Cellular plasticity and gene expression variability: from drug tolerance to oncogenesis, and back

Jean-Pascal Capp

Maître de conférences (Associated Professor), **INSA / University of Toulouse**

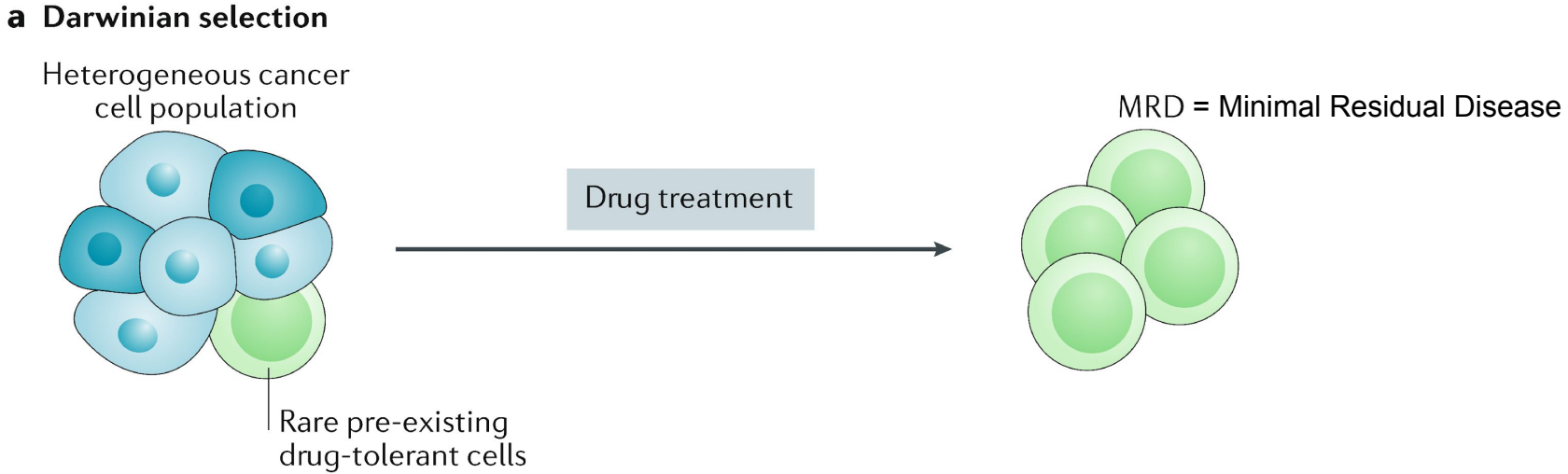
Toulouse Biotechnology Institute
Bio & Chemical Engineering

www.toulouse-biotechnology-institute.fr

Darwinian selection upon treatment

Historically, most of the focus in understanding resistance to therapy has been on genetic drivers of drug resistance, including mutations that enable bypassing target inhibition through impaired binding of the drug, activation of downstream effectors in the same signalling pathway or engagement of alternative survival pathways.

Boumahdi & de Sauvage, 2020, Nat Rev Drug Discov



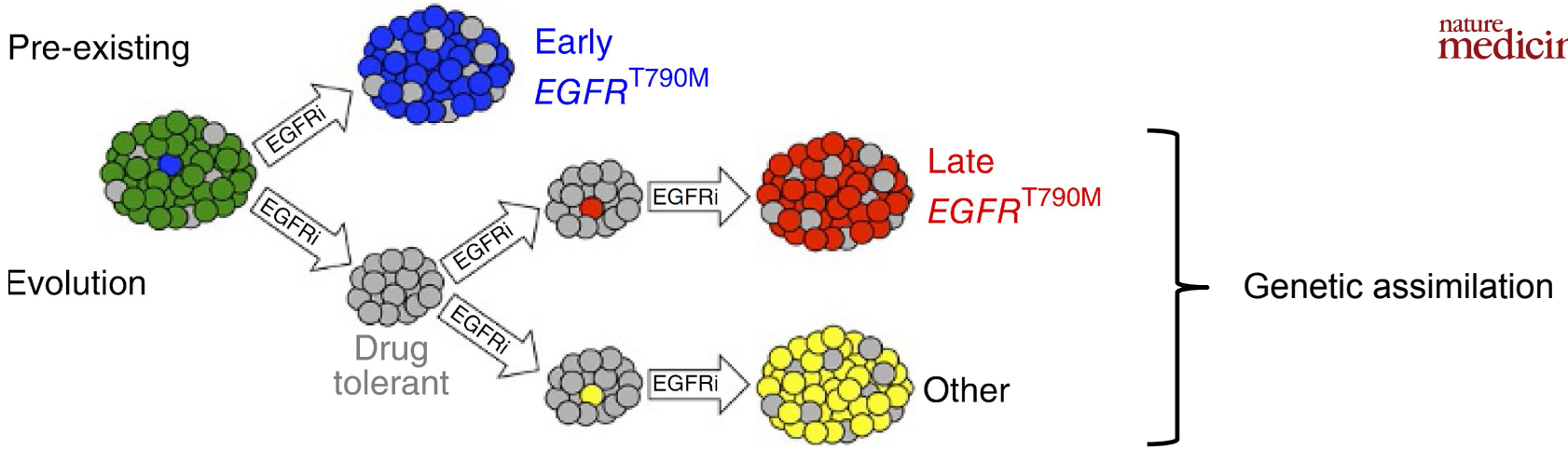
Darwinian selection upon treatment

Pre-existing or acquired?

Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition

Aaron N Hata[1,2,14], Matthew J Niederst[1,2,14], Hannah L Archibald[1], Maria Gomez-Caraballo[1], Faria M Siddiqui[1], Hillary E Mulvey[1], Yosef E Maruvka[1,3], Fei Ji[4], Hyo-eun C Bhang[5], Viveksagar Krishnamurthy Radhakrishna[5], Giulia Siravegna[6,7], Haichuan Hu[1], Sana Raoof[1,2], Elizabeth Lockerman[1], Anuj Kalsy[1], Dana Lee[1], Celina L Keating[5], David A Ruddy[8], Leah J Damon[1], Adam S Crystal[1,13], Carlotta Costa[1,2], Zofia Piotrowska[1,2], Alberto Bardelli[6,7], Anthony J Iafrate[9], Ruslan I Sadreyev[4,9], Frank Stegmeier[5], Gad Getz[1,3,9,10], Lecia V Sequist[1,2], Anthony C Faber[11,12] & Jeffrey A Engelman[1,2]

