Human mismatch repair, drug-induced DNA damage, and secondary cancer

Peter Karran a,*, Judith Offman a, Margherita Bignami b

a Cancer Research UK, London Research Institute, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts EN6 3LD, UK
b Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Received 29 September 2003; accepted 8 October 2003

Abstract

DNA mismatch repair (MMR) is an important replication error avoidance mechanism that prevents mutation. The association of defective MMR with familial and sporadic gastrointestinal and endometrial cancer has been acknowledged for some years. More recently, it has become apparent that MMR defects are common in acute myeloid leukaemia/myelodysplastic syndrome (AML/MDS) that follows successful chemotherapy for a primary malignancy. Therapy-related haematological malignancies are often associated with treatment with alkylating agents. Their frequency is increasing and they now account for at least 10% of all AML cases. There is also evidence for an association between MMR deficient AML/MDS and immunosuppressive treatment with thiopurine drugs. Here we review how MMR interacts with alkylating agent and thiopurine-induced DNA damage and suggest possible ways in which MMR defects may arise in therapy-related AML/MDS.

Keywords: MMR; DNA damage; Cancer

1. Introduction

The contribution of defective mismatch repair (MMR) to the development of human cancer has been acknowledged for more than a decade (for recent review see [1]). Tumours with the microsatellite instability (MSI) that is characteristic of inactive MMR occur in both familial and sporadic settings. In the former, genetic inactivation of MMR by combined germline and somatic mutations is represented by the widely studied HNPCC syndrome. In sporadic cancers, the MMR defect is generally the result of epigenetic gene silencing (see for example [2,3]). More recently, attention has been focused on the interaction between MMR and DNA damage. Active MMR is implicated in the lethal effects of some DNA damage and abrogated MMR is clearly linked to a type of drug resistance or DNA damage tolerance [4,5]. Because an MMR defect does not confer any obvious selective growth advantage whereas tolerance to DNA damage might, it has been suggested that selection for resistance to endogenous or environmentally produced DNA damage may contribute to the development of MMR defective tumours [4,6]. Chemotherapy provides one of the most obvious examples of deliberately induced DNA damage and most anticancer therapeutic agents inflict it, either directly or indirectly. There is a growing awareness that MMR defects are widespread among secondary cancers that arise after successful chemotherapy. Acute myeloid leukaemia/myelodysplastic syndrome (AML/MDS) is one of the most common forms of secondary cancer and now comprises around 10% of AML cases [7]. We have suggested [8] that this AML/MDS may sometimes reflect the clonal expansion of cells with significant resistance to therapy-inflicted potentially lethal DNA damage. In this review, we examine the circumstantial and experimental evidence for this proposal. We also review recent findings relating cellular MMR capacity and responses to drug-induced DNA damage that may shed new light on the association of MMR defects with therapy-related cancer.

* Corresponding author. Tel.: +44-207-269-3870; fax: +44-207-269-3812.
E-mail address: peter.karran@cancer.org.uk (P. Karran).

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doi:10.1016/j.biochi.2003.10.007
2. The role of MMR in spontaneous mutation avoidance

MMR—along with DNA replication proof-reading—ensures an acceptably low level of spontaneous mutation. Both MMR and proof-reading correct the misinsertion errors committed by the replicative DNA polymerases and restore normal Watson–Crick base pairs. The MMR pathway also prevents recombination between slightly divergent DNA (homologous) sequences with a degree of non-identity that exceeds a defined threshold. MMR is a DNA excision repair pathway. In common with nucleotide excision (NER) and base excision repair (BER), recognition and removal of the targeted sequence—in this case a mispair or structural anomaly produced by DNA strand misalignment—precedes its replacement by non-semiconservative DNA synthesis. The MMR repair tract is long—one or two orders of magnitude longer than those associated with NER or BER, respectively. MMR is particularly adept at correcting structural anomalies that occur during the replication of repetitive mono- or dinucleotide regions of DNA. If left uncorrected, these aberrant structures—generally envisioned as single or tandem bases that become extrahelical because of misalignment of reiterative template and daughter DNA strands—cause heritable increases or decreases in the length of the repetitive tract. In expressed genes, this results in frameshifts and in truncated, inactive proteins. In non-expressed microsatellite regions, it causes MSI. Frameshifts in repetitive regions of expressed genes and MSI are the defining features of MMR deficiency.

