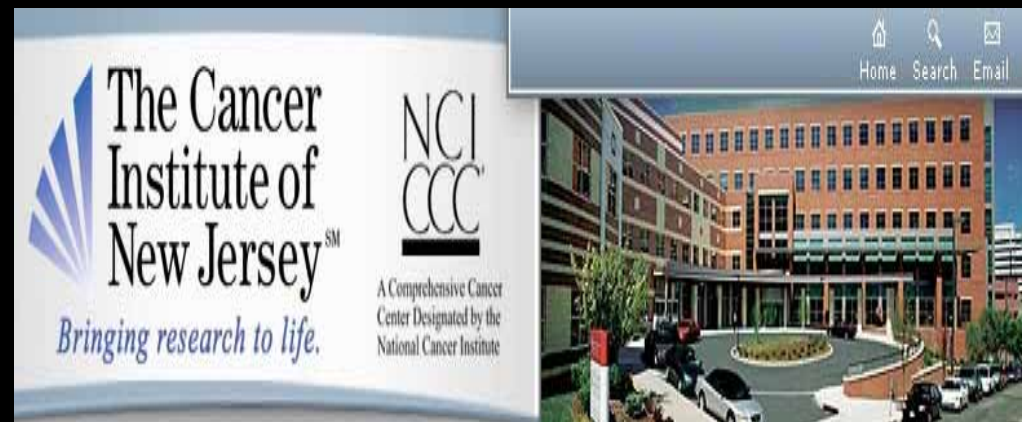
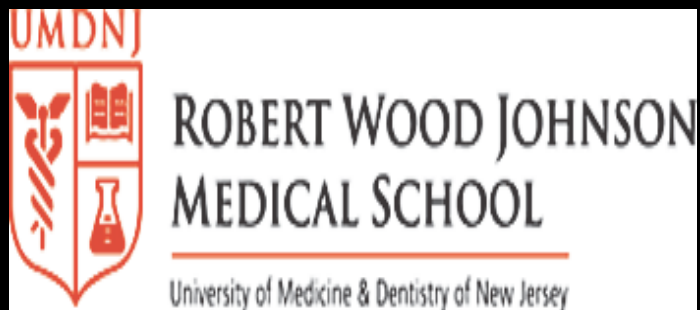


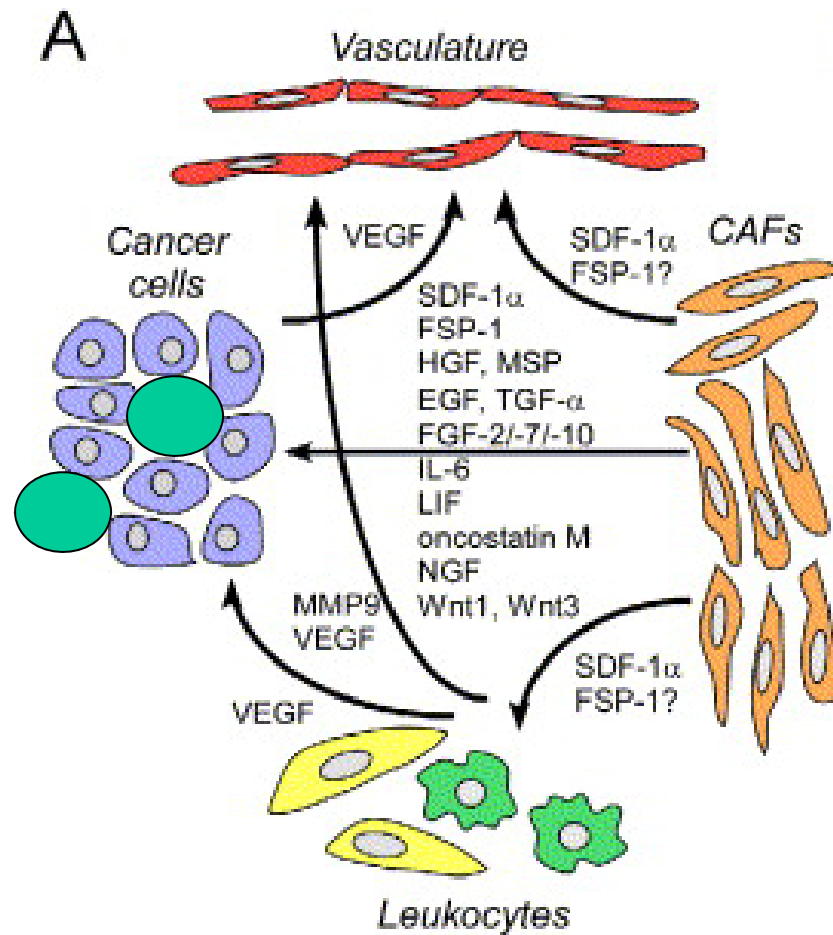
# Stem cells and Cancer

John Glod

Lehigh University  
December 2, 2009



# The Tumor Microenvironment



HYBRID

**SWEET CORN**

PEACHES AND CREAM  
BI-COLOR

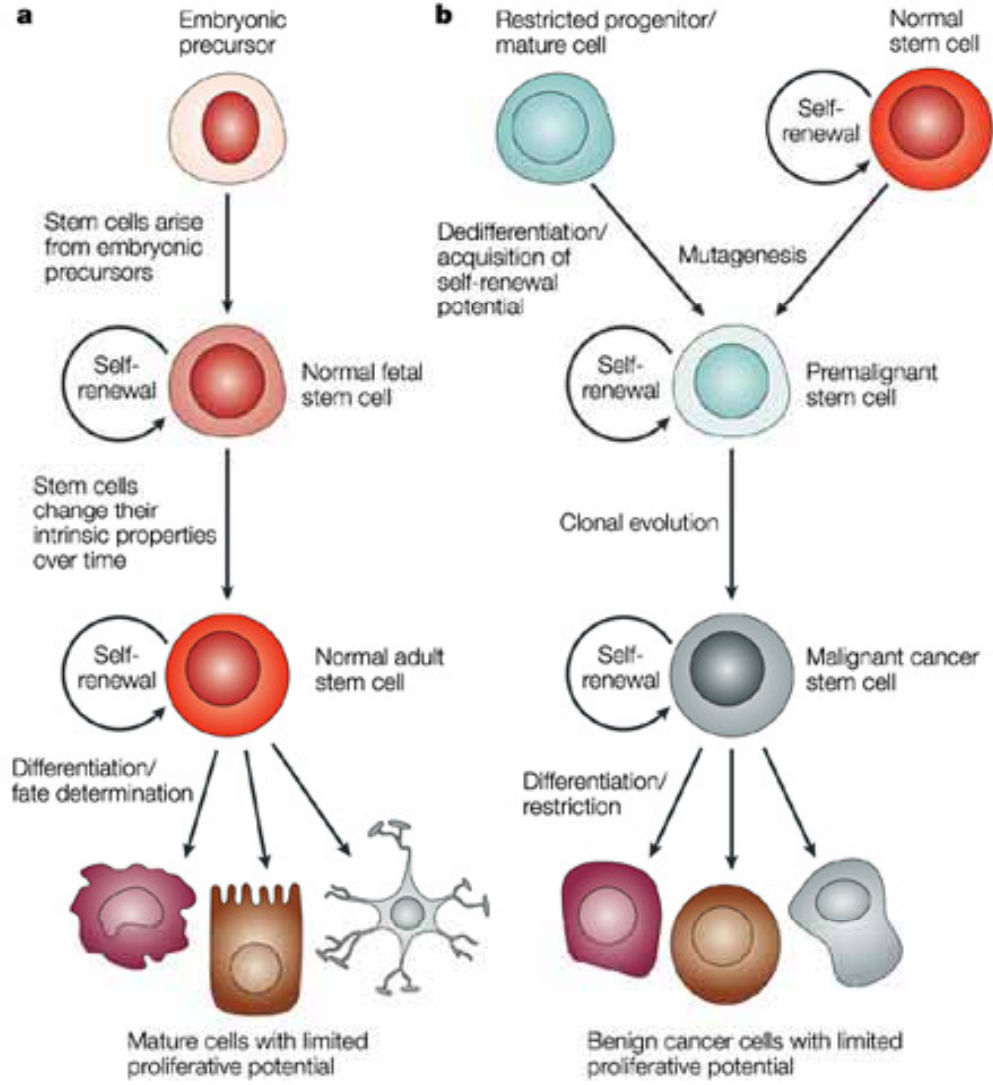
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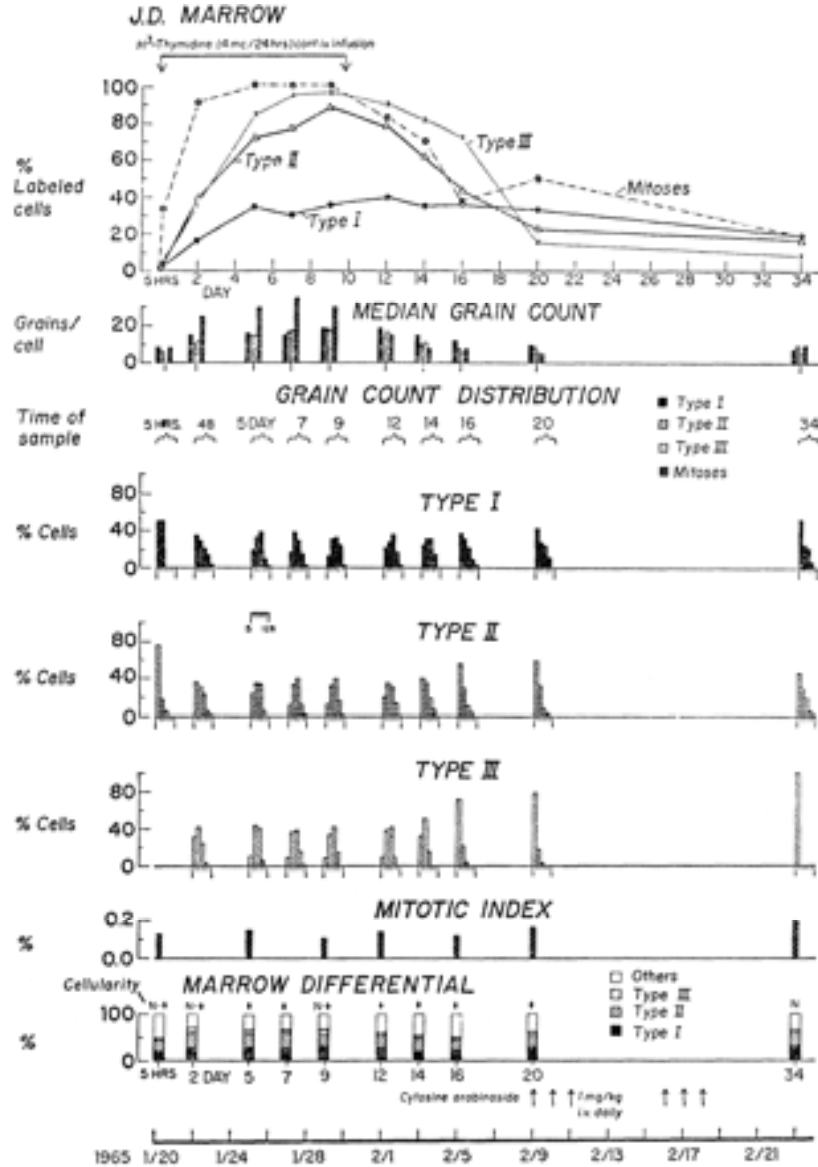




# Cancer Stem Cells

- A small group of cells within the larger tumor bulk is responsible for the maintenance of tumor replication.
- This small percentage of Cells has properties that are different from the bulk of the tumor cells.
- These properties include limitless replication potential and the ability to differentiate into multiple different cell types.

