INVAZIVITA A METASTÁZOVÁNÍ

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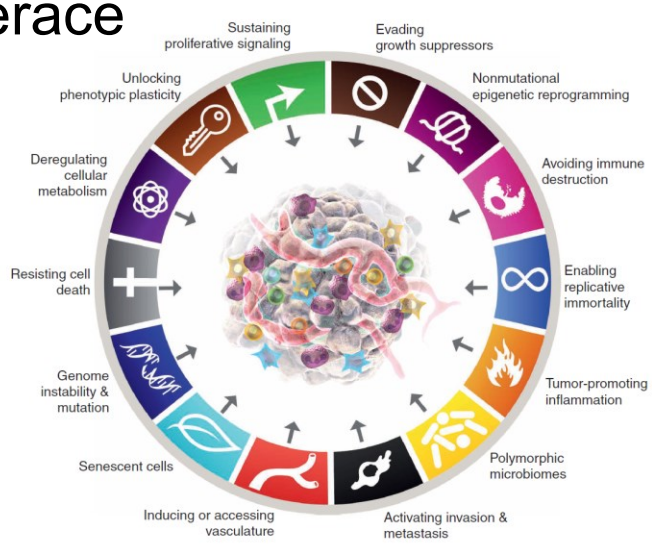
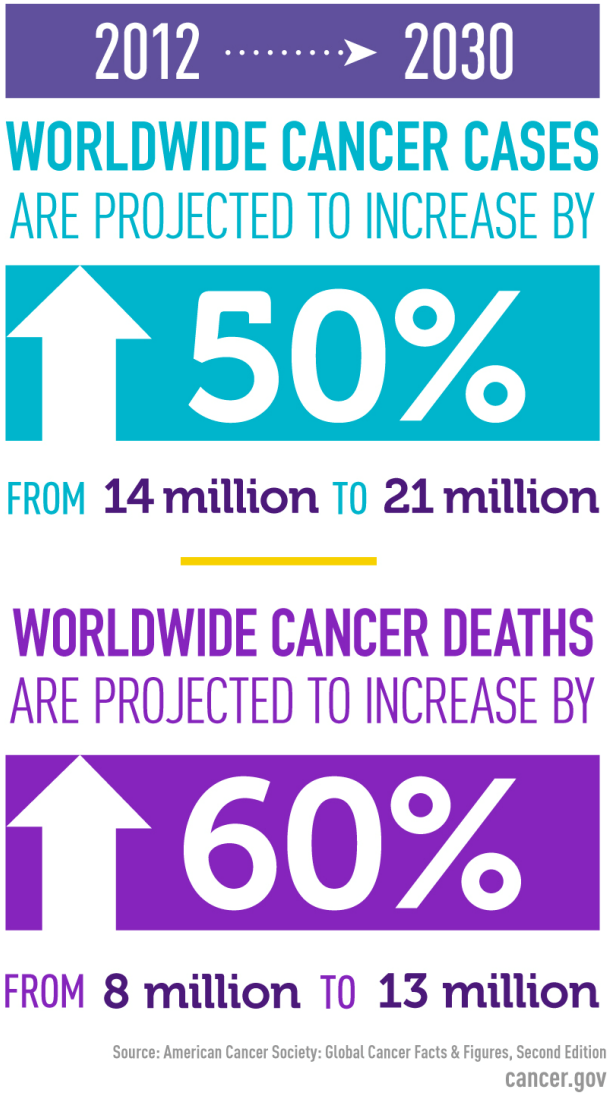
Typické znaky nádorové buňky

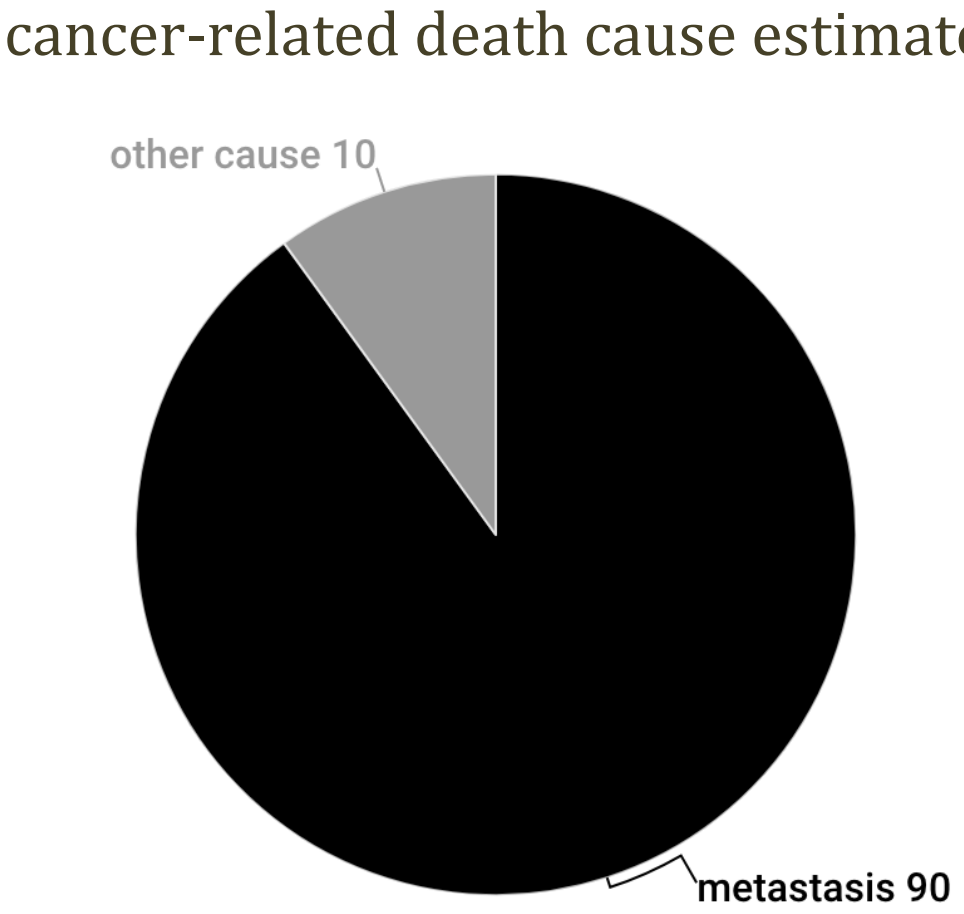
- podpůrné proliferační signály
- deregulace supresorů růstu/proliferace
- odolnost k buněčné smrti
- neomezená replikace
- neoangiogeneze
- **invaze a metastázování**
- mutace a genomická nestabilita
- zánět
- přestavba energetického metabolismu
- únik před zničením imunitním systémem
- *senescence*
- ***plasticita***
- *epigenetika*
- *mikrobiom*

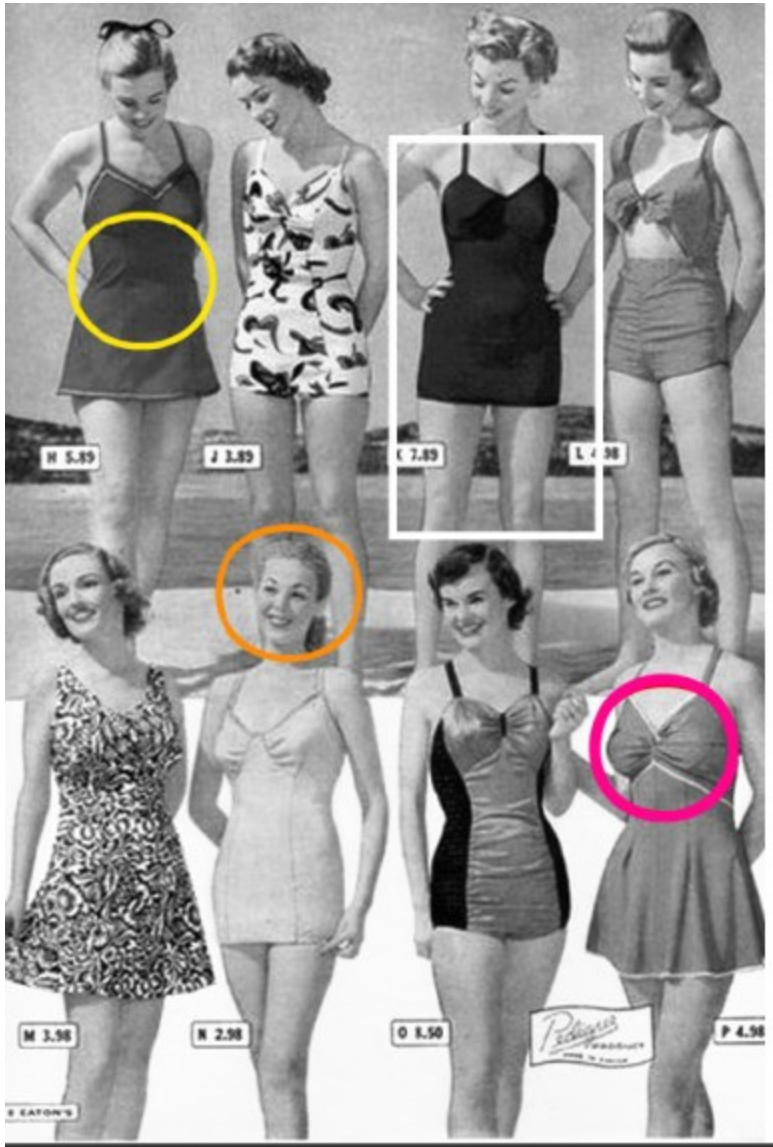


Douglas Hanahan & Robert A. Weinberg: Hallmarks of Cancer: Next Generation, Cell, 2011
Douglas Hanahan: Hallmarks of Cancer: New Dimension, Cancer Discovery, 2022

Proč je rakovina tak devastující?







MOST COMMON
BREAST CANCER
METASTASES

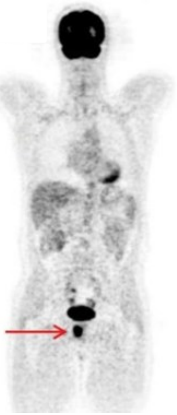
liver
bone
brain
lungs

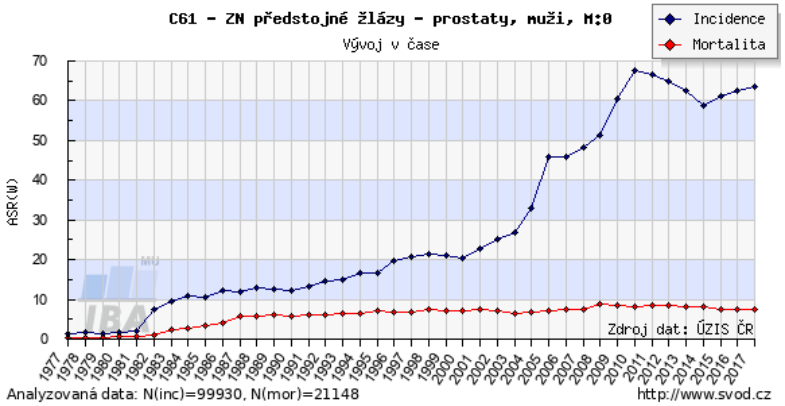
FB: AdvocatesForBreastCancer T: @BreastCancerABC

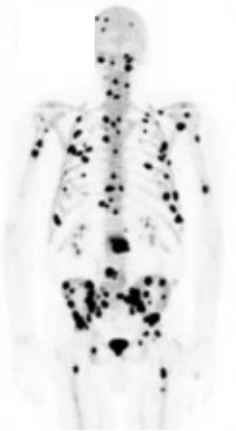
ADVOCATES FOR BREAST CANCER

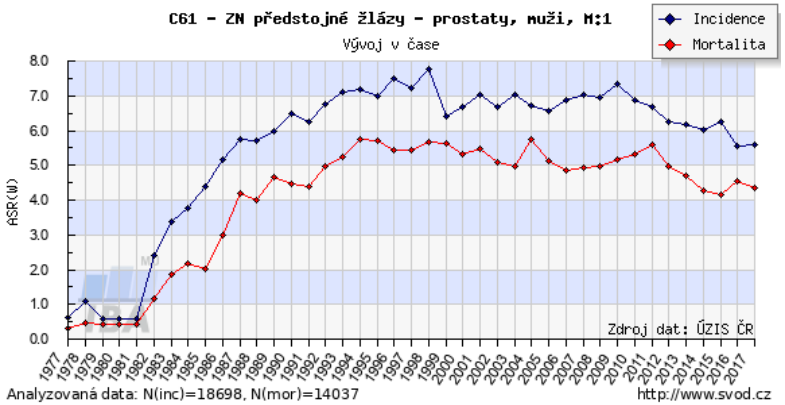
Proč je rakovina tak devastující?

Nádory prostaty

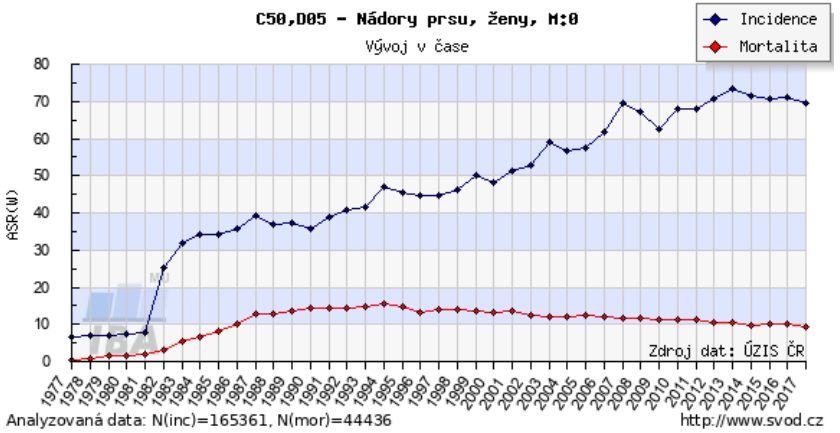


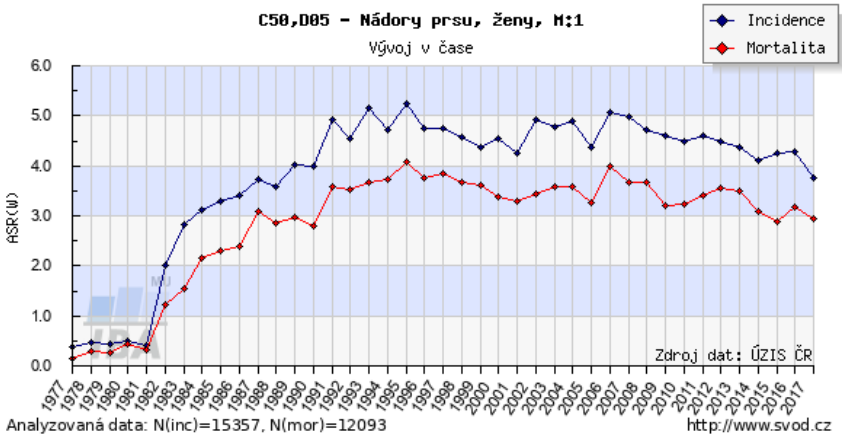






Nádory prsu



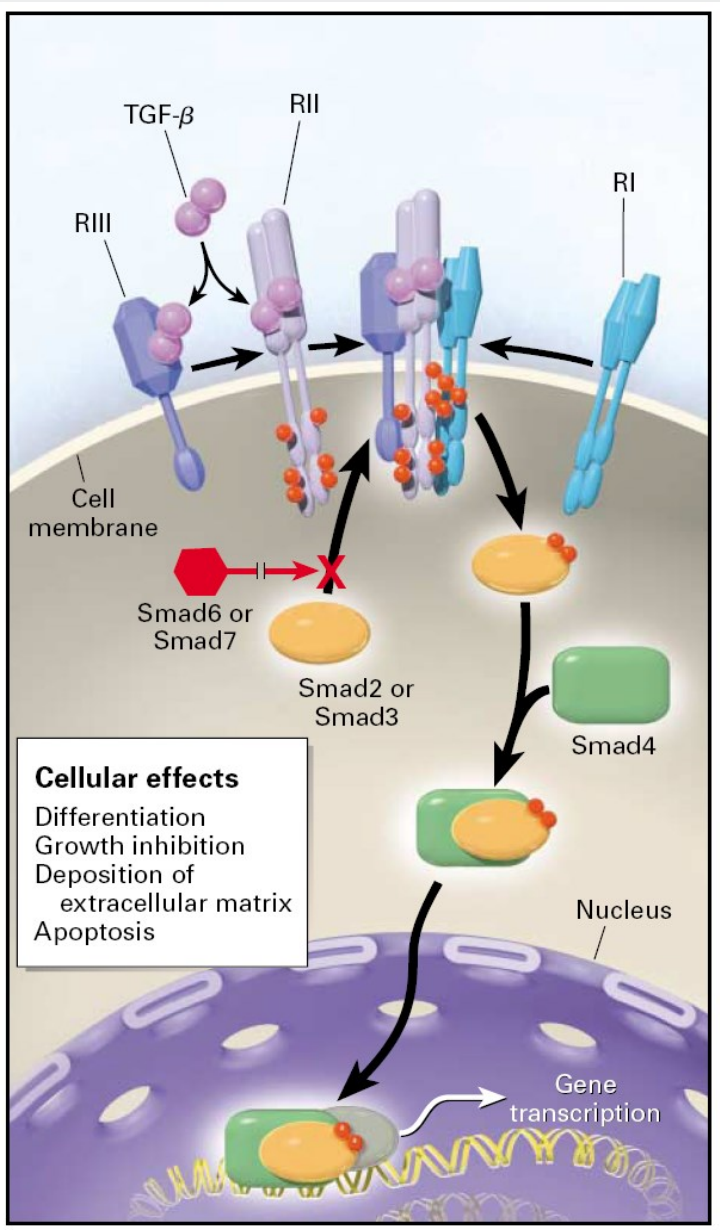


Transforming growth factor - β (TGF- β)

TGF-β rodina ~ TGF- βs, activins, bone morphogenic proteins (BMP)

TGF-β_1

- pleiotropní cytokin
- negativní regulátor



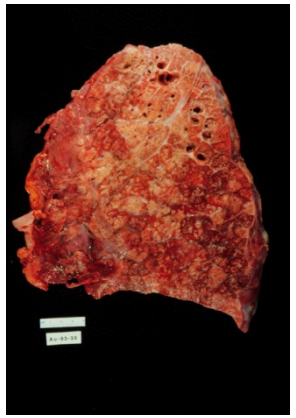
Epstein, F.H., N Engl J Med 2000; 342:1350-1358, 2000

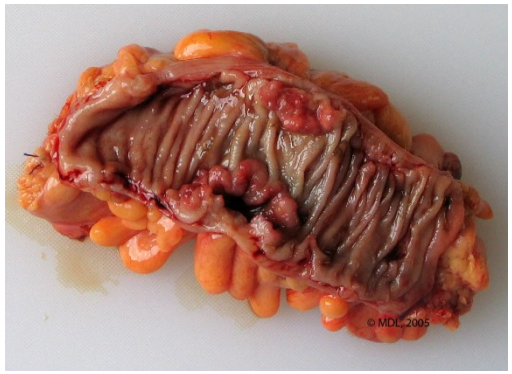
Biologické funkce TGF-β

- Hraje klíčovou úlohu během embryogeneze;
- reguluje proliferaci, diferenciaci, buněčnou smrt, motilitu, adhezi (v závislosti na buněčném typu) = ovlivňuje homeostázu;
- reguluje expresi extracelulární matrix;
  - indukuje fibrilární kolagen a fibronectin;
  - inhibuje degradaci ECM (inhibicí MMPs a indukci TIMPs).

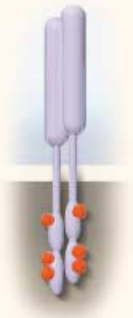
Role TGF-β v rozvoji patologických stavů

- Fibróza
  - deregulace exprese ECM prostřednictvím indukce proliferace fibroblastů a jejich myofibroblastového fenotypu.
- Nádorová onemocnění
  - ztráta citlivosti epiteliálních buněk k inhibičnímu působeni TGF-β;
  - indukce angiogeneze.



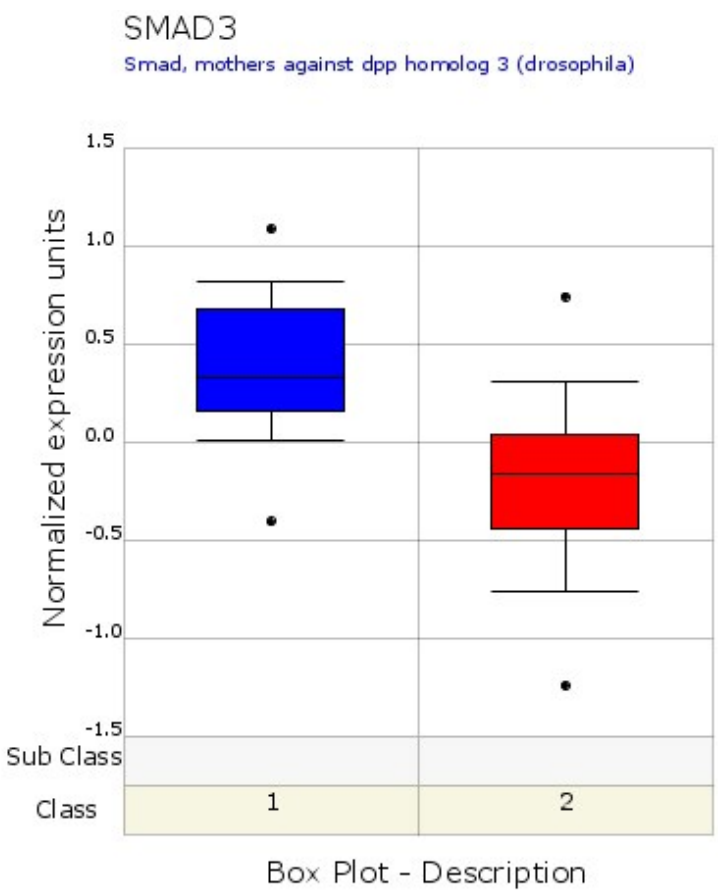


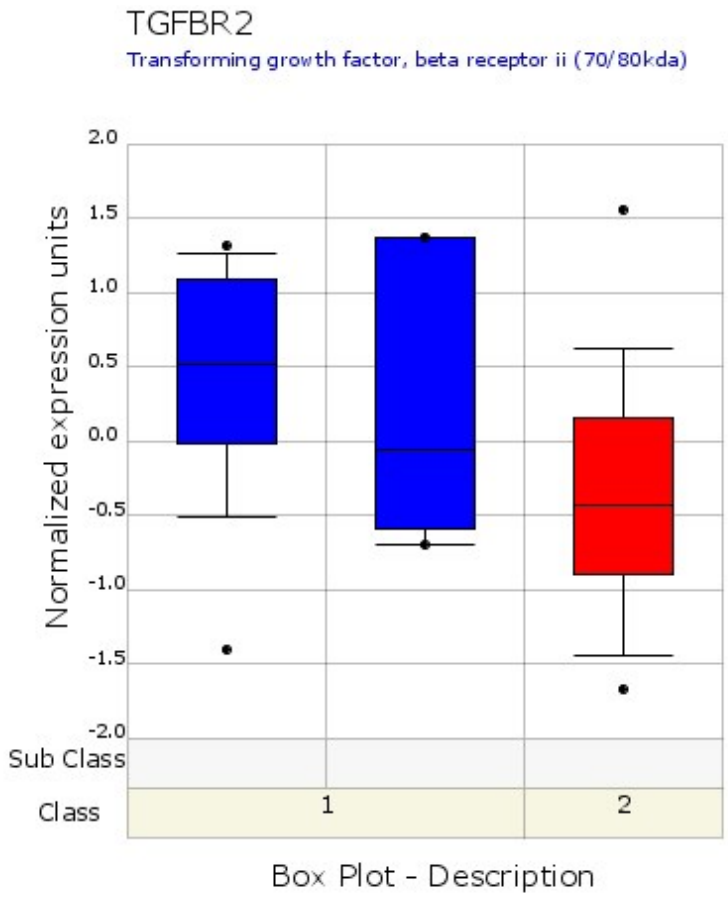
Role TGF-β v carcinogenezi

TGF-β signaling component	TGF-β	Endoglin	Type II receptors	Type I receptors	Smad2	Smad4
						
Cancers (somatic mutations)	Increased expression leads to enhanced invasion and metastasis		Colorectal (30%) Gastric (15%) Endometrial Prostate Breast Lung Hepatic Pancreatic Cervical Glioma Head and neck	Breast (16%) Pancreatic Biliary Cervical Chronic lymphocytic leukemia	Colorectal (11%) Lung (7%) Hepatocellular	Pancreatic (50%) Colorectal (30%) Lung (10%) Breast Prostate Ovarian Head and neck Esophageal Gastric Bladder Hepatocellular Renal cell
Other diseases (germ-line mutations or polymorphisms)	Fibrosis Hypertension Osteoporosis Atherosclerosis	Hereditary hemorrhagic telangiectasia	Atherosclerosis			Familial juvenile polyposis

