FISH for the confirmation of the cancerous origin of cells on a microsieve

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Introduction

Circulating tumor cells, CTCs, are cells which are released from a primary tumor and travel through the blood and could form metastasis. These cells can be isolated from blood of patients with metastatic cancer on base of their expression of EpCAM protein.[1] However, some CTCs lack EpCAM and are therefore not captured this way. By passing blood through a microsieve, these cells can be captured and can further be analyzed.[2]

Previous research on identification of cells from patients' blood on a microsieve using immunological staining showed that there are some cells present on a microsieve with an epithelial origin. These cells might be CTCs, but this needs confirmation. One way to confirm the cancerous origin of these cells is FISH.

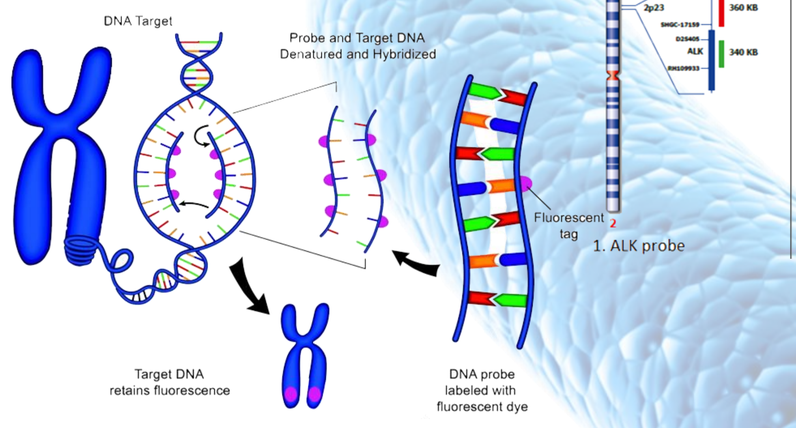

Figure 1: The principle of FISH:
Target DNA is first denatured by heat. At a lower temperature, the probe with fluorescent labels can hybridize to its single stranded target DNA. When using fluorescent microscopy, these signals can be visualized. [3]

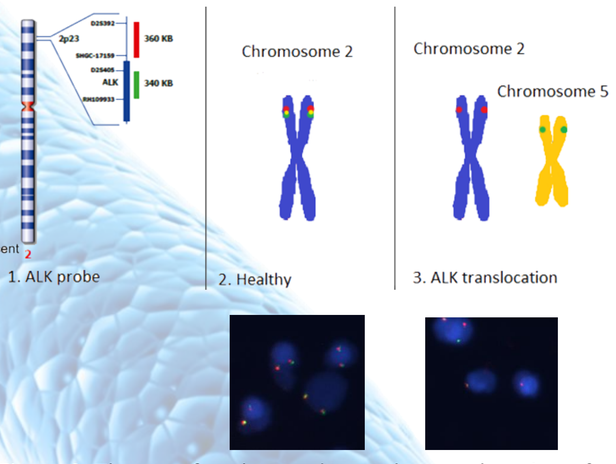

Figure 2: The set of probes to detect the translocation of the ALK gene consists of two probes. One probe, labelled with PE, hybridizes to a part of chromosome 2. The other probe, labelled with FITC, hybridizes to the ALK gene. In healthy cells, these probes hybridize close together The translocation in CTCs is visible as a separate red and green dot.

Aim

The aim of this research is to detect a heterozygous ALK translocation from chromosome 2 to chromosome 5 in CTCs from patient blood on a microsieve and therefore confirm the cancerous origin of these cells. For this, FISH first needs to be optimized on a microsieve.

Methods

Filtration

10.000 tumor cells, of different origins, were spiked in 7.5 mL whole blood and were fixed overnight in CellSave. Blood with these cells were passed through a microsieve using a pressure of -100mBar. After washing and permeabilization of the cells, the cells were stained on the microsieve with anti CD45-PerCP (white blood cell marker), anti-cytokeratin-PE (epithelial cell marker) and Hoechst (DNA marker).

