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Shingo Fujii, Robert Fuchs

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1 **Accidental Encounter of Repair Intermediates in Alkylated DNA May Lead to Double-Strand**
2 **Breaks in Resting Cells**

3

4 Shingo Fujii^{1*} and Robert P. Fuchs^{2*}

5

6 ¹ Cancer Research Center of Marseille, Department of Genome Integrity, CNRS UMR7258,
7 Inserm U1068, Institut Paoli-Calmettes, Aix Marseille Univ, Marseille, France

8 ² SAS bioHalosis, Marseille, France

9 * Correspondence: shingo.fujii@inserm.fr (SF); robert.fuchs@biohalosis.com (RF)

10

11 **Abstract**

12 In the clinic, chemotherapy is often combined with surgery and radiation to increase the chances
13 of curing cancers. In case of glioblastoma (GBM), patients are treated with a combination of radio-
14 therapy and TMZ over several weeks. Despite its common use, the mechanism of action of the
15 alkylating agent TMZ has not been well understood when it comes to its cytotoxic effects in tumor
16 cells that are mostly non dividing. The cellular response to alkylating DNA damages is operated
17 by an intricate protein network involving multiple DNA repair pathways and numerous check-point
18 proteins that are dependent on the type of DNA lesion, the cell type, and the cellular proliferation
19 state. Among the various alkylating damages, special focus has been directed towards O⁶-
20 methylguanine (O⁶-mG). Indeed, this lesion is efficiently removed via direct reversal by O⁶-
21 methylguanine-DNA methyltransferase (MGMT). As the level of MGMT expression was found to
22 be directly correlated with TMZ efficiency, O⁶-mG was identified as the critical lesion for TMZ mode
23 of action. Initially, the mode of action of TMZ was proposed to be as follows: when left on the
24 genome, O⁶-mG lesions form O⁶-mG : T mismatches during replication as T is preferentially mis-
25 inserted across O⁶-mG. These O⁶-mG : T mismatches are recognized and tentatively repaired by post-
26 replicative mismatched DNA correction system (i.e., the MMR system). There are two models

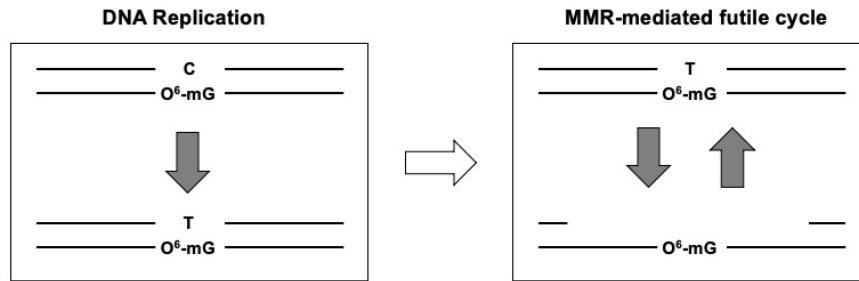
27 (futile cycle and direct signaling models) to account for cytotoxic effects of the O⁶-mG lesions, both
28 depending upon the functional MMR system in replicating cells. Alternatively, to explain cytotoxic
29 effects of alkylating agents in non-replicating cells, we have proposed a “repair accident model”
30 whose molecular mechanism is dependent upon crosstalk between the MMR and the base
31 excision repair (BER) systems. The accidental encounter between these two repair systems will
32 cause the formation of cytotoxic DNA double-strand breaks (DSBs). In this review, we summarize
33 these non-exclusive models to explain cytotoxic effects of alkylating agents and discuss potential
34 strategies to improve the clinical use of alkylating agents.

35
36 **Keywords:** Chemotherapy; DNA damages; DNA repairs; DNA double-strand breaks; Repair
37 Accident model

38
39 **Introduction**

40 Chemotherapy is an indispensable approach to tackle a variety of diseases in hospitals [1,2].
41 Whether a chemical during chemotherapy is effective largely depends upon the cellular response
42 that deals with the chemical. The representative chemical during the chemotherapy is generally
43 alkylating agents leading to DNA damages [2,3]. Human cells have a variety of defense
44 mechanisms to fix the DNA damages by means of intrinsic DNA repair systems. DNA damages
45 induced by alkylating agents are usually repaired in three distinct categories of DNA repair
46 pathways: direct reversal repair, base excision repair (BER) and mismatch repair (MMR). For
47 instance, the direct reversal repair system includes direct conversion of the O⁶-methylguanine (O⁶-
48 mG) lesion to guanine through the suicidal enzymatic reaction of O⁶-methylguanine-DNA
49 methyltransferase (MGMT), direct conversion of 1-methyladenine (1-mA) and 3-methylcytosine
50 (3-mC) lesions to the adenine and cytosine, respectively, through the enzymatic reaction of
51 alkylated DNA repair protein B homolog (ALKBH) proteins. While, repair via direct reversal is
52 obviously the best way to maintain genetic integrity, only a small number of DNA lesions are

