HHS Public Access

Author manuscript

Biochim Biophys Acta Rev Cancer. Author manuscript; available in PMC 2022 August 01.

Published in final edited form as:

Biochim Biophys Acta Rev Cancer. 2021 August ; 1876(1): 188567. doi:10.1016/j.bbcan.2021.188567.

Obscurin: a multitasking giant in the fight against cancer

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Abstract

Giant obscurins (720–870 kDa), encoded by *OBSCN*, were originally discovered in striated muscles as cytoskeletal proteins with scaffolding and regulatory roles. Recently though, they have risen to the spotlight as key players in cancer development and progression. Herein, we provide a timely prudent synopsis of the expanse of *OBSCN* mutations across 16 cancer types. Given the extensive work on *OBSCN*'s role in breast epithelium, we summarize functional studies implicating obscurins as potent tumor suppressors in breast cancer and delve into an *in silico* analysis of its mutational profile and epigenetic (de)regulation using different dataset platforms and sophisticated computational tools. Lastly, we formally describe the *OBSCN-Antisense-RNA-1* gene, which belongs to the long non-coding RNA family and discuss its potential role in modulating *OBSCN* expression in breast cancer. Collectively, we highlight the escalating involvement of obscurins in cancer biology and outline novel potential mechanisms of *OBSCN* (de)regulation that warrant further investigation.

Keywords

OBSCN ; obscurin; *OBSCN Antisense RNA 1* ; long non-coding RNA; tumor suppressor; tumorigenesis; metastasis; mutational analysis; epigenetic regulation; (hyper)methylation; breast cancer

I. Introduction

Mounting evidence has implicated *OBSCN* in the predisposition and development of several types of cancer [1–4]. In particular, *OBSCN* and *TP53* were found as the only common genes mutated in breast and colorectal cancers among >13,000 candidate genes examined [1]. While *TP53* is likely the most-well studied gene in cancer development and progression

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Conflict of Interests

The authors declare no conflict of interests.

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[5], the involvement of *OBSCN* in tumorigenesis has remained elusive, possibly due to its relative recent identification in 2001, the originally erroneous assumption that it is muscle-specific, and its molecular complexity due to its gigantic size [6–10].

OBSCN is located on human chromosome 1q42.13 spanning ~170 kb [7], giving rise to multiple isoforms ranging in size from 40–870 kDa via exon shuffling, different ribosomal entry sites and usage of distinct start codons [7, 8, 11, 12]. Giant obscurins (720–870 kDa) comprising the prototypical obscurin-A (~720 kDa) and the kinase-bearing obscurin-B (~870 kDa) are the best characterized isoforms (Fig. 1A), while intermediate (260–600 kDa) and small (40–260 kDa) obscurins remain understudied [9, 11]. Giant obscurins A and B share the same modular architecture consisting of immunoglobulin (Ig) and fibronectin-III (Fn-III) domains followed by an array of signaling motifs, including a calmodulin-binding IQ motif and a tripartite cassette consisting of tandem Src homology 3 (SH3), Rho-Guanine nucleotide exchange factor (RhoGEF) and pleckstrin homology (PH) domains, but differ in their extreme COOH-termini with obscurin-A containing a non-modular COOH-terminus bearing ankyrin binding sites (ABD) and obscurin-B harboring two active Ser/Thr kinase domains that belong to the Myosin Light Chain Kinase (MLCK) family (Fig. 1A) [7].

Most of our understanding about the structure, regulation, binding partners and (patho)physiological roles of giant obscurins stems from studies in striated muscles, including *C. elegans* where the obscurin homolog and founding member of the obscurin family, called UNC-89, was first discovered [10, 13–16]. Accordingly, giant obscurins are abundantly expressed in both skeletal and cardiac muscles where they wrap around the sarcomeric cytoskeleton and localize in specialized domains of the sarcolemma, while smaller obscurins have been reported in the nucleus [11, 17–22]. Given their molecular diversity and presence in multiple locations in muscle cells, obscurins have been shown to play both structural and regulatory roles by serving as scaffolds during myogenesis, contributing to the sarcomeric alignment of the sarcoplasmic reticulum membranes, acting as mechanosensors, mediating cell adhesion, and modulating Ca^{2+} homeostasis [10, 13, 18, 20–31]. Thus, it is not surprising that mutations in *OBSCN* have been causatively associated with different forms of skeletal and cardiac myopathies [32, 33].

During the last decade, accumulating evidence has shown that obscurins are not restricted to striated muscles but are expressed in non-muscle tissues, too (Fig. 1B) [6, 11]. This notion was prompted by independent large-scale sequencing studies identifying a collection of somatic and, in some instances, germline mutations in *OBSCN* across multiple cancers [1–4], and was further corroborated by work from our group providing compelling evidence indicating that giant obscurins possess tumor suppressor functions in breast epithelial cells [34–38].

Herein, we will comprehensively review the existing literature regarding the involvement of *OBSCN* in cancer formation and progression by: i. providing a synopsis of the currently known *OBSCN* mutations documented across 16 cancer types, ii. highlighting its tumor suppressor function in breast epithelial cells, where it has been primarily studied mechanistically, iii. discussing the transcriptional/epigenetic regulation of *OBSCN* in cancer with special emphasis on hypermethylation, and iv. revealing a previously unknown level of

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molecular complexity of the *OBSCN* locus, pertaining to the generation of antisense long non-coding (lnc) obscurin RNAs. Of note, a glossary including the definitions of not commonly used genetic and clinical terms is included.

II. Regulation and expression profile of *OBSCN*

In addition to being a large gene spanning 170 kb, *OBSCN* has a complex structure containing 119 exons [7, 8]. Interestingly, splice donor and acceptor sites are compatible for Ig domains 3 to 66, suggesting a high potential for exon shuffling and generation of splice variants containing different numbers of Ig/Fn-III domains that may span a wide range of molecular weights. Consistent with this notion, the *OBSCN* pre-mRNA was shown to be among 18 gene transcripts that were directly bound by RBM20, an RNA-binding protein modulating pre-mRNA splicing [39, 40]. Further analysis indicated that RBM20 binding to *OBSCN* pre-mRNA induced exon suppression, likely resulting in the generation of different size transcripts [39, 40]. Moreover, whole transcriptome termini site sequencing of gastrocnemius muscle from wild type and a knock-out mouse model of monophosphate activated protein kinase (AMPK) subunit α2 revealed up-regulated *OBSCN* mRNA levels in the latter via utilization of alternative polyadenylation sites [41]. Thus, exon shuffling and use of alternative polyadenylation sites may explain, at least in part, the presence of multiple and often distinct immunoreactive obscurin bands detected across different tissues, organs and cell lines [7, 8, 11].

Detailed analysis of the expression profile of *OBSCN* using publicly available human RNA-seq databases (GTExPortal Data Source) indicated that it is nearly ubiquitously present among different tissues and organs, although its relative abundance may vary considerably (Fig. 1B). Consistent with this, our earlier work demonstrated the presence of obscurin proteins in multiple tissues and organs of rodent origin, including striated muscles, brain, skin, kidney, liver, spleen, and lung where they localize to the plasma membrane, in cytoplasmic puncta and the nucleus [11]. Further evaluation of the expression profile of obscurins in human cell lines showed that they are highly expressed in normal breast, colon and skin epithelial cell lines, however their levels are dramatically decreased in the respective cancer cell lines [37]. Accordingly, obscurins displayed a preferential accumulation at cell-cell junctions, perinuclear/cytoplasmic puncta coinciding with the Golgi apparatus, and the nucleus in normal breast epithelial cells [37]. These findings were further corroborated by the abundant expression of obscurins in biopsies from normal human breast epithelium and their drastic reduction in adjacent invasive ductal carcinomas (IDC) of advanced grade (i.e., grade 2 or higher) [34].

Although the precise mechanisms resulting in loss of *OBSCN* expression during tumorigenesis remain elusive, given the high mutational prevalence and purported tumor suppressor role of the gene (discussed in detail below), it is highly likely that its loss conforms with the classic Knudson “two-hit” model involving an initial event leading to inactivation of one allele followed by a second event resulting in loss of heterozygosity, and thus functional inactivation of both alleles [42]. Such events may include inactivating mutations, genetic rearrangements, and/or epigenetic silencing, which we discuss below.

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III. Mutational analysis of *OBSCN* across different cancer types

Modification of gene expression and thus of protein functionality is widely credited for as one of the major underlying drivers of cell oncogenesis [43]. In this section, we summarize the litany of all 245 reported *OBSCN* mutations across various cancer types (Supplemental Table 1), which are presented in a "head-to-toe" manner except for breast cancer which is discussed last given the considerable literature regarding *OBSCN's* role. Moreover, we theorize on the potential genetic, molecular, and/or biochemical significance of the identified mutations where appropriate and highlight areas in which more research is required to generate such conjecture.

III-1. Brain Cancer:

Current data from 44 cases of malignant low-grade glioma (LGG), primarily of astrocyte origin, revealed the presence of 37 *OBSCN* mutations, with 36 being point mutations and 1 a complete gene deletion. These 36 point mutations comprised 25 missense, 2 nonsense, and 3 synonymous mutations in coding exons in addition to 2 mutations in introns. Interestingly, the 1 gene deletion occurred secondary to a point mutation in a splice region that resulted in erroneous splicing and complete loss of all exon transcription. Likewise, 69 cases of the more aggressive glioblastoma (GBM) tumors, also of astrocyte origin, were found to contain 87 *OBSCN* mutations, all of which were single base point mutations [44]. Importantly, both the LGG and GBM tumors that contained *OBSCN* mutations cluster into a larger group of tumors with high intratumor heterogeneity that correlates with worse prognosis, including a shorter interval before recurrence [44]. Although the great majority of *OBSCN* mutations identified in brain (and other types of cancer) are somatic, a germline mutation, R4558H, involving a highly conserved amino acid residue in the 2nd FN-III domain has been described in a GBM tumor biopsy; of note, this mutation was originally identified as a somatic mutation in breast and colorectal cancers [3].

III-2. Oral Squamous Cell Carcinoma:

Whole exome sequencing of human oral squamous cell carcinoma cell lines of pharyngeal and tongue origin has identified a total of 7 *OBSCN* missense mutations [45]. Similarly, whole exome sequencing of mouse pre-neoplastic oral stem cell clones obtained via laser capture microdissection from K14CreER^{TAM};ROSA26 female mice (a tamoxifen-inducible lineage tracing model for the keratin 14 promoter present solely in basal squamous stem cells of the tongue and skin) has identified 25 missense, 1 frameshift, and 1 disruptive inframe deletion in *OBSCN* [46]. However, as in other forms of cancers, the functional and clinical ramifications of these mutations are still elusive.

