BIO 302:
MARCH 4 & 6, 2014

WEEK 8 LECTURE 1:
CANCER AS A COMPLEX ADAPTIVE SYSTEM

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Cancer as a Complex Adaptive System (CAS)

- genotoxic insult(s), mutations and genome instability in cancer (drivers)
- the progressive evolution of phenotypic heterogeneity in different clones within tumors
- selection pressures on clonal fitness, adaptation and the evolution of the metastatic process
- tumor progression as a complex co-evolutionary interaction between dynamic changes in tumor behavior and host responses
Genotoxic Insult(s), Mutations and Genome Instability: The Drivers of Tumor Initiation and Progression
The Lengthy Timeframes for Progression in Different Human Cancers

- **NORMAL**
- **INITIATED**
- **MILD**
- **MODERATE**
- **SEVERE**
- **CIS**
- **CANCER**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Progression Stage</th>
<th>Timeframe</th>
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<tbody>
<tr>
<td>Colon</td>
<td>Adenoma</td>
<td>5–15 years</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>Dysplastic oral leukoplakia</td>
<td>6–8 years</td>
</tr>
<tr>
<td>Cervix</td>
<td>CIN 1</td>
<td>9–13 years</td>
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<tr>
<td>Cervix</td>
<td>CIN 3/CIS</td>
<td>10–20 years</td>
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<tr>
<td>Lung (smokers)</td>
<td>20–40 pack-years</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>Atypical hyperplasia</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>PIN</td>
<td>≥10 years</td>
</tr>
<tr>
<td>Breast</td>
<td>DCIS</td>
<td>6–10 years</td>
</tr>
<tr>
<td>Prostate</td>
<td>PIN</td>
<td>3–15 years</td>
</tr>
</tbody>
</table>

Figure 11.8a The Biology of Cancer (© Garland Science 2014)
Cancer Genomes: A Formidably Complex Catalog of Genomic Changes and Molecular Network Disruptions
Cancer Genes Identified in 4,742 Human Tumors

M. S. Lawrence et al. (2014) Nature 505, 495
Copy Number Alteration in 5135 Tumors from 14 Solid Tumor Types

From: G. Iyer et al. (2013) JCO 31, 3133
Genotoxic Insult(s), Accumulation of Mutations and Progressive Genome Instability

Figure 8.5a  The Biology of Cancer (© Garland Science 2014)
The Prevalence of Somatic Mutations Across Human Cancer Types

survey of 5 million mutations from over 7000 cancer cases from 30 different cancer types

highly variable mutation load
- 0.001 per DNA megabase (Mb) to 400 per Mb

childhood cancers carry fewest mutations

cancers related to chronic mutagenic exposure exhibit highest mutation burden
- lung cancer (tobacco expose), malignant melanoma (UV exposure)
The Shift from Cancer Diagnosis by Histopathology to Molecular Profiling of Perturbed Gene Networks and Altered Molecular Signaling Pathways

Different Tumor Subtypes Arise in the Same Cell Lineage via Different Genomic Perturbations and Alterations in Molecular Signaling Networks
The Evolution of the Classification of Non-Small Cell Lung Cancer (NSCLC)

From: T. Li et al. (2013) JCO 31, 1039
Emergence of Different Tumor Cell Clones and Subclones with Tumor Progression

continued accumulation of genomic alterations generates cancer cell clones and subclones with different genomic alterations and phenotypes (heterogeneity)
Emergence of New Clones With Different Phenotypes (Heterogeneity) During Tumor Progression

- chemical
- virus
- hormone
- nutrition

initiation/promotion

base state

tumor progression and clonal diversification

- driver mutations
- clonal diversification
- emergence of D^r and/or immune escape variants

Adapted from A. Barker and K. Buetow
Intratumor Genetic Heterogeneity in Multiple Regions of Primary Clear Cell Tumor and Three Metastases (Perinephric and Chest Wall) in RCC

From: M. Gerlinger et al. (2012) NEJM 366, 883
Intra-Lesional Variation in Somatic Gene Rearrangements in Three Treatment-Naïve Stage III/IV Epithelial Ovarian Cancer Patients

Adapted From: M. Hoogstraat et al. (2014) Genome Res. 24, 200
Clonal Heterogeneity in Cancer

