Comprehensive Study of Human FBXW7 Deleterious nsSNP's Functional Inference and Susceptibility to Gynaecological Cancer

Archana Vasuki.K
Sathyambama Institute of Science and Technology: Sathyabama Institute of Science and Technology
Jemmy Christy.H (jemmyjacob@gmail.com)
Sathyabama University https://orcid.org/0000-0002-5538-4270

Research Article

Keywords: FBXW7, cancer, uterine carcinosarcoma, chemoresistance, variants

Posted Date: September 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-692732/v1

Abstract

Cancer is one of the world's major causes of mortality, and it plays a most important role in the world's declining life expectancy. F-box and WD-40 domain protein 7 (FBXW7), a typical participant of the F-box family of proteins, has been considered as an antitumor protein and one of the maximum deregulated ubiquitin-proteasome system proteins in uterine carcinosarcoma, endometrial clear cell carcinoma and cervical carcinoma with the greatest prevalence of alterations. FBXW7 variants with known clinical significance, as well as nsSNP's in the F-Box and WD40 domains, were evaluated using functionality prediction web resources. Upon analysing the seventy-three deleterious nsSNP's impact on protein stability and function, we identified that forty-one nsSNP's of WD40 domain and three of F-Box domain imply decreased stability of the FBXW7 structure. Next to TP53 and PTEN, FBXW7 was reported with the highest percentage of arginine substitution among mutations related to cancer. The current research concentrated on two arginine residue locations (Arg465, Arg505) within the WD40-repeat domain, which is vital for substrate binding. Computational analysis revealed that significant deviation in stability and structural configuration of mutants R505L, R465H, R465P, R505G, R505C, R465C R505S and R505L structures. Protein–protein interaction network of FBXW7 populated with promising hub proteins NOTCH1, c-Myc, CCNE1, STYX, KLG5, SREB1, NFKB2, SKP1, CUL1, thus alteration in the FBXW7 leads to aberration in their signalling pathways as well as their substrate binding ability makes this protein as attractive target for personalized therapeutic intervention.

Introduction

Cancer is one of the prominent reasons for mortality globally, including increased life expectancy. GLOBACAN 2020, a Global Cancer Observatory statistics statement, publicised that breast cancer and cervical cancer are more predominant amongst the Indian population [1]. The disruption of various essential biochemical pathways and biological processes, including ubiquitination, is known to cause cancer. Proteasomal breakdown mediated via the ubiquitin-proteasome system (UPS) is the predominant eukaryotic proteolytic activity for more than 80% of proteins that control the cell cycle, cell proliferation, and death [2]. Therefore, the aberration of the UPS paves the way for cancer induction. Ubiquitination occurs when the ubiquitin protein binds to the target protein, causing enzymes like ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase to act (E3).A ubiquitin ligase (E3) attaches to substrate proteins and then causes them to be degraded by another enzyme called the 26S proteasome. Variation in ubiquitin ligase E3 function has been discovered to be one of the important contributors in the initiation and progression of cancer, according to research [3]. Among the various forms of E3 ubiquitin ligases, the SCF (Skp1-Cullin1-F-box) complex, which comprised of the scaffold protein Cullin1 (Cul1), the RING finger protein Rbx1, the linker protein S phase kinase-associated protein 1 (Skp1), and the F-box protein, has been extensively studied [4]. In humans, there are 69 F-box proteins, each of which has an F-box motif in its amino-terminal region and a carboxyl terminal containing WD-tryptophan and aspartic acid or leucine-rich repeats. -LRR [5]. Thus, the F-box is categorized as FBXW-F-box combined with WD repeats, FBXO-F-box with no motifs, FBXL-F-box coupled with LRR. F-box with 7 tandem WD40 repeats (FBXW7), which is known for its oncogenic substrates' recognition and targeting to facilitate ubiquitin-mediated degradation in many human malignancies. Furthermore, recent research has revealed their influence on chemotherapy resistance [6]. Phosphorylation of the conserved FBXW7 phosphodegron motifs on the substrates is essential for FBXW7 to bind with and target them for destruction. FBXW7 is known for its participation in the oncoprotein targets cyclin E, c-JUN, c-MYC, NOTCH-1, and MCL-1 in ubiquitylation and proteasome destruction. Knock out research findings revealed that almost ninety proteins were targeted as substrates by FBXW7. FBXW7 targets are mostly

transcription factors or important signalling molecules that control a variety of cellular activities, such as primary proliferation and tumour growth. FBXW7 mutations owing to chromosomal deletion or mutation, as well as promoter hypermethylation, are common in a variety of human malignancies [7]. In at least 16 percent of human endometrial tumours, FBXW7 has been found to be mutated. These mutations were found in the protein's amino-terminal region or the substrate-binding domain [8]. Though the most prevalent endometrial cancer (EC) histotype, endometrioid EC, can often be cured with hysterectomy, serous EC is an uncommon category that is coupled with metastases, relapse, therapy sensitivity, and poor prognosis [9]. The tumour suppressor FBXW7 is typically mutated in serous ECs compared with other clinically aggressive subtypes [8,12]. Nearly 15%-29% of serous ECs, 11-39% of uterine carcinosarcomas, 13-25% of clear cell endometrial cancers, and 0%-15% of endometrioid endometrial cancers were reported with somatic mutations in FBXW7 [10,11]. As a result, it has been hypothesized that reduced expression or deletion of FBXW7 in breast cancer leads to an accumulation of oncogenic transcription factors, which are key regulators of proliferation, apoptosis, and eventually transformation [13]. Mutations not only assist the oncogenic substrate accumulation but also direct the resistance phenotype of T-ALL cell lines in response to a gamma-secretase inhibitor [14]. These data show that FBXW7 could be exploited as a potential target for overcoming chemo resistance in a variety of cancers, not merely as a biomarker for predicting chemotherapy effectiveness. As a result, we evaluated sequence and structure-based bioinformatics protein stability indicator techniques to anticipate the effect of the mutation on FBXW7 protein stability to get extensive structural and mechanistic insight of wild type to mutant FBXW7 protein structures. The results of these computational investigations suggest that structural changes caused by missense mutations may affect FBXW7's functional activity, which will aid in the development of inhibitors.

Materials And Methods

TCGA based expression analysis and mutant screening of FBXW7 gene

The expression of FBXW7 in different cancers was higher when compared with 21 other cancer types in the TCGA database. Acute myeloid leukaemia had the lowest mRNA expression of FBXW7. FBXW7 expression in OSC was the highest among the 21 types of cancers documented in the TCGA. Data on FBXW7 mutational frequency was gathered from databases such as the Single Nucleotide Polymorphism database [15] and the Catalogue of Somatic Mutations in Cancer (COSMIC), which, based on the reference database GRCh37/hg19, includes both healthy (controls) and disease populations with gynaecological cancers. In addition, we utilized the CLINVar database to screen the listed mutants and their clinical significance among gynaecological cancer patients [16].

Screening of nsSNPs prevalence in FBXW7 functional domain

Through functional study of protein families, InterPro, a domain screening tool, calculates a protein's domains and active sites. It projected three FBXW7 functional domains: FBOX domain (278–235), WD40-Repeat-containing domain (376–659), and FBOX-LIKE domain (282–325) and showed that all ten clinically significant nsSNPs were in the WD40-Repeat-containing domain [17]. nsSNP’s localized in both F box and WD40 domain were considered for further study.

Screening of deleterious mutant’s functional effects in FBXW7

SIFT, PANTHER, PolyPhen-2 and SNPs & GO, PROVEAN, and PredictSNP were used to assess the functional effects of the variant retrieved from the clinvar database, as well as SNPs located in the F box and WD40 domain.

This validated the findings' precision and rigour, and we classified those variations as harmful to all six programmes. The SIFT algorithm calculates the impact of amino acid substitutions using sequence homology to uncover both helpful and hazardous variants. Substitution of amino acids in specific residue positions with a probability of 0.05 is thought to be deleterious and intolerant, whereas substitution with a probability greater than 0.05 is thought to be tolerant [18]. The PANTHER programme categorises proteins according to their evolutionary links, molecular activities, and interactions with other proteins. It assesses modifications using position-specific evolutionary conservation scores derived from the alignment of multiple evolutionarily related proteins [19]. Based on sequence-based characterisation, PolyPhen-2 predicts the functional impact of amino acid changes on FBXW7 structure and functions [20]. The SNPs & GO server uses support vector machines (SVM) to evaluate human disease-related variants [21]. PROVEAN is a web server that evaluates the functional impact of the listed amino acid changes based on sequence homology. PROVEAN's cut-off value is set to -2.5. Amino acid substitutions that above the cut-off threshold were considered harmful [22]. PredictSNP, a consensus classifier, predicts and annotates SNPs using the Protein Mutant Database and the UniProt database [23].

Analysing the effect of the mutant's impact on FBXW7 structural stability

A mutation alters the structure and stability of a protein in general. As a result, we used I-Mutant, a web server that uses a support vector machine to predict the stability of a protein after it has been modified. This database makes use of the ProTeam-derived dataset, which is the most comprehensive collection of research data on protein mutations. It computes the Gibbs free energy of native and mutant structures to evaluate free energy transformations. We submitted the clinvar based 10 nsSNPs as well as SNPs localized in the F box and WD40 domain of FBXW7 in FASTA format to assess the mutant's stability [24].

Analysing protein evolutionary conservation

ConSurf, which uses a Bayesian technique to analyse phylogenetic relationships between homologous sequences, was used to estimate the evolutionary conservation of amino acids in the protein sequence. Conservation scores with a colour system were used here to identify conserved areas, which are then assigned to various nine-grade scales. Variable conservation scores range from 1 to 4, intermediate conservation scores range from 5 to 6, and conserved conservation scores range from 7 to 9. For further analysis, we looked at the FBXW7 mutant's distribution and their score for further analysis [25].

Structure analysis of wild type and mutant models.

The 3D structure of native FBXW7 (2OVP) was retrieved from the Protein Data Bank [26]. The deleterious mutants were discretely substituted into the native sequence and 3D models for all the FBXW7 mutants were generated. The molecular configuration and its flexibility represent the main key properties of a protein molecule's biological function. To circumvent the practical difficulties surrounding laboratory techniques, we decided to generate the conformation flexibility of the wild and mutant FBXW7 protein using the CABS-flex web server. This server analysed the near native dynamics of globular proteins using a coarse-grain protein modelling approach to generate complete knowledge about the protein structure, conformational alterations, and residue level fluctuations. Structural comparisons between native and mutant models were explored based on an RMSF graph using CABSflex 2.0 [27]. FBXW7-WD40 repeat containing domain location mutant models and wild protein structures were generated using swisspdb-viewer and RMSD was predicted by PYMOL [28].

