DNAMismatch Repair System Imbalances in Breast Adenocarcinoma

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Abstract. DNA mismatch repair system (MMR) is considered a leading genetic mechanism in stabilizing DNA structure and maintaining its function. DNA MMR is a highly conserved system in bacteria, prokaryotic, and eukaryotic cells, and provides the highest protection to DNA by repairing micro-structural alterations. DNA MMR proteins are involved in the detection and repair of intra-nucleotide base-to-base errors inside the complementary DNA strand recognizing the recently synthesized strand from the parental template. During DNA replication, a spectrum of errors including base insertion, deletion, and miss-incorporation negatively affect the molecule's structure and its functional stability. A broad spectrum of genomic alterations such as promoter hypermethylation, mutation, and loss of heterozygosity (LOH) in MMR genes including predominantly hMLH1, hMSH2, hMSH3, hMSH6, hPMS1, and hPMS2 lead to their loss of base-to-base error repairing procedure. Microsatellite instability (MSI) refers to the DNA MMR gene alterations that are observed in a variety of malignancies of different histological origins. In the current review, we present the role of DNA MMR deficiency in breast adenocarcinoma, a leading cancer-based cause of death in females worldwide.

Breast cancer (BrCa) is the prominent type of cancer in females worldwide and the second leading cause of cancer-related death, behind lung carcinoma (1). Concerning pathology categorization, primary breast carcinoma demonstrates a variety of types including ductal, lobular, medullary, mucinous, adenoid cystic, and papillary correlated to medium or high metastatic potential (2). Regarding its etiology, several socio-demographic and genetic risk factors (i.e., age, chronic smoking, and alcohol consumption), ovarian hormone over-expression/exposure to drug diethylstilbestrol (DES) or radiation therapy, combined or not with reproductive history and potential family history (inherent gene mutations) have been proposed to be involved in its development (3, 4). Additionally, BrCa is characterized by genetic diversity and epigenetic heterogeneity (5, 6). Specifically, altered expression...
of crucial molecules such as estrogen (ER) and progesterone (PR) receptors, HER2/neu, ki-67 proliferation marker, and p53 suppressor gene is detected (7). For this reason, BrCa categorization based on these proteins is critical for optimal oncological handling by designing and applying specific targeted and chemotherapeutic regimens. Besides them, new immunotherapy strategies are applied alone or in combination with radiotherapy (8, 9). HER2/ER/PR gene expression signatures are critical for the development of sporadic BrCa, whereas BrCa genes, such as BRCA1/2, are involved in hereditary/familial cases (10, 11). Interestingly, other genetic micro-markers include microsatellite instability (MSI), which refers to specific DNA mismatch repair (MMR) genes that are responsible for the detection and repair of nucleotide dissimilarities in the DNA (12). MSI is detected in a variety of malignancies independently of their histological origin (13). In the current molecular review, we describe the function and the role of DNA MMR deficiency in BrCa.

DNA Mismatch Repair System (DNA MMR): Landscape and Mechanisms

DNA is characterized by a high – level structural stability that secures its multiple normal functions. There are different genetic mechanisms that provide a stable micro-environment inside the molecule including DNA MMR, which plays a leading role. DNA MMR is a highly conserved and efficient system in a series of prokaryotic and eukaryotic cells (14). The ability of the corresponding genes to detect and repair DNA base errors is crucial for its homeostasis. The MMR system distinguishes the newly synthesized DNA strand from the parental, which is used as a template. A broad spectrum of intra-nucleotide errors such as base deletion, insertion, and mis-incorporation occur during DNA replication and recombination. Concerning mismatches, they are implicated in a base tautomerization process that takes place in the G2 phase. G/T or A/C pairing that represents frequently detected base abnormalities is repaired by firstly recognizing the deformity, focal excision of the invalid base, and its replacement with the appropriate one. In fact, a significant number of bases (extended to hundreds or thousands of pairs) must be excised from the newly synthesized DNA strand in comparison to the initial template for preventing inappropriate base matching (15).

Significant human DNA MMR gene homologues including MLH1, MSH2, MSH3, GTBP/MSH6, PMS1, and PMS2 are located on chromosomes 2, 3, 5, and 7. Furthermore, PCNA, RPA, HMG1, RFC DNA ligase I, and DNA polymerase delta genes are implicated in DNA structural stability and function, interacting with histone and chromatin domains. Specific genomic alterations such as germline mutations, usually accompanied by allelic loss (loss of heterozygosity-LOH), or epigenetic changes including promoter hypermethylation lead to loss of the expression of MMR genes. For this reason, base errors are not repaired (16). Inherited as well as acquired deficiencies in DNA MMR genes lead to MSI, a genetic phenomenon frequently detected in hereditary (familial) and sporadic colorectal carcinoma (CRC) types.

