Stem Cells and Cancer

CELLULAR & MOLECULAR BIOLOGY OF CANCER - PATH G4500-001

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Structure of the Class

1. the concept of “stem cells”
2. the concept of “cancer stem cells”
3. biological implications of the “cancer stem cell” model
4. clinical implications of the “cancer stem cell” model
Historical origins of the word “stem cell” [#1]

Ernst Haeckel is famous for having introduced the concept: “Ontogenesis recapitulates phylogenesis”

The early stages of embryonic development are very similar across species

Ernst Haeckel, *Anthropogenie*, 1874

Images from: the *Wellcome Library, London*
Historically, Ernst Haeckel first used the word “stem cell” to identify an ancestor/progenitor cell that stands at the “stem” of a genealogic tree, used to depict either evolutionary (i.e. phylogenetic) or developmental (i.e. ontogenetic) processes.

Phylogenetic tree of life
(i.e. evolution of life on Earth)

Ontogenetic tree of life
(i.e. embryonic development)
Current use of the word “stem cell”

The meaning remains essentially intact today, where it is mainly used in developmental biology, to identify a cell capable to generate and sustain over time a specific set of diversified cell populations whose aggregate interaction leads to the formation of either:

a) an entire living organism:
   - the zygote (totipotent capacity)
   - embryonic stem cells (pluripotent capacity)

b) a specific subset of its organs and tissues:
   - adult stem cells (oligopotent or multipotent capacity)
Many tissues have a “hierarchical” organization and undergo a continuous process of cell turnover.

- **long-term self-renewing stem cells**
- **short-term multipotent progenitors**
- **oligolineage precursors**
- **mature cells**

Specialized cell types often short-lived undergo turnover during lifetime (with variable kinetics)
Stem Cell Properties

Stem cells are defined by three key properties:

1) The ability to give rise to a functionally heterogeneous progeny of cells, which progressively specialize according to a hierarchical process (differentiation);

2) The ability to form new stem cells with identical, intact differentiation potential, thus maintaining the stem cell pool (self-renewal);

3) The ability to modulate the two previous properties according to environmental stimuli and genetic constraints (homeostatic control).
Examples of normal tissues that are sustained by adult stem cell populations [#1].

Hematopoietic system

Examples of normal tissues that are sustained by adult stem cell populations [#2].

Intestinal epithelium

Tumors are histological “caricatures” of corresponding normal tissues

Dalerba et al., Nature Biotechnology, 29:1120-1127, 2011
Tumors are histological “caricatures” of their corresponding normal tissues.

Figure 7. Rectal Cancer [...] Great similarity with the normal mucosa. Goblet cells.

don Hansemann, Die mikroskopische Diagnose der bösertigen Geschwülste [The microscopic diagnosis of malignant tumors] (1897)
Tumor tissues act as “histological caricatures” of their normal counterparts.

The three-dimensional architecture and cell composition of neoplastic tissues usually mirrors that of the corresponding normal tissues of origin, and includes a variety of cell types.
Key points [#1]

• cancer tissues are frequently heterogeneous in cell composition; this diversity often mirrors the repertoire of specialized cell types found in normal counterparts;

• thus, in many instances, cancer tissues are not simply amorphous collections of transformed cells, but complex three-dimensional structures that retain architectural features of their parent tissues;

amorphous tumor mass

vs.

architecturally complex tumor mass
The “Cancer Stem Cell” hypothesis

If tumor tissues are “caricatures” of normal ones (and retain many of their architectural features), then do they also contain a pathological (e.g. mutated) stem cell population that sustains their long-term growth?

In other words, are malignant tissues the product of pathological stem cells?

