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Studying a Complex Tumor—Potential and Pitfalls

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Abstract

Glioblastoma multiforme (GBM) is a histopathologically heterogeneous disease with few treatment options. Therapy based on genomic alterations is rapidly gaining popularity because of the high response rate and high specificity. DNA copy number and exon sequencing studies of GBM samples have revealed recurrent genomic alterations in genes such as *TP53*, *EGFR* and *IDH1* but to date this has not resulted in novel GBM therapies. Identification of expression subtypes have resulted in new insights such as the association between genomic abnormalities and expression signatures. This review describes the types of genomic studies that have been performed and that are underway, the most prominent results and the implications of genomic research for development of clinical treatment modalities.

Keywords

Glioblastoma; genomics; sequencing; personalized medicine; expression profiling; molecular subtypes

Background

Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. Currently, less than 3% of GBM patients in the United States and Europe will survive more than 5 years¹. Care of GBM patients is in dire need of better treatment options. The current standard of care for GBM therapy is based on treatment with the alkylating agent temozolomide and ionizing radiation, which is aimed at causing DNA damage in the fast growing tumor cells. Methylation of the promoter of methyltransferase gene *MGMT* is a predictor of temozolomide response, but lack of alternative treatment options renders this biomarker clinically ineffective². In the current era wherein whole genome sequencing technology is a reality, using targeted agents based on tumor specific genetic alterations has become feasible and provides a hopeful avenue towards new GBM treatment modalities^{3, 4}.

High throughput genomic experiments have been broadly applied to catalogue somatic alterations that affect this fatal disease. Genomic characteristics, such as gene expression, somatic copy number alterations and somatic gene mutations have been interrogated and correlated to clinical factors^{5–26}. Because of its dim prognosis, GBM was selected as the

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first cancer type studied by The Cancer Genome Atlas (TCGA) project, initially launched in 2005. TCGA aims to generate a comprehensive catalog of genomic abnormalities through application of whole genome copy number arrays, expression arrays, methylation arrays, exome sequencing, microRNA arrays on a large cohort of patient tumor samples, combined with high quality clinical data. TCGA has qualified over 500 GBM tumor samples with matching normal tissue samples for multidimensional genomic interrogation, which is aimed to be completed in 2012 (<http://cancergenome.nih.gov>).

In this review paper, we discuss the recent advances of our understanding of GBM, inspired by TCGA and other studies that resulted from the analysis of high dimensional genomic data.

Pathology and intratumoral heterogeneity of GBM

The histopathological diagnosis of glioblastoma, also known as stage IV glioma, requires presence of anaplastic glial cells, brisk mitotic activity, and vascular proliferation and/or necrosis^{16, 27, 28}. Inter-observer variation in pathology review is rare for the diagnosis GBM but has been reported for lower grade gliomas^{29–31}. However, significant intratumoral heterogeneity is found which may have a profound impact on the accuracy of genome alteration identification. A subset of GBMs shows intratumoral variation in the level of *MGMT* promoter methylation, suggesting the presence of a mixture of different clonal populations³². Investigation of gene expression profiles revealed differences between different samples of the same tumor, which were unrelated to differences in histology⁶. Comparison of genome wide copy number of different areas of the same tumor showed that glioblastomas may harbor genetic alterations common to all areas analyzed, but also area specific alterations^{33, 34}. The same GBM may harbor different TP53 mutations in different tumor locations³⁵.

These data suggest that GBM can contain both ancestral genomic abnormalities as well as genomic alterations unique to different areas of the same tumor. Such a tumor population structure can be the result of a clonal evolution process, in which selective pressure continuously results in growth advantages for the better adapted clones³⁶. Not every GBM appears to contain regional heterogeneity, allowing the possibility for alternative theories, such as the cancer stem cell theory. In this model, each cell develops from a cancer stem cell and is therefore genomically homogeneous except for sets of mutations acquired during the differentiation process from stem cell to bulk tumor cell³⁷. It seems plausible that both models are not mutually exclusive and can be found in GBM.

