Corso di Oncologia Molecolare AA 2020-2021

CELLULE STAMINALI DEL CANCRO (CSC)

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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

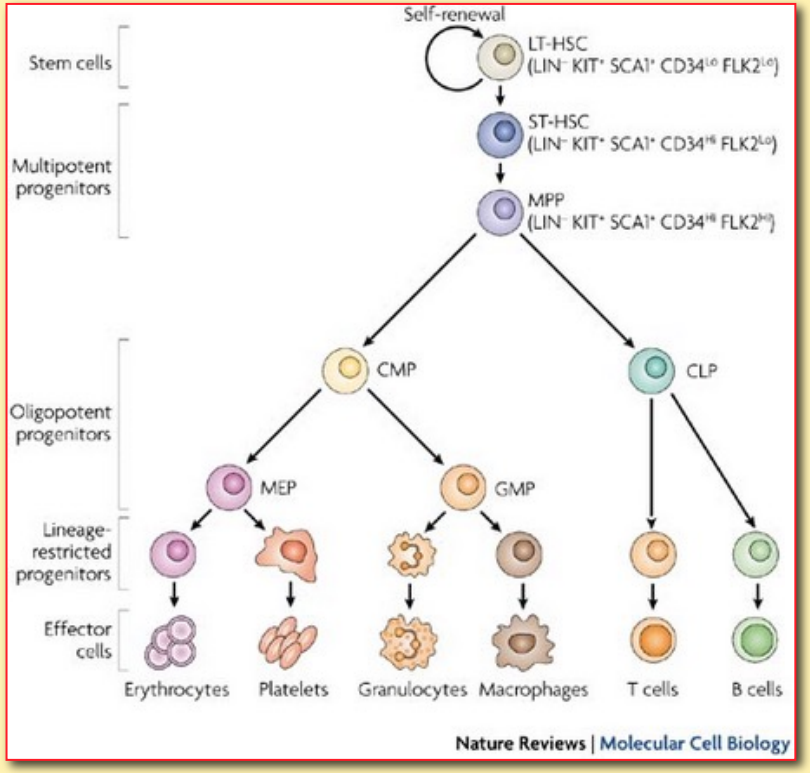
Test di tumorigenicità in vivo

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

Isolamento **prospettivo** di CSC basandosi sulle proprietà principali delle cellule staminali



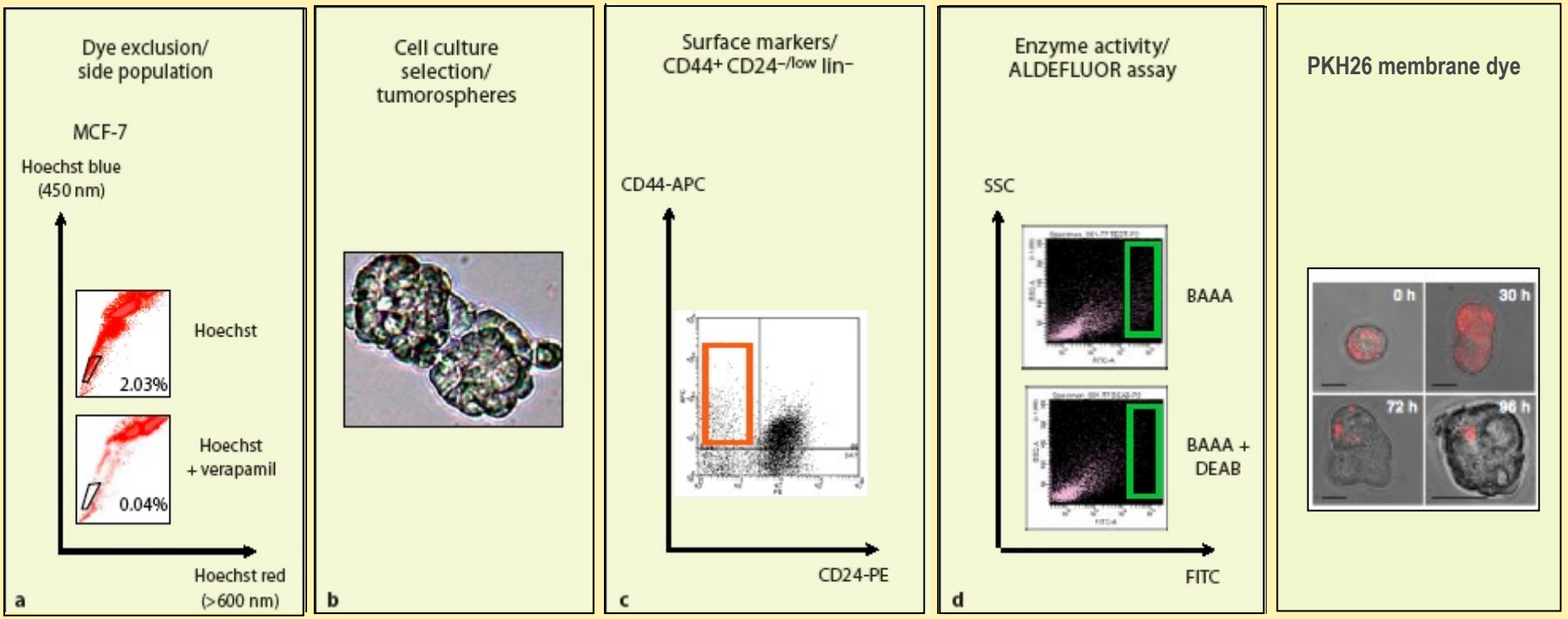
CSC

- Auto-rinnovamento
- Potenziale differenziativo
- Poteziale 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, detossficazione etc.



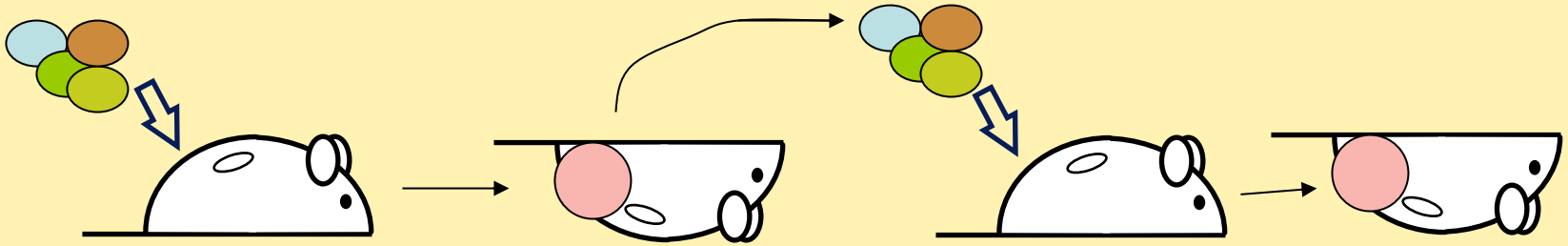
Adattato da Charaffe-Jauffret et al, Pathobiol 2008

Pece et al. Cell 2010

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

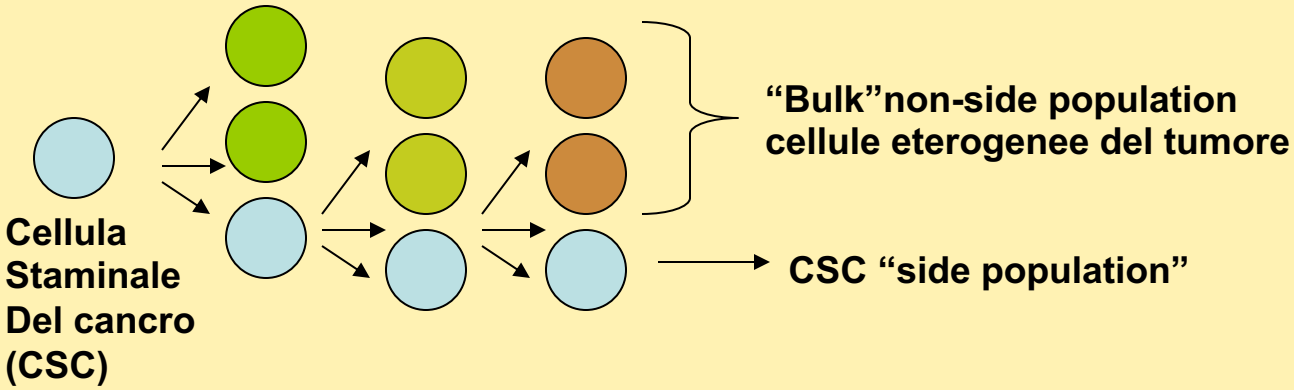
PRIMO trapianto ***SECONDO trapianto***

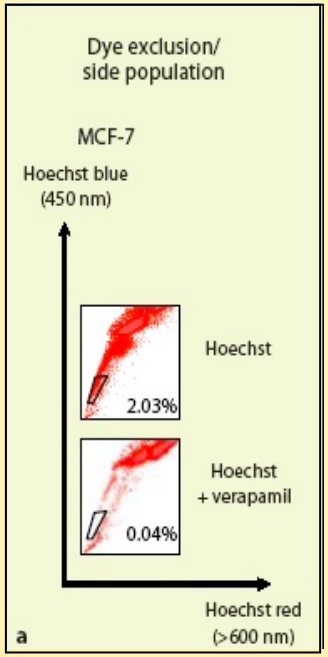
Cellule tumorali primarie

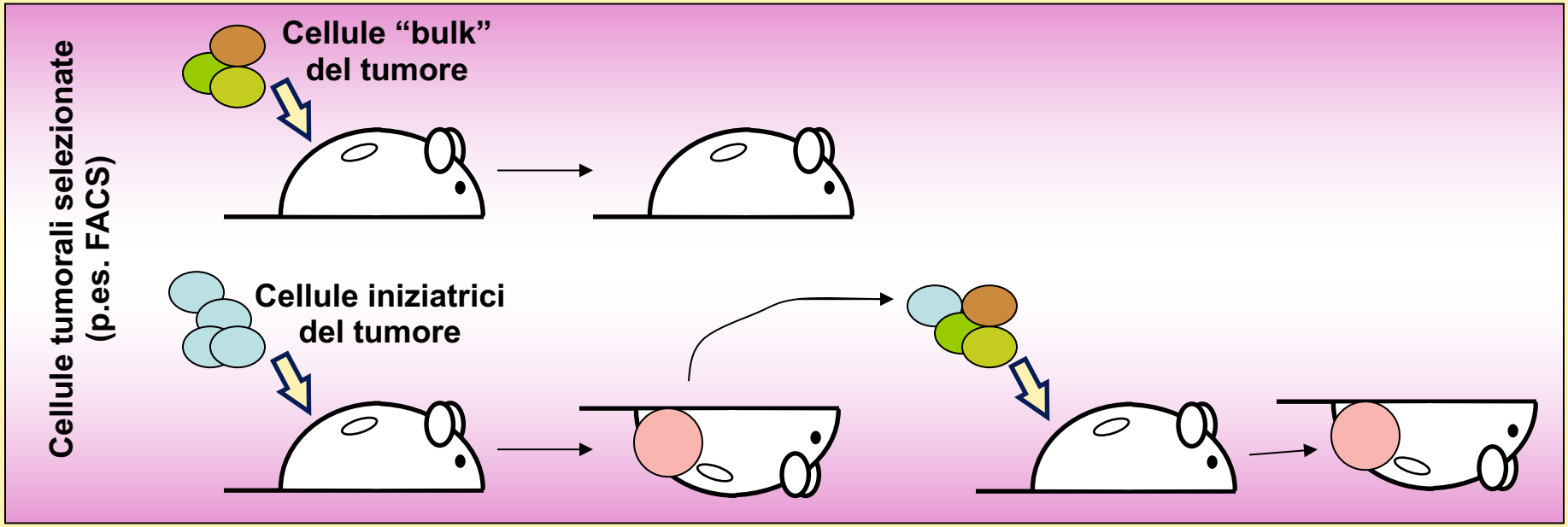


Tecniche di purificazione ed isolamento ***prospettivo*** di cellule staminali del cancro

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







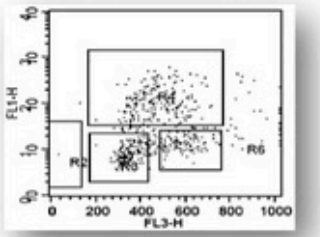
Identification of novel markers for CSC

Isolation based on stemness properties

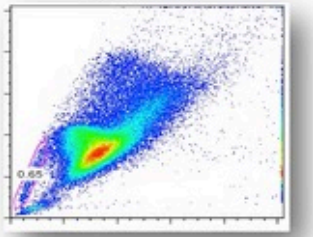
Primary cell lines & fresh tumors from Mouse & Man

