ID: 00788
Type: POSTER
Topic: 6. Liquid Biopsy

Evaluating the metastatic potential of CTC-clusters in breast cancer by in vitro and in vivo assays.

Inés Martinez[1] , Pablo Hurtado[1] , Carmen Abuín[1] , Laura Sánchez[2] , Rafael López[1] , Roberto Piñeiro[1]

1) Roche-CHUS Joint Unit 2) Genetics Department, Veterinary Faculty, Universidad de Santiago de Compostela

Breast cancer is the most frequent tumour type in women. It is a very heterogeneous disease, which hampers treatment efficiency. Most of the deaths related to cancer are due to metastasis, a complex and multi-step process by which new tumour foci are established in regions of the body located far from the primary tumour. The main steps of the progression to a metastatic state are: individual or collective cellular migration from the primary tumour, intravasation to the blood stream, cell survival, extravasation, colonization and cell growth in distant tissues. Circulating tumour cells (CTCs) are those cells released into the blood stream from the primary tumour and have the potential to seed new metastatic lesions. Therefore, CTCs analysis from a peripheral blood sample (or liquid biopsy) is an interesting alternative to the conventional histology biopsy to study tumour development. These CTCs can travel either as single cells or in groups called CTC-clusters, which are believed to have a greater metastatic potential and are associated with poor prognosis in breast cancer patients. However, the actual contribution of CTC-clusters to metastasis is still unknown and further research is needed. The objective of our research team is the functional characterisation of the clusters with the aim to identify differential capacities between single CTCs and CTC-clusters by using *in vitro* models of CTC-clusters generated from human breast cancer cell lines. To this purpose, we functionally compare single CTCs to CTC-clusters on *in vitro* and *in vivo* (zebrafish) assays which simulate different steps of the metastatic process in order to understand their role and contribution to metastasis. We use human breast cancer tumour cells lines labelled with enhanced Green Fluorescent Protein (eGFP). These cells were spiked into a peripheral blood sample from a healthy donor and recovered by a ficoll density gradient for downstream studies. This process mimics the circulation of CTCs through the blood stream during metastasis. The recovered cells were cultured overnight in suspension to allow the formation of large groups of cells, which were differentially disaggregated in order to form two cell populations, representing single CTCs and CTC-clusters. We performed migration assays using Transwell inserts to study the number of migrated cells towards an FBS gradient and through the porous membrane. We also made adhesion and invasion assays to and through the endothelium by adding a monolayer of HUVEC cells. Furthermore, soft agar colony formation assays were performed to evaluate the clonogenic potential of both populations. Initial *in vitro* results are showing that our CTC-clusters have differential properties typically associated with malignance, like migration capacity, adhesion to endothelial cells and invasion through endothelium or tumourigenicity, when compared to single CTCs. These differential properties between single CTCs and CTC-clusters could partially be the underlying causes explaining the contribution of CTC-clusters to metastasis. Besides, we are setting up a xenograft model in zebrafish (*Danio rerio*) embryos in which our CTC models (single CTCs and CTC-clusters) are injected into the blood stream of the fish in order to track *in vivo* tumour spread and metastasis. Preliminary result are showing that CTC-clusters can survive in the circulation of the fish and efficiently disseminate to seed metastases. Therefore, our CTC-cluster model is a promising model to study the biology of these groups of cells and their contribution to metastasis.