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cMYC Expression in Infiltrating Gliomas: Associations with *IDH1* Mutations, Clinicopathologic Features and Outcome

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Abstract

Gliomas are among the most frequent adult primary brain tumors. Mutations in *IDH1*, a metabolic enzyme, strongly correlate with secondary glioblastomas and increased survival. *cMYC* is an oncogene also implicated in aberrant metabolism, but its prognostic impact remains unclear. Recent genotyping studies also showed SNP variants near the *cMYC* gene locus, associate with an increased risk for development of *IDH1/2* mutant gliomas suggesting a possible interaction between *cMYC* and *IDH1*.

We evaluated nuclear *cMYC* protein levels and *IDH1* (R132H) by immunohistochemistry in patients with oligodendroglioma/oligoastrocytomas (n=20), astrocytomas (grade II) (n=19), anaplastic astrocytomas (n=21) or glioblastomas (n=111). Of 158 tumors with sufficient tissue, 110 (70%) showed nuclear *cMYC* immunopositivity – most frequent (95%, χ^2 p=0.0248) and intense (mean 1.33, ANOVA p=0.0179) in anaplastic astrocytomas versus glioblastomas (63%) or low grade gliomas (74%). *cMYC* expression associated with younger age as well as p53 immunopositivity (OR=3.6, p=0.0332) and mutant *IDH1* (R132H) (OR=7.4, p=0.06) among malignant gliomas in our cohort. Independent analysis of the publically available TCGA glioblastoma dataset confirmed our strong association between *cMYC* and mutant *IDH1* expression. Both *IDH1* (R132H) and *cMYC* protein expression were associated with improved overall survival by univariate analysis. However, *cMYC* co-expression associated with shortened time to malignant transformation and overall survival among *IDH1* (R132H) mutants in both univariate and multivariate analyses. In summary, our findings suggest that *cMYC* may be associated with a unique clinicopathologic and biologic group of infiltrating gliomas and help mediate the malignant transformation of *IDH1* mutant gliomas.

Keywords

cMYC; *IDH1* (R132H); *IDH1*; Gliomas; Prognosis; Survival; glioblastoma; astrocytoma

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Introduction

Infiltrating/diffuse gliomas are pathologically classified as oligodendroglioma, astrocytoma or oligoastrocytoma with World Health Organization (WHO) grades II-IV. Based on the Central Brain Tumor Registry of the United States (CBTRUS), gliomas are the most frequent adult primary parenchymal brain tumors with an incidence of 6.03 per 100,000 adults per year, mainly comprised of glioblastoma, involving 3.19 per 100,000 adults per year [1]. Malignant gliomas are an important cause of cancer mortality in young adults [2].

Primary and secondary glioblastoma have distinct genetic profiles. Primary glioblastomas frequently show *epidermal growth factor receptor* amplifications and *p16^{INK4A}* deletions [2, 3]. Conversely, a mutation of *IDH1*, leading to an amino acid substitution at codon 32 of exon 4 (*IDH1* (R132H)), is a robust marker of secondary glioblastomas and associated with younger age [4–6]. *IDH1* (R132H) also correlates with methylation of O6-methylguanine-DNA methyltransferase (MGMT) promoter [7], which predicts improved response to alkylating agents [8, 9]. Chromosomal arms 1p and 19q co-deletion, typical of oligodendrogliomas, and *IDH1* mutations are important genetic aberrations strongly correlated with survival and therapeutic response in gliomas [2, 3, 10, 11].

