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Review

Biological Basis for Threshold Responses to Methylating Agents

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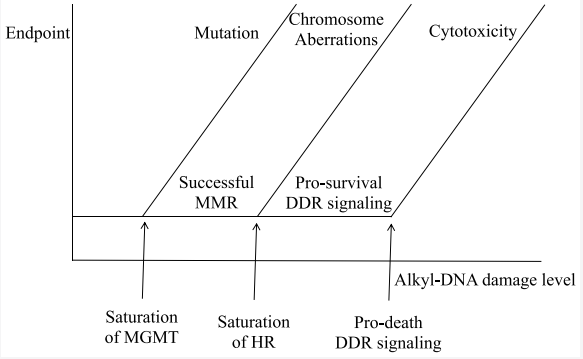
ABSTRACT: The cellular outcomes of chemical exposure are as much about the cellular *response* to the chemical as it is an *effect* of the chemical. We are growing in our understanding of the genotoxic interaction between chemistry and biology. For example, recent data has revealed the biological basis for mutation induction curves for a methylating chemical, which has been shown to be dependent on the repair capacity of the cells. However, this is just one end point in the toxicity pathway from chemical exposure to cell death. Much remains to be known in order for us to predict how cells will respond to a certain dose. Methylating agents, a subset of alkylating agents, are of particular interest, because of the variety of adverse genetic end points that can result, not only at increasing doses, but also over time. For instance, methylating agents are mutagenic, their potency, for this end point, is determined by the cellular repair capacity of an enzyme called methylguanine DNA-methyltransferase (MGMT) and its ability to repair the induceed methyl adducts. However, methyl adducts can become clastogenic. Erroneous biological processing will convert mutagenic adducts to clastogenic events in the form of double strand breaks (DSBs). How the cell responds to DSBs is via a cascade of protein kinases, which is called the DNA damage response (DDR), which will determine if the damage is repaired effectively, via homologous recombination, or with errors, via nonhomologous end joining, or whether the cell dies via apoptosis or enters senescence. The fate of cells may be determined by the extent of damage and the resulting strength of DDR signaling. Therefore, thresholds of damage may exist that determine cell fate. Such thresholds would be dependent on each of the repair and response mechanisms that these methyl adducts stimulate. The molecular mechanism of how methyl adducts kill cells is still to be fully resolved. If we are able to quantify each of these thresholds of damage for a given cell, then we can ascertain, of the many adducts that are induced, what proportion of them are mutagenic, what proportion are clastogenic, and how many of these clastogenic events are toxic. This review examines the possibility of dose and damage thresholds for methylating agents, from the perspective of the underlying evolutionary mechanisms that may be accountable.



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INTRODUCTION

The underlying biology of the cell will ultimately determine the effect of chemical exposure.[1] At each potential end point, along the pathway from exposure to cell death, the underlying biology of the cell will influence its sensitivity and, therefore, the dose at which the end point occurs. The first potential threshold will be the dose required to cause an increase in adducts. This will be influenced by several aspects of cell biology, including polymorphisms in metabolic enzymes that dictate how much

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of the administered chemical is potentially DNA-reactive.[2] Another factor affecting this adduct threshold will be efflux transportation and detoxification mechanisms that will need to be overcome.[3] Upon DNA reaction, the threshold of DNA repair will limit the mutagenic potency of an adduct and this mechanism has supportive data, particularly for alkylating agents, namely, the methylating agent *N*-methyl-*N*-nitrosourea (MNU) and the ethylating agent ethylmethanesulfonate (EMS).[4,5] This Review is an exploration into the thresholds of DNA damage and repair of methylating agents, in particular, and speculates on further thresholds in cytotoxicity.

