

Stem cells: The search for the holy grail by David Salomon



Historical Background

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

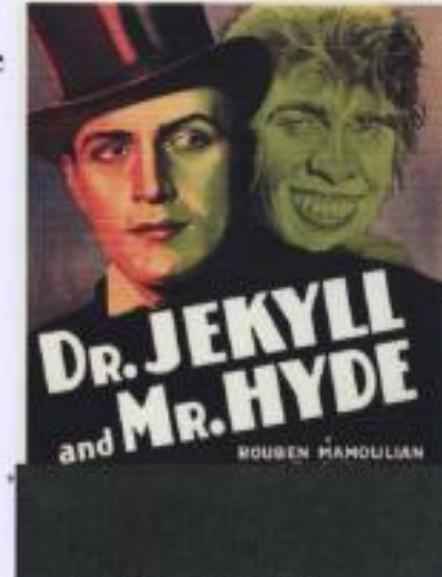
CANCER RESEARCH 48, 1994-2004, April 15, 1989

Perspectives in Cancer Research

Tumors as Caricatures of the Process of Tissue Renewal: Prospects for Therapy by Directing Differentiation¹

G. Barry Pierce and Wendell C. Speers

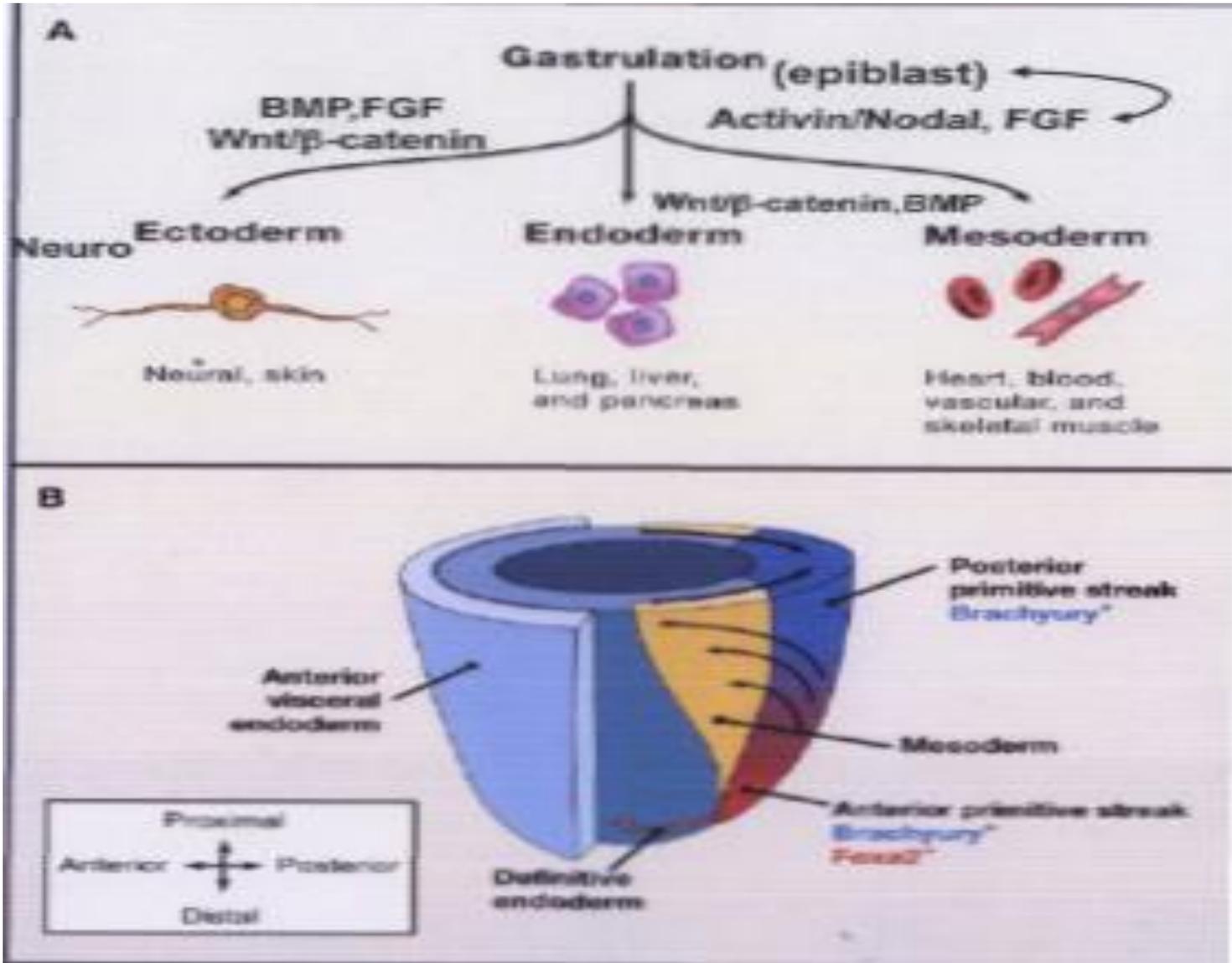
Department of Pathology, University of Colorado Health Sciences Center, Denver, Colorado 80262



-Embryonic microenvironment (Mintz/Hendrix) or the adult stem cell niche (Smith) can redirect or reprogram tumor cells to normal cellular lineage restriction and differentiation (Dominance of the Niche).

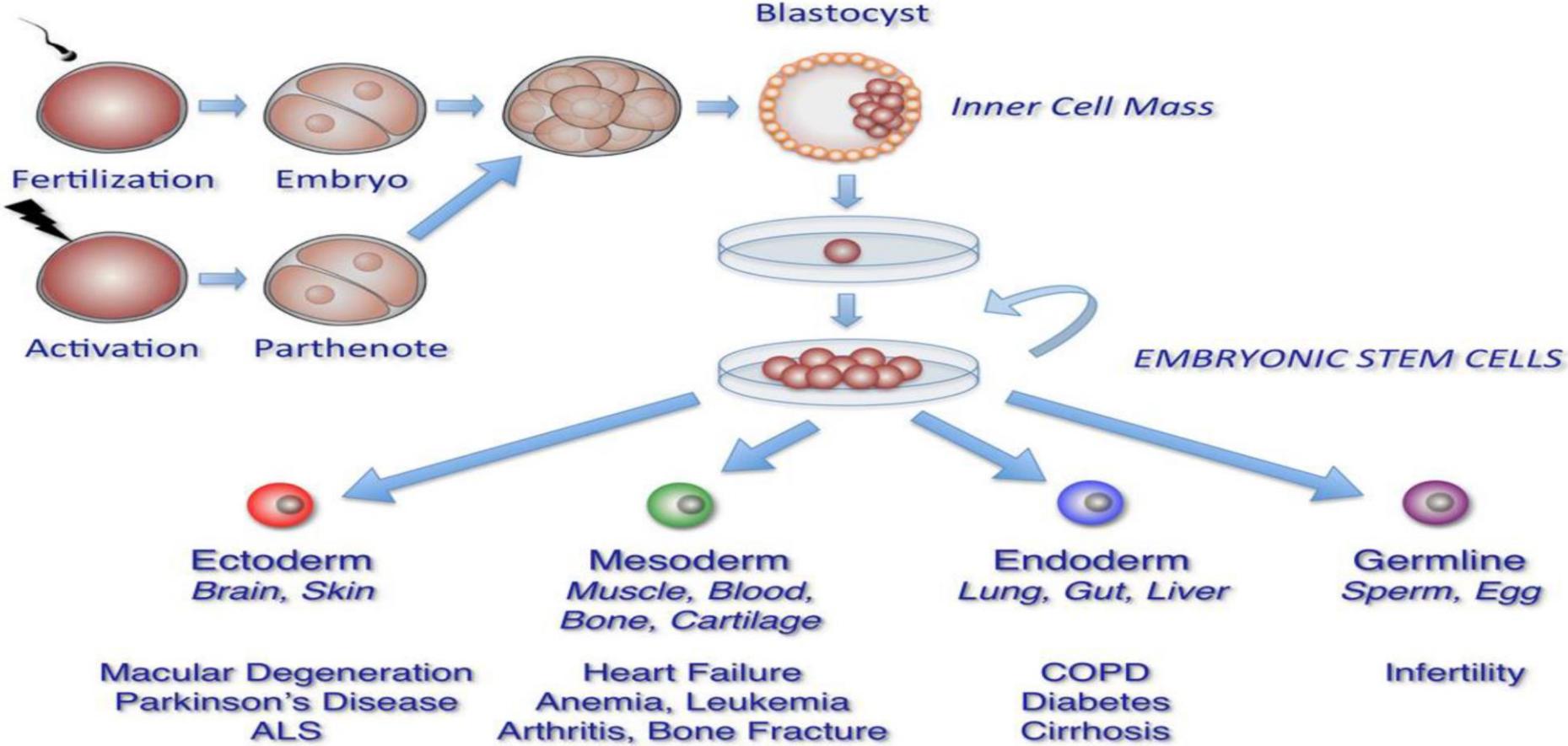
-Tumor microenvironment/niche can reprogram adult tissue stem cells and iPS cells to acquire properties of cancer stem cells (CSCs) or tumor initiating cells (TICs).

Germ Layer Formation



Embryonic stem cells

Embryonic Stem (ES) Cells



48 Genes overexpressed

Forty-eight Genes Overexpressed by
Microarray Analysis in hESCs compared to
Differentiated Cell Types in at least 40
studies

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

TDGF1 Teratocarcinoma-derived growth factor 1 9p21.31 Hs.365870 17 55.0

DPPA4 Developmental pluripotency associated 4 3q13.13 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 Methylene tetrahydrofolate dehydrogenase 1 14q24 Hs.435974 11 8.7

PPAT Phosphoribosyl pyrophosphate amidotransferase 4q12 Hs.331420 11 8.3

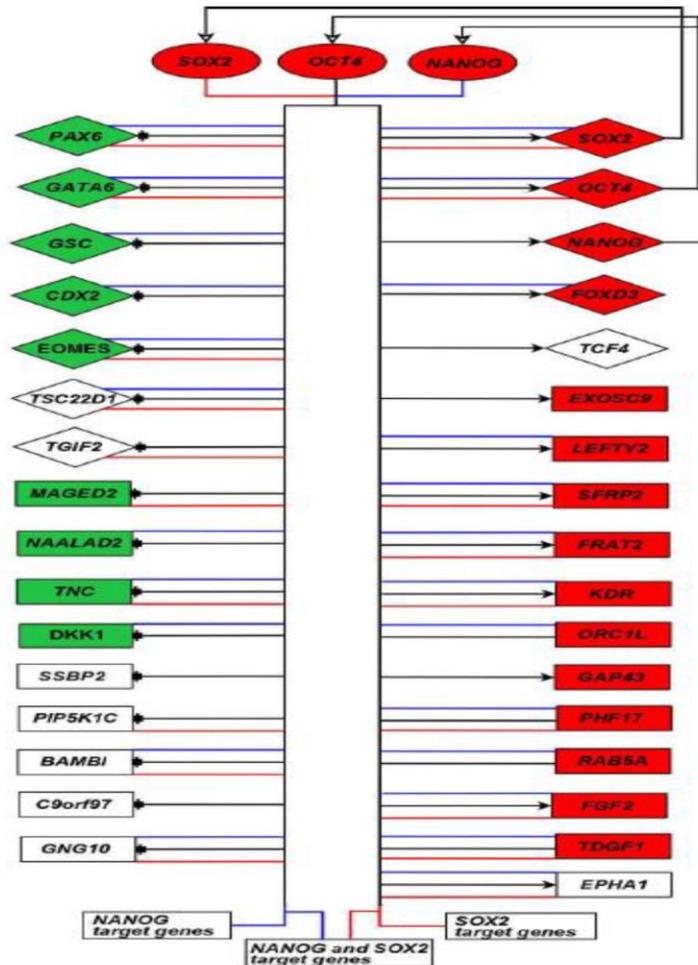
SLC16A1 AKR7 family pseudogene 1p12 Hs.75231 11 7.8

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

“Trinity/Triumverate”

Interdependent Cross Regulation of NOX genes and Their Targets

Interdependent Cross Regulation of NOS Genes and Their Targets



NOS-KM Single Target Genes

NOS-KM Single Target 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]).

Pathways regulated by Oct4

Pathways Regulated by Oct4 (PouF1)

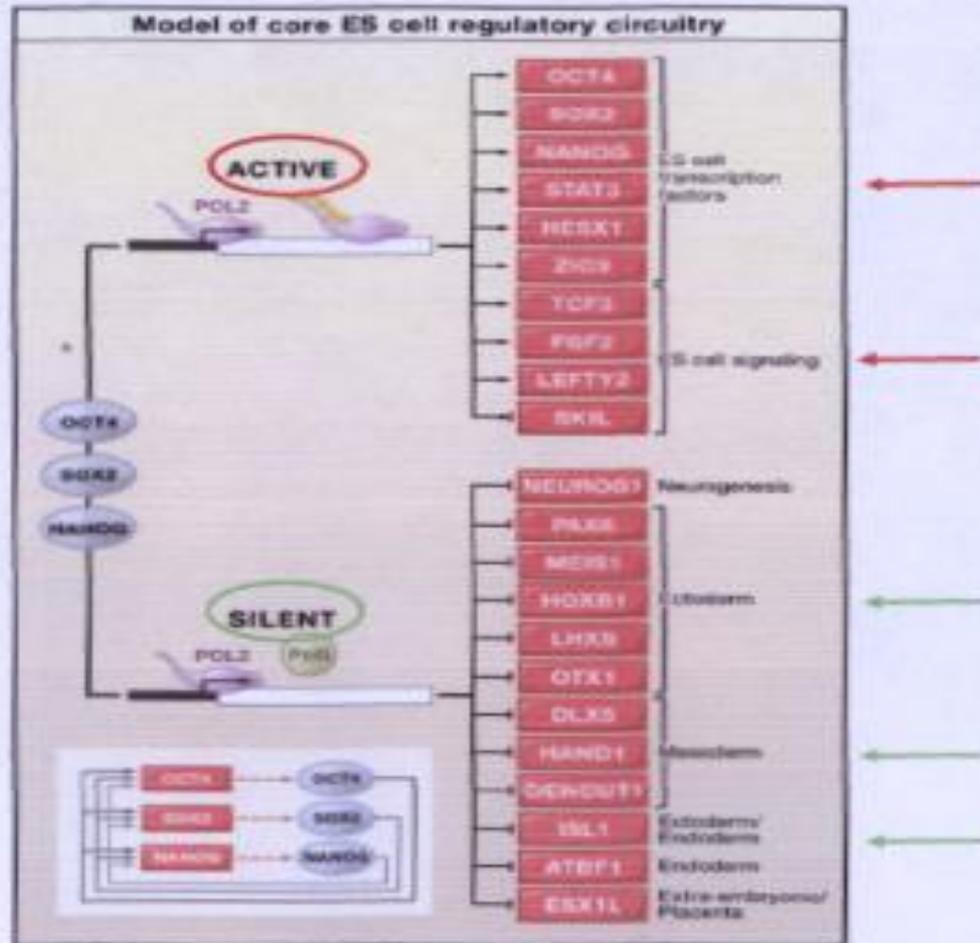
Table 1. Pathways of which gene components show significant expression changes between the OCT4 and EGFP knockdowns at 72 hours after transfection

KEGGID	Pathway description	Genes ^a	Z score ^b	p value ^c	OCT4-RNAi-UP ^d	EGFP-RNAi-UP ^e
hsa04610	Complement and coagulation cascades	25	2.973214	0.001473563	18	7
hsa00230	Purine metabolism	58	2.914994	0.001778544	22	36
hsa04510	Focal adhesion	64	2.708439	0.003380077	43	21
hsa04810	Regulation of actin cytoskeleton	69	2.6696	0.003797128	45	24
hsa04080	Neuroactive ligand-receptor interaction	49	2.660901	0.003896637	30	19
hsa03050	Proteasome	18	2.591246	0.004781487	4	14
hsa00190	Oxidative phosphorylation	58	2.582073	0.004910471	23	35
hsa04010	MAPK signaling pathway	92	2.476518	0.006633556	55	37
hsa04512	ECM-receptor interaction	27	2.25835	0.011961886	18	9
hsa00590	Prostaglandin and leukotriene metabolism	8	2.240448	0.012530885	7	1
hsa04520	Adherens junction	27	2.234325	0.012730806	19	8
hsa00251	Glutamate metabolism	16	2.171768	0.01493653	4	12
hsa00252	Alanine and aspartate metabolism	7	2.02837	0.021261181	1	6
hsa05110	Cholera: infection	13	1.991741	0.023199671	9	4
hsa00710	Carbon fixation	8	1.820364	0.034351738	2	6
hsa00400	Phenylalanine, tyrosine, and tryptophan biosynthesis	5	1.75292	0.039807826	1	4
hsa04630	Jak-STAT signaling pathway	40	1.693603	0.045170345	26	14
hsa00970	Aminoacyl-tRNA biosynthesis	9	1.599342	0.054872314	2	7
hsa04310	Wnt signaling pathway	52	1.575497	0.05757089	31	21
hsa04350	TGF- β signaling pathway	28	1.525685	0.063544186	18	10
hsa00440	Aminophosphonate metabolism	7	1.521278	0.064093066	2	5
hsa00052	Galactose metabolism	10	1.477977	0.069707001	8	2
hsa00260	Glycine, serine, and threonine metabolism	17	1.443812	0.074395975	5	12
hsa04110	Cell cycle	45	1.439163	0.075052216	21	24
hsa00640	Propanoate metabolism	15	1.306312	0.095723304	9	6
hsa04210	Apoptosis	30	1.30609	0.095761041	18	12

Pathways were taken from the KEGG database. Gene expression was compared in the OCT4 RNAi and EGFP RNAi at 72 hours, and the differences of array signals were used for computing Wilcoxon's paired signed rank test. Genes that were judged as nondetectable by the background value criterion were excluded from analysis. Shown are pathways with gene numbers >4, but it should be noted that the normal approximation is valid for sample sizes >25. The complete list of the pathway results is given in supplemental online Table 8.

