

#6

Aktivace vývojových mechanismů  
u dospělého organismu

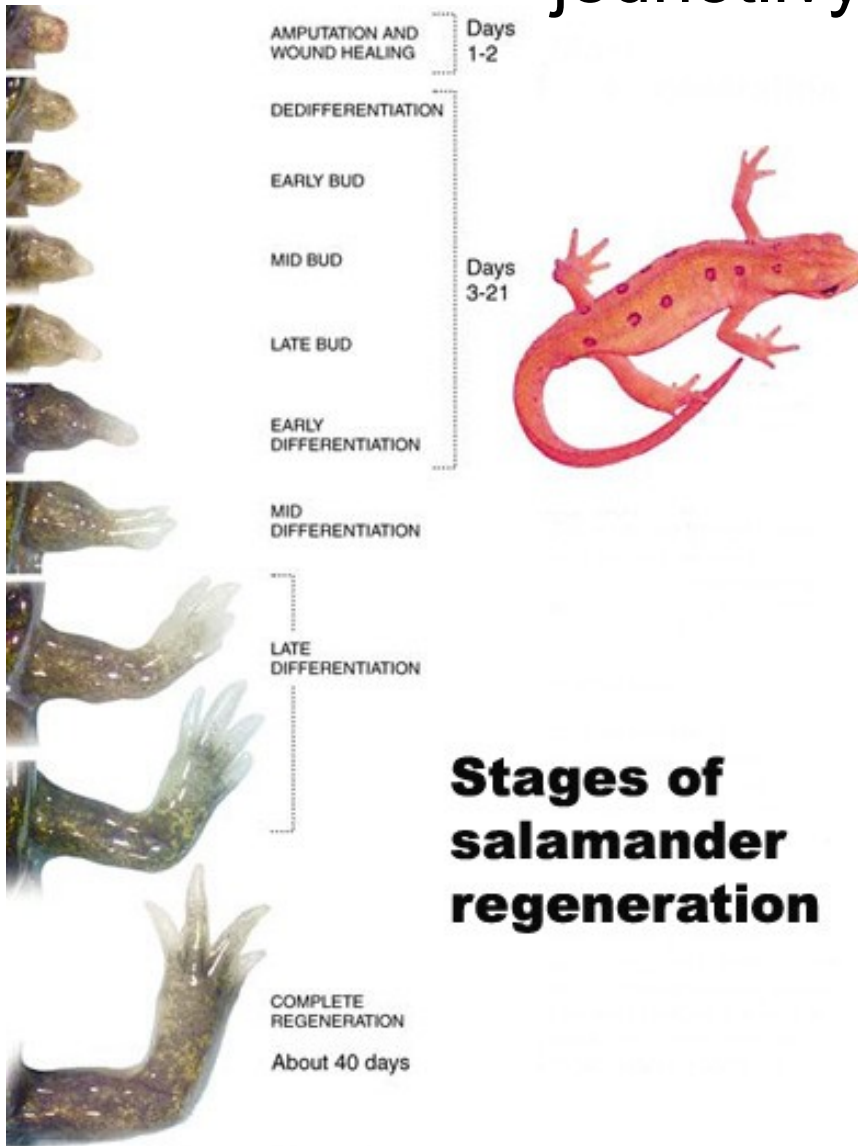
# Osnova:

- Kdy a proč se reaktivují procesy spjaté s embryonálním vývojem?
- Regenerace – buněčné a molekulární mechanismy
- Kmenové buňky – definice, ESCs, adult stem cells, stem cell niche
- When things go wrong – tvorba nádorů
- Cancer stem cell concept, markery, signální dráhy (...a jsme zpět u velké pětky)

# Kdy a proč se reaktivují procesy spjaté s embryonálním vývojem?

- odpověď je logická: tehdy, když je potřeba znovu vytvářet struktury poškozené či zničené tj. když je potřeba regenerovat
- v lidském organismu běžně regenerují celé tkáně – např. vlasové kořínky (doba „života“ 3-4 roky), krypty v tlustém střevě, epitel plic, krevní buňky nebo játra

# přesto se Schopnosti regenerace se liší mezi jednotlivými organismy



## Stages of salamander regeneration

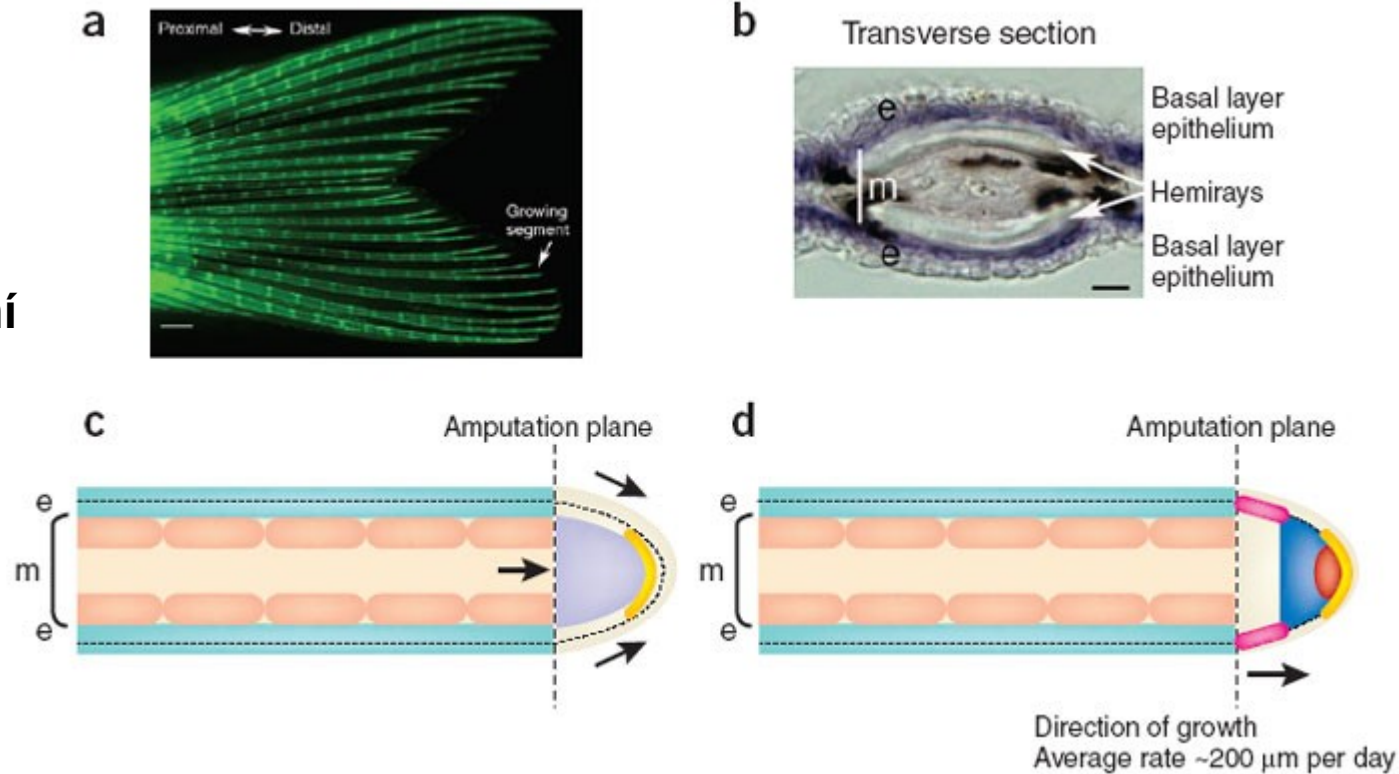
po amputaci



po 10 letech

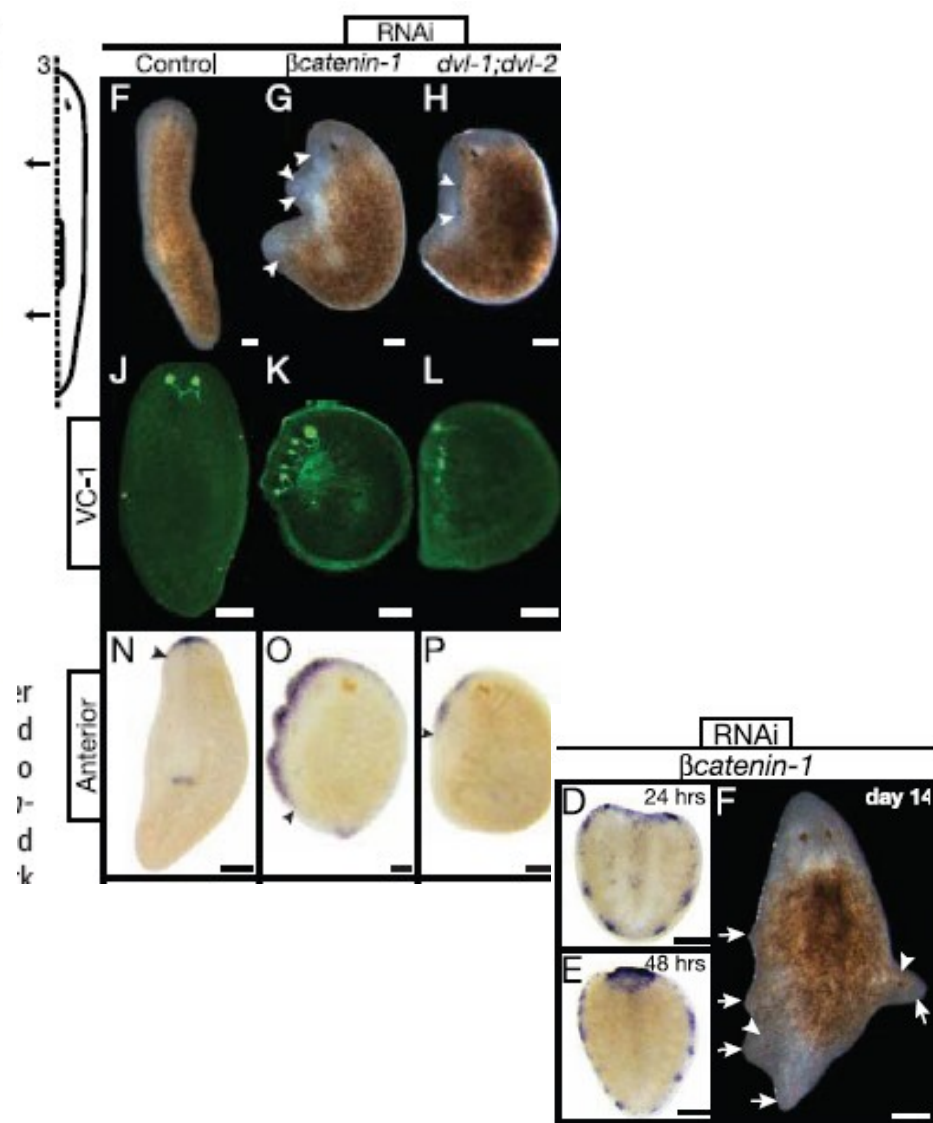
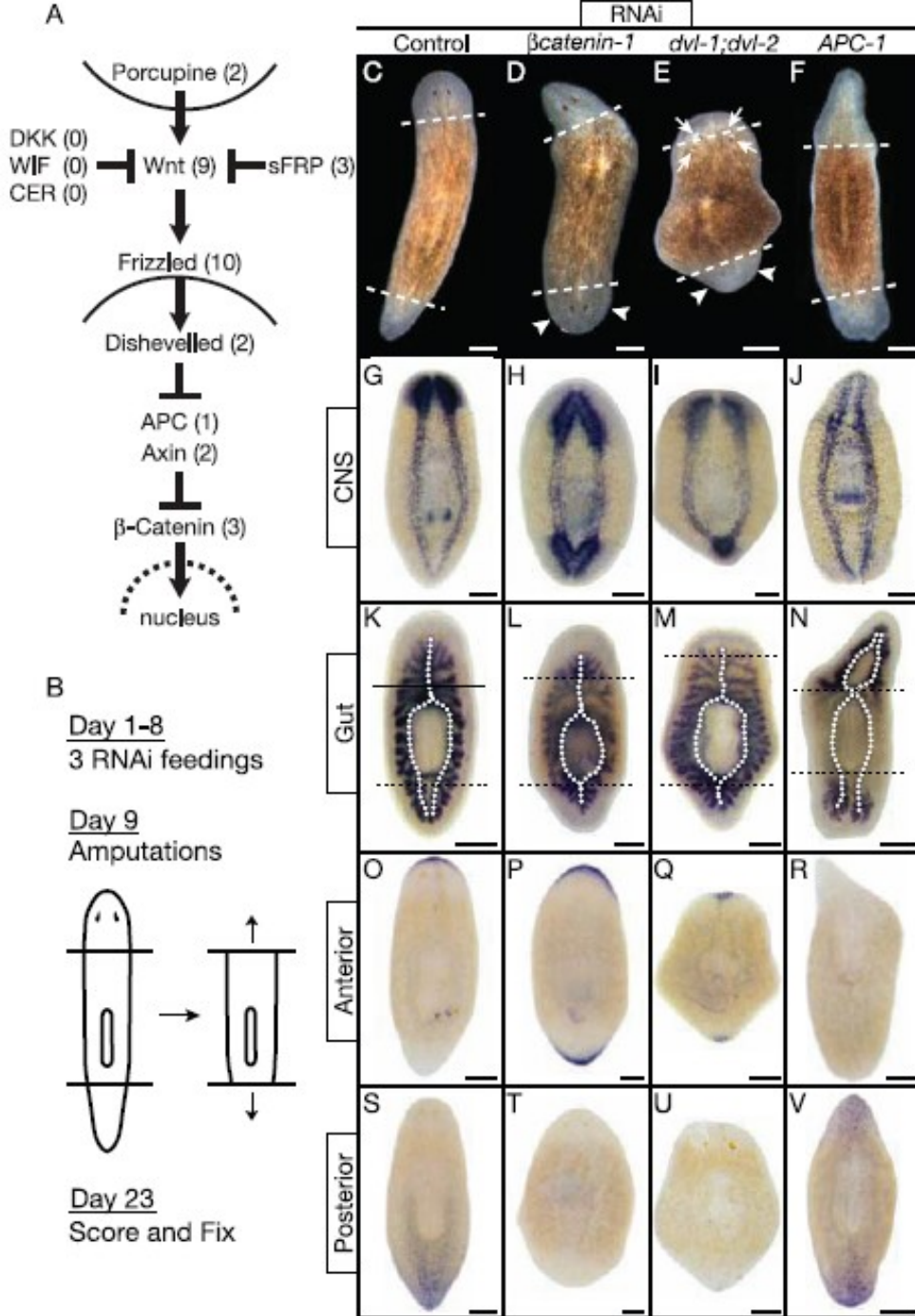


**Blastéma – masa nediferencovaných pluripotentních buněk, která se vytváří po poškození a dává vznik nové struktuře**



(a) Zebrafish caudal fin stained with calcein so that bony fin rays are visible. Scale bar, 200 μm. (b) Transverse section through a single fin ray. Hemirays (white arrows) are visible as crescents of bone surrounding mesenchymal tissue (fibroblasts, undifferentiated cells, artery, nerve, melanocytes). The bony rays are surrounded by an epithelium, and the basal layer of the epidermis has been labeled (purple stain and arrowheads). Scale bar, 15 μm. (c) Cartoon of a longitudinal section of a single amputated fin ray after formation of the immature blastema. The basal layer of the epithelium is indicated by the dotted line. Cells from the epithelium (light blue) cover the wound (side arrows), forming the wound epidermis and apical epidermal cap (AEC). Cells from the stump mesenchyme migrate distally (center arrow), forming the immature blastema (purple). (d) Cartoon of a longitudinal section of a single fin ray following blastemal reorganization. The basal layer of the epithelium is indicated by the dotted line, the AEC in yellow. The distal blastema (red) is distal to the proximal blastema (dark blue). Proliferating cells from the proximal blastema will migrate laterally, toward the basal epidermal layer (pink), and differentiate as bone forming cells. e, epidermis; m, mesenchyme.

