Published in final edited form as:

Wiley Interdiscip Rev Syst Biol Med. 2018 January ; 10(1): . doi:10.1002/wsbm.1398.

Targeting EGFR Co-dependent Signaling Pathways in Glioblastoma

Feng Liu and

National Research Center for Translational Medicine (Shanghai), State Key, Laboratory of Medical Genomics, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, 200025, P.R. China

Paul S. Mischel

Ludwig Institute for Cancer Research, Department of Pathology, Moores Cancer Center, University of California San Diego School of Medicine, La Jolla, California 92093, U.S.A

Abstract

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase (RTK) that is critical for normal development and function. EGFR is also amplified or mutated in a variety of cancers including in nearly 60% of cases of the highly lethal brain cancer glioblastoma (GBM). EGFR amplification and mutation reprogram cellular metabolism and broadly alter gene transcription to drive tumor formation and progression, rendering EGFR as a compelling drug target. To date, brain tumor patients have yet to benefit from anti-EGFR therapy due in part to an inability to achieve sufficient intratumoral drug levels in the brain, cultivating adaptive mechanisms of resistance. Here, we review an alternative set of strategies for targeting EGFR amplified GBMs, based on identifying and targeting tumor co-dependencies shaped both by aberrant EGFR signaling and the brain's unique biochemical environment. These approaches may include highly brain-penetrant drugs from non-cancer pipelines, expanding the pharmacopeia and providing promising new treatments. We review the molecular underpinnings of EGFR-activated co-dependencies in the brain and the promising new treatments based on this strategy.

Graphical Abstract

Conflict of interest

The authors declare no conflict of interest to this work.

Keywords

Epidermal growth factor receptor; EGFR; tyrosine kinase inhibitor; TKI; signal transduction; glioblastoma

Introduction

Glioblastoma (GBM) is the most common malignant tumor arising in the brain and is one of the most lethal of all cancers¹. Over the past 50 years, the standard of care for GBM—surgery, aggressive chemotherapy and radiation therapy—remains largely unchanged and the prognosis of GBM patients remains poor with a median survival of 12–15 months and a 5-year survival rate at less than 10%^{1,2}. The relative lack of therapeutic progress for GBM patients stands in stark contrast to the fact that GBM is now one of the most deeply characterized forms of cancer with clear and compelling molecular drug targets. Indeed, GBM was the first cancer profiled by the Cancer Genome Atlas (TCGA) project, and in depth genomic, transcriptomic, and epigenomic analyses of hundreds of samples have yielded a deep and comprehensive characterization of the somatic mutation landscape of GBM^{1,3}, potentially realizing the goal of using molecular characterization to guide precision therapy for individual patients⁴.

The epidermal growth factor receptor (*EGFR*) has emerged as a compelling GBM drug target⁵. *EGFR* alterations including focal amplification, point mutations, deletions and rearrangements, occur in approximately 60% of primary GBM³ and multiple monoclonal antibodies and small molecule inhibitors are available to specifically inhibit EGFR and its variants², promising to transform the care of GBM patients. However, these hopes have not been realized, as a relatively limited number of clinical trials have failed to demonstrate any benefit to patients⁶. How does one make sense of the disconnect between a highly recurrent genetic alteration in a cancer that shows dependence on that oncogene and the total lack of clinical benefit shown to date? Further, how does one use this information to develop more effective treatments? Here, we provide our perspective on these issues and discuss recently emerged new angles to tackle hyperactive EGFR-driven GBM.

