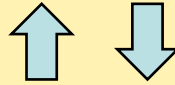


# Come si possono studiare le CSC?

Metodi di Isolamento/Purificazione/Arricchimento delle CSC per studiare le loro caratteristiche molecolari ed il loro comportamento

Isolamento "prospettivo" di CSC



Test di tumorigenicità in vivo



Analisi molecolari e fenotipiche

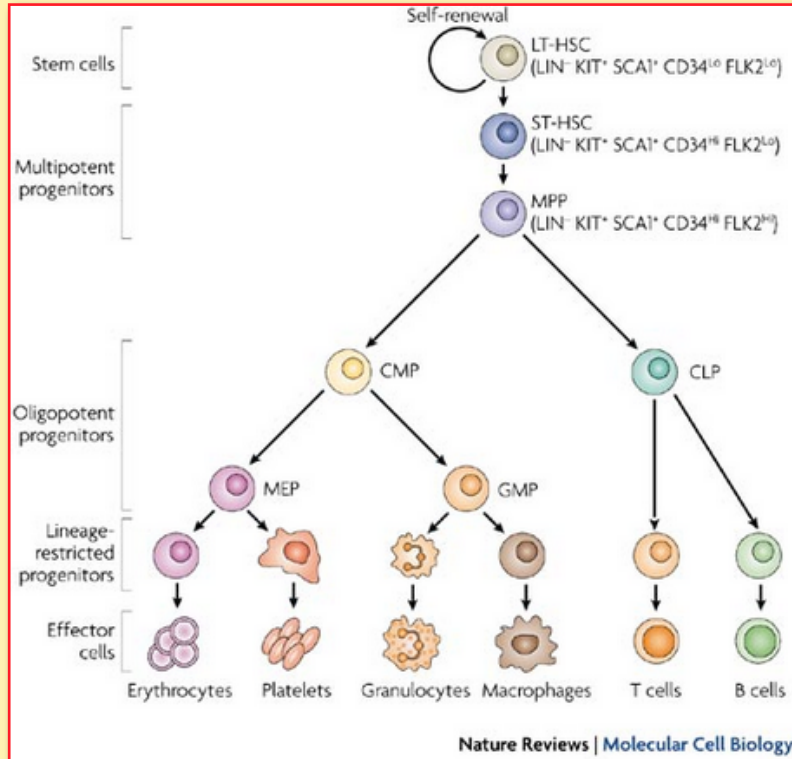


Trovare cratteristiche peculiari delle CSC e le differenze tra CSC e cellule staminali normali



Trovare marcatori per diagnosi e prognosi  
Sviluppo di farmaci e trattamenti che possano colpire le CSC e non le cellule staminali normali

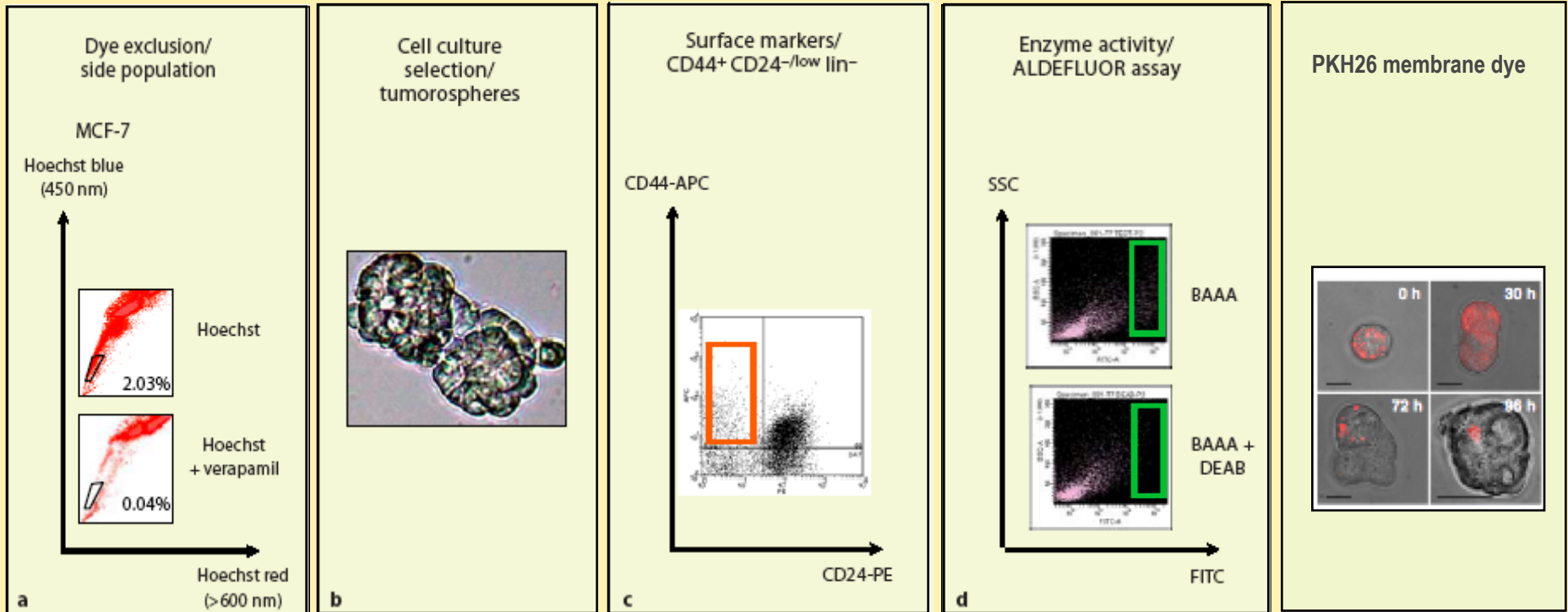
## CSC



- Auto-rinnovamento
- Potenziale differenziativo
- Potenziale proliferativo
- Quiescenza
- Marcatori di superficie specifici
  
- Chemioresistenza (meccanismi di detossificazione e pompe ad efflusso, quiescenza, etc.)

# Tecniche di purificazione ed isolamento *prospettivo* di cellule staminali del cancro

Si basano sulle proprietà caratteristiche di cellule staminali, quali quiescenza, meccanismi di esclusione di farmaci, detossificazione etc.



Adattato da Charaffe-Jauffret et al, *Pathobiol* 2008

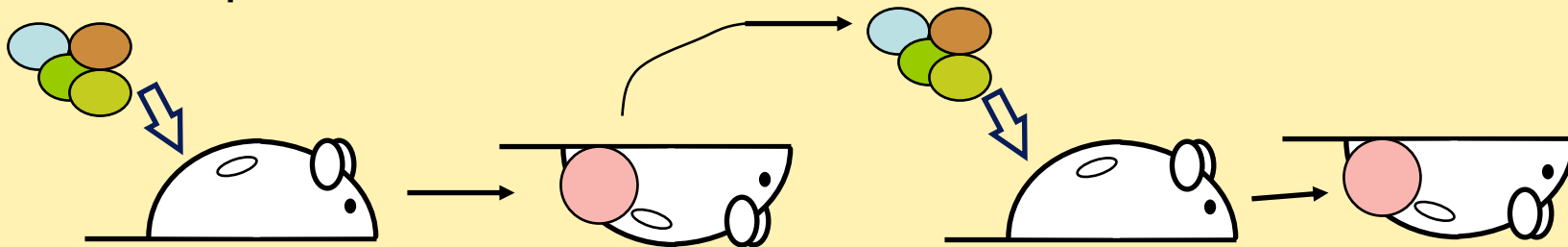
Pece et al. *Cell* 2010

# Trapianto seriale di cellule tumorali in topi immunocompromessi: Dimostrazione della tumorigenicità in vivo

*PRIMO trapianto*

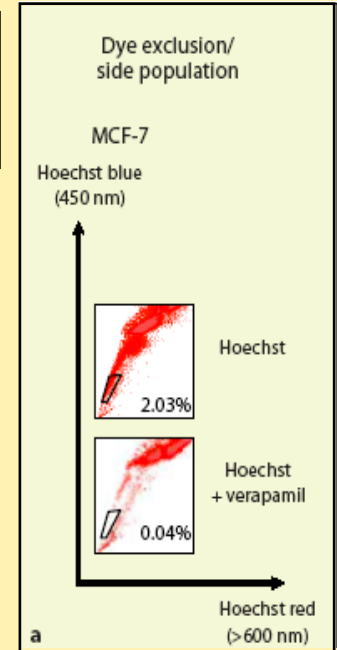
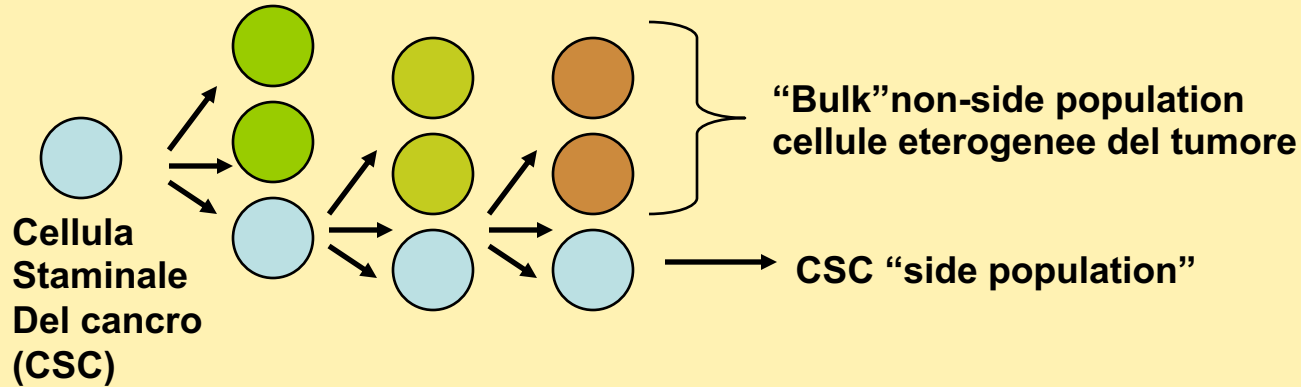
*SECONDO trapianto*

Cellule tumorali primarie

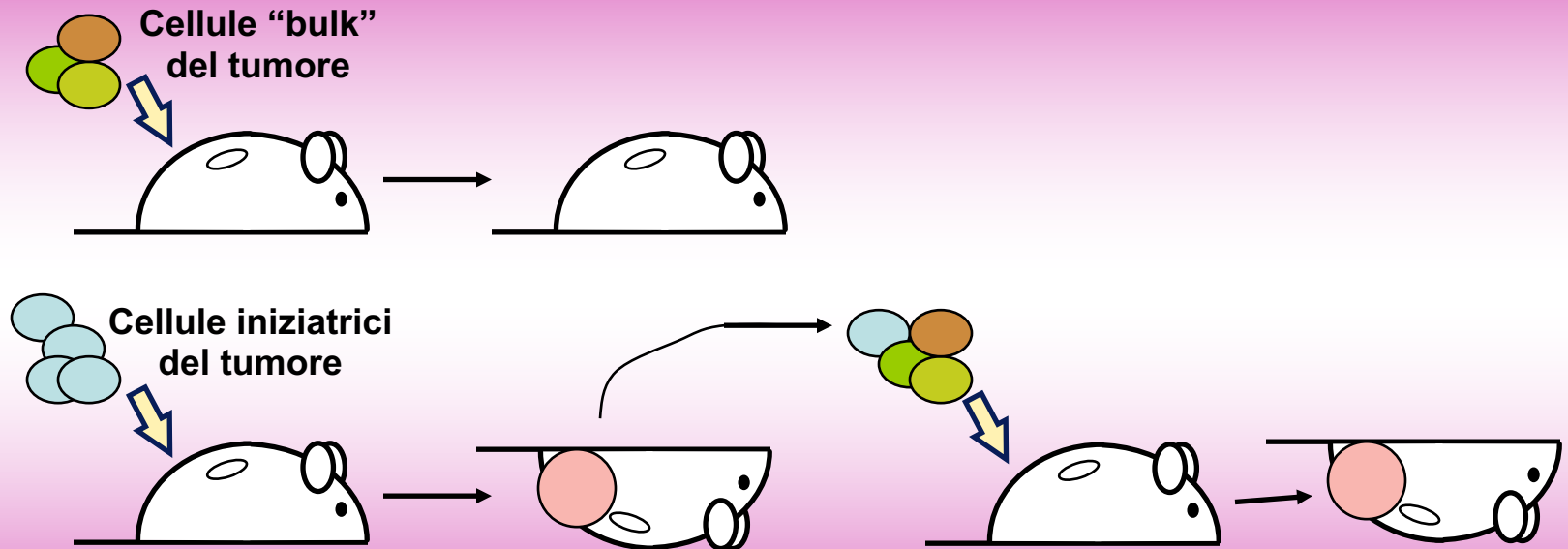


# Tecniche di purificazione ed isolamento *prospettivo* di cellule staminali del cancro

Definizione di una CELLULA STAMINALE TUMORALE  
= UNA CELLULA IN GRADO DI RICAPITOLARE TUTTA LA TUMORIGENESI SE  
IMPIANTATA IN UN OSPITE



Cellule tumorali selezionate  
(p.es. FACS)



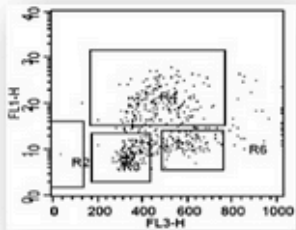
# Identification of novel markers for CSC

Isolation based on stemness properties

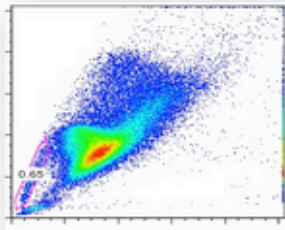
Primary cell lines & fresh tumors from Mouse & Man