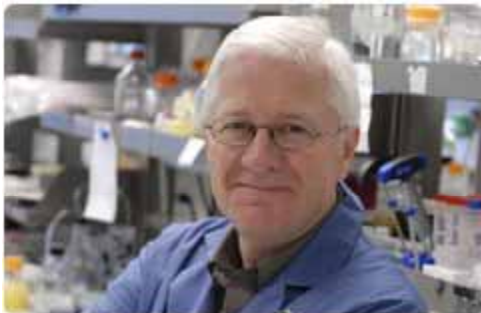
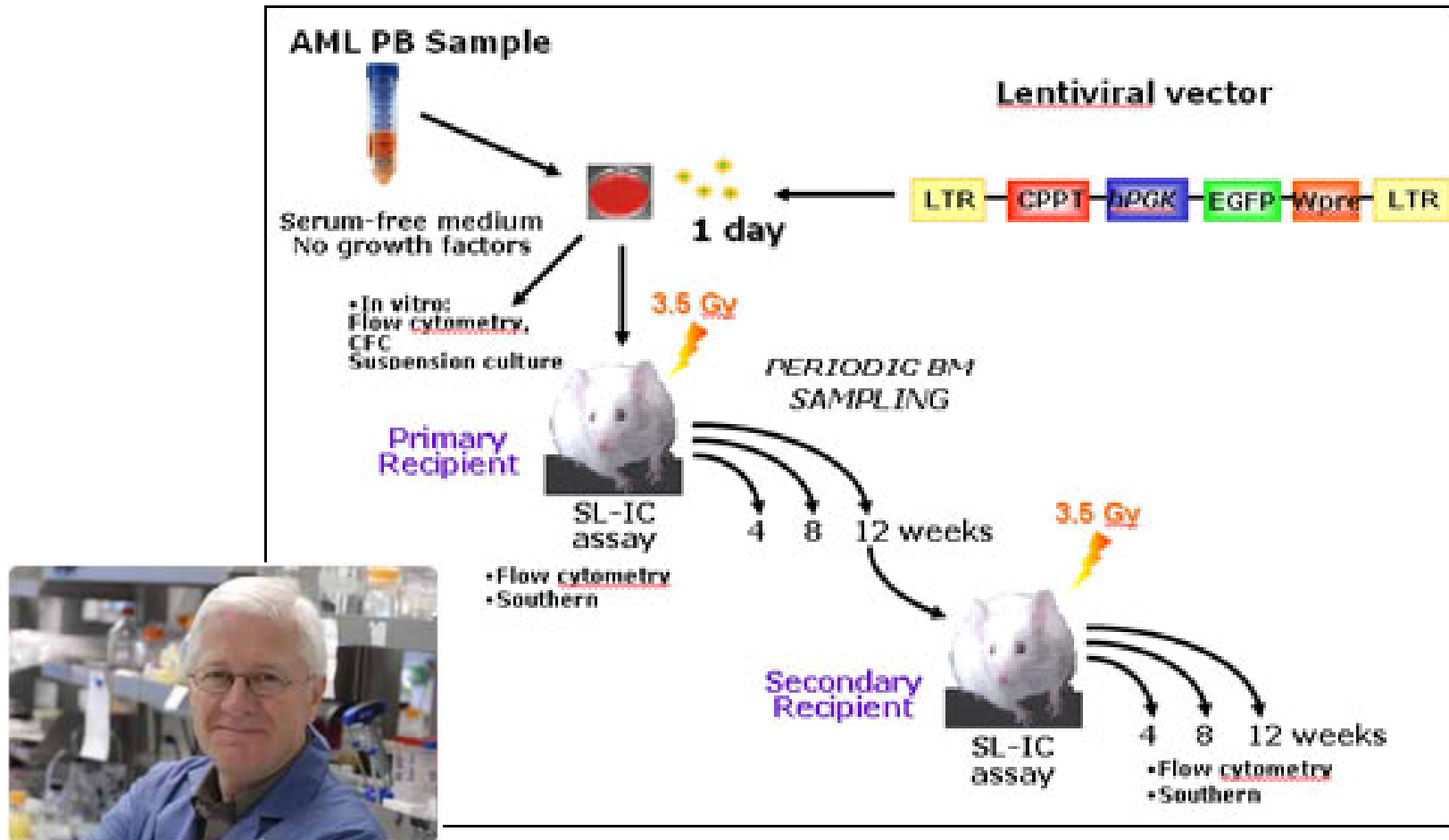
# The first Cancer Stem cell



**Figure 1. Labeling pattern of leukemic cells in marrow of patient 1.** Patient 1, a patient with acute myelomonocytic leukemia, received a continuous 10-day infusion of tritiated thymidine. Leukemic cells were arbitrarily divided into types I, II, and III based on increasing levels of morphologic maturity (type I indicates primitive blast forms; type III, most differentiated cells). At the end of the 10-day infusion, most type II and type III cells were labeled in both marrow (shown here) and blood (not shown), but only 40% of type I cells were labeled, reflecting their slow proliferative rate. Many of the type I cells remained highly labeled for over 3 weeks after infusion.

Clarkson BD. Review of recent studies of cellular proliferation in acute leukemia. *Natl Cancer Inst Monogr.* 1969;30:81-120.

A cell initiating human acute myeloid leukaemia after transplantation into SCID mice  
 Tsvee Lapidot Christian Sirard Josef Vormoor Barbara Murdoch Trang Hoang Julio Caceres-Cortes Mark Minden Bruce  
 Paterson Michael A. Caligiuri & John E. Dick  
*Nature* 367, 645 - 648 (1994) | doi:10.1038/367645a0.



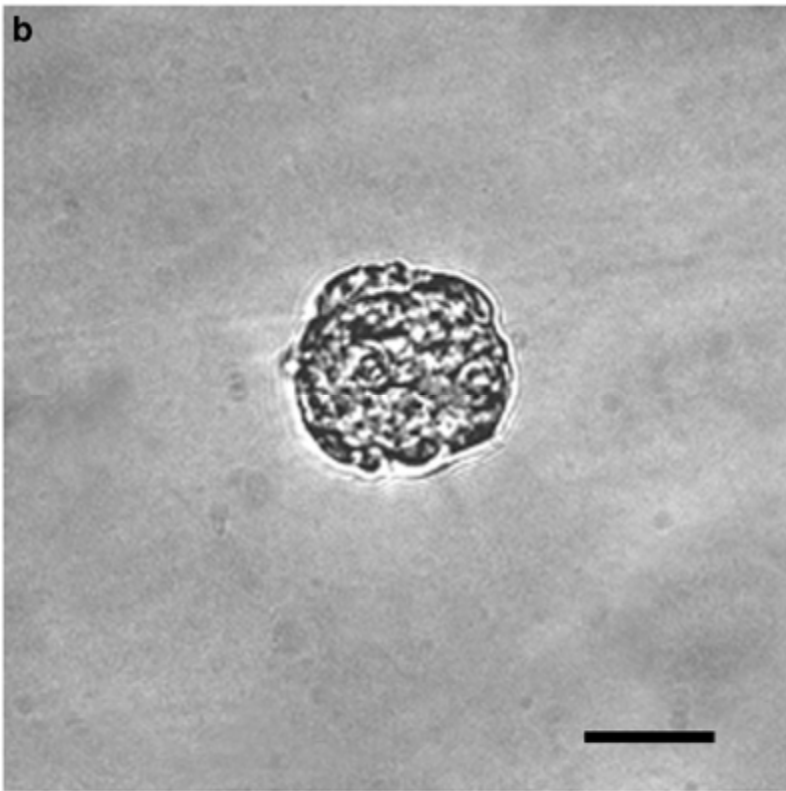
AML initiating cells represented approximately 1 in 10<sup>6</sup> of the total leukemia cells, they could be characterized by cell surface markers.



# Example

## Glioblastoma Multiforme

- Most common primary brain tumor in adults.
- Poor prognosis.
- Approximately 50% of patients will have some tumor response with radiation therapy and chemotherapy but will have tumor recurrence.

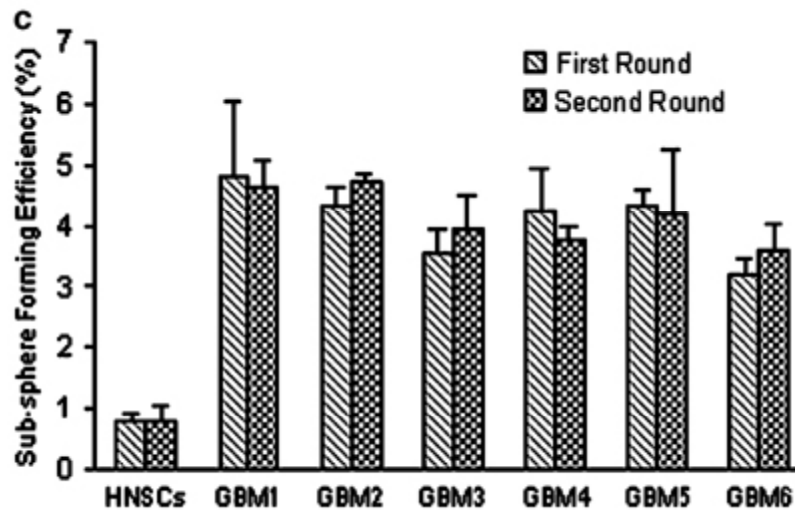


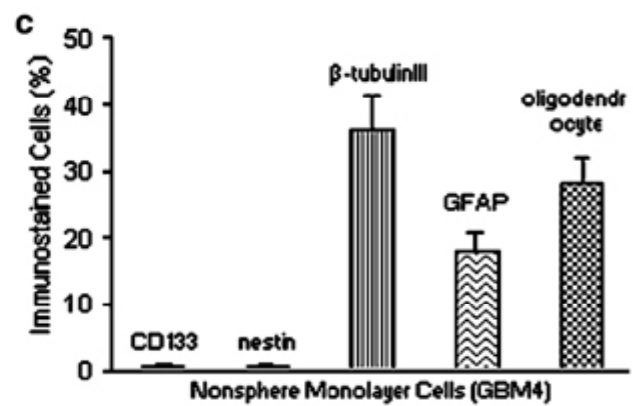
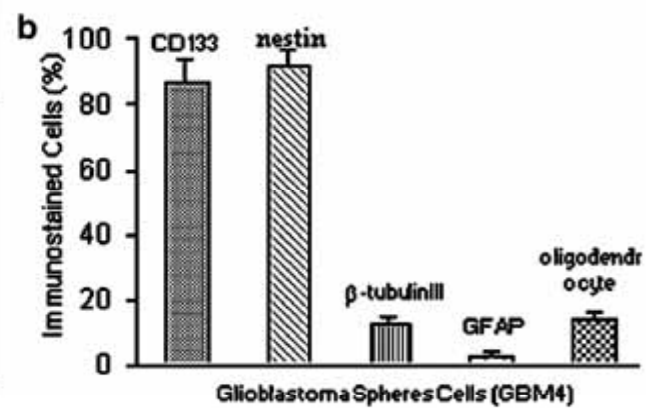
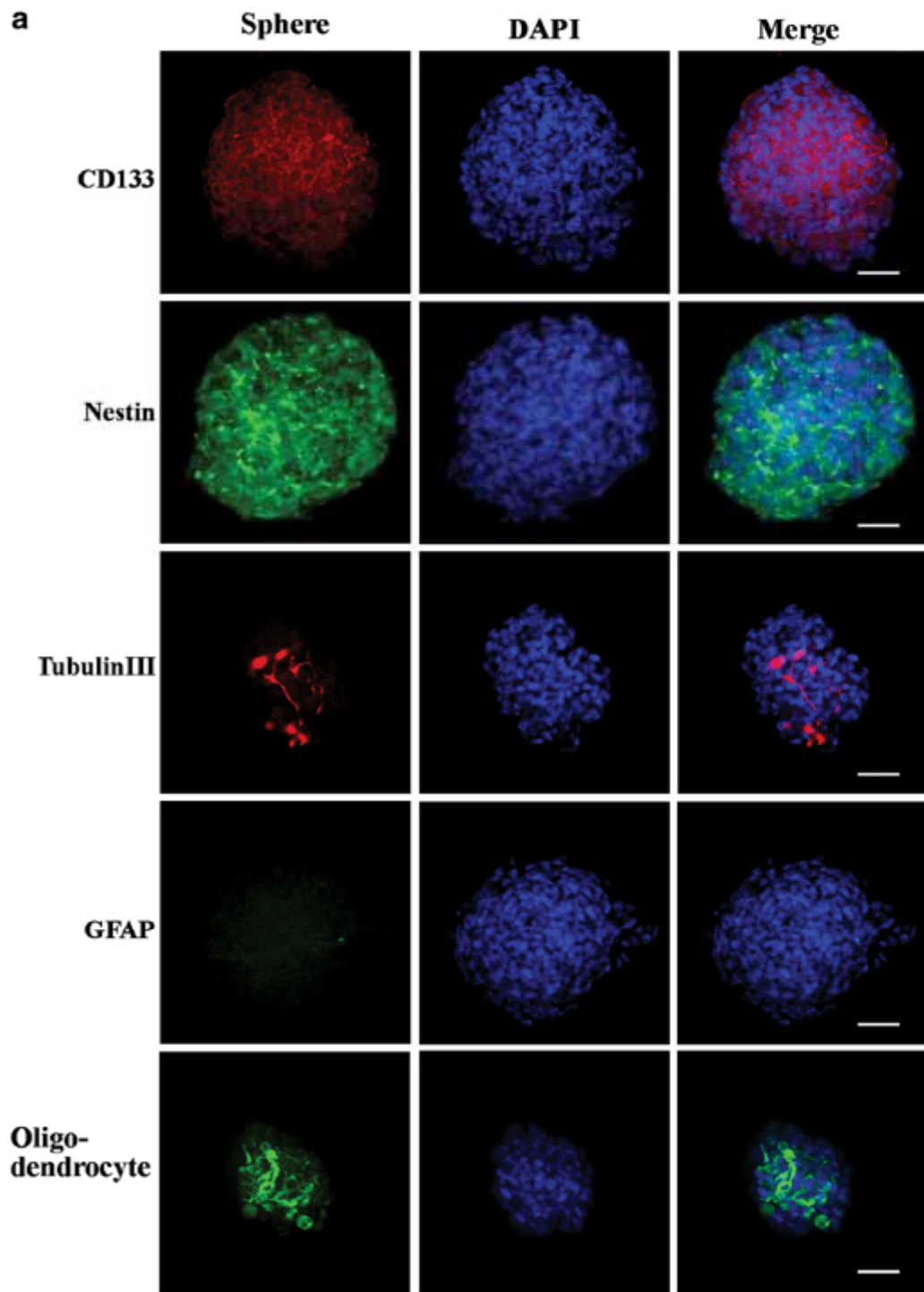
*Oncogene* (2004) **23**, 9392–9400. doi:10.1038/sj.onc.1208311

