News & Views

THE EMBO JOURNAL

Cancer: leaping the E-cadherin hurdle

George T Chen & Marian L Waterman

Aberrant activation of the Wnt signaling pathway is a common cause of colon cancer and other tumor types, accomplishing many of the hallmarks of cancer including sustained proliferative signaling, replicative immortality, reprogrammed metabolism, angiogenesis, and invasion. Yet, the dominant mutation that leads to chronic Wnt signaling in colon cancer is quite different from the spectrum of mutations that activate Wnt signaling in other tumor types. In this issue of *The EMBO Journal*, Huels *et al* (2015) focus on the influential role E-cadherin plays in shaping these differences.

See also: **DJ Huels *et al*** (September 2015)

Wnt-based oncogenesis derives from chronic recruitment of β-catenin to Wnt target genes and their consequent overexpression. β-catenin is the key signaling component that links Wnt:Frizzled:Lrp receptor events at the plasma membrane with gene regulatory activities in the nucleus. It does so by translocating through nuclear pores to interact with TCF/LEF transcription factors that are bound to Wnt response elements in enhancers and promoters of target genes. Normal cells keep the Wnt/β-catenin transcriptome suppressed, in part, through rapid capture of β-catenin by the "destruction complex." The destruction complex is a large, multi-subunit entity that uses a tumor suppressor subunit, adenomatous polyposis coli (*APC*), to coordinate CK-1α and GSK3 phosphorylation of serine/threonine residues in the N-terminus of β-catenin, marking the protein for ubiquitin-dependent degradation. Hence, in non-signaling normal cells, β-catenin is markedly unstable and virtually undetectable in the cytoplasm or nucleus. The one safe harbor from capture and degradation are E-cadherin-based adherens junctions at the plasma membrane of epithelial cells. In fact, β-catenin was first identified as a component of adhesion—its binding to the cytoplasmic tail of cadherin being essential for α-catenin-dependent linkage to the actin cytoskeleton (McCrea *et al*, 1991; reviewed in Fagotto, 2013). Multiple studies have probed for possible connections between the pool of β-catenin associated with E-cadherin and the pool that translocates into the nucleus, asking whether these are separate pools or whether they are functionally linked and coordinately regulated for Wnt signaling. According to the experimental outcomes, E-cadherin sequesters β-catenin and limits Wnt signaling, it provides a reserve pool to ready cells for signaling, and it plays a direct nuclear role in modulating Wnt signaling (reviewed in McCrea *et al*, 2015). However, these studies used cell lines or developmental model systems to probe the E-cadherin:β-catenin connection; few studies have used mouse models of cancer.

Huels *et al* (2015) used mice in which destruction complex components such as APC or GSK3α/β kinase isoforms could be deleted or the N-terminal phospho-destruction coding sequences in exon 3 of β-catenin could be excised (β-catenin^{ex3}—a so-called activating mutation because the protein is unable to be phosphorylated/ubiquitinated and therefore accumulates). The authors compared proliferation phenotypes in the intestine, Wnt independence using crypt organoid culture systems, and the rate of tumor occurrence. Homozygous loss of APC or GSKα/β kinases, or homozygous β-catenin$^{ex3/ex3}$ expression all led to the same hallmarks of oncogenic Wnt signaling in the small intestine and the colon (referred to in the study as the crypt progenitor phenotype). In contrast, heterozygous expression of β-catenin$^{ex3/+}$ did not produce transformation phenotypes until much later and then only weakly in the small intestine [a phenotype also observed by other groups (Leedham *et al*, 2013)]. If β-catenin$^{ex3/+}$ heterozygosity was introduced directly in crypt stem cells, CPC lesions occurred more aggressively in the small intestine, but the colon remained seemingly clear. These data were interpreted to mean that the barrier to tumor development in the colon requires an increased "dose" of nuclear β-catenin—achieved only by complete loss of the destruction complex or homozygous β-catenin$^{ex3/ex3}$ expression. The question is—what constitutes the barrier, the threshold? What is the hurdle to cell transformation?

