

# STEM CELLS

“Search for the Holy Grail”



# Historical background

## Historical Background

- Beatrice Mintz and Barry Pierce (1970-1985)
- Oncogeny partially recapitulates Ontogeny in an inappropriate temporal and spatial context (eg.embryonal carcinomas).

[CANCER RESEARCH 48, 1996-2004, April 15, 1988]

### *Perspectives in Cancer Research*

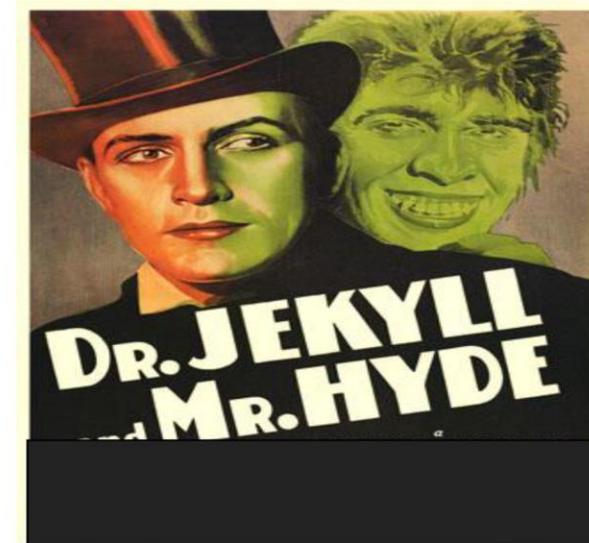
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#### **Tumors as Caricatures of the Process of Tissue Renewal: Prospects for Therapy by Directing Differentiation<sup>1</sup>**

**G. Barry Pierce and Wendell C. Speers**

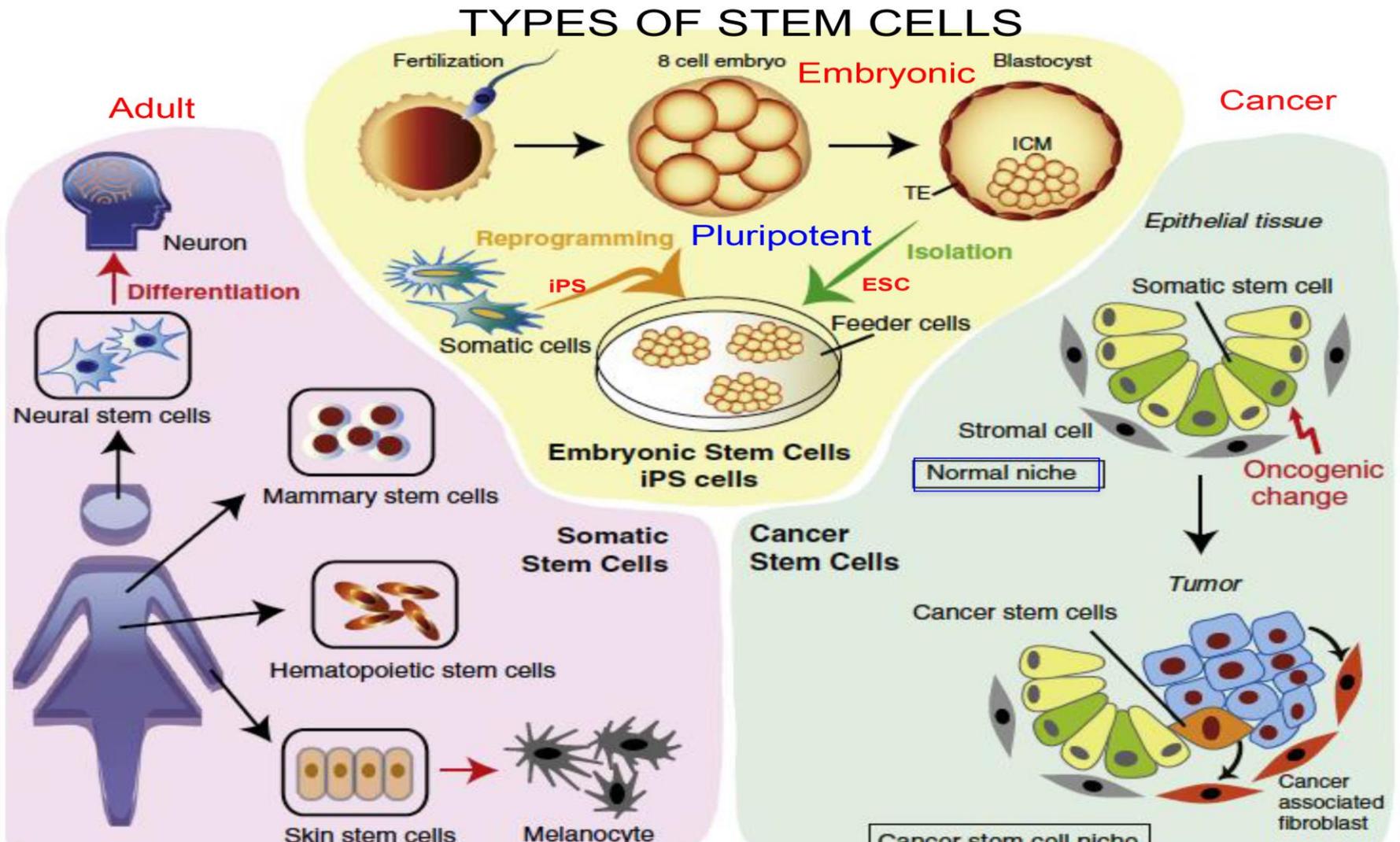
*Department of Pathology, University of Colorado Health Sciences Center, Denver, Colorado 80262*

Normal stem cell      Cancer stem cell



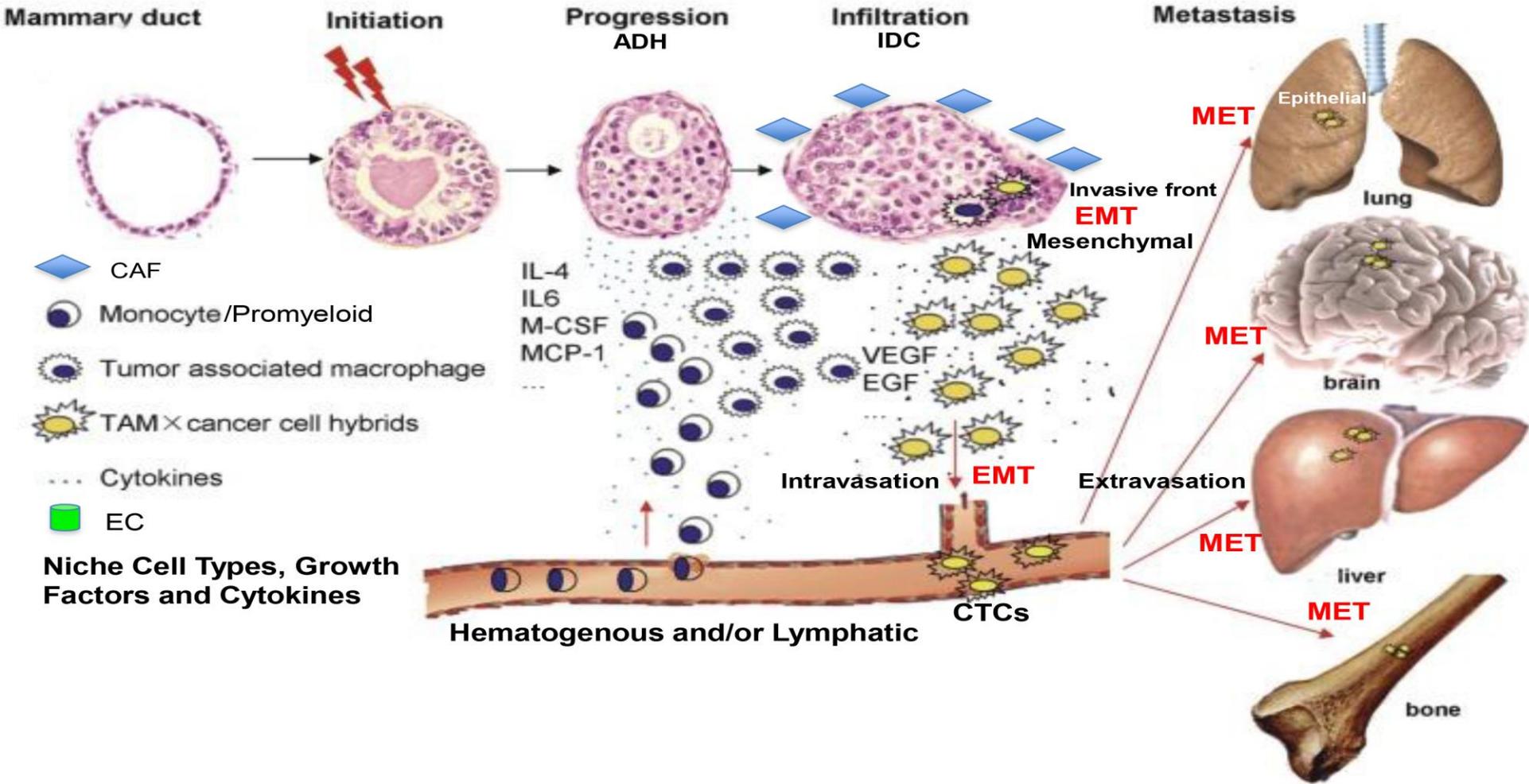
- The embryonic microenvironment or the adult stem cell niche can reprogram tumor cells to normal cellular lineage restriction and differentiation (Dominance of the Niche).
- The tumor microenvironment/niche can reprogram adult tissue stem cells and iPS cells to acquire properties of cancer stem cells (CSCs)/tumor initiating cells (TICs).

# Types of stem cells



# Mammary cancer progression

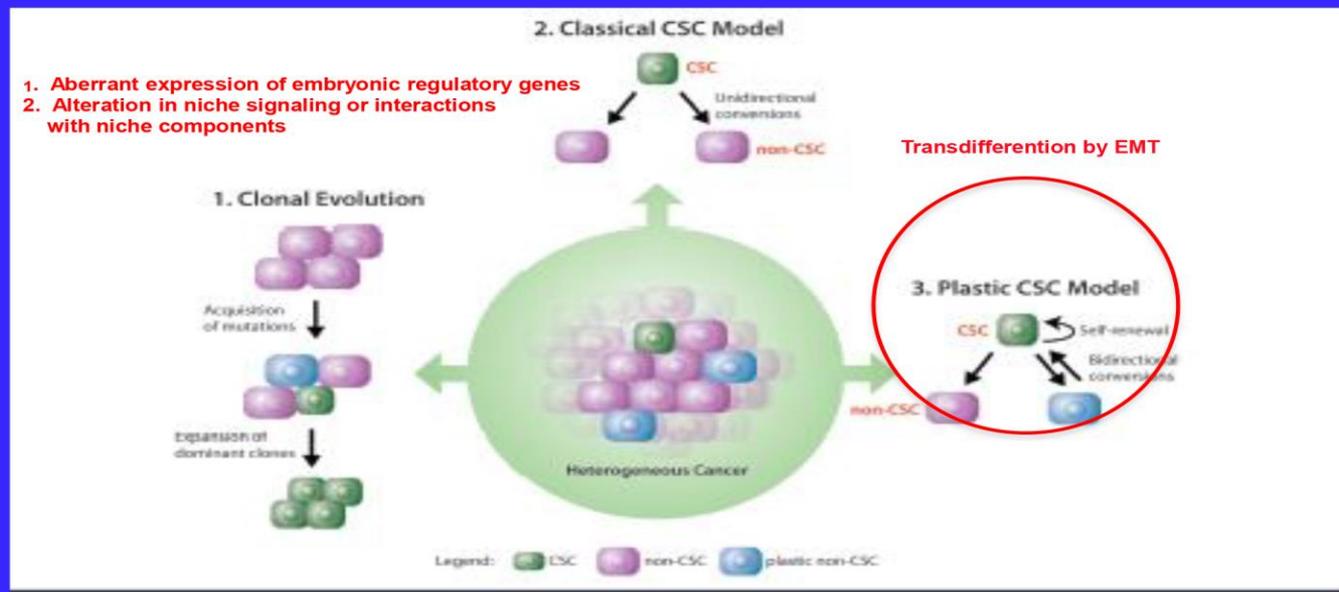
## MAMMARY EPITHELIAL CELL CARCINOMA PROGRESSION



# Cancer Heterogeneity

## Models for Cancer Heterogeneity

1. Any tumor cell has the potential to be a cancer stem cell (CSC) after immortalization and subsequent transformation (Stochastic or Clonal Model).
2. Only a small definable and preexisting subset of cancer cells are CSCs that have the ability to self renewal and to give rise to the full spectrum of the original tumor phenotype. CSCs can arise from the transformation of either normal tissue stem cells or progenitor/transit amplifying cells (CSC or Hierarchical Model).



# CSC Frequency

## Factors Regulating the Frequency and/or Representation of CSCs in a Tumor

1. Tumors can arise from populations of normal tissue stem or progenitor cells.
2. Genetic and epigenetic modifications that CSCs have temporally and spatially accumulated can contribute to the process of tumor progression and metastatic dissemination (intravasation and extravasation).
3. Contextual signals (autocrine and/or paracrine) that the CSCs are exposed to either in the tumor itself and/or within the CSC niche/microenvironment can initiate and/or contribute to these progressive genetic and/or epigenetic alterations.
4. The immunological status of the host in which the tumor develops can positively or negatively modify these CSC phenotypes in a temporal and spatial context.

# CSC properties

## Properties Shared by Normal Tissue Stem Cells and Cancer Stem Cells

### – Self-renewal

- Tissue-specific stem cells are capable of **self-renew via assymmetric cell division** throughout the lifetime of the animal to maintain specific differentiated properties of each organ or tissue.
- Cancer stem cells are the **tumor initiation population** that at limiting dilution through self-renewal initiate and maintain tumor growth (symmetric division).

### – Differentiation into phenotypically diverse cell types

- **Differentiate into a heterogeneous population** of cells that recapitulates the phenotype of the organ or the phenotype of the ~~organ or primary tumor. However, normal stem cells are able to~~ **for unlimited proliferation and usually undergo periods of regulated quiescence** which is not the case for cancer stem cells which can remain dormant for protracted periods and which ultimately contributes to their intrinsic chemo- and radio-resistance.

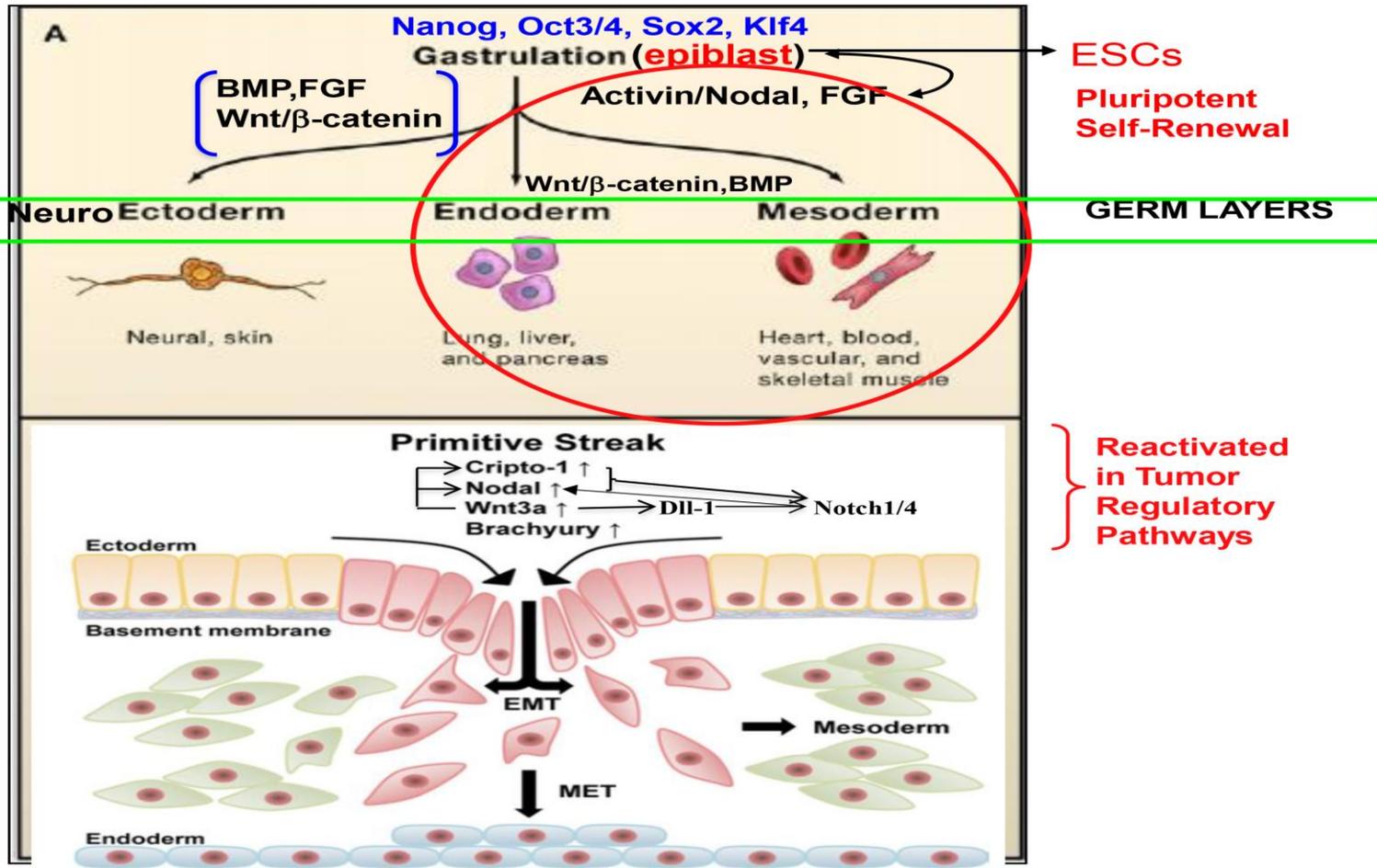
### – CSCs Share Regulatory Pathways with normal tissue stem cells

that can control self-renewal in normal stem cells or in the normal niche and that can become deregulated in cancer stem cells and/or the CSC niche both temporally and spatially.

