Glycans and Cancer/Stem Cells

Glycosaminoglcyan and Cancer/stem cells

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Learning Objectives

1. Develop conceptual understanding of Stem cells.
    1. Specifically understand similarities and differences between:
        1. Embryonic stem cells (ESC),
        2. Induced pluripotent stem cells (iPSC) and
        3. Cancer Stem Cells (CSC)

2. Understand the role of N/O-glycans in regulating Stem cells growth and fate.

3. Understand the clinical utility of N/O-glycans based stem cell biomarkers.

4. Recognize the role of integrated glycomics as a powerful tool to comprehensively characterize N/O-glycome of the stem cells.

Stem Cell Theory

Embryonic Stem cells (hESC or mESC)

Induced Pluripotent Stem Cells (iPSC)

Cancer Stem Cells (CSC)

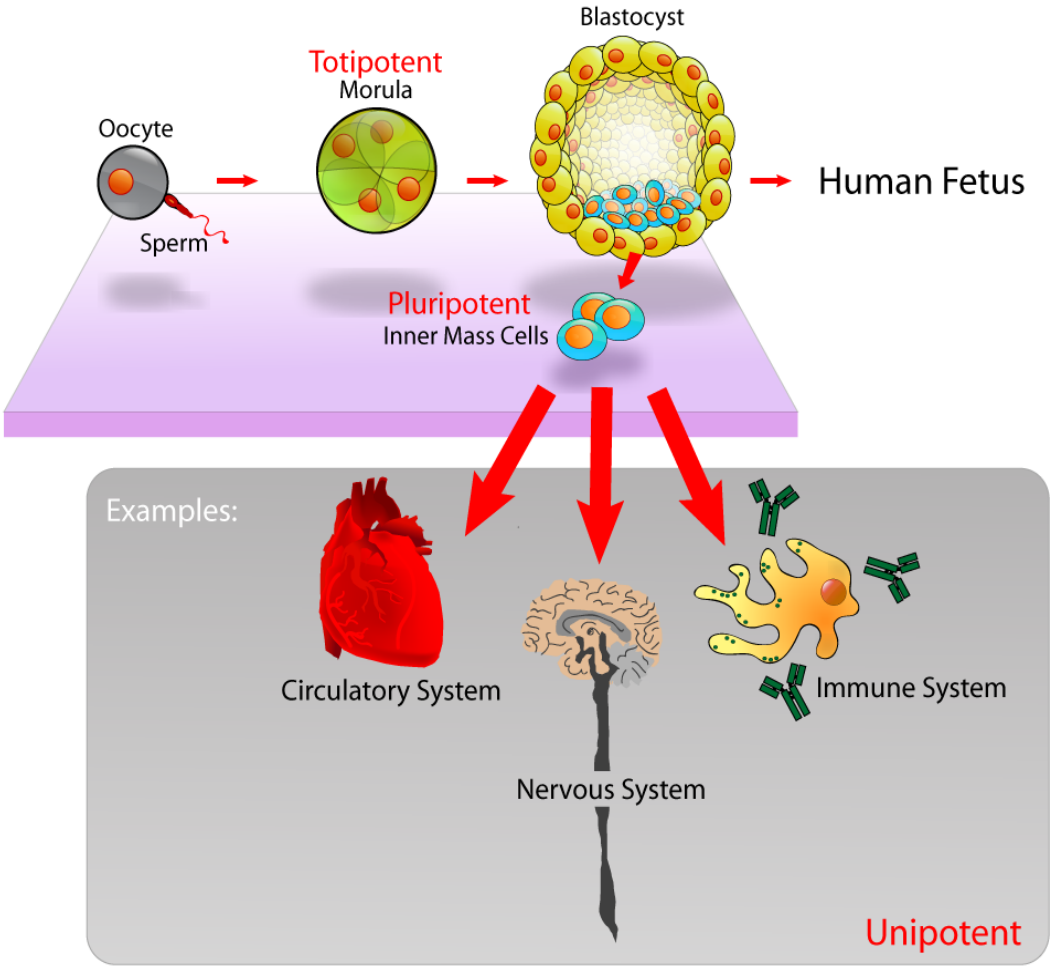
Stem Cells

Definition:

Stem cells are biological cells that possess the ability to differentiate into diverse specialized cell types as well as self-renew to produce more stem cells.

They are found in both embryos (embryonic stem cells) and/or adult tissues (fetal or adult stem cells) of all muticellular organisms.

Hierarchy of Stem Cells



Totipotent: embryonic and extra-embryonic tissues

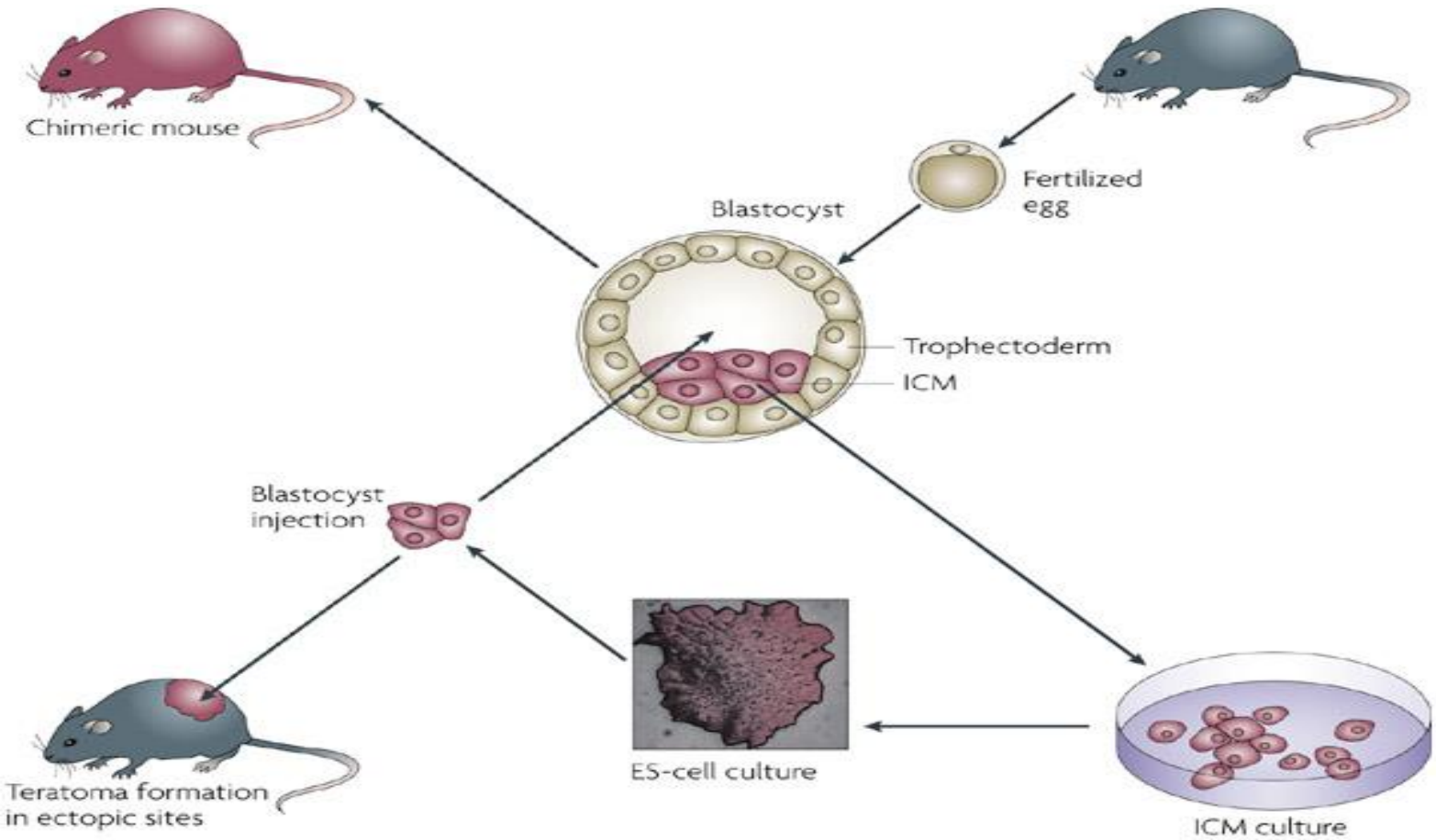
Pluripotent: all three embryonic germ layers

Mutipotent: closely related family of cells/organ type (e.g: mesenchymal stem cells)

Unipotent: only one cell type

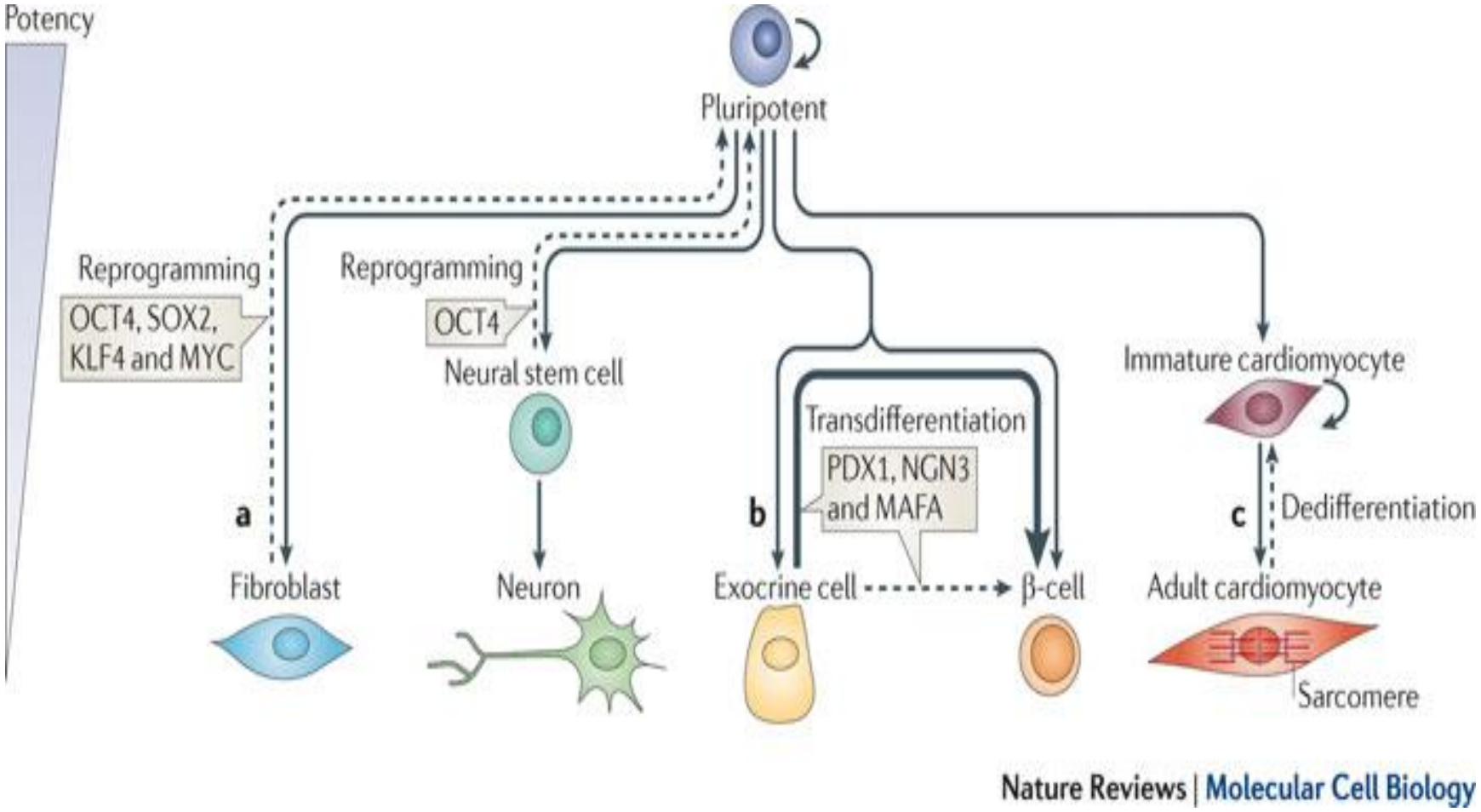
Adapted from Wikipedia- Stem cells

Derivation of Embryonic Stem Cells



Nature Reviews | Molecular Cell Biology

Reprogramming-Dedifferentiation



Induced Pluripotent Stem Cells (iPSC)

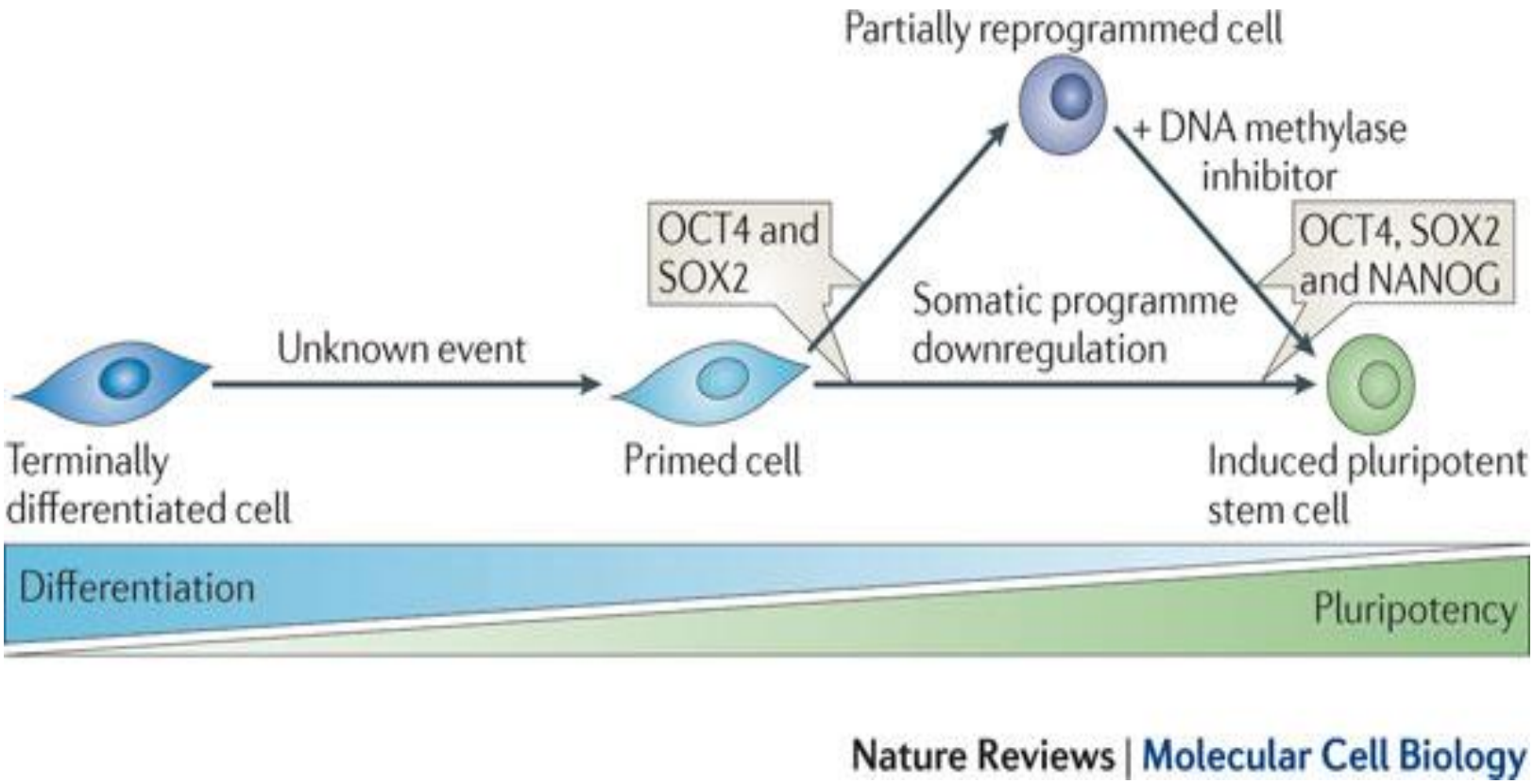
- Definition: iPSCs are pluripotent stem cells derived from differentiated cells by forced expression of reprogramming factors.

- 2012 Nobel Prize in Physiology and Medicine:

"for the discovery that mature cells can be reprogrammed to become pluripotent"

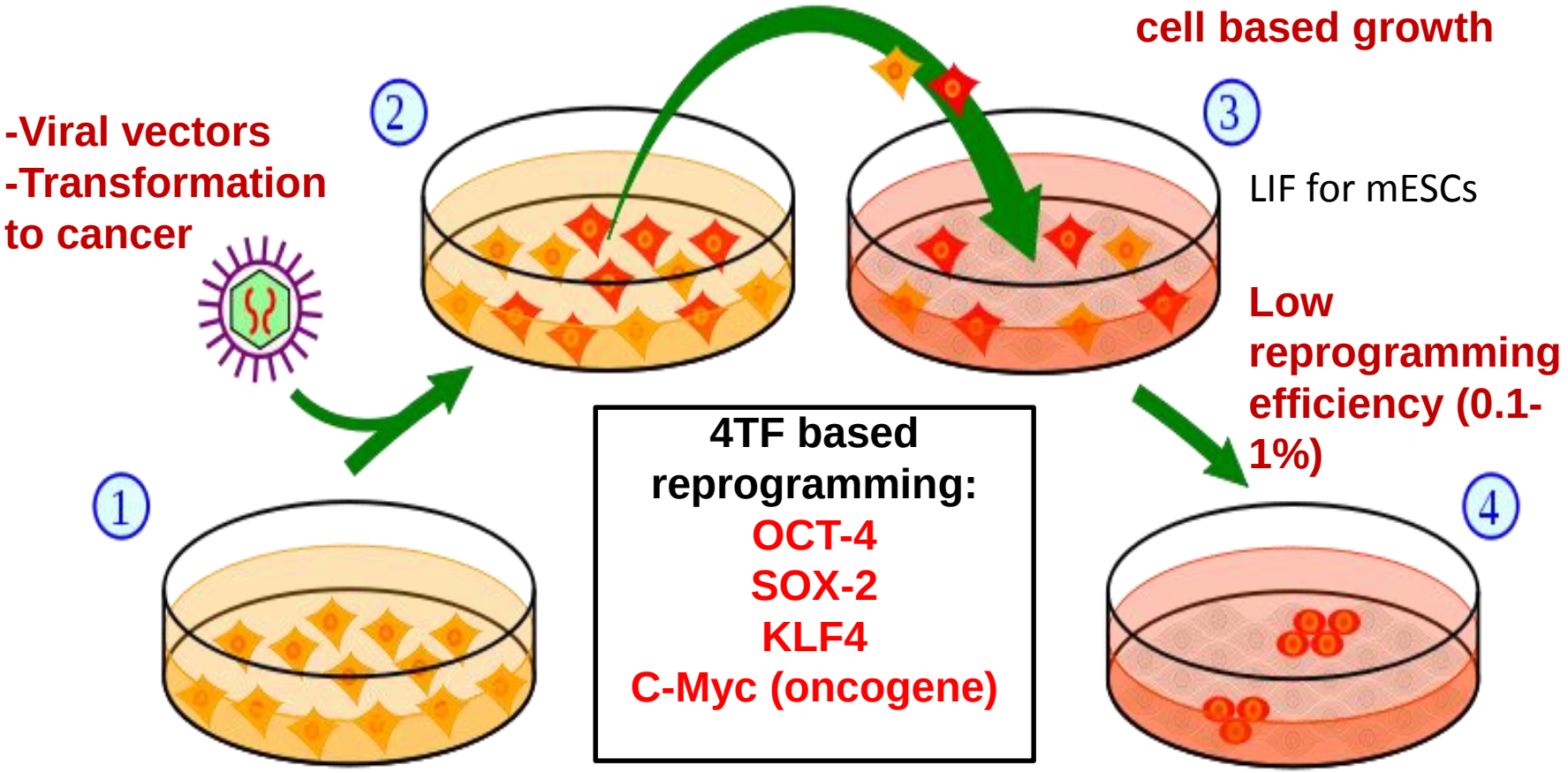
  - Shinya Yamanka- Japan
  - John Grudon-Cal Tech

