Published OnlineFirst June 30, 2009; DOI: 10.1158/0008-5472.CAN-09-1055

Sample Type Bias in the Analysis of Cancer Genomes

David A. Solomon,[1] Jung-Sik Kim,[1] Habtom W. Ressom,[1] Zita Sibenaller,[3] Timothy Ryken,[3] Walter Jean,[2] Darell Bigner,[4] Hai Yan,[4] and Todd Waldman[1]

[1]Department of Oncology, Lombardi Comprehensive Cancer Center and [2]Department of Neurosurgery, Georgetown University School of Medicine, Washington, District of Columbia; [3]Department of Neurosurgery, University of Iowa College of Medicine, Iowa City, Iowa; and [4]Preston Robert Tisch Brain Tumor Center, Duke University School of Medicine, Durham, North Carolina

Abstract

There is widespread agreement that cancer gene discovery requires high-quality tumor samples. However, whether primary tumors or cultured samples are superior for cancer genomics has been a longstanding subject of debate. This debate has recently become more important because federally funded cancer genomics has been centralized under The Cancer Genome Atlas, which has chosen to focus exclusively on primary tumors. Here, we provide a data-driven "perspective" on the effect of sample type selection on cancer genomics research. We show that, in the case of glioblastoma multiforme, primary tumors and xenografts are best for the identification of amplifications, whereas xenografts and cell lines are superior for the identification of homozygous deletions. We also note that many of the most important oncogenes and tumor suppressor genes have been discovered through the use of cell lines and xenografts, and highlight the lack of published evidence supporting the dogma that *ex vivo* culture generates artifactual genetic lesions. Based on this analysis, we suggest that cancer genomics projects such as The Cancer Genome Atlas should include a variety of sample types such as xenografts and cell lines in their integrated genomic analysis of cancer. [Cancer Res 2009;69(14):5630–3]

Introduction

After several decades in which cancer genomics research was performed in individual laboratories and funded by single-investigator grants, the field has recently been centralized and expanded under the auspices of The Cancer Genome Atlas (TCGA), which is performing integrated genomic analysis on a large number of samples from a wide range of common human tumor types. TCGA was initiated in December 2005, recently completed a 3-year pilot project [focused on glioblastoma multiforme (GBM), ovarian cancer, and lung cancer], and is currently organizing itself to begin the production phase of genomic analysis on a wider range of tumor types.

The procurement of high-quality cancer samples is the critical first step for cancer genomics projects such as TCGA. There are four principle types of human cancer samples available for such studies—primary tumors, primary cultures, primary xenografts, and established cell lines. The availability of each sample type is somewhat tumor type–specific (e.g., breast cancers do not efficiently form xenografts). Each of these sample types has unique advantages and disadvantages that are thought to affect the success of genomic analyses (see Supplementary Table S1).

Unlike other ongoing cancer genomics projects (1–3), TCGA has chosen to focus exclusively on the collection and analysis of primary tumor samples. This decision was based on considerations such as the fact that primary tumors can most easily be collected in large numbers in a prospective fashion, and the concern that *ex vivo* culture could induce artifactual genetic lesions. However, this decision was not based strictly on scientific data, as few (if any) published studies have directly evaluated the advantages and disadvantages of various sample types for genetic analysis.

We initially became interested in this issue of sample type selection for cancer genomics because, as TCGA was performing copy number analyses on GBM primary tumor samples (4), we were performing similar analyses on a panel of all four GBM sample types (5, 6). The results of these studies, described comprehensively for the first time in detail below, suggested that whereas primary tumors are an ideal sample type for the identification of genomic amplifications, they are inferior to xenografts and cell lines for the identification of genomic deletions. As such, this "perspective" will describe the effects of sample type on copy number analysis in GBM, examine the evidence supporting the widely accepted idea that cultured sample types contain artifactual genetic lesions, and review the role of different sample types in the history of cancer gene discovery.