53 repaired by direct reversal. BER pathways are responsible for the repair of a variety of additional
54 lesions, such as N3-methyladenine (N3-mA) and N7-methylguanine (N7-mG) [4–6]. If O⁶-mG
55 lesions escape direct reversal by MGMT, during replication they are efficiently converted into pre-
56 mutagenic O⁶-mG : T mispairs that were shown to be recognized by the post-replication mismatch
57 DNA repair pathway (MMR) [7,8]. Thymine opposite the O⁶-mG lesion is recognized as a DNA
58 replication error in the context of MMR because the thymine is located on the nascent strand.
59 During the process of MMR following the T-containing strand removal, T is frequently re-inserted
60 across template O⁶-mG triggering thus reforming the initial O⁶-mG : T mispair and triggering a
61 novel MMR attempt, leading to the so-called “futile MMR cycle”. These futile MMR cycling events
62 have tentatively been proposed to ultimately lead to DSBs [9] (Figure 1). However, the molecular
63 mechanisms supporting the formation of DSBs have remained elusive [10]. In contrast to detailed
64 knowledge of the molecular mechanisms operating for individual DNA repair pathways, relatively
65 little is known regarding crosstalks between repair pathways [6]. In order to obtain a more
66 integrated view about potential interference between DNA repair pathways that operate
67 simultaneously during exposure of DNA to alkylating agents, we implemented biochemical assays
68 in *Xenopus* egg extracts [11]. Based on our experimental results, we proposed a model during
69 which accidental interaction between BER and MMR pathways, simultaneously acting at closely
70 spaced lesions, leads to DSBs [11,12]. In addition to the futile cycle model, the model that we
71 propose adds a further dimension to the debate as it provides a molecular mechanisms of O⁶-mG
72 induced cytotoxicity and cell death in case of non-dividing cells that represent the vast majority of
73 cells in our bodies. In this review article, we will describe how the accidental encounter of two
74 independent repair events, MMR and BER, taking place simultaneously at closely-spaced
75 alkylation adducts in opposite DNA strands can lead to a DSB. In addition, we are suggesting that
76 a similar model for the cytotoxic effects for methylazoxymethanol (MAM), a hydrazine-related
77 chemical, with carcinogenic and neurotoxic potential [13–15].



78

79

80 **Figure 1.** The MMR-mediated futile cycle model driven by the persistent presence of the O⁶-mG

81 lesion on the genomic DNA. Alkylating agents such as temozolomide (TMZ), widely used in the

82 clinic to treat glioblastomas, induces a broad spectrum of adducts (lesions) on the genomic DNA.

83 In the MMR-mediated futile cycle model, the O⁶-mG lesion among alkylating agent-induced DNA

84 damages is exclusively focused as an only source related to alkylating agent-induced cell death

85 phenomenon. During DNA replication, thymine is preferentially incorporated opposite the O⁶-mG,

86 forming the O⁶-mG : T mismatch (left panel). This mismatch activates the MMR system. However,

87 the O⁶-mG lesion remains persistently present in the parental strand, resulting in a novel MMR

88 attempt which is futile by nature (right panel). Such iterative rounds of the MMR repair process

89 could ultimately lead to cell cycle arrest and apoptosis.

90

91

92 **Clinical use of temozolomide (TMZ)**

93 Alkylating DNA damages in genomic DNA result from cellular metabolic products (endogenous

94 alkylation) and from exogenous chemicals such as nitroso-compounds and chemotherapeutic

95 agents [4,6]. With respect to chemotherapy, the alkylating agent temozolomide (TMZ) is widely

96 used for the treatment of glioblastomas [1,2]. TMZ belongs to the group of triazine compounds

97 and is the predominant mono-functional DNA alkylating agent used in the treatment of

98 glioblastoma in combination with surgery and ionizing radiations [2,16,17]. During chemotherapy,