METHYLATING AGENTS

Methylating agents are a chemical class of genotoxicant that add methyl groups to nucleophilic sites in the DNA.[6] Members of this chemical class are diverse in chemical structure, reactivity, and source, whether that may be dietary, industrial, medical, or environmental. Each chemical produces a defined adduct spectra, which resembles a synonymous signature of damage, and therefore produces a characteristic mutational signature.[7] The methylating agent class can be further stratified based on their chemistry, molecular structure adduct spectra, and, therefore, their genotoxic and cytotoxic potency. This means that one methylating agent can be representative of many similarly acting methylating agents. Temozolomide (TMZ) is one such methylating agent; it is used in glioblastoma chemotherapy.[8] More specifically, TMZ is an SN_1-type methylating agent, which also describes similarly acting agents such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) and MNU. Both of these are typically confined to laboratory use, whereas other SN_1-type methylating agents, such as procarbazine and imidazole carboxamide, also known as dacarbazine (DTIC), are used in the clinic as effective chemotherapies against advanced cancer, such as melanoma, soft tissue sarcoma, and Hodgkin's disease/Hodgkin's lymphoma. Methylating agents have been well-studied and are currently well-used. However, if we have truly mastered their chemistry and completely understand the response of different types of treated cells, we would be able to eliminate cancer cells without inducing secondary, therapy-related acute myeloid leukemia.[9] The biological relevance of methylating agent treatment, regardless of whether the cell will die, continue to propagate but with mutations, or survive with their genome untouched or pristinely repaired can be predicted. This would be important not only in assessing risk of exposure to a certain dose, but also in tailoring chemotherapy dose to be effective and not to induce secondary genotoxicity events. Methylating agents induce the smallest alkyl adduct (CH_3). The situation is far more complex for alkyl adducts that are any bulkier than methyl adducts, even those with only one methylene ($-CH_2$) group (i.e., ethyl [$-CH_2CH_3$] adducts). Therefore, this review will only focus on methylating agents. Suffice to say that nonlinear dose thresholds have been identified for ethylating agents. Most overwhelmingly, compared to other SN_1-type ethylating agents, nonlinearity has been found for EMS, which is an SN_2-type ethylating agent.[10−12] Furthermore, the threshold was shown to have a biological basis in DNA repair.[4] It is possible that the genotoxic and cytotoxic potential of bulkier alkyl adducts (including those that have longer alkyl chains and side chains) are under the same biological constraints (i.e., detoxification[13] and DNA repair mechanisms[14,15]) as simpler alkyl adducts and agents. However, perhaps due to the paucity of data pertaining to the biochemistry and cellular response at low doses of bulkier alkyl adducts, it is difficult to draw firmer conclusions. A few studies have pointed to some potential points of biochemical difference (background levels and persistence[16]) between methyl adducts and bulkier adducts. For example, in one study, the background levels (and, therefore, the cellular tolerance [discussed later]) of 7-methylguanine (7-MeG; also referred to as N7-MeG in the literature) were much lower than 7-(2-hydroxyethyl)-guanine in the lymphocytes of the vast majority of the 34 individuals studied.[17] Whereas, an indirect comparison over two studies[18,19] showed the background levels of 7-MeG to be much higher than that of 7-ethylguanine (7-EtG). It is important not to extrapolate, because the biological threats that ethyl vs. methyl and larger vs. smaller adducts pose may differ significantly.

SN_1-TYPE ADDUCT SPECTRA

SN_1-type methylating agents, such as TMZ, directly damage the DNA by reacting with oxygen and nitrogen nucleophiles, forming adducts such as 3-methyladenine (3-MeA, known to be referred to as N3-MeA in the literature), 7-MeG and O^6-methylguanine (O^6-MeG) to a large extent, compared to other types of methylating agents, such as methylmethanesulfonate (MMS), an SN_2-type methylating agent. MMS has a weaker affinity for O^6 guanine and produce far fewer O^6-MeG adducts as a result.[20] The higher reactivity with exocyclic oxygen of guanine by SN_1-type methylating agents is the reason for their increased mutagenic potency, compared to SN_2-type methylating agents.[7] The complete adduct spectra of SN_1-type methylating agents has been compiled elsewhere.[21] Some of these adducts have been extensively studied, and their potential adverse effects on the replicating polymerase, and the resulting biological impact of such damage has been resolved. This work will focus on two of the best studied methyl adducts 7-MeG and O^6-MeG, but 3-MeA cannot be ignored. In many respects, 3-MeA is subject to the same repair processes as 7-MeG[21] and possibly O^6-MeG,[22] discussed later. However, 3-MeA also represents distinct methyl adduct biochemistry and may involve alternative, translesion processing.[23]

METHYLATING AGENT AND DNA REACTION

In the chemical exposure to biological effect journey, quantifying the levels of DNA adducts is an important biomarker of exposure.[24−26] Increasing evidence suggests that DNA methyl adducts have an exposure threshold.[27,28] However, the biological mechanisms are yet to be evidenced. The use of sensitive and specific analytical techniques, such as ultraperformance liquid chromatography−tandem mass spectrometry (UPLC-MS/MS),[28] will be crucial in further studies. However, the timing of the analysis post-exposure will be critical, not to confuse an exposure threshold with DNA repair.

THE REPAIR OF METHYL ADDUCTS

Each methyl adduct is a substrate for specialized repair. Generally, methyl adducts are substrates for excision repair mechanisms, that find, excise, and replace the damaged nucleotides. The adduct 7-MeG involves the action of base excision repair (BER), which is initiated by an adduct-specific glycosylase: *N*-methylpurine DNA glycosylase (MPG), also known as 3-alkyladenine DNA glycosylase (AAG). O^6-MeG is subject to direct repair by methylguanine methyltransferase (MGMT), which simply removes the methyl group, restoring guanine.[29,30] The adduct is also a substrate for mismatch excision repair (MMR). Direct repair is absolute; it simply

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removes the damage. Excision repair, on the other hand, causes additional damage during the repair procedure, either intentionally, as in the case of BER, or as a consequence of erroneous processing, as is the possible case in MMR. MMR is able to restore the DNA, through an excision pathway that recognizes the misaligned hydrogen bonds between O^6-MeG and its opposite base. However, the involvement of MMR has been shown to affect the replisome into stalling, with the potential to collapse into DSBs.[29,31] This has been attributed to the appearance of MMR-dependent single-stranded gaps in the DNA in successive cell cycles.[31,32] The DNA damage response (DDR) will then be initiated. One would imagine that it is in response to the strand breaks, but data suggests MMR directly activates the DDR.[33−35] The involvement of DDR signaling will be discussed later.

