



Trojantec

# Cancer Stem Cells

## The root of all evil...

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# Discordance of clinical outcomes

- Response
  - Quality of response (partial vs complete responses)
  - Kinetics of response
  - Duration of response
- Survival
  - None of these response criteria often correlates with survival
  - Most patients relapse and die of progressive disease

**What cells are responsible for relapse?**

# Cancer Stem Cell Hypothesis

The origin of cancers.....

- A) Stochastic, random model** where any tumour cell can become cancerous due to the activation of a series of oncogenes or the suppression of tumour suppressor genes; or the
  
- B) Hierarchical or cancer stem cell model** where the deregulation of the self-renewal process is brought about on a normal tissue stem cell or on a progenitor cell that acquired this stem cell property of self-renewal. Since these are clonal, not only do they generate more copies of themselves, but also more differentiated cancer cells

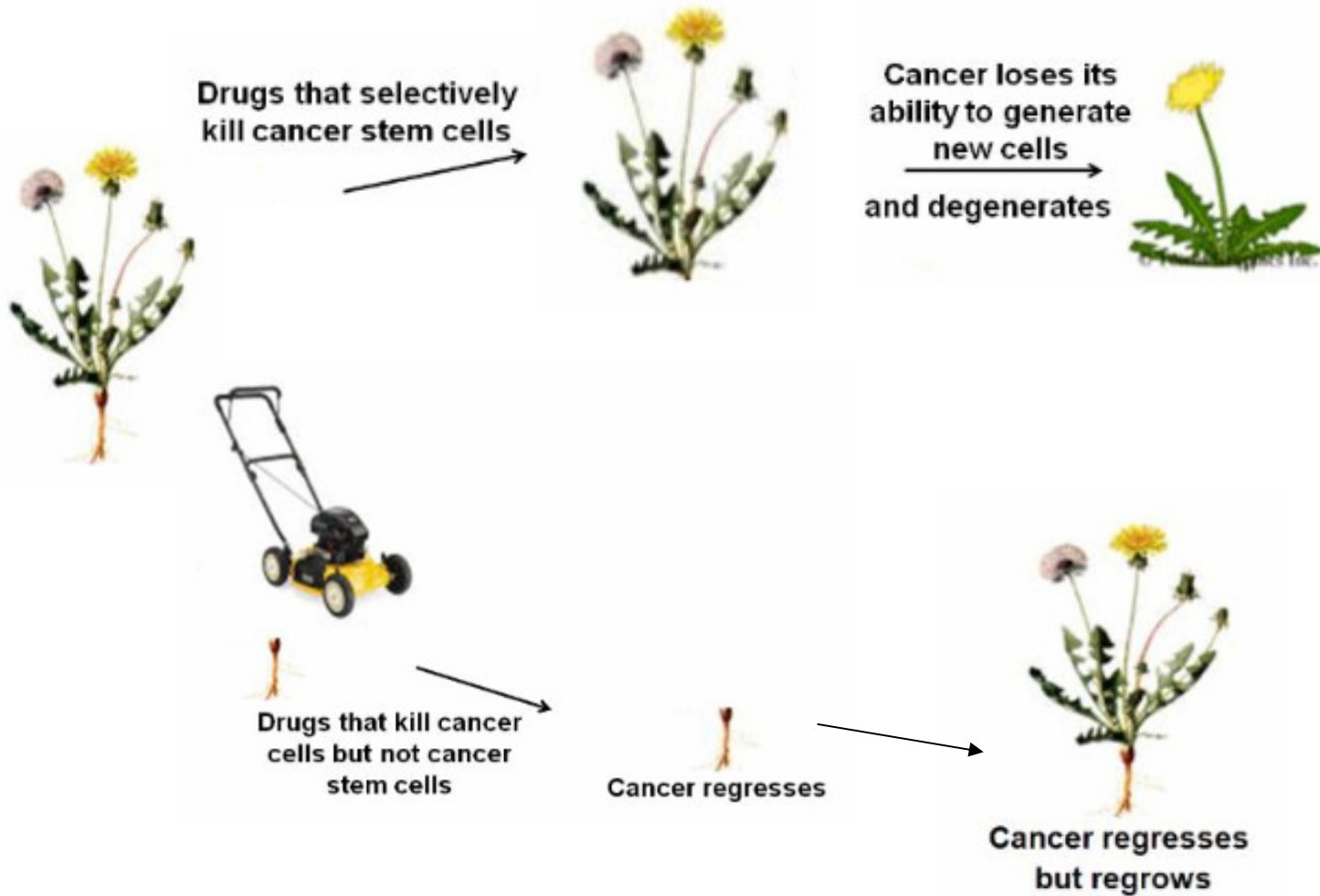
# Existence

Three key observations classically define the existence of a CSC population:

1. Only a minority of cancer cells within each tumor are usually endowed with tumorigenic potential when transplanted into immunodeficient mice.
2. Tumorigenic cancer cells are characterized by a distinctive profile of surface markers and can be differentially and reproducibly isolated from non-tumorigenic ones by means of flow cytometry or other immunoselection procedures.
3. Tumors grown from tumorigenic cells contain mixed populations of tumorigenic and non-tumorigenic cancer cells, thus recreating the full phenotypic heterogeneity of the parent tumor.

# Clinical Consequences


## The dandelion phenomenon



# Clinical Translation

- Optimal strategy

Sequentially target both cancer cells  
and then eliminate cancer stem cells

Debulk with  
high-dose  
treatments  Target stem cells

# Assumptions

- Biologically distinct from bulk tumour cells
- Share many features with normal stem cells
  - Asymmetric cell division
  - Give rise to differentiated progeny
  - Drug resistant due to properties that normally protect adult stem cells
- Combination of surface phenotype and functional properties may enhance isolation
- Therefore, one needs to identify the properties, appreciate any common regulatory mechanisms with normal SCs and find a potential therapeutic index

# What is the definition of a cancer stem cell?

- Definition 1 -
  - Self renewing, recapitulates all cell types in the original tumour, can give tumour in animals from a single cell
- Definition 2 –
  - The cell that survives chemotherapy and must be removed by a surgeon
  - Def 2 must incorporate Def 1 otherwise it is just a chemo resistant cell
- Definition 3 –
  - The cell that expresses a specific cell of markers
  - This definition must incorporate at least Def 1 as well

# Functional approach to defining a CSC

- General Traits
  - Rare
  - Quiescent
  - Self renewal
  - Recapitulates all cell types
  - Forms tumour from a single cell
  - Therapy resistance (chemotherapy/radiation)
- Surrogate “stemness” assays
  - Sphere formation
  - Niche adaptation
  - Self-renewal – *in vitro* single cell CFA
  - Self-renewal and regeneration after lethal dose of therapy (chemo, RT, ↓O<sub>2</sub>)
  - “Stemness” gene signature
  - Asymmetric division
  - Surface markers
- Demonstration with cell lines not enough
  - Primary tumours
  - Pleural effusions
  - Metastatic lesions

# Stem cell niche

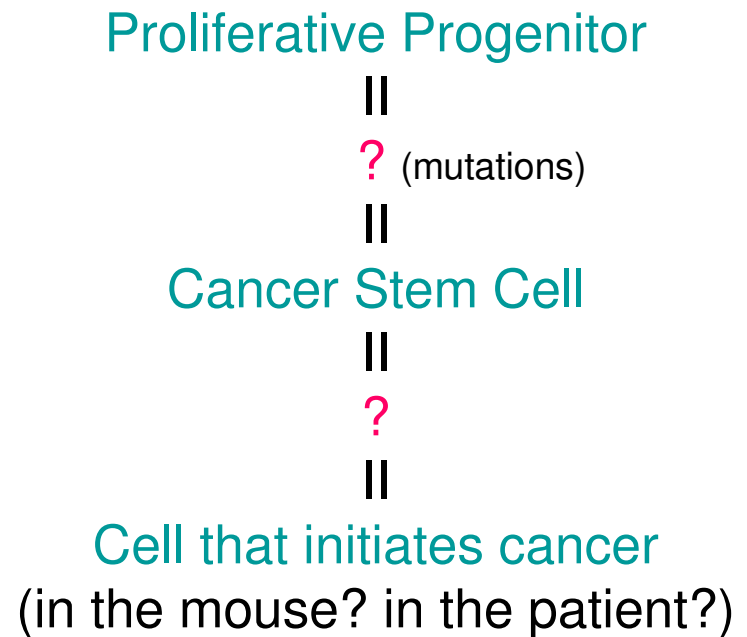
A restricted locale that supports self-renewing division of stem cells and prevents them from differentiating.