Prediction of structural effect of nsSNPs on human FBXW7 protein

We enlisted the help of HOPE to figure out how mutations affect protein structure. Project Have yOur Protein Explained (HOPE) is a web server that determines how point mutations in a protein sequence affect its structure [29]. Project HOPE predicted the hydrophobicity, charge, and size change between wild-type and mutant residue and the mutant model of the FBXW7 3D structure. Besides, we also employed Missense 3D [30] to confirm the precision and rigor of our result. The structural changes caused by an amino acid substitution are depicted in Model 3D.

Assessing the protein-protein interaction network of FBXW7

Asserting the connections between proteins is vital to maintaining the homeostasis of the living system. The reactome database-based molecular interaction networks of the human FBXW7 protein with other linked proteins were visualised using Cytoscape, a free Java-based software application [31]. The Cytoscape GUI platform was used to visualise interacting networks of various types of protein [32].

Results & Discussion

For most of the pathogenic variants, a strongly destabilizing mutation corresponds to the loss of function, whereas a modest change in stability may generate changes in protein conformation affecting the binding affinity with interacting molecules (protein, RNA and DNA). The influence of amino acid modifications on protein stability, on the other hand, is critical information for precision medicine [33].

TCGA based expression analysis and alterations in the FBXW7 gene

To have an overview of FBXw7 expression in different solid tumours, we analysed its expression in samples from the TCGA, which revealed that FBXw7 is coupled with differential expression as well as cervical, endometrial, and ovarian cancer in women (**Fig. 1**).

Retrieving nsSNPs.

We used the NCBI dbSNP database to evaluate reported FBXW7 variants, which include SNPs in the intronic region, 49,125 SNPs, and 701 SNPs in the non-coding area. Within the coding sequence, there were nearly 394 missense (nsSNPs) and 257 synonymous SNPs. The current study included clinically significant nsSNPs and mutants to investigate their impact on the FBXW7 structure. In addition to that, uncharacterized nsSNPs were assessed for their structural and functional level impact (**Table.1**). In addition to that, 57 nSSNP's located in the F-box and WD40 domain were also included in the current study.

Detection of harmful substances SNPs

SIFT, PANTHER, PolyPhen-2, SNPs & GO, PROVEAN, and PredictSNP were used to screen the mutant's impact at the sequencing and structural levels. As per prediction, ten proposed variants with clinical significance are considered as deleterious variants in all computational prediction methods (**Table.2**). The F-box and WD40 domain variants' impacts were also predicted and tabulated.

Screening of mutant residues prevalence within FBXW7 domains

InterPro, a domain identification tool, uses the protein family's functional analysis concept to predict a protein's domains as well as its active sites. To assess the position of variants within the conserved domains of FBXW7, we used the InterPro tool, which can identify motifs and domains of a protein. It is projected that two functional domains of FBXW7, which are 1PR001810 indicate F-BOX domain (278-325), and IPR17986 indicates WD40-repeat-containing domain (376-659) and FBXW7 variants of current study were positioned on second domain (**Fig.2**). Within the propeller phosphorylation-binding region, three arginine Missense point mutant residues (R465, R479, and R505) prohibit Fbxw7 from binding substrate [33]. The F-box domain contains nsSNPs and a few premature truncation cause variants. So far, nearly twenty-one premature truncation variants have been reported in the WD40 domain. Sixty-eight nsSNPs were reported in the WD40 domain, five nsSNP's in the F-Box domain, thus their deleterious effect was assessed using consensus prediction results. (**Supplementary table.1**)

Exploring the impact of the nsSNPs on protein stability

I-Mutant is a tool that assesses the influence of mutations on protein stability using a neural network technique. I-Mutant calculates the degree of protein instability and displays the projected free energy change value (G) as well as the prediction's sign: decrease or increase. The ΔΔG value predicted by I-Mutant revealed that all Clinvar proposed variants decreased stability (**Table.3**). In addition, we examined all the nsSNPs found in both domains of FBXW7.The support vector machine-based algorithm used here effectively predicted the transformation in protein stability free energy of the proposed FBXW7 mutants. The elevated negative DDG score suggests it is highly deleterious. (**Supplementary table.2**)

Evolutionary conservation analysis

The ConSurf web server revealed the evolutionary conservation of amino acid residues in native FBXW7. We looked at the evolutionary conservation and solvent accessibility of the FBXW7 mutant's structural and functional residues. R465 and R505 were found to be exposed and functional, whereas both mutants' residues were buried and structural. All these mutant residues have a high level of conservation (**Table.4**). Furthermore, both residues are expected to be relatively conserved and exposed, whereas both mutants are anticipated to be conserved and buried (**Fig.3**).

Structural changes are reflected through the energy state of the FBXW7 mutant models in the SwissPDB viewer assessment. Mutant models were visualized using Pymol and RMSD values were tabulated (**Table.5**). The potential energy of the wild type FBXW7 structure was observed at -11346.87 kJ/mol following energy minimization. Similarly, other mutants showed that R465H (−17.713 kJ/mol), which considerably diminished total energy, but R465P (−17.161 kJ/ mol) (Fig. 5), R465L (−16,953kJ/mol), and R505H (−16.667 kJ/mol) were the top three mutants that impacted the FBXW7 structure by increasing the total energy after energy minimization (**Table.6**).

Structural effect of point mutation on human FBXW7 protein

The Project HOPE server revealed that substituting Glycine in R505G, R465G can cause FBXW7 protein stiffness to be disrupted. Furthermore, R505L, R465H, R465L and R465P can be mutations located within a specification that is frequent in the protein repeat and is known as WD3. The mutation into a new residue might interrupt this repeat and, consequently, their substrate binding capability (Fig.4). Interference with both salt bridge and hydrogen bonding was seen in R505C mutants. The ability of a protein structure to offer specific activities

depends on its flexibility and rigidity nature. Besides this, the R505H and R505S mutants expose distinct properties and so they might drastically alter the functional FBXW7 associating domain (Table.7). Missense 3D tool stated that R465L, R465C, R465G, R465H, R465P, R505L, R505C variant created a buried Proline that delivers constrained backbone conformation. Moreover, R465L, R465C, R465G, R465H, R465P, R505L, R505C interrupted the sidechain and main-chain H-bond (s) established by wild type buried Arginine residue and R505L, R505G and R505S substitution triggered the expansion of cavity size (Table.8). The R505C substitution caused a shift from buried to exposed states, with the ARG exposed (RSA 23.7%) and the introduced mutant CYS buried (RSA 5.1%). Similarly, in R505L, ARG is exposed (RSA 23.7%) and LEU is buried (RSA 6.7%). The R465P substitution converts the 'E' (extended strand in parallel and/or anti-parallel -sheet conformation) to the " (no secondary structure). nsSNPs found in both domains of FBXW7 were also evaluated for structural level changes and are included in the supplementary file.1

FBXW7 mutants' structural level impact on its molecular interactors

Cytoscape was employed to construct the protein interaction network of FBXW7 protein, and it projected that the F-box family of proteins is functionally linked with 311 proteins, among which the top ten hub proteins NOTCH1, c-Myc, CCNE1, STYX, KLF5, SREB1, NFKB2, SKP1, CUL1 were included for the current study to explore the FBXW7 structural variants impact protein protein interaction (Table.9). The Cytoscape based protein-protein interaction network was based on the graph theory parameters, namely degree, average shortest path length, betweenness centrality, closeness centrality, and neighbourhood connectivity (Fig.5).

F-box protein Fbw7 (gene FBXW7), which forms the E3 ubiquitin ligase complex SCFFbw7 with Skp1 and Cullin1 [34]. Uterine carcinosarcoma (UCS) is a biphasic, high-grade endometrial cancer with de-differentiated sarcoma and carcinoma features. UCS tumours are serous-like, according to next-generation sequencing, and common somatic mutations are found in them. According to next-generation sequencing, UCS tumours are considered as serious-like and observed with somatic mutations in certain genes, namely TP53, PIK3CA, FBXW7, PTEN, and ARID1A [35].

Metaplastic breast carcinoma (MBC) and Uterine carcinosarcoma (UCS) anchorage have repeated somatic genetic changes influencing TP53 and a few other genes associated with the PI3K, Wnt, and Notch pathways. The histologically dissimilar components identified in MBCs and UCSs were discovered to be clonally related, and, at least in some cases, their mesenchymal component presumably arose from the epithelial component. Despite this, a few differences, specifically genetic alterations, distinguish MBCs and UCSs, as well as the role of the corresponding pathway in tumour induction The frequency of FBXW7 and PPP2R1A mutations, HER2 augmentation, and the absence of HRD distinguish UCSs from MBCs [36]. Endometrial carcinoma (EC) is a clinically diverse disease with a wide range of histological subtypes, and this heterogeneity may play a role in the accumulation of genetic alterations in the mutations were linked to late-stage cancer, vascular invasion, and lymph node metastatic disease [37]. Reported studies have revealed that about 15%-29% of serous ECs, 11%-39% of uterine carcinosarcomas, 13%- 25% of clear cell endometrial cancers, and 0%-15% of endometrioid endometrial cancers show somatic mutations in FBXW7 [38]. Besides the most common serous ECs, somatic mutational hotspots are listed as 423, 465, 479, and 505 residues [39]. genes FBXW7, PTEN, PIK3CA, TP53, KRAS, CTNNB1, FBFR2, and RB1.FBWX7

The coding region of the FBXW7 gene contains isoform-specific 5-exons, a nuclear localization signal, an F-box motif, and a WD40 domain. The Phosphorylated substrates such as cyclin E, Notch, cJun, MYC, PS1, and SREBF

bind to this functional region. GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1) suppresses lipogenesis in cancer cells when mTOR complex 2 is inhibited [40]. WD40 repeats, generating a propeller shape that may be observed on the surface or lumen of the h-propeller structure and is embedded with R465, R479, and R505 residues. As a result, such point mutations could impair substrate binding and interfere with wild type FBXW7 activity, implying a central negative effect. The WDR protein FBXW7 has a part in human cancer since it is the prime aberrated protein in the ubiquitin / proteasome system (UPS) seen among cancer patients [41]. The tumour suppressor protein FBXW7 interacts to substrate protein via phosphorylation and initiate breakdown process. FBXW7 gene loss-of-function mutations cause an abnormal build-up of cyclin E, which is found in 18% of colorectal malignancies, 15% of uterine endometrial carcinoma, and 40% of uterine carcinosarcoma [41,42].Surprisingly, these changed residues lead to the outside of the WD40-sheet domain, signalling that they are close to FBXW7-interacting substrate proteins.R505H, R505L, R505S, R505G, R505C, R465L, R465P, R465H, R465G, R465C mutations in FBXW7 resulted in changes in hydrophobic behaviour and electrostatic surface interactions, as well as a change in substrate binding.