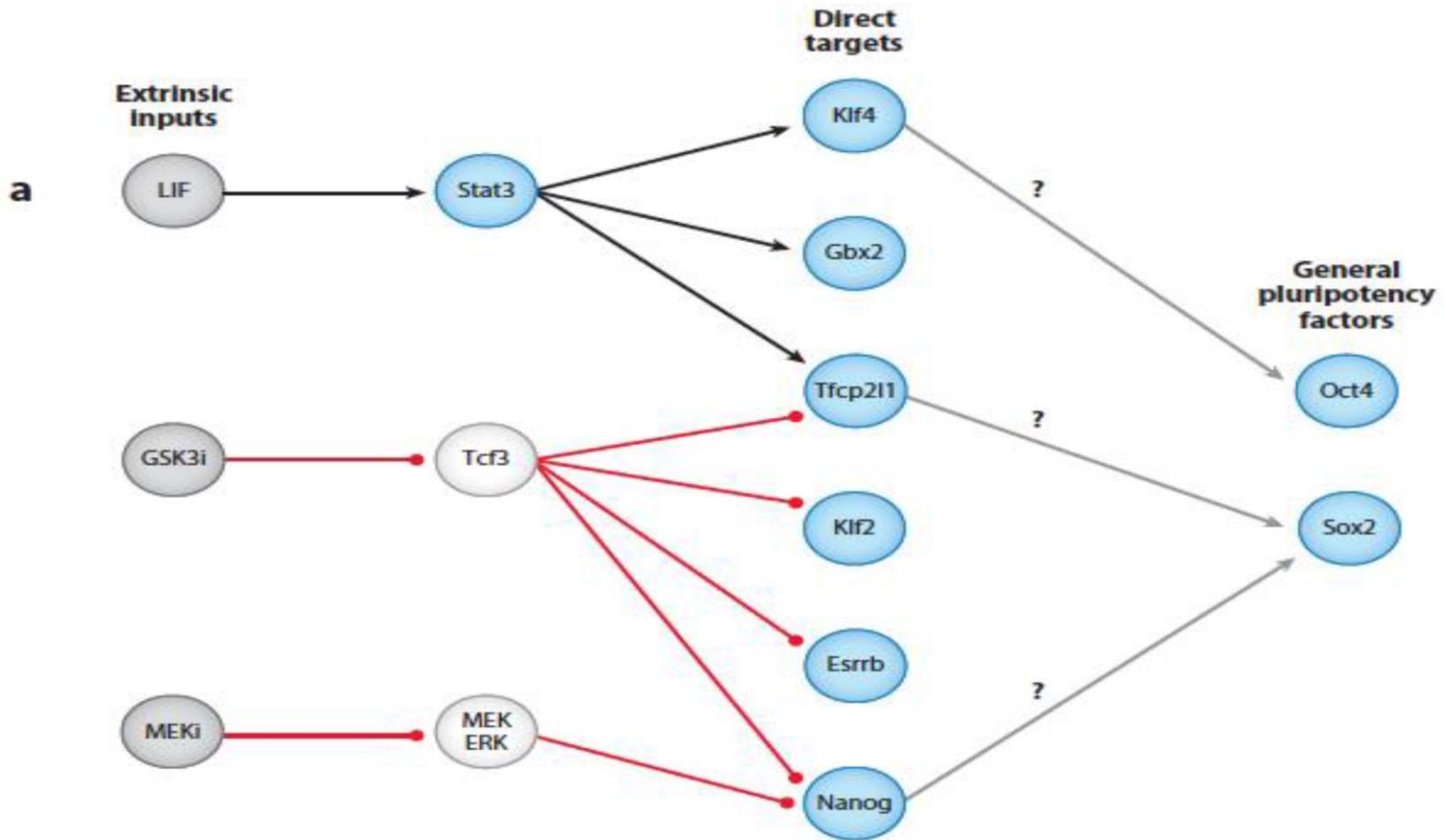
CO-OCCUPIED NOS TARGETS THAT ARE ACTIVATED OR REPRESSED

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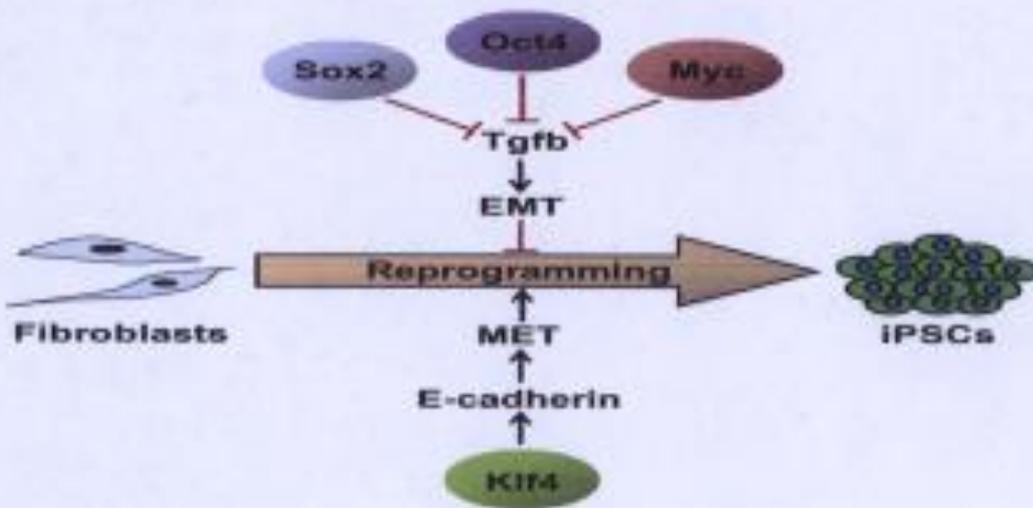
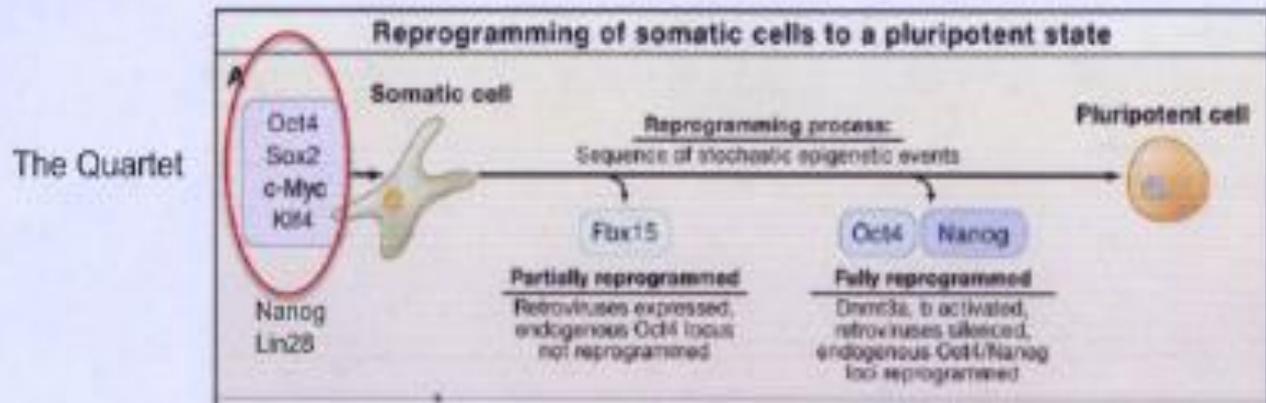
Signaling Pathways

Signaling Pathways Regulating NOS Gene Expression



Induction of Pluripotential Stem Cells (iPSCs) from Differentiated Adult Somatic Cells

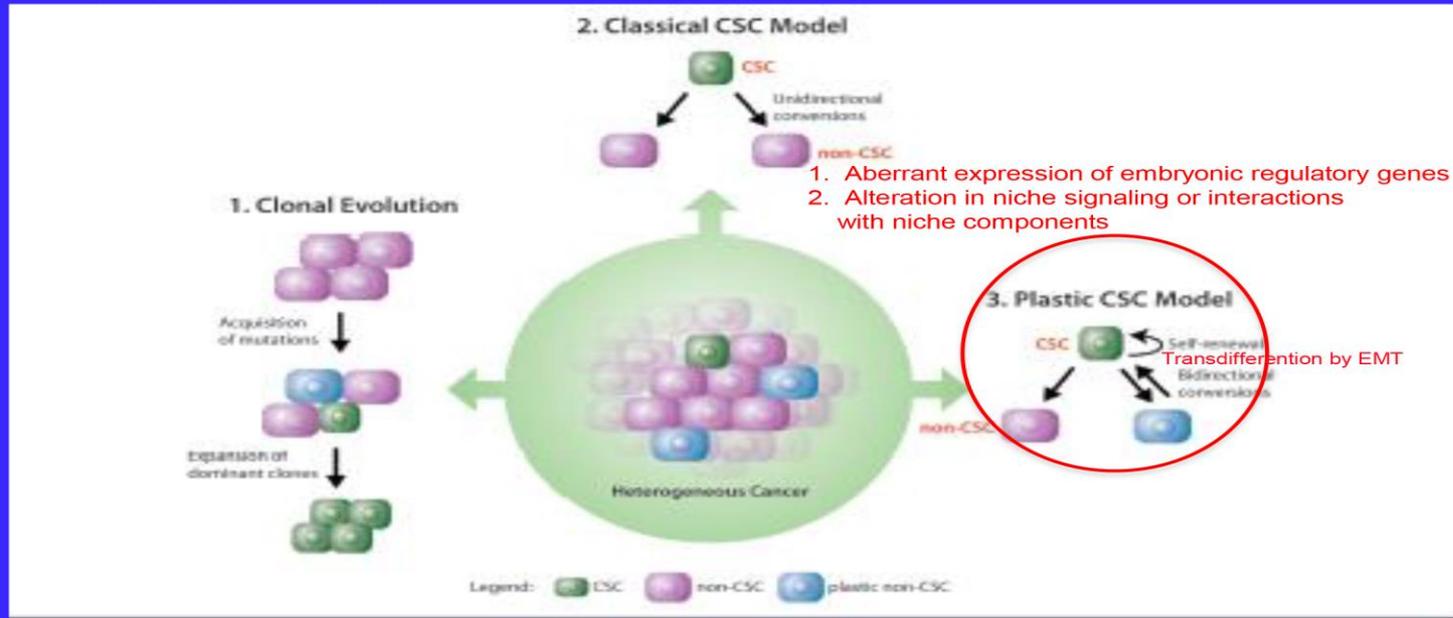
Induction of Pluripotential Stem Cells (iPSCs) from Differentiated Adult Somatic Cells



Cancer Heterogeneity

Two General Models for Cancer Heterogeneity

1. All tumor cells in a random fashion have the potential to be cancer stem cells after transformation (Stochastic or Clonal Model)
2. Only a small definable and preexisting subset of cancer cells are cancer stem cells that have the ability to proliferate indefinitely and that arise from the transformation of normal tissue stem cells or progenitor/transit amplifying cells (CSC or Hierarchical Model)

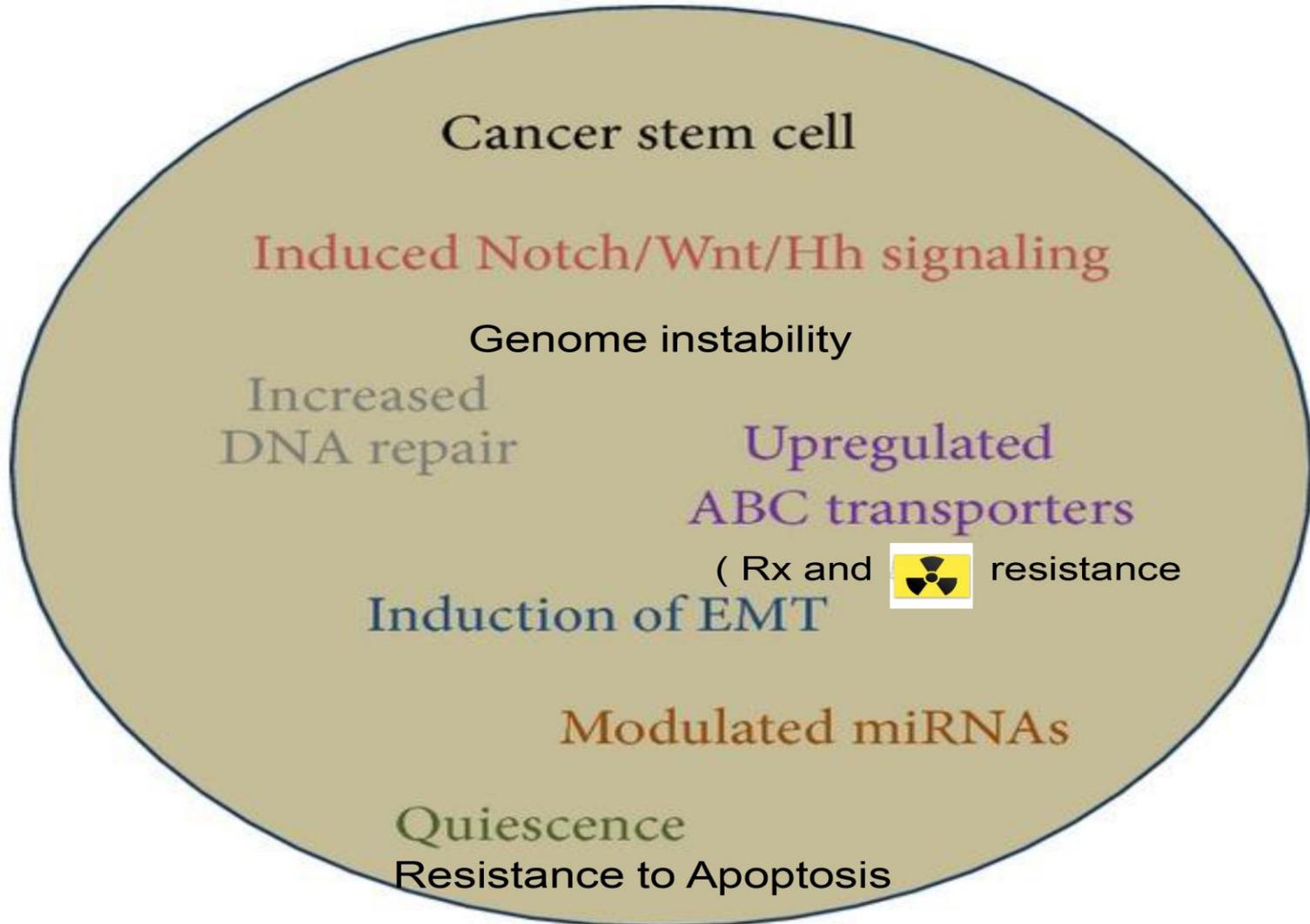


Factors regulating CSCs in a Tumor

Factors Regulating the Frequency or Representation of CSCs in a Tumor

1. Normal cell of origin from which the tumors arise
2. Genetic and epigenetic modifications that the tumor cells have accumulated during tumor progression
3. Contextual signals that the CSCs experience in the tumor niche/microenvironment.
4. The immunological status of the host in which the tumor develops.

