PERSONALIZED MEDICINE: Empowering Light Microscopy & the Pathologist

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OBJECTIVES

- Evolving role of Pathologists in contemporary health care….threats & opportunities…..
- Evolving Personalized Health Care paradigms… empowering it……
- Evolving role of Digital Pathology……enabling it……
DISCLOSURES & PERSPECTIVES

• Digital Pathology: Early adopter, not an expert
• Surgical Pathologist: end-user
• Educator: Training next generation of pathologists
• Researcher: Discovery of novel biomarkers into clinical practice
• Medical Director: Large full service anatomic, clinical & molecular pathology lab
• Chairman: Role of Pathology & Lab Medicine in an academic medical center & career development of faculty
HISTORY OF OUR SPECIALTY

WE STAND AT AN UNPRECENDENTED IMPORTANT CROSS ROAD IN MEDICINE, PARTICULARLY IN OUR SPECIALTY

Marcus Cicero (106-43 B.C.)

Not to know what happened before one was born is to always remain a child

INACTION IS NOT AN OPTION INNOVATE, CHANGE & ADOPT

Famous Roman Orator
HIPPOCRATES

• Humorism - doctrine of the four temperaments
• Paradigm of western medicine for 2000 years

460 B.C. – 370 B.C.
Father of Western Medicine
EVOLUTION OF OUR ROLE

• **Era of Autopsy Pathology** – Curious physicians (3000 B.C. – early 1900s) – Germanic Era

• **Era of Surgical Pathology** – Branched out from Surgery (early to mid-1900s) – American Era

• **Era of Personalized Medicine** – Integrated Anatomic and Clinical Pathology (turn of the century) – Global Era
ERa of the Autopsy: 3000 B.C – 1900’S A.D.

• **Autopsia**: *to see for oneself*

• Began in ancient Egypt & Greece

• Largely performed by curious treating physicians

• Foundation of anatomical basis of disease
THE LIGHT MICROSCOPE

Antonie van Leeuwenhoek
(1632-1723)

Max Magnification: 2000X
• The 1st catalogue in the National Library of Medicine is by James Wilson in 1819
• In the mid to late 1800’s surgeons performed macroscopic evaluation
  – *To advance their surgical technique*
  – *To determine benign vs malignant*
  – *There was no formal reporting*
  – *If specimen was interesting – sent to medical museum*
THE BIRTH OF PHOTOMICROSCOPY

• Nitrocellulose film in the 1880’s
• Photomicroscope in 1895

• Early images

Frog kidney
American Medical Museum
THE ERA OF HEMATOXYLIN AND EOSIN

• Began with the exploration of the new world
• Hematoxylin derived from logwood tree dye used for fabrics (*haematoxylum campechianum*)
• First successfully used in 1865
FOUNDATIONS OF PATHOLOGY

- Rudolf Virchow (1821-1902)
- Made microscopy an integral part of the practice of pathology
- *Die Cellularpathologie* (1858)
  - Proposed the concept that changes at the cellular level lead to disease
The Near Death of Surgical Pathology

- In 1887, Frederick III, the German Emperor, developed a throat lesion.
- Morel MacKenzie, a British ENT surgeon, brought to Berlin – biopsied the lesion (one of the first uses of biopsy).
- A semi-retired Rudolph Virchow brought in to review slides – interpreted as benign hyperplastic verrucous lesions.
- Emperor’s lesions recurred, condition worsened.
- Waldeyer diagnosed as a carcinoma.
- Emperor died from complications of laryngeal cancer - shock waves in the medical community in Germany & Britain.