The mutational landscape of EGFR in cancer

EGFR is one of four members of the human epidermal growth factor receptor (HER) family (the others are *HER1/ErbB2/neu*, *HER3/ErbB3*, *HER4/ErbB4*)⁷. It is also a founding member of the receptor tyrosine kinase (RTK) family, each having dual functions encoded in two distinct domains at opposite sides of the cell membrane—an extracellular domain serving as binding site for specific ligand (EGF and TGF- α), and an intracellular domain containing ligand-dependent tyrosine kinase activities⁸. Once bound by a ligand, adjacent EGFRs dimerize and activate each other's tyrosine kinase activities. This results in EGFR autophosphorylation in several tyrosine (Y) residues, including Y992, Y1045, Y1068, Y1148 and Y1173⁹. Additionally, activated EGFR phosphorylates other proteins through the latter's phosphotyrosine-binding SH2 domains and, in doing so, triggers three major intracellular signaling pathways: 1) the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway, 2) the RAS/RAF/MAPK pathway, and 3) the janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway. These intracellular signaling pathways eventually relay the signal to the nucleus, where transcription factors are mobilized—often by post-translational mechanisms such as protein phosphorylation—to regulate the transcriptional landscape of the genome¹⁰ (Figure 1).

Consistent with a role of signaling activator, oncogenic mutations of *EGFR* found in cancers are primarily amplification and/or gain-of-mutations (Figure 2a)³. Regional DNA amplification typically includes the entire *EGFR* locus (located in the 7p1214 region of chromosome 7) that encompasses 28 exons spanning 193 kb (Figure 2b). This leads to abnormally high expression of EGFR and ensuing elevated downstream pathway activity³. In addition, hyperactivation of EGFR can result from point mutations (including intragenic deletions) that relieves the receptor's dependency on extrinsic ligands. In GBM, one hotspot of intragenic alternations occurs in the exons encoding EGFR's extracellular (EC) domain, which produces truncated yet constitutively active EGFR (e.g. the EGFR vIII variant, or EGFRvIII) (Figure 2b)¹¹. Although amplified and mutated EGFRs may be sufficient to drive tumor growth, recent evidence suggests that they may also interact with other receptors to drive tumor growth¹². Furthermore, hyperactive EGFR can be produced by mutations in EGFR's intracellular tyrosine kinase domain (KD); although such mutations are more common in other cancers such as the non-small cell lung cancers (NSCLC), they are rare in GBM^{3,13}.

Challenges in directly targeting EGFR in GBM

The oncogenic function of EGFR in GBM was supported by preclinical studies using both animal models and patient derived cancer cell lines. For example, the constitutively active EGFRvIII variant was required for the growth of malignant tumors mimicking the pathogenesis of GBM in mice carrying *ink4a* null mutation¹⁴ (null mutation of *CCND4A*, the human homolog of *ink4a/Arf*, is the most common somatic alternations in GBM, which encodes an inhibitor of the cell cycle³). Likewise, EGFRvIII was essential for aggressive proliferation of patient derived GBM cell lines when the latter were cultured in vitro and transplanted in mice¹⁵. On the other hand, acute loss of EGFRvIII using genetic means (i.e. using a tetracycline-induced turn-off promoter) or small molecule inhibitors can cause

significant cell death of GBM cell lines carrying *EGFR* mutation, suggesting that these cells depend on hyperactive EGFR signaling for survival¹⁵. Together, these studies provided a compelling case for targeting EGFR and its mutant variants for the treatment of GBM carrying such mutations.

So far a dozen drugs have been developed to target EGFR in tumors that largely fall into two classes: monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs)². But despite of success of some anti-EGFR therapy in other cancers (e.g. a TKI named gefitinib has been approved for the treatment of NSCLC with EGFR alteration¹³), none of these anti-EGFR therapeutics successfully passed the phase III clinical trial of GBM¹⁶. The lack of efficacy of these drugs on GBM is complex, which includes both technical challenges of delivering drugs into the brain and other intrinsic resistance mechanisms, as discussed below.