NEJM 342, **18** (2000) 1350-1358

Role TGF-β v carcinogenezi

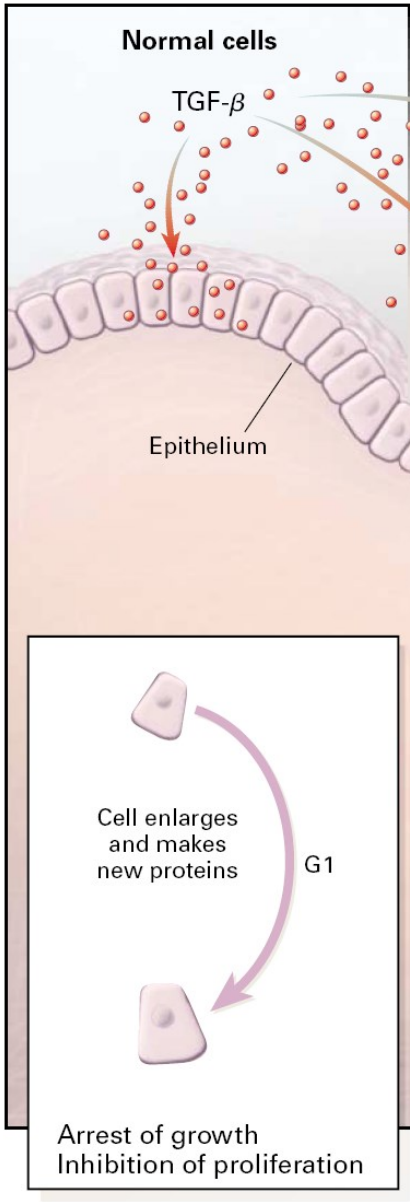




Prostate – normal vs. cancer

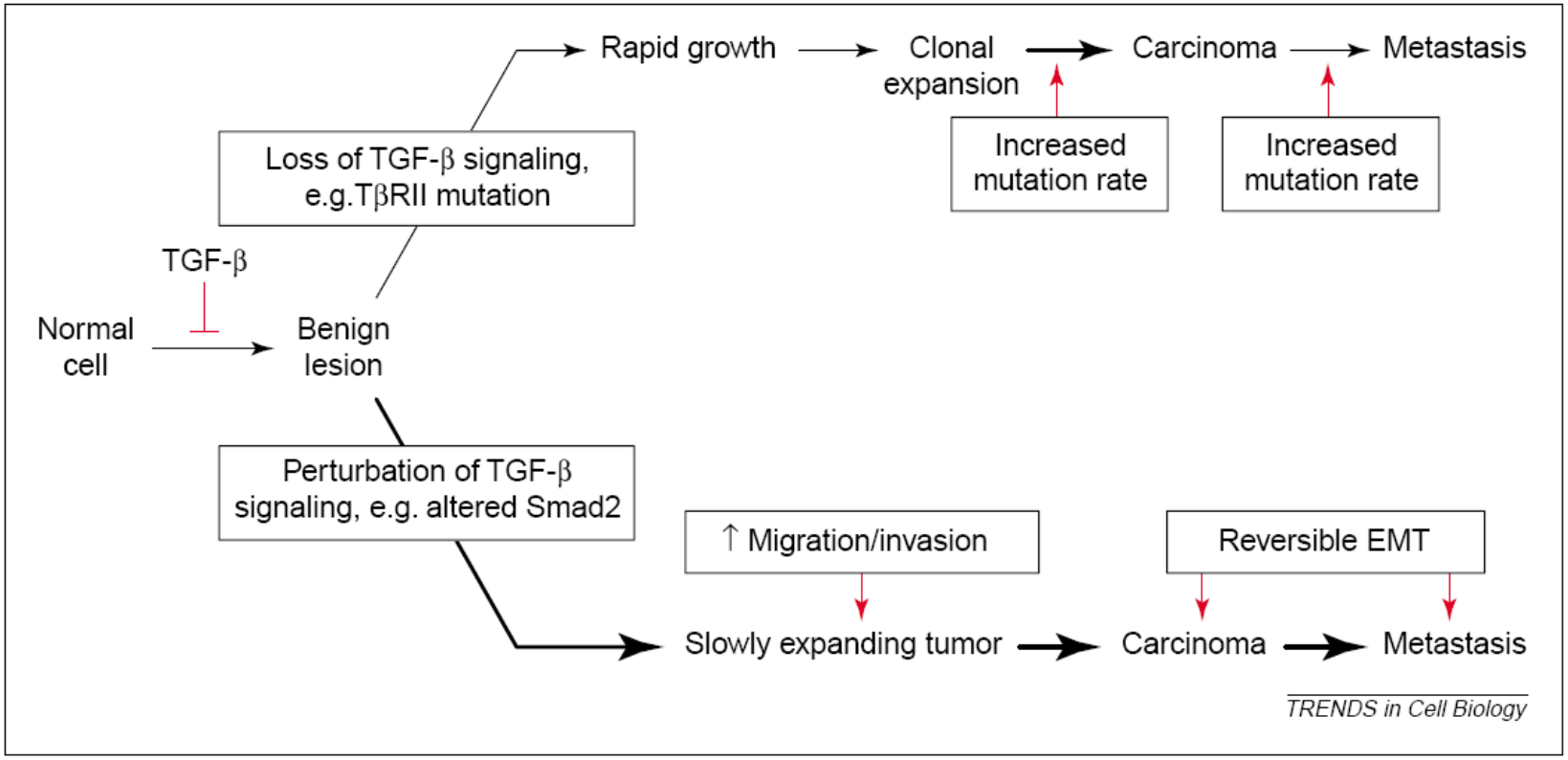
normal, hyperplasia vs. cancer

Role TGF-β v carcinogenezi



NEJM 342, **18** (2000) 1350-1358

Role TGF-β v carcinogenezi



TRENDS in Cell Biology Vol.11 No.11 2001

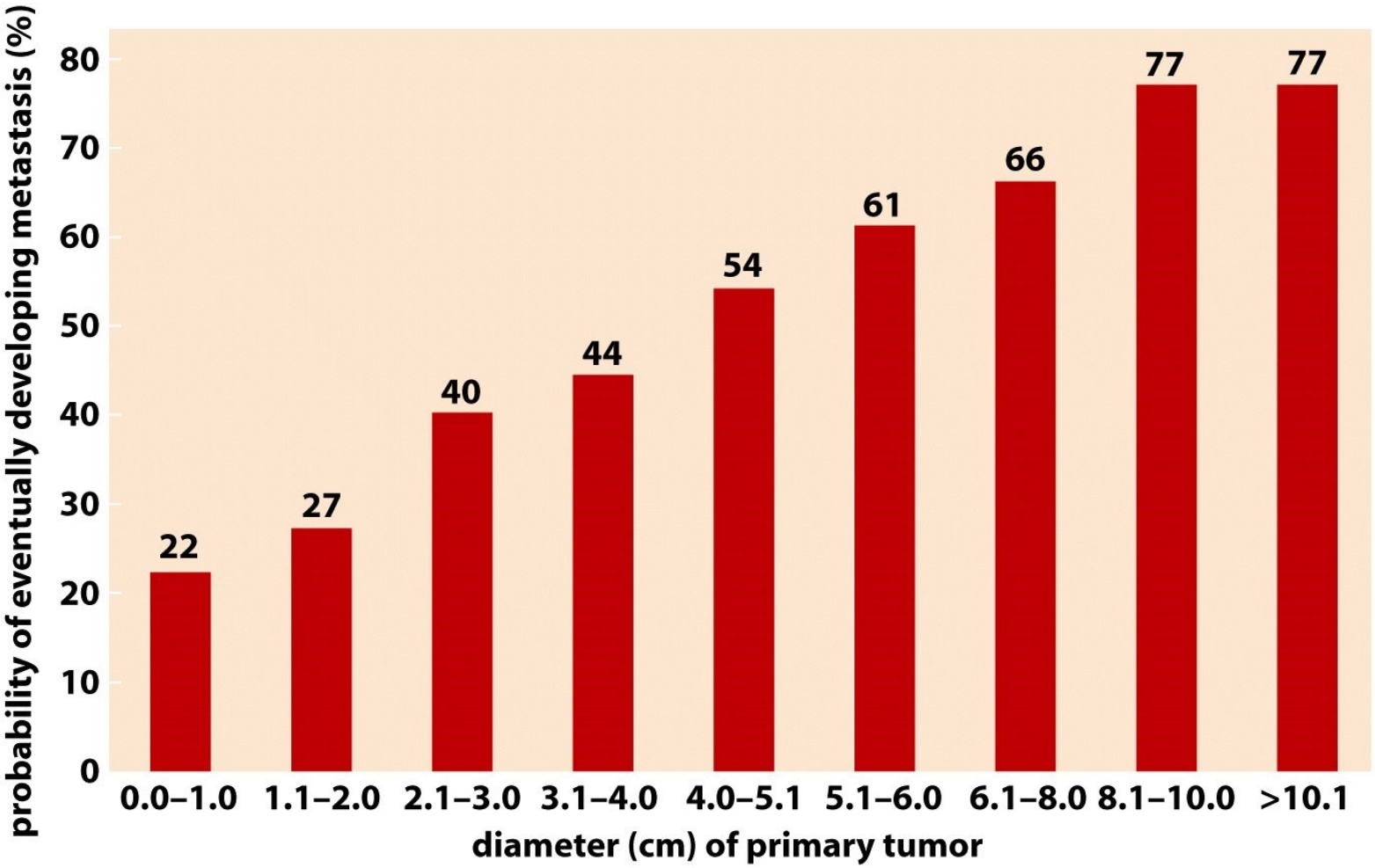


Figure 14.3 *The Biology of Cancer* (© Garland Science 2007)

Mechanismy karcinogeneze

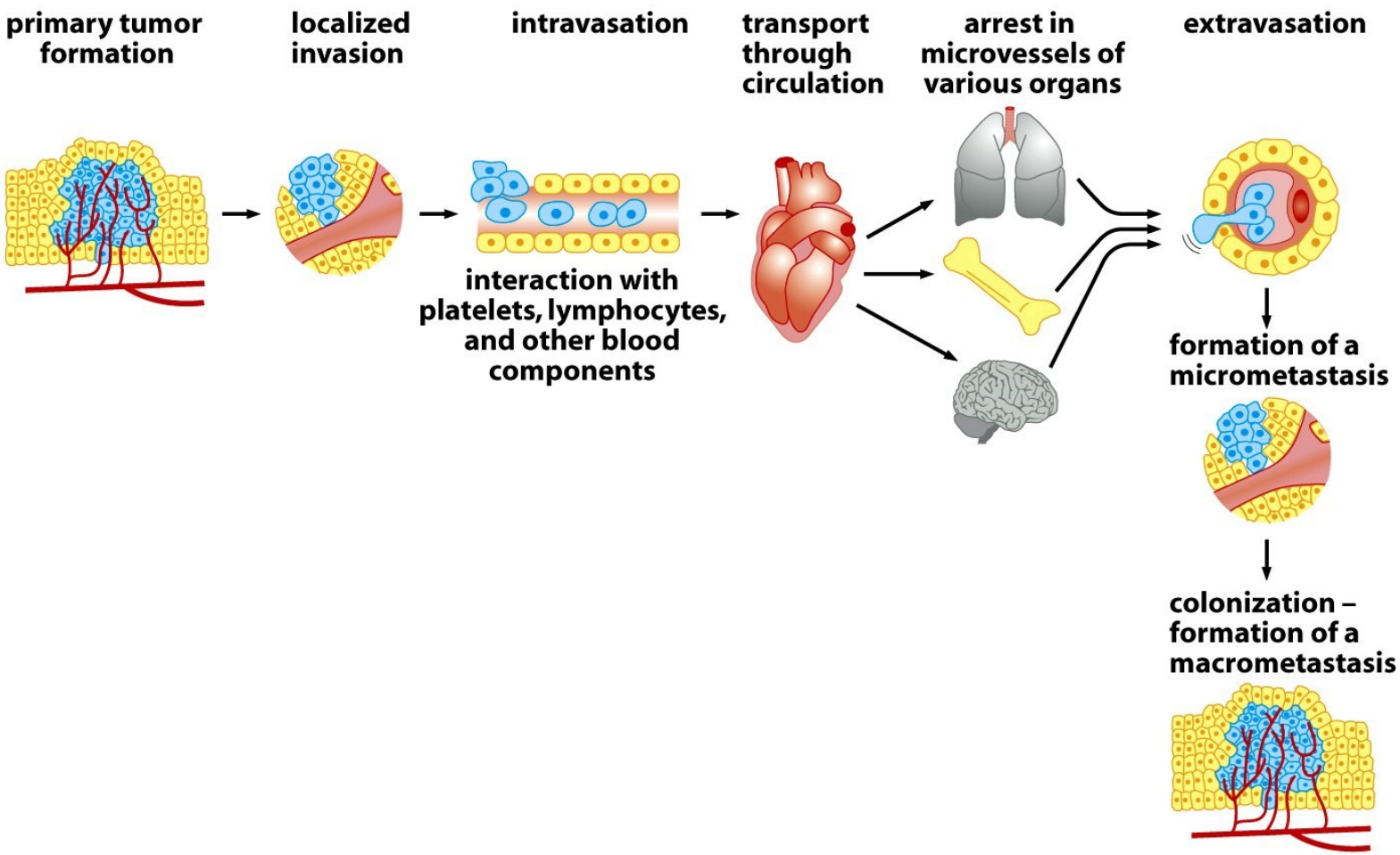


Figure 14.4 *The Biology of Cancer* (© Garland Science 2007)

Mechanismy karcinogeneze

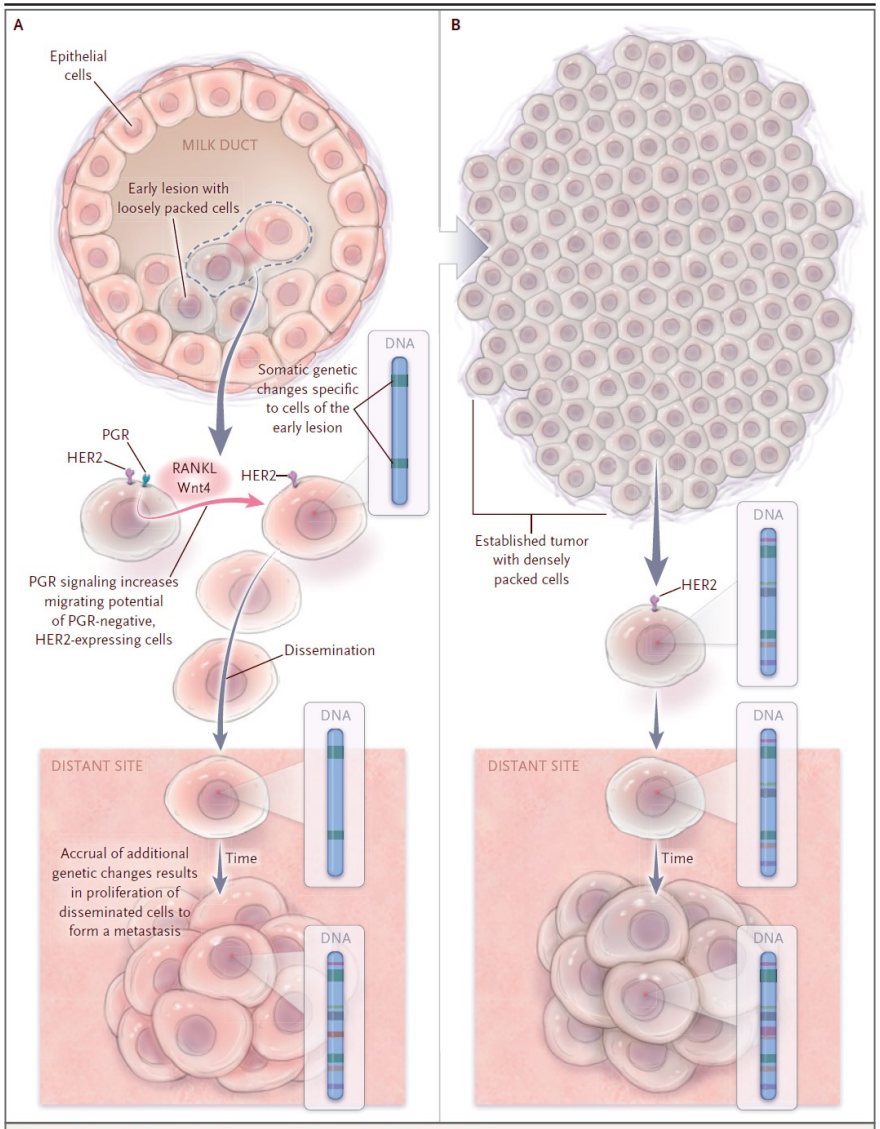


Figure 1. A Model of Parallel Progression after Early Dissemination.

Recent experiments with a mouse model of human epidermal growth factor receptor 2 (HER2)–positive breast cancer[1,2] have uncovered events critical to the dissemination of cancer cells from the early lesion (Panel A) and cells of the established tumor (Panel B). The cells in the early lesion are loosely arranged; some of them express the progesterone receptor (PGR) in addition to HER2. These PGR-positive cells secrete soluble factors, such as receptor activator of nuclear factor-$\kappa\beta$ ligand (RANKL) and Wnt4, that induce the migration of PGR-negative cells from the lesion. The cells in the established tumor (Panel B) are packed more tightly, typically do not express the PGR, and are less likely to metastasize. The cells that disseminate from established tumors to the bone marrow carry many of the genetic variants, such as loss of chromosome 8p, that are found in established tumors, whereas those that disseminate from early lesions do not and instead evolve in parallel with the primary tumor and other disseminated cells.

Lokální invaze a intravazace

- Invaze do cév, závislá na degradaci ECM
- Závislá na proteázové aktivitě
- Nádorové buňky mohou produkovat své vlastní proteázy (MMP-2, -9) nebo kooptují stromální buňky a využívají jejich aktivity

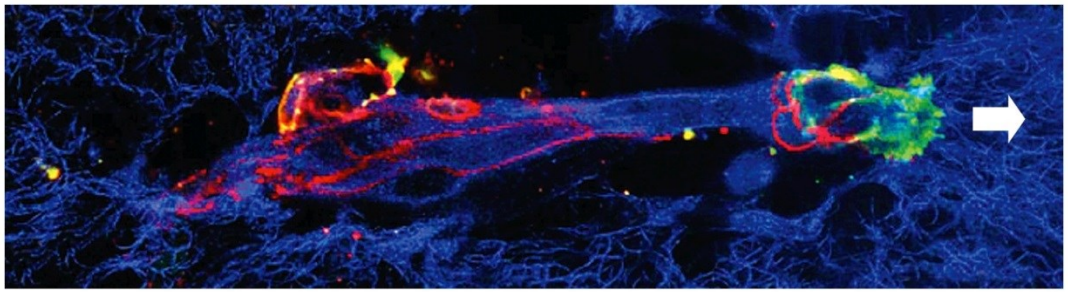


Figure 14.6a The Biology of Cancer (© Garland Science 2014)

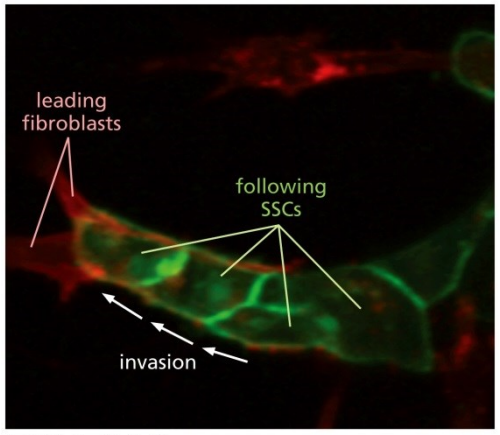


Figure 14.6b The Biology of Cancer (© Garland Science 2014)

Tumor cells, fibroblasts

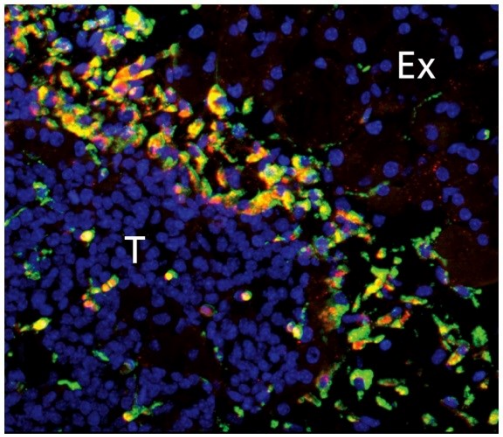


Figure 14.6c The Biology of Cancer (© Garland Science 2014)

Macrophages, cathepsin

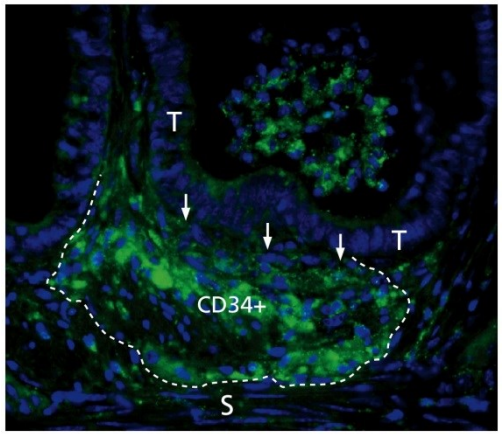


Figure 14.6d The Biology of Cancer (© Garland Science 2014)

Immature myeloid cells

Pohyb buněk

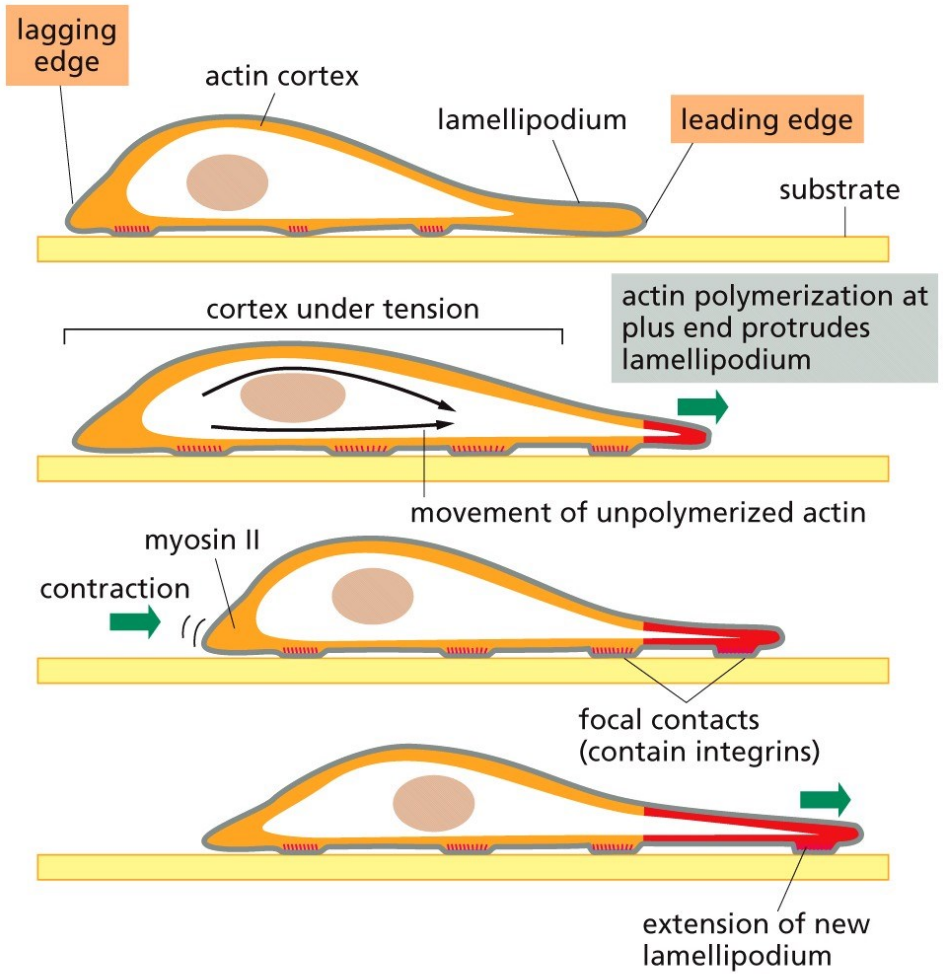


Figure 14.36 The Biology of Cancer (© Garland Science 2014)

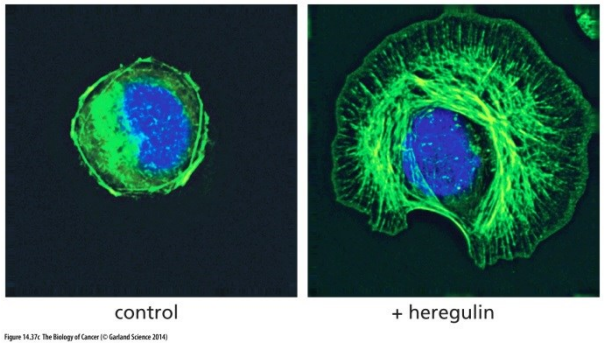


Figure 14.37: The Biology of Cancer (© Garland Science 2014)

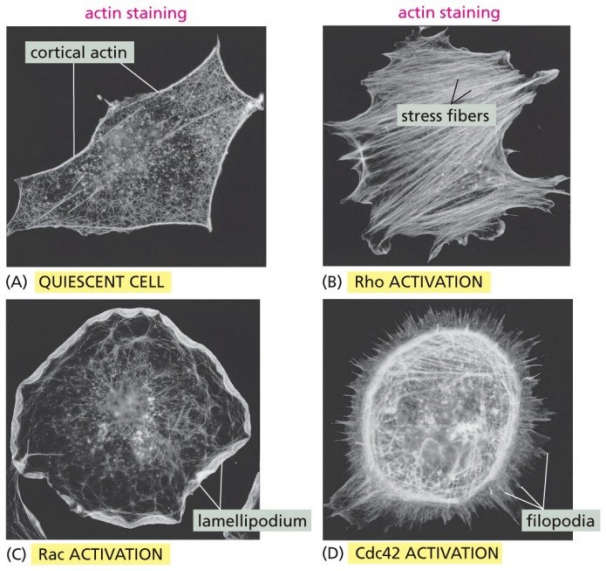


Figure 14.39 The Biology of Cancer (© Garland Science 2014)

Pohyb buněk

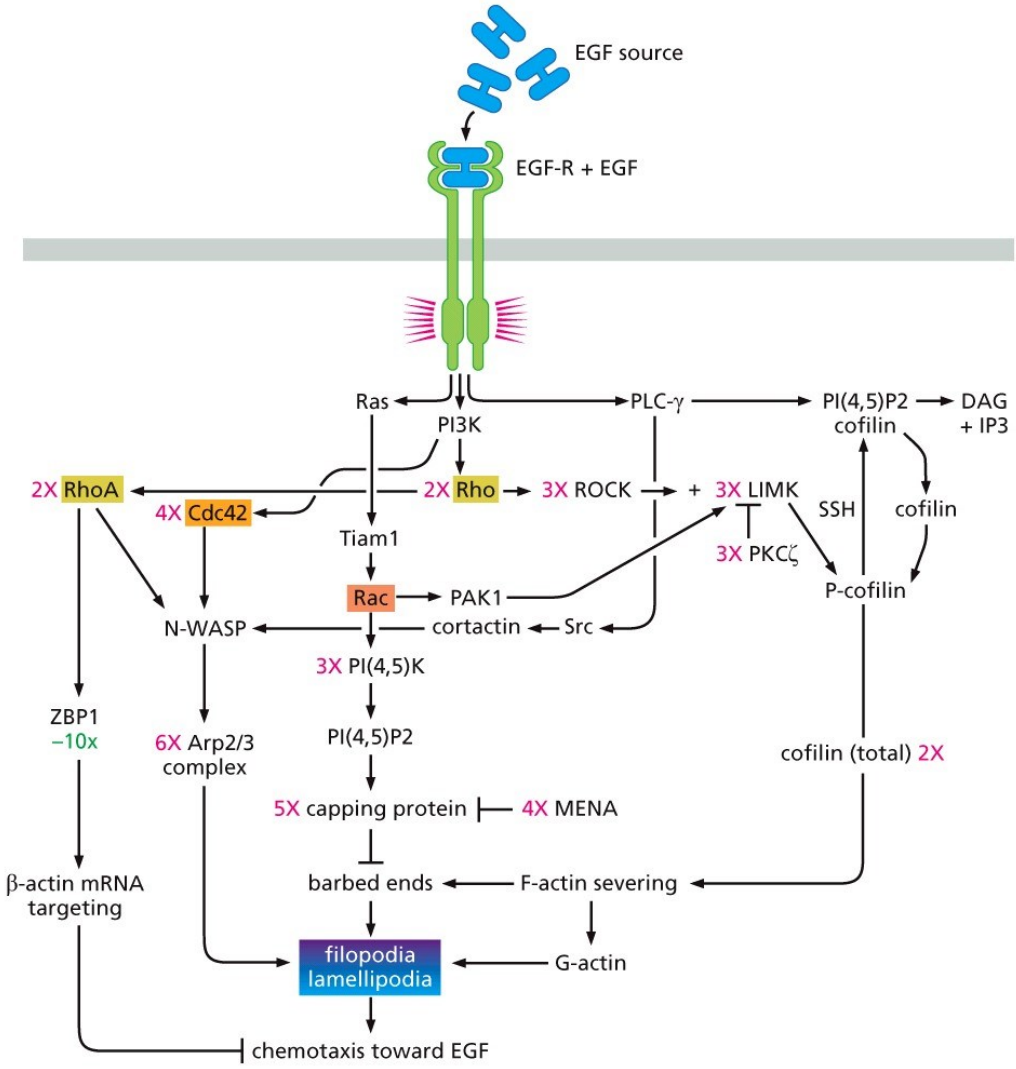


Figure 14.40 The Biology of Cancer (© Garland Science 2014)

Transport, cirkulace

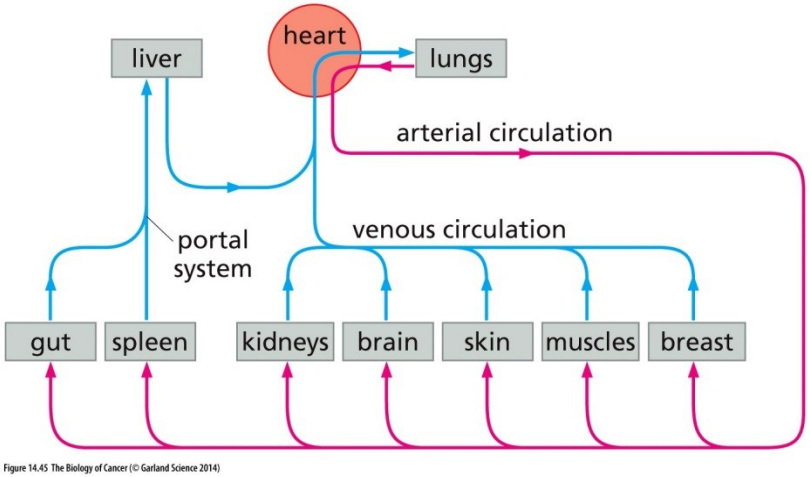
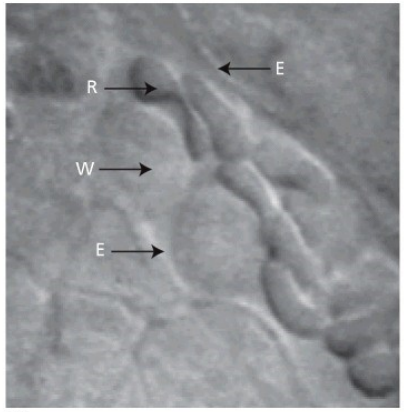
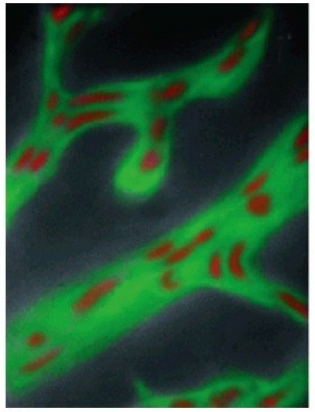


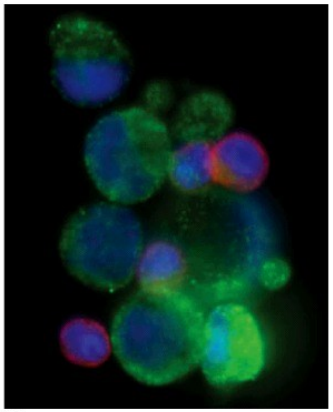
Figure 14.45 The Biology of Cancer (© Garland Science 2014)



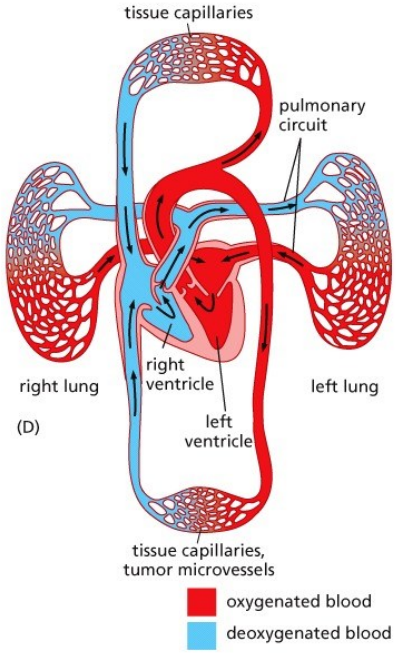
(A)



(B)



(C)