Cancer stem cell



Niche environment

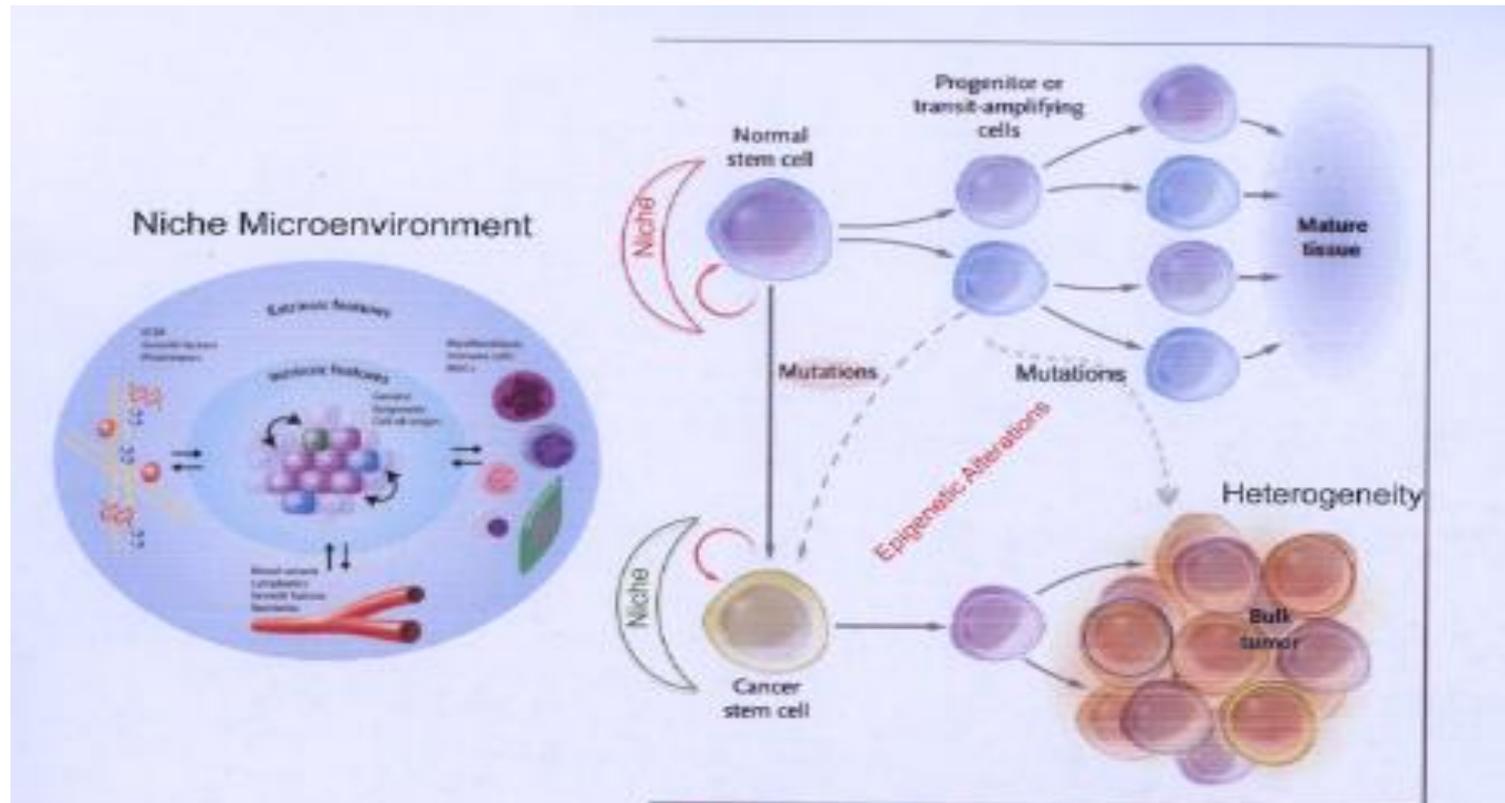
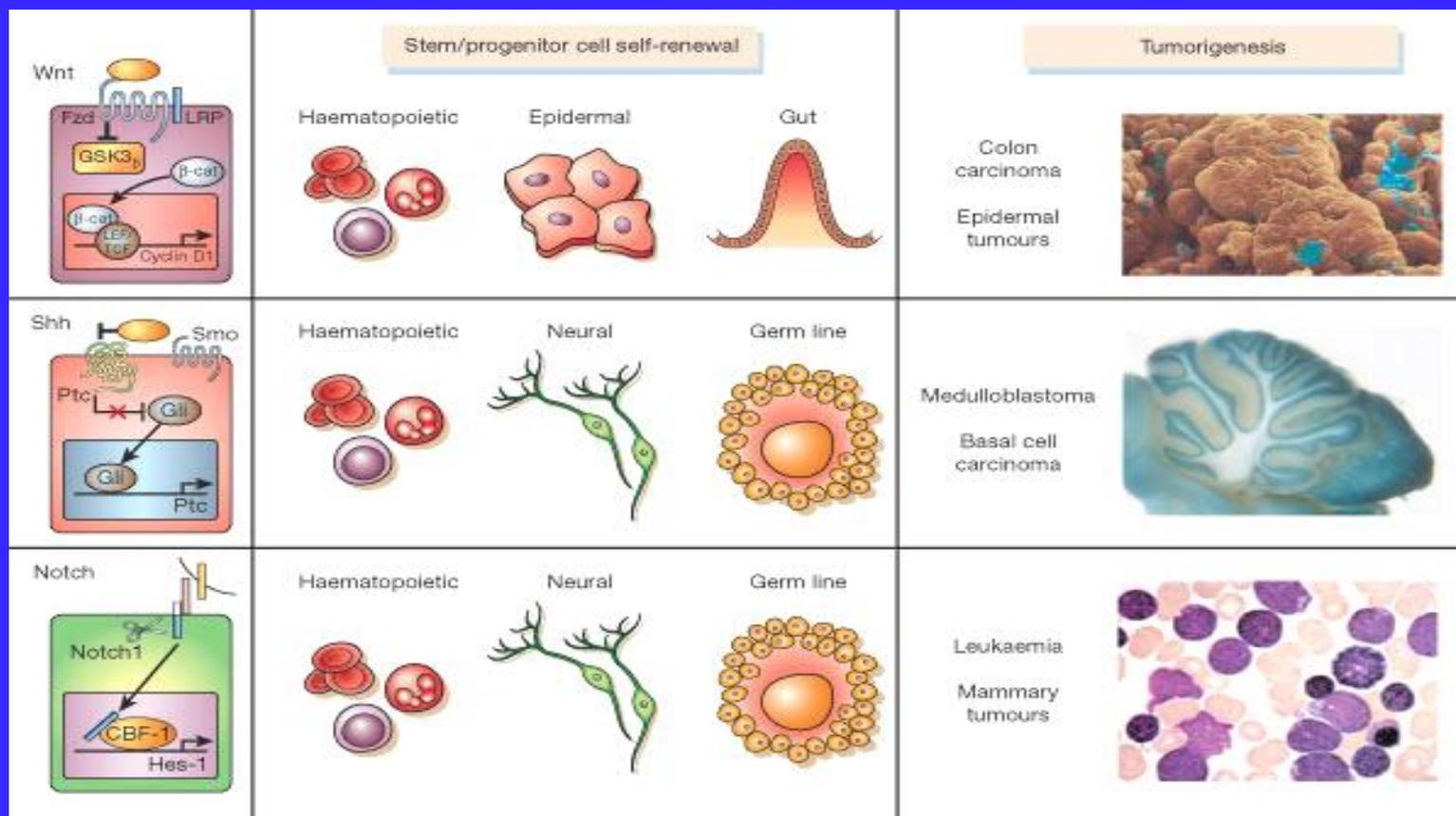


Figure 2. Stem-Cell Systems.

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), multilineage 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.

Pathways Involved in Self-Renewal that are Deregulated in Cancer Stem Cells 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).



Tumor grade

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 OncoPrint Cancer Microarray database.

Tissue	Oct4	Sox2	Klf4	c-MYC
Lymphoma	no	no	no	yes
Leukemia	yes	no	yes	yes
Myeloma	no	no	yes	yes
Adrenal	no	no	no	no
Bladder	yes	yes	no	no
Blood	no	no	no	no
Brain	yes	yes	yes	yes
Breast	no	no	no	yes
Cervix	no	no	no	no
Chondrosarcoma	no	no	no	no
Colon	no	yes	no	yes
Endocrine	no	no	no	no
Endometrium	no	no	no	no
Esophagus	no	no	no	no
Gastric	no	no	no	no
Head-Neck	no	no	no	yes
Liver	no	yes	no	no
Lung	yes	yes	no	yes
Melanoma	no	no	no	no
Mesothelioma	no	no	no	no
Multi-cancer	no	yes	no	no
Muscle	no	no	no	no
Neuroblastoma	no	no	no	no
Oval	no	no	no	no
Others	no	no	no	no
Ovarian	yes	no	no	no
Pancreas	yes	no	no	yes
Parathyroid	no	no	no	no
Prostate	yes	yes	yes	yes
Rectum	no	no	no	no
Renal	yes	no	no	yes
Salivary-gland	no	no	no	yes
Sarcoma	no	no	no	no
Seminoma	yes	yes	no	yes
Skin	no	no	no	no
Testis	yes	yes	yes	no
Thyroid	no	no	no	no
Uterus	no	no	no	no

Table 2 Tumor Grade

Association Sox2, Oct4, Klf4 and c-Myc with tumor grade.

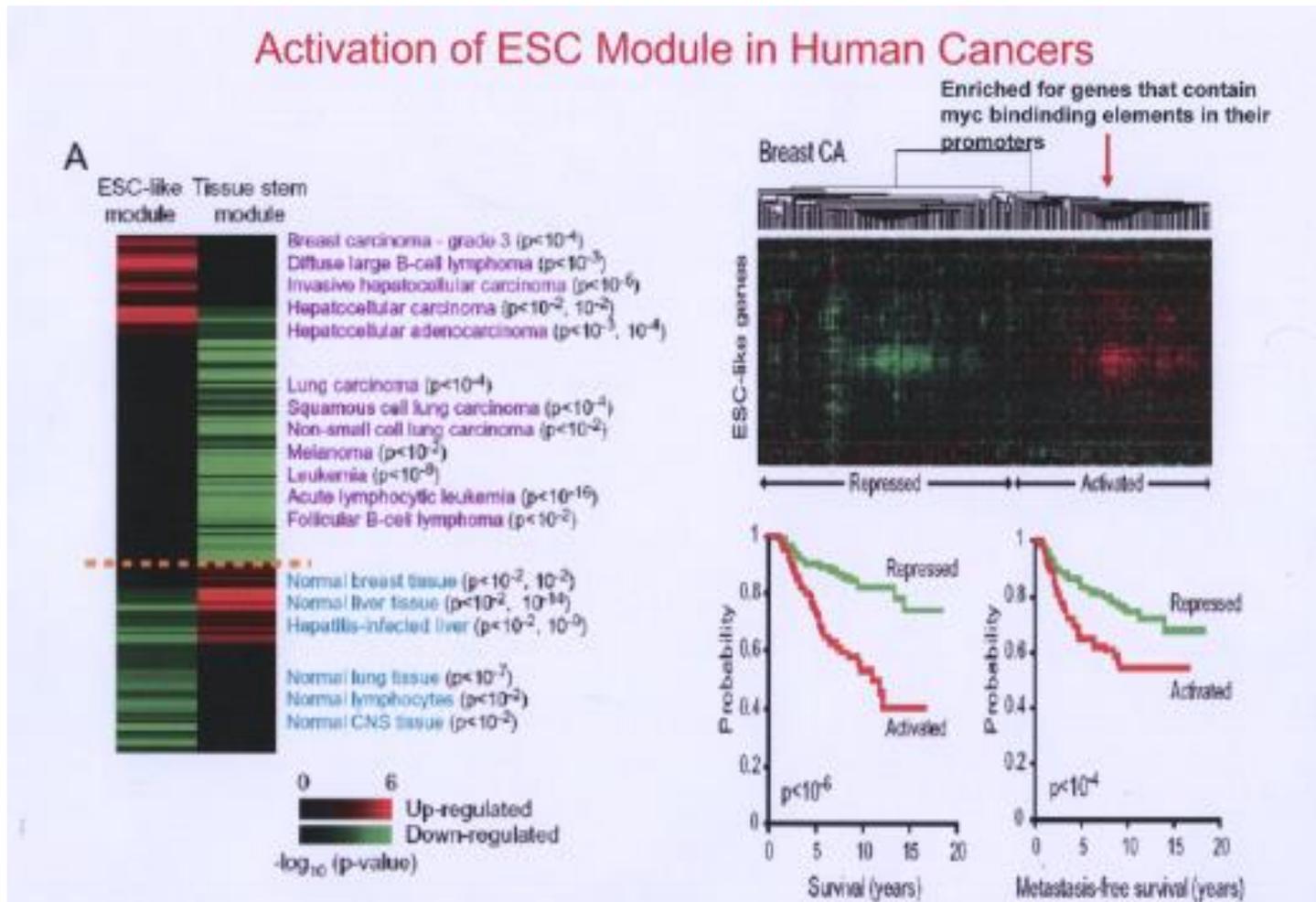
Cancer type	Sox2	Oct4	Klf4	c-Myc
Bladder	+			
Brain	+	+	+	
Breast	+	+		
Cervix	+			
Colon	+			
Endometrium	+			
Head-Neck	+	+		
Lung	+			+
Lymphoma	+			+
Melanoma	+		+	+
Ovarian	+		+	+
Pancreas	+		+	+
Prostate	+		+	+
Renal	+			+
Sarcoma	+	+		+
Thyroid	+			+

Table 3 Prognosis *

Association of Sox2, Oct4, Klf4 and c-Myc with prognosis in cancer.

Cancer type	Sox2	Oct4	Klf4	c-Myc
Bladder				
Brain				+
Breast				+
Colon				
Head-Neck				
Leukemia				
Liver				
Lung				+
Lymphoma				+
Melanoma	+			+
Myeloma	+			+
Ovarian	+			
Prostate				
Renal				

Activation of ESC Module in Human Cancers



A similar correlation was observed in primary liver, lung, prostate and gastric carcinomas.

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

230,000 women/year with 39,000 deaths

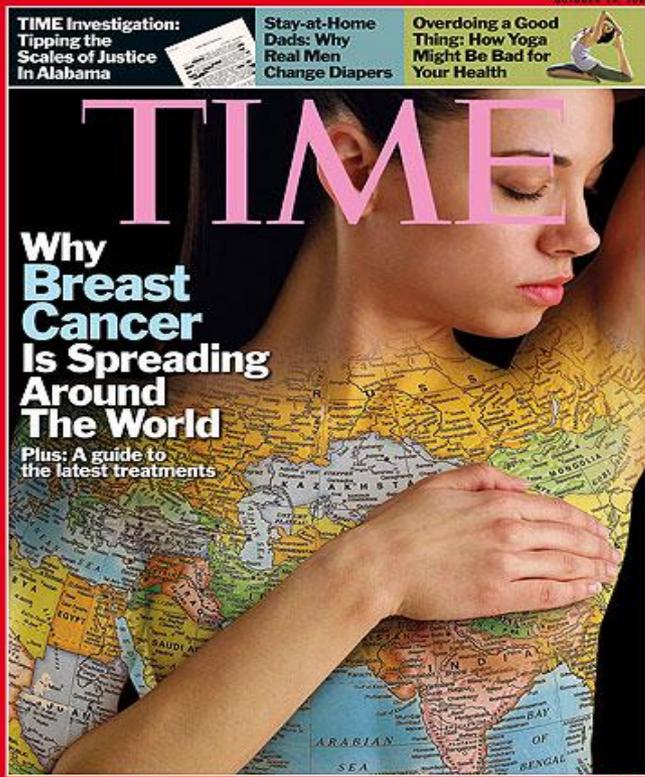
Lifetime risk of 1 out of 8 women

Early menarche (E2 exposure)

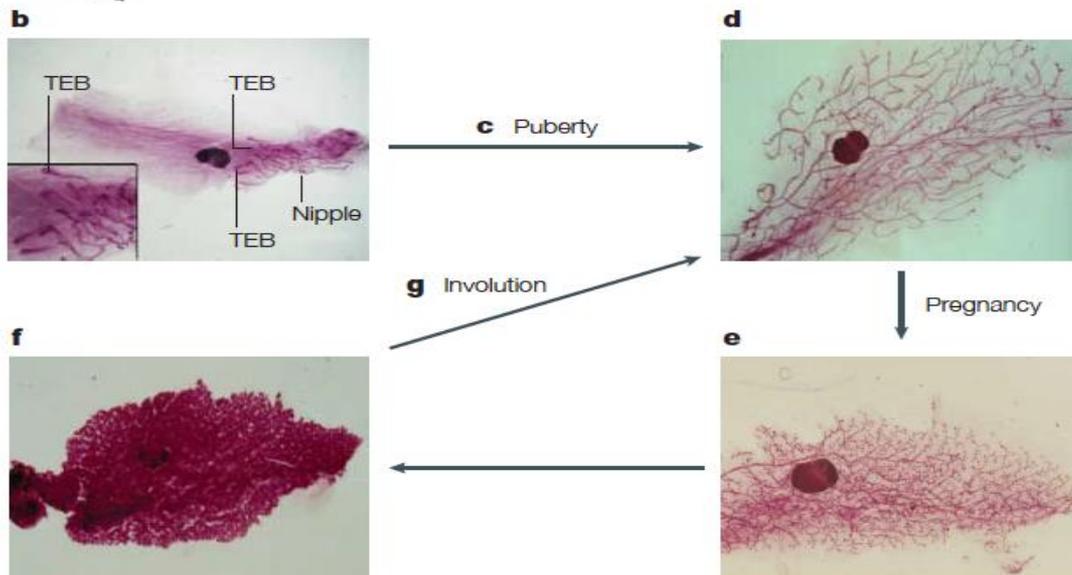
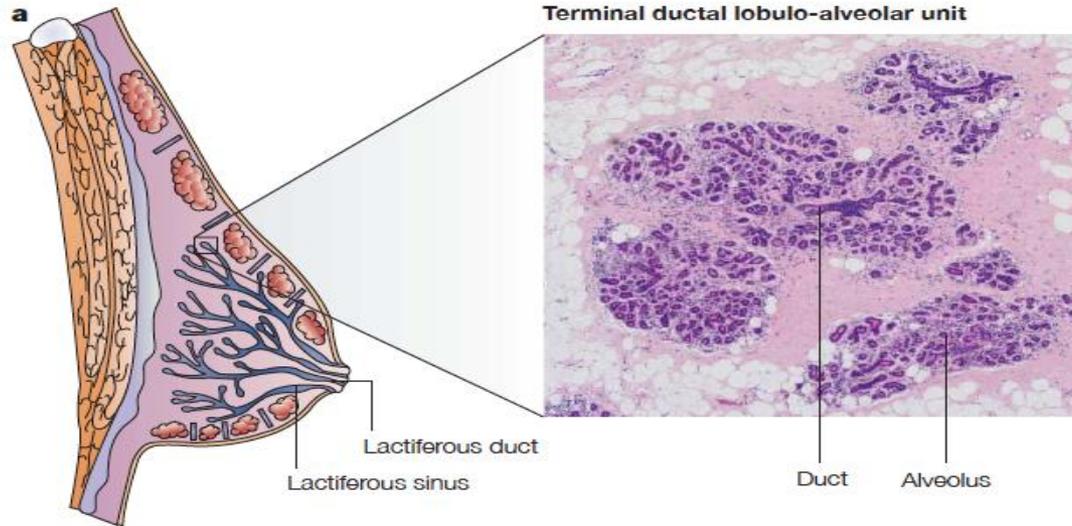
Nulliparity or late parity