Comparative Copy Number Analysis of Diverse GBM Sample Types

In an effort to experimentally address issues in sample type selection for cancer genomics projects, copy number analysis was performed on 58 GBM samples derived from all four GBM sample types—primary tumors, primary cultures, primary xenografts, and established cell lines.[5] Copy number data from an additional panel of 50 cell lines were also analyzed.[6]

Initially, we identified amplifications and deletions of the major GBM oncogenes and tumor suppressor genes (Table 1*A*; Supplementary Table S2). There was a substantial discrepancy in the frequency of oncogene amplification between sample types. For

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Todd Waldman, Lombardi Comprehensive Cancer Center, E304 Research Building, 3970 Reservoir Road, Northwest, Washington, DC 20057. Phone: 202-687-1340; Fax: 202-687-7505; E-mail: waldmant@georgetown.edu.

doi:10.1158/0008-5472.CAN-09-1055

[5] These data were generated using Affymetrix 250K *Nsp*I SNP arrays and analyzed using dChip, a publicly available software program (http://biosun1.harvard.edu/complab/dchip/). These data have been reported on previously (5, 6), and the raw and processed data sets have been deposited into the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), accession number GSE13021.

[6] Copy number data for a panel of 50 malignant glioma cell lines using Affymetrix SNP 6.0 arrays was generated by the Cancer Genome Project of the Wellcome Trust Sanger Institute, and is publicly available at http://www.sanger.ac.uk/genetics/CGP.

Published OnlineFirst June 30, 2009; DOI: 10.1158/0008-5472.CAN-09-1055

Table 1. Significant sample type effects on copy number alterations in GBM

(A)

	206 TCGA primary tumors	12 primary tumors	10 primary cultures	15 xenografts	21 cell lines	50 Sanger CGP cell lines
High-level amplifications						
EGFR	43%	**83%**	**10%**	40%	**5%**	**2%**
CDK4/6	16%	8%	0%	13%	5%	**4%**
MDM2/4	15%	17%	10%	13%	10%	**4%**
PDGFRA	11%	17%	0%	7%	0%	**2%**
CCND1/D2/D3	4%	8%	0%	7%	5%	2%
Genomic deletions						
CDKN2A/B	55%	58%	60%	**87%**	**81%**	**70%**
PTEN	8%	8%	10%	7%	14%	**18%**
CDKN2C	3%	0%	10%	**27%**	**19%**	**20%**
NF1	2%	0%	0%	7%	5%	**8%**

(B)

Genomic deletions					
CDKN2A	1.0 ± 0.3	**0.6 ± 0.1**	**0.3 ± 0.2**	**0.2 ± 0.1**	**0.3 ± 0.1**
PTEN	1.0 ± 0.3	0.7*	0.2*	0.2*	**0.5 ± 0.4**
CDKN2C	1.1 ± 0.3	—	0.4*	1.1 ± 0.2	**0.5 ± 0.3**
NF1	1.4 ± 0.2	—	—	0.3*	0.3*

NOTE: *A,* percentage of tumor samples with focal (<10 Mb) genomic deletion and high-level (copy number >7) focal amplification of the indicated gene loci. *B,* mean copy number and SD at the indicated gene loci in those tumor samples with focal genomic deletion. Two-tailed unpaired *t* test analysis was used to compare the statistical significance of any difference in frequency of copy number alteration (*A*) and mean copy number (*B*) between the TCGA primary tumors and other GBM tumor samples. Statistically significant differences ($P < 0.05$) in frequency (*A*) and copy number means (*B*) are highlighted in boldface. —, no samples with focal genomic deletion at the indicated gene loci. *, less than three samples with genomic deletion, no SD calculation or *t* test analysis possible.

example, amplification of *EGFR* was commonly found in primary tumors and xenografts, but rarely found in primary cultures and cell lines. This phenomenon of loss of amplifications in GBM cell lines has been previously described but was thought to be specific to *EGFR* (7, 8). However, our data indicate that amplification of other GBM oncogenes such as *PDGFRA*, *CDK4*, and *MDM4* is similarly lost during *in vitro* culture, and suggest that primary tumors and xenografts are the best sample type for the identification of novel amplicons containing candidate oncogenes.