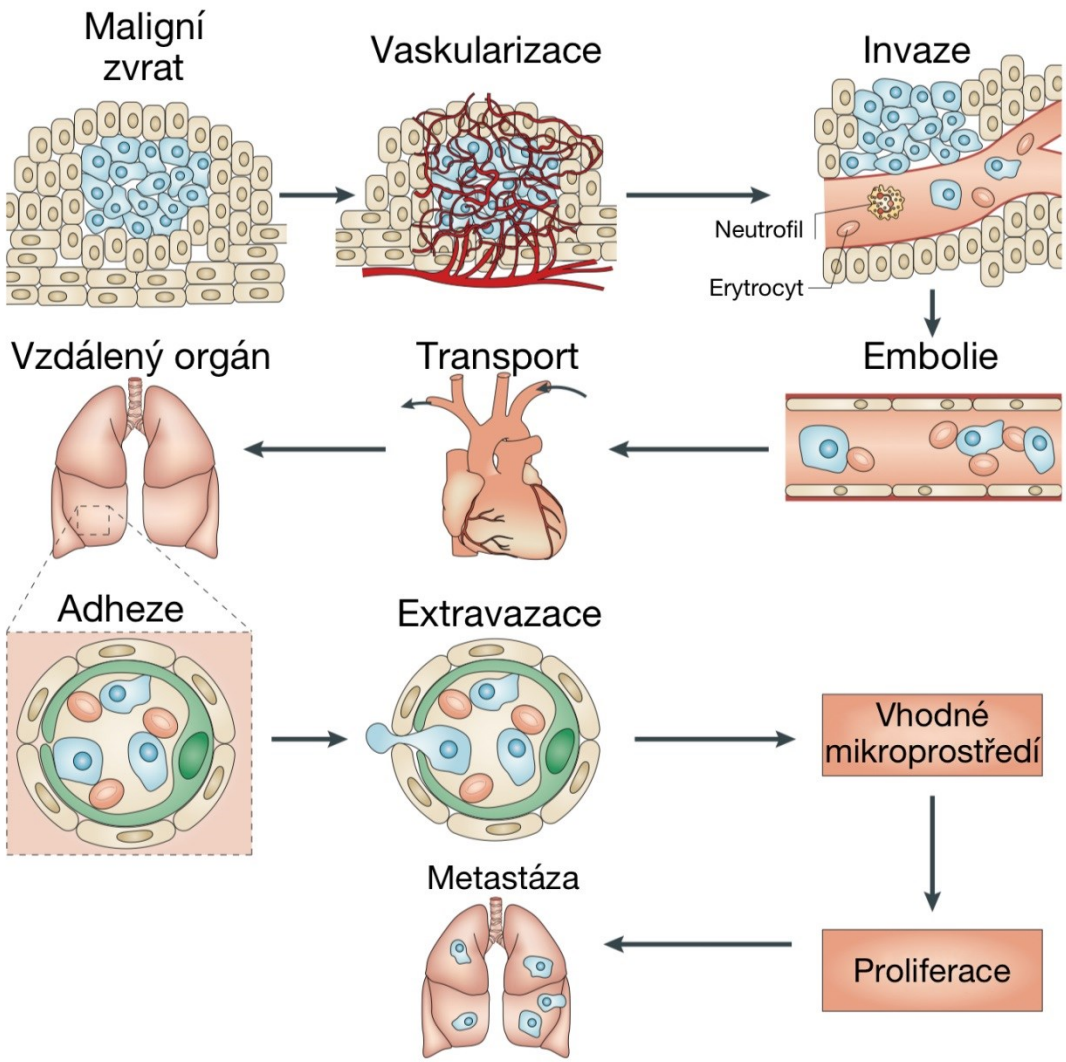


(D)

Figure 14.8 The Biology of Cancer (© Garland Science 2014)

Metastatická kaskáda

Cirkulující nádorové buňky (CNB) – klíčová úloha



Francia et al., *Nat. Rev. Cancer* (2011)

Deletion of *Nalcn (Sodium leak channel, non-selective)* from mice that lacked oncogenic mutations and never developed cancer caused shedding of epithelial cells into the blood at levels equivalent to those seen in tumor-bearing animals. These cells trafficked to distant organs to form normal structures including lung epithelium, and kidney glomeruli and tubules. Thus, NALCN regulates cell shedding from solid tissues independent of cancer, divorcing this process from tumorigenesis and unmasking a potential new target for antimetastatic therapies.

nature genetics

ARTICLES

https://doi.org/10.1038/s41588-022-01182-0

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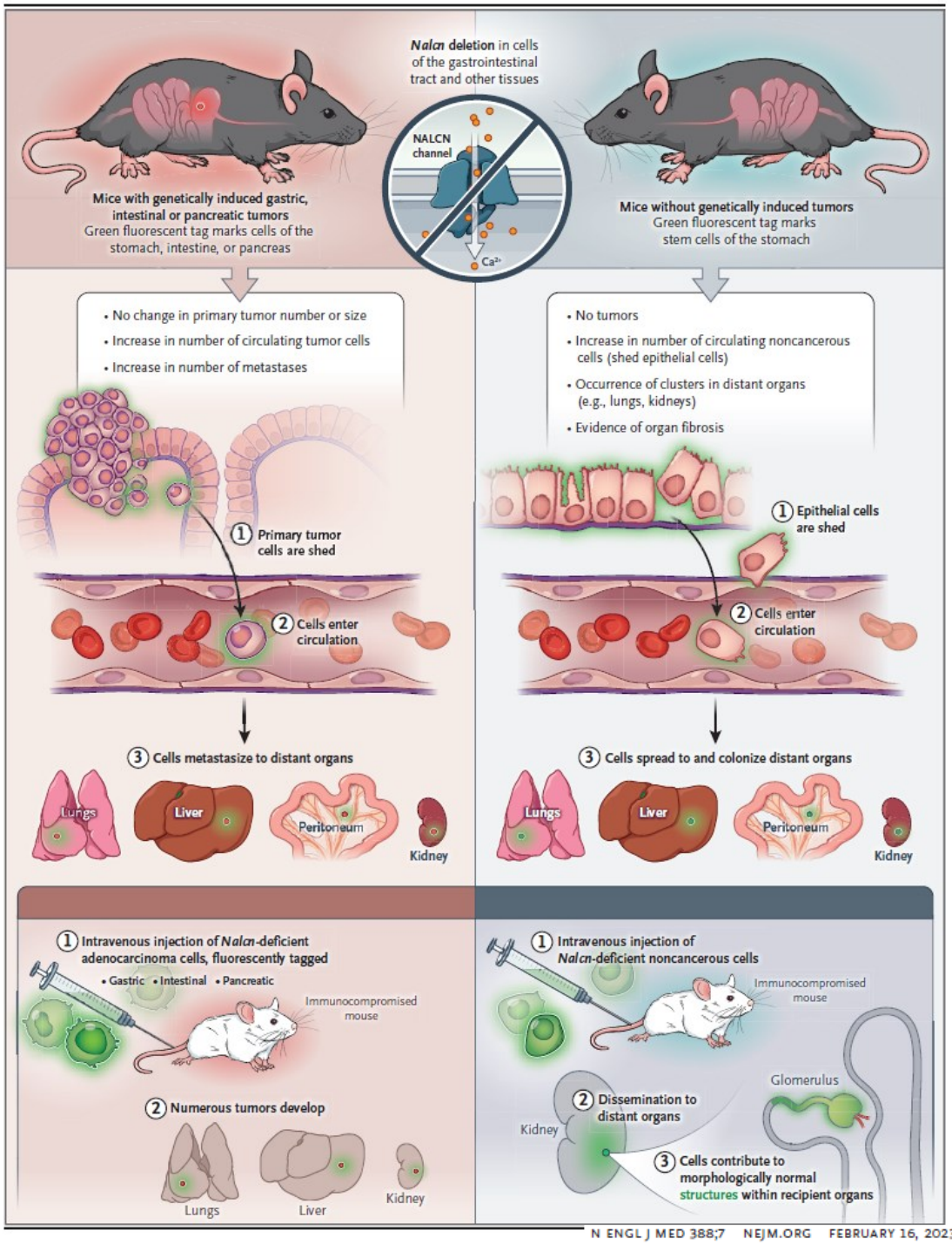
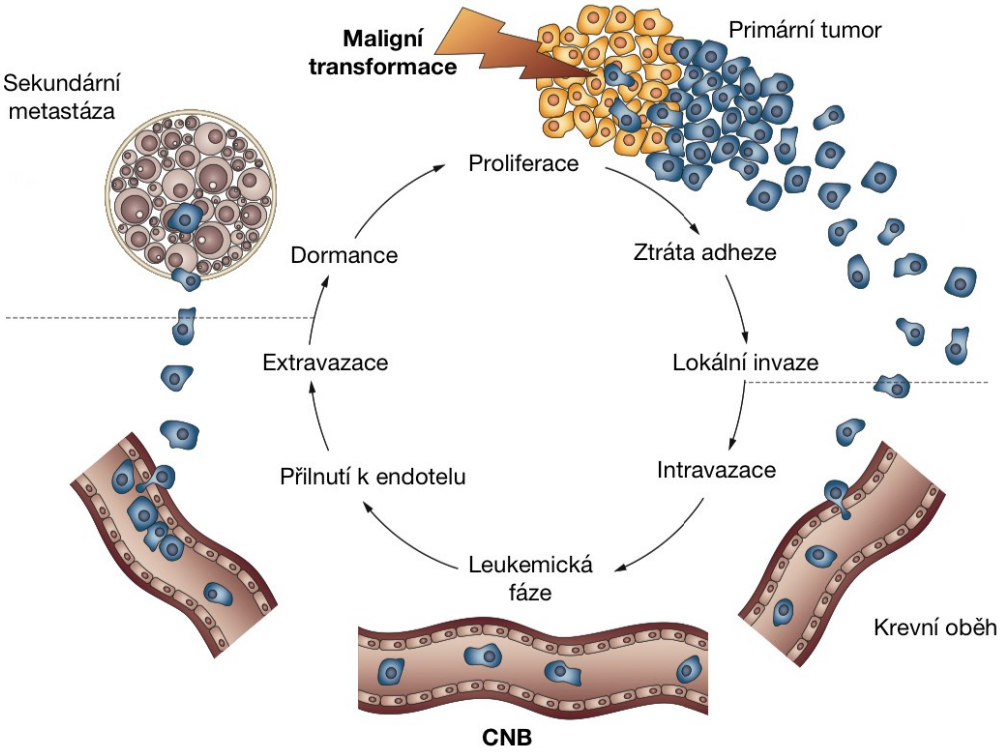
The NALCN channel regulates metastasis and nonmalignant cell dissemination

Eric P. Rahrmann[1], David Shorthouse[2], Amir Jassim[1], Linda P. Hu[1], Mariaestela Ortiz[3], Betania Mahler-Araujo[4], Peter Vogel[5], Marta Paez-Ribes[1], Atefeh Fatemi[1], Gregory J. Hannon[1], Radhika Iyer[6], Jay A. Blundon[7], Filipe C. Lourenço[1], Jonathan Kay[8], Rosalynn M. Nazarian[9], Benjamin A. Hall[2], Stanislav S. Zakharenko[7], Douglas J. Winton[1], Liqin Zhu[10] and Richard J. Gilbertson[1,11]



Proč se cirkulujícími nádorovými buňkami zabývat?

- 90% úmrtí spojených se solidními nádory – **metastáze**
  - Šíření primárně krví
- Klinicky významné
  - „Liquid biopsy“
  - Průběh terapie
  - Prognostický znak
  - Specifické mutace → cíle terapie



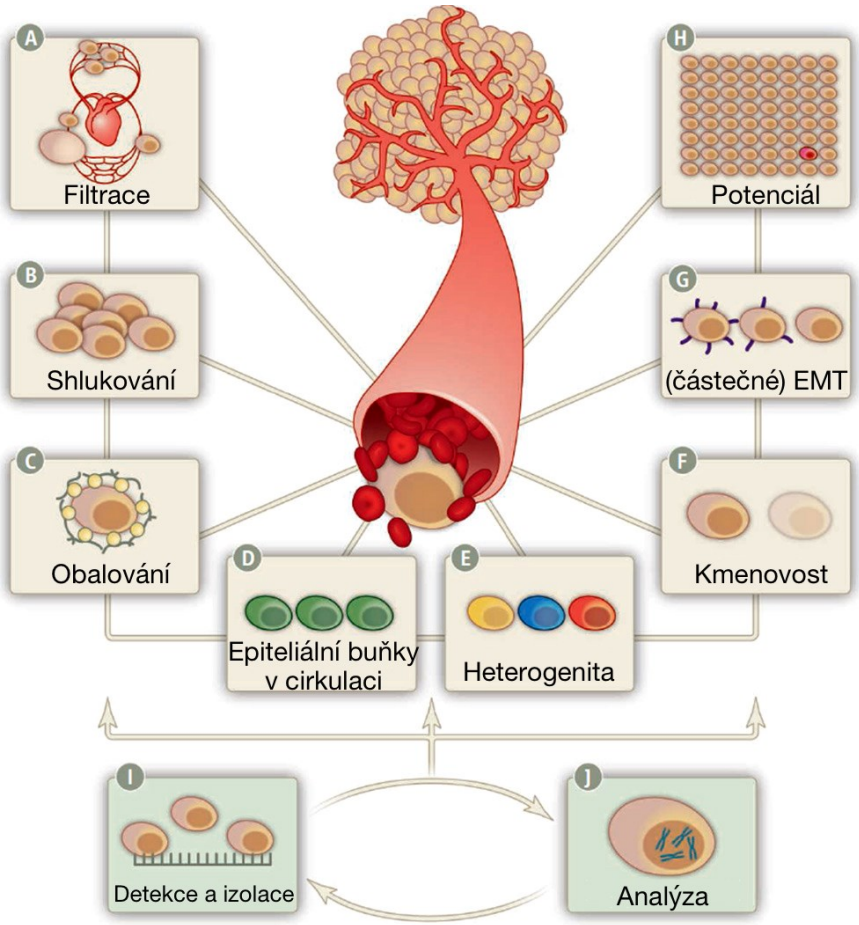
Schilling, *et al.*, *Nat. Rev. Urol.* (2012)

Vlastnosti cirkulujících nádorových buněk

- Překonání anoikis
- Změna fenotypu

- 1g (10^9 buněk) tumor – uvolnění 10^6 buněk/24 h
  - **1 CNB na 100 mil krevních buněk**
- Poločas života: 1 – 2 hod

- Velikost a deformovatelnost
- Exprese povrchových znaků
  - Možnosti detekce



Hayes et al., *Science* (2010)

Detekce nádorových cirkulujících buněk

Table 2. List of commercially available CTC enrichment technologies

Method	Company	Enrichment	Validation	Advantages	Limitations	Refs
ApoStream®	ApoCell	Combined microfluidic and microelectronic: CTCs are enriched based on the differential dielectric property compared with PBMCs	Quantitative immunofluorescence	Antibody-independent capture of viable CTCs available for downstream analysis	Larger leukocytes with similar dielectric property like CTCs may get captured. Similarly, CTCs of the same size as leukocytes may not be captured	[95,96]
CellCollector®	GILUPI	Affinity: CTCs are isolated using functionalized polymer surface stainless steel wire with anti-EpCAM antibodies	Immunocytochemical staining	*In vivo* technology. Steel inserts capture CTCs directly from patients without blood draws	Cannot isolate EpCAM$^-$CTCs	[97–99]
CellSearch®	Menarini-Silicon Biosystems	Immunomagnetic: EpCAM$^+$ CTCs are enriched by antibody nanoparticles	Fluorescence imaging	US FDA-approved for detection and enumeration of CTCs in metastatic breast, prostate, and colorectal cancer patients	Failed to enrich EpCAM$^-$CTCs. CTCs are not viable	[100]
CellSieve™	Creatv Microtech Inc.	Size: CTCs are enriched by filtering cells having diameter of <7 μm using microfilters	Multiple options can be exploited since cells are not fixed	Recovery of CTC clusters and immune-cancer fusion cells along with CTCs	Larger leukocytes and fibroblast contamination	[35]
ClearCell®FX	Clearbridge BioMedics	Size: CTCs are enriched by automated microfluidic chip	Multiple options can be exploited since cells are not fixed	Label-free CTCs readily available for downstream analysis	Loss of CTCs due to red blood cell lysis step	[101]
DEPArray™	Menarini Silicon Biosystems	Combined microfluidic and microelectronic: CTCs are enriched by dielectrophoretic movement and trapped in electronic cages	Fluorescence imaging	Individual CTCs from heterogeneous population of cells can be isolated for downstream analysis allowing both EpCAM$^{+/-}$ CTC characterization	Pre-enrichment and staining are required for rare cells like CTCs before loading to cartridge	[102]
EasySep™ Direct Human CTC Enrichment Cocktail	Stemcell Technologies	Immunomagnetic: CTCs are enriched by depleting hematopoietic cells and platelets with a cocktail of antibodies	Multiple options can be exploited since cells are not fixed	Cells are not fixed and viable, which keeps multiple options open, such as culturing, characterization, injecting to animals, etc. Both EpCAM$^{+/-}$ CTCs are enriched	Unable to achieve complete depletion of leukocytes and other cells	[103]
EasySep™ Human CD45 Depletion Kit	Stemcell Technologies	Immunomagnetic: CTCs are enriched by depleting CD45$^+$ cells	Multiple options can be exploited since cells are not fixed	Isolated cells are immediately available for downstream applications such as flow cytometry, culture, or DNA/RNA extraction	Depletion of only CD45$^+$ cells does not yield homogeneous CTCs	[104]
ISET® Technology	Rarecells	Size: leukocytes and other cells are removed by pressure-controlled filtration system, thereby enriching CTCs	Immunofluorescence	Fixed CTCs are processed with a specific ISET buffer and can be collected for downstream processing	Larger leukocytes may be captured, limiting downstream analysis of CTCs	[105]

Table 2. (continued)

Method	Company	Enrichment	Validation	Advantages	Limitations	Refs
IsoFlux™	Fluxion	Combined microfluidic and immunomagnetic: CTCs are enriched by anti-EpCAM antibody magnetic beads in microfluidic cartridges	Fluorescent imaging	High recovery of EpCAM$^+$ CTCs	Only EpCAM$^+$ CTCs are enriched	[106]
OncoQuick®	Greiner Bio-One	Size and density: CTCs are enriched by density gradient centrifugation over a porous barrier	Multiple options can be exploited since cells are not fixed	One-step enrichment of CTCs by simple centrifugation	Due to sparse numbers of CTCs, no visibility of distinct interphase layer that leads to loss of tumor cell on collection. Platelet contamination is another challenge	[107]
Parsortix® PR1	Angle	Size and deformability: CTCs are enriched by automated filtration cassette that separates leukocytes	Multiple options can be exploited since cells are not fixed	CTCs can be enumerated by in-cassette staining or harvested for downstream analysis	Possible contamination of uncompressible large cells like fibroblasts	[108]
RosetteSep™ CTC Enrichment Cocktail Containing Anti-CD36/CD56	Stemcell Technologies	Immunodensity: unwanted cells are crosslinked by an antibody cocktail that increases density and is pelleted by centrifugation	Multiple options can be exploited since cells are not fixed	Enriched CTCs are readily available for various downstream applications	Additional centrifugation step may result in loss of few CTCs	[12,61]
RosetteSep™ Human CD45 Depletion Cocktail	Stemcell Technologies	Immunodensity: unwanted cells are depleted by tetrameric antibody complex followed by density gradient centrifugation	Multiple options can be exploited since cells are not fixed	Enriched CTCs are readily available for various downstream applications.	Depletion of only CD45$^+$ cells does not remove other cell types	[46]
VTX-1	Vortex Biosciences	Size: automated microfluidic technology is adopted to enrich CTCs	Multiple options can be exploited since cells are not fixed	Label-free CTCs readily available for culture or characterization	Recovery of only 60–70% CTCs	[109]

Trends in Cancer

CellPress

Review

Preclinical models to study patient-derived circulating tumor cells and metastasis

Kanve N. Suvilesh,[1,*] Yariswamy Manjunath,[1,2] Klaus Pantel,[3] and Jussuf T. Kaifi[1,2,4,*]

Příklad: Filtrace

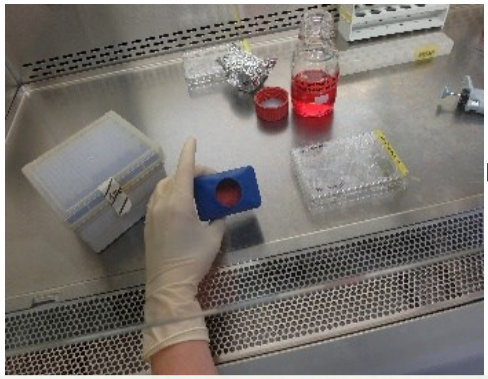
- CNB: epiteliální původ → větší velikost
- Platformy: **MetaCell**, CellSieve, Celsee,…

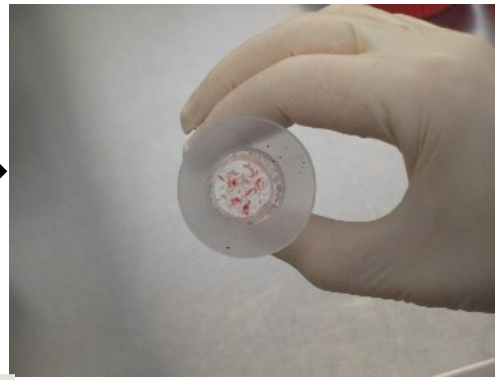
Buňky	Průměr [μm]
Erytrocyty	6 - 8
Granulocyty	12 – 15
Monocyty	15 – 25
Lymfocyty	7 – 10, 14 – 20
CNB	17 - 52

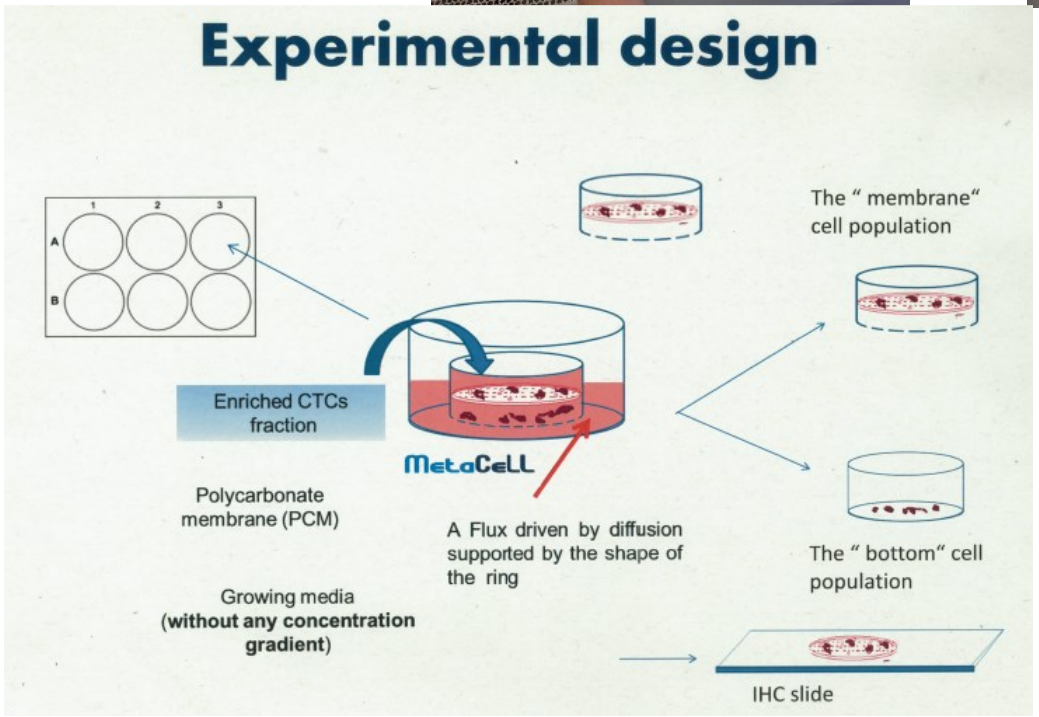
- **Výhody** – nezávislost na povrchových znacích
  - Heterogenní populace
  - Není nutná aktivace receptorů
  - Nativní stav
- **Nevýhody**
  - Možný překryv s leukocyty
  - Nutné využít dalších znaků (CD45)
  - Různá velikost CNB?

Příklad: Filtrace

- polycarbonate membrane with 8 μm pores (CTCs over 20 μm)
- capilary force-driven filtration

