CK1δ: a pharmacologically tractable Achilles' heel of Wnt-driven cancers?

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Abstract: Aberrant Wnt signaling has been widely accepted to be a key driver of a subset of human cancers and a heavily scrutinized molecular pathway for the development of personalized medicine. In a recently published issue of *Science Translational Medicine*, Rosenberg and coworkers reported that the delta isoform of the CK1 family of serine/threonine kinases (CK1δ), an important mediator of intracellular Wnt signaling, is amplified and overexpressed in human breast tumors. They further demonstrated that pharmacological inhibition of CK1δ is efficacious for these cancers and implicate β-catenin signaling as a key target of CK1δ. In this perspective, we will discuss the salient features of this novel anti-cancer therapeutic approach and the challenges that lie ahead to translate it into a viable treatment option for cancer patients.

Keywords: CK1δ; Wnt; β-catenin; cancer; small molecule inhibitor

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The intimate relationship between Wnt signaling and cancer was first revealed by Nusse and Varmus when they observed that the stable overexpression of Wnt1 induces spontaneous mammary hyperplasia and tumors in mice some three decades ago (1). The oncogenic property of Wnt ligands is due, in part, to their involvement in the inhibition of the β-catenin destruction complex that leads to the stabilization and nuclear translocation of β-catenin for transactivation of growth-promoting genes (2). Since then, aberrant Wnt signaling has been implicated in an ever-growing list of human cancers, including breast tumors [as reviewed in (3)]. Breast cancer is a clinically heterogeneous disease and it can be broadly classified into three major therapeutic groups, based on the molecular expression profile of the estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2/neu) receptors. While great strides have been made in the treatment of ER+ as well as HER2+ breast tumors via endocrine and targeted therapies, limited treatment options are available to combat the basal-like triple-negative breast cancers (TNBCs) (4). Propelled by such an unmet medical need, Rosenberg and colleagues pursued a study with the aim to further identify drivers and cell signaling networks in TNBCs and HER2+ breast cancers that could be targeted via pharmacological means.

Rosenberg *et al.* focused on the CK1 family of serine/threonine kinases that consists of six human isoforms (α, δ, ε, γ1, γ2 and γ3). Members of this kinase family regulate many cellular processes that are implicated in cancer, including Wnt, NFAT, Hedgehog, and Hippo signaling, membrane trafficking, cytoskeleton maintenance, DNA replication, DNA damage response, RNA metabolism and circadian rhythm [as reviewed in (5,6)]. Rosenberg *et al.* examined transcript expression of CK1 isoforms in breast cancer datasets from The Cancer Genome Atlas (TCGA). They found that *CK1δ* is widely overexpressed within a subset of breast tumors across all major classes, while *CK1ε* overexpression is restricted to the basal-like subclass. Notably, both *CK1δ* and *CK1ε* transcripts are also highly upregulated in the unclassified breast tumors, but the functional implications of this finding remains to be elucidated. The authors attributed the upregulation of *CK1δ*

to copy number gains of the *CSNK1D* locus found in 36% of breast tumors, with increased frequencies in the basal-like and luminal B subtypes. They demonstrated that CK1δ, not CK1ε, proteins are highly expressed in a panel of human breast cancer cell lines and human breast tumor specimens. Analyses of additional TCGA cancer datasets also revealed *CSNK1D* copy number amplifications in more than 70% of papillary renal cell carcinoma and in approximately 50% of bladder tumors, and these somatic alterations of *CSNK1D* correlated with the enhanced CK1δ expression. Thus, CK1δ has the opportunity to be a cancer driver.

Testing if CK1δ activity is important in these cancers, Rosenberg *et al.* found that the growth of CK1δ-high breast cancer cells and several tumor xenografts in mice are selectively blocked by SR-3029, a nanomolar dual inhibitor of CK1δ and CK1ε that their group previously developed (7). They analyzed the drug-treated cells by flow cytometric-based cell death assays and found that SR-3029 selectively triggered rapid apoptosis of the CK1δ-high breast cancer cells. These finding were confirmed by RNA interference (RNAi)-mediated depletion of CK1δ/ε in the CK1δ-high breast cancer cells. They further showed that SR-3029 or inducible short hairpin RNA (shRNA)-mediated knockdown of CK1δ markedly suppressed the growth of orthotopic TNBC tumor xenografts, and this effect could be rescued by exogenous expression of shRNA-resistant CK1δ. Consistent with the breast cancer cell line models, SR-3029 significantly inhibited the growth of a primary patient-derived xenograft (PDX) model by triggering tumor cell apoptosis. These data suggest that the SR-3029-induced apoptosis in these cancer cells is solely mediated through CK1δ. Notably, long-term daily intraperitoneal dosing with SR-3029 (20 mg/kg over 48 days) appeared to be well tolerated by mice, indicating that global inhibition of CK1δ is unlikely to elicit overt systemic toxicity. SR-3029 is therefore a promising new agent that appears to be both effective and well tolerated in preclinical models of CK1δ-high breast cancer.

