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Research review paper

A role for peptides in overcoming endosomal entrapment in siRNA delivery — A focus on melittin

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ABSTRACT

siRNA has the possibility to revolutionize medicine by enabling highly specific and efficient silencing of proteins involved in disease pathogenesis. Despite nearly 20 years of research dedicated to translating siRNA from a research tool into a clinically relevant therapeutic, minimal success has been had to date. Access to RNA interference machinery located in the cytoplasm is often overlooked, but must be considered when designing the next generation of siRNA delivery strategies. Peptide transduction domains (PTDs) have demonstrated moderate siRNA transfection, which is primarily limited by endosomal entrapment. Strategies aimed at overcoming endosomal entrapment associated with peptide vectors are reviewed here, including osmotic methods, lipid conjugation, and fusogenic peptides. As an alternative to traditional PTD, the hemolytic peptide melittin exhibits the native capacity for endosomal disruption but causes cytotoxicity. However, appropriate packaging and protection of melittin with activation and release in the endosomal compartment has allowed melittin-based strategies to demonstrate both in vitro and in vivo safety and efficacy. These data suggest that melittin's membrane disruptive properties can enable safe and effective endosomolysis, building a case for melittin as a key component in a new generation of siRNA therapeutics.

Contents

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1. Introduction

1.1. RNA interference by siRNA

RNA interference (RNAi) refers to an evolutionarily conserved mechanism for post-transcriptional control of protein expression in which short double-stranded RNAs target specific messenger RNA (mRNA) for degradation, thus decreasing protein translation (Eccleston and Eggleston, 2004). Tuschl et al. were the first to demonstrate that RNAi can be artificially induced by the delivery of exogenous small interfering RNA (siRNA) (Elbashir et al., 2001). siRNAs are short 21–23 base pair duplex RNA oligonucleotides with 5′-phosphorylated ends and 2-nucleotide 3′ overhangs. The "antisense" strand shares sequence complementarity to a target mRNA, while the "sense" strand serves as a bystander. When delivered into the cytoplasm of a cell, siRNA can co-opt the native RNAi machinery and induce assembly of the RNA induced silencing complex (RISC). The RISC unwinds siRNA, binds the antisense strand, and cleaves the sense strand, allowing the RISC to bind and cleave targeted mRNA based on the sequence of the antisense strand (Sakurai et al., 2011) (Fig. 1). This selective degradation of mRNA provides an avenue to decrease the expression of proteins involved in disease pathogenesis. Indeed, initial experimental siRNA therapeutics appeared just three years after the discovery of siRNA (McCaffrey et al., 2002). Recently, clinical trials have been initiated for the use of siRNA in ocular, renal, and hepatic diseases with limited success (Haussecker, 2012).

1.2. Cellular barriers to the therapeutic use of siRNA

On a cellular level, efficient and non-toxic siRNA delivery to the cytoplasm remains a major impediment to the use of siRNA as a therapeutic. siRNAs are large (~21 kDa), highly charged macromolecules, which cannot directly translocate across the hydrophobic core of the cell membrane (Overhoff and Sczakiel, 2005). Moreover, the barrier provided by impermeable membrane bilayers not only applies to direct translocation from the extracellular milieu into the cytoplasm, but also applies to cytosolic access of siRNA enclosed in endocytic vesicles (Detzer and Sczakiel, 2009; Gilleron et al., 2013). Entrapment of siRNA in endocytic compartments not only prevents siRNA from reaching the cytosol, but also accelerates siRNA degradation due to the harsh acidic environment encountered during endosome–lysosome trafficking (Fig. 2) (Wang and Thanou, 2010).

This barrier represents a major impediment in the delivery of biological therapeutics to the cytoplasm, especially when packaged with peptide transduction domains (El-Sayed et al., 2009). Strategies to achieve endosomolysis have traditionally been based on osmotic agents, fusogenic lipids, and fusogenic peptides. The application of these methodologies to peptide-mediated siRNA delivery will be reviewed here. In addition, novel strategies enabled by the membrane-disruptive properties of melittin appear to negate the need for secondary endosomolytic methodologies and will be presented as an alternative to traditional peptide-based siRNA delivery strategies.

2. Cationic peptides for siRNA transfection

Given the challenges associated with traditional siRNA delivery methodology, there is clearly a need for siRNA delivery technology to enable endosomal escape with minimal cytotoxicity. With the observation that the Trans-Activator of Transcription (TAT) peptide from HIV could directly translocate across cell membranes to trans-activate the viral promoter in tissue culture, cell penetrating peptides (CPPs) have become a widely utilized tool for delivery of therapeutics (Frankel and Pabo, 1988). CPPs have been recruited for delivery of cargoes ranging from small molecules to large proteins (Heitz et al., 2009). With their hypothesized ability to bypass the cellular membrane, CPPs were expected to enable cytoplasmic delivery of siRNA, while avoiding the endosomal compartment. Although this hope has ultimately proven unfounded, peptides remain a viable option for siRNA delivery thanks to a relative lack of cytotoxic effects.

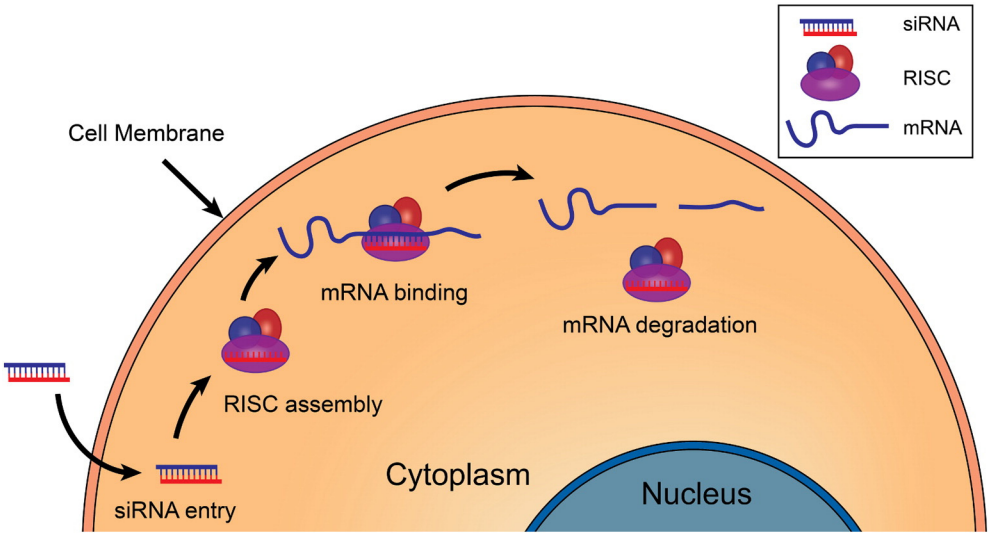

Fig. 1. siRNA can induce mRNA degradation and gene silencing if delivered into the cytoplasm.

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