# Embryonic genes

## EMBRYONIC GENES THAT REGULATE GERM LAYER FORMATION

Temporal  
Spatial  
Concentration  
dependent  
( Morphogenic  
Gradients )



# Gene overexpression

Forty-eight **Genes** Overexpressed by  
Microarray Meta Analysis in **hESCs** compared  
to Differentiated Cell Types in at least **40**  
**studies** and Involved in **Pluripotency and Self-  
Renewal**

**POU5F1 (Oct3/4) POU domain, class 5, transcription factor 1 6p21.31 Hs.249184 20**

**TDGF1 Teratocarcinoma-derived growth factor 1 3p21.31 Hs.385870 17 55.0**

**DPPA4 Developmental pluripotency-associated 4 2q43.13 Hs.317659 16 30.1**

**LIN28 Lin-28 homolog (C. elegans) 1p36.11 Hs.86154 16 24.8**

**NANOG Nanog homeobox 12p13.31 Hs.329296 15 88.9**

**DNMT3B DNA (cytosine-5)-methyltransferase 3 beta 20q11.2 Hs.251673 15 27.8**

**TERF1 Telomeric repeat binding factor (NIMA-interacting) 1 8q13 Hs.442707 15**

**SEMA6A Semaphorin 6A 5q23.1 Hs.156967 15 12.3**

**M6PR Mannose-6-phosphate receptor (cation dependent) 12p13 Hs.134084 15 10.6**

**SNRPN Small nuclear ribonucleoprotein polypeptide N 15q11.2 Hs.525700 15 7.3**

**FLJ10884 Hypothetical protein FLJ10884 1p31.3 Hs.562195 14 260.7**

**LEFTY1 Left-right determination factor 1 1q42.1 Hs.278239 14 34.1**

**GAL Galanin 11q13.2 Hs.278959 14 21.4**

**SEPHS1 Selenophosphate synthetase 1 10p14 Hs.124027 14 6.3**

**GABRB3 Gamma-aminobutyric acid (GABA) A receptor, beta 3**

**15q11.2-q1 Hs.302352 13 15.3**

**SOX2 SRY (sex determining region Y)-box 2 3q26.3-q27 Hs.518438 13 15.3**

**LECT1 Leukocyte cell derived chemotaxin 1 13q14-q21 Hs.421391 12 37.2**

**LOC90806 Similar to RIKEN cDNA 2610307I21 1q32.3 Hs.157078 12 14.6**

**BUB1 BUB1 budding uninhibited by benzimidazoles 1  
homolog**

**2q14 Hs.469649 12 11.2**

**PSIP1 PC4 and SFRS1 interacting protein 1 9p22.3 Hs.493516 12 5.4**

**INDO Indoleamine-pyrrole 2,3 dioxygenase 8p12-p11 Hs.840 11 34.4**

**HELLS Helicase, lymphoid-specific 10q24.2 Hs.546260 11 19.3**

**GPC4 Glypican 4 Xq26.1 Hs.58367 11 15.4**

**ITGB1BP3 Integrin beta 1 binding protein 3 19p13.3 Hs.135458 11 15.3**

**CYP26A1 Cytochrome P450, family 26, subfamily A,  
polypeptide 1**

**10q23-q24 Hs.150595 11 14.2**

**MCM5 MCM5 minichromosome maintenance deficient 5 22q13.1 Hs.517582 11 11.9**

**MTHFD1 Methylenetetrahydrofolate dehydrogenase 1 14q24 Hs.435974 11 8.7**

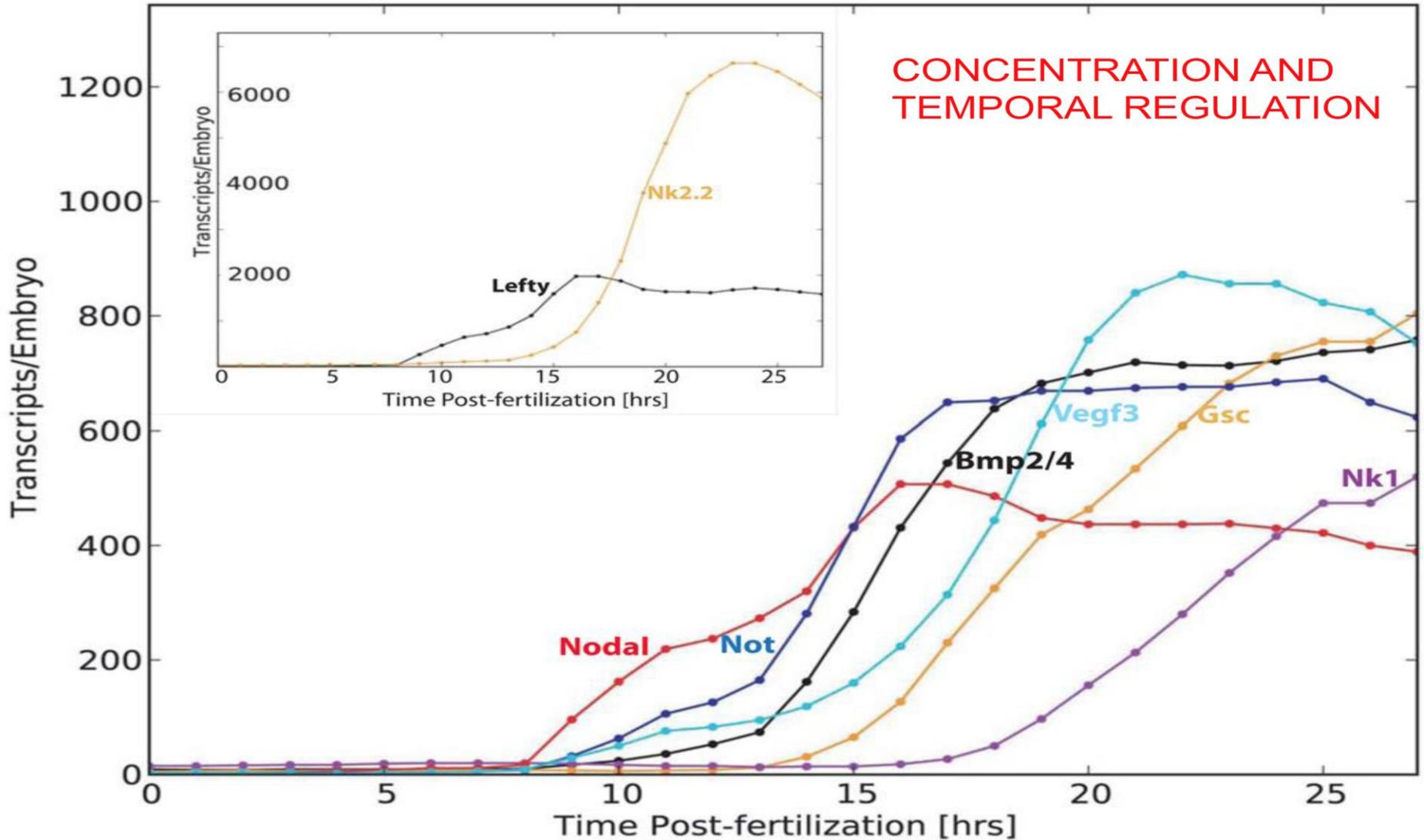
**PPAT Phosphoribosyl pyrophosphate amidotransferase 4q12 Hs.331420 11 8.3**

**SLC16A1 AKR7 family pseudogene 1p12 Hs.75231 11 7.8**

**NASP Nuclear autoantigenic sperm protein (histone-binding) 1p34.1 Hs.319334 11**

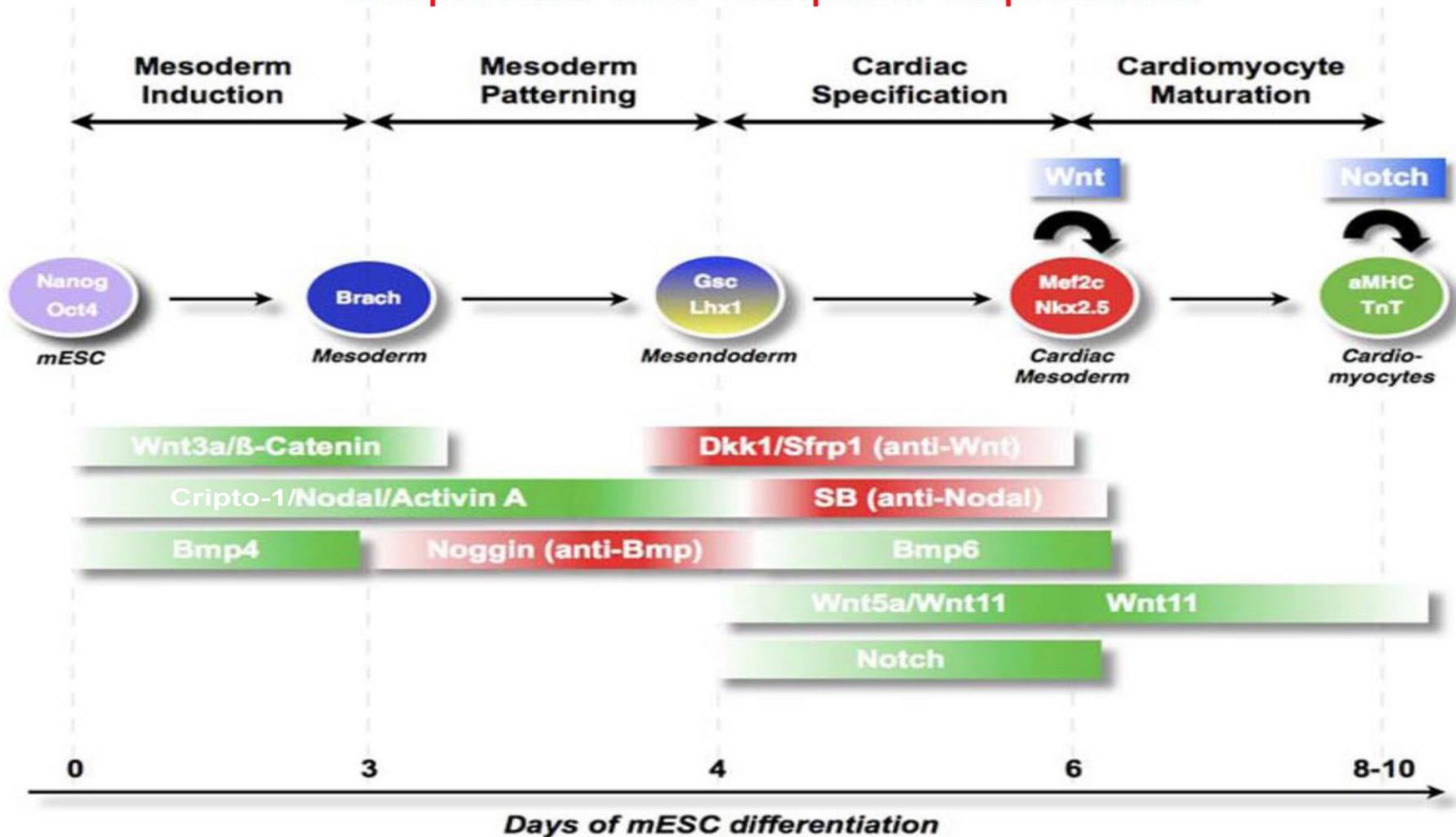
**“Trinity/Triumverate”  
N(NANOG), O(OCT4) AND  
S(SOX2)**

# CSC factors



# ESC differentiation

## Sequential and Temporal Expression



# Target genes

## Target Genes Co-Occupied by NOS Genes

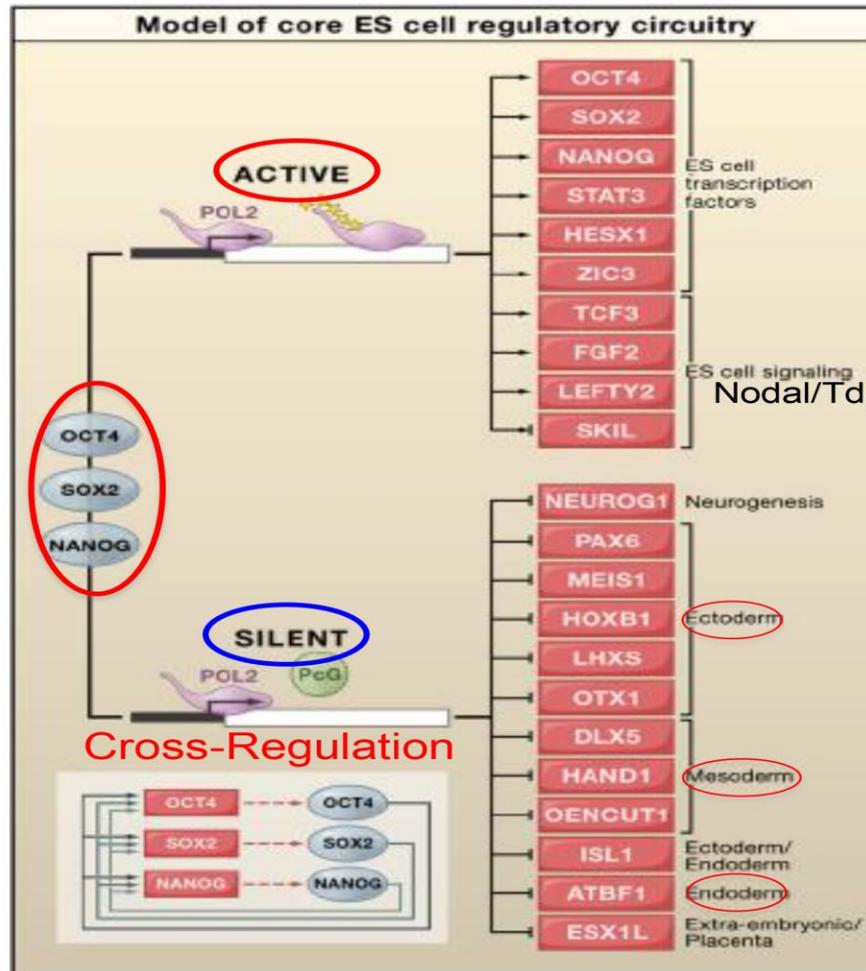
**Table 1.** Numbers of promoters occupied by transcription factors in ES cells

Protein	Number of promoters
Nanog	1284
Sox2	819
Dax1	1754
Nacl	804
Oct4	783
Klf4	1790
Zfp281	601
Rex1	1543
Myc	3542

Data from Kim et al. (2008 [© Elsevier]).

# Non Target Genes

CO-OCCUPIED NOS TARGET GENES THAT ARE ACTIVATED OR REPRESSED IN ES CELLS



← ESC Maintenance

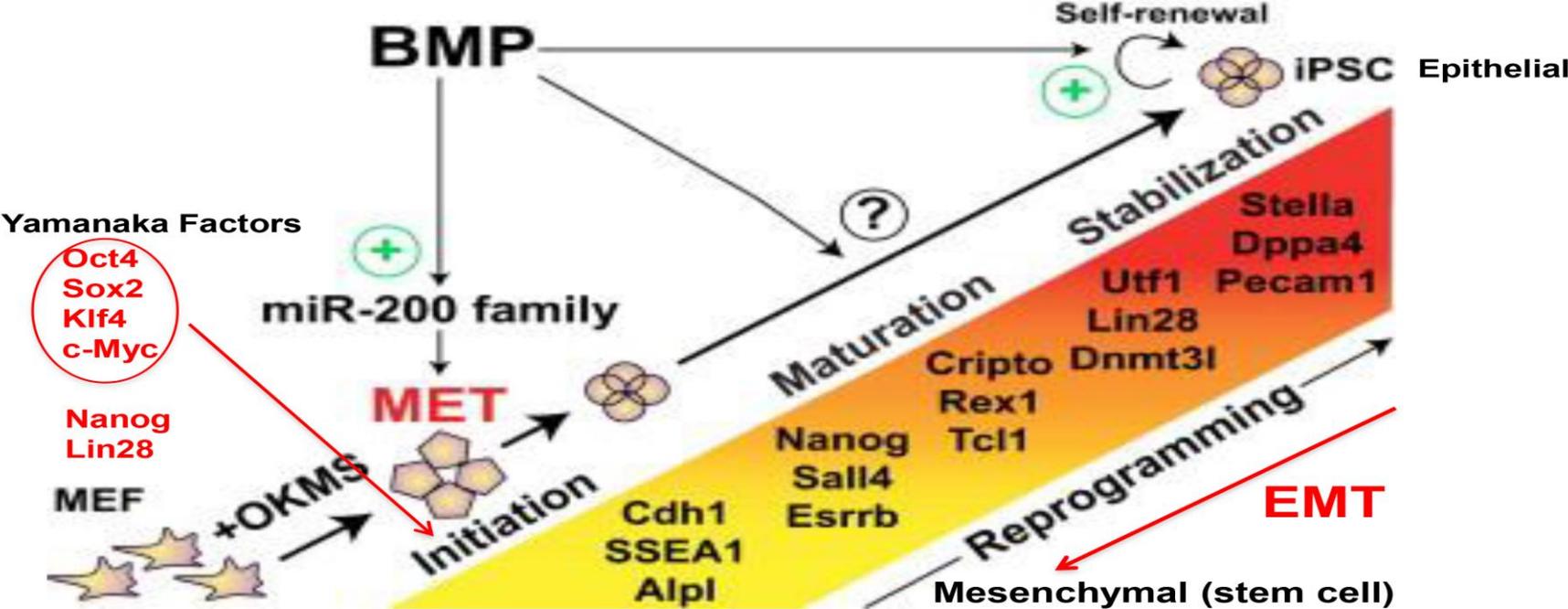
← Nodal/Tdgf1 (Cripto-1)

← Tissue Specific Stem Cell Maintenance and Initiation of Differentiation

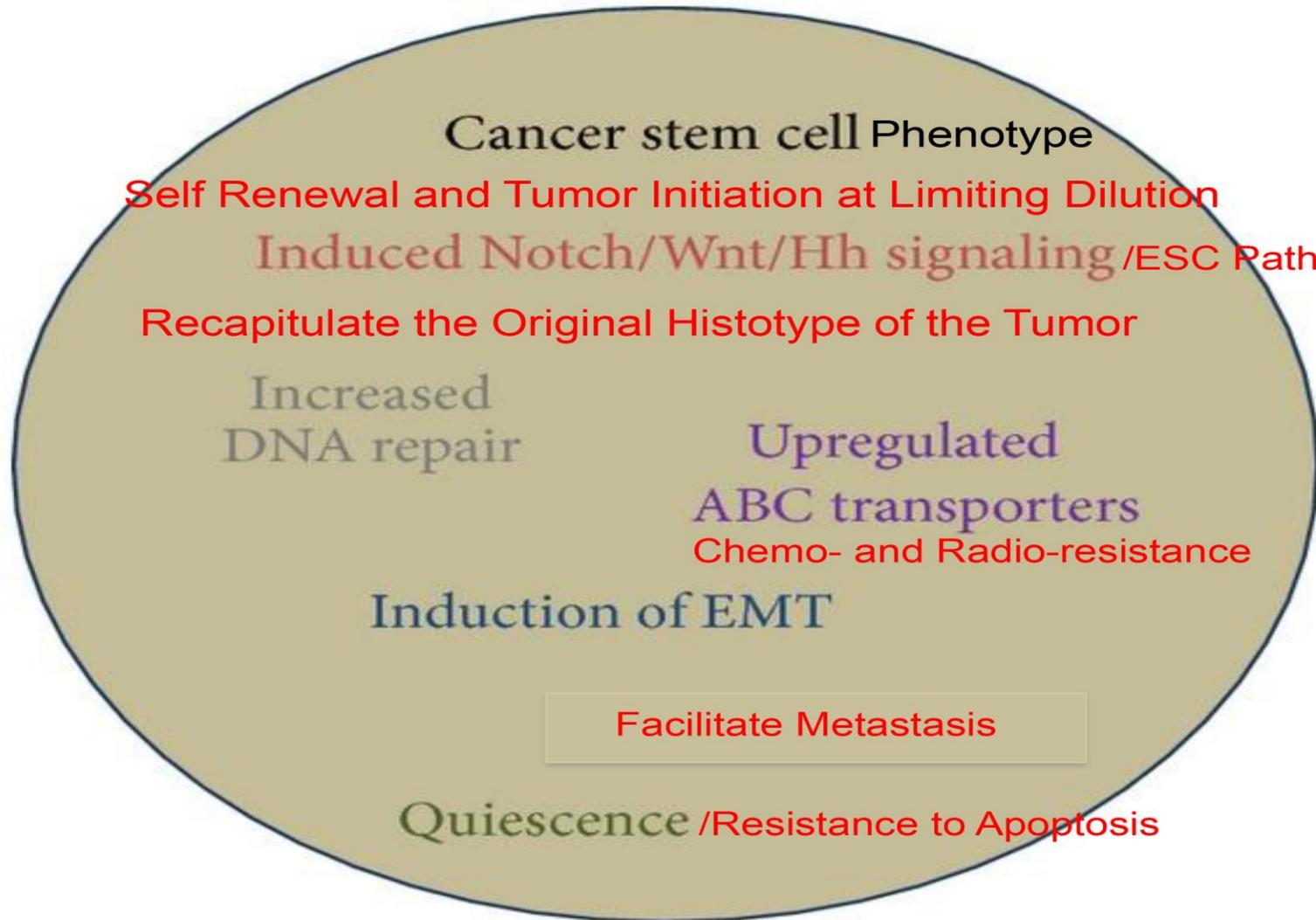
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# Reprogramming cells