FBXW7 mutants increases cancer-originating cell action in association with Notch1 oncogenes. Through skin carcinogenesis, FBXW7 regulates keratinocyte proliferation and differentiation, utilising both repressive and stimulatory signals, primarily through maintaining the proliferation-enhancing drive of c-Myc and the tumor-suppressive action of NOTCH [43]. Inactivation of FBXW7 has been shown to increase tumour resistance to anti-tubulin chemotherapeutic drugs. Furthermore, in cancer types that enhance resistance to gamma-secretase inhibitors, deletion of FBXW7 has been revealed to be a crucial prognostic marker (GSIs). These promising findings show that targeting NOTCH and/or FBXW7 to overcome MDR is a viable option. The biological importance of NOTCH and FBXW7 dysregulation in inducing MDR in tumours is discussed in the following sections [44,45]. Still, deregulation of FBXW7 and NOTCH activity can occur because of circumstances such as a mutation that disrupts the homeostatic state, leading to neoplastic transformation.

A recent study emphasises the specific variants, like FBXW7 R505L, localized in WD repeat 4 of the Fbxw7 protein. The Exon 9 based variant R465C of FBXW7 was reported numerous times for missense mutation occurrence (18.6%). Likewise, R465H and R505C were the next most familiar FBXW7 missense mutations (16.3% each) among the gynaecological conditions. Fbxw7 is rendered inactive by R505L, as evidenced by the activation of the NOTCH pathway in cultured cells [46] and the inability to bind substrates [47]. Another damaging mutation, R505C, is discovered in the WD repeat 4 of the Fbxw7 protein. R505C disrupts FBXW7-substrate interaction and impairs FBXW7 substrate degradation, leading to increased Notch intracellular domain and Myc expression [42], aberrant subnuclear localization [48], and decreased KLF5 degradation [49]. Mutant R465C is found in the Fbxw7 protein's WD repeat 3. In R465C mice, Fbxw7 protein function is lost, as indicated by a lack of Fbxw7-substrate interaction and poor substrate degradation by Fbxw7, resulting in prolonged Notch1 intracellular domain and Myc expression [46], as well as decreased degradation of Klf5 [49]. In culture, aberrant subnuclear localization resulted in lower inhibition of migration, invasion, and colony formation compared to wild-type Fbxw7 [48,50]. The WD repeat 3 of the Fbxw7 protein contains R465H (which corresponds to R385H in isoform 2. R465H prevents FBXW7 substrate degradation by preventing FBXW7-substrate contact, resulting in extended NICD and MYC expression [46], as well as KLF5 degradation [49]. H468R is in the WD repeat 3 of the Fbxw7 protein. H468R causes the Fbxw7 protein to lose function, as evidenced by the inability to induce the degradation of cyclin E, c-Myc, Mcl-1, and Braf in cultured cells [51], confers resistance to some BET inhibitors in cultured cells [52], and causes impaired NICD degradation in cultured cells, potentially leading to increased Notch1 signalling [53]. R479H is found in the Fbxw7 protein's WD repeat domain. This variation results in FBXW7 substrate degradation and a lack

of FBXW7-substrate interaction, resulting in Notch-driven reporter activation [52]. R479Q is present in the WD repeat 3 of the Fbxw7 protein. R479Q prevents FBXW7 from degrading substrates, resulting in prolonged Notch1 intracellular domain and Myc expression [46,53], as well as aberrant subcellular nuclear localization and loss of Notch1 intracellular domain binding in culture [48]. The FBXW7 R505H mutation is found in Fbxw7's WD repeat 4. R505H has been found in sequencing studies [54, 55, 56], but it has not been biochemically described. Therefore, its impact on the function of the Fbxw7 protein is unknown. R505G is found in the Fbxw7 protein's WD repeat 4. In cell culture studies, variant R505G causes enhanced proliferation, migration, invasion, and colony formation [50], implying that the Fbxw7 protein function will be lost. R465L is found in the Fbxw7 protein's WD repeat domain 3. This variant results in FBXW7 substrate degradation and a lack of FBXW7-substrate interaction, resulting in Notch-driven reporter activation [57]. The WD repeat domain of the Fbxw7 protein contains R465P. Other R465 hotspots inactivate Fbxw7, but R465P has yet to be explored. As a result, R465P is predicted to result in function loss [46, 57, 58]. R465Y has not been detected in the WD repeat domain of the Fbxw7 protein, however other R465 hotspots inactivate Fbxw7. As a result, R465Y is likely to cause function loss [46, 57, 58]. The WD repeat domain of the Fbxw7 protein contains R479L. This mutation causes the Fbxw7 protein to lose function, as seen by increased ubiquitination and lower protein stability [59]. R479P is found in the Fbxw7 protein's WD repeat domain. In culture, R479P causes enhanced proliferation, migration, invasion, and colony formation [50], implying that Fbxw7 protein function will be lost.

NFκB2 precursor protein is recognised as one of the main Fbw7 substrates. We identified NFκB2 as a downstream ubiquitin substrate of Fbw7 since it is a physiological interactor of SCFFbw7 [60]. Furthermore, the transcription factor nuclear factor kappa B (NF-kB2) is well-known for controlling cell survival, tumour invasion, and treatment resistance via the regulation of many oncogenic gene products [61]. Tumour suppressor FBXW7 is a component of the SCF (Skp1, Cullin 1, F-box protein) ubiquitin ligase complex, which regulates the degradation of a variety of substrates that, if not correctly regulated, might contribute to carcinogenesis. We show that FBXW7 mutations increase phosphorylated SRC-3, Cyclin E1, and c-MYC levels. Increased amounts of phosphorylated proteins have been linked to recurrent FBXW7 mutations, the majority of which occur in druggable pathways [62]. STYX has been associated to colorectal cancer cell proliferation, migration, invasion, and apoptosis, and several studies have suggested that STYX serves as a latent oncogene that inhibits apoptosis in colorectal and breast cancer, particularly via interacting with the FBXW7 protein. Collectively, these explanations reveal that the STYX/FBXW7 axis is engaged in the promotion of EC cells and might participate in another tumour development as well by controlling the NOTCH-mTOR signalling pathway. And hence, STYX being involved in numerous cancers, its part in modifying the NOTCH-mTOR interaction via FBXW7 permits further than consideration [63].

FBXW7 is a substrate differentiation factor of an E3 ubiquitin ligase of the SKP1-cullin-F-box (SCF) type that is responsible for the ubiquitin-dependent degradation of cyclin E. (encoded by CCNE1). When all uterine serous carcinomas were evaluated together, molecular genetic anomalies in the cyclin E pathway caused by FBXW7 point mutations, FBXW7 deletions, or CCNE1 amplification were found in more than half of them. Stimulation of cyclin E, whether by preventing its ubiquitin-dependent protein degradation owing to FBXW7 mutations or by elevating its expression due to gene amplification, may play a key role in uterine serous carcinoma carcinogenesis. SNP array analysis and immunohistochemistry based CCNE1 expression variation analysis also ensure the same functionality [64]. Zhao *et al.,* discovered that FBW7 phosphorylates GSK3 and recruits KLF5 for ubiquitin-mediated proteasomal destruction [65]. Aberrant expression of FBW7 diminishes the KLF5 protein level and its half-life, while deactivation of FBW7 surges the KLF5 protein and half-life [66]. These two groups' findings support

the idea that FBW7 is a critical negative regulator regulating KLF5-mediated cell proliferation. Mutations in FBXW7 increase KLF5 expression.

FBXW7 variants impact on chemoresistance:

FBXW7's antitumor effects are mostly achieved through controlling the network of proteins degradation, many of the members of proteins such as cyclin E, c-Myc, and Notch have oncogenic functions, Mutated FBXW7 is also known to induce oncoprotein stabilisation in tumours, resulting in chemoresistance induction. As a result, FBXW7 protein downregulation may contribute to tumour development and chemoresistance. As a result, FBXW7 has been recommended as a possible therapeutic target for improving chemotherapeutic drug sensitivity and efficacy [67]. The FBXW7 mutation, which primes the dysfunctional FBXW7 by producing missense mutations in three arginine residues (R465, R479, and R505), could be the basis for the accumulation of a wide range of substrates, which could be essential in chemoresistance [68]. Richter *et al* identified FBXW7 as a new FBXO45 substrate and investigated its function in cancerous cells [69]. In another study, researchers found that blocking FBXO45-arbitrated FBXW7 depletion could help with drug resistance in chemotherapeutic treatment by increasing mitotic cell death. In other research, some mutations may affect the degradation of FBXW7 targets differently than the hot area arginine mutations (R465, R479, and R505). Design of small inhibitors that target specific downstream signalling pathways and/or affect FBXW7 substrate preference could be aided by a better understanding of the conformational changes that generate these symptoms [70]. Earlier research has investigated the role of Fbw7 in cancer chemoresistance in a variety of cancers [71,72]. c-Myc, nuclear factor erythroid 2-related factor 2, myeloid leukaemia cell differentiation protein Mcl-1 (Mcl-1), and transcription factor SOX-9 are all participating in chemoresistance in cancers such pancreatic cancer, gastric cancer, and colorectal cancer [73,74]. According to Tong J et al, FBXW7 mutations in colorectal cancer cells impede Mcl-1 degradation, boosting the development of resistance to regorafenib-based targeted therapy [75]. FBXW7 ablation in ovarian cancer cells inhibited c-Myc degradation, which was like the findings in colon cancer cell lines. This renders the cells more resistant to vincristine-induced cell death. On the one hand, the above-mentioned research studies underlined the crucial role of FBXW7 in chemotherapeutic drug therapeutic effects, while on the other hand, they provided much-needed information for developing viable techniques to increase cancer cell sensitivity to vincristine [76]. Standard chemotherapy was shown to be more resistant in FBXW7-deficient leukaemia-initiating cells (LICs), but imatinib was found to be more sensitive [77]. Combining FBXW7 genetic ablation and imatinib is more effective than any of these approaches alone in cancer animal models, according to studies. When all these aspects are considered, it becomes evident that FBXW7 plays an important role.

