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COMMENTARY

For a PDK1 inhibitor, the substrate matters

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More than 20 protein kinases are directly activated by 3-phosphoinositide-dependent kinase 1 (PDK1), which is a central component of the pathways that regulate cell growth, proliferation and survival. Despite the importance of PDK1 in cell signalling, highly selective PDK1 inhibitors have not been described. In this issue of the *Biochemical Journal*, Dario Alessi's group and their collaborators at GlaxoSmithKline report GSK2334470, a potent and selective PDK1 inhibitor. They show that this compound blocks the phosphorylation of known PDK1 substrates, but surprisingly find that the potency and kinetics of inhibition vary for different PDK1 targets. This substrate-specific inhibition has implications for the development of PDK1 inhibitors as drugs.

Key words: kinase inhibitor, phosphoinositide 3-kinase (PI3K), ribosomal S6 kinase (RSK), p70 ribosomal S6 kinase (S6K), serum- and glucocorticoid-induced protein kinase (SGK).

If the kinome had a social hierarchy, then the AGC family would be the aristocracy. The AGC family is named after its prototypical members: protein kinase A, protein kinase G and protein kinase C (PKC) [1]. These kinases were the first shown to be regulated by second messengers such as calcium, lipids and cyclic nucleotides. The AGC family includes a total of 60 kinases and many of these enzymes regulate processes such as cell growth, proliferation and survival, in response to second messenger signals. Extensively studied AGC kinases include Akt and 3-phosphoinositide-dependent kinase 1 (PDK1), which are regulated by the lipid products of phosphoinositide 3-kinase (PI3K), and p70 ribosomal S6 kinase (S6K), which controls protein translation downstream of mammalian target of rapamycin (mTOR).

PDK1 is the AGC kinase responsible for regulating the activity of the other kinases in the AGC family. It does this by phosphorylating a sequence known as the T-loop which is critical for kinase activation. The T-loop is situated adjacent to ATP on the C-terminal lobe of the kinase, and T-loop phosphorylation induces a conformational change that activates the kinase domain for catalysis. PDK1 directly phosphorylates the T-loop of at least 23 AGC kinases, including various isoforms of Akt, PKC, S6K, ribosomal S6 kinase (RSK), and serum- and glucocorticoid-regulated kinase (SGK) [1]. Thus, a large fraction of the AGC family is under the direct control of PDK1 activity.

How does PDK1 differentially regulate so many different kinases? PDK1 undergoes autophosphorylation on its T-loop and therefore is constitutively active. However, PDK1 activity against specific substrates requires additional signals that prime those targets for PDK1 phosphorylation. For many AGC kinases, this involves phosphorylation of a sequence known as the hydrophobic motif. The hydrophobic motif is phosphorylated by a kinase other than PDK1 (in many cases, mTOR) and then binds to a specific cleft on PDK1 known as the PIF (PDK1-interacting) pocket. Binding of the phosphorylated hydrophobic motif to the PIF pocket enables PDK1 to phosphorylate its substrate kinase on the T-loop.

Given the unique position of PDK1 upstream of many protein kinases, a selective PDK1 inhibitor would be a valuable research tool. It is therefore surprising that such compounds have not been described. The only putatively selective PDK1 inhibitors published to date were a series of aminopyrimidines reported by 2005 [2]. A representative of this class, BX-795, was shown to inhibit PDK1 with an *in vitro* IC_{50} of 6 nM and displayed selectivity in a counterscreen against ten kinases. However subsequent studies revealed that BX-795 potently inhibits several kinases other than PDK1, including ERK8 (extracellular-signal-regulated kinase 8), MNK2 (MAPK-integrating protein kinase 2), Aurora B and C, MARK3 (microtubule-affinity-regulating kinase 3), IKKε (inhibitory κB kinase ε) and TBK1 (TANK-binding kinase 1) [3], and some of the cellular effects of BX-795 have been attributed to inhibition of these secondary targets [4,5]. Thus the wait for a highly selective PDK1 inhibitor continued.

A report in this issue of the *Biochemical Journal* indicates that this wait may be over. Najafov et al. [6] describe GSK2334470, a small molecule that inhibits PDK1, with an *in vitro* IC_{50} value of $\sim$15 nM, and shows impressive selectivity against a panel of 110 protein and lipid kinases. Importantly, GSK2334470 displays little or no activity against the seven kinases identified as secondary targets of BX-795 and shows good selectivity against several AGC kinases closely related to PDK1, suggesting that this compound has a high degree of specificity. GSK2334470 will soon be commercially available and should find widespread use as a research tool to perturb PDK1 signalling.