Obesity (premenopausal with a 70% increase in risk)

Family history/5-10% (BRCA1 and BRCA2)

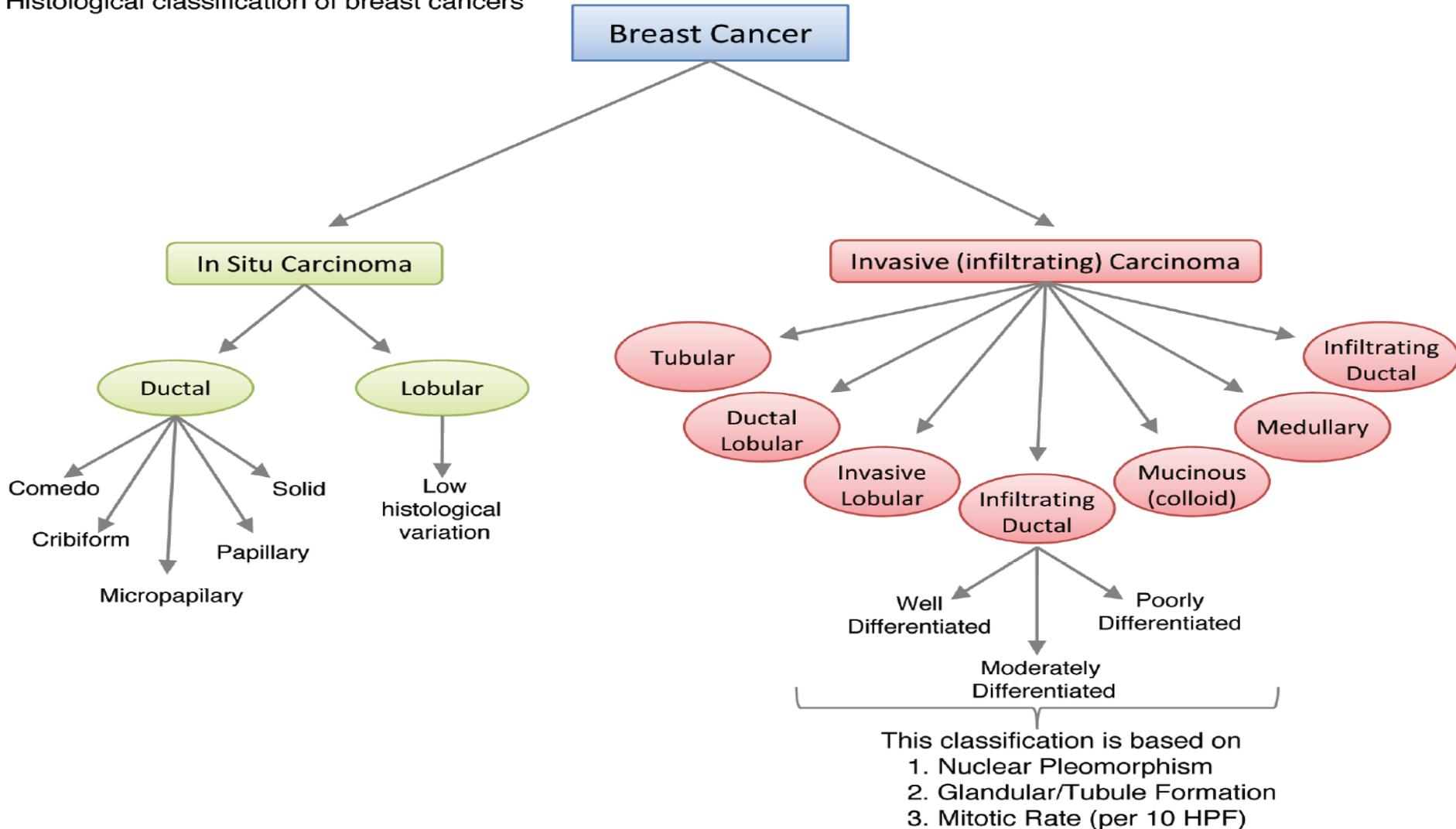


Breast anatomy

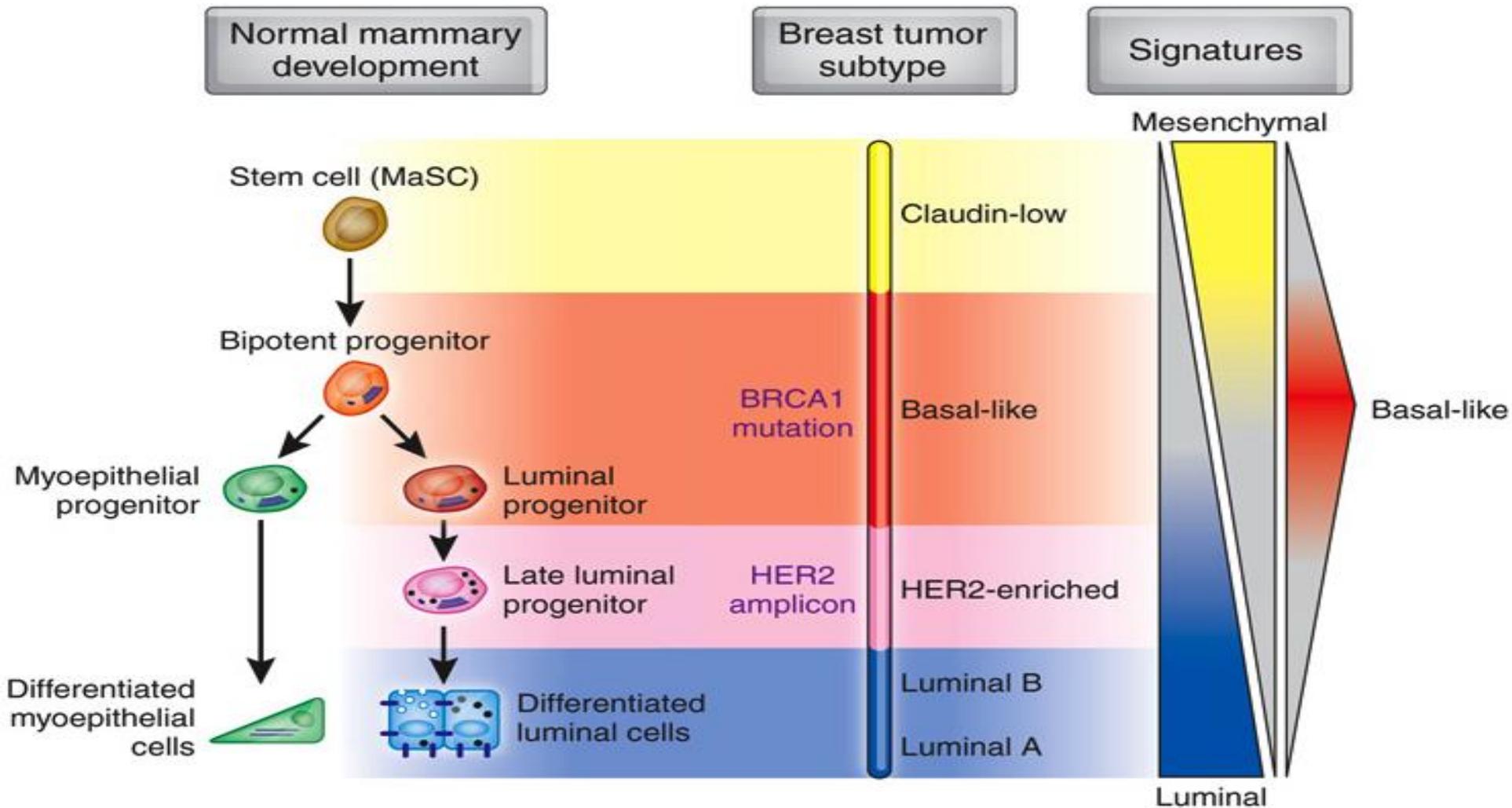


Histological classification of breast cancer

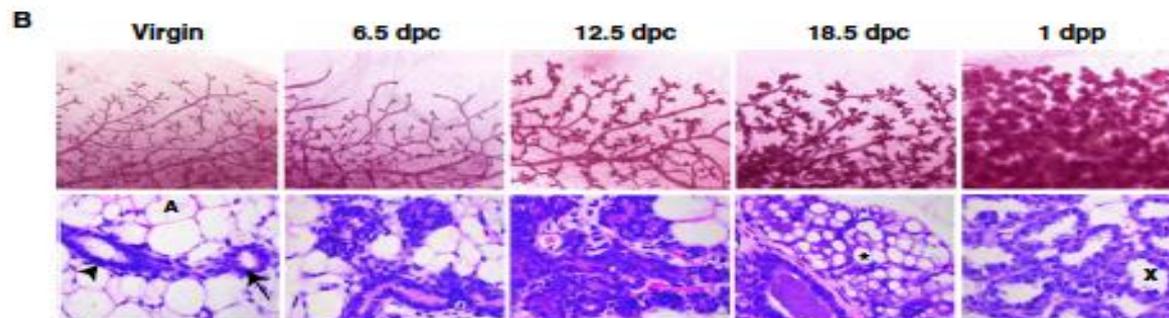
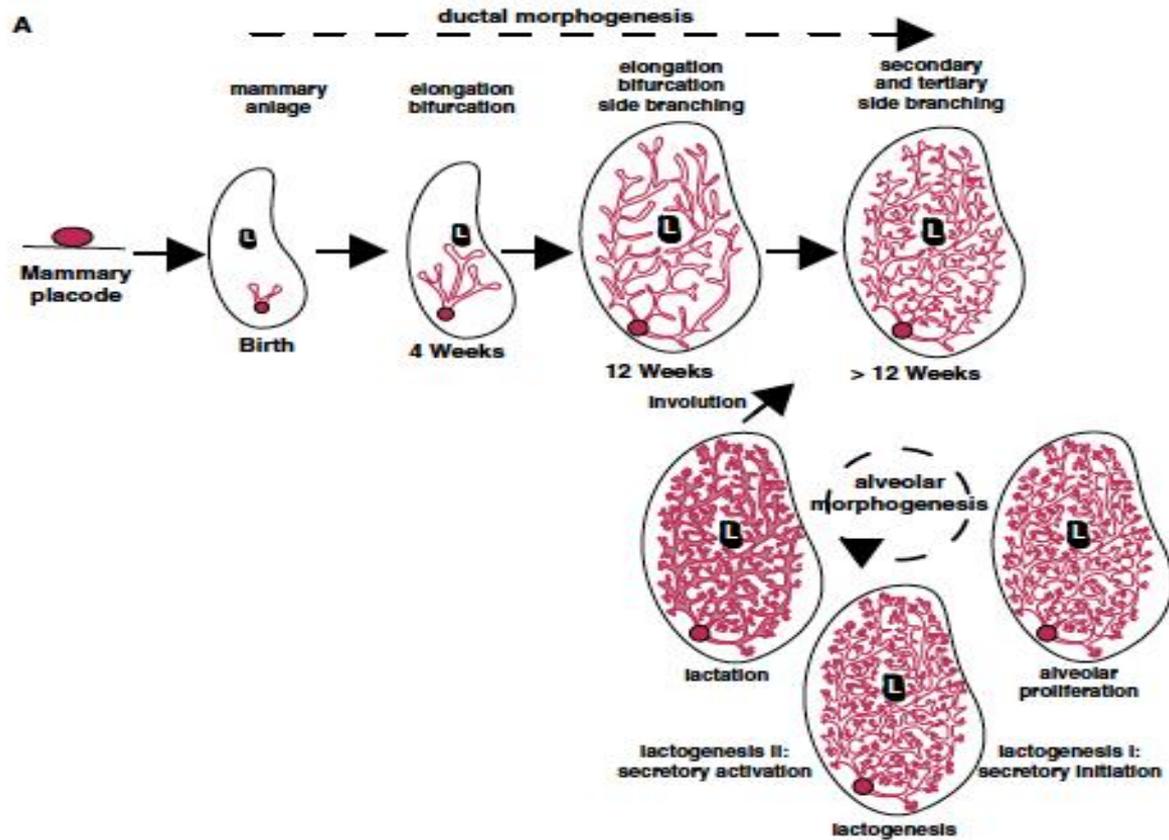
Histological classification of breast cancers



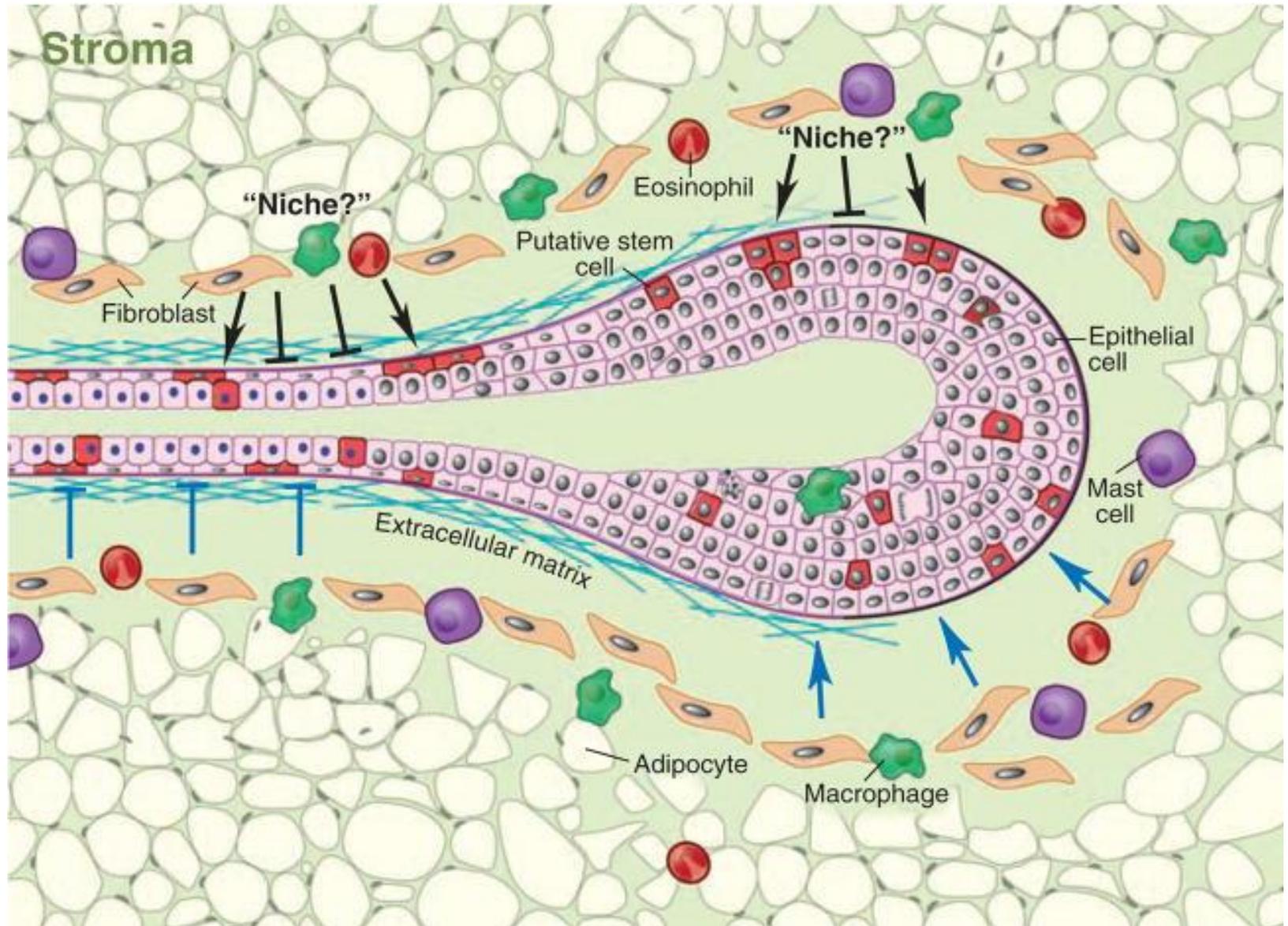
Molecular Classification and Subtypes of Breast Cancer



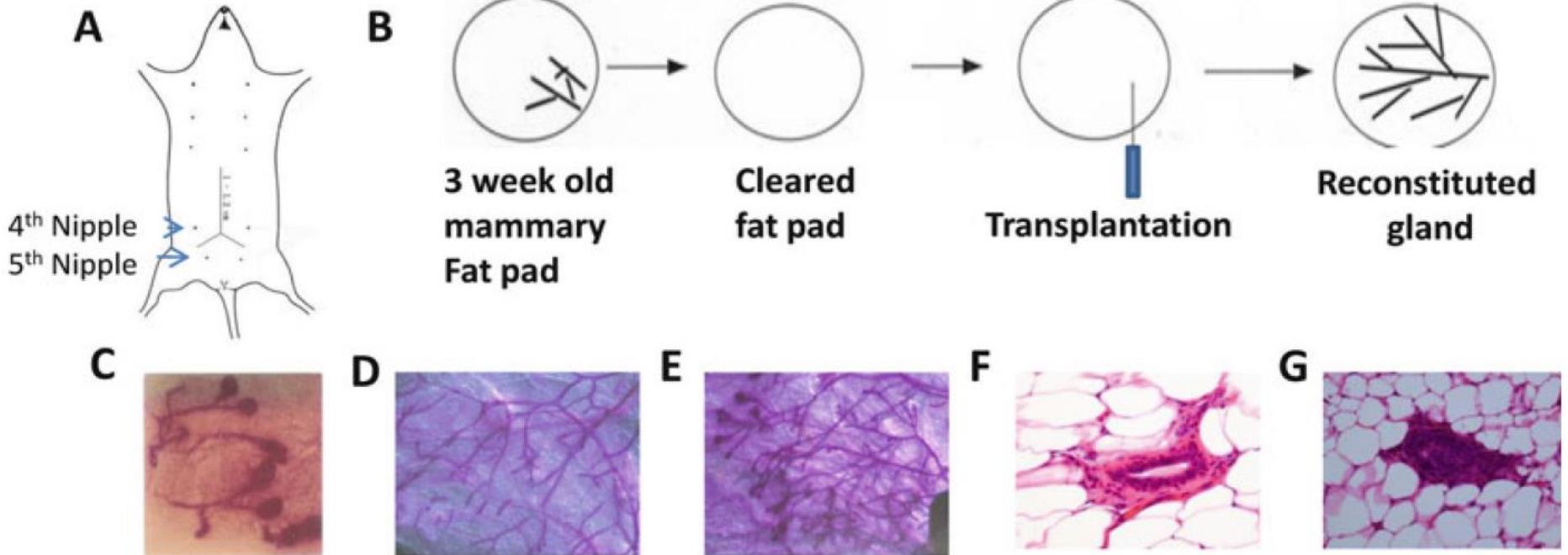
Postnatal Mouse Mammary Gland Development



