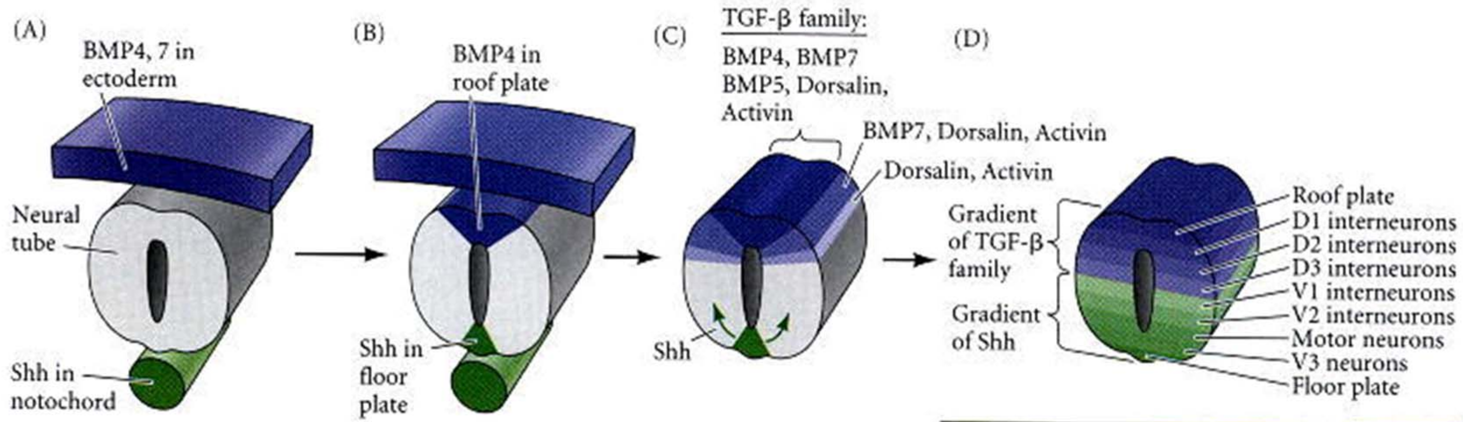
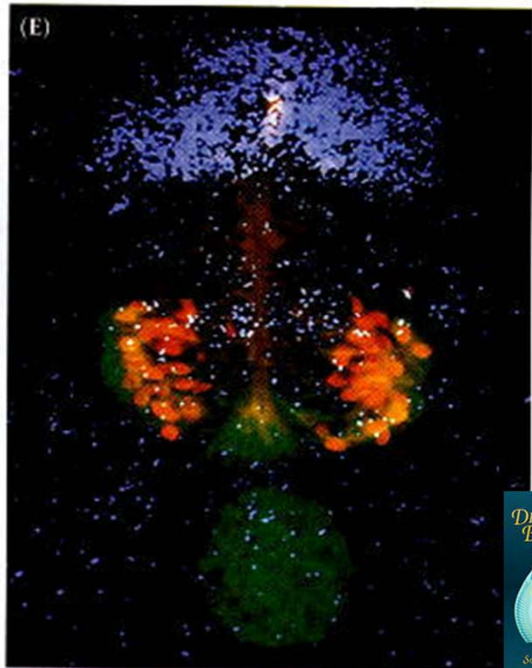
Transformující růstový faktor – β: rozmanitost přenosu signálu a funkce

