requirements for JAK2 activation in the nucleus and the process guiding plasma membrane-bound active JAK2 to the nucleus. However, basal phosphorylation of histone H3Y41 would hint at some redundancy in this pathway that may not require JAK2.

It has been apparent for some time that STAT5 cannot be the only major transcriptional effector of JAK2 and other substrates of this kinase have been characterized with various impact on gene expression. The identification of histone H3Y41 as a target of JAK2-dependent HP1α regulation adds a new dimension to the field of JAK2 biology. These findings will help to unravel some of the dynamics within the intricate signaling networks required for early hematopoiesis. As correctly pointed out by Dawson et al. (2009), the role of the HP1α binding region in nucleosome mobility and stability as well as that of HP1α itself in mitotic recombination may explain some of the genomic instability associated with malignancies containing active JAK2 (Fernandes et al., 2009; Plo et al., 2008). Nevertheless, it seems likely that other factors might regulate HP1α and the degree of JAK2 requirement is not entirely clear. It will need to be carefully determined whether these novel interactions of JAK2 with histone H3Y41 open up new opportunities for targeted therapeutic approaches that may benefit patients with hematologic neoplasms or other malignancies that involve deregulated JAK2 activity. Finally, it is possible that other members of the Janus kinase family (JAK1, JAK3, and TYK2) may have analogous functions in the nucleus and that should be examined.

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PARsing the Phrase "All in for Axin"—Wnt Pathway Targets in Cancer

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DOI 10.1016/j.ccr.2009.10.007

Genetic alternations resulting in constitutive stabilization of β-catenin and altered transcription of β-catenin/TCF-regulated genes are found in many cancers. A recent *Nature* paper offers insights into the role of tankyrase in regulating the Wnt/β-catenin pathway and suggests that compounds targeting tankyrase's poly-ADP-ribosylation (PARsylation) activity may hold promise for cancer therapy.

Defects in conserved signaling pathways are well known to play key roles in the origins and behavior of essentially all cancers. Mutations affecting the Wnt signaling pathway underlie the pathogenesis of cancers, including upwards of 80%–90% of colorectal cancers (CRCs) (MacDonald et al., 2009). The Wnt proteins are a conserved family of secreted molecules with pleiotropic and context-specific effects on cells (MacDonald et al., 2009). In the canonical or β-catenin-dependent Wnt pathway, Wnts regulate the level and subcellular localization of β-catenin. In the absence of an activating Wnt signal, glycogen synthase kinase 3β (GSK3β) collaborates with the AXIN and APC (adenomatous polyposis coli) proteins and other factors to phosphorylate β-catenin at its amino (N)-terminal domain. The phosphorylated β-catenin is recognized and ubiquitinated by a complex containing a β-transducin repeat-containing protein (βTrCP), then degraded by the proteasome. Wnt binding to the Frizzled-low density lipoprotein-related protein (LRP)-5/6 coreceptor complex on the cell surface inhibits the AXIN/GSK3β complex and stabilizes the free pools of β-catenin. In the nucleus, β-catenin can bind to T cell factor (TCF) transcriptional regulators along with other cofactors and modulate transcription of various genes. Mutational mechanisms with major contributing roles in stabilizing β-catenin in human cancer include inactivation of APC or AXIN1 or

activating (oncogenic) mutations in β-catenin's N-terminal domain (MacDonald et al., 2009), all of which lead to altered transcription of β-catenin/TCF-regulated genes in the absence of exogenous Wnt signals.

In spite of the great progress in understanding the Wnt pathway and its mutations in cancers, successes have been limited with respect to illuminating strategies for therapeutic targeting of this pathway. A recent paper in the journal *Nature* not only offers important new insights into proteins regulating Wnt signaling but also further supports the approach to antagonize β-catenin levels and localization via small molecules (Huang et al., 2009). Huang and colleagues used a high throughput screen to identify a small molecule XAV939 that interfered with Wnt-stimulated transcription. They found that XAV9393 blocked Wnt-stimulated accumulation of β-catenin by increasing the levels of the AXIN1 and AXIN2 proteins. Subsequent work by the authors established that XAV939 regulates AXIN levels via inhibition of tankyrases 1 and 2 (TNKS1 and TNKS2), both of which are members of the poly(ADP-ribose) polymerase (PARP) protein family (Hsiao and Smith, 2008). TNKS1/2 bind directly to AXIN proteins and appear to regulate AXIN levels via poly-ADP-ribosylation (PARsylation) and ubiquitination. Evidence that the TNKS proteins regulate the Wnt pathway in physiological settings was also obtained, via a Wnt signaling-dependent zebrafish model of fin regeneration. Moreover, XAV939 was shown to inhibit growth of the APC-defective CRC cell line DLD-1, and the antiproliferative effect of XAV939 was abrogated when AXIN1/2 levels were depleted in these cells by siRNA approaches. An independent study from Chen and colleagues had uncovered a compound termed IWR-1 in a high throughput screen that acted by an unknown mechanism to stabilize AXIN and block β-catenin accumulation (Chen et al., 2009). The Huang et al. group showed that the IWR-1 compound, like XAV393, exerted its effects via inhibition of TNKS1/2 PARsylation activity.

