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Chapter 3

Epimutation in DNA Mismatch Repair (MMR) Genes

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1. Introduction

Generally, disease susceptibility is determined based on changes not only in DNA sequences but also in the activities of genes and chromosomal regions. Epigenetic regulation has attracted attention as a mechanism underlying changes of activities of genes and chromosomal regions. Epigenetic modification regulates gene activity and is essential for cell division and histogenesis. Genetically, phenotype diversity of identical cells is thought to be caused by differences in epigenetic profiles. Epimutations have also recently been recognized as the first step of tumorigenesis of cancers and are thought to be direct dispositions to cancers [1].

2. What is epimutation?

Epimutation affects one or both alleles and decreases the gene product by inhibiting transcription. Tumor cells are typical examples of the results of epimutation that occurs at a high frequency in mammals. Epimutation in cancer generally occurs in somatic cells with tumor progression. Various epimutations are present in cancers and are frequently observed in tumor suppressor genes [1-4].

Germline epimutation which occurs in germ cells is defined as those changes maintained in fertilization and embryogenesis and present in all somatic cells in the mature body. Transmission of epigenetic characteristics through generations has been reported. The cancer risk is similar in individuals carrying a germline epimutation. However, epimutation is not nec-

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essarily inherited, and inheritance patterns that do not follow Mendel's laws have been reported [5-8]. Complete elimination of epimutation in spermatogenesis has also been shown [9]. Only inheritance of maternal epimutation has been confirmed, suggesting that elimination of epimutation in oogenesis is less likely to occur [8-9]. Several genomic imprinting-associated somatic cell abnormalities are thought to be caused by germline epimutation [4]. Constitutional epimutation is defined as those changes observed in all tissues in the body due to occurrence in an early step of embryogenesis before differentiation into the three germ layers. Not all cells possess this type of epimutation, leading to a mosaic pattern at the cell level, and it is unclear if this epimutation is transmitted from the previous generation. All epimutation types are a first step leading to tumorigenesis and may be direct causes of carcinogenesis [1].

3. Germline epimutation and disease

Epimutation is not only involved in cancer, but is also observed in genomic imprinting (Table 1). Since a gene transmitted from one parent is selectively expressed in genomic imprinting, a hereditary disease develops when the gene is defective, even though the allelic gene is normal. The characteristic phenotype of genomic imprinting is maintained by imprinting control centers (ICs). ICs are short sequences present in the gene to be imprinted. Hemiallelic methylation of ICs results in transcription of the other allele, controlling imprinting [1]. Diverse gene aberrations in these ICs, such as micro defects, have been discovered, and these are considered to be the causes of epimutations observed in very rare neurobehavioral congenital familial diseases such as Angelman syndrome (AS), Prader-Willi syndrome (PWS), and Beckwith-Wiedemann syndrome (BWS). PWS is characterized by hypotonia in the neonatal period, increased appetite, overeating and subsequent obesity after infancy, characteristic desires, mild mental retardation, and hypoplasia of the external genitalia. In contrast, AS is characterized by severe mental retardation, epilepsy, and awkward movement. However, the causative genetic locus is located in the q11-q13 region on the long arm of chromosome 15 in both diseases. PWS and AS are caused by chromosomal 15q11q13 deletion in many cases, but there are a few cases of imprinting mutation causing abnormal genomic imprinting. In imprinting mutation, the parental chromosome is normal, but the imprinting of 15q11-q13 is changed to the opposite pattern. Familial cases of imprinting mutations are known, and minute deletions upstream of the *SNURF-SNRPN* gene, which has ICs in PWS and AS, have been described [10]. However, ICs are resistant to minute changes or contain several extra elements, and most imprinting mutations are thought to occur due to epimutation after fertilization [11].