Microsatellites correspond to nucleotide sequences of 1 to 5 base pairs that are repeated typically 15–30 times and characterized by instability. Based on extensive genetic analyses, thousands of them are detectable throughout the human genome. During DNA replication, an increased microsatellite number is responsible for the formation of small loops in any of the two DNA strands (17, 18). The introns of genes demonstrate a variety of inserted or deleted microsatellites. MSI is used as a very promising, novel biomarker for detecting DNA MMR deficiency in colorectal carcinomas CRCs. Additionally, MSI is detected in a broad spectrum of malignancies characterized by different histogenetic origin (19). In the current review we focused on DNA MMR deficiency in lung and oral cavity carcinomas to identify mechanistic similarities and differences.

DNA MMR Alterations in BrCa

Alterations or different variants in DNA MMR genes are correlated with an elevated risk for BrCa development. A study group explored the role of specific MSH2 variants in women without BRCA1/BRCA2 gene mutations (20). They detected a combination of two mutations (p. Ala272Val and p. Met592Val) and designated them as potentially pathogenic in sub-groups of patients. They also suggested these two MSH2 gene variants to be analyzed as early genetic biomarkers in women with a family history of BrCa. Additionally, the involvement of MSH2 in neoplastic transformation of the breast epithelia is under investigation. A study group focused on MSH2 function and interactions- analyzed the formation of the MutSα/MutSβ complex in the cytoplasm and nucleus (21). They reported a higher nuclear/cytoplasmic K ratio for MutSα during progression from normal to cancerous cells. For this reason, they proposed MutSα elevated expression as a sensitive biochemical marker for early prediction of BrCa. Furthermore, increased MSH2 protein expression levels have been identified in the mononuclear fraction of the peripheral blood of BrCa patients (22). A study group detected high MSH2 expression in BrCa tissue sections in patients with increased mononuclear concentration associated also with lymph node metastasis. DNA MMR deficiency in familial/hereditary BrCa is correlated to specific single nucleotide polymorphisms (SNPs). A molecular analysis revealed two genetic alterations, MUTYH_rs3219489 and MSH2_rs2303428 variant alleles, which are associated with increased BrCa prevalence (23). Similarly, another study co-analyzed the potential impact of MMR genes (MLH1, MSH, and MSH3) on BrCa incidence as crucial genetic predisposition factors (24). They observed that XPC-Ala499Val, XPF-Arg415Gln, XPG-Asp1104His, and MLH1-Ile219Val SNPs in
MSH2 and MLH1 increased BrCa risk. Concerning the correlation between DNA MMR gene alterations and the histological origin of malignancies, a study analyzed the role of variations in MLH1, MSH2, and MSH6 in cancerous tissues derived from the colon, breast, ovaries, endometrium, and thyroid (25). They observed that MSH6 mutations followed by mutations in MLH1, MSH2 demonstrated the highest incidence in breast carcinomas. Additionally, germline monoallelic mutations in MSH6 and MHS2 were associated with inherited breast-ovarian carcinoma.

Estrogen/progesterone positive BrCas represent a specific category and the impact of DNA MMR gene mutations is under investigation. A study explored the potential usefulness of MMR gene testing in these tumors (26). Similarly, another study group applied a DNA MMR gene panel (MLH1, MSH2, MSH6, and PMS2) in familial BrCa cases. They detected eight pathogenic variants in the examined genes (27). Furthermore, MMR-deficient BrCas demonstrated resistance to specific chemotherapeutic agents such as aromatase inhibitors, but sensitivity to others (i.e., palbociclib) (28, 29).

A study group showed that MMR-deficient cases characterized by MSH2, MSH6, MLH1, and PMS2 loss of expression are eligible for an alternative treatment protocol based on CDK4 inhibitors (30). In conjunction, MMR-deficiency combined or not with epigenetic silencing of the gene O6-alkylguanine-DNA methyltransferase (MGMT) and BRCA1/2 gene mutations seem to negatively affect the response rates to specific chemotherapeutic agents in sporadic and hereditary BrCas (31). In BrCa cases related to Lynch syndrome (LS), especially in the triple-negative ones, the frequency and impact of DNA MMR deficiency is under investigation. LS is an autosomal dominantly inherited disorder derived from germline mutations in DNA MMR genes. A study group analyzing a significant number of them observed increased MSI and MLH1 promoter hypermethylation (32). Two studies showed higher DNA MMR deficiency in BrCa mutation carriers than in non-carriers (33, 34). Additionally, the involvement of germline mutations in DNA MMR genes in LS cases was the subject of another molecular study. By applying targeted next-generation sequencing (TNGS), a study group explored the mutational profile of MLH1, MSH2, MSH6, EPCAM, and PMS2 genes. They reported a high frequency of mutations in these genes in hereditary BrCa cases (35). Interestingly, another study analyzed LS-BrCa cases in a specific female population (Japanese) and observed a high MSI, especially in ER/PR+/HER2- cases (36). Besides LS-depended BrCa cases, carcinomas that demonstrate inactivation of critical suppressor genes - including phosphatase and tensin homolog (PTEN)- also involve DNA MMR deficiency (37, 38). A study group -based on this observation- formed a diagnostic algorithm to evaluate PTEN expression in MMR positive BrCas. Furthermore, specific MLH1 gene polymorphisms (rs63749795 and rs63749820) lead to MLH1 down-regulation and expression loss correlated to a high risk of BrCa onset (39). Concerning alternative molecules and mechanisms that are implicated in DNA repair, ubiquitination mediated by the ubiquitin-conjugating enzyme E2W (UBE2W) plays a major role in carcinomas including breast (40). A study group reported high UBE2W expression in patients with DNA MMR deficiency and suggested the molecule as a potential reliable biomarker for immune infiltration.

