HIV protease inhibitors and nuclear lamin processing: Getting the right bells and whistles

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One of the most notable successes of rational pharmaceutical design has been the development of drugs that inhibit the protease cleaving the polyproteins of the HIV into their structural and catalytic protein components (1, 2). Several of these inhibitors are currently used as components of "highly active antiretroviral therapy" for treating HIV infection and AIDS (2, 3). Given the similar chemistry involved in all peptide bond hydrolysis reactions, as well as the different sequence contexts of the polyprotein cleavage sites, the ability of these drugs to specifically target the HIV protease and not the hundreds of other proteases required for human health—digestive enzymes, enzymes of the coagulation pathway, enzymes of the complement cascade, proteases involved in protein maturation and transport—is truly remarkable. However, in a recent issue of PNAS, Coffinier *et al.* (4) demonstrate that these drugs do inhibit at least one unrelated protease and that this inhibition could conceivably account for some of the side effects of these pharmaceuticals. They show that HIV protease inhibitors, including lopinavir, a first-line widely used member of the saquinavir family, can also inhibit ZMPSTE24, a distinct protease involved in the conversion of farnesylprelamin A to lamin A, a key structural component of the nuclear lamina (Fig. 1). In recent years, genetic defects in the conversion of farnesylprelamin A to lamin A in humans have been shown to cause severe progeroid disorders (e.g., restrictive dermopathy, Hutchinson–Gilford progeria syndrome) (6–8). These findings suggest that the inhibition of prelamin A processing by HIV protease inhibitors may result in some of the same disease phenotypes observed in the genetic prelamin A-processing disorders.

The prelamin A translation product is modified by an intriguing series of enzymatic reactions that transiently form a short isoprenylated and methyl-esterified C-terminal tail that is subsequently clipped off to generate mature lamin A (8). These reactions include the S-isoprenylation of the C-terminal–CSIM sequence by protein farnesyltransferase, the cleavage of the peptide bond between the farnesylcysteine and serine residues, the methylation of the C-terminal α-carboxylate group of the farnesylcysteine residue, and finally the upstream cleavage of a peptide bond to release a 15-aa farnesylated C-terminal fragment. It is unclear why such a complicated path is taken; it would be possible to encode the same mature lamin A protein simply with an earlier stop codon. Presumably, the farnesylated and methylated tail of prelamin A assists in the proper targeting of lamin A to the nuclear lamina or functions in the assembly of the complexes of lamins A, C, B1, and B2 (6, 8). However, failure to remove the tail from prelamin A can compromise the integrity of the nuclear lamina, leading to "blebbing" and folds in the nuclear envelope (9). The structurally abnormal nuclear envelope may then send cells on a downward course leading to the multiple disease phenotypes characteristic of the progeroid aging syndromes. An additional puzzling feature of lamin biology remains to be explained: mice entirely lacking lamin A and prelamin A proteins are quite healthy and manifest only trivial structural abnormalities in the nuclear envelope (10).