A functional stem cell niche must have three components:

1. Localised signaling cells and extracellular matrix controlling stem cell behaviour
2. A specified effective range of signaling
3. Stem cells

# Some questions to consider

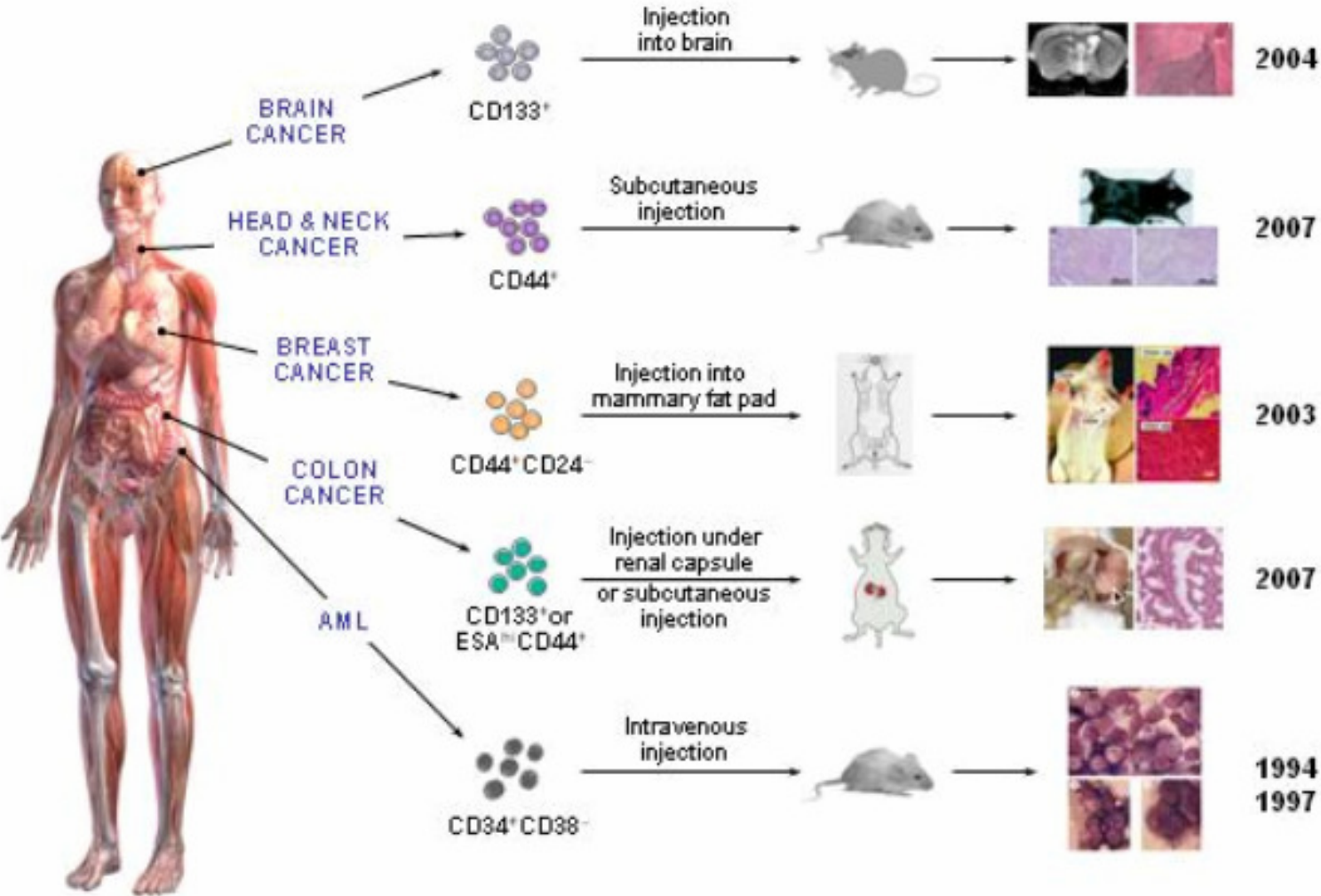
- Are any of these true?
- Are ALL of them true, some of the time?
- Are the assays problematic?
- Better term than “cancer stem cell”?



# Identification of cancer stem cells

- In 1997, Dick et al isolated the first CSC in myeloid leukemia using cell surface marker expression and the ability of human leukemia cells to engraft in nonobese diabetic severe combined deficiency (NOD-SCID) mice and be passaged serially.
- Two important tools integral to the isolation and study of cancer stem cells, fluorescence-activated cell sorting (FACS) and establishment of human tumor xenograft models in immunocompromised mice, were highlighted in this study and subsequently adapted by biologists studying solid organ malignancies.

