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Leading-Edge Pulmonary Gene Therapy Approached by Barrier-Permeable Delivery System: A Concise Review on Peptide System

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The lack of effective treatments for pulmonary diseases poses a global health burden. The direct local gene therapy serves as one of the alternative administrations to treat pulmonary diseases. Compared with the conventional viral/nonviral system, the peptide–vector-mediated in vivo lung gene therapies exhibit various benefits. However, the related clinical trials are still in their infancy. The major obstacle to the pulmonary delivery of gene cargoes may be the barriers from various extracellular mucosal layers and intracellular membranes. This review highlights the recent development of peptide-based gene delivery systems and their applications. The peptide designing rules for the barrier-permeable pulmonary gene delivery are described first. After briefly summarizing how oligopeptides facilitate the local gene therapy in lung tissue with the focus on cell-penetrating peptides, the local delivery system of the polypeptide and several alternative hybridizing systems of the peptide with other types of materials are discussed. Finally, the blueprint and the remaining challenges in peptide designations are discussed before they enter into the real translation process.

1. Introduction

Pulmonary diseases, including chronic diseases and communicable infections, contribute to the primary mortality or morbidity among the disease-caused death globally.[1,2] Although the necessity to figure health and economic burden was led by pulmonary diseases, the development of next-generation clinical-available respiratory therapeutics remains strikingly deficient.[3] To treat such unmet medication demands, the respiratory society highlighted that the pulmonary site is a target endogenously suitable for gene therapy as the progression of many innate, acquired, or infectious lung diseases ensued from the inadequate gene layout in diseased cells.[4] For example, several clinical trials for treating cystic fibrosis (CF) were proved effectively by targeting a common mutation, deletion of residue F508, and more than 2000 disease-leading mutants.[5] Delivering the therapeutic nucleic acid directly to the diseased pulmonary cells could reduce, supply, or edit related gene sequences, resulting in inhibition of the growth of specific pathologies. For the translation of gene therapy, a promising platform for gene delivery plays a crucial role in developing effective and safe therapeutics for preventing and recovering pulmonary diseases.

The early attempts of gene delivery relied on the virus-based system. However, the low-loading scope and the safety consideration (e.g., the high immunogenicity) eventually hampered its clinical translation.[6] Various nonviral gene vectors have been developed in the past several decades such as polymeric nanoparticles, liposomes, dendrimers, mesoporous materials, lipids, peptide, and quantum dots.[7–11] Although the nonviral vectors have paved multiple cornerstones in gene delivery fields, the harasses still exist because of the limitation of some nonviral vectors, such as the stability, biodegradability, and unwanted extracellular or intracellular entrapment of materials.[12,13] Among various nonviral vectors, peptide-based gene delivery technologies have drawn increased attention in the past few decades owing to their potential to settle forenamed problems.[14,15] To date, they have been applied in immune adjuvant, anticancer therapy, molecular imaging, and organelle targeting.[16–19] Multiple peptide-based gene delivery systems have been developed, including cell-penetrating peptide (CPPs)-facilitated transfection, virus-mimic peptide particles, polypeptide nanostructure, and the hybrid peptide system assisted by polymers or lipids. Several cutting-edge peptide-based vectors open a new adventure for efficacious gene transfection. For instance, Jiang and co-workers developed a nanofibrous hydrogel that formed via the self-assembly of a short peptide to condense human immunodeficiency virus (HIV) plasmid and elicit a systemic anti-HIV immune response.[20]

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/anbr.202200113.

DOI: 10.1002/anbr.202200113

Miserez and co-workers recently discovered phase-separating peptides for direct cytosolic nucleic acid delivery with impressive gene transfection efficiency.[21]

We sought to highlight the recent significant peptide-mediated pulmonary gene delivery or therapy systems in this minireview (**Table 1**). First, we introduced the biological barriers biomacromolecular gene cargoes may encounter, emphasizing the pulmonary barrier and related peptide design roles. Next, we described the recent pioneering works using CPPs-based delivery systems for pulmonary gene therapy. Then, we discussed the recent applications of the polypeptide in gene delivery. After introducing the pure peptide-based methodologies in this field, we further enumerated several state-of-art peptide-based hybrid systems. Finally, we provided a brief outlook to discuss the challenges and future directions.

2. Barriers for Pulmonary Gene Delivery and Peptide-Based Solution

2.1. Pulmonary Barriers

The classical peptide-based pulmonary gene delivery vector must achieve fast transporting in the lung environment, maintaining stability, high cellular uptake, and effective escaping from endosomal entrapment. Although conventional gene vectors are capable for cytosolic gene expression in vitro, the extracellular biological barriers in lung structure inevitably afford these nanoscale gene cargo more scrupulous obstructions.[22] The mucus layer on the airway epithelial cells is the first obstacle for incoming inhalants. Two main natural features render the mucus layer a nonspecifically adhesive network, with an average pore size of 200 nm, to thwart passby nanostructures.[23] One of them counts for the physical obstruction generated from the complicated sterical composition.[24] The other is the intensive hydrophobic/electrostatic interaction provided by mucin protein.[25] Right beneath the mucus gel layer is the periciliary layer (PCL). This layer is taken over by membrane-spanning mucins and mucopolysaccharides tightly restricted to the airway surface, which surrounds the cilia to form a periciliary brush for clearing the incoming nanostructure via the beating cilia (**Figure 1**).[26,27] Moreover, in several obstructive pulmonary diseases, the PCL undergoes collapse due to the enlargement of the osmotic modulus in the mucus layer.[28] For alveoli-targeting inhalants, their transfection efficiency might be lessened by two alveolar barriers, pulmonary surfactant and alveolar macrophage. The reported studies summarized that type II alveolar epithelial cells secrete pulmonary surfactants, which could disturb the stability of cationic vectors and cause unwanted vector aggregation.[29,30] Unfortunately, most lung injuries would boost the production of such surfactants (Figure 1).[31] The other barrier in alveolar space is the clearance led by macrophage uptake. Such uptake via phagocytosis was largely affected by the size and the surface charge of the nanostructure, as the nanostructure with cationic charge and size between 500 nm and 6 μm is favored to be cleared.[32] When infection or inflammation occurs, alveolar macrophages would be recruited to alveoli and fulfill the duty to uptake damaged cells and invading pathogens, which will concurrently clear nanostructured gene therapeutics (Figure 1).[33,34]

The harsh pulmonary environment requires a peptide delivery system to adopt extra lung-adaptable concerns. Providentially, numerous modification strategies or peculiar peptide entities were evolved for encountering pulmonary hurdles. Hanes' group first practiced the leading-edge PEGylation strategy by generating mucus penetration particles to overcome the mucus barrier. They functionalized polymer-based nanoparticles, peptides, or protein nanocages with a high density of polyethylene glycol (PEG) and maintained efficient mucus penetration on the airway or tumor tissue.[35–37] Inspired by this, more mucus-inert or

