A network perspective on macrophage phenotypes in the tumour microenvironment

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ABSTRACT

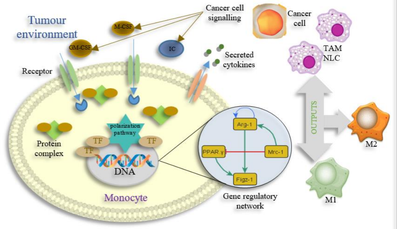
Background: The Tumour Microenvironment (TME) is the collection of cells in and surrounding cancer cells in a tumour including a variety of immune cells, especially neutrophils and monocyte-derived macrophages. In a tumour setting, macrophages cover a spectrum between a tumour suppressive (M1) or tumour promoting (M2) state. The biology of macrophages found in tumours (Tumour Associated Macrophages, TAMs) remains unclear, but understanding their impact on tumour progression is highly important.

Materials and Methods: We perform a comprehensive analysis of a macrophage polarization network, through inserting two lines of enquiry: **(i)** inference of macrophage polarization network based on literature- and data-driven methods, and **(ii)** dynamical modelling of macrophage polarization in the presence of different extra-cellular inflammatory and pro-tumoural stimuli.

Results: Model simulations recapitulate documented macrophage phenotypes while enabling the identification of the specific profile of TAMs in TME. Specifically, we identify the regulatory interactions and pathways that lead to a TAM polarization state and the role of knock-outs in the polarization states. This perturbation analysis can lead to the identification of new therapeutic targets and gives specific predictions regarding the cross-talk between cells in TME that determine the TME dynamics. This model expands our understanding of the mechanisms that determine macrophage polarization and it constitutes a valuable reasoning tool to delineate new experiments.

INTRODUCTION

- In a TME, cell – cell communication determines the TME dynamics;
- Pro-tumoural signals can shift the innate immune system towards a tumoural enhancing state, leading to tumour progression and proliferation;
- Macrophage polarization states are defined by a high degree of plasticity and identifying the pathways that lead to a Tumour Associated Macrophages (TAMs) state remains a challenge.



AIMS

- *Understanding TME dynamics and the mechanisms that drive the immune system infiltration through the implementation of dynamical models on gene regulatory networks;*
- *Running simulations to identify the action of specific pathways*

MATERIALS AND METHODS

1. Regulatory network inference of macrophage polarization

- Reference network: Regulatory network of macrophage polarization into two main polarization phenotypes (M1, M2) (A. Palma et al, 2018);
- Network extension: TF activities were calculated by using Dorothea and Viper Bioconductor R packages. The TFs with the highest variability between M1, M2 and TAM phenotypes were selected (Figure 1 a, b).

2. Dynamical model implementation

- Based on the regulatory network in Figure 1, the Boolean functions for each component were manually curated from literature;
- Numerical simulations were performed using BoolNet R package: we applied a synchronous updating method to detect the attractors, considering all the possible combinations of initial conditions;
- The categorization of the attractors into polarization states, that would correspond to macrophage phenotypes, was done by performing two separate methods: (1) supervised categorization, i.e. literature based, and (2) unsupervised clustering, by applying hierarchical clustering algorithms on the attractor similarity matrix (Jaccard similarity index).

RESULTS

REGULATORY NETWORK OF MACROPHAGE POLARIZATION

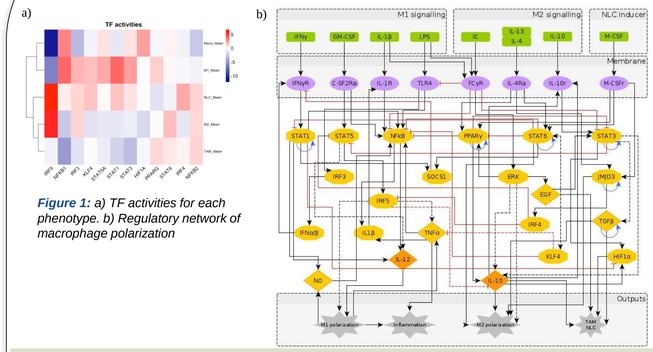


Figure 1: a) TF activities for each phenotype. b) Regulatory network of macrophage polarization