DNA MMR Deficiency in BrCa Immunotherapy Strategies

A variety of targeted therapies – monoclonal antibodies (mAbs) and tyrosine-kinase inhibitors (TKIs), which disrupt signal transduction pathways – have been applied in BrCa patients (41, 42). Besides them, novel immune checkpoint inhibitors that regulate immune system function are considered very promising agents (43). Programmed cell death-1 (PD-1) gene - located on chromosome 2 (gene locus: 2q37.3) - encodes a cell surface membrane protein of the immunoglobulin superfamily, which acts as an immune-inhibitory receptor implicated in tumor immune escape (44). PD-1 interacts with two potential ligands, PD-L1 and PD-L2 trans-membrane proteins (45). Programmed cell death ligand-1 (PD-L1), also known as CD274 (cytogenetic band: 9p24.1), is expressed in epithelial and hematopoietic cells as well as in thymus gland parenchyma. Furthermore, dendritic cells express PD-L1 modifying self-reactive T cell function. The PD-1/PD-L1 system promotes inhibition of T lymphocyte proliferation, survival, and cytokine release. Additionally, it induces tumor-specific T-dependent apoptosis and CD4+T cell differentiation. In fact, it enhances the resistance of tumor cells to cytotoxic T lymphocyte (CTL) lineage attack. PD-L1 over-expression induces inflammation in malignancies. Malignant cells also escape the host immune system by PD-L1 – mediated suppression of T cell activation (46).

The role of DNA MMR deficiency in anti-PD-L1 immunotherapy strategies is a novel and interesting field for research in BrCa (47). A study group reported low levels of MLH1 and PMS2 protein expression without hypermethylation of MLH1 gene promoter (48). Because a low MMR deficiency rate was detected in the examined malignancies, they did not propose a routine screening in these patients. Similarly, another study showed a few LS BrCa cases characterized by MMR deficiency, but one of them demonstrated strong response to anti-PD1 therapy (49). Another study group investigated the correlation between MMR-deficiency and PD-L1 in BrCa cases (50). They detected sub-groups of patients with complete or partial loss of MMR and/or high tumor-infiltrating lymphocytes (TILs) especially in triple-negative BrCas that were considered eligible for immunotherapy. Additionally, a series of studies
explored the impact of DNA MMR deficiency and MSI rates on PD-L1 expression in BrCas (51-54). They reported absence of DNA MMR deficiency and MSI in the examined cases, whereas PD-L1 positivity was prominent in TILs. In addition to these observations, other studies identified extremely low frequency of MSI in these malignancies with loss of MLH1/PMS2 proteins. They also concluded that in high-level TIL BrCas, MSI-H was absent. Furthermore, another study group focused on the role of potential germline mutations in MMR genes in the development of LS-related BrCa. They detected specific mutational signatures in DNA MMR genes correlated to PD-L1 positive cases (54).

In conclusion, a broad spectrum of genomic alterations such as promoter hyper methylation, mutations, and LOH in the MMR genes including predominantly hMLH1, hMSH2, hMSH3, hMSH6, hPMS1, and hPMS2 have been detected in BrCas. These alterations create specific MSI/MMR genetic signatures in subgroups of patients affecting potentially the response rates to targeted/immuno-therapeutic regimens. Understanding the nature and mechanisms of DNA MMR deficiency in BrCa cases, is a very interesting, significant, and promising field in BrCa molecular oncology.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors’ Contributions

GIE, ET: design of the study, ET, EF, MA, GIE, DD: manuscript writing: SM, DS, DP, CD: academic advisors: LM, PF, SM: collection and management of references’ data. All Authors read and approved the final manuscript.

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