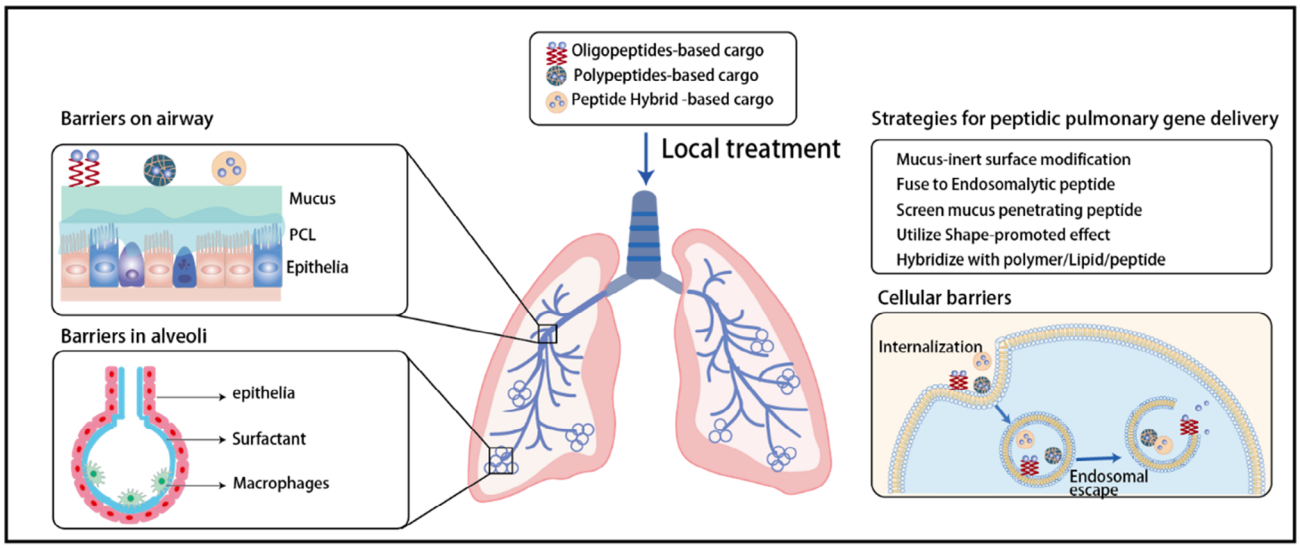

Figure 1. Schematic illustration of the pulmonary biological and cellular barrier-impeding peptide-mediated gene delivery. Pulmonary barriers composed of mucus layer and PCL at the airway and macrophage and surfactants at alveoli. Successful cellular penetration requires peptides to achieve effective cell internalization and efficient endosomal escape.

mucolytic-building blocks (e.g., mannitol or fluorocarbon) have been developed by conjugating oligopeptides or polypeptides to achieve mucus penetration successfully.[38] However, contrary concerns were elicited from the fact that some lung disease patients possessed anti-PEG antibodies at the pulmonary site.[39,40] In fact, the peptide itself could also be the mucus-penetrating agent. Ghosh's group screened several low-immunogenic peptide sequences that can help the cargoes across CF mucus by phage-display technology.[41,42] Except aforementioned surface modification, the shape-promoted effect in peptide-based pulmonary delivery and nebulization development was also demonstrated in Disher's and Collier's groups.[43,44] Because anisotropic nanorod or filamentous structure performed larger penetration through mucus pore and largely avoid pulmonary clearance, compared to spherical nanostucture.[45,46] To avoid the macrophage clearance in alveolar site, a series of stealth modifications applied on polymeric nanomedicine would benefit the peptide system as well, including PEGylation, conjugating with zwitterionic polymers. Recently, an important peptide-based "stealth" strategy was developed, as Disher and co-workers synthesized a new species of "self" peptide to mimic the "marker of self," CD47, and inhibit undesired phagocytic clearance.[47] Overall, in recent years, peptide society eyeballed the rising number of peptide nanostructures to overcome aforementioned barriers and inspired the future design.[48]

2.2. Cellular Barriers

After penetrating the extracellular barriers, the gene cargoes may be further barricaded by cellular barriers (Figure 1). First, before the cytosolic or nuclear cargo release, the peptide vector should interact with the cell membrane and switch on downstream internalization pathways. Contradictorily, extracellular barriers and cell membrane penetration may require inimical physicochemical properties, such as converse hydrophobicity or surface charge. Some stimulus–responsive peptide sequences cracked this predicament as the moieties for the extracellular mission were cleaved or transferred by the extracellular enzyme (e.g., alkaline phosphatase and matrix metallopeptidase) and acidity or external physical stimuli.[49–51] After cellular internalization, those nanostructures tend to be trapped in endosomes and then undergo degradation.[52] As endocytosis is the main pathway to uptake biomacromolecules containing nucleic acids, therefore, designing capacitating sufficient and fast endosomal escape is crucial to peptide-based vector design. To the best of our knowledge, such a solution was approached by peptide-mediated endosomal pore formation, membrane fusion, and pH-activated endosomalysis.[53,54]

3. Positively Charged Peptides Deliver Nucleic Acid to the Lung

Positively charged peptides hold the ability to compact and facilitate the cellular uptake of negatively charged nucleic acids. Conjugating a positively charged sequence to a self-assembled peptide helped to earn extra gene binding and delivery efficiency in deep tissues. For example, the conjugation of a tripeptide (Lys–Arg–Lys) at the C-terminal of a surfactant-like peptide allowed local BCL2 silencing in the deep brain using assembled nanofibers.[55] Recently, a family of receptor for advanced glycation end products (RAGEs)-binding peptides (RBPs) offered the majority of pulmonary prospects. Lee and co-workers introduced the early pulmonary usage of RBPs that relied on its anti-inflammatory property for treating acute lung injury (ALI). RBP works as the cationic antagonist to the RAGE, the activation of which mediates further lung inflammation.[56,57] They put forward a dual anti-inflammation therapy for ALI treatment using an inhalable nanocomplex consisting of the anti-inflammatory adiponectin plasmid and RBP in 2019.[58] Compared with a commercially available polymeric carrier, the RBP-mediated gene therapy promisingly mitigated lung inflammation, without leading to side effects. Such encouraging efficacy was explained as the low-positive charge of RBP attenuating the interaction between nanocomplex and the negatively charged mucus.[58] Two years later, Yin and co-workers published a lung-injury-aimed propeptide-reinforced system based on the RBP-cis-aconitic amide (RC).[59] Realizing that the positive charge of RBP still failed to maximize the transfection rate due to unwanted mucus entrapment, Yin engineered the RBP peptide with an inflammation-instructed charge reversal property.[59] Specifically, the RBP first underwent acidic shedding to hold a negative surface charge for successful mucus penetration.[59] When it reaches the diseased air space, the acidity of inflamed alveoli could turn negatively charged RC back to positively charged RBP for subsequent cellular/endosomal membrane penetration (**Figure 2**).[59] With this charge-reversal functionality, RC afforded ≈80% TNF-α mRNA knockdown, thus avoiding further cytokine storm in ALI.[59,60]

