Tumor cell heterogeneity: cellular differences within a single neoplasm

Tumor heterogeneity: Causes and consequences

- Monoclonality of tumors
- Heterogeneity of tumors
- Experimental and theoretical evidence for clonal diversity in tumor progression
- Cancer models: clonal evolution, stochastic, cancer stem cell hierarchy, or combined
Knowing the nature of cancer from our 200 years battle with cancer

- **En bloc resection** (19th and early 20th centuries): a minority of patients could be cured by surgical removal of their tumors alone. too much for small tumors and too little for large tumors that had already metastasized.

- **Radiation therapy** (began in 1950): Only about a third of all cancers could be cured by the use of surgery and radiation, alone or together.

- Proof of cure by **chemotherapy** on childhood leukemia and advanced Hodgkin’s disease in adults (mid-1960). By 1991, the rate of death from breast cancer began to fall, a trend that has continued from availability of multiple effective chemotherapeutic agents and hormone treatments, improved diagnostic tools for early diagnosis, and intelligently designed clinical trials.


- **Immunotherapy**: therapeutic antibody to inhibit growth factor receptors on the surface of cancer cells, rituximab for the treatment of B-cell lymphomas in 1997, bevacizumab for blocking blood vessel supplies of cancer growth. **Immunomodulatory agents** (Interleukin-2) caused regression of invasive metastatic disease (1985)

Monoclonality of tumors

- Starch gel electrophoresis assay on G6PD showed that all of the cancer cells in a tumor arising in a G6PD heterozygous patient express either one or the other form of this enzyme (Fialkow, N. Engl. J. Med. 291:26–35, 1974.)

- Analysis of immunoglobulin showed replacement of heterogeneous immunoglobulin populations by a single antibody species (termed an M-spike) in multiple myeloma, indicating arising from a single clonal population of antibody-secreting tumor cells. (“The Biology of Cancer”, Robert Weinberg)
Heterogeneity of tumors

- Intra-tumor phenotypic heterogeneity (Cellular morphology, gene expression (including the expression of cell surface markers and growth factor and hormonal receptors), cellular metabolism, motility, and angiogenic, proliferative, immunogenic, and metastatic potential)
- Genetic heterogeneity (the number of genes and chromosomes and the structure and epigenetic (methylation)

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Non-heritable sources of diversity in tumor cell populations

- **Cancer stem cells**

  These cells are able to self-renew and to give rise to phenotypically diverse nontumorigenic daughter cells with limited division properties that can differentiate and that compose the bulk of the tumor.

References:


Non-heritable sources of diversity in tumor cell populations

- Phenotypic plasticity

**Epithelial-mesenchymal transition (EMT)**

**References:**


**Endothelial transdifferentiation**

**References:**


Heritable heterogeneity

- Intra-tumor clonal diversity: prognostic factor of tumor progression
- Chromosome instability: the sources of clonal heterogeneity
- The biological impact of clonal heterogeneity to cancer therapy
Genetic clonal diversity predicts progression to esophageal adenocarcinoma

A, diploid, **LOH in TP53 and D9S1121**
B, aneuploid (3.7N), **LOH in TP53 and D9S1121**
C, tetraploid, **LOH in TP53 and D9S1121**
D, diploid, **LOH in TP53, D9S925, D9S1121 and D9S1118**
E, diploid, **LOH in TP53**
F, diploid with no LOH).
G, diploid, LOH at D9S2169 and D9S1121
H, diploid, LOH at D9S2169, D9S935 and D9S1118
I, diploid, LOH at D9S2169, D9S935, D9S925 and D9S1118; J, diploid with no LOH).
K, diploid, LOH at D9S935, D9S925 and D9S1118
L, diploid, **LOH in TP53**
M, diploid, LOH in all markers on chromosome 9p
K and L differ by two LOH events in four informative loci, resulting in a divergence of 0.27.
Accumulation of viable, clonal genetic variants is a greater risk factor for progressing to cancer than a recent homogenizing clonal expansion.
Transgenic oncogenes induce oncogene-independent cancers with individual karyotypes and phenotypes


Analyzed the karyotypes and phenotypes of mammary carcinomas of mice with transgenic SV40 tumor virus- and hepatitis B virus-derived oncogenes.

