A comprehensive genomic meta-analysis identifies confirmatory role of OBSCN gene in breast tumorigenesis

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ABSTRACT

The giant multifunctional protein “OBSCURIN” is encoded by OBSCN gene and is mostly expressed in cardiac and other skeletal muscles responsible for myofibrils organization. Loss of OBSCURIN affects the entire downstream pathway proteins vital for various cellular functions including cell integration and cell adhesion. The OBSCN gene mutations are more frequently observed in various muscular diseases, and cancers. Nevertheless, the direct role of OBSCN in tumorigenesis remains elusive. Interestingly, in clinical breast cancer samples a significant number of function changing mutations have been identified in OBSCN gene. In this study, we identified a significant role of OBSCN by conducting an integrative analysis of copy number alterations, functional mutations, gene methylation and expression data from various BRCA cancer projects data available on cBioPortal and TCGA firebrowse portal. Finally, we carried out genetic network analysis, which revealed that OBSCN gene plays a significant role in GPCR, RAS, p75 or Wnt signaling pathways. Similarly, OBSCN gene interacts with many cancer-associated genes involved in breast tumorigenesis. The OBSCN gene probably regulates breast cancer progression and metastasis and the prognostic molecular signatures such as copy number alterations and gene expression of OBSCN may serve as a tool to identify breast tumorigenesis and metastasis.

INTRODUCTION

Cancer in humans is a huge health burden in modern era worldwide. In recent years cancer genomics is assumed as a latest advancement in cancer research, which starts from disease identification and leads to personalized therapy. Owing to this scientific advancement, several key genetic elements have been identified and characterized in various cancer types. In human carcinogenesis events, cells lose their adhesion, integrity and others morphological characteristics and gain the invasive and migratory properties leading to cellular transitions which are called epithelial-to-mesenchymal transition (EMT), which is the most crucial step in initiating cancer metastasis [1, 2]. Large number of genes and their corresponding proteins are involved in EMT associated pathway including OBSCURIN. The giant OBSCURIN is encoded by unique gene called OBSCN located on chromosome 1 at loci q42 [3]. The giant OBSCURIN has two isoforms namely OBSCURIN-A & B [4–6]. The OBSCURIN-A comprises of immunoglobulin (Ig) and fibronectin type-III domains located in the amino terminal while in carboxyl terminal contains several signaling domains including Isoleucin & Glutamine calmodulin-binding (IQ), SRC homology-3 (SH3) domains, pleckstrin homology (PH) and a Rho-guanine nucleotide exchange factor (Rho-GEF) domains which are scattered in the non-modular sequences. The OBSCURIN-B or myosin light chain kinase (MLCK) isoform contains two serine/threonine kinase (STK) domains, which replace the non-modular carboxyl terminus of OBSCURIN-A. Additional to these domains, ERK kinase domain exists for phosphorylation along with two Ig domains [3, 4, 7, 8]. Every OBSCURIN domain regulates vast number of cellular and functional roles such as cell adhesion, migration and cell morphology, etc. Apart from these functional roles, OBSCURIN-A/B are also involved in cellular co-ordination that prevents cells from going in an EMT process [4, 9].
MLCK family domains chiefly maintain the cellular organizations and contractility [10]. Moreover, a huge number of copy number alterations and mutations were observed in OBSCN gene in many cancer types, although, OBSCN is highly mutated in breast cancer. The main study is to uncover the functional mutations and the active role of OBSCN in breast tumorigenesis. A few studies on this gene also proved that, the reduced or altered OBSCN gene expression largely disturbs the cellular integration and activates cancer initiation; therefore, OBSCN may function as a tumor suppressor gene and prevent cellular transition [11, 12]. OBSCN gene is mostly expressed in many cancer types although the role of functional association in cancer is still uncertain. Several studies on OBSCN gene mutations revealed potential roles of OBSCURIN in melanoma, glioblastoma, colorectal, lung, breast and pancreatic cancer [13–15]. Also, our recent study on breast cancer driver mutations genes also emphasized OBSCN as one among the 63 top candidate driver genes [16]. Here we will mainly focus on copy number variation, non-silent mutations, promoter methylation followed by epigenetic changes and their consequences in breast cancer formation.