3. How MMR processes DNA mismatches?

Mismatch correction is initiated by the binding of one of two mismatch recognition heterodimers, which have partially overlapping specificities. hMutSα comprising the hMSH2 and hMSH6 proteins, has a particular affinity for single base mispairs and structural anomalies involving mono- or dinucleotide repeats. hMSH2 forms a second heterodimer with the hMSH3 protein. This hMutSβ complex, which accounts for around 10% of the cell’s hMSH2, has a preference for rather more complex structures. A degree of functional redundancy between the hMutSα and hMutSβ heterodimers ensures that correction of all common replication errors is initiated efficiently but since most of the major replication errors are recognised by hMutSα, hMSH2 and hMSH6 are more important than hMSH3 in this process. Mismatch recognition precedes the recruitment of hMutLα, a heterodimer of hMLH1 and hPMS2. This rather enigmatic factor appears to provide a molecular coupling between the recognition factors and the downstream proteins involved in the excision and replacement steps of repair. Although there are similar factors, notably hMutLβ, comprising hMLH1 and the related hMLH3, there appears to be less redundancy in this step of repair. For most MMR events, the important proteins are hMSH2, hMLH1, hMSH6, and hPMS2.

4. MMR processes some damaged DNA bases into lethal lesions

hMutSα recognises, and MMR processes, some types of DNA damage. DNA O6-methylguanine (O6-meGua) is selectively lethal in MMR proficient cells. Cells that do not express O6-meGua-DNA methyltransferase (MGMT) that directly reverses DNA O6-meGua by in situ demethylation are extremely sensitive to methylating agents like N-methyl-N-nitrosurea (MNU) or N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). Full resistance to—but not repair of—DNA O6-meGua is restored by inactivating one of the genes encoding a component of hMutSα or hMutLα [4]. This phenomenon is known as methylation tolerance. MMR processes DNA that contains O6-meGua base pairs. In an in vitro MMR system, O6-meGua:T pairs in which O6-meGua in the nicked strand of a circular DNA heteroduplex (analogous to the daughter strand) is paired to T in an uninterrupted strand (analogous to the template), are efficiently corrected to A:T pairs. O6-meGua:C pairs in the same configuration are restored to G:C with a lower efficiency that is consistent with their poorer recognition by hMutSα [9,10]. Although strictly not a biologically relevant experiment because the O6-meGua is not in the template DNA strand, it is nevertheless important because it establishes the principle that O6-meGua base pairs provoke full processing by MMR—not just recognition. When the methylated base is in the daughter DNA strand, processing proceeds to a successful conclusion. When the miscoding O6-meGua is in the template strand and not, therefore, designated for removal, it is easy to envisage how complications might arise to prevent completion of processing and generate unresolved intermediates. We have suggested that the inability of O6-meGua to form a structurally acceptable base pair with incoming nucleotides during the repair process underlies the cytotoxicity of the O6-meGua/MMR interaction. The interaction of MMR with O6-meGua is quite selective. MMR is not implicated in lethal processing of other common DNA methylation products, such as 7-meGua or 3-meAde, that are produced along with O6-meGua. Whatever the mechanism of DNA lesion recognition by hMutSα, MMR clearly does not provide a general DNA damage recognition system.

In addition to extreme tolerance of DNA methylation damage, MMR defective cells are resistant to the base analogue 6-thioguanine (6-TG) [11,12]. The 6-TG resistance conferred by an MMR defect is substantial (about 5–10-fold). The toxicity of 6-TG requires its conversion into 6-TG nucleotides and incorporation into nucleic acids, particularly into DNA. Surprisingly, it is a methylated form of DNA 6-TG, 6-thiomethylguanine (S6-meGua), produced by rare in situ methylation, that interacts with MMR [13]. S6-meGua-containing pairs, like O6-meGua-containing base pairs, are recognised by hMutSα [14]. Furthermore, just as O6-meGua:T is a better substrate for hMutSα recognition and for correction, the S6-meGua:T pair is recognised more efficiently by hMutSα than S6-meGua:C. Both S6-meGua:T and
O\textsuperscript{6}-meGua:T base pairs can only be formed by replication of the methylated bases. This requirement for replication to produce the ‘DNA damage’ that provokes DNA damage responses—including MMR—is consistent with the delayed biological effects of methylating agent [15] or 6-TG [16] treatment. It seems likely that the interaction of MMR with DNA methylation damage simply reflects the recognition of replication ‘errors’. In order to interact with MMR, the initial DNA lesions must be capable of replication and the products must bear sufficient resemblance to a normal replication error to trigger hMutS\textsuperscript{a} recognition and subsequent processing. Of the numerous DNA lesions produced by MNU and MNNG, only O\textsuperscript{6}-meGua, meets these criteria. Other methylated bases are either not efficiently replicated (3-meAde) or are not perceived as mismatches when they are (7-meGua, O\textsuperscript{2}-meThy). Significantly, the ethyl counterpart of O\textsuperscript{6}-meGua, O\textsuperscript{6}-ethylGua, which is not efficiently bypassed during DNA replication, is not processed by MMR into a cytotoxic lesion and repair defective cells are only slightly tolerant to N-ethyl-N-nitrosourea (ENU) and not significantly to chloroethylating agents [17].