99 following oral administration, TMZ is rapidly converted into the active metabolite MTIC [5-(3-
100 methyl-1-triazeno) imidazo-ole-4-carboxamide] by cellular metabolic processes. After that, the
101 MTIC spontaneously decomposes into a methyldiazonium ion which directly reacts with genomic
102 DNA at the N7 position of guanine (N7-mG), the N3 position of adenine (N3-mA), and the O⁶
103 position of guanine (O⁶-mG) as well as minor adducts at the N1 position of adenine (1-mA) and
104 the N3 position of cytosine (3-mC). In order to repair the diversity of alkylating DNA damages,
105 human cells utilize at least three DNA repair processes, the BER system, the MMR system and
106 direct reversal repair proteins such as MGMT [18] and the ALKBH proteins [5,6]. Intriguingly,
107 whereas the O⁶-mG lesion represents a minor fraction (i.e., <10%) among all TMZ-induced
108 alkylating DNA lesions, these lesions turn out to be the most cytotoxic and mutagenic. Related to
109 the cytotoxic effects, it is observed that DSBs trigger apoptosis after TMZ treatment [19]. The
110 significant adverse effects of the O⁶-mG lesion were demonstrated through studies involving the
111 direct reversal repair protein MGMT. When MGMT is highly expressed in cells, alkylating agent
112 induced cell death phenomenon is largely blocked, thus highlighting the cytotoxic effect of O⁶-mG
113 lesions. In contrast, upon loss of MGMT expression, cells become highly sensitive to alkylating
114 agents [10,20]. These studies indicated that the presence of the O⁶-mG lesion on genomic DNA
115 is a critical factor for TMZ-mediated cancer therapy. Therefore, different approaches aimed at
116 maintaining O⁶-mG lesions on genomic DNA, such as depleting or inhibiting the MGMT protein,
117 have been developed [20]. As an alternative mechanism leading to TMZ resistance, it has been
118 reported that an efficient homologous recombination (HR) pathway can rescue glioblastoma-
119 derived tumor cells from TMZ-mediated cytotoxic effects [21,22].

120

121 **The MMR-mediated futile cycle model driven by the persistent presence of the O⁶-mG lesion**
122 **in the template strand**

123 Based upon the critical observation that O⁶-mG is the lesion that is responsible for cell death, led
124 to the so-called futile (or abortive) MMR cycle model [8,23] (Figure 1). When O⁶-mG lesions

125 escape direct MGMT-mediated reversal, the replicative DNA polymerase predominantly
126 incorporates thymine and, to a lesser extent, cytosine opposite the O⁶-mG lesion on the template
127 DNA during the DNA replication. The resulting base-pairing geometry remains essentially
128 unperturbed, and the T : O⁶-mG base pair evades the proofreading function associated with the
129 replicative DNA polymerase. Frequent in-corporation of T opposite the O⁶-mG lesion turns out to
130 be mutagenic, inducing GC to AT transitions [24]. Since O⁶-mG lesions, do not prevent progression
131 of DNA replication, the recurrent occurrence of O⁶-mG : T mispairs leads to MMR-mediated
132 cytotoxicity via a so-called “futile cycle model” as follows. As the replicative DNA polymerase re-
133 incorporates thymine opposite the O⁶-mG lesion thus re-forming the O⁶-mG : T mispair that is
134 recognized as a DNA replication error in the context of the post-replication mis-match DNA repair
135 pathway. The thymine on the nascent strand in the O⁶-mG : T mispair is recognized by the MutS α
136 complex composed of MSH2 and MSH6 proteins as a replication error. The MutS α complex in
137 turn recruits the MutL α complex composed of MLH1 and PMS2 proteins. After that, an
138 endonuclease activity associated with the MutL α complex introduces a nick(s) in the T-containing
139 nascent strand leading to the formation of a large single stranded DNA gap containing a persistent
140 O⁶-mG lesion. During MMR synthesis, thymine is frequently re-incorporated opposite the O⁶-mG
141 lesion [24], thus forming again the O⁶-mG : T mismatch base pair, thus the concept of abortive or
142 futile mismatch repair cycles [8,25] as proposed over 40 years ago [23]. The intrinsic property of
143 MMR to remove the nascent strand, inevitably leads to the permanence of the O⁶-mG lesion in
144 the parental strand. This situation is quite unique to O⁶-mG when compared to base analogs such
145 as 2-AP or BrdU that get incorporated in the nascent strand forming mismatched which are
146 efficiently removed by the MMR system. Within the framework of the futile cycle model, with
147 respect to cytotoxicity, it is supposed that the repeated rounds of excision and re-synthesis will
148 eventually lead to activation of the ATR/CHK1 signaling cascade and the onset of apoptosis
149 although no precise mechanism is available yet [4,6,26,27].