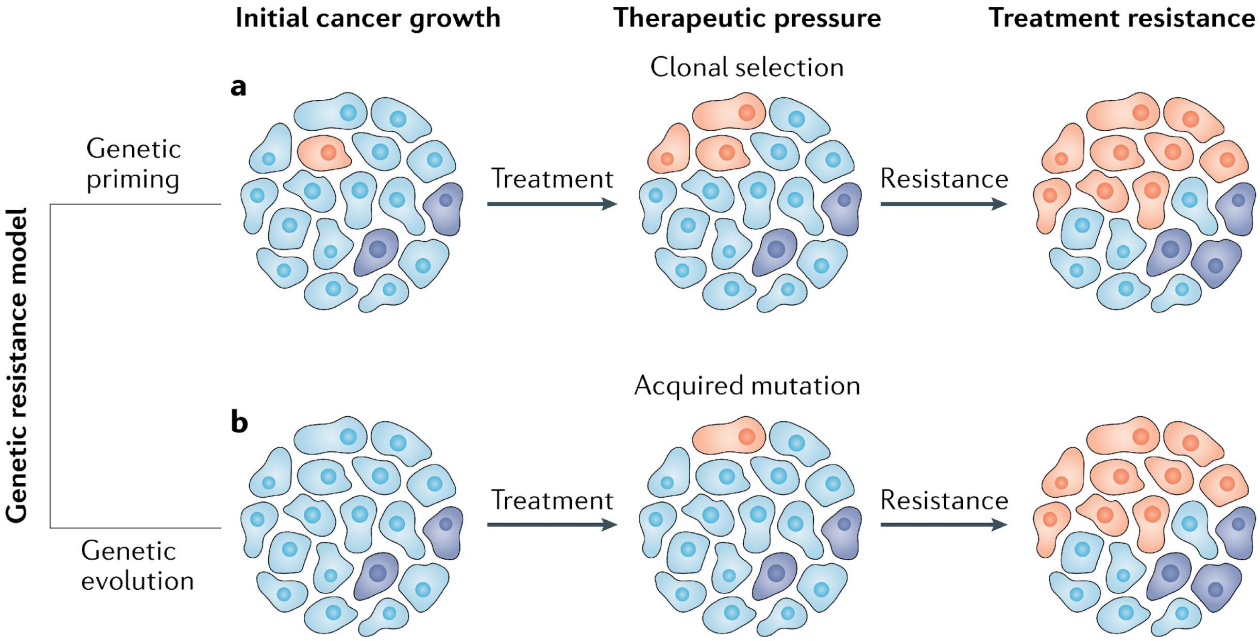
nature medicine 2016



Clinically relevant drug-resistant cancer cells can both pre-exist and evolve from drug-tolerant cells.

Darwinian selection upon treatment

The emergence of these mechanisms of resistance is the result of either the selection of rare pre-existing genetic alterations upon drug treatment or the acquisition of *de novo* mutations in tolerant cells during treatment.



Marine et al, 2020, Nat Rev Cancer

Drug tolerance / persistence

Drug-tolerant persisters (DTP), a subpopulation of cancer cells that transiently survives lethal drug exposures, were first described in non-small-cell lung cancer (NSCLC):

Cell
2010

A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations

Sreenath V. Sharma,[1] Diana Y. Lee,[1] Bihua Li,[1] Margaret P. Quinlan,[1] Fumiyuki Takahashi,[1] Shyamala Maheswaran,[1] Ultan McDermott,[1] Nancy Azizian,[1] Lee Zou,[1] Michael A. Fischbach,[1] Kwok-Kin Wong,[2] Kathleyn Brandstetter,[2] Ben Wittner,[1] Sridhar Ramaswamy,[1] Marie Classon,[1,3,*] and Jeff Settleman[1,3,*]

While the vast majority of cells in a cultured NSCLC cell population were rapidly killed on exposure to therapy, a rapid and transient accumulation of viable or residual ‘drug-tolerant’ cells was observed with kinetics and frequency that could not be explained by mutational mechanisms. Notably, as opposed to drug resistance, drug tolerance manifested itself as a state in which tumour cells could transiently survive but not proliferate on treatment.

Marine et al, 2020, Nat Rev Cancer

Drug tolerance / persistence

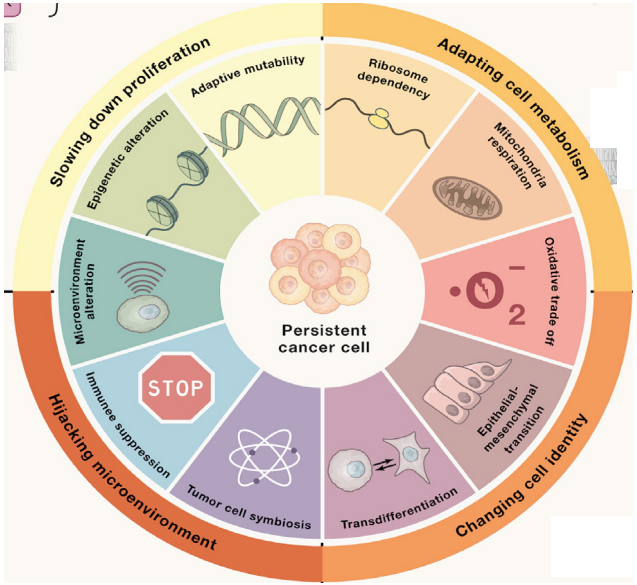
The understanding that non-mutational mechanisms may have a role in tumour relapse has prompted multiple studies focused on identifying factors that contribute to overall persister fitness.

CellPress 2020 Cell Leading Edge

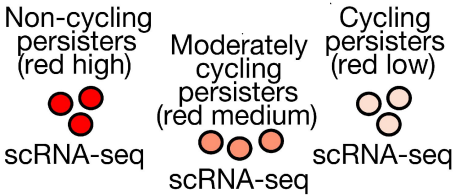
Review

Persistent Cancer Cells: The Deadly Survivors

Shensi Shen,[1,*] Stéphan Vagner,[3,4,5,*] and Caroline Robert[1,2,6,*]



Recently: identification of metabolic and expression adaptations that may facilitate cell cycle re-entry in a rare subset of persister cells.



Article

Cycling cancer persister cells arise from lineages with distinct programs

Nature, 2021

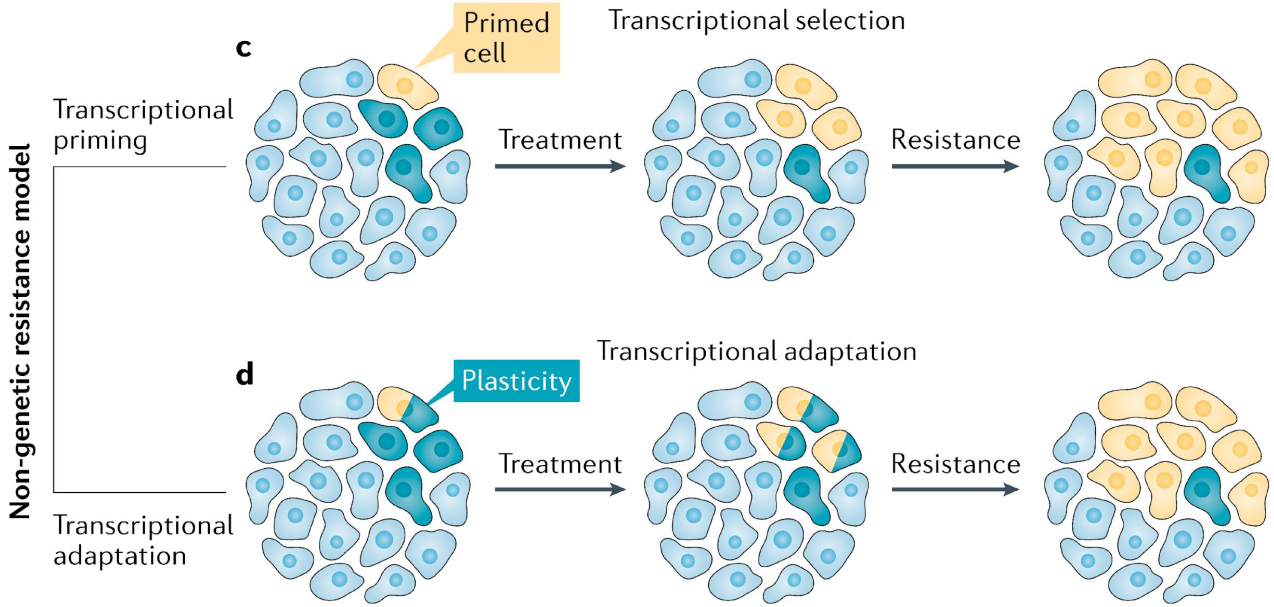
https://doi.org/10.1038/s41586-021-03796-6
Received: 1 June 2020
Accepted: 2 July 2021
Published online: 11 August 2021

Yaara Oren[1,2], Michael Tsabar[1,3,4,20], Michael S. Cuoco[1,20], Liat Amir-Zilberstein[1], Heidie F. Cabanos[5,6], Jan-Christian Hütter[1], Bomiao Hu[7], Pratiksha I. Thakore[1,18], Marcin Tabaka[1], Charles P. Fulco[8,19], William Colgan[8], Brandon M. Cuevas[1], Sara A. Hurvitz[9,10], Dennis J. Slamon[9,10], Amy Deik[11], Kerry A. Pierce[11], Clary Clish[11], Aaron N. Hata[5,6], Elma Zaganjor[12], Galit Lahav[3], Katerina Politi[13,14], Joan S. Brugge[2,15,21]✉ & Aviv Regev[1,16,17,18,21]✉

tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

Drug tolerance / persistence

Although DTP cells are seen to rapidly emerge on therapeutic challenge, it remains unclear whether these DTP cells represent an enrichment of a specific ‘primed’ drug-tolerant state or whether it occurs through active, therapeutically induced, non-genetic reprogramming.



