

INVAZIVITA A METASTÁZOVÁNÍ

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Otázky

- Proč je autokrinní signalizace destabilizující faktor pro normální tkáně?
- Jaké mechanismy mohou buňky uplatňovat pro snížení citlivosti k působení růstových faktorů?
- Jaké molekulární mechanismy mohou vést ke konverzi protoonkogenu na onkogen?

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



Proč je rakovina tak devastující?

2012> 2030

WORLDWIDE CANCER CASES
ARE PROJECTED TO INCREASE BY

↑ 50%

FROM 14 million TO 21 million

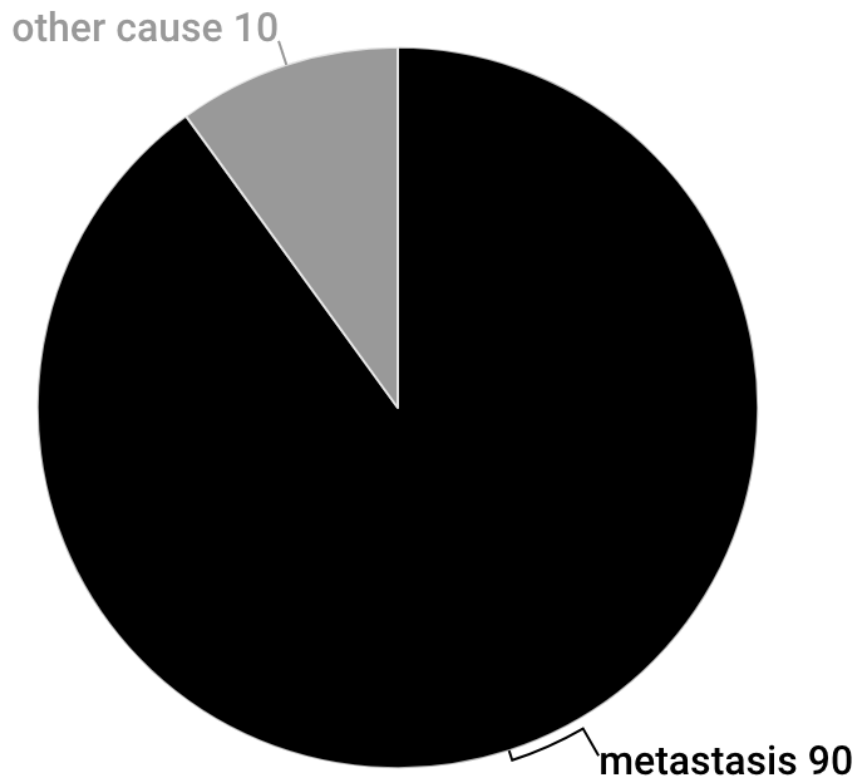
WORLDWIDE CANCER DEATHS
ARE PROJECTED TO INCREASE BY

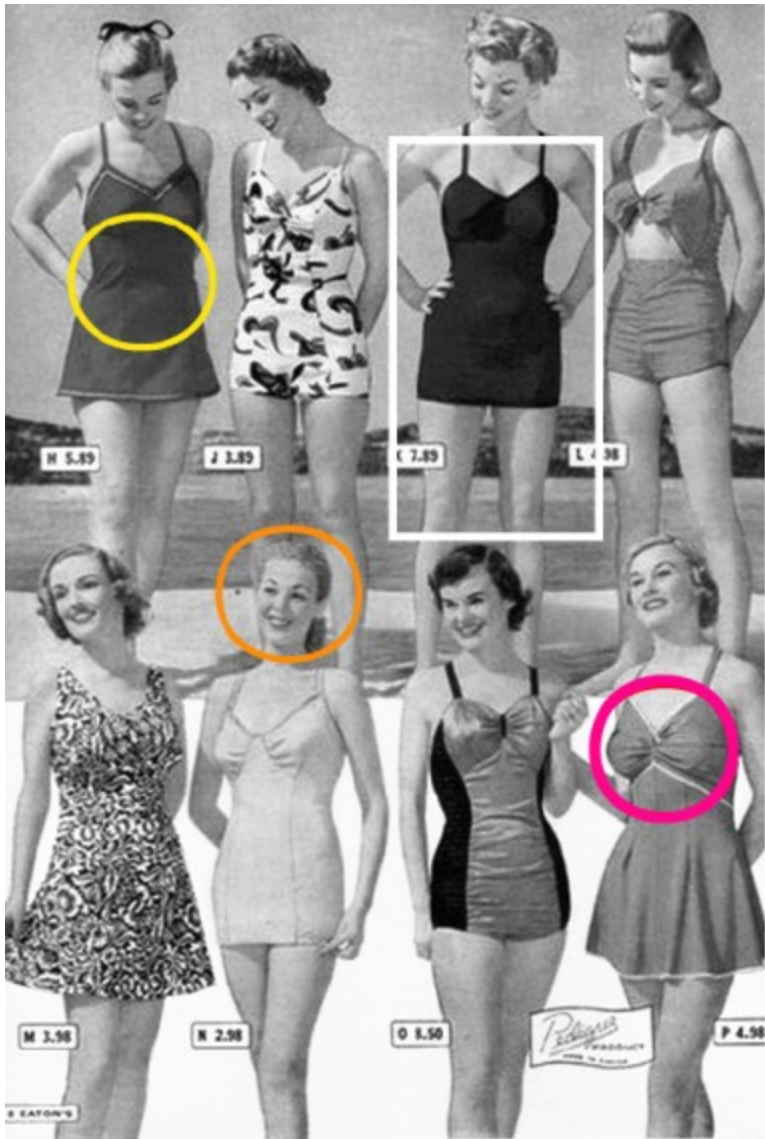
↑ 60%

FROM 8 million TO 13 million

Source: American Cancer Society: Global Cancer Facts & Figures, Second Edition
cancer.gov

cancer-related death cause estimate





MOST COMMON
BREAST CANCER
METASTASES



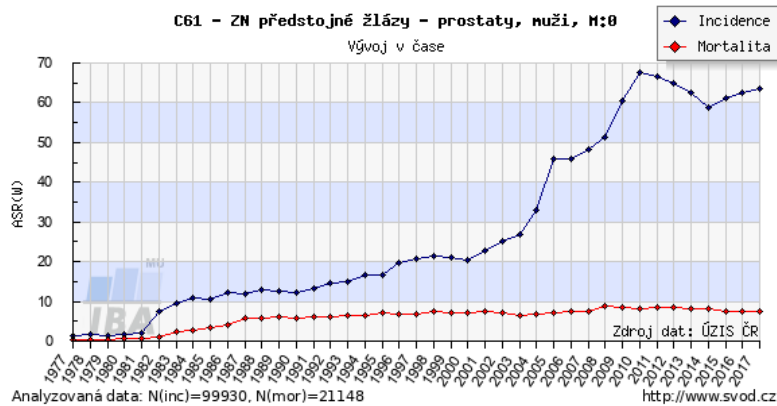
liver
bone
brain
lungs

FB: [AdvocatesForBreastCancer](#) T: [@BreastCancerABC](#)

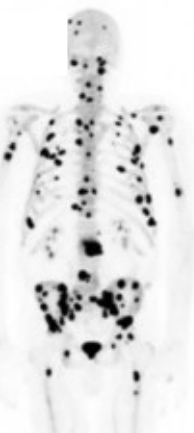
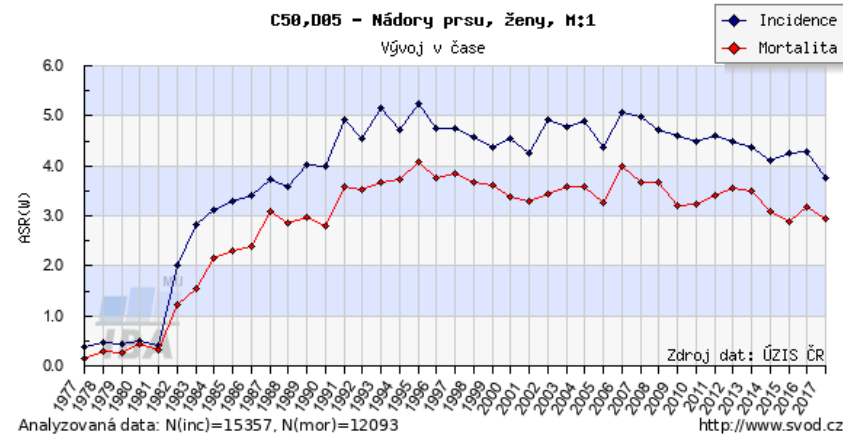
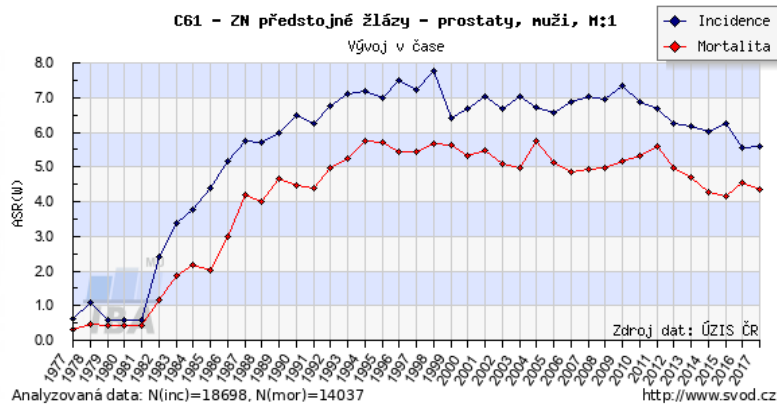
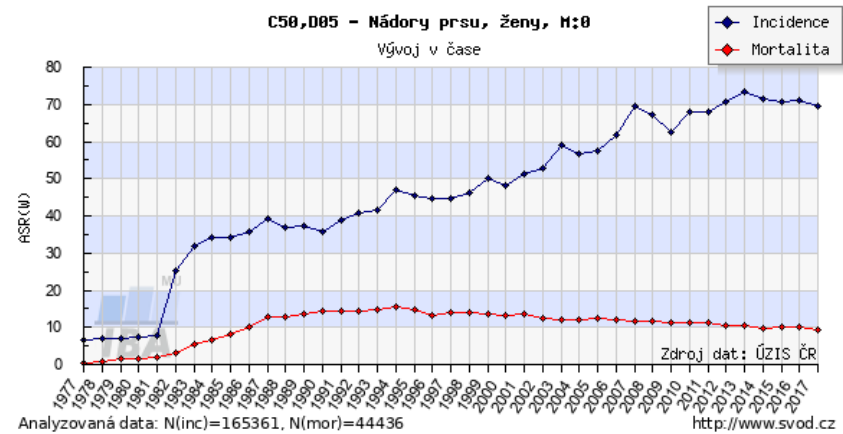
ADVOCATES FOR BREAST CANCER

Proč je rakovina tak devastující?

Nádory prostaty



Nádory prsu

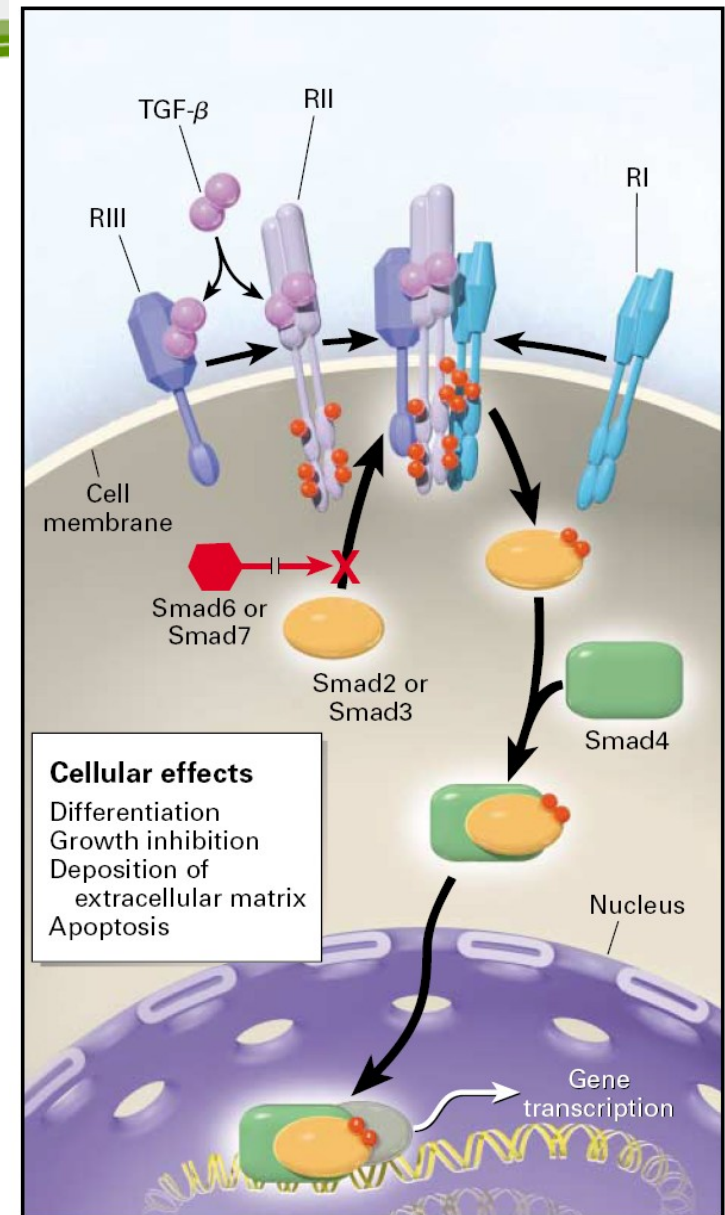


Transforming growth factor - β (TGF- β)

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

TGF- β_1

- pleiotropní cytokin
- negativní regulátor



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 působení TGF- β ;
 - indukce angiogeneze.



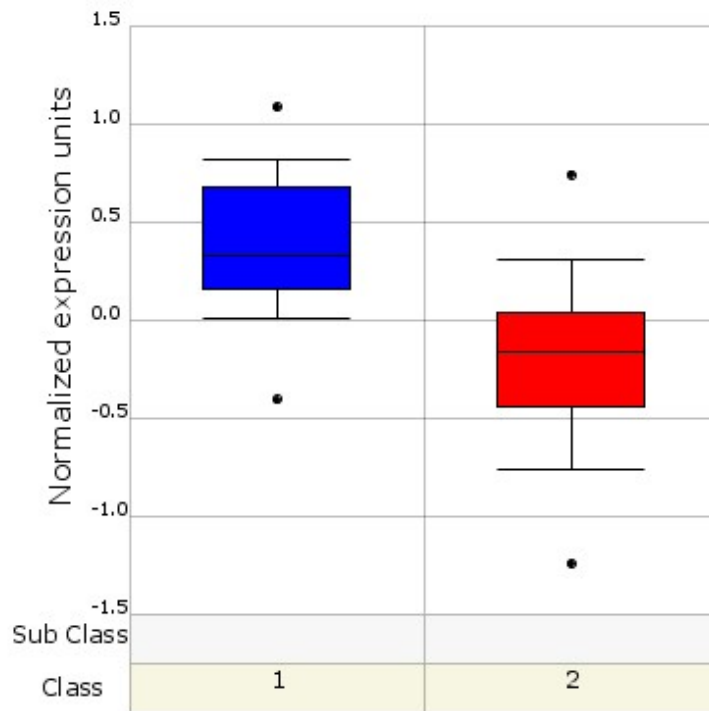
Role TGF- β v carcinogenezi

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

Role TGF- β v carcinogenezi

SMAD3

Smad, mothers against dpp homolog 3 (drosophila)

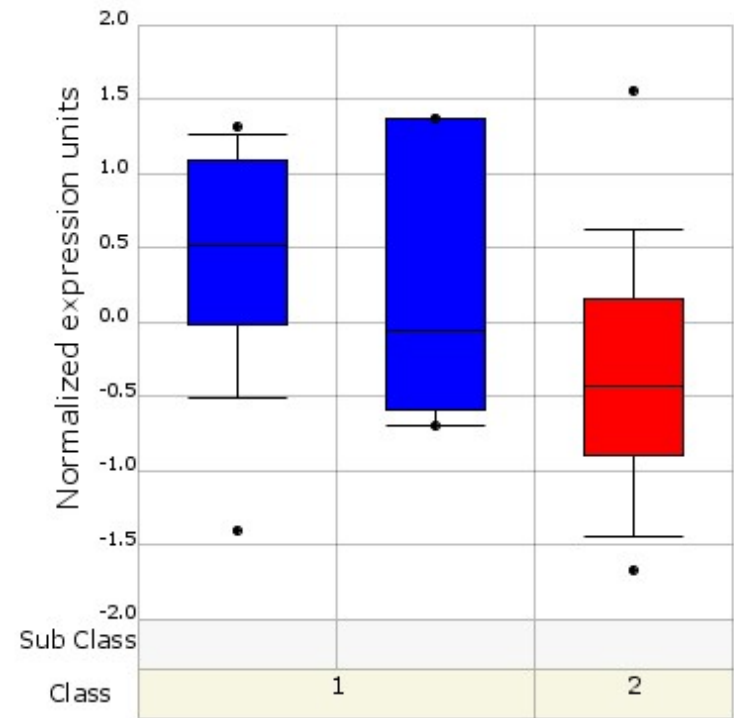


Box Plot - Description

Prostate – normal vs. cancer

TGFBR2

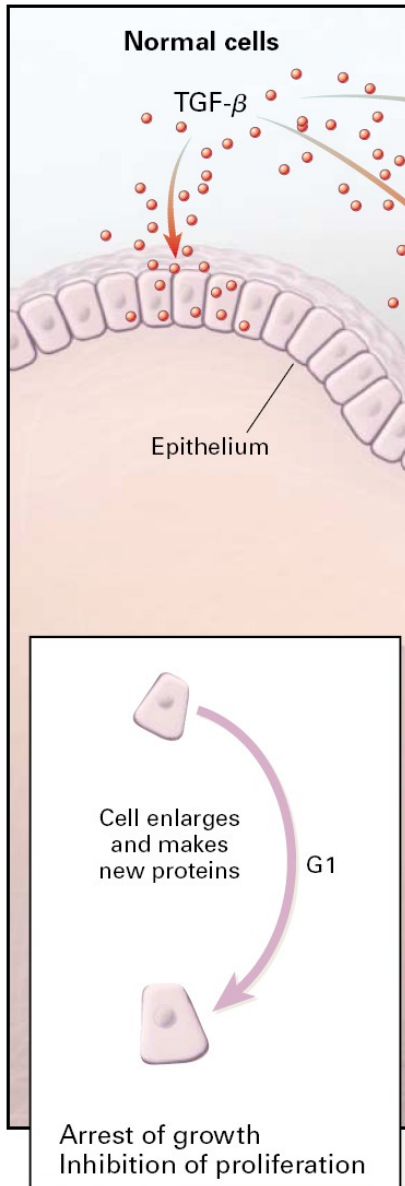
Transforming growth factor, beta receptor ii (70/80kda)



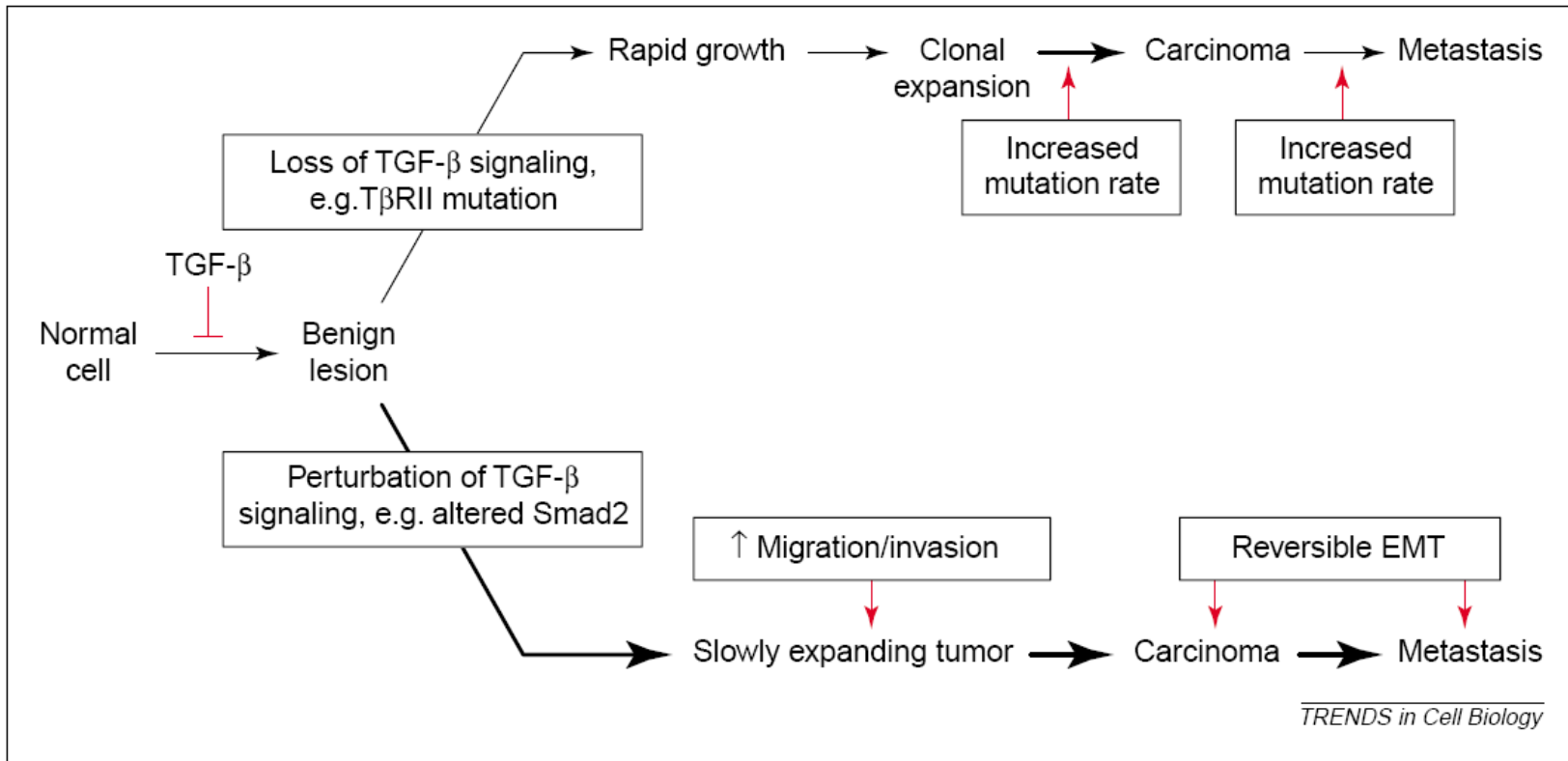
Box Plot - Description

normal, hyperplasia vs. cancer

Role TGF- β v carcinogenezi



Role TGF- β v carcinogenezi



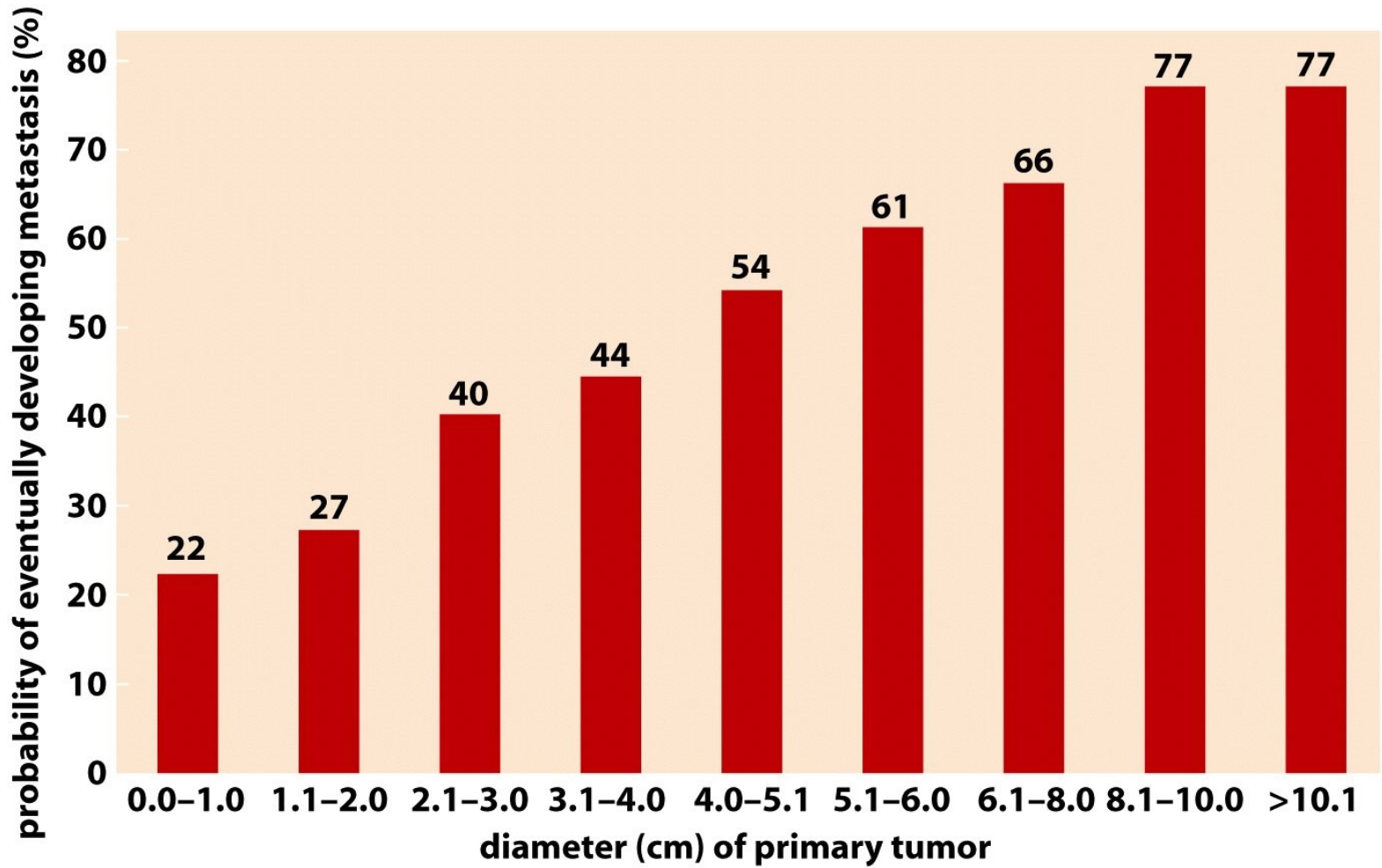


Figure 14.3 *The Biology of Cancer* (© Garland Science 2007)