5. Some MMR processing of damaged DNA bases is not associated with cell death

In addition to substrates containing O\textsuperscript{6}-meGua and S\textsuperscript{6}-meGua, purified hMutS\textsuperscript{a} is able to bind to synthetic DNA duplexes containing various other single DNA lesions including 8-oxoguanine (8-oxoGua) [18], UV photoproducts [19], 1,2-dipurinyl cisplatin intrastrand crosslinks [10,20], C8-guanylaminofluorenes or acetylaminofluorenes [21], and benz(a)pyrene adducts [22]. Binding in vitro does not necessarily indicate that these lesions are processed in vivo, however, and the extent to which MMR is involved in converting diverse DNA lesions into lethal damage has been the subject of debate. As indicated in the preceding section, we believe that a significant degree of lethal processing only occurs with a limited range of damaged bases of which O\textsuperscript{6}-meGua and the closely related S\textsuperscript{6}-meGua are the only unequivocally documented representatives. Lethal processing of other DNA lesions may differ mechanistically and is generally of minor significance since the survival advantages conferred by inactive MMR are slight.

On the other hand, there is increasing evidence that MMR does process a variety of DNA lesions, with the notable exceptions of O\textsuperscript{6}-meGua produced by treatment with methylating agents and S\textsuperscript{6}-meGua introduced via 6-TG, this need not result in significant lethality. It can, however, have a major impact on mutation and MMR deficient cells are at increased risk of being mutated by DNA damaging treatments to which they are not significantly tolerant. We will call this enhanced susceptibility to DNA damage-induced mutation that is superimposed on the already high spontaneous mutation rate of MMR defective cells a supermutator phenotype. We suggest that it might be more appropriate to direct attention to the drug-related supermutator phenotype of MMR defective cells rather than the minor effects on cell survival that characterise the majority of DNA damage/MMR interactions.

6. MMR defects, DNA damage and cancer

It has been proposed that some form of DNA damage might be involved in the development of cancer in HNPCC...
individuals [4,6]. The suggestion was advanced to confront the tricky problem that loss of MMR appears incompatible with the clonal evolution of tumours in which successive mutational events each confer a growth advantage. In the absence of selective pressure, inactivation of MMR might be expected to be neutral or even to impart a loss of fitness [34]. Until these putative sources of DNA damage have been identified, a role for DNA damage in the development of repair deficient primary cancers remains speculative. There is, however, mounting evidence to implicate DNA damage in the development of MMR defective secondary cancers.

7. Therapy-related AML/MDS with microsatellite instability

7.1. Methylating agents and thiopurines—selection of drug-resistant cells

Secondary AML/MDS is much more likely to be MSI+ than de novo AML and it is generally accepted that around 50% of therapy-related AML/MDS is MSI+ (Table 1). (To put this figure into perspective, the incidence of MSI in sporadic colorectal cancer—where it is considered to be prevalent—is usually estimated to be around 15% [35,36]). The vast majority of patients with secondary cancer have received some kind of chemo- and/or radiation therapy. Importantly, ≤5% of de novo AML/MDS cases are MSI. This firmly implicates DNA damaging therapeutic treatment in the development of most MMR defective AML/MDS. Repair defects tend to be associated with chemotherapy rather than radiation treatment [8]. There is no particular association with AML subclass.

Therapy-related AML/MDS is also associated with thiopurine therapy for non-malignant conditions. 6-TG, 6-mercaptopurine (6-MP) and azathioprine (AZA, a 6-MP prodrug) are immunosuppressants. They are prescribed for autoimmune conditions, including rheumatoid arthritis and colitis. Some of the reports linking AML/MDS and AZA treatment for autoimmune disorders are listed in Table 2. Extended treatment with AZA, usually combined with steroids and cyclosporin A, has also been almost mandatory in immunosuppressive regimes for organ transplant patients. We have recently demonstrated a considerably increased risk of AML among cardiac and renal transplant patients. Significantly, the majority of transplant-related AML/MDS cases examined were MSI+ (Offman et al., submitted for publication). Although there is considerable evidence that immunosuppression per se plays an important part in the development of virally associated cancer in transplant patients (see for example [37]), this does not provide a ready explanation for the high frequency of MSI+ AML/MDS. Since 6-TG treatment selects MMR defective clones from cultures of human cells, we have suggested that transplant-related MSI+ AML/MDS may reflect the selection and clonal expansion of rare thiopurine resistant MSI+ myeloid precursor cells [38] (Fig. 1).

Recent experimental evidence provides some support for the suggestion that the clonal expansion of MMR defective myeloid cells is indeed favoured by drug treatment. When mice with reconstituted bone marrow containing a mixture of wild-type and msh2−/− cells were treated with temozolomide—a clinical methylating drug—the proportion of MMR deficient marrow cells was significantly increased [39]. The same experiments also provided evidence for the reduced fitness of msh2−/− cells in the absence of this particular selective pressure. It would certainly be interesting to examine the impact of thiopurines in the same experimental system.