# Identification of cancer stem cells



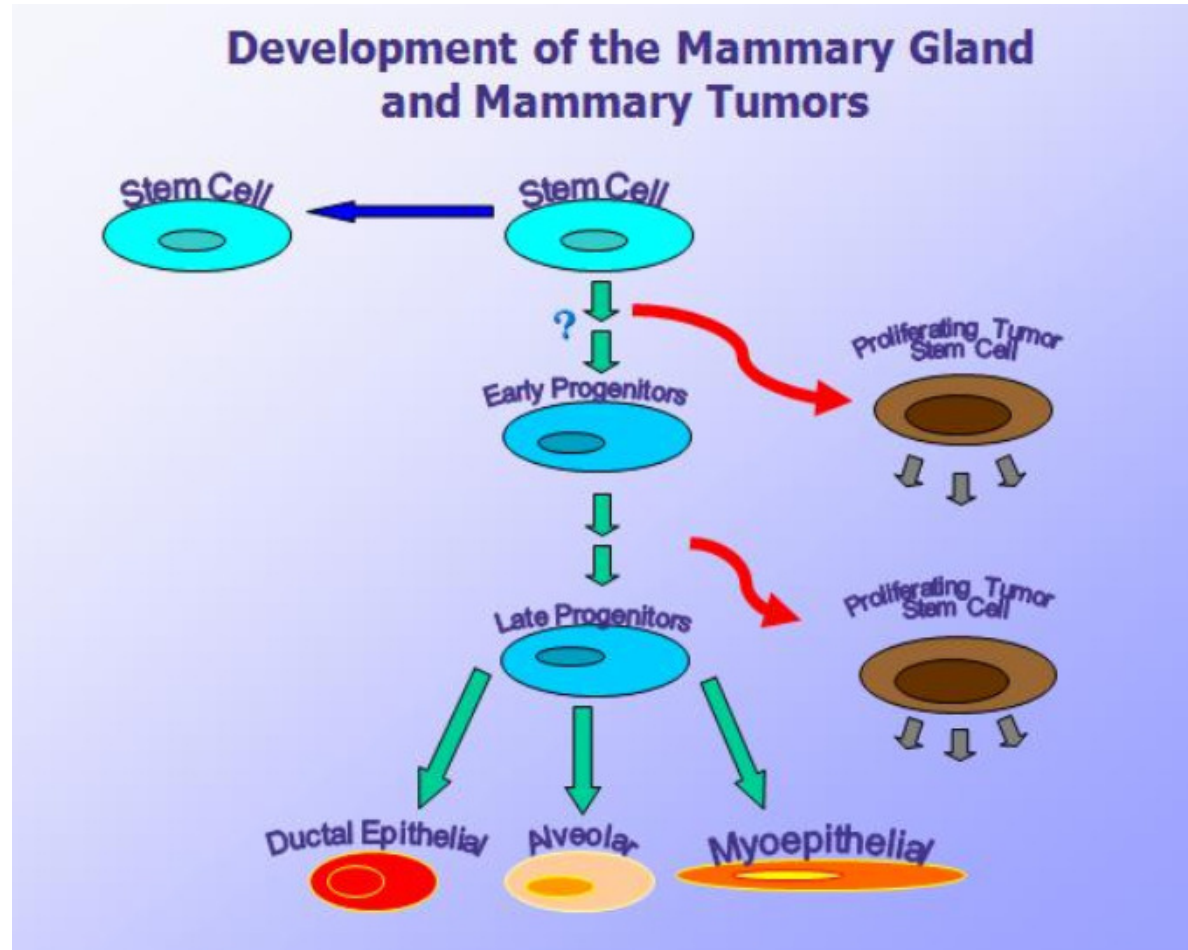
# Breast

- The mammary gland begins development during embryogenesis but after exposure to hormonal changes during puberty and pregnancy undergoes extensive further development.
- Hormonal changes are key regulators in the cycles of proliferation, differentiation, apoptosis and remodelling associated with pregnancy, lactation and involution following weaning.
- These developmental processes within the breast epithelium can be explained by the presence of a long-lived population of tissue-specific stem cells.
- The longevity of these stem cells makes them susceptible to accumulating genetic change and consequent transformation.

# Breast

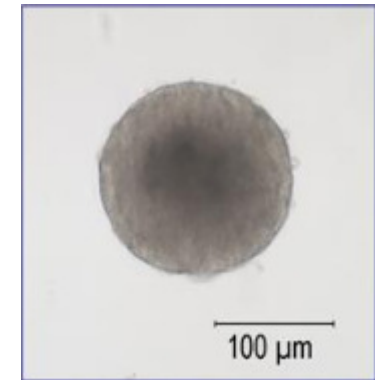
- The first epithelial solid organ cancer stem cell was identified in breast cancer by Al-Hajj et al.
- This study reported a phenotypically distinct and relatively rare population of tumor cells with the cell surface marker expression of CD44<sup>+</sup>CD24<sup>-</sup>/low epithelial-specific antigen (ESA)<sup>+</sup> that were highly tumorigenic and possessed the ability to form tumors that recapitulated the patient's tumor in immunocompromised mice.