cMYC is an oncoprotein with numerous downstream targets (reviewed in [12]). In human gliomas and model systems, cMYC plays a role in stem cell transformation and maintenance [13, 14]. However, cMYC also affects metabolic pathways (reviewed in [15]). Some of cMYC downstream activated targets contribute to the “Warburg effect”, a key metabolic property of numerous cancers. cMYC activates genes responsible for glutamine dependence, instead of glucose, in hypoxic and glucose-deficient tumors, such as gliomas outgrowing their blood supply [12]. Recent studies also suggest a key role for IDH1 in aberrant metabolism and proliferation [7]. IDH1 and IDH2 participate in a reversed tricarboxylic acid cycle that provides citrate as substrate for production of alpha-ketoglutarate by wild type IDH1 wild type. Both cMYC and IDH1/2 help mediate the high rate of lipid synthesis required by proliferating tumor cells [12, 16–19]. Furthermore, recent genotyping studies showed SNP variants mapping to 8q24.21, near the cMYC gene locus, associated with increased risk for development of IDH1/2 mutant gliomas [20], which suggests an interaction in the pathogenesis of gliomas at the genetic level.

The goal of this study was to assess the association of cMYC expression with important clinical and pathologic variables in archival tissue of human diffuse gliomas, as well as an effect on prognosis in our cohort and publically available databases.

Methods

Study Population

Following appropriate institutional review board approval (IRB#NA_00015113), archival formalin-fixed, paraffin-embedded tissue was obtained from the Johns Hopkins Hospital Department of Pathology. We analyzed 171 gliomas from 156 patients classified according to 2007 WHO guidelines [2], including 20 oligodendrogliomas/oligoastrocytomas (O/OA), 19 astrocytomas (A), 21 anaplastic astrocytomas (AA) and 111 glioblastomas (GBM). Prior or subsequent pathology and/or clinical reports were reviewed for evidence of malignant transformation, defined as pathologically-confirmed progression from a WHO grade II to either WHO grade III or IV gliomas. Malignant gliomas included grade III and IV. Radiologic progression was not part of this definition.

The median age at diagnosis was 39 years, including 119 (76%) adults and 37 (24%) pediatric cases with median age of 44 (range 26 to 86) and 13 years (range 2 months to 21

years), respectively. For glioblastomas (N=101), median age was 44 years (range 2 months to 86 years), including 43 (65%) adults and 23 (35%) pediatric cases with median ages 56 and 13 years, respectively. Demographics are summarized in Supplemental Table S1.

Tissue microarrays and Immunohistochemistry

Two tissue microarrays (TMAs) were constructed from 160 gliomas and controls according to standard procedures at the TMA core facility [21]. Tissue from 11 additional gliomas was included in the analyses. For each tumor, four cores measuring 0.6 mm were compiled in the TMA. Only tumors containing ≥ 2 evaluable cores were analyzed.

For cMYC immunohistochemistry, a validated rabbit monoclonal antibody (Epitomics, catalog #1472-1) was diluted 1:200 in PBS [22]. As previously described [23], each tumor was scored by intensity of cMYC protein nuclear staining using a 4-tiered scoring system (0:no staining; 1:low staining; 2:moderate staining; 3:marked staining) by two of the authors (YO and CGE) blinded to clinical variables or status of other molecular markers (Figure 1A–D). To test for IDH1 status, only immunohistochemistry was performed with an anti-IDH1 (R132) antibody (clone H09, Dianova, Hamburg, Germany; 1:50). IDH1 immunoreactivity was scored as positive or negative (Figure 1E–F). Immunohistochemical data was obtained from a previously published study [24] which used ATRX (Sigma-Aldrich, St. Louis, MO, USA, 1:600) and p53 antibodies (clone BP53-11, Ventana, Tucson, AZ, USA; prediluted).

Fluorescence *in situ* Hybridization (FISH)

FISH was performed on TMA sections using a commercially available locus-specific identifier probe targeting *cMYC* at chromosomal region 8q24 (SpectrumOrange, Abbott Molecular/Vysis, Des Plaines, Ill) with a corresponding centromere 8 control (CEP8, SpectrumGreen, Abbott Molecular), using previously published methods [25]. At least 50 non overlapping nuclei per tumor were enumerated. *cMYC* gain was defined as 30% of cells containing ≥ 3 signals plus a target/control probe ratio of greater than 1.30, as previously established [26]. Chromosome 8 gain was defined as ≥ 3 signals for the target and control probes in >30% of cells. Alternative lengthening of telomeres (ALT) data, obtained through telomere specific FISH, from a previously published study was used for additional correlations [24, 27].

