Abstract

Introduction: Circulating tumor cells (CTC) and disseminated tumor cells (DTC) are responsible for the development of metastasis. Detection of CTC from peripheral blood (so-called "liquid biopsy") may contribute to the diagnosis and choice of therapy in patients in whom it is not possible to obtain tissue directly from the tumor. Monitoring the effect of surgical treatment and detecting early recurrence of cancer are other potential uses for CTC.

Aim: The aim of our work was to isolate CTC/DTC from blood or peritoneal lavage of patients with various types of solid tumors and then try to cultivate them *in vitro* and to describe their cytomorphological characteristics. We targeted the following hypotheses:

I. Do CTCs occur in different types of solid tumors?
II. Does the presence of CTC reflect cancer stage?
III. Is it possible to use CTC for molecular characterization of cancer?
IV. Is it possible to visualize CTC/DTC in a viable status?

Methods: CTC/DTC detection was performed in a total of 288 patients, including 24 patients with pancreatic cancer, 165 with breast cancer, 98 with colorectal cancer and 1 with a neuroendocrine tumor of the small intestine. A cell size separation protocol (MetaCell®) was used to enrich CTC. After simple filtration of blood through a polycarbonate membrane with pores, the captured cells (greater than 8 µm) were incubated *in vitro* for a short time (3–5 days) or for a long time (more than 5 days). After short-term incubation, cells were stained by histochemical staining, immunohistochemical staining with specific antibodies to identify cell organelles, nuclear staining or vital fluorescent staining. The presence of CTC was evaluated on the basis of cytomorphological characterization according to defined histopathological criteria. In a portion of the samples (n = 43) CTC were detected by molecular analysis (qPCR). Gene expression analysis was performed in breast cancer patients using a multimarker panel of genes associated with tumorigenic or therapeutic potential. Twenty patients were monitored regularly during treatment for HER2 and ER status. The incidence of CTC/DTC was compared with the stage of cancer in a cohort of patients.

Results: The presence of CTC was demonstrated in 216 (75.3%) of total patient cohort. CTC/DTC were further incubated as both short-term and long-term cell cultures. Some isolated CTC/DTC (colorectal cancer and neuroendocrine tumors) were grown *in vitro* for more than 6 months. CTC positivity did not correlate with disease stage, tumor size, or lymph node

involvement. The same percentage of CTC positivity was observed in metastatic and non-metastatic (66.7% vs. 66.7%) patients with pancreatic cancer. The characteristics of the CTC changed during the observed period of ongoing disease. The most significant finding was that the status of HER2 and ER in the CTC may differ from the status of these receptors in the primary tumor.

Conclusion: The size-based isolation method used allowed us to introduce viable CTC cell cultures *in vitro* in patients with various types of solid tumors. After CTC enrichment, we were able to visualize and further analyze these living tumor cells during the treatment of patients.