How tumor cell identity is established and maintained?

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VII Simposium Bases Biológicas del Cáncer y Terapias Personalizadas
Salamanca, 21-22 de Mayo, 2015
Why early stages in cancer are important?

Oncogene-cell target interaction

Clinical malignant tumor mass
“billion-cell threshold”
(Oncology remission means 0 \( \text{---} \) \( 10^9 \) cells)
Risk assessment
Noninvasive screening for early-stage disease
Detection and localization
Disease stratification and prognosis
Response to therapy
Screening for disease recurrence
Why is important to know the etiology of cancer?

The identical twin on the right was given various treatments as a child for acute lymphoblastic leukaemia.
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?
Early decisions in cancer: reprogrammed tumor cell fates

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

Human cancer development

Cancer within a tissue

Therapeutic target CSC

normal tissue

mouse and human normal stem cells are similar
Outline

1- Current model of cancer

2- Tumoral epigenetic stem cell reprogrammimg 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?
Classical model for the role of human cancer gene defects in tumour cell fate specification

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

Cobaleda & Sanchez-García, BioEssays, 2009
Alternative view in which the oncogenic lesion acts on stem/progenitor cells by imposing a given, oncogene-specific, tumour-differentiated cell fate.
Review

Function of oncogenes in cancer development: a changing paradigm

Carolina Vicente-Dueñas1,2, Isabel Romero-Camarero1,2, Cesar Cobaleda3,* and Isidro Sánchez-García1,2,*

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Tumour-associated oncogenes induce unscheduled proliferation as well as genomic and chromosomal instability. According to current models, therapeutic strategies that block oncogene activity are likely to selectively target tumour cells. However, recent evidences have revealed that oncogenes are only essential for the proliferation of some specific tumour cell types, but not all. Indeed, the latest studies of the interactions between the oncogene and its target cell have shown that oncogenes contribute to cancer development not only by inducing proliferation but also by developmental reprogramming of the epigenome. This provides the first evidence that tumorigenesis can be initiated by stem cell reprogramming, and uncovers a new role for oncogenes in the origin of cancer. Here we analyse these evidences and propose an updated model of oncogene function that can explain the full range of genotype–phenotype associations found in human cancer. Finally, we discuss how this vision opens new avenues for developing novel anti-cancer interventions.

cells. This has been reflected in the therapeutic approaches employed in the clinic to treat the patients: with very few exceptions, anti-cancer treatments are targeted at the mechanisms of abnormal tumoural growth. These problems result in the eventual failure of therapy, that is often accompanied by the development of drug resistance and by metastatic dissemination. For this reason, an urgent goal of cancer research is to understand how to counteract the mechanisms that underlie the ability of normal cells to become cancer cells in the first place. The complexity of the properties of cancer cells was distilled by Hanahan and Weinberg (2011) into ‘nine essential alterations in cell physiology that collectively dictate malignant growth’. Cancer cells are the foundation of the disease: they initiate the tumours and drive cancer progression forward, and they are the ones carrying the oncogenic and tumour suppressor mutations that define cancer as a genetic disease (Hanahan and Weinberg, 2011). However, we still do not understand sufficiently well the underlying mechanisms leading to the origin of these cells, so as to have a sizable impact on cancer mortality (Jemal et al., 2009). As a result, our progress is incremental and largely empirical, leading only to slight improvements in treatments, surgical interventions or radiation regimes. These may provide some benefit, but they seem unable of bringing the disease itself to an end.

Thus, a complete understanding of the cancer process requires a more detailed knowledge of the mechanisms giving rise to neoplastic growth, and is a prerequisite, not only for the understanding of the genesis of human cancer but also for the identification of the molecular events respon-
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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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 multiplicity of action (MDR) transcriptome of six cancer types, in established cancer cell lines (grown in monolayer, 3D scaffold, or xenograft) and clinical samples, either containing > 75% tumor cells or micro-dissected. The MDR transcriptome was determined a priori based on an extensive curating 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 survival across all-targeted cancer cell lines evaluated. More troubling, however, were the data showing that all of the cell lines, grown either in vitro or in vivo, bear more resemblance to each other, regardless of the tissue 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 vitro cancer models and the use of primary tumor models in which gene expression can be manipulated and small molecules tested in a setting that more 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 mechanism 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 88 encoded in the human genome (16, 17). Moreover, besides classical drug efflux, it has also been demonstrated that some of these transporters may mediate the intracellular acq
**Human Cancer tissue**