Additional circumstantial evidence implicating selection of MSI+ myeloid precursor cells in the development of MSI+ AML/MDS is listed below:

i. Therapy-related AML/MDS is particularly associated with treatment of Hodgkin’s disease and non-Hodgkin’s lymphoma. Standard therapy for these conditions has often included methylating agents [40].

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| Table 1: Microsatellite instability in therapy-related and de novo AML/MDS |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Author                      | Number of markers analysed | t-AML/MDS % MSI or Number of cases | de novo AML/MDS % MSI or Number of cases |
| Ben-Yehuda et al. [70]      | 8                          | 94 or 16                      |                                            |
| Boyer et al. [71]           | 14 including Bat26          | 0 or 14 or 0                 | 34 or 0                                  |
| Horiike et al. [72]         | 12 including Bat26          | 20 or 10                     |                                            |
| Das-Gupta et al. [73]       | 11                          | 21 or 9 or 19                | 10 or 21 or 21                       |
| Sheikhhia et al. [74]       | 12 including Bat26          | 53 or 19 or 21               | 21 or 63                          |
| Olipitz et al. [69]         | 7 or 13 including Bat26     | 14 or 37                     | 37 or 37                                         |
| Casorelli et al. [8]        | 5 including Bat26          | 67 or 25                     | 25 or 0                                  |
| Worrollow et al. [42]       | Bat25, Bat26, Bat16        | 38 or 46                     | 46 or 0                                  |
| Sill et al. [76]            | 12                          | 0 or 20                      |                                            |
| Ohyashiki et al. [77]       | 7                          | 9 or 43                      |                                            |
| Indraccolo et al. [78]      | 10                          | 10 or 29                     |                                            |
| Rimsza et al. [79]          | Bat25, Bat26, Bat40        | 0 or 90                      |                                            |
| Olipitz et al. [75]         | 6 including Bat26          | 0 or 20                      |                                            |
| Ribeiro et al. [80]         | 5                          | 0 or 14                      |                                            |

* Mostly elderly de novo AML/MDS patients.
Table 2
AML/MDS in patients treated for autoimmune disorders