TCGA dataset

IDH1 mutational data, *cMYC* locus copy number alterations, and Z-normalized *cMYC* mRNA expression data from The Cancer Genome Atlas (TCGA) was downloaded from the cBio portal (<http://www.cbioportal.org/>) on 12/11/2012. The mRNA values indicate the number of standard deviations away from the mean of expression in the reference population (Z-score). Per the cBio portal, the reference population is either all tumors that are diploid for the gene in question, or, when available, normal adjacent tissue. The corresponding clinical information was downloaded from TCGA portal (<http://tcga-data.nci.nih.gov/tcga/>) [28]. *cMYC* amplification/gain was assessed per current TCGA algorithm.

Statistical Analyses

Statistical analysis was performed using GraphPad Prism 5.03 software (www.graphpad.com) and JMP 8.0.2 SAS statistical software (www.jmp.com). For continuous variables, mean and standard deviations or median were used for parametric or nonparametric distributions, respectively. Frequency and percentage were used for nominal variables. Analyses for TMA immunohistochemistry and TCGA mRNA expression utilized Chi-square, Fisher's exact test, ANOVA or two-way t-tests as appropriate. Percent survival

was calculated using the product limit (Kaplan-Meier) method in GraphPad Prism. Curves were compared using the Log-Rank test (Mantel-Haentzels). The continuous variable of Z-normalized cMYC mRNA expression in TCGA was divided using the 75% quartile (0.6097) as cut-off for stratified overall and progression-free survival analyses. Logistic and linear regression, and proportional hazards were calculated for multivariate analyses. Differences were considered significant at $p \leq 0.05$.

Results

cMYC nuclear protein levels associated with IDH1 (R132H) and strong p53 co-expression

For 158 biopsies with sufficient TMA samples, 110 (70%) showed nuclear cMYC protein expression. Fifteen patients had two ($n=14$) or three ($n=1$) sequential biopsies with generally concordant cMYC immunoreactivity across samples and WHO grade (Figure 1G–J). Conversely, cMYC was uniformly negative in gray and white matter, including 8 cores of non-neoplastic brain included in the TMA and brain parenchyma infiltrated by tumor.

IDH1 (R132H) immunoreactivity was evident in 47/158 (30%) of gliomas. IDH1 (R132H) was expressed less frequently in glioblastomas versus other gliomas (9% vs. 62–65%, Fisher's Exact $p < 0.0001$; $N=160$) and comparable to reported rates of 11% in glioblastoma [4]. Compared to IDH1 (R132H) negative malignant gliomas, IDH1 (R132H) mutants co-expressed cMYC more frequently [63/97 (65%) vs. 22/25 (88%), Fisher's exact $p=0.028$] and intensely [mean=0.85 vs. 1.20, t -test $p=0.022$] (Figure 2A–B).

Nuclear labeling of p53 was assessed in 109 malignant astrocytomas and correlated with IDH1 (R132H) and cMYC immunoreactivity. Strong p53 immunoreactivity was detected in 8/17 (47%) of cMYC-positive WHO grade III astrocytomas, while no p53 expression was available for the solitary cMYC negative anaplastic astrocytoma. Among WHO grade IV glioblastomas, strong p53 expression was significantly more frequent among cMYC-positive tumors (24/61 (39%) vs. 4/31 (13%); Pearson's $\chi^2=6.8$, $p=0.0092$). Among glioblastomas, strong p53 expression was also more frequent among IDH1 (R132H) mutants (7/8 (87.5%) vs. 21/87 (24%), Pearson's $\chi^2=14.1$, $p=0.0002$).

