MGMT in glial carcinogenesis. Roles from prevention to treatment

İlhan Elmaci[a], Meric A. Altinoz[b], Eylem Burcu Kahraman Ozlu[c], Ramazan Sari[a], Ozlem Er[d], Cumhur Gokhan Ekmekci[e], Burcu Turkgenc[f], Alp Ozpinar[g], Emily Hacker[g] and Aysel Ozpinar[b]

Many investigations exist regarding the effect of the DNA repair enzyme *MGMT* (O^6-methylguanine- DNA-methyltransferase)-encoding gene methylation on the antineoplasticity of temozolomide in glioblastoma patients. However, there exist surprisingly lesser studies regarding the associations between MGMT enzyme biochemistry with glial carcinogenesis. MGMT involves in risk of malignancies associated with ionizing radiation, smoking, exposure to polycyclic aromatic hydrocarbons, chlorinated solvents, vinylchloride and hairdyes. All these factors are also proposed to link with gliomagenesis, yet MGMT interactions with these carcinogens in gliomagenesis are not studied yet. In future, MGMT sequencing may be employed in vulnerable populations working in industries associated with exposure to these carcinogens to develop preventive strategies. Given that MGMT is involved in DNA repair, a polymorphism may simultaneously modify the risk of gliomas while enhancing temozolomide cytotoxicity in both marrow and tumor cells. *European Journal of Cancer Prevention* XXX: 000–000 Copyright © 2022 Wolters Kluwer Health, Inc. All rights reserved.

Introduction

Gliomas constitute a high percentage of adult primary brain tumors and account for about 81% of malignant and 31% of all brain and central nervous system (CNS) tumors. Prognosis of high-grade gliomas is poor, especially for Grade-4 glioma glioblastoma (GBM), where the median survival is around 14 months (Armstrong *et al.*, 2009; Altinoz *et al.*, 2017). In the current standard of treatment, the chemotherapeutic temozolomide (TMZ, 75 mg/m^2) and adjuvant radiotherapy are administered to GBM patients with a new diagnosis. TMZ is an alkylating antineoplastic that functions by its conversion to monomethyl-triazeno-imidazole carboxamide at physiologic pH (Sharma *et al.*, 2009). 7-week TMZ-chemotherapy and concomitant radiotherapy may exert toxicity with an incidence of grade-3 or grade-4 neutropenia and thrombocytopenia in about 4 and 3% of patients, respectively (Sylvester *et al.*, 2011). Female GBM patients are more sensitive to TMZ-induced myelotoxicity (Armstrong *et al.*, 2009). For the percentage who develops considerable myelotoxicity, delays in treatment, infectious complications and even death may occur (Armstrong *et al.*, 2009). The cytotoxicity of TMZ results in part from DNA strand breaks induced by the addition of an alkyl moiety to the guanine's O^6-position (Remington *et al.*, 2009). O^6MeG (O^6-methylguanine) adducts are repaired through the irreversible transfer of the methyl adduct to the active site cysteine (Cys145) in MGMT (O^6-methylguanine-DNA-methyltransferase) enzyme (Bugni *et al.*, 2007). Numerous studies exist which showed that promoter site methylation and subsequent silencing of *MGMT* gene expression enhances glioblastoma sensitivity to TMZ. However, much fewer investigations can be found on the association of *MGMT* with gliomagenesis and on the association of *MGMT* single nucleotide polymorphisms (SNPs) with TMZ myelotoxicity and tumoricidal cytotoxicity (Bugni *et al.*, 2007; Armstrong *et al.*, 2009; Remington *et al.*, 2009; Sylvester *et al.*, 2011; Altinoz *et al.*, 2017). Therefore, we outline the role of *MGMT* in oncogenesis with particular emphasis on gliomagenesis, roles of *MGMT* SNPs in TMZ myelotoxicity and tumoricidal efficacy, which are important both in future prevention and treatment strategies of glial tumors.

Biochemistry of *MGMT* gene and MGMT enzyme in cancer

The native MGMT enzyme may induce several pathways including (a) protecting benign cells (all somatic cells, including marrow progenitors and peripheral