Optimization of sphere cell culture conditions

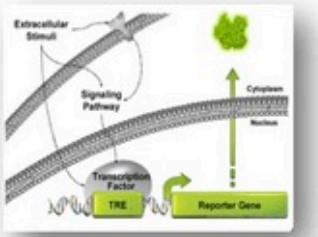
Resistance to chemotherapy



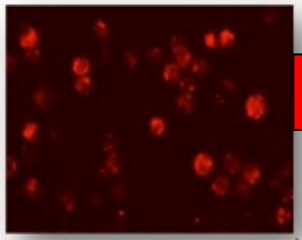
SP cells



Stemness reporter



Label retaining cell assay

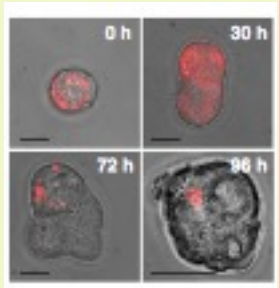


Identify distinct / overlapping populations / enrich populations

→ Membrane Proteomics / Phage Display Technology

→ Functional characterization of subpopulations

PKH26 membrane dye



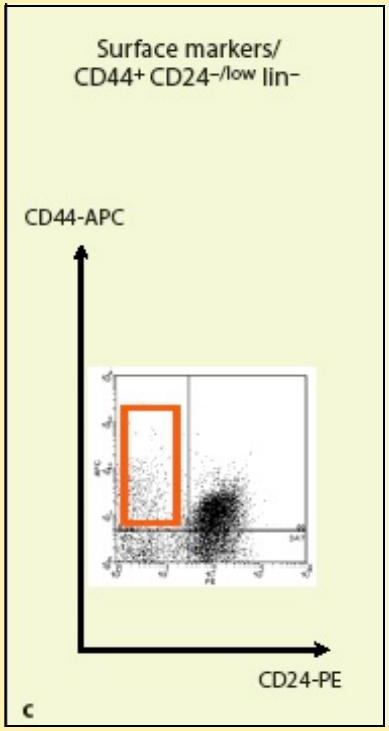
Pece et al. Cell 2010

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)



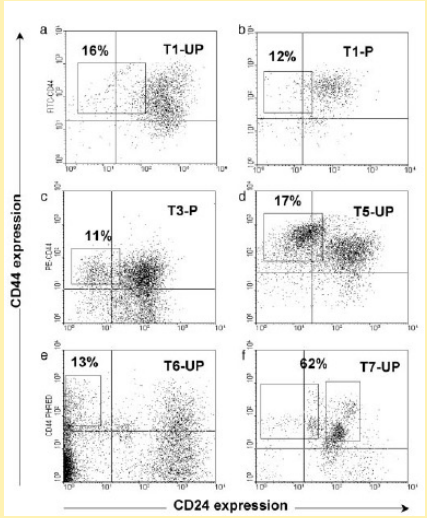


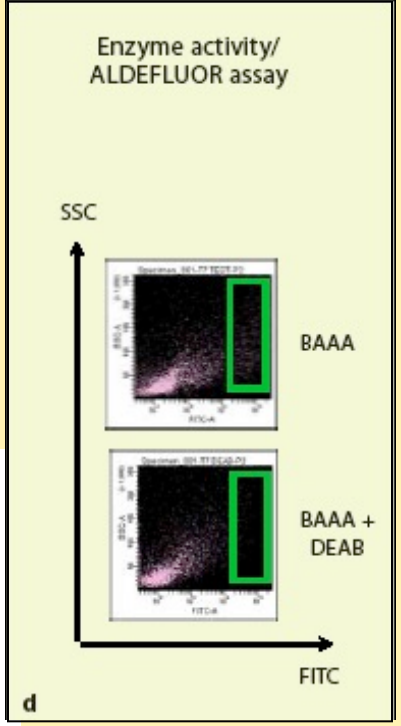
Table 3. Tumorigenic breast cancer cells were highly enriched in the ESA+CD44+CD24−/low population

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

Cell Stem Cell
Article

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

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



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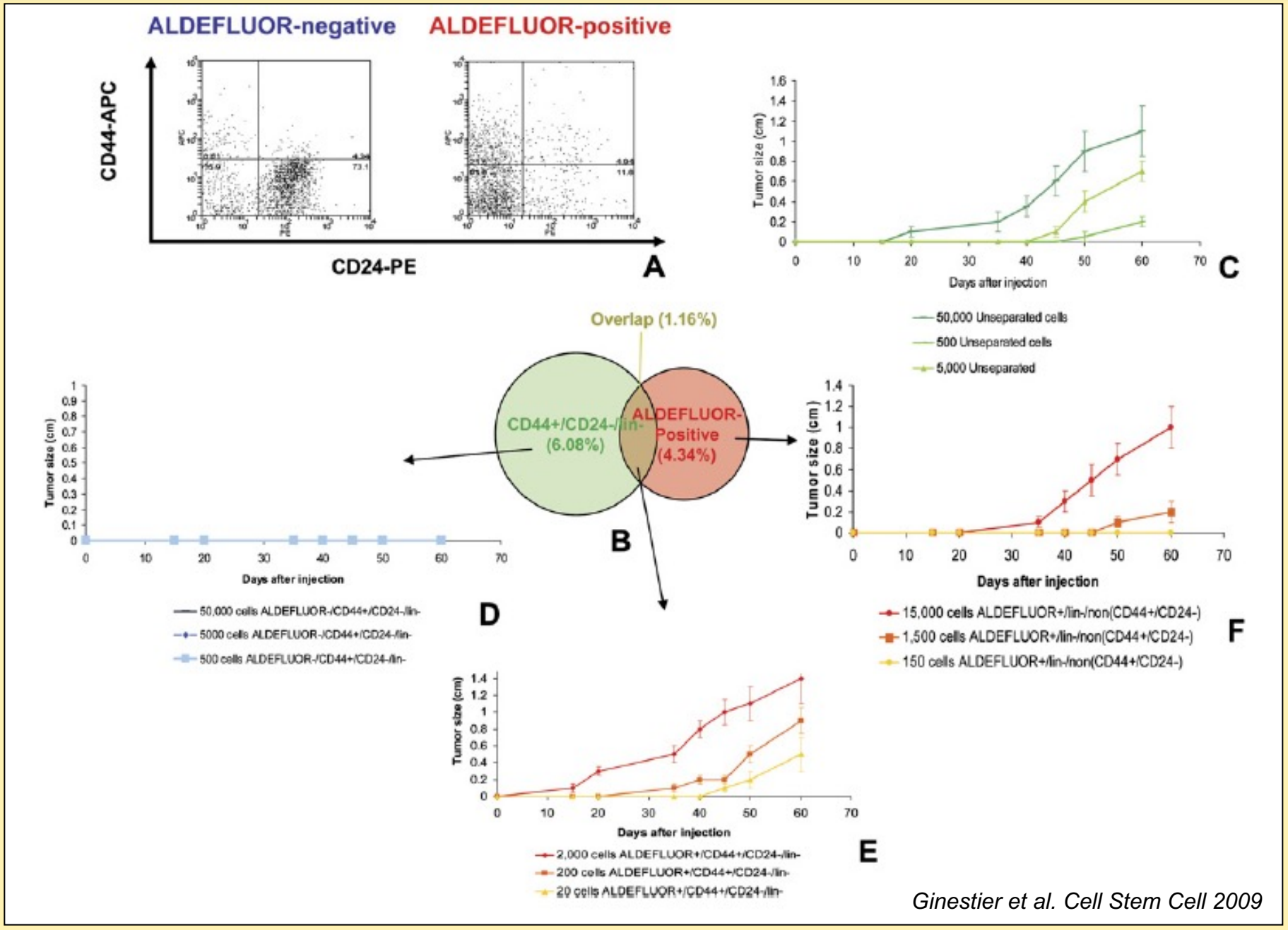
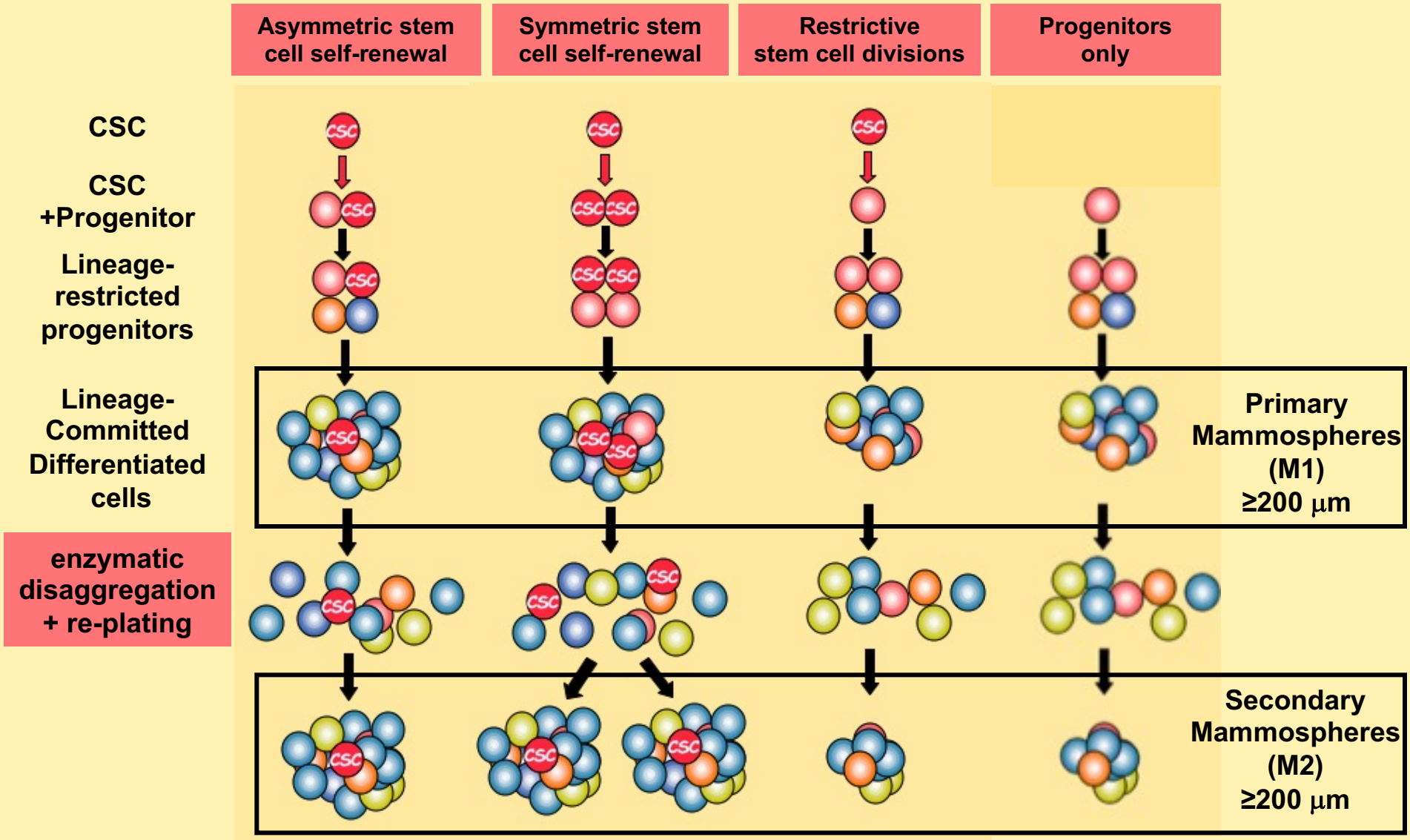
ALDEFLUOR™ is optimized for the detection of ALDHbr hematopoietic cells in human blood and bone marrow, but it can also be used with non-hematopoietic cells. For a full list of ALDEFLUOR™ products, please visit our website at www.stemcell.com.

