Molecules and Cells

Minireview

Mutation Hotspots in the β-Catenin Gene: Lessons from the Human Cancer Genome Databases

Sewoon Kim and Sunjoo Jeong*

Graduate Department of Bioconvergence Science and Technology, Dankook University, Jukjeon, Yongin, Gyeonggi 16890, Korea
*Correspondence: sjsj@dankook.ac.kr
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Mutations in the β-catenin gene (*CTNNB1*) have been implicated in the pathogenesis of some cancers. The recent development of cancer genome databases has facilitated comprehensive and focused analyses on the mutation status of cancer-related genes. We have used these databases to analyze the *CTNNB1* mutations assembled from different tumor types. High incidences of *CTNNB1* mutations were detected in endometrial, liver, and colorectal cancers. This finding agrees with the oncogenic role of aberrantly activated β-catenin in epithelial cells. Elevated frequencies of missense mutations were found in the exon 3 of *CTNNB1*, which is responsible for encoding the regulatory amino acids at the N-terminal region of the protein. In the case of metastatic colorectal cancers, in-frame deletions were revealed in the region spanning exon 3. Thus, exon 3 of *CTNNB1* can be considered to be a mutation hotspot in these cancers. Since the N-terminal region of the β-catenin protein forms a flexible structure, many questions arise regarding the structural and functional impacts of hotspot mutations. Clinical identification of hotspot mutations could provide the mechanistic basis for an oncogenic role of mutant β-catenin proteins in cancer cells. Furthermore, a systematic understanding of tumor-driving hotspot mutations could open new avenues for precision oncology.

Keywords: β-catenin, cancer genome database, hotspot mutations

INTRODUCTION

β-Catenin is an important co-activator downstream of the oncogenic Wnt signaling pathway, so mutations in the β-catenin gene (*CTNNB1*) have been implicated in oncogenesis (Korinek et al., 1997; Morin et al., 1997; Polakis, 2012b). Recently, large-scale cancer databases, such as The Cancer Genome Atlas (TCGA) pan-cancer analysis project, have leveraged systemic analyses on genome, exome, and transcriptome data from all types of cancers (Blum et al., 2018; Hutter and Zenklusen, 2018; Tomczak et al., 2015). Multidimensional cancer genome data are available on cBioPortal, an open platform for cancer genome analysis and visualization (Cerami et al., 2012; Gao et al., 2013). In this review, we have employed pan-cancer genome databases to analyze the current status of β-catenin gene (*CTNNB1*) mutations to identify mutation hotspots and to re-evaluate the oncogenic roles of specific β-catenin mutant proteins. An extensive review on the clinical aspects of the β-catenin protein is beyond the scope of this mini review, so we have provided a brief introduction regarding the basic biology of the β-catenin protein.

A BRIEF INTRODUCTION TO THE β-CATENIN PROTEIN

β-Catenin is a multitasking protein involved in transcription

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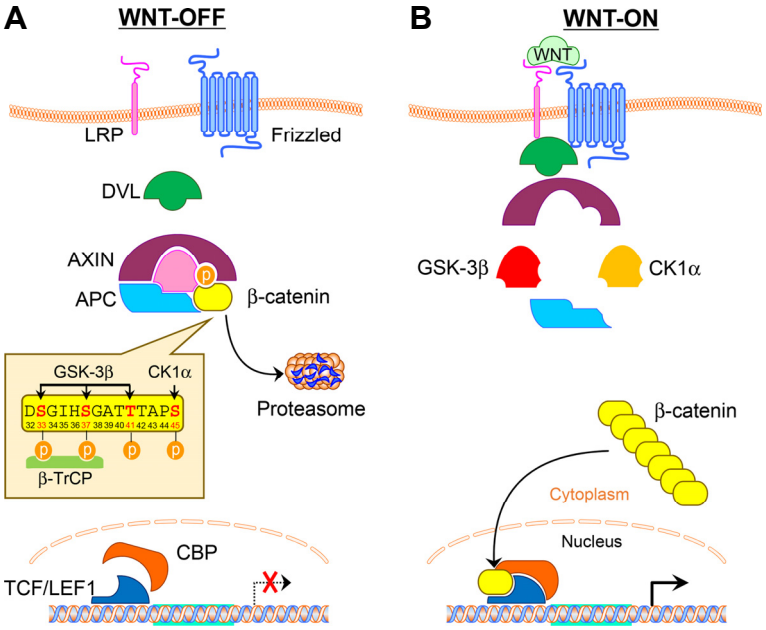

Fig. 1. A schematic diagram of the Wnt signaling pathway. (A) Wnt-off. In the absence of Wnt stimulation, β-catenin is phosphorylated by CK1α and GSK3β followed by ubiquitin-proteasome mediated proteolysis. (B) Wnt-on. Upon Wnt stimulation, the destruction complex is not functional, so the β-catenin protein is translocated into the nucleus and acts as a transcriptional co-activator to regulate oncogenic target genes. APC, Adenomatous polyposis; DVL, Disheveled.

and cell adhesion (Hur and Jeong, 2013; Kumar and Bashyam, 2017; Valenta et al., 2012). In particular, β-catenin is an important co-activator of Wnt target genes, such as *cyclin D1* and *c-myc* (Korinek et al., 1997; Morin et al., 1997). However, in differentiated cells, where Wnt signaling is off, the central regulatory mechanism for β-catenin is sequential phosphorylation in the N-terminal region followed by ubiquitin-mediated proteolysis (Fig. 1A). Casein Kinase-1α phosphorylates the S45 residue and primes subsequent phosphorylation on T41/S37/S33 by GSK-3β, leading to the binding of ubiquitin E3 ligase β-transducin repeats-containing proteins (β-TrCP) at the N-terminal region (D32 to S37) in a phosphorylation-dependent manner (Hart et al., 1998; Liu et al., 2002). Specific phosphorylation and ubiquitination occur in the APC/Axin complex, termed as the β-catenin destruction complex (Stamos and Weis, 2013). In contrast, the destruction complex functions no more, so the level of the β-catenin protein in the cytoplasm increases following Wnt activation (Fig. 1B). The mechanism by which Wnt signaling stabilizes β-catenin needs to be better understood in the aspect of the β-catenin destruction complex (Kim et al., 2013; 2015; Li et al., 2012; Taelman et al., 2010). Finally, Wnt-stimulated β-catenin is translocated into the nucleus, where it acts as transcriptional co-activator with DNA binding TCF/LEF proteins and activates many developmentally important, cancer-related and pathogenic genes (Nusse and Clevers, 2017).

