

Novel Somatic and Germline Mutations in Cancer Candidate Genes in Glioblastoma, Melanoma, and Pancreatic Carcinoma

Asha Balakrishnan,¹ Fonne E. Bleeker,^{1,2} Simona Lamba,¹ Monica Rodolfo,⁴ Maria Daniotti,⁴ Aldo Scarpa,⁶ Angela A. van Tilborg,^{2,3} Sieger Leenstra,² Carlo Zanon,¹ and Alberto Bardelli^{1,5}

¹Laboratory of Molecular Genetics, The Oncogenomics Center, Institute for Cancer Research and Treatment, University of Torino Medical School, Candiolo, Italy; Departments of ²Neurosurgery and ³Neuropathology, Academic Medical Center, University of Amsterdam, the Netherlands; ⁴Department of Experimental Oncology, Istituto Nazionale Tumori; ⁵FIRC Institute of Molecular Oncology, Milan, Italy; and ⁶Department of Pathology, Section of Anatomic Pathology, University of Verona, Verona, Italy

Abstract

A recent systematic sequence analysis of well-annotated human protein coding genes or consensus coding sequences led to the identification of 189 genes displaying somatic mutations in breast and colorectal cancers. Based on their mutation prevalence, a subset of these genes was identified as cancer candidate (CAN) genes as they could be potentially involved in cancer. We evaluated the mutational profiles of 19 CAN genes in the highly aggressive tumors: glioblastoma, melanoma, and pancreatic carcinoma. Among other changes, we found novel somatic mutations in *EPHA3*, *MLL3*, *TECTA*, *FBXW7*, and *OBSCN*, affecting amino acids not previously found to be mutated in human cancers. Interestingly, we also found a germline nucleotide variant of *OBSCN* that was previously reported as a somatic mutation. Our results identify specific genetic lesions in glioblastoma, melanoma, and pancreatic cancers and indicate that CAN genes and their mutational profiles are tumor specific. Some of the mutated genes, such as the tyrosine kinase *EPHA3*, are clearly amenable to pharmacologic intervention and could represent novel therapeutic targets for these incurable cancers. We also speculate that similar to other oncogenes and tumor suppressor genes, mutations affecting *OBSCN* could be involved in cancer predisposition. [Cancer Res 2007;67(8):3545–50]

Introduction

Cancer is a multistep, polygenic disease caused by accumulation of genetic alterations in oncogenes or tumor suppressor genes resulting in neoplastic transformation. Increasing evidence suggests that new and effective targets for diagnosis and therapy could be identified by mutation profiling of cancer genomes. A systematic analysis of 13,023 well-annotated human protein-coding genes, known as consensus coding sequences,⁷ was recently carried out by Sjöblom et al. (1). This strategy led to the identification of 189 genes displaying somatic mutations. To distinguish genes likely to contribute to tumorigenesis from those in which passenger mutations occurred by chance, the authors used a statistical approach that allowed the definition of a subset of candidate cancer (CAN)

genes. To determine the significance of these findings in cancers other than breast and colon, we analyzed a subset of CAN genes in glioblastoma multiforme, melanoma, and pancreatic ductal adenocarcinoma (PDAC). These tumor types are known to be highly malignant and resistant to treatment. For example, metastatic melanoma has a poor prognosis, with a median survival of 6 to 9 months (2). Glioblastoma multiforme is the most common brain tumor in adults and kills patients within a median of 14 months after diagnosis, even after surgical resection, radiotherapy, and concomitant chemotherapy (3). PDAC is highly aggressive and resistant to conventional and targeted therapeutic agents, resulting in a dismal 5-year survival rate of 3% to 5% (4).

In this study, we present the mutational profile of 19 CAN genes in glioblastoma multiforme, melanoma, and PDAC. The candidate genes were selected either because they displayed a mutation frequency above 10%, or because multiple mutations affecting a single amino acid residue were previously found in the same gene (1). Specifically, we examined the following genes and exons in which mutations have been recently described: *ABCA1*, *ADAMTSL3*, *ATP8B1*, *CUBN*, *DIP2C*, *EGFL6*, *EPHA3*, *EPHB6*, *FBXW7*, *FLNB*, *GNAS*, *MACF1*, *MLL3*, *OBSCN*, *PKHD1*, *SPTAN1*, *SYNE1*, *TECTA*, and *ZNF668*. Details on genes and exons analyzed can be found in Supplementary Table S1.

