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Author F.E. Bleeker
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General discussion

Mutational profiling in cancer

Cancer is caused by disruption of the regulation of cell proliferation, differentiation, and programmed cell death, and is genetically determined. The discovery of yet unknown oncogenes and tumor suppressor genes, whose function is causally altered during tumorigenesis, remains a major goal of modern cancer research, as these genes (also called the cancer genome), and the pathways they control, are the most potential targets for anticancer drug development.

Recently, the 'diseasome' was presented, describing a collection of 1.284 genetic diseases and the associated 1.777 genes.[1] A human disease network and a disease gene network were generated for genetic, both inheritable and not-inheritable disorders, which showed remarkable overlap in their distribution and links. In several disorders, including cancer, caused by mutations in anyone of a few distinct genes, the encoded proteins participate in the same cellular pathways, molecular complexes or functional modules. Different disease phenomena, like different cancer types, had somatic mutated oncogenes and particularly tumor suppressor genes as *TP53* and *PTEN* in common.[1]

In fact, originally, it was hoped that there would be a small, finite number of deregulated genes that cause various types of cancer, as *TP53*, *PTEN*, *BRAF*, *EGFR* and *PIK3CA.*[2-4] However, mutational profiling of different cancer genomes has pinpointed many new cancer candidate genes (CAN) genes and now most studies support the notion that the majority of genes that cause cancer contribute to cancer at low frequency and in a limited tumor spectrum.[5,6] The true number of CAN genes is still unknown, and although only a limited amount of cancer genomes has been studied thus far, more than 1% of the human genome is implicated to be involved in carcinogenesis when mutated.[7]

The most extensive cancer genome mutation analysis studies have sequenced ~20.000 well-annotated coding sequences. These studies have been performed on samples of breast and colorectal cancer (CRC),[6] glioblastoma[8] and pancreas ductal adenocarcinoma (PDAC).[9] Only small sets of tumors of one cancer type have been studied thus far. As a consequence CAN genes mutated at a frequency of less than 10-15% can easily have been missed in the cancer mutation profile studies. Therefore, studies of larger tumor sets are necessary to complete the genomic landscape of distinct types of cancer. Because CAN genes are usually not shared by different types of cancer, these studies have to be performed on all cancer types.

The prevalence of somatic mutations in the various types of cancer differs. Hematologic malignancies generally contain fewer non-synonymous somatic mutations than solid tumors.[3,10] In a cytogenetically normal acute myeloid leukemia (AML) genome, ten genes have been found to be somatically mutated in a non-synonymous manner,[10] whereas in breast, CRC, glioblastoma and PDAC cancers, on average 63 mutations of this type were identified per sample.[6,8,9] Even, between different types of solid tumors differences in mutation numbers have been found. Mutations have been found more frequent in colon, lung, ovarian and gastric cancer, compared to glioblastoma, breast and testis cancer.[3]

Distinct mutation spectra have been observed in the different cancer types, which can provide insights into cancer etiology.[3] In lung cancer, a high proportion of C:G>A:T transversions is found, which is compatible with the mutagenic effects of tobacco carcinogens.[3] Furthermore, C:G>T:A transitions at dinucleotides other than CpC have been found frequently in glioblastoma, melanoma and lung cancer,[3] suggesting common underlying mutagenic mechanisms. After temozolomide treatment, the observed mutation spectrum in glioblastoma samples is different: the tumors have acquired excess of C:G>T:A transitions at CpC dinucleotides.[8,11] This has been observed in other mutational signatures as well after treatment with alkylating agents.[11,12] In addition, pre-treated samples have a hypermutation phenotype, likely caused by defects in the DNA mismatch repair gene *MSH6*.[11,13] In addition, this phenotype may explain glioblastoma progression after temozolomide treatment.[13]