150

151 **The MMR-mediated direct signaling model complementing the futile cycle model**

152 There is a complementary model termed “direct signaling model” that has been proposed to
153 explain the resulting onset of apoptosis induced by the presence of the O⁶-mG lesion dependent
154 upon the DNA replication. It is suggested that recognition of the O⁶-mG : T mismatch by MMR
155 complexes MutS α and MutL α , further elicits the recruitment of the DNA damage response proteins
156 ATR, ATRIP and TopBP1 resulting in activation of the DNA damage checkpoint response (DDR)
157 [28,29]. In that model, the MutS α complex directly interacts with ATR, TopBP1, and Chk1 while
158 the MutL α complex interacts with TopBP1 [30]. In conclusion, the DDR checkpoint signal cascade
159 is activated via the activation of the MMR system, triggering the onset of cell cycle arrest and
160 apoptosis. Both models (“futile cycle” and “direct signaling”) seem to fit the observation that normal
161 stem cells exhibit MMR-dependent DDR signaling in response to O⁶-mG : T mismatches within
162 the first S phase [31]. Overall, both O⁶-mG lesion-induced cell death models are in good
163 agreement with published features, namely that a functional MMR pathway is indispensable [9],
164 that impaired MGMT is essential [18] and that the occurrence of DNA strand breaks is positively
165 correlated [10]. However, neither of these models can explain why the MMR-dependent DDR
166 response requires two rounds of DNA replication in cancer cells [31].

167

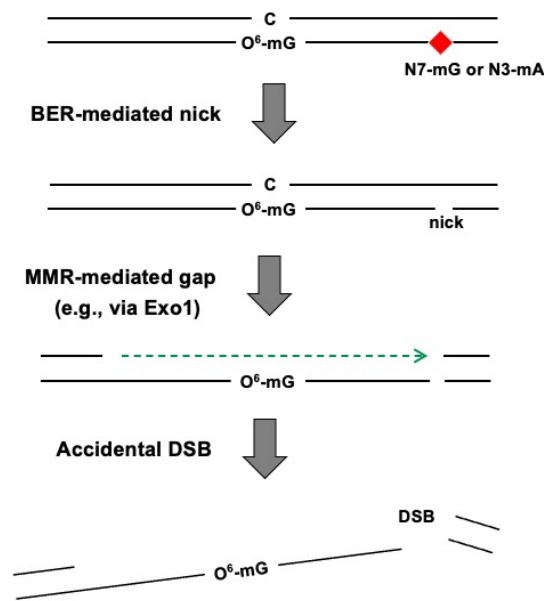
168 **The DNA repair accident model to explain the cytotoxic effects of the alkylating agent**

169 In combination with surgery and radiation during the clinical treatment of glioblastoma, the
170 alkylating agent TMZ is used as a chemotherapeutic agent. Despite its many years of clinical use,
171 the mode of cytotoxic action of TMZ remains elusive. Like other alkylating agents, TMZ induces a
172 broad spectrum of DNA lesions, including N7-mG (70-75%), N3-mA (8-12%), and O⁶-mG (8-9%).
173 As described above, distinct DNA repair systems deal with these lesions, namely BER for N7-mG
174 and N3-mA lesions, direct reversal repair via MGMT protein or the MMR system for the O⁶-mG
175 lesion. As discussed above, the cytotoxic mode-of-action of alkylating agents such as TMZ is

176 believed to result from MMR-mediated “futile cycling” and/or “direct signaling” at O⁶-mG : T
177 mispairs. Furthermore, in addition to MMR, BER-derived repair intermediates have been proposed
178 contribute to the cytotoxic effects of TMZ [32–34]. Alternative models involving crosstalk between
179 the BER and the MMR systems [35] or accumulation of BER repair intermediates [32] suggest the
180 occurrence of DNA double-strand breaks (DSBs) to explain cytotoxicity.

181 In contrast to the aforementioned models (“futile cycling” and “direct signaling”) both of
182 which require replication to form O⁶-mG : T mismatches (S phase), the vast majority of cells in
183 glioblastoma tumors are non-dividing or quiescent [36]. Therefore, investigating the mechanism
184 of action of TMZ in the absence of replication is of critical importance. To this end, we analyzed
185 the fate of TMZ damaged DNA in extracts (Xenopus egg extracts) in the absence of replication.
186 For that purpose, a recently developed plasmid DNA pulldown approach, termed IDAP (*for*
187 *Isolation of DNA Associated Proteins*), was implemented. This approach, which aims at capturing
188 nucleoprotein complexes formed on specific plasmids in nuclear extracts, was shown to be highly
189 efficient and versatile [37–39]. Briefly, the core aspect of this methodology is to immobilize, on
190 magnetic beads, a circular plasmid containing the DNA-of-interest. Plasmid immobilization is
191 mediated by means of a specific oligonucleotide able to form a triple helix (TFO probe) with a
192 cognate double-stranded DNA sequence present on the plasmid; the other extremity of the TFO
193 probe harbors a biotin moiety that interacts with streptavidin-conjugated magnetic beads. To
194 implement this approach in case of alkylation lesions, plasmid DNA treated by N-methyl-N-
195 nitrosourea (MNU: a TMZ mimic) was incubated with cell-free extracts prepared from *Xenopus*
196 *laevis* eggs under non-replicating conditions. In this methodology, many proteins were isolated
197 irrespective of the presence or the absence of DNA damages. Following identification of the
198 captured proteins by means of mass spectrometry (MS) analysis, a variety of proteins were
199 specifically recruited by the presence of MNU-induced DNA damages compared to undamaged
200 plasmid. Interestingly, most MMR core proteins were highly enriched despite the absence of DNA
201 replication. Biochemical assays were subsequently implemented to validate the MS data. It was

