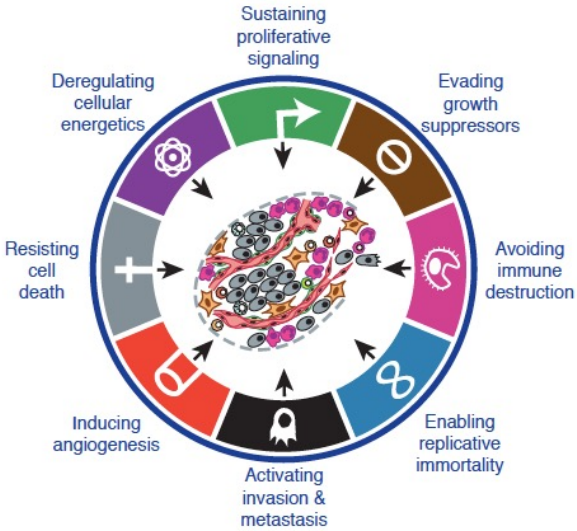
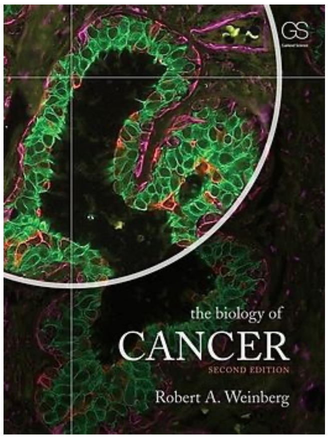
The hallmarks of cancer - BIO-392

Hanahan & Weinberg, 2011
Cell 144:646-674
(see Moodle, week 1)



Recommended textbook:

Titel: The Biology of Cancer
2nd edition, 2014
Garland Science
Autor: Robert A. Weinberg
EAN: 9780815345282
ISBN: 978-0-8153-4528-2



Introduction to oncology: Outline Constam part

Core hallmark capabilities:

March 5:	Sustained proliferation I
March 12:	Sustained proliferation II
April 2:	Evading growth suppression
April 9:	Establishing replicative immortality
April 23:	Spring break
May 7:	Activating invasion and metastasis
May 14:	Evading apoptosis

Today

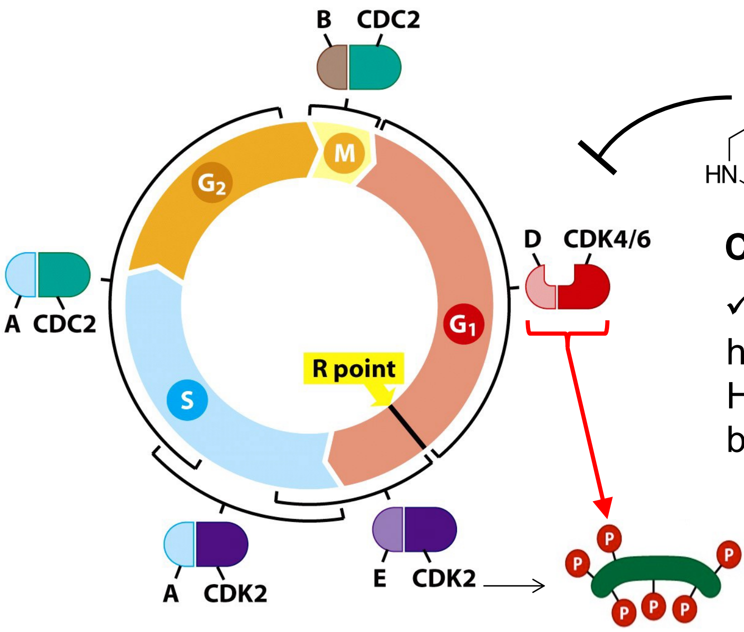
TODAY

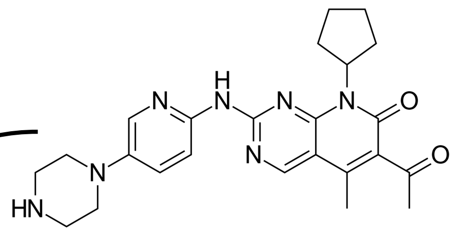
Hallmark capability 1: Sustained proliferative signaling

Weinberg, selected parts of chapters 5 & 6

- ☑ Receptor tyrosine kinases (RTK)
  - ✓ Discovery
  - ✓ Oncogenic mutations
  - ✓ Therapeutic inhibitors
- ☑ RTK signal transduction
  - ✓ Ras family of small GTPases
  - ✓ Drugging oncogenic KRAS
  - ✓ PI3K/Akt signaling (Ras effectors)
- ❑ JAK/STAT signaling & inhibitors
- ❑ Wnt/β-catenin signaling in colon cancer

Why not simply block CDK4 and CDK6?





CDK4/6i (Palbociclib, Pfizer)

✓FDA-approved in 2017 for hormone receptor-positive, HER2-negative metastatic breast cancer

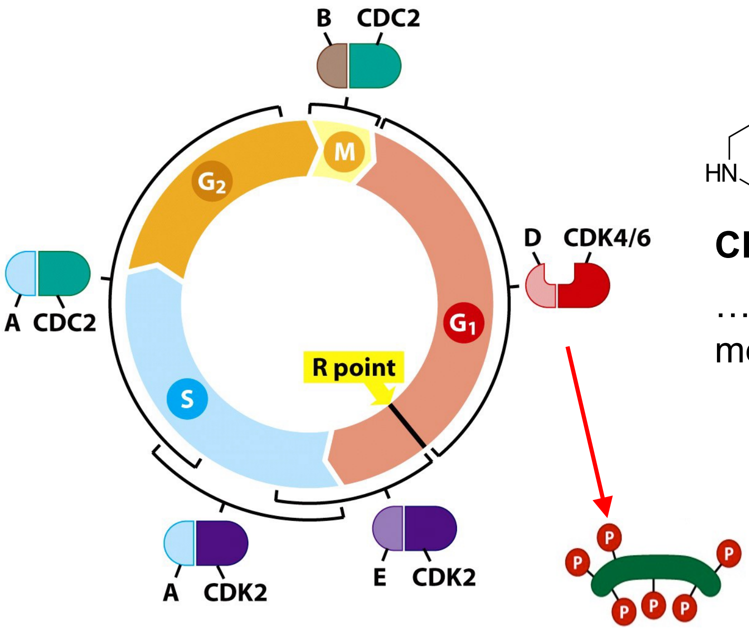
FREE ACCESS | Colorectal Cancer | January 19, 2022

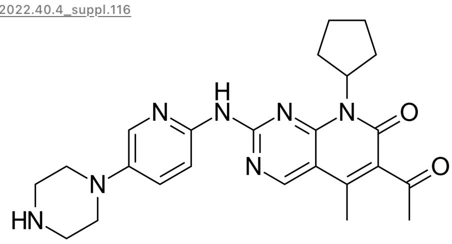
A randomized phase II trial of MEK and CDK4/6 inhibitors vesus tipiracil/trifluridine (TAS-102) in metastatic *KRAS/NRAS* mutant (mut) colorectal cancer (CRC).

2022

Authors: Michael Sangmin Lee, Tyler J. Zemla, Kristen Keon Ciombor, Autumn Jackson McRee, Mehmet Akce, Shaker R. Dakhil, Brandy L. Jaszewski, Fang-Shu Ou, Tanios S. Bekaii-Saab, and Scott Kopetz | AUTHORS INFO & AFFILIATIONS

Publication: Journal of Clinical Oncology • Volume 40, Number 4_suppl • https://doi.org/10.1200/JCO.2022.40.4_suppl.116

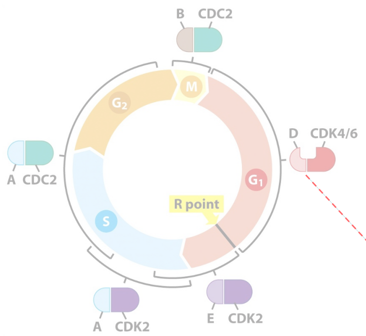


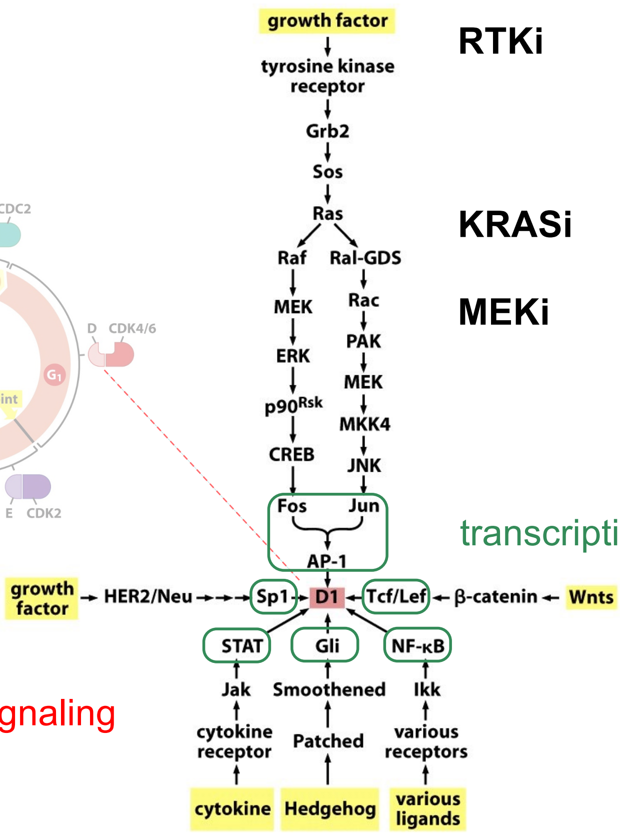


CDK4/6i (Palbociclib, Pfizer)

…but ineffective, e.g. in metastatic colorectal cancer

Targeting upstream regulators of cyclin D expression ?





RTKi

KRASi

MEKi

transcription factors

JAK/STAT signaling

JAKi ?

Figure 8.11b *The Biology of Cancer* (© Garland Science 2007)

We have seen:
RTKs transphosphorylate upon ligation by homodimeric ligands

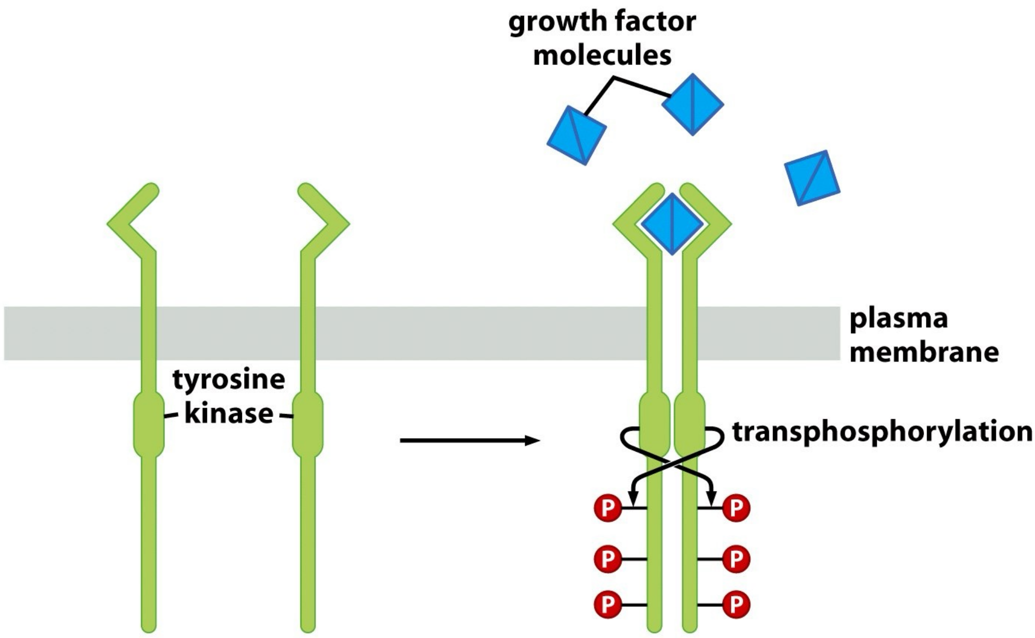
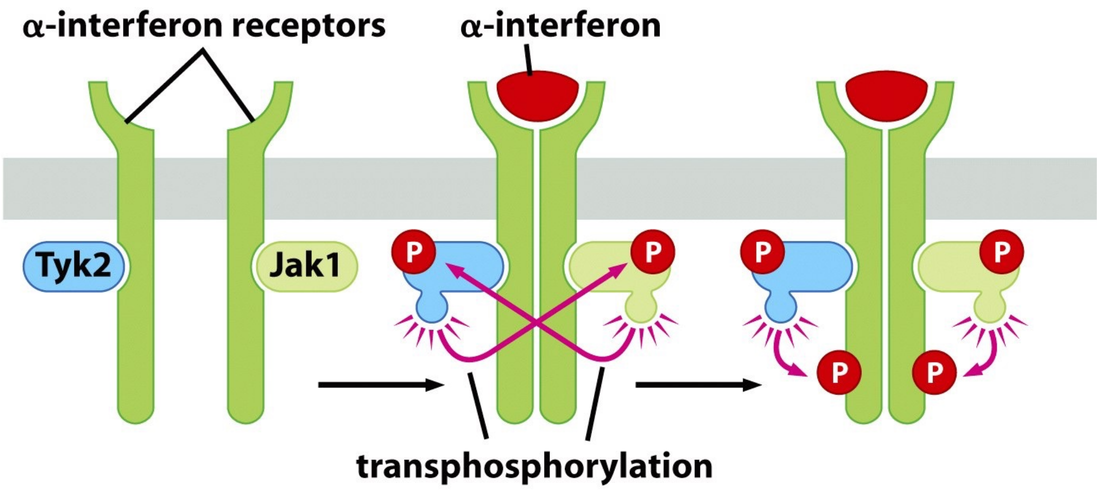