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



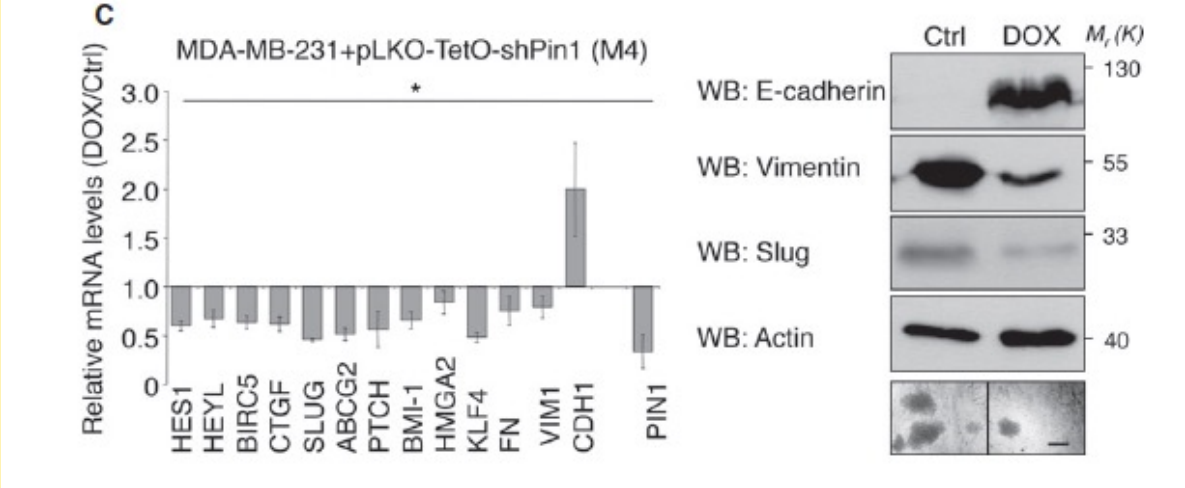
Ginestier et al. Cell Stem Cell 2009

Esperimento di formazione di sfere tumorali (*tumorspheres*)



Adapted from Dontu et al., Breast Cancer Res. 2004

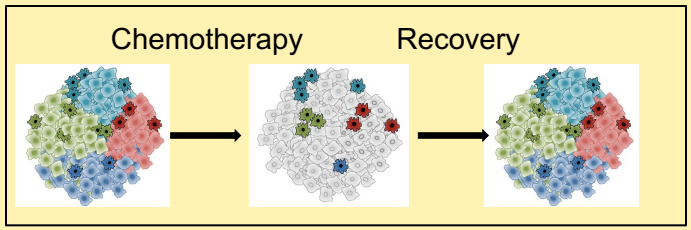
Analisi di marcatori di EMT e staminalità



Rustighi, Zannini et al., EMBO Mol Med. 2014
Campaner, Rustighi et al., Nat Commun. 2017)

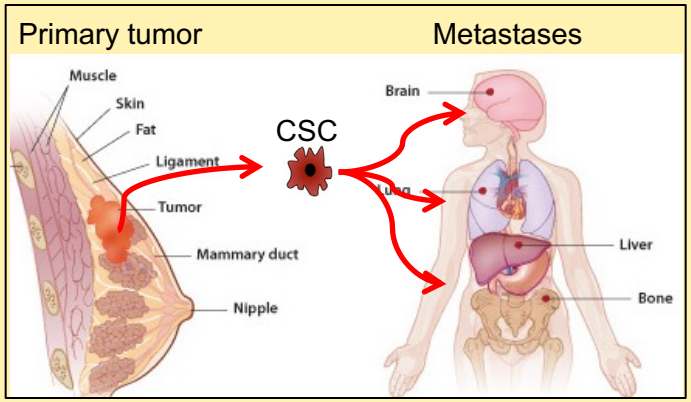
Possiamo sfruttare la conoscenza delle caratteristiche molecolari e fenotipiche delle CSC ai fini clinici

Le CSC sono responsabili della progressione tumorale e delle recidive



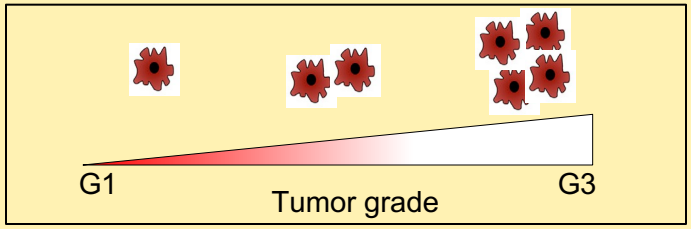
Le CSCs sono **insensibili alle chemiotherapie e sono alla base del** "dandelion phenomenon"

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

Yin et al. Journal of Experimental & Clinical Cancer Research 2014, 33:49
http://www.jeccr.com/content/33/1/49

Journal of Experimental & Clinical Cancer Research

RESEARCH Open Access

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

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

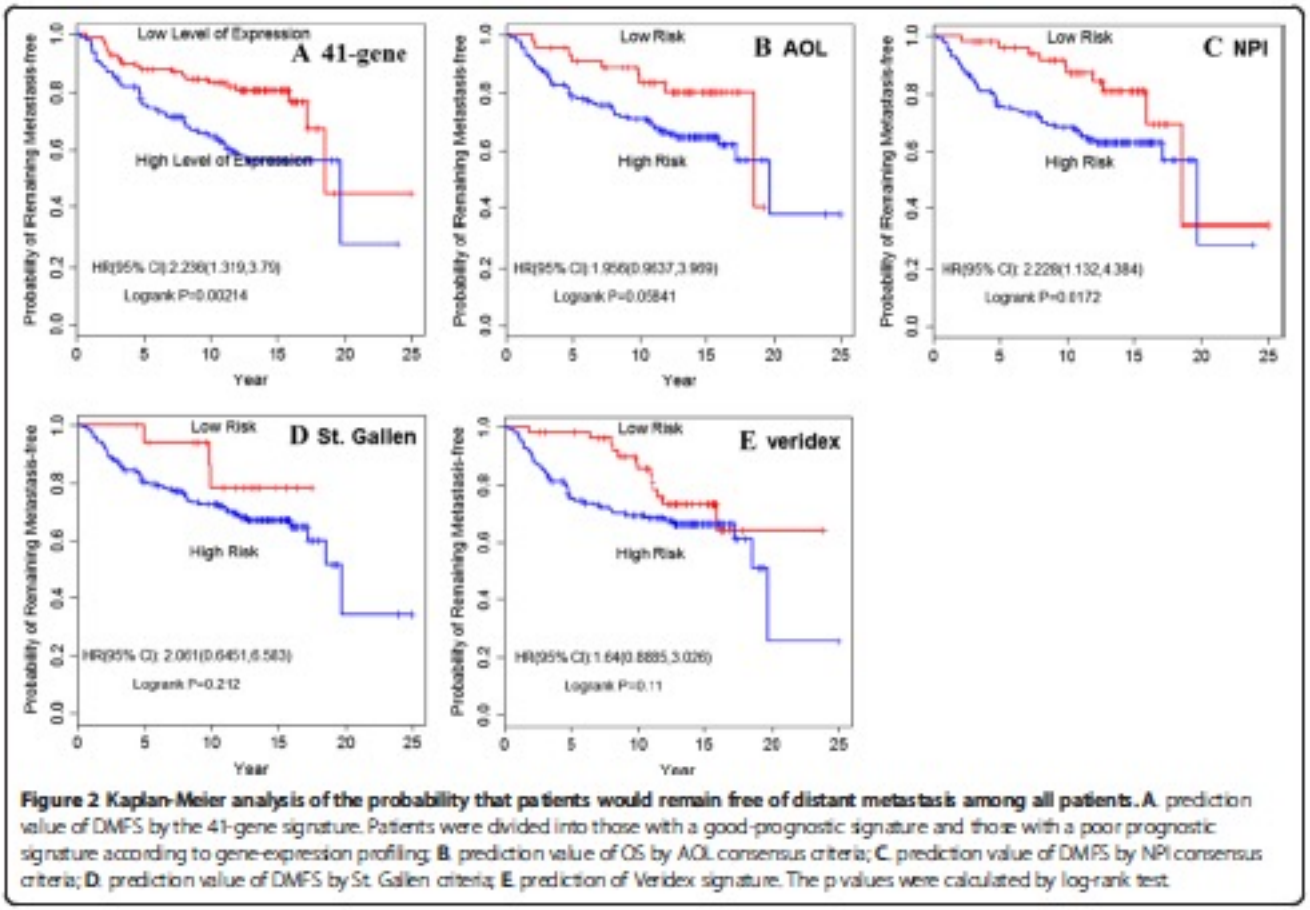


Figure 2 Kaplan-Meier analysis of the probability that patients would remain free of distant metastasis among all patients. A: prediction value of DMFS by the 41-gene signature. Patients were divided into those with a good-prognostic signature and those with a poor prognostic signature according to gene-expression 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.

Significato clinico delle CSC

Potenziale tumorigenico

- Le CSC garantiscono il mantenimento della cellula staminale e la produzione di cellule della massa tumorle “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 chemioterpici come ad esempio:

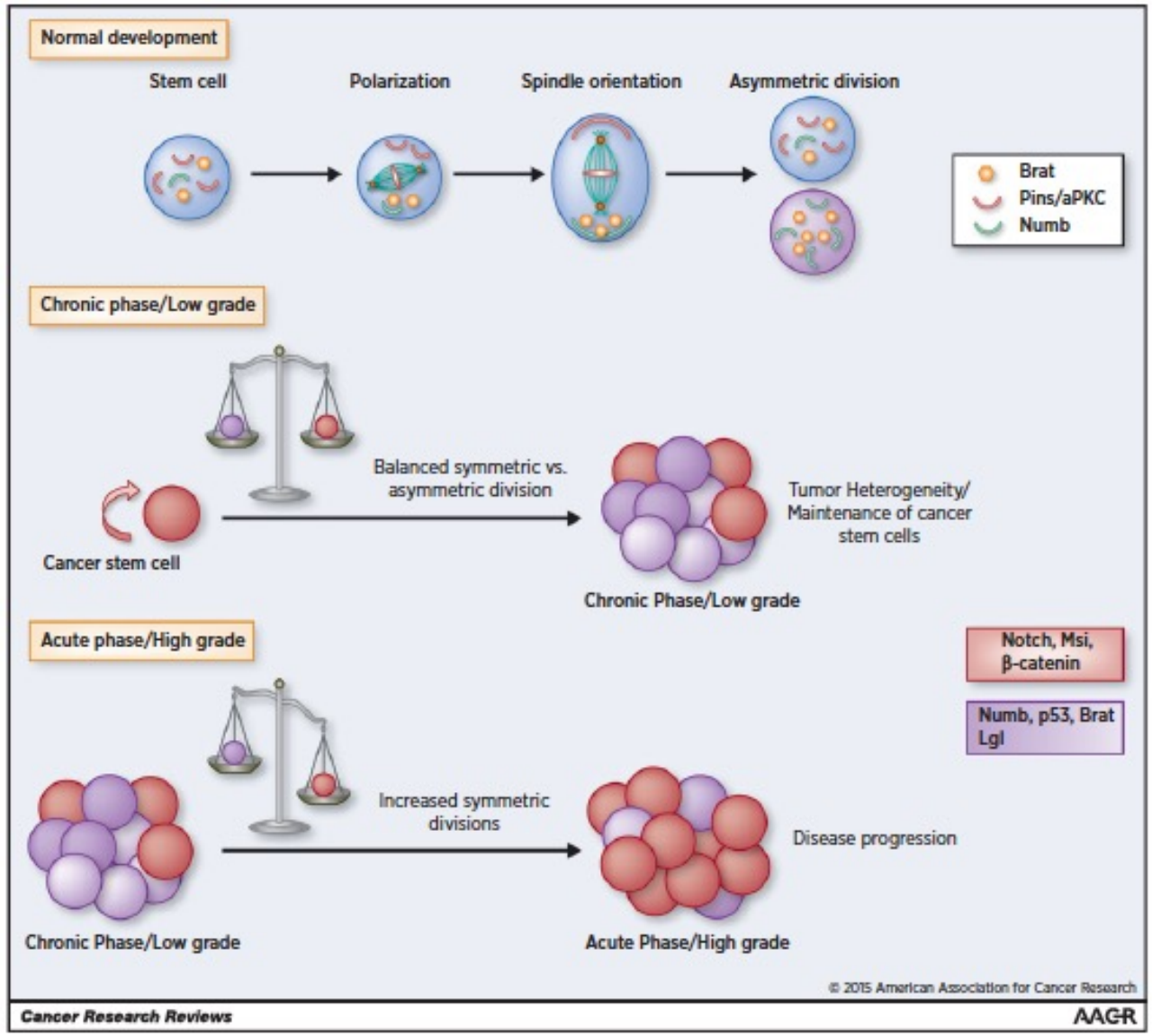
- Aumento dei livelli di proteine della famiglia BCL-2
- Proteine "multiple drug resistance"

