	1,90	MSS				
Case8	1,00	MSS				
Case9	1,00	MSS		p.Q10*[c]		p.T597S[a]
Case10	2,20	MSS		p.T724fs[b]		p.R20Q[a]
Case11	1,90	MSS	c.791-2A>T[c]		p.F1088fs[c]	p.Q781fs[c],p.E744fs[b], p.D699fs[b]
Case12	1,20	MSS	p.I159fs[b],c.790+1G>A[c], p.E429*[c],p.R659*[c]	p.C176*[b]		
Case13	2,90	MSS				
Case14	0,00	MSS		p.Q76*[c]	p.R1334Q[c]	
Case15	1,90	MSS				
Case16	83,80	MSI-High			p.F1088fs[c]	
Case17	3,20	MSS				
Case18	0,00	MSS				
Case19	1,90	MSS	c.790+1G>A[c]			
Case20	3,00	MSS				
Case21	2,00	MSS				p.R20Q[a]
Case22	5,70	MSS				
Case23	0,00	MSS				
Case24	1,90	MSS				
Case25	2,00	MSS				
Case26	0,90	MSS				
Case27	1,00	MSS	p.Q327*[c]	c.646-2A>T[b], p.K449*[c],p.L762*[c]	p.K218*[c],p.F1088fs[c], p.Y1287*[c]	p. V397fs[b]
Case28	2,80	MSS				
Case29	1,10	MSS	p.G336fs[c],p.Y379fs[b],	p.M253fs[c]		
Case30	3,60	MSS				
Case31	1,00	MSS				
Case32	0,90	MSS				
Case33	0,90	MSS				
Case34	3,80	MSS				
Case35	3,10	MSS		p.Q593*[c]		
Case36	1,10	MSS		p.H785fs[b]	p.P781fs[b]	
Case37	4,30	MSS	p.V384D[a]			
Case38	0,00	MSS				p.M622I[a], p.R20Q[a]
Case39	1,90	MSS		p.K82*[c]		
Case40	3,30	MSS		p.R406*[c]		
Case41	0,00	MSS				

[a] Benign variant according to ACMG Classification

[b] Likely Pathogenic variant according to ACMG classification

[c] Pathogenic variant according to ACMG classification

* stop gained mutation

fs: frame shift mutation

In addition, no variants were detected in PMS2 gene in endometrial tumors Six variants were detected in each of the MSH6 and MLH1 genes (Table 1). Of the 41 colorectal tumors in the study, 2 (Case4, Case16) were in the MSI-High category and the others were in the MSI-Stable category. Case4 had an MSI of 45.40 and Case16 had an MSI of 83.80. The mean MSI value of colorectal tumors was 4.89. In 19 tumors, no variants in MMR genes were detected Interestingly, only the pathogenic p.F1088fs variant in the MSH6 gene was detected in two tumors in the MSI-high category. The same variant was also present in an endometrial tumor (Case14). In colorectal tumors, MSH2 (12 variants) was the most common variant, while MSH6 (5 variants) was the least common (Table 2). In addition, the pathogenicity status of the variants detected in MMR genes was performed according to the American College of Medical Genetics and Genomics (ACMG) classification. The most common mutation types detected in MMR genes in both colorectal, endometrial and ovarian tumors were frame-shift and stop-gained mutations.

Discussion

The MMR system consists of a family of enzymes (MLH1, MSH2, MSH6 and PMS2) that detect and correct errors that occur spontaneously during DNA replication, such as incorrect single base pairs or short duplications and deletions (29-33). MMR proteins are expressed in normal tissues and show positive nuclear staining immunohistochemically. Tumors showing loss of a single MMR protein as a result of inactivation of one or more MMR genes are referred to as MMR deficiency (dMMR) (34). Microsatellite instability is a state of hypermutability caused by genetic or epigenetic inactivation of DNA mismatch repair genes. The presence of MSI therefore represents phenotypic evidence that MMR genes are not functioning normally. In contrast, Chalmers et al. showed that MSI-H was more closely associated with gastrointestinal and gynecologic cancers and that the majority of MSI-H samples (83%) had high TMB. However, only 16% of the samples with high TMB were shown to be MSI-H. The co-occurrence of these two phenotypes was highly dependent on the type of cancer.