4. CPP as the Pulmonary Gene Delivery System

CPPs are widely utilized as the delivery vehicle for transporting various cargoes, such as proteins, hydrophobic molecules, or nucleic acids.[61,62] In the pulmonary field, CPPs were proved to be capable of delivering therapeutics to the respiratory tract through local administration and are beneficial for the recovery of multiple pulmonary diseases.[60,63] The positively charged CPPs bind negatively charged nucleic acids through electrostatic interaction and enhance the cellular internalization of nucleic acids through its membrane penetration specialty. In addition, the present studies revealed that the CPPs are responsible for the cargo's permeabilization through extracellular biological barriers (e.g., the blood–brain barrier).[64] The first identified CPP sequence (TAT) was discovered in 1988 from HIV.[65,66] Subsequently, CPPs have attracted extensive interest because of the emergence of several new types of CPPs in the gene delivery field, such as TAT-derived peptides, arginine-rich peptides, or lysine-rich peptides.[61]

Although frequently applied in numerous in vitro transfections, few projects reported pulmonary gene delivery using CPPs. The most straightforward strategy to enhance pulmonary transfection is to fuse the existing peptide to other functional moieties, for example, a transfection enhancer. Krissansen and co-workers planned to utilize a new class of CPPs named Xentry for mRNA transfection in pulmonary cells, which failed to provide competitive mRNA transfection efficiency in cancer

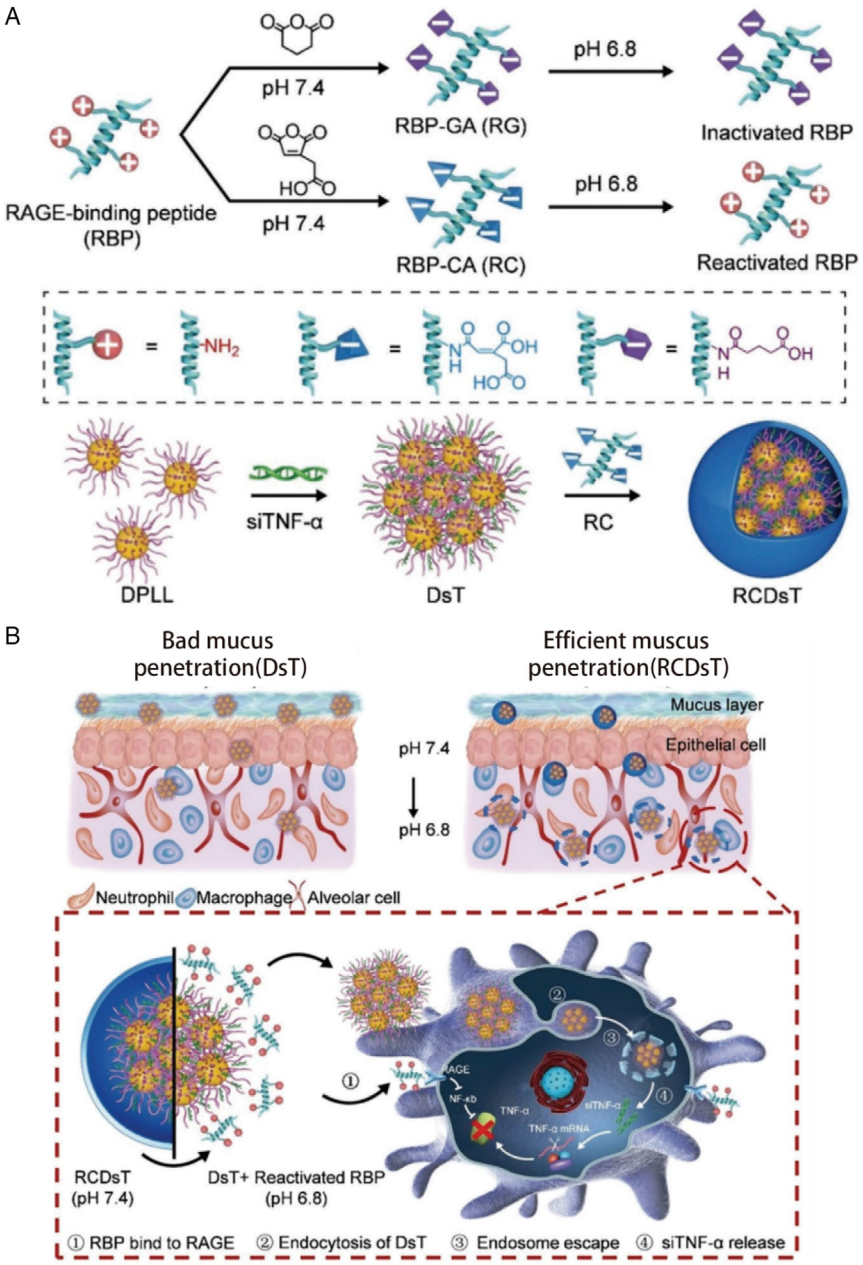

Figure 2. A) The schematic illustration of an acid-sensitive propeptide-based nanocomplex with charge-reversal capability. B) The scheme of the nanocomplex enabled efficient mucus and macrophage membrane penetration process. The negatively charged surface allowed efficient mucus penetration due to electrostatic repulsion, whereas the positive charge of RBP was retrieved due to acidity in inflamed alveoli. Reproduced with permission.[50] Copyright 2021, Wiley-VCH.

cells.[67] To address this problem, they fused Xentry to truncate the human protamine peptide and generated Xentry protamine fusion peptide (XP) as protamine can stabilize DNA in the sperm.[67] The other obstacle that needs to be improved is that the innate immune responses toward exogenous nucleic acid cargo may lead to undesirable intracellular clearance and a downturn in the transfection rate.[67] Thus, the same group further screened a transfection enhancer, named toll-like receptor (TLRs) antagonist, to suppress such an immune response.[67] Interestingly, after adding 20 μm of TLR antagonist E6446, XP could convey the CF transmembrane regulator (CFTR) mRNA into CFTR-deficient HEK293T cells with an increased expression (up to 62%) compared with the commercially available reagent.[67] Another strategy is to conjugate CPPs by targeting peptides for specific gene delivery. Zahid and co-workers discovered two lung-targeting peptides based on their original artificial cardiac-targeting peptide (APWHLSSQYSRT) by alanine screening.[68] These two peptides, S7A (APWHLSAQYSRT)

and R11A (APWHLSSQYSAT), could be taken up by human bronchial epithelial cells through a nonclathrin-dependent endocytic process within 30 min.[68] Furthermore, the ex vivo imaging studies suggested that R11A could maintain the robust pulmonary accumulation at 15 min postsystemic administration at a dosage down to 1 mg kg^{-1} in mice.[68] To evaluate whether the best-performed R11A could potentiate anti-COVID therapy, the authors also synthesized a more physiologically stable cyclic R11A (cR11A) and conjugated it to siRNA against SARS-CoV-2 spike proteins.[68] The resultant siRNA–peptide conjugate could be cleaved in VERO76 cells and inhibit SARS-CoV-2 replication ($EC90 = 64 \pm 0.2$ μm).[68]