leukocytes) against the mutagenic activity, carcinogenicity and toxicity of alkylating agents, including chemotherapeutics, environmental/occupational carcinogens, and mutagens and (b) modifying the sensitivity of cancer cells to chemotherapeutic chloroethylating and methylating agents (Altinoz *et al.*, 2017). MGMT enzyme exerts the highest activity towards O^6MeG but can also repair other O^6-alkyl lesions, including butyl, pyridyloxobutyl, benzyl, ethyl and chloroethyl adducts (Bugni *et al.*, 2007). MGMT has an important role in the defense against alkylating molecules that generate O^6-alkylguanine in DNA which are termed O^6-alkylating agents (O^6-AA). O^6-AA are environmental carcinogens and they are also formed endogenously during inflammation, normal cellular metabolism and are being utilized in cancer treatment (Kaina *et al.*, 2007). The locus of the *MGMT* gene encoding *MGMT* enzyme resides on chromosome 10q26 and spans >170 kb. *MGMT* includes five exons, four of which encode, with a transcribed mRNA length of less than 1 kb (Altinoz *et al.*, 2017). The MGMT enzyme itself reverses alkylation damage and consists of 207 amino acids. After the repair of a single nucleotide adduct, each MGMT molecule is inactivated and degraded; hence, it is a suicide enzyme (Remington *et al.*, 2009). Via cleavage of the methyl adducts, the MGMT enzyme restores the affected guanine nucleotides to normal. If this fails to happen, O^6-methylguanines pair erroneously with thymine during DNA replication, resulting in G:C > A:T transition mutations in the DNA, which contribute to carcinogenesis. This is a negative consequence of *MGMT*-silencing; oddly, the same mechanism can be beneficial in cancer treatment. Because TMZ induces DNA damage via guanine methylation, ineffective repair of these nucleotide adducts due to dysfunctional *MGMT* enzyme variants or *MGMT* gene silencing could cause accumulation of the intolerable amount of DNA mutations (e.g. DNA double-strand breaks) which would ultimately cause the death of tumor cells. Chronic exposure to alkylating/methylating agents can lead to increased MGMT activity, which protects from spontaneous G:C to A:T transition mutations. Besides DNA damage, expression of the MGMT can be induced by Protein Kinase-C, cyclic AMP, glucocorticoids and via interaction of several transcriptional factors, including activator proteins 1 and 2 (AP-1 and AP-2) and SP1 with its promoter region (Sharma *et al.*, 2009). The *MGMT* gene harbors various SNPs which influence enzyme activity *in vivo* (Sylvester *et al.*, 2011). Significant marrow suppression could occur by the partial or complete inactivation of the MGMT enzyme in leukocytes (Sylvester *et al.*, 2011). As suggested, epigenetic silencing of specific promoters – such as with methylation – may reduce the expression of *MGMT* and increase the efficacy of antineoplastic drugs, including TMZ (Sylvester *et al.*, 2011).

MGMT in carcinogenesis and glial oncogenesis

Suppression of DNA stability and cancer suppressor genes via promoter methylation is common in human malignancies (Candiloro *et al.*, 2009). *MGMT* gene methylation and its subsequent silencing of expression might precede and predispose to carcinogenesis. Lack of MGMT enzyme expression and subsequent loss of its function will cause a mutator phenotype, as alkylation damage from environmental sources is common and consequently, alkylated guanine may mispair with thymine during replication of DNA (Candiloro *et al.*, 2009). When the carcinogen 4-(methyltyrosine)- 1-(3-pyridyl)-1-butanone (NNK)-induced chromosomal injury was assessed in lymphocytes obtained from 114 individuals, it was found that chromosomal abnormalities were modified by the *MGMT* genotype (Bugni *et al.*, 2007). Loss of *MGMT* expression occurs in many cancers, including glioma, lymphoma, prostate and breast cancer, as well as retinoblastoma which is caused by promoter methylation, a feature also witnessed *in vitro* in cancer cells lacking MGMT enzyme activity (Sharma *et al.*, 2009). Here, it shall be also noted that discrepancies between level of *MGMT* expression and *MGMT* promoter methylation status were encountered in up to 20% of glioblastoma patients which indicates additional methylation-independent regulatory mechanisms of *MGMT* expression (Fogli *et al.*, 2016). There is a general trend, for both women and the elderly, of more frequent *MGMT* methylation in cancerous tissues. Cells with either rs12917 or rs2308321 SNPs of the *MGMT* gene are more prone to NNK-induced chromosomal injury, and cells with both variants are even more vulnerable (Bugni *et al.*, 2007). *MGMT* knock-out mice are mildly prone to spontaneous mutagenesis and carcinogenesis, yet they are highly susceptible to the carcinogenic actions of alkylating agents (Bugni *et al.*, 2007).

Somatic *MGMT* inactivation is associated with G>A transitions in human malignancies and the dominant *TP53* mutation type in gliomas is base pair transition (e.g. G>A and C>T) (Wiencke *et al.*, 2005). O^6-alkylation of guanine frequently induces these changes and alkylating agents act as carcinogens on neural tissues (Wiencke *et al.*, 2005). In humans, the brain is more vulnerable to

Table 1 MGMT interactions with chemical carcinogens

MGMT's possible interactions with chemical carcinogens	
Polycyclic aromatic hydrocarbons	Anthracene, benz[a]anthracene, chrysene, phenanthrene, naphthalene, acenaphthene, acenaphthylene, fluoranthene, benzo[b]fluoranthene, fluorone and pyrene
Chlorinated solvents	Carbon tetrachloride, trichloroethylene, tetrachloroethylene, and methylene chloride