Reprogramming of Fibroblast to iPSC

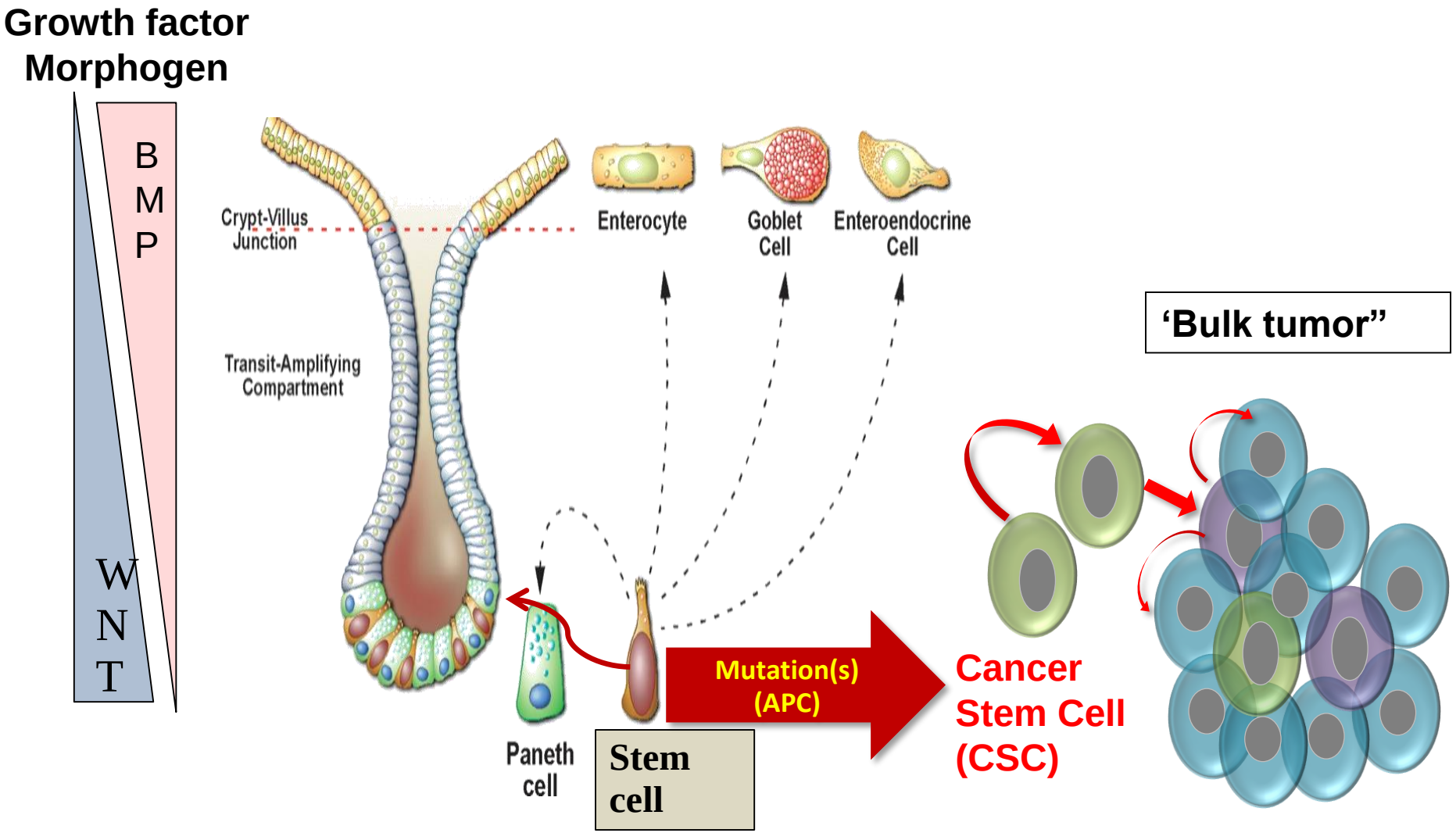


Nature Reviews | Molecular Cell Biology

Reprogramming of Fibroblast to iPSC

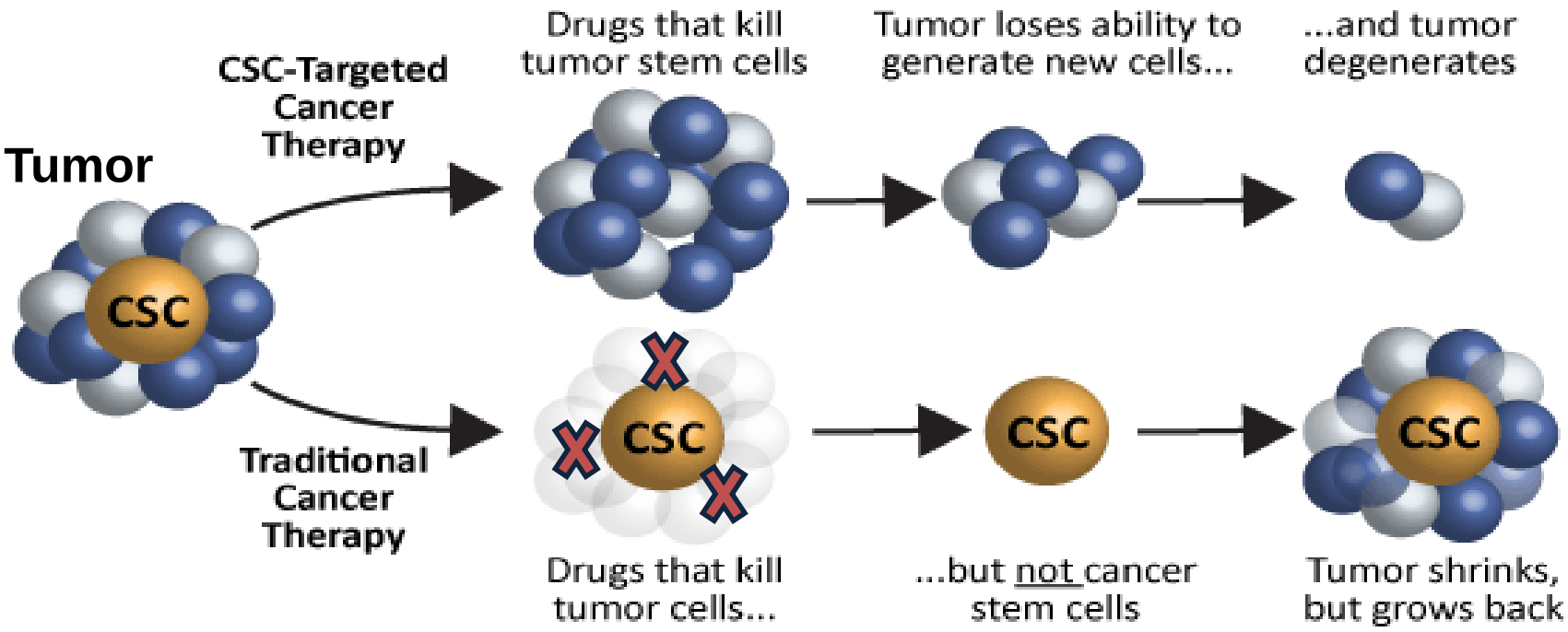


Intestinal Stem Cells
Adult vs. Cancer Stem Cells



Barker N et al. Genes Dev. 2008;22:1856-1864

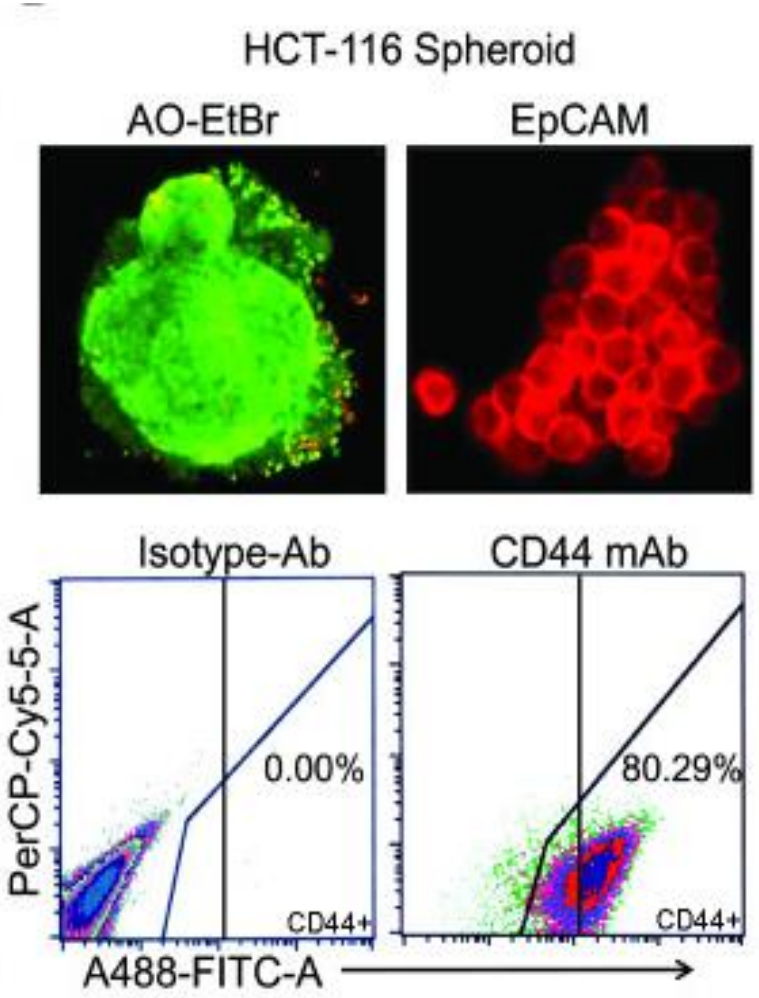
CSCs: Targets of Cancer Therapies

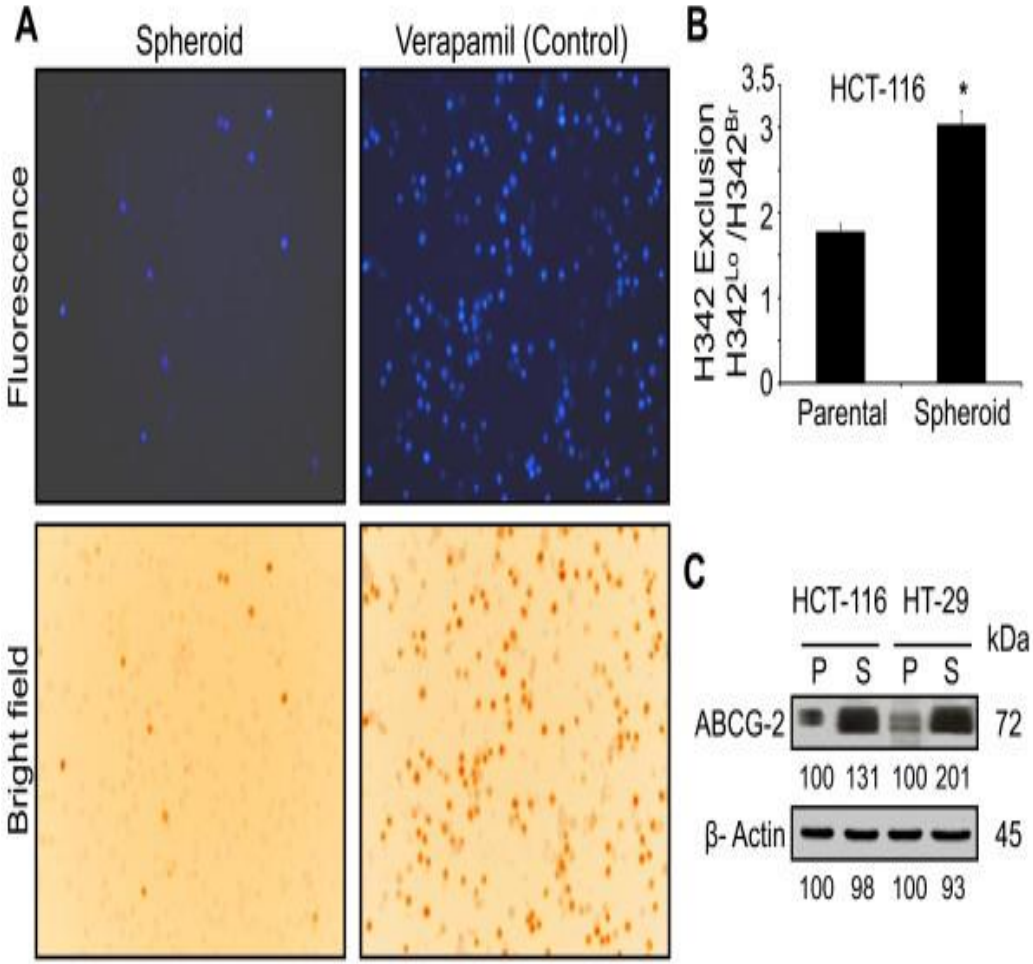


Adapted from: http://www.macrogenics.com/Platforms-cancer_stem_cells_csc.html

Characterization of Cells in Stem cell media

Chemoresistant HCT-116 Spheroid in SCM

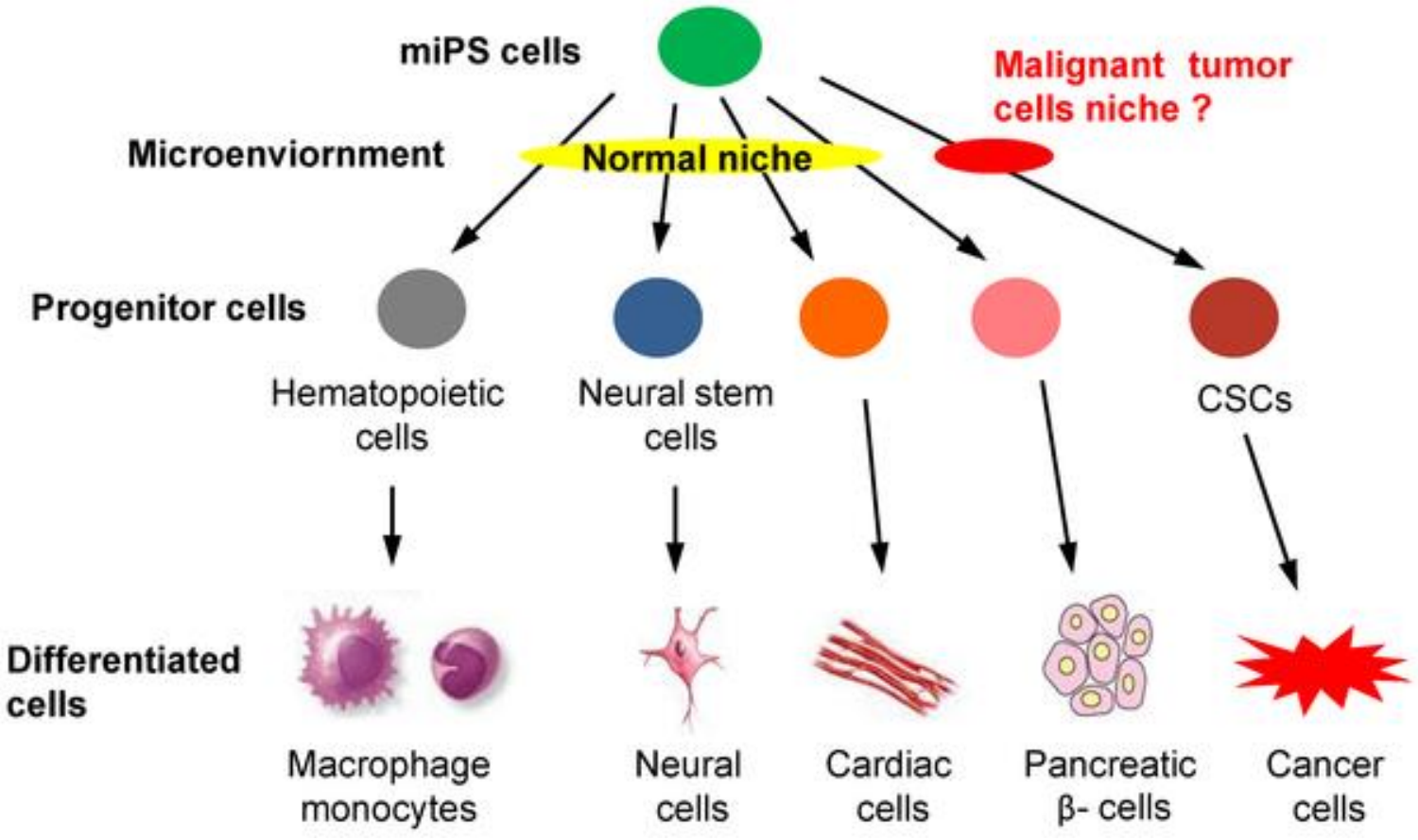




Colon CSC Markers

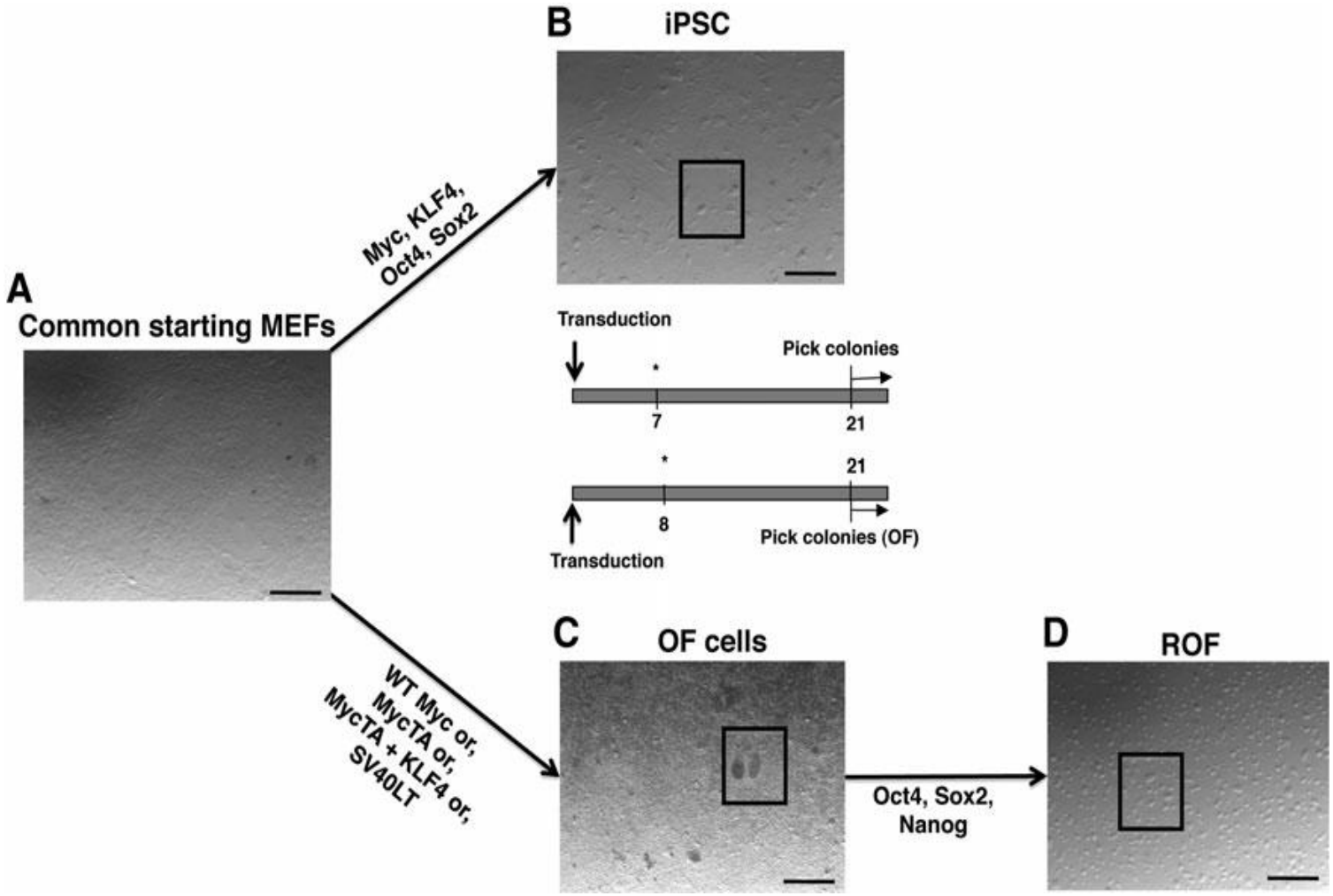
Relationship between ESC/iPSC and CSC

iPSC: a common source of adult & Cancer Stem Cells



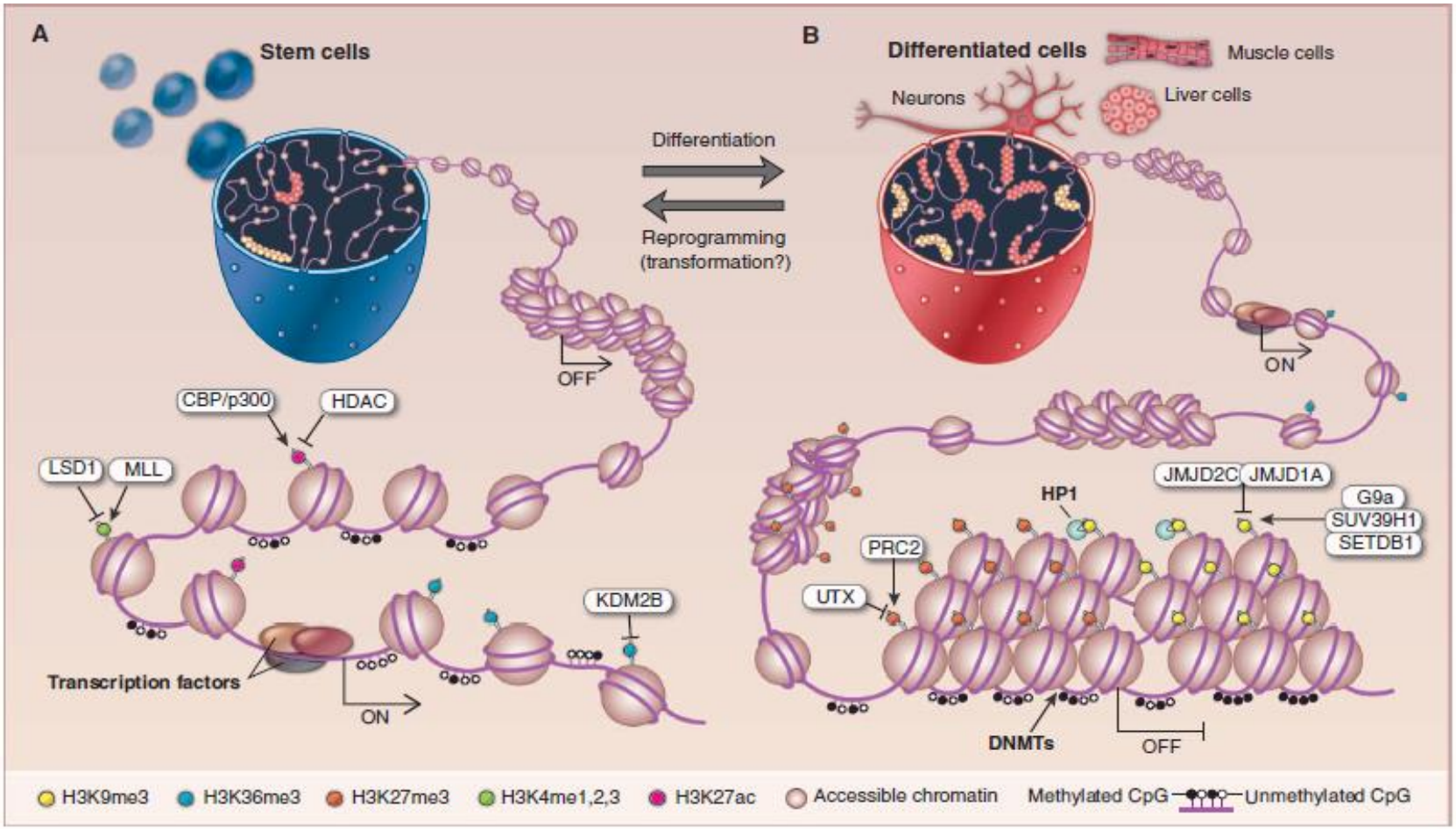
Chen L, et al. (2012) PLoS ONE

Can CSCs be reprogrammed into iPSC?