Multivariate logistic regression revealed a strong interaction between cMYC-expression and WHO grade (DF 2, Likelihood-Ratio $\chi^2=6.7$, $p=0.0343$), so associations of cMYC with p53 and IDH1 (R132H) were stratified by WHO grade. Among malignant gliomas, cMYC expression independently associated with both strong p53 (OR=3.6, $p=0.0332$) and mutant IDH1 (R132H) (OR=7.4, $p=0.06$) expression. Among glioblastomas, cMYC expression was most strongly associated with p53 immunoreactivity (OR=3.9, $\chi^2=4.7$, $p=0.0297$).

Associations of cMYC nuclear protein levels with specific clinicopathologic glioma subtypes

We then assessed associations between cMYC nuclear protein levels and specific diffuse glioma subtypes. cMYC immunoreactivity was significantly more frequent [20/21 (95%), χ^2 $p=0.024$] and intense [mean=1.33, SD=0.66, ANOVA $p=0.017$] in anaplastic astrocytomas, compared to glioblastomas [64/102 (63%); mean=0.81, SD=0.78] and low grade gliomas [26/35 (74%); mean=0.97, SD=0.67] (Figure 2C–D). Among glioblastomas, frequency of cMYC nuclear reactivity was higher in secondary glioblastomas [13/17 (77%)], but failed to reach statistical significance (X^2 $p=0.17$).

In univariate analyses, cMYC protein expression associated with younger age at diagnosis. Median age at diagnosis for cMYC-positive gliomas was 37 years versus 44 years for cMYC-negative gliomas [t -test $p=0.0290$]. Similar findings were noted in our glioblastoma cohort [$N=101$, median 34 vs. 52 years, respectively; $p=0.0271$] (Supplemental Figure S1).

As previously reported, compared to IDH1 (R132H) negative gliomas, IDH1 (R132H) immunoreactivity correlated with younger age at diagnosis (26.5 vs. 44 years, $p < 0.0001$), especially glioblastomas in adults (35.5 vs. 55 years, t -test $p = 0.0127$). Not surprisingly, multivariate linear regression revealed a strong interaction between age at diagnosis and WHO grade (DF 2, $\Sigma\chi^2 = 151$), so associations of age with cMYC, p53 and IDH1 (R132H) expression were stratified by WHO grade. Among malignant gliomas, age at diagnosis tended to correlate with strong p53 expression ($R^2 = 4.4$, $p = 0.0648$), but did not reach statistical significance for cMYC ($R^2 = 3.2$, $p = 0.1833$). Age and p53, cMYC and IDH1 (R132H) expression, thus, seem tightly associated.

Previous reports indicate significant associations between *IDH1* mutations and the alternative lengthening of telomeres (ALT) phenotype and ATRX loss in astrocytomas [29–31]. In univariate analysis, among malignant gliomas, cMYC expression was associated with the ALT phenotype ($\chi^2 = 3.96$, $p = 0.0467$), but not with ATRX loss ($p = 0.7854$). In multivariate analyses, there was a non-significant trend towards an association between the ALT phenotype and cMYC among WHO grade III gliomas ($\chi^2 = 2.78$, $p = 0.0955$), but best explained by its strong association with IDH1 (R132H) ($\chi^2 = 24.2$, $p < 0.0001$) and p53 reactivity ($\chi^2 = 14.3$, $p = 0.0002$). Results are summarized in Supplemental Table S2.

Associations between cMYC nuclear protein levels, mRNA Expression and DNA Copy Number

In the MSKCC and Rembrandt cohorts, gain of chromosome 8q, including the *cMYC* gene, correlated with improved overall survival [32]. Thus we performed FISH analysis in a subset of our cohort using probes targeting the cMYC locus and Ch 8 centromere. Our analysis revealed whole chromosome 8 gain/polysomies in 4/6 (66.7%) cMYC negative and 5/14 (35.7%) cMYC immunoreactive gliomas (Fisher's Exact $p = 0.3359$). We detected no high level *cMYC* amplifications. cMYC protein nuclear level was not associated with chromosome 8 gain among 20 cases tested.