- **intratumoral** (same patient)
  - ‘zonal’ heterogeneity in primary tumor
  - presence of different clones in separate metastases

- **inter-patient variation** (same subtype of tumor in different patients)
continued accumulation of genomic alterations and generation of clones and subclones with different genomic alterations and phenotypes

CLONAL HETEROGENEITY IN CANCER: THE LARGEST OBSTACLE TO SUCCESSFUL THERAPY
Tumor Cell Heterogeneity:
The Omnipresent and Greatest Challenge in Cancer Therapy
Tumor Cell Heterogeneity: The Omnipresent and Greatest Challenge in Cancer Therapy

More details Lecture Week II
Cancer as an Evolutionary Process
Clonal Selection (Fitness): Robustness, Adaption and Evolvability

- continuous acquisition of heritable genetic variation in individual tumor cells and generation of clonal progeny
- action of natural selection on the resulting phenotypes (selection and fitness)
- cumulative acquisition and selection of traits that confer autonomous unlimited replication, high survival and eventual progression to invasion and disseminated metastasis
The Complex Phenotypes for Cancer Cell Survival and Metastasis

Genome Instability in Cancer

- tumor initiation and progression via accumulation of mutations and other genomic alterations that confer selective growth advantage
- driver mutations
- passenger mutations
Making Sense of Cancer Genomic Data

- oncogenes
- suppressor genes
Cancer

Oncogene Activation
- overdrive of positive growth signals

Tumor Suppressor Gene Inactivation
- escape from growth control signals for natural tissue homeostasis
Cancer: Unchecked Proliferation

- "activating" (gain-of-function) mutations in proto-oncogenes
- freedom from the negative feedback that impose control on proliferation in normal tissues
- prototypic examples: RAS, PI3-kinase, MYC, RAF
Multiple Mechanisms for Activation of Oncogenes

(a) Point mutation
(b) Gene amplification
(c) Chromosomal translocation
(d) Local DNA rearrangements
(e) Insertional mutagenesis

Abnormal (hyperactive) protein
Excess normal protein
Translocated chromosome
Excess normal protein
Abnormal (hyperactive) protein
Proto-oncogene
DNA
Insertion
Deletion
Inversion or transposition
Viral DNA
Abnormal (hyperactive) proteins
Excess normal protein
Chromosomal Rearrangements and Gene Fusions in Cancer

- fusion of oncogenes with a second gene

Adapted From: T. H. Rabbitts et al. (2009) Cell 137, 391
Activation of Different Oncogenes in Cancers Arising in the Same Cell Type (Tumor Subtypes)
Heterogeneity of Driver Oncogenes in NSCLC

Frequencies of Molecular Alterations in CRC and Responsiveness to Cetuximab or Panitumumab

Two Classes of Tumor Suppressor Genes

**Gatekeepers**
- normally act to restrain cell proliferation
- loss of function mutations can lead to excessive proliferation/tumor formation
  - e.g. RB, NF2, TP53 and PTEN

**Caretakers**
- normally function in DNA repair and chromosome sorting
- loss of function mutations contribute to genomic instability
  - e.g. APC, BRCA1, BRCA2
Cancer Predisposition Genes

Retinoblastoma (RB)

Adenomatosis Polyposis Coli (APC)
BRCA 1 and 2 as Tumor Suppressor Genes: Different Mutations May Confer Different Risks

- substantially increased lifetime risks of breast and ovarian cancers but only small risk of increased pancreatic cancer
- loss-of-function mutations in central gene region confer higher risk of ovarian cancer versus breast cancer than mutations at gene end regions
TP53 mutation prevalence by tumor site

- Ovary
- Colorectum
- Head & neck
- Esophagus
- Lung
- Skin
- Pancreas
- Stomach
- Liver
- Bladder
- Brain
- Breast
- Uterus
- Soft tissues
- Lymph nodes
- Prostate
- Endocrine glands
- Bones
- Kidney
- Hematop. system
- Cervix

% of tumors with p53 mutation

Figure 9.4 The Biology of Cancer (© Garland Science 2014)
Making Sense of Cancer Genomic Data

**driver mutations**
- oncogenes
- suppressor genes

**passenger mutations**
- changes in genome and gene regulatory networks that are not causal for cancer initiation and progression
- accumulate with increasing genome instability as tumor progresses
- may still contribute to adaptive survival advantage
  - drug efflux transporters
How Does Genome Instability Generate the Complex Behavior (Phenotypes) of Tumor Cells?