**Optimization of sphere cell culture conditions**

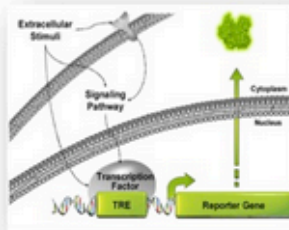
Resistance to chemotherapy



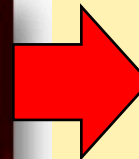
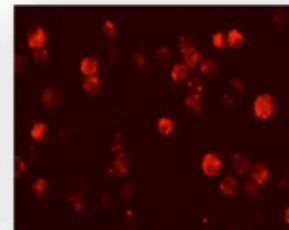
SP cells



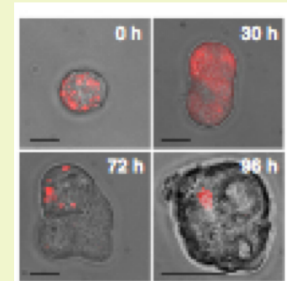
Stemness reporter



Label retaining cell assay



PKH26 membrane dye



**Identify distinct / overlapping populations / enrich populations**

→ Membrane Proteomics / Phage Display Technology

→ Functional characterization of subpopulations



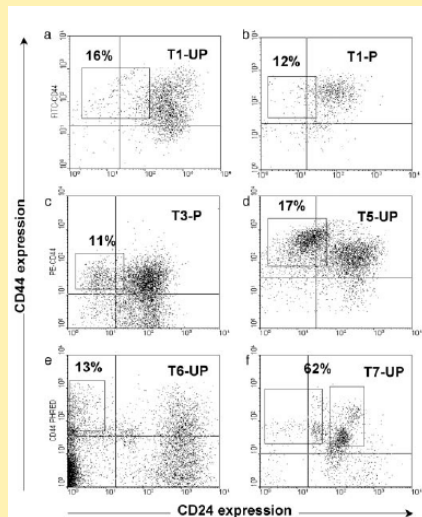
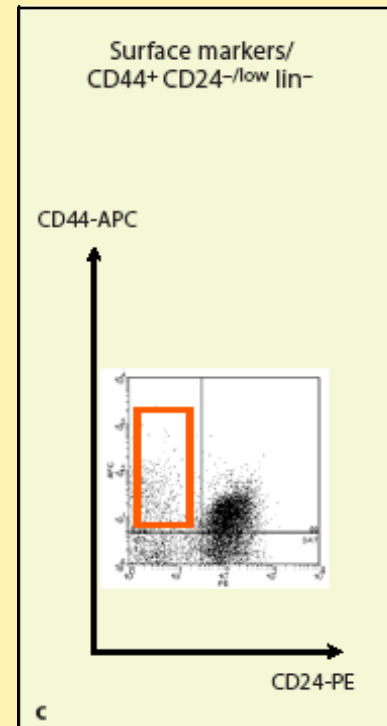
*Pece et al. Cell 2010*

# Prospective identification of tumorigenic breast cancer cells

Muhammad Al-Hajj\*, Max S. Wicha\*, Adalberto Benito-Hernandez†, Sean J. Morrison\*\*§, and Michael F. Clarke\*\*¶

Departments of \*Internal Medicine and †Pathology, Comprehensive Cancer Center, ‡Department of Developmental Biology, and §Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109

Communicated by Jack E. Dixon, University of Michigan Medical School, Ann Arbor, MI, January 16, 2003 (received for review December 18, 2002)



**Table 3. Tumorigenic breast cancer cells were highly enriched in the ESA<sup>+</sup>CD44<sup>+</sup>CD24<sup>-/low</sup> population**

	Tumors/injections									
	5 × 10 <sup>5</sup>	10 <sup>5</sup>	5 × 10 <sup>4</sup>	2 × 10 <sup>4</sup>	10 <sup>4</sup>	5 × 10 <sup>3</sup>	10 <sup>3</sup>	500	200	100
<b>Mouse passage 1</b>										
Unsorted	8/8	8/8	10/10		3/12		0/12	—	—	—
CD44 <sup>+</sup> CD24 <sup>+</sup>	—	—	—	0/10	0/10	0/14	0/10	—	—	—
CD44 <sup>+</sup> CD24 <sup>-/low</sup>	—	—	—	10/10	10/10	14/14	10/10	—	—	—
CD44 <sup>+</sup> CD24 <sup>-/low</sup> ESA <sup>+</sup>	—	—	—	—	—	—	10/10*	4/4	4/4	1/6
CD44 <sup>+</sup> CD24 <sup>-/low</sup> ESA <sup>-</sup>	—	—	—	—	—	—	0/10*	0/4	0/4	0/6
<b>Mouse passage 2</b>										
CD44 <sup>+</sup> CD24 <sup>+</sup>	—	—	—	—	0/9	—	—	—	—	—
CD44 <sup>+</sup> CD24 <sup>-/low</sup>	—	—	—	—	9/9	—	—	—	—	—
<b>Patients' tumor cells</b>										
CD44 <sup>+</sup> CD24 <sup>+</sup>	—	0/3	0/4	0/8	1/13	0/2	—	—	—	—
CD44 <sup>+</sup> CD24 <sup>-/low</sup>	—	3/3	4/4	—	11/13	1/1	—	—	—	—
CD44 <sup>+</sup> CD24 <sup>-/low</sup> ESA <sup>+</sup>	—	—	—	—	—	2/2	2/2	—	—	—
CD44 <sup>+</sup> CD24 <sup>-/low</sup> ESA <sup>-</sup>	—	—	—	—	—	2/2 <sup>†</sup>	0/2	—	—	—

# ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome

Christophe Ginestier,<sup>1</sup> Min Hee Hur,<sup>2</sup> Emmanuelle Charafe-Jauffret,<sup>3</sup> Florence Monville,<sup>3</sup> Julie Dutcher,<sup>1</sup> Marty Brown,<sup>1</sup> Jocelyne Jacquemier,<sup>3</sup> Patrice Viens,<sup>3</sup> Celina G. Kleer,<sup>1</sup> Suling Liu,<sup>1</sup> Anne Schott,<sup>1</sup> Dan Hayes,<sup>1</sup> Daniel Birnbaum,<sup>3</sup> Max S. Wicha,<sup>1</sup> and Gabriela Dontu<sup>1,\*</sup>

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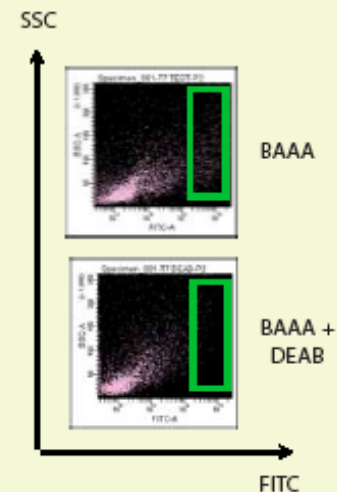
FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.

## Product Description

ALDEFLUOR™ is a reagent kit that is used to identify human cells that express high levels of the enzyme aldehyde dehydrogenase (ALDH). The activated ALDEFLUOR™ Reagent, BODIPY-aminoacetaldehyde (BAAA), is a fluorescent non-toxic substrate for ALDH, which freely diffuses into intact and viable cells. In the presence of ALDH, BAAA is converted into BODIPY-aminoacate (BAA), which is retained inside the cells. The amount of fluorescent reaction product is proportional to the ALDH activity in the cells and is measured using a flow cytometer. Viable ALDH-bright (ALDH<sup>br</sup>) cells can, in principle, be isolated using a cell sorter. Active efflux of the reaction product is inhibited by an efflux inhibitor in the ALDEFLUOR™ Assay Buffer. A specific inhibitor of ALDH, diethylaminobenzaldehyde (DEAB), is used to control for background fluorescence.

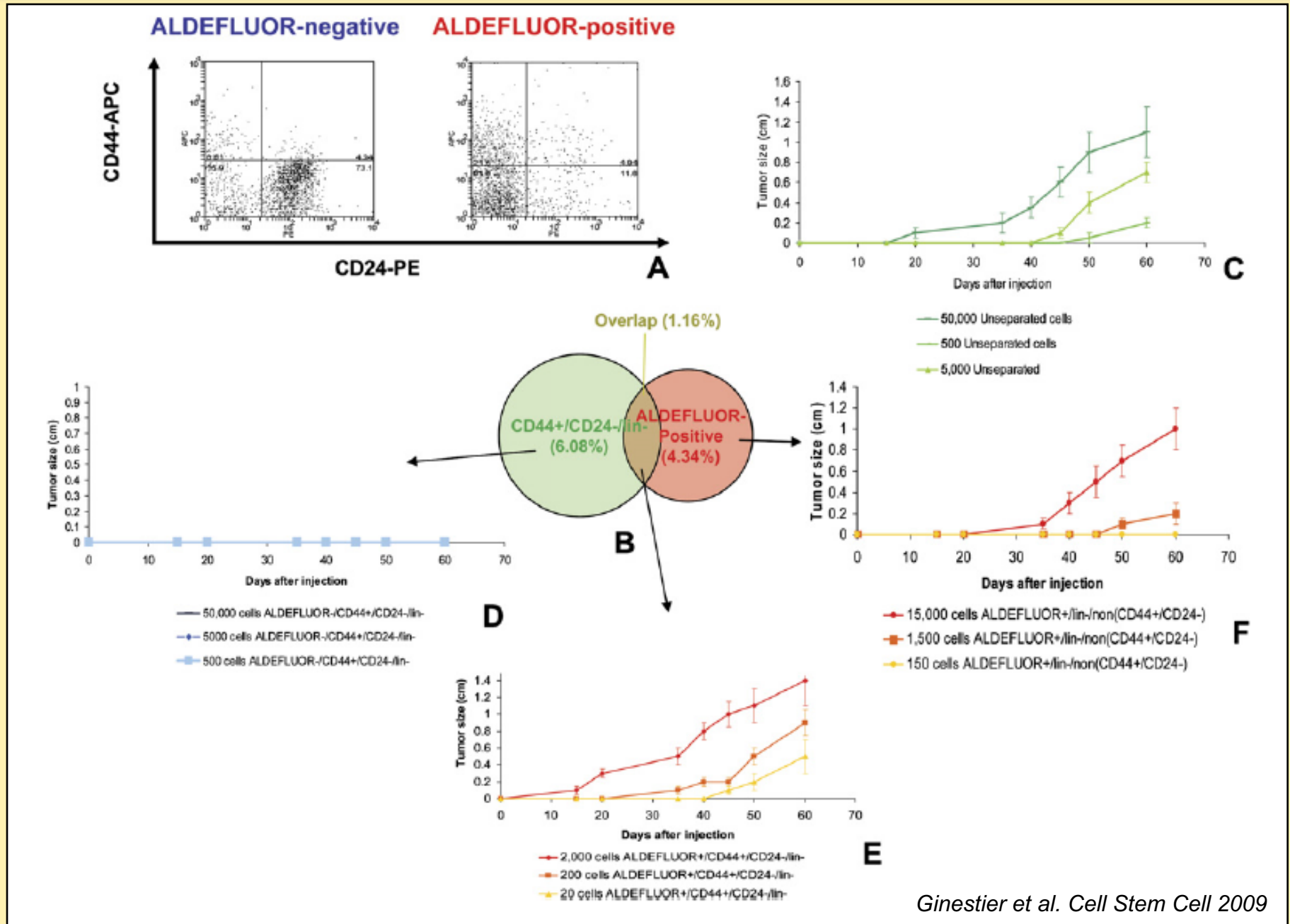
ALDEFLUOR™ is optimized for the detection of ALDH<sup>br</sup> hematopoietic cells in human blood and bone marrow, but it can also be used with non-hematopoietic cells. For a full list of ALDEFLUOR™ products, please visit our website at [www.stemcell.com](http://www.stemcell.com).

