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Applications of emerging molecular technologies in glioblastoma multiforme

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Abstract

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. Median survival from the time of diagnosis is less than a year, with less than 5% of patients surviving 5 years. These tumors are thought to arise through two different pathways. Primary GBMs represent *de novo* tumors, while secondary GBMs represent the malignant progression of lower-grade astrocytomas. Moreover, despite improvements in deciphering the complex biology of these tumors, the overall prognosis has not changed in the past three decades. The hope for improving the outlook for these glial-based malignancies is centered on the successful clinical application of current high-throughput technologies. For example, the complete sequencing of the human genome has brought both genomics and proteomics to the forefront of cancer research as a powerful approach to systematically identify large volumes of data that can be utilized to study the molecular and cellular basis of oncology. The organization of these data into a comprehensive view of tumor growth and

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progression translates into a unique opportunity to diagnose and treat cancer patients. In this review, we summarize current genomic and proteomic alterations associated with GBM and how these modalities may ultimately impact treatment and survival.

Keywords

genomics; glioblastoma; molecular signatures; proteomics

Disease of glioblastoma

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults [1,2]. These tumors are thought to arise from astrocytes or their progenitor cells, which are a type of glial cell that serve a supportive and protective function within the CNS. GBMs most often occur between the fourth and sixth decade of life, with younger patients having a better prognosis than the elderly. The tumor is usually seen as a focal lesion, although it frequently extends across the white matter tract of the corpus callosum into the contralateral hemisphere, thus giving the appearance of the so-called ‘butterfly GBM’, or may even appear as multifocal owing to diffuse infiltration of the brain by neoplastic glia. The presenting symptoms of disease include headaches, seizures or focal neurological deficits. As the name would suggest, GBM has a varied appearance microscopically. It is a highly cellular tumor with pleomorphic, basophilic nuclei and either indistinct cytoplasmic borders or plump, pink cytoplasm with fibrillary backgrounds. Varying amounts of mitoses, necrosis and capillary endothelial proliferation are common.

The survival with GBM is 1 year on average [3-6]. This survival pattern has not improved in some time, although radiation and chemotherapy appear to extend the life of the patient. While drugs are available to help treat GBM, they often have minimal effect, and doctors usually have time to try only one or two treatments before the disease causes severe impairment. GBMs feature many genetic variations that affect their response to different treatments. Researchers are trying to identify these genetic factors and to determine how they affect the disease in order to distinguish which patients are the most likely to benefit from specific drugs. In the following sections, we briefly review cancer genomics/proteomics and summarize the respective fields as they apply to GBM.

Cancer genomics

Genomics is based on the complete analysis of the human genetic code obtained by high-throughput techniques that provide a wide perspective of gene expression. As genomics involves the identification of genes and the process of how they work, the field is divided into two arms: structural and functional genomics. The concomitant detailed analysis of both the sequence data and the functional annotation of each gene has led to large volumes of data. In turn, this information has been used to provide a foundation for oncologic applications: from understanding tumor processes to better comprehending the expression patterns between patients. This has opened new perspectives for clinical research with regards to improving the diagnosis/prognosis in oncology and allowing treatment responses to be monitored. The technological platform used to interrogate these processes is DNA microarrays. Specifically, the microarray technology can be broadly categorized into high-density and low-density DNA microarrays. High-density platforms generate enormous amounts of data, which allows the expression levels of thousands of genes or the genotypes of thousands of single nucleotide polymorphisms (SNPs) to be determined. A multitude of high-density platforms are available that differ in terms of probe content, design, deposition technology, labeling and hybridization protocols. However, two major platforms for high-density microarray manufacture are in

common use. The first utilizes robotic deposition or 'spotting' of DNA molecules, while the second uses short oligonucleotides synthesized *in situ*. These high-density approaches are important to obtain global patterns of gene expression and are hampered by low sensitivity and high cost. As a result, low-density arrays function as validation tools that provide simplicity, good reproducibility, easy data management and low cost. Two categories of these arrays are custom-made spotted cDNA or oligonucleotide arrays with a limited number of particular genes or real-time PCR microfluidic arrays. As these platforms are becoming standard technologies in clinical oncology, continued advances in genomics promises to impact the management and outcomes of cancer patients.

Genomic profiling of GBM

The applications of DNA microarray technology have been successfully applied to GBM. Since the initial implementation of DNA microarrays in the 1990s [7], the PubMed database search terms 'glioblastoma and DNA microarrays' identified approximately 161 English published papers (seven reviews) that have been designed from 1 January, 1980, until 1 June, 2008, to study this malignancy utilizing DNA arrays. Reviewing the relevance of DNA arrays to GBM, they can be placed into the general categories of tumor-grade patterns, biomarkers for survival prediction and therapeutic targets. Moreover, using these and conventional molecular methodologies, several genetic alterations that regulate key pathways in the initiation and progression of GBM have been identified. These alterations involve the amplification of genes, such as EGF receptor (*EGFR*), mutation of genes, such as *p53*, and loss of heterozygosity (LOH) or deletion on chromosome 10q where several putative tumor-suppressor genes involving *PTEN* are located and are generally related to either activation of signaling pathways downstream of tyrosine kinase receptors or disruption of cell cycle arrest pathways. The most studied genetic alterations and their corresponding signaling pathways in GBM are briefly summarized.

Loss of heterozygosity

Loss of heterozygosity is the most frequent genetic alteration in GBM. A study performed in a series of 220 primary GBMs showed 75% of LOH on 10q, 47% on 9p, 29% on 19q and 19% on 1p [8]. It should be noted that 10q contains *PTEN* and other potential tumor-suppressor genes, such as *MXII*, which suggests that loss of this region may contribute to the development or malignant progression of GBM [9-11]. Indeed, a study has correlated LOH on 10q with shorter survival of patients with GBM [12]. In addition, because LOH on 1p and 19q is a common marker of oligo dendroglioma, (a lower-grade glioma), and oligodendroglioma with 1p/19q deletions shows particular sensitivity to radiotherapy and chemotherapy, it may have practical importance to identify the subset of GBMs with recognizable oligodendroglial features [13].