Karel Souček

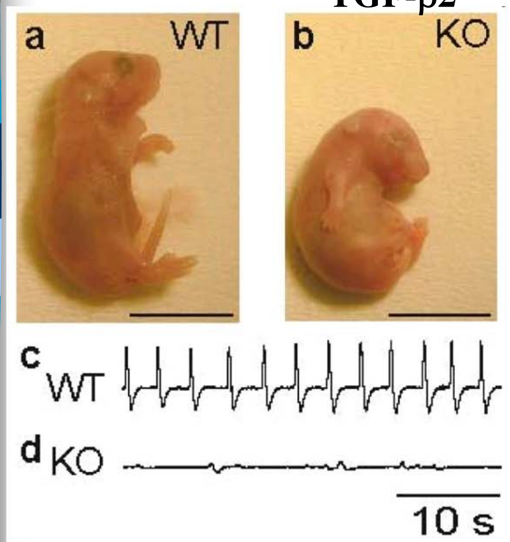
Bi6051 Molekulární fyziologie živočichů

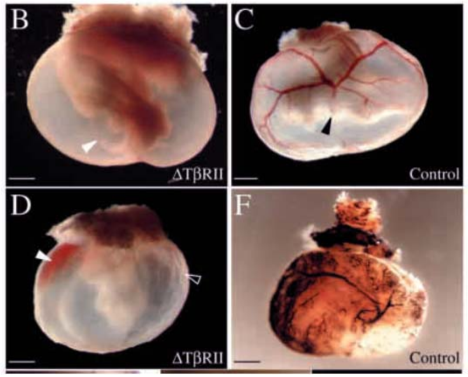
TGF-β rodina je důležitá v regulaci procesů raného vývoje, …





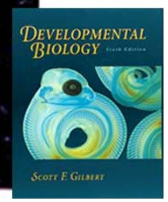
TGF-β2



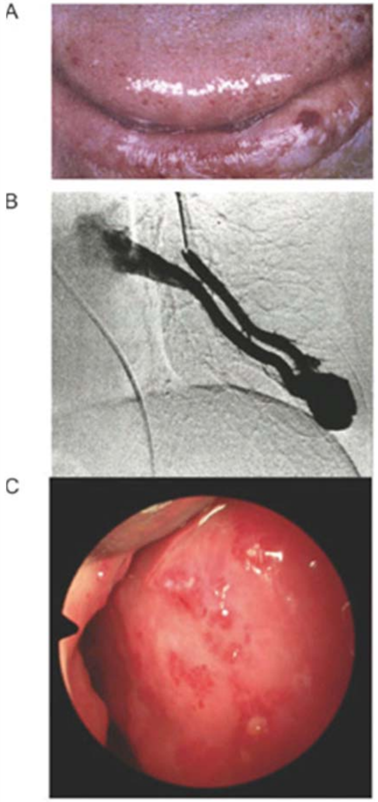


Goumans et al. *Development* 1999:126

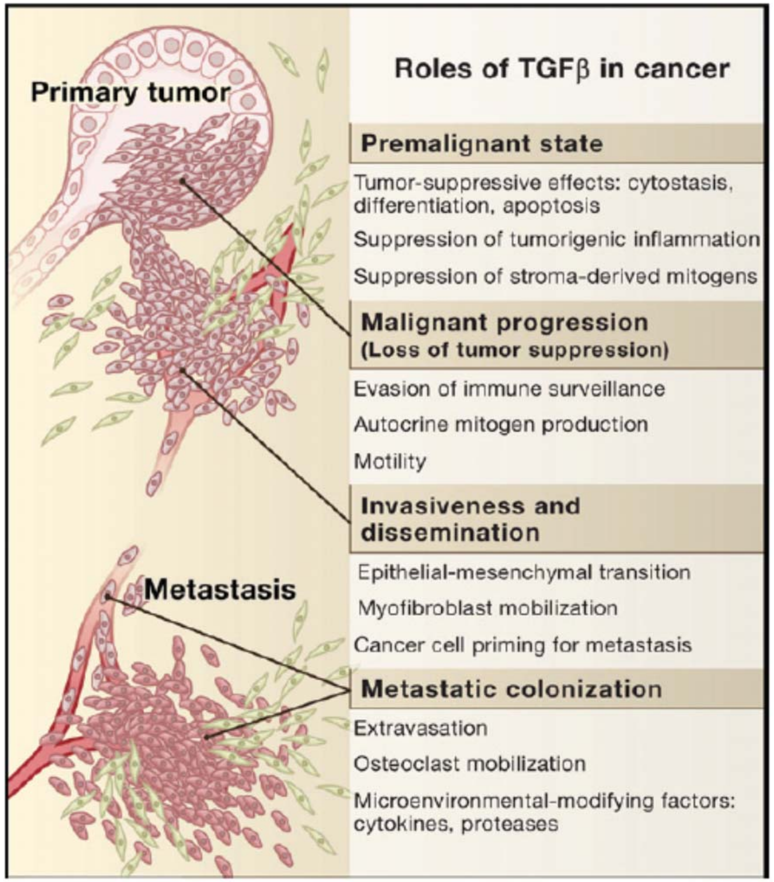
Heupel *et al. Neural Development* 2008 **3**:25



