# CANCER STEM CELL BIOLOGY and CANCER VACCINES
# (3)

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Fall-2021

# Cancer Stem Cell Signaling Pathways

## Self-renewal activity and Signaling pathways in CSCs

- Similar signaling pathways regulate the self-renewal activity in ESCs, SSCs, and CSCs.

- ESCs are pluripotent and can differentiate into any specialized cell in the body, while SSCs have more limited differentiation capacities. ESCs can be maintained in culture for long periods of time without losing their undifferentiated state. On the contrary, SSCs cultured in vitro differentiate rapidly, indicating that environmental and internal signals are fundamental for SSCs differentiation process and self-renewal.

- It is suggested that internal signals directed by genetic and epigenetic processes, and external signals composed of secreted cytokines and growth factors can change the fate of SSCs.

- In tumor environments, plasticity, usually mediated by microenvironmental signals, is very important mean for gaining excessive SCs self-renewal properties.

- Many of the mutations found in tumors are involved in the activation of self-renewal pathways in one way or another.

- In cancer, cells' multiple self-renewal pathways can not only be enhanced but also become continuously activated.

- Self-renewal program activation promotes tumor progression and metastasis by generating a high cell turnover and production of progenitors.

- Thus, a pathogenic self-renewal over differentiation balance in tumors further worsen the process of mutation accumulation.

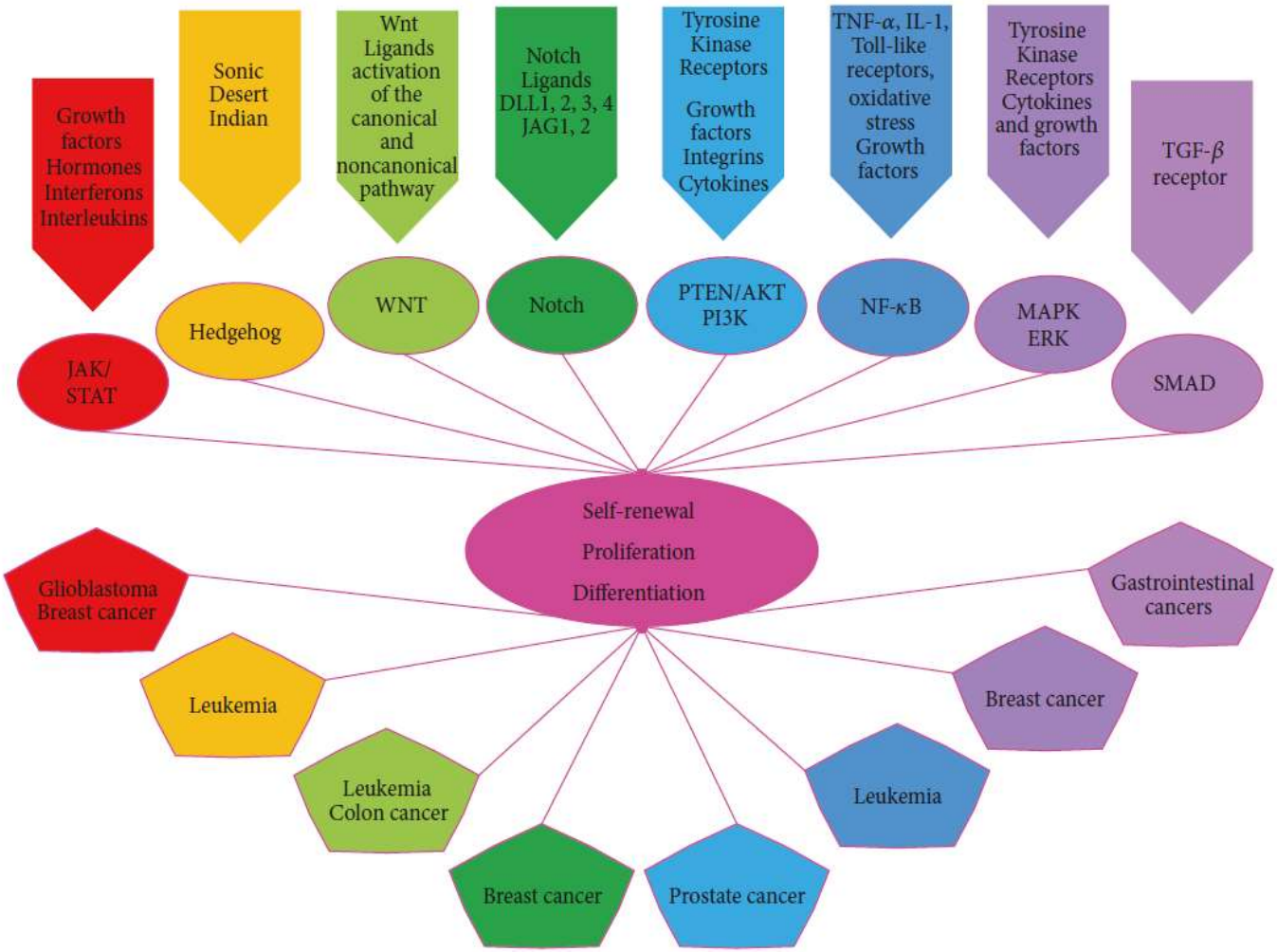
## Self-renewal activity and Signaling pathways in CSCs

- The self-renewal activity of SSCs is highly regulated by different signaling pathways but this tight regulation is lost in CSCs.

- These specific pathways such as

i. **Wnt/ β-catenin**,
ii. **Jack/Stat**,
iii. **TGF-β**,
iv. **Notch**, and
v. **Sonic Hedgehog** are deregulated in CSCs.

- CSCs have the ability to use these self-renewal pathways to drive tumor dissemination.




Aponte et al., 2017

# Role of Wnt/β-catenin pathway in CSCs

- The Wnt signaling pathway belongs to a family of secreted glycoproteins that have several functions, including *regulating proliferation, differentiation, and patterning throughout embryonic development*. Wnt drives symmetrical cell divisions in stem cells.

- Other components of the Wnt pathway include molecules of the Wnt secretory machinery, *Wnt co-receptors, new components of the β- catenin degradation machinery, and nuclear co-factors*.

- Mutations in Wnt genes or defects in their signaling pathway cause specific developmental defects during embryonic development leading to certain human diseases and cancer during adulthood.