Next, we evaluated public TCGA datasets for correlations between *IDH1* mutations and *cMYC* mRNA expression or copy number alterations in glioblastoma. *cMYC* copy number correlated with mRNA expression (Supplemental Figure S2). Similar to our immunohistochemical results, *cMYC* mRNA expression was significantly higher in *IDH1*-*R132* mutants versus *IDH1* wild type glioblastomas (median 0.9565 versus 0.0305, respectively; t -test $p = 0.0007$) (Figure 3). All heterozygous cMYC deletions were detected in *IDH1* wild types, while 7/14 (50%) of *IDH1*-*R132* mutated and 23/203 (11%) of *IDH1* wild types showed cMYC amplification ($p < 0.0001$). cMYC gene dosage, while associated with cMYC mRNA expression in TCGA, did not correspond with cMYC protein level or severe disease in our glioma cohort in contrast to medulloblastoma suggesting that other factors may affect nuclear cMYC protein levels.

Outcome analyses

Next we explored the prognostic significance of cMYC and IDH1 (R132H) expression. Due to the limited number of cMYC-negative anaplastic gliomas, the impact of cMYC expression on overall survival was assessed in combination with glioblastomas. cMYC immunoreactivity correlated with improved survival in malignant gliomas [median 24 vs. 17 months, $p = 0.0068$] in univariate analysis (Figure 4A), particularly in adult [median 21 vs. 13 months, $p = 0.0072$] but not pediatric [$p = 0.2558$] glioblastomas. IDH1 (R132H) expression associated with improved survival compared to IDH1 (R132H) negative malignant gliomas [median 42 vs. 16 months, $p = 0.0026$], irrespective of cMYC expression (Figure 4B), suggesting the impact of cMYC expression on survival depends on *IDH1* mutation status. In univariate analyses, cMYC immunoreactivity showed a non-significant trend for improved

survival for IDH1 (R132H) negative malignant gliomas ($p=0.06$) (Figure 4C). In multivariate analyses, there was again a strong interaction between WHO grade and overall survival (DF2, Likelihood Ratio $\chi^2=38.8$, $p<0.0001$), so subsequent analyses were stratified by WHO grade. Among malignant gliomas, neither cMYC or p53 status nor age was associated with survival among IDH1 (R132H) negative tumors. However, both age (RR=22.3, $p=0.0107$) and cMYC expression (RR=51.6, $p=0.0251$), but not strong p53 expression (RR=0.5, $p=0.1785$), were associated with increased risk of death among IDH1 (R132H) mutants in malignant gliomas. In addition, IDH1 (R132H) expression correlated with prolonged time to pathologically confirmed, malignant transformation. Interestingly, cMYC expression correlated with reduced time to malignant transformation in IDH1 (R132H) mutants ($p=0.02$) (Figure 5A).

Next we evaluated the impact of *cMYC* mRNA expression or gene amplification and *IDH1* mutations on survival in the glioblastoma TCGA dataset. As previously reported, *IDH1* (R132) mutations correlated with increased progression-free and overall survival. Despite trends similar to our cohort, we detected no association with progression-free or overall survival once stratifying by both *IDH1* mutation and *cMYC* mRNA expression (using the 75% quartile as cut-off). We also detected a non significant trend between *cMYC* amplification and improved survival among *IDH1*-R132 mutant glioblastomas ($p=0.07$) (Supplemental Figure S3). TCGA analyses were limited by sample size as only 14/185 (7.6%) glioblastomas had *IDH1* mutations. These findings suggest that although the clinical significance of cMYC nuclear protein levels is difficult to separate from other well established glioma prognostic factors (age, tumor grade, *IDH1* mutations), it may be an adverse prognostic factor in the smaller subset of *IDH1* mutant gliomas.