<table>
<thead>
<tr>
<th>Author</th>
<th>Number of cases</th>
<th>Primary disease/transplant</th>
<th>Sex</th>
<th>Total dose Aza/MP</th>
<th>Other treatment</th>
<th>Duration Aza (months)</th>
<th>Latency (months)</th>
<th>AML FAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maldonado et al.</td>
<td>1</td>
<td>Pyoderma Gangrenosum</td>
<td>M</td>
<td>2.5 mg/kg per day</td>
<td>Amphotericin B</td>
<td>9</td>
<td>45</td>
<td>AL NOS</td>
</tr>
<tr>
<td>Cobau et al.</td>
<td>1</td>
<td>RA/Vas</td>
<td>M</td>
<td>~50 g Aza</td>
<td>CTX, MTX, Pred</td>
<td>24</td>
<td>144</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Silvergleid and Schrier</td>
<td>1</td>
<td>Chronic active hepatitis</td>
<td>M</td>
<td>147 g Aza</td>
<td>Pred</td>
<td>47</td>
<td>53</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Roberts and Bell</td>
<td>1</td>
<td>Renal disease (oedema and proteinuria)</td>
<td>M</td>
<td>~387 g Aza</td>
<td>Pred, CTX</td>
<td>~84</td>
<td>86</td>
<td>AMML</td>
</tr>
<tr>
<td>Seidenfeld et al.</td>
<td>2</td>
<td>RA</td>
<td>F</td>
<td>113 g Aza</td>
<td>Gold, steroids, salicylates, CTX, Salicylates, steroids, phenylbutazone, chloroquine Steroids, ACTH</td>
<td>34</td>
<td>168</td>
<td>AL NOS</td>
</tr>
<tr>
<td>Cobau et al.</td>
<td>1</td>
<td>RA</td>
<td>M</td>
<td>~50 g Aza</td>
<td>Pred</td>
<td>24</td>
<td>144</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Silvergleid and Schrier</td>
<td>1</td>
<td>RA</td>
<td>F</td>
<td>99 g Aza</td>
<td>Steroids, ACTH</td>
<td>22</td>
<td>360</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Alexon and Brandt</td>
<td>1</td>
<td>RA/SLE</td>
<td>M</td>
<td>52 g Aza</td>
<td>Aspirin, colin/sodium salicylate, indomethacin, hydroxychloroquin, penicillamine, digoxin, diuretics, Pred</td>
<td>10</td>
<td>31</td>
<td>AMML</td>
</tr>
<tr>
<td>Vissmans et al.</td>
<td>1</td>
<td>SLE</td>
<td>F</td>
<td>272 g Aza</td>
<td>Pred</td>
<td>72</td>
<td>112</td>
<td>Subacute myelomonocytic leukaemia</td>
</tr>
<tr>
<td>Pikler et al.</td>
<td>1</td>
<td>SLE, CKT</td>
<td>F</td>
<td>~180 g Aza</td>
<td>Pred, ATG, Solu Medrol, radiotherapy+perhaps other cytotoxic drugs</td>
<td>45</td>
<td>49</td>
<td>AML M5</td>
</tr>
<tr>
<td>Matteson et al.</td>
<td>1/530</td>
<td>RA</td>
<td>M</td>
<td>Aza (dose ns)</td>
<td>Steroids</td>
<td>Ns</td>
<td>216</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Vasquez et al.</td>
<td>1</td>
<td>SLE</td>
<td>F</td>
<td>27 g Aza</td>
<td>Steroids, sulphasalazine, tetracycline, sulphasalazine, pred, ferrous/zinc sulphate</td>
<td>3</td>
<td>22</td>
<td>AML M5</td>
</tr>
<tr>
<td>Krishnan et al.</td>
<td>1</td>
<td>Polymyositis</td>
<td>M</td>
<td>~520 g Aza</td>
<td>Pred, thyroxine replacement</td>
<td>96</td>
<td>96</td>
<td>AML M0</td>
</tr>
<tr>
<td>Dombret and Maroleau</td>
<td>2</td>
<td>Crohn’s disease</td>
<td>M</td>
<td>Aza (dose ns), 6-MP (dose ns)</td>
<td>Steroids</td>
<td>Ns</td>
<td>216</td>
<td>AML M1</td>
</tr>
<tr>
<td>Heizer and Peterson</td>
<td>1</td>
<td>Crohn’s disease</td>
<td>M</td>
<td>~687 g MP</td>
<td>Steroids, sulphasalazine, tetracycline, sulphasalazine, pred, ferrous/zinc sulphate</td>
<td>3</td>
<td>22</td>
<td>AML M5</td>
</tr>
<tr>
<td>Kwong et al.</td>
<td>3</td>
<td>RA</td>
<td>F</td>
<td>260 g Aza</td>
<td>Anti-inflammatory agents</td>
<td>101</td>
<td>180</td>
<td>AML M2</td>
</tr>
<tr>
<td>Arnold et al.</td>
<td>1</td>
<td>Dermatomyositis</td>
<td>F</td>
<td>109 g Aza</td>
<td>Pred, enalapril, bendroluazide</td>
<td>24</td>
<td>27</td>
<td>AML M2</td>
</tr>
<tr>
<td>Asten et al.</td>
<td>6/1289</td>
<td>RA</td>
<td>F</td>
<td>Aza (dose ns)</td>
<td>Clb</td>
<td>28</td>
<td>95</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Mauritson et al.</td>
<td>7</td>
<td>Arth</td>
<td>F</td>
<td>140 g Aza</td>
<td>MTX, Clb</td>
<td>70</td>
<td>70</td>
<td>AML M5</td>
</tr>
<tr>
<td>Asten et al.</td>
<td>6/1289</td>
<td>RA</td>
<td>F</td>
<td>Aza (dose ns)</td>
<td>Clb</td>
<td>25</td>
<td>40</td>
<td>AML NOS</td>
</tr>
<tr>
<td>Kwong et al.</td>
<td>3</td>
<td>SLE</td>
<td>F</td>
<td>37 g Aza</td>
<td>CTX, allopurinol</td>
<td>15</td>
<td>54</td>
<td>AML M2</td>
</tr>
<tr>
<td>Arnold et al.</td>
<td>1</td>
<td>SLE</td>
<td>F</td>
<td>89 g Aza</td>
<td>Steroids</td>
<td>90</td>
<td>108</td>
<td>AML M3</td>
</tr>
<tr>
<td>Vismans et al.</td>
<td>1</td>
<td>SLE</td>
<td>M</td>
<td>Aza (dose ns)</td>
<td>Steroids, sulphasalazine, tetracycline, sulphasalazine, pred, ferrous/zinc sulphate</td>
<td>142</td>
<td>444</td>
<td>AML NOS</td>
</tr>
</tbody>
</table>

F, female; M, male; ns, not specified; MP, 6-mercaptopurine; Aza, azathioprine; MTX, methotrexate; CTX, cyclophosphamide; ACTH, adrenocorticotropic hormone; Pred, prednisone; ATG, antithymocyte globulin; Clb, chlorambucil; Gn, glomerulonephritis;VAS, vasculitis; CTD, connective tissue disease; SLE, Systemic Lupus Erythematosus; RA, rheumatoid arthritis; Arth, arthrosis/arthritis; CKT, Cadaver kidney transplant; PMR, polymyalgia; MS, multiple sclerosis; RARS, refractory anaemia with ringed sideroblasts; RAEB, refractory anaemia with excess of blasts; AL, acute leukaemia; AMML, acute myelomonocytic leukaemia; AML, acute myeloid leukaemia; AML NOS, acute myeloid leukaemia not otherwise specified.
ii. Myeloid cells have low levels of the MGMT that provides the first-line protection against the cytotoxicity of methylating drugs. This makes the bone marrow particularly sensitive to methylating agents and vulnerable to selection for inactive MMR\[41\].