202 revealed that proteins of both the MMR and the BER systems are active on the damaged DNA
 203 treated by MNU. From the biochemical data, we concluded that when MMR and BER repair
 204 processes operate independently on lesions located in the same DNA molecule in opposite
 205 strands, a double-strand break may result from the accidental encounter of these two repair
 206 intermediates. [11]. We refer to such an event as a “repair accident” (Figure 2) [12].
 207



208
 209
 210 **Figure 2.** Repair Accident model. In case of treatment with an alkylating agent such as TMZ during
 211 chemotherapy, a variety of DNA damages appear and are repaired by multiple DNA repair
 212 pathways. Indeed, distinct DNA repair systems work independently dependent upon their
 213 substrate specificities. The BER system mainly acts at the N7-mG or the N3-mA lesion, while the
 214 core MMR proteins recognize not only the O^6 -mG : T base pair but also the O^6 -mG : C base pair
 215 as shown by our work [11]. Therefore, even in non-dividing or quiescent cells treated by alkylating
 216 agents, when an N7-mG or an N3-mA lesion are closely located with the O^6 -mG lesion (e.g., within
 217 several hundred nucleotides), the accidental encounter of BER and MMR derived repair
 218 intermediates were shown to lead to DSB when they occur within the same time frame. In non-

219 dividing cells treated by an alkylating agent, the “repair accident model” scenario is as follows:
220 when the MMR system recognizes the O⁶-mG : C base pair, the mechanism of initiation of the
221 MMR reaction is presently unknown. It is likely that the strand discrimination signal is provided by
222 a BER-mediated nick equally likely to occur in either strand. This is in contrast to the situation that
223 occurs during replication where MMR is directed toward excision of the nascent strand. In any
224 case, Exo1-mediated strand degradation or helicase unwinding assists the DNA gap formation. If,
225 within the same timeframe, the MMR-mediated gap formation process encounters, in the opposite
226 strand, a nick resulting from an independent BER repair intermediate in, a DSB will result.

227

228 **Genotoxic impact of methylazoxymethanol (MAM)**

229 MAM, which is a hydrazine-related chemical, is naturally found in foods including mushrooms and
230 plants, possessing carcinogenic and neurotoxic potential (i.e., linked to cancer and neurological
231 disease) following metabolic activation in the liver [13–15]. Like TMZ, the activated MAM induce
232 a variety of DNA damages (e.g., O⁶-mG, N7-mG, 8-oxo-G), resulting in activation of multiple DNA
233 repair systems including the MMR and the BER systems. Whereas the MAM-treated postmitotic
234 cells undergo apoptosis or non-apoptotic cell death, the mechanism of action remains to be elusive.
235 Given the similarity, between MAM and TMZ, in adduct formation, we suggest that cytotoxic effects
236 of MAM in the postmitotic cells might be achieved within the framework of the “repair accident
237 model” described above.

238

239 **Discussion**

240 From numerous studies in numerous cellular and animal models, it has been concluded that the
241 cytotoxicity of alkylating agents, including the most crucial therapeutic agent TMZ, is mainly due
242 to O⁶-mG lesions. The cytotoxic cascade depends on both MMR and DNA replication. To explain
243 these features, two models (futile cycle and direct signaling models) have been proposed. In these
244 models, the target of the MMR system is the O⁶-mG : T mispair formed during the DNA replication

245 [8]. However, since these models cannot explain all of the experimental observations, it suggests
246 that there may be a missing piece to fully explain the alkylating agent-induced cytotoxic effects. In
247 particular, the proposed models require DNA replication, a feature that is not encountered in most
248 glioblastoma tumor cells that are non-dividing or quiescent [36]. In order to explain the intricate
249 phenomena involved in alkylating agent-induced cytotoxic effects, a crosstalk between the MMR
250 and the BER systems has been suggested [33–35]. The “repair accident model” proposed here
251 suggests a mechanism for the formation of cytotoxic DSBs in the absence of the DNA replication
252 by virtue of an accidental encounter of MMR and BER repair intermediates.

253 When considering the clinical use of alkylating agents based on the repair accident model
254 (Figure 2), as DSBs are formed as a consequence of concomitant processing of lesions by
255 proteins of both the MMR and BER systems, enhancement of the cytotoxic effects may be
256 achieved by partially impairing these repair systems, namely by slowing down the latter events
257 such repair synthesis or ligation steps in either BER and/or MMR.

