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Aberrant kynurenine signaling modulates DNA replication stress factors and promotes genomic instability in gliomas

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Abstract

Metabolism of the essential amino acid L-tryptophan (TRP) is implicated in a number of neurological conditions including depression, neurodegenerative diseases, and cancer. The TRP catabolite kynurenine (KYN) has recently emerged as an important neuroactive factor in brain tumor pathogenesis, with additional studies implicating KYN in other types of cancer. Often highlighted as a modulator of the immune response and a contributor to immune escape for malignant tumors, it is well known that KYN has effects on the production of the co-enzyme nicotinamide adenine dinucleotide (NAD^+), which can have a direct impact on DNA repair, replication, cell division, redox signaling, and mitochondrial function. Additional effects of KYN signaling are imparted through its role as an endogenous agonist for the aryl hydrocarbon receptor (AhR), and it is largely through activation of the AhR that KYN appears to mediate malignant progression in gliomas. We have recently reported on the ability of KYN signaling to modulate expression of human DNA polymerase kappa ($\text{hpol } \kappa$), a translesion enzyme involved in bypass of bulky DNA lesions and activation of the replication stress response. Given the impact of KYN on NAD^+ production, AhR signaling, and translesion DNA synthesis, it follows that dysregulation of KYN signaling in cancer may promote malignancy through alterations in the level of endogenous DNA damage and replication stress. In the following perspectives article, we discuss the connections between KYN signaling, DNA damage tolerance, and genomic instability, as they relate to cancer.

Graphical Abstract

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Keywords

Aryl hydrocarbon receptor; cancer treatment; DNA replication; glioma/glioblastoma; kynurenine; polymerase; translesion DNA synthesis

Aberrant kynurenine (KYN) signaling in gliomas promotes malignancy

L-tryptophan (TRP) is an essential amino acid in humans that has important neuroactive properties. Most TRP (~95%) in the body is metabolized to KYN by one of three enzymes: tryptophan 2,3-dioxygenase (TDO; also called TDO2), indoleamine 2,3-dioxygenase 1 (IDO1) and 2 (IDO2) (Figure 1).¹ KYN is a precursor to both neuroprotective and neurotoxic molecules, such as 3-hydroxy-L-kynurenine (3-HK, neurotoxic), 3-hydroxyanthranilic acid (3-HAA, neurotoxic), kynurenic acid (KYNA, neuroprotective), picolinic acid (PIC, neuroprotective), and quinolinic acid (QUIN, neurotoxic).² Metabolites of the KYN pathway (KP) act as *N*-methyl-D-aspartate (NMDA) receptor agonists/antagonists, glutamate receptor antagonists, and can contribute to the production of free radicals in the central nervous system (CNS).¹ As discussed below, KYN is an endogenous agonist for the aryl hydrocarbon receptor (AhR), a transcription factor best known for its role in activation of genes following exposure to xenobiotics. Moreover, production of the co-enzyme nicotinamide adenine dinucleotide (NAD⁺), which is used in many biological pathways including ATP production, p53-mediated stress responses and DNA repair, depends on the KYN metabolite QUIN. QUIN has been shown to increase the production of free radicals, leading to oxidative stress, DNA damage, and increased Poly(ADP-ribose) polymerase 1 (PARP-1) activity.^{3–5} NAD⁺ influences DNA repair and gene expression through its role as a substrate for PARP-1.⁶ Poly(ADP-ribosylation) of acceptor proteins near sites of DNA damage influences the recruitment of DNA repair factors. PARP-1 activity also influences gene expression (through interactions with transcriptional co-activators) and cellular energy balance (through modulation of cellular ATP levels). Thus, aberrant KYN signaling and subsequent formation of the NAD⁺ precursor QUIN can act through PARP-1 to modulate genomic integrity.

