...κοινή γαρ η τύχη και το μέλλον αόρατον.
(../fortune appears the same for all and the future is invisible.)

Isocrates, 380 BCE

What is past, is prologue..

WILLIAM SHAKESPEARE, The Tempest

The future is already here!

William Gibson, Neuromancer.
Pathology Today

• **As Medical Practice:** Pathology covers all aspects of diagnostic methods on solid or fluid tissues based on laboratory techniques. It also covers the methodologies to transmit diagnostic data in an interpretable way to other physicians directly involved with patient treatment. Pathology manages Blood Banks for diagnostic and therapeutic applications. In that capacity, it also manages Tissue Banks for research or diagnostic purposes. Cellular therapies from Tissue Banks or Blood Banks are increasingly becoming part of Pathology.

• **As Research Discipline:** Boundaries between disciplines in biomedical research are becoming ever more fluid and all definitions apply only to a central core concept. Each discipline benefits more from the overlaps with the other disciplines than from experiences derived exclusively from the central core. The central core of Pathology is **Tissue Pathobiology.** This covers cellular or biochemical or genetic processes related to tissue functions. Such processes provide the foundations for understanding disease mechanisms. These topics are not exclusive province of pathology practitioners. In general, however, Pathology as a research discipline is best defined at its core as the understanding of the normal tissue and cell mechanisms which, when they deviate, result in a disease process.
Pathology Practice: Nothing lost in Translation!

- The integrated efforts of many pathologists have led to standardized diagnostic procedures, which were derived from inspired but not standardized discoveries of biomedical research.

- **Examples:**
  - Histology and associated techniques developed more than a century ago leading to the myriads of systematic classification of histopathology, and becoming the core of Anatomic Pathology.
  - Humoral immunology leading to serology, immunopathology, ELISA assays in Clinical Chemistry, Immunohistochemistry in Anatomic Pathology.
  - Biochemistry and Enzymology leading to Clinical Chemistry
  - All of Microbiology and Virology leading to diagnostic aspects of the disciplines.
  - Molecular Biology and Molecular Genetics lent nucleic acid based techniques which led to yesterday’s revolution (and today's establishment!) of Molecular Diagnostics.
  - Computer based automation and microelectronics had multiple impacts. The automated testing laboratory (ATL), without which no hospital (or even small private medical group!) can function, is a result.
Emergent New Technologies impacting Pathology

- Molecular Diagnostics at the histology level.
- Digital Diagnostics
- Bedside diagnostics
- Nanosensors
- Pharmacogenomics
Tumor Cell Heterogeneity and its Effect on Tumor Behavior in Human Colorectal cancer

Mona F. Melhem, MD
Professor, Department of Pathology
VA Medical Center of Pittsburgh
p53 Immunohistochemical heterogeneity of Colorectal Adenocarcinoma

- Normal Crypts
- Tumor (1) p53 IHC (-)
- Tumor (2) p53 IHC (+)
- Tumor (3) mixed p53 IHC (+)/IHC (-)
LCM of Colorectal Adenocarcinoma

Before LCM

p53 IHC (-)

Remove (-) cells

After LCM #1

p53 IHC (+)

Remove (+) cells

After LCM #2
SSCP Mutational Analysis Scheme

Example:

p53 exon 8 from 2 select cases

-Vertical sequencing MDE gel, radiolabeled DNA.

Case 6

p53 (-)  p53 (+)  Norm.

→ p53 exon 8 (~200 bp)
Tissue based FISH studies of chronic lymphocytic leukemia/small lymphocytic lymphoma demonstrate chromosomal abnormalities in 100% of cases.