EGF receptor

Amplification of the *EGFR* gene (located on chromosome 7p12) occurs in 34-50% of primary GBMs [8,14-17] and rarely in secondary GBMs [16,18]. Overexpression of EGFR is also more common in primary GBMs (>60%) than in secondary GBMs (<10%) [18]. All primary GBMs with *EGFR* amplification show EGFR overexpression and 70-90% of those with EGFR overexpression have *EGFR* amplification [16,19]. *EGFR* gene rearrangements and expression of their aberrant protein products are common in GBM. Five common variants that harbor exon or N- or C-terminal deletion, and a small number of variants consisting of a variety of tandem duplications of exons, as well as missense and insertion mutations, have been identified, of which the variant 3 (EGFRvIII) containing an in-frame deletion of exons 2-7 within the extracellular ligand-binding domain is the most frequent type [20]. The joining of exons 1-8 creates a novel tertiary conformation of the extra cellular domain that lacks ligand-binding

ability. As a result, EGFRvIII is not activated by its ligand; however, it is constitutively activated, thus leading to constitutive long-term signaling.

The EGFR and its ligands are variably expressed from embryogenesis, throughout brain development and into adulthood, and these factors are involved in the proliferation, migration, differentiation and survival of all CNS cell types and their precursors [20,21]. Aberrant activation of EGFR-mediated signal-transduction pathways have been found in GBM, which may be caused by the aforementioned genetic alterations and contribute to the induction of glial transformation and progression. For example, in the absence of ligand binding, the constitutively active EGFRvIII causes the activation of downstream the lipid kinase PI3K/Akt and MAPK pathways [22-25] and is, therefore, thought to confer cell proliferation advantages and to increase cell survival by inhibiting apoptosis [26,27]. An *in vitro* assay has shown that expression of extracellular matrix components and metalloproteases was enhanced in EGFRvIII-expressing GBM cells, and the authors confirmed that the mutant EGFR did make GBM cells both more motile and invasive [28].

PTEN

The tumor-suppressor gene, *PTEN*, is located at 10q23.3, whose function is frequently lost in GBM due to LOH at this locus of chromosome 10q or mutations (15-40%) [29-32]. The mutations occur almost exclusively in primary GBMs [16,19].

The PTEN protein encoded by the *PTEN* gene is a phosphatase that removes phosphate groups from both proteins [33] and lipids [34]. One of its primary cellular targets is the phosphatidylinositol-3,4,5-trisphosphate, a plasma membrane lipid that is produced during cellular signaling events by the action of PI3K [35]. PTEN removes the phosphate group on the D3 position of the inositol ring, the same position where PI3K deposits a phosphate group after it is activated. Thus, PTEN serves as a negative regulator of the PI3K pathway, and loss of PTEN function results in constitutive activation of the PI3K pathway. PTEN loss has been correlated with higher activated Akt levels in glioma cells [32].

The protein phosphatase activity of PTEN is involved in cell migration, a malignant phenotypic change that contributes to the morbidity and mortality of the advanced glioma. One mechanism for PTEN to regulate migration has been demonstrated to be the direct dephosphorylation of focal adhesion kinase (FAK) [36]. A very recent study suggests that PTEN regulates glioma cell migration through its control of FYN and RAC GTPase downstream of $\alpha_v\beta_3$ integrin engagement in a PI3K/Akt-independent manner [37]. It has been demonstrated that the ability of PTEN to control cell migration is dependent on the protein phosphatase activity of PTEN and on the dephosphorylation at a single residue, Thr³⁸³, and loss of the protein phosphatase activity by mutation of this residue accelerates the migration of GBM cells [38].

While PTEN has been assigned a tumor-suppressor function, a recent study showed that it has tumor-promoting properties in the setting of gain-of-function *p53* mutations [39]. PTEN restoration to GBM cells harboring gain-of-function *p53* mutations leads to induction of cell proliferation and inhibition of cell death, possibly via inhibition of mut-p53 degradation by murine double minute (MDM)2 and direct stabilization of mut-p53 protein. Conversely, inhibition of endogenous PTEN in glioma cells expressing mutp53 leads to inhibition of cell proliferation and inhibition of *in vivo* tumor growth.

p53/MDM2/p14^{ARF}

The *p53* tumor-suppressor gene mutations occur in two-thirds of secondary GBMs and the majority of these were seen at previous biopsy, in cases when the tumor presented as a lower-grade malignancy [40], while a lower frequency of *p53* mutations (<30%) is seen in primary

GBM [16]. Amplification and overexpression of MDM2 occurs in 6-12% of GBMs [8,40]. GBM also has frequent $p14^{ARF}$ deletion or methylation (36-58%) and loss of $p14^{ARF}$ expression (76%) [8,42].

p53 is a transcription factor that is induced in response to diverse stresses, including DNA damage, overexpressed oncogenes and various metabolic limitations, and can either induce cell cycle arrest or apoptosis. After stress, the activity of p53 is blocked by its crucial negative regulator, MDM2, via ubiquitin-dependent degradation while reaccumulation and activation of p53 is achieved through the inactivation of MDM2 by the binding of $p14^{ARF}$ [43]. This switch system for the p53 signaling pathway is disrupted in many tumors, including GBM, due to $p53$ mutation, amplification or overexpression of MDM2, or loss of expression of $p14^{ARF}$, thus, blocking p53 activity and leading to uncontrolled cell proliferation and tumor formation.

Recent findings showing that p53 regulates the proliferation, differentiation and survival of stem cells further highlight the importance of p53 in GBM suppression. There is growing evidence that GBMs may be generated and maintained by a small subset of cancer stem cells and these stem cells display some features of normal neural stem cells, such as the potential for self-renewal. This suggests that molecular mechanisms controlling neural stem cell proliferation and GBM initiation and growth may be shared [44,45]. Meletis *et al.* showed that p53 is expressed in the neural stem cell lineage in the adult brain, and knockdown of p53 led to increased self-renewal of neural stem cells by increasing cell proliferation and decreasing apoptotic cell death, implicating p53 as a suppressor of tissue and cancer stem cell self-renewal [46]. Through analysis of the neural stem cell transcriptome, the authors further demonstrated that p53 suppresses the cell self-renewal by regulating the expression of p21, a known negative regulator of stem cell self-renewal. Further evidence for the role of p53 in stem cell differentiation was provided by Lin *et al.* who demonstrated that p53 induced differentiation of mouse embryonic stem cells by downregulation of Nanog, a gene involved in self-renewal [47].

p16^{INK4a}/RB

In addition to the p53 pathway, there is another important cell cycle-controlling route, the p16^{INK4a}/retinoblastoma (RB) pathway. Homozygous deletion or methylation of $p16^{INK4a}$ gene locus and complete absence or loss of RB expression was detected in 34 and 47% of GBM cases, respectively [42]. Another study also observed that 56% of GBMs lack $p16^{INK4a}$ expression [48].

