Monitoring of coxsackievirus replication in clones of NIH 3T3 cell line

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INTRODUCTION: Type B coxsackieviruses (CVB) belong to genus *Enterovirus* of the family *Picornaviridae*. CVs are small positive-stranded RNA viruses, important human pathogens that cause both acute and chronic diseases. These viruses induce lytic infections, their cytopathic effect (CPE) includes morphological changes and destruction of the host cell monolayer *in vitro*. Their viral RNA can remain persistent for prolonged times in cells and organs post infection. Literature reports show moderate replication of enteroviruses in NIH 3T3 cells.

AIM: to study the replication of CVB in NIH 3T3 cells with resistance to Puromycin alone or along with Blastomycin in combination with truncated variant of the Dicer ribonuclease.

RESULTS: morphological changes were absent in early passages of the infected cells as compared to the mock-infected control of the NIH 3T3 cell lines. Rounding and cell disformation of cells was observed after 3 or4 passages. PCR analyses showed presence of the viral RNA without CPE.

MATERIALS and METHOD: Genetically modified clones of NIH 3T3 cells:
2801196: NIH3T3 cells, SpCas9, clone#E8, Puro^R
2801222: NIH3T3 cells, SpCas9#E8 + $^{HRBlast\text{-}HA\text{-}Dcro\_v3}$, clone#4, Puro^R +Blast^R
2801225: NIH3T3 cells, SpCas9#E8 + $^{HRBlast\text{-}HA\text{-}DcrO\_v3}$, clone#15, Puro^R +Blast^R
2801170: NIH3T3 cells, SpCas9#E8 + $^{HRBlast\text{-}HA\text{-}Dicer\ SOM\_v3}$, clone#C6, Puro^R +Blast^R
Virus: CVB3 (Nancy strain). Previously passaged in Vero cells (monkey kidney epithelial cells), with a titer of $10^{6.75}TCID_{50}$ and with a 0.1 multiplicity of infection.
The cells were observed for the CPE till 5 days post infection (p.i.).
Presence of viral RNA: Reverse transcriptase polymerase chain reaction (PCR) and Nested-PCR.
Staining: Monolayer fixed with 4% formalin and stained with hematoxylin-eosin.
To confirm viral persistence, we followed 3 different protocols for processing the infected cells.

CONCLUSIONS: NIH 3T3 cells 2801196 showed virus persistence to passage number 4 and viral RNA was absent on further passages. Clones 2801225, 2801170 with knockout mutations did show persistence of viral RNA to passage levels 3 and clone 2801222 did not support viral RNA persistance at all.
We suggest that the virus may not replicate but the RNA may persist in the cells till 4 number of passages.
Our results demonstrate the possibility of the development of persistent viral infection of NIH 3T3 cells. Pathogenesis of viral infections is affected not only the host immune response, but an altered viral replication and transcription.

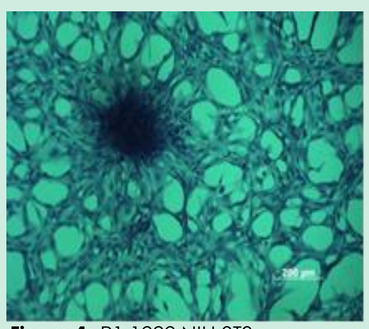
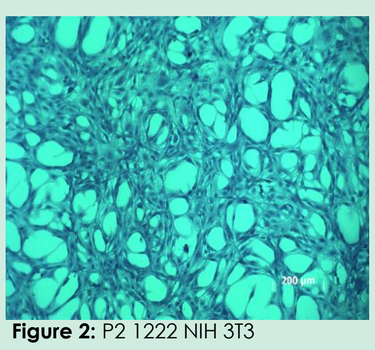


Figure 1: P1 1222 NIH 3T3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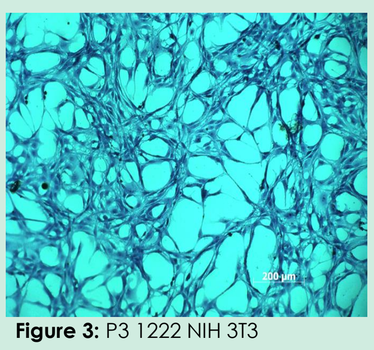
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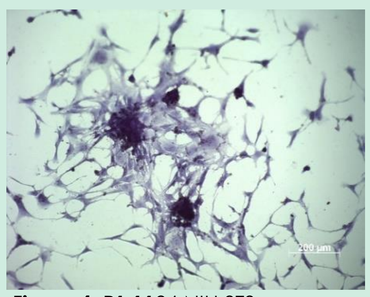
Figure 4: P1 1196 NIH 3T3