If this hypothesis is correct, what are its biological implications and how can we test it?
Biological implications of the “Cancer Stem Cell” hypothesis

1) Origin of cell heterogeneity in cancer tissues
   Is the cellular diversity observed within tumor tissues generated only by genetic mechanisms (e.g. random mutations) or also by epigenetic mechanisms (e.g. multi-lineage differentiation)?
   **genetic vs. epigenetic diversity**

2) Functional hierarchy in cancer cell populations
   Are the different types of cancer cells found within malignant tissues also different in their capacity to sustain long-term tissue expansion and proliferation?
   **tumorigenic vs. non-tumorigenic populations**
Sources of cell heterogeneity in cancer tissues [#1]

“stochastic” model (random mutation)

clone #1

clone #2

clone #3

differences between various types of cancer cells are caused by differences in the repertoire of genetic mutations (i.e. co-existence of different genetic sub-clones);

the heterogeneity is genetic

“cancer stem cell” model (multi-lineage differentiation)

lineage cell type #1

lineage cell type #2

lineage cell type #3

differences between various types of cancer cells are caused by differential activation of specialized gene-expression programs that “enforce” specific cell identities (i.e. differentiation);

the heterogeneity is epigenetic
In women, each cell randomly “inactivates” one of the two X chromosomes.

In women, adult tissues are mosaics with regard to X chromosome expression.

What about tumor tissues in women?

Strayer and Rubin – Chapter 5 “Neoplasia”.

Monoclonal origin of human cancer

Stanley M. Gartler (1923 - present)

Professor Emeritus: University of Washington
Post-doctoral fellow: Columbia University

Indirect evidence of multi-lineage differentiation in human hematological malignancies

CML (chronic myelogenous leukemia)

- Long-term self-renewing stem cells
- Short-term multipotent progenitors
- Oligolineage precursors
- Mature cells

Monoclonal expansions of either A or B G6PD alleles (in female patients)


Philip Fialkow (1934-1996)

Image: University of Washington
IMPORTANT: the two models are not mutually exclusive, the two mechanisms co-exist and act together to generate cell diversity... but with different functional implications!
Implications of different sources of cell diversity

"stochastic" model
(random mutation)

clone #1

clone #2

clone #3

The different types of cancer cells (i.e. the different genetic clones within the tumor mass) are all endowed with long-term growth and proliferation capacity. Each of them can expand as a distinct monoclonal population.

"cancer stem cell" model
(multi-lineage differentiation)

Only a subset of cancer cells, the "cancer stem cells" (CSC), is endowed with long-term growth capacity, while other tumor cell types have limited proliferative capacity. Tissues originated from CSC contain multiple cell types as a result of multi-lineage differentiation.
Experimental approach to test the “cancer stem cell” model

Primary tumor specimen → Dis-aggregation into single-cell suspension → Staining with monoclonal antibodies → Visualization of different cell types by flow cytometry

Evaluation of differential tumorigenic properties by injection in NOD-SCID mice + Evaluation of the ability to recreate the phenotypic heterogeneity of parent tumors
Experimental validation of the “cancer stem cell” model

Colon Cancer
Selection of **surface markers** for differential isolation by flow cytometry of **mature/immature cell types** in the human normal colon epithelium.
Sorting and injection strategy for differential tumorigenicity experiments based on EpCAM/CD44 in human Colon Cancer.

Dalerba et al., P.N.A.S., 104:10158-10163 (2007)
Reconstitution of parental EpCAM/CD44 expression profile in tumors grown from sorted EpCAM\textsuperscript{high}/CD44\textsuperscript{+} cells.

Dalerba et al., PNAS, 104:10158-10163 (2007)
Morphology and differentiation of human CRC tumors grown from EpCAM\textsuperscript{high}/CD44\textsuperscript{+} cells.

Dalerba et al., PNAS, 104:10158-10163 (2007)
Limitations of classic transplantation experiments based on flow cytometry [#1]

Due to obvious ethical reasons, transplantation experiments using human cells are performed in xenogeneic hosts (i.e. immuno-deficient mice).

Are differences in the tumorigenic capacity of different tumor cell types due to an incompatibility of specific cell types with the mouse microenvironment?
Mouse models of epithelial “Cancer Stem Cells”

Breast Cancer (MMTV-Wnt-1)
*Cho et al., Stem Cells, 26:364-371 (2008)*

Skin Cancer (squamous cell carcinoma)

DMBA/TPA-induced Skin Carcinoma

- **CD34^{neg}, KRT10^{+}**: non-tumorigenic
- **CD34^{+}, KRT10^{neg}**: tumorigenic

Limitations of classic transplantation experiments based on flow cytometry [#2]

Most experiments are performed using high numbers of purified cells ($10^2$-$10^3$ cells/dose).