FREQUENCY OF GENOMIC ALTERATIONS IN THE *CTNNB1* GENE IN CANCERS

Small-scale targeted gene analysis demonstrates mutations in the β-catenin gene (*CTNNB1*) in some cancers (Polakis, 2007; 2012b). Large-scale β-catenin mutational landscape was revealed from clinical sequencing of 10,000 prospective cancer patients by the Memorial Sloan Kettering-Integrated

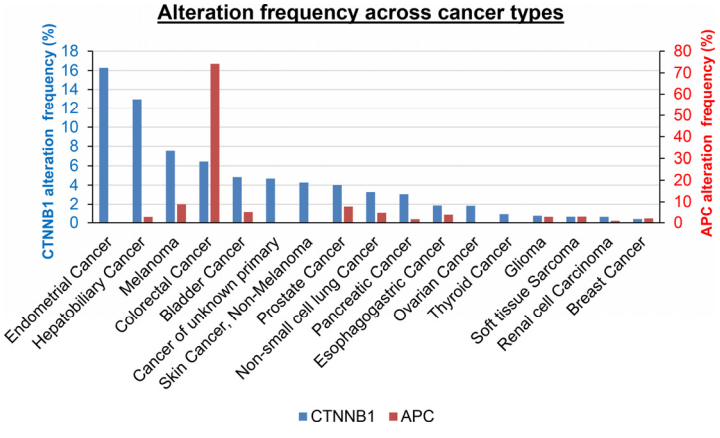

Fig. 2. The alteration frequency of CTNNB1 and APC across cancer types. Data obtained from the MSK-IMPACT pan-cancer study on cBioportal (www.cbioportal.org).

Table 1. The alteration frequency of CTNNB1 in endometrial, liver, and colorectal cancer

Cancer type	Sequencing data source	No. Sequenced	No. Alteration (%)	No. Exon3-mut (%)	Reference
Endometrial cancer	Endometrial Cancer (MSK, 2018)	187	27 (14.4)	25 (13.4)	Soumerai et al., 2018
	Uterine Corpus Endometrial Carcinoma (TCGA, Nature 2013)	240	71 (29.6)	63 (26.3)	Cancer Genome Atlas Research et al., 2013a
	Uterine Carcinosarcoma (TCGA, PanCancer Atlas)	56	1 (1.8)	0 (0.0)	Cancer Genome Atlas Research et al., 2013b
	Uterine Clear Cell Carcinoma (NIH, Cancer 2017)	16	0 (0.0)	0 (0.0)	Le Gallo et al., 2017
Liver cancer	Liver Hepatocellular Carcinoma (TCGA, PanCancer Atlas)	353	95 (26.9)	78 (22.1)	Cancer Genome Atlas Research et al., 2013b
	Liver Hepatocellular Carcinoma (AMC, Hepatology 2014)	231	53 (22.9)	46 (19.9)	Ahn et al., 2014
	Liver Hepatocellular Carcinoma (RIKEN, Nat Genet 2012)	25	3 (12.0)	3 (12.0)	Fujimoto et al., 2012
	Hepatocellular Carcinomas (Inserm, Nat Genet 2015)	243	87 (35.8)	76 (31.3)	Schulze et al., 2015
	Hepatocellular Adenoma (Inserm, Cancer Cell 2014)	30	13 (43.3)	11 (36.7)	Pilati et al., 2014
Colorectal cancer	Colorectal Adenocarcinoma (TCGA, Nature 2012)	212	11 (5.2)	1 (0.5)	Cancer Genome Atlas, N. et al., 2012
	Colorectal Adenocarcinoma (Genentech, Nature 2012)	72	5 (6.9)	2 (2.8)	Seshagiri et al., 2012
	Colorectal Adenocarcinoma (DFCI, Cell Reports 2016)	619	31 (5.0)	8 (1.3)	Giannakis et al., 2016
	Metastatic colorectal cancer (MSK, Cancer Cell 2018)	1099	84 (7.6)	19 (1.7)	Yaeger et al., 2018
	Colon Adenocarcinoma (TCGA, PanCancer Atlas)	389	27 (6.9)	15 (3.9)	Cancer Genome Atlas Research et al., 2013b
	Rectum Adenocarcinoma (TCGA, PanCancer Atlas)	137	8 (5.8)	0 (0.0)	Cancer Genome Atlas Research et al., 2013b

*Data obtained from the listed cancer studies on cBioportal (www.cbioportal.org)

Table 2. Status of mutations in cancer cell lines harboring activating mutations of CTNNB1

Cancer type	Cell Line	Mutations				
		CTNNB1	APC	TP53	BRAF	KRAS
Colorectal cancer	SW48	S33Y	R2714C			
	CCK81	T41A	Y159C	P278H	S273N	
	SNU407	T41A			R726C	G12D
	HCT116	S45del				G13D
	LS180	S45F	R1788C		D211G	G12D
Gastric cancer	KE39	D32N		V272L		
	AGS	G34E				G12D
	SNU719	G34V				
	OCUM1	S45C				
Endometrial cancer	HEC265	D32V, X561_splice	P1233L			
	HEC6	D32V				V160A
	HEC108	S37P, D207G	S678G, A2388V, T2514I	P151H		
	JHUEM2	S37C				
	SNGM	S37P	A2V			G12V
Lung cancer	MORCPR	S33L	P865L, A2122dup	P152Rfs*18		G13C
	SW1573	S33F				G12C
	LXF289	T41A		R248W		
	HCC15	S45F, Y670*	D2796G	D259V		
Liver cancer	HUH6	G34V		N239D, A159D		
	SNU398	S37C				
Melanoma	SKMEL1	S33C			V600E	
	COLO783	S45del		P27L	V600E	