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

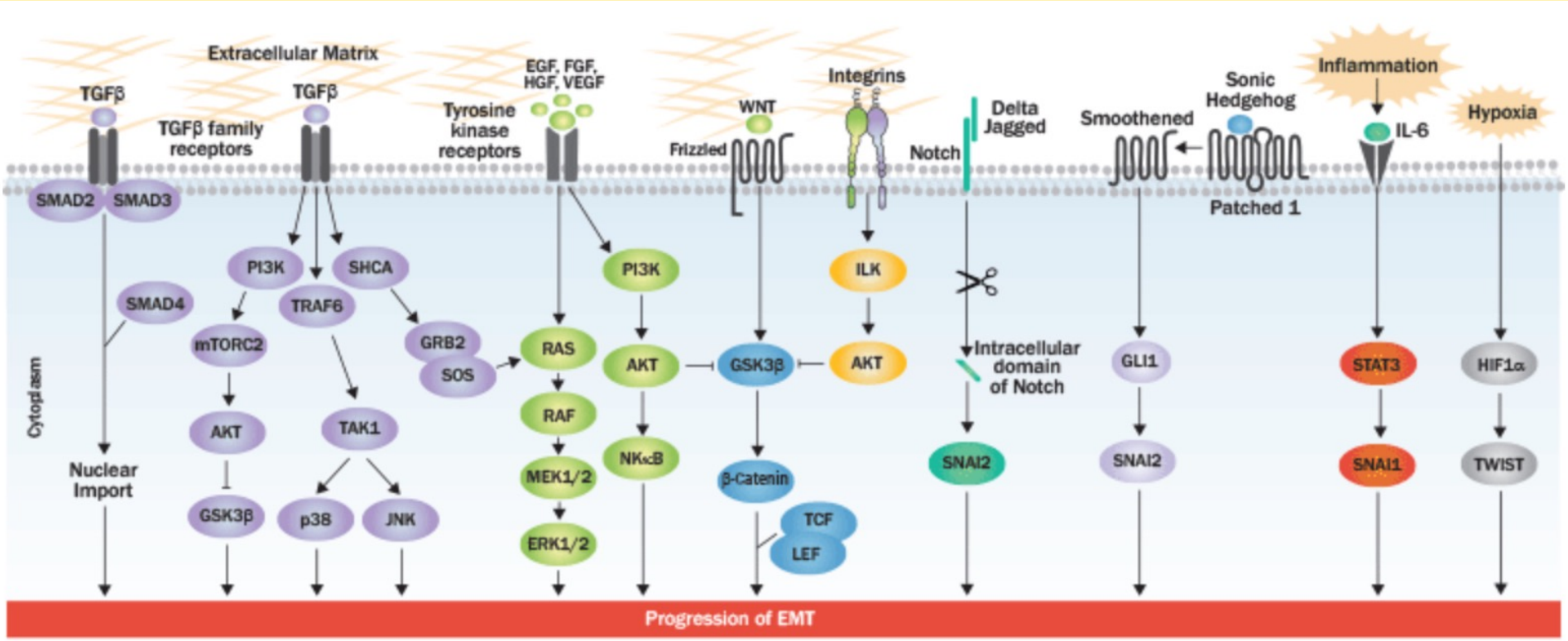
SCOPRIRE QUALI SONO I MECCANISMI MOLECOLARI E LE VULNERABILITA' SPECIFICHE DELLE CSC

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

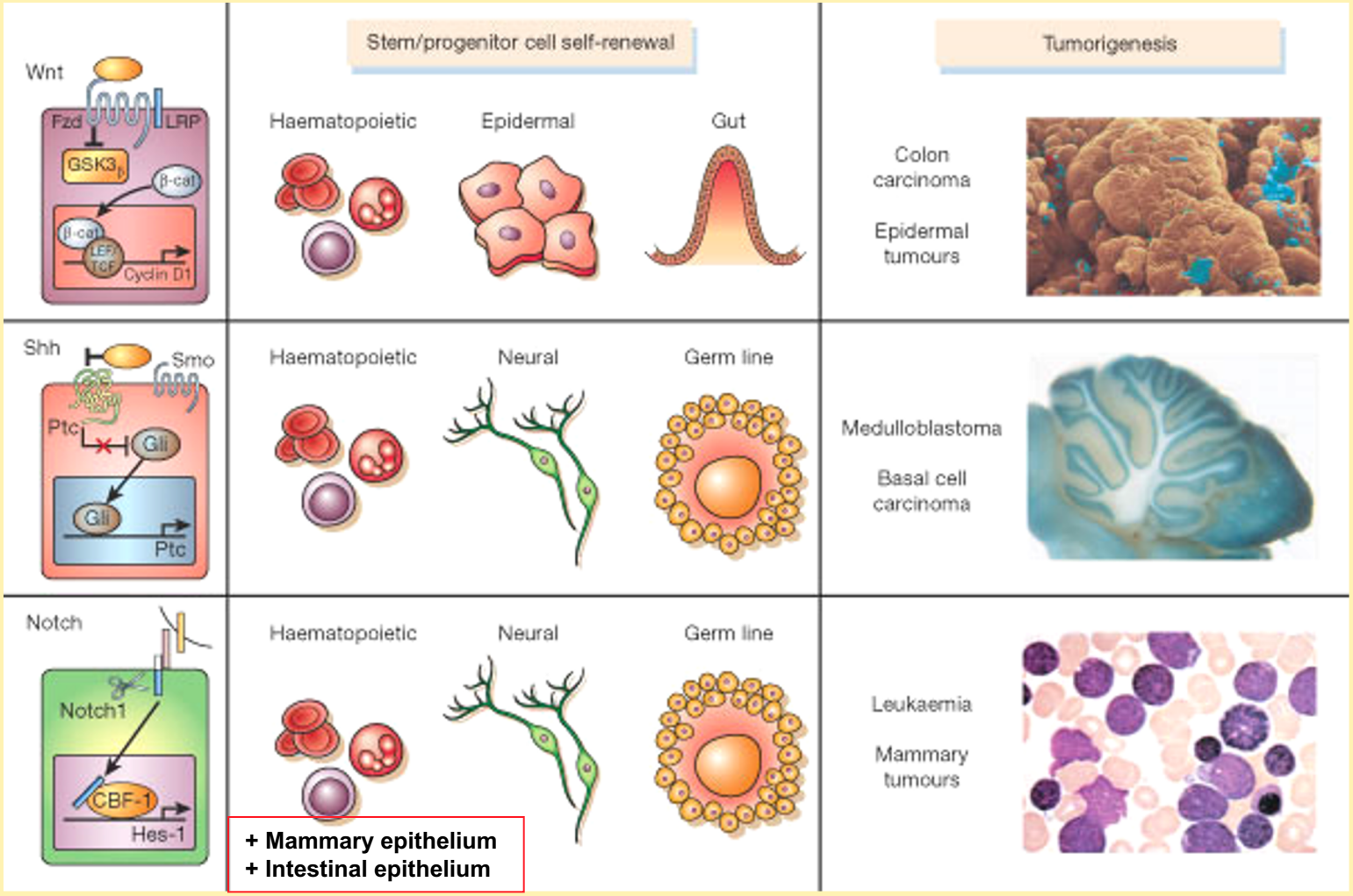


Bajaj et al., Cancer Res 2015

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

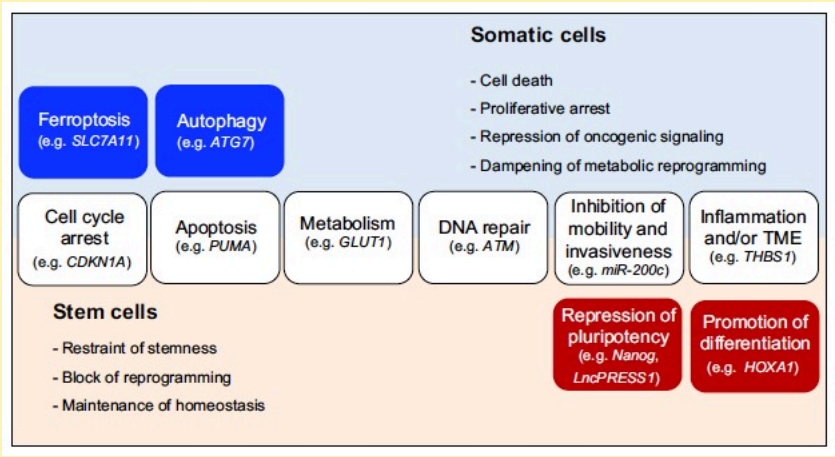


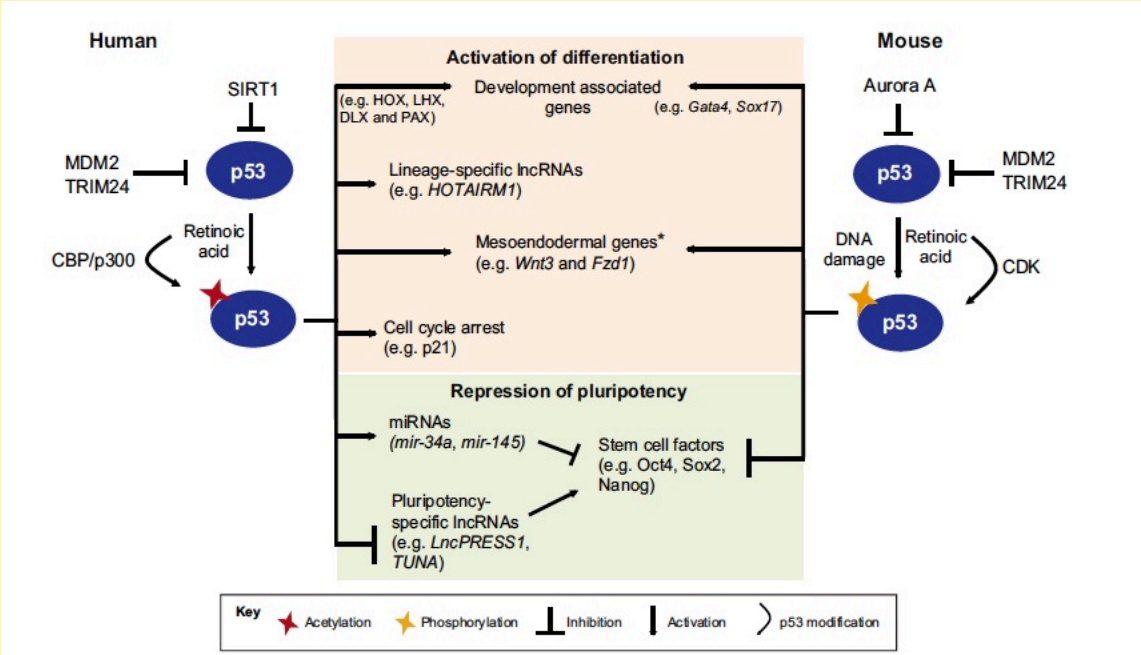
Bersagli per colpire le CSC: Pathways coinvolte nel *self-renewal* e deregolate nel cancro



Adapted from Reya et al. Nature 2001

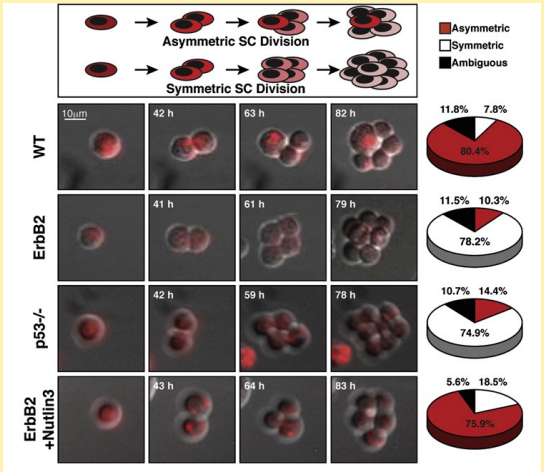
Ruolo di p53 nel differenziamento cellulare: un ulteriore modalità di soppressione tumorale