BWS is a congenital disease with a high reported risk of embryonal fetal tumors, such as Wilms tumor, hepatoblastoma, and rhabdomyosarcoma. The p15.5 region on the short arm of chromosome 11 (11p15.5) has been identified as the causative locus. There are two imprinting domains in 11p15.5: the C*yclin-dependent kinase inhibitor 1C/KCNQ1 opposite antisense transcript 1(CDKN1C/KCNQ1OT1)* domain and the *Insulin-like growth factor 2(IGF2)/H19* domain, and expression of the imprinting gene near the domain is controlled by the respective imprinting

regulation region. *CDKN1C* expression is decreased due to DNA hypomethylation of the *CDKN1C/KCNQ1OT1* domain in about 30-50% of BWS cases, and *IGF2* expression is enhanced due to DNA hypermethylation of the *IGF2/H19* domain in about 5-10% [12]. Silver-Russell syndrome (SRS) is characterized by intrauterine growth restriction and severe failure to thrive after birth, and epimutation of the *H19* gene in 11p15.5 is the cause of this disease [13]. *IGF2* and *H19* are regulated by a common enhancer present in the terminal end of the short arm of chromosome 11. Normally, sperm-derived *H19–DMR* is methylated and ovum-derived *H19–DMR* is not methylated. The enhancer acts on *IGF2* because CTCF protein cannot bind to methylated DMR in the former case, whereas it acts on *H19* because CTCF protein binds to non-methylated DMR in the latter. Hypomethylation of sperm-derived H19-DMR due to epimutation causes the gene to behave similarly to the maternal domain and induces underexpression of *IGF2* and overexpression of *H19*, causing SRS due to *IGF2* underexpression [14]. Thus, these diseases are thought to develop due to aberration in ICs.

Gene name	Epimutation type	Disease
hMLH1	germline, constitutional	Lynch syndrome
hMSH2	germline	Lynch syndrome
DAPK1	unknown	B-cell CLL
HBA2	unknown	α-Thalassemia
BRCA2	constitutional	Sporadic breast cancer
KIP2/LIT1	unknown	Beckwith-Wiedemann syndrome
IGF2	unknown	Beckwith-Wiedemann syndrome
H19	unknown	Silver-Russell syndrome

Table 1. Epimutation and disease

Epimutation also occurs due to genomic changes, such as insertion, deletion, and changes in the length of tandem repeat sequences, which are termed copy number variations (CNVs) [15]. In α-thalassemia, another well-known epimutation-associated disease, the deleted region of the *LUC7-like (LUC7L)* gene is close to an α-globin gene, *hemoglobin alpha 2 (HBA2)*, leading to methylation of the *HBA2* gene promoter [16].

4. Epimutation of DNA mismatch repair genes

A study on familial cancer showed that a gene group inactivated by mutation in characteristic regions produces a predisposition to cancer. Mutation of a tumor suppressor gene, *Retinoblastoma (RB)*, provided the first evidence of a causative gene in hereditary cancer [17]. Subsequently, Nishishou et al. reported mutation of *Adenomatous polyposis coli (APC)* in familial adenomatous polyposis [17] and Hussussian et al. found mutation of *Cyclin-dependent kinase inhibitor2A (CDKN2A)* in familial melanoma [19]. As more mutations have been iden-

tified in tumor suppressor genes, the various cancer-associated mechanisms of these genes have been elucidated. Relationships of *Breast cancer susceptibility gene 1(BRCA1)*, *MutL protein homolog 1 (MLH1)*, and *MutS homologue 2 (MSH2)*, all of which are DNA repair genes (DNA mismatch repair: MMR), with predispositions to familial cancers have also been found. Mutation-induced gene inactivation in hereditary cancer is recessively inherited and many carriers have no abnormal phenotype. However, the cancer prevalence shows marked dominant inheritance because mutation, inactivation, and loss of heterozygosity readily occur in the normal allele [1].

Methylation of *RB* was the first reported cancer-inducing epimutation [19-20]. Later, methylation of many other oncogenes, such as *Von Hippel-Lindau (VHL), MLH1, APC,* and *BRCA1,* was shown in sporadic cancers [22-24]. *VHL* mutation is related to primary ciliary function, hemostasis of the extracellular matrix, tumor metabolism, and particularly to clear cell carcinoma [25]. Vaziri et al. examined the *VHL* gene in an analysis of the clonal relationship between the primary tumor and metastatic lesions of clear cell carcinoma in 10 patients. The gene status differed between the primary tumor and the metastatic lesions in 4 patients. In addition, even when the *VHL* genotype differed in another renal primary tumor or among several metastatic lesions within a patient, the *VHL* germline genotype in adjacent normal tissue was always the wild-type germline *VHL* gene in the primary tumor. These findings indicated that the status of *VHL* may differ between the primary tumor and metastatic lesions in clear cell carcinoma [26].