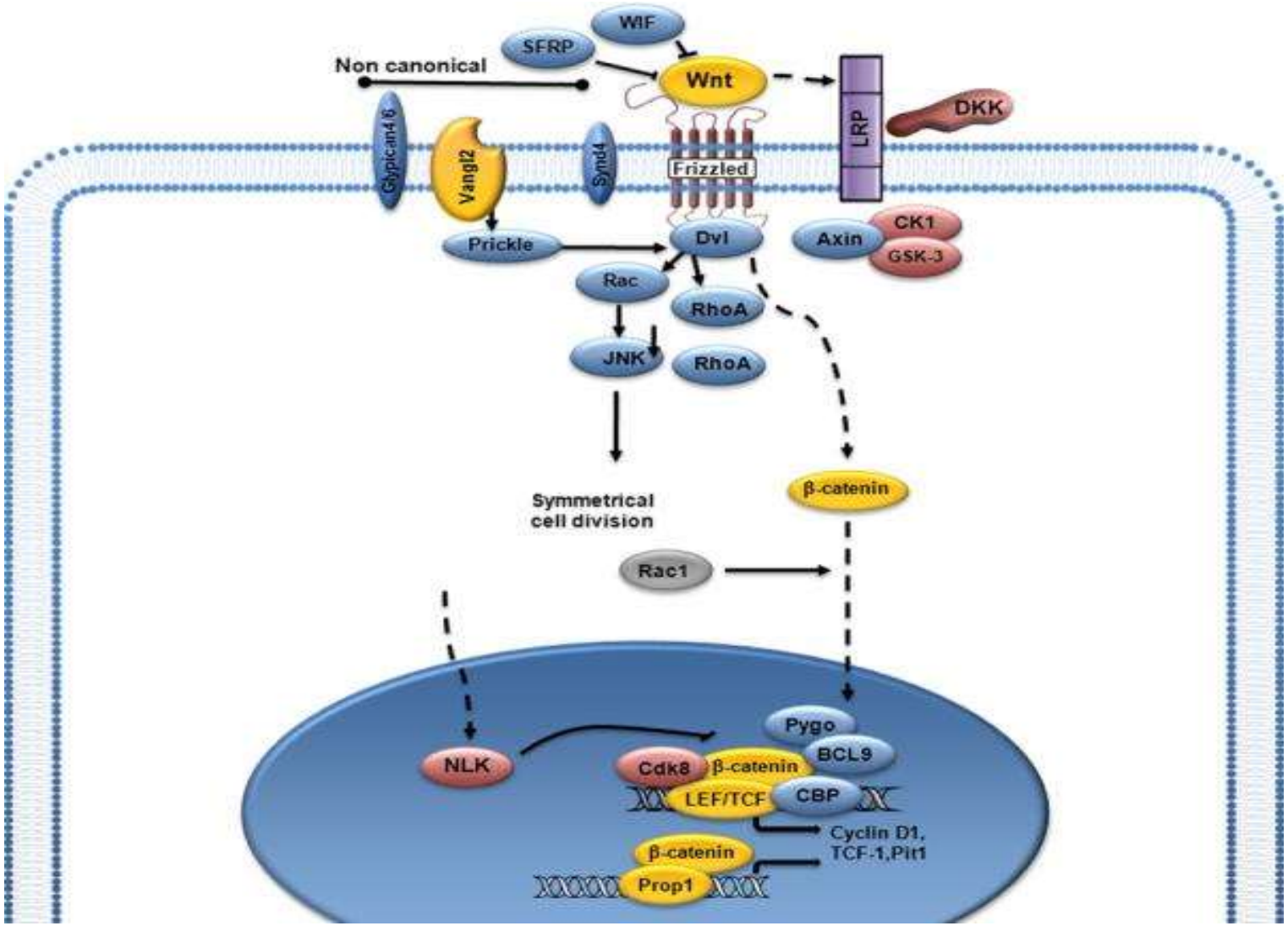
- Abnormal expression of Wnt ligand proteins has been observed in different types of tumors in osteosarcoma, hematological malignancies, breast cancer, and non-small cell lung cancer.

# Role of Wnt/β-catenin pathway in CSCs

- Wnt signaling has been shown to be deregulated in leukemic stem cells compared to somatic hematopoietic stem cells.

- In AML, deregulated activation of the Wnt/β-catenin pathway induces cell proliferation by turning on genes encoding oncoproteins and cell-cycle regulators.

- The Wnt family of genes encodes 19 glycoprpteins that bind frizzled (Fzd) receptors.


WIF
SFRP
Non canonical
Wnt
LRP
DKK
Glypican4/6
Vangl2
Synd4
Frizzled
Prickle
Dvl
Axin
CK1
GSK-3
Rac
RhoA
JNK
RhoA
Symmetrical
cell division
β-catenin
Rac1
NLK
Pygo
BCL9
Cdk8
β-catenin
LEF/TCF
CBP
Cyclin D1,
TCF-1,Pit1
β-catenin
Prop1

Yu et al., 2012

# STAT signaling deregulation and CSCs induction

- STAT ⟹ Signal transducers and activators of transcription

- Involve tyrosine phosphorylation by Janus family tyrosine kinases (JAKs) that further allow STAT protein dimerization and nuclear translocation and, finally, expression of target genes.

- JAK-STAT signaling pathway regulates:

✓ somatic cell differentiation,

✓ proliferation,

✓ immune response, and

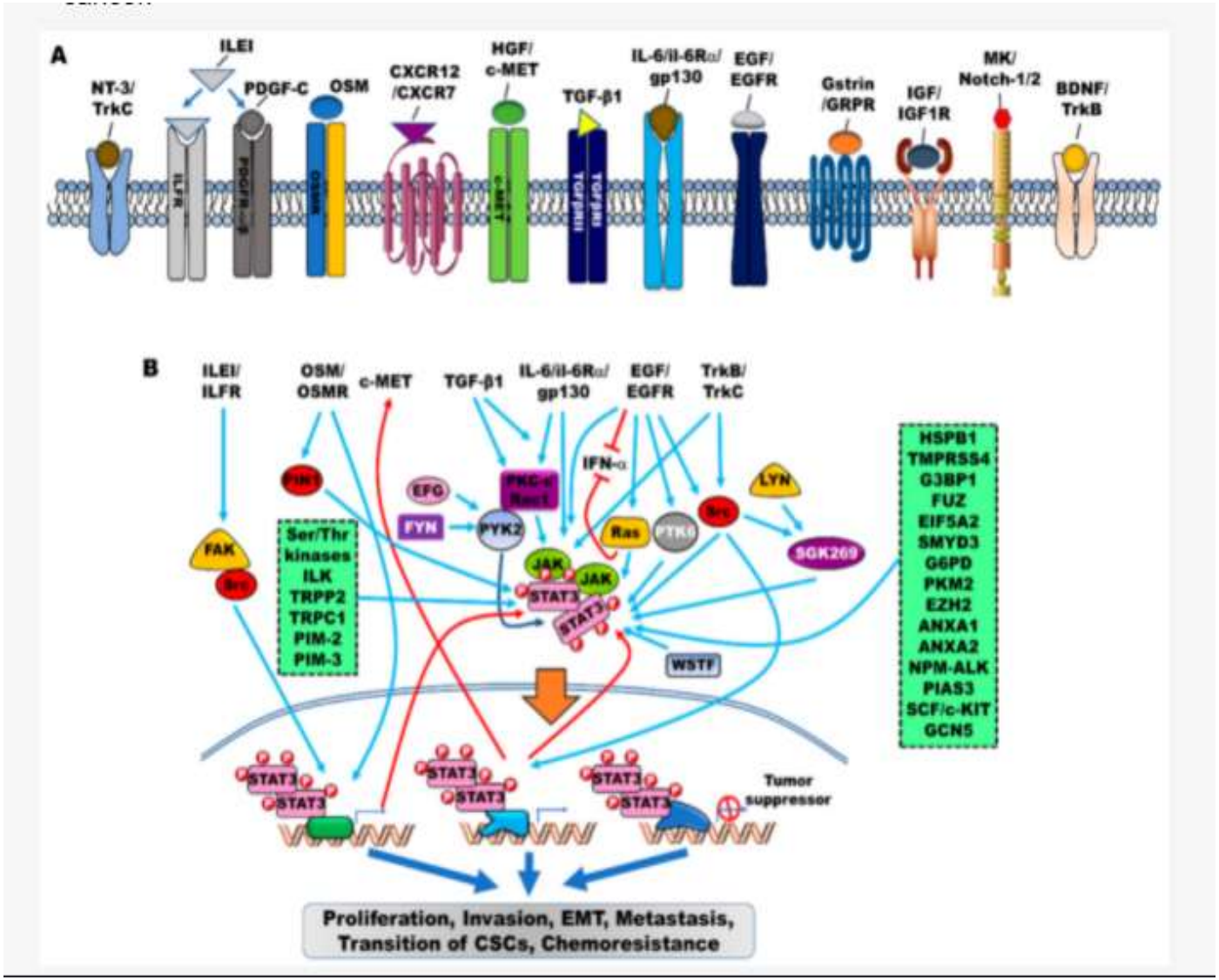
✓ apoptosis

# STAT signaling deregulation and CSCs induction

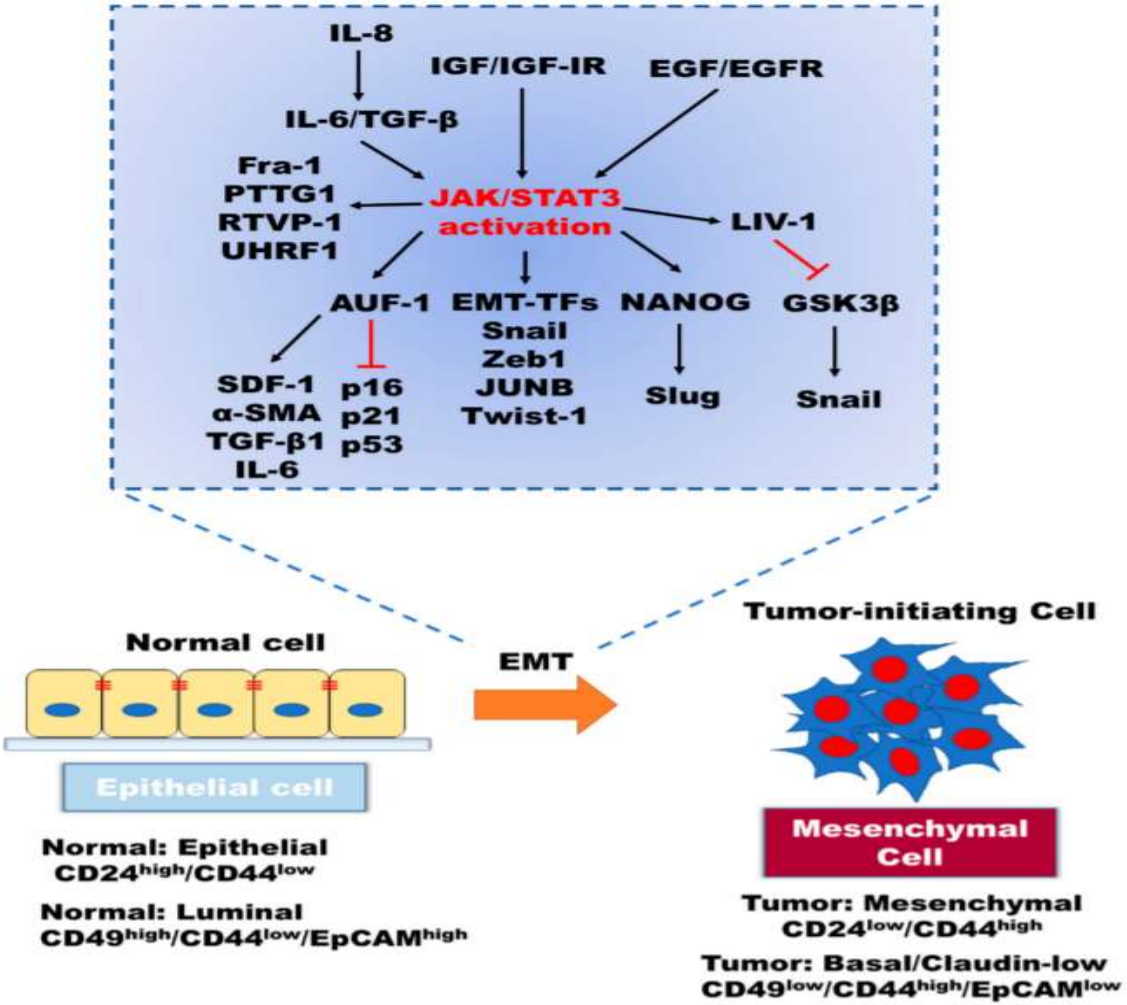
- STAT signaling pathway has also been shown to regulate both stem cell-renewal and tumorigenesis, and abnormal activation of the STAT pathway induces cell transformation and oncogenesis of many cancer types.

- This deregulation of STAT pathway can cause the inhibition of differentiation pathways and induce stem cell self-renewal.

- It has been shown that STAT signaling plays a role in different stem cell niches in SSCs and CSCs.