Out of fifty-eight nsSNP's reported within the WD40 domain of FBXW7, ten were related to gynaecological cancer and had clinical significance; the remaining sixty deleterious nsSNPs were assessed for their mutational impact on FBXW7 and tabulated in the **supplementary file 1.** Our comprehensive study assessed all identified deleterious nsSNPs localized in the WD40 domain decreased stability of the FBXW7 structure. Only three of the five nsSNPs within the F-box domain have been shown to have a negative impact on the FBXW7 structure. The significance of arginine substitution among malignancies was revealed by proteins with the highest ratio of arginine substitution events in the CCLE database. Arginine has highly unusual properties studies showed that arginine is most frequently lost in mutations in cancer. Out of forty-three arginine residues twenty-six substitutions reported in FBXW7.TP53 and PTEN are the other proteins with arginine substitutions. In our studies we found twelve nsSNP's as deleterious out of eighteen arginine substitutions within the domain region of FBXW7.Increasing evidence about the FBXW7 variant's role in cancer induction and chemoresistance emphasizes the need for this kind of

comprehensive study, which would be helpful in refining the currently available therapeutic regimen for gynaecological cancer.

Conclusion

FBXW7 is known for its ubiquitylation and proteasome degradation function towards its oncoprotein targets, namely cyclin E, c-JUN, c-MYC, NOTCH-1, and MCL-1. A knockout study revealed that nearly ninety proteins were targeted as substrates by FBXW7.Many of these substrates are transcription factors or important signalling molecules that control a variety of cellular processes, resulting in tumour growth and development. Mutations in FBXW7 frequently occur in various human cancers and are reported to have a chemoresistance association also. The current intracellular study of all functional FBXW7 mutants and reported nsSNPs with deleterious effects sheds light on the structural level impact on the FBXW7 structure. Positively charged basic residues, such as arginine, are typically seen ionised at neutral pH on protein surfaces in these residues, electrostatic contact or surface charge-charge interaction is thought to contribute to protein. stability. In addition to hydrogen bonds, electrostatic phenomena such as salt bridges play an important role in protein stability. A salt bridge or ion pair is formed when oppositely charged residues such as Arg, Glu, Asp, His, and Lys contact. As a result, substituting other amino acids for arginine has an impact on the development of salt bridges with neighbouring amino acids. This is the first comprehensive in silico investigation of all functional nsSNPs found in both FBXW7 domains. Our findings in this current study will provide a guide to the role of FBXW7 variants' role in diagnostic and therapeutic interventions.

Declarations

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors. Hence, no formal consent is required.

Consent to Participate All the authors have given the consent to participate in the present research concept.

Consent to Publish All authors have read the final manuscript and gave the consent for publishing the manuscript.

Authors Contributions JCH has reviewed and compiled the paper and conceived and designed the protocol. AVK helped in understanding the bioinformatic tools incorporated in the paper. AVK helped in understanding the mechanism of FBXW7 mutants which helped in the compilation of the manuscript. JCH helped in reviewing the relevant papers and compilation of paper.

Funding No funding received for the present work

Competing Interests The authors declare that they have no conflicts of interest.

Availability of data and materials The authors declare that the data and materials are transparent.

References

1. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

2. Wei, D.; Sun, Y (2010). Small RING Finger Proteins RBX1 and RBX2 of SCF E3 Ubiquitin Ligases: The Role in Cancer and as Cancer Targets. Genes Cancer 1, 700–707. https://dx.doi.org/10.1177%2F1947601910382776
3. Diaz, V.M.; de Herreros, A.G.(2016) F-box proteins: Keeping the epithelial-to-mesenchymal transition (EMT) in check. Semin. Cancer Biol. , 36, 71–79. https://doi.org/10.1016/j.semcancer.2015.10.003
4. Gong, J.; Cao, J.; Liu, G.; Huo, J.R.(2015) Function and mechanism of F-box proteins in gastric cancer (Review). Int. J. Oncol. , 47, 43–50.https://doi.org/10.3892/2015 .2983
5. Davis, R.J.; Welcker, M.; Clurman, (2014) B.E. Tumor suppression by the Fbw7 ubiquitin ligase: Mechanisms and opportunities. Cancer Cell , 26, 455–464. https://doi.org/10.1016/j.ccell.2014.09.013
6. Yan L, Lin M, Pan S, et al.(2020) Emerging roles of F-box proteins in cancer drug resistance. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. Mar; 49:100673. https://1016/j.drup.2019.100673.
7. Yumimoto, K.; Nakayama, K.I. Recent (2020) insight into the role of FBXW7 as a tumor suppressor. Semin. Cancer Biol., 67 Pt 2, 1–15. https://doi.org/10.1016/j.semcancer.2020.02.017
8. Spruck, C.H.; Strohmaier, H.; Sangfelt, O.; Muller, H.M.; Hubalek, M.; Muller-Holzner, E.; Marth, C.; Widschwendter, M.; Reed, S.I. (2002) hCDC4 gene mutations in endometrial cancer. Cancer Res. , 62, 4535–4539.
9. Sun, D.; Shen, Y.; Wang, S.H.; Xiang, Z.W.; Xie, Y.S.; Jiang, X.(2010) Effects of UO-126 on proliferation and fbw7 expression of HeLa cells. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi , 26, 138–140. https://doi.org/3390/cancers11020246.
10. Zhou, C.; Shen, L.; Mao, L.; Wang, B.; Li, Y.; Yu, H.(2015) miR-92a is upregulated in cervical cancer and promotes cell proliferation and invasion by targeting FBXW7. Biochem. Biophys. Res. Commun. , 458, 63–69 https://doi.org/10.1016/j.bbrc.2015.01.066
11. Liu, F., Zou, Y., Wang, F., Yang, B., Zhang, Z., Luo, Y., Liang, M., Zhou, J., & Huang, O. (2019). FBXW7 Mutations Promote Cell Proliferation, Migration, and Invasion in Cervical Cancer. Genetic testing and molecular biomarkers, 23(6), 409–417. https://doi.org/10.1089/gtmb.2018.0278
12. Garcia-Dios DA, Lambrechts D, Coenegrachts L, Vandenput I, Capoen A (2013), Webb PM. High-throughput interrogation of PIK3CA, PTEN, KRAS, FBXW7 and TP53 mutations in primary endometrial carcinoma. Gynecol. Oncol. ; 128:327–334. https://doi.org/10.1016/j.ygyno.2012.11.037
13. Wang Y, Yu M, Yang JX, Cao DY, Shen K, Lang JH.(2018) Clinicopathological and survival analysis of uterine papillary serous carcinoma: a single institutional review of 106 cases. Cancer Manag Res.; 10:4915-4928. https://doi.org/10.2147/S179566
14. Urick ME, Bell DW. (2019) Clinical actionability of molecular targets in endometrial cancer. NatRevCancer.;19(9):510-521.https://doi.org/10.1038/s41568-019-0177x
15. Kitts A, Sherry S. (2002) The Single Nucleotide Polymorphism Database (dbSNP) of Nucleotide Sequence Variation. 2002 Oct 9 [Updated 2011 Feb 2]. In: McEntyre J, Ostell J, editors. The NCBI Handbook [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); -. Chapter 5. Available from: Smigielski, E. M., Sirotkin, K., Ward, M., & Sherry, S. T. (2000). dbSNP: a database of single nucleotide polymorphisms. *Nucleic acids research*, *28*(1), 352–355. https://doi.org/1093/nar/28. 1.352
16. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Jang W, Karapetyan K, Katz K, Liu C, Maddipatla Z, Malheiro A, McDaniel K, Ovetsky M, Riley G, Zhou G, Holmes JB,

Kattman BL, Maglott DR. (2018) ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res . Jan 4. https://doi.org/10.1093/nar/gkx1153

17. Blum M, Chang H, Chuguransky S, Grego T, Kandasaamy S, Mitchell A, Nuka G, Paysan-Lafosse T, Qureshi M, Raj S, RichardsonL, Salazar GA, Williams L, Bork P, Bridge A, Gough J, Haft DH, Letunic I, Marchler-Bauer A, Mi H, Natale DA, Necci M, Orengo CA, Pandurangan AP, Rivoire C, Sigrist CJA, Sillitoe I, Thanki N, Thomas PD, Tosatto SCE, Wu CH, Bateman A and Finn RD (2020) The InterPro protein families and domains database: 20 years on. Nucleic Acids Research, Nov, (doi: 10.1093/nar/gkaa977) https://doi.org/10.1093/nar/gkaa977
18. Kumar P, Henikoff S, Ng PC. (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. Jul;4(7):1073–81. Pmid:19561590 https://doi.org/10.1038/nprot.2009.86
19. Tang H, Thomas PD (2016). PANTHER-PSEP: predicting disease-causing genetic variants using position-specific evolutionary preservation. Bioinformatics.;32(14):2230–2. Pmid:27193693 https://doi.org/10.1093/Bioinformatics /btw222
20. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. (2010) A method and server for predicting damaging missense mutations. Nat Methods.;7(4):248–9. Pmid:20354512 https://doi.org/10.1038/nmeth0410-248
21. Capriotti, R. Calabrese, P. Fariselli, P.L. Martelli, R.B. Altman, R. Casadio (2013) WS-SNPs&GO: a web server for predicting the deleterious effect of human protein variants using functional annotation,BMC Genomics, 14 , p. S6 https://doi.org/10.1186 /1471-2164-14-S3-S6
22. Choi Y, Chan AP. (2015) PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. Aug 15;31(16):2745-7. doi: 10.1093/bioinformatics/btv195. Epub 2015 Apr 6. PMID: 25851949; PMCID: PMC4528627.https://doi.org/10.1093/bioinformatics/btv195
23. Bendl, J., Stourac, J., Salanda, O., Pavelka, A., Wieben, E. D., Zendulka, J., et al. (2014). PredictSNP: Robust and accurate consensus classifier for prediction of disease-related mutations. PLoS Computational Biology, 10(1), e1003440. https://doi.org/10.1371/journal.pcbi.1003440
24. Capriotti E, Fariselli P, Casadio R. (2005) I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res., 33 (Web Server issue): W306-W310.https://doi.org/10.1093/nar/gki375
25. Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T., & Ben-Tal, N. (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucleic acids research, 44(W1), W344–W350. https://doi.org/10.1093/nar/gkw408
26. Hao, B., Oehlmann, S., Sowa, M. E., Harper, J. W., & Pavletich, N. P. (2007). Structure of a Fbw7-Skp1-cyclin E complex: multisite-phosphorylated substrate recognition by SCF ubiquitin ligases. Molecular cell, 26(1), 131–143. https://doi.org/10.1016/j.molcel.2007.02.022
27. Kuriata, A.*, Gierut, A.M.*, Oleniecki, T., Ciemny, M.P., Kolinski, A., Kurcinski, M., Kmiecik, S. (2018), CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures.Nucleic acids research.https://doi.org/10.1093/nar/gky356
28. The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.
29. Venselaar, H., Te Beek, T. A., Kuipers, R. K., Hekkelman, M. L., & Vriend, G. (2010). Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC bioinformatics, 11, 548. https://doi.org/10.1186/1471-2105-11-548