Suboptimal dosing

Needlessly to say, no drug can produce a clinical benefit unless it reaches its target site above the level required to effectively suppresses its intended molecular target. This is unfortunately a major obstacle in treating GBM as they are semi-insulated from the circulatory system by the blood brain barrier. Drugs of large molecular weight, such as anti-EGFR mAbs, are largely blocked from reaching GBM. Additionally, while usually manifested as diffusive mass, GBM is solid tumor and is recalcitrant for small molecule drugs to penetrate into the inner tumor cells^{1,5}. These features of GBM present an unusually high bar to effectively applying drugs to GBM patients. Even drugs shown to penetrate the brain in pre-clinical studies frequently don't reach sufficient intratumoral levels to kill tumor cells, in part because poor brain-plasma ratios result in far lower maximal tolerated dose that is needed for full target engagement in the brain¹⁵. Indeed, the failure of several phase I/II clinical trials using small molecule EGFR TKIs was due to suboptimal dosing in the brain with roughly 15% target inhibition observed in a "window of opportunity" study of GBM patients treated with lapatinib¹⁵. Moreover, loss of PTEN, a phosphatase that opposes EGFR signaling through PI3K-AKT pathway, in GBM limits the ability of EGFR tyrosine inhibitors to suppress downstream signaling, further contributing to therapeutic resistance^{17,18}.

Bypass pathway activation and RTK switching

As a consequence of incomplete target engagement in the tumor, drug tolerant GBM cells are able to engage other signaling pathways to activate the same downstream signaling effectors. For example, EGFR inhibition by erlotinib in GBM resulted in potent activation of PDGFR β , which can drive tumor growth by triggering the same ensemble of protein kinase cascades (i.e. PI3K/AKT, RAS/MAPK, and JAK/STAT pathways)¹⁹. Release of feedback inhibition may be a wide-spread phenomenon, as EGFR inhibition can also lead to upregulation of the MET kinase, or release of cytokines such as TGF β and IL6²⁰. As a result of these homeostatic mechanisms, combinatorial therapies are required to suppress bypass pathway signaling and drug resistance⁵.

Dynamic exchange of intra- and extra-chromosomal DNA

A more recently realized challenge in targeting EGFR stems from the surprising discovery that resistance to EGFR TKIs in GBM can be mediated by reversible loss of extrachromosomal DNA (ecDNA)²¹ (Figure 3). It has long been observed that extra-chromosomal DNA entities, known as double-minutes (DM), exist within cancer cells²². Recently, we showed that frequently nearly all amplified and/or mutated *EGFR* is present on ecDNA and, in response to EGFR TKI treatment, loss of EGFRvIII from ecDNA renders GBM cells insensitive to TKIs, promoting drug resistance. Remarkably, upon drug withdrawal, EGFRvIII levels rapidly rise as the mutation reappears on ecDNA to drive rapid tumor growth²¹. The reemerged ecDNA at the end of erlotinib treatment eventually repopulate the tumor mass with cells expressing high levels of EGFRvIII²¹.

Our recent study also showed that the frequency and scope of ecDNA in cancer is far greater than initially thought²³. Previous studies had suggested that ecDNA was a rare event in cancer, occurring in 1.4% of cancers according to the Mitelman database of chromosomal abnormalities²⁴. Through integrated genomic, cytogenetic and bioinformatics analyses, we demonstrated that nearly half of human cancers contain ecDNA and that amplified oncogenes are found either entirely on ecDNA, or on ecDNA and abnormal regions of the chromosome that may be derived from ecDNAs²³. Further, because of random segregation of oncogenes amplified on ecDNA relative to chromosomal amplification, tumors develop far higher copy number variation of oncogenes and much greater intratumoral genetic heterogeneity, contributing to accelerated cancer evolution and potentially therapeutic resistance²³. These surprising findings challenge our understanding of the “Map” of cancer genome abnormalities. In particular, current attempts to localize amplified oncogenes in cancer rely on using short reads from next generation sequencing (NGS) technologies and mapping them back to presumed locations based on the normal human reference genome. This widely used bioinformatic analysis strategy assumes that genes are all in the tumor chromatin. Our recent findings, however, suggest that not only oncogene copy number, but also the subnuclear localization of oncogene DNA²³ may potentially contribute to tumor development and progression, as well as drug resistance (Figure 3)²¹.

