Exploring the Biomarker Potential of Circulating Tumour Cells in Patients with Hepatocellular Carcinoma: FGFR4 and FGF-19 Expression Levels in Liver Cancer Cell Lines

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Introduction

Hepatocellular Carcinoma (HCC) is the 2nd commonest cause of cancer death and it is rising in the UK . Research has recently uncovered a number of novel targeted treatments for HCC patients, which are now in clinical trials. Fibroblast Growth Factor Receptor 4 (FGFR4) and its ligand FGF-19 are seen to be generally over-expressed in a significant portion HCC patients, which suggests that the FGF-19-FGFR4 pathway is critically involved in the development of HCC (1). Thus, determining if these 'targets' or biomarkers are detectable in Circulating Tumour cells (CTCs) in patients' blood would provide prognostic value and aid in treatment stratification for HCC patients. To detect the targets, primary antibodies are used to attach a secondary fluorescent marker to be visualised and quantified. These primary antibodies are first optimised and then tested on liver cancer cell lines using the ImageStream Flow Cytometer to determine the individual biomarker expression levels in the cell lines. With these results, we can then know if FGFR4 and FGF-19 are highly expressed in liver cancer cells and further research on detecting these biomarkers in patients' blood can be conducted.

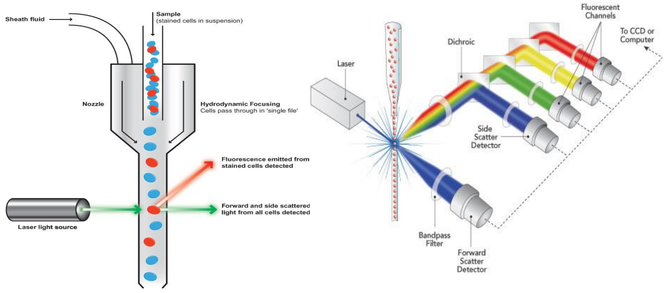

Figure 1: Illustration of the how the ImageStream Flow Cytometer work. The stained cells are first passed through a narrow tube. The cells and fluorophores will then be excited by lasers and the signals given off by the cells will be detected by fluorescent channels (2).

Research Aims

- Optimise the concentration of primary antibody, temperature and duration of incubation and fixing agent for both FGFR4 and FGF-19.
- Determine the expression level of FGF-19 and FGFR4 in liver cancer cell lines.

Systemic Literature Review

- The Google Scholar and PubMed database was used as search databases.
- The search terms include 'Hepatocellular carcinoma', 'cell lines', 'biomarker' and 'flow cytometry'.
- Studies which reported positive and negative cell lines were included in the study.
- Studies which compared staining protocols were also included in the study.

Optimisation of Primary Antibody

- Before the primary antibodies were used in a biomarker panel for targeted treatment stratification in patient samples, optimisation was carried out to determine the staining protocol to be used in liver cancer cell lines and patient samples.
- In order to determine each antibody's sensitivity and selectivity, a series of optimisation steps were carried out on positive and negative controls, which are cell lines that are positive or negative for the given biomarker.

Biomarker	Primary Antibody	Variable	Optimum of variable
FGFR4	FGFR4 (C-16) sc-124, Rabbit Polyclonal IgG	Positive and Negative Controls	Positive Cell Line Negative Cell Line
		Antibody concentration: 1:100, 1:200, 1:500	1:500
		Fixing method: Methanol, Formalin	Both
		Normal Parameter of Time and Temperature	1 Hour at Room Temperature
		Blocking Agent: BSA only, BSA and 5% Goat Serum	BSA and 5% Goat Serum
FGF-19	FGF19 (W12) sc-73984, Mouse Monoclonal IgG	Positive and Negative Controls	Positive Cell Line Negative Cell Line
		Antibody Concentration: 1:50, 1:100, 1:200	1:100
		Fixing Method: Methanol, Formalin	Both
		Normal Parameters of Time and Temperature	1 Hour at Room Temperature
		Blocking Agent: BSA only, BSA and 5% Goat Serum	BSA and 5% Goat Serum

Table 1: Optimisation of FGFR4 and FGF19 Primary Antibodies based on different variables and the optimum variable.

Staining of Liver Cancer Cell Lines

- Before the collection of cell line cells, the cells are first cultivated in an incubator at 37 °C with appropriate media to proliferate, or divide. The cells are then collected and counted using a haemocytometer.
- 500,000 cells from each cell line are then stained with either FGFR4 or FGF-19 primary antibody.
- They are next stained with Texas Red Secondary antibody which binds to the primary antibody. This secondary antibody has a fluorophore attached to it. The fluorophore fluoresce when excited by a laser with a particular wavelength given off by the flow cytometer.
- They are finally added with DAPI fluorescent stain which binds strongly to DNA.

