Comparative Analysis of EpCAM High-Expressing and Low-Expressing Circulating Tumor Cells with Regard to their Clonal Relationship and Clinical Value

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Background & objectives

Circulating tumor cells (CTCs) are mainly enriched based on the epithelial cell adhesion molecule (EpCAM). Although it was shown that an EpCAM low-expressing CTC fraction is not captured by such approaches, knowledge about its prognostic and predictive relevance and its relation to the EpCAM positive CTC subpopulation is lacking.

To close this gap of knowledge we developed an immunomagnetic assay to enrich CTCs EpCAM-independently enabling the comparison of EpCAM high expressing and EpCAM low expressing CTCs obtained from the same patient.

Methods

CTCs were enriched from blood samples of metastatic breast cancer patients using antibodies targeting Trop-2 and CD-49f. Their EpCAM expression was determined by fluorescence microscopy and EpCAM high expressing and low expressing CTCs were isolated as single cells. Their genomic DNA was amplified and analyzed regarding chromosomal aberrations and predictive mutations. Additionally, we compared enrichment of CTCs using this antibody mix with the EpCAM based enrichment using the CellSearch system.

Results

The combined application of antibodies against Trop-2 and CD-49f achieved a synergistic effect on the CTC yield. Patients with EpCAM high-expressing CTCs had a worse overall and progression free survival. EpCAM high- and low-expressing CTCs presented similar chromosomal aberrations and mutations indicating a close evolutionary relationship. A sequential enrichment of CTCs from the EpCAM depleted fraction yielded a population of CTCs not captured EpCAM dependently but harboring predictive information.

Conclusion

Our data indicate that EpCAM low-expressing CTCs could be used as a valuable tumor surrogate material – although they may be prognostically less relevant than EpCAM high-expressing CTCs – and have particular benefit if no CTCs are detected using EpCAM dependent technologies.