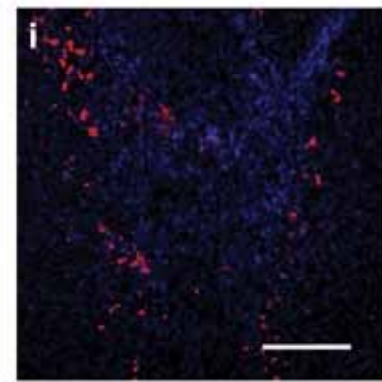
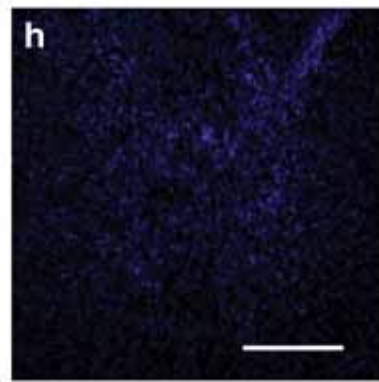
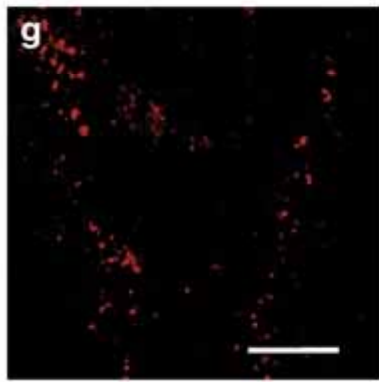
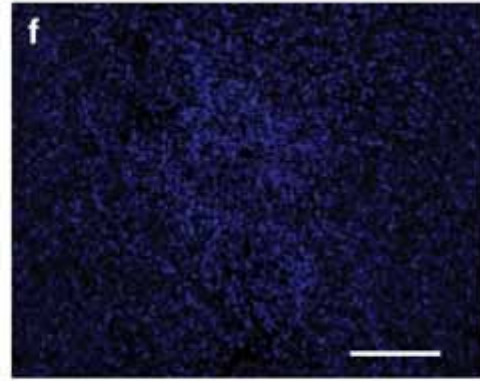
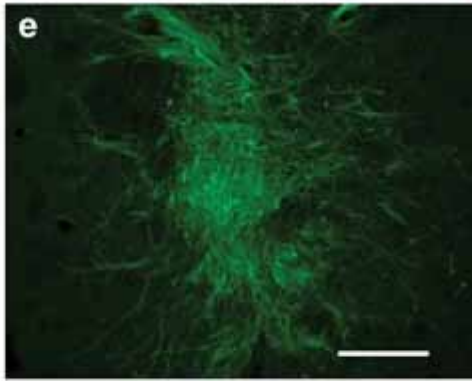
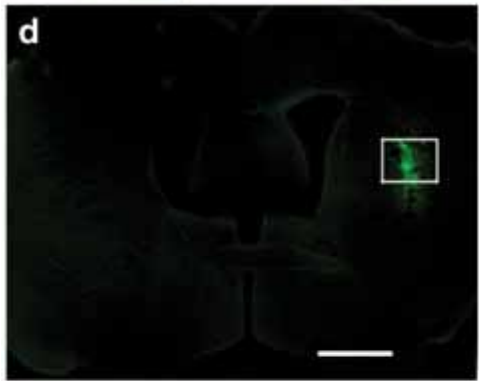
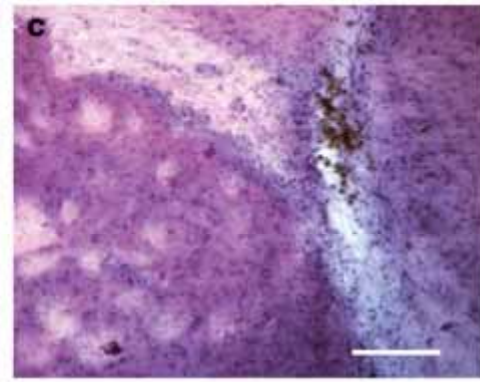
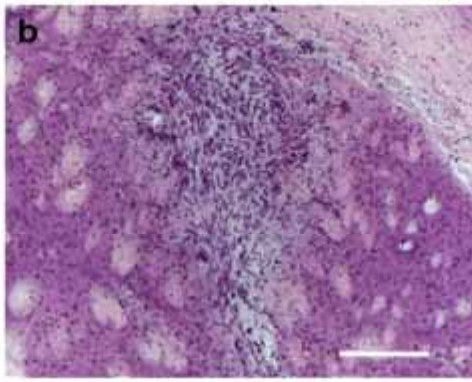
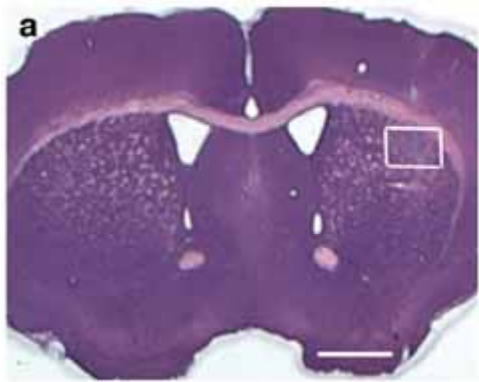
Published online 22 November 2004

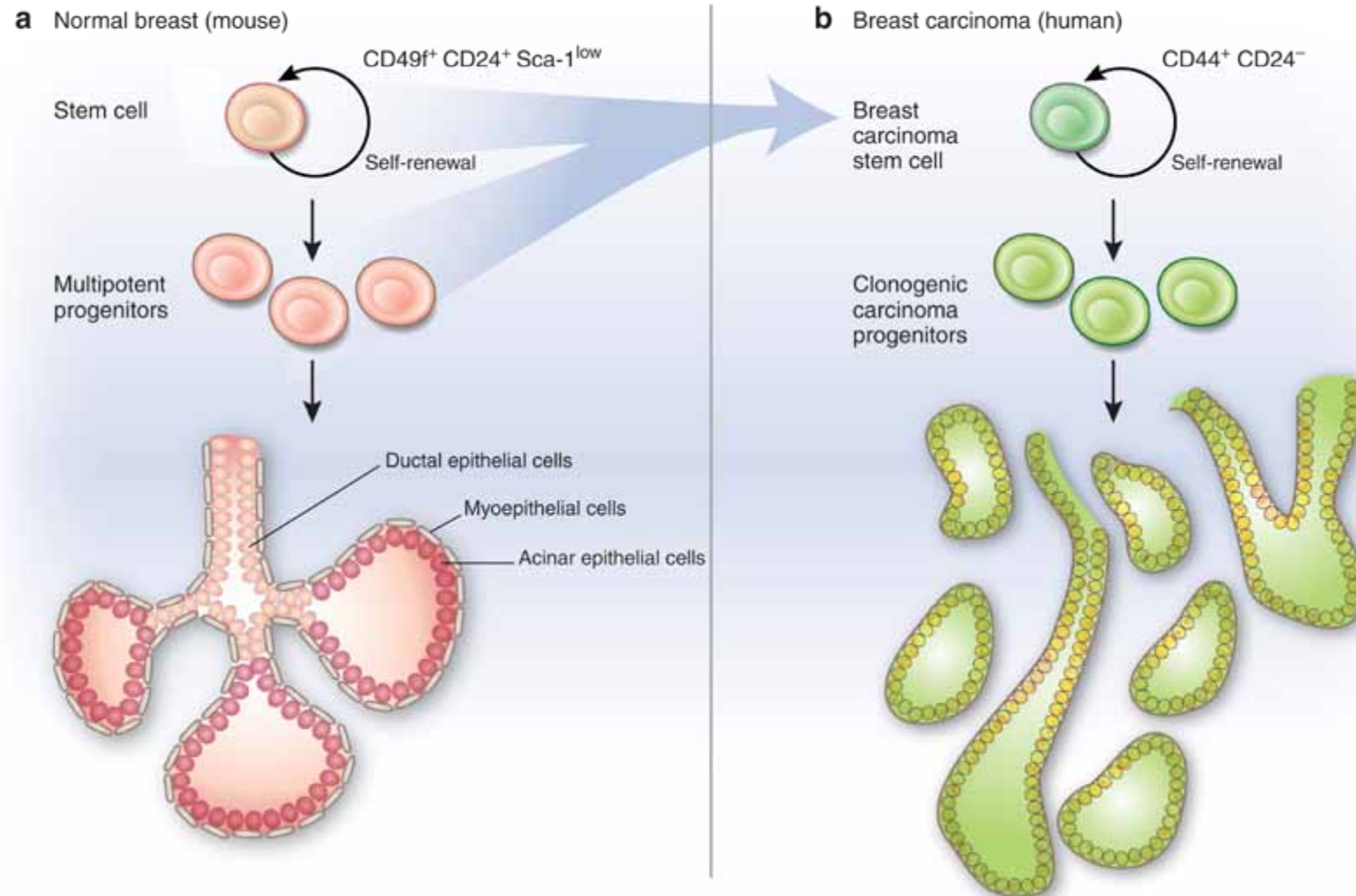
**Isolation of cancer stem cells from adult glioblastoma  
multiforme**

Xiangpeng Yuan<sup>1</sup>, James Curtin<sup>2</sup>, Yizhi Xiong<sup>3</sup>, Gentao Liu<sup>1</sup>,  
Sebastian Waschmann-Hogiu<sup>2</sup>, Daniel L Farkas<sup>2</sup>, Keith L Black<sup>1</sup>  
and John S Yu<sup>1</sup>









*Laboratory Investigation* (2006) **86**, 1203–1207. doi:10.1038/labinvest.3700488; published online 30 October 2006

**The cancer stem cell hypothesis: a work in progress**

Brenton Thomas Tan<sup>1,\*</sup>, Christopher Yongchul Park<sup>1,\*</sup>, Laurie Elizabeth Ailles<sup>1</sup> and Irving L Weissman<sup>1</sup>

# Other Cancer Stem Cells

- Breast Cancer
- Leukemia
- Prostate Cancer
- Lung Cancer

# What are the clinical implications?

- Even if a therapy kills most of the cancer cells that are present, they may not be the cells that should be targeted to cure the patient.
- Cancer stem cells have properties that are different than the bulk of cells in a tumor (resistance to chemotherapy, a slower division rate, etc.)

HYBRID

**SWEET CORN**

PEACHES AND CREAM  
BI-COLOR

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# THE LANCET.

A Journal of British and Foreign Medicine, Surgery, Obstetrics, Physiology,  
Chemistry, Pharmacology, Public Health, and News.

THE  
DISTRIBUTION OF SECONDARY GROWTHS  
IN CANCER OF THE BREAST.

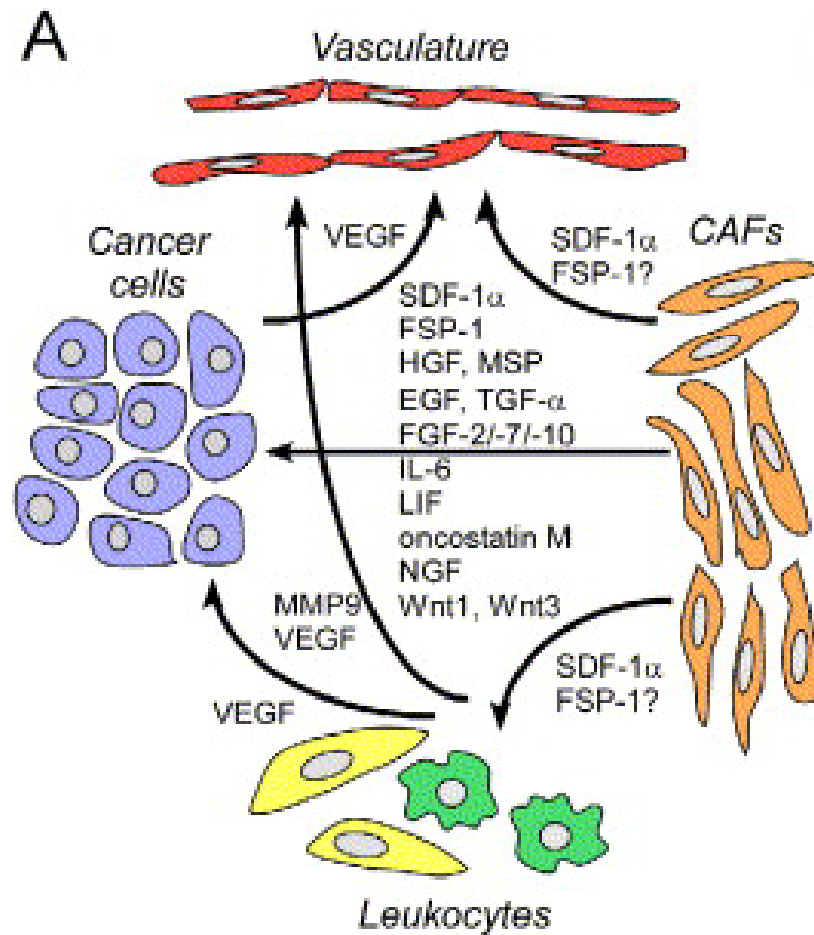
BY STEPHEN PAGET, F.R.C.S.,  
ASSISTANT SURGEON TO THE WEST LONDON HOSPITAL AND THE  
METROPOLITAN HOSPITAL.

