WORKING TITLE

Developing an R package for the high-throughput analysis of cell-cell interaction between T cells and tumour cells

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1. Summary

It is widely known that cancer is still one of the leading causes of death in Western society [citation]. Its high mortality is predominantly caused by tumor resistance agains available therapies [citation]. To combat this resistance, new therapies have been developed from which the most recent and promising therapeutic approach are immunotherapies which have shown great success in the past [citation]. Immunotherapies make use of engineered or innate immune cells, especially T cells, to eliminate malignant tumor cells. Still, many cancer types remain resistant. To overcome this, there follows a high need to understand the crucial factors behind therapy resistance and efficient T cell killing.

To elucidate the mechanisms of efficient T cell killing, [Introduction on OVA tumor models, live cell imaging, staining, fix-while-filming]

To achieve meaningful results, large data sets and their statistical analysis are pivotal. To enable a large-scale analysis of tumor and T cell interaction, this thesis aims at developing an R package to automatically evaluate cell dynamics in live cell imaging movies. This would enable a quantitative analysis of cell-cell contacts on big sample sets. Besides, the R packages proposes to correlate dynamical cell data to immunological staining results. To put it in a nutshell, this thesis' goal is to heighten the explanatory power of available live cell imaging and immunological staining techniques by enabling large-scale analyses and correlation of dynamical to immunological staining methods.

To achieve an efficient analysis of live cell imaging data, [... More details on the R package and workflow].

We show that [...]

2. Introduction

- Why T cells are crucial for tumour elimination
- Crucial factors for T cell killing and the relevance of cell dynamics
- Tools to investigate cell dynamics
- Software to analyze cell tracks

2.1 T cells are crucial for tumour elimination

- Hallmarks of Cancer
- Escaping immune system
- T cell killing
- Tumor cell elimination by the body always by T cells (I think)

2.2 CAR T cells as arising tumour therapy

- Introduction to CAR T cells
- Potential of CAR T cells and shortcomings so far
- Approaches to improve CAR T cells
- Tools to evaluate CAR T cell efficiency

2.3 The relevance of cell dynamics

- Advantages of static methods (FACS, sequencing, immuno-stainings)
- Shortcomings of static methods
- Benefit of cell dynamics
- Results so far about cell dynamics
- Aim of this thesis

Cell-cell interaction is essential for tumour killing

- What is known about cell-cell interaction
- Why it is important to investigate cell-cell interaction
- What we would like to know about cell-cell interaction

This is the question central to this thesis. How do the dynamics of T cell behaviour correlate to tumour killing efficiency? More specifically, this thesis builds a tool for a large-scale analysis of cell-cell interaction between T cells and tumour cells. Furthermore, we combine our results on dynamical data to findings on tumour killing efficiency from immunological methods.

2.4 Tools to investigate cell dynamics

Requires recognizing the cell and then characterizing its movement. Live Cell Imaging

2.4.1 Cell segmentation

- Stardist
- CellPose

2.4.2 Cell tracking

- LAP tracker
- Kalman tracker

2.4.3 Cell-cell contacts

- Available tools
- Our needs

3. Methods

- Overview
- Imaging details
- Segmentation
- Tracking
- Export to RStudio
- Cell-cell contact computation
- Extracting more features
- Quality control (CelltrackR)
- Data analysis

3.1 Work flow

3.2 Extracting features

For a given set of cell tracks, we would like to observe meaningful features of the cell movement and activity. To acquire the cell motility data, tumour cells (B16F10-H2BmCherry-OVA) and T cells (OT1 GFP) were seeded into a 3D collagen gel and imaged over time for several hours at a rate of 90 seconds per image. Afterwards, the images were segmented and cell tracks were obtained using an image analysis tool, e.g., the TrackMate Plugin for ImageJ or Imaris 3D.

The cell tracks have the following structure: Each .csv-file is a table with the columns "ID", "time point", "x-" , "y-" and "z"-coordinate. We have separate .csv-files for the tracks from tumour cells and the tracks from T cells.

3.3 Developing the R package *cellcontacts*

3.3.1 Computing cell-cell contacts

3.3.2 Identifying dying tumour cells

3.4 Tools for quality control

4. Results

4.1 Segmentation and tracking of cells

4.2 Computing cell-cell contacts

4.3 Benchmarking computation time

4.4 Investigation of killing efficiency

- Identifying dead cells

4.5 Investigating the induction of cell senescence

- Visualizing cell size with regard to cell-cell contacts

5. Discussion

5.1 Biological interpretation

5.2 Working title: Comparison to available tools