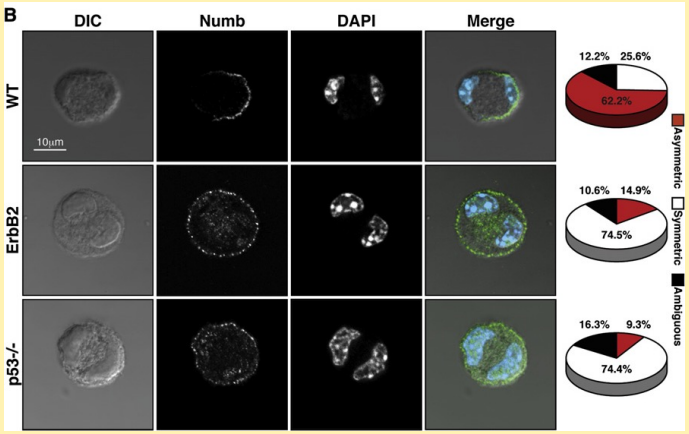


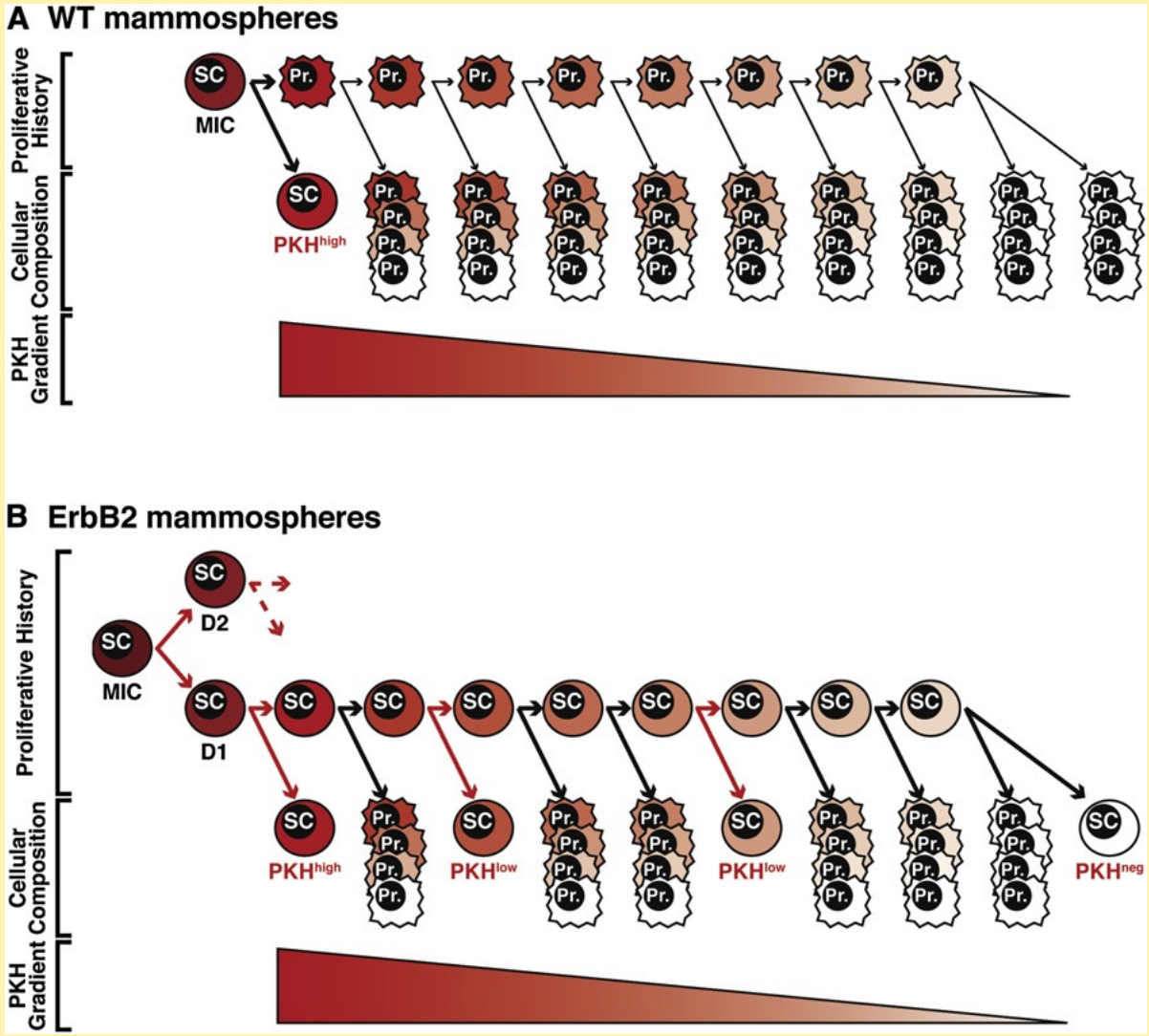


Jain & Barton Development 2018

p53 regola le divisioni asimmetriche delle cellule staminali della mammella

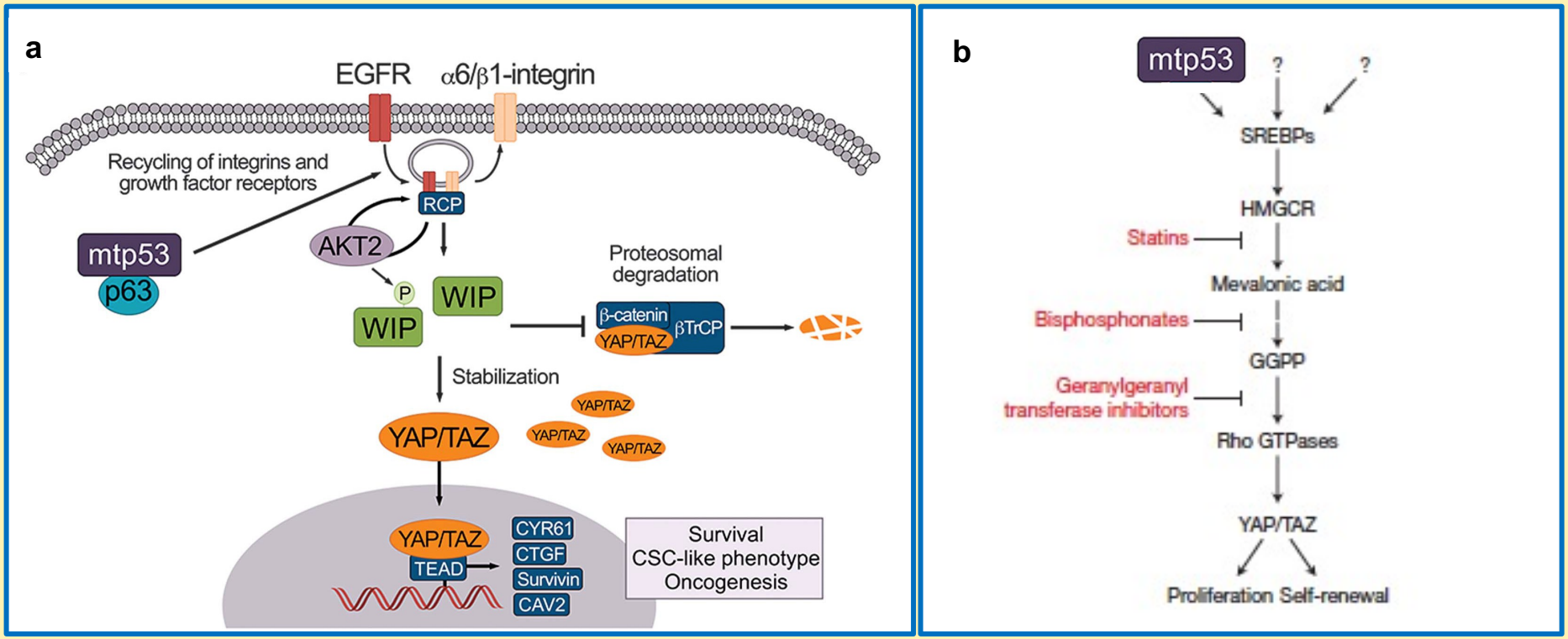






Cicalese et al., Cell 2011

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



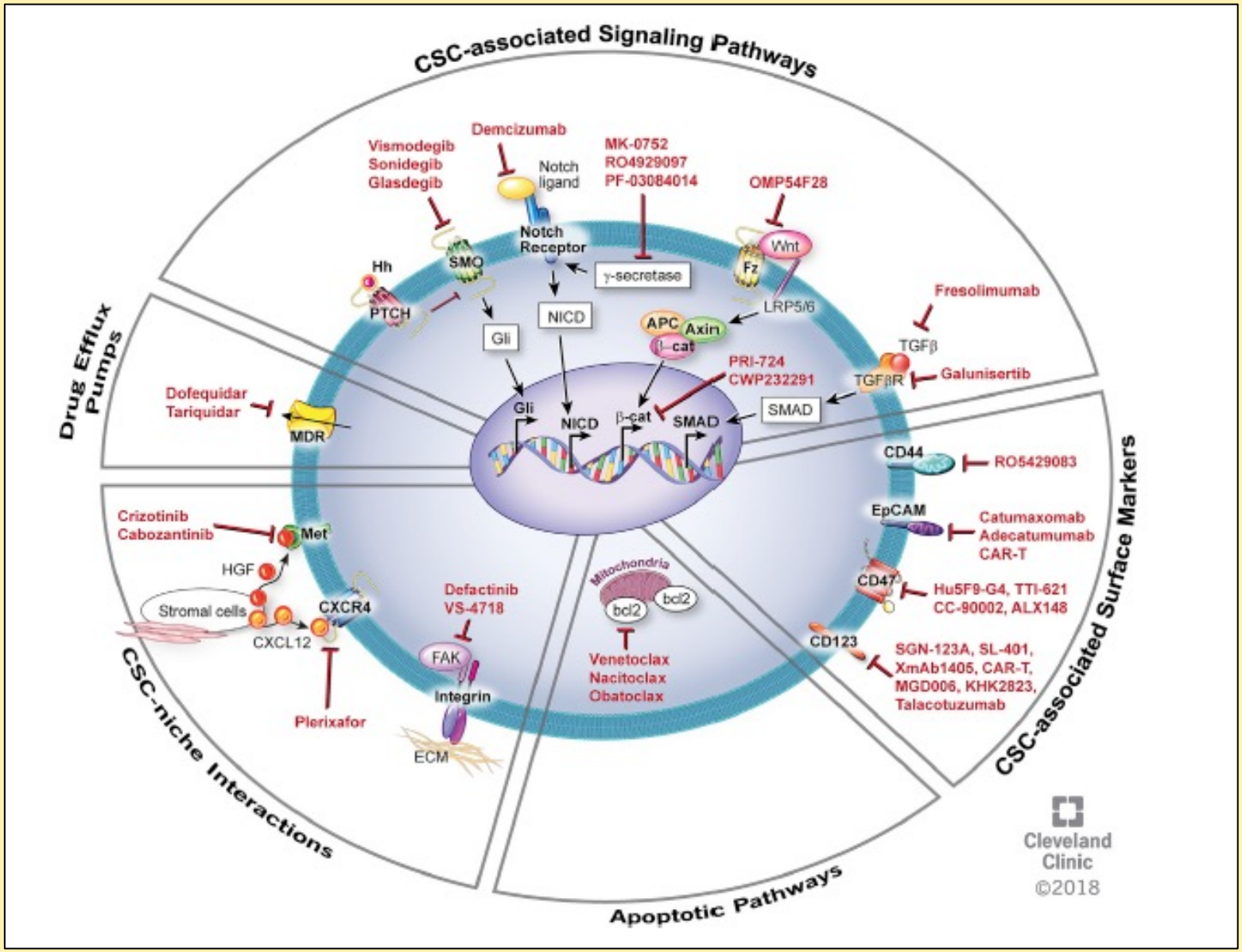
Muller et al., Cell 2009

Escoll et al., Oncogene 2017

Freed-Pastor et al., Cancer Cell 2012

Sorrentino et al., Nat Cell Biol 2014

Terapie anti-CSC (I)



Saygin et al., Cell Stem Cell 2019

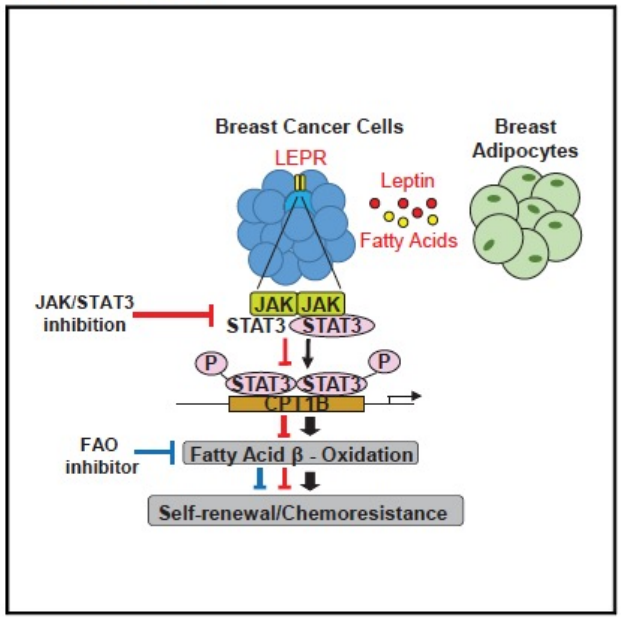
Vulnerabilità selettive delle CSC: metabolismo lipidico alterato

Article

Cell Metabolism

JAK/STAT3-Regulated Fatty Acid β-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 β-oxidation (FAO), promoting breast cancer stemness and chemoresistance. Blocking FAO and/or depleting leptin re-sensitize 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

