Minireview

Tumour–stroma interactions in colorectal cancer: converging on β-catenin activation and cancer stemness

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Sporadic cases of colorectal cancer are primarily initiated by gene mutations in members of the canonical Wnt pathway, ultimately resulting in β-catenin stabilisation. Nevertheless, cells displaying nuclear β-catenin accumulation are nonrandomly distributed throughout the tumour mass and preferentially localise along the invasive front where parenchymal cells are in direct contact with the stromal microenvironment. Here, we discuss the putative role played by stromal cell types in regulating β-catenin intracellular accumulation in a paracrine fashion. As such, the tumour microenvironment is likely to maintain the cancer stem cell phenotype in a subset of cells, thus mediating invasion and metastasis.

Keywords: colorectal cancer; β-catenin; cancer stem cells; stroma; fibroblast

In the intestinal tissue architecture, epithelial cells lining the luminal surface are tightly regulated to ensure homeostasis. Epithelial cell renewal is fuelled by an adult stem cell compartment localised at the bottom of the crypt (Potten and Loeffler, 1990; Barker et al., 2007). As cells migrate upwards, after a transient proliferative phase, the epithelium differentiates into specialised cell types including absorptive enterocytes, mucus secreting goblet cells, and enteroendocrine cells. This migratory process with concomitant differentiation is finalised when cells reach the top of the crypt where they are exfoliated into the lumen upon apoptosis. In the upper gastrointestinal tract, Paneth cells represent an exception as they move downwards while differentiating. Overall, cell renewal, proliferation, and differentiation are coupled to positional localisation along the crypt-to-villus axis. In fact, this positional regulation of proliferation and differentiation can be correlated to gradients in the degree of activity of several signalling pathways known to govern stemness and differentiation, including Wnt/β-catenin, bone morphogenetic protein (BMP), Notch, and transforming growth factor-β (TGFβ) (Crosnier et al., 2006). The mesenchyme plays a complex role in the positional gradient of signalling ligand availability. Intestinal subepithelial myofibroblasts are specialised stromal cells that form a continuous sheet directly localised underneath the mucosa. These myofibroblasts contribute to epithelial cell function by providing mechanical support and secreting key signalling ligands. Thus, the intimate interaction between the parenchyma and mesenchyme ensures proper tissue function, balancing cell renewal, and differentiation.

Activation of canonical Wnt signalling characterises the base of the intestinal crypt as shown by the nuclear β-catenin localisation crypt cells (Figure 1). Note that Paneth cells in the small intestine also show nuclear β-catenin accumulation as previously reported (Van Es et al., 2003). Moving upwards along the crypt-to-villus axis, terminal differentiation coincides with the more restricted membrane-bound β-catenin localisation and its absence in the nucleus and cytosol (Figure 1). Recently, it has been reported that the BMP antagonists, gremlin 1, gremlin 2, and chordin-like 1, are selectively expressed by crypt-based myofibroblasts and smooth muscle cells (Kosinski et al., 2007). Moreover, Gremlin 1 was shown to activate Wnt/β-catenin signalling in normal rat intestinal epithelial cells, thus indicating that stromal-derived factors regulate Wnt/β-catenin-signalling activity in the intestinal stem cell niche.