**In vivo experimental model of tumoural stem cell reprogramming**

Genetic defect is present in both CSC and differentiated tumor cells

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
SOLVING CANCER
YOU CAN'T CURE WHAT YOU DON'T UNDERSTAND

\[(X + Y = -C) (X + Y = -C) (X + Y = -C) (X + Y = -C)\]
How to restrict oncogene expression to the stem cells

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)

Oncogene

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

<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 leukemia</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

Diagram showing various cancers and their genetic markers, such as BCR-ABL, EWS-FLI-1, SYT-SSX2, MALT1, MafB, and HGAL, associated with stem/progenitor cells.
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

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The Journal of Clinical Investigation 121(1) | January 2013

Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival

"Ashley Hohman,1,2 Gun-Vigdis Helgason,3 Milad S. Alizadeh,4 Bin Zhang,4 Svend E. Woywod,5 Elaine K. Allen,1 Francesc E. Nicollin,1 Casimil Millet-Tiffard,6 Paul Bhatia,2 Valeria D. Brumell,7 Stuiften Schuirmen,1 and Tessa L. Holtys5

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

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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

Janine-Claude Chemin,1,2 Marie-Laure Bonnat,2 Nathalie Soral,1 Angéline Bertrand,1 Marie-Claudia Meinrath2 Serge Tichet,1 Michael Meul,1 Anne-Lise Bernauvex-Griscelli,7 Françoise Guillot,1,4 and Al G. Turlin1,3

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

Can this hypothesis be extrapolated to other malignancies?
Ewing sarcoma

Synovial sarcoma

B-cell hyperplasia

Multiple myeloma

MALT lymphoma

ABC-DLBCL

CML

Stem/Progenitor cell

BCR-ABL_p210

EWS-FLI-1

SYT-SSX2

MafB

MALT1

HGAL

Other pathologies?

Oncogene. 2012.

Genes & development. 2010.

Cell Cycle. 2009.

Nature communications. 2014.

Proc Natl Acad Sci USA. 2012.
Cell Cycle. 2012.

Cell Cycle. 2012.

Embo J. 2012.
Cell Cycle. 2012.
Cell Cycle. 2012.

Cellular origin of bladder neoplasia and tissue dynamics of its progression to invasive carcinoma

Kunyoo Shin\textsuperscript{1,8}, Agnes Lim\textsuperscript{2}, Justin I. Odegaard\textsuperscript{3}, Jared D. Honeycutt\textsuperscript{4}, Sally Kawano\textsuperscript{1}, Michael H. Hsieh\textsuperscript{5} and Philip A. Beachy\textsuperscript{1,2,6,7,8}

Understanding how malignancies arise within normal tissues requires identification of the cancer cell of origin and knowledge of the cellular and tissue dynamics of tumour progression. Here we examine bladder cancer in a chemical carcinogenesis model that mimics muscle-invasive human bladder cancer. With no prior bias regarding genetic pathways or cell types, we prospectively mark or ablate cells to show that muscle-invasive bladder carcinomas arise exclusively from Sonic hedgehog (\textit{Shh})-expressing stem cells in basal urothelium. These carcinomas arise clonally from a single cell whose progeny aggressively colonize a major portion of the urothelium to generate a lesion with histological features identical to human carcinoma \textit{in situ}. \textit{Shh}-expressing basal cells within this precursor lesion become tumour-initiating cells, although \textit{Shh} expression is lost in subsequent carcinomas. We thus find that invasive carcinoma is initiated from basal urothelial stem cells but that tumour cell phenotype can diverge significantly from that of the cancer cell of origin.
Identification of cancer initiating cells in K-Ras driven lung adenocarcinoma

Sara Mainardi, Nieves Mijimolle, Sarah Francoz, Carolina Vicente-Dueñas, Isidro Sánchez-Garcia, and Mariano Barbacid