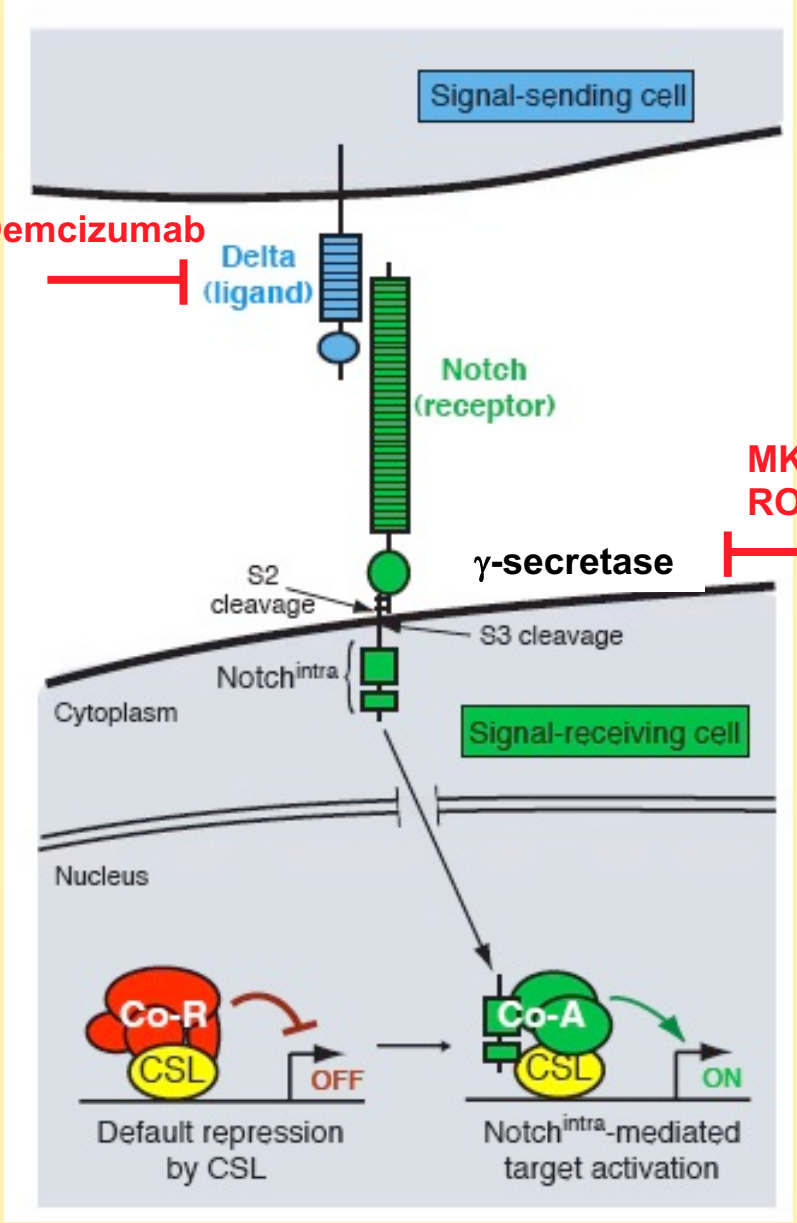
ARRESTED DEVELOPMENT?

Notch Emerges as New Cancer Drug Target

By Ken Garber

JNCI, 2007

The Notch receptor signaling pathway



Lai, Curr Biol 2004

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.)

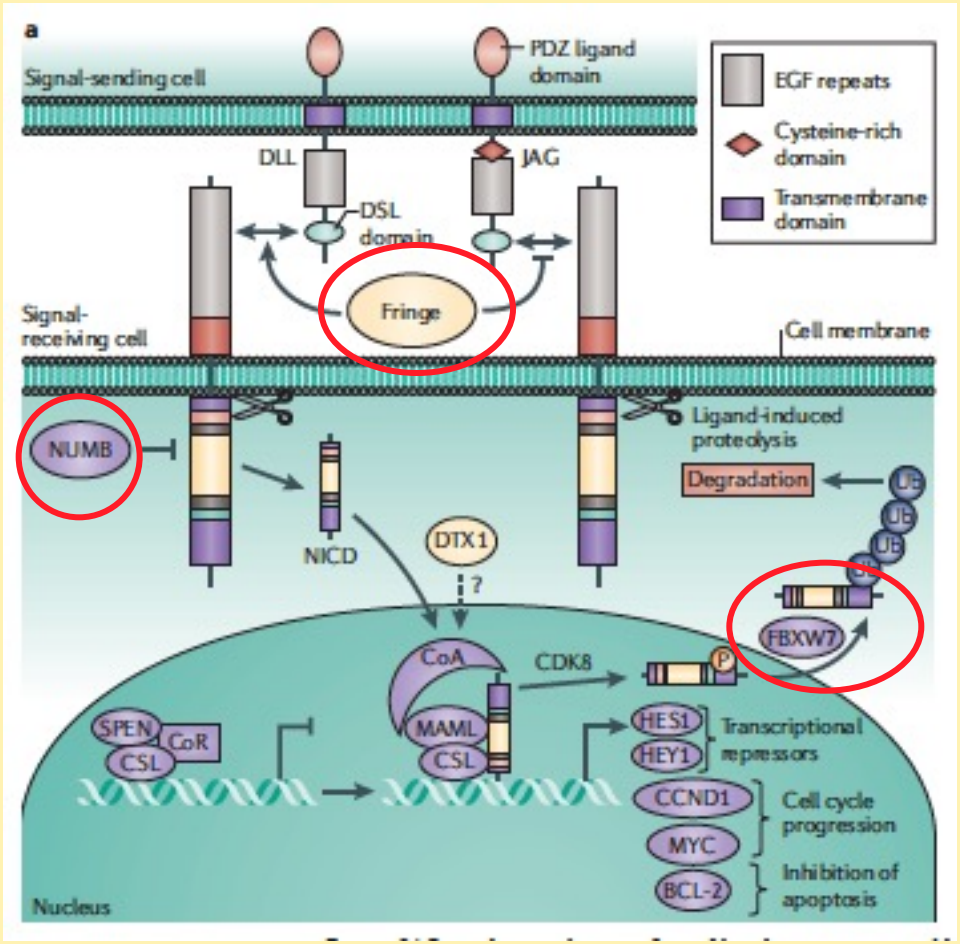
Cross-talks with many pathways (Ras, TGFbeta, HIF-1 alpha, NF-kappaB)

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

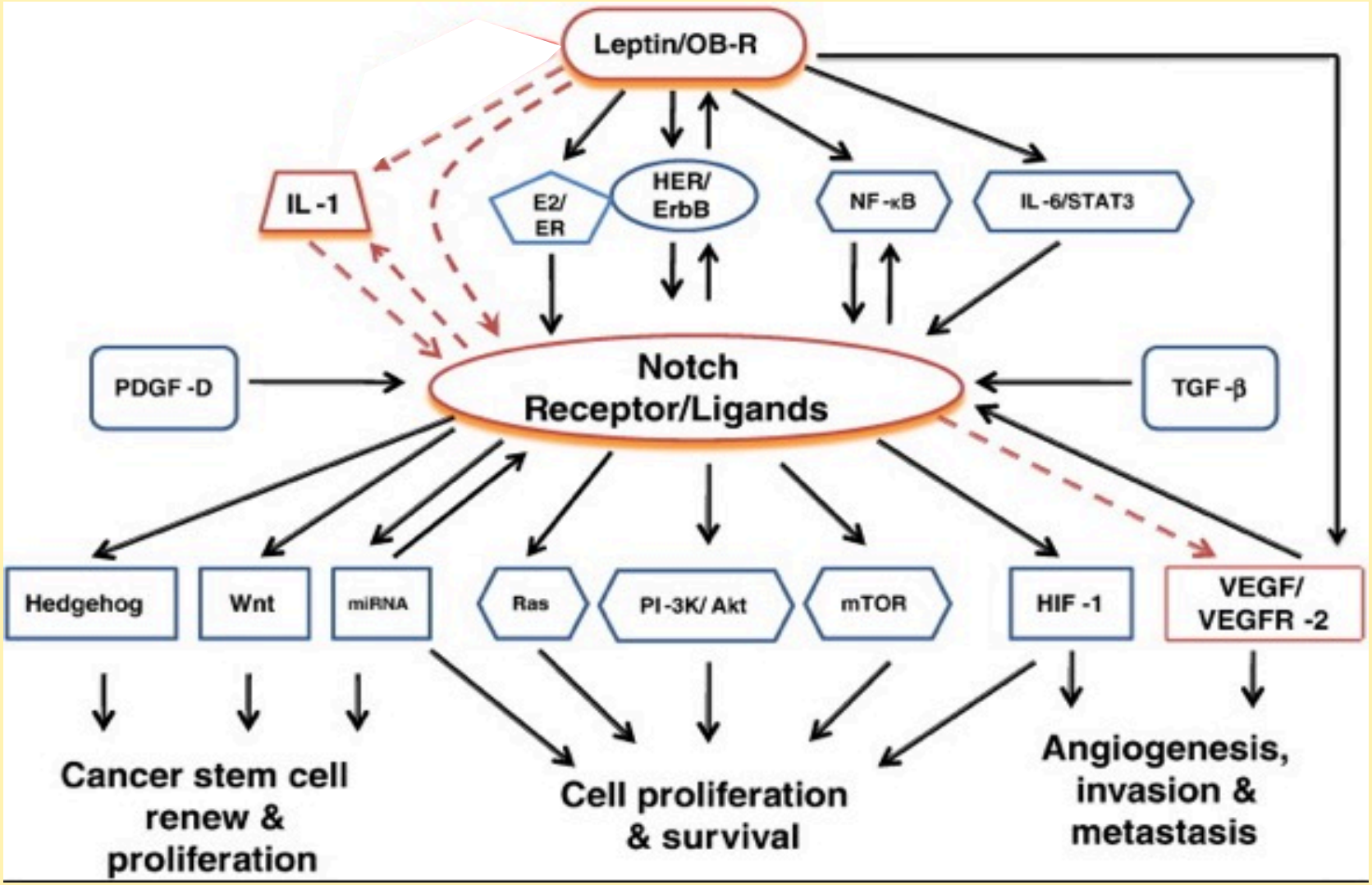
Notch4
c-myc
NF-kappaB2
PPAR
GATA-2
Snail
Fattori trascrizionali: crescita e differenziamento

ABCG2
Bcl-2
Mediatori di chemioresistenza

p21
Deltex
Regolatori negativi

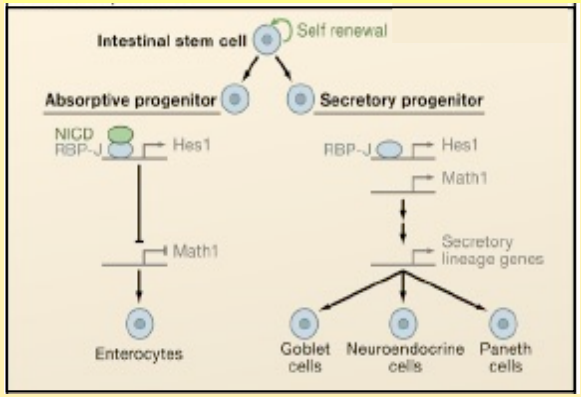
E. Lai, Curr Biol 2004

Interazione di Notch con altre pathways

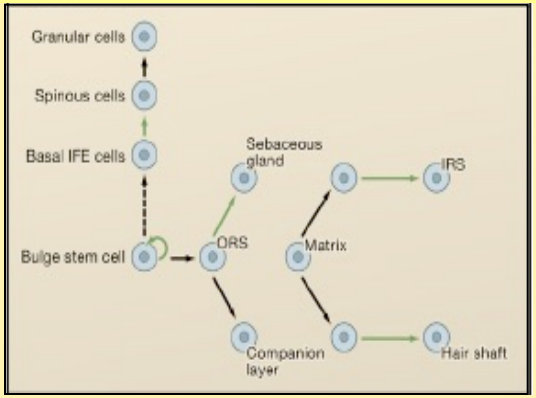


Notch controlla il *self-renewal* e differenziamento di cellule epiteliali nell'uomo