Mutations and expression of OBSCN: genetic predisposition to tumorigenesis

Recent advances in high throughput sequencing support genomics in a magnificent way. In every cancer, vast numbers of genes are mutated, therefore the identification of real genetic elements responsible for carcinogenesis becomes quiet challenging. OBSCN gene is frequently and consistently mutated in various cancers with a strong correlation with breast, colorectal and other female related cancers. Recent studies revealed that TP53 and OBSCN genes are highly mutated among 189 candidate genes in breast and colorectal cancers [13, 17]. However, scientific background on OBSCN mutation and its impacts are limited in comparison to the other key genes involved in breast cancer [18, 19]. Mutations in OBSCN gene (> 15%) are observed in breast cancer patient samples published by TCGA. Furthermore, a recent study on OBSCN gene in breast cancer revealed that the loss of giant OBSCURIN protein increases the cell migration with more metastatic characteristics [20].

However, recent studies revealed that OBSCURIN expression loss causes functional abnormality, which increases the probability of cancer in human breast epithelial cells. Perry et al. (2012) found that knocking down OBSCN significantly affected breast epithelial cells in both growth and biological property, such as cellular adhesions, cell-cell communications, etc. As a result, expression level of other interlinked proteins such as E-cadherin, α and β-catenin involved in cell-cell junctions were largely affected. Meanwhile, an elevated level of p120-catenin prevents the cell adhesion and stimulates mesenchymal proteins, which stimulate cellular morphogenesis leading to metastasis [21–23]. The actin and microtubule cytoskeleton dysregulation is responsible for cellular transformation and migration found in many cancers and activation and deactivation of Rho-GTPase family proteins majorly regulates actin filaments through Ras homolog gene family, member A (RHOA) signaling. The loss of OBSCN caused RHOA signaling impairment leading to breast cancer initiation, progression and metastasis. [12, 24–28]. The consequent event of OBSCN loss may also affect tubulin microtentacles (McTN) formation. McTN is the key process in metastatic breast cancer and it increases metastatic probability and endothelial coupling followed by circulating tumor cells (CTC) [29–31].

Similarly, low levels of epithelial proteins accelerate phenotypic changes in cells, which further affect focal adhesion and increase of F-actin dynamics in cell-cell contacts [28]. Another interesting study proved that, an active oncogene, K-RAS in the OBSCN knockdown cells increases tumorigenesis probability [9]. The OBSCN down regulation also affected RHOA-mediated pathways leading to dramatic reduction of Rho activated kinase (ROCK) and its targeting proteins such as myosin light chain (MLC), lim kinase (LimK) and coflin [4, 8, 22, 29, 30]. In this study, we reviewed and analyzed OBSCN gene and its association with breast cancer. The high throughput sequencing data for breast cancer mutations in clinical samples were retrieved from various breast cancer projects available in eBioPortal (www.cbioportal.org), Pan-Cancer (https://www.synapse.org), mRNAseq and Methylation data were retrieved from Firebrowse (http://firebrowse.org/) data portal, which provides multi-tier cancer data analysis. The analyses of the overall mutation rates and the mutational patterns of OBSCN gene, as well as their impact on downstream pathways may help us to predict feasible drug targets for breast cancer.

The OBSCN mutations in human cancers using high throughput analysis

Apart from breast cancer, OBSCN gene is widely mutated in many other cancer types as well (Supplementary Figure 1) (www.cbioportal.org). Among the various cancer types breast cancer cases are observed with significant numbers of OBSCN mutations (n = 819) with average mutation frequency of 18%. The overview of this work and study selection are given in the Figure 1. The overall mutations percentage of OBSCN gene in various breast cancer projects are illustrated in Figure 2 (www.cbioportal.org). The large numbers of functional mutations were observed and OBSCN copy number analyses were performed using GISTIC program and many copy gain, amplification and few deletion mutations were observed in major breast cancer projects data (Figure 3). From the overall DNA copy number analysis, we found that OBSCN
Figure 1: Flow diagram summarizing selection and validation of the present meta-analysis on OBSCN gene.

Figure 2: Overall OBSCN gene mutations across various breast cancer projects data of cBioportal. (A) number of OBSCN mutations/copy number variations found in each project; (B) Distribution of OBSCN gene mutations across the patient samples (%).

Figure 3: GISTIC copy number analyses of OBSCN gene in various breast cancer projects found with more number of gain and amplification mutations.
gene had more number of copy gain and amplification mutations and these variations may potentially affect the giant OBSCURINs protein level and their subsequent expression.

