Commentary

Aptamers as Viral Replication Antagonists

Nicola J. Stonehouse

School of Molecular and Cellular Biology, Faculty of Biological Sciences and Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom

Abstract | Aptamers are nucleic acids, selected *in vitro* to specifically bind target molecules. They can therefore be useful as molecular tools and also as novel therapeutic agents. This commentary describes RNA aptamers selected to bind to the RNA-dependent-RNA polymerase of foot-and-mouth disease virus. The aptamers are able to inhibit the function of the enzyme both *in vitro* and in the context of a sub-genomic replicon and highlight the future potential of this technology in many aspects of virus research as well as in diagnostics and therapeutics.

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***Correspondence** | Stonehouse, N.J., University of Leeds, UK; **E-mail** | n.j.stonehouse@leeds.ac.uk
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Aptamers are single-stranded oligonucleotides that fold into complex structures and bind target molecules in a conformation-dependent manner. Because of the high affinity of binding, aptamers have the ability to modulate the function of target molecules and therefore have therapeutic potential (for review, see Brody and Gold, 2000). Examples of the potential of this technology include the aptamer Macugen (Pegaptanib) which gained clinical approval to treat age-related macular degeneration in 2004, and G-rich DNA oligonucleotides (e.g. AS1411) with anti-proliferative properties in cancer cells. The paper by Forrest et al describes the use of RNA aptamers to inhibit the replication of foot-and-mouth disease virus (FMDV).

Foot-and-mouth disease (FMD) is an acute, systemic vesicular disease in cloven-hoofed animals resulting from infection by FMDV (for review, see Jamal and Belsham, 2013). Major outbreaks in Taiwan (1997) and the UK (2001) had serious consequences for agriculture and tourism, with losses to the UK estimated in excess of £8 billion. The disease remains endemic in many parts of Asia, South America, Africa and the Middle East, including countries bordering on Europe, such as Turkey. Although severe trade restrictions are imposed on countries where the disease is endemic, outbreaks do occur elsewhere. The problem with controlling the spread of the disease is mainly due to high infectivity and transmissibility of the virus, and is complicated by the ability of the virus to develop an asymptomatic carrier state. Because of the economic importance and highly infectious nature of FMDV, work with the virus is restricted to high containment facilities; however, sub-genomic replicons can be safely used to study many aspects of virus replication.

In common with other picornaviruses, the FMDV genome is a positive stranded RNA molecule comprising a large single open reading frame (ORF) containing structural followed by non-structural genes, flanked by untranslated regions. The RNA-dependent-RNA polymerase (RdRp) $3D^{pol}$ is one of the key proteins in viral RNA replication and therefore a potential antiviral target.

The paper by Forrest et al reports the use of a FMDV

replicon to study replication in BHK-21 cells in the presence of potential inhibitory aptamers. The replicon includes all of the non-structural genes and a green fluorescent protein/puromycin N-acetyltransferase (GFP/PAC) reporter gene cassette in place of the structural genes. A description of the design and synthesis of the pGFP-PAC replicon employed is reported in Tulloch, et al., 2014. Protein expression (as monitored by GFP reporter expression) acts as a direct real-time measure of replication. Continual production of $3D^{pol}$ facilitates replication of the replicon RNA to provide increasing levels of template for GFP expression, reaching maximal levels after approximately 8 h. This rapid expression of GFP is consistent with the fast replication rate of FMDV and is mirrored by a change in the morphology of the cells.

The selection of aptamers to $3D^{pol}$ and their characterisation *in vitro* has been reported previously (Ellingham, et al., 2006; Bentham, et al., 2012). Forrest et al demonstrate that these molecules could be modified to increase their stability while maintaining effective inhibition *in vitro*. Furthermore, (as demonstrated by live cell imaging and immuno-blotting) the molecules were able to inhibit replication in BHK-21 cells. The dose-dependent inhibition was maximal between 125-500 nM; at which normal cell morphology was retained. The molecules used in this study were small (31-33 nucleotides), predicted to possess minimal double-stranded regions and no 5' triphosphate (Doble, et al., 2014; Ellingham, et al., 2006). It was therefore unlikely that they would trigger RIG-I/MDA5 interferon signaling (Belyaeva, et al., 2014). Consistent with this, the $3D^{pol}$ aptamers were well-tolerated in cells and able to reverse the cytotoxic effects of replicon expression.

It has also been demonstrated that small aptamer RNAs can be delivered passively to cells without the need for transfection reagents (Doble, et al., 2014), thus opening up possibilities for the therapeutic use of aptamers in a topical/localised manner. Furthermore, the inherent flexibility of RNA and conformational changes that occur upon target recognition, lead to possible applications for aptamers in diagnostics, alongside more conventional antibodies. Therefore, the RNA aptamers described here, together with aptamers to other parts of the FMDV replication complex, are not only useful molecular tools in the study of replication, but have possible applications in diagnostics and therapeutics in the future. Furthermore, aptamers have been generated to a variety of viral proteins including the E6 and E7 oncoproteins of human papillomavirus (Nicol, et al., 2011 and 2013, Belyaeva, et al., 2014), HCV protease NS3 (Fukuda, et al,. 2004) and polymerase NS5B (Biroccio, et al., 2002) SARS coronavirus helicase (Jang, et al., 2008) H5N1 influenza HA (Cheng, et al., 2008) and several HIV proteins including gp120, reverse transcriptase and the trans-activator protein Tat (James, 2007). This opens up many avenues for development of tools for the investigation of protein function as well as novel therapeutic agents against viral diseases in the future.

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