The answer lies with E-cadherin. Using proximity ligation assays to quantitatively measure E-cadherin:β-catenin complexes in individual intestinal epithelial cells, much greater levels were detected on the cell surface of colon epithelium than the small intestine. Reduction of E-cadherin (via removal of one *Cdh1* allele) dramatically increased the oncogenicity of heterozygous β-catenin$^{ex3/+}$, not only in the small intestine, but importantly, also in the colon—a phenotype similar to homozygous APC loss or homozygous β-catenin$^{ex3/ex3}$. Thus, lowering the levels of E-cadherin created less of a hurdle for β-catenin$^{ex3/+}$ to transform colon epithelial cells. While it has been known for some time that overexpression of E-cadherin in colon cancer cell lines interferes with Wnt signaling (Orsulic *et al*, 1999; Gottardi *et al*, 2001), the Huels and Ridgway study is an important *in vivo* demonstration that E-cadherin plays a tumor suppressor-like role directly linked to β-catenin, and as such, the actual levels of E-cadherin determine the β-catenin threshold for cell transformation.

Do these observations have relevance to human cancer? One might wonder because

University of California, Irvine, Irvine, CA, USA. E-mail: marian.waterman@uci.edu
DOI 10.15252/embj.201592757 | Published online 17 August 2015

mice and humans have different patterns of tumorigenesis in the gut. Mice tend to develop tumors in the small intestine not the colon, whereas the reverse is true in humans. Clearly, other factors distinguish mice from humans, including environmental factors such as the gut microbiome or other molecular and morphological differences. Nevertheless, this study focuses the spotlight on the influence that E-cadherin can wield—meaning, anything that changes the level or activity of E-cadherin will affect the threshold that β-catenin must exceed in order to cause transformation, a relationship that might track with the spectrum of mutations that emerge in different types of tumors. Huels *et al* (2015) offer up a dramatic example. Solid pseudopapillary carcinoma (SPT) of the pancreas is a type of cancer wherein more than 90% of the tumors have a heterozygous activating mutation in β-catenin—a mutation functionally equivalent to the weak β-catenin$^{ex3/+}$ mutation. Interestingly, SPT cells are poorly adhesive and have low levels of E-cadherin (Tang *et al*, 2007). Using the proximity ligation assay to take a closer look at E-cadherin:β-catenin complexes on SPT cell membranes, the authors observed that in every case, these complexes were significantly diminished (approximately fivefold) compared to normal cells. Thus, Wnt activation in SPT is achieved by a combination of β-catenin stabilization and E-cadherin downregulation. Downregulation of E-cadherin is one way to lower the threshold, but sequence analysis of cancer genomes reveals multiple tumor types with significant rates of E-cadherin mutations and/or mutations that activate β-catenin, a pattern that is especially evident in endometrial and breast tumors (Fig 1).

In principle, any genetic mutation that increases the levels of β-catenin in the nucleus should cause aberrant activation of the Wnt transcriptome and increased pressure for cell transformation. Yet, cancer genome studies show that colon cancer is unique in that nearly all of the Wnt-activating mutations are inactivation mutations of APC, not activating mutations in β-catenin