Regarding DNA repair genes, methylation of *MLH1* and *MSH2* has been reported to cause Lynch syndrome (hereditary non-polyposis colorectal cancer (HNPCC)). This methylation is also known as a predisposition to characteristic cancers, such as those in the endometrium, small intestine, and ovary, in addition to colon cancer. Both genes encode mismatch repair proteins and inactivation of these proteins is thought to induce microsatellite instability (MSI) in tumors [27]. MSI frequently occurs in endometrial cancer and accumulation of MSI-induced gene mutations plays a major role in carcinogenesis [28]. It has since been discovered that *MLH1* may also be methylated in sporadic colorectal cancer. In an investigation of methylation of the *MLH1* promoter in 110 patients with sporadic early-onset colorectal cancer, Auclair et al. found methylation in 55 (50%) and also observed decreased *MLH1* expression due to hypermethylation, which was present in 7.4% of all patients, suggesting that constitutional epimutation is the fundamental mechanism inducing early-onset colorectal cancer [29]. The phenotype of sporadic colorectal cancer with *MLH1* methylation is the same as that of mismatch repair defects, and the clinicopathological characteristics are similar to those of a hereditary tumor. *MLH1* methylation occurs in sporadic colorectal cancer at a high frequency [23] and is strongly related to cancers showing the CpG island methylator phenotype (CIMP). Methylation of CpG islands, which are characteristic of promoter regions, has been shown to occur at a high frequency in CIMP-positive cancer [30]. These cancers arise mainly from the ascending colon and have a particularly high incidence in elderly women.

Gazzoli et al. first demonstrated that *MLH1* may be methylated in peripheral blood, as in tumors, in colorectal cancer patients [31]. In an investigation of 14 Lynch syndrome patients

with MSI, no mismatch repair gene methylation was noted in any patient, but hypermethylation (about 50%) of *MLH1* was discovered in normal blood DNA in a 25-year-old female patient [31]. This allelic methylation in unrelated tissue derived from the embryologically different germ layer indicated that the methylation may be constitutional or germline. No conclusion could be reached with regard to the heredity of this epimutation because no mutation was detected in parental tissue, but the occurrence of methylation so early in life is of interest. A later study clarified that constitutional methylation occurs in colorectal cancer patients with hemiallelic methylation of *MLH1* [32], in 2 colorectal cancer patients. Tissues from parents were unavailable, but no methylation was observed in tissues in 4 of 5 children of these patients.

It remains unclear whether constitutional epimutation is transmitted from the mother or father or occurs *de novo* in early embryogenesis [1]. Crepin et al. investigated constitutional epimutations of *MLH1* and *MSH2* and defective *EPCAM* in 134 germline mutation-free patients with suspected Lynch syndrome, and found *MLH1* constitutional epimutation in 2 patients. One was a female patient, and her 2 children (one male and one female) developed early-onset colorectal cancer, suggesting that *MLH1* constitutional epimutation is related to inheritance. In addition, somatic cell *BRAF* mutation was found in one child, indicating that cancers in patients *with MLH1* constitutional epimutation are similar to *MSI-high* sporadic cancers [33]. In addition to reports supporting inheritance from the mother, Goel et al. described cases of epimutation of the paternal allele, in which analysis of the genotype showed that the inactivated T allele was inherited from the father [34]. Miyukura et al. showed that complete methylation of the *MLH1* promoter region plays an important role in inactivation of *MLH1* in sporadic colorectal cancer patients with high MSI [35]. This complete methylation was induced in both alleles, and methylation upstream of the *MLH1* promoter region was also observed in normal large intestinal mucosa adjacent to the cancer in one-third of colorectal cancer patients with complete methylation [36]. Subsequently, Miyukura et al. surveyed methylation of the *MLH1* promoter region in peripheral blood lymphocytes in 30 patients with sporadic early-onset colorectal cancer or multiple primary cancers, and found complete methylation of the *MLH1* promoter region in peripheral blood lymphocytes (PBLs) in 4 patients (early-onset sporadic colorectal cancer: 2, multiple cancers including colorectal cancer: 1, multiple cancers including cancer of the uterine body: 1) [37]. This was hemiallelic methylation. In one of the patients with early-onset sporadic colorectal cancer, no methylation was detected in a sister's PBLs. MSI was confirmed in all patients and methylation was also observed in the normal large intestine, gastrointestinal mucosa, endometrium, and bone marrow in 3. Interestingly, loss of heterozygosity (LOH), loss of the G allele of the *MLH1* locus in somatic cells, and biallelic methylation were observed when both alleles of *MLH1* in colorectal cancer were investigated, and these findings are consistent with the germline epimutation-associated cancerization mechanism based on Knudsen's "two hit" hypothesis proposed by Suter et al. (Figure 1) [31]. Furthermore, according to Kantelinen et al., variants of uncertain significance (VUS) of the mature hereditary MMR gene present in some colorectal cancer patients may form pairs with other MMR gene VUS and indirectly induce MMR deficiency. An analysis of 8 pairs of MMR gene mutations carried by cancer patients showed aberrations in 2 pairs. Pairs with *MSH2* may increase the cancer risk by reducing the repair