*Gal: AAP, 2001: In Search of the Origins of Modern Surgical Pathology*
The Era of Pathology in the U.S. 1900’s…

Department of Pathology & Lab Medicine

- Surgical Pathology by Surgeons
- Laboratory Hematology by Hematologists
- Biochemistry in Nephrology & Endocrinology
- Microbiology in Infectious Diseases
ELECTRON MICROSCOPE

- Co-invented by Germans, Max Knoll and Ernst Ruska in 1931
- Co-awarded the Nobel Prize for Physics in 1986
- Maximum magnification up to 2 million times
1941: Coons identified pneumococci using a direct fluorescent method

1979: Peroxidase-antiperoxidase technique

1980s: Use of the avidin and biotin complex

1990s: Widespread use in surgical pathology

- objective
Surgical Pathology Approach

Gross

Microscopy

Clinical Information

Ancillary studies

PATHOLOGIC DIAGNOSIS
SELECT CONTRIBUTIONS OF PATHOLOGISTS & THE LIGHT MICROSCOPE

- Classification of all neoplasms into carcinoma and its specialized types, blastomas, germ cell tumors, gliomas and lymphoma morphotypes
- Concept of grading and staging
- Classification of specific inflammatory diseases of the kidney, lung, GI tract, etc.
- Identification of specific infectious diseases e.g. the AIDS complex, PML, CMV, etc.
- Recognition of distinct entities
  - Myositis ossificans
  - Gonadal blastoma
  - GVHD
  - PEComa
  - Desmoplastic Round Cell Tumor
ERA OF SUBSPECIALIZATION

- **Clinical Pathology**
  - Chemistry
  - Microbiology
  - Transfusion Medicine
  - Cytogenetics

- **Anatomic Pathology**
  - Neuropathology
  - Renal Pathology
  - Dermatopathology
  - Surgical Pathology
    - Subspecialized sign outs

Molecular Pathology

Hematopathology
PRESENT
# Personalized Diagnostics in Anatomic Pathology

## Estimated Deaths 2011

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>85,600</td>
<td>71,340</td>
<td>Lung &amp; bronchus</td>
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<td>Prostate</td>
<td>33,720</td>
<td>39,520</td>
<td>Prostate</td>
<td>39,520</td>
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<td>Colon &amp; rectum</td>
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<td>18,300</td>
<td>Pancreas</td>
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<td>15,460</td>
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<tr>
<td>Leukemia</td>
<td>12,740</td>
<td>9,570</td>
<td>Non-Hodgkin lymphoma</td>
<td>9,570</td>
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<tr>
<td>Esophagus</td>
<td>11,910</td>
<td>9,040</td>
<td>Leukemia</td>
<td>9,040</td>
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<tr>
<td>Urinary bladder</td>
<td>10,670</td>
<td>8,120</td>
<td>Uterine Corpus</td>
<td>8,120</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>9,750</td>
<td>6,330</td>
<td>Liver &amp; intrahepatic bile duct</td>
<td>6,330</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,270</td>
<td>5,670</td>
<td>Brain &amp; other nervous system</td>
<td>5,670</td>
</tr>
</tbody>
</table>

**All Sites** 300,430 100%  **All Sites** 271,520 100%
PERSONALIZED MEDICINE: CANCER

- **Breast** - established paradigm
- **Lung** - exciting hot story
- **Prostate** - in need of a breakthrough
38 YEAR OLD WOMAN WHO UNDERWENT MAMMOGRAPHY
Symptoms-based

- Symptomatic diagnosis, prescription & monitoring
- Treatment targets selected based on largest population
- Blockbuster drug for all patients effective in only 40-60% and can have adverse drug reactions (ADR)
- Reactive

This patient
- Radical surgery
- Standard chemotherapy
- Prognosis based on population statistics

One size fits all
Personalized Medicine

- Sequencing of the human genome (2003)
- New paradigm for medicine based on gene-based knowledge combined with health information technology: Personalized Medicine
- 3 billion DNA base pairs
- 30,000 genes
- 500,000 protein characterize the human genome
• **Predict** our individual susceptibility to disease
• **Provide** more useful & person specific tools for preventing disease
• **Detect** the onset of disease at the earliest moments
• **Preempt** the progression of disease
• **Target medicines** and dosages more precisely and safely to each patient.

