DIAGNOSIS: ANATOMY

BRAIN CANCER

LUNG CANCER

COLON CANCER

MUSCLE CANCER
DIAGNOSIS: HISTOLOGY

- BRAIN CANCER
- LUNG CANCER
- COLON CANCER
- MUSCLE CANCER
DIAGNOSIS: HISTOLOGY

- DEFINITIVE DIAGNOSIS OF CANCER
- RECOGNITION OF SUBTYPES
- PLATFORM FOR CONTINUOUS DIAGNOSTIC REFINEMENT
- BASIS OF ALL CLINICAL DECISION MAKING
- BASIS OF ALL CLINICAL CANCER RESEARCH
DIAGNOSIS: HISTOLOGY

BRAIN CANCER

QUESTION:

HAS THE PROGRESSIVE SUBDIVISION OF CANCERS INTO PRECISELY DEFINED ENTITIES DISTRACTED US FROM THE POTENTIAL IMPORTANCE OF COMMONALITIES AMONG TUMORS WHICH CUT ACROSS DIFFERENT HISTOTYPES?
NEW ADVANCES IN CANCER DIAGNOSIS BASED ON THE EVER DEEPENING UNDERSTANDING OF CANCER BIOLOGY AND ADVANCEMENTS IN BIOTECHNOLOGY

GOALS

• BIOLOGICALLY INFORMED
  - CANCER DIAGNOSIS
  - CLINICAL DECISION MAKING

• PRECISION CANCER THERAPY
FROM DNA STRUCTURE TO THE HUMAN GENOME

1953 UNDERSTANDING GENOME FUNCTION
TECHNOLOGY DEVELOPMENT 2001
“...in every cell there is a specific arrangement for inhibiting ...and definite chromosomes which inhibit division.... Tumors would arise if those inhibiting chromosomes were eliminated”

T. Boveri 1902

“What can be the nature of the generality of neoplastic changes... for the steplike alterations that they frequently undergo. A favorite explanation has been that oncogenes cause alterations in the genes of the body, somatic mutations as these are termed. But numerous facts, when taken together, decisively exclude this supposition. ”

P. Rous  Nobel Prize Lecture 1966
It is taken as axiomatic that alterations in the cancer genome substantially determine the malignant phenotype, and that characterizing these will yield correspondingly substantial insights into tumor biology.
NORMAL CHROMOSOMES
INTEGRATED CANCER GENOMICS

- Gene Expression
- Gene Copy Number
- Transcription Factor Activity
- Chromatin Modification
- DNA Methylation
- Sequence Variation

Cancer Genome
CAN CANCER TYPES BE DEFINED BY THEIR GENE EXPRESSION PROFILE?
Tumor Classification Model: Small Blue Round Cell Tumors

- Ewing Sarcoma
- Neuroblastoma
- Rhabdomyosarcoma
- Lymphoma

Khan J 2001
SEPARATION OF FOUR TUMOR TYPES BY GENE EXPRESSION

Lymphoma
RMS
NBL
EWS
GENE EXPRESSION SIGNATURES OF 11 TUMOR TYPES

Baird K 2005
GENE EXPRESSION SIGNATURES OF 11 TUMOR TYPES

- DFS
- ES
- GIST
- HM
- LEI
- LIPO
- MFH
- OS
- PNS
- RM
- SYN

156.4981  v-kit Hardy-Zuckerman 4 feline sarcoma
88.4149  adenosine monophosphate deaminase (isoform aconitase 2, mitochondrial"
42.1622  "guanylate cyclase 1, soluble, alpha 3"
20.8935  four and a half LIN domains 2
18.6057  chromosome 21 open reading frame 6
18.1963  "protein kinase, cAMP-dependent, regulatory"
15.5833  "phosphoinositide-3-kinase, catalytic, butyrylcholinesterase"
13.457  "erythrocyte membrane protein band 4.1"
13.2547  "melanoma antigen, family A, 10"
13.1497  p300/CREB-associated factor
12.6072  HIV-1 Rev binding protein-like
11.7322  "tumor necrosis factor receptor superfamily, member 8"
11.4732  Homo sapiens mRNA: cDNA DIFLZp954P116 (f"
11.0377  "lectin, galactoside-binding, soluble, SpA transcription factor"
10.4359  "protein tyrosine phosphatase, receptor"
10.0924  "plasminogen activator, tissue"
10.0622  "factor X activator, plasma"

...
IMPLICATIONS OF TUMOR EXPRESSION PROFILING

• Powerful tool for class discovery.

• Data can be used to develop diagnostic and prognostic clinical tests.

• Identify genes for transition to clinical assays such as immunohistochemistry, flow cytometry, or Q-RT-PCR.
GASTROINTESTINAL STROMAL TUMOR (GIST)

- Derived from the interstitial cells of Cajal
- Model for targeted therapy (KIT, PDGFRA)
- Not all GISTs carry these mutations.
• SUBSET WHICH LACKS THESE MUTATIONS; OFTEN PEDIATRIC & HEREDITARY.

• MAINLY GASTRIC.

• HEREDITARY CASES MAY BE ASSOCIATED WITH OTHER TUMORS (PARAGANGLIOMA) AND MAY CAUSED BY MUTATIONS IN SDH subunit genes.

• SOME CASES FIT “CARNEY TRIAD” (GIST, PARAGANGLIOMA, PULMONARY CHONDROMA).