The fact that GBMs can be polygenomic tumors that maintain multiple clonal populations has important implications for the design of studies that genomically interrogate GBM samples and clinical trials³⁸. Ultra deep sequencing or assaying multiple tumor sections may be a requirement to adequately characterize GBM samples. Importantly, although targeted molecular cancer therapies such as gefitinib treatment of *EGFR* kinase domain mutations in lung adenocarcinoma are highly effective, acquired therapy resistance remains an important clinical problem and may be the result of tumor polyclonality^{4, 39}.

Gene expression profiling identifies prognostic and subtype GBM signatures

Several groups have turned to gene expression profiling to classify morphologically indistinguishable gliomas into disease relevant subtypes^{9, 13, 14, 16, 18, 23, 40}. Supervised expression profiling studies aim to establish signatures that recognize specific subsets of tumor samples, and this study design has been applied to improve the prediction of survival

over histopathology, to predict response to temozolomide therapy, and to measure EGFR activation^{9, 14, 16, 40}. Unsupervised studies have been aimed at grouping all tumor samples into naturally occurring expression subtypes^{13, 18, 23}. Phillips et al. used a cohort of 107 grade III and grade IV astrocytoma samples to define a 35 gene signature that defined subclasses named Proneural, Mesenchymal and Proliferative (Figure 1A)¹⁸. Importantly, the Proneural subclass, containing the majority of grade III astrocytomas, showed prolonged survival compared to the Mesenchymal and Proliferative subclasses even when grade III cases were excluded.

We analyzed 202 expression profiles available through TCGA to establish four GBM subtypes which we termed Proneural, Neural, Classical and Mesenchymal (Figure 1C)²³. Our approach differed from the results reported by Phillips et al in two important ways: 1) only GBMs were included and 2) 1,740 variably expressed genes, not specifically related to survival, were used to define the clusters. The patterns of these subtypes were well recapitulated by an independent validation data set consisting of 260 samples, including those analyzed by Phillips et al^{9, 10, 18, 41}.

Single sample gene set enrichment analysis (ssGSEA) is a method that measures the activation level of a gene signature in an individual expression profile, independent of other profiles in the same data set. Combined analysis of the Phillips et al and TCGA data sets using both sets of gene signatures and ssGSEA shows that the Phillips et al cohort contains few samples characterized by the Classical signature, potentially prohibiting the identification of the Classical subgroup on the basis of this data set. Interestingly, the Phillips et al Proneural subset resembles a mixture of TCGA Proneural and Neural classes. The Phillips Proliferative class contains samples from both the TCGA Proneural and Classical samples. Both studies identified a Mesenchymal subtype which recapitulates a very similar set of samples.

Currently, gene expression subtypes have no direct clinical relevance due to the lack of alternative treatment options. However, the existence of transcriptomic classes suggests that each GBM cannot be treated similarly and that characterization of patient samples prior to inclusion into clinical trials may provide important new insights into why certain patients are more or less responsive to certain therapies. Examples of these are the efficacy of angiogenesis inhibitors specifically in the mesenchymal class, which signature suggests strong activation of angiogenesis gene sets, and the application of inhibitors of the mammalian target of rapamycin (mTOR) protein in the proneural group, whose expression phenotype suggests that PI(3)K/AKT pathway plays an important role.

Methylation profiling defines a G-CIMP phenotype

Genome wide DNA methylation profiling identified a subset of 9% of GBMs with a hypermethylated phenotype, known as the G-CIMP¹⁵. G-CIMP GBMs are younger at time of diagnosis, show significantly prolonged survival and typically express the Proneural expression signature. Interestingly, the G-CIMP subclass entirely explains the increased survival associated with the Proneural subtype. This observation holds for both Verhaak classification²³ and Philips classification¹⁸, suggesting that this methylation defined subtype is more robust in predicting prognosis than the gene expression based subtypes.