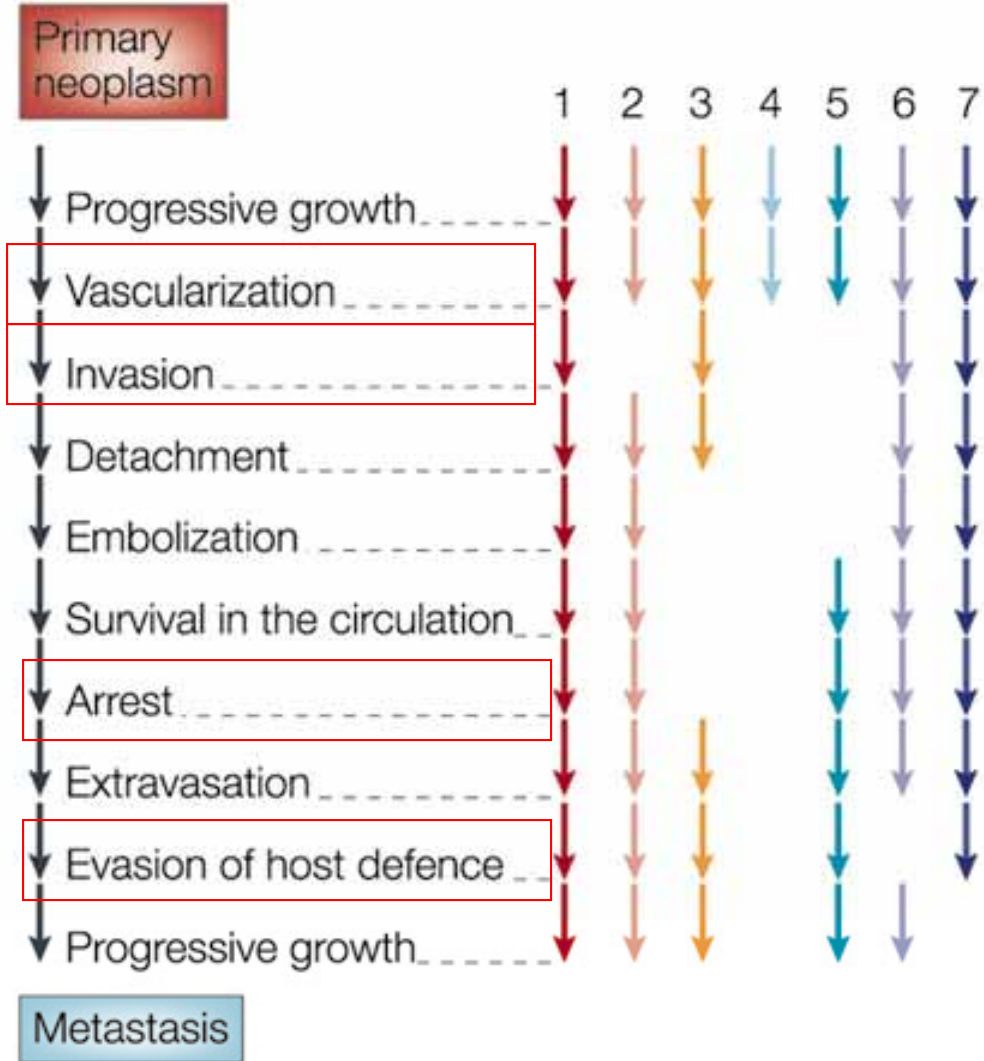
AN attempt is made in this paper to consider "metastasis" in malignant disease, and to show that the distribution of the secondary growths is not a matter of chance. It is urged both by Langenbeck and by Billroth that the question ought to be asked, and, if possible, answered: "What is it that decides what organs shall suffer in a case of disseminated cancer?" If the remote organs in such a case are all alike passive and, so to speak, helpless—all equally ready to receive and nourish any particle of the primary growth which may "slip through the lungs," and so be brought to them,—then the distribution of cancer throughout the body must be a matter of chance. But if we can trace any sort of rule or sequence in the distribution of cancer, any relation between the character of the primary growth and the situation of the secondary growths derived from it, then the remote organs cannot be altogether passive or indifferent as regards embolism.



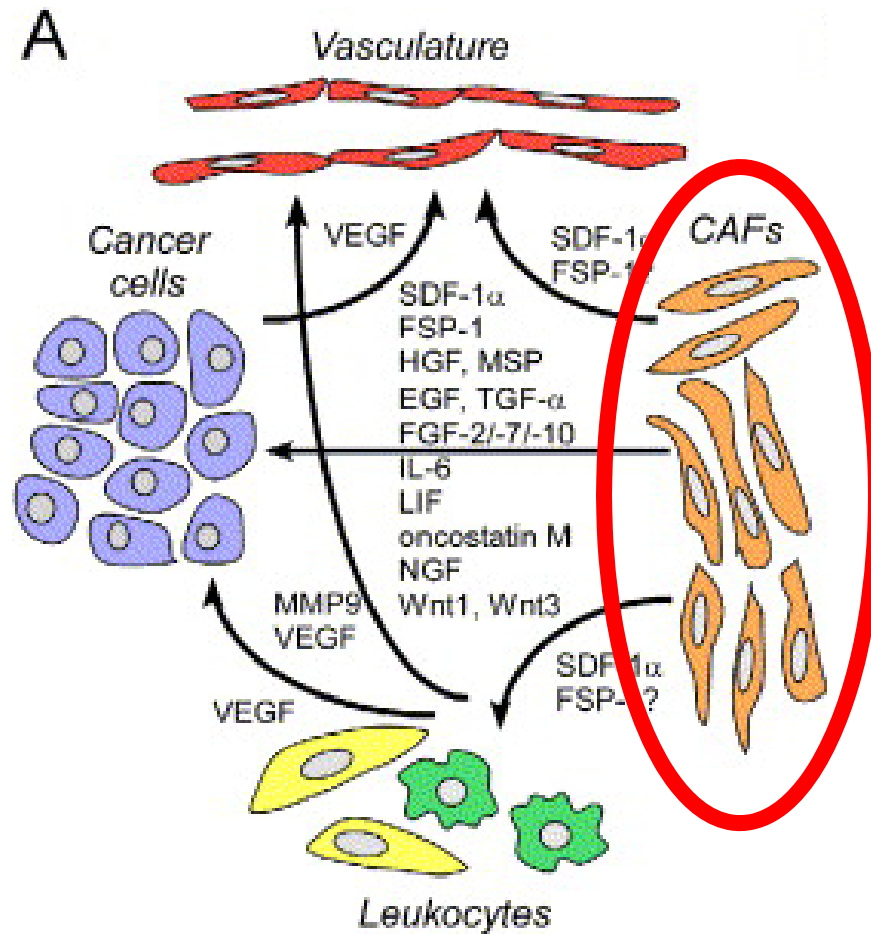
Nature Reviews | Cancer

# The Tumor Microenvironment





# The Tumor Microenvironment

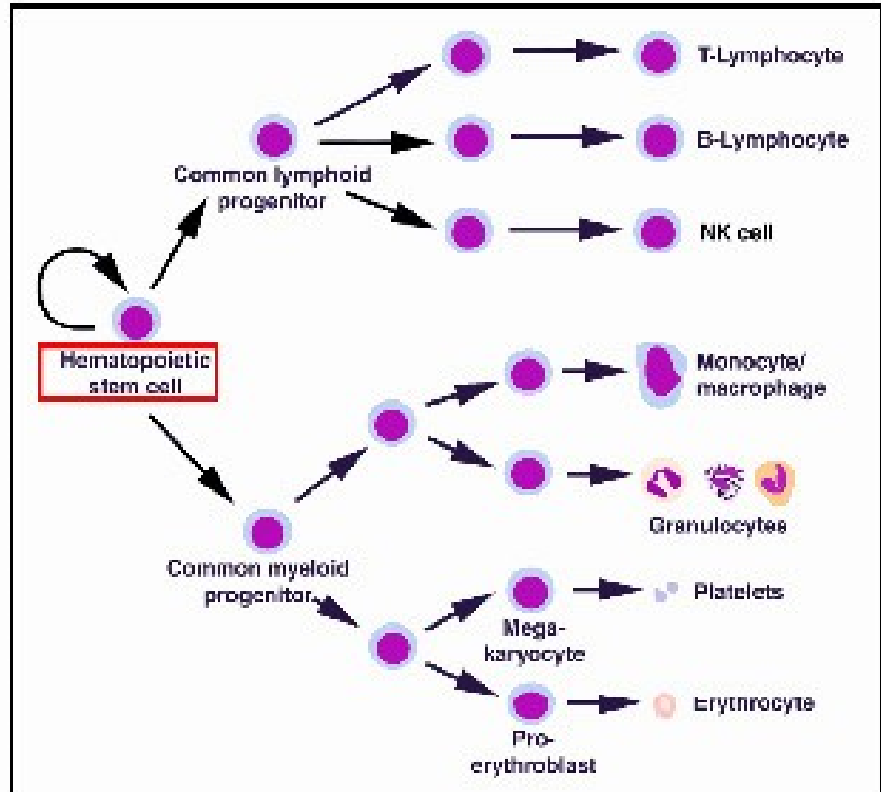


# Questions about the tumor stroma

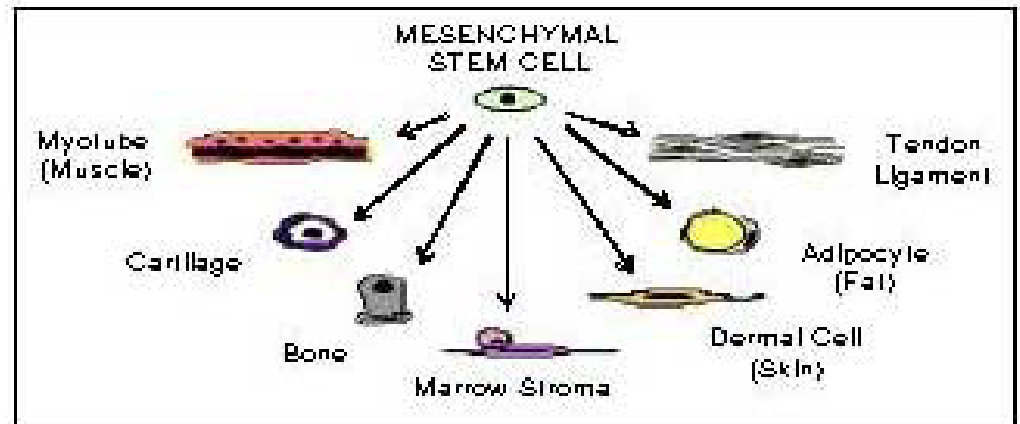
- Where does it come from?
- How does it get there?
- What does it do?