ability of the *wild-type MSH2* by half. Two *MSH6* mutations were MMR defects [38]. *MLH1* VUS has also been reported to influence mRNA transcription and impair MMR activity [39].

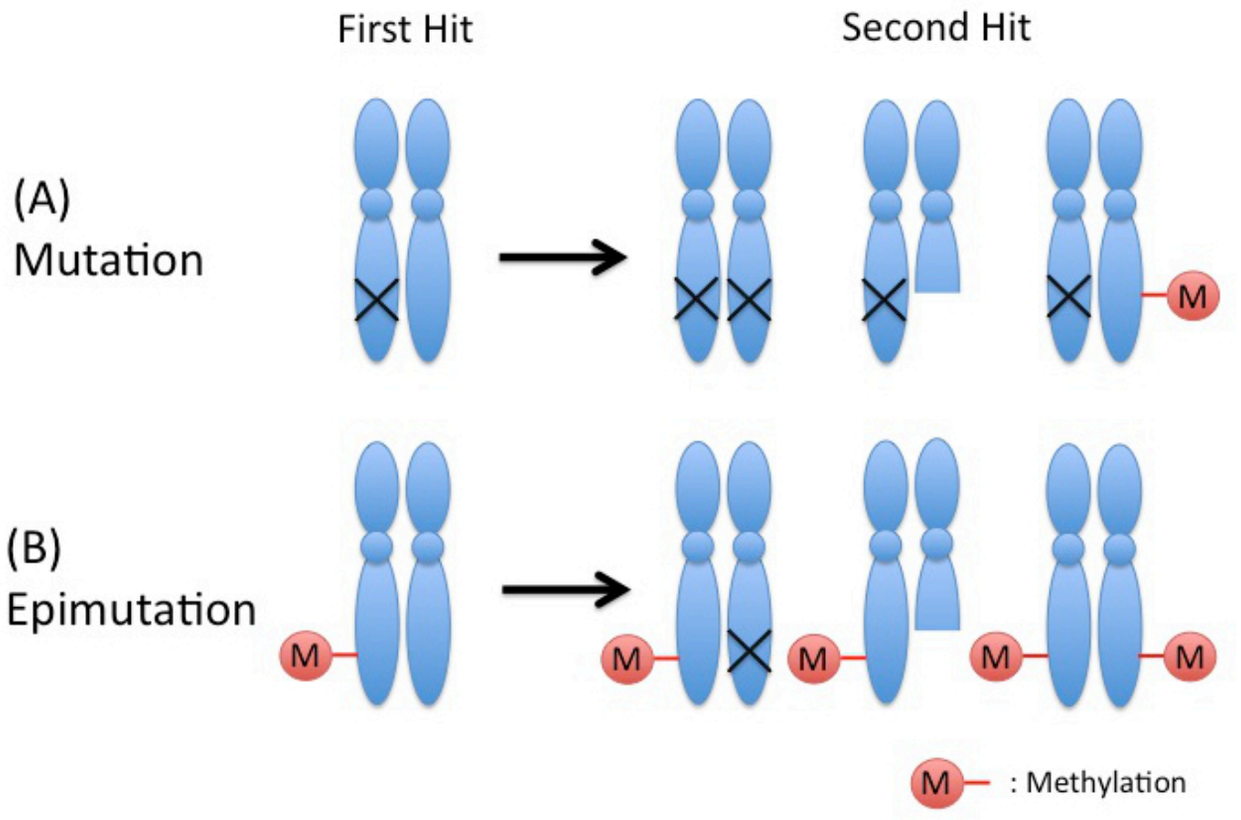


Figure 1. Mechanisms of epimutation in induction of cancer. (A) Germ cell mutation of tumor suppressor genes. (B) Germ cell epimutation of tumor suppressor genes. Somatic cell mutation, heterozygote loss, and other allele epimutations are triggers that induce tumorigenesis.