Enzyme activity/  
ALDEFLUOR assay

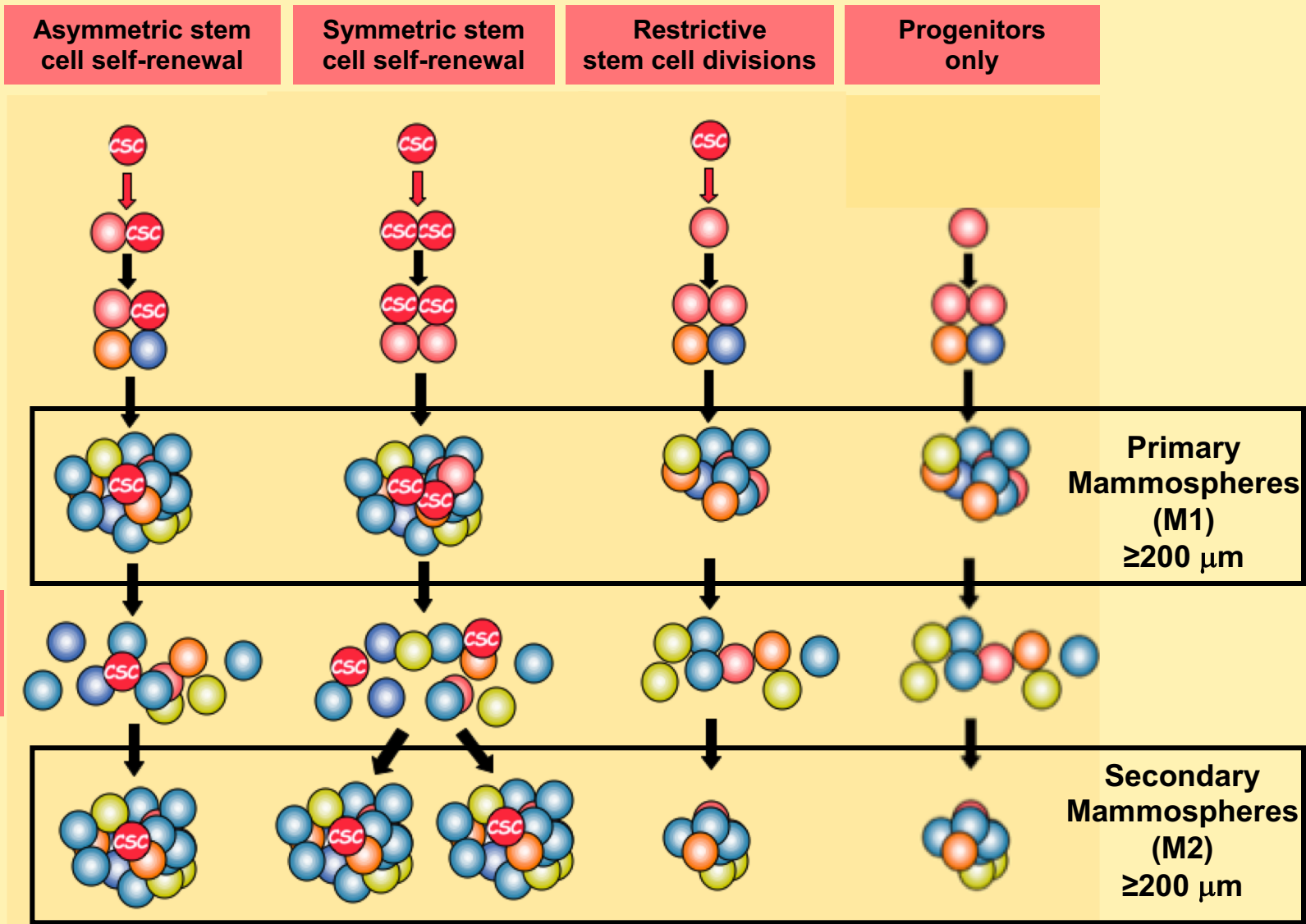




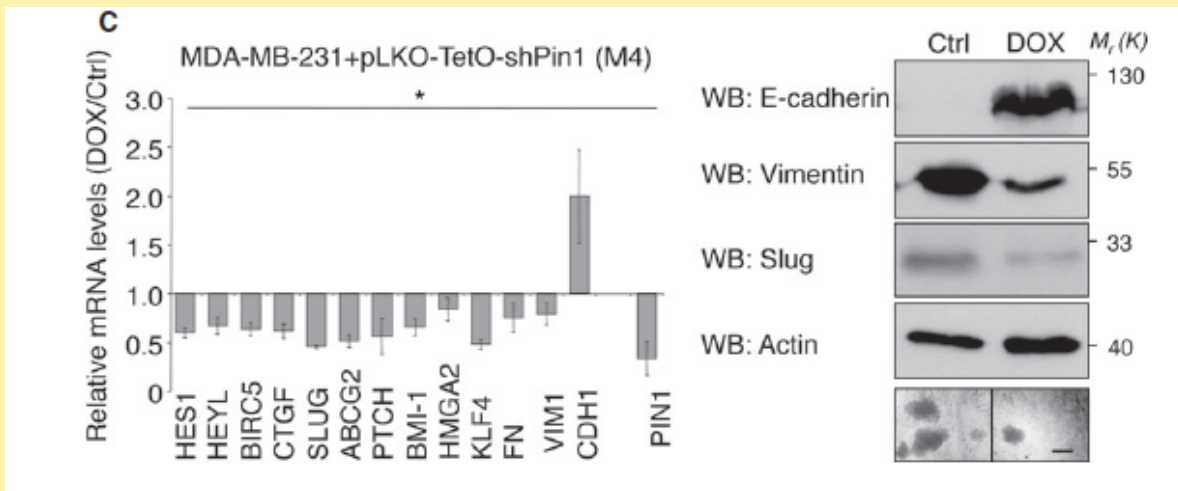
# I marcatori per cellule staminali non sono univoci: esistono diverse popolazioni di CSC



# Esperimento di formazione di sfere tumorali (*tumorspheres*)

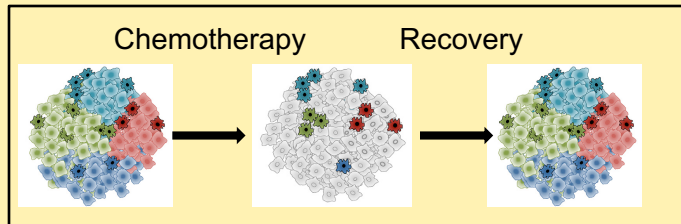


# Analisi di marcatori di EMT e staminalità



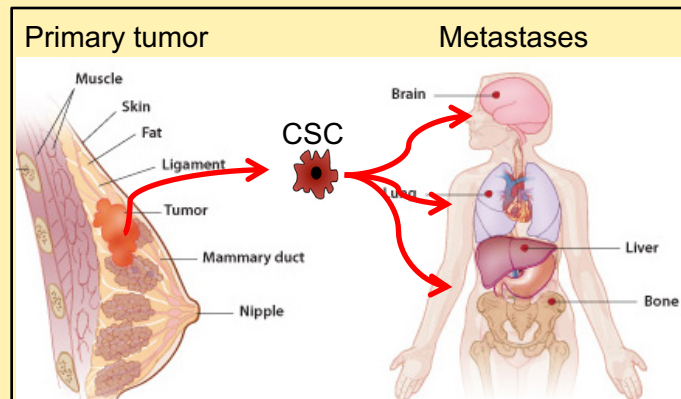
Possiamo sfruttare la conoscenza delle caratteristiche molecolari e fenotipiche delle CSC ai fini clinici

# Le CSC sono responsabili della progressione tumorale e delle recidive



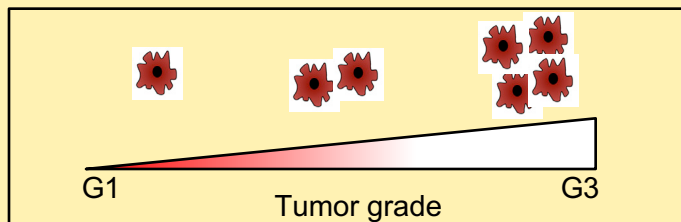
Le CSCs sono **insensibili alle chemioterapie** e sono alla base del “dandelion phenomenon”

*Kreso and Dick, 2014; Dean 2009; Dean 2005*



Le CSCs mostrano caratteristiche di transdifferenziamento (ad es. **EMT**) e sono responsabili della disseminazione metastatica. Induzione di EMT in cellule epiteliali della mammella induce un fenotipo CSC.

*Oskarsson 2014; Mani 2008*



I carcinomi della mammella più aggressivi sono arricchiti di **CSCs**.

*Pece 2010; Ben-Porath 2008*

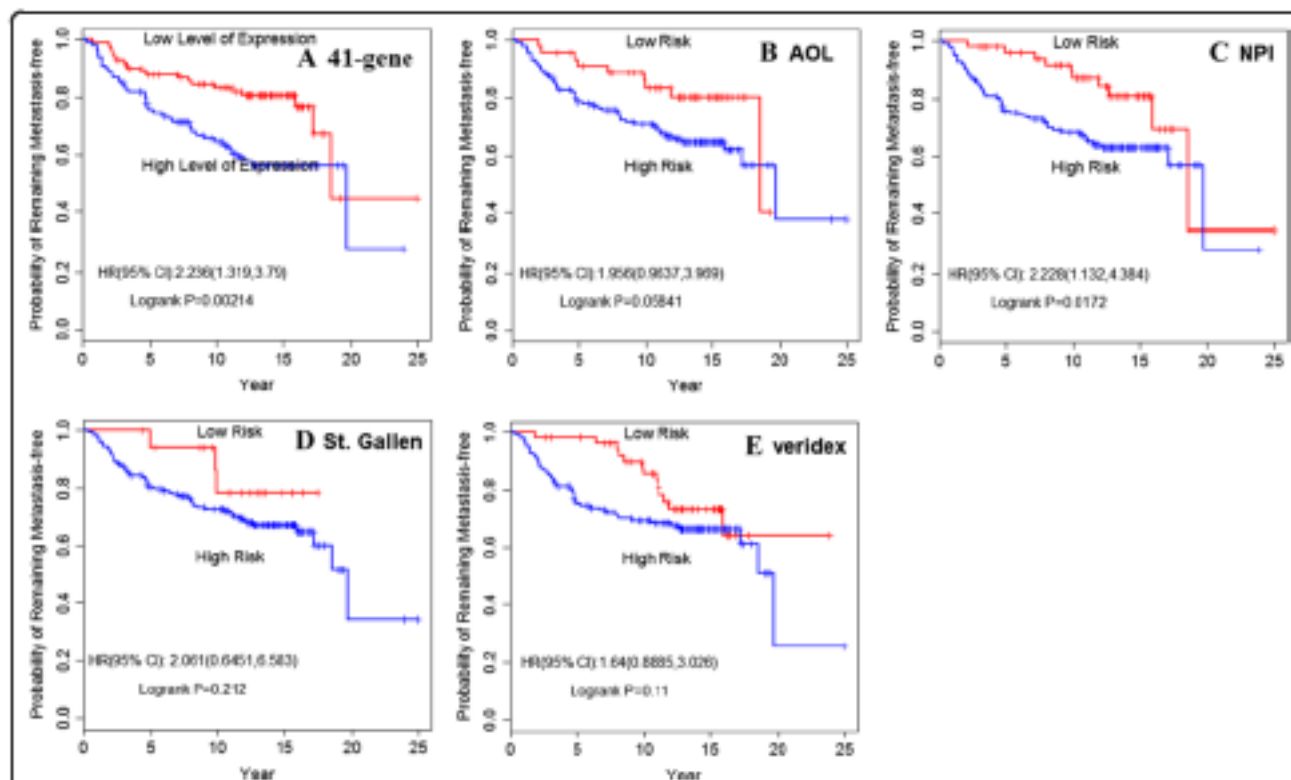


RESEARCH

Open Access

# A 41-gene signature derived from breast cancer stem cells as a predictor of survival

Zhi-Qiang Yin<sup>1†</sup>, Jian-Jun Liu<sup>1†</sup>, Ying-Chun Xu<sup>1</sup>, Jian Yu<sup>2</sup>, Guo-Hui Ding<sup>2</sup>, Feng Yang<sup>1</sup>, Lei Tang<sup>1</sup>, Bao-Hong Liu<sup>2</sup>, Yue Ma<sup>1</sup>, Yu-Wei Xia<sup>1</sup>, Xiao-Lin Lin<sup>1</sup> and Hong-Xia Wang<sup>1,3\*</sup>



**Figure 2** Kaplan-Meier analysis of the probability that patients would remain free of distant metastasis among all patients. **A**, prediction value of DMFS by the 41-gene signature. Patients were divided into those with a good-prognostic signature and those with a poor prognostic signature according to gene-expression profiling; **B**, prediction value of OS by AOL consensus criteria; **C**, prediction value of DMFS by NPI consensus criteria; **D**, prediction value of DMFS by St. Gallen criteria; **E**, prediction of Veridex signature. The p values were calculated by log-rank test.

## **Potenziale tumorigenico**

- Le CSC garantiscono il mantenimento della cellula staminale e la produzione di cellule della massa tumorale “differenziata” (bulk).

## **Resistenza intrinseca alle radio- e chemioterapie**

- Quiescenza;
- Meccanismi di resistenza/sopravvivenza potenziati in CSC proliferanti (anti-apoptotici, pompe ad efflusso, antiossidanti, riparazione del DNA, interazioni con la nicchia etc.).

## **Correlazione con parametri clinici (impatto prognostico e terapeutico)**

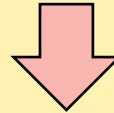
- Frequenza di CSC nel tumore è associata con aggressività in molti tumori;
- Aumento del pool di CSC dopo chemioterapia e diminuita sopravvivenza dei pazienti;
- Valore prognostico di firme molecolari delle CSC;
- Bersagli di terapie specifiche.

FALLIMENTI DELLE CHEMIO O RADIOTERAPIE >> RECIDIVE, METASTASI

**QUALI CELLULE DEVONO ESSERE COLPITE DALLE TERAPIE ANTI-TUMORALI?**

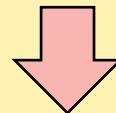
Terapie anti-proliferative non hanno senso per le cellule staminali del cancro, vista la loro quiescenza e l'attivazione di meccanismi, che le rendono resistenti al trattamento con chemioterapici:

- Aumento dei livelli di proteine della famiglia BCL-2
- Proteine "multiple drug resistance"



**Terapie anti-CSC:**  
Contrastare meccanismi di chemioresistenza  
Contrastare l'attivazione di pathway dello sviluppo ("Terapie differenzianti")  
etc.