Figure 5.15 *The Biology of Cancer* (© Garland Science 2007)

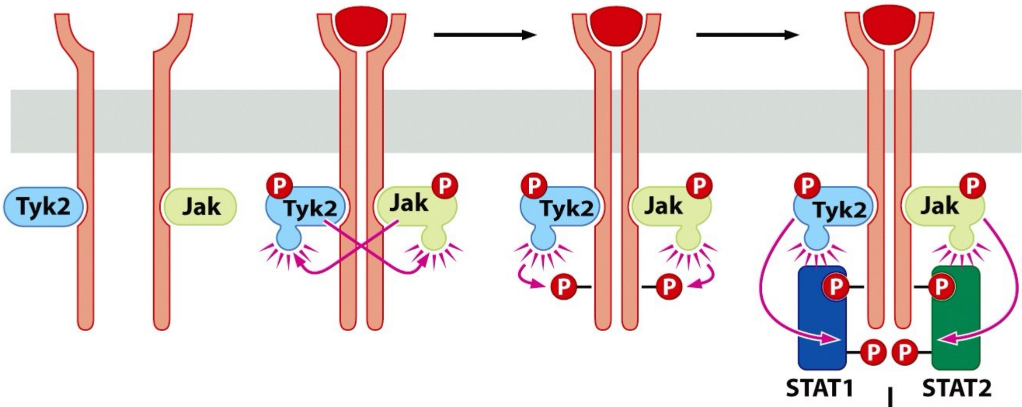
A variation on the theme:
Cytokine receptors associate with cytoplasmic Janus kinases



Cytokine receptors function like RTKs, except that they have "outsourced" their TK domain to a family of interacting partners, the JAKs (Tyk2, JAK2, 2 and 3)

Figure 5.20 *The Biology of Cancer* (© Garland Science 2007)

Phosphorylation by JAKs induces nuclear translocation of STATs



- receptor phosphorylation by JAK family members creates docking sites for STAT1 and STAT2 (Signal Transducer and Activator of Transcription)
- subsequent phosphorylation of the STATs triggers their nuclear translocation, where they directly regulate target genes

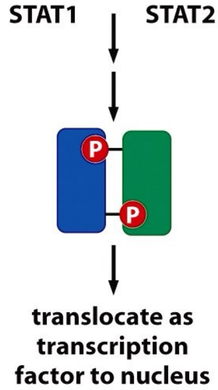
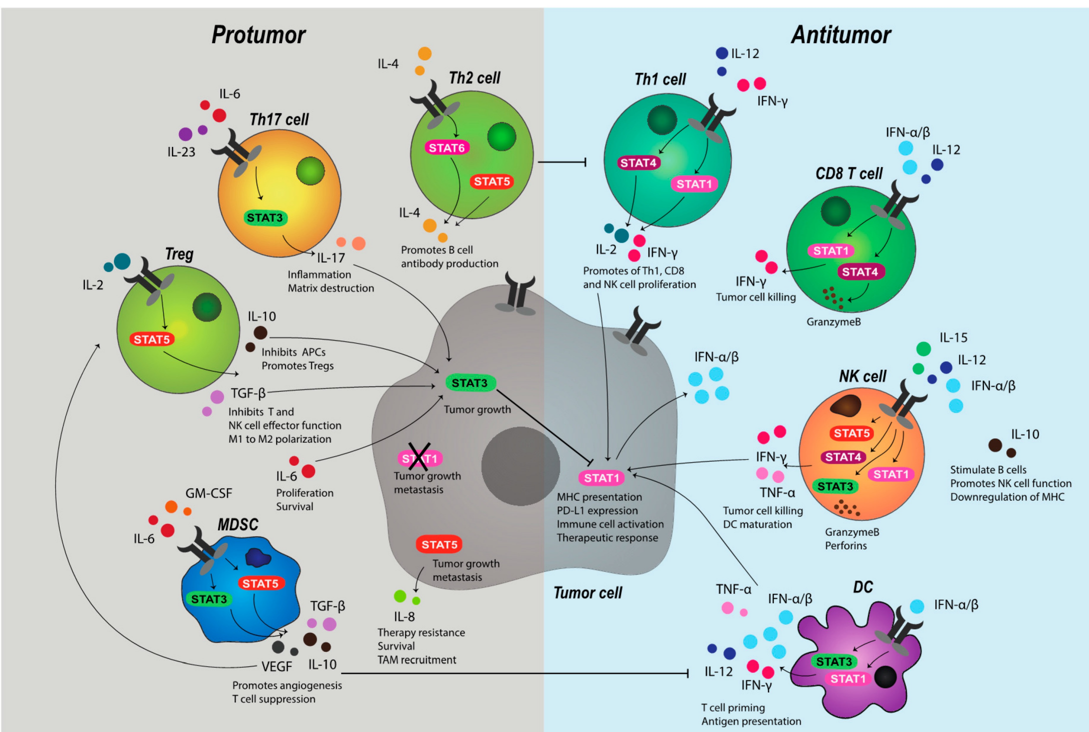


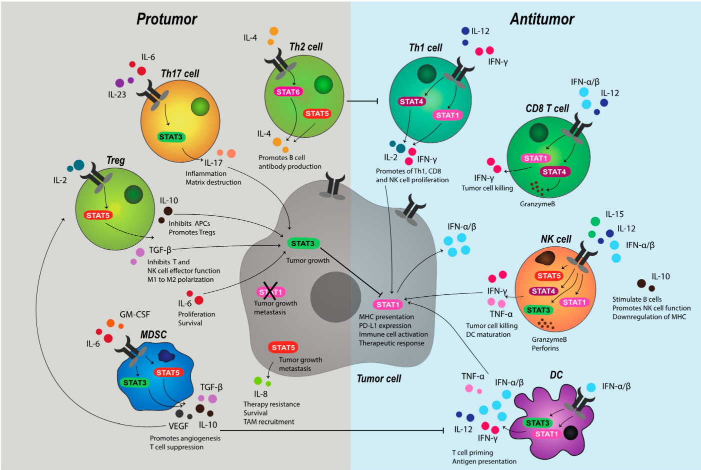
Figure 6.22 *The Biology of Cancer* (© Garland Science 2007)

Beyond cell cycle regulation: STATs have *numerous* pro- and anti-tumor functions



Owen et al. 2019, Cancers 11(12)

No clinically approved JAK inhibitors yet



→ JAK inhibitors as anti-cancer therapeutics?

"…JAKinibs that have shown promise in preclinical and early patient trials have failed to enter the clinic due to high toxicity and off-target immune-suppression,…"

Owen et al. 2019, Cancers 11(12)

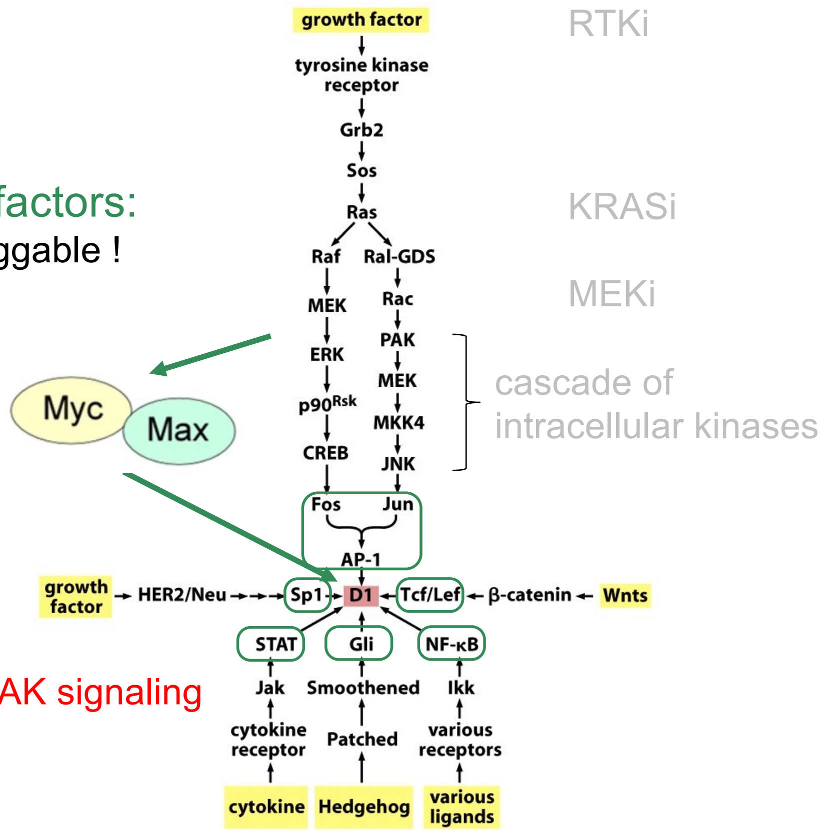
Agent	Disease(s)	Phase	Status*	ClinicalTrials.gov identifier(s)
Ruxolitinib + Chidamide	Peripheral Blood Stem Cell Transplantation	Phase 2	Recruiting	NCT05088226
				NCT04582604
Ruxolitinib + Radiation and Temozolomide	Glioma	Phase 1	Active, not recruiting	NCT03514069
Ruxolitinib + Trastuzumab	Metastatic HER2 Positive Breast Cancer	Phase 1/2	Completed	NCT02066532
Itacitinib + Everolimus	Classical Hodgkin Lymphoma	Phase 1/2	Recruiting	NCT03697408
Itacitinib + Low-Dose Ruxolitinib	Myeloproliferative Neoplasms (MPN)	Phase 2	Completed	NCT03144687
Itacitinib + Osimertinib	Non-Small Cell Lung Cancer	Phase 1/2	Active, not recruiting	NCT02917993
Itacitinib + Alemtuzumab	T-Cell Prolymphocytic Leukemia	Phase 1	Recruiting	NCT03989466
Itacitinib + Ibrutinib	Diffuse Large B-Cell Lymphoma	Phase 1/2	Completed	NCT02760485
Itacitinib + Corticosteroids	Acute Graft-versus-host disease	Phase 3	Completed	NCT03139604
Itacitinib + Gemcitabine and Nab-Paclitaxel	Pancreatic Cancer	Phase 1/2	Completed	NCT01858883
Itacitinib + Dabrafenib and Trametinib	Melanoma	Phase 1	Active, not recruiting	NCT03272464
Itacitinib + Pembrolizumab	Colorectal Cancer	Phase 1	Completed	NCT02646748
Fedratinib + Decitabine	Myeloproliferative Neoplasms (MPN)	Phase 1	Recruiting	NCT05524857
Fedratinib + Nivolumab	Myelofibrosis	Phase 2	Recruiting	NCT05393674
Decitabine + Ruxolitinib or Fedratinib	Accelerated/Blast Phase Myeloproliferative Neoplasms	Phase 2	Recruiting	NCT04282187

Hu et al. 2023, Frontiers in Bioengineering and Biotechnology 11

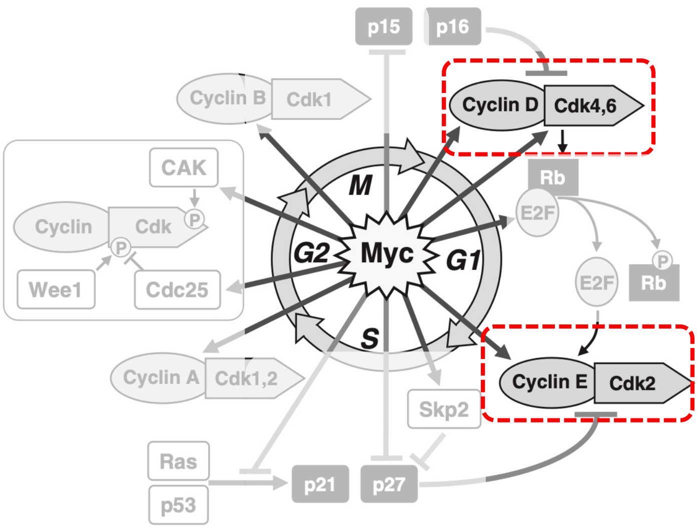
Targeting alternative regulators of cell cycle genes: MYC ?

Transcription factors:
No longer undruggable !

Future
cancer drugs ?