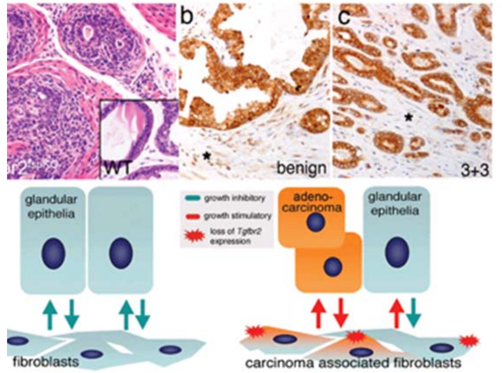
…a pro udržení homeostázy



ALK1 mutation => Clinical symptoms of hereditary hemorrhagic telangiectasia (HHT) include **(A)** bleedings in tongue and lower lip, **(B)** arteriovenous malformations (pulmonary angiogram is shown), and **(C)** nasal telangiectases (courtesy of Dr U Geisthoff).
Cell Research (2009) **19**:116



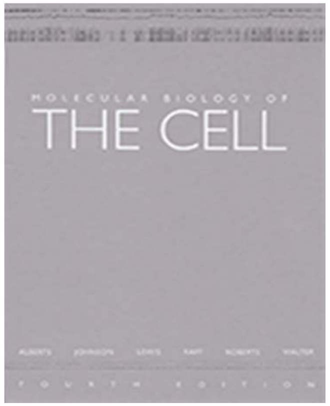
Joan Massague, Cell *134*, July 25, 2008



Placencio VR, Sharif-Afshar A-R, Li X, et al. 2008. Stromal transforming growth factor-beta signaling mediates prostatic response to androgen ablation by paracrine Wnt activityTGF-beta responsiveness of the stroma dictates prostatic sensitivity to androgen ablation. Cancer Res 68:4709-4718.

Li X, Placencio V, Iturregui JM, et al. 2008. Prostate tumor progression is mediated by paraacrine TGF-beta/Wnt3 a signaling axis. Oncogene 27:7118-30.

Signálová transdukce: základní principy



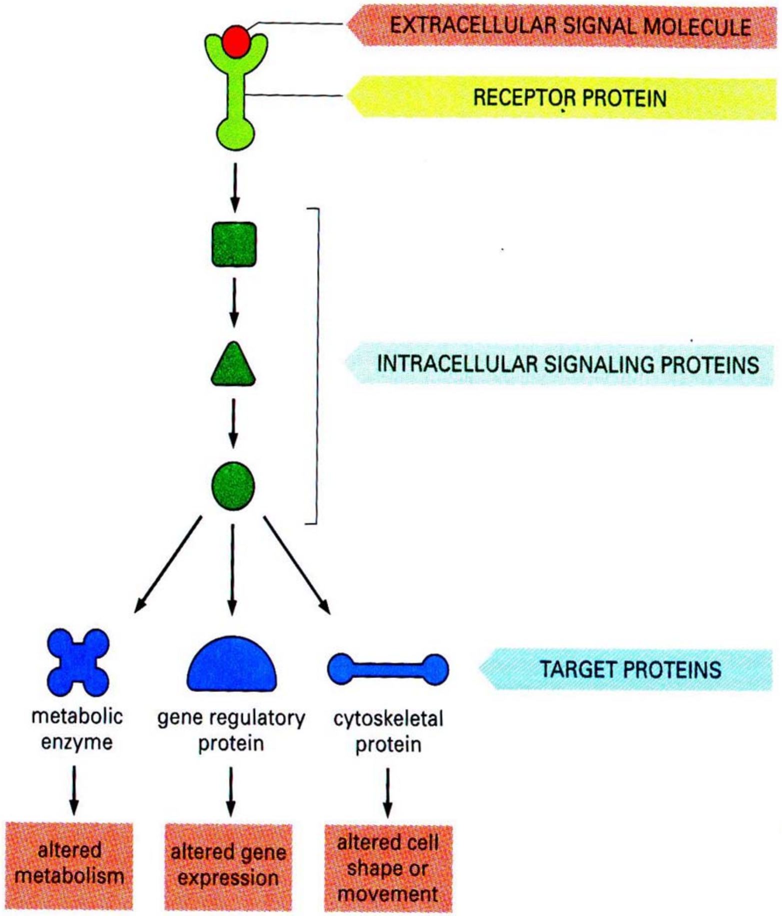
http://www.ncbi.nlm.nih.gov/books/NBK21054/