Genomics- health information technology-evidence/clinical delivery
MOLECULAR TESTING ON BREAST CANCER
Molecular Testing on Breast Cancer Risk Stratification

Fresh Tumor Tissue

Tumor DNA/RNA

Labeled Tumor cDNA/cRNA

Tumors genetic signature stratifies patients risk for recurrence/metastasis (Examples include: Oncodx™ – 21 gene, Mammaprint™ – 70 gene signature)

RT-PCR EXPRESSION PROFILING

Recurrence Score vs. Distant mets

Low risk

High risk
STRATIFICATION OF TUMORS

Breast cancer -

- Triple negative (15%) (basal phenotype) - no targeted therapy
- HER2 positive (15%) – Herceptin, T-DM1
- Luminal, ER, PR positive (70%) – Hormonal therapies

ER, PR status
HER2 status

CK5/6,EGFR(+)
Her2 (+/-)
Her2 (-)
PERSONALIZED MEDICINE

Based on genetic testing and detection of variation in production of enzyme that metabolizes Tamoxifen

Genetic signature determines correct drug and dose - *pharmacogenomics*
• Patient had family history of breast cancer.

• Ashkenazi Jewish heritage.

• Underwent sequencing for BRCA1 and BRCA2 mutations.

• Patient detected to be BRCA2 mutation positive.

• Increased surveillance in opposite breast with choices for chemoprevention and prophylactic surgery.

  Increased risk of laryngeal, melanoma and pancreas cancers.
SYMPTOMS VS. GENETIC-BASED MEDICINE

**Symptoms-based**

- Symptomatic diagnosis, prescription & monitoring
- Treatment Targets selected based on largest population
- Blockbuster drug for all patients effective in only 40-60% and can have adverse drug reactions (ADR)
- Reactive

One size fits all
SYMPTOMS VS. GENETIC-BASED PROSPECTIVE CARE

**Genetic-based**

- Molecular Diagnosis
- Risk-stratification by molecular
- Drug-targeted therapy
- Less or no ADR
- Molecular monitoring of disease
- Preventive

**Our patient**

- Local resection
- Personalized risk-stratification
- Targeted therapy
- Prevention and prophylaxis

The right treatment for the right person at the right time with the right dose for right outcome and improved quality of life.

Our patient...
Histologic and Molecular Correlations
Travis, Journal Thoracic Oncology, 2011

- Small cell carcinoma
- Non-small cell lung carcinoma (NSCLC)
  - LCNEC
  - SCC
  - AdenoSCC
  - AdenoCa
  - LC

LCNEC: Large cell neuroendocrine, LC: Large cell carcinoma, SCC: Squamous cell carcinoma, AdenoSCC: Adenosquamous
Molecular Classification of Pulmonary Adenocarcinoma

Histology: Adenocarcinoma or Other Non-squamous

EGFR mutation analysis

+ TKI treatment

- EML4-ALK translocation

+ Young male
   light or non-smoker
   Acinar, papillary, mucinous

- ALK inhibitor Crizotinib

Chemotherapy

Travis, Journal Thoracic Oncology, 2011
Molecular Classification of Pulmonary Adenocarcinoma

Adenocarcinoma

Non-mucinous Asians, non-smokers

EGFR & KRAS 10-30%

AIS, MIA, LPA, Solid, Papillary Micropapillary

BRAF 5%

Micropapillary 20%

KRAS 80-100%

No EGFR mut

Solid Variant Mucinous Non-Asians, smokers

Papillary, Lepidic 5%

Travis, Journal Thoracic Oncology, 2011
PERSONALIZED DIAGNOSTICS IN ANATOMIC PATHOLOGY

• Similar evolving paradigms in
  • Hematopoetic malignancies
  • Colorectal carcinoma
  • Melanomas (BRAF Mutations)
  • Gliomas (1p- in oligos and MGMT methylation in astrocytoma)
Current Determination of Prognosis

- Digital Rectal Exam
- Serum PSA
- Amt. of tumor in biopsy
- Gleason score
Limitations of Prognostication & Therapy Selection

- Subjective assessment
- Broad distinctions
- Lack of predictive power at the individual level

Traditional approach—One size fits all
Molecular Stratification of PCa Pts.