258 In addition to the pre-existing two models (futile cycle and direct signaling models), the
259 repair accident model will become the third model to compensate the weak points of the pre-
260 existing two models that are incompatible with the cytotoxic effects in non-dividing or quiescent
261 cells. Furthermore, the idea of DSB formation in the repair accident model may become a
262 molecular basis to explain the cytotoxic effects of MAM inducing cancer and neurological disease
263 in non-dividing or quiescent cells

264

265 **Author Contributions:** Conceptualization, S.F.; writing—original draft preparation, S.F.; writing—
266 review and editing, R.F. and S.F. All authors have read and agreed to the published version of the
267 manuscript.

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270 **References**

- 271 1. Bobola, M.S.; Kolstoe, D.D.; Blank, A.; Silber, J.R. Minimally Cytotoxic Doses of
272 Temozolomide Produce Radiosensitization in Human Glioblastoma Cells Regardless of MGMT
273 Expression. *Molecular Cancer Therapeutics* 2010, 9, 1208–1218, doi:10.1158/1535-7163.MCT-
274 10-0010.
- 275 2. Strobel, H.; Baisch, T.; Fitzel, R.; Schilberg, K.; Siegelin, M.D.; Karpel-Massler, G.;
276 Debatin, K.-M.; Westhoff, M.-A. Temozolomide and Other Alkylating Agents in Glioblastoma
277 Therapy. *Biomedicines* 2019, 7, 69, doi:10.3390/biomedicines7030069.
- 278 3. Doak, S.H.; Jenkins, G.J.S.; Johnson, G.E.; Quick, E.; Parry, E.M.; Parry, J.M.
279 Mechanistic Influences for Mutation Induction Curves after Exposure to DNA-Reactive
280 Carcinogens. *Cancer Research* 2007, 67, 3904–3911, doi:10.1158/0008-5472.CAN-06-4061.
- 281 4. Fu, D.; Calvo, J.A.; Samson, L.D. Balancing Repair and Tolerance of DNA Damage
282 Caused by Alkylating Agents. *Nat Rev Cancer* 2012, 12, 104–120, doi:10.1038/nrc3185.
- 283 5. Fedeles, B.I.; Singh, V.; Delaney, J.C.; Li, D.; Essigmann, J.M. The AlkB Family of
284 Fe(II)/ α -Ketoglutarate-Dependent Dioxygenases: Repairing Nucleic Acid Alkylation Damage and
285 Beyond. *Journal of Biological Chemistry* 2015, 290, 20734–20742,
286 doi:10.1074/jbc.R115.656462.
- 287 6. Soll, J.M.; Sobol, R.W.; Mosammaparast, N. Regulation of DNA Alkylation Damage
288 Repair: Lessons and Therapeutic Opportunities. *Trends in Biochemical Sciences* 2017, 42, 206–
289 218, doi:10.1016/j.tibs.2016.10.001.
- 290 7. Gerson, S.L. MGMT: Its Role in Cancer Aetiology and Cancer Therapeutics. *Nat Rev*
291 *Cancer* 2004, 4, 296–307, doi:10.1038/nrc1319.
- 292 8. Jiricny, J. The Multifaceted Mismatch-Repair System. *Nat Rev Mol Cell Biol* 2006, 7,
293 335–346, doi:10.1038/nrm1907.
- 294 9. Kat, A.; Thilly, W.G.; Fang, W.H.; Longley, M.J.; Li, G.M.; Modrich, P. An Alkylation-
295 Tolerant, Mutator Human Cell Line Is Deficient in Strand-Specific Mismatch Repair. *Proc. Natl.*
296 *Acad. Sci. U.S.A.* 1993, 90, 6424–6428, doi:10.1073/pnas.90.14.6424.
- 297 10. Kalamegham, R.; Warmels-Rodenhiser, S.; MacDonald, H.; Ebisuzaki, K. O⁶-
298 Methylguanine-DNA Methyltransferase-Defective Human Cell Mutant: O⁶-Methylguanine, DNA
299 Strand Breaks and Cytotoxicity. *Carcinogenesis* 1988, 9, 1749–1753,
300 doi:10.1093/carcin/9.10.1749.
- 301 11. Fuchs, R.P.; Isogawa, A.; Paulo, J.A.; Onizuka, K.; Takahashi, T.; Amunugama, R.; Duxin,
302 J.P.; Fujii, S. Crosstalk between Repair Pathways Elicits Double-Strand Breaks in Alkylated DNA
303 and Implications for the Action of Temozolomide. *eLife* 2021, 10, e69544,
304 doi:10.7554/eLife.69544.

