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Quantitative and immunohistochemical detection of breast cancer cells in blood samples

Background

Disseminated tumor cells (DTCs) in bone marrow and circulating tumor cells (CTCs) in blood are found in patients with epithelial carcinomas (breast cancer) and are often correlated with poor prognosis of the disease.
In current models circulating tumor cells (CTCs) dissolved from the primary tumor are thought to be responsible for the occurrence of metastases.
However, the detection of CTCs is still a technical challenge.
In this study, two methods for tumor cell detection of patients' samples are presented (Real-Time-PCR and immunohisto-chemical staining). Both are known methods with a high sensitivity and a spread marker panel.

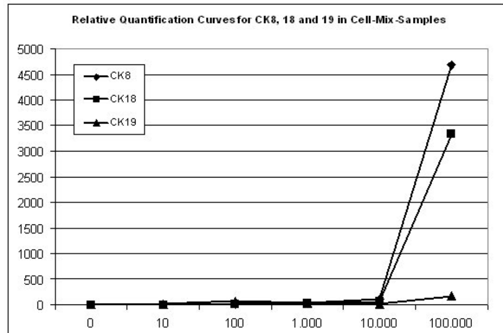

Figure 1: Relative Quantification Curves

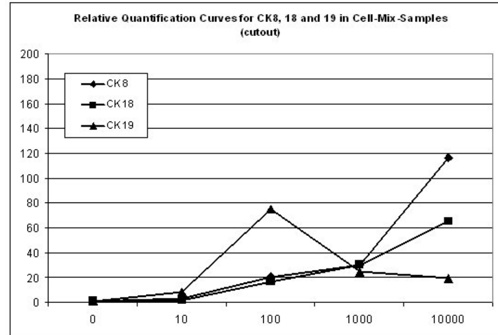

Figure 2: Enlargement of region between 0 and 10.000 cells added per ml/sample

Patients and Methods

For the implementation of both methods, different breast cancer cell lines have been used (Cama-1, MCF-7; ZR-75-1). For Real-Time-PCR, blood samples of a healthy donors were spiked with different cell counts (0, 10, 100, 1000, 10.000 and 100.000) per ml blood sample. Total RNA was isolated from the samples, reversely transcribed into cDNA and used for TaqMan Real-Time-PCR reaction with probes against CK8, 18 and 19, while 18S was used as reference. Relative Quantification Curves were drawn by MicrosoftTM Excel®.
For immunohistochemical staining, cytospins were prepared from spiked blood samples, fixed with acetone, air dried and stained with antibodies against Her2- and Thomsen-Friedenreich-Antigen (CD176). In a second staining step fluorescently labelled secondary antibodies were applied. Nuclei were counterstained with DAPI, TF-Antigen was stained by Cy2 and Her2-Antigen by Cy3. The staining was controlled and documented by an epifluorescence microscope.

Results

The curve of Relative Quantification for MCF-7 and ZR-75-1 cells shows an increasing slope starting from 1000 cells (Fig.1). For the Cama-1 cell line this trend is already seen from 10-100 cells (Fig.2). In ZR-75-1 all three genes analysed reveal this trend, whereas in Cama-1 and MCF-7 cells a strong increase in Relative Quantification is especially seen for CK8 and 18.
In the immunohistochemical staining, the cells were considered as tumour cells if they showed staining with the antibody-combinations used (Fig.3). Stained cells were counted and recovery rates were determined. For ZR-75-1, 17 of 30 cells which were spiked in the blood samples were recovered. For MCF-7, 18 cells were found in average, and for Cama-1 23 cells were located per slide. The recovery rates calculated from these numbers are 56,6% and 60,0% for ZR-75-1 and MCF-7, for Cama-1 the recovery rate reaches 76,6% (see Table1).

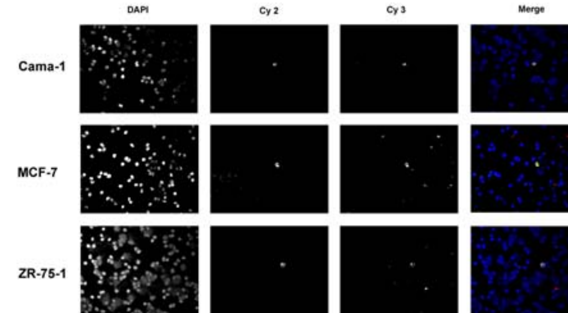

Figure 3: Staining patterns in variouis blood samples spiked with cell line cells

Table1: Rates of TF, Her-2 or both positive staining in various cell lines

	Cama-1	MCF-7	ZR-75-1	Cell-Mix
TF (CD176)	28 (93,3%)	21 (70%)	22 (73,3%)	28 (93,3%)
Her-2 (CD 340)	26 (86,6%)	18 (60,0%)	23 (76,6%)	25 (83,3%)
TF / Her-2	23 (76,6%)	18 (60,0%)	17 (56,6%)	25 (83,3%)

Conclusion

It seems that Cama-1 cells are a better model than MCF-7 and ZR-75-1 for Real-Time PCR quantification of mamma carcinoma tumor cells in blood samples. MCF-7 and ZR-75-1 cells tend to react more likely immunologically with blood cells of the donor (agglutination between blood cells and cancer cells). The Cama-1 cell line shows also advantages in the detection of tumor cells using immunohistochemical staining. Therefore it will be necessary to test both methods on patient samples to proof their benefit.

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