Cancer and tumor markers

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EGFR, KRAS AND BRAF MUTATION ANALYSIS IN INDIVIDUALLY DEPOSITED SINGLE CIRCULATING EPITHELIAL TUMOR CELLS (CETCS) USING THE COBAS® Z 480 ANALYZER

Z. Dorothea[1], P. Monika[1], P. Ulrich[1], P. Katharina[1]
[1]*Transfusion Center*

BACKGROUND: Targeted therapies directed specifically against somatic mutations enhancing the activity of signalling pathways have been shown to improve outcome compared with cytotoxic chemotherapies in patients with advanced tumors carrying the respective mutations. Using maintrac®, a non-dissipative approach avoiding enrichment steps, CETCs can be detected and individually isolated in almost all patients with lung and colon cancer and melanoma at different times of disease and therefore can provide a liquid biopsy to monitor the course of disease. We, here, report on the successful analysis of multiple isolated cells from individual patients for gene mutations in tumor driver genes EGFR, KRAS and BRAF.

MATERIALS and METHODS: Blood from patients with non-small cell lung cancer, colon cancer and malignant melanoma was analyzed for cells positive for epithelial cell adhesion antigen (EpCAM) using the maintrac® approach, which avoids cell selection, and an image analysis system or laser scanning cytometry for detection. Between 8-20 EpCAM positive cells from each patient were isolated individually using a semiautomated capillary approach and deposited one by one into micro cups. The DNA was subsequently amplified by whole genome amplification and assayed using either the cobas® EGFR Mutation Test, the cobas® KRAS Mutation Test or the cobas® BRAF V600 Mutation Test.

RESULTS: DNA could be amplified from all individually isolated cells. An EGFR mutation was detected in 17% of isolated tumor cells from a patient with non-small cell lung cancer, the KRAS Mutation was detectable in 28% of cells from a patient with colon cancer and the BRAF Mutation in 50% and 100 % of evaluable cells, respectively, from two patients with melanoma.

CONCLUSIONS: Individually isolating CETCs from the peripheral blood from patients with non-small cell lung cancer, colon cancer and melanoma allows not only to detect driver mutations but also to determine the frequency of mutated cells. This proves, that at least part of the CETCs are from the tumor. They can, in the future, be used as markers of response to the action of drugs and contribute insight into how resistance may be acquired.