Multiple oncogenic roles of nuclear beta catenin

Abbreviated title: Oncogenic β-catenin

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Abstract

β-catenin is essential for embryonic development and required for cell renewal/regeneration in adult life. Cellular β-catenin exists in three different pools: membranous, cytoplasmic and nuclear. In this review, we focus on functions of the nuclear pool in relation to tumorigenesis.

In the nucleus, β-catenin functions as both activator and repressor of transcription in a context dependent manner. It promotes cell proliferation and supports tumor growth by enhancing angiogenesis. β-catenin mediated signalling regulates cancer cell metabolism and is associated with tumor initiating cells (TICs) in multiple malignancies. In addition, it functions as both pro- and anti-apoptotic factor besides acting to inhibit recruitment of inflammatory anti tumor T-cells. Thus, β-catenin appears to possess a multifaceted nuclear function that may significantly impact tumor initiation and progression.

Keywords: Epithelial-mesenchymal transition; Tumor initiating cells; Tumor microenvironment; Cancer cell metabolism; Anti-tumor immunity

1. Introduction

β-catenin, a 781 amino acid protein, is well known as the effector molecule of canonical Wnt signalling. It was discovered (along with α and γ-catenin) as an E-cadherin associated protein in the late 1980s (Ozawa et al. 1989). Human β-catenin is a highly conserved protein having 67% identity to its Drosophila homolog Armadillo, whereas only six amino acids differ between the human and Xenopus proteins (as reviewed in Shapiro & Weis 2009). The central region (residues 141–664) consists of twelve Armadillo (ARM) repeats (R1–12), flanked by well-defined N- and C-terminal domains (NTD and CTD). β-catenin binding partners interact with the ARM repeats R3–R9 and form salt bridges with amino-acid residues Lys312 and Lys435, whereas rest of the ARM repeats strengthen the interactions (Huber et al. 1997). Terminal domains (NTD and CTD) may also contribute to the binding (Solanas et al. 2004).

During embryonic development, β-catenin regulates cell fate determination and body axis patterning in all metazans (as reviewed in Clevers 2006 and van Amerongen & Nusse 2009). It is also required for cell renewal/regeneration and tissue homeostasis in later stages of animal life. Cellular β-catenin exists in three different pools: membranous, cytoplasmic and nuclear (summarized in figure 1). Freshly synthesized β-catenin localises to Adherens Junctions (AJs) via interaction with E-cadherin (membranous pool) whereas excess β-catenin is captured by a destruction complex (cytoplasmic pool). In the presence of a sub-optimally functioning destruction complex, excess β-catenin translocates into the nucleus (nuclear pool) (as reviewed in Grigoryan et al. 2008). Conformation change and interaction with different binding partners may contribute to its varied functions as discussed in detail below. Of note, β-catenin is associated with various pathological conditions like cancer, neurodegenerative disorders and osteoporosis (as reviewed in MacDonald et al. 2009). In this review, we will focus on function of β-catenin in the different pools in relation to cancer biology, with particular emphasis on the nuclear pool.

2. Three cellular pools of β-catenin and tumorigenesis

2.1 The membranous pool
As a structural protein localized to the cell membrane, β-catenin plays crucial role in maintaining cell adhesion. Using the entire ARM domain, it interacts with the cytoplasmic domain of E-cadherin and connects α-catenin to E-cadherin; the N-terminus of β-catenin (residues 120-147) forms the binding site for α-catenin. Binding of α-catenin to β-catenin distorts the continuity of the first ARM repeat and creates a hinge region which in turn permits β-catenin to bind both E-cadherin and α-catenin (Pokutta et al. 2000). The β-catenin - α-catenin complex links cadherin to the actin cytoskeleton (as reviewed in Takeichi 1995 and Morin 1999); this link is crucial for assured cadherin-mediated cell adhesion (as reviewed in Shapiro & Weis 2009). The catenin-cadherin interaction is dynamic in nature and its disintegration promotes release of β-catenin from the membrane (as reviewed in Heuberger & Birchmeier 2010). β-catenin release from membrane is promoted by protease mediated cadherin (Ito et al. 1999) or β-catenin (Abe & Takeichi 2007) cleavage. β-catenin phosphorylation at Tyr142 by the Fer/Fen tyrosine kinase notably diminishes α-catenin binding and thus weakens its adhesive function (Piedra et al. 2003). Perturbed cadherin-catenin interaction results in loss of cell adhesion causing polarized epithelial cells to ‘transform’ into motile mesenchymal cells; a phenomena popularly termed epithelial-mesenchymal transition (EMT) (as reviewed in Martin et al. 2013 and Huber et al. 2005). EMT plays a crucial role during embryonic development (as reviewed in Thiery et al. 2009) as well as in tumor invasion and metastasis (as reviewed in Lamouille et al. 2014). In contrast, Tyr654 phosphorylation reduces β-catenin binding to cadherin and may direct β-catenin into cytoplasmic and nuclear pools (Piedra et al. 2001). Phosphorylation driven β-catenin release (from membrane) is counter balanced by phosphatases (protein tyrosine phosphate 1B, PT1B) that dephosphorylate β-catenin at Tyr654 (Balsamo et al. 1996).

