Published OnlineFirst May 20, 2015; DOI: 10.1158/1078-0432.CCR-15-0458

Clinical Cancer Research

Growth Factor Receptor Fusions Predict Therapeutic Sensitivity

Manmeet S. Ahluwalia[1] and Jeremy N. Rich[1,2]

Dysregulated growth factor pathways promote tumor growth in many cancers, but receptor-targeting strategies frequently offer limited benefit despite activation by receptor overexpression or amplification. In contrast, tumors harboring growth factor receptor fusions display exquisite dependence on receptor activity, providing predictive markers for patient response to inform precise oncology treatment. *Clin Cancer Res; 21(14); 3105–7. ©2015 AACR.*

See related article by Di Stefano et al., p. 3307

In this issue of *Clinical Cancer Research*, Di Stefano and colleagues (1) discuss the use of RT-PCR-sequencing as a sensitive and specific method to identify *FGFR–TACC* fusion genes in patients with grade 2–4 gliomas. In their study, *FGFR3–TACC3*-positive patients treated with a FGFR inhibitor derive clinical benefit.

Theodor Boveri proposed that acquired chromosomal abnormalities have an important role in the initiation of carcinogenesis a century ago (2). Growth factors promote a myriad of potentially oncogenic activities; therefore, it is not surprising that growth factor receptors and downstream effectors are commonly altered in cancers through transcriptional upregulation, gene amplification, activating mutation, and genomic fusion events. Fusion genes are generated when two previously separate genes rearrange and fuse together, resulting in a hybrid gene. The first fusion gene described in cancer cells was the Philadelphia chromosome in chronic myeloid leukemia (CML) discovered in 1960 as a result of the work of Nowell and Hungerford (3). Four decades later, the extraordinary activity of imatinib against CML harboring the *BCR–ABL1* fusion exemplified precision targeting of genetic targets as an effective therapeutic approach in oncology (4). In solid tumors, the *EML4–ALK* fusion reported in 4% to 7% of lung cancers has been successfully targeted by crizotinib and ceritinib (5).

The extension of imatinib activity to gastrointestinal stromal tumors, which harbor constitutive activation of the KIT receptor tyrosine kinase, appeared to confirm the broad potential for sustained tumor control with targeted therapeutics (6). Unfortunately, sustained responses to kinase inhibitors have often proved the exception, rather than the rule. Epidermal growth factor receptors (EGFR) are altered in the majority of glioblastoma, the most prevalent primary intrinsic brain tumor, but EGFR antagonists have repeatedly failed in clinical trials (7). Even when a promising mutational signature appeared to predict success (8), further studies failed to validate EGFR inhibitor efficacy in glioblastoma (9). Failure of EGFR antagonists and numerous other kinase inhibitors in glioblastoma has tempered enthusiasm for receptor-targeting approaches until a new wave of discoveries of novel fusion events.

Singh and colleagues (10) first reported in-frame fusions of the tyrosine kinase coding domains of fibroblast growth factor receptor (*FGFR*) genes (*FGFR1* or *FGFR3*) to transforming acidic coiled-coil (*TACC*) coding domains of *TACC1 or TACC3* in 3% of glioblastoma (Fig. 1). A flurry of reports has demonstrated *FGFR* family fusions in bladder, lung, breast, thyroid, oral, and prostate cancer. In an *in vivo* model, oral administration of an *FGFR* inhibitor resulted in prolonged survival of mice harboring intracranial *FGFR3–TACC3*-initiated glioma. Hence, a subset of glioblastoma patients that harbor the *FGFR–TACC* fusions could derive benefit from targeted FGFR kinase inhibition. FGFR inhibitors for *FGFR3–TACC3*-positive glioblastomas may have large therapeutic indices due to the relatively low levels of wild-type *FGFR3* within the brain. FGFR3 inhibitors would presumably target only the neoplastic compartment expressing *FGFR3–TACC3*, while sparing normal healthy tissues.