Genomic analysis identifies new oncogenes and tumor suppressor genes in GBM

Copy number gains and activating mutations of proto-oncogenes provide proliferative advantage to tumor cells whereas deletions and disrupting mutations targeting tumor

suppressor genes remove barriers that suppress cell growth. An overview of genes that have been repeatedly reported to be genomically altered in GBMs shows that similar numbers of amplified and deleted genes have been identified so far, suggesting that no specific emphasis on activation of oncogenes versus deactivation of tumor suppressors genes exists (Table 1). Early on, cytogenetic studies have shown the nearly uniform co-occurrence of heterozygous loss of chromosome 10, leading to reduced transcript levels of cell cycle regulator *PTEN*, and low level gain of chromosome 7, which harbors receptor tyrosine kinase (RTK) signaling gene *EGFR* and the proto-oncogene *MET*^{10, 20, 42}. Whole genome copy number analysis by TCGA and others allowed the study of these abnormalities in much higher detail, as well as the pinpointing of new genomic alterations. Interestingly, these analyses showed that genes and proteins can be activated or de-activated through multiple mechanisms. For instance, the gene *EGFR* is focally amplified at high levels in 30–40% of GBM. A subset of *EGFR*-amplified tumors contain *EGFR* point mutations, whereas another subset maintains activating deletions of exons 2–7 (*EGFR* variant III or *EGFRvIII*). Both the deletion and point mutations are found in the extracellular domain. Deletion of the p16 locus which contains the genes *CDKN2A* and *CDKN2B* often co-occurs with focal high level *EGFR* amplification, but few mutations in these genes have been found. Germline single nucleotide variants near the p16 locus have been identified that increase the risk of glioma^{43, 44}.

Exon sequencing analysis allows identification of different types of mutations, such as single nucleotide variants (SNVs) that lead to altered amino acids, alternative stop codons, alternative mRNA splice sites, alternative transcription start sites and small insertions and deletions. This methodology was applied by TCGA, who used Sanger sequencing to analyze 91 samples and 601 genes which found both known mutations such as in *TP53* and *EGFR*, as well as novel mutations such as in *PIK3R1* and *NF1*²⁰. At the same time, a group at Johns Hopkins University performed exome wide sequencing on 22 samples, similarly recognizing known mutations as well novel mutations, most notably in the isocitrate dehydrogenase gene *IDH1*⁸. TCGA is currently performing genome wide characterization of its entire cohort of approximately 500 samples, scheduled for completion in 2012. Identification of sequence variants is dependent on the tumor purity of a sample, as well as the allele frequency of a mutation. For instance, if a gene is amplified and a mutation is present in only one out of four alleles, and the tumor purity is 80%, the fraction of sequence reads of that position that covers the mutation is 22%. SNV discovery is further affected by mutations that may only be present in a subset of the cells in a tumor. Effective mutation discovery thus requires sufficient sequencing depth to account for these factors, but may aid to assess the clonality of a tumor sample through comparison of allele frequencies.

Genome wide sequencing can additionally identify intra- and interchromosomal rearrangements. This methodology was applied to the U87 glioma cellline and showed that there was only a limited overlap in comparison to TCGA results, suggesting an intrinsic difference in the genetic context of this cell line and human primary GBM samples⁴⁵.

Integrated analysis of genomic data

The TCGA data set presented a unique opportunity to characterize gene expression subtypes by genomic abnormalities²³. A number of significant associations between subtype and gene alterations were found, which suggests that the expression pattern is an important determinant of the growth enhancing ability of specific genomic alterations. An example of this is the preference of *PDGFRA* amplifications and mutations to occur within the Proneural expression group. The Proneural signature resembles the signature of normal oligodendrocyte progenitor cells, whereas *PDGFRA* protein expression is an important marker of normal oligodendrocyte development⁴⁶. Moreover, a recent study suggests the

oligodendrocyte progenitor as a cell of origin for Proneural GBMs⁴⁷. PDGFR α activation may thus be an important step in the ability to maintain a progenitor like cell state for tumors arising from the oligodendrocyte lineage⁴⁶.