Upon constitutive activation of the Wnt signalling route, intestinal homeostasis is disturbed, paving the way for pathogenesis. Indeed, the vast majority of sporadic colorectal cancer cases is caused by constitutive Wnt activation due to mutations in either the APC tumour suppressor or the β-catenin (CTNNB1) oncogene (Fodde et al., 2001). Loss of APC function leads to destabilisation of the ‘destruction complex’, a multiprotein complex encompassing three scaffold proteins, APC, Axin1, and Axin2 (conductin), and two kinases, glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1). The complex binds and phosphorylates β-catenin at serine and threonine residues, thus targeting it for ubiquitinisation and proteolytic degradation. In contrast, oncogenic mutations in β-catenin render it resistant to Ser/Thr phosphorylation and proteolytic degradation. Upon its cytoplasmic stabilisation and subsequent nuclear translocation, β-catenin binds to members of the TCF/LEF family of transcription factors, thus modulating expression of a broad range of target genes (http://www.stanford.edu/~rnusse/pathways/targets.html). Although the presence of these initiating mutations predicts nuclear β-catenin accumulation throughout the tumour mass, heterogeneous intracellular distributions are observed within primary colorectal tumours and their metastases. In particular, tumour cells located at the invasive front and those migrating into the adjacent stromal tissue are earmarked by nuclear β-catenin accumulation (Brabletz et al., 1998, 2001; Figure 1). Hence, different levels of Wnt/β-catenin-signalling activity are likely to reflect tumour heterogeneity and to underlie
malignant behaviour (Gaspar and Fodde 2004; Brabletz et al., 2005a).

Several intrinsic (cell autonomous and/or autocrine) and extrinsic (paracrine, derived from the tumour microenvironment) factors may explain the observed heterogeneity of Wnt/β-catenin-signalling activity within the tumour mass (Fodde and Brabletz, 2007). Here, we discuss stromal factors likely to play a role in the heterogeneous β-catenin intracellular localisation and signalling activity in tumour cells. As such, the tumour microenvironment may drive tumour growth and even selectively support a subset of tumour cells, the cancer stem cells (CSCs), thus actively contributing to malignancy.

STROMAL CELLS AFFECTING TUMOUR GROWTH, NUCLEAR β-CATENIN ACCUMULATION, AND CANCER STEMNESS

As stated above, stromal regulation significantly contributes to the preservation of normal tissue architecture. Myofibroblasts, for example, are not only tightly associated with the intestinal epithelium thus ensuring homeostasis through reciprocal interactions, but are also essential for wound healing upon tissue injury, when they are transiently enriched and activated (Gabbiani, 2003). Expression of α-smooth muscle actin (α-SMA) characterises these myofibroblasts and underlies contractile force tension that facilitates healing. Myofibroblasts produce a variety of growth factors, prostaglandins, cytokines, chemokines, and extracellular matrix components that facilitate tissue repair and survival. Myofibroblasts arise through a multitude of processes, including transdifferentiation of resident fibroblasts, epithelial-to-mesenchymal transition (EMT) of parenchymal cells, recruitment, and differentiation of pericytes (progenitor cells localised at vascular sinuses), and from bone marrow-derived circulating immature fibrocytes (Desmouliere et al., 2004). Upon completion of the wound healing process, myofibroblasts revert back to their dormant state. In fact, tumorigenesis has been described as a condition comparable to an open wound of chronic nature (Dvorak, 1986). Accordingly, fibroblasts are one of the most abundant cell types in the stromal microenvironment associated with solid tumours (Adegboyega et al., 2002; De Wever and Mareel, 2003; Kalluri and Zeisberg, 2006). In response to the malignant lesion within the epithelial compartment, stromal fibroblasts become morphologically 'activated'. Similar to the wound-healing process, an activated response of the tumour stroma may initially be triggered in an attempt to restore tissue homeostasis. However,
as the tumour progresses, the microenvironment is more likely to become a 'partner in crime' in malignancy. A subset of tumour stromal fibroblasts, also referred to as cancer-associated fibroblasts (CAFs), peritumoral fibroblasts, reactive stromal fibroblasts, tumour-associated fibroblasts, or myofibroblasts, acquire distinct phenotypic characteristics. These cells share many of the properties of normal myofibroblasts such as α-SMA expression and increased production of growth factors, and of a variety of matrix remodelling proteases, which facilitate migration and invasion of the tumour cells (De Wever and Mareel, 2003; Desmouliere et al., 2004; Mukaratirta et al., 2005). In view of their specific growth promoting effects, CAFs are primary candidates for locally modulating Wnt/β-catenin signalling, resulting in heterogeneous phenotype cells that can homing to and form secondary outgrowths. This may be of particular importance in view of the CSC hypothesis, which predicts that only a subset of tumour cells, displaying stem cell characteristics, will be successful in invading surrounding tissues and forming metastases in secondary organs. We have previously postulated that cancer stemness may be conferred by specific levels of β-catenin activation in colorectal cancer (Brabletz et al., 2005a; Fodde and Brabletz, 2007). Stromal cells may play a significant role by providing a supportive microenvironment that constitutes a 'congenial soil' for tumour cells, becoming a 'partner in crime' in malignancy. A subset of tumour cells, sampled in colorectal cancer cohorts (Pages et al., 2006), have been reported to support tumour growth, including tumour-associated fibroblasts (CAFs), peritumoral fibroblasts, reactive stromal fibroblasts, also referred to as cancer-associated fibroblasts (CAFs), and adoptive immune cells may confer both tumour-growth-supporting and adoptive immune effects due to their immune suppressive function to mediate self-tolerance, prevent autoimmunity, and enable the presence of a commensal bacterial flora in the intestine (Powrie, 2004). Regulatory T cells have been reported to be increased in peripheral blood and infiltrating lymphocytes among colorectal cancer patients (Ling et al., 2007). The increased presence of these Tregs sets the stage for immune evasion by tumour cells. Several other tumour-infiltrating immune cells have been reported to support tumour growth, including tumour-associated immune cells and adipocytes modulating tumour growth