Technical aspects that need to be considered in mutation analysis

The quality of mutational profiling studies is crucial to reach correct conclusions. Many techniques have been developed for the detection of mutations.[14,15] A major limitation of all sequencing techniques is the inability to detect mutations in a small number of cells in a wild type background. Direct or Sanger sequencing is considered the standard method at present.[14] When a mutation is assumed, an independent PCR is imperative to confirm the presence of the mutation. Artifacts in sequences can occur during the enzymatic PCR performed by DNA polymerase. Particularly, PCR errors can occur when small amounts of DNA are used after extraction from paraffin-embedded tissue, as described in **Chapter 8** of this thesis.[16] Direct sequencing is only useful when the fraction of mutated alleles is larger than 25%,[17] because mutations in a smaller fraction generally cannot be distinguished from background in sequencing chromatograms.[18] When a mutation is assumed to be present in a small fraction of cancer cells after an independent confirmation of the PCR, cloning of the PCR product can help to assess whether the mutation is present or not. During cloning, the wild-type (wt) and the smaller fraction of mutated alleles are segregated, and only a single DNA sequence (wt or mutated) can integrate into the plasmid. Therefore, when both wt and mutated alleles are present, both have to be inserted into plasmids in the same fraction in which the mutated allele is present in the tumor (**Chapter 3**). When primers for PCR are designed in regions containing single nucleotide polymorphisms (SNPs), mutations can be missed. Heterozygosity for SNPs within the PCR primer annealing sequence can cause preferential amplification of the matched chromosome and hence suppress the signal from the alternate allele on the mismatched chromosome.[19] To circumvent this problem, primers ideally are designed in regions not containing SNPs.

The human genome probably contains 20,000-25,000 genes. However, only 1.5% of the 3.08 Gb human genome consists of protein-encoding DNA.[20] The remainder consists of introns, non-coding RNA genes, functional elements such as promoters and other non-coding intergenic DNA,[20] which have been once referred to as 'junk' DNA.[21] Due to the occurrence of repetitions, sequencing has not been able for 1% of the transcriptionally active DNA and 10% of the genome that lies within the gene-poor heterochromatin thus far.[20] The non-gene-encoding DNA may contain mutations that we can only speculate about at present.

New 'next generation' sequencing techniques are being developed. These technologies will probably provide the opportunity to sequence this previously impenetrable DNA. Furthermore, they will provide the opportunity for faster and more economic whole genome sequencing.[22] For a limited number of cases, unbiased whole genome sequencing has been performed, including James D. Watson,[23] J. Craig Venter,[24] a Homo sapiens neanderthalensis[25] and a cytogenetically normal AML patient.[10]

Tissue-specificity and validation of mutations

Mutational profiling of the cancer genome has identified many novel mutations and new CAN genes in various types of cancer. Tissue-specific mutation patterns have been observed for various CAN genes, e.g. *AKT1* and *IDH1*.[26,27] Why some genes are mutated in specific cancer types remains an unsolved issue but its solution is relevant for basic and clinical cancer research. Two obvious explanations are that the proteins require unique contexts to act effectively as tumor suppressor or oncoproteins, or that the expression of genes is controlled in a tissue-specific manner and therefore only mutations in a specific gene with the appropriate expression characteristics dominate in the corresponding tumors.[28] Functional validation of mutations may help to resolve this issue, as was recently shown for *Hras* and *Kras* mutations in mice lung tumors.[29]

A higher ratio of non-synonymous to synonymous mutations is an indicator of selection pressure during tumorigenesis.[3] This can be applied to large mutation analysis studies and also for individual genes with a high mutation rate.[3] Two classes of somatic non-synonymous mutations occur in cancer genomes. 'Driver' mutations, which confer growth advantage to the cells, are causally implicated in cancer development and therefore cells with the mutated allele are positively selected. 'Passenger' mutations are biologically neutral and are not subjected to selection.[3] Discrimination between 'driver' and 'passenger' mutations is a challenge and the statistical methods used have been much under debate.[30-32] To determine the effect of mutations, in silico prediction of cancer association of mutations can be helpful. However, these programs are only indicators. For example, in a computational tool the oncogenic hotspot mutation *PIK3CA*^{E545K} was predicted as 'likely not cancer-associated'.[33] Consequently, functional validation of identified mutations is obligatory. Mutation screens can rapidly be performed. However, the functional validation takes much more time and effort. In particular for genes mutated at low frequency, the question whether their mutations are 'drivers' or 'passengers', makes the functional validation vital. Paradoxically, these genes are not rapidly subjected to functional validation because attention is focused on genes that affect many patients and/or different tumor types. In addition, functional validation of mutations in large genes such as *TTN* and *OBSCN*, that encode for the family of giant sarcomeric signaling proteins,[34] is very demanding. Mutations in these genes have been found in different tumor types.[3,5] For a while, mutations in *TTN* have been thought to be 'passenger' mutations occurring in these genes due to their extensive lengths.[35] Now, the mutations really seem to be overrepresented in these genes and might indicate 'drivers'.[3] Therefore, the question remains whether these mutations are 'drivers' or 'passengers'. This issue will not be resolved until the mutations in these genes are evaluated in biological experiments.