III-3. Gastrointestinal Tract Cancers:

Gastric cancers, 90% of which are approximated to be adenocarcinomas, develop via multifactorial processes that may include bacterial or Epstein-Barr viral infection, host genetic polymorphisms and dietary factors [47]. One of the best documented bacterial causes of gastric cancer is chronic *Helicobacter pylori* (*H. pylori*) infection shown to drive tumorigenesis through multiple mechanisms, including hypermethylation of key tumor suppressor genes and inflammation producing radical oxygen species that cause DNA

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mutations [47]. Whole-exome gene sequencing of gastric tumor biopsies revealed the presence of 4 non-synonymous *OBSCN* mutations [48]. Interestingly, 3 of these 4 mutations occurred in a sole tumor sample that exhibited marked microsatellite instability [48]. Similarly, multiple missense mutations in *OBSCN* were reported in stomach adenocarcinomas [49], while 7 novel *OBSCN* mutations were found in colorectal tumors that functionally cluster with mutations in genes modulating cellular motility and adhesion [1]. Interestingly, *in vitro* analysis using Kinome capture array and subsequent deep sequencing of gastric cell lines showed a high co-mutational incidence of *OBSCN* and *TP53* and revealed the presence of 9 non-synonymous *OBSCN* mutations [50].

Evaluation of tumor biopsies from accessory organs of the gastrointestinal tract has also revealed the presence of *OBSCN* mutations. Specifically, whole-exome sequencing identified 2 somatic non-synonymous point mutations in salivary gland mucoepidermoid carcinoma biopsies [51]. Moreover, 2 somatic point mutations and 1 amplification mutation were identified in pancreatic ductal adenocarcinoma (PDAC) biopsies [52, 53], in addition to 1 non-synonymous point mutation in an adjacent pancreatic intraepithelial neoplasia (PEN) [53]. Notably, each of these mutations was predicted to be damaging via SNPEffect analysis [53]. Shifting from gene-based to protein expression alterations, serum electrospray mass profiling of sera derived from patients with stage IIB PDAC revealed a decrease in obscurin peptides compared to normal tissue [54], although the presence of *OBSCN* mutations was not investigated as a possible cause of reduced mRNA and/or protein expression. Regardless, the combined incidence of *OBSCN* mutations and reduced expression in separate PDAC samples convincingly suggests a potent role of *OBSCN* in pancreatic tumorigenesis.

Moreover, changes in obscurin protein expression have been documented in hepatocellular carcinomas. Mass spectrometry combined with bioinformatics analysis indicated that obscurin peptides display a 3-fold higher abundance in the more metastatic MHCC97H hepatocellular carcinoma cell line compared to the less metastatic MHCC97L cell line [55]. It is important to note, however, that these findings cannot distinguish whether the identified peptides correspond to the giant (which appear to have tumor suppressive properties at least in breast cancer; please see below), intermediate or small obscurins, possibly implying distinct roles for the different obscurin isoforms in cancer formation and progression.

Alterations in *OBSCN* expression were also observed in stromal cancers in the abdomen, which can be divided into gastrointestinal stromal tumors (GIST) and leiomyosarcomas (LMS). GIST originate from the same cell lineage as the neuromuscular pacemaker interstitial cells of Cajal, primarily arising in the stomach and small intestine [56]. On the other hand, LMS are derived from a smooth muscle lineage, mainly affecting the gastrointestinal tract, uterus, and inferior vena cava [57].Given that GIST and LMS, unlike other forms of cancer, have potential overlapping sites of primary origin, improved histological evaluation that distinguishes between the two types is required. Following initial microarray screening for gene pairs that can reliably distinguish GIST from LMS tumors, RT-PCR analysis confirmed that *OBSCN* mRNA expression compared to *C9orf65* (a poorly characterized gene located on chromosome 9 whose protein product may interact with the Ca^{2+}-binding protein reticulocalbin 3) is increased in GIST but decreased in LMS [4]. Thus, it has been suggested that the ratio of *OBSCN*:*C9orf65* may be used as a new diagnostic tool

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in gastric tumors to aid the differentiation of GIST *vs* LMS. Importantly, GIST tumors respond well to the receptor-tyrosine kinase inhibitor imatinib while LMS tumors fail to do so, rendering them clinically more difficult to treat [58, 59]. Consequently, we note the correlation of the lower *OBSCN*:*C9orf65* expression ratio in the clinically more resilient LMS compared to GIST. Although the diagnostic potential of the *OBSCN*:*C9orf65* ratio is an exciting observation with possible translational implications, the mutational profile of *OBSCN* and its contribution to tumorigenesis in stromal tumors remain elusive.

III-4. Wilms Tumor:

Though not widely reported, tumors of the kidney have been also shown to contain correlational *OBSCN* mutations. A patient with Wilms tumor and contralateral nephrogenic rest, who lacked the characteristic 11p15 Wilms Tumor 1 (*WT1)* gene mutation, was found via FISH mapping of a t(1;7) breakpoint to contain a germline constitutional balanced chromosomal translocation of t(1;7)(q42;p15) [60]. This translocation results in a minor 2bp 'GA' deletion that bisects the first intron of the *OBSCN* gene in 1q42 [60]. It was theorized that splice variant changes to the *PTH B1* gene in 7p15 may drive the formation of Wilms tumor since only one copy of *OBSCN* was affected, resulting in no loss of heterozygosity [60]. Nevertheless, the identification of germline *OBSCN* mutations in patients with Wilms tumor and GBM warrants further investigation, especially given the molecular complexity of the gene.

III-5. Renal Cell Carcinoma:

Evaluation of 288 papillary renal cell carcinoma (pRCC) biopsies demonstrated the presence of 18 non-synonymous missense mutations in *OBSCN* in >20 samples, which correlated with higher *OBSCN* transcript levels [61]. Using Kaplan-Meier survival analysis, it was determined that increased *OBSCN* mRNA levels in pRCC associate with poor survival outcome [61]. Similar to pRCC biopsies, clear cell renal cell carcinoma (ccRCC) biopsies, a clinically more aggressive subtype of RCC, also contained *OBSCN* mutations [61]. Although the pathophysiological significance of the increased *OBSCN* transcripts in pRCC biopsies containing *OBSCN* mutations is speculative at this time, it is possible that this may be a compensatory or adaptive cellular response early in the process of tumorigenesis that does not necessarily result to increased (mutant) protein expression. Consistent with this notion, *OBSCN* transcript levels are notably increased in the MCF7 breast epithelial cancer cell line, although the protein is nearly absent [37].

III-6. Female Reproductive Cancers:

OBSCN mutations have been found in both ovarian and uterine cancers. Sequencing analysis of 31 epithelial ovarian cancer biopsies followed by filtering of non-pathogenic (e.g., synonymous) nucleotide variants identified 15 different mutated tumor suppressor genes exhibiting potential pathogenicity [62]. *OBSCN*, along with the well-known *TP53* and *BRAC1* tumor suppressors, was found to contain 2 non-synonymous mutations [62]. Moreover, whole exome sequencing of uterine serous carcinomas identified 2 somatic mutations in *OBSCN*, including a base deletion and a T to A conversion [63]. As the impact of these non-silent mutations on the expression profile of *OBSCN* is currently unknown, further investigation is required to ascertain their functional and clinical significance.

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III-7. Prostate Cancer:

Clinically, prostate cancer can be divided into androgen sensitive and insensitive subtypes, with the latter most often presenting as recurrent prostate cancer post-initial therapy, conferring lower survival rates [64]. Using a human xenograft prostate cancer mouse model, a lentiviral-mediated insertional mutagenesis screen identified 2 proviral integration sites within *OBSCN* specific to the more aggressive androgen-independent prostate cancer (AIPC) subtype [65]. These integration sites were separated by ~30.3 kilobases with the first potential integration site occurring in intron 9 and the second in exon 16, suggesting reduced obscurin mRNA levels [65]. Consistent with this, Oncomine microarray analysis of AIPC biopsy data from the TCGA database revealed reduced *OBSCN* mRNA levels compared to normal prostate tissue [65], while *OBSCN* was shown to be significantly hypermethylated at intragenic CpG sites in the androgen sensitive 22Rv1 prostate cancer cell line [66]. Moreover, use of the ServExpress biomarker tool, which compiles prevalently mutated genes among thousands of published datasets that likely serve as biomarkers and correlates their expression with subsequent survival data, indicated that the combination of deleterious mutations in *OBSCN* along with *FAM83H* (Family With Sequence Similarity 83 Member H), *CLDN7* (Claudin 7), and *ARFGAP3* (ADP Ribosylation Factor GTPase Activating Protein 3) predicts a higher recurrence risk of prostate cancer after prostatectomy [65].

In addition to alterations in *OBSCN* levels, 1 intronic, 2 nonsense, and 10 missence mutations have been identified in *OBSCN* in prostate cancer biopsies along with 2 complete gene deletions [2, 65, 67–69]. More interesting, however, was the discovery of a unique fusion product with the first break-site occurring at the 5'-UTR of *GATAD2B* encoding the transcriptional repressor P66-Beta, ~45 Kb upstream of the coding start site, and the second break-site occurring in the *OBSCN* promoter, ~3 Kb from the translation start site, resulting in the generation of a *GATAD2B-OBSCN* hybrid transcript [68]. Although further investigation is needed to examine the impact of the aforementioned mutations in the expression profile of *OBSCN*, the identification of complete gene deletion and the generation of a *GATAD2B-OBSCN* fusion transcript suggest reduced expression and/or functionality of the gene at least in some prostate tumors, consistent with its purported tumor suppressor role in breast epithelium (please see below).

III-8. Dermatologic Cancers:

Of the three major dermatologic cancer subtypes, including basal cell carcinoma, squamous cell carcinoma and melanoma, *OBSCN* mutations have been reported in the latter [3]. Specifically, a non-passenger somatic missense mutation (E4574K) residing in Fn-III 60 was discovered in a patient with melanoma [3]. Previously, in the context of skeletal muscle differentiation, obscurin depletion in zebrafish embryos was shown to markedly reduce fibronectin matrix organization [70]. In melanoma, fibronectin 1 has been postulated to regulate tumor cell proliferation and metastasis [71]. Although additional work is needed to establish any functional connection, the presence of the E4574K mutation in obscurin domain Fn-III 60 is intriguing in the context of melanoma specifically.

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III-9. Breast Cancer:

In 2006, Sjöblom *et al.* published a seminal comprehensive study aiming to identify mutated consensus coding sequences in breast and colorectal cancers [1]. More than 13,000 genes were screened, yielding 189 “candidate” genes exhibiting high prevalence of somatic mutations in both cancer types, with 122 of those “candidate” genes found in breast cancer alone [1]. Remarkably, *OBSCN* was identified as one of only two candidate genes (the other being *TP53*) commonly mutated in both tumor types. Five homozygous *OBSCN* mutations were reported in breast cancer that functionally clustered with genes altering cellular motility and adhesion [1]. These included 3 missense mutations in exons 26, 54, and 55, 1 nonsense mutation in exon 69, and 1 base pair deletion in exon 10, all of which encode Ig domains, except for exon 69, which encodes the kinase 1 domain [1]. Following-up on these initial findings, our group pioneered the study of obscurins in breast cancer formation and progression, taking advantage of our expertise on the unique biochemistry of these gigantic proteins.