Mechanism of carcinogenesis

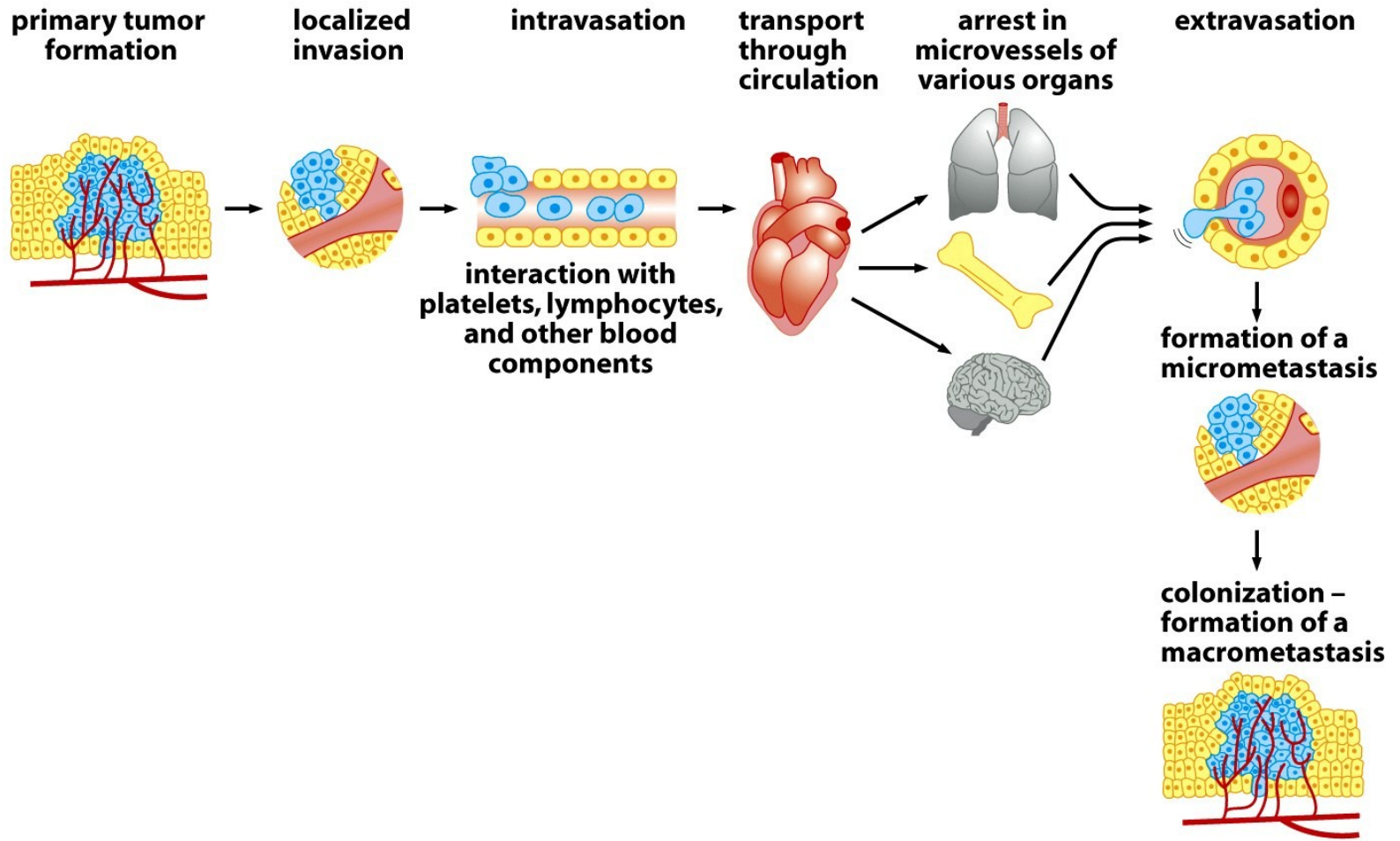


Figure 14.4 *The Biology of Cancer* (© Garland Science 2007)

Mechanism of carcinogenesis

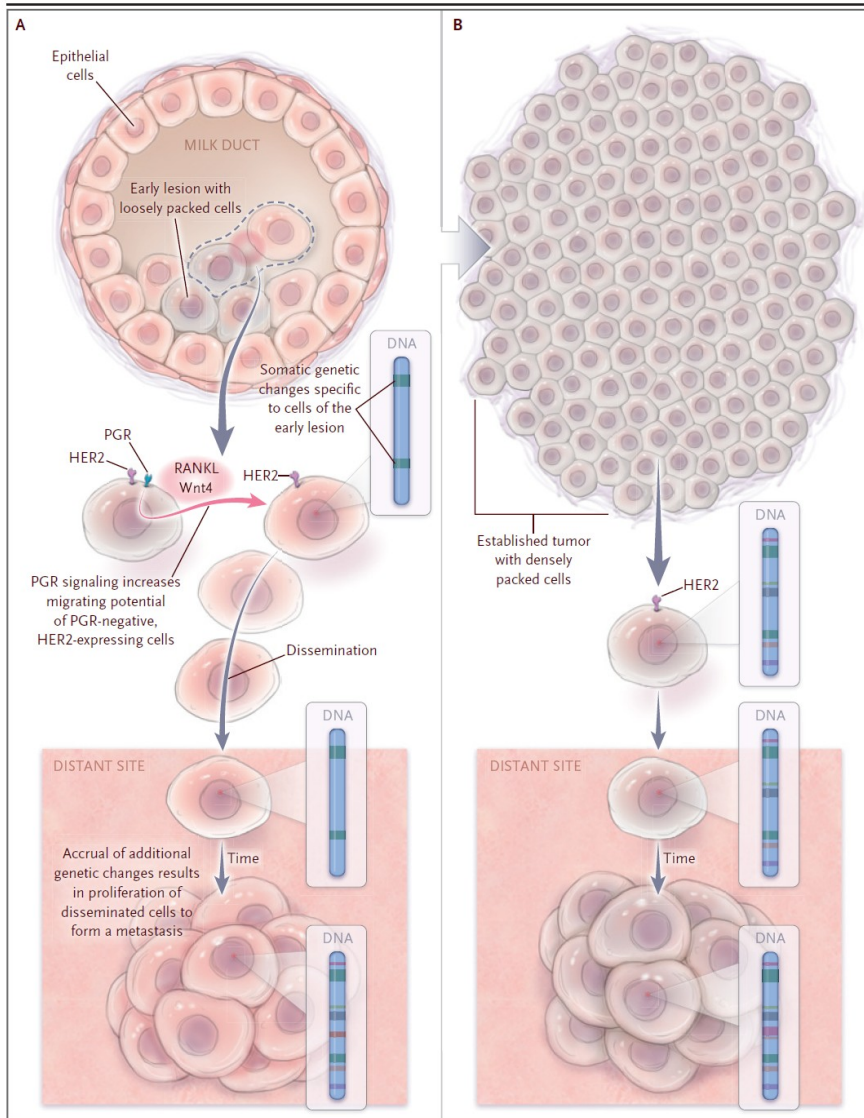


Figure 1. A Model of Parallel Progression after Early Dissemination.

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- κ B 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

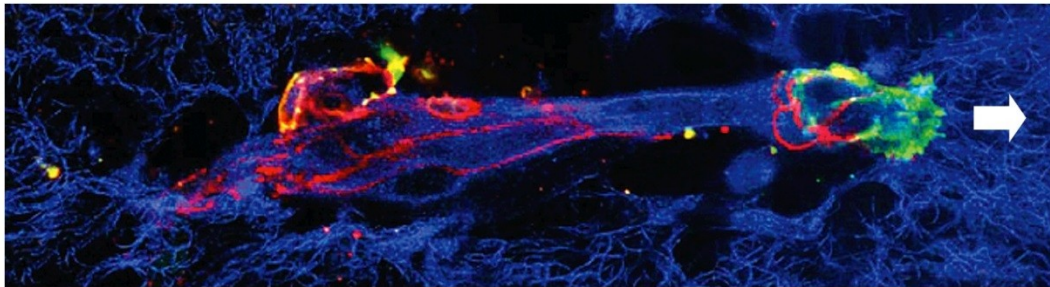


Figure 14.6a The Biology of Cancer (© Garland Science 2014)

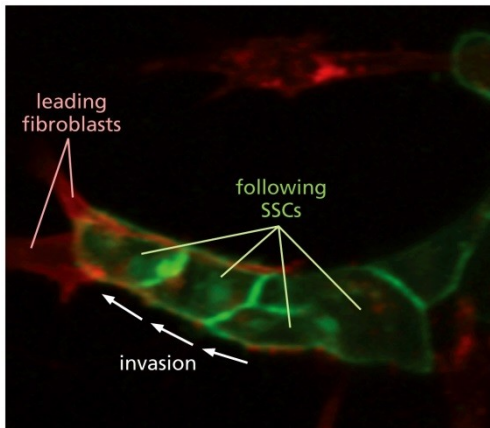


Figure 14.1b The Biology of Cancer (© Garland Science 2014)

Tumor cells, fibroblasts

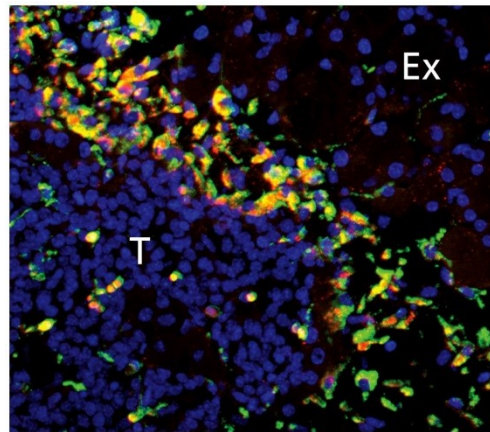


Figure 14.4c The Biology of Cancer (© Garland Science 2014)

Macrophages, cathepsin

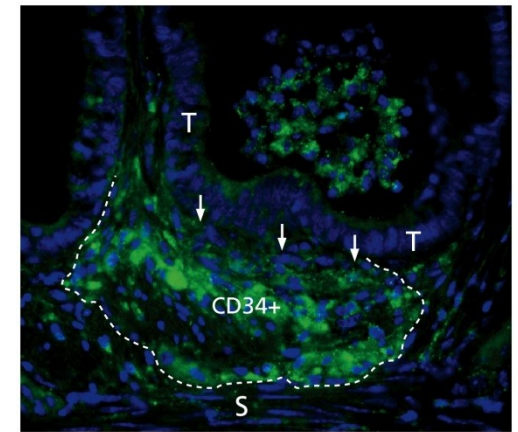


Figure 14.6d The Biology of Cancer (© Garland Science 2014)

Immature myeloid cells

Pohyb buněk

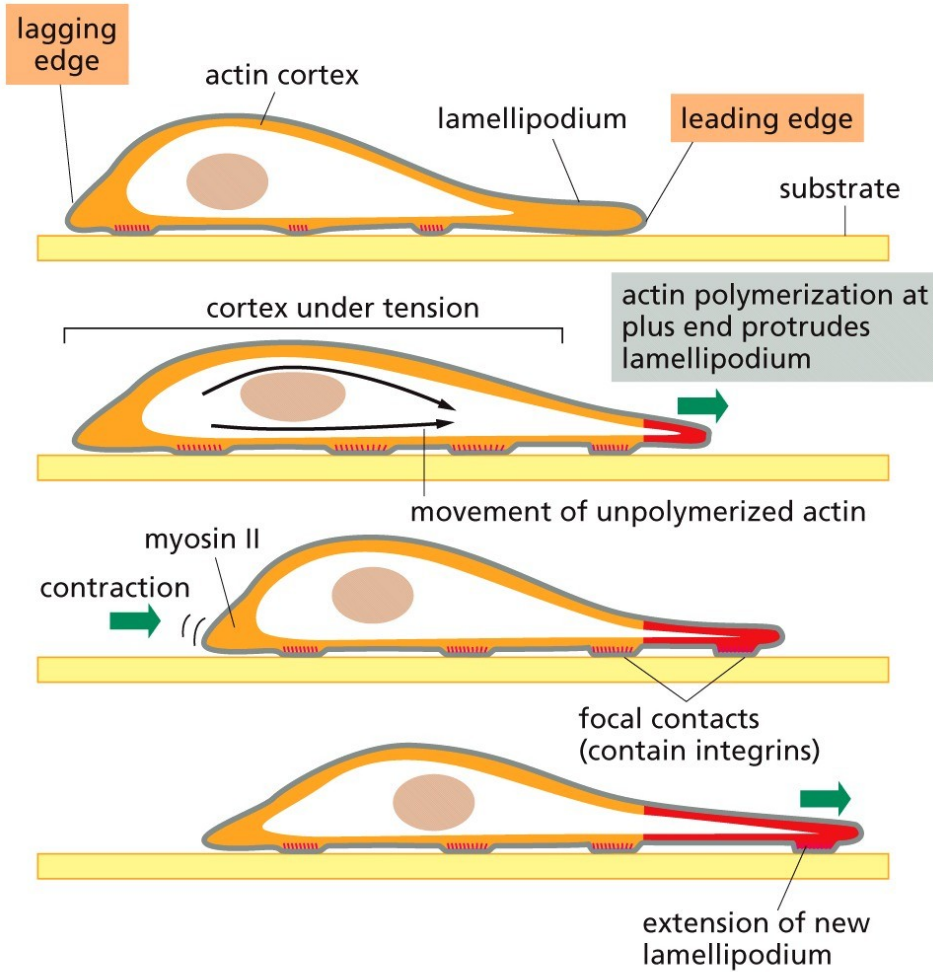
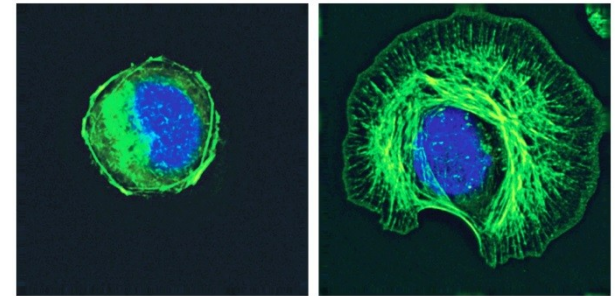


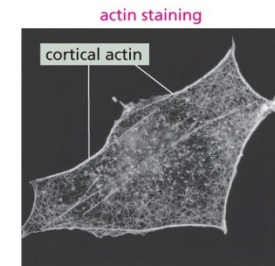
Figure 14.36 The Biology of Cancer (© Garland Science 2014)



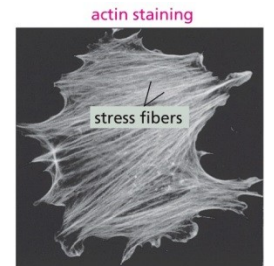
control

+ heregulin

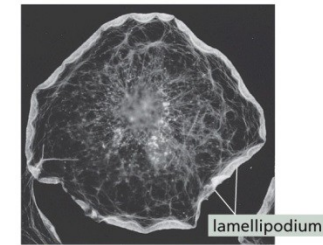
Figure 14.37a The Biology of Cancer (© Garland Science 2014)



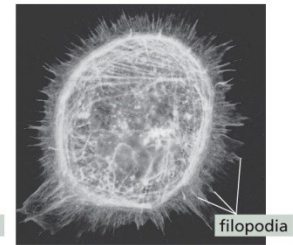
(A) QUIESCENT CELL



(B) Rho ACTIVATION



(C) Rac ACTIVATION



(D) Cdc42 ACTIVATION

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

Pohyb buněk

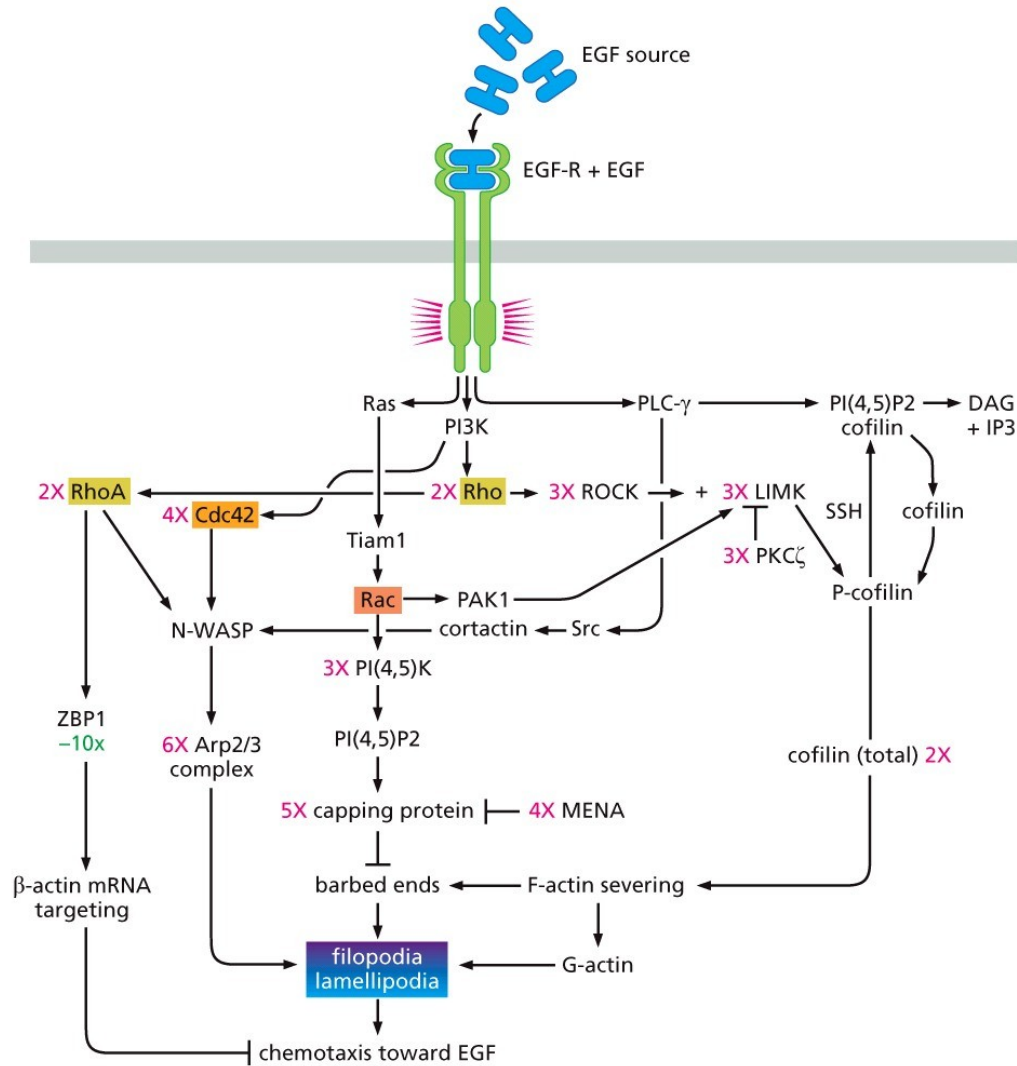


Figure 14.40 The Biology of Cancer (© Garland Science 2014)