A
ILEI
NT-3/
TrkC
PDGF-C
OSM
CXCR12
/CXCR7
HGF/
c-MET
TGF-β1
IL-6/il-6Rα/
gp130
EGF/
EGFR
Gstrin
/GRPR
IGF/
IGF1R
MK/
Notch-1/2
BDNF/
TrkB
B
ILEI/
ILFR
OSM/
OSMR
c-MET
TGF-β1
IL-6/il-6Rα/
gp130
EGF/
EGFR
TrkB/
TrkC
IFN-α
PIM1
EFG
FYN
PKC-ε
Rac1
PYK2
Ras
PYK2
Src
LYN
FAK
Src
Ser/Thr
kinases
ILK
TRPP2
TRPC1
PIM-2
PIM-3
JAK
JAK
STAT3
STAT3
SGK269
WSTF
HSPB1
TMPRSS4
G3BP1
FUZ
EIF5A2
SMYD3
G6PD
PKM2
EZH2
ANXA1
ANXA2
NPM-ALK
PIAS3
SCF/c-KIT
GCN5
STAT3
STAT3
STAT3
STAT3
STAT3
STAT3
Tumor
suppressor
Proliferation, Invasion, EMT, Metastasis,
Transition of CSCs, Chemoresistance

Jin, 2020


IL-8
IGF/IGF-IR
EGF/EGFR
IL-6/TGF-β
Fra-1
PTTG1
RTVP-1
UHRF1
JAK/STAT3
activation
LIV-1
AUF-1
EMT-TFs
Snail
Zeb1
JUNB
Twist-1
NANOG
GSK3β
SDF-1
α-SMA
TGF-β1
IL-6
p16
p21
p53
Slug
Snail
Normal cell
EMT
Tumor-initiating Cell
Epithelial cell
Mesenchymal Cell
Normal: Epithelial
CD24high/CD44low
Normal: Luminal
CD49high/CD44low/EpCAMhigh
Tumor: Mesenchymal
CD24low/CD44high
Tumor: Basal/Claudin-low
CD49low/CD44high/EpCAMlow

Jin, 2020

# TGF-β pathway in CSCs

- TGF-β is mostly known for its role in tissue repair and maintenance in SSCs.

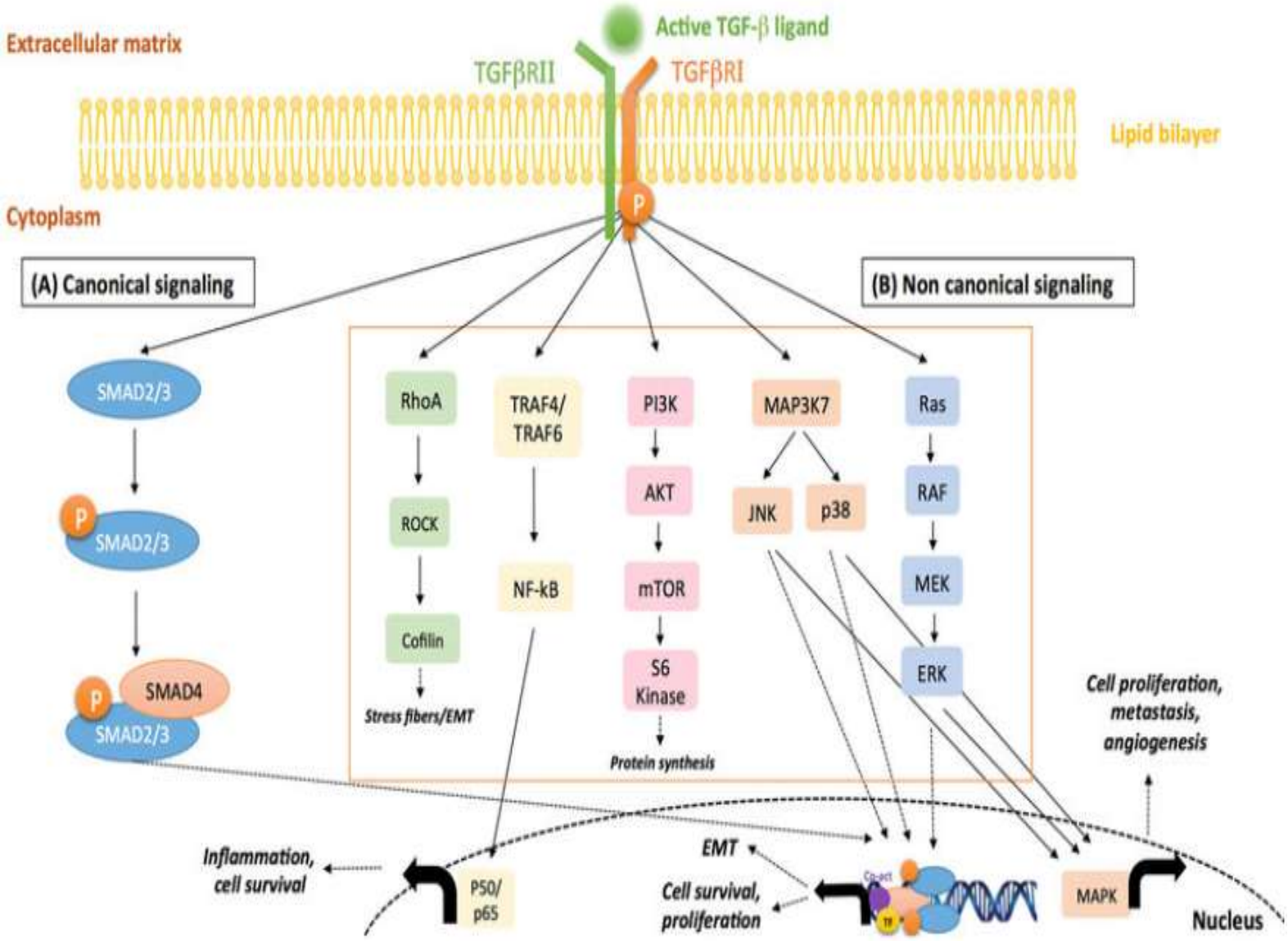
- Together with CSCs, TGF-β participates in the initiation and development of various tumors, and in the acquisition of CSC-like properties.

# TGF-β pathway in CSCs

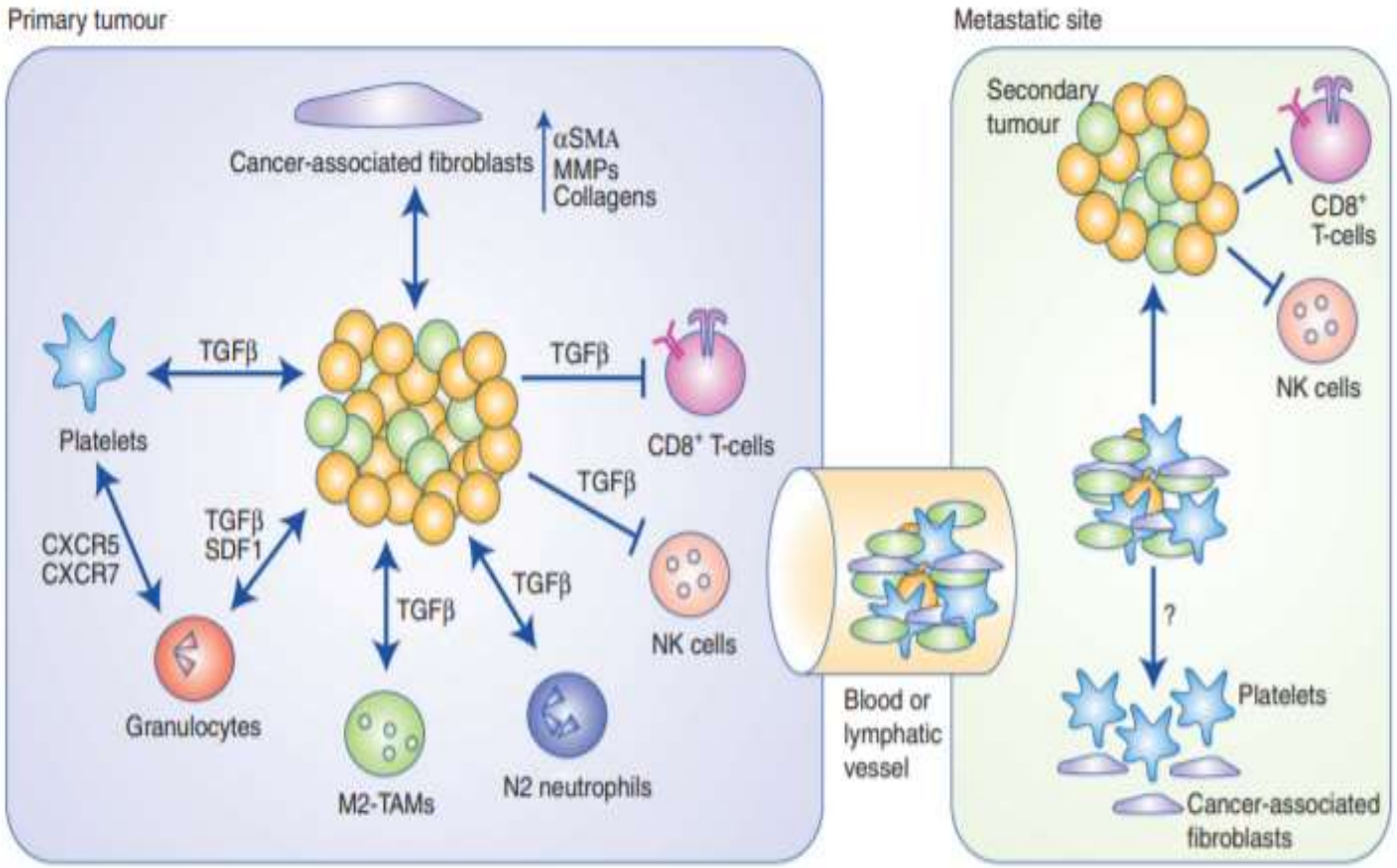
- TGF-β signaling is initiated upon TGF-β ligand interaction with type II and type I transmembrane serine/threonine kinase receptors on the cell surface which induces oligomerization of the receptor kinases and phosphorylation of the cytoplasmic signaling molecules Smad2 and Smad3 for the TGF-β/activin pathway.

- The activated Smad complexes are translocated into the nucleus together with other nuclear co-factors to regulate the transcription of target genes.

- Interestingly, the loss of function of certain Smads is observed during tumorigenesis of pancreatic and colorectal cancers as well as other cancer types.




Costanza et al., 2017




Bellomo et al., 2016

# Notch Signaling in CSC

- This pathway is mainly involved in cell to cell communication, tissue differentiation, and self-renewal of stem cells.
- Notch pathway is deregulated in different cancers, and it is believed that Notch regulates the formation of cancer stem cells and the initiation of EMT phenotype.
- Notch activation contributes to expansion of a variety of stem and early progenitor cells.

# Notch Signaling in CSC

- Notch signaling is initiated by the interaction between a Notch ligand and a Notch receptor expressed on the surface of neighboring cells.

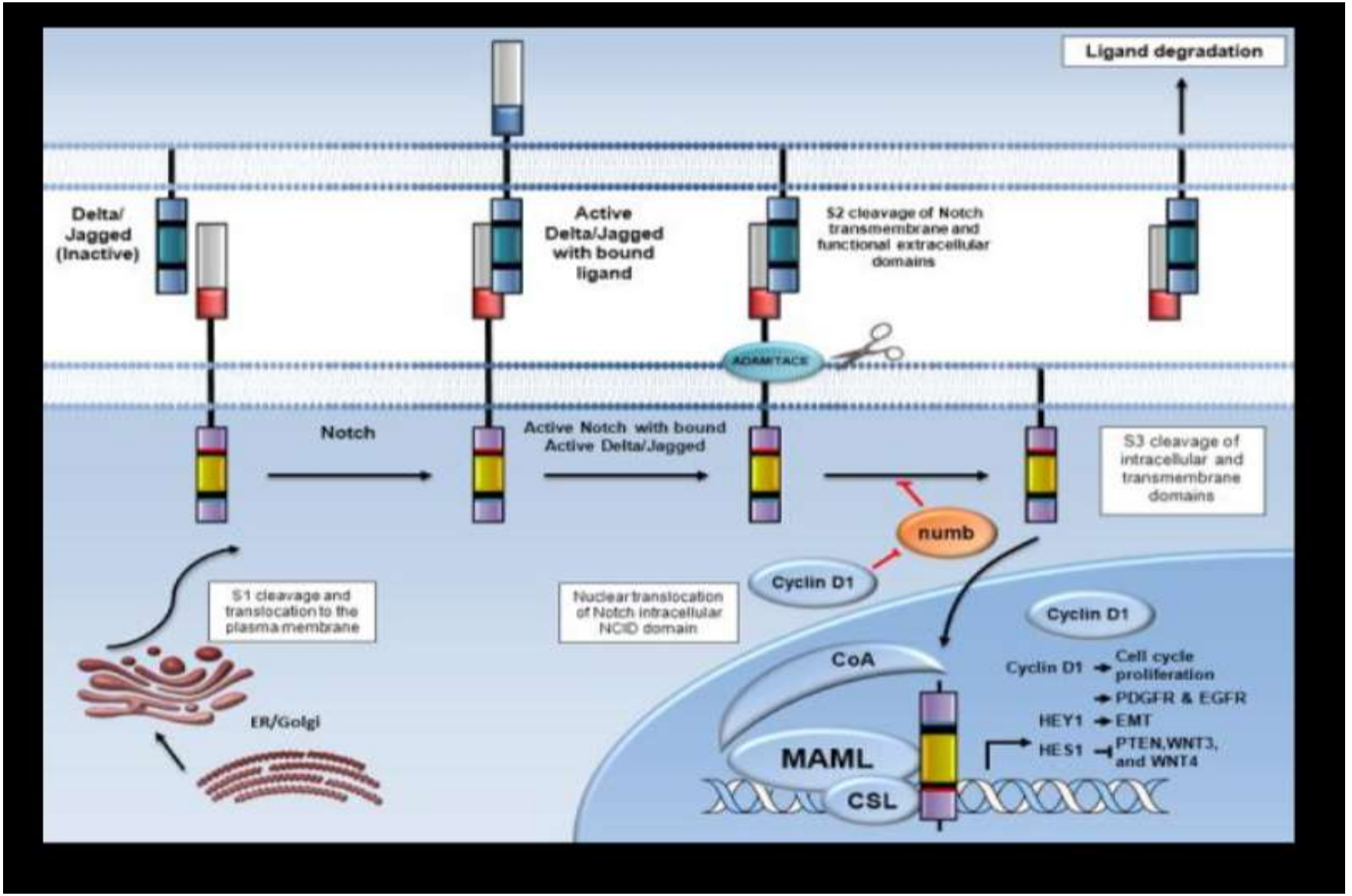
- In mammals, the Notch pathway is formed by five canonical type I transmembrane ligands, including:

✓ Delta-like ligands (DLLs), DLL1, DLL3, and DLL4;

✓ two Jagged proteins, Jagged1 and Jagged2; and

- four Notch transmembrane receptors, Notch1–4.

- This interaction triggers a two-step proteolytic cleavage of the receptor, mediated by an ADAM/TACE (tumor necrosis factor alpha converting enzyme) metalloproteases. ADAM10 and ADAM17 interact with nuclear factors to regulate target gene expression that regulates cell differentiation.


Ligand degradation
Delta/
Jagged
(Inactive)
Active
Delta/Jagged
with bound
ligand
S2 cleavage of Notch
transmembrane and
functional extracellular
domains
ADAM/TACE
Notch
Active Notch with bound
Active Delta/Jagged
S3 cleavage of
intracellular and
transmembrane
domains
numb
Cyclin D1
S1 cleavage and
translocation to the
plasma membrane
Nuclear translocation
of Notch intracellular
NCID domain
Cyclin D1
CoA
Cyclin D1 → Cell cycle proliferation
→ PDGFR & EGFR
HEY1 → EMT
HES1 ⊣ PTEN, WNT3, and WNT4
ER/Golgi
MAML
CSL

Yu et al., 2012

# Sonic Hedgehog Signaling in CSC

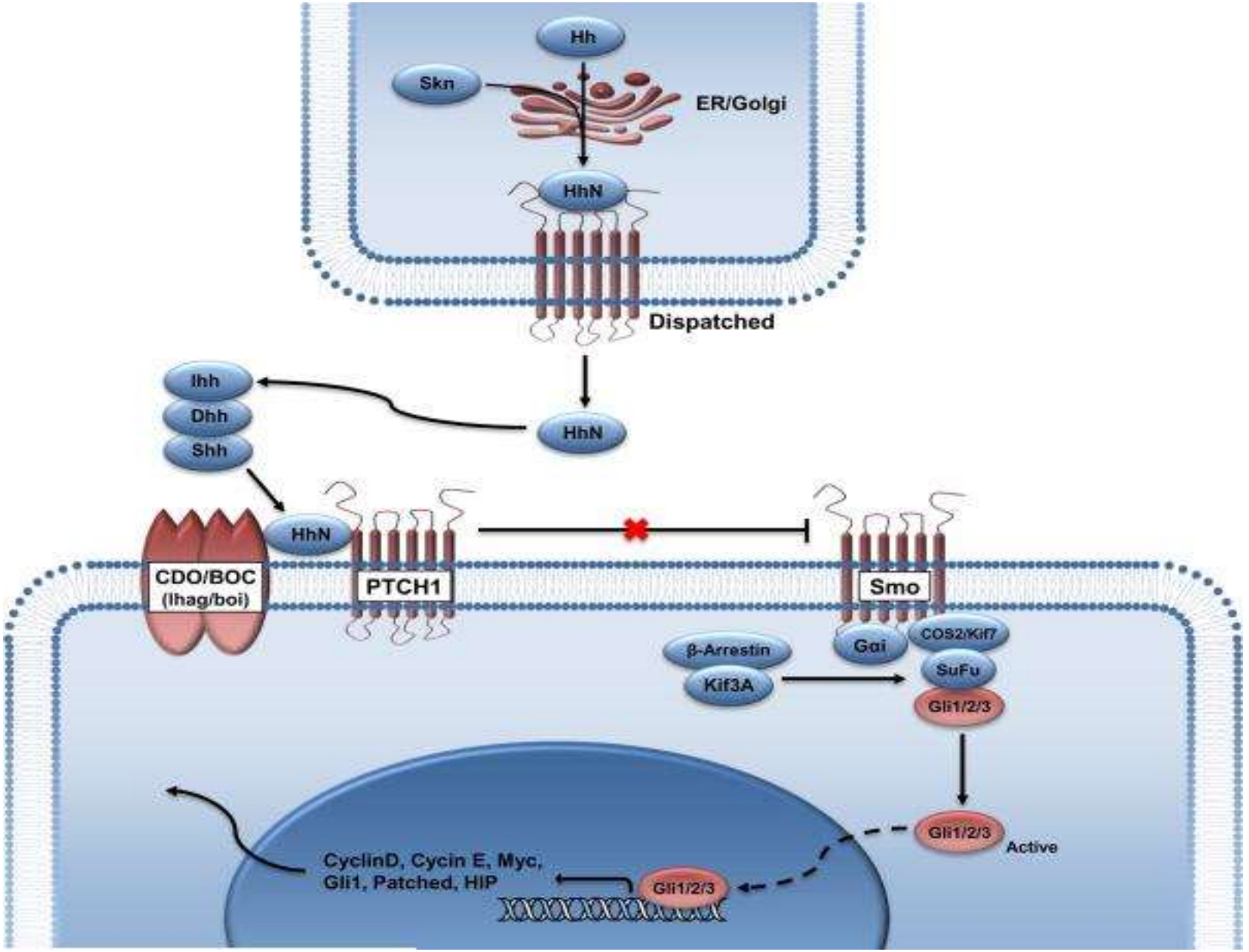
- Hh signaling pathway forms one of the networks of *major regulators of cell differentiation, proliferation, and cell polarity.*

- This pathway is a fundamental part of embryonic development. Mutations generated in the Hedgehog pathway resulted in defective axial patterning, causing a cephalic disorders.

- Alterations in Hh signaling pathway were also associated with cancer development.

- Hh signaling has been shown to be crucial for the maintenance and expansion of CSCs. Deregulation of Hh signaling has been linked with development of CSC formation and EMT development.

- Hh, like the Wnt signaling pathway, is one of the major regulators of cell differentiation and proliferation.


Hh
Skn
ER/Golgi
HhN
Dispatched
Ihh
Dhh
Shh
HhN
HhN
CDO/BOC
(Ihag/boi)
PTCH1
Smo
β-Arrestin
Kif3A
Gαi
COS2/Kif7
SuFu
Gli1/2/3
Gli1/2/3
Active
CyclinD, Cycin E, Myc,
Gli1, Patched, HIP
Gli1/2/3

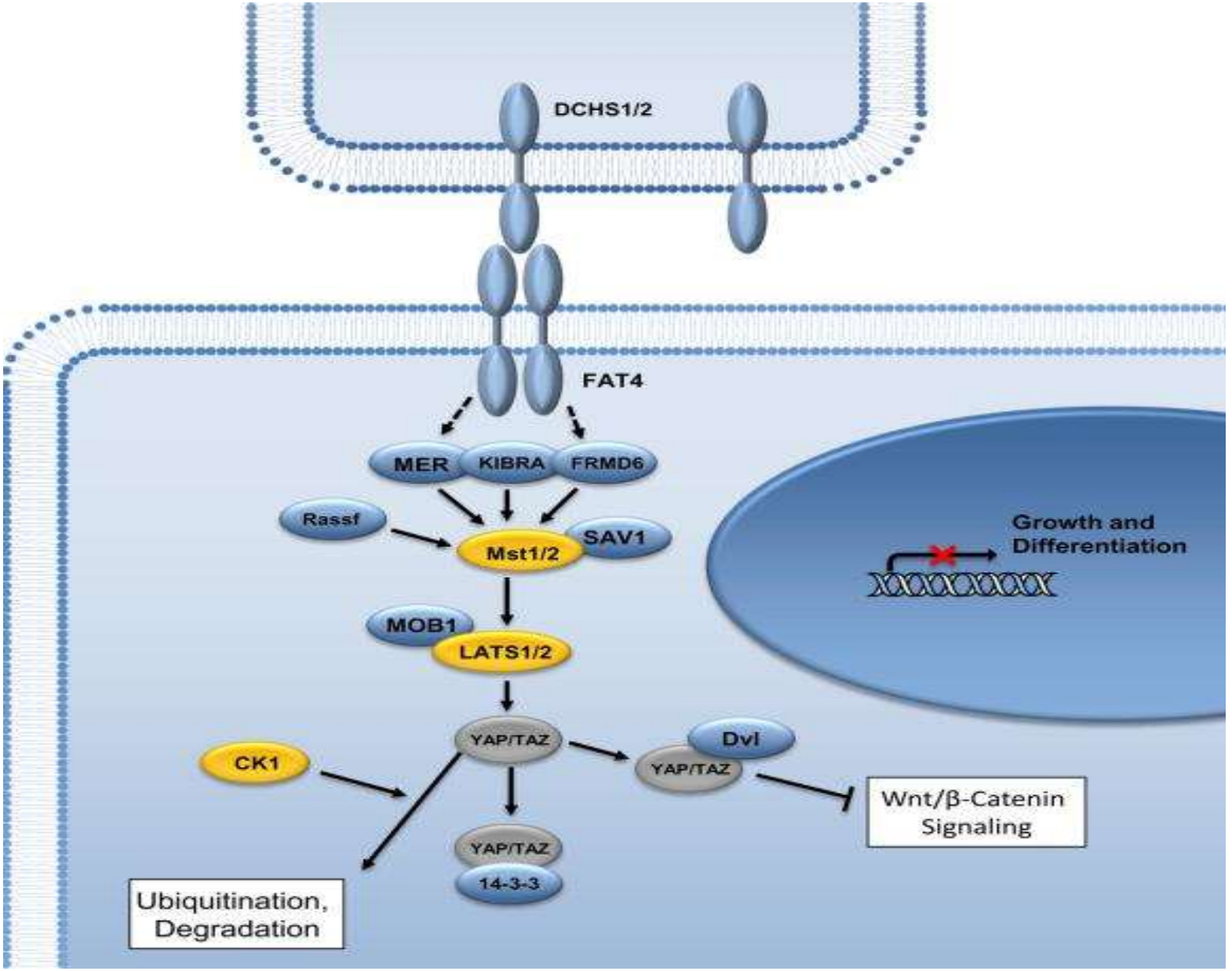
Yu et al., 2012

# Hippo Pathway

- Although primarily involved in determining organismal size and functioning as a tumor suppressor pathway, recent studies have demonstrated a role for the Hippo pathway in stem cells.

- The hippo pathway component YAP prevents ESCs differentiation.

- High YAP expansion in a perivascular cancer stem cell compartment, suggest a role for YAP in CSC maintenance in the Hippo pathway.


DCHS1/2
FAT4
MER
KIBRA
FRMD6
Rassf
Mst1/2
SAV1
MOB1
LATS1/2
Growth and
Differentiation
YAP/TAZ
CK1
Dvl
YAP/TAZ
Wnt/β-Catenin
Signaling
YAP/TAZ
14-3-3
Ubiquitination,
Degradation

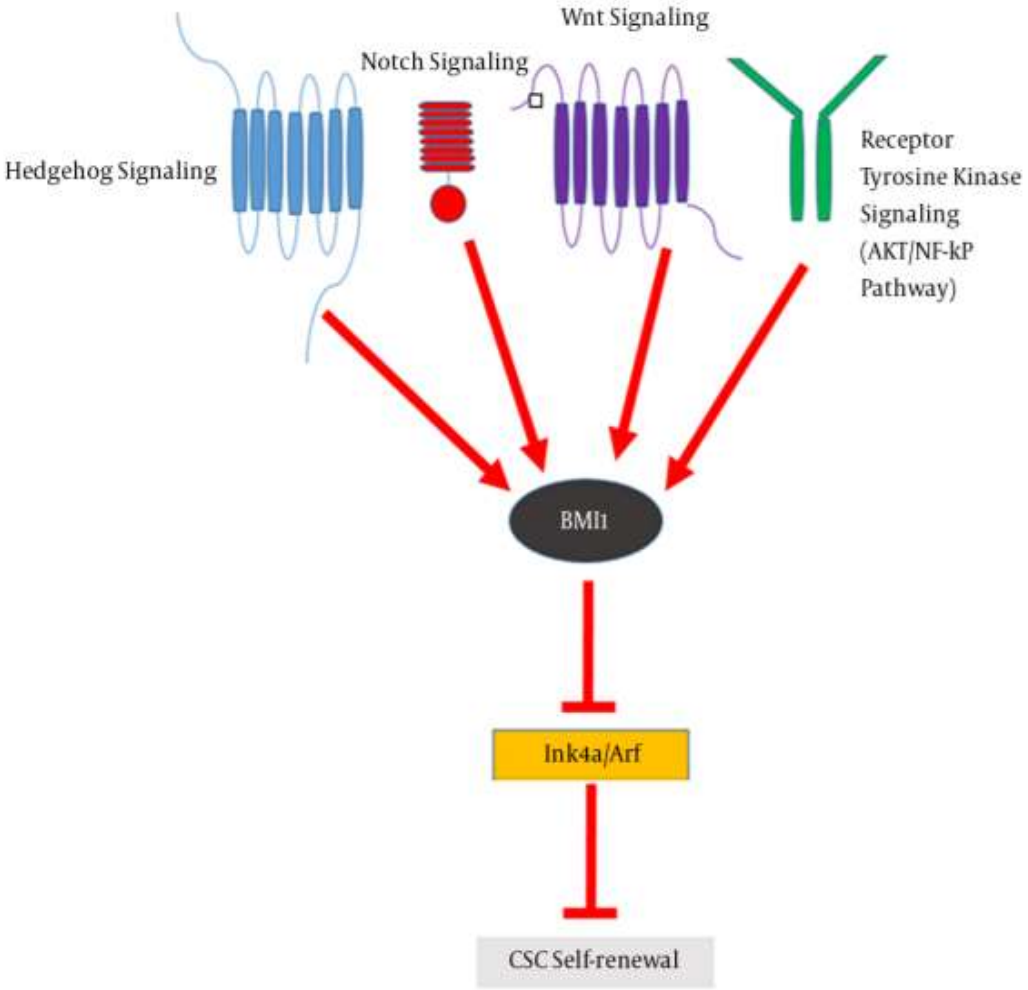
Yu et al., 2012

# Bmi-1 Pathway

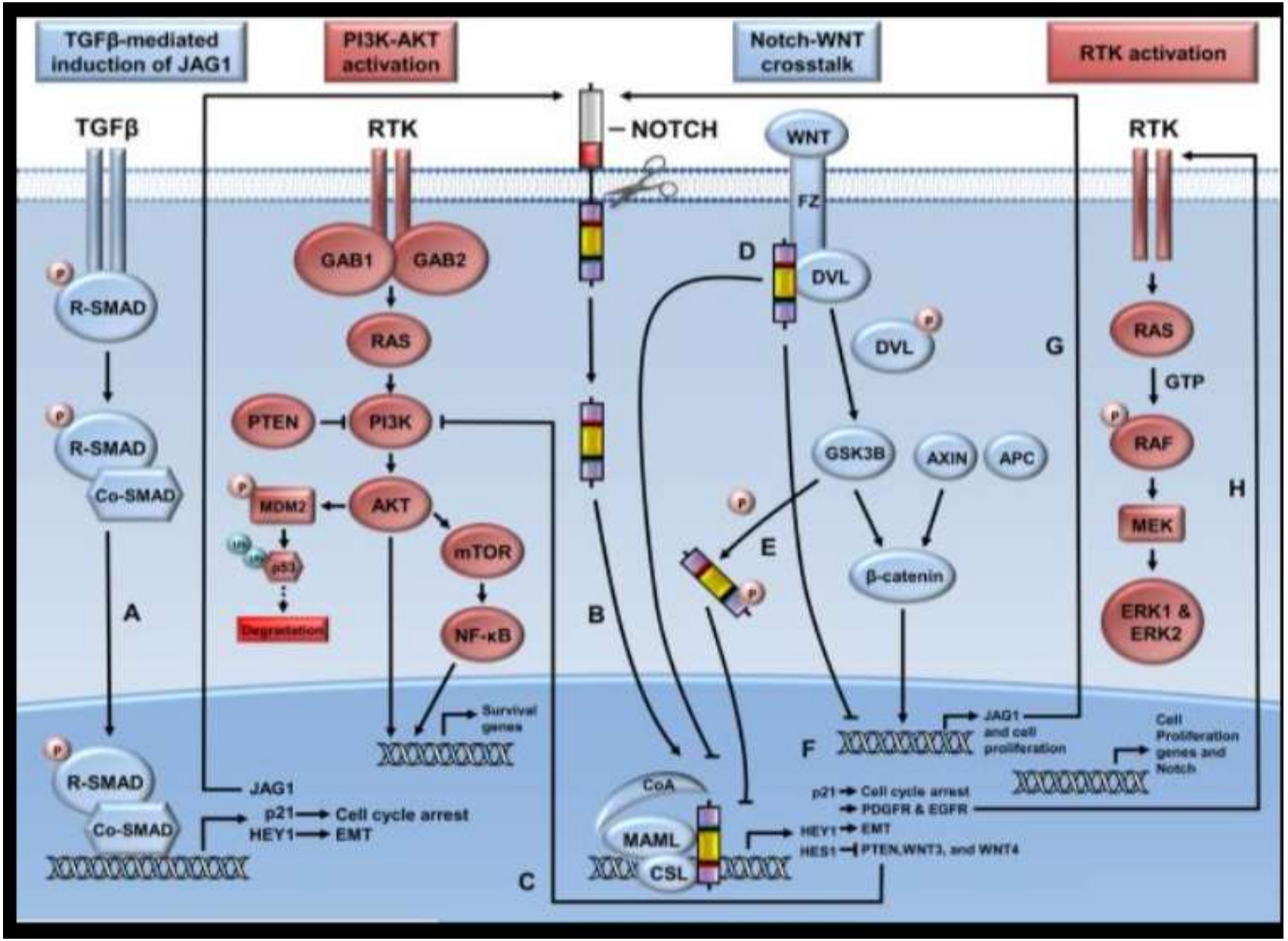
- Recently it has been identified that Bmi-1 is a vital regulator of the self-renewal of both ordinary, leukemic SCs and neuronal SC.

- It has also been suggested that there is a link between Bmi-1 and mammary carcinogenesis.

- Although Bmi-1 mediated stem cell self-renewal mechanism was not clear but P-16 silenced by Bmi-1. But, P-16 partially mediates the effects of Bmi-1 proteins in neural stem cells, which suggests that other factors might take part in Bmi-1's effects on SC self-renewal.




Soheilifar et al., 2018


TGFβ-mediated induction of JAG1
PI3K-AKT activation
Notch-WNT crosstalk
RTK activation
TGFβ
RTK
NOTCH
WNT
FZ
RTK
GAB1
GAB2
D
DVL
DVL
R-SMAD
RAS
G
RAS
GTP
R-SMAD
PTEN
PI3K
GSK3B
AXIN
APC
RAF
Co-SMAD
MDM2
AKT
E
H
MEK
mTOR
β-catenin
A
B
ERK1 & ERK2
NF-κB
Survival genes
F
JAG1 and cell proliferation
Cell Proliferation genes and Notch
R-SMAD
JAG1
CoA
p21 → Cell cycle arrest
→ PDGFR & EGFR
Co-SMAD
p21 → Cell cycle arrest
HEY1 → EMT
MAML
HEY1 → EMT
HES1 → PTEN,WNT3, and WNT4
C
CSL

Yu et al., 2012

- **Having the knowledge of how CSC pathways are regulated in relation to cell differentiation, renewal, and quiescence opens the possibility of targeting CSCs specific pathways without affecting normal cells!!**

# References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# To be Continued…