iii. A rare polymorphism (–6 exon 13 T → C) in the DNA binding domain of hMSH2 that is expected to reduce its MMR efficiency is particularly associated with tAML/MDS after methylating agent chemotherapy [42].

iv. The bone marrow is also more sensitive to thiopurines than other tissues and myelotoxicity is an established dose-limiting factor. This defines bone marrow cells as likely targets for thiopurine-induced selection [43,44].

v. A low level of thiopurine methyltransferase (TPMT) is associated with an increased risk of thiopurine-related AML/MDS. TPMT catalyses the catabolism of thiopurines. Patients with a low level accumulate higher levels of 6-TG nucleotides and, presumably, DNA 6-TG [45].

vi. Although thiopurines and methylating drugs are very different—the former react directly with DNA whereas the latter are scavenged by the purine salvage pathway for metabolic incorporation into DNA—they are united by a shared ability to form methylated DNA bases that interact with MMR and promote MMR inactivation.

vii. Therapy-related AML/MDS after alkylating agent treatment is associated with particular karyotypic abnormalities involving loss of all or part of chromosomes 5 and 7 [46,47]. Similar chromosome 5/7 changes occur frequently among thiopurine-related AML/MDS cases. Thus, if the documented ability of methylating agents and 6-TG to select for MMR defective variants of cultured human tumour cells applies in myeloid cells of patients, it can provide a plausible explanation for the association of MSI with methylating agents and by thiopurines (the supermutator phenotype). The contribution from drug-induced mutation might be particularly significant: cancer patients undergo numerous cycles of treatment and thiopurine immunosuppression for organ transplant patients is lifelong.
7.2. Other alkylating agents—MMR defects and enhanced drug-induced mutation and carcinogenesis

Direct selection of drug-resistant MSI\(^+\) myeloid cells provides a coherent explanation for MSI\(^+\) AML/MDS after treatment with methylyating agents or thiopurines. This is clearly not the full story because MSI\(^+\) therapy-related AML/MDS is not confined to patients treated with these drugs. It occurs following treatment with other alkylating agents to which a MMR defect may not furnish a significant selective survival advantage. Therapy frequently involves simultaneous treatment with a combination of different agents. Although it is possible that a significant selective growth advantage might accrue from their additive effects, recent findings from experiments with MMR deficient KO mice suggest a more plausible alternative hypothesis. They indicate that, even in the absence of a selective growth advantage, the supermutator phenotype of MMR defective cells may have a significant impact on cancer.

MMR KO mice are highly susceptible to tumour induction by methylyating agents. MNU treatment increases the already high incidence of lymphoma in msh2\(^{-/-}\) mice [48]. Similarly, treatment with dimethylhydrazine, an acknowledged colon carcinogen, produces more lymphoma and colorectal tumours in msh2\(^{-/-}\) mice than in their MMR proficient counterparts [49]. There is increasing evidence that this increased susceptibility to drug-induced cancer is not confined to agents to which MMR defects confer tolerance, however. For example, the supermutator phenotype of ENU treated msh2\(^{-/-}\) mouse cells is paralleled by a heightened susceptibility of msh2\(^{-/-}\) mice to tumour induction by the same drug [28].

The apparently paradoxical interaction between MMR and NER defects in UVB-induced skin cancer in mice may provide a second example of the contribution of the DNA damage related supermutator phenotype to carcinogenesis. Although MMR defects are not associated with significant cellular UV resistance, msh2\(^{-/-}\) mice nevertheless display a predisposition to UV light induced skin tumours that is additive with xpa\(^{-/-}\) or xpc\(^{-/-}\) [50, 51]. An increased susceptibility of msh2\(^{-/-}\) skin cells to mutation by UV provides a simple explanation for this enhanced cancer susceptibility. Finally, chronic inflammation-related colitis is associated with a higher incidence of colorectal cancer and high-grade dysplasia in msh2\(^{-/-}\) mice than in wild-type animals [52]. Although the involvement of DNA lesions in this chemically induced inflammation has not been formally demonstrated, it seems likely that oxidised DNA bases are an important factor. This may be another example of the impact of a DNA damage related supermutator phenotype on the early events in carcinogenesis.

Thus, it seems feasible that the supermutator phenotype that accompanies DNA damage in a MMR defective cell will contribute to the development of MSI\(^+\) therapy-related cancer (Fig. 1). For most types of DNA damage, the contribution of enhanced survival to selection of the MSI\(^+\) myeloid clone will be minimal. The hypermutability of drug-treated MSI\(^+\) myeloid cells significantly increases the probability that they, rather than their MMR proficient counterparts, will sustain the advantageous mutations/genetic rearrangements that confer loss of growth control. The resulting AML/MDS will be MSI\(^+\).