7MEG-ENDOGENOUS AND EXOGENOUS LEVELS

7-MeG is, by far, the most abundant methyl adduct induced as part of the adduct spectra.[20] This adduct is naturally present at very high levels, as measured in rat liver,[36] and it was thought that exogenous exposure to a methylating agent would add only a negligible amount of 7-MeG, compared to the pre-existing, natural levels. This led to the assumption that 7-MeG is innocuous. 7-MeG and also 3-MeA have been said to be susceptible to spontaneous depurination, but under physiological conditions, the process is incredibly slow[37] and, therefore, unlikely to pose a significant toxic effect. However, 7-MeG and 3-MeA are labile to more rapid depurination through enzymatic catalysis[38] by an initiating BER glycosylase, which, in this case, is MPG.[39] In 2014, a revealing paper showed that the depurination of 7-MeG converts 7-MeG to a polymerase-blocking event leading to the stalling and eventual collapse of the replication forks into recombinogenic and toxic DSBs. This was shown to be entirely dependent on the presence of MPG.[40] As previously stated, MPG is also able to operate on 3-MeA and, therefore, 3-MeA may also be involved here. Distinguishing the effects between 7-MeG and 3-MeA adducts would be difficult in this instance. With this evidence, it suggests that the level of the initiating glycosylase determines the clastogenic potency of 7-MeG (and also 3-MeA), as opposed to the adducts themselves. This explains how such a high level can naturally exist, if only a proportion of all the 7-MeG adducts in the genome become depurinated at any one time. The 7-MeG level must be set by a flux of newly induced damage, MPG-mediated depurination, and successful completion by BER. Stimuli that influence the level of depurination may then influence cellular tolerance to this adduct. Upon high levels of exogenous exposure, there is an imbalance in this flux. The increase in 7-MeG does not pose a problem in itself, but the basal level of glycosylase creates more sites of depurination than the downstream BER enzymes can repair. Thus, increased levels of 7-MeG, at increasing exogenous doses, creates an increased burden of repair intermediates, which saturate the downstream BER enzymes, and lead to genomic instability in the form of DSBs (chromosome breaks), as an effect of glycosylase-BER uncoupling. Of course, the downstream BER enzymes will also have the burden of damage from other glycosylases, such as those that respond to oxidative lesions, i.e., OGG1 alias MUTYH,[41] which may also be a factor in this decoupling.

The possibility exists that the activity of the MPG glycosylase determines how many more 7-MeG adducts a cell can tolerate, and perhaps it is the ratio of active glycosylase enzymes to downstream BER enzymes that dictates cellular resistance to this adduct. Indeed, an experimentally induced imbalance of this MPG:BER ratio increased genomic instability.[42−45] In this instance, it is reasonable to conclude that perhaps the threshold of resistance against 7-MeG is set by the basal and synchronized level of MPG and BER enzymes. Indeed, MPG was shown to act as the defense against DSBs, measured via micronuclei (acting as a proxy for unrepaired DSBs) in AHH-1 cells at low doses of the SN_2-type methylating agent, MMS.[4]

O^6-MEG; SUCCESSFUL REPAIR, POINT MUTATIONS, OR DNA BREAKS?

The biological response and effect to methyl adducts is perhaps best known for O^6-MeG, although there are still some ambiguities. The adduction of exocyclic oxygen places a miscoding potential on guanine, which accounts for the high level of guanine to adenine transitions observed following exposure to SN_1-type methylating agents.[46,47] The involvement of MGMT has been shown to defend against the mutagenicity of MNU in vitro[5] and also in vivo against azoxymethane (AOM), another SN_1-type methylating agent.[48] The same outcome would be expected for an end point measuring clastogenicity, as MGMT would effectively preclude O^6-MeG-induced DSBs, although this has not been substantiated. Similarly, whether MMR is involved in low-dose protection has not yet been resolved. One would anticipate that its involvement in genotoxic end points may be difficult to resolve, given its propensity to induce further damage, in the form of DSBs, akin to the situation of imbalanced BER and 7-MeG. It could be hypothesized that higher levels of MMR enzymes could promote clastogenicity of even low doses of SN_1-type methylating agents.

THE ADVERSE POTENTIAL OF 3-MEA: THE INFLUENCE OF TLS

This adduct is mutagenic and clastogenic in very similar respects to both 7-MeG and O^6-MeG, as eluded to previously. One could therefore anticipate that an amount of 3-MeA adducts would pose the same genotoxic potential as the same amounts of 7-MeG and O^6-MeG in a DNA repair competent cell. However, the involvement of translesion synthesis (TLS) over this adduct, in addition to TLS over any subsequent MPG-catalyzed apurinic site,[49] one would imagine that this adduct would pose the same or even a higher mutagenic potential, but a lower clastogenic potential than both 7-MeG and O^6-MeG, respectively. It is reasonable to postulate that TLS would, therefore, have different protective effects at low doses, depending on the genotoxic end point being measured. How many adducts/apurinic sites resubject to TLS is unknown. On this note, the relative toxicities of each adduct would be dependent on the proficiencies of each adduct-specific DNA repair mechanism, which would dictate the likelihood of presenting with a clastogenic lesion and potentially toxic lesion, such as a DSB. Of course, the toxic potential of a DSB will also have a biological basis and will be dependent on the cellular response to this specific insult. This will also dictate the effect of exposure to methylating chemicals.

