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.
“Evolution” of “Cancer Stem Cells” over disease progression?

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).

“Cancer Stem Cell” model of Metastasis.

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:


Dalerba et al., Annual Review of Medicine, 58:267-284 (2007)
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 cell” populations 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:

Breast Cancer Stem Cells Vs Normal Breast Epithelium

Strategy designed to maximize the content of prognostically relevant information, including:

1) tumor-specific genes
2) cancer stem cell-specific genes

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.

population:
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]

Dalerba et al., Nature Biotechnology, 29:1120-1127, 2011
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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<tbody>
<tr>
<td>202831_at</td>
<td>GPX2</td>
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<tr>
<td>206387_at</td>
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<td>219418_at</td>
<td>NHEJ1</td>
<td>5.38</td>
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</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

C

<table>
<thead>
<tr>
<th>Pathological Grade</th>
<th>CDX2 status</th>
<th>% CDX2$^{\text{neg}}$</th>
<th>OR$^1$ (95% CI$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1/G2 (n = 316)</td>
<td>CDX2$^{\text{neg}}$</td>
<td>23</td>
<td>7.3% (23/316)</td>
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<tr>
<td></td>
<td>CDX2$^{\text{pos}}$</td>
<td>293</td>
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<tr>
<td>G3/G4 (n = 50)</td>
<td>CDX2$^{\text{neg}}$</td>
<td>25</td>
<td>50% (25/50)</td>
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<tr>
<td></td>
<td>CDX2$^{\text{pos}}$</td>
<td>25</td>
<td></td>
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</tbody>
</table>

$^1$OR: odds ratio; $^2$CI: confidence interval

Pearson's Chi-squared Test

$\chi^2 = 69.15$

$\text{p} < 0.001$ ***

Dalerba et al., NEJM, 374:211-222 (2016)