Targeting EGFR-associated co-dependency pathways essential for GBM survival

From the above discussion, one conclusion is inescapable: while EGFR is a compelling target in GBM, the current suite of anti-EGFR therapeutics is not likely to be effective, at least in the way they are currently dosed and delivered. Therefore, our recent studies have focused on trying to understand the biological consequences of EGFR signaling with the goal of identifying potentially exploitable therapeutic vulnerabilities, particularly the ones that are shaped by the unique biochemical and structural environment of the brain. This idea, sometimes called non-oncogene addiction²⁵ or non-oncogene co-dependency²⁶, has begun to show promise as a new approach for developing more effective treatments for GBM.

EGFR mutation-dependent metabolic reprogramming

One of the primary functions of growth factor receptors is to enable cells to take up and utilize nutrients, including for anabolic metabolism, from the environment. In 1924, the German physiologist Otto Warburg noticed that cancer cells undergo anaerobic glycolysis even in the presence of ample oxygen²⁷. Subsequent studies have shown that this biochemical adaptation, known as “Warburg effect”, is tightly linked towards shuttling glucose derived carbons towards anabolic metabolism²⁸, although amino acids (e.g. glutamine) and lipids are also actively taken up by tumor cells to drive rapid growth²⁸. In GBM, hyperactivated EGFR signaling elevates expression of c-MYC, a central regulator of the Warburg effect via both the PI3K-AKT-mTORC1 pathway, as well as an AKT-independent activity of the mTORC2 complex²⁹. Thus, even in the case of anti-EGFR inhibition being intercepted by the null mutation of *PTEN*, anaerobic glycolysis can be suppressed by mTOR inhibitors²⁹ (Figure 4a).

EGFR-induced transcription factor network and epigenome remodeling

A further downstream effect of hyperactive EGFR signaling is the change of gene regulatory activities in the nucleus. Recently, the explosive growth of the fields of cancer genomics and epigenomes have spurred the development of many small molecule agents for the interrogation of the biochemical function of chromatin modifiers and remodelers, some of which have exhibited appealing properties in killing tumors driven by elevated transcriptional activities³⁰. Through integrated epigenome and transcriptome analyses, we showed that EGFR mutations remodel the activated enhancer landscape of GBM, promoting tumorigenesis through a *SOX9* and *FOXG1*-dependent transcriptional regulatory network³¹ (Figure 4b). Critically, we found that these two transcription factors converge on a c-Myc-dependent, BET-bromodomain inhibitor JQ1-sensitive gene expression program³¹. JQ1 can cause regression of cancers whose survival rely on active transcription of c-MYC, such as AML, T-ALL, mixed lineage leukemia (MLL), diffusive large B cell lymphoma, medulloblastoma, and KRAS-mutant non-small cell lung cancer³². In GBM, EGFRvIII-expression promoted sensitivity to JQ1-induced apoptosis³¹, suggesting that EGFR mutated GBMs are “addicted” to dysregulated transcriptional program that can be pharmacologically targeted³².

EGFR mutation-dependent reliance on cholesterol uptake in the brain

Glucose is a fundamental “ingredient” for cell growth, hence the rationale for dietary treatment of cancer based on the ketogenic diet³³. Rigorous studies are now underway to test this hypothesis³⁴. However, glucose is not the only cell resource that matters. In fact, targeting other metabolic pathways, including lipid and cholesterol metabolism, provides promising new directions for therapy. Importantly, one of the most salient feature of the brain’s biochemical environment is the lipid milieu, raising the possibility of finding an organ-site dependent co-dependency for therapeutic exploitation²⁵.