*Mutation data obtained from Cancer Cell Line Encyclopedia (Novartis/Broad, Nature, 2012) on cBioportal (www.cbioportal.org).
#Abbreviation: del, deletion; dup, duplication; fs, frame shift; splice, splice site mutation; *, stop codon

Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) (Zehir et al., 2017). Figure 2 shows the frequency of *CTNNB1* alterations across tumor types. A high frequency of *CTNNB1* mutations are found in endometrial (16%), hepatobiliary (12%), melanoma (7%), and colorectal (6%) cancers. It is noteworthy that the frequency of *APC* mutations is much higher (approximately 70%) than that of *CTNNB1* in colorectal cancer, but *CTNNB1* and *APC* mutations exist in the exclusive manner. The alteration frequency of *CTNNB1* in endometrial, liver, and colorectal cancer from other genomic analysis networks is compiled in Table 1. In addition, cancer cell lines with mutations in *CTNNB1* are summarized in Table 2.

Endometrial cancer

Wnt/β-catenin pathway has been linked to endometrial cancer. Loss of APC function in the mouse endometrium induces nuclear β-catenin accumulation in uterine hyperplasia and squamous cell metaplasia. Although APC loss alone does not lead to malignant transformation, APC loss enhances endometrial tumorigenesis driven by PTEN loss (van der Zee et al., 2013). The majority of *CTNNB1* alterations occur in endometrial carcinoma, but not in carcinosarcoma or clear cell carcinoma as indicated in Table 1 (Cancer Genome Atlas Research et al., 2013a; Cancer Genome Atlas Research et al., 2013b; Le Gallo et al., 2017; Soumerai et al., 2018). In endometrial cancer cases from TCGA, the alterations of *CTNNB1* or *APC* genes are 30% or 12% of 240 patients, respectively (Cancer Genome Atlas Research et al., 2013a). Loss of APC function arises from truncation of the gene, but the frequency is only 6% in endometrial cancer. Thus, in endometrial cancer, *CTNNB1* mutations, rather than *APC* mutations, might be direct driver mutations. Recently, 245 endometrial cancer patient samples were sequenced using 46-200 gene panels. *CTNNB1* mutations appear more frequently in low-grade (grades 1-2) and early-stage (stages I-II) patients. More significantly, the patients harboring *CTNNB1* mutations are associated with worse recurrence-free survival (Kurnit et al., 2017).

Hepatocellular carcinoma (HCC)

Liver cancer is the seventh most common cancer and the fourth leading cause of cancer mortality worldwide (Bray et al., 2018). However, treatment options are still limited for patients with advanced HCC due to the heterogeneity of genome alterations. Genome-wide studies have been carried out to identify driver genes responsible for tumorigenesis. SNP array analysis of 125 HCC cases have identified that four genes (*CTNNB1* (32.8%), *TP53* (20.8%), *ARID1A* (16.8%), and *AXIN1* (15.2%)) are altered in more than 10% of the samples (Guichard et al., 2012). Whole exome sequencing analysis with 231 early-stage HCC Korean patient samples identified recurrent somatic mutations in *CTNNB1* (23%) and *TP53* (32%) (Ahn et al., 2014). In addition, *CTNNB1* and *TP53* were found to be frequently altered in a large cohort of HCC patient samples (Cancer Genome Atlas Research et al., 2013b; Fujimoto et al., 2012; Schulze et al., 2015). The *CTNNB1* gene is also frequently altered in hepatocellular adenoma (HCA) (Pilati et al., 2014). β-catenin transgenic mouse models have been used to define a function of β-catenin in HCC tumorigenesis (Nejak-Bowen and Monga, 2011). Ectopic expression of either wild-type or mutant β-catenin is not sufficient to induce tumorigenesis (Harada et al., 2002; Nejak-Bowen et al., 2010). In some cases, β-catenin may accelerate tumorigenesis in cooperating with activated Ha-Ras (Harada et al., 2004) or heterozygote deleted *Lkb1* (Miyoshi et al., 2009).

Colorectal cancer (CRC)

Wnt/β-cat signaling plays an important role in the tumorigenesis of CRC (Polakis, 2012b). In particular, alteration of *APC*, a negative regulator in Wnt signaling, is found in approximately 70% of CRC patients. Most *APC* alterations are truncation mutations, which cannot facilitate the proteolysis of β-catenin. In addition, loss of heterozygosity is frequently found in colorectal cancers. As shown in Table 1, genetic alterations also occurred in the *CTNNB1* gene (5% of TCGA, 5% of DFCI, 6.9% of Genentech) (Giannakis et al., 2016; Seshagiri et al., 2012). Several studies reported that β-catenin has oncogenic activity in CRC cells, so the inhibition of β-catenin by gene targeting or knockdown resulted in growth inhibition of colorectal cancer cells (Cancer Genome Atlas, 2012; Green et al., 2001; Kim et al., 2002; Roh et al., 2001).

MUTATION HOTSPOTS IN EXON 3 (ENCODING THE N-TERMINAL REGION) OF THE β-CATENIN GENE

The β-catenin protein is composed of three domains: an N-terminal domain (~130 aa), a central domain (residue 141-664) made of 12 Armadillo (Arm) repeats and a C-terminal domain (~100 aa) (Fig. 3A). The central domain of the protein, the Arm repeats domain, forms a rigid rod-like structure and interacts with many binding proteins (Xu and Kimelman, 2007). However, it has been difficult to determine the structure of the terminal regions (N- and C-terminals) of β-catenin, so they are likely to be flexible and could be intrinsically disordered (Xing et al., 2008). Interestingly, the N-terminal region of the β-catenin protein is encoded by exon 3 (amino acid residues 5-80) of *CTNNB1*, so the N-terminal mutations can also be referred to as exon 3 mutations. *CTNNB1* mutation hotspots were statistically analyzed by the Sorting Intolerant From Tolerant (SIFT) and the Polymorphism Phenotyping (PolyPhen) (Adzhubei et al., 2010; Naus, 1982; Sim et al., 2012).