Alterations in MSH6 are found in many solid tumors, including somatic mutations in colorectal and endometrial cancer (35). Inactivating mutations in MSH6 cause MMR deficiency (dMMR) and microsatellite instability (MSI). MSH6 inactivating mutations cause loss of function of the MSH6 protein and are associated with mismatch repair deficiency (dMMR) and high microsatellite instability (MSI-H) in solid tumors (35-37). Pembrolizumab (FDA, Health Canada, TGA, TFDA, NCCN), nivolumab (FDA, Swissmedic, TFDA, NCCN) and nivolumab in combination with ipilimumab (FDA, Swissmedic, TFDA, NCCN) are approved and recommended for certain patients with colorectal cancer with high microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR). In our study, the R1005* variant of MSH6 gene was detected in ovarian tumors. According to the Catalogue of Somatic Mutation in Cancer (COSMIC) database, this variant has not been defined in ovarian tumors, but has been found in endometrial tumors (38). In addition, the c.3261delC (F1088fs) variant in the MSH6 gene was detected in endometrial, ovarian and colorectal tumor samples in our study, whereas this variant was identified only in colorectal tumors in COSMIC. In addition, 13 other MSH6 variants that we identified in different tumor types in our study were not available in the COSMIC database. The c.3261delC variant is predicted to cause a frameshift starting at codon 1088 that alters the amino acid sequence of the protein and leads to a premature stop codon at position 1089. This change has subsequently been shown to result in a truncated or missing protein and loss of function. In a study, heterogeneous MSH6 IHC staining was demonstrated in a colon adenocarcinoma tumor and molecular analysis revealed the presence of two MSH6 frameshift variants (c.3261delC and c.3261dupC) in areas of MSH6 protein loss compared to areas where MSH6 was preserved. In addition, it has also been reported that the c.3261delC. variant is present in tumors with gastric cancer and in four out of five cases where the MMR protein was lost, MSI-H was found in tumors with the variant (39).

As with MSH6, mutations inactivating MSH2, PMS2 and MLH1 are associated with MMR deficiency in somatic context (dMMR) and microsatellite instability (MSI-H) (35-37). In our study, variants in MSH2 gene were found only in colorectal tumors and not in other tumors. However, none of the 12 different variants we detected in MSH2 gene in colorectal tumors were registered in the COSMIC database. In our study, a total of 13 different variants were detected in the PMS2 gene in ovarian and colorectal tumors, of which the M622I variant was available in COSMIC and was found in colorectal tumors as in our samples. In addition, while the R20Q variant was detected in both ovarian and colorectal tumors in our samples, we observed that it

was detected in ovarian tumors in COSMIC. The other 11 variants we detected in the PMS2 gene were not present in COSMIC. In our study, we found a total of 15 different variations in MLH1 gene in endometrial and colorectal tumors of these, 6 were registered in the COSMIC database. While R265C and Q327* variants were detected in endometrial tumors in our samples, we observed that they were detected in colorectal tumors in COSMIC database. In addition, Q328*, V384D and c.790+1G>A variants that we detected in colorectal tumors in our study were also detected in colorectal tumors in the COSMIC database. We also found that the R659* variant, which we detected in colorectal tumors in our study, was present in both endometrial and colorectal tumors in COSMIC. The other 9 variants we detected in MLH1 gene were not registered in COSMIC.

Although the NCCN guideline recommends that TMB high and MSI-H biomarkers should be taken into consideration for response to immunotherapy in cancer patients, studies in the literature have emphasized that tumor agnostic cancer patients show variable responses to immunotherapy and approximately half (30-50%) exhibit resistance to treatment despite having TMB high and MSI-H biomarkers (34). In addition, in our study, although frame-shift and stop gained mutations were detected in 23 tumors that would cause protein deficiency in MMR genes, MSI-H was not detected in 21 tumors except two colorectal tumors as expected. Therefore, the results of our study emphasise the need to define new predictive biomarkers clinically within the framework of algorithms to predict response to immunotherapy and determine prognosis.

Ethics Committee Approval

The study was carried out with the permission of Selçuk University Faculty of Medicine Ethics Committee. (Date: 03.07.2024, Decision No: E-70632468-050.04- 785159).

Informed Consent

This study was designed retrospectively and consent forms were also obtained from the patients.

Conflict of Interest Statement

The author have no conflicts of interest to declare.

Financial Disclosure

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References

1.Pardoll D. Cancer and the immune system: basic concepts and targets for intervention. Semin Oncol. 2015;42:523–538.

2.Oiseth SJ, Aziz MS. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. J Cancer Metastasis Treat. 2017;3:250–261.

3.Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–674.

4.Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1–10.

5.Gasparri ML, Ruscito I, Taghavi K, Farooqi AA, Papadia A, Foraccetti C, et al. Molecular oncology: underlying mechanisms and translational advancements. Switzerland: Springer; 2017. p. 193–204. ISBN: 978-3-319-53081-9.

6.Couzin-Frankel J. Cancer immunotherapy. Science. 2013;324:1432–1433.

7.Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med. 2009;11:42–65.

8.Latham A., Srinivasan P., Kemel Y., Shia J., Bandlamudi C., Mandelker D., Middha S., Hechtman J., Zehir A., Dubard-Gault M., et al. Microsatellite Instability Is Associated with the Presence of Lynch Syndrome Pan-Cancer. J. Clin. Oncol. 2019;37:286–295.

