The Future of Pathology: Systems Pathology

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The Practice at Present

• Practice is fragmented

• Diagnostician vs. Therapist

• Pathologist as diagnostician must integrate, a cumbersome activity at present
Factors

- Human brains adept at pattern-recognition but not at extracting and processing quantitative information
- 400 year history of descriptive and correlative data
- New technology slow to emerge
- Sanction of experiential practice
- Eminence-based rather than evidence-based
Top Down (Network Topology)

Bottom Up (Molecular Structure)

Combined Causal Action

Complex Properties of the System

Variational Modification of Molecular Ensemble

Dynamic Modification

Properties Shift Through Time

Shift Through Time
Forces

• Rapid progress in technologies
• Progress in computational sciences
• Complex sciences come of age in biology
• Singularity of the individual
• Globalization
• Health care economics
The Thesis

Systems Pathology represents a practical adaptive response to the forces of change.

Systems Pathology seeks to understand perturbed physiological systems and complex pathologies in their entirety by integrating all levels of functional and morphological information into a coherent model. It enables the design and testing of effective intervention and preventive measures. It is practiced by a combination of bottom-up data collection, often comprehensive (omics) and top down computational modeling and simulation.
Integrated Tumor Biology in the Clinic

Person epidem history genetic

Lesion histopath in situ probes

Molecular in situ probes htp techs

Normalize data sets

Machine learning tools

Superclassifier elements to predict Nat Hx - Rx -

Personalized Predictive Medicine

multiparameter Bayesian Vector Machines
MOLECULAR EVENTS ASSOCIATED WITH PROSTATE TUMOR PROGRESSION

Normal epithelium → Prostatic intraepithelial neoplasia (PIN) → Invasive carcinoma → Metastasis

- PSMA Overexpression
- Loss of basal cells
- Loss of p27
- Loss of 10q PTEN
- Loss of 13q RB
- Loss of 17p p53
- NFkB Overexpression
- Hdm2 Overexpression
- AR Overexpression

(Adapted from a scheme courtesy of Dr. C. Abate-Shen)
PTEN AND P-AKT STATUS IN PROSTATE CANCER

Normal | PIN | PrCa-1 | PrCa-2

Pten

P-Akt (Ser473)

Growth
Apoptosis

(Cordon-Cardo et al, Submitted 2006)
SEGMENT, CLASSIFY, FEATURE EXTRACTION

Original image

Segmented image

Feature Statistics

Classified image
NUCLEI

- Nuclei Candidate Objects are classified as Nuclei using
  - fuzzy rules with the membership function defined for normalized red channel and blue ratio values:

\[
\tilde{\xi}_B = \frac{\tilde{Y}_B}{\tilde{Y}_R + \tilde{Y}_G + \tilde{Y}_B}
\]

is blue channel ratio value

\[
\frac{\tilde{Y}_R - \tilde{Y}_B}{\tilde{Y}_B + \tilde{Y}_G + \tilde{Y}_B} \leq -0.015
\]
LUM-CELL
CK18

BAS-CELL
CK14
p63

GENERAL
PSA
PSMA
AMACR
AR

SIGNALING
EGFR / Her-2
IGFBP
SHH
HOX

PROLIFERATION
Ki67
PTEN
pAKT
pmTOR
Cyc D
p27
pMAPK
STAT3

SURVIVAL
BCL2
BAX
BAD

EC / ANGIOG
CD34
VEGF
FLK
VEGFR3

INVASION
EZH2
ECAD
MMP-9
Ep-Cam

STROMA / INFLAMM
Vimentin
CD45
CD68

CLINICOPATHOLOGICAL AND MORPHOMETRIC DATA

(Cordon-Cardo - Submitted 06)
NEW ADVANCES IN MULTIPLEXING PROTEIN DETECTION
KI-67 AND AR IMMUNO-DETECTION SYSTEM
SPECTRAL IMAGING - PROCESSING

520 nm – 560 nm

Composite Image

Spectral Profile

Unmixing

Grayscale Tiff

Tissue AF

RBC

CK18
ASSESSMENT OF SPECTRAL PROFILES

Example: FITC
520 nm – 570 nm

Spectral Profile

- CK18
- Red Blood Cells
- Tissue Background Fluorescence
- Glass
DYNAMIC RANGE AND UNMIXING PARAMETERS