Transport, cirkulace

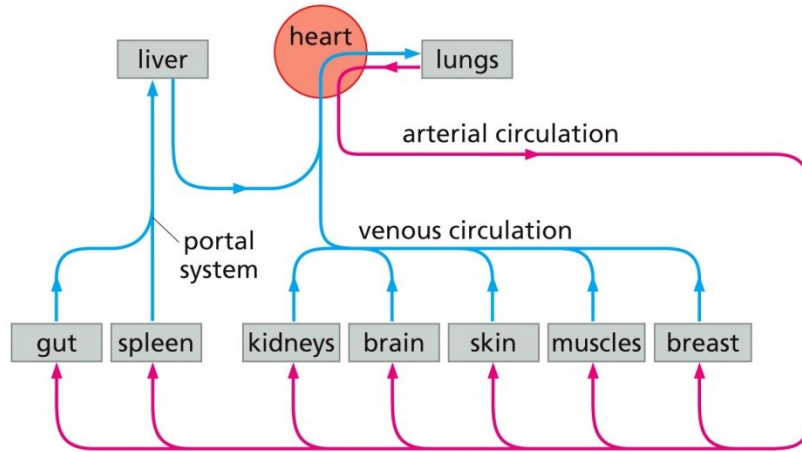
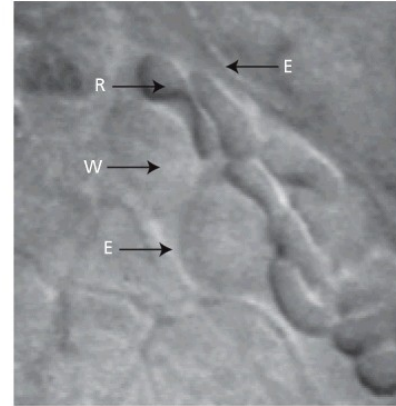
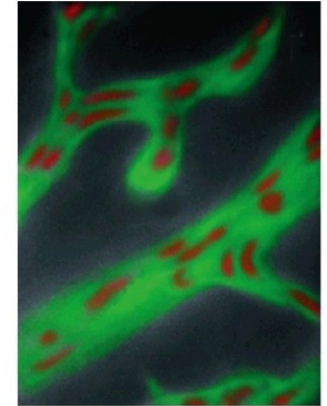


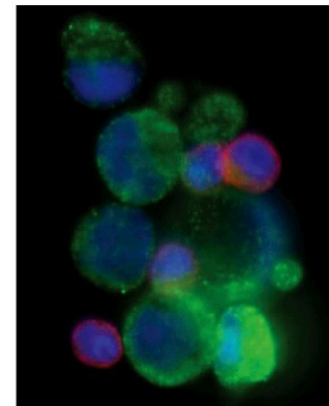
Figure 14.45 The Biology of Cancer (© Garland Science 2014)



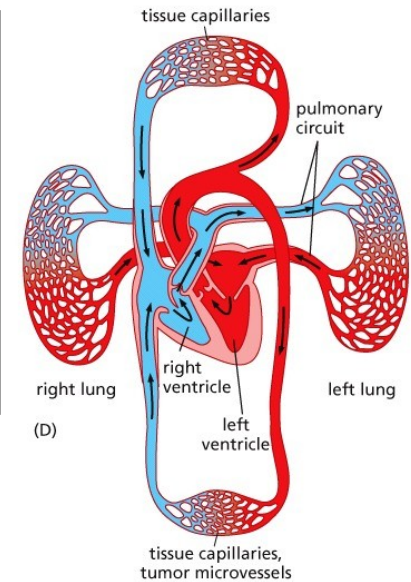
(A)



(B)



(C)



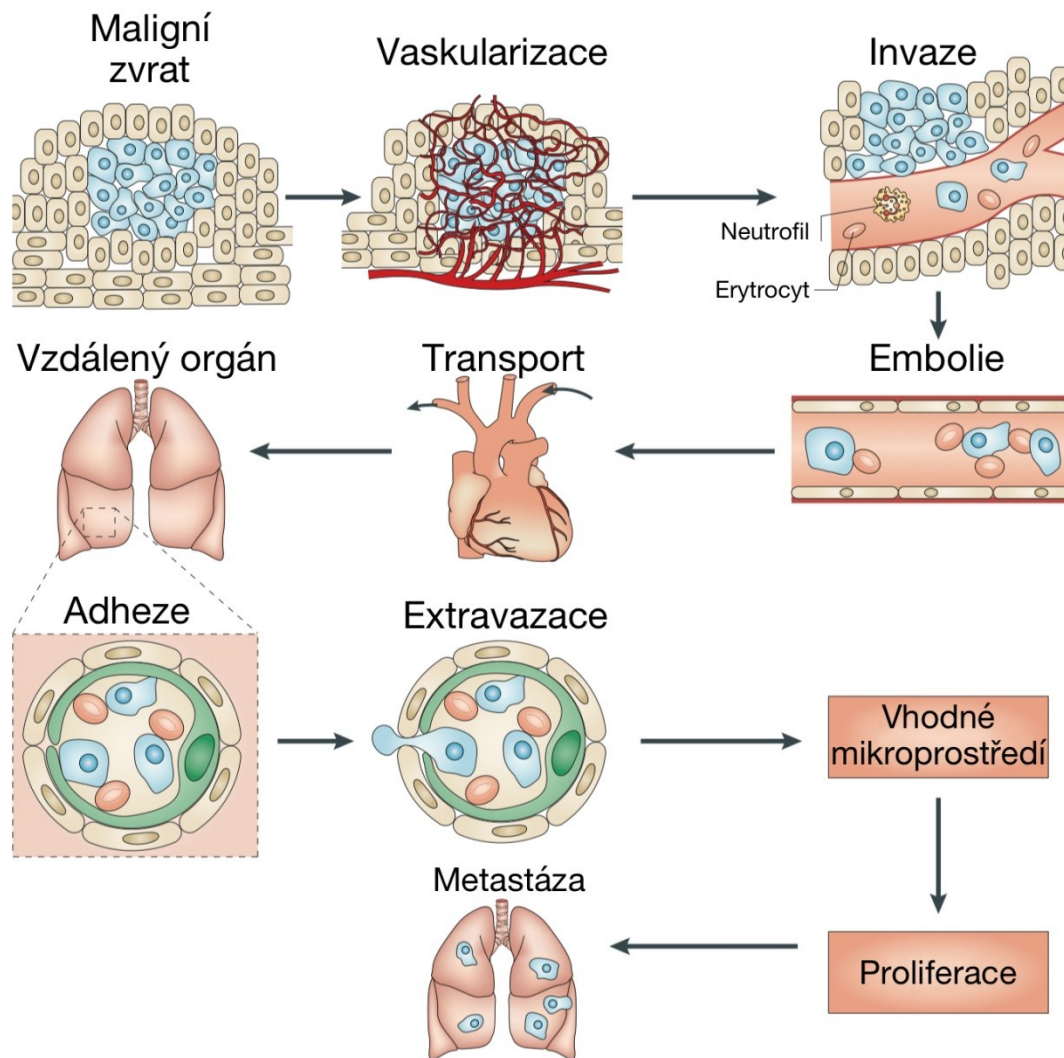
(D)

■ oxygenated blood
■ deoxygenated blood

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

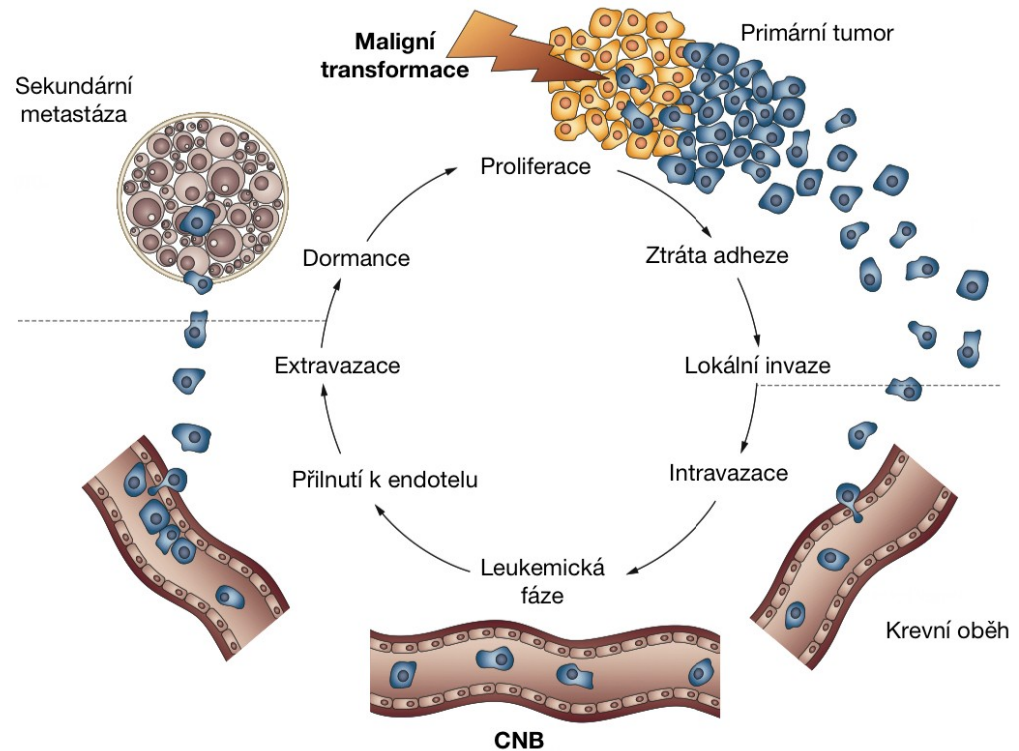
Metastatická kaskáda

Cirkulující nádorové buňky (CNB) – klíčová úloha



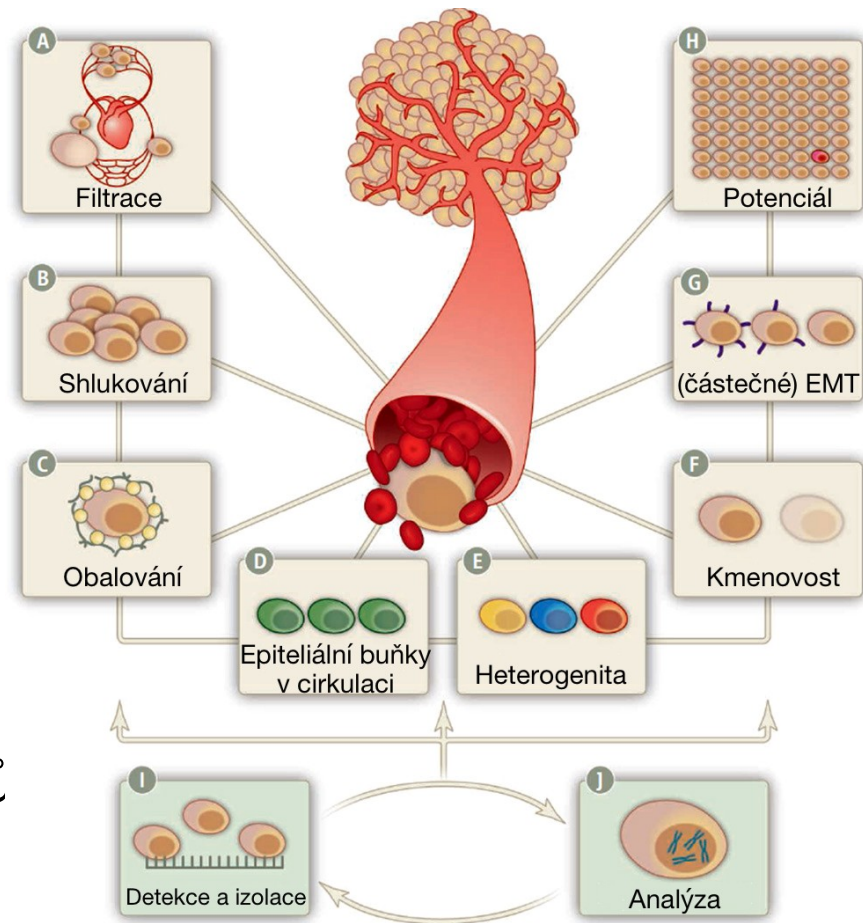
Proč se cirkulujícími nádorovými buňkami zabývat?

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



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



Detekce nádorových cirkulujících buněk

A) Systémy využívající detekce biologických vlastností CNB					
Systém	Druh nádorového onemocnění	Princip	Možnosti následné analýzy	Výrobce	Reference
AdnaTest	prostata, prso, tlusté střevo, ovaria,	imunomagnetická separace EpCAM+ → lýza → izolace RNA	RT-PCR	Qiagen	[URL1]
CellCollector	plicé, prso, tlusté střevo, prostata	zachycení EpCAM+ buněk <i>in vivo</i> pomocí sondy potažené protilátkami proti EpCAM zaváděné přímo do paže pacienta	molekulární charakterizace, kultivace	Gilupi	[URL2]
CellSearch	metastázující: prostata, prso, tlusté střevo	imunomagnetická separace EpCAM+ → permeabilizace → značení na DNA (DAPI), CK, CD45 → jako CNB jsou vyhodnoceny CD45-, DAPI+, CK+	stanovení prognózy (validováno)	Veridex	[URL3]
CTC chip	plicé, jícen, prostata, prso, tlusté střevo, aj.	krev protéká přes mikrofluidní čip se sloupečky s EpCAM protilátkami	molekulární charakterizace, kultivace	-	(Sequist <i>et al.</i> 2009)
HD-CTC	metastázující: prostata, prso, pankreas	lýza erytrocytů → permeabilizace → značení na CK, CD45, DNA (DAPI) → vyhodnocení softwarem	morfologické znaky a cytopatologické znaky, identifikace shluků CNB	-	(Marrinucci <i>et al.</i> 2012)

B) Systémy využívající detekce fyzikálních vlastností CNB					
Systém	Druh nádorového onemocnění	Princip	Možnosti následné analýzy	Výrobce	Reference
Akustický	melanomy, karcinomy	Průchod mikrofluidním kanálem, vystavení akustickým vlnám → různé vlastnosti (velikost, deform., hustota,...) → různé vychýlení	molekulární charakterizace, kultivace	-	(Li <i>et al.</i> 2015)
Apostream	různé	separace na základě dielektrických vlastností	molekulární charakterizace, kultivace,	Apocell	[URL4]
Celsee	prostata, prso, tlusté střevo	mikrofluidní, separace pomocí filtračních komůrek	DNA/RNA FISH, Kultivace,	DeNovo Sciences	[URL5]
CellSieve	různé	filtrace za nízkého tlaku	molekulární charakterizace, kultivace,	Creatv microtech	[URL6]
MetaCell	různé	filtrace usnadněná kapilární silou	histologie, enzym. aktivita	MetaCell	[URL7]
Cluster chip	metastázující: melanomy, prso, prostata	mikrofluidní, pomalý průtok přes systém sloupců	molekulární charakterizace, izolace shluků CNB	-	(Sarioglu <i>et al.</i> 2015)
OncoQuick	karcinomy, melanomy	gradientová centrifugace	molekulární charakterizace, kultivace,	Greiner BioOne	[URL8]
Spirální mikrofluidní	různé (>12 μm)	hydrodynamické oddělení na základě velikosti	molekulární charakterizace, kultivace, izolace shluků CNB	-	(Khoo <i>et al.</i> 2015)

Detekce nádorových cirkulujících buněk

Table 1

Circulating tumor cell (CTC) isolation technologies. Relevant performance characteristics of the discussed CTC isolation technologies. Capture efficiency refers to the percentage of cells isolated in cell spike experiments with cancer cell lines in whole blood. Purity refers to the captured number of target cells as opposed to captured non-target cells as expressed either as a percentage or log depletion. Blank spaces indicate that this metric was not provided by the reference

Technology	Year	Capture efficiency	Purity	Throughput	Clinical verification	References
CellSearch	2004	85.50%	Low		Breast, bladder, colorectal, gastric, lung, ovarian, pancreatic, prostate, renal	[3]
CTC Chip	2007	>60%	50%	1 mL/h	Breast, colon, lung, pancreatic, prostate	[8,11,43**]
GEDI	2009	78–85%	68%	1 mL/h	Breast, gastric, pancreatic, prostate	[12,13,38]
HTMSU	2008	94.50%		1.6 mL/h	Pancreatic (PDX mouse)	[14,39]
HT-CTC Chip	2014		86%	1.38 mL/h	Prostate	[15]
NanoVelcro	2011	95%		0.5 mL/h	Lung	[16*]
Hb Chip	2010	92%	14%	1.2 mL/h	Prostate	[17,41]
LbL Hb Chip	2015	96%	High		Breast, lung	[18]
Oncobean	2014	82.7–100%	Higher with increased flow rates	Up to 10 mL/h	Breast, lung, pancreatic	[19]
GO Chip	2013	94.20%	High	1 mL/h	Breast, lung, pancreatic	[20**]
CTC-iChip	2013	77.8–98.6%	2.5–3.5 log depletion	8 mL/h	Breast, colorectal, lung, pancreatic, prostate	[21**,42**,44**]
VeriFAST	2014	90%			Lung	[22]
SB microfilter	2014	78–83%	$1.7\text{--}2 \times 10^3$	Around 5 mL/h	Tested in mouse model	[26]
FMSA device	2014	92.6%	1.4×10^4	Around 45 mL/h	Breast, colorectal, and lung	[27*,40]
Vortex technology	2014	10–20%	57–95% for clinical samples		Lung, breast	[29]
Multiplex spiral device	2013	>85%	10%	3 mL/h	Lung	[34]
ApoStream (DEP)	2011	70%	Reduction of WBCs 99.33% ± 0.56% (2–3 log depletion)	1 mL/h	Prostate, breast, lung, hepatocellular, bladder	[35]
taSSAW	2013	>83%	Around 90% removal rate of WBCs (1 log depletion)	1.2 mL/h	Lung	[36,37*]

Příklad: Filtrace

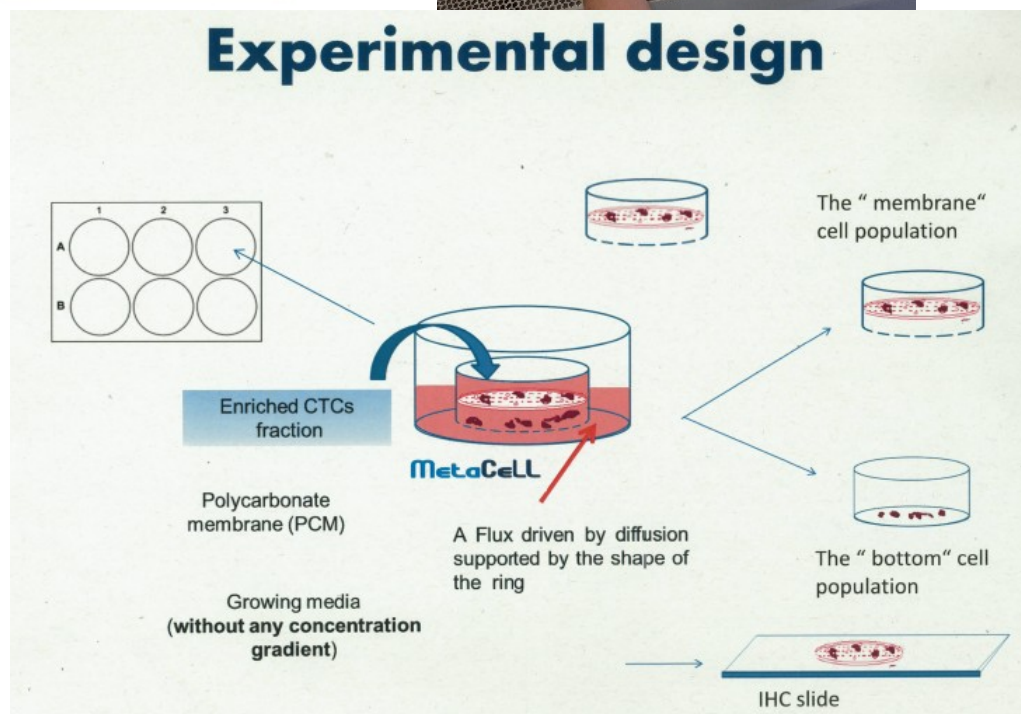
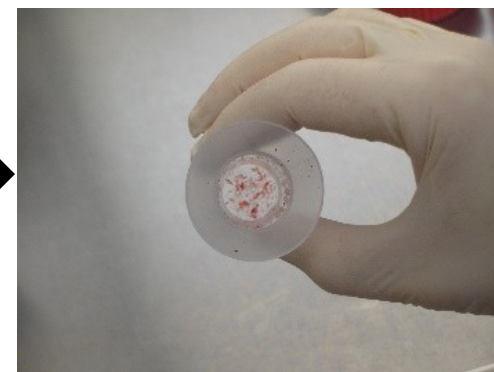
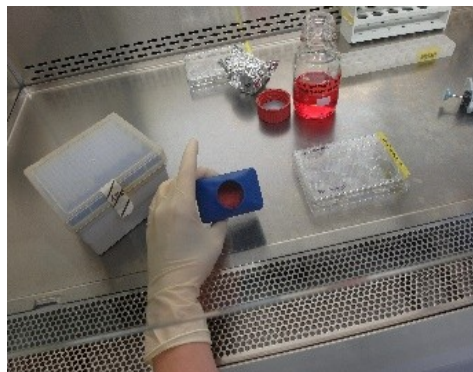
- CNB: epiteliální původ → větší velikost
- Platformy: **MetaCell**, CellSieve, Celsee,...

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

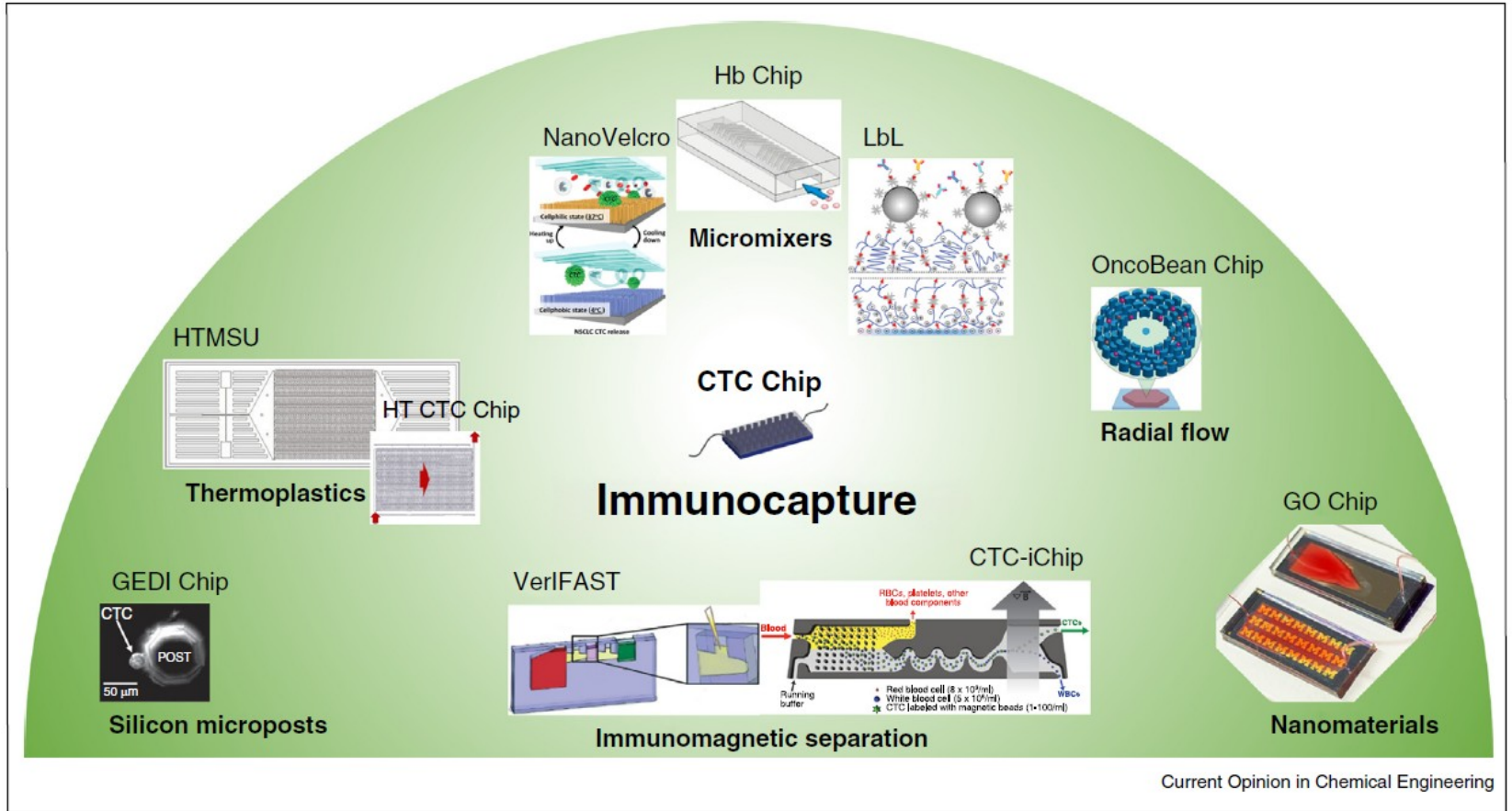
- **Výhody** – nezávislost na povrchových znacích
 - Heterogenní populace
 - Není nutná aktivace receptorů
 - Nativní stav
- **Nevýhody**
 - Možný překryv s leukocyty
 - Nutné využít dalších znaků (CD45)
 - Různá velikost CNB?

