

OVERVIEW OF MISMATCH REPAIR PATHWAY

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ABSTRACT

BACKGROUND

DNA mismatch repair (MMR) has an exclusive chapter in maintaining DNA replication fidelity, mutation avoidance and genome stability. At mechanistic level MMR is quite complex in eukaryotes than prokaryotes. In recent years, many MMR-deficient human cancers specifically hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome (LS), and associated endometrial cancer are allied to mutations in MMR genes. Most of the proteins involved in this pathway are predictably tumour suppressors and defects in the MMR genes convey a strong predisposition for cancer. This review summarizes the literature on MMR, emphasising the role of different MMR proteins in the repair pathway.

KEY WORDS

Mismatch, MSH2, PMS2, Repair.

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BACKGROUND

DNA mismatch repair pathway plays a pivotal role in maintaining the genomic integrity during DNA replication, repair and recombination by preventing DNA base mismatches, insertions or deletions. The integrity of the genomic structure is contingent on the precision with which DNA replication is carried out and post replication check out or surveillance. DNA polymerases proofread the replicated DNA and errors are corrected, tailed subsequently by quality control via DNA mismatch repair (MMR) pathway which restitutes the errors that had dodged the DNA polymerase proofreading. MMR makes out mispaired bases, chemically modified bases and insertion-deletion loops that arise during DNA replication and initiates DNA damage response pathway thus reducing the number of replication-associated errors and prevents mutations from becoming permanent in dividing cells. Shortcomings in MMR increase the rate of spontaneous mutations and microsatellite instability (MSI) resulting in hereditary and sporadic cancers in human cells. MSI is due to insertion-deletion loops subsequent to gain or loss of short repeat units within microsatellite sequence.

Mismatch Repair Proteins

The basic MMR machinery involves two families of proteins - MutS and MutL convoluted to form heterodimers in contrast to bacteria, which function as homodimers. Several phases of human MMR pathway are analogous to that in bacteria. Voluminous studies have been done in bacteria with still enigmatic areas in human MMR repair mechanism which is much more complex.

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The elementary method of operation is an excision-repair in which the recently synthesized strand is inspected for discrepancies and the section of the strand containing the inappropriate base is excised and resynthesized, expending a number of suitable DNA repair proteins.⁽¹⁾

Six MutS homologs are well-known in eukaryotes and these form three MutS complexes - MutS α , MutS β , and MutS γ . MutS α is a heterodimer of MSH2-MSH6 (also known as G:T-binding protein, GTBP); MutS β is a heterodimer of MSH2-MSH3 both of which are crucial for mispair recognition and initiation during the initial stages of MMR mechanism. A third MSH complex, MutS γ is a heterodimer of MSH4-MSH5. It has an imperative role in meiotic recombination and is not elucidated in any MMR-related function. MLH1, MLH3, PMS1, and PMS2 are the 4 recognized human MutL homologs. MLH1 heterodimerizes with PMS2, PMS1, or MLH3 to form MutL α (MLH1-PMS2), MutL β (MLH1-PMS1), or MutL γ (MLH1-MLH3). Of which homologs MutL α has an obligatory role in MMR whereas MutL γ is critical in meiosis, but no specific biological role has been identified for MutL β but is reflected as an add-on associate in MMR. Higher levels of MMR proteins are appreciated in multiplying cells than resting cells as replication errors primarily occur in proliferating cells and MMR is framed to correct these.^{(1),(2)}

Mismatch Repair Pathway

Recognition of Mismatched DNA

MutS α makes out base-base mismatches, single base-pair insertion/deletion loops whereas MutS β recognizes larger insertion/deletion loops i.e. more than one base-pair but less than 12- base-pair.⁽³⁾ The crystalline edifice of bacterial DNA has provided an understanding of the mechanism of mispair recognition by MutS. Each monomer of the homodimer contains six structural domains with discrete functions. Domain I corresponds to the mispair recognition by binding to the G: T mismatch and to the unpaired T; by way of only one subunit creating an asymmetry in the dimer. Eukaryotic, MutS is asymmetric because of the presence of heterodimers MSH2-MSH6 and MSH2-MSH3. MSH6 and MSH3 are

suggested to be the subunits involved in recognition of DNA mismatch. By lacking a large segment of domain, I, MSH4 and MSH5 are meiosis-specific and are not involved in mismatch recognition. The connector domain or the domain II interacts with MutL, domain III typifies the core domain and divides DNA binding and nucleotide binding sites, the periphery of the DNA clamp forms domain IV; domain V encompasses the ATPase domain and dimerization sites; and domain VI or helix-turn-helix domain (HTH) is obligatory for dimerization at moderate protein concentrations. In eukaryotes, similar to MutS, domain V of MSH6 and MSH2 contain ATPase domains that bind ATP with different kinetics and affinities.⁽⁴⁾