Cholesterol does not readily cross the blood-brain barrier, yet 20% of the body’s cholesterol pool resides in the brain³⁵ and is synthesized largely de novo by astrocytes³⁶. Interestingly, to fuel rapid growth, GBM cells upregulate the low-density lipoprotein receptor (LDLR) to enhance cholesterol while concurrently suppressing de novo synthesis of cholesterol and

oxysterols (Figure 4c). De novo synthesis of cholesterol requires 26 units of NADPH, a key resource for tumor cells that is required for a wide range of biosynthetic reactions and for buffering ROS³⁷. Thus, cholesterol uptake rather than synthesis is a highly adaptive strategy for GBM cells. Further, because GBM cells do not engage the cholesterol synthesis pathway, they are not subject to the usual feedback mechanisms that converge on HMG-coA reductase^{38,39}. In normal cells, cholesterol is processed into oxysterols, endogenous ligands of the Liver X receptors (LXR), which are also transcription factors that drive expression of a wide array of target genes, including the E3 ubiquitin ligase IDOL and the ATP-binding cassette (ABC) transporter ABCA1. IDOL degrades LDLR to limit cholesterol uptake and ABCA1 pumps cholesterol out of the cell, thus keeping cholesterol levels in homeostatic balance^{40–43}. Our study indicates that GBM cells do not process cholesterol into oxysterols, and are thus freed from this negative feedback regulation^{44,45}. This seemingly parasitic behavior of GBM cells coupled to disabling of negative feedback systems makes them well adapted to grow in the brain, but it also renders them especially and selectively vulnerable to exogenous LXR agonists, some of which are clinically available and are highly brain penetrant with acceptable brain plasma ratios⁴⁶.

Conclusion

As one of the archetypical oncogenes, EGFR has served as a paradigm for the study of cancer biology and anti-cancer drug development for more than half a century⁴⁷. Yet, up to recently, many hidden aspects of its role in regulating tumor development are still beginning to come to light, with the underlying mechanisms waiting for further investigation. For example, little is known about the factors involved in the formation, disappearance, and reappearance of extra-chromosomal EGFR DNA before, during, and after anti-EGFR therapy²¹. Despite of tremendous progress in the field of human genomics, studies are still ongoing to reach a coherent model to predictably interfere with cancer-specific gene expression programs.

One sobering lesson of studying cancers is the realization that cancer cells are relentless in acquiring the ability to develop drug resistance mechanisms over the course of treatment. Indeed, the multiple lines of investigation to exploit EGFR mutation co-dependent pathways were necessitated by the multitude of means of GBM cells to escape the reliance on EGFR signaling for survival. While these directions have already led to the discovery of novel therapeutics that are better at penetrating the blood-brain barrier, potentially with more potent therapeutic efficacy, it is notable that further studies are required to evaluate the long-term effect of these drugs on GBM in vivo

Nevertheless, one may remain cautiously optimistic that studying these open questions might lead to improvement of anti-EGFR therapy in GBM. Past studies on EGFR and related RTKs have yielded a large intracellular molecular interaction network underlying the inner working of cells at both normal physiological conditions and in cancer. In the future, powerful computational algorithms are required to use these data to faithfully model the dynamic features of these signaling networks⁴⁸. Such study will provide deeper insights into the relationship between tumor cell's behavior and EGFR activation, a prerequisite to evaluating how EGFR inhibition can be exploited to influence a tumor's reliance on EGFR

signaling. Additionally, recent years have seen the rise of pharmacogenomics that aims to systematically identify the relationship between a cell's genotype and its sensitivity to various drugs^{49,50}. By comparing a cell line's genome sequence and its sensitivity to various drugs, this area of research has the potential to comprehensively define the genetic makeup of the cell line that determine its response to each drug⁵⁰. Thus, pharmacogenomics may provide the long-desired knowledge to stratify patients according to their likelihood to targeted cancer therapy, such as the use of EGFR TKIs⁵¹.