Besides stromal fibroblasts, the tumour microenvironment consists of a variety of cell types capable of modulating tumour growth and possibly cancer stemness. Tumour-infiltrating innate and adaptive immune cells may confer both tumour-growth-promoting and -inhibiting effects (de Visser et al., 2006). Intuitively, increased infiltration of T cells should correlate with improved tumour clearance and prognosis, as recently reported in colorectal cancer cohorts (Pages et al., 2005; Clarke et al., 2006; Galon et al., 2006). However, a subtype of T cells, termed regulatory T cells (Tregs), are likely to exert tumour-growth-promoting effects due to their immune suppressive function to mediate self-tolerance, prevent autoimmunity, and enable the presence of a commensal bacterial flora in the intestine (Powrie, 2004). Regulatory T cells have been reported to be increased in peripheral blood and infiltrating lymphocytes among colorectal cancer patients (Ling et al., 2007). The increased presence of these Tregs sets the stage for immune evasion by tumour cells. Several other tumour-infiltrating immune cells have been reported to support tumour growth, including tumour-associated immune cells and adipocytes modulating tumour growth

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showed that immature myeloid cells (iMCs) are recruited from the bone marrow to the tumour invasion front of compound heterozygous cis-Apc+/Smad4−/−/Smad4+/− mice with invasive intestinal adenocarcinoma. These CD34+ iMCs promote tumour growth by expression of the matrix metalloproteinases, MMP9 and MMP2, and the CC-chemokine receptor 1 (CCR1), and migrate to the invasive front. CX-1, a highly metastatic human colorectal cancer cell line, has shown that tumour cells express TNFα and form an inflammatory microenvironment. Atget et al. (2004) reported that activation of the transcription factor NFκB (nuclear factor-κB), a key mediator of inflammation, has a critical role in the development of tumours resulting from chronic inflammation or exogenous mutagens, induced by exposure to dextran sulphate sodium salt and Azoxymethane (AOM). Moreover, genetic ablation of MyD88, a signalling adaptor of Toll-like receptors in the innate immune system, was shown to reduce mortality due to intestinal tumorigenesis in Apc+/Smad4−/− mice (Rakoff-Nahum and Medzhitov, 2007). In fact, Auguste et al. (2007) have shown that liver metastases formation coincides with an inflammatory, TNFα-mediated, host organ response. This inflammatory reaction upregulates cell adhesion molecules in the liver stromal microvasculature and supports tumour cell arrest and extravasation in a metastatic mouse model induced by intrasplenic injection of the highly metastatic human colorectal cancer cell line, CX-1. Finally, adipocytes have also been reported to promote proliferation of colon cancer cells (Amemori et al., 2007).