The significant copy number alterations are observed and their outcomes are predicted using GISTIC copy number analysis [32]. The overall mutation profiles of OBSCN gene in breast cancer shallow loss, diploid, gain and high level amplifications are classified based on distinctive copy number profiles and their clinical settings [33, 34]. This analysis also revealed a huge number of gain and high level amplification mutations in OBSCN gene found in invasive ductal carcinoma (IDC), followed by invasive lobular carcinoma (ILC) and so on. These results indicated that OBSCN gene might play an active role in invasion of cancer cells to distant organs and other metastatic processes. The subsequent mRNA expression analysis was performed using breast cancer patient data and based on the median Log2 value, the OBSCN gene is seen down regulated in majority of the mutated TCGA data. To confirm the OBSCN gene expression we also compared with other well-known breast cancer genes such as TP53, PIK3CA, ARID1A, BRCA1, etc. and found that these genes had closer expression levels and it indicated that OBSCN gene mutations may also have a positive correlation with breast cancer dispositions (Figure 4) (https://cran.r-project.org/); (www.cbioportal.org); (https://cancergenome.nih.gov/). In addition to that, OBSCN methylation profiles were also assessed using breast cancer methylation data by calculating beta value and found OBSCN gene apparently hyper-methylated. Then we compared methylation profiles of OBSCN gene over various cancer types and found the gene is hyper-methylated in breast cancer data, which indicates its methylation may also play an important role in disease etiology. We used promoter methylated and gene expression samples of OBSCN gene from TCGA wanderer (http://maplab.imppc.org/wanderer/). The OBSCN methylation and gene expression was compared with normal (n = 98) and tumor (n = 720) samples at cg09411356 and interestingly, the hyper methylation on OBSCN gene significantly reduced gene expression in tumor than the normal samples. The detailed methylation profile of single probe and its corresponding gene expression and comprehensive comparison of methylation pattern over major cancer types is depicted in Figure 5. (https://xenabrowser.net/#). The downregulation of OBSCN may affect cell-cell adhesion of breast epithelial cells and cells undergo EMT process [12]. Further we analyzed the expression of OBSCN gene in various immunohistochemical breast cancer molecular subtypes. Nearly 73% cases found with luminal A (46.9%) and luminal B (26.0%) type of disease followed by other subtypes including basal (10.7%), Her2(8.9%), normal (3.78%) and claudin-low (3.56%). These molecular subtypes classifications distinctly emphasized, OBSCN is vital gene may involve in the cell proliferation. Moreover, several previous studies on OBSCN gene mutations and its association with other cancers were identified by intensive literature search and the detailed information such as cancer types, mutation profiles, co-mutating genes, amino acid variations and supporting citations are tabulated (Table 1).

Functional analysis of differentially expressed OBSCN gene in breast cancer

Mutations in the OBSCN gene largely affect various interlinked proteins involved in cell adhesion and integration process [52]. Functional mutations in OBSCN largely affects E-cadherin, α-catenin and β-catenin expressions which initiate epithelial cells dispersions and subsequently increase the level of F-actin followed by cell migration [20, 53–55]. Actin filaments are vital for cellular mechanistic amoeboid movements accelerated by filapodia or pseudopodia like structures formed in cytoplasmic regions [56, 57]. A recent study showed that OBSCN functional gene mutations are responsible for the loss of vital protein expressions [21]. The loss of OBSCN appears to be consequent event in primary to metastatic breast cancer majorly affecting cellular integration. The protein kinases (PKs) are from the larger protein family involved in various cellular, structural and functional mechanisms in humans. The Serine/Threonine Kinase (STK) is one of the important protein kinase involved in cellular cytoskeletal and integration roles such as cell proliferation, differentiation and apoptotic process, etc. [58, 59]. The STK actively binds with p120-catenin and regulates cadherin facilitated intercellular adhesions and E-cadherin-p120 bound complex also plays a crucial role in maintaining the equilibrium of cadherin levels [60, 61]. The OBSCN mutations affect E-cadherin level, lead to flux in adherens junctions and increase cell disintegration followed by cell movement [62]. The OBSCN gene mutations heavily affect STK protein activity and also act as a ligand for small ankyrin1 (sANK1). sANK1 are integral proteins to the fundamental spectrin-actin cytoskeleton involved in cellular movement activation and proliferation, which cause metastasis [63]. Moreover, OBSCN gene also acts as a kinome regulatory element that regulates few other kinase family proteins in either direct or indirect manner.