One stationary model⁽⁵⁾ and two moving models i.e. translocation model⁽⁶⁾ and the other called as molecular switch or sliding model⁽⁷⁾ have been proposed to explain the pathway of communication between DNA and MMR proteins. Recent studies are in favor of the sliding model which proposes that soon after DNA replication the heterodimer of MSH2-MSH6, is tethered to ADP and in an open configuration, scans the newly synthesized DNA for mispairs. Upon encountering a mispair, the heterodimer is anchored by Phe-X-Glu to the minor groove of the duplex at the mismatch site to ensign the assembly or nearby localization of the excision repair machinery. When the entire system is cumulated, then the exchange of ADP→ATP would be prompted and Phe-X-Glu is released from the site, because of changes in affinity. Then it shifts to a closed, sliding clamp that diffuses along the DNA. The MutS α moving in the 5' > 3' directions will eventually signal subsequent repair events. In both models, the role of MutL in downstream signaling and detailing of strand excision is not properly elaborated.^{(8),(9),(10)}

Strand Discrimination

The intermittent gaps or strand discontinuities in DNA in between the 5' or 3' ends of Okazaki fragments on lagging strand of DNA could discriminate parental from daughter strand and persuade the MMR reaction on the nascent strand.^{(11),(12),(13)} PCNA is loaded onto DNA at the pre-existing nick by RFC. PCNA is perhaps a multifunctional matchmaker protein that aids MMR apparatus to distinguish nascent and template strands. Moreover, PCNA intensifies the mismatch-binding specificity of MSH2-MSH6, and also contributes to the conveyance of MSH2-MSH6 onto the mismatched DNA alongside interacting with the mismatch excision enzyme EXO1.⁽¹⁴⁾

Recruitment of Repair Enzymes

The DNA: MutS α : ATP complex recruits the MutL α to form a ternary complex. This complexing is through its interaction with MutS α . MutL α serves as a connecting link between recognition and excision of a mismatch as it activates the endonuclease activity of PMS2. MutL α then diffuses along the DNA until it encounters PCNA that is loaded at a 3' terminus of a nearby nick by RFC. It then displaces the DNA polymerase and proliferating cell nuclear antigen (PCNA) from the nascent daughter strand, and recruits exonuclease I. The PMS2 subunit of MutL α possesses a cryptic endonuclease activity and brings about additional single strand breaks into the pre-nicked strand as soon as triggered.

Excision of the Mismatch

The heading of excision in 5' → 3' or 3' → 5' directions is determined by the position of the nick qualified to the

mismatch. Ternary clamps that propagate upstream from 3' → 5' terminus come across RFC that is tacked at the 5' terminus of the strand break and in due course displace it by loading EXO1. The degradation of the strand in a 5' → 3' direction commences once the exonuclease is activated. The single-stranded gap is stabilized by RPA which is a single strand binding protein and is a prerequisite to finish the excision process once the mismatch is removed. At this point, the activity of EXO1 is suppressed by MutL α , thus terminating DNA excision. Ternary Clamps that diffuse downstream 5' → 3' terminus come across PCNA molecule that is allied to the 3' terminus of the strand break. After several events of recruitment and loading, EXO1 is activated resulting in collapse of the region between the preliminary discontinuity and the mismatch. RFC that is allied at the 5' terminus of the break precludes further degradation in the 5' → 3' direction i.e. away from the mismatch. DNA bound RPA and MutL α constrain EXO1 activity the minute after the mismatch is removed. An extent of over 1,000 nucleotides in a single strand of DNA can be excised by EXO1 which is a 5' → 3' exonuclease extending from the nick to the mismatch and terminating about 150 nucleotides past the mismatch.⁽¹⁵⁾

DNA Resynthesis

Replicative polymerase Pol δ is then conscripted by the DNA bound PCNA to the 3' terminus of the preliminary discontinuity. This complex seals the gap and DNA ligase I patches up the remaining nick to complete the restorative process.^{(16),(17)}

