braries can be readily applied to select from a wide variety of possible peptides.

Whether the parent Tat peptide shows the penetrating capability of other known CPPs is uncertain.[12,16] The RGD peptide has long been used to facilitate the transport of bioactive molecules through adsorptive endocytosis.[17] However, comparison of short oligolysine peptides to that of equivalent length polymers of arginine showed that oligoarginine was much more efficient in carrying GFP into cultured cells.[18] Conversely, the ability of short oligolysine peptides, from 6 to 12 residues in length, has been shown to be more efficient than oligoarginine in carrying larger macromolecules (60—500 kDa) into cultured cells. In this study, the 435B and 439A peptides contained hardly any basic amino acids but still displayed an ability to mediate cell penetration more efficiently than the parent Tat peptide. This result indicated that the cell penetrating activity of CPPs is controlled not only by the electrical charge but also by the structural characteristics. Practically, it is known that the amino acid component and associated tertiary structure of peptides are essential for cell penetrating activity.[19] We did not examine our peptides on a structural level; however, the results presented here suggest the importance of hydrophobicity, as hydrophobic amino acids were enriched by sequential biopanning.

It has been theorized that the ionic interaction between positively charged Arg residues in these CPPs and the negatively charged phosphate head group of the membrane lipid bilayer plays a key role in CPP membrane interaction.[20] However, the exact mechanism by which these CPPs operate is still largely unknown. Work performed by several investigators has shown that Tat binds heparin and that this heparin/Tat interaction involves the basic domain of Tat.[21] Meanwhile, previous study showed that the histidine residues of peptide sequence might enhance an endosomal escape of the cargo.[22] In this study, the 435A and 439B peptides did not show enrichment of basic amino acids, but did exhibit an increase in proportion of hydrophobic amino acids. Several numbers of histidine residues were included in 435B and 439A peptide sequence compared to native TAT peptide. Accordingly, it is possible that these peptides do not penetrate through the binding of cell surface receptors such as HSPG but escape from endosome efficiently. Whereas the conformation of these peptides should be examined, our results suggest that the 435B and 439A peptides penetrate the cell membrane independently of cell surface receptor.

In this study, the transduction efficiency was observed to be different between the peptides fused with PSIF and those labeled with FITC. We think there are two ideas to explain this discrepancy. Firstly, the molecular size and structure of the respective cargo is different. Secondly, there is some possibility that the intracellular kinetics is different between the parent Tat peptide and our peptides. It is thought that the parent Tat peptide is transferred to nuclei after penetrating the cell membrane, while our mutant peptide-PSIF conjugate diffuses throughout the cytoplasm. We are currently examining the intracellular kinetics of these peptides in an effort to resolve this issue.

In this study, our peptides have a unique sequence compared to preexisting CPPs. These peptides are able to introduce a large molecule into the intracellular space more efficiently than the parent Tat peptide, the latter which is known to have a high level of transduction ability. Using these peptides, efficient introduction of large molecules to the cytoplasm is accomplished. As such, one could readily conceive of using these peptides to target disease-related proteins, revealed from extensive-omic analysis. Furthermore, our peptides can be used as analytical tools to explore the mechanism(s) of peptide penetration.

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