Vinylchloride (chloroethene)
Phenylenediamines and paraphenylenediamine (hairdye ingredients)
O^6-alkylating agents [O^6-AA] that generate O^6-alkylguanine in DNA

alkylating agents than other tissues because the activity of the MGMT enzyme is comparatively lowest in the brain among different organs (Zawlik *et al.*, 2009). Before 19 weeks of gestational age, fetal brain MGMT levels were low, indicating that exposure to endogenous and exogenous alkylating xenobiotics before that period may, due to vascular permeability, result in enhanced vulnerability to glial oncogenesis in later life (Sharma *et al.*, 2009). Besides the *P53*, *MGMT* methylation is associated with mutations of *k-ras*, methylation of the *CDKN1A* gene encoding p21, and the *CDKN2A* gene, encoding p16 (Sharma *et al.*, 2009). Methylation of MGMT promoter is found in 51.3–66% of glioblastomas (Liu and Jiang 2017). Very recently, it was shown that an insertion variant of *MGMT* (rs10659396) increases the risk of glioma by reducing MGMT expression by disrupting a STAT1-binding site (Huang *et al.*, 2021). There also exists immunohistochemical data which showed lesser protein expression of MGMT in high-grade versus low-grade gliomas (Sharma *et al.*, 2009) but such studies are always open to criticism because immunohistochemical antibodies may also bind to enzyme variants produced due to inhibiting or over-activating mutations. Here, it shall also be mentioned that MGMT expression can also be found to be increased in various malignancies, including gliomas (Sharma *et al.*, 2009). Hypothetically, this feature may associate with the fact that these tumors may try to cope with the inherent and chemotherapy-induced DNA damage and sustain their survival. Increased *MGMT* gene expression in glioma tissues following chemotherapy could be through the selection of high *MGMT*-expressing cells under the cytotoxic action of chemotherapy as selective survival of tumor cells expressing higher levels of *MGMT* during alkylating agent therapy may alter the *MGMT* status in recurrent state (Yu *et al.*, 2020). Another factor is the localization of glioblastomas. For instance, glioblastomas juxtaposing the subventricular zone exert increased stem cell features and poorer progression-free survival (PFS) and overall survival while expressing higher levels of MGMT enzyme (Steed *et al.*, 2020). A subset of recurring gliomas harbors *MGMT* genomic rearrangements that cause overexpression of MGMT enzyme, independently from alterations in *MGMT* promoter methylation (Oldrini *et al.*, 2020). By utilizing the clustered regularly interspaced palindromic repeats (CRISPR)/Cas9 technology, some of these *MGMT* rearrangements were created in glioma cells which revealed that these *MGMT* genomic rearrangements lead to TMZ-resistance *in vitro* and *in vivo* (Oldrini *et al.*, 2020).

MGMT SNP's in carcinogenesis, TMZ myelotoxicity, and tumoral response to TMZ chemotherapy

Transcriptional regulation of *MGMT* occurs in a 1.2 kb fragment of the gene, which includes the first untranslated exon (Leng *et al.*, 2011). A 59 bp enhancer necessary for efficient *MGMT* promoter functioning was determined between the exon 1 and intron 1 boundary along with an SNP (rs16906252) residing at the boundary between the exon 1 and enhancer (Leng *et al.*, 2011). This common *MGMT* promoter rs16906252 is strongly associated with *MGMT* methylation and is also associated with the absence of MGMT expression in malignancies (as assessed by immunohistochemical). Healthy people harboring the T allele of rs16906252 were shown to be prone to somatic methylation of *MGMT* by analyses on peripheral blood monocyte DNA from 89 healthy subjects ($P = 0.00005$) (Candiloro *et al.*, 2009). Promoter reporter assays in malignant lung cell lines revealed that the haplotype harboring the T allele of rs16906252 has decreased promoter activity in comparison to the wild-type sequence. Such SNPs may activate site-specific transcriptional repressors which then attract DNA methyltransferases, causing de novo DNA methylation and epigenetic silencing of gene expression via abnormal CpG island methylation. As mentioned, loss of MGMT expression occurs by epigenetic silencing in various malignancies, including >30% of colorectal, head and neck and brain cancers. Specifically, a correlation between methylation and the T allele of the rs16906252 SNP in colorectal carcinomas was described (Bugni *et al.*, 2007; Candiloro *et al.*, 2009). *MGMT* methylation may be simultaneous with wide CpG methylation defined as the CpG island methylator phenotype. The lowered transcription and expression seen for the rs16906252 SNP was decided to be a predisposing factor for *MGMT* methylation (Leng *et al.*, 2011).

Rapkins *et al.*, (2015) analyzed two independent TMZ-treated glioblastoma cohorts – one Australian ($n = 163$) and the other American ($n = 159$). They found that the T allele of the rs16906252 SNP was associated with higher glioma risk and this risk further increased when the cases were classified by the existence of *MGMT* methylation (Rapkins *et al.*, 2015). On the other hand, the same SNP was linked to *MGMT* gene methylation and low expression of MGMT enzyme and was associated with significantly better survival in TMZ-treated patients. Intriguingly, this better survival was encountered for both MGMT methylated and nonmethylated GBM (Rapkins *et al.*, 2015). To reveal the mechanism of rs16906252 conferring a survival advantage, they made constructs with either the T nucleotide at the rs16906252 SNP position or the wild-type promoter sequence and transfected these into GBM cell lines, U251 and U87 (Rapkins *et al.*, 2015). A decrease of ~30% in normalized promoter reporter activity was encountered in both cell lines transfected with the T allele (Rapkins *et al.*, 2015). They speculated that the variation in transcriptional extent between the two genetic alleles may be caused by the changed binding of a nuclear factor or complex to the *MGMT* enhancer-promoter, which in turn could change nucleosome occupancy at the transcriptional initiation site (Rapkins *et al.*, 2015). The authors suggested

that a genotype analysis at the rs16906252 SNP may be a valuable adjunctive test to *MGMT* methylation assessment of GBM, which could reveal a subset of patients with unmethylated *MGMT* that would also benefit from TMZ (Rapkins *et al.*, 2015).