Retrospective Analysis of MMR Proteins in Malignancy

Inactivation of MMR protein function is well known to have numerous biological consequences, including genome instability, resistance to DNA damaging agents including chemotherapeutic drugs,⁽¹⁸⁾ altered class switch recombination and somatic hypermutation of immunoglobulin genes,⁽¹⁹⁾ emergence of pathogenic bacteria, infertility, promotes TNR expansion^{(20),(21)} and increased susceptibility to cancer.⁽²²⁾ Imbalance in the expression of MMR proteins essentially an increased copy number are linked to enhanced mutation rates and development of cancer. Heterocomplexes of MMR proteins, MSH2: MSH6 and MLH1: PMS2 display a pattern of increased expression within the nucleus from G₁ through S phase and remain relatively high into G₂ phase.^{(23),(24)} MSH2 levels increase during the replicative and post-replicative levels of the cell cycle that is at least 12-fold increase in proliferating cells in contrast to resting cells.⁽²⁵⁾

Pereira et al,⁽²⁶⁾ investigated the expression of MSH2 in head and neck squamous cell carcinoma (HNSCC) and established a down-regulation in 9% of the samples. Their scrutiny revealed that reduced MSH2 expression in HNSCC tissues was associated with poor overall survival rate and might contribute to high genomic instability resulting in a worse prognosis. Supporting this PMS2-deficient mouse have shown to have decreased MMR efficiency, increased microsatellite instability, as well as an increased susceptibility to develop sarcomas and lymphomas (Baker et al. 1995; Prolla et al. 1998).

Increased expression of MSH2 in tongue squamous cell carcinoma (SCC) was significantly associated with reduced depth of invasion, no evidence of muscular invasion

prompting the fact that MSH2 increase may be an elicitation of efforts to repair DNA damage in order to impede the advancement of the tumour. But on the contrary, there was also a significant association with shorter disease-free patients and diffuse tumour shape suggesting a highly invasive tumour pattern. Based on these results it was concluded that MSH2 may not be directly related to prognosis but as this protein is related to many other processes, this increase may be linked to their activation.⁽²⁷⁾

With the increase in grades of Oral squamous cell carcinoma (OSCC) that is as the tumour becomes less differentiated MSH2 expression is decreased,⁽²⁸⁾ contrariwise P53 expression increases.⁽²⁹⁾ Studies have shown that MutS α and MutL α deficient cells are defective in cell cycle arrest in response to DNA damage probably because the cells deficient in MMR fail to phosphorylate p53 and p73 emanating subversive mutations. Majority of erstwhile data suggest that MMR deficiency is associated with increasing grades i.e. more aggressive tumours and poor prognosis. In contrary to this Kato et al., 2015⁽³⁰⁾, established that MMR deficiency was indicative of a good prognosis in endometrial cancers probably due to sensitivity to adjuvant radiotherapy. Furthermore, high MSH6 was associated with an increased risk of death from primary melanoma.⁽³¹⁾ MutS α i.e. MSH6 and MSH2 complex overexpression also obliges as an independent prognostic factor for poor overall survival in patients with OSCC although higher hMSH6 expression was only associated with poor prognosis, whereas MSH2 expression was not (Wagner et al., 2016)⁽³²⁾.

Immunohistochemical staining with MSH2 and MLH1 in samples obtained from low and high risk areas of development of oral cancer in smokers and non-smokers, disclosed normal expression in the basal and suprabasal layers of the squamous epithelium and MSH2 immunoreactivity was not directly influenced by demographic factors, tobacco smoking or inflammation.⁽³³⁾ Majority of OSCC tissue samples analyzed showed low to no immunohistochemical expression of MLH1 or MSH2 and promoter hypermethylation of MLH1 or MSH2 was detected in 50% of the DNA.⁽³⁴⁾ MSH2 with different methylation status in OSCC or head and squamous cell carcinoma was also reported in a number of other studies.^{(35),(36),(37)}

The expression of MMR proteins was not significantly different between benign and malignant salivary gland tumours.^{(38),(39)} The expression of MMR proteins was lower in severe dysplasia.^(40,41) Moreover, their expression also decreased in potentially malignant disorders like leukoplakia.^{(42),(43),(28)} Lower expression of MMR proteins was evidenced in oral melanomas⁽⁴⁴⁾ which also correlated with high aneuploidy ratio.⁽⁴⁵⁾ The utility of a two-antibody panel i.e. PMS2 and MSH6 in colorectal carcinoma and extraintestinal tumours such as intestinal carcinomas, endometrial carcinomas, and skin sebaceous neoplasms was reviewed and its applicability was stamped by a loss of expression of MLH1 or PMS2 and MSH2 or MSH6 in almost all the cases.⁽⁴⁶⁾ The incidence of cancer was high in germline mutation carriers of DNA mismatch repair genes. Most noteworthy was the involvement of 8 or more organ sites, signifying a need to develop methods to screen for extracolonic cancer also.⁽⁴⁷⁾