Immunoevasion, Metastasis and Drug Resistance as the Three Most Clinically Relevant (and Dangerous) Phenotypes in Cancer Cell Behavior

Understanding the Altered Molecular Signaling Networks that Drive These Aspects of Tumor Behavior and as Targets for Diagnostics (Dx) and Therapeutics (Rx)
Defining Disease Mechanisms at the Molecular Level

Mapping the Disruption of Molecular Signaling Networks

Profiling Altered Patterns of Information Flow in Signaling Pathways and Understanding Their Contribution to Disease Requires A Systems-Based Approach
The Overly Simplistic and Deterministic Dangers of a Genome-Sequence Centric Perspective

Genes For ....

The Over-Simplified Perspective That Whole Exome-and Whole Genome-Sequencing Will Reveal the Full Etiology of Disease Pathogenesis and Transform Treatment Options
Profiling Changes in Biological Signaling Networks in Cancer: Understanding Cancer Requires a Holistic “Systems” Approach

- Genome sequence data alone will not provide a sufficiently complete picture for either Dx or Rx decisions
  - Need to understand cancer as a complex multi-component process

- Mapping disruption in signaling pathways requires profiling of multiple aspects of both genotypic and phenotypic changes
Elucidation of Disease Mechanisms Will Require More than Genome Sequence Information: The Need for Comprehensive Knowledge of Disruption of Gene Regulatory Networks

- mutations
- amplifications, deletions, copy number variation
- chromosomal translocations
- aneuploidy

- epigenetic modifications

- the non-coding transcriptome and gene regulation
  - the increasing complexity of regulation of gene expression by diverse RNA species
The Epigenome: Reading The Second Genomic Code

- chromatin structure and access to DNA
- DNA methylation
Epigenome

**the epigenome**

- Dynamic modulation of gene expression without change in the primary sequence of nucleotide bases
- Regulation of gene expression (activation, silencing) by modification of chromatin structure and histones
- Dynamic changes in cytosine methylation

**the epitranscriptome**

- Dynamic and reversible chemical modification of RNA
  - e.g. N6-methyladenosine
Epigenetic Gene Silencing

Epigenetic Gene Activation

green line = expressed; red line = silent; * = DNA methylation
Figure 7.18 The Biology of Cancer (© Garland Science 2014)
Growing Recognition of the Complexity of the RNA Universe in Regulating Gene Expression

- pervasive transcription of >90% of the genome with less than 2% encoding protein-coding genes
- human transcriptome
  - 21,000 protein-coding genes
  - 9,000 small RNAs
  - 10,000-32,000 long non-coding RNAs (lncRNAs)
  - 11,000 pseudogenes
- extensive crosstalk and interactions between different RNA species
Post-Transcriptional Regulation of Gene Expression

gene transcript(s) → messenger RNA

- block by miRNAs
  - competitive capture of miRNAs (RNA sponges)
  - by IncRNAs, competitive endogenous (ceRNAs) and circular RNAs (circRNAs)

- ribosome

control of protein expression levels
Individual Variation, Genome Complexity and the Challenge of Genotype-Phenotype Predictions

Junk No More: Pervasive Transcription
- alternate transcription
- (co)splicing
- SNPs, CNVs
- pseudogenes
- indels, SVs
- ncRNAs
- phasing
- epistasis
- imprinting
- silencing

recognition of the complexity of genome organization and regulation

Cell-specific Molecular Interaction Networks

Perturbed Networks and Disease
The Multiplicity of Genomic, Transcriptomic, Epigenomic and Signaling Pathway Alterations in AML Illustrate Why Genome Sequencing Alone is Only One Part of the Molecular Profile Needed for Guiding Diagnosis and Therapy

From: S-J. Chen et al. (2013) Nature Genetics 45, 586
GBM Expression Subtypes and TF and miRNA-TF Regulatory Networks