THRESHOLD LEVELS OF DSBS AND DNA DAMAGE SIGNALING

As with the adducts, DSBs can also be repaired, although their repair is via complex recombination mechanisms—homologous recombination (HR) and nonhomologous end-joining (NHEJ)—and involves the activation of the DDR.[50−53] Much like MGMT, MMR, MPG, and BER, for example, the role of HR

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and NHEJ is as a barrier against mutation; however, for these processes, the mutation is the result of adduct clastogenicity (i.e., DSBs).[54] Therefore, one would expect a threshold level of methylating agent in order to saturate the recombination processes and result in a clastogenic threshold, possibly observed as structural chromosomal aberrations or unrepaired DSBs. Such genotoxic end points have not yet been investigated in the context of thresholds. One can anticipate the effects of HR and NHEJ on the genotoxicity, and even the cytotoxicity, of a methylating agent more confidently than the effects of DDR signaling. The DDR is a multienzyme pathway composed of ataxia-telangiectasia (ATM), ATM and Rad3-related (ATR), checkpoint one and two (Chk1 and Chk2), and p53, among many others. The effect is the orchestrated control of cell cycle checkpoints and recombinational repair that leads to cell survival. However, under certain conditions, that remain to be fully defined, the DDR, and the proteins within it (for example, p53)[55] elicit cell death.[56] This may be the biochemical link between DNA damage and cell death.[57] The DDR pathway can be activated by MNNG-induced DSBs.[58] Intriguingly, the cellular outcome was shown to be dependent on dose and time. In the study, the DDR displayed distinct signaling dynamics, which is a phenomenon that my research group is interested in investigating. The question exists: at what point (dose and time) do the DSBs become toxic, and is this dependent on DDR signaling dynamics? A recent study[59] has highlighted the importance of the DDR in survival against DSBs. The study provides evidence of the role of ATM in the survival of mouse embryonic stem cells by promoting the repair of DSBs via HR and circumventing the need for NHEJ, which produced toxic chromosome fusions.[59] It is unknown how these fusions killed the cells and whether pro-repair functions of ATM and HR can become saturated at increasing doses (an increasing demands for DSB repair), leading to an accumulation of these fusions. Of course, the pro-survival functions of the DDR are well-known.[60] However, the DDR also has an apoptotic branch of the cascade that leads to cell death.[61] The possibility that a given level of DSBs (i.e., a damage threshold within the DDR) is needed to activate the pro-death branch of the cascade[62−64] and turn off repair is under investigation. The activation of the pro-death branch of the cascade may be a contributory factor, together with functional levels of the featured classical repair pathways,[65−67] in determining cell sensitivity to the clastogenicity of the adducts and the subsequent toxicity of the DSBs. This warrants further investigation, particularly in the context of cancer cell sensitization.

CONCLUDING REMARKS

The pathway of genotoxicity from drug exposure to adverse end point requires as much thought with regard to chemistry as it does of the biology of the cell. Our understanding of the chemical interaction of genotoxicants with cellular components, not just the DNA, coupled with the analytical methods employed to measure such interactions, has been incredibly revealing. It has led us to the point that we can say the dose is the genotoxic and cytotoxic poison, or not, and why. This depend, of course, on the biology of the cell. We should now be moving towards a position to be able to predict if a dose will be a poison, and in what way (mutagen, clastogen, toxicant) for a given cell type. The relationships of dose and end point are quantifiable. So, for a given biological system, we would be able to predict how many molecules are needed to saturate detoxification mechanisms, how many adducts will be repaired, how many will be mutagenic, and how many adducts are cytotoxic. This requires an understanding of the biological functions of the cell, enzyme polymorphisms for example, which will determine repair efficiencies or detoxification capabilities. In an era of personalized medicine and pharmacogenomics, this is personalized genotoxicity and cytotoxicity.

THE START OF SOMETHING SPECIAL

It started with a very casual conversation. I was approaching the end of my final year of undergraduate studies in Genetics and Dr. George Johnson said "what about a Ph.D.?" There was an opening in his working group, which was part of the DNA damage group, together with Professors Shareen Doak and Gareth Jenkins, at Swansea University. I learned so much about teamwork and sharing ideas from these incredible people and from being in such a collegiate, supportive environment.

