SINGLE-CELL ANALYSIS UNCOVERS A NOVEL INFLUENZA A VIRUS-DERIVED DEFECTIVE INTERFERING PARTICLE FOR ANTIVIRAL THERAPY

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Single-cell analysis of virus-infected cells (Heldt and Kupke et al., 2015) enables the characterization of individual highly productive cells, which may support strategies to improve cell culture-based vaccine production. However, the definition of poor producer single cells can also yield valuable information. Here we show that low-productive single Madin-Darby canine kidney (MDCK) cells, infected with influenza A virus (IAV) of strain A/PR/8/34 (PR8), were affected by a yet unrecognized form of defective-interfering particle (DIP). Conventional DIPs (cDIPs) typically contain a deleted form of the viral genome and are therefore unable to reproduce in an infection. However, upon complementation by the co-infection with fully infectious standard virus (STV), interference with the normal viral life cycle can be observed. Interestingly, considering their ability to suppress STV replication, cDIPs are of growing interest for clinical application, i.e. for their use as antivirals (Dimmock and Easton, 2014).

Single-cell infection experiments revealed a surprisingly high variability in IAV replication with progeny virus yields that ranged from 0 to roughly 1000 plaque-forming units (PFU) per cell. Intriguingly, low-productive cells (0-10 PFU) displayed an abnormal phenotype, which was caused by the co-infection of a subpopulation of virus, in the following termed OP7 virus. Sequences of the genomic viral RNA (vRNA) of OP7 virions showed a significant amount of nucleotide substitutions in one of the eight vRNA segments, affecting its promotor, encoded proteins and virus packaging signals. We showed that these alterations were all directed towards the predominant genomic replication and packaging of the mutated vRNA over other genome segments. Concurrently, OP7 virions lacked a large fraction of other vRNAs, which constitute its defect in virus replication. Finally, co-infection experiments showed strong interference of OP7 virus with IAV replication, as indicated by a dramatic reduction in the infectivity of released virions. This interference was directed against relevant homologous and heterologous IAV strains, including strains of the current influenza season. Furthermore, we demonstrated interference in human cell lines.

Therefore, OP7 virions are a novel form of IAV-derived DIPs with a non-deleted but mutated genomic RNA segment. First, it seems reasonable to investigate the presence of OP7 virions in seed virus preparations, as they can reduce virus titers in a production process, similar to cDIPs (Frensing et al., 2014). Second, OP7 virus may be used for antiviral therapy. As they are not able to reproduce on their own, they may be administered to organisms with no harm. The presence of OP7 virions can then inhibit the propagation of IAV of a natural infection. In addition, the induction of the innate immune response, observed upon infection with OP7 virus, can even further promote the antiviral effect. In the future, the design of efficient production systems for OP7 virions and the execution of animal trials may facilitate its utilization as a novel antiviral agent.

References

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Patent

Patent pending for usage of OP7 virions as an antiviral agent