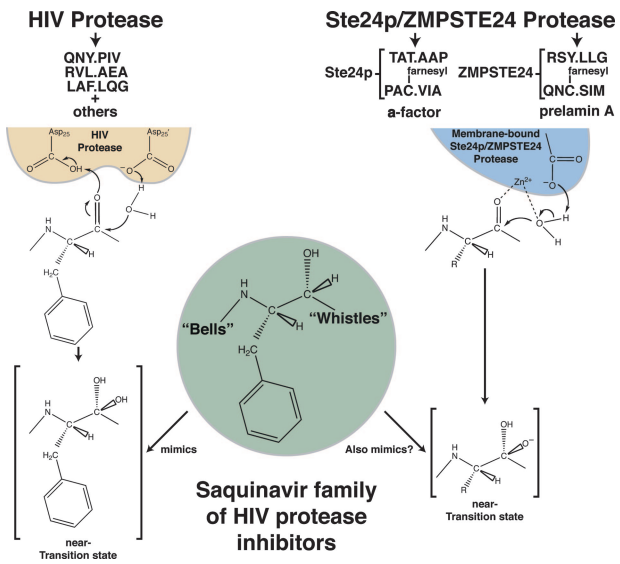

Fig. 1. The saquinavir family of HIV aspartyl protease inhibitors, including lopinavir and atazanavir, can also affect the zinc metalloprotease involved in the maturation of prelamin A to lamin A (4). These inhibitors were designed as transition state inhibitors for HIV-encoded peptidyl substrates with a phenylalanine residue in the P1 site (1) and contain different chemical groupings at their ends, designated here as "bells" and "whistles." The HIV protease is a soluble member of the aspartyl family of proteases; its active site is at the dimeric interface where the side-chain carboxylate of Asp-25 in one subunit functions as a base to deprotonate the hydrolytic water molecule, and the side-chain carboxylic acid in Asp-25 of the other subunit functions as an acid to activate the carbonyl group of the scissile peptide bond to create a transition state with tetrahedral geometry and structure that is matched by the inhibitor structure. Although the configuration at the –CH–OH– sites in lopinavir and atazanavir is *S* and that in saquinavir is *R*, the hydroxyl groups in all of these compounds can mimic one or the other of the two hydroxyl groups in the tetrahedral near-transition state. ZMPSTE24 is a membrane-associated *z*inc *m*etallo*p*rotease where a likely mechanism involves the metal ion binding to the hydrolytic water to lower its pK_a and to the scissile carbonyl oxygen to activate its carbon electrophile (5). Although there are similarities in the use of a carboxylate group and in the overall structure of the presumed transition state, it comes as a surprise that a specific HIV protease inhibitor would also inhibit ZMPSTE24 (4). This figure was prepared by Brian D. Young (University of California, Los Angeles).

The link between HIV protease inhibitors and lamin function was first proposed by Caron *et al.* (11). One of the side effects of HIV protease inhibitors is a type of partial lipodystrophy characterized by a redistribution of adipose tissue resulting from loss of fat in the face, arms, and legs, and gain of fat in the trunk, particularly a characteristic "buffalo hump" in

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the back of the neck. A similar phenotype is seen in patients with missense mutations in *LMNA*, the gene encoding prelamin A and lamin C. Of note, progeroid disorders caused by the accumulation of prelamin A are characterized by a striking loss of adipose tissue (12). Caron *et al.* (11, 13) found an increase in the level of prelamin A in mouse preadipocyte cell lines treated with the HIV protease inhibitors nelfinavir and indinavir. Using a different set of HIV protease inhibitors, including lopinavir, atazanavir, and tipranavir, Coffinier *et al.* (4) have now biochemically identified the zinc metalloprotease ZMPSTE24 as the processing step that is affected by HIV protease inhibitors (Fig. 1). Caron *et al.* (11) also found altered nuclear localization of the sterol regulatory element-binding protein-1 (SREBP-1) in cells treated with HIV protease inhibitors. Cleavage of SREBP-1 by the site-2 protease allows this transcription factor to travel to the nucleus and regulate lipid metabolism. The site-2 protease, like ZMPSTE24, is an integral membrane metalloprotease of the endoplasmic reticulum (14). It would be interesting to see whether HIV protease inhibitors also affect this enzyme and whether some of the changes in lipid metabolism in patients taking HIV protease inhibitors could be explained by effects on SREBP metabolism.

The ZMPSTE24 protease, located in the endoplasmic reticulum, is solely responsible for the cleavage of the C-terminal 15-aa domain, although it can also catalyze the removal of the carboxyl-terminal–SIM sequence. Abnormal processing of prelamin A by the loss of this cleavage reaction has been observed in humans in both restrictive dermopathy and Hutchinson–Gilford progeria syndrome and now as a result of drug therapy. In patients with restrictive dermopathy, ZMPSTE24 activity is absent, leading to an accumulation of farnesylprelamin A (6, 8). In Hutchinson–Gilford progeria syndrome, a 50-aa internal deletion in prelamin A removes the key cleavage site for ZMPSTE24, leading to an accumulation of a mutant form of farnesylprelamin A (9). With the current study, it is clear that treatment with front-line HIV protease inhibitors also blocks lamin A biogenesis, perhaps leading to an accumulation of farnesylprelamin A in the tissues of treated patients (4). Indeed, recent studies by Caron *et al.* (13) have revealed prelamin A accumulation in the adipose tissues of patients treated with the HIV protease inhibitors nelfinavir and indinavir.