How can we be absolutely certain that, indeed, the various cell types found in secondary tumors arise from the multi-lineage differentiation of a single cell, and not from the parallel expansion of multiple genetic clones?
Lineage tracing experiments on “Cancer Stem Cells”

Analysis of a well-differentiated human colon cancer tissue reveals the presence of multiple epithelial cell types.

Subcutaneous injection into a NOD/SCID/IL2RY+/- mouse and generation of a monoclonal EGFP+ colon cancer tissue.

Analysis of monoclonal EGFP+ colon cancer xenograft tissues reveals the presence of multiple epithelial cell types, closely recapitulating the full cellular diversity of the parent tissue.

Monoclonality of cancer tissues is verified by confirmation of a unique lentivirus integration site.

Isolation of a single (n= 1) EGFP+ human colon CSC (EpCAMhigh/CD44+).

Infection of cancer cells with a lentivirus encoding EGFP.

Dissociation into a single-cell suspension.
Generation and characterization of a monoclonal colon cancer xenograft, from a single (n = 1) “cancer stem cell”

<table>
<thead>
<tr>
<th>Xenograft line</th>
<th>10,000</th>
<th>5,000</th>
<th>1,000</th>
<th>500</th>
<th>100</th>
<th>50</th>
<th>10</th>
<th>5</th>
<th>1</th>
<th>frequency (95% CE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM-COLON#4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/62 (1/44 – 1/89)</td>
</tr>
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</table>

UM-COLON#4 clone 8
Lin⁻ phenotypic populations
Tested for tumorigenicity

<table>
<thead>
<tr>
<th>Cell dose and tumor formation in NOD/SCID/IL2Rγ⁻ mice (EpCAM+/CD44+)</th>
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<tr>
<td>Xenograft line</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>UM-COLON#4</td>
</tr>
<tr>
<td>UM-COLON#8</td>
</tr>
</tbody>
</table>

Key points [#2]

- the cellular diversity of cancer tissues frequently recapitulates the physiological lineage composition of their normal counterparts;

- the cellular diversity of cancer tissues is frequently epigenetic in origin, and arises as the result of a multi-lineage differentiation process, reminiscent of a stem cell hierarchical system;

- often, within cancer tissues, only a specific subset of cancer cells is endowed with the capacity to initiate the growth of a secondary tumor when transplanted; tumors originated from these cells, however, recapitulate the repertoire of heterogeneous cells types found in the parent tissues; these cells are defined “cancer stem cells”;
Integrated view of cell heterogeneity in cancer tissues

“stochastic” model (random mutation)
- Genetic sub-clone #1
- Genetic sub-clone #2
- Genetic sub-clone #3

“cancer stem cell” model (multi-lineage differentiation)
- Lineage cell type #1
- Lineage cell type #2
- Lineage cell type #3

Integrated model
- Sub-clone #1 (undergoing multi-lineage epigenetic differentiation)
- Sub-clone #2 (undergoing multi-lineage epigenetic differentiation)
- Sub-clone #3 (undergoing multi-lineage epigenetic differentiation)
Conceptual implications of the “cancer stem cell” theory for “evolutionary” or “darwinian” models of tumor progression

The “units of selection” in cancer tissues are individual “cancer stem cells” (i.e. the cells with self-renewal capacity) as opposed to any individual cell.
Origin and biological identity of “cancer stem cells”

1) “cancer stem cells” vs. normal stem cells

Are “cancer stem cells” mutated variants of normal stem cells (that have lost normal homeostatic controls) or do they correspond to more mature, differentiated cell types (that have aberrantly acquired the capacity to self-renew)?

2) one vs. multiple “cancer stem cell” identities

Are “cancer stem cell” populations homogeneous or heterogeneous in their epigenetic identities (i.e. do they encompass one or multiple cell types)?
Theoretical considerations in support of a stem cell origin of cancer tissues.