rs1625649 SNP located at the *MGMT* promoter exists in 37.5% of glioblastomas in Taiwanese patients. Homozygous rs1625649 (AA genotype) was associated with an increased *MGMT* methylation and a reduced MGMT protein expression, albeit without statistical significance. In glioblastoma patients with *MGMT* methylation, subjects with homozygous rs1625649 (AA genotype) were associated with total absence of MGMT protein expression in a significant manner and had a longer PFS than the patients with wild type rs1625649 (CC genotype) or heterozygous rs1625649 (CA genotype). The impact on survival was found to be significant in multivariate analyses. The polymorphism rs1625649 is associated with enhanced lung cancer risk in an additive interaction with smoking; on the other hand, transfection experiments with glioma cells demonstrated that this polymorphism reduces expression of *MGMT* gene explaining better survival in patients harboring this SNP. A detrimental SNP increasing carcinogenesis acted beneficially in terms of enhanced chemotherapy responses.

MGMT rs12197 SNP was found to associate with higher DNA adducts in lung cancer patients who smoke despite this SNP was claimed to increase DNA repair efficacy of the MGMT enzyme (Martínez-Ramírez *et al.*, 2019). rs12917 polymorphism does not affect the risk of gastric cancers and melanomas, yet it is associated with increased risk of bladder and prostate carcinoma and of breast cancer in heavy smokers (Sharma *et al.*, 2009). Paradoxically, this polymorphism is also associated with a reduced risk of endometrial cancers (Sharma *et al.*, 2009). Even more peculiarly, studies exist for both increased and reduced risk of head and neck carcinomas in subjects carrying rs12917 SNP (Kiczmer *et al.*, 2018). In similar, while some studies showed no effect of this SNP on lung cancer risk, some other studies showed a risk-increasing effect of this allele (Martínez-Ramírez *et al.*, 2019; Sharma *et al.*, 2019). The likely reasons of contradictory or even dual effects are discussed in a previous review publication of our study group (Altinoz *et al.*, 2017). Similar to the situation encountered in head and neck tumors, both L84F and F84L variants of MGMT enzyme are found to correlate with increased risk of gliomas, which is difficult to explain (Adel Fahmideh *et al.*, 2014; Altinoz *et al.*, 2017). Based on data from the online databases, Sheng *et al.*, (2018) conducted an updated meta-analysis in 2018 where they included a total of 54 case-control studies (21 010 cases and 34 018 controls) for a series of pooling analyses. The T/T allele of the SNP rs12917 was associated with an increased risk in cancer compared with C/C [$P < 0.001$; odds ratio (OR) = 1.29] or with C/C+C/T ($P < 0.001$; OR = 1.32) (Sheng *et al.*, 2018). They revealed similar positive findings in the subgroups 'Caucasian', and 'glioma' (all $P < 0.05$; OR > 1) (Sheng *et al.*, 2018). Begg's and Egger's tests demonstrated that the results were free of publication bias, and sensitivity analysis indicated the stability of the pooling results suggesting that the T/T genotype of *MGMT* rs12917 may be linked to an increased vulnerability to malignancies overall, especially glial tumors, in the Caucasian population (Sheng *et al.*, 2018).

Radiation, risk of gliomas and *MGMT*
The strongest established and proven risk factor for glial cancers is ionizing radiation (Bauchet and Ostrom 2019). Besides the role of high dose ionizing radiation as a CNS carcinogen in children and adults, low doses of radiation may also increase the risk of childhood nervous system cancers (Berlivet *et al.*, 2020). Berlivet *et al.*, (2020) studied the role of natural background radiation (γ-radiation and radon) in childhood central nervous system tumors in a recent and very large study. They analyzed 5471 childhood CNS cancer cases registered for a period of 12 years and found an association between γ-radiation (incident rate ratio = 1.12 per 50 nSv/h) and pilocytic astrocytomas (Berlivet *et al.*, 2020). MGMT involves in radiation-triggered carcinogenesis. Lonjou *et al.*, (2017) studied the association of 141 polymorphisms located in 43 DNA repair genes in 254 controls and 75 papillary thyroid carcinoma cases in the Gomel region in Belarus. All cases were younger than 15 years when the Chernobyl radiation power plant accident happened. Three gene polymorphisms out of the studied 43 DNA repair genes were associated with thyroid carcinoma. One of these was the intronic SNP rs2296675 in *MGMT* which is associated with an increased risk of papillary thyroid carcinoma (Lonjou *et al.*, 2017). Sandler *et al.*, (2018) determined whether germline polymorphisms in 52 DNA repair genes are associated with developing thyroid cancer after diagnostic radiation exposure. Three polymorphisms in two different DNA repair genes were associated with thyroid cancer following radiation exposure. One of these was the *MGMT* rs12769288 polymorphism (Sandler *et al.*, 2018). Currently, there exists no data on how *MGMT* polymorphisms influence the risk of glial tumors in association with radiation exposure.