# Diagram of stem cell lineages in bone marrow

Hematopoietic Lineage  
Differentiation potential of hematopoietic stem



Mesenchymal Lineage  
Differentiation potential of MSC

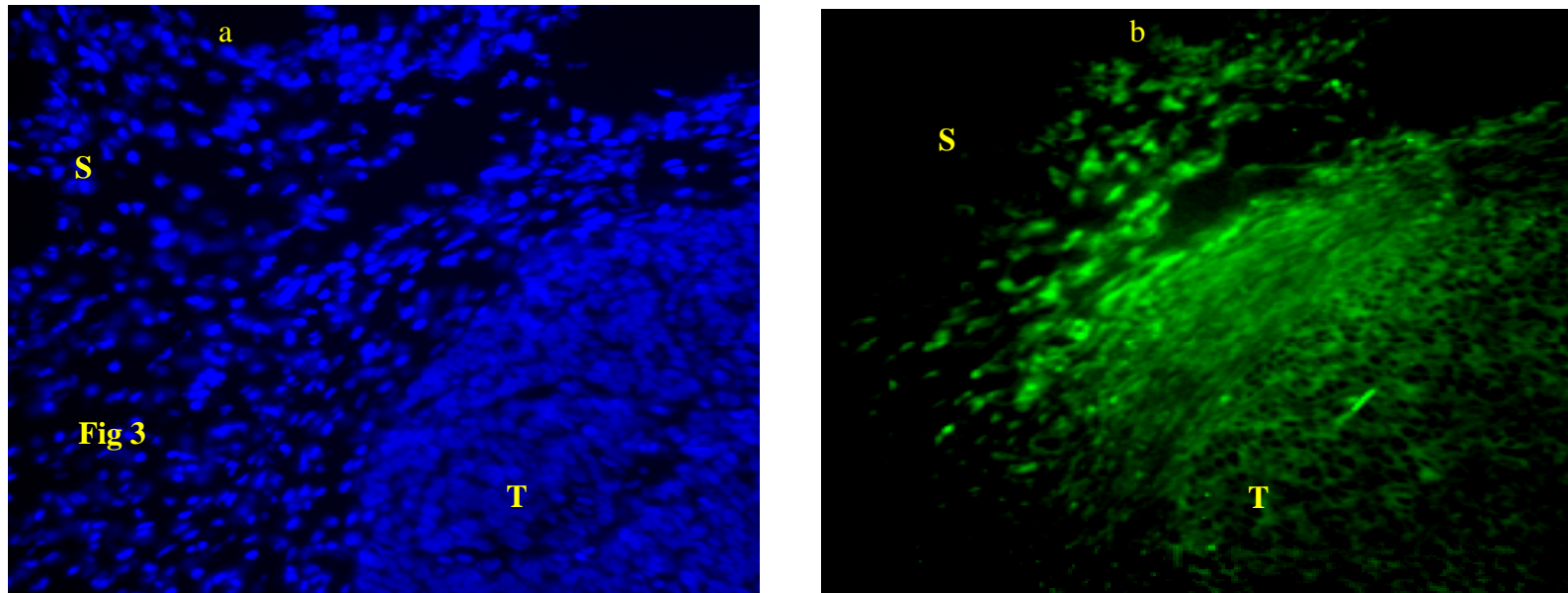




# Multipotent Mesenchymal Stromal Cells

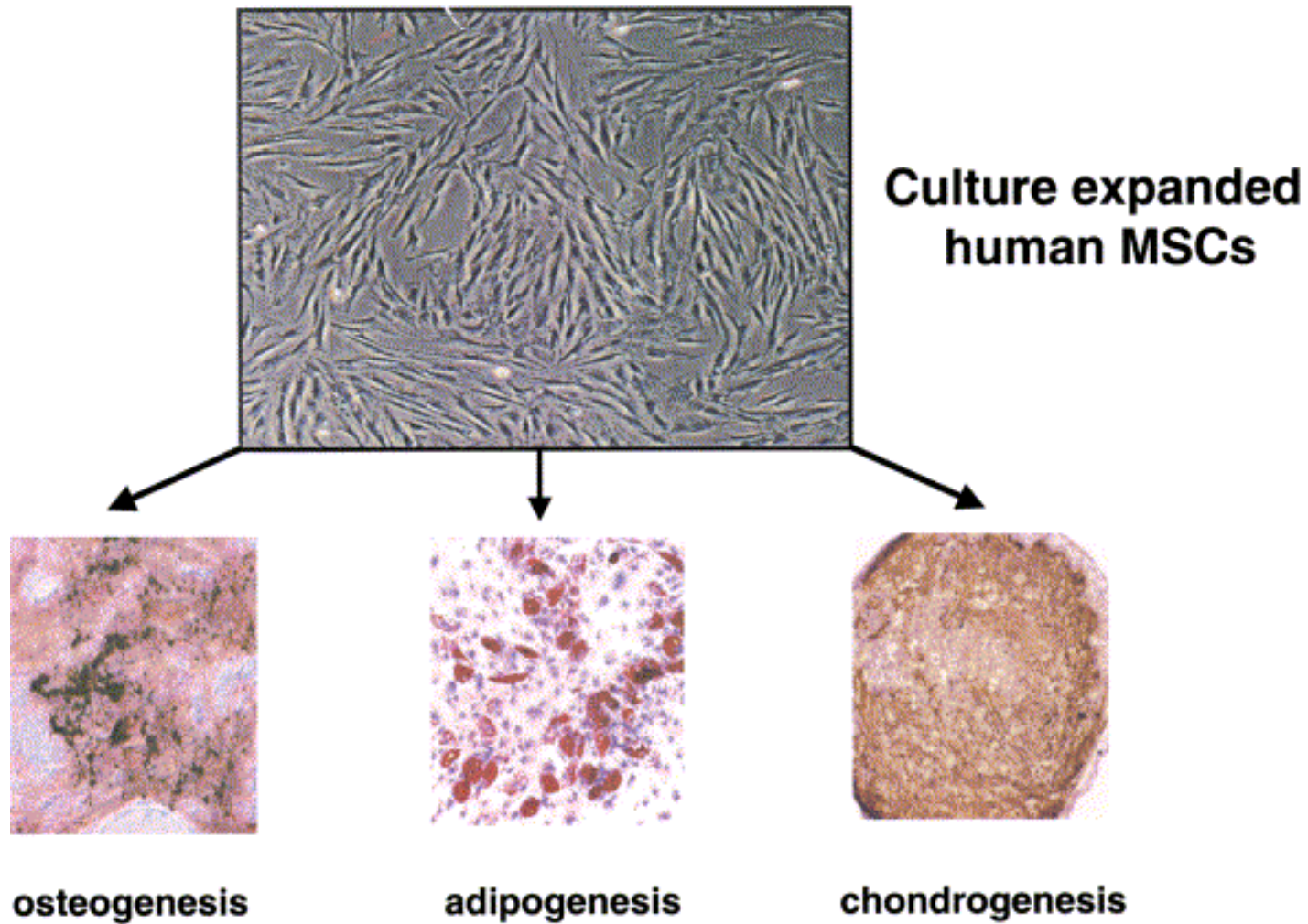
- Nonhematopoietic cells of mesenchymal origin found in the bone marrow.
- Friedenstein described the isolation and characterization of MSCs in 1980.
- In vitro adherent cells derived from long-term bone marrow cultures.

# Migration of MSCs to tumor site in vivo

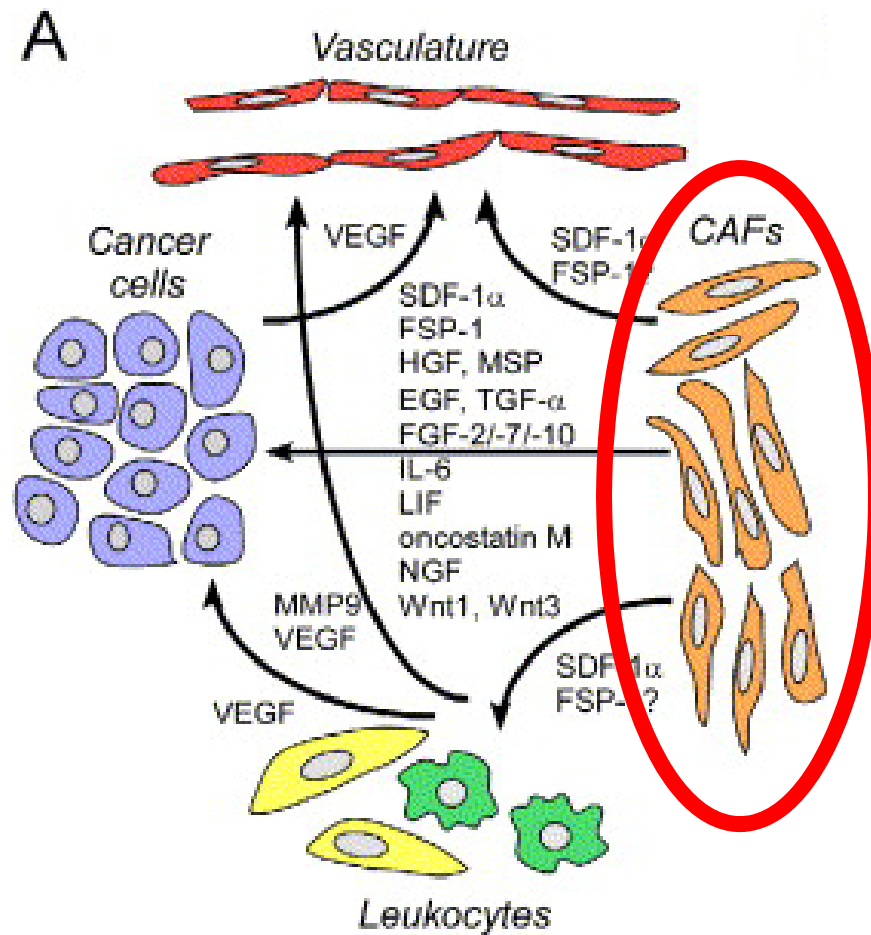


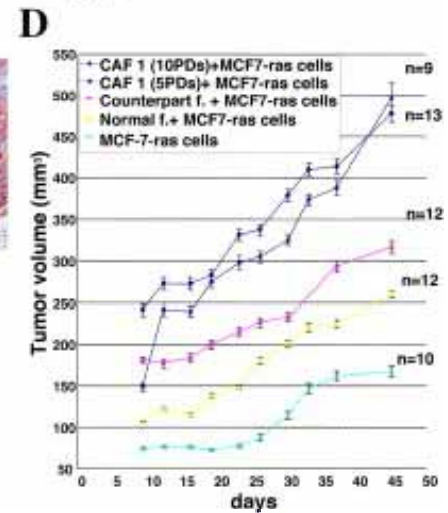
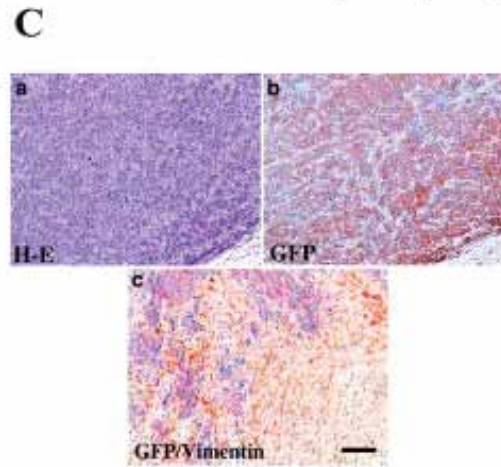
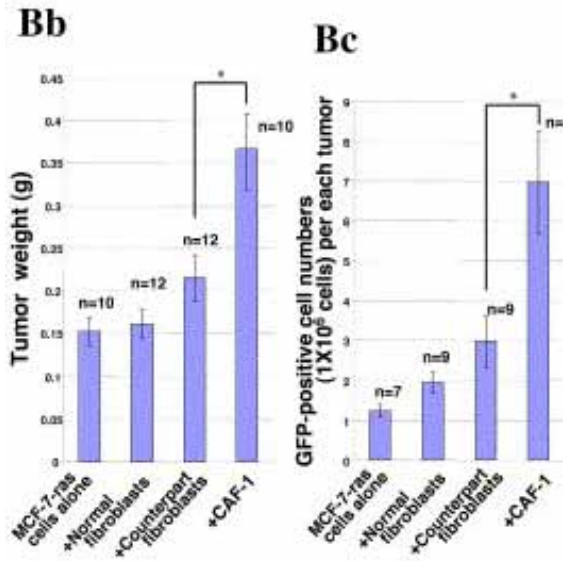
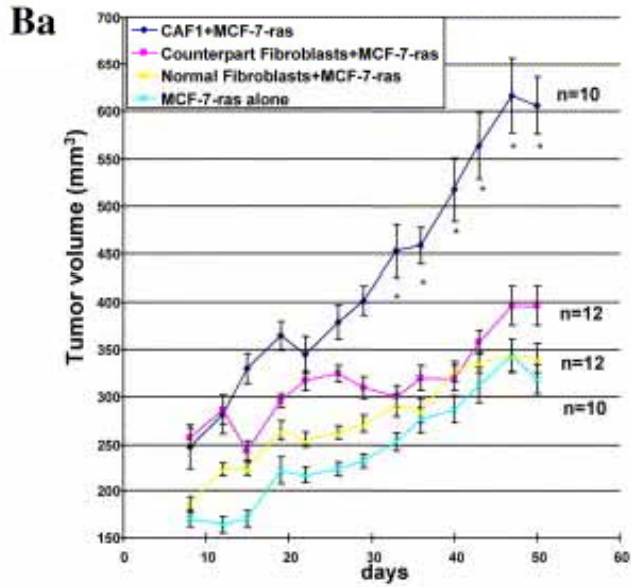
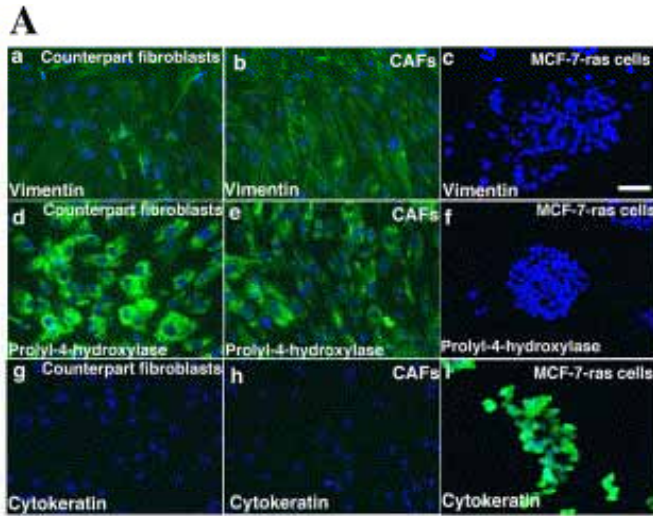
*CFDA-SE labeled MSCs (panel b) were detectable in the TMEN (marked as S in Fig 3). DAPI staining (panel a) revealed that the MSCs surrounded the tumor mass (marked as T in both panels Fig 3).*