2.2 The cytoplasmic pool

Free β-catenin in the cytoplasm, when recognized by a multi-protein destruction complex, is first phosphorylated at S45 by casein kinase 1 alpha (CK1α) and then at S33, S37 and T41 by glycogen synthase kinase 3 beta (GSK3β) (as reviewed in Heuberger & Birchmeier 2010) (figure 1) and targeted for proteasome mediated degradation by β-TrCP, an E3-ubiquitin ligase (as reviewed in Shamos & Weis 2013). Surprisingly, not all cytoplasmic β-catenin undergoes degradation. A fraction of phosphorylated β-catenin localizes to centrosomes and regulates proper mitotic spindle establishment (Chilov et al. 2011; Kaplan et al. 2004) (figure 1). In the beginning of mitotic spindle formation, β-catenin participates in centrosome cohesion and dissociation and its depletion obstructs de novo formation of microtubules (Huang et al. 2007). During cell division, a centromeric serine/threonine-protein kinase NEK2 (NIMA-Related Kinase 2), regulates β-catenin stability at centrosomes (Bahmanyar et al. 2008). NEK2 phosphorylates β-catenin at GSK3β phosphorylation sites and competes with β-TrCP to block β-TrCP and β-catenin interaction leading to inhibition of β-catenin proteosomal degradation, which results in accumulation of phosphorylated β-catenin at centrosomes (Mbom et al. 2014). During interphase, phospho-β-catenin localizes mostly to the mother centriole; whereas localization is favoured towards daughter centriole during mitosis (Fuentesalba et al. 2008; Huang et al. 2007). Taken together, above findings suggest a role of phosphorylated β-catenin in centrosome and microtubule functioning. It is not clear how β-catenin mediated centromeric cohesion ensures proper spindle formation. Future studies on β-catenin interacting proteins located to centrosomes can give insight into its role in regulating mitotic spindles.
2.3 The nuclear pool

