CCR New Strategies

Clinical Cancer Research

New Strategies in the Molecular Targeting of Glioblastoma: How Do You Hit a Moving Target?

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Abstract

Cancer is a molecularly complex, genomically unstable disease. Selection for drug-resistant mutations, activation of feedback loops, and upregulation of cross-talk pathways provide escape routes by which cancer cells maintain signal flux through critical downstream effectors to promote therapeutic resistance. Attempts to target signal transduction pathways in cancer may therefore require investigators to aim at a moving target. We need to anticipate the routes of resistance to guide the selection of drugs that will lead to durable therapeutic response. In this *New Strategies* article, we discuss the challenges imposed by the complexity and adaptive capacity of cancer and suggest potential new diagnostic strategies to more effectively guide targeted cancer therapy. We focus on glioblastoma, the most common malignant primary brain tumor of adults. Glioblastoma is a model for a pathway-driven, molecularly heterogeneous cancer for which new genomic insights obtained through The Cancer Genome Atlas are ripe for integration with functional biology and incorporation into new molecular diagnostic assays. *Clin Cancer Res; 17(1); 6–11.* ©*2011 AACR.*

Background

The challenge of targeted therapy

In a 2006 perspective in *Nature*, Reuben Shaw and Lew Cantley suggested that "[a]s more drugs that target specific components of signal transduction pathways become available and as we increase our knowledge of the complexity of these signaling networks, the burden of selecting the right drug combinations for each individual cancer patient will ultimately shift to the pathologist who must identify the underlying defect in each tumor" (1). These words crisply embody the vision of personalized cancer medicine, and they also impose a formidable challenge. Finding the underlying defect becomes a significant challenge when genomic surveys identify a landscape of hundreds of mutated genes within each individual tumor.

Further, targeting the signal transduction pathways that a tumor needs to proliferate and survive is like trying to strike a moving target. Interconnectivity between oncogenic signaling pathways enables cancer cells to evade targeted therapy by maintaining signal flux to downstream effectors (2–4) through both genetic (5) and nongenetic (6) mechanisms. Selection of cancer cells bearing kinase inhibitor–resistant mutations can promote resistance to signal transduction inhibitors, as has been demonstrated for chronic myelogenous leukemia patients treated with imatinib, and non–small cell lung cancer patients treated with erlotinib or gefitinib (7–10). On top of this already robust repertoire of escape tools, radiation, and chemotherapy, the frontline treatments received by virtually all glioblastoma (GBM) patients greatly increase background mutation rates (11), providing ample opportunity for development and selection of therapeutically resistant clones.

The challenges of developing more effective targeted therapies and combination therapy strategies are significant. However, we are also in a time of unprecedented opportunity. A confluence of circumstances now makes GBM an ideal model in which to consider the challenges of personalized therapy. GBM was one of the first cancers to be sequenced by The Cancer Genome Atlas (TCGA), which provided a window into its mutational landscape (11, 12). Independently, powerful mouse genetic models of GBM have been developed that show the importance of the very same mutations identified by TCGA in the development and progression of GBM (13–22). In addition, GBM is a paradigmatic example of intratumoral cellular and molecular heterogeneity, and recent data suggest that tumor cell subpopulations, either as cancer stem cells or through clonal selection, may be important in the development of resistance to therapy (23–25). Finally, the GBM research community is well organized and highly collaborative, which has facilitated the performance of molecularly guided clinical trials in which the effect of targeted agents on their intended signaling pathways has been quantified

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and correlated with biological and clinical outcomes. The thesis of this *New Strategies* article is that investigators will need to integrate the mutational landscape of cancer with functional studies, particularly those conducted in patients, to translate the molecular catalog into better treatment in the clinic.

We propose three steps to address the challenge of developing more effective personalized therapy for GBM patients. Step 1 builds on the work of TCGA to expand and refine the molecular catalog of GBM. Step 2 requires the development of an interactive dynamic network map of GBM that is heavily informed by studying patients in well-designed clinical trials in which molecular endpoints are carefully analyzed. Step 3 necessitates the development of tools that can help determine the effect of drugs on signaling networks in defined tumor cell subsets, with resolution to the single-cell level. To develop more effective personalized treatments for GBM patients, it is essential to gain a better understanding of the functional biology of these interactive pathways, in part by following the steps outlined above and integrating them with studies in model systems.