EGFR signaling is an important regulator of astrocyte reactivity and proliferation⁴⁸. Interestingly, the Classical subtype shows high activation of normal astrocyte progenitor signatures, and is hallmarked by high level amplifications of *EGFR* and *EGFR* abnormalities such as the vIII deletion, point mutations and c-terminal deletions. An association between the Classical subtype and a cell of origin is yet to be reported but may reveal the importance of the transcriptomic context for *EGFR* abnormalities.

Heterozygous deletion of putative tumor suppressor *NFKBIA* rarely occurs within tumor samples expressing the Classical signature but is frequently found in non-Classical tumors, again suggesting the importance of the expression pattern in which the *NFKBIA* abnormalities are found¹¹.

The availability of multidimensional genomic data sets facilitated identification of the striking co-occurrence of the G-CIMP methylation phenotype with mutations in the *IDH1*¹⁵. This finding raises important questions on the mechanism of action of *IDH1* associated gliomagenesis and whether the G-CIMP profile is the result of the *IDH1* mutation, vice versa, or whether they are otherwise related. The G-CIMP phenotype/*IDH1* mutation is found at high frequency amongst lower grade gliomas which may explain the significant improvement in outcome that this phenotype confers, even within GBMs. Like the majority of lower grade tumors, *IDH1* mutated/G-CIMP tumors predominantly express the Proneural gene signature.

Examination of co-occurring and mutually exclusively occurring genomic abnormalities showed that glioblastomas nearly universally (1) circumvent cell cycle inhibition through genetic alterations to the RB1 pathway, (2) evade apoptosis by blocking the TP53 pathway and (3) maintain enhanced proliferation properties through activation of the RTK/phosphatidylinositol-3-OH kinase (PI(3)K) pathway^{20, 24}. Although mutations, deletions and amplifications in the individual members of these pathways had been identified to occur at significantly higher rates than random chance, thereby suggesting their importance for GBM, integrated analysis of combined copy number and mutation patterns resulted in the important new insight that GBMs require at least one alteration in each of these three pathways, and the striking mutual exclusivity of genomic abnormalities within the RTK, *TP53* and *RB1* pathways^{20, 24}.

The emphasis on these core pathways has resulted in the identification of novel pathway members and therapeutic opportunities. Co-deletion of *CDKN2A* and *CDKN2C* sensitizes glioma cells to treatment with RB1 pathway inhibitors targeting CDK4 and CDK6⁴⁹. Integrated network modeling of DNA copy number and transcriptome data allowed the identification of new TP53 pathway related genes such as *NDN*⁵⁰. A similar analysis specific to the Mesenchymal subtype revealed STAT3 and C/EBP β as master network regulators of mesenchymal transformation¹².

Clinical developments

Personalized therapy based on the genetic characterization of a given patient's tumor offers great potential in cancer treatment. These therapies that target the molecular drivers of cancers offer high efficacy and more palatable side effect profiles compared to standard chemotherapies. Successes include imatinib which treats chronic myeloid leukemia with its characteristic translocations causing the fusion protein BCR-ABL and gefitinib for non small cell lung cancers with point mutations in the intracellular kinase domain of

EGFR^{3, 4, 39, 51}. Activating mutations and amplifications are easier to block, than it is to reinstall the activity of disrupting mutations and deletions.