Remarkably, the sole downregulation of obscurins transformed the normal MCF10A breast epithelial cells to tumorigenic by eliciting epithelial to mesenchymal (EMT) transition, up- and down-regulating the expression of anti- and pro-apoptotic genes, respectively, following exposure to DNA-damaging agents, and enabling them to escape anoikis [34, 37]. Obscurin-depleted cells exhibited significantly increased growth, motility (both in 2-dimensional, 2D, substrata and 3D-like confined spaces), invasion, stemness, and microtentacle-forming potential *in vitro* [34, 38]. More importantly, loss of giant obscurins from MCF10A breast epithelial cells rendered them less susceptible to treatment with paclitaxel, a mainstay clinical chemotherapy employed to treat breast cancer, as they exhibited increased survival and cell attachment capabilities *in vitro*, suggesting that loss of obscurins may represent a substantial selective advantage for breast cancer cells during metastasis [34, 38]. Consistent with these *in vitro* findings, obscurin-depleted MCF10A breast epithelial cells stably expressing the K-Ras oncogene generated robust primary tumors in a subcutaneous model and effectively colonized the lungs in an experimental metastasis model [34]. Taken together, these studies indicated that obscurins may play tumor suppressor roles in normal breast epithelial cells, and that their loss potentiates tumorigenesis and metastasis.

Critical to deciphering the tumor suppressor function of obscurins in breast epithelial cells has been the elucidation of the molecular pathways that they regulate, especially considering the multiple signaling motifs that they contain in their COOH-termini (Fig. 1A). Consistent with the preferential ability of the obscurin RhoGEF motif to bind and activate RhoA [27, 72], obscurin-depleted MCF10A cells exhibited decreased levels of active RhoA accompanied by reduced phosphorylation of major RhoA effectors modulating actomyosin contractility and actin filament stabilization/turn-over [38]. As such, obscurin-deficient MCF10A cells contained fewer and shorter actin stress fibers and displayed reduced contractility and focal adhesion size and density, in agreement with their increased migratory, invasive and reattachment capabilities [34, 36, 38]. These findings are in accordance with Sorting Nexin 9 (SNX9), a key membrane trafficking protein, transduced MDA-MB-231 breast cancer cells in which downregulation of RhoA promoted cell invasion *in vitro* and metastasis *in vivo* [73]. Predicted to integrate different signaling cascades via

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their distinct signaling motifs and subcellular distributions [6, 10, 13], loss of giant obscurins from breast epithelial cells resulted in upregulation of the phosphoinositide 3 kinase (PI3K) pathway [35], in addition to downregulation of the RhoA axis [38], primarily mediated via AKT2. Importantly, chemical and/or molecular inhibition of the PI3K/AKT2 cascade suppressed the tumorigenic potential of obscurin-depleted MCF10A cells as it reversed EMT, and significantly reduced their growth, migratory and invasive potentials *in vitro* [35], suggesting that obscurins may act upstream of the PI3K/AKT2 axis. Biochemical validation of this notion came from *in vitro* binding studies indicating that the PH domain of obscurin specifically interacts with the SH3 domain of the p85 regulatory subunit of PI3K with a K_D in the low nM range (~50 nM) suggesting a strong interaction [35]. Thus, it is likely that in normal breast epithelial cells, obscurins may modulate the activity of PI3K through direct binding to the PI3K/p85 regulatory subunit. The notion that obscurins may act upstream of the RhoA and PI3K pathways in breast epithelial cells is further substantiated by studies in striated muscle cells where gain-of-function experiments indicated that the obscurin RhoGEF and PH motifs are involved in the modulation of growth responses via the regulation of downstream targets of the RhoA and PI3K axes [22, 27]. Considering that the COOH-terminus of obscurins is a hub of signaling motifs, while the NH_2-terminus and middle portion may provide binding sites for diverse proteins via the array of tandem Ig and FN-III domains, it is conceivable that in addition to RhoA and PI3K/AKT2 pathways, alterations to additional cascades may contribute to enhanced tumorigenicity. As such, work from our group demonstrated that obscurin kinase-1 directly binds and phosphorylates the cytosplasmic domain of N-cadherin [23], a bona fide marker of EMT, possibly playing key roles in cell adhesion and the regulation of the underlying cytoskeleton. Thus, the more we learn about these multitasking giants, the more intriguing their (patho)biology turns out to be.

Taken together, the literature details the accumulation of both somatic and germline *OBSCN* mutations across multiple cancer types and provides evidence for its role as tumor suppressor, reinforcing the notion that genetic alterations in the previously elusive *OBSCN* gene may potentiate tumorigenesis by mediating its loss. Importantly, recent analysis of 17 TCGA datasets of different cancer types revealed a scattering of mutations across the *OBSCN* gene, and identified 3 statistically significant mutational hotspots including Ig19, the 2nd FN-III domain (domain 50), and the kinase1-Ig59 linker region [2]. Consistent with these findings, a systematic overlay of the currently reported 224 *OBSCN* mutations across the 16 different cancer types discussed above indicates that the 3 previously identified hotspots in addition to the RhoGEF motif contain the highest number of non-synonymous *OBSCN* variants (Fig. 1C). The pathophysiological relevance of these hotspots and/or individual mutations in impacting protein expression and role in driving tumorigenesis, however, are major outstanding questions in *OBSCN*'s biology.

IV. *OBSCN* deregulation in cancer

The causative relationship between reduced obscurin protein expression and tumorigenesis in breast cancer in conjunction with the numerous *OBSCN* mutations identified across different cancer types holds a magnifying glass to the molecular etiologies of *OBSCN* deregulation. In the following sub-sections, we describe three key molecular mechanisms

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that may account for alterations in *OBSCN* expression in cancer (and possibly other diseases, too), including: i. genetic alterations, ii. epigenetic modifications, and iii. regulation via *OBSCN-Antisense RNA 1 (OBSCN-AS1)* encoding long non-coding obscurin anti-sense RNA variants.

IV-1. Genetic alterations:

Using a combination of >10 different computational tools, Rajendran and Deng identified *OBSCN* as a novel candidate driver gene in breast cancer [74]. Specifically, a total of 956 candidate driver genes were identified after an initial comprehensive analysis using Driver DB associated tools, which after intensive filtering and evaluation using data from the IntOGen prediction, COSMIC, cBioPortal and OASIS databases yielded 63 driver genes, including *OBSCN* [74]. Following up on these findings, the same authors interrogated the possible role of *OBSCN* in breast tumorigenesis by performing a systematic *in silico* analysis assessing the presence of copy number alterations, mutational prevalence, and methylation/expression profile of the gene [75]. Using the Genomic Identification of Significant Targets in Cancer (GISTIC) algorithm, the authors primarily described gain and amplification mutations for *OBSCN* and to a lesser extent shallow deletions across 5 breast cancer projects [75]. Moreover, using the cBioPortal tool an average mutational frequency of 18% was determined for *OBSCN* in breast cancer [75]. In accordance with these findings, we found several genetic alterations in *OBSCN* across different cancer types using the TCGA PanCancer Atlas Studies datasets (Fig. 2A) available through cBioPortal [76, 77]. Such alterations included missense and nonsense mutations, amplifications, deep deletions, and less commonly fusions. Interestingly, 11% of all queried patient samples (i.e., 1152 out of 10953), covering 33 cancer types, carried genetic alterations in *OBSCN* with mutational frequency ranging from 0.6% in well-differentiated thyroid cancer to >30% in undifferentiated stomach adenocarcinoma (Fig. 2A). In breast cancer specifically, *OBSCN* was altered in 12.55% of queried invasive breast carcinoma samples across all molecular subtypes (i.e., in 136 out of 1084 patients) with 8.21% (i.e., 89 patients) exhibiting amplification, 3.51% (i.e., 38 patients) containing non-synonymous mutations, 0.55% (i.e., 6 patients) harboring multiple alterations, and 0.28% (i.e., 3 patients) displaying deep deletions (Fig. 2B). As a reference metric, *PTEN*, a well-known and extensively characterized tumor suppressor gene involved in the regulation of the PI3K/AKT axis, was altered in 12% of queried patient samples (i.e., in 1325 out of 10953) across the 33 cancer types included in the analysis, and in ~11% of invasive breast carcinomas (i.e., in 109 out of 996 samples) with the majority of alterations being mutations and deep deletions (Fig. 2B).

Paradoxically for a purported tumor suppressor gene whose transcript and/or protein levels are decreased, the most common genetic alteration seen in *OBSCN* in invasive breast carcinoma is amplification (8.21%) (Fig. 2B). Studies across different cancer types have shed light to this paradox as they have found changes in the expression levels of only 10–63% of genes residing in amplified regions and 14–62% of genes located in deleted regions [78]. Consistent with these observations, 62% of DNA amplifications identified in breast cancer cell lines and tumors have been associated with elevated expression of 54 genes exhibiting a 2-fold increase in DNA copy number that is linked to a 1.5-fold increase of mRNA levels [79]; interestingly, *OBSCN* is not listed as one of those 54 genes although its

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locus is commonly amplified (8.21%) according to our *in silico* analysis (Fig. 2B). Further evidence supporting the lack of a necessary correlation between gene amplification and enhanced mRNA expression comes from evaluation of 37 breast tumor samples using a linear regression model that indicated only 7–12% of variations in mRNA levels can be directly attributed to gene copy number alterations [79]. Even in the setting of entire chromosome and chromosomal arm gain or loss, some genes within the affected region may still exhibit normal expression or counterintuitively downregulated genes may reside in DNA gain regions, and *vice versa* [78]. Consistent with this, 50% of genes with significant expression changes are located in regions unaffected by genomic imbalances in a prostate carcinogenesis animal model [80]. Moreover, 14% of downregulated genes mapped to regions of DNA gain and 9% of upregulated genes occurred in regions of DNA loss in cell lines generated from the above model, while only 1 gene, *MMP-9*, exhibited >2-fold upregulation in cell lines containing 3–7 copies of chromosomal arm 20q where it resides [80]. Thus, it becomes apparent that changes in DNA copy number do not necessarily correlate with altered gene expression and may not be sufficient to override the transcriptional control mechanisms that regulate gene expression [80].

