European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 02, Issue 04, 2014

Structure-based Engineering of Sabin 2 Poliovirus Polymerase to Alter Replication Fidelity

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Abstract

Picornaviruses cause a wide range of ailments, including myocarditis, poliomyelitis, and vesicular lesion type diseases. Excellent vaccines exist for several of them, and the development of the live-attenuated oral polio vaccine (OPV) provided an efficient and cost-effective avenue for successful poliovirus eradication in the majority of the world. However, one hurdle for developing successful live-attenuated vaccines lies with the viral RNA-dependent-RNA-polymerase (RdRP) enzyme whose low replication fidelity allows for reversion of attenuated viruses to disease causing variants. Improving the replication fidelity of RdRPs is an attractive avenue for virus attenuation because it may curtail such reversion issues. We have previously solved the crystal structures of several picornaviral polymerase-RNA complexes that show the structural changes taking place within these polymerases during active site closure and catalysis (Gong et al., 2010, 2013). Based on this, we engineered a panel of fidelity variant coxsackievirus B3 polymerases that caused reduced infectivity and attenuated virus growth in mice (Gnädig et al., PNAS, 2012). We hypothesize that such modulation of polymerase fidelity via structure based protein engineering can provide an effective platform to improve the design of live-attenuated vaccines. To investigate this further we have generated over a dozen mutations in the poliovirus Sabin 2 strain polymerase and carried out in vitro biochemical assays to show that these can either increase or decrease polymerase fidelity while having minor effects on elongation rates and processivity. The fidelity modulation can arise from single point mutations, multi-site mutations that replace entire groups of interacting residues, or from grafting in structurally homologous sequences from related polymerases.

Keywords:

and we are now seeking to test these variant polymerases in an infectious virus context