Materials and Methods

Twenty-three human glioblastoma multiforme samples and the matched normal DNA were obtained from the tumor bank maintained by the Departments of Neurosurgery and Neuropathology at the Academic Medical Center (Amsterdam, The Netherlands). The glioblastoma cell line U87MG, officially known as an astrocytoma grade 3 cell line, was provided by Dr. Chris van Bree (Department of Radiotherapy, Academic Medical Center, Amsterdam, The Netherlands). One of the changes we identified in *EPHA3* (K500N) occurred in U87MG, for which no matched normal is available. Therefore, the somatic status of this mutation could not be ascertained. The melanoma and PDAC tumor samples and matched normals were obtained from the tumor banks maintained by the Department of Experimental Oncology, Istituto Nazionale Tumori, Milan, Italy and the Department of Pathology, Section of Anatomic Pathology, University of Verona, Verona, Italy, respectively (Table 1). Genomic DNA was isolated as previously described (5), except for the PDAC samples that were isolated using DNeasy Blood & Tissue kit (Qiagen, Milan, Italy). For samples in which mutations were found, matching between germline and tumor DNA was verified by direct sequencing of 26 single nucleotide polymorphism (SNP) at 24 loci (data not shown).

PCR and sequencing primers were designed using Primer 3⁸ and synthesized by Invitrogen/Life Technologies, Inc. (Paisley, England;

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

A. Balakrishnan and F.E. Bleeker contributed equally to this study.

Requests for reprints: Alberto Bardelli, Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment, University of Torino, Medical School, Str prov 142 Km 3.95, Candiolo (TO), ZIP 10060, Italy. Phone: 39-119933235; Fax: 39-11993225; E-mail: a.bardelli@unito.it.

©2007 American Association for Cancer Research.
doi:10.1158/0008-5472.CAN-07-0065