The adaptive mechanisms that are responsible for the disconnect between copy number alterations and gene expression levels are poorly understood. Interesting findings from a newly developed platform-independent method of Transcriptional Adaptation to Copy Number Alterations (TACNA) profiling suggest that non-genetic mechanisms might be involved [81]. DNA methylation was shown to be a potential mechanism buffering or counteracting the effect of copy number alterations in the expression profile of a subset of genes [81]. Along these lines, it was postulated that aberrant expression of genes that are normally under tight non-genetic adaptive control could lead to more aggressive tumor phenotypes [81]. Thus, it is plausible that *OBSCN* may be one of those genes that is genetically amplified due to chromosomal arm gain, but its expression levels remain unchanged or are downregulated via adaptive mechanisms. In agreement with this notion, DNA copy number gains within chromosomal arm 1q (where *OBSCN* is located) are commonly observed in breast cancer cell lines (90%) and tumor biopsies (69%) [79], suggesting that *OBSCN* amplification seen in invasive breast carcinomas is likely due to 1q gain. To interrogate this, we used Breast Invasive Carcinoma datasets available via cBioPortal (TCGA, PanCancer Atlas), and found that *OBSCN* amplification most commonly occurred in breast cancer samples with 1q status gain (Fig. 2C). This suggests that the *OBSCN* amplification seen in breast cancer biopsies is likely due to chromosome arm 1q gain, but how this influences *OBSCN* expression is unknown. Along these lines, *OBSCN* mRNA levels do not appear to be drastically altered due to copy number alterations, with the exception of shallow deletions where they seem reduced (Fig. 2D). Thus, it is plausible that in breast cancer cells bearing *OBSCN* copy number alterations especially in the form of amplification, *OBSCN* is under tight adaptive transcriptional regulation that counteracts its upregulation, which would be disadvantageous (to cancerous cells) given its tumor suppressor function.

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IV-2. Epigenetic modifications:

Epigenetic modification of the genome plays key roles in the regulation of essential cellular processes, including chromosomal stability and transcription [82]. Specifically, epigenetic silencing is a complex and dynamic process that is commonly involved in the transcriptional inactivation of tumor suppressor genes during tumorigenesis [83]. As such, epigenetic reprogramming involves a variety of regional changes that include DNA methylation and post-translational histone modifications with DNA hypermethylation, loss of histone activating marks and gain of histone repression marks being commonly observed in epigenetically silenced genes [83].

DNA methylation involving the addition of a methyl (CH_3) group at the carbon-5 position of cytosine in CpG dinucleotides by DNA methyltransferases (DNMTs) is often observed during cancer formation and progression [82]. Consistent with this, promoter hypermethylation at CpG islands (i.e., regions enriched in CpG dinucleotides) has been associated with silencing of well characterized tumor suppressor genes, such as *CDH1* [84] and *BRCA1* [85]. The mechanism by which promoter (hyper)methylation is thought to suppress gene expression is via the recruitment of methyl-binding proteins (MBPs), which alter chromatin conformation and inhibit transcription factor recruitment and transactivation [86]. In breast cancer tumors, CpG hypermethylation has been predominantly found in upstream regulatory regions (i.e., within the promoter/enhancer elements, the 5' untranslated region, or the 1st exon) and has been correlated with reduced gene expression, whereas the gene body is typically hypomethylated [86]. Consistent with these observations, using methylation data available through TCGA Wanderer, *OBSCN* was found to be considerably hypermethylated in breast cancer (average beta value > 0.80 in a scale of 0–1) [75]. Importantly, breast cancer samples exhibiting *OBSCN* hypermethylation contained reduced levels of obscurin transcripts compared to paired normal samples [75].

Given the likely pathophysiological importance of methylation in regulating *OBSCN* expression, we performed CpG island analysis of *OBSCN* using the makeCGI R software package (http://www.haowulab.org/software/makeCGI/index.html), which identified a total of 27 CpG islands within a 20kb region upstream and downstream of the *OBSCN* transcription start site that included 4,108 individual CpG sites. To learn if the identified CpG sites are differentially methylated between breast invasive carcinomas and normal breast tissue samples, we used Wanderer (http://maplab.imppc.org/wanderer/), an interactive viewer tool that allowed us to explore the DNA methylation and expression profile of *OBSCN* [87]. We identified several CpG sites differentially methylated between invasive breast carcinoma and normal samples, with a number of them located within CpG islands (Fig. 3A; representative results for 25 CpG probes, out of 125 possible CpG probes spanning the entire *OBSCN* locus, are shown). Consistent with this, *OBSCN* expression was significantly reduced in invasive breast carcinomas compared to normal samples as indicated via analysis of Illumina HiSeq RNA-Seq data using Wanderer (Fig. 3B).

IV-3. OBSCN-Antisense RNA 1:

In addition to being genetically altered and/or epigenetically modulated during carcinogenesis, *OBSCN* may be regulated in a cis mechanism by *OBSCN-Antisense RNA 1*

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(*OBSCN-AS1)*, also known as C1orf145, formally described for the first time herein. *OBSCN-AS1* is an RNA gene located in chromosome 1q42.13 in the minus strand of the *OBSCN* gene and belongs to the long non-coding RNA (lncRNA) family. LncRNAs lack protein-coding potential and they differ from other non-coding RNAs in that they are >200 nucleotides in length. LncRNAs are classified based on their genomic location and orientation relative to a protein-coding gene [88]. Unlike protein coding transcripts, lncRNAs have relatively low evolutionary conservation, originate from short genes with few exons, are present in low levels, and exhibit tissue-specific expression [88, 89]. Similar to protein coding transcripts, lncRNAs are transcribed by RNA polymerase II, their expression is under the control of the gene's promoter and enhancer elements and can undergo analogous transcript processing such as 5' capping, 3' polyadenylation, and splicing [88, 89]. Importantly, lncRNAs have been shown to play key roles in the epigenetic modulation, transcriptional regulation, alternative splicing, mRNA stability, and post-transcriptional modification of their targets [90].

OBSCN-AS1 gives rise to two antisense lncRNA transcript variants that partially overlap with the protein-coding *OBSCN* transcripts at the 5' end (Fig. 4A). Specifically, *OBSCN-AS1* transcripts originate from the opposite strand of *OBSCN* within exon 3 and end upstream of the *OBSCN* transcription start site (Fig. 4A). *OBSCN-AS1* variant-1 consists of 4 exons and is 2884 bp long, whereas *OBSCN-AS1* variant-2 consists of 2 exons, is 981 bp long, and contains an alternate terminal exon compared to variant-1 (Fig. 4A). Currently, there are no studies regarding the expression profile or functional significance of *OBSCN-AS1* in health or disease, however given its genomic location it is plausible that it may be involved in modulating the expression of its protein-coding gene pair, *OBSCN*. A reliable indicator of lncRNA's mechanism of action is its subcellular localization [90]. Using the LncAtlas tool that provides insights into lncRNA localization based on RNA-sequencing databases [91] (http://lncatlas.crg.eu), we performed *in silico* analysis of the potential subcellular localization of *OBSCN-AS1* in the MCF7 breast cancer cell line, which predicted a preferential nuclear distribution (Fig. 4B). Although this finding needs to be experimentally verified, it suggests that *OBSCN-AS1* may modulate gene expression possibly by altering chromosomal structure through regulation of histone modification, transcription factor recruitment, RNA polymerase II binding, and alternative splicing, all of which are well-known mechanisms of actions of lncRNAs [90].

It has been previously shown that antisense and sense transcript expression is tightly correlated, and that positive expression correlations are more common than negative ones [92–94]. Our preliminary results using PLAIDOH [95], a computational method for functional prediction of lncRNAs, indicated a positive correlation between *OBSCN* and *OBSCN-AS1* expression (Pearson correlation coefficient=0.9989727, p-value=0.02886). Consistent with this, using cBioPortal we found amplification of *OBSCN-AS1* in 8.84% (i.e., in 88 out of 996 cases) of breast invasive carcinomas, often co-occurring with *OBSCN* amplification (Fig. 5A) and 1q status gain (Fig. 5B). Given these findings, we performed mutual exclusivity analysis of the *OBSCN-AS1* and *OBSCN* gene pair via cBioPortal and found a significant tendency of co-occurrence (Fig. 5C), suggesting that alterations in these genes tend to coincide in the same patient sample. Based on the fact that these two genes partially share a genomic locus and that amplification was the only genetic alteration our

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analysis showed for *OBSCN-AS1*, we infer that amplification copy number alterations in *OBSCN* and *OBSCN-AS1* co-occur in breast cancer patient samples likely with 1q status gain.

Concluding remarks and future perspectives

Obscurins, initially thought to be exclusively expressed in striated muscles, have been recently implicated in tumorigenesis given their purported tumor suppressor function. Leveraging our research expertise on obscurins, we have been delineating their role in breast cancer formation and progression. Our research has demonstrated that giant obscurins are abundantly expressed in normal breast epithelium, where they preferentially concentrate at the cell membrane, but are dramatically diminished in advanced stage breast cancer biopsies and tumor cell lines [34, 37]. Depletion of giant obscurins from normal breast epithelial cells induces epithelial-to-mesenchymal transition (EMT) and stemness, promotes survival in the presence of common chemotherapeutic agents, enhances cell migration and invasion, and results in alterations in major signaling cascades including the RhoA and PI3K/AKT axes [34, 35, 37, 38].

In this comprehensive review, we summarize the vast literature documenting *OBSCN* mutations across different cancer types and the growing evidence implicating *OBSCN* in cancer development. Undoubtedly, we have barely “scratched the surface” in trying to understand the impact and regulation of this giant and complex gene in tumorigenesis. Of note, we are cognizant about the increased likelihood for large genes like *OBSCN* to accumulate high numbers of mutations, whether these are passenger with questionable functional significance or driver providing a selective growth advantage. Nevertheless, given the 4 hotspots along the *OBSCN* gene (Fig. 1C), we postulate that at least a considerable number of the identified mutations may have important functional ramifications in driving and/or potentiating tumorigenesis.

Given our current knowledge on *OBSCN*’s involvement in cancer formation and progression along with its recently reported radioresistance in esophageal squamous cell carcinoma [96], we propose 8 main areas of future research to the scientific community that will help us tackle the biology of *OBSCN* in carcinogenesis:

- Investigate the expression profile and possible tumor suppressor role of *OBSCN* across different cancer types, with special emphasis on brain cancer where an overwhelming number of mutations has been described.
- Interrogate the possible interplay between *OBSCN* loss and breast cancer subtypes.
- Examine the impact of individual pathogenic variants and/or mutational hotspots in *OBSCN*.
- Elucidate the downstream signaling cascades that are altered due to *OBSCN* loss, mutation or deregulation; such studies should consider the potentially distinct and overlapping roles of giant obscurins A and B in modulating key signaling cascades via their unique and shared domains.

- Decipher the role of epigenetic regulation of *OBSCN* with particular attention on the impact of hypermethylation.
- Interrogate the role of *OBSCN-AS1* in *OBSCN* regulation in healthy and cancer cells.
- Determine how mutant *OBSCN* provides treatment resistance.
- Device novel and effective ways to restore obscurin expression and/or functionality using peptide therapy or gene editing with the ultimate goal of generating targeted therapies that can be used individually or in combination with current treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health/National Cancer Institute Interdisciplinary Training Program in Cancer Biology T32CA154274 to T.G. and the MPI R01CA183804 award to A.K.K.