There are a number of neurological pathologies associated with alterations in KYN signaling including Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, multiple sclerosis, and Parkinson's disease. The physiology and clinical implications related to KYN signaling in the CNS is complex and has been reviewed in detail elsewhere.¹ Needless to say, there are many aspects of how KYN and its metabolites affect neurodegenerative and autoimmune diseases that are under intense investigation as possible routes to new therapies. Likewise, the role of KYN signaling in cancer is multi-faceted and new molecular features continue to be reported. A landmark study in 2011 revealed a previously unrecognized connection between TDO-mediated elevation of KYN levels, subsequent activation of the AhR, and malignant progression in gliomas.⁷ Additionally, increases in the KYN/TRP ratio and/or increased expression of KYN pathway enzymes have been observed in adult T-cell leukemia/lymphoma,⁸ breast cancer,^{9, 10} cervical cancer,¹¹ colorectal cancer,¹² meningiomas,¹³ and non-small cell lung cancer.¹⁴ The effect of increased KYN signaling on tumor biology is multifaceted, but several metabolites of TRP catabolism, including KYN,

can promote malignant properties in cancer (Figure 1). TRP catabolism has been linked to an immunosuppressive effect in cancer both by depleting Trp, which induces T-cell hyporesponsiveness and apoptosis, and by accumulation of immunosuppressive trp catabolites, such as KYN.¹⁵ Elevated KYN levels can suppress allogeneic T-cell proliferation, promote tumor cell survival, as well as motility, in glioma cells.⁷ While many studies have focused on the role of KYN in immune escape, there is now evidence supporting the notion that aberrant KYN signaling can alter genome stability in gliomas through changes in DNA repair and replication factors.

Glioma pathobiology, molecular features, and clinical impact

Gliomas, which arise from glial cells, are the most common type of primary brain tumor.¹⁶ Glial cells provide support and protection for neurons. They are the most common cells in the brain and consist of three subtypes: astrocytes, oligodendrocytes, and ependymal cells. More than three quarters of gliomas are astrocytomas, which are generally thought to arise from astrocytes, although it is unclear if they arise from differentiated astrocytes, astroglial progenitors, or neural stem cells.¹⁶ Other types are oligodendrogliomas and ependymomas, and tumors consisting of mixed types also occur.¹⁶ The most malignant form of glioma, glioblastoma multiforme (GBM or simply glioblastoma), is the most common of all malignant brain and CNS tumors, accounting for 46.1% of these patients.¹⁶ It is predicted that > 12,000 people will be diagnosed with GBM in 2016.¹⁶ Symptoms vary widely depending on where the tumor is located and the rate of growth. Recurrence rate for GBM is approximately 90%.¹⁷ In addition to being the most prevalent type of malignant tumor in the central nervous system, gliomas are also one of the deadliest forms of cancer. Glioblastoma is defined by the World Health Organization (WHO) as a grade IV astrocytoma. The current one-year relative survival rate for GBM is 37.2% and for five years is 5.1%.¹⁶ The median survival time of GBM patients has hovered near 12 months for the past decade¹⁸ despite the intense pursuit of new therapeutic strategies.

Morphologically, GBMs are characterized by widespread infiltration throughout the brain, which often makes surgical removal difficult. Additional complications arise from the extreme resistance to chemo- and radio- therapies, destruction of normal brain tissue, uncontrolled cellular proliferation, high vascularization, and rampant genomic instability observed in GBMs.¹⁸ These characteristics are common to most cancers,¹⁹ however, the degree of genomic instability in particular is much higher than in other epithelial carcinomas.²⁰ As the name “multiforme” suggests, GBMs have significant intratumoral heterogeneity, which is also related to a high level of genomic instability. Understanding glioma-specific mechanisms promoting increased levels of replication stress and endogenous DNA damage is a vital area of research.

Aberrant DNA damage signaling and replication stress in gliomas

The poor prognosis associated with higher stage gliomas is due in part to the fact that these tumors, especially GBMs, generally exhibit little response to therapies, such as ionizing radiation and chemotherapy.¹⁸ This inherent resistance may be due to the fact that these cancers exhibit constitutive, widespread, and robust activation of the replication stress

response and DNA damage signaling.²⁰ Indeed, it has been shown that glioma cells with higher DNA damage response and cell cycle checkpoint activation are more resistant to irradiation than those with less activation.²¹ Since the prolonged presence of DNA breaks induces apoptosis or senescence, the ability to tolerate stress at the fork and repair DNA damage is essential for tumor cells to survive treatment with DNA damaging agents.^{22–24}