Missense mutations affecting the N-terminal region of the β-catenin protein

In most cancers, mutations are found in the N-terminal region of β-catenin, especially in exon 3 of β-catenin mRNA (Fig. 3B). In endometrial cancers, integrated analysis showed that exon 3 mutations in β-catenin mRNA are associated with an aggressive phenotype of low-grade and low-stage in younger women (Liu et al., 2014). These studies suggest that β-catenin mutations can be a prognostic marker for aggressive endometrial cancer. Additionally, in liver cancer, hotspot mutations in *CTNNB1* were deeply analyzed in a large cohort of patients from HCA to carcinoma (HCC). S45, K335, and N387 mutations result in weak activation of β-

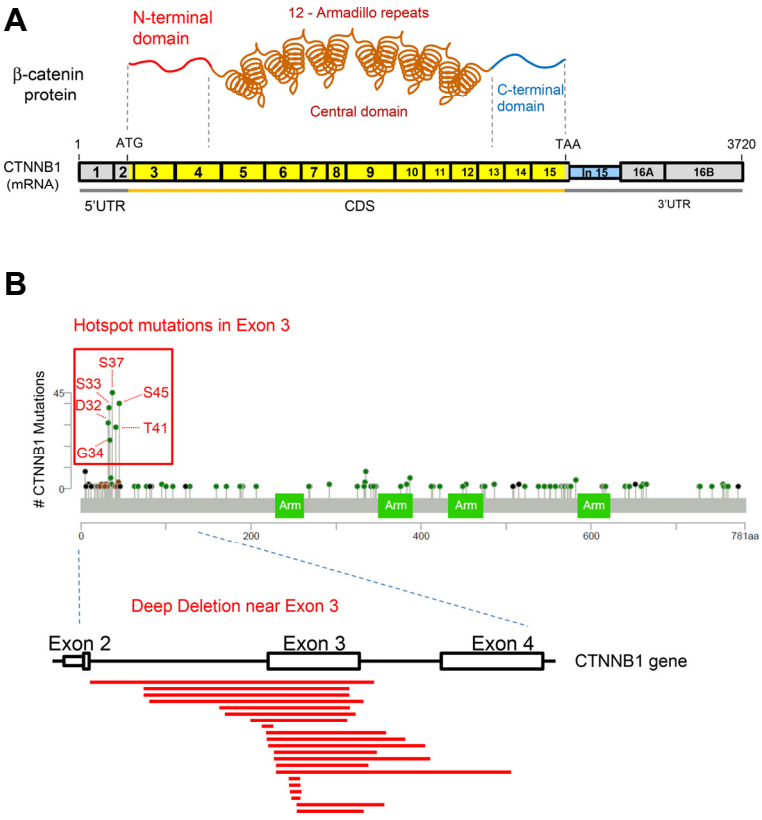

Fig. 3. Diagram of β-catenin protein domains and hotspot mutations. (A) A schematic diagram of the β-catenin protein and mRNA. UTR, untranslated region; CDS, coding sequence; ATG, translation start codon; TAA, translation stop codon. (B) Exon 3 hotspot mutations of *CTNNB1* are marked on the lollipop plot downloaded from the MSK-IMPACT pan-cancer study on cBioportal. Deep deletions near Exon 3 of *CTNNB1* pre-mRNA are indicated as red lines. Deletion data were obtained from metastatic colorectal cancer study (MSK) on cBioportal.

catenin and are frequently found in HCA. T41 mutations show relatively moderate activation. Exon 3 deletion and β-TrCP binding site (D32-S37) mutations show strong activation and are enriched in HCA/HCC borderline and HCC, respectively. Highly activated β-catenin is associated with malignant tumors, as evaluated by glutamine synthase staining. Although S45 mutations show weak activation, most S45 mutant alleles in HCC are duplicated, resulting in strong activation of β-catenin. This study suggests that HCA harboring high/moderate mutants or S45 mutants may be associated with malignant transformation (Rebouissou et al., 2016). Accelerated liver regeneration and hepatocarcinogenesis was also observed in mouse overexpressing S45 mutant β-catenin (Nejak-Bowen et al., 2010). In colorectal cancers, most somatic mutations are observed at D32, S33, G34, S37, T41, and S45 in exon 3 of β-catenin mRNA. These hotspot mutations have been shown to stabilize β-catenin by disturbing the phosphorylation-dependent ubiquitination, leading to tumorigenesis. S45 is a priming-phosphorylation site for Casein Kinase I alpha (CK1α) (Liu et al., 2002). S33, S37, and T41 are further phosphorylated by GSK3β. D32 and G34 is required to bind with β-TrCP, a component of ubiquitin E3 ligase for phosphorylated β-catenin (Aberle et al., 1997; Hart et al., 1998).

Exon 3-spanning in-frame deletion in metastatic colorectal cancers

Recently, prospective targeted sequencing was reported with metastatic and early-stage colorectal cancer patients of a large cohort study (Yaeger et al., 2018). In this MSK study, the frequency of *CTNNB1* alterations (8%) is slightly higher than that in TCGA cohort (5% of TCGA pan-cancer atlas), but in-frame deletion is highly enriched in the MSK cohort. This difference may be due to the distinct features between MSK and TCGA cohorts. The MSK cohort includes 47% of metastases that were not included in TCGA cohort, representing more aggressive and advanced disease. Activating hotspot mutations of β-catenin are more frequently occurred in microsatellite instability-high (MSI-H) tumors than in microsatellite stable (MSS) tumors (25% of MSI-H, 6% of MSS). Interestingly, however, exon 3-spanning in-frame deletions were identified only in MSS tumors and the nuclear staining of β-catenin was observed in tumors harboring in-frame deletions in *CTNNB1* (Yaeger et al., 2018).