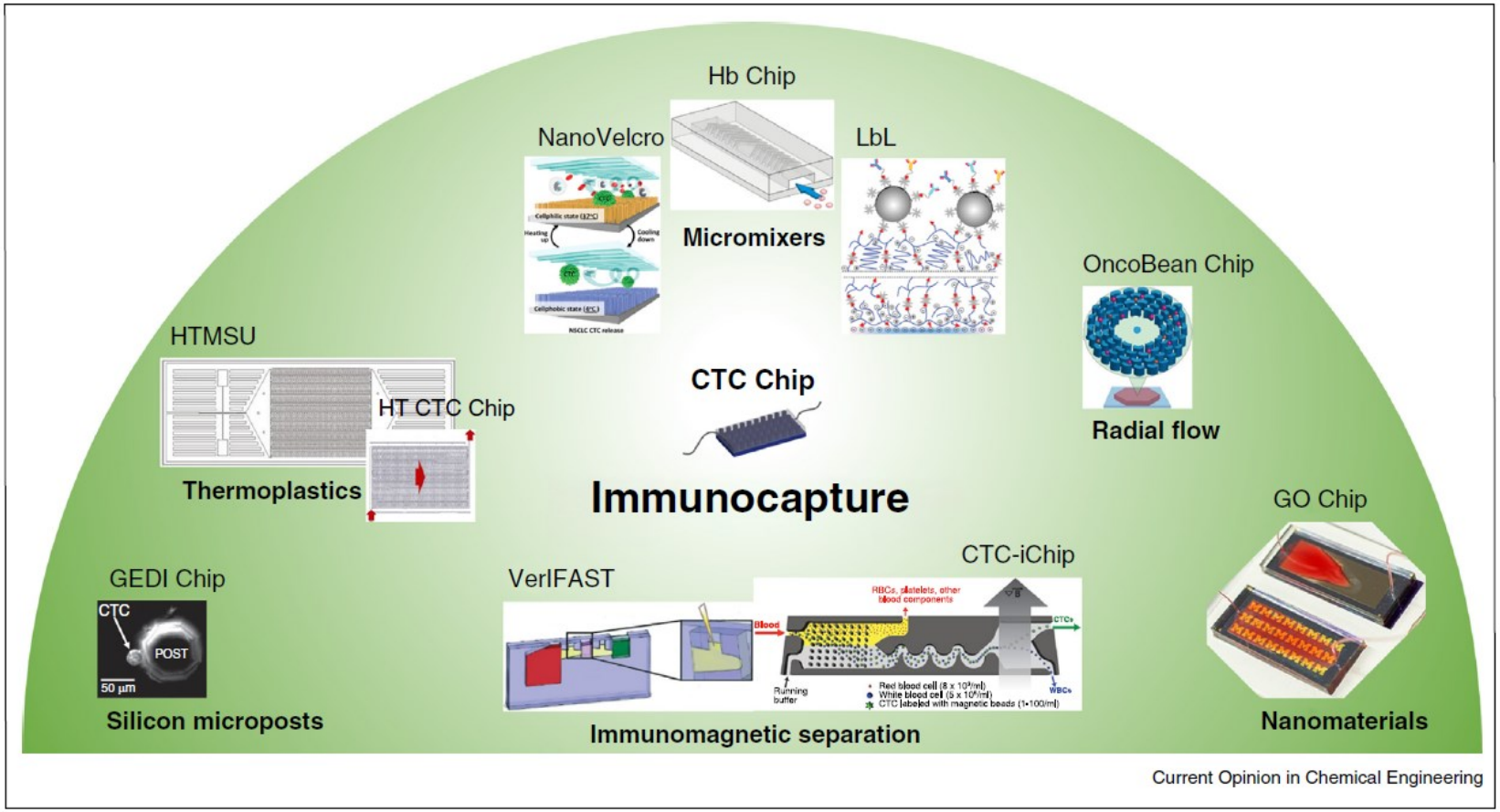




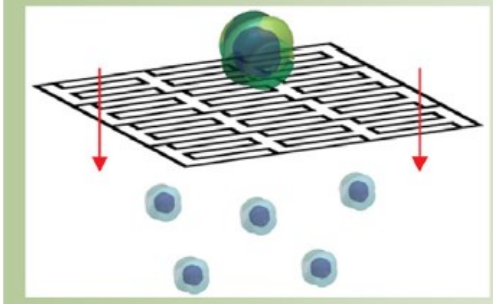


MetaCell

Příklad: mikrofluidní separace



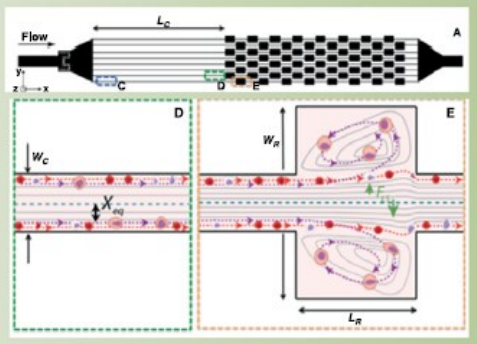
Příklad: mikrofluidní separace



Size Based Separation

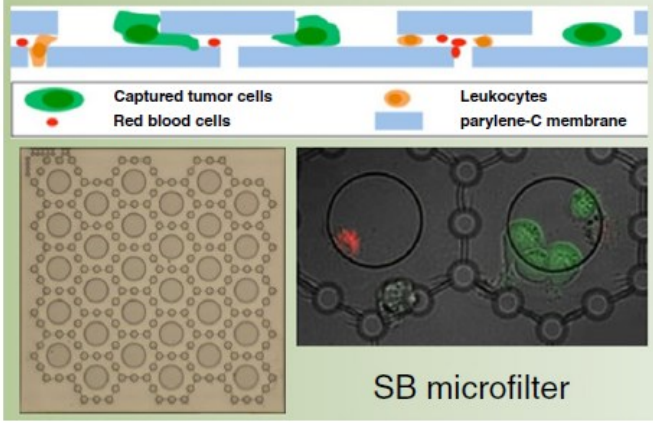
Microfilter

Inertial Effects

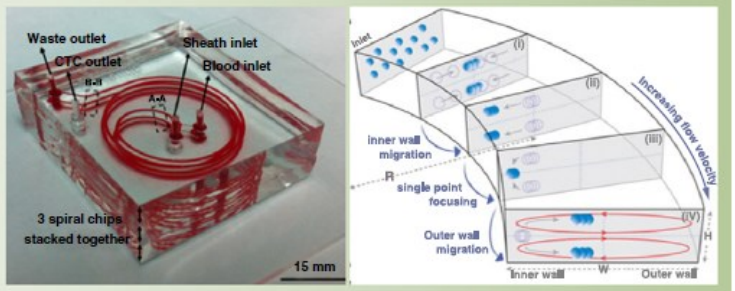


FMSA device

Vortex technology



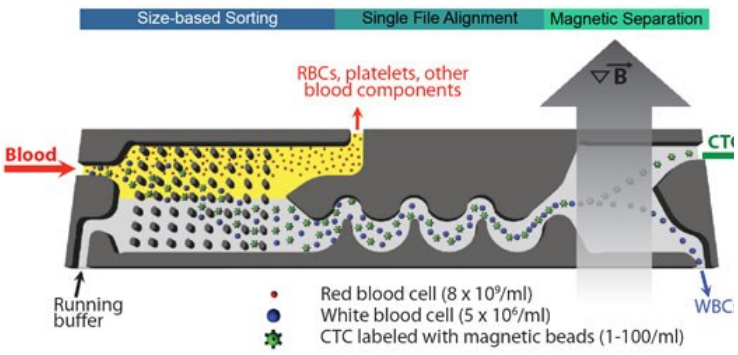
SB microfilter

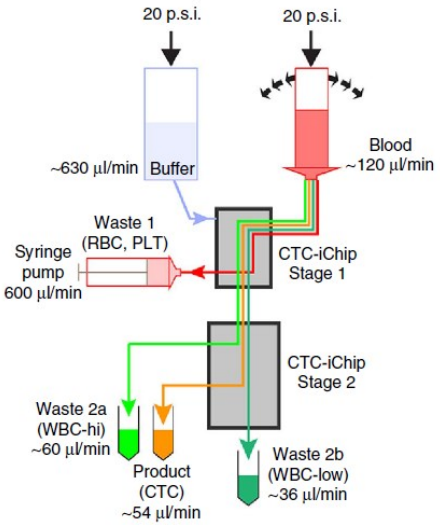


Spiral devices

Current Opinion in Chemical Engineering

Příklad: mikrofluidní separace





CTC-iChip running platform

PROTOCOL

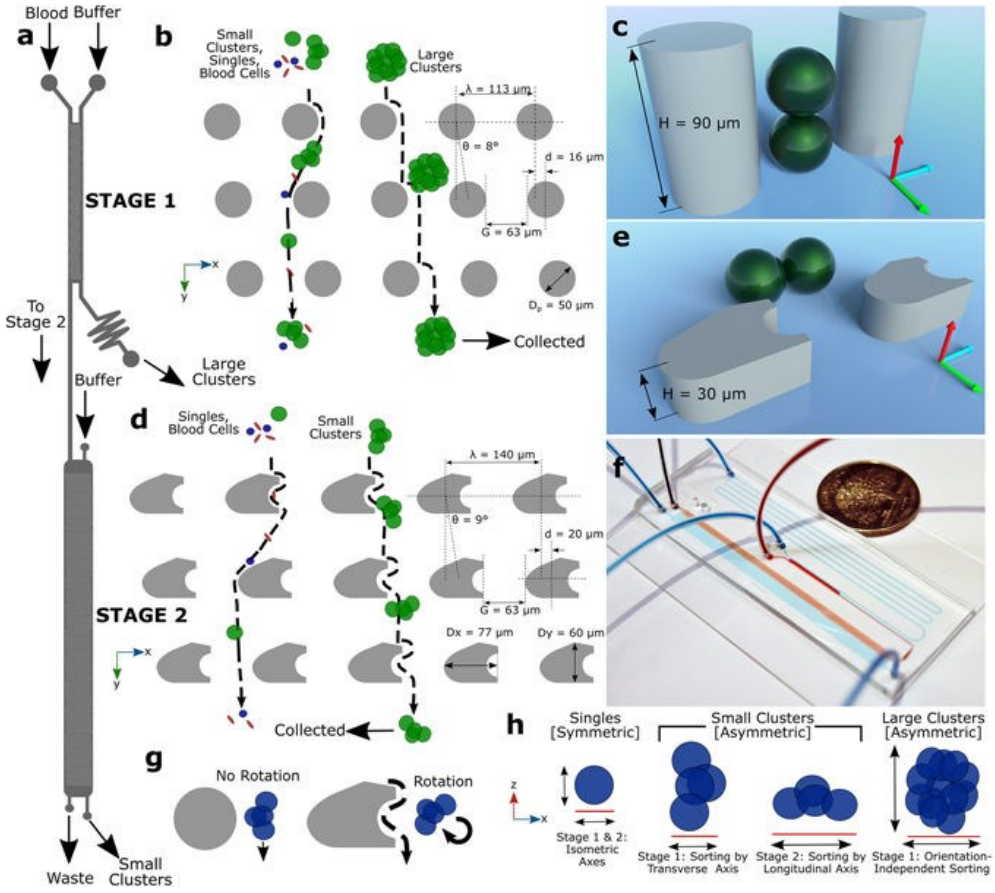
Microfluidic, marker-free isolation of circulating tumor cells from blood samples

Nezihi Murat Karabacak[1,4], Philipp S Spuhler[1,4], Fabio Fachin[1], Eugene J Lim[1], Vincent Pai[1], Emre Ozkumur[1], Joseph M Martel[1], Nikola Kojic[1], Kyle Smith[1], Pin-i Chen[1], Jennifer Yang[1], Henry Hwang[1], Bailey Morgan[1], Julie Trautwein[2], Thomas A Barber[1], Shannon L Stott[1,2], Shyamala Maheswaran[2], Ravi Kapur[1], Daniel A Haber[2,3] & Mehmet Toner[1]

[1]Department of Surgery and Center for Engineering in Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA. [2]Cancer Center, Massachusetts General Hospital, Boston, Massachusetts, USA. [3]Howard Hughes Medical Institute, Chevy Chase, Maryland, USA. [4]These authors contributed equally to this work. Correspondence should be addressed to M.T. (mtoner@hms.harvard.edu).

Published online 27 February 2014; doi:10.1038/nprot.2014.044

Příklad: mikrofluidní separace



SCIENTIFIC REPORTS

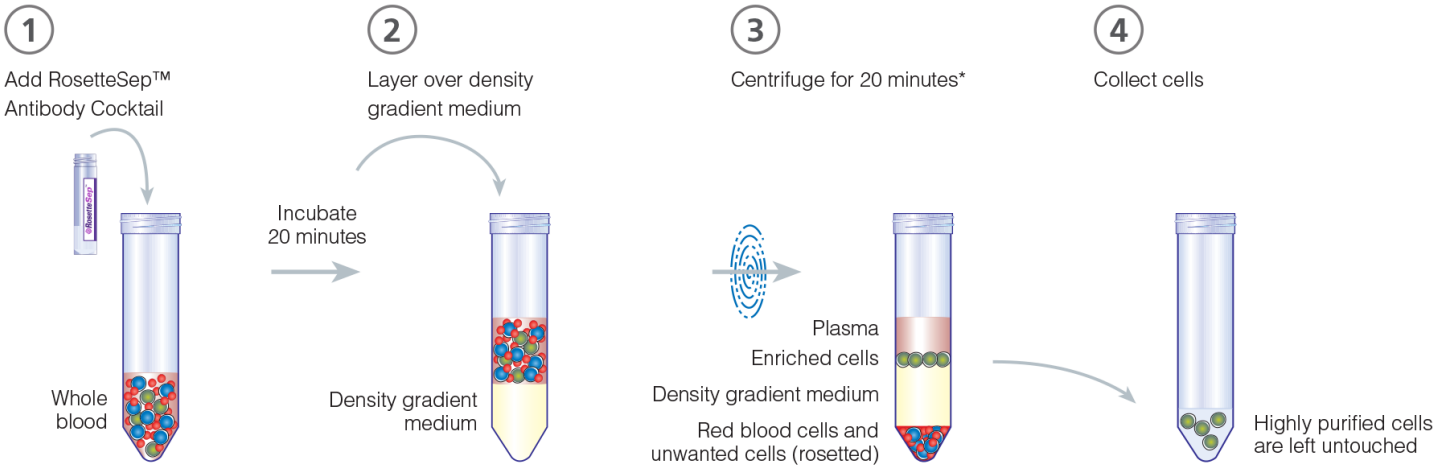
OPEN

Microfluidic Isolation of Circulating Tumor Cell Clusters by Size and Asymmetry

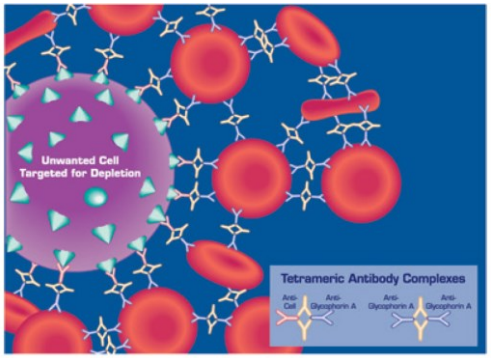
Sam H. Au[1,2], Jon Edd[1], Amy E. Stoddard[1], Keith H. K. Wong[1,2], Fabio Fachin[1], Shyamala Maheswaran[2,3], Daniel A. Haber[2,4,5], Shannon L. Stott[1,3,4], Ravi Kapur[1] & Mehmet Toner[1,2,6]

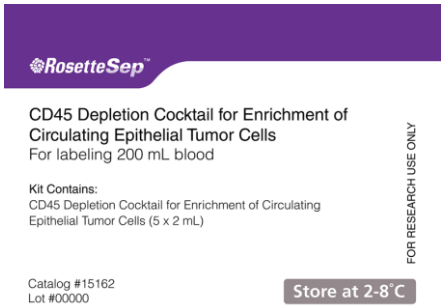
Received: 20 January 2017
Accepted: 27 March 2017
Published online: 26 May 2017

Izolace CTC pomocí deplece CD45+ buněk krve



*Use SepMate™ to reduce centrifugation time to 10 minutes with brake on.





RosetteSep™

Unique Immunodensity Cell Isolation

RosetteSep™ kits offer one-step enrichment of cells directly from human whole blood. By crosslinking unwanted cells to red blood cells (RBCs) present in the sample, CTCs are enriched during standard density gradient centrifugation. RosetteSep™ is easy to use, does not require additional equipment, reduces sample handling time and maximizes convenience. RosetteSep™ can be easily combined with **SepMate™**, a specialized isolation tube that standardizes and minimizes variability when isolating cells using density gradient centrifugation. Learn more at **www.RosetteSep.com** and **www.SepMate.com**.

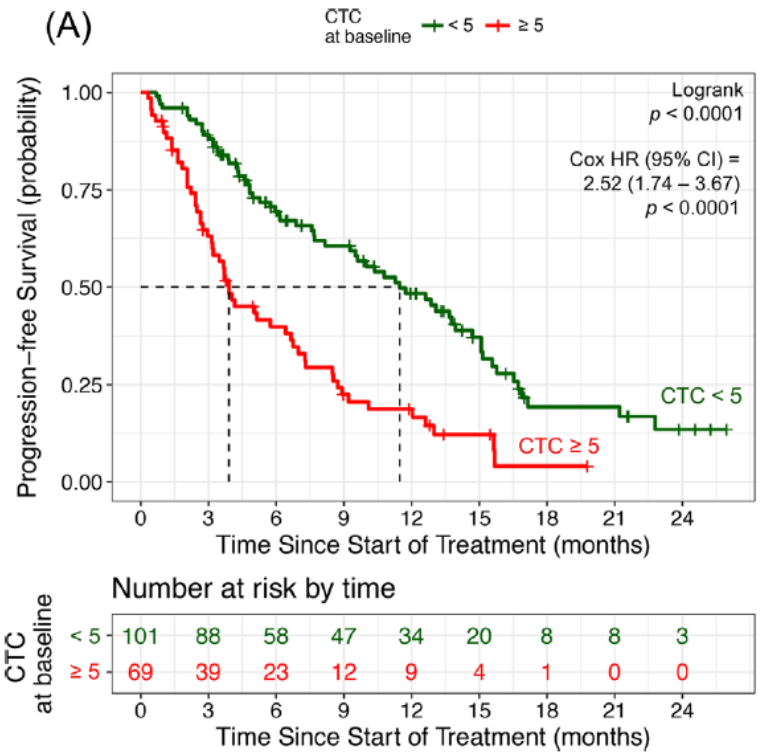
Klinické využití detekce cirkulujících nádorových buněk

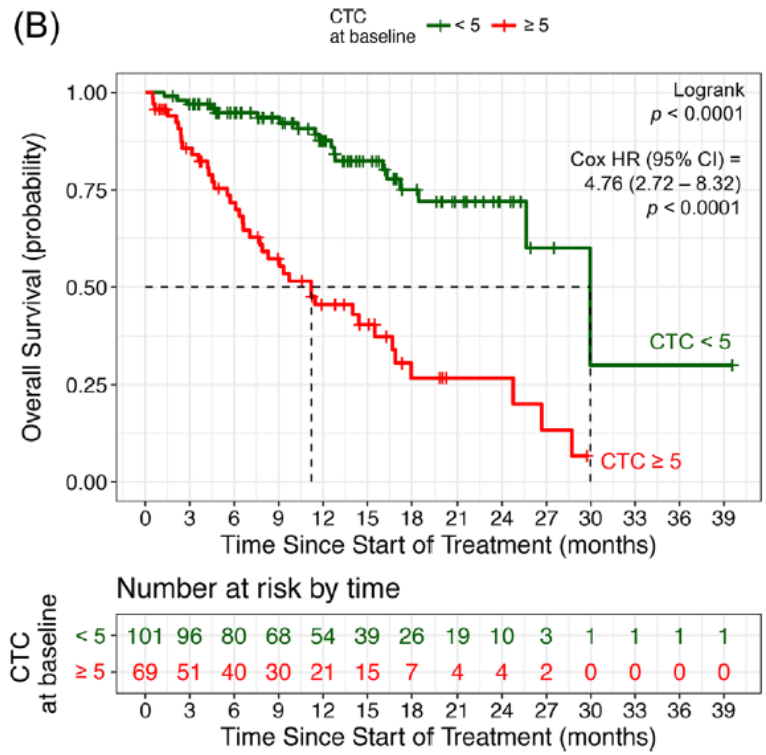
- Odhad prognózy pacienta
- **Monitoring průběhu onemocnění**
- Včasná detekce

- Metastázující karcinomy prsu a prostaty – hranice 5 CNB/7,5ml
- Metastázující karcinom tlustého střeva – hranice 3 CNB/7,5 ml
- CellSearch system Veridex – schváleno FDA

www.cellsearchctc.com

Množství cirkulujících nádorových buněk korelují s prognózou





Received: 21 September 2017 | Accepted: 9 January 2018
DOI: 10.1002/pros.23488

ORIGINAL ARTICLE

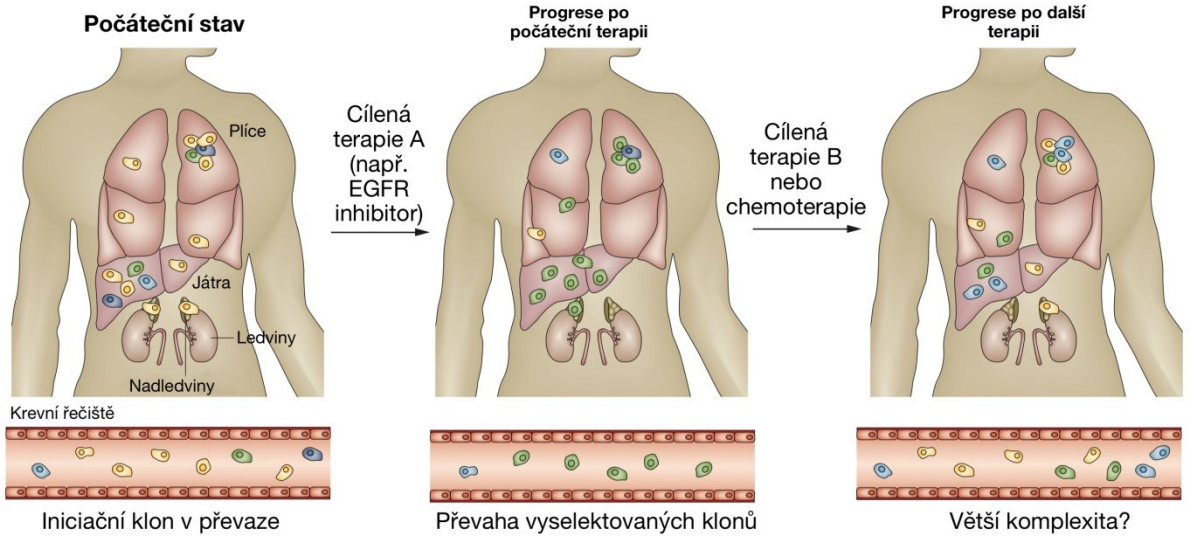
WILEY The Prostate

Circulating tumor cells and survival in abiraterone- and enzalutamide-treated patients with castration-resistant prostate cancer

Bram De Laere[1] | Steffi Oeyen[1] | Peter Van Oyen[2] | Christophe Ghysel[2] | Jozef Ampe[2] | Piet Ost[3] | Wim Demey[4] | Lucien Hoekx[5] | Dirk Schrijvers[6] | Barbara Brouwers[7] | Willem Lybaert[8] | Els Everaert[8] | Piet Van Kerckhove[7] | Daan De Maeseneer[9] | Michiel Strijbos[4] | Alain Bols[7] | Karen Fransis[5] | Nick Beije[10] | Inge de Kruijff[10] | Valerie van Dam[1] | Anja Brouwer[1] | Pieter-Jan van Dam[1] | Gert Van den Eynden[1,11] | Annemie Rutten[12] | Stefan Sleijfer[10] | Jean Vandebroek[12] | Steven Van Laere[1] | Luc Dirix[1,12]

Molekulární charakterizace CNB → cílená terapie

- Biopsie – identifikace mutací – zacílení terapie
- Uvolňovány i z metastáz → komplexita
- **Vývoj onemocnění** → chemorezistence, identifikace nových cílů
- Využití v budoucnu?

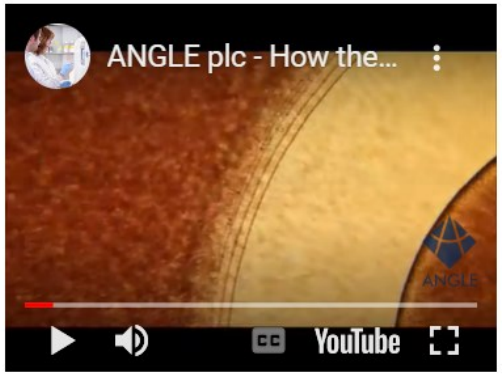


Krebs et al., *Nat. Rev. Clin. Onc.*, (2014)

Parsortix® Technology

- Pacientovi je odebrána tekutá biopsie (vzorek krve) - obvykle 10 ml do vakuové zkumavky EDTA.
- Vzorek se připojí k přístroji Parsortix. Není nutné žádné předběžné zpracování.
- Do přístroje se vloží filtrační kazeta Parsortix a systém se připraví k použití.
- Přístroj Parsortix automaticky propustí krev přes filtrační kazetu.
- CTC jsou zachyceny v kazetě Parsortix díky své větší velikosti a nižší stlačitelnosti než ostatní složky krve.

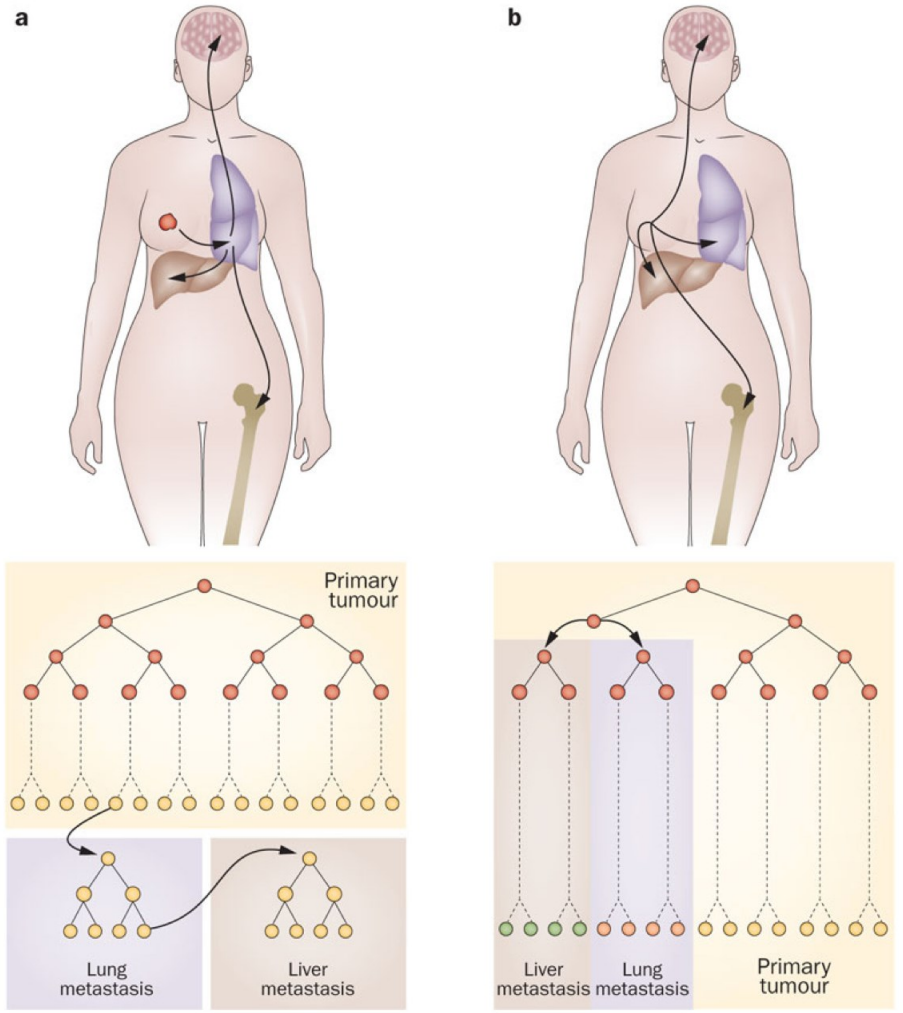
- První zdravotnický prostředek schválený FDA pro zachycení a sběr cirkulujících nádorových buněk (CTC) z krve pacientů s metastazujícím karcinomem prsu (MBC) pro následnou analýzu.



https://angleplc.com/parsortix-technology/how-it-works/

Cirkulující nádorové buňky – prekurzor metastáz

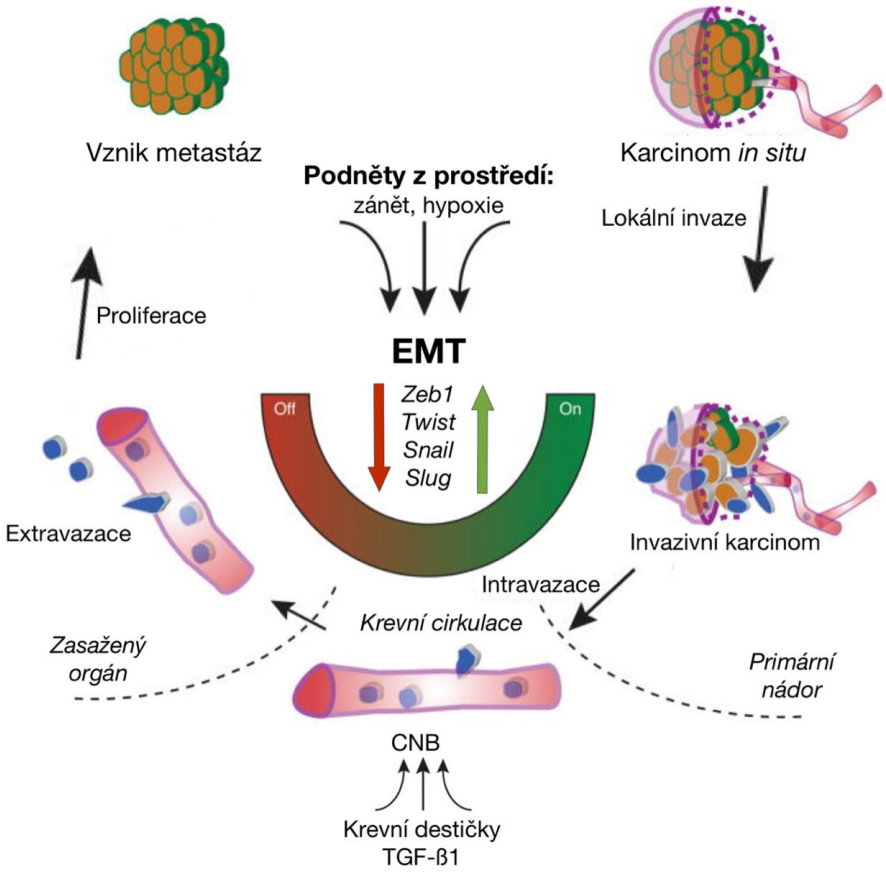
- Množství CNB nekoreluje s množstvím metastáz
- Rozsev nádorových buněk může probíhat v časných stádiích (b)
- K vytvoření metastáz nutná další stimulace – mikroprostředí, mutageneze
- Relaps: metastáze původem z rozesetých buněk (a)
- „Tumor self-seeding“
- Oblast intenzivního výzkumu



Naxerova et al., *Nat. Rev. Clin. Onc.* (2014)

Plasticita cirkulujících nádorových buněk

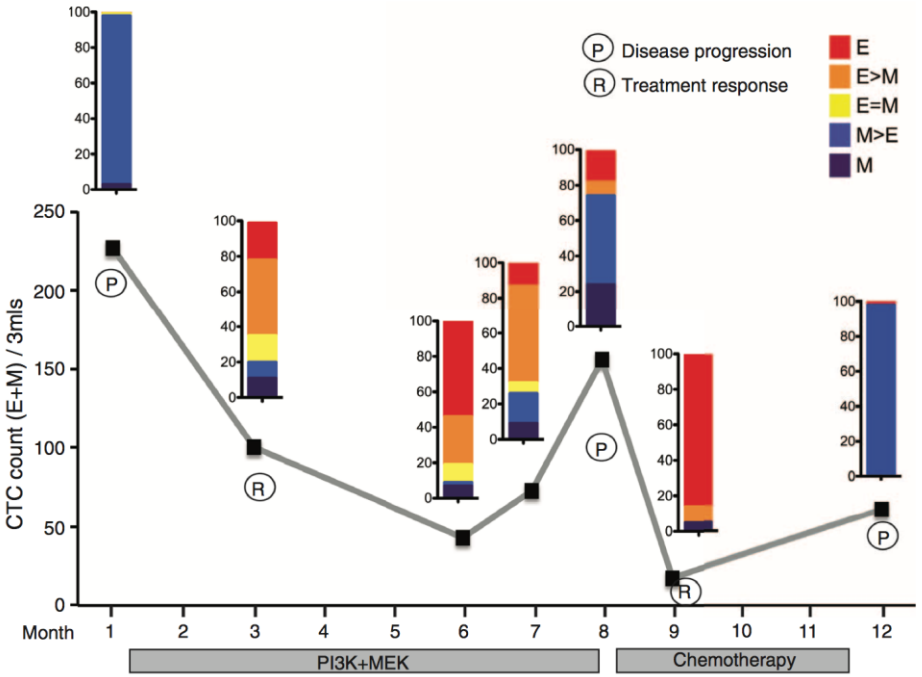
- Tvorbu metastáz ovlivňuje řada faktorů – mj. **plasticita CNB**
- **Epiteliálně-mezenchymální přechod**
  - Podíl na vzniku CNB
  - Vyšší motilita a invazivita
  - Vznik chemorezistence
  - Detailní mechanismy stále předmětem výzkumu
  - Význam popsán u řady karcinomů (prsu, prostaty, plic, tlustého střeva, vaječníků, atd.)