⁷ <http://www.ncbi.nlm.nih.gov/projects/CCDS/>

⁸ http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

Table 1. Detailed information on the samples included in this study

Tumor type	Sample	Age	Gender	Grade	Tumor source	Tumor DNA source	Matched normal source	
Glioblastoma multiforme	T1	39	F	4	Primary tumor	Primary tumor	Blood	
	T03	52	F	4	Primary tumor	Primary tumor	Blood	
	T12	74	F	4	Primary tumor	Primary tumor	Blood	
	T37	69	F	4	Primary tumor	Primary tumor	Blood	
	T39	37	M	4	Primary tumor	Primary tumor	Blood	
	T52	72	F	4	Primary tumor	Primary tumor	Blood	
	T70	76	M	4	Primary tumor	Primary tumor	Blood	
	T71	66	F	4	Primary tumor	Primary tumor	Blood	
	T78	29	M	4	Primary tumor	Primary tumor	Blood	
	T79	54	F	4	Primary tumor	Primary tumor	Blood	
	T80	47	M	4	Primary tumor	Primary tumor	Blood	
	T83	52	M	4	Primary tumor	Primary tumor	Blood	
	T85	31	F	4	Primary tumor	Primary tumor	Blood	
	T86	73	M	4	Primary tumor	Primary tumor	Blood	
	T90	64	F	4	Primary tumor	Primary tumor	Blood	
	T91	64	M	4	Primary tumor	Primary tumor	Blood	
	T94	57	F	4	Primary tumor	Primary tumor	Blood	
	T99	62	F	4	Primary tumor	Primary tumor	Blood	
	T104	69	M	4	Primary tumor	Primary tumor	Blood	
	T105	41	M	4	Primary tumor	Primary tumor	Blood	
	T107	39	F	4	Primary tumor	Primary tumor	Blood	
	T111	46	F	4	Primary tumor	Primary tumor	Blood	
	T112	48	M	4	Primary tumor	Primary tumor	Blood	
T113	40	M	4	Primary tumor	Primary tumor	Blood		
Melanoma	U87MG	44	F	3	Primary tumor	Cell line	Not available	
	2A	51	M	4	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	4A	55	F	2B/3C	Primary tumor	Short-term cultures	EBV immortalized B lymphocytes	
	5A	36	F	3B/3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	6A	43	M	3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	7A	22	F	3B/3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	8A	55	M	3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	9A	53	M	3B/3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	10A	52	M	3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	11A	69	M	3A/3B	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	12A	70	M	4	Cutaneous metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	13A	59	F	4	Cutaneous metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	14A	56	M	3	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	15A	30	M	4-M _{1c}	Colon metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	16A	45	F	3B/3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	17A	51	M	4	Cutaneous metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	18A	68	M	4	Cutaneous metastasis	Short-term cultures	Blood	
	19A	71	F	3C T _{4a} N ₃ M ₀	Cutaneous metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	20A	24	M	3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	21A	36	F	3B/3C	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	22A	39	F	3C	Nodal metastasis	Short-term cultures	Blood	
	23A	56	M	4-M _{1c}	Lung metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	24A	49	M	4	Cutaneous metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	25A	49	M	4	Cutaneous metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	26A	62	F	4-M _{1a}	Nodal metastasis	Short-term cultures	EBV immortalized B lymphocytes	
	Pancreatic ductal adenocarcinoma	360	76	F	T ₃ N ₀ /G ₂	Primary tumor	Xenograft	Duodenum
		362	54	M	T ₃ N _{1b} /G ₃	Primary tumor	Xenograft	Pancreas
369		61	M	T ₃ N ₀ /G ₂	Primary tumor	Xenograft	Not available	
370		52	F	T ₃ N _{1b} /G ₂	Primary tumor	Xenograft	EBV immortalized B lymphocytes	
371		57	M	T ₃ N ₀ /G ₃	Primary tumor	Xenograft	Duodenum	
374		62	F	T ₃ N ₀ /G ₃	Primary tumor	Xenograft	Pancreas	
375		59	M	T ₃ N _{1a} /G ₃	Primary tumor	Xenograft	EBV immortalized B lymphocytes	
377		52	M	T ₃ N _{1a} /G ₂	Primary tumor	Xenograft	Pancreas	

(Continued on the following page)

Table 1. Detailed information on the samples included in this study (Cont'd)

Tumor type	Sample	Age	Gender	Grade	Tumor source	Tumor DNA source	Matched normal source
	379	62	F	T ₃ N ₁ /G ₂	Primary tumor	Xenograft	Spleen
	380	57	M	T ₃ N _{1a} /G ₂	Primary tumor	Xenograft	Duodenum
	382	44	F	T ₃ N ₀ /G ₂	Primary tumor	Xenograft	Duodenum
	384	69	F	T ₃ N ₀ /G ₂	Primary tumor	Xenograft	Spleen

NOTE: Clinical information of the patient, tumor staging, and source of the genomic DNA used for the mutational analysis are indicated. Abbreviations: F, female; M, male.

Supplementary Table S1). PCR primers were designed to amplify the selected exons and the flanking intronic sequences, including splicing donor and acceptor regions, of the 19 cancer genes. PCR products were ~400 bp in length, with multiple overlapping amplimers for larger exons. PCRs were done in both 384- and 96-well formats in 5- or 10- μ L reaction volumes, respectively, containing 0.25 mmol/L deoxynucleotide triphosphates, 1 mmol/L each of the forward and reverse primers, 6% DMSO, 1 \times PCR buffer, 1 ng/ μ L DNA, and 0.01 unit/ μ L Platinum Taq (Invitrogen/Life Technologies). A touchdown PCR program was used for PCR amplification (Peltier Thermocycler, PTC-200, MJ Research, Bio-Rad Laboratories, Inc., Italy).

PCR conditions were as follows: 94°C for 2 min; three cycles of 94°C for 15 s, 64°C for 30 s, 70°C for 30 s; three cycles of 94°C for 15 s, 61°C for 30 s, 70°C for 30 s; three cycles of 94°C for 15 s, 58°C for 30 s, 70°C for 30 s; and 35 cycles of 94°C for 15 s, 57°C for 30 s, and 70°C for 30 s, followed by 70°C for 5 min and 12°C thereafter.