To identify which key cancer pathways are regulated by CK1δ and responsible for the anti-cancer effects of SR-3029, the authors first performed Ingenuity Pathway Analysis (IPA) on the TCGA datasets and identified 612 genes whose expression correlated with CK1δ. Several of these were Wnt pathway genes, although core β-catenin targets such as *AXIN2* were not identified. Presumably other pathways were also enriched in the correlating genes but were not described further. Given the many roles of CK1δ, there may be additional opportunities here. While Wnt pathway-activating mutations typical of other cancer types are rarely found in breast cancer (8), increased CK1δ activity might be a driver of β-catenin signaling in some cases.

In contrast to CK1α, CK1δ/ε has been shown to be a positive regulator of β-catenin signaling by regulating the stability of the destruction complex (9-12). To test if CK1δ is a critical positive regulator of the canonical Wnt/β-catenin signaling in CK1δ-high breast cancer, the team studied whether RNAi-mediated depletion or pharmacological inhibition of CK1δ has a negative impact on nuclear β-catenin activity in multiple CK1δ-high TNBC and luminal B subtypes of breast cancer cell lines. Consistent with published reports, the loss of CK1δ or its kinase activity markedly reduced the total abundance of β-catenin proteins, particularly its nuclear pool, as well as partially suppressed β-catenin/TCF-dependent gene transactivation in these breast cancer cells. The effects of SR-3029 could be rescued by expression of stabilized β-catenin *in vitro*, suggesting that SR-3029 indeed acts at least in part through the Wnt/β-catenin pathway. Whether stabilized β-catenin protects cancers in more rigorous xenograft models was not tested. The data are consistent with the role of CK1δ in Wnt signaling.

Rosenberg *et al.* further demonstrated that depletion or inhibition of CK1δ is sufficient to abolish ligand-induced activation of canonical Wnt/β-catenin signaling in the TNBC cells. These cancer cells are also sensitive to the loss of β-catenin, suggesting that the survival signals from ligand-induced activation of Wnt signaling are mainly mediated through β-catenin, although it is important to keep in mind that epithelial cells also use β-catenin for functions unrelated to Wnt signaling (13). Similarly, the abundance of nuclear β-catenin and expression of β-catenin/TCF-target genes are dramatically reduced in TNBC tumor xenografts derived from SR-3029-treated mice compared to vehicle-treated controls.

Some questions remain unanswered. Whether Wnt signaling drives the proliferation of human breast cancers *in vivo* remains unclear. The effects of SR-3029 on β-catenin appear robust, but this may not be the critical pathway *in vivo*. Furthermore, they find that CK1δ expression/activity correlated with *WNT3* and *WNT9A* transcripts, suggesting a feed forward loop where increased *WNT* expression would lead to increased β-catenin activity. However, we have reported that interruption of Wnt secretion has no effect on proliferation of most breast cancer cell lines, including MDA-MB-231, *in vitro* or orthotopic mouse models (14).

Taken together, these findings suggest that the CK1δ-regulated signaling circuits could represent a pharmacologically tractable Achilles' heel of a variety of human cancers, perhaps those driven by aberrant upregulation of Wnt ligands and/or its co-receptors. As CK1δ has many targets beyond Wnt signaling, it is likely that other effects of kinase inhibition also play a role in the activity of SR-3029. As always with small molecules, unanticipated off-target effects can always play a role (15). We and others have previously demonstrated that the pan-CK1δ/ε nanomolar inhibitor PF670462 lacks potent anti-cancer activity in a variety of tumor cell types/origin, including breast tumor cells (15,16). The superiority of SR-3029 over PF670462 in cancer cell growth inhibition could be attributed to its lack of off-target effects that might hinder the anti-cancer effect of CK1δ inactivation (7), or the presence of other targets that combine with CK1δ inhibition to produce efficacy. Given that CK1δ regulates a myriad of protein substrates like MDM2 (17) and p53 (18), it would be great interest to determine how the dysregulation of these proteins upon the loss of CK1δ or its kinase activity contributes to the growth inhibition of Wnt-driven cancer cells in future studies.

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Footnote

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