CONCLUSION

Large-scale analysis of pan-cancer genomic database revealed a high frequency of *CTNNB1* mutations in endometrial, liver, and colorectal cancers. In addition, mutations are frequently located near exon 3 of *CTNNB1*, which encode for the regulatory amino acids (D32, S33, G34, S37, T41, and S45) at the N-terminal region of the protein. Since the N-terminal region is highly unstructured and flexible, the contributions of N-terminal hotspot mutations from a structural perspective are not easy to comprehend (Dar et al.,

2017; Gottardi and Peifer, 2008; Xing et al., 2008). Rather, their contribution to cancer development should be understood in terms of their roles in normal and pathogenic epithelial cell states.

FUTURE PERSPECTIVES

Re-evaluating hotspot mutations

The high frequency of mutations affecting the GSK3β and β-TrCP-binding sites (D32, S33, G34, S37) can be explained by their roles in the β-catenin destruction complex (Megy et al., 2005; Stamos and Weis, 2013). However, higher frequencies of S45 and T41 mutations cannot be easily explained in terms of the residues for priming and relay kinases, respectively. In fact, recent study suggested the uncoupling of CK1α phosphorylation on S45 residue to GSK3β phosphorylation on S37/S33 residues. The phosphorylations on the T41/S45 residues of β-catenin were spatially uncoupled from the phosphorylated S33/S37/T41 (Maher et al., 2010). In addition, a previous study reported that the phosphorylations on S33/S37/T41 can occur in the absence of the phospho-S45 in colon cancer cells (Wang et al., 2003). In desmoid-type fibromatosis, protein stability and target genes for the S45F mutant are different from those of the wild-type β-catenin (Colombo et al., 2017). Moreover, the S45F mutation is associated with low efficacy of a cyclooxygenase-2 inhibitor in desmoid tumors (Hamada et al., 2014). It will be important to determine the oncogenic role of the S45 mutant β-catenin protein, as a type of mutation distinct from other mutant β-catenin proteins.

β-catenin in multiple protein complexes

β-Catenin protein was first discovered as a component of the adherens junction (Ozawa et al., 1989). Later, it is considered as a multitasking protein involved in transcription as well as in cell adhesion (Hur and Jeong, 2013; Kumar and Bashyam, 2017; Valenta et al., 2012). However, it should be noted that most β-catenin proteins reside in the adhesion complex near the plasma membrane in which it interacts with E-cadherin and α-catenin with high affinities (Huber and Weis, 2001). Multiple roles of β-catenin protein may come from multiprotein assembly forming distinct complexes in different intracellular locations (Xu and Kimelman, 2007). In the nucleus, β-catenin associates with DNA binding proteins, such as TCF/LEF and BCL9 (Graham et al., 2001; Sampietro et al., 2006). Collectively, the N-terminal region of β-catenin is critical for regulating the adhesion and transcription functions of the protein. Thus, the regulatory mechanism of phosphorylation may differ in distinct β-catenin complexes (Dar et al., 2016). Therefore, many questions arise as to whether the specific mutant β-catenin proteins can form a previously unknown complex, in addition to the adhesion, destruction, and transcription complexes (Fig. 4). We hope that the clinical information gained from the large cancer genome databases could facilitate the study of novel functions of β-catenin in RNA metabolism as an RNA-binding protein (Hur and Jeong, 2013; Kim et al., 2009; Kim et al., 2012; Lee and Jeong, 2006). To enhance our understanding of such novel functions, a systematic mutant β-catenin library could be developed to link the differential functional impacts to specific mutations in cancer. More functional studies on specific mutant β-catenin proteins will open up new avenues for elucidating the mechanisms underlying mutant β-catenin-mediated oncogenesis.

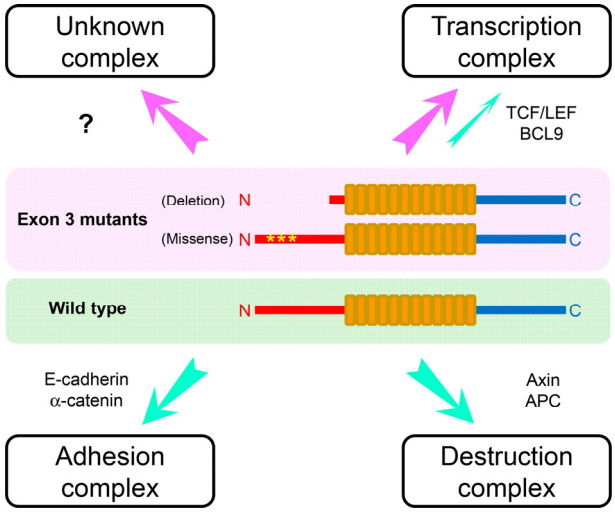

Fig. 4. Proposed model for the role of mutant β-catenin proteins in distinct complexes. The green indicates wild-type β-catenin protein and the pink indicates exon 3-mutated β-catenin proteins. In addition to the adhesion, destruction and transcription complexes with the indicated proteins, additional unknown protein complexes are likely to be formed by the mutant β-catenin proteins.

Novel therapeutic approach for mutant β-catenin proteins

β-Catenin protein has been a prime target for anti-cancer drug development, but some limitations may suspend successful drug development. In most cases, wild-type β-catenin protein have been utilized as a target protein and Wnt signaling activated transcription is used as a screening read-out (Cui et al., 2018; Krishnamurthy and Kurzrock, 2018; Polakis, 2012a). As a novel strategy, the information obtained for mutant β-catenin can be implemented for mutant-specific anti-cancer therapeutics, as utilized for mutant p53 proteins (Bykov et al., 2018; Kotler et al., 2018). Large-scale clinical analysis could provide important information on the functions of cancer-related proteins and cancer signaling, as shown here (Hyman et al., 2017). Therefore, future research should be directed toward a precision oncology strategy by identifying the molecular signature of cancer-related genes and exploiting cancer genome databases (Zehir et al., 2017).