At the time I joined, George was fairly recently out of his Ph.D. and a new principal investigator. I believe I had a more rewarding and fruitful experience as a result. He was personally supportive and gave me numerous professional opportunities. One being an introduction to Professor Bernd Kaina at UKEMS, held in Swansea in 2012. This shaped the next two years of my life, post-Ph.D. I had the incredible opportunity of working in Germany, in Professor Kaina's Institut für Toxikologie in Mainz. Professor Kaina and Dr Wynand Roos, a group leader there, are masters of the intricacies of chemical exposure and repair dynamics. Despite all that they had discovered, their thirst for more was incredible. There's always more to find out. At this point, I would like to mention some of those contemporary giants in this field, those whose shoulders I stand upon. Although not direct mentors of mine, Professors Leona D. Samson, Thomas G. Hofmann, Stephen P Jackson, Jiri Bartek, Penny Jeggo, and Geoff Margison continue to have an influence on my research through their insightful and groundbreaking publications.

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Notes

The author declares no competing financial interest.

Biography

Adam D. Thomas is a Senior Lecturer in Human Genetics and Genomics and early career researcher at UWE Bristol. He joined the academic team there in 2017, after completing his Ph.D. at Swansea University, Wales, and postdoctoral associate positions at the Johannes Gutenberg University of Mainz, Germany and Cardiff University, Wales. Adam is head of a newly established research team that wants to understand the underpinnings of the cellular response to low and high (toxic) doses of genotoxins and how signaling pathways dictate the fate of cells following chemical exposure.

REFERENCES

(1) Thomas, A. D., Fahrer, J., Johnson, G. E., and Kaina, B. (2015) Theoretical considerations for thresholds in chemical carcinogenesis. *Mutat. Res., Rev. Mutat. Res. 765*, 56−67.

https://dx.doi.org/10.1021/acs.chemrestox.0c00052
Chem. Res. Toxicol. 2020, 33, 2219−2224

