RNASeq Analysis of Glioma Tumors reveal Targetable Gene Fusions

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Abstract

Background: Fusions involving oncogenes have been reported in gliomas and may serve as novel therapeutic targets. We aim to use RNA sequencing to interrogate a large cohort of gliomas for targetable genetic fusions.

Methods: Gliomas were profiled using the ArcherDx FusionPlex Assay at a CLIA certified lab (Caris Life Sciences) and 52 gene targets were analyzed. Fusions with preserved kinase domains were investigated.

Results: Among 404 gliomas tested, 39 (9.7%) presented potentially targetable fusions, of which 24/226 (11%) of glioblastoma (GBM), 5/42 (12%) of anaplastic astrocytoma (AA), 2/25 (8%) of grade II astrocytoma and 3 of 7 (43%) of pilocytic astrocytoma (PA) harbored targetable fusions. In GBMs, 1 of 15 (6.7%) IDH-mutated tumors had a fusion while 22 of 175 (12.6%) IDH wild type tumors had fusions. 46 oligodendroglial tumors were profiled and no fusions were seen, which was lower than frequency of fusions in astrocytic tumors (34/300, p = 0.0236). The most frequent fusions seen involved FGFR3 (N = 12), including 10 FGFR3-TACC3 (1 AA, 6 GBM and 3 glioma NOS); 1 FGFR3-NBR1 (AA) and 1 FGFR3-BRAP (GBM). 11 fusions involving MET were seen, 10 in GBM and 1 in AA. The most common MET fusion was PTPRZ1-MET (1 in AA and 4 in GBM), followed by ST7-MET (N = 3, GBM), CAPZA2-Met (N = 2, GBM) and TPR-MET (N = 1, GBM). 8 NTRK fusions were seen; 1 involving NTRK1 (BCANNTRK1, PA), 6 NTRK2 (1 NOS1AP-NTRK2 in AA; GKAP1-NTRK2, KCTD8-NTRK2, TBC1D2-NTRK2 and SOSTM1-NTRK2, 1 each in GBM and 1 VCAN-NTRK2 in grade II astrocytoma) and 1 NTRK3 (EML4-NTRK3 in GBM). EGFR fusions (2 EGFR-SEPT14 and 1 EGFR-VWC2) were seen in 3 GBMs, BRAF in 2 (1 LOC100093631-BRAF in PA and 1 ZSCAN23-BRAF in glioma NOS) and PDGFRA (RAB3IP-PDGFRA, in GBM) in 1. C11orf95-RELA fusions were seen in 2 of 3 grade III ependymomas but not in the 2 grade II ependymomas.

Conclusions: We report targetable fusion genes involving NTRK, MET, EGFR, FGFR3, BRAF and PDGFRA including novel fusions that haven't been previously described in gliomas (e.g., EGFR-VWC2; FGFR3-NBR1). Fusions were seen in over 10% of astrocytic tumors, while none was seen oligodendrogliomas. Identification of such kinase associated fusion transcripts may allow us to exploit therapeutic opportunities with targeted therapies in gliomas.

Methods

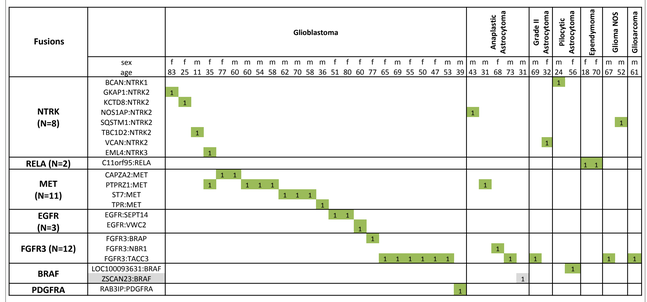
- Retrospective evaluation of 404 glioma tumor specimens tested by fusion analysis at a CLIA-certified lab (Caris Life Sciences, Phoenix, AZ)
- RNA-sequencing by ArcherDx fusion assay based on anchored multiplex PCR
- FusionPlex Solid Tumor Kit was used
- Anchored primers allow 53 genes to be analyzed as potential fusion partners
- In-frame fusions were analyzed
- Fusions involving protein kinases were predicted to have functional kinase domains