Due to compromised function of the destruction complex, β-catenin escapes degradation and translocates to the nucleus to participate in transcriptional reprogramming (Munemitsu et al. 1995; Iwao et al. 1998; Sparks et al. 1998) (figure 1). Nuclear translocation mechanism of β-catenin is not completely understood (as reviewed in Städeli et al. 2006). β-catenin cannot bind to the promoter(s) of its transcriptional targets, as it does not possess any DNA binding domain. It functions by binding to other DNA binding transcription factors (Xing et al. 2008) and dictates transcriptional activation of a plethora of genes (Behrens et al. 1996; Herbst et al. 2014). Many β-catenin transcriptional co-factors have been identified, which indicates complexity of β-catenin mediated transcription regulation. These mainly include proteins belonging to the T cell factor/lymphoid enhancer factor (TCF/LEF) family (as reviewed in Cadigan & Nusse 1997). Genes activated by the β-catenin-TCF complex regulate several biological processes ranging from cell proliferation to anti tumor immunity (Schuijers et al. 2014; Spranger et al. 2015). The consensus DNA binding motif of TCF has been derived based on several single base substitution studies (Tuupanen et al. 2009; Jason B. Wright 2010). Other non-canonical β-catenin partners include AP-1 (Naerri et al. 2005), Estrogen receptor alpha (Kouzmenko et al. 2004), Forkhead box protein G1/3/5a (Essers et al. 2005), HIF1-alpha (Kaidi et al. 2007), hB1F (Botrugno et al. 2004), Oct-4 (Zhang et al. 2013), p50 (Kim et al. 2005) and SOX-17 (Sinner et al. 2004). β-catenin also interacts with the androgen receptor and regulates expression of several genes (Truica et al. 2000; Yang et al. 2002). In colorectal cancer cells, β-catenin dependent transcription is inhibited by activation of vitamin D receptor to promote cell differentiation (Palmer et al. 2001; Shah et al. 2006). Similarly, retinoic acid receptor mediated inhibition of β-catenin signalling pathway is also validated (Easwaran et al. 1999). β-catenin functions as both activator and repressor of transcription. Using its C terminus, it brings transcription activating complexes to promoters of target genes to ensure efficient transcription (Hecht et al. 2000; Barker et al. 2001); whereas β-catenin mediated transcription repression mechanism is diverse and not well understood (as reviewed in Valenta et al. 2012). Recruitment of β-catenin to the promoter of E-cadherin in keratinocytes (Jamora et al. 2003) and p16INK4a in melanocytes (Delmas et al. 2007) represses their transcription.

The intestinal lumen epithelium undergoes rapid regeneration to maintain homeostasis. The lumen comprises sac like structures called crypts of Lieberkühn. Crypts harbour enterocytes, intestinal stem cells (ISCs) and paneth cells. Paneth cells play critical role in maintaining enterocyte and stem cell cohabitation (as reviewed in Clevers & Bevins 2013) whereas enterocyte functions mainly in nutrient absorption. ISCs divide in a highly controlled manner to replenish the lumen epithelium. Several studies have proposed a pivotal role for β-catenin signalling in functioning of ISCs. Nuclear β-catenin has been shown to localize near base of the intestinal crypts (and not in the apical region) (van de Wetering et al. 2002) and is essential for intestinal epithelium homeostasis (Fevr et al. 2007). Direct role of β-catenin in maintaining crypt homeostasis was shown in a study where Ephrin type-B receptor 3 (a β-catenin target) was found to be essential for proper allocation of paneth cells to the crypt bottom (Batlle et al. 2002).

Elevated nuclear levels of β-catenin are found in several cancers including colorectal (Cheah et al. 2002), adrenocortical (Gaujoux et al. 2011), endometrial (Scholten et al. 2003), glioblastoma (Liu et al. 2011), hair follicle (Doglioni et al. 2003) and hepatocellular
carcinoma (Liu et al. 2010). β-catenin exhibits heterogeneous distribution pattern in colorectal cancer; well differentiated cancer cells present at the centre of tumor possess membrane expression akin to normal colon epithelium whereas undifferentiated invasive fronts and stroma manifest nuclear expression (Brabletz et al. 1998; as reviewed in Fodde & Brabletz 2007). More importantly, β-catenin is associated with patient survival, however different studies report different direction of association. Nuclear stabilised β-catenin predicts poor (Li et al. 2013; Inagawa et al., 2002) as well as better prognosis (Hommura et al. 2002) in non-small cell lung carcinomas. Nuclear phospho β-catenin predicted better prognosis in colon cancer (Chung et al. 2001) whereas nuclear overexpressed β-catenin was associated with poor prognosis (Nazemalhosseini Mojarad et al. 2015; Kazem et al. 2014). The contradictory effect(s) of β-catenin on cancer prognosis is not surprising. Reduced expression is expected to de-stabilize Adherens junctions leading possibly to increase migration and invasion resulting in poor prognosis. In contrast, increased expression particularly in the nucleus is expected to promote tumorigenesis due to transcriptional activation of oncogenic targets, thus also potentially resulting in poor prognosis. In addition, different forms (phosphorylated vs non-phosphorylated) may have different effect on cancer prognosis. Moreover, its differential prognostication in different cancers may also stem from the presence/absence of cross-talk with other pathways/proteins.