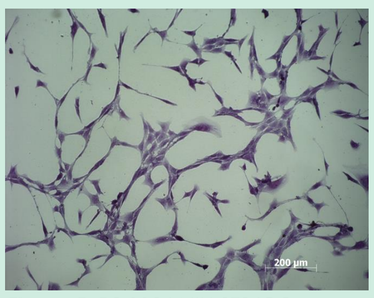
Figure 5: P2 1196 NIH 3T3/CO_2

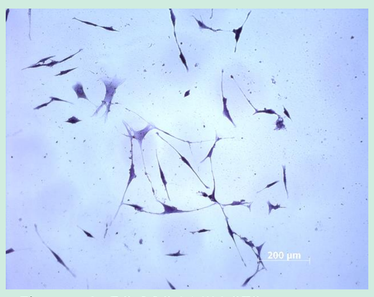
Figure 6: P2 1196 NIH 3T3

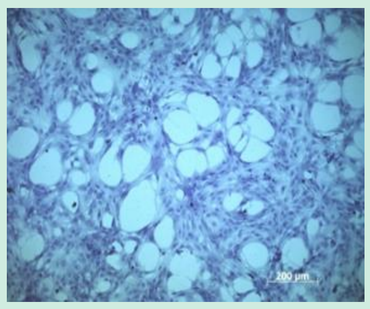
Figure 7: P3 1225 NIH 3T3

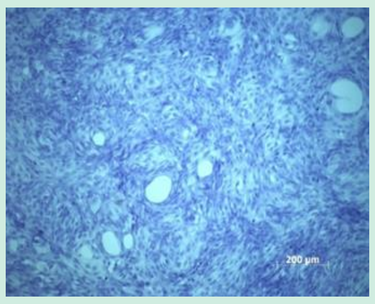
Figure 8: P4 1225 NIH 3T3

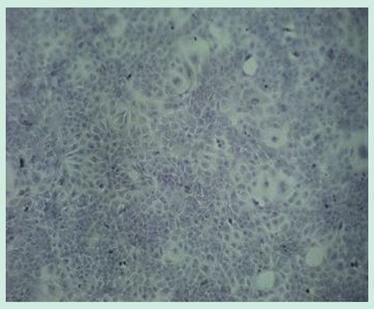
Figure 9: P2 1170 NIH 3T3

Figures: Cell morphology at different passage levels: although these cells are adherent cell lines, monolayers with empty "eyelids" were observed in all cell lines. In addition, 3T3-1222 cells also form dense cellular foci. We also compared cells under CO2 conditions, but did not affect cell growth.

Passage: from original (CVB3:Vero7, titer $10_{6,75}$) → NIH 3T3 cells				
Passage number of the virus (P)	**Cell characteristics.** Day post infection, morphological changes.	**PCR-C.** The pellet of the infected monolayer was washed with 2ml PBS, freeze thaw, centrifuge, and RNA isolated.	**PCR- S.** only infected cell supernatant when no CPE. Centrifuged and only the supernatant collected for PCR.	**PCR** direct Cells were not washed but directly processed by freeze thawing
P1, P2, P3, P4 (2801196)	Day 5, no CPE	positive	positive	positive
P5, P6(2801196)	Day 5, no CPE	negative	negative	negative
P1, P2, P3 (2801225)	Day 5, no CPE	positive	positive	positive
P4, P5 (2801225)	Day 5, no CPE	negative	negative	negative
P1, P2, P3 (2801170)	Day 5, no CPE	positive	positive	positive
P4, P5, P6 (2801170)	Day 5, no CPE	negative	negative	negative
P1, P2, P3 (2801222	Day 5, no CPE	negative	negative	negative
p.i.= post infection; m.l = monolayer				

Table: We also made an attempt to adapt the virus to the cells by blind passages in the NIH 3T3 cells. The table shows the results of the virus passages on the NIH 3T3 cells.

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