Methods for functional validation of cancer alleles are manifold and can be performed *in vitro* and *in vivo*. The classic *in vitro* model to test the oncogenic potential of a novel CAN gene is the ectopical expression in immortalized mouse embryonic fibroblast 3T3 cells in a transformation assay.[36] Expression of the mutated oncogenes is regulated under the control of a retroviral or lentiviral promoter. Therefore, the gene is transcribed artificially in a non-natural abundant manner. A condition which more closely resembles the situation in human cancer can be recapitulated by targeted homologous recombination.[37] Different cancer mutations can be introduced (or 'knocked in') into the genome of untransformed human cells by stable modification of the corresponding genomic locus.[38,39] The major advantage of this approach is that a cancer allele is expressed under its own promoter, and therefore resembles human cancer much more adequately. Although this 'knock in' (for oncogenes) or 'knock out' (for tumor suppressor genes) technique is time consuming, an optimal model system is generated for functional validation of mutations and has been shown to be useful in drug screening approaches.[39,40] The 'knock-in' of reporter genes such as fluorescence proteins expressed under the regulation of the promotor of the target gene, makes the model even more attractive. This approach is currently being used to generate *PTEN* reporter cells as *PTEN* is commonly lost in a heterozygous manner in cancer, including glioblastoma.

An important question that needs to be addressed is how many mutations are required to transform a 'normal' human cell? The general idea was that approximately five mutations was the minimum.[41] However, with the recent identification of ~63 mutated genes in different types of solid tumors, the question arises whether all these mutations, each associated with a small survival advantage for the cells, drive tumor progression[42] or whether oncogenesis is based on a limited number of 'driving' mutations which are accompanied by a large number of passenger mutations. Tumor progression models can be made by homologous recombination, to study the correct order of introducing oncogenic alleles and/or knocking out tumor suppressor genes.

Mutational profiling of glioblastoma

The Cancer Genome Atlas (TCGA) Research Network recently sequenced 601 genes in 91 glioblastoma samples, including 137 genes encoding kinases.[43] In addition, the samples have been evaluated for methylation, gene expression and copy number alterations.[43] This study provided an extensive overview of mutated genes known to be involved in gliomagenesis (such as *TP53*, *EGFR*), but also revealed new genes. For example, *ERBB2*, *NF1* and *PIK3R1* were found to be mutated at considerable frequencies. All this data provided an updated network view of the pathways involved in gliomagenesis.[43] In addition, Parsons et al.[8] sequenced 20.661 genes, including all kinase genes, in 11 tumors and found 21 genes to be mutated at a considerable frequency in the discovery screen. For these 21 genes, mutation analysis was performed on another 83 glioblastoma samples, and these samples were also assessed for copy number alterations and gene expression changes. Interestingly, the most striking finding of this unbiased approach is the mutation frequency of 12% in *IDH1,*[8] a gene encoding isocitrate dehydrogenase 1, that was not sequenced by the TCGA.[43]

These two different approaches - a study with a large number of samples providing a complete overview for the limited number of genes and a study profiling a large number of genes in a limited number of samples – show the options we had, when we started mutational profiling of glioblastoma as described in **Chapter 3** of this thesis. We performed mutational profiling in a large set of tumors, as not to miss genes mutated at a low frequency. As a consequence, the number of genes that could be sequenced was limited. As kinases are implicated in many aspects of carcinogenesis and several have already been validated as targets for drug therapy with promising responses in cancer,[44,45] we particularly focused on kinases. Kinase genes previously found to be mutated in other types of cancer were selected, and specifically exons in which mutations had been found. The exons sequenced in our study were similar to 230 mutation spots in 19 genes that were screened in a high-throughput oncogene mutation profiling study in which PCR was followed by MALDI-TOF mass spectrometry.[46] However, in our study and in the study of Thomas et al.[46] a novel mutational hotspot was not identified in glioblastoma.