Signálová transdukce: základní principy

Komunikace mezi buňkami

- příjem signálu
- přenos signálu uvnitř buňky
- změna chování (fenotypu) na základě zpracování signálu (ů)

Jednoduchá intracelulární signální dráha aktivovaná vnější signální molekulou ovlivňuje odpověď buňky.



Signální molekula

↓ **vazba**

receptor

↓ **aktivace**

Intracelulární signální dráha

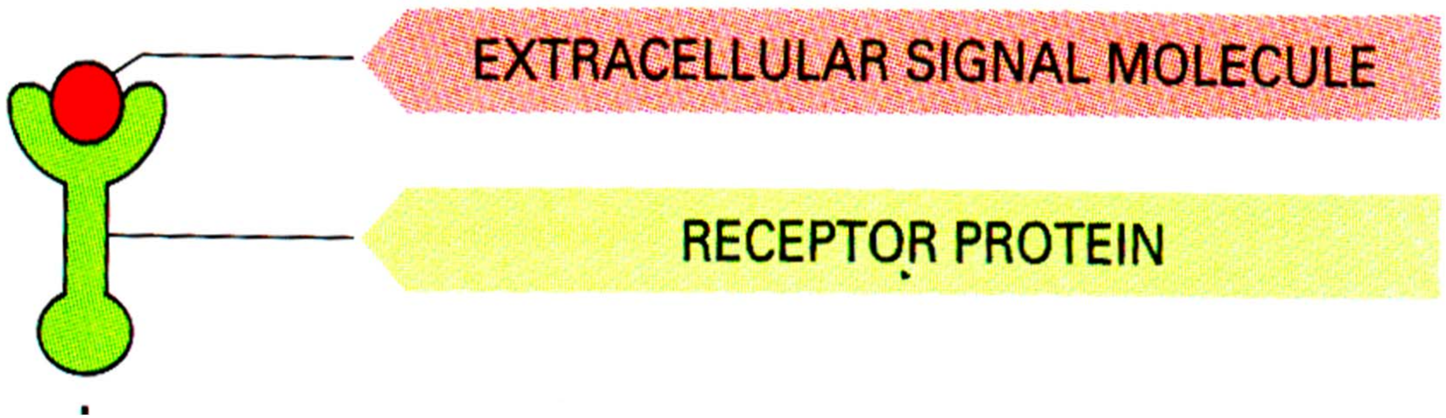
↓

Cílový protein (komplex proteinů)

↓

Změna chování (fenotypu) buňky

Základní principy: signální molekuly a jejich receptory



- Buňky mohou komunikovat prostřednictvím řady různých molekul

  Příklady různých signálních molekul:
  Proteiny, malé peptidy, aminokyseliny, retinoidy, mastné kyseliny a jejich deriváty, plyny

- Signální molekuly jsou produkovány signalizující buňkou
- Bez ohledu na typ signálu odpovídá cílová buňka pomocí receptoru který specificky váže signální molekulu a iniciuje vlastní odpověď.

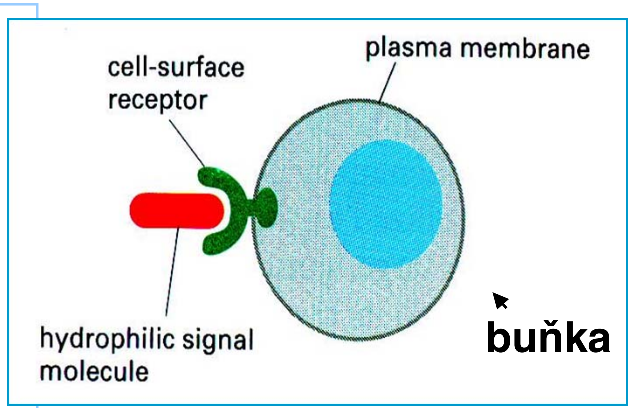
Základní principy: signální molekuly a jejich receptory

➢Extracelulární signál

-rozpoznán pomocí specifického receptoru.

