CANCER

Wnt/β-Catenin and MAPK Signaling: Allies and Enemies in Different Battlefields

Daniele Guardavaccaro and Hans Clevers*

Two papers published in *Science Signaling* reveal extensive crosstalk between Wnt/β-catenin and mitogen-activated protein kinase (MAPK) signaling in cancer. Although both studies describe previously unknown links between these two signaling pathways, the relationship between Wnt/β-catenin and MAPK signaling depends on the specific cellular context. Indeed, in melanoma, hyperactivated MAPK signaling down-regulates the Wnt/β-catenin signal transduction cascade, thereby establishing a negative crosstalk between the two signaling pathways. In contrast, in colorectal cancer, stimulation of the Wnt/β-catenin pathway leads to activation of the MAPK pathway through Ras stabilization, representing an example of positive crosstalk. Moreover, activation of Wnt/β-catenin signaling has context-dependent functions that trigger opposing effects on tumor growth. In melanoma, aberrant activation of Wnt/β-catenin signaling may have anti-oncogenic functions by promoting programmed cell death; by contrast, in the intestine, Wnt/β-catenin signaling drives malignant transformation. Thus, there is no single correct way to target the Wnt/β-catenin pathway for all cancers.

Signal transduction cascades transduce extracellular signals into cellular responses. Modifications of proto-oncogenic signaling pathways contribute to the acquisition of cancer traits. When two well-studied proto-oncogenic pathways—the Wnt/β-catenin pathway and the mitogen-activated protein kinase (MAPK) pathway—are deregulated, they play prominent roles in cancer pathogenesis.

Wnt/β-catenin signaling controls normal embryonic development and regulates self-renewal in various adult tissues [reviewed in (*1*, *2*)]. This signal transduction pathway is initiated by the binding of Wnt ligands to receptor complexes that include the transmembrane receptors of the Frizzled family. In the absence of Wnt ligands, the "destruction complex," which includes a scaffolding core composed of two tumor suppressor proteins, Axin and Adenomatous Polyposis Coli (APC), enables two protein kinases—glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1)—to phosphorylate β-catenin, triggering its ubiquitylation by the $SCF^{\beta TrCP}$ ubiquitin ligase and subsequent degradation by the proteasome. When a Wnt ligand interacts with a Frizzled receptor, a complicated sequence of events leads to the inactivation of the destruction complex and accumulation of β-catenin. The latter interacts with transcription factors of the TCF/LEF1 family to regulate the expression of Wnt target genes, which regulate various biological processes (such as proliferation, fate determination, and differentiation) in a tissue-specific fashion. Mutations in the genes encoding β-catenin, Axin, or APC, which occur in diverse human tumors, lead to accumulation of β-catenin and transactivation of TCF/LEF1 transcription factors.

The MAPK cascade, which consists of three sequentially activated protein kinases [reviewed in (*3*–*5*)], is activated by the GTP-bound Ras protein, a small guanosine triphosphatase (GTPase) for which oncogenic mutations have been reported in one-third of all human cancers [reviewed in (*6*)]. Ras activates the kinase Raf and its close relative BRAF, both of which phosphorylate and activate the second kinase in the cascade, MEK. In turn, MEK phosphorylates and activates the third kinase, ERK. The latter phosphorylates a number of cytoplasmic substrates (such as regulators of translation) as well as nuclear targets (such as transcription factors). Constitutive activation of the Ras-Raf-MEK-ERK signaling cascade has been reported in a large number of human cancers, where it induces growth-promoting genes, regulates cell adhesion and migration, and causes changes in cell shape.