Overall, these data indicate that the host inflammatory response is a key mediator of tumour survival, extravasation, and metastasis formation. In fact, a broad spectrum of diverse cell types from within the tumour microenvironment may contribute to the modulation of β-catenin activation and cancer stemness, thus promoting intestinal tumour progression and even initiation. In this regard, tumour progression and cancer stemness may significantly be determined by a context-dependent modulation of β-catenin activity in colorectal cancer.

**HYPOXIA- AND OXIDATIVE STRESS-INDUCED SELECTION OF TUMOUR CELLS DISPLAYING NUCLEAR β-CATENIN ACCUMULATION AND CANCER STEMNESS**

Besides the specific stromal cell types, other factors from within the tumour microenvironment are likely to play significant roles in promoting cancer stemness and malignant behaviour through β-catenin nuclear accumulation and signalling. Hypoxia underlies progressive tumour growth in the majority of solid tumours (Sullivan and Graham, 2007). During tumour growth, certain areas are exposed to reduced oxygen tension due to a disturbed microcirculation and inadequate blood supply. Hypoxic conditions are often found in the invasive front of colorectal carcinomas in association with stabilisation of the HIF1α (hypoxia-inducible factor-1α) transcription factor (Sivridis et al., 2005). HIF1α stabilisation results in transcriptional regulation of a variety of target genes, including the proangiogenic factors vascular endothelial growth factor and PDGF (Koukourakis et al., 2006). In fact, Cleven et al. (2007), showed that expression of HIF1α in the stromal compartment correlates with poor prognosis in colorectal cancer. Moreover, loss of MUTYH function, a DNA glycosylase involved in base excision repair caused by oxidative stress, results in increased susceptibility to spontaneous and oxidative stress-induced (by the oxidative reagent KbrO3) intestinal tumorigenesis (Sakamoto et al., 2007). These data indicate that hypoxia and oxidative stress play a pivotal role in colorectal cancer progression. Notably, Kaidi et al. (2007) reported that HIF1α binds directly to β-catenin in the nucleus, thus linking hypoxia-induced cellular changes to β-catenin activation.

**BIOLOGICAL EFFECTS OF NUCLEAR β-CATENIN ACCUMULATION**

The nuclear accumulation of β-catenin observed in colorectal cancer cells distributed along the invasive front may not only reflect a specific level of canonical Wnt activity but also indicate the activation of additional signalling pathways. It has been shown that in the nucleus, β-catenin binds to a broad spectrum of transcription factors other than TCF and LEF and modulates a plethora of downstream targets possibly contributing to cancer stemness and malignancy (Table 1). As stated above, hypoxia induces stabilisation of HIF1α and its interaction with β-catenin, thereby competing with TCF/LEF1 transcription factors for β-catenin binding in colorectal cancer cells (Kaidi et al., 2007). Stabilisation and binding of HIF1α to β-catenin results in inhibition of Wnt reporter activity, induction of cell-cycle arrest, survival, and cellular adaptation, and is likely to contribute to the malignant and invasive behaviour of tumour cells exposed to reduced oxygen tension. Similarly, oxidative stress stimulates β-catenin binding to the Forkhead box O transcription factors, inducing cell-cycle arrest and survival (Essers et al., 2005).

Through interaction with Smads (including Smad1, Smad3, and Smad4), β-catenin may also coregulate a subset of common TGFβ, BMP, and Wnt target genes (Nishita et al., 2000; Hussein et al., 2003; Chakladar et al., 2005; Hu and Rosenblum, 2005). Transforming growth factor-β and BMP signalling are known to be important regulators of epithelial cell function. Synergism among TGFβ, BMP, and Wnt signalling pathways may represent a significant determinant of malignant behaviour in tumour cells. β-Catenin binding to Smad7, an inhibitory molecule induced upon TGFβ pathway activation as part of a negative feedback loop, has also been reported to be rate limiting for TGFβ-induced apoptosis (Edlund et al., 2005) and induces proteolytic degradation of β-catenin (Han et al., 2006). When c-Jun, a stress- and growth factor-induced transcription factor, is recruited to the TCF/LEF1/β-catenin complex, synergistic effects on intestinal tumorigenesis are observed (Nateri et al., 2005). Also, gut-specific deletion of c-Jun decreased tumour multiplicity and increased life span in the ApcEtim mouse model for intestinal cancer. Recently, both c-Jun and its known homodimerisation partner, c-Fos, were reported to bind directly to β-catenin (Toualbi et al., 2007). Therefore, binding of β-catenin to different interaction partners in the nucleus may direct both TCF/LEF1-dependent and -independent transcriptional regulation.