Expression of cyclin D and multiple other cell cycle genes is stimulated by *the MYC proto-oncogene*



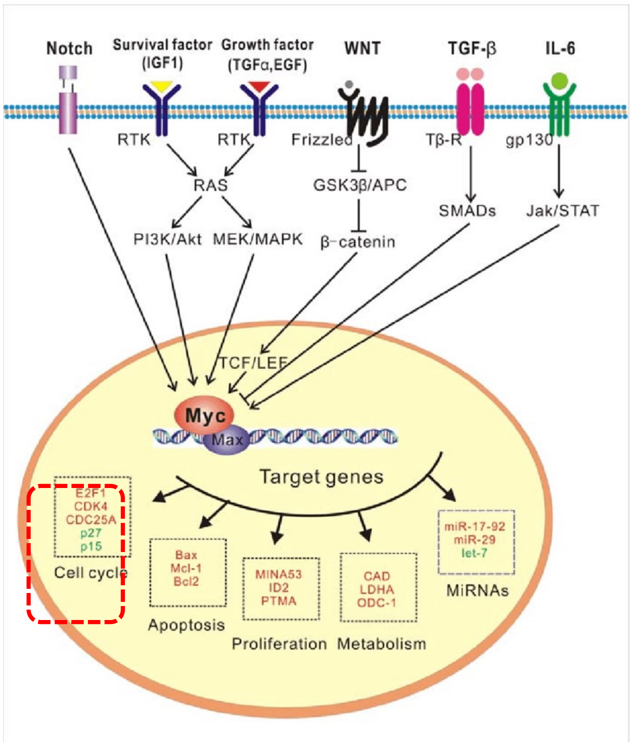
- Oncogenes: Genes that promote cancer when mutated or amplified
- “Proto-oncogenes”: Wild-type genes with the *potential* to become oncogenic upon upregulation, or upon gene amplification or mutation

Current Pharmaceutical Design 20(42):6543-54

Blockade of MYC will target multiple hallmarks

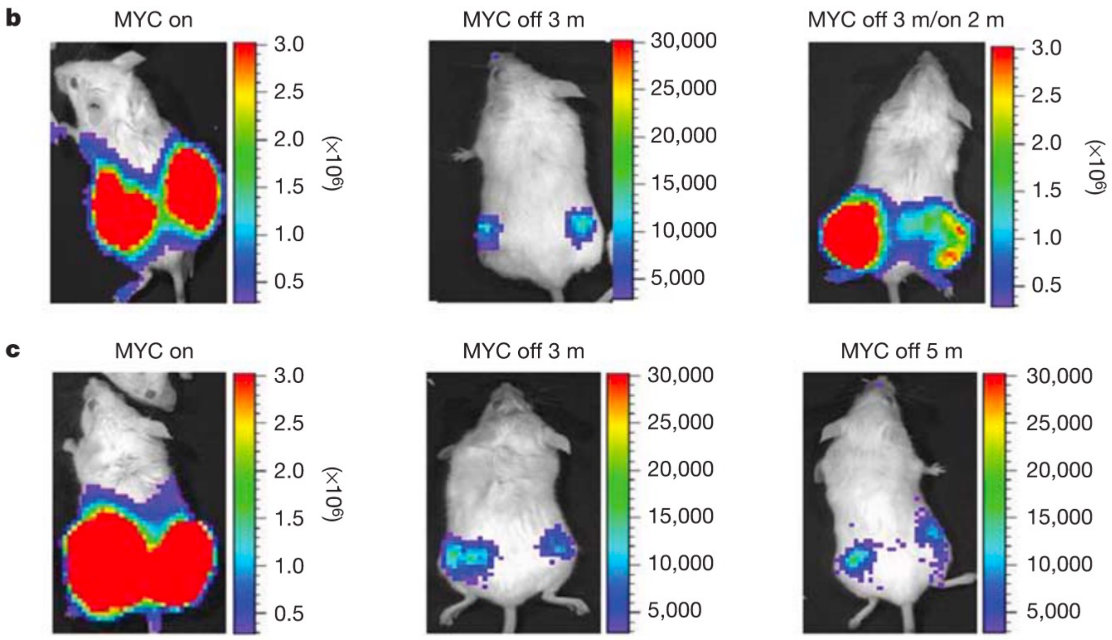
What is MYC?

- Avian myelocytomatosis viral oncogene homolog (MYC)
- One of the four "Yamanaka factors" (cell reprogramming): Thousands of target genes
- Upregulated in most cancer types, e.g. due to altered growth factor signaling
- Promotes *multiple* cancer hallmarks, incl. proliferation



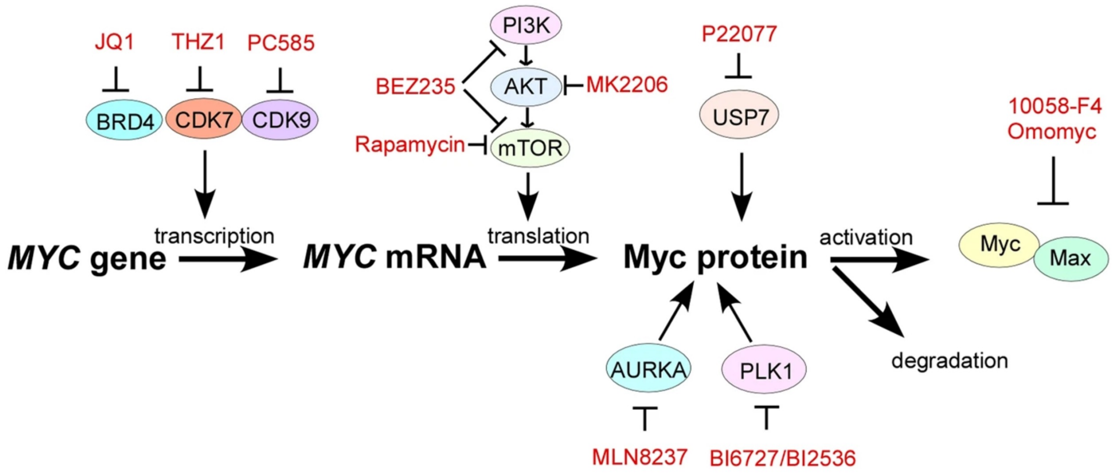
Huang et al. 2014, Current Pharmaceutical Design 20(42):6543-54

Addiction of mouse hepatocellular carcinoma to transgenic MYC overexpression



Shachaf et al. 2004, Nature 431:1112-1117

Therapeutic strategies under development to target MYC



- Omomyc: A cell-penetrating miniprotein (truncated bHLH)
- Blocks binding of MYC to its obligate partner MAX
- An Omomyc transgene can eradicate cancer in multiple tissues in mouse models, regardless of their driver mutations → *huge potential !*

Chen et al. 2018, Sig Transduct Target Ther 3:5

nature medicine

Article

https://doi.org/10.1038/s41591-024-02805-1

MYC targeting by OMO-103 in solid tumors: a phase 1 trial

Stay tuned…

Received: 7 February 2023

Accepted: 4 January 2024

Published online: 06 February 2024

Check for updates

Elena Garralda[1,7], Marie-Eve Beaulieu[2,7], Víctor Moreno[3], Sílvia Casacuberta-Serra[2], Sandra Martínez-Martín[2], Laia Foradada[2], Guzman Alonso[1], Daniel Massó-Vallés[2], Sergio López-Estévez[2], Toni Jauset[2], Elena Corral de la Fuente[4], Bernard Doger[3], Tatiana Hernández[3], Raquel Perez-Lopez[1], Oriol Arqués[1], Virginia Castillo Cano[2], Josefa Morales[2], Jonathan R. Whitfield[1], Manuela Niewel[2], Laura Soucek[1,2,5,6] & Emiliano Calvo[4]

A dose-escalation phase 1 study in all-comers solid tumors:

- ✓ safety and preliminary signs of drug activity
- ✓ target engagement
- ✓ biomarkers predicting response

Garralda et al. 2024, Nat Med, https://doi.org/10.1038/s41591-024-02805-1

TODAY

Hallmark capability 1: Sustained proliferative signaling

Weinberg, selected parts of chapters 5 & 6

- ☑ Receptor tyrosine kinases (RTK)
- ☑ RTK signal transduction
- ☑ JAK/STAT signaling

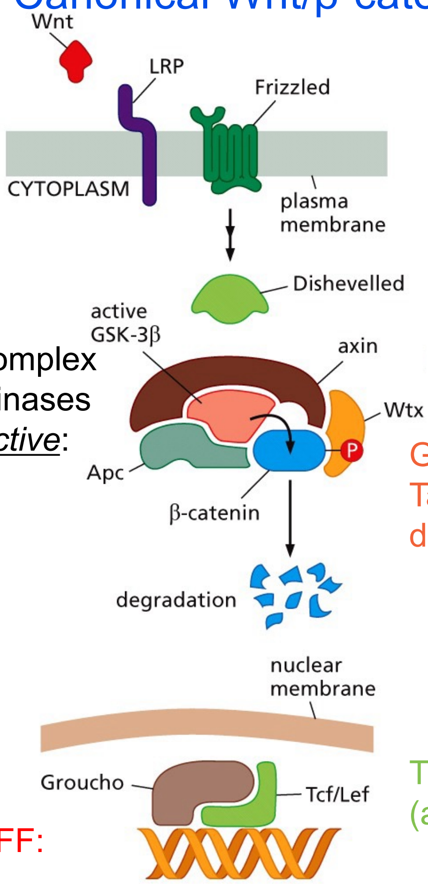
- ❑ Wnt/β-catenin signaling
  - Role in intestinal stem cells & in colon cancer
  - Target genes in stem cells include MYC
  - Cancer cell differentiation therapy

Canonical Wnt/β-catenin signaling pathway

Wnt OFF

Essential to memorize !

β-catenin destruction complex of APC and kinases (GSK3β) is *active*:

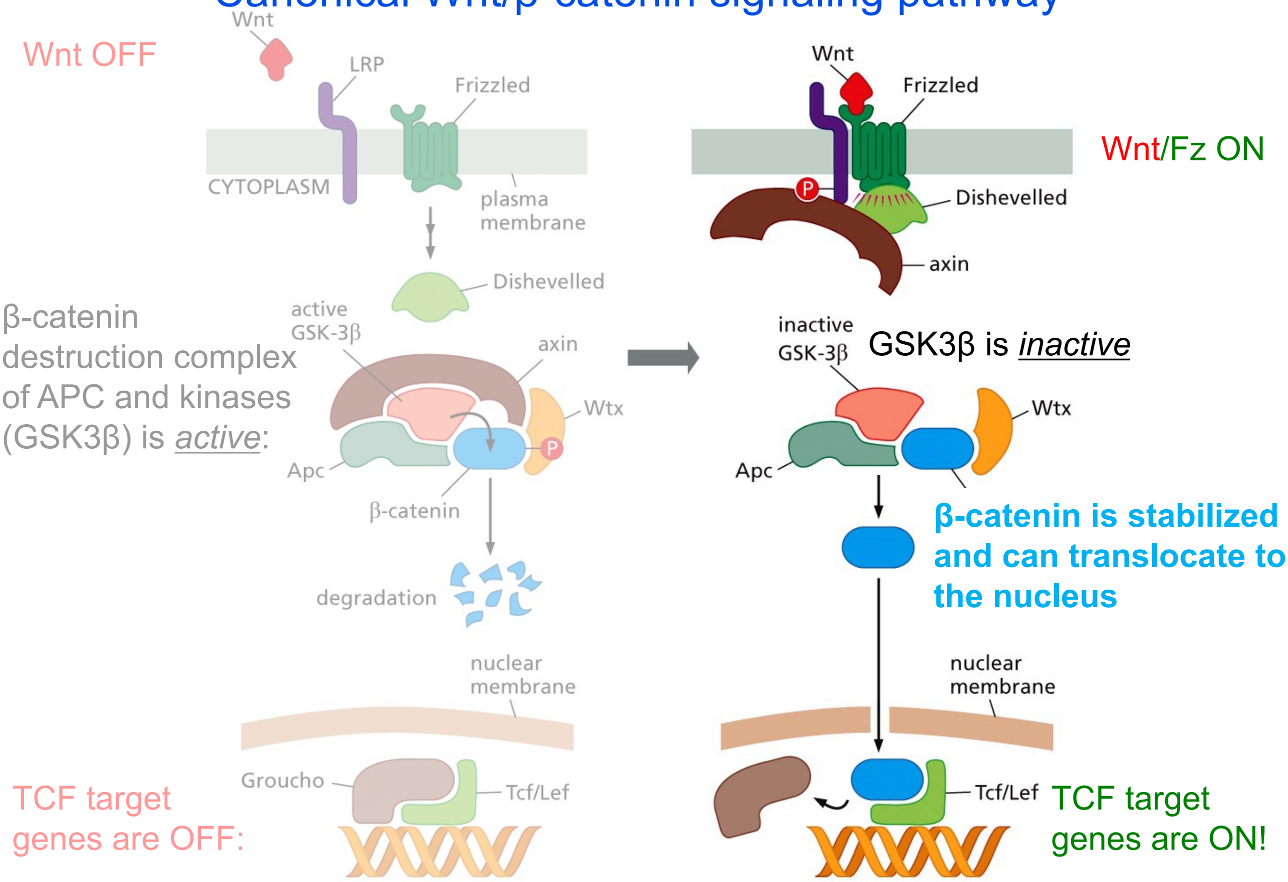


Glycogen synthase kinase 3 β: Targets β-catenin for proteasomal degradation by phosphorylating it

TCF target genes are OFF:

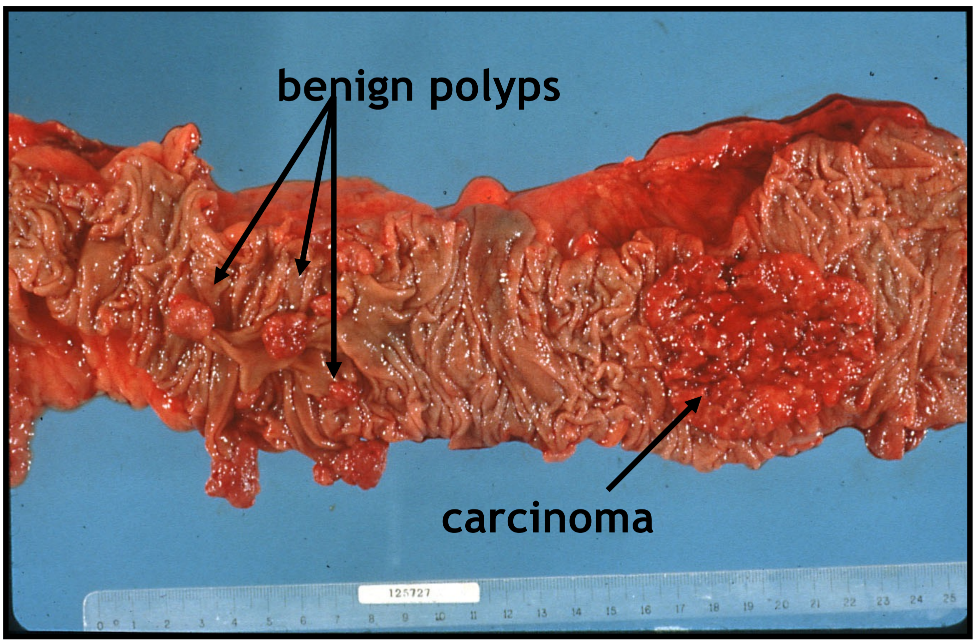
T cell factor (TCF) proteins (aka Lymphoid Enhancer Binding Factor)