Elevated levels of replication stress and DNA damage markers in gliomas have been observed before the onset of chemotherapy, suggesting that these events are intrinsic to the biology of the disease.²⁰ Both low- and high-grade human gliomas exhibit elevated γ H2AX levels, activation of the ATM-Chk2-p53 pathway, and increased 53BP1 foci formation.²⁰ Chk1 activation, Rad17 phosphorylation, replication protein A (RPA) foci, and ssDNA gaps are all indicative of ongoing elevated replication stress in GBMs and are thought to be a major source of genomic instability in these tumors.²⁰ There is evidence of elevated oxidative damage to DNA (i.e. increased 8-oxoguanine) in some GBM cell lines and tumors.²⁰ However, the presence of high γ H2AX staining in tumor sections with low 8-oxoguanine staining indicates that oxidative damage is not the main source of DNA damage inducing the replication stress response.²⁰ Interestingly, the highest level of DNA damage signaling has been observed in grade II astrocytomas (based on γ H2AX staining).²⁰ Typically, DNA damage response activation is thought to act as a barrier to tumor progression, and this does seem to be the case in early stages of glioma. However, the fact that mutant p53 was most prevalent in GBM tissue sections with high constitutive DNA damage response activation supports a model where persistent replication stress selects for mutations that facilitate escape from cell cycle checkpoints.²⁰ Selection through mutation is dependent upon mechanisms that promote tolerance of DNA damage and the resolution of replication stress.

Translesion DNA synthesis: a central mediator of DNA damage tolerance

DNA damage and replication stress responses play an essential role in helping maintain genomic stability through the activation of pathways that cause cell cycle arrest when DNA damage is detected, allowing the damage to be repaired.²⁴ However, if there is unrepaired damage or damage that occurs during S-phase, lesions may be bypassed in lieu of actual repair to preserve fork stability and ensure completion of replication and avoid prolonged stalling, which can lead to fork collapse, double strand break formation, and increased genomic instability.²⁵ The direct bypass of lesions occurs through a process known as translesion DNA synthesis (TLS). Evolution has retained multiple different types of DNA polymerases (pols) that are classified into different families, including A, B, C, D, X, Y, and reverse transcriptases.²⁶ TLS is carried out by a group of specialized DNA pols, of which the Y-family members are the most versatile at bypassing a wide-variety of lesions (Figure 2A). In humans, the Y-family consists of pol eta (pol η), pol iota (pol ι), pol kappa (pol κ), and Rev1.²⁷ The B-family member pol zeta (pol ζ) is also important for many TLS events.^{28–30}

TLS pols are recruited to sites of DNA damage/replication stress to allow direct bypass of lesions (Figure 2B). Recruitment of the Y-family pols to the replication fork is a complicated process and some aspects (e.g. order of recruitment, extent of protein-protein interactions, cell-cycle dependence) are not completely understood.^{25, 31} Like most other DNA pols, Y-

family members possess a short PCNA interacting peptide (or PIP box), which facilitates binding to a hydrophobic patch on the sliding clamp.^{32, 33} The interaction with PCNA through the PIP box has been shown to stimulate Y-family pol activity (at least for some members), but the stimulation is likely related to a decreased rate of dissociation from the DNA substrate and not due to altered conformational dynamics.^{32, 34–36}

Ubiquitination of PCNA is important for the recruitment of Y-family pols in certain instances. Y-family pols contain ubiquitin-binding domains that are important for interactions with ubiquitinated PCNA that stabilize the TLS pols at the fork.^{37–41} Replication fork stalling can lead to uncoupling of pol and helicase action, which can generate regions of ssDNA that are recognized as signs of replication stress. Formation of ssDNA gaps leads to the activation of Rad18 and cleavage of USP1, a de-ubiquitinating isopeptidase that removes the ubiquitin from mono-ubiquitinated PCNA.⁴² Rad18 binds Rad6 and this complex catalyzes the mono-ubiquitination PCNA^{25, 31}, increasing the affinity of PCNA for TLS pols.^{37, 38, 43–45} There is a constant cycle of PCNA ubiquitination/deubiquitination, and it is through the degradation of the deubiquitinase USP1 in response to ATR activation and subsequent phosphorylation of the Chk1 kinase that the balance is shifted towards stable ubiquitinated-PCNA.⁴⁶ In addition to post-translational regulation of TLS, there is evidence that Y-family pols track with replication forks in the absence of damage signaling (i.e. in the absence of ATR activation and subsequent stabilization of Ub-PCNA).^{47, 48} This so-called “on the fly” TLS likely serves to help bypass endogenous barriers to replication, such as G-quadruplexes, and the basal level of DNA damage that results from normal cellular metabolism.^{48, 49}