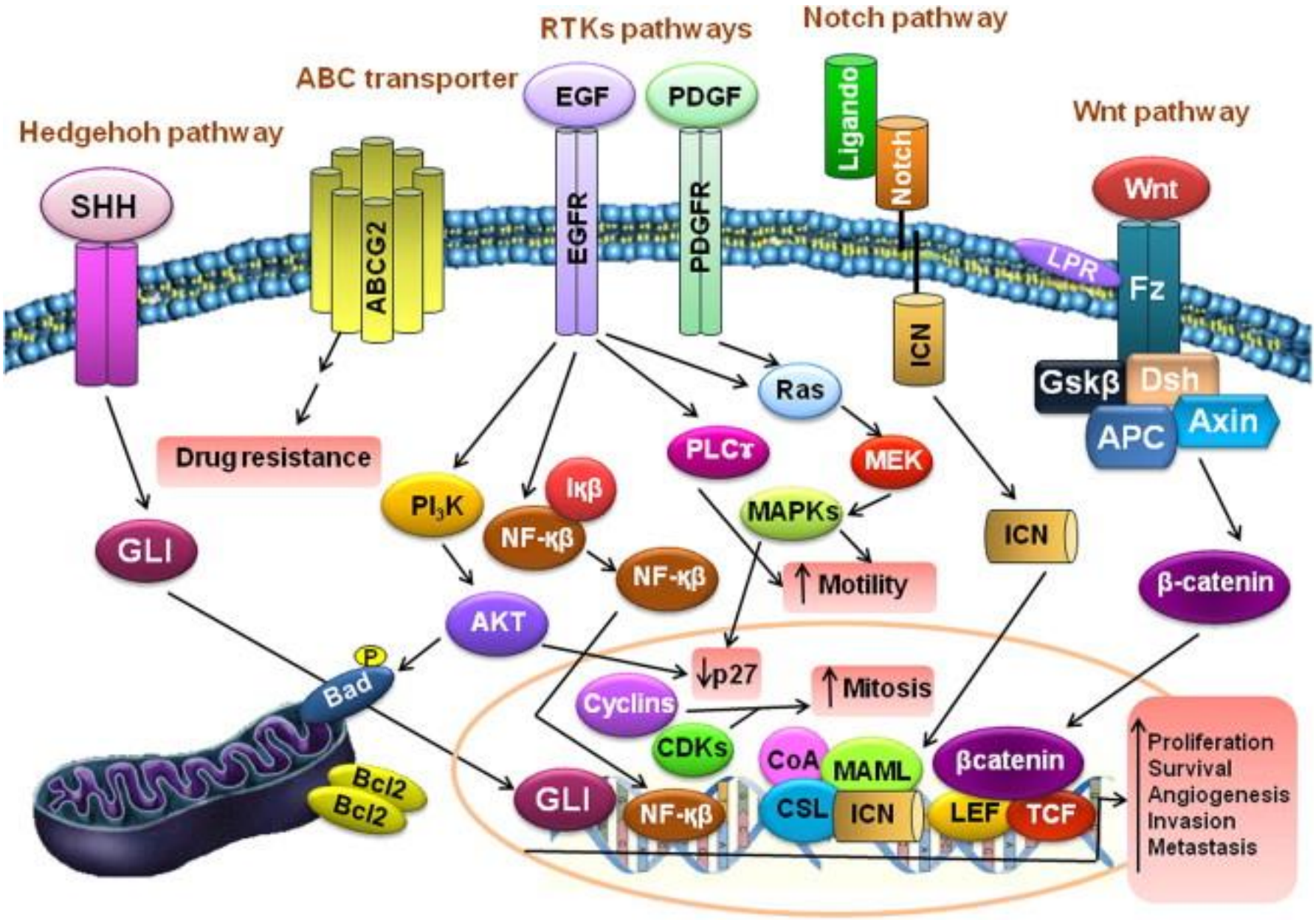


Riggs JL et al. Stem cell Dev. 2012

Epigenetic Process Dictates Stem Cell Fate

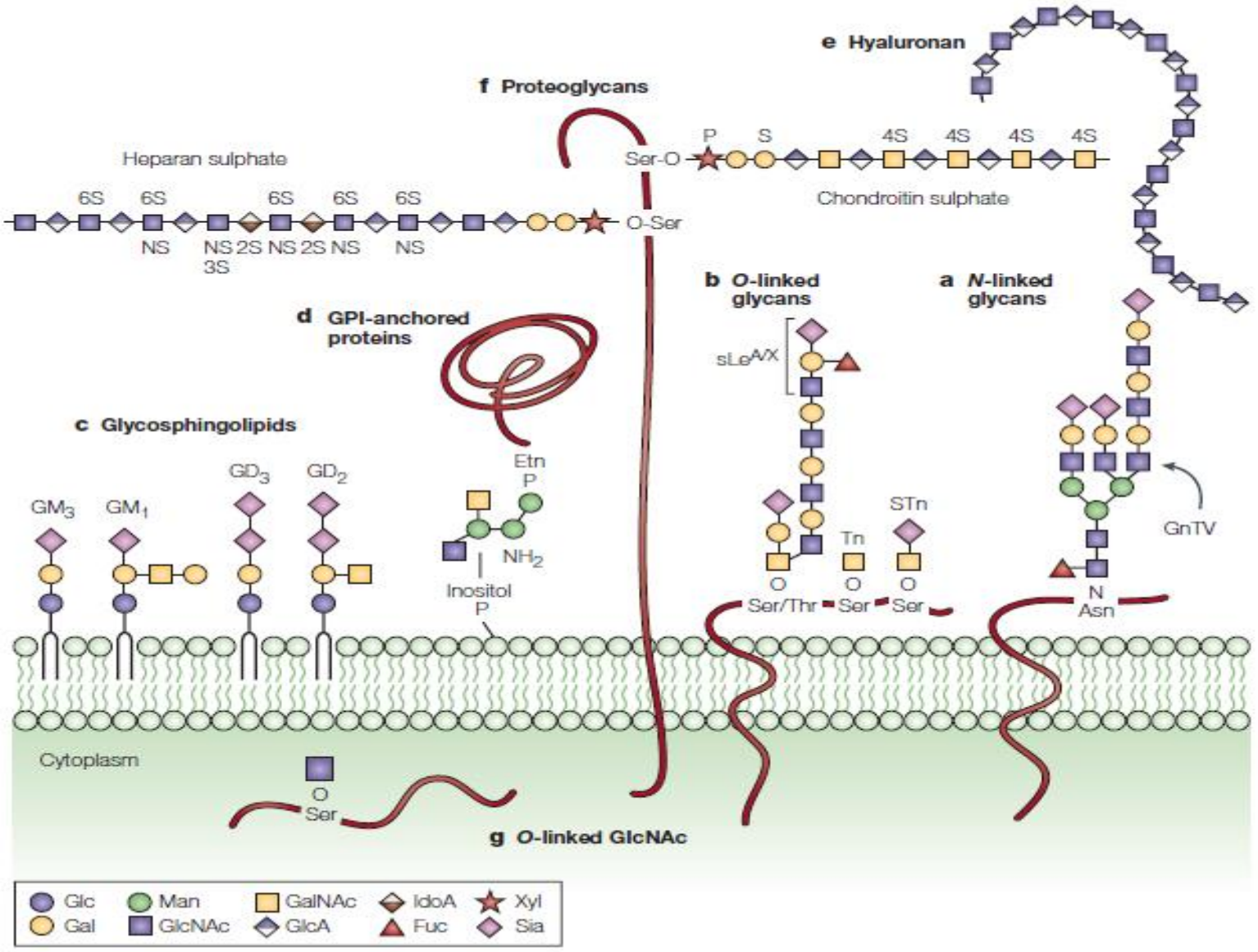


Epigenetic Reprogramming in Cancer
Mario L. Suvà *et al.*
Science **339**, 1567 (2013);


RTKs pathways
Notch pathway
ABC transporter
Hedgehoh pathway
Wnt pathway
EGF
PDGF
Ligando
Notch
SHH
ABCG2
EGFR
PDGFR
ICN
LPR
Wnt
Fz
Gskβ
Dsh
APC
Axin
Ras
Drug resistance
PLCγ
MEK
PI3K
Iκβ
NF-κβ
MAPKs
ICN
GLI
NF-κβ
↑Motility
β-catenin
AKT
P
Bad
↓p27
Cyclins
↑Mitosis
CDKs
CoA
MAML
βcatenin
Bcl2
Bcl2
GLI
NF-κβ
CSL
ICN
LEF
TCF
↑Proliferation
Survival
Angiogenesis
Invasion
Metastasis

Role of N/O-glycans in regulating ES/iPSCs and CSCs Growth and fate

Glycans



Fuster et al. Nature Reviews Cancer, 2005

Glycans in CSCs

- Glycan structures are altered in cancer compared to their normal counter parts.

- CSCs likely exhibit a unique glycoprofile compared to the ‘bulk’ cells.

- Biological and clinical implications:
  - Most clinically used tumor markers are abnormal glycans on proteins. (bio-markers). N-glycans can serve as biomarkers to identify and isolate CSCs.

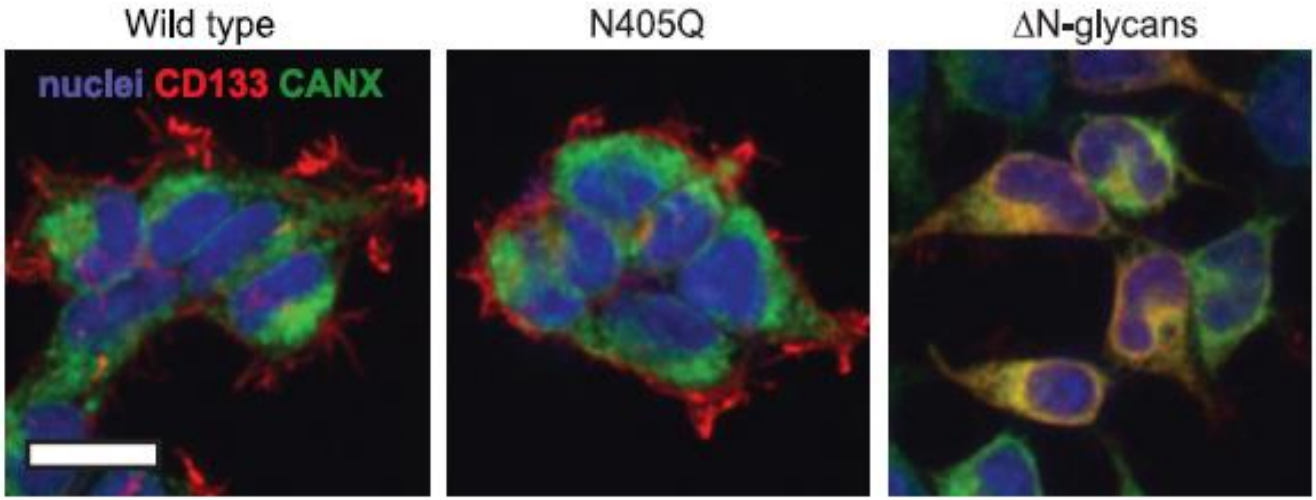
  - Altered glycosylation may regulates stem cell phenotype. Novel therapies can be developed against these glycotargets. (functional role)

Glycan markers of CSCs

Table 2 Carbohydrate stem cell markers

Marker	Description	Expression on stem cell-like populations	References
H type 1	SSEA-5, stage-specific embryonic antigen-5; carried on proteins Fucα1-2Galβ1-3GlcNAcβ1-	PSC, iPSC; CSC (germ cell carcinomas)	1
CD15	Lewis X, SSEA-1, stage-specific embryonic antigen-1; carried on lipids or proteins Galβ1-4[Fucα1-3]GlcNAcβ1-3Galβ1-	ESC, NSC, MSC; CSC (globlastomas)	2-7
CD60a	GD3; ganglioside NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ1-	NSC; CSC (differentiated germ cell carcinomas, melanomas)	7, 8
CD77	Gb3, P^k antigen, Burkitt lymphoma antigen (BLA); globoside Galα1-4Galβ1-4Glcβ1-	CSC (Burkitt lymphoma, breast cancer, germ cell carcinomas)	8, 9
CD173	H type 2; carried on proteins or lipids Fucα1-2Galβ1-4GlcNAcβ1-	ESC cell lines, HProgC, MSC	9-11, 13
CD174	Lewis Y; carried on proteins or lipids Fucα1-2Galβ1-4[Fucα1-3]GlcNAcβ1-	HProgC; CSC (breast cancer)	9, 11
CD175	Tn antigen; carried on proteins GalNAcα1-	ESC cell lines; onfFN	12,13
CD176	TF, Thomsen-Friedenreich antigen, core-1; carried on proteins Galβ1-3GalNAcα1-	ESC; CSC (diverse carcinomas and leukemias); onfFN	12-14
GD2	OFA-I-2; ganglioside GalNAcβ1-4[NeuAcα2-8NeuAcα2-3]Galβ1-4Glcβ1-	NSC, MSC; CSC (differentiated germ cell carcinomas, breast cancer, melanomas)	7, 8, 10, 15
Gb4	Globoside GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-	CSC (germ cell carcinomas)	8
Gb5	SSEA-3, stage-specific embryonic antigen-3; globoside Carries TFβ (the β-anomer of TF) Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-	ESC, MSC, iPSC; CSC (breast cancer, germ cell carcinomas)	4, 8, 16-19
Sialyl-Gb5	SSEA-4, stage-specific embryonic antigen-4, GL7; globoside NeuAcα2-3Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-	ESC, MSC, iPSC, ProgC (breast); CSC (germ cell carcinomas)	4, 8, 16, 17, 19-21
Globo-H	Carried on proteins or lipids Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Gal-	CSC (breast cancer)	18
TRA-1-60	Tumor-recognition antigen; carried on protein Sialylated keratan sulfate proteoglycan	ESC, MSC; CSC (teratocarcinomas)	4, 19, 22

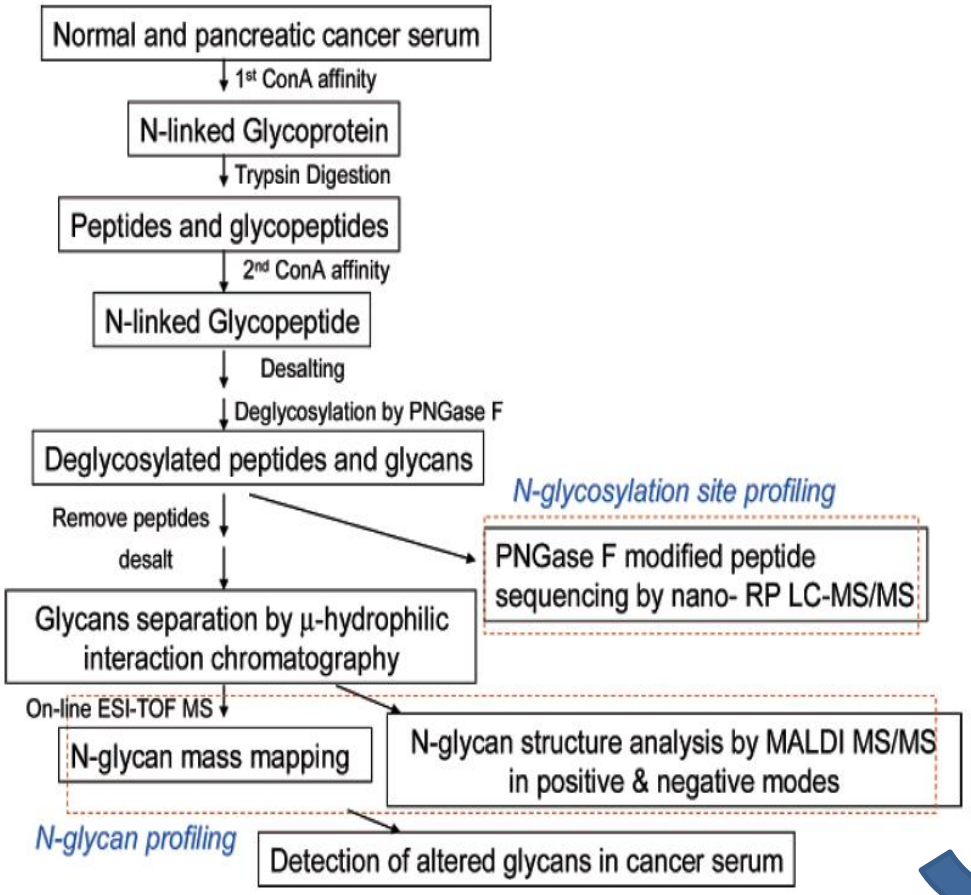
N-glycosylation of CD133 is required for its detection as CSC biomarker

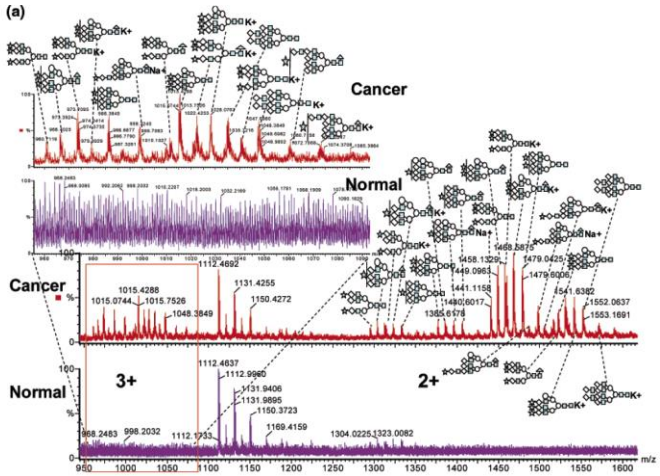


Mak et al. JBC, 2011:41046

N-glycan profiling of serum-Non-invasive biomarkers of cancer

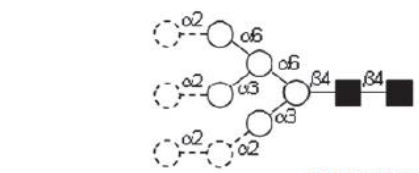
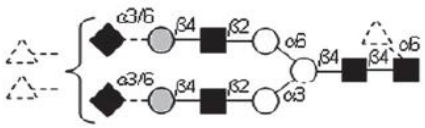
1. Branched N-glycans
2. Fucosulation
3. Sialylation

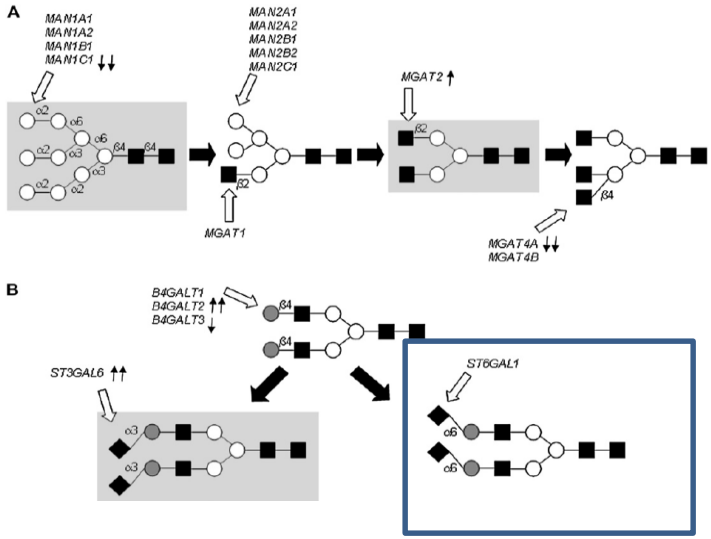




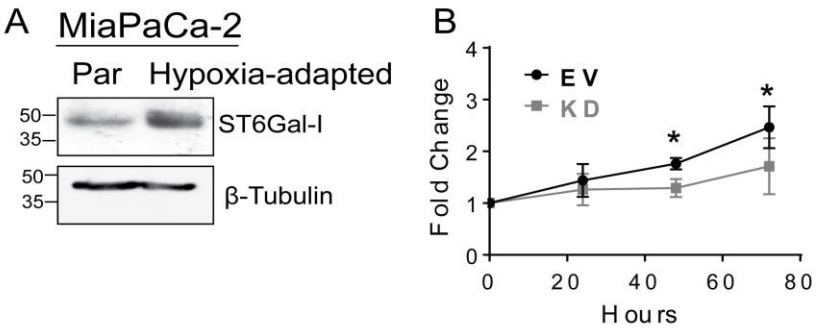
Zhou J et al. J Proteome Res. 2006: 1126

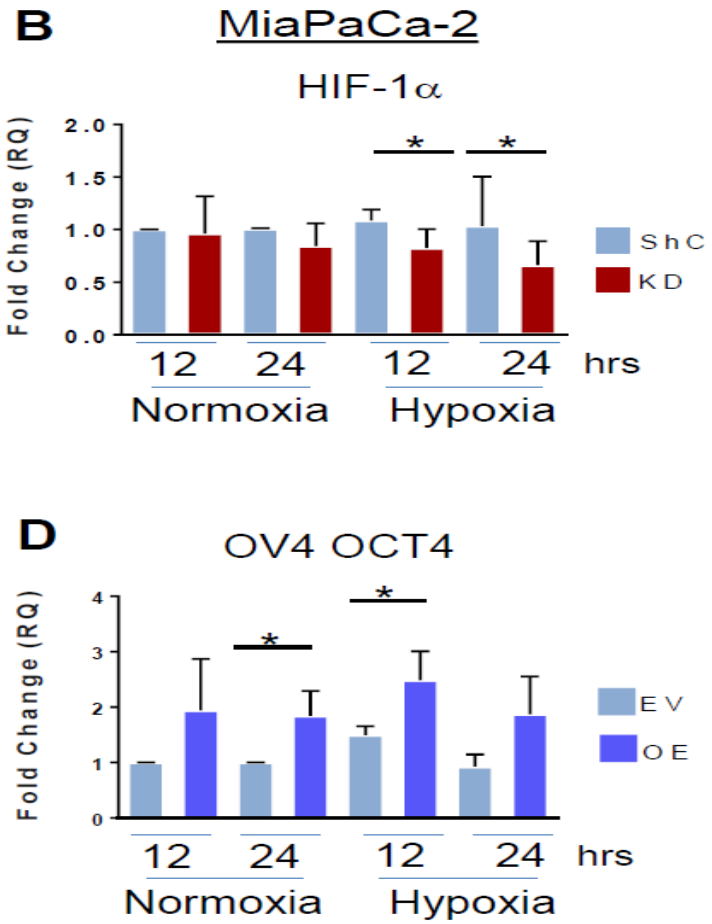
Circulating CD133+ (CSCs) display a unique N-glycan profile

	CD133+ cells	CD133- cells
 H(5-9)N2*	69%	64%
 S(1-2)H5N4F(0-3)*	46%	39%



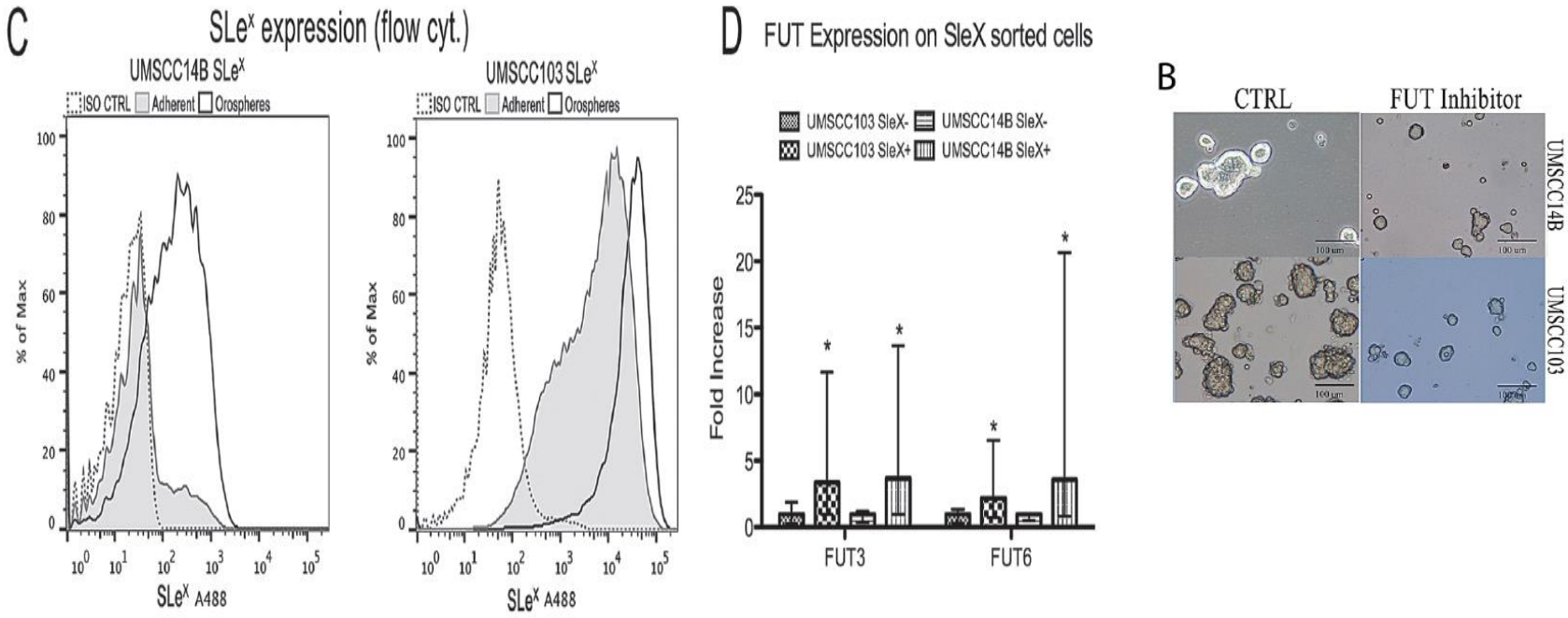
Role of ST6GAL1 in induction of Hypoxic response