From: M. S. Carro et al. (2010) Nature 463, 318

From: G. Wu et al. (2010) Genome Biology 11:R53 pg. 10

From: M. S. Carro et al. (2010) Nature 463, 318

The Complex Ecology of Tumor Progression and Metastasis

The Tumor Microenvironment: Molecular Signaling Between Cancer Cells, Immune Cells and Tissue Components
The Complex Ecology of Malignant Neoplasms

- multiple cell types and inter-cellular molecular communication activities in the tumor microenvironment(s)
- tumor cell-tumor cell interactions
- tumor cell-host stroma interactions
- tumor cell-host defense interactions
- tumor cell-host interactions in different body organ environments for successful metastasis
- effect of treatment(s) on tumor cells, host defense systems and the host microbiome
The Complex Microenvironment of Neoplasms

From L. J. Kleinsmith, *Principles of Cancer Biology*. Copyright (c) 2006 Pearson Benjamin Cummings.
Reprogramming Energy Metabolism in Cancer Cells
The Warburg Effect

- pioneering work of Otto Warburg (1927)
- normal dominance of oxidative metabolism under aerobic conditions (Krebs cycle)
- normal cells shift to glycolysis under anaerobic conditions
  - redirect pyruvate away from mitochondrial oxidation to reduce pyruvate to lactate
- tumor cells metabolize glucose to lactate in both aerobic and anaerobic environments
  - support survival and proliferation in microenvironments with limited oxygenation (hypoxia) due to impaired vascular supply
• possible role of reactive oxygen species (ROS) released by tumor cells in causing tumor-associated host fibroblasts to switch to aerobic glycolysis
  – increased production of lactate and pyruvate as metabolites to fuel cancer cells
• similar reprogramming of adipocytes in fat tissue
  – production of free fatty acids (FFA) as tumor nutrients
insufficient blood supply, hypoxia, increased acidity and impaired nutrient supply in growing tumors trigger necrosis and release of pro-inflammatory signals

- stimulus to recruit host immune cells who then release further pro-proliferation signals exploited by tumor cells to promote survival and progression
The Tumor Microenvironment Hijacking: Host Responses by Tumor Cells to Promote Proliferation and Progression

- Tumors secrete growth factors and chemoattractants for host inflammatory and immune cells
- Pro-inflammatory cytokines released by these host cells promote tumor progression
  - Suppress apoptosis
  - Promote cell cycle progression/cell replication
  - Angiogenesis
  - Induce epithelial-mesenchymal transition (EMT) markers
The Tumor Microenvironment

From: Ray-Coquard Future Oncol. (2013) 9, 12 Suppl. 1, 11
Tumor-Mediated Suppression of Host Anti-Cancer Immune Responses

- Tumors recruit T regulatory cells (Treg) and myeloid-derived suppressor cells (MDSC).
- These host immune cells suppress the ability of cytotoxic killer T cells to detect and destroy tumor cells.

More Details in Lecture Week II
Invasion and Metastasis: The Start of the Deadly Phase of Cancer Progression

basal cell carcinoma

Invasion Without Metastasis

lung

colorectal

breast

prostate

Invasion and Metastasis

glioblastoma
Macrophages and the Tumor Microenvironment

- tumor-associated macrophages (TAM)
- switch of macrophages from M1 to M2 phenotype in normal inflammation and then switch back
- in tumors and obesity macrophages remain in M2 state
- role of TAM in early steps in metastasis (intravasation)
A Perverse Molecular Conversation: Tumor Cell Signals That Stimulate Host Macrophages to Promote Tumor Growth and Spread

1. Cancer cell releases microvesicles that contain miRNA-21 and miR-29.

2. Macrophages take up the microvesicles releasing miR-21 and miR-29 into the cytoplasm where they are taken up by endosomes.

3. In the endosome, miR-21 and miR-29 bind with toll like receptors, causing the macrophage to release interleukin 6 (IL-6) and tumor necrosis factor (TNF) alpha.