During limb growth in vertebrates, an essential first step is the establishment of 'signaling centers', localized areas in the limb bud that contribute to the mechanisms regulating outgrowth and pattern formation by secreting signaling molecules such as Fgfs, Wnts, transforming growth factor (TGF)-s or bone morphogenetic proteins (BMPs), and hedgehogs (Hh). In the zebrafish, gene profiling studies have shown that the expression levels of these and other signaling molecules are regulated during the course of regeneratio

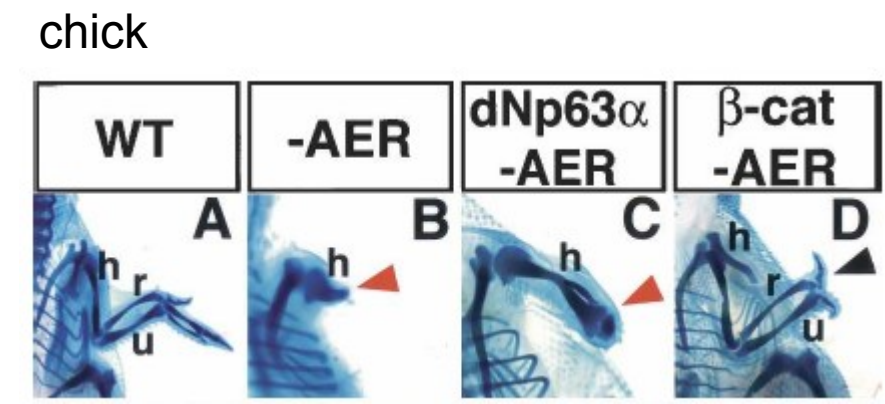
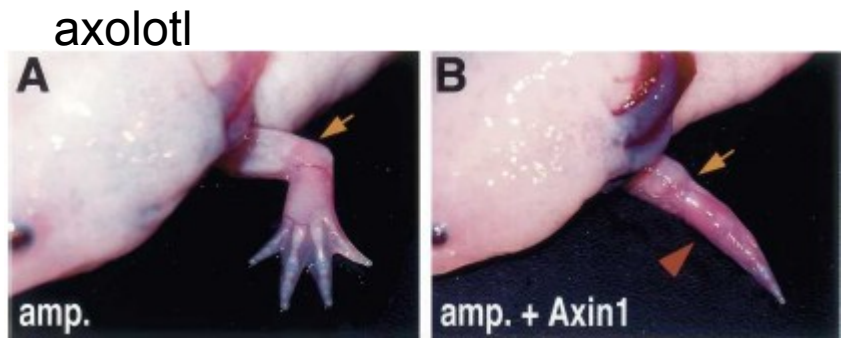


## $\beta$ -Catenin Defines Head Versus Tail Identity During Planarian Regeneration and Homeostasis

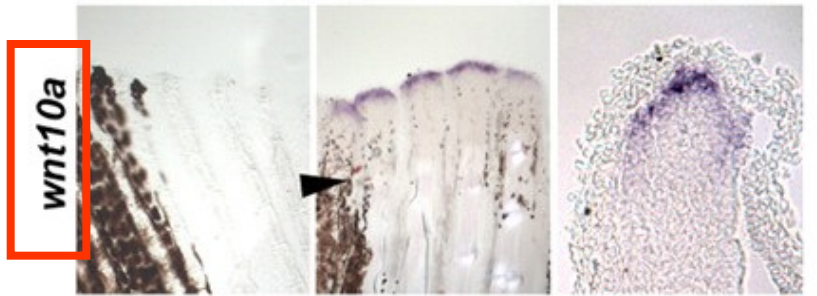
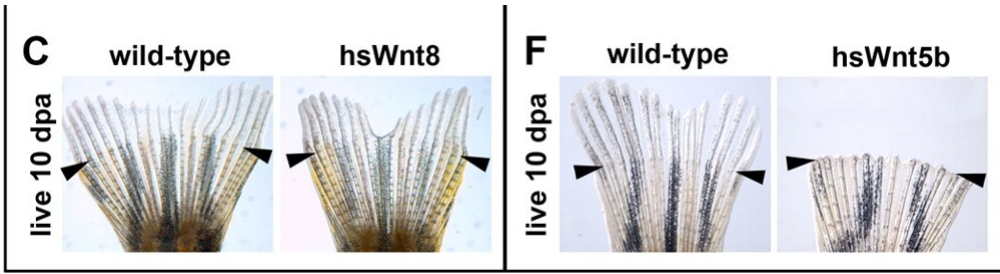
Kyle A. Gurley, Jochen C. Rink, Alejandro Sánchez Alvarado\*

SCIENCE VOL 319 18 JANUARY 2008

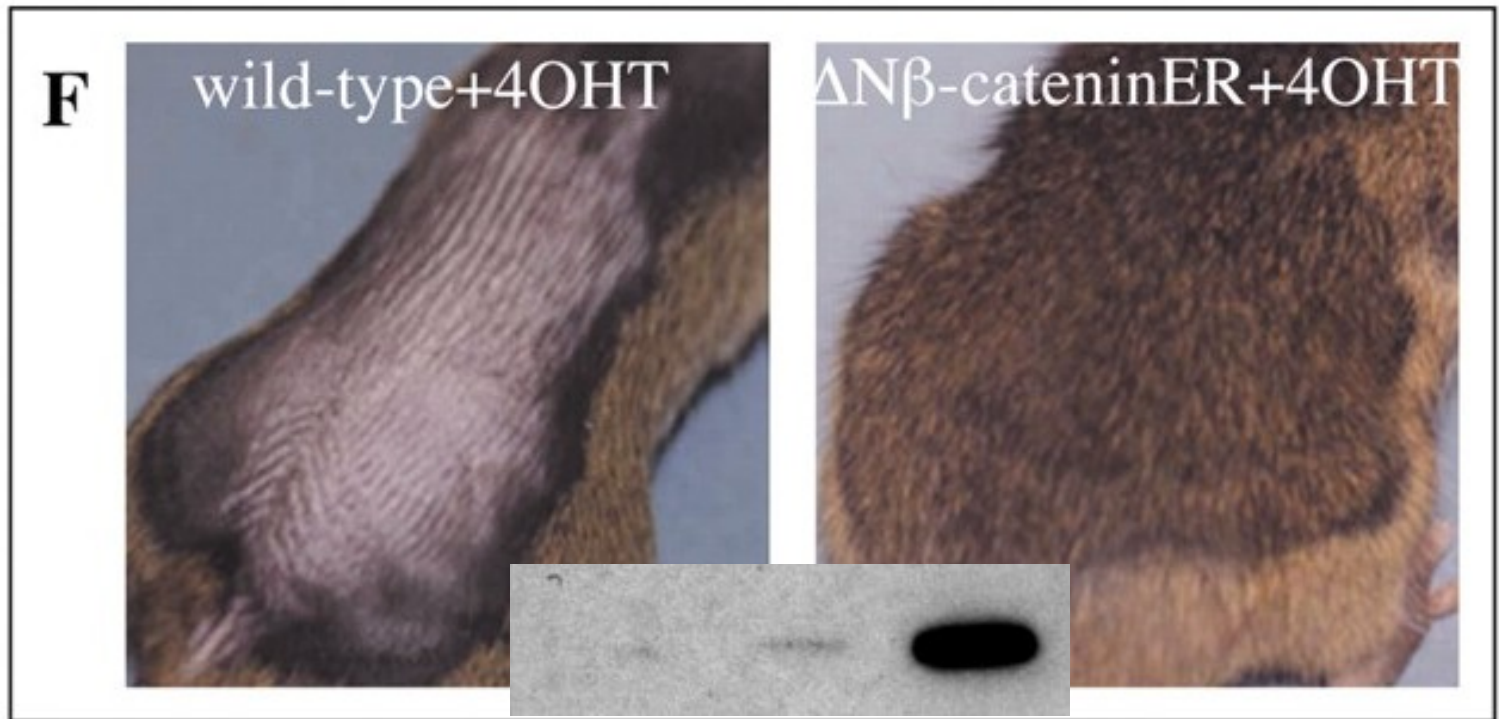
**Morphogenetic pathways (canonical Wnt signalling, Hedgehog, TGF, Notch) are required for regeneration in multiple organisms**



**zebrafish**

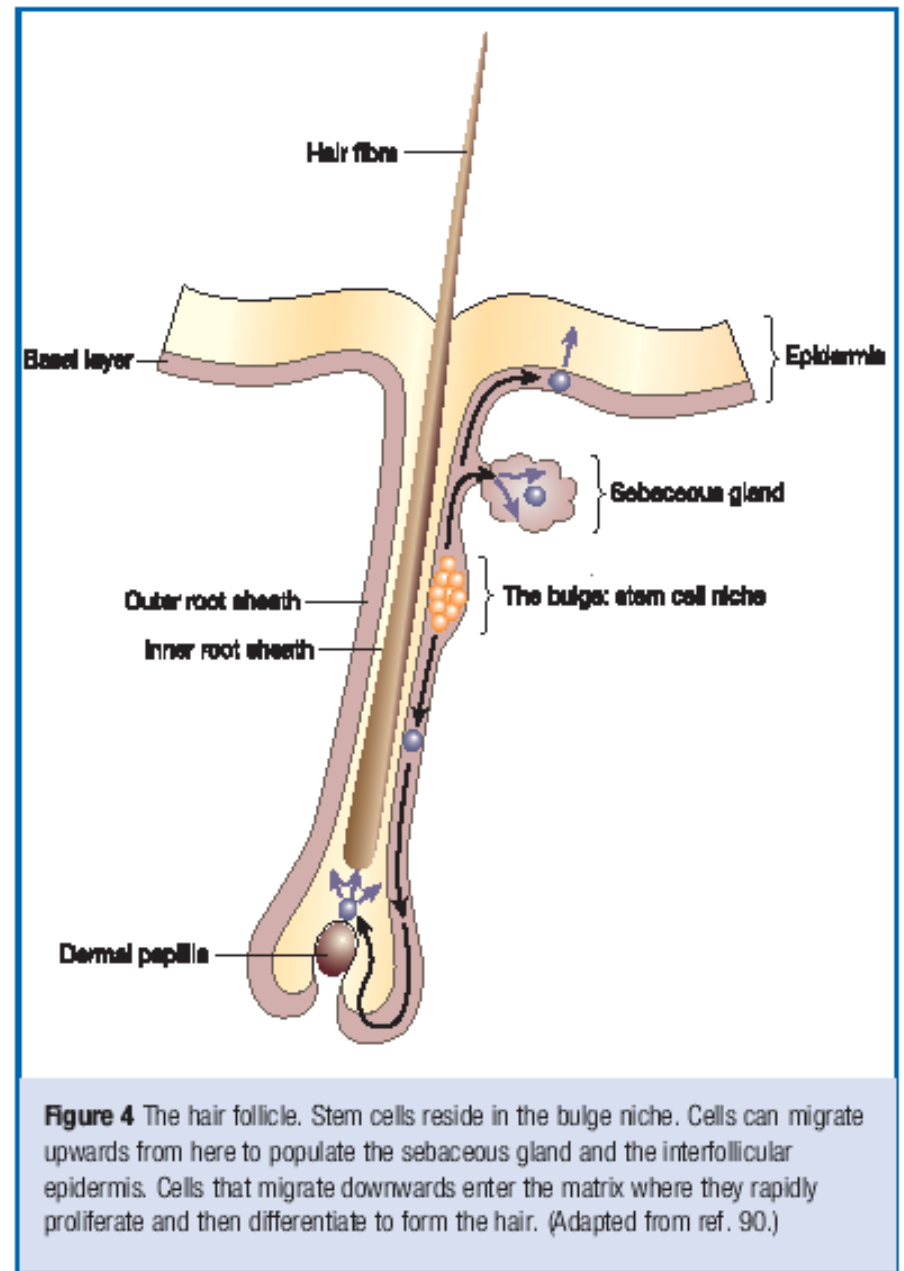
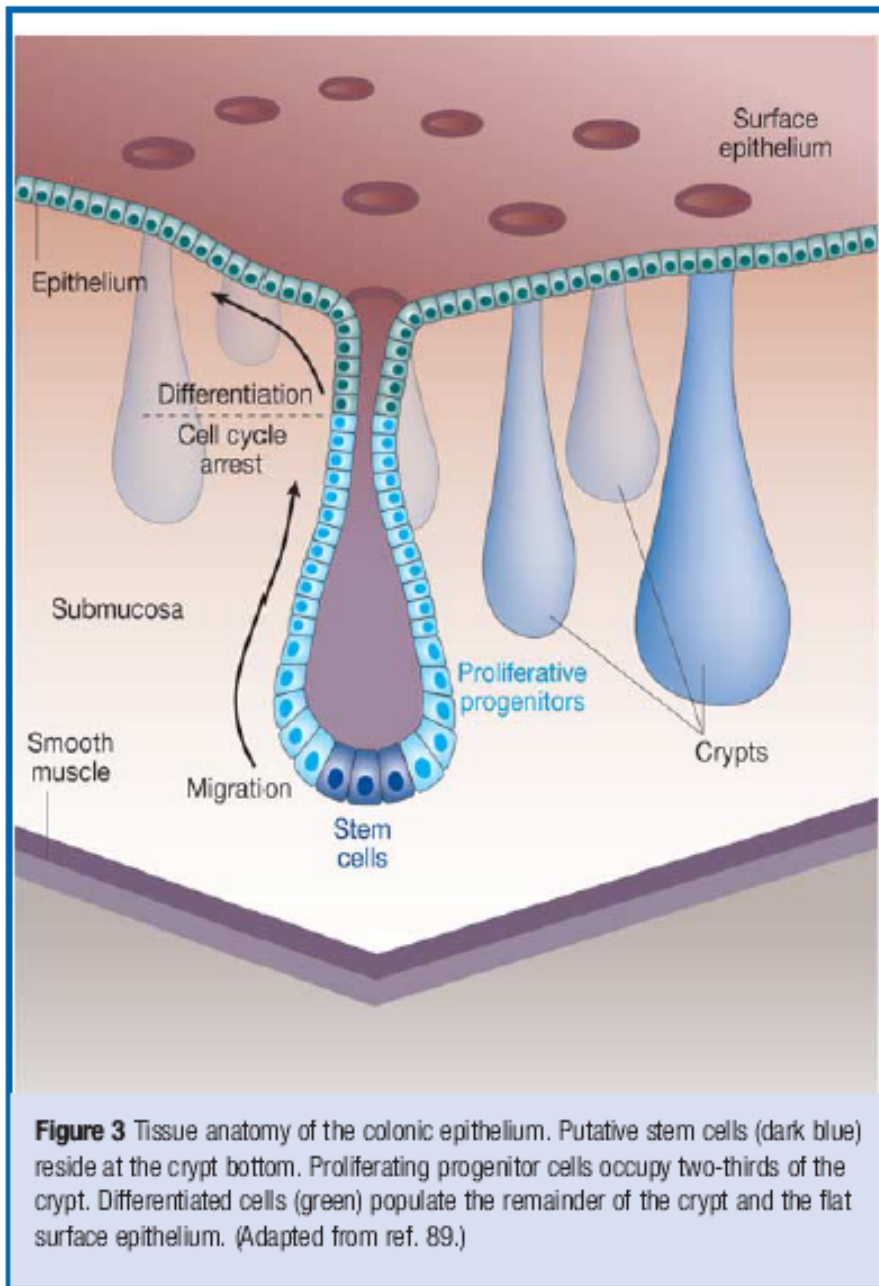


Stejné mechanismy aktivují regeneraci i u savců a lidí.



Lo Celso, C. L. et al. Development 2004;131:1787-1799





**Reya & Clevers 2005, Nature**

# Kmenové buňky

a) sebeobnova (selfrenewal)

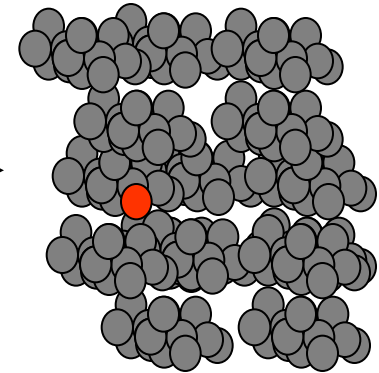
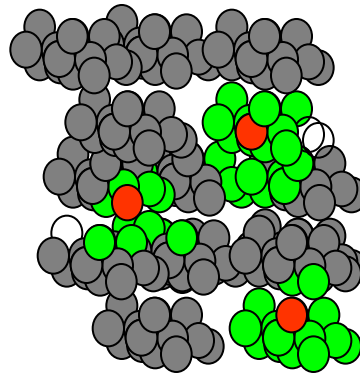
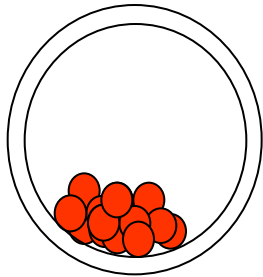
b) multipotence

