Protein Cell 2011, 2(11): 855–857
DOI 10.1007/s13238-011-1124-0

Protein & Cell

NEWS AND VIEWS

Progress in targeted delivery of siRNA to combat Coxsackievirus

Anju Gautam ✉

Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
✉ Correspondence: anjela628723@gmail.com

Cardiovascular disease is one of the major causes of human death and has been linked to different risk factors. Several pathogens have been found to be involved in myocardial disorders. Among these, Coxsackievirus B3 (CVB3) is one of the most important causes of virus-induced myocarditis (Henke et al., 2003). CVB3 is a member of the genus Enterovirus, within the family Picornaviridae. The importance of CVB3 is reflected in the data from the World Health Organization global surveillance of viral diseases, where the coxsackie virus B was ranked the number one cause of clinically evident cardiovascular diseases (Grist and Reid, 1993). Clinically, CVB3 infections are associated with different forms of subacute, acute, and chronic myocarditis (Woodruff, 1980; Reyes and Lerner, 1985). The infections may cause cardiac arrhythmias and acute heart failure, while in some cases the myocardial inflammation may persist chronically and progress to dilated cardiomyopathy (Kandolf, 1993; Rose, et al., 1993; Martino, et al., 1994; Cetta and Michels, 1995), requiring heart transplantation, or death.

The CVB3 genome is a positive single-stranded RNA virus and infects host cells through a coxsackie and adenovirus receptor (CAR) (Bergelson et al., 1997). It is approximately 7.4 kb in length and has a single open reading frame, which is flanked by 5′ and 3′ untranslated regions (UTR). The 5′ UTR contains a highly structured internal ribosome entry site that directs viral translation initiation (Yang et al., 1997; Liu et al., 1999). The 3′ UTR contains three stem-loops followed by a poly (A) tail. The genomic RNA can serve as a template for viral RNA transcription to synthesize more copies of positive genomic RNA through a negative-strand intermediate (Yuan et al., 2005). This viral genome encodes four capsid proteins VP1–VP4 and seven nonstructural proteins including two proteases 2A and 3C, and an RNA-dependent RNA polymerase 3D. CVB3 RNA can be directly translated into a single polyprotein, which is subsequently processed mainly by viral proteases 2A and 3C to produce mature structural and nonstructural proteins for viral replication (Klump et al., 1990). The entire replication cycle of CVB3 from entry of the host cell to release of progeny virus takes approximately 6 to 8 h. Although the life cycle of CVB3 appears clear, yet a specific antiviral therapy for coxsackievirus-mediated diseases is currently not available. Regarding this issue, the plus-stranded RNA genome of CVB3 highlights an exciting possibility to cure or attenuate virus-mediated disease by applying RNAi based antiviral therapeutic strategy.

A relatively new approach for delivering siRNA is described in an earlier study by Yuan and colleagues and examines the effects of RNAi on CVB3 replication using five siRNAs targeting different regions of CVB3 genomic RNA (Yuan et al., 2005). Small interfering RNAs (siRNAs) are short, double stranded RNA (dsRNA) molecules that can target mRNA of a specific sequence for degradation via a cellular process known as RNA interference (RNAi) (Elbashir et al., 2001; Fire, et al., 1998; Vaucheret, et al., 2001; Baulcombe, 2002). The efficacy of siRNA is also influenced by its evolutionarily conserved natural mechanism and has been shown to be more potent, specific and versatile compared to mRNA targets, and thus provides enormous opportunity for rational drug discovery. In this process, siRNA incorporates into an RNA-induced silencing complex (RISC) that recognizes and cleaves the target sequence (Dorsett et al., 2004). Yuan and colleagues, for the first time, achieved 92% inhibition of Coxsackievirus B3 replication, synthesizing a siRNA targeting the CVB3 protease 2A gene. They demonstrated an inhibition of CVB3 replication in Hela cells using wild-type CVB3 virus together with employing an ongoing viral infection in a dose dependent manner for long term treatments (8–48 h) (Yuan et al., 2005). The author also reported the inhibition of CVB3 in HL-1 mouse cardiomyocyte cell lines for 48 h post infection at a MOI of 10, thereby showing that siRNA exhibits greatest potency in inhibiting CVB3 replication. To investigate the issue of which viral RNA strand is responsible for the site of sequence complementation with siRNA, Yuan and colleagues approached to site-directed mutagenesis. They found

