Stem cell reprogramming as a driver of cancer: Implications in its development and treatment

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Tumour cellular identity (tumour cell fate)

If cellular fate was immovable, cancer would not be possible, since no new lineages could be generated other than the normal, physiological one.

Which is the impact that oncogenes have in establishing the identity of the tumour cell?

Clinical malignant tumor mass
“billion-cell threshold”
(Oncology remission means 0 ---- 10⁹ cells)
Cancer within a tissue

Therapeutic target

CSC

human cancer development

Genetic program:
- specific cancer cell targets,
- biomarkers,
- predict cancer response, etc

mouse and human normal stem cells are similar

Early decisions in cancer: reprogrammed tumor cell fates
Outline

1- Current model of cancer

2- Tumoral epigenetic stem cell reprogramming hypothesis

3- Experimental validation and clinical application

4- Implications in the development and treatment of cancer
Current model of cancer

- Heterogenous tumor cell composition.
- Initiating genetic alteration is present in both CSC and differentiated tumor cells.
- Homogenous mode of action for oncogenes within cancer cells.
- Brief inactivation of oncogenes can cause cancer remission in model systems: oncogene addition
- However, unfortunately, the therapies based on this cancer model fail to eradicate tumours in humans.

Do the oncogenes have a mode of action that is not homogeneous throughout the cancer cell population?
Traditionally, the human cancer genetic defects have been thought to act on cells already committed to a differentiation program, in such a way that the tumoural phenotype is derived from that of the initial differentiated target cell.
Presumptive Cellular Origins of Chromosomal translocations in Human B-cell malignancies

Assignment of human B-cell malignancies to their normal B-cell counterparts
Alternative view in which the oncogenic lesion acts on stem/progenitor cells by imposing a given, oncogene-specific, tumour-differentiated cell fate.
The tumoural stem cell reprogramming hypothesis

Tumoural reprogramming: the process by which the initial oncogenic lesion(s) can ‘reset’ the epigenetic and/or transcriptome status of an initially healthy cell (the cancer cell-of-origin), therefore establishing a new, pathological differentiation program ultimately leading to cancer development, where the oncogenic lesion(s) does not need to be present anymore once the initial cancer fate-inducing change has taken place.
We reasoned that a similar organization could be happening for cancer formation (hypothesis-driven research project).
In vivo experimental model of tumoral stem cell reprogramming

To be able to demonstrate this lack of homogeneity in the mode of action of oncogenes throughout the biological history of the tumor, it would be necessary to dissect and isolate the function that the oncogene is playing at the earliest stages of the disease, at the level of the cell-of-origin.

Redefining the relevance of established cancer cell lines to the study of mechanisms of clinical anti-cancer drug resistance

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Edited by Ina Partan, National Cancer Institute, National Institutes of Health, Bethesda, MD, and approved October 10, 2011 (received for review July 21, 2011)

Although in vitro models have been a cornerstone of anti-cancer drug development, their direct applicability to clinical cancer research has been uncertain. Using a state-of-the-art Taqman-based quantitative RT-PCR assay, we investigated the mutational resilience (MDR) transcriptome of six cancer types, in established cancer cell lines (grown in monolayer, 3D scaffold, or in xenograft) and clinical samples, either containing >75% tumor cells or micro-dissected. The MDR transcriptome was determined a priori based on an extensive curatorial of the literature published during the last three decades, which led to the enumeration of 188 genes. No correlation was found between clinical samples and established cancer cell lines. As expected, we found up-regulation of genes that would facilitate survived across all tumour cell lines evaluated. More troubling, however, were data showing that all of the cell lines, grown either in vitro or in vivo, bear more resemblance to each other, regardless of the issue of origin, than to the clinical samples they are supposed to model. Although cultured cells can be used to study many aspects of cancer biology and response of cells to drugs, this study emphasizes the necessity for new in vivo cancer models and the use of primary tumour models in which gene expression can be manipulated and small molecules tested in a setting that closely mimics the in vivo cancer microenvironment so as to avoid radical changes in gene expression profiles brought on by extended periods of cell culture.

characterized, we chose to use them, and additional cancer cell lines, to assess the relevance of cultured cell lines in the study of clinical multidrug resistance (MDR) mechanisms (12). Over the past 30 y, in vitro studies have led to the enumeration of close to 400 genes whose expression affects response to chemotherapy (13). Among those genes, ATP-binding cassette (ABC) transporters, a superfamily of 48 highly homologous members classified in seven subfamilies, have an important role in the pharmacokinetic mechanisms mediating MDR by exporting chemotherapeutic agents from the cell (14, 15). Although the roles of 13 ABC transporters in MDR have been fully characterized, recent studies suggest the involvement of up to 30 members of the ABC family in the human genome (16, 17). Moreover, besides classical drug efflux, it has also been demonstrated that some of these transporters that mediate the intracellular sequestration of chemotherapeutic drugs (18–20). This intracellular sequestration is the case for ABCA3, which was recently found to be overexpressed in clinical samples of childhood ALL, and correlated with poor response to treatment (21). The establishment of a specific and sensitive standard assay, capable of discriminating highly homologous genes, is critical to a better understanding of MDR mechanisms. We and others have shown that Taqman Low Density Array (TLDA) provide the most sensitivity and specificity in measuring the expression patterns of ABC transporter genes (22, 23). Therefore, we chose to conflate such a platform to study multidrug resistance.
Human Cancer tissue

- Genetic defect is present in both CSC and differentiated tumor cells

In vivo experimental model of tumoural stem cell reprogramming

- Genetic defect is only present in CSC

Might cancer stem cells initially arise through a reprogramming-like mechanism?