Treatment

- ETS gene fusion
- PTEN deletion
- C-MYC gain/amplification
- Loss of 8p21
- AR gene amplification
- MET over-expression
- VEGFR2 over-expression

Active Surveillance

GS 6

GS 6
mTOR Is Activated in PCa

Growth Factors

PI3 Kinase

PTEN

~50% of prostate cancers

Growth Factors

New Blood Vessel Formation

mTOR Inhibitor

mTOR

Inhibit Apoptosis

Growth

Miltefosine

AKT

Promote Survival
Active Surveillance

Radiation

Surgery

Targeted Therapy

Active Surveillance
Histopathology
Microscopy of a tumor in the kidney

Papillary Renal Cell Carcinoma
The tumor is confined to the kidney (>95%)

The tumor by IHC is positive for CK7 and racemase (>90%)

The tumor shows trisomy 7, 17 and -1 (>90%)

The tumor has a >90% 5 year survival

Metastatic tumors respond to sunitinib therapy
FUTURE
Empowering Light Microscopy & the Surgical Pathologist… *the journey continues*……

**Personalized Health Care**
Advances in understanding the clinical significance of genomic, & proteomic signatures for diagnostic, prognostic & predictive markers

**Disruptive technology**
Digital Pathology

**Enabling technologies**
High throughput & multiplexing protein, RNA, DNA analysis (microarrays, sequencing, nanotechnology)

**Medical & Bioinformatics**
Data mining, integration & reporting
Decision making algorithms

**Disruptive technology**
Digital Pathology
STAGES OF DIGITAL PATHOLOGY (DP): EMPOWERING THE PRACTICING SURGICAL PATHOLOGIST

- **Stage I: Current DP capabilities:** archival, research, basic slide scanning, image analysis and telepathology

- **Stage II: DP equivalent to or better than the glass slide:** integration & more universal adoption

- **Stage III: DP extension beyond the light microscope:** quantification, multiplexing, subcellular localization of proteomic, genomic, functional capabilities

- **Stage IV: DP beyond the cellular microscopic level:** macroscopic/clinical level to integrated in vitro- vivo diagnostics
STAGE I: CURRENT DP CAPABILITIES

Image Capture (limited adoption)
- Digital recuts
- Image enhanced reports
- Biopsy sign out
- Quality assurance

Consultation
- 2nd Opinions
- Frozen Section
- Remote sign out

Signal Quantification
- IHC – image analysis algorithm
- FISH

Education
- Teaching sets
- Tumor boards & clinician consultation
- CME & examinations

Research
- Morphologic control for archive tissue
- Image analysis for TMA – qualitative and quantitative
STAGE II: DP EQUIVALENT TO OR BETTER THAN THE GLASS SLIDE

Integration into pathologists current workflow:

• Standards for WSI
• Speed
• Objective quantification of pathologic parameters
• Annotation
• Real time consultation networks
• Rapid retrieval of archived cases for comparison
• Side by side comparisons of H&E & special stains
• Integration with LIS
• Integration with EMR
Surgical Pathology Diagnosis

Architecture
Cytology
Adjunctive features
Clinical Information

Human Cognition

Diagnosis
Subjective Interpretation
Unique, point in time process which is sometimes precisely irreproducible
Digital Pathology
Data Objectivity and Persistence

- Persistent data set available for analysis by multiple software algorithms
Helpful Quantification “Apps”
• Mitoses / rare events
• Nuclear anaplasia
• Architecture (Gleason grading)
• Nucleolar size (Fuhrman grading)
• Identifying plasma cells
• Counting eosinophils or mast cells
• Other repetitive tasks…
STAGE III: DP EXTENSION BEYOND THE LIGHT MICROSCOPE

Computer Assisted Diagnosis
Objective recognition - extract “features” from H&E stained slides that are clinically relevant

- Systematic, reproducible and quantifiable “feature” recognition based on mathematical algorithms
- Diagnostic “Apps” through virtual integration of “features” with histologic and immunohistochemical data

STAGE III: DP EXTENSION BEYOND THE LIGHT MICROSCOPE

Dynamic functional cell imaging

• Stem cells (cells with metastatic potential or resistant to therapy)
• Virtual cellular motility & metastasis

Predictive multiplex biomarker panels

• Single cell analysis and subcellular localization
• Multispectral protein and RNA detection (in situ detection 3-5 markers in pathways)
Boosted-Bayesian Multi-Resolution Workflow Algorithm