Retinoblastoma protein inhibits cell proliferation by restraining the G₁-S transition through the regulation of E2F-responsive genes. Hypophosphorylated RB binds and inhibits the E2F transactivation domain in G₀ and early G₁ and phosphorylation of RB by cyclin D-Cdk4/Cdk6 releases E2F, which then induces genes that mediate S-phase entry. The pRB-E2F interaction can be disrupted by loss of RB expression or by inappropriate RB phosphorylation due to loss of the $p16^{INK4a}$ inhibitor of Cdk4/Cdk6 [49-51].

To this end, knowledge of these genetic events responsible for GBM provides important biotherapeutic targets. For example, the extensive research on EGFR in GBM has led to the development of inhibitors of this target. As a result, rational design of therapies antagonistic to EGFR has been launched into Phase I/II studies [52]. While the results of anti-EGFR strategies demonstrate selected benefit for some patients [53], the maturing discipline of pharmacogenomics will address the relationships between genetic variation and interindividual differences with respect to drug response. Thus, we anticipate that genomics will have a significant impact on individualizing medical care for GBM.

Epigenetic changes

A better understanding of the epigenetic changes that occur within GBM has added to the available chemotherapeutic agents employed in the treatment of the disease. Temozolamide (TMZ), an oral alkylating agent, has demonstrated a benefit in the treatment of a subset of GBM patients [54-57]. The mechanism of action of TMZ involves the alkylation of the O-6 position of guanine within DNA strands producing interstrand cross-links. These links are cytotoxic and lead to apoptosis. The adducts created by TMZ are reversible through the action of the DNA-repair enzyme, O-6-methylguanine DNA methyltransferase (MGMT). The presence of MGMT leads to a decrease in the effectiveness of TMZ. Conversely, a decrease in the expression of MGMT is beneficial for the action of TMZ. A decrease in MGMT expression occurs through hypermethylation of CpG islands in the promoter region of the *MGMT* gene, leading to silencing of transcription and a decrease in the amount of gene product. In one study, methylation of the *MGMT* promoter region was present within 47.7% of GBM samples, with this feature being more common in secondary GBMs compared with primary GBMs [54].

Several clinical trials have demonstrated the benefit of TMZ in patients with GBM. The largest study, a multi-institutional randomized trial encompassing 573 patients, demonstrated an improved median survival of 14.6 months when TMZ was added to the treatment regimen, compared with a median survival of 12.1 months without the medication [57]. A separate subset analysis of this trial examined the survival of those in whom the methylation status of the *MGMT* promoter could be determined. Those patients with a methylated *MGMT* promoter had a significantly better median survival when they received TMZ compared with those that did not (21.7 vs 15.3 months) [58].

Cancer proteomics

The human genome consists of approximately 30,000 different genes [59], yet these components do not adequately characterize the molecular pathways of an organism. For these reasons, the functional delineation of genes (functional genomics) has been undertaken at the protein level. The corresponding set of protein species that constitute the human body is estimated at 1,000,000 [60]. Proteomics refers to the entire complement of proteins expressed by cells and takes into account the alterations in protein expression, post-translational modifications, protein-protein interactions, protein structure and splice variants that occur within living cells. The importance of this field to clinical oncology is underscored by the fact that comparative proteomics between healthy and malignant states can be exploited to generate an ensemble of proteins or a protein profile for characterizing cancer development and progression. Moreover, the identified proteins may provide important tumor biomarkers or be utilized for novel therapeutic target development. Currently, electrophoresis, mass spectrometry (MS), liquid chromatography-tandem MS, surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS and protein arrays are technologies used to separate, identify, characterize and comparatively quantify the expression of hundreds of proteins in living cells or tissues. Common electrophoretic methods include 2D polyacrylamide gel electrophoresis (PAGE) and fluorescent 2D differential gel electrophoresis (DIGE). As 2D-PAGE forms the foundation for protein analysis and allows for high-resolution separation, this technique remains one of only a few methods that are able to routinely detect protein modifications. DIGE technology allows the differentially regulated proteins to be viewed as changed in color; protein extracts are labeled with fluorescent cyanine dyes and then coelectrophoresed on the same 2D electrophoresis gel. This technique further strengthens the 2D platform by the use of a reference sample, known as an internal standard, which comprises equal amounts of all biological samples in the experiment. MS-based protein-identification strategies rely on proteolytic digestion of proteins into peptides before introduction into the

mass spectrometer. Digestion of proteins into similar-sized peptides helps to overcome the solubility and handling problems associated with proteins and creates peptide fragments that are easily ionized in the mass spectrometer. Peptide ions are first measured as intact fragment ions, then selected based on their mass-to-charge ratio (m/z) and subject to collisionally induced dissociation (CID) in a process known as tandem MS (MS/MS). Computer algorithms are implemented to use the CID fragmentation patterns of sample peptides to determine the sequence of the peptide and this sequence information is used to search against theoretical spectra generated from protein and nucleotide databases. A new proteomic technique based on serum proteomic pattern diagnostics via SELDI-TOF MS uses mass spectral patterns to diagnose cancer. Lastly, protein arrays using a variety of biochips represent innovative advances that are uncovering new cancer biomarkers and have the potential to overcome the complexity and heterogeneity of malignancy.

Proteomic profiling of GBM

In light of the growth of proteomic approaches to oncology, review of scientific publications to GBM is limited compared with genomics. In all, we identified 29 English language articles (three reviews) using the terms 'glioblastoma and proteomics' or 'glioblastoma and proteome' in the PubMed database (1 January, 1980, until 1 August, 2008). We expectantly see an increase in proteomic-related papers from 2003 and 2004 (four articles in total) to a total of 15 manuscripts in 2006 and 2007. In comparison, a combination of the terms 'brain cancer', 'glioma', 'proteome' and 'proteomics' generated 146 articles (33 reviews). Articles were then assessed for their relevance towards the field. The relative dearth of publications exemplifies that the proteome has remained largely unexplored for GBM discovery and, additionally, shows an undefined potential as the field matures with respect to GBM.

Nevertheless, proteomic efforts have made distinctive contributions as they relate to GBM. Diagnosis of gliomas is based almost exclusively on tissue histology. This approach can be highly subjective with significant interobserver variability due to the heterogeneous and diffuse growth patterns of these tumors. Diagnoses are dependent on the relative weighting of specific morphological features and the experience of individual pathologists. Proteomics offers a more objective strategy to more accurately classify human gliomas.