The precise application of FGFR targeting for patients with the relevant fusions will require rapid and accurate detection of these fusion events. Selection of methods utilized to diagnose the fusion genes is informed by the type of fusion. Fusion genes resulting from translocations are usually diagnosed by fluorescence *in situ* hybridization (FISH), detecting abnormal chromosomes within the cell, e.g., *BCR–ABL1* fusions in chronic CML. Microarray techniques are also used to diagnose fusion genes that arise as a result of overexpression of one of the fusion partners, e.g., *TMPRSS2–ERG* fusions in prostate cancer (11). Next-generation whole transcriptome sequencing aids in diagnosis of fusions genes by analyzing the different reads from fusion gene as compared with normal tissue. Polymerase chain reaction (PCR) with primers flanking the fusion junction followed by sequencing informs the diagnosis of fusion gene if both fusion partner genes are present on analysis. In the current issue, Di Stefano and colleagues describe the detection, characterization, and inhibition of *FGFR–TACC* fusions in glioma with wild-type isocitrate dehydrogenase 1 (IDH1). Due to the close proximity of FGFR3 and TACC3 on chromosome 4p16.3, FISH detection of *FGFR3–TACC3* rearrangements is not optimal using currently

[1]Burkhardt Brain Tumor Neuro-Oncology Center, Neurological Institute, Cleveland Clinic, Cleveland, Ohio. [2]Department of Stem Cell Biology and Regenerative Medicine, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio.

Corresponding Author: Manmeet S. Ahluwalia, Taussig Cancer Center, Cleveland Clinic, 9500 Euclid Avenue, S73, Cleveland, OH 44195. Phone: 216-444-6145; Fax: 216-444-0924; E-mail: ahluwam@ccf.org

doi: 10.1158/1078-0432.CCR-15-0458

Published OnlineFirst May 20, 2015; DOI: 10.1158/1078-0432.CCR-15-0458

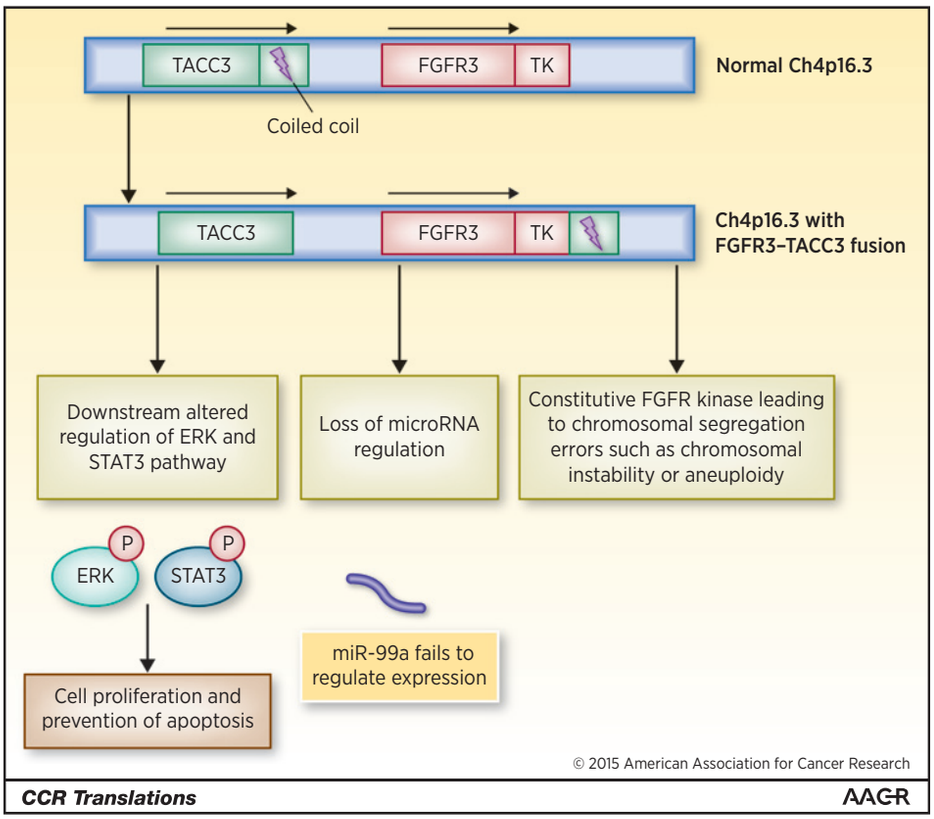

Figure 1.
Downstream impact of FGFR–TACC fusion oncogene.

