TUTOR | Ivana Kurelac

TITOLO DEL PROGETTO
Optimizing circulating tumor cell detection protocols for liquid biopsy in cancer

DESCRIZIONE DEL PROGETTO
During the past ten years, circulating tumor cells (CTCs) have received enormous attention as new biomarkers for early diagnosis of tumors via liquid biopsy. Although CTCs are already used in numerous clinical trials, their clinical utility is still under investigation (Alix-Panabières C, Nature Reviews Cancer 14, 623–631, 2014). Despite the large number of approaches proposed to detect and classify CTCs, e.g. by examining their physical and/or biological properties, the accurate identification of CTCs still remains an unaccomplished goal. The biggest problem to be tackled is the very small amount of CTCs with respect to the other components of the bloodstream (red blood cells, white blood cells and platelets), so that the ideal diagnostics system has to be capable of analyzing the blood flow with significantly high-throughput and remarkable identification accuracy to minimize the number of false positives. CTC detection methods most often involve screening of several molecular markers. However, cancer cells are highly heterogenous, differing among each other in cell surface marker expression, even in a tumor from the same patient. Thus, marker-based CTC detection may still result in false negative diagnosis. Moreover, the use of immune-based markers increases the complexity and costs of CTC detection methods. Thus, there is a need for discrimination of CTCs based on a marker-independent system. A new approach has been proposed recently for CTC discrimination, based on their physical properties, such as size, volume, density, morphology and inner organelle distribution (Villone MM, Lab on Chip, 18(1), 126-131, 2018). The technology is being developed by a group of chemists and physicists from CNR in Naples, and includes an innovative opto-microfluidic platform for full-3D single cell analysis in tomography through quantitative phase imaging to map the 3D refractive index of the flowing cells. Once optimized, this tomographic approach would result in a cheaper and more informative technique, which could potentially be translated to all solid cancers. The current phase of the tomographic system development requires creating dictionaries of CTCs versus other blood cell types, through machine learning process that involves flow-through cell recordings. Moreover, in order to increase the throughput of the analysis, the system currently requires CTC enrichment.

Objectives
The aims of this project are:
1) Generation of cell dictionaries that will allow CTC discrimination from the other blood cell populations
2) Identification of the optimal CTC enrichment approach for faster tomography-based CTC identification.

Methodology (descrizione del campione, principali tecniche utilizzate, aspetti biostatistici, fattibilità…)
A set of cancer and normal cells will be analyzed, including thyroid cancer TPC1, normal thyroid Nthy, ovarian cancer SKOV3, normal ovarian epithelium HoSE, renal cancer UOK and normal kidney HK2 cells. For dictionary generation, the cell suspensions will be prepared (approx 30-40,000 cells/100ul) for flow-through recordings by the tomograph. Moreover, blood samples from healthy donors will be collected and cell fractions prepared to separate red blood cells from leukocytes. Their suspension (approx 30-40,000 cells/100ul) will also be prepared for tomograph recordings. In parallel, differences in organelle morphology will be compared between cancer, normal epithelium and blood cell populations (red blood cells and leukocytes). Features like size, number, structure, granularity and organelle/cell ratio will be evaluated by confocal microscopy. Differences will be evaluated by Fisher’s exact and Chi-square tests.

For CTC enrichment experiments, test samples will be prepared by “spiking” 10ml of peripheral blood (PB) from healthy donors with known numbers (1, 10, 100 and 1000) of cancer cells labelled with green fluorescent protein (GFP). Such “artificial” CTC/PB standards will be processed by Ficoll density centrifugation separation, Size-based Metacell filtering, Milteny magnetic CD45 negative CTC selection or by Parsortix. The number and viability of recovered GFP+ cells will in parallel be defined by fluorescent microscope and/or flow cytometry.
contingency tables will be set up and Fisher’s exact test used to estimate sensitivity (true positive rate), specificity (true negative rate), positive/negative predictive values and likelihood ratios for each tested method.

**Risultati attesi**

By month 12 it is expected that the preparation of the dictionaries discriminating cancer from normal cells is completed and that the optimal approach for CTC enrichment is identified.

**DESCRIZIONE DELLE ATTIVITÀ DELL’ASSEGNISTA**

The candidate is expected to have expertise in molecular and cell biology, with good knowledge on microscopy, flow cytometry and cell culture techniques. Introduction to the use of Parsortix and other CTC enrichment methods will be provided during the first bimester of the project, as well as handling of human samples.

Primary objective 1: Generation of CTC versus blood cell dictionaries. Tasks will include (i) organelle characterization in normal versus cancer cells by confocal microscopy (months 1-4), (ii) analysis of the correlation between morphologic features and cancer/normal cell phenotype (months 5-6), (iii) preparation of the samples for dictionary generation (months 1-12).

Primary objective 2: Optimization of CTC enrichment protocols. Tasks will include (i) GFP labelling of the cells (months 1-3) (ii) preparation of the artificial CTC standards to be used as golden standard (months 4-10), (iii) setting up conditions for enrichment techniques (months 1-10), (iv) evaluation of specificity and sensitivity of tested methods (months 11-12).

The candidate will be required to manage the collaboration with chemists and physicists from CNR in Naples, manage the submission of the ethical committee (month 1), participate in data analysis, present the results on lab meetings and congresses, and contribute to scientific paper writing.

_Scheda attività assistenziale (se prevista)_

**ATTIVITÀ ASSISTENZIALI DELL’ASSEGNISTA/ N. ORE SETTIMANA**

Non è prevista attività assistenziale.

**AZIENDA SANITARIA PRESSO CUI SI SVOLGERA L’ATTIVITA**