Optimisation of a Reporter Cell Line for Assessing Neutralising Antibody Titres to Hepatitis C Virus

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Introduction
Neutralising antibodies to hepatitis C virus (HCV) are studied to characterise the determinants of HCV virulence and for vaccine and immunotherapy development. To discover useful antibodies, patients are screened for HCV neutralising activity by incubating their serum with a lab strain of HCV, and incubating this with susceptible cells. Days later the cultures are stained to reveal infected cells and the amount of infecting virus is expressed as focus forming units (FFU). This method is expensive and time-consuming. The Huh7-J20 reporter cell line, provided by Dr A Patel, may provide a cheaper and faster alternative. When this line is infected with HCV, the NS3/4A viral protease cleaves a reporter protein, releasing secreted alkaline phosphatase (SEAP) into the culture supernatant, which can be measured by spectrophotometry.

Aim: To optimise the Huh7-J20 cell line for use in neutralising antibody assays.

Results

1. Huh7-J20 cells express the fusion protein
The Huh7-J20 cell line contains a fusion protein of EGFP which anchors SEAP within uninfected cells allowing visualisation of cells expressing the fusion protein.

Figure 1: Fluorescent images of uninfected Huh7-J20 cells (left, green is EGFP) and Huh7 cells (middle) with DAPI stained nuclei (purple), x20. GFP distribution within the Huh7-J20 cell line by FACS (right).

2. Cell density affects SEAP secretion

Figure 2: SEAP levels at 4 days of culture of Huh7-J20 infected with 1000 FFU HCV (orange, n = 3). Uninfected monolayers (blue, n = 2). SEAP was highest at 1-1.5×10⁴ cells/well (P<0.01) corresponding to 50-70% monolayer confluency.

3. SEAP levels increase with incubation time of infected monolayers

Figure 3. The length of infection time at which Huh7-J20 gives strong SEAP levels was investigated using 1000 FFU, (orange) compared with uninfected cells (blue). SEAP was detected at 3 days post infection, with 4 days giving a robust signal.

4. Supernatant SEAP levels increase with virus dose

Figure 4. Cultures were infected with a range of FFU and SEAP assayed on day 4 after infection. The SEAP assay was incubated for different periods of time to determine the effect on titres. As little as 50 FFU HCV can be detected with a longer SEAP incubation. A routine infection of 1000 FFU gives an adequate absorbance reading at 24 hours.

5. Absorbance and chemiluminescence SEAP detection

Figure 5: An inexpensive absorbance SEAP detection assay was compared with a commercial chemiluminescence assay for sensitivity and proved satisfactory.

6. Neutralisation assays
The Huh7-J20 cell line detected HCV neutralising activity in human serum as confirmed with purified antibody from 1 donor (Fig 6) and also in plasma from 3 other patients. One gave a higher response by this reporter assay than expected. This donor will be tested in parallel by both assays to determine if the titre is assay specific.

Figure 6. SEAP activity from an infection neutralising assay using purified immunoglobulin from a HCV neutralising donor (blue) and uninfected patient plasma (orange).

Summary
The Huh7-J20 cell line was optimised for maximum SEAP secretion on HCV infection. It detects the activity of neutralising antibodies within human serum in a sensitive and high-throughput manner.

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Methods
Plasma: from patients with HCV genotypes 2, 3 or 4 kindly provided by Dr J Flexman, Royal Perth Hospital. Serial dilutions of plasma were incubated for 1 hour at 37°C with HCV JFH-1 virus (genotype 2a), then added in duplicate to Huh7-J20 monolayers in a 96 well tray.

Huh7-J20 infection assay optimisation: Huh7-J20 cells were seeded at 0.5 - 4.5×10⁴ cells/well in a 96 well tray. The next day cells were incubated with 50-3500 FFU HCV for 1-3 hours before replacing with fresh medium. The cell supernatant was collected and assayed for SEAP.

SEAP detection: Supernatant was heated at 65°C for 20 minutes to inactivate endogenous SEAP. 150µL of p-nitrophenylphosphate dissolved in diethanolamine buffer was added to supernatant samples in a 96 well tray. Blank controls were included. The plate was incubated at 37°C for 24 hours before reading absorbance at 405 nm. The Great EscAPl™ SEAP Chemiluminescence Kit 2.0 (Clontech) was used following the manufacturer’s protocol.