8. The impact of reduced levels of MMR on various MMR-related functions

Recent findings indicate that changes in MMR protein levels do not have a uniform influence on the different processes in which MMR is involved. The differential impact of reduced MMR capacity has particular implications for MMR defective cells exposed to DNA damaging drugs, and possibly for the development of treatment related malignancy.

There is no evidence of haploinsufficiency for mismatch correction in humans [53, 54]: normal tissue from both hMSH2 and hMLH1 heterozygous individuals is fully MMR competent and does not exhibit MSI. An early study did not find any evidence for increased sensitivity to drug-induced chromosome aberrations in lymphoid cell lines established from HPNCC family members bearing either an hMLH1 or hMLH1 mutation [55]. More recently, it has been shown that hMSH2 protein levels are around 50% of normal in lymphoblastoid cells established from hM SH2 heterozygotes. This does not dramatically compromise their MMR and there is a very modest reduction in mismatch correction activity and mismatch binding by cell extracts. These cells are, however, significantly tolerant to the methylating agent temozolomide [56]. Thus, for lethal processing of DNA O\(^6\)-meGua, there is evidence of haploinsufficiency for hMLH2. (Curiously, this does not apply to hMLH1. hMLH1 protein levels in heterozygous cells are not significantly lower than normal, MMR is unaffected, and they are not methylation tolerant.) Experiments in which MSI\(^+\) cell lines have been engineered to express a low level of the missing MMR activity provide additional evidence that reduced MMR capacity is compatible with efficient DNA mismatch correction but not with DNA damage processing. Thus, 20% of the normal level of hMSH6 expressed in defective HCT15 cells significantly ameliorated their mutator phenotype and MSI. This low level of expression also largely restored normal levels of in vitro mismatch binding and repair capability. Despite the restoration of reasonably effective MMR, the extreme methylation tolerance of the HCT15 cells was unaffected [57]. Methylation tolerance is also associated with reduced levels of hMutLa. A low level of hMLH1 expression in MMR-defective 293 human embryonic kidney cells was sufficient to correct their MSI\(^+\) and fully complemented the mismatch correction defect of cell extracts. The same low hMLH1 levels did not restore MNNG sensitivity and the cells retained full methylation tolerance [58].}

A reduced level of the mouse msh2 protein also has a different impact on DNA damage processing and correction.
of replication errors. The effect of heterozygosity on drug resistance is less apparent in mouse cells. msh2+/– cells do not have an overt methylation tolerant phenotype [59] but they are, however, more resistant than msh2+/+ cells to the induction of sister chromatid exchanges by MNU treatment—a very sensitive indicator of MMR-mediated processing of DNA O6-meGua [60]. A more profound reduction in msh2 protein is associated with methylation tolerance. msh2+/– cells that express 10% of the WT level of msh2 (msh2low) are fully MNNG tolerant indicating that the corresponding level of mMutSα is insufficient to initiate lethal processing of O6-meGua. The minimal msh2 expression in msh2low– cells nevertheless reverses their mutator phenotype, suppresses homologous recombination, and prevents MSI. Significantly, in addition to methylation tolerance, msh2low– cells retain the supermutator phenotype of msh2+/– cells and are particularly susceptible to mutation induction by MNNG treatment [61].

Although data are scarce, there is some evidence that heterozygous KO MMR mice may be more susceptible to drug-induced cancer. Heterozygous animals are not generally regarded as more susceptible than wild-type mice to death from spontaneous cancer although msh2+/– mice develop more tumours than msh2+/+ animals. The excess tumours tend to be non-gastrointestinal and appear to retain the active copy of msh2 [62]. Heterozygosity for msh2 affects carcinogen susceptibility. DMH treatment produced more tumours and a somewhat different spectrum of tumour types in msh2+/– compared to msh2+/+ animals [49]. On the other hand, examination of mutation frequencies in colonic crypts of drug-treated mhs2+/– mice did not provide evidence of increased sensitivity to induced mutagenesis. MNU treatment induces more thymic lymphoma in mgmt+/–mlh1+/– mice and these animals are less sensitive than the mgmt+/–mlh1+/+ controls to the lethal effects of the drug [63]. MNU treatment also causes significantly more gastrointestinal tumours in pms2+/– mice than in pms2+/+ animals. In this case there was no effect on overall survival, however, and mice of both genotypes died at the same rate [64]. There are two significant caveats, however. Mouse cells are often intrinsically more methylation tolerant than human cells and inactivation of MMR increases their methylation resistance only marginally [65]. This is consistent with the less dramatic impact of heterozygosity for MMR functions on methylation tolerance in mouse cells. In the absence of carcinogen treatment, MMR heterozygous mice are not notably prone to gastrointestinal cancer. In this regard, they are not a particularly good analogy for human heterozygotes in HNPCC. In contrast, MMR null mice more faithfully recapitulate the cancer predisposition of MMR null humans [66–68]. Thus, although homozygous MMR deficient mouse and human cells share many properties, it may be inappropriate to extrapolate from the heterozygous mouse to humans.

