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 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-carboxylic 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).

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
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 carboxy-terminal–SIM sequence. Abnormal processing of prelamin A by the loss of this terminal–SIM sequence. Abnormal processing of prelamin A by the loss of this terminal 15-aa domain, although it can

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 non-polar 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.