Step 1: Build and refine the molecular catalog

The ability to catalog the landscape of somatic DNA mutations in tumor tissue from individual patients may well represent the most important advance toward personalizing therapy. Whole-genome sequencing technologies will make it technically and financially feasible to obtain individual cancer genomes in the near future (26). Genomic surveys of the mutational landscape of GBM provide an important resource to the community and show that mutations occur most commonly in genes whose protein products regulate the core signaling pathways that control cell growth (11, 27). Such studies have identified many of the mutations and copy number alterations that are already known to be important in GBM, as well as other types of cancer, including EGFR amplification and mutation, PTEN loss, p53 mutation, and CDKN2A and CDKN2B loss and mutation (28–30). Surveys have also identified previously unrecognized alterations such as NF1 loss and IDH1 mutation (11). Most importantly, studies using this comprehensive integrated approach have shown that the mutations and copy number alterations cluster along core pathways, including (*a*) receptor tyrosine kinase/RAS/PI3K signaling, (*b*) p53 signaling, and (*c*) pRB signaling. Cooperative coactivation of these core pathways gives rise to GBM in a wide range of mouse genetic models (13–22), providing scientifically gratifying, functional support for the importance of cooperation among these core pathways in the formation, maintenance, and progression of GBM. Integration of diverse types of molecular data, linking transcriptional signatures, methylation patterns, and signal transduction pathway profiles with genomic subtypes based on core pathway alterations has therefore opened up a window into a systems-biology–level understanding of GBM, potentially yielding an array of therapeutic targets (12, 31). Additional core pathways and their modifiers may soon be uncovered. However, the extent of genomic complexity seems daunting. A recent study of the genome at 30× coverage of U87, the most commonly studied GBM in vitro cell line model, revealed a striking degree of complexity: 512 genes were homozygously mutated, including single nucleotide variations, small insertions and deletions, microdeletions, and interchromosomal translocations (26). The challenge of translating this myriad of DNA alterations into drug targets will require not only an intense investment in the bioinformatic infrastructure but also a commitment to sequence clinical samples, including before and after treatment in patients treated with targeted agents in clinical trials. It will also require a serious commitment to conduct functional experiments in both *in vitro* and *in vivo* models to elucidate the targets and to understand the molecular context that determines therapeutic response.

Step 2: Develop an interactive dynamic network map, in part by studying patients

Even at this relatively early stage of cancer genomics, it appears that just knowing the mutational landscape (i.e., the catalog of mutations) will not be sufficient for guiding more effective personalized cancer therapy. Despite the compelling biological plausibility, the small-molecule inhibitors that target key proteins within these core pathways (i.e., the EGFR inhibitors erlotinib and gefitinib, and the mTOR complex inhibitor rapamycin and its analogs) have failed to demonstrate clinical benefit for GBM patients, resulting in only a few clinical responses of very short duration (32–35). One potential explanation for this disconnect is that other mutations and core pathways that have yet to be uncovered will prove to be more suitable molecular targets. This is possible; however, the frequency of alterations of the currently identified core pathways, their importance across many cancer types, and their clear role in promoting tumor formation and progression in mouse genetic models strongly suggest that the currently identified candidates are among the most compelling therapeutic targets.

Another possibility arises from the recognition that a core pathway consists of a complex interacting network of proteins, not single linear pathways. Therefore, therapeutic resistance to small-molecule inhibitors may be achieved through cross-talk within and/or between core pathways to maintain signal flux to key downstream effectors, even if the "right" molecules are targeted. Our own work in identifying mechanisms of resistance to EGFR inhibitors (35), as well as that of others (6, 36, 37), clearly demonstrates this point: maintained signal flux through PI3K appears to be a common denominator for alternative mechanisms of resistance to EGFR-targeted therapies. Therefore, understanding the static architecture of the mutationally activated linear pathways is not likely to be sufficient for guiding treatment. Instead, we will need to develop a map of the dynamic interactive architecture of the target signaling pathways to develop more effective combination therapies.