- 305 12. Fujii, S.; Sobol, R.W.; Fuchs, R.P. Double-Strand Breaks: When DNA Repair Events
306 Accidentally Meet. *DNA Repair* 2022, 112, 103303, doi:10.1016/j.dnarep.2022.103303.
- 307 13. Kisby, G.E.; Fry, R.C.; Lasarev, M.R.; Bammler, T.K.; Beyer, R.P.; Churchwell, M.;
308 Doerge, D.R.; Meira, L.B.; Palmer, V.S.; Ramos-Crawford, A.-L.; et al. The Cycad Genotoxin
309 MAM Modulates Brain Cellular Pathways Involved in Neurodegenerative Disease and Cancer in
310 a DNA Damage-Linked Manner. *PLoS ONE* 2011, 6, e20911, doi:10.1371/journal.pone.0020911.
- 311 14. Kisby, G.E.; Spencer, P.S. Genotoxic Damage During Brain Development Presages
312 Prototypical Neurodegenerative Disease. *Front. Neurosci.* 2021, 15, 752153,
313 doi:10.3389/fnins.2021.752153.
- 314 15. Spencer, P.S.; Kisby, G.E. Role of Hydrazine-Related Chemicals in Cancer and
315 Neurodegenerative Disease. *Chem. Res. Toxicol.* 2021, 34, 1953–1969,
316 doi:10.1021/acs.chemrestox.1c00150.
- 317 16. Jiapaer, S.; Furuta, T.; Tanaka, S.; Kitabayashi, T.; Nakada, M. Potential Strategies
318 Overcoming the Temozolomide Resistance for Glioblastoma. *Neurol. Med. Chir.(Tokyo)* 2018,
319 58, 405–421, doi:10.2176/nmc.ra.2018-0141.
- 320 17. Choi, S.; Yu, Y.; Grimmer, M.R.; Wahl, M.; Chang, S.M.; Costello, J.F. Temozolomide-
321 Associated Hypermutation in Gliomas. *Neuro-Oncology* 2018, 20, 1300–1309,
322 doi:10.1093/neuonc/noy016.
- 323 18. Karran, P.; Bignami, M. Self-Destruction and Tolerance in Resistance of Mammalian Cells
324 to Alkylation Damage. *Nucl Acids Res* 1992, 20, 2933–2940, doi:10.1093/nar/20.12.2933.
- 325 19. Maksoud, S. The DNA Double-Strand Break Repair in Glioma: Molecular Players and
326 Therapeutic Strategies. *Mol Neurobiol* 2022, 59, 5326–5365, doi:10.1007/s12035-022-02915-2.
- 327 20. Dolan, M.E.; Moschel, R.C.; Pegg, A.E. Depletion of Mammalian O6-Alkylguanine-DNA
328 Alkyltransferase Activity by O6-Benzylguanine Provides a Means to Evaluate the Role of This
329 Protein in Protection against Carcinogenic and Therapeutic Alkylating Agents. *Proc. Natl. Acad.*
330 *Sci. U.S.A.* 1990, 87, 5368–5372, doi:10.1073/pnas.87.14.5368.
- 331 21. Roos, W.P.; Batista, L.F.Z.; Naumann, S.C.; Wick, W.; Weller, M.; Menck, C.F.M.; Kaina,
332 B. Apoptosis in Malignant Glioma Cells Triggered by the Temozolomide-Induced DNA Lesion
333 O6-Methylguanine. *Oncogene* 2007, 26, 186–197, doi:10.1038/sj.onc.1209785.
- 334 22. Gil del Alcazar, C.R.; Todorova, P.K.; Habib, A.A.; Mukherjee, B.; Burma, S. Augmented
335 HR Repair Mediates Acquired Temozolomide Resistance in Glioblastoma. *Molecular Cancer*
336 *Research* 2016, 14, 928–940, doi:10.1158/1541-7786.MCR-16-0125.
- 337 23. Karran, P.; Marinus, M.G. Mismatch Correction at O6-Methylguanine Residues in *E. Coli*
338 DNA. *Nature* 1982, 296, 868–869, doi:10.1038/296868a0.

