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β-catenin in metastatic melanoma – the smoking gun reloaded

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The canonical Wnt signaling pathway, regulated largely through the efficiency by which the multifunctional protein β-catenin is stabilized, has long been associated with embryonic development, adult homeostasis, and stem cell function. Without Wnt signaling, β-catenin is held in a 'destruction complex' (along with adenomatous polyposis coli (APC), Axin, glycogen synthase kinase 3β, and casein kinase 1α), phosphorylated, and targeted for proteasomal degradation by an E3 ubiquitin ligase. Wnt binding to members of the Frizzled and low-density lipoprotein receptor-related protein families activates Dishevelled, disrupting the complex, thus preventing β-catenin phosphorylation/ubiquitination. Consequently, active β-catenin accumulates in the cytoplasm and then translocates to the nucleus, where it binds to members of the T-cell factor/lymphoid enhancer factor (TCF/LEF) family. The consequence is transcriptional activation of key regulatory genes such as *myc*, *cyclin D1,* and *microphthalmia-associated transcription factor* (*MITF*). Such 'master' regulatory nodes are often conscripted by aspiring cancer cells, and β-catenin is no exception.

Nearly 15 yr ago, the oncogenic nature of β-catenin was first revealed, the 'Smoking Gun' of Mark Peifer's cancer crime scene (Peifer, 1997). Since then, its prominent role in tumors of the colon, breast, prostate, and ovary has been well documented; β-catenin can be drawn to a life of crime by mutations in the degradation-specific phosphorylation sites, or more frequently through loss of APC function. However, despite the fact that melanoma was one of the first cancer types associated with β-catenin's guilt (Rubinfeld et al., 1997), its role in this deadly disease has remained elusive (reviewed by Lucero et al., 2010). For example, almost a quarter of melanoma cell lines harbor activating β-catenin mutations, compared to only 5% of primary uncultured melanomas. Now, the paper by Marcus Bosenberg and colleagues (Damsky et al., 2011) uses a genetically engineered mouse (GEM) model system to provide evidence for a β-catenin signaling role(s) in melanoma metastasis.

Damsky et al. employed a previously described robust GEM melanoma model driven by two events common in the human disease: expression of the activating mutant $Braf^{V600E}$ and inactivation of Pten (Dankort et al., 2009). These events are triggered genetically in the conditional model (hereafter termed *Pten*/*Braf*) by a melanocyte-specific Cre driver and topical application of 4-hydroxytamoxifen (4-OHT) to perinatal mice. Upon induction, mice rapidly develop melanomas, with common metastasis to lymph nodes and modest lung metastases. Damsky et al. examined the effect(s) of stabilized β-catenin on tumorigenesis in *Pten*/*Braf* mice using the $Ctnnb1^{loxex3}$ allele (Harada et al., 1999), which, upon 4-OHT exposure, switches expression of wild-type β-catenin to that of a constitutively activated β-catenin harboring stabilizing exon 3 phosphorylation site mutations. Relative to in *Pten*/*Braf* mice, these mice experienced a significant reduction in survival, as well as sharply elevated metastasis in the lymph nodes, lung, bowel, and spleen. In contrast, inhibiting β-catenin function by incorporation of Cre-dependent homozygous null alleles ($Ctnnb1^{-/-}$) significantly delayed the onset of melanoma, dramatically reduced metastasis, and enhanced survival in the *Pten*/*Braf* background.

Because this conditional mouse model permits genetic initiation of melanoma via the simultaneous 4-OHT-mediated activation of Braf and β-catenin and inactivation of Pten in a

temporally and spatially specific manner, Damsky et al. could ask whether the metastatic consequences of β-catenin activation were different in perinatal versus adult melanocytes. Notably, although they found that activated β-catenin could clearly stimulate metastatic potential in adult as well as perinatally initiated melanocytes, there were quantitative and qualitative differences. Expression of stabilized β-catenin in *Pten*/*Braf* pups resulted in metastatic melanoma in 100% of lymph nodes, 60% of bowels, and 10% of spleens, and there were well over 300 lesions per lung. In contrast, induction in adult *Pten*/*Braf* mice produced fewer than 10 metastatic lesions per lung, a lower tumor burden in lymph nodes (Bill Damsky and Marcus Bosenberg, personal communication), and no other metastatic sites. As a disproportionately high percentage of melanocytic cells in perinatal mouse skin are progenitors with stem-like properties, these data are consistent with the concept that the cell of origin is a major determinant of disease manifestation.

The authors went on to show that β-catenin plays an important role in the melanoma differentiation state, both in adult and pup treatments; mice harboring activated β-catenin develop more heavily pigmented tumors, while those arising in the absence of β-catenin were less differentiated with a more nerve sheath-like appearance. Accordingly, melanomas arising in the context of mutant β-catenin exhibited an upregulation in MITF, a transcriptional target of β-catenin/LEF, as well as multiple melanocyte differentiation markers. The functional relationship between pigmentation/differentiation and metastatic capability is of significant interest, and mechanisms underlying this relationship remain unresolved.

The relevance of this murine study to the human condition was demonstrated using gene set enrichment analysis (GSEA) of transcriptome assessment. The most differentially expressed genes in the *Pten*/*Braf* mouse melanomas, with and without activated β-catenin, were used to perform transcriptome GSEA on analogous Pten-inactivated/Braf mutant human melanoma cell lines, with and without activated β-catenin. The result was a statistical concordance of samples based on β-catenin stabilization. Importantly, the converse approach was performed on the mouse expression data using the most differentially expressed genes in human melanoma samples, with or without activated β-catenin, again yielding a significant enrichment. Clearly, activated β-catenin drives broadly similar gene expression changes in mouse and human melanoma, many of which are associated with melanocyte differentiation. Surprisingly, however, although Akt and Wnt downstream pathways were predictably stimulated in mouse melanomas arising in the context of stabilized β-catenin, MAPK activation was not elevated based on pErk1/2 levels. Added to what was already appreciated about key pathways driving the Pten/Braf model, these new data help fill out the molecular landscape for metastatic melanoma, including a possible role for the prolyl isomerase PIN1. These findings also strongly suggest that further molecular classification of mutant BRAF melanomas and more detailed analyses of the relationship between Wnt and MAPK signaling are warranted.

The importance of canonical Wnt signaling has been a point of some contention in the melanoma research community, at least relative to some other cancer types; however, in their study, Damsky et al. provide definitive genetic evidence that, in the *Pten*/*Braf* mouse model, stabilized β-catenin facilitates melanoma development and progression. Additionally, endogenous β-catenin was shown to be required for melanomagenesis. The *Pten*/*Braf* mouse represents an excellent model of human melanoma, comprising genetic lesions that are highly relevant to a prominent subset of human melanomas. However, it is now imminently clear that multiple melanoma subtypes can arise depending upon factors such as environmental exposure, cell of origin, heritable susceptibility, and accrual of driver mutations (Whiteman et al., 2011). It is therefore likely that the neoplastic consequences of aberrant Wnt signaling in general, and mutationally stabilized β-catenin in particular, will be

highly contextual. If β-catenin-associated pathways are to be considered as therapeutic targets in metastatic melanoma, the first hurdle will be to reproducibly identify the subpopulation of patients most likely to benefit from such targeting. The fact that β-catenin is intracellular and harbors no discernable enzymatic activity places it squarely in the 'undruggable' category of targets; however, upstream and downstream accomplices are numerous and may prove more tractable. Although the jury is still out on the value of β-catenin in melanoma therapy, Damsky et al. have provided the incentive to revisit the crime scene and reexamine the evidence.

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