2.3.1 Nuclear β-catenin and tumor proliferation and growth

Sustained cell proliferation is a hallmark of cancer cells (as reviewed in Hanahan & Weinberg 2011). Activation of β-catenin in mouse intestinal villi causes increased proliferation which in turn leads to adenomatous lesions and polyposis (Harada et al. 1999). β-catenin activation promotes cell proliferation through cell cycle progression (G1 to S) (as reviewed in Davidson & Niehrs 2010). Possibly, G1 progression takes place through upregulation of the β-catenin transcriptional target, c-Myc and cyclin D1 (He et al. 1998; Shuttman et al. 1999). c-Myc serves a dual function in G1 progression. It upregulates cyclin D1 (Daksis et al. 1994) while repressing p21 and p27 expression (Gartel et al. 2001; Yang et al. 2001). Other β-catenin transcriptional targets that induce tumor cell proliferation and growth are listed in Table 1. Small interfering RNA-mediated β-catenin knockdown restrains colon cancer cell growth in vitro and in vitro (Verma et al. 2003; Xu et al. 2010). In mouse model studies, constitutively active β-catenin was shown to promote tumor growth in prostate cancer (Pearson et al. 2009; Yu et al. 2011). Mutationally activated β-catenin causes pilomatricoma (tumor originating from hair matrix and hair germ) (Fuchs et al. 1999). Expression of constitutively active β-catenin provides growth enhancement to myeloma cells in vitro (Deksen et al. 2004). Nuclear stabilized β-catenin is associated with proliferation in hepatocellular carcinoma (Inagawa et al. 2002; Nhieu et al. 1999). The positive effect of β-catenin on tumor proliferation and growth is brought about by activation of a variety of other genes with different target genes being pivotal in different cancers. Though several previous studies had suggested possible cross-talk between Wnt/β-catenin and EGFR signalling pathways, the study by (Guturi et al. 2012) provided clinching evidence for β-catenin-mediated transcriptional activation of EGFR that further resulted in activation of cell proliferation in prostate cancers cells. The complex and multi-level cross-talk between these two pathways was also validated in Glioblastoma (as reviewed in Paul et al. 2013) as well as in epithelial tissue regeneration (as reviewed in Georgopoulos et al. 2014). Similarly, melanoma growth is brought about by Microphthalmia-associated transcription factor
(MITF), a β-catenin target (Widlund et al. 2002). Further, MITF was shown to directly interact with β-catenin resulting in transcriptional induction of specific genes involved in both melanocyte and melanoma development (Schepsky et al. 2006). Finally, more recent studies revealed an intricate relationship between Wnt signalling and oncogenic role of MITF in Melanocyte (and melanoma) development (Ploper et al. 2015). Under Wnt7A activation, β-catenin regulates ovarian tumor growth (Yoshioka et al. 2012) and proliferation (as reviewed in Arend et al. 2013). Further, β-catenin supports tumor growth by enhancing angiogenesis, through regulation of vascular endothelial growth factor (Mann et al. 1999). Interestingly, both Wnt signalling and oncogenic mutant KRAS were shown to transcriptionally activate VEGF in precursor colon cancer lesions (Zhang et al., 2001).

Similarly, β-catenin transcriptional activity could enhance VEGF expression in hepatocellular carcinoma (Qu et al. 2014).