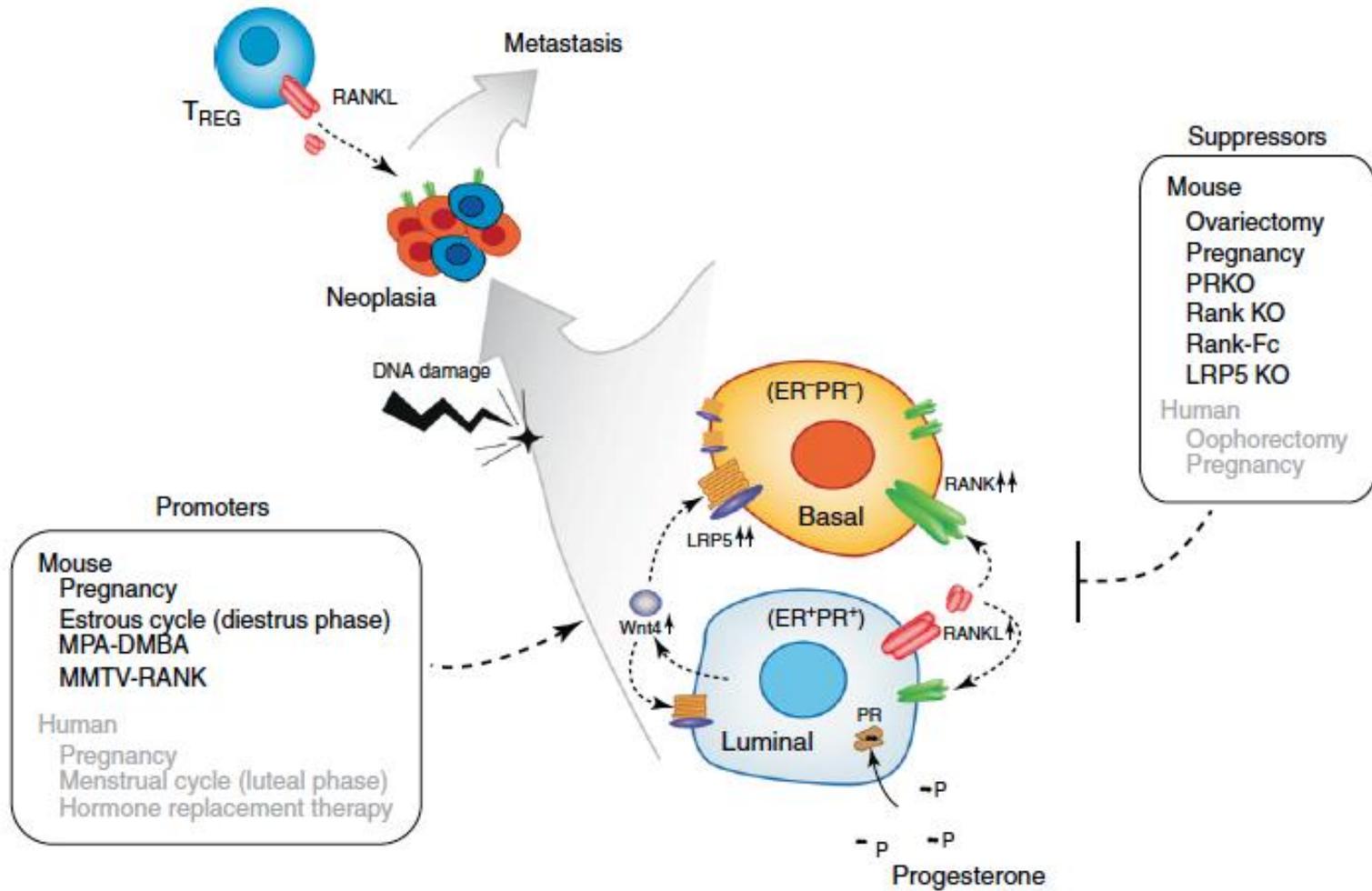
Virgin Mouse Mammary Terminal End Bud (TEB)



Mammary Fat Pad Transplantation for Mammary Gland Reconstitution



Autocrine and Paracrine Effectors of MaSC Development



Assays for Assessing Cancer Stem Cell Activity

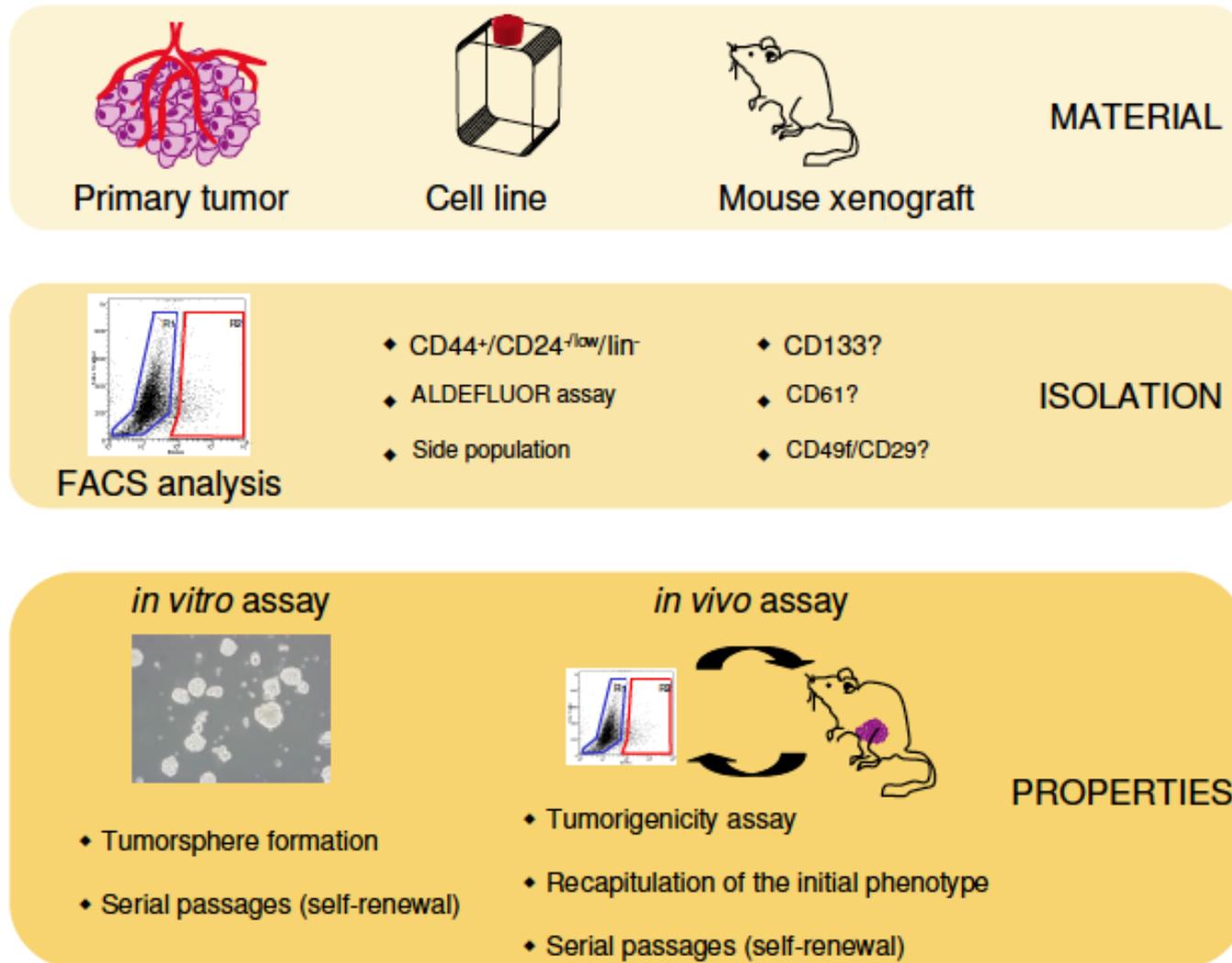


Figure 1
Markers and model for breast cancer stem cell studies. The main assays, markers and models used to study breast cancer stem cells are schematically represented. Models and assays rely on the main stem cells properties that are self-renewal ability and differentiation potential. The various markers illustrate the great phenotypic diversity of the cancer stem cell population.

Mammary epithelial cells

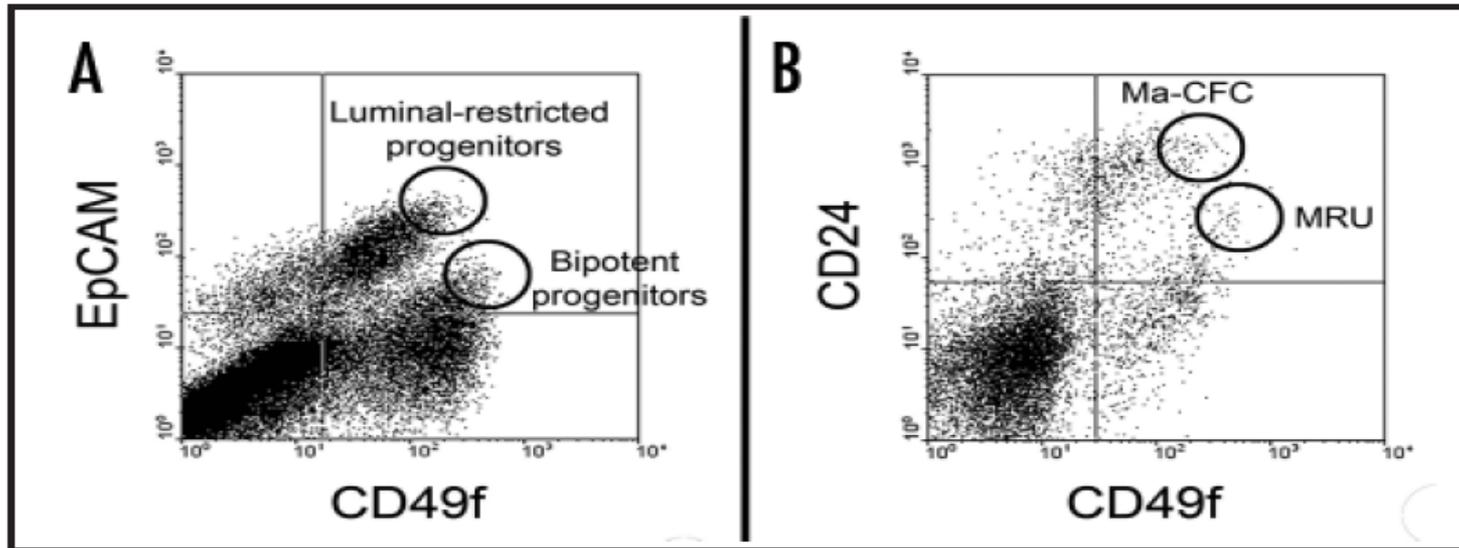


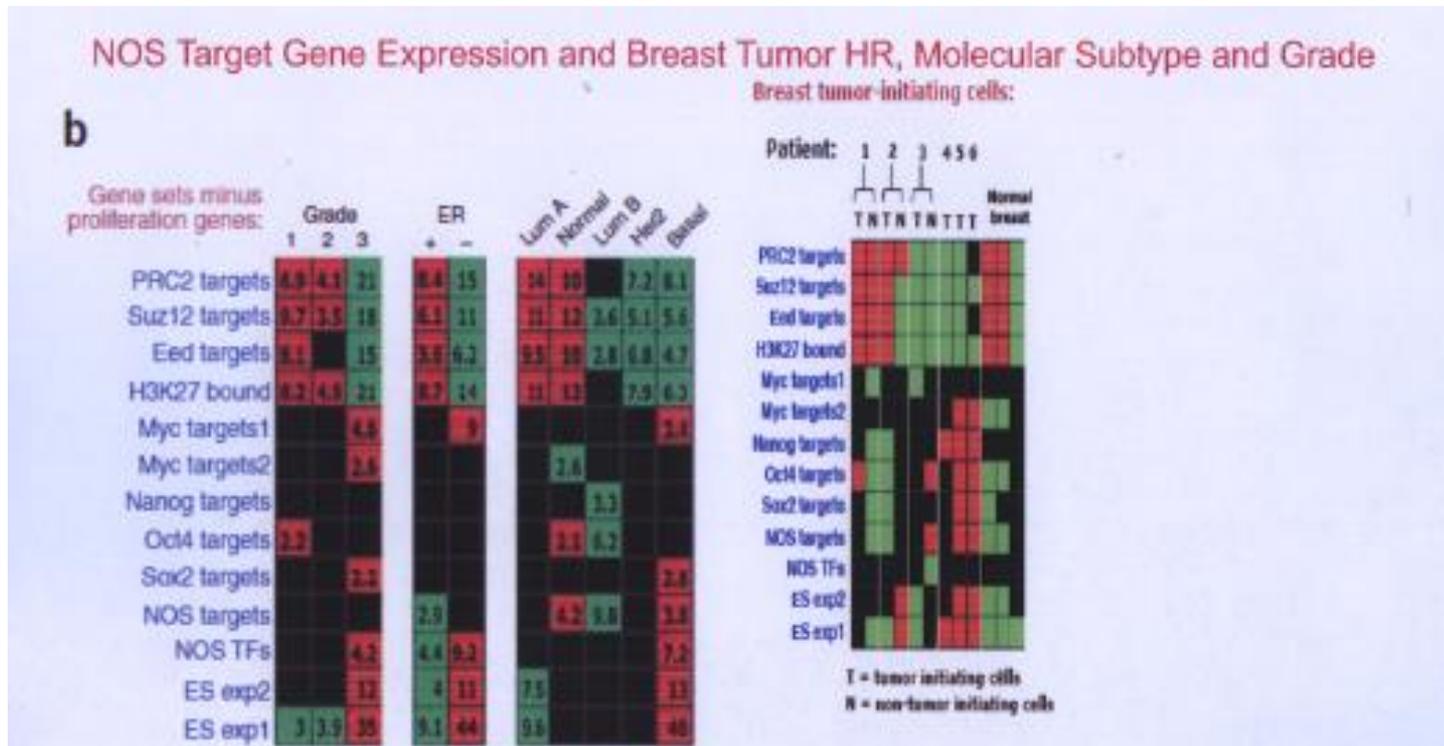
Figure 3. FACS profiles of phenotypically defined subsets of mammary epithelial populations. (A) Human mammary epithelial cells from three-day cultures of primary cells stained with specific antibodies to detect cell surface expression of EpCAM and CD49f as described in reference 9. Subpopulations that are enriched for progenitors that generate pure luminal cell colonies and multi-lineage colonies in vitro are indicated. (B) Freshly isolated mouse mammary cells were depleted of hematopoietic and endothelial cells and then stained with specific antibodies to detect cell surface expression of CD24 and CD49f as described in reference 11. The subpopulation that is enriched for the progenitors (Ma-CFCs) that generate colonies in adherent in vitro colony assays as well as a subpopulation that is highly enriched for mammary stem cells (MRUs) are indicated.