Gene interaction analysis revealed that OBSCN gene is positively correlated with protein binding and cell transition associated genes

The OBSCN gene mutations have significant relationship with genes responsible for cellular structural and functional roles. Also, the expression of OBSCN gene is positively correlated with few other genes such as TOP1MT, MYC, TARBP1, ADCK3, TRIM17, and
Figure 4: Comparative evaluation of \textit{OBSCN} gene expression pattern with other known breast cancer genes shows \textit{OBSCN} gene has similar expression pattern with some of the known breast cancer driver genes.

Figure 5: (A) \textit{OBSCN} methylation profiles of normal \((n = 98)\) and breast tumor samples \((n = 720)\) comparison; (B) Gene expression comparison between normal and tumor samples; (C) Comparison of methylation profiles showed that \textit{OBSCN} gene largely hypermethylated in breast cancer than other cancer types.
RND2. Initially we used metastatic breast cancer data (Metastatic Breast cancer-France 2016) for network analysis and revealed many participating genes such as RHOT2, RAC3, RHOD, AKT3, PAK1, PKD1, AKT2, RIPK2, ERBB2, PRKD1, etc. which are positively correlated with amplification mutations and have strong association with cell proliferation, differentiation, tumorigenesis and other metastasis features. Similarly, we used British Columbia Xenografts (British Columbia, Nature 2014) data linked with many interesting genes such as RHOB, ITSN1, RHOD, ERBB2, AKT3, BRAF, CDK1, MASTL along with eight more genes which were highly correlated with cell signaling and cell migration functions. The breast invasive carcinoma (TCGA, Provisional) data showed that the OBSCN had strong interaction with PAK1 gene through RHOU and RAC3 genes. The breast cancer oncogene, PAK1, acts as a hub gene connecting two sub networks. In first sub network, PDPK1 gene a central gene linked with other significant genes such as AKT3, ERBB2, SGK3, PRKCB, PRKCG, etc. and the second sub network is connected with STK3, AURKA, CSNK1D, NEK2, etc. The PAK1 and PDPK1 genes are strongly associated with breast tumorigenesis and cell migration in metastatic event [64, 65]. Along with the afore-mentioned genes, OBSCN is interconnected with other genes such as PAK2, AKT1, TP53, NEK2, WNK1, CSNK1D, etc. which are frequently mutated in invasive breast carcinoma (Nature 2012) (Cell 2015). The detailed OBSCN mutation specific network neighbors were identified using functional protein association network using STRING (http://string-db.org/). Additional pathway specific networks were also used to identify and validate the genes involved in OBSCN associated pathway using PCViz (http://www.pathwaycommons.org/) and detailed networks are illustrated in Figure 6.

**OBSCN genetic alterations lead to cell survival and switches towards EMT**

Substantial evidences propose that OBSCN functional mutations may have strong associations with Wnt signaling regulated by a series of activators along with β-catenin [66]. In the nucleus, β-catenin binds to transcription activation elements (TCF/LEF), leading to the activation of the target genes (specifically CCND1, c-MYC, and FN1) responsible for the regulation of cell proliferation and differentiation [67–69]. Mutational event of OBSCN gene majorly affects β-catenin downstream signaling leading to lower down or over expression of genes such as CCND1, c-MYC, and FN1, which stimulates several types of cancers including breast, ovarian, lung, pancreatic, colorectal, and uterine carcinoma [70–75]. The elevated level of fibronectin or loss of proteins such as E-CADHERIN, B-CATENIN, etc. may cause cellular