The goal of creating a dynamic interactive network map presents a formidable challenge. It will require the development of quantitative tools to measure many nodes in a complex signaling network in patient tissue samples, and, more importantly, it mandates the application of these tools to patient samples treated with targeted agents in the clinic. This requires a significant intellectual and financial investment in developing the infrastructure to measure the engagement of new agents with their intended molecular targets (38) and to assess their impact broadly on signal transduction in patients. In fact, this step is as essential a component in the development of personalized treatments as illuminating the molecular catalog of each patient. More sensitive molecular diagnostic tools that can quantify the effect of new agents on different cellular subpopulations within a tumor are needed. Further, a new level of collaboration among clinicians, biologists, translational scientists, and bioinformatics/statistics experts in designing clinical trials must be realized to make this possible. It will also require an unprecedented level of collaboration between academics and partners in the pharmaceutical industry to help develop clinical trials with molecular endpoints. As an example, in the next section we discuss a study in which we uncovered the dynamic architecture of key signaling pathways by studying patients enrolled in a clinical trial.

An illuminating example. In the trial discussed here, only a small subnetwork was studied in a small subset of patients. However, the lessons learned suggest that in-depth molecular analyses of patient samples and the effect of drugs on their intended pathway targets in carefully designed clinical trials, even of incomplete and limited networks of signaling molecules, may provide critical insights that lead to better treatments. The mammalian target of rapamycin, mTOR, emerged for many reasons as a compelling molecular target in cancer. PI3K signaling is hyperactivated in nearly 90% of GBMs, and mTOR kinase is one of its critical effectors. mTOR exists in two signaling complexes. As part of complex 1 (mTORC1), mTOR is an important downstream effector of PI3K/Akt activity linking growth factor signaling with protein translation and cellular proliferation. As part of complex 2 (mTORC2), mTOR is a critical activator of Akt. Rapamycin and its derivatives are highly effective at blocking mTORC1 signaling. Rapamycin and its analogs were shown to be remarkably effective in preclinical mouse GBM models (39–41) and subsequently were given to patients as an immunosuppressive therapy, generating hope for more effective targeted therapy for GBM patients. However, phase II studies of a single-agent rapamycin analog in recurrent GBM multiforme demonstrated no clinical efficacy (33, 42). How can this disconnect between the compelling nature of the target and the dismal record in the clinic be reconciled?

We reasoned that for targeted agents that inhibit the activity of specific signaling pathways, such as the mTORC1/S6K1/S6 signaling axis, assays to assess the adequacy of pathway inhibition in patients must be incorporated into the design, interpretation, and implementation of clinical trials. In a close multidisciplinary collaboration involving clinicians, biologists, translational scientists, and bioinformatics/statistic experts, we conducted a small, pilot phase I/II neoadjuvant clinical trial of rapamycin in patients with relapsed PTEN-negative GBM at three dose cohorts. Salvage surgical resection is often part of the clinical management of GBM patients who relapse after standard upfront therapy (which typically consists of surgical resection followed by adjuvant radiation and chemotherapy). This gave us an opportunity to design a molecularly guided clinical trial that included molecular selection criteria and enabled molecular analysis of the effect of rapamycin on mTOR signaling *in vivo*. Rapamycin was orally administered to patients before a scheduled tumor resection, with the primary goals of defining a dose required for mTOR target inhibition and assessing potential antiproliferative effects on tumor cells. Upon initial clinical presentation and biopsy evaluation, the PTEN status within the tumor tissue was determined. Upon relapse after standard upfront therapy, patients whose tumors were determined to be PTEN deficient at initial biopsy received a 10-day course of rapamycin, at three dose cohorts (2, 5, and 10 mg/day twice a day) followed by surgical excision. Intratumor drug levels, mTORC1 signaling (as measured by S6 phosphorylation), and cellular proliferation were measured and compared between surgery 1 and surgery 2 in rapamycin-treated patients, and in a set of similarly matched control patients with relapsed GBMs who did not receive rapamycin treatment.