In addition to interactions with PCNA, the Y-family pols can interact with each other to help recruit and stabilize the TLS pols near sites of damage. For example, Rev1 is capable of binding pol κ , pol η and pol ι , as well as pol ζ .^{50–53} Rev1 also interacts with PCNA and is thought to serve as a scaffold between PCNA and the other TLS pols.^{40, 54–56} Both pol η and ι are localized in replication foci in response to DNA damage.^{31, 57} Ubiquitination of PCNA increases the number of contacts between these enzymes and PCNA through their ubiquitin-binding domains.^{25, 32} Pol κ , which has two ubiquitin binding domains²⁵, is thought to be recruited to the replication fork when PCNA is mono- or poly-ubiquitinated in response to specific types of damage or during prolonged periods of replication stress.^{25, 60–62} Polyubiquitination of PCNA occurs through the actions of Rad18, which is able to bind not only to Rad6 but also to the Rad5 E3 ligase⁶³, and the Rad5/MMS2/UBC13 complex.^{25, 31}

Due to their unusually spacious catalytic sites, TLS pols can accommodate many different lesions that cause high-fidelity replicative pols to stall.²⁷ The bypass of lesions by Y-family pols can occur in either an “error-free” manner, where the correct nucleotide is inserted opposite the damage, or in an “error-prone” manner, with the wrong nucleotide inserted. TLS pols lack exonuclease activity and, therefore, cannot readily correct any errors they may make.^{48, 62, 64, 65} The fidelity of the bypass depends upon several factors, such as the type of DNA lesion and the catalytic attributes of the specific pol carrying out bypass.⁴⁸ For example, Rev1 promotes proficient and error-free synthesis through N2-adducts on guanines because this enzyme is specialized for the incorporation of dCTP through pairing with

arginine in its active site rather than the template base.⁶⁶ Similarly, hpol κ has been shown to play an important role in the accurate bypass of bulky-N2-dG adducts and cell survival following exposure to benzo[a]pyrene.^{67–69} The recruitment of pol κ to sites of benzo[a]pyrene dihydrodiol epoxide-induced replication stress is mediated by the action of the Rad18 E3 ligase⁷⁰

In addition to the role for pol κ in catalyzing bypass of DNA lesions, there is also evidence that pol κ DNA synthesis is important for Chk1 activation.⁷¹ Phosphorylation of Chk1 following treatment with hydroxyurea was decreased in pol κ -deficient cells relative to cells expressing pol κ .⁷¹ The activity of pol κ has been shown to be involved in the formation of short replication intermediates in regions undergoing replication stress.⁷¹ These intermediates possess free 5' termini that are crucial for the binding of the 9-1-1 complex to DNA through Rad9.⁷² It is, therefore, not surprising that pol κ depletion also affects recruitment of the Rad9 subunit to chromatin.⁷¹ The 9-1-1 complex consists of three proteins-Rad9, Hus1, and Rad1-that interact in a heterotrimeric complex.⁷³ This complex binds to DNA in response to replication stress and facilitates the activation of Chk1 through phosphorylation by the ATR kinase. Chk1 regulates S-phase progression, G2/M arrest, activation of DNA repair, TLS, and mediates apoptotic signaling.^{73, 74} Thus, one would predict that one consequence of hpol κ over-expression would be constitutive activation of the RSR.

Mis-regulation of TLS occurs in cancer, is related to disease progression, and can affect response to treatment

Y-family pols have been linked both to suppressing mutations in some instances and to promoting mutagenesis in others.^{64, 75–78} This balance between protective and deleterious effects is likely a major reason evolution has retained multiple regulatory mechanisms that either allow or disallow TLS pols access to the fork. Perhaps not surprisingly, DNA damage tolerance components have been reported to be mis-regulated in many different cancers^{76, 79, 80} and are involved in resistance to many chemotherapeutics^{75, 81, 82} (Table 1).