REPROGRAMMING ADULT DIFFERENTIATED SOMATIC CELLS TO AN ESC-LIKE STATE



# CSC Phenotype



# CSC Proliferation

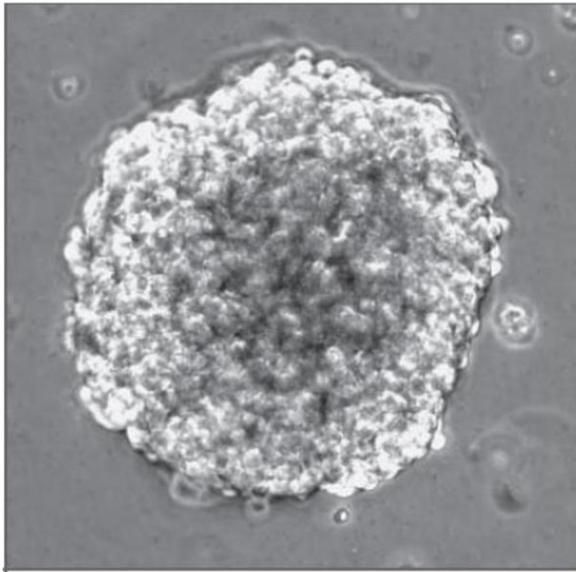


Figure 4. Tumorsphere

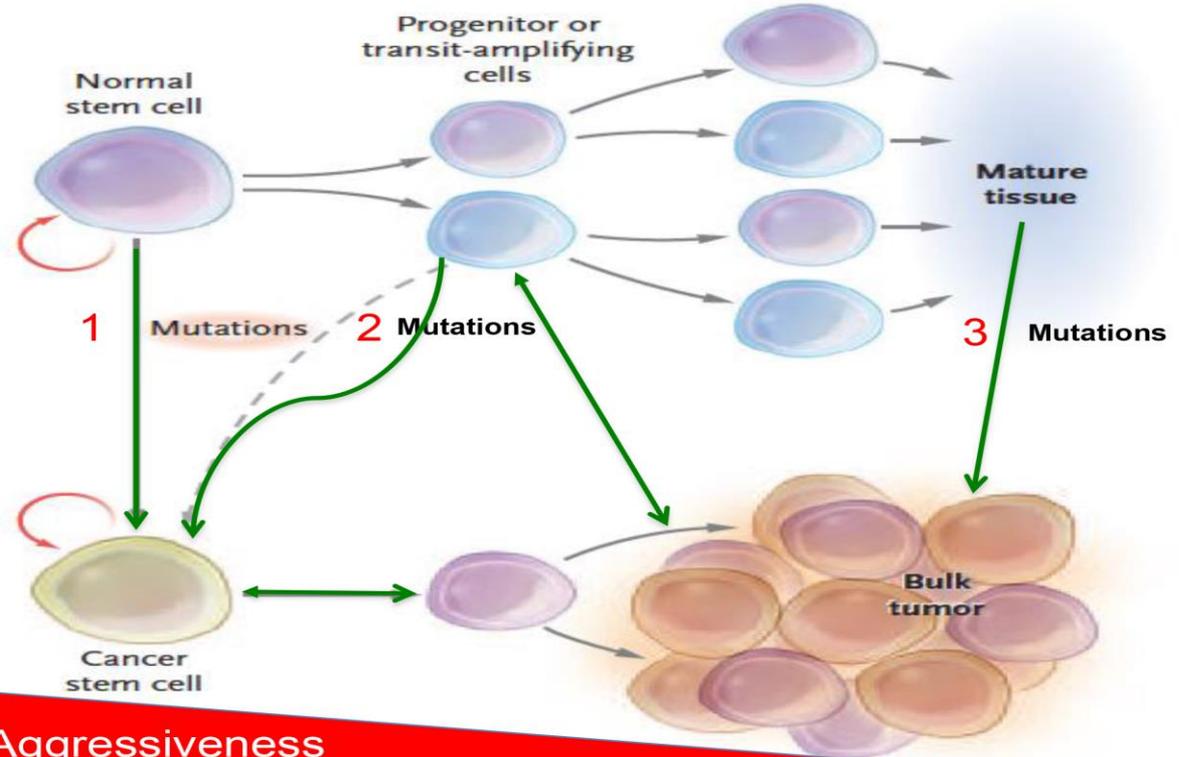
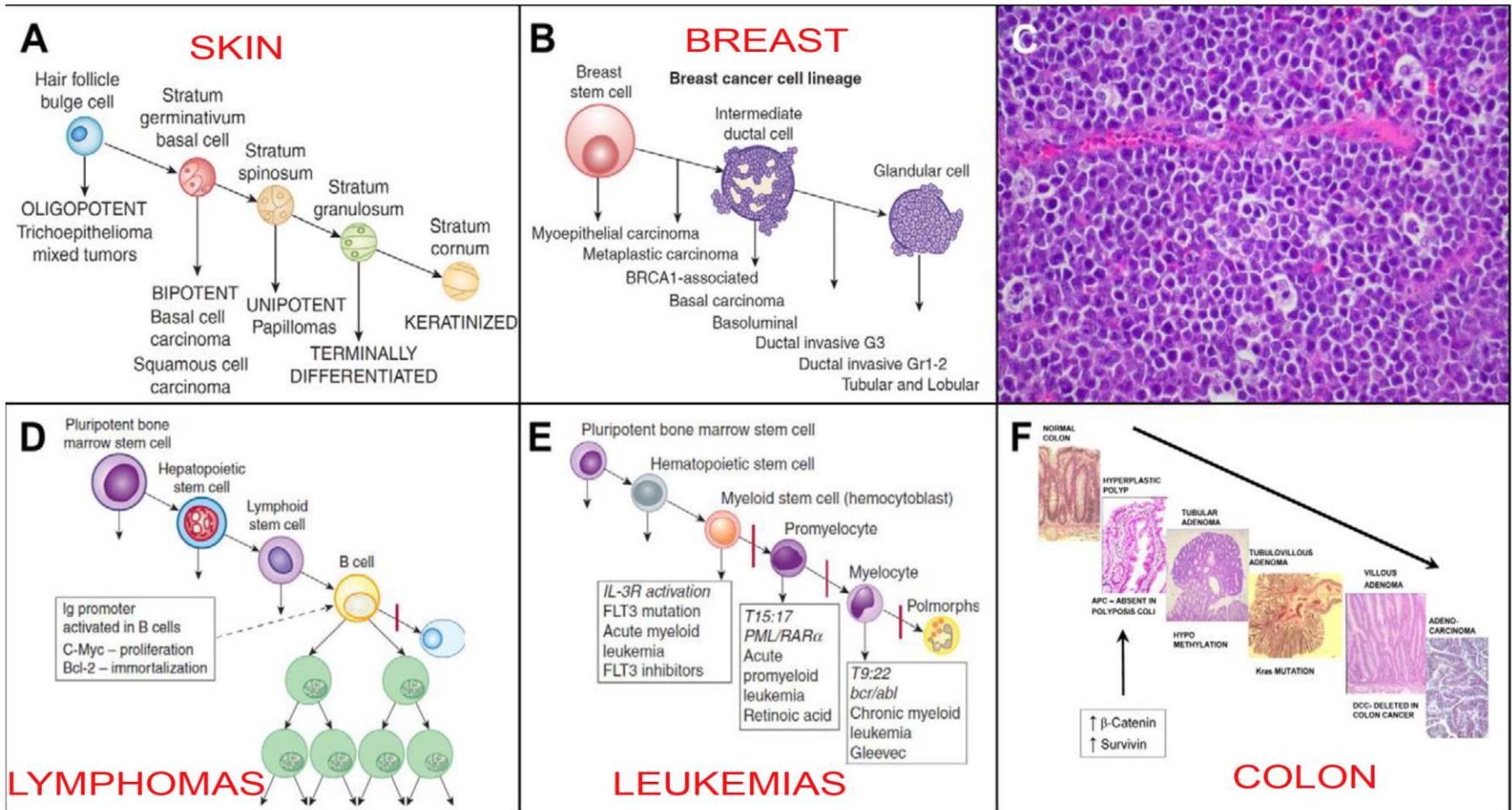


Figure 2. Stem-Cell Systems.

Normal tissues arise from a central stem cell that grows and differentiates to create progenitor and mature cell populations. Key properties of normal stem cells are the ability to self-renew (indicated by curved arrow), multi-lineage potential (indicated by cells of different colors), and extensive proliferative capacity. Cancer stem cells arise by means of a mutation in normal stem cells or progenitor cells, and subsequently grow and differentiate to create primary tumors (the broken arrow indicates that specific types of progenitors involved in the generation of cancer stem cells are unclear). Like normal stem cells, cancer stem cells can self-renew, give rise to heterogeneous populations of daughter cells, and proliferate extensively.

# Hierarchical Lineage

## Hierarchical Lineage Models of Cancer



# ESC Signatures

**Table 2. Studies of embryonic stem cell signatures in cancer NOS OR NOS TARGET GENES**

Study	Gene sets used in the study	Gene set generated by:	Tested cancers
Ben-Porath <i>et al.</i> [23]	ES cell expression profiles, Nanog, Oct4, Sox2 targets, Myc targets, and PRC targets	ES-cell-specific gene expression, and factor occupancy	Breast, glioma, and bladder cancers
Wong <i>et al.</i> [22]	ES-cell-like module, adult stem cell module	Gene module map [81]	Liver, breast, prostate, gastric, and lung cancers
Schoenhals <i>et al.</i> [26]	Nanog, Oct4, Sox2, and Klf4	ES-cell-specific gene activity in multiple cancers	Multiple cancers
Kim <i>et al.</i> [20]	Core module, PRC module, and Myc module	Factor co-occupancy within transcription sub-networks	Myeloid/lymphoid leukemia, bladder and breast cancers
Mizuno <i>et al.</i> [25]	ES cell, iPS cell, PRC2, and p53 ES cell signatures	Gene expression profiles	Breast cancer
Shats <i>et al.</i> [24]	CSR signature	Combined gene sets	Breast and lung cancers, adenocarcinoma, and medulloblastoma

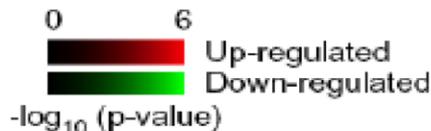
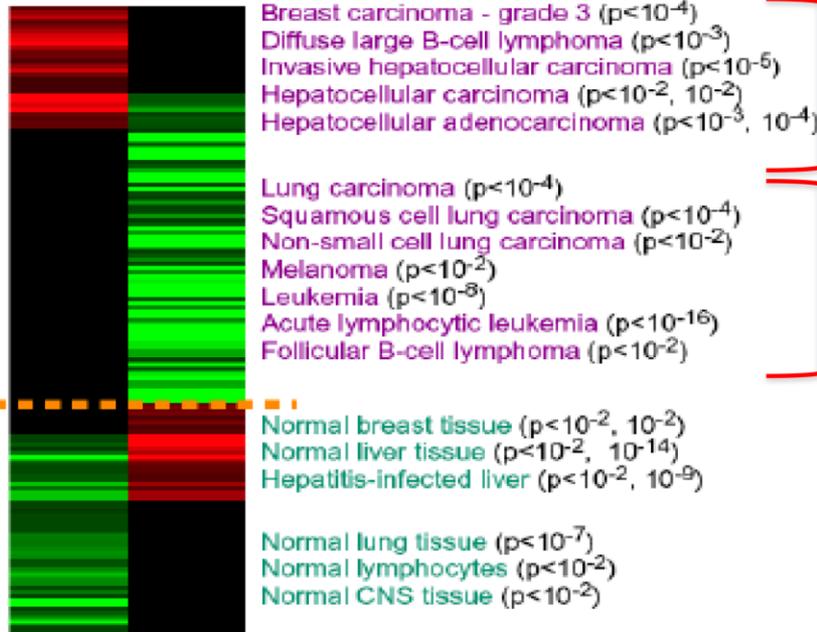
# Cancer Signatures

**Table 1**

Over-expression of Sox2, Oct4, KLF4 and c-MYC in human tumor types to that of their normal tissue counterparts using publicly available gene expression data, including the Oncomine Cancer Microarray database.

**A**

ESC-like module    Tissue stem module



**Table 2**

## Tumor Grade

Association Sox2, Oct4, KIF4 and c-Myc with tumor grade.

Cancer type	Sox2	Oct4	KIF4	c-Myc
Bladder				
Brain	+	+	+	
Breast	+	+	+	+
Cervix	+			
Colon	+			
Endometrium	+			
Head-Neck	+	+		
Lung				+
Lymphoma				+
Melanoma			+	+
Ovarian			+	+
Pancreas				+
Prostate			+	
Renal	+			
Sarcoma	+	+		+
Thyroid	+			

**Table 3**

## Prognosis

Association of Sox2, Oct4, KIF4 and c-Myc with prognosis in cancer.

Cancer type	Sox2	Oct4	KIF4	c-Myc
Bladder				
Brain				+
Breast				+
Colon	+	+		
Head-Neck				
Leukemia				
Liver				
Lung	+	+		+
Lymphoma		+		+
Melanoma	+			
Myeloma	+			+
Ovarian	+			
Prostate				
Renal	+	+		

# Breast cancer

## Breast Cancer: A Heterogeneous Disease (Six Molecular and Clinical Subtypes)



- 250,000 women/year with 39,000 deaths
- Second most frequent type of cancer in women
- Lifetime risk of 1 out of 8 women
- Early menarche (E2 exposure)
- Nulliparity or late parity
- **Obesity (premenopausal with a 70% increase in risk)**
- Family history/5-10% (BRCA1 and BRCA2 mutations)

# Molecular classification

Molecular classification of breast cancers

Undifferentiated  
Metaplastic

Breast Cancer

Differentiated

ER<sup>-</sup>, Claudin-  
3/4/7<sup>low</sup>, vimentin<sup>+</sup>,  
E-cadherin<sup>low</sup>, Zeb1<sup>+</sup>

Claudin Low  
(12-14%)

ER<sup>-</sup>, PR<sup>-</sup>, Her2<sup>-</sup>,  
K5/14<sup>+</sup>, EGFR<sup>+</sup>

Basal Like  
(15-20%)

Her2 enriched  
(10-15%)

Her2<sup>+</sup>,  
ER<sup>-</sup>

Normal Breast  
Like

Adipose tissue  
gene signature<sup>+</sup>

Luminal B  
(~20%)

ER<sup>low</sup>, Her2<sup>low</sup>,  
proliferation<sup>high</sup>

Luminal A  
(~40%)

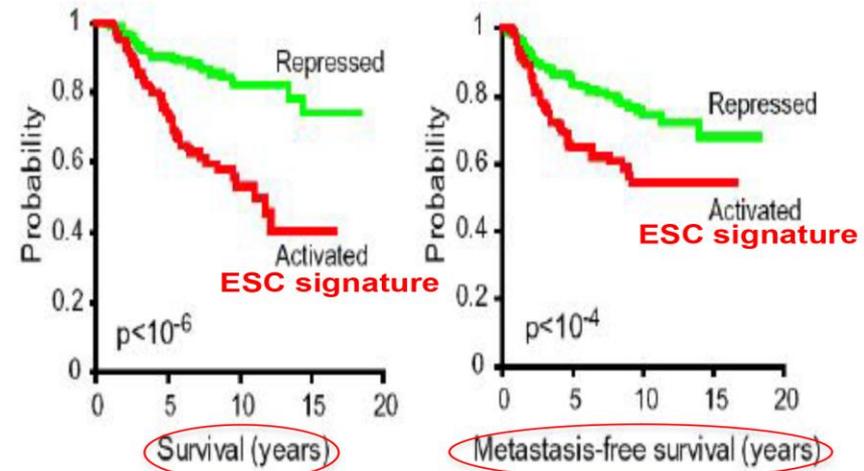
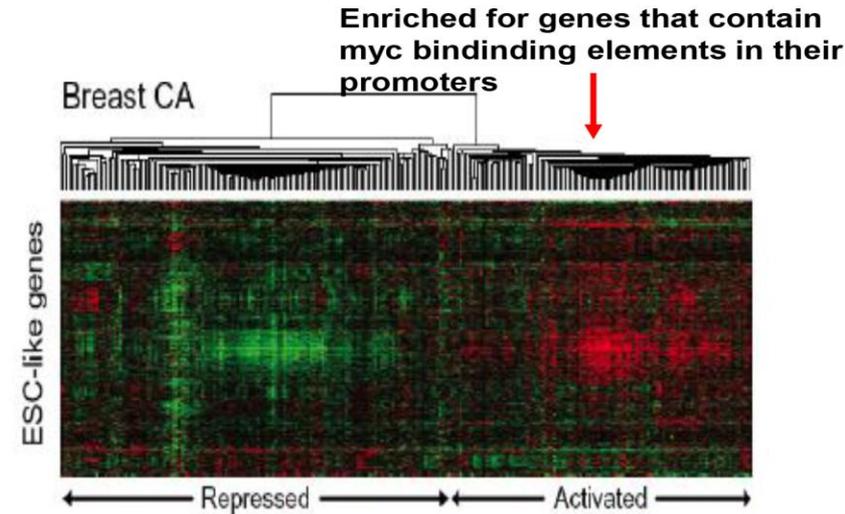
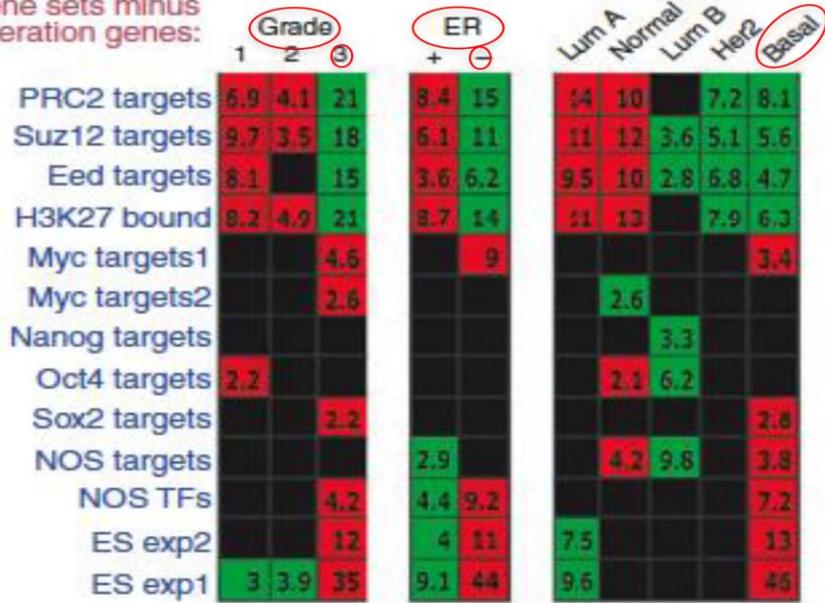
ER<sup>high</sup>,  
Her2<sup>low</sup>

