

INVAZIVITA A METASTÁZOVÁNÍ

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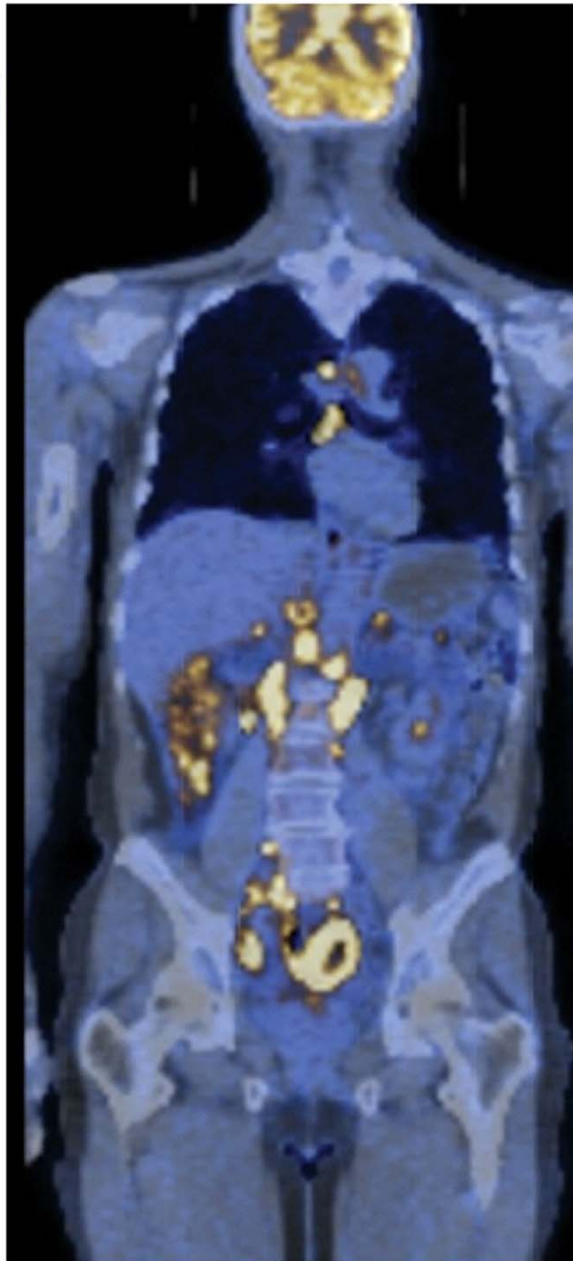
Otázky na 20.3.

- ▶ Proč je autokrinní signalizace destabilizující faktor pro normální tkáň?
- ▶ Jaké mechanismy mohou buňky uplatňovat pro snížení citlivosti k působení růstových faktorů?
- ▶ Jakou roli hrají integriny v procesu buněčné motility?
- ▶ 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





Whole-body scan of a patient with metastatic non-Hodgkin's lymphoma (NHL). This is a fusion image of a CT (computed X-ray tomography) scan of the body's tissues (*gray, blue*) and a PET (positron-emission tomography) scan in which the uptake of radioactively labeled fluorodeoxyglucose (FDG) in various tissues (*yellow*) has been detected. FDG uptake indicates regions of high glucose uptake associated with aerobic glycolysis. The activity associated with the brain is normal. However, the yellow spots in the abdominal regions indicate multiple NHL metastases.

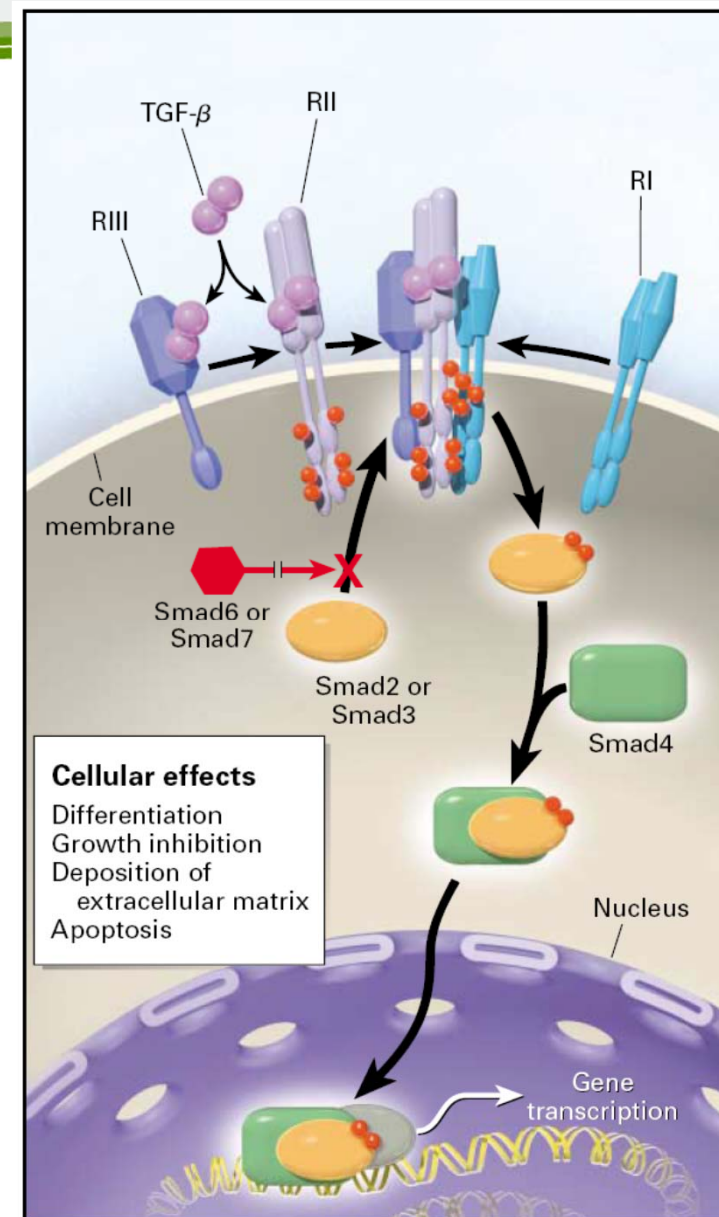
Figure 14.1 *The Biology of Cancer* (© Garland Science 2007)

Transforming growth factor - β (TGF- β)

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

TGF- β_1

- pleiotropní cytokin
- negativní regulátor

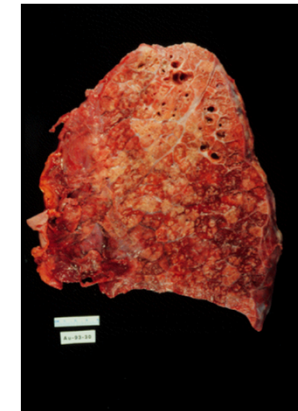


Biologické funkce TGF- β







- ▶ 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.



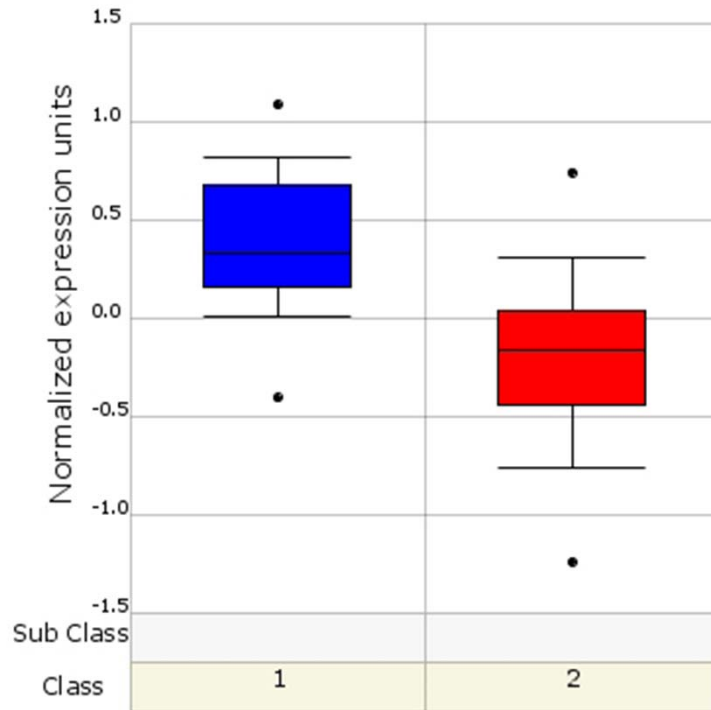
Role TGF- β v carcinogenezi

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)

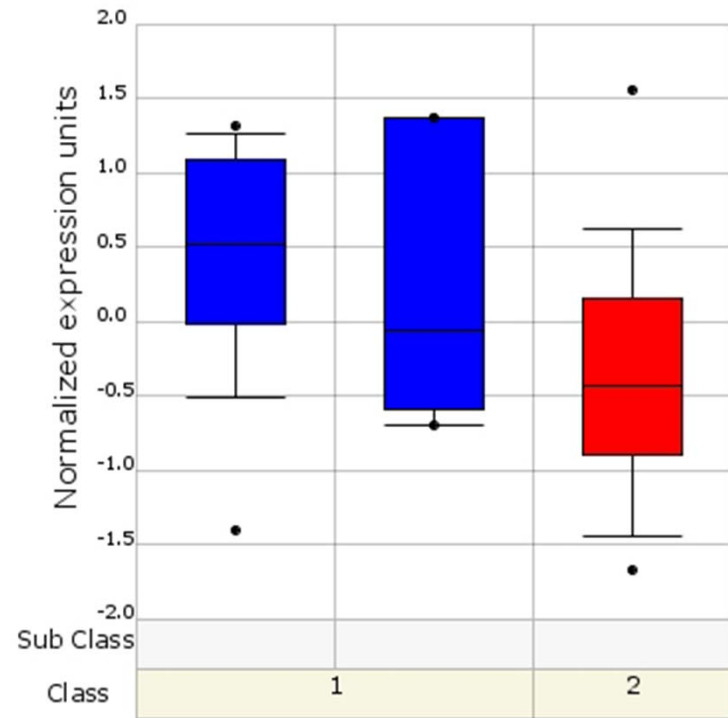


Box Plot - Description

Prostate – normal vs. cancer

TGFBR2

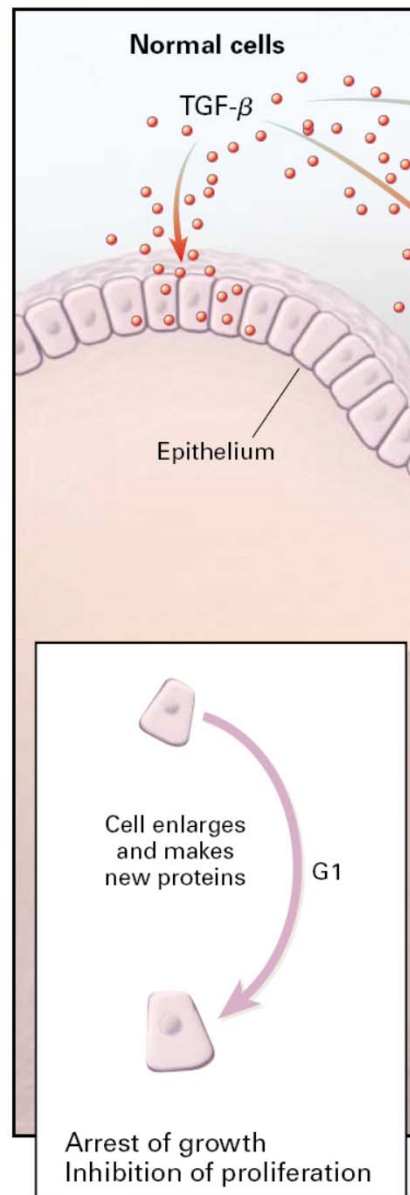
Transforming growth factor, beta receptor ii (70/80kda)



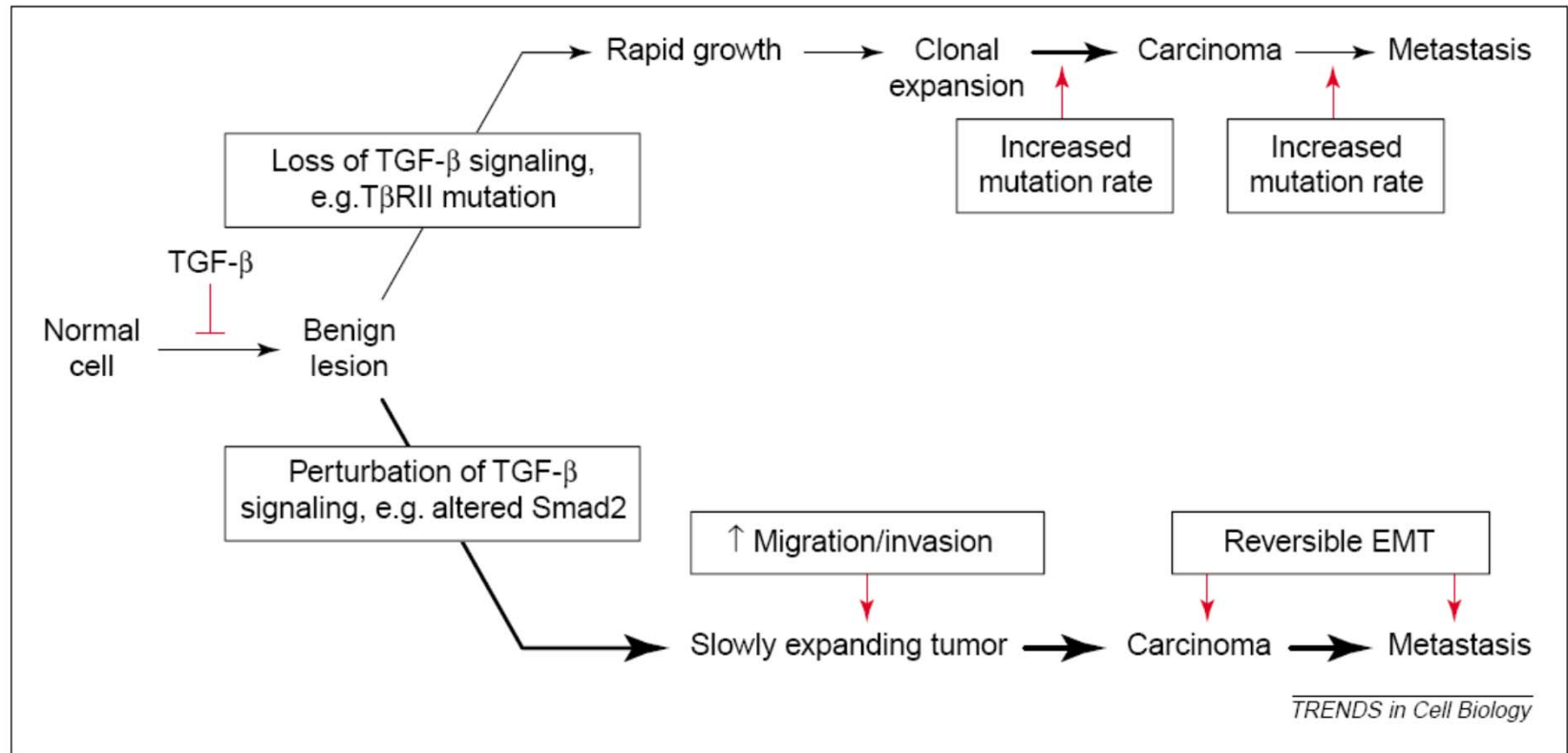
Box Plot - Description

normal, hyperplasia vs. cancer

Role TGF- β v carcinogenezi



Role TGF- β v carcinogenezi



Mechanismy karcinogeneze

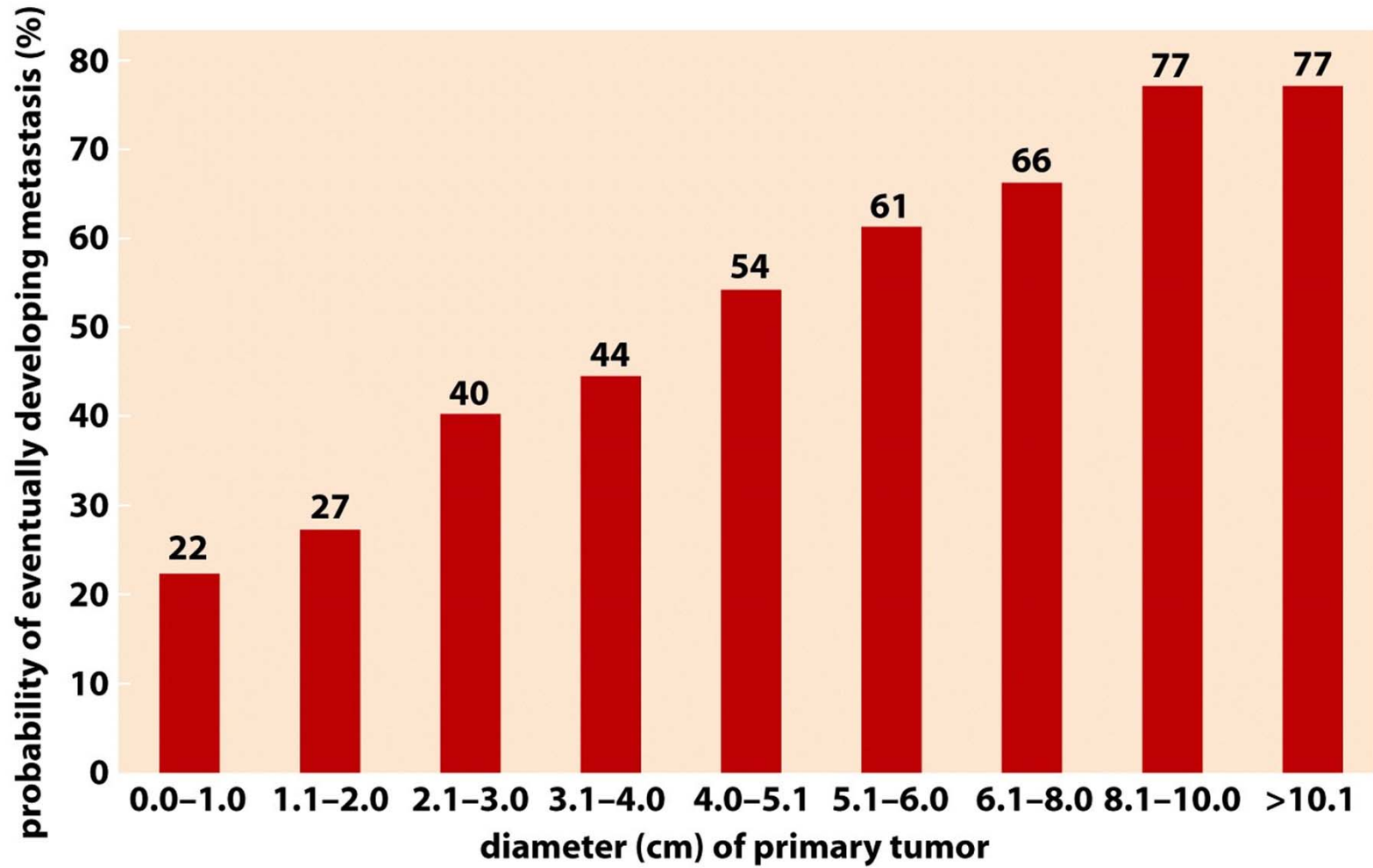


Figure 14.3 *The Biology of Cancer* (© Garland Science 2007)