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REFERENCES

Aberle, H., Bauer, A., Stappert, J., Kispert, A., and Kemler, R. (1997). β-catenin is a target for the ubiquitin-proteosome pathway. EMBO J. *16*, 3797-3804.

Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. Nat. Methods. *7*, 248-249.

Ahn, S.M., Jang, S.J., Shim, J.H., Kim, D., Hong, S.M., Sung, C.O., Baek, D., Haq, F., Ansari, A.A., Lee, S.Y., et al. (2014). Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. Hepatology *60*, 1972-1982.

Blum, A., Wang, P., and Zenklusen, J.C. (2018). SnapShot: TCGA-analyzed tumors. Cell *173*, 530.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R.L., Torre, L.A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. *68,* 394-424.

Bykov, V.J.N., Eriksson, S.E., Bianchi, J., and Wiman, K.G. (2018). Targeting mutant p53 for efficient cancer therapy. Nat. Rev. Cancer *18*, 89-102.

Cancer Genome Atlas, N. (2012). Comprehensive molecular characterization of human colon and rectal cancer. Nature *487*, 330-337.

Cancer Genome Atlas Research, N., Kandoth, C., Schultz, N., Cherniack, A.D., Akbani, R., Liu, Y., Shen, H., Robertson, A.G., Pashtan, I., Shen, R., et al. (2013a). Integrated genomic characterization of endometrial carcinoma. Nature *497*, 67-73.

Cancer Genome Atlas Research, N., Weinstein, J.N., Collisson, E.A., Mills, G.B., Shaw, K.R., Ozenberger, B.A., Ellrott, K., Shmulevich, I., Sander, C., and Stuart, J.M. (2013b). The cancer genome atlas pan-cancer analysis project. Nat. Genet. *45*, 1113-1120.

Cerami, E., Gao, J., Dogrusoz, U., Gross, B.E., Sumer, S.O., Aksoy, B.A., Jacobsen, A., Byrne, C.J., Heuer, M.L., Larsson, E., et al. (2012). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. *2*, 401-404.

Colombo, C., Belfiore, A., Paielli, N., De Cecco, L., Canevari, S., Laurini, E., Fermeglia, M., Pricl, S., Verderio, P., Bottelli, S., et al. (2017). β-catenin in desmoid-type fibromatosis: deep insights into the role of T41A and S45F mutations on protein structure and gene expression. Mol. Oncol. *11*, 1495-1507.

Cui, C., Zhou, X., Zhang, W., Qu, Y., and Ke, X. (2018). Is β-catenin a druggable target for cancer therapy? Trends Biochem. Sci. *43*, 623-634.

Dar, M.S., Singh, P., Mir, R.A., and Dar, M.J. (2017). β-catenin N-terminal domain: An enigmatic region prone to cancer causing mutations. Mutat. Res. *773*, 122-133.

Dar, M.S., Singh, P., Singh, G., Jamwal, G., Hussain, S.S., Rana, A., Akhter, Y., Monga, S.P., and Dar, M.J. (2016). Terminal regions of β-catenin are critical for regulating its adhesion and transcription functions. Biochim. Biophys. Acta *1863*, 2345-2357.

Fujimoto, A., Totoki, Y., Abe, T., Boroevich, K.A., Hosoda, F., Nguyen, H.H., Aoki, M., Hosono, N., Kubo, M., Miya, F., *et al.* (2012). Whole-genome sequencing of liver cancers identifies etiological influences on mutation patterns and recurrent mutations in chromatin regulators. Nat. Genet. *44*, 760-764.

Gao, J., Aksoy, B.A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S.O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E., *et al.* (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci. Signal. *6*, pl1.

Giannakis, M., Mu, X.J., Shukla, S.A., Qian, Z.R., Cohen, O., Nishihara, R., Bahl, S., Cao, Y., Amin-Mansour, A., Yamauchi, M., *et al.* (2016). Genomic correlates of immune-cell infiltrates in colorectal carcinoma. Cell Rep. *15*, 857-865.

Gottardi, C.J., and Peifer, M. (2008). Terminal regions of β-catenin come into view. Structure *16*, 336-338.

Graham, T.A., Ferkey, D.M., Mao, F., Kimelman, D., and Xu, W. (2001). Tcf4 can specifically recognize β-catenin using alternative conformations. Nat. Struct. Biol. *8*, 1048-1052.

Green, D.W., Roh, H., Pippin, J.A., and Drebin, J.A. (2001). β-catenin antisense treatment decreases β-catenin expression and tumor growth rate in colon carcinoma xenografts. J. Surg. Res. *101*, 16-20.

Guichard, C., Amaddeo, G., Imbeaud, S., Ladeiro, Y., Pelletier, L., Maad, I.B., Calderaro, J., Bioulac-Sage, P., Letexier, M., Degos, F., *et al.* (2012). Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat. Genet. *44*, 694-698.

Hamada, S., Futamura, N., Ikuta, K., Urakawa, H., Kozawa, E., Ishiguro, N., and Nishida, Y. (2014). CTNNB1 S45F mutation predicts poor efficacy of meloxicam treatment for desmoid tumors: a pilot study. PloS one *9*, e96391.

Harada, N., Miyoshi, H., Murai, N., Oshima, H., Tamai, Y., Oshima, M., and Taketo, M.M. (2002). Lack of tumorigenesis in the mouse liver after adenovirus-mediated expression of a dominant stable mutant of β-catenin. Cancer Res. *62*, 1971-1977.

Harada, N., Oshima, H., Katoh, M., Tamai, Y., Oshima, M., and Taketo, M.M. (2004). Hepatocarcinogenesis in mice with β-catenin and Ha-ras gene mutations. Cancer Res. *64*, 48-54.

Hart, M., Concordet, J.-P., Lassot, I., A;bert, O., Santos, E., Durand, H., Perret, C., Rubinfeld, B., Margottin, F., Benarous, R., *et al.* (1998). The F-box protein β-TrCP associates with phosphorylated β-catenin and regulates its activity in the cell. Curr. Biol. *9*, 207-210.

