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# Activity-Based Profiling for Drug Discovery

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**Activity-based protein profiling (ABPP) is emerging as a game-changing tool for drug discovery, target validation, and basic biology. In this issue, Chang et al. (2011) report the ABPP-facilitated discovery of JW480, a highly selective potent and orally bioavailable inhibitor of monoalkylglycerol ether hydrolase KIAA1363 that dramatically impairs in vivo growth of human prostate cancer cell lines.**

Identification and characterization of the functionally diverse enzyme complexes that coordinate and control all cellular processes is among the most important challenges of the postgenomic era. Quantitative understanding of dynamic enzyme activity, integrated from the cell up to the whole organism, is an essential step toward a unified model of life, and a powerful tool in the increasingly complex search for viable drug targets across all diseases. To understand enzyme function, we must decipher the emergent chemistry of proteins, and the application of chemical technologies to this challenge has proven particularly powerful, giving rise to the vibrant multidiscipline of chemical proteomics. An ultimate objective of this emerging field is to profile all types of enzymatic activity in whole organisms, a process commonly termed Activity-Based Protein (or Proteome) Profiling (ABPP), presenting some fascinating challenges in chemical biology (Heal et al., 2011). ABPP has origins in work from the 1980s, labeling the active site of proteases, but it is only recently that it has matured into a versatile and powerful platform technology. The Cravatt laboratory is a recognized ABPP pioneer, having demonstrated profiling across a remarkably broad range of enzyme classes. In a notable recent study, a search for selective inhibitors of members of the serine hydrolase (SH) superfamily (Bachovchin et al., 2010) was implemented using competitive ABPP, in which synthetic molecules compete with the probe for binding to the target enzyme. In this case, an SH-directed fluorophosphonate-rhodamine (FP-Rh) probe was used to profile over 70 hydrolases against more than 150 carbamate inhibitors, ultimately resulting in compounds selective toward single or small groups of SHs. Competitive ABPP is a perfect fit to the aspirations of modern drug discovery, allowing fine tuning of inhibitor selectivity and potency against numerous enzymes in parallel, directly in the native complexity of the proteome. Furthermore, even inhibitors that are selective for or against uncharacterized enzymes for which substrates have not yet been reported can be developed utilizing this methodology. The implications for understanding inhibitor on- and off-targets in vivo during drug development are evident, and ABPP holds great promise for avoiding drug attrition due to toxicity or efficacy failures in late-stage clinical trials.

In the current issue, Ben Cravatt and coworkers (Chang et al., 2011) report the development of JW480, a potent and selective carbamate-based inhibitor of KIAA1363 (also known as AADACL1), which is a membrane-bound 2-acetyl monoalkylglycerol ether hydrolase. KIAA 1363 is a member of the aforementioned abundant and diverse SH superfamily, which includes esterases, thioesterases, lipases, amidases, and proteases. Several SHs are implicated in the development and progression of tumors (Nomura et al., 2010a), but unfortunately the biological and physiological functions for many of these potential pharmacological targets remain poorly understood (Simon and Cravatt, 2010). Increased activity of KIAA1363 results in the overproduction of monoalkylglycerol ethers (MAGEs), which in turn are converted into lysophospholipids that stimulate survival, mobility, and aggressiveness of cancer cells (Chiang et al., 2006). KIAA1363 is the second SH enzyme reported recently by the same group to lead to overproduction of protumorigenic lipids. In the former study, a combination of ABPP, proteomic, and lipidomic analyses revealed a key role for monoacylglycerol lipase (MAGL) in leveling these fats (Nomura et al., 2010b).

The current study evolves from this work, focusing on the KIAA1363-MAGE pathway in prostate cancer cells with recently discovered lead compounds (Bachovchin et al., 2010), providing a starting point for rational design of more potent and selective analogs. In initial experiments, increased activity of KIAA1363,