Marine et al, 2020, Nat Rev Cancer

Drug tolerance / persistence

Cell Stem Cell
Article

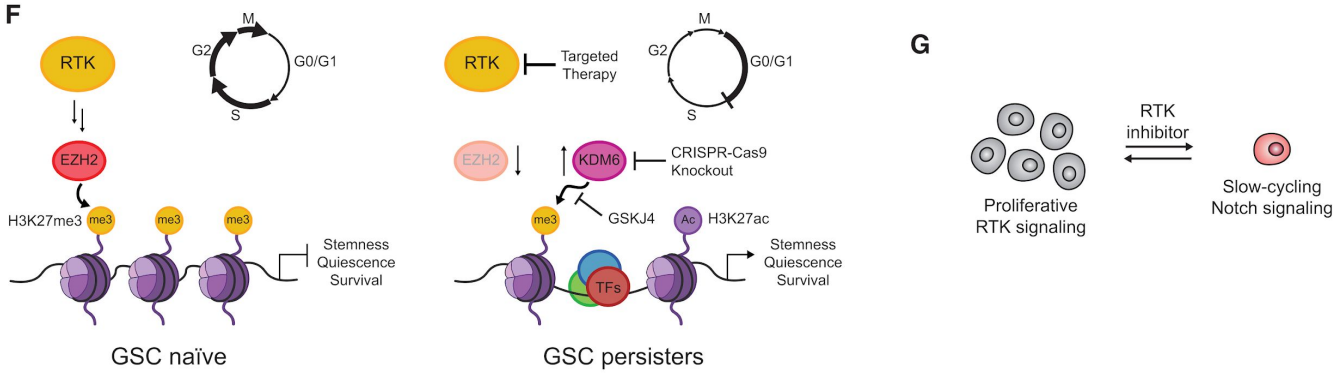
2017

CellPress

Adaptive Chromatin Remodeling Drives Glioblastoma Stem Cell Plasticity and Drug Tolerance

Brian B. Liau,[1,2,7] Cem Sievers,[1,2,7] Laura K. Donohue,[1,2] Shawn M. Gillespie,[1,2] William A. Flavahan,[1,2] Tyler E. Miller,[5,6] Andrew S. Venteicher,[1,2,3] Christine H. Hebert,[1,2] Christopher D. Carey,[4] Scott J. Rodig,[4] Sarah J. Shareef,[1,2] Fadi J. Najm,[1,2] Peter van Galen,[1,2] Hiroaki Wakimoto,[3] Daniel P. Cahill,[3] Jeremy N. Rich,[5,6] Jon C. Aster,[4] Mario L. Suvà,[1,2] Anoop P. Patel,[1,2,3,*] and Bradley E. Bernstein[1,2,8,*]

"We show that patient-derived GSCs can evade RTK inhibition and other anti-proliferative therapies by regressing to a slow-cycling, Notch-dependent state. We also establish that the GSC persister transition is characterized by widespread remodeling of repressive chromatin."



"We present a reversible epigenetic transition that enables GSCs to transition between proliferative and slow-cycling states, which may enable GBM tumors to propagate, adapt, and persist in the face of environmental and therapeutic pressures."

Dynamic fluctuation

DTPs were also identified in colon cancer, melanoma, especially in:

LETTER

Nature, 2017

doi:10.1038/nature22794

Rare cell variability and drug–induced reprogramming as a mode of cancer drug resistance

Sydney M. Shaffer[1,2], Margaret C. Dunagin[1], Stefan R. Torborg[1,3], Eduardo A. Torre[1,2], Benjamin Emert[2,4], Clemens Krepler[5], Marilda Beqiri[5], Katrin Sproesser[5], Patricia A. Brafford[5], Min Xiao[5], Elliott Eggan[2], Ioannis N. Anastopoulos[2], Cesar A. Vargas-Garcia[6], Abhyudai Singh[6,7], Katherine L. Nathanson[2], Meenhard Herlyn[5] & Arjun Raj[1,8]

Very rare melanoma cells that transiently display high expression of resistance genes prior to drug exposure were more likely to survive than parental cells. These drug-tolerant cells could give rise to sensitive cells when the drug was removed, indicating that this drug-refractory state is transient rather than genetically heritable. These findings support the idea that DTPs are not a pre-existing, defined subpopulation but rather arise stochastically from a dynamically fluctuating cell population.

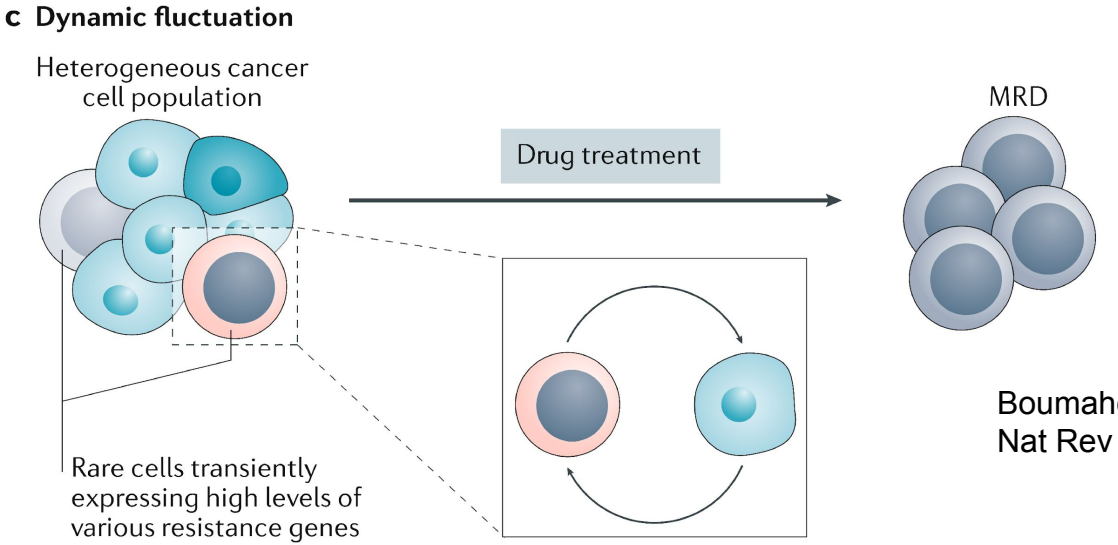
Marine et al, 2020, Nat Rev Cancer

Dynamic fluctuation

After adding drug, the percentage of resistance genes expressed increased, demonstrating a progressive transformation of the transcriptome as cells became stably resistant.

Shaffer et al, 2017, Nature

Privileged model:



Boumahdi & de Sauvage, 2020, Nat Rev Drug Discov

Expression of resistance markers fluctuates in cancer cells and, at treatment initiation, the rare cells that express high levels of various resistance genes will survive and then stably express a resistance gene signature.