Hence, in view of this observed promiscuity for nuclear transcription factors (Table 1), β-catenin is likely to represent a central node where different signals converge and are subsequently coordinated to regulate tissue homeostasis under physiological conditions and cancer stemness in the context of tumour–stroma interactions. Because the putative β-catenin interaction partners are themselves regulated by extracellular stimuli, it is plausible that the subsequent effects on β-catenin activation and possibly cancer stemness are modulated in a context-dependent manner. In fact, β-catenin has been reported to interact directly with several growth factor receptors, including EGFR (epidermal growth factor receptor, ErbB1), Met (the receptor for HGF), TGFRII (the receptor that is activated upon TGF stimulation), and KIT (the receptor for stem cell factor; Hoschuetzky et al., 1994; Monga et al., 2002; Tian and Phillips, 2002; Kajiguchi et al., 2008). These interactions result in β-catenin Tyr phosphorylation, stabilisation, and increased transcriptional activity.
| Protein                                      | Interaction and biological significance                                                                                                                                                                                                 | Reference                                                                 |
|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| 14-3-3-z                                   | Binds β-catenin and stabilises it, Akt dependent                                                                                                                                                                                                                                               | Tian et al (2004)                                                          |
| Akt                                         | Phosphorylates β-catenin at S552 and increases cytoplasmic pool, increases binding to 14-3-3, increases transcriptional activity                                                                                                                                                             | Fang et al (2007)                                                          |
| AR (androgen receptor)                      | Binds β-catenin, augments AR activity, inhibits TCF-dependent transcription                                                                                                                                                                                                                  | Yang et al (2002)                                                          |
| API1 and Smad3/4                            | Complex with β-catenin and TCFLEF1 to activate gastrin target gene                                                                                                                                                                                                                           | Chakladar et al (2005)                                                    |
| BCL9 (Legless)                              | Binds β-catenin and TCFLEF1, increases transcription, Pygopus dependent                                                                                                                                                                                                                   | Kramps et al (2002)                                                       |
| B9L/BCL9-2 (BCL9-like protein)              | Binds β-catenin, increases transcription, induces EMT                                                                                                                                                                                                                                     | Adachi et al (2004); Brembeck et al (2004)                                |
| Brp-1 (chromatin remodelling factor)        | Binds β-catenin, increases transcription                                                                                                                                                                                                                                                      | Barker et al (2001)                                                       |
| c-Jun (phosphorylated)                      | Binds β-catenin and TCFLEF1 in a JNK- and β-catenin-dependent manner, knockdown decreases tumour multiplicity in APC-/- mice                                                                                                                                                               | Nateri et al (2005); Toulalbi et al (2007)                                |
| c-Fos                                       | Binds β-catenin, decreases transcription, induces EMT                                                                                                                                                                                                                                       | Toulalbi et al (2007)                                                    |
| CARMI (coactivator-associated arginine methyltransferase) | Binds β-catenin, increases transcription                                                                                                                                                                                                                                                     | Koh et al (2002)                                                          |
| CBP (CREB-binding protein)                  | Binds β-catenin, increases transcription, regulates pre-mRNA splicing                                                                                                                                                                                                                        | Takekawa and Moon (2000)                                                  |
| cdx1 and cdx2 (homeodomain transcription factors) | Decreases β-catenin Tyr phosphorylation, decreases transcription, induces E-cadherin adhesion                                                                                                                                                                                               | Guo et al (2004); Ezaki et al (2007)                                      |
| Chibby (nuclear protein)                    | Binds β-catenin, increases transcription, regulates pre-mRNA splicing                                                                                                                                                                                                                      | Takekawa and Moon (2000)                                                  |
| CREB (cyclic AMP response element binding protein) | Binds β-catenin, increases expression of WISP-1 (Wnt-1-induced secreted protein 1)                                                                                                                                                                                                        | Xu et al (2000)                                                           |
| cul4B (Cullin4B/E3-ubiquitin ligase)         | Binds β-catenin and induces its proteolytic degradation                                                                                                                                                                                                                                         | Tripathi et al (2007)                                                    |
| Duplin (axis duplication inhibitor)          | Binds β-catenin in nucleus and inhibits transcription                                                                                                                                                                                                                                         | Sakamoto et al (2000)                                                    |
| EBP50 (PDZ-containing protein)              | Binds β-catenin, increases transcription                                                                                                                                                                                                                                                      | Shibata et al (2003)                                                    |
| emerin (nuclear membrane protein)           | Binds β-catenin resulting in its cytoplasmic retention and decreased transcriptional activity                                                                                                                                                                                                    | Markiewicz et al (2006)                                                  |
| Erα (estrogen receptor)                     | Binds β-catenin, increases transcription                                                                                                                                                                                                                                                      | Kozmienko et al (2004)                                                   |
| ezh2 (enhancer of zeste homolog 2, polycomb group protein) | Binds β-catenin and Erα, regulates pre-mRNA splicing                                                                                                                | Shi et al (2007)                                                          |
