5-Hydroxymethylcytosine Profiling Identifies Differential Targeting in IDH1 Mutant Versus IDH1 Wild-Type High-Grade Gliomas

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Introduction

Gliomas demonstrate epigenetic dysregulation highlighted by the Glioma CpG-Island Methylator Phenotype (G-CIMP) seen in IDH1 mutant tumors. IDH1 mutation perturbs the balance between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) by inhibiting TET-mediated active demethylation. 5mC has been firmly implicated in oncogenesis. More recently, 5hmC has been identified as dysregulated and contributing to the malignant phenotype in human cancers. Despite this, the role 5hmC plays in IDH1 mutant gliomas remains poorly understood.

Methods

We examined for 5hmC profiles in high grade (WHO III/IV) IDH1 mutant (n = 12) and IDH1 wild-type (n = 9) tumors through parallel processing of samples using bisulfite (BS) and oxidative bisulfite (OxBS) conversion, with subsequent analysis on the Illumina MethylationEPIC Beadchip platform. Probes within the highest top 1% beta-value as well as differentially hydroxymethylated regions (DHMR) between IDH1 cohorts were identified. Hydroxymethylation profiles were correlated with gene expression measured using the Affymetrix Human Gene 2.0 ST array platform.

Results

Mean 5hmC beta-values were 4.6%% and 3.8% for IDH1 mutant and wild-type tumors, respectively. Top 1% and DHMR probes demonstrated increased 5hmC among IDH1 mutants. 5hmC enriched for enhancer and super-enhancers. Among G-CIMP target genes, 22/50 were hydroxymethylated in our IDH1 mutant cohort, suggesting that 5hmC contributes to their overall methylation. Gene expression was strongly associated with gene body 5hmC. 48 genes differentially expressed between IDH1 cohorts showed a positive Spearman correlation between 5hmC and gene expression, in particular for genes upregulated in IDH1 mutant tumors.

Conclusions

Our study represents the first quantitative genome-wide locus-specific study examining 5hmC comparing IDH1 mutant versus wild-type gliomas. Focal gains of 5hmC targeting gene bodies and regulatory regions, and associated with over-expressed genes, implicates 5hmC as

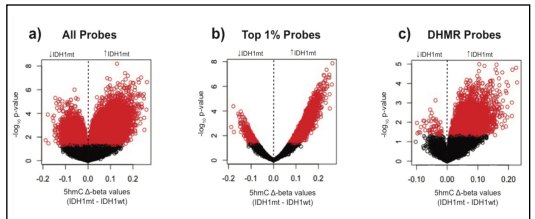
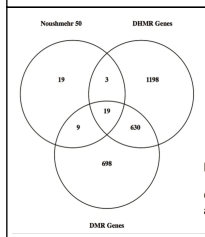


Fig. 1 Volcano plots

Volcano plots depicting the difference between average beta value between *IDH1* mutant vs *IDH1* wild-type for a) all probes, b) Top 1% probes, and c) DHMR probes. For probes with the greatest 5hmC abundance and differentially hydroxymethylated probes, IDH1 mutant tumors posses increased 5hmC. Probes with $p < 0.05$ are highlighted in Red.

Table 1: Tumor sample characteristics

	IDH1 mutant cohort	*IDH1* wild type cohort
Pathology (WHO Grade)		
Anaplastic Astrocytoma (III)	6 (50%)	0 (0%)
High-grade glioma (III/IV)	3 (25%)	0 (0%)
GBM (IV)	3(25%)	9 (100%)
IDH1 Mutation Status		
R132H	11 (91.7%)	0 (0%)
R132C	1 (8.3%)	0 (0%)
Age at diagnosis		
Median	40.6	58.8
Range	26 - 54	37 - 77
Sex		
Male	7 (58.3%)	5 (55.6%)
Female	5 (41.7%)	4 (44.4%)



	overlap	p-value
DHMR vs Noushmehr	22/50	P <= 7.918 e-11
DMR vs Noushmehr	28/50	P <= 1.898 e-20

Fig.2 5hmC contributes to G-CIMP gene methylation

Overlap between the G-CIMP 50 gene list (Noushmehr 50) and gene targets of DHMRs and DMRs.

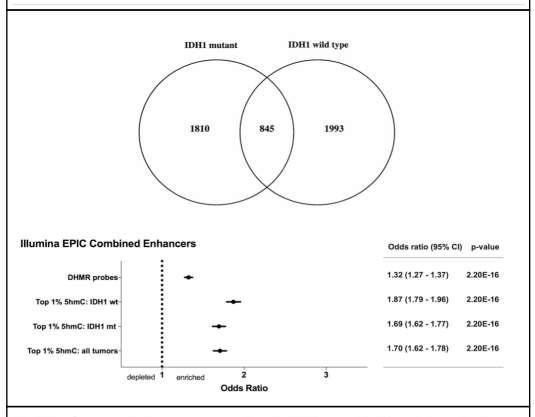
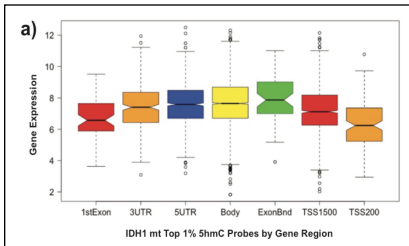


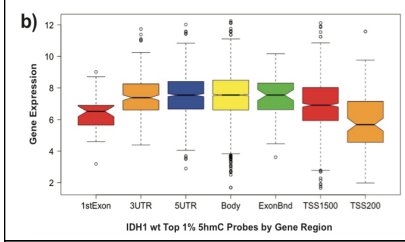
Fig.3 5hmC targets distinct enhancer regions in *IDH1* mt and *IDH1* wt high-grade gliomas

Top: Venn diagram depicting enhancer-related probes among top 1% 5hmC probes. Bottom: Forest plots depicting Odds Ratios and 95% confidence intervals for enriched enhancer targeting by top 1% 5hmC probes.