- embryonální kmenové (ES)
- tkáňově specifické

blastocysta

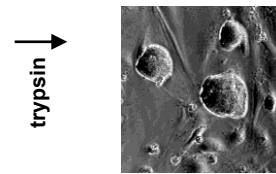
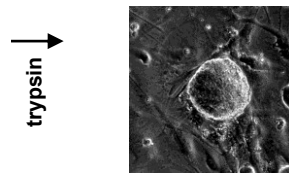
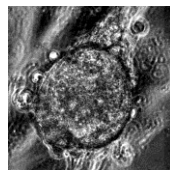
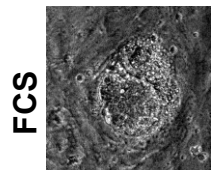
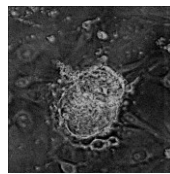
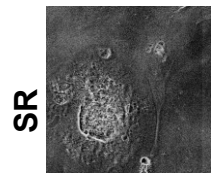
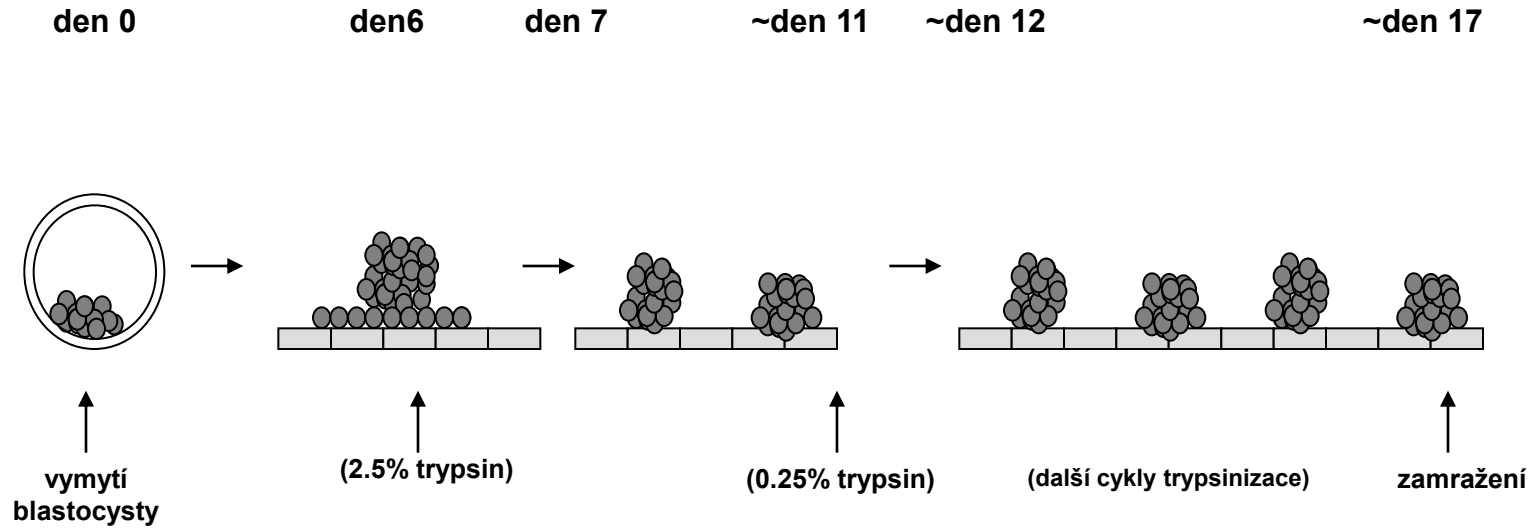
vyvíjející se tkáň (orgán)

dospělá tkáň



# Příprava embryonálních kmenových buněk:

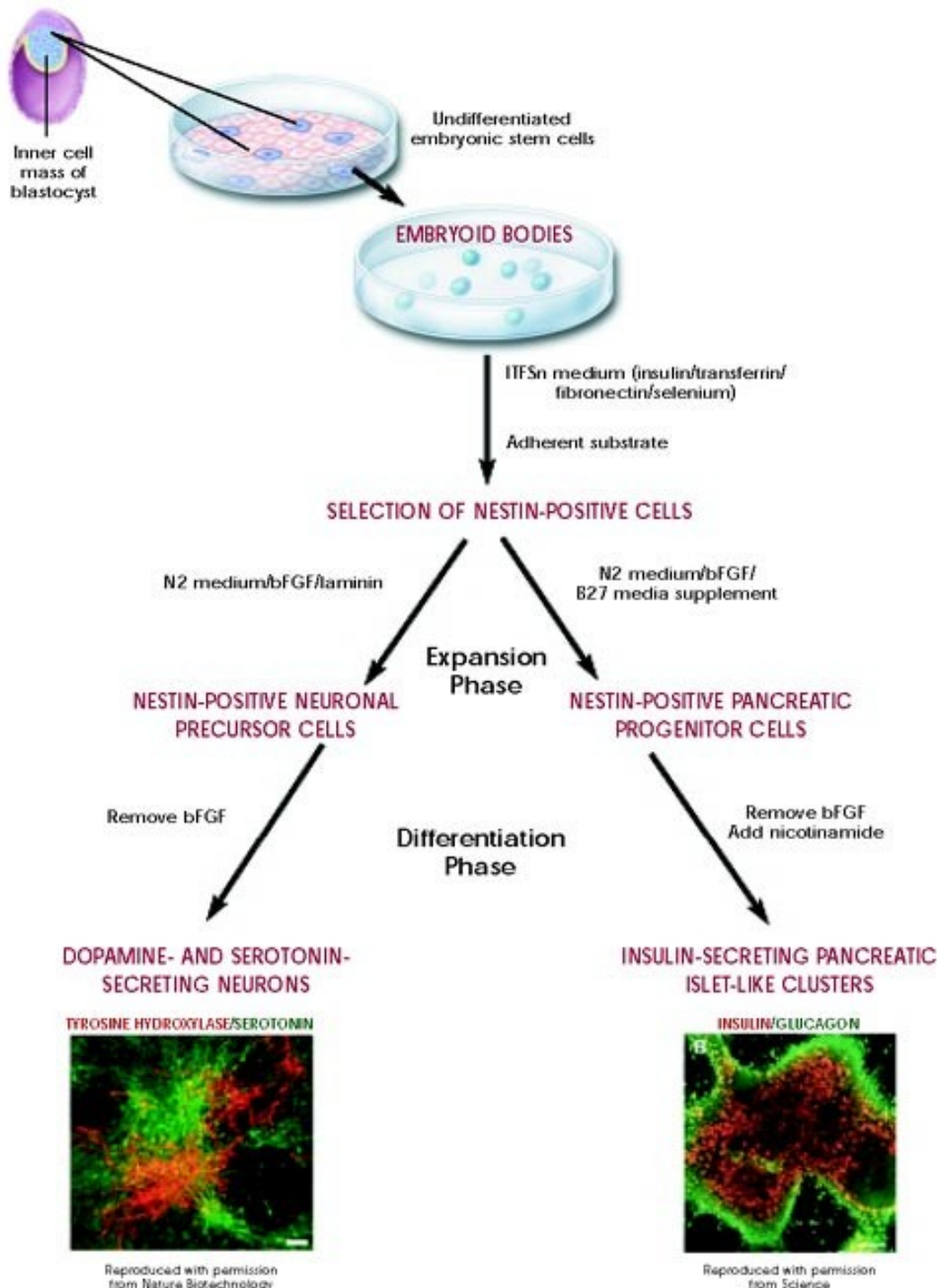
Bryja V. et al.: An efficient method for the derivation of mouse embryonic stem cells. Stem Cells, April 2006)



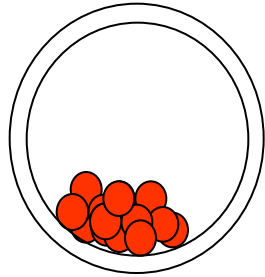
↓ trypsin

žádné kolonie připomínající ES buňky



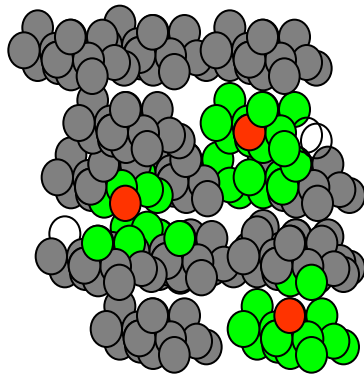


# In vivo

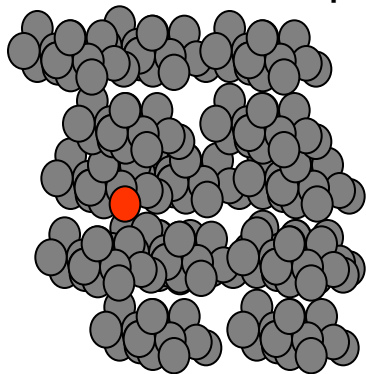


blastocysta

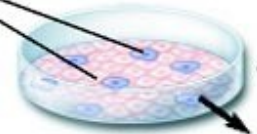
vyvíjející se tkáň (orgán)



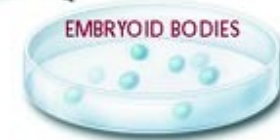
dospělá tkáň



# In vitro



Undifferentiated embryonic stem cells



ITF5n medium (insulin/transferrin/  
fibronectin/selenium)  
Adherent substrate

SELECTION OF NESTIN-POSITIVE CELLS

N2 medium/bFGF/laminin

N2 medium/bFGF/  
B27 media supplement

Expansion  
Phase

NESTIN-POSITIVE NEURONAL  
PRECURSOR CELLS

NESTIN-POSITIVE PANCREATIC  
PROGENITOR CELLS

Differentiation  
Phase

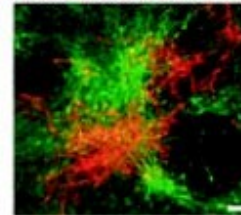
Remove bFGF

Remove bFGF  
Add nicotinamide

DOPAMINE- AND SEROTONIN-  
SECRETING NEURONS

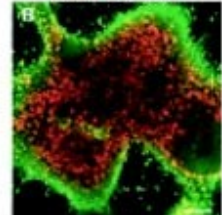
INSULIN-SECRETING PANCREATIC  
ISLET-LIKE CLUSTERS

TYROSINE HYDROXYLASE/SEROTONIN



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INSULIN/GLUCAGON

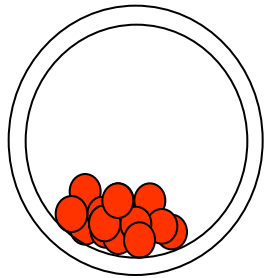


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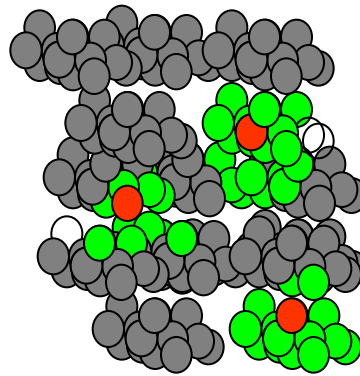
embryonální vývoj

ontogeneze

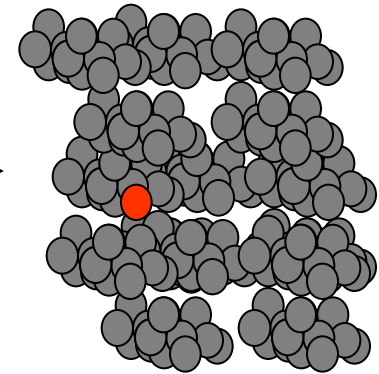
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vyvíjející se tkáň (orgán)

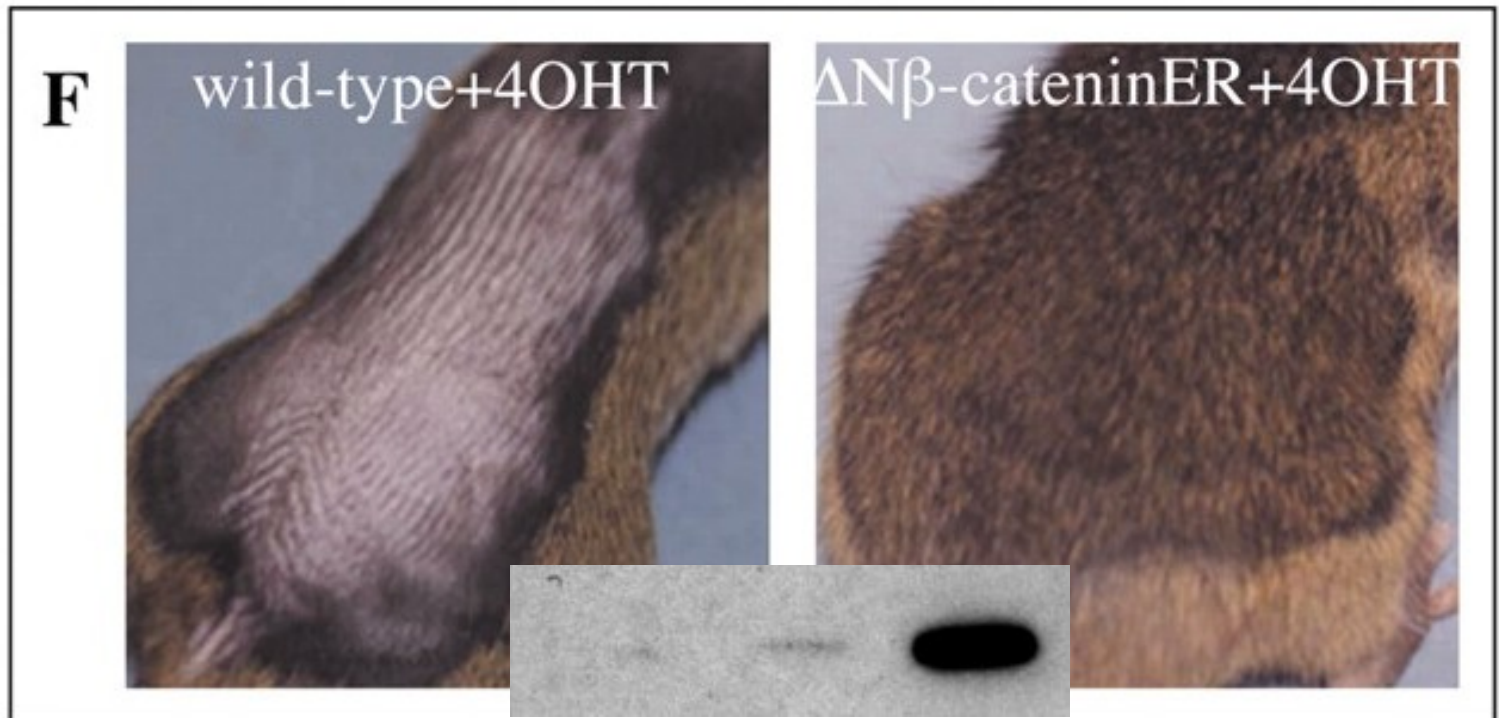


dospělá tkáň





Klíčové regulátory (Velká Pětka): Wnt, Shh, BMP/TGF, RTK (FGF), Notch

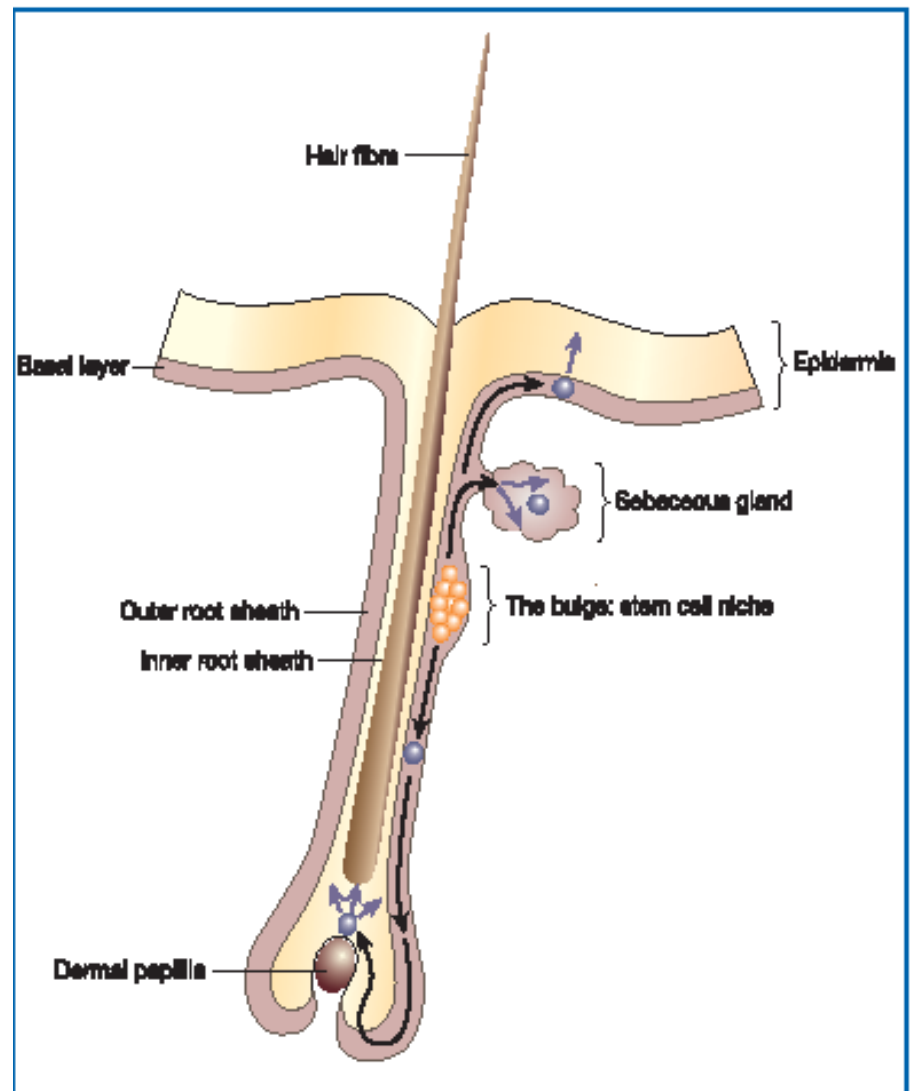




Kde jsou adult stem cells a jak  
vypadají?