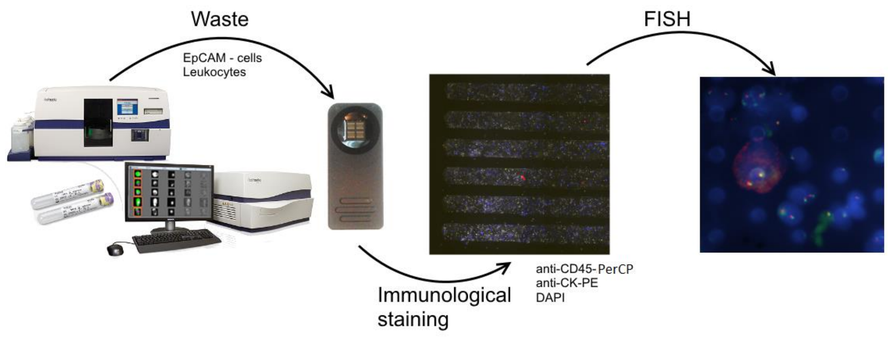

Figure 3: Experimental approach. Waste from the CellSearch AutoPrep system, containing EpCAM negative cells, is passed through a microsieve. After immunological staining of these cells, possible CTCs can be detected.
To confirm the cancerous origin of these cells, FISH using specific probes can be applied onto the microsieve.

Fluorescent in-situ hybridization

10µl probe with a concentration of 4 ng/µl was applied on the upside and underside of the microsieve. The microsieve was then covered with a coverslip and sealed with Fixogum. DNA was denatured on a humidified chamber at 85˚C for 10 minutes, followed by hybridization overnight at 37˚C. After hybridization, stringency washes were applied on the microsieve. Cells were counterstained using 5µl counterstain with DAPI (DNA marker).

Fluorescence microscopy

Images were taken using the Nikon Eclipse 400 epi-fluorescent microscope, equipped with filters for detection of the used fluorochromes.

Results

The image of FISH on a microsieve containing a CTC among leukocytes is visible in figure 4. Here, one possible CTC with the ALK translocation and one leukocyte without the ALK translocation are found.

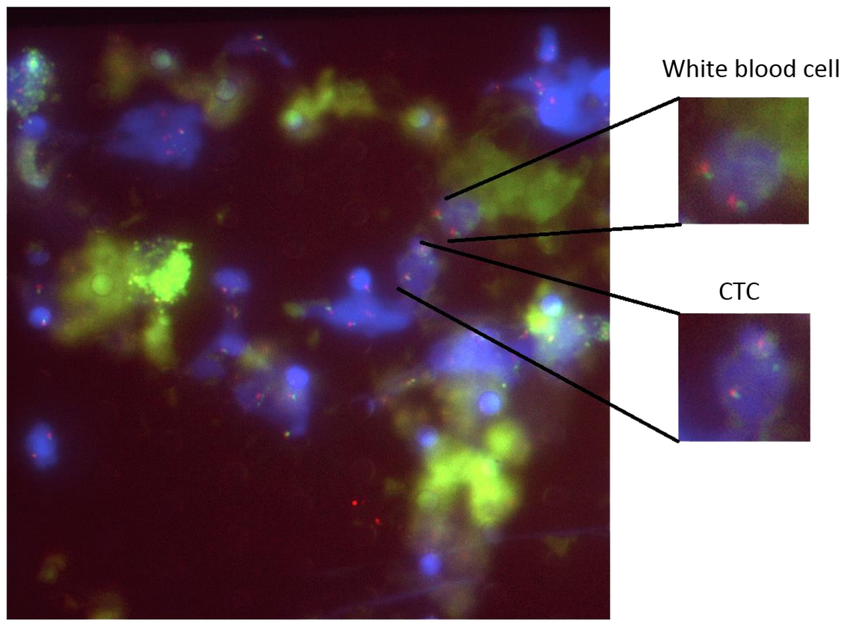

Figure 4: Microsieve after FISH. Overlay of DAPI (DNA stain, blue), PE (red) and FITC (green). Cell with ALK translocation and a leukocyte are enlarged. The leukocyte doesn't contain a translocation, visible as a red and green dot close together. The CTC does contain a heterozygous translocation, visible as a separate red and green dot.

Figure 5 shows the characteristics of other found CTCs and leukocytes. This figure shows that not all cells with ALK translocation are positive for cytokeratin staining, but are all negative for CD45 staining. The leukocytes which have been found are positive for CD45 and negative for cytokeratin staining.