- (1) a given transgene induced diverse carcinomas with individual karyotypes and phenotypes
- (2) these karyotypes coevolved with newly acquired phenotypes such as drug resistance
- (3) 8 of 12 carcinomas were transgene negative.

Activated oncogenes destabilize karyotypes and are dispensable in cancers, function indirectly, like carcinogens.

Carcinogenesis is a form of speciation and that individual karyotypes maintain cancers as they maintain species.
Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells

Benefit of tumor from heterogeneity

- **Synergize tumor growth**

Benefit of tumor from heterogeneity

- **Genetic clonal diversity predicts progression to esophageal adenocarcinoma**

  Cancers can be viewed from an ecological perspective that focuses on interactions of organisms with their environment and among each other. When applying an ecological perspective to human cancers, subclonal populations of tumor cells that differ in heritable traits are considered distinct “species,” whereas infiltrating normal cells, extracellular matrix, vessels, etc., are considered the environment.

- **Chromosomal instability, a source of clonal heterogeneity, confers intrinsic multidrug resistance**


Hsp90 stress potentiates rapid cellular adaptation through induction of aneuploidy

CIN: source of drug resistance of cancer cells


Facts about cytogenetic chaos in carcinomas

1) Carcinomas are defined by a non-random distribution of chromosomal gains and losses;
2) The distribution of these imbalances is tumor specific;
3) Tumor specific chromosomal gains and losses occur before the transition to invasive disease;
4) These imbalances are not present in normal cells;
5) Such imbalances often comprise entire chromosome arms or chromosomes;
6) Aberrations emerging early in disease progression are usually maintained at advanced stages of the disease, in metastases, and in tumor-derived cell lines;
7) Specific aneuploidies are the basis for the clonal evolution and expansion of precancerous lesions;
8) The majority of structural aberrations result in genomic copy number changes, i.e., balanced translocations, the hallmark of hematological malignancies, are rare

non-random distribution of chromosomal gains and losses

Fig. 1. Distribution of chromosomal gains and losses in cervical and colorectal carcinomas. The distribution of genomic imbalances is tumor specific. The results are normalized to n = 10. Note that essentially all cervical carcinomas carry a gain of chromosome arm 3q, and colorectal carcinomas are defined by a recurrent gain of chromosomes 7, 8q, 13, and 20q, and losses of chromosomes 8p, 17p, and 18.
Fig. 2. Composite of CGH profiles from 22 GBM cases. Each line depicts the chromosomal region lost (red) and gained (green) in a single case.

Box 1 | The mitotic checkpoint: a safeguard to protect against aneuploidy
Aneuploidy: hallmark of Cancer

Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer
http://cgap.nci.nih.gov/Chromosomes/Mitelman

Aneuploidy

Cellular genetic heterogeneity

Maintenance tumor heterogeneity with an equilibrium optimal for tumor growth

- Complex interplay of various types of cells.
- Mutations in landscaper genes, alterations promoting angiogenesis and mutations enabling the cell to fight off the immune system.
Cancer initiation and progression

Chromosome instability (CIN)

Targeting the cause or consequences of aneuploidy therapeutically.
Cancer evolution theories/models
Clonal evolution / stochastic model

Alternative cancer models

CANCER CLONE DIVERSITY AND PROPAGATION

- chromosomal instability

Sustains cancer
Self-renews
Target for therapy

MODELS

• Stem cell
  (fixed; developmentally hierarchical)
  or
• Stochastic
  (random, variable)
  or
• Dominant sub-clone
  (dynamic, sub-clone genetic advantage and selection)
Schematic view of monoclonal and multiclonal models of tumor progression
Evolution in a hypothetical case of Barrett’s esophagus
An ecosystem describes the physical and biological components of an environment in relation to each other as a unit.


Concepts in tumor evolution

- **Cellular genetic heterogeneity**: Genetic instability will lead to clonal diversity only if the genetic variants are viable and can expand into detectable clones.