Příklad: Filtrace

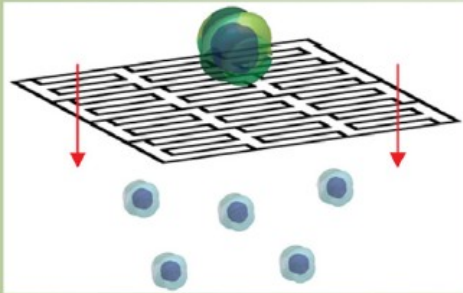
- polycarbonate membrane with 8 μm pores (CTCs over 20 μm)
- capillary force-driven filtration



Příklad: mikrofluidní separace



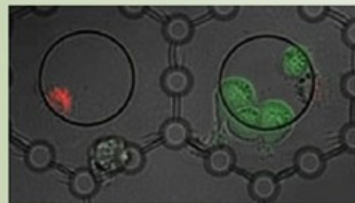
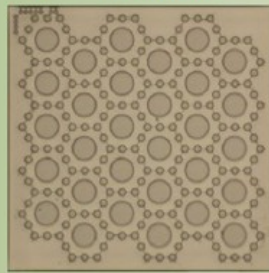
Příklad: mikrofluidní separace



Microfilter

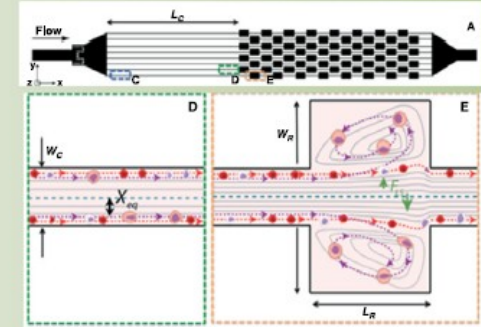
Size Based Separation

FMSA device

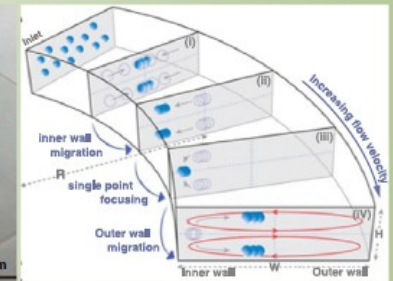
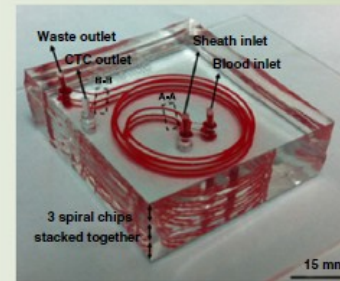


SB microfilter

Inertial Effects

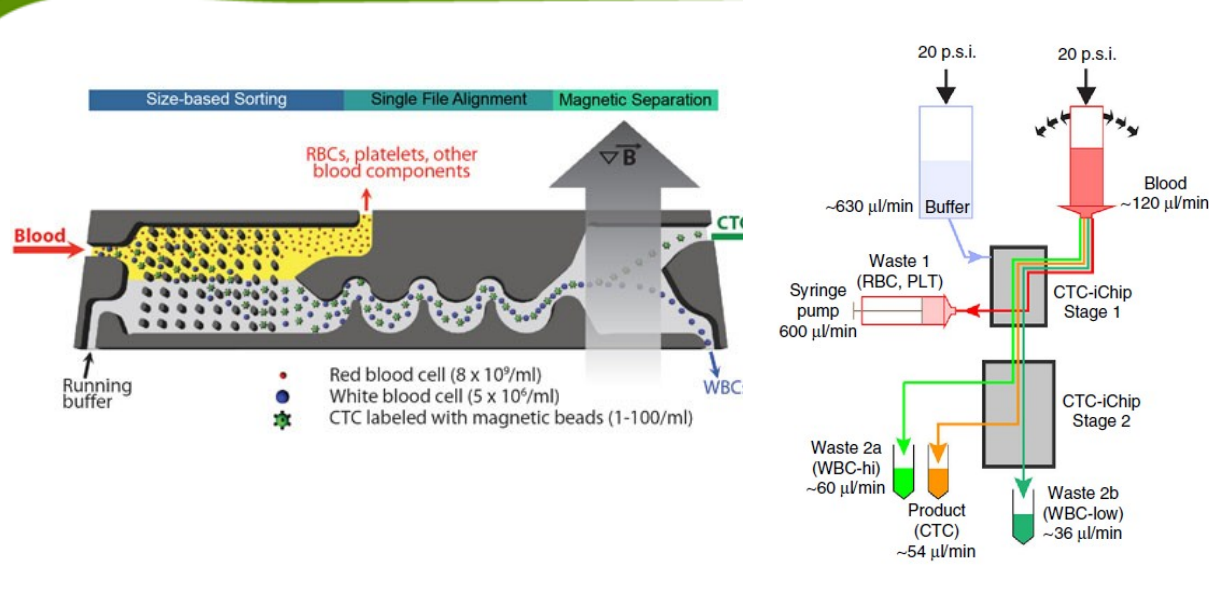


Vortex technology



Spiral devices

Příklad: mikrofluidní separace



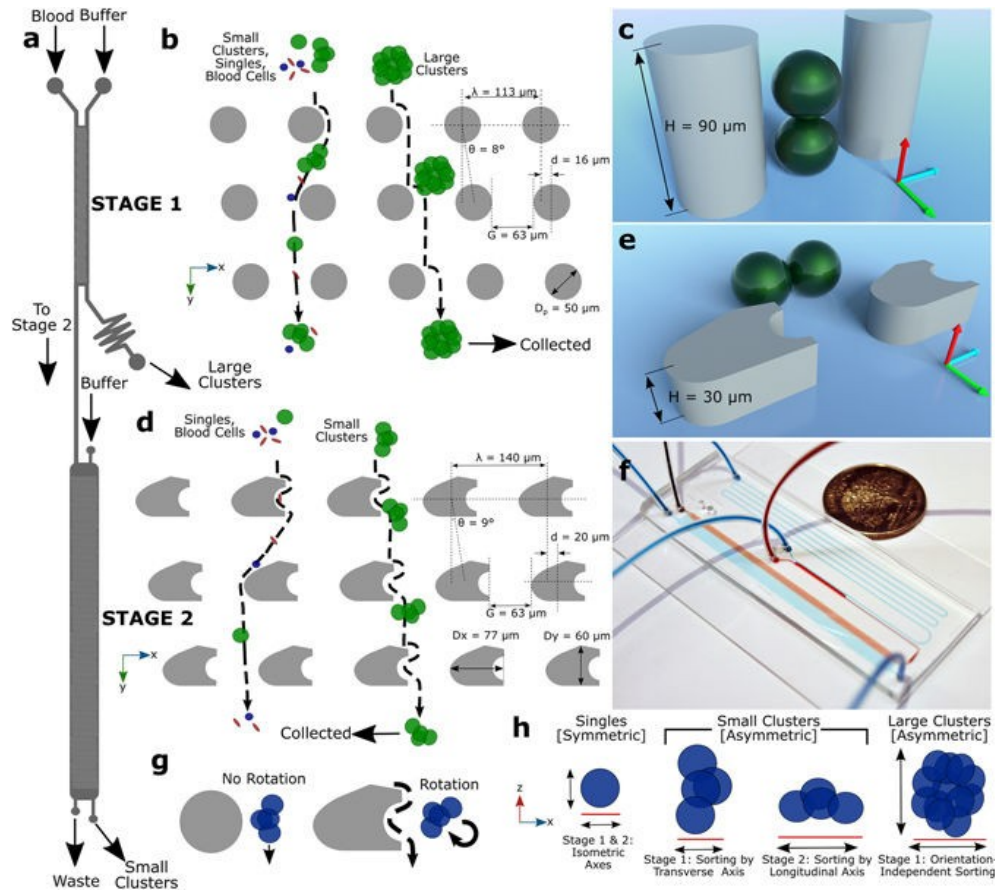
PROTOCOL

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

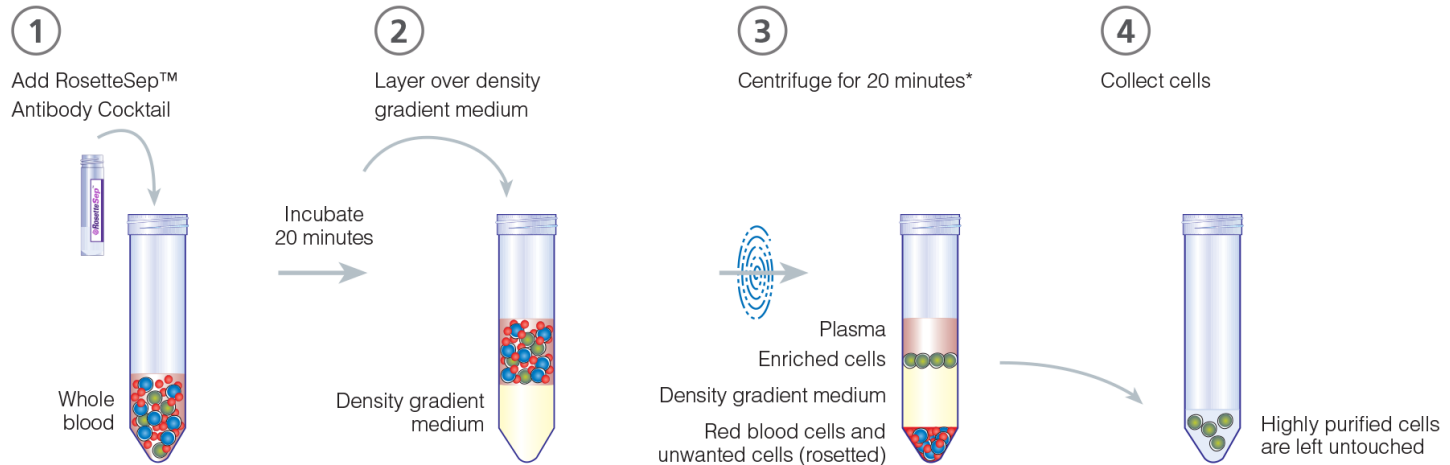
Nezihi Murat Karabacak^{1,4}, Philipp S Spuhler^{1,4}, Fabio Fachin¹, Eugene J Lim¹, Vincent Pai¹, Emre Ozkumur¹, Joseph M Martel¹, Nikola Kojic¹, Kyle Smith¹, Pin-i Chen¹, Jennifer Yang¹, Henry Hwang¹, Bailey Morgan¹, Julie Trautwein², Thomas A Barber¹, Shannon L Stott^{1,2}, Shyamala Maheswaran², Ravi Kapur¹, Daniel A Haber^{2,3} & Mehmet Toner¹

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

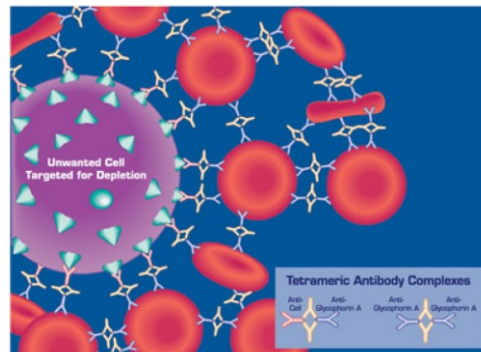
Příklad: mikrofluidní separace



Izolace CTC pomocí deplece CD45+ buněk krve



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



RosetteSep™

CD45 Depletion Cocktail for Enrichment of Circulating Epithelial Tumor Cells
For labeling 200 mL blood

Kit Contains:
CD45 Depletion Cocktail for Enrichment of Circulating Epithelial Tumor Cells (5 x 2 mL)

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FOR RESEARCH USE ONLY

Store at 2-8°C



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.

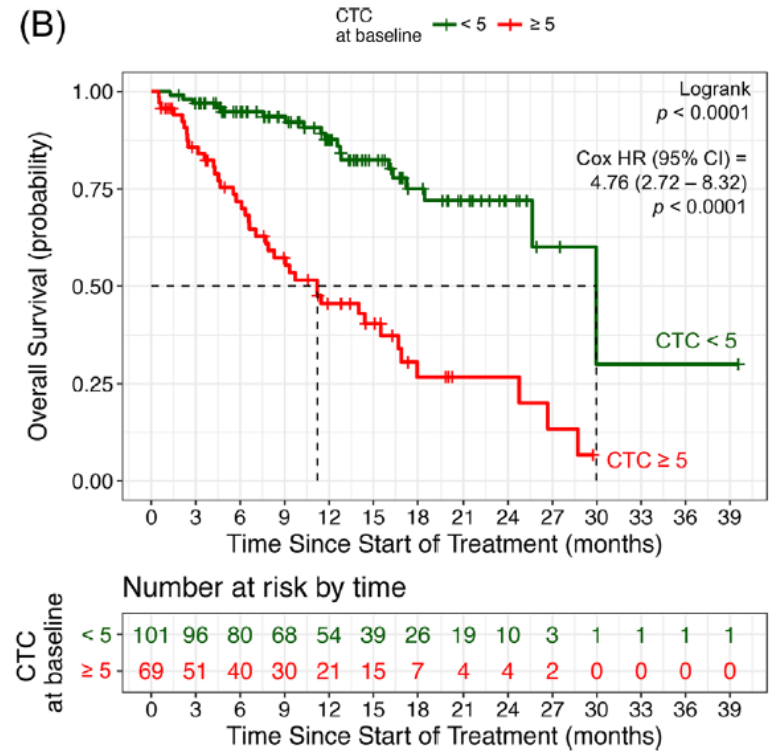
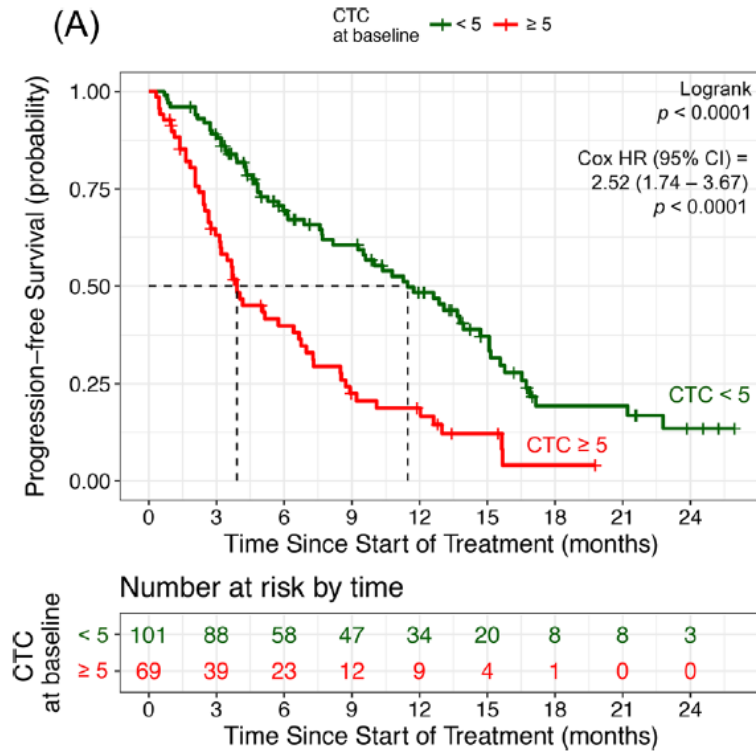
Klinické využití detekce cirkulujících nádorových buněk

- 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



Množství cirkulujících nádorových buněk koreluje s prognózou



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

ORIGINAL ARTICLE

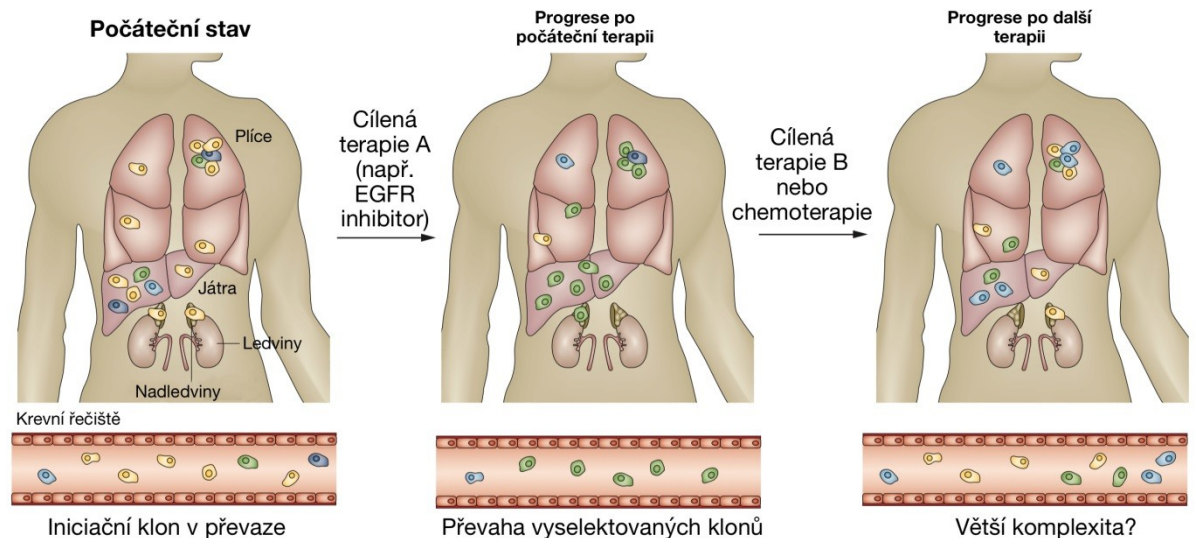
WILEY [The Prostate](#)

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

Bram De Laere¹ | Steffi Oeyen¹ | Peter Van Oyen² | Christophe Ghyse² | Jozef Ampe² | Piet Ost³ | Wim Demey⁴ | Lucien Hoekx⁵ | Dirk Schrijvers⁶ | Barbara Brouwers⁷ | Willem Lybaert⁸ | Els Everaert⁸ | Piet Van Kerckhove⁷ | Daan De Maeseneer⁹ | Michiel Strijbos⁴ | Alain Bols⁷ | Karen Fransis⁵ | Nick Beijer¹⁰ | Inge de Krujff¹⁰ | Valerie van Dam¹ | Anja Brouwer¹ | Pieter-Jan van Dam¹ | Gert Van den Eynden^{1,11} | Annemie Rutten¹² | Stefan Sleijfer¹⁰ | Jean Vandebroek¹² | Steven Van Laere¹ | Luc Dirix^{1,12}

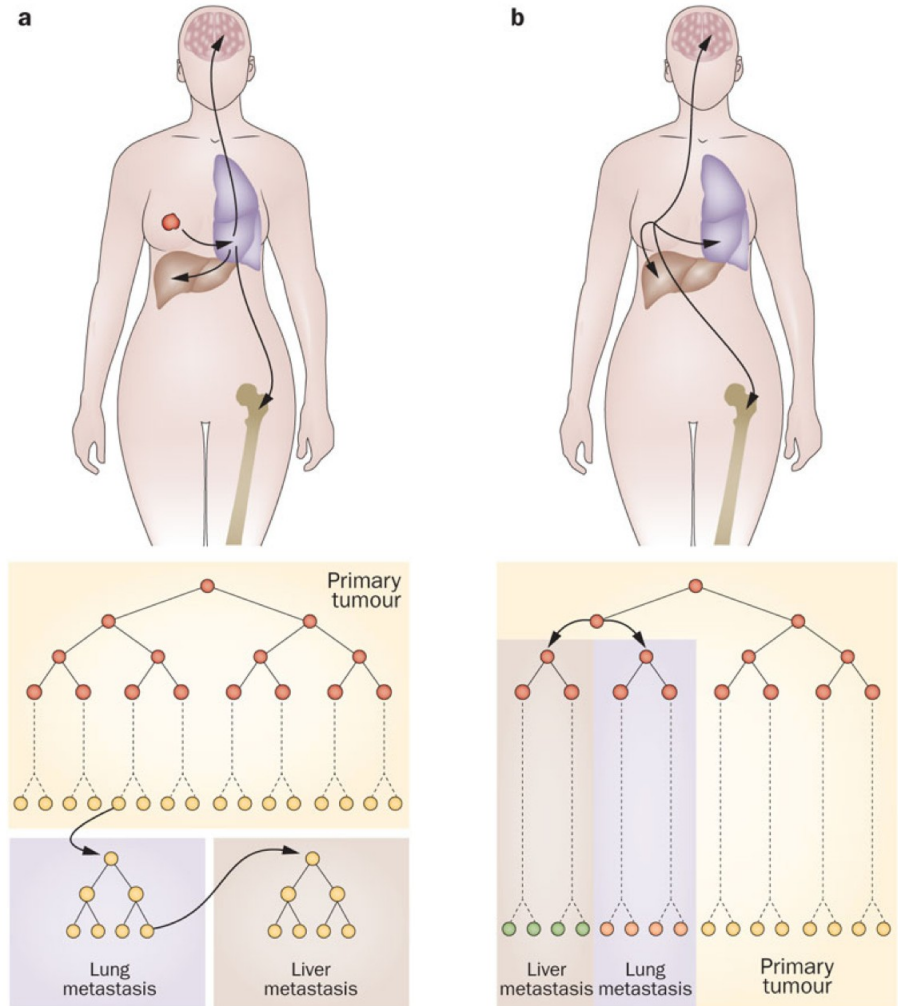
Molekulární charakterizace CNB → cílená terapie