BOOLEAN MODEL

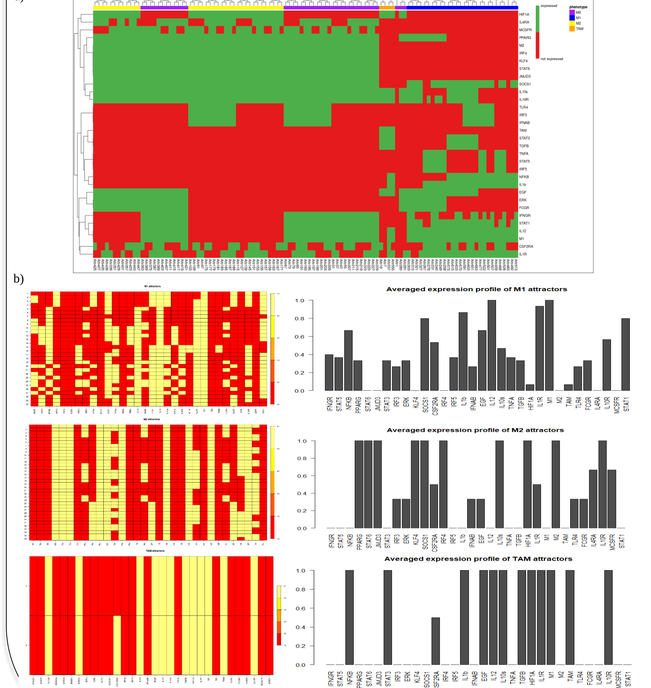


Figure 2: a) Heatmap of all attractors obtained from the Boolean model. b) (left) Clusters corresponding to each phenotype (M1, M2 and TAM). (right) Averaged expressions of each phenotype attractors.

RESULTS

PRELIMINARY RESULTS: CLUSTERING THE ATTRACTOR SPACE

Jaccard dissimilarity index $= \frac{S_{10}+S_{01}}{S_{10}+S_{01}+S_{11}}$

Hypothesis: Attractors that correspond to the same polarization state, will have a small dissimilarity index

Procedure: Calculate the Jaccard index between attractors and apply HCA on the indexes matrix.

Results: 4 main clusters are identified. Cluster 1 correspond to all components in OFF state and it is no longer considered in analysis.

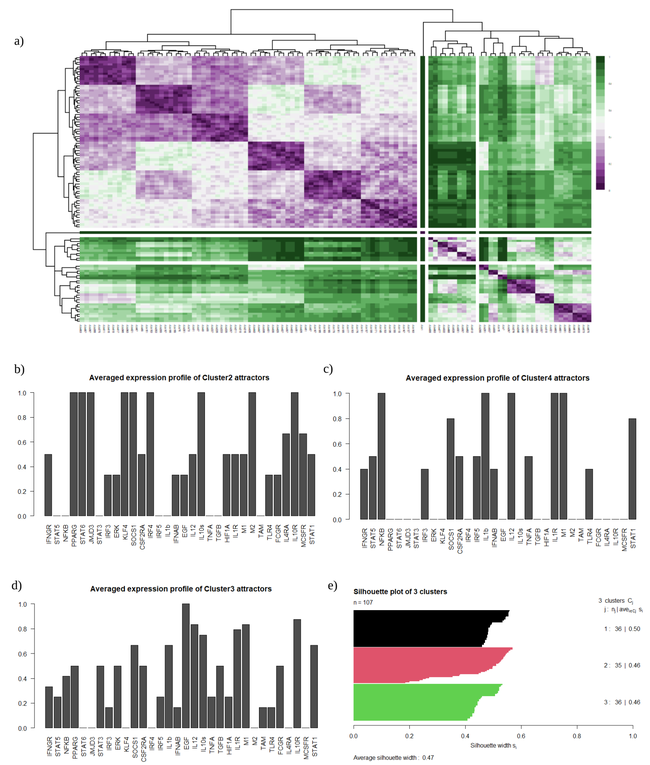


Figure 2: a) Heatmap of Jaccard indeces between all attractors. b), c), d) Averaged values of each cluster . e) Silhouette plot of clustering score

Observing the polarization states, conditions:
M1: IL12 (ON) and at least one of among STAT1, STAT5, NF-κB (ON)
M2: IL10, STAT3, PPARγ, JMJD3 (ON)
NLC: TGFβ, HIF1α (ON)

M1 and M2 drivers

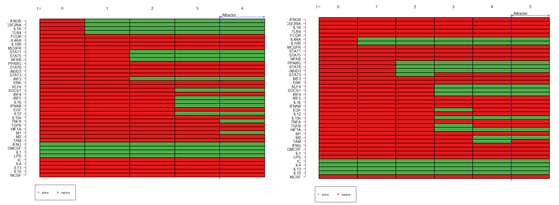


Figure 3: (left) Switching OFF the M2 extracellular signals lead to a single polarization state corresponding to M1 phenotype, (right) Switching OFF the M1 extracellular signals lead to a single polarization state corresponding to M2 phenotype

CONCLUSIONS

- The Boolean model captures known polarization states of macrophages in different extracellular environments, while it identifies TAMs as a separate polarization state, driven by pro-tumoural stimuli in the TME.
- Capturing unknown polarization states requires the development of more data-driven techniques of both network inference and analyzing the simulations' results.
- In silico results show that the Boolean model explains the polarization pathways that can are observed in M1 or M2 stimuli, thus opening the perspective for more knock-out simulations, in order to identify the key components that lead to TAM formation.

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