Caddyshack therapeutics: overcoming glioblastoma adaptation

David H. Gutmann

Department of Neurology, Washington University School of Medicine, St Louis, Missouri

Corresponding Author: David H. Gutmann, MD, PhD, Department of Neurology, Washington University School of Medicine, Box 8111, 660 South Euclid Avenue, St. Louis MO 63110 (gutmann@wustl.edu).

See the article by McNeill et al on pages 1469–1480.

Under normal physiologic conditions, cell growth is tightly regulated, thus allowing cells to respond appropriately to local environmental cues. These cellular responses typically involve transmembrane receptors (eg, receptor tyrosine kinases [RTKs]) that operate through small-molecule effectors to regulate cell proliferation, differentiation, death, and migration. Following receptor-mediated effector activation, inhibitory circuits are initiated that dampen or limit signal duration, leading to termination of mitogenic drive. In genetically transformed cells, this orderly series of events can be disrupted by disabling these normally-activated inhibitory feedback systems. The mechanisms underlying these disruptions may result from mutational gains or losses that converge on mitogenic pathways or from transcriptional or metabolic changes that affect the milieu in which these pathways function. As such, the context in which a signaling pathway operates in normal tissues may be markedly different in cancer cells, highlighting one reason why rationally chosen targeted therapies may underperform in human clinical trials.

In glioblastoma (GBM), the RTK/phosphoinositide-3-kinase (PI3K)/mitogen-activated protein kinase (MAPK) axis is commonly hyperactivated, supporting the therapeutic use of kinase inhibitors that block RTK, PI3K, or MAPK signaling. Unfortunately, single-agent kinase inhibitors have yielded disappointing outcomes in clinical trials, often due to the emergence of drug resistance. With the development of escape mechanisms, treatment-insensitive clones develop, resulting in a never-ending game of “whack-a-mole.” McNeill and colleagues describe one adaptive strategy employed by GBM cells to evade long-term growth suppression from monotherapy kinase inhibition—via activation of other kinases that circumvent dependence on the targeted mitogenic pathway, and thus maintain cancer cell growth.¹ For these studies, they employed a combination of simian virus 40 large T-antigen immortalized astrocytes transformed with oncogenic Kras and phosphatase and tensin homolog loss and patient-derived GBM xenografts to examine the impact of drug potency on target inhibition, alternate pathway activation,

and efficacy. By targeting both the PI3K and MAPK pathways together, McNeill and colleagues observed synergistic effects in vitro and in vivo, leading to GBM cell death, an effect not observed with single therapies alone. Based on these findings, the researchers suggest that monitoring genome-encoded protein kinase (kinome) dynamics may be a valuable tool to identify cellular adaptations amenable to combination therapies.

This study is instructive in several respects. First, it extends findings made in other cancers to glioblastoma, supporting the notion that cancer adaptations represent Lamarckian changes necessary for the survival of neoplastic cells in the face of chemotherapy. Second, it underscores the fundamental differences between constitutive mitogenic pathway signaling that results from mutational activation versus RTK pathway activation as a consequence of growth factor binding to its cognate receptor. While the latter proceeds in an orderly fashion with tightly controlled kinetics, the former subjects the cell to a constant flood of mitogenic signaling. The cellular response to a tonic level of pathway hyperactivity is to establish compensatory circuits, as well as to shift the dependence from a single mitogenic effector system (eg, mitogen-activated protein kinase kinase [MEK]) to other effector pathways (eg, Akt). This response limits addiction to one single overactive growth control pathway, enabling cells to survive in the face of nonphysiological signaling overdrive. However, the diminished functioning of proteins that normally limit the amplitude and duration of growth factor signaling, coupled with aberrant activation of parallel pathways, create multiple escape routes and paradoxical responses to targeted inhibition of kinase molecules.

Third, and perhaps most important, the concept that the kinome can evolve to maintain cellular fitness further expands our understanding of the complex mechanisms that cancer cells leverage to rewire their intracellular machinery.^{2,3} In this regard, the result of effector molecule activation that follows growth factor binding to a single RTK can be profoundly altered in the presence of coexisting cellular or molecular changes (Fig. 1). We

Fig. 1 Tumor adaptation. (Left panel) In normal cells, the binding of an extracellular growth factor (GF) to its cognate RTK initiates a cascade of activated intermediates (eg, Ras, Raf, MEK), which culminates in cell cycle progression and the orderly enactment of specific transcriptional programs. The signal is quickly dampened by negative feedback loops (eg, Sprouty). Additionally, there are growth factors in the local microenvironment that cannot transmit their growth-promoting signals due to lack of appropriate growth factor receptor expression. (Right panel) In the context of neoplasia, the playing field is markedly different as a result of aberrant expression of receptors that can now bind to available growth factors and activate other signaling pathways (eg, PI3K, Akt), which interact (either positively or negatively) with activated RTK circuits to create inhibitory feedback and positive feed-forward loops. The negative feedback loops in cancer cells are sometimes attenuated by reduced expression of Sprouty (or other inhibitory proteins). Additionally, mutational activation of RTKs or proto-oncogenes ("mut"), as well as loss of tumor suppressor gene (TSG) expression ("loss"), can converge on the same pathways or lead to increased activity of other growth control pathways. Epigenetic reprogramming at the level of chromatin or transcriptional regulation can also lead to deregulated expression of both positive and negative regulators of mitogenic signaling pathways, while changes in mitochondrial function (endoplasmic reticulum stress responses) and cell metabolism could alter the intracellular milieu in which these signaling effectors operate. Lastly, the local microenvironment in cancer is different from that found in normal tissues, with the elaboration of growth factors from stromal elements (eg, cancer-associated immune system cells). These stromal factors influence the activity of signaling effectors and change intracellular pathway wiring in unpredictable ways, resulting in adaptive responses to molecularly targeted treatments and culminating in tumor escape.

envision that the simple circuitry, as revealed using in vitro systems in response to defined ligands, may not translate well to tumor cells when analyzed in the context of alterations in the extracellular milieu, subcellular structures (eg, mitochondria), transcriptional programs, or deregulated activity of other intracellular effector molecules. As such, the impact of activation of a particular kinase, and the consequence of its inhibition, are heavily influenced by these conditions (contextual signaling).⁴ In cancer cells, changes in mitochondrial dynamics,⁵ cell metabolism,⁶ and epigenetic programming (transcriptional outputs)⁷ each create new cellular contexts in which signaling pathways operate. Similarly, mutational activation of proto-oncogenes, or inactivation of tumor suppressor genes, converges on many of the same signaling pathways to amplify or attenuate their responses.⁸ In addition, aberrant expression or mutational activation of RTKs can influence shared signaling intermediate function, but could also lead to the activation or suppression of other

signaling pathways that interact to influence responses to kinase inhibitors: aberrant RTK or growth factor expression enables cancer cells to establish new mitogenic circuits not found in nonneoplastic cells, thus permitting escape routes to avoid targeted inhibition.⁹ Finally, adaptive mechanisms may develop as a consequence of productive interactions between cancer cells and non-neoplastic (stromal) cells in the tumor microenvironment.¹⁰ Not only are these stromal cells dynamic in their capacity to change in response to alterations in the local environment, but kinase inhibitors selected for their anticancer cell effects could have opposite effects on stromal cells, leading to paradoxical increases in cancer cell growth, migration, or survival.

Taken together, it becomes increasingly important to appreciate the ever-changing microcosm that is the tumor, and to employ therapeutic strategies that target the tumor ecosystem using novel dosing schedules and multiple convergence points (nodes). In this regard, we need to consider

the temporal order of treatments that target different core cancer-sustaining pathways (nodal disruptors) so that we might best squelch the development of escape mechanisms. Additionally, inhibitors that work on different components of this ecosystem (stromal cells, transcriptional programs, mitogenic signaling intermediates) will be required to maximally induce tumor cell death, and, at the same time, suppress the emergence of resistant clones. As we deepen our understanding of the determinants that promote the maintenance and progression of the cancer ecosystem, improved nodal disruptors will undoubtedly be identified, which when used in combination with molecularly targeted or conventional therapies, may afford the greatest likelihood of achieving optimal outcomes for deadly brain tumors.

Funding

D.H.G. is supported by a Research Program Award from the National Institute of Neurological Disorders and Stroke (1-R35-NS097211-01).

Acknowledgment

The text is the sole product of the author and no third party had input or gave support to its writing.

Conflict of interest statement. D.H.G. reports no competing disclosures or conflicts of interest.

References

1. McNeill RS, Canoutas DA, Stuhlmiller TJ, et al. Combination therapy with potent PI3K and MAPK inhibitors overcomes adaptive kinome resistance to single agents in preclinical models of glioblastoma. *Neuro Oncol.* 2017;19(11):1469–1480.
2. Johnson GL, Stuhlmiller TJ, Angus SP, Zawistowski JS, Graves LM. Molecular pathways: adaptive kinome reprogramming in response to targeted inhibition of the BRAF-MEK-ERK pathway in cancer. *Clin Cancer Res.* 2014;20(10):2516–2522.
3. Bertacchini J, Guida M, Accordi B, et al. Feedbacks and adaptive capabilities of the PI3K/Akt/mTOR axis in acute myeloid leukemia revealed by pathway selective inhibition and phosphoproteome analysis. *Leukemia.* 2014;28(11):2197–2205.
4. Smithson LJ, Anastasaki C, Chen R, Toonen JA, Williams SB, Gutmann DH. Contextual signaling in cancer. *Semin Cell Dev Biol.* 2016;58:118–126.
5. Ma XH, Piao SF, Dey S, et al. Targeting ER stress-induced autophagy overcomes BRAF inhibitor resistance in melanoma. *J Clin Invest.* 2014;124(3):1406–1417.
6. Haq R, Shoag J, Andreu-Perez P, et al. Oncogenic BRAF regulates oxidative metabolism via PGC1alpha and MTF. *Cancer Cell.* 2013;23(3):302–315.
7. Matkar S, Sharma P, Gao S, et al. An epigenetic pathway regulates sensitivity of breast cancer cells to HER2 inhibition via FOXO/c-Myc axis. *Cancer Cell.* 2015;28(4):472–485.
8. Nissan MH, Pratilas CA, Jones AM, et al. Loss of NF1 in cutaneous melanoma is associated with RAS activation and MEK dependence. *Cancer Res.* 2014;74(8):2340–2350.
9. Kentsis A, Reed C, Rice KL, et al. Autocrine activation of the MET receptor tyrosine kinase in acute myeloid leukemia. *Nat Med.* 2012;18(7):1118–1122.
10. Chung AS, Wu X, Zhuang G, et al. An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. *Nat Med.* 2013;19(9):1114–1123.