Tsai et al., *Cancer Cell*, (2012) - upraveno

Epiteliálně-mezenchymální přechod

- U CNB popsán epiteliální i mezenchymální fenotyp
- M+ buňky – spojeny s progresí onemocnění
- Dynamické změny

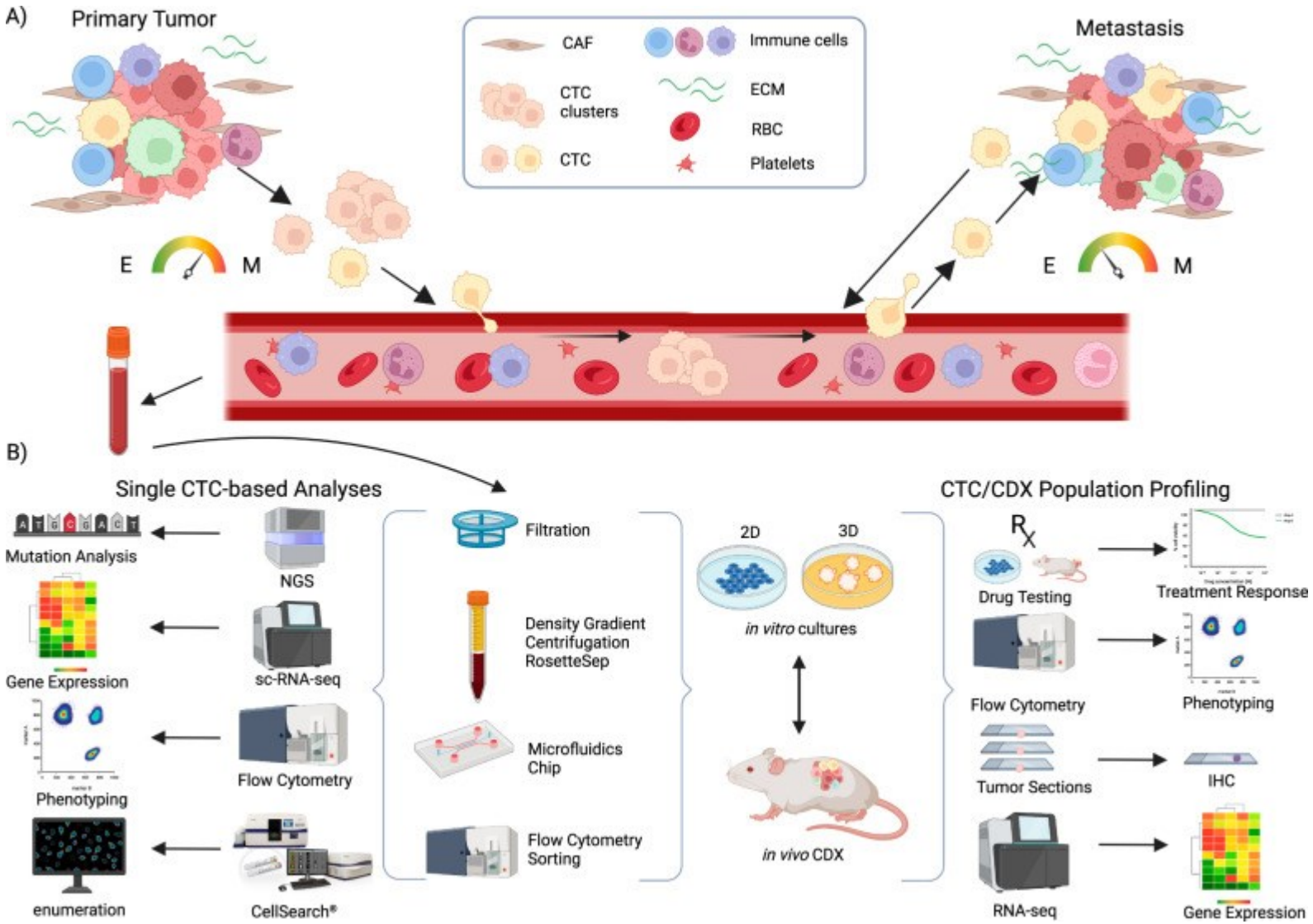


Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition

Min Yu,[1,6*] Aditya Bardia,[1,3*] Ben S. Wittner,[1] Shannon L. Stott,[1,2] Malgorzata E. Smas,[1] David T. Ting,[1] Steven J. Isakoff,[1,3] Jordan C. Ciciliano,[1] Marissa N. Wells,[1] Ajay M. Shah,[2] Kyle F. Concannon,[1] Maria C. Donaldson,[1] Lecia V. Sequist,[1,3] Elena Brachtel,[1,4] Dennis Sgroi,[1,4] Jose Baselga,[1,3] Sridhar Ramaswamy,[1,3] Mehmet Toner,[2,5] Daniel A. Haber,[1,3,6†] Shyamala Maheswaran[1,5†]

Yu et al., *Science*, (2014)

Experimentalní modely a CTCs



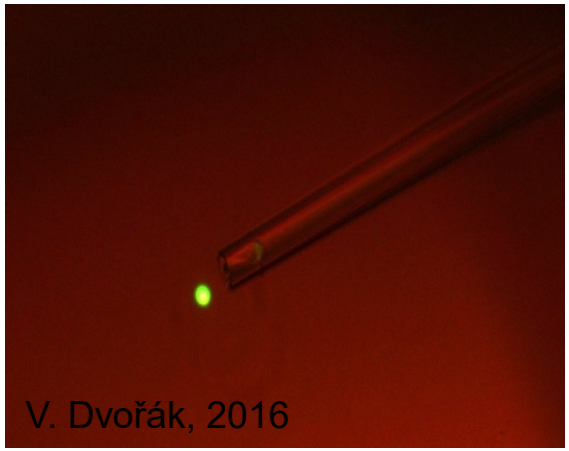
Cell Death Dis. 2023 Aug; 14(8): 530.

Cirkulující nádorové buňky: shrnutí

Nádorové buňky uvolněné do cirkulace

- Klíčová úloha ve vzniku metastáz
- Heterogenita a plasticity

- Detekce
  - Oblast intenzivního výzkumu
- Klinicky významné
  - Počet koreluje s prognózou
  - Molekulární charakterizace – personalizace medicíny


V. Dvořák, 2016

Extravazace

- Penetrace buněk do okolní tkáně
- Interakce nádorové buňky se stěnou cévy
- Možnost proliferace uvnitř lumen cévy

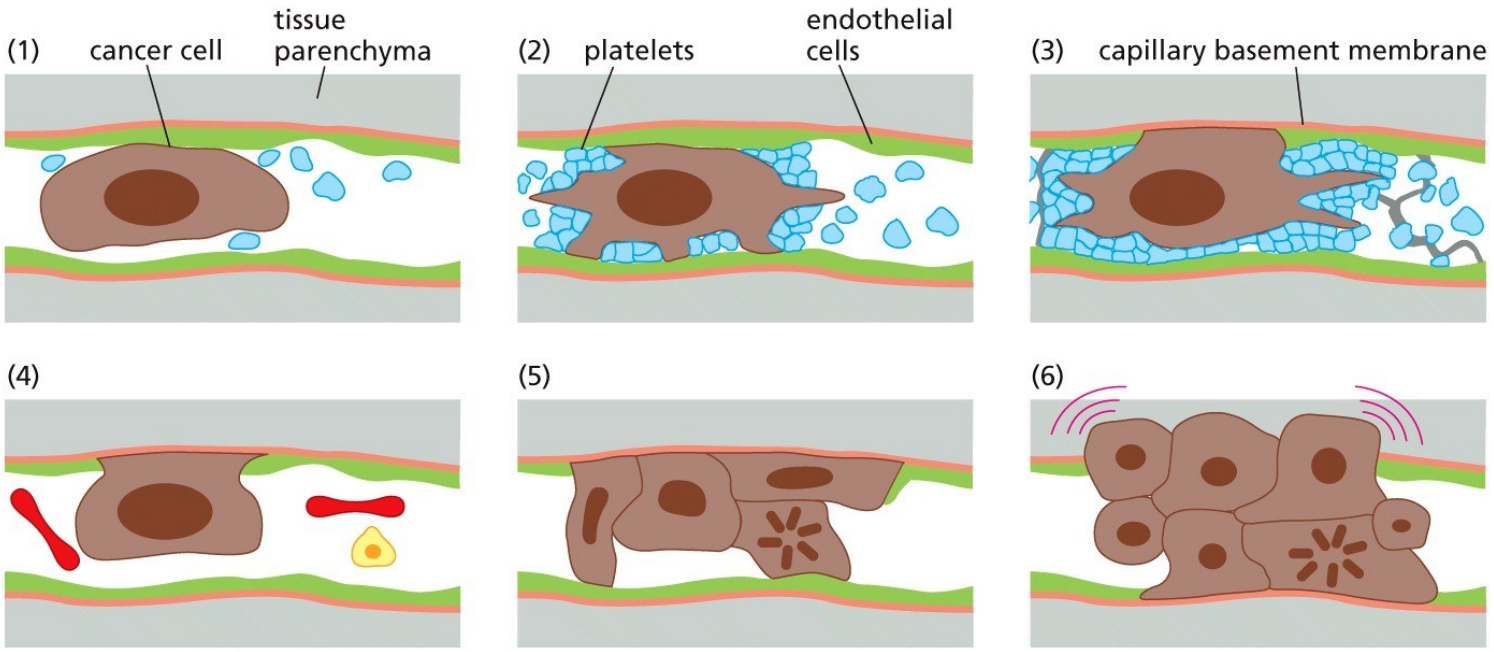


Figure 14.9d The Biology of Cancer (© Garland Science 2014)

Kolonizace

- Velice nízká účinnost
- Dormantní mikrometastázy
- Metastatický relaps

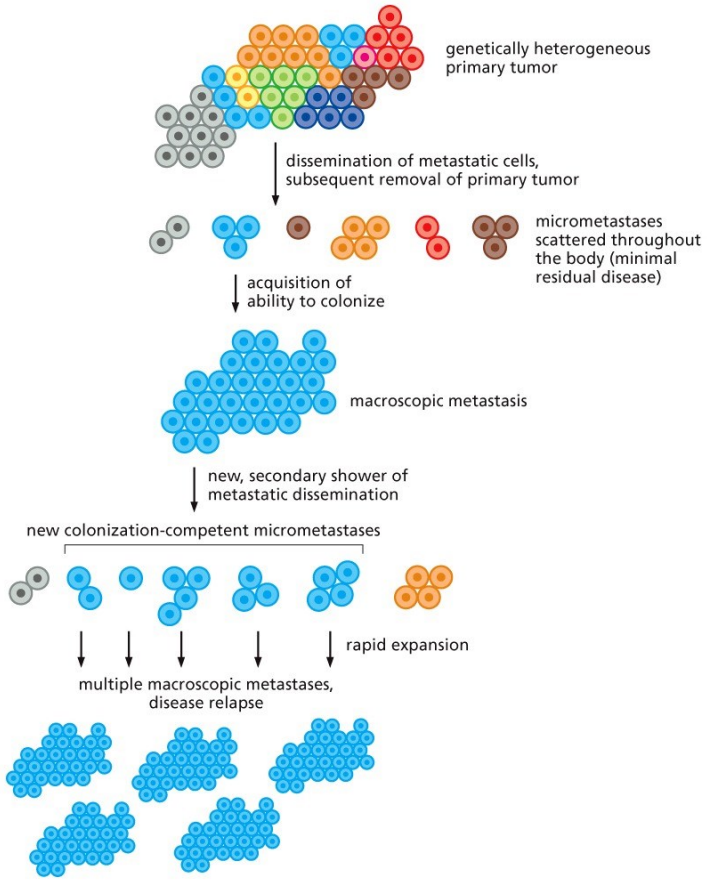


Figure 14.11c The Biology of Cancer (© Garland Science 2014)

CK+ buňky v kostní dřeni

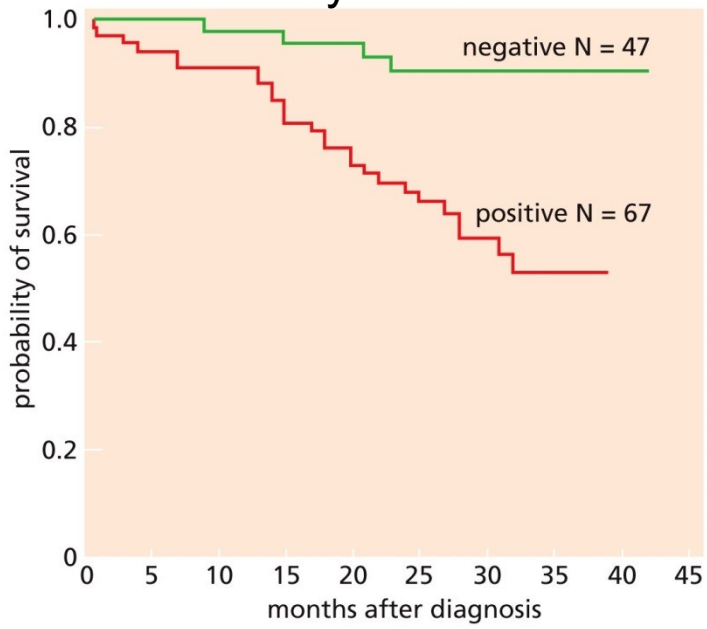
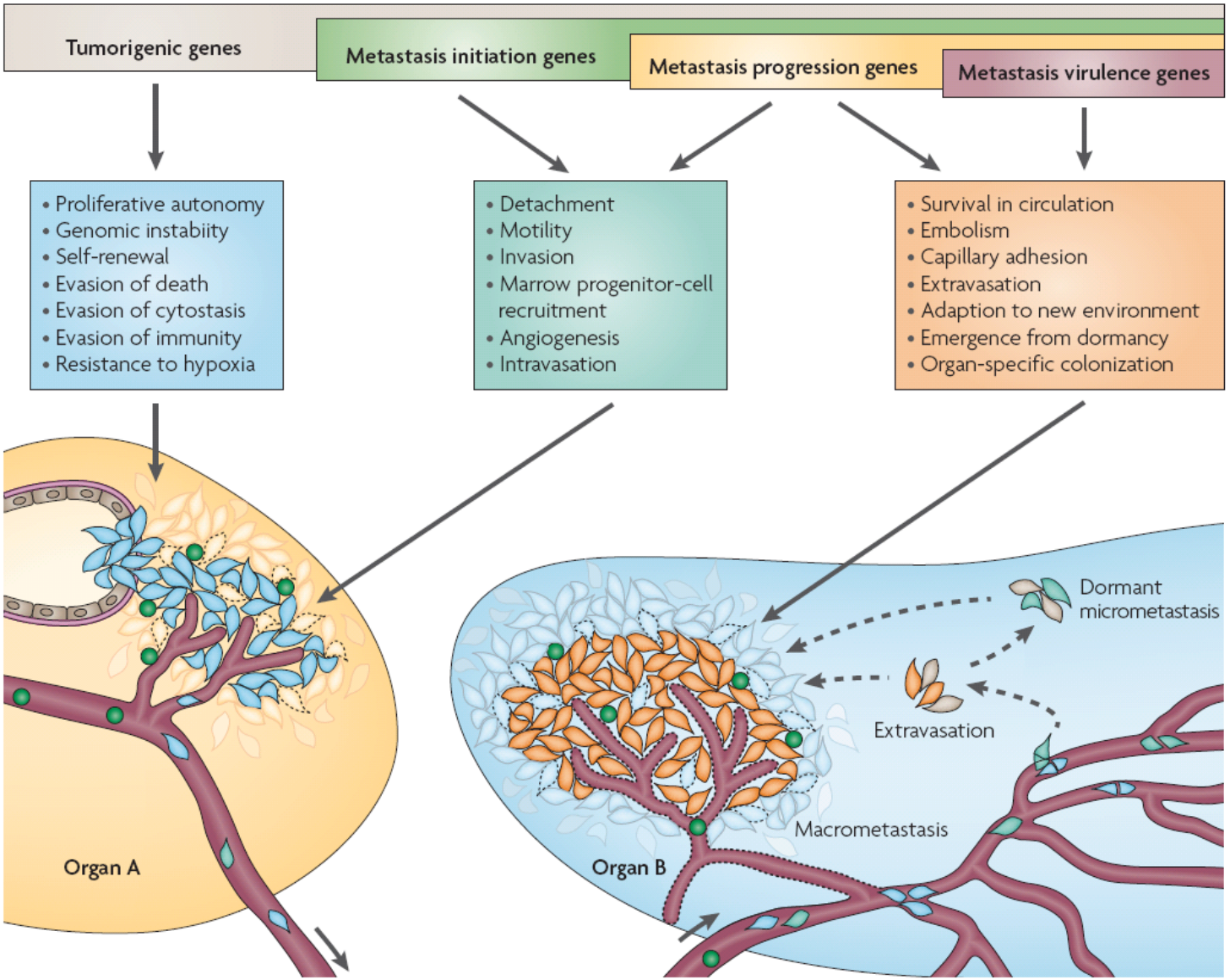


Figure 14.10d The Biology of Cancer (© Garland Science 2014)

Genetic determinants of cancer metastasis

Don X. Nguyen and Joan Massagué



Epithelial-Mesenchymal Transition (EMT)
Epithelial-Mesenchymal Plasticity (EMP)

- Změna buněčného fenotypu spojená se ztrátou adheze a zvýšením motility

Table 14.1 Examples of EMTs during mouse embryonic development

Process	Transition	
	From	To
Gastrulation	epiblast	mesoderm
Prevalvular mesenchyme in the heart	endothelium	atrial and ventricular septum
Neural crest cells	neural plate	neural crest cells, which can yield bone, muscle, peripheral nervous system
Somitogenesis	somite walls	sclerotome
Palate formation	oral epithelium	mesenchymal cells
Müllerian duct regression	Müllerian tract	mesenchymal cells

Adapted from P. Savagner, *BioEssays* 23:912–923, 2001.

EMT & nádory

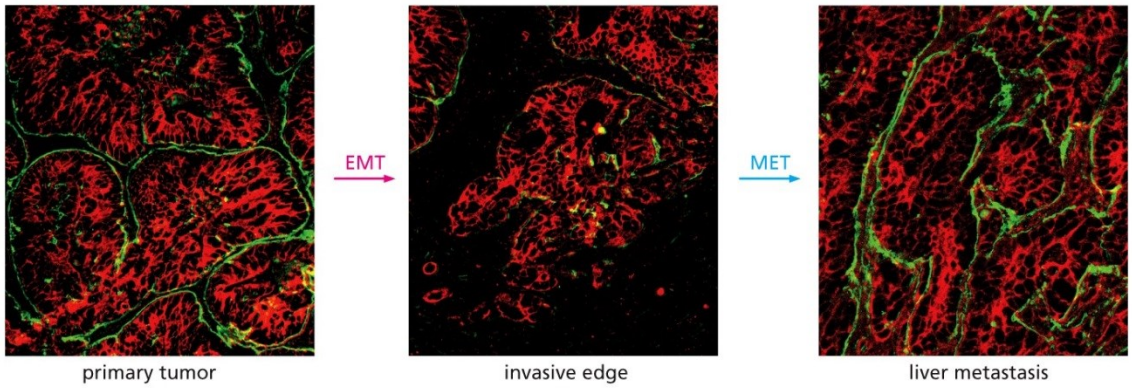


Figure 14.18a The Biology of Cancer (© Garland Science 2014)

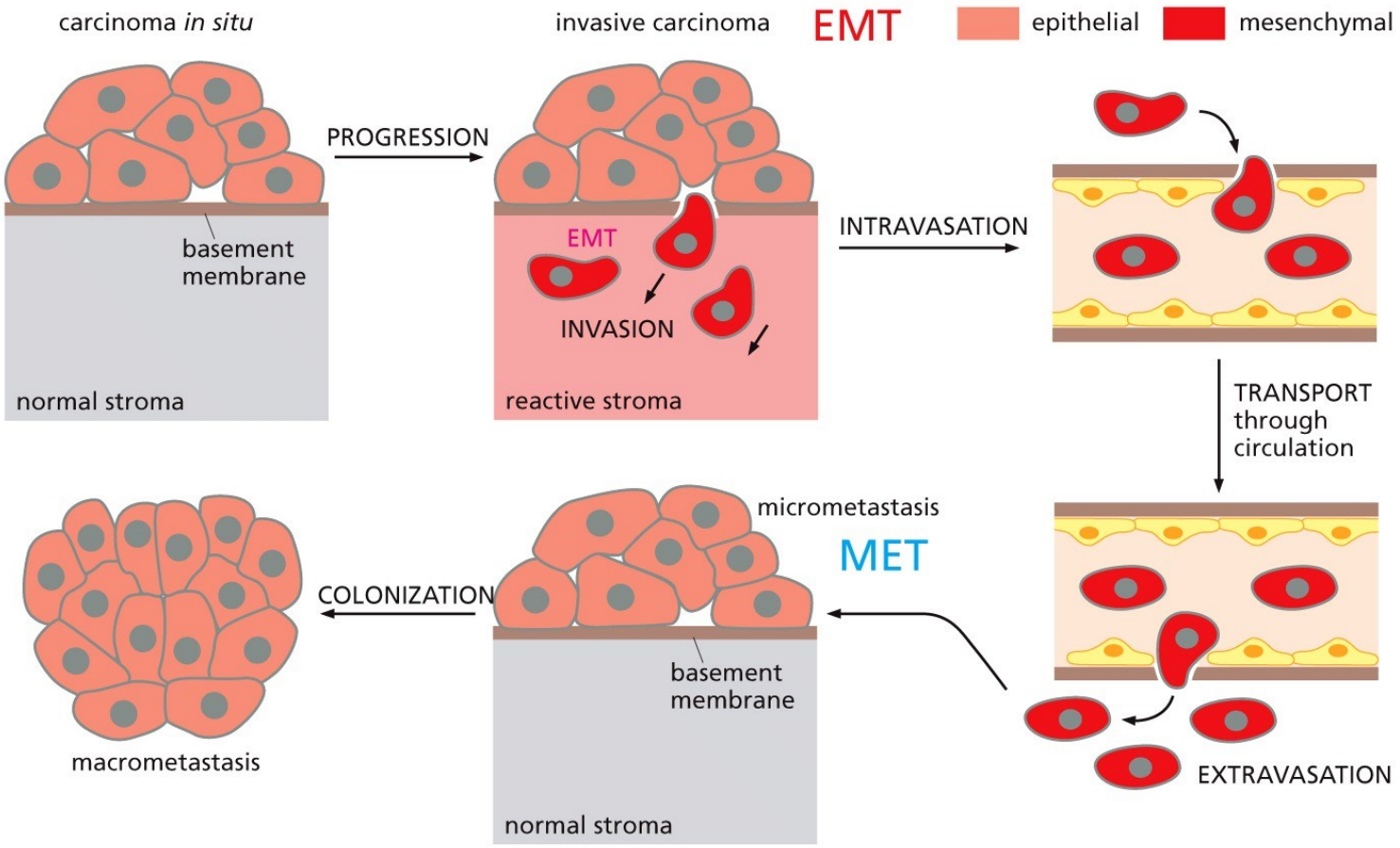
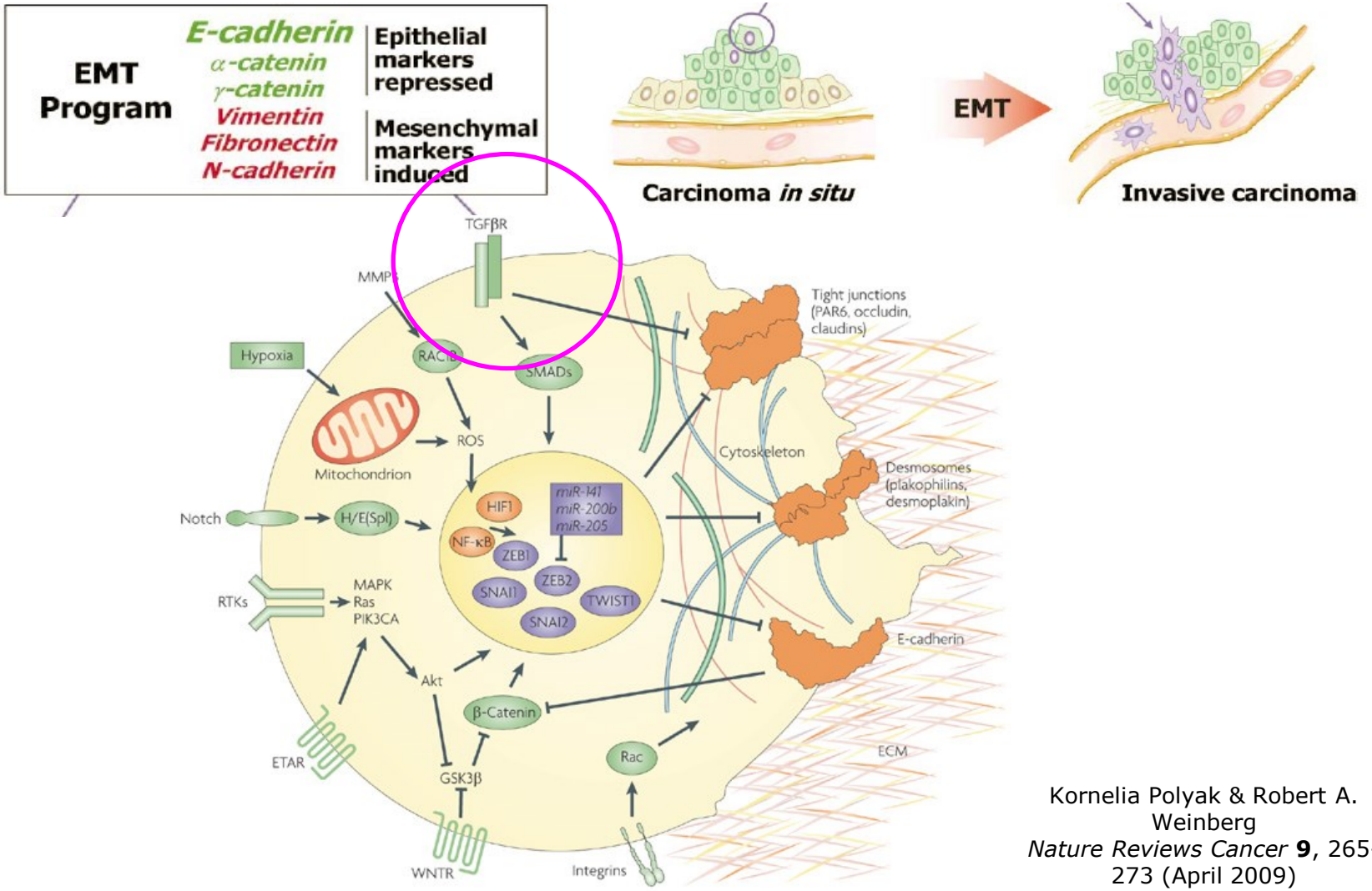


Figure 14.18b The Biology of Cancer (© Garland Science 2014)

Znaky a regulátory EMT

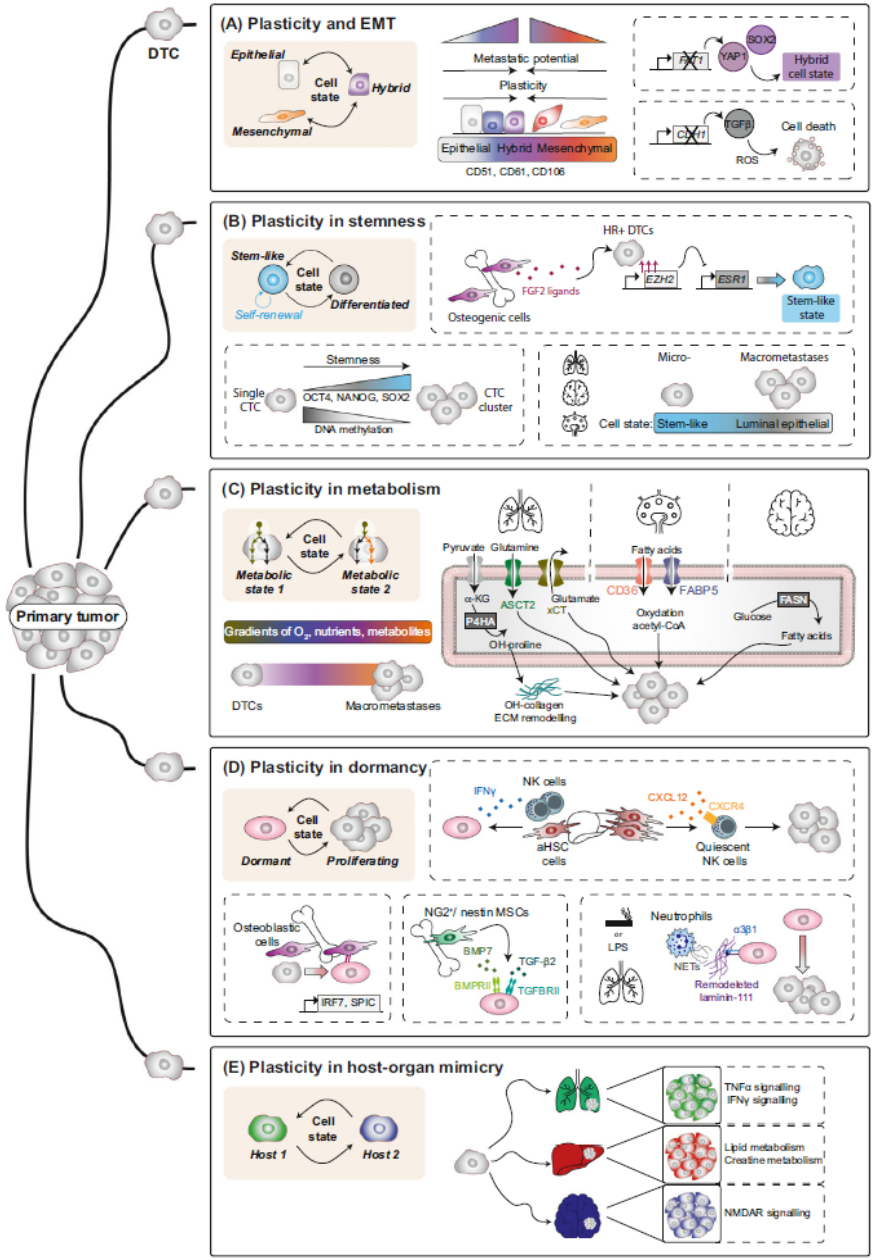


Kornelia Polyak & Robert A. Weinberg
Nature Reviews Cancer **9**, 265-273 (April 2009)