# NOS target genes

Activation of ESC Module/NOS Target Genes in Human Breast Cancers

b

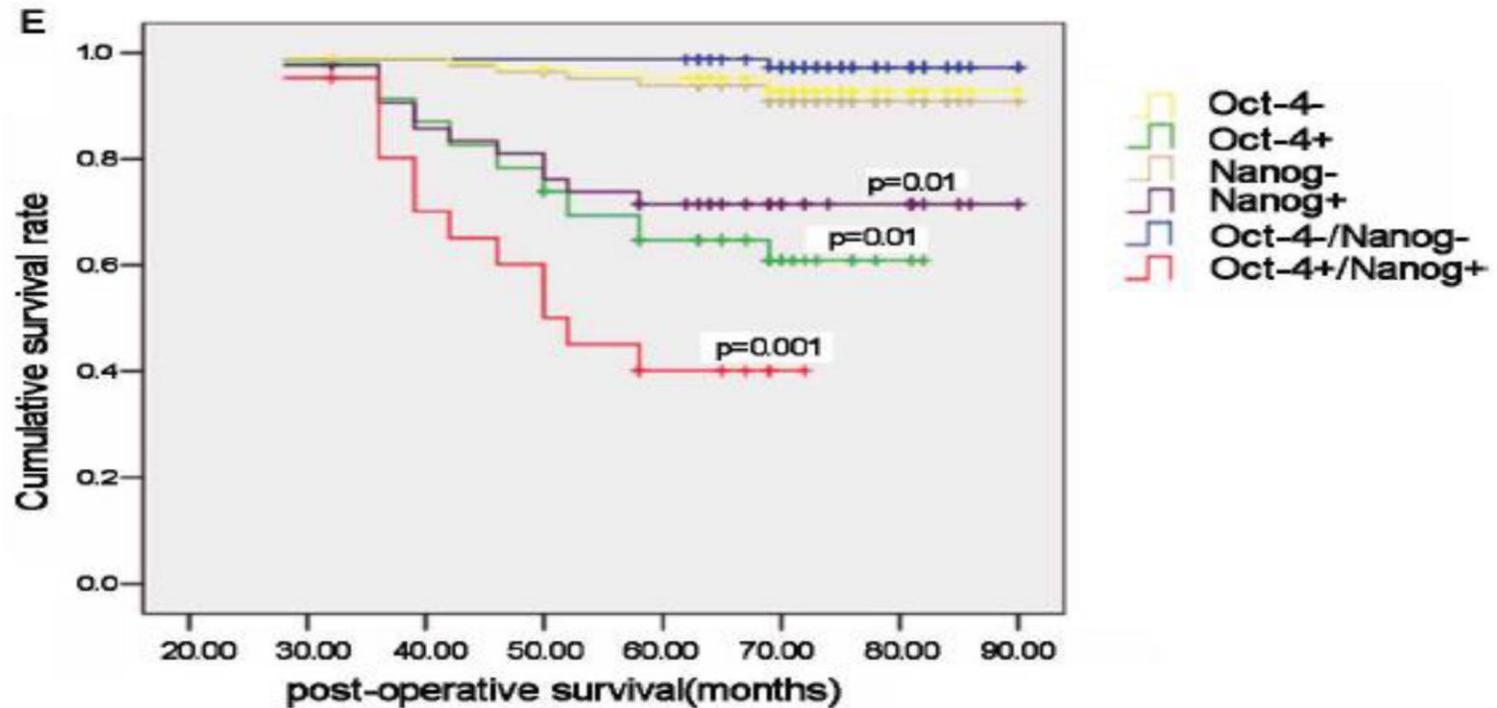
Gene sets minus proliferation genes:



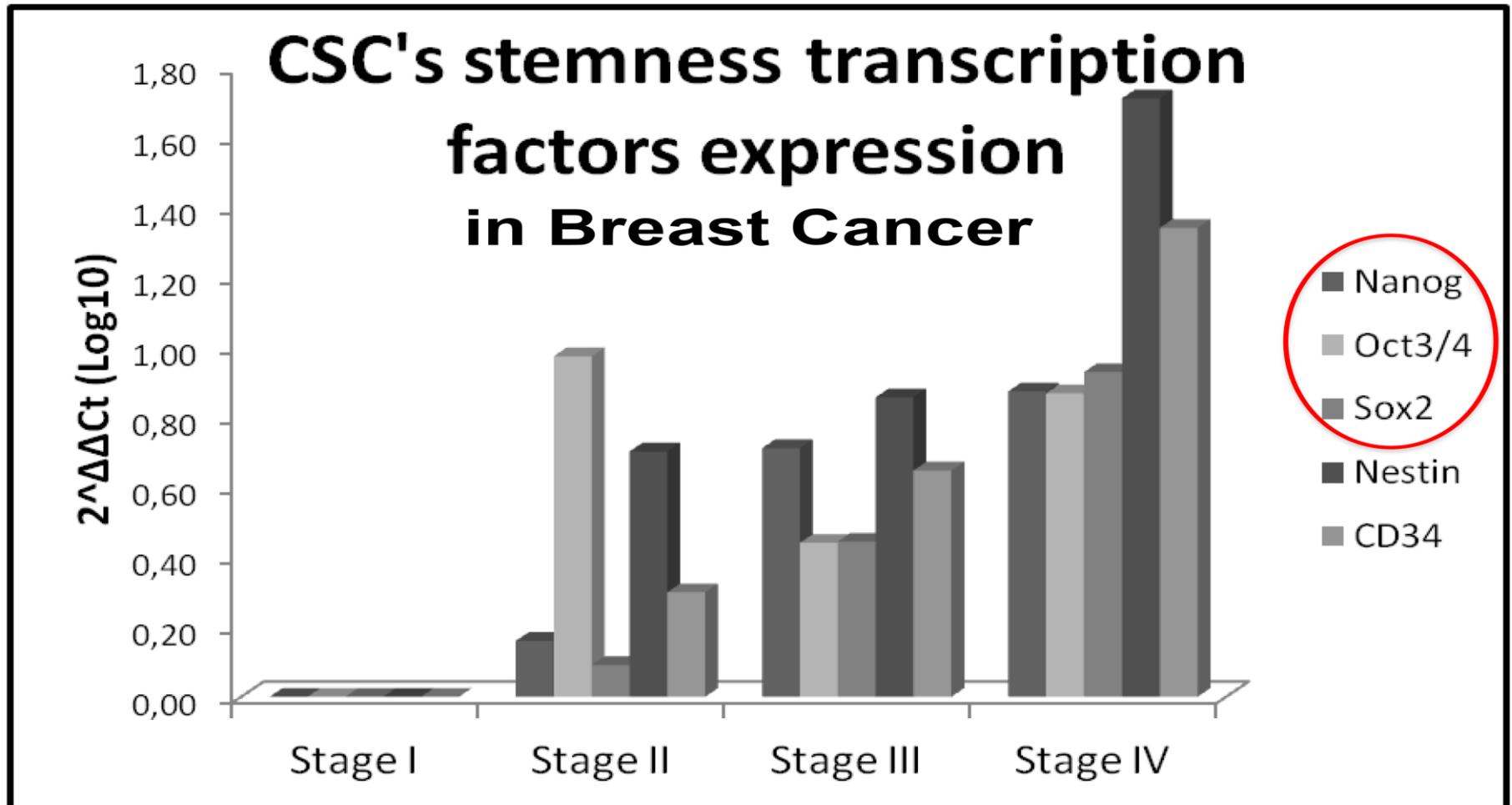
Similar correlations have been observed in primary liver, lung, prostate and gastric carcinomas.

# Gene Expression

## Embryonic Gene Expression in Breast Cancer and Overall Survival



# Transcription factors



# Survival curves

E6.5/Adult/EMT-signatures, Survival Curves, Recurrence-Free Survival in Breast Cancer

Signature →

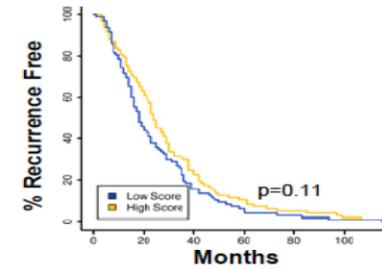
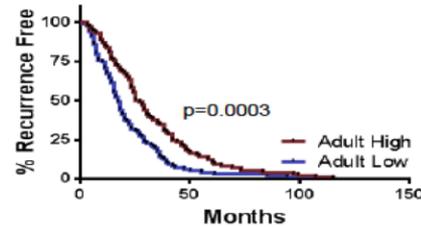
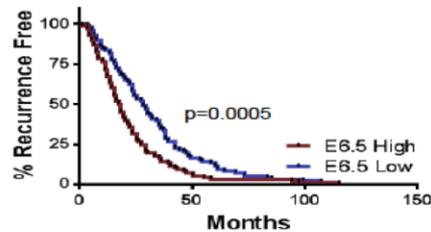
E6.5

Adult (differentiation)

EMT\*\*

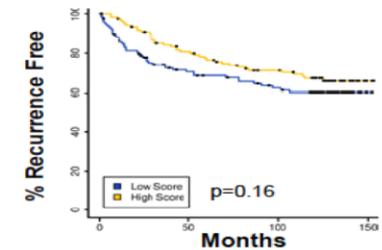
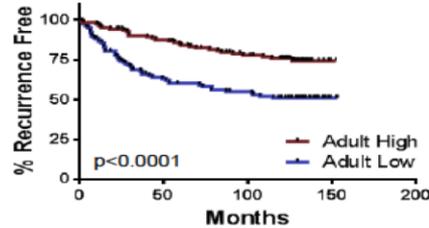
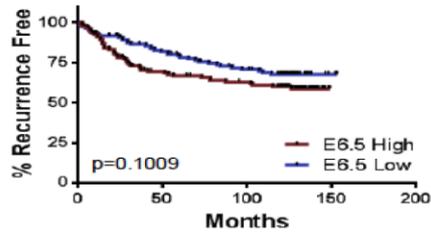
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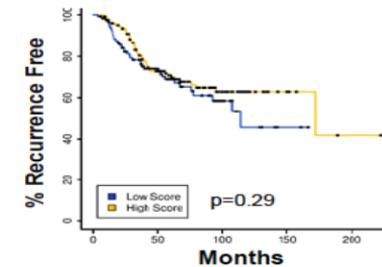
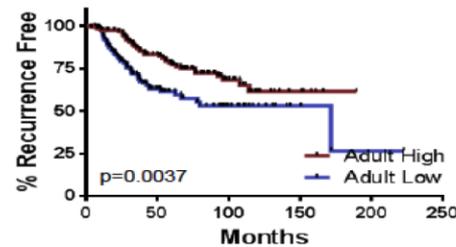
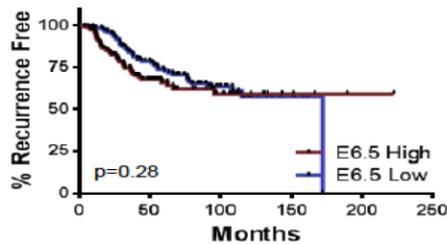
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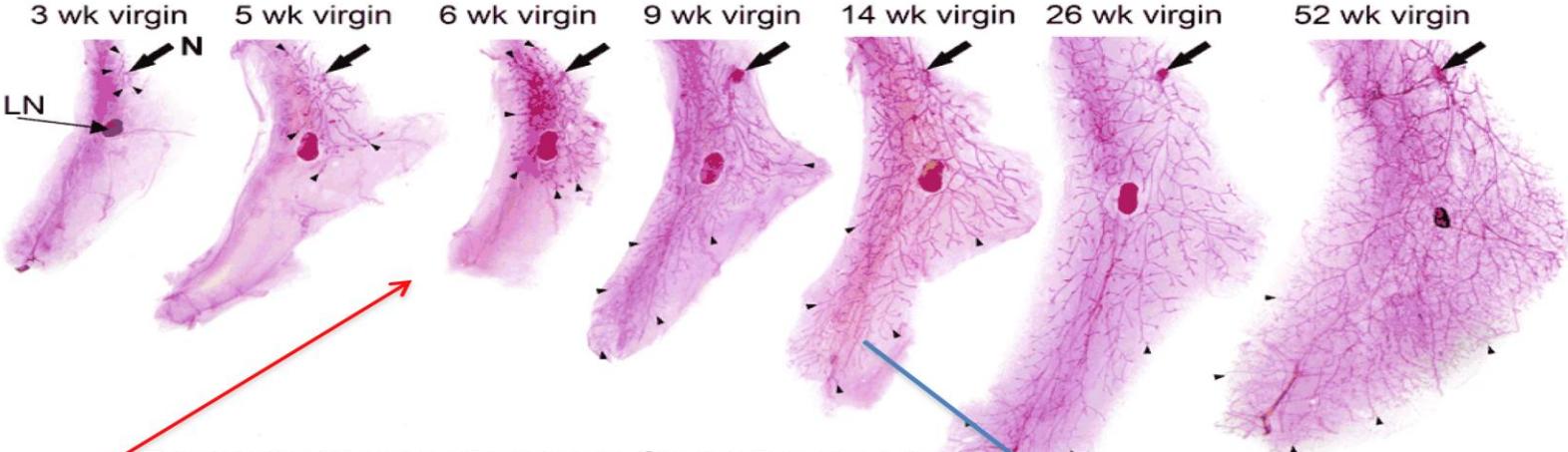


GSE21653

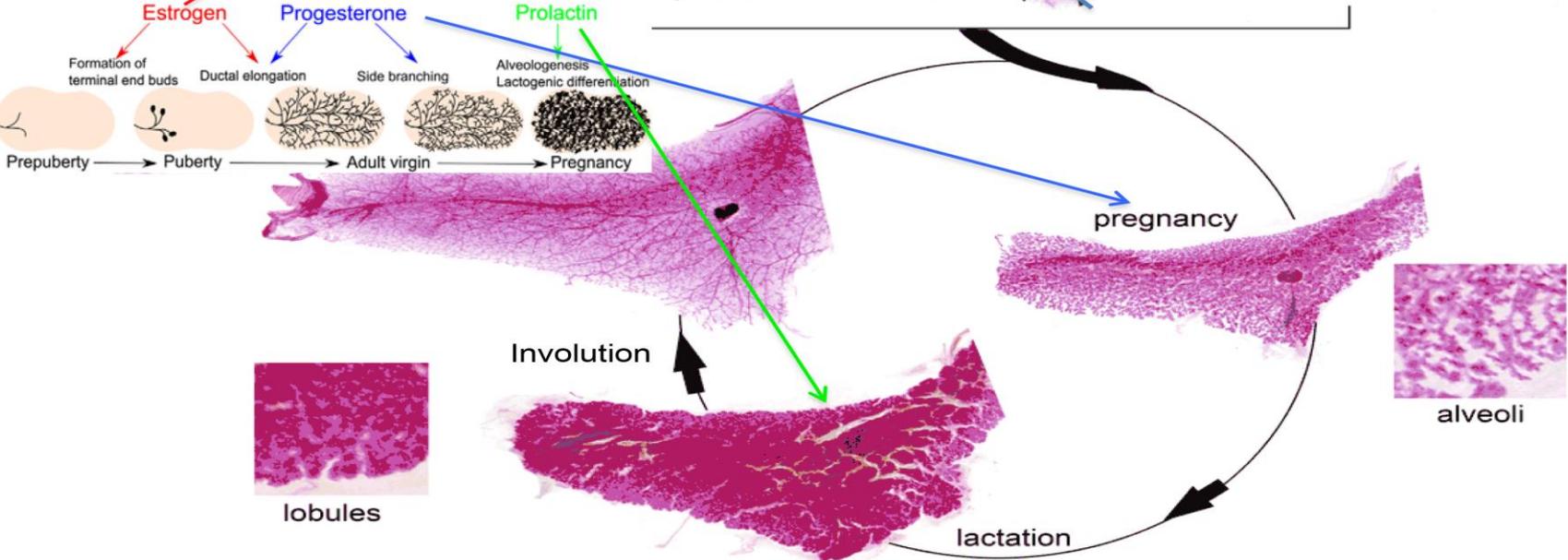
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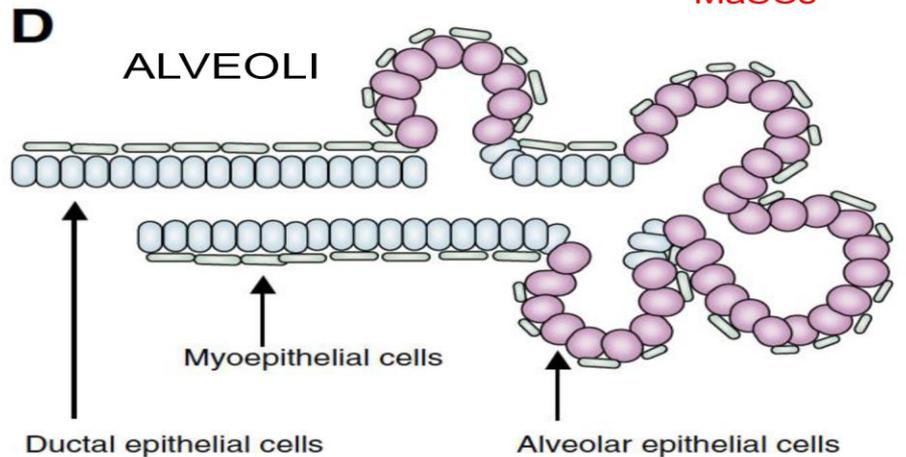
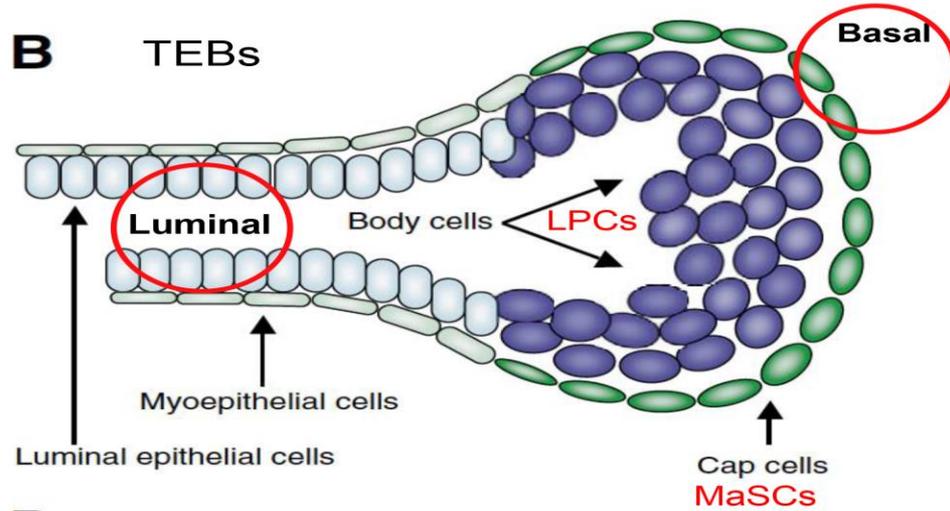
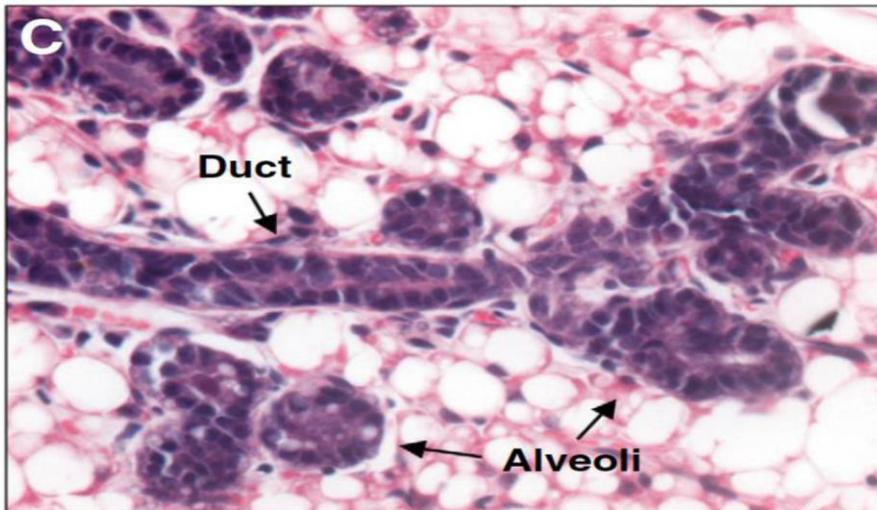
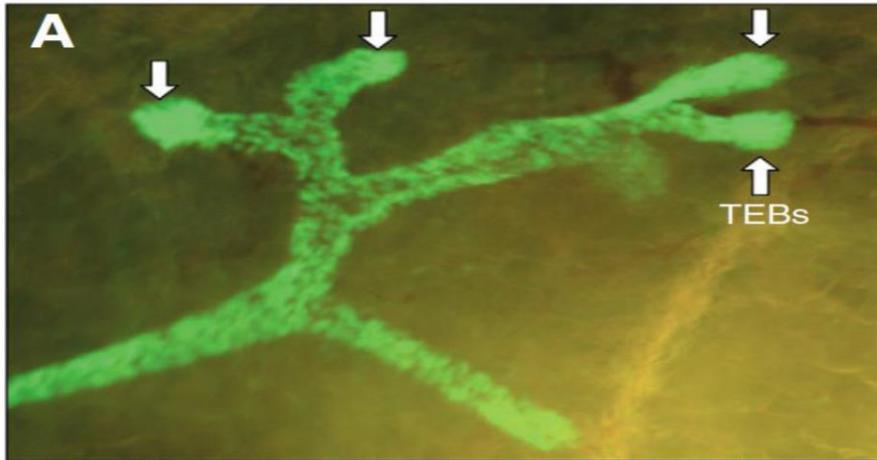
# Mammary Gland Development