30. Khanna, T., Hanna, G., Sternberg, M.J.E. & David, A. (2021) Missense3D-DB web catalogue: an atom-based analysis and repository of 4M human protein-coding genetic variants. Human Genetics, 140(5), 805-812. DOI: 10.1007/s00439-020-02246-z https://dx.doi.org/10.1007%2Fs00439-020-02246-z
31. Jassal B, Matthews L, Viteri G, Gong C, Lorente P, Fabregat A, Sidiropoulos K, Cook J, Gillespie M, Haw R, Loney F, May B, Milacic M, Rothfels K, Sevilla C, Shamovsky V, Shorser S, Varusai T, Weiser J, Wu G, Stein L, Hermjakob H, D'Eustachio P. (2020) The reactome pathway knowledgebase. Nucleic Acids Res. Jan 8;48(D1):D498-D503. https://doi.org/1093/nar/gkz1031.
32. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker (2003) T.Cytoscape: a software environment for integrated models of biomolecular interaction networks.Genome Research Nov; 13(11):2498-504 https://doi.org/10.1101/gr.1239303
33. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, et al. (2010) A method and server for predicting damaging missense mutations. Nat Methods 7: 248–249 https://doi.org/10.1038/nmeth0410-248
34. Shimizu, N.T. Nihira, H. Inuzuka, W. Wei (2018) ,Physiological functions of FBW7 in cancer and metabolism.Cell Signal, 46, pp. 15-22FBXW7 variants impact among the gynaecological cancer https://doi.org/10.1016/j.cellsig.2018.02.009
35. Matsuzaki, S., Klar, M., Matsuzaki, S., Roman, L. D., Sood, A. K., & Matsuo, K. (2021). Uterine carcinosarcoma: Contemporary clinical summary, molecular updates, and future research opportunity. Gynecologic oncology, 160(2), 586–601. https://doi.org/10.1016/j.ygyno.2020.10.043
36. Moukarzel, L. A., Ferrando, L., Da Cruz Paula, A., Brown, D. N., Geyer, F. C., Pareja, F., Piscuoglio, S., Papanastasiou, A. D., Fusco, N., Marchiò, C., Abu-Rustum, N. R., Murali, R., Brogi, E., Wen, H. Y., Norton, L., Soslow, R. A., Vincent-Salomon, A., Reis-Filho, J. S., & Weigelt, B. (2021). The genetic landscape of metaplastic breast cancers and uterine carcinosarcomas. Molecular oncology, 15(4), 1024–1039. https://doi.org/10.1002/1878-0261.12813
37. Watanabe, T., Nanamiya, H., Kojima, M., Nomura, S., Furukawa, S., Soeda, S., Tanaka, D., Isogai, T., Imai, J. I., Watanabe, S., & Fujimori, K. (2021). Clinical relevance of oncogenic driver mutations identified in endometrial carcinoma. Translational oncology, 14(3), 101010. https://doi.org/10.1016/j.tranon.2021.101010
38. Urick ME, Bell DW (2020). Proteomic profiling of FBXW7-mutant serous endometrial cancer cells reveals upregulation of PADI2, a potential therapeutic target. Cancer Med. Jun;9(11):3863-3874. doi: 10.1002/cam4.3013. Epub 2020 Apr 5. PMID: 32248654; PMCID: PMC7286459. https://doi.org/10.1002/cam4.3013
39. Getz G, Gabriel SB, Cibulskis K (2013), et al. Integrated genomic characterization of endometrial carcinoma. Nature.;497(7447): 67-73. https://doi.org/10.1038 /nature12113
40. Li, S., Oh, Y. T., Yue, P., Khuri, F. R., & Sun, S. Y. (2016). Inhibition of mTOR complex 2 induces GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1) and suppresses lipogenesis in cancer cells. Oncogene, 35(5), 642–650. https://doi.org/10.1038/onc.2015.123
41. Davis RJ, Welcker M, Clurman BE (2014) . Tumor suppression by the Fbw7 ubiquitin ligase: mechanisms and opportunities. Cancer Cell;26:455-64. https://doi.org/10.1016/j.ccell.2014.09.013
42. Siu KT, Rosner MR, Minella AC (2012). An integrated view of cy-clin E function and regulation. Cell Cycle;11:57-64. https://doi.org/10.4161/cc.11.1.18775
43. Ishikawa Y, Hosogane M, Okuyama R, et al. (2013) Opposing functions of Fbxw7 in keratinocyte growth, differentiation and skin tumorigenesis mediated through negative regulation of c-Myc and NOTCH.

Oncogene.;32(15):1921-1932. https://doi.org/10.1038/onc.2012.213.

44. Yeh C, Bellon M, Nicot C. (2018) FBXW7: a critical tumor suppressor of human cancers. Mol Cancer.;17:115.https://doi.org/10.1186/s12943-018-0857-2
45. Yumimoto K, Nakayama KI. (2020) Recent insight into the role of FBXW7 as a tumor suppressor. Semin Cancer Biol.;67(Pt 2):1-15; https://doi.org/10.1016/j.semcancer.2020.02.017
46. O'Neil, J., Grim, J., Strack, P., Rao, S., Tibbitts, D., Winter, C., Hardwick, J., Welcker, M., Meijerink, J. P., Pieters, R., Draetta, G., Sears, R., Clurman, B. E., & Look, A. T. (2007). FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. The Journal of experimental medicine, 204(8), 1813–1824. https://doi.org/10.1084/jem.20070876
47. Ye, M., Zhang, Y., Zhang, X., Zhang, J., Jing, P., Cao, L., Li, N., Li, X., Yao, L., Zhang, J., & Zhang, J. (2017). Targeting FBW7 as a Strategy to Overcome Resistance to Targeted Therapy in Non-Small Cell Lung Cancer. Cancer research, 77(13), 3527–3539. https://doi.org/10.1158/0008-5472.CAN-16-3470
48. Close, V., Close, W., Kugler, S. J., Reichenzeller, M., Yosifov, D. Y., Bloehdorn, J., Pan, L., Tausch, E., Westhoff, M. A., Döhner, H., Stilgenbauer, S., Oswald, F., & Mertens, D. (2019). FBXW7 mutations reduce binding of NOTCH1, leading to cleaved NOTCH1 accumulation and target gene activation in CLL. Blood, 133(8), 830–839. https://doi.org/10.1182/blood-2018-09-874529
49. Zhang, X., Choi, P. S., Francis, J. M., Gao, G. F., Campbell, J. D., Ramachandran, A., Mitsuishi, Y., Ha, G., Shih, J., Vazquez, F., Tsherniak, A., Taylor, A. M., Zhou, J., Wu, Z., Berger, A. C., Giannakis, M., Hahn, W. C., Cherniack, A. D., & Meyerson, M. (2018). Somatic Superenhancer Duplications and Hotspot Mutations Lead to Oncogenic Activation of the KLF5 Transcription Factor. Cancer discovery, 8(1), 108–125. https://doi.org/10.1158/2159-8290.CD-17-0532
50. Liu, F., Zou, Y., Wang, F., Yang, B., Zhang, Z., Luo, Y., Liang, M., Zhou, J., & Huang, O. (2019). FBXW7 Mutations Promote Cell Proliferation, Migration, and Invasion in Cervical Cancer. Genetic testing and molecular biomarkers, 23(6), 409–417. https://doi.org/10.1089/gtmb.2018.0278
51. Yeh, C. H., Bellon, M., Wang, F., Zhang, H., Fu, L., & Nicot, C. (2020). Loss of FBXW7-mediated degradation of BRAF elicits resistance to BET inhibitors in adult T cell leukemia cells. Molecular cancer, 19(1), 139. https://doi.org/10.1186/s12943-020-01254-x
52. Malyukova, A., Dohda, T., von der Lehr, N., Akhoondi, S., Corcoran, M., Heyman, M., Spruck, C., Grandér, D., Lendahl, U., & Sangfelt, O. (2007). The tumor suppressor gene hCDC4 is frequently mutated in human T-cell acute lymphoblastic leukemia with functional consequences for Notch signaling. Cancer research, 67(12), 5611–5616. https://doi.org/10.1158/0008-5472.CAN-06-4381
53. Chang, C. C., Lin, H. H., Lin, J. K., Lin, C. C., Lan, Y. T., Wang, H. S., Yang, S. H., Chen, W. S., Lin, T. C., Jiang, J. K., & Chang, S. C. (2015). FBXW7 mutation analysis and its correlation with clinicopathological features and prognosis in colorectal cancer patients. The International journal of biological markers, 30(1), e88–e95. https://doi.org/10.5301/jbm.5000125
54. Ling, C., Wang, L., Wang, Z., Xu, L., Sun, L., Yang, H., Li, W. D., & Wang, K. (2015). A pathway-centric survey of somatic mutations in Chinese patients with colorectal carcinomas. PloS one, 10(1), e0116753. https://doi.org/10.1371/journal.pone.0116753
55. Liu, Y., Easton, J., Shao, Y., Maciaszek, J., Wang, Z., Wilkinson, M. R., McCastlain, K., Edmonson, M., Pounds, S. B., Shi, L., Zhou, X., Ma, X., Sioson, E., Li, Y., Rusch, M., Gupta, P., Pei, D., Cheng, C., Smith, M. A., Auvil, J. G., …

Mullighan, C. G. (2017). The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nature genetics, 49(8), 1211–1218. https://doi.org/10.1038/ng.3909