| FHL2 (four and a half of LIM-only protein 2, LIM coactivator) | Binds β-catenin, increases transcription                                                                                                                                                                                                                                                       | Wei et al (2003); Martin et al (2002)                                     |
| FOXO (insulin- and oxidative stress signaling-induced transcription factor) | Binds β-catenin resulting in increased FOXO target gene transcription                                                                                                                                                                                                                     | Essers et al (2005)                                                      |
| FUS (fusion/translocated in liposarcoma, TLS) | Binds and increases β-catenin, regulates pre-mRNA splicing                                                                                                                                                                                                                                     | Sato et al (2005)                                                        |
| GRIP1 (p160 coactivator of AR)             | Binds β-catenin, augments AR activity                                                                                                                                                                                                                                                        | Li et al (2004)                                                           |
| Groucho/TLE (transcriptional repressor)     | Binds β-catenin and is subsequently displaced from TCF/LEF1                                                                                                                                                                                                                                  | Daniels and Weis (2005)                                                  |
| HIF-1α (hypoxy inducible factor)            | Binds β-catenin, competes with TCFLEF1, induces survival and cellular adaptation to hypoxia                                                                                                                                                                                                                 | Kaidi et al (2007)                                                      |
| hARD1 (human arrest defective 1, acetyltransferase) | Binds and acetylates β-catenin, increases transcription                                                                                                                                                                                                                                    | Lim et al (2006)                                                          |
| IκBα (inhibitor of MyoD Family a)          | Binds β-catenin, relieving IκBα-mediated gene repression                                                                                                                                                                                                                                      | Pan et al (2005)                                                         |
| ICAT (inhibitor of β-catenin and TCF-4)     | Binds β-catenin, represses transcription                                                                                                                                                                                                                                                     | Tago et al (2000)                                                        |
| IKKα (IκB kinase α)                         | Binds β-catenin, inhibits its ubiquitination, increases transcription                                                                                                                                                                                                                         | Lamberti et al (2001)                                                    |
| IKKβ (IκB kinase β)                         | Binds β-catenin, inhibits transcription                                                                                                                                                                                                                                                       | Lamberti et al (2001)                                                    |
| LRH-1 (orphan nuclear receptor)            | Binds β-catenin, induces proliferation                                                                                                                                                                                                                                                       | Botrugno et al (2004)                                                    |
| LZF52 (leucine zipper tumor suppressor 2)   | Binds β-catenin, inhibits transcription                                                                                                                                                                                                                                                       | Thysen et al (2006)                                                    |
| Mediator (MED12 subunit)                    | Binds β-catenin, increases transcription                                                                                                                                                                                                                                                      | Kim et al (2006)                                                         |
| Mif1 (microphthalmia-associated transcription factor) | Binds β-catenin and competes with TCFLEF1 to activate mif target genes, important for melanocyte development                                                                                                                   | Schepsky et al (2006)                                                   |
| NfκB, p50 subunit                           | Binds β-catenin, decreases NFκB DNA binding, transactivation activity, regulates TNFα-induced CRP (C-reactive protein, acute-phase response protein) expression                                                                                                                                 | Deng et al (2002); Sun et al (2005); Choi et al (2007)                    |
| Nurr1 (orphan nuclear receptor)             | Binds β-catenin in ES cells, upregulates Nanog                                                                                                                                                                                                                                                  | Katagawa et al (2007)                                                   |
| oct3/4                                      | Binds β-catenin upon PDGF-induced Tyr phosphorylation of p68, increases transcription and EMT                                                                                                                                                                                                  | Takao et al (2007); Yang et al (2006)                                     |
| p68 (DEAD box family of RNA helicases)      | Binds and acetylates β-catenin, increases transcription                                                                                                                                                                                                                                         | Sun et al (2000); Hecht et al (2000)                                      |
| p300                                        | Binds β-catenin, increases transcription, Pogopyus dependent                                                                                                                                                                                                                                   | Mosemann et al (2006)                                                   |
| Parafibromin (component of polymerase-associated factor 1 (PAF1) complex) | Binds β-catenin, increases transcription, Pogopyus dependent                                                                                                                                                                                                                                 |                                             |
| Pin1 (prolyl isomerase)                     | Binds β-catenin, displaces it from APC, stabilises it and induces transcription, overexpressed in human tumours                                                                                                                                                                                 | Ryo et al (2001)                                                         |
| Pbx2 (bicoid-related transcription factor)  | Induced by Wnt/Dvl/β-catenin, increases transcription                                                                                                                                                                                                                                          | Kioussi et al (2002)                                                    |
| Poxin52 (nuclear protein)                   | Binds β-catenin, increases transcription, Pogopyus dependent                                                                                                                                                                                                                                   | Bauer et al (1998)                                                      |
### Table 1 (Continued)

