Mesenchymal markers: the new avenue for circulating tumor cells detection

Mariacristina Ciccioli1, Natalia Bravo-Santano2, Amy Davis1, Jolie Lewis1, Ross Malcolm2, Anne-Sophie Pailhes-Jimenez1
1ANGLE Europe Limited, 10 Nugent Road, Surrey Research Park, Guildford, Surrey GU2 TAF United Kingdom

Introduction

Most CTC isolation systems are based on epitope-dependent CTC capture using epithelial markers. However, it is known that tumor cells can undergo epithelial-to-mesenchymal transition (EMT) when extravasating from the primary tumor to enter the bloodstream and eventually establish distant metastases (Figure 1). Epitope-dependent CTC detection platforms have limited sensitivity for detection of mesenchymal CTCs, leading to the inability to capture potentially clinically relevant CTCs, particularly in cancers with high mesenchymal phenotypes such as non-small cell lung cancer (NSCLC). In this study, we evaluated the performance of a research use only assay developed for the identification of epithelial and mesenchymal CTCs enriched by the Parsortix® System, a label-independent microfluidic device that isolates cells based on their size and compressibility.

Workflow

Analytical performance of the assay was assessed by spiking known numbers of breast cancer cell lines into blood samples from 12 healthy volunteers. The contrived samples were processed as per the workflow in Figure 2. SKBR3 cells were used as a positive control for epithelial markers and as a negative control for mesenchymal markers. HS 578T cells were used as a positive control for mesenchymal markers and as a negative control for epithelial markers. The sensitivity, specificity and consistency of the assay were defined as follow:

- Sensitivity = % of cancer cells stained for the positive expressed marker
- Specificity = 100 – (% cancer cells stained for the negative expressed marker)
- Consistency= Coefficient of variation (CV%) of fluorescent signal intensity across donors

Performance of the assay using clinical samples was assessed on 107 healthy volunteers (HV), 47 metastatic breast cancer (MBC) patients, and 48 non-small cell lung cancer patients (NSCLC) processed as depicted in Figure 2.

Results

• ANGLE’s EMT assay showed a high degree of analytical sensitivity (97-98%) and specificity (96-98%), as well as good consistency (CV=22-25%), which is illustrated in Figure 3A. Results from clinical samples confirmed this finding, showing high specificity (96%). CTC-like cells were observed in only 4% (4/107) of the healthy volunteers, of which 2 declared suspected endometriosis at the time of blood donation, indicating the possible presence of Circulating Endometrial Cells as a Biomarker for Endometriosis. Chin Med J (Engl), 2017. The assay identified ≥1 CTC in 31% of the NSCLC patients and 40% of the MBC patients (Figures 3B and 3C). CTC clusters were observed in 63% of the CTC-positive MBC patients and 33% of the CTC-positive NSCLC patients. Cluster size ranged from 2-22 CTCs (Figures 3B and 3C).

• Phenotypically, more CTCs were detected only mesenchymal markers were harvested from NSCLC patients compared to MBC patients, 38% vs 25% respectively (Figures 3B and 3C). A larger proportion of CTCs expressing both epithelial and mesenchymal markers were detected in both cancer types (59% in NSCLC and 74% in MBC) and a small percentage of the CTCs harvested expressed just the epithelial markers.

• In MBC, the positivity rate for CTC presence was significantly higher in Stage IV compared to Stage III patients, and a significantly higher number of EMT CTCs was captured in patients diagnosed with squamous cell carcinoma compared to those diagnosed with adenocarcinoma.

Conclusions

• ANGLE’s EMT assay successfully allowed for the identification of epithelial AND mesenchymal CTCs in the population of cells harvested by the Parsortix® System from blood samples of MBC and NSCLC patients.

• This study highlights the importance of the inclusion of mesenchymal markers into CTC characterization, as a large proportion of the CTCs harvested by the Parsortix® System would have been missed using an epithelial-only based approach, particularly in NSCLC patients.