Up to now, CPP-mediated gene therapy has embraced more translational concerns. In terms of that, few respiratory pharmacists are trying to develop gene-loaded CPP dry powder or spray to confer clinical applications.[69,70] Lam's group employed spray drying and spray freeze drying to manufacture an inhalable mRNA-loaded peptide dry powder. In detail, the group grafted PEG12 to a cationic peptide (KL4) that is derived from surfactant protein B.[69] This PEG12KL4/mRNA formulation showed sufficient mRNA expression in A549 cells (**Figure 3**B). Encouragingly, this inhalable particle achieved acceptable mRNA release in the deep lung region of locally treated mice (Figure 3A) because of its suitable aerodynamic properties for respiratory deposition (Figure 3C).[69] McCarthy's group recently recount the effect of each process parameter in manufacturing inhalable CPPs/gene dry powder using a delivery peptide (N-WEARLARALARALARHLARALARALRACEA-C) (RALA) peptide as a vehicle and D-mannitol as an excipient.[70] They adopted a factorial design to optimize this spray-dried formulation to encapsulate pEGFP-N1, in which the optimized nanoparticle (<200 nm) maintained 61–89% DNA recovery, and the encapsulation efficiency was larger than 65%.[70]

The state-of-the-art gene therapy focuses on remedial genome editing via CRISPR/Cas system.[71,72] Regretfully, the cytosolic delivery of gene and recombinant protein via CPPs was largely impeded due to the endosomal sequestration.[73,74] To overcome this hindrance, David Guay's group developed a novel sort of membrane-permeabilizing amphiphilic peptide (CPP-endosomal leakage domain (ELD)) and achieved swift cytosolic delivery of CRISPR-Cas9 in hard-to-modify natural killer (NK) cells. In their engineering, the fusion of HIV-TAT variant PTD4 sequence (YARAAARQARA) to an endosomolytic peptide CM18 (KWKLFKKIGAVLKVLTTG) generated the optimal delivery vehicle 6His-CM18-PTD4. This peptide could convey Cpf1-NLS ribonucleoprotein (RNP) and induce 27% genome editing in NK cells. Furthermore, the resultant peptide promoted direct homologous recombination when coshipping multiple CRISPR/Cas RNP systems in HELAs.[75] Based on this successful transfection in NK cells, Guay's group proved the feasibility of RNP delivery to the hard-to-transduce respiratory tract via CPP-ELD peptides in 2019.[76] Their best-performed peptide candidate s10 contains three components: length, hydrophobicity/charge, and linker (Figure 3D): 1) the incorporation of five highly hydrophobic residues in the CM18-derived domain; 2) the optimization hydrophilicity and cationic of PTD4 sequence; and 3) a linker (GGSGGGS) between these two domains. Formulating by simple incubation of s10 with Cas RNP, this peptide mediated 26% indel frequency (Figure 3F) in non-CF human primary airway epithelial by delivering Cas12a RNP targeting CFTR intron 22-23 (Figure 3E).[76] Furthermore, they investigated whether the intranasal administrations of s10 and loxP-targeting Cas9 RNP could enable pulmonary gene editing in ROSAmT/mG mice (Figure 3G).[76] The results suggested that the RNP distribution pattern in the mice lung was constrained into airway epithelia, where s10 completed $13 \pm 2\%$ editing efficiency on large airways and $12 \pm 1\%$ editing efficiency on small airways (Figure 3H,I).[76,77] Apart from that, this s10-mediated gene therapy did not lead to acute toxicity, which is manifested by the absence of abnormal immune cell infiltration in bronchoalveolar lavage (BAL).[76]

5. Efficient Pulmonary Gene Delivery Aided by the Polypeptide

The pulmonary delivery efficiency of gene therapy during the diseased stage could be further weakened due to the appearance of the thickened mucus layer and the enhanced immune cell clearance.[77] One strategy to such a thickened airway was characterized recently, in which the researchers endowed the polycationic peptide vector with the ability to overcome the mucus layer and cell membrane by guanidination and fluorinated bifunctional modification on the side chain of peptides.[38] The rationale for this design could be elucidated as fluorination was reported to enhance the serum stability of biomacromolecule-containing cargoes due to fluorocarbon's lipophobic/hydrophobic property.[78] Yin and co-workers synthesized a series of fluorinated polypeptides based on the disparate number of fluorine atoms (m) and graft ratios (x mol%) on the side chains of polypeptides.[38] Subsequently, the local administration of optimal P3F16/siTNF-α or P7F7/siTNF-α polyplexes provided promising anti-inflammation efficacy in a mice model of lipopolysaccharides-challenged ALI.[38] The level of pulmonary TNF-α and related proinflammatory cytokines dwindled by more than 90%.[38] Hence, several previously exacerbating pathological symptoms recovered to the average level, including blood pH, PaO_2, interstitial edema, and thickening of the alveolar wall.[38] This satisfactory anti-inflammation efficacy shall be credited to the solid mucus penetration ability of fluorinated polypeptide (**Figure 4**A).[38] Herein, specific convincible evidences were listed. First, their fluorescent imaging suggested that P3F16 and P7F7 provided the most powerful siRNA internalization (22–25 folds) in an air-interfaced culture of Calu-3 cells covered with a monolayer of mucus (Figure 4B).[38] Meanwhile, multiparticle tracking indicated that the fluorinated polypeptides P3F16 and P7F7 exhibited larger diffusion in 5% CF mucus compared with their counterpart (Figure 4C).[38] Yin clarified that the mechanism of enhanced penetration is due to the fluorination-contracted surface energy and created steric hindrance to prevent mucin absorption sustainedly (up to 4 h in mucin solution).[38]

6. Efficient Pulmonary Gene Delivery Aided by Peptide Hybrid System

Upon the dilemma that vectors containing single-component cationic peptides were hard to fit more than one barrier, a broad