# Breast



# Assays

- Mammosphere Assay – takes advantage of the property of all stem cells that are anchorage-independent. Suspension of cells grown in matrigel into ‘mammospheres’.
- Aldehyde dehydrogenase – used as a marker. ↑ activity in progenitor cells (not very reliable)
- CD44+, CD24- markers
- BRCA1 and PTEN mutated
- Notch and Hedgehog upregulated
- Combined, as little as 20 cells are tumourigenic



# Leukemia

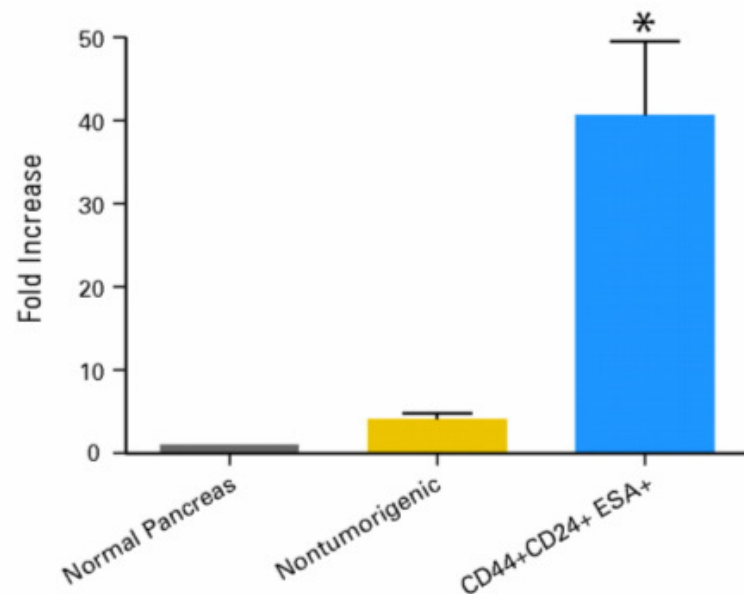
- It is widely accepted that the CD34+CD38-Lin- fraction of human bone marrow cells contains a HSC population, whereas the CD38+ and Lin+ fractions contain more committed progenitors and mature elements
- In the 1970s it was shown that that diseases as diverse as chronic myeloid leukemia (CML), acute myeloid leukemia (AML), essential thrombocythemia, and polycythemia vera were all characterized by the expansion of a monoclonal population of cells that contained multiple lineages of differentiated mature blood elements
- In the early 1990s, it was identified in AML that only a small percentage (1:100) of leukemic cells in clonogenic assays had the clonogenic potential to create a colony
- CD34+CD38-Lin- 1% of these had grafting potential and retained differentiative capacity

# Brain

- Brain tumors are diverse group of neoplasms that remain a formidable cancer problem that has seen few therapeutic advances during the last 40 years.
- Human brain tumors contain a minority population of cells that have potent tumorigenicity, a property not shared by the bulk of the tumor cells.
- This tumorigenic subpopulation has many of the properties that can be ascribed to a stem cell, namely the expression of neural stem-cell markers, and more importantly, the properties of self-renewal, extensive proliferation, and the ability to differentiate into more mature neural lineages.
- CD133 was identified as a marker that was a good candidate for testing the clonogenic abilities of distinct brain tumor subpopulations.
- As few as 100 CD133+ human brain tumor cells were capable of initiating fatal infiltrative tumors in mice after orthotopic transplantation. Injection of 100,000 CD133- cells did not lead to tumor formation.
- CD133+ human glioblastoma cells have been shown to be resistant to radiation therapy, retaining a clonogenic and tumorigenic potential. CD133+ cells increase in number after irradiation of glioblastomas cells in culture and in tumors growing in vivo. The CD133+ cells undergo similar DNA damage to those of their CD133- counterparts, but they show a better ability to repair strand breaks, through a more potent activation of DNA-damage checkpoint mechanisms.

# Pancreas

- Pancreatic cancer has the worst prognosis of any major malignancy, and the annual death rate due to this disease approximates its annual incidence rate.
- Single CD44+CD24+ESA+ cells have been shown to form spheres termed tumorspheres which can be passaged multiple times without loss of 'tumorsphere' forming capability, and in so demonstrating self-renewal capacity *in vitro*.
- Human pancreatic adenocarcinomas display increased hedgehog pathway activity.