PCR products were purified using AMPure (Agencourt Bioscience Corp., Beckman Coulter S.p.A, Milan, Italy). Cycle sequencing was carried out using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) with an initial denaturation at 97°C for 3 min, for 28 cycles at 97°C for 10 s, 50°C for 20 s, and 60°C for 2 min. Sequencing products were purified using CleanSeq (Agencourt Bioscience, Beckman Coulter) and analyzed on a 3730 DNA Analyzer, ABI capillary electrophoresis system (Applied Biosystems). Sequence traces were analyzed using the Mutation Surveyor software package (SoftGenetics, State College, PA).

Results and Discussion

A recent large-scale sequencing effort led to the identification of somatic mutations in more than 150 *CAN* genes in breast and colon cancer (1). The detailed mutational status of these genes in other cancer types remains to be determined. In this study, we examined the mutational profile of 19 *CAN* genes (Supplementary Table S1) in a panel of 24 glioblastoma, 24 melanomas, and 12 pancreatic cancers samples. The clinical information associated with these tumors is described in Table 1. For each gene, all exons in which somatic mutations had previously been identified were analyzed. Exon specific primers were designed to amplify and sequence the coding region, and at least 15 intronic bases at both the 5' and 3' ends, including the splicing donor and acceptor sites (Supplementary Table S1).

A total of 7,798 PCR products, spanning 1.47 Mb of tumor genomic DNA, were generated and subjected to direct sequencing. A total of 2,743 nucleotide changes were identified during this initial screening. Changes previously described as SNPs were excluded from further analyses.⁹ To ensure that the observed mutations were not PCR or sequencing artifacts, amplicons were

independently re-amplified and re-sequenced in the corresponding tumors. All verified changes were re-sequenced in parallel with the matched normal DNA to distinguish between somatic mutations and SNPs not previously described. This approach led to the identification of eight novel somatic mutations and one germline change in five *CAN* genes (Table 2). Three missense somatic mutations were found in *EPHA3*, two of which (K500N and A971P) were found in glioblastoma multiforme. The third *EPHA3* mutation (G228R) was observed in a melanoma sample that also contained a missense mutation in *OBSCN* (E4574K). Two mutations were found in *MLL3* in a glioblastoma multiforme (3614Ddel) and a PDAC sample (P1863A). In addition, one mutation each was found in *FBXW7* (R473fs*23) and *TECTA* (P802S) in two melanoma samples. The observed mutation rate was higher than the expected passenger mutation rate ($P < 0.01$, binomial distribution; ref. 6). In addition, we found a germline variant of a previously reported somatic mutation (R4558H) in *OBSCN* in a glioblastoma multiforme patient.

One of the most interesting genes found mutated is the Ephrin receptor A3 (*EPHA3*). *EPHA3* is a member of the Ephrin receptor family, which forms the largest subgroup of the receptor tyrosine kinases. Ephrin receptors and their ligands (Ephrins) are essential for a variety of biological processes and are implicated in tumor growth and survival (reviewed in ref. 7). *EPHA3*, or human eph/elk-like kinase, maps to chromosome 3p11.2, a region frequently affected in different cancers (8). Mutations in *EPHA3* in have been described in lung and colon cancer (9, 10).¹⁰ Here, we report for the first time an *EPHA3* mutation in melanoma. This mutation (G228R) occurs in a cysteine-rich linker region of the extracellular domain. Interestingly, this region is evolutionary conserved and may be important in determining the binding affinity to its particular ligand type. EphA2 receptor antagonists have remarkable anti-angiogenic and antitumor effects, suggesting that the EphA signaling pathway represents an attractive novel target for cancer therapy (11). The tyrosine kinase activity of the EphA3 receptor may be therapeutically targeted. Therefore, for this gene only, we extended our analysis to all coding exons. Two additional *EPHA3* mutations (K500N and A971P) were found in glioblastoma multiforme. The K500N affected the second fibronectin type-III domain, whereas A971P (Fig. 1A) occurred in the sterile α -motif region. Both domains are highly conserved throughout evolution, suggesting that the changes might affect critical functions of this gene.