Polycylic aromatic hydrocarbons, smoking, risk of gliomas and *MGMT*
Polycyclic aromatic hydrocarbons (PAHs) are aromatic molecules with two or morfe fused benzene rings in their structure which do not possess substituents and heteroatoms on the ring system (Sahoo *et al.*, 2020). PAHs composed of up to four rings are named light PAHs and those that possess more than four rings are called heavy PAHs which are more toxic and stable than the light PAHs. In general, the increase in the angularity and size

of a PAH molecule increases the electrochemical stability and hydrophobicity (Sahoo *et al.*, 2020). The examples of PAHs are anthracene, benz[a]anthracene, chrysene, phenanthrene, naphthalene, acenaphthene, acenaphthylene, fluoranthene, benzo[b]fluoranthene, fluorone and pyrene among many others (Sahoo *et al.*, 2020). Several industrial activities that cause the production of PAHs include iron, steel and aluminium manufacturing; tar distillation, production of coke, coal gasification, carbon black, asphalt manufacturing and road paving, production of rubber tires; utilizing or manufacturing of fluids in metalworking and activity of natural gas or coal power stations, airport work (Sram *et al.*, 2016). Early studies demonstrated glioma associations with rubber and plastic industries. Preston-Martin *et al.*, (1989) obtained information and detailed occupational histories about potential risk factors during interviews with 272 men (ages 25–69 years) with a primary brain malignancy and with 272 matched neighbor controls. More glioma cases were found to work in the hot processes using plastics (9/1) and in the rubber industry (discordant pairs 6/1) (Preston-Martin *et al.*, 1989). Some carcinogenic PAHs are genotoxic that trigger mutation to initiate cancers; others are not mutagenic and are instead, involved in the promotion and progression of cancers. During PAH metabolism, versatile reactive and unstable intermediates of PAHs are produced which attack DNA, leading to cellular transformation (Shimada 2006). Epoxide hydrolase and P450 cytochrome enzymes convert PAHs to carcinogenic metabolites, PAH-diols, which are further metabolized again by P450s to ultimate carcinogenic metabolites, PAH diol-epoxides, or by aldo-keto reductase to active PAH o-quinones (Shimada 2006). PAHs are also activated by peroxidases and P450 to active radical cations that covalently attach to DNA. Mutagenic metabolites of PAHs include quinones, diol-epoxides and radical PAH cations which bind to specific sites in DNA, causing bulky complexes called DNA adducts which can be unstable or stable (Ewa and Danuta 2017). Stable adducts cause errors in DNA replication, whereas unstable adducts interact with the DNA, removing a purine base (either guanine or adenine), which can transform genes into oncogenes (such as H-ras) if they are not properly repaired (Cavalieri and Rogan 2004). PAH metabolites, such as quinones can also cause sustained production of reactive oxygen species that may independently cause DNA injury (Monks and Jones 2002; Idowu *et al.*, 2019). PAHs also possess endocrine-disrupting effects and immunotoxicity which may contribute to carcinogenesis (Sun *et al.*, 2021).

Many PAHs exist in oil and coal deposits and are also formed by the thermal decomposition of organic matter. For instance, they are formed in incinerators and engines or when biomass burns in fires. PAHs primarily exist in natural resources, such as fossil fuels and bitumen (Rehman *et al.*, 2020). The major environmental sources of PAHs are from human activity: combustion of biofuels, such as crop or dung residues and wood-burning. Lower-temperature combustion, such as tobacco smoking generally generates PAHs with lower molecular weight whereas high-temperature industrial processes tend to generate PAHs possessing higher molecular weights. PAH molecules with five or more rings have low volatility and lower solubility in water; thus they are dominantly in the solid-state, yet exist as bound to particulate air pollution, sediments and soils. Human exposure across the world varies depending on factors, such as fuel types in cooking, smoking rates, industrial processes, pollution controls on power plants and vehicles. In countries, burning solid fuels, such as biofuels and coal in residentials for heating and cooking is a major global source of PAH emissions (Berthiaume *et al.*, 2021). In industrial countries, people who smoke, or who are exposed to second-hand tobacco smoke, are among the most exposed groups. In these groups, tobacco smoke constitutes about 90% of PAH levels in the homes of smokers. The role of smoking regarding the risk of glial cancers is demonstrated by a recent and very large epidemiological study (Ahn *et al.*, 2020). By utilizing data from the Korean National Health Insurance System, 9 811 768 people over 20 years old without any cancer history in 2009 were followed for a median follow-up of 7.31 years until 2017 (Ahn *et al.*, 2020). In total 6100 glioma cases were determined. After adjusting for confounders, current smokers were shown to exert a higher risk of developing malignant gliomas in comparison to never-smokers. This association was stronger for those who smoked ≥20 cigarettes daily (hazard ratio = 1.50; CI, 1.36–1.64). Importantly, the risk of developing malignant gliomas increased with increasing pack-years of smoking in a dose dependent manner (the hazard ratio increased to 1.68 for ≥ 50 pack-years of smoking) (Ahn *et al.*, 2020).