We know now that most of the CAN genes are not shared by different cancer types, and even when they are shared, they often have a tissue-specific mutation pattern. This phenomenon can be appreciated from the mutation analysis of *ERBB2* in cancer. A mutational study in lung cancer had shown mutations in the kinase domain of *ERBB2.*[47] Therefore, our mutation analysis included the exons coding for the kinase domain of ERBB2, but did not reveal any mutations in 128 samples. An unbiased mutational analysis of the complete coding sequence of the *ERBB2* gene in 206 tumors resulted in mutations in 7.6% of the samples.[43] Interestingly, all mutations were located in the extracellular domain of *ERBB2*, analogous to its family member *EGFR.*[48] This example demonstrates that unbiased sequencing of the complete genes is required. Moreover, sequencing has to be performed in a high number of samples, otherwise low mutation frequencies are likely to be missed, as occurred for *ERBB2* in other studies.[8,11]

Another important issue that needs to be addressed in the coming years is the origin of glioblastomas. For different types of cancer, including glioblastoma, cancer stem cells (CSCs) have been identified, based on the expression of stem cell markers.[49] Thus far, it is uncertain which is the best marker for glioblastoma CSCs.[50] Another question is whether the CSCs arise from developmentally stalled neural progenitor cells or from dedifferentiated terminally differentiated cells (reviewed in [51]) and furthermore, whether either one of these may transform to a malignant phenotype by mutations. CD133^{+} glioblastoma CSCs have been expression-profiled and seem more resistant towards therapies,[52,53] *MGMT* methylation has been assessed in CSCs,[54] but, thus far, no mutational profiling of CSCs has been described.

PI3K-AKT pathway mutations in glioblastoma and therapeutic options

Many of the alterations identified in glioblastoma cluster in three pathways: the PI3K-AKT pathway (50%), downstream of the receptor tyrosine kinases (altered 88% in total), P53 (64-87%) and RB (68-78%) signaling.[8,43] Most alterations occur in a mutual exclusive fashion: alterations within one tumor affect only a single gene in a pathway, suggesting that different genes in a pathway are functionally equivalent.[8,43] Many of the kinases found to be mutated in glioblastoma are thought to play a role upstream or in the PI3K-AKT pathway. Kinase genes with frequent mutations are *EGFR*, *ERBB2*, *PIK3CA* and *PIK3RB*[18,43] as listed in **Table 1**. *EGFR* is amplified in 40% of glioblastomas[55] and mutated in an additional 40% of glioblastomas. Activating mutations in *EGFR* are found as point mutations or large deletions in the extracellular part of the receptor (*EGFRvIII*).[48] In 8% of glioblastoma samples the EGFR family member *ERBB2* gene contains mutations, which are also predominantly located in the extracellular part of the tyrosine kinase receptor.[43] In 40% of glioblastomas, the tumor suppressor gene *PTEN* is mutationally and/or transcriptionally inactivated.[56] The PI3K enzyme is composed of a regulatory and a catalytic subunit and has been found to be altered in ~20% of glioblastomas.[8,43] Both *PIK3CA* – coding for p110α - the catalytic subunit of PI3K, and *PIK3R1* – coding for P85α – the PI3K regulatory subunit, are found to be mutated.[8,43] Mutations in both genes are predicted to cause constitutive activation of PI3K. PI3K phosphorylates PIP2 into PIP3 that is responsible for activation of the PI3K-AKT pathway.[57] Normally, PTEN dephosphorylates PIP3 into PIP2, and inhibits activation of the downstream pathway member AKT. Genes encoding for AKT have not been found to be mutated,[58,59] but are amplified in 2% of glioblastomas.[43] Moreover, mutations in *IRS1* and *FOXO* have been found with a low incidence in glioblastoma.[8,43] In conclusion, many members of the AKT/PI3K pathway have been identified by mutational profiling and can be considered to be potential therapeutic targets (**Figure 1**).