Dynamic fluctuation

nature biotechnology

ARTICLES

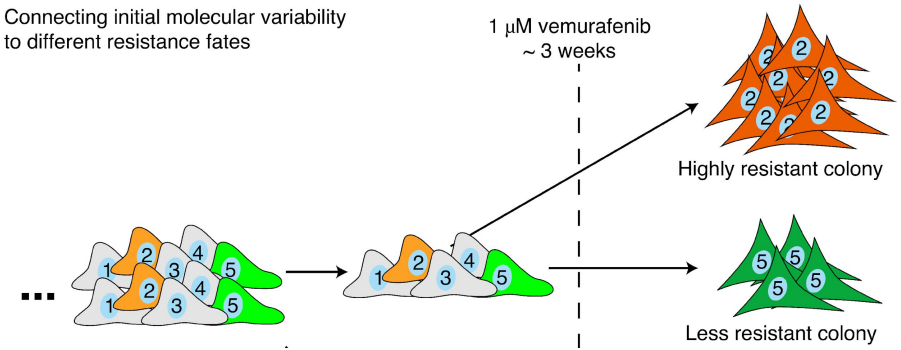
https://doi.org/10.1038/s41587-021-00837-3

Check for updates

2021

Variability within rare cell states enables multiple paths toward drug resistance

Benjamin L. Emert[1], Christopher J. Cote[2,3], Eduardo A. Torre[4], Ian P. Dardani[3], Connie L. Jiang[5], Naveen Jain[5], Sydney M. Shaffer[3,6,7] and Arjun Raj[2,3]



"We have here revealed the existence of a rich set of rare subpopulations within seemingly homogenous cells, several of which can lead to phenotypically distinct fates. Despite the population having a clonal origin and being grown in homogeneous cell culture conditions, these subpopulations spontaneously emerge via transient cell-state fluctuations that can persist for several cell divisions (5–6 generations)"

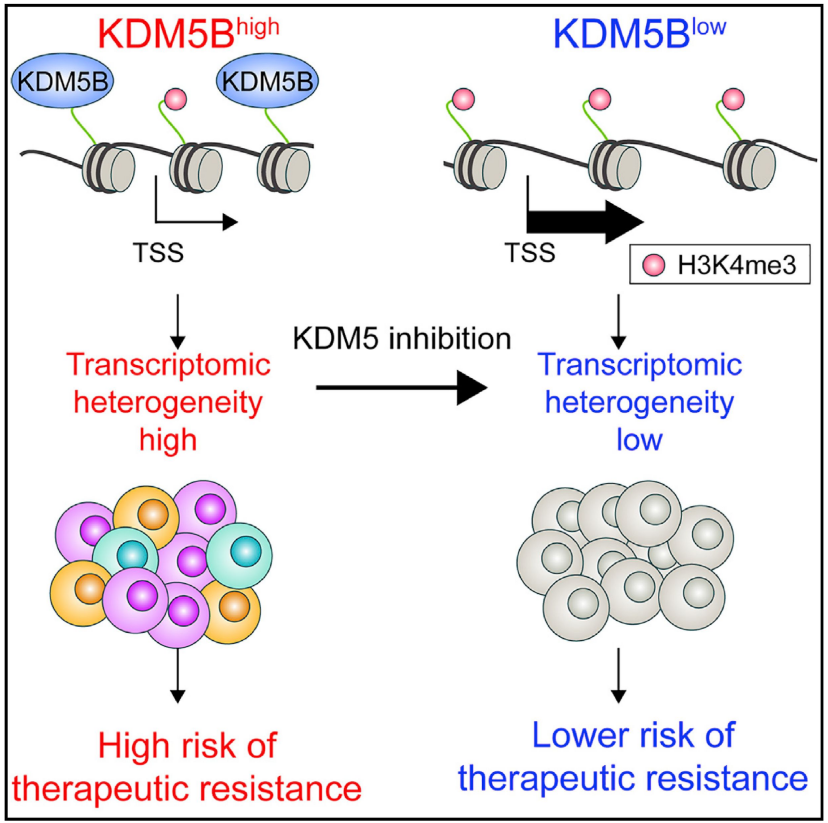
The role of gene expression variability and cellular plasticity

Cancer Cell
Article

2018

KDM5 Histone Demethylase Activity Links Cellular Transcriptomic Heterogeneity to Therapeutic Resistance

Kunihiko Hinohara,[1,2,16] Hua-Jun Wu,[3,4,5,16] Sébastien Vigneau,[6,7] Thomas O. McDonald,[3,4,5,8] Kyomi J. Igarashi,[6,7,13] Kimiyo N. Yamamoto,[3,4,5] Thomas Madsen,[3,4,5] Anne Fassl,[6,7] Shawn B. Egri,[9] Malvina Papanastasiou,[9] Lina Ding,[1,2] Guillermo Peluffo,[1,2] Ofir Cohen,[1,9] Stephen C. Kales,[10] Madhu Lal-Nag,[10] Ganesha Rai,[10] David J. Maloney,[10,14] Ajit Jadhav,[10] Anton Simeonov,[10] Nikhil Wagle,[1,2,9] Myles Brown,[1,2,11,12] Alexander Meissner,[5,9,15] Piotr Sicinski,[6,7] Jacob D. Jaffe,[9] Rinath Jeselsohn,[1,2] Alexander A. Gimelbrant,[6,7] Franziska Michor,[3,4,5,8,9,12,*] and Kornelia Polyak[1,2,8,9,11,12,17,*]



The role of gene expression variability and cellular plasticity

ARTICLES
https://doi.org/10.1038/s41588-020-00749-z
nature genetics
Check for updates

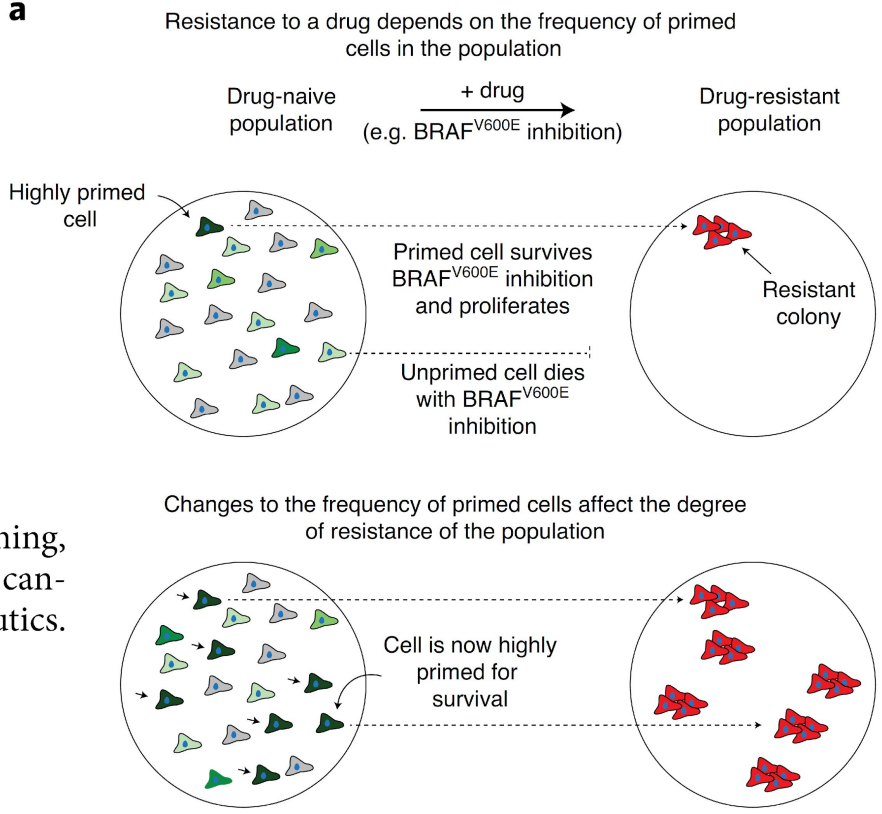
Genetic screening for single-cell variability modulators driving therapy resistance

Eduardo A. Torre[1], Eri Arai[2,10], Sareh Bayatpour[3,10], Connie L. Jiang[4,10], Lauren E. Beck[3], Benjamin L. Emert[5], Sydney M. Shaffer[3,6], Ian A. Mellis[5], Mitchell E. Fane[7], Gretchen M. Alicea[7], Krista A. Budinich[2], Ashani T. Weeraratna[7,8], Junwei Shi[2] ✉ and Arjun Raj[3,9] ✉

2021

We have demonstrated, using high-throughput genetic screening, that there are genetic factors that can alter cellular plasticity in cancer cells, thereby affecting their resistance to targeted therapeutics.

a



The role of gene expression variability and cellular plasticity

Important role of gene expression variability and cellular plasticity in drug resistance.
What about oncogenesis?