Canonical Wnt/β-catenin signaling pathway

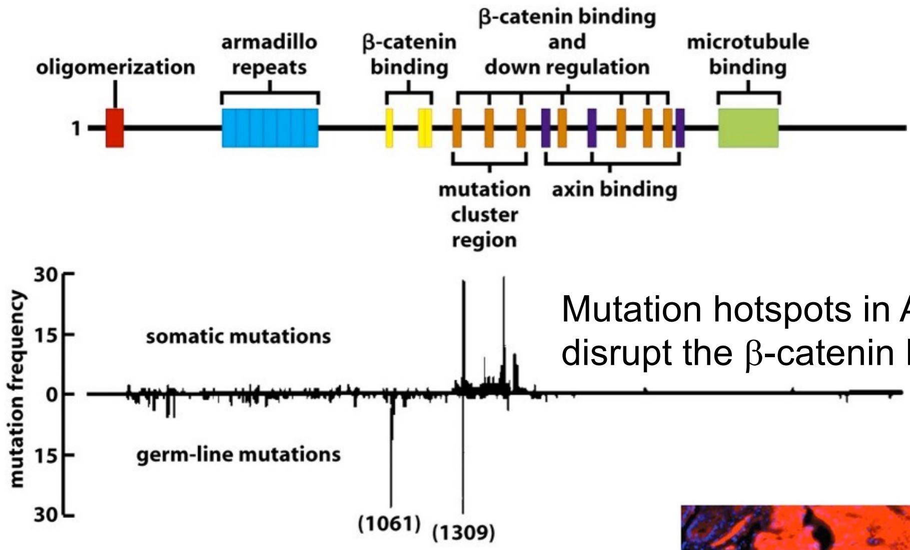


Familial Adenomatous Polyposis (FAP)

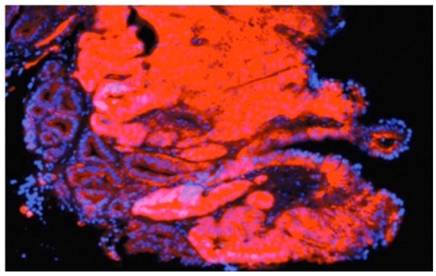
Adenoma: a benign tumor of glandular origin
Polyposis: hundreds of benign small polyps
>90% risk for colorectal carcinoma (CRC) before age of 50y



Mutations in Adenomatous Polyposis Coli (APC) protein



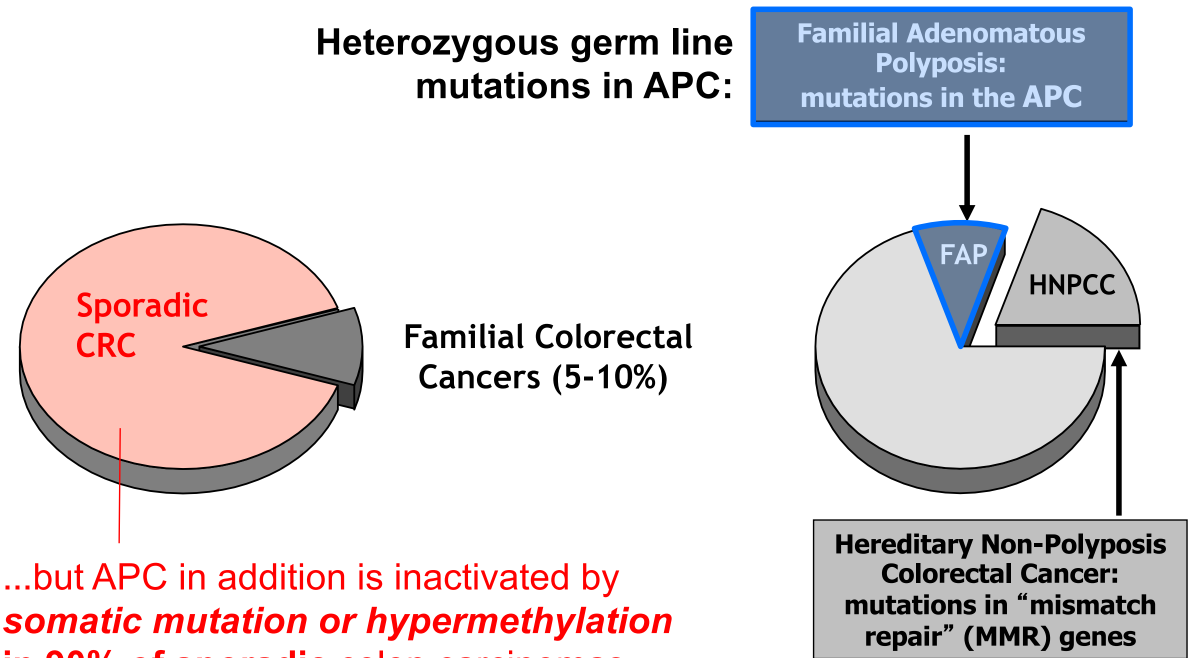
Mutation hotspots in APC structure disrupt the β-catenin binding region!



Red: *β-catenin in colon of Apc$^{Min/+}$ mice*
Blue: *nuclei of normal cells*

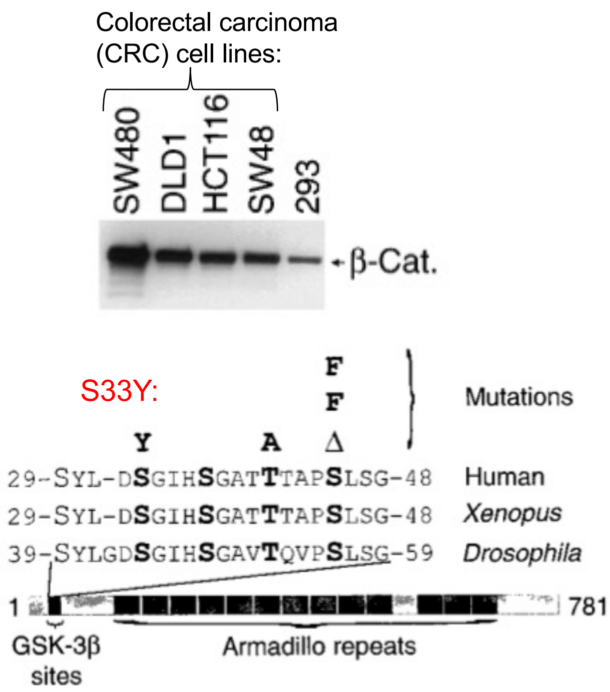
Figure 7.22+25 *The Biology of Cancer* (© Garland Science 2007)

Familial Adenomatous Polyposis (FAP) accounts for (only) 1% of all hereditary colorectal cancers...



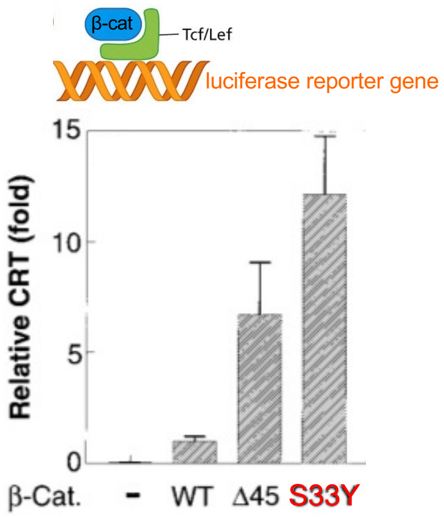
...but APC in addition is inactivated by ***somatic mutation or hypermethylation*** **in 90% of sporadic** colon carcinomas.

In some APC^{WT} colorectal cancers and melanoma, β-catenin itself is mutated at GSK3β phosphorylation sites



Morin et al. 1997 Science

e.g. S33Y mutation or S45 deletion stabilizes β-catenin in SW480 colon carcinoma cells and potentiates the induction of a Tcf reporter gene compared to wild-type:



Concept:
Oncogenes and tumor suppressor genes

function	type of alteration[1]	examples
oncogene	gain-of-function (GoF)	EGFR RAS β-catenin
tumor suppressor	loss-of-function (LoF)	APC NF-1 } not yet PTEN } covered

[1] can include genetic mutations *or* epigenetic alterations

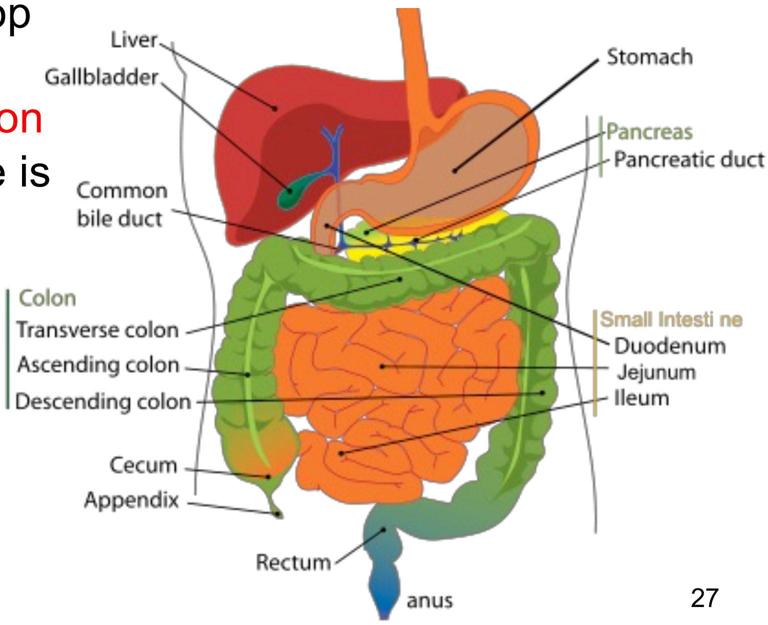
The relative impact of such alterations on a given cancer type depends on the role of the affected pathway in the corresponding normal tissue (example: WNT/β-catenin signaling).

Relative contribution of different signaling pathways to cancer: Dependent on the tissue?

- FAP patients are also at risk for a broad spectrum of extra-colonic manifestations

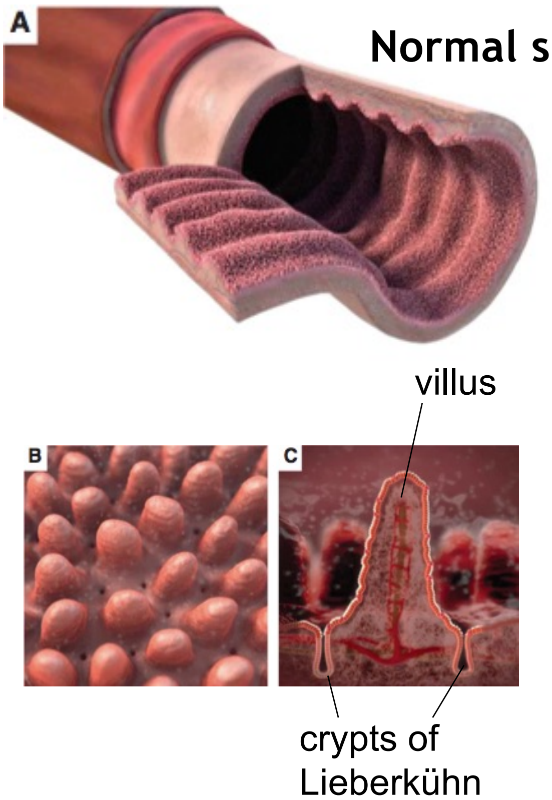
- Predisposition to develop adenomatous polyps is most pronounced in colon and rectum (penetrance is close to 100% already <30 years after birth)

- Why is hyperactive β-catenin most oncogenic in the colon (human) or intestine (mouse) ?



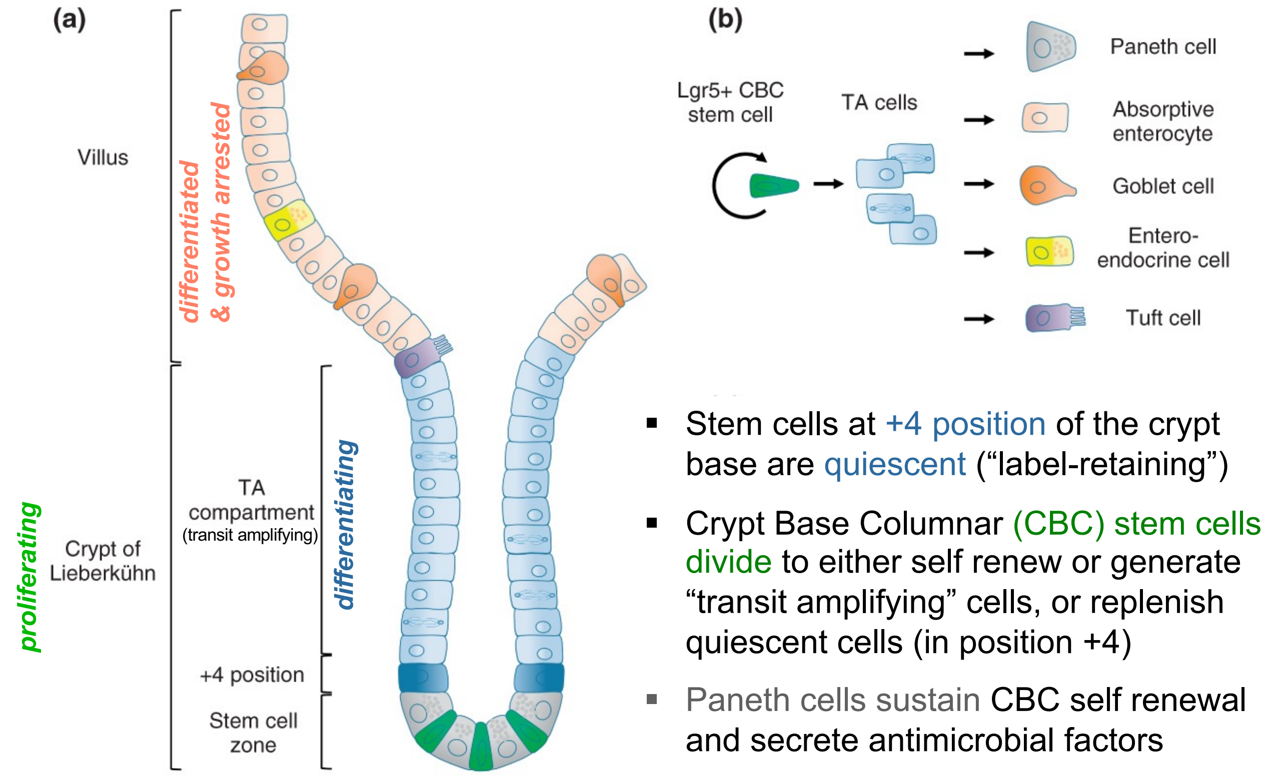
Crypt-villus architecture of intestine and colon

Normal small intestinal architecture



Adenomatous polyp:

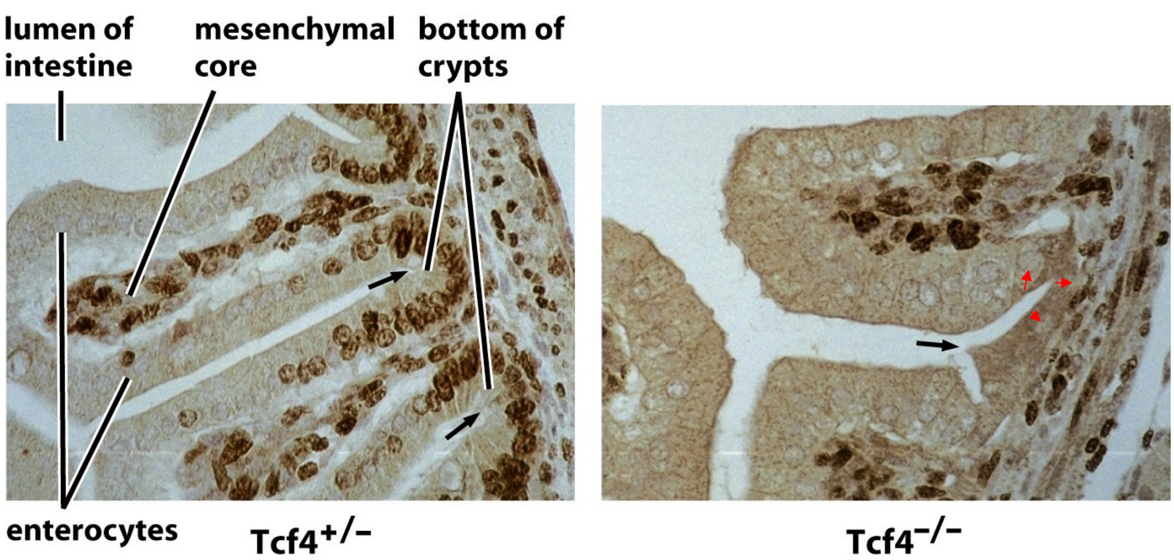
Tissue homeostasis in normal intestine



- Stem cells at +4 position of the crypt base are quiescent ("label-retaining")
- Crypt Base Columnar (CBC) stem cells divide to either self renew or generate "transit amplifying" cells, or replenish quiescent cells (in position +4)
- Paneth cells sustain CBC self renewal and secrete antimicrobial factors

P Rizk *et al. WIREs Syst Biol Med* 2012. Copyright © 2012 Wiley Periodicals, Inc.

Loss of Wnt/β-catenin/TCF signaling depletes crypt stem cells

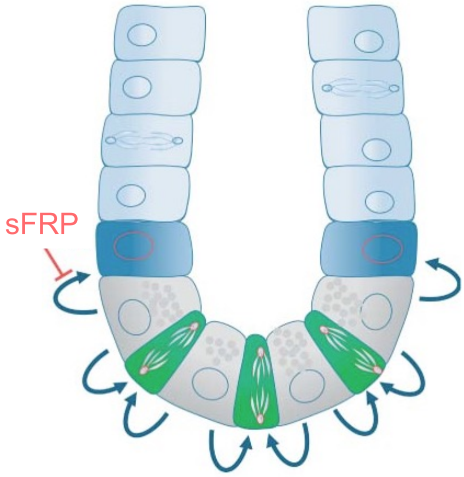


⇒ immunostaining of the S-phase marker protein Ki67 reveals cell proliferation in wild-type crypts

⇒ Loss of Ki67 staining and progressive loss of enterocytes in Tcf4 KO revealed that canonical Wnt signaling is essential for crypt stem cell proliferation

Figure 7.24b *The Biology of Cancer* (© Garland Science 2007)

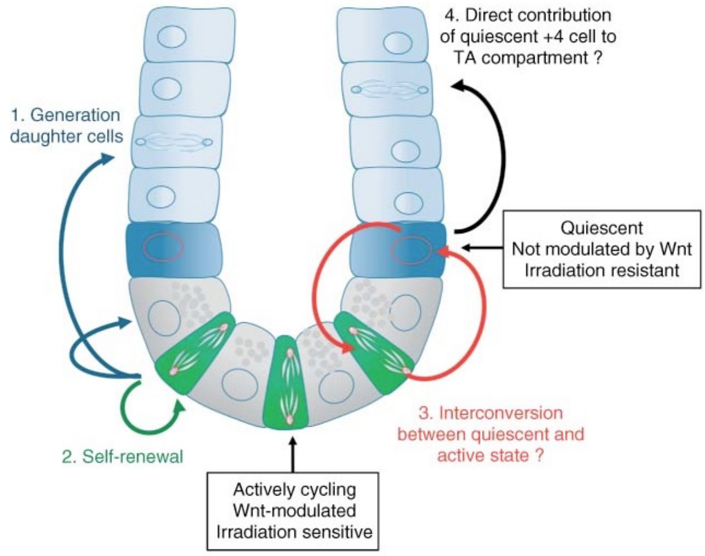
CBC stem cells are wired to respond to WNT/β-catenin already in healthy crypts



Factors secreted by
Paneth cells (stem cell niche):

Wnt3 **Wnt11**	**EGF** **TGFα**	Dll4
↓	↓	↓
Fz	**EGFR**	**Notch**

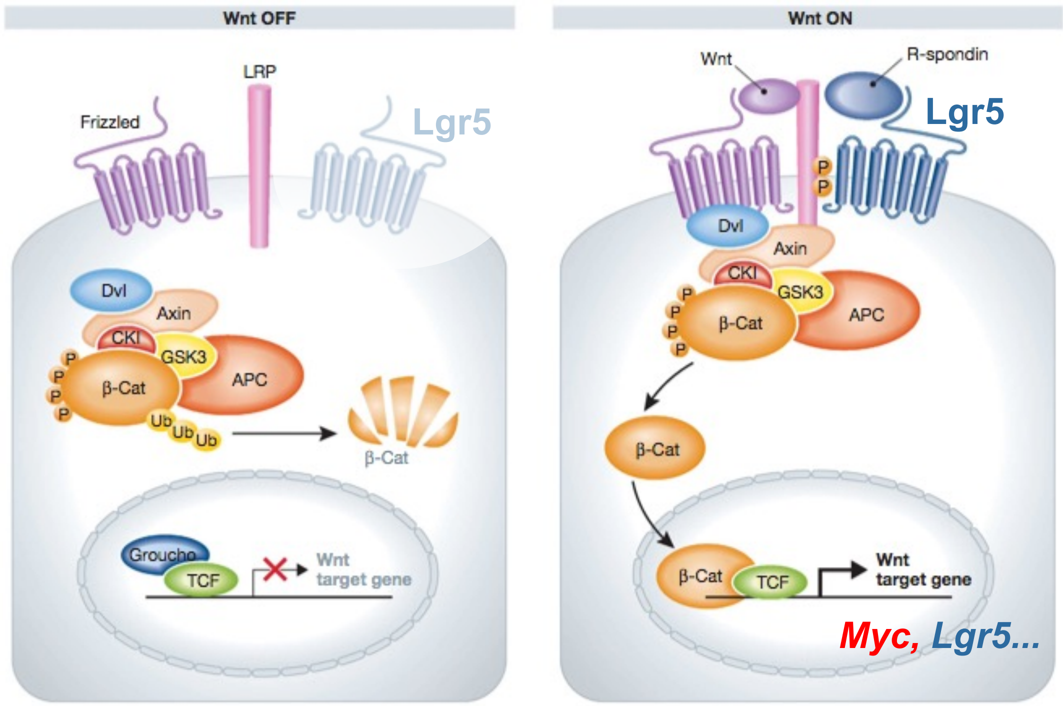
(receptors in CBC stem cells)



The control of stem cell *proliferation* and *quiescence* by WNT and its antagonist 'secreted frizzled-related protein' (sFRP) govern *normal intestinal tissue homeostasis*

P Rizk *et al. WIREs Syst Biol Med* 2012. Copyright © 2012 Wiley Periodicals, Inc.

Leucine-rich repeat containing G protein-coupled receptors (Lgr)

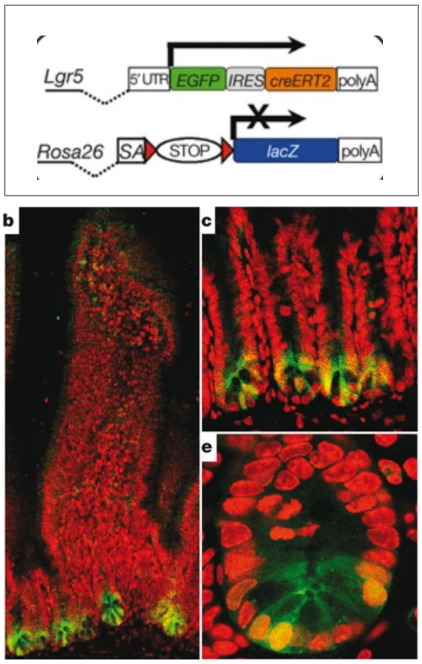


- Binding of Rspo to Lgr5 blocks Fzd endocytosis into lysosomes.
- *Positive feedback* by Lgr5 is essential for *sustained* Wnt/Fz signaling

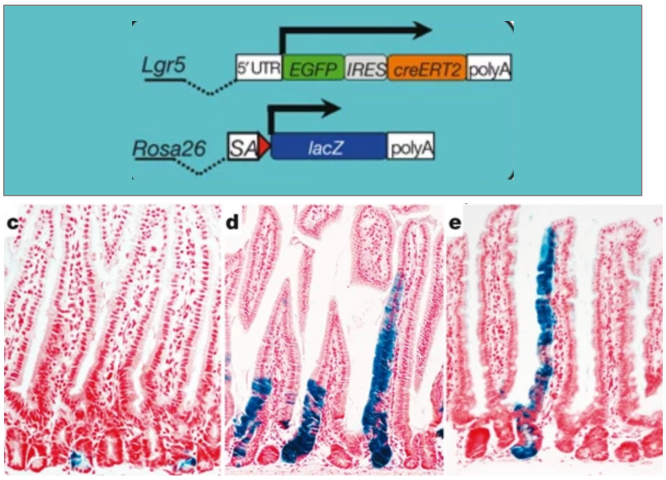
Schuijers & Clevers 2012 Embo J

Genetic lineage tracing of the CBC progeny using *Lgr5*CreIRESeGFP

+ drug (tamoxifen) to activate CreERT2



→ Lgr5 expression marks CBC cells



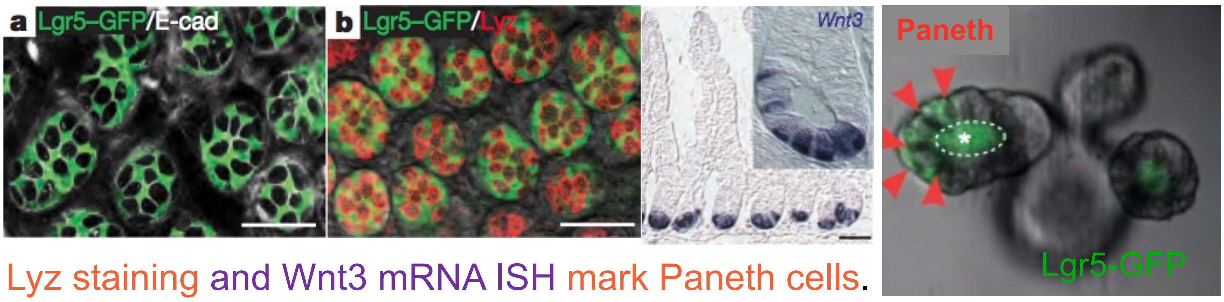
1 day 5 days 60 days

tamoxifen

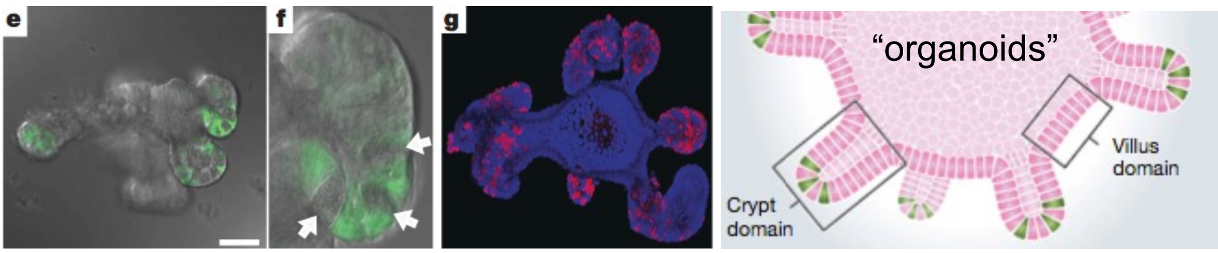
→ Excision of lox-stop-lox cassette activates the histological marker lacZ (blue staining)

Barker *et al. 2007,* Nature 449:1003-1007

Sustained Wnt signaling maintains CBC proliferation *in vitro*



Lyz staining and Wnt3 mRNA ISH mark Paneth cells.
Lgr5::GFP transgene marks Wnt-receiving CBC stem cells.
Paneth cells sustain growth of CBC-derived organoids upon coculture.