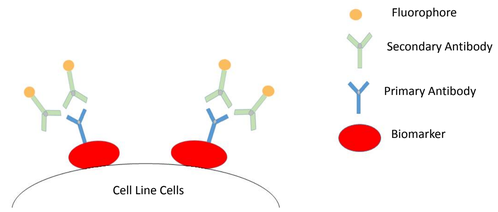

Figure 2: Illustration of the how the primary and secondary antibody attach to the antigen and fluorophore respectively.

Results and Discussion

- The cell line cells are run through the ImageStream flow cytometer to collect data regarding the biomarker expression levels.
- The IDEAS® software is then used to analyse and compare the biomarker expression levels of each cell line.

FGFR4

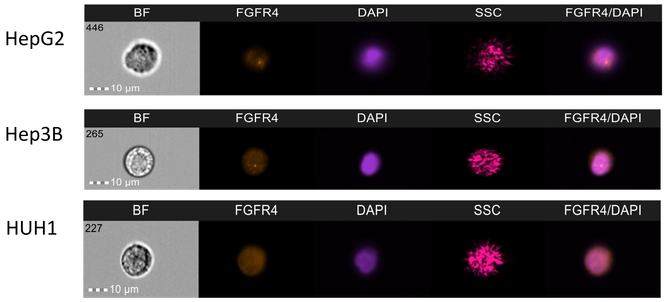

Figure 3: Cell Line Expression Images. From the figure above, it can be seen that cells in the cell lines above do express FGFR4 to a certain degree . The DAPI signal depicts the degree of nuclear density, which is largely increased in cancer cells compared to normal cells.

FGF-19

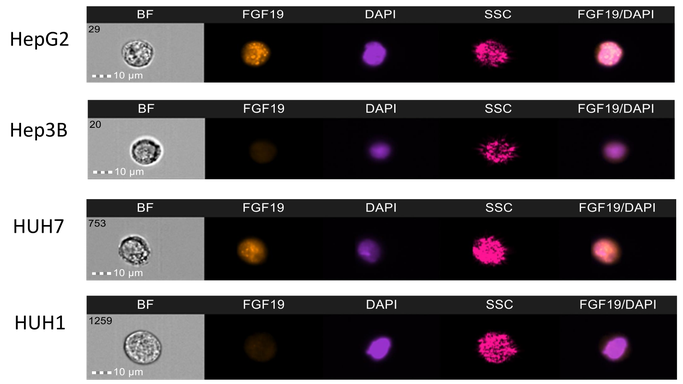

Figure 4: Cell Line Expression Images. From the figure above, it can be seen that the cells express FGF19. It can also be seen that these cell lines have a varying degree of expression levels by looking at the intensity of the FGF19 stain.

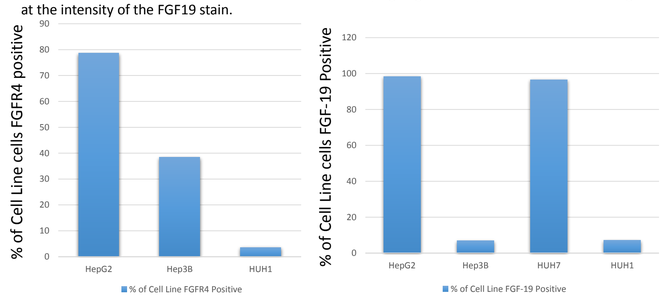

Figure 5: Percentage of Cell Line Biomarker Positive. From the graphs above, it is seen that there are varying degree of positivity in total cells. HepG2s highly express both FGFR4 and FGF-19 while HUH-1s only express slightly. This suggests that the biomarkers are amplified differently in tumours of different patient.

Conclusion

- The primary antibodies are sensitive in detecting some of the liver cancer cell line cells. Thus, the primary antibodies can be used in a biomarker panel in detecting CTCs in patients' blood.
- However, the specificity of the biomarker should be tested on cell lines other than liver cancer cell lines to determine if it is a liver cancer cell specific biomarker or just a cancer cell specific biomarker.

References: (1) Mellor, H. R. (2014). "Targeted inhibition of the FGF19-FGFR4 pathway in hepatocellular carcinoma; translational safety considerations." Liver Int **34**(6): e1-9.
(2) DR. Debby Burshtyn. "Flow Cytometry@FoMD; What is flow cytometry."