Finally, the mechanistic link between EGFR mutation and the epigenome provides a means to intercept the resistance mechanism acting upon the EGFR receptor in the cell membrane or its effectors in the cytoplasm. This raises the interesting possibility that EGFR amplification and/or hyperactivating mutations can be used as biomarkers to stratify patient groups that will most likely benefit from epigenetic therapy using compounds like JQ1³¹. Moreover, it suggests that epigenetic therapy can be used to treat relapsed tumors after the first-line anti-EGFR therapy. As the search for novel epigenetic therapeutics continues, it is hopeful that more options will be available in the future to counter attack anti-EGFR resistant tumors.

Acknowledgments

This work is supported by Shanghai Pujiang Program (17PJ1405800) and Shanghai Jiao Tong University School of Medicine Gao Feng Gao Yuan Program (F.L.), and by grant from the National Institute for Neurological Diseases and Stroke (NS73831), the Defeat GBM Program of the National Brain Tumor Society, the Ben and Catherine Ivy Foundation, and generous donations from the Ziering Family Foundation in memory of Sigi Ziering (P.S.M.).

References

1. Cloughesy TF, Cavenee WK, Mischel PS. Glioblastoma: from molecular pathology to targeted treatment. *Annu Rev Pathol*. 2014; 9:1–25. [PubMed: 23937436]
2. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med*. 2008; 358:1160–74. [PubMed: 18337605]
3. Brennan CW, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013; 155:462–77. [PubMed: 24120142]
4. Chin L, Gray JW. Translating insights from the cancer genome into clinical practice. *Nature*. 2008; 452:553–63. [PubMed: 18385729]
5. Furnari FB, Cloughesy TF, Cavenee WK, Mischel PS. Heterogeneity of epidermal growth factor receptor signalling networks in glioblastoma. *Nat Rev Cancer*. 2015; 15:302–10. [PubMed: 25855404]
6. Padfield E, Ellis HP, Kurian KM. Current Therapeutic Advances Targeting EGFR and EGFRvIII in Glioblastoma. *Front Oncol*. 2015; 5:5. [PubMed: 25688333]
7. Cohen S, Ushiro H, Stoscheck C, Chinkers M. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. *J Biol Chem*. 1982; 257:1523–31. [PubMed: 6276390]
8. Erneux C, Cohen S, Garbers DL. The kinetics of tyrosine phosphorylation by the purified epidermal growth factor receptor kinase of A-431 cells. *J Biol Chem*. 1983; 258:4137–42. [PubMed: 6101263]
9. Downward J, Parker P, Waterfield MD. Autophosphorylation sites on the epidermal growth factor receptor. *Nature*. 1984; 311:483–5. [PubMed: 6090945]
10. Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol Syst Biol*. 2005; 1 2005 0010.

