Precision Pathology
- A new frontier

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“Surgeons know nothing but do everything.
Internists know everything but do nothing.
Pathologists know everything and do everything but too late.”

The idea of precision
THE PRECISION MEDICINE INITIATIVE
“Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best they can to individuals. You can match a blood transfusion to a blood type — that was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard? What if figuring out the right dose of medicine was as simple as taking our temperature?”

- President Obama, January 30, 2015
Precision Medicine

• Personalized medicine
• Individualized medicine

It's health care tailored to EACH individual.
Precision medicine – why now?

• Current issues of modern medicine
  • Nonspecific toxic treatment of malignancy
  • Clinical trial based therapeutic plans

• Some targeted therapies being proved more effective
  • APL and all-trans retinoid acid (ATRA)
  • CML and Imatinib (Gleevec)

• Cutting-edge technologies now available
  • Next-generation sequencing (NGS), Omics and AI
  • Humanized antibodies and small molecules
Current issues: therapy-related cancers

Ionizing Radiation - Cancers
Chemotherapy - Myelodysplasia

- Hodgkin's Disease,
- Retinoblastoma,
- Acute Lymphocytic Leukemia,
- Wilms Tumor,
- Pediatric Sarcomas,
- Upper Aerodigestive Tract Cancers,
- Breast Cancer
- Prostate Cancer
- Testicular Cancer
- Pancreas/Gastric Cancer
- Colorectal Cancer
- Endometrial/Ovarian Cancer
- Skin Cancer

Targeted therapy being proved effective

• Some examples:
  - Vitamin C and Scurvy
  - Insulin and Diabetes
  - APL and all-trans retinoic acid (ATRA)
  - CML and Imatinib (Gleevec)
Vitamin C and Scurvy
Insulin and Diabetes

JULY 27, 1921
The Insulin A hormone is isolated by Dr. Frederick Banting & Charles Best.

Insulin a chain

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Banting House National Historic Site
Chronic myeloid leukemia and Imatinib

*weisses blut* (white blood) = leukemia
Chronic myeloid leukemia and Imatinib
Chronic myeloid leukemia and Imatinib
Cutting-edge tools of clinical laboratories

• Next generation sequencing (NGS)
• Omics:
  • Genomics
  • Transcriptomics
  • Proteomics
  • Kinomics
  • Metabolomics
• Biosensors
• Artificial intelligence (AI): deep thinking and machine learning
• Humanized antibodies and small molecules
Next-generation DNA sequencing

1. Library preparation
2. Clonal amplification
3. Cyclic array sequencing

DNA fragmentation and in vitro adaptor ligation

emulsion PCR

bridge PCR

Pyrosequencing
Sequencing-by-ligation
Sequencing-by-synthesis

454 sequencing
SOLiD platform
Solexa technology
Next generation sequencing

1. Fragments
2. Add adaptors
3. Attach to flowcell
4. Bind to primer
5. PCR extension
6. Dissociation
7. Cluster formation
8. Sequencing
9. Signal scanning
Difference between Sanger sequencing and NGS

**Sanger Sequencing**
- Amplification of region of interest by PCR
- Chain termination PCR using fluorescently labeled ddNTPs
- PCR fragments are separated based on size and the DNA sequence is read by the order in which fluorescent signals are detected

**Second Generation Sequencing**
- Shearing of genomic DNA and ligation of adaptors
- Hybridization of fragments to solid surface, followed by amplification of fragments to form clusters
- Fluorescently labeled nucleotides are incorporated while highly sensitive cameras record the sequence of lights
- Sequence reads can be used for de novo assembly of the genomic sequence, or be mapped back to a known genome and quantitated
Omics

- Genome
- DNA
- Transcriptome
- RNA
- Proteome
- Proteins
- Metabolome
  - Sugars
  - Nucleotides
  - Amino acids
  - Lipids (Lipidome)

Metabolites

Phenotype/Function

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Pathology
Metabolomics

Sample collection
Treatment, disease vs control

Sample preparation

Sample analysis
Mass spectrometry (MS) or Nuclear magnetic resonance (NMR)

Data acquisition

Data analysis and interpretation
Multivariate analysis, e.g. PCA

Score Plot

Loading Plot
Metabolomics

Metabolite extraction → Data acquisition → Mass spectrometry → Features selection

Pathway analysis & reconstruction → Identification & quantitation of metabolites
Magnetic nanoparticle biosensor

Sequence of steps for the auto-assembly of magnetic immunoassay
Magnetic nanoparticle biosensor assay
AI concept
Deep thinking and machine learning
AI in action in Pathology

Precision Pathology

• Unlike traditional pathology, which applies pattern recognition, precision pathology employs cutting-edge techniques to identify the etiological factors of a disease and optimize treatment by focusing on the therapeutic targets.
  - It provides molecular targets in the disease for specific therapy.
  - It provides molecular evidence in the patient for optimal clinical outcome.