## Postnatal Mouse Mammary Gland Development

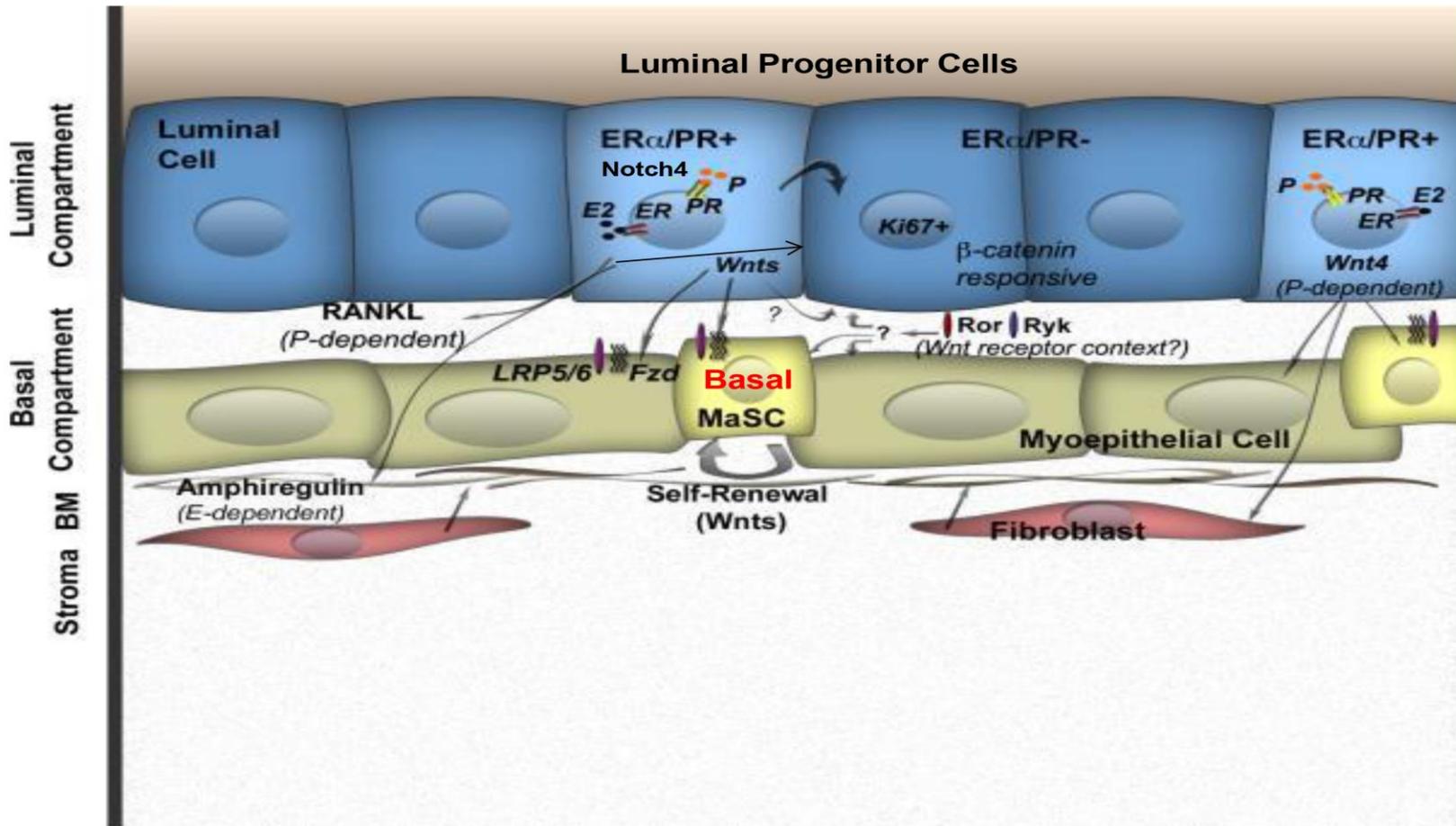


# Breast morphology



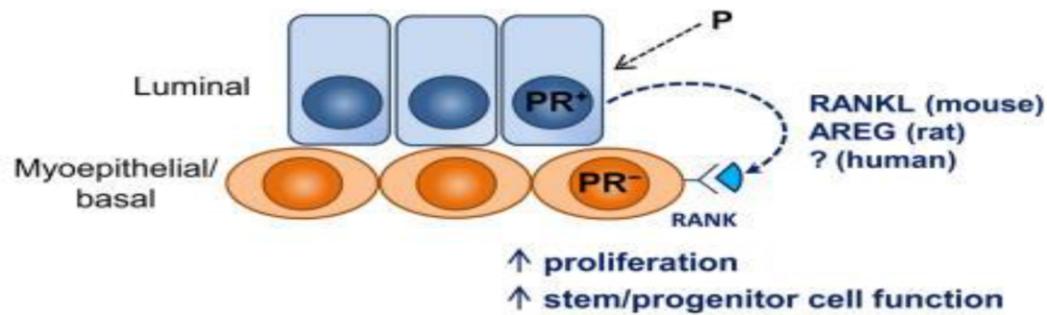
# Mammary Cell Communication

## Paracrine Communication between Different Mammary Cell Types



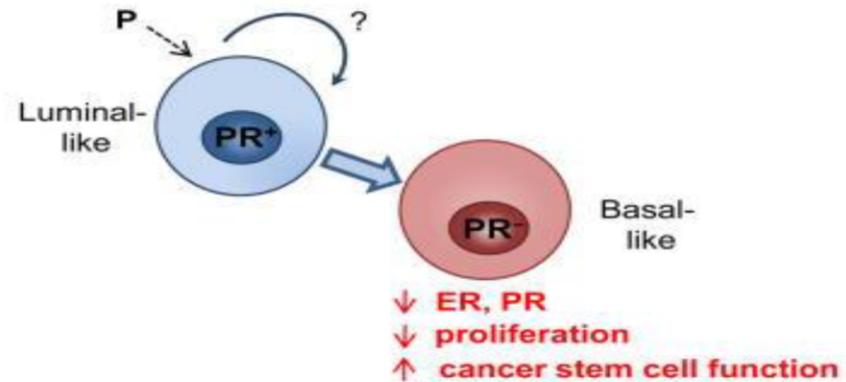
# Signaling

## Paracrine signaling



Normal mammary gland cells

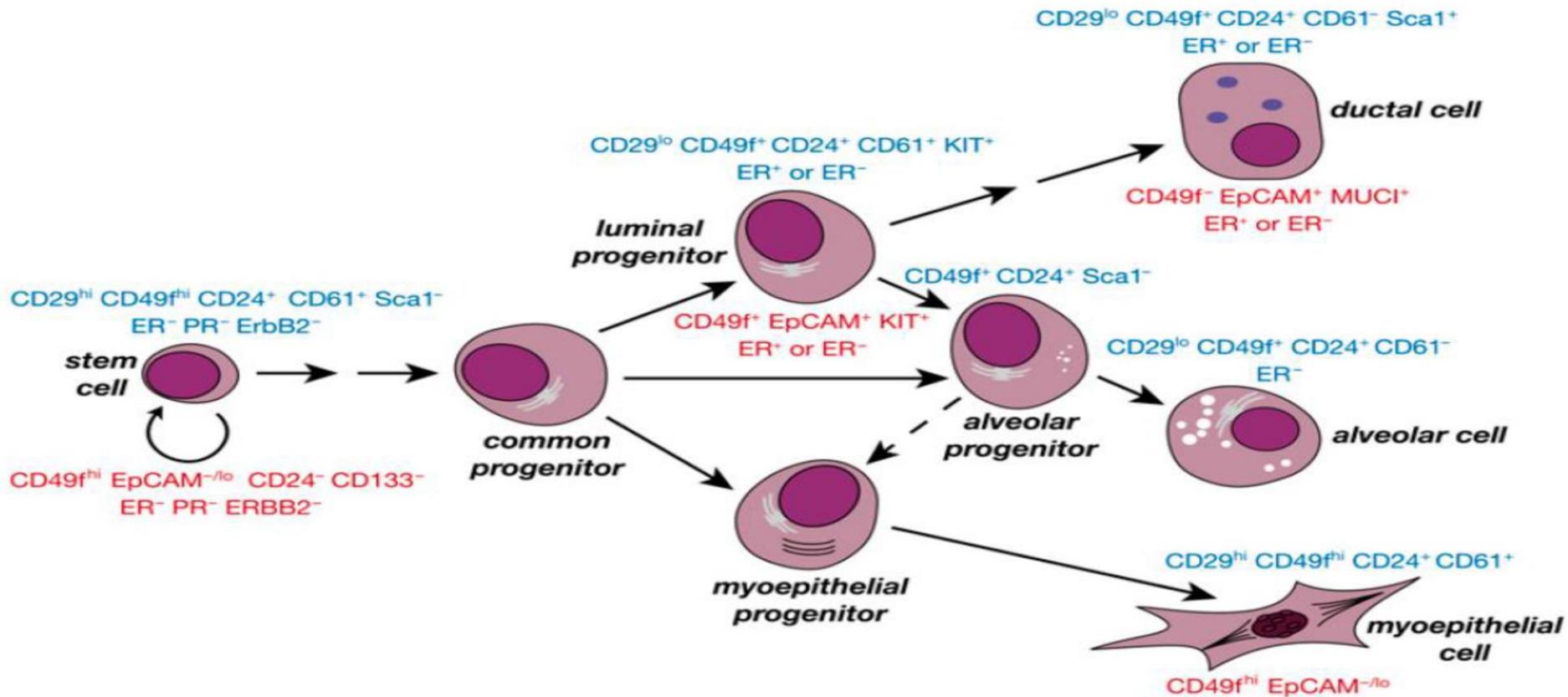
## Autocrine signaling



Human breast cancer cells

# Epithelial cell lineages

## MOUSE MAMMARY EPITHELIAL CELL LINEAGES



# Stem Cell Markers

**Table1.** Cancer stem marker and their distribution

Marker	Tumor type	Marker	Tumor type
CD133	Brain[85] Prostate [86] Pancreas [ 87] Melanoma [88] Colon [88] Liver [89] Lung [19] Ovary [90]	ALDH	Breast [91] Lung [92] Head and neck [25] Colon [93] Liver [17] Pancreas [94] Gastric [95] Prostate [96]
CD44	Colon [97] Breast [8] Prostate [13] Pancreatic [98]	ABCB5	Melanoma [99]
ABCG2	Pancreas [100] Lung [101] Limbal epithelium [102] Brain [103] prostate [104] Liver [105] Ovarian [106] Retinoblastoma [107]	CD90	T-acute lymphoblastic leukemia [108] Gliomas [109] Liver [110]

# Surface Markers

## Commonly Used Surface Markers to Identify Mouse and Human Mammary Stem Cells

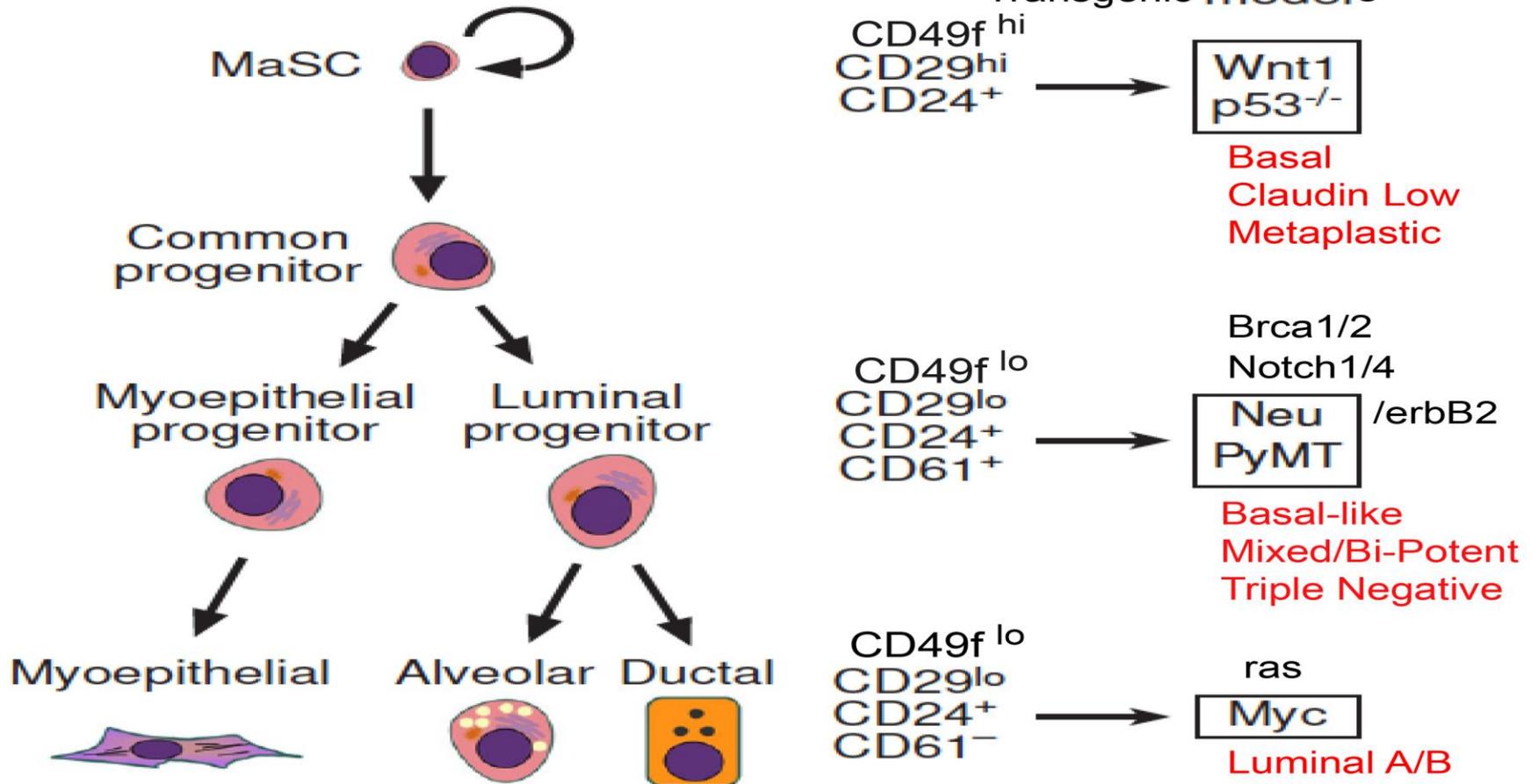
Mammary Gland Stem Cells	Marker <i>Integrins</i> $\beta 1$ $\alpha 6$ $\beta 3$
Mouse	CD24, CD29, CD49f, CD61, Sca-1.
Human	ALDH1, c-KIT, CD10, CD24, CD44, CD49f, CD90, CD133, EpCAM, MUC-1 <i>HA receptor</i>

