Applied Digital Pathology in Colon and Lung Cancer Research

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Content

• Whole slide analysis to inform molecular pathology

• Multiparametric tissue analysis
  ✓ Next-generation TMA (ngTMA)
  ✓ Multimodality immunoprofiling
  ✓ Ex vivo drug testing
Understanding disease -

_Different levels of information_

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Andreas Vesalius
_De humani corporis fabrica_
_(On the fabric of the human body)_
Basel, 1543

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Rudolf Virchow
_Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebslehre_
_(Cellular pathology in relation to physiology and pathology)_
Berlin, 1859
Understanding disease - Different levels of information

Cellular pathology – Classification, staging, grading

Molecular pathology – Precision medicine
Complex phenotypes captured on histological slides are used for staging and grading of malignant tumours...

...but have not been sufficiently connected to the multi-dimensional biomarkers that are driving personalized therapy.

«We only see what we know»
Johann Wolfgang von Goethe [1749-1832]
Consequence:
Deficits in cost efficient selection methods for molecular testing, loss of prognostic and predictive information

Solution:
Multi-scale, multi-disciplinary, and integrative approaches connecting pathology, computational image analysis and clinical prediction models
Understanding disease -
Different levels of information

Molecular testing
High costs of some tests
Limited availability of the biological material
Test to result: days-weeks

Histology / image analysis
Inexpensive
Available from standard workup
Test to result: hours-days
Tumour annotation for molecular analysis

**Workflow**

1. **Surgical specimen / biopsy**
2. **Histopathology**
3. **Tumour annotation**
   - determine cellularity
4. **DNA/RNA extraction**
5. **Analysis, Interpretation**
6. **Integrated report**

**Manual annotation**

**Estimation of % tumour**
Tumour annotation for molecular analysis

Problems

High interobserver variability: Two pathologists annotating a colorectal cancer section
Problems

Two pathologists estimate % tumour cell content
(n=100 colorectal cancer cases)

Observer 1 vs Observer 2

$r = 0.3$
Tumour annotation for molecular analysis

 Workflow

- Surgical specimen / biopsy
- Histopathology
- Tumour annotation
determine cellularity
- DNA/RNA extraction
- Analysis, Interpretation
- Integrated report

Manual annotation
Estimation of % tumour

- Tissue segmentation by machine learning
  - Nuclear segmentation
  - Exact quantification of neoplastic AND non-neoplastic elements
  - Extraction of cell-level features
  - Extraction of subcellular features
Tumour annotation for molecular analysis

Clinical relevance

1. Improved NGS data analysis
2. Precise correlation of morphological and molecular data
3. Molecular pathology research
Tumour annotation for molecular analysis

Pilot study

(Slides 13-26: unpublished data)
Digital pathology to complement NGS

Multi-region sequencing

(Slides 27-28: unpublished data)
Content

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  ✓ Next-generation TMA (ngTMA)
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  ✓ Ex vivo drug testing
Collaboration

Translational Research Unit, Institute of Pathology, University of Bern, Switzerland

Head: Prof. Dr. Inti Zlobec, PhD
Caroline Hammer
Dr. José A. Galván, PhD
Dr. Irene Centeno, PhD
Dr. Magdalena Skowronska, PhD
Carmen Cardozo
Patricia Ney
Stefan Reinhard
Sandrine Ruppen
Liliane Schöni

www.ngtma.com
www.compath.ch
tru@pathology.unibe.ch
Aim: To address shortfalls of conventional TMA design using digital pathology.

A) To improve **technical accuracy** (capture of the correct histologic areas from the donor blocks).

B) To improve **analytical accuracy** (concordance with the tissue of origin).