11. Huang HS, et al. The enhanced tumorigenic activity of a mutant epidermal growth factor receptor common in human cancers is mediated by threshold levels of constitutive tyrosine phosphorylation and unattenuated signaling. *J Biol Chem.* 1997; 272:2927–35. [PubMed: 9006938]
12. Jahani-Asl A, et al. Control of glioblastoma tumorigenesis by feed-forward cytokine signaling. *Nat Neurosci.* 2016; 19:798–806. [PubMed: 27110918]
13. Paez JG, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science.* 2004; 304:1497–500. [PubMed: 15118125]
14. Bachoo RM, et al. Epidermal growth factor receptor and Ink4a/Arf: convergent mechanisms governing terminal differentiation and transformation along the neural stem cell to astrocyte axis. *Cancer Cell.* 2002; 1:269–77. [PubMed: 12086863]
15. Vivanco I, et al. Differential sensitivity of glioma- versus lung cancer-specific EGFR mutations to EGFR kinase inhibitors. *Cancer Discov.* 2012; 2:458–71. [PubMed: 22588883]
16. Taylor TE, Furnari FB, Cavenee WK. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. *Curr Cancer Drug Targets.* 2012; 12:197–209. [PubMed: 22268382]
17. Mellinghoff IK, Cloughesy TF, Mischel PS. PTEN-mediated resistance to epidermal growth factor receptor kinase inhibitors. *Clin Cancer Res.* 2007; 13:378–81. [PubMed: 17255257]
18. Mellinghoff IK, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med.* 2005; 353:2012–24. [PubMed: 16282176]
19. Akhavan D, et al. De-repression of PDGFR β transcription promotes acquired resistance to EGFR tyrosine kinase inhibitors in glioblastoma patients. *Cancer Discov.* 2013; 3:534–47. [PubMed: 23533263]
20. Logue JS, Morrison DK. Complexity in the signaling network: insights from the use of targeted inhibitors in cancer therapy. *Genes Dev.* 2012; 26:641–50. [PubMed: 22474259]
21. Nathanson DA, et al. Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. *Science.* 2014; 343:72–6. [PubMed: 24310612]
22. Vogelstein B, et al. Cancer genome landscapes. *Science.* 2013; 339:1546–58. [PubMed: 23539594]
23. Turner KM, et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. *Nature.* 2017
24. Fan Y, et al. Frequency of double minute chromosomes and combined cytogenetic abnormalities and their characteristics. *J Appl Genet.* 2011; 52:53–9. [PubMed: 21107781]
25. Galluzzi L, Kepp O, Vander Heiden MG, Kroemer G. Metabolic targets for cancer therapy. *Nat Rev Drug Discov.* 2013; 12:829–46. [PubMed: 24113830]
26. Raj L, et al. Selective killing of cancer cells by a small molecule targeting the stress response to ROS. *Nature.* 2011; 475:231–4. [PubMed: 21753854]
27. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009; 324:1029–33. [PubMed: 19460998]
28. Pavlova NN, Thompson CB. The Emerging Hallmarks of Cancer Metabolism. *Cell Metab.* 2016; 23:27–47. [PubMed: 26771115]
29. Masui K, et al. mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO acetylation and upregulation of c-Myc. *Cell Metab.* 2013; 18:726–39. [PubMed: 24140020]
30. Shortt J, Ott CJ, Johnstone RW, Bradner JE. A chemical probe toolbox for dissecting the cancer epigenome. *Nat Rev Cancer.* 2017; 17:268.
31. Liu F, et al. EGFR Mutation Promotes Glioblastoma through Epigenome and Transcription Factor Network Remodeling. *Mol Cell.* 2015; 60:307–18. [PubMed: 26455392]
32. Bradner JE, Hnisz D, Young RA. Transcriptional Addiction in Cancer. *Cell.* 2017; 168:629–643. [PubMed: 28187285]
33. Woolf EC, Syed N, Scheck AC. Tumor Metabolism, the Ketogenic Diet and beta-Hydroxybutyrate: Novel Approaches to Adjuvant Brain Tumor Therapy. *Front Mol Neurosci.* 2016; 9:122. [PubMed: 27899882]
34. Erickson N, Boscheri A, Linke B, Huebner J. Systematic review: isocaloric ketogenic dietary regimes for cancer patients. *Med Oncol.* 2017; 34:72. [PubMed: 28353094]
35. Dietschy JM. Central nervous system: cholesterol turnover, brain development and neurodegeneration. *Biol Chem.* 2009; 390:287–93. [PubMed: 19166320]