# Breast cancer subtypes

Mouse Mammary Cell Lineages Related to Breast Cancer Subtypes

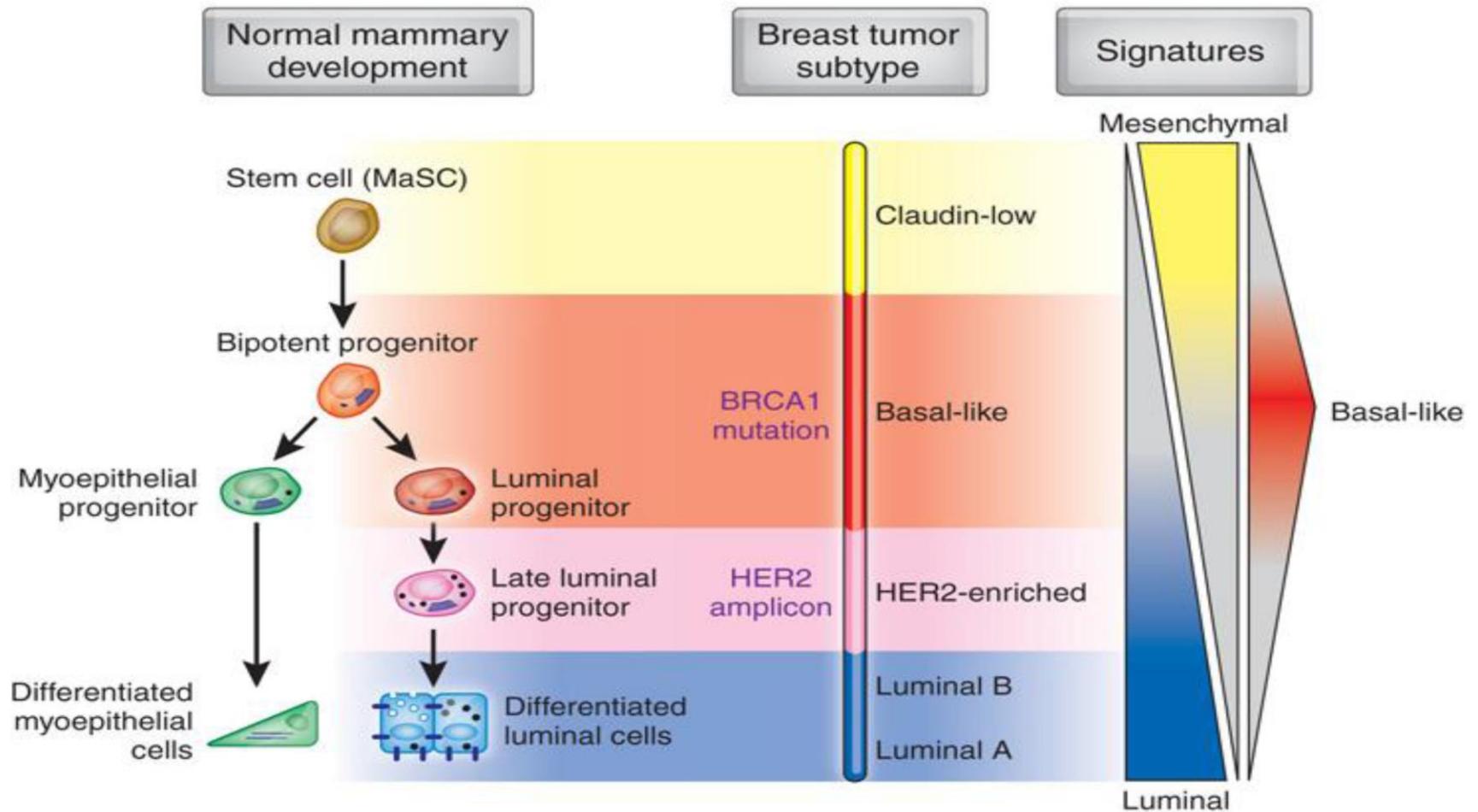
Most similar mouse tumor

Transgenic models



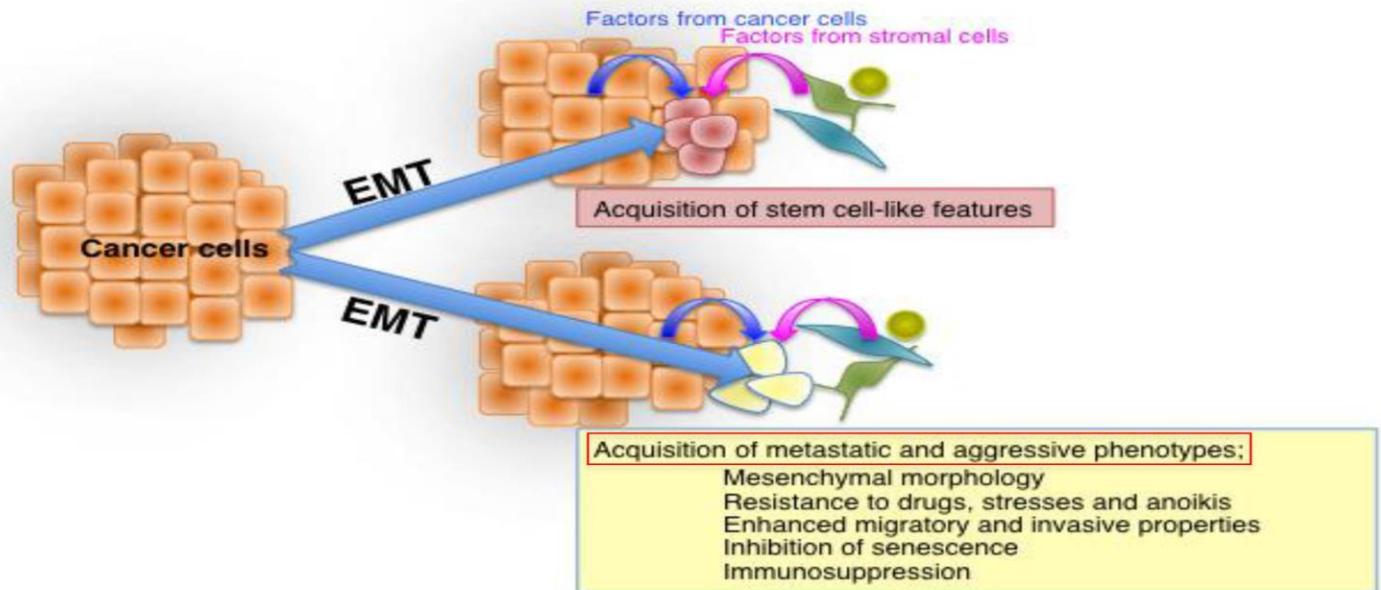
# Cell lineages

HUMAN MAMMARY EPITHELIAL CELL LINEAGES RELATED TO BREAST CANCER SUBTYPES

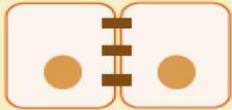


# Origins of CSC

## Mechanistic Origins of Cancer Stem Cells



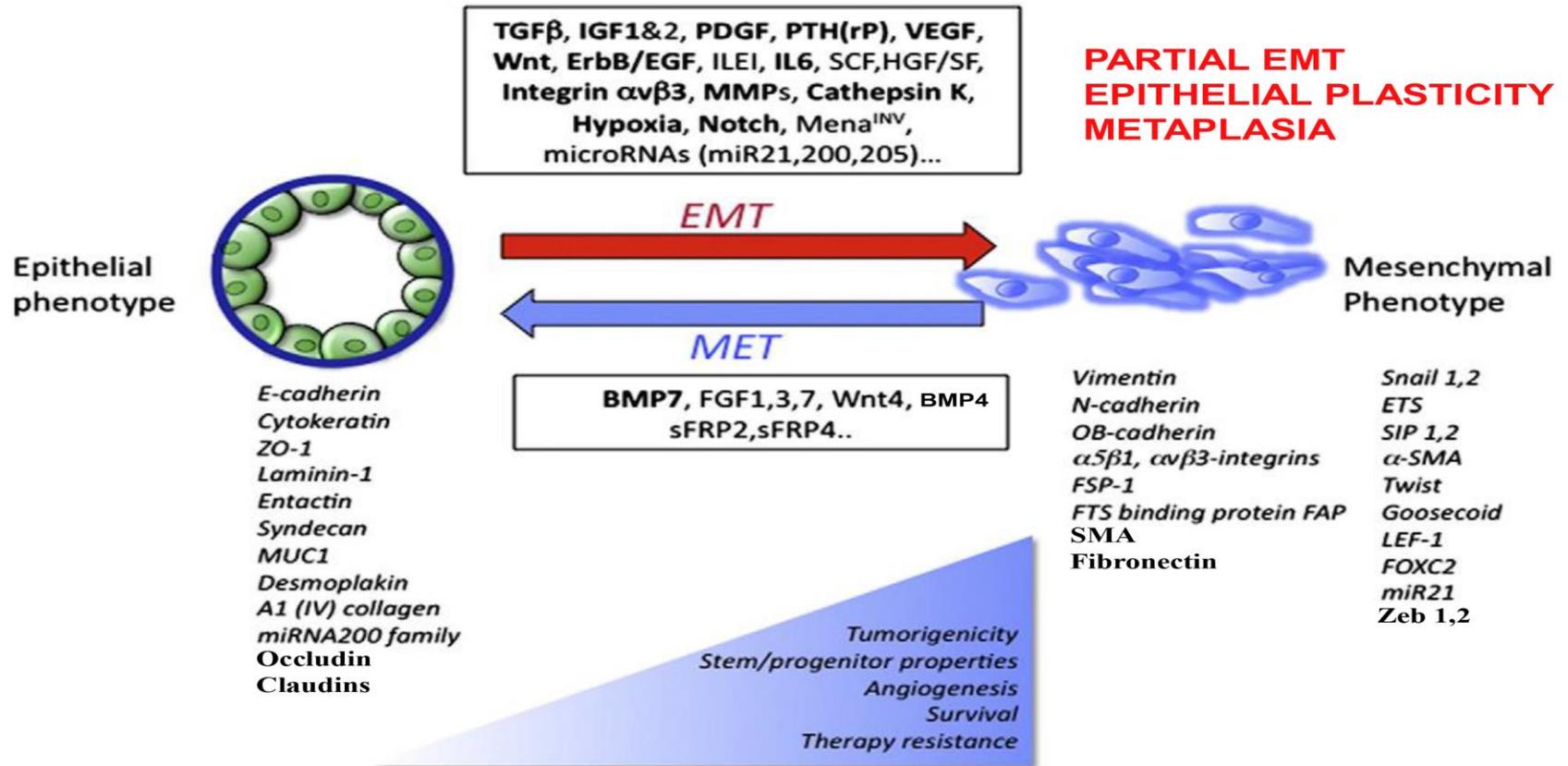
# EMT transition

	Epithelial-type	EMT MET	Mesenchymal-type
<b>Molecular signature</b>	<ul style="list-style-type: none"> <li>• E-cadherin</li> <li>• MUC1</li> <li>• Cytokeratin</li> <li>• Laminin-1</li> <li>• Desmoplakin</li> <li>• Zona-occludens 1 (ZO-1)</li> <li>• Syndecan</li> </ul>		<ul style="list-style-type: none"> <li>• Vimentin</li> <li>• Fibronectin</li> <li>• N-cadherin</li> <li>• FSP-1 (S100A4)</li> <li>• Alpha smooth muscle actin (<math>\alpha</math>SMA)</li> <li>• Twist</li> <li>• Snail</li> <li>• Slug</li> <li>• FOXC2</li> <li>• ZEB2</li> </ul>
<b>Phenotypic traits</b>	<ul style="list-style-type: none"> <li>• Apical-basal polarity</li> <li>• Polarity complexes</li> <li>• Adherens junctions</li> <li>• Cohort migration</li> </ul>		<ul style="list-style-type: none"> <li>• Front-back polarity</li> <li>• Spindle-like morphology</li> <li>• Protease-dependent invasion</li> <li>• Therapy resistance</li> <li>• Stem cell traits</li> <li>• Anoikis resistance</li> <li>• Inhibition of senescence</li> </ul>
<b>General morphology</b>			

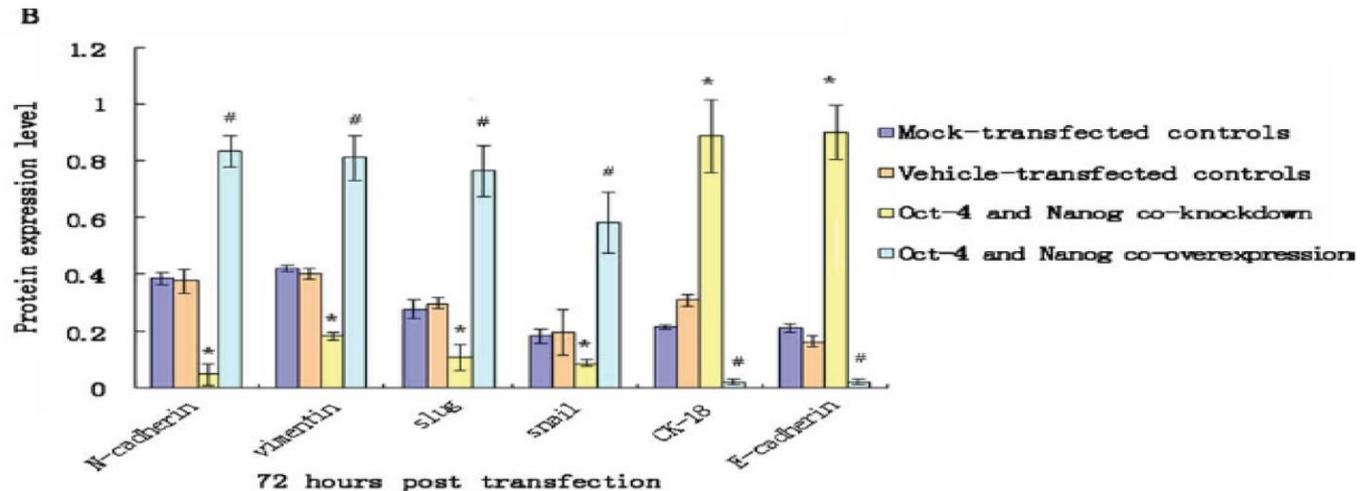
Gastrulation, Neural Crest Cell formation, Cardiogenesis, Wound Healing, Fibrosis and Cancer

# EMT Factors

Markers and Factors Associated with or Facilitating EMT and MET



# EMT and CSC.



**Figure 3: Western blot analyses of relative expression levels of epithelial-mesenchymal transition (EMT)-related genes in cancer stem cells (CSC) following modulating Oct-4 and/or Nanog expression *in vitro*.** CSC were transfected with mock or Oct-4 and Nanog siRNAs, vehicle, or Oct-4 and Nanog-expressing plasmids for 72 h. The relative expression levels of EMT-related genes in the different groups of cells were characterized at the indicated time points post-stimulation by western blot assays. Data shown are representative images (A) or are expressed as the means  $\pm$  standard deviation of the relative levels of each protein to the control GAPDH at 72 h post-transfection (B) from 3 separate experiments. A similar pattern of the relative levels of targeting proteins to the control GAPDH were detected in the different groups of CSC at 24 h post-stimulation (data not shown). A: The mock-transfected CSC; B: The vehicle-transfected CSC; C: Oct-4- and Nanog-silenced CSC; D: Oct-4- and Nanog-overexpressing CSC. \* $p < 0.05$  vs. mock-transfected CSC; # $p < 0.05$  vs. vehicle-transfected CSC.

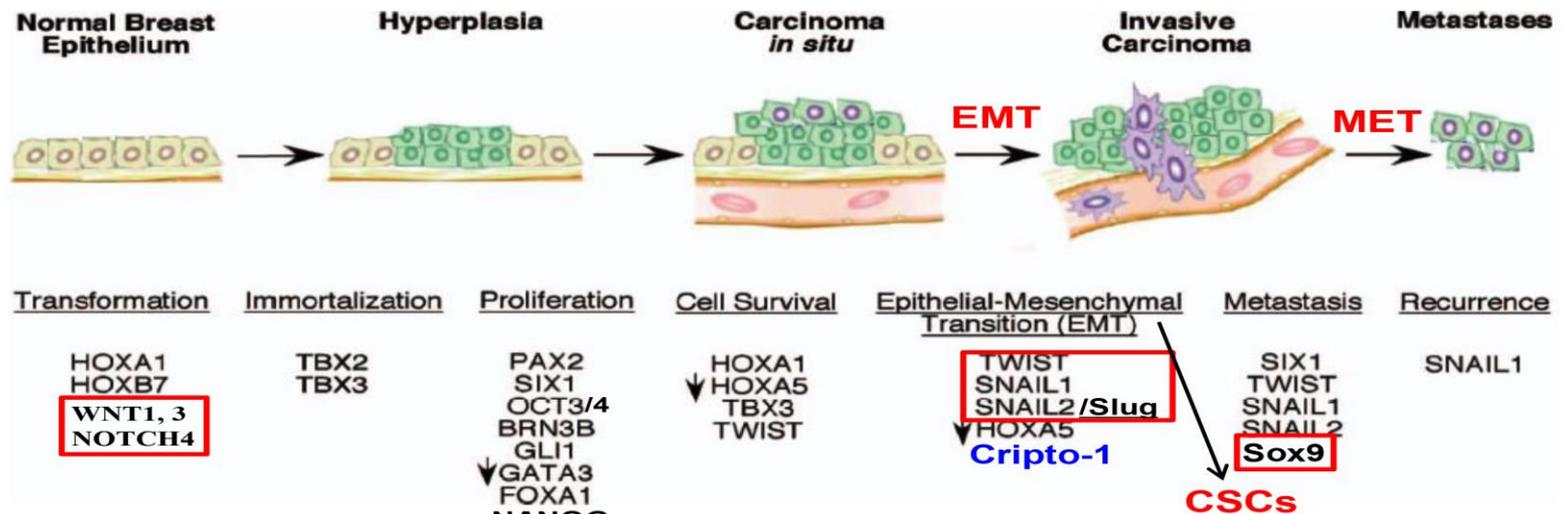
**Table 1**

Listing of cell lines and experimental conditions for which EMT commitment was found to be associated with a gain in stem-cell like properties.

Cell line	Genetic alterations	EMT-inducer
HMEC-hTERT	SV40 T/t	SNAIL1, TWIST1
HMEC-hTERT	SV40 T/t, H-RAS <sup>G12V</sup>	-
MCF-10A, MCF-7	-	TWIST1
MCF-10A, MCF-7	-	TWIST2
MCF-10A, MMC	-	TGF- $\beta$ , TNF- $\alpha$
FaDu, OECM-1	-	HIF1 $\alpha$ , TWIST1, BMI1
Pten <sup>-/-</sup> hepatic tumor derived cell line	-	TGF $\beta$ , SNAIL1

# Embryonic genes

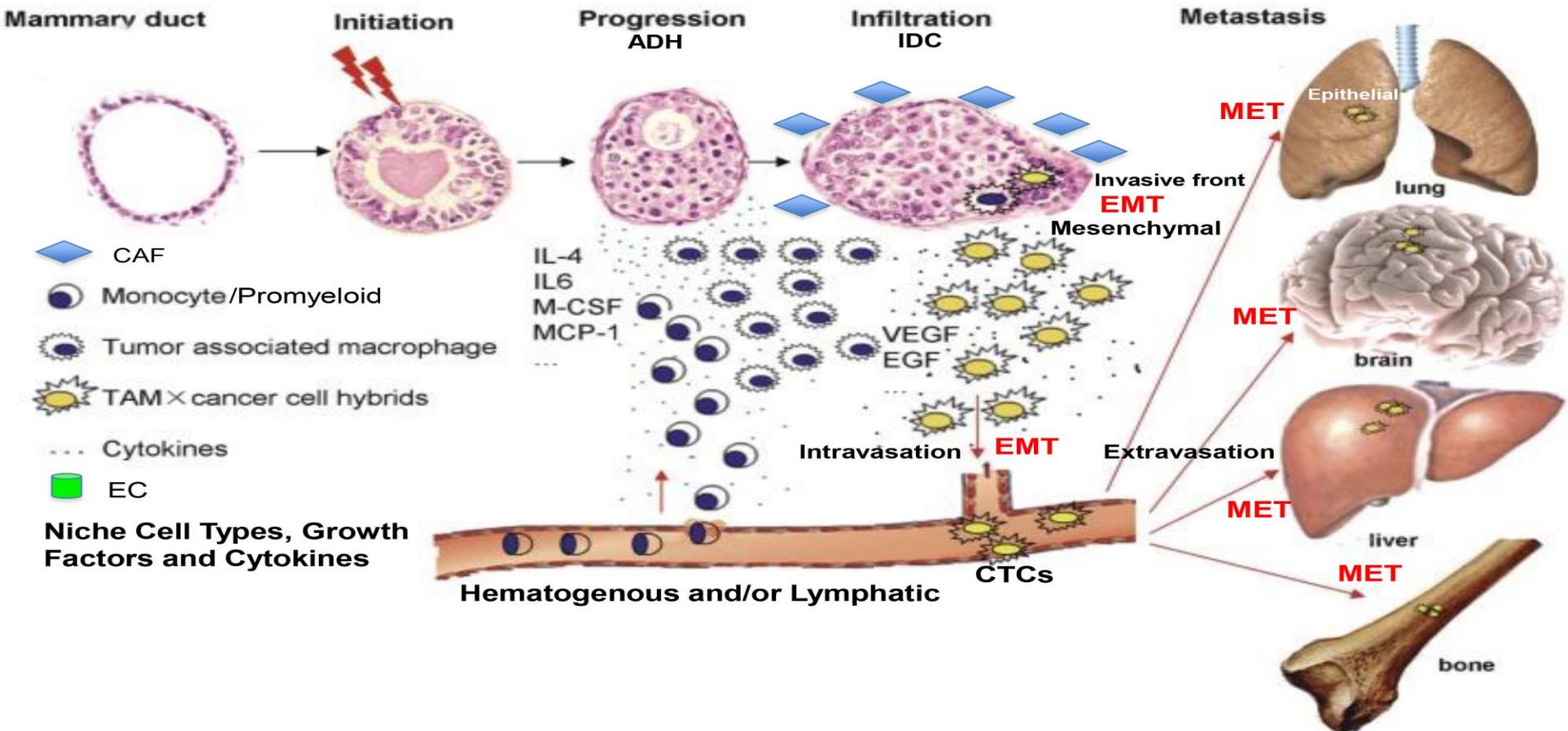
## Embryonic Genes Associated with Breast Cancer Progression



**Figure 1.** Schematic diagram of the different stages of breast carcinogenesis. Breast carcinomas are derived either from mammary epithelial cells that line the milk ducts (invasive ductal carcinomas = IDC) or from mammary epithelial cells that form the milk-secreting end buds of the mammary glands (invasive lobular carcinomas = ILC). Carcinogenesis begins with the hyperplastic growth of normal mammary epithelial cells and develops from benign to abnormal hyperplastic stages into pre-cancerous non-invasive breast lesions (carcinoma *in situ*). Tumors become malignant when they progress into invasive stages (invasive carcinoma). Tumor progression is accompanied by increased cell survival, neo-vascularization and epithelial-mesenchymal transition (EMT) of primary mammary epithelial tumor cells leading to blood stream intravasation and disseminate of cancer cells to other tissues. Embryonic transcription factors implicated in human breast cancer and the neoplastic changes they control during breast carcinogenesis are shown.

# Mammary carcinoma progression

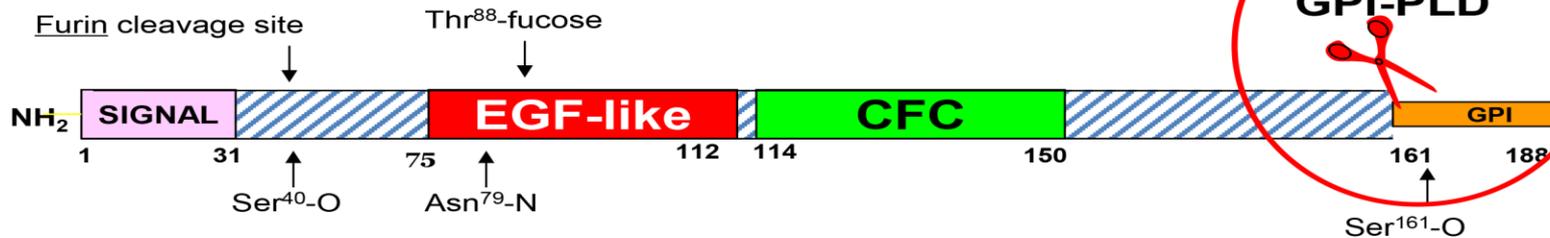
## MAMMARY EPITHELIAL CELL CARCINOMA PROGRESSION



# CRIPTO-1 Family

## CRIPTO-1/TDGF-1/CFC-2 Family

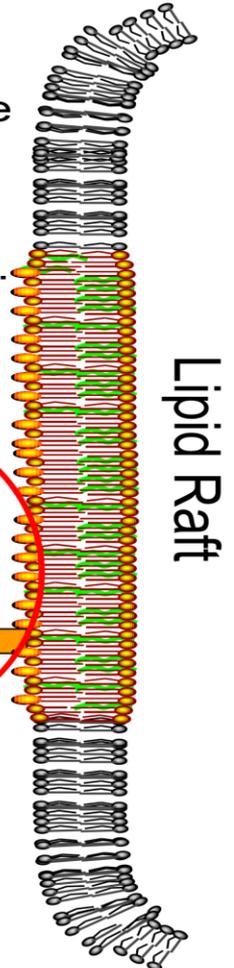
- CRIPTO-1 is a cell surface **GPI-linked glycoprotein** that can function either *in cis* (autocrine) or *in trans* (paracrine) either membrane tethered in microvesicles such as exosomes or as a soluble isoform.
- CRIPTO-1 can be cleaved from mammalian cells by endogenous GPI-PLD.
- Multifunctional adapter or chaperone protein that binds to several different signaling proteins (Nodal, Grp78, Lrp5/6 and Notch).