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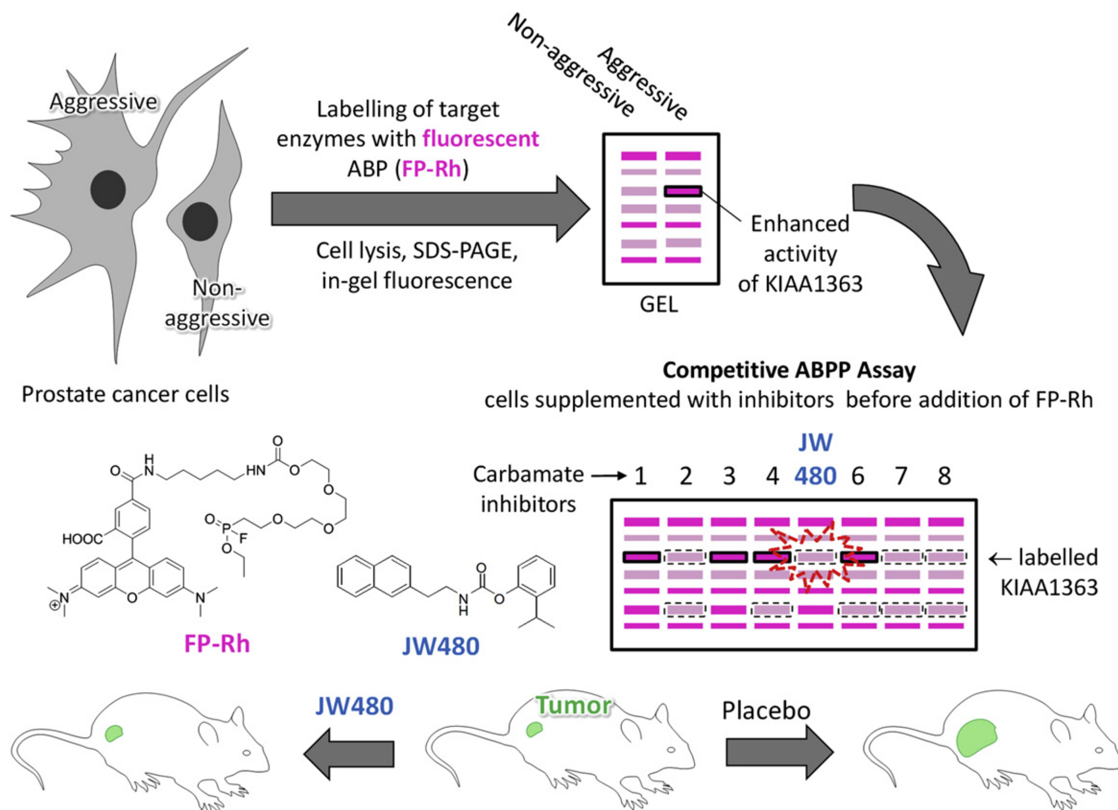


**Figure 1. Discovery of JW480: Activity-Based Protein Profiling in Action**
ABPP-assisted discovery of selective inhibitor JW480 against serine protease KIAA1363, the activity of which is upregulated in aggressive cancer cells. Orally administered JW480 also significantly impaired the growth of tumor in a mouse xenograph model.

measured with the FP-Rh probe and 1D PAGE-fluorescence readout (Figure 1), was shown to correlate with elevated levels of MAGE in aggressive cancer cell lines. Next, a sizable library of carbamates was designed and tested, ultimately resulting in JW480, a highly selective and potent ($IC_{50}$ = 6 nM) mechanism-based inhibitor of KIAA1363 in living aggressive prostate cancer cells. The authors leveraged competitive and quantitative variants of ABPP technology to demonstrate that the inhibitory action of JW480 (1 $\mu$M) is maintained in these cells for at least 48 hr with no signs of off-target reactivity, with the exception of carboxylesterase ES1, a cross-reactivity target common for carbamates. Furthermore, highly selective inhibition of mouse brain KIAA 1363 by JW480 can be achieved in vivo, which has striking potential implications in the context of brain tumor progression (Albert and Anderson, 1977) and lipid signaling in the CNS. Finally, in an aggressive prostate cancer model (Figure 1), severe reduction, but not complete blockage, of tumor growth was observed in mice to which JW480 was administered orally.

The data presented make a strong case for JW480 as a near-ideal tool for further studies on the cellular biochemistry of hydrolase KIAA1363 and the lipid signaling network within which it is embedded. The authors have delivered a useful and practical compound that is readily synthesized, orally bioavailable, and CNS penetrant, with important potential applications extending beyond cancer to CNS and metabolic disorders (Homan et al., 2011). Most importantly to scientists trying to understand lipid signaling, by exploiting the power of ABPP, the authors have gone much further than traditional biochemical approaches permit to validate the on-target effect of their compounds and eliminate potential off-target effects. In future investigations, it will be interesting to see whether bio-orthogonally tagged or fluorescent analogs of JW480 can be used to cross-confirm selectivity in a manner not biased toward the SH superfamily and for in vivo imaging studies of KIAA1363 activity. The other standout feature of this work is the elegant combination of activity-based proteomics with targeted lipidomics, resulting in new information about the role of the target enzyme in the network as a whole. Taking this a step further, potential feedback of aberrant lipid metabolism into changes in post-translational lipidation of proteins is an intriguing prospect for future exploration, since aberrant protein lipidation is also implicated in the progression of cancer (Heal and Tate, 2010). As ABPP becomes integrated with other high-throughput postgenomic and postproteomic tools, a multidimensional analytical platform is now on the horizon that has the potential to revolutionize both modern drug discovery and our fundamental understanding of living systems.