Cancer originates as the result of the progressive accumulation of multiple genetic mutations over several years (Fearon and Vogelstein, 1990). Thus, stem cells represent the ideal targets for malignant transformation, as they are long-lived and have the opportunity to accumulate sequential mutations over time.
“Cancer stem cells” is an operational definition.

Three observations define the existence of a “Cancer Stem Cell” population:

1. Only a subset of cancer cells within each tumor is endowed with tumorigenic capacity and can be serially transplanted into immunodeficient mice.

2. Tumorigenic cancer cells are characterized by a distinctive profile of surface markers and can be differentially and reproducibly isolated from non-tumorigenic ones by flow cytometry.

3. Tumors grown from tumorigenic cells contain mixed populations of both tumorigenic and non-tumorigenic cancer cells, thus recreating the full phenotypic heterogeneity of the parent tumor.

Thus, the concept of “Cancer Stem Cells”...

1) it is a “working tool” to dissect the functional hierarchy of cancer populations;
2) it implies the fulfillment of two stem-cell properties (i.e. self-renewal and differentiation), but it does not imply the origin of “cancer stem cells” from normal stem cells.
Limitations of transplantation experiments based on flow cytometry [#3]

Flow cytometry provides only limited knowledge about molecular identity, functional status and heterogeneous composition of “cancer stem cells”.

a) are they homogeneous or heterogeneous populations?

b) which cell types do they contain?

c) are they composed by immature or mature cell types?
1) Primary tissues are collected from surgical specimens and dis-aggregated into single-cell suspensions.

2) Single-cell suspensions are stained with fluorochrome-conjugated monoclonal antibodies and analyzed by flow cytometry.

3) Single-cells from selected phenotypic populations are sorted into individual 96-well PCR plates.

4) RNA is reverse transcribed and loaded into a microfluidic chip.

5) 9,216 (96x96) single-cell PCR reactions are run in parallel using a microfluidic chip.

6) Individual cell real-time PCR curves are analyzed and converted into gene-expression levels. Individual cells are associated into distinct subsets using statistical clustering algorithms.

Analysis and graphic display of single-cell PCR data.

1) For each of the 9,216 (96x96) SINCE-PCR reactions, an amplification curve is generated and an individual threshold cycle (Ct) value is calculated.

2) Ct values are normalized and color-coded. Normalized Ct values (Ct\text{norm}) are obtained by subtracting from the raw Ct value (Ct) the mean Ct value for the same gene on the whole sample (Ct\text{mean}) and then by dividing by three times the standard deviation of the same gene’s Ct values distribution (3SD\text{Ct}). Results are color-coded using increasingly darker shades of red for high expression values (Ct < Ct\text{mean}), increasingly darker shades of green for low expression values (Ct > Ct\text{mean}) and grey for lack of expression (Ct > 40).

3) Gene-expression results are plotted using hierarchical clustering algorithms available in the MATLAB software (MathWorks Inc.). Hierarchical clustering is performed on both cells and genes, to visualize simultaneously cells with similar expression patterns and genes with similar expression profiles.

Dalerba et al., Nature Biotechnology, 29:1120-1127 (2011)
Analysis by flow cytometry of the human colon epithelium: purification of “top-crypt” vs. “bottom-crypt” cell populations.

EpCAM

CD66a
CEACAM1

CD44

Dalerba et al., Nature Biotechnology, 29:1120-1127, 2011
Single-cell PCR analysis of human normal colon epithelium


Goblet cells
- MUC2+
- TFF3^{high}

Enterocytes
- CA1+
- SLC26A3+

Novel populations
- GUCA2B+
- OLFM4+
- CA2^{high}

CBC (columnar basal cells)
- LGR5+
- ASCL2+
Single-cell PCR analysis of a monoclonal colon cancer xenograft originated from a single (n = 1) “cancer stem cell”

Dalerba et al., Nature Biotechnology, 29:1120-1127, 2011
Key points [#3]

- “cancer stem cell” populations, as currently defined using flow cytometry and operational criteria, are often heterogeneous and contain multiple cell types, including immature progenitor populations;

- it is unclear whether all or only some of the cell types within the currently defined “cancer stem cell” populations are endowed with “cancer stem cell” properties;

- it also remains unclear whether the epigenetic identity of “cancer stem cells” corresponds to that of normal stem cells or of more differentiated, specialized cell types.
Most likely, the molecular identity of “cancer stem cells” evolves during disease progression.