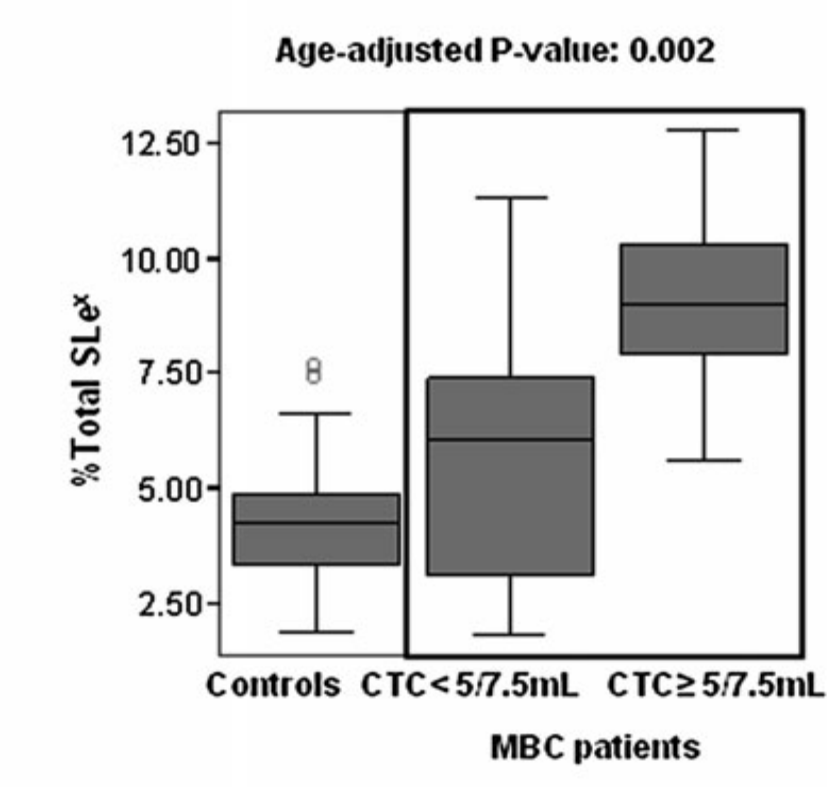
Jones, R.B. et al. JBC 2018

Sialyl Lewis^x expression is a marker of oral squamous cell CSCs



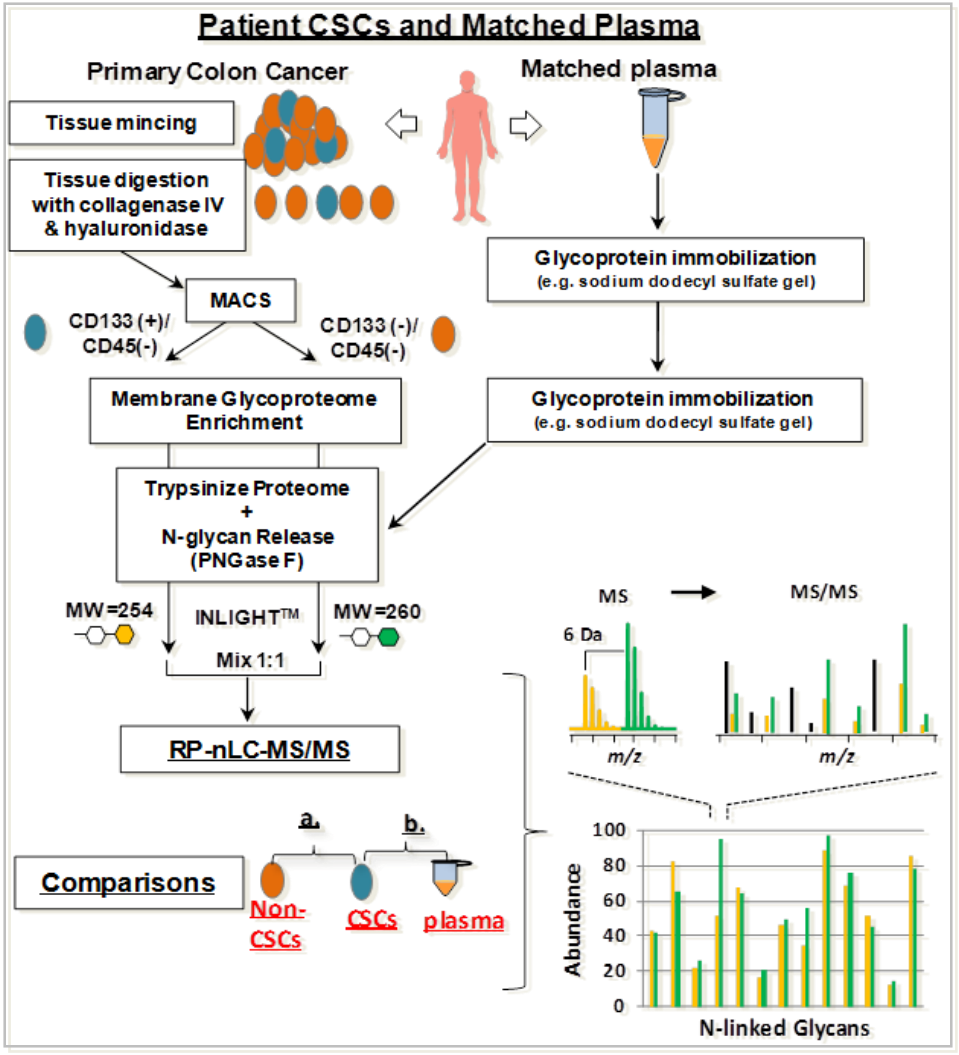
Desiderio et al. Oncotarget, 2014:71

Sialyl Lewis^x expression in circulation correlates with CTC in breast cancer

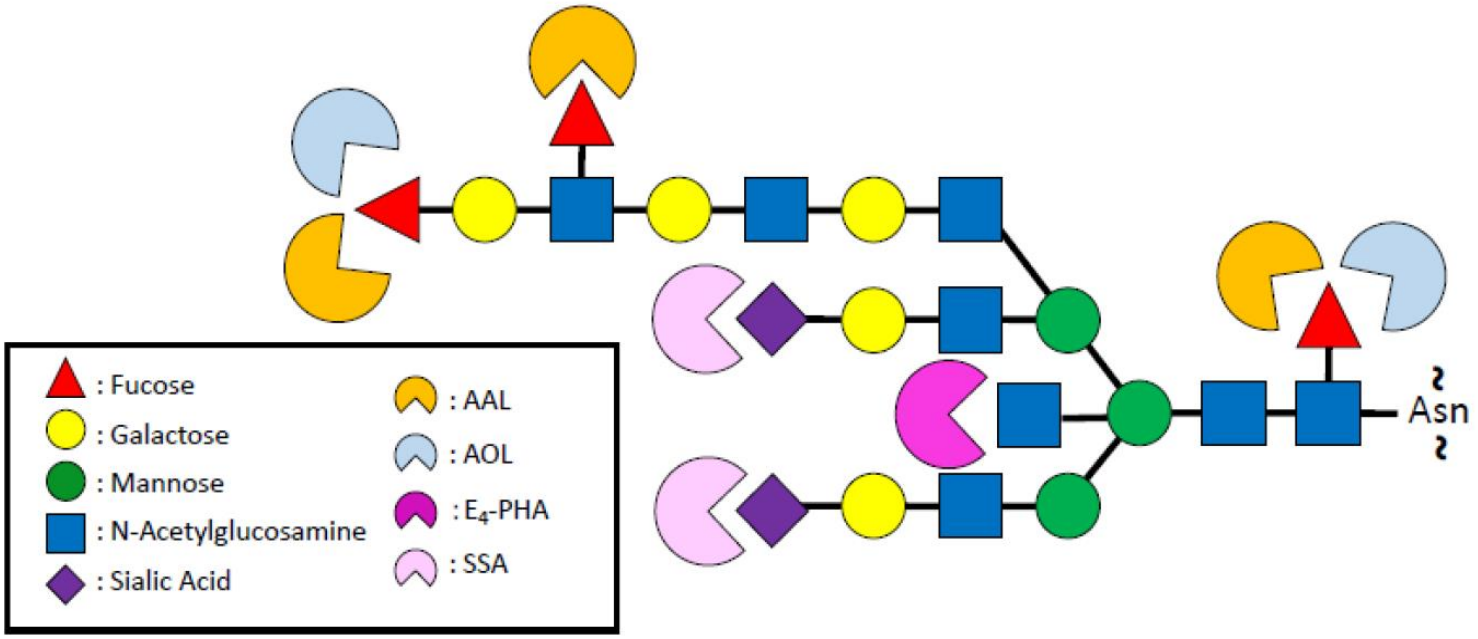


Saldova et al. Annals of Oncology, 2014

Development of non-invasive signature of CSCs



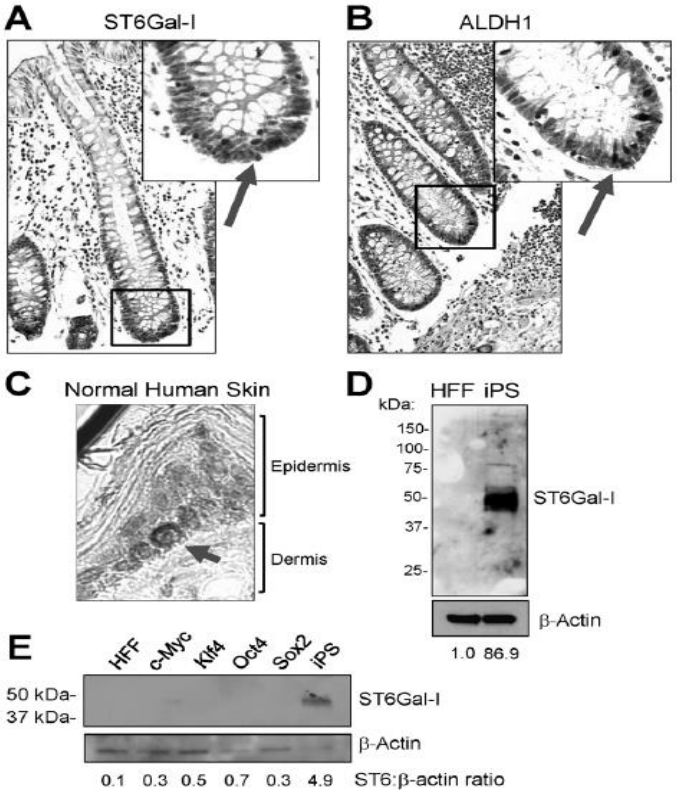
Lectins can serve as novel tools to isolate CSCs



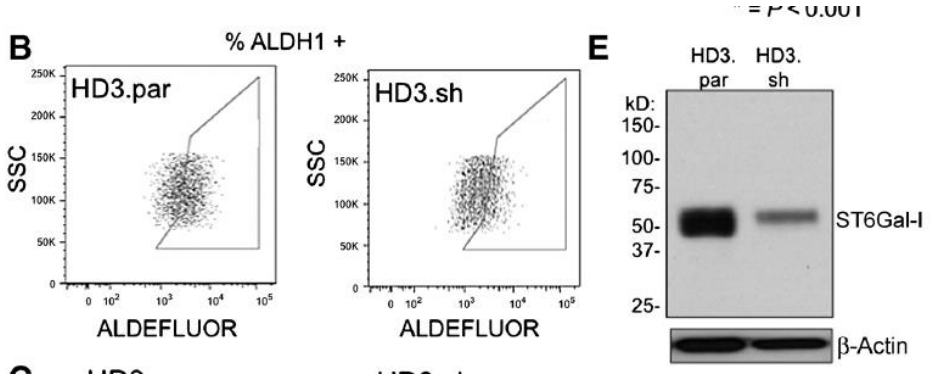
Swanonbori A et al, proteomics, 2016 (ahead of print)

Aberrant N-glycan synthesis in stem cells

Adult stem cells

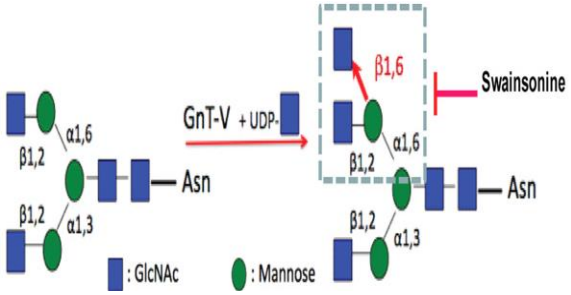


CSCs

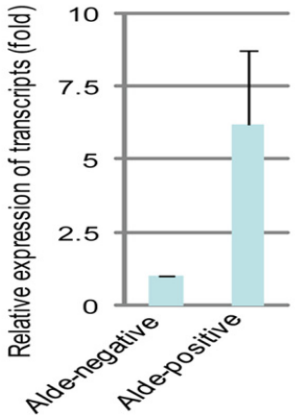


Swindall et al. Cancer Res, 2013: 2368

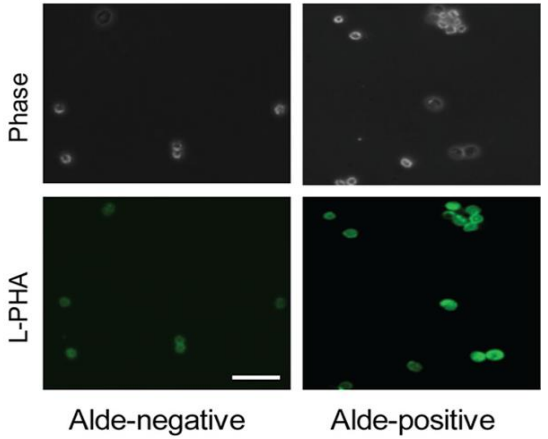
Branched N-glycans regulate colonic CSCs growth

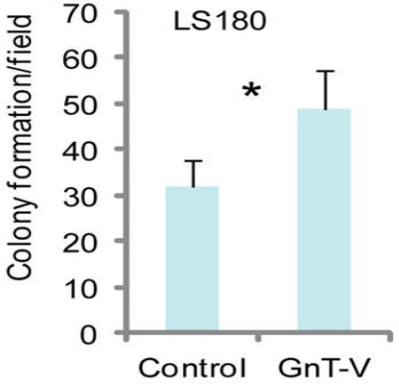


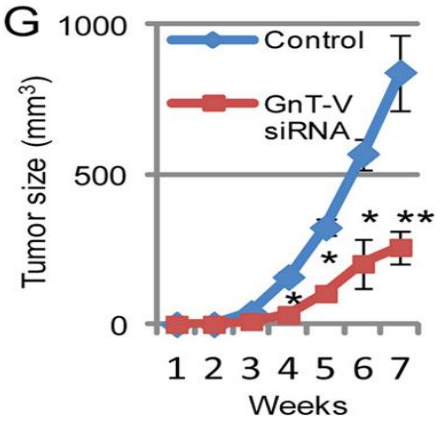
GnT-V expression



(1,6) branched N-linked Glycans

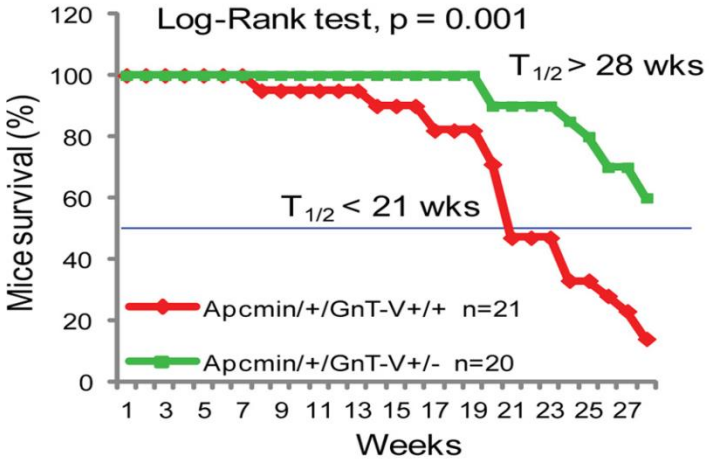


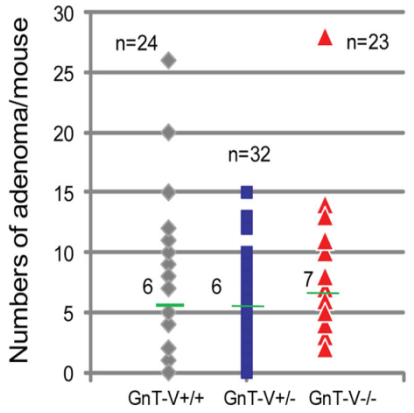


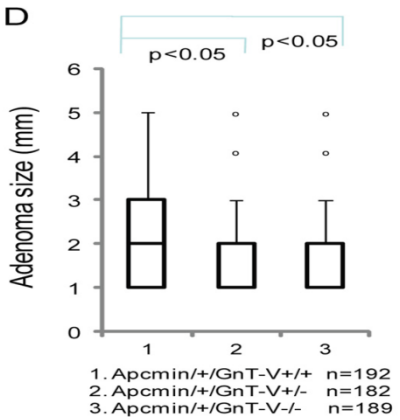


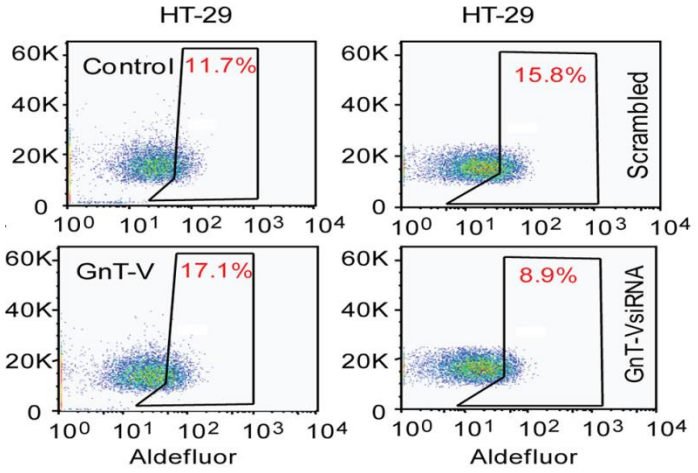
Guo H et al. JBC, 2014: 31534

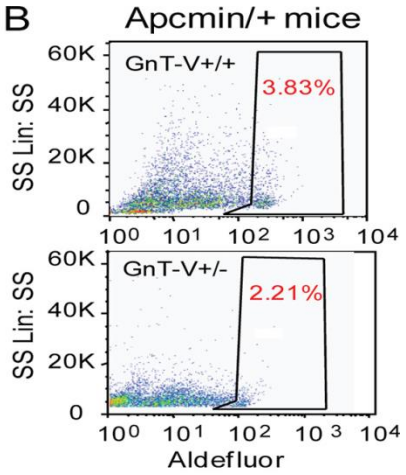
Branched N-glycans regulate CSCs phenotype in Apc min mice model



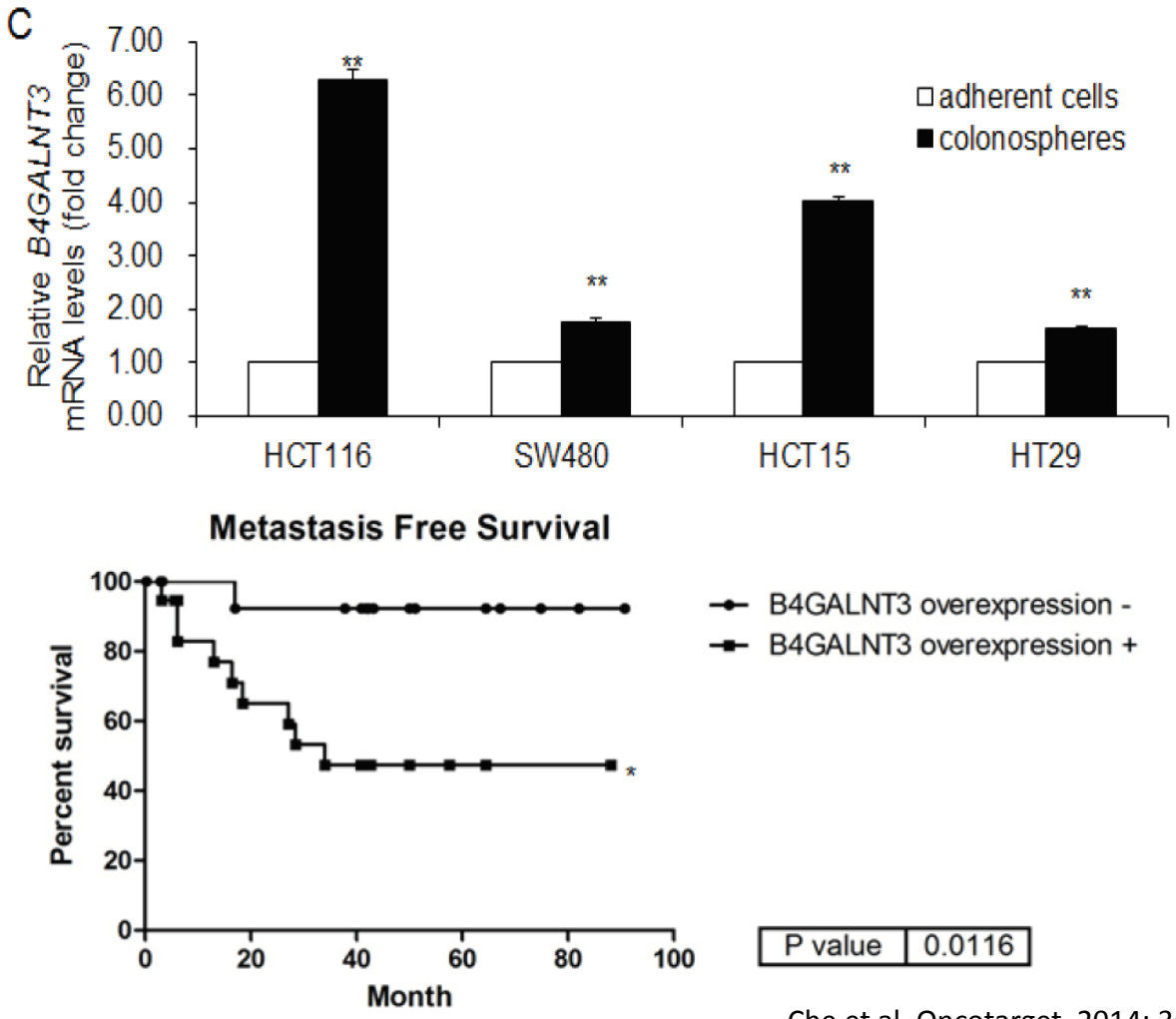






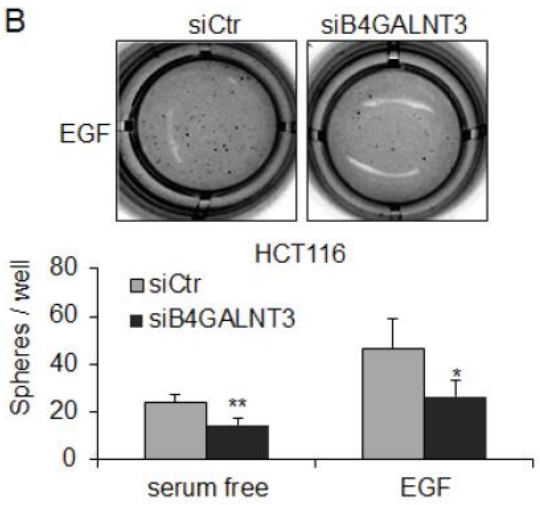


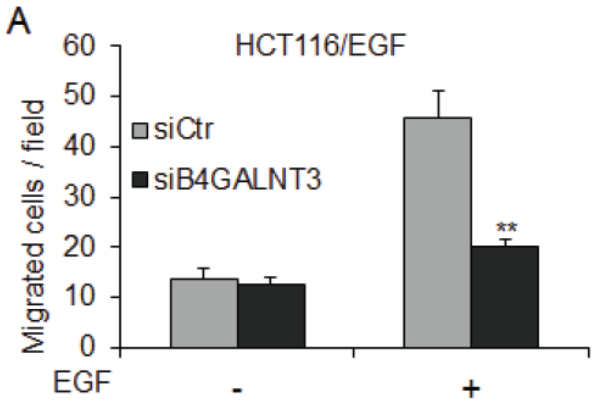
LacdiNac modified N-glycan in colonic CSCs