Mechanismy karcinogeneze

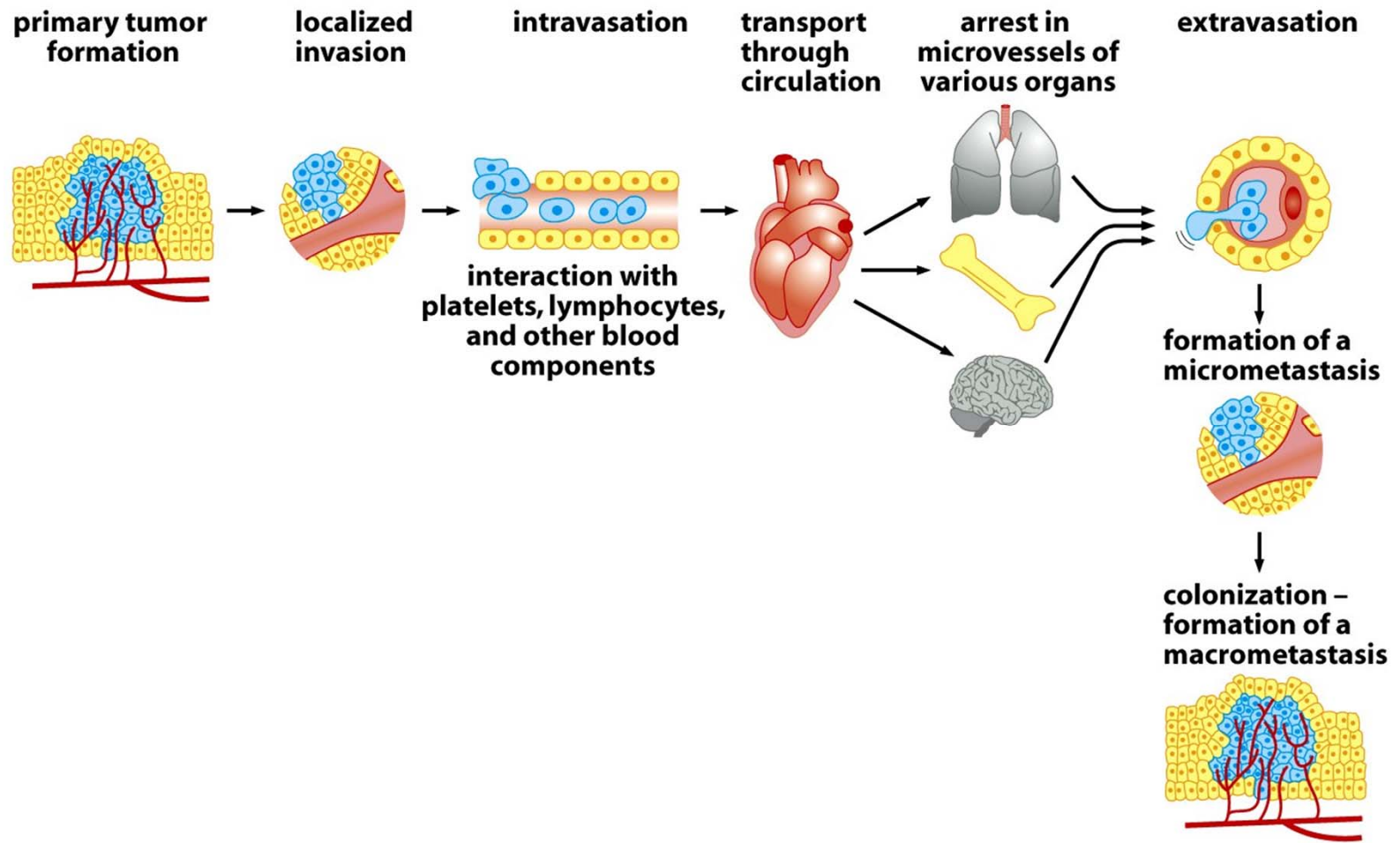


Figure 14.4 *The Biology of Cancer* (© Garland Science 2007)

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

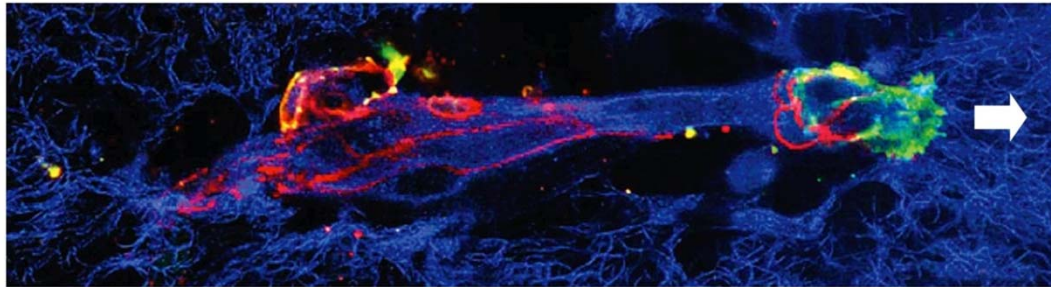


Figure 14.6a The Biology of Cancer (© Garland Science 2014)

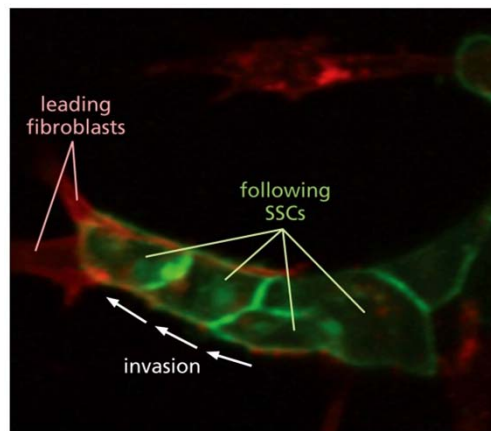


Figure 14.6b The Biology of Cancer (© Garland Science 2014)

Tumor cells, fibroblasts

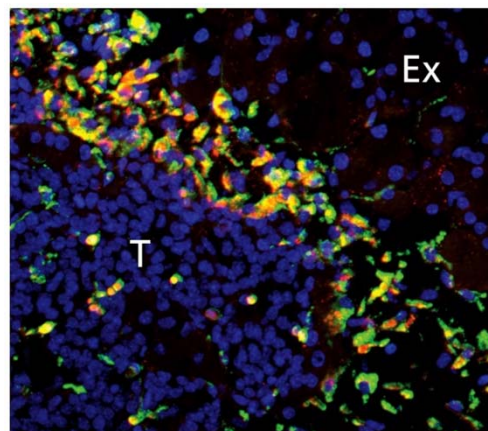


Figure 14.6c The Biology of Cancer (© Garland Science 2014)

Macrophages, cathepsin

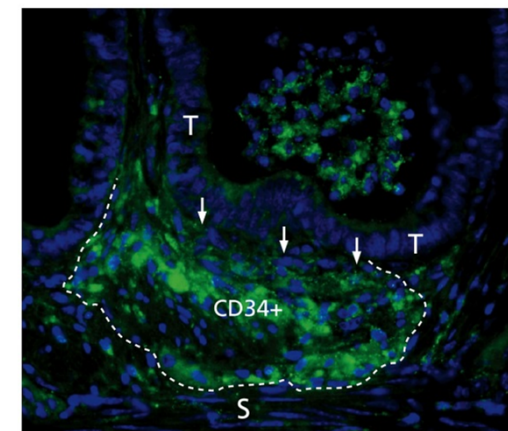


Figure 14.6d The Biology of Cancer (© Garland Science 2014)

Immature myeloid cells

Pohyb buněk

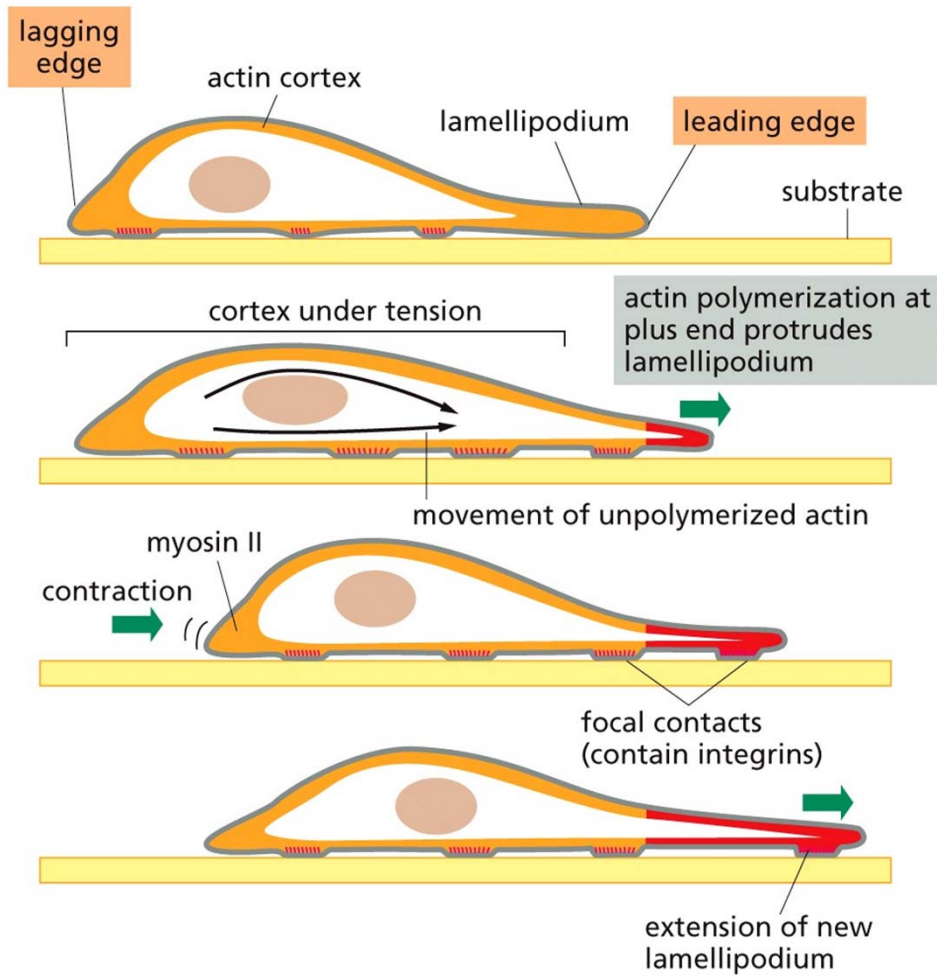


Figure 14.36 The Biology of Cancer (© Garland Science 2014)

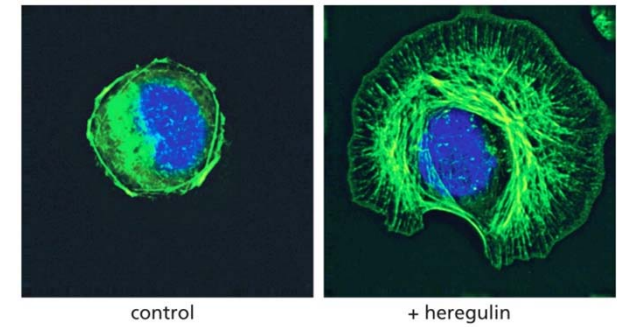


Figure 14.37c The Biology of Cancer (© Garland Science 2014)

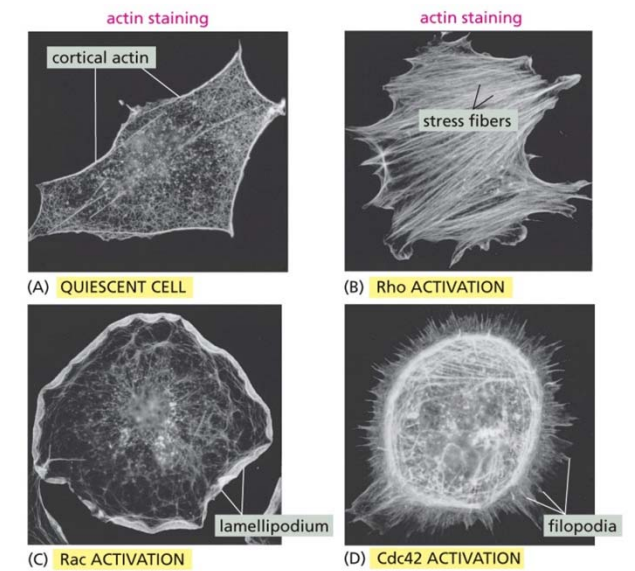


Figure 14.39 The Biology of Cancer (© Garland Science 2014)

Pohyb buněk

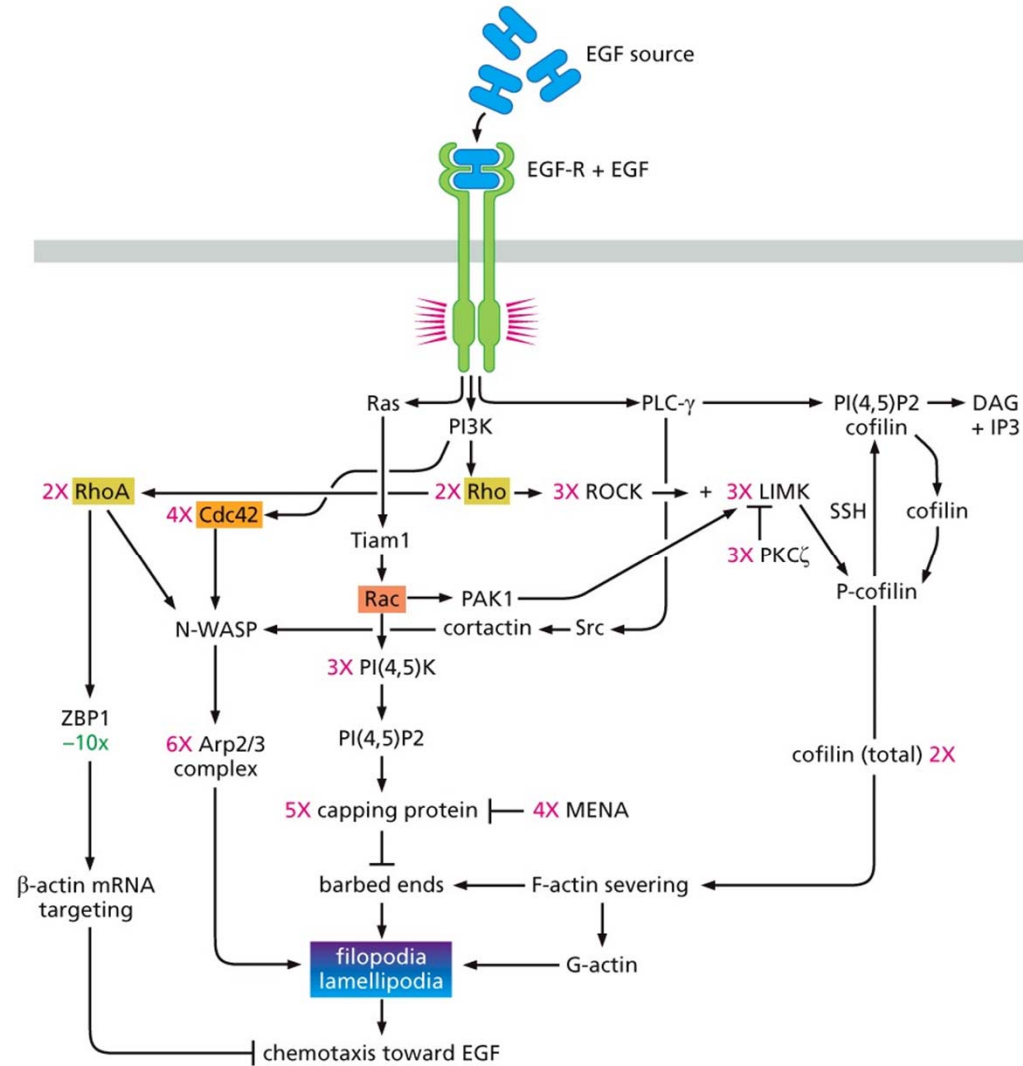


Figure 14.40 The Biology of Cancer (© Garland Science 2014)

Transport, cirkulace

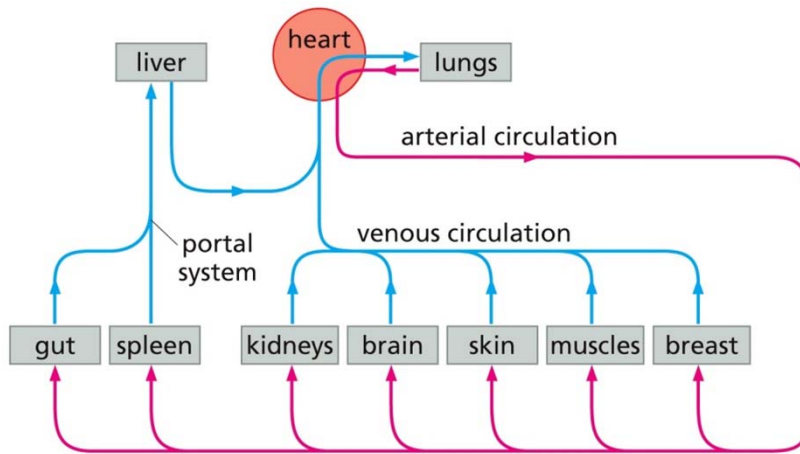
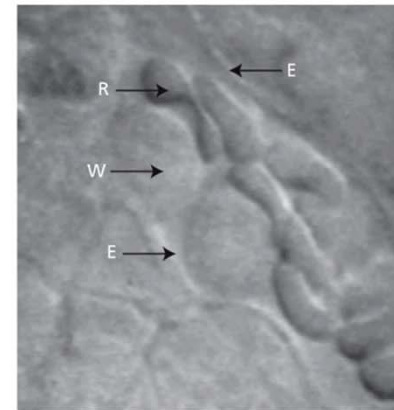
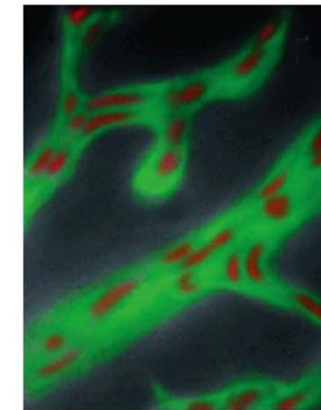


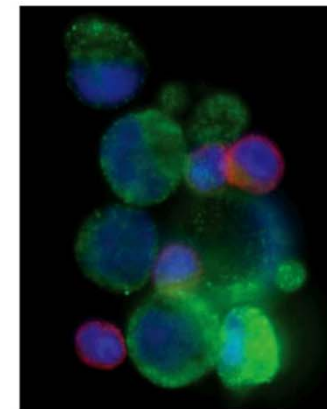
Figure 14.45 The Biology of Cancer (© Garland Science 2014)



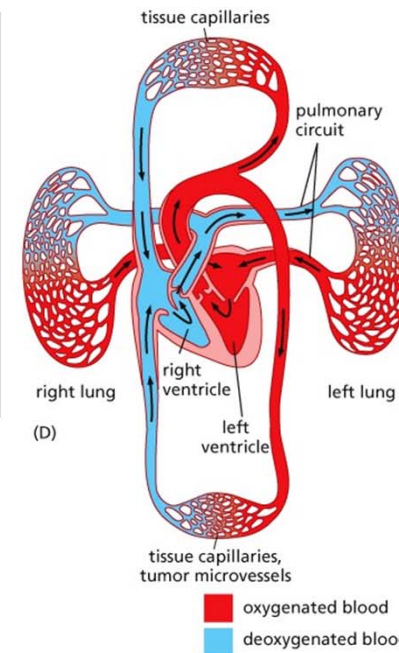
(A)



(B)



(C)

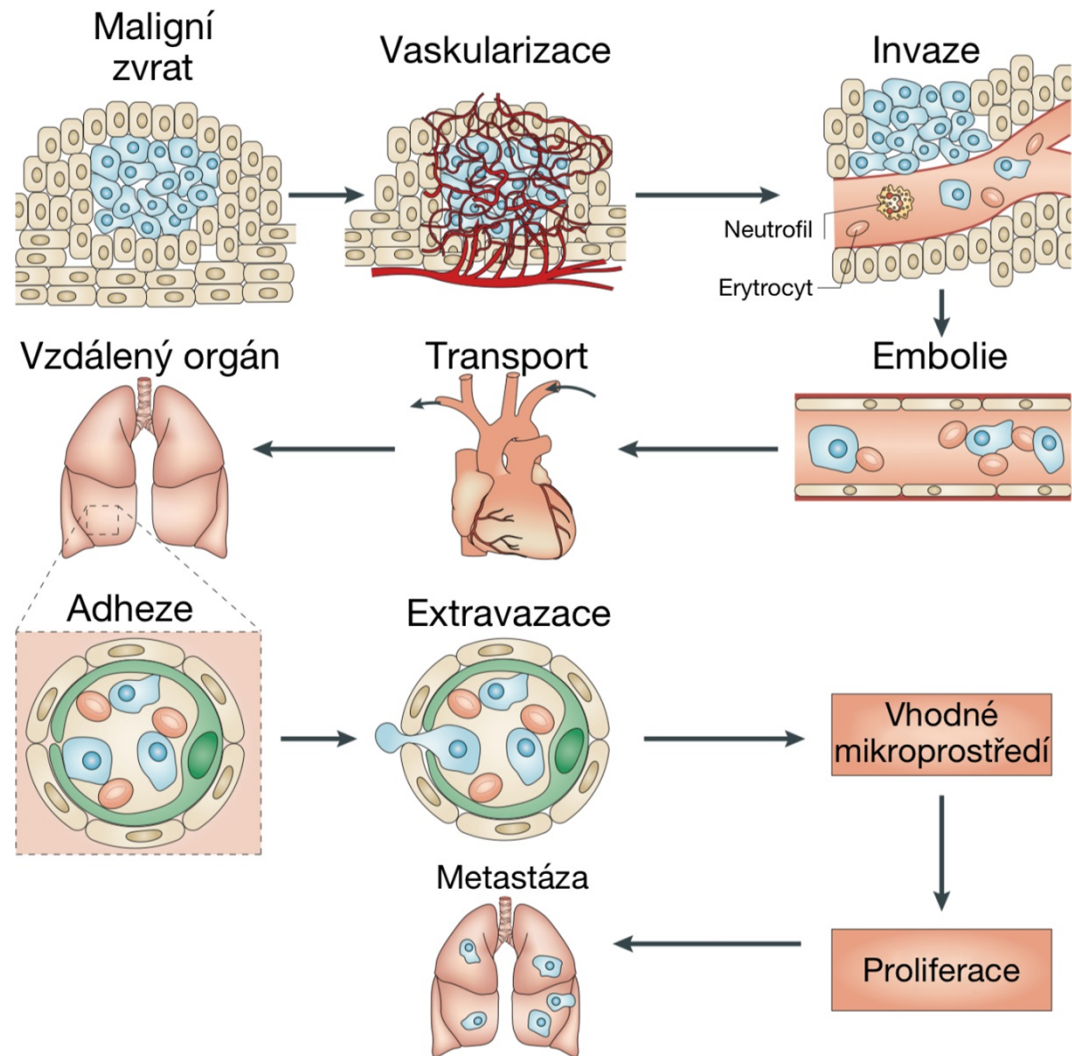


(D)

