Comprehensive genomic characterization defines human glioblastoma genes and core pathways

The Cancer Genome Atlas Research Network*

Human cancer cells typically harbour multiple chromosomal aberrations, nucleotide substitutions and epigenetic modifications that drive malignant transformation. The Cancer Genome Atlas (TCGA) pilot project aims to assess the value of large-scale multi-dimensional analysis of these molecular characteristics in human cancer and to provide the data rapidly to the research community. Here we report the interim integrative analysis of DNA copy number, gene expression and DNA methylation aberrations in 206 glioblastomas—the most common type of primary adult brain cancer—and nucleotide sequence aberrations in 91 of the 206 glioblastomas. This analysis provides new insights into the roles of ERBB2, NF1 and TP53, uncovers frequent mutations of the phosphatidylinositol-3-OH kinase regulatory subunit gene PIK3R1, and provides a network view of the pathways altered in the development of glioblastoma. Furthermore, integration of mutation, DNA methylation and clinical treatment data reveals a link between MGMT promoter methylation and a hypermutator phenotype consequent to mismatch repair deficiency in treated glioblastomas, an observation with potential clinical implications. Together, these findings establish the feasibility and power of TCGA, demonstrating that it can rapidly expand knowledge of the molecular basis of cancer.
Background

- Comprehensive sequencing of cancer genomes now feasible
- Median survival just over 1 year in GBM
- TCGA (The Cancer Genome Atlas) analyzed Glioblastoma
- Used primary Glioblastoma for this study
- 2 decades of traditional study have implicated 3 major pathways
- Genome-wide analysis may give a more comprehensive view of the GBM genome
Methods

- 52 page supplement lists complete methods in detail (n = 537)
- Used primary GBM with >80% Tumor Nuclei and <50% necrosis (n = 234)
- Extracted DNA/RNA and performed quality control (n = 147)
- 206 were screened for copy number, expression and DNA methylation
- Of those, 143 had blood/tissue DNA for comparison
- 21 were also post-treatment glioblastomas
Copy Number Alterations

- Measured by 3 microarray platforms
- Confirmed previously known alterations (i.e., EGFR amplification)
- Identified new alterations (NF1 deletions, Akt3 amplifications)
- ~76% of alterations confirmed on expression level
Patterns of Somatic Mutation

- 91 samples from 143 were chosen
- Had normal tissue controls
- 72 untreated, 19 treated
- Sequenced 601 genes for mutations
- Background mutation rate 1.4 untreated vs 5.8 for treated
Hypermutator Phenotype

- Standard treatment of Glioblastoma is resection + XRT + temazololamide
- MGMT methylation status is prognostic of response to temazololamide (methylated = better response)
- 7 hypermutator phenotypes
  - 3/7 s/p temazololamide
  - 4/7 s/p lomustine or combination
- 6/7 had mutations in MMR genes
Figure 1 | Significant copy number aberrations and pattern of somatic mutations. 

a. Frequency and significance of focal high-level CNAs. Known and putative target genes are listed for each significant CNA, with ‘Number of genes’ denoting the total number of genes within each focal CNA boundary.

b, c. Distribution of the number of silent (b) and non-silent (c) mutations across the 91 glioblastoma samples separated according to their treatment status, showing hypermutation in 7 out of the 19 treated samples.

d. Significantly mutated genes in 91 glioblastomas. The eight genes attaining a false discovery rate <0.1 are displayed here. Somatic mutations occurring in untreated samples are in dark blue; those found in statistically non-hypermuted and hypermutated samples among the treated cohort are in respectively lighter shades of blue. Numbers of events in each group are noted.
NF1 and EGFR Family Mutations

- NF1 controversial in GBM
- 19 NF1 mutations in 13/91 samples
- 30/206 heterogeneous deletions
- Likely inactivating
- EGFR has common vIII activating mutant, identified on genomic profiling
- 41/91 with alterations, mutations
- ERBB2 (Her2/neu) mutations identified in 13 samples 11/91 patients
Somatic PI3K Mutations

- PI3K complex comprised of p110α (PIK3CA) and regulatory component p85α (PIK3R1)
- PIK3CA mutations found to interact with regulatory PIK3R1 site
- PIK3R1 mutations not well known in GBM
- Found mutations (9/91) likely interfere with binding to regulatory site
Figure 3 | **PIK3R1** and **PIK3CA** mutations in glioblastoma. **a**, The locations of mutations found in TCGA tumours are indicated above the backbone. ABD, adaptor binding domain; RBD, Ras binding domain; C2, membrane-binding domain; nSH2, N-terminal SH2 domain; iSH2, inter-SH2 domain; cSH2, C-terminal SH2 domain. **b**, Three mutations found in the interaction interface of the C2 domain of p110α with iSH2 of p85α. Two residues of p85α, D560 and N564, are within hydrogen-bonding distance of the C2 residue of p110α, N345.
MGMT Methylation and MMR

- Cancer specific mutations of CpG were measured relative to normal brain
- Promoter methylation of MGMT linked to GBM sensitivity to alklyating agents
- 19/91 samples showed MGMT promoter methylation
- MGMT methylation status correlated with hypermutator phenotype
- Mutations consistent with inability to repair damaged guanine residues caused by treatment
Integrative Analysis Begins to Define GBM Core Pathways

- Mapped unequivocal genomic alterations onto respective major pathways
- 3 major pathways involved, RTK, p53, RB1
- 59%, 70%, 66% respectively by copy number data alone in 206 samples
- In 91 samples with sequencing data, 88%, 78%, 87%
- Statistical trend toward mutual exclusivity in each pathway
- Multiple pathways implicated in each sample, 74% had derangements of all 3
Figure S8. Signaling Pathway Alterations (DNA Copy Number and Mutations, n=91)
Conclusions

• TP53, RB1 and RTK pathways involved in GBM pathogenesis
• Major mutations pin-pointed, suggests therapeutic targets
• i.e. tumors with PTEN deletions may benefit from a PI3K inhibitor, whereas tumors with Akt3 activation may be refractory
• In MGMT methylated patients treated with temazolamide, may develop hypermutator phenotype