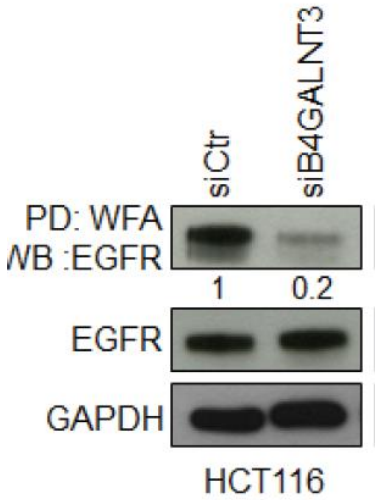


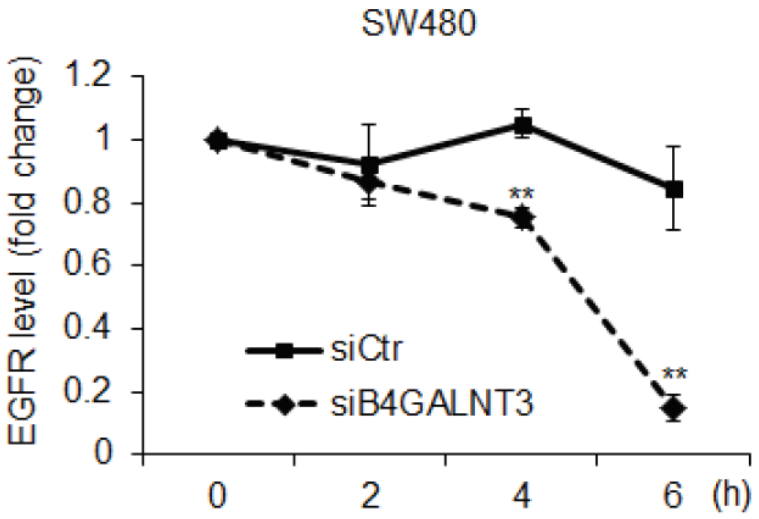
Che et al. Oncotarget, 2014: 3673

LacdiNac modification of EGFR regulates colonic CSCs properties

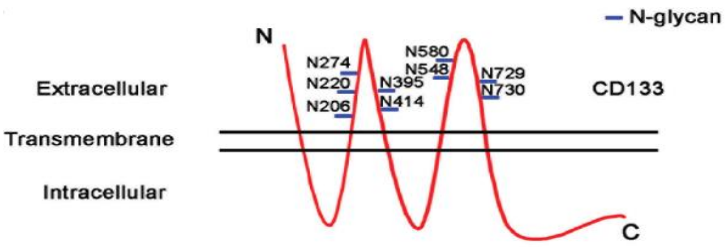


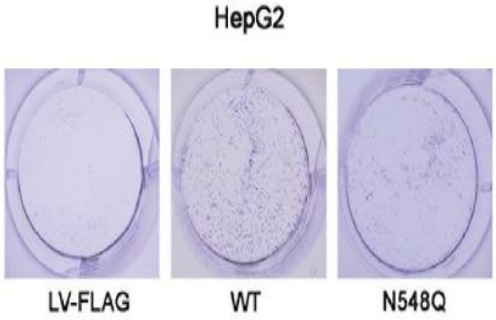


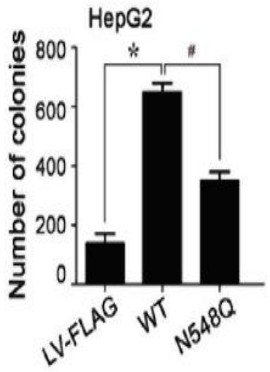


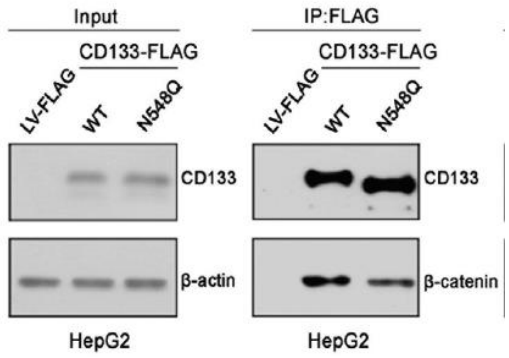


Site specific N-glycosylation regulates function of CSC marker protein



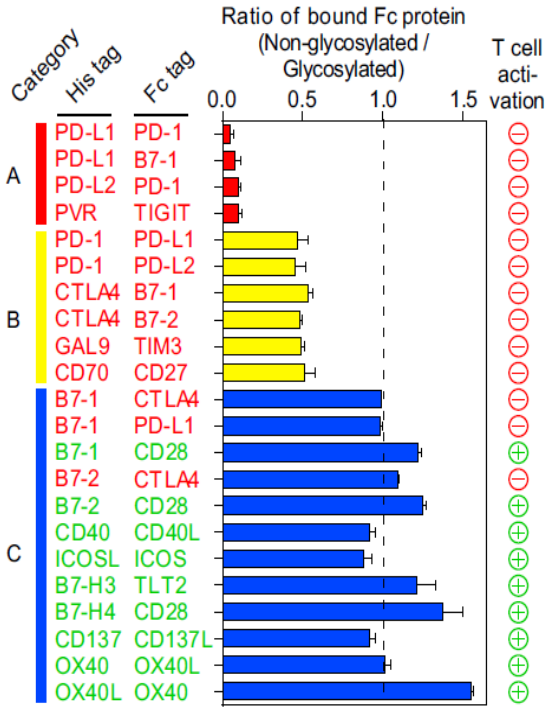


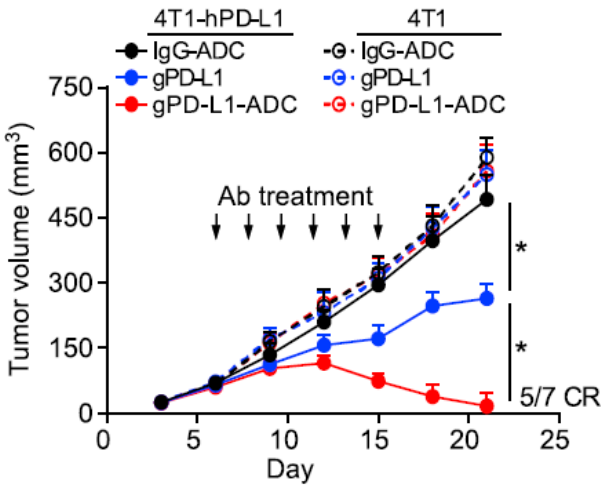


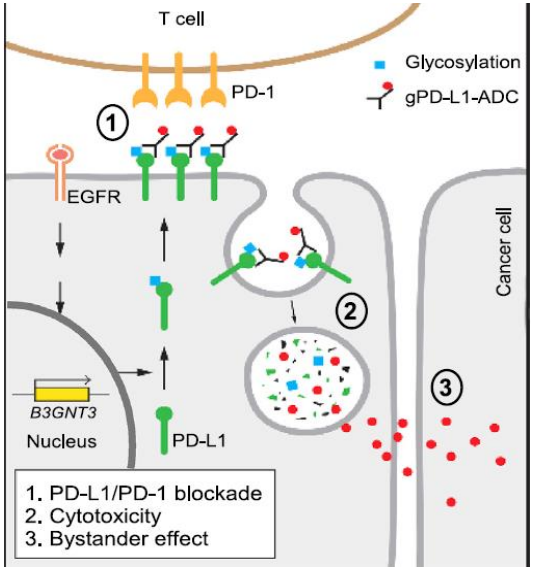


Liu Y et al. Oncotarget, 2015:20650

Glycosylation of immune checkpoint inhibitors and its impact on antitumor immunity



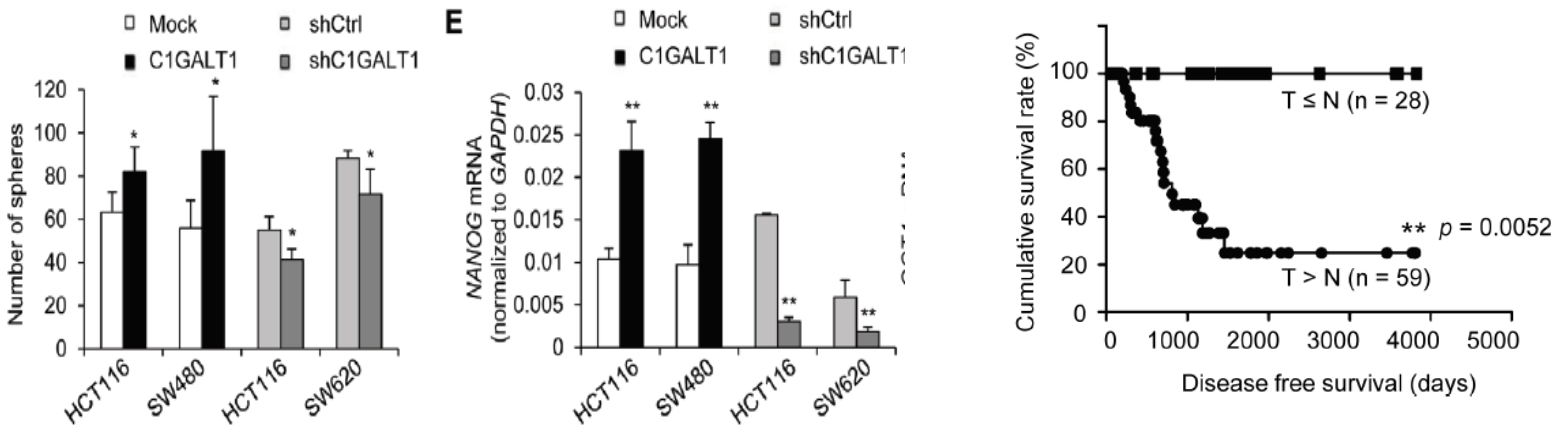




Li et al. 2018 Cancer Cell 33, 187–201

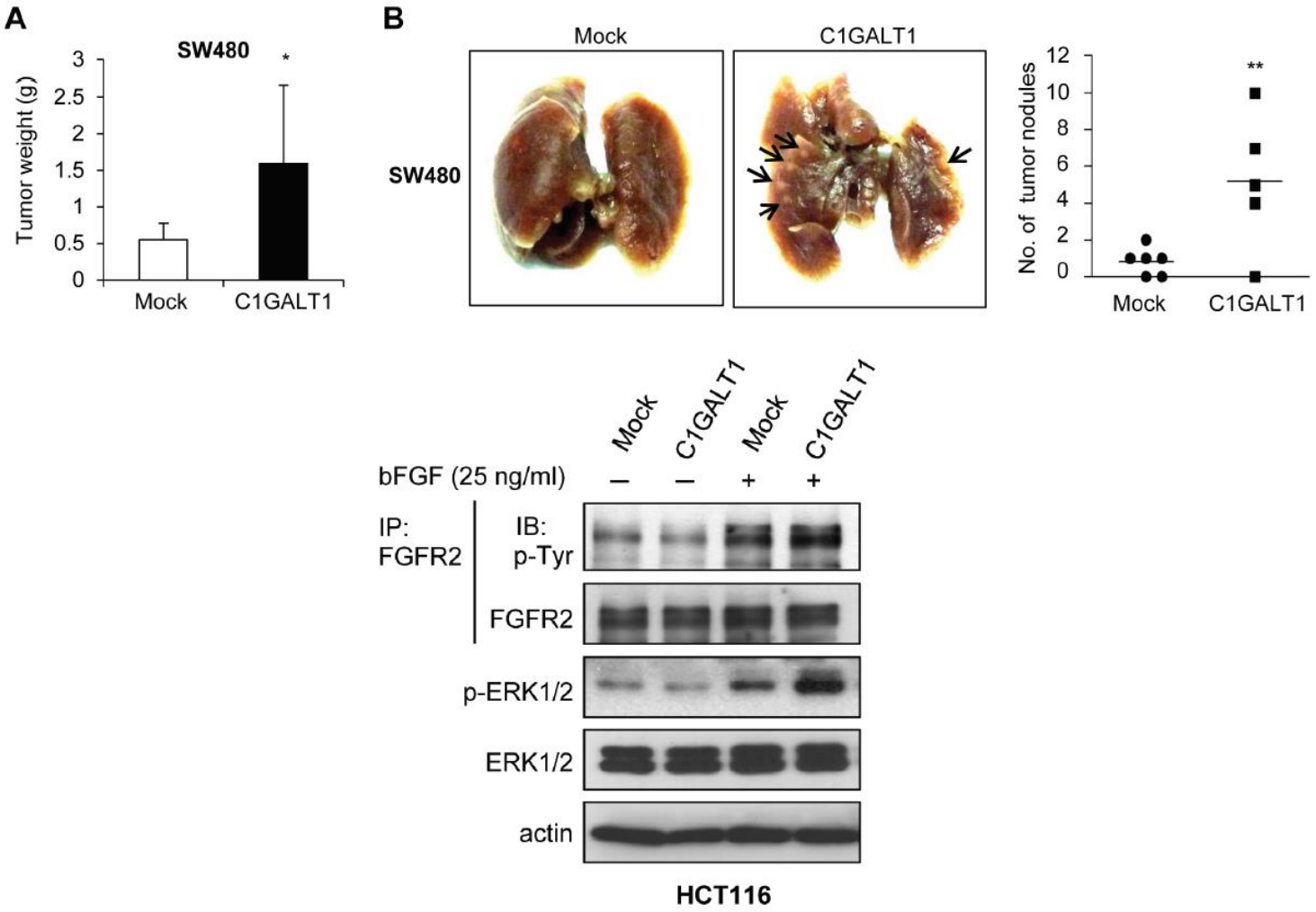
Aberrant O-glycan synthesis in CSCs

C1GALT1 expression in colon cancer is increased compared to normal tissue



Hung et al. Oncotarget, 2014: 2096

Aberrant O-glycan synthesis promotes FGFR signaling and cancer metastasis



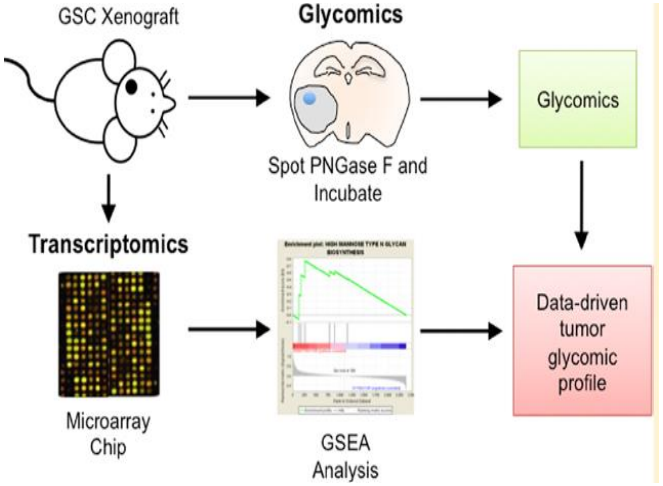
Integrated glycomics

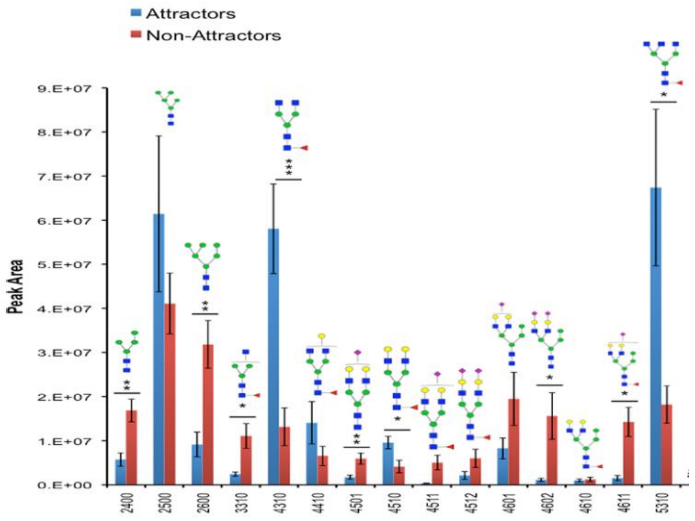
Integrated glycomics of stem cells

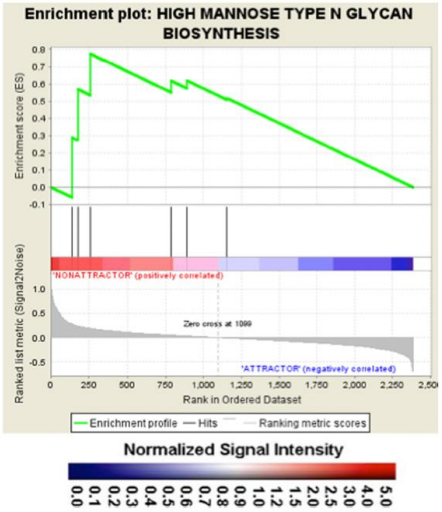
- Multidisciplinary approach to characterize structure, function, and regulatory aspect of aberrant glycosylation in CSCs or other stem cells.

- Simultaneous macromolecularomics followed by data integration.

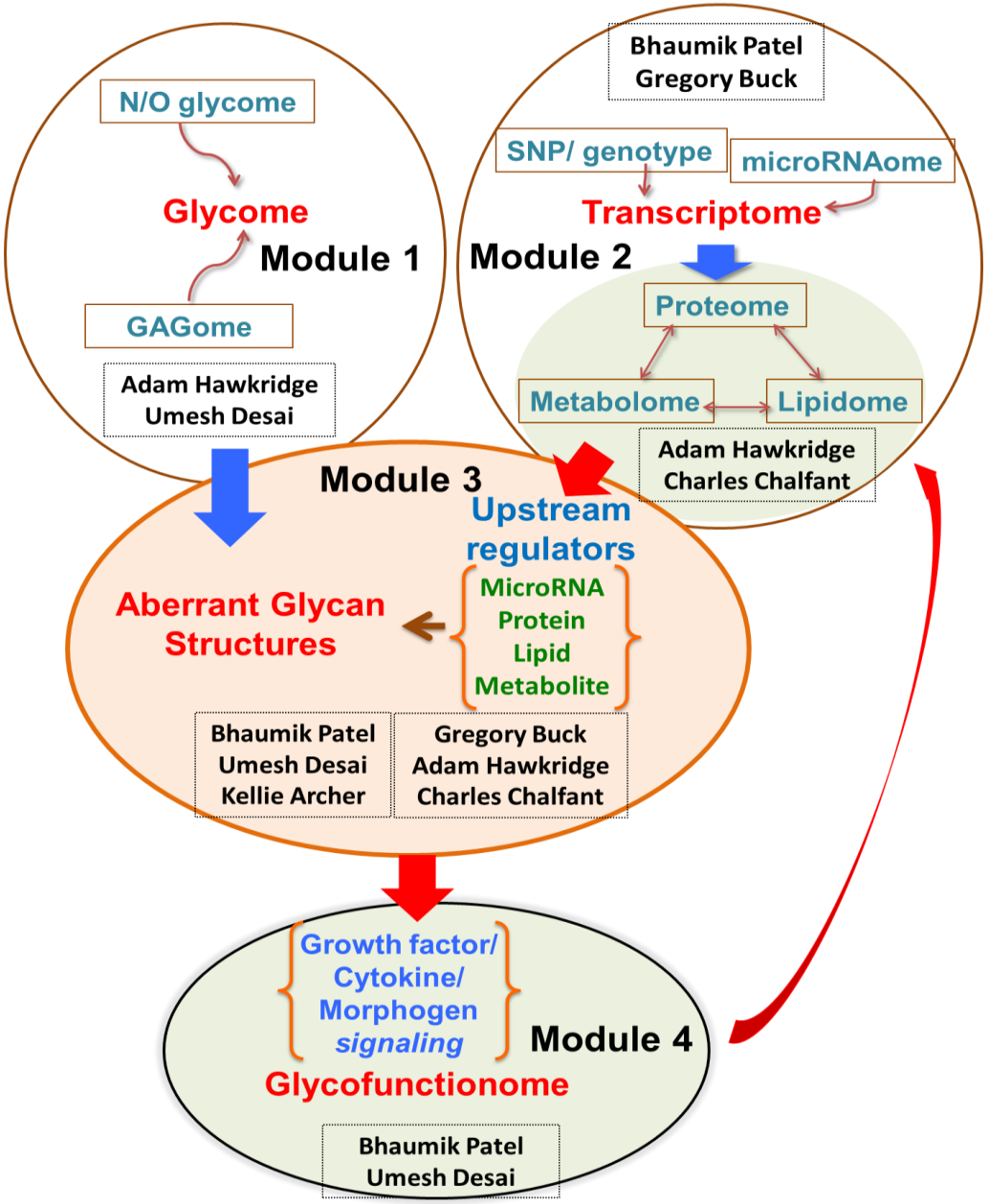
High mannose type N-glycan determine homing ability of hMSCs







‘Integrated CSC Glycomics’



Conclusions

- Stem cells show differential expression of N/O-glycans on proteins. Hence, they can serve as powerful biomarkers to identify and isolate various stem cells.

- Aberrant N/O-glycome of the CSCs regulate their function.

- Integrated glycomics is a powerful tool to comprehensively characterize stem cell glycome with respect to structure, function, and their upstream regulators.

Learning Objectives

- Understand the role of sulfated glycosaminoglycans (GAGs) such as HS, CS, and DS in regulation of stem cell growth, self-renewal, and fate.

- Understand the role of hyaluronan in regulation of cancer stem cells (CSCs) growth.