Discussion

Using immunohistochemistry to assess nuclear protein levels, we correlated cMYC expression with pathological and clinical outcomes in human gliomas. Our main finding is the association of cMYC expression with mutant IDH1 (R132H) expression and strong p53 nuclear labeling in malignant gliomas. Previous studies suggest a role for IDH1 and cMYC in aberrant metabolism and proliferation. In both glioma, melanoma and leukemia cells, cMYC and IDH1 sustain high energy demands via a reversed TCA cycle and modified lipid synthesis in glucose deficiency or hypoxia [12, 16–18]. cMYC protein was previously correlated with increased proliferative index and grade in gliomas [33, 34]. Additionally, recent genotyping studies showed SNP variants mapping to 8q24.21, near the *cMYC* gene locus, associated with increased risk for development of *IDH1/2* mutant gliomas [20], which further suggests their interaction in gliomagenesis.

Univariate analyses of IDH1 (R132H) expression confirmed prior associations with younger age, secondary glioblastomas and improved survival [4, 5, 8–11, 35]. Interestingly, cMYC co-expression predicted decreased time to malignant transformation among IDH1 (R132H) mutants, suggesting the prognostic significance of cMYC may depend on *IDH1* mutation status. The role of cMYC in transformation to anaplasia and poor prognosis is better defined in embryonal tumors – the most common malignant brain neoplasm in children. Specifically, increased *cMYC* mRNA correlates with high-grade features in experimental models and anaplastic/large cell subtypes of human medulloblastoma [23]. These morphologic features are uniformly high-risk [36]. Although it is unclear how *IDH1* mutations affect cMYC protein nuclear levels, strong evidence supports *IDH1* mutations as early events in infiltrating gliomas and links IDH1 to profound metabolic and epigenetic effects [37] that putatively may affect cMYC expression downstream (Figure 4B). Concurrent cMYC expression, in turn, may promote transcription of genes associated with metabolism, including mitochondrial biogenesis and glutaminolysis. Interestingly, prior

studies demonstrated growth inhibition by inhibiting glutaminase in the context of *IDH1* mutations [38].

Among *IDH1* (R132H) mutants with better prognosis, both age and cMYC protein co-expression predicted decrease survival among malignant gliomas, consistent with prior associations with increased proliferation and WHO grade [23, 33, 34, 39]. One caveat is that all of these associations were limited by the rarity of cMYC-negative *IDH1* (R132H) mutants, and therefore will require validation in larger cohorts. Interestingly, *cMYC* amplification showed a non significant trend for improved survival among *IDH1* (R132) mutated TCGA glioblastomas, although we found no *bona fide* *cMYC* amplifications in the limited cases tested by FISH in our cohort. Interestingly, 8q gain associated with a favorable molecular subtype and improved survival in MSKCC and Rembrandt astrocytoma-restricted cohorts [32]. In contrast to TCGA results but in support of our findings, a prior aCGH study of oligodendrogliomas, characterized by a high frequency of *IDH1/2* mutations, 8q gain was a feature in a subset (17%) and associated with worse outcome [40]. The marked post-transcriptional regulation of cMYC, including protein degradation and nuclear translocation [12], may explain differences in the prognosis of cMYC protein, mRNA and copy number variations. A recent study identified *CIC* and *FUBP1* as putative tumor suppressor genes frequently mutated in oligodendrogliomas [41]. *FUBP1*, a negative regulator of cMYC [42], may partly explain cMYC overexpression in oligodendrogliomas in the absence of copy number alterations. MicroRNAs may also regulate cMYC protein levels in other tumors [43–45]. These findings suggest that the relationship between gene dosage, mRNA levels and nuclear protein levels is not straightforward. The impact of cMYC on survival among *IDH1* (R132H) mutants should, thus, account for protein, mRNA and gene dosage variations separately.