The studies of Coffinier *et al.* (4) depended on high-quality lamin A/C Western blots and careful biochemical assays. Their Western blots resolved not only lamin A and prelamin A but also the farnesylated and nonfarnesylated forms of prelamin A. They showed, convincingly, that the HIV protease inhibitors lead to the accumulation of farnesylprelamin A but not nonfarnesylated prelamin A. They also showed that HIV protease inhibitors interfered with the enzymatic activity of ZMPSTE24 but had no effect on other enzymes in the pathway, such as protein farnesyltransferase, the isoprenylcysteine carboxyl methyltransferase, or RCE1, another prenylprotein endoprotease.

Coffinier *et al.* (4) used concentrations of lopinavir (20 μM) that are similar to therapeutic plasma levels in humans (15), so it is likely that their results are physiologically relevant. Drug concentrations needed for ZMPSTE24 inhibition are clearly higher than for inhibition of the HIV protease. However, AIDS therapy requires complete inhibition of HIV protease activity, necessitating very high therapeutic levels that can partially inhibit ZMPSTE24 and give rise to small amounts of unprocessed intermediates that may be toxic (9). The data presented by Coffinier *et al.* appear to show that atazanavir, a newly approved HIV protease inhibitor of the saquinavir family, results in less farnesylprelamin A accumulation than lopinavir. It is intriguing that atazanavir has been associated with milder degrees of lipodystrophy (16). They also found that a tipranavir, an HIV protease inhibitor not in the saquinavir family, is even a better *in vitro* inhibitor of ZMPSTE24 activity than lopinavir (4). It is unclear whether tipranavir results in increased lipodystrophy, but it does cause increased plasma levels of triglycerides (17).

Why might the HIV protease inhibitors interfere with ZMPSTE24 protease activity? As shown in Fig. 1, the transition states for peptide bond cleavage with each enzyme share similarities that may allow the HIV drugs to mimic both of them. Both proteases cleave a limited number of peptide bonds in linkages often connecting hydrophobic amino acids, but their specificity cannot be explained by the amino acid sequences themselves (18). Rather, it appears that both enzymes recognize some combination of conformation and sequence of their substrate polypeptide chains. It is possible that a large part of the answer depends on the chemical groupings attached to the central core of the inhibitor structure; these groups are labeled "bells" and "whistles" in Fig. 1 because they are apparently not crucial to mimicking the transition state but are important in binding. These generally nonpolar groups allow drug entry into cells and may also facilitate their binding to integral membrane enzymes such as ZMPSTE24.

Many questions remain about the relationship between the ZMPSTE24 inhibition observed in these experiments and the side effects of HIV protease inhibitors. It will be of interest to monitor the accumulation of prelamin A in different tissues of patients and to further correlate the presence of side effects with ZMPSTE24 inhibition, especially with new generations of HIV protease inhibitors. It will be particularly intriguing to see whether variations in ZMPSTE24 expression levels can explain why some patients taking HIV protease inhibitors have mild side effects, whereas others have severe side effects. Hopefully, at some point, HIV protease inhibitors will be found with just the right "bells" and "whistles," strictly limiting their action to the intended target.

Many human proteases represent attractive targets for drug therapy (19). Approximately 400 genes encoding proteases have been identified in the human genome, and it has been estimated that the total number may rise to include ≈500 of the 30,000 human genes (20). Some 70 different human proteases are now being investigated as pharmaceutical targets (20), and what we learn from HIV protease and ZMPSTE24 may be very instructive in these pursuits.

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