Glioblastoma patients have been treated with EGFR inhibitors; the response rate was 15-20%.[60] This limited response is likely caused by molecular events downstream of EGFR that activate the oncogenic PI3K-AKT signaling pathway. The clinical response to EGFR inhibitors was found to be associated with co-expression of EGFRvIII and PTEN in recurrent glioblastomas,[60] but not in combination with chemoradiation for newly glioblastomas[61] or in glioblastomas treated with erlotinib and TMZ diagnosed.[62] Probably mutational events in *PIK3CA*, *PIK3R1*, *IRS1* and *FOXO* can contribute to resistance for EGFR inhibitors. The proteins encoded by these genes may form therapeutic targets. PI3K inhibitors are currently being tested in phase I/II clinical trials, and rapamycin showed encouraging results in *PTEN*-deficient glioblastoma patients.[63]

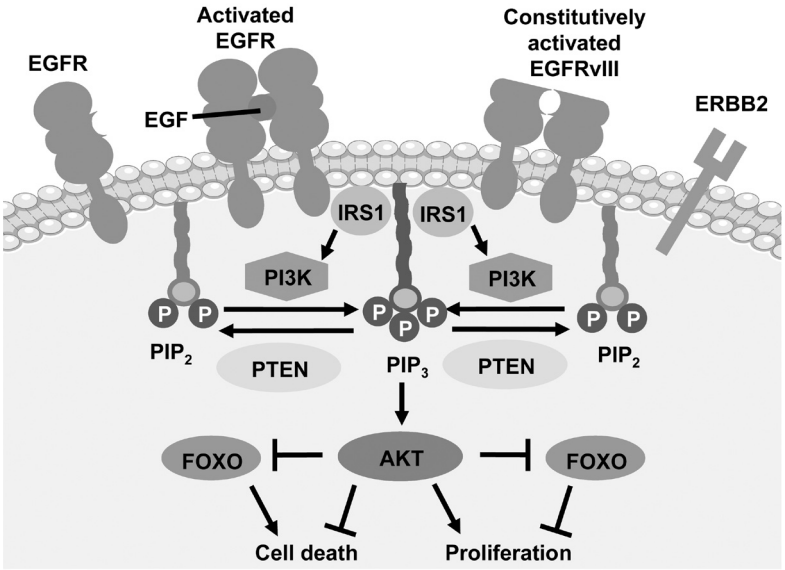

Figure 1. Simplified representation of the PI3K-AKT signaling pathway

Mutational inactivation of IDH1 in glioblastoma and therapeutic options

In addition to the identification of mutated kinase genes, novel somatic mutations were found in other genes as well. Some of these genes have not been linked to cancer before. An overview of frequently mutated genes in glioblastoma is shown in **Chapter 11** of this thesis. Of particular interest is the identification of somatic mutations in *IDH1*.[8] *IDH1* encodes isocitrate dehydrogenase 1,[64] a cytoplasmic and peroxisomal enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate,[65] with concomitant production of NADPH.[66] Mutations in *IDH1* appeared to be specific for some types of gliomas,[27,67,68] with a high incidence in low-grade gliomas.[67,68] Recently, mutations affecting *IDH2*, another $NADP^+$ dependent isocitrate dehydrogenase, but located at the mitochondria, have also been described at lower frequency in gliomas.[68] Mutations in both *IDH1* and *IDH2* affect evolutionarily highly conserved amino acid arginines at position R132 and R172, respectively. This arginine is localized in the substrate binding site of IDH1/2, where hydrophilic interactions between the arginine and both the α- and ß-carboxylate of isocitrate are formed.[69] The alterations likely affect these interactions, as the mutations inactivate both IDH1 and IDH2 *in vitro*[68] and *ex vivo*. The role of IDH1 in gliomagenesis may be related to its NADPH production, which is necessary to tackle oxidative stress that induces DNA damage.[70] The fact that mutational inactivation of IDH1/2 provides a survival advantage to glioma patients may be explained by increased apoptosis induced by inactivation of IDH,[70] and warrants further functional validation of *IDH* mutations. A very recent study suggests a link between IDH1 and Hypoxia inducible factor-1α (HIF-1α).[71] Mutationally altered metabolic enzymes are thought to contribute to tumor growth by stimulating the HIF-1α pathway and tumorigenesis.[71] This has been observed for other tumor types with mutations in Krebs cycle enzymes Fumarate hydratase (*FH*) and Succinate dehydrogenase (*SDH*) as well,[72,73] and may provide therapeutic options for gliomas by targeting HIF-1α or α-ketoglutarate.