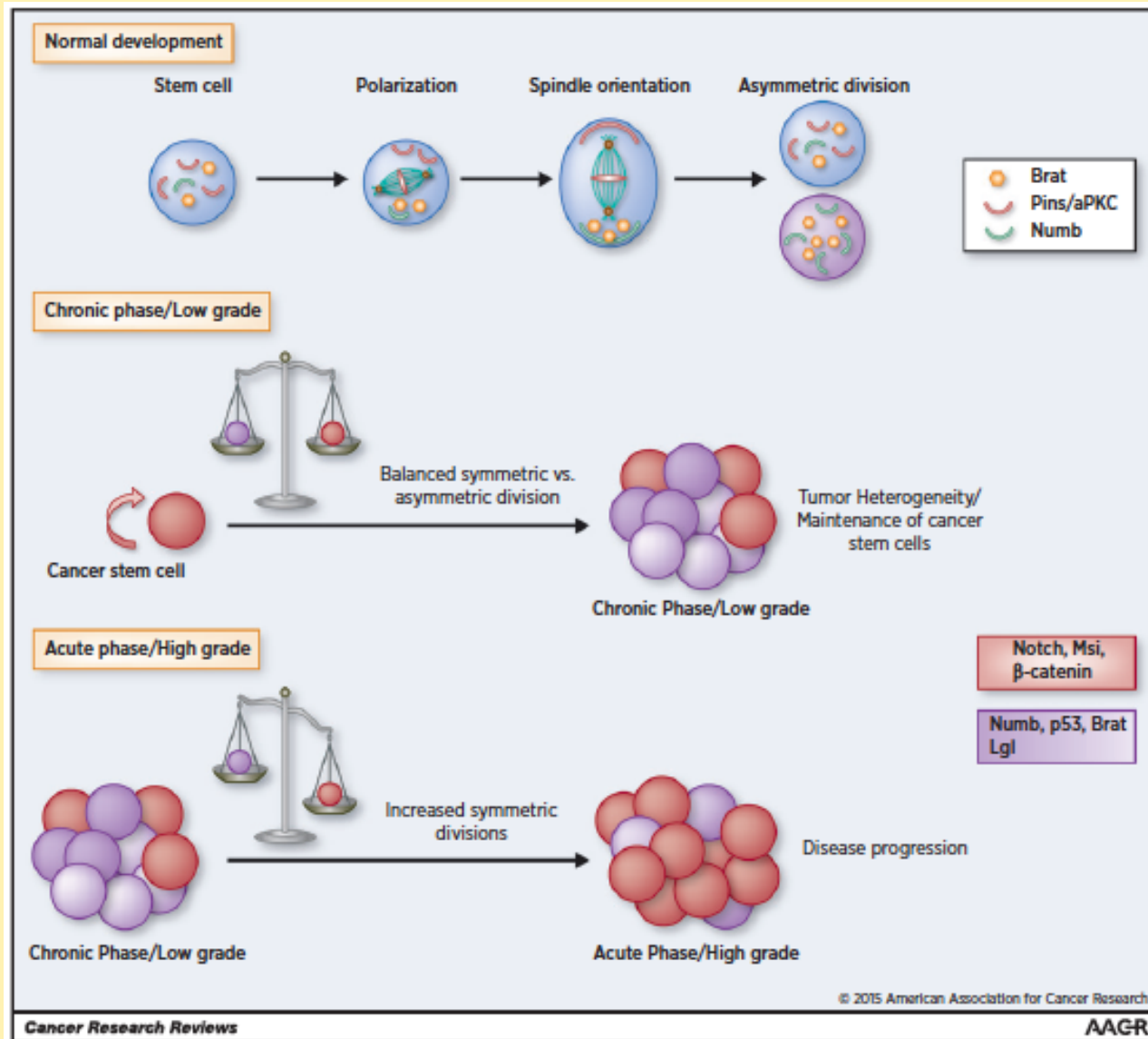
**+ CHEMIOTERAPIE CONVENZIONALI (CONTRO IL "BULK")  
+ TERAPIE A BERSAGLIO SPECIFICO**



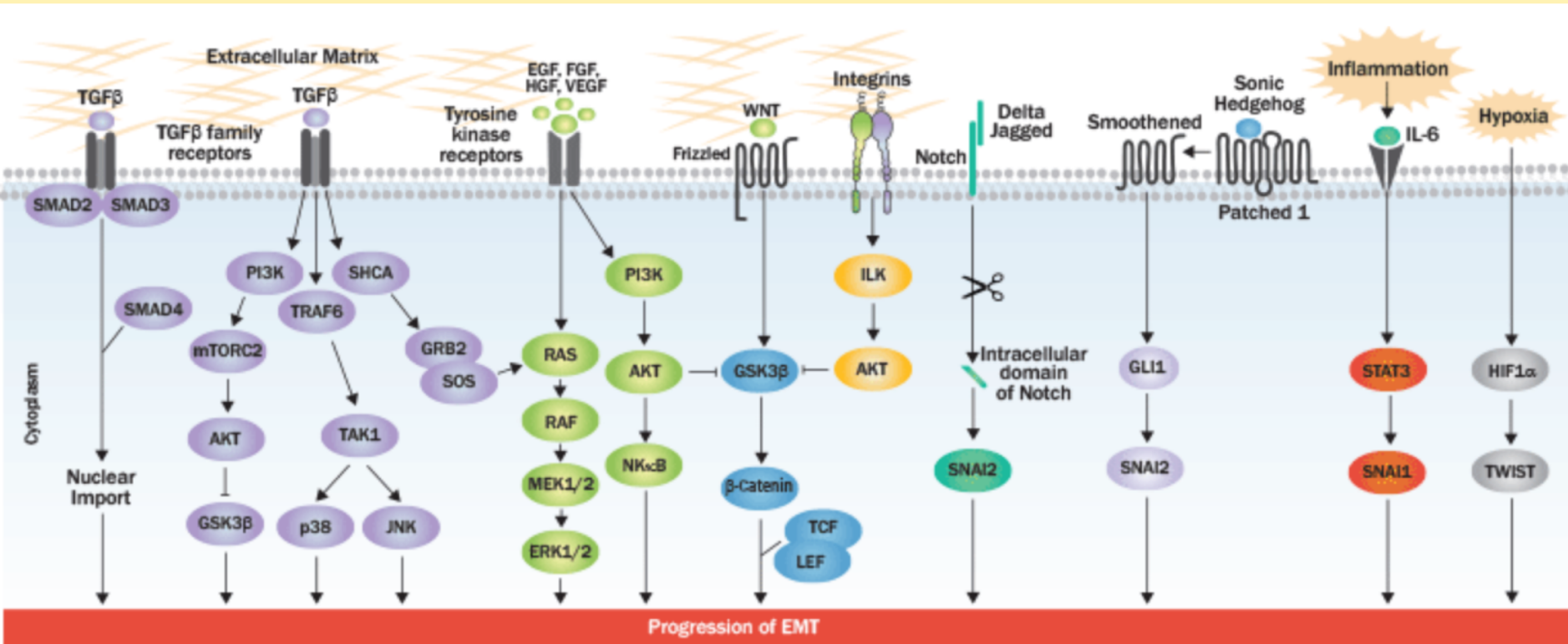
**SCOPRIRE QUALI SONO I MECCANISMI MOLECOLARI E LE  
VULNERABILITA' SPECIFICHE DELLE CSC**



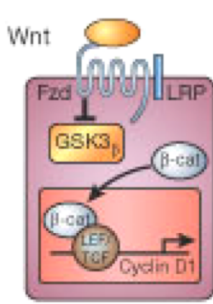

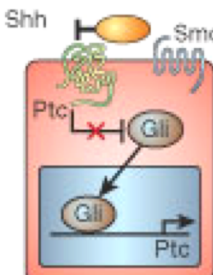
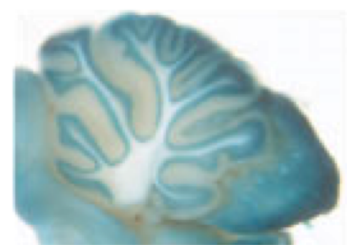
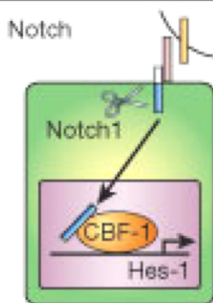
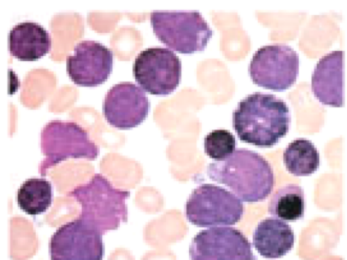
# Mantenimento delle CSC e della massa tumorale dipende da alterazioni di pathway specifiche: nuovi bersagli per la terapia anti-CSC?



# Bersagli per colpire le CSC: Pathways coinvolte nella EMT e deregolate nel cancro



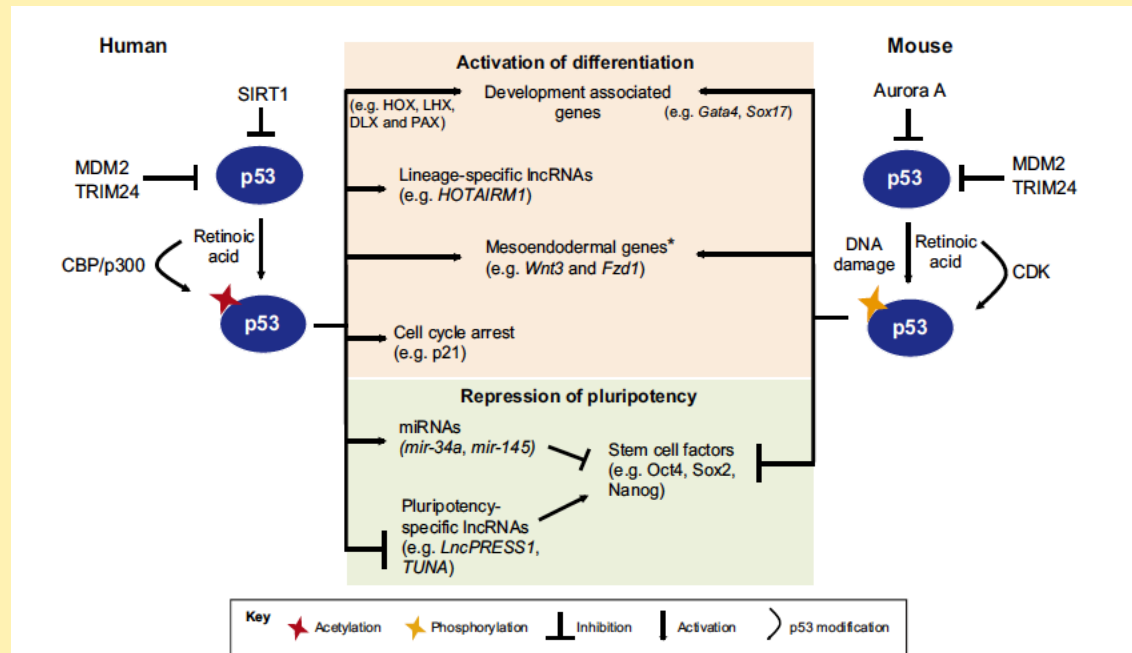
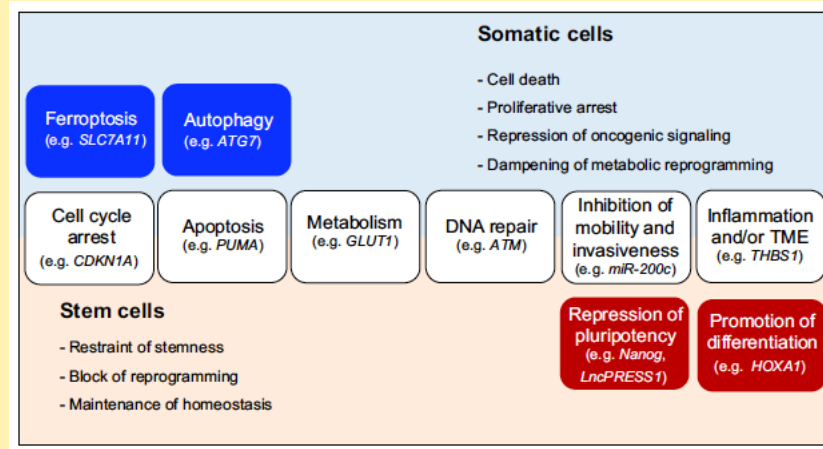
# Bersagli per colpire le CSC: Pathways coinvolte nel *self-renewal* e deregolate nel cancro

	Stem/progenitor cell self-renewal			Tumorigenesis	
	Haematopoietic	Epidermal	Gut	Colon carcinoma	
				Epidermal tumours	
	Haematopoietic	Neural	Germ line	Medulloblastoma	
				Basal cell carcinoma	
	Haematopoietic	Neural	Germ line	Leukaemia	
				Mammary tumours	
				<div style="border: 1px solid red; padding: 5px; display: inline-block;">                     + Mammary epithelium                      + Intestinal epithelium                 </div>	

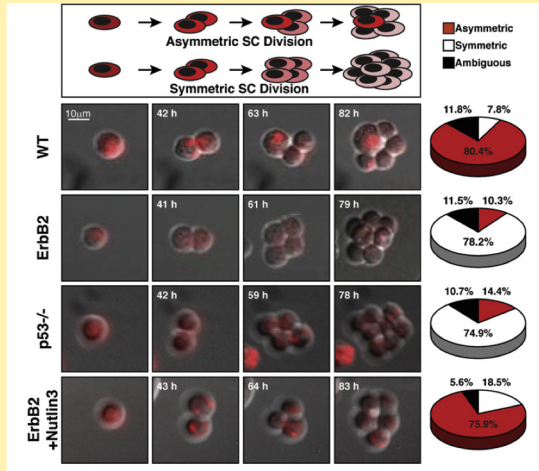
*Adapted from Reya et al. Nature 2001*

Wnt, Shh, and Notch pathways have been shown to contribute to the self-renewal of stem cells and/or progenitors in a variety of organs, including the haematopoietic and nervous systems. When dysregulated, these pathways can contribute to oncogenesis. Mutations of these pathways have been associated with a number of human tumours, including colon carcinoma and epidermal tumours (Wnt), medulloblastoma and basal cell carcinoma (Shh), and T-cell leukaemias (Notch).