High microsatellite instability (MSI) was associated with high frequency of absence or abnormal expression of MSH2,

MSH6 and PMS2 proteins in isolation or in combination.^{(47),(48)} Gill et al,⁽⁴⁸⁾ also suggested that loss of PMS2 was more likely linked to a germline mutation rather than to the presence of a somatic aetiology. Alternatively, mutations in MLH1 can also secondarily lead to loss of PMS2 expression while retaining MLH1 immunohistochemical expression. High frequency of PMS2 deficiency is evidenced in CRC's when compared to other proteins possibly because (a) in most of the studies the screening was focused on subjects belonging to families with an obvious history of CRC, (b) PMS2 staining was not included in many screening studies based on the unfounded credence of PMS2 having a minor role in MMR, and (c) mutation detection was complicated by the presence of PMS2 pseudogenes. MSH6 mutations are associated with markedly lower cancer risks than MLH1 or MSH2 mutations.^{(49),(50)} The magnitude of colorectal cancer risk in MLH1 and MSH2 mutation carriers was same, whereas MLH1 carriers had a slightly higher risk of endometrial cancer^{(51),(52)} MSH2 deficiency was associated with more extracolonic cancer types like stomach, small bowel, and prostate in comparison to the families with MLH1 or MSH6 defects.

It has been established that colorectal cancers (CRC) tend to arise in field defects that are deficient in DNA repair and deficiencies in expression of PMS2, ERCC1 and XPF proteins are frequently early, and often coordinated with progressive stages of colon cancer.⁽⁵³⁾ MLH1/MSH2 immunoreactivity in CRC was related to several pathologic features like the tumour site, tumour type, grade of differentiation, nodal status, Crohn's like lymphoid reaction, DNA ploidy pattern and p53 protein expression. Thus MLH1/MSH2 immunoreactivity can be used as a prognostic evaluator for the management of stage II and III colorectal cancer patients.⁽⁵⁴⁾ Reduced MSH2 protein expression has also been shown to be of unfavorable prognostic value in prostate cancer,⁽⁵⁵⁾ soft tissue sarcoma, and biliary tract carcinoma.

Lynch syndrome or hereditary nonpolyposis colorectal cancer (CRC) is an inherited autosomal dominant disorder characterized by the development of CRC and other visceral malignancies due to deleterious germline mutations in the DNA mismatch repair (MMR) genes like MLH1, MLH2, MSH6, and PMS2. De Jesus-Monge et al analyzed MLH1 and MSH2 proteins by IHC and determined 4.3% prevalence of MMR deficiency among 164 Puerto Rican patients with the majority of cases attributable to Lynch syndrome, and this low prevalence rate than other population was attributed to partial MMR protein testing. Lynch syndrome-related CRC was characterized by microsatellite instability and absence of MMR protein expression in the associated tumour.^{(56),(52)} Most of the studies in the literature are pertaining to high-risk population predisposing to CRC and hence Lynch syndrome is underdiagnosed in the general population. Abnormal IHC staining results should be pursued with additional genetic testing irrespective of families meeting the clinical criteria for the diagnosis of Lynch syndrome.⁽⁵⁷⁾ Erstwhile data indicate that MMR deficiency is associated with progression of carcinogenesis and more aggressive tumours.

Thus, the cardinal task of MMR is to correct the replication errors and a flawed MMR would marshal elevated spontaneous mutations especially transitions, transversions, and frameshifts. More recently, a surfeit number of cancers

including colon, endometrium, prostate, head and neck, stomach, ovarian, breast, and pancreatic have been associated with defects in the MMR mechanism. Few of the MMR proteins are also widely-used as a diagnostic biomarker for human cancers. Apart from increased susceptibility to cancer due to genome instability inactivation of MMR proteins are acknowledged to have numerous biological consequences.

Conclusion and Perspectives

In summary, this review highlights the precise mechanism of MMR and its essential role in upholding genome stability. Diverse proteins work hand in hand in each phase of MMR to sustain the accuracy of DNA. Implication of MMR proteins in a number of tumours reflects the need to elucidate it from a clinical view point.

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