Conclusion and future perspectives

In conclusion, mutational profiling studies of cancer genomes have identified novel mutations in cancer-causing genes. The mutations vary from tumor to tumor, but the most important mutations tend to be linked within the same signaling pathways. It suggests that cancer treatment cannot be focused on single genes, but rather on pathways. Most of these pathways were already known to be involved in cancer. A novel gene mutated in gliomas is *IDH1*, which pinpoints metabolic pathways to be involved in gliomagenesis. In addition to the predictive and prognostic aspect of some mutations, the activation of pathways has been shown to predict prognosis as well.[74,75] A comprehensive understanding of the genetic effects underlying cancer can only be gained by integrating mutational analysis studies with analysis of copy number alterations, translocations, expression and methylation profiling for each single cancer sample. It can be expected that within a decade advances in techniques will enable us to genotype every individual tumor[76] at reasonable costs and within a limited timeframe. If drug design, drug development and clinical trials could also be performed more rapidly than nowadays, it might eventually become possible that every patient receives a tailor-made cocktail of inhibitors and drugs to target pathways deregulated in his or her tumor.[76,77] When treatment-induced resistance does occur, it should be possible to alter the cocktail. As only a limited number of altered genes has been identified thus far, future mutation profiling studies are required to identify more CAN genes, markers and therapeutic targets. Mutational profiling of cancer genomes has just begun….

Kinase	Amino acid mutation	Frequency	Type of mutation
ABL2	P487L		Missense
ADRBK2	G337D		Missense
AKT3	G171R		Missense
ALPK3	V1541I		Missense
ATM	G138R		Missense
ATM	V245A		Missense
ATR	R2533*		Nonsense
AURKB	V57M		Missense
AXL	P304S		Missense
BCR	A138T		Missense
BCR	D451N		Missense
BRAF	K601E		Missense
BRAF	T310I		Missense
BRAF	V600E	2	Missense
BRD2	G30E		Missense
BRD2	P714L		Missense
BRD2	W91*		Nonsense
BTK	H491Y		Missense
CDC2L6	A395V		Missense
CDC7	G119E		Missense
CDK2	P45L		Missense
CDK3	S106N		Missense
CDK5	G16R		Missense
CDK6	3'UTR		3'UTR
CHEK1	E320K		Missense
CHEK1	R160H		Missense
CHEK1	Splice Site		Splice Site
CHKA	G344E		Missense
CHUK	R133*		Nonsense
CSK	P45S		Missense
CSNK1E	G415R		Missense
DMPK2	S280F		Missense
DYRK1B	A71A		Missense
DYRK2	P198L		Missense
DYRK3	R205K		Missense
EGFR	A289T		Missense
EGFR	A289V	4	Missense
EGFR	A597P	2	Missense
EGFR	C219G		Missense
EGFR	C620Y		Missense
EGFR	E866D		Missense
EGFR	G598V	4	Missense
EGFR	L210Q		Missense
EGFR	L62R		Missense
EGFR	P589L		Missense
EGFR	R222C		Missense
EGFR	R252C		Missense
EGFR	R680G		Missense
EGFR	S703F		Missense
EGFR	Splice Site		Splice Site
EGFR	T263P		Missense
EGFR	V651M		Missense
EPHA2	G111D		Missense
EPHA2	P817L		Missense
EPHA3	K500N		Missense
EPHA3	A971P		Missense

