Aurora A: Target Invalidated?

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Aurora kinases are important mitotic regulators, and Aurora kinase inhibitors are under investigation as treatments for cancer. An ongoing debate in the field is which Aurora kinase is the better drug target. A new study (Girdler et al., 2008, in this issue of *Chemistry & Biology*) pushes the case forward for Aurora B.

Drug discovery often requires a full commitment of effort while in possession of rather less than a full understanding of the ultimate goal. In particular, unambiguous validation of a drug target is generally difficult to achieve, and scientists from different disciplines often have different ideas about what constitutes useful target modulation, a key step toward biological validation. In part, this is because the phenotypes observed can depend on the methodology used (Weiss et al., 2007). Ultimately, practical validation of a biological target molecule as a small-molecule drug target requires first the identification of a suitable small molecule and only then a full assessment of its mode of action. Therefore, these experiments are undertaken fairly late in the discovery process, sometimes even during clinical trials, and do not always give the expected result. One might conclude that the target and the drug must validate one another.

A new and exceptionally active target for anticancer drug discovery is the Aurora kinases. There are two major human Aurora kinases, Aurora A and Aurora B (Carmena and Earnshaw, 2003). The interest in Aurora kinases as potential therapeutic targets was stimulated by the finding that Aurora A is amplified or overexpressed in diverse tumor types and that transfection with Aurora A is transforming in some cell lines (Bischoff et al., 1998). Despite the very high sequence identity of the Aurora A and Aurora B kinase domains, the two proteins have distinct mitotic functions, and each kinase is essential for proper mitotic progression and cellular viability. Aurora A localizes predominantly to the centrosome, and Aurora A loss of function disrupts bipolar spindle formation. Aurora B localizes during early mitosis first to chromosomes and then to the inner centromere. At this stage, Aurora B is required for spindle assembly checkpoint (SAC) function. Later in mitosis, Aurora B moves to the spindle midzone, and it is required for completion of cytokinesis.

Despite much progress toward understanding basic aspects of Aurora kinase function, there are indications of an interrelationship that is not well understood. Modest overexpression of Aurora A causes little change in the kinetics of mitotic entry, but instead appears to interfere with the SAC (Jiang et al., 2003) and cytokinesis (Meraldi et al., 2002), both of which depend on Aurora B function. Similar results were obtained when two different ''kinase-dead'' mutants of Aurora A were overexpressed (Jiang et al., 2003; Meraldi et al., 2002; Littlepage and Ruderman, 2002). In contrast, induction of kinase-dead variants of Aurora A appears to inhibit Aurora A function only in the absence of endogenous wild-type Aurora A (Girdler et al., 2006). Taken together, these studies indicate that cells may require low levels of Aurora A function to progress through mitosis. Furthermore, Aurora A overexpression may not produce a simple Aurora A gain of function, but instead it may dominantly interfere with the more sensitively balanced Aurora B (Carmena and Earnshaw, 2003). To further complicate matters, the transforming activity of Aurora A does depend on catalytic activity but is not further enhanced by kinase-activating mutations (Bischoff et al., 1998). Because some aspects of Aurora disregulation do not require the kinase activity of Aurora A, it is not obvious that these processes can be targeted by a small molecule, much less what the desired selectivity profile might be.

Fortunately, potent and structurally diverse small molecule Aurora inhibitors have arrived on the scene. These molecules produce cellular effects indicating Aurora A loss of function (Hoar et al., 2007), Aurora B loss of function (Ditchfield et al., 2003), or both (Tyler et al., 2007). Examples from each class have been shown to inhibit tumor growth in multiple xenograft models and have entered human clinical trials. Is this the end of the story? As it happens, the story is not so simple.

Stephen Taylor and colleagues (Girdler et al., 2008) have taken on the question that is so seldom asked: Does the inhibitor really act through the intended target? It is usually straightforward to show that a small-molecule drug inhibits a target in a cellular or in vivo context. However, this does not mean that the therapeutic benefit (e.g., tumor growth inhibition) is caused by inhibition of this target. In the kinase area, it is especially difficult to demonstrate causality, as most kinase inhibitors are far from perfectly selective (Karaman et al., 2008). The inhibitor studied by the Taylor group (Girdler et al., 2008) is ZM447439, originally discovered at AstraZeneca (Ditchfield et al., 2003). ZM447439 was observed to most closely phenocopy Aurora B loss of function in cellular assays, despite inhibiting Aurora A and Aurora B in enzymatic assays.

The mechanistic connection between Aurora B and ZM447439 was strengthened in a follow-up study (Girdler et al., 2006). Tetracycline-inducible expression of wild-type Aurora kinases or their kinase-dead versions was established in stable cell lines. Consistent with the earlier studies noted here, expression of the inactive Aurora A mutant had little effect on cellular growth, and cells remained capable of colony formation. In contrast, expression of an inactive Aurora B mutant strongly inhibited cell growth and reduced the number of colonies. The effects on cell number in culture and colony outgrowth were similar to those observed upon treatment with ZM447439. A closely

related inhibitor with greater potency against Aurora B (and 100-fold selectivity against Aurora A) produced all the same phenotypic effects as ZM447439, but at a lower concentration consistent with its greater enzymatic potency. These results support that ZM447439 inhibits Aurora B (and not Aurora A) but stop short of a full demonstration that Aurora B is the target through which ZM447439 blocks cellular proliferation and colony outgrowth.

The new study addresses this issue and adds provocative questions of its own (Girdler et al., 2008). The authors begin by selecting for clones of HCT-116 colon cancer cells that have acquired resistance to ZM447439. They identify several mutant alleles of Aurora B. They then demonstrate that the mutant variants of Aurora B are sufficient to confer resistance to ZM447439 by establishing stable tetracycline-inducible cell lines for each variant. Induction of mutant Aurora B, but not wild-type, allows colony outgrowth in the presence of ZM447439. This establishes that Aurora B is the relevant target of ZM447439 antiproliferative activity. Even had the authors stopped at this point, it would have been an important contribution.

However, the authors also test the effects of two other Aurora kinase inhibitors against the Aurora B mutant cell lines. VX-680 (MK-0457) is an inhibitor of both Aurora kinases in cellular assays (Tyler et al., 2007), and MLN8054 shows selectivity for Aurora A when used at 1 μM in cellular assays (Hoar et al., 2007). The first puzzling result is that the Aurora B mutation confers resistance to VX-680 in a colony outgrowth assay. This is consistent with the known activity of VX-680 against Aurora B. However, it also suggests that the clearly observable inhibition of Aurora A by VX-680 does not produce a correspondingly strong antiproliferative effect. The story becomes still more puzzling: the ZM447439-resistant cell line remains fully sensitive to the Aurora A-selective inhibitor MLN8054 (Girdler et al., 2008).

What do these data imply about Aurora A as an anticancer target? First, it is difficult to understand how inhibition of Aurora A by two different active-site inhibitors could produce entirely different results. It is possible that the cellular readouts for Aurora A inhibition do not reflect full occupancy by inhibitor. Accordingly, one possibility suggested by the authors is that the extent of inhibition may be less in the case of VX-680. It is well worth comparing the two inhibitors directly in mechanistic cellular assays to explore this further. A more provocative possibility is that the antiproliferative activity of MLN8054 is driven through a target other than Aurora A. MLN8054 appears to be a much more selective kinase inhibitor than VX-680 (Karaman et al., 2008), so it is conceivable that it has an important target that is not a kinase. Despite the strong circumstantial evidence linking Aurora A to cancer, it would appear that there is still more to do before Aurora A can be validated as definitively as Aurora B for pharmacological intervention.

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