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Multiple functions of a glioblastoma fusion oncogene

Ivan Babic, Paul S. Mischel

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Commentary

RNA sequencing facilitates the discovery of novel gene fusions in cancer. In this issue of the *JCI*, Parker et al. identify an *FGFR3-TACC3* fusion oncogene in glioblastoma and demonstrate a novel mechanism of pathogenicity. A miR-99a binding site within the 3′–untranslated region (3′-UTR) of *FGFR3* is lost, releasing *FGFR3* signaling from miR-99a–dependent inhibition and greatly enhancing tumor progression relative to WT *FGFR3*. These results provide compelling insight into the pathogenicity of a novel fusion oncogene and suggest new therapeutic approaches for a subset of glioblastomas.

apical ENaC abundance largely through signaling mechanisms that do not require Nedd4-2. This conclusion derives not only from the current paper (8), but also from work showing that aldosterone responsiveness of ENaC in the rectum is increased rather than decreased when Nedd4-2 is deleted (18) and that aldosterone still regulates ENaC trafficking in the kidney when PY motifs are deleted in vivo (4). One possibility, suggested by the current results, is that Nedd4-2–mediated ubiquitylation primarily accelerates ENaC degradation within the cell (step iii in Figure 1A) rather than its removal from the membrane (step ii in Figure 1A). Secondly, Nedd4-2 appears to play a physiologically significant and nonredundant role in modulating NCC abundance. That said, it should be pointed out that SGK1 also activates NCC through pathways independently of Nedd4-2 (16) and that other NCC modulators, such as WNK4, may also have large effects on NCC activity. Finally, this work shows again why it is so important to test mechanistic models in vivo. While the results are sometimes confusing and can challenge accepted dogma, their relevance to human health and disease is beyond question. An intriguing clinical implication of the paper by Ronzaud and colleagues (8) is that manipulating Nedd4-2 activity might be a novel approach to treating Gitelman syndrome, an ionic imbalance disease linked to reductions in membrane NCC (19). As Nedd4-2 may inhibit both membrane removal and intracellular degradation, its inhibition might increase NCC abundance enough to ameliorate the troubling symptoms of the disease.

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Address correspondence to: David H. Ellison, Division of Nephrology and Hypertension, CH12R, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, Oregon 97239, USA. Phone: 503.494.8490; Fax: 503.494.5330; E-mail: ellisond@ohsu.edu.

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Multiple functions of a glioblastoma fusion oncogene

Ivan Babic and Paul S. Mischel

Ludwig Institute for Cancer Research, UCSD, La Jolla, California, USA.

RNA sequencing facilitates the discovery of novel gene fusions in cancer. In this issue of the *JCI*, Parker et al. identify an *FGFR3-TACC3* fusion oncogene in glioblastoma and demonstrate a novel mechanism of pathogenicity. A miR-99a binding site within the 3′–untranslated region (3′-UTR) of *FGFR3* is lost, releasing *FGFR3* signaling from miR-99a–dependent inhibition and greatly enhancing tumor progression relative to WT *FGFR3*. These results provide compelling insight into the pathogenicity of a novel fusion oncogene and suggest new therapeutic approaches for a subset of glioblastomas.

Genomic technologies are transforming our knowledge about the mutational landscape of cancer. For glioblastoma, the most common and lethal form of adult primary brain cancer, integrated DNA, transcriptional, and epigenetic analyses have identified copy number alterations, mutations, tumor transcriptional and epigenetic subclasses, and potential new drug targets (1–5). Recent progress in next-generation sequencing technologies, including RNA sequencing, provides a powerful new platform adding to this integrated toolkit. Researchers are now able to identify and quantify changes in both coding and noncoding RNA; identify alternative splicing events; and detect expressed mutations, SNPs, gene