# Prostředí kmenových buněk (stem cell niche)

vlasový kořínek

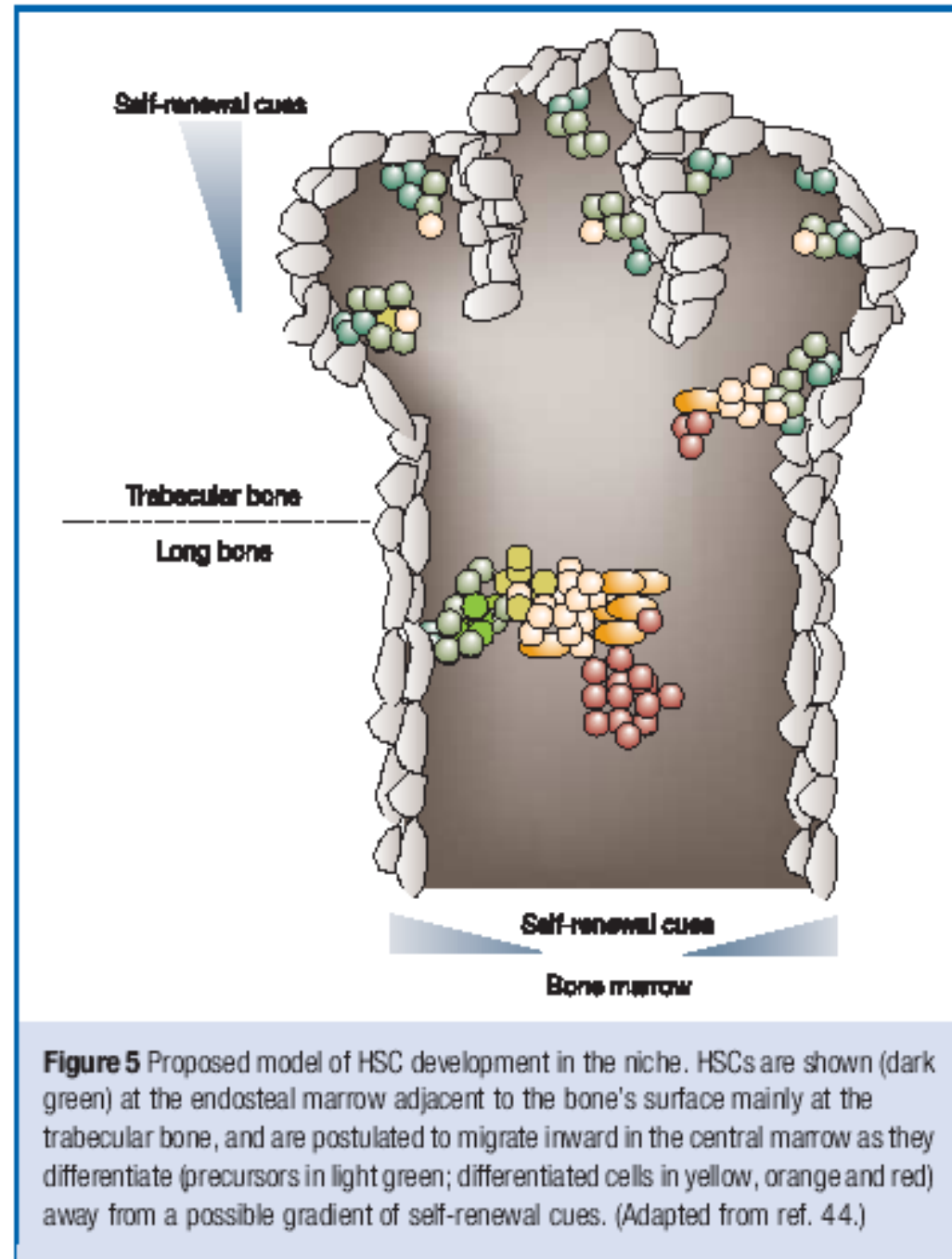


**Figure 4** The hair follicle. Stem cells reside in the bulge niche. Cells can migrate upwards from here to populate the sebaceous gland and the interfollicular epidermis. Cells that migrate downwards enter the matrix where they rapidly proliferate and then differentiate to form the hair. (Adapted from ref. 90.)

Reya & Clevers 2005, Nature

# Prostředí kmenových buněk (stem cell niche)

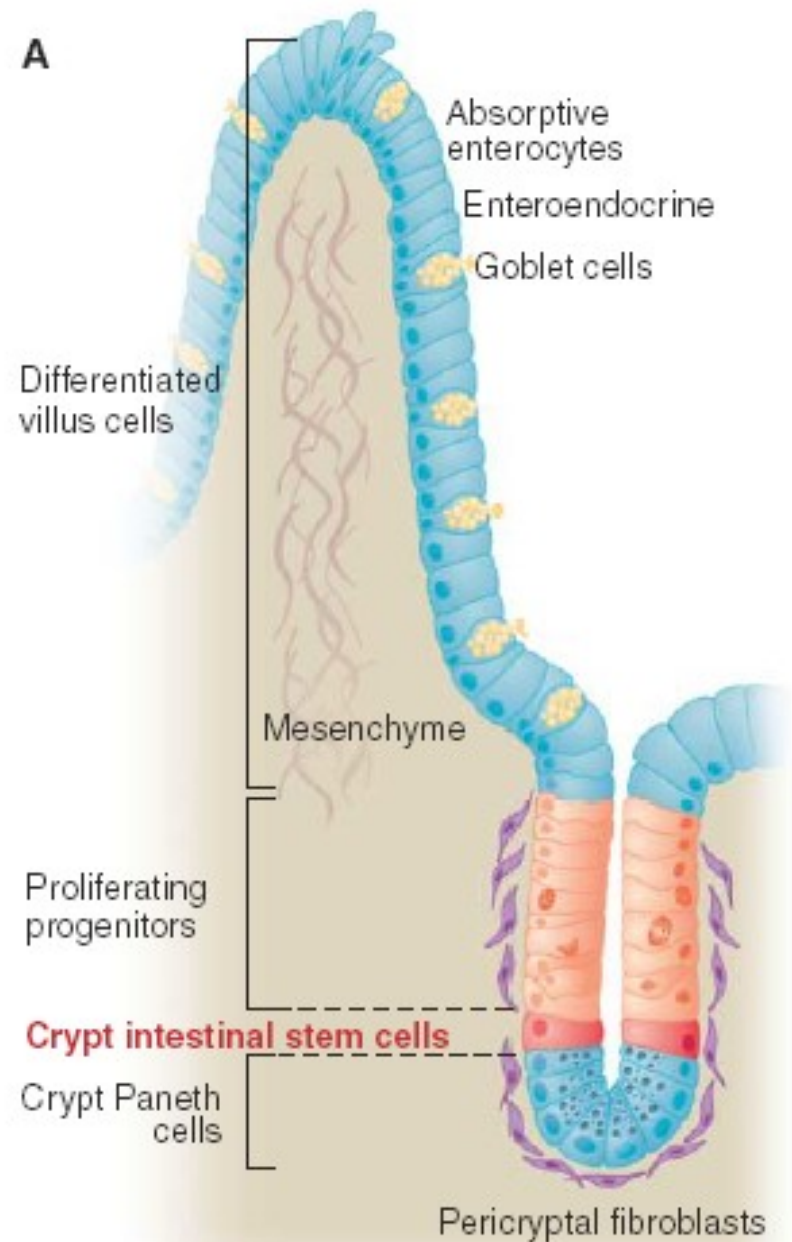
kostní dřeň



Reya & Clevers 2005, Nature

# Prostředí kmenových buněk (stem cell niche)

střevní epitel

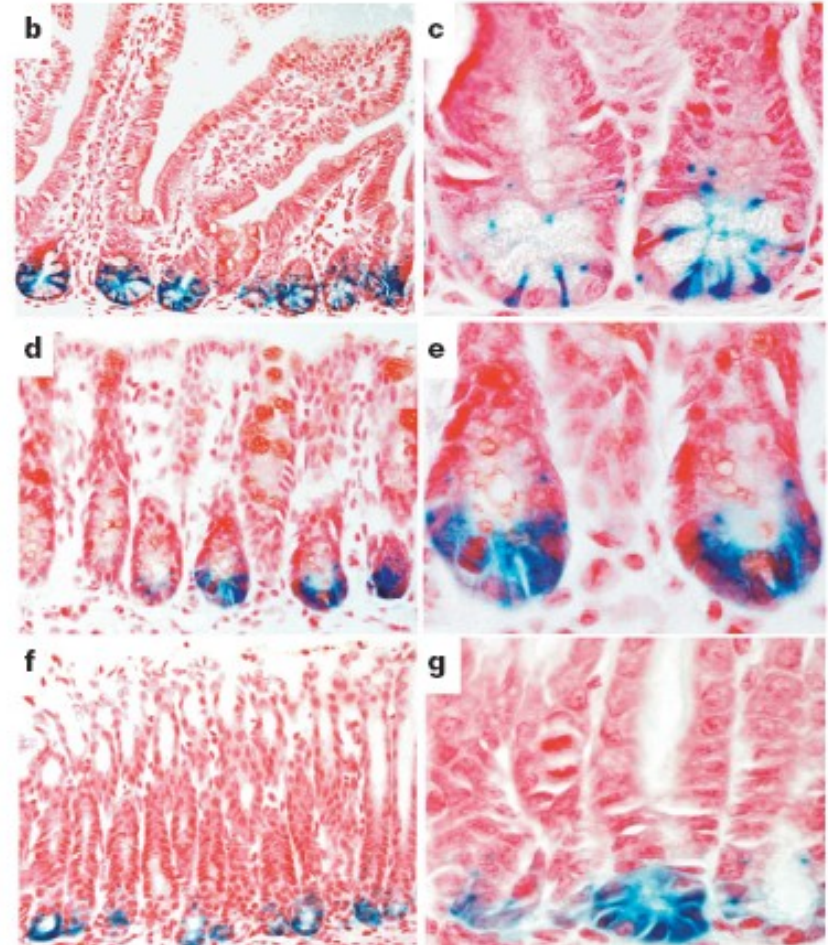
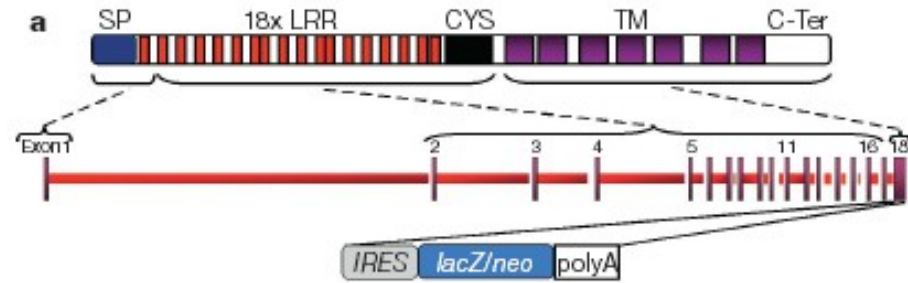


# Prostředí kmenových buněk (stem cell niche)

střevní epitel – latest developments  
aneb jak opravdu na to (Barker et al.,  
Nature, October 2007)

A. Příprava transgenní myši č. 1 za  
účelem zjistit, kde je nový potenciální  
stem cell marker exprimován (in vivo  
expression profiling). *Lgr5* je  
exprimován specificky v buňkách ve  
spodní části krypty.

**Figure 3 | Restricted expression of an *Lgr5-lacZ* reporter gene in adult mice.** **a**, Generation of mice carrying *lacZ* integrated into the last exon of the *Lgr5* gene, removing all transmembrane (TM) regions of the encoded *Lgr5* protein. Neo, neomycin resistance cassette; SP, signal peptide; LRR, leucine-rich repeat region; C-Ter is carboxy terminus. **b–h**, Expression of *Lgr5-LacZ* (blue) in selected adult mouse tissues. **b, c**, In the small intestine, expression is restricted to six to eight slender cells intermingled with the Paneth cells at the crypt base. **d, e**, In the colon, expression is confined to a few cells located at the crypt base. **f, g**, Expression in the stomach is limited to the base of the glands.



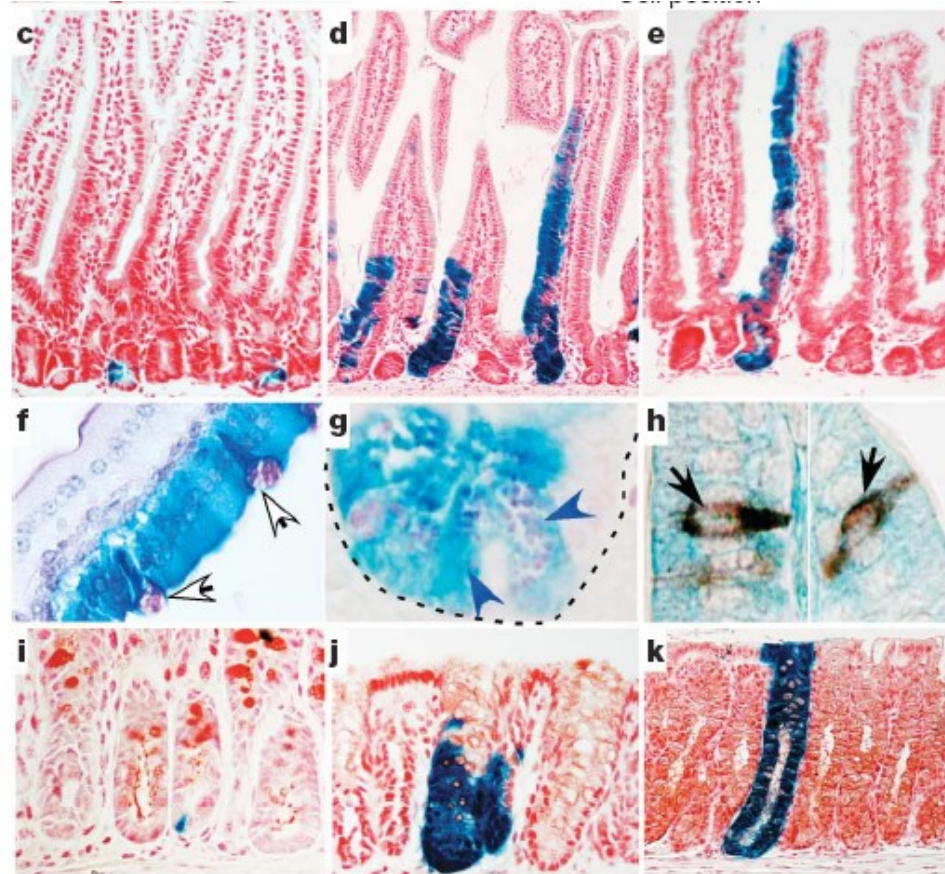
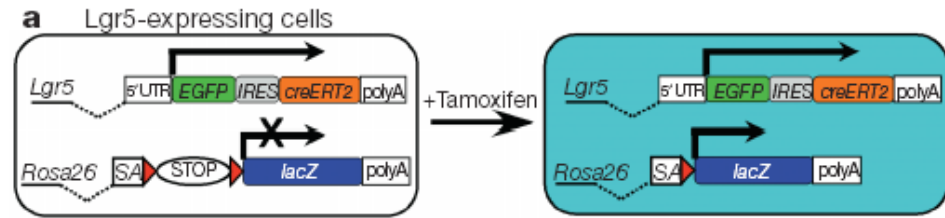
# Prostředí kmenových buněk (stem cell niche)

střevní epitel – latest developments  
aneb jak opravdu na to (Barker et al.,  
Nature, October 2007)

B. Příprava transgenní myši 2, 3 a 4  
za účelem zjistit, co všechno vzniká z  
Lgr5-pozitivních buněk (Lgr5+  
lineage tracing). Lgr5 pozitivní buňky  
dávají vzniknout všem částem  
buněčného epitelu.

**Figure 5 | Lineage tracing in the small intestine and colon.** a, *Lgr5-EGFP-IRES-creERT2* knock-in mouse crossed with *Rosa26-lacZ* reporter mice 12 h after tamoxifen injection. b, Frequency at which the blue cells appeared at

