18. PHARMACOLOGIC INHIBITION OF MTOR ACTIVITY ATTENUATES GLIOMA CELLS INVASION BY DOWNREGULATING NEUROPILIN-1 EXPRESSION

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The mammalian target of rapamycin mTOR controls a spectrum of cellular events such as initiation of translation, ribosome biogenesis, and cell growth and proliferation. Pharmacologic inhibition of mTOR activity by rapamycin has been shown to elicit antitumor activity possibly through G_1 cell cycle arrest and inhibition of VEGF expression. Currently, an analogue of rapamycin, RAD001, is being used in clinical trial for different cancers including gliomas. Glioblastoma multiforme is a malignant tumor that is extremely refractory to therapy because of rapid growth and local invasive potential of these tumors. In this study, we sought to examine the effect of antagonizing mTOR activity by RAD001 on glioma tumor invasion in vitro. Four different glioma cells were treated with RAD001 for 72 h before harvesting for Western blotting, RT-PCR, microarray, and in vitro Matrigel gel invasion analyses. Glioma cells were stably transfected with NRP-1 expression and control vector followed by G418 selection. The resulting G418-resistant cells were examined for their NRP-1 expression and in vitro invasion propensity. Glioma cell lines treated with RAD001 resulted in a reduction of mTOR downstream target genes expression such as S6K1 and the eukaryotic initiation factor 4E-binding protein 1. Inhibition of mTOR activity with RAD001 in tumor cells also leads to G_1 cell cycle arrest and reduction in VEGF secretion consistent with previously documented studies. Importantly, invasion propensity of tumor cells is greatly inhibited by RAD001 as assessed in an *in vitro* Matrigel invasion assay. Interestingly, RAD001 treatment does not affect either the expression or the activity of MMP2 in tumor cells. Using Affymetric microarray analyses we discovered the expression of neuropilin-1 (NRP-1) is decreased by RAD001. NRP-1, initially found to be involved in axon growth during neuronal development, is expressed in both tumor cells and endothelial cells. It has been shown that NRP-1 is a co-receptor for $VEGF_{165}$ and controls cell motility. Semi-quantitative PCR further confirmed the microarray results, which suggests that RAD001 affects NRP-1 mRNA expression. Exogenous NRP-1 expression significantly increases glioma cells invasion and promotes anchorage-independent growth. Our results demonstrate that anticancer activity of RAD001 may be a combination of growth arrest, antiangiogenesis, and anti-invasion effects. Furthermore, the potential anti-invasion activity of RAD001 is likely through controlling cell motility by inhibiting NRP-1 expression.

19. POLYMORPHISMS IN DNA REPAIR GENES AND SUSCEPTIBILITY TO PRIMARY INTRACRANIAL BRAIN GLIOMAS

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Enzymes in base excision (BER), nucleotide excision (NER), double strand break/recombination (DSB/RR), mismatch (MMR), and direct-damage DNA repair pathways are important in the repair of diverse types of DNA damage. Polymorphisms in many of the genes encoding these enzymes have been identified as risk factors for environmentally and occupationally caused cancers. We evaluated the associations of polymorphisms in BER (*ADPRT* V762A, *APEX* D148E, *MUTYH* Ex1+8A>C>G/T, *OGG1* S326C, *POLB* IVS11-235A>G, *XRCC1* R399Q, R280H, R194W, *LIG1* Ex2-24C>T, *PCNA* IVS1-124C>T), NER (*ERCC2* D312N, K751Q, *ERCC4* R415Q, *ERCC5* H1104D, *RAD23B* A249V, *LIG1* Ex2-24C>T, *PCNA* IVS1-124C>T), DSB/RR (*NBS1* Q185E, *RAD52* Y415stop, *XRCC2* R186H, *XRCC3* T241M, *XRCC4* N298S), MMR (*MLH1* I219V, *MSH2* G322D), and direct-damage repair (*MGMT* I143V, R178K, L84F) as risk factors for primary intracranial gliomas in the Upper Midwest Health Study, a population-based case-control study including rural residents of four states with high glioma incidence. Glioma cases (N = 798) were identified from hospitals, private physicians, and registries. Control participants (N = 1175) were stratified samples of licensed drivers and HCFA enrollees. Questionnaires elicited occupational and environmental exposures. DNA was obtained from 451 controls with no self-reported cancer and from 316 cases. TaqMan and MGB Eclipse methodology were used to characterize genotypes. In unadjusted analyses, a polymorphism in *ADPRT* (V/V 67% of controls, 75% of cases, odds ratio [OR] 1.48, 95% confidence interval [CI] 1.07–2.04) had a statistically significant association with glioma, and polymorphisms in three other genes showed associations with glioma of borderline statistical significance: (1) *RAD23B* A/V + V/V, 32% of controls, 38% of cases, OR 1.32, CI 0.97–1.78; (2) *ERCC5* H/H, 61% of controls, 68% of cases, OR 1.33, CI 0.98–1.78; and (3) *XRCC4* N/N 74% of controls, 80% of cases, OR 1.37, CI 0.97–1.94. For each DNA repair pathway, multivariate logistic analyses included all polymorphisms in the pathway plus ever/never living on a farm and ever/never smoking, as surrogates for occupational and environmental exposure. Adjusting for these factors did not change odds ratios substantially. Our results should be confirmed in additional glioma case-control studies. Future analyses of our data will include assessing the risk of DNA repair polymorphisms under specific exposure conditions, such as exposures to pesticides, solvents, and UV light.