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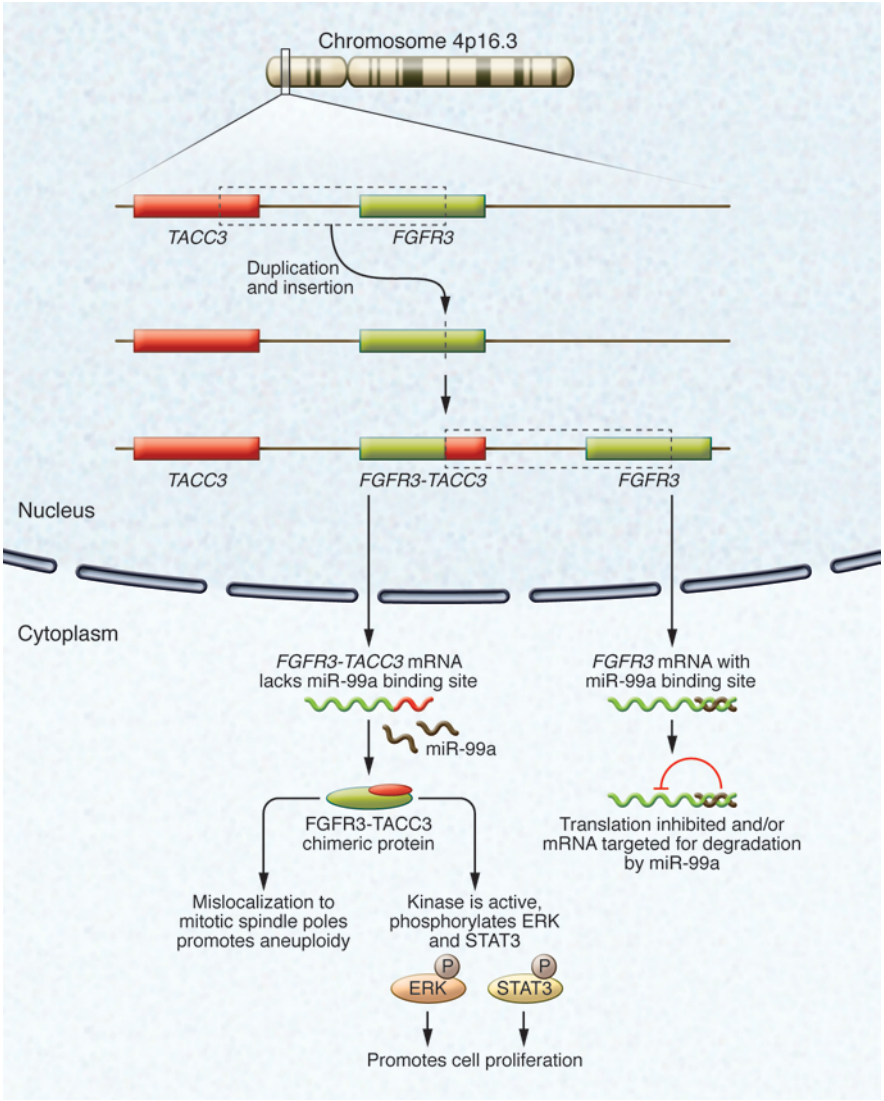

Figure 1
A tandem duplication event results in the formation of an *FGFR3-TACC3* fusion product. Here, Parker et al. demonstrate that the fusion transcript lacks a miR-99a binding site, resulting in increased expression (12). In addition, *FGFR3-TACC3* fusion activates ERK and STAT3 signaling and enhances tumor progression. Previous work also demonstrated that localization of this fusion protein to the mitotic spindles promotes aneuploidy (13).

translocations, and fusion transcripts. Importantly, the identification of novel fusion proteins may provide new insights into the biology of this dreadful disease.

Gene fusions and cancer

Fusion genes occur when parts of two genes combine during a chromosomal rearrangement, resulting in expression of a chimeric protein, a process whose importance in cancer is well recognized. *BCR-ABL1* in chronic myelogenous leukemia (CML; ref. 6), *PML-RARA* in acute promyelocytic leukemia (APL; reviewed in refs. 7, 8), *EML4-Alk* in non–small-cell lung cancer (NSCLC; reviewed in ref. 9), *EWS-FLI* in Ewing sarcoma (10), and *TMPRSS2-ERG* in prostate cancer (11) are paradigmatic examples. Fusion oncogenes are not common in cancer in general, but their importance in understanding cancer biology is disproportionately large, providing some of the most compelling examples of successful targeted therapies for selected cancer subtypes. Mechanistic insights gleaned by studying the *BCR-ABL1* and *PML-RARA* oncogenes have translated into near-cures for two previously deadly types of cancer: imatinib for the treatment of CML and combined arsenic trioxide and retinoic acid for the treatment of APL (6, 7). Of note, the finding of *EML4-Alk* fusion and its rapid translation into clinical benefit for NSCLC patients treated with crizotinib brings new hope that the insights gained from studying fusions may not be limited to the rarer types of molecularly homogeneous hematopoietic cancers, such as CML and APL. Thus, discovery of a new fusion in glioblastoma is an exciting and important development.