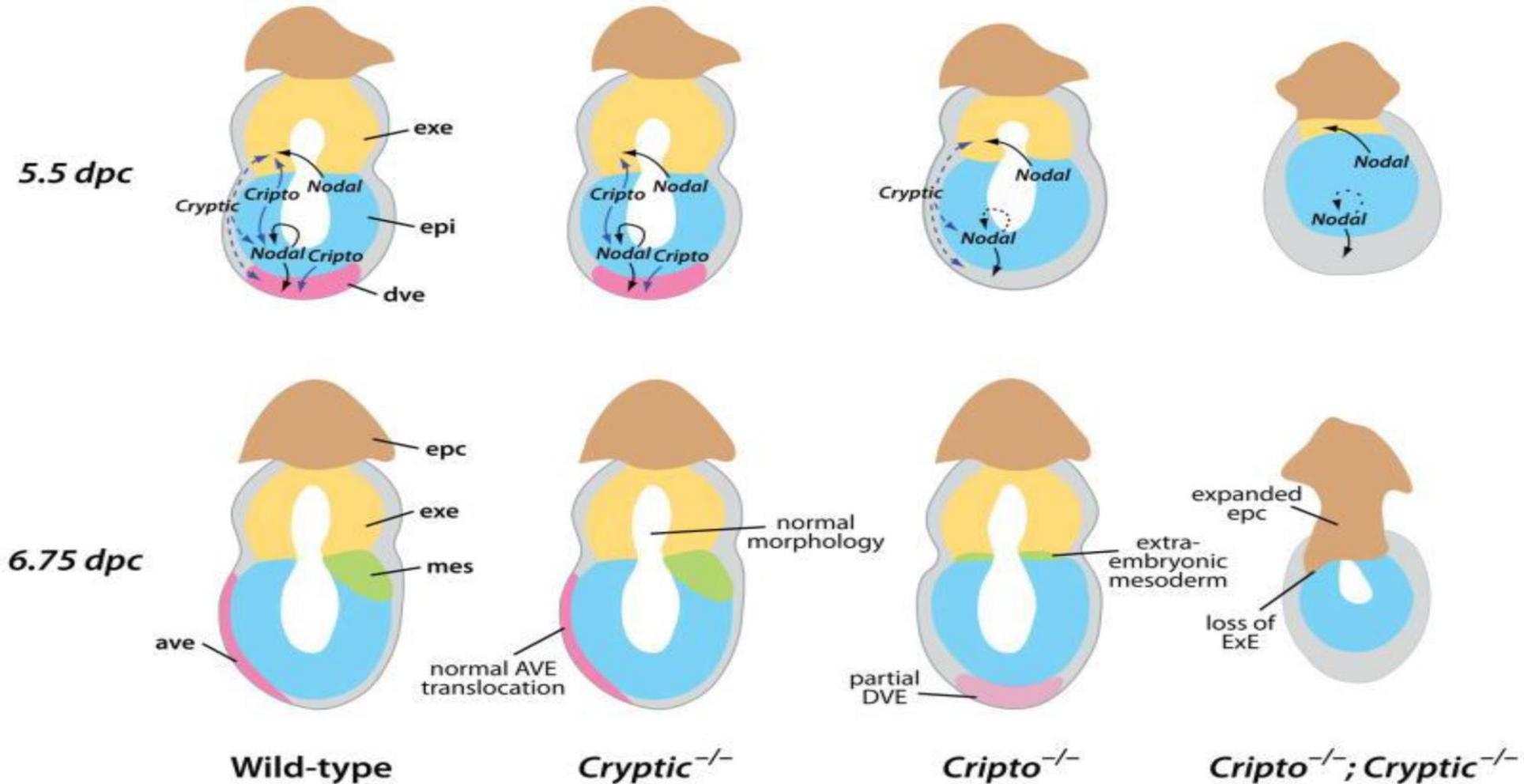
## EGF-CFC Family

Apical Surface

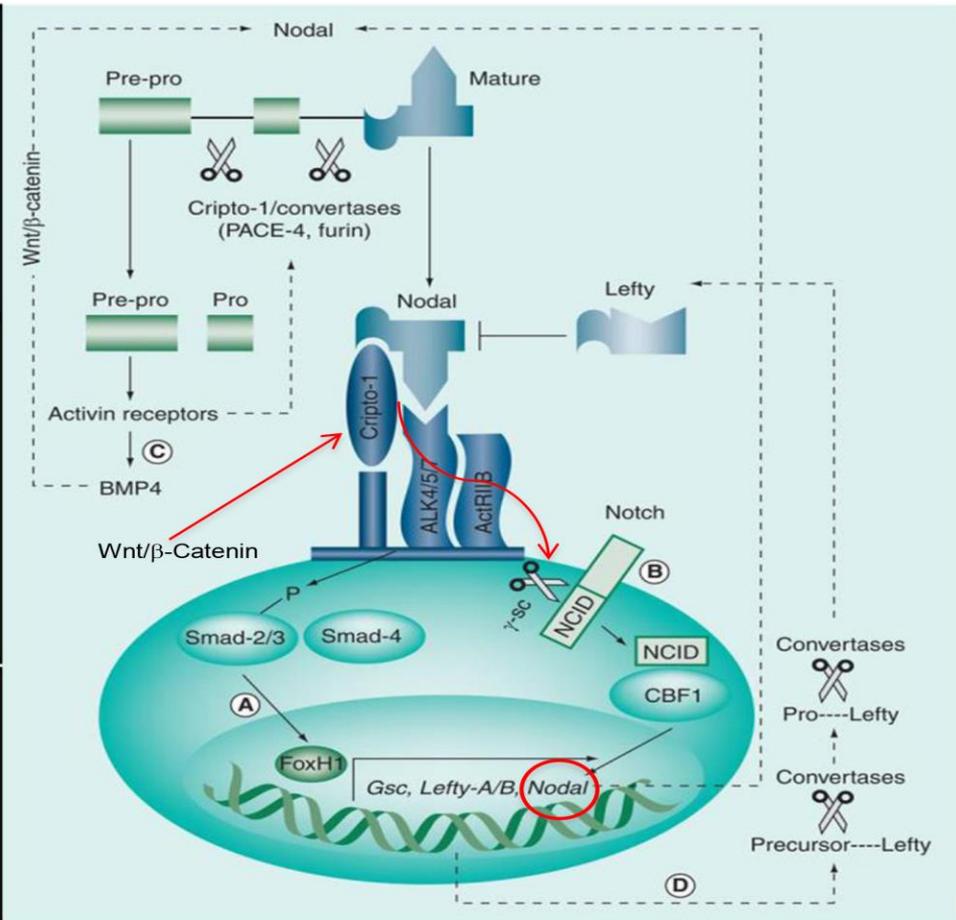
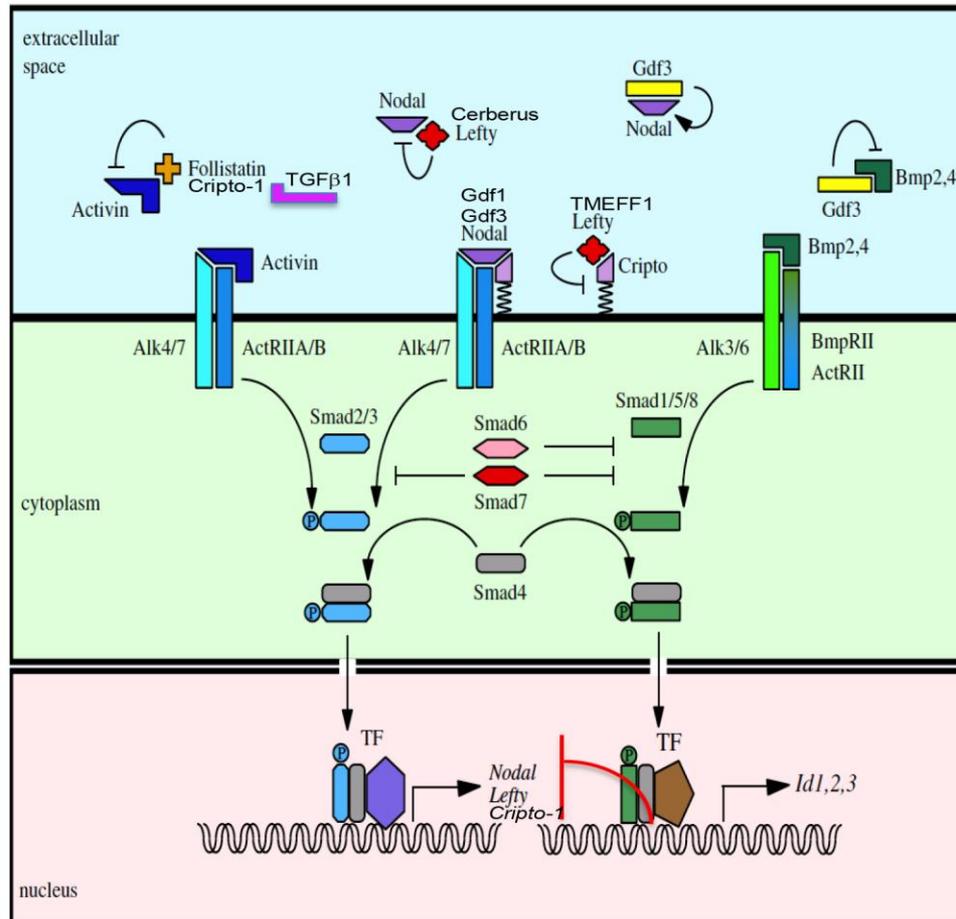
Lipid Raft



# Morphology



# Cellular signaling



# CRIPTO-1 Expression

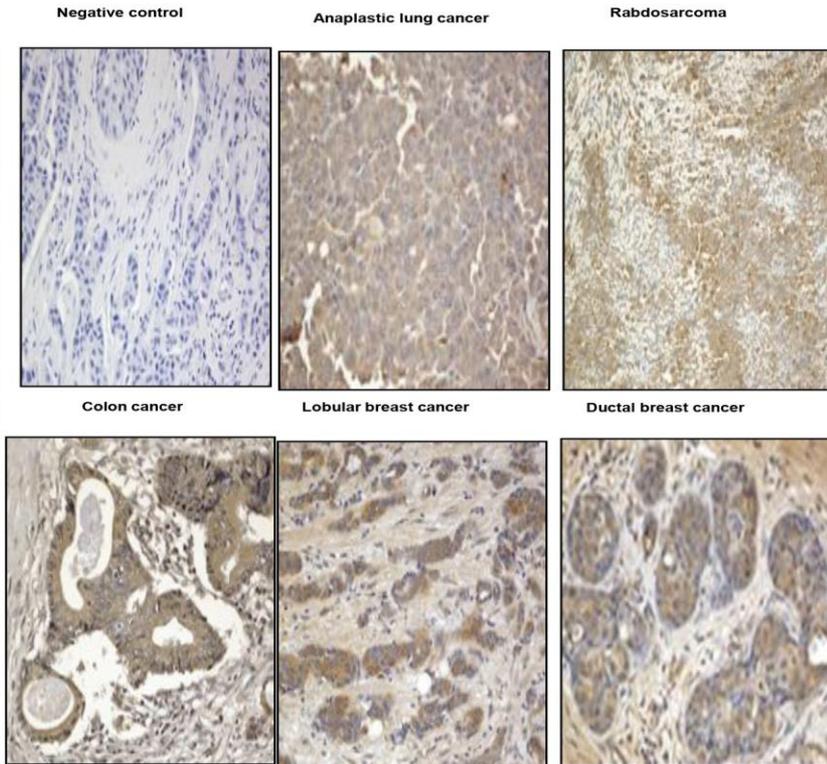
## EXPRESSION OF CRIPTO-1 IN HUMAN TUMORS

Table 2. Cripto-1 expression in human tumors.

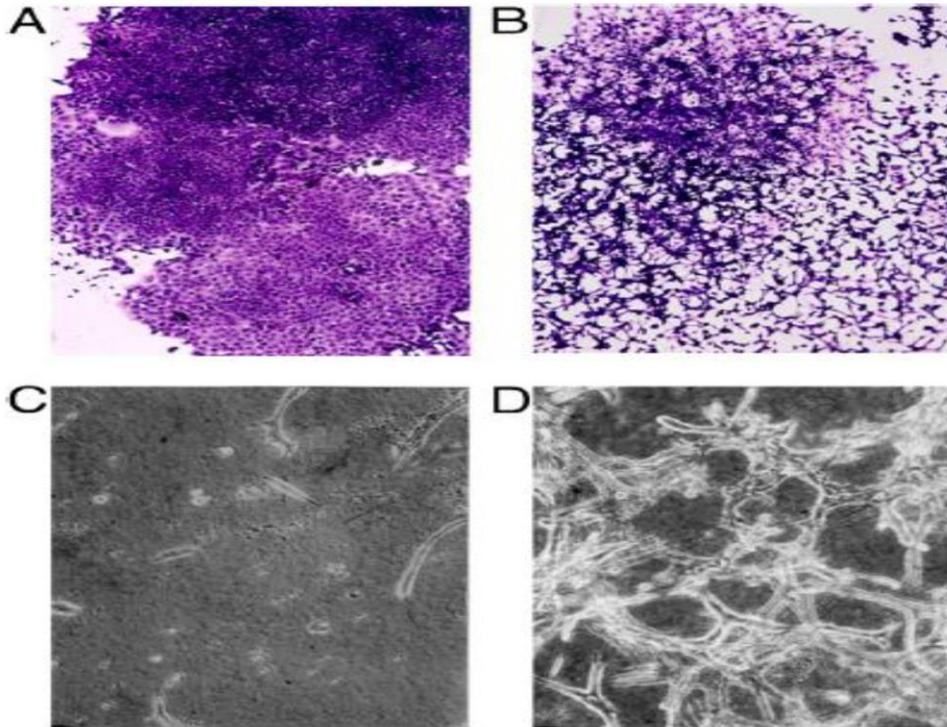
Tumor tissues	Cripto-1 expression in tumors (%)	Methods of detection	Ref.
Basal cell carcinoma	80	Semiquantitative RT-PCR	[97]
Bladder	60	IHC	[102]
Breast	47–82 <sup>†</sup>	IHC and RT-PCR	[85–87,91]
Cervix	26–53	IHC and semiquantitative RT-PCR	[82,95]
Colon	30–84 <sup>†</sup>	IHC and northern blot and qRT-PCR	[72–76]
Endometrium	50–71 <sup>†</sup>	IHC and semiquantitative RT-PCR	[82,83]
Gall bladder	68 <sup>†</sup>	IHC	[81]
Lung (non-small-cell)	91	IHC	[103]
Nasopharynx	54–76	IHC and RT-PCR	[101]
Ovary	47–71	IHC and RT-PCR	[93,94]
Pancreas	44–100	IHC and northern blot and qRT-PCR	[78–80]
Stomach	35–78 <sup>†</sup>	IHC and RT-PCR	[67–70]
Testis			
– Nonseminomas	100	IHC and northern blot	[104]
– Seminomas	31	IHC and northern blot	[104]
Uterus	69	IHC	[96]
Uveal melanoma	77–94	IHC	[99]

<sup>†</sup>Cripto-1 expression correlates with tumor histological grade.

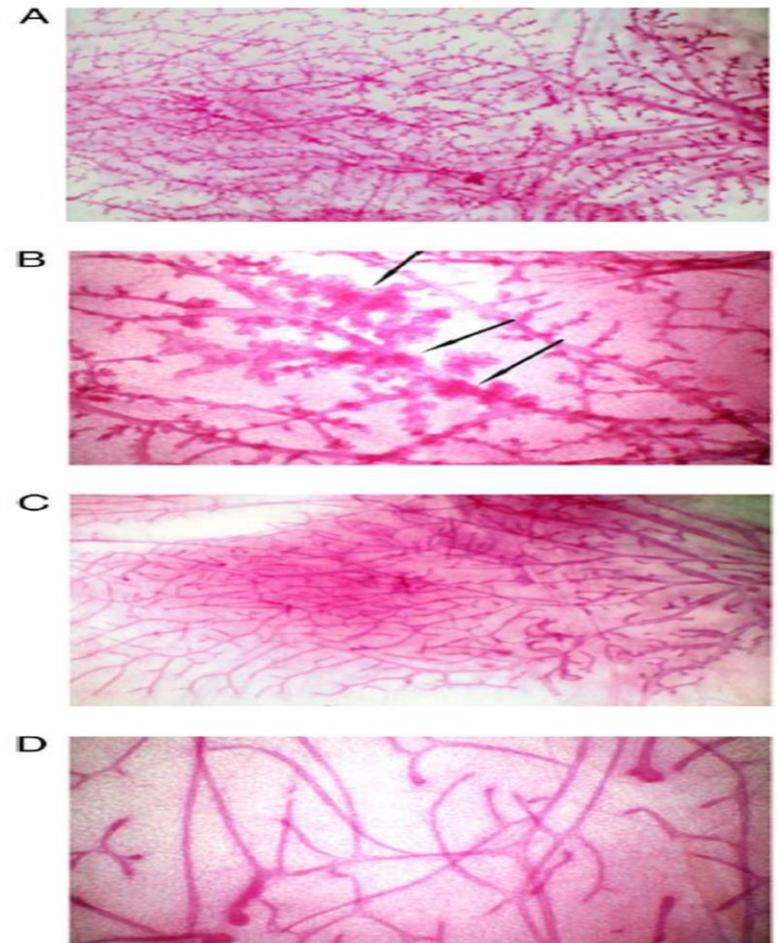
IHC: Immunohistochemistry; qRT-PCR: Quantitative reverse transcription PCR; RT-PCR: Reverse transcription PCR.