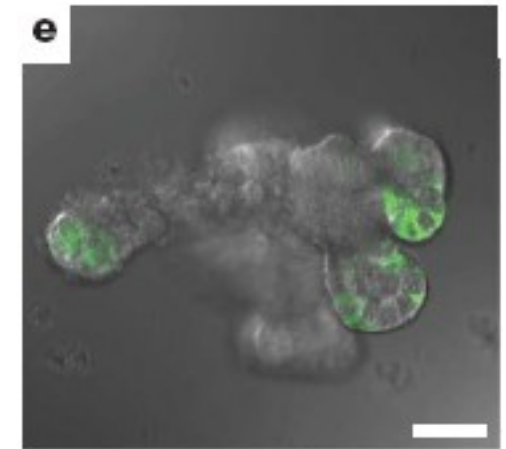
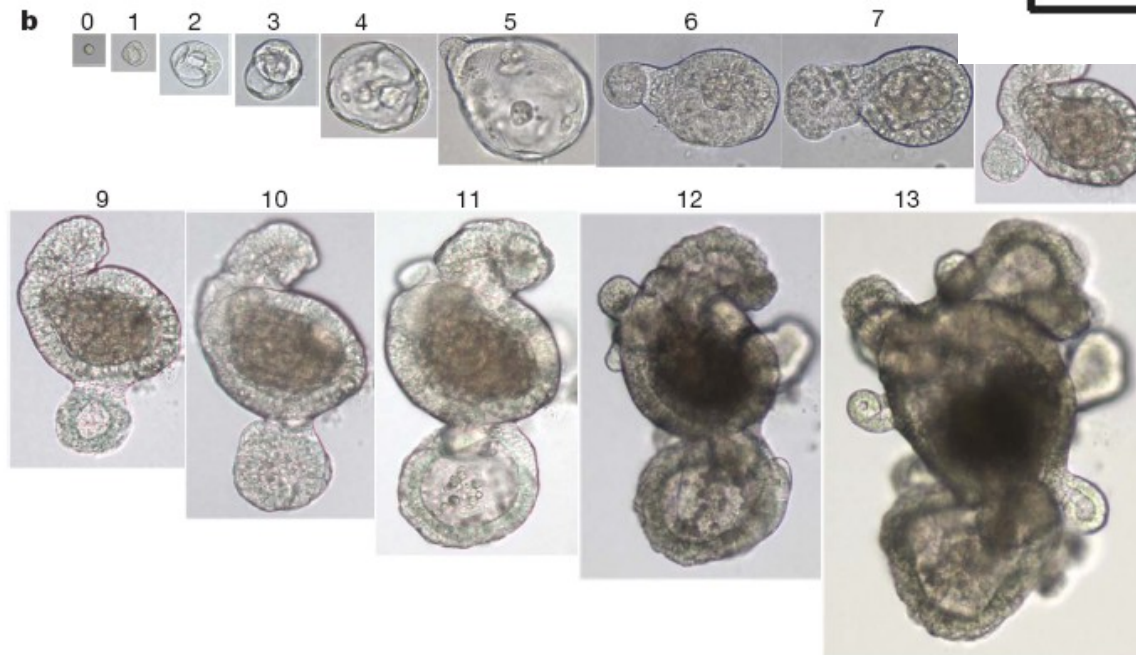
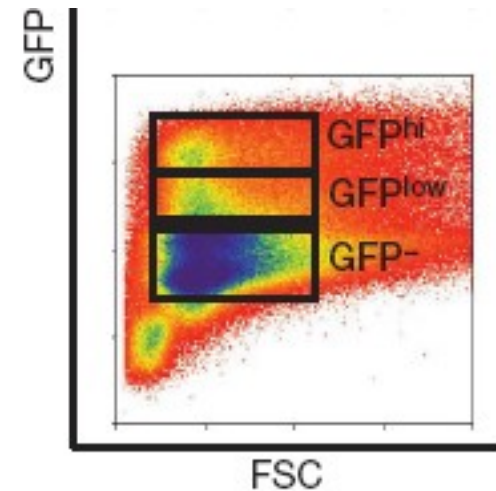
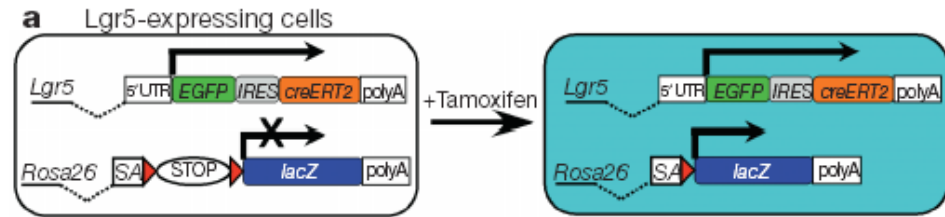
carrying activated Cre. c–e, Histological analysis of LacZ activity in small intestine 1 day after induction (c), 5 days after induction (d) and 60 days after induction (e). f–h, Double-labelling of LacZ-stained intestine using PAS demonstrates the presence of goblet cells (f, white arrows) and Paneth cells (g, blue arrows) in induced blue clones. Double-labelling with synaptophysin demonstrates the presence of enteroendocrine cells within the induced blue clones (h, black arrows). i–k, Histological analysis of LacZ activity in colon 1 day after induction (i), 5 days after induction (j) and 60 days after induction (k).



# Prostředí kmenových buněk (stem cell niche)

střevní epitel – latest developments  
aneb jak opravdu na to (Barker et al.,  
Nature & Sato, Nature 2009)

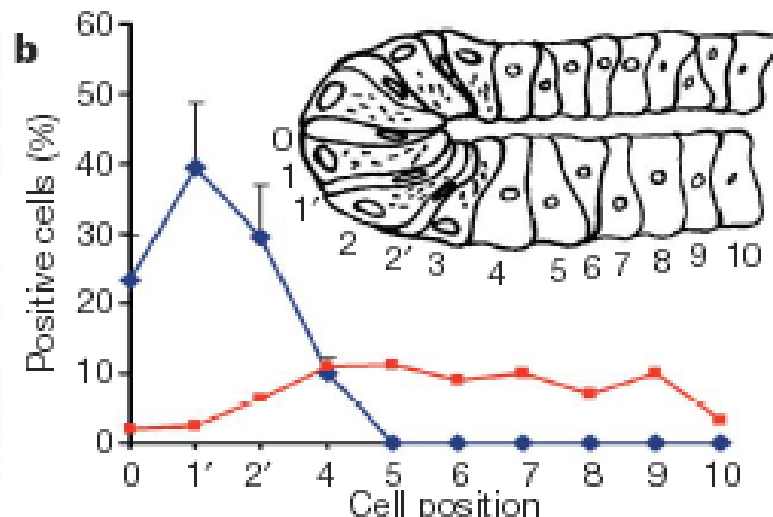
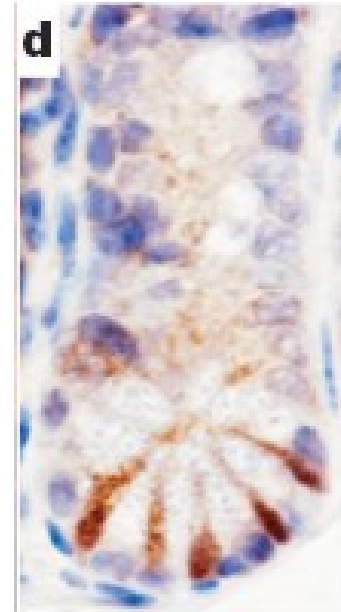
C. Lgr5 pozitivní buňky in vitro dávají  
vzniknout kompletní villus-crypt  
strukturu in vitro (Doposud se to s  
žádnými jinými buňkami nepodařilo)



# Prostředí kmenových buněk (stem cell niche)

**střevní epitel – latest developments  
aneb jak opravdu na to (Barker et al.,  
Nature, October 2007)**

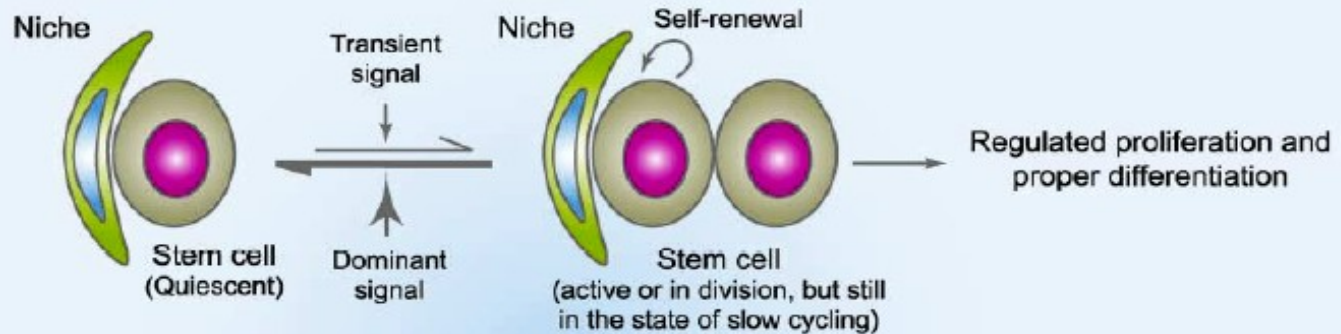
**D. Závěr: Kmenové buňky epitelu  
tlustého i tenkého střeva jsou  
protáhlé, dříve nepovšimnuté buňky,  
v relativní pozici 1', 2' a 3' od spodu  
krypty. Všechno co se říkalo předtím  
byly jen žvásty ☺**



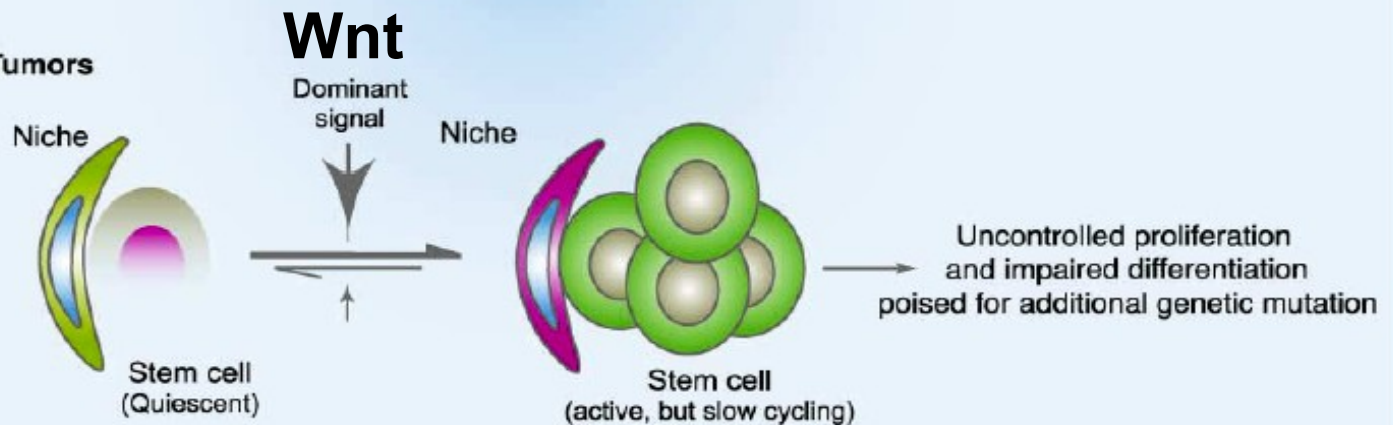


# Prostředí kmenových buněk (stem cell niche)

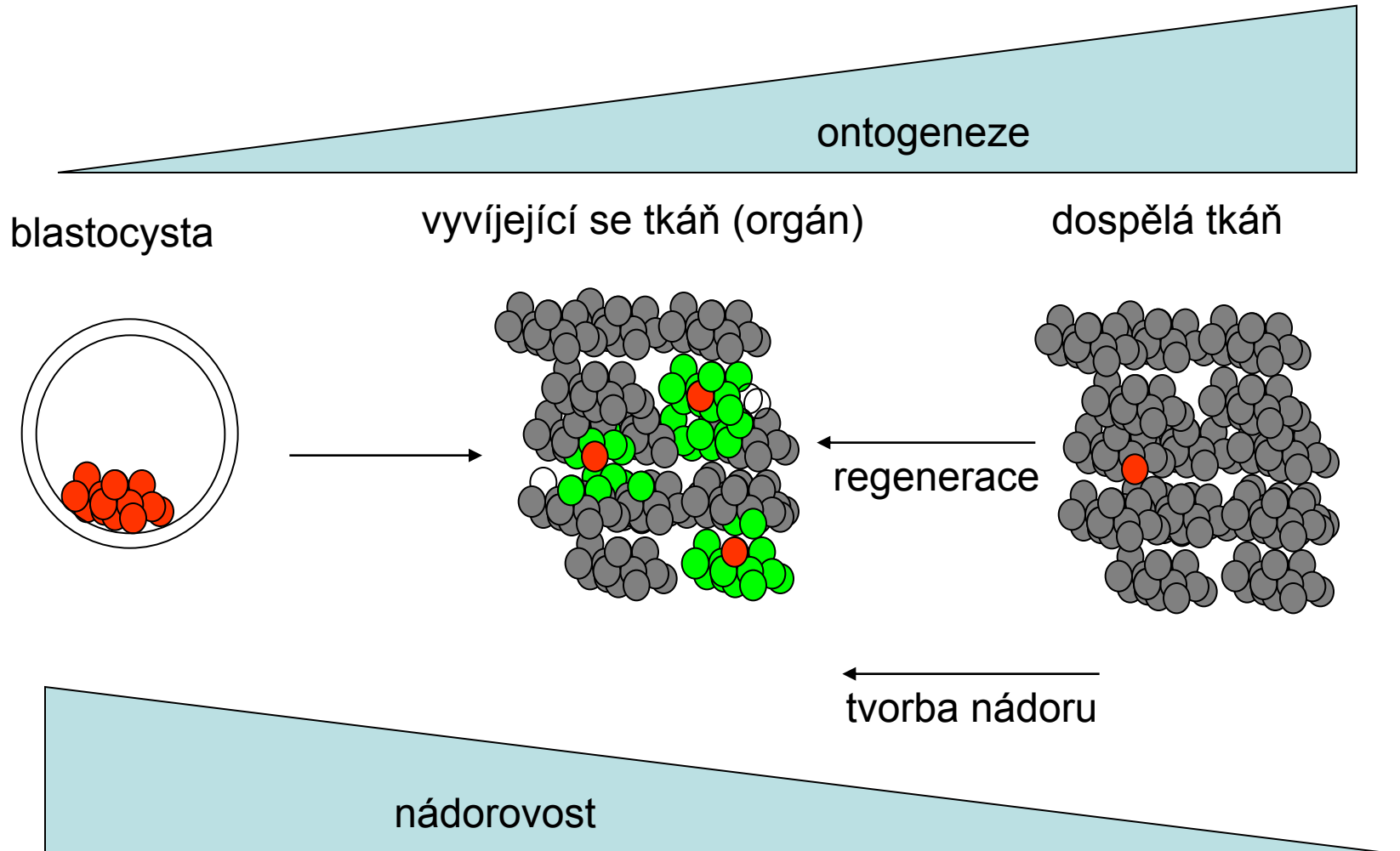
## Under Normal Physiological Conditions



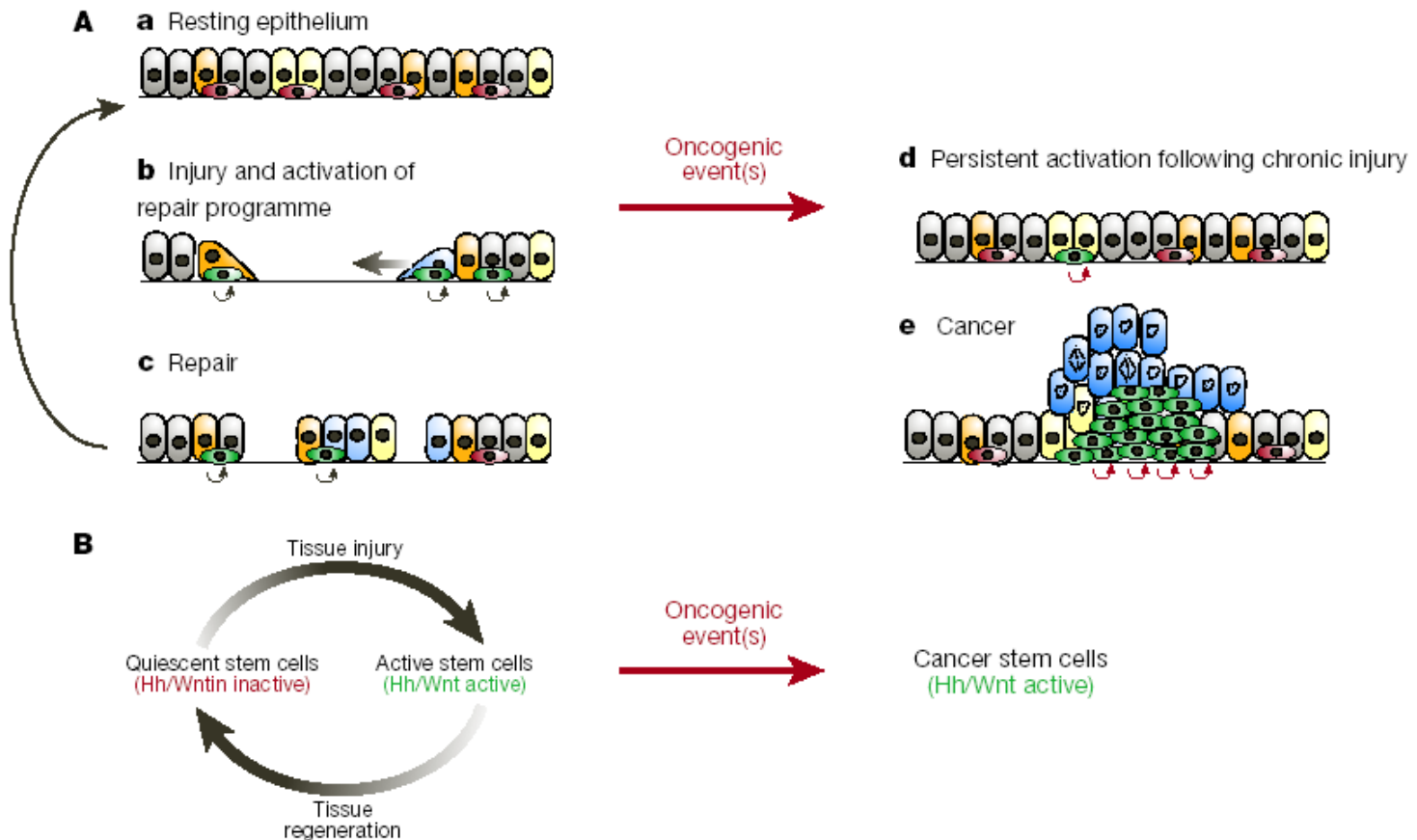
## In Cancers or Tumors



# Cancer stem cell hypothesis



# Aktivace nádorových kmenových buněk jako důsledek chronického poškození a regenerace

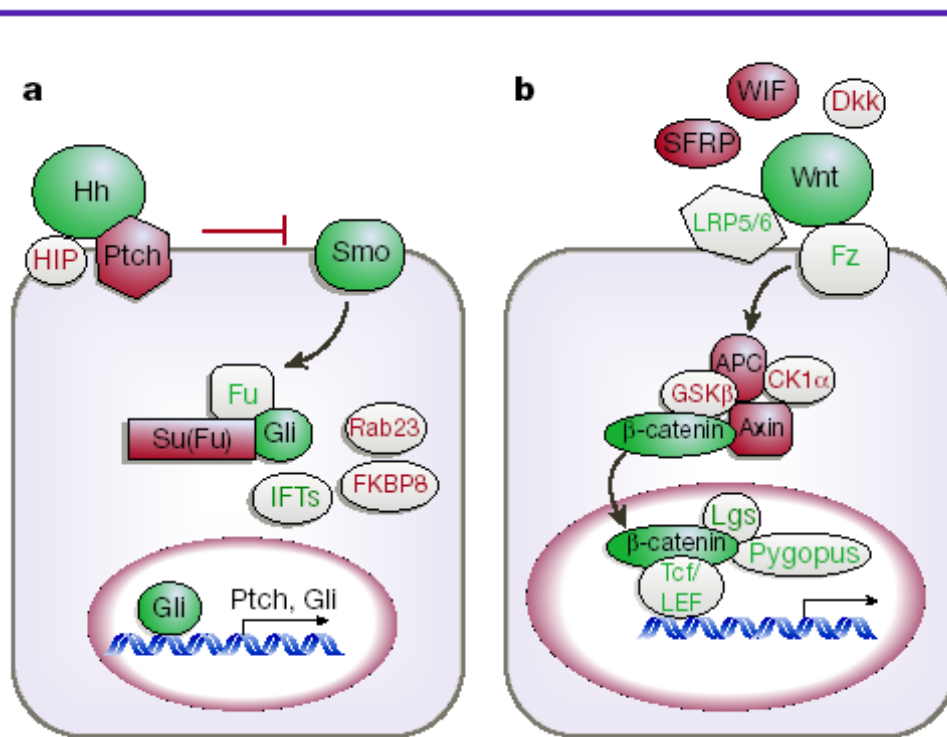


**Figure 2** Model for carcinogenesis resulting from persistence of a state of injury repair.

**A.** Cellular events of epithelial repair. **a.** Resting epithelium with several differentiated cell phenotypes (brown, orange, and yellow) derived from tissue stem cells, now quiescent (red). Pathways such as Hh and Wnt signalling pathways that have a role in the renewal of stem cells are not active. **b.** Epithelial defect resulting from acute injury. Loss of epithelial continuity activates a repair program which is driven by Hh or Wnt signalling. This program results in the acquisition by epithelial cells of a more mesenchymal phenotype, including flattening and movement of cells (straight arrow) to cover the wound, activation (green), and expansion of stem cells through renewal divisions (curved arrows). **c.** The wound is repaired, first by rapid cell movement, and then by restoration of cell numbers resulting from the amplification of stem cells and