that only one point mutation in the middle of the antisense strand could eliminate the anti-CVB3 activity, whereas the corresponding mutation on the sense strand did not interfere with the viral replication, suggesting that the negative-strand RNAs produced during viral replication are not the direct target of siRNA. This result might be explained by the fact that the replicating negative strands of virus only exist as a double stranded form in the vesicles (Fields et al., 2001); thus, they are less likely to be accessible to siRNAs. Conversely, the positive strand is the recognition site for RNAi, which forms complementary base pairs with the antisense strand of the siRNA (Yuan et al., 2005). Based on this finding, the researcher suggests that the match between the positive strand of viral RNA and antisense strand of siRNA is critical for RNA interference. Although this new strategy has exciting potential, one of the major barriers for application is the non-specific distribution of drugs in the body after administration.

To overcome this barrier, Zhang, Yuan and colleagues (Zhang at al., 2009) in their latest study, demonstrated the feasibility of delivering siRNAs to CVB3 susceptible target cells using ligand-linked packaging RNA as a vehicle, lowering the possibilities of unspecific binding. This novel approach used folate as a ligand for delivering siRNA/2A to Hela cells and constructed folate-labeled chimeric pRNA-siRNA/2A monomers and heterodimer complexes demonstrating that pRNA-siRNA/2A achieved a similar strong anti-CVB3 activity, which indicates that siRNA/2A carried by pRNA can be properly folded into dsRNA helix and processed by a dicer to release functional siRNA/2A after entering the cells. In addition, the viral plaque assay and MTS assay revealed that treatment with pRNA-siRNA/2A could maintain approximately 95% cell survival compared with cells treated with the pRNA vector control, which was < 20% cell survival (Zhang at al., 2009). They showed for the first time that ligand-mediated delivery of siRNA carried by pRNA vector could successfully inhibit viral replication.

To date, there is no clinically proven specific treatment available for viral myocarditis and dilated cardiomyopathy. The ultimate treatment strategy for patients with dilated cardiomyopathy is heart transplantation. The work done by Yuan, Zhang and colleagues demonstrates the possibility of developing new anti CVB3 treatments provided that the application of RNAi can inhibit viral infection by targeting viral or cellular genes. First, they suggested the delivery of siRNA targeting CVB3 protease 2A gene and showed 92% strong inhibition against CVB3 virus. Merl et al. (2005) reported a similar siRNA delivery system involving fluorescence labeled siRNA and CVB3. Racchi et al. (2009) also supported the work of Yuan et al. and favored the concept that protease 2A is a particularly good target for CVB3. Secondly, Yuan and colleagues designed pRNAs, tremendously broadening the understanding of delivering this therapeutic molecule to the target cells via the pRNA vehicle. This lab continues to advance knowledge in this area of study. In 2011, Ye et al. (Ye et al., 2011) took on the challenge of overcoming the drug resistance caused by viral escape mutants by designing artificial microRNAs to inhibit the CVB3 viral replication, and delivering them into the cells by using folate-linked pRNA as a vehicle, targeting 3′ UTR region of virus CVB3. The author found that artificial microRNAs targeting 3′ UTR region of CVB3 are tolerable to target mutations, and effective in inhibiting replication of CVB3, signifying that the pRNA ligand-mediated delivery of microRNA and siRNA strategy holds promising advantages to treat CVB3 viral replication and is the first successful attempt to deliver microRNAs to target cells using the pRNA vector.

The work done by Yuan, Zhang and colleagues and many others is an important example of a rationally designed siRNA and a new approach for drug delivery system to overcome drug resistance—a key strategy for improving success rates of targeting viral infections. Future work evaluating the efficacy of using siRNA and ligands guiding drug specific delivery as drug vehicles for siRNA in large animals, as well as more detailed analysis of the immune response, will be needed for clinical translation of this technology.

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