Of note, this loss of oncogene amplification during tissue culture seems to be tumor type–specific, as there are examples of tumor types in which oncogenes are amplified at a similar frequency in both cultured and uncultured samples. For example, *MYC* or *MYCN* are amplified in 28 out of 37 neuroblastoma cell lines (76%),[7] comparable to that observed in neuroblastoma primary tumor samples (9).

There was also a discrepancy in the frequency of identifiable tumor suppressor gene deletions between sample types. For example, deletions of the *CDKN2A/B* locus were identifiable in a much higher fraction of xenografts and cell lines than in primary tumors and primary cultures (Table 1*A*; Supplementary Table S2). Importantly, this disparity was not limited to CDK inhibitors, but was also present for *PTEN*, *NF1*, and *PTPRD*. In the case of *PTPRD*, deletions in primary tumors were very rarely identified, and therefore TCGA did not sequence the gene in their GBM pilot project (4). It was only the use of additional sample types that enabled the identification of frequent deletions and somatic mutations of this emerging tumor suppressor gene in GBM (6).

To determine whether the presence of admixed nonneoplastic cells and intratumoral genetic heterogeneity was responsible for impeding the identification of deletions in primary tumor samples, we analyzed *CDKN2A/B* and *CDKN2C* in both a first passage xenograft and the primary tumor from which it was derived. Deletions of both loci were present in the xenograft, but were largely masked in the primary tumor by the presence of admixed nonneoplastic cells and intratumoral genetic heterogeneity (5, 10). This same observation is evident when comparing copy numbers at each of the major tumor suppressor genes—deletions in primary tumors are more difficult to identify because their average copy number is significantly higher and their boundaries are less discrete (Table 1*B*; Fig. 1; Supplementary Table S3).

Taken together, these data indicate that xenografts and cell lines are superior to primary tumors for the identification of genomic deletions. The presence of nonneoplastic cells and heterogeneity in even the most homogeneous tumor types such as GBM results in substantial "noise" in the analysis, which hinders the identification of deletions and leads to a high rate of false-negatives. Such noise would be expected to pose similar problems in other cancer genomics assays as well, including DNA sequencing.

[7] Copy number data for a panel of 37 neuroblastoma cell lines using Affymetrix SNP 6.0 arrays was generated by the Cancer Genome Project of the Wellcome Trust Sanger Institute, and is publicly available at http://www.sanger.ac.uk/genetics/CGP.