2.3.2 Nuclear β-catenin and EMT and metastasis

EMT plays an essential role during cancer progression and metastasis (as reviewed in Hay 2005; and Thiery et al. 2009). β-catenin mediated EMT induction plays an important role in tumor progression in several cancers including squamous cell carcinoma (Taki et al. 2003) and CRC (Brabletz et al. 2005). A key feature of EMT is replacement of E-cadherin by N-cadherin at the cell membrane (as reviewed in Zeisberg et al. 2009) which in turn perturbs cadherin-catenin interaction; β-catenin can then be released from the cell membrane and translocate to nucleus, if it escapes cytosolic degradation (as reviewed in Heuberger & Birchmeier 2010). Twist (a basic helix-loop-helix transcription factor), a transcriptional target of β-catenin (Howe et al. 2003), downregulates E-cadherin expression to facilitate tumor metastasis (as reviewed in Kang & Massague 2004). Snail1, Snail2 (Slug) and ZEB1 are additional E-cadherin transcriptional repressors which function in a β-catenin dependent manner (Conacci-Sorrell et al. 2003; as reviewed in Barrallo-Gimeno & Nieto 2005; Sanchez-Tillo et al. 2011). Several additional transcriptional targets of β-catenin have been identified that regulate EMT, cell migration and tumor metastasis (Table 1). p68 RNA helicase mediated nuclear translocation of β-catenin stimulates EMT in cultured cancer cells (Yang et al. 2006; as reviewed in He 2006). Suppression of β-catenin signalling is also associated with differentiation of colonic epithelial cells (Mariadason et al. 2001). Epithelial cell polarity was restored in a colorectal cancer cell line upon repression of β-catenin mediated gene transactivation (Naishiro et al. 2001). Despite increasing evidence of role of epithelial-mesenchymal transition in cancer metastasis, methods of treating same remains limited. Better understanding of exact role of β-catenin in this important process may help in development of therapy targeted against metastasis.

2.3.3 Nuclear β-catenin and tumour-initiating cells (TICs) and tumor micro-environment (TME)

TICs (previously called as cancer stem cells) are a sub-population of tumor cells having capacity of self-renewal (to generate tumor) and to differentiate into any cell type within tumor to promote growth and metastasis (as reviewed in Jordan et al. 2006; and Reya et al. 2001). β-catenin mediated signalling is associated with TICs in multiple malignancies, including Breast (Lamb et al. 2013), Colon (Shenoy et al. 2012), Gastric (Yong et al. 2016), and Glioblastoma (Kaur et al. 2013). Several β-catenin target genes, listed in Table 1, serve as prominent markers for TICs. PCNA-associated factor (PAF) activates β-catenin
transcriptional target (Jung et al. 2013) and regulates cell plasticity to maintain breast cancer cell stemness (Wang et al. 2016). β-catenin mediates drug resistance in Mixed Lineage Leukemia TICs (J. Yeung et al. 2010) and its loss impairs renewal of Chronic myelogenous leukemia stem cells (Zhao et al. 2007). c-Kit mediated β-catenin regulation enhances self-renewal and expansion of TICs to promote ovarian tumorigenesis (Chau et al. 2013). β-catenin was shown to maintain ovarian cancer spheroid culture and promote tumor formation via ALDH1A1 (Condello et al. 2015). In hepatocellular carcinoma, TGFβ activated β-catenin induces an early liver progenitor phenotype and promotes tumor recurrence (Zulehner et al. 2010). WNT16B mediated β-catenin signaling in prostate TME promotes prostate cancer cell survival and tumor progression (Sun et al. 2012); whereas miR-320 mediated β-catenin downregulation, supresses stem like properties (Hsieh et al. 2013). By regulating Telomerase (TERT, a direct target of β-catenin) expression, β-catenin may help in maintaining telomere length of TICs, thus promoting their maintenance (Hoffmeyer et al. 2012).

Generally, cancer cells are surrounded by stromal cells which include various immune cells, endothelial cells, fibroblasts, and mesenchymal stem cells (MSCs) (as reviewed in Friedl & Alexander 2011). Interplay between cancer and stromal cells creates the TME, which plays an essential role during all stages of tumorigenesis (as reviewed in Mbeunkui & Johann 2009). In esophageal cancer, tumor associated fibroblasts secrete Wnt2 into the tumor milieu, to promote β-catenin mediated signalling in adjacent malignant cells for tumor progression (Fu et al. 2011). Ectopic expression of β-catenin in breast cancer associated fibroblasts increases proliferation of co-cultured cancer cells (Verghese et al. 2011).

Overall, these studies underscore the role of β-catenin signalling in regulating TICs and TME to promote tumor growth. With the advent of organoid cultures, upcoming studies may provide more insight into role of β-catenin in regulating TME and its interplay in tumorigenesis.