When these malignancies are examined histologically, they are assigned tumor grades based on their level of differentiation. A common classification scheme is the one employed by the WHO in which astrocytomas are categorized as WHO I-IV, with grade IV being GBM. The qualities that are examined in order to make these categorizations include measures of nuclear pleomorphism, mitoses, endothelial cell proliferation and necrosis [1]. In questionable cases, proliferation markers, such as Ki-67, have been used via immunohistochemistry. The distinction between grades is a clinically important one as it offers prognostic value. Through direct analysis of tissue samples using matrix-assisted laser desorption ionization MS, Schwartz *et al.* obtained protein profiles of various grades [61]. These profiles offer a more objective and rigorous measure than the use of individual markers in tumor grading.

The highest grades of gliomas often exhibit a degree of dedifferentiation that makes distinction of cellular origin extremely difficult. This is the case for astrocytomas and oligodendrogliomas. Currently, there are no immunohistochemical markers that have been reliably developed for oligodendrogliomas. In its highest grade, it shares features common with anaplastic astrocytomas and, in these cases, the term 'mixed glioma' has been used [1]. While histologically it may be difficult to distinguish these tumors, proteomics has been able to differentiate GBM from other tumors of glial origin. Bouamrani *et al.* were able to use SELDI-TOF MS on frozen tissue samples to find three protein biomarkers that were expressed in GBMs but not in oligodendrogliomas [62].

In addition to providing profiles that distinguish tumor grade and cell type, proteomics can be used to provide additional information in the form of mechanism of tumor formation. As previously mentioned, there are both primary and secondary pathways through which GBMs occur. A recent study was able to identify distinct protein profile differences between primary and secondary GBMs [63]. In total, 11 proteins were identified that were exclusively found either in primary or secondary tumors.

Besides histopathological methods, other venues of diagnosis in GBM include the use of cranial imaging. Computed tomography (CT) and MRI are often utilized in establishing the diagnosis of GBM. The presence of mass effect is easily visualized, and surrounding normal brain tissue often demonstrates the presence of edema. The 'leaky' nature of the newly formed vasculature within GBM is demonstrated with the injection of intravenous contrast. This gives rise to areas of contrast enhancement and nonenhancement. The exact differences between these two areas are not always appreciated under histopathological examination. In a rather novel study, Hobbs *et al.* used SELDI-TOF MS to examine the protein-expression profiles in GBM patients who had undergone gadolinium contrast-enhanced T1-weighted MRI prior to surgical resection [64]. They determined that the protein expression profiles in non-enhancing areas had very similar protein expression patterns among all GBM patients, while the contrast-enhancing areas exhibited distinct protein expression profiles, even within the same patient. These data raise interesting possibilities in the diagnosis of GBM. The relative similarity in protein profiles of the nonenhancing regions seen on MRI may markers that can confirm the diagnosis of GBM. The different protein-expression profiles seen in the contrast-enhancing regions observed on MRI may offer markers of prognostic value.

There are several areas where the conventional methods of diagnosis, including tissue histology and cranial imaging, fall short, the first being that they rely on preservation of tissue architecture; that is to say that normal and atypical cell features must be compared and contrasted in spatial relation to each other rather than examination of small groupings of cells. The potential advantage proteomics offers over conventional methods is that it does not require large samples of tissue nor does it need to have the architecture of the tissue intact. This benefit is of most importance in anatomical locations where minimally invasive biopsies are preferred.

One of the major areas of application of proteomics in the past decade has been the search for serum biomarkers. These proteins can be useful as screening tools as is the case of prostate specific antigen and prostate cancer [65]. Other proteins, such as carcino embryonic antigen, cancer antigen (CA)-19-9 and CA-125, have important roles in monitoring responses to therapy and detecting recurrences in colon, pancreatic and ovarian cancers [66].

Petrik *et al.* have found that serum levels of α_2 -Heremans-Schmid glycoprotein (AHSG) were predictive of survival in patients with GBM [67]. By taking serum samples from 200 patients ranging from normal controls to patients with various grades of astrocytomas and using SELDI-TOF MS, they were able to identify a 2.740-kDa protein whose expression was significantly decreased as the tumor grade increased. Using MS/MS, this protein was identified as AHSG, a common component of plasma. Normal levels of AHSG were found to correlate with an improved survival.

Within GBM there is a great need for the discovery of these serum biomarkers. Given the expense of brain imaging modalities, such as CT and MRI, and the relatively high incidence of this disease, a serum marker with sensitivity high enough for screening purposes would be a most valuable tool. In addition to screening and diagnosis, serum markers can also provide a great impact in the field of predicting preoperative survival. Prior to the Petrik *et al.* study, the only available preoperative predictors of survival for GBM patients were age, Karnofsky performance scores and patterns of enhancement and necrosis seen on MRI [3,68,69]. Being

able to preoperatively predict a better outcome would justify more aggressive neurosurgical resections, as there is a significantly improved survival if greater than 98% of the gross tumor volume can be removed [3].

Expert commentary

The past decade has seen a vast increase in the number of repositories of genetic information available to GBM researchers. Between two of the larger array databases, Gene Expression Omnibus (GEO) and Oncomine, over 1000 distinct gene-expression profiles for brain cancers can be examined. A significant portion of the Cancer Genome Atlas, a grand effort to map the entire spectrum of genetic alterations in human cancers, is devoted to GBM.

The importance of integrating molecular information into clinical treatments has increasingly gained attention as more targeted, tailored and effective therapies can be implemented into care-management algorithms. The Repository for Molecular Brain Neoplasia Data (REMBRANDT) is a joint collaboration between the National Cancer Institute and National Institute of Neurological Disorders and Stroke to create a bioinformatics database that integrates genomic and clinical data from clinical trials of patients with gliomas. By combining molecular data including gene, comparative genomic hybridization and SNP arrays with clinical data, such as survival and response to treatment, this tool will allow physician scientists to make appropriate treatment decisions.