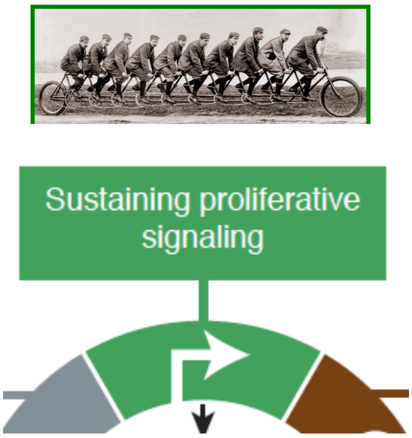
Recombinant Wnt3a (+EGF +Noggin +Notch agonist) can substitute for Paneth cells to maintain CBC proliferation (EdU incorporation, magenta)

Sato et al. 2009 Nature; Sato et al. 2011 Nature

TODAY

Hallmark capability 1: Sustained proliferative signaling

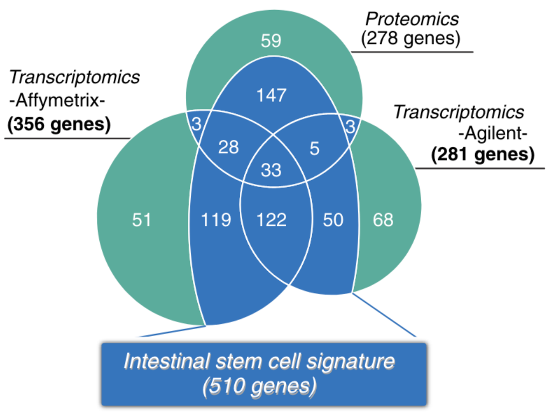
Weinberg, selected parts of chapters 5 & 6



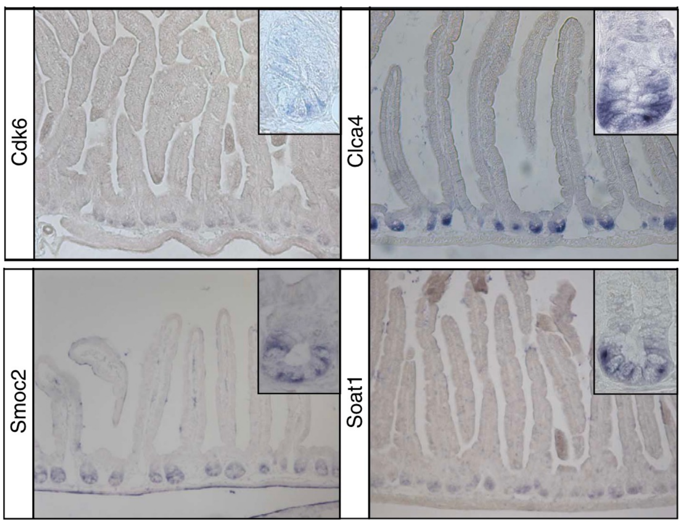
- ☑ Receptor tyrosine kinases (RTK)
- ☑ RTK signal transduction
- ☑ JAK/STAT signaling

- ❑ Wnt/β-catenin signaling
  - ✓ Role in intestinal stem cells & in colon cancer
  - Target genes in stem cells include MYC
  - Cancer cell differentiation therapy

Transcriptome analysis of intestinal stem cells



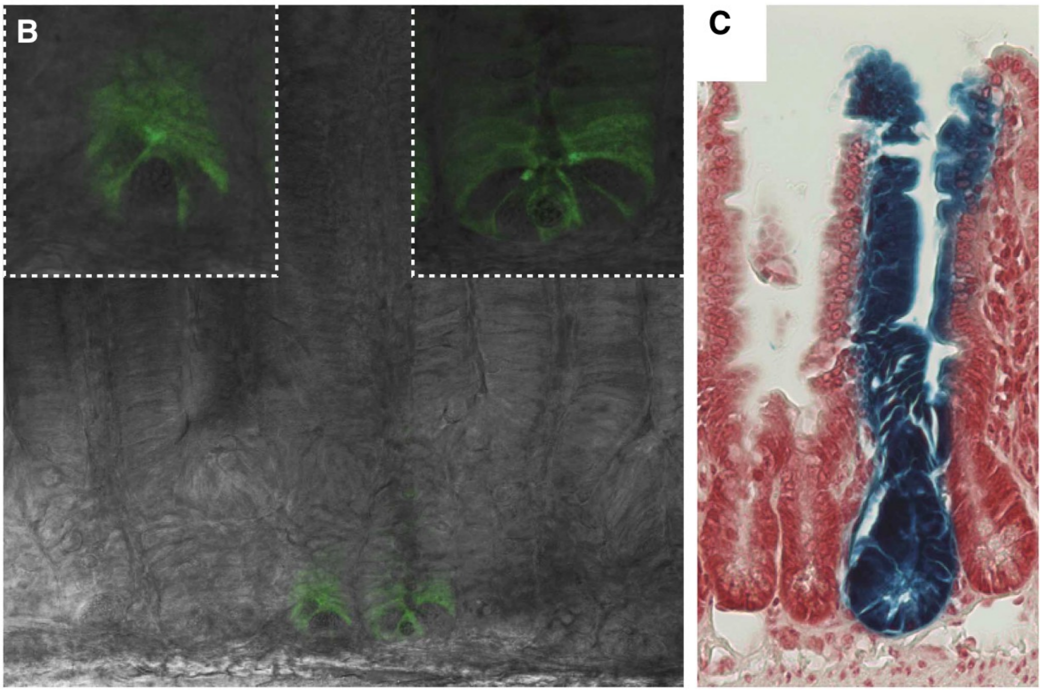
Validation by mRNA in situ hybridization



Muñoz al. 2012, Embo J 31:3079-3091

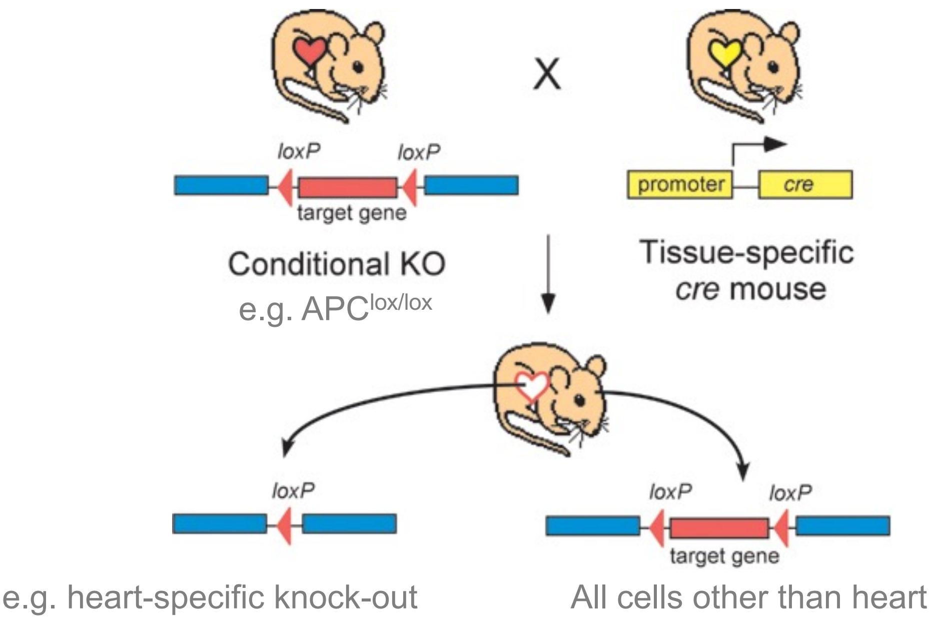
Genetic lineage tracing of normal intestinal stem cell progeny using lox-stop-lox Rosa26-lacZ reporter mice

Smoc2-EGFP-ires-CreERT2/R26RLacZ mice

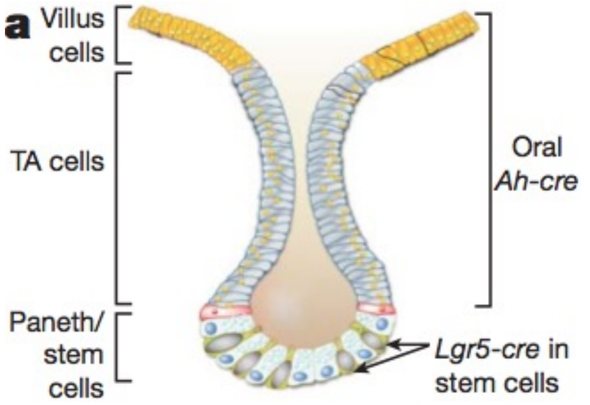


Muñoz al. 2012, Embo J 31:3079-3091

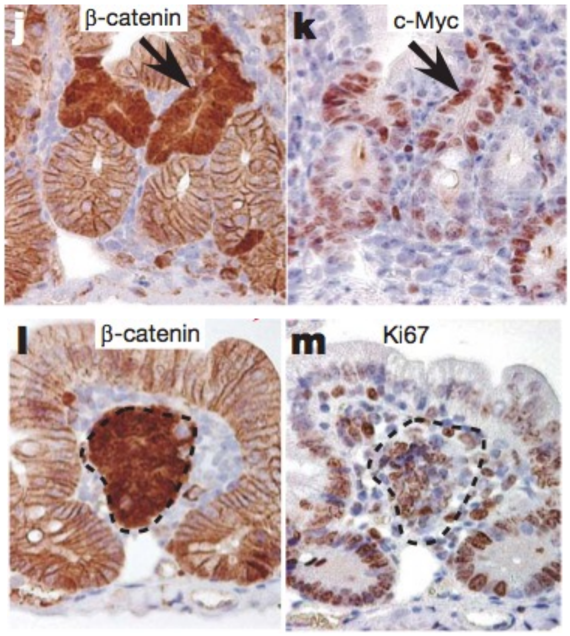
Mouse tumor models: Cell/tissue-specific gene *deletion* using the Cre/lox technique



Identification of Lgr5+ CBC as tumor-initiating cells



Myc:



Lgr5-CreERT2; APC$^{lox/lox}$ mice:

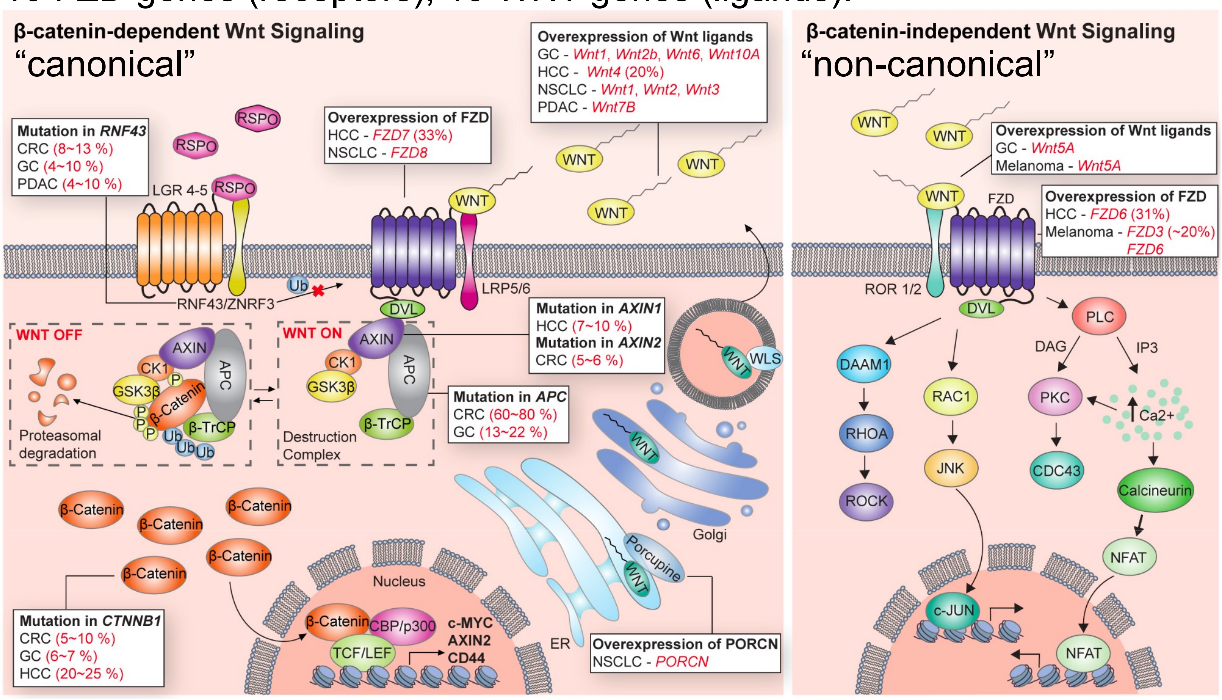
→ "**Constitutive**" β-catenin signaling in long-lived Lgr5+ CBC induces the *c-Myc* oncogene → Proliferation (Ki67)

By contrast, APC deletion by Ah-Cre <u>*in TA cells*</u> *rarely* induces adenomas

Barker et al. 2009 Nature

Overview of WNT pathway alterations by cancer type

10 FZD genes (receptors), 19 WNT genes (ligands):



Not intended for memorization !

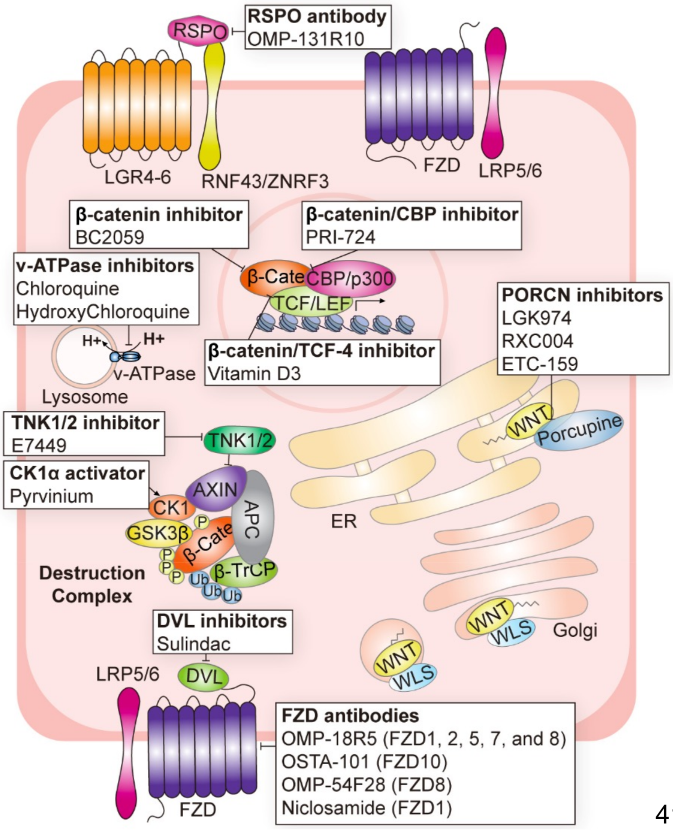
Park et al. 2023, Cells 12(8):1110

WNT pathway-targeting compounds in clinical trials

Several drugs in clinical trials, but still no FDA-approved WNT pathway inhibitor so far !

Major obstacle:

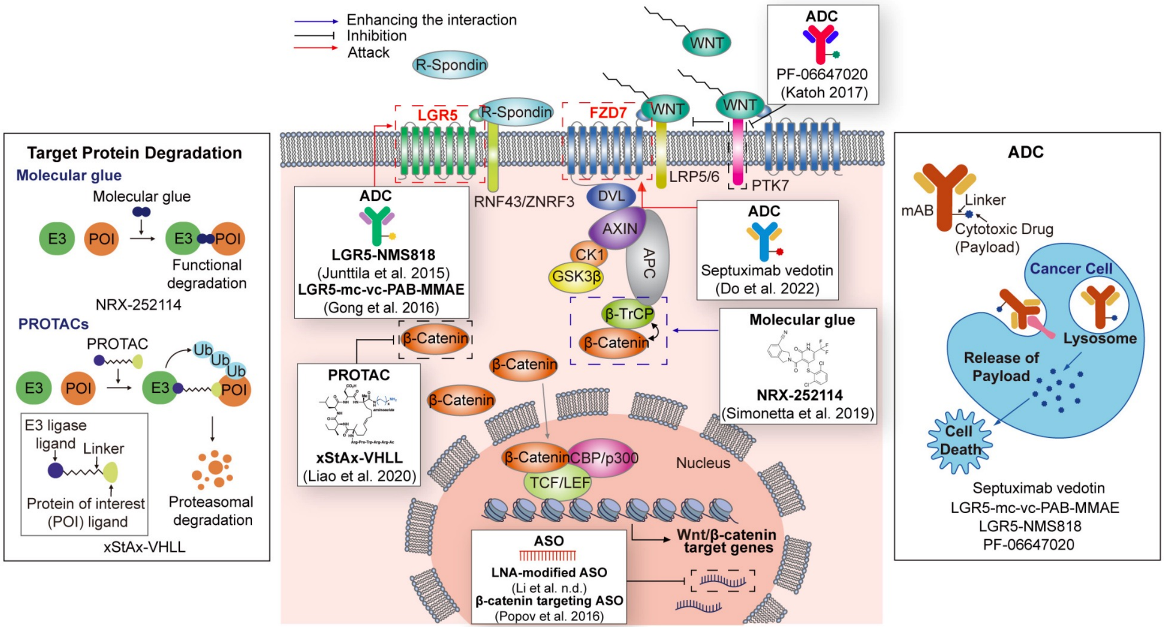
Side effects due to essential roles of Wnt signaling in stem cells and tissue homeostasis.



Park et al. 2023, Cells 12(8):1110

The latest wave of WNT-targeting strategies

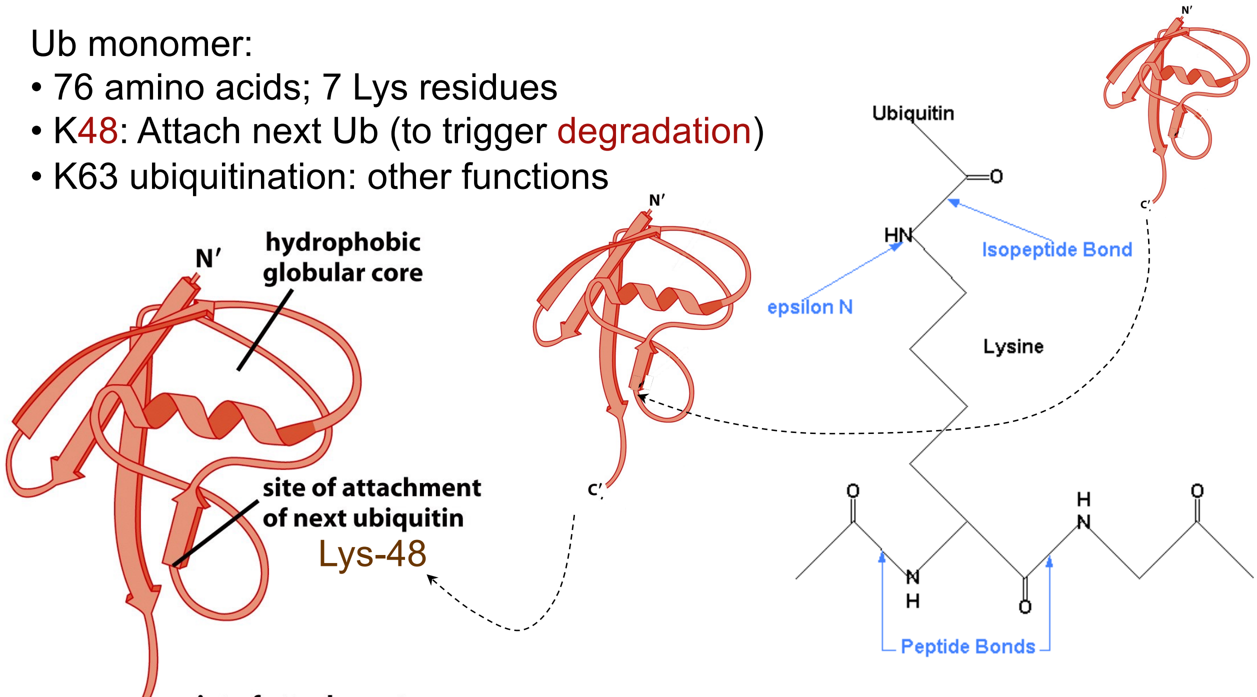
- ADC: Antibody drug-conjugates
- Molecular glues
- PROTAC: PROteolysis TArgeting Chimera (a bifunctional molecule)



Park et al. 2023, Cells 12(8):1110

Mono- and polyubiquitination involves isopeptide bonds

Ub monomer:
- 76 amino acids; 7 Lys residues
- K48: Attach next Ub (to trigger degradation)
- K63 ubiquitination: other functions

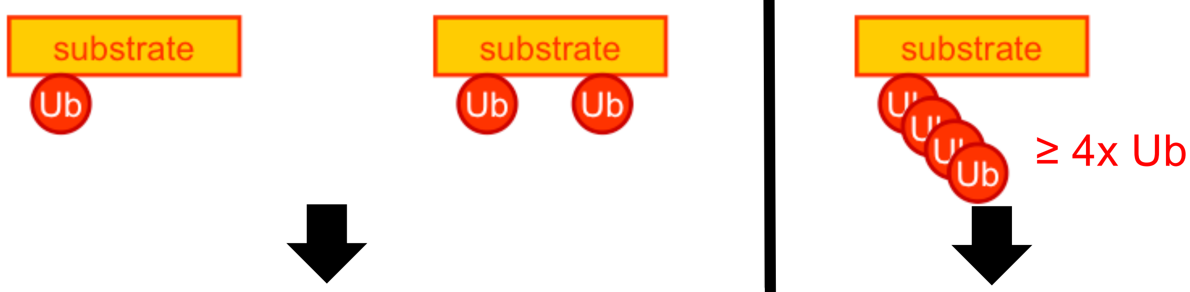


C-terminal glycine forms isopeptide bond with ε-amino group of target lysine side chains

Figure 7.26b *The Biology of Cancer* (© Garland Science 2007)

Different types of ubiquitination

MONO-Ub | **MULTI-Ub** | **POLY-Ub**



Various effects:

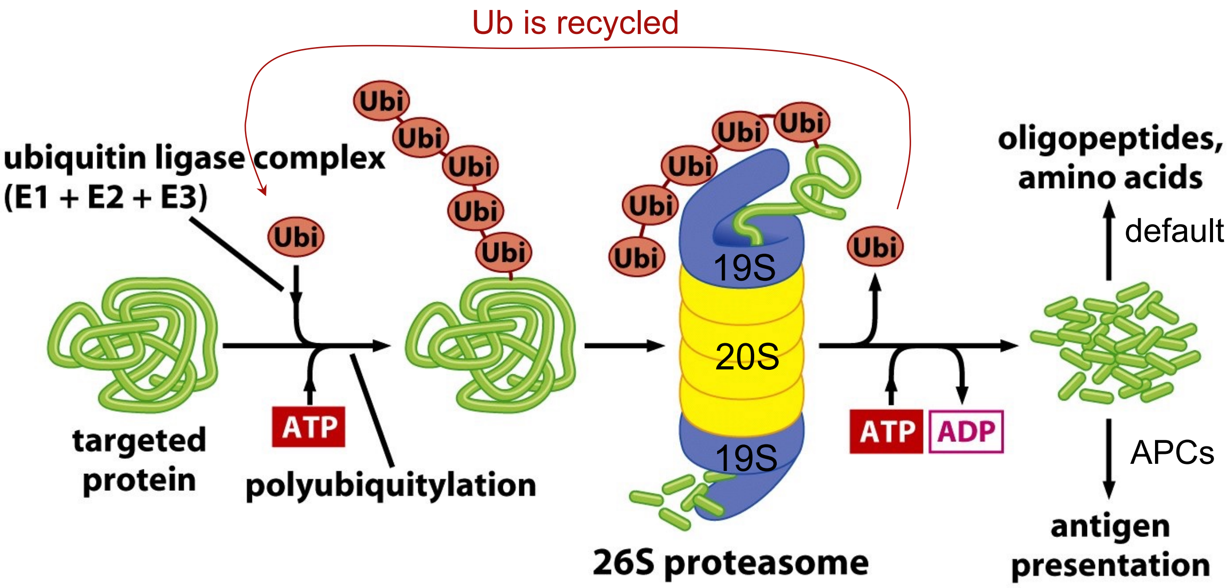
- can change protein conformation
- mediates association to Ub-binding motif (UBM)-containing proteins such as Hrs
- triggers endocyosis of RTKs & other cell surface receptors to target them to *lysosomes*

Degradation by 26S proteasome

Cyclins, β-catenin, TP53, SMADs, …

Or (rarely):
Proteolytic processing
(e.g. NF-kB)

K48 polyubiquitin marks proteins for proteasomal degradation

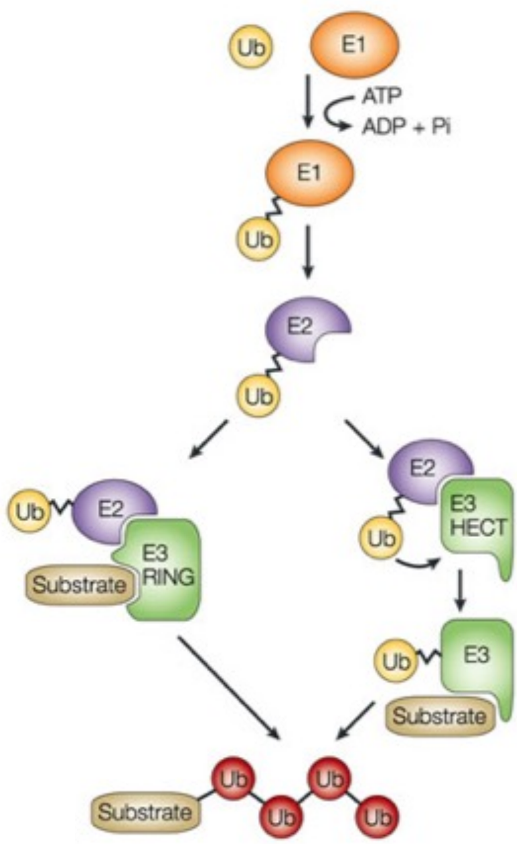


ATP-dependent ubiquitination

19S: multiple regulatory subunits; ATP-dependent unfolding of target proteins
20S: several proteolytic subunits

Figure 7.26a *The Biology of Cancer* (© Garland Science 2007)

Ubiquitination is mediated by specific Ub ligases



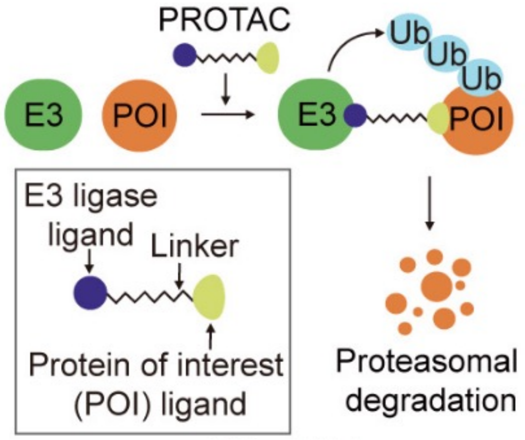
E1: Activation of the C-terminus of ubiquitin (thioester bond)

approx. 20 E2 ligases

> 500 substrate-specific E3 ligases,
two classes:

a) RING domain (**adaptor**)
b) HECT domain (**enzyme**)
=> substrate specificity