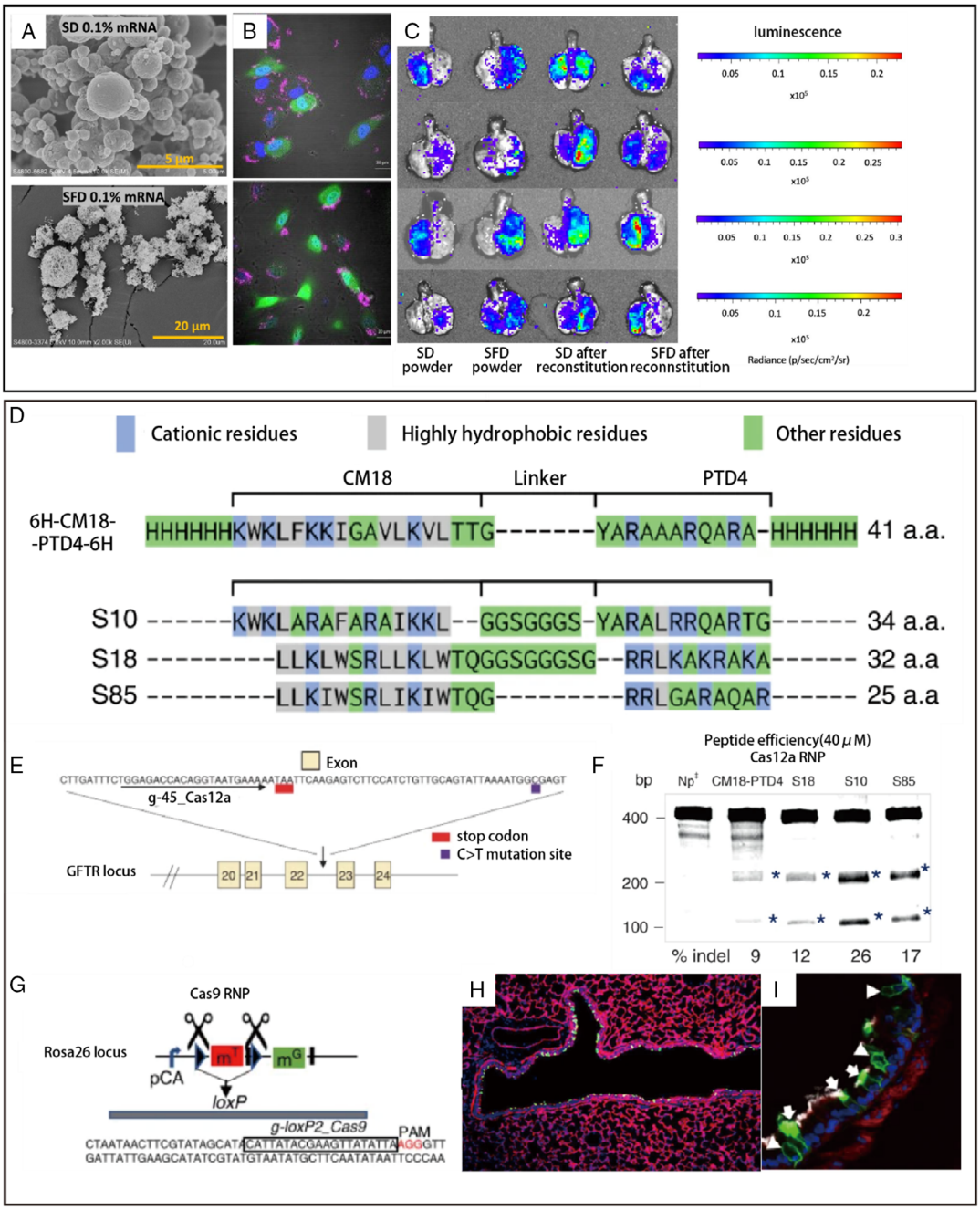

Figure 3. A) Representative SEM images of spray-dried (upper image) and spray-freeze-dried (lower image) formulations of PEGylated KL4 peptide and mRNA. B) Confocal laser scanning microscopy (CLSM) images of the PEG12KL4 peptide-mediated intracellular distribution of Cy5-labeled mRNA and enhanced green fluorescent protein expression (green). Upper image: 4 h post-transfection; lower image: 24 h post-transfection. C) Bioluminescence imaging indicating pulmonary mRNA expression postintratracheal injection of powder aerosol or reconstituted liquid aerosol. Reproduced with permission.[69] Copyright 2019, Elsevier. D) Amino acid sequences of shuttle peptides for RNP delivery to airway epithelia. E) Scheme of the targeted sequence of Cas12a guide RNA in CFTR locus containing 3849 + 10C > T mutation. F) Gene editing efficiency of Cas12aRNPs in CFTR locus mediated by shuttle peptides. G) Scheme of gRNA-aimed sequences in ROSAmT/mG mice. H) Fluorescence image of large airway after 7 days postintranasal injection of s10 and RNP cargo. Green fluorescent protein (GFP) (green). I) Colocalization of GFP with marker of ciliated cells. α-tublin (white). Reproduced with permission.[76] Copyright 2019, Nature Publishing Group.

range of peptide-based hybrid systems thrived and started to guide the new way out. The peptide could generally hybridize with lipid, polymer, or other peptides to earn additional versatility.

6.1. Peptide–Lipid Hybrid System

With the participation of a third component, peptides could afford responsibility other than gene compaction. For example,