❖hydrofilní: neprojde plazmatickou membránou – vazba na povrchový receptor

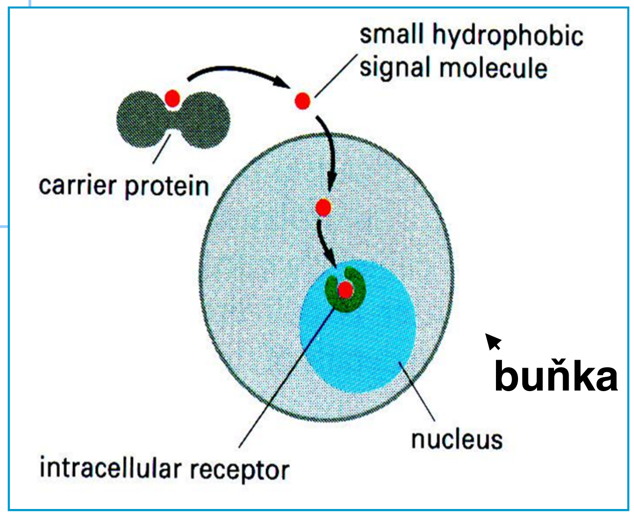
příklad: růstové faktory



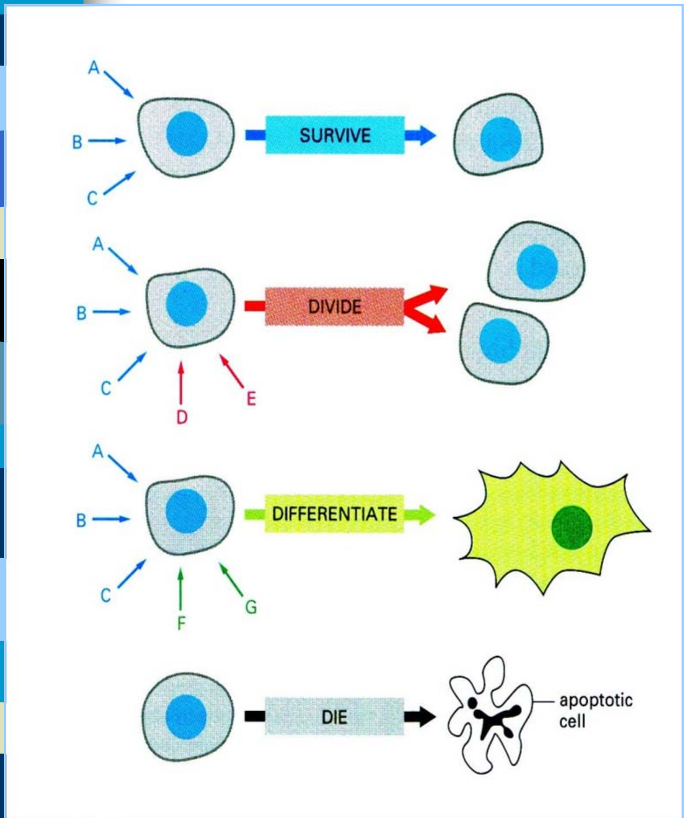
❖hydrofóbní: prochází buněčnou membránou a přímo reguluje aktivitu intracelulárního receptoru.

-transportován pomocí nosiče

příklad: steroidní hormony, vitamin D



- **Každý buněčný typ je „naprogramován“ odpovídat na specifickou kombinaci signálních molekul.**



Typická savčí buňka je vystavena kombinaci stovek různých signálů -selektivní odpověď

- Každá buňka má určitý set receptorů
- Signální molekuly mnohdy fungují v kombinaci s dalšími

Základní principy buněčné signalizace

Tři skupiny proteinových <u>povrchových</u> receptorů:

- **spřažené s iontovými kanály**
- **spřažené s G-proteiny**
- **spřažené s enzymatickou aktivitou**