C) To facilitate the use of TMA technology for **hypothesis driven analysis of tumour resections, and expand to cell lines, explant cultures, biopsies and animal models of human disease**.
Methods

Digital planning and design

- TMA
- Molecular analysis
- Extra punches

www.ngTMA.com
Methods

Digital slide and tissue block alignment

Strong technical and analytical accuracy

TMA Grandmaster (3DHistech)

Digital TMA analysis

Virtual grids, high throughput, direct link to clinical data
Digital TMA analysis
Better data quality and improved reproducibility

Manual
1-2 markers at a time
Standardisation is difficult
Reproducibility is variable

Digital
Many markers simultaneously
Standardisation is easy → CE-IVD/FDA certification
Excellent reproducibility → better data and study quality

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15/12/2017, VH Koelzer
• Whole slide analysis to inform molecular pathology

• Multiparametric tissue analysis

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Multiparametric tissue analysis

Decoding the immune contexture

Adapted from Galon, et al. *Immunity*, 2013

- Immunoscore
- Type
- Density
- Location

- Immune-contexture
- Cellular composition
- Signature of rejection
- Effector molecules
- Cytokines
- Adhesion and migration
- Molecular markers
- Immuno-genomics
- Epigenetics
Multiparametric tissue analysis

Decoding the immune contexture

Study design

T-effector cell infiltration

Cytotoxic effector molecules

Interferon signaling gene expression

Cell adhesion

Epigenetic regulation (miRNA profiling)

Colorectal cancer patients, n=335

Clinical data: Patient gender, age, performance status, tumor size, location, pre- and post-operative therapy information, 5-year overall and disease-free survival time

Histopathological data: pT, pN, pM, L, V, Pn, G, R, histological subtype, tumor border configuration, tumor budding

Molecular data: Tumor microsatellite-instability status

Exclusion criteria: Pre-operative treatment n=51 or insufficient material n=43

n=241 Next generation tissue microarray construction

Immunohistochemistry for STAT1, IRF-1, IRF-5, perforin, granzyme B, ICAM-1, CD8

In situ hybridization for miR-34a, miR-93, miR-146a

Digital analysis of protein and miRNA expression. Correlation of STAT-1, IRF-1 and IRF-5 expression with miRNAs, markers of immune activation and clinicopathological features

Koelzer VH, Sokol L, et al. *OncoImmunology, 2017*
Multiparametric tissue analysis

Digital immunoprofiling using IHC and miRNA ISH

Definition of an “activated” or “quiescent” immune contexture based on digital scoring of interferon signalling gene (ISG) expression by HALO™.

Correlation with digital analysis for

- T-effector cell infiltration
- Cytotoxic effector molecules
- Cell adhesion markers

Koelzer VH, Sokol L, et al. *Oncoimmunology, 2017*
Multiparametric tissue analysis

*Digital immunoprofiling using IHC and miRNA ISH*

Patients with a high ISG score:

- **Strong CD8 and CD3 infiltration into tumours**
- **Increased expression of granzyme B and perforin**
- **Increased expression of T-cell adhesion molecules (ICAM-1)**
- **10-fold lower chance of distant metastasis.**

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<th>p value</th>
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Koelzer VH, Sokol L, et al. *OncoImmunology*, 2017

15/12/2017, VH Koelzer
Multiparametric tissue analysis

Digital immunoprofiling using IHC and miRNA ISH

Epigenetic control:
miR-34a and miR-93 in the CRC microenvironment are negatively correlated with the interferon signaling genes STAT1 (HR=0.39, p=0.006) and IRF-1 (HR=0.48, p=0.051).

Koelzer VH, Sokol L, et al. *OncoImmunology*, 2017

15/12/2017, VH Koelzer
Conclusions (II)

A) Immune activated phenotype

B) Immune quiescent phenotype

Koelzer VH, Sokol L, et al. *OncoImmunology, 2017*
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Project examples

*Ex vivo drug testing*

(Slides 30-37: unpublished data)
Conclusions (III)

• ngTMA coupled to digital pathology is a powerful platform for high throughput tissue analysis and pre-clinical drug testing.

• Multimodality profiling of ex-vivo samples can stratify drug responsive vs. non-responsive tumours.

• Broad applicability of digital pathology to clinical and pre-clinical research.
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Thank you

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