(2) Preissner, S. C., Hoffmann, M. F., Preissner, R., Dunkel, M., Gewiess, A., and Preissner, S. (2013) Polymorphic cytochrome P450 enzymes (CYPs) and their role in personalized therapy. *PLoS One 8* (12), e82562.
(3) Paumi, C. M., Ledford, B. G., Smitherman, P. K., Townsend, A. J., and Morrow, C. S. (2001) Role of multidrug resistance protein 1 (MRP1) and glutathione S-transferase A1−1 in alkylating agent resistance. Kinetics of glutathione conjugate formation and efflux govern differential cellular sensitivity to chlorambucil versus melphalan toxicity. *J. Biol. Chem. 276* (11), 7952−7956.
(4) Zaïr, Z. M., Jenkins, G. J., Doak, S. H., Singh, R., Brown, K., and Johnson, G. E. (2011) N-methylpurine DNA glycosylase plays a pivotal role in the threshold response of ethyl methanesulfonate-induced chromosome damage. *Toxicol. Sci. 119* (2), 346−358.
(5) Thomas, A. D., Jenkins, G. J. S., Kaina, B., Bodger, O. G., Tomaszowski, K.-H., Lewis, P. D., Doak, S. H., and Johnson, G. E. (2013) Influence of DNA repair on nonlinear dose-responses for mutation. *Toxicol. Sci. 132* (1), 87−95.
(6) Warwick, G. P. (1963) The mechanism of action of alkylating agents. *Cancer Res. 23*, 1315−1333.
(7) Jenkins, G. J. S., Doak, S. H., Johnson, G. E., Quick, E., Waters, E. M., and Parry, J. M. (2005) Do dose response thresholds exist for genotoxic alkylating agents? *Mutagenesis 20* (6), 389−398.
(8) Strobel, H., Baisch, T., Fitzel, R., Schilberg, K., Siegelin, M. D., Karpel-Massler, G., Debatin, K.-M., and Westhoff, M.-A. (2019) Temozolomide and other alkylating agents in glioblastoma therapy. *Biomedicines 7* (3), 69.
(9) Sill, H., Olipitz, W., Zebisch, A., Schulz, E., and Wölfler, A. (2011) Therapy-related myeloid neoplasms: Pathobiology and clinical characteristics. *Br. J. Pharmacol. 162* (4), 792−805.
(10) Doak, S. H., Jenkins, G. J., Johnson, G. E., Quick, E., Parry, E. M., and Parry, J. M. (2007) Mechanistic influences for mutation induction curves after exposure to DNA-reactive carcinogens. *Cancer Res. 67*, 3904−3911.
(11) Gocke, E., and Muller, L. (2009) In vivo studies in the mouse to define a threshold for the genotoxicity of EMS and ENU. *Mutat. Res., Genet. Toxicol. Environ. Mutagen. 678*, 101−107.
(12) Klapacz, J., Pottenger, L. H., Engelward, B. P., Heinen, C. D., Johnson, G. E., Clewell, R. A., Carmichael, P. L., Adeleye, Y., and Andersen, M. E. (2016) Contributions of DNA repair and damage response pathways to the non-linear genotoxic responses of alkylating agents. *Mutat. Res., Rev. Mutat. Res. 767*, 77−91.
(13) Thier, R., Lewalter, J., Kempkes, M., Selinski, S., Brüning, T., and Bolt, H. M. (1999) Haemoglobin adducts of acrylonitrile and ethylene oxide in acrylonitrile workers, dependent on polymorphisms of the glutathione transferases GSTT1 and GSTM1. *Arch. Toxicol. 73* (4−5), 197−202.
(14) Peterson, L. A. (2010) Formation, repair, and genotoxic properties of bulky DNA adducts formed from tobacco-specific nitrosamines. *J. Nucleic Acids 2010*, 284935.
(15) Pottenger, L. H., Boysen, G., Brown, K., Cadet, J., Fuchs, R. P., Johnson, G. E., and Swenberg, J. A. (2019) Understanding the importance of low-molecular weight (ethylene oxide- and propylene oxide-induced) DNA adducts and mutations in risk assessment: Insights from 15 years of research and collaborative discussions. *Environ. Mol. Mutagen. 60* (2), 100−121.
(16) Goth, R., and Rajewsky, M. F. (1974) Persistence of O^6-ethylguanine in rat-brain DNA: correlation with nervous system-specific carcinogenesis by ethylnitrosourea. *Proc. Natl. Acad. Sci. U. S. A. 71* (3), 639−643.
(17) Zhao, C., and Hemminki, K. (2002) The *in vivo* levels of DNA alkylation products in human lymphocytes are not age dependent: an assay of 7-methyl- and 7-(2-hydroxyethyl)-guanine DNA adducts. *Carcinogenesis 23* (2), 307−310.
(18) Mustonen, R., and Hemminki, K. (1992) 7-Methylguanine levels in DNA of smokers' and non-smokers' total white blood cells, granulocytes and lymphocytes. *Carcinogenesis 13* (11), 1951−1955.