While these contemporary tools have provided a wealth of molecular information, what is becoming clearer is that no definitive genetic profile exists for GBM, given the number of diverse genetic alterations present within the tumor. Instead, many of these pathways interact with, and modify, each other. For example, PTEN can either inhibit or promote GBM cell proliferation, depending on whether *p53* is wild-type or mutated [39]. Another example of this type of complex existing in GBM has recently been reported based on the function STAT3 plays in mediating either a prooncogenic or tumor-suppressive role, depending on the mutational profile of the tumor [70]. STAT3 can form a complex with the oncoprotein EGFR variant, EGFRvIII, in the nucleus and, thereby, mediate EGFRvIII-induced glial transformation, whose function is opposed to its well established pro-oncogenic role. As multiple protein complexes act to elicit a range of biological responses in GBM, efforts aimed at uncovering inter-related pathways may come from analyzing other high-throughput parameters.

Developments in other 'omics'-based techniques, such as transcriptomics and metabolomics, can be used in a strategy to advance and accelerate the discovery of other correspondent components involved in GBM tumorigenesis. Transcriptomics refers to the set of all transcripts or mRNA molecules produced in cells, thus providing a tool for deciphering gene-expression networks. The genome-wide measurement of mRNA expression levels has provided a unique perspective to the classification of gliomas. Hanash and colleagues applied this method to distinguish between high- and low-grade gliomas and identified a subset of novel genes (celladhesion and splice factor genes) that showed a relatively high expression between these two grades of tumor [71]. Interestingly, transcriptomics has led to the identification of a novel category of RNAs (miRNAs) that denote noncoding protein RNAs that have been implicated in the pathogenesis of many malignancies, including human GBM [72]. Several groups have demonstrated the upregulation of miRNAs 21 and 221/222 and the set of down regulated miRNAs 7, 128 and 181a/b/c correlated with this malignancy [73-76]. Recent evidence also indicates miRNAs are involved in the modulation of protein-interaction networks, thereby highlighting the cellular regulation these sequences have on biological processes [77]. As a result of the significance of miRNAs in cancer, they are generating appeal as miRNA-based therapies as a consequence of preclinical success in the treatment of various malignancies

[78]. In particular, anti-miR-21 oligonucleotide miRNA was shown by Corsten and associates to significantly reduce the growth of human glioma in combination with the cytotoxic agent soluble TNF-related apoptosis-inducing ligand [79]. The ongoing development of anti-miRNA molecules (antagomirs and oligonucleotides) appears hopeful as an anticancer remedy [80, 81]. In summary, miRNAs represent important elements derived from the transcriptome that play a profound role in tumor development, cell regulation and potent diagnostic/therapeutic tools in the armamentarium against GBM.

Metabolic profiling is the high-throughput measurement and analysis of metabolites (metabolomics) that is aimed at uncovering the relationship between metabolism and physiological processes [82]. This technologic platform is quickly becoming an expanding area of cancer research as it has the applicability to aid in tumor diagnosis through the discovery of new markers, improve taxonomic classification by generating accurate metabolic tumor profiles and aid in the identification of novel anticancer targets and screening of compounds for antiproliferative effects [83]. With respect to human brain cancer, Griffin and Kauppinen reviewed the relevant features of metabolomics to various brain tumors using proton nuclear magnetic resonance spectroscopy (^1H MRS) and MS [84]. The description of specific metabolic patterns unique to neurones, glial and meningeal cells based on ^1H MRS unequivocally permits the discrimination between brain cancer types/grades, and these methods have been used to monitor/characterize brain tumors both *ex vivo* and *in vivo*. As metabolic profiling seeks to complement other profiling efforts, an anticipated dilemma will be to successfully develop databases of metabolite concentrations in cancer cells, such that a set of standard conditions are established that will be comparable and integrated with data from the other levels of functional genomic analysis. The Consortium on Metabonomic Toxicology project is an example of database management in this specialty, in which the study of approximately 150 model liver and kidney toxins will eventually allow the generation of expert systems where liver and kidney toxicity can be predicted for model drug compounds [85]. A similar undertaking for oncology, based on this framework, would aid in providing an expanded metabolic perspective of GBM.

Moreover, large-scale omics approaches used to comprehend the internal cellular environment culminating in GBM formation and growth may only provide unrefined gene or protein activity. Subsequently, it has been suggested that these constraints can be overcome by integrating these distinct biological platforms into global functional organization systems [86]. Systems biology is the coordinated study of a living system through the integration of theory, computational modeling and experimentation [87]. As delineated by van der Greef *et al.*, the development of these networks must overcome domain-specific barriers, including statistical analysis, data integration and bioinformatics [88]. One statistical challenge is exemplified by the fact that false-positive rates may be excessive due to the larger total number of measured analytes versus the number of available distinct samples; however, methods have been developed to account for significance while taking into account the enormous amounts of data [89,90]. Problems with data integration exist secondary to data heterogeneity as a result of the disproportionate number of products being derived from each biologic platform; analyses involving correlative networks have emerged to address these sources of variance [91,92]. Lastly, the optimal bio-statistical approach may be difficult to apply in the face of inadequate databases for metabolites and transient variability of these analytes. While these hurdles denote problems inherent to relational database establishment, this achievement potentially translates into an interweaving of data obtained from distinct large-scale technologies using systems biology that will help establish the global interplay of pathways involved with GBM and lay the foundation for driving novel therapies and streamline clinical trials in cancer.

Five-year view

Genome sequencing coupled with technological advances promises to make the postgenomic era an exciting and prosperous time for revolutionizing care for GBM. The high-throughput cataloguing of differentially regulated transcripts, proteins and metabolites associated with GBM will allow for accelerated understanding and characterization of this disease. Consequently, the opportunities for formulating new concepts in the treatment of this malignancy will come from integrating data obtained from genomic, transcriptomic, proteomic and metabolomic levels. In fact, the application of systems biology is an advancing discipline being utilized to contextualize and interpret large diverse sets of biological data and, in turn, promote patient-tailored therapy by assimilating biologic-expression patterns with diagnosis, treatment and clinical data. While these efforts may take more than the projected 5-year view, we believe the scientific milieu is primed for enabling the systems development for GBM biomarker identification, early detection of disease-causing alterations and specific therapeutic targeting for GBM. Current technological achievements clearly have the potential to be out-done by what awaits to be accomplished in the not too distant future and, ultimately, augment the clinical care for patients with GBM.