Results

Table 1: Patient characteristics

	Tumor Type	Total N	Average Age (years)	Female N	Male N
Astrocytoma	Glioblastoma	226	57	92	134
	Grade III Astrocytoma	42	47	16	26
	Grade II Astrocytoma	25	43	11	14
	Pilocytic Astrocytoma	7	23	1	6
Oligodendroglioma	Grade III Oligodendroglioma	11	48	7	4
	Grade II Oligodendroglioma	14	46	9	5
	Oligodendroglioma, NOS	8	46	2	6
Ependymoma	Grade III Ependymoma	3	41	2	1
	Grade II Ependymoma	2	54	1	1
	Subependymoma	1	59		1
Mixed	Oligoastrocytoma (Grade II or III)	13	46	9	4
Other	Gliosarcoma	9	57	7	2
	Ganglioglioma	4	37	1	3
Unclear	Grade III Glioma, NOS	4	38	3	1
	Grade II Glioma, NOS	4	58	1	3
	Glioma, NOS	31	48	10	21
	Grand Total	**404**	**52**	**172**	**232**

Table 2: Landscape of targetable fusions found. Green squares indicate that the gene sequence at the breakpoint was investigated and the preservation of the kinase domain was confirmed and the fusion event was confirmed to be in-frame. Gray: ZSCAN23: BRAF breakpoint indicates that the fusion is not in-frame, therefore excluded for further analysis



Results, continued

Figure 1: Distribution of targetable fusions in histological groups of CNS tumors investigated. A star indicates that 11% of astrocytomas were found to have a fusion vs. no fusion was found in the 33 oligodendrogliomas studied, p<0.05.

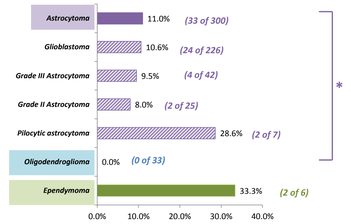
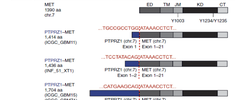


Figure 2: Details of targetable fusions found

2A: MET fusions found

N	Fusion Product	Fusion partner: break point	MET: break point	Histology	Reported previously?
2	CAPZA2-MET	CAPZA2 (exon 1)	MET: full length protein preserved	Glioblastoma	• Gastric cancer • Kidney cancer • Endometrial • Lung cancer
5	PTPRZ1-MET	PTPRZ1 (exon 2, exon 8, exon 1)		Glioblastoma N=4 Anaplastic astrocytoma N=1	Pediatric glioblastoma
3	ST7-MET	ST7 (exon 1)		Glioblastoma	No
1	TPR-MET	TPR (exon 36)	MET: exon 15 (codon 1010)	Glioblastoma	Yes



Recurrent MET fusions reported in pediatric glioblastomas; one patient treated with crizotinib had a short-lived response.
(*Bender et al. 2016, Nature Medicine)

•All 5 PTPRZ1-MET fusions in this study have been previously reported*; Exon 1 in 3 cases, Exons 2 and 8 in one each

•MET kinase domain preserved in predicted fusion products

•Potential Therapies: crizotinib, glesatinib, tepotinib

2B: NTRK fusions found

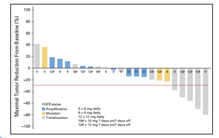
N	Fusion Product	Fusion Partner: break point	NTRK: break point	Histology	Reported Previously?
1	BCAN-NTRK1	BCAN: exon 12	NTRK1: exon 10	Pilocytic astrocytoma	GBM, low-grade glioneuronal tumor
1	GKAP1-NTRK2	GKAP1: exon 9	NTRK2: exon13	Glioblastoma	No
1	KCTD8-NTRK2	KCTD8: exon 1	NTRK2: exon13	Glioblastoma	No
1	NOS1AP-NTRK2	NOS1AP: exon 9	NTRK2: exon 11	Anaplstic astrocytoma	No
1	SQSTM1-NTRK2	SQSTM1: exon 5	NTRK2: exon 13	Glioma, NOS	Yes
1	TBC1D2-NTRK2	TBC1D2: exon 6	NTRK2: exon 12	Glioblastoma	No
1	VCAN-NTRK2	VCAN: exon 6	NTRK2: exon 10	Grade II astrocytoma	No
1	EML4-NTRK3	EML4: exon 2	NTRK3: exon 13	Glioblastoma	recurrent congenital fibrosarcoma