The critical test of any kinase inhibitor is how well it inhibits its target in cells, and the authors assessed the ability of GSK2334470 to block the phosphorylation of a range of PDK1 substrates. As expected, they found that GSK2334470 blocked the T-loop phosphorylation and functional activation of the AGC kinases SGK, Akt, S6K and RSK2. However, there was surprising variability in the potency and kinetics of inhibition of these different PDK1 targets. GSK2334470 blocked Akt phosphorylation under conditions that moderately activated PI3K signalling, but failed to do so in response to a strong stimulus such as IGF-1 (insulin-like growth factor 1). RSK2 phosphorylation was inhibited by GSK2334470, but only after extended incubation with the drug (for 8–24 h). By contrast,

Abbreviations used: IGF-1, insulin-like growth factor 1; mTOR, mammalian target of rapamycin; PDK1, 3-phosphoinositide-dependent kinase 1; PI3K, phosphonositide 3-kinase; PIF, PDK1-interacting fragment; PKC, protein kinase C; PTEN, phosphatase and tensin homologue deleted on chromosome 10; RSK, ribosomal S6 kinase; SGK, serum- and glucorticoid-regulated kinase; S6K, p70 ribosomal S6 kinase.

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PDK1 phosphorylation of SGK and S6K was inhibited rapidly and potently.

What accounts for the differential sensitivity of these PDK1 substrates to the same drug? In the case of Akt, it appears that under conditions that strongly activate the PI3K pathway, even a small fraction of the available PDK1 kinase activity is sufficient to fully phosphorylate Akt. Since no drug inhibits 100 % of the activity of its target, the residual PDK1 that escapes drug inhibition in this setting is able to fully activate the pathway. In principle, any kinase inhibitor could be rendered ineffective in this way, but in practice, most kinase inhibitors block their targets robustly, regardless of the cellular context. Indeed, the fact that kinase inhibitors work so reliably suggests that evolution has tuned signalling pathways so that most kinases become just sufficiently active to do their job in response to any given stimulus [7]. But Najafov et al. [6] show that this is not always the case.

The authors provide experimental data to support this model. PDK1 phosphorylation of Akt, but not of other AGC kinases, is regulated directly by interaction with the phosphoinositides PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2. These lipids are produced by PI3K and recruit Akt and probably PDK1 to the plasma membrane, thereby increasing their local concentration. Binding of PtdIns(3,4,5)P_3 to Akt also induces conformational changes that facilitate PDK1 phosphorylation. Together, these PtdIns(3,4,5)P_3-dependent mechanisms can increase dramatically the efficiency of PDK1 phosphorylation of Akt [8,9]. Mutants of Akt (ΔPH-Akt) or PDK1 (PDK1^{K465E}) that cannot bind to phosphoinositides do not experience the same rate enhancements in response to PtdIns(3,4,5)P_3 and therefore would be predicted to be more sensitive to GSK2334470 inhibition in cells. Consistent with this reasoning, the authors find that GSK2334470 is able to inhibit the IGF-1-induced phosphorylation of ΔPH-Akt, but not wild-type Akt. They likewise show that GSK2334470 inhibits Akt phosphorylation more potently in PDK1$^{K465E/K465E}$ embryonic stem cells than in matched embryonic stem cells that express wild-type PDK1. These data support a model in which the strength of the upstream signal determines whether a PDK1 inhibitor can block Akt phosphorylation.

While Akt phosphorylation was inhibited weakly by GSK2334470, RSK2 phosphorylation was blocked slowly, requiring 8–24 h in two different cell lines. This was in contrast with PDK1 targets such as S6K, which were dephosphorylated within minutes of commencement of drug treatment. Slow dephosphorylation of RSK2 was also observed in a study utilizing a PDK1^{L159G} mutant that is sensitive to an inhibitor analogue [4]. In that setting, PDK1 inhibition also resulted in slow RSK dephosphorylation over 24 h of drug treatment. The simplest explanation for these data is that the phosphatase responsible for dephosphorylating the T-loop of RSK is unusually inactive against that site, resulting in slow phosphate turnover. While it is a truism that every phosphorylation reflects a balance between the activity of a kinase and a phosphatase, in most cases the role of the phosphatase is ignored. These data are a reminder that individual phosphorylation sites can have widely different half-lives *in vivo*.

What does all of this mean for the development of PDK1 inhibitors as drugs? The PI3K pathway is frequently activated in tumours as a result of mutation of either PTEN (phosphatase and tensin homologue deleted on chromosome 10) or PIK3CA (PDK1 itself is rarely mutated). A PDK1 inhibitor could block this PI3K pathway activation and thereby have anti-tumour activity, a strategy that has been encouraged by the finding that a hypomorphic mutant of PDK1 can prevent tumorigensis in PTEN$^{+/-}$ mice [10]. However, it remains unclear which PI3K effectors are most important for human tumours. If Akt is the critical kinase downstream of PI3K, then PDK1 may be a poor target, since the present data suggest that high concentrations of a PDK1 inhibitor would be necessary to prevent Akt activation. In that case, inhibiting PI3K or Akt directly would make more sense. On the other hand, if PI3K activation drives tumorigenesis through a kinase such as S6K, then PDK1 inhibitors may be attractive, since they could inhibit S6K activation at concentrations that do not completely block Akt signalling. This would minimize the metabolic side-effects associated with Akt inhibition.

For basic scientists, these findings are a reminder that there is always something new to learn from the use of a selective drug that targets an important signalling protein. This is true even for a kinase such as PDK1, which has been carefully scrutinized for years using biochemical and genetic approaches. As the numerous selective kinase inhibitors currently being developed by the pharmaceutical industry make their way into the hands of basic researchers, these insights are likely to continue.

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