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



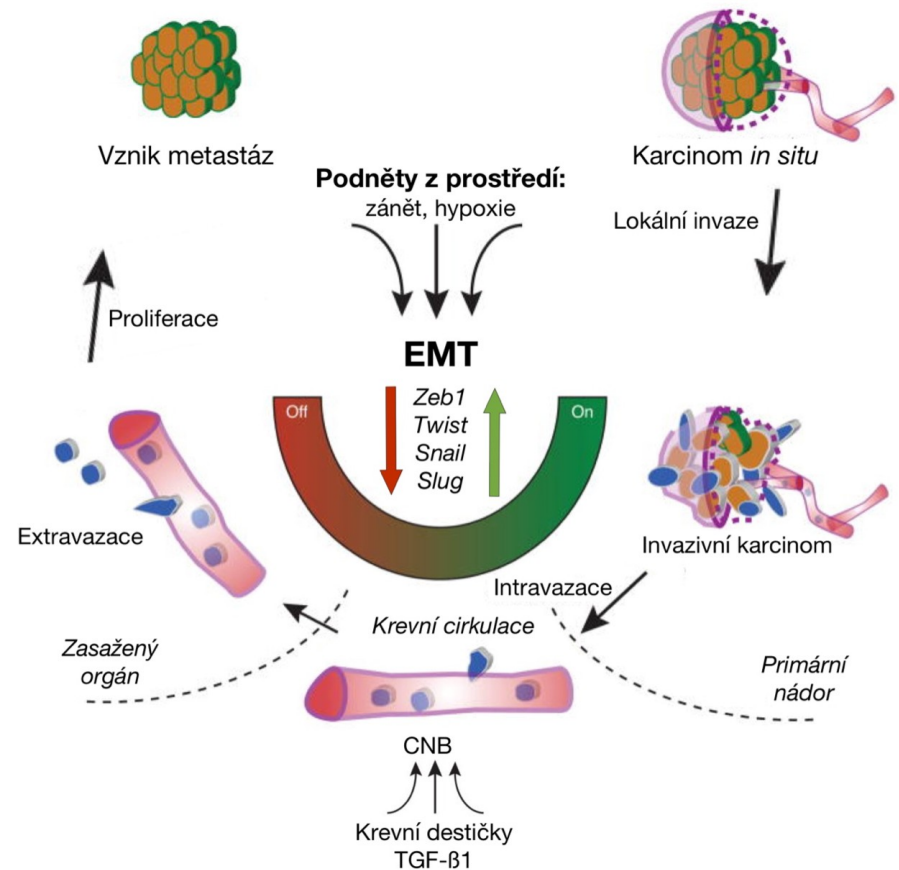
Cirkulující nádorové buňky – prekurzor metastáz

- 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 rozestých buněk (a)
- „Tumor self-seeding“
- Oblast intenzivního výzkumu



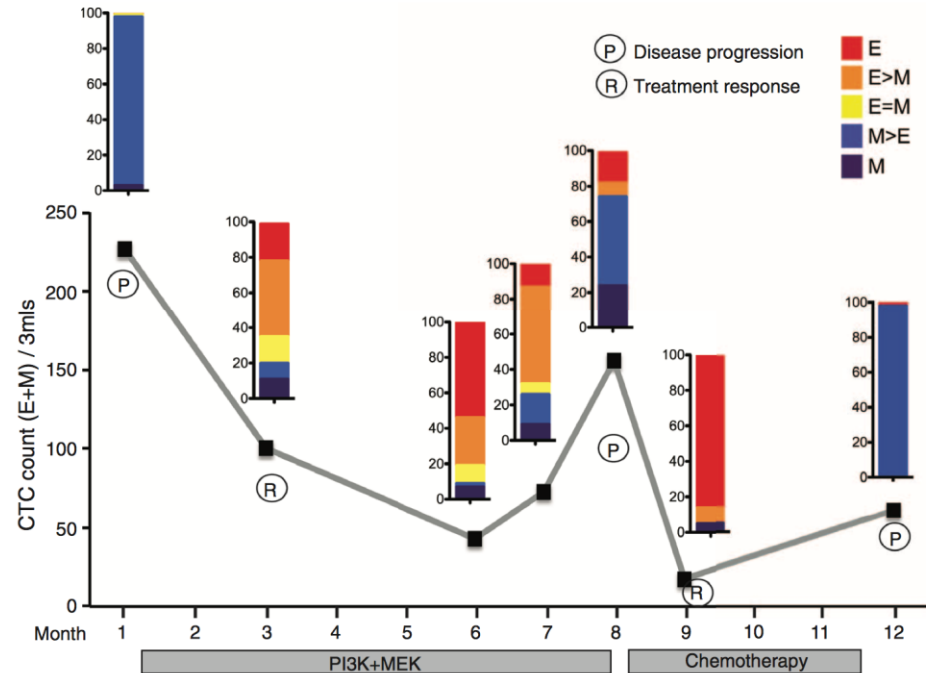
Plasticita cirkulujících nádorových buněk

- 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 (prsů, prostaty, plic, tlustého střeva, vaječníků, atd.)



Epiteliálně-mezenchymální přechod

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



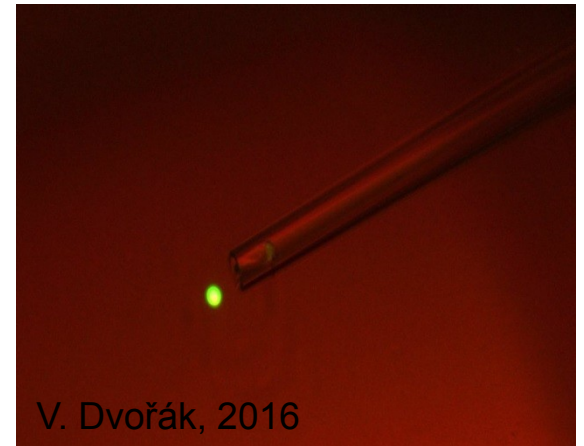
Circulating Breast Tumor Cells Exhibit Dynamic Changes in Epithelial and Mesenchymal Composition

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,³ 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†}

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

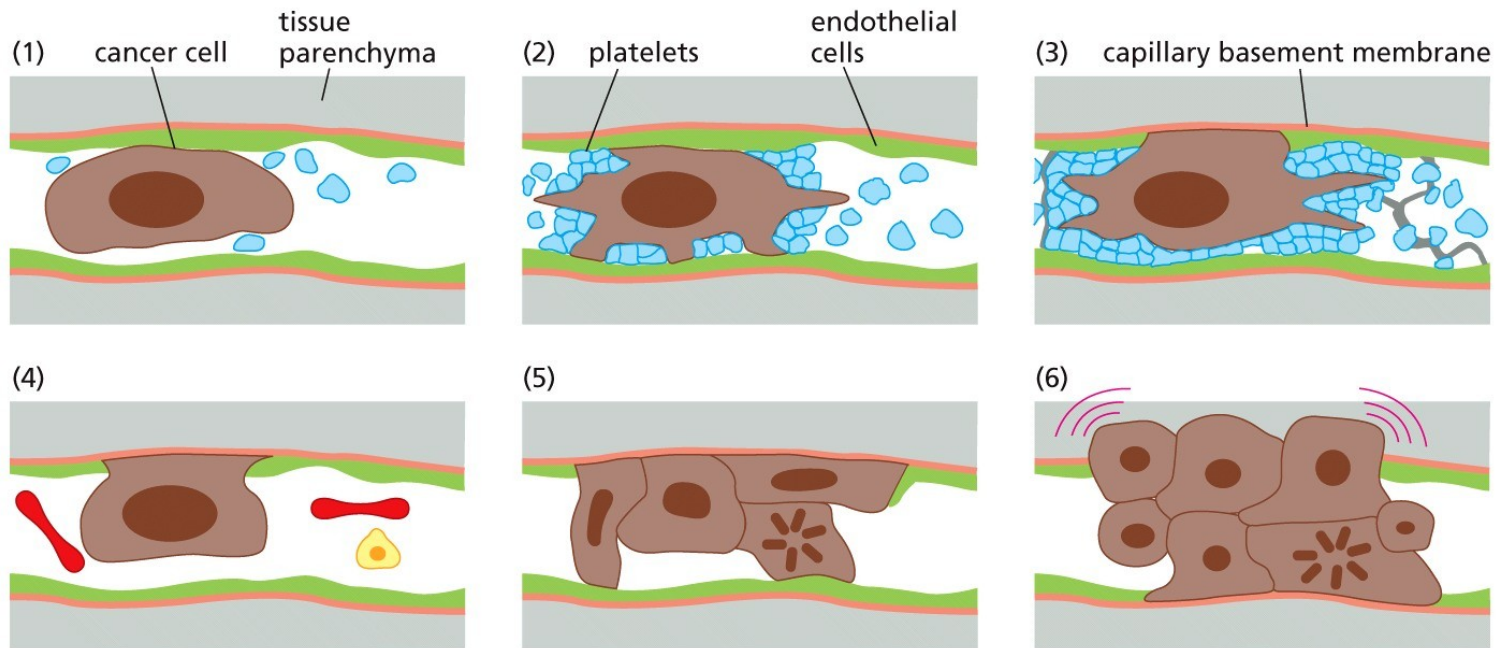


Figure 14.9d The Biology of Cancer (© Garland Science 2014)

Kolonizace

- Velice nízká účinnost
- Dormantní mikrometastázy
- Metastatický relaps

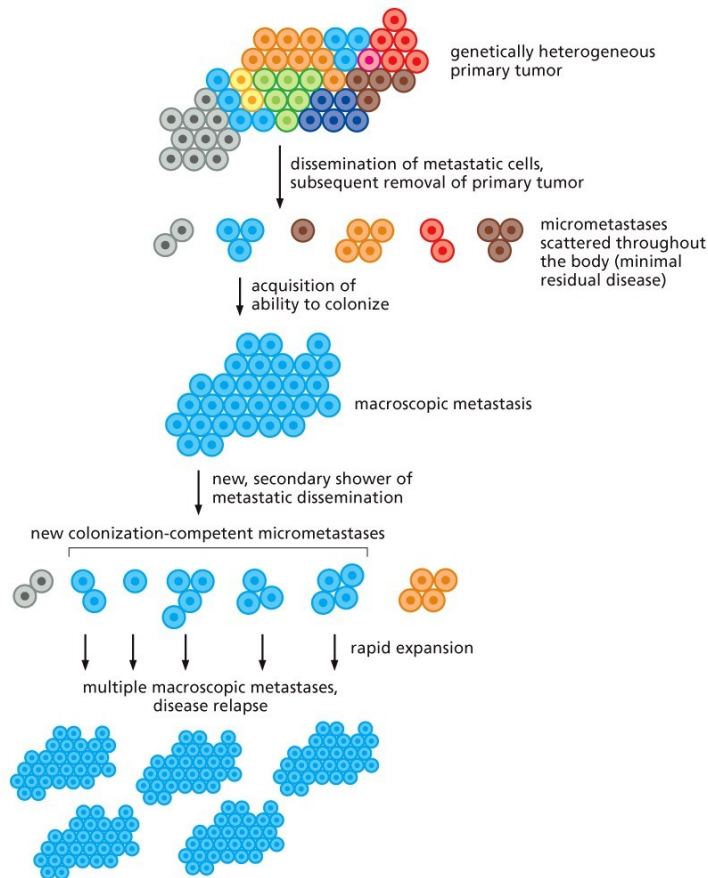


Figure 14.11c The Biology of Cancer (© Garland Science 2014)

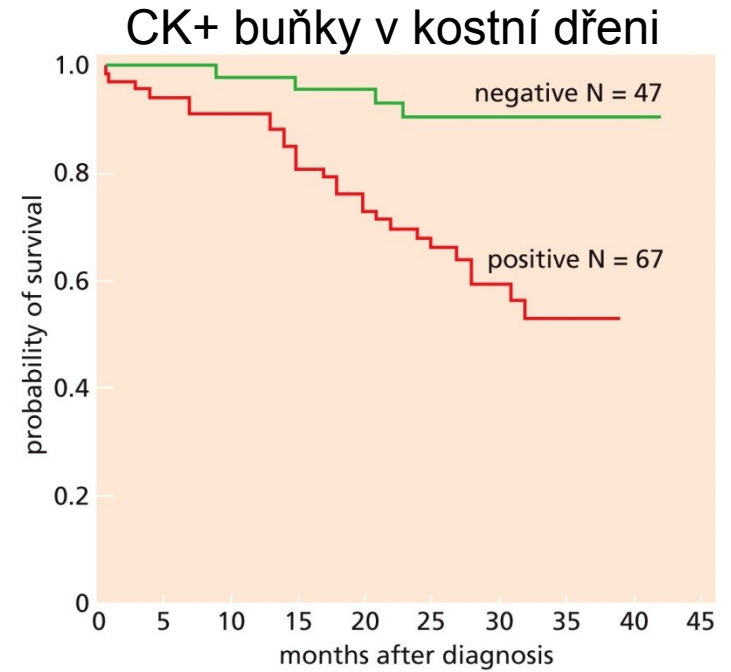
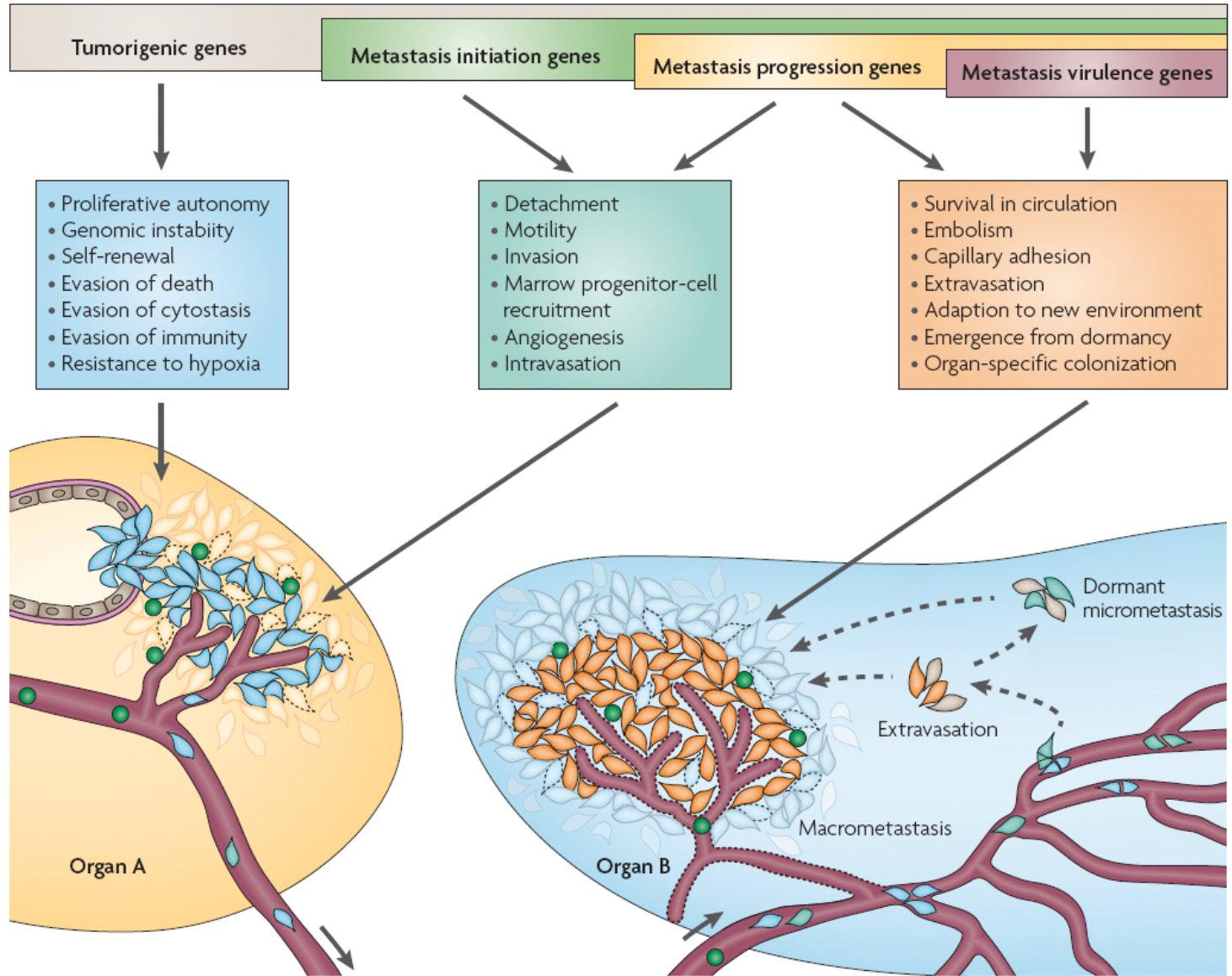


Figure 14.10d The Biology of Cancer (© Garland Science 2014)



Epithelial-Mesenchymal Transition (EMT)

- 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

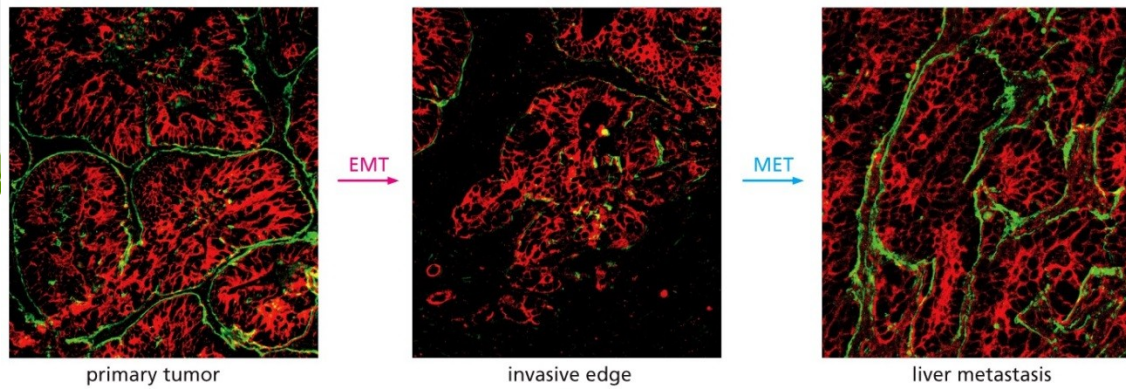


Figure 14.18a The Biology of Cancer (© Garland Science 2014)

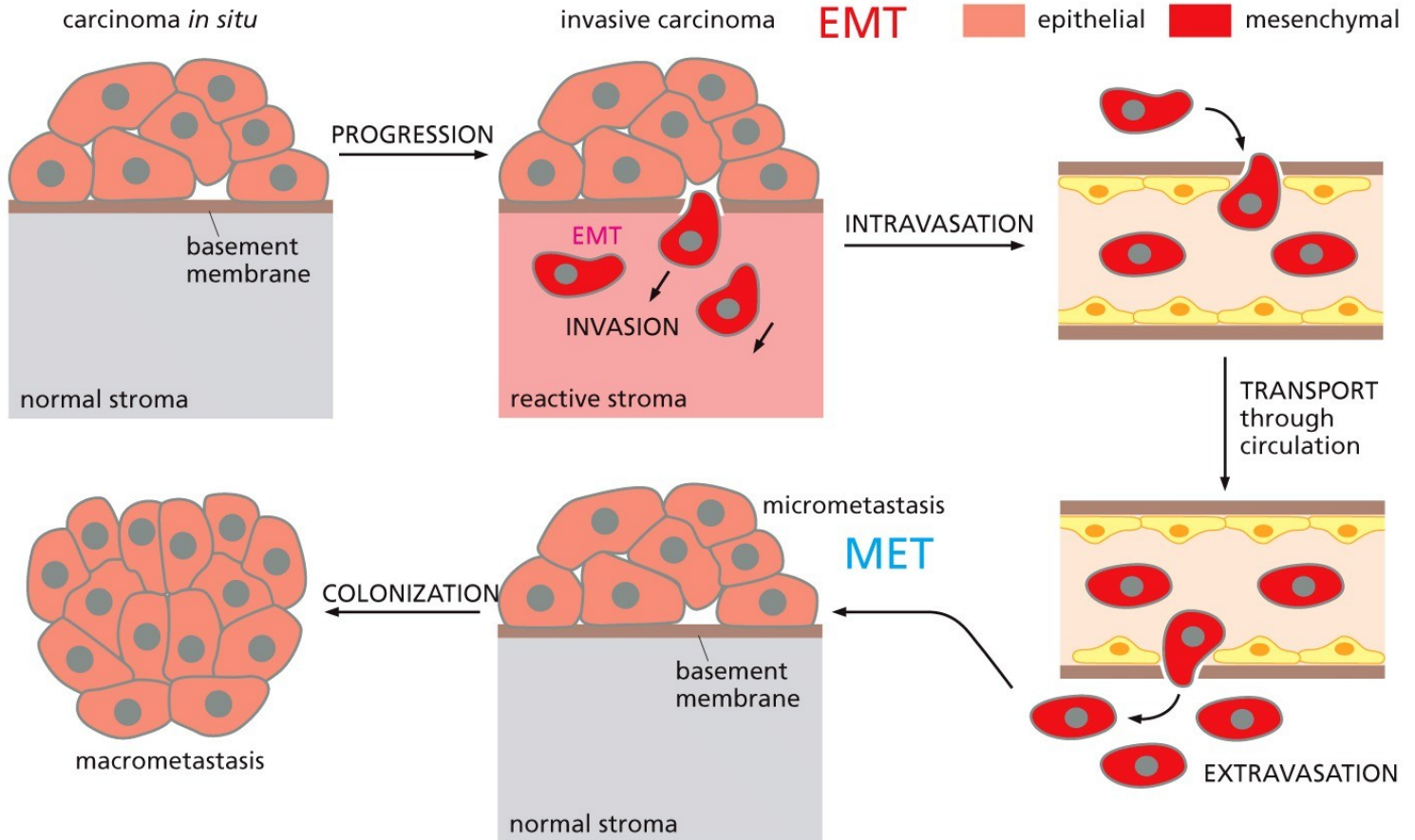
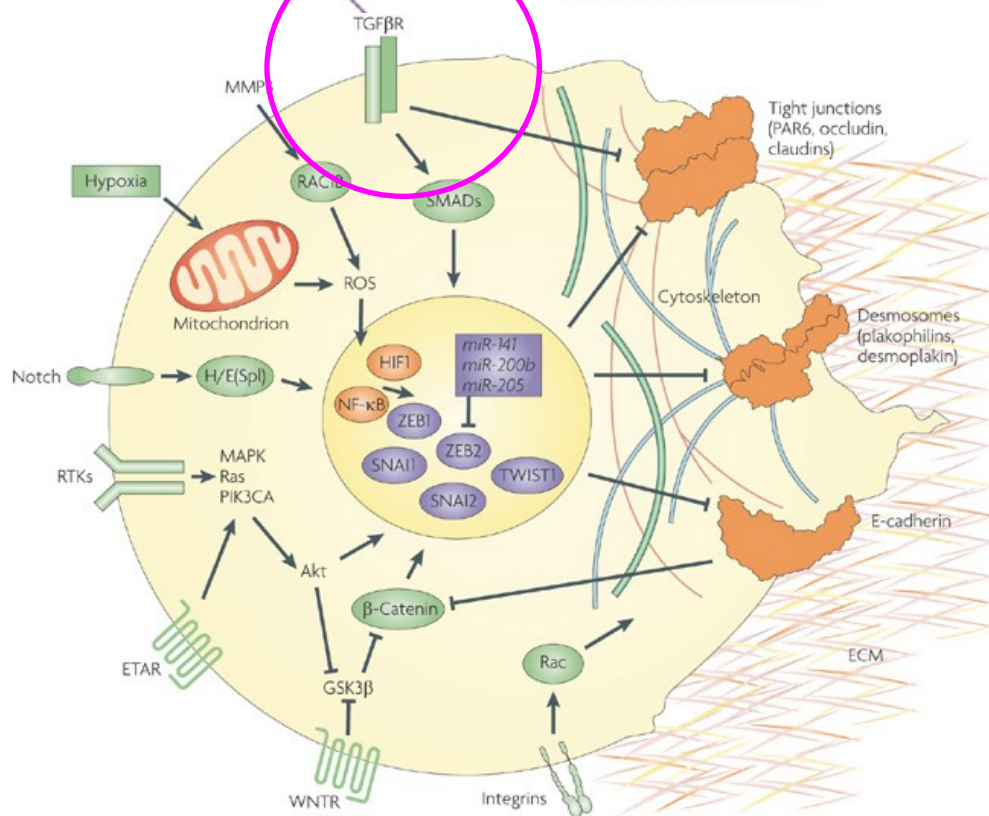
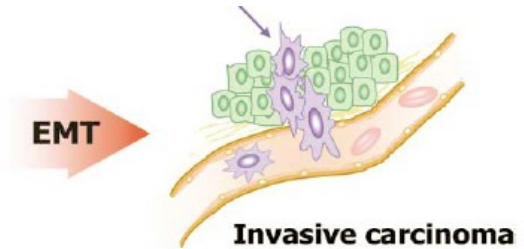
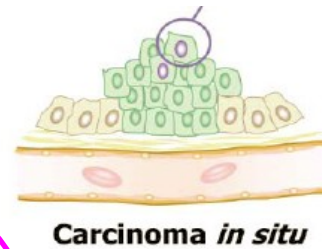