available methods. The authors developed an RT-PCR assay to identify the known and possibly novel variants of *FGFR1–TACC1* and *FGFR3–TACC3* fusions that retain the mRNA sequences coding for the key FGFR–TK and TACC domains required for the oncogenic activity of the fusion protein. Critically, confirmation of the in-frame breakpoint was performed by Sanger sequencing.

Three of 85 wild-type IDH1/2 grade 2–3 gliomas (3.5%) harbored *FGFR3–TACC3* fusions; however, none of 126 IDH1/2–mutant tumor samples demonstrated evidence of *FGFR3–TACC3* fusions. Seventeen of the 584 glioblastoma (2.9%) harbored *FGFR–TACC* rearrangements. These findings are consistent with the 3% incidence of *FGFR–TACC* rearrangements in glioblastoma described earlier (10). *IDH* wild-type grade 2–3 gliomas (diffuse glioma and anaplastic glioma) have similar prevalence of FGFR–TACC fusions to that of glioblastoma, suggesting an early mutation consistent with a tumor driver. The authors report considerable structural variability among *FGFR3–TACC3* fusion isoforms, with five of the identified variants occurring only in individual cases. In addition, the investigators identified 6 new fusion transcripts that have not been reported before. Collectively, these results suggest that *FGFR* fusions may serve as early tumor drivers in the absence of *IDH1* mutations and that the direct contribution *TACC* to oncogenic function may be modest.

Recent work from the comprehensive analysis of 293 grade 2 and 3 gliomas using multiple genomic and proteomic platforms from The Cancer Genome Atlas reported three superclusters of these tumors (12). Group 1 tumors are wild-type for IDH1/IDH2; group 2 tumors are *IDH1/IDH2* mutant with chromosome *1p/19q* intact; and group 3 harbors *IDH1/IDH2* mutations with codeletion of chromosome *1p/19q*. The IDH wild-type group of grade 2 or 3 gliomas have a glioblastoma-like phenotype: focal gains of *EGFR*, *CDK4*, and *MDM4*, mutations in NF1, EGFR, and PTEN, and a poor median survival, compared with those who harbor *IDH1/IDH2* mutations. Di Stefano and colleagues also report that *FGFR–TACC* rearrangements are mutually exclusive with *IDH1/2* mutations and *EGFR* amplification whereas co-occur with *CDK4* amplification and *MDM2* to a lesser extent. In this era of precision medicine and targeted therapy, knowledge of these molecular characteristics will help enrich the future trials with patients who harbor appropriate mutations and that are likely to derive benefit from agents targeting the mutation.

Di Stefano and colleagues further describe the clinical benefit they observed when they used JNJ-42756493, an inhibitor of FGFR1–4, in the treatment of 2 patients whose tumors harbored the fusion gene FGFR3–TACC3. This preliminary clinical activity supports further evaluation of FGFR inhibition in *FGFR–TACC*-positive patients. Previous trials of FGFR inhibitors, such as ninetanib (13) or dovitinib (14), in unselected recurrent glioblastoma did not demonstrate meaningful clinical activity. However, an ongoing phase II study of BGJ398 in recurrent glioblastoma enriches patients that harbor amplification, translocation, or activating mutation in *FGFR1*, *2*, *3*, or *4* (NCT01975701) and may address the clinical utility of these agents in this patient population. Whether the success in targeting *BCR–ABL1* in leukemia and in the *EML4–ALK* fusion in lung cancer will be replicated for the FGFR inhibitors in gliomas with FGFR fusions remains to be determined, but new hope for targeted therapies against growth factor pathways is emerging for these tumors.