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

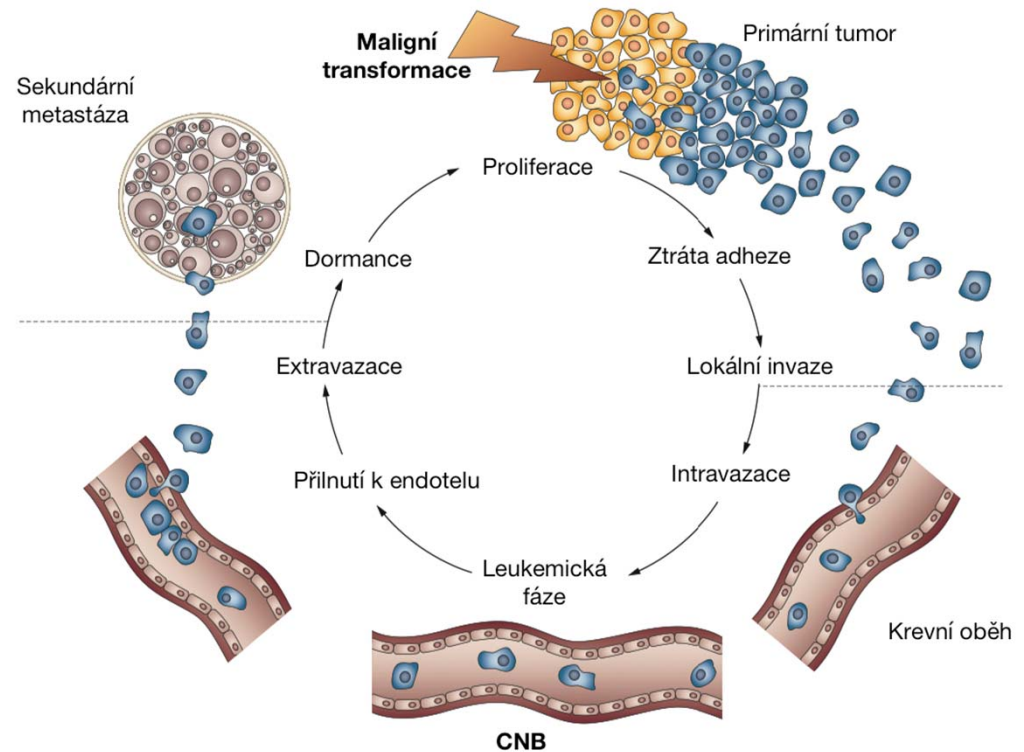
Metastatická kaskáda

Cirkulující nádorové buňky (CNB) –
klíčová úloha



Proč se cirkulujícími nádorovými buňkami zabývat?

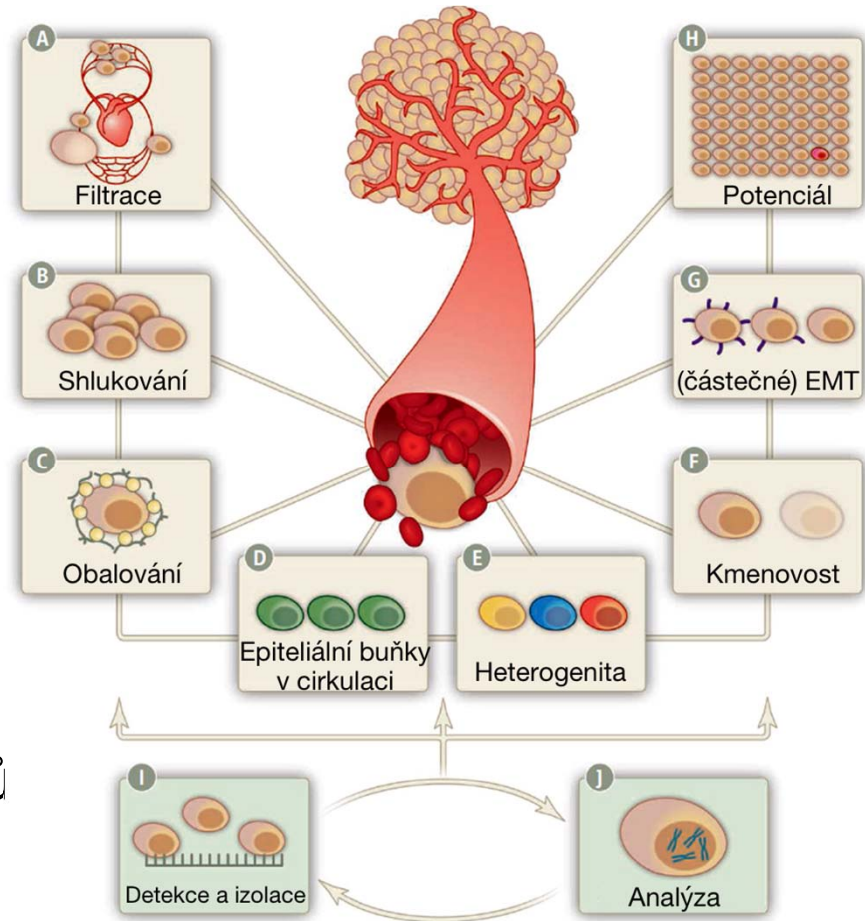
- 90% úmrtí spojených se solidními nádory – **metastáze**
 - Šíření primárně krví
- Klinicky významné
 - „Liquid biopsy“
 - Průběh terapie
 - Prognostický znak
 - Specifické mutace → cíle terapie



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



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	plicí, 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	plicí, 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]
Celsec	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**]
VerlFAST	2014	90%			Lung	[22]
SB microfilter	2014	78–83%	$1.7-2 \times 10^3$	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 $99.33\% \pm 0.56\%$ (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

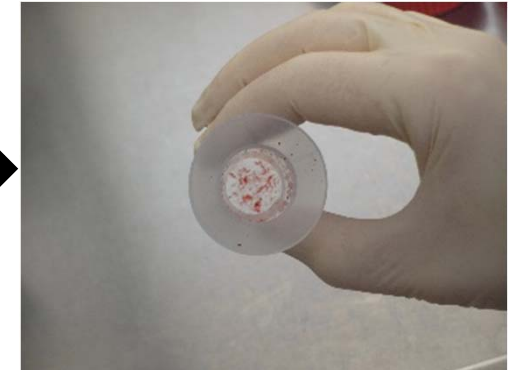
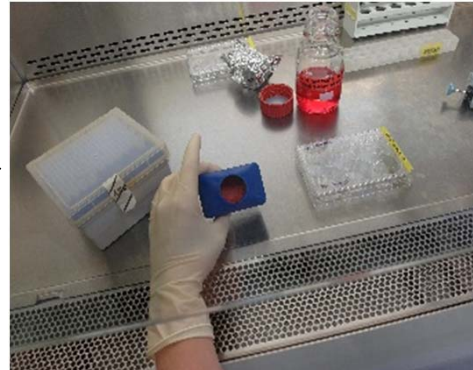
- CNB: epiteliální původ → větší velikost
- Platformy: **MetaCell**, CellSieve, Celsee,...

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

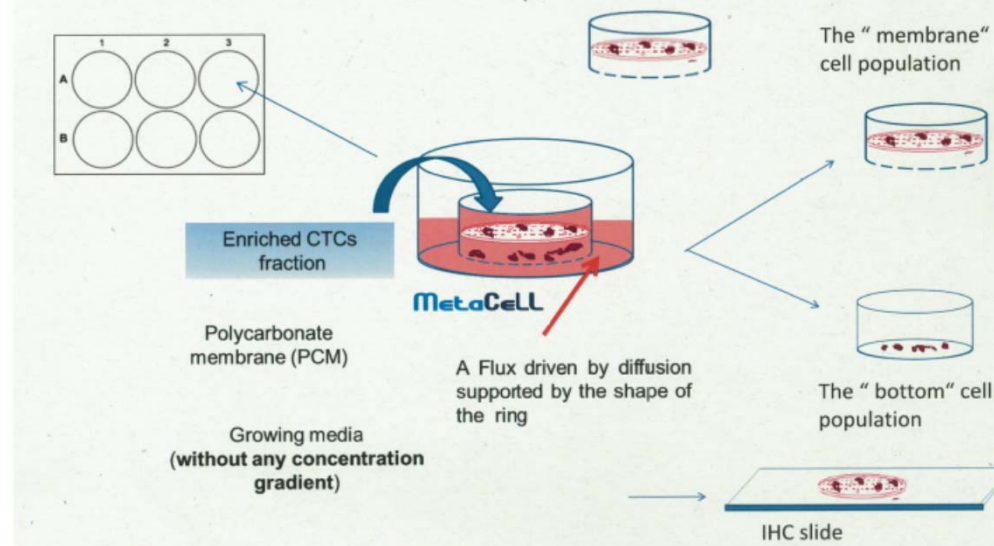
- **Výhody** – nezávislost na povrchových znacích
 - Heterogenní populace
 - Není nutná aktivace receptorů
 - Nativní stav
- **Nevýhody**
 - Možný překryv s leukocyty
 - Nutné využít dalších znaků (CD45)
 - Různá velikost CNB?

Příklad: Filtrace

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

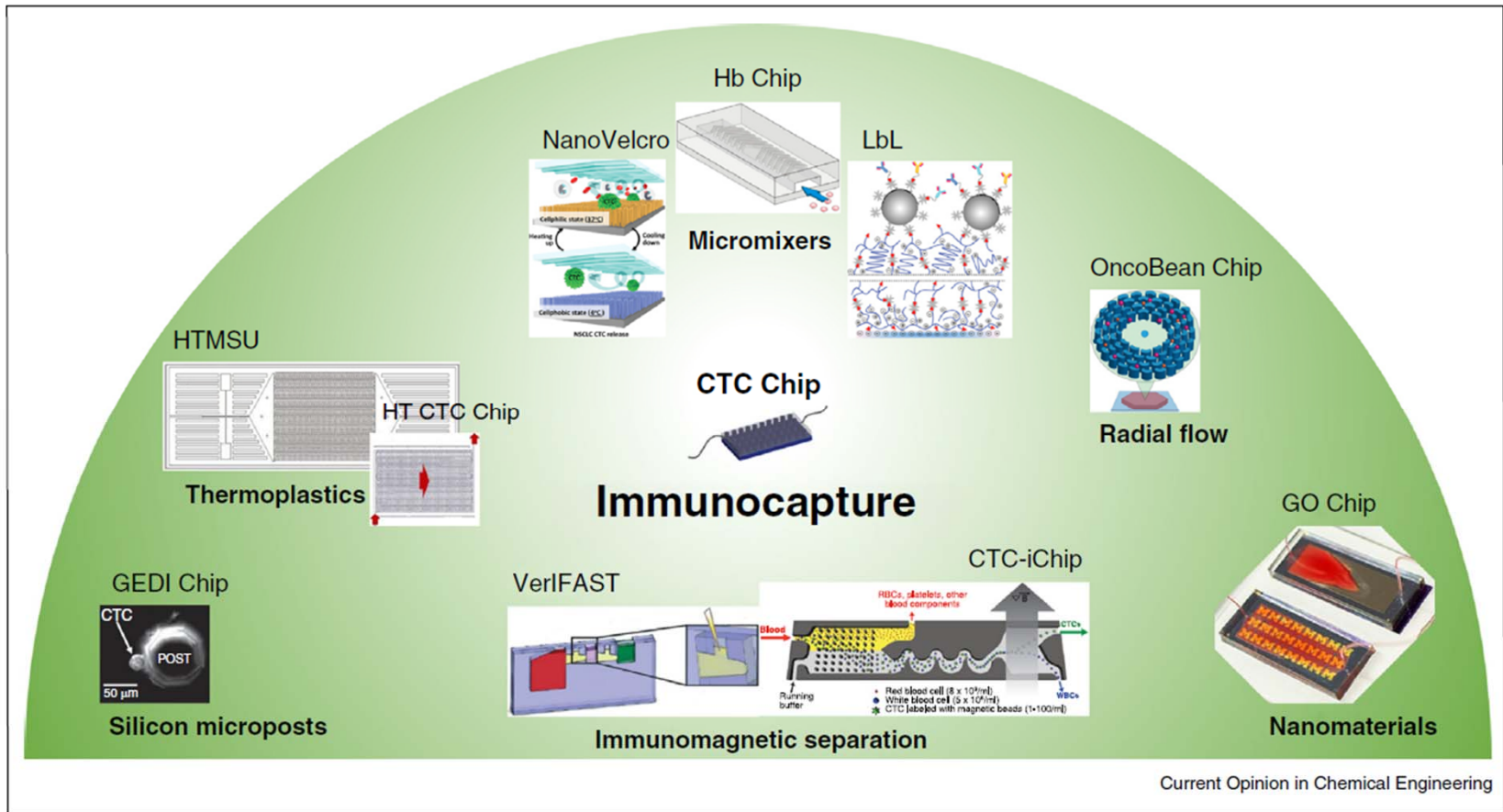


Experimental design

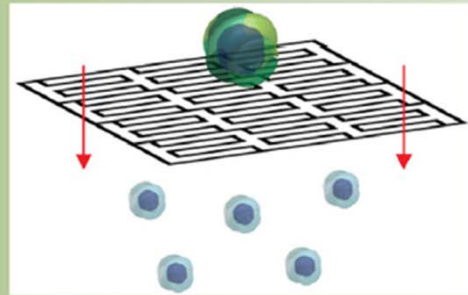


MetaCell

Příklad: mikrofluidní separace



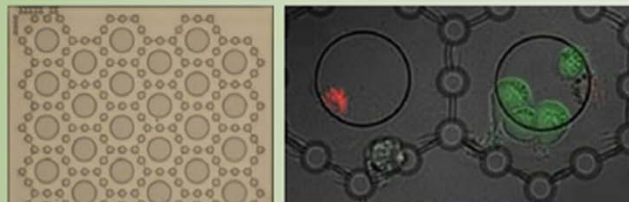
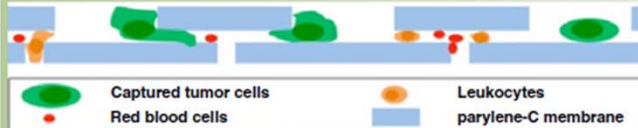
Příklad: mikrofluidní separace



Size Based Separation

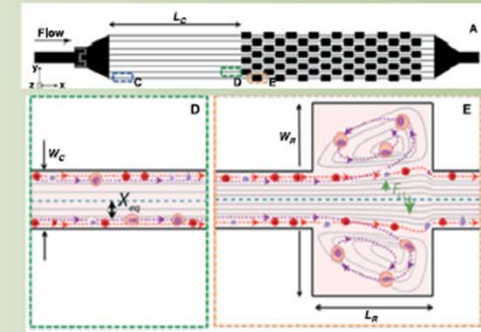
Microfilter

FMSA device

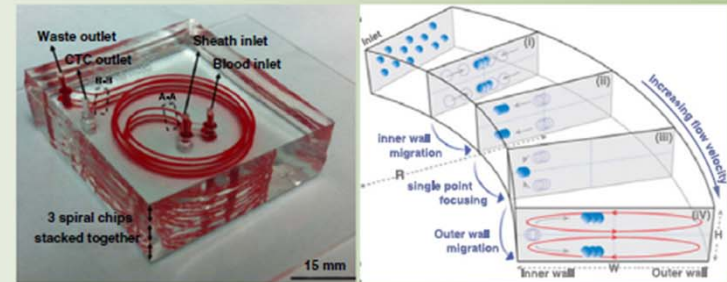


SB microfilter

Inertial Effects

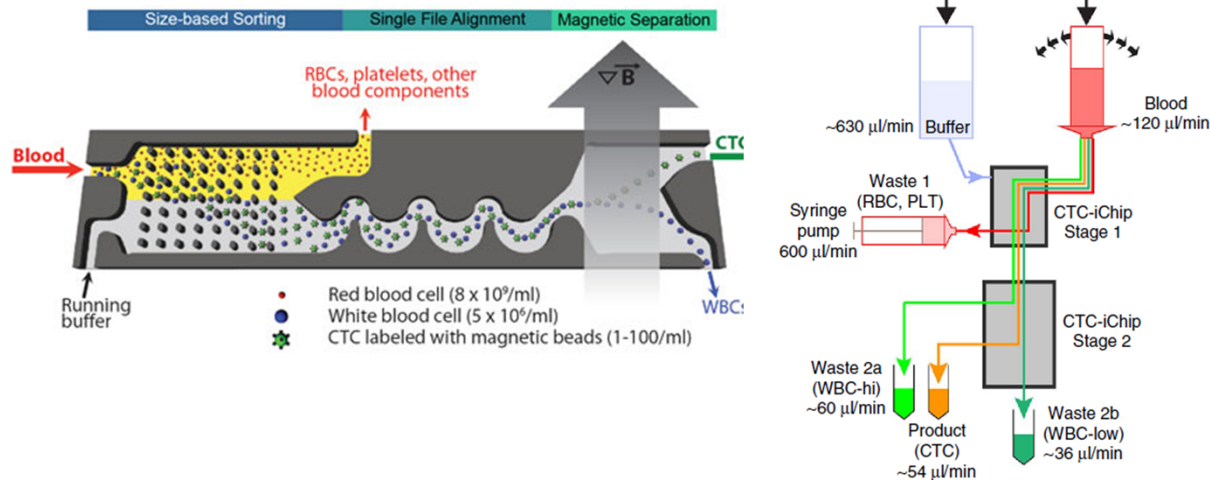


Vortex technology



Spiral devices

Příklad: mikrofluidní separace



CTC-iChip running platform

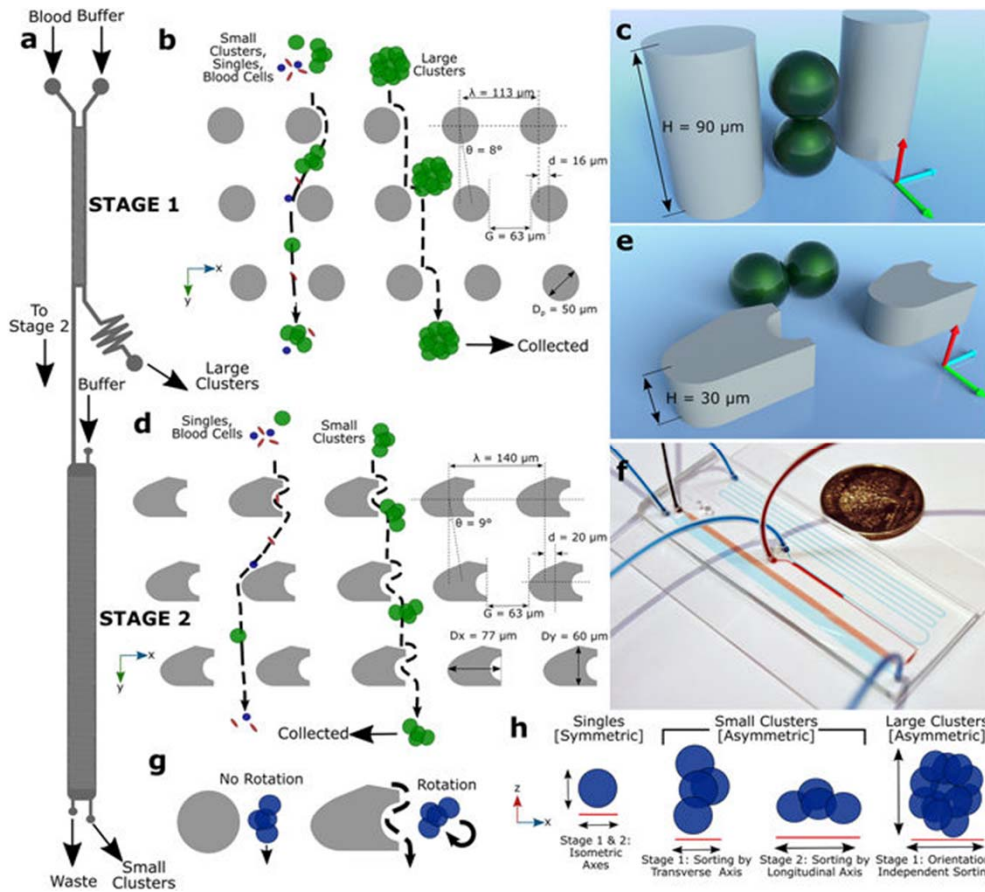
PROTOCOL

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

Nezihi Murat Karabacak^{1,4}, Philipp S Spuhler^{1,4}, Fabio 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