- Learn about the therapeutic applications of GAGs and/or GAG mimetics in targeting CSCs

Role of GAGs in regulation of ES self-renewal and diffrentiation

Physiological Functions of GAGs

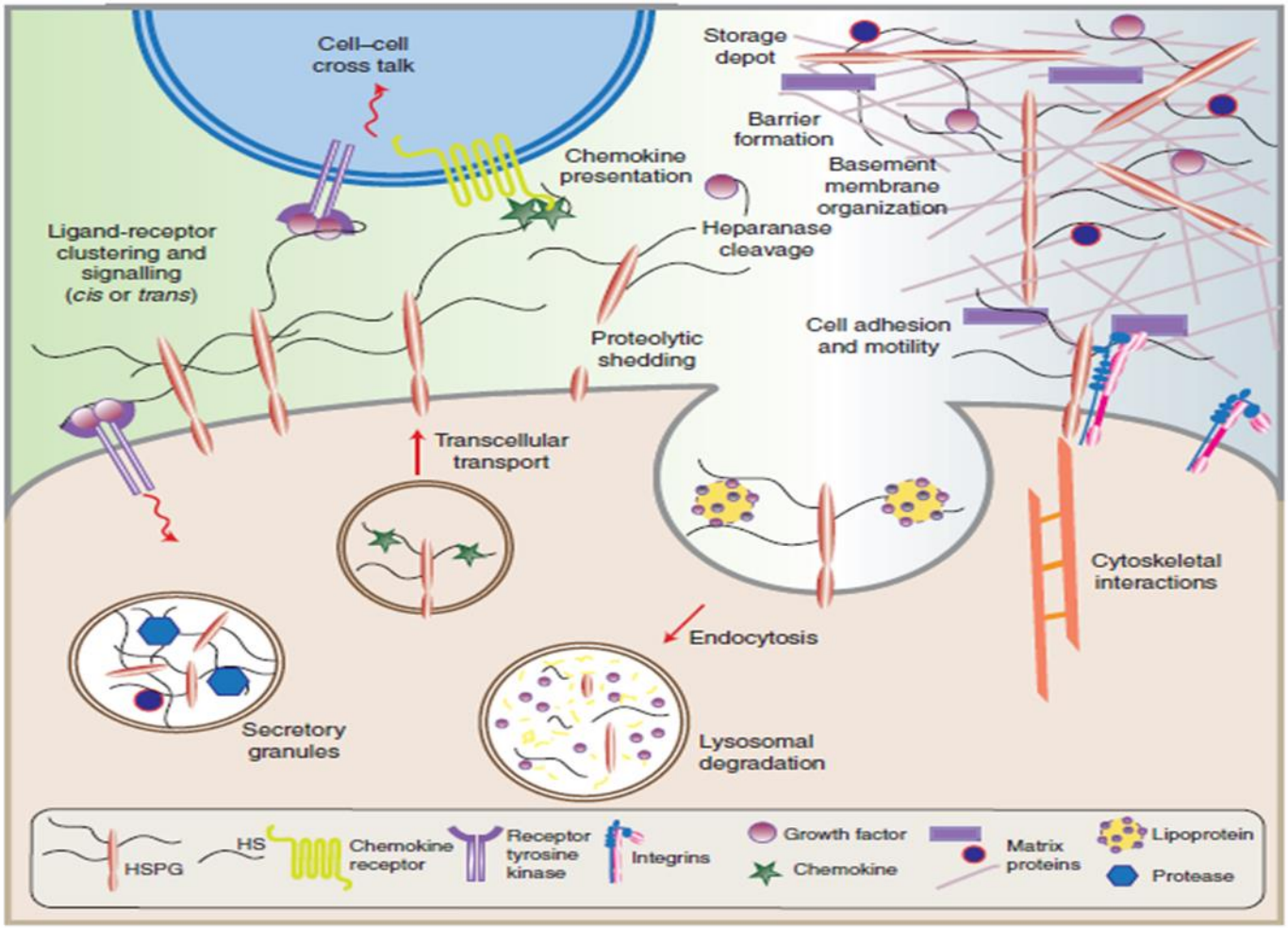
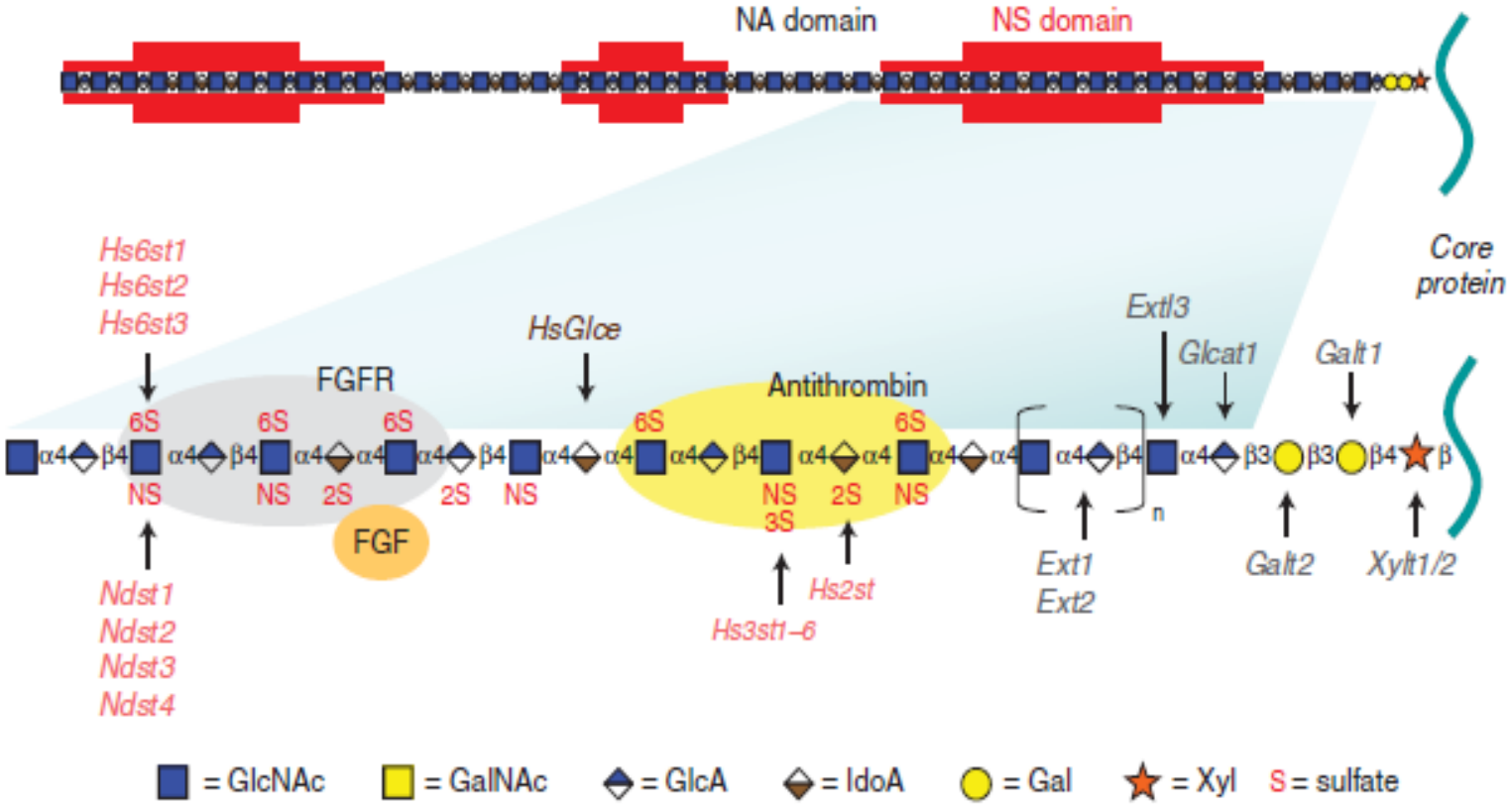


Figure 1. HSPGs have multiple activities in cells and tissues. (Adapted from Bishop et al. 2007; reprinted with permission from Nature Publishing Group © 2007.)

Role of Heparan Sulfate in various cellular processes

- HSPGs serve as depot for morphogen, cytokines, growth factors etc.
- HSPGs can act as low affinity receptors for morphogen and create morphogen gradient
- HSPGs can act as co-receptors for various tryosine kinase growth factor receptors.
- HSPGs are involved in endocytic processes that regulate ligand concentration and regulate growth as well as morphogen gradient during development.

Enzymes Involved in Sulfated GAGs Synthesis and modifications



Sarrazin t al. Cold Spring Harbor Perspective in Biology, 2011

PG & GAG syntetic enzymes in Stem Cell Biology

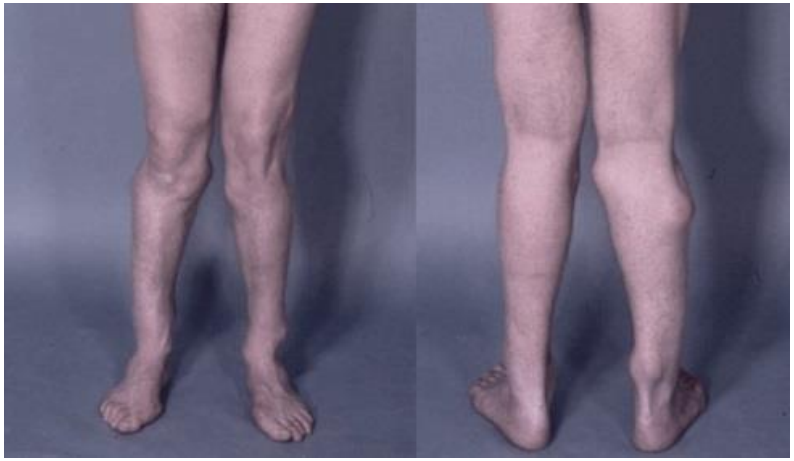
Proteoglycan/GAG syntheic enzymes	Mutant Phenotype	Stem Cell Signaling
Glypican-3	Simpson-Golabi-Behmel Syndrome (Somatic overgrowth)	Increased SHH signaling Increased WNT/-β catenin signaling
Glypican-6	Omodysplasia	? Likely impaired morphogen growth signaling.
EXT1$^{-/-}$	increased ES self-renewal	Impaired FGF signaling
EXT1 siRNA	decreased self-renewal	Increased FGF signaling due to altered sulfation
Ndst1/2$^{-/-}$	impaired ES differentiation	FGF signaling
H3ST loss	increased ES self-renewal	FAS-Caspase signaling targeting NANOG

Simpson–Golabi–Behmel syndrome

- Mutation in Glypican-3
- Dysregulation of morphogen signaling e.g. Sonic hedgehog and Wnt.
- 2-3 standard deviation increase in weight, height, or head circumference above the average for sex and age.
- Increased risk of childhood neoplasm.

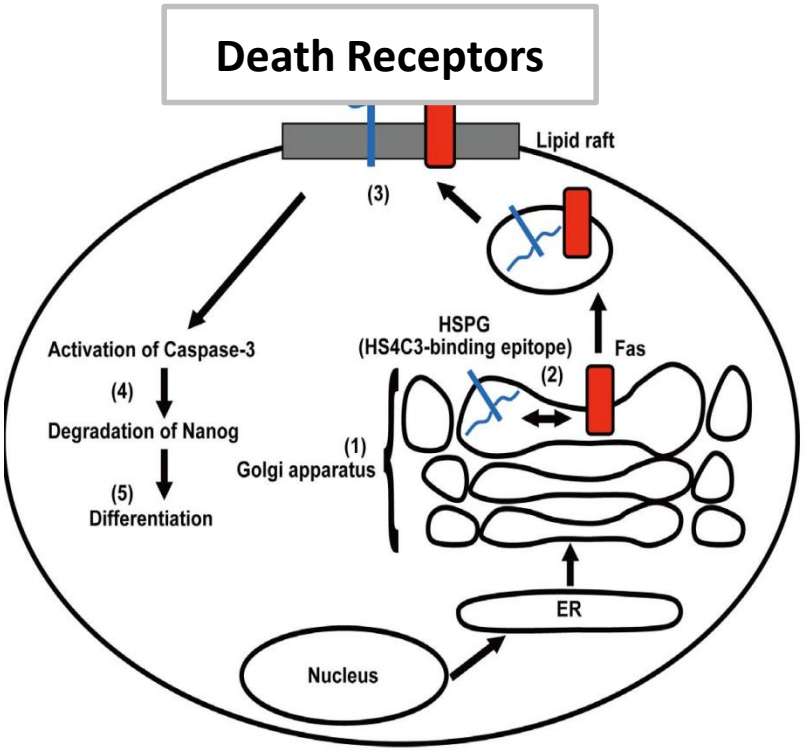
Hereditary multiple exostoses

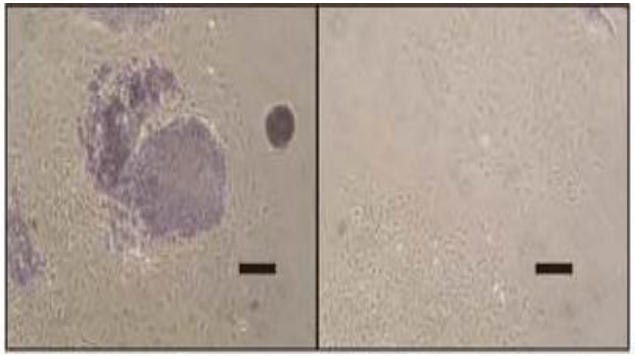
- Mutations in EXT1, EXT2, or EXT3
- Impaired GAG synthesis
- Altered FGF signaling



HS sulfation pattern and stem cells differentiation

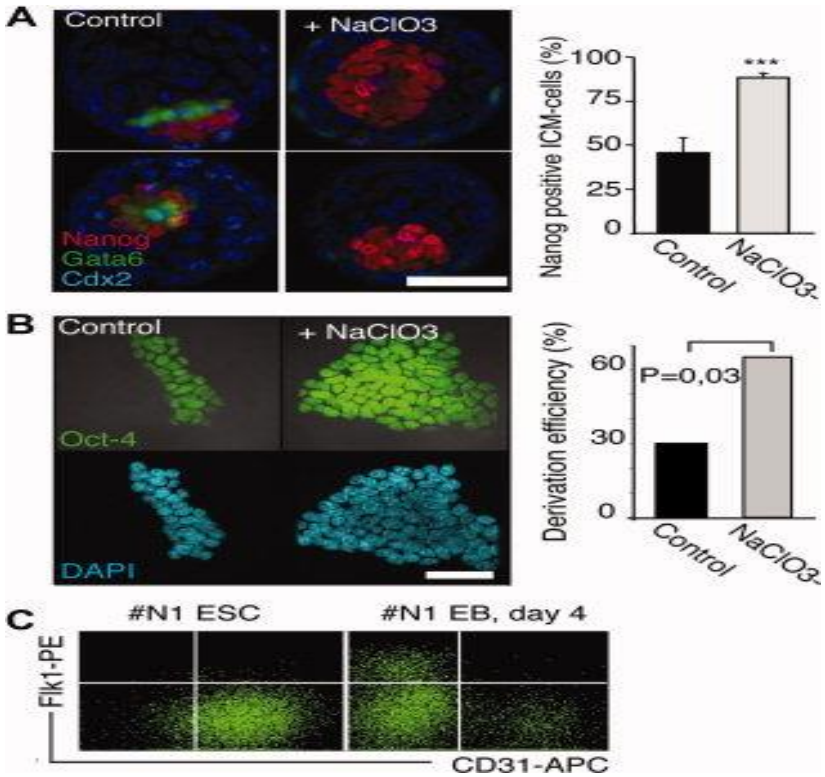
- HS3ST regulates 3-O sulfation on Glucosamine of HS
- This modification is critical for HS's ability to activate death receptor signaling
- Absence of HS3ST results in impaired death receptor signaling resulting in

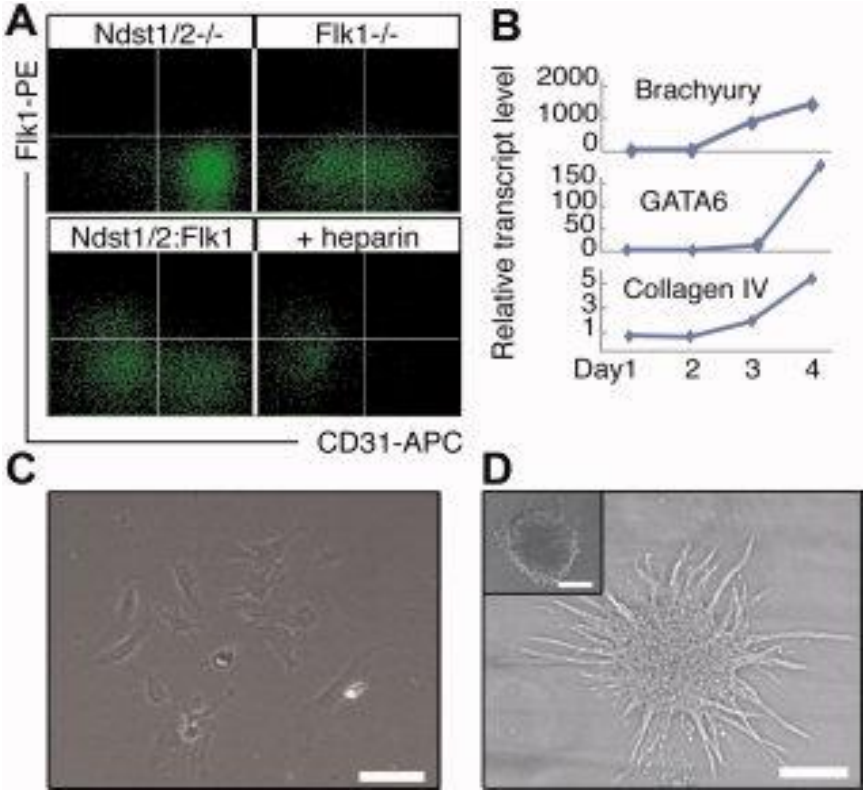




Hirano et al. PLOS One, 2012

Modulation of HS sulfation affects ES Self-renewal and differentiation





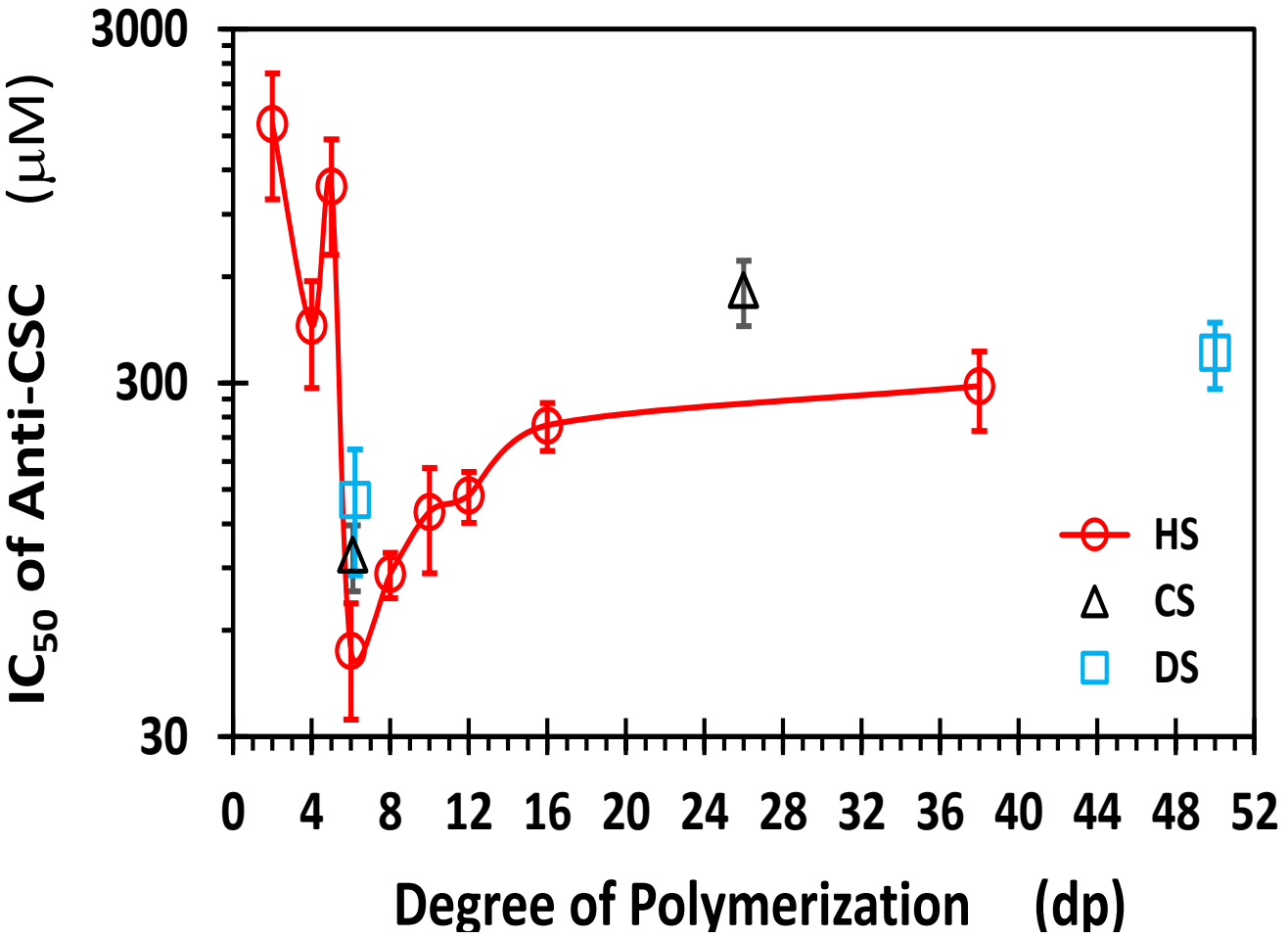
Sodium Chlorate-an inhibitor of HS sulfation- promotes ES self renewal

Exogenous heparin rescues HS sulfation deficient cells from block in differentiation

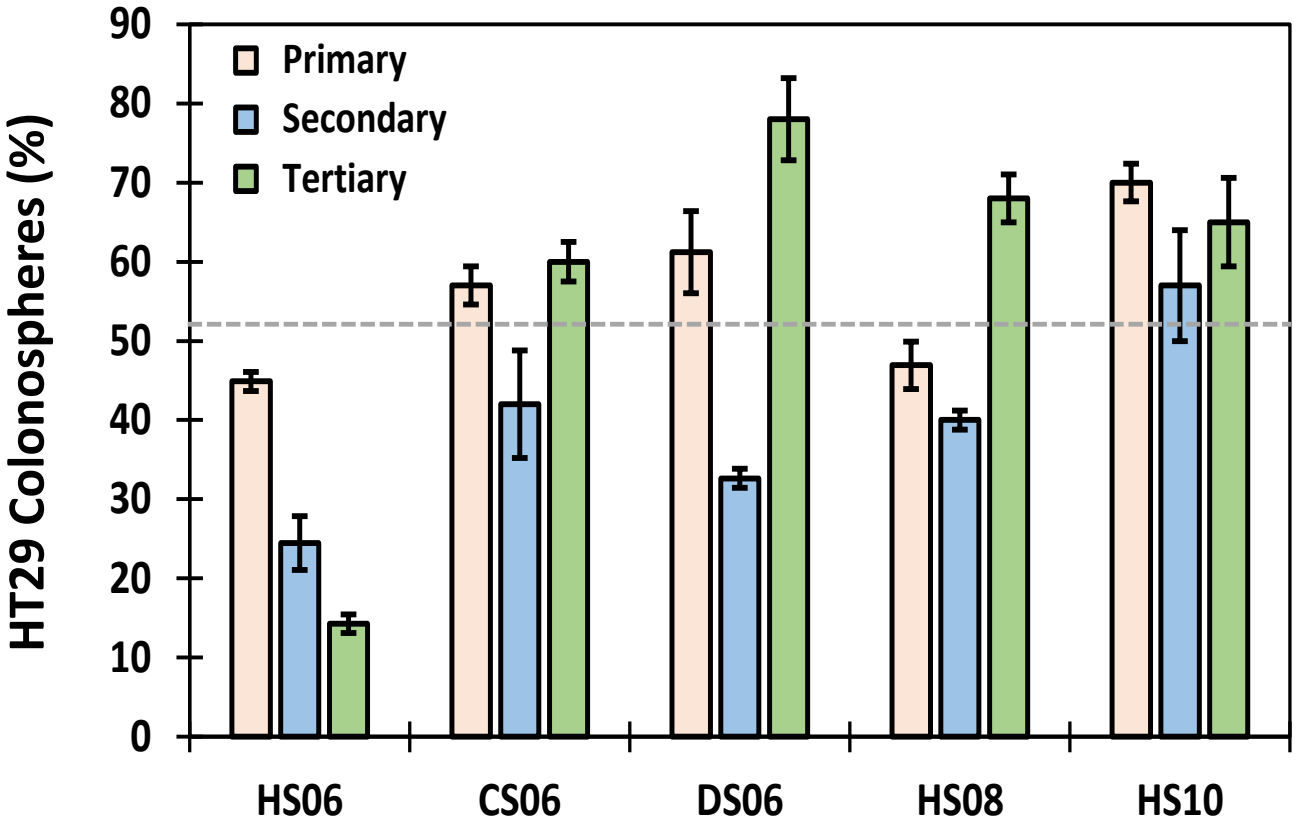
STEM CELLS. Volume 28, Issue 2, pages 191-200, 23 NOV 2009 DOI: 10.1002/stem.265
http://onlinelibrary.wiley.com/doi/10.1002/stem.265/full#fig2