| Protein | Interaction and biological significance | Reference |
|---------|----------------------------------------|-----------|
| PPARγ (peroxisome proliferator-activated receptor) | Binds β-catenin, decreases membrane bound and cytoplasmic fraction | Sharma et al (2004); Liu et al (2004) |
| PRA1 (Prenylated Rab acceptor 1) | Binds β-catenin, inhibits transcription | Kim et al (2006) |
| prol1 (Proprietor of P1) | Binds β-catenin, activates expression of lineage-determining transcription factor Pit1, represses the lineage-inhibiting transcription factor Hesx1 via TLE/Reptin/HDAC1 corepressor complexes | Olson et al (2006) |
| Pygopus | Complexes with β-catenin and TCF/LEF1 in a Legless-dependent manner | Kramps et al (2002); Thompson et al (2002) |
| RanBP3 (Ran binding protein 3) | Cofactor of chromosome region maintenance 1 (CRM1)-mediated nuclear export | Hendriksen et al (2005) |
| RAR (retinoic acid receptor) | Binds β-catenin in retinoid-dependent manner; competes for binding with TCF/LEF1 | Easwaran et al (1999) |
| Reptin52 (homologue of pontin52) | Binds β-catenin and Pontin52, inhibits transcription | Bauer et al (2003) |
| RXR (retinoid X receptor) | Binds β-catenin, targets it for proteolytic degradation | Xiao et al (2003) |
| SHP-1 (protein-tyrosine phosphatase) | Binds β-catenin and inhibits transcription in intestinal epithelial cells | Duchesne et al (2003) |
| Smad1 | Complexes with β-catenin and TCF/LEF1 resulting in increased myc expression | Hu and Rosenblum (2005) |
| Smad3 | Binds β-catenin and TCF/LEF1 | Labbe et al (2000); Jian et al (2006) |
| Smad4 | Interacts with TCF/LEF1 (strong) and β-catenin (weak), coregulates TGFβ/Wnt common target genes | Nishita et al (2000) |
| Smad7 | Binds β-catenin, important for TGFβ-induced apoptosis and targets β-catenin for proteolytic degradation | Edlund et al (2005); Han et al (2006) |
| Sox4 | Binds and stabilises β-catenin and TCF/LEF1 | Sinner et al (2007) |
| Sox9 | Binds β-catenin and targets it for degradation | Akiyama et al (2004) |
| Sox17 | Binds β-catenin and TCF/LEF1, targets it for proteolytic degradation and transcription | Sinner et al (2007) |
| TAK1 (MAPKKK) and NLK (Nemo-like kinase) | Interact with and phosphorylate TCF/LEF1 and β-catenin, inhibit DNA binding capacity | Ishitani et al (1999) |
| Teashirt (zinc finger protein) | Binds to armidillo (Drosophila homologue of β-catenin), activated by Wingless | Gallet et al (1998) |
| TCFs | Binds β-catenin | Molenaar et al (1996); Song and Gelmann (2005) |
| TIF2/GRIP1 (transcriptional intermediary factor-2/glucocorticoid receptor interacting protein-1) | Binds β-catenin and increases binding affinity to AR | |
| TOPO IIα (DNA topoisomerase IIα) | Binds β-catenin, increases transcription | Saito et al (2005); Huang et al (2007) |
| VDR (vitamin D receptor) | Binds β-catenin in a vitamin D-dependent manner; competes for binding with TCF/LEF1 | Pålmer et al (2001) |
| XSox1(7αβ/β and Xsox3 | Binds β-catenin and inhibit transcription | Zom et al (1999) |