the differentiation of their progeny. Subsequently, either epithelial continuity and patterning is restored, Hh and Wnt signalling ceases, and the stem cell compartment returns to quiescence (**a**); or oncogenic event(s) may trap a stem cell in an activated state of continuous renewal, which is driven by autonomous Wnt or Hh signalling (**d**). Further genetic or epigenetic change in such a persistently activated stem cell (curved red arrows) might produce a cancer stem cell (green) which is capable of aggressively propagating a cancer (**e**). This may result from enhanced proliferation and production of more cancer stem cells as well as from differentiated cancer cells (blue). **B.** Stem cells cycle between quiescence and activity as a consequence of Hh/Wnt driven responses to injury. Oncogenic event(s) may trap activated stem cells in a permanent state of Hh/Wnt driven activity, resulting in cancer stem cells.

# Klasické morfogenetické dráhy (Wnt, Hh, Notch a další) regulují regeneraci, tkáňové specifické kmenové buňky i nádory

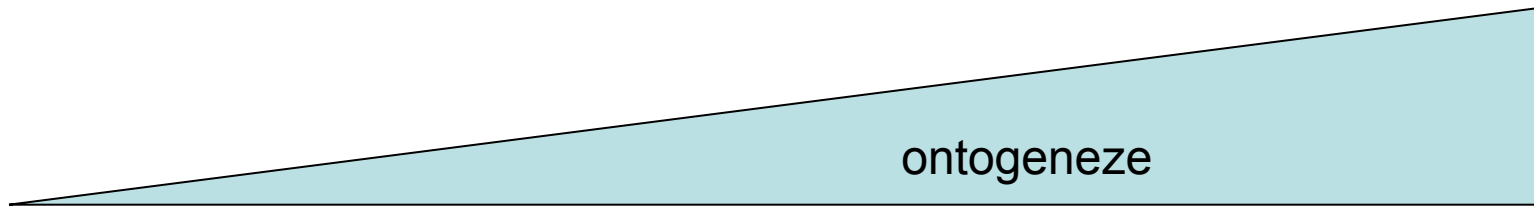


**Figure 1** Hh and Wnt signalling pathways. Simplified views of the Hh and Wnt signalling pathways, with emphasis on components implicated in cancer or tissue regeneration. Green and red colours denote pathway components with primarily positive or negative roles, respectively, in pathway activation. Shaded components have been causally implicated in tumorigenesis (see Table 2 and text; more complete pathway descriptions are available in refs 32–34 for Hh and refs 17, 46 for Wnt). **a**, Activation of the Hh signalling pathway is initiated by binding of a Hh ligand to Ptch. This lifts suppression of Smo, activating a cascade that leads to the nuclear translocation of Gli and the activation of target genes. HIP is a membrane protein that antagonizes pathway activity by binding to Hh ligands, and Fu, Su(Fu), Rab23, FKBP8 and the IFTs (intraflagellar transport proteins) act downstream of Ptch and Smo to regulate Gli. The function of Rab23, FKBP8 and the IFTs outside the CNS is not established. HIP, Hh-interacting protein; Rab23, a member of the Rab family of GTPases; FKBP8, a member of the FK506-binding protein family. **b**, The Wnt signalling pathway is activated by binding of Wnt ligands to their receptors Fz and LRP5/6, leading to the release of  $\beta$ -catenin from the degradation complex and facilitating its entry into the nucleus, where it regulates target gene transcription through association with Tcf/LEF, Legless (Lgs) and Pygopus. SFRP, Wnt inhibitory factor; Dkk, Dickkopf; GSK3 $\beta$ , glycogen synthase kinase 3 $\beta$ ; CK1a, casein kinase 1a.

Table 2 **Hh and Wnt pathways in cancer**

Tissue	Tumour	Evidence of pathway involvement	References
<b>Hh pathway</b>			
Brain	Medulloblastoma	Tumorigenesis by inactivation of <i>PTCH</i> ; allograft and cell-line growth inhibition by cyclopamine; inhibition of autochthonous tumour growth by synthetic small molecule antagonist	37, 81; reviewed in 6
		Tumorigenesis by inactivation of <i>Su(fu)</i>	86
	Glioma	<i>Gli</i> amplification; growth inhibition of some cell lines by cyclopamine	87, 88
Skin	Basal cell carcinoma	Tumorigenesis by inactivation of <i>PTCH</i> ; <i>in vivo</i> tumorigenesis by expression of activating form of <i>SMO</i> or by <i>Shh</i> overexpression and <i>in vitro</i> growth inhibition by synthetic Hh pathway antagonist; inhibition of human tumour growth topical cyclopamine	82, 83; reviewed in 6
Muscle	Rhabdomyosarcoma	Tumorigenesis by inactivation of <i>PTCH</i>	reviewed in 6
Oesophagus	Adenocarcinoma	Cell-line growth inhibition by cyclopamine, Hh blocking antibody	42
Stomach	Adenocarcinoma	Cell-line growth inhibition by cyclopamine, Hh blocking antibody	42
Pancreas	Adenocarcinoma	Xenograft and cell-line growth inhibition by cyclopamine, Hh blocking antibody; tumour initiation (in mouse) by <i>Shh</i> overexpression	42, 43
Biliary tract	Adenocarcinoma	Xenograft and cell-line growth inhibition by cyclopamine, Hh blocking antibody	42
Lung	Small-cell lung cancer	Xenograft and cell-line growth inhibition by cyclopamine, Hh blocking antibody	41
Prostate	Adenocarcinoma	Xenograft and cell-line growth inhibition and suppression of metastasis by cyclopamine; increased xenograft growth by <i>Shh</i> and <i>Gli</i> overexpression	29, 89, 90
Bladder	Urothelial carcinoma	Increased tumour induction (in mouse) by alkylating agent in <i>Ptch</i> heterozygote	91
Oral cavity	Squamous cell cancer	Growth inhibition of cell lines by cyclopamine;	92
<b>Wnt pathway</b>			
Colon	Adenocarcinoma	Tumorigenesis by inactivation of APC, Axin; tumorigenesis by stabilization of $\beta$ -catenin; epigenetic inactivation of SFRPs	47; reviewed in 45
Liver	Hepatoblastoma	Tumorigenesis (in mouse) by inactivation of APC and by stabilization of $\beta$ -catenin	reviewed in 45
Blood	Multiple myeloma	Cell-growth inhibition by dominant negative TCF4; growth stimulation by Wnt ligand	93
Hair follicle	Pilomatricoma	Tumorigenesis (in mouse) by overexpression of $\beta$ -catenin	reviewed in 45
Bone	Osteosarcoma	<i>Dkk3</i> and <i>LRP5</i> expression inhibits tumour cell growth <i>in vitro</i>	94, 95
Lung	Non-small-cell carcinoma	Apoptosis and cell-growth inhibition by short interfering RNA and a blocking antibody against Wnt2	96
Pleura	Mesothelioma	Apoptosis and cell-growth inhibition by transfection of SFRP	97

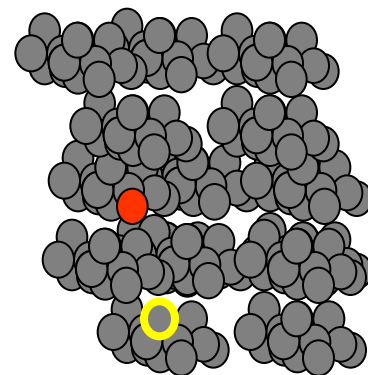
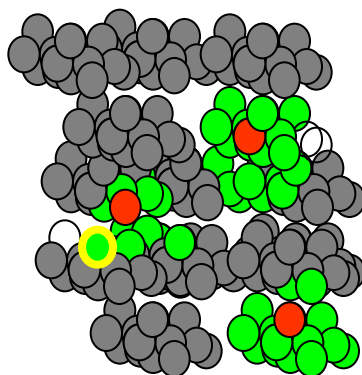
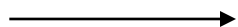
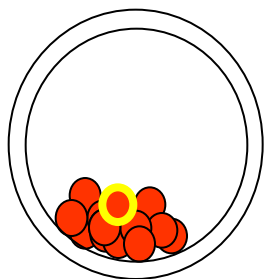
Emphasis is placed on functional data showing a requirement for pathway activation in tumour formation and/or tumour cell growth. (See Fig. 1 and text for gene abbreviations.)



blastocysta

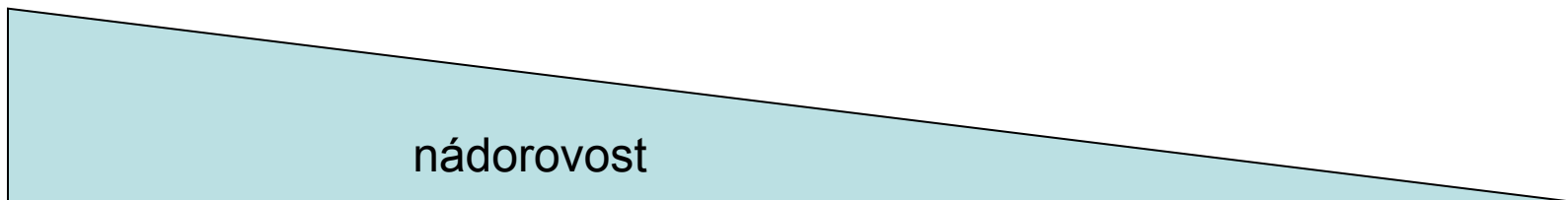
vyvíjející se tkáň (orgán)

dospělá tkáň



Počet mutací nutných  
pro vznik nádoru:

0



# Teratoma



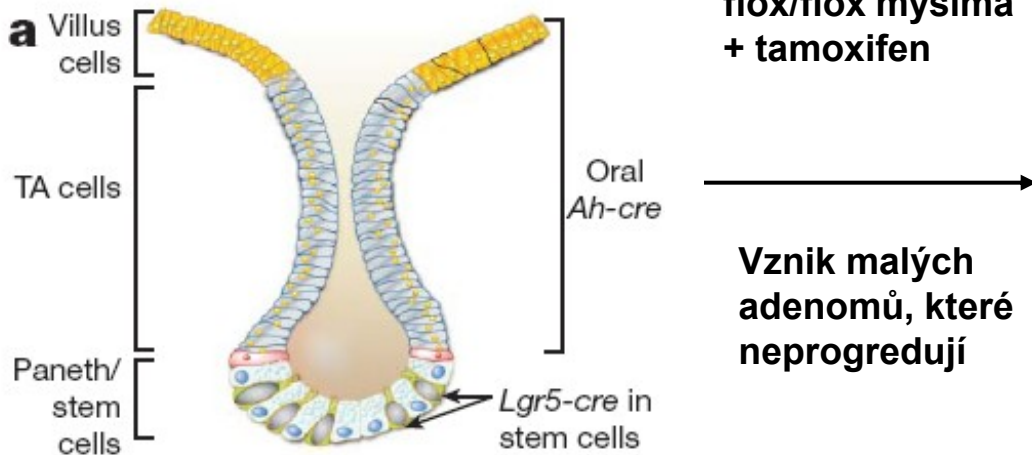
# Je to pravda?

Počet  
pro v

nádorovost

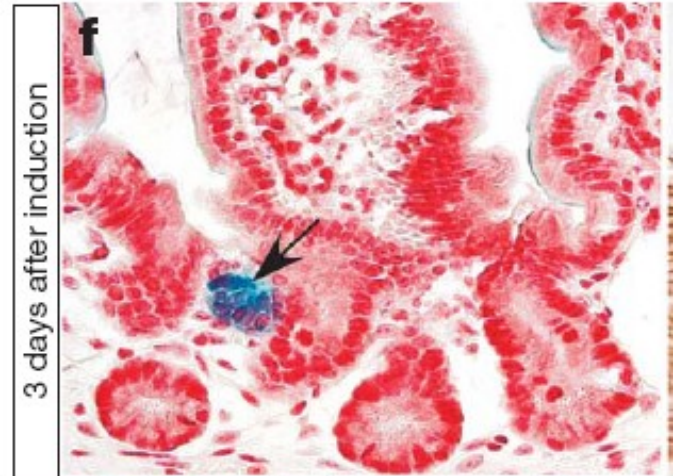


# Experimentální důkaz:

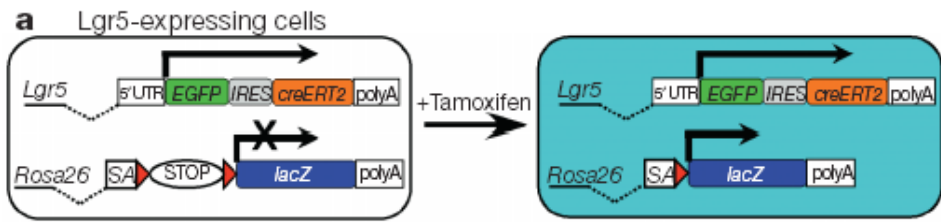


Zkřížení s APC flox/flox myšima + tamoxifen

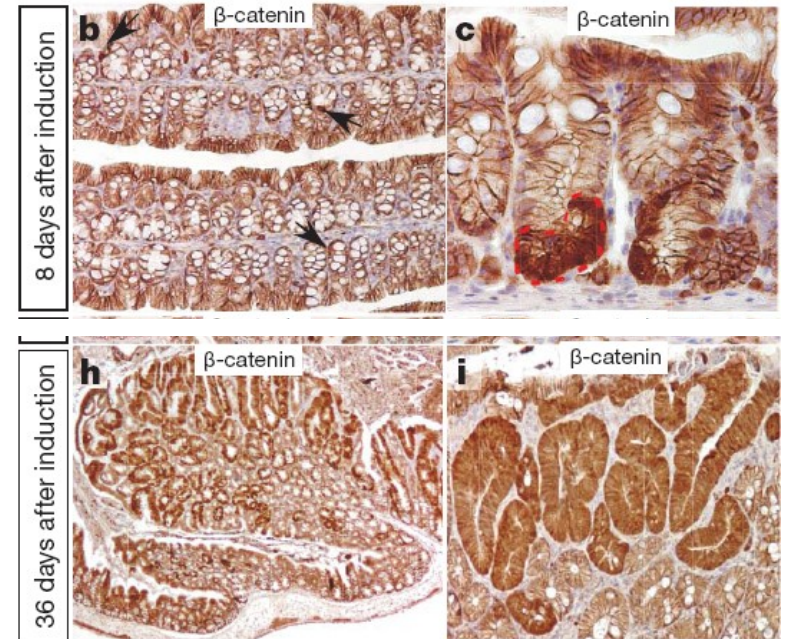
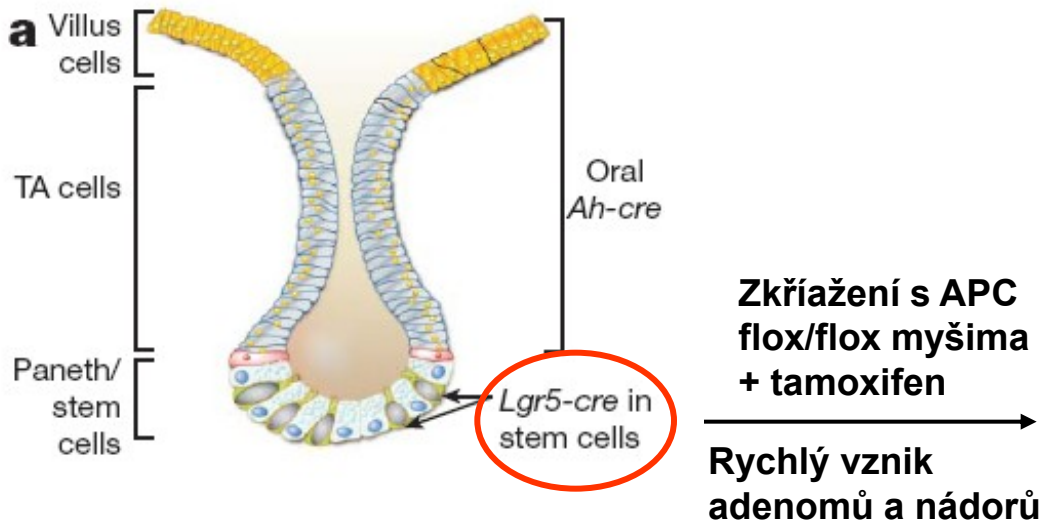
Vznik malých adenomů, které neprogredují