(19) Chen, H. J., and Liu, Y. F. (2013) Simultaneous quantitative analysis of N3-ethyladenine and N7-ethylguanine in human leukocyte deoxyribonucleic acid by stable isotope dilution capillary liquid chromatography-nanospray ionization tandem mass spectrometry. *J. Chromatog. A 1271* (1), 86−94.
(20) Beranek, D. T. (1990) Distribution of methyl and ethyl adducts following alkylation with monofunctional alkylating agents. *Mutat. Res., Fundam. Mol. Mech. Mutagen. 231* (1), 11−30.
(21) Bobola, M. S., Kolstoe, D. D., Blank, A., Chamberlain, M. C., and Silber, J. R. (2012) Repair of 3-methyladenine and abasic sites by base excision repair mediates glioblastoma resistance to Temozolomide. *Front. Oncol. 2*, 1−9.
(22) Blank, A., Bobola, M. S., Gold, B., Varadarajan, S., Kolstoe, D. D., Meade, E. H., Rabinovitch, P. S., Loeb, L. A., and Silber, J. R. (2004) The Werner syndrome protein confers resistance to the DNA lesions N3-methyladenine and O^6-methylguanine: implications for WRN function. *DNA Repair 3* (6), 629−638.
(23) Monti, P., Traverso, I., Casolari, L., Menichini, P., Inga, A., Ottaggio, L., Russo, D., Iyer, P., Gold, B., and Fronza, G. (2010) Mutagenicity of N3-methyladenine: a multi-translesion polymerase affair. *Mutat. Res., Fundam. Mol. Mech. Mutagen. 683* (1−2), 50−56.
(24) Sharma, V., Collins, L. B., Clement, J. M., Zhang, Z., Nakamura, J., and Swenberg, J. A. (2014) Molecular dosimetry of endogenous and exogenous O^6-Methyl-dG and N7-Methyl-G adducts following low dose [D_3]-Methylnitrosourea exposures in cultured human cells. *Chem. Res. Toxicol. 27* (4), 480−482.
(25) Swenberg, J. A., Fryar-Tita, E., Jeong, Y.-C., Boysen, G., Starr, T., Walker, V. E., and Albertini, R. J. (2008) Biomarkers in toxicology and risk assessment: informing critical dose-response relationships. *Chem. Res. Toxicol. 21* (1), 253−265.
(26) Swenberg, J. A., Lu, K., Moeller, B. C., Gao, L., Upton, P. B., Nakamura, J., and Starr, T. B. (2011) Endogenous versus exogenous DNA adducts: Their role in carcinogenesis, epidemiology, and risk assessment. *Toxicol. Sci. 120* (Suppl. 1), S130−S145.
(27) Ji, Z., Lebaron, M. J., Schisler, M. R., Zhang, F., Bartels, M. J., Gollapudi, B. B., and Pottenger, L. H. (2016) Dose-response for multiple biomarkers of exposure and genotoxic effect following repeated treatment of rats with the alkylating agents, MMS and MNU. *Mutagenesis 31* (3), 297−308.
(28) Kraus, A., McKeague, M., Seiwert, N., Nagel, G., Geisen, S. M., Ziegler, N., Trantakis, I. A., Kaina, B., Thomas, A. D., Sturla, S. J., and Fahrer, J. (2019) Immunological and mass spectrometry-based approaches to determine thresholds of the mutagenic DNA adduct O^6-methylguanine *in vivo*. *Arch. Toxicol. 93*, 559−572.
(29) Kaina, B., Christmann, M., Naumann, S., and Roos, W. P. (2007) MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. *DNA Repair 6* (8), 1079−1099.
(30) Mitra, S. (2007) MGMT: a personal perspective. *DNA Repair 6* (8), 1064−70.
(31) Mojas, N., Lopes, M., and Jiricny, J. (2007) Mismatch repair-dependent processing of methylation damage gives rise to persistent single-stranded gaps in newly replicated DNA. *Genes Dev. 21* (24), 3342−3355.
(32) Quiros, S., Roos, W. P., and Kaina, B. (2010) Processing of O^6-methylguanine into DNA double-strand breaks requires two rounds of replication whereas apoptosis is also induced in subsequent cell cycles. *Cell Cycle 9* (1), 168−178.
(33) Kaina, B. (2003) DNA damage-triggered apoptosis: Critical role of DNA repair, double-strand breaks, cell proliferation and signaling. *Biochem. Pharmacol. 66*, 1547−1554.
(34) Stojic, L., Mojas, N., Cejka, P., Di Pietro, M., Ferrari, S., Marra, G., and Jiricny, J. (2004) Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Dev. 18* (11), 1331−1344.
(35) Stojic, L., Cejka, P., and Jiricny, J. (2005) High doses of SN1 type methylating agents activate DNA damage signaling cascades that are largely independent of mismatch repair. *Cell Cycle 4* (3), 473−477.
(36) Park, J. W., and Ames, B. N. (1988) 7-Methylguanine adducts in DNA are normally present at high levels and increase on aging: Analysis