Mechanismy karcinogeneze

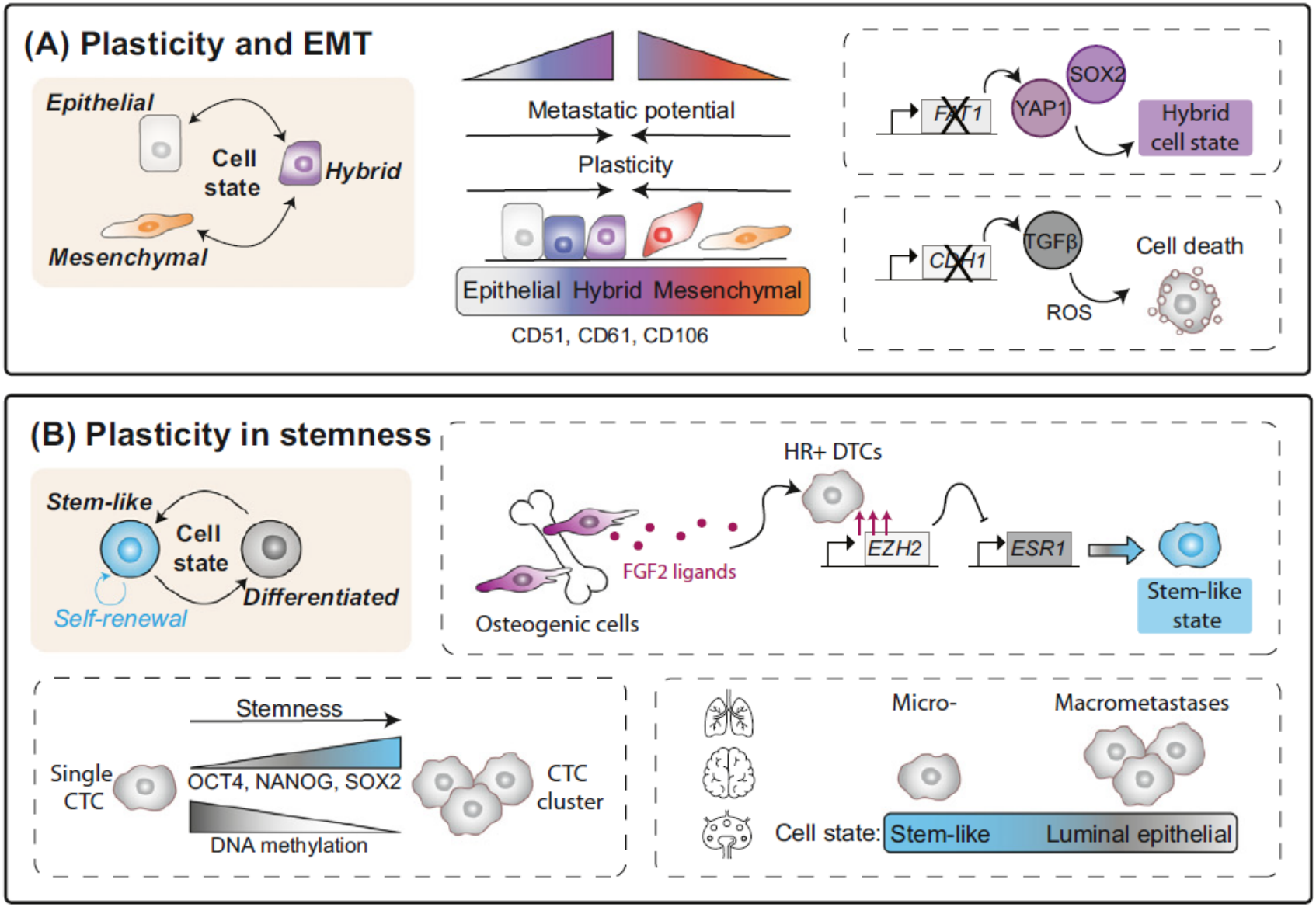
Key figure

Phenotypic plasticity of disseminated tumor cells during metastatic colonization

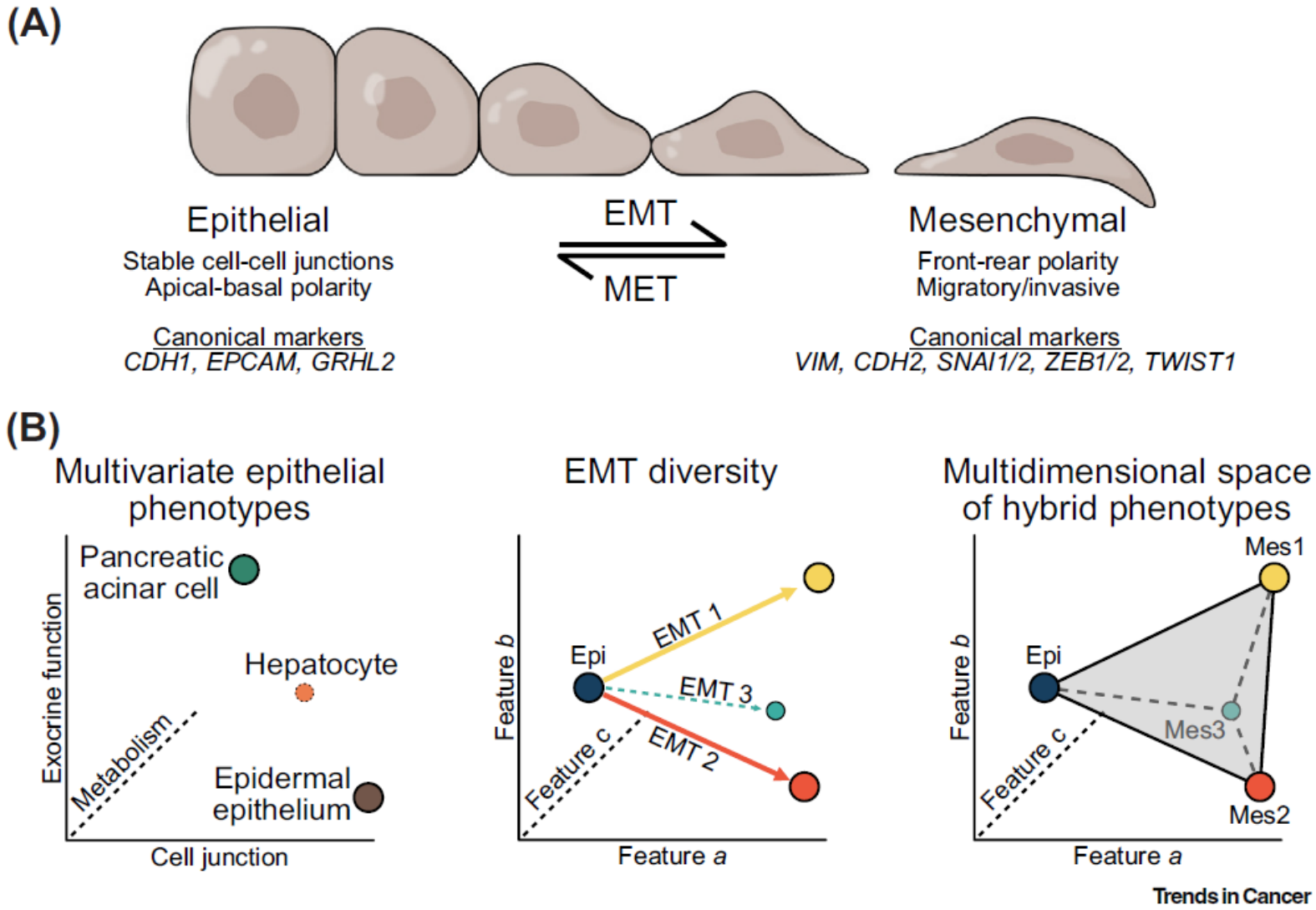


Trends in Cell Biology

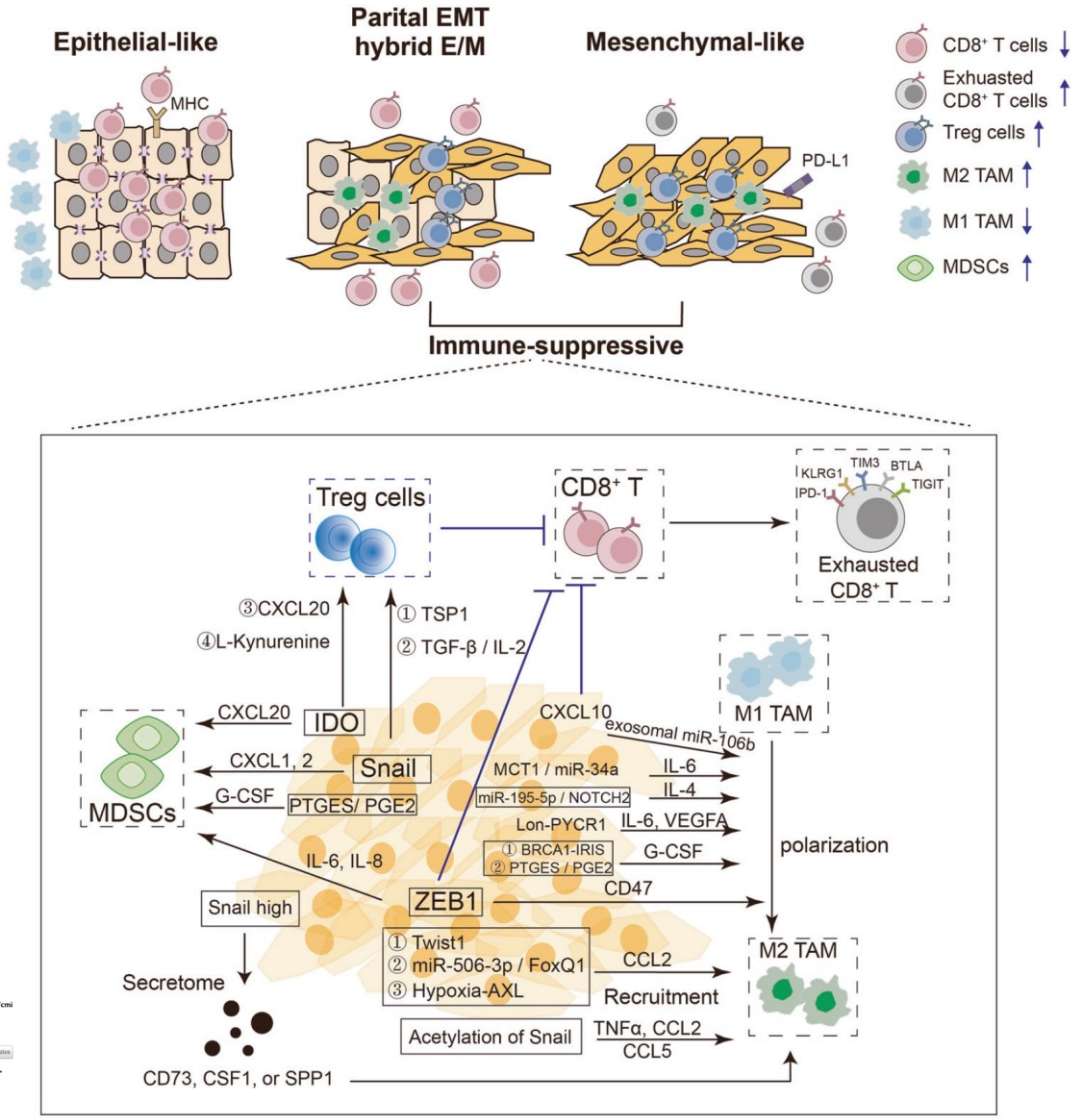
Mechanismy karcinogeneze



Nádorová plasticita

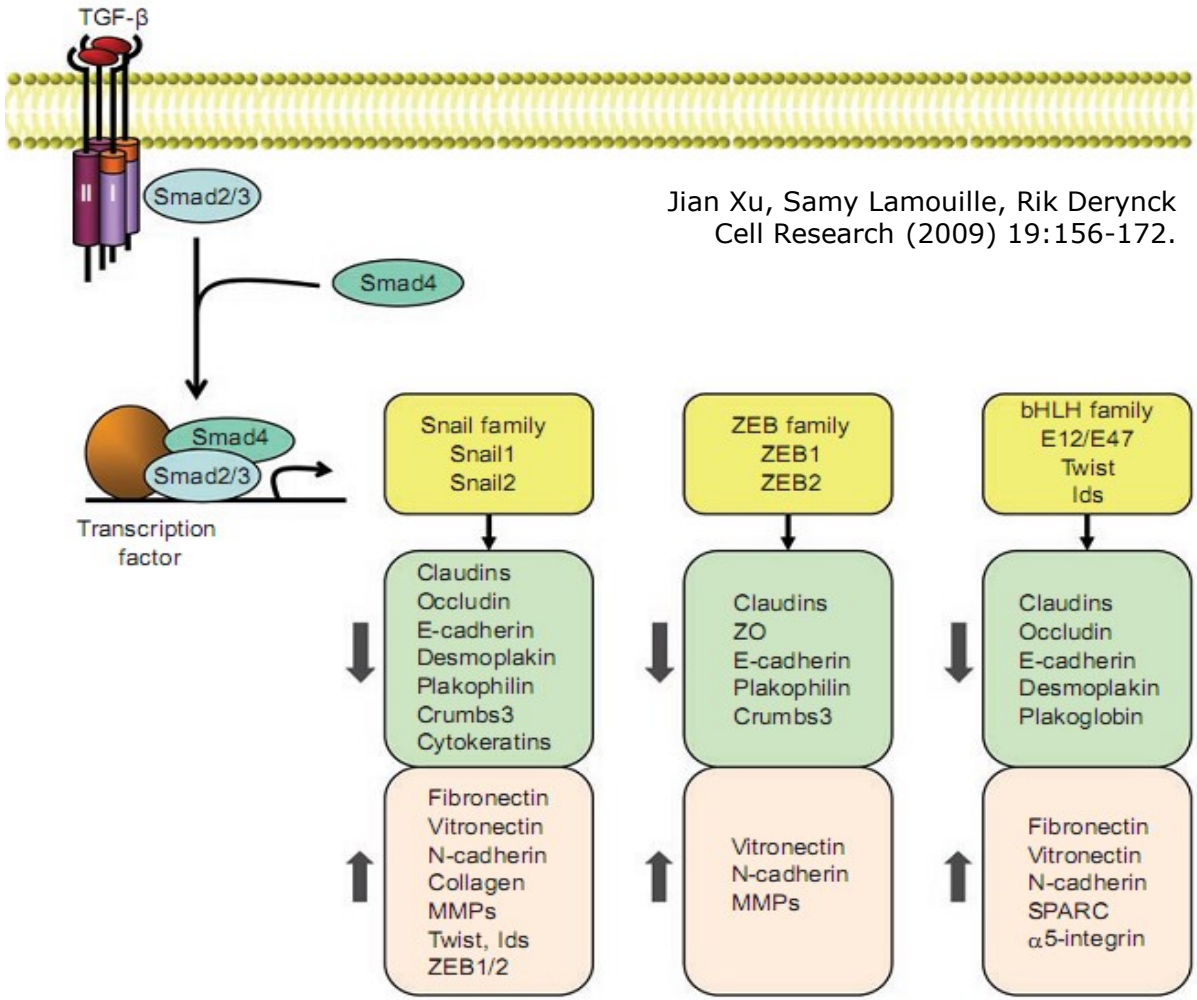


EMP ovlivňuje interakce mezi nádorovými buňkami a imunitními buňkami a utváří imunosupresivní TME.



CMI
www.nature.com/cmi
REVIEW ARTICLE OPEN
Harnessing epithelial-mesenchymal plasticity to boost cancer immunotherapy
Yuanzhuo Gu[1,4], Zhengkui Zhang[2,3,4] and Peter ten Dijke[1]✉

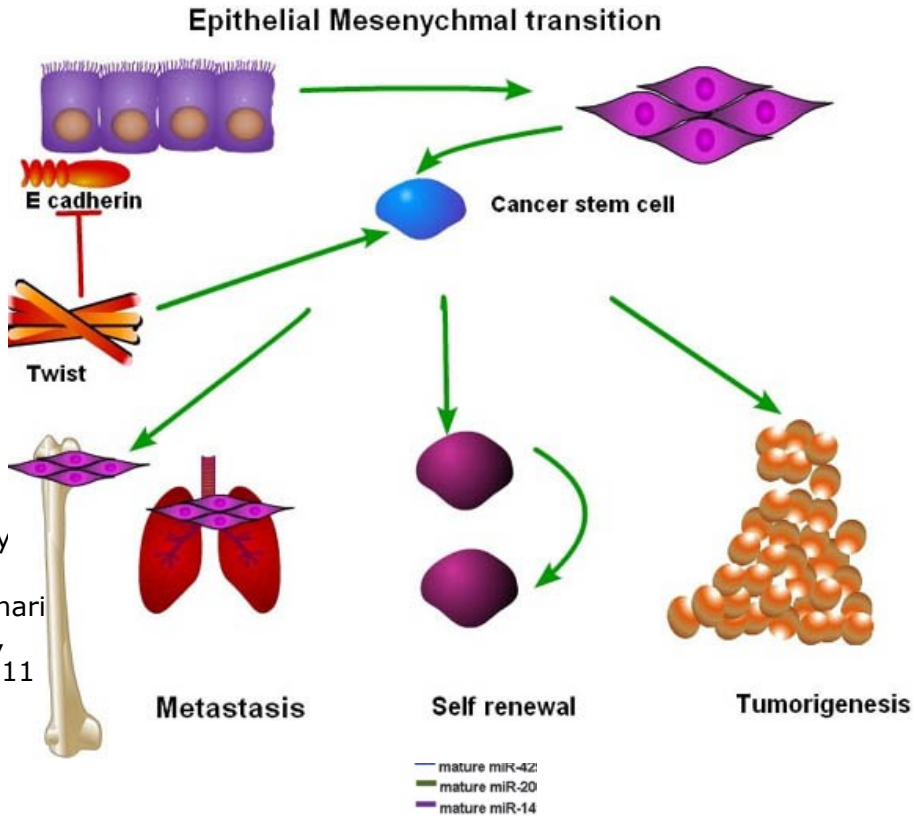
Transforming growth factor-β (TGF-β)

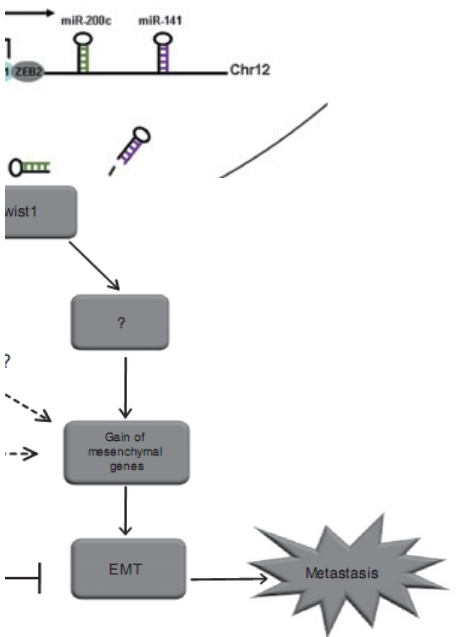


Jian Xu, Samy Lamouille, Rik Derynck
Cell Research (2009) 19:156-172.

Klíčové objevy v EMT a rakovině

- EMT creates cells with cancer stem cell characteristics
  Mani SA, et al., Cell. 2008 May 16;133(4):704-15.
- Cross-regulation between ZEB1/2 and miR-200 family
  Gregory PA, et al., Nat Cell Biol. 2008 May;10(5):593-601.
- Cross-regulation between Twist and Slug





Esmeralda Casas, Jihoon Kim, Andrés Bendesky, et Cancer Res; 71(1) January 2011
Prachi Jain, Suresh K. Alahari Frontiers in Bioscience 16, 1824-1832, January 1, 2011

H Zhang, Y Li and M Lai *Oncogene* **29**, 937-948 (18 February 2010)

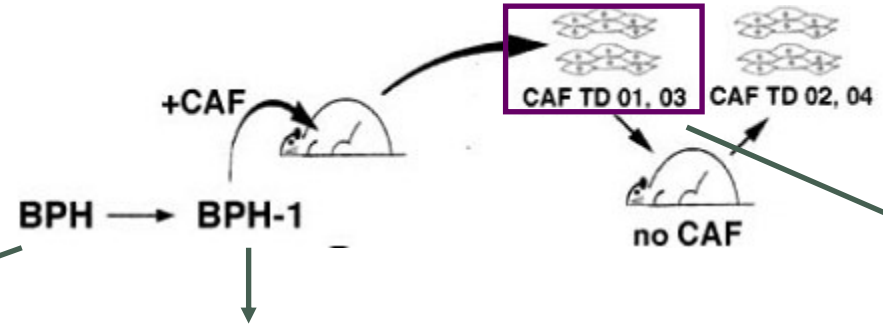
Experimentální přístupy

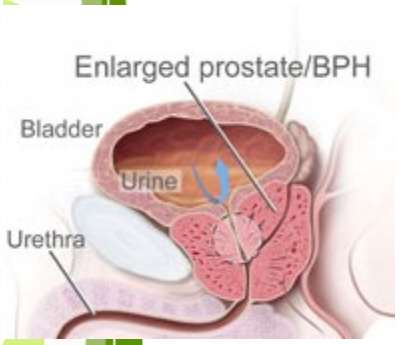
ESTABLISHMENT AND CHARACTERIZATION OF AN IMMORTALIZED BUT NON-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELL LINE: BPH-1

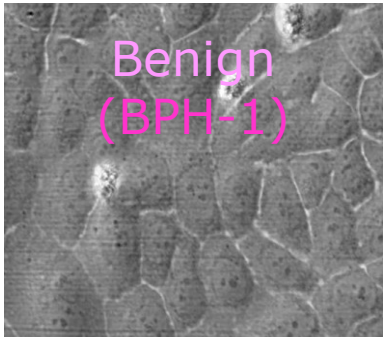
S. W. HAYWARD, R. DAHIYA, G. R. CUNHA, J. BARTEK, N. DESHPANDE, AND P. NARAYAN

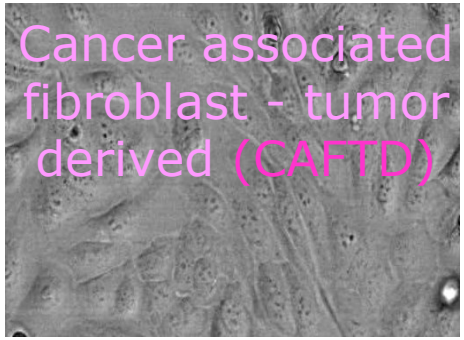
Malignant Transformation in a Nontumorigenic Human Prostatic Epithelial Cell Line[1]

Simon W. Hayward,[2] Yuzhuo Wang, Mei Cao, Yun Kit Hom, Baohui Zhang, Gary D. Grossfeld, Daniel Sudilovsky, and Gerald R. Cunha









EMT

TGF-β

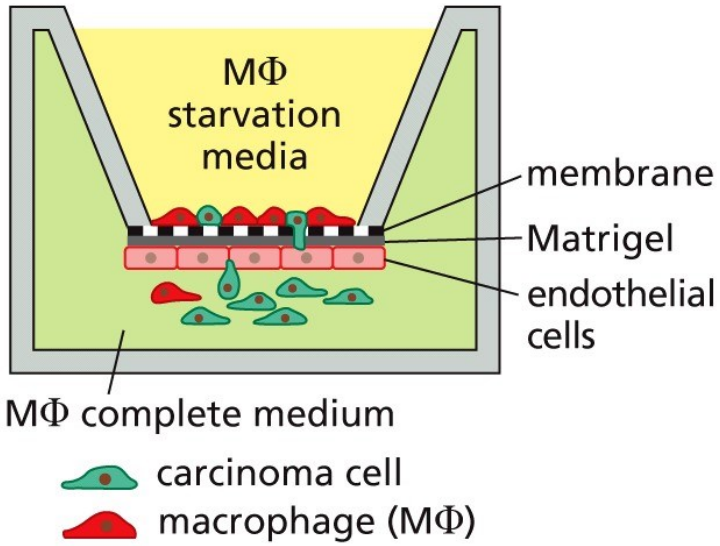
EMT

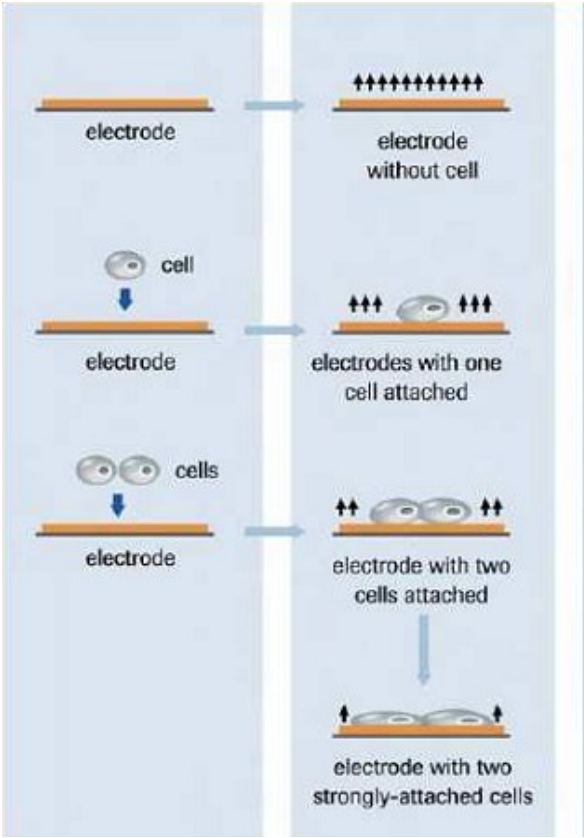
TGF-β

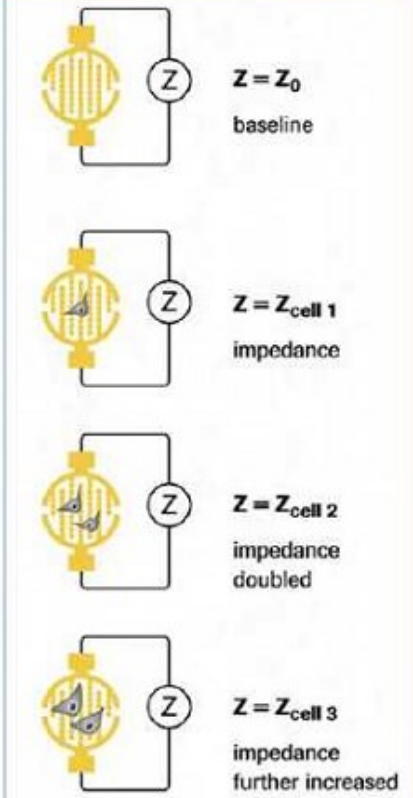
EMT

- EMT markers
- EMT regulators
- Cell shape and behavior

Analýza migračního potenciálu







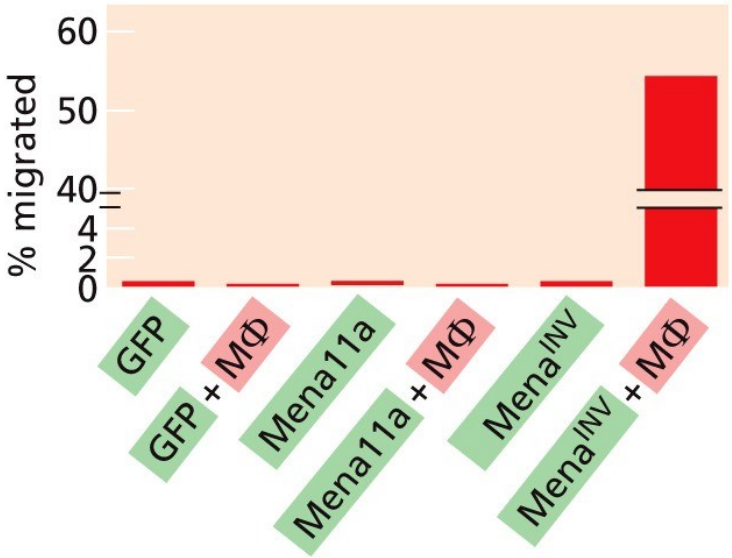
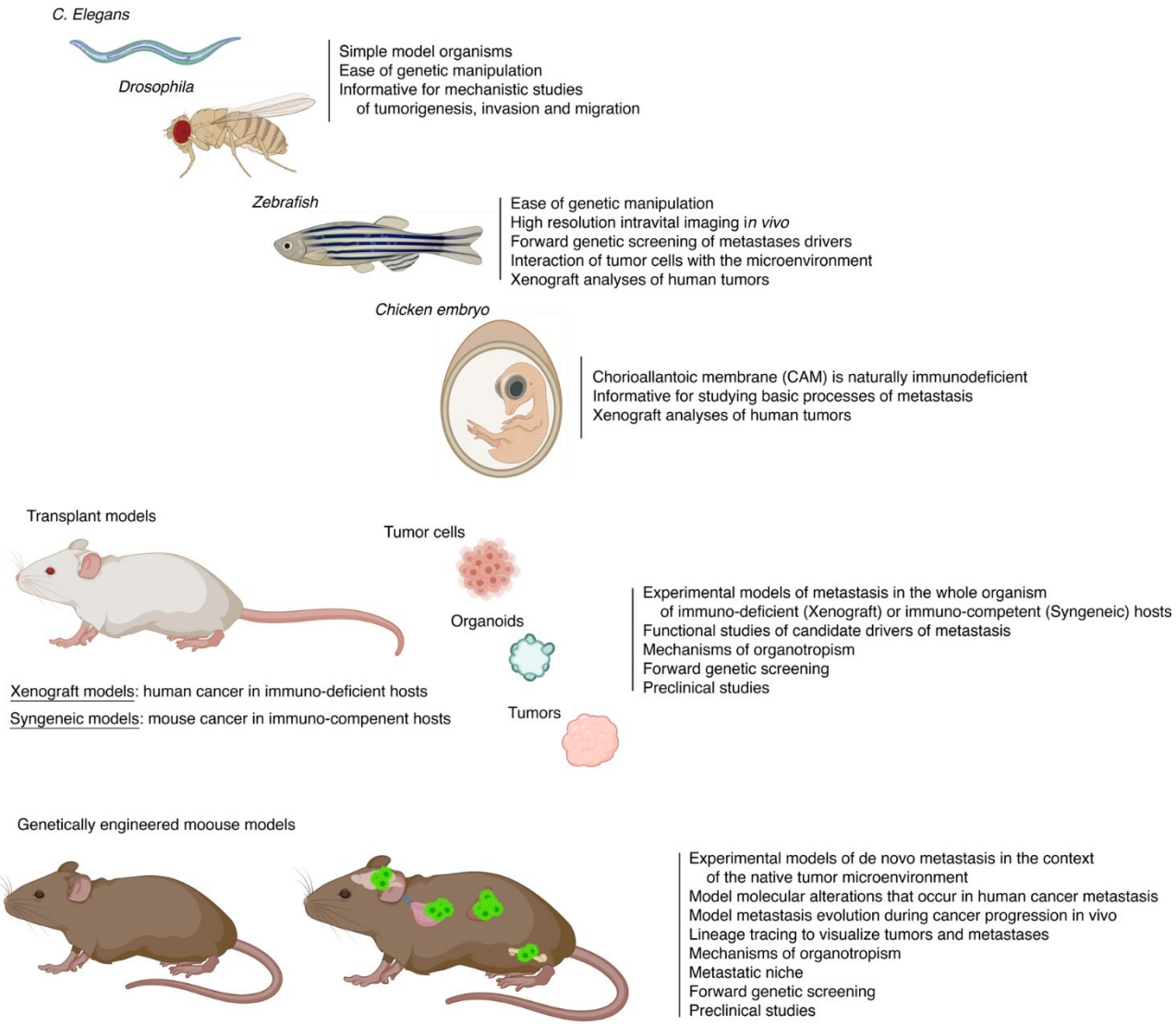