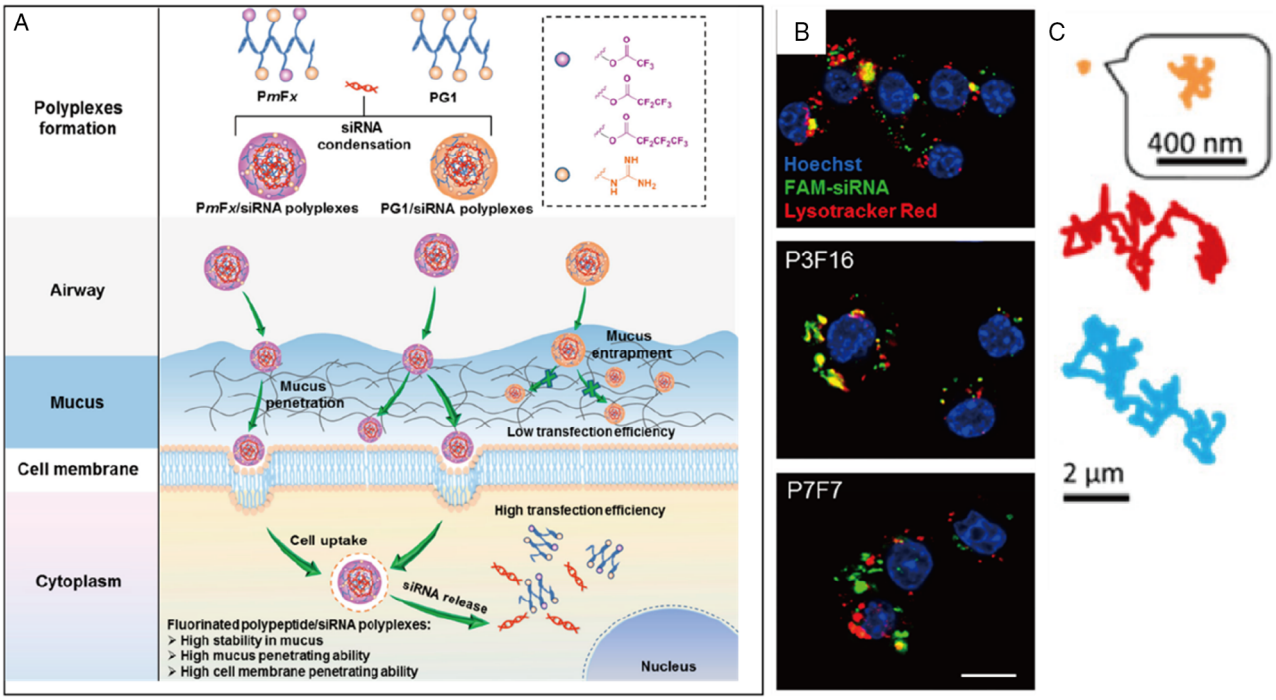

Figure 4. A) Schematic illustration of fluorinated and guanidinated polypeptides-enabled transmucus and transmembrane siRNA delivery in the respiratory tract. B) CLSM images of cells treated with FAM-siRNA-loaded polyplexes. FAM-siRNA (green), Lysotracker, and Hoechst (blue). C) Multiparticle tracking of polypeptide-based nanocomplex in the human CF mucus. PG1 (orange), P3F16 (red), and P7F7 (blue). Reproduced with permission.[38] Copyright 2020, American Chemical Society.

in the hybrid system enclosing lipid materials, the peptides usually comprised the targeting moiety covalently or noncovalently. Several targeting peptides (e.g., arginylglycylaspartic acid families) have long been conjugated to lipids for cell targeting and facilitating the deep penetration in solid cancers.[79,80] Except for covalent linkage, noncovalent binding was also favored. Hart's group recently introduced a lipid–peptide hybrid system via noncovalent formulation. This study integrates lung–epithelial-targeting peptides (K16GACSERSMNFCG) into DOTMA/DOPE system. The formulated hybrid particle could quickly diffuse through porcine gastric mucus at a rate of 1046.1 ± 47.6 ng cm^{-2}.[81] They similarly formulated the same peptide with siRNA-loaded liposome to generate the LPR particle, whose morphology fit the aerodynamics of a qualified inhalant. This LPR was proved responsible for inhibiting the hyperactivity of the ENac channel in CF via siRNA-mediated silencing.[82] This formulation was demonstrated inhalable as the ENac silencing capability could be retained after nebulization.[82] Furthermore, a recent report in 2021 showed that the lipid particle is competent for nebulized pulmonary mRNA delivery.[83] We anticipate that peptidic moiety could benefit those inhalable lipid particles via various functionalities rather than targeting.

6.2. Peptide–Polymer Hybrid System

For smooth pulmonary biological penetration, the most direct and prevalent polymer hybridization is PEGylation or conjugation with PEG-containing polymer.[84] Although this polymer modification provides the so-called mucus-inert property, several studies doubted these conjugated entities as such decorations might weaken the cellular internalization due to the comprised intercommunication with the cell membrane.[85] This limitation is addressable using the multielement hybrid system because the hybrid system is easily accessible to more than one building block, implanting mucus penetration vectors an extra cell penetration function or targeting ability. Dixon's team recently focused on the glycosaminoglycan (GAG)-binding enhanced transduction (GET) because they could promote endocytosis by the interaction between peptides with membrane heparan sulfate.[86] They introduced a peptide–polymer hybrid nanoparticle for the synchronously enhanced mucus and cell membrane penetrating by grafting CPP with GET peptide and PEGylation (**Figure 5**A), which showed a tunable (Figure 5B, sC) pulmonary transfection rate.[37]

However, the covalent linkage of the original peptide to numerous blocks could escalate the entire complexity and then bring unpredictable harmful degradation products. Therefore, the noncovalent binding might figure out this problem. Rosenecker and co-workers published a translational-favored nanoparticle system aiming at genome restoration in CF.[87] An FDA-approved copolymer poloxamine 704 (T704) was applied in their engineering for its acknowledged biocompatibility and the capability for mucus penetration, which is proved in the clinical trial of T704-assisted plasmid therapy for CF.[88] Most essentially, a synthetic peptide was formulated with the original T704 system by noncovalent interactions to enlarge the