To be able to demonstrate this lack of homogeneity in the mode of actions of oncogenes throughout the biological history of the tumour.

This still unexplored possibility would have major implications for our understanding of the genesis and treatment of cancer.
“What I cannot create, I do not understand”

Richard P. Feynman
Nobel Prize in Physics 1965

Written on his blackboard at time of his death, in 1988
How to restrict oncogene expression to the stem cells

Contribution of CSC to cancer biology?

The key feature of these Sca1 mice is that they express an oncogene under the control of a promoter that is expressed in a population of stem/progenitor cells, but is switched off after lineage commitment.
Constitutive Stem-Cell Restricted Oncogene Expression

Mouse Stem-cell-restricted gene (Transgenic Vector)

A cancer without Oncogene??

Modified allele in all mouse cells

Oncogenic alteration restricted to Stem/Progenitor Cells

Dis Model Mech. 2010 Mar-Apr;3(3-4):149-55
### Oncogene-induced plasticity and CSC

#### Stem cell compartment

- **CSC proliferating cells**

#### Differentiated cells

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Genetic product</th>
<th>Tumour type</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(12;16)(q13;p11)</td>
<td>FUS-DDIT3</td>
<td>Myxoid Liposarcoma</td>
</tr>
<tr>
<td>t(16;21)(p11;q22)</td>
<td>FUS-ERG</td>
<td>Acute myeloid leukaemia</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABLp190</td>
<td>B acute lymphoblastic leukaemia</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABLp210</td>
<td>Chronic myeloid leukaemia</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>BCR-ABLp230</td>
<td>Chronic neutrophilic leukaemia</td>
</tr>
<tr>
<td>t(?;3)(?;q27)</td>
<td>?+ BCL6</td>
<td>DLBCL/ Follicular lymphoma</td>
</tr>
</tbody>
</table>
Reprogramming in malignancies originated from stem cells
In vivo experimental model of tumoural stem cell reprogramming

1- Proof of principle experiment

2- Chronic myeloid leukemia (CML) stem cells are not oncogene addicted and the therapies that biochemically target BCR-ABL do not eliminate them (CML stem cells).

3- First animal model anticipating human clinical results in the CSC field

4- Results were confirmed in human patients two years later
Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity

Annie S. Corbin, Anupriya Agrawal, Marc Loriaux, Jorge Cortes, Michael W. Deininger, and Brian J. Druker

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The Journal of Clinical Investigation: http://www.jci.org, Volume 121, Number 1, January 2011


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**A.H., G.V.M., M.S., S.K., and T.C. contributed equally to this study.

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Persistence of leukemia stem cells in chronic myelogenous leukemia patients in prolonged remission with imatinib treatment

Su Chu, Tinisha McDonald, Allen Lin, Sajata Chakraborty, Qin Huang, David S. Snyder, and Ravi Bhatia

Division of Hematopoietic Stem Cell and Leukemia Research, Department of Hematology and Hematopoietic Cell Transplantation, and Department of Pathology, City of Hope National Medical Center, Duarte, CA.

The online version of this article contains a data supplement.

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Brief report

Leukemic stem cell persistence in chronic myeloid leukemia patients with sustained undetectable molecular residual disease

Juan-Claudio Chemello, Maria-Luana Bonnat, Nathalie Soral, Angéline Bertrand, Maria-Claudia Meunier, Serge Fichelson, Michael Mallius, Annelise Berneuille-Girardelli, François Guillot, and Ali G. Turhan

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The online version of this article contains a data supplement.

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New concept of the human cancer as a Reprogramming-like Disease

Can this hypothesis be extrapolated to other malignancies?
B cell development

B-ALL
(Tel-Aml1; BCR-ABLp190)

CML
(BCR-ABLp210)

Lymphoma MALT
(Malt1)

Multiple Myeloma
(MafB)

B-ALL
(Tel-Aml1; BCR-ABLp190)

CML
(BCR-ABLp210)

B-ALL
(Tel-Aml1; BCR-ABLp190)

CML
(BCR-ABLp210)

B cell lymphoma
(HGAL)

B cell lymphoma
(HGAL)

B cell lymphoma
(Lmo2)

B cell lymphoma
(Lmo2)
Ewing sarcoma

Synovial sarcoma

EWS-FLI-1

BCR-ABLp210

Stem/Progenitor cell

MALT lymphoma

MALT1

MafB

Multiple myeloma

HGAL

B-cell hyperplasia

Other pathologies?