Lee, C. J. et al. J Clin Oncol; 26:2806-2812 2008

# Colon

It has been demonstrated that CD133+ colon CSCs were resistant to cell death induced by the chemotherapeutic agents oxiplatin and fluorouracil, and that this resistance was mediated by expression of interleukin (IL)-4 by the CD133+ colon CSCs.

Treatment with an IL-4 receptor antagonist or anti-IL-4 neutralizing antibody strongly enhanced the antitumor efficacy of these chemotherapeutic drugs through selective sensitization of CD133+ cells

# Based on Stem Cell Markers

- **Breast:** CD44+, CD24-/low, Lin-  
Al-Hajj M, et al., Proc Natl Acad Sci 2003
- **Brain:** CD133+  
Singh SK et al., Nature 2004
- **Bone Sarcoma:** Stro-1+, CD105+, CD44+  
Gibbs CP et al., Neoplasia 2005
- **Lung:** Sca-1+, CD45-, Peca,-, CD34+  
Kim CF et al., Cell 2005
- **Melanoma:** CD20+  
Fang D. Cancer Res 2005
- **Prostate:** CD44+, CD133+,  $\alpha 2\beta 1$ h1  
Collins AT et al., Cancer Res 2005

# Stem cell self renewal

## Putative pathways

- Developmental signaling pathways (hedgehog, wnt, notch, bmi)
- Telomerase
  
- **Do these processes regulate CSC?**
- **Can they serve as anticancer targets?**

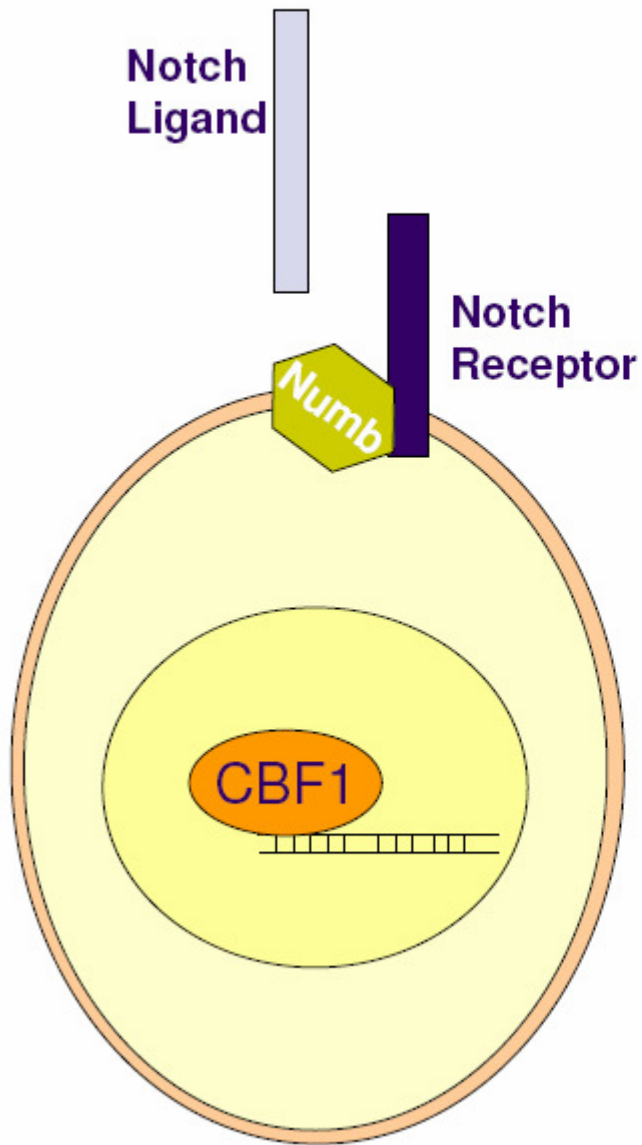
# Hedgehog signaling

- Mammals have three Hedgehog homologues, of which Sonic hedgehog is the best studied. The pathway is equally important during vertebrate embryonic development.
- In knockout mice lacking components of the pathway, the brain, skeleton, musculature, gastrointestinal tract and lungs fail to develop correctly.
- Recent studies point to the role of hedgehog signaling in regulating adult stem cells involved in maintenance and regeneration of adult tissues. The pathway has also been implicated in the development of cancers.
  - Activating mutations along components of the signaling pathway: medulloblastoma, basal cell carcinoma, rhabdomyosarcoma
  - Aberrant up-regulation of the pathway: lung, upper GI, prostate cancers
  - Activation of the Hedgehog pathway leads to an increase in Snail protein expression and a decrease in E-cadherin and Tight Junctions. Hedgehog signaling also appears to be a crucial regulator of angiogenesis and thus metastasis