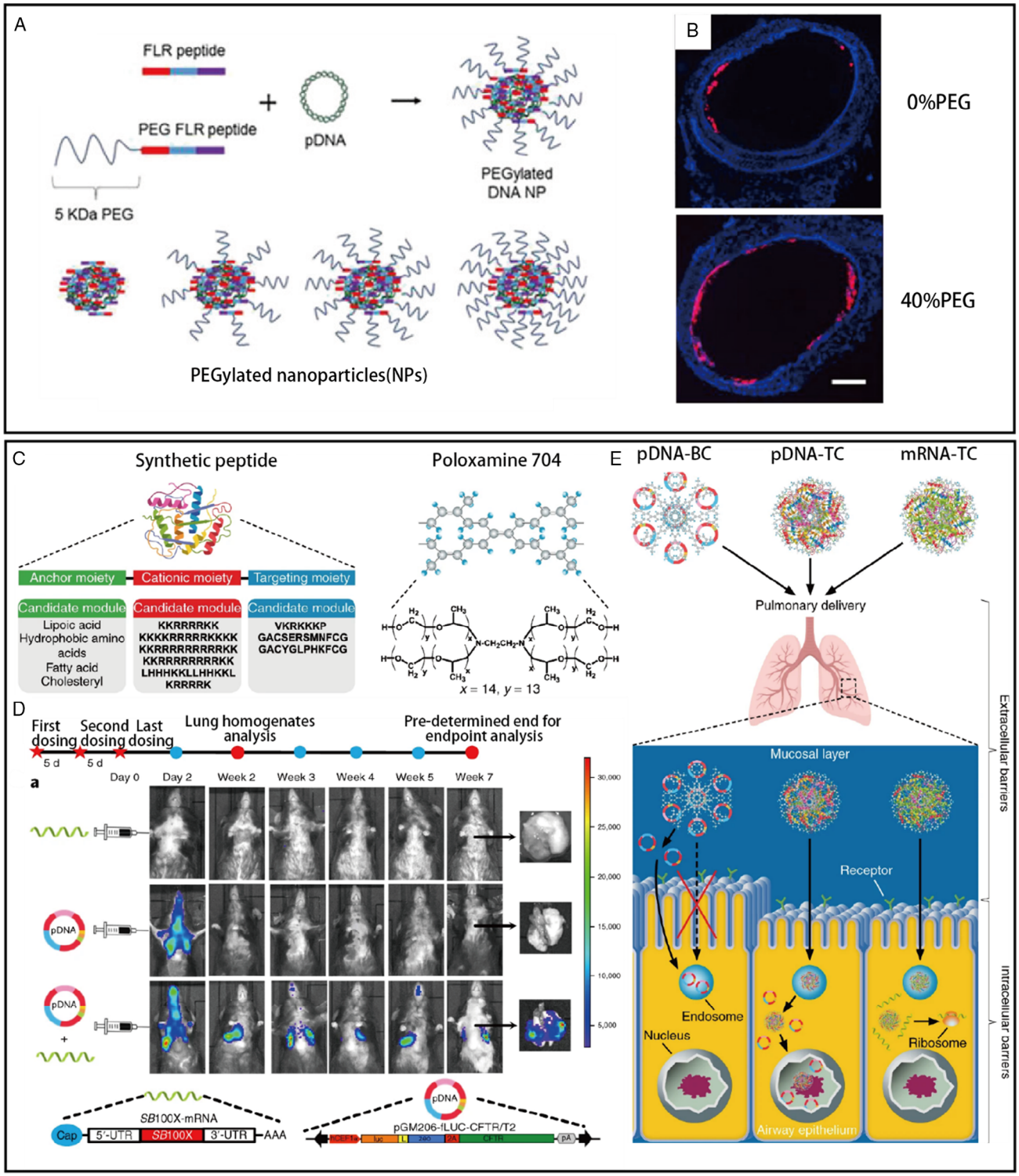

Figure 5. Pulmonary-applicable peptide systems hybridized with polymers. A) Schematic illustration of the gene cargo formulation blending PEGylated or non-PEGylated GET transfection peptide FGF2B LK15 8R peptides and pDNA. B) Representative images of the distribution of the peptide-based nanoparticles on the airway. The formulation bearing 40% PEGylated peptide showed enhanced accumulation on airway epithelia. Reproduced with permission.[37] Copyright 2018, Elsevier. C) Molecular structure of the multimodular peptide and poloxamine 704 (T704). D) The scheme of the proposed pulmonary delivery and intracellular delivery of the binary or ternary formulation. E) The experiment scheme and bioluminescence images illustrate the long-term luciferase expression mediated by SB transposon system-loaded ternary complex. Reproduced with permission.[87] Copyright 2019, Nature Publishing Group.

extracellular and intracellular transportation simultaneously because the individual usage of T704 might result in discontented cellular internalization and endosomal escape[87] (Figure 5D). The combination allowed each component to self-assemble synergistically to form ternary complexes (Figure 5F).[87] The peptide addition granted the ternary self-assembly a monodispersed uniform spherical shape with a near neutrally charged surface compared with the even morphologies of the gene/T704 binary system.[87] Next, in their initial in vitro and in vivo pursuit, they optimized ternary complex mediated long-term CFTR expression with pDNA and mRNA signals (at least 2 weeks) in CFBE-delF cells.[87] The bioluminescence imaging conceded significant enlargement of pDNA or mRNA expression in the lung of CF mice without causing lung inflammation. Compared with the T740 or peptide-assisted binary system, the augment in transfection is due to two aspects. First, peptide addition enables more diverse cellular uptake pathways.[87] Second, T704 protects the cargoes against pulmonary clearance.[87] These solid results further stimulated a long-term pulmonary CFTR expression study, as the mRNA and pDNA cotransfected mice showed a greater gene-related luciferase signal and CFTR level in the lung at 18 weeks postlast local injection (Figure 5E).[87] The emergence of this versatile peptide–polymer hybrid nanoparticle was highly valued, as it ensures the safe and lasting expression of therapeutic protein and broadens the possible clinical usage targeting other organs based on certain peptide modifications.

6.3. Peptide–Peptide Hybrid System

Except for the nanocomplexes or nanoparticles listed previously, peptide holds the promising potential to form hydrogels as a topical therapy on various organs.[89] One frequent usage is topically applying peptide-based hydrogels on the myocardium to promote cardiomyocyte regeneration or vascularization in myocardial infarction.[90,91] In addition, we have sighted these peptide hydrogels that provided regenerative recovery in other mucus-harbored tissues, including brain and ocular tissue.[92–94] In the pulmonary area, a unique pulmonary surface-anchorable peptide hydrogel system was introduced to the ex vivo study on porcine lung by Hoang and Schneider last year.[95] This surface-filled hydrogel (SFH) has drawn special attention due to its special surface-fill property to fit the pleural cavity, caused by surgical debulking of mesothelioma, and then released miRNA 215 or 206