Of particular interest, the mis-regulated expression of hpol κ has been observed in multiple types of cancer.^{79, 80, 83–85} For example, higher stage gliomas have been found to over-express DNA damage tolerance components.⁸⁶ Over-expression of hpol κ was observed in 23 out of 40 glioma patients (57.5%) and hpol ι in 11 of 40 (27.5%), while there was no observable change in hpol η expression between glioma patients and normal tissue.⁸⁴ Additionally, 72 out of 104 (69.2%) formalin-fixed, paraffin embedded human glioma specimens had positive hpol κ staining and 33 (31.7%) were positive for hpol ι . Importantly, hpol κ expression is correlated with advanced stages and hpol κ expression has been identified as an independent prognostic indicator of a poor outcome for glioma patients.⁸⁶ It is important to note that over-expression of hpol κ , either in animals or cell culture, has been shown to induce DNA breaks, stimulate homologous recombination, and promote aneuploidy.⁷⁶ Also, aberrant recruitment of hpol κ to the replication fork leads to a decrease in replication fork speed and an increase in genomic instability.⁸⁷ The manifestation of these

properties related to aberrant expression of hpol κ in cancer patients would certainly be detrimental.

Mis-regulation of Y-family pols has been linked to increased resistance to chemotherapies. An increase in hpol η expression, for example, causes an increased resistance to the chemotherapeutic cis-diamminedichloroplatinum(II) (cisplatin or CDDP).^{75, 88, 89} Replicative pols α (pol α), δ (pol δ), and ϵ (pol ϵ) cannot copy past intrastrand cross-linking CDDP adducts.⁹⁰ Nucleotide excision repair (NER) is known to play a major part in the removal of CDDP-induced intra- and inter-strand crosslinks;^{91–94} however, pol η appears to play a significant role in bypass of unrepaired intrastrand platinum adducts.^{75, 88, 89, 95} Knockdown of hpol η with siRNA hypersensitized cells to cisplatin;⁸⁹ and SV40-transformed fibroblasts from an XP-V patient were found to be significantly more sensitive to CDDP than clones that expressed recombinant wild-type pol η .⁷⁵ XP-V cells had more S phase arrest induced by CDDP treatment than cells expressing pol η .^{75, 96} CDDP treatment of SV40-transformed normal fibroblast (MRC5) cells also led to an increase in the number of cells with nuclear pol η foci.⁷⁵ Additionally, XP-V cells treated with CDDP had a reduction in the length of nascent DNA strands and increased DNA damage signaling compared to CDDP treated cells expressing pol η .⁹⁶

The expression of pol η has also been found to correlate with poor response to oxaliplatin.⁹⁷ Oxaliplatin has a very similar mechanism to CDDP, causing intra- and inter-strand platinum crosslinks. In tumor tissue from metastatic gastric adenocarcinoma patients, positive pol η expression was only found in 28.75% (23 out of 80), and this expression was moderate.⁹⁷ However, expression of pol η was linked to an increase in resistance to oxaliplatin and a decrease in patient survival (8 months median survival for pol η positive versus 14 months for pol η negative; $P < 0.001$).⁹⁷

Although often associated with bypass of bulky DNA adducts, there are several studies implicating pol κ in the bypass of adducts formed following exposure to methylating agents. For example, mouse pol $\kappa^{-/-}$ cells are hypersensitive to methyl methanesulfonate (MMS).⁹⁸ A separate study found that expression of hpol κ could rescue a repair-deficient yeast strain exposed to MMS.⁹⁹ Additional work led to the conclusion that hpol κ aids in the bypass of adducts following treatment with the methylating agent methyl nitrosourea (MNU).¹⁰⁰ Treatment of HeLa cells with MNU leads to co-localization of hpol κ and PCNA, indicating that hpol κ is recruited to the replication fork to help bypass MNU-induced DNA lesions.¹⁰⁰ In that study, knock down of hpol κ increased the cytotoxicity of MNU, but not MMS, in MMR-deficient HeLa cells.¹⁰⁰ These results conflict with the work done with MMS and mouse pol κ -deficient cells, as well as other studies implicating pol κ in the tolerance of MMS-induced DNA damage. While there is no direct evidence to suggest that hpol κ modulates the efficacy of the commonly used chemotherapeutic temozolomide (TMZ), it is noteworthy that pol κ has been implicated in providing resistance to the same types of DNA lesions imparted by TMZ.