Cell-to-cell random phenotypic fluctuations originating from stochastic gene expression (the so-called gene expression noise, or variability) are nowadays considered as a major contributor in the differentiation processes in multicellular organisms.

For example, a genome-wide transcriptional variability precedes cell fate decisions in haematopoietic progenitor cells.

LETTERS

Nature, 2008

Transcriptome-wide noise controls lineage choice in mammalian progenitor cells

Hannah H. Chang[1,2,3], Martin Hemberg[4]†, Mauricio Barahona[4], Donald E. Ingber[1,5] & Sui Huang[1]†

Indeed, highly variable gene expression patterns would be the necessary ground on which developmental “choices” can then be made, the number of expressed genes per cell being a hallmark of developmental potential.

RESEARCH ARTICLE

Science, 2020

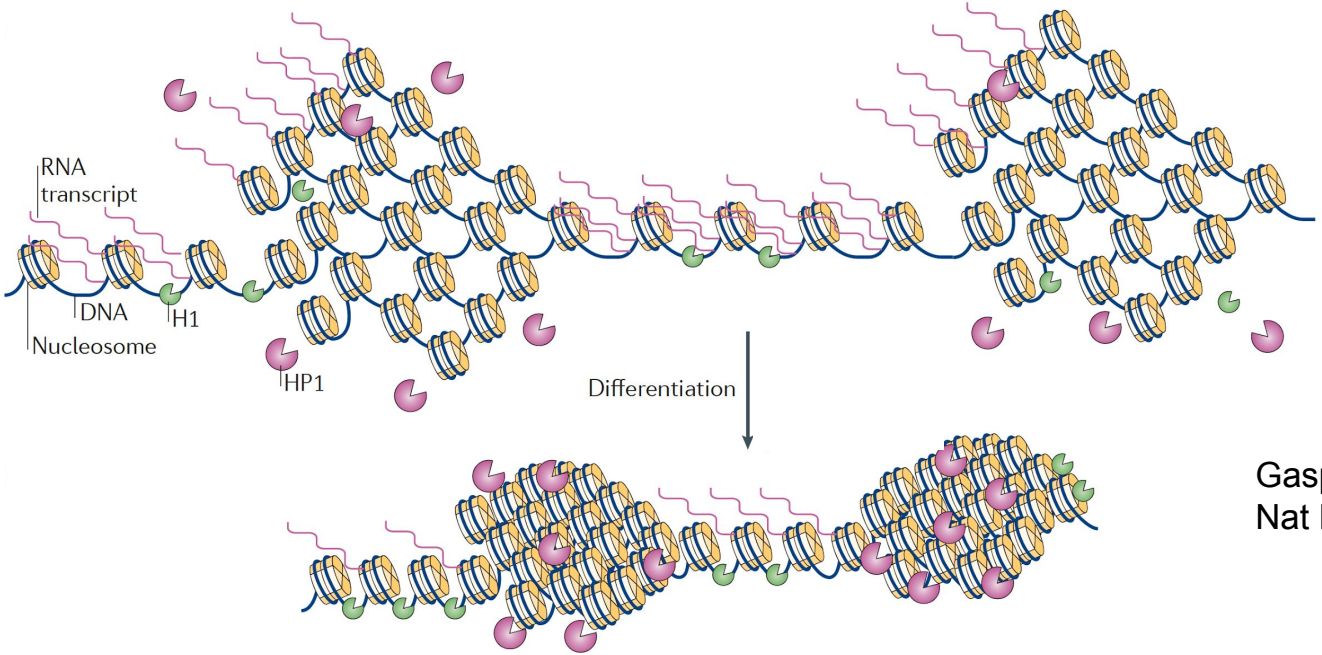
RESEARCH METHODS

Single-cell transcriptional diversity is a hallmark of developmental potential

Gunsagar S. Gulati[1]*, Shaheen S. Sikandar[1]*, Daniel J. Wesche[1], Anoop Manjunath[1], Anjan Bharadwaj[1], Mark J. Berger[2]†, Francisco Ilagan[1], Angera H. Kuo[1], Robert W. Hsieh[1], Shang Cai[3], Maider Zabala[1]‡, Ferenc A. Scheeren[4], Neethan A. Lobo[1]‡, Dalong Qian[1], Feiqiao B. Yu[5], Frederick M. Dirbas[6], Michael F. Clarke[1,7], Aaron M. Newman[1,8]§

The role of gene expression variability and cellular plasticity

Thus, transcriptional diversity, defined as the number genes expressed in a cell, decreases during differentiation. This phenomenon is linked to the progressive reduction in chromatin accessibility during lineage commitment. Therefore, it can now be proposed that the looser chromatin in less-mature cells permits a wider sampling of the transcriptome, whereas chromatin accessibility and transcriptional diversity are restricted in more-differentiated cells as they specialize.



Gaspar-Maia et al, 2011,
Nat Rev Mol Cell Biol

The role of gene expression variability and cellular plasticity

Decreased gene expression variability and homogeneization of expression profiles during differentiation.

Statistical Mechanics of Pluripotency

Ben D. MacArthur[1,*] and Ihor R. Lemischka[2]

Cell, 2013

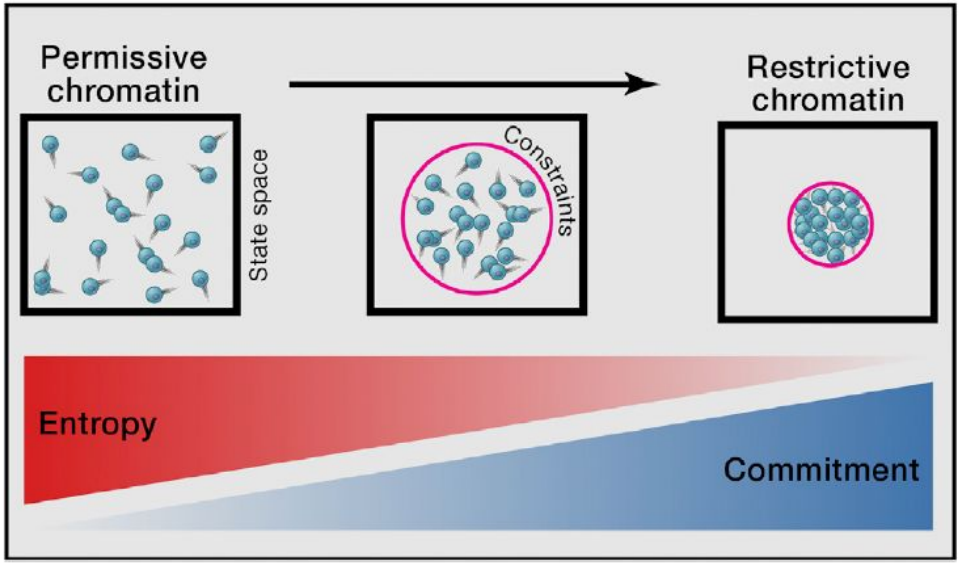


Figure 1. Entropy and Developmental Potency

The permissive regulatory architecture of PSCs imposes weak constraints on mRNA/protein expression, giving rise to high-entropy expression patterns within the population. As differentiation progresses expression patterns become more tightly constrained and population entropy decreases.