The concept of precision pathology

• It is a deep-thinking approach to define a pathological condition with a panel of molecular changes at DNA, RNA, protein and metabolite levels.

• Precision pathologists identify therapeutic targets in a unique biological ecosystem based on the NGS and Omics to design the optimal therapeutic plan.
Molecular Pathology vs. Precision Pathology

• Although both are utilizing molecular techniques, but the former focuses on diagnosis and the latter focuses on therapy.
  • Molecular pathology provides supporting evidence for the diagnosis of diseases.
  • Precision pathology provides therapeutic targets and pharmacomics for optimally managing patients.
More steps and more likely errors…

Pathologic changes → Disease

Molecular abnormalities → Traditional pathology

Therapy → Disease
Yes…

Pathologic changes -> Disease

Molecular abnormalities -> Traditional pathology

Molecular abnormalities -> Precision pathology

Disease -> Therapy

Precision pathology -> Therapy
Traditional Pathology vs. Precision Pathology

Specimen → Histology → Pathology → Diagnosis → Treatment plan → Therapy

Metabolomics + Genomics + Artificial Intelligence → Treatment plan
Impacts on medical education

• Medical training:
  • Changes in pathology, pharmacology, oncology, and therapeutics

• Residency training:
  • Shifting from recognizing different pathological entities to identifying the molecular changes for each pathological conditions
How to hit the target?
Molecular basis of precision pathology

• Cancer “Driver” genes (targets)
Driver vs. Rider

• Driver genes – Oncogenes, mutated tumor suppressor genes:
  • Oncogenes: ABL1, BRAF, cyclin D1, EGFR/Her-2, KRAS, PIK3CA, RARA
  • Tumor suppressors: APC, PTEN, p53, RB

• Rider genes – Tissue specific genes and reactive genes
  • Tissue specific: cytokeratin, CD3, CD7, CD13, CD20
  • Reactive: ILs, cytokines, chemokines

• The strategy is to target the “Driver”.

Molecular basis of precision pathology

- Cancer “Driver” genes (targets)
- Node theory – key joints of signal transduction network (targets)
Node Theory

Signal transduction network in a cell
Connecting nodes - key players in the network
Structural “hole” – the shunt of signals
BCR-ABL1

\[ t(9;22)(q34;q11.2) \]
BCR-ABL1

ATP-binding competitors

BCR-ABL1

Substrate

Imatinib mesylate

Substrate

Effector
Molecular basis of precision pathology

• Cancer “Driver” genes (targets)
• Node theory – key joints of signal transduction network (targets)
• Small molecules specifically targeting the “driver genes” and the “nodes” (arrows)
Drugs for precision therapeutics

• EGFR inhibitors:
  • gefitinib  small molecule  reversible
  • erlotinib  small molecule  reversible
  • lapatinib  small molecule  reversible
  • canertinib small molecule  irreversible
  • Neratinib  small molecule  irreversible
  • osimertinib small molecule  irreversible
  • cetuximab  humanized antibody
  • necitumumab humanized antibody
  • panitumumab humanized antibody

• BCR-ABL1 inhibitor – imatinib

• mTOR inhibitor – Rapamycin

• BRAF inhibitor – vemurafenib (Zelboraf), dabrafenib (Tafinlar), and encorafenib (Braftovi)
Molecular basis of precision pathology

• Cancer “Driver” genes (targets)
• Node theory – key joints of signal transduction network (targets)
• Small molecules specifically targeting the “driver genes” and the “nodes” (arrows)
• Individual unique metabolism that affects drug effects and toxicities (environment: distance, wind, visibility, etc.)
Pharmacomics

• Pharmacogenomics:
  • P450 family: CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP3A4, and CYP3A5

• Pharmacoproteomics:
  • Drug-binding proteins: CBG, SHBG
  • Drug receptors

• Pharmacometabolomics:
Future Healthcare Team

To precision medicine