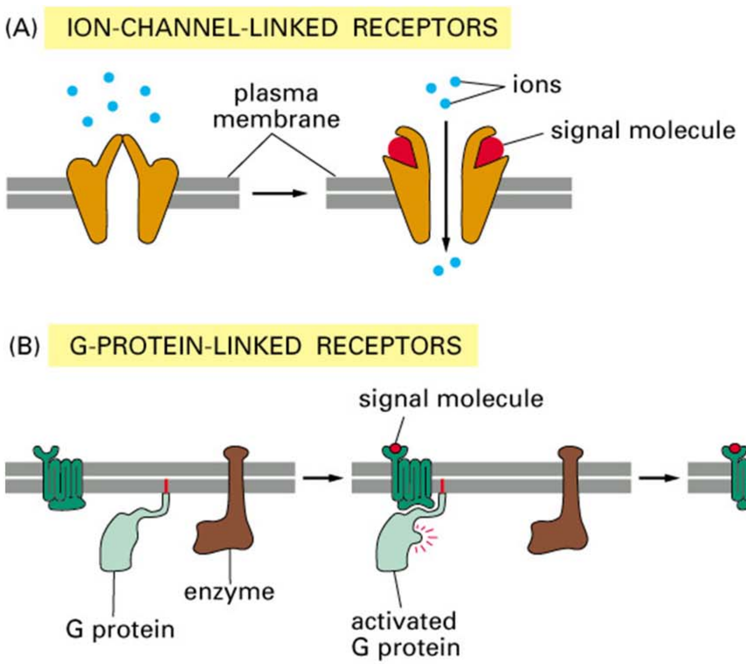


Figure 15–15 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

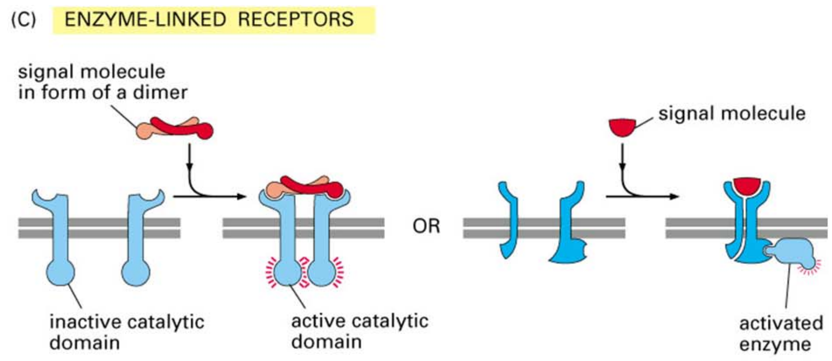


Figure 15–15 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Buněčné povrchové receptory

- **Působí jako přenašeče signálu: vážou signální molekulu s vysokou afinitou a konvertují tento extracelulární signál a jednu nebo více intracelulárních signálních drah vedoucí k odpovědi na buněčné úrovni (změna fenotypu atp.).**

- **Aktivovaný povrchový receptor spouští kaskádu fosforylací vedoucí k přenosu signálu buňkou až k jádru.**