36. Bjorkhem I, Meaney S. Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol.* 2004; 24:806–15. [PubMed: 14764421]
37. Vander Heiden MG, et al. Metabolic pathway alterations that support cell proliferation. *Cold Spring Harb Symp Quant Biol.* 2011; 76:325–34. [PubMed: 22262476]
38. Bjorkhem I. Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain. *J Intern Med.* 2006; 260:493–508. [PubMed: 17116000]
39. Brown MS, Goldstein JL. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J Lipid Res.* 1980; 21:505–17. [PubMed: 6995544]
40. Chen J, Zhang X, Kusumo H, Costa LG, Guizzetti M. Cholesterol efflux is differentially regulated in neurons and astrocytes: implications for brain cholesterol homeostasis. *Biochim Biophys Acta.* 2013; 1831:263–75. [PubMed: 23010475]
41. Repa JJ, et al. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science.* 2000; 289:1524–9. [PubMed: 10968783]
42. Venkateswaran A, et al. Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXR alpha. *Proc Natl Acad Sci U S A.* 2000; 97:12097–102. [PubMed: 11035776]
43. Zelcer N, Hong C, Boyadjian R, Tontonoz P. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. *Science.* 2009; 325:100–4. [PubMed: 19520913]
44. Guo D, et al. The AMPK agonist AICAR inhibits the growth of EGFRvIII-expressing glioblastomas by inhibiting lipogenesis. *Proc Natl Acad Sci U S A.* 2009; 106:12932–7. [PubMed: 19625624]
45. Guo D, et al. EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. *Sci Signal.* 2009; 2:ra82. [PubMed: 20009104]
46. Villa GR, et al. An LXR-Cholesterol Axis Creates a Metabolic Co-Dependency for Brain Cancers. *Cancer Cell.* 2016; 30:683–693. [PubMed: 27746144]
47. Cohen S. Origins of growth factors: NGF and EGF. *J Biol Chem.* 2008; 283:33793–7. [PubMed: 18697735]
48. Liang W, et al. Network meta-analysis of erlotinib, gefitinib, afatinib and icotinib in patients with advanced non-small-cell lung cancer harboring EGFR mutations. *PLoS One.* 2014; 9:e85245. [PubMed: 24533047]
49. Lucas AT, Madden AJ, Zamboni WC. Challenges in preclinical to clinical translation for anticancer carrier-mediated agents. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* 2016; 8:642–53. [PubMed: 26846457]
50. Iorio F, et al. A Landscape of Pharmacogenomic Interactions in Cancer. *Cell.* 2016; 166:740–54. [PubMed: 27397505]
51. Belizario JE, Sangiuliano BA, Perez-Sosa M, Neyra JM, Moreira DF. Using Pharmacogenomic Databases for Discovering Patient-Target Genes and Small Molecule Candidates to Cancer Therapy. *Front Pharmacol.* 2016; 7:312. [PubMed: 27746730]
52. Gao J, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal.* 2013; 6:pl1. [PubMed: 23550210]

Figure 1. Structure and function of the EGFR signaling pathway

The membrane-bound EGFR can activate three major branches of protein kinase pathway, leading to profound changes in both the cytoplasm and the nucleus to influence cellular phenotype at tissue and organismal levels.

Figure 2. Genetic alterations of EGFR in cancers

(a) The alteration frequency of EGFR across cancers surveyed by The Cancer Genome Atlas consortium (www.cbioportal.com)⁵². (b) In primary GBM, EGFR amplification mutation often occur in the same tumor.

Figure 3. Intracellular mechanisms of resistance against EGFR-targeted therapy

Anti-EGFR therapy can be compromised by feedback mechanisms that maintain intracellular RTK signaling activities, and by dynamic exchanges of between extra- and intra-chromosomal EGFR DNA, which replenishes EGFR expression levels in GBM cells.

Figure 4. Studying non-oncogene co-dependency provides novel drug targets

(a) Hyperactivated EGFR signaling activities converge on the elevation of c-MYC to promote anaerobic glycolysis (Warburg effect) in GBM cells. This effect can be suppressed by dual inhibition of PI3K and mTOR inhibitor(s). (b) Amplified and hyperactivated EGFR can also stimulate thousands of distal cis-regulatory elements called enhancers to promote a cancer-driving gene expression program. Small molecule compound such as JQ1 can intercept this epigenomic reprogramming activity by inactivating an enhancer-binding transcription cofactor BRD4. (c) In the brain, hyperactivated EGFR increases cholesterol uptake in GBM cells. LXR agonist like LXR-623 suppresses cholesterol uptake by activating an endogenous cholesterol homeostasis pathway involving IDOL, which degrades LDLR, and ABCA1, which transports cholesterol out of the cell.