In conclusion, our study showed frequent co-expression of *IDH1* (R132H) and cMYC nuclear protein by immunohistochemistry in our malignant glioma cohort and with mRNA expression and copy number alterations in the TCGA glioblastoma cohort. cMYC protein nuclear expression was most prominent among anaplastic astrocytomas. The independent prognostic significance of cMYC is still unclear since it is difficult to dissociate from *IDH1* mutations, a more robust, prognostic factor in gliomas. In addition, the number of *IDH1* (R132H) positive, cMYC negative cases was relatively small. However, our data support a role for cMYC protein as an adverse prognostic factor among malignant gliomas expressing mutant *IDH1*. Future evaluation of the prognostic and predictive value of cMYC expression at the DNA, mRNA or protein levels should allow for stratification by *IDH1* mutation status and WHO grade and via prospective ascertainment of response to therapy and survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. cMYC nuclear protein levels in infiltrating gliomas

Each sample was scored on a 4-tiered scoring system based on intensity of cMYC nuclear staining: “negative” (0), “low intensity” (1)(A), “moderate intensity” (2)(B) and “marked intensity” (C). Non-neoplastic brain samples were uniformly negative (D). Mutant IDH1 (R132H) immunohistochemistry was scored as positive (E) or negative (F). Representative H&E examples of the same patient demonstrating transformation from a WHO grade II astrocytoma (G) to an anaplastic (WHO grade III) astrocytoma, characterized by easily identifiable mitotic figures (arrows)(H). cMYC nuclear labeling was concordant and did not change during transformation from grade II(I) to grade III(J) in this tumor.

Figure 2. cMYC nuclear immunoreactivity in Malignant Gliomas and Frequent Co-Expression of IDH1 (R132H) mutation

A: cMYC protein is frequently co-expressed with the IDH1 (R132H) mutation in malignant gliomas, but not among low grade gliomas (Contingency Tables, Fisher's Exact). **B:** cMYC protein is more intensely expressed among IDH1 (R132H) mutant compared to IDH1 (R132H) negative malignant gliomas (mean 1.2 versus 0.85, Mann Whitney t-test p =0.0225). cMYC nuclear immunoreactivity varied by histopathologic group, with anaplastic astrocytomas having greatest proportion (**C**, Contingency Table) and intensity (**D**, ANOVA) of cMYC immunoreactivity.

Figure 3. IDH1-R132 Mutations Correlate with Copy Number Alterations and Relatively Higher cMYC mRNA Expression in the TCGA Glioblastoma Cohort

A: The contingency table of IDH1 mutation status versus copy number alterations shows no *cMYC* deletions among IDH1-R132 mutants and a higher frequency of IDH1-R132 mutants (7/30, 23.3%) among glioblastomas with *cMYC* amplification compared to those with no copy number alterations (7/171, 4.1%; Fisher's Exact $p=0.0002$). **B:** IDH1-R132 mutants expressed *cMYC* mRNA at higher levels compared to IDH1 wild type glioblastomas. The values for *cMYC* mRNA expression indicate the number of standard deviations away from the mean of expression in the reference population (Z-score).

Figure 4. IDH1 (R132H) and cMYC Expression Associate with Overall Survival in Malignant Gliomas

A: cMYC protein expression correlated with improved survival in malignant gliomas, but may reflect the strong correlation with the IDH1 (R132H) mutation. **B:** The IDH1 (R132H) mutation was independently associated with improved overall survival among malignant gliomas. **C:** cMYC protein nuclear positivity showed a non-significant trend for improved survival among IDH1 (R132H) negative malignant gliomas ($p=0.0634$). Survival analysis of cMYC in the IDH1 (R132H) subset was limited by the small number of cMYC negatives. Percent survival was calculated using the product limit (Kaplan-Meier) method. Curve comparisons were evaluated using the log rank test (Mantel-Haentzels).

Figure 5. cMYC co-Expression Correlates with Malignant Transformation in IDH1 (R132H) mutant gliomas

WHO grade II gliomas with pathologically-confirmed malignant transformation to either WHO grade III or IV (n=44) gliomas were stratified by cMYC and IDH-R132H immunopositivity. cMYC protein nuclear immunopositivity correlated with shortened time to malignant transformation among IDH1 (R132H) mutants, but has no effect on rate of malignant transformation among IDH1 (R132H) negative gliomas [p=0.0240, Kaplan Meier] (**A**). Hypothetical pathways, based on our observational data and others (7,12,15), that may explain the growth advantage provided by increased cMYC protein levels in the context of IDH1 mutant gliomas. (**B**).