Figure 14.18b The Biology of Cancer (© Garland Science 2014)

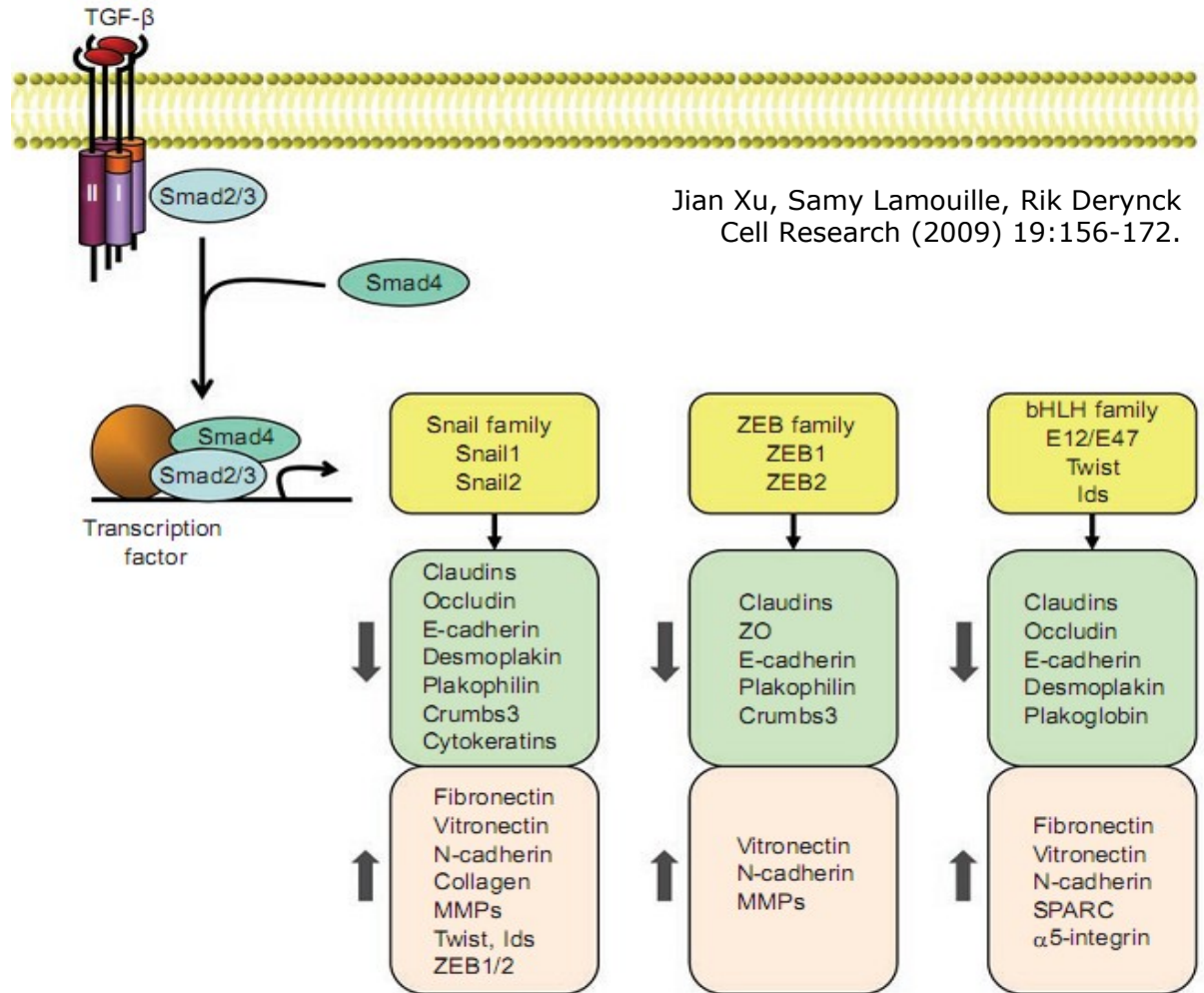
Znaky a regulatory EMT

EMT Program	<i>E-cadherin</i>	Epithelial markers repressed
	<i>α-catenin</i> <i>γ-catenin</i>	
	<i>Vimentin</i> <i>Fibronectin</i> <i>N-cadherin</i>	Mesenchymal markers induced



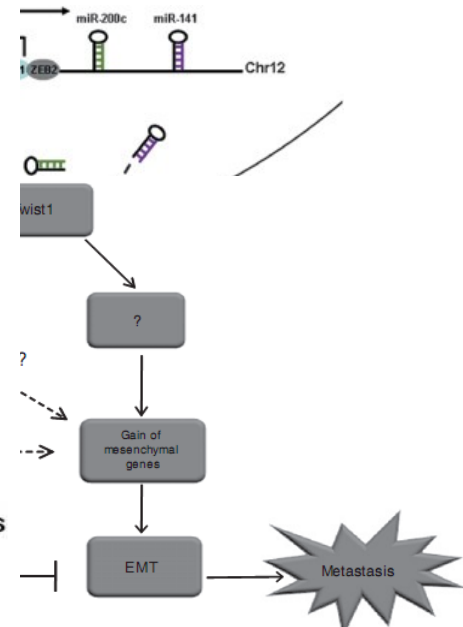
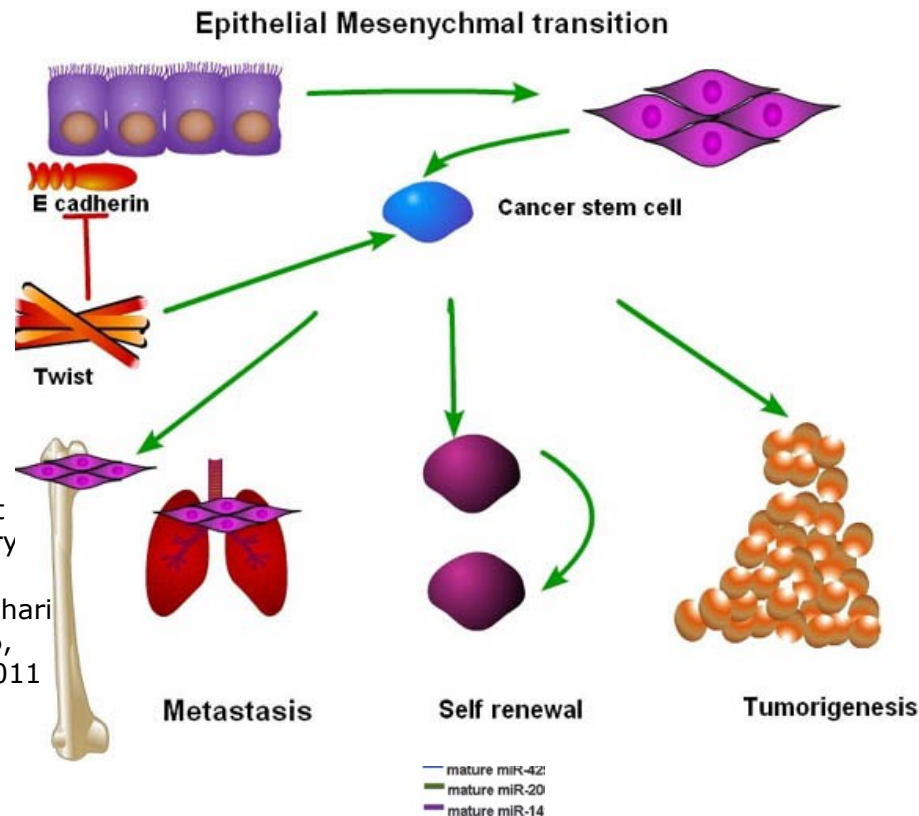
Kornelia Polyak & Robert A. Weinberg
Nature Reviews Cancer **9**, 265-273 (April 2009)

Transforming growth factor- β (TGF- β)



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)

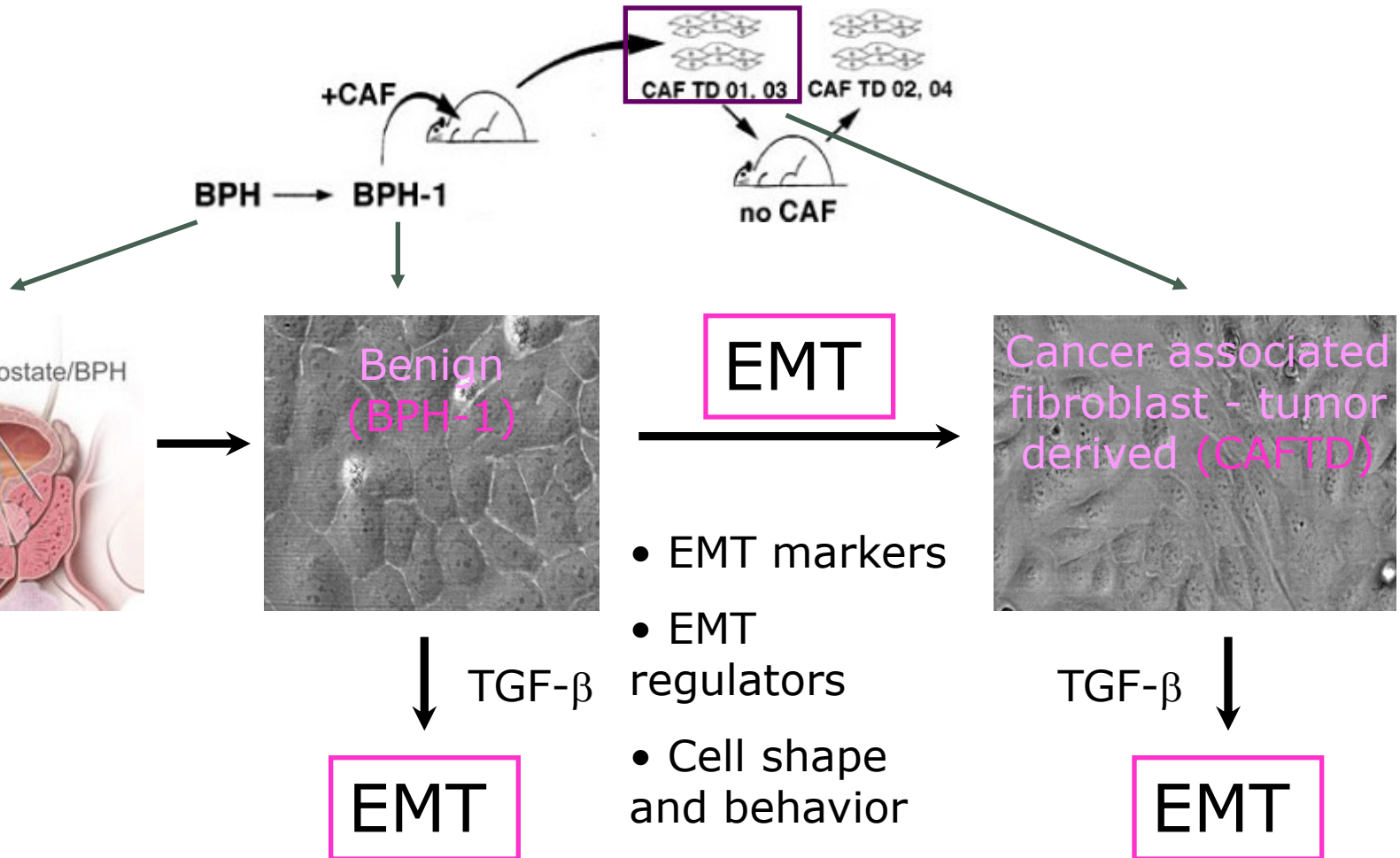
Experimentální přístupy

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

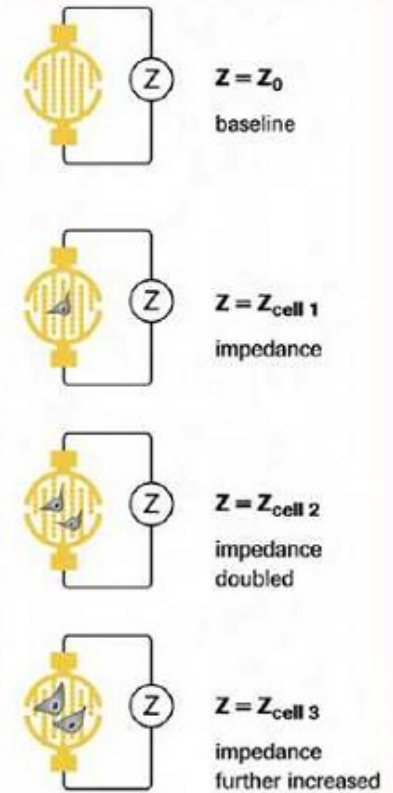
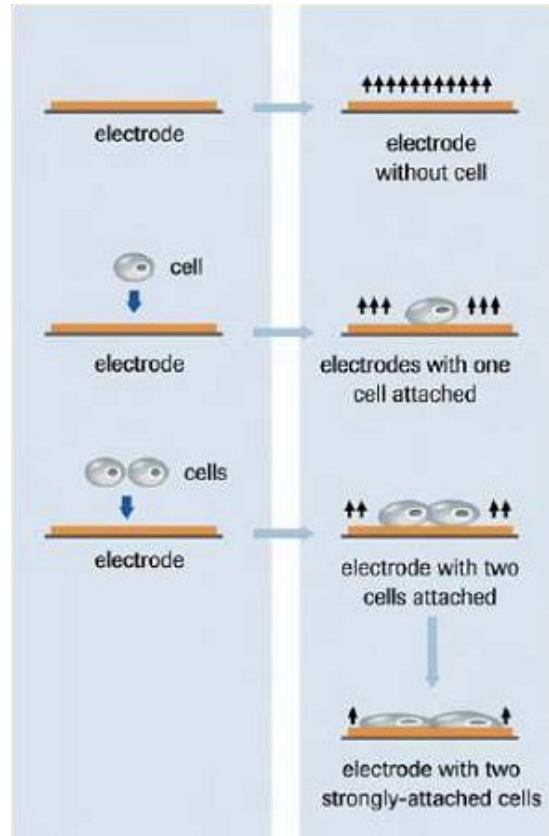
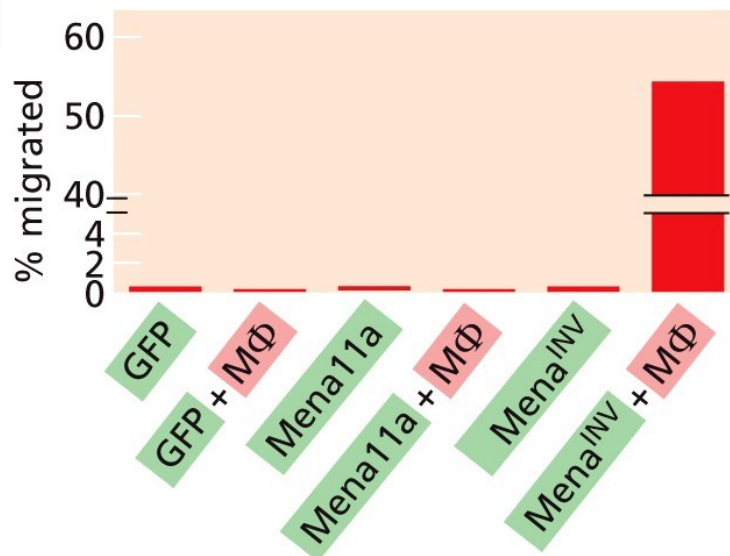
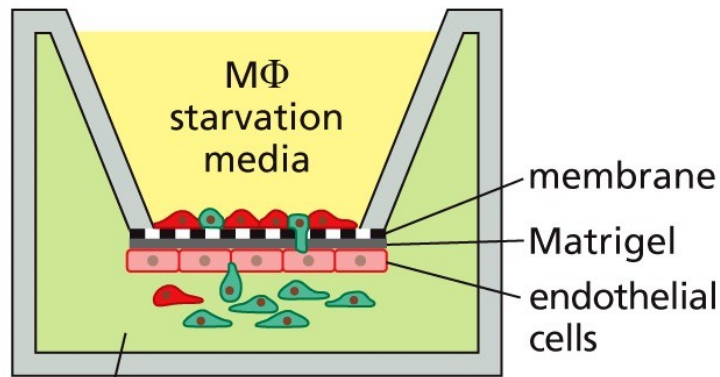
S. W. HAYWARD, R. DAHIYA, G. R. CUNHA, J. BARTEK, N. DESHPANDE, AND P. NARAYAN

Malignant Transformation in a Nontumorigenic Human Prostatic Epithelial Cell Line¹

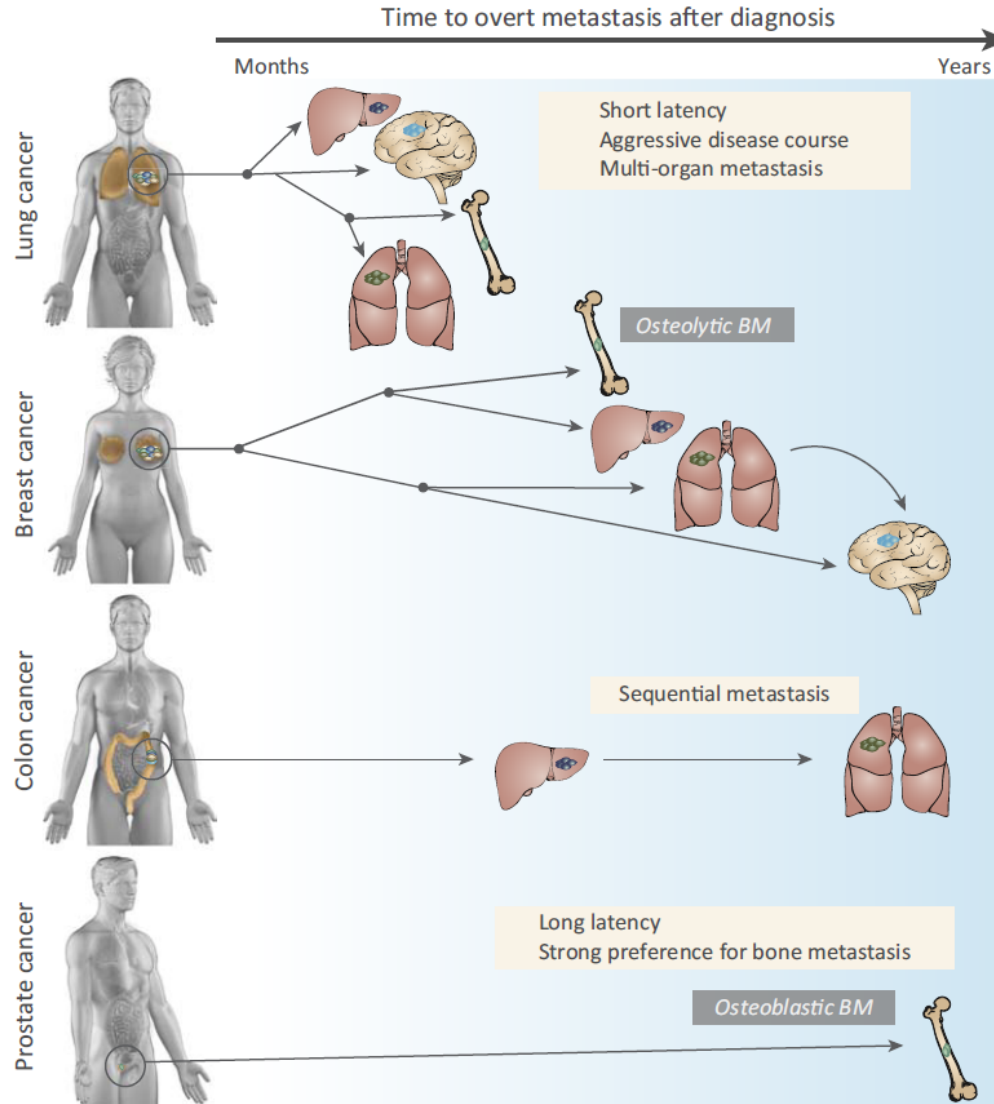
Simon W. Hayward,² Yuzhuo Wang, Mei Cao, Yun Kit Hom, Baohui Zhang, Gary D. Grossfeld, Daniel Sudilovsky, and Gerald R. Cunha



Analýza migračního potenciálu



Diseminace solidních nádorů



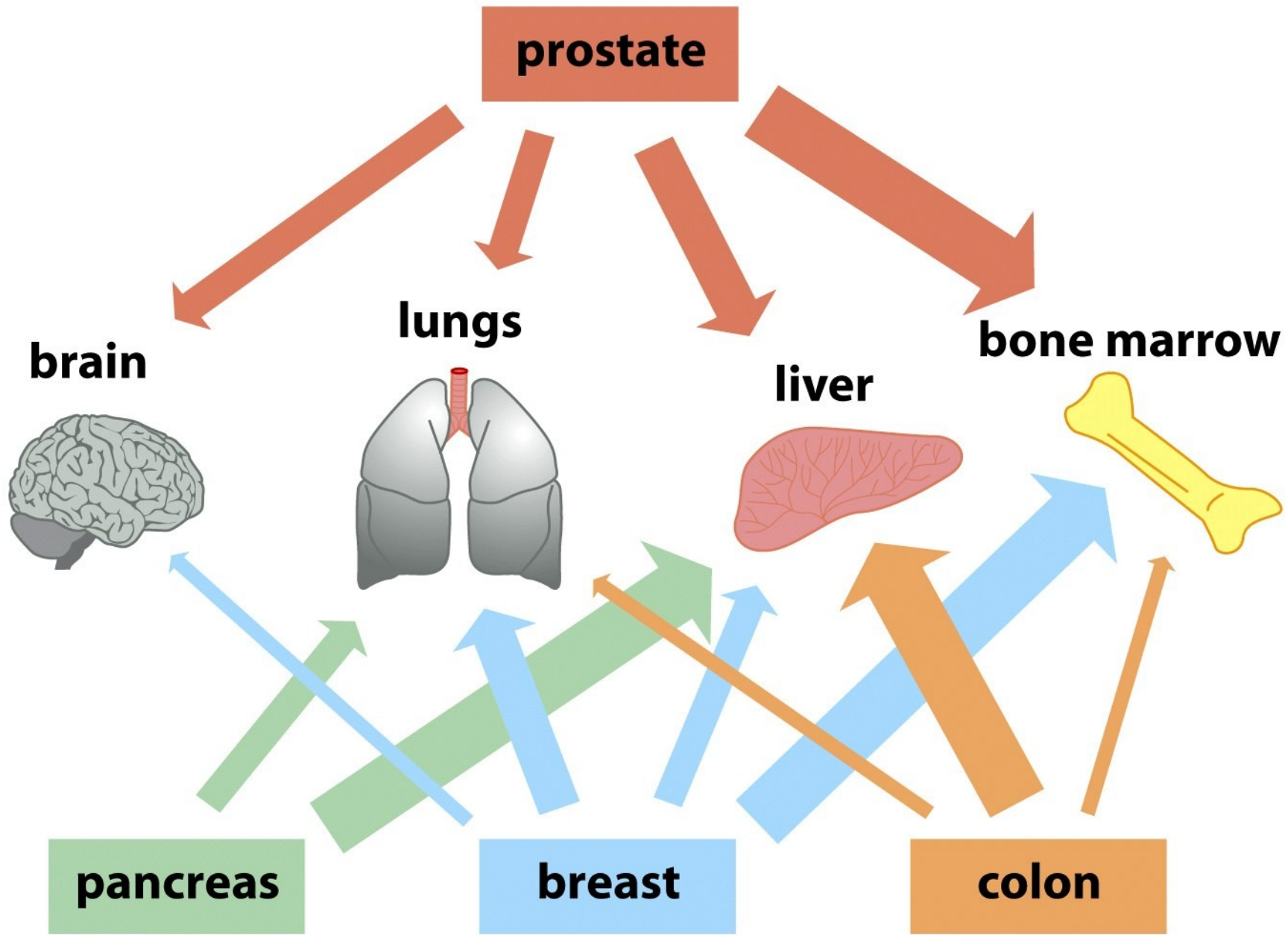


Figure 14.42 *The Biology of Cancer* (© Garland Science 2007)