- 339 24. Warren, J.J.; Forsberg, L.J.; Beese, L.S. The Structural Basis for the Mutagenicity of O 6
340 -Methyl-Guanine Lesions. *Proc. Natl. Acad. Sci. U.S.A.* 2006, 103, 19701–19706,
341 doi:10.1073/pnas.0609580103.
- 342 25. Wang, J.Y.J.; Edelman, W. Mismatch Repair Proteins as Sensors of Alkylation DNA
343 Damage. *Cancer Cell* 2006, 9, 417–418, doi:10.1016/j.ccr.2006.05.013.
- 344 26. Gupta, D.; Heinen, C.D. The Mismatch Repair-Dependent DNA Damage Response:
345 Mechanisms and Implications. *DNA Re-pair* 2019, 78, 60–69, doi:10.1016/j.dnarep.2019.03.009.
- 346 27. Li, G.-M. Mechanisms and Functions of DNA Mismatch Repair. *Cell Research* 2008, 18,
347 85–98, doi:10.1038/cr.2007.115.
- 348 28. Yoshioka, K.; Yoshioka, Y.; Hsieh, P. ATR Kinase Activation Mediated by MutS α and
349 MutL α in Response to Cytotoxic O6-Methylguanine Adducts. *Molecular Cell* 2006, 22, 501–510,
350 doi:10.1016/j.molcel.2006.04.023.
- 351 29. Li, Z.; Pearlman, A.H.; Hsieh, P. DNA Mismatch Repair and the DNA Damage Response.
352 *DNA Repair* 2016, 38, 94–101, doi:10.1016/j.dnarep.2015.11.019.
- 353 30. Liu, Y.; Fang, Y.; Shao, H.; Lindsey-Boltz, L.; Sancar, A.; Modrich, P. Interactions of
354 Human Mismatch Repair Proteins MutS α and MutL α with Proteins of the ATR-Chk1 Pathway.
355 *Journal of Biological Chemistry* 2010, 285, 5974–5982, doi:10.1074/jbc.M109.076109.
- 356 31. Gupta, D.; Lin, B.; Cowan, A.; Heinen, C.D. ATR-Chk1 Activation Mitigates Replication
357 Stress Caused by Mismatch Re-pair-Dependent Processing of DNA Damage. *Proc. Natl. Acad.*
358 *Sci. U.S.A.* 2018, 115, 1523–1528, doi:10.1073/pnas.1720355115.
- 359 32. Fortini, P.; Rosa, S.; Zijno, A.; Calcagnile, A.; Bignami, M.; Dogliotti, E. Methoxyamine
360 Modification of Abasic Sites Protects CHO Cells from the Cytotoxic and Mutagenic Effects of
361 Oxygen Alkylation. *Carcinogenesis* 1992, 13, 87–93, doi:10.1093/carcin/13.1.87.
- 362 33. McNees, C.J.; Conlan, L.A.; Tennis, N.; Heierhorst, J. ASCIZ Regulates Lesion-Specific
363 Rad51 Focus Formation and Apoptosis after Methylating DNA Damage. *EMBO J* 2005, 24,
364 2447–2457, doi:10.1038/sj.emboj.7600704.
- 365 34. Agnihotri, S.; Gajadhar, A.S.; Ternamian, C.; Gorlia, T.; Diefes, K.L.; Mischel, P.S.; Kelly,
366 J.; McGown, G.; Thorncroft, M.; Carlson, B.L.; et al. Alkylpurine–DNA–N-Glycosylase Confers
367 Resistance to Temozolomide in Xenograft Models of Glio-blastoma Multiforme and Is Associated
368 with Poor Survival in Patients. *J. Clin. Invest.* 2012, 122, 253–266, doi:10.1172/JCI59334.
- 369 35. Simonelli, V.; Leuzzi, G.; Basile, G.; D’Errico, M.; Fortini, P.; Franchitto, A.; Viti, V.; Brown,
370 A.R.; Parlanti, E.; Pascucci, B.; et al. Crosstalk between Mismatch Repair and Base Excision
371 Repair in Human Gastric Cancer. *Oncotarget* 2017, 8, 84827–84840,
372 doi:10.18632/oncotarget.10185.

- 373 36. Das, S.; Srikanth, M.; Kessler, J.A. Cancer Stem Cells and Glioma. *Nat Rev Neurol* 2008,
374 4, 427–435, doi:10.1038/ncpneuro0862.
- 375 37. Fuchs, R.P.; Isogawa, A.; Paulo, J.A.; Fujii, S. Identification of Proteins Specifically
376 Assembled on a Stem-Loop Composed of a CAG Triplet Repeat. *DNA* 2023, 3, 109–118,
377 doi:10.3390/dna3020009.
- 378 38. Isogawa, A.; Fuchs, R.P.; Fujii, S. Versatile and Efficient Chromatin Pull-down
379 Methodology Based on DNA Triple Helix Formation. *Sci Rep* 2018, 8, 5925, doi:10.1038/s41598-
380 018-24417-9.
- 381 39. Isogawa, A.; Fuchs, R.P.; Fujii, S. Chromatin Pull-Down Methodology Based on DNA
382 Triple Helix Formation. In *DNA Electrophoresis*; Hanada, K., Ed.; *Methods in Molecular Biology*;
383 Springer US: New York, NY, 2020; Vol. 2119, pp. 183–199 ISBN 978-1-07-160322-2.