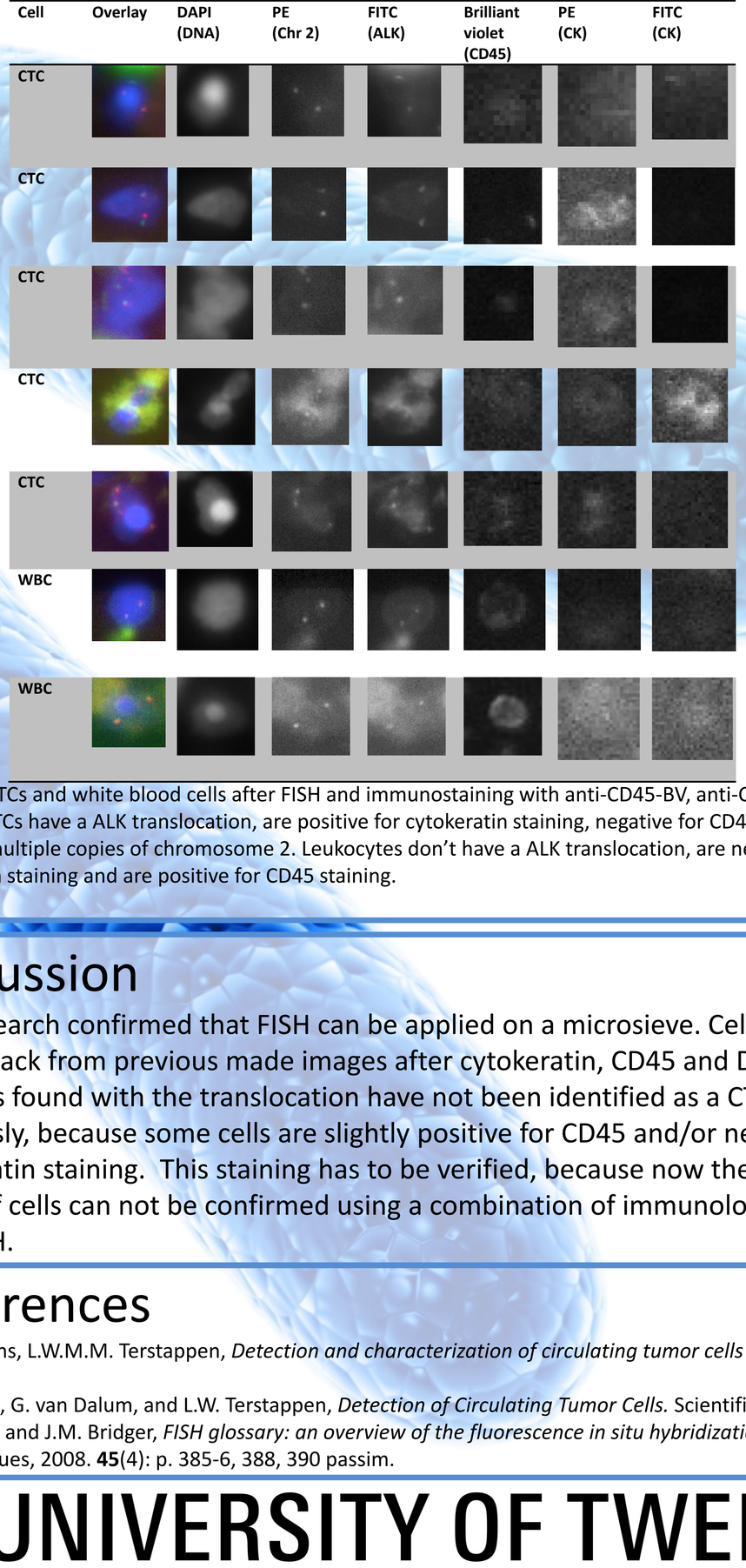

Figure 5: CTCs and white blood cells after FISH and immunostaining with anti-CD45-BV, anti-CK-PE and anti-CK-FITC. CTCs have a ALK translocation, are positive for cytokeratin staining, negative for CD45 staining and can have multiple copies of chromosome 2. Leukocytes don't have a ALK translocation, are negative for cytokeratin staining and are positive for CD45 staining.

Discussion

This research confirmed that FISH can be applied on a microsieve. Cells can be traced back from previous made images after cytokeratin, CD45 and DNA staining. The cells found with the translocation have not been identified as a CTC previously, because some cells are slightly positive for CD45 and/or negative for cytokeratin staining. This staining has to be verified, because now the cancerous origin of cells can not be confirmed using a combination of immunological staining and FISH.

References

[1] F. Coumans, L.W.M.M. Terstappen, *Detection and characterization of circulating tumor cells by the CellSearch approach.*

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[3] Volpi, E.V. and J.M. Bridger, *FISH glossary: an overview of the fluorescence in situ hybridization technique.* Biotechniques, 2008. **45**(4): p. 385-6, 388, 390 passim.

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