People can be occupationally exposed to PAHs during work involving wood-burning, fossil fuels and their derivatives and diesel exhaust. These PAHs may associate with glial carcinogenesis in association with occupation. When all cases of CNS primitive neuroectodermal tumors (PNET), medulloblastomas and astrocytomas before 6 years of age diagnosed between 1990 and 2007 from the California Cancer Registry were analyzed, exposures to air toxins during gestation and infancy were found for 34 medulloblastoma, 43 PNET and 106 astrocytoma cases (von Ehrenstein *et al.*, 2016). Exposure to PAHs during the first year of life was found to be positively associated with astrocytoma (von Ehrenstein *et al.*, 2016). Volk *et al.*, (2019) utilized nationwide register data of Danish Cancer Registry and analyzed between paternal and maternal perinatal employment in industries with diesel engine exhaust and CNS cancers in children aged ≤19 years (who were diagnosed between 1968 and 2016) . In this wide analysis, they demonstrated increased risks for CNS cancers and astrocytoma for maternal employment in jobs with exposure to diesel engine exhaust (Volk *et al.*, 2019).

In an early observational study, Alexander *et al.*, (1980) investigated 18 deaths due to primary brain malignancy among male workers at a Texas petrochemical plant from 1965 to 1980. Median employment period was determined as 21 years and median latency was determined as 24 years. In total 15 of 18 tumors were glioblastoma multiforme which was evaluated as an unusual histologic proportion. Analyses demonstrated an enhanced brain cancer risk twice expected among 6800 white males employed at the petrochemical factory (Alexander *et al.*, 1980).

Thomas *et al.*, (1987) conducted a case-referent study on the risk of brain tumors among workers in refining and chemical manufacturing of petroleum products. They identified 300 brain tumor cases of white men (aged equal to or greater than 30 years) with a pathologic diagnosis of mixed glioma with astrocytic cells, astrocytoma and glioblastoma multiforme. The risk of astrocytic tumors was elevated among the subjects with production or maintenance jobs in petroleum refining (OR, 1.7); yet oddly, it decreased with the duration employed (Thomas *et al.*, 1987). In a case-control study, 78 astrocytoma cases have been compared with 92 population and 197 clinical controls with a detailed questionnaire to obtain information about residential, occupational and environmental exposure (Olin *et al.*, 1987). Examinations regarding individuals or groups of chemicals revealed subtle differences between controls and cases. Nonetheless, the questions 'living near a petrochemical plant' or 'working at an airfield' revealed increased risks compared to both control groups (Olin *et al.*, 1987). Morrison *et al.*, (1992) conducted a cohort study of the mortality experience of 156 242 male Canadian farmers between 1971 and 1987. A significant association was determined between enhanced fuel-oil expenditures and the risk of dying of glioblastomas (Morrison *et al.*, 1992). In a study analyzing occupational histories of 879 glioma cases and 864 controls in the San Francisco Bay Area, ever held or longest occupations of 1 year or more for all glial and nonglial cancers and controls were compared (Krishnan *et al.*, 2003). Statistically significant or two-fold or higher increased OR were determined overall and in men among those with longest-held occupations, as firefighters, motor vehicle operators and painters (Krishnan *et al.*, 2003). It is well known that PAHs are among chemicals which are exposed to painters and firefighters (Myong *et al.*, 2018; Hwang *et al.*, 2021).