Allelic methylation is noted in many cases of Lynch syndrome, but there are some exceptions. Wu et al. investigated germline methylation of *MLH1* in 140 gastric cancer patients with a familial medical history. *MLH1* promoter methylation was detected in peripheral blood DNA in only 0.7% of the gastric cancer patients, and the methylation pattern of these patients was mosaic. Mosaic germline epimutation of *MLH1* occurs in familial gastric cancer, although the incidence is low [40]. Hitchins et al. found allelic *MLH1* epimutation in 2 cases in an investigation of constitutional *MLH1* methylation in white blood cell DNA in 122 ethnically diverse South African subjects aged ≤50 years old with early-onset colorectal cancer, with a few alleles showing a mosaic pattern [41].

Epimutation is not always inherited and inheritance patterns that do not follow Mendel's laws have been reported [5-8]. Complete elimination of epimutation in spermatogenesis has also been shown. Only inheritance of maternal epimutation has been found in previous re-

ports, suggesting that elimination of epimutation in oogenesis is less likely to occur [8-9]. In a cohort study of 160 Lynch syndrome patients without germline mutation of mismatch repair genes, constitutive *MLH1* methylation was induced in only one patient, and no *MLH1* methylation was found in the parents or siblings of this patient, indicating that clinicopathological characteristics are better indices than familial medical history for identification of constitutional epimutation of tumor suppressor genes in cancer patients [5]. In addition, Pineda et al. reported that it is useful to screen for *MLH1* methylation in lymphocyte DNA in patients with Lynch syndrome-related tumor with early *MLH1* methylation to judge the presence of epimutation [42].

Epimutation is also related to chronic lymphocytic leukemia (CLL), in which apoptosis of leukemia cells is strongly inhibited. Apoptosis inhibition in CLL is caused by enhanced B-cell lymphoma 2 (BCL2) production and methylation of the *Death-associated protein kinase1* (*DAPK1*) promoter region [44]. *DAPK1* was identified as a familial tumor suppressor gene and the *DAPK1* promoter region is methylated in CLL [44]. This methylation increases Homeobox B7 (HOXB7) protein binding upstream of the promoter region and 75% of *DAPK1* genes in the allele are downregulated. Methylation-induced *DAPK1* inactivation causes both familial and sporadic CLL, whereas hypomethylation of *DAPK1* in peripheral blood mononuclear cells (PBMCs) of healthy subjects has been reported [45]. An association of this hypomethylation with CLL has yet to be shown.

A recent study showed that a specific MMR gene is involved in regulation of cellular dynamics, such as apoptosis. Therefore, the action of specific MMR gene expression of *MSH2* and *MLH1* may also be important in resistance to cytotoxic drugs used in chemotherapy, such as cisplatin [46]. However, it has also been shown that MMR inactivation is not related to inherent cisplatin resistance of cells, suggesting that MMR inactivation may have a role in acquired drug resistance [47]. Involvement of impairment of the MMR pathway in aging of hematopoietic stem and precursor cells has also been reported. Kenyon et al. investigated MSI and MMR gene expression in hematopoietic stem, precursor, and colony-forming cells, and found that there were many *CD34(+)* precursors with MSI lacking *MLH1* expression and protein in hematopoietic colony-forming cells in subjects aged ≥45 years old, compared to younger subjects [48].

There have been many reports on the relationship of breast cancer with *BRCA1* mutation. Armes and Lakhani et al. showed that breast cancer arising in patients with germline *BRCA1* mutation has histological characteristics such as a high mitotic count and lymphocyte infiltration. This morphology is now referred to as the basal-like type, and Foulkes et al. found that this type accounted for 80-90% of cancers arising in germline *BRCA1* mutation carriers [49]. Methylation in the *BRCA1* promoter region in sporadic breast cancer was subsequently discovered [50] and this led to many studies on the association between *BRCA1* mutation and methylation. Under the hypothesis that a sporadic tumor with *BRCA1* methylation should be similar to tumors with *BRCA1* mutation if *BRCA1* methylation induces tumorigenesis, Cattear and Morris et al. reported that sporadic tumors with *BRCA1* methylation have pathological characteristics similar to those of hereditary breast cancer with *BRCA1* mutation [51].