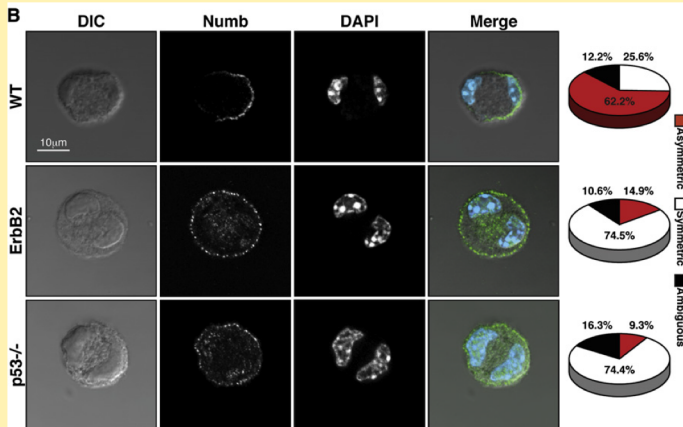
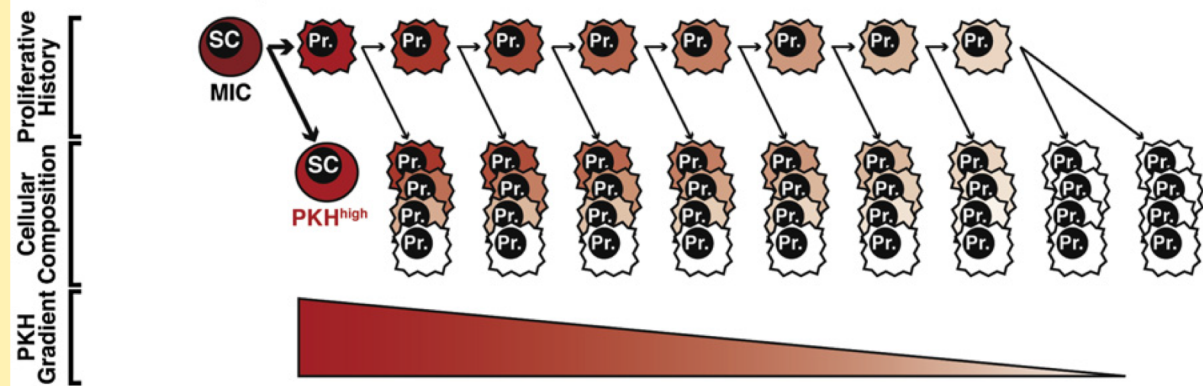
# Ruolo di p53 nel differenziamento cellulare: un'ulteriore modalità di soppressione tumorale



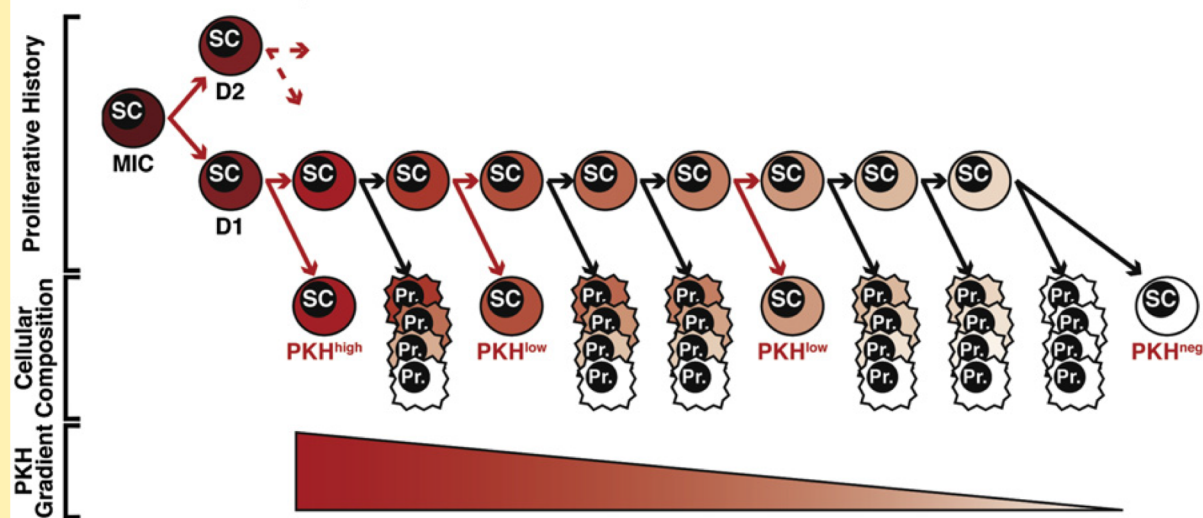
# p53 regola le divisioni asimmetriche delle cellule staminali di mammella



## A WT mammospheres

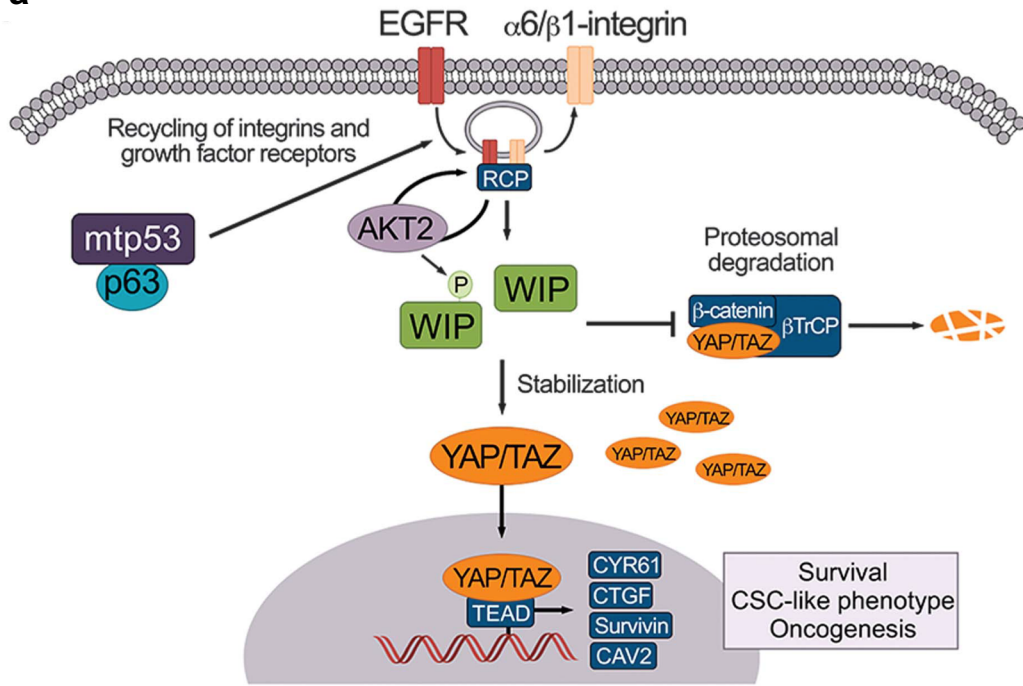


## B ErbB2 mammospheres

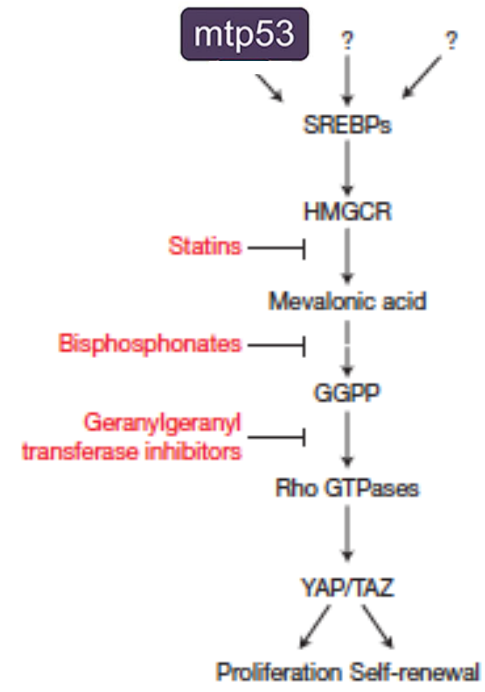


# Forme mutate di p53 (mtp53) cooperano nell'induzione di YAP/TAZ e CSC

**a**



**b**



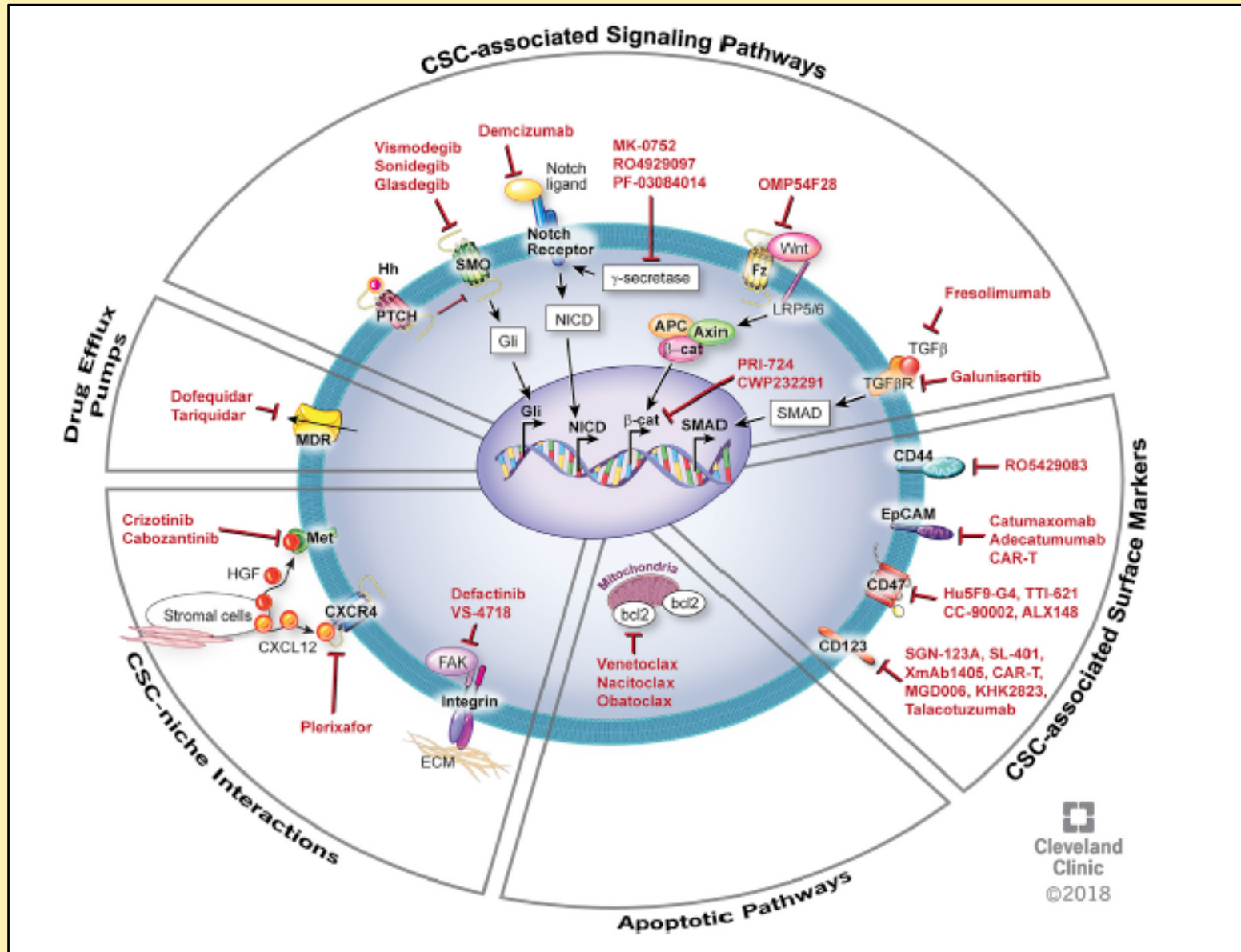
*Muller et al., Cell 2009*

*Escoll et al., Oncogene 2017*

*Freed-Pastor et al., Cancer Cell 2012*

*Sorrentino et al., Nat Cell Biol 2014*

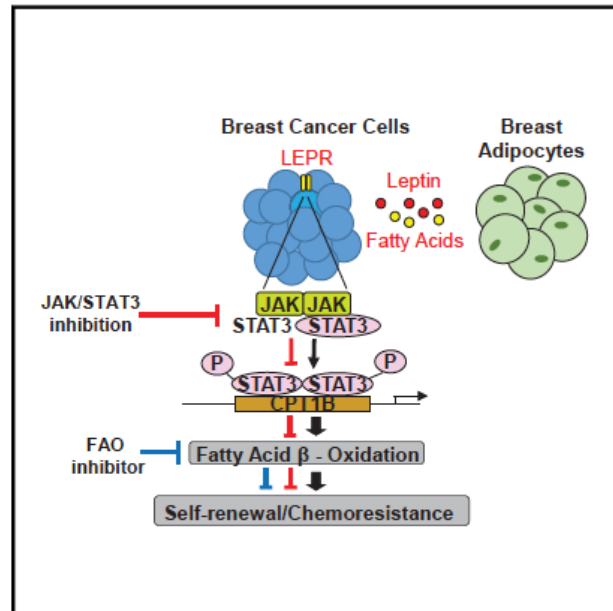
# Therapie anti-CSC (I)



## Cell Metabolism

### JAK/STAT3-Regulated Fatty Acid $\beta$ -Oxidation Is Critical for Breast Cancer Stem Cell Self-Renewal and Chemoresistance

Graphical Abstract



Authors

Tianyi Wang,  
Johannes Francois Fahrmann,  
Heehyoung Lee, ..., Samir Hanash,  
Richard Jove, Hua Yu

Correspondence

hyu@coh.org

In Brief

Cancer stem cells play an important role in cancer development and chemoresistance. Wang et al. show that leptin-JAK/STAT3 regulates lipid metabolism through fatty acid  $\beta$ -oxidation (FAO), promoting breast cancer stemness and chemoresistance. Blocking FAO and/or depleting leptin resensitize cancer cells to chemotherapy while reducing cancer stemness *in vivo*.