Key issues

- The high incidence and aggressive nature of glioblastoma multiforme (GBM) represents a significant challenge in cancer therapeutics.
- Despite a better understanding of the genetic changes associated with GBM, there has been little improvement in survival over the past several decades.
- Identification of specific molecular targets in GBM using genomics has led to prognostic gene signatures.
- Proteomics promises to yield complementary functional information and will contribute to the development of GBM specific fingerprints.
- Genomics and proteomics are not the only pieces to understanding GBM, the additional high-throughput applications of transcriptomics and metabolomics will advance the mining strategy for this disease.
- Systems biology will aid in integrating these high-dimensional platforms (genomics, transcriptomics, proteomics and metabolomics) to uncover translational applications for GBM.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. Frosch, MP.; Anthony, DC.; Girolami, UD. The Central Nervous System. In: Kumar, V.; Abbas, AK.; Fausto, N., editors. Robbins and Cotran Pathologic Basis of Disease. 7th Edition. Elsevier Saunders; PA, USA: 2005. p. 1401-1410.
2. Rosell R, de Las Peñas R, Balaña C, et al. Translational research in glioblastoma multiforme: molecular criteria for patient selection. *Future Oncol* 2008;4(2):219–228. [PubMed: 18407735]
3. Lacroix M, Abi-Said D, Fourney DR, et al. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J. Neurosurg* 2001;95(2):190–198. [PubMed: 11780887]

4. Buckner JC. Factors influencing survival in high-grade gliomas. *Semin. Oncol* 2003;30(Suppl 19):10–14. [PubMed: 14765378]
5. DeAngelis LM. Brain tumors. *N. Engl. J. Med* 2001;344(2):114–123. [PubMed: 11150363]
6. Walker MD, Alexander E Jr, Hunt WE, et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas: a cooperative clinical trial. *J. Neurosurg* 1978;49(3):333–343. [PubMed: 355604]
7. Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995;270(5235):467–470. [PubMed: 7569999]
8. Houillier C, Lejeune J, Benouaich-Amiel A, et al. Prognostic impact of molecular markers in a series of 220 primary glioblastomas. *Cancer* 2006;106(10):2218–2223. [PubMed: 16568472]
9. Thorough analysis of the genetic changes associated with glioblastoma multiforme (GBM) and their impact on survival.
9. Karlom AE, James CD, Boethius J, et al. Loss of heterozygosity in malignant gliomas involves at least three distinct regions on chromosome 10. *Hum. Genet* 1993;92(2):169–174. [PubMed: 8370584]
10. Rasheed BK, McLendon RE, Friedman HS, et al. Chromosome 10 deletion mapping in human gliomas: a common deletion region in 10q25. *Oncogene* 1995;10(11):2243–2246. [PubMed: 7784070]
11. Fults D, Pedone CA, Thompson GE, et al. Microsatellite deletion mapping on chromosome 10q and mutation analysis of MMAC1, FAS, and MXI1 in human glioblastoma multiforme. *Int. J. Oncol* 1998;12(4):905–910. [PubMed: 9499454]
12. Homma T, Fukushima T, Vaccarella S, et al. Correlation among pathology, genotype, and patient outcomes in glioblastoma. *J. Neuropathol. Exp. Neurol* 2006;65(9):846–854. [PubMed: 16957578]
13. Stupp R, Hegi ME, van den Bent MJ, et al. Changing paradigms—an update on the multidisciplinary management of malignant glioma. *Oncologist* 2006;11(2):165–180. [PubMed: 16476837]
14. Wong AJ, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B. Increased expression of the epidermal growth factor receptor gene in malignant gliomas is invariably associated with gene amplification. *Proc. Natl Acad. Sci. USA* 1987;84(19):6899–6903. [PubMed: 3477813]
15. Ekstrand AJ, Sugawa N, James CD, Collins VP. Amplified and rearranged epidermal growth factor receptor genes in human glioblastomas reveal deletions of sequences encoding portions of the N- and/or C-terminal tails. *Proc. Natl Acad. Sci. USA* 1992;89(10):4309–4313. [PubMed: 1584765]
16. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. *Am. J. Pathol* 2007;170(5):1445–1453. [PubMed: 17456751]
18. Discusses the various genetic pathways that result in GBM formation.
17. Ruano Y, Mollejo M, Ribalta T, et al. Identification of novel candidate target genes in amplicons of glioblastoma multiforme tumors detected by expression and CGH microarray profiling. *Mol. Cancer* 2006;5:39. [PubMed: 17002787]
18. Watanabe K, Tachibana O, Sata K, Yonekawa Y, Kleihues P, Ohgaki H. Overexpression of the EGF receptor and *p53* mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. *Brain Pathol* 1996;6(3):217–223. [PubMed: 8864278]
19. Tohma Y, Gratas C, Biernat W, et al. *PTEN (MMAC1)* mutations are frequent in primary glioblastomas (*de novo*) but not in secondary glioblastomas. *J. Neuropathol. Exp. Neurol* 1998;57(7):684–689. [PubMed: 9690672]
20. Nicholas MK, Lukas RV, Jafri NF, Faoro L, Salgia R. Epidermal growth factor receptor-mediated signal transduction in the development and therapy of gliomas. *Clin. Cancer Res* 2006;12(24):7261–7270. [PubMed: 17189397]
21. Wong RW, Guillaud L. The role of epidermal growth factor and its receptors in mammalian CNS. *Cytokine Growth Factor Rev* 2004;15(23):147–156. [PubMed: 15110798]
22. Moscatello DK, Holgado-Madruga M, Emlet DR, Montgomery RB, Wong AJ. Constitutive activation of phosphatidylinositol 3-kinase by a naturally occurring mutant epidermal growth factor receptor. *J. Biol. Chem* 1998;273(1):200–206. [PubMed: 9417065]
23. Lorimer IA, Lavictoire SJ. Activation of extracellular-regulated kinases by normal and mutant EGF receptors. *Biochim. Biophys. Acta* 2001;1538(1):1–9. [PubMed: 11341977]