Hedenfalk et al. also showed that the overall phenotypes of the gene were similar between the two breast cancer types [52]. Tumors accompanied by *BRCA1* methylation have a high grade, are negative for estrogen and progesterone receptors, and have a high incidence in young women. These features are referred to as *BRCA1*-like characteristics. Hedenfalk et al. also found *BRCA1* methylation at high frequencies of 67% in medullary carcinoma and 55% in mucinous carcinoma, and these histologic types were noted at high frequency in family lines carrying *BRCA1* mutations [52]. Recently, Snell et al. discovered methylation of the *BRCA1* promoter region in normal tissue of breast cancer patients with the *BRCA1*-like characteristic histologic type [53]. No germline mutation of *BRCA1* or *BRCA2* was detected in these patients. These findings suggest constitutional epimutation of *BRCA1* in breast cancer patients. It is thought that *BRCA1* methylation is the first hit and subsequent deletion of both *BRCA1* genes then leads to the characteristic tumor pathology [1].

MMR gene mutation-induced breast cancer in Lynch syndrome has also recently been described by Buerki et al. [54] in an investigation of 70 unrelated families with Lynch syndrome. The subjects were 632 females, of whom 51 and 40 carried *MLH1* and *MSH2* mutations, respectively. MMR impairment was detected in 85.7% (6/7) of molecular test-applicable breast cancer patients. Combined with information from related reports, *MSI* was present in 70.3% (26/37) of breast cancer patients with *MLH1* or *MSH2* mutation, and altered MMR protein expression was noted in 72.7% (16/22) [54]. Lotsair et al. also found that the ratio of breast cancer cases with MMR protein deficiency and *MSI*-induced MMR impairment was markedly higher in MMR mutant cases than in a non-mutant group. These findings suggest that MMR dysfunction is closely related to the development of breast cancer in Lynch syndrome. However, the development pattern and onset age of breast cancer in patients with MMR mutation are similar to those in general breast cancer patients without mutation. Moreover, the frequency of MMR protein deficiency is lower than those in other Lynch syndrome-related cancers [55].

5. Epimutation and Lynch syndrome

Lynch syndrome (HNPCC) is a typical familial tumor transmitted through autosomal dominant inheritance, and is observed in about 3% of cases of colorectal cancer [56]. MMR gene aberration is involved in carcinogenesis in Lynch syndrome. Six types of MMR genes have been cloned: *MSH2, MLH1, MutS protein homolog 3 (MSH3), MutS protein homolog 6 (MSH6), Postmeiotic segregation increased 1 (PMS1),* and *Postmeiotic segregation increased 1 (PMS2)*. Mutations of 3 of these genes (*MSH2, MLH1,* and *MSH6*) in family lines with Lynch syndrome have been reported [57], with *MSH2* and *MLH1* aberrations accounting for about 90%, and *MSH6* and *PMS2* gene aberrations accounted for only 7 and 1% of cases, respectively [57]. Thus, *MLH1* and *MSH2* mutations are particularly associated with Lynch syndrome. These mutations are also predispositions to cancers in the endometrium, small intestine, and ovary [1]. Both genes encode mismatch repair proteins, and inactivation of these proteins is thought to induce MSI in tumors [27]. Since microsatellites (short-tandem repeats, STRs) are

generally present in non-coding regions, mutations in STRs do not lead to abnormal protein production. However, some STRs are present in regions with important genes, such as those encoding *BCL2-associated X protein (BAX)*, which is involved in apoptosis induction, *Insulin-like growth factor 2 receptor (IGF2R)*, which is associated with inhibition of cell proliferation, and mutations in these regions are thought to be involved in cancerization of cells [1].

Typical cases of Lynch syndrome-related ovarian cancer develop early, and the tumor is FIGO cancer stage I and non-serous in many cases [58]. Grindedal et al. reported that the prognosis of Lynch syndrome-related invasive ovarian cancer is better than that of invasive cancer in patients carrying a *BRCA1/2* mutation [59]. Regarding endometrial cancer, Shih et al. investigated MMR protein deficiency in 56 women aged ≤40 years old with endometrial cancer, and found abnormal MMR in 9 cases. The families of these 9 patients had a medical history of Lynch syndrome; the mean BMIs were 23.4 and 31.2 in the patients with and without abnormal MMR, respectively; the stage was I in 80% of the cases in the patients without abnormal MMR, but ≥II in 90% of those with abnormal MMR; muscular layer and lymph vascular invasions were noted in many cases with abnormal MMR; and the 5-year/5-year exacerbation-free survival rate was 70% [60]. Many pathological aspects of familial endometrial cancer are unclear despite the high malignancy, and an effective screening method has yet to be established.

