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UNIVERSITÀ DEGLI STUDI DI TORINO

Doctoral Program in Bioengineering and Medical-Surgical Sciences (37th Cycle)

RabAnalyser: a computational framework to explore the cell-to-cell variability of Rab GTPases organization

Simone Tealdi

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Supervisor(s):

Prof. Gianluca Ciardelli, Supervisor

Dr. Carlo Cosimo Campa, Co-Supervisor

Prof. Clara Mattu, Co-Supervisor

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Summary

Cancer cells rely on cell-to-cell variability for their evolution. This variability arises from both genetic and non-genetic variations, which prime cancer cells for survival in fluctuating environments. Genetic cell-to-cell variability emerges from differences in the genomic sequence among cells within the same population. In this context, mutations and recombination are recognized as sources of cancer cell variability, thereby facilitating the evasion of therapeutic pressure and enabling adaptation to changing conditions. In contrast, transcriptional and proteomic changes in the abundance of transcripts and proteins are sources of non-genetic cell-to-cell variability. Recently, additional factors have been implicated in the regulation of cancer cell variability, including the spatial geometry and architecture of cells within tissues, as well as the accumulation of distinct cellular metabolites. Collectively, these findings highlight that the mechanisms underlying cancer cell variability span the entire spectrum of molecular processes and cellular behaviours.

Rab GTPases constitute the largest subfamily of the Ras superfamily of monomeric G proteins and are associated with specific membrane-bound compartments within the endomembrane system. These proteins cycle between a cytosolic, guanosine diphosphate (GDP)-bound inactive state and a membrane-associated, guanosine triphosphate (GTP)-bound active state. In their active form, Rab GTPases assemble into protein clusters on membranes, where they act as organizational hubs that coordinate organelle transport, membrane tethering, and fusion. These clusters vary in their spatial distribution, size, shape, and abundance within the cell, collectively contributing to the subcellular organization of Rab GTPases, which is increasingly recognized as critical for membrane trafficking. Dysregulation of Rab-mediated pathways, including alterations in subcellular organization, has been implicated in multiple aspects of cancer progression and tumorigenicity, such as the sustainment of proliferative signalling, evasion of growth suppression, enhanced receptor recycling, activation of invasion and metastasis, reprogramming of tumour metabolism, and immune evasion. However, much of our knowledge about the subcellular organization of Rab GTPases is based on population-averaged measurements. How and whether this organization varies across individual cells within a population is unclear.

In this thesis, we developed RabAnalyser, a computational framework designed to analyse fluorescence microscopy images by quantifying and comparing similarity between individual cells based on the intensity, shape, size, and spatial localization of spots corresponding to fluorescently labelled Rab GTPases. RabAnalyser takes as input microscopy images of fluorescently labelled cells to extract quantitative features describing subcellular organization Of Rab GTPases. Systematic literature review and experimental validation identified a minimal, yet essential, set of 11 features, sufficient to classify Rab organization effectively. RabAnalyser couples the conventional assessment of both size and intensity of Rab-positive spots to the analysis of their geometrical and positioning features, thus providing for the first time a more profound characterization of Rab GTPase subcellular organization. Kolmogorov-Smirnov statistic is used to compare feature distributions at single cell level, resulting in the identification of distinct cell subpopulations characterized by different Rab GTPase organization. Then, to measure the features characterizing each subpopulation, the computational framework provides as outputs: UMAP-based clustering plots, statistical analysis graphs and machine learning-based classification plots.

We first applied RabAnalyser to quantify and compare similarity of glioblastoma cells based on subcellular organization of Rab11 GTPase. The framework revealed that these cells were grouped

in four distinct subpopulations, thereby indicating cell-to-cell variability in subcellular organization of Rab11 GTPase. Then, we showed that cell-to-cell variability was also observed in response to Nocodazole treatment, an anti-mitotic agent that interferes with polymerization of microtubules. RabAnalyser identified distinct subpopulations based on subcellular organization of Rab11 GTPases and revealed that the drug redistributed cells across these groups. Specifically, Nocodazole induced an enrichment of cells within subpopulations where spatial features of Rab11-positive spots contributed more strongly to the differences between cells. Therefore, the anti-mitotic agent enhanced population heterogeneity by generating distinct groups of cells characterized by either a denser or sparser spatial distribution of Rab11-positive spots.

Our results demonstrate that RabAnalyser enables the study of the cell-to-cell variability from fluorescence images characterized by spotty signals. Moreover, we provide, for the first time, a computational framework specifically designed to analyse membrane trafficking at single-cell level, an essential yet previously underexplored biological process in existing analytical tools.