There exists substantial evidence that MGMT activation occurs in response to PAH exposure. Diesel engine exhaust (DEE) is a well-established carcinogen to humans with its high PAH content (Zhang *et al.*, 2016). Changes in DNA methylation regarding DNA damage response (DDR)-related genes may potentially affect DEE exposure-related carcinogenesis. In 112 non-DEE-exposed workers and 117 DEE-exposed workers, Zhang *et al.*, (2016) evaluated urinary levels of six monohydroxylated PAHs (OH-PAHs). They also detected the methylation levels of the *MGMT* gene via bisulfite-pyrosequencing. They revealed that workers exposed to DEE exerted significantly lesser mean promoter methylation levels of MGMT ($P < 0.001$). In all nonsmoking workers and study subjects, elevated quartiles of urinary OH-PAHs were linked to hypomethylation of *MGMT* ($P < 0.05$). Additionally, *MGMT* methylation level demonstrated a negative correlation with cytokinesis-block micronucleus assay measured in the same workers (all $P < 0.05$) suggesting that *MGMT* gene activation occurs in response to mutagenic events of PAH exposure (Zhang *et al.*, 2016). Xing *et al.*, (2020) explored the gene expression and epigenetic changes in response to PAH exposure and analyzed MGMT expression and trimethylated Lys-36 of histone-H3 (H3K36me3) in peripheral blood lymphocytes of 173 coke-oven workers and 94 nonexposed workers (Xing *et al.*, 2020). The PAH-exposed group exerted increased DNA damage and enhanced expression of *MGMT* ($P < 0.001$). Expression of MGMT was positively correlated with modification of H3K36me3. Cell culture studies utilizing human bronchial epithelia exposed to extracts of coke-oven emissions also revealed that H3K36me3 is required for the expression of *MGMT* after PAH exposure (Xing *et al.*, 2020). PAH-DNA adducts are also associated with polymorphisms of *MGMT*. Zienolddiny *et al.*, (2006) determined PAH-DNA adducts in healthy lung tissue from 211 subjects and found that *MGMT* rs2308327 SNP (Lys178Arg) were more frequent in those with PAH-DNA adduct levels lower than the mean. Polymorphisms of the *MGMT* gene also affect bulky DNA adducts in subjects who smoke. Molina *et al.*, (2013) revealed a significant association between higher formation of DNA adducts in smokers and MGMT rs12917 (Phe/Phe) haplotype ($P = 0.0215$). Thus, it is not surprising to encounter data suggesting *MGMT* SNPs affect the smoking-associated risk of cancers (Christmann and Kaina 2012). Despite there existing substantial data regarding the association of PAHs, smoking and gliomas and role of *MGMT* in PAH carcinogenicity, there exists no current data on how the *MGMT* genotypes would link PAHs and smoking to the risk of gliomas.

Vinyl chloride, risk of gliomas and *MGMT*

Vinyl chloride harboring the chemical formula $H_2C = CHCl$ is a colorless organochloride gas with a sweet odor that is also named chloroethene. It is a major industrial chemical, produced about 13 billion kilograms per year and is mainly used to produce its polymer, polyvinyl chloride. In global production, vinyl chloride is among the top 20 petroleum-derived chemicals (petrochemicals). Vinyl chloride can also be formed by the breakdown of other chlorinated chemicals and then can enter the drinking water supplies and air. Epidemiologic data of occupational risk of neurologic cancers has been

demonstrated in a series of prospective investigations in the petrochemical industry, and vinyl chloride exposure was identified as a major occupational carcinogen for the CNS (Moss 1985).

MGMT also seems to involve in the genotoxicity of vinyl chloride. In 101 vinyl chloride-exposed workers, *MGMT* methylation was detected by methylation-specific PCR, and chromosome damage was determined by the cytokinesis-block micronucleus assay in peripheral blood lymphocytes (Wu *et al* 2013). They determined *MGMT* promoter methylation in 5 out of 49 chromosome-damaged subjects, but not in the chromosome nondamaged subjects and this difference was statistically significant ($P < 0.05$). No studies exist regarding the glial carcinogenicity of vinyl chloride and its association with MGMT.

Chlorinated solvents, risk of glioma and MGMT

Associations of human glial brain cancers were observed with exposure to chlorinated solvents carbon tetrachloride, trichloroethylene, tetrachloroethylene and methylene chloride which were strongest for the methylene chloride (also called dichloromethane) (Cocco *et al.*, 1994; Heineman *et al.*, 1994). This nonflammable, colorless volatile liquid is utilized as a solvent in paint removers but is also employed as a solvent in the production of pharmaceuticals, as a degreasing agent, as an ethane foam blowing agent, in aerosol formulations and in electronics manufacturing. Brain tumor patients with exposure to chlorinated solvents harbor significantly higher levels of passenger loss of heterozygosity mutations in comparison to sporadic brain tumor controls (Ellsworth *et al.*, 2012). *MGMT* SNPs considerably affect carcinogenesis associated with exposure to chlorinated solvents. Compared to women with no occupational exposure to chlorinated solvents, women exposed to these solvents exerted an increased risk of all non-Hodgkin lymphomas (NHL) if they carry the *MGMT* (rs12917) CT/TT genotype (OR = 3.05), but not among women with the *MGMT* (rs12917) CC genotype (OR = 1.02) (Jiao *et al.*, 2012). Similar interactions were noted between *MGMT* (rs12917) SNP with follicular lymphoma ($P < 0.005$) and diffuse large B-cell lymphoma ($P < 0.005$). We did not encounter any studies which linked *MGMT* genotype in risk of gliomagenesis associated with exposure to chlorinated solvents.

Hairdyes, risk of gliomas and MGMT

Neuberger *et al.*, (1991) investigated a brain cancer cluster in Missouri by utilizing two approaches to analyze associations with suspected risk factors. In a case-control study conducted in a rural town, they made interviews with cases and controls about risk factors. Additionally, they calculated the expected and observed proportion of brain malignancies by industry and occupation in Missouri decedents in a standardized proportional mortality investigation for the Missouri state. They found that hairdressers and cosmetologists had significantly elevated proportions of brain cancer (Neuberger *et al.*, 1991).