56. Goeppert, B., Toth, R., Singer, S., Albrecht, T., Lipka, D. B., Lutsik, P., Brocks, D., Baehr, M., Muecke, O., Assenov, Y., Gu, L., Endris, V., Stenzinger, A., Mehrabi, A., Schirmacher, P., Plass, C., Weichenhan, D., & Roessler, S. (2019). Integrative Analysis Defines Distinct Prognostic Subgroups of Intrahepatic Cholangiocarcinoma. Hepatology (Baltimore, Md.), 69(5), 2091–2106. https://doi.org/10.1002/hep.30493
57. Larson Gedman, A., Chen, Q., Kugel Desmoulin, S., Ge, Y., LaFiura, K., Haska, C. L., Cherian, C., Devidas, M., Linda, S. B., Taub, J. W., & Matherly, L. H. (2009). The impact of NOTCH1, FBW7 and PTEN mutations on prognosis and downstream signaling in pediatric T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Leukemia*, *23*(8), 1417–1425. https://doi.org/10.1038/leu.2009.64
58. Cherian, C., Devidas, M., Linda, S. B., Taub, J. W., & Matherly, L. H. (2009). The impact of NOTCH1, FBW7 and PTEN mutations on prognosis and downstream signaling in pediatric T-cell acute lymphoblastic leukemia: a report from the Children's Oncology Group. Leukemia, 23(8), 1417–1425. https://doi.org/10.1038/leu.2009.64
59. Akhoondi, S., Sun, D., von der Lehr, N., Apostolidou, S., Klotz, K., Maljukova, A., Cepeda, D., Fiegl, H., Dafou, D., Marth, C., Mueller-Holzner, E., Corcoran, M., Dagnell, M., Nejad, S. Z., Nayer, B. N., Zali, M. R., Hansson, J., Egyhazi, S., Petersson, F., Sangfelt, P., … Spruck, C. (2007). FBXW7/hCDC4 is a general tumor suppressor in human cancer. Cancer research, 67(19), 9006–9012. https://doi.org/10.1158/0008-5472.CAN-07-1320
60. Min, S. H., Lau, A. W., Lee, T. H., Inuzuka, H., Wei, S., Huang, P., Shaik, S., Lee, D. Y., Finn, G., Balastik, M., Chen, C. H., Luo, M., Tron, A. E., Decaprio, J. A., Zhou, X. Z., Wei, W., & Lu, K. P. (2012). Negative regulation of the stability and tumor suppressor function of Fbw7 by the Pin1 prolyl isomerase. Molecular cell, 46(6), 771–783. https://doi.org/10.1016/j.molcel.2012.04.012
61. Fukushima, H., Matsumoto, A., Inuzuka, H., Zhai, B., Lau, A. W., Wan, L., Gao, D., Shaik, S., Yuan, M., Gygi, S. P., Jimi, E., Asara, J. M., Nakayama, K., Nakayama, K. I., & Wei, W. (2012). SCF(Fbw7) modulates the NFkB signaling pathway by targeting NFkB2 for ubiquitination and destruction. Cell reports, 1(5), 434–443. https://doi.org/10.1016/j.celrep.2012.04.002
62. Arabi A., Ullah K., Branca R.M., Johansson J., Bandarra D., Haneklaus M., Fu J., Aries I., Nilsson P., Den Boer M.L., et al.(2012) Proteomic screen reveals Fbw7 as a modulator of the NF-kappaB pathway. Nat. Commun. ;3:976. doi: 10.1038/ncomms1975 https://doi.org/10.1038/ncomms1975
63. Kar, R., Jha, S. K., Ojha, S., Sharma, A., Dholpuria, S., Raju, V., Prasher, P., Chellappan, D. K., Gupta, G., Kumar Singh, S., Paudel, K. R., Hansbro, P. M., Kumar Singh, S., Ruokolainen, J., Kesari, K. K., Dua, K., & Jha, N. K. (2021). The FBXW7-NOTCH interactome: A ubiquitin proteasomal system-induced crosstalk modulating oncogenic transformation in human tissues. Cancer reports (Hoboken, N.J.), e1369. Advance online publication. https://doi.org/10.1002/cnr2.1369
64. Kuhn, E., Wu, R. C., Guan, B., Wu, G., Zhang, J., Wang, Y., Song, L., Yuan, X., Wei, L., Roden, R. B., Kuo, K. T., Nakayama, K., Clarke, B., Shaw, P., Olvera, N., Kurman, R. J., Levine, D. A., Wang, T. L., & Shih, I. (2012). Identification of molecular pathway aberrations in uterine serous carcinoma by genome-wide analyses. Journal of the National Cancer Institute, 104(19), 1503–1513. https://doi.org/10.1093/jnci/djs345
65. Liu, H. Li, S. Li, M. Shen, N. Xiao, Y. Chen, Y. Wang, W. Wang, R. Wang, Q. Wang (2010), The Fbw7/human CDC4 tumor suppressor targets proproliferative factor KLF5 for ubiquitination and degradation through multiple phosphodegron motifs. J. Biol. Chem., 285, (2010), 18858–18867.https://doi.org/10.1074/jbc.M109.099440

66. Zhao, H.Q. Zheng, Z. Zhou, C. Chen (2010), The Fbw7 tumor suppressor targets KLF5 for ubiquitin-mediated degradation and suppresses breast cell proliferation. Cancer Res., 70, (2010), 4728–4738.https://doi.org/10.1158/0008-5472.CAN-10-0040
67. Gong J, Zhou Y, Liu D, Huo J. 2018 F-box proteins involved in cancer-associated drug resistance. Oncol Lett. 2018;15:8891–8900 . https://dx.doi.org/10.3892%22018.8500
68. Tong, J., Tan, S., Zou, F., Yu, J., & Zhang, L. (2017). FBW7 mutations mediate resistance of colorectal cancer to targeted therapies by blocking Mcl-1 degradation. Oncogene, 36(6), 787–796. https://doi.org/10.1038/onc.2016.247
69. Richter KT, Kschonsak YT, Vodicska B, Hoffmann I. (2018) FBXO45-MYCBP2 regulates mitotic cell fate by targeting FBXW7 for degradation. Cell Death Differ. 2019;27:758–772. doi: 10.1038/s41418-019-0385-7.
70. Yeh CH, Bellon M, Nicot C.(2018) FBXW7: a critical tumor suppressor of human cancers. Mol. Cancer. 2018;17:115. doi: 10.1186/s12943-018-0857-2.
71. Inuzuka, H., Shaik, S., Onoyama, I., Gao, D., Tseng, A., Maser, R. S., Zhai, B., Wan, L., Gutierrez, A., Lau, A. W., Xiao, Y., Christie, A. L., Aster, J., Settleman, J., Gygi, S. P., Kung, A. L., Look, T., Nakayama, K. I., DePinho, R. A., & Wei, W. (2011). SCF(FBW7) regulates cellular apoptosis by targeting MCL1 for ubiquitylation and destruction. Nature, 471(7336), 104–109. https://doi.org/10.1038/nature09732
72. Suryo Rahmanto, A., Swartling, F. J., & Sangfelt, O. (2016). Targeting SOX9 for degradation to inhibit chemoresistance, metastatic spread, and recurrence. Molecular & cellular oncology, 4(1), e1252871. https://doi.org/10.1080/23723556.2016.1252871
73. Yada, M., Hatakeyama, S., Kamura, T., Nishiyama, M., Tsunematsu, R., Imaki, H., Ishida, N., Okumura, F., Nakayama, K., & Nakayama, K. I. (2004). Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. The EMBO journal, 23(10), 2116–2125. https://doi.org/10.1038/sj.emboj.7600217
74. Biswas, M., Phan, D., Watanabe, M., & Chan, J. Y. (2011). The Fbw7 tumor suppressor regulates nuclear factor E2-related factor 1 transcription factor turnover through proteasome-mediated proteolysis. The Journal of biological chemistry, 286(45), 39282–39289. https://doi.org/10.1074/jbc.M111.253807
75. Tong J, Wang P, Tan S, Chen D, Nikolovska-Coleska Z, Zou F, Yu J and Zhang L:(2017) Mcl-1 degradation is required for targeted therapeutics to eradicate colon cancer cells. Cancer Res. 77:2512–2521. 2017. View Article: Google Scholar : PubMed/NCBIhttps://doi.org/10.1158/0008-5472.CAN-16-3242
76. Wertz IE, et al (2018). Sensitivity to antitubulin chemotherapeutics is regulated by MCL1 and FBW7. Nature. 2011;471(7336):110–4. https://doi.org/10.1038/nature09779
77. Takeishi, S., & Nakayama, K. I. (2014). Role of Fbxw7 in the maintenance of normal stem cells and cancer-initiating cells. British journal of cancer, 111(6), 1054–1059. https://doi.org/10.1038/bjc.2014.259

Tables

Table 1: FBXW7 Variant's with gynaecological cancer clinical significance

Name	Protein change	Clinical significance
c.1514G>A (p.Arg505His)	R505H	R505H is found in the Fbxw7 protein's WD repeat 4. R505H has been found in sequencing studies, however it has not been biochemically described, therefore its impact on the function of the Fbxw7 protein is unknown.
c.1514G>T (p.Arg505Leu)	R505L	R505L is found in the Fbxw7 protein's WD repeat 4. Fbxw7 is rendered inactive by R505L, as evidenced by the activation of the NOTCH pathway in cultured cells and the inability to bind substrates.
c.1513C>A (p.Arg505Ser)	R505S	R505S mutation is found in the Fbxw7 protein's WD repeat 4.
c.1513C>G (p.Arg505Gly)	R505G	R505G is found in the Fbxw7 protein's WD repeat 4. In culture, R505G causes enhanced proliferation, migration, invasion, and colony formation, which is expected to result in the loss of Fbxw7 protein function.
c.1513C>T (p.Arg505Cys)	R505C	R505C is found in the Fbxw7 protein's WD repeat 4. R505C causes a lack of FBXW7-substrate interaction and affects FBXW7 substrate degradation, leading in a prolonged Notch intracellular domain and Myc expression with abnormal subnuclear localization and reduced Klf5 degradation.
c.1394G>T (p.Arg465Leu)	R465L	R465L lies within the WD repeat domain 3 of the Fbxw7 protein. R465L confers a loss of FBXW7-substrate interaction and impairs substrate degradation by FBXW7, resulting in activation of a Notch-driven reporter.
c.1394G>C (p.Arg465Pro)	R465P	R465P lies within the WD repeat domain of the Fbxw7 protein. R465P has not been characterized however, other R465 hotspots inactivate Fbxw7, and therefore, R465P is predicted to lead to a loss of function.
c.1394G>A (p.Arg465His)	R465H	R465H (corresponds to R385H in isoform 2) lies within the WD repeat 3 of the Fbxw7 protein. R465H confers a loss of FBXW7-substrate interaction and impairs substrate degradation by FBXW7, resulting in sustained NICD and MYC expression and also has impaired degradation of Klf5.
c.1393C>G (p.Arg465Gly)	R465G	No published Literature
c.1393C>T (p.Arg465Cys)	R465C	R465C lies within WD repeat 3 of the Fbxw7 protein (UniProt.org). R465C confers a loss of Fbxw7 protein function as demonstrated by a loss of Fbxw7-substrate interaction and impaired substrate degradation by Fbxw7, resulting in sustained Notch1 intracellular domain and Myc expression, impaired degradation of Klf5, aberrant subnuclear localization relative to wild-type Fbxw7 in culture, and reduced suppression of migration, invasion, and colony formation in culture.