Metastatický tropismus

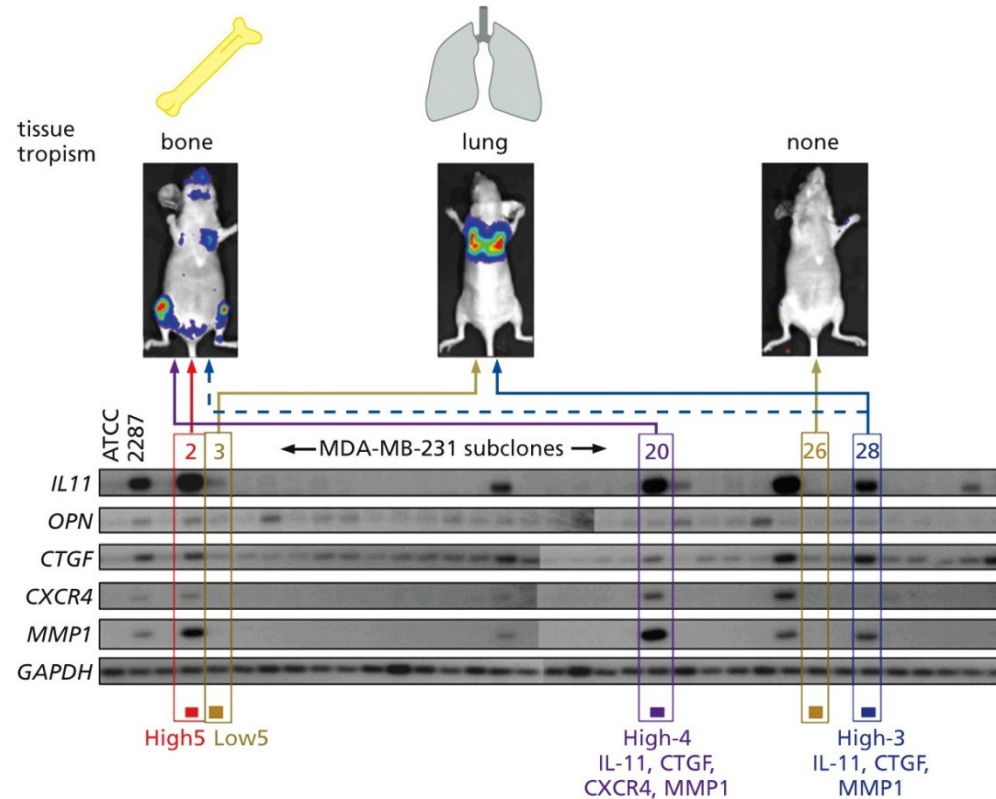
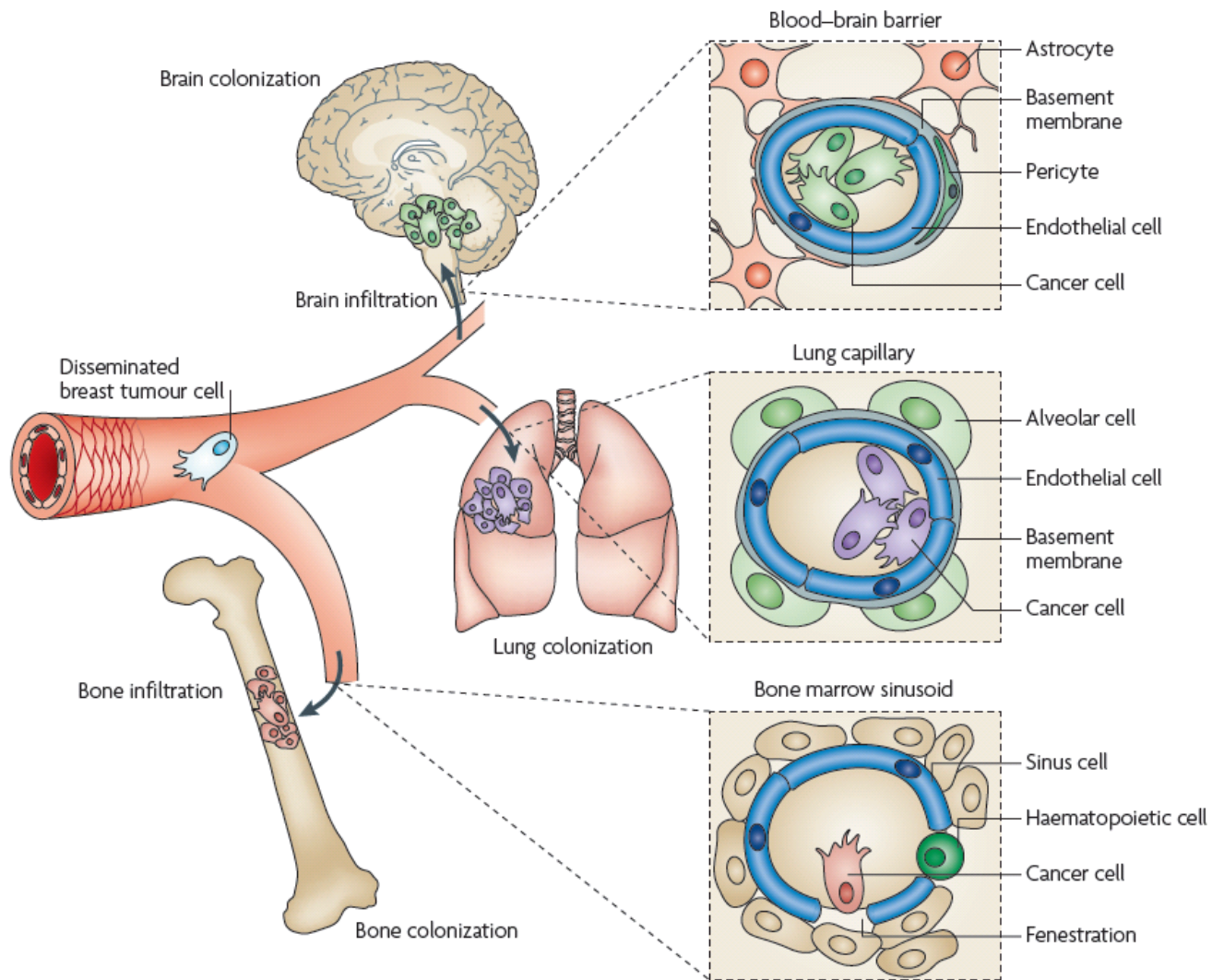


Figure 14.53 The Biology of Cancer (© Garland Science 2014)

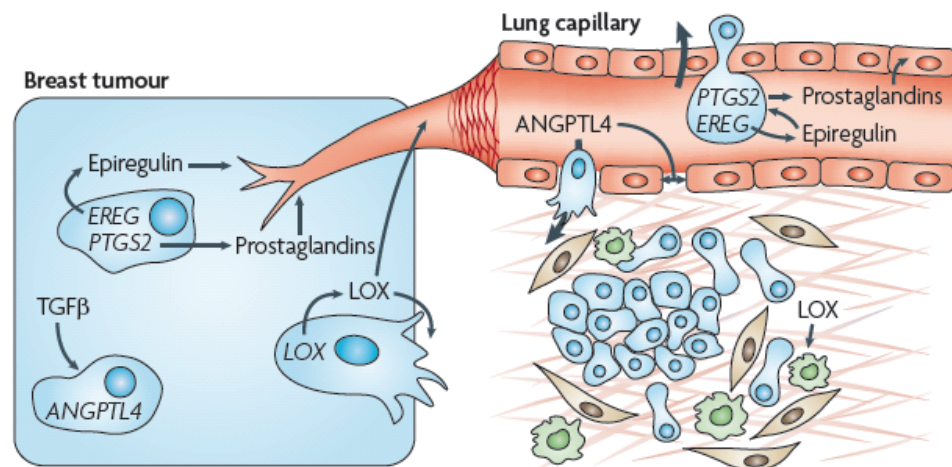
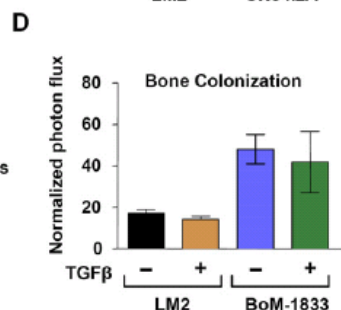
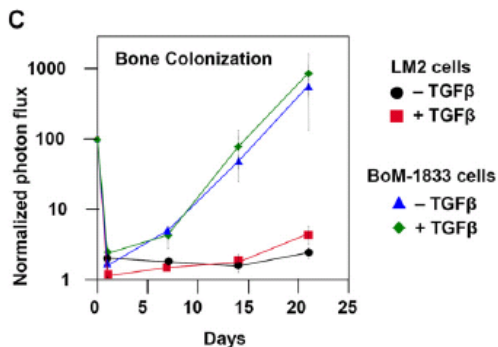
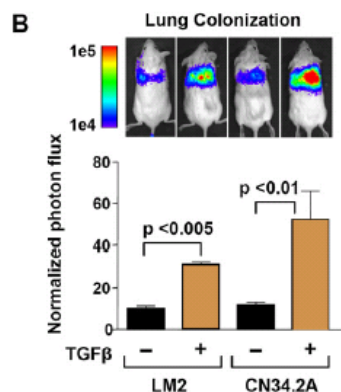
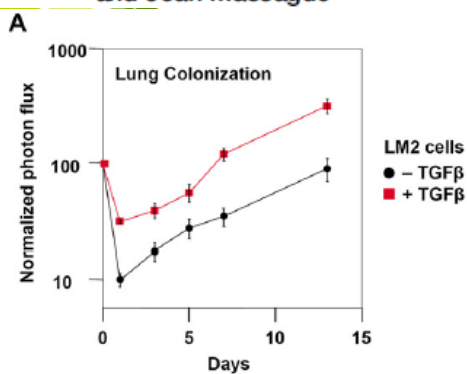
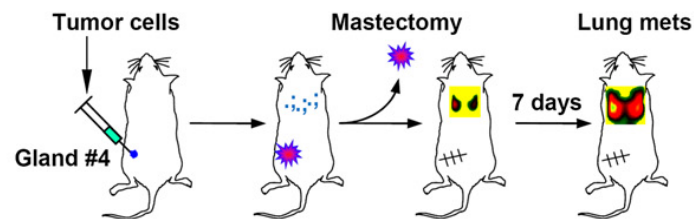
- Thirty-three cells from a large population of human MDA-MB-231 cells were each expanded into a clonal population in culture. The mRNA expression pattern of each subclone was analyzed (columns, arrayed left to right) using probes for the mRNAs of five genes—*IL11* (interleukin-11), *OPN* (osteopontin), *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



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

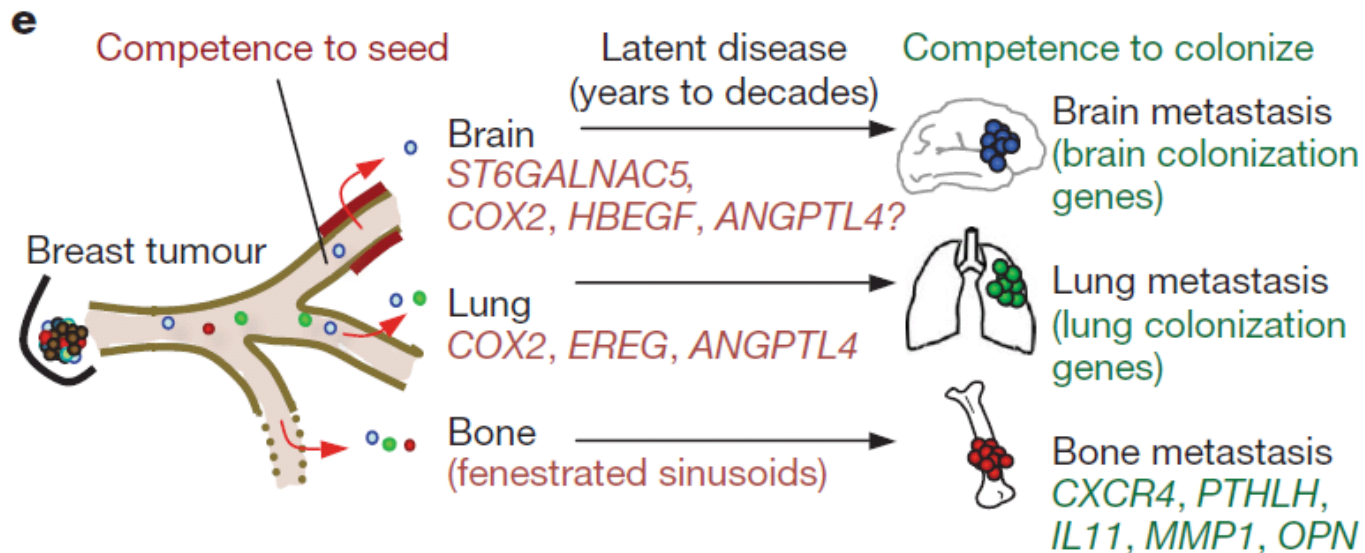
David Padua,¹ Xiang H.-F. Zhang,¹ Qionqing Wang,¹ Cristina Nadal,⁵ William L. Gerald,² Roger R. Gomis,⁴ and Joan Massagué^{1,3,*}



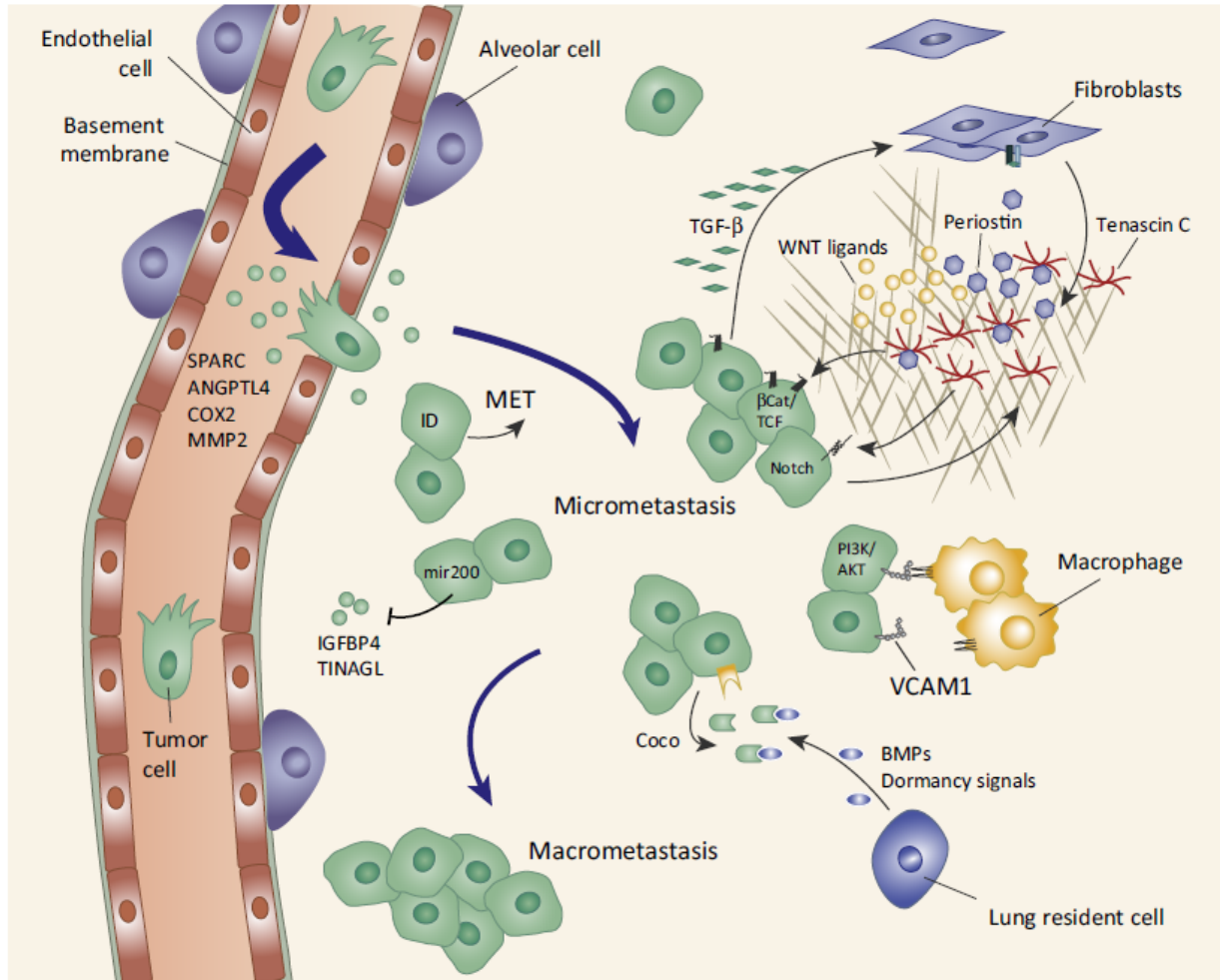
LETTERS

Genes that mediate breast cancer metastasis to the brain

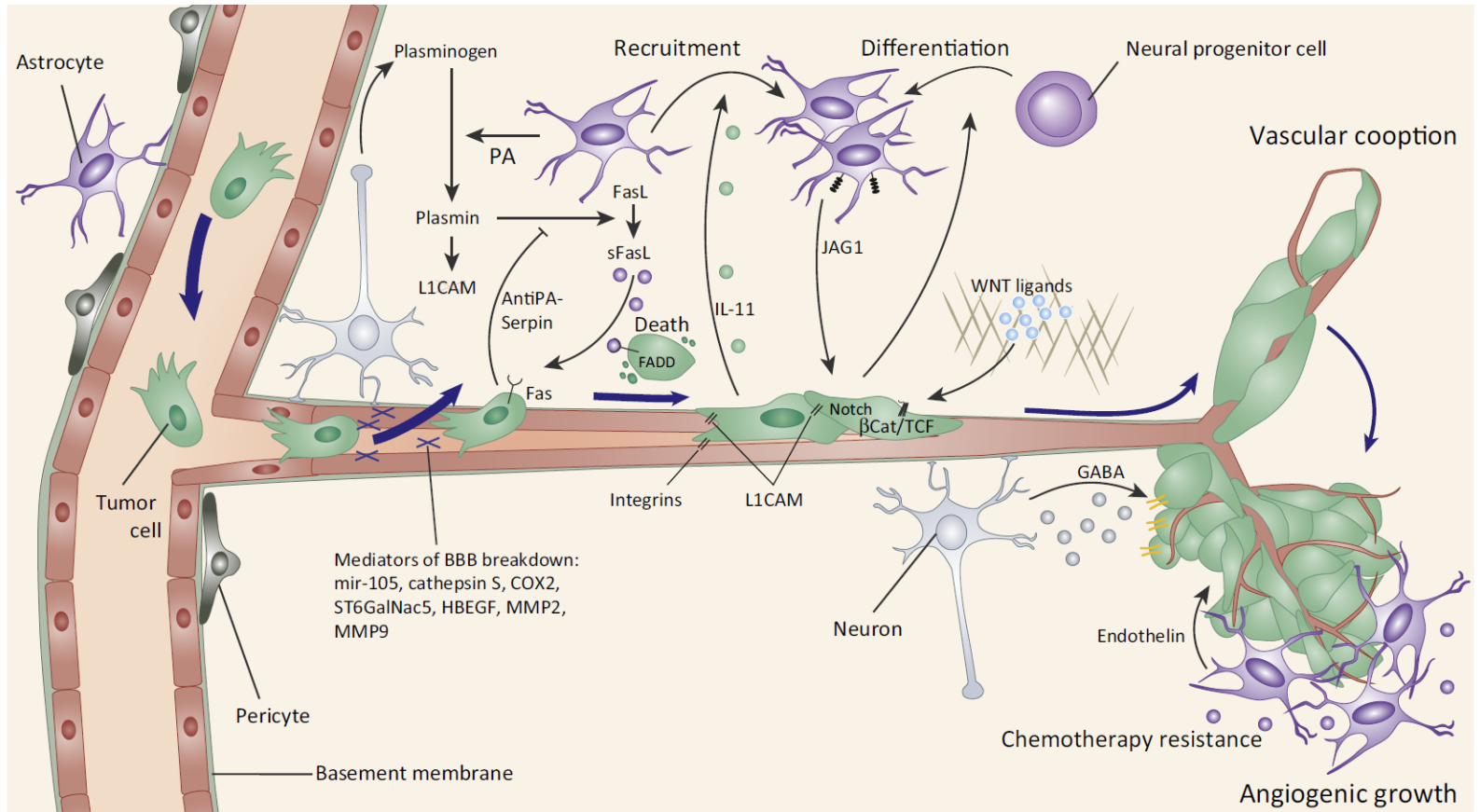
Paula D. Bos¹, Xiang H.-F. Zhang¹, Cristina Nadal^{1†}, Weiping Shu¹, Roger R. Gomis^{1†}, Don X. Nguyen¹, Andy J. Minn², Marc J. van de Vijver³, William L. Gerald⁴, John A. Foekens⁵ & Joan Massagué^{1,6}