KYN activation of AhR promotes up-regulation of hpol κ

While numerous reports have shown that Y-family pols exhibit altered levels in many different cancers, there are very few studies investigating the mechanisms promoting mis-regulation of TLS. Several studies have examined the effect of post-translational modifications on PCNA, as well as ubiquitination of the Y-family pols themselves in regulating these enzymes.^{37, 38, 101–103} Other studies have examined transcriptional regulation of Y-family pols. For example, the promoter region of the gene encoding for pol κ contains stimulating protein-1 (Sp1) and cyclic AMP-responsive element (Cre)-binding sites.⁸⁰ Mutations at either one of the Cre-binding sites or the Sp1-binding site leads to a decrease in pol κ transcription.⁸⁰ Loss of these regulatory factors was implicated in the down-regulation of hpol κ observed in colorectal cancer. Additionally, a series of important studies established that expression of the *POLK* gene in mammals is stimulated following exposure to molecules, such as 3-MC and B[a]P, that activate the AhR pathway.^{104–106}

The AhR is a basic helix-loop-helix transcription factor that is best known for its role in the response to xenobiotics via induction of cytochrome P450 expression.¹⁰⁷ AhR activation is also known to play a role in developmental processes, as well as inflammation and tumorigenesis.^{7, 108} It is activated by polycyclic aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD), benzo[a]pyrene (B[a]P), 7,2-dimethylbenz[a]anthracene (DMBA), and 3-methylcholanthrene (3MC).^{107–111} Importantly, KYN is an endogenous AhR agonist. As noted above, the most widely known AhR target genes are the cytochrome P450s CYP1A1 and CYP1B1,¹¹¹ which are involved in phase I metabolism of xenobiotics and the bio-activation of certain carcinogens. The bio-activation of some AhR ligands can generate reactive intermediates capable of forming covalent DNA adducts, which require Y-family pol activity to bypass. For example, hpol κ has been shown to accurately bypass B[a]P-N2-dG adducts.^{68, 69, 76, 105} Mouse cells lacking pol κ were three times more sensitive to B[a]P.⁶⁹ Additionally, the mutation rate in pol κ -deficient cells was 10-fold higher than in wild-type cells following exposure to B[a]P, with pol κ -deficient cells producing more G-to-T mutations in response to B[a]P treatment.⁶⁹ A separate study of mouse embryo fibroblasts suggested that at least two-thirds of B[a]P-N2-dG adducts in these cells were bypassed by pol κ and again found that cells lacking pol κ were much more prone to mutagenesis.¹¹² These results are consistent with a role for pol κ in cellular responses to bulky DNA adducts that can result from bio-activation of AhR agonists.

Given the relationship between AhR signaling and pol κ , we were intrigued by the study building a case for aberrant stimulation of AhR activity by KYN in the promotion of malignancy in glioblastomas.⁷ We hypothesized that the AhR pathway might be the major pathway leading to the over-expression of hpol κ reported in higher stage gliomas (Figure 3). Indeed, we conclude from our studies that the AhR signaling pathway does regulate hpol κ mRNA and protein levels in GBM cells, as treatment with the AhR agonist 3MC increased hpol κ expression and treatment with an AhR antagonist decreased its expression.¹¹³ Furthermore, blocking KYN signaling in GBM-derived cells with a small-molecule inhibitor of TDO (680C91) led to a dramatic decrease in hpol κ protein levels.¹¹³

We went on to hypothesize that the mis-regulation of hpol κ through aberrant activity of TDO promotes the inherent genomic instability that is often seen with GBM. To assess DNA damage in GBM-derived cells, we measured micronuclei (MN) formation. Inhibition of TDO activity resulted in decreased MN formation in multiple GBM-derived cell lines. Similar decreases in MN formation were observed when AhR signaling was inhibited with the small-molecule CH-223191, as well as following RNAi-mediated knock-down of hpol κ expression.¹¹³ Simultaneous inhibition of TDO activity and AhR activation did not result in an additive effect on MN formation. Similarly, combining siRNA-mediated knock-down of hpol κ with inhibition of either TDO activity or AhR activation failed to reduce MN levels beyond those observed for inhibition of individual components of the triad, suggestive of these processes functioning together to influence genomic stability in glioma cells (i.e. TDO activity $\rightarrow$ AhR signaling $\rightarrow$ hpol κ expression).¹¹³ It is reasonable to assume that KYN signaling will be found to affect additional DNA damage and replication stress response factors. If such an assumption proves true, then it would go some way towards explaining why blocking KYN signaling has been found to act synergistically with genotoxic anti-cancer drugs in multiple models of cancer.^{114–118}