Mammary surface markers

Table 1. Commonly Used Surface Markers to Identify Mouse and Human Mammary Stem Cells

Mammary Gland Stem Cells	Marker
Mouse	CD24, CD29, CD49f, CD61, Sca-1.
Human	ALDH1, c-KIT, CD10, CD24, CD44, CD49f, CD90, CD133, EpCAM, MUC-1

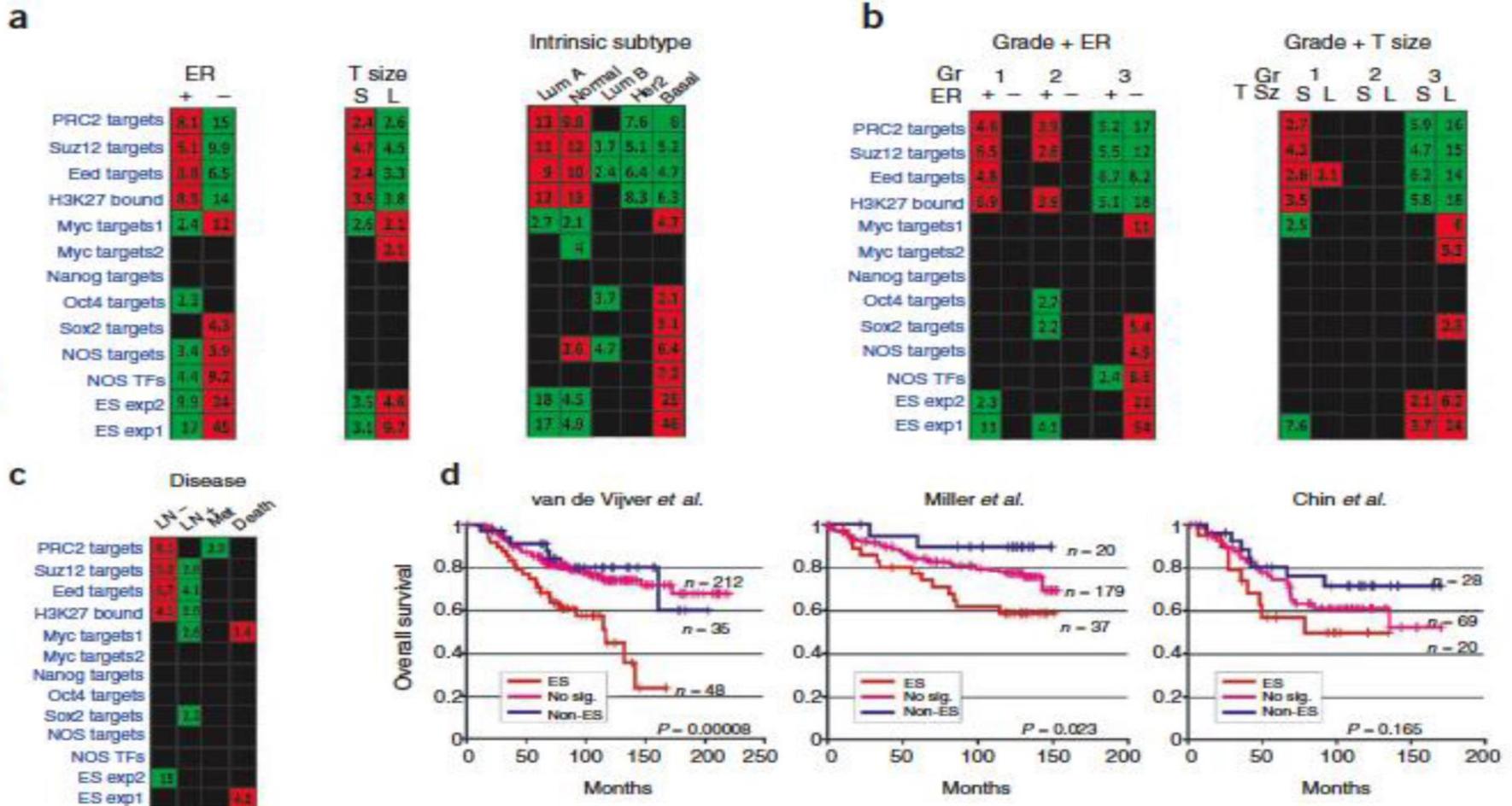
NOS Target Gene Expression and Breast Tumor HR, Molecular Subtype and Grade



Supplementary Figure 3. Gene-set enrichments in breast cancer tumor-initiating and non-tumor-initiating cell fractions. Gene set enrichments in the tumor-initiating ($CD44^{high}/CD24^{low}$) fraction (T) and the non-tumor initiating fraction (N) isolated from 3 individual breast tumors (1,2,3) (Ref. 43). Also included are the tumorigenic fraction from 3 additional tumors (4,5,6) and three normal breast samples profiled in this study.

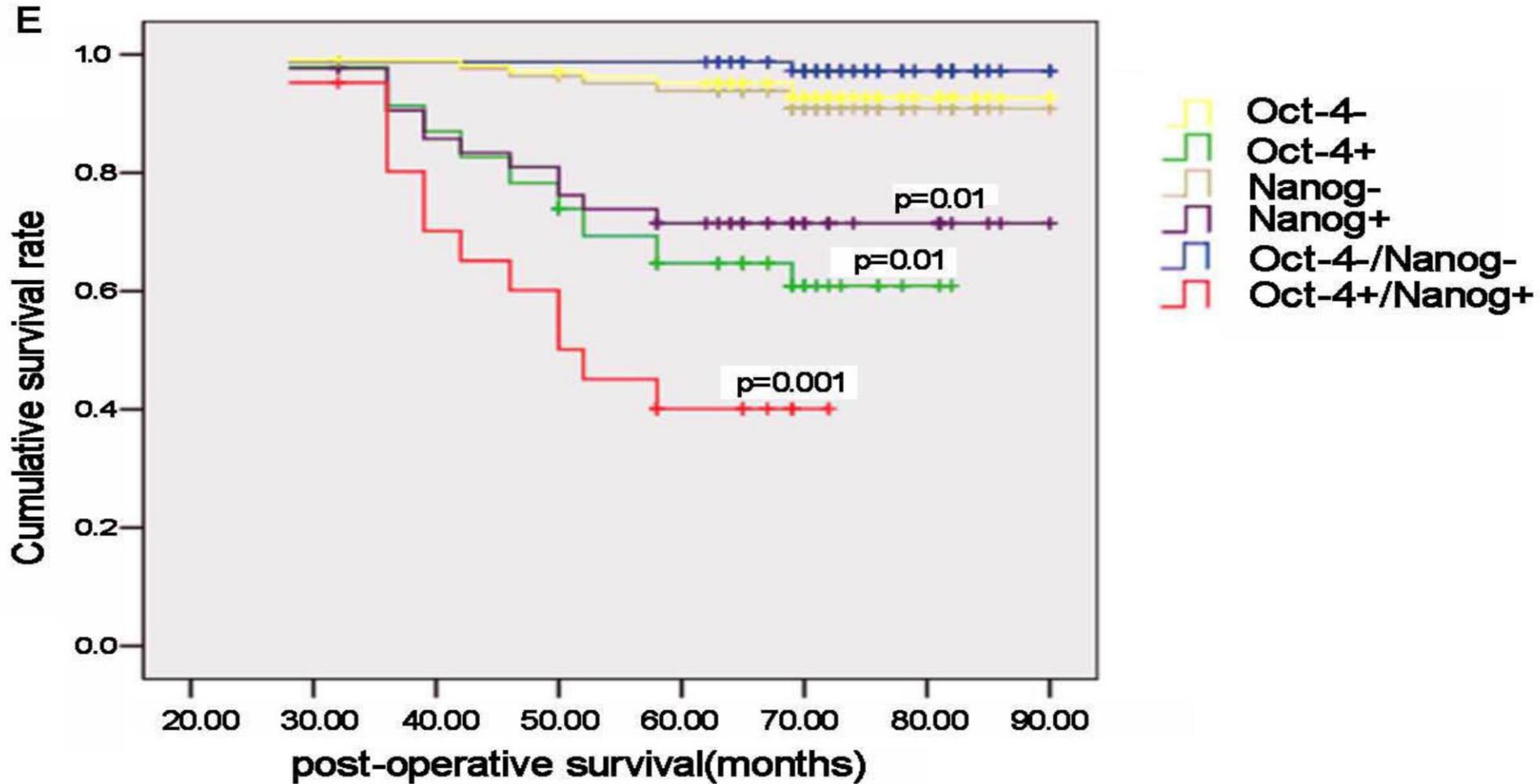
NOS signature in Breast Cancer

NOS Signature in Breast Cancer Relative to ER status, Tumor Size, Subtype and Grade versus Survival



NOS genes versus survival

Expression of NOS Genes in Breast Tumors versus Survival



Cancer stem marker and their distribution

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]

Therapeutic implications

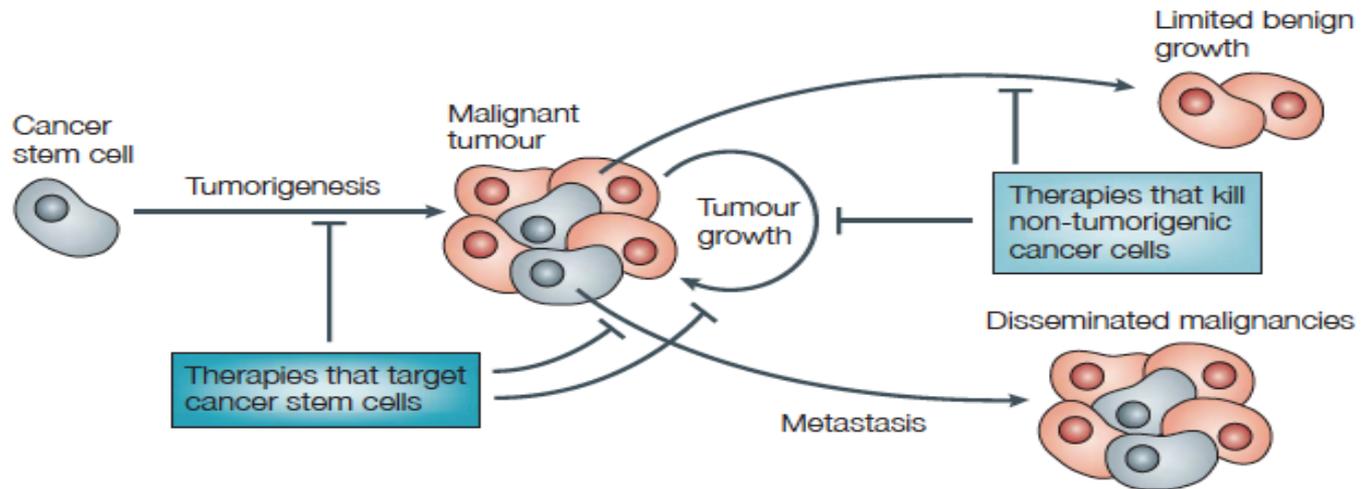
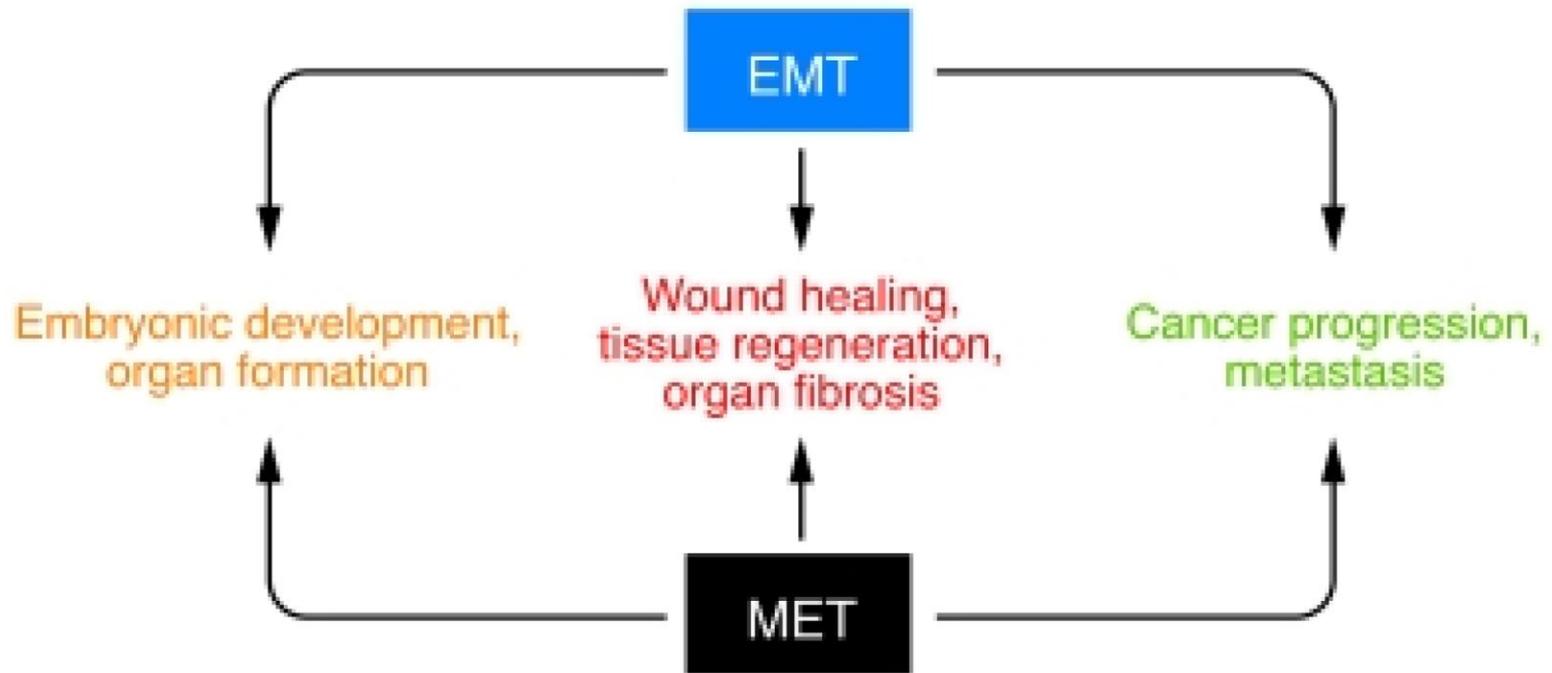


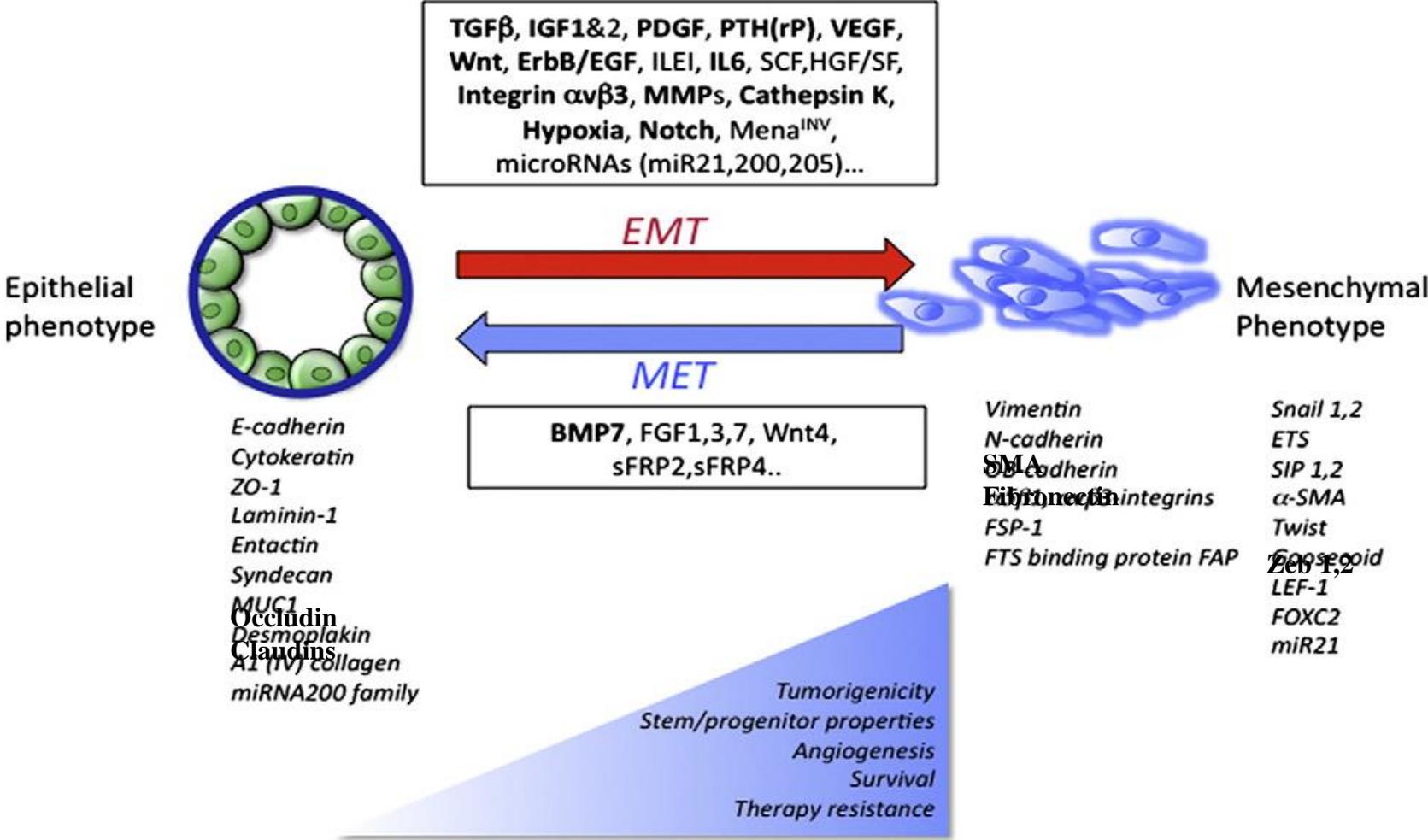
Figure 2 | **Therapeutic implications of cancer stem cells.** Cancer stem cells (grey) self-renew and differentiate within tumours to form additional cancer stem cells as well as non-tumorigenic cancer cells (orange), which have limited proliferative potential. As the tumour grows, these cells can either undergo limited benign growth or form disseminated malignancies. Therapies that kill, induce differentiation or prevent the metastasis of cancer stem cells represent potential cures. Therapies that kill primarily non-tumorigenic cancer cells can shrink tumours, but will not cure the patient because the cancer stem cells will regenerate the tumour. By prospectively identifying and characterizing cancer stem cells it might be possible to identify more effective therapies. The intrinsic differences in tumorigenic potential among cancer cells might also explain why it is possible to detect disseminated solid cancer cells in patients that never develop metastatic disease. The identification and characterization of cancer stem cells should therefore also lead to diagnostic methods that can distinguish between disseminated tumorigenic and non-tumorigenic cells, as well as provide a better understanding of the mechanisms that regulate migration of cancer stem cells.