Glossary

Deep deletion
deep loss, possibly a homozygous deletion

Shallow deletion
shallow loss, possibly a heterozygous deletion)

Constitutional balanced chromosomal translocation
a chromosomal translocation occurring in all body tissues, without loss or gain of genetic material

Non-passenger somatic missense mutation
an oncogenic driver missense mutation that is not a germline mutation

Contralateral nephrogenic rest
Wilms‟ tumor precursor lesion located in the contralateral kidney relative to the primary tumor

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Fig. 1: Domain architecture, transcript abundance and mutational profile of *OBSCN*.

(A) Schematic depiction of the domain architecture of giant obscurin isoforms A and B, adapted from [6]. **(B)** *OBSCN* expression profile across normal tissues and organs obtained from the Genotype-Tissue Expression (GTEx) project (GTExPortal; https://gtexportal.org/home/); expression values are shown in transcripts per million (TPM). Box blots are shown as 25th, 50th (median) and 75th percentiles; points are displayed as outliers if they are above or below 1.5 times the interquartile range. **(C)** Graphic representation of the 224 *OBSCN* mutations located in coding regions across 15 cancer types; obscurin-B (the largest obscurin

isoform; accession number NP_001092093.2) was used for domain-assignment of the reported mutations. Mutations residing in domains Ig1 through Ig57 are present in all 3 giant obscurin isoforms (A, B, and theoretical 1C). The 4 regions containing the highest number of mutations are denoted with arrowheads, with the “Kin1-Ig59 linker” region containing the most non-synonymous variants among them.

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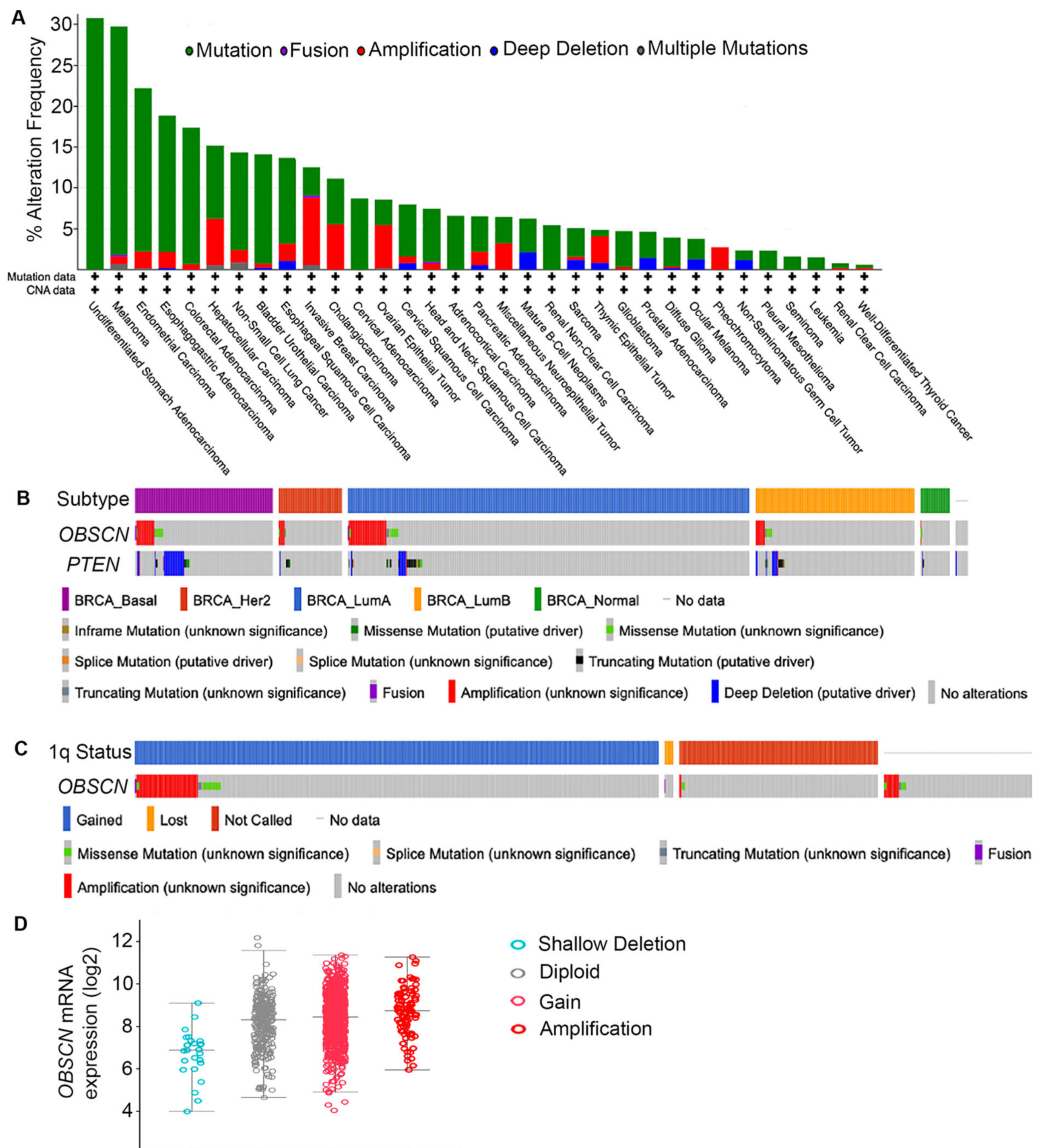

Fig. 2: Analysis of *OBSCN* alterations and expression levels across different cancer types using the TCGA PanCancer Atlas Studies datasets available through cBioPortal (https://www.cbioportal.org).

(A) The average *OBSCN* mutational frequency was determined across 33 cancer types to be ~11% ranging between 0.6% in well-differentiated thyroid cancer to >30% in undifferentiated stomach adenocarcinoma. **(B)** Evaluation of the genetic and copy number alterations in *OBSCN* and *PTEN* in invasive breast cancer according to molecular subtype revealed a 12.55% and 11% overall alteration frequency, respectively, with amplification being the most common alteration for *OBSCN* and deep deletion for *PTEN.* **(C)** Copy

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number alterations in *OBSCN* in invasive breast carcinoma samples sorted according to 1q status showing that the majority of *OBSCN* amplifications are observed in samples with 1q gain. **(D)** Plot of *OBSCN* mRNA expression *vs* putative *OBSCN* copy number alteration (from GISTIC) in breast invasive carcinomas (994 samples from cbioportal) showed minimal changes in *OBSCN* transcript levels, with the exception of shallow deletion where they appear reduced.

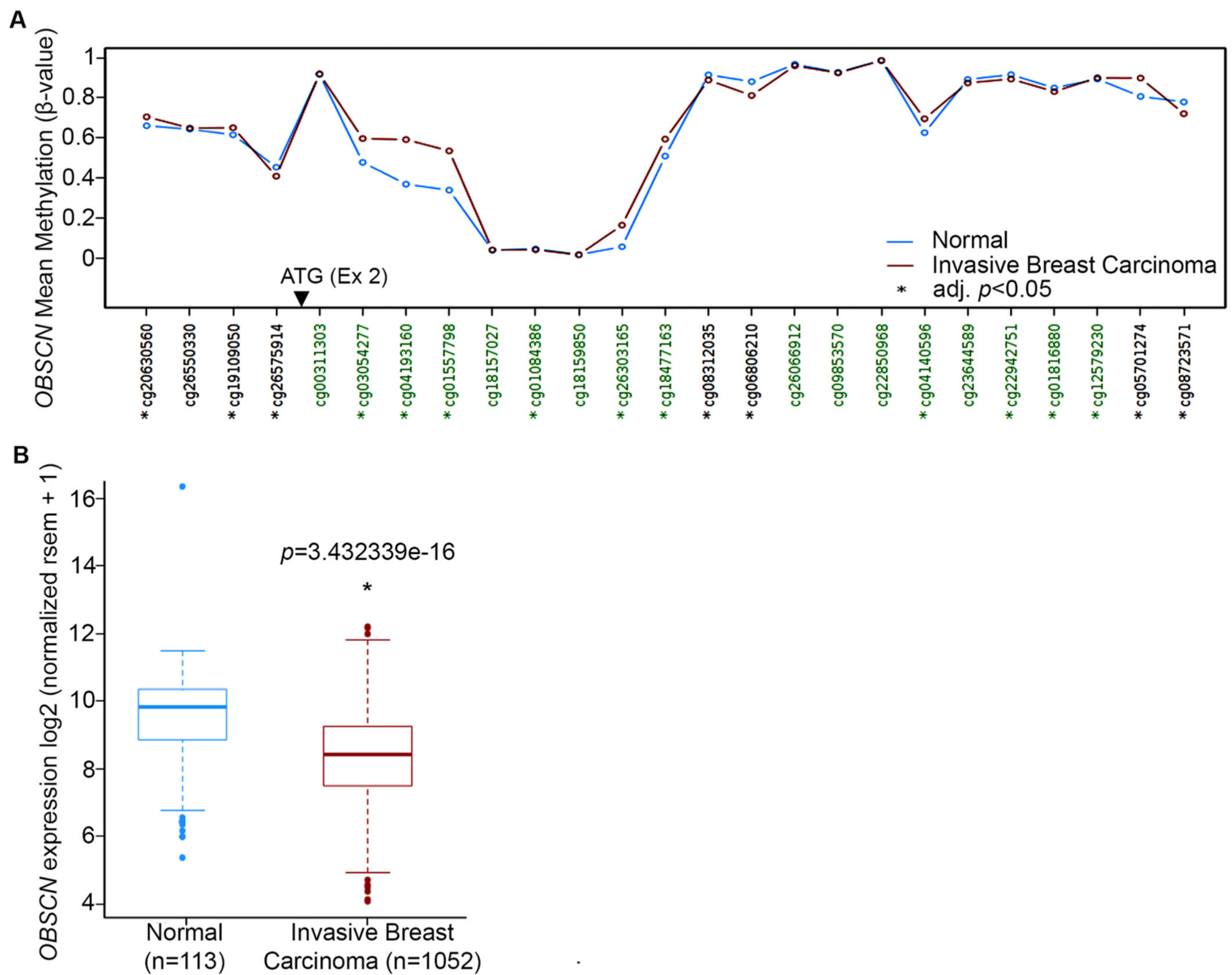

Figure 3: Differential methylation profile of *OBSCN* in normal and breast invasive carcinoma samples.