Kinase	Amino acid mutatio	Frequency	Type of mutation
EPHA4	P332L		Missense
EPHA5	Splice Site		Splice Site
EPHA6	G777E		Missense
EPHA6	V280L		Missense
EPHA7	R69Q		Missense
EPHA7	S207F		Missense
EPHB1	E698*		Nonsense
EPHB6	E860K		Missense
ERBB2	C311R		Missense
ERBB2	C334S		Missense
ERBB2	D326G		Missense
ERBB2	Splice Site		Splice Site
ERBB2	E321G		Missense
ERBB2	L49H		Missense
ERBB2	N319D		Missense
ERBB2	T216S		Missense
ERBB2	V750E		Missense
ERBB2	V777A	2	Missense
ERBB3	S1046N		Missense
ERN1	P336L		Missense
ERN1	Q780*		Nonsense
ERN1	S769F		Missense
FGFR1	K656E		Missense
FGFR1	N546K		Missense
FGFR1	R576W		Missense
FGFR2	Q212K		Missense
FGFR3	E466K		Missense
FLT1	R781Q		Missense
FLT1	G727C		Missense
FLT1	K427N		Missense
FLT1	S818N		Missense
FLT3	A627T		Missense
FLT4	E989D		Missense
FRAP1	P2476L		Missense
FRAP1	A492T		Missense
FYN	W290*		Nonsense
GAK	Splice Site		Splice Site
GUCY2F	G568D		Missense
H11	G67S		Missense
IKBKE	Splice Site		Splice Site
ILK	D138G		Missense
INSR	A20T		Missense
INSRR	G1065E		Missense
IRAK1	V412M		Missense
ITPKB	Splice Site		Splice Site
KDR	V1041M		Missense
KIAA1804	H890P		Missense
KIAA1804	M894T		Missense
KIT	A599T		Missense
LATS1	V719I		Missense
LATS2	G909R		Missense
LRRK1	fs		Ins/del
LTK	L485L		Missense
LTK	A321V		Missense
LYN	A119V		Missense
MAP3K11	R659H		Missense

Kinase	Amino acid mutation	Frequency	Type of mutation
MAP3K2	M568I		Missense
MAP3K6	E1186K		Missense
MAP3K8	A387T		Missense
MAP3K9	D994N		Missense
MAP4K4	fs		Ins/del
MAPK13	V184M		Missense
MAPK7	A590T		Missense
MAPK8	G177R		Missense
MAPK9	G35R		Missense
MAPK9	V91M		Missense
MAPKAPK3	P28S		Missense
MARK3	I655_K657del		Ins/del
MARK4	Splice Site		Splice Site
MET	Splice Site		Splice Site
MET	W540*		Nonsense
MGC8407	Splice Site		Splice Site
MINK1	A554V		Missense
MINK1	P513L		Missense
MLCK	G601E		Missense
MYLK2	S72N		Missense
MYO3A	fs		Ins/del
MYO3B	R579K		Missense
NEK10	I95V		Missense
NEK11	L170L		Missense
NLK	A331T		Missense
NRBP2	V315M		Missense
NRK	R1374X		Missense
NTRK3	D405N		Missense
NTRK3	N95S		Missense
PCTK2	L310L		Missense
PDGFRA	G829R		Missense
PDGFRA	I1050T		Missense
PDGFRA	S1048fs*3		Nonsense
PDGFRA	W349C		Missense
PDGFRB	A74T		Missense
PDGFRB	L986F		Missense
PDK2	G342R		Missense
PDK2	P182S		Missense
PDPK1	S94S		Missense
PIK3C2B	E1144K		Missense
PIK3CA	M2V	2	Missense
PIK3CA	C420R	2	Missense
PIK3CA	delE110		Missense
PIK3CA	E545A		Missense
PIK3CA	E545K		Missense
PIK3CA	F83S		Missense
PIK3CA	G1049S		Missense
PIK3CA	G118D		Missense
PIK3CA	H1047L		Missense
PIK3CA	H1047R		Missense
PIK3CA	H1047Y		Missense
PIK3CA	L866F		Missense
PIK3CA	LWGIHLM10del		Ins/del
PIK3CA	M1043V		Missense
PIK3CA	N345K		Missense
PIK3CA	P18del		Ins/del