The role of gene expression variability and cellular plasticity

Both biological and physical considerations allow arguing for a theory that considers the acquisition of differentiated features as the result of decreased cellular stochasticity linked to the appearance of dynamically evolving environmental constraints acting at the cell (through compartmentation and mesoscopic structures such as chromatin, nuclear membrane, etc), tissue (through cell-cell interactions and communications) and organism (through endocrine, immunity and blood networks) levels.

frontiers in Cell and Developmental Biology 2020 HYPOTHESIS AND THEORY published: 23 July 2020 doi: 10.3389/fcell.2020.00659

Check for updates

A Darwinian and Physical Look at Stem Cell Biology Helps Understanding the Role of Stochasticity in Development

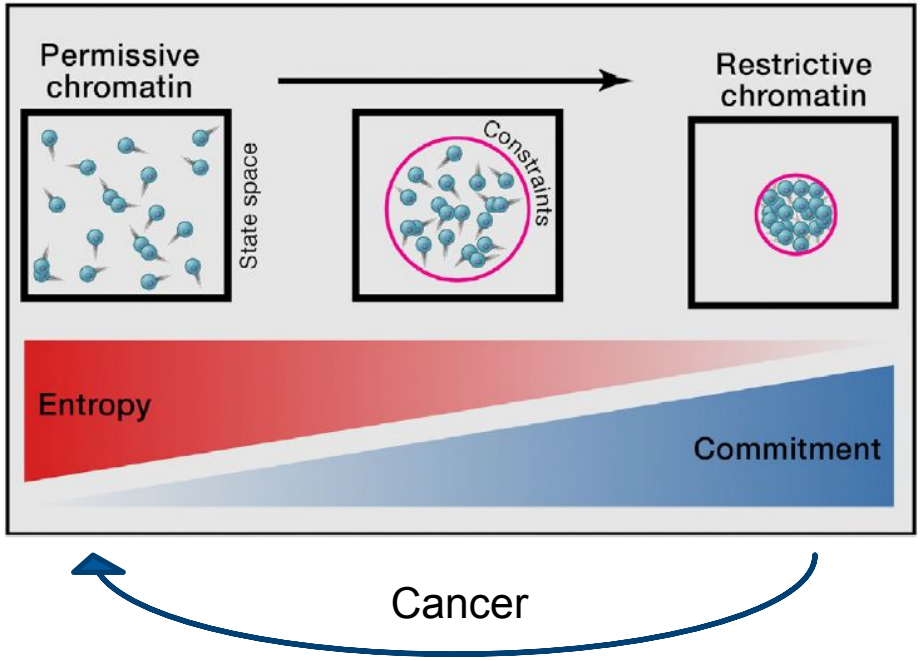
Jean-Pascal Capp[1] and Bertrand Laforge[2]*

Generation of a differentiated state as a constrained random process: randomness is provided by the stochastic dynamics of biochemical reactions while the environmental constraints, including cell inner structures and cell-cell interactions, drive the system toward a stabilized state of equilibrium.

tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

The role of gene expression variability and cellular plasticity

And in cancer ?



Mac Arthur & Lemischka, Cell, 2013

Cancer = removal of the microenvironmental control, disruption of the normal cellular interactions?

⇒ Increased cellular stochasticity (= increased entropy)

Tissue-disruption-induced cellular stochasticity at the origin of cancer

Tissue disruption increases stochastic gene expression thus producing tumors: Cancer initiation without driver mutation

Jean-Pascal Capp

Int J Cancer, 2017

Any disruption of the tissue equilibrium should able to produce phenotypic plasticity

⇨ In this case, differentiation and quiescence can no longer be maintained because of the stochastic nature of gene expression which will be no more ‘controlled’ by the cellular microenvironment

Tissue-disruption-induced cellular stochasticity at the origin of cancer

Initiation = failure of the microenvironmental control

But increase of the probability that precancerous cells are more aggressive more rapidly with mutagenic agents and hereditary predispositions

Increased probability that a tumor forms, but only if the correct tissue microenvironment is not maintained

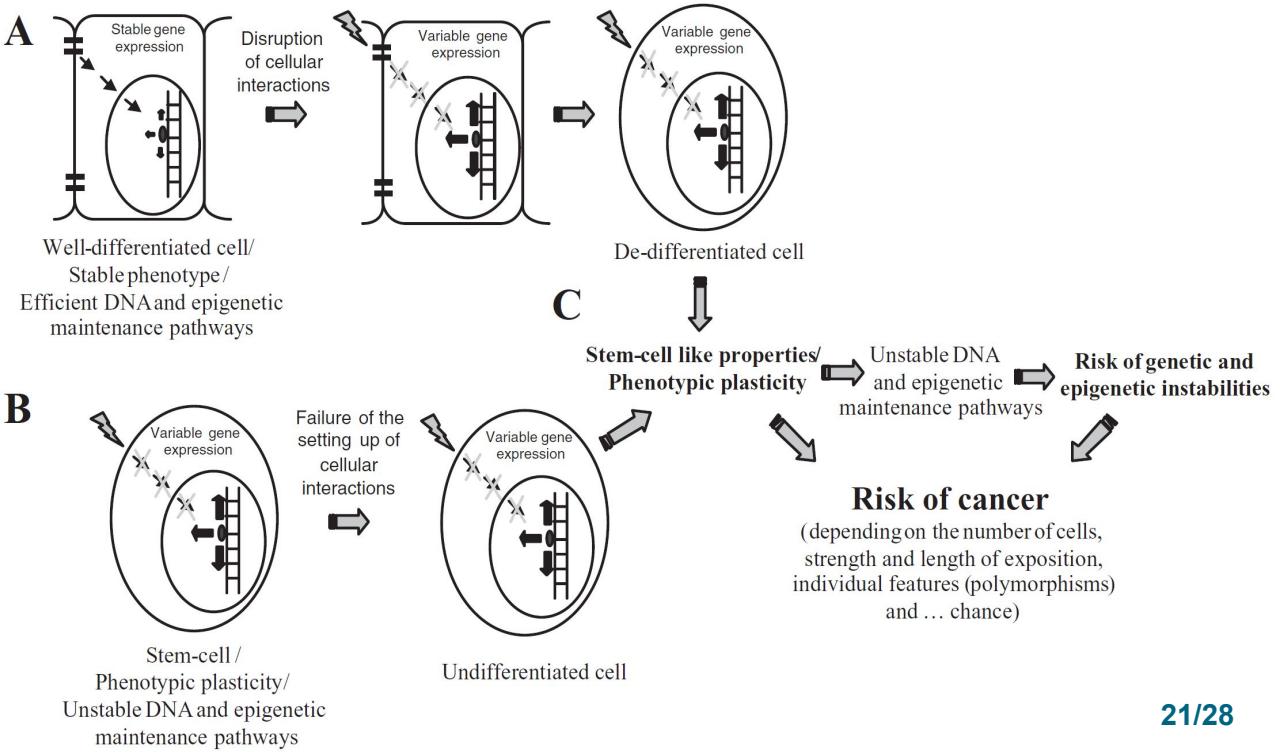
Reversed perspective :
disruption of tissue equilibrium is the initial event, and genetic alterations are promoting factors

Tissue-disruption-induced cellular stochasticity at the origin of cancer

Stochastic gene expression, disruption of tissue averaging effects and cancer as a disease of development

Jean-Pascal Capp

BioEssays, 2005



Tissue-disruption-induced cellular stochasticity at the origin of cancer

Many more intriguing observations can be explained by this model, e.g. the fact that the frequency of cancer stem cells (CSC) depends on the micro-environment…

Efficient tumour formation by single human melanoma cells

Nature, 2008

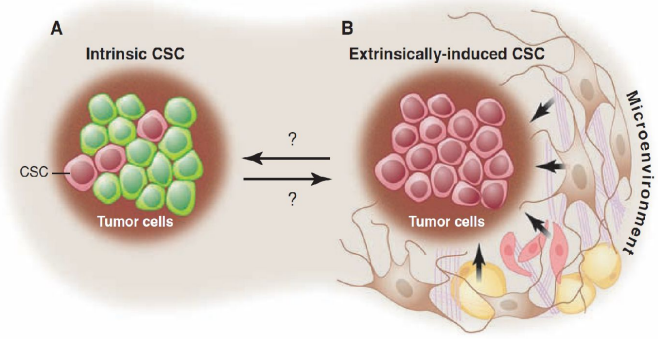
Elsa Quintana[1]*, Mark Shackleton[1]*, Michael S. Sabel[2], Douglas R. Fullen[3], Timothy M. Johnson[4] & Sean J. Morrison[1]

25% of CSC, without any specific molecular marker

The Increasing Complexity of the Cancer Stem Cell Paradigm

Jeffrey M. Rosen[1] and Craig T. Jordan[2]

Science, 2009



Hindawi
Journal of Oncology
Volume 2019, Article ID 5189232, 10 pages
https://doi.org/10.1155/2019/5189232

Hindawi

Review Article

Cancer Stem Cells: From Historical Roots to a New Perspective

J Oncol, 2019

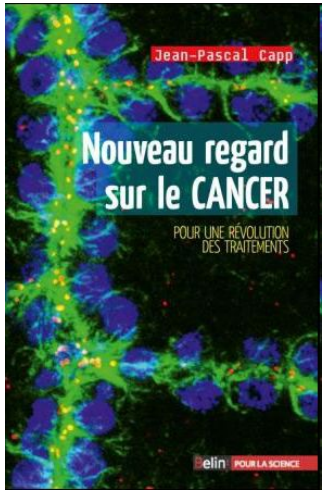
Jean-Pascal Capp

tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

Tissue-disruption-induced cellular stochasticity at the origin of cancer

… and also:

- the normalizing effect of an healthy micro-environment,
- the presence of oncogenic mutations in healthy tissues,
- the absence of driver mutations in precancerous lesions,
- the epigenetic and gene expression alterations that precede cancerization,
- …



Belin, 2012
Preface Jean-Jacques Kupiec
Postface Gilles Favre

The example of multiple myeloma

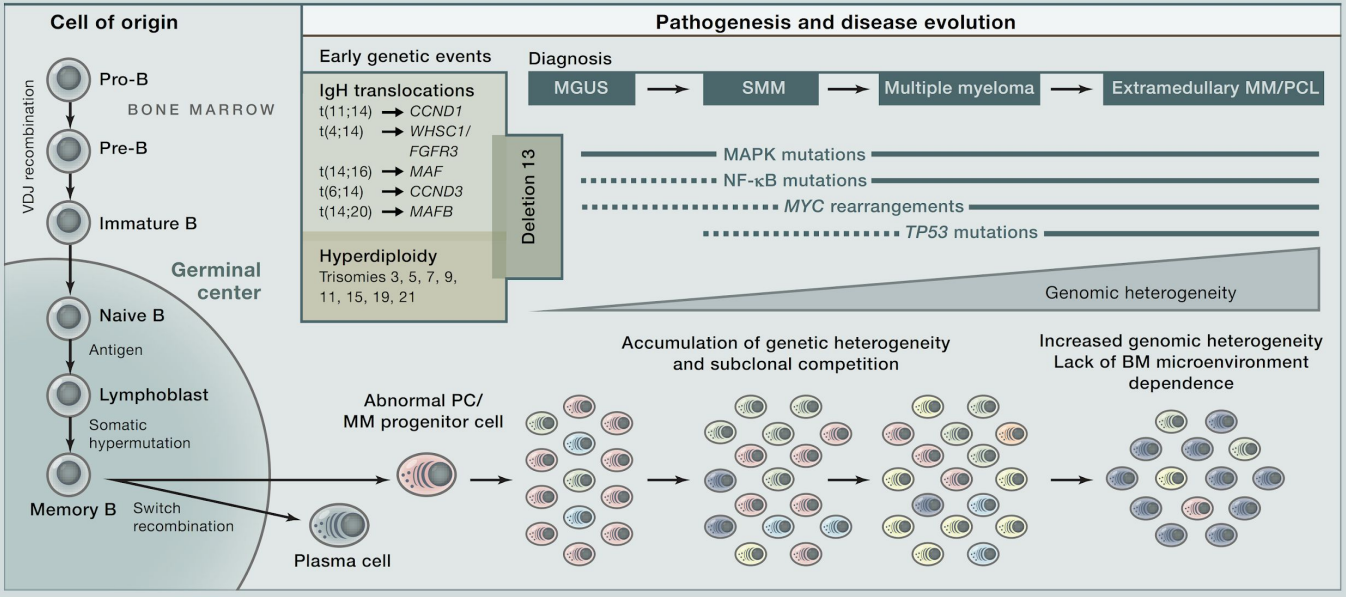
Classical genetic model:

2015

SnapShot: Multiple Myeloma

Esteban Braggio, K. Martin Kortüm, A. Keith Stewart
Division of Hematology-Oncology, Mayo Clinic, Scottsdale, AZ 85259, USA

Cancer Cell



The example of multiple myeloma

frontiers in Oncology

2018

HYPOTHESIS AND THEORY
published: 10 September 2018
doi: 10.3389/fonc.2018.00355

Multiple Myeloma Exemplifies a Model of Cancer Based on Tissue Disruption as the Initiator Event

Jean-Pascal Capp [1] and Régis Bataille [2]*

cancers

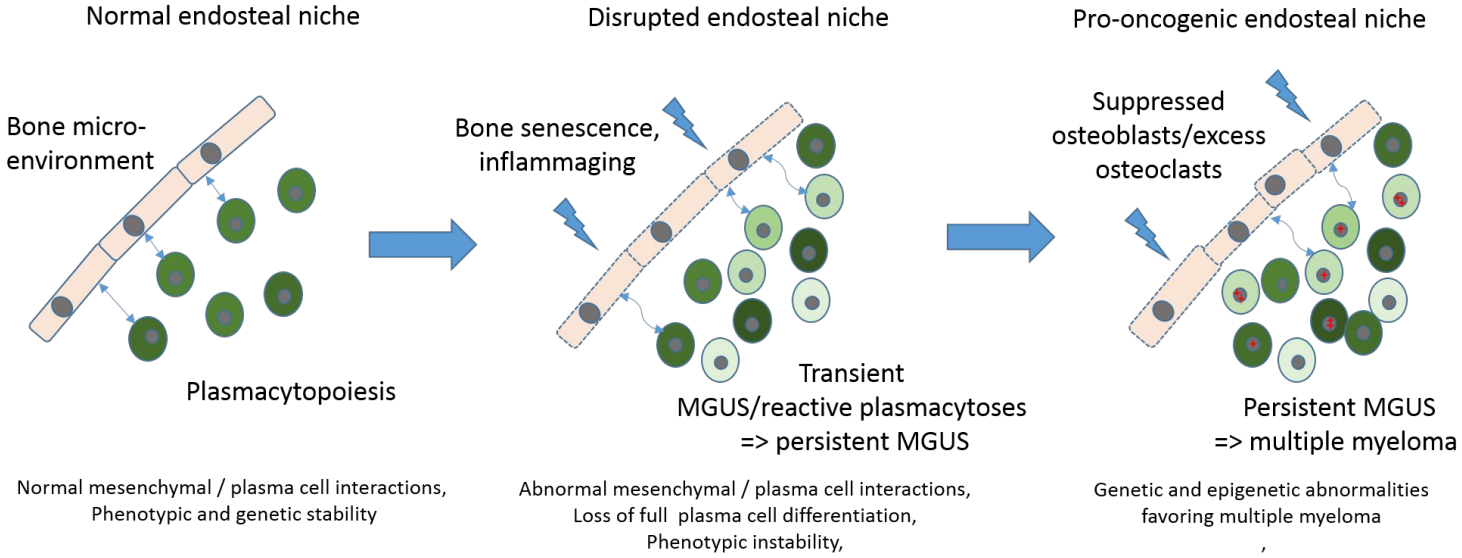
2020

MDPI

Perspective

Multiple Myeloma as a Bone Disease? The Tissue Disruption-Induced Cell Stochasticity (TiDiS) Theory

Jean-Pascal Capp [1,*] and Régis Bataille [2]



Capp & Bataille, Crit Rev Oncol Hematol, in revision

tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

Common origins with aging?