It is clear that more studies are needed to define the properties of human cells with heterozygous or significantly reduced MMR levels. If the preliminary indications that a low MMR capacity does not impair normal repair functions but does incapacitate lethal processing of drug-induced DNA damage are substantiated, these findings have significant implications for therapy-related cancer. If human and mouse cells do maintain considerable reserve MMR capability, the limits of MMR capacity are unlikely to be breached by the ‘normal’ substrates of MMR—replication errors or mis-paired recombination intermediates. Exposure to cytotoxic drugs is not a ‘normal’ situation. Cells with a reduced MMR capacity resemble those in which repair is completely abrogated, they are resistant to and/or hypermutable by drug-induced DNA damage. Thus, although fully competent at repair of replication errors, individuals with reduced MMR will be at increased risk of cancer following drug treatment (Fig. 1). It is easy to envisage that MMR capacity might be reduced by low penetrance mutations or by certain combinations of MMR gene polymorphisms. Importantly, because normal MMR functions would not be overtly impaired, the cancer cells would not have a spontaneous mutator phenotype and the tumour would not be MSI+. Thus, subtle MMR defects—from reduced MMR protein expression—that might not necessarily contribute sufficiently to primary malignancy to be classified as HNPCC, may nevertheless have a significant impact on therapy-related cancer. These subtle MMR deficiencies might be invisible to current analytical approaches.

9. Concluding remarks

In this brief review, we have addressed the increasingly apparent association of MMR defects and secondary cancer. Because most therapeutic treatments produce DNA damage, we have summarised recent studies of the interaction of MMR with DNA damage. It is clear that normal and MMR repair defective cells respond differently to DNA damage. This has important implications for therapy-related cancer. In extreme cases—represented by methylating drugs or thiopurines—a combination of high level resistance and mutability associated with a repair deficiency provides a plausible explanation for the association of MSI with tAML/MDS. For other drugs, the findings that MMR defective cells are hypermutable by DNA damaging agents to which they are not significantly tolerant should shift the emphasis away from the, often small, changes in DNA damage tolerance that accompany inactivation of MMR and focus attention on the increased susceptibility of repair defective cells to DNA damage-induced mutation. It remains to be seen whether this supermutator phenotype may be sufficient in itself to contribute significantly to the high frequency of MSI among therapy-related AML/MDS cases.

The second factor that has implications for therapy-related cancer is the differential impact of reduced—but not zero—repair capacity on the response to ‘normal’ DNA mismatches and to DNA damage. A significantly reduced MMR capability is probably not detrimental under normal circumstances but it may be following exposure to DNA damaging
drugs. Since the frequency of hMSH2 heterozygotes in the general population is quite high [36], and there is also evidence that some hMSH2 polymorphisms may be associated with less than full activity, it is obviously important to extend the interesting preliminary studies on methylating agents and to look at the responses of cells from heterozygous individuals or those with MMR gene polymorphisms to the toxic and mutagenic effects of other therapeutic drugs. From a clinical perspective, the high frequency of MSI among tAML/MDS cases is now well established. As a next step, retrospective studies to examine the frequency of MMR gene (particularly hMSH2) heterozygotes and the incidence of MMR gene polymorphisms among individuals who developed tAML/MDS would be worth considering. Already there are some intriguing observations. Olipitz et al. [69] reported that the primary tumour of three of four patients who developed MSI tAML, was also MSI. If these findings are confirmed on a wider scale, they have important implications for chemotherapy in individuals heterozygous for a MMR gene. There is also a recent report that a particular hMSH2 polymorphism is significant overrepresented in tAML/MDS following methylating agent treatment [40].

AML/MDS arises in myeloid precursor/stem cells. We have summarised the evidence that suggests that myeloid cells are likely to be particularly vulnerable to methylating agents and thiopurines. What is needed now is a thorough examination of the DNA repair capabilities of myeloid precursor/stem cells. Ideally, this would address the question of the variation of DNA repair, and particularly MMR, capacity in individual members of the precursor population. For example, is there evidence of a significant frequency of cells with reduced or null MMR activity? Are the NER and BER pathways fully active in removing the potentially mutagenic DNA lesions that, if left unrepaired, would have particularly severe effects in cells with compromised MMR? Do significant changes in repair capacity accompany differentiation into the various myeloid cell types? In order to follow up these intriguing preliminary findings, careful quantitation of DNA repair levels in the potential target myeloid cell populations is a clear priority.

References


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