While, in the early stages of neoplastic transformation, the first round of mutations is likely to occur in the long-lived stem cell compartment, it is also possible that, during disease progression, a second round of mutations might disable controls in self-renewal pathways and “unleash” self-renewal in rapidly dividing multi-potent progenitors.

Does the “Cancer Stem Cell” hypothesis have implications for the modeling of metastasis?
Traditional model of Metastasis.

The “classic” model predicts that primary tumors and autologous metastases will display substantial differences (e.g. different repertoires of genetic mutations).

The “cancer stem cell” model predicts that primary tumors and autologous metastases will show substantial degrees of similarity.

Indeed, this has been a common (though somewhat unexpected) experimental finding:

Clinical implications of the “cancer stem cell” hypothesis

1) “Cancer stem cells” and tumor aggressiveness

Is the cell composition of tumor tissues associated to a more or less aggressive disease? Are tumors with abundant “cancer stem cells” more aggressive?

2) “Cancer stem cells” and response to treatments

Are the different types of cancer cells found within malignant tissues also different in their sensitivity to various types of anti-tumor therapies?
Exploring a “cancer stem cell” approach to the prognostic stratification of human malignancies.

Is the cell composition of malignant tissues, in terms of mature vs. immature cell types, associated with different prognosis?

Dalerba et al., Nature Biotechnology, 29:1120-1127 (2011)
Exploiting “cancer stem cells” to develop a prognostic tool for human Breast Cancer patients

Gene-expression arrays:

<table>
<thead>
<tr>
<th>Class</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td>DPF2, CASP8, BCL2</td>
</tr>
<tr>
<td>Calcium-ion binding</td>
<td>SCN5A, SWAP70, KIAA0276</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>C10orf9, C10orf7, ALKBH1, TOB2</td>
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<tr>
<td>Cell-surface receptor</td>
<td>XPR1, CD59, LRP2</td>
</tr>
<tr>
<td>Chemotaxis</td>
<td>PLP2, MAPK14, CXCL2</td>
</tr>
<tr>
<td>Collagen catabolism</td>
<td>MMP7</td>
</tr>
<tr>
<td>Differentiation</td>
<td>MGP, MLF1, FLNB</td>
</tr>
<tr>
<td>Ion-channel activity</td>
<td>SCN1M</td>
</tr>
<tr>
<td>Membrane protein</td>
<td>HSPC163, C3orf18, MGCG4399, CDW92, TMC4, ZDHHC2, TICAM2, KDEL3</td>
</tr>
<tr>
<td>Metabolism</td>
<td>GNPDA1, THEM2, DBR1, FLJ20709, FLJ10774, C16orf33, GAPD, LDHA, MR-1, LARS, GTPBP1, PRSS16, WFC2C, A1IM1, DHR65, DHR54, MGCI5429, MGCG45840, ECHD2C, GOL Gin67, AFUR51, KIAA0436, CYP4V2, ITTV1</td>
</tr>
<tr>
<td>Methyltransferase</td>
<td>ICMT, DNMT3A, HMN1, METTL7A, METTL2</td>
</tr>
<tr>
<td>Morphology</td>
<td>VIL2, TPD52, ARPC</td>
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<tr>
<td>Nucleotide binding</td>
<td>NOL8, ASF, RAD23B, SRP54, HSAP2, PBP, THAP2, CIRBP, SNRPN, KIAA0052</td>
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<tr>
<td>Proliferation</td>
<td>SSR1, ERBB4, EMPI, CHPT1, LRPA1</td>
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<tr>
<td>Protein binding</td>
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<td>Protein kinase</td>
<td>STK39, PAK2, CSNK2A1, PILRB, ERN1, SGK1, WEE1, MAST4, C1orf17</td>
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<tr>
<td>Protein transport</td>
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<td>Signal transduction</td>
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<td>Transcription factor</td>
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</table>

186 genes

Invasiveness Gene Signature (IGS)

The IGS is associated with poor prognosis in human Breast Cancer.