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# A Case of Cross-Reactivity

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**Studies using chemical inhibitors have suggested that p38 MAP kinase is a key regulator of Wnt/β-catenin signaling. In this issue, Verkaar et al. (2011) show that cross-reactivity of p38 inhibitors with casein kinase Iδ/ε is responsible for Wnt/β-catenin pathway inhibition.**

Wnt proteins are members of an evolutionarily conserved family of signaling molecules that play a central role in embryonic development and adult tissue homeostasis (Logan and Nusse, 2004). Given this wide array of functions, it is not surprising that the deregulation of Wnt signaling is associated with a range of human diseases (Clevers, 2006); chief among these is cancer, providing a major incentive to define the molecular mechanism of Wnt signaling and to find inhibitors that can be used to target the Wnt pathway in cancer treatment.

In recent years, a detailed outline of the Wnt signaling mechanism has emerged. Wnt can signal through several distinct pathways, but the major pathway related to stem cell maintenance and cancer is the so-called canonical Wnt/β-catenin pathway (MacDonald et al., 2009). A central player in this pathway is β-catenin, which interacts with TCF transcription factors to co-activate the expression of Wnt target genes. In the absence of the Wnt ligand, β-catenin levels are downregulated by a destruction complex that consists of the scaffold protein Axin, the APC tumor suppressor protein, and the kinases casein kinase Iα (CKIα) and GSK3β, which phosphorylate β-catenin to target it for proteasome-mediated degradation. Binding of Wnt to its receptors Frizzled and LRP5/6 inhibits destruction complex function, leading to stabilization and translocation of β-catenin to the nucleus. Although the mechanism of destruction complex inhibition is still poorly understood, it is clear that phosphorylation of Disheveled (Dvl) by casein kinase Iδ/ε (CKIδ/ε) is an important step in this process (Bryja et al., 2007; Gao et al., 2002). Recently, the MAP kinase p38 has been implicated in Wnt/β-catenin signaling as well (Bikkavilli et al., 2008).

To identify low molecular weight compounds that inhibit the Wnt/β-catenin pathway, Verkaar et al. (2011) developed an elegant β-galactosidase complementation assay to detect translocation of β-catenin into the nucleus. In the study published in this issue of *Chemistry & Biology*, they applied this method to screen a relatively small collection of a little over 2000 compounds and found that two compounds, TAK-715 and AMG-548, potently inhibit the Wnt-dependent stabilization of β-catenin (Verkaar et al., 2011). TAK-715 and AMG-548 were previously characterized as inhibitors of p38 MAP kinase, which would be in agreement with the reported role of p38 in the Wnt/β-catenin pathway (Bikkavilli et al., 2008). However, further characterization of TAK-715 and AMG-548 using a panel of kinases revealed that these compounds also inhibit CKIδ/ε. This finding prompted the question of whether the two compounds might inhibit Wnt/β-catenin signaling through CKIδ/ε instead of p38. Consistent with this notion, it was found that selective p38 inhibitors that do not cross-react with CKIδ/ε have no effect on Wnt/β-catenin signaling, raising doubts about the role of p38 in this signaling pathway. Indeed, Verkaar et al. (2011) found that knockdown of p38 did not interfere with the Wnt-dependent phosphorylation of Dvl, and that p38 was not activated after Wnt stimulation.

This study has several important implications. First, it shows that results obtained with kinase inhibitors should be interpreted with caution and that potential cross-reactivity with other kinases should be rigorously tested. Second, the study provides compelling evidence that p38 MAP kinase is not involved in Wnt/β-catenin signaling. Finally, TAK-715 and AMG-548 are clinically evaluated compounds that can now be repositioned for the treatment of Wnt-dependent tumors. As TAK-715 and AMG-548 target the Wnt pathway at the level of CKIδ/ε, it is unlikely that they will be effective in tumors harboring mutations in downstream