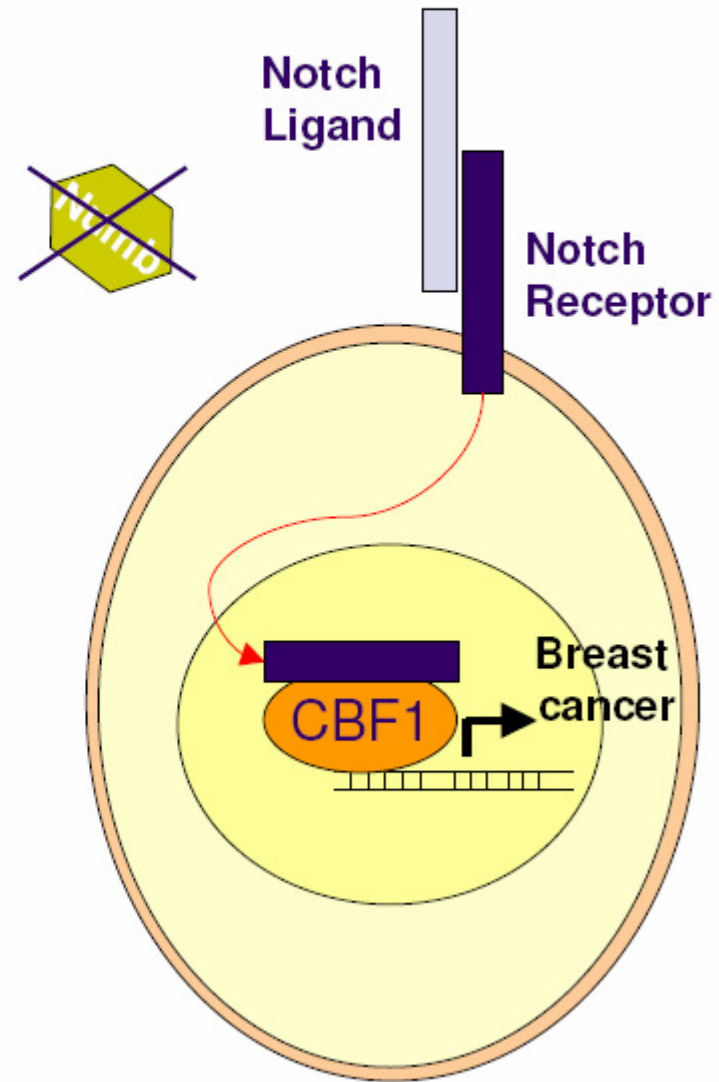
# Notch and cancer

- Notch signaling is an evolutionary-conserved pathway in vertebrates and invertebrates which is involved many developmental processes, including cell-fate decisions, apoptosis, proliferation, and stem-cell self-renewal.
- Cancers with over-expression of Notch signalling
  - T-cell acute lymphoblastic leukaemia
  - Cervical cancer
  - Basal cell carcinoma
  - Small-cell lung carcinoma
  - Breast cancer
  - Prostate cancer
  - Neuroblastoma

## Notch Signalling Inhibition



## Notch Signalling Activation



# Telomerase

## Stem cells and cancer

- **Telomerase**
  - Maintains the ends of linear chromosomes
  - Inhibition leads to progressive chromosome shortening at every cycle and after a critical limit cellular senescence follows
- **Normal stem cells**
  - High activity in stem/progenitor compartments (recently shown that skin stem cells have the longest telomeres)
  - Dyskeratosis congenita- mutations affecting reverse transcriptase activity
    - Exhaustion of normal HSC and aplastic anaemia (no blood forming ability)

# Telomerase

## Stem cells and cancer

- **Cancer**
  - Telomerase activity common feature in cancer
  - Multiple myeloma – telomerase activity and telomere length correlate with clinical outcomes
- **Telomerase-based therapeutics**
  - Inhibitors of telomerase RT activity
  - Telomere-disrupting agents
  - Telomerase-based vaccines

# Therapeutic Challenges

- Current methods to assess clinical efficacy may not predict activity against CSCs
- Major obstacles to direct clinical assessment of anti-CSC activity:
  - Identification of new markers for tracking CSCs
  - Validation of surrogate endpoints of drug efficacy
  - Improved resolution of molecular imaging
- Greater emphasis on preclinical development
  - Novel drug discovery platforms using relevant cell populations
  - Functional validation of activity in *in vivo* models

