A new approach for screening of colorectal cancer circulating tumor cells (CTC) using EpCAM-pluriBeads

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Introduction

The presence of adequate methodological tools is the most important prerequisite for successful achievement of actual goals. Developing of methods for circulating tumor cells (CTC) screening is the key problem in cancer research. Typically, appearance of CTCs in blood indicates a poor prognosis in cancer development. Recently, several innovative approaches to detect rare cells in blood were developed. Some of them are based on physical or biological properties of epithelial cells. For example, epithelial cell adhesion molecule (EpCAM) antigen (an oncogenic signaling molecule for cancer cells) is being used as a target for immunotherapy treatment of human carcinomas.

Here we are presenting a new approach of colon carcinoma circulating tumor cells (CTC) screening using pluriBeads carrying a tumor-associated EpCAM antibody. This method is based on a non-magnetic cell separation technology. It does not require any sample pre-treatment. EpCAM-pluriBeads can be added directly to a whole blood sample. The method is also suitable for single cell isolation from different biological fluids. Moreover, its sensitivity can be additionally increased via raising of the sample volume. The bound EpCAM-positive colon carcinoma cells can be easily involved in further molecular-genetic experiments aiming detection of their mutation status. In case of colon carcinoma, KRAS mutation status is predictive tool of response to anticancer therapy. Thus, the new method can be judged as a fast and effective instrument for early cancer diagnostics

Materials and methods

a. Known number of Caco-2 colon carcinoma cells were spiked in fresh human EDTA whole blood, then EpCAM-pluriBeads were added and mixed

b. 30 minutes incubation under gentle agitation

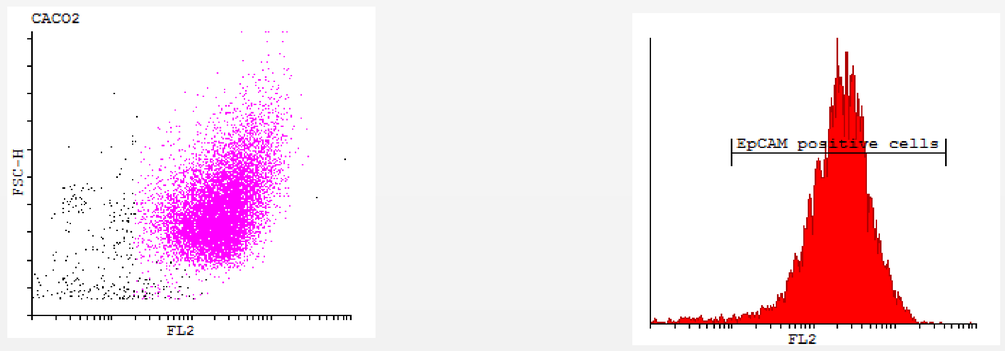
c. captured target cells were separated via appropriate sieves

d. Target cancer cells remained on the sieve

e. molecular-genetic and cell culture approaches for study of cancer cells
- RNA/DNA isolation
- In situ pcr
- Cell culture experiments

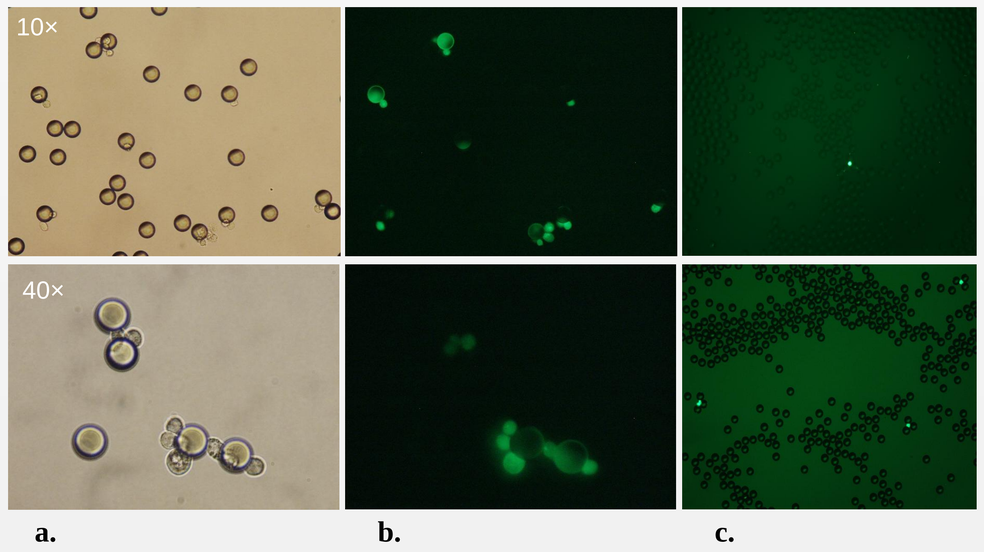
Fig.1. Protocol for detection of colon carcinoma cells in blood by cell spiking.