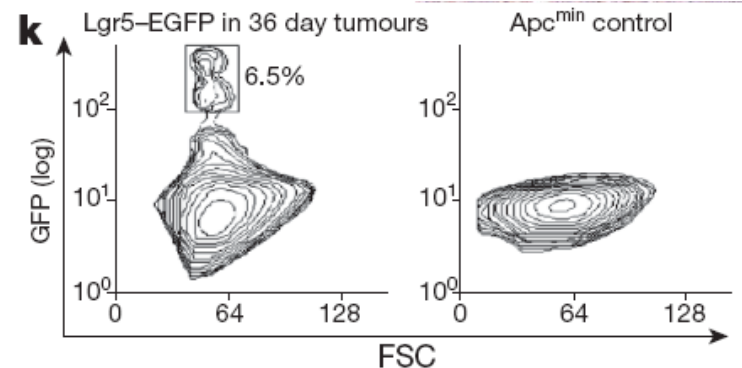
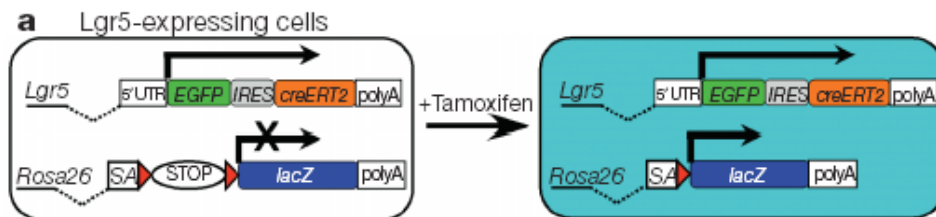
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# Nekontrolovaná aktivace kmenových buněk má fatální následky

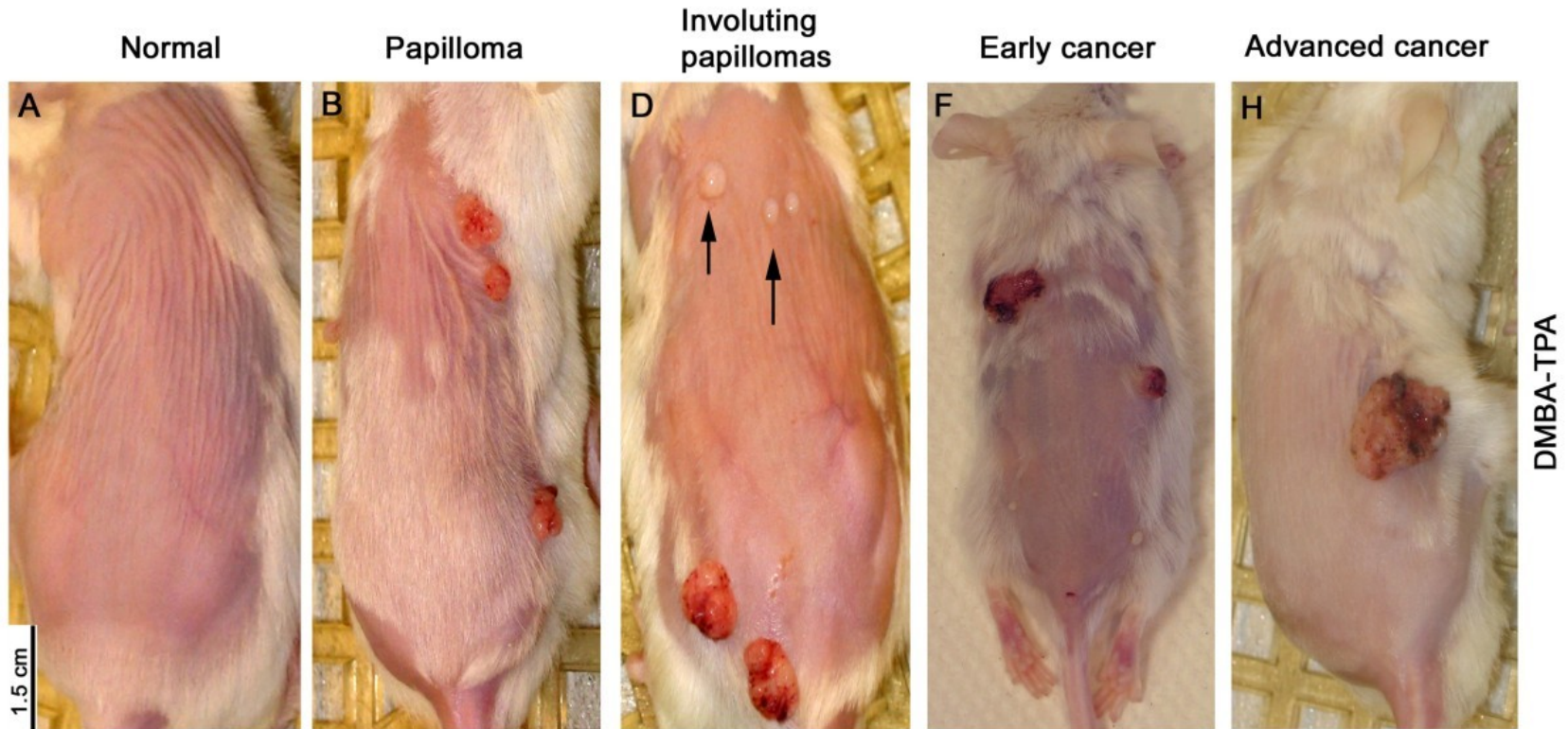


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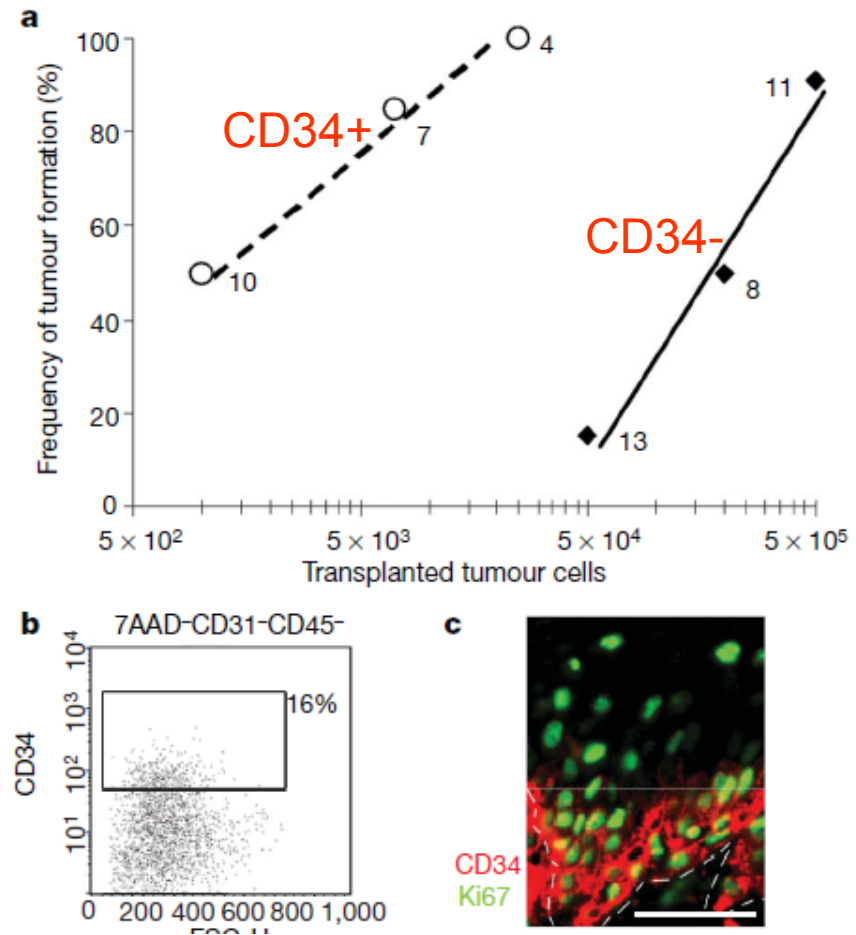


# DMBA/TPA-induced skin carcinogenesis

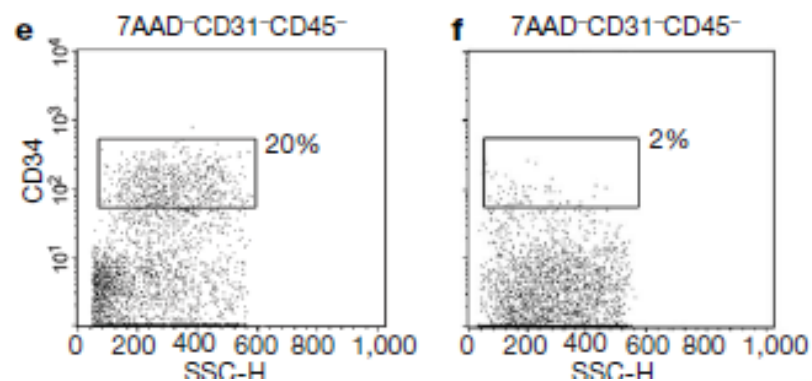
- benign papillomas, which in some cases progress into squamous cell carcinoma (SCC)



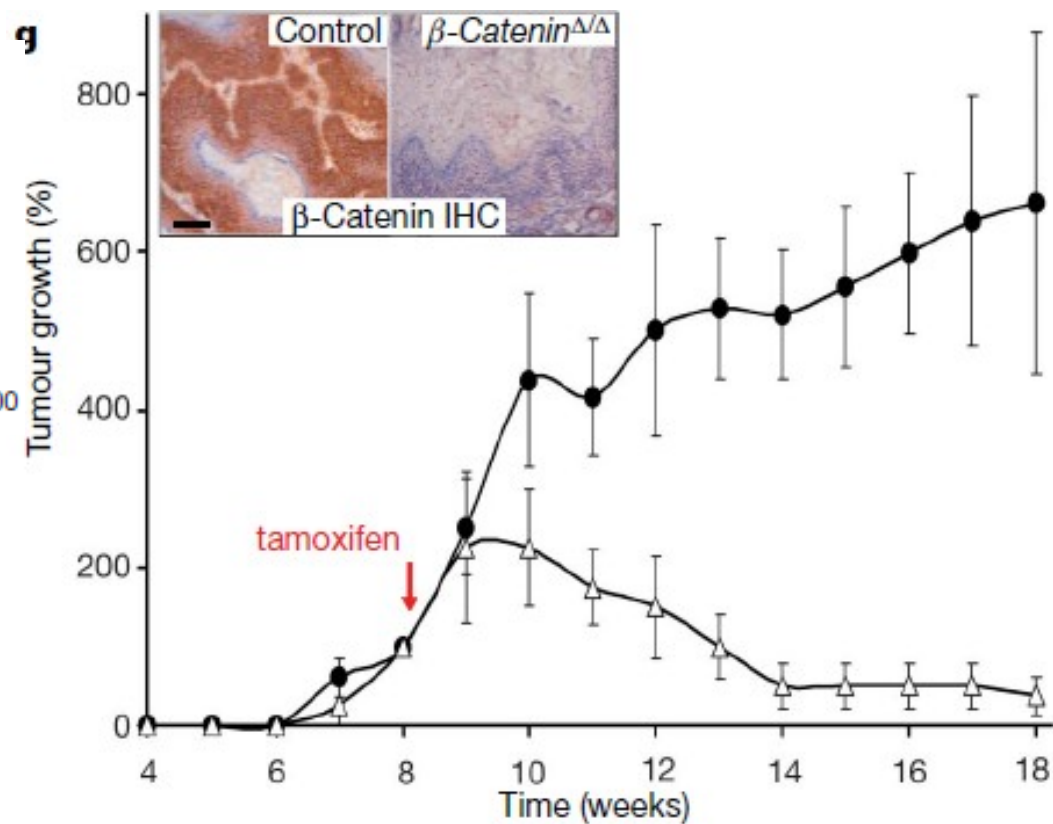
## DMBA/TPA-induced skin carcinogenesis



**Figure 2 | Cancer stem cells efficiently initiate secondary tumours that recapitulate the organization of the primary tumour. a**, Diagram summarizing the frequency of tumour formation in orthotopic tumour transplants using unsorted cells (filled diamonds) or CD34<sup>+</sup> cells (open circles) in varying amounts. The *n* value for each point is shown. **b**, Abundance of CSCs (CD34<sup>+</sup>7AAD<sup>-</sup>CD31<sup>-</sup>CD45<sup>-</sup>) in secondary tumours derived from orthotopic transplantations of CD34<sup>+</sup> cells.



characterized by abundant keratinization. **e, f**, Abundance of CD34<sup>+</sup> cells in control tumours (**e**) and  $\beta$ -catenin-deficient tumours (**f**) two weeks after induction of deletion. **g**, Tumour regression after tamoxifen-induced  $\beta$ -catenin deletion in established skin tumours ( $n = 6$ ; control (filled circles),  $K14$ -creER<sup>T2</sup>: $\beta$ -catenin<sup>+/-</sup>; mutant (open triangles),  $K14$ -creER<sup>T2</sup>: $\beta$ -catenin <sup>$\Delta/\Delta$</sup> ). Tumour numbers at 8 weeks were set to 100%. Error bars show



Malanchi et al. 2008, Nature

**Table 1 The table lists the size of the CSC population in different tumor types as determined by using the indicated markers**

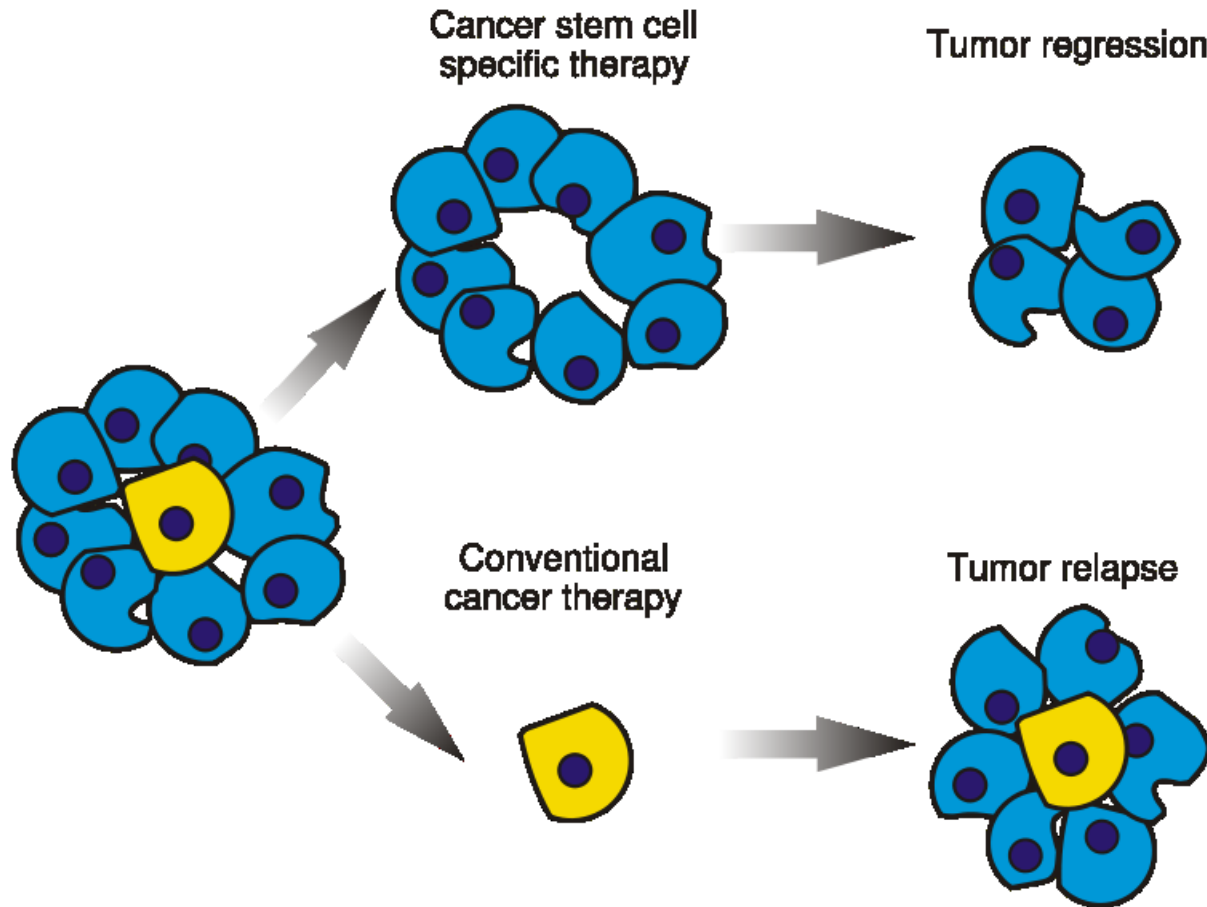
Cancer type	Reference	Stem cell marker	Abundance (%)	Transplanted cells (efficiency in %)
Acute myeloid leukemia	[1]	CD34 <sup>+</sup> CD38 <sup>-</sup>	0.02–1	100 000 (ND)
Breast	[2]	CD44 <sup>+</sup> CD24 <sup>low</sup>	11–35	5000 (100)
	[2]	CD44 <sup>+</sup> CD24 <sup>low</sup> ESA <sup>+</sup>	2.5–5	1000 (100)
	[3]	ALDH <sup>high</sup>	3–10	500 (100)
		CD44 <sup>+</sup> CD24 <sup>low</sup> ALDH <sup>high</sup>	0.9–1.2	20 (100)
Brain (medulloblastoma and glioblastoma)	[4]	CD133 <sup>+</sup>	19–29	100 (100)
Colon	[5]	CD133 <sup>+</sup>	2–24	100 (25), 500 (83)
Liver	[6]	CD90 <sup>+</sup>	0.7–6.2	5000 (50)
Lung	[7]	CD133 <sup>+</sup>	0.4–7	10 000 (ND)
Melanoma	[8]	ABC B5 <sup>+</sup>	1.6–20.4	100 000 (50)
Prostate	[9]	CD44 <sup>+</sup> $\alpha$ 2 $\beta$ 1 <sup>high</sup> CD133 <sup>+</sup>	0.1–0.3	ND
	[10]	CD44 <sup>+</sup> CD24 <sup>low</sup>	ND	100 (ND)
Pancreas	[11]	CD44 <sup>+</sup> CD24 <sup>+</sup> ESA <sup>+</sup>	0.2–0.8	100 (50) <sup>a</sup>
Head and neck SCC	[12]	CD44 <sup>+</sup>	10–35	5000–10 000 (50)
Skin SCC <sup>b</sup>	[13 <sup>**</sup> ]	CD34 <sup>+</sup>	13–20	1000 (50)

The minimal number of cells inducing secondary tumor formation upon transplantation is included, as well as the efficiency of tumor formation with this number of cells.

