Caspase Inhibition, Specifically

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In this issue of *Structure*, Grütter and coworkers report mutagenesis of ankyrin repeat proteins to generate the first specific, tight-binding caspase inhibitor selectively targeting caspase-2 (Schweizer et al., 2007). Interestingly, this inhibitor operates by an allosteric mechanism that does not target the enzyme active site.

Proteases participate in most cell fate decisions and behaviors. Additionally, they provide therapeutic targets in several diseases. Proteases are attractive drug targets primarily because they are easy to assay and screen with compound libraries, and it is estimated that about 5% of drug targets pursued by the pharmaceutical industry are proteases (Lindsay, 2003). Historically, protease inhibitor drugs have met with substantial success. Angiotensin converting enzyme inhibitors and HIV protease inhibitors are prominent examples; however, there have been equally notable disasters, as with the first generation of drugs targeting metalloproteinases (Coussens et al., 2002). One main reason for the failures is off-target effects because traditional strategies for targeting proteases produce compounds that are often hampered by limited specificity, leading to the inhibition of unintended proteases in vivo with potentially devastating results.

The problem is pretty easy to understand. The hotspot in a protease is its active site, and most small molecule screens are going to hit there. Thus, it represents an almost overwhelmingly tempting target for pharmaceutical chemists, who have developed a wealth of chemical probes to target enzyme catalytic centers. However, individual protease families contain members that due to evolution and sheer architectural requirements have overlapping specificities and identical catalytic mechanisms, impeding the development of active site-directed reagents that can discriminate among them. Even though in rare cases a lack of specificity may be an advantage (Abdulla et al., 2007), in most instances therapeutic doctrine states that the more specific a drug, the less off-target possibilities and the more likely the ultimate therapy will prove effective without side effects. Thus, the central problem in targeting enzymes such as proteases is lack of specificity that can be achieved by employing active site-directed strategies. This problem is exemplified perfectly by the caspases, a group of proteases that are involved in inflammation and apoptosis (Fuentes-Prior and Salvesen, 2004). Too much apoptosis, mediated by distinct caspases, results in inappropriate death of postmitotic cells such as neurons, liver cells, and cardiomyocytes. Consequently, there is great interest in achieving specific and tight binding inhibition of one of the several caspases that constitute the known apoptotic pathways to alleviate acute and chronic conditions thought to be due to inadvertent apoptosis.

From this perspective, it is wise to heed the lessons of nature (which of course has already mastered this elusive task), since the stringent regulation of caspases by endogenous inhibitors is considered pivotal in controlling cell fate decisions. Some viruses even recognized caspase inhibition as a means to defeat infected host cell attempts to commit suicide. To date, three naturally occurring caspase inhibitors have been extensively examined: the orthopox virus protein CrmA, the baculovirus inhibitor p35, and the human protein XIAP (Stennicke et al., 2002). CrmA and p35 are both viral "suicide inhibitors" that offer themselves up as bait to an attacking protease. They get cleaved very much like a substrate, but then trap the protease's catalytic machinery. Matching the needs of the virus, these inhibitors directly target caspase active sites and show little specificity for individual caspases. In contrast, however, the endogenous multidomain human caspase inhibitor XIAP shows exquisite specificity. One domain acts as an absolutely specific inhibitor for caspase-9, and a separate domain only targets caspase-3 and its close paralog caspase-7. None of the other eight caspases are inhibited by XIAP. Notably, the mechanisms that deliver these stringent specificities are not based on directly targeting the caspase active sites, but rather use surface regions that are specific for interaction with the individual caspases, leading either to blocked access to the enzyme in the case of caspases-3 and -7 or to allosteric inhibition in the case of caspase-9 (Riedl and Shi, 2004). The lesson is clear—to achieve specificity you must avoid the actual active site and rather deploy strategies that target parts of the protein that control formation of the active enzyme.