### 2.3.4 Nuclear β-catenin and chromosomal instability

Chromosomal instability (CIN) is an early event in tumorigenesis (Nowak et al. 2002; as reviewed in Grady 2004) characterised by structural abnormalities in chromosomes and/or change in their dosage (aneuploidy) (Geigl et al. 2008; as reviewed in Pikor et al. 2013). CIN is an important hallmark of cancer (as reviewed in Negrini et al. 2010; Lengauer et al. 1997). Accumulation of genomic alterations is suggested to be an important cause of clonal heterogeneity (as reviewed in Sieber et al. 2003 and Janssen & Medema 2013). Several studies have highlighted nuclear transcriptionally active β-catenin to be an important driver if CIN. Wnt-signalling mediated aberrant activation of β-catenin causes CIN in colon cancer (Hadjihannas et al. 2006; Mårtensson et al. 2007) and T-cell lymphomas (Dose et al. 2014). In cancer cells, the β-catenin target AXIN2 modulates mitotic spindle check point by interacting with spindle check point regulator Polo-like kinase 1 (PLK1) to induce gain or loss of chromosomes, thus generating CIN (as reviewed in Hadjihannas & Behrens 2006). In gastric tumors, anaphase bridge index (an indicator of CIN) is in concordance with nuclear β-catenin expression; β-catenin signalling deregulates G2/M progression and promotes escape from mitotic arrest and apoptosis to generate CIN (Aoki et al. 2007). The β-catenin target c-Myc generates CIN via ROS mediated DNA damage and promotes aneuploidy through its targets Mad2 and BubR1 (as reviewed in Prochownik & Li 2016 and Schwartzman et al. 2013).
2010). Activated c-Myc also induces chromosomal structural aberrations like fusion of centromere and telomere, chromosome breaks, deletion and translocation to trigger CIN via c-myc activation (as reviewed in Kuzyk & Mai 2014). Altogether, β-catenin appears to promote CIN by modulating target gene expression. Though the importance of CIN in tumor progression in solid tumors is well established, the role of β-catenin in generating CIN is not understood in detail.

2.3.5 Nuclear β-catenin and cancer cell metabolism

A cancer cell reprograms its metabolism in order to facilitate growth and survival (as reviewed in Pavlova et al. 2016; Warburg 1956). The most well studied metabolic process in cancer cell is the manifestation of increased aerobic glycolysis (‘Warburg effect’) (as reviewed in Vander Heiden et al. 2009). The Warburg effect describes an oft noted observation in a cancer cell wherein generation of energy in the form of ATP is achieved predominantly using aerobic glycolysis followed by fermentation of lactic acid in the cytosol rather than through the ‘time-consuming’ TCA cycle in the mitochondria; the latter being the norm in normal cells. Several β-catenin transcriptional targets that regulate cancer cell metabolism are listed in Table 1. β-catenin along with its co-activator Pyruvate kinase M2 (PKM2) induces myc expression, which in turn promotes the Warburg effect (Yang et al. 2012). In breast cancer, β-catenin regulates mitochondrial respiration and glucose metabolism by inducing expression of pyruvate carboxylase (a key enzyme of anaplerosis) and by suppressing cytochrome C oxidase (an integral enzyme of electron transport chain, essential for oxidative phosphorylation) activity (Lee et al. 2012). β-catenin transcriptional target Pyruvate dehydrogenase kinase 1 promotes aerobic glycolysis in colon cancer cells (Pate et al. 2014). β-catenin targets are also involved in fatty acid and glutamine metabolism in ovarian adenocarcinoma (Sherwood 2015). There is also evidence of β-catenin itself being regulated by oxidative stress. In breast cancer cells, reactive oxygen species (ROS) were shown to promote β-catenin-FOXO3a interaction resulting in decrease of TICs and tumorigenicity (Dong et al. 2013). Furthermore, specific nutrients are reported to modulate β-catenin signalling in cancer cells; glucose enhances β-catenin signalling through its acetylation (Chocarro-Calvo et al. 2013). Taken together, above studies give insight into the different modes of β-catenin signalling integration with cancer cell metabolism. Future studies on β-catenin signalling will shed light on the manipulation process of altered tumor cell metabolism to support cancer progression.