Role of GAGs in regulation of CSCs growth and self-renewal

Effect of sulfated GAGs on CSCs growth



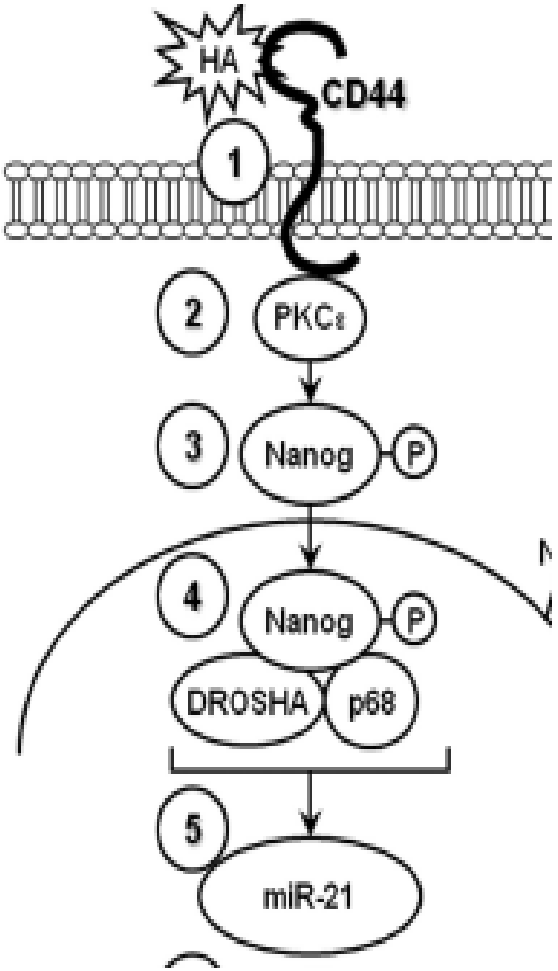
Effects of sulfated GAGs on CSCs self-renewal

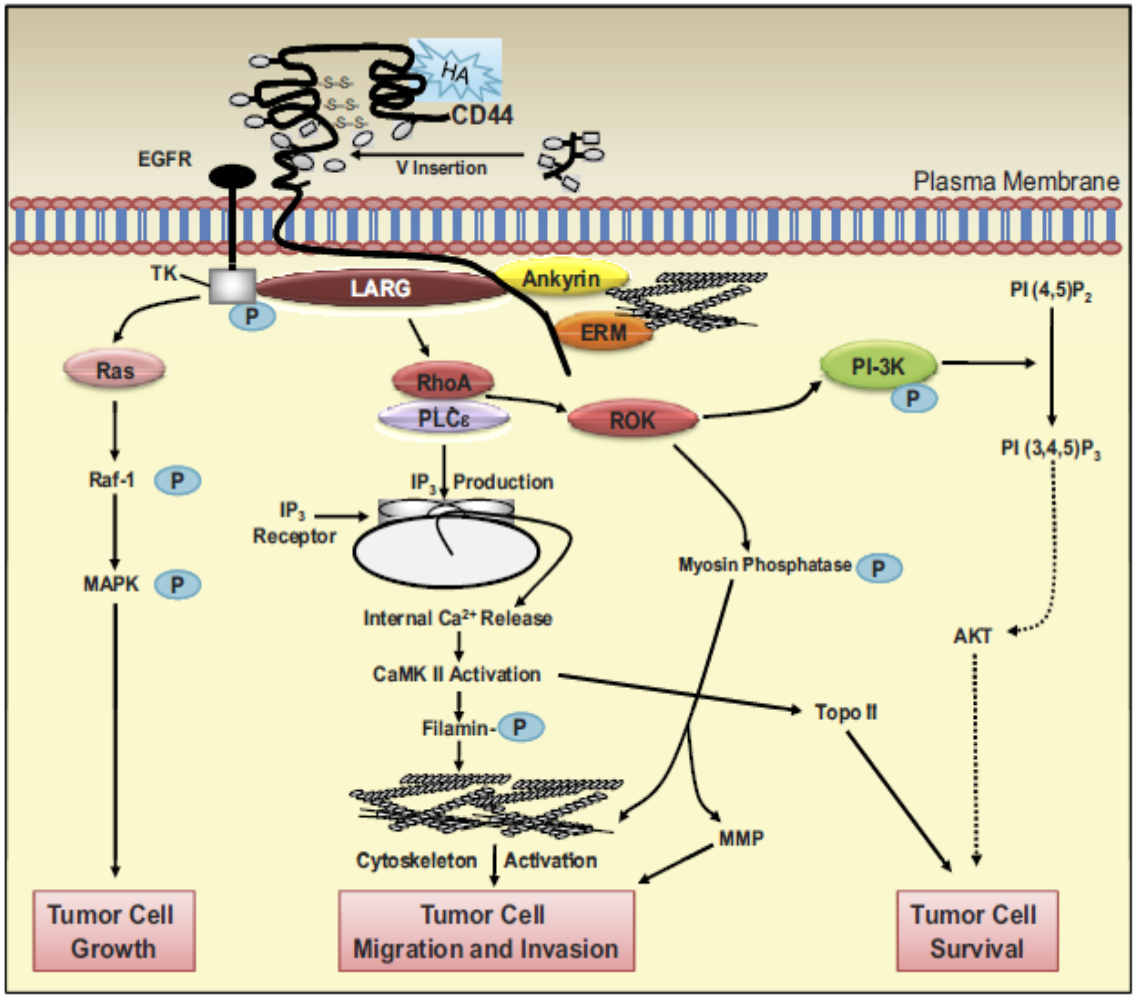


Hyaluronan and cancer

- Many classes of malignant tumors express high levels of hyaluronan.
- In breast and pancreatic cancers, hyaluronan is usually enriched in the tumor-associated stroma.
- Hyaluronan promotes cancer growth through:
  - Promotes tissue hydration, which can facilitate movement of cells through tissues.
  - Participates in tumor cell–matrix interactions that facilitate or inhibit tumor cell survival and invasion.
  - It interacts with several types of cell-surface receptors, especially CD44 promoting CSC signaling

CD44 and Hyluronan

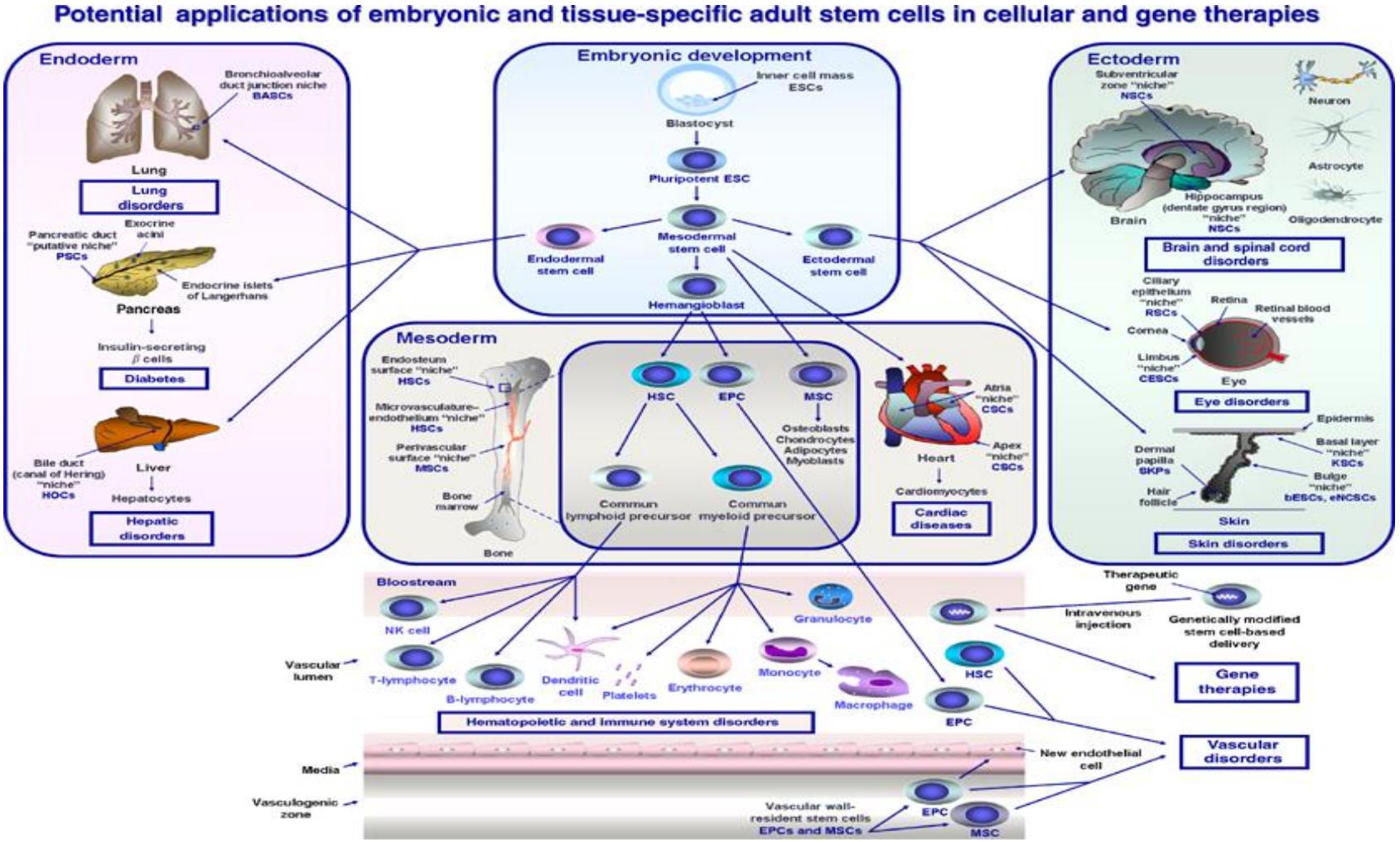




Bourguignon et al. JBC 2009

Therapeutic Applications of Manipulation of GAGs in ES/iPSCs and CSCs growth.

Therapeutic Applications of Stem Cells Therapy

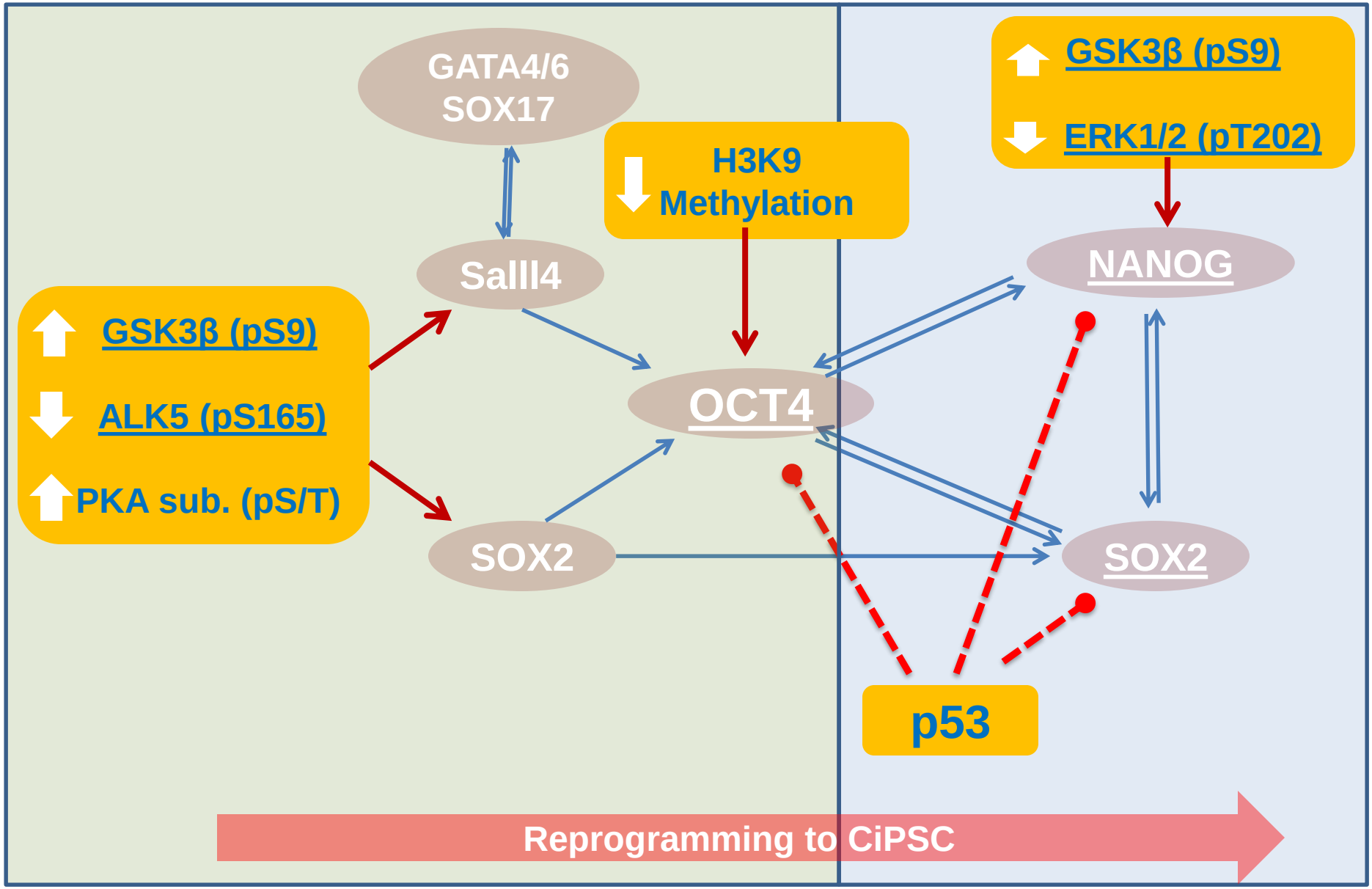


Mimeault M et al. CLINICAL PHARMACOLOGY & THERAPEUTICS , 2007: 252

Challenges in use of hESCs and iPSCs-

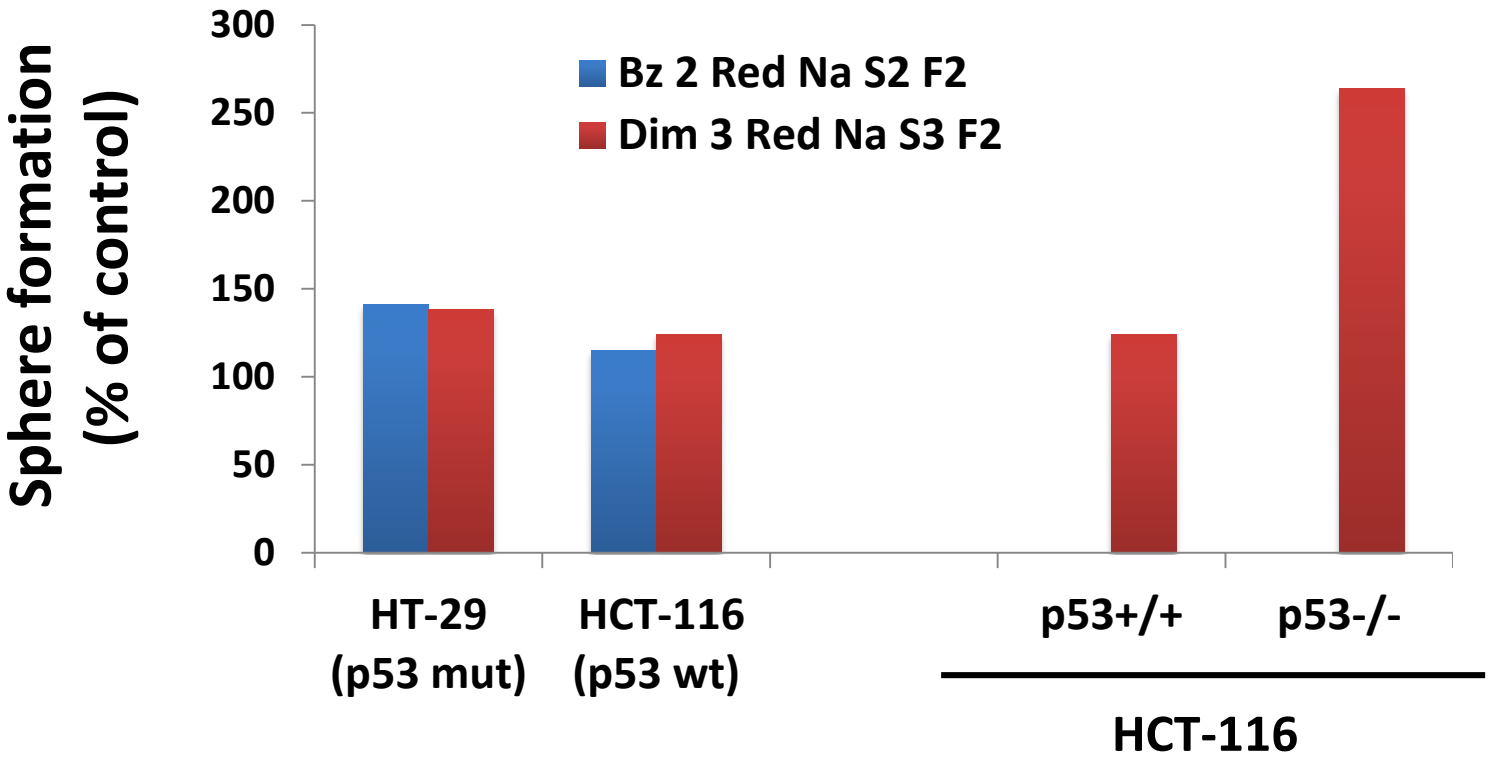
1) Low efficiency of reprogramming
2) Technical challenges in growing them in feeder free conditions.
3) Technical challenges in directed differentiation ex vivo.
4) Teratoma formation
5) Potential for cancer from cells derived from iPSCs/hESCs (i.e. formation of cancer stem cells)

Chemical Approach for Generation of iPSCs

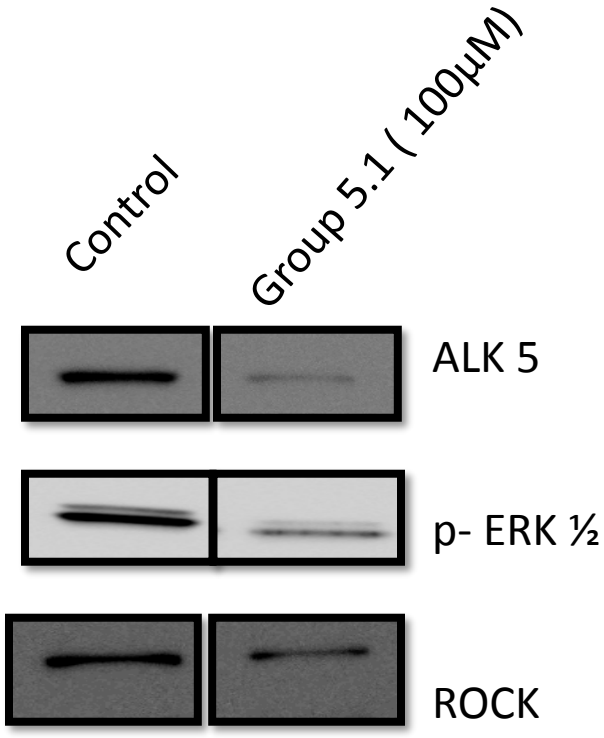


Hou P et al. Science; 2013

NSGM promote Stem Cell Growth

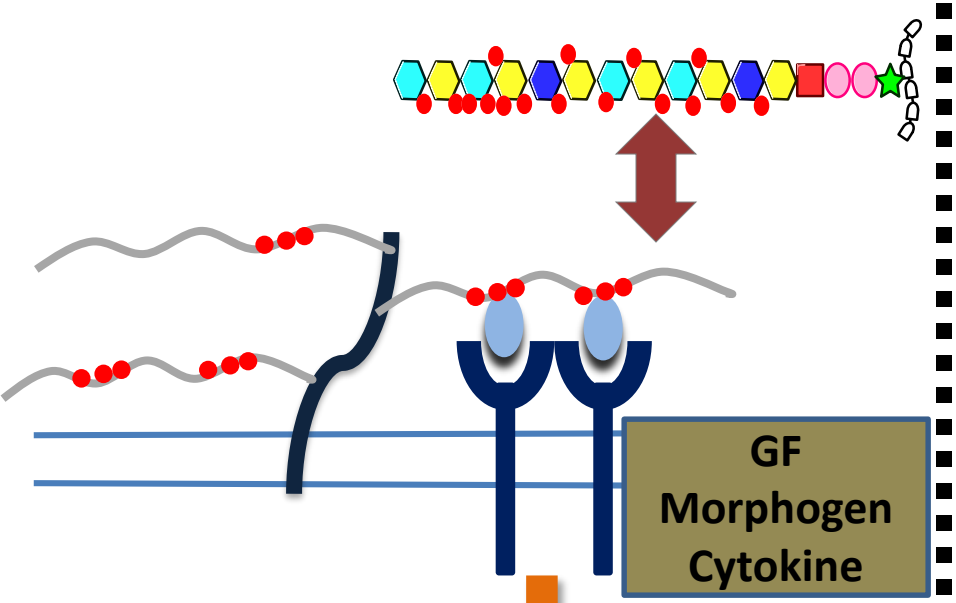


NSGMs modulates Essential ES pluripotency signaling



N.Patel, 2013

Glycosaminoglycans (GAGs)

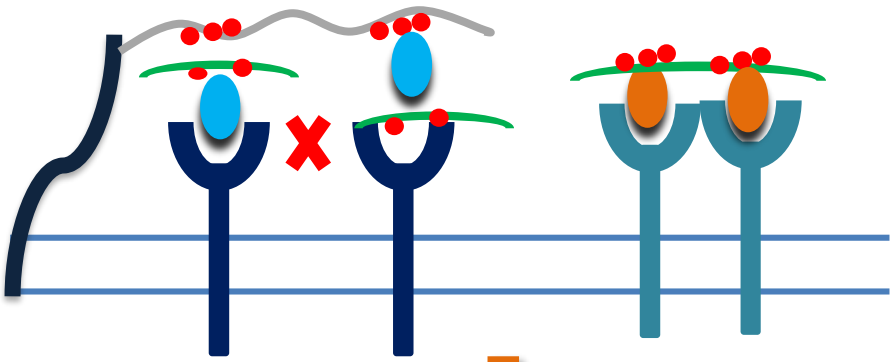


Intracellular Signaling

CSC Growth and Self-renewal

Hypothesis

GAG Mimetic



Altered Intracellular Signaling

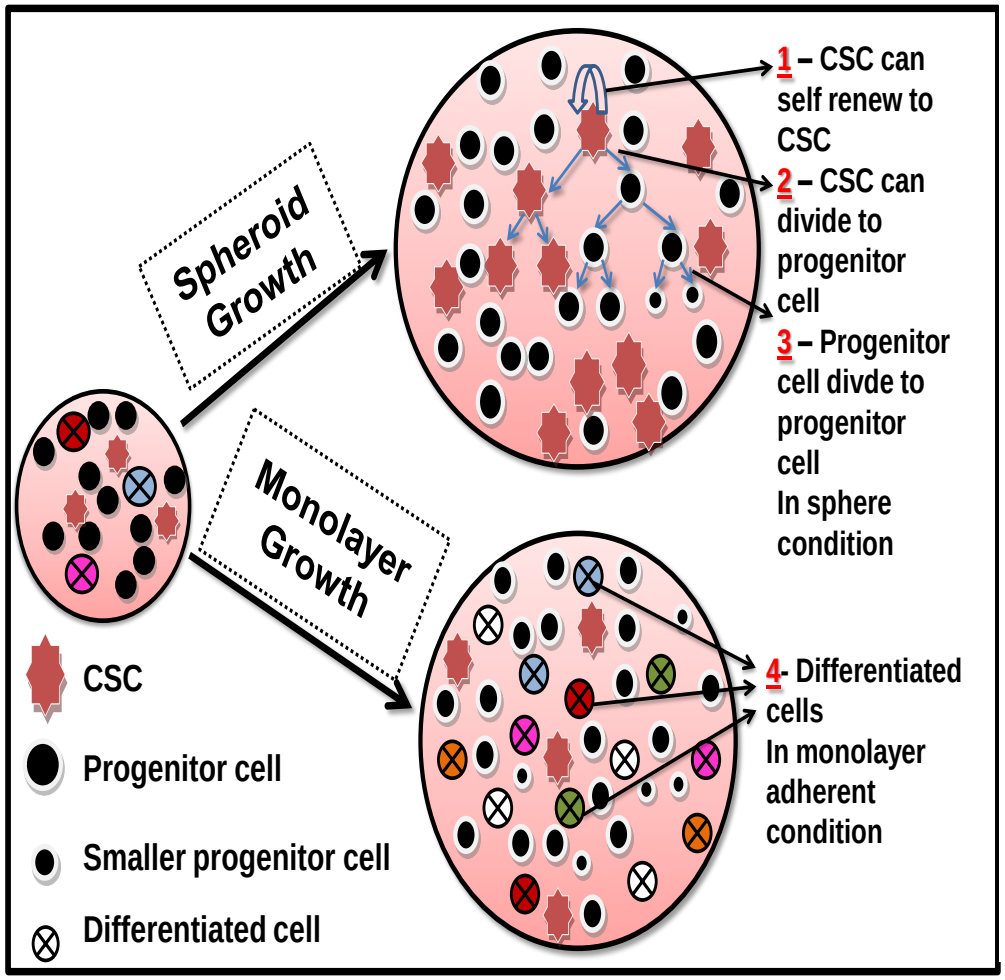
CSC Growth and Self-renewal

Select GAG derivatives/mimetics in Clinical Development for cancer treatment

	Matrix Remodeling enzymes	**Growth Factor/ Morphogens**			
	Heparanase	HGF	FGF	EGF	BMP
Natural GAG /derivatives					
Heparin/ LMWH		+	+		
M402		+	+		
Synthetic Saccharide based GAG mimetics					
PI-88	+		+		
PG-545	+	+	+	+	