Kinase	Amino acid mutation	Frequency	Type of mutation
PIK3CA	R88Q	2	Missense
PIK3CG	A445V		Missense
PIK3R1	D560Y		Missense
PIK3R1	DKRMNS560del		Ins/del
PIK3R1	E439del		Ins/del
PIK3R1	G376R		Missense
PIK3R1	H450_E451del		Ins/del
PIK3R1	KS459delN		Ins/del
PIK3R1	N564K		Missense
PIK3R1	R574delKDERPILDVVDSKRCSAKEVERVVGQ*		Nonsense
PIK3R1	T576del		Ins/del
PIK3R1	W583del		Ins/del
PIK3R4	V1045I		Missense
PIM1	N269S		Missense
PINK1	P215L		Missense
PIP5K1A	G453D		Missense
PRKCA	P613S		Missense
PRKCB1	D631N		Missense
PRKCB1	V496M		Missense
PRKCBP1	D726N		Missense
PRKCD	R67C		Missense
PRKCN	V716M		Missense
PRKCZ	D230N		Missense
PRKCZ	E574K		Missense
PRKCZ	R260H		Missense
PRKD2	D411N		Missense
PRKDC	P2022S		Missense
PRKDC	P2531S		Missense
PRKDC	S3457F		Missense
PRKDC	Splice Site		Splice Site
PRKG1	L647L		Missense
PRKG2	E402K		Missense
PRKG2	L136M		Missense
PTK2	A612V		Missense
RAGE	G177G		Missense
RET	R330W		Missense
RIPK3	R37R		Missense
RIPK4	P222Q		Missense
ROCK1	E670_I671del		Ins/del
ROR2	A271T		Missense
ROR2	E519K		Missense
ROS1	Splice Site	2	Splice Site
ROS1	V1666I		Missense
RPS6KA3	L608F		Missense
RPS6KA3	L381H		Missense
RPS6KB	Splice Site		Splice Site
RPS6KC1	Q741*		Nonsense
SGK	K152K		Missense
SGK2	R152G		Missense
SgK494	D359N		Missense
SgK495	Q271*		Missense
SNF1LK	G211S		Missense
SNF1LK2	A635V		Missense
SNF1LK2	G497E		Missense
SRPK2	G243D		Missense
SRPK2	G317D		Missense

Kinase	Amino acid mutation	Frequency	Type of mutation
STK33	W401X		Missense
STK36	W184*		Missense
STK39	I208T		Missense
STYK1	V395I		Missense
STYK1	L316F		Missense
TAF1	G73E		Missense
TAF1	S1654T		Missense
TAF1L	H1549Y		Missense
TAF1L	C965Y		Missense
TAOK1	N639S		Missense
TAOK3	R490C		Missense
TBK1	A535T		Missense
TGFBR2	A204D		Missense
TGFBR2	I120L		Missense
TLK1	L636F		Missense
TNK2	A50T		Missense
TNK2	R147*		Nonsense
TNK2	W138*		Nonsense
TRIB3	T60I		Missense
TRIB3	C96C		Missense
TRIM24	R391H		Missense
TRIM33	K1057*		Nonsense
TRIM33	M580I		Missense
TRIM33	P885S		Missense
TRIO	A156_L157insP		Ins/del
TRPM6	T1218I		Missense
TRPM7	V1519I		Missense
TRRAP	D3788G		Missense
TRRAP	E2017K		Missense
TRRAP	P3199S		Missense
TRRAP	V2301I		Missense
TTN	A5502T		Missense
TTN	G16596V		Missense
TTN	P17151S		Missense
TTN	V6096I		Missense
TYRO3	A709T		Missense
ULK1	Splice Site		Splice Site
WEE1	G160S		Missense
WNK1	G1765S		Missense
WNK2	A1267T		Missense

Table 1. Identified mutations in kinases in glioblastoma
283 Non-synonymous somatic mutations or somatic mutations affecting splice sites identified in 106 kinases in glioblastoma. Frequency indicates the number of times that mutation has been found. Abbreviations: Del, deletion; Fs, frameshift; Ins, insertion.

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