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Chemical and Structural Biology to Direct the Repurposing of Sulindac

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Thousands of drugs are currently on the market for the treatment of human diseases, and even hundreds more are in clinical trials. Although the molecular drug–target interactions, especially in the case of enzyme inhibitors, are well understood in most cases, effective treatments often remain plagued by a plethora of unwanted systemic effects due to interactions with known or unknown secondary targets.

Almost ten years ago, the completion of the Human Genome Project sparked a new era in genome analysis; it laid the foundation for the dissection of disease-related genetic alterations and fostered the development of next-generation medicines. Soon, however, it became evident that genetic information alone is insufficient to bridge the genotype–phenotype gap, since individual genes and gene products—proteins—are not self-contained functional entities in cells. Proteins operate and function at the cellular and molecular level by interacting to form complex networks, pathways and signaling cascades. The strict regulation of the temporal and spatial aspects of protein function is essential to sustain life and highlights the complexity of living organisms.

From a pharmaceutical point of view, it becomes even more complex when one tries to further develop small-organic molecules into biologically active drugs that are selective for a target protein in this vast array of signaling networks and can be used for the treatment of diseases. Over the past 20 years, an improved understanding of aberrantly regulated signaling processes has led to the development of small molecules that specifically target unwanted enzyme activities. In the case of cancer biology, a milestone was set with the approval of the tyrosine kinase inhibitor imatinib (Gleevec) for the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GISTs), representing the dawn of targeted tumor therapy. Stimulated by this initial success, targeted approaches to specifically perturb protein function at the cellular level are moving to the forefront of kinase inhibitor research.

One of these complex networks is the Wnt signaling pathway, a regulatory pathway that is crucial for cell development and growth. Abnormal activation or disregulation of Wnt signaling is connected to tumor development in several cancer types, including colorectal and prostate cancer, making this pathway an attractive target for chemical biology and medicinal chemistry research. The protein Dishevelled (Dvl) is recognized as a key player in the Wnt signaling pathway and transduces signals by interacting with the Wnt receptor (Frizzled) via its PDZ domain, ultimately modulating downstream events, such as the β-catenin and T cell factor-dependent transcription of genes associated with tumorogenesis regulation.[1] Moreover, disruption of Dvl function by RNAi interference or by mutagenesis prevented tumor growth, specifically highlighting the role for Dvl and the Wnt signaling pathway in tumor growth.[2]

Interestingly, recent evidence has accumulated that suggests some nonsteroidal anti-inflammatory drugs (NSAIDs), which were originally developed to inhibit cyclooxygenase enzymes (COX1/2) to treat inflammation and pain, might also show an effect in the treatment and prevention of cancer. Although this has not yet been convincingly shown in clinical studies, the arylacetic acid sulindac (Clinoril) and some of its metabolites were proposed to modulate the Wnt signaling pathway by interacting with Dvl through an as yet unknown molecular mechanism. Stimulated by these findings, Lee et al.[3] set out to better understand the molecular mechanism by which sulindac and its metabolite, sulindac sulfone, modulate Wnt signaling by analyzing their binding characteristics to the PDZ domain of Dvl using protein NMR spectroscopy. This approach is similar to that used to confirm binding of sulindac derivatives to a Ras binding site, resulting in disruption of the Ras–Raf interaction. These studies also confirmed inhibition by several sulindac derivatives using Ras pathway-selective phenotype-based cell screening.[4,5]

Employing these strategies, Lee et al.[3] observed chemical-shift perturbations in titration experiments of the ^{15}N-labeled Dvl PDZ domain with increasing concentrations of sulindac, indicating dose-dependent compound binding to a site also known to accommodate the native ligand of Dvl (Figure 1). 2D NOE NMR spectroscopy with the doubly labeled (^{15}N, ^{13}C) PDZ domain of Dvl allowed the 3D structure of the complex to be determined and revealed the exact binding mode of sulindac to the peptide-binding pocket (Figure 2). The structure also helped to explain that the side chain of a particular Arg residue (Arg 322), found only in the PDZ domain of Dvl, serves as a crucial selectivity filter, thus giving specificity to sulindac binding. In a competitive binding assay, the authors showed that sulindac competes with the C terminus of the Wnt signaling antagonist Dapper for binding to the PDZ domain of Dvl, and thereby disrupts protein–protein interactions. The biological effect of sulindac as an inhibitor of canonical Wnt

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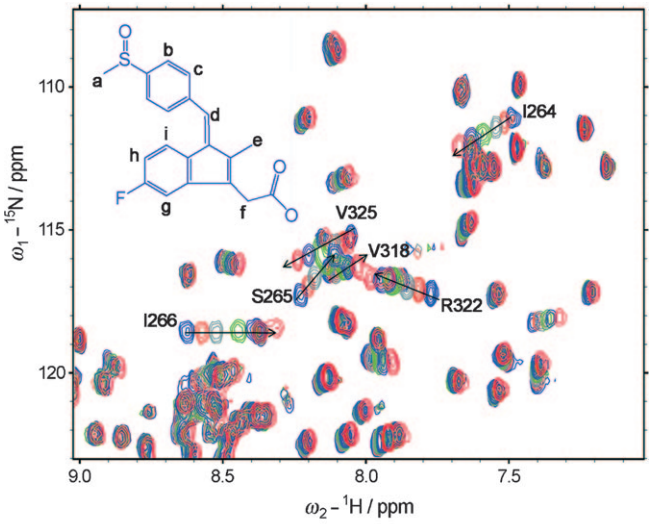

Figure 1. ^{15}N-HSQC spectra of the PDZ domain of Dishevelled at various concentrations of sulindac. Significant changes in the chemical shifts of the backbone resonances upon sulindac binding indicates interactions with the highlighted amino acids. Reproduced with permission from Reference [3]. Copyright 2009, Wiley-VCH.

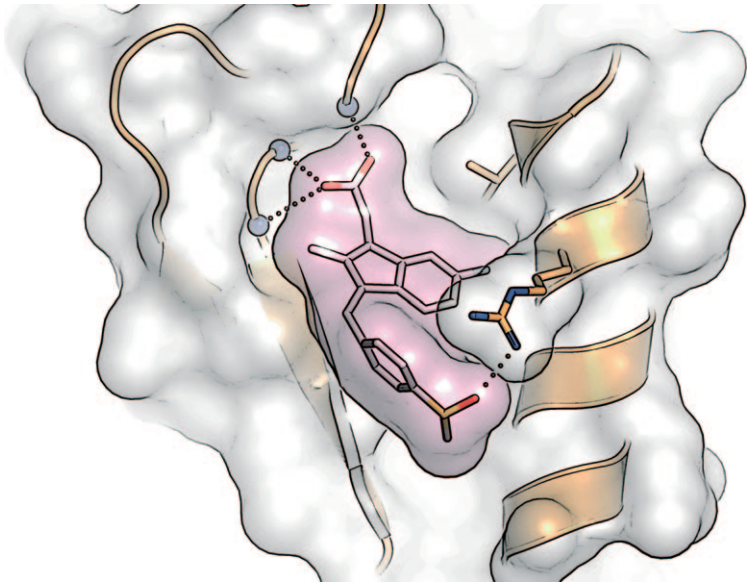

Figure 2. Solution structure of sulindac binding to the Dvl PDZ domain. The carboxylate of sulindac forms hydrogen bonds to the backbone of the protein. The side chain of Arg 322 forms a hydrogen bond to the methylsulfinylmethane group; this interaction does not occur in any other PDZ domain and serves as a critical selectivity determinant.

signaling at the cellular level was then confirmed by in vivo experiments, which study early development and cellular differentiation in Xenopus embryos.

The systematic analysis by Lee et al. involved a combination of structural and chemical biology to demonstrate that the indicated chemoprotective anticancer effects of sulindac might not only be due to the perturbation of prostaglandin biosynthesis (inhibition of COX1/2) but by direct interference with protein–protein interactions in the Wnt signaling pathway. Although sulindac and its metabolites are only weak binders to the PDZ domain of Dvl, with affinities in the mid-micromolar range, the provided solution structure of the Dvl-PDZ–sulindac complex (PDB code: 2kaw), as well as a series of recently published crystal structures of human Dvl2-PDZ in complex with inhibitory peptides,[1] opens up the possibility to rationally design more potent inhibitors as tool compounds to probe the biology of Dvl, and to chemically validate Wnt signaling pathway inhibition for therapeutic uses.

A second interesting aspect of the work by Lee et al. is that it brings the concept of "repurposing" currently available drugs to the forefront. Given the recent slowdown in newly approved drugs entering the market, such approaches may open new doors in drug research and redirect chemistry efforts to modulate and give further specificity to known compounds, which may have been otherwise overlooked.

The repurposing approach to drug discovery generally involves: 1) the use of new biological understanding to discover new mechanisms of action for known compounds; or 2) the discovery of a new role for an already existing target in another disease. In the former case, a detailed understanding of gene-product function in the complexity of a living cell, or better at the organism level, is crucial for the development of innovative next-generation drugs or the repurposing of known drugs. As seen in the work of Lee et al.,[3] modern chemical-biology research facilitates the use of small molecules to disrupt biological systems and offers new opportunities to dissect complex network architectures within the environment of a living cell.[6]

A study by Blanchard and co-workers extends this concept further by showing that the combination of two known drugs, the antibiotic meropenem and the β-lactamase inhibitor clavulanate, is surprisingly effective against extensively drug-resistant tuberculosis (XDR-TB).[7] Similarly, a recent study by Sos et al.[8] reports the use of a chemogenomic approach to highlight the dependency of the PI3K and MAPK pathways to a subset of upstream genomic aberrations in cancer, which led to the conclusion that the combination of a known PI3K inhibitor with a known MEK inhibitor might be beneficial for patients whose tumors harbor tyrosine kinase receptor oncogenes.

Novelty in drug discovery is often judged by the development of therapeu-

tic agents from new chemical scaffolds to hit known disease targets, or that work by a new mechanism-of-action. The idea of "novelty" in drug discovery can be loosely defined as the discovery of new therapeutic alternatives to treat disease. In light of the recent advances in drug repurposing, this approach has the potential to identify new ways to treat disease, while simultaneously circumventing the challenges and time required to develop new drugs. It is very likely that we will start to see more research focusing on known compounds that are rather promiscuous and have known off-target effects. Since unwanted side effects of a compound for one condition may actually turn out to be beneficial for another condition, the identification of these other targets and pathways would be of great interest and, although it may not lead to the discovery of novel drugs in the traditional sense, it may nonetheless reveal novel therapeutic strategies for treating various diseases.

Keywords: cancer · chemical biology · medicinal chemistry · NMR · protein–ligand interactions

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