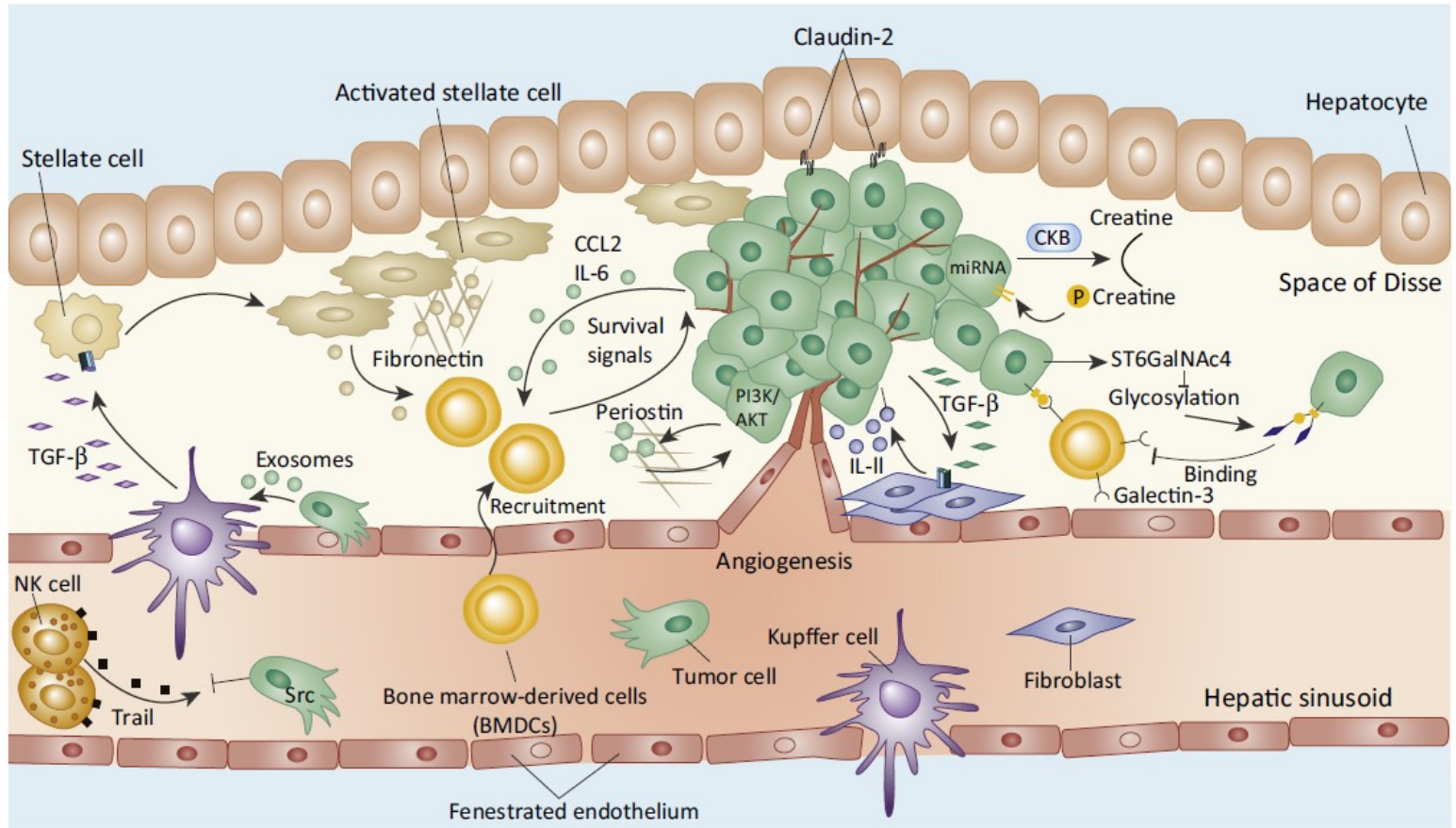
Metastatická kolonizace plic



Metastatická kolonizace mozku



Metastatická kolonizace jater



Osteotropní metastáze

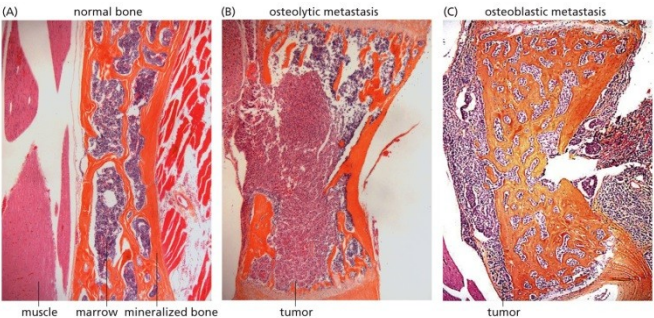
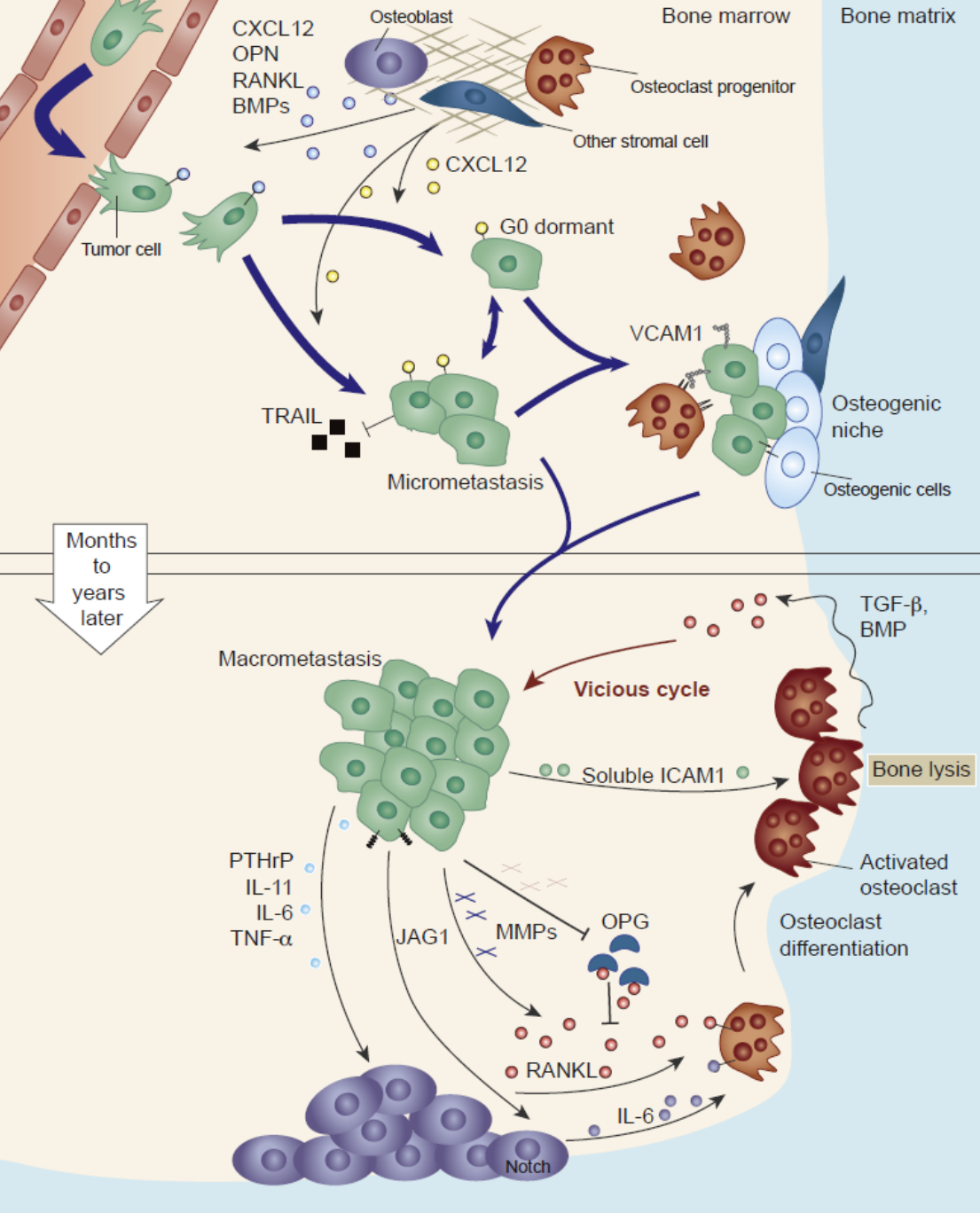


Figure 14.47abc The Biology of Cancer (© Garland Science 2014)

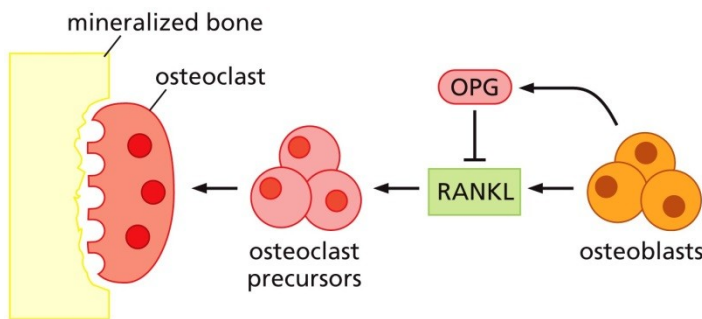


Figure 14.48a The Biology of Cancer (© Garland Science 2014)

Osteotropní metastáze

- ▶ Parathyroid hormone-related peptide (PTHrP)
- ▶ Behém 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

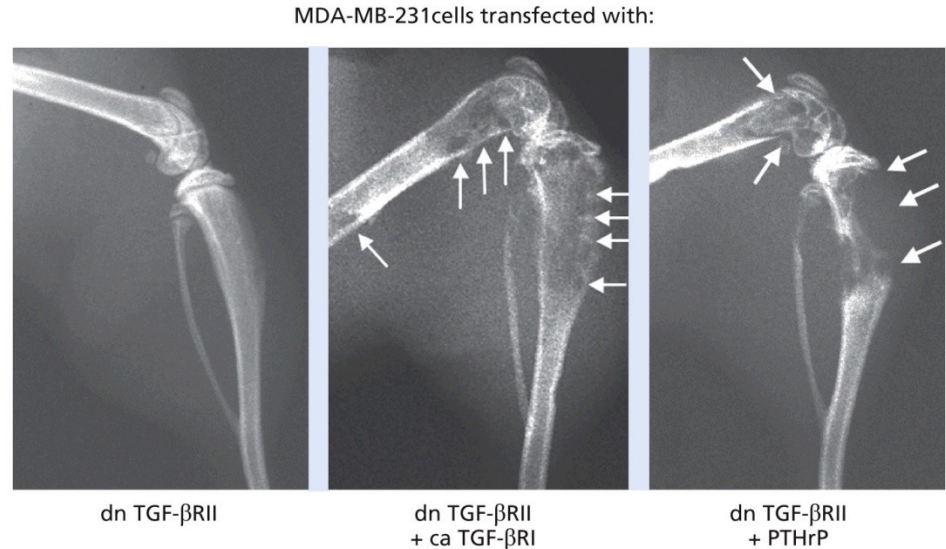


Figure 14.49 The Biology of Cancer (© Garland Science 2014)

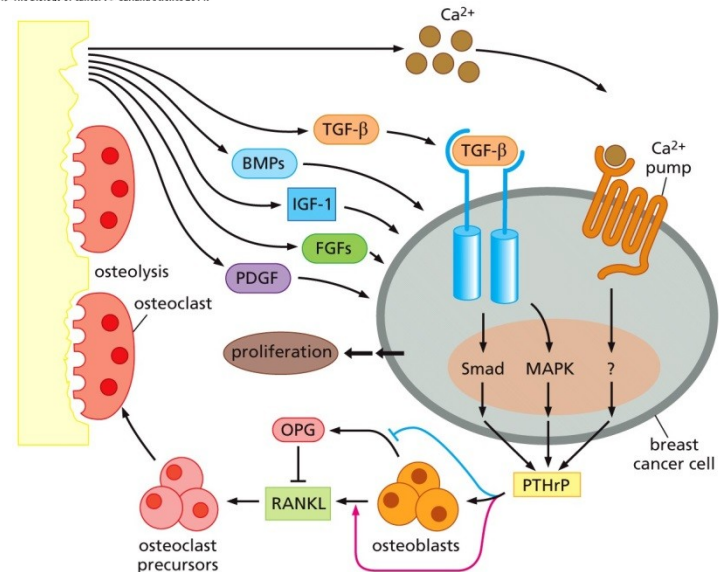
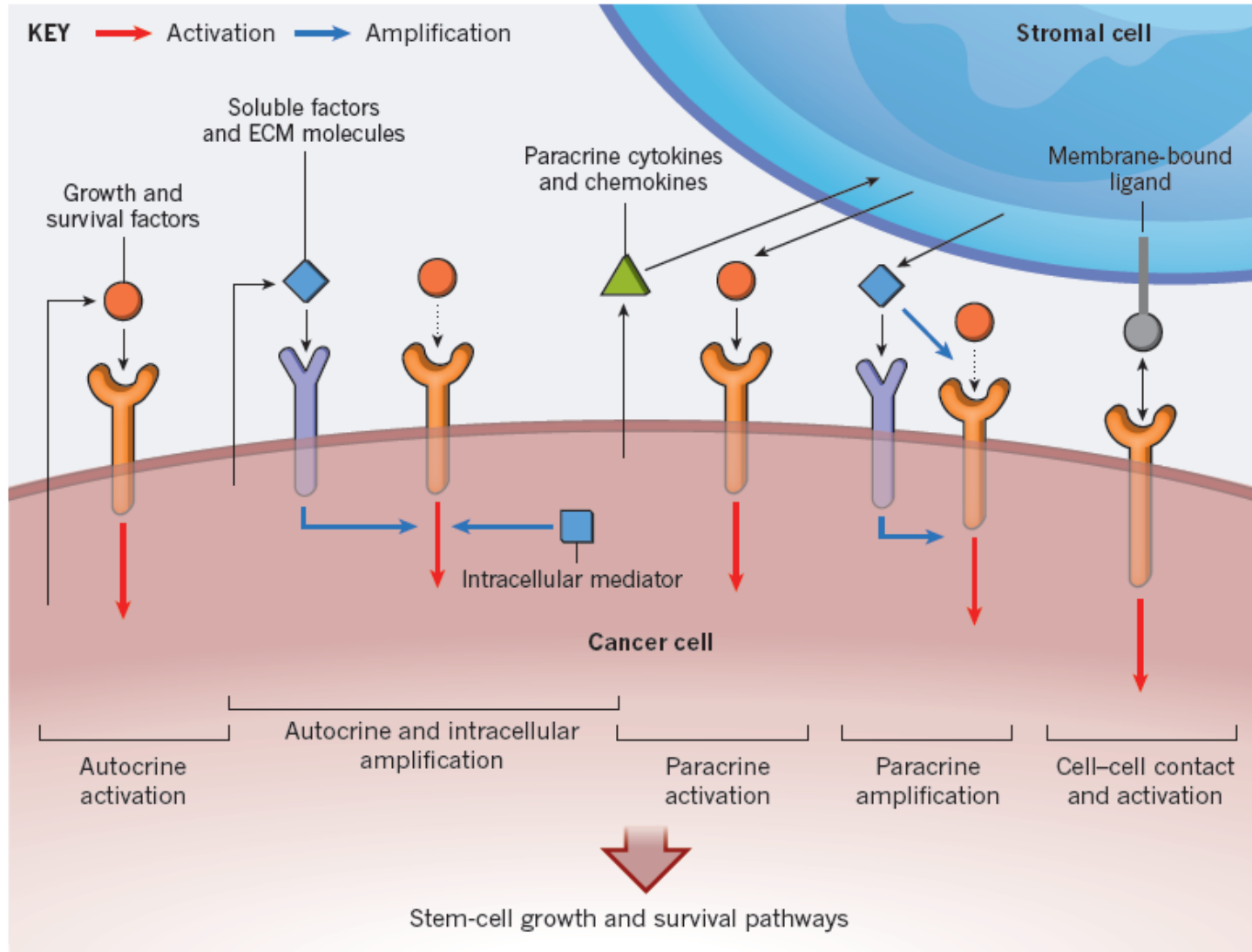
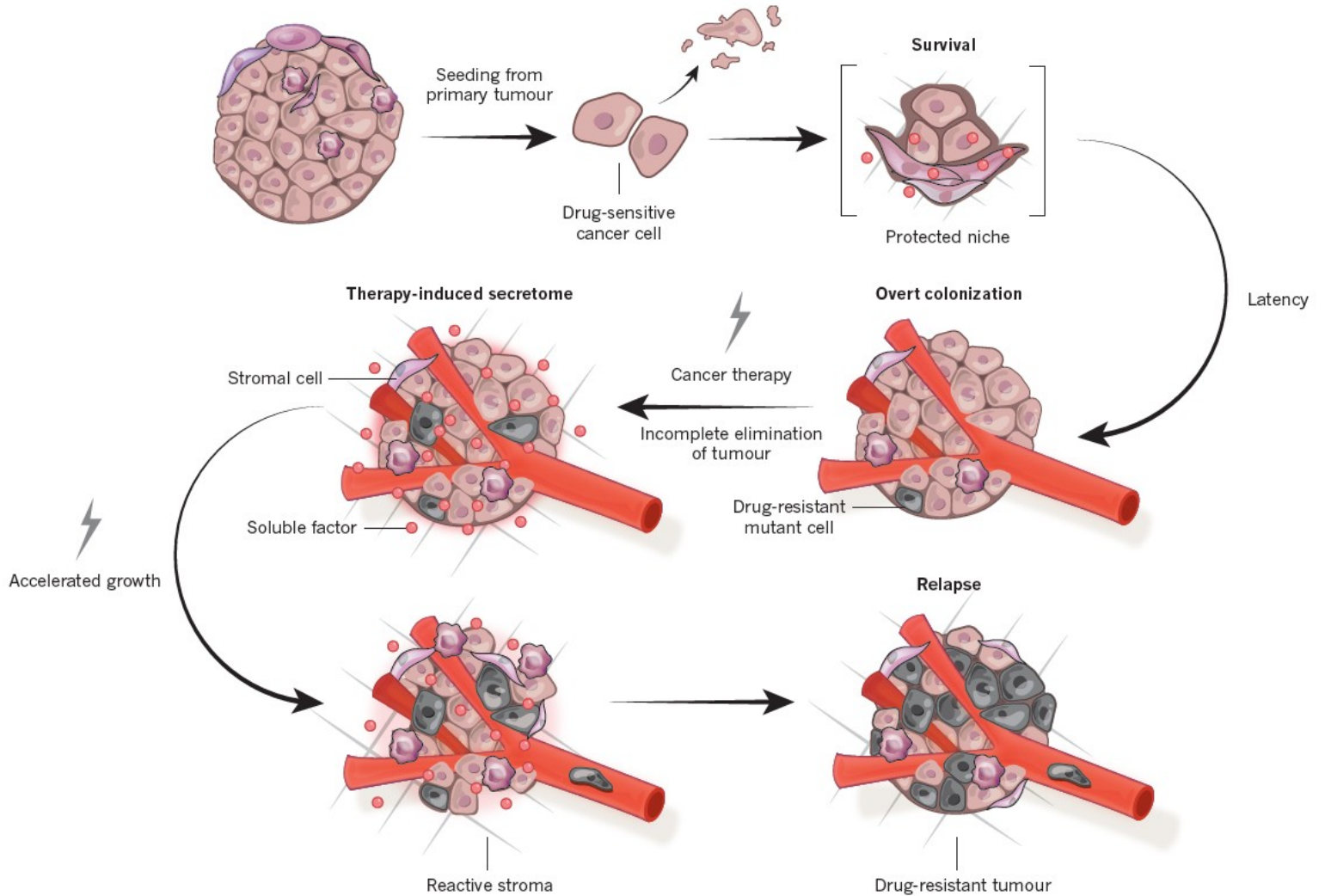


Figure 14.48b The Biology of Cancer (© Garland Science 2014)

Aktivace růstových signálů

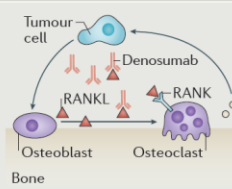
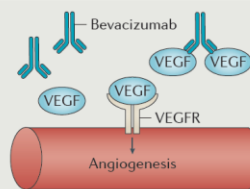
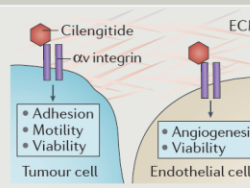
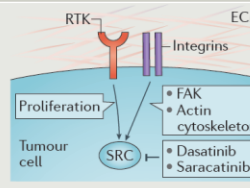


Metastáze před a po terapii



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
<p>Denosumab</p> <p>Monoclonal antibody to RANKL</p> 	<p>RANKL activates osteoclasts and promotes bone destruction; denosumab reduced bone resorption in mice expressing human RANKL²⁹</p>	<p>SREs* in metastatic setting; adjuvant trials used time to first bone metastasis or fracture³⁰⁻³³</p>	<p>FDA approved for prevention of SREs in solid tumours; approved as adjuvant therapy in prostate cancer</p>	
<p>Bevacizumab</p> <p>Monoclonal antibody to VEGF</p> 	<ul style="list-style-type: none"> • Bevacizumab inhibited corneal angiogenesis and lymphangiogenesis²⁴⁴ • In multiple cancer xenograft models, bevacizumab reduced primary tumour growth rates and, in some studies, enhanced survival. Reduced angiogenesis and vessel normalization was observed²⁴⁵ • Prevention or, less frequently, abrogation of metastasis^{246,247} 	<ul style="list-style-type: none"> • Recurrent ovarian cancer, PFS^{35,36} • Metastatic colorectal cancer, OS^{260,261} • Metastatic or resistant HER2⁺ breast cancer, PFS³⁸ • Metastatic renal cancer, PFS²⁶² • Glioblastoma, OS, PFS²⁶³ • Advanced lung cancer, OS³⁷ • Adjuvant therapy in triple-negative breast cancer, DFS⁴¹ 	<ul style="list-style-type: none"> • 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 	
<p>Cilengitide</p> <p>$\alpha v \beta 3$ and $\alpha v \beta 5$ integrin peptide inhibitor</p> 	<ul style="list-style-type: none"> • Stabilization of glioma growth and angiogenesis. Synergistic inhibition of glioma with TMZ⁶¹⁻⁶⁴ • Synergy with therapeutics in melanoma primary tumour growth⁶³, synergy with radio-immunotherapy in breast cancer tumour growth²⁴⁸ • Inhibition of metastasis⁶² • Synergy with verapamil increased angiogenesis and reduced metastasis²⁴⁹ 	<ul style="list-style-type: none"> • Phase III CENTRIC EORTC, with radiation therapy and TMZ, for glioma, OS. Newly diagnosed glioma, same combination, recurrence⁶⁵ • Phase II trials in melanoma and lung and prostate cancers, PFS⁶⁶⁻⁶⁸ 	<p>All advanced trials were negative</p>	
<p>Dasatinib and saracatinib</p> <p>SRC kinase and BCR-ABL kinase inhibitor</p> 	<ul style="list-style-type: none"> • Inhibition of CML models²⁵⁰ • Inhibition of primary tumour growth in multiple model systems, as monotherapy or in combination²⁵¹⁻²⁵³ • Prevention of metastasis in multiple cancer model systems²⁵⁴⁻²⁵⁸, but not osteosarcoma²⁵⁹ • Inhibition of prostate cancer growing in bone and bone remodelling^{62,63} 	<ul style="list-style-type: none"> • Cytogenetic response end points for CML • Response for advanced solid tumours⁷¹⁻⁸⁰ • OS in Phase III prostate cancer⁸⁷ 	<ul style="list-style-type: none"> • 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- κ B; 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.

Shrnutí

- 90% úmrtí na nádorová onemocnění souvisí s metastázováním
- Invazivní kaskáda zahrnuje: lokální invazi, intravazaci, transport, extravazaci, formování 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

Otázky

- ▶ Jaké je možné vysvětlení nízké účinnosti kolonizace mikrometastáz?
- ▶ Jaký mechanismus může vysvětlit korelaci mezi velikostí nádoru a prognózou vzniku metastáz?
- ▶ Jaké důkazy podporují zapojení EMT v nádorové patogenezi, jaké jsou naopak proti?