2.3.5 Nuclear β-catenin and programmed cell death and autophagy

Evading programmed cell death (apoptosis) is an essential process during malignant transformation (Hanahan & Weinberg 2011). Nuclear β-catenin increases cancer cell proliferation and protects it against apoptosis (He et al. 1998; Tetsu et al. 1999). In a rat myocardial infarction model, β-catenin was shown to promote anti-apoptotic signalling via induction of VGEF, BCL-2 and BIRC5 (Kaga et al. 2006). β-catenin signalling has been shown to block cytochrome c release to inhibit apoptosis in colorectal cancer cells (Chen et al. 2001). In contrast, knockdown of β-catenin disrupts mitochondrial membrane potential to induce apoptosis (Hsu et al. 2014). In metastatic melanoma cell lines, downregulation of β-catenin induces apoptosis via reduction in the expression of anti-apoptotic genes (Bcl-2, Mcl-1) and the cell cycle regulator Cyclin D1 (Sinnberg et al. 2011). However, contradictory studies also exist, where β-catenin was shown to promote apoptosis. In melanoma cells, Wnt
mediated β-catenin activation promotes TRAIL-dependent apoptosis through increased pro-apoptotic (BCL2L11 and BBC3) and decreased anti-apoptotic (MCL1) protein levels (Zimmerman et al. 2013). Activation of β-catenin promotes apoptosis in hematopoietic progenitor cells through the intrinsic mitochondrial pathway (Ming et al. 2012). Upon overexpression, β-catenin induces apoptosis in colon cancer cell lines, independent of its LEF1 (transcriptional cofactor) dependent function (Kim et al. 2000; Lu et al. 2012). The β-catenin target c-myc enhances mitochondrial dependent apoptotic signal (as reviewed in Hoffman & Liebermann 2008) and induces apoptosis (as reviewed in McMahon 2014) through activation of cdc25A in growth factor depleted cells (Galaktionov et al. 1996). It is not surprising that β-catenin, being an oncogene, exhibits a contradictory role in regulating apoptosis. It is a normal cellular defence mechanism to induce apoptosis upon aberrant, untimely or very high level activation of an oncogene, as is already known for other oncogenes such as c-Myc (as reviewed in Hoffman and Liebermann, 2008, oncogene); this could explain the positive effect of β-catenin on apoptosis. However, transcriptional targets of β-catenin have specific roles in inhibiting apoptosis in a context dependent manner. Thus, β-catenin functions as both pro-apoptotic and anti-apoptotic factor.

Autophagy is a catabolic process that maintains cellular homeostasis and is known to both promote and suppress tumor growth (as reviewed in Yang et al. 2011 and Mathew et al. 2007). β-catenin appears to negatively regulate autophagy in cancer. It regulates basal and stress induced autophagy by suppressing autophagosome (a key structure in autophagy) formation. Whereas, in a negative feedback-loop, autophagy induces proteasome independent β-catenin degradation to inhibit its signalling (Petherick et al. 2013). Suppression of β-catenin pathway induces autophagy in breast TICs (Fu et al. 2014) as well as in prostate cancer cells (Lin et al. 2015). The role of β-catenin in cellular autophagy has not been explored in detail thus far. Given the importance of autophagy in tumorigenesis and the preliminary results from work done during past few years, the link between β-catenin and autophagy is worth exploring.

2.3.6 Nuclear β-catenin and anti-tumor immunity

β-catenin signalling plays a significant role in immune cell biology (as reviewed in Staal et al. 2008). Growing literature supports role of tumor intrinsic β-catenin signalling in anti-tumor immunity (Spranger et al. 2015; Sweis et al. 2016). Intrinsic β-catenin signalling positively correlates with T-cell exclusion in cutaneous melanoma (Spranger et al. 2015). In addition, constitutively active β-catenin excludes T-cell infiltration response against tumor antigens in mouse model of melanoma (Spranger et al. 2015). Overexpression of β-catenin inhibits melanoma-specific cytotoxic T cell mediated IFN-γ production in an IL-10 dependent manner and suppresses dendritic cell activity in vivo (Yaguchi et al. 2012). These studies suggest the involvement of β-catenin signalling in suppression of anti-tumor immunoresponse. In muscle-invasive urothelial bladder cancer, β-catenin signalling is present in non-T-cell-inflamed tumors, an immunotherapy resistant type (Sveis et al. 2016). Further studies on tumor intrinsic β-catenin signalling and immune response interplay may provide more insight into this emerging and exciting area of β-catenin biology.