Examination of the DNA methylation profile of *OBSCN* in normal and breast invasive carcinoma samples using data from the Illumina Infinium Human Methylation450 (450K) BeadChip array and the interactive viewer Wanderer (http://maplab.imppc.org/wanderer/). **(A)** Representative results for 25 CpG probes out of 125 possible CpG probes spanning the entire *OBSCN* locus in chromosome (chr) 1 are shown; the exact locations of the first (cg20630560) and last (cg08723571) CpG probes shown are chr 1: 228208157–228208158 and chr 1: 228220101– 228220102, respectively. The first 4 CpG probes are located upstream of the *OBSCN* translation start site (ATG located in exon 2 at chr 1: 228211784) and the other 21 CpG probes are within the gene body of *OBSCN*. CpG probes recognizing individual sites are shown in black while those located within CpG islands are shown in green; statistically significant differentially methylated sites between normal and breast carcinoma samples are denoted with an asterisk (*; adj. p<0.05, Wilcoxon Rank Sum Test with Benjamini and Hochberg adjustment). **(B)** *OBSCN* expression is significantly reduced

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in invasive breast carcinoma samples compared to normal samples (*; p=3.432339e-16, Wilcoxon Rank Sum Test).

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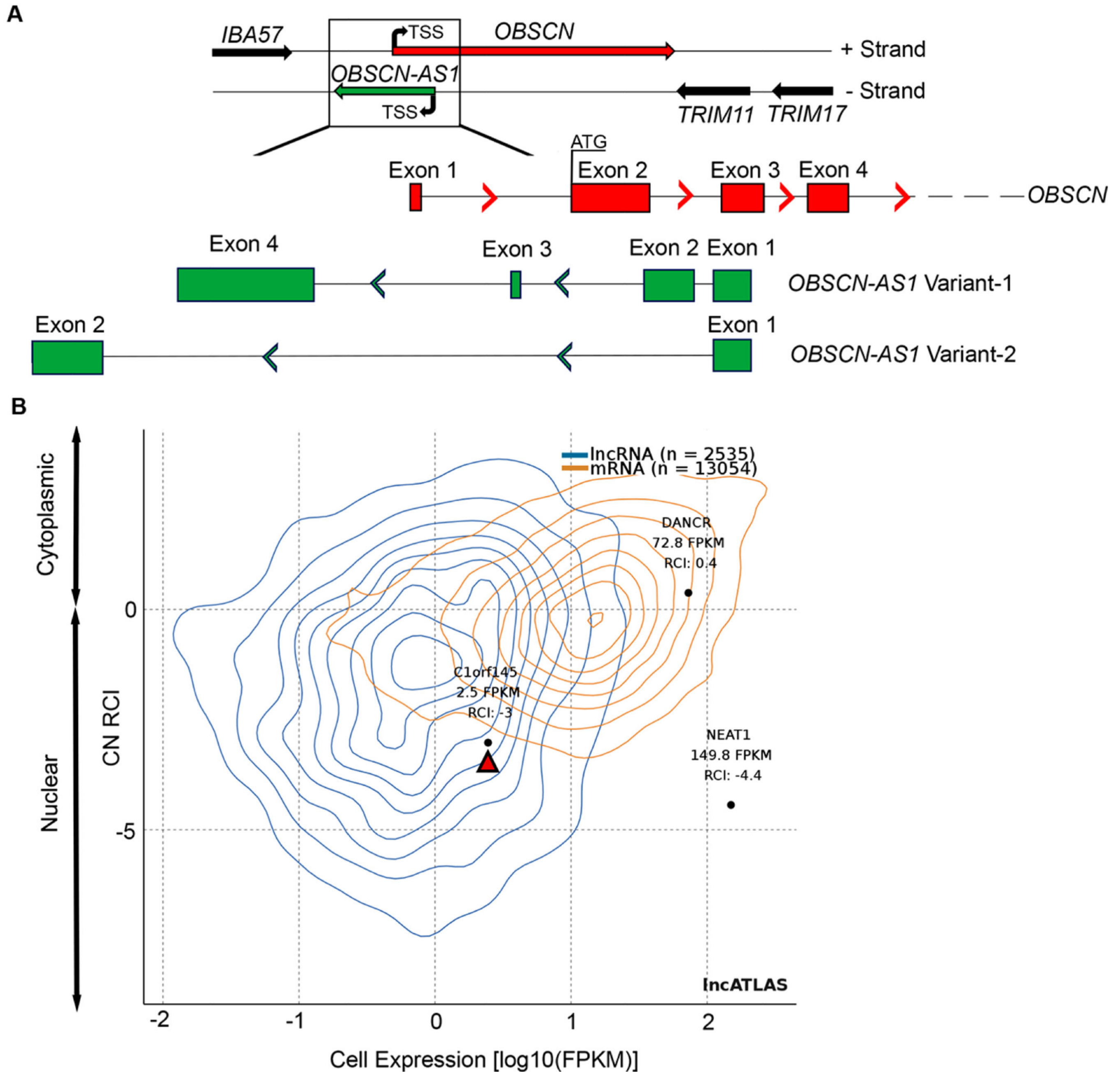

Figure 4: Schematic representation of the *OBSCN-AS1* locus and subcellular distribution.
(A) Schematic illustration of the *OBSCN-AS1* genomic locus. *OBSCN-AS1*, *TRIM11*, and *TRIM17* are encoded by the (−) strand, while *OBSCN* and *IBA57* are encoded by the (+) strand. **(B)** *In silico* evaluation of the subcellular localization of *OBSCN-AS1/C1orf145* in the MCF7 breast cancer cell line determined using LncAtlas (http://lncatlas.crg.eu). The preferential concentration (CN) of a given gene in the nucleus or the cytoplasm is expressed as Relative Concentration Index (RCI), which is a comparison of the gene concentration per unit mass of RNA between the two compartments. An RCI<0 indicates that a particular gene is more concentrated in the nucleus compared to the cytoplasm and *vice versa*. *OBSCN-*

AS1/C1orf145 (Ensemble gene ID: ENSG00000162913) exhibits a preferential nuclear localization with an RCI value of −3; *NEAT1* and *DANCR* lncRNA genes exhibit nuclear (RCI: −4.4) and cytoplasmic (RCI: 0.4) distributions, respectively, and were used as reference genes in our analysis.

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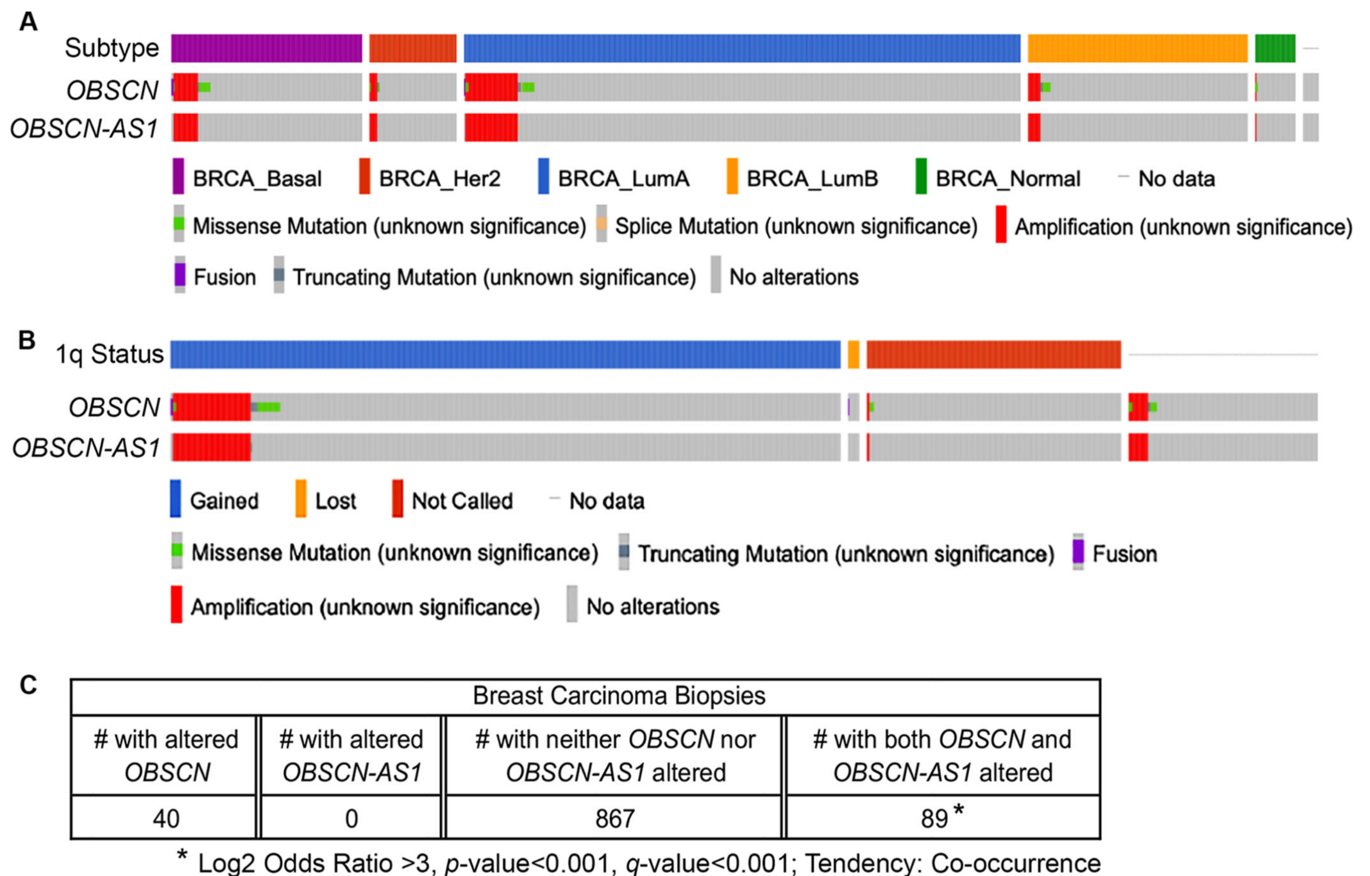

Breast Carcinoma Biopsies			
# with altered *OBSCN*	# with altered *OBSCN-AS1*	# with neither *OBSCN* nor *OBSCN-AS1* altered	# with both *OBSCN* and *OBSCN-AS1* altered
40	0	867	89 *

* Log2 Odds Ratio >3, *p*-value<0.001, *q*-value<0.001; Tendency: Co-occurrence

Figure 5: Analysis of *OBSCN-AS1* alterations and expression levels in breast invasive carcinoma. *OBSCN-AS1* genetic and copy number alterations were evaluated in breast invasive carcinoma using the Breast Invasive Carcinoma (TCGA, PanCancer Atlas) dataset available through cBioPortal. **(A)** Evaluation of genetic alterations in *OBSCN-AS1* and *OBSCN* in invasive breast carcinoma samples according to subtype revealed an 8.84% (i.e., in 88 out of 996 cases) and 12.55% overall alteration frequency, respectively, with amplification being the most common alteration in both genes. **(B)** Genetic alterations in *OBSCN-AS1* and *OBSCN* in invasive breast carcinoma samples sorted according to 1q status showing that the majority of amplification alterations in both genes are observed in samples with 1q gain. **(C)** Mutual exclusivity analysis of the *OBSCN-AS1* and *OBSCN* gene pair indicate a co-occurrence tendency in invasive breast carcinoma samples (*p*-value <0.001, one-sided Fisher Exact Test; *q*-value <0.001, Benjamini-Hochberg FDR correction).