TRAINING SET

(a) Slide Digitization
(b) CaP Extent Annotation By An Expert
(c) Constructing Image Pyramid
(d) Multi-Resolution Feature Extraction

(i) Evaluation
(h) Mask Out Benign Regions At Next Scale
(g) AdaBoost Training
(f) Training Weak Bayesian Learners

Feature Modeling

TEST CASE

Transactions on Biomedical Engineering Jul 2010 Doyle et al
STAGE IV: INTERGATED CLINICAL, MACROSCOPIC, IN VITRO & IN VIVO STUDIES

GENETICALLY ENGINEER HEMATOPOETIC STEM CELLS IN CULTURE BY EGFR RECEPTOR REPORTER GENES

STEM CELLS CONSTITUITIVELY EXPRESS REPORTER GENE

INJECT IN PATIENT CELLS LOCALIZE TO TARGET

REPORTER PROBE INJECTETED DETECTS LOCALIZED TARGET PROVIDES INVIVO INFORMATION ON TUMOR CELL FUNCTIONALITY
Role of the Pathologist

Traditional
“Guardian of the paraffin”

Contemporary
Guardian of the RNA, DNA and Protein
Consultant & Chief Informatician
Diagnostic Pathologist

- reviews slides
- generates reports

Diagnostic Oncologist

- Participates in multidisciplinary care
- Integrates morphologic, molecular & outcome data
- Data generators & interpreters
Surgical Pathology Approach (1870s - 2000)

Gross

Microscopy

Clinical Information

Ancillary studies

PATHOLOGIC DIAGNOSIS
Contemporary Pathology Approach
(2000-2020)

Anatomic pathology

Clinical pathology

MOLECULAR DIAGNOSTIC SIGNATURE

Bio-medical informatics

Molecular pathology
Futuristic Disease Management Approach (2020...)

Pathology & Lab Medicine
In vitro Diagnostics

Imaging (Radiology)
In vivo Diagnostics

Bio-medical informatics

Medical Oncology Therapeutics

DIAGNOSTIC & THERAPEUTIC ONCOLOGIST
(Disease Management Team)
WE STAND AT AN UNPRECEDENTED IMPORTANT CROSS ROAD IN MEDICINE, PARTICULARLY IN OUR SPECIALTY

ALL IN THIS ROOM ARE INVOLVED IN AN EXCITING TRANSFORMATION OF OUR SPECIALTY..... TOGETHER, LETS MAKE HISTORY HAPPEN

INACTION IS NOT AN OPTION INNOVATE, CHANGE & ADOPT

HAVE A GREAT PATH VISIONS 2011 CONFERENCE!!!!!!!
CREDITS/WEBSITES

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Pathology and Laboratory Medicine

Individualized Diagnosis at the Speed of Right
LIMITATIONS OF PATHOLOGY THROUGH THE LIGHT MICROSCOPE

- Largely subjective and eminence-based
- Slides yield 2 dimensional view
- Limited ability for multiplexing of biomarkers
- Limited ability for quantification
- Operational
  - Slide storage
  - Slide portability
Autopsy-based experience: first to emphasize patient care should be based on anatomical diagnosis
FOUNDATIONS OF PATHOLOGY

• Karl Rokitansky (1804-1878)
• Championed the autopsy as performed today
• Performed over 30,000 & supervised over 70,000 autopsies
• Created an institute of centralized autopsy separate from clinical practice
STAGE IV: IN-VIVO IMAGING

Live cell imaging underneath skin:

- Normal skin
- Mole

20μm below epidermis

Probe based confocal laser endoscopy:

- Sophisticated probe handling and image interpretation
- Real time diagnosis
- Improved accuracy of sampling for histologic confirmation


Multiplexing of biomarkers with subcellular localization of proteomic, genomic, functional capabilities

Multispectral protein and RNA detection for:

• Improved prediction of prognosis and treatment response
  Detecting subpopulations of treatment resistant cells and identifying cytotoxic targeted therapy options

• Functional cell imaging
  - Stem cell imaging: Simultaneous chromatin configuration and transcriptional activity with IHC
  - Activation of signaling networks
  - Virtual cellular motility and metastatic potential

• Multiplex marker detection in small biopsies and FNAs