Published OnlineFirst June 30, 2009; DOI: 10.1158/0008-5472.CAN-09-1055

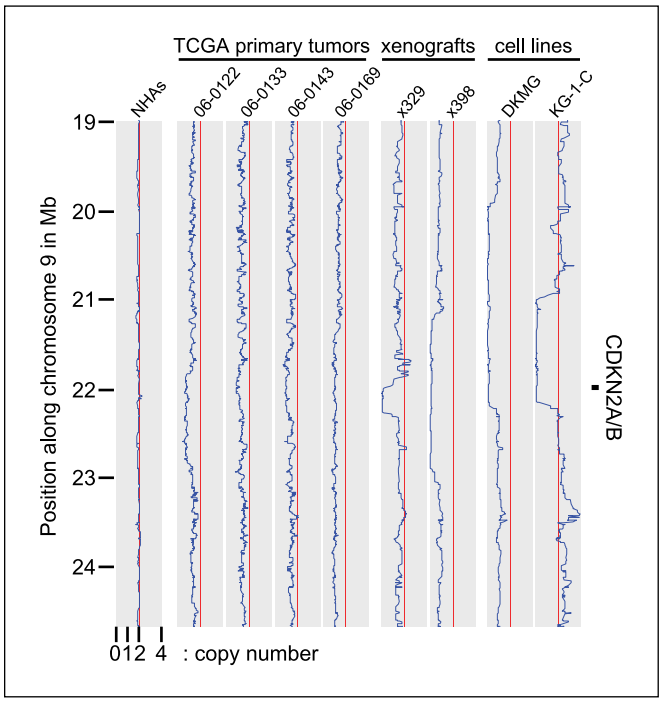

Figure 1. Copy number plots along chromosome 9p for four TCGA primary tumors (reported to have homozygous deletion of CDKN2A/B), two xenografts, two cell lines, and normal human astrocytes (*NHAs*). Each of the depicted xenografts and cell lines have homozygous deletion of the CDKN2A/B locus with copy number <0.2, whereas the four TCGA primary tumors have hemizygous/heterogeneous deletion with copy numbers of 1.07, 1.32, 1.22, and 1.29 for TCGA-06-0122, TCGA-06-0133, TCGA-06-0143, and TCGA-06-0169 respectively.

No Evidence of Artifactual Genetic Lesions Caused by *Ex vivo* Culture

Many cancer researchers favor using primary tumors rather than cultured samples because of the widespread belief that *ex vivo* culture can lead to the accumulation of spurious genetic alterations. Concerns of this type reached a pinnacle 15 years ago, when there was substantial controversy about whether the recently identified deletions and mutations of the $p16^{INK4a}$ tumor suppressor gene could be artifacts of *ex vivo* culture (11, 12). After substantial high-profile debate, this concern was eventually refuted and it is now universally accepted that $p16^{INK4a}$ is one of the most commonly inactivated tumor suppressor genes in human cancer. However, such concerns remain firmly entrenched in the minds of most cancer researchers.

To test whether these concerns are valid, we catalogued all the copy number alterations present in each of our 58 samples. Strikingly, there were no examples of recurrent deletions or amplifications present exclusively in cultured samples. Additionally, if *ex vivo* culture specifically enriches for cells with deleted tumor suppressor genes, one would similarly expect culture to enrich for cells with amplified oncogenes. Yet as we show in Table 1*A*, *ex vivo* culture leads to a decrease in oncogene amplification in GBM cells, not the predicted increase.

Next, a comprehensive search of the literature was performed in an effort to identify studies that document copy number alterations and/or mutations present exclusively in cultured samples but not in primary tumors. Although we were able to identify several studies which showed expression differences between primary tumors and cultured samples (13, 14), we were unable to identify any studies documenting genetic lesions unique to cultured samples.

In contrast, Jones and colleagues recently provided remarkably strong evidence in support of the idea that cultured samples faithfully recapitulate the genetic profile present in the tumor from which they were derived. In their study, 287 of 289 mutations (99.3%) initially discovered in human colon cancer xenografts and cell cultures were similarly present in the primary tumors from which the cultured samples were derived (15). These data indicate that *ex vivo* culture of colon tumors does not lead to the formation or accumulation of spurious genetic aberrations.

Based on these findings, we believe that there is little convincing evidence to support the dogma that *ex vivo* culture leads to artifactual deletions, amplifications, and somatic mutations. As such, the risk of failing to identify deletions in human cancer samples due to an exclusive focus on primary tumors is likely to be substantially greater than the risk of identifying spurious genetic events by including other sample types in the analysis. This is

Table 2. Sample types used in the initial discovery of major somatically altered cancer genes

Gene	Tumor type(s)	Sample type(s)	Reference
Oncogenes			
HRAS	Bladder carcinoma	Cell lines	(17)
KRAS	Colon carcinoma	Cell lines	(18)
NRAS	Neuroblastoma	Cell lines	(19)
MYC	Myeloid leukemia	Cell lines	(20)
EGFR	Glioma	Primary tumors	(21)
CTNNB1 (β-catenin)	Colon carcinoma	Cell lines	(22)
BRAF	Melanoma, others	Cell lines	(23)
PIK3CA	Colon carcinoma	Xenografts and primary cultures	(24)
Tumor suppressors			
RB1	Bladder carcinoma	Cell lines	(25, 26)
TP53	Colon carcinoma	Xenografts	(27)
CDKN2A ($p16^{INK4a}$)	Multiple	Cell lines	(11)
SMAD4	Pancreatic carcinoma	Xenografts	(28)
PTEN	Multiple	Cell lines, xenografts, and primary cultures	(29, 30)