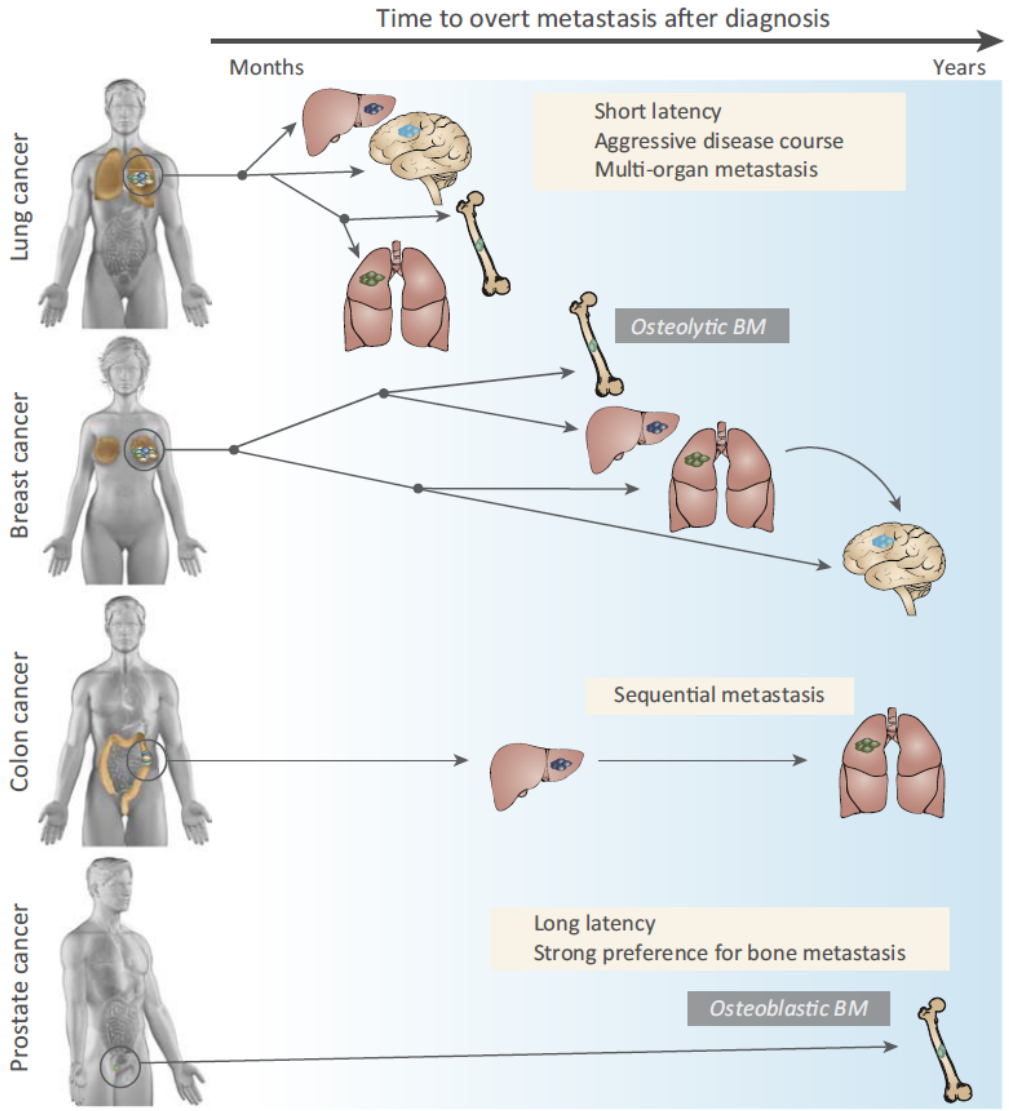
Figure 14.41c The Biology of Cancer (© Garland Science 2014)

Modelové organismy pro studium metastázování



Trends Cancer. 2021 Oct; 7(10): 916–929.

Diseminace solidních nádorů



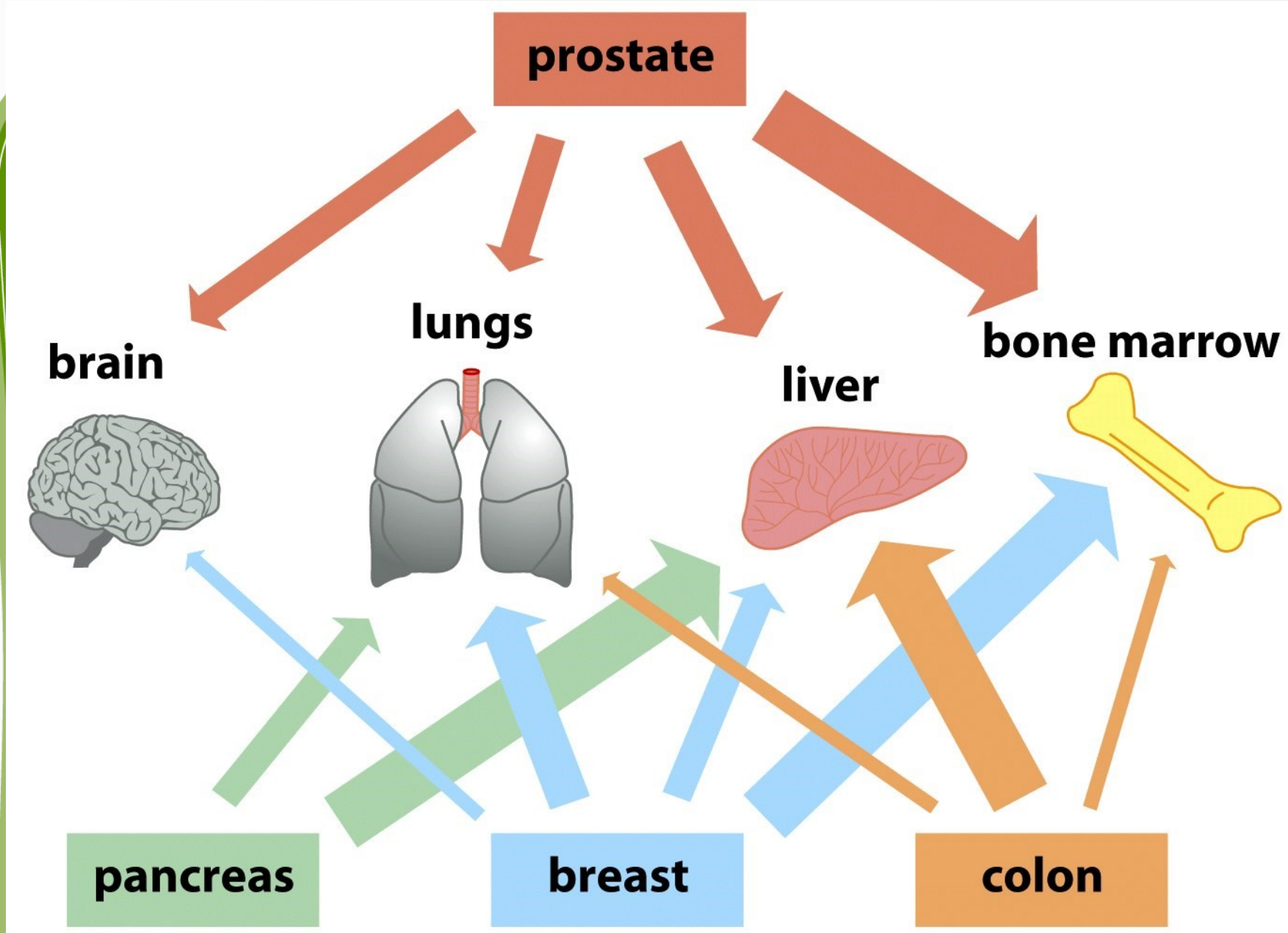


Figure 14.42 *The Biology of Cancer* (© Garland Science 2007)

Metastatický tropismus

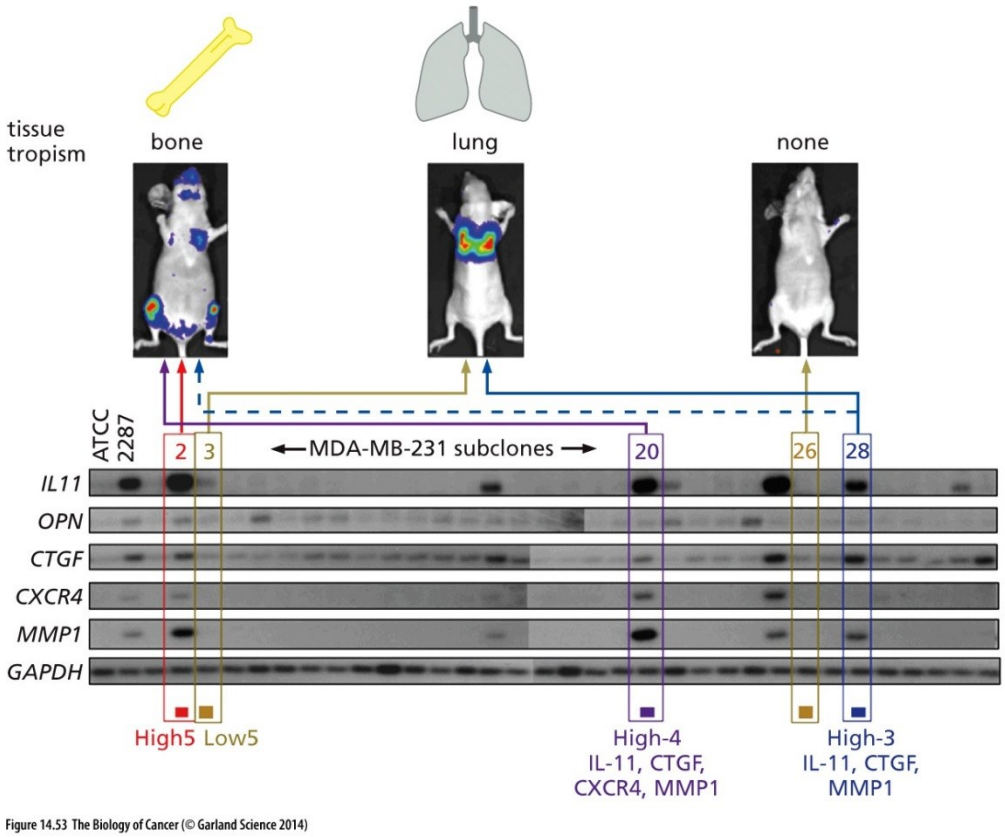
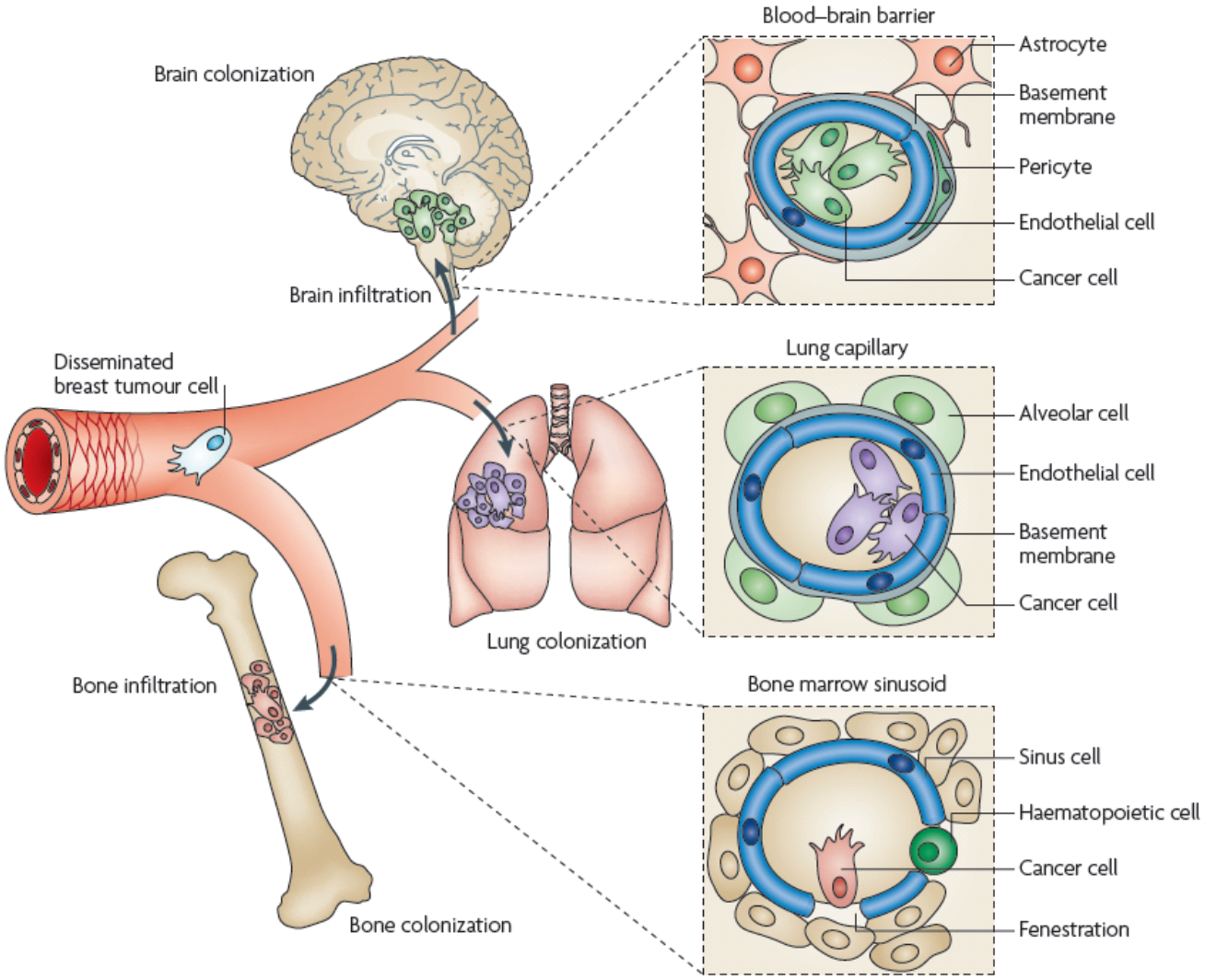


Figure 14.53 The Biology of Cancer (© Garland Science 2014)

- Thirty-three cells from a large population of human MDA-MB-231 cells were each expanded into a clonal population in culture. The mRNA expression pattern of each subclone was analyzed (*columns, arrayed left to right*) using probes for the mRNAs of five genes—*IL11* (interleukin-11), *OPN* (osteopontin), *CTGF* (connective tissue growth factor), *CXCR4* (chemokine receptor 4), and *MMP1* (matrix metalloproteinase-1)—and, as loading control, a probe for *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) mRNA. In addition, the expression patterns of the original tumor cell population (ATCC, *left column*) and a subcloned cancer cell population termed 2287 (which was selected for its ability to generate osteolytic metastases; *2nd column*) were analyzed. The five experimental genes were chosen because of their overexpression in osteotropic metastatic cells and their known biological properties in promoting osteolytic metastases. Clone 2 cells (*red box*), when injected into the arterial circulation of mice, showed a tendency to produce osteotropic metastases, as indicated by *in vivo* imaging; these cells expressed high levels of all five experimental mRNAs. Clone 3 cells (*yellow box*), in contrast, expressed low levels of all five mRNAs and preferentially formed lung metastases. And clone 26 genes (*yellow box*), which expressed essentially none of these mRNAs, formed no metastases at all. Moreover, when otherwise poorly metastatic cells were forced to express combinations of three of these genes, they acquired the ability to form bone metastases efficiently (*not shown*), pointing to the causal role of these genes in forming these metastases. Metastases were visualized through the presence of a luciferase gene in the tumor cells, which causes cells to release a bioluminescent signal. (From Y. Kang et al., *Cancer Cell* 3:537–549, 2003.)

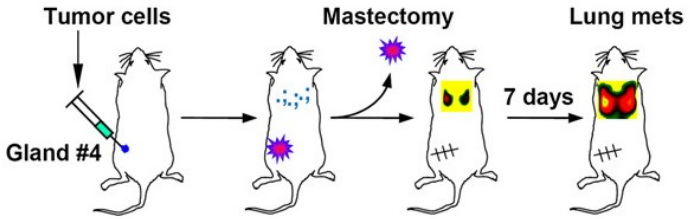
Orgánově specifické bariéry



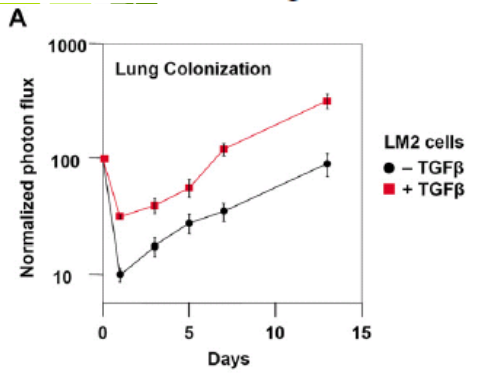
Cell

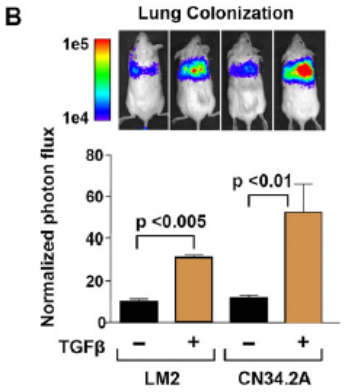
Cell 133, 66–77, April 4, 2008

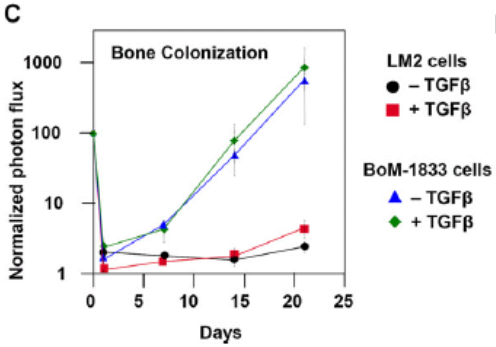
TGFβ Primes Breast Tumors for Lung Metastasis Seeding through Angiopoietin-like 4

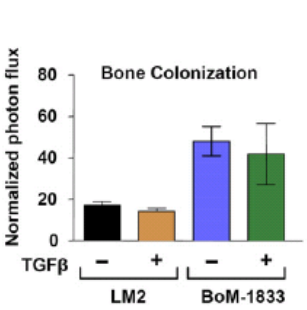


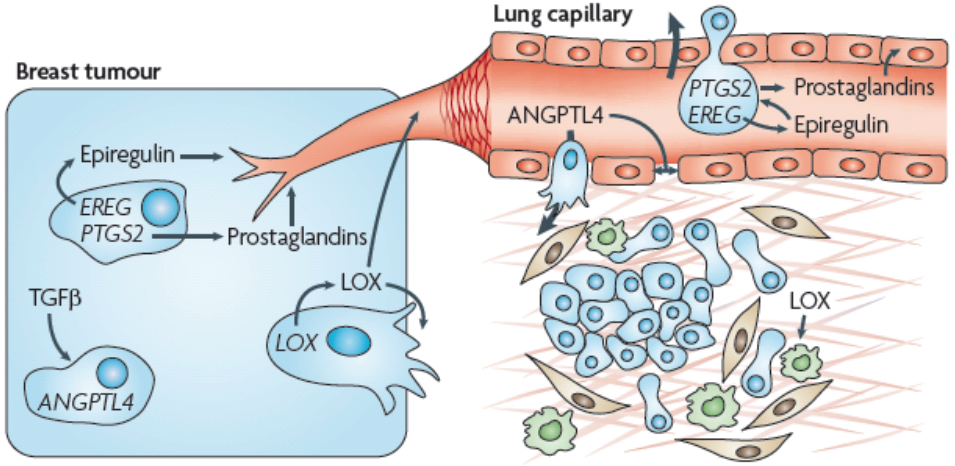
David Padua,[1] Xiang H.-F. Zhang,[1] Qiongqing Wang,[1] Cristina Nadal,[5] William L. Gerald,[2] Roger R. Gomis,[4] and Joan Massagué[1,3,*]









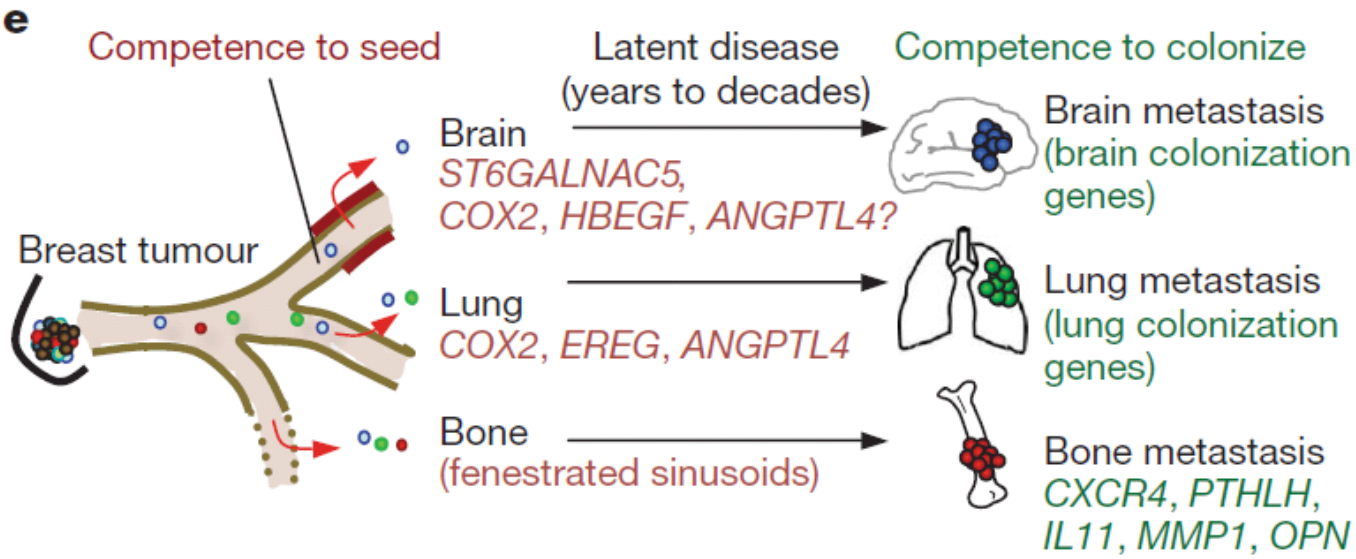


Vol 459|18 June 2009|doi:10.1038/nature08021 nature

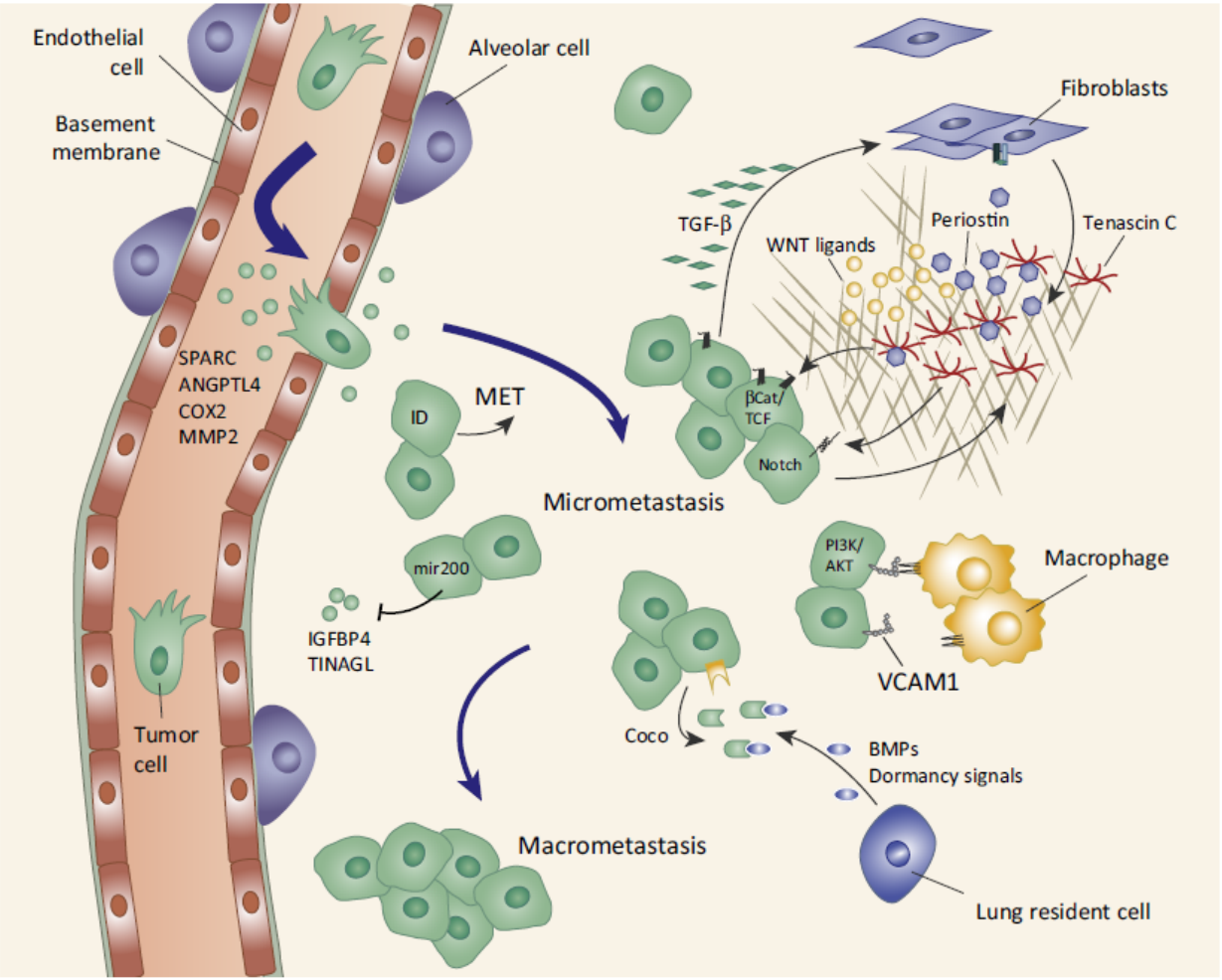
LETTERS

Genes that mediate breast cancer metastasis to the brain

Paula D. Bos[1], Xiang H.-F. Zhang[1], Cristina Nadal[1]†, Weiping Shu[1], Roger R. Gomis[1]†, Don X. Nguyen[1], Andy J. Minn[2], Marc J. van de Vijver[3], William L. Gerald[4], John A. Foekens[5] & Joan Massagué[1,6]