Lynch syndrome cases with epimutation of the *MLH1* or *MSH2* promoter region in blood cells without morbid MMR gene mutation have recently been discovered, showing that germline *MLH1* epimutation causes Lynch syndrome. Takahashi et al. reported that *MLH1* protein expression was deficient in Lynch syndrome patients carrying a germline mutation in the 5' splice site of *MLH1*, and that mutation of this intron of *MLH1* induced aberrant splicing, influencing the onset of Lynch syndrome [62]. In family lines with *MSH2* methylation, germline mutation of the *Epithelial cell adhesion molecule (EPCAM)* gene present upstream of *MSH2* has been reported to be the cause of epimutation. *EPCAM* is strongly expressed in epithelial tissue and cancers [63] and a defective 3'-terminal of this gene causes read-through to *MSH2*, resulting in hypermethylation of the CpG island promoter [64]. Interestingly, no *MSH2* methylation in any other cancer has been reported to date. In contrast to the allelic methylation found in many patients with constitutional methylation of *MLH1*, allelic methylation of *MSH2* occurs in only about 50%. This methylation level is also dependent on the tissues examined. Unlike *MLH1* epimutation, inheritance of *MSH2* methylation following Mendel's laws has been reported. In Lynch syndrome caused by these epimutations, methylation levels vary among epimutation carriers in the same family line and among tissues within the same patient [1]. In addition, the *MLH1* and *MSH2* mutations show racial differences. In a comparison of Asian and Western subjects based on International Society of Gastrointestinal Hereditary (InSiGHT) data, Wei et al. found differences in mutations in the regions containing *MLH1* and *MSH2*, with some mutations found to be more frequent or to be present only in Asian subjects [65]. This indicates the importance of consideration of racial differences in evaluating mutations in screening [65].

6. Conclusion

Epimutation has diverse characteristics: some epimutations are inherited or eliminated in embryogenesis, while others are inherited in patterns that do not follow Mendel's laws. Cancers associated with epimutations include Lynch syndrome (HNPCC), familial colorectal cancer, CLL, breast cancer, and ovarian cancer. Defined histological characteristics of epimutation-associated tumors have been suggested, and it is possible that the histologic type of cancers will ultimately be identifiable based on the methylation pattern detected in normal tissue, which may reduce the need for invasive tests such as tumor tissue biopsy [1]. Furthermore, elucidation of differences in the methylation pattern between healthy subjects and cancer patients may facilitate low-invasive cancer risk evaluation in healthy individuals.

To develop these techniques, it will be important to identify the causes of methylation. The extent of variation of methylation in normal somatic cell tissues within an individual is unclear, but conservation of the methylation pattern in an individual has been shown [1]. Different DNA methylation patterns in monozygotic twins have been observed, and the difference increased as the twins lived in different environments [66]. Aging-dependent methylation of non-methylated CpG islands has also been shown, and it has been suggested that metabolite ingestion can influence methyl metabolism, such as metabolism of folic acid, choline, vitamin B12, and betaine, and change the methylation pattern. In particular, the influence of environmental factors in early embryogenesis may serve as a predisposition to cancers and other diseases associated with epigenetic changes [67]. Methylation is influenced by environmental factors and aging, in addition to inheritance, as described above, and further studies on the association of these factors with epimutation are required.

Improvement of epigenetic aberration has also been attempted through induction of re-expression of tumor suppressor genes, with some success using DNA methyltransferase (DNMT) inhibitors, azacitidine and decitabine, for blood malignant tumors [68]. However, intense epigenetic therapy using a DNMT inhibitor and a histone deacetylase (HDAC) inhibitor concomitantly did not achieve complete chromosome remodeling, and stable gene re-expression was not obtained [9]. Moreover, reinhibition of re-expressed genes has occurred after suspension of epigenetic therapy in many studies. These findings indicate that there are many problems to be overcome in development of epigenetic therapy.

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