Published OnlineFirst June 30, 2009; DOI: 10.1158/0008-5472.CAN-09-1055

especially true because it is relatively trivial to determine whether an event initially discovered in cultured samples is similarly present in primary tumors, as was the case, for example, with the recent identification of *CDKN2C* as a GBM tumor suppressor gene (5, 16).

Cultured Samples Have Been Used in the Discovery of Most Oncogenes and Tumor Suppressors

Finally, we looked back through the modern history of cancer genetics to identify the sample types used to discover the most commonly altered oncogenes and tumor suppressor genes (Table 2). Notably, most somatically altered cancer genes that were not discovered via linkage analysis were initially identified using xenografts and cell lines. This includes p53, PTEN, $p16^{INK4a}$, K-Ras, PIK3CA, B-Raf, and others (11, 17–30). Based on this history, it seems prudent to include cultured samples in any cancer genomics initiative whose major goal is the identification of novel somatically altered cancer genes.

Conclusions

Here, we provide three rationales for the inclusion of cultured samples in TCGA and other cancer genomics efforts. First, we show that in the case of one major human tumor type, there are significant differences in the utility of different sample types for the identification of copy number alterations. Second, we document that there is little evidence supporting the popular notion that *ex vivo* culture of human tumors leads to spurious genetic alterations. And third, we show that most major somatically altered cancer genes discovered to date were identified using xenografts and cell lines. Based on these arguments, we believe it would be prudent for TCGA to include a range of sample types in their burgeoning analysis of cancer genomics. We also note that the use of cultured samples is supported by the Cancer Genome Project of the Wellcome Trust Sanger Institute and is within the agreed guidelines of the International Cancer Genome Consortium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 3/20/09; revised 5/6/09; accepted 5/13/09; published OnlineFirst 6/30/09.

Grant support: National Cancer Institute, American Cancer Society, and Georgetown University School of Medicine (T. Waldman).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

1. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153–8.
2. Parsons DW, Jones S, Zhang X, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807–12.
3. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801–6.
4. The Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 2008;455:1061–8.
5. Solomon DA, Kim JS, Jenkins S, et al. Identification of $p18^{INK4c}$ as a tumor suppressor gene in glioblastoma multiforme. Cancer Res 2008;68:2564–9.
6. Solomon DA, Kim JS, Cronin JC, et al. Mutational inactivation of PTPRD in glioblastoma multiforme and malignant melanoma. Cancer Res 2008;68:10300–6.
7. Bigner SH, Humphrey PA, Wong AJ, et al. Characterization of the epidermal growth factor receptor in human glioma cell lines and xenografts. Cancer Res 1990;50:8017–22.
8. Pandita A, Aldape KD, Zadeh G, Guha A, James CD. Contrasting *in vivo* and *in vitro* fates of glioblastoma cell subpopulations with amplified EGFR. Genes Chromosomes Cancer 2004;39:29–36.
9. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 1984;224:1121–4.
10. Solomon DA, Kim JS, Jean W, Waldman T. Conspirators in a capital crime: co-deletion of $p18^{INK4c}$ and $p16^{INK4a}/p14^{ARF}/p15^{INK4b}$ in glioblastoma multiforme. Cancer Res 2008;68:8657–60.
11. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. Science 1994;264:436–40.
12. Cairns P, Mao L, Merlo A, et al. Rates of p16 (MTS1) mutations in primary tumors with 9p loss. Science 1994;265:415–6.
13. Camphausen K, Purow B, Sproull M, et al. Influence of *in vivo* growth on human glioma cell line gene expression: convergent profiles under orthotopic conditions. Proc Natl Acad Sci U S A 2005;102:8287–92.
14. Stein WD, Litman T, Fojo T, Bates SE. A serial analysis of gene expression (SAGE) database analysis of chemosensitivity: comparing solid tumors with cell lines and comparing solid tumors from different origins. Cancer Res 2004;64:2805–16.
15. Jones S, Chen WD, Parmigiani G, et al. Comparative lesion sequencing provides insights into tumor evolution. Proc Natl Acad Sci U S A 2008;105:4283–8.
16. Wiedemeyer R, Brennan C, Heffernan TP, et al. Feedback circuit among INK4 tumor suppressors constrains human glioblastoma development. Cancer Cell 2008;13:355–64.
17. Taparowsky E, Suard Y, Fasano O, Shimizu K, Goldfarb M, Wigler M. Activation of the T24 bladder carcinoma transforming gene is linked to a single amino acid change. Nature 1982;300:762–5.
18. McCoy MS, Toole JJ, Cunningham JM, Chang EH, Lowy DR, Weinberg RA. Characterization of a human colon/lung carcinoma oncogene. Nature 1983;302:79–81.
19. Shimizu K, Goldfarb M, Suard Y, et al. Three human transforming genes are related to the viral ras oncogenes. Proc Natl Acad Sci U S A 1983;80:2112–6.
20. Collins S, Groudine M. Amplification of endogenous myc-related DNA sequences in a human myeloid leukaemia cell line. Nature 1982;298:679–81.
21. Libermann TA, Nusbaum HR, Razon N, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumors of glial origin. Nature 1985;313:144–7.
22. Morin PJ, Sparks AB, Korinek V, et al. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science 1997;275:1787–90.
23. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–54.
24. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science 2004;304:554.
25. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986;323:643–6.
26. Horowitz JM, Yandell DW, Park SH, et al. Point mutational inactivation of the retinoblastoma antioncogene. Science 1989;243:937–40.
27. Baker SJ, Fearon ER, Nigro JM, et al. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science 1989;244:217–21.
28. Hahn SA, Schutte M, Hoque AT, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 1996;271:350–3.
29. 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;275:1943–7.
30. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15:356–62.

Supplemental Table Legend

Supplemental Table 1. Description and features of the four major human cancer sample types.

Supplemental Table 2. Copy number alterations present in a panel of 50 malignant glioma cell lines. These copy number data were generated on Affymetrix 6.0 SNP arrays by the Cancer Genome Project of the Wellcome Trust Sanger Institute.

Supplemental Table 3. Mean copy number values reported by the TCGA of the genomic deletions in primary tumor samples containing homozygous deletions of CDKN2A (a), PTEN (b), CDKN2C (c), and NF1 (d).

Supplemental Table 1

Primary Tumors

- Tumor tissue obtained directly from the operating room
- Faithfully represent the genetic, epigenetic, and transcriptional profile of human tumors
- Contain admixed non-neoplastic cells
- Contain intratumoral genetic heterogeneity
- Often contain substantial necrosis
- Limited in quantity (*i.e.* individual tumors are non-renewable)
- Susceptible to ischemia and degradation due to delays between surgical resection and freezing/fixation

Primary Xenografts

- Primary tumors that have been implanted into immunodeficient mice and have re-grown into tumors
- Largely free of admixed non-neoplastic human cells
- Unlimited in quantity (*i.e.* xenografts can be repeatedly passaged)
- Some tumor types do not efficiency form xenografts
- Xenograft growth can require several months

Primary Cultures

- Early passage cultures of dissociated primary tumors
- Often contain admixed non-neoplastic cells
- Limited in quantity

Established Cell Lines

- Late passage cultures of dissociated primary tumors
- Do not contain admixed non-neoplastic human cells
- Unlimited in quantity (*i.e.* cell lines can be repeatedly passaged)
- Some tumor types do not efficiently form cell lines
- Establishment can require several months
- Matched normal tissue is often not available