9.Li X., Liu G., Wu W. Recent advances in Lynch syndrome. Exp. Hematol. Oncol. 2021;10:37.

10.Dominguez-Valentin M., Sampson J.R., Seppälä T.T., ten Broeke S.W., Plazzer J.-P., Nakken S., Engel C., Aretz S., Jenkins M.A., Sunde L., et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: Findings from the Prospective Lynch Syndrome Database. Genet. Med. 2020;22:15–25

11.Guan J, Lim KS, Mekhail T, Chang CC. Programmed death ligand-1 (PDL1) expression in the programmed death receptor-1 (PD-1)/PD-L1 blockade: a key player against various cancers. Arch Pathol Lab Med. 2017;141:851–861.

12.Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366:2443–2454.

13.Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366:2455–2465.

14.Lipson EJ, Forde PM, Hammers HJ, Emens LA, Taube JM, Topalian SL. Antagonists of PD-1 and PD-L1 in cancer treatment. Semin Oncol. 2015;42:587–600.

15.Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348:124–128.

16.Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371:2189–2199.

17.Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015;350:207–211.

18.Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, et al.

Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med. 2017;9:34.

19.Jamieson NB, Maker AV. Gene-expression profiling to predict responsiveness to immunotherapy. Cancer Gene Ther. 2017;24:134–140.

20.Palmieri G, Colombino M, Cossu A, Marchetti A, Botti G, Ascierto PA. Genetic instability and increased mutational load: which diagnostic tool best direct patients with cancer to immunotherapy? J Transl Med. 2017;15:17.

21.Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372:2509–2520.

22.Dudley JC, Lin MT, Le DT, Eshleman JR. Microsatellite instability as a biomarker for PD-1 blockade. Clin Cancer Res. 2016;22:813–820.

23.Naboush A, Roman CA, Shapira I. Immune checkpoint inhibitors in malignancies with mismatch repair deficiency: a review of the state of the current knowledge. J Investig Med. 2017;65:754–758.

24.Samowitz WS, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. Cancer Epidemiol Biomarkers Prev. 2001;10:917–923.

25.Hiroyuki Y, Yoshiyuki W, Tadateru M, Kohzoh I, Fumio I. Microsatellite instability in cancer: a novel landscape for diagnostic and therapeutic approach. Arch Toxicol. 2020;94:3349–3357.

26.Keitaro S. Concordance analysis of microsatellite instability status between polymerase chain reaction based testing and next generation sequencing for solid tumors. Sci Rep. 2021;11:20003.

27.Hirotsu Y, Nagakubo Y, Amemiya K, Shinozaki E, Mochizuki H, Omata Masao. Microsatellite instability status is determined by targeted sequencing with MSIcall in 25 cancer types. Clin Chim Acta. 2020;502:207–213.

28.Yamamoto H, Imai K. An updated review of microsatellite instability in the era of next-generation sequencing and precision medicine. Semin Oncol. 2019;46:261–270.

29.Palomaki GE, McClain MR, Melillo S, Hampel HL, Thibodeau SN, Schaefer G. EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. Genet Med. 2009;11:42–65.

30.Shia J, Ellis NA, Klimstra DS. The utility of immunohistochemical detection of DNA mismatch repair gene proteins. Virchows Arch. 2004;445:431–441.

31.Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn. 2008;10:293–300.

32.Lynch HT, Snyder CL, Shaw TG. Milestones of Lynch syndrome: 1895–2015. Nat Rev Cancer. 2015;15:181–194.

33.Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010;138:2073–2087.

34.Liisa C. Microsatellite instability: a predictive biomarker for cancer immunotherapy. Appl Immunohistochem Mol Morphol. 2018;26:e15–e21.

35.Haraldsdottir S, Hampel H, Tomsic J, Frankel WL, Pearlman R, de la Chapelle A, et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. Gastroenterology. 2014;147:1308-1316.e1.

36.Gelsomino F, Barbolini M, Spallanzani A, Pugliese G, Cascinu S. The evolving role of microsatellite instability in colorectal cancer: a review. Cancer Treat Rev. 2016;51:19-26.

37.Mensenkamp AR, Vogelaar IP, van Zelst-Stams WA, Goossens M, Ouchene H, Hendriks-Cornelissen SJ, et al. Somatic mutations in MLH1 and MSH2 are a frequent cause of mismatch-repair deficiency in Lynch syndrome-like tumors. Gastroenterology. 2014;146:643-646.e8.

38.McCarthy AJ, Capo-Chichi JM, Spence T, Grenier S, Stockley T, Kamel-Reid S, et al. Heterogenous loss of mismatch repair (MMR) protein expression: a challenge for immunohistochemical interpretation and microsatellite instability (MSI) evaluation. J Pathol Clin Res. 2019;5:115-129.

39.Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. Nucleic Acids Res. 2019;47:D941-D947.