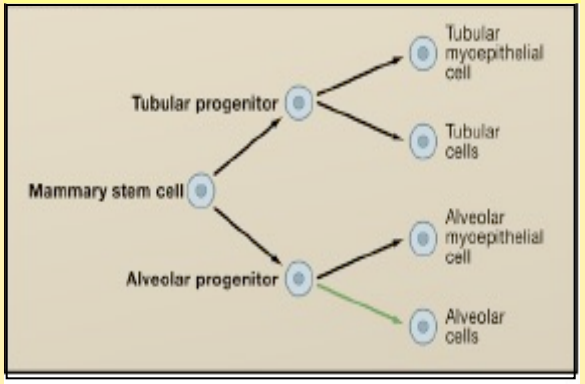
Intestinal epithelium



Skin epithelium



Mammary gland



Blanpain, Cell 2007

Meccanismi di attivazione oncogenica di Notch

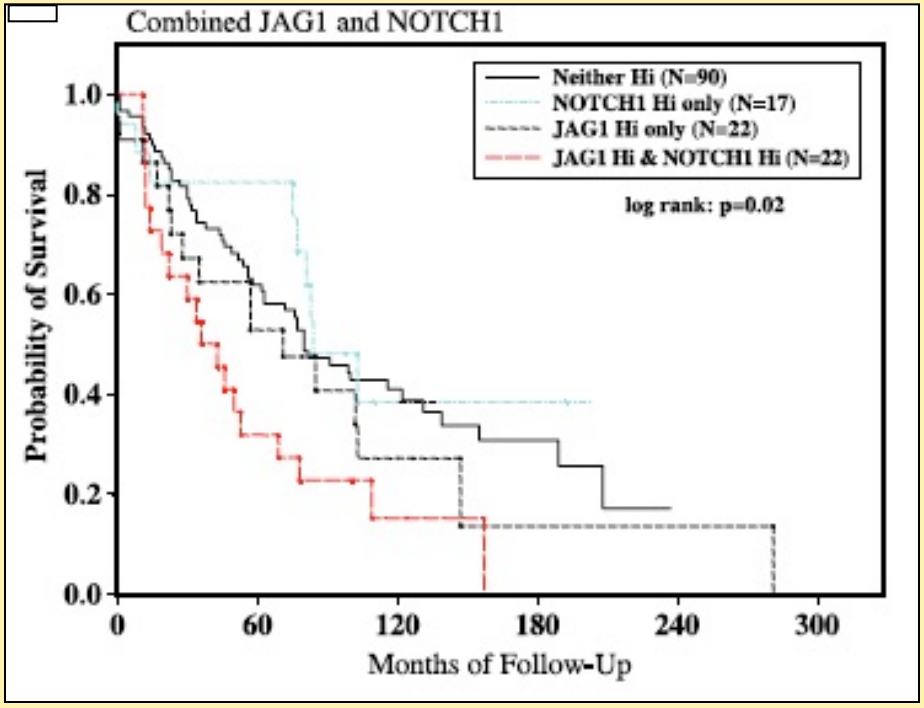
Attivazione deregolata nel cancro alla mammella

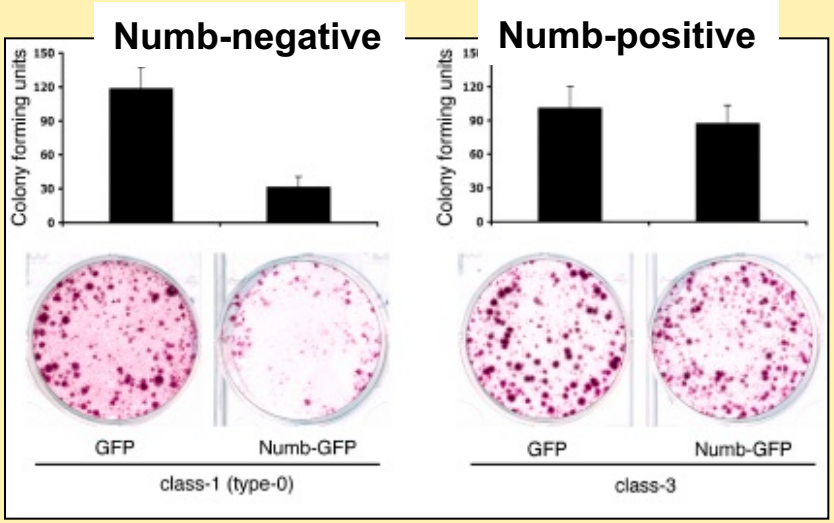
High-level Coexpression of JAG1 and NOTCH1 Is Observed in Human Breast Cancer and Is Associated with Poor Overall Survival

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

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

Salvatore Pece,[1,2] Michela Serresi,[1] Elisa Santolini,[1] Maria Capra,[1,3] Esther Hulleman,[1] Viviana Galimberti,[1] Stefano Zurrida,[1] Patrick Maisonneuve,[1] Giuseppe Viale,[1,2] and Pier Paolo Di Fiore[1,2,3]

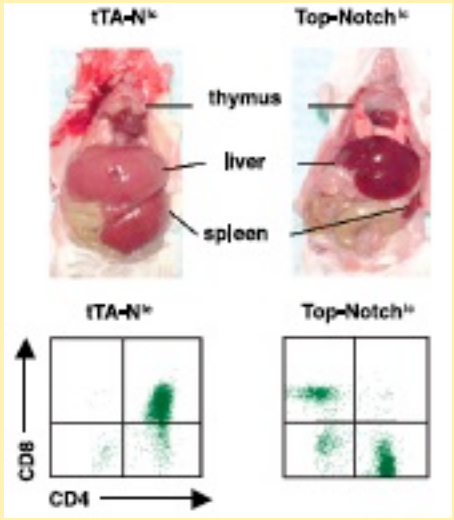


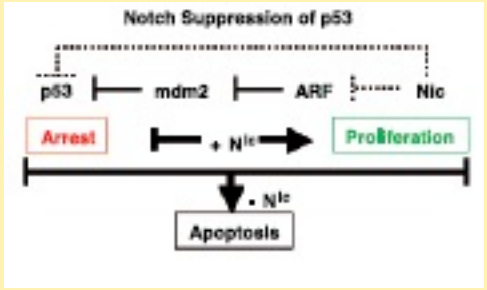


Re-expression of Numb selectively suppresses growth in Numb-negative tumors

Meccanismi oncogénici di Notch: repressione dell'attività di p53

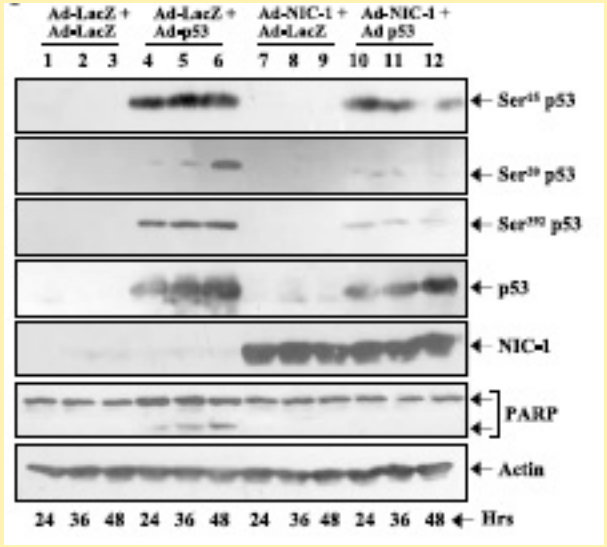
Notch suppresses p53 in Lymphomagenesis

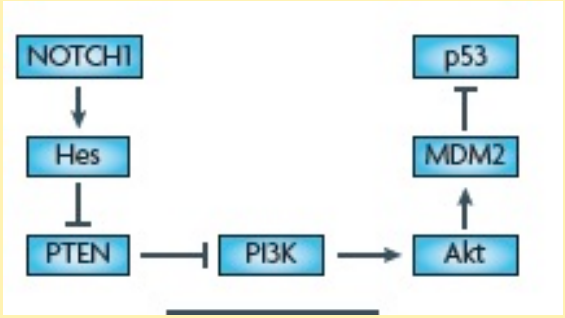




Beverly et al, Cancer Res 2006

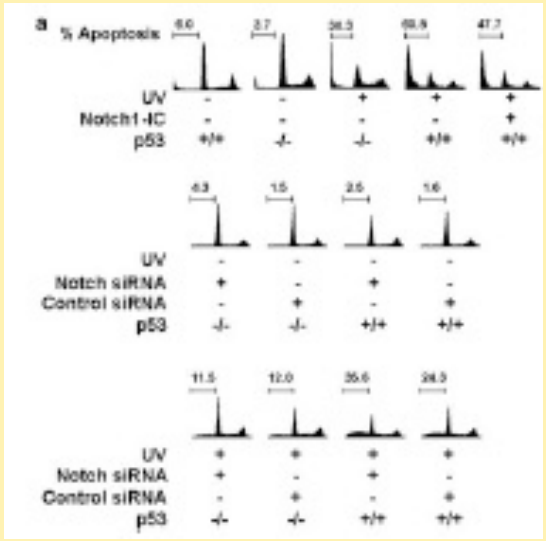
Activated Notch1 inhibits p53 function in H460 cells through PI3K/Akt pathway





Mungamuri et al, Cancer Res 2006

Activated Notch1 interacts with p53 to inhibit its phosphorylation and transactivation



Notch signaling downregulates p53-dependent apoptosis

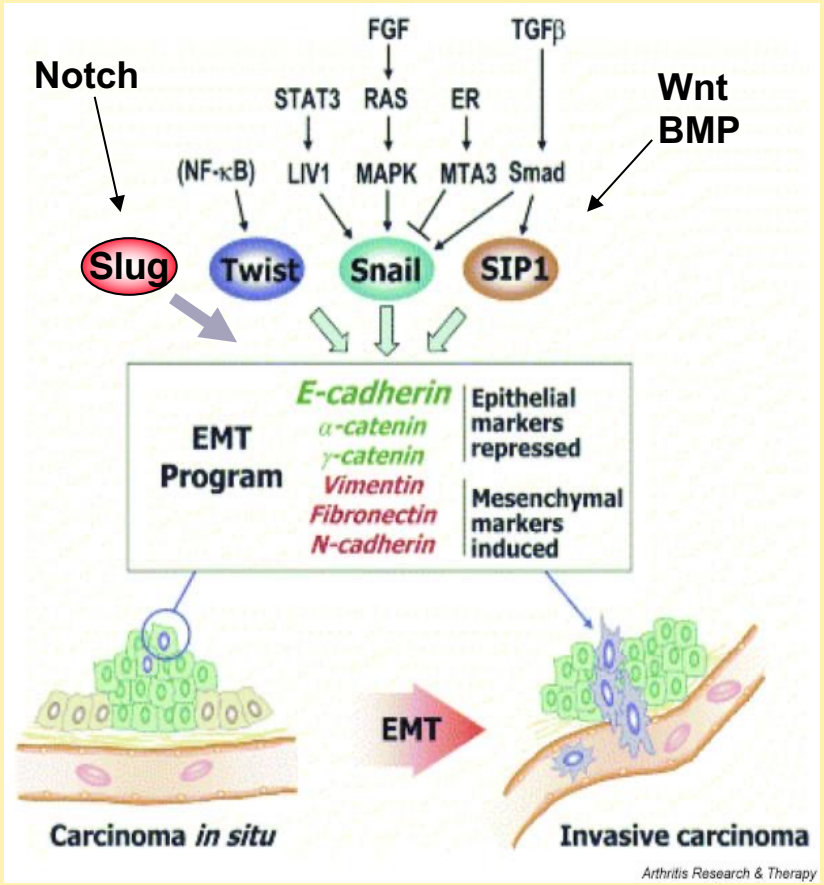
Kim S.B. et al, Cell Death & Differ 2006

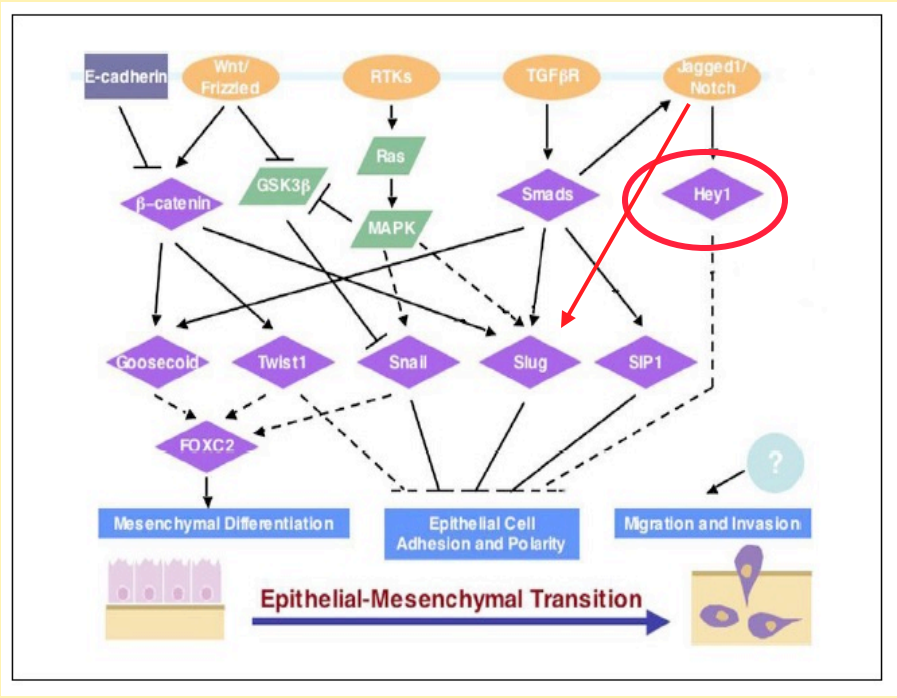
Meccanismi oncogénici di Notch: induzione di EMT