ABC-DLBCL

CML

Genes & development. 2010.

Nature communications. 2014.

Proc Natl Acad Sci USA. 2012.

Cell Cycle. 2012.


Cell Cycle. 2009.

Oncogene. 2012.

Embo J. 2012.

Cell Cycle. 2012.

Cell Cycle. 2012.

Cancer as a result of tumoral epigenetic stem cell reprogramming

DNA damage

p53 loss should accelerate the tumor reprogramming process
Tumour suppressors can act as barriers for tumoural stem cell reprogramming

**Sca1-MafB MM development**

HSC → CSC → REPROGRAMME D CELLS → TUMOR CELLS

**Sca1-MafB MM development in the absence of p53**

HSC → CSC → REPROGRAMMED CELLS → TUMOR CELLS
Are there evidences of tumoral epigenetic stem cell reprogramming??

- CSC compartment
- Tumor differentiated cells
- Normal stem cell compartment
- Normal differentiated cells
Genome-scale DNA methylation maps of stem cells and mature B cells in mice predicts human cancer organization.

Whether this mechanism is involved in the genesis of human cancers was presently not known, but recent results confirmed similar cellular hierarchy in human MM patients.
Xbp1s-Negative Tumor B Cells and Pre-Plasmablasts Mediate Therapeutic Proteasome Inhibitor Resistance in Multiple Myeloma

Chunghee Leung-Hagesteijn,1 Natalia Erdmann,1 Grace Chung,1 Jonathan J. Keats,2,3 A. Keith Stewart,1 Donna E. Pierce,1,4 Kim Chih Chung,1 and Rodger E. Tiedemann1,5

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http://dx.doi.org/10.1016/j.ccr.2013.08.009

Significance

PLs, including bortezomib, are a mainstay of treatment for MM but fail to cure. Previously reported in vitro resistance mechanisms have not been validated in the clinic and reflect an artifact of cell culture. An alternative PI resistance mechanism is described here that occurs in patients with MM; because this differs from in vitro resistance reports, the need for clinical confirmation of in vitro drug resistance models is highlighted. Our results reveal that MM cells tolerate XBP1 inactivation, which contributes to therapeutic resistance, suggesting that XBP1 inhibitors may prove ineffectual in MM. Furthermore, an extensive progenitor organization is revealed in primary MM. Our results suggest that to achieve cure, treatment strategies must better address early MM progenitors.

Clinical Cancer Research

Stemness of B cell progenitors in multiple myeloma bone marrow

Yi Yang, Jumai Shi, Giulia Tolomelli, Hongwei Xu, Jiliang Xia, He Wang, Wai Zhou, Yi Zhou, Satyabrata Das, Zhumin Gu, Dana Lavasseri, Fenghuang Zhan and Guido Tricot

RARα2 expression confers myeloma stem cell features

Kelly Boucher, Nancy Parquet, Raymond Wider, et al.
Clin Cancer Res Published OnlineFirst September 17, 2012
Tumour stem cell reprogramming largely relies on epigenetic modifications. These, unlike genetic changes, can be erased, manipulated, and reinitiated, therefore implying that anti-tumour reprogramming strategies can provide a new window of opportunity to interfere with the cancer fate-inducing change.
CSCs do not have oncogene addition

Oncogenes cannot be used as a target to kill CSCs

BUT, Tumour stem cell reprogramming is a specific CSC target

Could we use it to prevent/kill CSCs?
**p53 RESTORATION KILLS PRIMITIVE LEUKEMIA CELLS IN VIVO AND INCREASES OVERALL SURVIVAL OF LEUKEMIC MICE**

**TIMEPOINT 1**

15 weeks (timepoint 1)

Two Genomes Influence Every Cancer Patient

Germline: Host Genome
- Systemic pharmacokinetics
- Drug toxicity (normal tissue)

Somatic: Cancer Genome
- Cellular pharmacodynamics
- Drug sensitivity (tumor)

Diagnosis
Relapse/metastasis
Genetic background affects susceptibility to tumoral stem cell reprogramming

These results demonstrate for the first time that tumoral stem cell reprogramming fate is subject to polymorphic genetic control

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of mice</th>
<th>No. with CML(%)</th>
<th>No.with B-cell leukemia</th>
<th>No. with T-cell lymphoma</th>
<th>NO TUMORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>23</td>
<td>23(100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B6/FVB</td>
<td>35</td>
<td>10(28,5)</td>
<td>6(17,2)</td>
<td>0</td>
<td>19(54,3)</td>
</tr>
<tr>
<td>FVB</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>11(100)</td>
<td>0</td>
</tr>
</tbody>
</table>
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Thank you for your attention!!!!