- **Clonal genetic heterogeneity**: e.g. Increased Shannon diversity but lack of increased divergence in TP53 LOH epithelium suggests that TP53 LOH increases generation of viable genetic variants that may derive from a recent common ancestor, hence TP53 LOH epithelium is clonal. Clonal diversity predicts progression to cancer, although whether cellular diversity predicts progression remains an open question.
Mathematical models describe the process of cancer initiation and progression

- Cancer is principally caused by mutations in cancer-susceptibility genes, which include oncogenes, tumor-suppressor genes (TSGs) and genes causing genetic instability.
- Cancer arises when a single cellular lineage receives multiple mutations.
- Small lesions without genetic instability can take a very long time to inactivate the next TSG, whereas the same lesions with genetic instability pose a much greater risk for cancer progression.
- Knudson’s two-hit hypothesis is compatible with the idea that one mutation occurs in the first allele of the TSG and one mutation occurs in a CIN gene. The mutation inactivating the second TSG allele is not rate-limiting in a CIN cell.
- Because of the tremendous acceleration of loss of heterozygosity in CIN cells, it is very likely that most cancers, which require inactivation of at least two TSGs in rate-limiting scenarios, are initiated by CIN mutations, even if CIN has a severe cost in terms of somatic fitness.

Classes of CIN

- Class 1 — trigger CIN if one allele of the gene is lost (haploinsufficiency): MAD2, STAG2
- Class 2 — trigger CIN if one allele is mutated in a dominant (negative) fashion: BUB1
- Class 3 — require mutations in both alleles to trigger CIN (recessive at the cellular level): BRCA1
The role of aneuploidy in promoting and suppressing tumors
Aneuploid can cause changes of over- and under expressing thousands of normal genes

Potential mechanisms to generate aneuploid cells

Aneuploidy: cause or consequence of cancer?

- Most cancer cells are aneuploid (Fact)
- Chr8 duplication cause APL (Fact)
- Transgenic oncogenes initiate carcinogenesis by inducing aneuploidy (Fact)
- Cancers are clones of autonomous cells defined by individual karyotypes, much like species (Theory)
- Despite such karyotypic evidence for causality, three to six synergistic mutations, termed oncogenes, are generally thought to cause cancer (Theory)
Enhancing CIN to kill tumor cells

- Elevating the frequency of chromosome mis-segregation sensitized cancer cell to low doses of taxol (Janssen et al. *Proc Natl Acad Sci U S A.* 2009;106(45):19108-19113.)

- Anaphase catastrophe is a target for cancer therapy: pharmacologic inhibition of cyclin dependent kinase 2 (Cdk2) combined with a microtubule inhibitor to kills tumor cells with more than 2 centrosomes (Galimberti et al. *Clin Cancer Res.* 2011;17(6):1218-1222.)

- Inducer of cancer cell polyploidy (Tovar et al. *Cell Cycle.* 2010;9(16):3364-75.)
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<thead>
<tr>
<th>Year</th>
<th>Discovery or Event</th>
<th>Relative Survival Rate</th>
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<tbody>
<tr>
<td>1863</td>
<td>Cellular origin of cancer (Virchow)</td>
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<td>1889</td>
<td>Seed-and-soil hypothesis (Paget)</td>
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<td>1914</td>
<td>Chromosomal mutations in cancer (Boveri)</td>
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<td>1937</td>
<td>Founding of NCI</td>
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<td>1944</td>
<td>Transmission of cellular information by DNA (Avery)</td>
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<td>1950</td>
<td>Availability of cancer drugs through Cancer Chemotherapy National Service Center</td>
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<td>1953</td>
<td>Report on structure of DNA</td>
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<td>1975</td>
<td>Hybridomas and monoclonal antibodies</td>
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<td>Cellular origin of retroviral oncogenes</td>
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<td>2005</td>
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<td>2006</td>
<td>Tumor stromal interaction</td>
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The mechanisms of cancer in maintaining tumor heterogeneity

- autonomous
- environmental
- system
Student lab presentations

The roads to aneuploidy
Time: 1/22/13

Centrosome aberrations
Presenter 1:

Cause from environmental stress
Presenter 2:

Suggested references

Control of chromosomal instability
Time: 1/24/12

Genes in genome guidance, mis-match repair
Presenter 3:

Genes in kinetochore-microtubule dynamics
Presenter 4:
Presenter 5:

Suggested references
2 student lab presentations—Thursday

Tetraploidy, aneuploidy, and senescence, CIN-based therapy

Time: 1/29/13

Presenter 6:
Presenter 7:
Presenter 8:

Suggested references


Cancer cell plasticity and tumor microenvironment

Time: 1/31/13

Epithelial-mesenchymal transition (EMT)

Presenter 9:

Endothelial transdifferentiation

Presenter 10: