Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

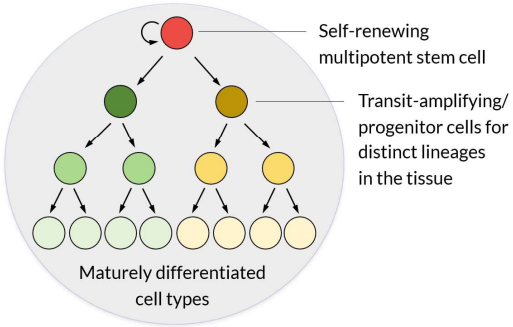
Cancer Stem Cells

Wenjun Guo, Ph.D.

Associate Professor
Department of Cell Biology
Albert Einstein College of Medicine
New York City, USA

1

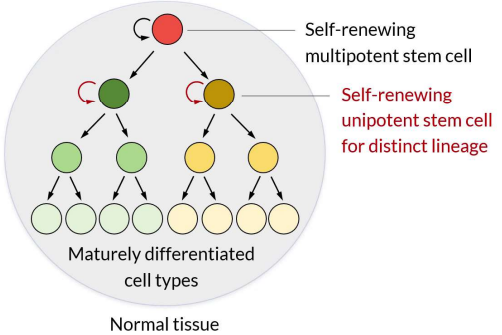
Multipotent stem cells maintain tissue homeostasis



2

Lineage-restricted unipotent stem cells

Some tissues can be maintained by lineage-restricted unipotent stem cells



3

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

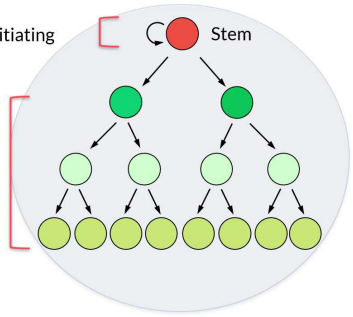
Early concept of cancer stem cells (CSCs)

Tumors as caricatures of the process of tissue renewal

Stem-like, tumor-initiating

Stem

Increasingly differentiated, non-tumor-initiating



Tumor

4

Pierce J. B. and Speers W.C. *Cancer Res.* 1988; **48**(8): 1996-2004.

Discovery of CSCs in leukemia

Acute myeloid leukemia

FACS sorting

CD34+ CD38–

CD34+ CD38+

CD34–

Transplantation to immunodeficient mice

Re-initiate leukemia

No leukemia

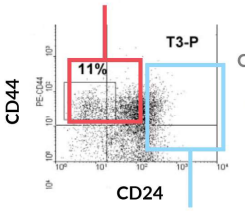
1 in 2.5 X 10^5 leukemia cells can reinitiate cancer

5

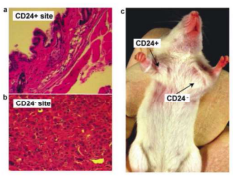
Lapidot T. *et al., Nature.* 1994; **367**: 645–648.

Identification of breast CSCs

100 cells can form tumor



Orthotopic injection



Tens of thousand cells can not form tumor

Breast CSCs are enriched in $CD44^{high}CD24^{low}$ cell population

6

Al-Hajj M. *et al., Proc Natl Acad Sci U S A.* 2003; **100**(7): 3983-3988.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Identification of glioma CSCs

Glioblastoma or medulloblastoma

↓ FACS sorting

CD133+ → Re-initiate glioma

CD133– → No tumor

7

Singh SK. *et al., Nature.* 2004; **432**(7015):396-401.

CSCs have been identified in many other cancer types

Colon cancer

Prostate cancer

Ovarian cancer

Squamous cell carcinoma

Pancreatic cancer

Liver cancer

...

8

In some cancer types, a large portion of tumor cells have tumorigenic potential

Melanoma

↓ Single cell transplantation to deeply immunodeficient NSG mice

25% cells can re-initiate tumor growth

These tumors may not follow the hierarchical CSC model or have very shallow hierarchies

9

Quintana E. *et al., Nature.* 2008; **456**(7222): 593-598.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Commonly used markers for identifying CSCs

Cell surface markers

- CD44, CD133, CD90, CD34, CD271 ...
- Convenient, but not specific to CSCs
- Need to use in combination
- Not all cell surface markers are functionally important for CSCs function/activity

10

Commonly used markers for identifying CSCs

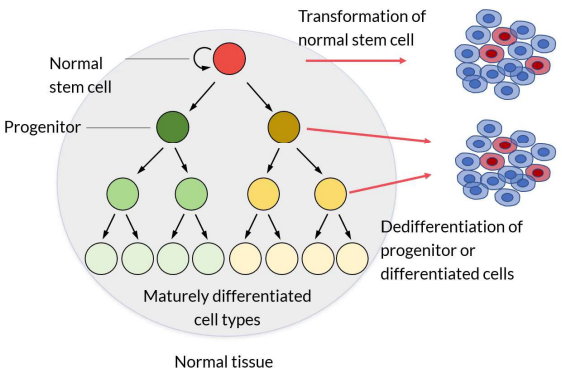
Cell surface markers

High ALDH activity

Reporters of stem cell signalling pathways

- Wnt
- Notch
- STAT3
- Msi2
- Nanog
- Oct4/Sox2

Possible origins of CSCs



11

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

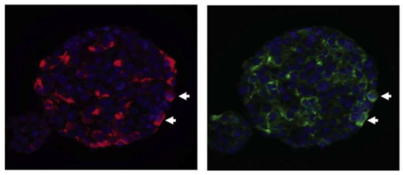
Detecting CSC activity by *in vitro* clonogenic assays

Tumor sphere culture

Neural and memory stem cells can grow into spheroid structures when cultured in suspension in the presence of different growth factors

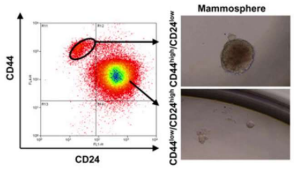
12

Detecting CSC activity by *in vitro* clonogenic assays



Stem

Non-adherent culture



Stem

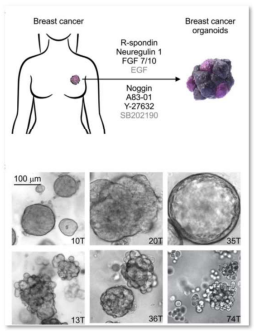
Non-stem

Mani *et SA. al., Cell.* 2008; **133**(4): 704-715.

Measuring CSC activity by *in vitro* clonogenic assays

Organoid culture

Culture in extracellular matrix, *e.g.* **Matrigel**



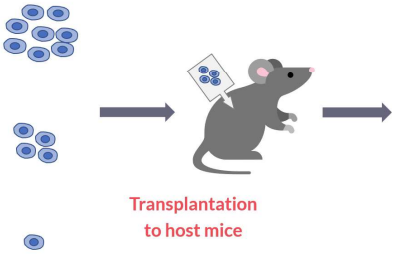
13

Sachs *et al., Cell.* 2018; **172**(1-2): 373-386.e10.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Measure CSC frequency by limiting dilution transplantation

Different doses of cells



Transplantation to host mice

- Measure tumor onset
- Calculate CSC frequency by limiting dilution analysis

14

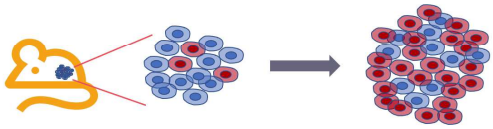
Studying CSC function by lineage tracking *in vivo*

- Transplantation assays take tumor cells out of their native microenvironment and inject them into a foreign environment
- This may not reflect the fate of the cells in an unperturbed tumor microenvironment
- The ability of cancer cell to grow into a new tumor in a new host may depend on its ability to adapt to the new foreign environment
- **Lineage tracing approach -** can measure CSCs function and fate in an unperturbed tumor microenvironment

15

Studying CSC function by lineage tracking *in vivo*

Labelled CSCs contribute to tumor growth



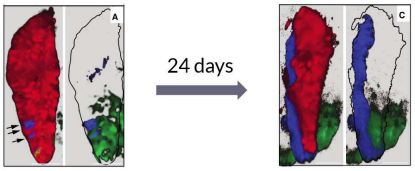
Genetically label CSCs with detectable markers

Measure CSC activity in native tumor microenvironment

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Lineage tracing identifies Lgr5+ cells as intestinal adenoma CSC

LGR5 is a stem cell marker which was initially identified in normal intestinal stem cells



Some Lgr5+ CSCs were labelled by CFP

CFP+ CSCs generate tumor mass over time

16

Schepers *et al.*, *Science*. 2012; **337**(6095): 730-5.

Studying CSC function by cell ablation *in vivo*

Cell ablation

- One can specifically express in CSCs an inducible suicide gene
- This can be done by utilizing CSC promoter to drive the expression of suicide genes or toxin transporters

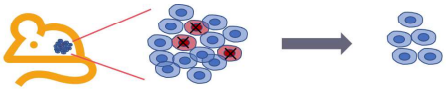
17

Studying CSC function by cell ablation *in vivo*

Cell ablation

Ablate CSCs with suicide genes or toxins

Tumor regression



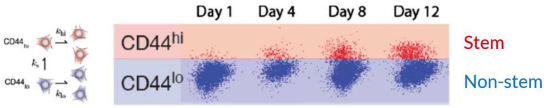
This could suggest high levels of plasticity within the tumor mass

Determine whether non-CSCs can be converted to CSCs

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Cellular plasticity contributes to the conversion of non-CSC to CSC

Breast cancer cells in culture

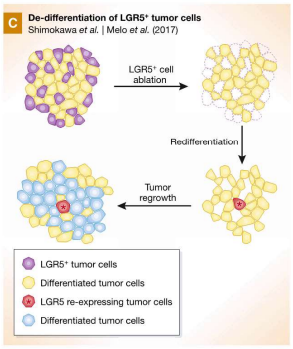


Non-CSCs can regenerate CD44^{high} stem cell population

18

Chaffer CL. *et al., Proc Natl Acad Sci U S A.* 2011; **108**(19): 7950-7955.

Non-CSCs can generate CSCs *in vivo* upon CSC ablation



There is a fair amount of plasticity between CSC and non-CSC

Non-CSC can revert to CSC, especially when CSC were irradicated or injured

In order to effectively treat cancer, one needs to **irradicate CSCs** but also **block the conversion of non-CSCs into CSCs**

19

Dieter SM. *et al., EMBO Mol Med.* 2017; 9(7): 856-858.
Shimokawa M. *et al.*, 2017; *Nature.* **545**(7653): 187-192. de Sousa e Melo F. *et al., Nature.* 2017; **543**(7647): 676-680.

Contribution of CSCs to metastasis

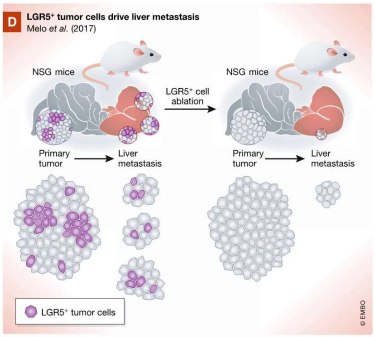
Tumor metastasis is a process in which cancer cells re-initiate a new tumor in a foreign organ

One would imagine that CSCs are needed for metastasis formation

20

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Contribution of CSCs to metastasis



Dieter SM. *et al., EMBO Mol Med.* 2017; 9(7): 856-858.
Melo F. *et al., Nature.* 2017; **543**(7647): 676-680.

Contribution of CSCs to metastasis

- Tissue microenvironment at the distant organ may be less reactive than that of the primary tumor
- Primary tumor microenvironment is highly-active and provides the necessary signals to convert non-CSCs to CSCs
- Can non-CSCs convert to CSCs during metastasis? This would explain how metastasis occurs years after the removal of the primary tumor

Dieter SM. *et al., EMBO Mol Med.* 2017; 9(7): 856-858.
Melo F. *et al., Nature.* 2017; **543**(7647): 676-680.

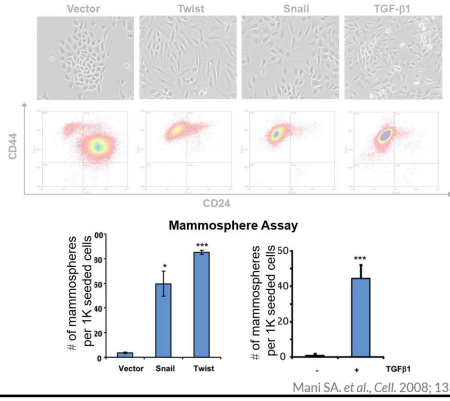
Epithelial-mesenchymal transition (EMT) and CSC plasticity

- EMT is closely associated with the induction of stemness
- EMT was initially identified during embryonic development where it is involved in establishing three germ layers and generating diverse cell types
- During EMT epithelial cells will lose their epithelial markers and properties and gain mesenchymal features

21

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

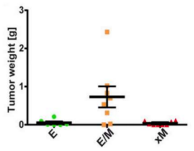
Epithelial-mesenchymal transition (EMT) and CSC plasticity

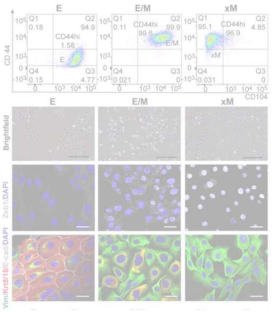


Mani SA. *et al., Cell.* 2008; 133(4): 704-715.

EMT cells are associated with higher CSC activity

- Cells can reside at a spectrum between fully epithelial and fully mesenchymal
- EMT cells are associated with higher CSC activity



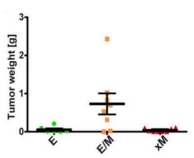


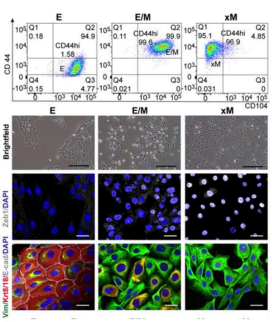
22

Kroger C. *et al., Proc Natl Acad Sci U S A.* 2019; 116(15): 7353-7362.

EMT cells are associated with higher CSC activity

- The cells with hybrid EMT features are likely to have the highest plasticity





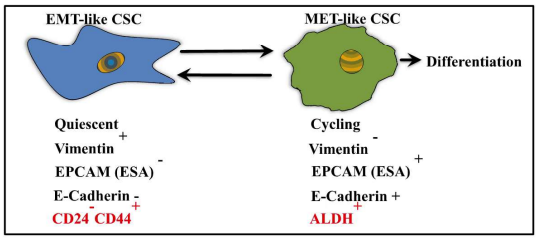
Kroger C. *et al., Proc Natl Acad Sci U S A.* 2019; 116(15): 7353-7362.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Distinct CSC populations could exist in the same tumor

Breast cancer

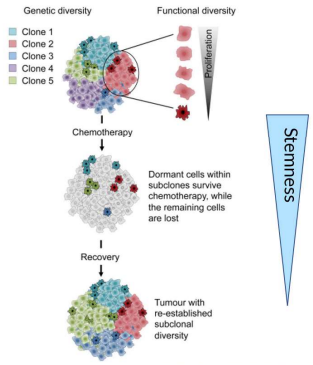
Invasive edge | Tumor interior



23

Liu S. *Cell. et al.*, 2013; 2(1): 78-91.

Contribution of the CSC model to intratumor heterogeneity

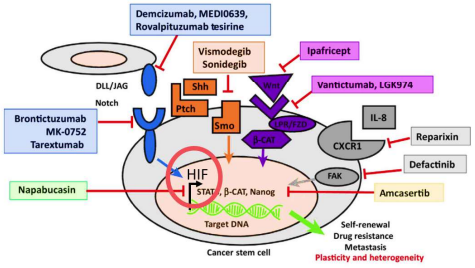


24

Kreso A. and Dick JE. *Cell.* 2014; 14(3): 275-291.

Control of CSCs by developmental signaling pathway

- Wnt
- Notch
- Hedgehog
- Hypoxia-inducible factor (HIF)



CSC-associated signaling pathways

25

Ramos EK. *et al., Trends Cancer.* 2017; 3(11): 780-796.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Control of CSC by epigenetic regulators

Promoting cancer stemness

Polycomb Repressor Complex 1 (PCR1)	Bmi1: in various cancer types
Polycomb Repressor Complex 2 (PCR2)	EZH2: in various cancer types
JARID1B	Melanoma
LSD1	

26

Control of CSC by epigenetic regulators

Suppressing cancer stemness

TET2	AML
DNMT3A	AML

Metabolic regulators for CSCs

- CSCs often have unique metabolic profiles
- CSCs can use glycolysis or oxidative phosphorylation, depending on the tumor type and microenvironment
- Some CSCs are dependent on glycolysis and fatty acid metabolism

27

Dong C. *et al.*, Cancer cell. 2013; **23**(3): 316-331.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Metabolic regulators for CSCs

Breast cancer example

FBP1 ↓

↓

Glycolysis ↑

↓

ROS ↓

↓

Cancer stemness ↑

Dong C. *et al.*, Cancer cell. 2013; **23**(3): 316-331.

Niche regulation of CSCs

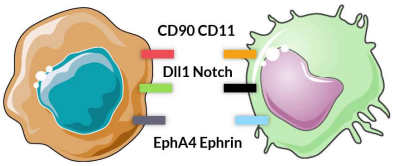
The niche means the microenvironment surrounding the cells provided by specific cell types, extracellular matrices or soluble factors

28

Lu H. *et al.*, *Nat Cell Biol.* 2014; 16(11): 1105-1117.
Chakrabarti R. *et al.*, *Science.* 2018; 360(6396): eaan4153.

Niche regulation of CSCs

Interaction of breast CSCs with macrophages



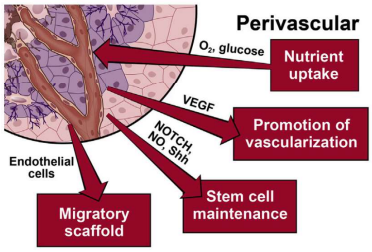
CSC Macrophage

Lu H. *et al.*, *Nat Cell Biol.* 2014; 16(11): 1105-1117.
Chakrabarti R. *et al.*, *Science.* 2018; 360(6396): eaan4153.

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Niche regulation of CSCs

Peri-vascular niche for glioma stem cells



29

Prager BC. *et al., Cell.* 2019; 24(1):41-53.

CSC properties causing chemo- and radio-therapy resistance

Mechanisms rendering CSCs therapeutic resistant are still under investigation, however, some include:

Upregulation of drug-efflux pumps

Enhanced DNA-repair capacity

Enhanced protection against ROS

Ability to adopt a quiescent state

Increased cell plasticity, e.g. EMT

30

Evasion or suppression of anti-tumor immunity by CSCs

1. Downregulation of MHC1 and antigen presenting machinery
2. Downregulation of NK cell receptor ligands
3. Upregulation of immune checkpoint molecules
4. Recruitment of immunosuppressive cells by paracrine signalling

31

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA

Therapeutic strategies targeting CSCs

- Inhibiting CSC signaling pathways → Wnt, Notch, Hedgehog
- Targeting epigenetic regulators key to CSCs
- Targeting aberrant metabolism of CSCs
- Targeting CSC niche
- Immunological targeting of CSC antigens → CAR-T cells against GD2
- Differentiation therapy - convert CSCs to non-CSCs → All-trans retinoic acid for PML-RARα-induced acute promyelocytic leukemia

32

Therapeutic strategies targeting CSCs

Challenge

↓

Targeting CSCs

BUT

Sparing normal stem cells

Summary

- Basic introduction of CSC biology
- CSC is a rapidly evolving and progressing field

33

Dr. Wenjun Guo – Albert Einstein College of Medicine, USA