

RESEARCH HIGHLIGHT

Virtual drug design: Skp1–Skp2 inhibition targets cancer stem cells

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The dysregulation of pathways regulating cellular function is a frequent hallmark of cancer and the development of specific pathway inhibitors that alter tumor growth and progression are the focus of multiple recent studies. E3 ubiquitin ligases are a large group of diverse protein enzymes that specifically target proteins for clearance, and their importance to normal cellular function is illustrated in the many diseases associated with their loss of function or inappropriate targeting. S-phase kinase-associated protein 2 (Skp2) is an F box protein that plays critical roles in cell-cycle progression, senescence, metabolism, and acts as an Skp1–Cullin-1–F box (SCF) ubiquitin ligase substrate recognition factor. Overexpression of Skp2 is associated with poor prognosis and metastasis in many cancers and is a well validated drug target. In a recent report, Chan *et al.* have identified an Skp2 inhibitor that selectively impairs Skp2 E3 ligase activity using an integrated virtual high-throughput drug screening and experimental validation approach. This Skp2 inhibitor restricts cancer stemness and potentiates sensitivity to chemotherapeutic agents in multiple animal tumor models. These findings identify a new novel small molecule that targets the Skp2 and reduces tumor growth by attenuating aerobic glycolysis and inducing cellular senescence.

The F box protein S-phase kinase-associated protein 2 (Skp2) is one of the four subunits of the Skp1–cullin-1–F box (SCF) ubiquitin E3

ligase complex that can trigger the ubiquitination and degradation of several protein substrates. E3 ubiquitin ligases facilitate the final step of ubiquitin attachment to their specific target protein substrates for proteasome degradation. In the Skp2 SCF complex, Skp2 binds to Skp1, a linker protein, which binds to cullin-1. Skp2 targets several cell-cycle regulator and tumor suppressor proteins, including p27, for ubiquitin-mediated degradation and positively regulates the cell-cycle. Several studies have implicated an oncogenic role for Skp2 and overexpression of Skp2, which leads to increased degradation of p27 and subsequent unchecked cellular proliferation, has been observed in several tumor types. Conversely, Skp2 deficiency triggers p27-dependent cellular senescence and apoptosis. In previous studies, Chan and colleagues^{1,2} demonstrated that the SCF Skp2 complex could also trigger non-proteolytic K63-linked ubiquitination of Akt. The Akt pathway is a critical regulator of cell survival and metabolism. Akt ubiquitination results in enhanced Akt membrane localization and phosphorylation, which increases Akt activation. Akt activation results in aerobic glycolysis, enabling rapid generation of ATP and biomaterials to sustain cancer cell hyperproliferation. Considering that tumor cells express low levels of p27 and activated Akt, specifically disrupting Skp2 E3 ligase function could serve as a promising cancer treatment strategy by reducing both p27 degradation and cell glycolysis, thereby selectively inhibiting cancer cell survival.

Virtual drug screening utilizes informatics approaches to first define hot spot residues and binding pockets and then identify potential inhibitors to block the protein–protein interactions. From these virtual arrangements, predictions can be made as to which molecules will bind most effectively, while those with low binding affinities are excluded. This method can efficiently sift through

hundreds of thousands of candidate molecules to identify a workable number of potential compounds that target the protein of interest. These potential inhibitors will then be taken to the wet lab for validation. Recently the identification of compounds that could specifically target and inhibit E3 ligase activity has generated much enthusiasm. Wu *et al.*³ previously utilized an *in silico* structure-based virtual ligand screening strategy to identify compounds that could specifically inhibit the Skp2-mediated degradation of p27 by disrupting phosphor–p27 and Skp2–Cks1 interaction. These small molecules were shown to interrupt Skp2–p27 interaction, resulting in reduced ubiquitination and increased stability of p27. Furthermore, the compounds were capable of inhibiting p27- and Skp2-dependent cell-cycle progression in several cancer cell lines, resulting in decreased proliferation. However, these compounds were highly specific to the Skp2–p27 interface and did not disrupt formation of the Skp1–Skp2 complex, suggesting that the degradation of other SCF Skp2 ligase targets would remain unaffected.

In a recent paper in *Cell*, Chan and colleagues⁴ utilized a high throughput virtual screening approach to identify residue ‘hot spots’ along the Skp2–Skp1 interaction surface. These hot spots are residues along the Skp2–Skp1 interaction interface that contribute more significantly to their binding. The authors identified 19 such potential residues and grouped them according to proximity to the N-terminus or the C-terminus of Skp2. Inhibitors were screened *in silico* using their previously developed high-performance computing-based molecular docking scheme, termed HiPCDock,⁵ for chemical compounds predicted to bind at either or both sites that might block the binding of Skp1 to Skp2. The candidate inhibitors were screened for their ability to prevent Skp2 and Skp1 interaction

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in vitro with a glutathione S-transferase pull-down assay. Biochemical analyses in prostate cancer cell lines verified that candidate compound #25 could stabilize expression of Skp2 canonical substrates p27 and p21, as well as inhibit the activation of Akt. Further experiments in cell lines indicated that compound #25 was specific to Skp2 E3 ligase activity, but not other F box proteins.

Cancer stem cells (CSCs) or cancer initiating cells express stem cell markers and are capable of self-renewal and asymmetric cell division. Several studies have postulated that CSCs can be the cells of origin for cancers and are responsible for tumor progression and metastasis. Additionally, tumor relapse is thought to be caused by the survival of small CSC populations that resist conventional treatment. Chan and colleagues showed that Skp2 inactivation reduced the CSC population in prostate cancer cell lines and inhibited the ability of prostate CSCs to form prostate spheres, suggesting that Skp2 inhibition suppressed the self-renewal capacity of CSCs. In xenograft experiments, the Skp2 inhibitor compound #25 enhanced prostate tumor response to doxorubicin or cyclophosphamide. Furthermore, they demonstrated that treatment of established lung or prostate

tumor xenografts with compound #25 induced an upregulation of p27, p21 and apoptosis and a decrease in cellular proliferation. Glycolysis regulators Akt and Glut1 were also downregulated, suggesting that Skp2 inactivation could attenuate aerobic glycolysis in xenograft tumors.

The authors identified a diverse set of compounds using an *in silico* high-throughput virtual screening method to quickly sift through a large chemical database containing 120 000 commercially available compounds. Among them, 25 were selected to be validated in the wet lab for their specificity in preventing Skp2–Skp1 interaction, inhibiting Skp2 SCF E3 ligase activity toward ubiquitination of p27 and Akt, and repression of tumor xenograft growth. Chan and colleagues also revealed a critical role for Skp2 in governing CSC properties, including their capacity for self-renewal. Pharmacological inactivation of Skp2 in tumors could confer sensitivity to chemotherapy and reduce tumor progression by disrupting the ability of CSCs to survive and resist treatment. Translation of Skp2 inhibition as a cancer treatment strategy to the clinic will require the identification of additional Skp2 target proteins disrupted by these small molecules, their effect on untransformed cells and

the potential for parallel degradation pathways to compensate for the loss of Skp2 function. This current study illustrates the power of using virtual molecular structure-based modeling, high-throughput *in silico* screening and experimental validation to develop highly specific targeting and inhibition of E3–substrate interactions. This study illustrates a promising new therapeutic strategy targeting Skp2 E3 ligase that could potentially be used to kill the elusive cancer stem cell.

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