SINGLE-CELL ANALYSIS OF INFLUENZA A VIRUS-INFECTED CELLS FOR THE OPTIMIZATION OF CELL CULTURE-BASED VACCINE PRODUCTION

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Key Words: influenza, single-cell analysis, vaccine manufacturing, mathematical modeling

Individual cells generally show a large cell-to-cell variability in their properties, such as the cell cycle stage or protein content. Moreover, cellular biochemical reactions are subject to stochastic effects which can increase cell-to-cell heterogeneity. Yet, how this variability affects virus replication, which comprises noisy reactions itself, remains largely elusive. We conducted single-cell analysis of influenza A virus (IAV)-infected cells to investigate cell-to-cell heterogeneity in virus replication in detail. Single Madin-Darby canine kidney cells, infected with influenza virus A/Puerto Rico/8/34 (H1N1), were isolated in 384-well plates by using a limiting dilution approach. After incubation, we quantified virus titers in the supernatant by the plaque assay and intracellular genomic viral RNAs (vRNAs) by real-time RT-qPCR. Our experiments reveal a surprisingly high variability in IAV replication. Progeny virus yields ranged from 1 to 970 plaque-forming units per cell and intracellular vRNA levels spanned three orders of magnitude. With the assistance of stochastic mathematical modeling, we show that two types of molecular noise affect virus titers: (i) extrinsic noise, which can arise by cell-to-cell variability and (ii) intrinsic noise, originating from stochastic effects during viral RNA synthesis. Furthermore, we demonstrate that the heterogeneity in IAV infection is apparently not generated by the genetic diversity of the infecting virus population; and defective interfering particles affected only the infectivity of progeny virions. In addition, our simulations suggest that random degradation of viral genomes can result in a large fraction of non-productive cells at a low multiplicity of infection. The observed large cell-to-cell variability in IAV replication supports the notion that population-derived measurement data do not accurately represent viral infections. Moreover, characterizing high-yielding cells at the single-cell level may enable us to derive strategies for the optimization of cell culture-based vaccine manufacturing processes.