Table 2: Consensus prediction of nsSNP's of FBXW7:

SNP Id	AA change	SIFT	Polyphen2	Panther	SNPs&GO	PROVEAN	Predict SNP
R505S	rs149680468	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R505C	rs149680468	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R505G	rs149680468	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R505L	rs149680468	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R505H	rs149680468	Deleterious	Damaging	Damaging	Disease	Deleterious	Non deleterious
R465L	rs1057519895	Deleterious	Damaging	Damaging	Disease	Deleterious	Non deleterious
R465P	rs1057519895	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R465H	rs1057519895	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R465G	rs867384286	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious
R465C	rs867384286	Deleterious	Damaging	Damaging	Disease	Deleterious	Deleterious

Table 3: Impact of nsSNPs on protein stability predicted by I-MUTANT 2.0

SNP ID	AA substitution	I MUTANT	Mutant RI	DDG-Free energy change value (kcal/mol)
R505S	rs149680468	Decrease	8	-1.19 kcal/mol
R505C	rs149680468	Decrease	4	-0.94 kcal/mol
R505G	rs149680468	Decrease	4	-1.41 Kcal/mol
R505L	rs149680468	Decrease	8	-0.14 Kcal/mol
R505H	rs149680468	Decrease	9	-1.30 Kcal/mol
R465L	rs1057519895	Decrease	7	-0.34 Kcal/mol
R465P	rs1057519895	Decrease	2	-0.46 Kcal/mol
R465H	rs1057519895	Decrease	8	-1.91 Kcal/mo
R465G	rs867384286	Decrease	7	-1.38 Kcal/mol
R465C	rs867384286	Decrease	3	-0.83 Kcal/mol

Table 4: Evolutionary conservancy of amino acids in FBXW7 analyzed by Consurf

SNP Id	Residue and Position	Conservation Score	Prediction
rs149680468	R505S	9	Highly Conserved and Exposed (F)
rs149680468	R505C	9	Highly Conserved and Exposed (F)
rs149680468	R505G	9	Highly Conserved and Exposed (F)
rs149680468	R505L	9	Highly Conserved and Exposed (F)
rs149680468	R505H	9	Highly Conserved and Exposed (F)
rs1057519895	R465L	9	Highly Conserved and Exposed (F)
rs1057519895	R465P	9	Highly Conserved and Exposed (F)
rs1057519895	R465H	9	Highly Conserved and Exposed (F)
rs867384286	R465G	9	Highly Conserved and Exposed (F)
rs867384286	R465C	9	Highly Conserved and Exposed (F)

Table 5: Pymol predictions for nsSNPs in FBXW7

AA substitution	Domain	RMSD
R505S	W40 Repeat Containing domain	0.00
R505C		0.00
R505G		0.00
R505L		0.035
R505H		0.003
R465L		0.035
R465P		0.094
R465H		0.7
R465G		0.00
R465C		0.00

Table 6: Swiss PDB Viewer Result

AA substitution	Presence of Clash / Hydrogen bond	Number of Rotamer	Total Energy after Energy Minimization Kcal/mol (SWISS PDB VIEWER)
Wild type			-255,16.217
R505S	Both	-1	-244,410.648
R505C	Hydrogen bond	-1	-230,78.449
R505G	Both	0	-229,77.867
R505L	Both	3	-215,40.178
R505H	Both	-2	-230,43.666
R465L	BOTH	-2	-230,77.969
R465P	Both	-2	-228,75.055
R465H	BOTH	-4	-221,96.342
R465G	BOTH	0	-229,77.867
R465C	BOTH	-2	-230,70.758

Table 7: Structural effect of mutants over FBXW7 protein

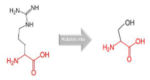
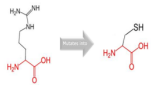
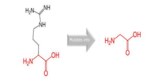
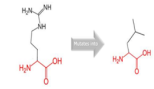
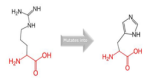
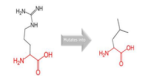
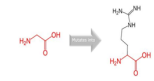
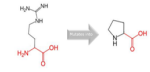
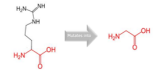
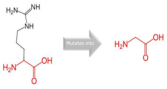
RESIDUE	Structure	Properties
R505S		➢ Mutant serine is Smaller than the arginine residue. ➢ Wild-type Arginine residue charge was POSITIVE, the mutant serine charge is NEUTRAL. ➢ Mutant serine residue is more hydrophobic than the wild-type residue. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 4. So Arginine into serine residue might disturb this repeat and consequently substrate binding function of WD40 domain
R505C		➢ Mutant residue cysteine is smaller than the wild-type Arginine residue. ➢ The wild-type Arginine residue charge was POSITIVE, the mutant cysteine residue charge is NEUTRAL. ➢ The mutant cysteine residue is located within a stretch of residues that is repeated in the protein, this repeat is named WD 4. The Arginine into cysteine residue might disturb this repeat and consequently substrate binding function of WD40 domain
R505G		➢ Mutant residue Glycine is smaller than the wild-type cysteine residue. ➢ Mutant residue Glycine is more hydrophobic than the Wild-Type cysteine residue. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 4. The cysteine into Glycine residue might disturb this repeat and consequently substrate binding function of WD40 domain. The mutation introduces a glycine at this position which is very flexible and can disturb the required rigidity of the protein at this position.
R505L		➢ Mutant residue leucine is smaller than the wild-type residue. ➢ The wild-type Arginine residue charge was POSITIVE, the mutant residue Leucine charge is NEUTRAL. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 4. The mutation into another residue might disturb this repeat and consequently substrate binding function of WD40 domain
R505H		➢ Mutant residue is bigger than the wild-type residue ➢ The wild-type residue charge was POSITIVE, the mutant residue charge is NEUTRAL. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 4. The mutation into another residue might disturb this repeat and consequently substrate binding function of WD40 domain
R465L		➢ Mutant residue is smaller than the wild-type residue. ➢ The wild-type residue charge was POSITIVE, the mutant residue charge is NEUTRAL. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 3. The mutation into another residue might disturb this repeat and consequently substrate binding function of WD40 domain
R465P		➢ The mutation is located within Ras-associating domain, which can disturb this domain and abolish its function. ➢ Wild-type residue is a glycine, mutation of this glycine can abolish flexibility function.
R465H		➢ The mutant residue is smaller than the wild-type residue. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 3. The mutation into another residue might disturb this repeat and consequently substrate binding function of WD40 domain.
R465G		➢ Mutant residue is smaller than the wild-type residue. ➢ Wild-type residue charge was POSITIVE whereas, mutant residue is NEUTRAL. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 3. The mutation into another residue might disturb this repeat and consequently substrate binding function of WD40 domain. The mutation introduces a glycine at this position. Glycine is very flexible and can disturb the required rigidity of the protein at this position.
R465C		➢ Mutant residue cysteine is smaller than the wild-type Arginine residue. ➢ Wild-type Arginine residue charge was POSITIVE, the mutant cysteine residue charge is NEUTRAL. ➢ The mutation is located within a stretch of residues that is repeated in the protein, this repeat is named WD 4. Arginine into another cysteine residue might disturb this repeat and consequently substrate binding function of WD40 domain.

Table 8: Structural effect of 10 nsSNPs over FBXW7 protein using Missense3D tool

AA substitution	Result Analysis
R505S	No structural damage predicted
R505C	This substitution results in a change between buried and exposed state of the target variant residue. ARG is exposed (RSA 23.7%) and CYS is buried (RSA 5.1%)
R505G	No structural damage predicted
R505L	This substitution results in a change between buried and exposed state of the target variant residue. ARG is exposed (RSA 23.7%) and LEU is buried (RSA 6.7%)
R505H	No structural damage predicted
R465L	No structural damage predicted
R465P	This substitution changes 'E' (extended strand in parallel and/or anti-parallel β-sheet conformation) to ' ' (no secondary structure).
R465H	No structural damage predicted
R465G	No structural damage predicted
R465C	No structural damage predicted

Table.9: FBXW7 associated proteins and their Network parameters

Protein name	Degree	Average Shortest Path Length	Betweenness Centrality	Closeness Centrality
NOTCH 1(Neurogenic locus notch homolog protein 1)	10	36.66	90	10
c-Myc(MYC Proto-Oncogene)	1	2.177	0	5.5
CCNE1(G1/S-specific cyclin-E1)	1	2.177	0	5.5
STYX(Serine/Threonine/Tyrosine Interacting Protein)	1	2.177	0	5.5
KLF5(Kruppel Like Factor 5)	1	2.177	0	5.5
SKP1(S-phase kinase-associated protein 1)	1	2.177	0	5.5
NFKB2(Nuclear Factor Kappa B Subunit 2)	1	2.177	0	5.5
CUL1(Cullin 1)	1	2.177	0	5.5
SREB1(sterol regulatory element-binding protein 1)	1	2.177	0	5.5