Because the Wnt/β-catenin signaling pathway is oncogenic in most cancers, yet anti-oncogenic and a marker for good prognosis in melanoma, Biechele and colleagues (*7*) searched for previously unknown regulators of Wnt/β-catenin signaling in melanoma. They used an RNA interference–based genetic screen for protein kinases, whose silencing synergized with Wnt in stimulating β-catenin transcriptional activity in a human melanoma cell line. Using this screen, they unexpectedly found that silencing of BRAF enhanced β-catenin activity in the presence of Wnt. This finding is particularly interesting because oncogenic mutations in BRAF occur in about 60% of human melanomas (*8*–*10*). The most frequent BRAF mutation in melanoma is $Val^{600} \rightarrow Glu$ (V600E). BRAF(V600E) induces cell proliferation, survival, and invasion in melanoma cells and stimulates tumor angiogenesis. Remarkably, small-molecule compounds such as PLX-4720 that selectively inhibit oncogenic BRAF display potent activity against melanoma (*11*, *12*). Biechele and colleagues found that PLX-4720 treatment of a melanoma cell line that harbors the BRAF(V600E) mutation enhanced Wnt/β-catenin signaling. The oncogenic activity of BRAF(V600E) in melanoma results mostly from the activation of the MAPK pathway. Accordingly, Biechele *et al*. found that inhibition of MEK, the kinase phosphorylated and stimulated by BRAF, also enhanced Wnt/β-catenin signaling.

To examine the underlying molecular mechanism responsible for the inhibitory effect of BRAF on the Wnt/β-catenin signaling pathway, the authors focused on two components of the β-catenin destruction complex, Axin and GSK3. In melanoma cells, inhibition of BRAF combined with Wnt/β-catenin activation caused proteasome-dependent degradation of Axin, inhibition of GSK3, and consequent dephosphorylation of β-catenin on the GSK3 target sites.

The relationship between the Wnt/β-catenin and MAPK signaling pathways was also investigated by Jeong and colleagues (*13*), although within the different context of intestinal tumorigenesis. In contrast to the opposing effects of Wnt/β-catenin and MAPK signaling found in melanoma, in colon cancer, Wnt/β-catenin and MAPK signaling synergized in a combined effort to promote transformation. Jeong *et al*. found that the same negative regulators of Wnt/β-catenin signaling analyzed by Biechele and colleagues, the destruction complex com-

Hubrecht Institute-KNAW and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT Utrecht, Netherlands.

***Corresponding author. E-mail: h.clevers@hubrecht.eu**

ponents Axin and GSK3, along with the E3 ubiquitin ligase $SCF^{\beta TrCP}$, conspired to trigger not only the proteolysis of β-catenin but also the degradation of Ras. As a result, in colon cancer, aberrant activation of Wnt/β-catenin signaling caused the stabilization of both β-catenin and Ras through the inactivation of the destruction complex.

The effects on tumor growth of the activation of Wnt/β-catenin signaling in the two different contexts, melanoma and colon cancer, are also strikingly different. In melanoma, stimulation of Wnt/β-catenin signaling synergized with the ability of BRAF inhibitors to reduce tumor cell size. This reduction in tumor size was due to apoptotic cell death mediated by Bim, a proapoptotic BH3-only protein. In addition, Wnt/ β-catenin signaling seemed to act as a key determinant of the apoptotic response induced by inhibition of BRAF and the downstream MAPK cascade. In contrast, in the intestine, Jeong *et al*. found that aberrant activation of Wnt/β-catenin signaling promotes cancer development by triggering stabilization of both β-catenin and Ras, the latter of which activates the MAPK cascade.