# Clinical implications

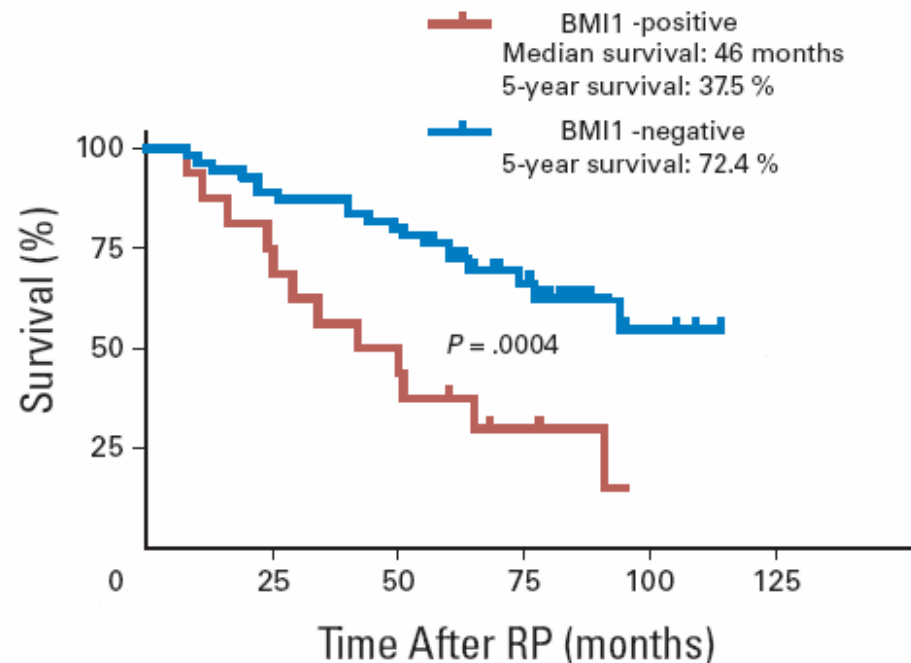
- **Prognostic relevance –**
- Profiled CD24<sup>-</sup> /low/CD44<sup>+</sup> and CD24<sup>+</sup>/CD44<sup>+</sup>/- cell populations from multiple breast tumors and concluded that a gene expression signature characteristic of breast cancer stem cells is associated with shorter distant metastasis-free and overall survival
- Treatment of mice transplanted with human AML cells with activating anti-CD44 antibody markedly reduced leukemic repopulation

# Clinical implications

- Gene expression signatures (GESs) associated with the “stemness” state of a cell might be informative as molecular predictors of cancer therapy outcome
- A stemness CTOP algorithm was generated comprising a combination of nine signatures which demonstrates high prognostic accuracy for a majority of patients in retrospective supervised analysis of large cohorts of breast, prostate, lung, and ovarian cancer patients, suggesting that therapy-resistant and -sensitive tumors develop within genetically distinct stemness/differentiation programs driven by engagement of specific pathways.

# Clinical implications

- *Bmi-1* is a transcriptional repressor that regulates stem cell self-renewal through the repression of important cell cycle regulatory genes in the INK-4A/ADP ribosylation factor (ARF) complex, p16 INK-4A, and p19 ARF. It also induces telomerase activity.
- It is expressed in all primary myeloid leukemia and leukemic cell lines, in non-small-cell lung cancer, human breast carcinomas and breast cancer cell lines, human medulloblastomas, prostate carcinomas, and GI cancers, as well as prostate cancer and metastases.
- Kaplan-Meier survival analysis of 71 prostate cancer patients with distinct levels of positive BMI1-high-expressing cells in primary prostate tumors
- Prostate cancer patients with more than 1% of positive BMI1-high-expressing cells manifested statistically significant increased likelihood of therapy failure after radical prostatectomy



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REVIEW ARTICLE

“Stemness” Genomics Law Governs Clinical Behavior of  
Human Cancer: Implications for Decision Making in  
Disease Management

*Gennadi V. Glinsky*

# Conclusion

- Development of a concise catalog of gene expression changes comprising approximately 300 human genes divided into nine signatures and reflecting a transcriptional pathology of stemness/differentiation pathways associated with therapy-resistant phenotypes of human solid tumors.
- One of the significant advantages of availability of such a catalog of 'stemness' pathways relevant to human cancer is the potential to exploit this information for a therapeutic gain in the effort to target clinically lethal states of malignant phenotypes.

**THANK YOU**