⁹ <http://www.ensembl.org>

¹⁰ <http://www.sanger.ac.uk/genetics/CGP/cosmic/>

Table 2. Mutations identified in *CAN* genes

Gene	Nucleotide change	Amino acid change	Zygosity	Sample
<i>EPHA3</i>	c. 907 G>A	p.G228R	Heterozygous	23A
<i>EPHA3</i>	c. 1725G>T	p.K500N	Heterozygous	U87
<i>EPHA3</i>	c. 3136 G>C	p.A971P	Heterozygous	T78
<i>MLL3</i>	c. 5767 C>G	p.P1863A	Homozygous	369
<i>MLL3</i>	c.11020-11022delGAT	p.3614Ddel	Heterozygous	T70
<i>TECTA</i>	c. 2404 C>T	p.P802S	Heterozygous	8A
<i>FBXW7</i> *	c. 1566 del A	p.R473fs*23	Heterozygous	19A
<i>OBSCN</i>	c.13791G>A	p.E4574K	Heterozygous	23A
<i>OBSCN</i> [†]	c.13673G>A	p.R4558H	Heterozygous	T1

NOTE: The genes and the type of mutations found are listed alongside the samples in which they were found. The nucleotide position of each mutation corresponds to the position of that change in the coding sequence of each gene, where position 1 is the A of the ATG. Zygosity for the mutations is shown.

*The deleted nucleotide is part of a codon formed by the last two bases of exon 9 and the first base of exon 10; therefore, a heterozygous deletion of the second last base of exon 9 causes a frameshift in exon 10 and leads to a premature stop codon.

†This germline mutation in sample T1 in glioblastoma multiforme was previously reported as a somatic mutation (1).

We report for the first time somatic mutations in the interdomain regions of mixed-lineage leukemia 3 (*MLL3*) gene in glioblastoma multiforme and PDAC cancer. *MLL3*, also designated as “homologous to ALR” (*HALR*), is a member of the *TRX/MLL* gene family and maps to 7q36, a chromosome region that is frequently deleted in myeloid leukemia (12). Members of the *MLL* family are often targets for translocations in leukemias, leading to oncogenic fusion proteins that are associated with an extremely poor prognosis (13). *TRX/MLL* members serve as tumor suppressors, act as chromatin regulators, and play an important role during development. Interestingly, amplification of *MLL2* has been reported in glioblastoma and pancreatic carcinoma cell lines (14). Therefore, our results indicate that multiple members of the *MLL* family can be deregulated via different oncogenic mechanisms in these two cancer types.

The third gene in which we detected mutations was tectorin- α (*TECTA*) that was found altered in a melanoma sample. *TECTA* is

the major non-collagenous component of the tectorial membrane of the inner ear. Mutations in *TECTA* have been shown to be responsible for autosomal dominant non-syndromic hearing impairments and a recessive form of sensorineural pre-lingual non-syndromic deafness (15). The identified mutation, P802S (Fig. 1B), lies in the second von Willebrand factor type D domain and has not been described in any type of deafness. No previous association between *TECTA* and cancer has been reported.

The mutational profiling of *FBXW7* resulted in the identification of the R473fs*23 mutation in a melanoma sample. F-box and WD-40 domain protein 7 (*FBXW7* or *hCDC4*) is part of an ubiquitin ligase complex that targets molecules, such as cyclin E, Notch, c-Jun, and c-Myc, for degradation (16). Inactivating mutations in *FBXW7* have been previously described in a variety of human tumors and cancer cell lines. The nonsense mutation we found (R473fs*23) is located in the third of seven highly conserved WD40 repeats, known to serve as a protein binding platform. It gives rise