Breast Cancer patients whose tumor is characterized by a transcriptional profile similar to the IGS are characterized by reduced overall and disease-free survival.

*early breast cancer patients* from the Netherlands Cancer Institute (NKI database)

Using differentiation markers to classify human malignancies.

**KRT20**
Cytokeratin 20

**CA1**
Carbonic Anhydrase 1

[The Human Protein Atlas]

Expression of differentiation genes associates with patient survival.

Dalerba et al., Nature Biotechnology, 29:1120-1127, 2011
Using **Boolean logic** to identify early markers of epithelial differentiation in the human colon epithelium

**Boolean condition:**

"$X^{\text{low}}$ implies $\text{ALCAM}^{\text{high}}$"

Levin et al., Gastroenterology, 139:2072-2082 (2010)

Dalerba et al., NEJM, 374:211-222 (2016)
Relationship between **CDX2** and **ALCAM** mRNA expression values in human colon cancer.

Genes fulfilling the "$X^{low}$ implies $ALCAM^{high}$" Boolean implication (FDR: $< 10^{-4}$)

<table>
<thead>
<tr>
<th>Affymetrix® U133A probe set</th>
<th>Gene Symbol</th>
<th>Dynamic Range</th>
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<td>219418_at</td>
<td>NHEJ1</td>
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</tr>
</tbody>
</table>

Dalerba et al., NEJM, 374:211-222 (2016)
Prognostic role of CDX2 mRNA expression
(discovery dataset: GSE14333, GSE17538, GSE31595, GSE37892)

Dalerba et al., NEJM, 374:211-222 (2016)
Immunohistochemistry for CDX2 protein expression

CDX2\textsuperscript{neg} colorectal carcinoma

CDX2\textsuperscript{pos} colorectal carcinoma

\[ \begin{array}{|c|c|c|c|}
\hline
\text{Pathological Grade} & \text{CDX2 status} & \% \text{CDX2}\textsuperscript{neg} & \text{OR}\textsuperscript{1} \ (95\% \text{ CI}\textsuperscript{2}) \\
\hline
G1/G2 \ (n = 316) & 23 & 293 & 7.3\% \ (23/316) & 1 \\
G3/G4 \ (n = 50) & 25 & 25 & 50\% \ (25/50) & 12.7 \ (6.3-25.6) \\
\hline
\end{array} \]

\textsuperscript{1}OR: odds ratio; \textsuperscript{2}CI: confidence interval

Pearson's Chi-squared Test

\( \chi^2 = 69.15 \)

\( p < 0.001 \) ***

Dalerba \textit{et al}., NEJM, 374:211-222 (2016)
Prognostic role of CDX2 in Stage-II patients

Dalerba et al., NEJM, 374:211-222 (2016)
Predictive role of CDX2 expression on preferential benefit from adjuvant chemotherapy (Stage-II)

CDX2\textsuperscript{neg} tumors

CDX2\textsuperscript{+} tumors

Dalerba et al., NEJM, 374:211-222 (2016)
Implications of the CSC model for the design and evaluation of anti-tumor treatments

a) Treatment is broadly cytotoxic, but does not specifically target self-renewing cancer cells.
   - Short-term outcome: Tumor is grossly reduced in size, but eventually relapses driven by self-renewing cancer cells.
   - Long-term outcome:

b) Treatment targets all self-renewing cancer cells.
   - Short-term outcome: Tumor progressively exhausts its growth potential.
   - Long-term outcome:
“Stem Cell” working models to explain tumor resistance to classic anti-neoplastic agents (i.e. chemotherapy, radiotherapy)

Classical anti-tumor agents (e.g. agents toxic to proliferating cells in the S or M phase of the cell cycle)
Examples:
- Vinca alkaloids
- Anti-metabolites
- Topo-isomerase inhibitors
- Ionizing radiation