Inhibition of FAO preferentially eliminates BCSCs

JAK/STAT3 activates FAO through transcription of CPT1B

Adipocyte-derived leptin is critical for JAK/STAT3-FAO in BCSCs

Targeting FAO/leptin inhibits BCSCs, chemoresistance, and breast tumor growth



The Notch receptor signaling pathway:  
an appealing target for cancer therapy

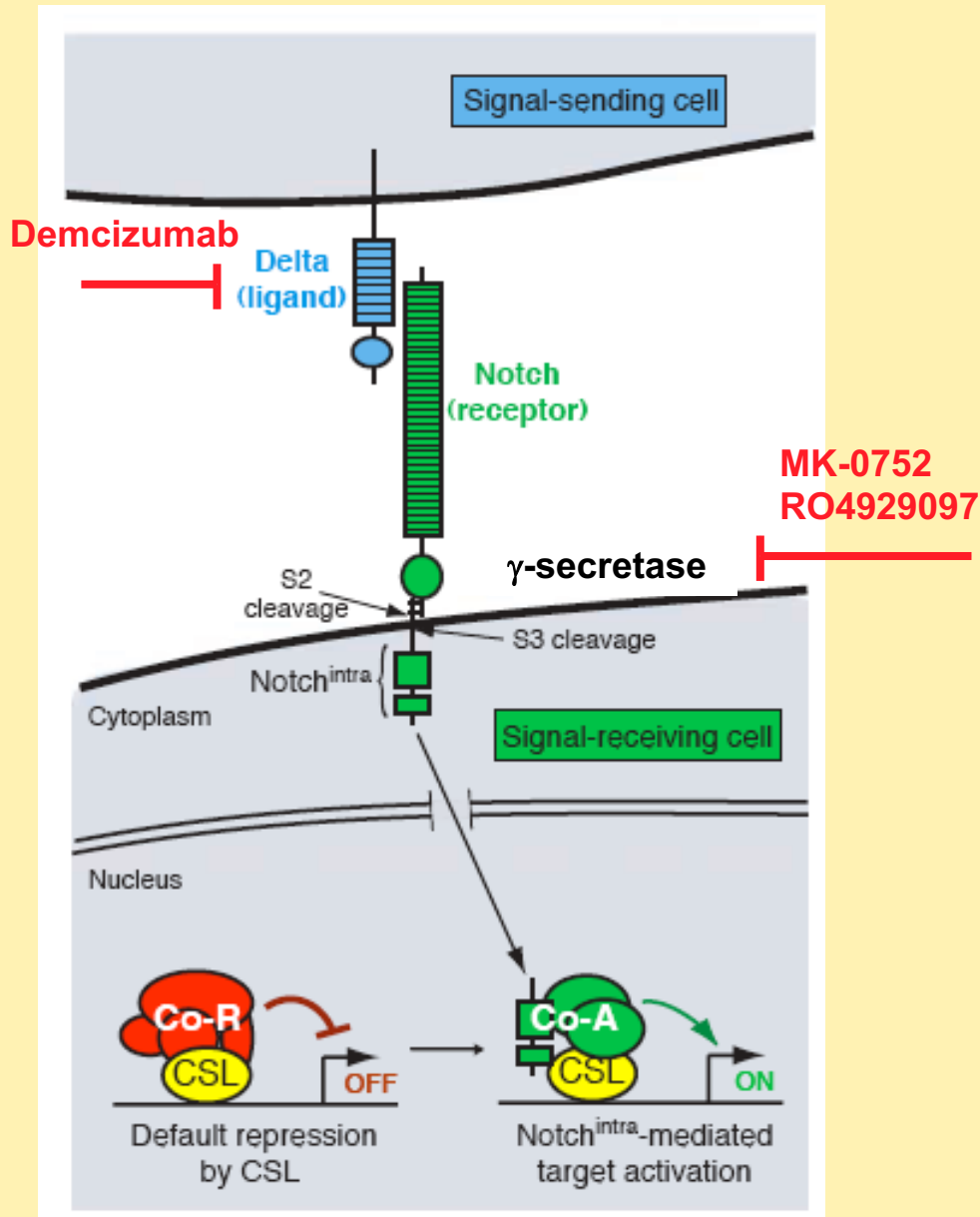
*ARRESTED DEVELOPMENT?*

## **Notch Emerges as New Cancer Drug Target**

By Ken Garber

*JNCI, 2007*

# The Notch receptor signaling pathway



Mediates short-range signals that control many cell fate decisions, such as

- Stem cell renewal/Asymmetric cell division
- Inhibition of differentiation/Differentiation
- Proliferation/Growth promotion or arrest
- Inhibition of Apoptosis
- Movement

In multiple tissues....but is cell/context dependent!

Depends on cell-cell contact

Cooperates with developmental pathways (Wnt, SHH, RTKs etc.)

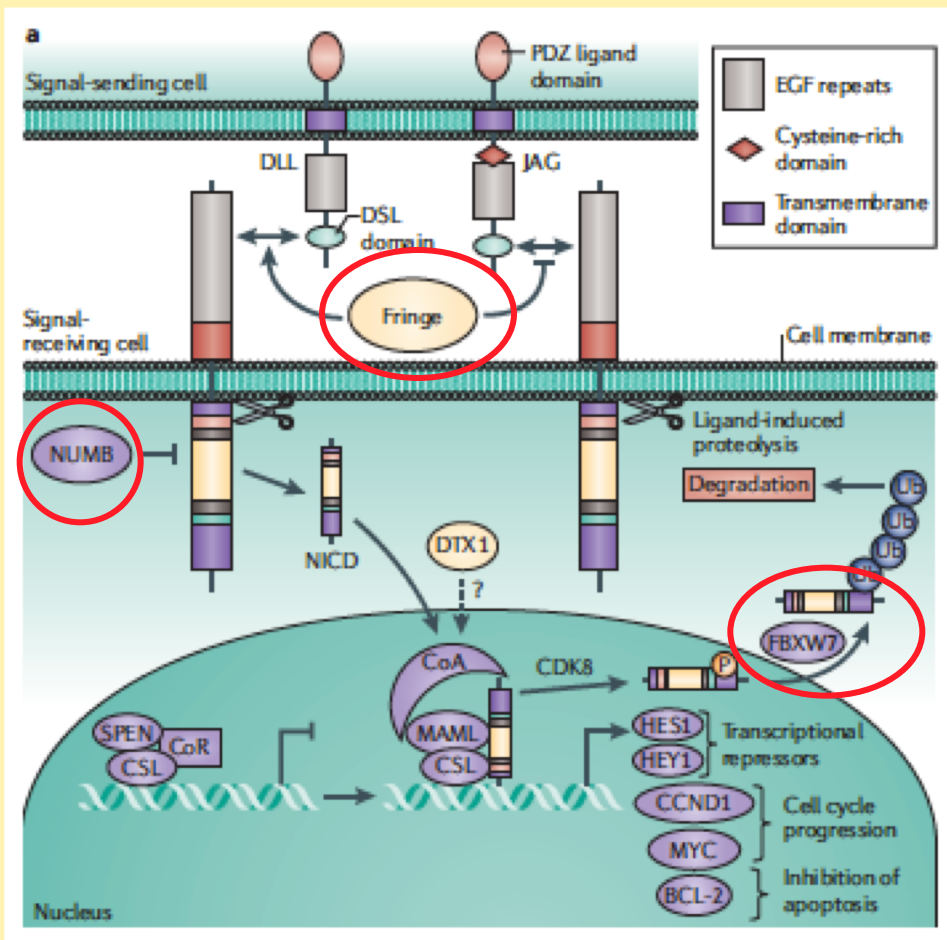
Cross-talks with many pathways (Ras, TGFbeta, HIF-1 alpha, NF-kappaB)

Misregulation of Notch has been linked to Cancer and to developmental disorders.