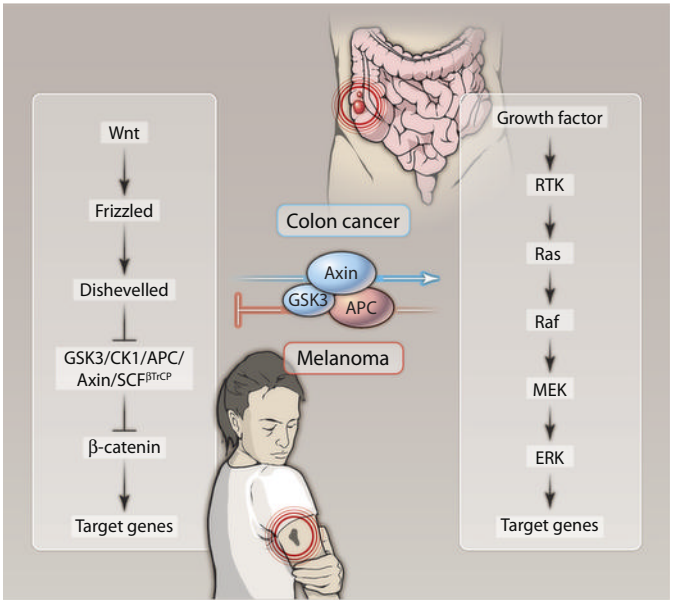

Fig. 1. Crosstalk between Wnt/β-catenin and MAPK signaling in cancer. In colon cancer, stimulation of Wnt/β-catenin signaling (**left**) inhibits the activity of the destruction complex, indicated by the scaffold proteins Axin and APC and the kinase GSK3, leading to Ras stabilization and consequent activation of the downstream MAPK signaling cascade (**right**). In melanoma, increased MAPK signaling stabilizes Axin, which inhibits Wnt signaling. RTK, receptor tyrosine kinase.

A point raised by both studies is that Axin seems to play a decisive role in controlling the interplay between Wnt/β-catenin and MAPK signaling. This is particularly intriguing because Axin is the concentration-limiting component of the destruction complex (*14*). Although additional mechanisms cannot currently be ruled out, these findings support a model in which Axin is the key node for coordination between Wnt/β-catenin and MAPK signaling (Fig. 1). Additional work is required to identify the molecular mechanisms controlling Axin turnover. In this regard, it would be interesting to determine whether RNF146, a RING-domain E3 ubiquitin ligase implicated in targeting Axin for degradation (*15*), plays a role in the interplay between the Wnt/β-catenin and MAPK signaling cascades.

Both studies have strong implications for the development of combination therapies for melanoma and colon cancer. Indeed, the paper by Biechele and colleagues suggests that melanoma patients might benefit from simultaneous inhibition of BRAF(V600E) and the downstream MAPK cascade, along with the activation of Wnt/β-catenin signaling. In contrast, in intestinal tumorigenesis, inhibition of both MAPK signaling and Wnt/β-catenin signaling may be most effective.