## Human MSCs -- Mesenchymal Differentiation in vitro



# The Tumor Microenvironment



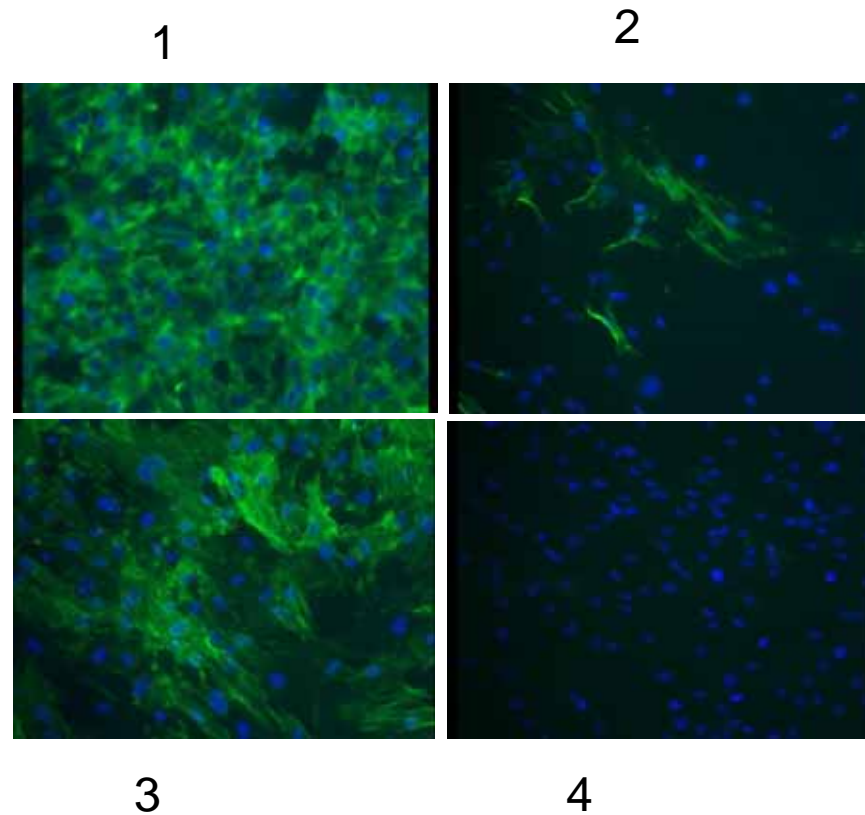


Orima et al Cell 2005

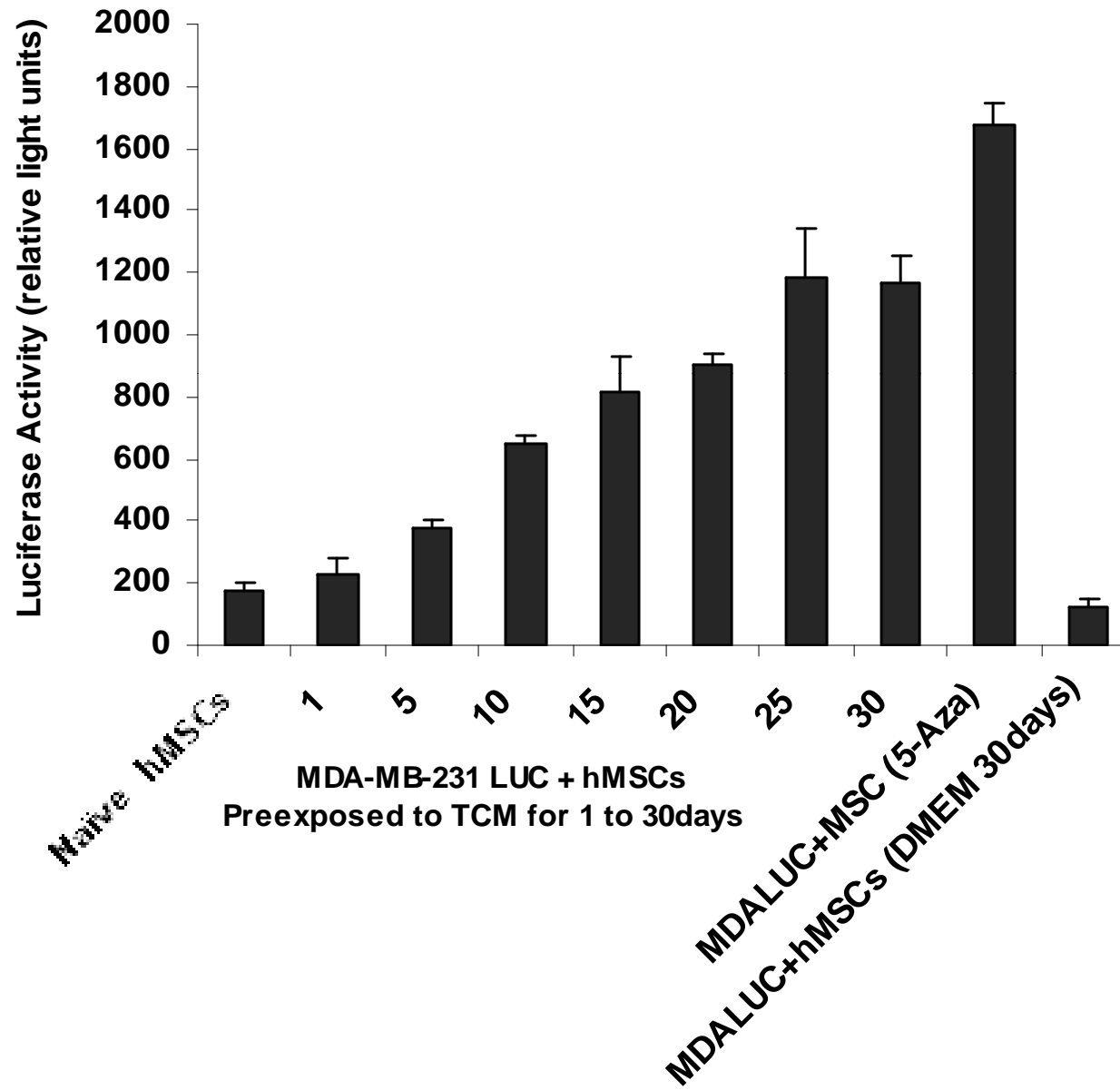
# Are MSCs the Source of CAFs?

- CAFs may be derived from the bone marrow
- MSCs look like fibroblasts
- MSCs localize to solid tumors
- Both MSCs and CAFs produce SDF-1

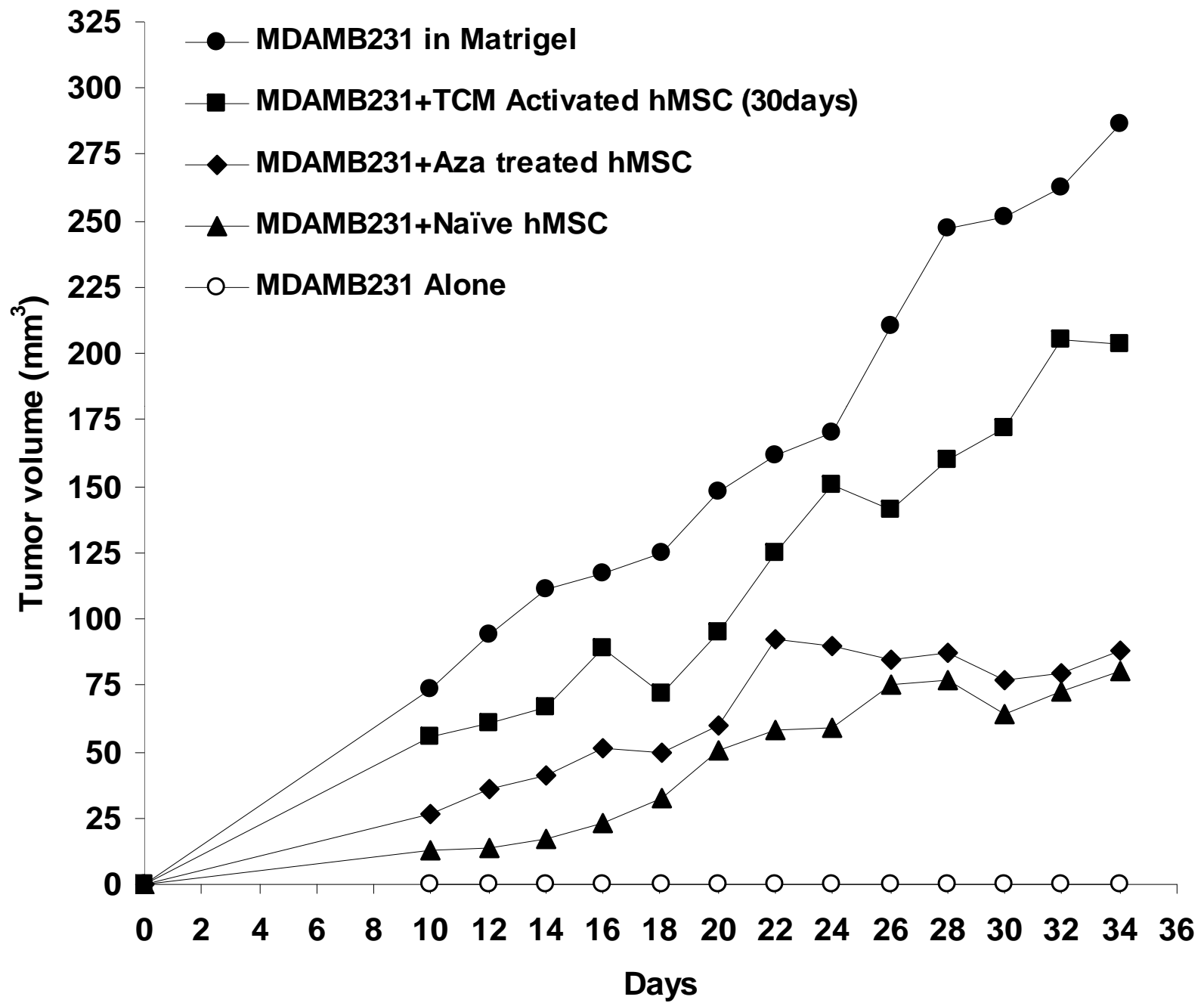
# hMSCs express alpha smooth muscle actin following exposure to tumor cell CM



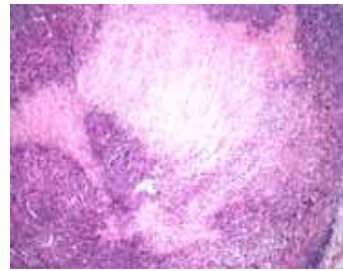
1: NIH 3T3 cells; 2: hMSCs in TCM 6days; hMSCs plus 5aza; hMSCs neg control