Cancer Progression

Developmental Processes Usurped in Cancer Progression



EMT



EMT facilitates metastatic spread of cancer cells

EMT Facilitates Metastatic Spread of Cancer Cells

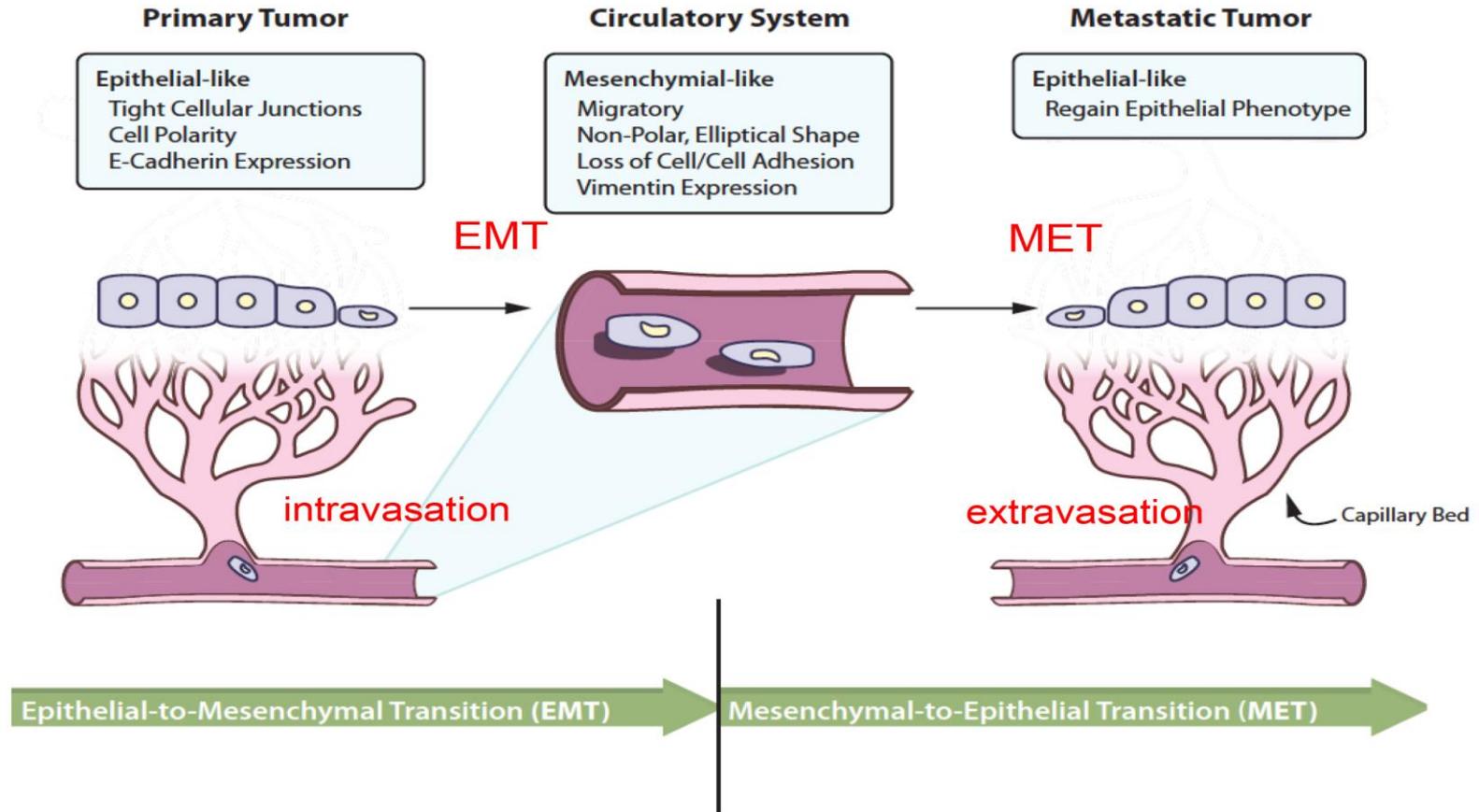
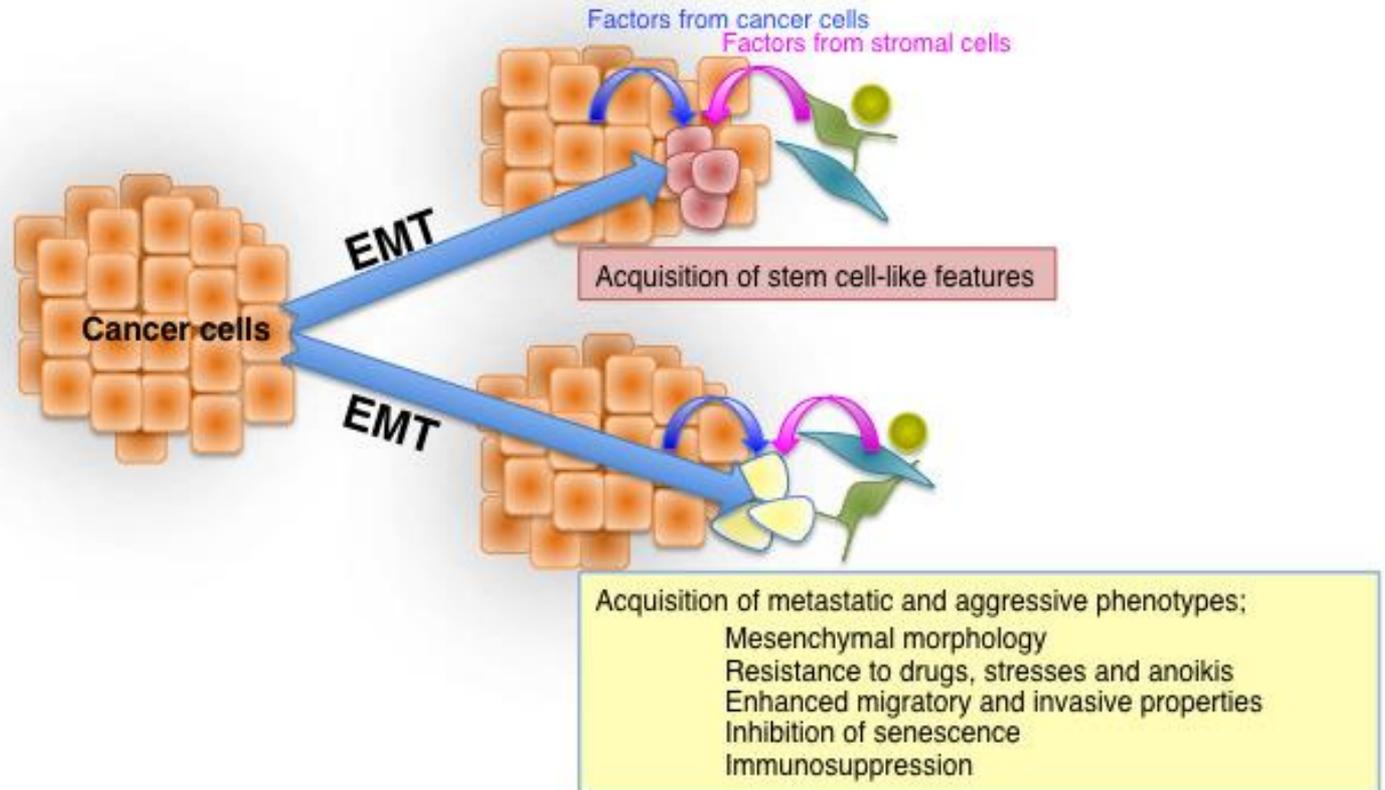


Figure 1. Epithelial-to-mesenchymal transition, mesenchymal-to-epithelial transition, and the migration of cancer stem cells. In the presence of stimulatory signaling (that is, Hedgehog (Hh), Notch, Wnt, transforming growth factor (TGF)- β) primary tumor cells may undergo epithelial-to-mesenchymal transition (EMT), a process where cells suppress E-cadherin expression and lose their tight membrane junctions. Cells can acquire a mobile phenotype and migrate into the circulatory system by entering capillary beds. Exiting the circulatory system at a distant anatomical site, cells undergo the reverse process of mesenchymal-to-epithelial transition (MET), reacquiring their original non-mobile epithelial-like phenotype.

EMT Induces a Cancer Stem Cell Phenotype on Differentiated Tumor Cells

Figure 1



Nanog and Oct4 Induction of EMT Target Genes in Breast CSCs

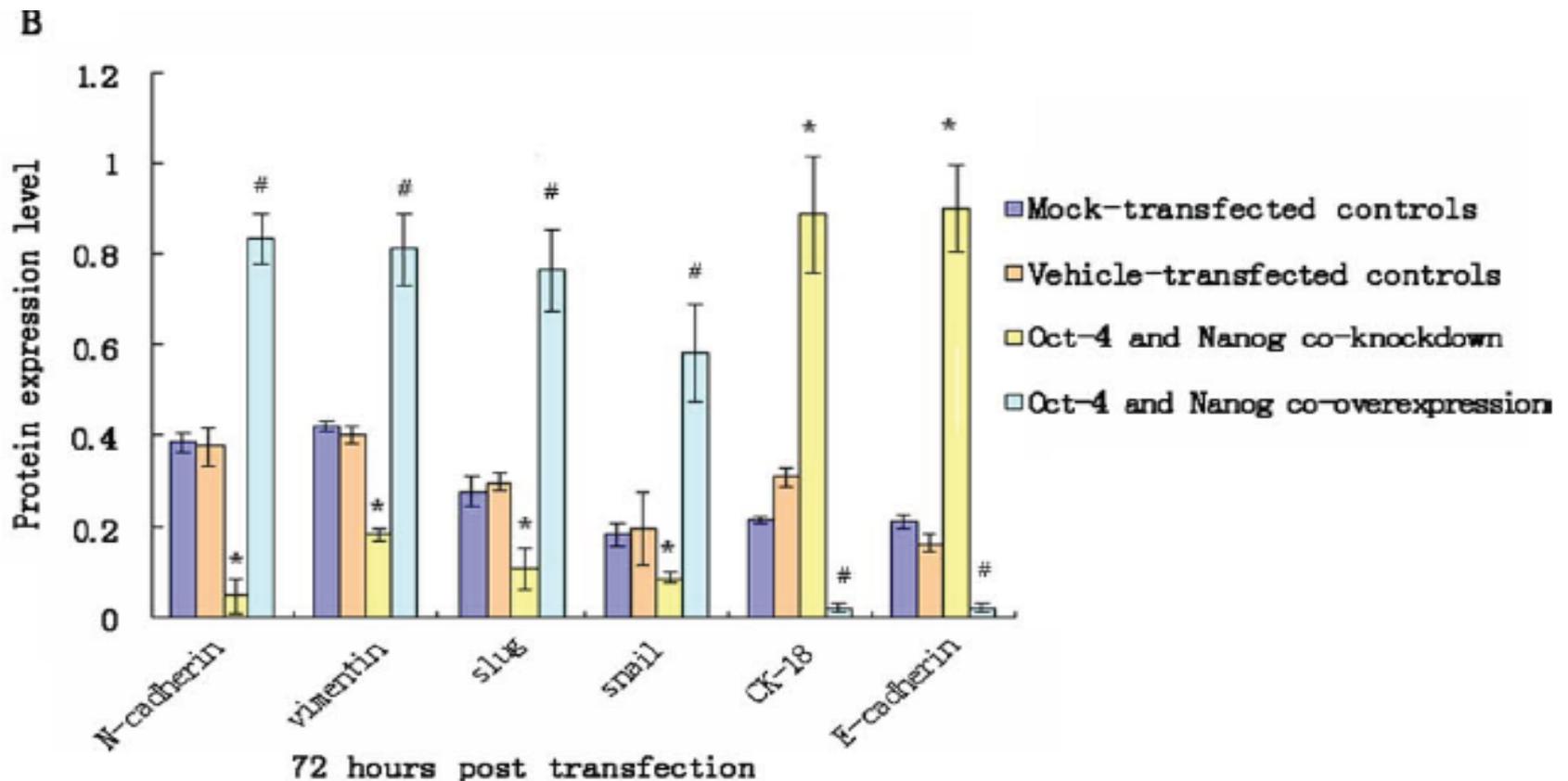
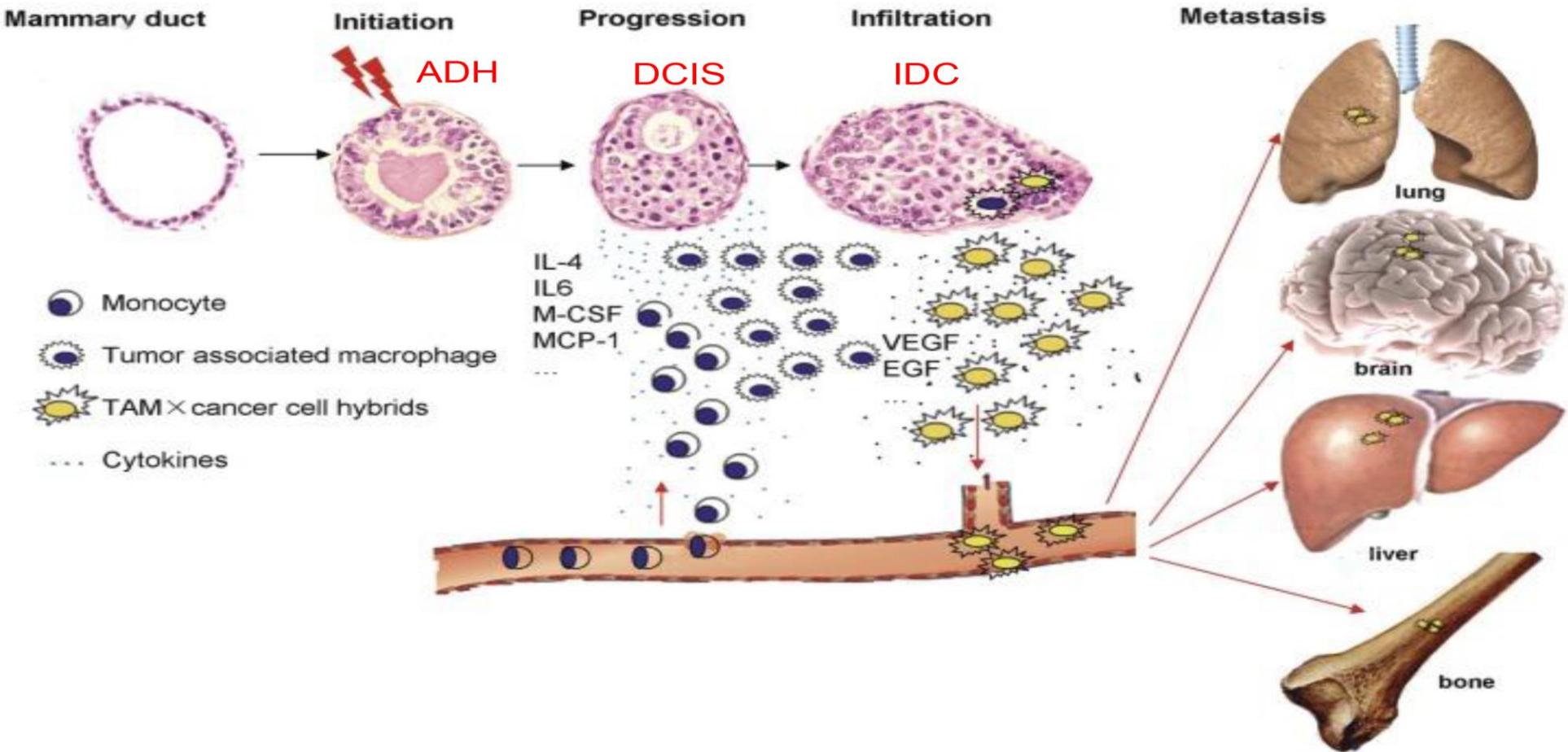


Figure 3: Western blot analyses of relative expression levels of epithelial-mesenchymal transition (EMT)-related genes in cancer stem cells (CSC) following modulating Oct-4 and/or Nanog expression *in vitro*. CSC were transfected with mock or Oct-4 and Nanog siRNAs, vehicle, or Oct-4 and Nanog-expressing 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.