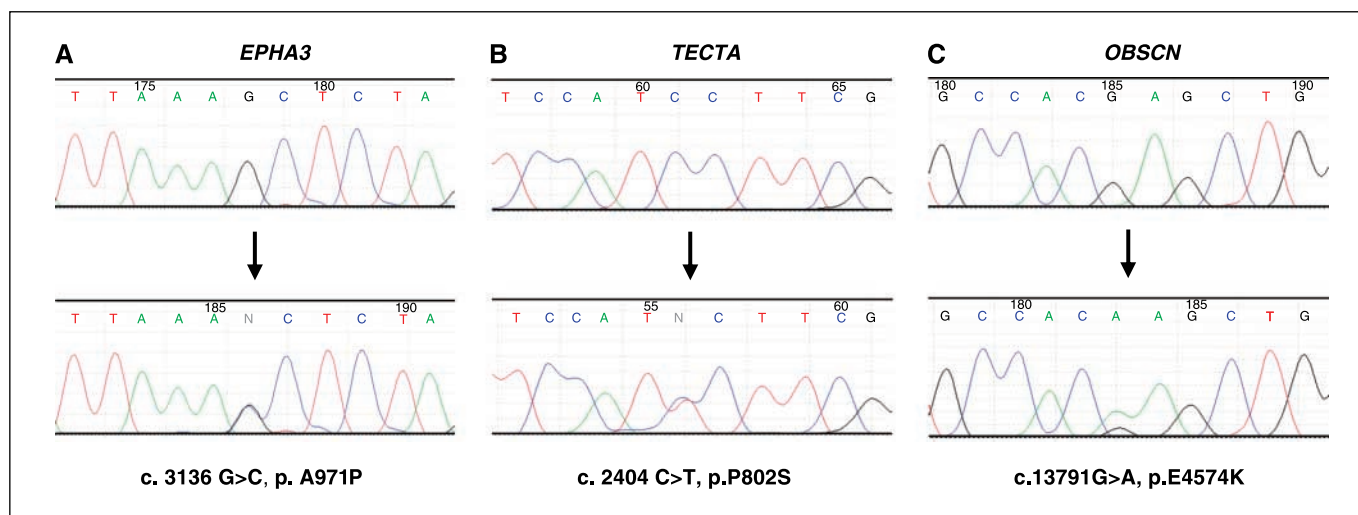


Figure 1. Examples of somatic mutations in *EPHA3*, *TECTA*, and *OBSCN*. *Bottom*, chromatogram of the sequence of a tumor sample; *top*, chromatogram of the matched normal. *Arrows*, location of missense somatic mutations. Nucleotide and amino acid alterations are below the traces. Numbers above the sequences are part of the software output. *A*, *EPHA3* mutation in glioblastoma multiforme. *B*, *TECTA* mutation in melanoma. *C*, *OBSCN* mutation in melanoma.

to a premature stop codon, resulting in truncation of the protein, thereby potentially interrupting the binding to its substrate (17). This change may, therefore, have direct functional implications, especially considering the putative haploinsufficient nature of this tumor suppressor gene (16).

One of the most intriguing results of this analysis is the identification of novel somatic and germline mutations in *OBSCN*. *OBSCN* encodes a RhoGEF protein that interacts with cytoskeletal calmodulin and titin and is part of the giant sarcomeric signaling protein family of myosin light chain kinases. Different isoforms have been described, containing functionally interesting domains, including two serine-threonine kinase domains, with a potential role in signal transduction (18). The somatic mutation E4574K (Fig. 1C) affects the fibronectin type-III domain 3, which is highly conserved throughout evolution. Thus far, *OBSCN* has been mainly known for its role in cardiac and skeletal muscle, where it is required for the assembly and organization of sarcomeres and the sarcoplasmic reticulum (18). Mutations in other genes encoding the giant muscle proteins titin (*TTN*) and nebulin (*NEB*) have been associated with cardiac and skeletal myopathies in humans. Interestingly, *OBSCN* was reported to be interrupted by a t(1;7)(q42;p15) breakpoint, in a Wilms' tumor patient with thrombocytopenia-absent radius syndrome-like symptoms (19). The recent identification of *OBSCN* somatic mutations in multiple cancer types indicates that this gene may play a role in different diseases (1).¹⁰ It is also interesting to note that of the 189 *CAN* genes identified in colon and breast, only *TP53* and *OBSCN* were common to both tumor types. The identification of mutations in *OBSCN* and *TTN* in multiple cancer types, which now include melanoma and glioblastoma, suggests that the cellular functions of these partner molecules could be related to a common tumor progression mechanism.