FGFR3-TACC3 gene fusion in glioblastoma

In this issue, Parker et al. used whole-transcriptome RNA sequencing (RNA-seq) to look for fusions formed as a result of chromosomal translocations (12). Analysis of 48 glioblastoma samples and 43 low-grade glioma samples obtained from the United States and China, as well as analysis of the large Cancer Genome Atlas dataset, revealed the presence of a fusion composed of FGF receptor 3 (*FGFR3*) with transforming acidic coiled-coil 3 (*TACC3*). The authors demonstrated that fusion occurred via a tandem duplication event and detected it in 8.3% of glioblastoma patients. It was not detected in any

of the low grade glioma samples. Importantly, *FGFR3-TACC3* fusions were mutually exclusive with *EGFR*, *PDGFR*, or *MET* genetic alterations, the receptor tyrosine kinase alterations commonly detected in glioblastoma. Taken together, these findings suggest an important and specific role for *FGFR3-TACC3* in promoting glioblastoma growth.

This study by Parker and colleagues independently validates the very recent report by Singh et al. (13), which identified *FGFR3-TACC* fusions in a small subset of glioblastomas. While independent validation is essential, the real excitement of this study lies more in its identification of the novel and potentially targetable mechanism of pathogenicity created by the *FGFR3-TACC3* fusion.

FGFR3 encodes a receptor tyrosine kinase that is commonly mutated in bladder and cervical cancer (14). *FGFR3* engages downstream signaling cascades that are commonly activated in cancer, including PI3K-Akt and Ras-Mek-Erk signaling (15). In glioblastoma, a role for *FGFR3* had not been previously established, although recent work suggests that it can phosphorylate PTEN to promote glioblastoma resistance to EGFR tyrosine kinase inhibitors (16). *TACC3* encodes a centrosomal protein involved in mitosis (17) that is overexpressed in lung and colon carcinomas and in multiple myeloma (18). What are the mechanisms of its pathogenicity, and in what ways are the activities of this fusion protein greater than the sum of its parts?

Singh et al. demonstrated that the transforming capacity of *FGFR3-TACC3* is related to its localization to the mitotic spindle, where it causes chromosomal missegregation and aneuploidy. Importantly, they showed that this requires FGFR kinase activity, because a pan-FGFR tyrosine kinase inhibitor abrogated the fusion protein's effects on chromosomal instability, reversing aneuploidy (13). Thus, Singh and colleagues identified one intriguing mechanism by which *FGFR3-TACC3* fusion can promote tumorigenicity.

In the present study, Parker and colleagues provide an alternative and entirely new view of a mechanism driving *FGFR3-TACC3* pathogenicity (Figure 1). They conclude that the pathogenicity of *FGFR3-TACC3* is mediated, at least in part, through loss of the miR-99a binding site. MicroRNAs typically bind the 3′–untranslated region (3′-UTR) of a transcript and can repress translation and/or promote degradation of that transcript (19). Changes in the 3′-UTR, due to alternative splicing or shortening through alternative cleavage, can significantly affect mRNA translation (20, 21), resulting in enhanced expression of transcripts insensitive to microRNA regulation. This may promote tumor development and/or progression (20, 21). Parker and colleagues demonstrated that a miR-99a binding site in the 3′-UTR of *FGFR3* was lost during fusion of *FGFR3* with *TACC3*, causing greatly increased *FGFR3* expression, an effect that was counteracted by reintroduction of the 3′-UTR of *FGFR3* in the presence of miR-99a (12). Importantly, *FGFR3-TACC3* fusion was demonstrated to preferentially engage ERK and STAT3 signaling and to enhance tumor progression in vivo relative to WT *FGFR3*, which suggests that the fusion creates a specific gain of function.

New approaches

Glioblastoma is now one of the most intensely studied of all cancers at the molecular level; however, the mapping of the mutational landscape has yet to be successfully leveraged to yield better treatment for patients. Obtaining a mechanistic understanding of this mutational landscape, particularly with regard to clarifying function of specific mutations, may go a long way toward transforming basic science knowledge into clinical benefit for patients. Here, Parker and colleagues have taken important steps toward developing a functional understanding of the consequences of the *FGFR3-TACC3* fusion in glioblastoma (12). Their study, taken together with the work of Singh et al. (13), demonstrates the importance of the *FGFR3-TACC3* fusion in glioblastoma, which suggests that the gain of function created by the *FGFR3-TACC3* fusion is greater than the sum of its parts.

The current study also raises several questions for further investigation. First, what are the mechanisms by which the FGFR3 component of the fusion protein differentially engages downstream signaling, enabling it to more effectively activate the ERK and STAT3 signaling cascades? Second, the data from Singh et al. suggest a critical role for the TACC3 component of the fusion protein in regulating aneuploidy in a fashion dependent on FGFR3 signaling; how is that regulated? Finally, what are the therapeutic implications? Will glioblastomas bearing *FGFR3-TACC3* fusions respond to pharmacologic inhibition with FGFR3 inhibitors, when they become available? Efforts to elucidate the mechanisms by which fusion oncogenes promote their oncogenic effects, even if they are rare, have yielded remarkable therapeutic insights. We expect that similar deep investigation into these pathogenic mechanisms, and others that will be discovered through RNA sequencing, will also reveal new therapeutic strategies.