| Disease Types | Most common co-mutation with OBSCN gene | Major mutations type | Specific Amino acid variations | References |
|---------------|----------------------------------------|----------------------|-------------------------------|------------|
| Breast Cancer | TP53                                   | Missense             | NA                            | [13, 21, 35]|
| Lung Cancer; Respiratory Disease | TP53, ARID1A, PTEN, KRAS, MYC, PIK3CA, BRAF, EGFR, NRAS | Missense             | NA                            | [36, 37]|
| Gastrointestinal Stromal tumor and Leiomyosarcoma | C9orf65, TTN, SPEG | Missense             | NA                            | [15, 38, 39]|
| Colorectal cancer | TP53                                   | Missense             | NA                            | [13, 35]|
| Prostate cancer | CLDN7, STRA13, FLNA EAM83H, CLDN7, ARFGAP3, KDM2A | Missense             | NA                            | [40, 41]|
| Melanoma | EPHA3, TTN | Somatic Missense | E4574K | [14]|
| Cervical/ Endometrial and Ovarian cancer | Tp53, PIK3CA, FBXW7, NEB, Dnah11, ORA12, RNFIN, SPTA1, UB2, UTRN, BSN | Missense             | NA                            | [42]|
| Liver & Pancreatic cancer | ATPF1, TRPM4, ML13, ARID1A, ARID1B, ARID2, SOS1, MROH1 | Missense             | NA                            | [14, 19, 43, 44]|
| Glioblastoma | TTN | Germline Missense | R4558H | [13, 14]|
| Esophageal Cancer | C9orf65, TP53, ARID1A, MUC17 | Missense             | NA                            | [45]|
| Sarcoma | C9orf65, PRUNE2 | Missense             | NA                            | [15]|
| Nephroblastoma and other kidney cancers | PTHB1 | Missense             | NA                            | [46, 47]|
| Cardiomyopathy | TTN, MYH7, DSP, VCL, LAMA4, MYOM1, TNNC1, TNNH | Truncating Mutation | NA                            | [47–51]|

Table 1: The OBSCN gene mutations and their associations with various cancer types
proliferation followed by EMT, which render patients overall survival. Henceforth, \textit{OBSCN} gene is believed to be an indirect regulator of fibronectin expression in breast and colon cancer [72, 76]. The fibronectin malfunction may also affect vital proteins such as integrin and other transmembrane receptors responsible for cell-cell contact, extracellular matrix (ECM) communications, cell adhesion and accommodate several ligands such as collagen, laminin and vitronectin. Fibronectin is an important protein which activates integrin and is involved in the regulation of cellular signal transduction, cell proliferation, gene expression and cytoskeletal reorganization [77]. Hence, the \textit{OBSCN} gene mutation perhaps affects the regulation of β-catenin and other regulatory elements in Wnt signaling pathway largely affects mainly the downstream genes and proteins expressions involved [78]. Interestingly in metastatic cancer, \textit{OBSCN} mutation observed with new list of interacting genes in Ras protein family members such as \textit{RHOD}, \textit{RHOT2} and \textit{RAC3} are involved in major functions such as reorganization of actin cytoskeleton, GTPase activity, formation of lamellipodia, respectively [79–81, 99]. The \textit{RAC3} gene is an important gene activates \textit{PAK1}, which is vital to stimulates F-actin formation leads to cell migration. Similarly, \textit{PAK1} activates another important proliferation related protein, aurora kinase (\textit{AURKA}) and it inhibits \textit{IKB-α}, which inhibits apoptosis through NF-κB and \textit{CIAP1} genes [82, 83]. The detailed \textit{OBSCN} gene involved cancer-associated pathway illustrated in Figure 7. The Panther database (http://pantherdb.org/) was used to further validate gene

Figure 6: (A) Mutation specific \textit{OBSCN} functional association network and its interacting network partners identified using STRING database; (B) Gene specific interactions pathways were identified using pathway commons network visualizer and many interacting partners of \textit{OBSCN} are cancer-associated genes.

Figure 7: The \textit{OBSCN} gene involved either direct or indirect pathway of GPCR, Ras, p75 or Wnt signaling. (In GPCR pathway, \textit{OBSCN}-RhoGEF is simultaneously regulates mDIA, Rho and their downstream genes which are involved in cell differentiation and lamellipodia formation; the other chain of \textit{OBSCN}, MLCK and its downstream pathway activates MLC leads to formation of stress fiber which cause cell differentiation and survival; On the other way RhoA activated from p75 (NTR) pathway also involved in Ras pathway regulates down regulates MLCK; in Wnt signaling \textit{OBSCN} disequilibrium leads to β-catenin loss which leads to carcinogenesis.
ontology based molecular functions analysis and further confirmed by evaluating fold enrichment scores [84]. The OBSCN gene and their networks neighbors were involved in binding and cell regulatory mechanisms including cell adhesion and binding with several receptors and signaling molecules (Figure 8).