12 cen (D12Z3) 13q14.3 (D13S319)/13q34 (control)

**Normal:**
- 2 orange
- 2 aqua
- 2 green

**Trisomy 12:**
- 2 orange
- 2 aqua
- 3 green

**Deletion 13q (single):**
- 1 orange
- 2 aqua
- 2 green

**Deletion 13q (double):**
- 0 orange
- 2 aqua
- 2 green

D: deletion (1: single, 2: double); T: trisomy; R: translocation; N: normal
SISH HER2 (Black)
IHC HER2 (Red)
Amplified & Over-Expressed

David Dabbs
Magee Women’s Hospital
Human Genome: Impact on Diagnostic Pathology

• The decipherment of the human genome has widened the pathway of connecting genomic alterations to neoplastic development, genetic diseases, susceptibility risks. These alterations can be precisely determined by applying standard MDx techniques.

• Most of these discoveries are not yet connected to biological therapies. As this occurs, standard diagnostic tests need to be developed, in order to determine whether patients will benefit from specific therapies.

• Current therapeutic modalities often lead to different results in individuals with the same disease in the same stage. New genomic data with highlighting of polymorphisms lead to determination of diagnostic parameters for defining individually tailored treatment protocols.
Fifty-four recurrent somatic regions of deletion were identified, with the minimal deleted regions typically measuring less than 1 megabase (Mb) in size, and twenty-four of the deletions containing only a single gene (Supplementary Table 10). None were present in the germline samples. Although technical aspects of the methodology used might theoretically lead to false-positive or false-negative results, fluorescence in situ hybridization (FISH) and/or quantitative polymerase chain reaction (qPCR) confirmatory studies (described below) validated each of the examined lesions. The recurring deletions included 3p14.2 (FHIT) 2, 6q16.2-3 (including CCNC) 3, 9p21.3 (two regions involving CDKN2A (ref. 4) and MLLT3), 12p13.2 (ETV6) 5, 11q23 (including ATM) 6, 13q14.2 (RB1) 7 and 13q14.2-3 (including mir-16-1 and mir-15a) 8. In addition, deletions of other tumour associated genes not previously implicated in ALL were identified including LEF1, BTG1 and ERG. The most notable observation, however, was the identification of genomic alterations in genes that regulate B-lymphocyte differentiation in 40% of B-progenitor ALL cases.

To obtain a comprehensive registry of genetic lesions in ALL, we examined DNA from the leukaemic cells (blasts) of 242 cases of paediatric ALL (Supplementary Table 1) using Affymetrix single nucleotide polymorphism (SNP) arrays that interrogate over 350,000 loci and permit identification of copy number changes at an average resolution of less than 5 kilobases (kb). In addition, paired copy number.

The new Affymetrix® Genome-Wide Human SNP Array 6.0 features more than 1.8 million markers for genetic variation, including more than 906,600 single nucleotide polymorphisms (SNPs) and more than 946,000 probes for the detection of copy number variation. The SNP Array 6.0 enables high-performance, high-powered and low-cost genotyping.
Interlaboratory Performance of a Microarray-Based Gene Expression Test to Determine Tissue of Origin in Poorly Differentiated and Undifferentiated Cancers


From the Department of Pathology,* Virginia Commonwealth University, Richmond, Virginia; the Clinical Genomics Facility and Department of Pathology;† University of Pittsburgh, Pittsburgh, Pennsylvania; the Department of Pathology;§ Stanford University, Stanford, California; and Pathwork Diagnostics,§ Sunnyvale, California

Clinical workup of metastatic malignancies of unknown origin is often arduous and expensive and is reported to be unsuccessful in 30 to 60% of cases. Accurate classification of uncertain primary cancers may improve with microarray-based gene expression testing. We evaluated the analytical performance characteristics of the Pathwork tissue of origin test, which uses expression signals from 1668 probe sets in a gene expression microarray, to quantify the similarity of tumor specimens to 15 known tissues of origin. Sixty archived tissue specimens from poorly and undifferentiated tumors (metastatic and primary) were analyzed at four laboratories representing a wide range of preanalytical conditions (eg, personnel, reagents, instrumentation, and protocols). Cross-laboratory comparisons showed highly reproducible results between laboratories, with correlation coefficients between 0.95 to 0.97 for measurements of similarity scores, and an average 93.8% overall con-
Gene Expression patterns in Liver Cancer