<sup>a</sup> Some growth from non-CSC population.

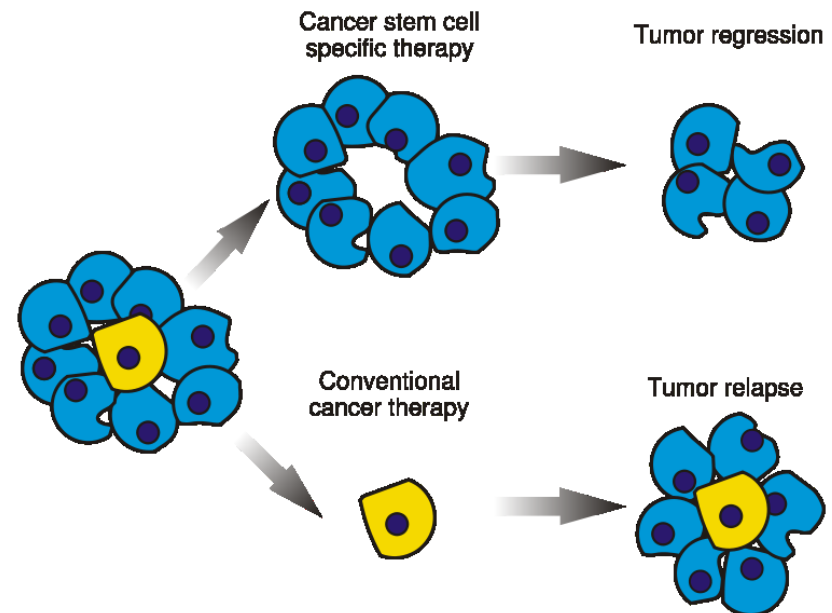
<sup>b</sup> Mouse; ND, not determined; SCC, squamous cell carcinoma.

# Cancer stem cell based therapy

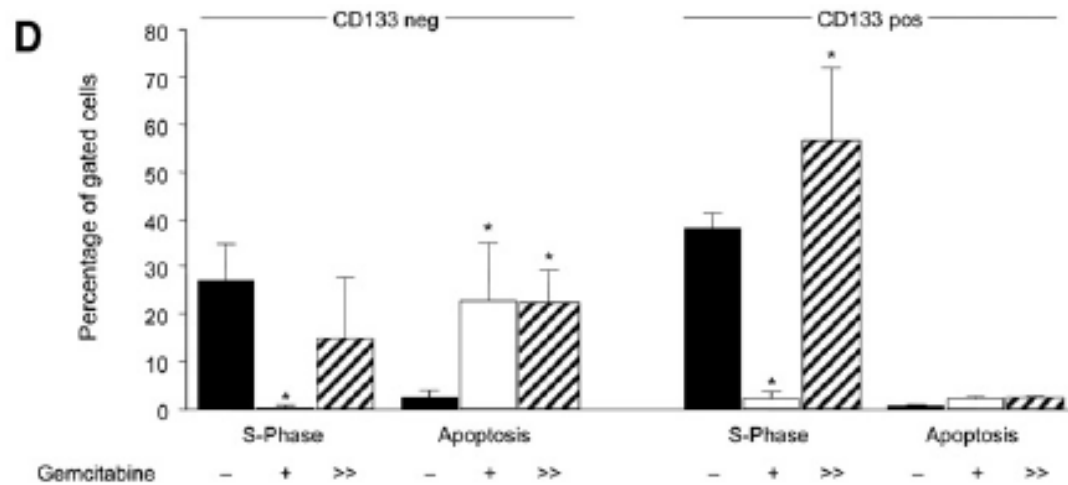
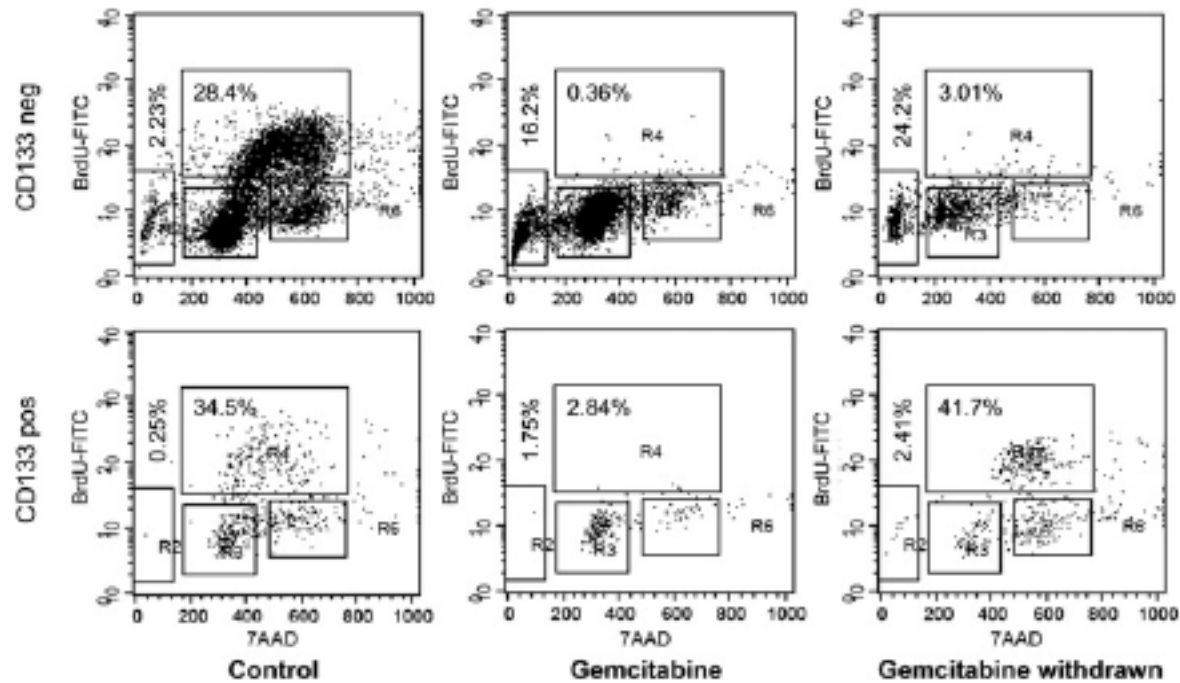
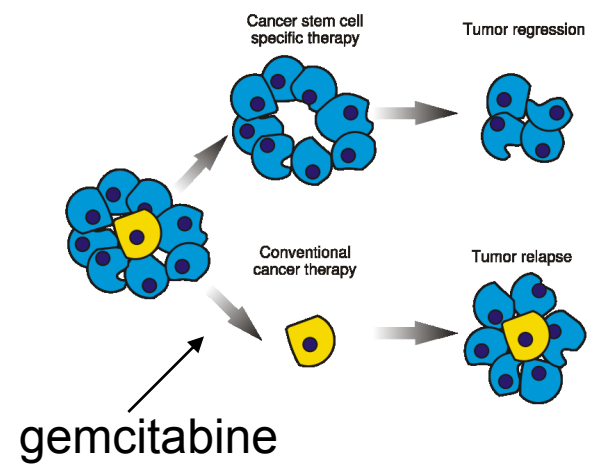


# Cancer stem cell based therapy - naděje pro nemocné s nádorem slinivky?

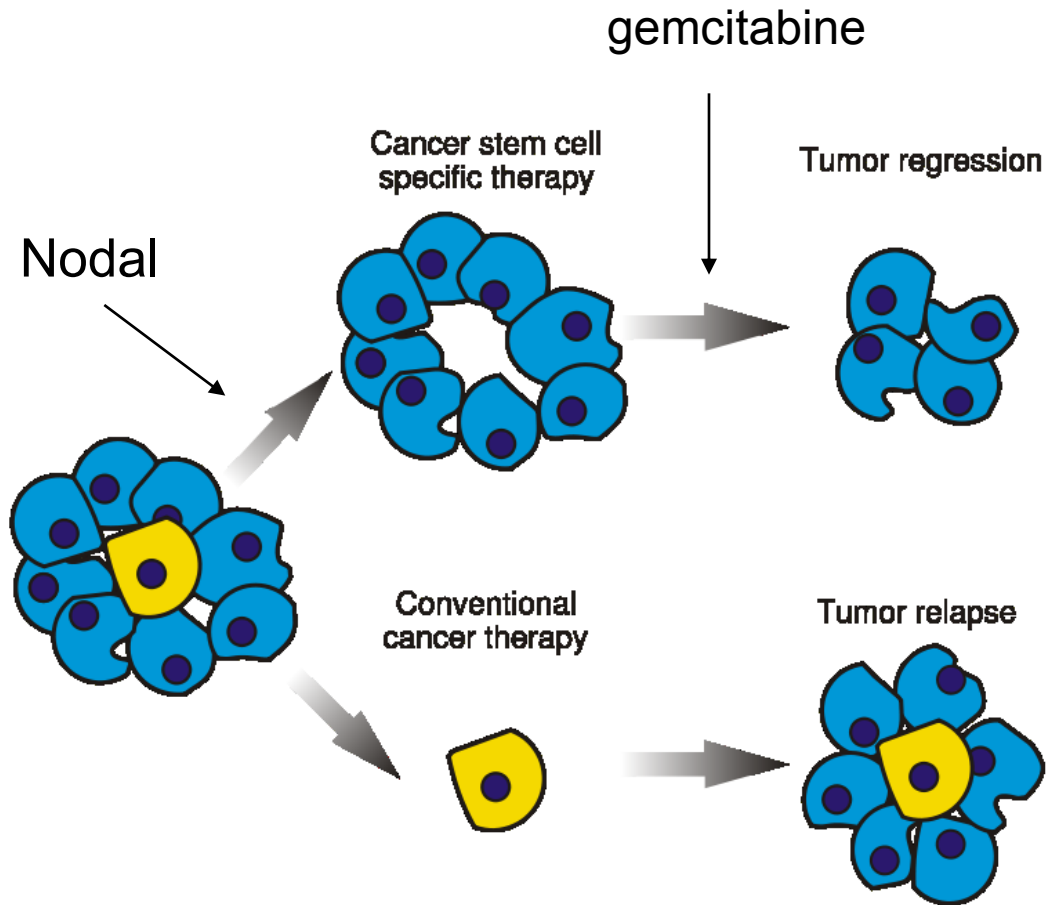
- Pancreatic adenocarcinoma - čtvrtý nejčastější důvod úmrtí u pacientů nádorových onemocnění
- Median survival - 4-6 months
- 5-year survival - 1%
- Treated with gemcitabine - does not work



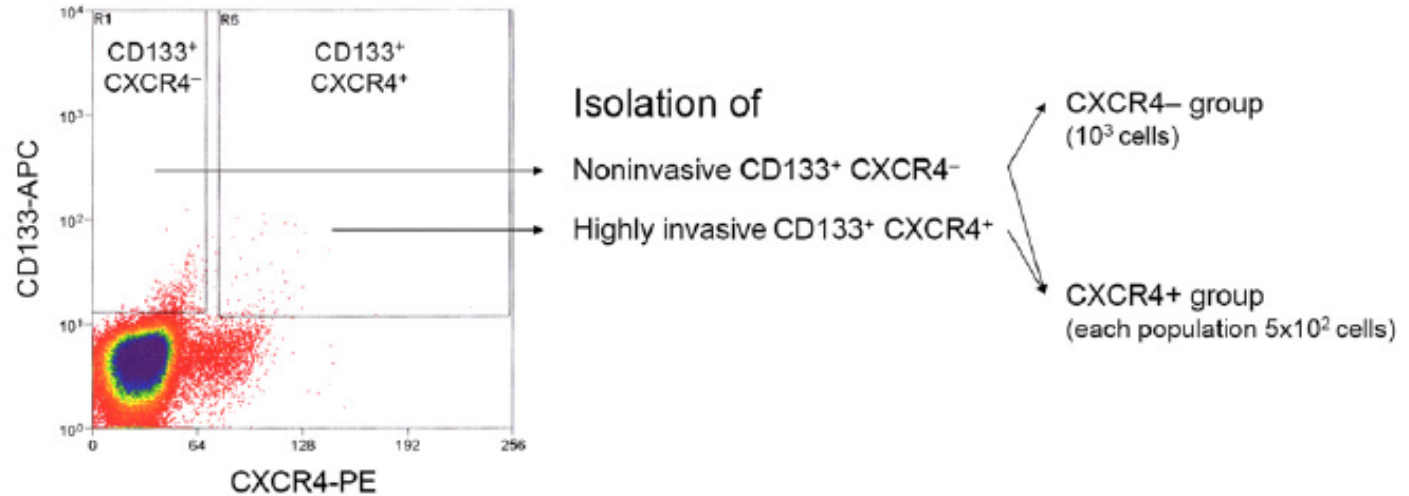




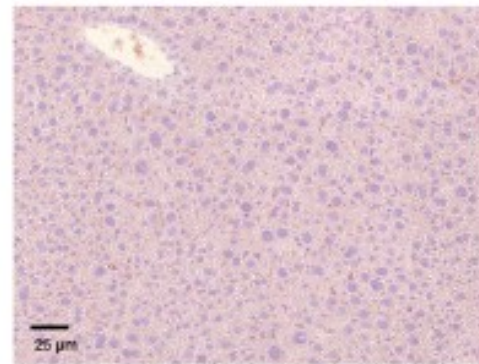
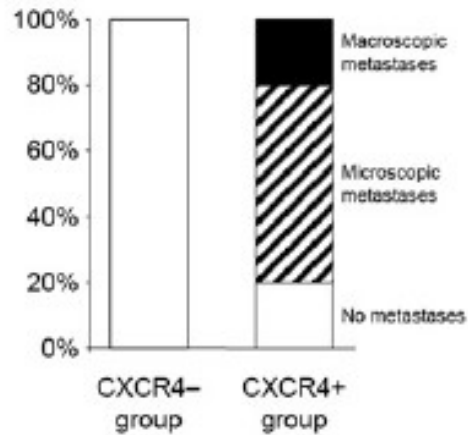
# Terapie budoucnosti?



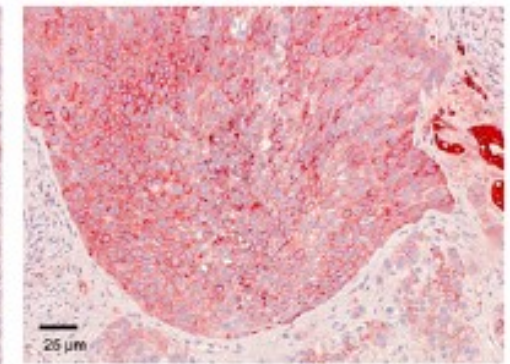
# Metastasis driving cancer stem cells



**D**



CXCR4<sup>-</sup> group



CXCR4<sup>+</sup> group

SDF1/CXCL12 - chemokin, ligand exprimovaný v plicích, játrech, kostní dřeni a lymfatických uzlinách; tj. hlavních cílech metastáz