**Mutational tolerance profiles and their consequences in proteome level**

Recent studies on OBSCN mutations uncover key amino acids mutations, which are crucial factor for many human diseases including cardiomyopathy and cancers. [49, 51, 85]. In this study, we also attempted to know the deep impact of OBSCN mutations including the proteome level. The OBSCN gene mutational protein variants retrieved from Ensembl genome browser (http://asia.ensembl.org/) and their effects were predicted using Variant Effect Predictor (VEP) tool (http://asia.ensembl.org/Tools/VEP) and wANNOVAR (http://wannovar.wglab.org/) [86, 87]. The SIFT (http://sift.jcvi.org/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) tools were used to achieve the most reliable protein variants prediction, and we also considered few other variants impact prediction algorithms such as Log ratio test (LRT), Mutation Taster (http://www.mutationtaster.org/), Mutation Assessor (http://mutationassessor.org/r3/), FATHMM (http://fathmm.biocompute.org.uk/) and Variant effect scoring tool 3 (VEST3) (http://karchinlab.org/apps/appVest.html), etc. Most of the abovementioned tools predict the variants and their impacts based on sequence changes and very few tools also consider structural changes [88–96]. The variants predicted by the maximum number of tools were used to filter and used for further analysis (Figure 9) (http://compbio.berkeley.edu/proj/variant/manual.html). From the overall analysis, it was

![Figure 8: Molecular functional classification analysis of OBSCN gene and its network neighbor genes showed that OBSCN gene majorly involved in binding activity with various molecules.](image)

![Figure 9: (A) SIFT and POLYPHEN-2 programs were used to predict the most common and significant OBSCN mutational impact variants; (B) To validate the high-impact variants and their mutations impact are predicted using 10 potential impact prediction tools.](image)
seen that OBSCN gene had a low tolerance against few functional mutations (total of 251 protein variants), half of the variants (~48%) exhibited deleterious and probably damaging effects (Supplementary Table 1), and rest of the variants came in deleterious & possibly damaging and deleterious with benign categories [95, 97, 98]. The overlapping mutational impact variants were taken and further analyzed the somatic mutations using COSMIC database. The detailed OBSCN mutational impact on protein levels are emphasized using lollipop plot of maftools (Bioconductor R-package) and amino acids variations and their impacts are indicated in various colors representations along with mutations count (Figure 10) (https://github.com/PoisonAlien/mafTools). In addition, the key mutational variants, types, amino acid variations and impacts in domain levels are listed in Table 2.

**OBSCN mediated targeted anti-cancer therapy: a present and future perspectives**

Several interesting genes are interlinked with tumorigenesis including OBSCN gene, which is considered the key gene among them. The OBSCN gene may act as unique target for anti-cancer therapy in breast and other cancers, since it is having multiple interactions with intra and inter-cellular levels of numerous interconnecting proteins. In breast cancer targeted anti-cancer therapy, several key genes are targeted such as CTNND1, CDC42 and DVL. Over expression of P120-CATENIN tremendously inhibited several signaling molecules such as RHOA, RAC1 and CDC42 which are involved in the regulation of numerous cellular functions [100]. Similarly, downregulation of CDC42 initiated cancer. Interestingly OBSCN gene is an active target regulating CDC42 downstream pathway accounting for the tumorigenesis [101]. DVL (Dishevelled homology1) protein phosphorylation are vital phenomenon accelerated by CK1ε and RIPK4 in Wnt signaling pathway [102]. Hence, reduced β-catenin level and LRP6 association facilitates further downstream signaling of β-catenin, which activates transcription factors/lymphoid enhancer-binding factors (TCF/LEF) involved in numerous cellular regulations and cell migrations. An alternative and most interesting target is DVL expression responsible for calmodulin-dependent protein kinase (CamK-II) activation followed by expression of F-actin. The F-actin is the key protein involved in cell division, contraction and locomotion and altered F-actin dynamics is most vital feature for epithelial to mesenchymal transition (EMT) of metastatic breast cancer. Hence, anti-cancer drug targets may actively repress DVL and it may potentially block F-actin dynamics, which inhibits EMT process in cancer cells, and the overall phenomena, is indirectly regulated by OBSCN gene.