Supplemental Table 2

	Focal (<10 Mb) genomic deletions					High-level (copy number >7) focal amplifications								
cell line	**CDKN2A**	**CDKN2B**	**PTEN**	**CDKN2C**	**NF1**	**EGFR**	**CDK4**	**CDK6**	**MDM2**	**MDM4**	**PDGFRA**	**CCND1**	**CCND2**	**CCND3**
8-MG-BA														
A172	homozygous	homozygous	homozygous											
AM-38	homozygous	homozygous												
Becker			homozygous	homozygous										
CAS-1	homozygous	homozygous												
CCF-STTG1	hemizygous	hemizygous						amplified	amplified					amplified
D-245MG	homozygous	homozygous	homozygous	homozygous										
D-247MG	homozygous	homozygous												
D-263MG	homozygous	homozygous		hemizygous										
D-336MG	homozygous	homozygous	homozygous											
D-392MG	homozygous	homozygous												
D-397MG	homozygous	homozygous												
D-423MG							amplified							
D-502MG	homozygous	homozygous												
D-538MG	homozygous	homozygous												
D-542MG														
D-566MG	homozygous	homozygous												
DBTRG-05MG	homozygous	homozygous												
DK-MG	homozygous	homozygous				amplified			amplified					
GAMG	homozygous	homozygous												
GB-1	homozygous	homozygous		hemizygous										
GMS-10			hemizygous		homozygous									
H4	homozygous	homozygous	homozygous											
KALS-1														
KINGS-1	homozygous	homozygous		homozygous	hemizygous									
KNS-42														
KNS-81-FD	homozygous	homozygous												
KS-1	homozygous	homozygous												
LN-405														
M059J														
MOG-G-CCM														
MOG-G-UVW														
NMC-G1	homozygous	homozygous												
no-10			homozygous											
no-11					homozygous									
SF126	homozygous	homozygous												
SF268	homozygous	homozygous												
SF295	homozygous	homozygous												
SF539			homozygous											
SK-MG-1	homozygous	homozygous			homozygous									
SNB19	homozygous	homozygous		hemizygous										
SNB75	hemizygous	hemizygous												
SW1088	homozygous	homozygous	homozygous											
SW1783											amplified			
T98G	homozygous			homozygous										
U-118-MG	homozygous	homozygous												
U-87-MG	homozygous	homozygous		homozygous										
U251*	homozygous	homozygous		homozygous										
YH-13	homozygous	homozygous												
YKG-1	homozygous	homozygous		homozygous										
Total events	**35**	**34**	**9**	**10**	**4**	**1**	**1**	**1**	**2**	**0**	**1**	**0**	**0**	**1**
Frequency	**70%**	**68%**	**18%**	**20%**	**8%**	**2%**	**2%**	**2%**	**4%**	**0%**	**2%**	**0%**	**0%**	**2%**

*U251 cell line is synonymous with B2-17 cell line that was also analyzed.

Supplemental Table 3a

Tumor #	CDKN2A	mean copy number
02-0001	homo del	1.236
02-0004	homo del	1.475
02-0009	homo del	0.701
02-0014	homo del	0.664
02-0015	homo del	0.866
02-0016	homo del	0.974
02-0021	homo del	1.697
02-0023	homo del	1.001
02-0024	homo del	0.757
02-0027	homo del	0.980
02-0034	homo del	0.998
02-0037	homo del	0.819
02-0038	homo del	0.948
02-0043	homo del	0.615
02-0047	homo del	1.079
02-0048	homo del	0.733
02-0057	homo del	1.592
02-0064	homo del	1.176
02-0068	homo del	1.155
02-0071	homo del	1.064
02-0085	homo del	1.457
02-0086	homo del	1.172
02-0089	homo del	1.099
02-0104	homo del	0.694
02-0106	homo del	0.876
02-0111	homo del	1.422
02-0115	homo del	1.293
02-0116	homo del	0.661
02-0260	homo del	0.776
02-0269	homo del	1.214
02-0281	homo del	0.794
02-0285	homo del	1.484
02-0289	homo del	1.074
02-0290	homo del	0.988
02-0317	homo del	0.853
02-0324	homo del	unavailable
02-0325	homo del	1.432
02-0326	homo del	1.385
02-0333	homo del	0.709
02-0422	homo del	0.942
02-0430	homo del	0.911
02-0439	homo del	2.137