• NCI PEDIATRIC GIST CLINIC ESTABLISHED TO STUDY THESE PATIENTS.
Miettinen et al. 2012
EPigenetic Landscape

Waddington 1954
DISTINCT METHYLATION PATTERN IN SDH DEFICIENT TUMORS

Killian et al. Cancer Discovery 2013
DISTINCT METHYLATION PATTERN IN SDH DEFICIENT TUMORS

Killian et al. *Cancer Discovery* 2013
INTERACTION BETWEEN SDH DEFICIENCY AND TET2 DEMETHYLATION

- SDH COMPLEX
- Inner Membrane
- Succinate
- Fumarate
- TET2
- 5-mC, 5-OHmC
- α-KG, Succinate
Genetic Features of SDHx mutant tumors:

- Deleterious mutation in an SDH subunit.
- Frequent LOH of second allele.
- Pervasive remodeling of the epigenome.
- Few copy number changes.
Most SDH deficient tumors are readily classified by DNA sequencing, but there remains a significant group with no molecular diagnosis.

**Question:** What is going on in those patients?

**Approach:** Differential DNA methylation and gene expression analyses of SDHx wt vs. SDHx mutant tumors.
Whole Genome Janus Plot
Differential DNA Methylation and Gene Expression
SDHwt vs. SDHx

Killian et al. 2014
SDHC EXPRESSION SILENCING IN SDHx-WT GIST TUMORS
INTEGRATION OF DNA SEQUENCE, DNA METHYLATION AND GENE EXPRESSION CONTRIBUTES TO A DEFINITIVE DIAGNOSIS IN THE MAJORITY OF SDH DEFICIENT GIST PATIENTS.

IN TUMORS WHICH LACK SDHx MUTATION, SDHC IS TYPICALLY INACTIVATED BY A HIGHLY SPECIFIC EPIMUTATION.

GIST PATIENTS WITH SDHC EPIMUTATION MAY BE SUSCEPTIBLE TO PARAGANGLIOMA AND PULMONARY CHONDROMA.
PROTEIN ALTERING MUTATIONS IN CANCER

SPECTRUM OF COMPLEXITY

0  5  10  20  >100  >1000 mutations

Heme. MTC  ER+ breast  TNBRCA  DNA REPAIR DEFECTS
           Ped. cancers  COLON  MUTAGEN EXPOSURES
SPECIFIC ALTERATIONS OF THE CANCER GENOME ARE TUMOR DRIVERS THAT POINT THE WAY TOWARD PRECISION THERAPY

• Chronic Myelogenous Leukemia

  Philadelphia chromosome (Nowell 1960)  
  Translocation joining ch9 and ch21 (Rowley 1973)  
  BCR-ABL fusion gene (Grosveld 1984)  
  Imatinib treatment (Druker 1996)

• Does CML define a paradigm for cancer cure?
  
  - Specific driver gene needed for cancer cell survival.
  
  - Therapeutic agent which can target that gene.
BIOLOGY AND OUTCOME

TUMOR BIOLOGY

OUTCOME

THERAPEUTIC INTERVENTION
BIOLOGY AND OUTCOME

TUMOR BIOLOGY

MEDICAL INTERVENTION

OUTCOME

MEDICAL INTERVENTION

OUTCOME
BIOLOGY AND OUTCOME

BCR-ABL → OUTCOME

ABL INHIBITOR

EGFR MUTATION → OUTCOME

EGFR INHIBITOR
DOES THE CML PARADIGM GENERALIZE TO OTHER CANCERS?

Successes:

• Acute Promyelocytic Leukemia with PML-RARA fusion
• Gastrointestinal Stromal Tumor with KIT or PDGFRA mutations
• Breast cancers with ERBB2 amplification
• Colon cancers with EGFR mutations
• Lung cancers with ALK fusions
• Melanoma with BRAF mutations

But:

• In most common adult cancers, CML-like results are not achievable with a single drug even when a “druggable” target gene is present.

• A large proportion of cancers do not have a gene which fits the CML paradigm.
DOES THE CML PARADIGM GENERALIZE TO OTHER CANCERS?

Successes:

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And:

- Many of the altered genes that have been successfully targeted in cancers where they occur frequently are also observed at low frequency in diverse cancers and/or rare cancers.

- Could known targetable mutations be useful as molecular markers to guide precision therapy out of the disease context in which they were originally validated?
INCORPORATING DNA SEQUENCING INTO CANCER DIAGNOSIS

• It is now practical to sequence DNA from small clinical samples quickly and accurately.

• Sequencing can be on any scale from whole genome to individual genes.

• Sequencing a panel of “driver” genes which could be targeted for therapy or which would aid definitive diagnosis is now clinically accessible.

• The broad utility of tumor DNA sequencing is currently a subject of ongoing clinical research, but sequencing is already standard-of-care in specific clinical situations.
LINEAR MODEL OF TUMOR PROGRESSION

Gene 1
Gene 2
Gene 3
Gene 4

classification
hyperplasia
premalignant
premalignant
invasiveCA
CANCER AS A BRANCHING CLONAL DISEASE

TUMOR GROWTH

RECURRENCE/METASTASIS
CANCER AS A CLONAL DISEASE

CLONAL HETEROGENEITY CAN BE QUANTITATED BY TUMOR GENOME SEQUENCING.

TUMOR GROWTH  |  RECURRENCE/METASTASIS
GENOMIC ONCOLOGY
EVALUATION AND LONGITUDINAL FOLLOW-UP

PRESENTATION:
Biopsy: T/N GENOME SEQ
RNA-Seq
Methyl-Seq

• CLASSIFICATION
• OUTLIER GENE EXPRESSION
• COPY NUMBER/SMALL MUTATIONS
• NEOANTIGEN PREDICTION
• TRANSLOCATIONS
• PATHWAY/TARGET PREDICTION
• CLONALITY ANALYSIS
• GERM LINE PHARMACOGENOMICS

RECURRENCE:
Biopsy: T/N GENOME SEQ
RNA-Seq
Methyl-Seq

• REPEAT ANALYSES AS AT DX
• RETARGET BASED ON ACTIVE CLONE(S)