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Preliminary study on expression of human Siat7e gene in VERO cell line to improve influenza virus titer

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Introduction: Over 30 years, Vero cell line has been used for producing viral vaccines. Recently Vero cell-based H5N1 vaccine is undergoing clinical trials. One of the limiting aspects in scaling up the virus production in these continuous cell lines is the fact that these cells are anchorage-dependent and thus require surface adhesion to proliferate. The human sialyltransferase [*siat* 7e (ST6GalNac V)] was identified as one of the genes that play a critical role in controlling the degree of cell adhesion which could enhance influenza virus propagation.

Material and methods: The full-length of human sialyltransferase gene (siat7e) was amplified with primers containing restriction sites for XhoI and HindIII. The PCR product was cloned into pEGFP-N1 expression vector upstream of GFP gene sequence, confirmed using restriction enzyme analysis, colony PCR and sequencing. Vero cells was transfected with the siat7e-pEGFP-N1 construct using lipofectamine 2000 (Invitrogen) under manufacture's protocol. The cell clonal selection was performed using selective medium containing G418 which routinely replaced every 3 to 4 days for a period of time. Stably transfected Vero-siat7e-expressing cells were evaluated using Inverted immunofluorescence microscopy.

Results & Conclusion: Anchorage-dependent Vero cells exhibited changes in cell-cell adhesion and cell spreading behavior following the incorporation of the Siat7e gene. The Vero-siat7e-expressing cells also lost the ability to form tight junctions with the adjacent cells. Microscopic analysis at the end of the growth showed that the parental Vero cells were aggregated, while the siat7e-expressing, on the other hand, appeared with 100% viability and were suspended primarily as individual cells with fluorescent signal.

It is the first study of human siat7 gene transfection in the WHO candidate cell for virus propagation in order to cell-based vaccine production in emergency situation.

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