Dosage-dependent GOF, LOF

# Regolazione e attivazione trascrizionale a valle di Notch

Lista crescente di geni bersaglio:  
Mediatori delle funzioni di Notch



HES-1/6  
HERP-1/3

**Repressori trascrizionali:  
Ruolo anti-differenziativo**

c-erb b2  
Cyclin D1

**Mediatori della proliferazione  
cellulare**

Notch4  
c-myc  
NF-kappaB2  
PPAR  
GATA-2  
Snail

**Fattori trascrizionali: crescita e  
differenziamento**

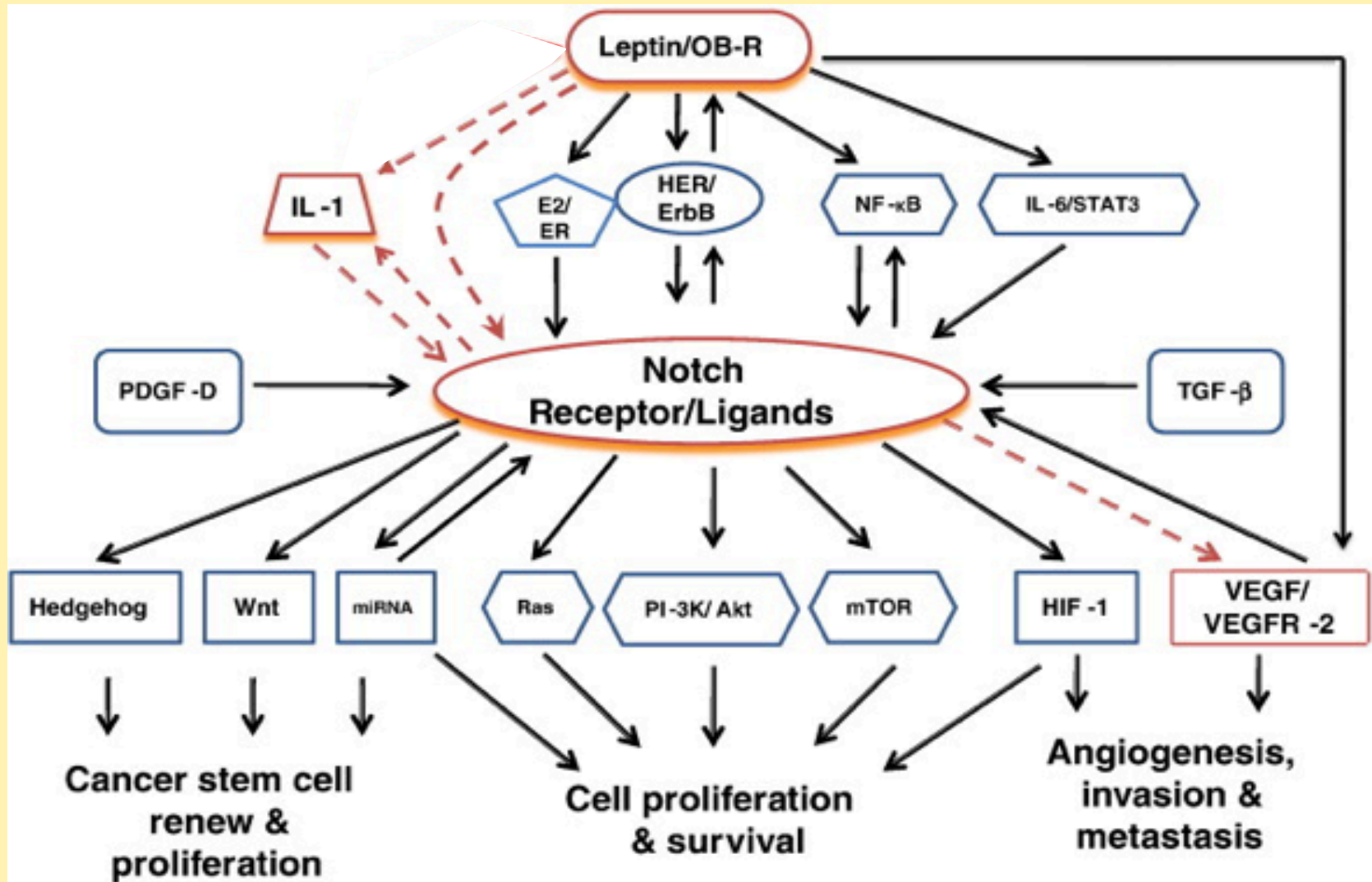
ABCG2  
Bcl-2

**Mediatori di chemioresistenza**

p21  
Deltex

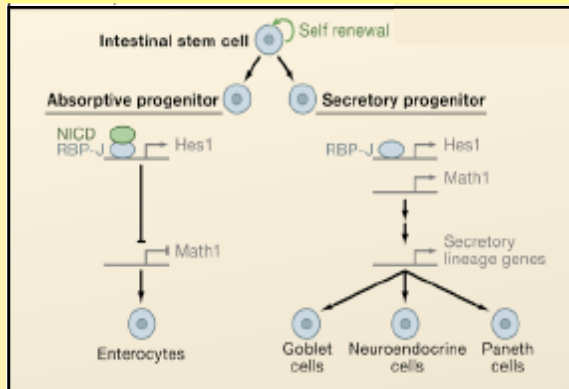
**Regolatori negativi**

# Interazione di Notch con altre pathways

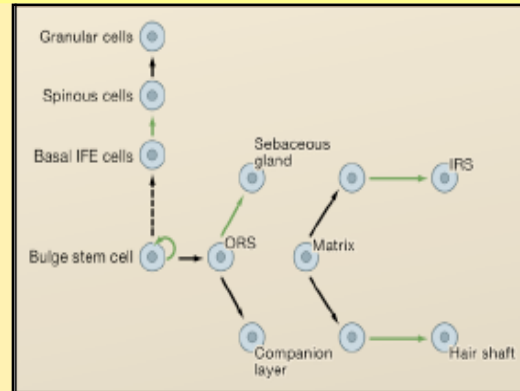


# Notch controlla il *self-renewal* e differenziamento di cellule epiteliali nell'uomo

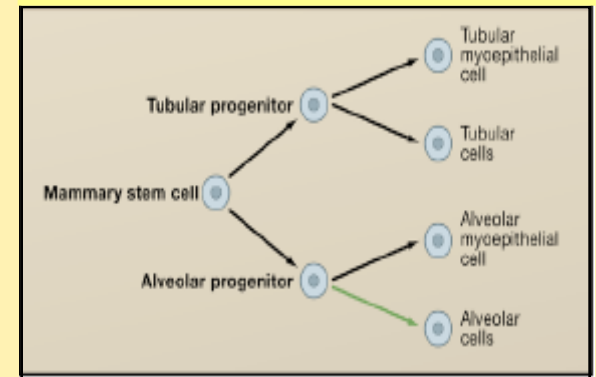
## Intestinal epithelium



## Skin epithelium



## Mammary gland



**Blanpain, Cell 2007**

# Meccanismi di attivazione oncogenica di Notch

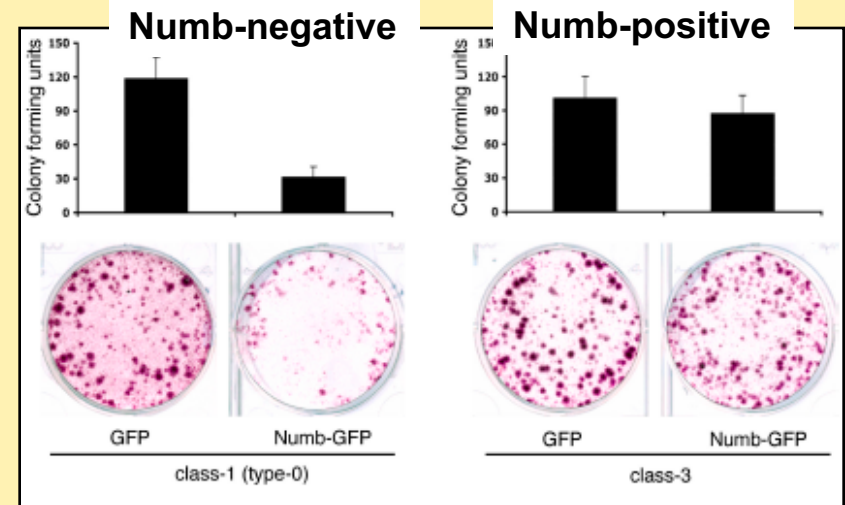
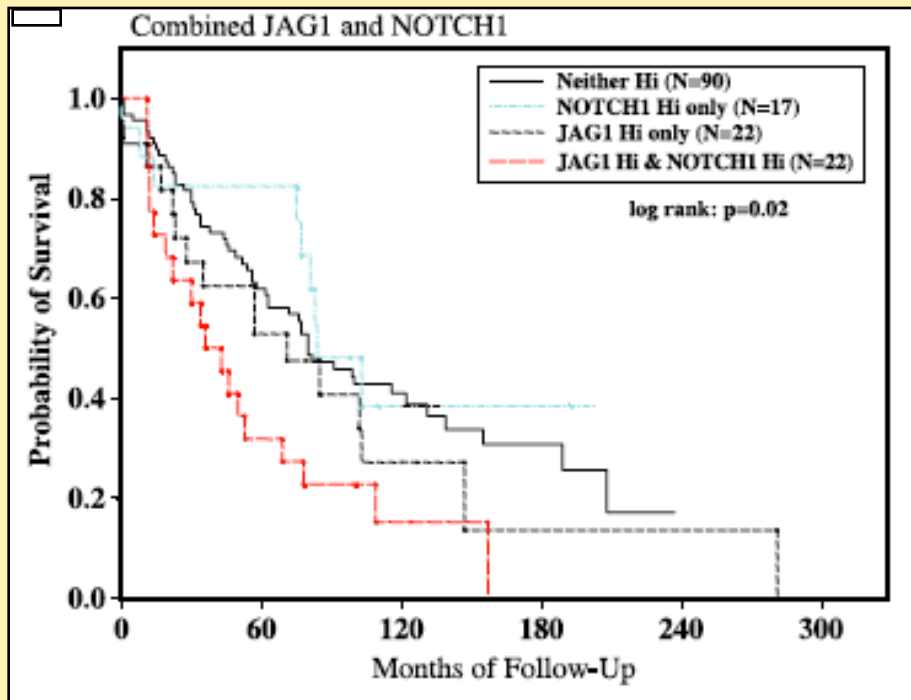
## Attivazione deregolata nel cancro alla mammella

### High-level Coexpression of JAG1 and NOTCH1 Is Observed in Human Breast Cancer and Is Associated with Poor Overall Survival

Michael Reedijk,<sup>1,2,3</sup> Silvia Odorcic,<sup>1</sup> Lynn Chang,<sup>1</sup> Hui Zhang,<sup>1</sup> Naomi Miller,<sup>4</sup> David R. McCready,<sup>3</sup> Gina Lockwood,<sup>5</sup> and Sean E. Egan<sup>1,6</sup>

### Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis

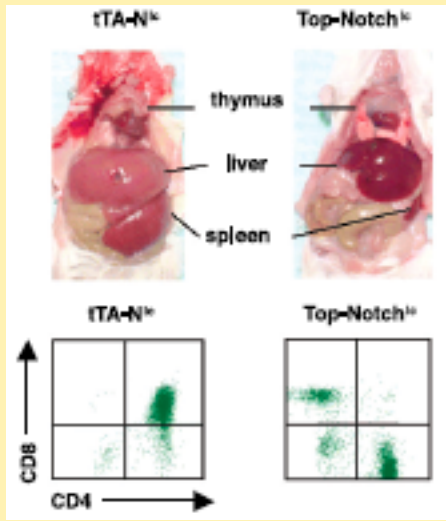
Salvatore Pece,<sup>1,2</sup> Michela Serresi,<sup>1</sup> Elisa Santolini,<sup>1</sup> Maria Capra,<sup>1,3</sup> Esther Hulleman,<sup>1</sup> Viviana Galimberti,<sup>1</sup> Stefano Zurrada,<sup>1</sup> Patrick Maisonneuve,<sup>1</sup> Giuseppe Viale,<sup>1,2</sup> and Pier Paolo Di Fiore<sup>1,2,3</sup>



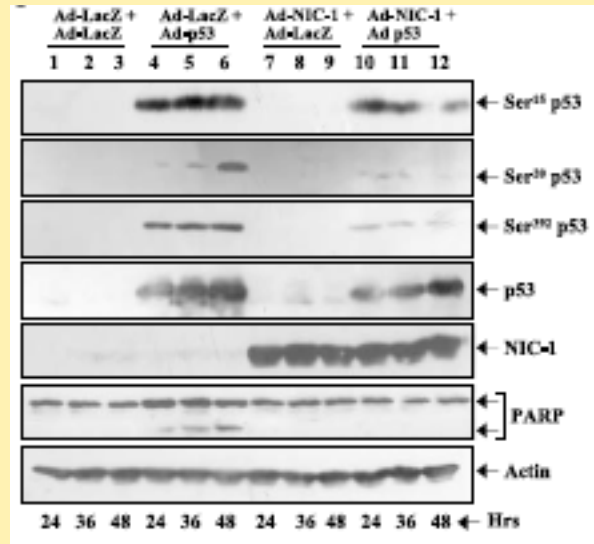
Re-expression of Numb selectively suppresses growth in Numb-negative tumors

# Meccanismi oncogénici di Notch: repressione dell'attività di p53

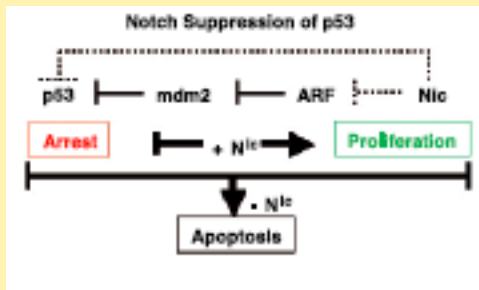
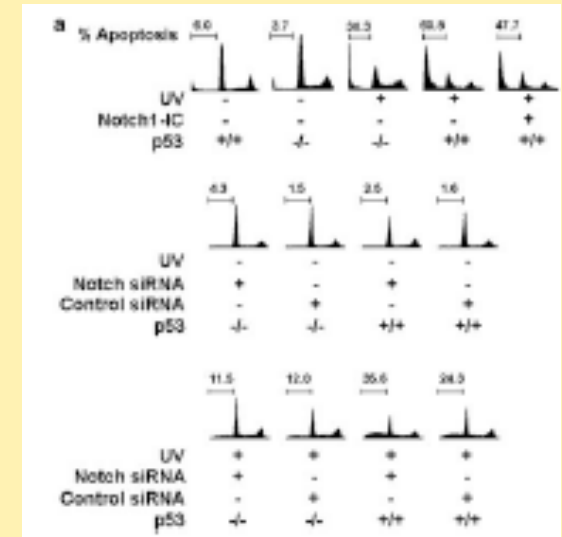
Notch suppresses p53 in Lymphomagenesis



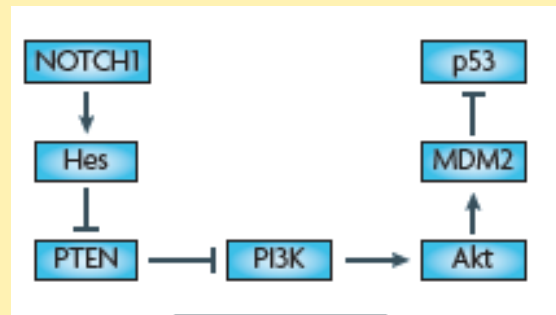
Activated Notch1 inhibits p53 function in H460 cells through PI3K/Akt pathway



Activated Notch1 interacts with p53 to inhibit its phosphorylation and transactivation



Beverly et al, Cancer Res 2006



Mungamuri et al, Cancer Res 2006

Notch signaling downregulates p53-dependent apoptosis

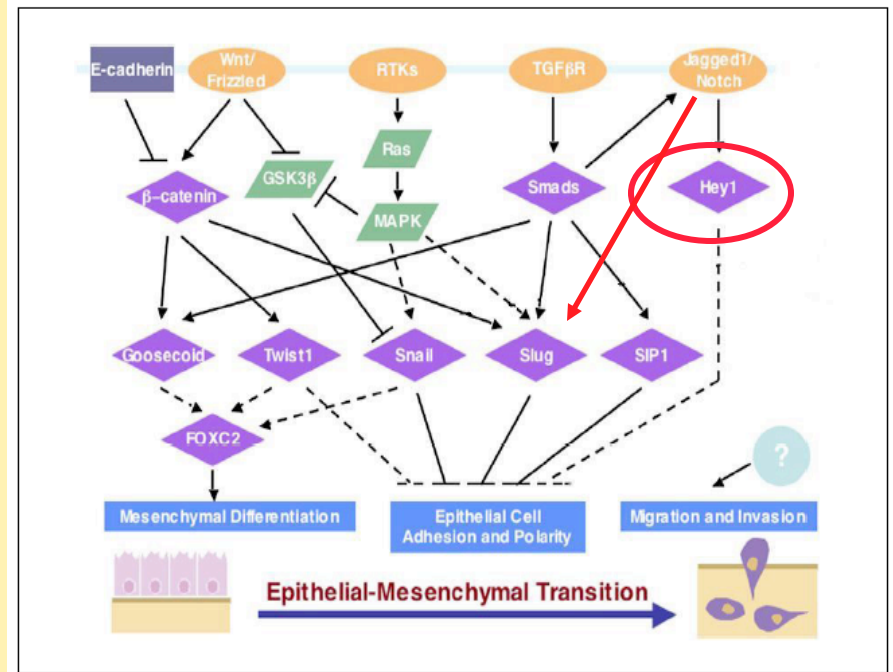
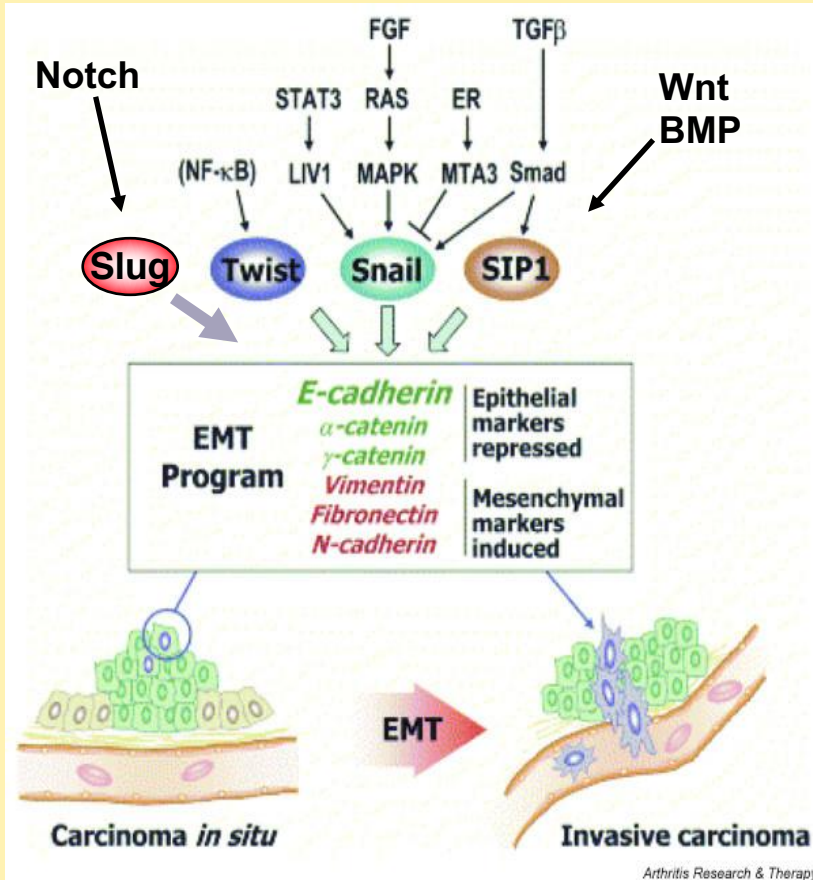
Kim S.B. et al, Cell Death & Differ 2006