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Příklad: mikrofluidní separace



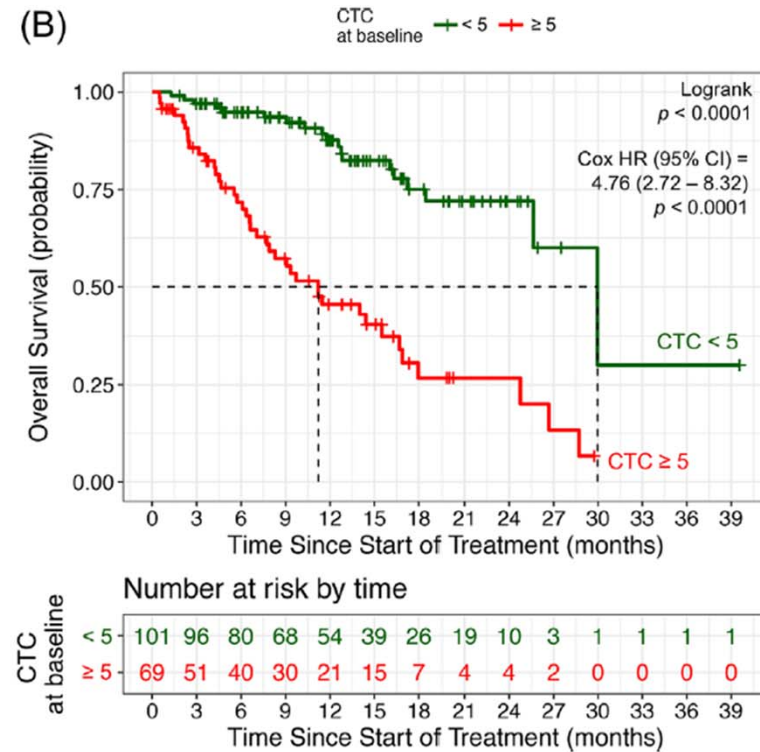
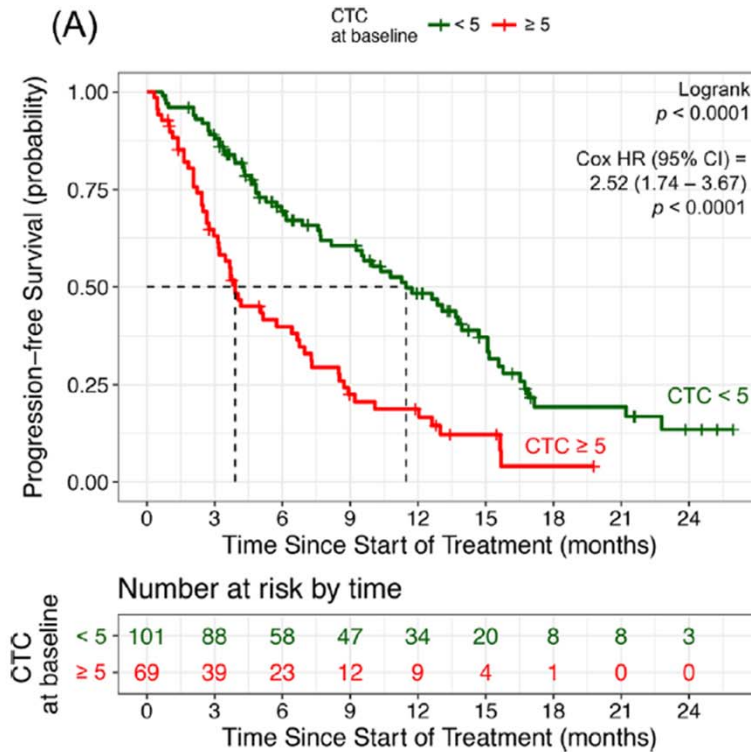
Klinické využití detekce cirkulujících nádorových buněk

- 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



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ORIGINAL ARTICLE

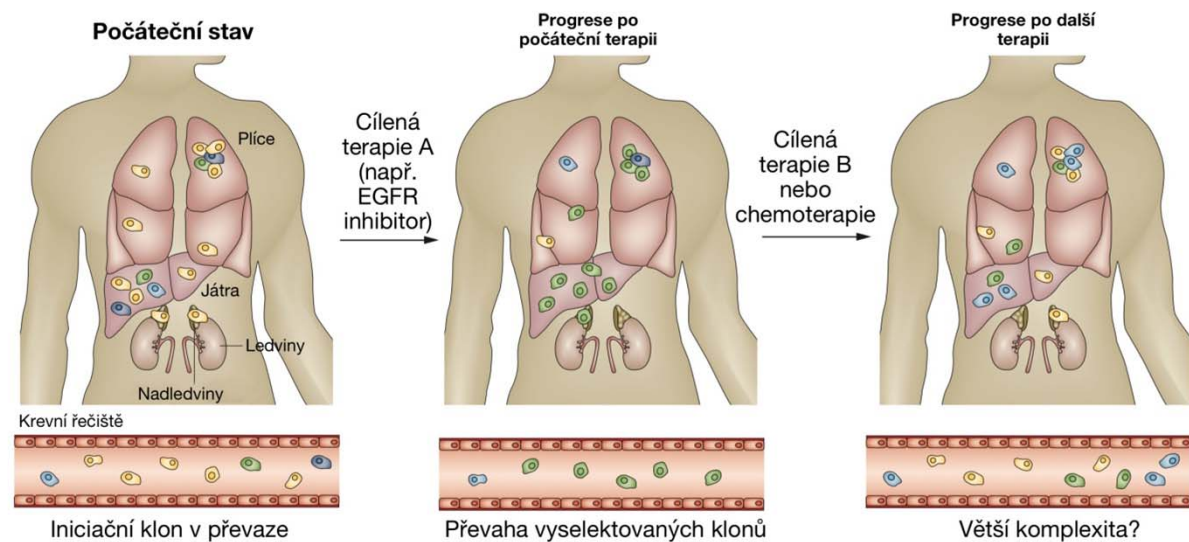
WILEY [The Prostate](#)

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 Ghysel² | 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 Beije¹⁰ | Inge de Kruijff¹⁰ | 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}

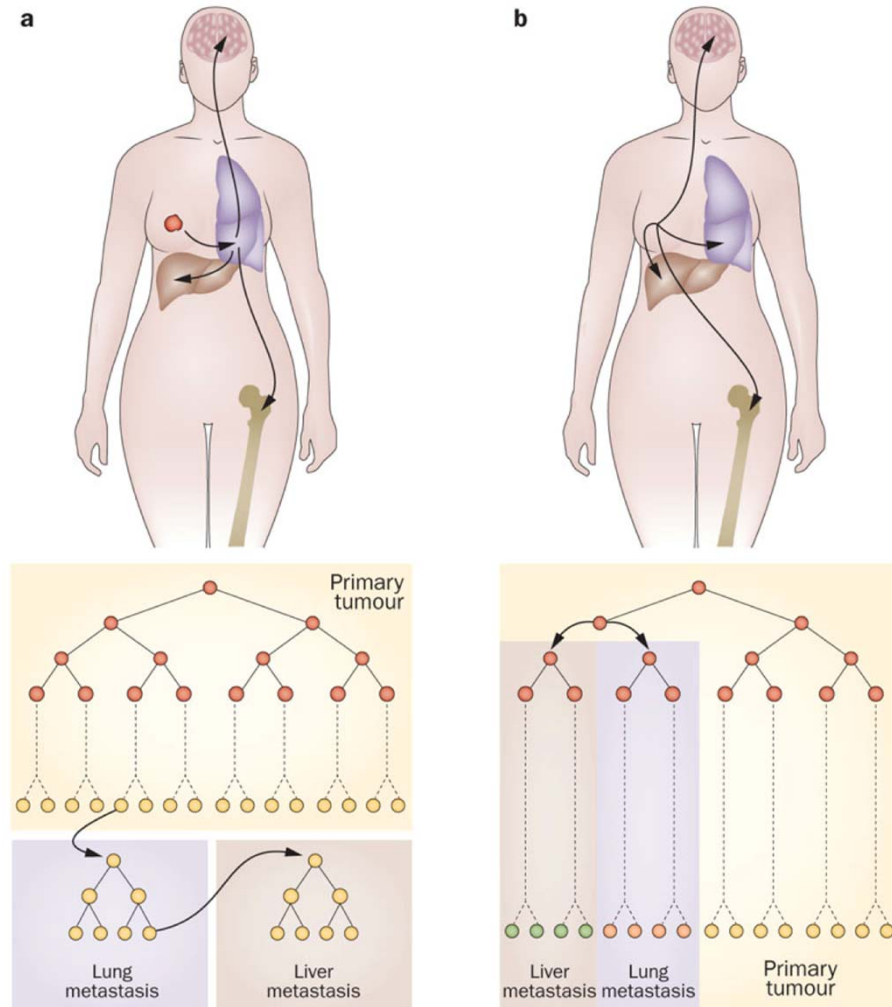
Molekulární charakterizace CNB → cílená terapie

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



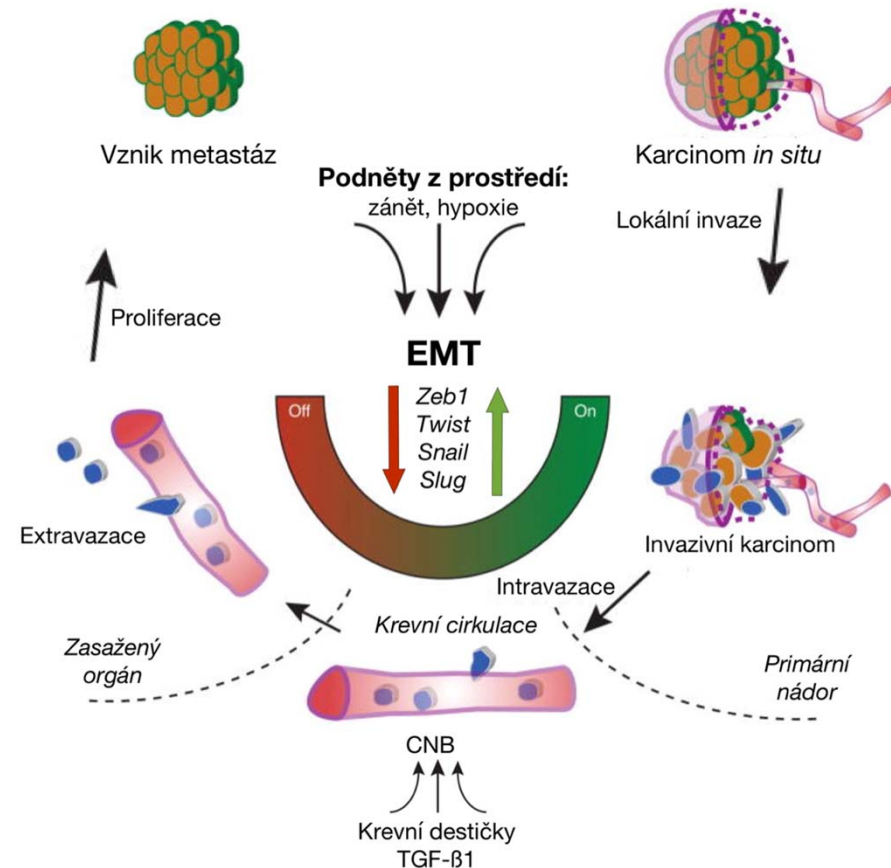
Cirkulující nádorové buňky – prekurzor metastáz

- 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 rozsetých 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 (prsu, prostaty, plic, tlustého střeva, vaječníků, atd.)

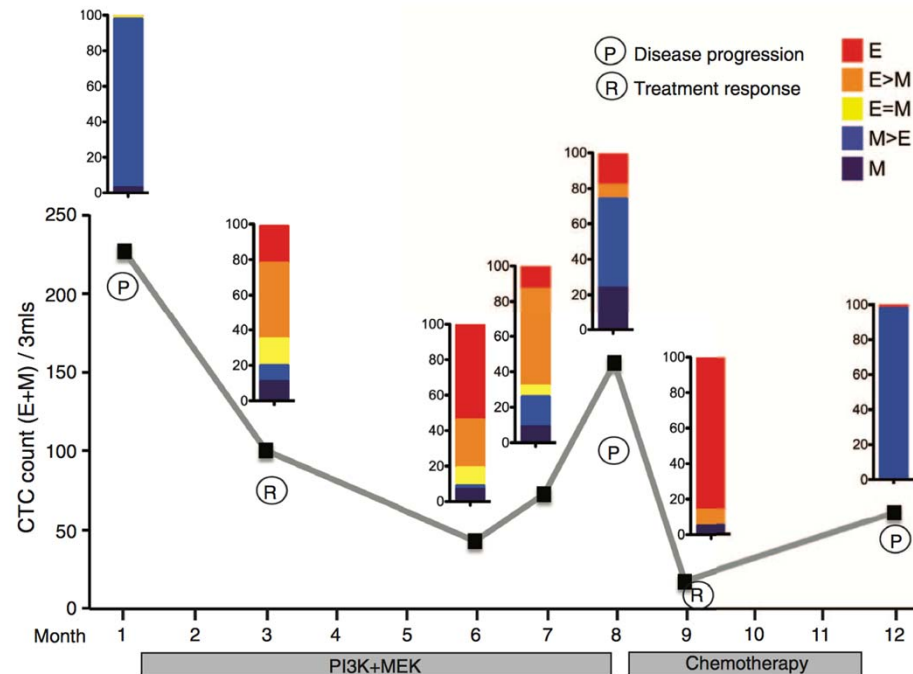


Epiteliálně-mezenchymální přechod

- 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

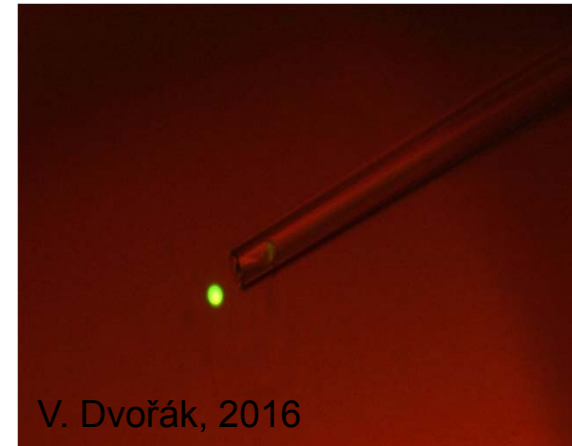
Min Yu,^{1,6*} Aditya Bardia,^{1,3*} Ben S. Wittner,¹ Shannon L. Stott,^{1,2} Malgorzata E. Smas,¹ David T. Ting,¹ Steven J. Isakoff,^{1,3} Jordan C. Ciciliano,¹ Marissa N. Wells,¹ Ajay M. Shah,² Kyle F. Concannon,¹ Maria C. Donaldson,¹ Lecia V. Sequist,^{1,3} Elena Brachtel,^{1,4} Dennis Sgroi,^{1,4}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



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

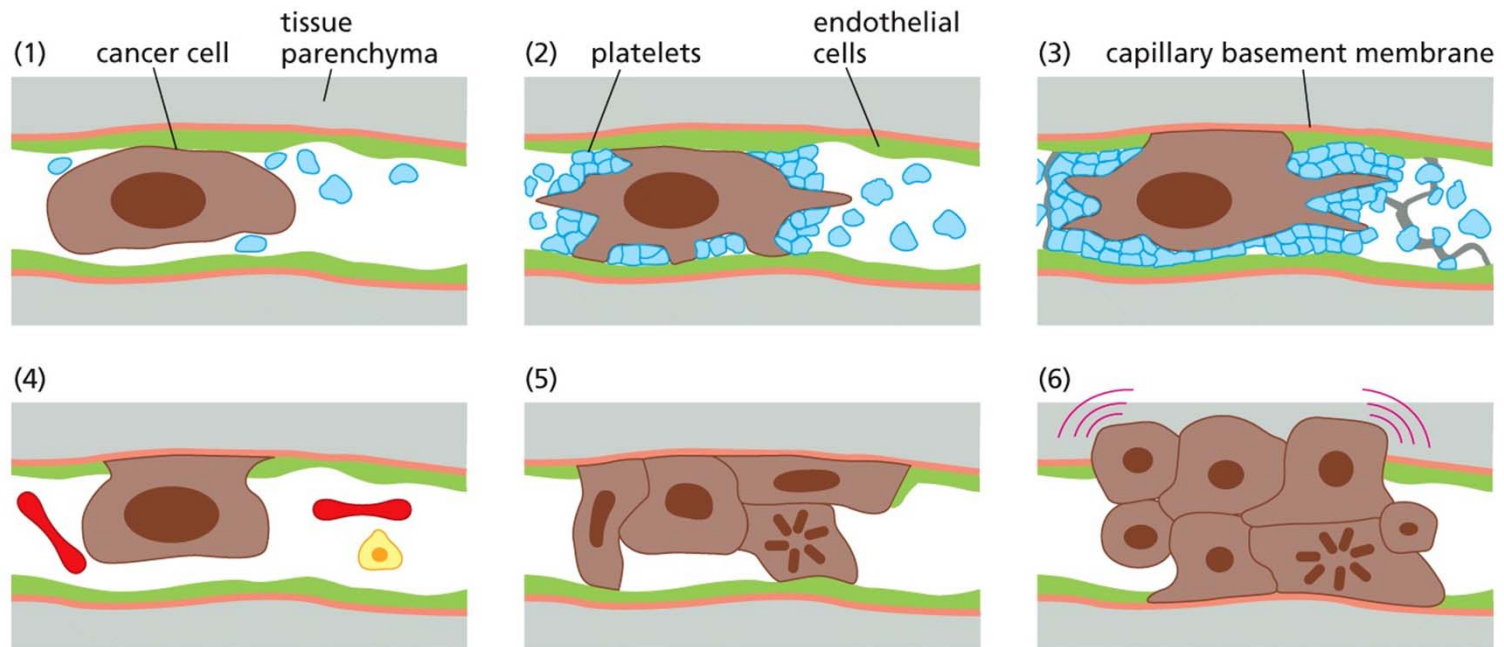


Figure 14.9d The Biology of Cancer (© Garland Science 2014)

Kolonizace

- Velice nízká účinnost
- Dormantní mikrometastázy
- Metastatický relaps

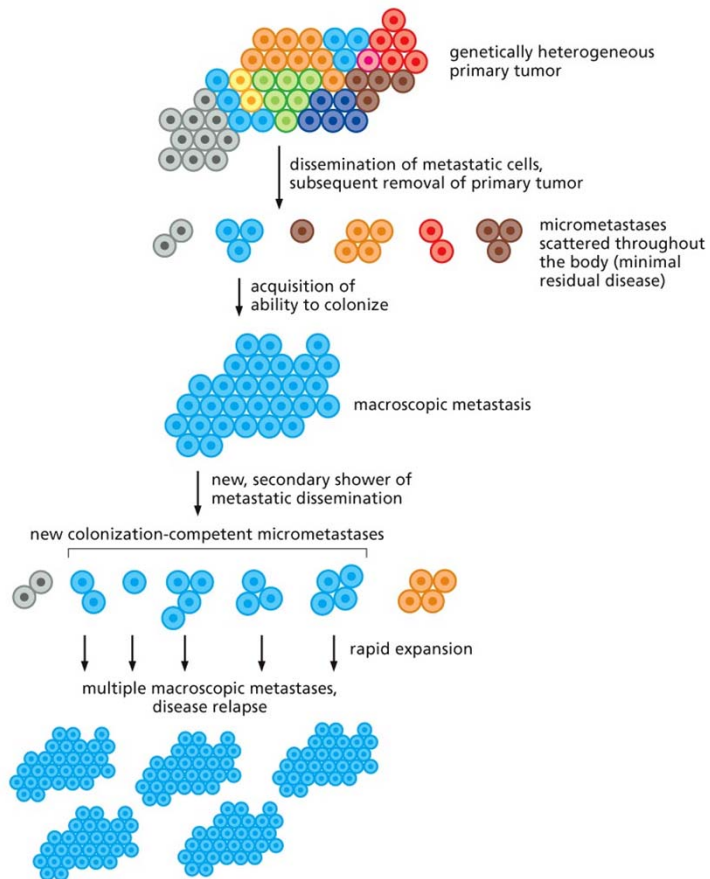


Figure 14.11c The Biology of Cancer (© Garland Science 2014)