DOI: 10.1002/bies.202000140

PROBLEMS & PARADIGMS

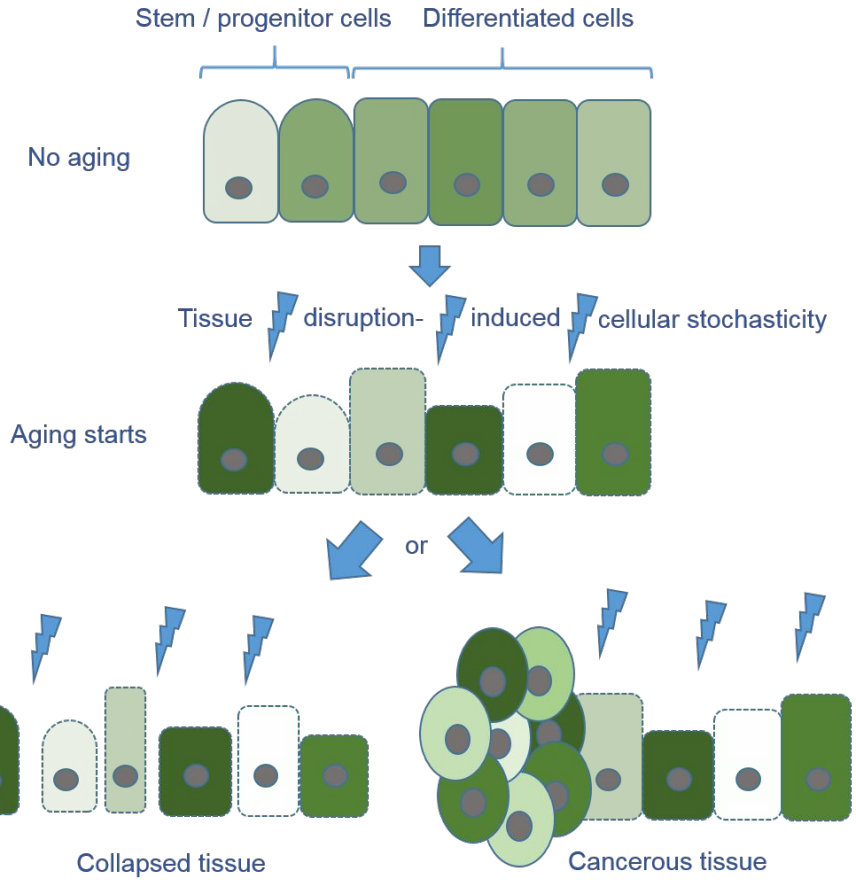
Prospects & Overviews

BioEssays WILEY

Tissue-disruption-induced cellular stochasticity and epigenetic drift: Common origins of aging and cancer?

Jean-Pascal Capp[1] | Frédéric Thomas[2]

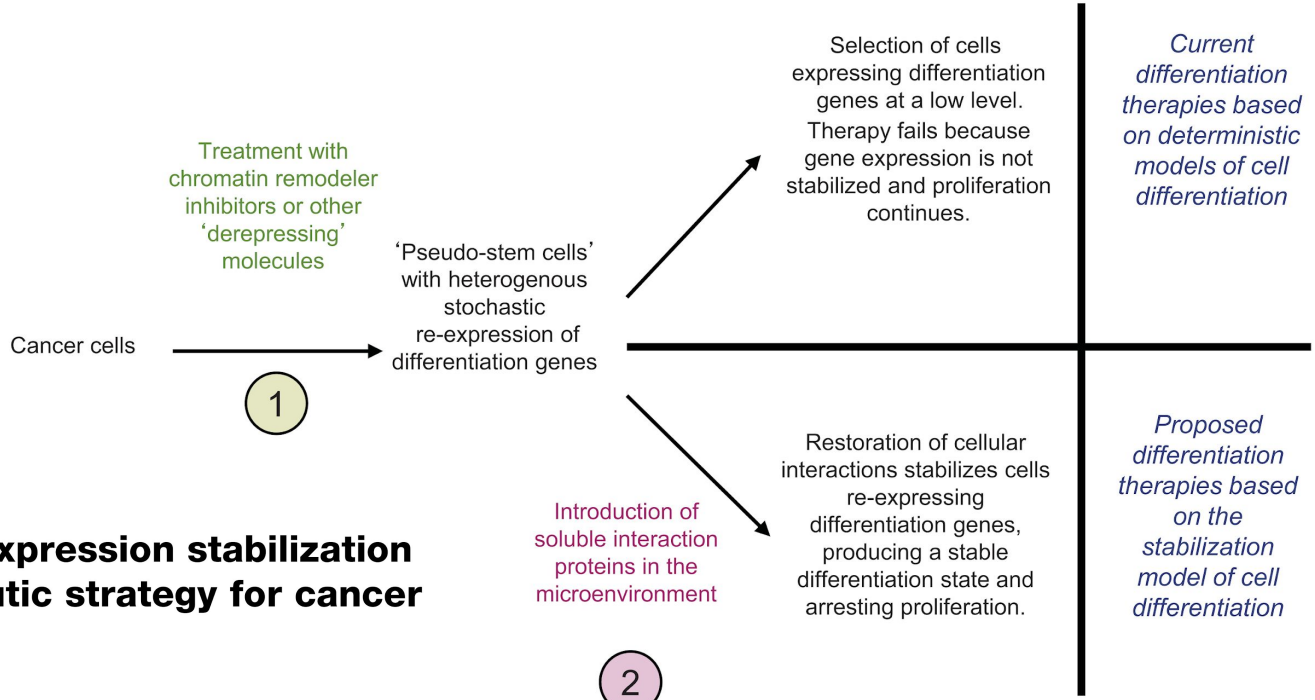
2021



tbi Toulouse Biotechnology Institute Bio & Chemical Engineering

Conclusion: back to drug resistance

Cancer cells probably remain intrinsically unstable and plastic because they do not interact with their native microenvironment anymore. In this perspective, targeting driver genetic events is clearly not sufficient because this phenotypic instability and plasticity would allow them to counteract these treatments. On the contrary, searching for molecules that stabilize gene expression and thus help to restore full differentiation and quiescence appears to be a valid alternative. Only molecules that interact with the cancerous cells and "mimic" their original microenvironment would be able to phenotypically stabilize them.

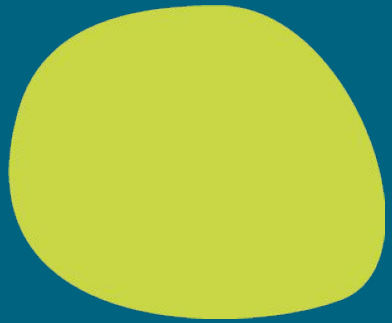


Ideas & Speculations

Stochastic gene expression stabilization as a new therapeutic strategy for cancer

Jean-Pascal Capp

BioEssays, 2012



Thank you

Toulouse Biotechnology Institute
Bio & Chemical Engineering