Modulation of genomic maintenance through inhibition of KYN signaling as a potential anti-cancer treatment in gliomas

The first treatment employed for gliomas is typically surgery, but diffuse infiltration throughout the brain in advanced stages of the disease makes a surgical cure impossible. Radiation and chemotherapies are then used to kill as many of the remaining tumor cells as possible, and combining these treatments have succeeded in extending the survival time for some GBM patients.^{119–124} Some of the most exciting progress in the development of new treatments for advanced stage brain tumors is the use of oncolytic poliovirus (PVSRIPO) infused directly into the tumor, although this treatment is still in clinical trials.¹²⁵ The majority of GBM patients continue to be treated with some combination of radiation and/or chemotherapy.

TMZ is one of the few genotoxic anti-cancer drugs able to cross the blood-brain barrier, and is one of the most common chemotherapeutics used to treat GBM.^{124, 126, 127} TMZ is a pH-dependent pro-drug that hydrolyzes to form a reactive methyldiazonium ion.¹²⁸ The active form of TMZ primarily reacts with guanine to produce unstable N7-methyl-2'-deoxyguanine (m7dG) and stable O⁶-methyl-2'-deoxyguanine (O⁶-MeG) adducts.¹²⁹ In the absence of repair by glycosylases, the spontaneous depurination of m7dG produces abasic sites that, like O⁶-MeG, are both cytotoxic and mutagenic.¹³⁰ Numerous studies in tumor cell lines and patients have correlated high levels of O⁶-MeG repair with poor response to TMZ and other DNA alkylating agents.^{130–134} Repair of O⁶-MeG occurs through the action of the protein alkylguanine alkyltransferase (AGT, also referred to as methylguanine methyltransferase, MGMT), which irreversibly transfers the methyl group from the O6 atom of O⁶-MeG to a reactive cysteine residue.^{135–137} Hydroxyurea, procarbazine, cisplatin, and nitrosamines, such as carmustine, lomustine, and nimustine are among the other genotoxic agents often used to treat GBM.¹³⁸ Ultimately, all of these genotoxic agents fail to eliminate

GBM from the patient's body. Therefore, it is of utmost importance that new routes to effective treatments for GBM patients are pursued and validated.

It is a well-known fact that increased DNA repair/damage tolerance can limit the effectiveness of genotoxic anti-cancer treatments. It is possible that modulation of the KYN pathway could be used to enhance existing genotoxic treatments by diminishing the ability of tumors to handle DNA damage (Figure 3). There are several clinical trials testing IDO inhibitors in cancer patients either alone or in combination with existing drugs, including trials to treat both adult and pediatric brain tumors with a combination of indoximod and TMZ (NCT02502708 and NCT02052648). TDO has, thus far, received less attention than IDO in therapies attempting to reverse immune-suppression in cancer, but it is emerging as an important target for new strategies to treat glioblastoma. A potential weakness in treatments for GBM that combine IDO inhibitors with TMZ is related to the fact that TDO seems to be the major source of KYN production in these tumors.⁷ Future clinical studies will likely include radiation with chemotherapy and inhibition of KYN signaling, as pre-clinical models have shown positive results when IDO-blocking drugs were combined with chemo- and radio-therapy.^{115–117} Finally, the rationale for combining inhibitors of the KYN pathway with existing anti-cancer drugs is solely based on the idea that TDO/IDO activity modulates immunologic responses. These studies might benefit from a greater understanding of the effect that inhibiting KYN signaling has upon genomic maintenance in tumors. Additional investigations should explore the effect of inhibiting the KYN pathway on DNA damage and replication stress responses *in vivo* to examine whether effects observed in cell culture are also manifest in whole organisms. Ultimately, exploring the relationship between KYN signaling and genomic maintenance in malignant gliomas may aid in the design of new and better therapeutic strategies for what continues to be a deadly disease.