Huber, A.H., and Weis, W.I. (2001). The structure of the β-catenin/E-cadherin complex and the molecular basis of diverse ligand recognition by β-catenin. Cell *105*, 391-402.

Hur, J., and Jeong, S. (2013). Multitasking β-catenin: from adhesion and transcription to RNA regulation. Anim. Cells Syst. *17*, 299-305.

Hutter, C., and Zenklusen, J.C. (2018). The Cancer Genome Atlas: Creating Lasting Value beyond Its Data. Cell *173*, 283-285.

Hyman, D.M., Taylor, B.S., and Baselga, J. (2017). Implementing Genome-Driven Oncology. Cell *168*, 584-599.

Kim, H., Vick, P., Hedtke, J., Ploper, D., and De Robertis, E.M. (2015). Wnt signaling translocates Lys48-linked polyubiquitinated proteins to the lysosomal pathway. Cell Rep. *11*, 1151-1159.

Kim, I., Kwak, H., Lee, H.K., Hyun, S., and Jeong, S. (2012). β-Catenin recognizes a specific RNA motif in the cyclooxygenase-2 mRNA 3'-UTR and interacts with HuR in colon cancer cells. Nucleic Acids Res. *40*, 6863-6872.

Kim, J.S., Crooks, H., Foxworth, A., and Waldman, T. (2002). Proof-of-principle: oncogenic β-catenin is a valid molecular target for the development of pharmacological inhibitors. Mol. Cancer Ther. *1*, 1355-1359.

Kim, M.Y., Hur, J., and Jeong, S. (2009). Emerging roles of RNA and RNA-binding protein network in cancer cells. BMB Rep. *42*, 125-130.

Kim, S.E., Huang, H., Zhao, M., Zhang, X., Zhang, A., Semonov, M.V., MacDonald, B.T., Zhang, X., Garcia Abreu, J., Peng, L., *et al.* (2013). Wnt stabilization of β-catenin reveals principles for morphogen

receptor-scaffold assemblies. Science *340*, 867-870.

Korinek, V., Barker, N., Morin, P.J., van Wichen, D., de Weger, R., Kinzler, K.W., Vogelstein, B., and Clevers, H. (1997). Constitutive transcriptional activation by a β-catenin-Tcf complex in APC-/- colon carcinoma. Science *275*, 1784-1787.

Kotler, E., Shani, O., Goldfeld, G., Lotan-Pompan, M., Tarcic, O., Gershoni, A., Hopf, T.A., Marks, D.S., Oren, M., and Segal, E. (2018). A systematic p53 mutation library links differential functional impact to cancer mutation pattern and evolutionary conservation. Mol. Cell *71*, 178-190 e178.

Krishnamurthy, N., and Kurzrock, R. (2018). Targeting the Wnt/β-catenin pathway in cancer: Update on effectors and inhibitors. Cancer Treat Rev. *62*, 50-60.

Kumar, R., and Bashyam, M.D. (2017). Multiple oncogenic roles of nuclear β-catenin. J. Biosci. *42*, 695-707.

Kurnit, K.C., Kim, G.N., Fellman, B.M., Urbauer, D.L., Mills, G.B., Zhang, W., and Broaddus, R.R. (2017). CTNNB1 (β-catenin) mutation identifies low grade, early stage endometrial cancer patients at increased risk of recurrence. Mod. Pathol. *30*, 1032-1041.

Le Gallo, M., Rudd, M.L., Urick, M.E., Hansen, N.F., Zhang, S., Program, N.C.S., Lozy, F., Sgroi, D.C., Vidal Bel, A., Matias-Guiu, X., *et al.* (2017). Somatic mutation profiles of clear cell endometrial tumors revealed by whole exome and targeted gene sequencing. Cancer *123*, 3261-3268.

Lee, H.K., and Jeong, S. (2006). β-Catenin stabilizes cyclooxygenase-2 mRNA by interacting with AU-rich elements of 3'-UTR. Nucleic acids Res. *34*, 5705-5714.

Li, V.S., Ng, S.S., Boersema, P.J., Low, T.Y., Karthaus, W.R., Gerlach, J.P., Mohammed, S., Heck, A.J., Maurice, M.M., Mahmoudi, T., *et al.* (2012). Wnt signaling through inhibition of β-catenin degradation in an intact Axin1 complex. Cell *149*, 1245-1256.

Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G.-H., Tan, Y., Zhang, Z., Lin, X., and He, X. (2002). Control of β-cateninphosphorylation/degradation by a dual-kinase mechanism. Cell *108*, 837-847.

Liu, Y., Patel, L., Mills, G.B., Lu, K.H., Sood, A.K., Ding, L., Kucherlapati, R., Mardis, E.R., Levine, D.A., Shmulevich, I., *et al.* (2014). Clinical significance of CTNNB1 mutation and Wnt pathway activation in endometrioid endometrial carcinoma. J. Natl. Cancer Inst. *106*.

Maher, M.T., Mo, R., Flozak, A.S., Peled, O.N., and Gottardi, C.J. (2010). β-catenin phosphorylated at serine 45 is spatially uncoupled from β-catenin phosphorylated in the GSK3 domain: implications for signaling. PloS one *5*, e10184.

Megy, S., Bertho, G., Gharbi-Benarous, J., Baleux, F., Benarous, R., and Girault, J.P. (2005). Solution structure of a peptide derived from the oncogenic protein β-Catenin in its phosphorylated and nonphosphorylated states. Peptides *26*, 227-241.

Miyoshi, H., Deguchi, A., Nakau, M., Kojima, Y., Mori, A., Oshima, M., Aoki, M., and Taketo, M.M. (2009). Hepatocellular carcinoma development induced by conditional β-catenin activation in Lkb1+/- mice. Cancer Sci. *100*, 2046-2053.

Morin, P.J., Sparks, A.B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K.W. (1997). Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science *275*, 1787-1790.

Naus, J.I. (1982). Approximations for distributions of scan statistics. J. Am. Stat. Assoc. *77*, 177-183.