Intratumoral rapamycin concentrations sufficient to inhibit mTOR *in vitro* were achieved in all patients, even those given the lowest dose. However, the magnitude of mTORC1 inhibition in tumor cells varied significantly from patient to patient (from 10% to 80% inhibition of S6 phosphorylation). Reduction in tumor cell proliferation (as measured by Ki67 staining) *in vivo* was significantly related to the degree of inhibition of mTORC1 signaling. Inhibition of >50% resulted in significantly inhibited tumor cell proliferation; in contrast, lower levels of mTOR inhibition did not translate into cytostatic response in patients. Further, tumor cells that were removed from rapamycin-resistant patients who were cultured *ex vivo* were found to be highly sensitive to the drug. Thus, resistance to rapamycin was not cell autonomous; rather, the lack of cytostatic response in patients represented a failure of the drug to fully access its target *in vivo*. These results suggest a different interpretation of the clinical failure of rapamycin in GBM patients, namely, that the lack of efficacy is a consequence of incomplete inhibition rather than an injudicious choice of molecular target.

mTORC1 is both a positive regulator of PI3K/Akt signaling from growth factor receptors and a negative regulator of PI3K pathway activation when signal flux through PI3K is high. This ensures homeostatic regulation of PI3K activity in healthy cells (43). Therefore, derepression of mTORC1-mediated feedback by treatment with rapamycin could paradoxically result in more rapid clinical progression by promoting PI3K activity in GBM patients treated with rapamycin.

To test this possibility, rapamycin treatment was reinstituted in these patients after surgery and they were monitored for progression. Strikingly, rapamycin treatment led to Akt activation in seven of 14 patients, presumably due to loss of negative feedback, which was associated with a significantly shorter time to progression during postsurgical maintenance rapamycin therapy (43). These results highlight the importance of pathway cross-talk in determining response to targeted therapy and suggest a rational next step toward combination therapy (i.e., dual mTOR/PI3K inhibition). Of equal importance, this study shows that measuring the effect of the drug on its target signal transduction pathway and on other proteins in the signaling network is feasible and can provide insights into the mechanisms of therapeutic resistance.

Step 3: Dissect intratumoral cellular and molecular heterogeneity

Human cancer is not a homogeneous population of cells; rather, it is a complex interactive microenvironment composed of different cell types (i.e., cancer cells, inflammatory cells, vascular cells, and support cells). Interactions among these different cells may be critical for tumor development, maintenance, and resistance to treatment (44). The cancer cells within a tumor also demonstrate considerable morphological, phenotypic, and physiological heterogeneity, varying greatly in their biological aggressiveness (45). Cancer stem cell subpopulations (46, 47) and phenotypic plasticity (i.e., the result of interactions between a cell's genotype and the local molecular signals it receives from the microenvironment, including through epigenetic changes; refs. 45, 48) provide complementary nongenetic routes toward increasing intratumoral cellular and molecular heterogeneity (45). Other nongenetic factors appear to profoundly influence the response of cancer cell subpopulations to treatment, potentially promoting therapeutic resistance (49). Therefore, it may be necessary to identify the molecular signals in multiple tumor cell subpopulations to more effectively guide targeted treatment (50). In addition, clonal genetic diversity (51), which can arise from the interaction of a rare mutation with local selection pressures, provides a powerful heritable component to intratumoral heterogeneity that can promote resistance to targeted therapies. This concept has been powerfully demonstrated by the emergence of resistance-promoting BCR-ABL kinase domain mutations in chronic myelogenous leukemia patients treated with imatinib and by resistance-promoting EGFR T790M kinase domain mutations in lung cancer patients treated with erlotinib and gefitinb (7–10).

Of great potential clinical relevance, a study by the Settleman group (52) showed the transient acquisition of a drug-tolerant phenotype in a low frequency of individual cancer cells within a tumor. This phenotype may give rise to tumor cells that are strikingly resistant to diverse anticancer agents. The molecular mechanisms underlying this transient drug-tolerant phenotype are only beginning to be understood; however, an altered chromatin state mediated by the histone demethylase Jarid1A has been implicated (52). Whether this mechanism is generalizable to many types of cancer, and whether it is clinically relevant remain to be determined. However, this important study raises the critical possibility that subpopulations of tumor cells may acquire a resistant phenotype and that molecular markers could potentially identify these resistant subpopulations. Further studies will be needed to develop more effective ways to target them.