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ABBREVIATIONS

AhR	aryl hydrocarbon receptor
GBM	glioblastoma multiforme
Kyn	kynurenine
TDO	tryptophan-2,3-dioxygenase
TLS	translesion DNA synthesis
TMZ	temozolomide

Trp tryptophan

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Biographies

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Figure 1. KYN signaling can have multifaceted effects on gliomas. Major steps in the breakdown of TRP through the KYN pathway are shown and important properties are noted. The effects of certain intermediates in the KYN pathway on glioma biology are listed in the box to the right.

Figure 2.

Translesion DNA synthesis is a central mediator of DNA damage tolerance. **A.** Prototypical finger, palm, thumb, and little finger domains for the human Y-family members are shown in blue, red, green, and purple, respectively. The N-clasp of hpol κ and N-digit of hRev1 are shown in orange. Distinctive structural features allow the enzymes to accommodate unique nt base pairs (bp) and facilitate bypass of blocks to replication (PDB codes: eta – 4DL2; kappa – 2W7P; iota – 3EPG; hRev1 – 2AQ4). **B.** Replisomes encounter a barrier to replication, stalling B-family replicative pols (B-pol) (upper left panel). Immediate “on-the-fly” bypass of lesions occurs via binding of TLS pols, such as Y-family pols and pol ζ , to Rev1, and through interactions with PCNA. If the replication fork slows and stalls, resulting in binding of ssDNA by RPA and activation of RSR mechanisms, ATR-mediated USP1 degradation allows levels of Ub-PCNA to increase. Levels of ubiquitinated PCNA (Ub-PCNA) are increased by Rad6/Rad18 activity and decreased by USP1 activity. Y-family pol expression and post-translational modifications are also altered to promote increased cellular concentrations of the TLS pols, further amplifying the strength of Y-family pol association with Ub-PCNA and promoting TLS past the block to replication (lower right panel).

Figure 3.

KYN signaling modulates genomic stability in gliomas and is a viable target for new therapeutic strategies. **A.** The cartoon schematic is shown to illustrate the apparent relationship between KYN signaling, AhR activation, DNA damage tolerance, and genome maintenance in gliomas. **B.** Targeted therapies that inhibit KYN signaling could attenuate the malignant properties of gliomas by reducing endogenous levels of DNA damage and inhibiting pathways that promote resistance to genotoxic treatments, such as radio- and chemo-therapy.

Table 1

Notable bypass properties of the human Y-family pols and roles in cancer

Notable bypass properties			Role in cancer	
Rev1	•	Incorporates dCMP opposite dG and abasic sites ⁶⁶	•	Germline missense variants associated with an increased risk for cervical cancer ¹³⁹
	•	Acts as a scaffold protein for the other Y-family pols ⁶⁴	•	Facilitates bypass of platinum-induced DNA damage ¹⁴⁰
Pol κ	•	Accurately and efficiently bypasses several bulky minor groove adducts, such as benzo-[a]-pyrene-N2-dG adducts ⁶⁸	•	Decreased level of transcripts in human colorectal tumors ⁸⁰
	•	Has additional roles in NER ¹⁴¹	•	Increased expression in gliomas ⁸⁶
Pol η	•	Bypasses T-T cyclobutane pyrimidine dimers efficiently and with the same accuracy as undamaged DNA ¹⁴³	•	Increased expression in lung cancer ¹⁴²
	•	Accurately bypasses 8-oxo-dG ^{144, 145}	•	Non-functional pol η (in XPV) predisposes for sunlight-induced skin cancer ¹⁴⁷
	•	Involved in homologous recombination (HR)-mediated repair of double-strand breaks ¹⁴⁶	•	Increased expression in ovarian cancer stem cells ¹⁴⁸
Pol ι	•	Specialized Hoogsteen base-pairing mechanism of action ¹⁴⁹	•	Important means of bypassing platinum-induced DNA damage ^{75, 82, 89, 95, 96}
	•	Replicates template dT in a highly error-prone manner ¹⁵⁰	•	Pol η -deficient cells are sensitive to doxorubicin ⁸¹
Pol ν	•	Specialized Hoogsteen base-pairing mechanism of action ¹⁴⁹	•	Increased expression in gliomas ⁸⁶
	•	Replicates template dT in a highly error-prone manner ¹⁵⁰	•	Increased expression in breast cancer cell lines but not in breast cancer patient samples ¹⁵¹