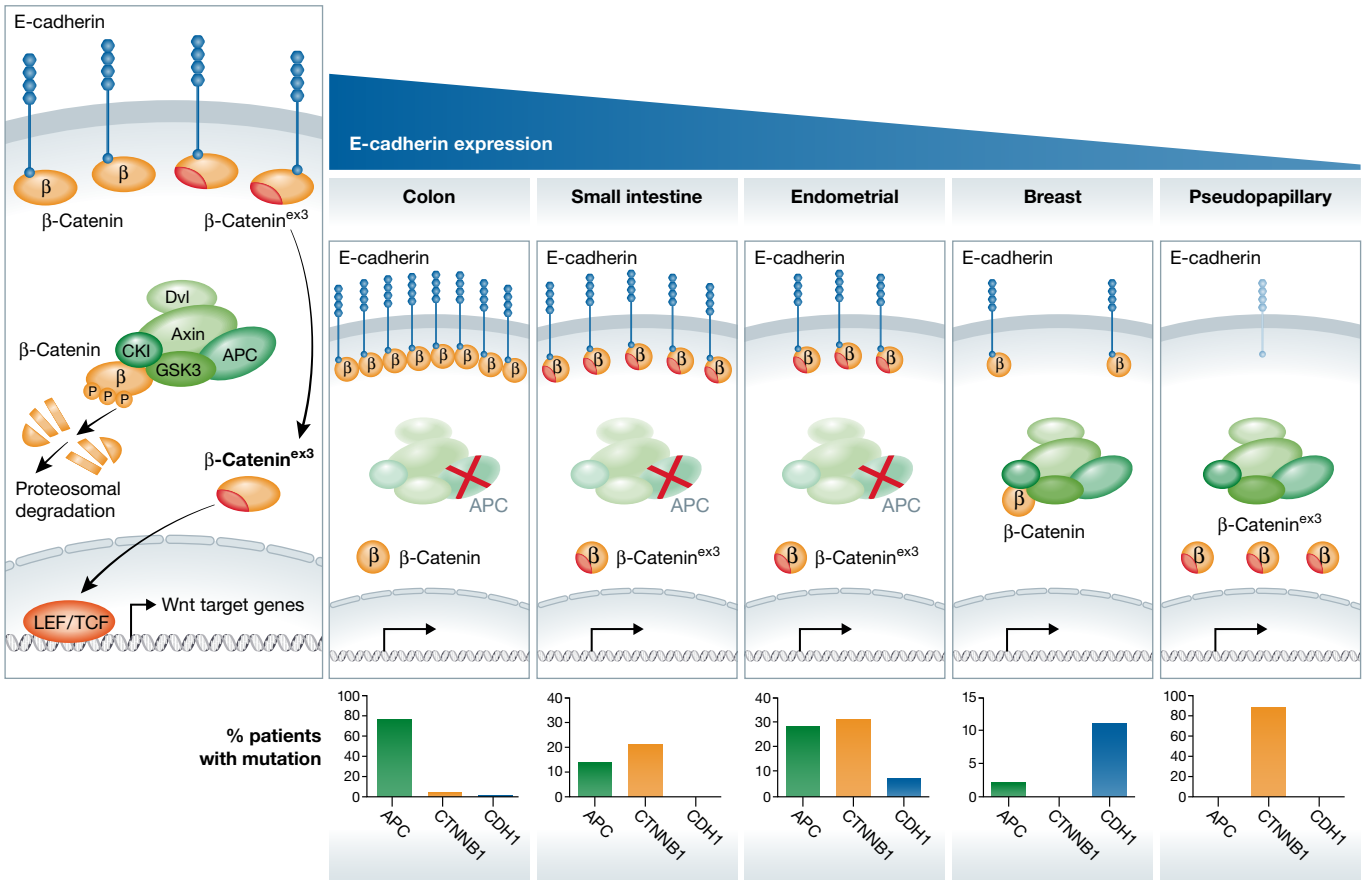

Figure 1. E-cadherin levels and β-catenin availability in tumors.
In wild-type cells, β-catenin is captured and phosphorylated by the destruction complex, a multi-subunit structure that contains the tumor suppressor adenomatous polyposis coli (APC). Phosphorylation of N-terminal serine and threonine residues of β-catenin marks it for ubiquitination and proteasomal degradation. Deletion or missense mutations of N-terminal coding sequences in exon 3 (a stabilizing, activating mutation denoted here as β-catenin^{ex3}), prevent the destruction complex from degrading β-catenin, allowing accumulated protein to translocate to the nucleus to drive expression of Wnt target genes. The adherens junction protein E-cadherin can sequester both wild-type and mutant β-catenin, opposing nuclear translocation and Wnt target gene activation. Results from the Huels, Ridgway *et al* study show that in cancers with high levels of E-cadherin, homozygous loss of function of APC and/or homozygous β-catenin^{ex3} mutations are needed to exceed the threshold established by the E-cadherin sink and drive pathway activation. In other cancers where E-cadherin is mutated (e.g., breast cancer) or the protein is downregulated (e.g., solid pseudopapillary tumors in the pancreas, SPT), heterozygous β-catenin^{ex3} mutations are likely sufficient to exceed the E-cadherin threshold. Patient mutation rates were collected from: Forbes *et al*, 2014; Lawrence *et al*, 2014; Yeang *et al*, 2008.

or inactivating mutations in E-cadherin (Fig 1). Clearly, mutant APC provides advantages to intestinal crypt cells that are not as relevant to Wnt-active cancer cells in other tissues. It is tempting to speculate that loss of APC might be better at supplying sufficient β-catenin to surmount the colon-specific threshold set by E-cadherin. However, if that alone were the reason, APC mutations would be appearing or dominating more often in cancers where thresholds are lower. Instead, the dominance of APC mutations in colon cancer and the concept of an E-cadherin threshold might be relevant in light of the unique architecture of intestinal crypts. A recent study of stem cell dynamics revealed that loss of one *APC* allele in a crypt stem cell provides a measurable survival advantage over neighboring wild-type stem cells, leading to a small but significant rate of "fixation" or "retention" and subsequent clonal establishment of mutant stem cells in the crypt (Vermeulen *et al*, 2013). A second study in human colon found that loss of APC increased the rate of crypt fission 10-fold, fission being the duplication and splintering off of a new daughter crypt (Baker *et al*, 2014). Since fission of a mutant crypt is likely to be the formation event of an adenoma, this event and the fitness fixation of mutant stem cells are key features of colon cancer initiation. Could E-cadherin play a role in these dynamics? Is it possible that maintenance of E-cadherin is important for mutant stem cell survival or the fission process even though it sets a high bar for activation of the Wnt pathway? Indeed, unlike other cancers where E-cadherin levels are downregulated or the gene is mutated, E-cadherin is wild type and robust expression levels are maintained in early-stage colon cancer. Downregulation of E-cadherin typically appears at later stages and mostly in regions such as the invasion front or in metastatic break-away cells (Brabletz *et al*, 2001). It would be interesting to compare the stem cell dynamics of crypts in the mouse models used in the Huels and Ridgway study—asking whether or not mutations in β-catenin$^{ex3/+}$ and/or *Cdh1* afford the same level of fitness to stem cells and crypts as APC mutations. Whatever the outcome, this *in vivo* study clearly highlights the importance of E-cadherin in shaping the genetic profile of tumors and it underscores how vital it is to fully understand its regulation and functions.