Notwithstanding the elegance and sophistication of the study, does the paper herald the emergence of a new era for the therapeutic targeting of the Wnt pathway in cancer? At the risk of being viewed as a skeptic, I would offer the short answer "maybe." The papers of Huang et al. and Chen et al. offer further compelling support for earlier work that showed both APC and AXIN function as key scaffold proteins for β-catenin but AXIN protein levels are limiting under physiological conditions (MacDonald et al., 2009, and references therein). Another notable advance is that the enzymatic function of TNKS1/2 enhances their candidacies as "druggable" targets in the Wnt pathway. To date, much of the research on inhibiting the Wnt pathway has focused on interfering with protein-protein interactions, either Wnt-receptor interactions or β-catenin binding to TCF proteins or other nuclear cofactors, such as CREB-binding protein (CBP) (Barker and Clevers, 2007, and references therein). Besides the obvious challenges associated with identifying small molecules that potently interfere with protein-protein interactions, constitutive β-catenin dysregulation in cancer arises most frequently from mutational defects downstream of Wnt-receptor interactions and thus would be minimally affected by ligand-receptor antagonists. Transcription factors and cofactors often interact with multiple nuclear proteins; compounds that antagonize their interaction with β-catenin might also interfere with their interaction with other critical proteins. Given this background, TNKS1/2 joins a small list of downstream Wnt pathway factors with presumptive enzymatic function, such as the chromatin remodeling-associated factor Brg1 (Barker and Clevers, 2007) and the kinases CDK8 and TNIK (Firestein et al., 2008; Mahmoudi et al., 2009), which could perhaps be targeted in cancer cells for therapeutic effect.

The utility of compounds, like XAV939, that stabilize AXIN1/2 would presumably be limited to cancer cells with upstream ligand-receptor defects or APC defects, or perhaps also those with defects in one of the two, but not both, AXIN proteins. Cancer cells that express a mutant oncogenic β-catenin protein would presumably be resistant to AXIN stabilization. Moreover, a major assumption underlying cancer therapy with compounds that stabilize AXIN proteins and in turn antagonize β-catenin is that advanced cancer cells with β-catenin defects are highly dependent on continued dysregulation of β-catenin. To date, the limited data to implicate dysregulated β-catenin as a critical factor in the continued growth and survival of advanced cancer cells reveal differences from one cell line to another (Kim et al., 2002, and references therein). At this point, the argument that antagonizing dysregulated β-catenin function in advanced cancer cells will have broad and profound therapeutic effects is far from certain. The availability of compounds like XAV939 and IWR-1 should help to advance knowledge of the range and types of cancers and precancers that might be amenable to β-catenin antagonism and possible mechanisms for its resistance.

Various unsettled issues with respect to the action of XAV939 on cancer cells include uncertainties about whether its growth inhibitory action reflects effects of AXIN stabilization on β-catenin versus other AXIN-interacting proteins. Elevated levels of AXIN1 have been shown to potently inhibit the growth of HCT116 cells, which express a mutant β-catenin but are not dependent on it for growth and survival (Satoh et al., 2000; Kim et al., 2002). Moreover, AXIN proteins may even have positive roles in cancer progression in some settings, such as in epithelial-mesenchymal transition via stabilization of the Snail1 transcriptional repressor (Yook et al., 2006). There is also much to be learned about the role of TNKS1/2 function in normal and cancer cells. TNKS proteins are evolutionarily conserved and function in telomere length regulation and sister telomere cohesion, GLUT4 vesicle translocation, and possibly also mitotic spindle pole regulation (Hsiao and Smith, 2008). As such, targeting of TNKS1/2 might be expected to have varied effects on cells. Notably, inhibition of TNKS1 resulted in synthetic lethal effects in cells with BRCA1 or BRCA2 defects, due apparently to exacerbation of the centrosome amplification phenotype associated with BRCA deficiency (McCabe et al., 2009).

An implied promise of the enormous progress achieved in our understanding of the molecular pathogenesis of cancer is that a plethora of new therapeutic targets will be revealed. The new findings on TNKS1/2 function in regulating AXIN and the potential of the TNKS1/2 proteins as therapeutic targets highlight the power of innovative chemical genetics and proteomics approaches as well as conceptual challenges that need to be addressed before directed targeting of cancer cells

with Wnt pathway defects becomes a reality.

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