Mass Cytometry: A Novel Flow Cytometry Modality to Study Tumor Immunology

Bernd Bodenmiller
Institute of Molecular Life Sciences
University of Zurich

www.bodenmillerlab.org
Metastatic cascade:
Tumor cell intrinsic and...
...tumor cell extrinsic processes

Tumor microenvironment

Cells of the tumor microenvironment?

Microenvironment of the metastatic process?
Overview

1. Mass cytometry
Quantitative single cell analysis: Flow cytometry coupled to mass spectrometry

Cell fixation & permeabilization → Cell staining with antibodies

→ Cell type, signaling and functional state in a targeted manner

Mass cytometer provides 135 measurement channels
Replace fluorophores …

…with metals and atomic mass spectrometry

~100 isotopes available for mass cytometry
Quantitative single cell analysis

Mass cytometry: current status

Viability, size, DNA content, cell cycle

51 parameters simultaneously

1,000 cells per second

100 copies/cell detection limit

100s samples/day

>400 validated antibodies

Cells are atomized

No live cell information

Antibody availability
Overview

1. Mass cytometry
2. Imaging mass cytometry
Mass cytometry:
Relies on cell suspensions
Workflow imaging mass cytometry

### State of the art

<table>
<thead>
<tr>
<th></th>
<th>Markers</th>
<th>Resolution</th>
<th>Speed (0.5mm x 0.5mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Published</td>
<td>32 (100)</td>
<td>1000 nm</td>
<td>20 Hz / ~3 hrs</td>
</tr>
</tbody>
</table>

Validated antibodies

High resolution images similar to IF microscopy

Current

Version 2.0

H3, Vimentin, HER2
Quantitative single cell analysis and segmentation for tissue images exploiting multiplexed data


→ File with phenotypes, morphology and neighborhoods
Quantitative single cell analysis of microenvironment:
Breast cancer tissue microarray

Per patient:
- Tumor core
- Invasive front
- Metastatic site(s)
- Normal tissue

→ All patients with clinical information
Inter- and intra-patient tumor heterogeneity: luminal A HER2+ patients

ER

HER2

CK8/18

E-Cad.

Low  Expression level  High
Microenvironment of macrophages?

→ TAMs #1 have no distinct TME
→ TAMs #2 induce signaling in their TME
TAM #3 show EMT phenotype in their microenvironment

Vimentin^+\text{/}EpCAM^+ double positive cells indicate EMT

These markers indicate cells undergoing EMT
Features stratifying patient groups

Samples grade 1 tumors

Samples grade 3 tumors

Data clustering

Feature extraction

PAMR/GLMNET

Regularized learning

Differential populations

Features of the most stratifying cell cluster

Of the TME

Cell phenotype

→ Use cell types, spatial features and cell-cell interactions as biomarkers
3. Summary
Quantitative single cell analysis

Summary

Screening

Any other biology

Biomedical profiling

Systems biology

Tissue imaging

Quantitative single cell analysis

Acknowledgements: My team
Acknowledgements: Collaborators

D. Guenther
H. Wang
B. Hattendorf
D. Grolimund
Buhmann lab

Z. Varga (USZ)

Swiss Cancer League