4. Cancer cells take up IL-6/TNFalpha, facilitating tumor invasion and metastasis.

Adapted from Frontiers: OHSU James CCC Winter 2014
Entry of Metastatic Cancer Cells Into The Circulation (Intravasation) and Promotion by Tumour-Associated Macrophages

Tumor Cell-Platelet Interactions in Promoting Dissemination and Extravasation

- protect against lysis by NK cells; increase adhesion to endothelial cells; recruitment of WBC that release pro-inflammatory cytokines

The Plasticity of Epithelial Cancer Cells: Switching (Transition) Between Epithelial and Mesenchymal Phenotypes

Epithelial: Mesenchymal Transition (EMT) and Mesenchymal: Epithelial Transition (MET) as Core Features of Metastatic Spread and Tumor Dormancy
Formation of the Germ Layers in Embryogenesis: Endoderm, Mesoderm and Ectoderm
Migration of Neural Crest Cells and Formation of Different Cell Types in Multiple Body Locations
Epithelial (E)- Mesenchymal (M) Transition (EMT)

- Acquisition of M-like properties by E cells
- Physiological processes
  - Migration of mesodermal cells during embryonic gastrulation
  - Delamination of neural crest cells from dorsal neural tube
  - Migration to multiple locations and subsequent differentiation into diverse cell lineages
- Cancer
  - Proposed crucial role in invasion and metastasis of epithelial malignancies
  - Potential for repeated cycles of EMT and reversal (MET)
The Epithelial-mesenchymal Transition in the Invasion and Metastasis of Malignant Epithelial Cancers

1. EMT signals at tumor margin
2. Partial EMT state facilitates motility and invasion into stroma
3. Mesenchymal phenotype facilitates intravasation and anoikis resistance during dissemination and extravasation
4. Migrating cancer stem cell
5. Survival and dormancy at distant site
6. Exit from dormancy and early colonization
7. Colonization: proliferation and formation of macrometastasis

Principal Patterns of Metastatic Dissemination in the Major Epithelial Cancers

Figure 14.43 The Biology of Cancer (© Garland Science 2014)
“When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil… While many researchers have been studying ‘the seeds,’ the properties of ‘the soils’ may reveal valuable insights into the metastatic peculiarities of cancer cases.”

Stephen´ Paget 1889
Cancer Metastasis: Key Questions

- what determines the different anatomic distribution of solid tumor metastases?
- is every cancer cell in a malignant tumor capable of causing metastases?
- is metastatic capability a property of only certain cell subpopulations/clones present in the primary tumor?
- do cells in metastases continue to spread (secondary metastases)?
- are future sites of metastatic colonization ‘primed’ by host cells to promote conditions for metastatic cell survival?
Tumor Dormancy Versus Metastatic Progression

Summary and Key Points
Cancer

determinants of clonal fitness: robustness, adaptability, evolvability

emergence of clones with diverse genotypes and phenotypes with tumor progression and metastasis

genotoxic insult(s), genome instability and dysregulation of molecular signaling networks in different cell types
Cancer determinants of clonal fitness: robustness, adaptability, evolvability

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Dynamics of Host: Tumor Co-evolution and Rx-Effects

Intra-and Inter-patient Variation Within Same Tumor Subtype

Tumor Subtypes in the Same Cell Type

SELECTION

HETEROGENEITY

DRIVERS
Cancer

Sustained Tumor Growth, Progression and Metastasis and Resistance to Treatment

determinants of clonal fitness: robustness, adaptability, evolvability

emergence of clones with diverse genotypes and phenotypes with tumor progression and metastasis

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Dynamics of Host:Tumor Co-evolution and Rx-Effects

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Tumor Subtypes in the Same Cell Type

SELECTION

HETEROGENEITY

DRIVERS
The ‘Fitness’ of the Metastatic Clone: “The Decathlon Phenotype”

- survival in the primary tumor
- invasion of surrounding tissue
- epithelial-mesenchymal transition
- intravasation
- survival in the circulation
- extravasation
- colonization of new metastatic site
- mesenchymal-epithelial transition
- evasion of destruction by host immune defenses
- resist therapeutic assaults
Determinants of clonal fitness: robustness, adaptability, evolvability.

Sustained Tumor Growth, Progression and Metastasis and Resistance to Treatment

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Cancer: A Complex Adaptive System

Heterogeneity

Drivers
Cancer as a Complex Adaptive System (CAS)

Lecture 2

- mapping the dynamics of the evolution of clones with genotypic and phenotypic differences (heterogeneity) during progressive tumor growth and metastatic spread

- how knowledge of the co-evolution of tumor behavior and host responses is essential to design improved diagnostic and therapeutic approaches in cancer