Published OnlineFirst May 20, 2015; DOI: 10.1158/1078-0432.CCR-15-0458

Disclosure of Potential Conflicts of Interest

M.S. Ahluwalia reports receiving commercial research grants, through his institution, from Boehringer Ingelheim, Eli Lilly/ImClone Systems, Novartis, Spectrum Pharmaceuticals, and TRACON Pharmaceuticals; speakers bureau honoraria from Sigma-Tau Pharmaceuticals; and is a consultant/advisory board member for Caris Life Sciences, Genentech/Roche, and Incyte. No potential conflicts of interest were disclosed by the other author.

Authors' Contributions

Conception and design: M.S. Ahluwalia, J.N. Rich
Development of methodology: M.S. Ahluwalia
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.S. Ahluwalia
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.S. Ahluwalia
Writing, review, and/or revision of the manuscript: M.S. Ahluwalia, J.N. Rich
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.S. Ahluwalia
Study supervision: M.S. Ahluwalia

Grant Support

J.N. Rich was supported by the NIH under award numbers CA154130, CA171652, CA169117, NS087913, and NS089272; the Research Programs Committees of Cleveland Clinic; and the James S. McDonnell Foundation.

Received March 27, 2015; accepted April 2, 2015; published OnlineFirst May 20, 2015.

References

1. Di Stefano AL, Fucci A, Frattini V, Labussiere M, Mokhtari K, Zoppoli P, et al. Detection, characterization, and inhibition of FGFR–TACC fusions in IDH wild-type glioma. Clin Cancer Res 2015;21:3307–17.
2. Harris H. Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. Preface. J Cell Sci 2008;121 Suppl 1:v–vi.
3. Nowell PC, Hungerford PA. A minute chromosome in human chronic granulocytic leukemia. Science 1960;3438:1497.
4. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001;344:1031–7.
5. Shaw AT, Engelman JA. Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med 2014;370:2537–9.
6. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002;347:472–80.
7. Rich JN, Reardon DA, Peery T, Dowell JM, Quinn JA, Penne KL, et al. Phase II trial of gefitinib in recurrent glioblastoma. J Clin Oncol 2004;22:133–142.
8. Mellinghoff IK, Wang MY, Vivanco I, Haas-Kogan DA, Zhu S, Dia EQ, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N Engl J Med 2005;353:2012–24.
9. van den Bent MJ, Brandes AA, Rampling R, Kouwenhoven MC, Kros JM, Carpentier AF, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. J Clin Oncol 2009;27:1268–74.
10. Singh D, Chan JM, Zoppoli P, Niola F, Sullivan R, Castano A, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. Science 2012;337:1231–5.
11. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
12. Yung WKA, Verhaak R, Cooper L, Salama S, Aldape K, Brat D. GE-41 comprehensive and integrative genomic characterization of diffuse lower grade gliomas. Neuro Oncol 2014;105(16 Suppl 5):GE41.
13. Norden AD, Schiff D, Ahluwalia MS, Lesser GJ, Nayak L, Lee EQ, et al. Phase II trial of triple tyrosine kinase receptor inhibitor nintedanib in recurrent high-grade gliomas. J Neurooncol 2015;121:297–302.
14. Ahluwalia MS, Papadantonakis N, Alva Venur V, Schilero C, Peereboom DM, Stevens G, et al. Phase II trial of dovitinib in recurrent glioblastoma. J Clin Oncol 2015 (suppl; abstr 2050).

Published OnlineFirst May 20, 2015; DOI: 10.1158/1078-0432.CCR-15-0458

Clinical Cancer Research

Growth Factor Receptor Fusions Predict Therapeutic Sensitivity

Manmeet S. Ahluwalia and Jeremy N. Rich

Clin Cancer Res 2015;21:3105-3107. Published OnlineFirst May 20, 2015.