Cancer Stem Cell (CSC)

a) multi-potent progenitors (not self-renewing)
b) mature - differentiated cancer cells

a) quiescent - G₀ state (leukemia)
b) high-level expression of:
- drug pumps (leukemia)
- enzymes for DNA repair (brain cancer)
- scavengers of ROS (breast cancer)
- anti-apoptotic pathways

a) multi-potent progenitors:
- high proliferation rates

b) mature - differentiated cancer cells:
- exposed to high intra-cellular concentrations of cytotoxic drugs
- exposed to high levels of ROS (“reactive oxygen species”)
- vulnerable to extensive DNA damage
- sensitive to apoptosis
Implications of the CSC model for the **design and evaluation** of anti-tumor treatments

Treatment **targets a specific lineage of cells**, not encompassing all self-renewing cancer cells

Tumor is disrupted in architecture, but eventually relapses driven by self-renewing cancer cells

"**Targeted**" therapies might be aimed at cell populations devoid of self-renewal capacity, leading to inconsequential clinical results.
Key points [summary #1]

1) Tumor tissues are frequently heterogeneous in cell composition; this diversity: a) is not only genetic, but also epigenetic in origin; b) it often mirrors the physiological diversity of specialized cell types found in their normal counterparts; c) can arise as the result of a multi-lineage differentiation process, reminiscent of a stem cell hierarchical system;

2) In tumor tissues, the capacity to form tumors upon transplantation is frequently restricted to a subset of cancer cells, operationally defined as “cancer stem cells”; the precise molecular identity of “cancer stem cells” is still under study, and probably evolves over time, during disease progression;
Key points [summary #2]

3) analysis by gene-expression arrays of a bulk tumor’s transcriptional profile and the identification of a high degree of similarity with a *gene-expression signature characteristic of “cancer stem cells”* is usually associated with a more aggressive disease and, possibly, a differential response to anti-tumor drugs;

4) anti-tumor treatments unable to target and eradicate “cancer stem cell” populations are likely unable to achieve long-term eradication of tumor tissues.
Final remarks

“Not everyone accepts the hypothesis of cancerous stem cells. Skeptics say proponents are so in love with the idea that they dismiss or ignore evidence against it. [...] the hypothesis was more akin to religion than to science.”

Gina Kolata
“Scientists Weigh Stem Cells’ Role as Cancer Cause”
The New York Times (December 21, 2007)
Suggested readings

• Ramalho-Santos M. and Willenbring M. 
  On the origin of the term “stem cell” 
  Cell Stem Cell, 1, 35–38 (2007)

• Weinberg R.A. 
  Multi-step Tumorigenesis 

• Rajendran P.S. and Dalerba P. 
  Theoretical and experimental foundations of the “cancer stem cell” model. 
  Chapter 1 - In: Cancer Stem Cells, 3-16 (2014)

• Dalerba P., Clarke M.F., Weissman I.L., Diehn M. 
  Stem Cells, Cell Differentiation and Cancer 
  Chapter 7 - In: Abeloff’s Clinical Oncology (5th edition), 98-107 (2013)
Conflict of interest (COI) disclosures

• Some of the “cancer stem cell” markers described in this presentation have been described in a patent held by one of my former academic institutions (University of Michigan) and licensed to a start-up pharmaceutical company (OncoMed Pharmaceuticals Inc.). As a result of the licensing agreement negotiated by the University of Michigan, I receive royalties in recognition of my role as a co-inventor. Aside from this, I have no other financial interests in the company (e.g. no paid consultant agreements, no industry-sponsored research grants, no ownership of stock-options).

• Some of the “single-cell genomics” techniques described in this presentation have been described in a patent held by one of my former academic institutions (Stanford University) and licensed to a start-up pharmaceutical company (Quanticel Pharmaceuticals Inc.). As a result of the licensing agreement negotiated by Stanford University, I was awarded an aliquot of restricted stock in the company, and I receive royalties in recognition of my role as a co-inventor. Aside from this, I have no other financial interests in the company (e.g. no paid consultant agreements, no industry-sponsored research grants).

• My spouse is an employee of Amgen, a pharmaceutical company which manufactures and sells an anti-EGFR monoclonal antibody (panitumumab) approved for the treatment of human colon cancer. As a result of her employment, my spouse owns stock in the company. Aside from this, I have no other financial interests in the company (e.g. no paid consultant agreements, no industry-sponsored research grants, no direct ownership of stock options).

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