02-0440	homo del	0.778
02-0446	homo del	1.150
02-0451	homo del	1.096
06-0122	homo del	1.068
06-0124	homo del	1.220
06-0125	homo del	0.757
06-0126	homo del	0.925
06-0127	homo del	0.975
06-0133	homo del	1.319
06-0137	homo del	0.185
06-0143	homo del	1.221
06-0145	homo del	0.410
06-0148	homo del	0.703
06-0154	homo del	1.037
06-0156	homo del	0.719
06-0158	homo del	1.010
06-0164	homo del	1.315
06-0166	homo del	0.984
06-0169	homo del	1.292
06-0171	homo del	1.202
06-0175	homo del	1.059
06-0179	homo del	1.566
06-0185	homo del	0.749
06-0187	homo del	0.787
06-0188	homo del	0.825
06-0194	homo del	1.436
06-0201	homo del	1.444
06-0208	homo del	1.040
06-0210	homo del	0.706
06-0211	homo del	1.096
06-0214	homo del	1.169
06-0219	homo del	0.815
06-0241	homo del	0.830
06-0394	homo del	1.666
06-0397	homo del	0.992
06-0410	homo del	0.755
06-0412	homo del	0.981
06-0413	homo del	0.797
06-0644	homo del	0.966
06-0646	homo del	0.467
06-0648	homo del	0.366
08-0244	homo del	0.925
08-0246	homo del	0.981
08-0345	homo del	0.942
08-0346	homo del	1.297
08-0347	homo del	1.196

08-0348	homo del	1.038
08-0353	homo del	0.882
08-0354	homo del	1.103
08-0355	homo del	0.964
08-0357	homo del	1.104
08-0358	homo del	0.258
08-0359	homo del	1.130
08-0360	homo del	1.493
08-0375	homo del	0.796
08-0390	homo del	1.468
08-0392	homo del	0.953
08-0509	homo del	1.398
08-0510	homo del	1.119
08-0511	homo del	1.942
08-0512	homo del	1.471
08-0514	homo del	0.948
08-0517	homo del	0.749
08-0518	homo del	0.645
08-0520	homo del	0.937
08-0521	homo del	0.970
08-0522	homo del	1.245
08-0525	homo del	0.915
08-0529	homo del	0.978
08-0531	homo del	0.830
12-0615	homo del	0.530
12-0620	homo del	0.858

Supplemental Table 3b

Tumor #	PTEN	mean copy number
02-0006	homo del	1.243
02-0034	homo del	1.035
02-0039	homo del	1.264
02-0043	homo del	0.716
06-0149	homo del	1.097
06-0214	homo del	1.188
06-0219	homo del	0.781
06-0414	homo del	0.976
06-0645	homo del	1.144
06-0646	homo del	0.465
06-0648	homo del	0.433
08-0347	homo del	0.897
08-0386	homo del	unavailable
08-0512	homo del	1.411
12-0615	homo del	0.526
12-0619	homo del	1.417

Supplemental Table 3c

Tumor #	CDKN2C	mean copy number
02-0074	homo del	0.788
02-0333	homo del	0.725
02-0446	homo del	1.165
06-0188	homo del	1.089
06-0194	homo del	1.531
06-0410	homo del	0.735
08-0360	homo del	1.497

Supplemental Table 3d

Tumor #	NF1	mean copy number
02-0055	homo del	1.173
02-0324	homo del	unavailable
06-0166	homo del	1.639
06-0206	homo del	1.284