In GBM, developing targeted therapies is complicated: we need to (1) identify true driver mutations and alterations while steering clear of the hundreds of non-driver "passenger" mutations that result from massive genomic instability of GBM; (2) effectively deliver drug to the tumor despite the blood-brain barrier; and (3) completely perfuse the heterogeneous tumor with high enough drug levels to affect the target. Additionally, successful targeted therapies for GBM might be particularly challenging to develop because of co-activation of multiple, independent, signaling cascades that might require hitting multiple targets in order to effectively kill the tumor^{20, 52}. Targeting multiple pathways with multiple agents in this way makes clinical trial design more difficult and trials take longer, since the dose limiting toxicities of each drug must be demonstrated, and there might be synergistic effects between toxicities from multiple targeted agents combined. The field is further muddled by clinical trials that do not require evidence of given pathway activation as an inclusion criterion for study entry for the given targeted therapy. Such trials fail to account for the fact that GBM is a heterogeneous disease in which targeted therapies will have minimal effects when the target is not the driver for the treated patient's tumor. While such data can still be used to study dose limiting toxicity, it is not helpful in extrapolating the potential utility of the drug. The last limitation to interpreting results of targeted therapies is the failure to select agents that truly cause cell death -- and not just senescence or decreased cell proliferation -- in their targeted cells in vitro. Although cell death might not be required for the utility of targeted agents when used in combination with other drugs, it should be a distinguishing feature of drugs that are moved to high priority for further clinical testing. On a practical level, assessing successful target shut down in brain tumor patients is complicated: in order to select the appropriate patients, tissue is required. However, to assess successful target shut down requires more tissue after a given time of treatment. This therefore requires a minimum of two brain surgeries. While the first surgery might only require a relatively low risk biopsy for tissue, sample bias as a result of intratumoral heterogeneity is further complicating when it comes to improving treatment of GBM. The success of targeted therapies depends on patient selection, either by histological and staining criteria that define the susceptible pathway targeted, or tumor signatures that accurately predict response. For example, one study sponsored by North Central Cancer Treatment Group and NCI is collecting protein expression, immunohistochemistry staining, and gene expression data on patients to identify the best candidates for successful EGFR inhibition.

Of the 579 GBM clinical trials in the US and Europe presently listed on <http://www.clinicaltrials.org>, a number aim to bring targeted therapies to GBM. Although far from an exhaustive, this list serves as an example of the depth of what is being tried, such as various targeted agents at different levels of action from the cell membrane down to cyclin dependent kinases.

Since EGFR and other RTK pathways are aberrant at the genomic level of a high fraction of tumors, these offer suitable targets for GBM therapies. Early trials of RTK-targeting were hampered by inadequate drug levels in the tumor or not assessing the tumor after treatment for target activity^{53, 54}. Erlotinib, which blocks internal tyrosine kinase activation of EGFR, cetuximab and nimotuzumab, which block ligand binding to EGFR, have been or are in trial⁵⁵⁻⁵⁷. These might be a hopeful strategy for *EGFR*-amplified tumors, but not necessarily tumors with constitutively activated EGFRvIII. Gefitinib, which effectively blocks EGFR with intracellular point mutations in lung adenocarcinoma, is under study although GBM is different from lung cancer in that the GBM EGFR mutations are typically extracellular and therefore the promise of gefitinib for GBM is less clear. PF00299804 is an irreversible pan ERBB family inhibitor that has shown efficacy in non small cell lung cancer models and is

being tried in GBM given its EGFR inhibition⁵⁸. Other RTKs are being targeted as well, for example, XL184 which targets MET in addition to VEGFR2, also known as KDR, and MEDI-575, an antibody that targets the platelet-derived growth factor-receptor PDGFR α ⁵⁹. Sunitinib targets PDGFR β as well as KDR^{60, 61}. Tyrosine kinases are the targets in a trial with E7050 in combination with E7080 in addition to KDR^{61, 62}. A trial of pazopanib, which blocks PDGFR, FLT1 (VEGFR1), KDR, (VEGFR2), FLT4 (VEGFR3), and c-KIT, did not prolong progression free survival in patients with recurrent GBM⁶³.

While *RAS* genes are not found to be frequently mutated in GBM, tipifarnib is a RAS farnesyltransferase inhibitor that is under investigation, after it failed to gain approval for several other cancers⁶⁴. Lonafarnib is a farnesyltransferase that inhibits post-translational modification of H-RAS, but not N-RAS or K-RAS⁶⁵.