EMT = epithelial-to-mesenchymal transition; FOXO = Forkhead box O; PDGF = platelet-derived growth factor; TGFβ = transforming growth factor-β. Proteins previously shown to directly bind to β-catenin in the nucleus are listed in alphabetical order together with a brief description and corresponding literature references. Please note that the list is admittedly incomplete as only direct binding partners have been included. Many other proteins have been excluded that do not directly bind to β-catenin but participate to its many complexes and may yet significantly affect its function.

### CONCLUSIONS

Despite the clear genetic prerequisite for mutations in downstream components of the Wnt/β-catenin-signalling pathway that result in its constitutive activation, heterogeneous intratumour expression and subcellular localisation of β-catenin is commonly observed in colorectal cancer. Tumour cells located at the invasive front display increased nuclear β-catenin accumulation, suggesting that this nonrandom intracellular distribution earmarks and underlies tumour heterogeneity and malignancy. Therefore, it has been postulated that β-catenin may play a significant role in cancer stemness, driving invasion and metastasis. β-Catenin regulation is already known to be important during homeostasis, as Wnt/β-catenin signalling governs several adult stem cell niches, including the intestinal crypt. The tumour microenvironment may play a central role in the malignant transformation of tumour cells by locally modifying β-catenin activity at the primary tumour site as well as preparing secondary organ sites for metastatic growth. Individual 'stromal signatures', that is, characteristic of stromal cell function, inflammation, and other stress-induced responses, may determine disease progression, responsiveness to different (adjuvant) therapeutic strategies, and, thus, prognosis and survival for colorectal cancer patients. Stromal cells have even been suggested as possible targets for tailor-made therapeutic interventions for intestinal tumorigenesis, rather than parenchymal cells. Here, we propose that the functional characterisation of additional β-catenin-binding partners in these alleged CSCs will improve our understanding of malignancy and invasion and open future perspectives for a metastasis-free survival to colorectal cancer patients.

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