Table 1:

Summary of OBSCN mutations documented in the literature across different cancer types. Reported mutations in OBSCN documented across 15 different forms of cancer, including the specific genome mutation location (as specified by the referenced NCBI human genome build), affected amino acid according to obscurin-B isoform (NCBI Ref. NP_001092093.2), mutation type, co-documented presence of non-synonymous TP53 mutations, and relevant reference. Under mutation location, we indicate those mutations that are homozygous (in bold) or heterozygous (italicized) when reported in the literature. Mutations belonging to introns within obscurin-B are reported as amino acid changes in respective domains and motifs belonging either to obscurin-A (Iso A; NCBI Ref. NP_443075.3) or to the larger theoretical obscurin isoform 1C, when possible (Iso 1C; NCBI Ref. NP_001258152.2). Abbreviations: MSI – Microsatellite Instability; NA – Not Applicable.

Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
Gastric Tumor (adenocarcinoma)	Patient Tumor Samples	Ch.1 228437718 C>T Ch. 1 228475577 G>A	1362 1723	Ig14-Ig15 Linker Ig18-Ig19 Linker	Missense	Associated-MSI NA	Yes	GRCh37 /hg19	[45]
		Ch. 1 228475577 G>A Ch. 1 228527746 G>A Ch. 1 228538596 A>G	3242 5786 6123	Ig35 RhoGEF Ig57		Associated-MSI NA Associated-MSI			
	YCC11 YCC16	**Ch.1 226528228** Ch.1 226536484	E1758Q F2809V	Ig19 Ig30		NA		NCBI36/ hg18	[47]
	YCC3 MKN1 MKN7 MKN28 (cell lines)	Ch.1 226626277 *Ch.1 226552790* Ch.1 226571278	G7059S R1147L A4511T	Kin1-Ig59 Linker Ig12 Ig49					
		Ch.1 226571278 **Ch.1 226625016**	A4511T A6727V	Ig49 Kin1-Ig59 Linker					
Stomach adenocarcinoma	TCGA Database Analysis	NA	NA	NA	Missense	NA	Yes	NA	[46]
Colorectal Cancer	CCDS Database	*Ch.1 224738933 C>T*	1136	Ig12	Missense	Functionally clustered with genes modulating cellular motility and adhesion	Yes	NCBI35/ hg17	[1]
		Ch.1 224768323 G>A Ch.1 224768443 G>A Ch.1 224769112 G>A Ch.1 224770933 G>A Ch.1	1752 1792 1930 2090 3983	Ig19 Ig19 Ig21 Ig23 Ig43-Ig44 Linker	Nonsense Missense Missense Missense Missense				

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Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
		224801358 G>A							
		Ch.1 224812011 G>A	R4558H	2nd FN-III	Missense				
Breast Cancer	CCDS Database	*Ch.1 22473 7790 delG*	1034	Ig11	Frameshift	Functionally clustered with genes modulating cellular motility and adhesion	Yes	NCBI35/ hg17	[1]
		Ch.1 224773206 C>T	2314	Ig26	Missense				
		Ch.1 224813617 G>A	4810	Ig51-IQ Linker	missense				
		Ch.1 224816488 G>A	5071	IG52-Ig53 Linker	missense				
		Ch.1 224833341 C>T	5713	RhoGEF	Nonsense				
Pancreatic Ductal Adenocarcinoma (PDAC)	Patient Tumor Samples	NA	NA	NA	1 Amplification 1 Missense	Associated with survival less than 25 months	Yes	GRCh38 /hg38	[49]
Pancreatic Intraepithelial Neoplasia (PEN)		Ch.1 228433195	C1188F	Ig13	Missense	Predicted to be damaging	Yes	GRCh37 /hg19	[50]
PDAC with associated PEN		Ch.1 228505380	R4593C	2nd FN-III					
Salivary Gland Mucoepidermoid	Patient Tumor Samples	Ch.1 226614003 C>T	C6263R	C-term.-ABD Linker (Iso A)	Missense	NA	Yes	NCBI36/ hg18	[48]
		Ch.1 226593203 C>T	F5704S	RhoGEF (GTPase interaction site)					
Low Grade Glioma (LGG)	TCGA Project ID: TCGA-LGG	Ch1: 228341613 G>A	W6011	PH-Ig56 Linker	Nonsense	Associated with higher intra-	Yes	GRCh38 /hg38	[41]
		Ch1: 228319145 C>T	A4798V	Ig51-IQ Linker	Missense	tumor heterogeneity,			
		Ch1: 228299403 G>A	S4452N	Ig46 (Iso 1C)	Missense	worse prognosis, shorter interval			
		Ch1: 228350934 C>T	P6137L	Ig.5.7	Missense	before			
		Ch1: 228340809 G>A	R5873H	RhoGEF	Missense	recurrence			
		Ch1: 228283581 C>T	T2939M	Ig32	Missense				

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Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
		Ch1: 228341480 G>A	R5967H	PH-domain	Missense				
		Ch1: 228303735 C>T	A4600V	Ig49 (Iso 1C)	Missense				
		Ch1: 228377157 C>T	R7715R	Kin2 domain	Synonymous				
		Ch1: 228217187 C>T	T851M	Ig8	Missense				
		Ch1: 228288739 G>A	R3397H	Ig36	Missense				
		Ch1: 228283725 C>T	R2987H	Ig3i	Synonymous				
		Ch1: 228377110 G>A	A7700T	Kin2 domain	Missense				
		Ch1: 228288794 C>T	C3415C	Ig36	Synonymous				
		Ch1: 228298707 C>T	L4400L	Ig46 (Iso 1C)	Synonymous				
		Ch1: 228299977 G>A	V4524M	Ig48 (Iso 1C)	Missense				
		Ch1: 228356185 C>T	T6232T	C-term - ABD Linker (Iso A)	Synonymous				
		Ch1: 228287954 G>T	V3269L	Ig.3.4	Missense				
		Ch1: 228321665 C>T	R4942W	Ig.5.1	Missense				
		Ch1: 228321626 G>A	A4929T	Ig.5.1	Missense				
		Ch1: 228304362 G>A	A4688T	Ig50 (Iso 1C)	Missense				
		Ch1: 228341479 C>T	R5967C	PH-domain	Missense				
		Ch1: 228273895 G>A	D1755N	Ig19	Missense				
		Ch1: 228323546 C>T	R5198C	Ig.5.3	Missense				

Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
		Ch1: 228214854 C>T	S528S	1st FN-III	Synonymous				
		Ch1: 228300082 G>T	D4559Y	Ig48 (Iso 1C)	Missense				
		Ch1: 228277849 C>T	R2284	Ig24-Ig25 Linker	Nonsense				
		Ch1: 228214853 C>A	S528Y	1st FN-III	Missense				
		Ch1: 228315995 G>T	E4387D	Ig.4.8	Missense				
		Ch1: 228243447 T>C	L1065P	Ig11-Ig12 Linker	Missense				
		Ch1: 228316744 G>A	A4441T	Ig49	Missense				
		Ch1: 228243285 C>T	T1011M	Ig.1.1	Missense				
		Ch1: 228293537 C>T	R3684R	Ig40	Synonymous				
		Ch1: 228244491 C>A	P1134Q	Ig12	Missense				
		Ch1: 228211935 C>T	A51V	I.g.1	Missense				
		Ch1: 228212775 (del AGCTC)	Intron	Splice region	Deletion				
		Ch1: 228268612 C>T	C1648C	Ig18	Synonymous				
		Ch1: 228279902 G>A	R2493Q	Ig.2.Z	Missense				
		Ch1: 228341615 G>A	NA	NA	Splice Donor				
		Ch1: 228333293 C>T	R5276C	Ig.5.4	Missense				
		Ch1: 228306988 C>T	A4005	Ig.4.4	Missense				
Glioblastoma (GBM)	TCGA Project ID:	Ch1: 228299327 C>A	Q4427K	Ig4Z (Iso 1C)	Missense				

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Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
	TCGA-GBM	Ch1: 228299502 C>G	S4485	Ig4Z (Iso 1C)	Nonsense				
		Ch1: 228299269 C>G	A4407A	Ig46-Ig4Z Linker (Iso 1C)	Synonymous				
		Ch1: 228259597 C>T	A1653V	Ig18	Missense				
		Ch1: 228337003 G>T	-		Splice acceptor				
		Ch1: 228217043 C>T	A803	Ig8-Ig9 Linker	Missense				
		Ch1: 228299988 A>C	R4527S	Ig.4.8 (Iso 1C)	Missense				
		Ch1: 228350929 G>A	P6135P	Ig57	Synonymous				
		Ch1: 228372962 G>A	S7395N	Kin1-Ig59 Linker	Missense				
		Ch1: 228214307 C>T	G464G	Ig5	Synonymous				
		Ch1: 228212516 C>T	R245C	Ig2-Ig3 Linker	Missense				
		Ch1: 228287889 G>A	R3247Q	Ig35	Missense				
		Ch1: 228217289 G>T	-		Splice donor				
		Ch1: 228362590 C>T	R6226	Ig57-Ig58 Linker	Nonsense				
		Ch1: 228278781 G>A	A2318	Ig25	Missense				
		Ch1: 228299383 C>G	L4445L	Ig47 (Iso 1C)	Synonymous				
		Ch1: 228295038 T>C	V3885A	Ig.4.2	Missense				
		Ch1: 228286862 G>A	D3123N	Ig34	Missense				
		Ch1: 228215644 C>T Ch1:	T637M L1938F	Ig7 Ig.2.1	Missense Missense				

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		228274700 C>T							
		Ch1: 228368359 G>A	Q6570Q	Kin1	Synonymous				
		Ch1: 228356258 C>T	Intron	-	-				
		Ch1: 228366556 C>A	L6445M	Ig.5.8	Missense				
		Ch1: 228316759 G>A	G4446S	Ig49	Missense				
		Ch1: 228299465 G>A	G4473R	Ig46 (Iso 1C)	Missense				
		Ch1: 228250017 C>T	A1362A	Ig15	Missense				
		Ch1: 228323438 G>A	D5162N	Ig.5.3	Missense				
		Ch1: 228374424 G>T	D7499Y	Ig.5.9	Missense				
		Ch1: 228336238 C>T	A5502V	Ig52-Ig53 Linker	Missense				
		Ch1: 228370048 G>A	E6692K	Kin1	Missense				
		Ch1: 228292010 G>A	G3484G	HjbHH	Missense				
		Ch1: 228341564 G>C	W5995S	PH-domain	Missense				
		Ch1: 228280782 G>A	R2728Q	Ig.2.9	Missense				
		Ch1: 228264295 C>T	R1589W	Ig.17.	Missense				
		Ch1: 228273990 C>T	S1786S	Ig19	Synonymous				
		Ch1: 228274409 G>A	R1883H	Ig.2.0	Missense				
		Ch1: 228299475 C>G	S4476	Ig46 (Iso 1C)	Nonsense				

