Fighting Hepatitis C Virus with Peptide Nanotubes

Ashraf Brik[1,2,*]
[1]Department of Chemistry
[2]National Institute For Biotechnology in the Negev
Ben-Gurion University of the Negev, Beer Sheva 84105, Israel
*Correspondence: abrik@bgu.ac.il
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There is an urgent need to develop effective therapies against hepatitis C virus with different modes of action from limited options already available. In this issue, Montero and coworkers present a detailed study on using peptide nanotubes to effectively block the entry of HCV into target cells.

It is estimated that more than 170 million people world-wide are infected with hepatitis C virus (HCV), and a large proportion of those infected may eventually develop acute and chronic hepatitis and hepatocellular carcinoma (Alter, 1997). These devastating numbers continue to put pressure on the scientific community to find a successful therapy against HCV. To date, there has been no general and effective solution. HCV belongs to the *Flaviviridae* family and comprises a single-stranded RNA genome, which corresponds to a 3000 amino acid polypeptide that is processed into at least 10 mature structural and functional viral proteins. Today, we have a very good understanding of the HCV life cycle and factors that lead to viral infections (Pawlotsky et al., 2007). The infection process is initiated by the attachment of the virus to the cell surface and entry followed by the uncoating of single-stranded RNA genome. Translation of HCV genome into polypeptide and cleavage by NS3/NS4A protease generates the viral proteins. The assembly of these virions and maturation in the ER lumen follows. After being transported to the cell surface, the virions are released into circulation and undergo postrelease maturation to become infectious. Because our understanding of several of these steps at the molecular level, various new classes of anti-HCV drugs are now being developed, such as NS3/4A protease and NS5B polymerase inhibitors.

Despite the fact that new therapies based on combinations of pegylated interferon with direct-acting antivirals such as ribavirin are now used to treat HCV patients, these drugs exhibit limited efficacy and serious side effects (Gelman and Glenn, 2011). Moreover, the dangers associated with the emergence of resistant variants, due to the inherent inaccuracy of the RNA polymerase and the selective pressures of antiviral drugs that favor drug resistance, requires the search for new therapies with unique modes of action, improved efficacy, and toxicity profiles. In this issue of *Chemistry and Biology*, Montero and coworkers (2011) report on a family of highly active cyclic peptides that assemble to nanotubes and specifically block the entry of all tested HCV genotypes into target cells at a post-binding step (Figure 1).

The Ghadiri group has pioneered the development of a class of cyclic peptides that is known to self-assemble in well-defined nanotube supramolecular structures (Ghadiri et al., 1993). Cyclic peptides contain an even number of alternating D- and L-α-amino acid residues with ring-shaped conformations. Under conditions that favor hydrogen bonding, e.g., lipid membranes, the donor and the acceptor of the amide backbone functionalities on either side of the ring are fully engaged in internal hydrogen bond networks, which assist in stacking these peptides to form nanotube like ensembles. Since this development, the Ghadiri group (Ashkenasy et al., 2006) has been engaged in using these nanotubes for various applications such as converting them to biomaterials with extended charge delocalized states for potential use as optical and electronic devices. Moreover, the group was able to design these cyclic peptides into antibacterial agents that are capable of selectively targeting bacterial membrane where they were shown to exert antibacterial activity by self-assembling and increasing the membrane permeability (Fernandez-Lopez et al., 2001).

In the current work, the utility of these nanotubes is extended to combat HCV. To achieve this, 144 sequences, known to be nontoxic in HeLa cells, were screened in a cell-based ELISA assay and nine cyclic amphiphilic octapeptides emerged as potential hits with EC_{50} between 8 to 16 μM. Studying the structural features that are required for antiviral activity of these peptides has revealed that the peptides are amphiphilic with net neutral charge structures, the antiviral activity is sequence dependant, and the cyclic structure and supramolecular assembly are required for the antiviral activity. This has been supported by several experiments such as preparing the linear or the *N*-methylated analogs of these peptides, which led to a significant loss of the antiviral activity. Studying the mode of action of these peptides indicated that they are likely to interfere with an early step of the infection without altering the binding of the virus to the target cell, but inhibiting a step in viral entry downstream of cell binding. These conclusions came in light of several observations. For example, peptides do not show any antiviral activity when added after the virus already entered the cell in the time-of-addition experiment, and HCV-pseudotyped lentiviral particle entry into Huh-7 cells was efficiently inhibited in a dose-dependent manner by the peptide nanotubes. Importantly, these peptides exhibited activity against all the tested HCV genotypes and were found to efficiently control viral spread in cell culture.