A total of 37 hepatocellular carcinomas and 7 hepatoblastomas were used for the study. Due to differences in the amount of tumor available, there were many cases in which adjacent tissue was not available (required for identification of tumor margins for diagnostic purposes). There were also cases in which the tumor size was very small and all required for diagnostic purposes, but tissue adjacent to the tumor was available. Overall, 32 samples of cirrhotic liver adjacent to the tumor were used for the study. Due to these considerations, each one of these “tissues adjacent to tumor” was investigated as a separate item, and not in relation to a specific tumor to which it may relate. The statistical analysis examined the “tissues adjacent to tumor” as a population of its own. Normal livers (from donor liver tissue) were obtained from 29 cases.
Figure 3C
Shortcomings: Gene expression computer algorithms can determine how many types of HCC exist but cannot reliably diagnose tumor from tumor-adjacent tissue.
Gene Arrays: are they going to change practice of Pathology?

• Before diagnostic algorithms become widely adopted, there needs to be some standardization of platforms and corroboration of published results by multiple groups.
• The “biology” of the results needs to be better understood (if cell cycle genes are all increased in anaplastic tumors, is this more reliable than a PCNA stain?)
• Impact on Tissue Banking: Need to collect more fresh tissue from which RNA can be extracted.
• Crude microdissection versus super-microdissection: Problems with RNA amplification distortions versus purity of cellular material.
• Gene alterations in surrounding non-malignant tissues often similar to the main tumors: The rebirth of the field effect! Can arrays reliably make the diagnosis between benign and malignant?
• Highly likely that arrays will strengthen but not replace the microscopic diagnosis between benign vs. malignant done by surgical pathologists! 😊😊😊😊 !!!!!!
• What do we teach our residents?
Optical histo-pattern recognition through machine learning

- If histopathology patterns are in large part the basis for histopathologic diagnosis, can these patterns be analyzed and assessed by artificial intelligence?
- Similar issues in relation to Radiology, Internal Medicine (robo-doc!), Surgery (robotic surgery) Pediatrics (kids like computers better than doctors!) and Psychiatry (?)
- Machine learning in histopathology: Assign codes/diagnoses in a standard language to digitized pathology images.
- Run thousands of such images through “untrained” computer and allow “machine learning” programs to assign binary morphologic attributes to specific codes/diagnoses.
- Use untested images on the educated computer and test for pattern recognition.
Low-power tissue segmentation: prostate cancer

From Dr. Rich Levenson, CRI
Low-power tissue segmentation: breast cancer

Green: normal breast
Red: ductal carcinoma
Yellow: stroma, inflammation and fat

From Dr. Rich Levenson, CRI

10X magnification
Mitosis Detection

From Dr. Rich Levenson, CRI
Digital Pathology: Getting the most from the “static” image

• **Current paradigm:**
  – Simple staining of histopathology sections (typically hematoxylin-eosin)
  – Apply immunohistochemical techniques to identify expressed proteins using specific antibodies (one antibody per section).
  Problems when multiple markers/sections are needed: Very small pieces of tissue, not enough for all markers; exfoliative cytology, no replicate slides.

• **New Paradigm:**
  • Apply multiple antibodies on the same section, using Quantum Dot photo-emitters attached to each antibody species.
  • Detect each antibody (emitting at different wavelength) using specific tuners.
  • Integrate data from multiple antibodies on the same histologic section.
  • Is there ANY staining of tissue needed? SPECTRAL PATHOLOGY!
Quantum Dots

CdSe core

ZnS shell

TOPO coating

TEM of a 4 nm CdSe quantum dot

Solutions of differently sized CdSe quantum dots

Fluorescent emission fingerprinting of polymer beads that contain CdSe quantum dots

Caption: Micrograph of pyramid-shaped quantum dots grown from indium, gallium, and arsenic. Each dot is about 20 nanometers wide and 8 nanometers in height.