20. A GLTSCR1 HAPLOTYPE IS ASSOCIATED WITH OLIGODENDROGLIOMA DEVELOPMENT

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Deletions of 19q have been associated with gliomas, especially oligodendrogliomas. In addition, oligodendrogliomas with 19q deletion have a better survival. We have previously described a 150-kb minimal deletion region in gliomas that maps to 19q13.33 that contains three novel candidate genes (*GLTSCR1*, *EHD2*, and *GLTSCR2*). Polymorphisms in loci near this deletion region (ERCC1, ERCC2, RAI, ASE-1, and D19S246) have been associated with basal cell, breast and lung carcinoma, and mixed oligoastrocytomas. A polymorphism within GLTSCR1 has recently been associated with prostate cancer aggressiveness. We have recently shown that a polymorphism in GLTSCR1 (SNP rs1035938) is associated with oligodendroglioma development. We have now evaluated five additional SNPs within GLTSCR1 using the same cohort of glioma cases and general controls. One of the polymorphisms (Novel 4 – A to G at position 304 from the putative transcription start site) is a novel SNP discovered during GLTSCR1 mutation screening studies of sporadic glioma specimens. Of these five SNPs, two (Novel 4 and rs1005911) were found to have borderline association with oligodendroglioma development by allele-based analysis (P for both = 0.052). However, the prevalence of the at-risk allele was significantly increased in patients whose oligodendroglioma have 19q deletion (P for Novel 4 = 0.025; P for rs1005911 = 0.014). By genotyping 10 CEPH families we were able to determine the most prevalent haplotypes for the six total GLTSCR1 SNPs we tested. We then assessed the prevalence of these haplotypes in the cases with glioma and the normal controls. One haplotype (haplotype 1, ACTCGG) was more prevalent in gliomas than in controls (27.5% vs. 20.4%, P = 0.067). The prevalence increased to 36.7% in gliomas with 19q deletion (P vs. controls = 0.009; P vs. gliomas without 19q deletion = 0.02). The increased prevalence of this haplotype in gliomas was primarily due to its prevalence in oligodendrogliomas (34%, P vs. controls = 0.010) and among the oligodendrogliomas, those with 19q deletion (45%, P vs. controls = 0.001; P vs. oligodendrogliomas without 19q deletion = 0.002). Interestingly, this haplotype is retained in 8 of 10 oligodendrogliomas with 19q deletion. Even though it had a low overall prevalence, another haplotype (ACCCGG) was significantly more prevalent in the controls than the cases (2% versus 0%; P = 0.035). The high-risk and low-risk haplotypes only differ by the presence of the GLTSCR1-exon-1 T allele (rs1035938). These preliminary data strongly suggest that a polymorphism (mutation) is in linkage disequilibrium with the GLTSCR1 ACTCGG haplotype and is associated with oligodendroglioma development, especially those with 19q deletion.

21. NON-ENZYME-INDUCING ANTIEPILEPTIC DRUG USE IN BRAIN TUMOR PATIENTS: EXPERIENCE WITH LEVETIRACETAM

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It has been estimated that 20% to 40% of brain tumor patients experience a seizure by the time of diagnosis, accounting for 60,000 patients annually. The hepatic enzyme-inducing medications such as phenytoin and carbamazepine have been the drugs of choice for treatment. There may be advantages to using non-enzyme-inducing drugs such as levetiracetam (LEV) to help decrease medication interactions. A retrospective analysis was completed using our brain tumor database that identified all brain tumor patients treated over the past three years with LEV. Patients were evaluated for tumor type, seizure type and frequency, and medication side effects. At the time of the abstract, 278 brain tumor patients were identified. The breakdown included 91 glioblastomas, 24 anaplastic astrocytomas, 15 anaplastic oligodendrogliomas, 13 mixed anaplastic gliomas, 24 low-grade astrocytomas, 46 low-grade oligodendrogliomas, 15 mixed low-grade gliomas, 19 meningiomas, 18 metastases, and 13 tumors defined as "other". Of the 278 patients, 10 (3.5%) stopped or dose reduced the LEV for behavioral

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