The studies described by Montero and coworkers (2011) represent another remarkable and exciting application of peptide nanotubes and open new front in the fight against HCV. In the assembled

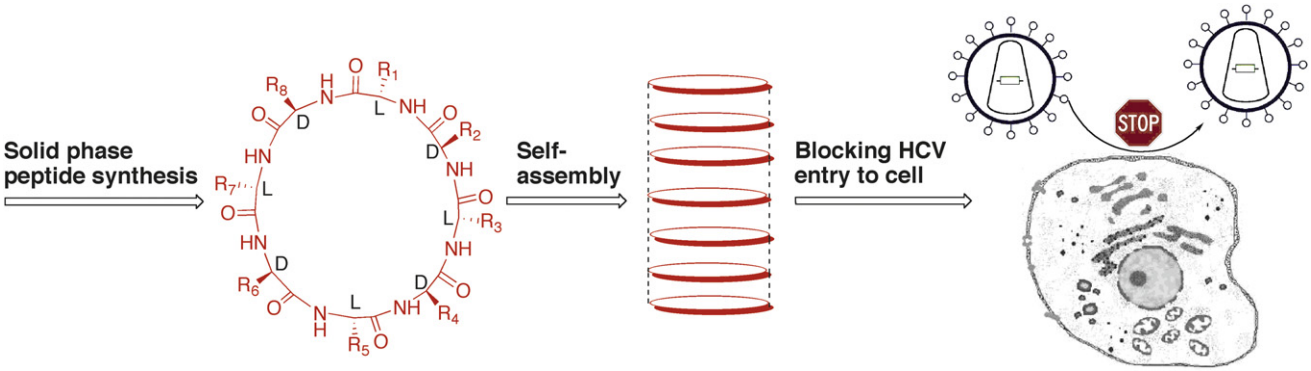

Figure 1. The Use of Peptide Nanotubes as Potential Therapeutics against HCV
The peptides comprising eight amino acids of alternating D- and L-α-amino acid residues are prepared using solid phase peptide synthesis. Upon interacting with cellular membranes, these peptides assemble into nanotubes and exert, in part, their antiviral activity by blocking the HCV entry to the cell. R_1–R_8, amino acid side chains; —, a hydrogen bond within the nanotube.

structure, the amino acid side chains are located on the outside surface of the ensemble, which should allow their functionalization without much interference with the supramolecular structures. For example, this could be useful to enhance their drug-like properties and allow specific labeling for a variety of mechanistic studies. Moreover, due to their relative ease of preparation and low molecular weight, these peptide nanotubes could potentially boost the current arsenal of antiviral compounds against other existing infectious diseases.

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Mitochondrial Complex III: Tuner of Autophagy

Shengkan Jin[1,*]
[1]Pharmacology Department, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854, USA
*Correspondence: jinsh@umdnj.edu
DOI 10.1016/j.chembiol.2011.11.004

Using chemical approaches, Ma et al. in this issue of *Chemistry & Biology* identify mitochondrial complex III as a specific positive regulator of autophagy. This study brings us a step closer to understanding the mechanism by which basal autophagy is coupled to cellular energy flux.

Autophagy is a membrane trafficking process leading to lysosomal degradation of cytoplasmic components. This evolutionarily conserved process serves two fundamental functions (Mizushima, 2005; Mizushima et al., 2008). First, the constitutive, or basal, autophagy allows cells to turnover long-lived proteins and organelles, providing an important mechanism for cellular maintenance. Second, autophagy can be dramatically elevated in response to stress conditions, in particular, nutrient deprivation. This allows cells to mobilize internal resources for maintaining cellular homeostasis during fluctuations of external environment. How nutrients and nutrient deprivation regulate autophagy has been extensively investigated; however, much less is known about the nature of basal autophagy.

One important question regarding basal autophagy is whether it is a passive repair process that responds to damaged or dysfunctional long-lived proteins or organelles, or if it is an active process that preemptively turns over cytoplasmic components. If the first scenario is true,