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

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

JEM 2007





Meccanismi oncogénici di Notch: angiogenesi e metastatizzazione

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

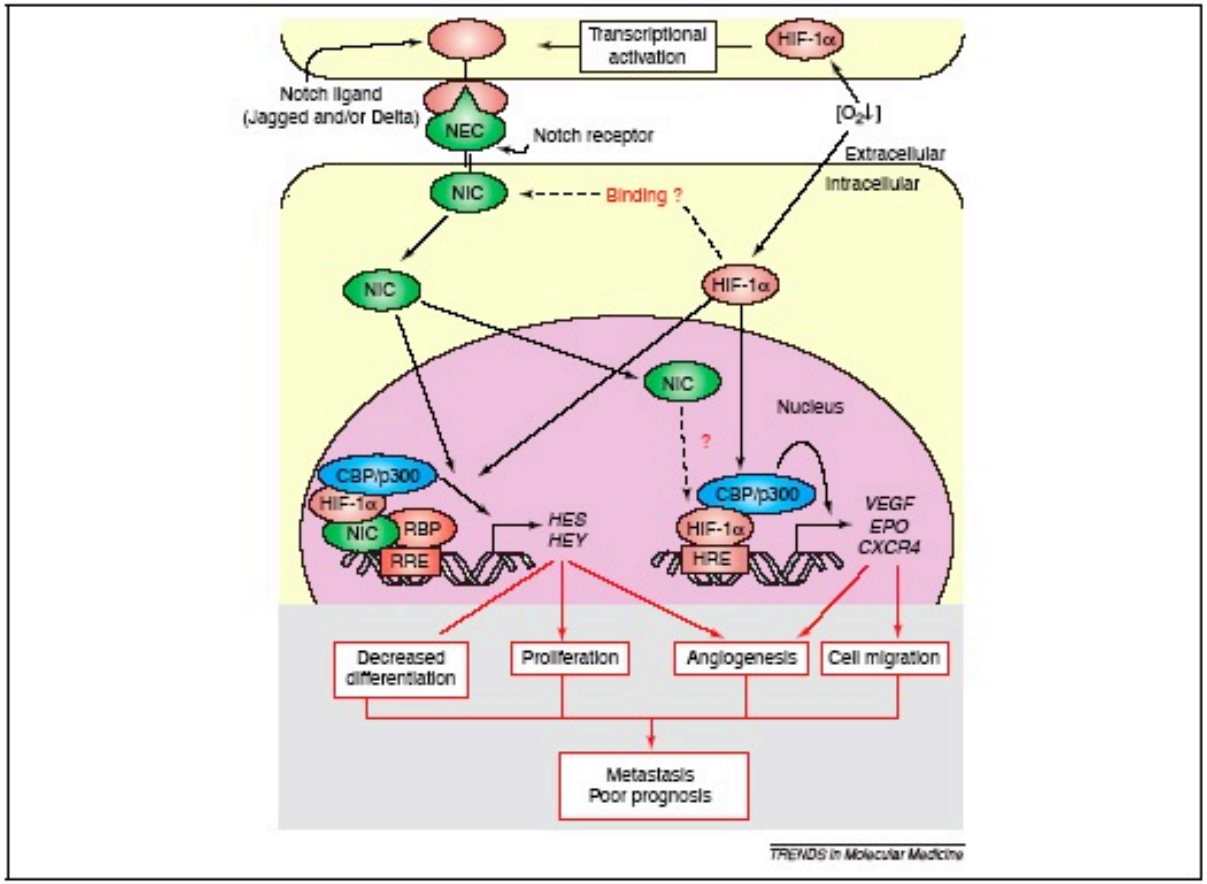


Figure 1. Effects of hypoxia and Notch signalling crosstalk on cancer growth and spreading. Decreased oxygen levels (hypoxia) that are often observed during solid-tumour growth can activate Notch signalling either at the processing level or at the transcriptional level of Notch target genes. Hypoxia 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-1α, 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, HIF response element; NEC, Notch extracellular domain; NIC, Notch intracellular domain; RRE, RBP-Jκ response element; VEGF, vascular endothelial growth factor.

Sainson & Harris, Trends in Mol Med 2006

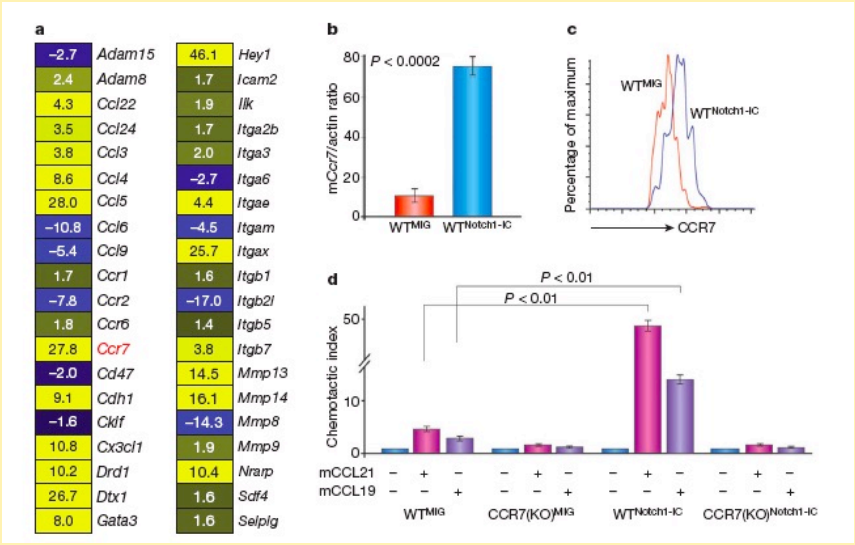
Meccanismi oncogénici di Notch durante la fase metastatica: Notch agevola il *homing* di cellule leucemiche nel cervello

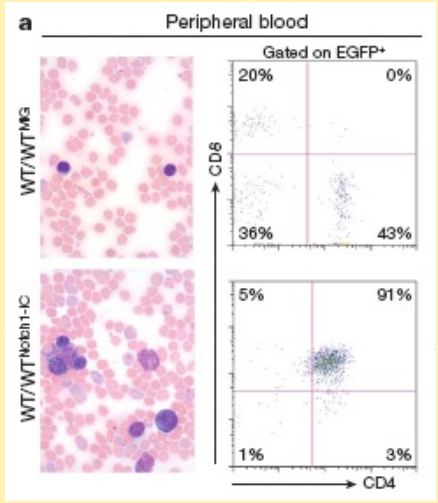
nature Vol 459 | 18 June 2009 | doi:10.1038/nature08020

LETTERS

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

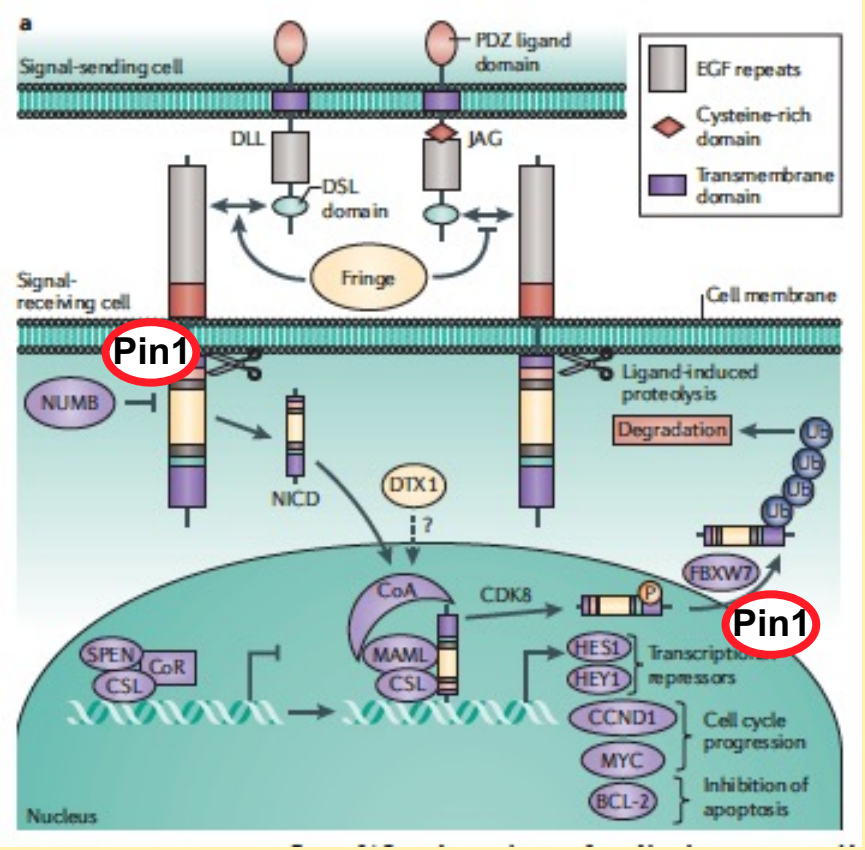
Silvia Buonamici[1,2], Thomas Trimarchi[1,2], Maria Grazia Ruocco[1,3], Linsey Reavie[1,2], Severine Cathelin[1,2], Brenton G. Mar[4], Apostolos Klinakis[5], Yevgeniy Lukyanov[1], Jen-Chieh Tseng[1], Filiz Sen[1,2], Eric Gehrie[6], Mengling Li[7], Elizabeth Newcomb[1], Jiri Zavadil[1], Daniel Meruelo[1],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



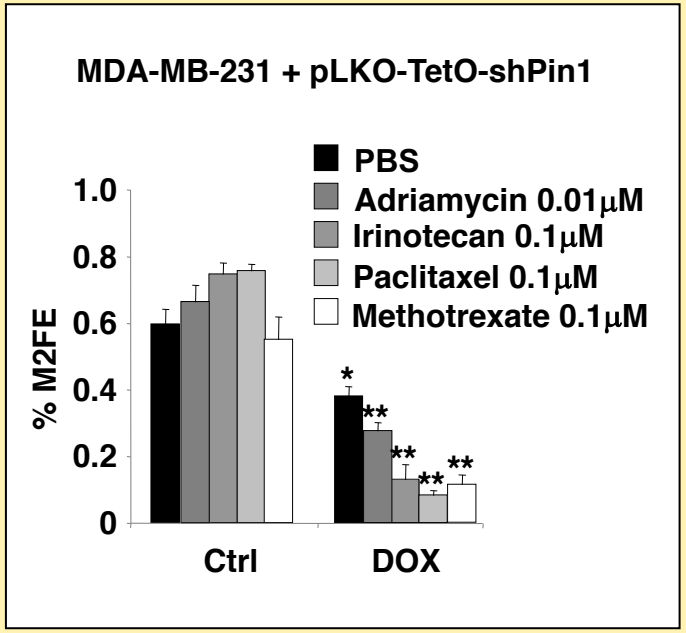


Notch ⟶ CCR7 (chemokine receptor) CCL19 ligand in the brain

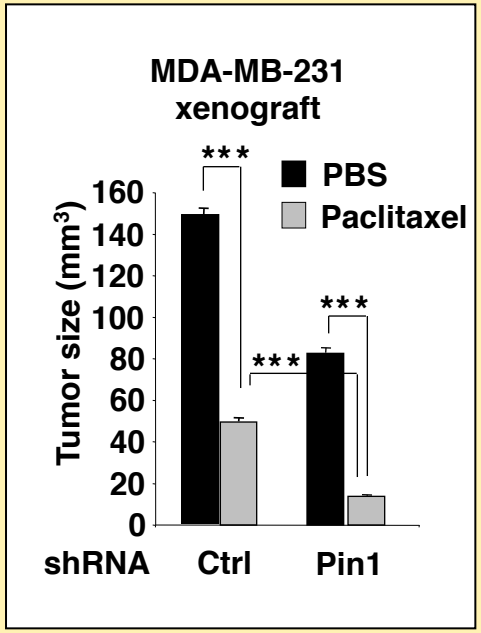
Ruolo della cooperazione tra la prolil-isomerasi Pin1 e Notch nella chemioresistenza

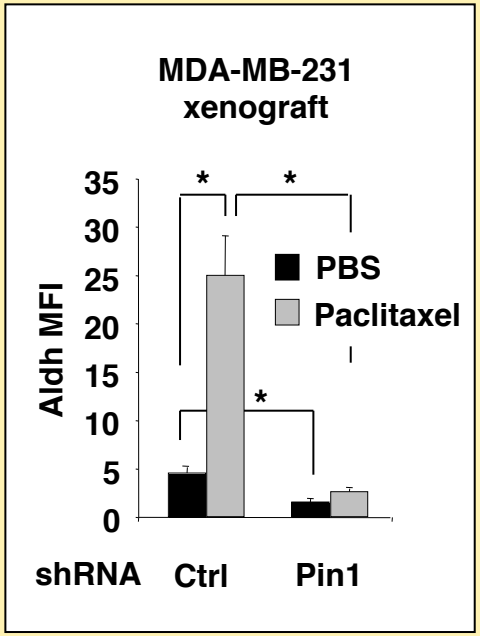


In vitro



In vivo





Rustighi, Zannini et al. EMBO Mol Med 2014