In addition to the E4574K somatic mutation, we also found another change (R4558H) in *OBSCN* in a glioblastoma multiforme sample. Exactly the same change had been previously found to be a somatic mutation by Sjöblom et al. (1). Surprisingly, however, we found that the same change was also present in the matched normal DNA (obtained from the blood of the same patient), indicating that it was a germline rather than a somatic mutation (Fig. 2). The R4558H mutation is not a common SNP as it is absent from the publicly available databases (20).¹¹ To further exclude the possibility that R4558H was a rare SNP, we sequenced an additional 359 human DNA samples again without finding this allele. Our results, therefore, suggest that the R4558H could be a germline cancer mutation. Importantly, the glioblastoma multiforme patient carrying the R4558H allele does not have a history of cardiac or skeletal muscle anomalies. This suggests that the R4558H mutation is not associated with the previously reported role of *OBSCN* in muscle tissues. The identification of germline and somatic mutations in *OBSCN* resembles what has been previously observed in other important cancer genes such as *PTEN*, *RET*, and *TP53*. In those cases, the very same mutations involved in the development of sporadic tumors were found to be cancer-predisposing when present in the germline. It is conceivable that germline changes in *OBSCN* might also similarly predispose to cancer.

In conclusion, our data identify novel genes and their specific molecular alterations involved in glioblastoma, melanoma, and

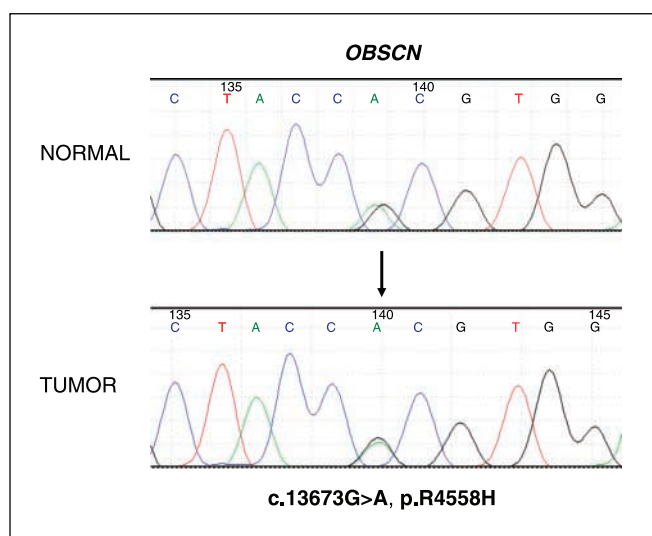


Figure 2. Germline mutation in *OBSCN*. Sequence of the *OBSCN* gene in sample T1 where the germline mutation R4558H was found. *Bottom*, chromatogram of the sequence of the glioblastoma sample; *top*, chromatogram of the respective matched normal. *Arrow*, location of the mutation. Nucleotide and amino acid alterations are below the trace.

pancreatic cancers. None of the somatic mutations described by Sjöblom et al. (1) were found in our analyses of glioblastoma multiforme, melanoma, and PDAC samples. These results, therefore, suggest that tumors have their own *CAN* genes, and only a few of the *CAN* genes are shared by different tumor types. In addition, the mutations themselves, rather than the genes, might be tumor specific. With the exception of P1863A in *MLL3*, all the mutations we found were in heterozygous state. Interestingly, this is similar to the mutation pattern observed by Sjöblom et al. The occurrence of heterozygous mutations is very common in oncogenes where they act in a dominant fashion. Alternatively, as previously shown for *FBXW7*, a haploinsufficient tumor suppressor, a heterozygous mutation may result in its inactivation. Because *TECTA* and *OBSCN* have not been previously associated with cancer, the effect of these mutations cannot presently be predicted. Some of the mutations we found affect genes (such as the tyrosine kinase *EPHA3*) that are clearly amenable to pharmacologic intervention and therefore could represent novel therapeutic targets for these untreatable cancers. Finally, we speculate that similar to other oncogenes and tumor suppressor genes, mutations affecting *OBSCN* could be involved in cancer predisposition.

Acknowledgments

Received 1/5/2007; revised 2/16/2007; accepted 3/2/2007.