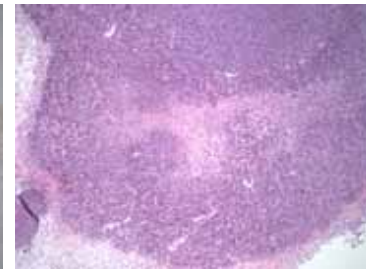




**Fibroblast Surface Protein and H&E**



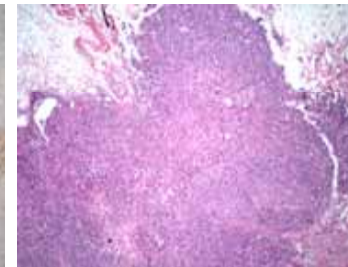
Naïve hMSCs



Matrigel



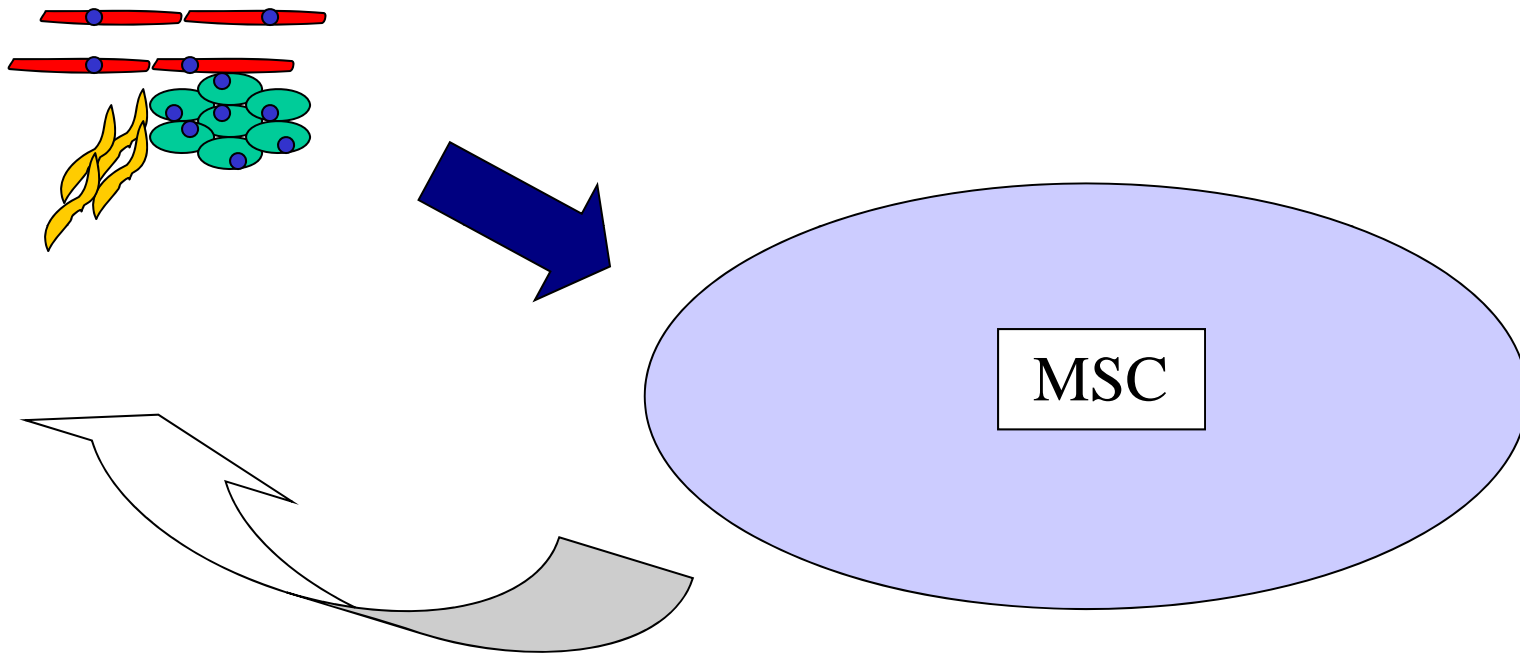
5-aza



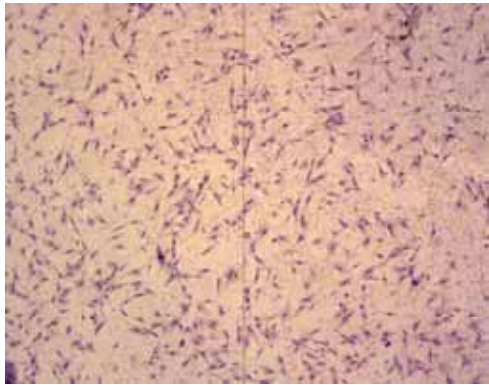
TCM

# Molecular mechanisms underlying activation of MSCs

## Tumor Microenvironment

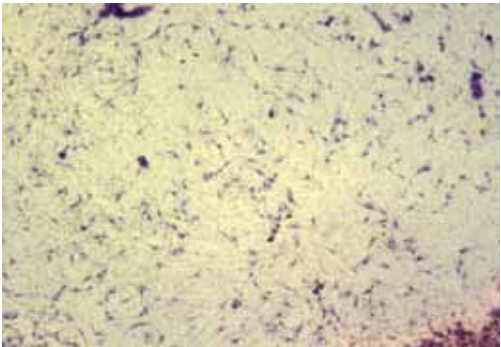


# MSCs migrate towards C85 colon tumor cells as well as to conditioned medium from these cells



**Cells and media**

**Conditioned media**



**Complete media**

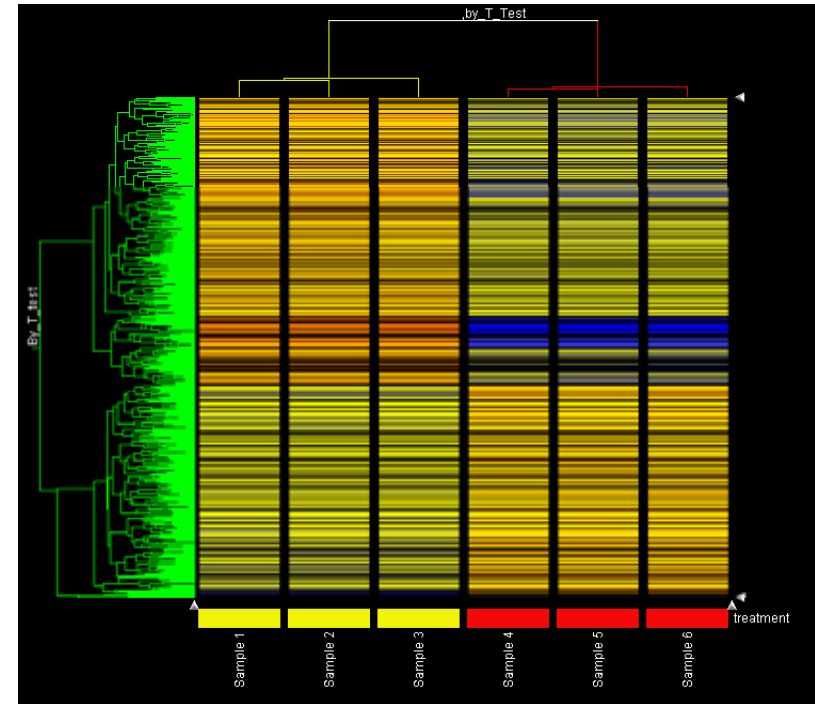
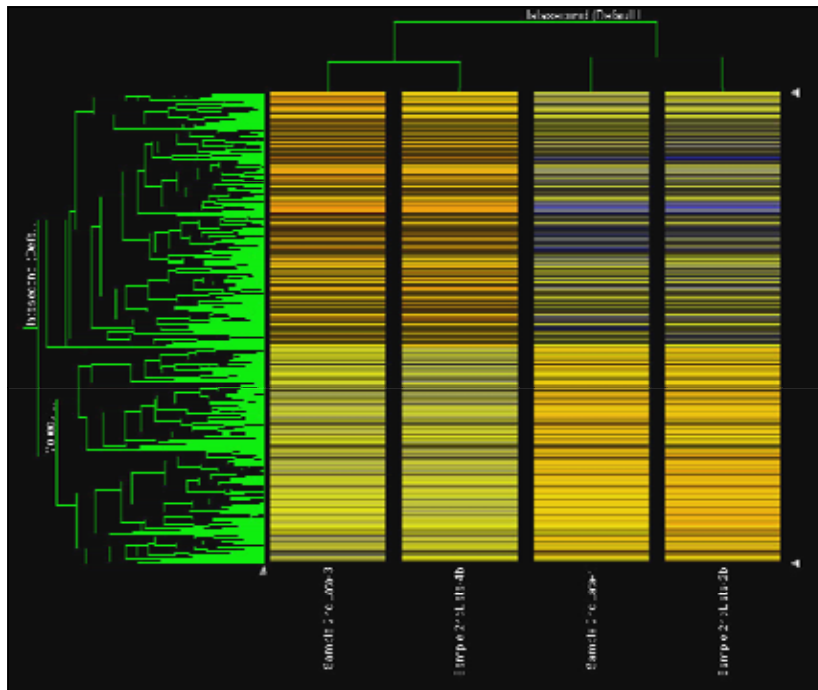


Can we identify patterns of gene expression that are specific for MSC chemotaxis to the tumor microenvironment?

# **cDNA microarray as a tool to investigate molecular basis of migration of MSCs**

MSCs are exposed to CM from tumor cells or control media, RNA isolated and processed for cDNA microarray

## cDNA micro array analysis of rMSCs exposed to CM from rat BM (left panel) as compared to CM from tumor cells (right panel)



The two migration conditions are shown side by side, genes upregulated for BM migration appear to be distinct from genes upregulated for tumor migration

Note similarity of gene quadrants up and downregulated in migrating versus non migrating cells

**Table 1b**

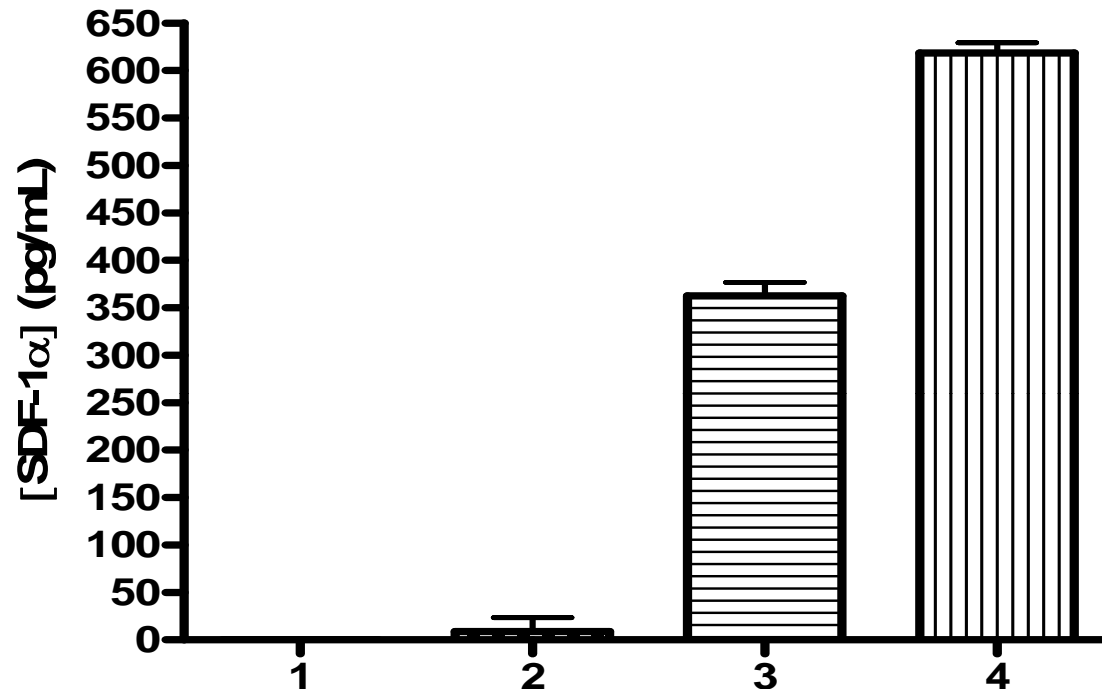
**Expression of mRNA levels of following genes are increased in MSCs exposed to Tumor CM but decreased in MSCs exposed to Bone marrow CM**

<b>Gene ID</b>	<b>Gene name</b>	<b>Fold Increase</b>
1387648_at	chemokine (C-X-C motif) ligand IX	184.93
1370634_at	cytokine-induced neutrophil chemoattractant-2	59.17
1368760_at	chemokine (C-X-C motif) ligand 2	20.03
1387655_at	chemokine (C-X-C motif) ligand 12, SDF-1	10.52
1368078_at	endothelial cell-specific molecule 1	9.68
1375951_at	thrombomodulin	3.33
1369884_at	fibroblast growth factor 7	3.03
1370968_at	nuclear factor kappa B p105 subunit	2.27

The table contains only functionally identified genes

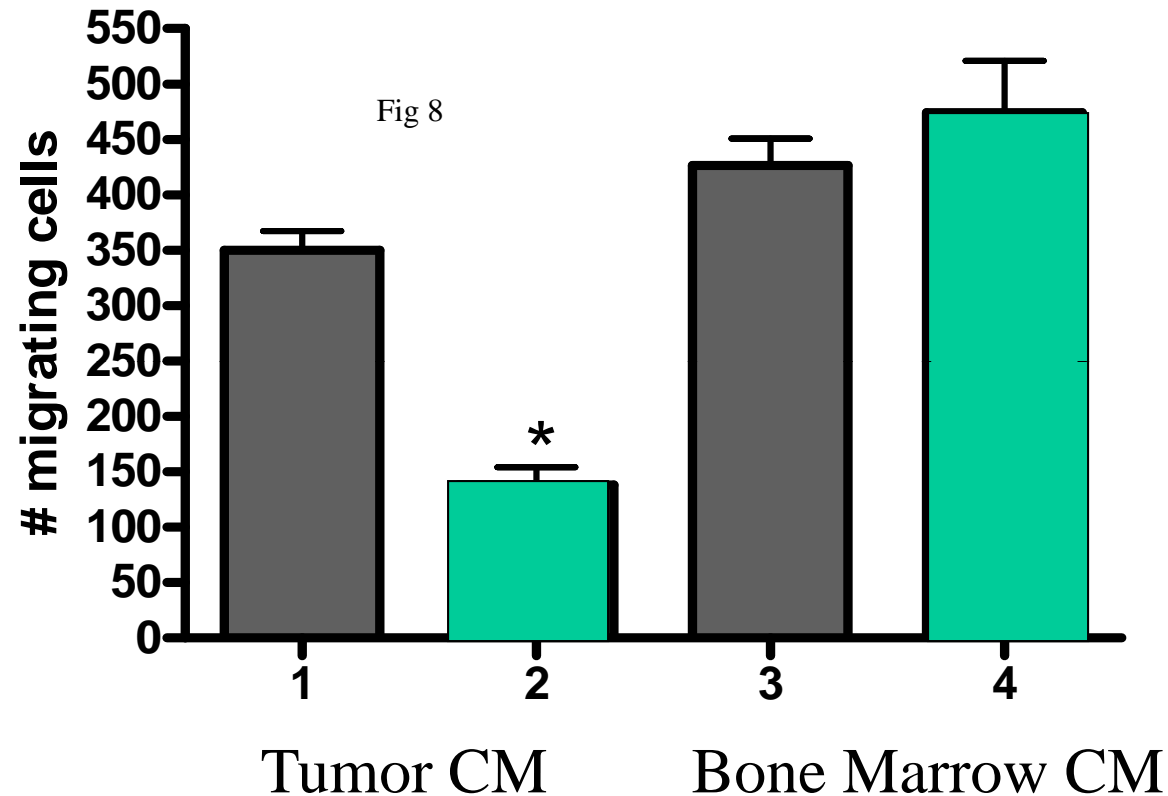


## Exposure to tumor cell CM leads to increased secretion of SDF-1 by rMSCs confirming the microarray data



Tumor cell conditioned medium (bar 1) and RPMI medium (bar 2) have barely detectable levels of SDF-1. Exposure of MSCs to RPMI+10% FBS for 16h (bar 3) and to tumor cell CM for 16 h (bar 4) leads to a significant increase in SDF-1 levels in secreted medium of MSCs in agreement with the cDNA microarray results. The difference between SDF-1 levels induced by RPMI+10%FBS and CM from tumor cells is statistically significant ( $p < 0.005$ , unpaired t test).