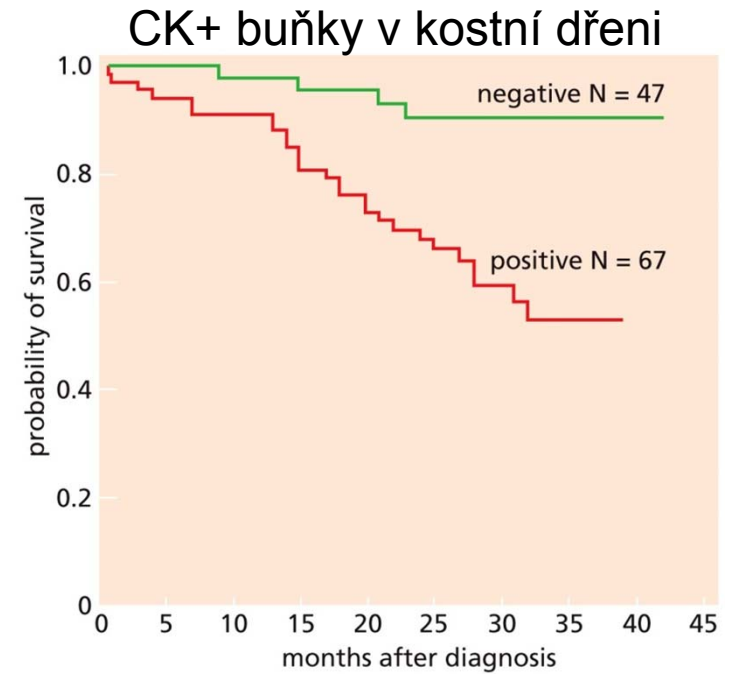
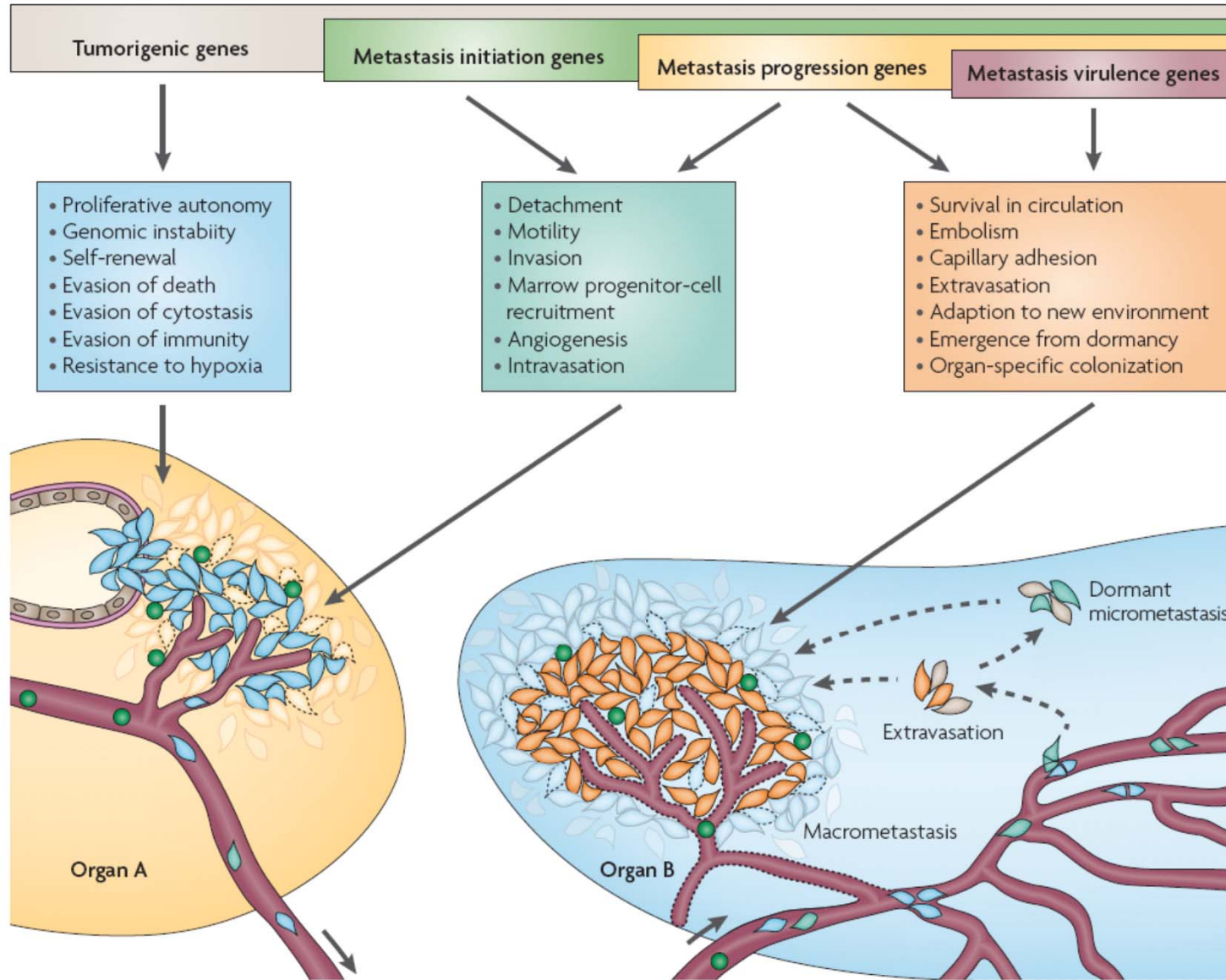


Figure 14.10d The Biology of Cancer (© Garland Science 2014)

Genetic determinants of cancer metastasis

Don X. Nguyen and Joan Massagué



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.

Mechanismy karcinogeneze

EMT & nádory

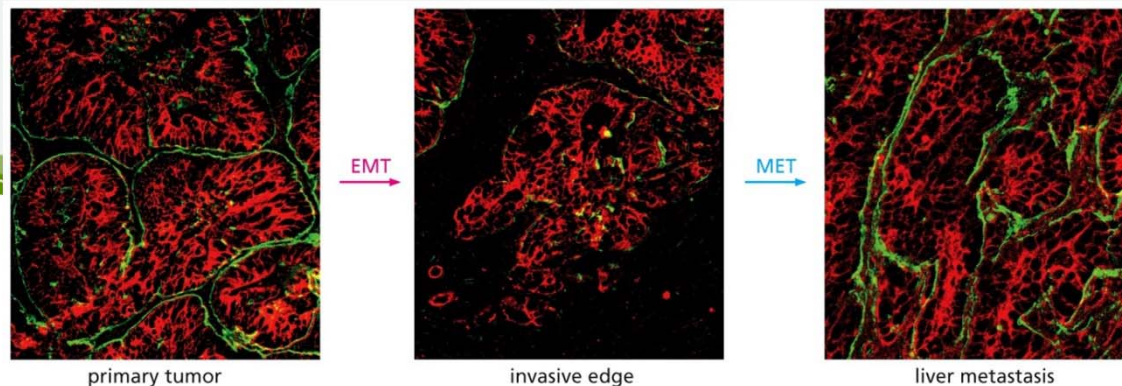


Figure 14.18a The Biology of Cancer (© Garland Science 2014)

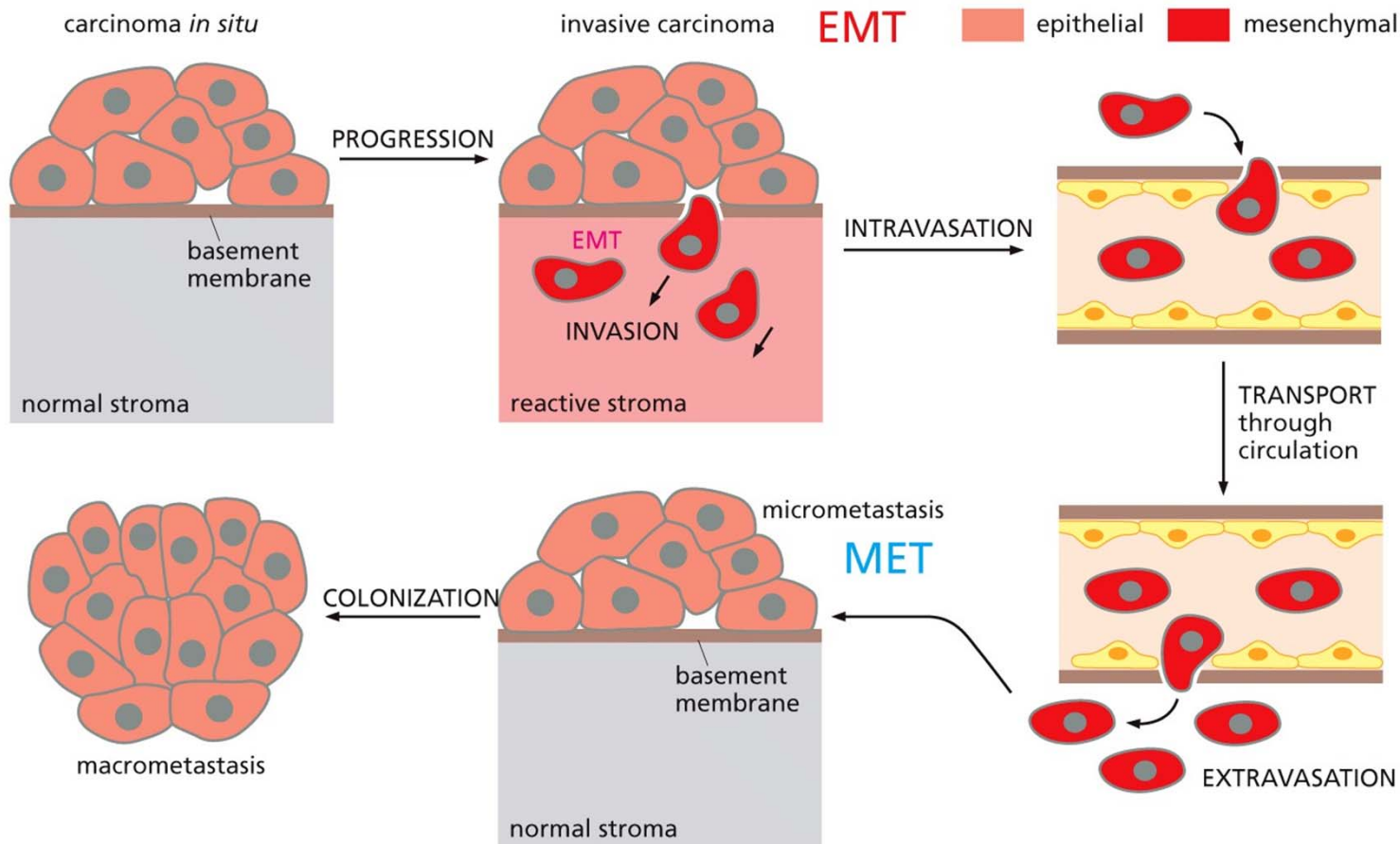
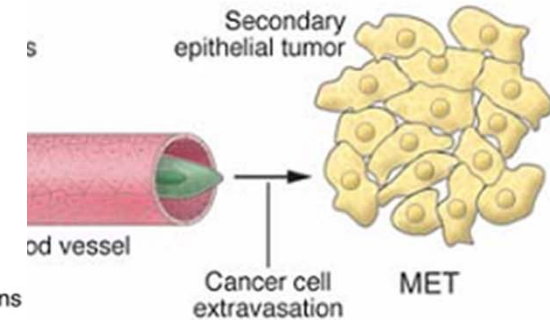
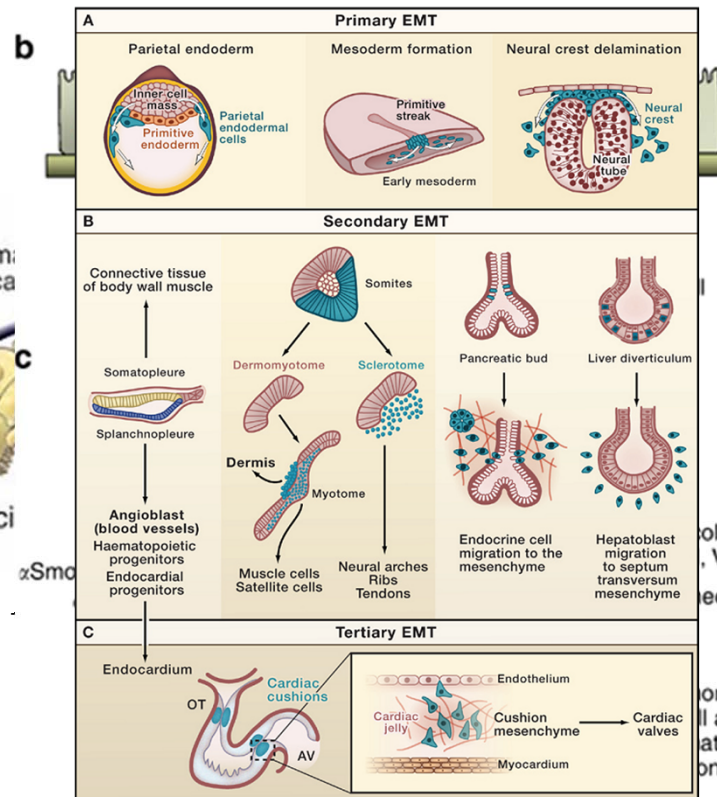
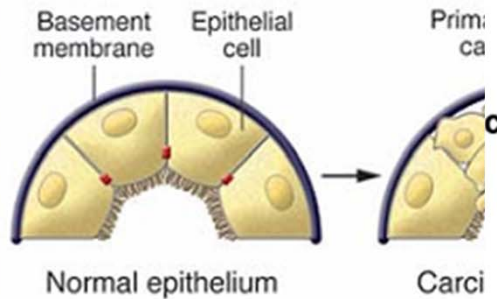


Figure 14.18b The Biology of Cancer (© Garland Science 2014)

Epithelial-to-mesenchymal transition (EMT)

- Reversible acquisition of migratory and invasive properties by epithelial cells
- Role in embryonic development, fibrosis, cancer



Kalluri, R. and R.A. Weinberg, 2004

transitions in development and disease. *Cell*, 2008, 139(5): p. 871-90.

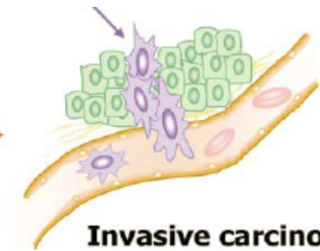
M S Simonson
Kidney International **71**,
846-854 (May (1) 2007)

Znaky a regulatory EMT

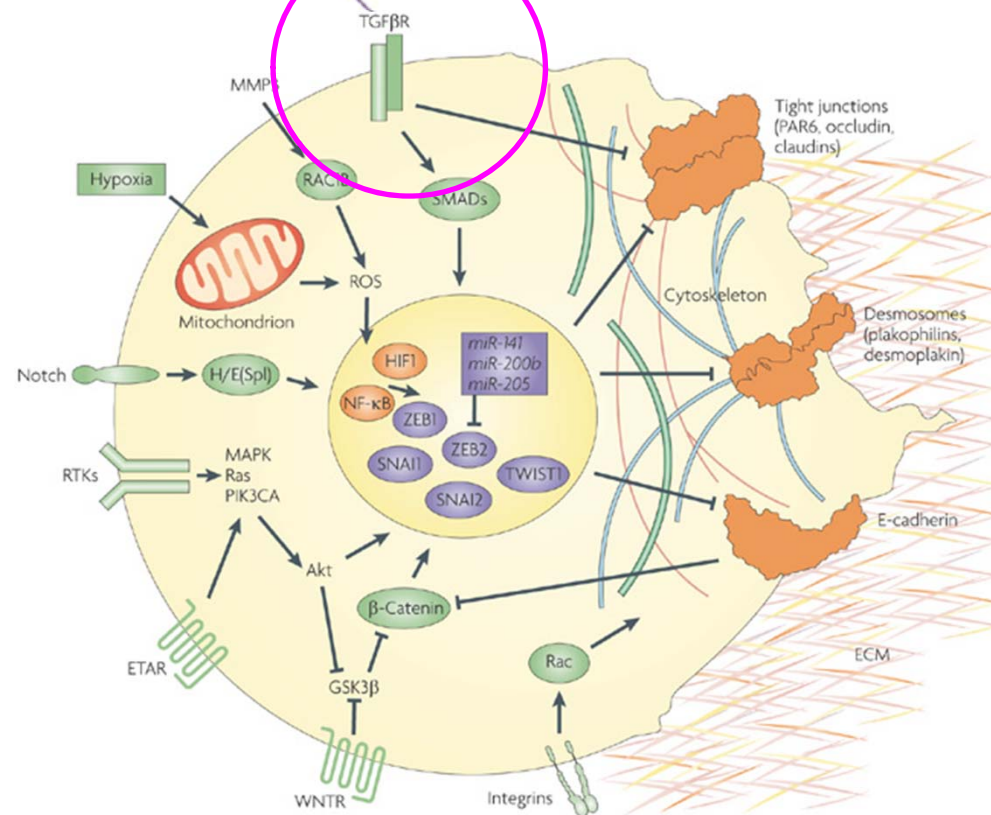
EMT Program	<i>E-cadherin</i>	Epithelial markers repressed
	<i>α-catenin</i> <i>γ-catenin</i>	
	<i>Vimentin</i> <i>Fibronectin</i> <i>N-cadherin</i>	Mesenchymal markers induced



Carcinoma in situ

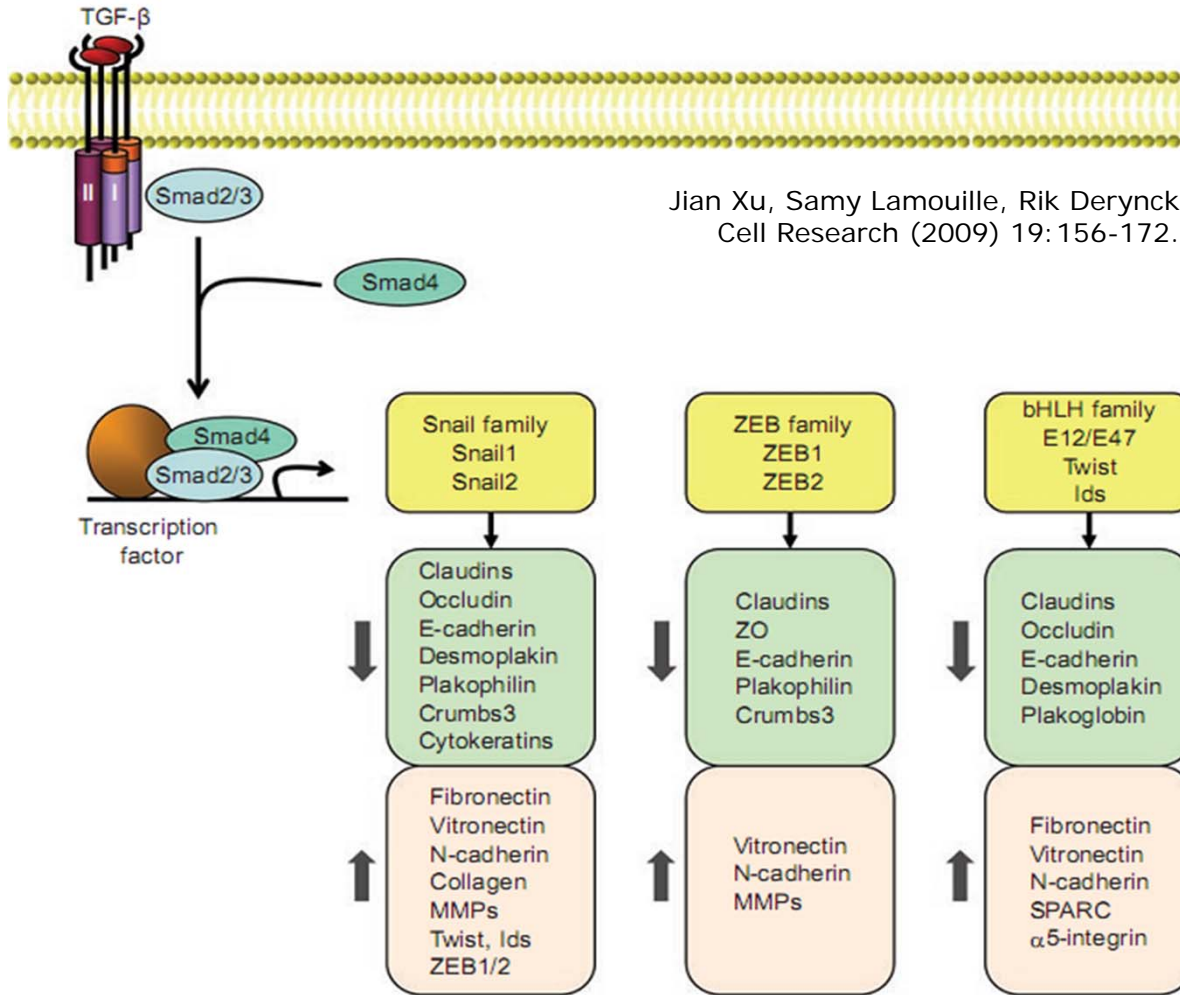


Invasive carcinoma



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

Transforming growth factor-β (TGF-β)