24. Li B, Yuan M, Kim IA, Chang CM, Bernhard EJ, Shu HK. Mutant epidermal growth factor receptor displays increased signaling through the phosphatidylinositol-3 kinase/AKT pathway and promotes radioresistance in cells of astrocytic origin. *Oncogene* 2004;23(26):4594–4602. [PubMed: 15077177]
25. Narita Y, Nagane M, Mishima K, Huang HJ, Furnari FB, Cavenee WK. Mutant epidermal growth factor receptor signaling down-regulates p27 through activation of the phosphatidylinositol 3-kinase/Akt pathway in glioblastomas. *Cancer Res* 2002;62(22):6764–6769. [PubMed: 12438278]
26. Nagane M, Coufal F, Lin H, Bogler O, Cavenee WK, Huang HJ. A common mutant epidermal growth factor receptor confers enhanced tumorigenicity on human glioblastoma cells by increasing proliferation and reducing apoptosis. *Cancer Res* 1996;56(21):5079–5086. [PubMed: 8895767]
27. Sugawa N, Yamamoto K, Ueda S, et al. Function of aberrant EGFR in malignant gliomas. *Brain Tumor Pathol* 1998;15(1):53–57. [PubMed: 9879464]
28. Lal A, Glazer CA, Martinson HM, et al. Mutant epidermal growth factor receptor up-regulates molecular effectors of tumor invasion. *Cancer Res* 2002;62(12):3335–3339. [PubMed: 12067969]
29. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat. Genet* 1997;15(4):356–362. [PubMed: 9090379]
30. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997;275(5308):1943–1947. [PubMed: 9072974]
31. Dahia PL. *PTEN*, a unique tumor suppressor gene. *Endocr. Relat. Cancer* 2000;7(2):115–129. [PubMed: 10903528]
32. Knobbe CB, Merlo A, Reifenger G. Pten signaling in gliomas. *Neuro-oncology* 2002;4(3):196–211. [PubMed: 12084351]
33. Myers MP, Stolarov JP, Eng C, et al. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proc. Natl Acad. Sci. USA* 1997;94(17):9052–9057. [PubMed: 9256433]
34. Maehama T, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem* 1998;273(22):13375–13378. [PubMed: 9593664]
35. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002;296(5573):1655–1657. [PubMed: 12040186]
36. Tamura M, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* 1998;280(5369):1614–1617. [PubMed: 9616126]
37. Dey N, Crosswell HE, De P, et al. The protein phosphatase activity of PTEN regulates SRC family kinases and controls glioma migration. *Cancer Res* 2008;68(6):1862–1871. [PubMed: 18339867]
38. Raftopoulou M, Etienne-Manneville S, Self A, Nicholls S, Hall A. Regulation of cell migration by the C2 domain of the tumor suppressor PTEN. *Science* 2004;303(5661):1179–1181. [PubMed: 14976311]
39. Li Y, Guessous F, Kwon S, et al. PTEN has tumor-promoting properties in the setting of gain-of-function *p53* mutations. *Cancer Res* 2008;68(6):1723–1731. [PubMed: 18339852]
40. Watanabe K, Sato K, Biernat W, et al. Incidence and timing of *p53* mutations during astrocytoma progression in patients with multiple biopsies. *Clin. Cancer Res* 1997;3(4):523–530. [PubMed: 9815715]
41. Reifenger G, Reifenger J, Ichimura K, Meltzer PS, Collins VP. Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. *Cancer Res* 1994;54(16):4299–4303. [PubMed: 8044775]
42. Nakamura M, Watanabe T, Klangby U, et al. p14^{ARF} deletion and methylation in genetic pathways to glioblastomas. *Brain Pathol* 2001;11(2):159–168. [PubMed: 11303791]
43. Toledo F, Wahl GM. Regulating the p53 pathway: *in vitro* hypotheses, *in vivo* veritas. *Nat. Rev. Cancer* 2006;6(12):909–923. [PubMed: 17128209]
44. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumor initiating cells. *Nature* 2004;432(7015):396–401. [PubMed: 15549107]

45. Galderisi U, Cipollaro M, Giordano A. Stem cells and brain cancer. *Cell Death Differ* 2006;13(1):5–11. [PubMed: 16123777]
46. Meletis K, Wirta V, Hede SM, et al. p53 suppresses the self-renewal of adult neural stem cells. *Development* 2006;133(2):363–369. [PubMed: 16368933]
47. Lin T, Chao C, Saito S, et al. p53 induces differentiation of mouse embryonic stem cells by suppressing Nanog expression. *Nat. Cell Biol* 2005;7(2):165–171. [PubMed: 15619621]
48. Puduvalli VK, Kyritsis AP, Hess KR, et al. Patterns of expression of Rb and p16 in astrocytic gliomas, and correlation with survival. *Int. J. Oncol* 2000;17(5):963–969. [PubMed: 11029499]
49. Flemington EK, Speck SH, Kaelin WG Jr. E2F-1-mediated transactivation is inhibited by complex formation with the retinoblastoma susceptibility gene product. *Proc. Natl Acad. Sci. USA* 1993;90(15):6914–6918. [PubMed: 8346196]
50. Helin K, Harlow E, Fattaey A. Inhibition of E2F-1 transactivation by direct binding of the retinoblastoma protein. *Mol. Cell Biol* 1993;13(10):6501–6508. [PubMed: 8413249]
51. Cobrinik D. Pocket proteins and cell cycle control. *Oncogene* 2005;24(17):2796–2809. [PubMed: 15838516]
52. Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J. Clin. Oncol* 2004;22(1):133–142. [PubMed: 14638850]
53. Prados MD, Lamborn KR, Chang S, et al. Phase I study of erlotinib HCl alone and combined with temozolomide in patients with stable or recurrent malignant glioma. *Neuro-oncology* 2006;8(1):67–78. [PubMed: 16443950]
54. Eoli M, Menghi F, Bruzzone MG, et al. Methylation of O6-methylguanine DNA methyltransferase and loss of heterozygosity on 19q and/or 17p are overlapping features of secondary glioblastomas with prolonged survival. *Clin. Cancer Res* 2007;13(9):2606–2613. [PubMed: 17473190]
55. Stupp R, Dietrich PY, Ostermann Kraljevic S, et al. Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. *J. Clin. Oncol* 2002;20(5):1375–1382. [PubMed: 11870182]
56. Hegi ME, Diserens AC, Godard S, et al. Clinical trial substantiated the predictive value of 0-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. *Clin. Cancer Res* 2004;10(6):1871–1874. [PubMed: 15041700]
57. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolamide in glioblastoma. *N. Engl. J. Med* 2005;352(10):997–1003. [PubMed: 15758010]
58. Stupp R, Mason WP, van der Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med* 2005;352(10):987–996. [PubMed: 15758009]
59. Landes ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001;409(6822):860–921. [PubMed: 11237011]
60. Humphery-Smith I. A human proteome project with a beginning and an end. *Proteomics* 2004;4(9):2519–2521. [PubMed: 15352225]
- 61•. Schwartz SA, Weil RJ, Thompson RC, et al. Proteomic-based prognosis of brain tumor patients using direct-tissue matrix-assisted laser desorption ionization mass spectrometry. *Cancer Res* 2005;65(17):7674–7681. [PubMed: 16140934]
64. Introduction into the clinical uses of proteomic profiling in GBM.
62. Bouamrani A, Ternier J, Ratel D, et al. Direct-tissue SELDI-TOF mass spectrometry analysis: a new application for clinical proteomics. *Clin. Chem* 2006;52(11):2103–2106. [PubMed: 16990423]
63. Furuta M, Weil RJ, Vortmeyer AO, et al. Protein patterns and proteins that identify subtypes of glioblastoma multiforme. *Oncogene* 2004;23(40):6806–6814. [PubMed: 15286718]
64. Hobbs SK, Shi G, Homer R, Harsh G, Atlas SW, Bednarski MI. Magnetic resonance image-guided proteomics of human glioblastoma multiforme. *J. Magn. Reson. Imaging* 2003;18(5):530–536. [PubMed: 14579395]
65. Stenman UH, Hakama M, Knekt P, Aromaa A, Teppo L, Leinonen J. Serum concentrations of prostate specific antigen and its complex with α_1 -antichymotrypsin before diagnosis of prostate cancer. *Lancet* 1994;344(8937):1594–1598. [PubMed: 7527116]
66. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. *Nat. Rev. Cancer* 2005;5(11):845–856. [PubMed: 16239904]