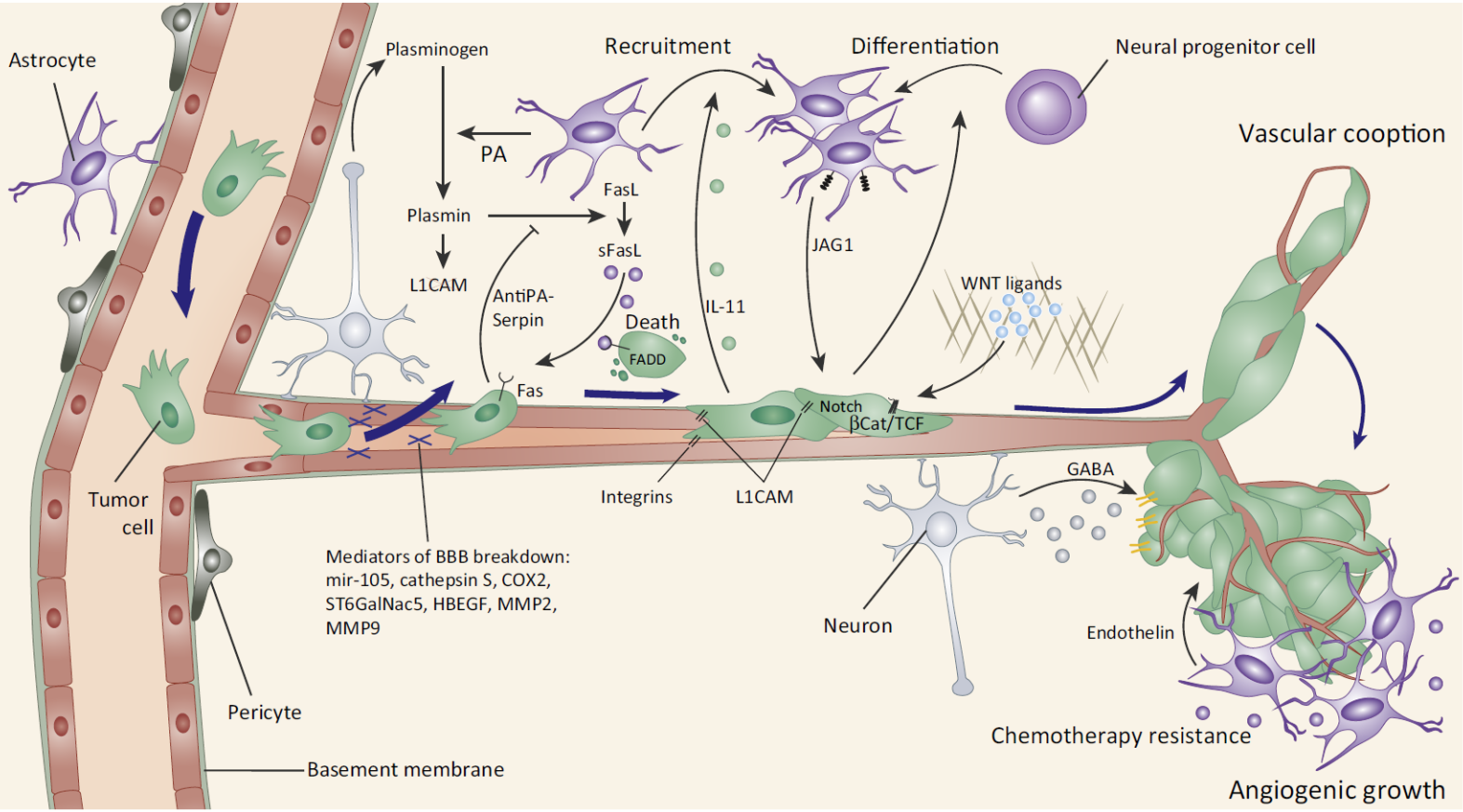


Metastatická kolonizace plic



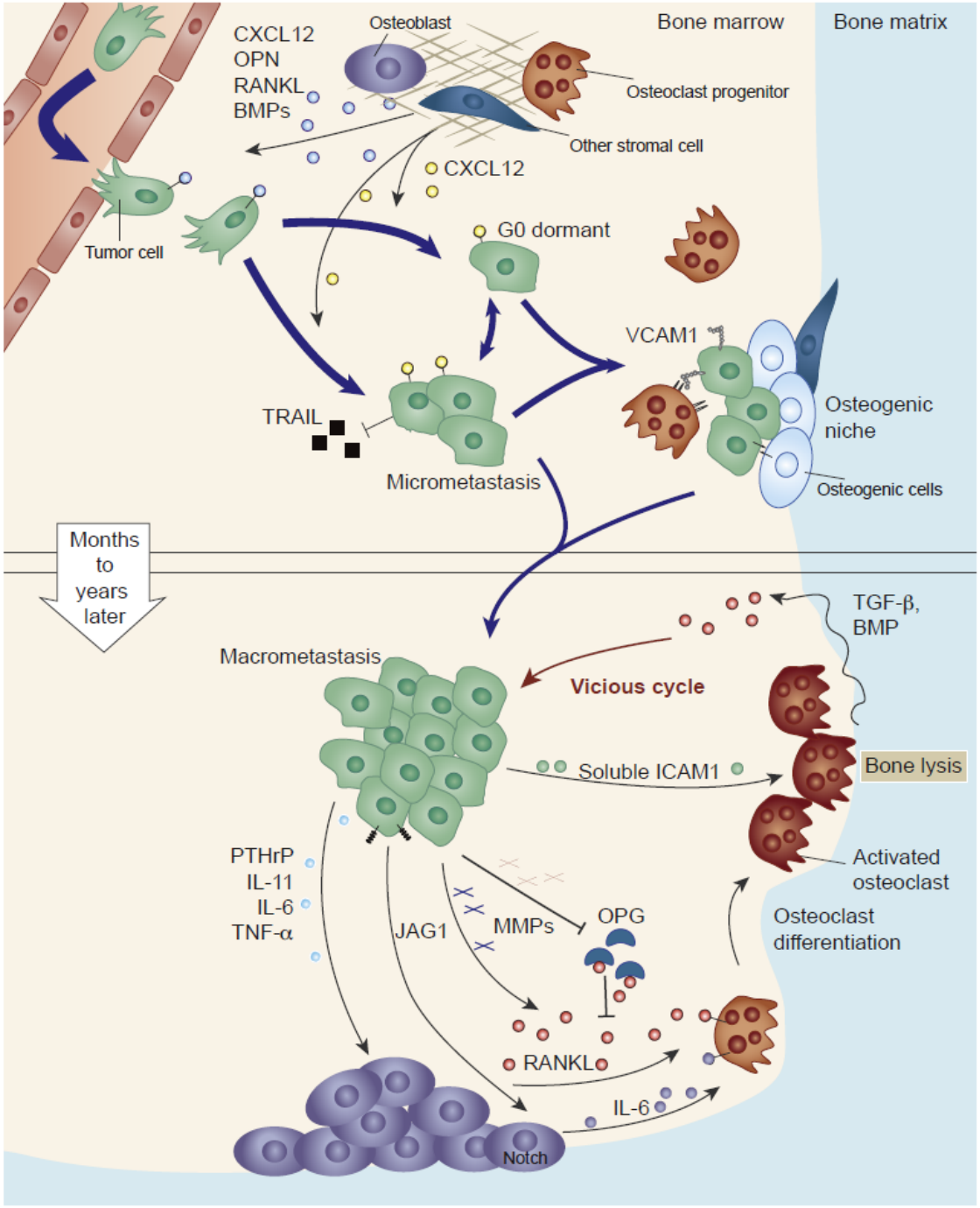
Trends in Cancer

Metastatická kolonizace mozku



Trends in Cancer

Osteotropní metastáze



Trends in Cancer

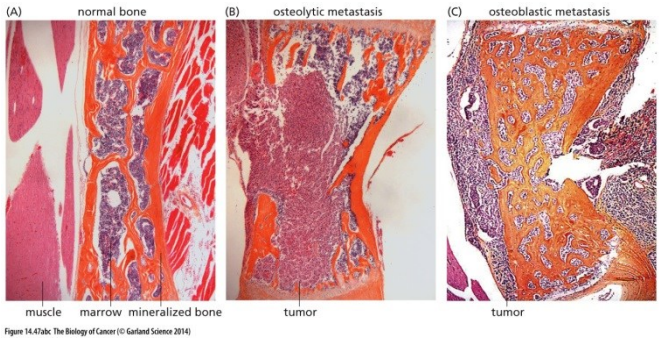


Figure 14.47abc The Biology of Cancer (© Garland Science 2014)

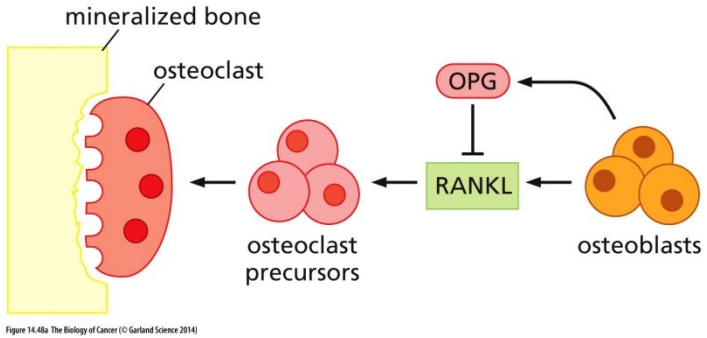


Figure 14.48a The Biology of Cancer (© Garland Science 2014)

Osteotropní metastáze

- Parathyroid hormone-related peptide (PTHrP)
- Během laktace produkován normálními MECs - mammary epithelial cells – > mobilizace vápníku
- Nádorové buňky - adaptují tento mechanismus – osteolýza kostí vede k uvolnění řady růsových faktorů stimulujících nádorové buňky

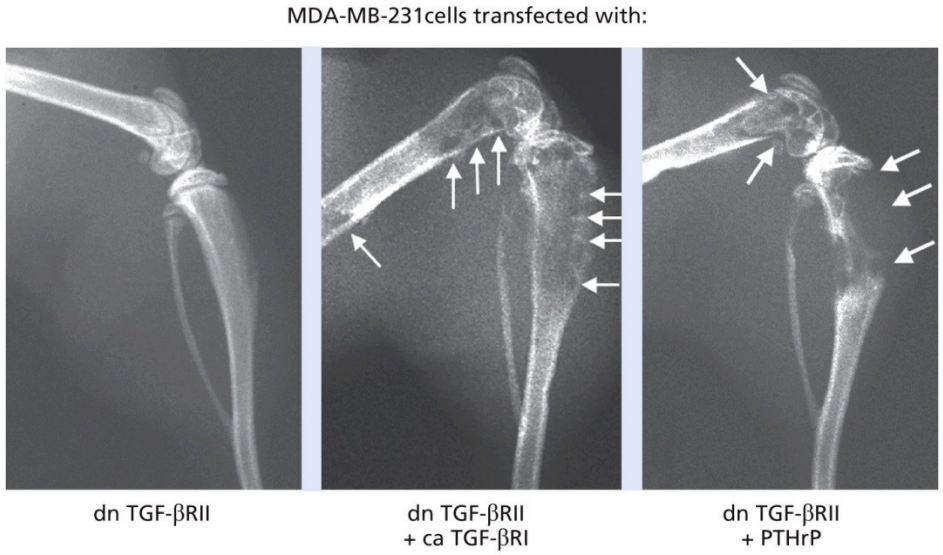


Figure 14.49 The Biology of Cancer (© Garland Science 2014)

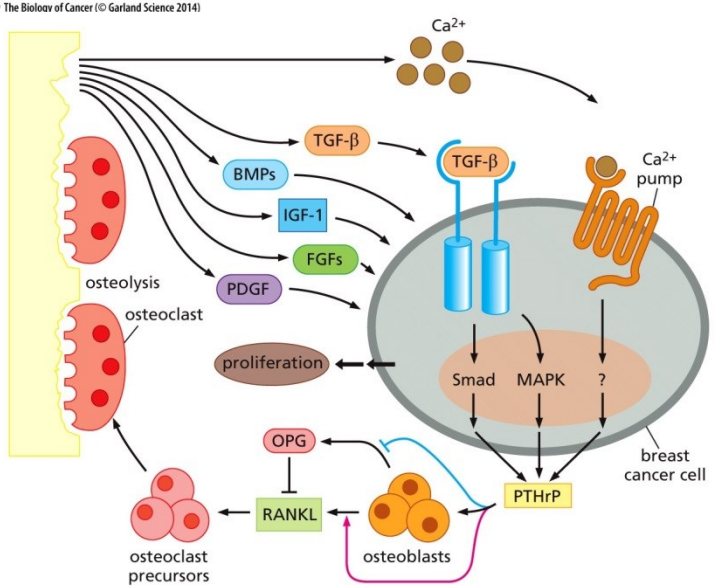
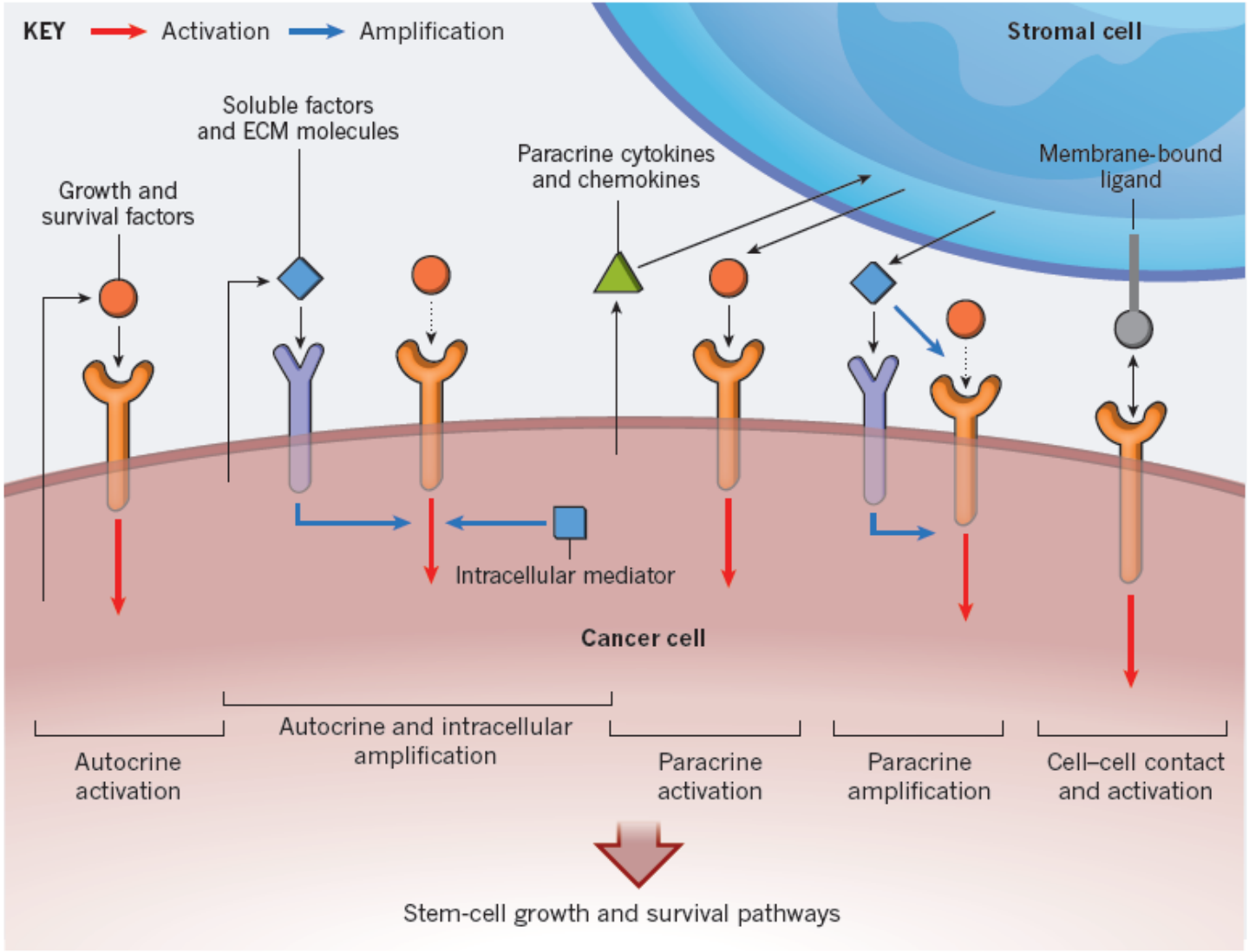
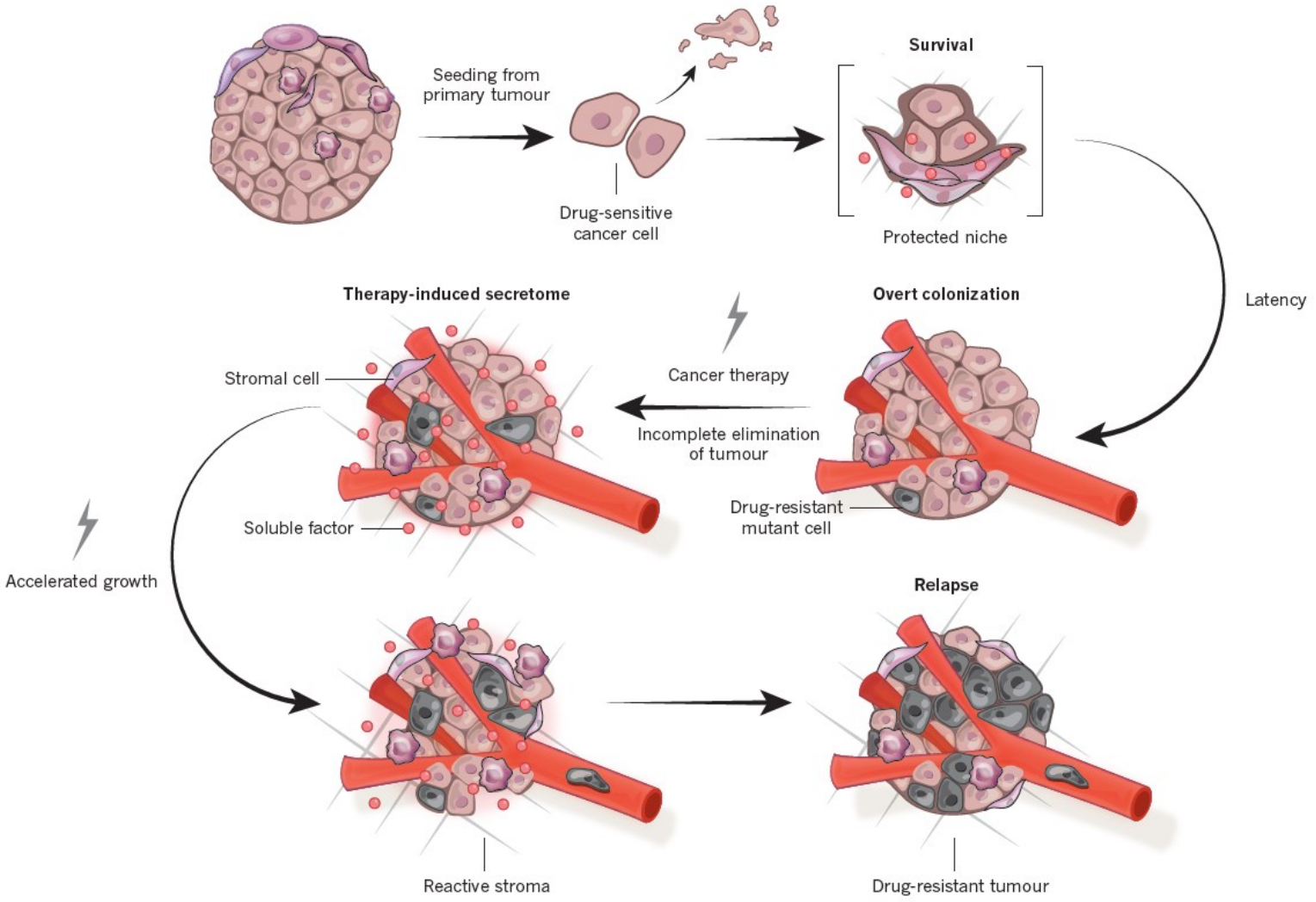


Figure 14.48b The Biology of Cancer (© Garland Science 2014)

Aktivace růstových signálů

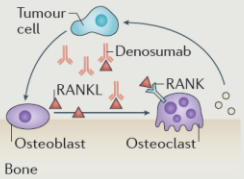
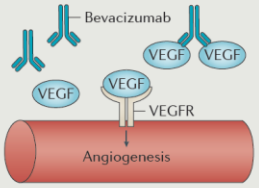
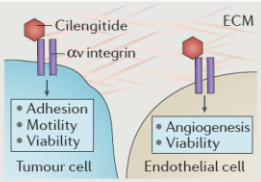
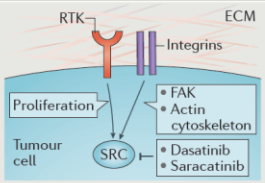


Metastáze před a po terapii



Terapie cílená na metastázování

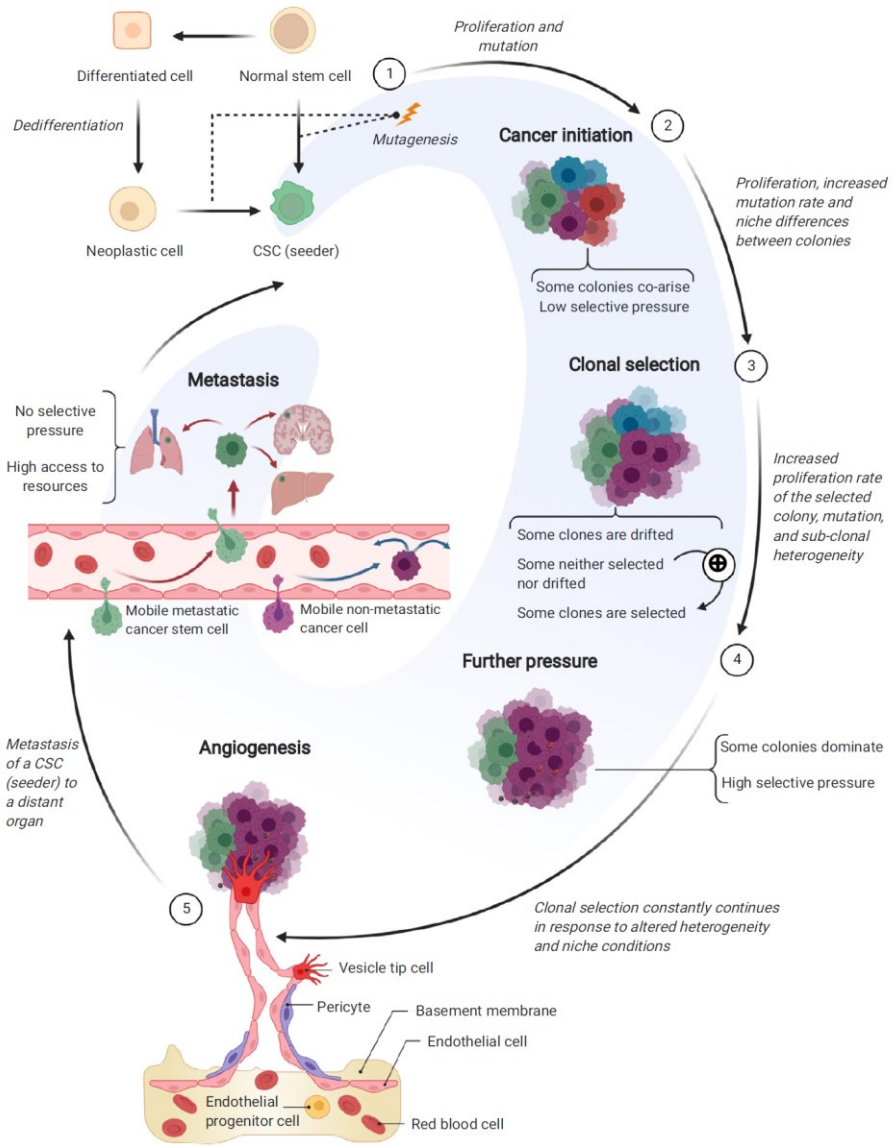
Table 1 | **Preclinical and clinical history of four metastasis-directed drug development efforts**

Description	Pathway	Preclinical validation	Pivotal trials and end points	Outcomes
Denosumab				
Monoclonal antibody to RANKL		RANKL activates osteoclasts and promotes bone destruction; denosumab reduced bone resorption in mice expressing human RANKL[29]	SREs* in metastatic setting; adjuvant trials used time to first bone metastasis or fracture[30–33]	FDA approved for prevention of SREs in solid tumours; approved as adjuvant therapy in prostate cancer
Bevacizumab				
Monoclonal antibody to VEGF		• Bevacizumab inhibited corneal angiogenesis and lymphangiogenesis[244] • In multiple cancer xenograft models, bevacizumab reduced primary tumour growth rates and, in some studies, enhanced survival. Reduced angiogenesis and vessel normalization was observed[245] • Prevention or, less frequently, abrogation of metastasis[246,247]	• Recurrent ovarian cancer, PFS[35,36] • Metastatic colorectal cancer, OS[260,261] • Metastatic or resistant HER2⁺ breast cancer, PFS[38] • Metastatic renal cancer, PFS[262] • Glioblastoma, OS, PFS[263] • Advanced lung cancer, OS[37] • Adjuvant therapy in triple-negative breast cancer, DFS[41]	• FDA approved for resistant ovarian, cervical and colorectal cancers, glioblastoma, also advanced or metastatic lung, colorectal and renal cancers • Revoked for metastatic breast cancer • Negative trials for first-line treatment of glioblastoma
Cilengitide				
αvβ3 and αvβ5 integrin peptide inhibitor		• Stabilization of glioma growth and angiogenesis. Synergistic inhibition of glioma with TMZ[61–64] • Synergy with therapeutics in melanoma primary tumour growth[63], synergy with radio-immunotherapy in breast cancer tumour growth[248] • Inhibition of metastasis[62] • Synergy with verapamil increased angiogenesis and reduced metastasis[249]	• Phase III CENTRIC EORTC, with radiation therapy and TMZ, for glioma, OS. Newly diagnosed glioma, same combination, recurrence[65] • Phase II trials in melanoma and lung and prostate cancers, PFS[66–68]	All advanced trials were negative
Dasatinib and saracatenib				
SRC kinase and BCR–ABL kinase inhibitor		• Inhibition of CML models[250] • Inhibition of primary tumour growth in multiple model systems, as monotherapy or in combination[251–253] • Prevention of metastasis in multiple cancer model systems[254–258], but not osteosarcoma[259] • Inhibition of prostate cancer growing in bone and bone remodelling[82,83]	• Cytogenetic response end points for CML • Response for advanced solid tumours[71–80] • OS in Phase III prostate cancer[87]	• FDA approved for CML and resistant ALL • Discontinued in advanced lung, ovarian, colorectal and breast cancers • Negative in prostate cancer Phase III trial with docetaxel • Multiple adjuvant trials terminated

ALL, acute lymphoblastic leukaemia; CML, chronic myelogenous leukaemia; DFS, disease-free survival; ECM, extracellular matrix; FAK, focal adhesion kinase; FDA, US Food and Drug Administration; OS, overall survival; PFS, progression-free survival; RANK, receptor activator of NF-κB; RANKL, RANK ligand; RTK, receptor tyrosine kinase; TMZ, temozolomide; VEGF, vascular endothelial growth factor. *Skeletal-related event (SRE) captures the deleterious effects of new lesions and progression of existing lesions to cause patient morbidity.

NATURE REVIEWS | CANCER

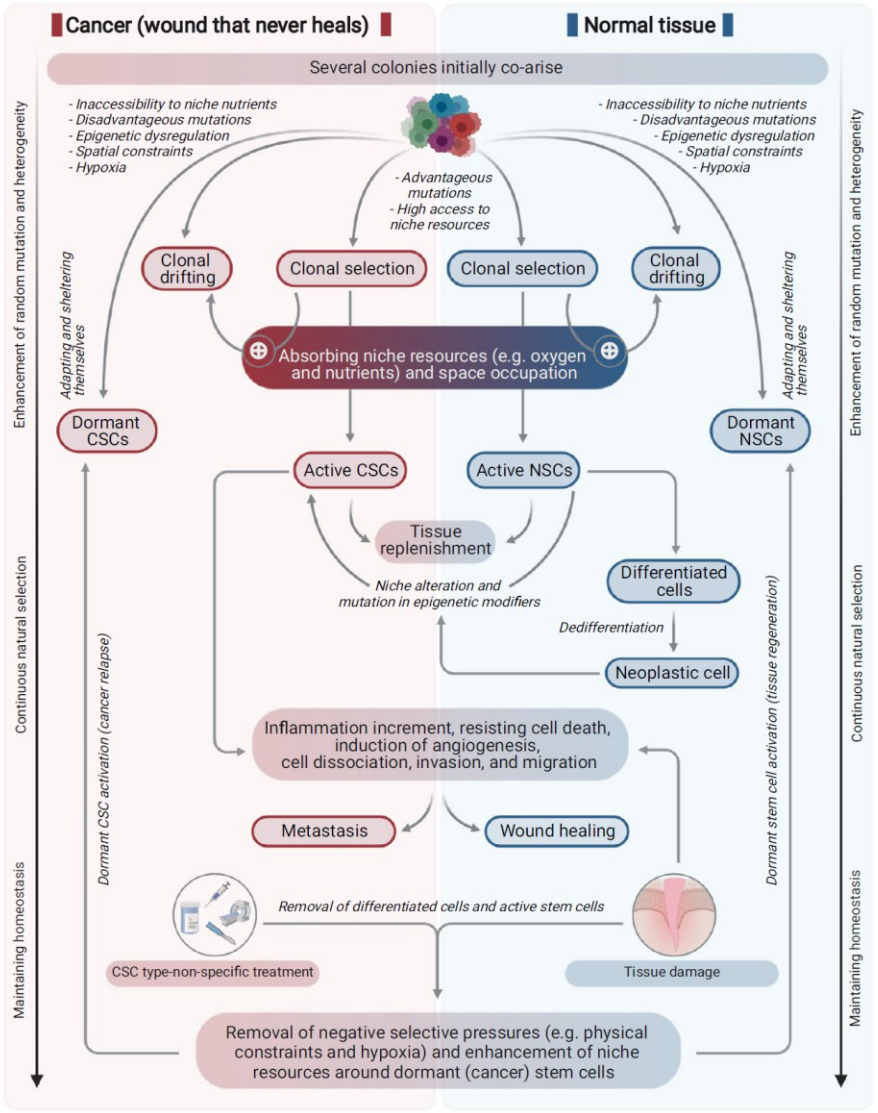
Selektivní tlak řídí proces selekce a driftingu během vývoje rakoviny



Trends in Genetics

https://doi.org/10.1016/j.tig.2023.01.007

Paralely v klonální selekci/driftu mezi vývojem rakoviny a normální tkáně a jejich přechodem.



https://doi.org/10.1016/j.tig.2023.01.007

Shrnutí

- 90% úmrtí na nádorová onemocnění souvisí s metastázováním
- Invazivní kaskáda zahrnuje: lokální invazi, intravazaci, transport, extravazaci, formovaní mikrometastáz a kolonizaci
- Nízká efektivita celé kaskády, nejméně efektivní je kolonizace
- EMT, řízena pleiotropními TF v různých fázích embryogeneze, adaptována během tumorigeneze
- Motilita je řízena malými GTPasami, Rho rodina
- Proteázy (MMP) umožňují invazi nádorových buněk, degradace ECM
- Tkáňový tropismus nádorových buněk lze v některých případech vysvětlit organizací oběhového systému, často prozatím neobjasněn