# CRIPTO-1 and Morphology

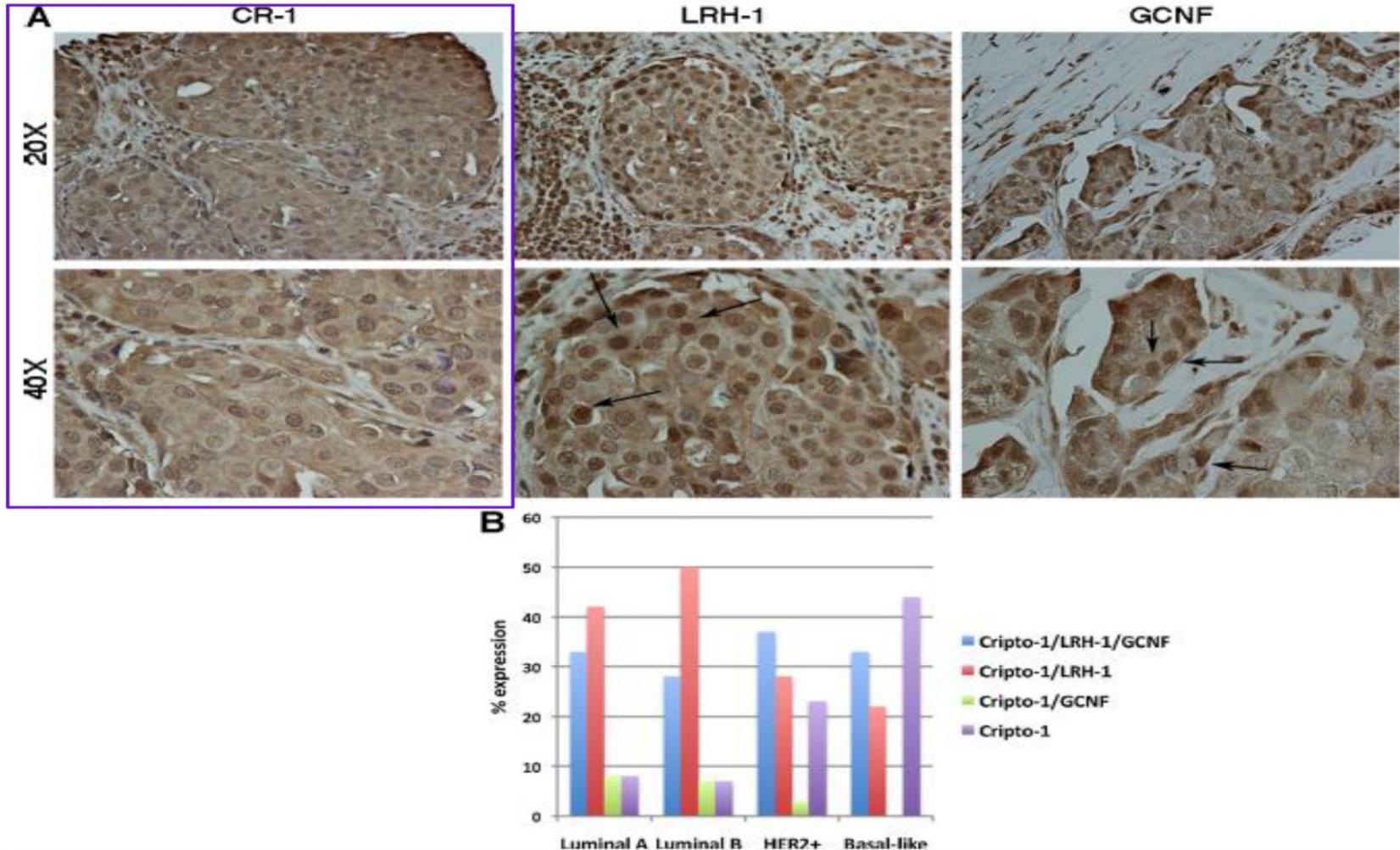


**Figure 3.** Effects of Cripto-1 on morphological characteristics and branching morphogenesis of mouse mammary epithelial cells. NOG-8 mouse mammary epithelial cells were grown on plastic as colonies in the absence (A) or presence (B) of recombinant Cripto-1 protein (50 ng/mL) (R&D Systems, Minneapolis, MN) for 8 days. Cripto-1 treatment induces scattering of peripheral epithelial cells, which acquire more fibroblastic-like morphological characteristics (B). Eph4 wild-type (C) and Eph4 Cripto-1 cells (D) were grown in collagen type I matrix for 2 weeks. Cripto-1 overexpression induces the extensive formation of branching duct-like structures that contain a lumen (D) compared with Eph4 control cells (C).



**Figure 4.** Whole mount histological features of mammary glands from nulliparous virgin MMTV-Cripto-1 and wild-type FVB/N mice. Virgin MMTV-Cripto-1 mammary glands (A and B) exhibit more secondary and tertiary ductal side branching compared with wild-type control mammary glands (C and D). Arrows, hyperplastic regions. Original magnification:  $\times 1$  (A and C);  $\times 5$  (B and D).

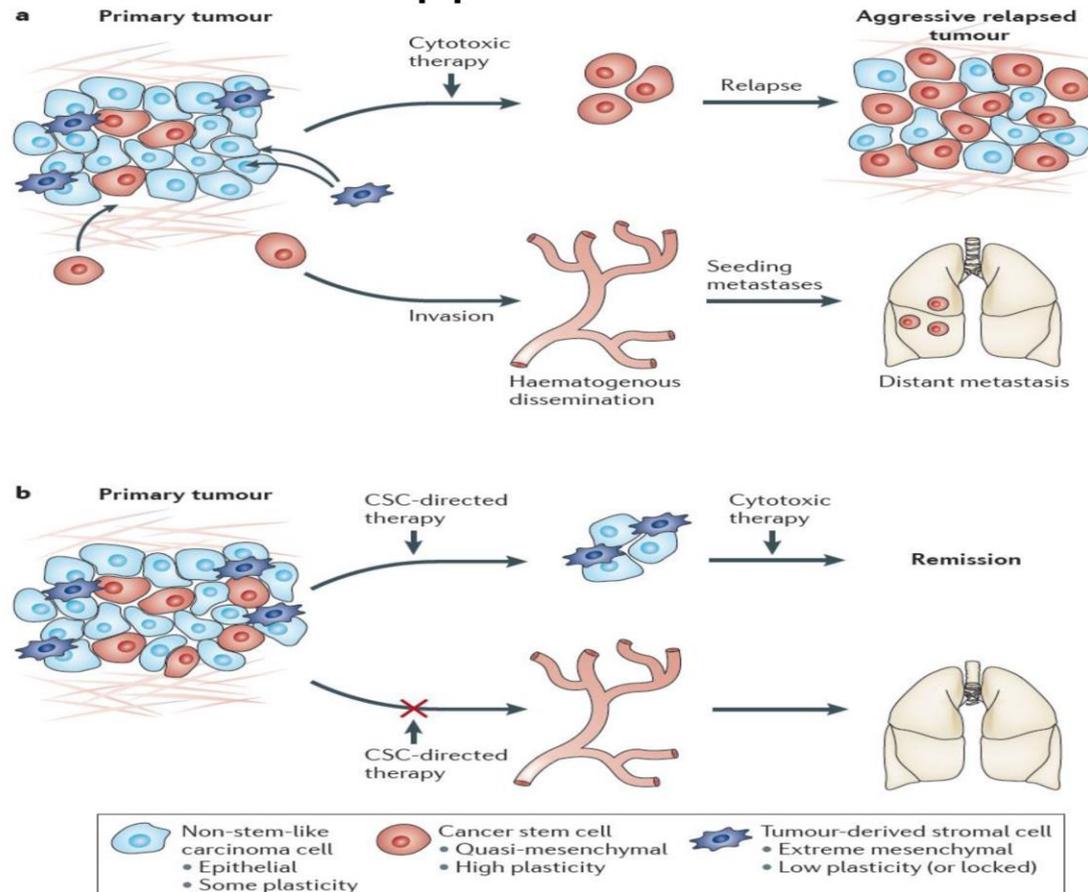
# CRIPTO-1 and DCIS



**Fig. 11.** Expression of CR-1, LRH-1, and GCNF in human invasive ductal breast carcinomas. **A:** Immunohistochemical analysis shows positive staining for CR-1, LRH-1, and GCNF. LRH-1 and GCNF show cytoplasmic and nuclear staining. Arrows are pointing to some cells with LRH-1 and GCNF nuclear staining. Original magnifications are 20X and 40X. **B:** Expression of CR-1, LRH-1, and GCNF in breast cancer molecular subtypes as assessed by immunohistochemical staining.

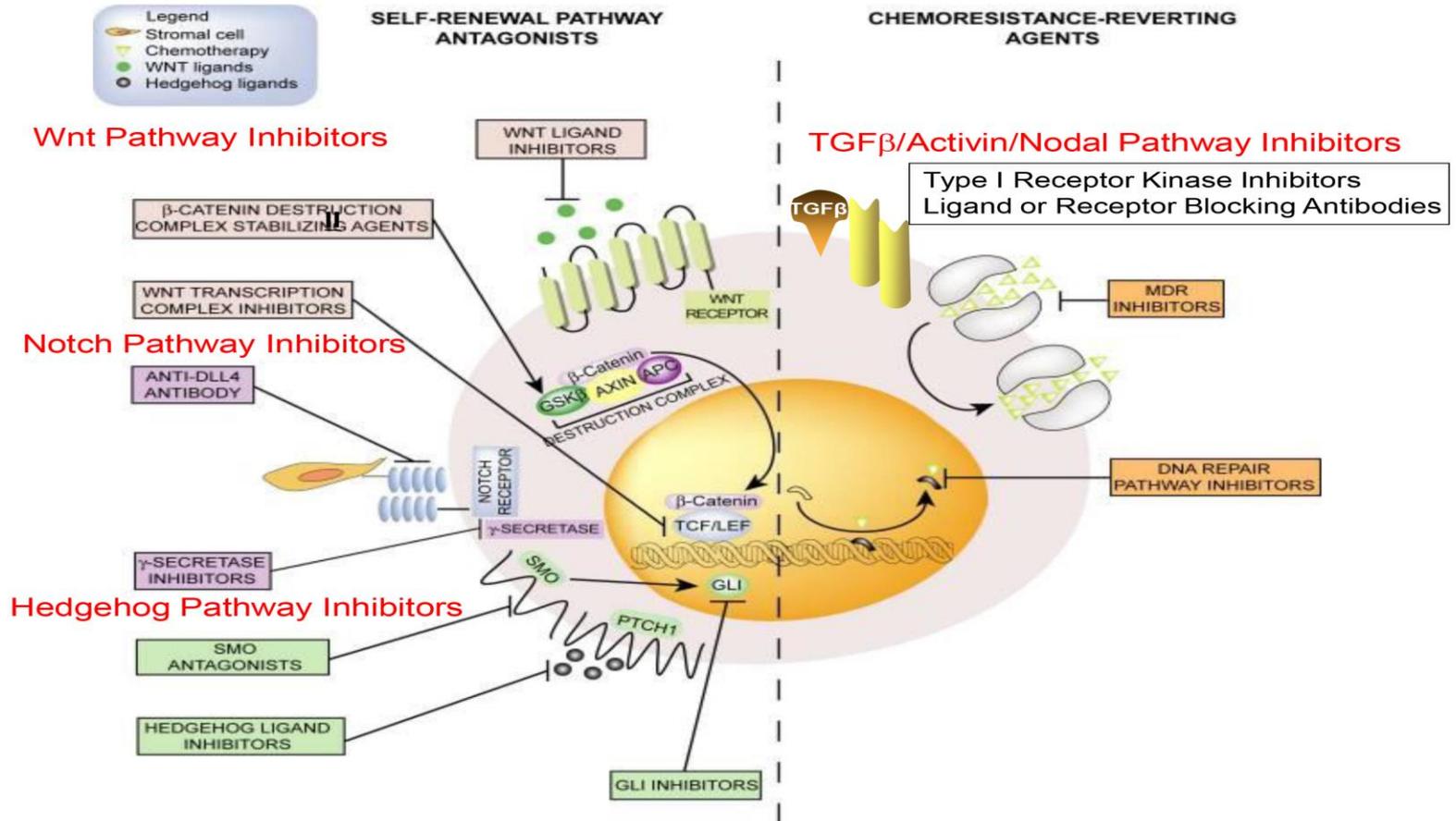
# Potential Treatments

## Potential Treatment Approaches to Breast Cancer



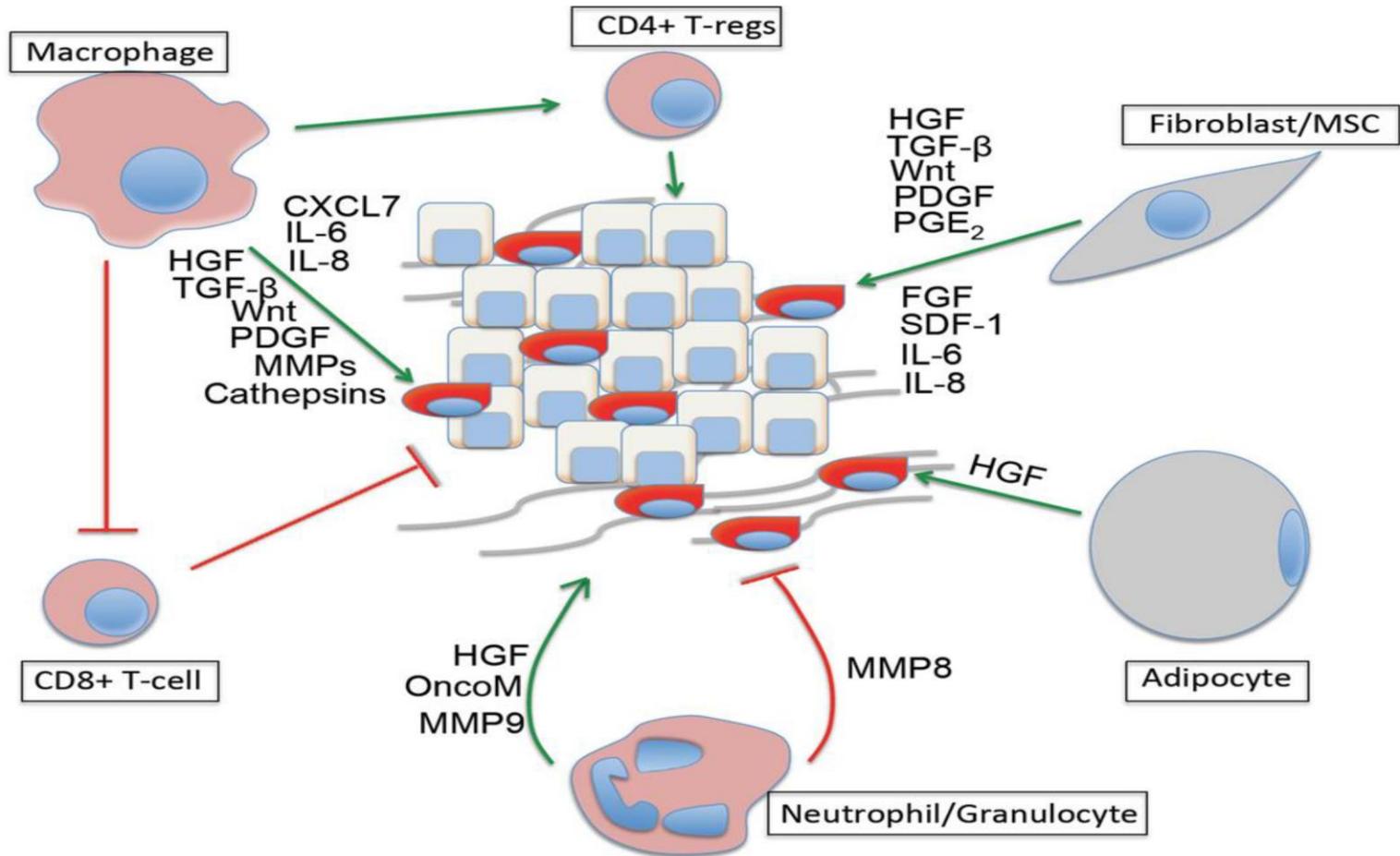
# CSC Signaling

## TARGETING CANCER STEM CELL SIGNALING PATHWAYS



# Cellular Niche

## Human Breast Cancer Cell Niche



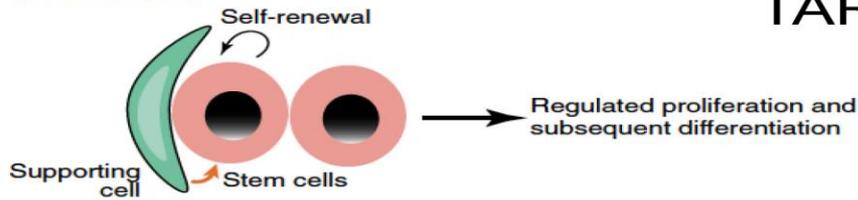
# Targeting niche

## TARGETING NICHES

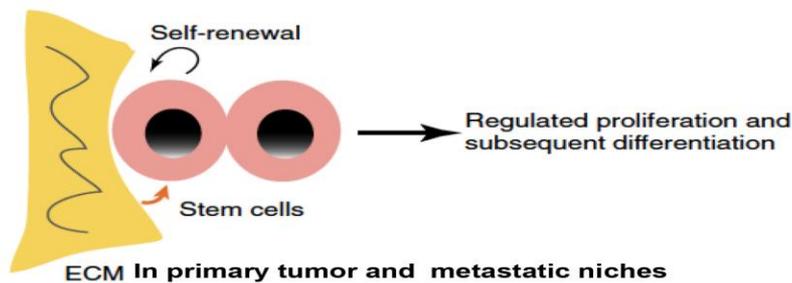
Current and potential therapeutics targeting tumor-stromal interactions in bone metastasis.

Therapeutic target	Mechanism	Agent
<i>FDA-approved therapies</i>		
Prenylation	Osteolysis inhibitor	Zoledronic acid
RANKL	Osteoclastogenesis inhibitor	Denosumab
CTLA4	Cytotoxic T cell activator	Ipilimumab
Cytotoxic cells	NK cell activator	Interleukin 2
<i>Potential therapies</i>		
EGFR	Osteoclastogenesis inhibitor	Gefitinib
PD1	Immunosuppression antagonist	CT-011
CD137	Immunosuppression antagonist	BMS-663513
Gal3	CTC attachment ligand mimic	Lactulose-l-leucine
c-MET	Pre-metastatic conditioning inhibitor	Tivantinib
CXCR4	Homing and dormancy inhibitor	AMD3100
CCL2	Homing and growth antagonist	Carlumab
ET1	Osteogenesis antagonist	Atrasentan
Metastatic cells	Suppressive pathway activator	Interferon 7
PSA+ cells	PSA based vaccine	PROSTVAC-VF
VWF	Platelet shielding inhibitor	ARC1779
$\beta_3$ -Integrin	Seeding and growth antagonist	RGD-mimetic/mAb
Cathepsin B	Osteolysis inhibitor	CA-074
c-FMS	Osteoclastogenesis inhibitor	Ki-20227
SRC	Proliferation inhibitor	Dasatinib
Cathepsin K	Osteolysis inhibitor	Odanacatib
TGF $\beta$	Osteoclastogenesis inhibitor	Ki26894, SD-208, LY2109761

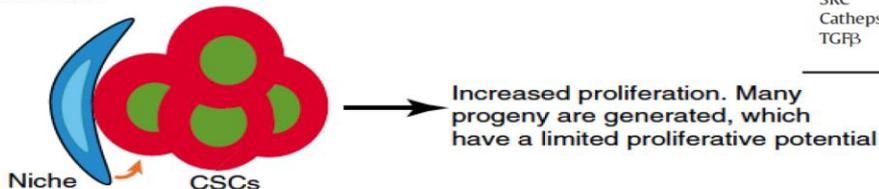
### A Cellular niche



### B Non-cellular niche



### C CSC niche



Key:  Secreted signals from niche

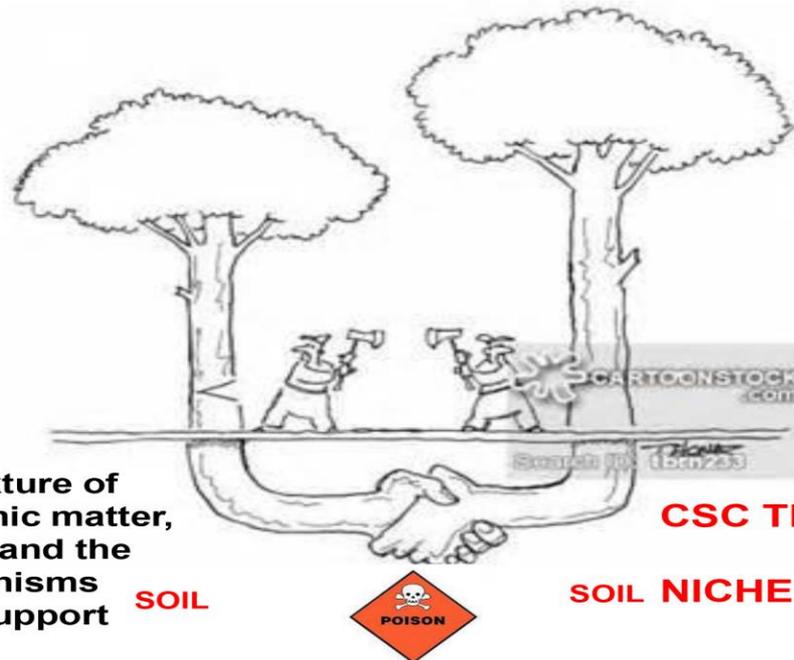
# Chemotherapy and Radiotherapy



**CURRENT CHEMOTHERAPY  
AND RADIOTHERAPY!!!!**



**+**



• Soil is the mixture of minerals, organic matter, gases, liquids, and the myriad of organisms that together support plant life.

**SOIL**

**CSC THERAPY**

**SOIL NICHE THERAPY**



# The End