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		Ch1: 228309503 G>A	G4319E	Ig.4.8	Missense				
		Ch1: 228356166 C>T	T6226T	Post-IgC2 (Iso A)	Synonymous				
		Ch1: 228321629 C>T	R4930C	Ig.5.2	Missense				
		Ch1: 228322033 G>A	A5064A	Ig52-Ig53 Linker	synonymous				
		Ch1: 228374637 G>T	K7516N	Ig.5.9	Missense				
		Ch1: 228306513 G>A	R3934H	Ig.4.3	Missense				
		Ch1: 228256816 C>T	A1492V	Ig16	Missense				
		Ch1: 228362653 T>C	S6247P	Ig57-Ig58 Linker	Missense				
		Ch1: 228286107 G>A	E3012K	Ig33	Missense				
		Ch1: 228280229 G>A	E2572K	Ig28	Missense				
		Ch1: 228317492 G>	E4530D	2nd FN-III	Missense				
		Ch1: 228287787 C>T	A3213V	Ig35	Missense				
		Ch1: 228299416 C>T	D4456D	Ig46 (Iso 1C)	Synonymous				
		Ch1: 228256772 G>A	K1477K	Ig16	Synonymous				
		Ch1: 228283679 C>A	L2972I	Ig.3.2	Missense				
		Ch1: 228377115 G>A	K7701K	Kin2 (active site)	Synonymous				
		Ch1: 228274346 T>C	V1862A	Ig.2.0	Missense				
		Ch1: 228307267 C>A	P4068T	Ig.4.5	Missense				

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		Ch1: 228217091 G>T	C819F	Ig.9.	Missense				
		Ch1: 228372050 G>T	S7091I	Kin1-Ig59 Linker	Missense				
		Ch1: 228360440 C>T	R4150K	Ig44 (Iso A)	Missense				
		Ch1: 228294182 G>A	R3721M	Ig53	Missense				
		Ch1: 228299364 G>T	R4439M	Ig46 (Iso 1C)	Missense				
		Ch1: 228288868 C>A	S3440Y	Ig37	Missense				
		Ch1: 228215613 G>A	G627S	Ig7	Missense				
		Ch1: 228278948 C>T	F2373F	Ig26-Ig27 Linker	Synonymous				
		Ch1: 228377190 G>A	P7726P	Kin2	Synonymous				
		Ch1: 228264301 C>A	L1591M	Ig17	Missense				
		Ch1: 228250089 C>T	D1386D	Ig15	Synonymous				
		Ch1: 228256757 G>A	E1472E	Ig.1.6	Synonymous				
		Ch1: 228318974 A>G	E4741G	Ig51-IQ linker	Missense				
		Ch1: 228321649 G>T	V4936V	Ig62	Synonymous				
		Ch1: 228243444 G>T	R1064L	Ig11-Ig12 Linker	Missense				
		Ch1: 228272029 G>A	E1932K	Ig19 (Iso 1C)	Missense				
		Ch1: 228288277 C>T	S3343L	Ig36	Missense				
		Ch1: 228287762 C>T	R3205C	Ig35	Missense				

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		Ch1: 228307359 C>T	A4098A	Ig45	Synonymous				
		Ch1: 228340089 G>A	Q5801Q	RhoGEF	Synonymous				
		Ch1: 228215734 C>T	T667M	Ig7	Missense				
		Ch1: 228323480 G>T	E5176	Ig53	Nonsense				
		Ch1: 228308293 C>T	L4217L	Ig46	Synonymous				
		Ch1: 228340064 C>T	S5793L	RhoGEF	Missense				
		Ch1: 228371950 C>T	P7058S	Kin1-Ig59 Linker	Missense				
		Ch1: 228287923 C>T	F3258F	Ig35	Synonymous				
		Ch1: 228317697 G>C	V4599L	2nd FN-III	Missense				
	Patient Tumor Sample	Ch.1 224812011 G>A	R4558H	2nd FN-III	Missense	Germline Mutation	Yes	NCBI35/ hg17	[3]
Wilms Tumor	Patient Tumor Sample	*t(1;7) (q42;p15), causing "GA" deletion*	Intron 1	-	-	Germline Mutation	No	NA	[57]
Epithelial Ovarian Cancer (EOC)	Patient tumor Sample	NA	NA	NA	2 Missense	NA	Yes	GRCh37 /hg19	[59]
Uterine Serous	Patient tumor Samples	Ch1: 226631126 A>T	E7656V	FNIII-Kin2 Linker	Missense	NA	Yes	NCBI36/	[60]
Carcinoma		Ch1: 226548617 –18 Del.	H3758	Ig43	Frameshift			hg18	
Melanoma	Patient Tumor Sample	NA	E4574K	2nd FN-III	Missense	NA	No	NCBI35/ hg17	[3]
Oral Squamous Cell Carcinoma	FaDu	*Ch1: 228464316 C>G*	P2129R	Ig23	Missense	NA	yes	HRCh37 /hg19	[42]
		Ch1: 228504650 G>A	R4509H	Ig49	Missense				

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	SCC-15	*Ch1: 228462074 G>A*	R1871H	Ig20	Missense				
		Ch1: 228469801 G>C	G2789R	Ig30	Missense				
	SCC-25	*Ch1: 228404305 G>A*	R760Q	Ig8	Missense				
		Ch1: 228467095 T>C	V2449A	Ig26-Ig27 Linker	Missense				
	SCC-4	*Ch1: 228464303 G>T*	A2125S	Ig23	Missense				
Pre-neoplastic oral stem cell clones	Laser Capture from K14CreER TAM;ROSA 26 female mice	NA	NA	NA	25 Missense 1 Frameshift 1 In-frame Del.	NA	No	NA	[43]
Androgen Independent Prostate Cancer (AIPC) Mouse Model	Human Xenograft Prostate Cancer	Ch1: 228264130 Ch1: 228233817	F1534 intron	Ig16-Ig17 Linker-	Pro-viral Integration Sites	Associated with co-documented reduced OBSCN gene mRNA expression in prostate cancer	NA	GRCh38 /hg38	[62]
	TCGA Provisional Database	NA	NA	3 missense 2 gene deletions	NA	NA	NA		
Prostate Adenocarcinoma	Patient Tumor Samples	Ch1: 228482633 T>C	Q3850	Ig42	Nonsense	NA	Yes	HRCh37 /hg19	[64]
		Ch1: 228509429 T>C	R4963C	Ig52	Missense				
		Ch1: 228400217 T>C	R245C	Ig2-Ig3 Linker	Missense				
		Ch1: 228559495 T>C	Q7006	Kin1-Ig59 Linker	Nonsense				
		Ch1: 228560317 A>G	V7280M	Kin1-Ig59 Linker	Missense				
		Ch1: 228470926 T>C	A2893V	Ig31	Missense				
		(Ch1: 153845910/	-	GATA2B (-) 5'-UTR/OBS	Fusion				[65]

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		Ch1: 228393182)		CN(+) promoter					
		Ch1: 226466172 C>T	S22L	Ig1	Missense	Specific to Castrate-Resistant Prostate Cancer	yes	NCBI36/ hg18	[66]
		Ch1: 226573337 C>T	T4754M	Ig51-IQ Linker	Missense				
		Ch1: 226573457 G>A	R4794H	Ig51-IQ Linker	Missense				
		Ch1: 226570139 C>T	Intronic	-	-	Not Castrate-Resistant Prostate Cancer	No		
Papillary Renal Cell Carcinoma (pRCC)	TCGA Project ID:TCGA KIRP	Ch1: 228299946 228299947insTT	L4514Ffs *6	Ig49 (Iso 1C)	Frameshift	NA	Yes	GRCh38 /hg38	[58]
		Ch1: 228321344 G>A	V4835I	IQ	Missense				
		Ch1: 228319074 G>A	E4744E	Ig50-IQ Linker	Synonymous				
		Ch1: 228274796 G>C	V1970L	Ig21	Missense				
		Ch1: 228371112 A>G	V6778V	Kin1-Ig59 Linker	Synonymous				
		Ch1: 228283995 G>T	Intron	-	-				
		Ch1: 228306533 G>A	G3941S	Ig43	Missense				
		Ch1: 228333313 delCAAG	K5282	Ig54	Frameshift				
		Ch1: 228273990 C>T	S1786S	Ig19	Synonymous				
		Ch1: 228280360 A>G	V2615V	Ig.2.8	Synonymous				
		Ch1: 228216595 228216596insA	A758Gfs *61	Ig8	Frameshift				
		Ch1: 228295010 C>A	Q3876K	Ig42	Missense				
		Ch1: 228340765 G>A	A5858A	RhoGEF	Synonymous				

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Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
		Ch1: 228351329 A>G	K6143R	Ig58					
		Ch1: 228359755 delGC...	R6288	C-term.-ABD Linker (Iso A)	Frameshift				
		Ch1: 228338043 G>A	A5634T	SH3	Missense				
		Ch1: 228303698 G>A	D4588N	Ig49 (Iso 1C)	Missense				
		Ch1: 228323416 G>A	M5154I	Ig53	Missense				
		Ch1: 228372866 C>G	A7363G	Kin1-Ig59 Linker	Missense				
		Chi: 228282151 G>T	E2806	Ig30	Nonsense				
		Ch1: 228333235 G>C	E5256D	Ig53-Ig54 Linker	Missense				
		Ch1: 228286154 G>C	R3027R	Ig3.3	Synonymous				
		Ch1: 228286837 C>A	T3114T	Ig34	Synonymous				
		Ch1: 228360418 delT	V6509	C.term - ABD (Iso A)	Missense				
		Ch1: 228271972 C>T	Q1913	IQ	Nonsense				
Clear Cell Renal Cell Carcinoma (ccRCC)	TCGA Project ID: TCGA-KIRC	Ch1: 228215780 G>C	L682L	Ig50-IQ Linker	Synonymous				
		Ch1: 228256790 C>T	S1483S	Ig21d	Synonymous				
		Ch1: 228317617 G>T	C4572F	Kin1-Ig59 Linker	Missense				
		Ch1: 228371834 C>A	P7019Q		Missense				
		Ch1: 228372058 T>C	L7094L	Ig43	Synonymous				
		Ch1: 228283536 A>G	N2924S	Ig54	Missense				

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Cancer Type	Source	Mutation Location	Obscurin Isoform B Amino Acid	Obscurin Isoform B Domain	Type of Mutation	Comments	Co-mut. TP53	Human Genome Build	Ref.
		Ch1: 228256792 T>C	F1484S	Ig19	Missense				
		Ch1: 228317618 C>T	C4572C	Ig28	Synonymous				
		Ch1: 228212585 G>A	E268K	Ig8	Missense				