https://dx.doi.org/10.1021/acs.chemrestox.0c00052
Chem. Res. Toxicol. 2020, 33, 2219−2224

by HPLC with electrochemical detection. *Proc. Natl. Acad. Sci. U. S. A. 85* (20), 7467−7470.
(37) An, R., Jia, Y., Wan, B., Zhang, Y., Dong, P., Li, J., and Liang, X. (2014) Non-enzymatic depurination of nucleic acids: Factors and mechanisms. *PLoS One 9* (12), e115950.
(38) Rubinson, E., Gowda, A., Spratt, T., Gold, B., and Eichman, B. (2010) An unprecedented nucleic acid capture mechanism for excision of DNA damage. *Nature 468* (7322), 406−411.
(39) Engelward, B., Dreslin, A., Christensen, J., Huszar, D., Kurahara, C., and Samson, L. (1996) Repair-deficient 3-methy-ladenine DNA glycosylase homozygous mutant mousecells have increased sensitivity to alkylation-induced chromosome damage and cell killing. *EMBO J. 15*, 945−952.
(40) Ensminger, M., Iloff, L., Ebel, C., Nikolova, T., Kaina, B., and Lobrich, M. (2014) DNA breaks and chromosomal aberrations arise when replication meets base excision repair. *J. Cell Biol. 206* (1), 29−43.
(41) Banda, D., Nunez, N., Burnside, M., Bradshaw, K., and David, S. S. (2017) Repair of 8-oxoG:A Mismatches by the MUTYH glycosylase: Mechanism, metals and medicine. *Free Radical Biol. Med. 107* (3), 202−215.
(42) Chou, W. C., Hu, L. Y., Hsiung, C. N., and Shen, C. Y. (2015) Initiation of the ATM-Chk2 DNA damage response through the base excision repair pathway. *Carcinogenesis 36* (8), 832−840.
(43) Coquerelle, T., Dosch, J., and Kaina, B. (1995) Overexpression of N-methylpurine-DNA glycosylase in Chinese hamster ovary cells renders them more sensitive to the production of chromosomal aberrations by methylating agents-a case of imbalanced DNA repair. *Mutat. Res., DNA Repair 336* (1), 9−17.
(44) Eyler, D. E., Burnham, K. A., Wilson, T. E., and O'Brien, P. J. (2017) Mechanisms of glycosylase induced genomic instability. *PLoS One 12* (3), e0174041.
(45) Frosina, G. (2000) Overexpression of enzymes that repair endogenous damage to DNA. *Eur. J. Biochem. 267* (8), 2135−2149.
(46) Loechler, E. L., Green, C. L., and Essigmann, J. M. (1984) *In vivo* mutagenesis by O^6-methylguanine built into a unique site in a viral genome. *Proc. Natl. Acad. Sci. U. S. A. 81* (20I), 6271−6275.
(47) Margison, G. P., Santibáñez Koref, M. F., and Povey, A. C. (2002) Mechanisms of carcinogenicity/chemotherapy by O^6-methylguanine. *Mutagenesis. 17* (6), 483−487.
(48) Fahrer, J., Frisch, J., Nagel, G., Kraus, A., Dorsam, B., Thomas, A. D., Reißig, S., Waisman, A., and Kaina, B. (2015) DNA repair by MGMT, but not AAG, causes a threshold in alkylation-induced colorectal carcinogenesis. *Carcinogenesis 36* (10), 1235−1244.
(49) Fronza, G., and Gold, B. (2004) The biological effects of N3-methyladenine. *J. Cell. Biochem. 91* (2), 250−257.
(50) Giglia-Mari, G., Zotter, A., and Vermeulen, W. (2011) DNA damage response. *Cold Spring Harbor Perspect. Biol. 3* (1), a000745.
(51) Jackson, S. P., and Bartek, J. (2009) The DNA-damage response in human biology and disease. *Nature 461* (7267), 1071−1078.
(52) Pannunzio, N. R., Watanabe, G., and Lieber, M. R. (2018) Nonhomologous DNA end-joining for repair of DNA double-strand breaks. *J. Biol. Chem. 293* (27), 10512−10523.
(53) Wright, W. D., Shah, S. S., and Heyer, W. D. (2018) Homologous recombination and the repair of DNA double-strand breaks. *J. Biol. Chem. 293* (27), 10524−10535.
(54) Roos, W. P., Nikolova, T., Quiros, S., Naumann, S. C., Kiedron, O., Zdzienicka, M. Z., and Kaina, B. (2009) Brca2/Xrcc2 dependent HR, but not NHEJ, is required for protection against O^6-methylguanine triggered apoptosis, DSBs and chromosomal aberrations by a process leading to SCEs. *DNA Repair 8* (1), 72−86.
(55) Mayo, L. D., Seo, Y. R., Jackson, M. W., Smith, M. L., Rivera Guzman, J., Korgaonkar, C. K., and Donner, D. B. (2005) Phosphorylation of human p53 at serine 46 determines promoter selection and whether apoptosis is attenuated or amplified. *J. Biol. Chem. 280* (28), 25953−25959.
(56) Liebl, M. C., and Hofmann, T. G. (2019) Cell fate regulation upon dna damage: p53 serine 46 kinases pave the cell death road. *BioEssays 41* (12), No. 1900127.
(57) Villunger, A., Michalak, E. M., Coultas, L., Müllauer, F., Böck, G., Ausserlechner, M. J., Adams, J. M., and Strasser, A. (2003) p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science 302* (5647), 1036−1038.
(58) Noonan, E. M., Shah, D., Yaffe, M. B., Lauffenburger, D. A., and Samson, L. D. (2012) O^6-Methylguanine DNA lesions induce an intra-S-phase arrest from which cells exit into apoptosis governed by early and late multi-pathway signaling network activation. *Integr Biol. (Camb). 4* (10), 1237−1255.
(59) Balmus, G., Pilger, D., Coates, J., Demir, M., Sczaniecka-Clift, M., Barros, A. C., Woods, M., Fu, B., Yang, F., Chen, E., Ostermaier, M., Stankovic, T., Ponstingl, H., Herzog, M., Yusa, K., Martinez, F. M., Durant, S. T., Galanty, Y., Beli, P., Adams, D. J., Bradley, A., Metzakopian, E., Forment, J. V., and Jackson, S. P. (2019) ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks. *Nat. Commun. 10*, 87.
(60) Curtin, N. J. (2012) DNA repair dysregulation from cancer driver to therapeutic target. *Nat. Rev. Cancer 12* (12), 801−17.
(61) He, Y., Roos, W. P., Wu, Q., Hofmann, T. G., and Kaina, B. (2019) The SIAH1-HIPK2-p53ser46 damage response pathway is involved in Temozolomide-induced glioblastoma cell death. *Mol. Cancer Res. 17* (5), 1129−1141.
(62) Conrad, E., Polonio-Vallon, T., Meister, M., Matt, S., Bitomsky, N., Herbel, C., Liebl, M., Greiner, V., Kriznik, B., Schumacher, S., Krieghoff-Henning, E., and Hofmann, T. G. (2016) HIPK2 restricts SIRT1 activity upon severe DNA damage by a phosphorylation-controlled mechanism. *Cell Death Differ. 23* (1), 110−122.
(63) Matt, S., and Hofmann, T. G. (2016) The DNA damage-induced cell death response: a roadmap to kill cancer cells. *Cell. Mol. Life Sci. 73* (15), 2829−2850.
(64) Roos, W. P., Thomas, A. D., and Kaina, B. (2016) DNA damage and the balance between survival and death in cancer biology. *Nat. Rev. Cancer 16* (1), 20−33.
(65) Fu, D., Calvo, J. A., and Samson, L. D. (2012) Balancing repair and tolerance of DNA damage caused by alkylating agents. *Nat. Rev. Cancer 12* (2), 104−120.
(66) McFaline-Figueroa, J. L., Braun, C. J., Stanciu, M., Nagel, Z. D., Mazzucato, P., Sangaraju, D., Cerniauskas, E., Barford, K., Vargas, A., Chen, Y., Tretyakova, N., Lees, J. A., Hemann, M. T., White, F. M., and Samson, L. D. (2015) Minor changes in expression of the mismatch repair protein msh2 exert a major impact on glioblastoma response to Temozolomide. *Cancer Res. 75* (15), 3127−3138.
(67) Nagel, Z. D., Kitange, G. J., Gupta, S. K., Joughin, B. A., Chaim, I. A., Mazzucato, P., Lauffenburger, D. A., Sarkaria, J. N., and Samson, L. D. (2017) DNA repair capacity in multiple pathways predicts chemoresistance in glioblastoma multiforme. *Cancer Res. 77* (1), 198−206.

https://dx.doi.org/10.1021/acs.chemrestox.0c00052
Chem. Res. Toxicol. 2020, 33, 2219−2224