PROteolysis TArgeting Chimera (a bifunctional molecule)



Goal & strategy:

Target your protein of interest (POI) for proteasomal degradation

…by connecting it to a specific "E3 Ub ligase"

…in this case via a *linker* that contains a specific E3-binding peptide ("ligand")

Or even *without* a ligand using a "molecular glue" that *directly* links the POI to its own known E3 ligase: **See today's exercise, question 5.**

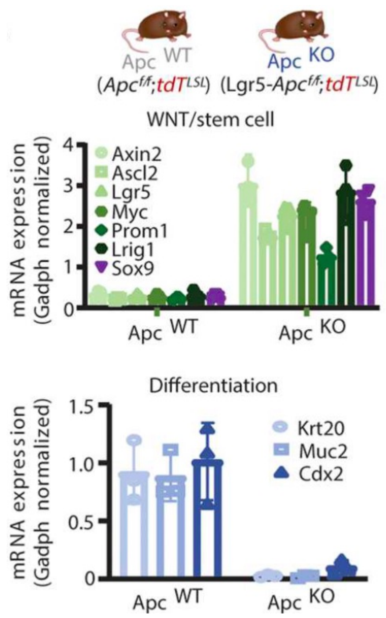
Other strategies to counteract pro-tumor functions of WNT

- Block essential *downstream* targets (e.g. MYC)

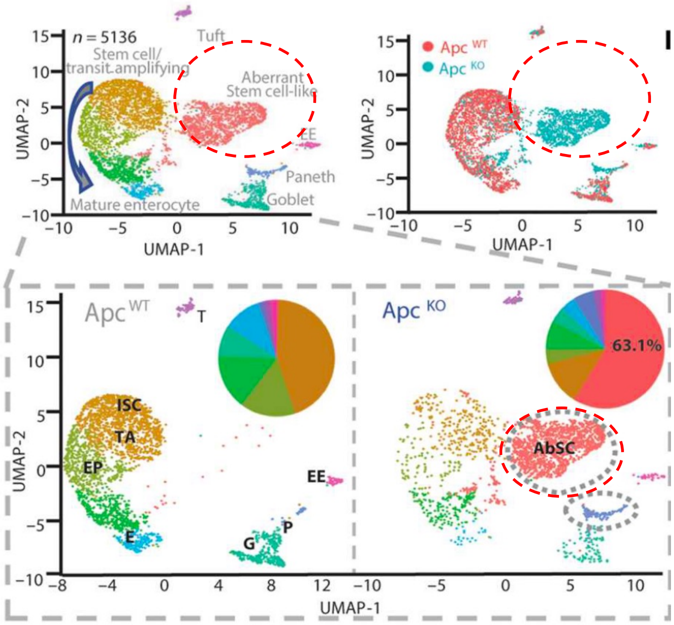
- Can we block other essential "stemness" factors to thereby force cancer stem cells to differentiate *despite* the presence of activated β-catenin?

APC$^{-/-}$ adenoma cells express an *aberrant* stem cell signature

RT-qPCR of genes of interest in sorted cells (tumor vs stroma):



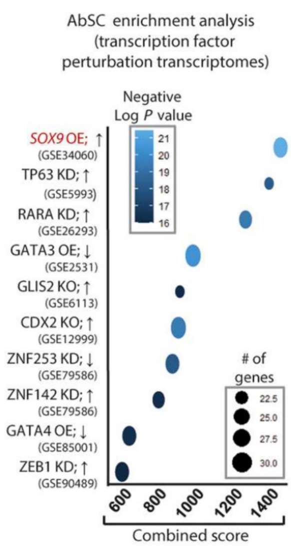
Single cell RNA-sequencing (scRNA-seq) analysis of adenoma transcriptome vs normal crypts:
→ Identification of a tumor-specific cluster of AbSC cells



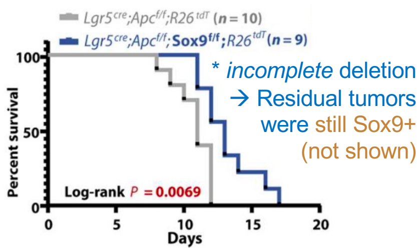
Bala et al. 2023, Science Advances 9:eadf0927

"Differentiation therapy" as a treatment approach to revert the cancer hallmark of sustained proliferation

AbSCs upregulated a Sox9-driven signature:

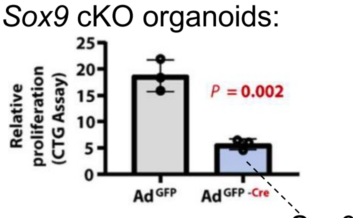


Kaplan-Meier curve of the survival of *Sox9* cKO hosts*:



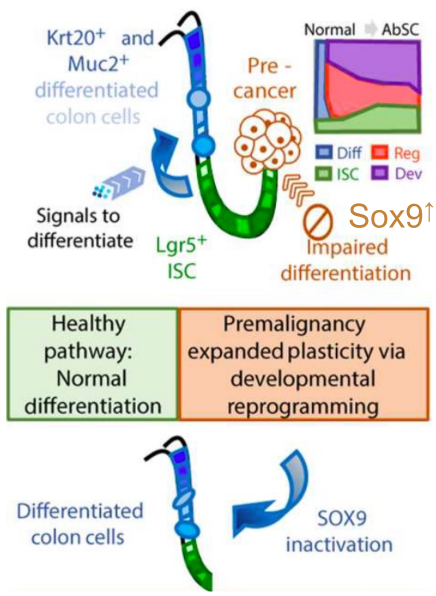
* *incomplete* deletion
→ Residual tumors were still Sox9+
(not shown)

Cell proliferation in *Sox9* cKO organoids:



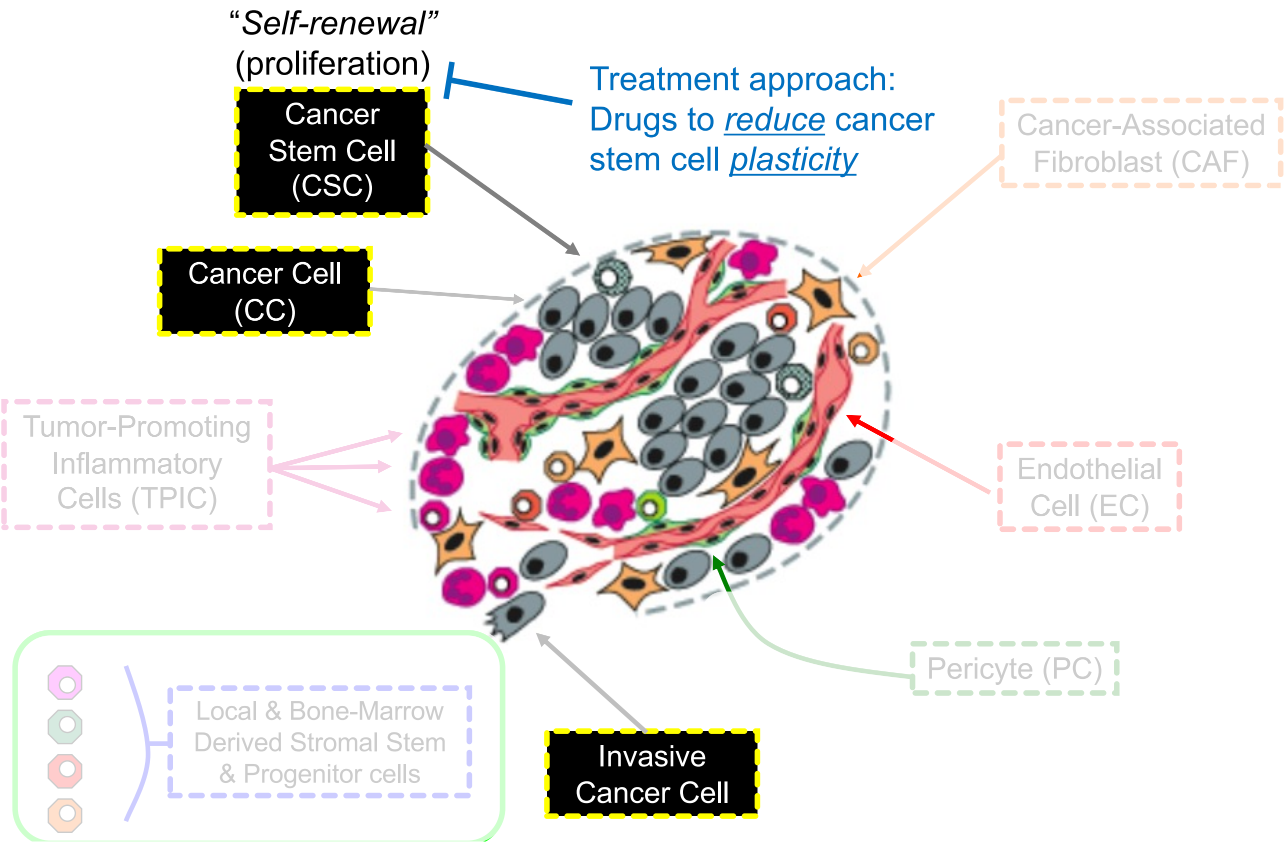
Sox9 deleted

Treatment approach to reduce cancer stem cell plasticity:

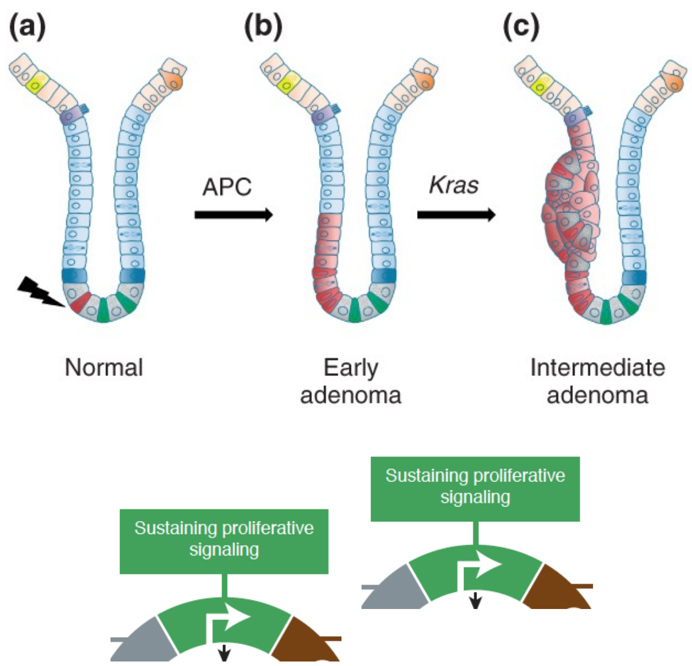


Bala et al. 2023, Science Advances 9:eadf0927

Genetic and epigenetic perturbations *within* cancerous cells or "cancer stem cells" clearly can initiate the process



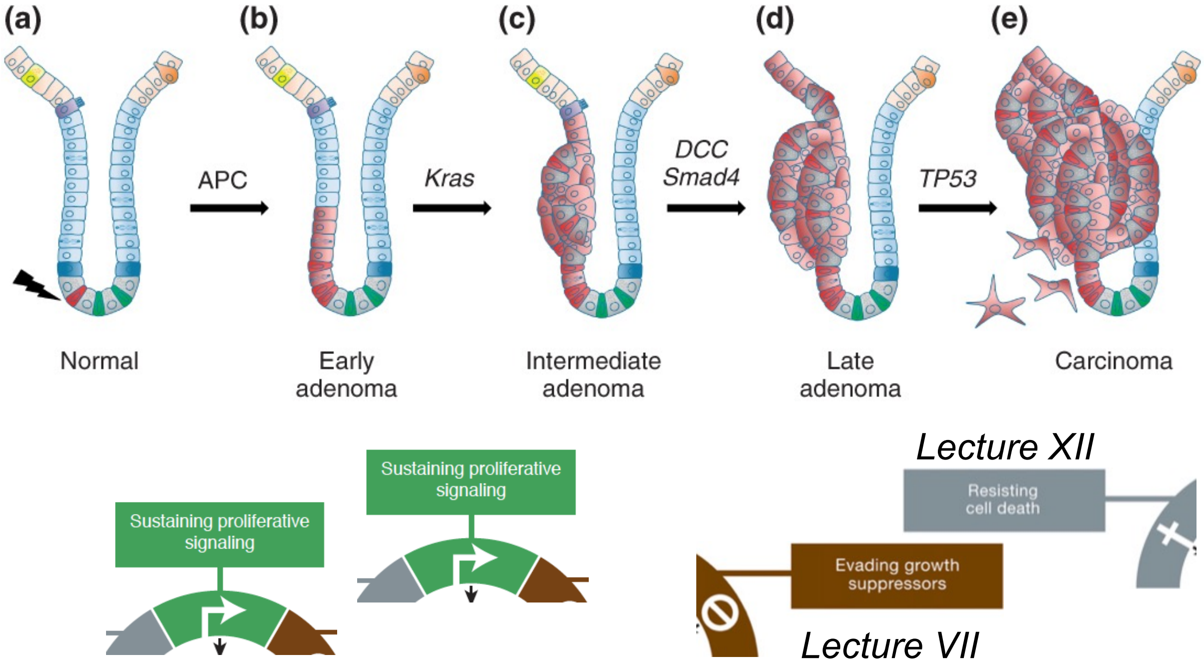
From normal tissue homeostasis to cancer hallmark capability: β-catenin hyperactivation initiates colorectal cancer



1. « **Constitutive** » **β-cat** signaling hinders APC mutant cells to exit the TA compartment of crypts

2. Addition of an oncogenic mutation in Kras promotes progression to a benign adenoma (polyp)

Progression to CRC requires additional hallmark capabilities



Questions?