Linear Response to Fluorescent Dyes

Dilution Series of Alexa Dyes

Test Unmixing of Fluorescent Dyes with 100% Spatial Overlap

Series of Dye-Dye Ratios

Measurement

Software Processing

Starting Dilution for Alexa 555 and 568 (from original stock)

Total Gray Value vs. Dye Concentration with CRI Nuance

Test Unmixing of Fluorescent Dyes with 100% Spatial Overlap
3 Fluorochromes with Cy3 filter

Unmixed plus Background Subtraction
CYTOPLASM SEGMENTATION AND CLASSIFICATION

MULTIPLEXED IMAGE

EXTRACTED FEATURE

NUMBER OF EPITHELIAL CELLS (DAPI+ CK18)
AVERAGE SIGNAL INTENSITY IN THE CYTOPLASM

INTENSITY DISTRIBUTION (%)

3+  2+  1+  Negative
VESSEL SEGMENTATION AND CLASSIFICATION

MULTIPLEXED IMAGE

EXTRACTED FEATURE

NUMBER OF LABELED VESSELS (CD34)
TOTAL VESSEL AREA, PERIMETER, LENGTH, WIDTH
MICROVESSEL AREA (AMVD)

INTENSITY DISTRIBUTION (%)

3+  2+  1+  Negative

Lumen
COMPUTATIONAL ANALYSIS OF ANGIOGENESIS AND MICROVESSEL DENSITY

Microvessel Density in Prostate Cancer is a Significant Independent Biomarker for PSA Recurrence
CHARACTERIZATION OF THE ANDROGEN RECEPTOR

ORIGINAL MULTIPLEXING

SEGMENTED IMAGE

- DAPI+ (All Cells)
- CK18+ Epith Cells
- CK18+AR+ Epith Cells
- AR+ Stroma Cells

(Cordon-Cardo - Submitted 06)
CHARACTERIZATION OF THE ANDROGEN RECEPTOR

(p=0.0001)

Biochemical Recurrence vs. Time (months)

AR Low

AR High

NL Epith Cells: CK18+AR+

Tm Cells: CK18+AR+AMACR+

AR- Stroma Cells  AR+ Stroma Cells

(Cordon-Cardo - Submitted 06)
MATHEMATICS OF COMPLEXITY
SVRc® - Learning and decision making from censored data.
PREDICTION OF TIME TO RECURRENCE AND RISK STRATIFICATION

n = 262

P < 0.001

Low-Risk (180 Pts., 171 Recurrence-Free)

High-Risk (82 Pts., 54 Recurrence-Free)

PREDICTIVE ACCURACY: 86% - SPECIFICITY: 81%; SENSITIVITY: 85%
PREDICTION OF TIME TO CLINICAL FAILURE – RISK GROUPS

PREDICTIVE ACCURACY: 92% - SPECIFICITY: 91%; SENSITIVITY: 90%

Low-Risk (257 Pts., 252 Clinical Failure-Free)

High-Risk (88 Pts., 63 Clinical Failure-Free)

n = 345

P < 0.0001
PROSTATE NEEDLE BIOPSY STUDIES

Comparing prostatectomy and needle Bx, about 90% correlation in biomarker distribution.

Preliminary predictive model of PSA recurrence, concordance index of 0.85.
CAUSES FOR ADVERSE OUTCOME IN CANCER

- LATE DIAGNOSIS OF DISEASE
- LIMITED BIOLOGY KNOWLEDGE
- SUBOPTIMAL TUMOR TARGETING
- INSUFFICIENT TUMOR DOSE
- POOR COORDINATION OF MULTIMODAL THERAPY

2006 Estimated US Cancer Cases

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Cases</td>
<td>720,280</td>
<td>679,510</td>
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INTEGRATED SYSTEMS PATHOLOGY APPROACH TO DISEASE MANAGEMENT

<table>
<thead>
<tr>
<th>RISK ASSESSMENT PREDICTION</th>
<th>EARLY DETECTION</th>
<th>DISEASE CATEGORIZATION TREATMENT PLANNING</th>
<th>FOLLOW-UP AND TREATMENT RESPONSE</th>
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<tbody>
<tr>
<td>MLDA</td>
<td>RT-PCR</td>
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<td>Molecular Cytology</td>
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<tr>
<td>SNPs</td>
<td>Proteomics</td>
<td>Morphometry/Multiplexing</td>
<td>MLDA</td>
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Liquid/Exfoliated Cells ➔ Tissue Biopsies/Samples ➔ Liquid/Exfoliated Cells

Molecular Imaging