# Meccanismi oncogénici di Notch: induzione di EMT

Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin

Kevin G. Leong,<sup>1,2,4</sup> Kyle Niessen,<sup>1,2,4</sup> Iva Kulic,<sup>1,2,4</sup> Afshin Raouf,<sup>3</sup> Connie Eaves,<sup>3,4,5,6</sup> Ingrid Pollet,<sup>1,2,5</sup> and Aly Karsan<sup>1,2,4,5</sup>

**JEM 2007**





# Meccanismi oncogénici di Notch: angiogenesi e metastatizzazione

Hypoxia (reduced oxygen levels has received considerable attention as an inducer of tumor metastasis)

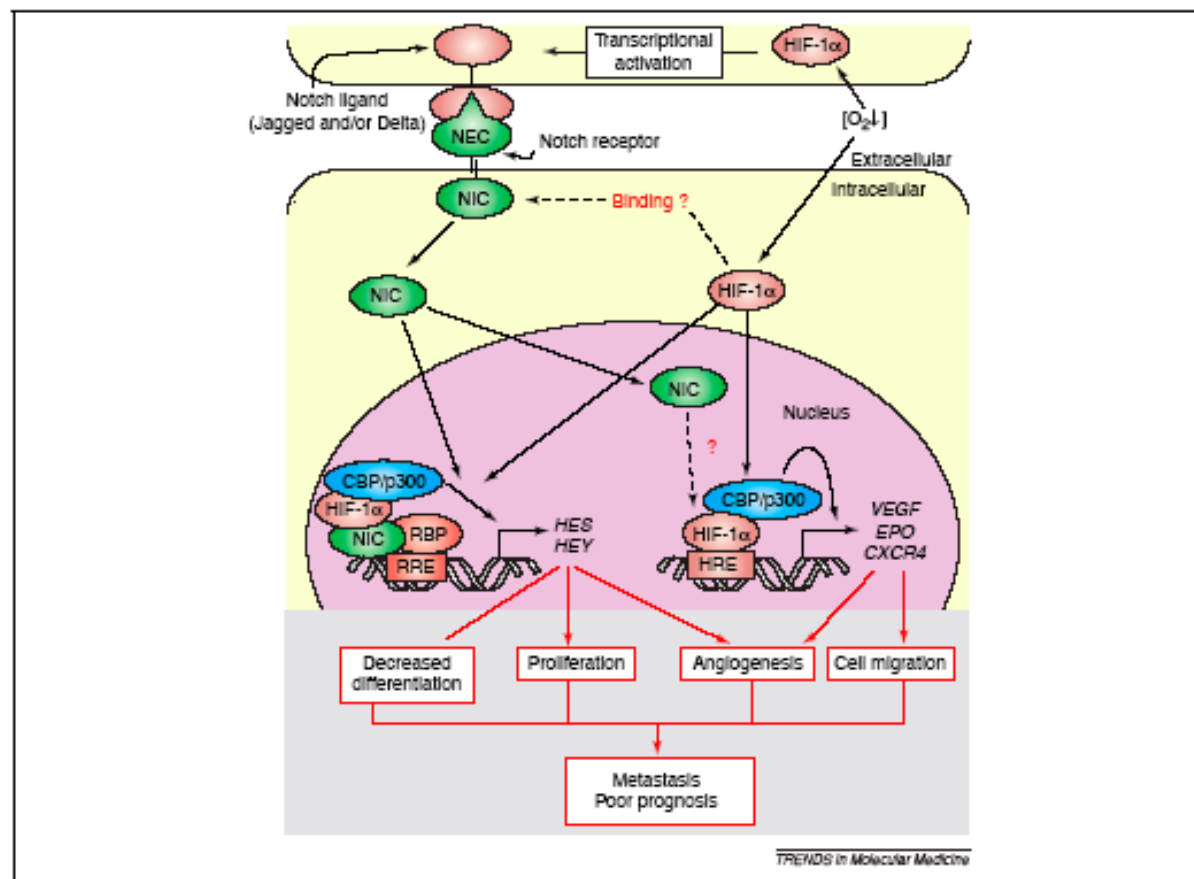


Figure 1. Effects of hypoxia and Notch signalling cross-talk on cancer growth and spreading. Decreased oxygen levels (hypoxia) that are often observed during solid-tumour growth can activate Notch signalling either at the processing level or at the transcriptional level of Notch target genes. Hypoxia can activate the expression of Notch ligands, thus inducing Notch signalling from neighbouring cells. The article by Gustafsson *et al.* [9] also demonstrated that hypoxia stabilizes HIF-1 $\alpha$ , which subsequently binds to NIC and activates transcription of Notch target genes. Altogether, coactivation of the HIF pathway and the Notch pathway results in transcriptional activation of genes regulating cellular differentiation, proliferation, angiogenesis and cell migration. In the context of tumour formation, such responses might result in additional tumour growth and metastasis. Abbreviations: EPO, erythropoietin; HIF, hypoxia-inducible factor; HRE, HF response element; NEC, Notch extracellular domain; NIC, Notch intracellular domain; RRE, RBP-1k response element; VEGF, vascular endothelial growth factor.

Sainson & Harris, *Trends in Mol Med* 2006

# Meccanismi oncogénici di Notch durante la fase metastatica: Notch agevola il *homing* di cellule leucemiche nel cervello

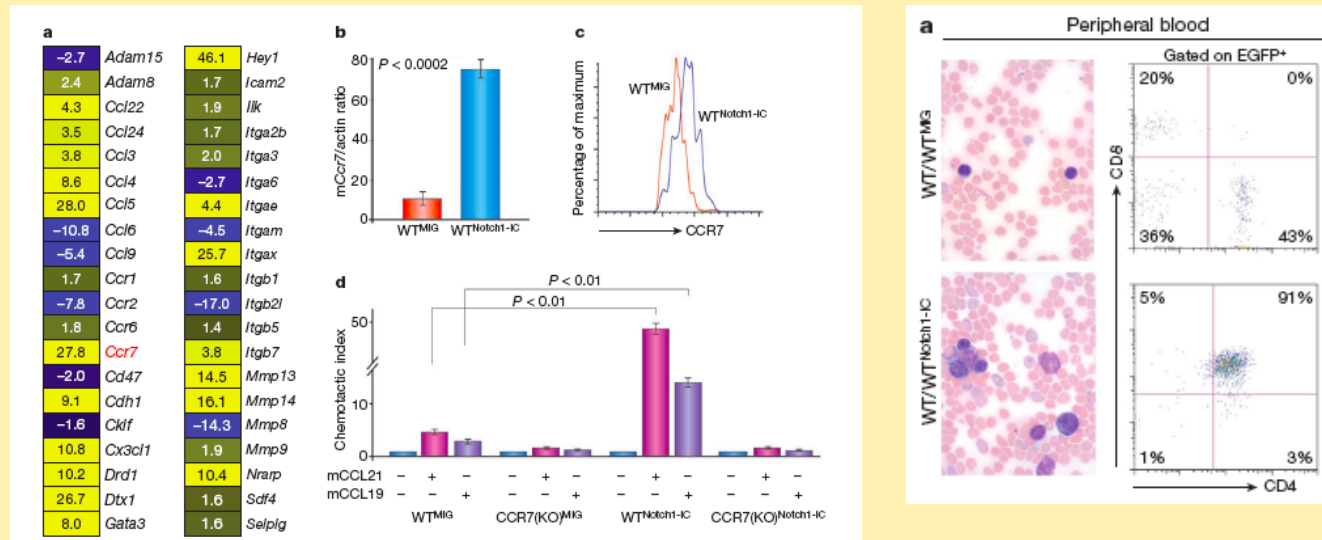
nature

Vol 459 | 18 June 2009 | doi:10.1038/nature08020

## LETTERS

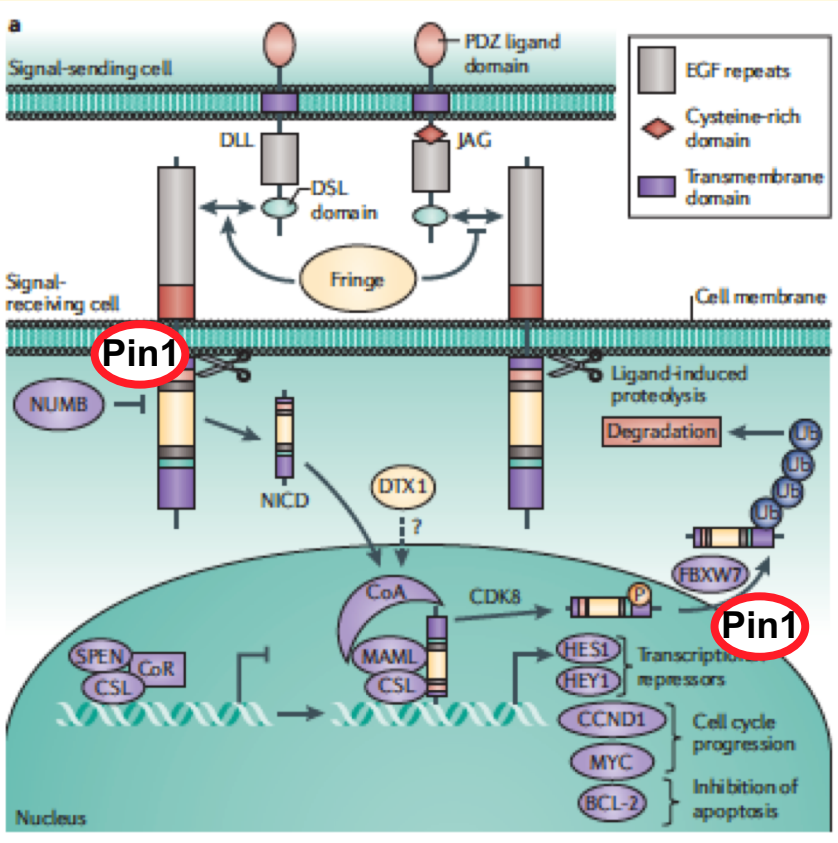
### CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia

Silvia Buonamici<sup>1,2</sup>, Thomas Trimarchi<sup>1,2</sup>, Maria Grazia Ruocco<sup>1,3</sup>, Linsey Reavie<sup>1,2</sup>, Severine Cathelin<sup>1,2</sup>, Brenton G. Mar<sup>4</sup>, Apostolos Klinakis<sup>5</sup>, Yevgeniy Lukyanov<sup>1</sup>, Jen-Chieh Tseng<sup>1</sup>, Filiz Sen<sup>1,2</sup>, Eric Gehrie<sup>5</sup>, Mengling Li<sup>7</sup>, Elizabeth Newcomb<sup>1</sup>, Jiri Zavadil<sup>1</sup>, Daniel Meruelo<sup>1</sup>, Martin Lipp<sup>8</sup>, Sherif Ibrahim<sup>1</sup>, Argiris Efstratiadis<sup>5</sup>, David Zagzag<sup>1</sup>, Jonathan S. Bromberg<sup>6</sup>, Michael L. Dustin<sup>1,3</sup> & Iannis Aifantis<sup>1,2</sup>



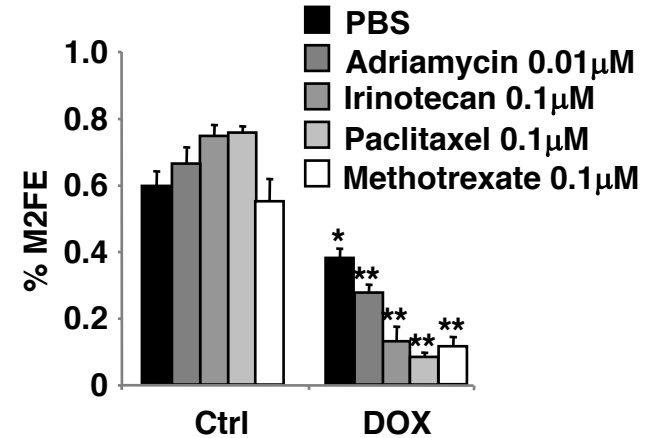
Notch → CCR7 (chemokine receptor)    CCL19 ligand in the brain

# Ruolo della cooperazione tra la prolil-isomerasi Pin1 e Notch nella chemioresistenza



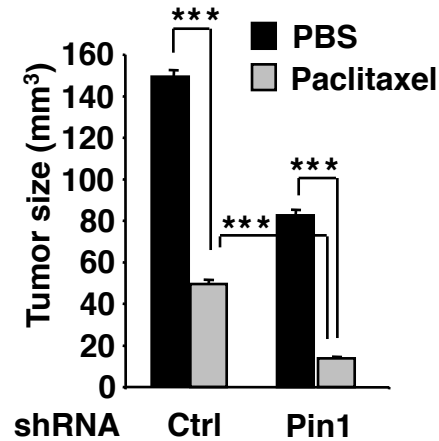
*In vitro*

MDA-MB-231 + pLKO-TetO-shPin1



*In vivo*

MDA-MB-231 xenograft



MDA-MB-231 xenograft