Kuijten *et al.*, (1992) analyzed parental occupations as possible risk factors for pediatric astrocytoma in a case-control study of 163 pairs under 15 years of age. Cases were determined through the cancer registries of eight hospitals in Pennsylvania, Delaware and New Jersey. Elevated – although NS odds ratios – were observed for some white-collar and professional occupations in case parents and for maternal occupation as a hairdresser (Kuijten *et al.*, 1992). Several studies demonstrated increased risks of NHL associated with increasing duration of hair dye use (Guo *et al.* 2014). Several hair dye ingredients, such as phenylenediamines and paraphenylenediamine, may act as carcinogens that can lead to DNA damage and cytogenetic alterations (Guo *et al.*, 2014). Particularly, some of the hair dye ingredients utilized before 1980 were declared to be more mutagenic, which provide plausibility for the epidemiologic studies that suggested an elevated risk of some NHL subtypes and overall NHL in women who began to use hair dye products before the year 1980 but not later (Guo *et al.*, 2014). Guo analyzed 24 SNPs in 16 DNA repair genes among 597 controls and 518 NHL cases and evaluated the associations between hair dye use and risk of common NHL subtypes. Women who began to use hair dyes before 1980 had a significantly elevated NHL risk, particularly for the follicular lymphoma subtype. *MGMT* genotypes rs12917 CC, rs2308321 AA and rs2308327 AA were associated with a higher risk of folicular lymphoma (Guo *et al.*, 2014). We did not find studies which associated the risk of gliomas with hair dye use according to the *MGMT* genotype.

How can we benefit from information about MGMT regarding prevention of gliomas?

Above, we mentioned that *MGMT* polymorphisms may profoundly interact with carcinogenesis associated with exposure to different types of carcinogens. Gene sequencings become cheaper as technologic advances are every day in progress. In near future, routine gene sequencing of *MGMT* may be employed in risky occupations where exposure to gliomagenesis-associated carcinogens takes place. If workers harbor the risk of carrying *MGMT* variants, higher preventive measures shall be taken to reduce the risk of gliomagenesis. These may include changing the workplace or even complete avoidance of work associated with exposure to carcinogens. Meanwhile, molecular advances, such as CRISPR/Casp9 technology makes the possibility of gene changing every day more feasible and more secure. This may allow changing risky *MGMT* variants with *MGMT* forms more potent in repairing DNA damage. This would allow avoiding chemotherapy myelotoxicity and marrow stem cell-transforming risks of carcinogens. In an elegant study, Neff *et al.*, (2005) demonstrated that

a gain-of-function mutant *MGMT* (meaning an *MGMT* variant encoding an MGMT enzyme isoform with more potent DNA repair capability) can selectively spare marrow cells against TMZ myelotoxicity without causing leukemia. In a clinically relevant large animal experiment, gene therapy with the P140K mutant of the *MGMT* was employed which encodes an MGMT isoform that provides resistance to the combination of the MGMT-inhibitor O^6-benzylguanine and TMZ or nitrosourea drugs, such as carmustine. Two dogs were given *MGMT*(P140K)-transduced autologous CD34$^+$-selected marrow stem cells. Following stable engraftment, gene marking in granulocytes was detected to occur between 3 and 16%. Repeated exposure to TMZ and O^6-benzylguanine lead to a multilineage enhancement in gene-transduced repopulating cells with levels of more than 98% in granulocytes (Neff *et al.*, 2005). Overexpression of *MGMT*(P140K) hindered the prominent myelotoxicity normally occurring with this drug combination. Moreover, throughout the study, the hematopoiesis remained polyclonal, extrahematopoietic toxicity was very low and no myelodysplasia or leukemia was encountered (Neff *et al.*, 2005). Here, it shall be underlined that this study was conducted as early as in 2005; and more efficient and more secure gene changing strategies, such as CRISPR/Casp9 technology may confer better gene therapy to uncouple the tumoricidal benefits versus myelotoxic and proleukemic effects of DNA-poisoning antineoplastics.

Conclusion

In future, *MGMT* gene variants could be routinely screened in vulnerable populations working in jobs with exposure to carcinogens, such as ionizing radiation, polycyclic aromatic hydrocarbons, chlorinated solvents, vinylchloride and hairdyes. If risk-carrying variants will be detected, more intensive precautions and measures shall be taken to prevent and minimize the risk of gliomagenesis. Some polymorphisms of MGMT and accompanying methylation could decrease the functioning or presence of the MGMT enzyme, which enhances the risk of cancer and renders the patient's marrow susceptible to chemotherapy-induced myelosuppression. These are certainly detrimental effects. On the other side, decreased levels or lack of MGMT enzyme in cancer cells may also sensitize them to chemotherapy. We believe that – for the future – whole sequencing of the *MGMT* gene shall be routinely performed in high-grade glioma patients to reveal the exact contribution of each different SNP to the risk of gliomagenesis, myelosuppression and response to chemotherapy. Clarifying the effects of these polymorphisms on myelotoxicity versus tumoricidal activity of antineoplastics may also pave to develop novel and precise strategies for treatment. Additionally and likely, more importantly, better glioma prevention strategies may be employed in occupations conferring risks to glioblastoma by avoiding exposure to carcinogens, especially in workers harboring *MGMT* gene variants with improper DNA repair capability.

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