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

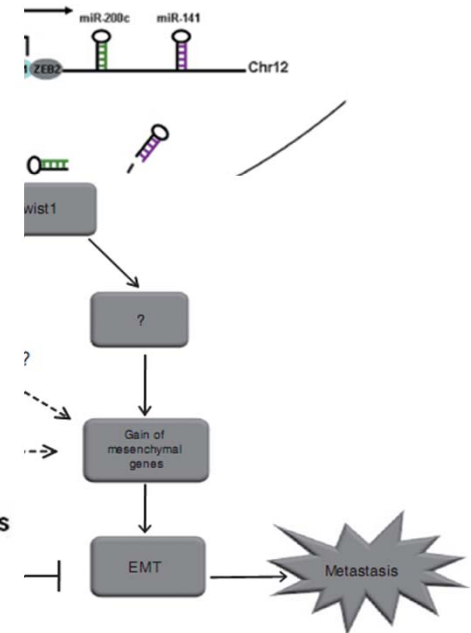
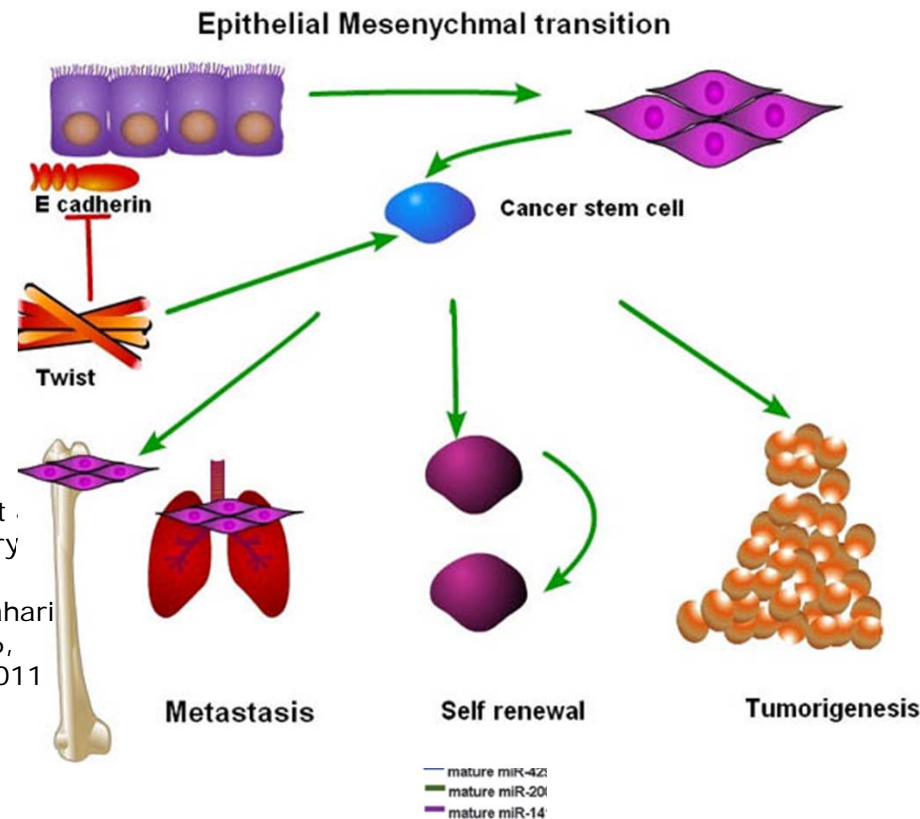
Klíčové objevy v EMT a rakovině

- 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 al. 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)



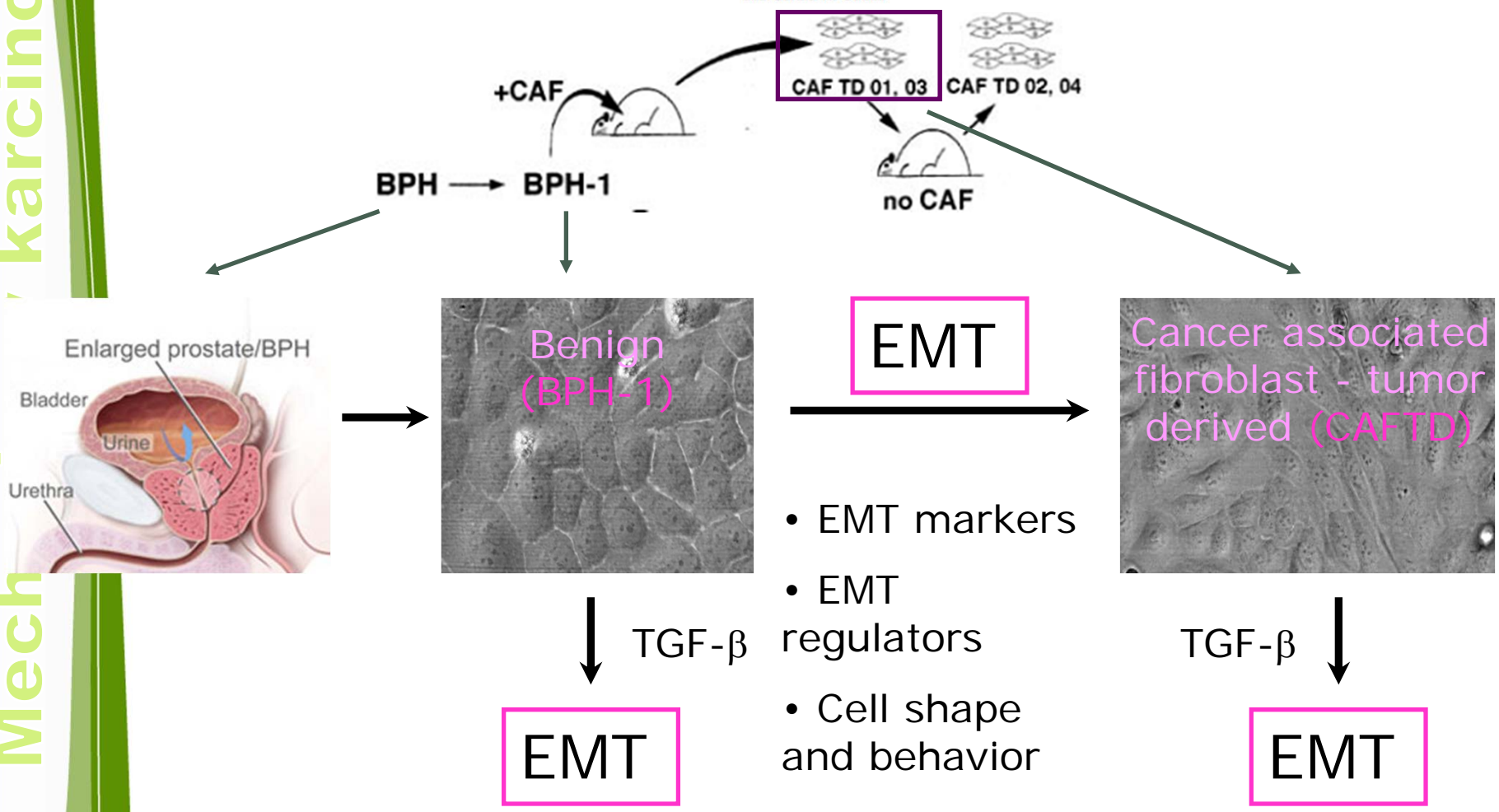
Experimentální přístupy

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

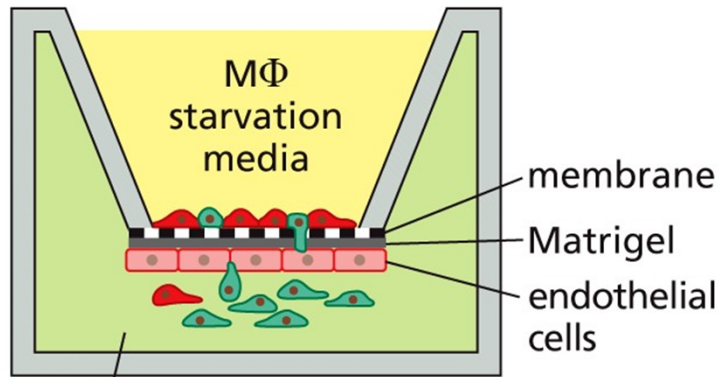
S. W. HAYWARD, R. DAHIYA, G. R. CUNHA, J. BARTEK, N. DESHPANDE, AND P. NARAYAN

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



MΦ complete medium
 carcinoma cell
 macrophage (MΦ)

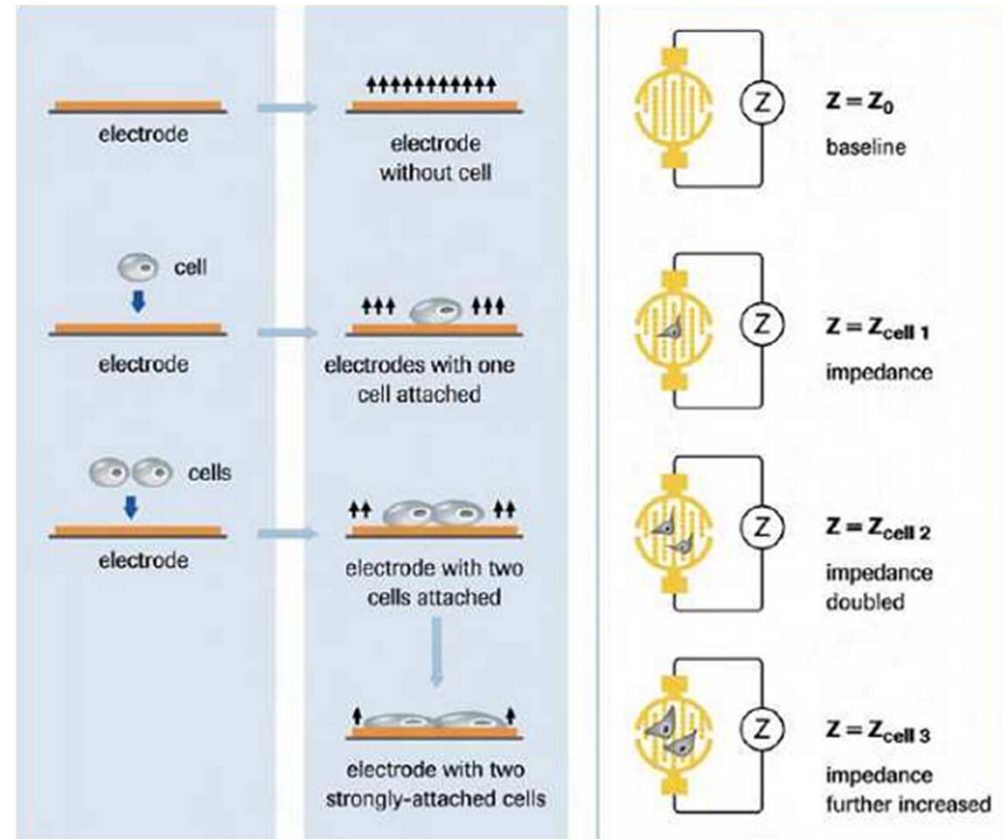
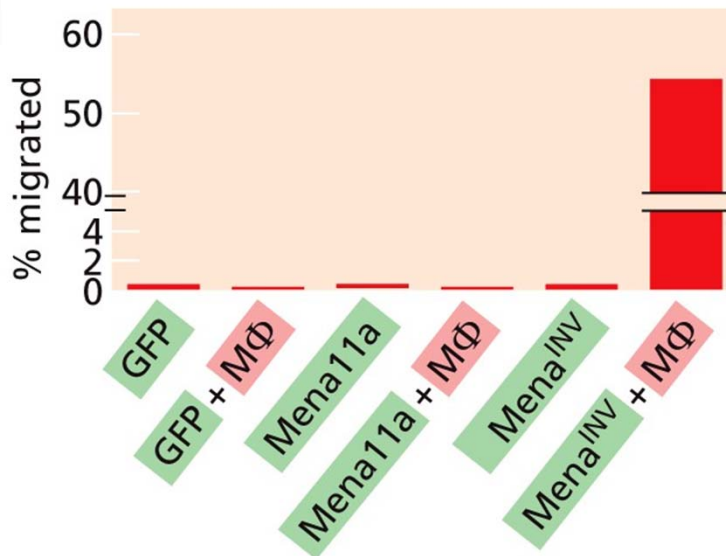
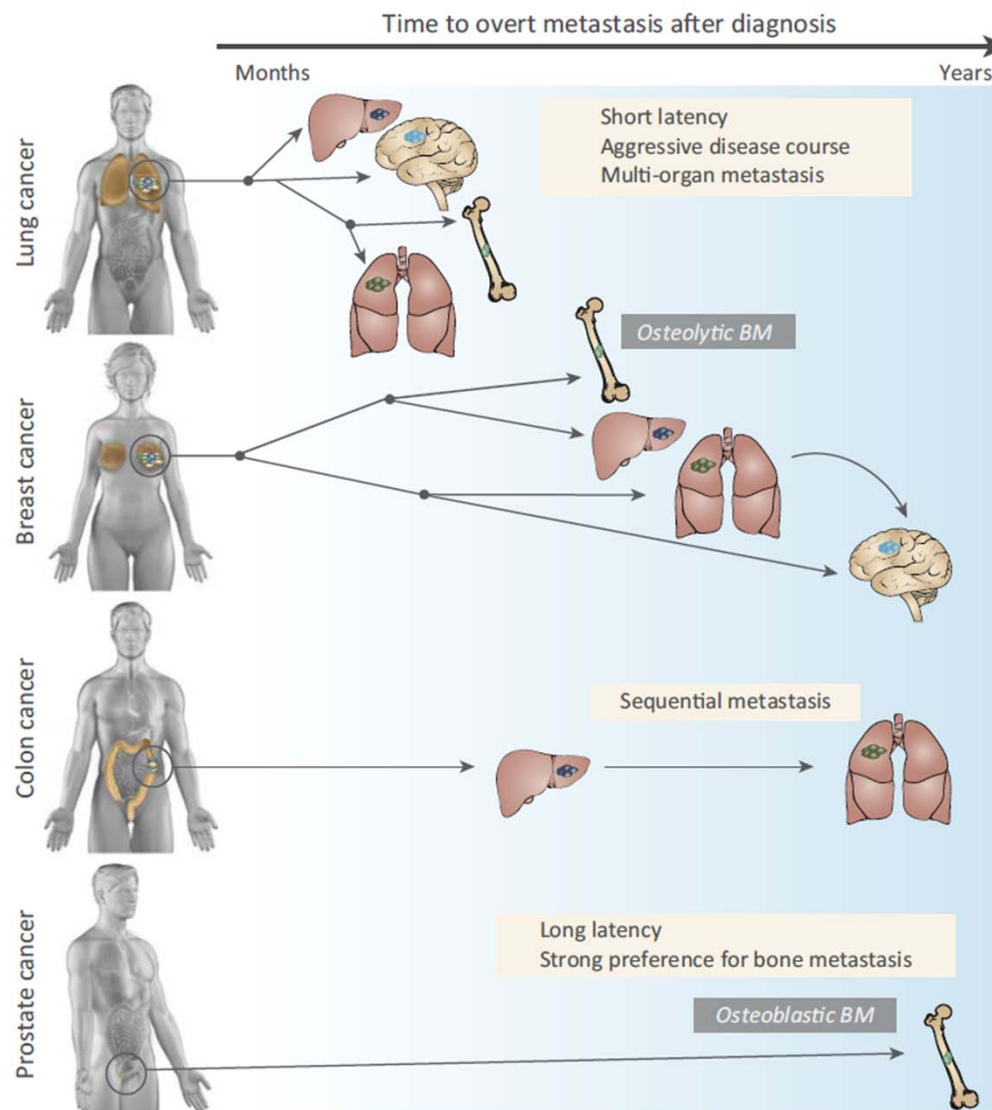


Figure 14.41c The Biology of Cancer (© Garland Science 2014)

Diseminace solidních nádorů



Mechanismy karcinogeneze

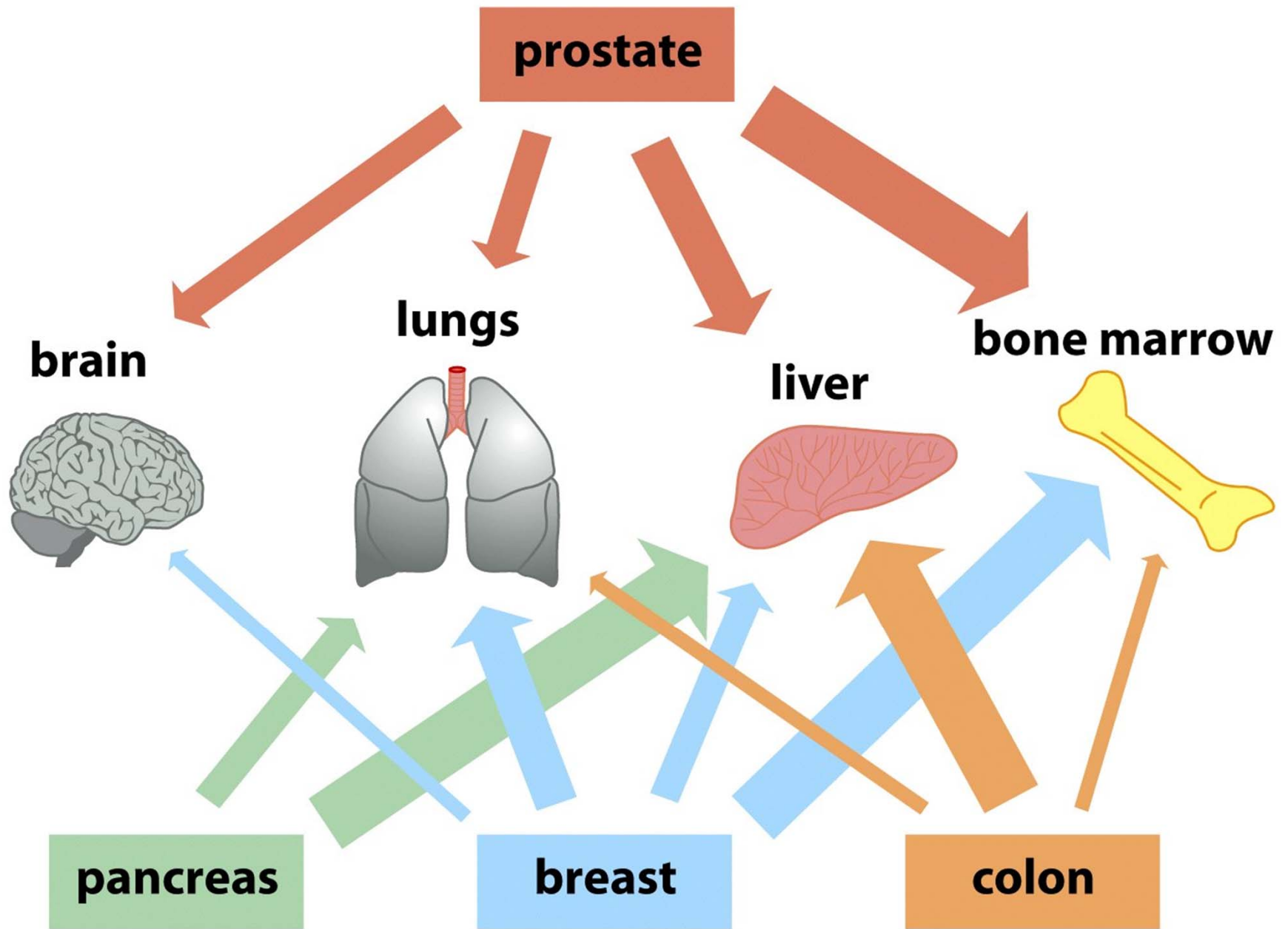


Figure 14.42 *The Biology of Cancer* (© Garland Science 2007)