IDH1 mutant gene body region probe association with top 20% most highly expressed genes:

OR 2.27
95% CI 2.14 – 2.42

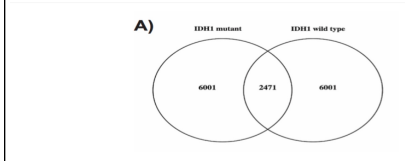


IDH1 wild-type gene body region probe association with top 20% most highly expressed genes:

OR 2.09
95% CI 1.95 – 2.21

Fig. 4 5hmC versus Gene Expression by Gene Region

a) Notched box plot depicting *IDH1* mt top 1% probe correlation with annotated target gene expression, stratified by probe gene region. b) Notched box plot *IDH1* wt top 1% probe correlation with annotated target gene expression, stratified by probe gene region. Hydroxymethylated probes associated with gene body regions (Body, ExonBnd, 3'UTR, 5'UTR) demonstrate statistically significant association with the top 20% most highly expressed genes in our tumor cohorts.



B)

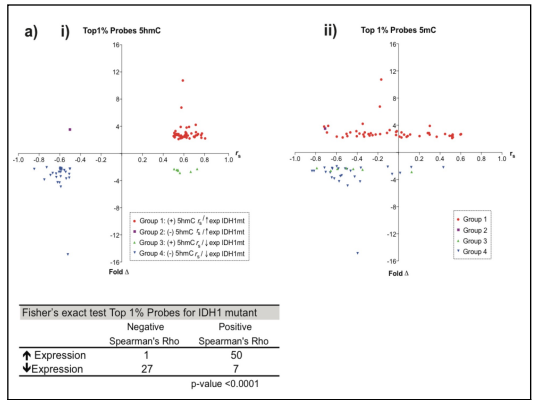
Top 1% Probe (IDH1 mutant and wild type) Pathway Analysis

Pathways	IDH1 mt Matched Genes (Total Genes)	IDH1 wt Matched Genes (Total Genes)
Common to IDH1 mt and wt		
Pathways in cancer	124 (397)**	134 (397)**
ERK signaling	306 (1176)**	299 (1176)**
RET signaling	248 (980)**	241 (980)**
Dopamine D2 receptor transactivation of EGFR	63 (164)**	63 (164)**
Unique to IDH1 mt		
NFAT and cardiac hypertrophy	107 (326)**	
Beta-adrenergic signaling	102 (308)**	
HGF signaling	73 (208)**	
Endometrial cancer	51 (124)**	
TGF-beta receptor signaling	55 (133)**	
Apoptotic pathways in synovial fibroblasts	194 (725)**	
Unique to IDH1 wt		
Wnt/Hedgehog/Notch		65 (180)**
G protein signaling regulation of P38 and JNK		40 (91)**
Ras signaling		99 (324)**
Angiotensin activation of ERK		73 (208)**
Phospholipase C pathway		137 (498)**
Axon guidance		63 (177)**

** corrected p-value less than or equal to 0.0001
* corrected p-value less than or equal to 0.05

Fig.5 Pathway analysis for top 1% 5hmC probe targets

A) Venn diagram depicting the degree of overlap for the top 1% 5hmC probes in *IDH1* mutant and *IDH1* wild-type tumors. B) Pathway analysis for top 1% 5hmC probe targets. C) Pathway analysis for differentially-hydroxymethylated region (DHMR) 5hmC probe targets.



Fisher's exact test Top 1% Probes for IDH1 mutant

	Negative Spearman's Rho	Positive Spearman's Rho
↑ Expression	1	50
↓ Expression	27	7

p-value <0.0001

Fig 6. 5hmC correlates with genes highly expressed in IDH1 mutant tumors

Spearman correlation coefficients (r_s) between 5hmC β-values for top 1% probes versus gene expression, for genes differentially expressed between *IDH1* cohorts were determined. Probes with $|r_s| \geq 0.5$ are graphed as a scatter plot showing r_s versus fold-change in gene expression. A significant positive Spearman correlation is identified between between probes with increased 5hmC and genes highly expressed in IDH1 mutants.

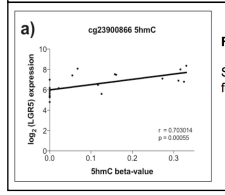


Fig 7. LGR5

Spearman correlation between 5hmC and expression for LGR5 ($r_s = 0.7$).

Conclusions

1) Among targets of increased 5hmC and DHMRs, IDH1 mutant tumors demonstrate increased 5hmC versus wild-type tumors
2) 5hmC contributes to overall "methylation" of G-CIMP target genes
3) Regions marked by high 5hmC abundance in IDH1 mutant and wild type gliomas enrich for unique enhancers targets
4) 5hmC targeting gene body regions is significantly associated with gene expression in both IDH1 mutant and wild-type tumors
5) Pathway analysis of 5hmC targets identifies pathways implicated in gliomagenesis, unique to IDH1 mutant and wild-type gliomas
6) 5hmC correlates positively with gene expression for genes implicated in glioma pathogenesis as well as novel candidate genes.

Acknowledgements

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