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Address correspondence to: Paul S. Mischel, Ludwig Institute for Cancer Research, University of California at San Diego, La Jolla, California 92093, USA. Phone: 858.534.6080; Fax: 858.534.7750; E-mail: pmischel@ucsd.edu.

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Old King Coal — molecular mechanisms underlying an ancient treatment for atopic eczema

W.H. Irwin McLean[1] and Alan D. Irvine[2,3,4]

[1]Centre for Dermatology and Genetic Medicine, Division of Molecular Medicine, Colleges of Life Sciences and Medicine, Dentistry and Nursing, University of Dundee, Dundee, United Kingdom. [2]Department of Paediatric Dermatology, Our Lady's Children's Hospital, Dublin, United Kingdom. [3]Clinical Medicine, Trinity College Dublin, Dublin, United Kingdom. [4]National Children's Research Centre, Our Lady's Children's Hospital, Dublin, United Kingdom.

Traditional remedies for common disorders have been known for centuries, but insight into their mechanism of action is often limited. In this issue of the *JCI*, Joost Schalkwijk's research group at the Radboud University Nijmegen Medical Centre in The Netherlands advances our understanding of why topical coal tar is an effective treatment for atopic dermatitis (AD), both rationalizing the use of this traditional medicine, and providing the scientific basis for new therapeutic approaches.

AD (also called "eczema") is the most common inflammatory skin condition, affecting about 20% of children in the developed world. AD is a classic complex trait where a combination of several genetic predisposing factors interact with environmental stimuli to trigger the disease. AD is frequently associated with high serum IgE and a Th2 immune response (1, 2). In 2006, a paradigm shift in the pathomechanistic understanding of AD took place when loss-of-function mutations were discovered in the *FLG* gene encoding the skin barrier protein filaggrin in the common monogenic skin disease ichthyosis vulgaris (dry, flaky skin) (3). Soon thereafter, these same filaggrin variants, which are carried by about 10% of populations of European ancestry and persist at high frequencies in other populations (4), were shown to be the major genetic predisposing factor in AD (5). Filaggrin-deficient animals were subsequently shown to have a "leaky" skin barrier, allowing passive percutaneous transfer of antigens, which trigger skin inflammation and an allergic immune response, analogous to AD in humans (6). This work showed that a primary skin barrier deficiency is at least one important factor in AD pathogenesis, although Th2 immunity is clearly also a major player (1).

Making the pitch for pitch

Coal tar has been used medically since ancient times. In his epic 5-volume work, Περὶ ὕλης ἰατρικῆς (Latin: *De Materia Medica*), the Greek physician, pharmacologist, and botanist Pedanius Dioscorides (circa 40–90 CE) chronicled many and varied herbal and other remedies in use at the time, including the "grime of a gymnasium wall." While this remedy for abrasions and ulcers does not currently enjoy popularity, his suggestion of the use of bitumen or asphalt/coal tar for "inflammation" (7) has maintained traction over the succeeding 2 millennia (8). For as long as modern dermatology departments have existed, liquor picis carbonis (LPC) has been a part of their working vocabulary, and preparations containing LPC are widely considered to be effective in the treatment of psoriasis and AD. Indeed, a recent systematic review provided evidence of the efficacy of 0.5%–5% LPC preparations for both these conditions (9).

Tar, asphalt, bitumen, and pitch are related substances consisting of complex mixtures of high molecular weight organic compounds that can be derived from heat distillation of plants, wood, petrochemicals, or coal. Pitch essentially functions as a solid but is really an incredibly viscous liquid that is estimated to have more than 100 billion times the viscosity of water (10). The world's longest continuously running laboratory experiment is The University of Queensland's "Pitch Drop Experiment" (10). Begun in 1927–1930 by Thomas Parnell (it took 3 years just for the pitch to settle into a glass funnel), droplets of pitch fall under gravity only about once a decade, with the next one expected this year. We live in exciting times!

A target identified

Although the terms are somewhat ambiguous and interchangeable, pitch tends to refer to the more solid substances in this group, and tar generally refers to the more liquefied products. Coal tar is an extremely viscous liquid obtained from dry-heating coal to temperatures in the range of 900°C to 1200°C, and is thought to consist of at least 10,000 distinct high molecular weight hydrocarbon and aromatic compounds,

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