Abstract

Colorectal cancer (CRC) is the third most common cancer worldwide; it is responsible for nearly 10% of all newly diagnosed cancers and is the second most cause of cancer related death in Europe. Biomarkers for therapy guidance, targeted therapy and survival prognosis are still limited. As CRC is a heterogeneous disease, different parts of the tumor might have varying molecular characteristics which may change during therapy or disease progression. Through solid biopsies and screenings, these local or temporal differences are impossible to monitor. To facilitate detection of these possible temporal changes, a regularly and non-invasively accessible biomarker is required for disease monitoring. Circulating tumor cells (CTCs) might represent such a biomarker as they have been shown to be fluid surrogates of the solid tumor. EpCAM positive CTCs have shown to be prognostic in CRC for survival, but their full potential has not yet been evaluated further. By using the High Definition Single Cell Analysis (HD-SCA) workflow, we were able to analyze the entire spectrum of CTCs and categorize them as the regular CTCs (HD-CTC), CTCs with a smaller nuclear area (CTC-Small), CTCs with low expression of epithelial marker cytokeratin (CTC-LowCK) and CTCs undergoing apoptosis and therefore releasing cell free DNA (CTC-cfDNA producing). In addition we observed and analyzed CTC clusters (CTCCs). The analysis included not only morphology and enumeration of CTCs, but also copy number variation (CNV) profiles on single-cell level.

In the first part of this study we focused on enumeration of all sub-categories of CTCs and CTCCs detected in the blood of stage IV CRC patients before surgery and in a follow-up draw. The goal was to characterize and define a subset of CTCs associated with metastases or reduced survival. Unlike previous publications we have not observed an association of the regular CTC (HD-CTCs) category with survival, but we observed that the CTC-Small category in the follow-up draw was associated to overall survival (OS, p=0.040). These findings are not concordant with common literature. This might be caused by the non-EpCAM based detection method or it could be specific for the Czech patient cohort.

However, the number of CTCCs per ml blood in the pre-resection draw was associated with shorter OS (p=0.021). We also detected an association of metastatic status at the time of CRC diagnosis (M1 vs. M0) with higher average amount of cells per cluster (p=0.035) which indicates, that larger CTCCs may be drivers for metastases.

In the second part of this study we extracted and analyzed single HD-CTCs from the pre-resection draws and also single cells from tumor tissue touch preparations. We amplified the whole genome and used next-generation sequencing to compare CNV profiles. The goal was to study clonality within CTCs and distinguish cancer promoting CTCs from less invasive and non-proliferating CTCs. Also, we wanted to

compare CNV profiles and clones of tissue samples with those of CTCs to gain knowledge about tumor evolution in CRC. After analysis of 136 single HD-CTCs from 11 CRC patients we could not detect any clonality (two or more single cells showing similar CNV changes). Compared to that, analysis of single cells from CRC touch preparations revealed clonality in 83.3% of tested patients. Comparison between tissue cells from the primary tumor and the metastasis showed similar clonal profiles with minor adaptions in the hepatic metastasis.

Overall, we were able to detect and characterize four morphologically different groups of CTCs showing the high heterogeneity of CTCs in CRC patients. The missing association of HD-CTC counts with survival may be caused by the limitation of the cohort, but also be due to probable detection of endothelial cells and their missing differentiation from ‚real' CTCs. This study serves as a preliminary study for future projects focused on both, CTC detection in earlier stage CRC in a prospective observational study, and characterization of CTCs and cells within CTCCs through multiplex protein detection.