To date, almost all current drug development strategies that target caspases are based on active site-directed inhibitors. In some cases, this can be a valid strategy, such as for inhibition of the inflammatory caspases-1, -4, and -5 (Linton, 2005). These caspases have a considerably different specificity from the apoptotic caspases-2, -3, -6, -7, -8, -9, and -10. Thus, there is less concern about accidentally interfering with important apoptotic regulation. However, it is very difficult to obtain inhibitors that distinguish between caspases-2, -3,

and -7, or between caspases-6, -8, -9, and -10. Granted there are many reports of one or two orders of magnitude differences between inhibitor potency, but in reality nobody knows whether this is enough to avoid off-target effects. A very promising approach was proposed a few years ago for small molecules that target the interface between caspase-3 and -7 heterodimers, abolishing formation of the active enzymes by an allosteric mechanism (Hardy et al., 2004) This approach, while holding great promise, has yet to yield molecules that are practical inhibitors.

In this issue of *Structure*, Schweizer et al. (2007) present a new approach to address this specificity problem. The investigators used a strategy that provides a large library of protein sequences displayed on the surface of a designed ankyrin repeat protein (DARPin) to screen for inhibitors of caspase-2. This library, with a combinatorial complexity of up to 10^{10} individual sequences, allows sampling of a large amount of conformational space in a relatively confined volume, using three loops emanating from the ankyrin repeat scaffold for probing nooks and crannies on protein surfaces (Binz et al., 2003). Binding selection with this DARPin library resulted in a clone that, when expressed, inhibited caspase-2 with an overall subnanomolar K_i, and absolutely no inhibition of caspases-3, -7, -8, or 9 at 150 nM. These results mean that the DARPin inhibitor is the first synthetic caspase reagent that could be classified as essentially totally specific.

Recalling the principle of nature's approach to specificity, the mechanism of inhibitor binding is allosteric. The 3.2 Å resolution structure of the enzyme-inhibitor complex shows the ankyrin loops forcing relatively small changes in the caspase loops that surround the active site, freezing the enzyme in a nonproductive conformation. There are at least two key implications from this beautifully conducted study. First, specificity is achieved by an allosteric mechanism, where the inhibitor has no direct contact with the active site. Second, specific control can more readily be achieved when large volumes of conformational space are scanned. The latter means that it is unlikely that the DARPin strategy will ultimately lead to replacement of the protein structural motifs by small molecule analogs (or ultimately drugs). However, many reagents that would never make it to the clinic have proven irreplaceable as research tools to probe signaling pathways. From this perspective, we imagine that the caspase-2 specific DARPin, which can readily be expressed ectopically in recipient mammalian cells, will be invaluable in dissecting the role of caspase-2—probably the most controversial caspase in terms of its role in signaling death.

REFERENCES

Abdulla, M.H., Lim, K.C., Sajid, M., McKerrow, J.H., and Caffrey, C.R. (2007). PLoS Med. *4*, e14. 10.1371/journal.pmed.0040014.

Binz, H.K., Stumpp, M.T., Forrer, P., Amstutz, P., and Pluckthun, A. (2003). J. Mol. Biol. *332*, 489–503.

Coussens, L.M., Fingleton, B., and Matrisian, L.M. (2002). Science *295*, 2387–2392.

Fuentes-Prior, P., and Salvesen, G.S. (2004). Biochem. J. *384*, 201–232.

Hardy, J.A., Lam, J., Nguyen, J.T., O'Brien, T., and Wells, J.A. (2004). Proc. Natl. Acad. Sci. USA *101*, 12461–12466.

Lindsay, M.A. (2003). Nat Rev Drug Discov. *2*, 831–838.

Linton, S.D. (2005). Curr. Top. Med. Chem. *5*, 1697–1717.

Riedl, S.J., and Shi, Y. (2004). Nat. Rev. Mol. Cell Biol. *5*, 897–907.

Schweizer, A., Roschitzki-Voser, H., Amstutz, P., Briand, C., Gulotti-Georgieva, M., Prenosil, E., Binz, H.K., Capitani, G., Baici, A., Plückthun, A., et al. (2007). Structure *15*, this issue, 625–635.

Stennicke, H.R., Ryan, C.A., and Salvesen, G.S. (2002). Trends Biochem. Sci. *27*, 94–101.

