Functional analysis of live circulating tumor cells from triple negative breast cancer patients using TetherChip technology.

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Background & objectives

Microtentacles (McTN) formation represents an important mechanism of metastasis. TetherChip provides a new tool for the evaluation of McTN in Circulating Tumor Cells (CTCs). Triple-negative (TN) is the most aggressive breast cancer (BC) subtype, however, there is limited knowledge regarding the phenotypic and functional analysis of the CTCs from these patients. The present study aimed to isolate live CTCs of TNBC patients and characterize them regarding McTN formation and expression of important biomarkers such as GLU, VIM, PDL-1, and CTLA-4, before and after Vinorelbine treatment.

Methods

Cytotoxic effects of Vinorelbine were confirmed on TNBC cell lines (MDA-MB-231 and MDA-MB-436) and on a metastatic colon cancer patient-derived CTC-MCC-41 line by the MTT assay. CTCs from 20 TNBC patients were isolated and placed in TetherChip. Biomarker expression was identified by immunofluorescence staining and VyCap analysis. Observation of McTNs has been performed with WGA staining. Vinorelbine-induced apoptosis was evaluated based on the detection of M30-positive cells.

Results

Cell viability of MDA-MB 231 (83%), MDA-MB-436 (87%) and CTC-41 (77%) remained high after 1-h Vinorelbine treatment, whereas 24-h Vinorelbine treatment induced significant loss

of viability of MDA-MB-231 (56%) and CTC-MCC-41 (58%) cells but less significant loss of MDA-MB-436 (70%). Vinorelbine treatment (1 h) of live TNBC patients' CTCs led to the induction of apoptosis ($p = 0.010$). In addition, it caused a significant reduction of GLU and PD-L1 expression ($p < 0.001$), but not VIM and CTLA-4 expression. Interestingly, CTC numbers were significantly increased in TetherChips compared to the number of CTCs in patients' cytospins exactly after blood collection ($p < 0.001$), implying that our method of cell culture for 4-5 days followed by TetherChip analysis provides a useful tool for functional evaluation of CTCs. Formation of McTNs was observed in all cancer cell lines and TNBC patient CTCs, which were disrupted after 1 h of Vinorelbine treatment.

Conclusion

TetherChips is a new promising approach to the functional and real-time investigation of drug efficacy on isolated CTCs. Vinorelbine treatment could have anti-metastatic actions in TNBC patients by disrupting McTNs, inducing apoptosis and reducing the expression of GLU as well as PD-L1.