Figures

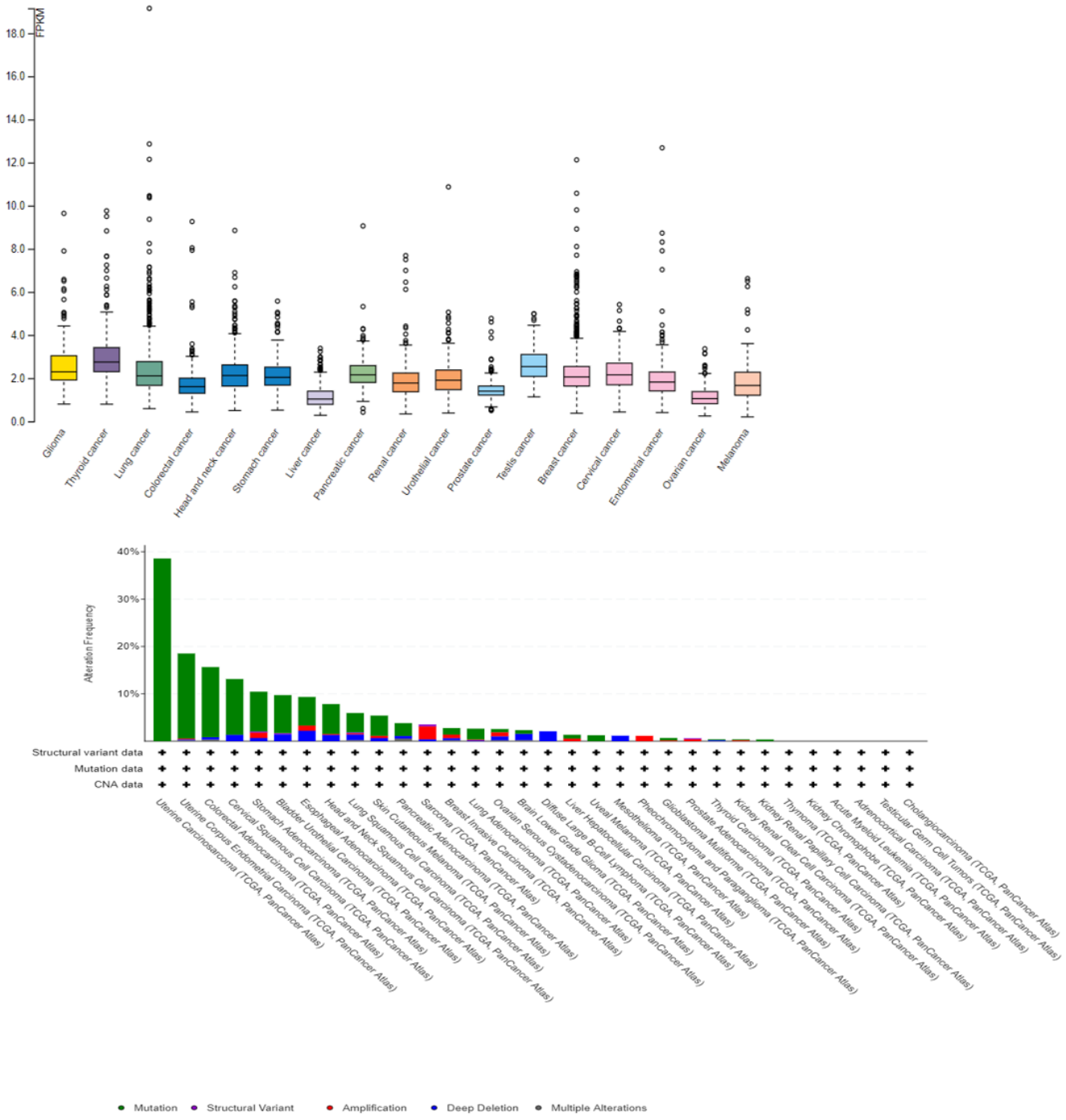


Figure 1

Frequency of FBXW7 expression in different types of solid tumors. RNA-seq data in 17 cancer types are plotted as median number fragments per kilobase of exon per million reads generated by The Cancer Genome Atlas (TCGA). Points are displayed as outliers if they are above or below 1.5 times the inter quartile range. The cancer types are color-coded according to which type of normal organ cancer originates from.

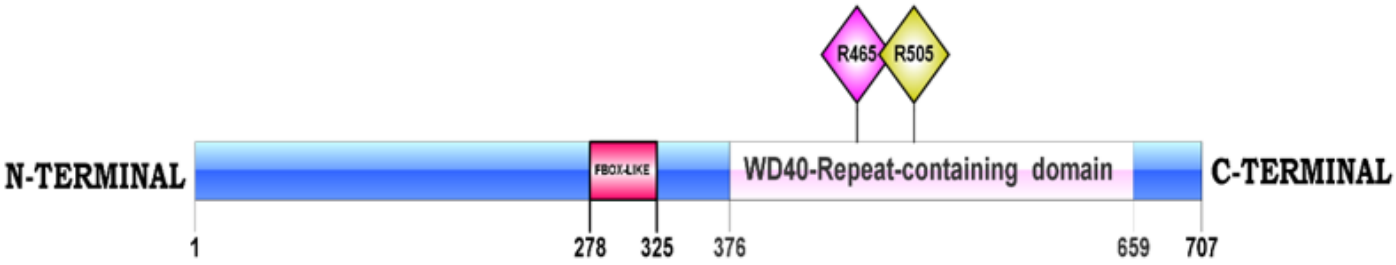


Figure 2

Domain identification of FBXW7 protein using InterPRO server. 1PR001810 indicates F-BOX domain (278-325), and IPR17986 indicates WD40-repeat-containing domain (376-659).

Figure 3

Evolutionary conservation profile of amino acid residues of FBXW7 as predicted by ConSurf. Almost all the nsSNPs primarily evaluated as deleterious belonged to the highly conserved regions. e: exposed residues according to the neural-network algorithm are indicated in orange letters. b: residues predicted to be buried are demonstrated via green letters. f: predicted functional residues (highly conserved and exposed) are indicated with red letters. s: predicted structural residues (highly conserved and buried) are demonstrated in blue letters. The black boxes indicate the deleterious nsSNPs related gynaecological cancer (R505H, R505L, R505S, R505G, R505C, R465L, R465P, R465H, R465G, R465C)

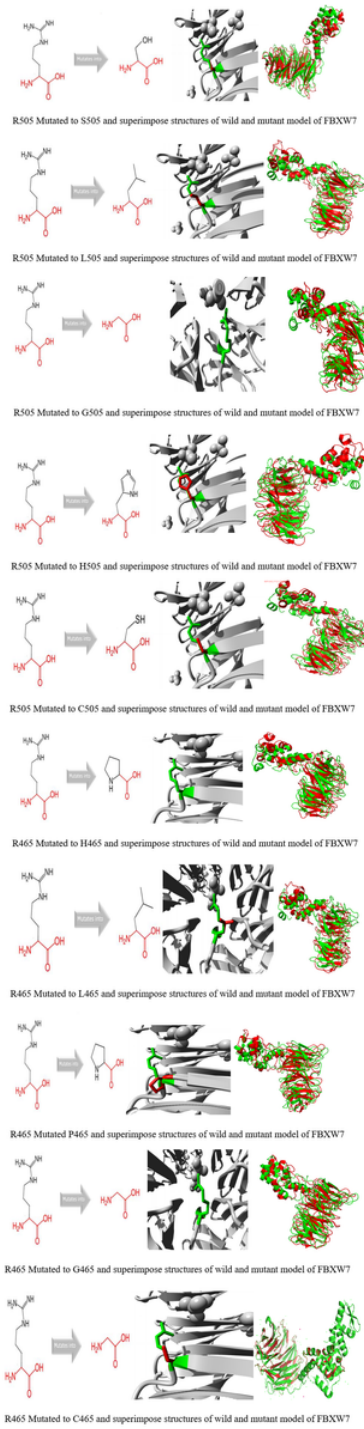


Figure 4

Structural alteration of the wild type of residue and mutant residue illustrated by Project Hope. The wild type of residue is presented as green, and the mutant residue is shown in red.

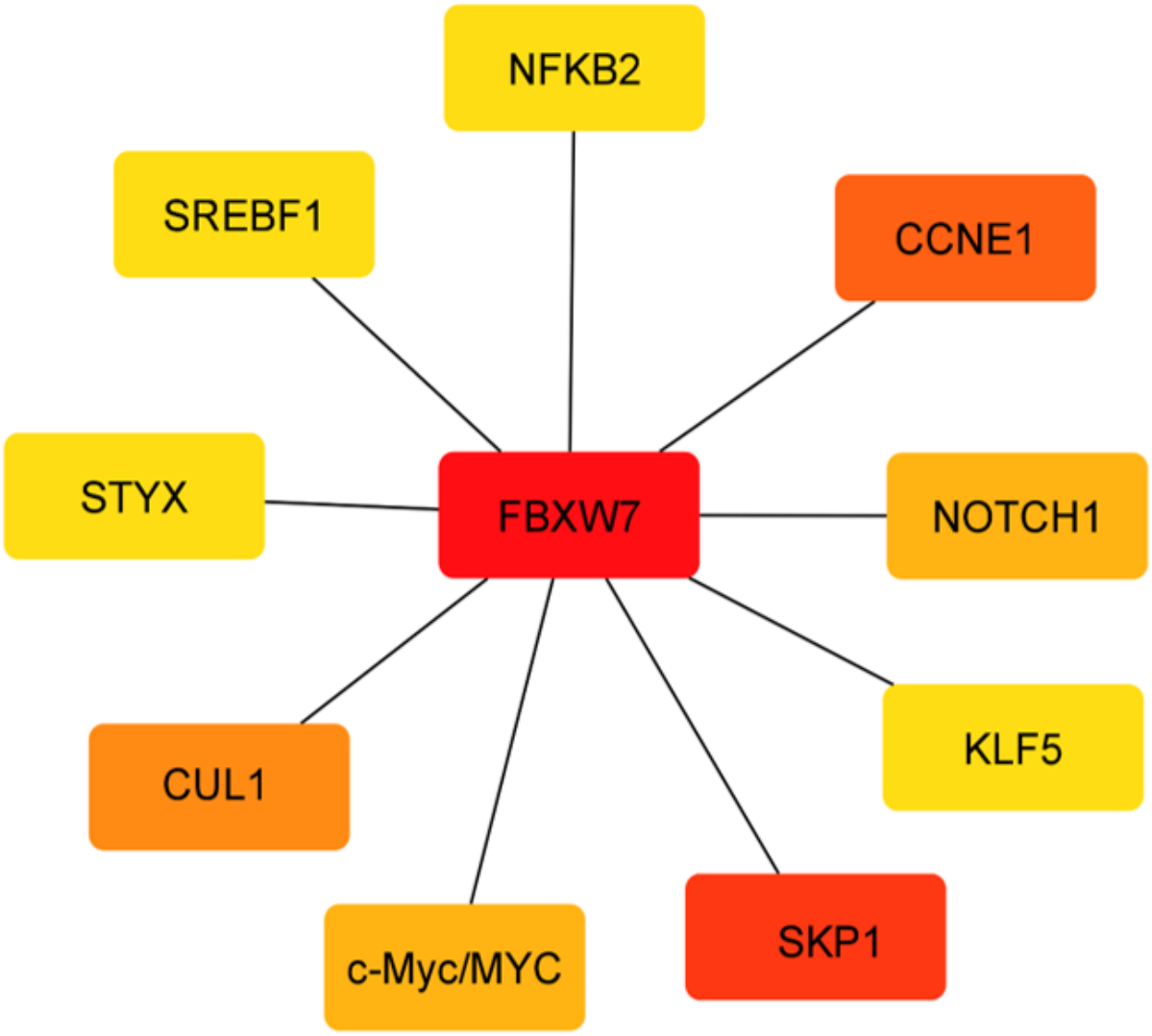


Figure 5

Protein- protein interacting network of FBXW7

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarytable1.docx