**Concluding remarks**

We have analyzed OBSCN gene mutation, expression and methylation data and the data revealed that OBSCN gene is one of most frequently mutated gene in various cancer types, especially in breast cancer. The OBSCN gene mutation may play an essential role during cancer initiation and progression, largely distressed by various kinds of mutations in gene level due to loss of heterozygosity, various oncogenic factors, intra and intercellular environmental stress. Mutations in OBSCN gene largely affects multiple properties of cells including cell adhesion and increase the integration and render cellular transitions and many more. Evaluation of OBSCN mutational status may help early prognosis of metastatic potential of breast cancer. Mutational, copy number and epigenetic analysis of OBSCN gene status may serve as a
| Genomic Coordinate | Reference and Variants | Mutation Type | Copy Number | Amino acid Variant | Major Domain Affected |
|-------------------|------------------------|---------------|-------------|--------------------|----------------------|
| 228402602         | C>G                    | Missense      | AMP         | T544S              | Fibronectin-III      |
| 228412169         | NA                     | Missense      | Gain        | V888G              | Ig-Domain            |
| 228432150         | T>G                    | Missense      | Gain        | V1212G             | Ig-Domain            |
| 228432150         | NA                     | Missense      | Gain        | V1120G             | Ig-Domain            |
| 228434244         | G>T                    | Missense      | Gain        | Q1243H             | Ig-Domain            |
| 228434340         | C>T                    | Missense      | Diploid     | T1382M             | Ig-Domain            |
| 228461665         | G>A                    | Missense      | Gain        | V1778M             | Fibronectin-III      |
| 228462221         | G->                    | Frameshift DEL| Del         | V2282fs            | Ig-Domain            |
| 228462225         | -*G                   | Frameshift INS| Gain        | Q2283fs            | Ig-Domain            |
| 228479785         | NA                     | Missense      | Gain        | G3509V             | Ig-Domain            |
| 228479785         | G>T                    | Missense      | Gain        | G3938V             | Ig-Domain            |
| 228480325         | T>C                    | Missense      | Gain        | G3693V             | Ig-Domain            |
| 228480325         | T>C                    | Missense      | Gain        | G3998R             | Ig-Domain            |
| 228480325         | NA                     | Missense      | Gain        | W3569R             | Ig-Domain            |
| 228481236         | C>T                    | Missense      | Gain        | W3753R             | Ig-Domain            |
| 228491624         | AC->                   | Frameshift DEL| Del         | L4419fs            | Ig-Domain            |
| 228492905         | G>C                    | Missense      | Gain        | S4538T             | Ig-Domain            |
| 228494273         | G>A                    | Missense      | Gain        | E4911K             | Ig-Domain            |
| 228494273         | NA                     | Missense      | Gain        | E3954K             | Ig-Domain            |
| 228494979         | NA                     | Missense      | AMP         | K4071N             | Ig-Domain            |
| 228497221         | G>A                    | Missense      | Gain        | V5282M             | Ig-Domain            |
| 228497221         | NA                     | Missense      | Gain        | V4325M             | Ig-Domain            |
| 228497221         | G>A                    | Missense      | Gain        | V5037M             | Ig-Domain            |
| 228503574         | G>A                    | Missense      | Gain        | E5059Q             | Ig-Domain            |
| 228506585         | T>C                    | Missense      | Gain        | V5668A             | SH3                  |
| 228506585         | NA                     | Missense      | Gain        | V4711A             | Ig-Domain            |
| 228506585         | T>C                    | Missense      | Gain        | V5423A             | Ig-Domain            |
| 228506947         | G>A                    | Missense      | Gain        | A5544T             | NA                   |
| 228509882         | G>A                    | Missense      | Gain        | E5826K             | NA                   |
| 228521392         | NA                     | Missense      | Gain        | S5322C             | Ig-Domain            |
| 228521466         | C>T                    | Missense      | Gain        | R6304W             | NA                   |
| 228521466         | NA                     | Missense      | Gain        | R5347W             | Ig-Domain            |
| 228521466         | C>T                    | Missense      | Gain        | R6059W             | Ig-Domain            |
| 228537694         | -*T                   | Frameshift INS| Gain        | Y6796fs            | NA                   |
| 228538601         | C>T                    | Nonsense      | Gain        | Q6838*             | Ig-Domain            |
| 228547542         | G>A                    | Missense      | Gain        | G6317R             | NA                   |
| 228556454         | G>T                    | Missense      | Gain        | R7312L             | STK-cat Domain       |
| 228559643         | C>A                    | Missense      | Gain        | P7767Q             | NA                   |
| 228559663         | C>T                    | Missense      | Gain        | P7774S             | Protein Kinase 2     |

*1Ig- Immunoglobulin. AMP-Amplification; Del-Deletion
tool for the prediction of targeted anti-cancer drugs, which will be helpful for targeted breast cancer therapy [11, 20]. Further functional studies on OBSCN gene in various model systems with gene overexpression and/or targeted disruption should also greatly facilitate these processes.

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CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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