•Potential Therapies: Entrectinib, Larotrectinib

Treatment of a glioneuronal tumor harboring a BCAN-NTRK1 fusion with a pan-TRK inhibitor resulted in a 60% volumetric tumor reduction, clinical benefit lasting 11 months*.
(*C. Alvarez-Breckenridge et al. npj Precision Oncology (2017) 1:5)

2C: FGFR fusions found

N	Fusion product	FGFR: break point	Fusion Partner: break point	Histology	Reported previously?
10	FGFR3-TACC3	FGFR3: exon 17	TACC3: exon 6, 8, 10, 11	Glioblastoma N=6 Anaplastic astrocytoma N=1 Grade II Astrocytoma N=1 Glioma NOS N=1 Gliosarcoma N=1	• Bladder cancer • GBM • HNSCC • Low grade glioma • Lung squamous cell carcinoma
1	FGFR3-BRAP	FGFR3: exon 17	BRAP: exon 5	Glioblastoma	No
1	FGFR3-NBR1	FGFR3: exon 17	NBR1: exon 7	Anaplastic astrocytoma	No

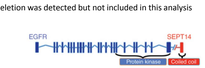


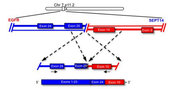
- Phase 1 trial of an oral pan-FGFR inhibitor generated 5/23 partial responses (one GBM) in FGFR altered patients; 16 pts had SD* (*Tabernero et al. J Clin Oncol 33:3401-3408)*
- Potential Therapies: NVP-BGJ398, LY2874455

2D: Additional fusions found

N	Fusion Product	Oncogene: break point	Fusion partner: break point	Other notable alterations	Reported previously?	Potential Therapy
2	EGFR-SEPT14		SEPT14: exon10	EGFR amp	yes	
1	EGFR-VWC2	EGFR: exon24	VWC2: exon4	EGFR amp	No	EGFR inhibitors (Erlotinib, lapatinib)
1	LOC100093631-BRAF	BRAF: exon10	GTF2IP4	No other alteration	No	BRAF inhibitors
1	RAB3IP-PDGFRA	PDGFRA: exon11	RAB3IP: exon1	PDGFRA amplification	No	PDGFRA inhibitors

*EGFRvIII deletion was detected but not included in this analysis





Previously reported genomic fusion of EGFR exon 25 with intron 9 of SEPT14. The same fusion seen in two cases.

Frattini, V et al (2013) Nature Genetics

Conclusions

- Targetable fusions are frequent events in various glioma subtypes
- 11% of astrocytic tumors harbor targetable fusions identified by RNA sequencing
- Further functional studies are needed to fully characterize the novel fusions found
- Identification of kinase-associated transcripts may allow us to exploit therapeutic opportunities with targeted therapies in gliomas in a systematic fashion

References

1. *Bender et al. 2016, "Recurrent MET fusion genes represent a drug target in pediatric glioblastoma." Nature Medicine,* 22(11):1314-1320
2. Alvarez-Breckenridge et al. 2017 "Clinical and radiographic response following targeting of BCAN-NTRK1 fusion in glioneuronal tumor" npj Precision Oncology (2017)
3. Tabernero J et al. 2015 "Phase I Dose-Escalation Study of JNJ-42756493, an Oral Pan-Fibroblast Growth Factor Receptor Inhibitor, in Patients With Advanced Solid Tumors." J Clin Oncol. 33(30):3401-8.
4. Frattini V, et al. 2013 "The integrated landscape of driver genomic alterations in glioblastoma." Nat Genet 45(10):1141-9.