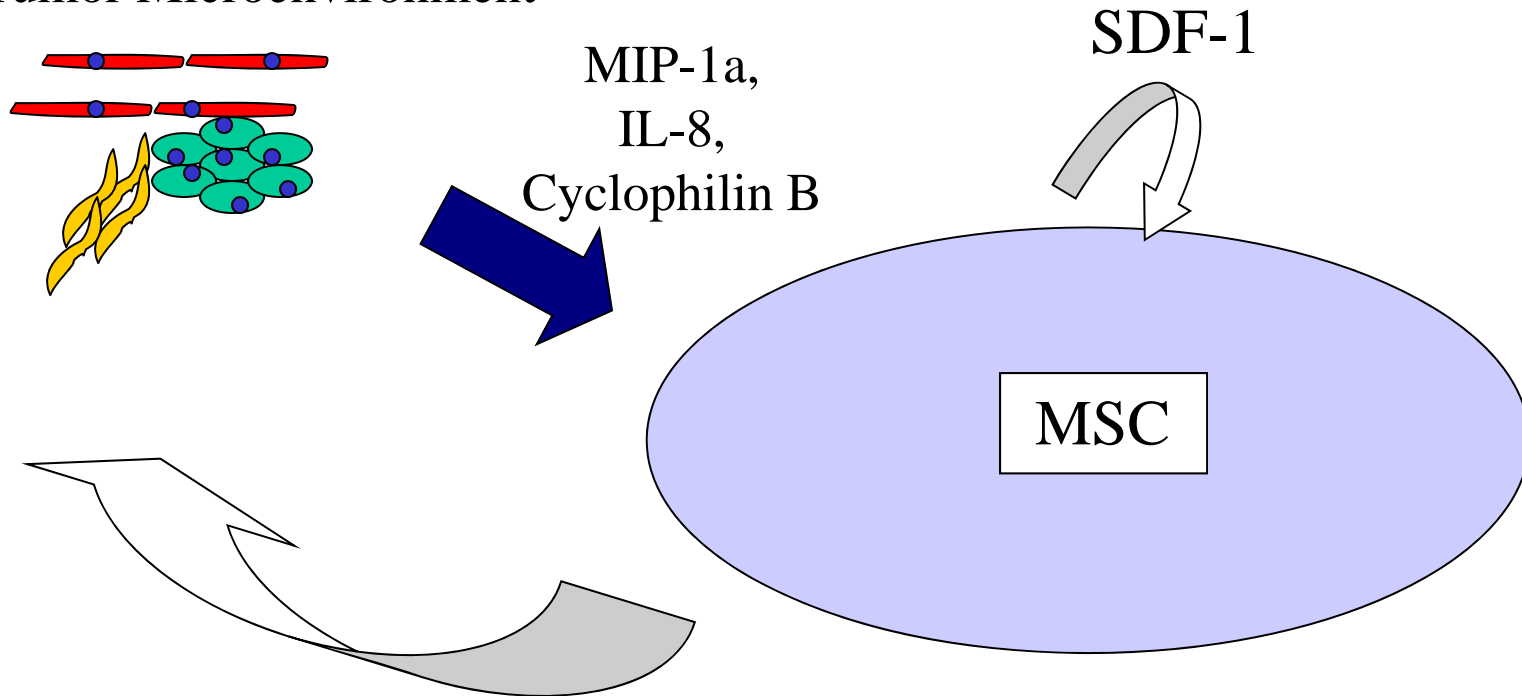
## Knockdown of SDF-1 inhibits migration of rMSCs to CM from tumor cells



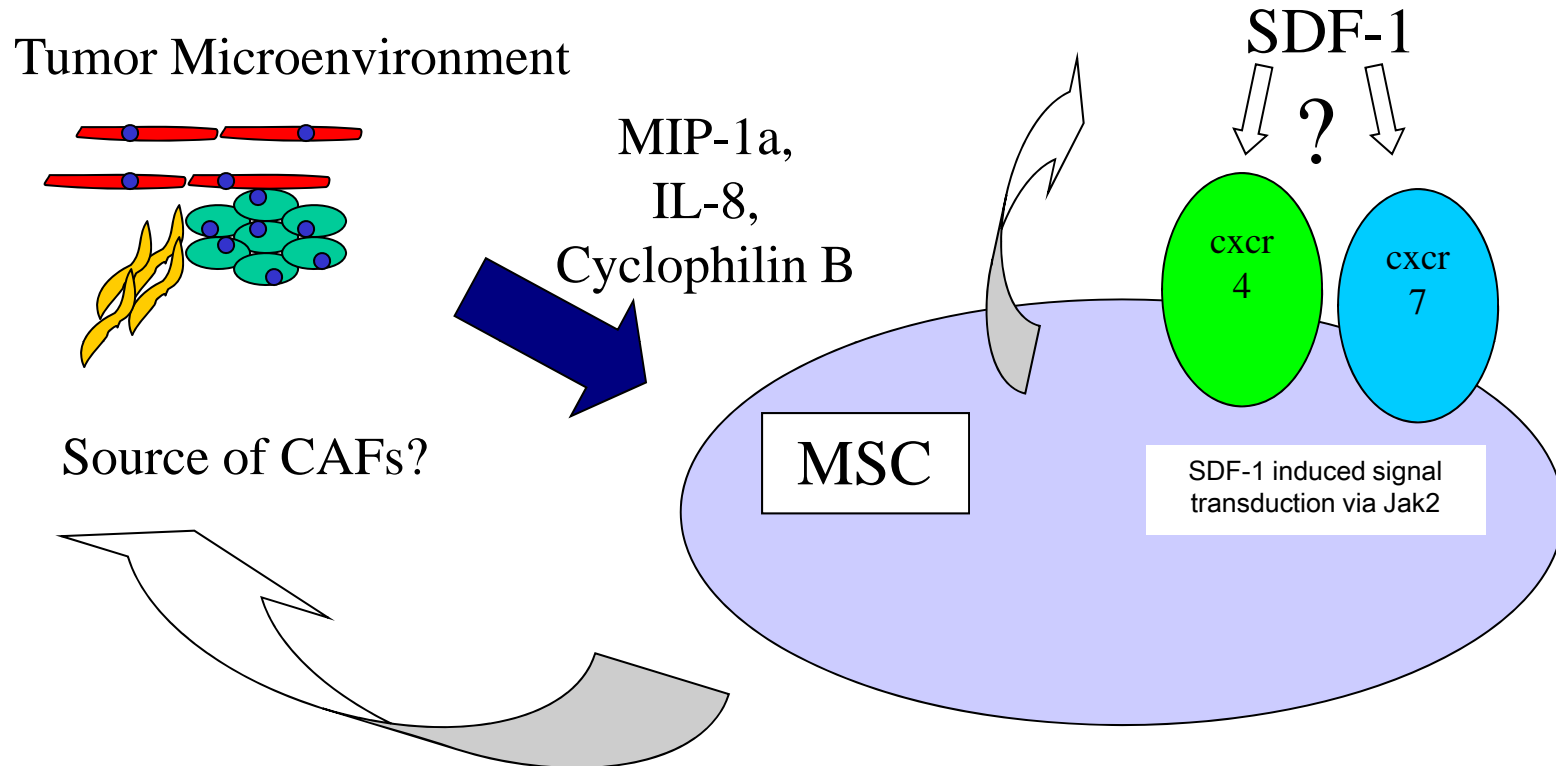
SDF-1 knockdown using 50nM siRNA inhibits migration of MSCs (bars 1 and 3) to CM from tumor cells (bar 2) but not to CM from bone marrow cells (bar 4).

# Molecular mechanisms underlying activation of MSCs

Tumor Microenvironment

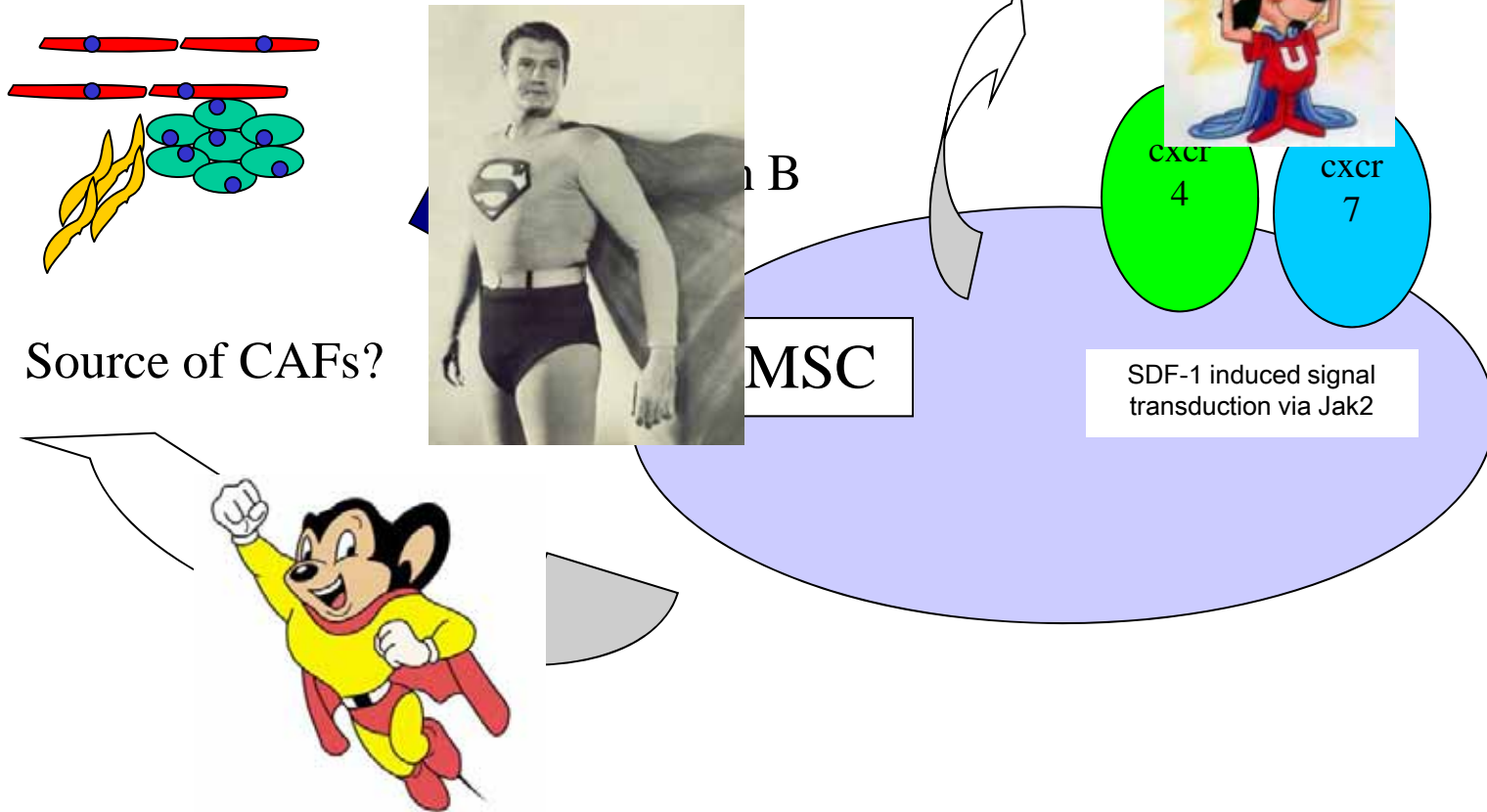


# Molecular mechanisms underlying activation of MSCs



# Treatment Targets involved in the Interaction between MSCs and other components of the Tumor microenvironment

## Tumor Microenvironment



# Summary

- The cellular components of tumors are heterogeneous.
- A very small population of cancer stem cells are probably responsible for the propagation of the neoplastic cell.
- Other progenitor cell populations are important in tumor growth and formation.