PEPTIDES FOR MACROMOLECULAR DELIVERY

Tech ID: 32434 / UC Case 2021-192-0

PATENT STATUS

Patent Pending

BRIEF DESCRIPTION

This invention includes:

1) Applying amphiphilic peptides (AP) (e.g. E5-TAT, INF7-TAT, or similar peptides) in novel scenarios for delivering CRISPR-Cas9 RNPs and performing genome editing in primary human cells of substantial clinical value (human pluripotent stem cells [HSPCs], T cells) and mouse neuronal progenitor cells in vitro or in vivo. These peptides have also been used for delivering plasmid DNA as a DNA vaccine into human cells in culture, and into mouse tissue in vivo to produce a robust immune response.

2) Novel peptide sequences (derivatives of E5-TAT or INF7-TAT) with improved properties and/or improved activity (relative to the founder peptides) in delivering cargo into target cells. The inventors have created a library of related sequences, all with distinct activity in delivering cargo to different cell types. These novel peptide sequences have been applied to the same scenarios as above, with improved outcomes compared to the parent peptides, E5-TAT or INF7-TAT, in genome editing, and may provide benefits in delivery of plasmid DNA as well. This is especially valuable when delivering to cell types that are notoriously difficult to transduce, such as HSPCs and T cells, leading to new therapeutic opportunities.

BACKGROUND

Biological macromolecules offer great potential as therapeutics but the greatest hurdle that remains is their efficient cell entry into target cell types. Cell entry is limiting for two very promising technologies, DNA vaccines and genome editing, and this invention aims to address the unmet need.

DNA vaccines would provide a rapid and inexpensive approach to vaccination for a wide range of viral pathogens, but have been hampered by poor delivery of the DNA into the target cells, resulting in an immune response not potent enough for effective vaccination. DNA vaccines involve delivering a plasmid which encodes a viral protein into the nucleus of human cells, where it can be transcribed and then expressed to be recognized by the immune system. In order to improve their efficacy, DNA vaccines have been delivered via in vivo electroporation, a painful process that requires specialized equipment and repeat dosing.

Genome editing holds immense therapeutic promise for correcting the genetic mutations underlying disease, or for preventing or treating non-genetic disease. Delivery of the genome editing enzymes, such as CRISPR-Cas9, into the cytosol or nuclei of cells in need of manipulation remains the largest hurdle.

Delivering CRISPR-Cas9 as a ribonucleoprotein (RNP) complex offers many advantages compared to other approaches (e.g. the use of viral vectors or lipid nanoparticles), but the RNP lacks an inherent method of cell entry. Amphiphilic peptides (AP) enable transduction of macromolecules into cells and therefore the inventors have aimed to apply APs to delivering cargo such as CRISPR-Cas9 RNPs as well as plasmid DNA to target cell types.

The activity of a specific AP in delivering cargo into the cytosol of a cell is often dependent on the specific cargo being delivered as well as the specific cell type. Therefore, the inventors have created libraries of related APs with diversity in their amino acid sequence in order to allow delivery of macromolecular cargo to a range of cell types, dependent on the specific application.

A peptide sequence derived from influenza hemagglutinin sequence, HA2, has been previously described and applied as an endosomolytic peptide (ELP). When the HA2 sequence is appended with TAT, a positively charged cell penetrating peptide sequence derived from the HIV TAT protein, the fusion peptide "HA2-TAT" is able to deliver macromolecular cargo across the cell membrane and also act as an ELP to allow endosomal escape. Derivatives of the HA2-TAT sequence with changes in the amino acid sequence of the peptide, such as the peptide "E5-TAT," has improved properties compared to HA2-TAT in solubility as well as delivering cargo into cells. The INF7-TAT peptide has similar properties, where INF7 is another glutamine-rich analog of HA2 with improved properties for endosomal escape. HA2 and its derivates (E5, INF7) with and without fusion to cell penetrating peptides (HA2-TAT, E5-TAT, INF7-TAT) have been applied to delivering macromolecular cargo such as proteins and nucleic acids into cells.

SUGGESTED USES

This invention can be used to facilitate:
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development of genome editing therapeutics, since it offers an alternative to electroporation, which works in the lab but not in a patient.

development of research reagents (e.g. transfection reagents).

ADVANTAGES

Compared to physical approaches:

Electroporation is often used to allow transduction of macromolecules (such as Cas9 RNP or plasmids) into cells. The strong electric pulse creates pores in the plasma membrane allowing the material to traverse. Electroporation has facilitated RNP-based cell manufacture in clinical trials involving T cells or human pluripotent stem cells (HSPCs) (with the latter resulting in many people being cured of hemoglobinopathies). However, electroporation is incompatible with in vivo administration of T cells or HSPCs, so its application is inherently limited. Other mechanical approaches (cell squeezing or sonication) are similarly incompatible with in vivo delivery. Electroporation is also being used to administer DNA vaccines in vivo but causes significant pain when administered and still requires multiple administrations for sufficient efficacy.

Our peptide-mediated approach offers numerous potential advantages over electroporation (and other mechanical approaches) in an in vivo context, as electroporation of cells requires very specialized equipment, highly concentrated cells and reagents, can result in substantial cell death, has sample to sample variability, and is a very costly and cumbersome addition to a workflow. In contrast, applying these peptides in conjunction with the material to be internalized by the cell is a facile approach that simply requires mixing of the reagents together, with no specialized manipulation of the cells.

This invention may be useful in vivo, as well. Macromolecular genome editing reagents (e.g. CRISPR-Cas9) are in a particle-size “sweet spot” (~12 nm) for intravenous delivery: small enough that biodistribution is optimized, but not so small that the reagents are rapidly cleared via the kidneys. APs are made from naturally-occurring amino acids and have an appealing toxicity profile. These reagents are amenable to large-scale manufacture and can enable intracellular delivery of macromolecules in many contexts.

Compared to viral delivery:

CRISPR-Cas9 and other technologies can be delivered via viral vectors into cells, where the genetic sequence encoding CRISPR-Cas9 and its guide RNA are packaged in the virus and expressed by the target cells. Viruses offer robust transduction, but have several disadvantages: manufacturing of engineered viruses at a scale relevant for therapeutics remains an immense technical challenge and is incredibly costly; viruses have a packaging limit that precludes large cargo; viruses themselves are highly immunogenic which causes issues for in vivo delivery; persisting expression of macromolecular cargo can result in immune responses against the cargo (e.g. CRISPR enzymes of microbial origin) as well as increasing unintended off-target effects in genome editing contexts.

Compared to other non-viral approaches:

Lipid nanoparticles can be used to package macromolecules such as CRISPR-Cas9 or plasmid DNA to perform cellular transduction. These technologies can be quite effective at transduction but come with the caveat of being relatively toxic to cells and tissues. When administered in vivo, lipid nanoparticles mostly enter liver tissue and tend not to reach all tissues in the organism efficiently (due to particle sizes of 50–100 nm). Delivering macromolecules (<20 nm in diameter) using peptides (perhaps tethered to the cargo) may allow targeting a broader range of tissues.

RELATED MATERIALS