- 67•. Petrik V, Saadoun S, Loosemore A, et al. Serum α_2 -HS glycoprotein predicts survival in patients with glioblastoma. *Clin. Chem* 2008;54(4):713–722. [PubMed: 18281421]
71. In-depth examination of the process of identifying and validating GBM serum markers.
68. Laws ER, Parney IF, Huang W, et al. Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the glioma outcomes project. *J. Neurosurg* 2003;99(3):467–473. [PubMed: 12959431]
69. Devaux BC, O'Fallon JR, Kelly PJ. Resection, biopsy, and survival in malignant glial neoplasms: a retrospective study of clinical parameters, therapy and outcome. *J. Neurosurg* 1993;78(5):767–775. [PubMed: 8468607]
70. de la Iglesia N, Konopka G, Puram SV, et al. Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. *Genes Dev* 2008;22(4):449–462. [PubMed: 18258752]
71. Hanash SM, Bobek MP, Rickman DS, et al. Integrating cancer genomics and proteomics in the post-genome era. *Proteomics* 2002;2(1):69–75. [PubMed: 11788993]
- 72•. Nicoloso MS, Calin GA. MicroRNA involvement in brain tumors: from bench to bedside. *Brain Pathol* 2008;18(1):122–129. [PubMed: 18226107]
77. Clinically oriented examination of miRNA uses in brain tumors.
73. Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res* 2005;65(14):6029–6033. [PubMed: 16024602]
74. Medina R, Zaidi SK, Liu CG, et al. MicroRNAs 221 and 222 bypass quiescence and compromise cell survival. *Cancer Res* 2008;68(8):2773–2780. [PubMed: 18413744]
75. Kefas B, Godlewski J, Comeau L, et al. MicroRNA-7 inhibits the epidermal growth factor receptor and the Akt pathway and is down-regulated in glioblastoma. *Cancer Res* 2008;68(10):3566–3572. [PubMed: 18483236]
76. Ciafré SA, Galardi S, Mangiola A, et al. Extensive modulation of a set of microRNAs in primary glioblastoma. *Biochem. Biophys. Res. Commun* 2005;334(4):1351–1358. [PubMed: 16039986]
77. Hsu CW, Juan HF, Huang HC. Characterization of microRNA-regulated protein-protein interaction network. *Proteomics* 2008;8(10):1975–1979. [PubMed: 18491312]
78. Liu G, Wong-Staal F, Li QX. Development of new RNAi therapeutics. *Histol. Histopathol* 2007;22(2):211–217. [PubMed: 17149694]
79. Corsten MF, Miranda R, Kasmieh R, Krichevsky AM, Weissleder R, Shah K. MicroRNA-21 knockdown disrupts glioma growth *in vivo* and displays synergistic cytotoxicity with neural precursor cell delivered S-TRAIL in human gliomas. *Cancer Res* 2007;67(19):8994–9000. [PubMed: 17908999]
80. Krutzfeldt J, Rajewsky N, Braich R, et al. Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature* 2005;438(7068):685–689. [PubMed: 16258535]
81. Weiler J, Hunziker J, Hall J. Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? *Gene Ther* 2006;13(6):496–502. [PubMed: 16195701]
82. German JB, Hammock BD, Watkins SM. Metabolomics: building on a century of biochemistry to guide human health. *Metabolomics* 2005;1(1):3–9. [PubMed: 16680201]
83. Lindon JC, Holmes E, Nicholson JK. Metabonomics and its role in drug development and disease diagnosis. *Expert Rev. Mol. Diag* 2004;4(2):89–99.
84. Griffin JL, Kauppinen RA. A metabolomics perspective of human brain tumours. *FEBS J* 2007;274(5):1132–1139. [PubMed: 17298437]
- 85•. Lindon JC, Nicholson JK, Holmes E, et al. Contemporary issues in toxicology the role of metabolomics in toxicology and its evaluation by the COMET project. *Toxicol. Appl. Pharmacol* 2003;187(3):137–146. [PubMed: 12662897]
91. Detailed look at metabolomic technologies and their applications.
- 86•. Kitano H. Systems biology: a brief overview. *Science* 2002;295(5560):1662–1664. [PubMed: 11872829]
93. Short but informative explanation of how to examine complex biological systems on a more integrated, functional level using current high-throughput technologies.
87. Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. *Annu. Rev. Genomics Hum. Genet* 2001;2:343–372. [PubMed: 11701654]

88. van der Greef J, Martin S, Juhasz P, et al. The art and practice of systems biology in medicine: mapping patterns of relationships. *J. Proteome Res* 2007;6(4):1540–1559. [PubMed: 17373844]
89. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Statist. Soc. B* 1995;57(1):289–300.
90. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl Acad. Sci. USA* 2001;98(9):5116–5121. [PubMed: 11309499]
91. Steuer R, Kurths J, Fiehn O, Weckwerth W. Interpreting correlations in metabolomic networks. *Biochem. Soc. Trans* 2003;31(Pt 6):1476–1478. [PubMed: 14641093]
92. Steuer R, Kurths J, Fiehn O, Weckwerth W. Observing and interpreting correlations in metabolomic networks. *Bioinformatics* 2003;19(8):1019–1026. [PubMed: 12761066]