Downstream of RTK and RAS signaling, there are multiple agents that target the PI3K pathway such as pan PI3K inhibitor BKM120⁶⁶. Multiple studies include sirolimus or rapamycin, which target mTOR⁶⁷. There is an ongoing trial that includes nelfinavir, a protease inhibitor used in the treatment of HIV that also interferes with Akt activity⁶⁸. Sorafenib is a RAF inhibitor that also targets PDGFR as well as VEGFR⁶⁹. An example of targeting the cell cycle alterations found in GBM genomes is the CDK4/CDK6 inhibitor PD0332991; patients must have activated Rb in order to be enrolled⁷⁰. There are agents in trial that affect less commonly aberrant pathways in GBM, like an attempt to target TGF β 2 with an antisense oligonucleotide, AP12009⁷¹. Lastly, a large number of trials have been focusing on targeting members of pathways that enable vascularization, like VEGF receptors. While in studies there might be improvements in imaging characteristics (decreased gadolinium enhancement on MRI) and the ability for patients to decrease their steroid dose (less brain edema with VEGF-targeting agents), so far, there has been minimal benefit in survival⁷². VEGFR status on the tumor cells is typically not used to decide whether a patient receives these agents and it possible that some subclasses of patients will be found to do better, such as those that express the angiogenesis marker. Interestingly, the Mesenchymal expression subtype signature contains several angiogenesis markers. An ongoing study of bevacizumab and temozolomide in older patients with newly diagnosed GBM, sponsored by UCLA and Genentech, is collecting tumor microarray data. Such studies are then able to ask questions like whether the Mesenchymal subclass signature predicts response to bevacizumab.

Lessons learned and future directions

Genomic experiments have accelerated our knowledge of GBM in the past decade. Microarray expression data allowed researchers to identify prognostic signatures and to stratify patients into molecular subtypes. Copy number and sequencing data analysis have identified the most frequent genomic alterations such as amplification of chromosome 7, heterozygous deletion of chromosome 10, *TP53* mutation, *EGFR* focal amplification and p16 homozygous deletion which may serve as potential drug targets. Methylation profiling surprisingly revealed the presence of a G-CIMP hypermethylation phenotype and co-occurrence with *IDH1* mutations. Combined, these results improved our understanding of the mechanistic characterizations of GBM.

However, the fact that the clinical outcome has not been much improved shows we are still in the curve. One problem we are facing now is how to combine the many outputs from genomic data analysis to generate novel therapeutic options, and to distinguish the real drivers behind gliomagenesis.

Large multi-institutional research projects like TCGA aim to shed light on this problem, by providing high quality multi-faceted genomic data on a curated set of 500 GBMs. The

number of samples interrogated allows not only to identify the most abnormalities in GBM as a whole, but to perform analysis such as network modeling and mutual exclusivity analysis on expression, methylation based subsets of GBM.

Even though GBM patients today still face a dim prognosis, the prospects for improving the outcome to this diagnosis are hopeful. The door has been opened for translational research in which clinical trials are directly connected to genomic characteristics. It is up to the current generation of researchers and clinicians to self organize into teams of clinical, biological and computational investigators as it clear that each expertise is required to change the bleak future of GBM patients.

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Figure 1. Single sample Gene Set Enrichment Analysis of the TCGA and Phillips et al expression data sets. Single sample Gene Set Enrichment Analysis (ssGSEA) assesses gene set activation levels based on gene expression levels of individual samples. The figure shows ssGSEA scores for the TCGA/Verhaak et al expression profiles ($n = 202$) and Phillips et al expression profiles ($n = 76$). Gene set scores were row-normalized to the same scale for each gene set.

A. ssGSEA scores of the three Phillips et al subtype signatures and Phillips expression profiles

B. ssGSEA scores of the four TCGA subtype signatures and Phillips et al expression profiles

C. ssGSEA scores of the four TCGA subtype signatures and TCGA expression profiles

D. ssGSEA scores of the three Phillips et al subtype signatures and TCGA expression profiles

TABLE 1

Gene Abnormalities in GBM.