Nejak-Bowen, K.N., and Monga, S.P. (2011). β-catenin signaling, liver regeneration and hepatocellular cancer: sorting the good from the bad. Semin. Cancer Biol. *21*, 44-58.

Nejak-Bowen, K.N., Thompson, M.D., Singh, S., Bowen, W.C., Dar, M.J., Khillan, J., Dai, C., and Monga, S.P.S. (2010). Accelerated liver regeneration and hepatocarcinogenesis in mice overexpressing serine-45 mutant β-catenin. Hepatology *51*, 1603-1613.

Nusse, R., and Clevers, H. (2017). Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell *169*, 985-999.

Ozawa, M., Baribault, H., and Kemler, R. (1989). The cytoplasmic domain of the cell adhesion molecule uvomorulin associates with three independent proteins structurally related in different species. EMBO J. *8*, 1711-1717.

Pilati, C., Letouze, E., Nault, J.C., Imbeaud, S., Boulai, A., Calderaro, J., Poussin, K., Franconi, A., Couchy, G., Morcrette, G., et al. (2014). Genomic profiling of hepatocellular adenomas reveals recurrent FRK-activating mutations and the mechanisms of malignant transformation. Cancer cell *25*, 428-441.

Polakis, P. (2007). The many ways of Wnt in cancer. Curr Opin Genet Dev *17*, 45-51.

Polakis, P. (2012a). Drugging Wnt signalling in cancer. EMBO J. *31*, 2737-2746.

Polakis, P. (2012b). Wnt signaling in cancer. Cold Spring Harbor perspectives in biology *4*.

Rebouissou, S., Franconi, A., Calderaro, J., Letouze, E., Imbeaud, S., Pilati, C., Nault, J.C., Couchy, G., Laurent, A., Balabaud, C., et al. (2016). Genotype-phenotype correlation of CTNNB1 mutations reveals different ss-catenin activity associated with liver tumor progression. Hepatology *64*, 2047-2061.

Roh, H., Green, D.W., Boswell, C.B., Pippin, J.A., and Drebin, J.A. (2001). Suppression of β-catenin inhibits the neoplastic growth of APC-mutant colon cancer cells. Cancer Res. *61*, 6563-6568.

Sampietro, J., Dahlberg, C.L., Cho, U.S., Hinds, T.R., Kimelman, D., and Xu, W. (2006). Crystal structure of a β-catenin/BCL9/Tcf4 complex. Mol. Cell *24*, 293-300.

Schulze, K., Imbeaud, S., Letouze, E., Alexandrov, L.B., Calderaro, J., Rebouissou, S., Couchy, G., Meiller, C., Shinde, J., Soysouvanh, F., et al. (2015). Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat. Genet. *47*, 505-511.

Seshagiri, S., Stawiski, E.W., Durinck, S., Modrusan, Z., Storm, E.E., Conboy, C.B., Chaudhuri, S., Guan, Y., Janakiraman, V., Jaiswal, B.S., et al. (2012). Recurrent R-spondin fusions in colon cancer. Nature *488*, 660-664.

Sim, N.L., Kumar, P., Hu, J., Henikoff, S., Schneider, G., and Ng, P.C. (2012). SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res. *40*, W452-457.

Soumerai, T.E., Donoghue, M.T.A., Bandlamudi, C., Srinivasan, P., Chang, M.T., Zamarin, D., Cadoo, K.A., Grisham, R.N., O'Cearbhaill, R.E., Tew, W.P., et al. (2018). Clinical utility of prospective molecular characterization in advanced endometrial cancer. Clin. Cancer Res. *24*, 5939-5947.

Stamos, J.L., and Weis, W.I. (2013). The β-catenin destruction complex. Cold Spring Harb. Perspect. Biol. *5*, a007898.

Taelman, V.F., Dobrowolski, R., Plouhinec, J.L., Fuentealba, L.C., Vorwald, P.P., Gumper, I., Sabatini, D.D., and De Robertis, E.M. (2010). Wnt signaling requires sequestration of glycogen synthase kinase 3 inside multivesicular endosomes. Cell *143*, 1136-1148.

Tomczak, K., Czerwinska, P., and Wiznerowicz, M. (2015). The cancer genome atlas (TCGA): an immeasurable source of knowledge. Contemp Oncol (Pozn) *19*, A68-77.

Valenta, T., Hausmann, G., and Basler, K. (2012). The many faces and functions of β-catenin. EMBO J. *31*, 2714-2736.

van der Zee, M., Jia, Y., Wang, Y., Heijmans-Antonissen, C., Ewing, P.C., Franken, P., DeMayo, F.J., Lydon, J.P., Burger, C.W., Fodde, R., et al. (2013). Alterations in Wnt-β-catenin and Pten signalling play distinct roles in endometrial cancer initiation and progression. J. Pathol. *230*, 48-58.

Wang, Z.H., Vogelstein, B., and Kinzler, K.W. (2003). Phosphorylation of β-catenin at S33, S37, or T41 can occur in the absence of phosphorylation at T45 in colon cancer cells. Cancer Res. *63*, 5234-5235.

Xing, Y., Takemaru, K., Liu, J., Berndt, J.D., Zheng, J.J., Moon, R.T., and Xu, W. (2008). Crystal structure of a full-length β-catenin. Structure *16*, 478-487.

Xu, W., and Kimelman, D. (2007). Mechanistic insights from structural studies of β-catenin and its binding partners. J. Cell Sci. *120*, 3337-3344.

Yaeger, R., Chatila, W.K., Lipsyc, M.D., Hechtman, J.F., Cercek, A., Sanchez-Vega, F., Jayakumaran, G., Middha, S., Zehir, A., Donoghue, M.T.A., et al. (2018). Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. Cancer cell *33*, 125-136 e123.

Zehir, A., Benayed, R., Shah, R.H., Syed, A., Middha, S., Kim, H.R., Srinivasan, P., Gao, J., Chakravarty, D., Devlin, S.M., et al. (2017). Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat. Med. *23*, 703-713.