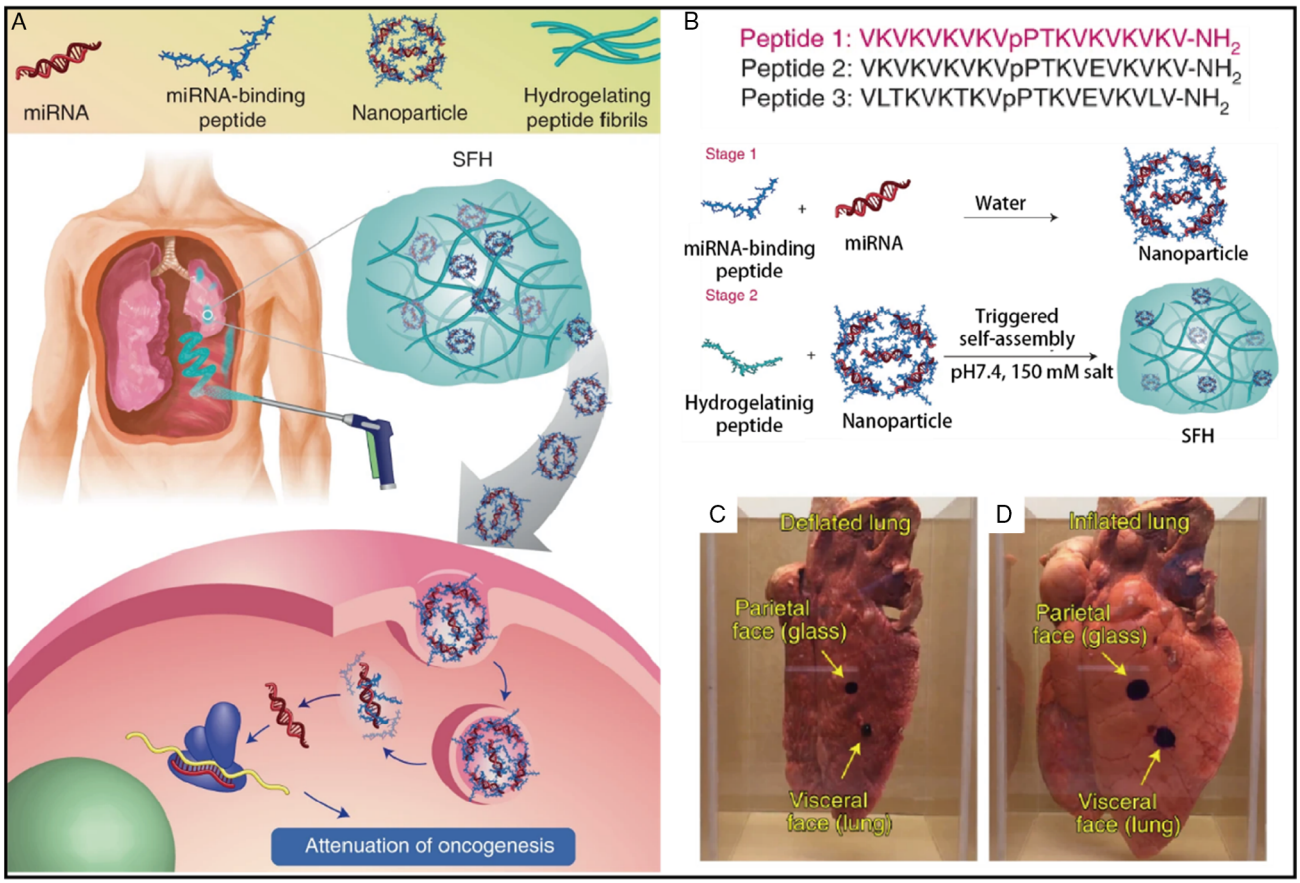

Figure 6. A) The spray of peptide-based SFH can treat mesothelioma located in the plural cavity. B) The molecular design of the disordered peptides (top) and the dual-stage preparation of SFH. First, the disordered peptide was complexed with the double-stranded miRNA. Second, a peptide-based hydrogel was formulated to encapsulate particles produced previously. C) SFH was delivered to deflate lung and glass chamber surfaces by the syringe to simulate its presence in the pleural cavity after cancer debulking. D) During lung reinflation, SFH owns the spread-fill property. Reproduced with permission.[95] Copyright 2021, Nature Publishing Group.

Table 1. Selected peptide-based systems for barrier-permeable pulmonary gene therapy.

Peptide-based pulmonary delivery vector	Gene	Disease	Dosing method	Type of peptide system	Animal model	References
RAGE binding peptide	pDNA	ALI	Inhalation	Positively charged peptides	BALB/c mice	[58]
RAGE binding peptide-cis-aconitic amide (RC)	siRNA	ALI	Intratracheal administration	Positively charged peptides	BALB/c mice	[59]
Xentry protamine fusion peptide (XP)	mRNA	CF	None	CPPs	None	[67]
CPP-fused cardiac targeting peptide for pulmonary targeting	siRNA	SARS-CoV-2 infection	i.v. injection	CPPs	CD1 mice	[68]
PEGylated KL4 peptide : the dry powder of KL4 peptide is attached to a linear PEG of 12-mers	mRNA	Healthy	Intratracheal administration	CPPs	BALB/c mice	[69]
CPPs RALA employed with factorial design	pDNA	None	Inhalation	CPPs	None	[70]
CPPs grated with endosomolytic peptide	CRISPR/Cas RNP	CF	Intranasal administration	CPPs	ROSAmT/mG transgenic mice	[76]
Fluorinated and guanidinated bifunctional helical polypeptides	siRNA	ALI	Intratracheal administration	Polypeptide	BALB/c mice	[38]
Lung epithelial-targeting peptides integrated into DOTMA/DOPE system	siRNA	CF	Oropharyngeal instillation	Peptide–lipid hybrid system	C57BL/6 mouse	[81]
Nanoparticle consists of lung epithelial-targeting peptide and cationic liposome	siRNA	CF	Oropharyngeal instillation	Peptide–lipid hybrid system	C57BL/6 mouse	[82]
CPP grafted with GET peptide and 5 kDa PEG	pDNA	CF	Intratracheal administration	Peptide–polymer hybrid system	BALB/c mice	[37]
Transfection platform composed of synthetic peptide and poloxamine 704	pDNA; mRNA	CF	Intratracheal administration	Peptide–polymer hybrid system	B6CF mice	[87]
SFH formed by RNA-binding peptide and hydrogelating peptide	miRNA	Pleural mesothelioma	Intrapleural administration	Peptide–peptide hybrid system	Porcine	[95]

(**Figure 6**A).[95] This SFH was hybridized in two steps: 1) intrinsically disordered peptides were engineered and complexed cooperatively with chemically modified double-stranded miRNA mimics to form nanoparticles and 2) a fibrillar peptide endorsing β-hairpin conformation was designed for surface attachment and encapsulating miRNA particles generated in the previous step (Figure 6B).[95] To validate its future clinical usage, SFH was extruded between the gap of the deflated porcine lung and glass chamber (mimicking chest wall).[95] Then, this SFH underwent compliant shape deformation during lung reinflation, suggesting the desired amenability within the pleural cavity (Figure 6C,D).[95] Moreover, the SFH was proved sprayable due to its shear-thin/recovery mechanical behavior, which further favored the accommodation in those hard-to-reach thoracic crevices by a simple spray.[95] In short, this proof-of-concept SFH offers a worth learning example, illustrating that the system hybridized with different peptide species is also qualified for gene binding and peripulmonary delivery.[95]

7. Conclusion and Perspectives

This minireview summarized the recently reported peptide-based delivery systems for safe and effective pulmonary nucleic acid therapy. We emphasized that a comprehensive understanding of the pulmonary biological barriers is crucial for guiding peptide engineering because peptides need to maintain stability in a harsh pulmonary environment and penetrate through those notorious barriers. Then, we introduced current peptide delivery systems for pulmonary gene delivery based on peptide species. Despite the guarantee of adopting these advanced peptide delivery systems for pulmonary-specific applications, there still are several main challenges to fix before the practical clinical uses. The current peptide-based platforms for pulmonary gene delivery mainly focus on treating CF. Although sharing similar features, such as mucus oversecretion, the presence of biological barriers at distant diseased tissue calls for tailored vector design for each type of lung disease is different. Moreover, the pulmonary barrier may vary in the wake of disease progress. For example, the PCL layer in CF lung would gradually dehydrate along with CF exacerbating from mild to a severe state.[96] Therefore, one workable peptide vector may become invalid when facing a more severe phenotype. Except for that, the divergent physiochemical properties of different nucleic acid therapeutics (e.g., siRNA, mRNA, pDNA, and Cas RNP) obligate specialized vector design. Consequently, a universal peptide vector providing a sufficient transfection rate is still unseen. The discovery of novel peptides for gene condensation and transportation might bank on diverse technologies, such as high-throughput phage display and an organoid-based in vitro screening. Overall, considering the present public devotion to the development of mRNA vaccine for pulmonary local administration during the COVID-19 pandemic, we are anticipating the forthcoming arising of new branches of peptide-based systems for pulmonary gene delivery.

Acknowledgements

D.C. and N.K. contributed equally to this work. This project was supported by the National Natural Science Foundation of China (82022038) and Westlake Education Foundation.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

gene delivery, lungs, mucus penetration, peptides, pulmonary therapy

Received: July 31, 2022
Revised: August 29, 2022
Published online: October 28, 2022

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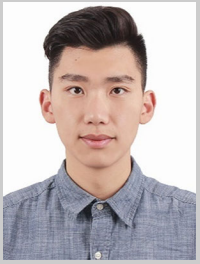

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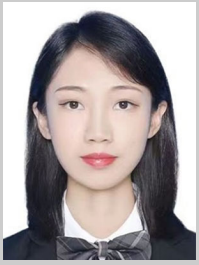

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