NIST
Cell cycle markers:

$p^{WAF-1/Cip1}$ -- inhibits proliferation
DAB (pseudo-colored green)

$p^{Kip1}$ -- suppression of proliferation
LPR

Ki67 -- proliferation marker
Vector VIP

Skin, FFPE (vessel malformation)

From Dr. Rich Levenson, CRI
Lymph node germinal center labeled with 5 quantum dots plus DAPI

From Dr. Rich Levenson, CRI
“Flow on a slide”

From Dr. Rich Levenson, CRI
Quantum Dots for Multiplexed Analysis

The ability of the QDs for multiplexed analysis of four toxins was demonstrated by Goldman and coworkers [83] using four different QDs having different emission wavelengths in a sandwich immunoassay with a single excitation source. Similarly, two spectrally different QDs were employed by Makrides and coworkers [84] for the detection of two proteins in a western blot assay. The multiplexed approach would be of extreme importance in the detection of various cancer biomarkers present at the targeted tumor site.
Virtual Microscopy

There are current technologies under rapid development which aim to create a digitized computer file from complete scanning of a stained tissue section. The digitized file will be amenable to examination at any spot on a computer screen at different magnifications. Typically 1 cm² of tissue consumes more bytes for virtual microscopy than a CT scan. This may replace the microscope as the basis for morphologic analysis of tissues. This may also facilitate transmission of digitized tissue files for concurrent examination by diagnostic pathologists at different sites. Current limitations (speed, accuracy) and efforts to overcome them.
Going beyond the microscope!
Proteomics and Microscopy

- To provide complete proteomics analysis connected to the histology of the tissue:
  - Cover frozen section with sinapinic acid
  - Overlay an “insect eye” mass spectrometer (parallel array of mass specs). Each aperture is 50 micron in diameter.
  - Collect all proteins (less than 30 kDa) out of each 50 micron section
  - Connect through the computer the distribution of every protein peak mapped against the histology of the frozen section.
Profiling/Imaging of Proteins in Tissues by MS for Molecular Discovery in Disease Research and as an Aid in Clinical Diagnoses

Richard M. Caprioli

Vanderbilt University School of Medicine
Nashville, TN
Acquire mass spectra

Slice frozen tissue on cryostat (~12 µm thick)

Thaw slice onto MALDI plate, allow to dry

Profiling

Imaging

Droplet

Apply matrix

Spray coating

Acquire mass spectra

Protein profiles

Protein images

m/z 18388
Profiling vs. Imaging
(Head and Neck Squamous Cell Carcinoma)
Human Glioma

m/z 11640, Calvasculin

m/z 41663, Actin

m/z 4965, Thymosin β.4

1 mm

Analysis of drugs in tissue by mass spectrometry

- Dose animal
  - orally
  - i.v.

- Remove tissue

- Cut frozen slice (12 μm)

- Apply matrix

- Analyze by MALDI MS/M
Image of OSI-774 in mouse tumor tissue

- Optical image of tissue slice
- MS/MS image of tissue slice
  m/z 394→278

- Mouse dosed at 100 mg/kg
- Tissue removed 16 hr after dose
- Resolution: 200 x 400 μm
- Area imaged: 106 mm²
Histology based proteomics: Practical applications for pathology practice.

- “Immunohistochemistry without antibodies?”
- When (soon, next 5-10 years?) all proteins are mapped on standard mass spec, this approach will allow complete molecular determination of ALL proteins present in a section, histologically mapped.
- The pathologist will be able to project on a screen mapped expression levels of any tumor marker.
- Concurrent mapping: A marker for endothelial cells and marker for a tumor marked together in different pseudo-colors can determine vascular invasion?
Realities

Implants

• Artificial organs and bio-hybrid organs are in use

• New understanding for monitoring and pathology of implant

Texas Heart Inst

Alan Wells
VC Lab. Medicine
Pathology
Cellular therapeutics

- Cell and tissue engineering in trials
- Requires pre-input monitoring of living cells
- Requires post-input cell monitoring and analyses
Point of Care Testing, Bedside Diagnostics and BEYOND!