4. Conclusion

In this review, we have summarized the important role of nucleus-restricted β-catenin in tumor biology. Nuclear β-catenin appears to function in a dosage and context dependent
manner. As a multifunctional protein, it regulates distinct biological processes by activating transcription of a plethora of genes in a context dependent manner, based on interaction with a wide range of partners. Our understanding of role of β-catenin in cancer has improved considerably. As described above, it appears to regulate several pro-tumorigenic processes within the cell including cell cycle, apoptosis, metabolism, etc. The importance of β-catenin signalling is not only limited to tumor cells, but studies have suggested a possible role in microenvironment and tumor-immunity as well. However, knowledge of its coordination across complex function(s) is still rudimentary. Therefore, a more meticulous perception of molecular mechanisms is needed to understand the full potential of the varied β-catenin dependent impact on tumorigenesis.

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Figure legends:

Figure 1. Diagrammatic representation of various cellular functions of β-catenin.
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Immune cell/Cancer associated fibroblasts

Extracellular space

Cytoplasm

Nucleus

β-Catenin  LRP5/6  Frizzled  E-Cadherin  Axin2  APC

GSK3β  Centrosome  Wnt ligand  Phosphorylation

Exosome  Poly-ubiquitination  TCF  Non-canonical partner
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<td>Zeb1</td>
<td>EMT</td>
<td>Colorectal</td>
<td>(Sanchez-Tillo et al. 2011)</td>
</tr>
<tr>
<td>BOP1, CKS2 and NFIL3</td>
<td>EMT</td>
<td>Colorectal</td>
<td>(Qi et al. 2015)</td>
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<tr>
<td>HEF1</td>
<td>Migration</td>
<td>Colorectal</td>
<td>(Li et al. 2011)</td>
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<tr>
<td>MMP2</td>
<td>EMT</td>
<td>T-cells</td>
<td>(Wu et al. 2007)</td>
</tr>
<tr>
<td>MMP9</td>
<td>Migration</td>
<td>Neural stem cells</td>
<td>(Ingraham et al. 2011)</td>
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<td>MMP7</td>
<td>Migration</td>
<td>Colorectal</td>
<td>(Brabletz et al. 1999)</td>
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<tr>
<td>MMP26</td>
<td>EMT</td>
<td>Epithelial cancers</td>
<td>(Marchenko et al. 2002)</td>
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<tr>
<td>S100A4</td>
<td>Metastasis</td>
<td>Colorectal</td>
<td>(Stein et al. 2006)</td>
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<tr>
<td>Tenascin C</td>
<td>EMT</td>
<td>Colorectal</td>
<td>(Beiter et al. 2005)</td>
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<td>CST1 and EDN3</td>
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<td>Ovarian</td>
<td>(Schwartz et al. 2003)</td>
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<td>ALDH1A1</td>
<td>TIC marker</td>
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<td>(Huang et al. 2009)</td>
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<td>CD24</td>
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<td>(Yeung et al. 2010)</td>
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<td>CD44</td>
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<td>Colorectal</td>
<td>(Zeilstra et al. 2008)</td>
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<td>Lgr5</td>
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<td>(Kemper et al. 2012)</td>
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<td>TERT</td>
<td>TIC marker</td>
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<td>(Hoffmeyer et al. 2012)</td>
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<td>Chromosomal Instability</td>
<td>Colorectal</td>
<td>(Hadjihannas et al. 2006)</td>
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<td>PDK1</td>
<td>Metabolism</td>
<td>Colorectal</td>
<td>(Pate et al. 2014)</td>
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<td>VGEF, BCL-2 and BIRC5</td>
<td>Anti-apoptotic</td>
<td>Rat model</td>
<td>(Kaga et al. 2006)</td>
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<td>MCL1, BCL2L11 and BBC3</td>
<td>Anti-apoptotic</td>
<td>Melanoma</td>
<td>(Zimmerman et al. 2013)</td>
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EMT, Epithelial-Mesenchymal transition; TIC, Tumor initiating cells