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Discordant Effects of Gag Cleavage Site Mutations on the IC_{50} of Protease Inhibitors and on HIV Replicative Capacity.

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Background

- HIV variants developing resistance to protease inhibitors accumulate mutations in the protease and in some cleavage sites (CS) in Gag.
- The best characterized of these CS mutations, most commonly seen in viruses with advanced resistance to protease inhibitors, are located in the neighborhood of the SP2 spacer peptide that separates the NC and p6 proteins in Gag (Figure 1B).
- These CS mutations have been found to partially compensate for resistance-associated loss of HIV replicative capacity (RC). However, the extent to which they also contribute to changes in IC_{50}, and the question of whether such IC_{50} changes are independent of changes in replicative capacity, remain to be fully elucidated.

Methods

- Gag, PR and RT sequences from 3 highly resistant viruses, obtained from patients of the ANRS 109 VISTA study, were cloned into pNL4-3. All 3 vriuses contained multiple mutations in PR (Figure 1A) and at least one CS mutation in Gag : A431V in viruses VIS13 and VIS18, I437V in virus VIS16 (Figure 1B).
- Two types of clones were constructed : BS clones, which carried the whole Gag-PR-RT sequences from patient virus, and XS clones, in which the only patient-derived sequences were PR and RT (Figure 1C).
- To ascertain the individual role of CS mutations in resistance and RC, CS mutations were individually removed from patient-derived Gag sequences in BS clones and individually introduced in the NL4-3Gag sequences of XS clones (Figure 1D).
- The IC_{50} and RC values of recombinant viruses and site-directed mutants was evaluated in a single-cycle assay.

Results

Figure 1 : PR and Gag sequences, construction of recombinant clones and of site-directed mutants

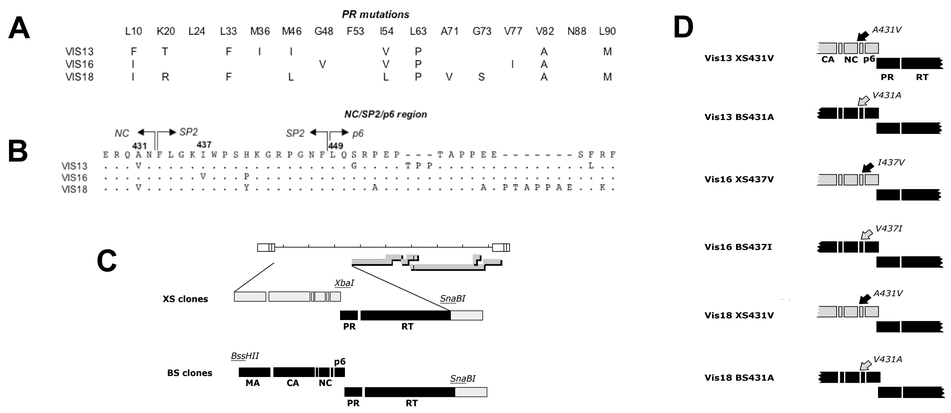


Figure 2 : IC_{50} of protease inhibitors (IDV, APV, LPV) on recombinant and mutant clones

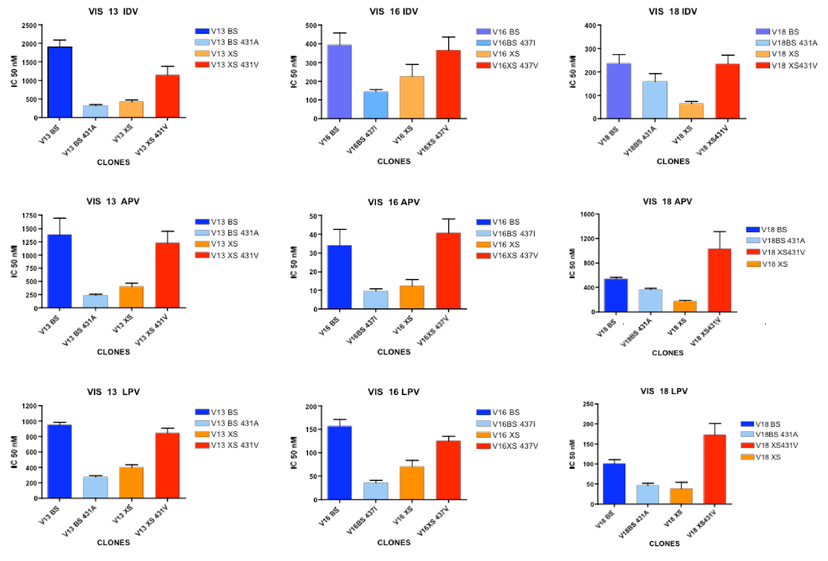
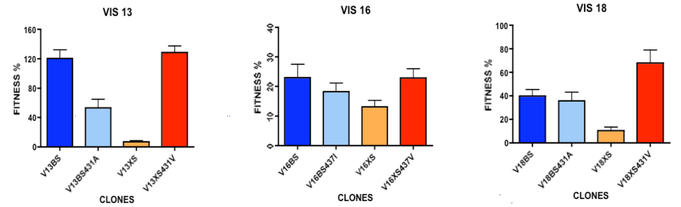


Figure 3 : Replicative capacity of recombinant and mutant clones



Summary

- The removal of CS mutations from BS clones produced dramatic reductions in IC_{50} for viruses VIS13 and VIS16 (Fig 2, ■ *vs* ■) to levels comparable to that of the XS (■) clones. These results revealed the remarkable importance of CS mutations in resistance, an effect carried without apparent involvement of other Gag sequences. This was not clearly the case for virus VIS18, in which the effect of reversion of CS mutation A431V was less prominent. In all 3 virus backgrounds, introduction of CS mutations (■) in XS clones restored resistance to BS levels
- In contrast to the IC_{50} results, reversion of CS mutations in BS clones only produced a partial reduction in RC compared to XS clones (Figure 3), indicating that Gag determinants outside of the p1 CS sequences may participate in correction of protease resistance-associated loss of RC. Surprisingly, however, introduction of CS mutations in the NL4-3-derived Gag sequences of XS clones fully restored RC to BS levels.
- Taken together, our results reveal that in viruses carrying multiple PR mutations, ***Gag CS mutations not only affect RC, but also strongly contribute to resistance***. Interestingly, their removal is sufficient to promote strong changes in IC_{50}, but not sufficient to promote comparable changes in RC. These findings suggest that ***CS mutations could play a direct role in resistance,*** but that ***they may work in concert with other Gag determinants to improve viral fitness***.

Acknowledgements

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