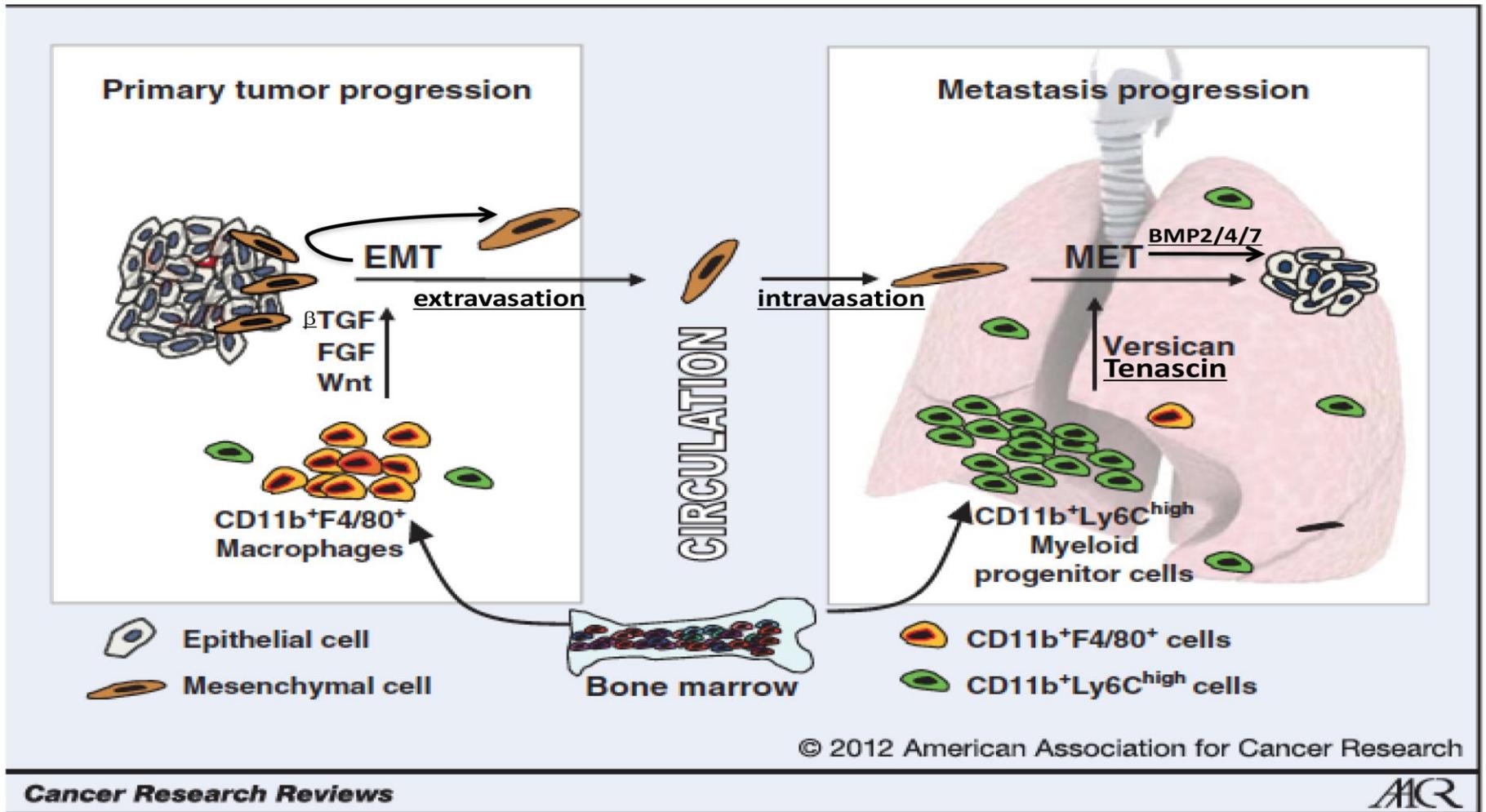
Breast Cancer Development

Breast Cancer Development

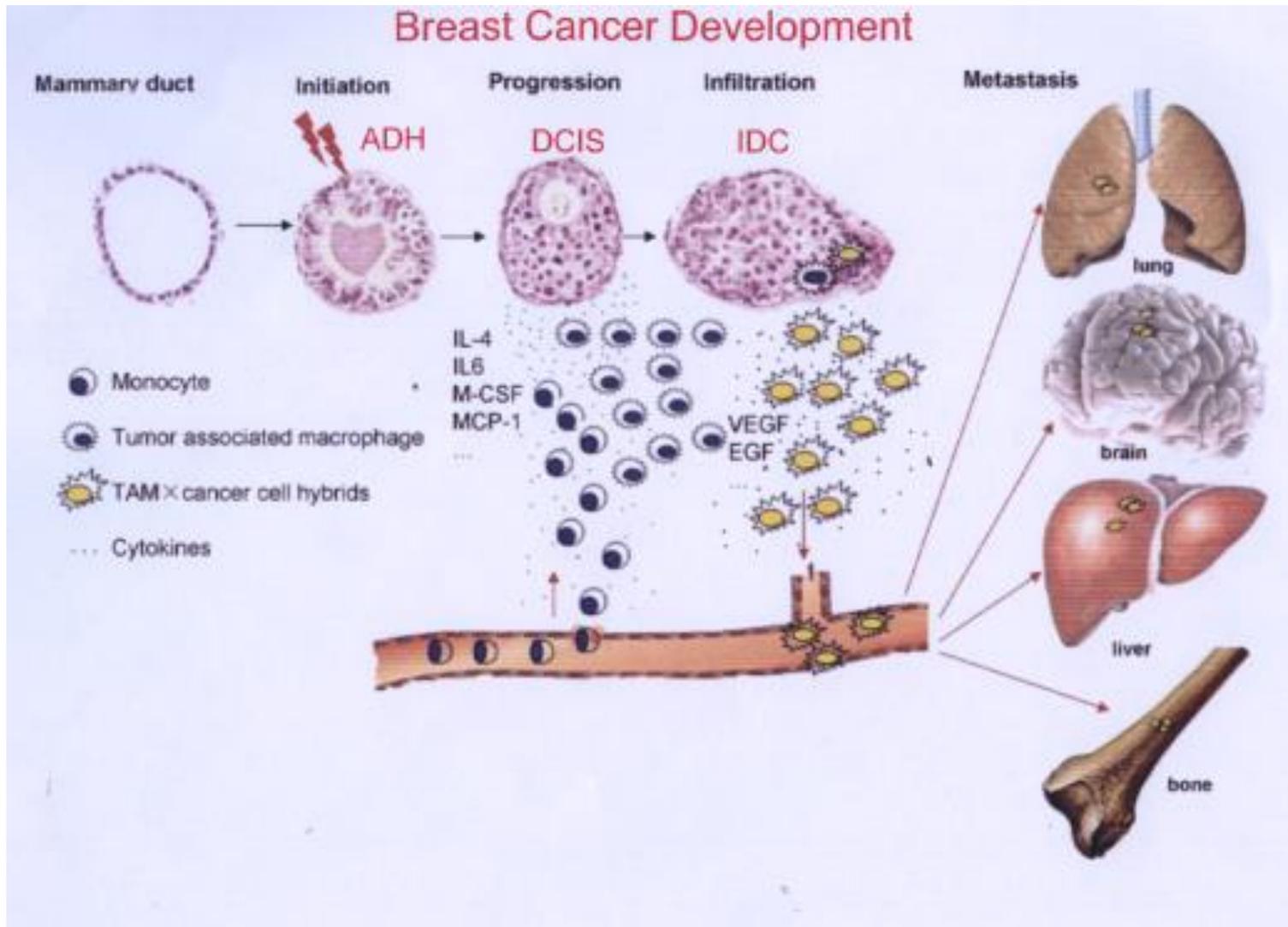


Breast Cancer Niche Factors

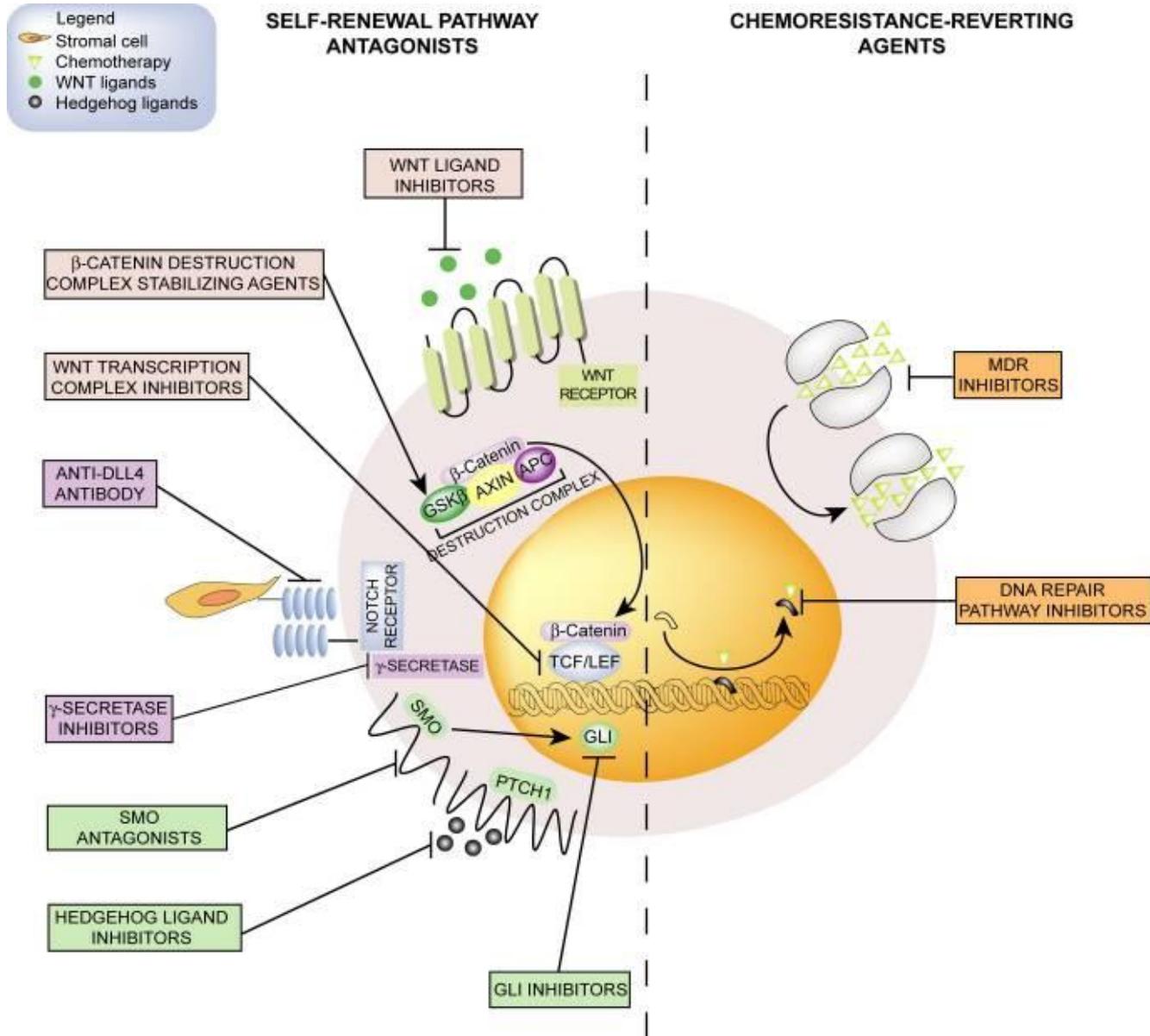
Primary Breast Cancer and Metastatic Niche Factors



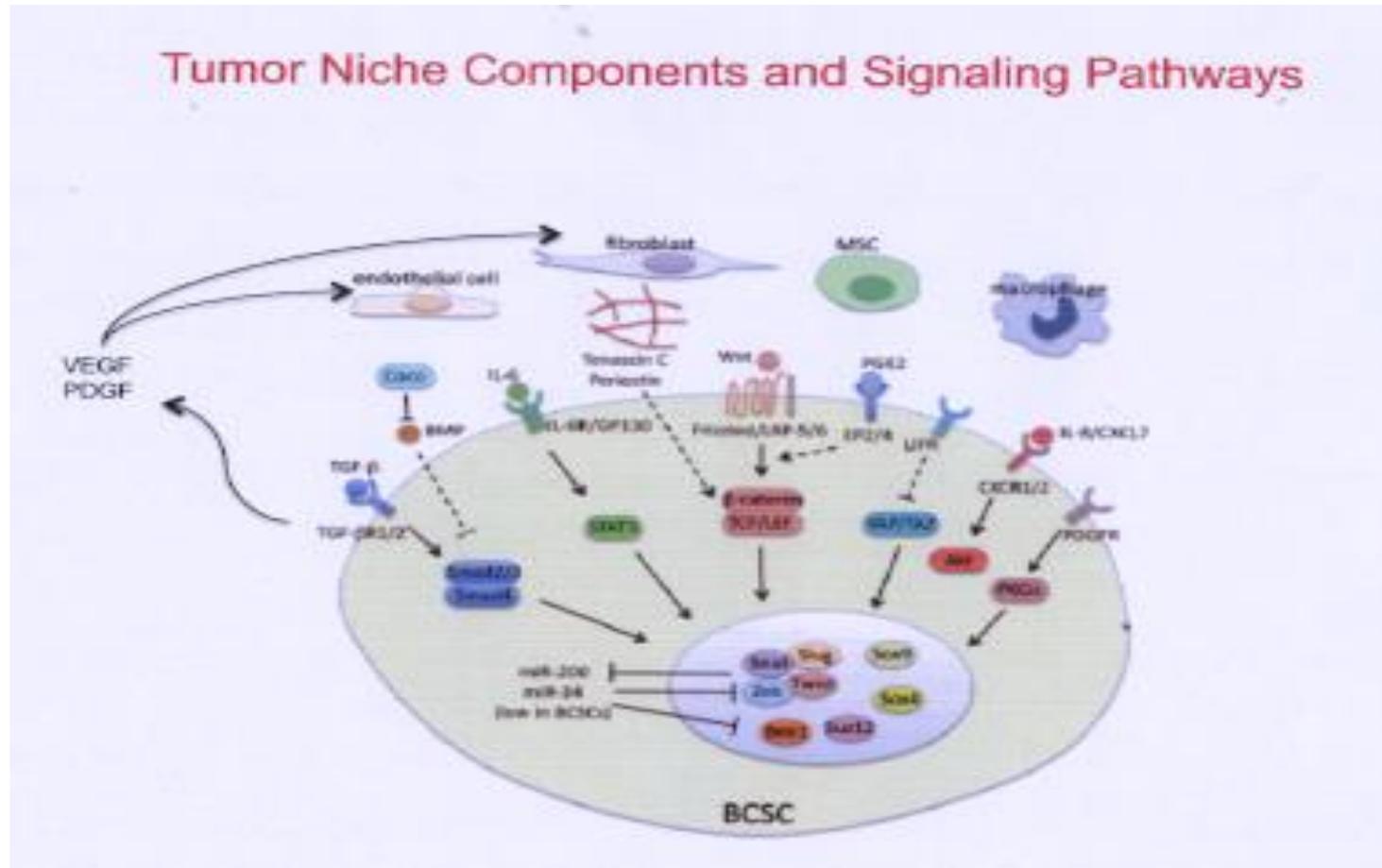
Breast Cancer Development



TARGETING CANCER STEM CELL SIGNALING PATHWAYS



Tumor niche components and signaling pathways



Therapeutic Targeting of CSCs, the Tumor Niche or Epigenetic Pathways

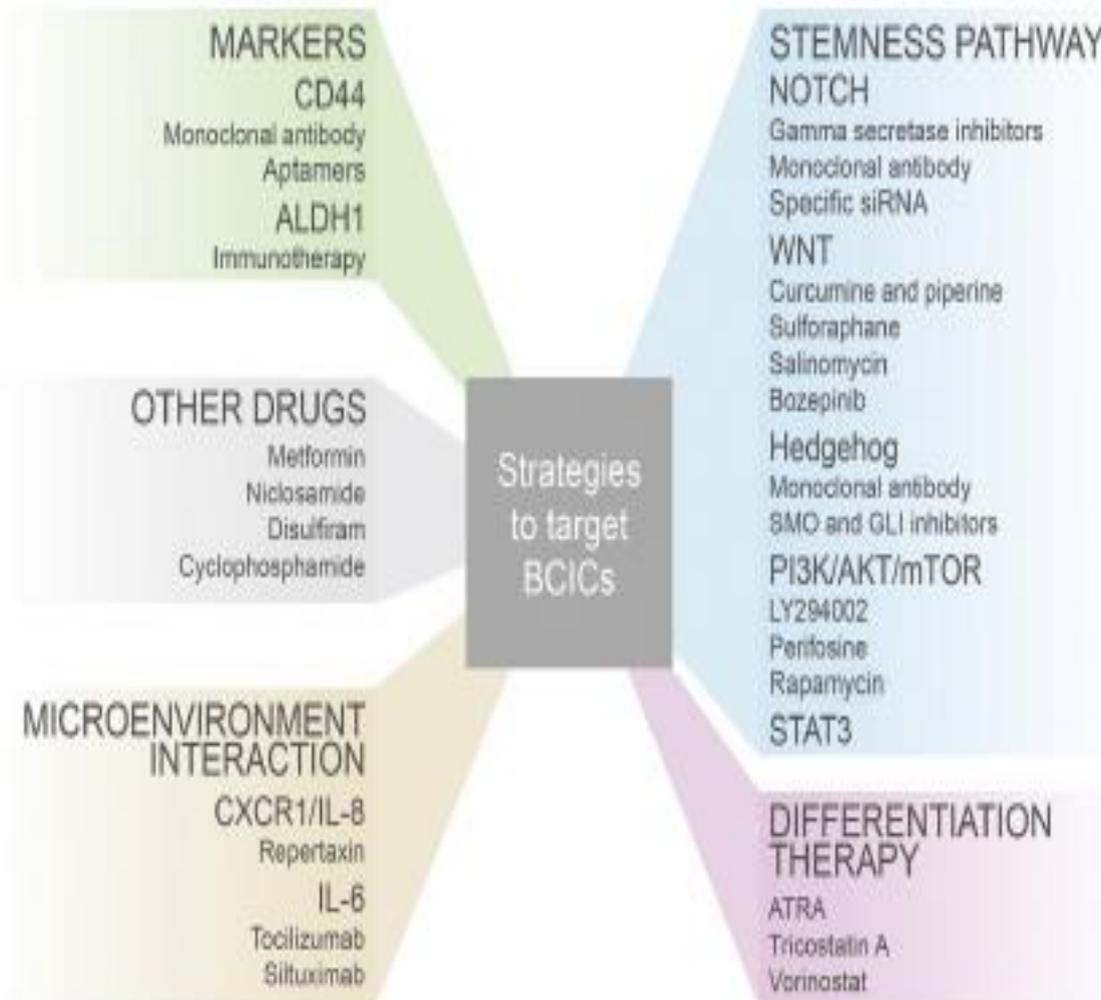


Fig. 1. Schematic diagram of emerging strategies and related compounds to target breast cancer-initiating cells (BCICs).