References

1. H. Clevers, Wnt/β-catenin signaling in development and disease. *Cell* **127**, 469–480 (2006).
2. P. Polakis, The many ways of Wnt in cancer. *Curr. Opin. Genet. Dev.* **17**, 45–51 (2007).
3. L. Chang, M. Karin, Mammalian MAP kinase signalling cascades. *Nature* **410**, 37–40 (2001).
4. M. Raman, M. H. Cobb, MAP kinase modules: Many roads home. *Curr. Biol.* **13**, R886–R888 (2003).
5. Y. D. Shaul, R. Seger, The MEK/ERK cascade: From signaling specificity to diverse functions. *Biochim. Biophys. Acta* **1773**, 1213–1226 (2007).
6. Y. Pylayeva-Gupta, E. Grabocka, D. Bar-Sagi, RAS oncogenes: Weaving a tumorigenic web. *Nat. Rev. Cancer* **11**, 761–774 (2011).
7. T. L. Biechele, R. M. Kulikauskas, R. A. Toroni, O. M. Lucero, R. D. Swift, R. G. James, N. C. Robin, D. W. Dawson, R. T. Moon, A. J. Chien, Wnt/β-catenin signaling and AXIN1 regulate apoptosis triggered by inhibition of the mutant kinase BRAFV600E in human melanoma. *Sci. Signal.* **5**, ra3 (2012).
8. M. S. Brose, P. Volpe, M. Feldman, M. Kumar, I. Rishi, R. Gerrero, E. Einhorn, M. Herlyn, J. Minna, A. Nicholson, J. A. Roth, S. M. Albelda, H. Davies, C. Cox, G. Brignell, P. Stephens, P. A. Futreal, R. Wooster, M. R. Stratton, B. L. Weber, BRAF and RAS mutations in human lung cancer and melanoma. *Cancer Res.* **62**, 6997–7000 (2002).
9. H. Davies, G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M. J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, R. Wilson, H. Jayatilake, B. A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G. J. Riggins, D. D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J. W. Ho, S. Y. Leung, S. T. Yuen, B. L. Weber, H. F. Seigler, T. L. Darrow, H. Paterson, R. Marais, C. J. Marshall, R. Wooster, M. R. Stratton, P. A. Futreal, Mutations of the BRAF gene in human cancer. *Nature* **417**, 949–954 (2002).
10. P. M. Pollock, U. L. Harper, K. S. Hansen, L. M. Yudt, M. Stark, C. M. Robbins, T. Y. Moses, G. Hostetter, U. Wagner, J. Kakareka, G. Salem, T. Pohida, P. Heenan, P. Duray, O. Kallioniemi, N. K. Hayward, J. M. Trent, P. S. Meltzer, High frequency of BRAF mutations in nevi. *Nat. Genet.* **33**, 19–20 (2003).
11. J. Tsai, J. T. Lee, W. Wang, J. Zhang, H. Cho, S. Mamo, R. Bremer, S. Gillette, J. Kong, N. K. Haass, K. Sproesser, L. Li, K. S. Smalley, D. Fong, Y. L. Zhu, A. Marimuthu, H. Nguyen, B. Lam, J. Liu, I. Cheung, J. Rice, Y. Suzuki, C. Luu, C. Settachatgul, R. Shellooe, J. Cantwell, S. H. Kim, J. Schlessinger, K. Y. Zhang, B. L. West, B. Powell, G. Habets, C. Zhang, P. N. Ibrahim, P. Hirth, D. R. Artis, M. Herlyn, G. Bollag, Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 3041–3046 (2008).
12. G. Bollag, P. Hirth, J. Tsai, J. Zhang, P. N. Ibrahim, H. Cho, W. Spevak, C. Zhang, Y. Zhang, G. Habets, E. A. Burton, B. Wong, G. Tsang, B. L. West, B. Powell, R. Shellooe, A. Marimuthu, H. Nguyen, K. Y. Zhang, D. R. Artis, J. Schlessinger, F. Su, B. Higgins, R. Iyer, K. D'Andrea, A. Koehler, M. Stumm, P. S. Lin, R. J. Lee, J. Grippo, I. Puzanov, K. B. Kim, A. Ribas, G. A. McArthur, J. A. Sosman, P. B. Chapman, K. T. Flaherty, X. Xu, K. L. Nathanson, K. Nolop, Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. *Nature* **467**, 596–599 (2010).
13. W.-J. Jeong, J. Yoon, J.-C. Park, S.-H. Lee, S.-H. Lee, S. Kaduwal, H. Kim, J.-B. Yoon, K.-Y. Choi, Ras stabilization through aberrant activation of Wnt/β-catenin signaling promotes intestinal tumorigenesis. *Sci. Signal.* **5**, ra30 (2012).
14. E. Lee, A. Salic, R. Krüger, R. Heinrich, M. W. Kirschner, The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol.* **1**, E10 (2003).
15. Y. Zhang, S. Liu, C. Mickanin, Y. Feng, O. Charlat, G. A. Michaud, M. Schirle, X. Shi, M. Hild, A. Bauer, V. E. Myer, P. M. Finan, J. A. Porter, S. M. Huang, F. Cong, RNF146 is a poly(ADP-ribose)-directed E3 ligase that regulates axin degradation and Wnt signalling. *Nat. Cell Biol.* **13**, 623–629 (2011).

10.1126/scisignal.2002921

Citation: D. Guardavaccaro, H. Clevers, Wnt/β-catenin and MAPK signaling: Allies and enemies in different battlefields. *Sci. Signal.* **5**, pe15 (2012).

Wnt/β-Catenin and MAPK Signaling: Allies and Enemies in Different Battlefields

Daniele Guardavaccaro and Hans Clevers

Sci. Signal. **5** (219), pe15.
DOI: 10.1126/scisignal.2002921