Synthetic Non-saccharide based GAG mimetics	Over 150 molecules developed by Dr. Desai

Identification of Selective anti-CSC agent- Tandem Screening # 1



		Spheroid Growth +	Spheroid Growth -
Monolayer Growth	+	A	B
	-	C	D

A → Targeting CSC and Non-CSCs

B → Targeting Selective Non-CSCs

C → Targeting Selective CSCs/ Progenitor

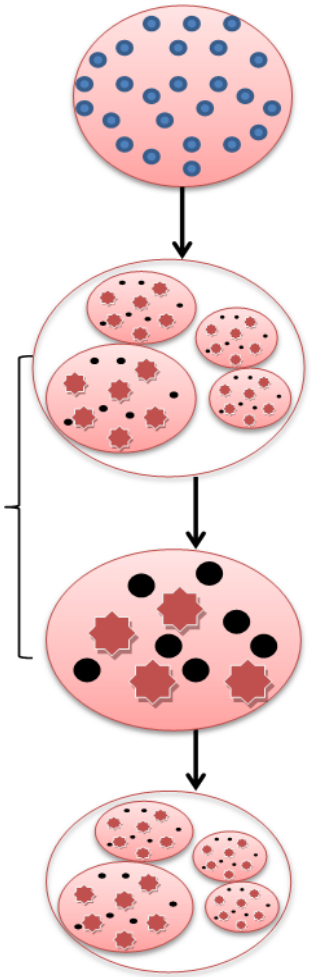
Identification of Selective anti-CSC agent- Tandem Screening # 2

2°/3° Colonosphere formation assay

Day 1 – Plate 100 cell/100 µl/ well followed by vehicle or treatment after 1 hour

Day 5- Average number of spheres counted, single cell suspension obtained for Secondary sphere

Day 10-Average number of secondary spheres counted



Primary Spheres

Targeting Selective CSCs / Progenitor

Secondary / Tertiary Spheres

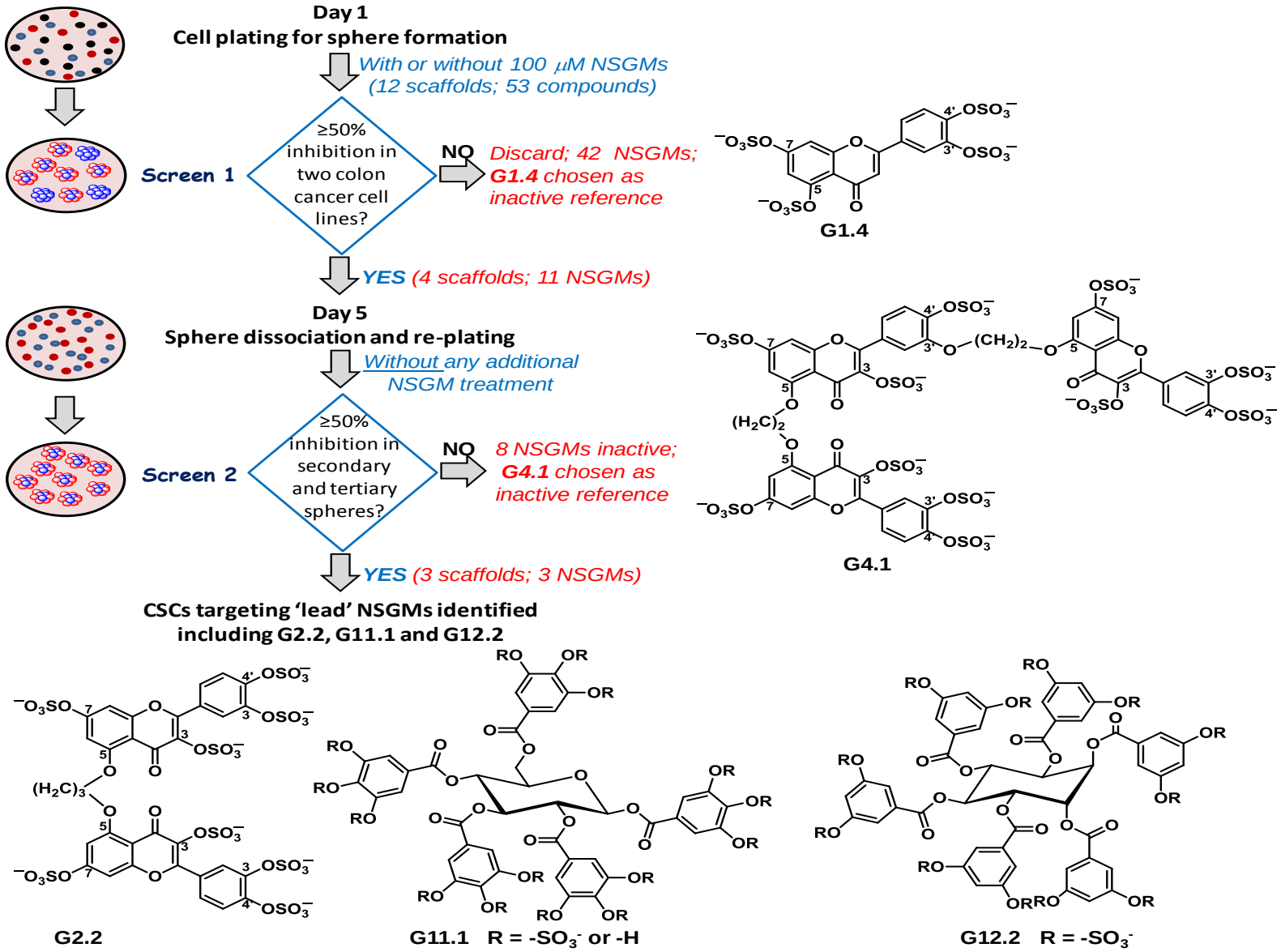
Growth Inhibition: Present

C1: Selectively targeting of self-renewing CSCs

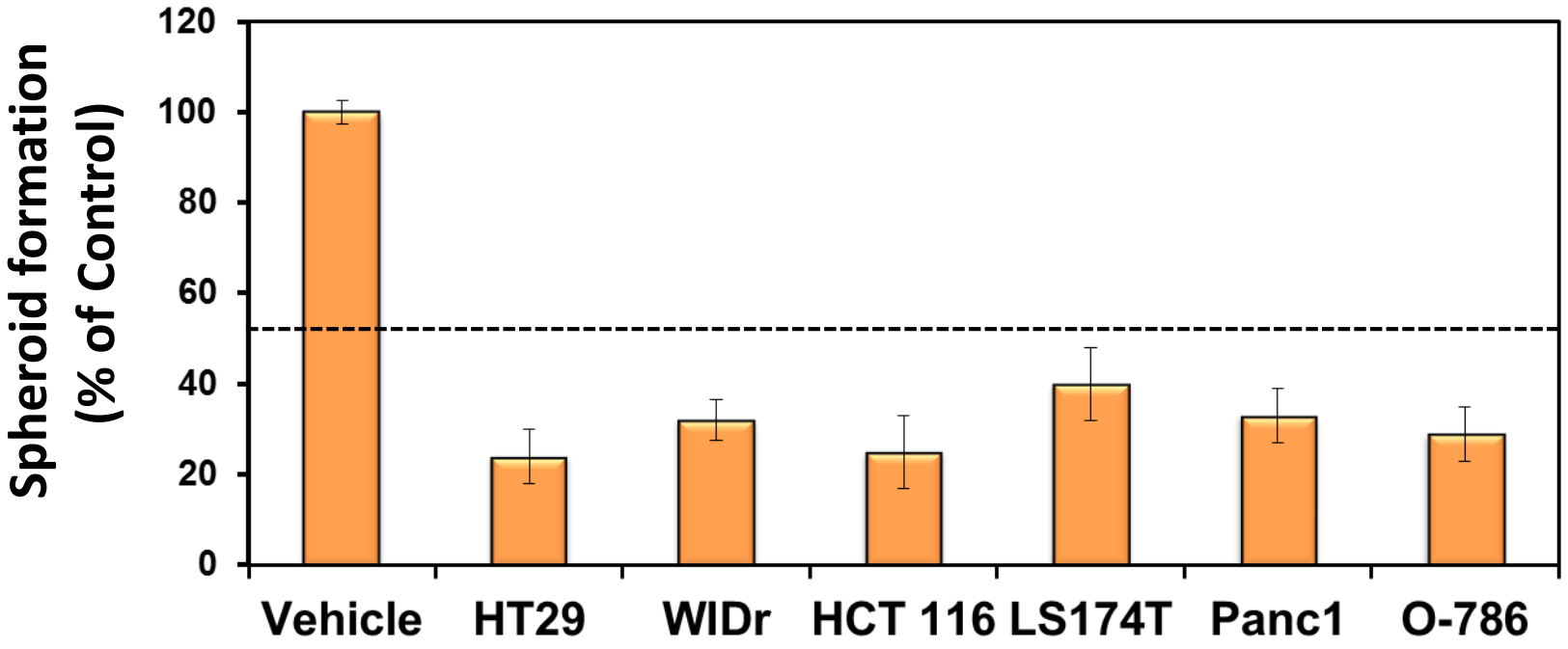
Growth Inhibition: Absent

C2: Targeting of progenitor cells

Tandem screening identified 3 NSGMs that Selectively Target CSCs



G2.2 Targets CSCs Irrespective of Genomics

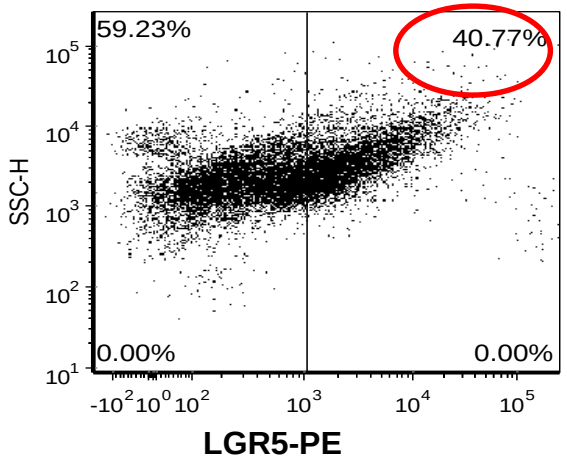


KRAS			■	■	■	
TP53	■				■	
BRAF	■	■				
B-Catenin			■	■		
APC	■					

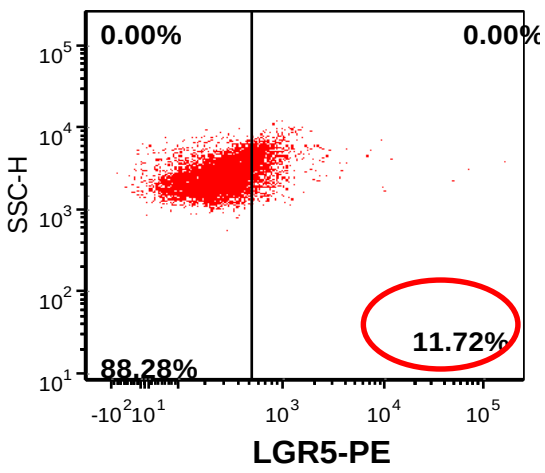
G2.2: in vivo anti-CSC effects

Intraperitoneal injection- three times a week up to 5 weeks

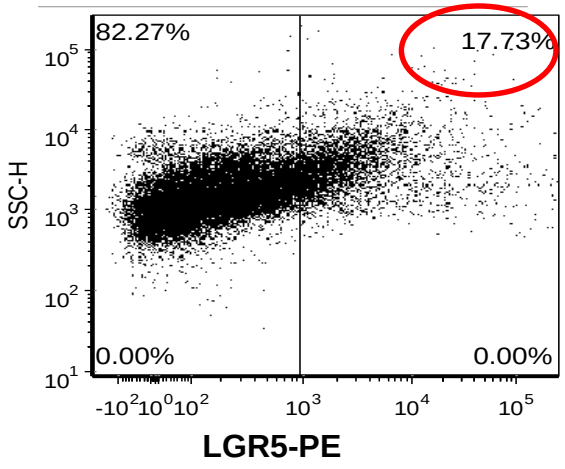
Control



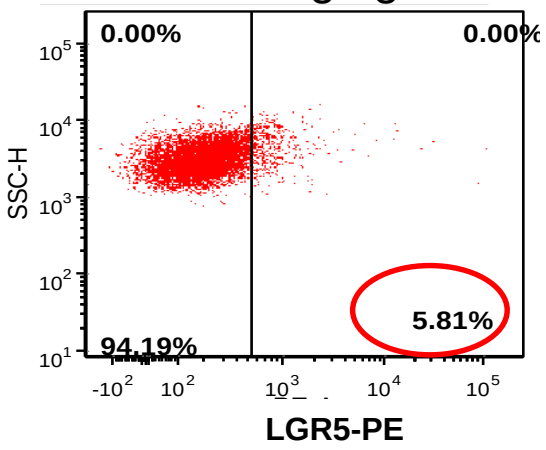
Control



10mg/Kg



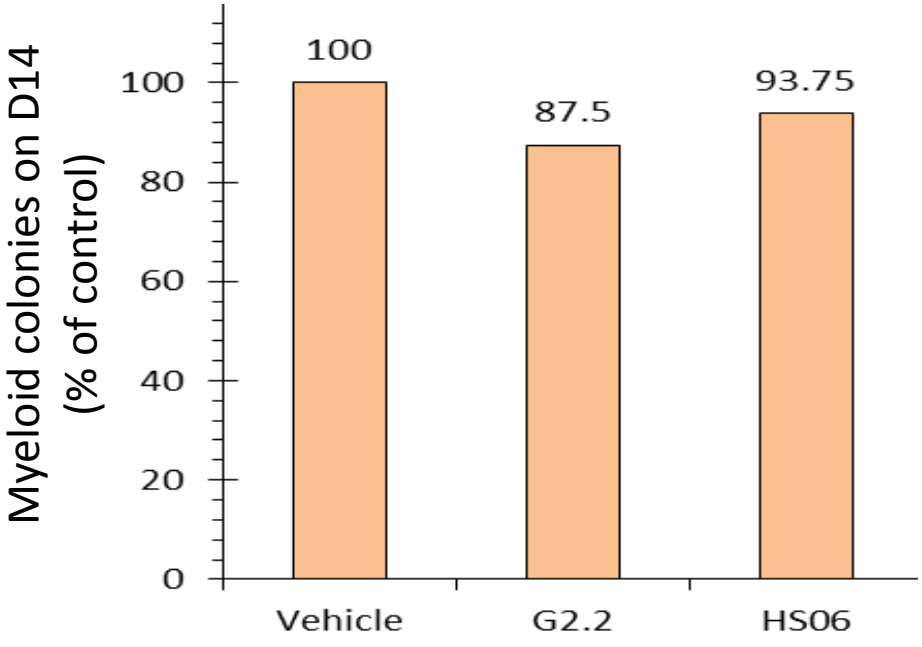
100mg/kg



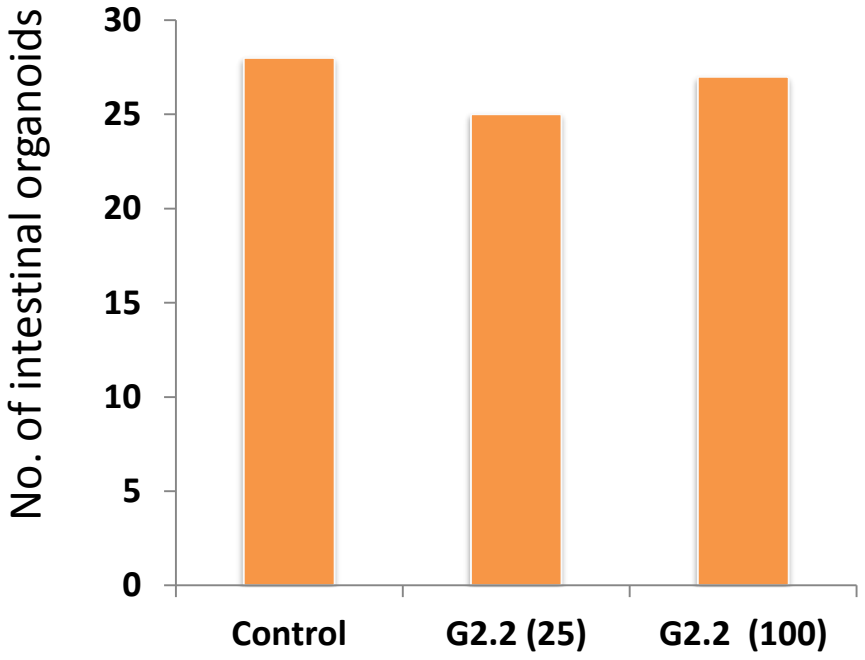
G2.2 toxicity

Adult hematopoietic and intestinal stem cells function

CD34 hematopoietic stem cell line



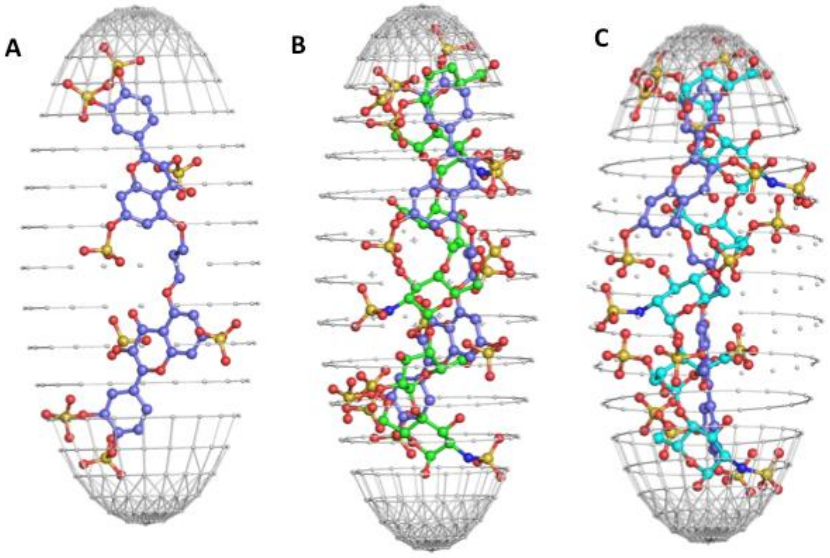
Mice intestinal epithelial cells- i.p. G2.2 or vehicle.



G2.2 is a mimic of HS06

Molecular dynamics simulation of G2.2 in water

Minimum volume enclosing ellipsoid

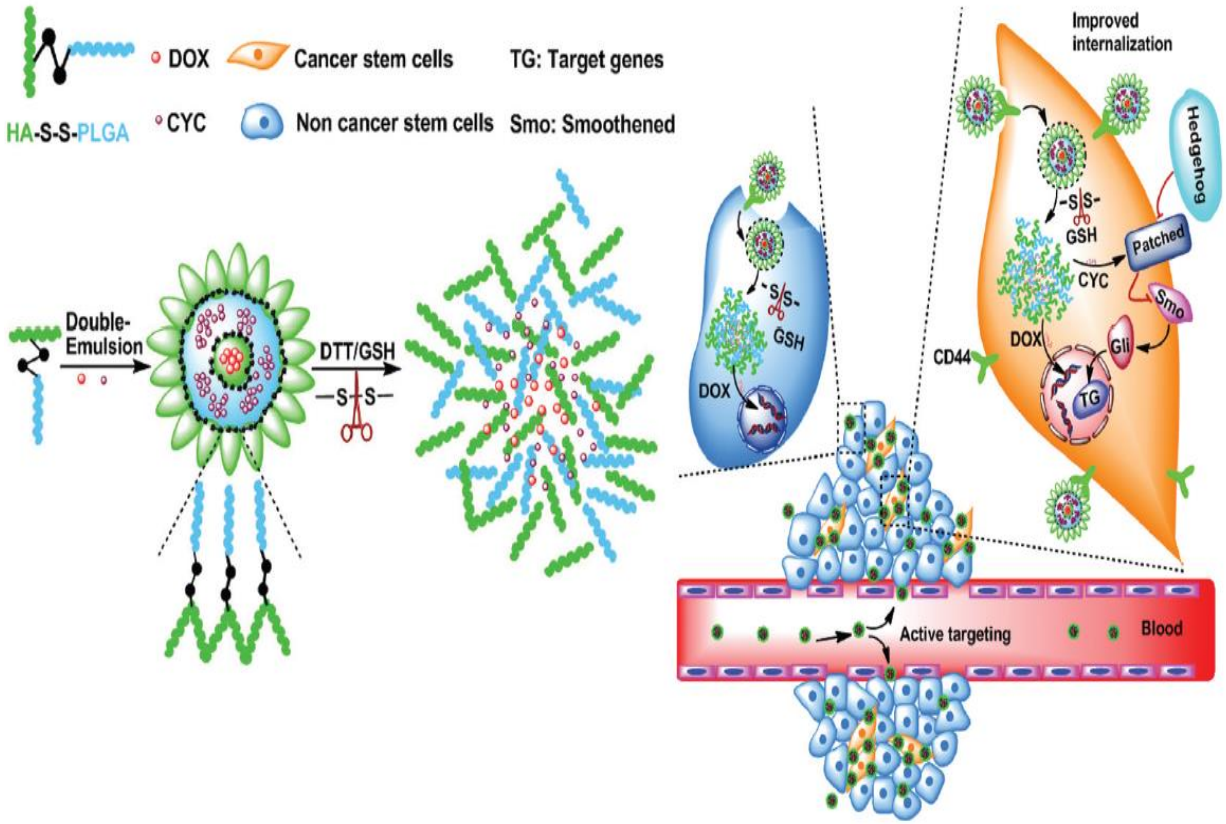


G2.2 **HS06**

Volume distribution

	G2.2	NMR Hexasaccharide	
	Avg	1HPN (^2S_O)	1HPN ($^1C_4$)
Vol	629.68	673.905	678.071
R1	5.05	6.120	5.97
R2	8.104	7.29	6.78
R3	15.52	15.11	16.70

Hyaluronan as carrier of anti-caner drugs to selectively target CSCs



Conclusions

- ✓ Glycosaminoglycans play a crucial role in stem cell growth and fate determination
- ✓ Glycosaminoglycan mimetics can potentially modulate ES/iPSC growth in culture defined growth conditions
- ✓ Use of Glycosaminoglycan mimetics as selective CSC targeting agents represent a paradigm shifting approach to cancer therapy.