Diagnostics and Nanotechnology

Integration Of Nanotechnology With Biology And Medicine Will Result In Major Medical Advances

ScienceDaily (Apr. 2, 2003) — NEW ORLEANS --

Until very recently, nanotechnologists — scientists who build devices and materials one atom or molecule at a time — concentrated almost entirely on electronics, computers, telecommunications, and materials manufacture. Now biomedical nanotechnology, in which bio-engineers construct tiny particles combining inorganic and biological materials — is pushing to the forefront of this rapidly advancing field of science.
Figure 1. (Left) Nanogen’s Nanochip device which employs the power of electric current to direct DNA probes to specific sites on the array. (Right) Close-up of DNA hybridization on three different sites. Pictures are courtesy of Cognoscenti Health Institute (3)
A new type of biopsy.....capture of circulating tumor cells!

Toner et al.,
NATURE| Vol 450| 20/27
December 2007

Figure 1 | Isolation of CTCs from whole blood using a microfluidic device.

**a,** The workstation setup for CTC separation. The sample is continually mixed on a rocker, and pumped through the chip using a pneumatic-pressure-regulated pump. **b,** The CTC-chip with microposts etched in silicon. **c,** Whole blood flowing through the microfluidic device. **d,** Scanning electron microscope image of a captured NCI-H1650 lung cancer cell spiked into blood (pseudo coloured red). The inset shows a high magnification view of the cell.
Pathology and Radiology

• Considerable overlap already, e.g. liver ultrasound vs. liver biopsies, “virtual autopsy: (a.k.a. virtopsy), etc.
• Collaboration between pathologists and radiologists for fine needle aspirates (FNA).
• Imaging analysis of resected whole organs.
• While imaging techniques will continue to provide higher resolution of the “anatomic geography” of specific diseases (tumors most prominently), the need to conduct genomic and biochemical analyses to precisely direct biologic therapies will continue well into this century and beyond…
• Nobody can see beyond “beyond”…
The amount of information that can be extracted from minute tissue and fluid samples using complex, automated and miniaturized devices will continue to increase.

Sophisticated computer-based algorithms will provide assistance in integration of all information.

Lab and tissue-based diagnostics will be increasing their capability to provide a safe guide to therapy.

Enhanced imaging capabilities will allow groups of pathologists to share information on tissue-based diagnostics.

Pathology practitioners, blending knowledge of histopathology, disease related molecular processes and lab diagnostics, will be the integrators of information related to the molecular, biochemical and cellular processes underlying the patient’s disease, complications and symptoms.

Will anybody, ever, be able to do without us?

Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET.

Naldini L, Vigna E, Narsimhan RP, Gaudino G, Zarnegar R, Michalopoulos GK, Comoglio PM.

Department of Biomedical Sciences & Oncology, University of Torino, School of Medicine, Italy.

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**MET Amplification Leads to Gefitinib Resistance in Lung Cancer by Activating ERBB3 Signaling**

Jeffrey A. Engelman,1,2,3 Kreshnik Zejnullahu,4,5 Tetsuya Mitsudomi,6 Youngchul Song,2,3 Courtney Hyland,4 Joon Oh Park,4,5 Neal Lindeman,7 Christopher-Michael Gale,3 Xiaojun Zhao,5 James Christensen,6 Takayuki Kosaka,6 Alison J. Holmes,4,5 Andrew M. Rogers,5 Federico Cappuzzo,9 Tony Mok,20 Charles Lee,7 Bruce E. Johnson,4,5 Lewis C. Cantley,2,5 Pasi A. Jänne3,5.