Results



a. **b.**

Fig. 2. a. b. Flow cytometric analysis of Caco-2 cells staining with anti-EpCAM-PE.



a. **b.** **c.**

Fig. 3. a. Colon carcinoma Caco-2 cells captured by EpCAM-pluriBeads. b. Captured colon carcinoma Caco-2 cells staining with calcein. c. Captured colon carcinoma Caco-2 cells spiked in 30 ml whole blood with 0.5 Mio EpCAM-pluriBeads

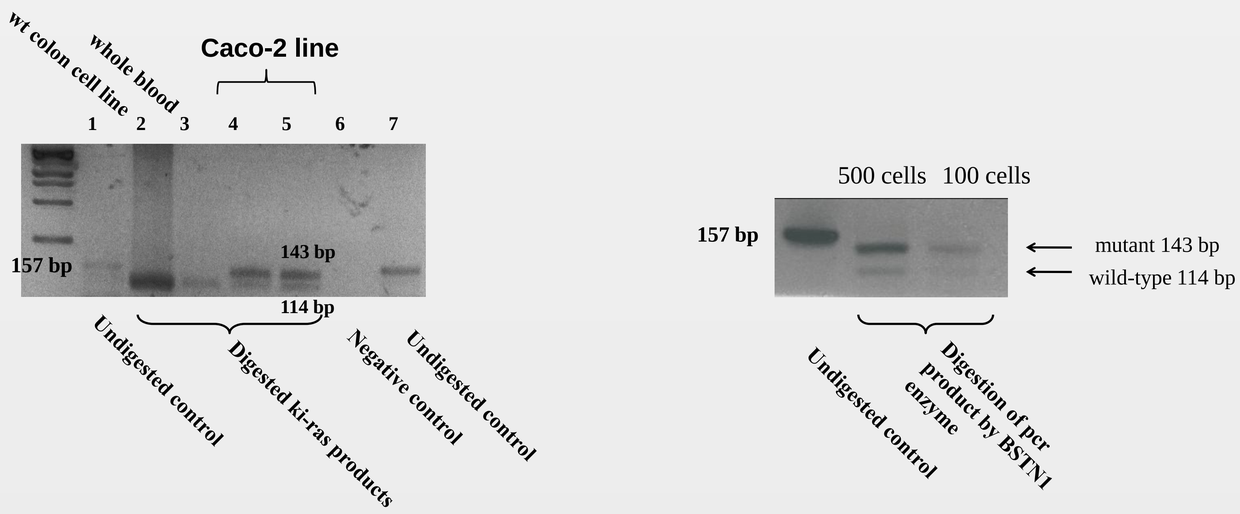


Fig. 4. Screening of colon carcinoma lines for the presence of mutation in 12th codone of k-ras oncogene by pcr using primers that introduce BSTN1 restriction enzyme sites into the PCR products. Bands at 114 bp (2 – wilde type colon cancer line, 3 – whole blood) show the presence of normal (wild type) sequence. Bands at 143bp (4, 5) lines show the presence of mutant k-ras sequence in Caco-2 line. Bands 1 and 7 – 157 bp – undigested product, line 6 – negative control.

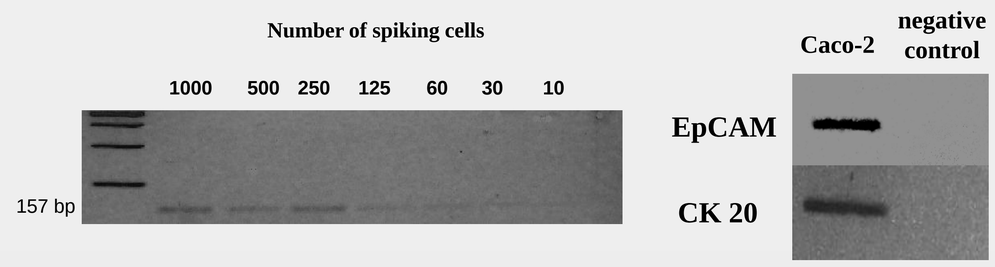


Fig.5. Sensitivity of pcr method for detection of k-ras protooncogene in known number of spiking Caco-2 cells.
b. Products of RT-PCR for EpCAM and CK20 mRNA isolated from the Caco-2 cells, captured by EpCAMbeads (as a negative control mRNA from whole blood was used)

Conclusions and perspectives

Here we developed a new approach for detection of circulating colon tumor cells in blood using EpCAM-pluriBeads. Captured cells are suitable for further molecular-genetic screening of specific markers, connecting with tumor formation. Developed „in situ immunobeads pcr“ method does not required preliminary RNA/DNA isolation and can effectively save the time of analysis. The further aim of this work is to increase the sensitivity of method via raising of the sample volume, varying of cell tumor lines and/or antibody specificity.

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