Metastatický tropismus

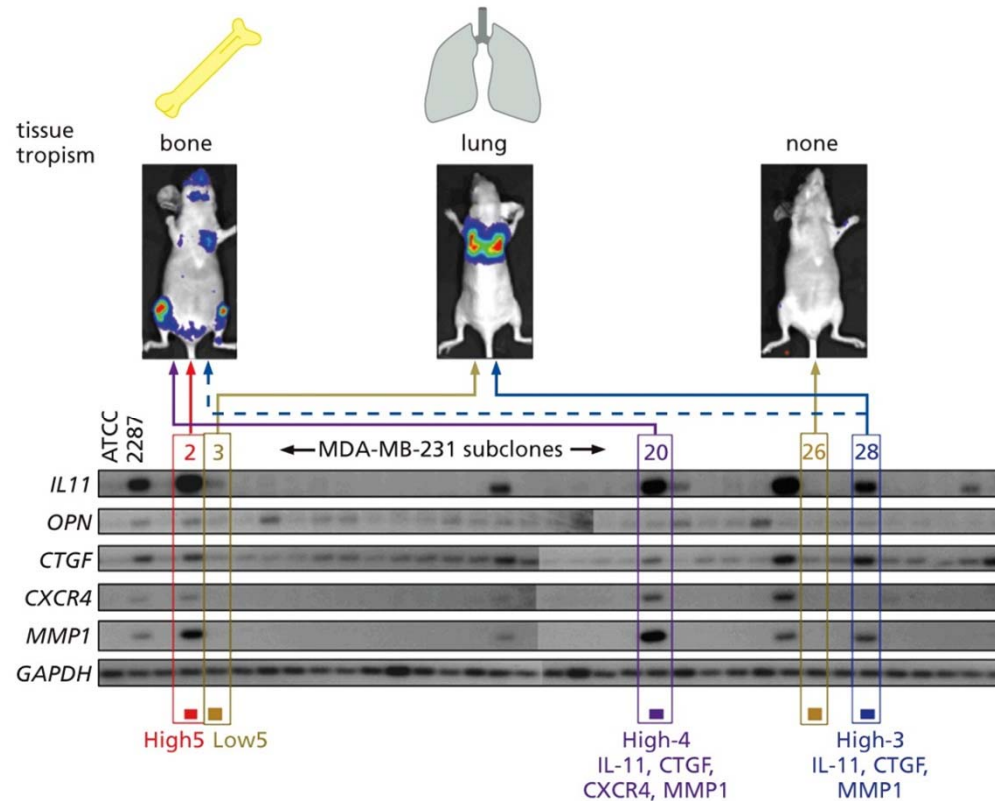
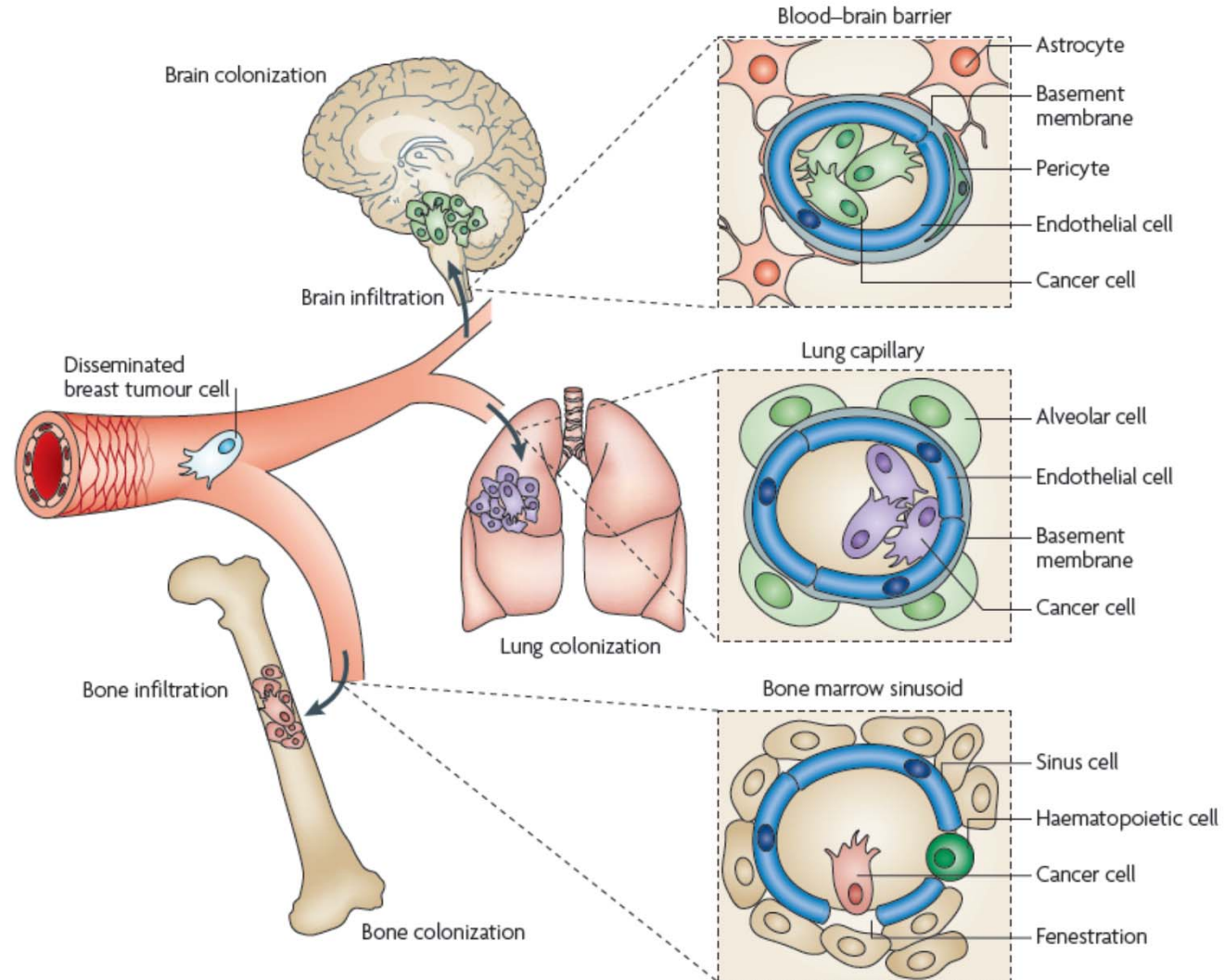


Figure 14.53 The Biology of Cancer (© Garland Science 2014)

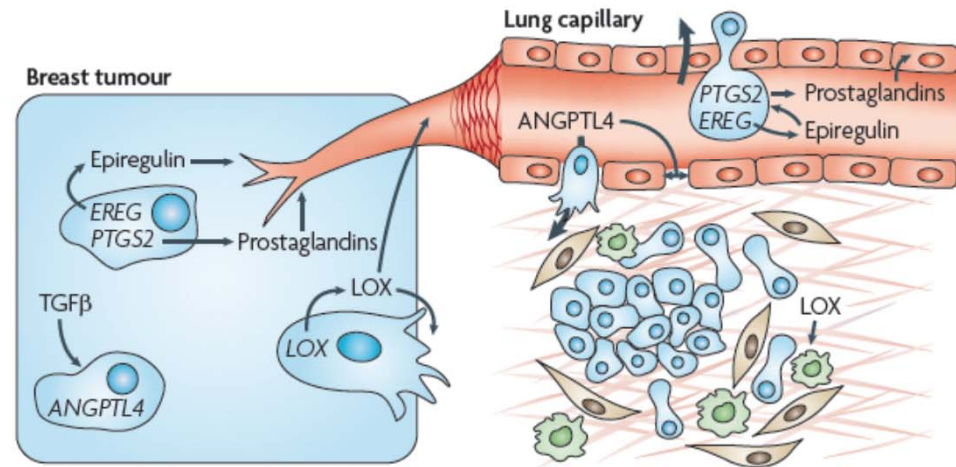
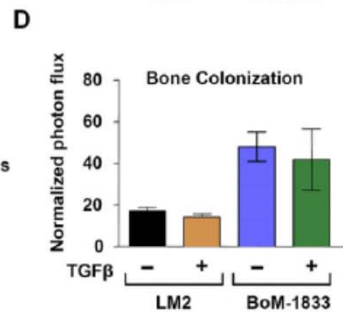
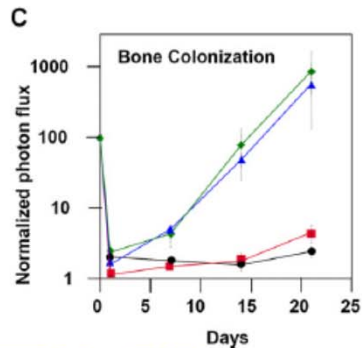
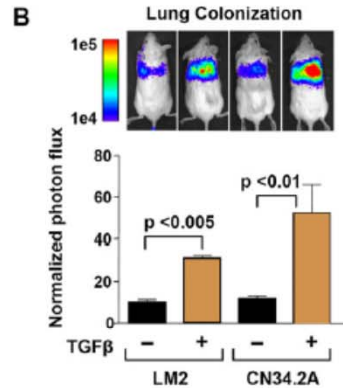
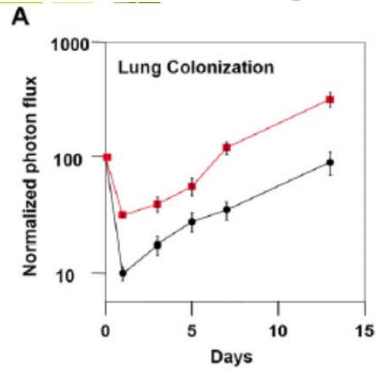
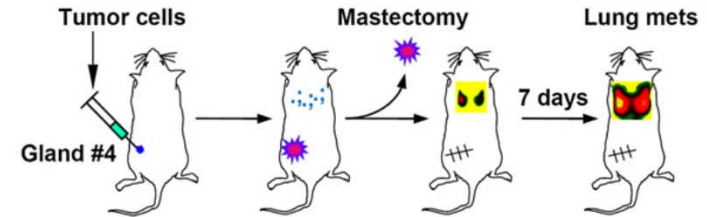
- Thirty-three cells from a large population of human MDA-MB-231 cells were each expanded into a clonal population in culture. The mRNA expression pattern of each subclone was analyzed (columns, arrayed left to right) using probes for the mRNAs of five genes—*IL11* (interleukin-11), *OPN* (osteopontin),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



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

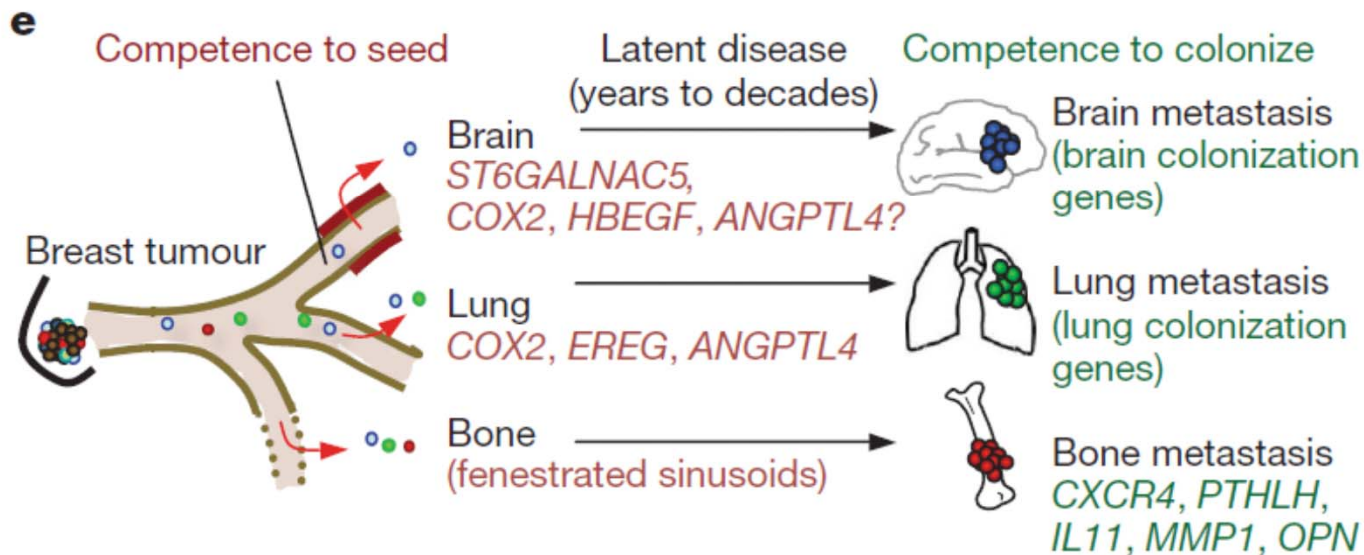
David Padua,¹ Xiang H.-F. Zhang,¹ Qiongqing Wang,¹ Cristina Nadal,⁵ William L. Gerald,² Roger R. Gomis,⁴ and Joan Massagué^{1,3,*}



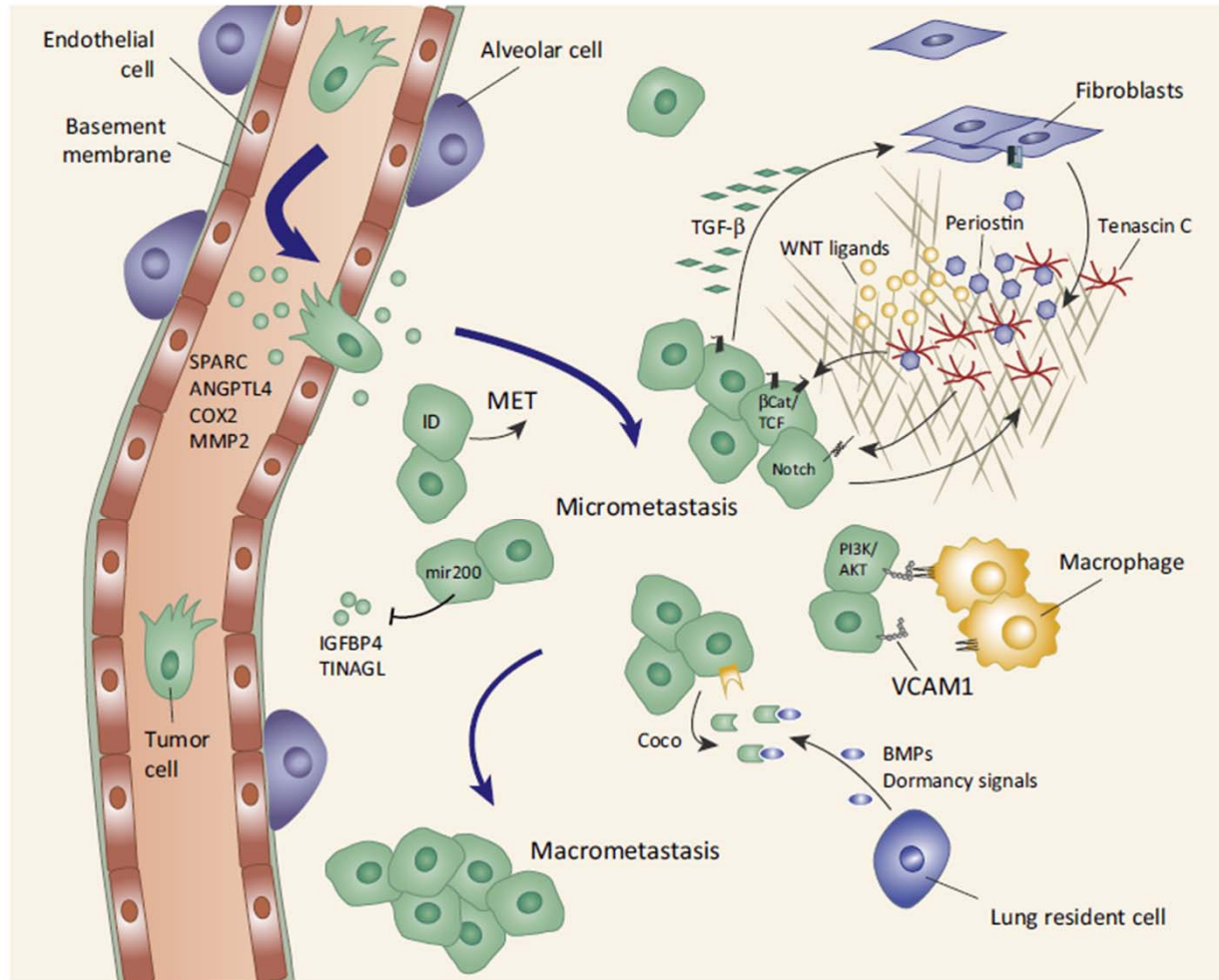
LETTERS

Genes that mediate breast cancer metastasis to the brain

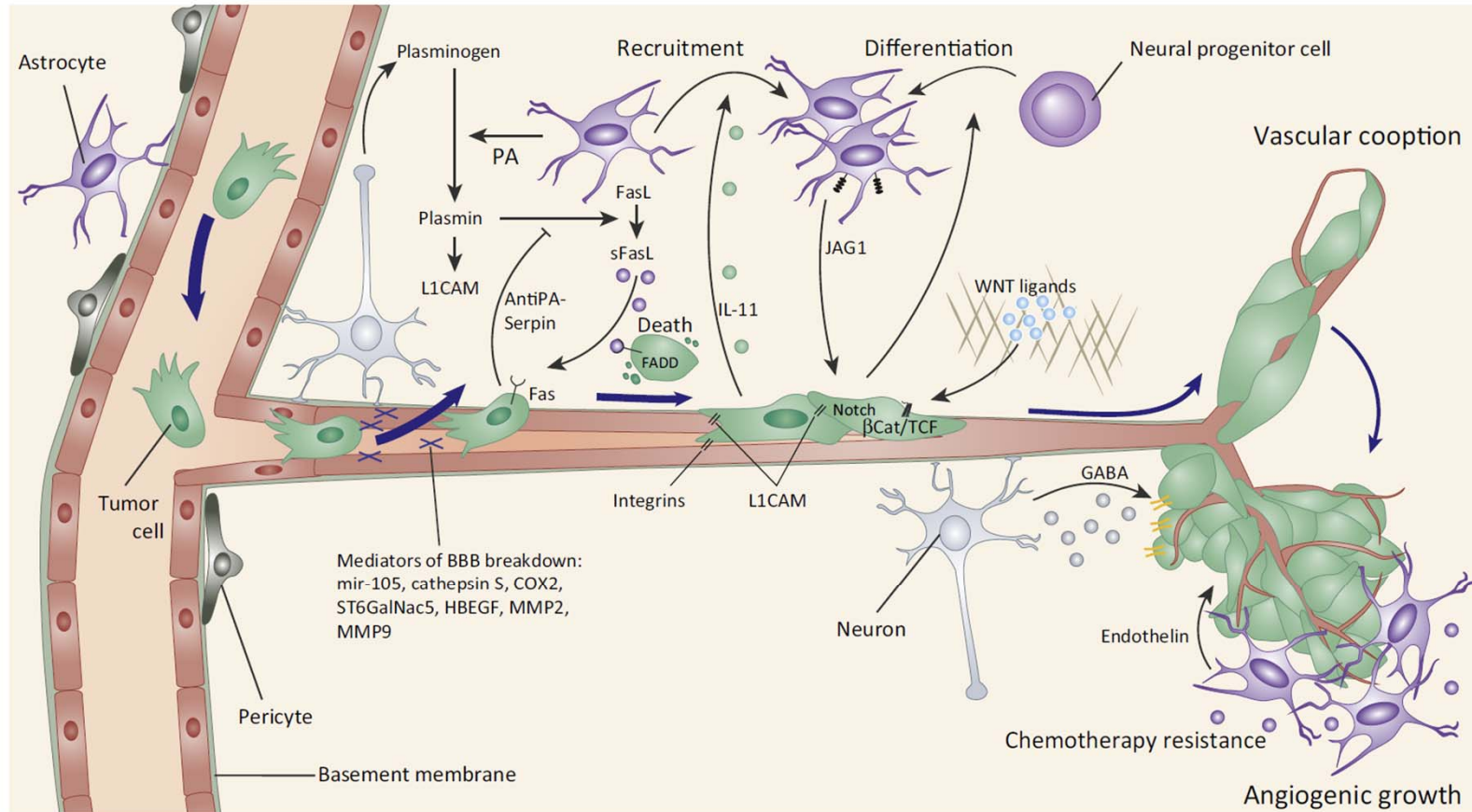
Paula D. Bos¹, Xiang H.-F. Zhang¹, Cristina Nadal^{1†}, Weiping Shu¹, Roger R. Gomis^{1†}, Don X. Nguyen¹, Andy J. Minn², Marc J. van de Vijver³, William L. Gerald⁴, John A. Foekens⁵ & Joan Massagué^{1,6}