References

Baker AM, Cereser B, Melton S, Fletcher AG, Rodriguez-Justo M, Tadrous PJ, Humphries A, Elia G, McDonal SA, Wright NA, Simons BC, Jansen M, Graham TA (2014) Quantification of crypt and stem cell evolution in the normal and neoplastic human colon. *Cell Rep* 8: 940 – 947

Brabletz T, Jung A, Reu S, Porzner M, Hlubek F, Kunz-Schughart LA, Knuechel R, Kirchner T (2001) Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci USA* 98: 10356 – 10361

Fagotto F (2013) Looking beyond the Wnt pathway for the deep nature of β-catenin. *EMBO Rep* 14: 422 – 433

Forbes SA, Beare D, Gunasekaran P, Leung K, Bindal N, Boutselakis H, Ding M, Bamford S, Cole C, Ward S, Kok CY, Jia M, De T, Teague JW, Stratton MR, McDermott U, Campbell PJ (2015) COSMIC: exploring the world's knowledge of somatic mutations in human cancer. *Nucleic Acids Res* 43: D805 – D811

Gottardi CJ, Wong E, Gumbiner BM (2001) E-cadherin suppresses cellular transformation by inhibiting β-catenin signaling in an adhesion-independent manner. *J Cell Biol* 153: 1049 – 1059

Huels DJ, Ridgway RA, Radulescu S, Leushacke M, Campbell AD, Biswas S, Leedham S, Serra S, Chetty R, Moreaux G, Parry L, Matthews J, Song F, Hedley A, Kalna G, Ceteci F, Reed KR, Meniel VS, Maguire A, Doyle B *et al* (2015) E-cadherin can limit the transforming properties of activating β-catenin mutations. *EMBO J* 34: 2321 – 2333

Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, Meyerson M, Gabriel SB, Lander ES, Getz G (2014) Discovery and saturation analysis of cancer genes across 21 tumour types. *Nature* 505: 495 – 501

Leedham SJ, Rodenas-Cuadrado P, Howarth K, Lewis A, Mallappa S, Segditsas S, Tomlinson IPM (2013) A basal gradient of Wnt and stem-cell number influences regional tumour distribution in human and mouse intestinal tracts. *Gut* 62: 83 – 93

McCrea P, Turck C, Gumbiner BM (1991) A homolog of the armadillo protein in Drosophila (plakoglobin) associated with E-cadherin. *Science* 254: 1359 – 1361

McCrea P, Maher M, Gottardi C (2015) Nuclear signaling from cadherin adhesion complexes. *Curr Top Dev Biol* 112: 1 – 60

Orsulic S, Huber O, Aberle H, Arnold S, Kemler R (1999) E-cadherin binding prevents beta-catenin nuclear localization and beta-catenin/LEF-1-mediated transactivation. *J Cell Sci* 112: 1237 – 1245

Tang WW, Stelter AA, French S, Shen S, Qiu S, Venegas R, Wen J, Wang HQ, Xie J (2007) Loss of cell-adhesion molecule complexes in solid pseudopapillary tumor of pancreas. *Mod Pathol* 20: 509 – 513

Vermeulen L, Morrissey E, van der Heijden M, Nicholson AM, Sottoriva A, Buczacki S, Kemp R, Tavare S, Winton DJ (2013) Defining stem cell dynamics in models of intestinal tumor initiation. *Science* 342: 995 – 998

Yeang CH, McCormick F, Levine A (2008) Combinatorial patterns of somatic gene mutations in cancer. *FASEB J* 22: 2605 – 2622