Functional analyses of CTCs in cancer patients

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Circulating tumor cells (CTCs) in blood are promising new biomarkers potentially useful for prognostic prediction and monitoring of therapies in patients with solid tumors including colon cancer. Moreover, CTC research opens a new avenue for understanding the biology of metastasis in cancer patients. However, an in-depth investigation of CTCs is hampered by the very low number of these cells, especially in the blood of colorectal cancer patients. Thus, the establishment of cell cultures and permanent cell lines from CTCs has become the most challenging task over the past year.

In 2015, we described for the first time the establishment of a permanent cell line from CTCs of one colon cancer patient. The cell line designated 'CTC-MCC-41' has been cultured for more than four years and cells have been characterized at the genome, transcriptome, proteome and secretome levels. This thorough analysis showed that CTC-MCC-41 cells resemble characteristics of the original tumor cells in the colon cancer patient and display a stable phenotype characterized by an intermediate epithelial/mesenchymal phenotype, stem-cell like properties and an osteomimetic signature indicating a bone marrow origin. Functional studies showed that CTC-MCC-41 cells induced rapidly *in vitro* endothelial cell tube formation and *in vivo* tumors after xenografting in immunodeficient mice. More recent results highlighted that CTC-MCC-41 cells display a very specific transcription program. Interestingly, among the 1,624 transcripts exclusively upregulated in CTC-MCC-41 samples compared to other colon cancer cell lines obtained from primary tumors or from metastatic sites, key genes related to energy metabolism, DNA repair and stemness genes were observed. Such data may supply insights for the discovery of new biomarkers to identify the most aggressive CTC sub-populations and for the development of new drugs to inhibit metastasis-initiator CTCs in colon cancer.

In fact, we could establish a unique biological material: 9 CTC-MCC lines from one metastatic colon cancer patient out of 168 during treatment and cancer progression. We obtained new data at the genomic and phenotypic level that suggest that CTCs cultured from sequential liquid blood biopsies during therapy have common traits but selection of treatment-resistant clones with distinct phenotypic characteristics was observed. Further studies with these CTC-MCC lines are in progress, evaluating their tumorigenicity in mouse models with resistance to specific drugs or analyzing the epigenetic contribution.

Our main goal will be to identify new pathways specific for metastasis-competent, therapy-resistant CTCs to be able to eradicate them with novel drugs.

Finally, we improved a new functional EPIDROP assay to detect viable CTCs at the single cell level. The principle and all advantages of this innovative technology will be presented at the ISMRC 2018.