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Detection and characterization of viable circulating tumor cells in solid tumors using the EPISPOT assay

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The enumeration and characterization of circulating tumor cells (CTC) in the peripheral blood and disseminated tumor cells (DTC) in the bone marrow may provide important prognostic information and in help to monitor efficacy of therapy. Since current assays cannot distinguish between apoptotic and viable CTC/DTC, we applied a novel functional test called 'EPISPOT' (EPithelial ImmunoSPOT) which detects proteins secreted/released/shed from viable single epithelial cancer cells after the depletion of $CD45^+$ cells, avoiding thus direct contact with the target cells and mostly a selection based on the expression of EpCAM. Cells are cultured for 24 hours on a membrane coated with antibodies that capture the secreted/released/shed proteins which are subsequently detected by secondary antibodies labeled with fluorochromes.

In breast cancer, we measured the release of CK19 and mucin-1 (MUC1) and demonstrated that many patients harbored viable DTC in bone marrow, even in patients with apparently localized tumors (stage M0: 54%). Preliminary clinical data suggest that patients with DTC releasing CK19 might have an unfavorable outcome. In addition, we studied the HER-2 status of CTC in the blood of 121 metastatic breast cancer patients. We showed that CTC can release HER-2, however, HER2-releasing CTC remains a rare event (4.4%).

In prostate cancer, we used prostate-specific antigen (PSA) secretion as marker and found that a significant fraction of CTC secreted fibroblast growth factor-2 (FGF-2), a known stem cell growth factor.

In colorectal cancer, we studied the gradient of CTC concentration between mesenteric (MB) and peripheral (PB) blood of colorectal cancer patients using the EPISPOT assay and the semi-automated FDA approved CellSearch system. Both assays revealed higher incidences of CTC in MB than PB, and in particular EpCAM+ CTC (detected by the CellSearch® System) appear to be trapped in the liver.

In conclusion, the EPISPOT assay offers a new opportunity to detect and characterize viable CTC/DTC in cancer patients and it can be extended to a multi-parameter analysis revealing a CTC/DTC protein fingerprint.

Biography

Catherine Alix-Panabières received her PhD degree in 1998 at the Institute of Virology, University Louis Pasteur, in Strasbourg (FranceDuring this last decade, Dr Alix-Panabieres has focused on optimizing new techniques of enrichment and detection of viable disseminating tumour cells in patients with solid tumors. She is the expert for the EPISPOT technology which is used to detect viable tumor cells in the peripheral blood and the bone marrow of patients with breast, prostate and colon cancer. She is an associate-professor in the Laboratory of Cellular and Hormonal Biology at the University Medical Center of Montpellier and recently, she built a new laboratory (Laboratory of Rare Human Circulating cells - Institute of Research in Biotherapy – University Medical Centre Montpellier, France).

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