Gene	Location	Mutation	Amplification	Deletion	Intragenic deletion	Methylation	Pathway	Reference
<i>CDKN2C</i>	1p32			hom 2% ²⁰			Cell cycle	20, 42, 54
<i>ERRFI1</i>	1p36			hom 3% ²⁰			RTK signaling	20, 43
<i>IGFBP2</i>	1p36.22			Y ⁴⁴			Cell migration and invasion	44
<i>CHD5</i>	1p36.31			het 30% ⁹⁴			Chromatin structure regulation	20, 45, 94
<i>MDM4</i>	1q32		7% ²⁰				p53 signaling	20, 80
<i>AKT3</i>	1q44		2% ²⁰				RTK signaling	20, 94
<i>MYCN</i>	2p24.3		2% ²⁰				Transcription regulation	7, 20
<i>LRP1B</i>	2q21.2			het 7% ⁴⁷	7% ⁴⁷		Signal transduction	20, 47, 95
<i>IDH1</i>	2q33.3	12% ⁸					Cellular respiration and metabolism	8, 48
<i>PIK3CA</i>	3q26.3	7% ²⁰	2% ²⁰				RTK signaling	20, 49
<i>SOX2</i>	3q26.3-q27		14% ⁹⁶				Cell differentiation	20, 96, 97
<i>PDGFRA</i>	4q12	3% ²³	13% ²⁰		3% ¹⁷		RTK signaling	20, 17, 23
<i>PIK3RI</i>	5q13.1	10% ²⁰					RTK signaling	8, 20
<i>PARK2</i>	6q25.2-q27	9% ⁵⁰		het 22% ⁵⁰ hom 2% ⁵⁰	2% ⁵⁰		Signal transduction	20, 50
<i>QKI</i>	6q26			het 25% ⁴⁷ hom 4% ⁴⁷			mRNA splicing, transportation/cell differentiation	20, 47
<i>EGFR</i>	7p12	18% ²⁰	42% ²⁰		20-30% ⁹⁸		RTK signaling	20, 51, 98
<i>CDK6</i>	7q21-q22		1% ²⁰				Cell cycle	20, 52
<i>MET</i>	7q31	2% ²⁰	4% ²⁰				RTK signaling	20, 53
<i>CDKN2A/2B</i>	9p21			hom 50% ²⁰			Cell cycle	20, 55
<i>PTPRD</i>	9p23-p24.3	6% ²²		40% ²²	Y ²²	37% ²²	Signal transduction	20, 22
<i>PTEN</i>	10q23.3	31% ²⁰		hom 8% ²⁰			RTK signaling	20, 56
<i>CDK4</i>	12q14		18% ²⁰				Cell cycle	20, 55, 57
<i>MDM2</i>	12q14.3-q15		14% ²⁰				p53 signaling	20, 58
<i>RB1</i>	13q14.2	10% ²⁰		hom 3% ²⁰			Cell cycle	20, 59

Gene	Location	Mutation	Amplification	Deletion	Intragenic deletion	Methylation	Pathway	Reference
<i>NFKB1A</i>	14q13			het 23% ¹¹			NF-kappa-B signaling	11
<i>TP53</i>	17p13.1	34% ²⁰		hom 1% ²⁰			Cell cycle/apoptosis	20, 60
<i>NF1</i>	17q11.2	14% ²⁰		hom 2% ²⁰			RTK signaling	20
<i>CCNE1</i>	19q12		Y ²⁰				Cell cycle	20
<i>BCL2L12</i>	19q13.3	Y ⁶¹					p53 signaling	61
<i>IL6</i>	7p21		42% ¹⁰⁰				Growth arrest and differentiation	62, 99, 100
<i>MGMT</i>	10q26					21% ²⁰	DNA repair	2, 20
<i>CCND2</i>	12p13		2% ²⁰				Cell cycle	20, 63

hom, homozygous; het, heterozygous; Y, reported but no specific percentage available.