Molecular characterization of EpCAM-positive disseminated cancer cells in prostate cancer

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Metastasis is the cause of more than 90% of cancer-related deaths. After resection of primary tumor, remaining disseminated cancer cells (DCCs) comprise founder cells of later arising lethal metastasis. DCCs are primary targets of adjuvant therapies. However, molecular features of DCCs are unknown, which explains the modest success of targeted adjuvant therapies. Therefore, our aim was to detect, isolate, and characterize DCCs from prostate cancer patients at molecular level.

DCCs can be found in bone marrow (BM) of cancer patients at frequency 10⁻6. They are usually detected using staining against epithelial markers (e.g. cytokeratins [CK] or epithelial cell adhesion molecule [EpCAM]). We have analyzed BM samples of three cohorts of PC patients for presence of single cytokeratin-positive (CK$^+$) or EpCAM$^+$ cells. These single cells were isolated and their genome and/or transcriptome subjected to whole-genome- (WGA) and/or whole-transcriptome-amplification (WTA). WGA products were analyzed using comparative genomic hybridization (CGH) and Sanger sequencing, and WTA products were analyzed using PCR, microarray analysis, and next generation sequencing.

Survival analysis showed that the presence EpCAM$^+$ cells in BM, as opposed to CK$^+$ cells, is associated with progression of the disease. CGH analysis revealed that EpCAM$^+$ and CK$^+$ cells show different levels of genome instability. Next, we focused on transcriptome analysis of EpCAM$^+$ cells. After limited transcriptome profiling, we found that EpCAM$^+$ DCCs co-express epithelial and BM-hematopoietic transcripts, thereby displaying unexpected transcriptome plasticity. Comprehensive transcriptome analysis of selected cells revealed that there are at least two subpopulations of EpCAM$^+$ DCCs, differing in abundance and identity of expressed transcripts, as well as in the level of genomic instability. Furthermore, these two subgroups displayed different mutation patterns. Finally, we analyzed transcripts specific for each group, and attempted to classify all cells of the collective into one of two groups.

Our data indicate that DCCs in prostate cancer show high level of heterogeneity at genome at transcriptome levels.