Grant support: Italian Association for Cancer Research (AIRC; A. Bardelli), Italian Ministry of Health (A. Bardelli), Italian Ministry of University and Research (A. Bardelli), Regione Piemonte (A. Bardelli), Compagnia di S. Paolo Foundation (A. Bardelli), Fondazione Cariverona (A. Scarpa), Fondazione Zanotto (A. Scarpa), Association for International Cancer Research UK (AIRC UK; A. Bardelli), European Union FP6, MCSCs contract 037297 (A. Bardelli) and PL018771 (A. Scarpa), Accelerate Brain Cancer Cure (A.A. van Tilborg), Netherlands Genomics Initiative Fellowship (F.E. Bleeker), and Stichting Jo Kolk Studiefonds (F.E. Bleeker).

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.

We thank Prof. Dirk Troost (Department of Neuropathology, Academic Medical Center) for histologic verification of all glioblastoma multiforme samples, Catherine Tighe for article editing, Dr. Theo Hulsebos (Department of Neurogenetics, Academic Medical Center) and the members of the Laboratory of Molecular Genetics, Institute for Cancer Research and Treatment for helpful discussions.

¹¹ <http://genome.ucsc.edu/cgi-bin/hgGateway>

References

1. Sjöblom T, Jones S, Wood LD, et al. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006;314:268–74.
2. Balch CM, Soong SJ, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 2001;19:3622–34.
3. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
4. Hezel AF, Kimmelman AC, Stanger BZ, Bardeesy N, Depinho RA. Genetics and biology of pancreatic ductal adenocarcinoma. *Genes Dev* 2006;20:1218–49.
5. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
6. Bielas JH, Loeb KR, Rubin BP, True LD, Loeb LA. Human cancers express a mutator phenotype. *Proc Natl Acad Sci U S A* 2006;103:18238–42.
7. Surawska H, Ma PC, Salgia R. The role of ephrins and Eph receptors in cancer. *Cytokine Growth Factor Rev* 2004;15:419–33.
8. Pejovic T. Genetic changes in ovarian cancer. *Ann Med* 1995;27:73–8.
9. Wood LD, Calhoun ES, Silliman N, et al. Somatic mutations of GUCY2F, EPHA3, and NTRK3 in human cancers. *Hum Mutat* 2006;27:1060–1.
10. Bardelli A, Parsons DW, Silliman N, et al. Mutational analysis of the tyrosine kinome in colorectal cancers. *Science* 2003;300:949.
11. Dobrzanski P, Hunter K, Jones-Bolin S, et al. Antiangiogenic and antitumor efficacy of EphA2 receptor antagonist. *Cancer Res* 2004;64:910–9.
12. Ruault M, Brun ME, Ventura M, Roizes G, De Sario A. MLL3, a new human member of the TRX/MLL gene family, maps to 7q36, a chromosome region frequently deleted in myeloid leukaemia. *Gene* 2002;284:73–81.
13. Popovic R, Zeleznik-Le NJ. MLL: how complex does it get? *J Cell Biochem* 2005;95:234–42.
14. Huntsman DG, Chin SF, Muleris M, et al. MLL2, the second human homolog of the *Drosophila* trithorax gene, maps to 19q13.1 and is amplified in solid tumor cell lines. *Oncogene* 1999;18:7975–84.
15. Petersen MB. Non-syndromic autosomal-dominant deafness. *Clin Genet* 2002;62:1–13.
16. Minella AC, Clurman BE. Mechanisms of tumor suppression by the SCF (Fbw7). *Cell Cycle* 2005;4:1356–9.
17. Rajagopalan H, Jallepalli PV, Rago C, et al. Inactivation of hCDC4 can cause chromosomal instability. *Nature* 2004;428:77–81.
18. Young P, Ehler E, Gautel M. Obscurin, a giant sarcomeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. *J Cell Biol* 2001;154:123–36.
19. Vernon EG, Malik K, Reynolds P, et al. The parathyroid hormone-responsive B1 gene is interrupted by a t(1;7)(q42;p15) breakpoint associated with Wilms' tumour. *Oncogene* 2003;22:1371–80.
20. Riva A, Kohane IS. A SNP-centric database for the investigation of the human genome. *BMC Bioinformatics* 2004;5:33.