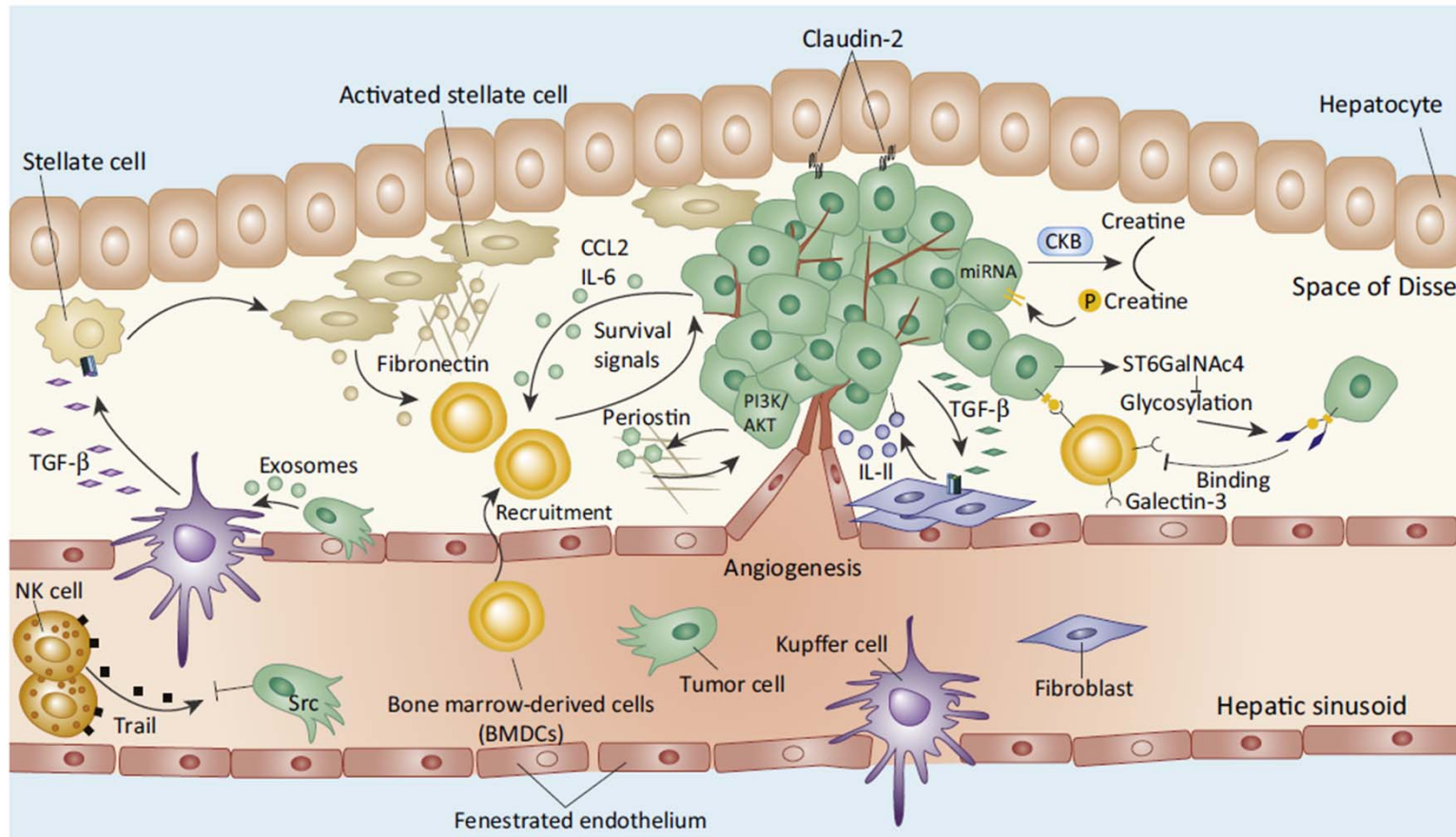
Metastatická kolonizace plic

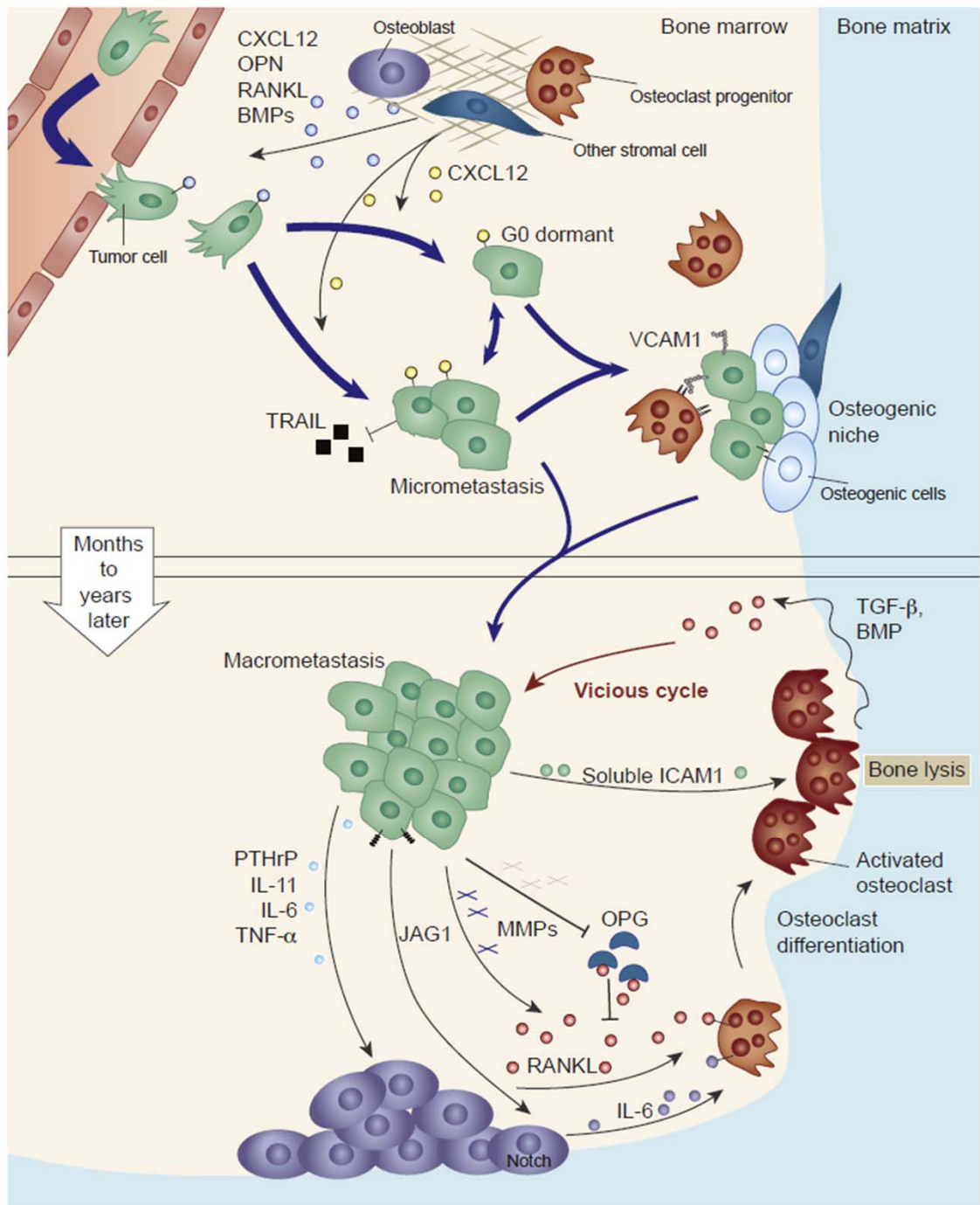


Metastatická kolonizace mozku

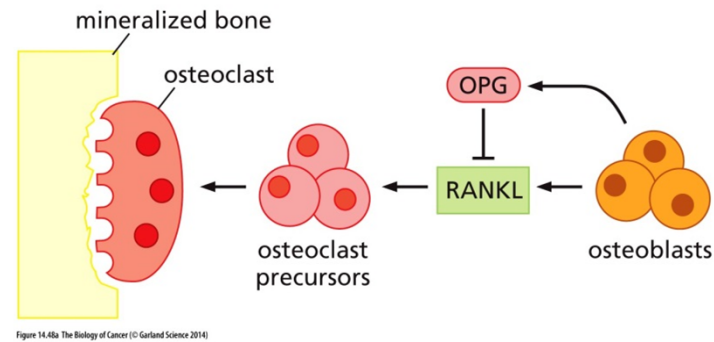
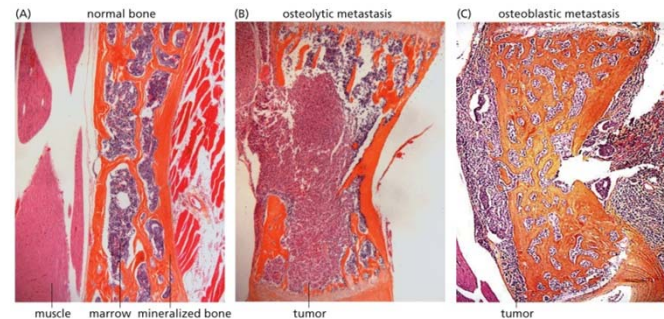


Metastatická kolonizace jater





Osteotropní metastáze



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

MDA-MB-231 cells transfected with:

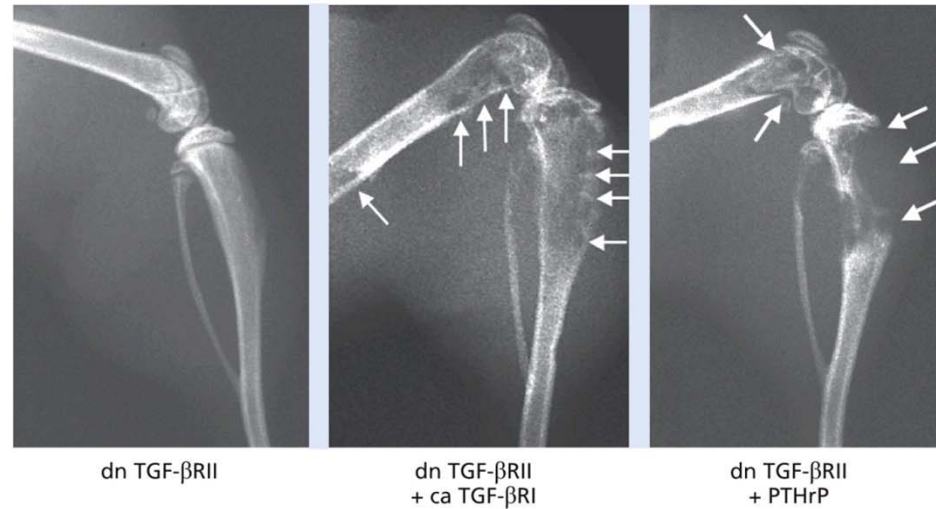


Figure 14.49 The Biology of Cancer (© Garland Science 2014)

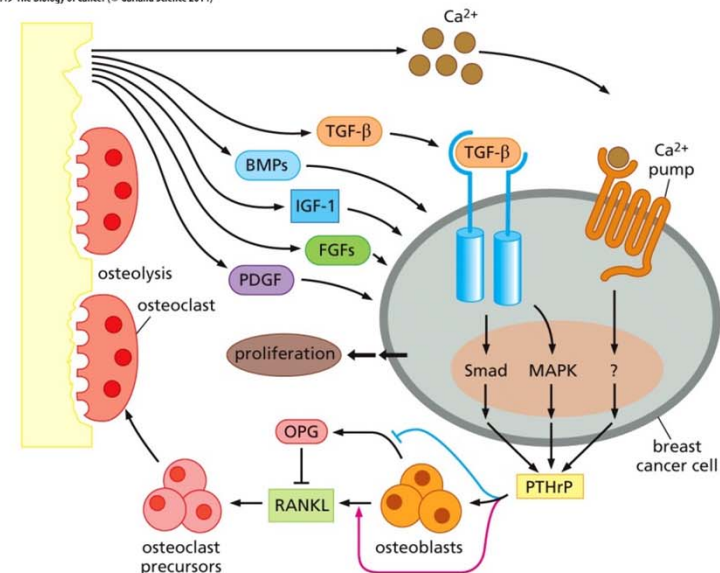
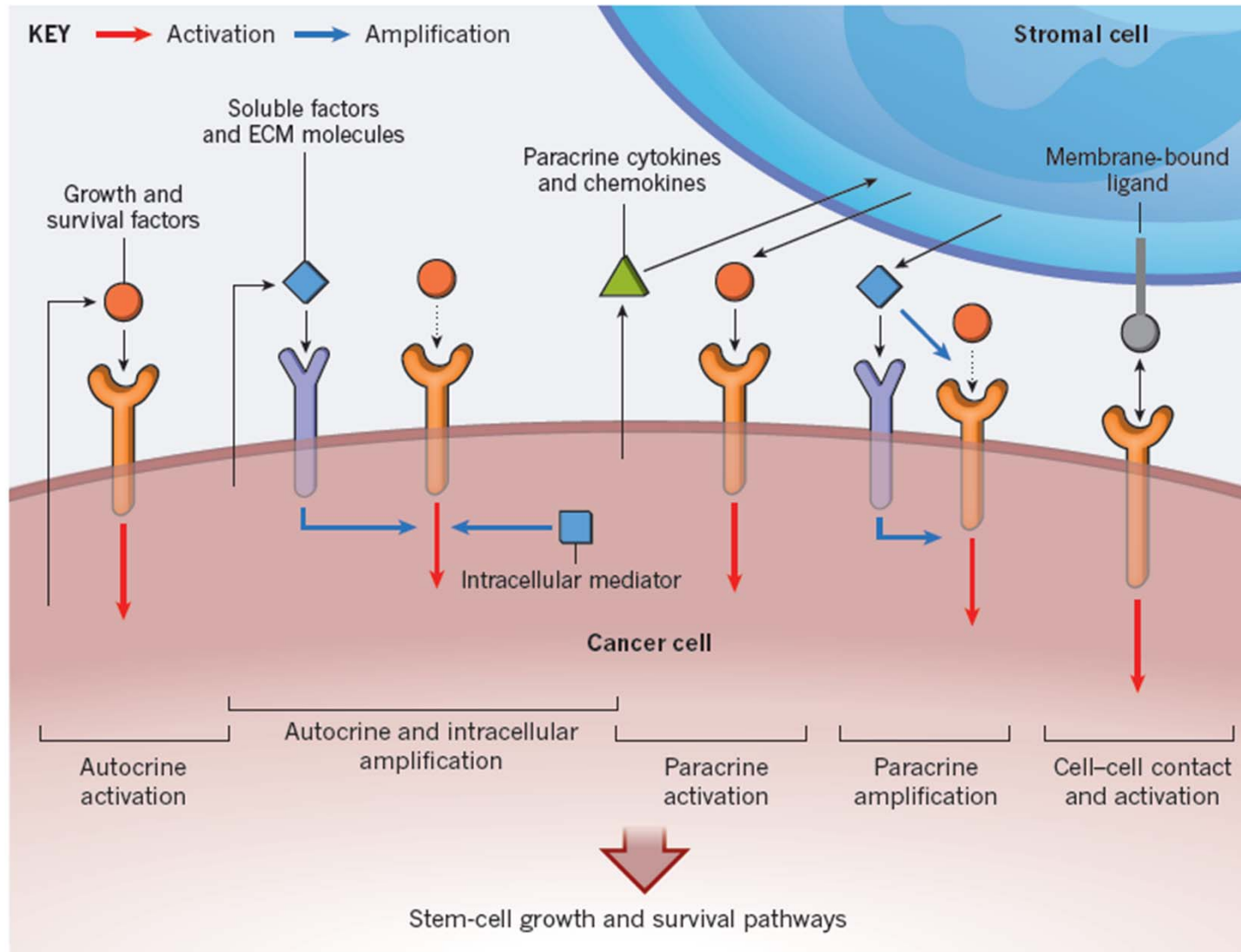
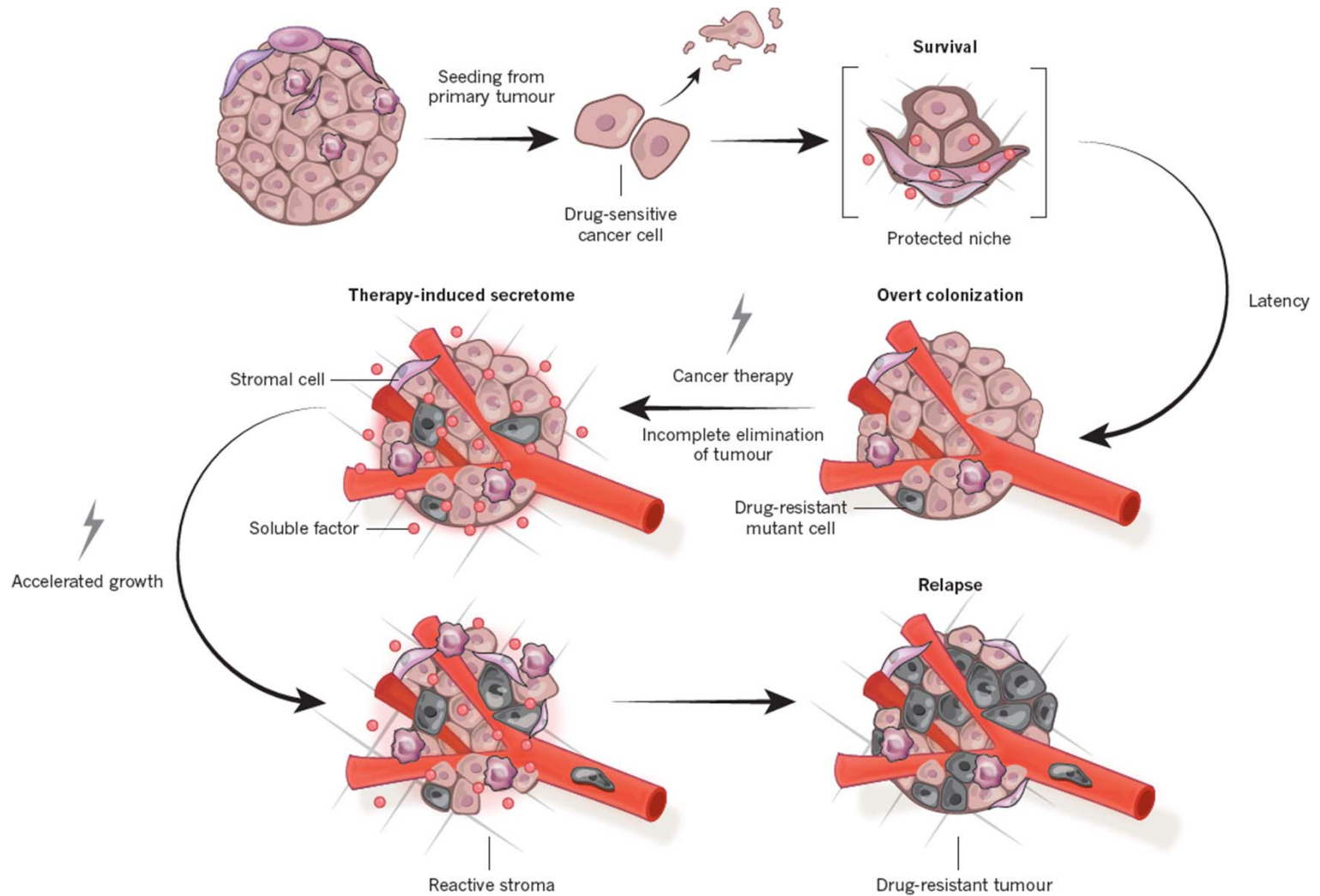


Figure 14.48b The Biology of Cancer (© Garland Science 2014)

Aktivace růstových signálů

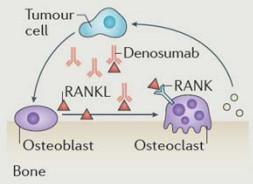
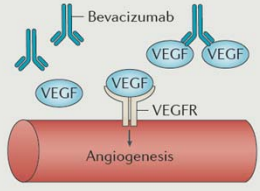
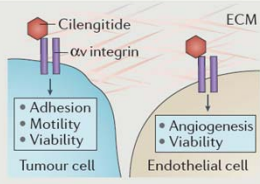
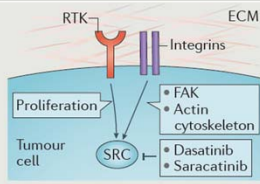


Metastázi před a po terapii



Terapie cílena 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	 <p>Bone</p>	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	 <p>Angiogenesis</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
Cilengitide $\alpha\beta 3$ and $\alpha\beta 5$ integrin peptide inhibitor	 <p>Tumour cell</p> <p>Endothelial cell</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 growth⁶¹, 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 	All advanced trials were negative
Dasatinib and saracatinib SRC kinase and BCR-ABL kinase inhibitor	 <p>Tumour cell</p> <p>ECM</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, RANKL 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?