On the Horizon

How do we as a community move forward to translate knowledge of the cancer genome into more effective treatment for patients? In this article, we have proposed three steps:

Step 1 builds on TCGA resources to extend and more fully integrate the molecular catalog of cancer mutations. Integration of diverse data types on this framework will rapidly refine the catalog to provide a more integrated view. It will also uncover new targets and potentially identify molecularly defined subsets of patients who may respond differentially to therapy. As the cost of fully sequencing individual genomes falls, it is even feasible to begin to consider the role of genome sequencing as a basis for tailoring therapy for individual patients in the future. The challenges will lie in extracting biologically meaningful pathways and targets, testing their function in appropriate models, developing agents that target them, and understanding the cellular context that determines response or resistance. We believe that many of the key pieces for this foundation are already in place. The work will be difficult, time-consuming, and laborious, but will likely lead to a direct impact in the clinic.

Step 2 requires a commitment to develop better molecular diagnostics to measure the engagement of drugs with their targets (38) and to quantitatively assess the impact of these agents on dynamic interactive signal transduction networks. A major thesis of this *New Strategies* article is that considerably more attention must be paid to this step before this unprecedented knowledge base can be translated into improved outcome for patients. This step represents a commitment to uncovering the dynamic interactive network map by studying patients in clinical trials with molecular endpoints. It will require innovative clinical trial designs in which patients are molecularly stratified for treatment and for which tissue is obtained both before treatment, during acute exposure to the drug to assess the engagement with the target pathway, and ultimately at the time resistance develops. The logistics of designing such a trial are challenging. We will need to justify the additional tissue sampling and we will have to demonstrate a benefit to patients from monitoring the effect of the drug on its intended target. Because such tissue sampling must be as low-risk as possible, smaller, less invasive biopsy approaches will be needed, necessitating an improved ability to extract maximal molecular information from very small samples. Finally, new molecular diagnostic tools

for quantitative, highly multiplexed, multiparameter measurement of signaling networks must be incorporated into the design, implementation, and interpretation of clinical trials. Although it seems quite difficult, this step provides an ideal foundation on which to build new and highly integrated forms of collaboration among clinicians, biologists, translational scientists, physical scientists, and bioinformatics experts, as well as between academics and industry.

Step 3 requires the development of technologies that can measure molecular signals in tumor cell subsets with resolution to the single-cell level and gauge the effect of new therapies on tumor cell subsets. Integration of tools arising from the physical sciences with genomic platforms and for analysis of tumor tissue from patients treated with targeted agents in clinical trials will augment our currently existing technological platforms [i.e., phospho-flow cytometry (50) and/or fluorescence in situ hybridization and immunohistochemistry applied to clinical samples (50)]. This in turn will (1) greatly increase our understanding of cancer as an adaptive microenvironment, (2) help identify mechanisms by which subsets of cells drive therapeutic resistance, and (3) facilitate the development of a dynamic interactive map to more effectively guide cancer treatment. A natural integration of steps 1–3 will help provide a better functional understanding of the biology of these interactive pathways. It will be critical to integrate these insights with studies in appropriate models to uncover how signaling networks dynamically interact. Integrating siRNA and small chemical screening library approaches in the context of genetically defined models (particularly ones that reference the molecular subsets being developed through TCGA) to identify synthetic lethal interactions, and illuminating the molecular linkages by which oncogenic signaling regulates altered cellular metabolism (53, 54) will greatly enrich our understanding of the biology of the disease. Furthermore, the results will likely provide critical therapeutic leads that can be translated into clinical benefit. In summary, this is a time of unprecedented opportunity and challenge. Disentangling the complexity of cancer, learning the rules of complex interactive signaling networks, and learning how best to treat the disease by studying patients are all within our grasp. Integration is key; there is much work to be done, but the map is already becoming clearer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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