Ubiquitous expression of a resident K-RasG12V oncogene in adult mice revealed that most tissues are resistant to K-Ras oncogenic signals. Indeed, K-RasG12V expression only induced overt tumors in lungs. To identify these transformation-permissive cells, we induced K-RasG12V expression in a very limited number of adult lung cells (0.2%) and monitored their fate by X-Gal staining, a surrogate marker coexpressed with the K-RasG12V oncogene. Four weeks later, 30% of these cells had proliferated to form small clusters. However, only SPC+ alveolar type II (ATII) cells were able to form hyperplastic lesions, some of which progressed to adenomas and adenocarcinomas. In contrast, induction of K-RasG12V expression in lung cells by intratracheal infection with adenoviral-Cre particles generated hyperplasias in all regions except the proximal airways. Bronchiolar and bronchioalveolar duct junction hyperplasias were primarily made of CC10+ Clara cells. Some of them progressed to form benign adenomas. However, only alveolar hyperplasias, exclusively made up of SPC+ ATII cells, progressed to yield malignant adenocarcinomas. Adenoviral infection induced inflammatory infiltrates primarily made of T and B cells. This inflammatory response was essential for the development of K-RasG12V-driven bronchiolar hyperplasias and adenomas, but not for the generation of SPC+ ATII lesions. Finally, activation of K-RasG12V during embryonic development under the control of a Sca1 promoter yielded CC10+, but not SPC+, hyperplasias, and adenomas. These results, taken together, illustrate that different types of lung cells can generate benign lesions in response to K-Ras oncogenic signals. However, in adult mice, only SPC+ ATII cells were able to yield malignant adenocarcinomas.

Significance

K-RAS oncogene-driven lung adenocarcinomas is one of the
Transient expression of Bcl6 is sufficient for oncogenic function and induction of mature B-cell
Seminars in
CANCER BIOLOGY
STEM CELL REPROGRAMMING AS A DRIVER OF CANCER

Guest Editor
ISIDRO SÁNCHEZ-GARCÍA

Volume 32. June 2015
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 → REPROGRAMME D CELLS

Sca1-MafB

Sca1-MafB MM development in the absence of p53

HSC → CSC → REPROGRAMMED CELLS → TUMOR CELLS

Sca1-MafB

p53−/−

Cell Cycle 2012, 11(20): 3896-3900 (issue cover)
Are there evidences of tumoral epigenetic stem cell reprogramming??
Identification of a cytosine hypomethylation signature in Sca1-Bcl6

Red points are the 323 CSC-specific differentially hypomethylated genes
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


Princess Margaret Cancer Centre, Toronto, ON M5G 2M9, Canada

Translational Genomics Research Institute, Phoenix, AZ 85034, USA

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

Clinical Cancer Research

Stemness of B cell progenitors in multiple myeloma bone marrow

Ye Yang, Jumal Shi, Giulia Tolomelli, Hongwei Xu, Jiijia Xia, He Wang, Wai Zhou, Yi Zhou, Satyabrata Das, Zhimin Gu, Dana Lievassier, Fenghuang Zhan and Guido Tricot

RARα2 expression confers myeloma stem cell features

2013 122: 1437-1447
Prepublished online July 11, 2013;
doi: 10.1182/blood-2013-02-492319

Clinical Cancer Research

Stemness of B cell progenitors in multiple myeloma bone marrow

Kelly Boucher, Nancy Parquet, Raymond Widen, 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?
Cancer progression also involves CSC evolution

Evolutionary speciation or ancestral tree, from Charles Darwin’s 1837 Transmutation notebook B

Semin Cancer Biol. 2010
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>
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<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>
Genetic approach to identify CSC maintenance genes and genes/loci responsible for different therapeutic response, etc.

Resistant strain (B6) x Susceptible Strain (FvB) → Population with high genetic variability primed for CANCER (inherited and/or acquired susceptibilities) → Different risk factor exposure susceptibility

F1 B6 x FvB → High CANCER phenotype variability (tumor phenotype and genetics)

Study genomic (SNPs) and epigenetic variability before and after to identify risk factors

Study physiological variability

Potential environmental risk factors/carcinogen to study gene-environment interactions
Thank you for your attention!!!!