Receptory spřažené s iontovými kanály

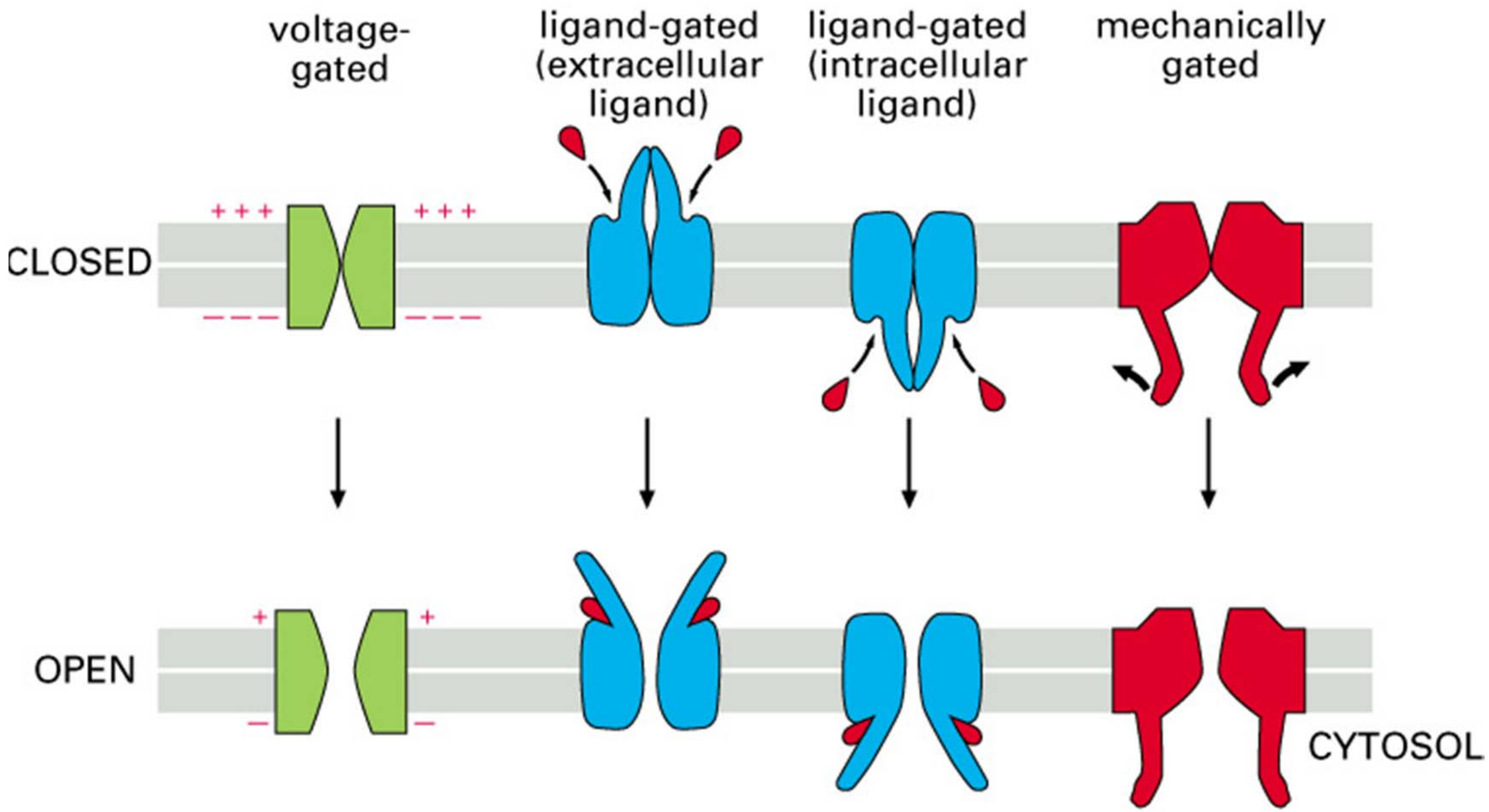


Figure 11–21. Molecular Biology of the Cell, 4th Edition.

Receptory spřažené s G-proteiny

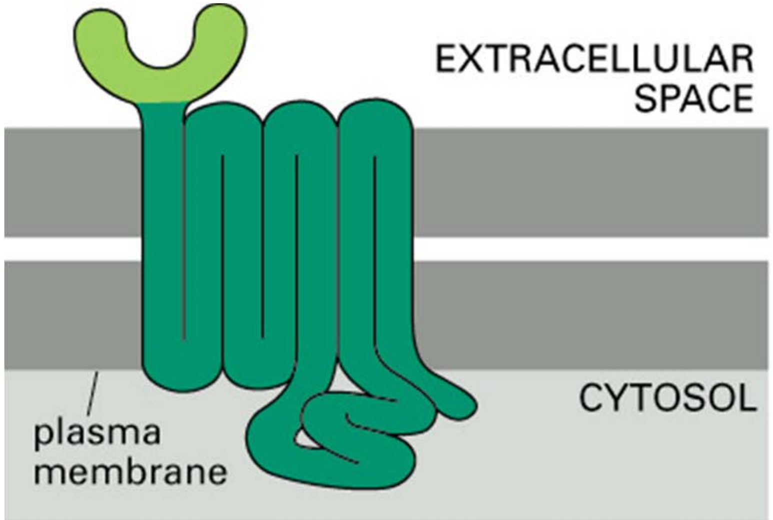


Figure 15–26. Molecular Biology of the Cell, 4th Edition.

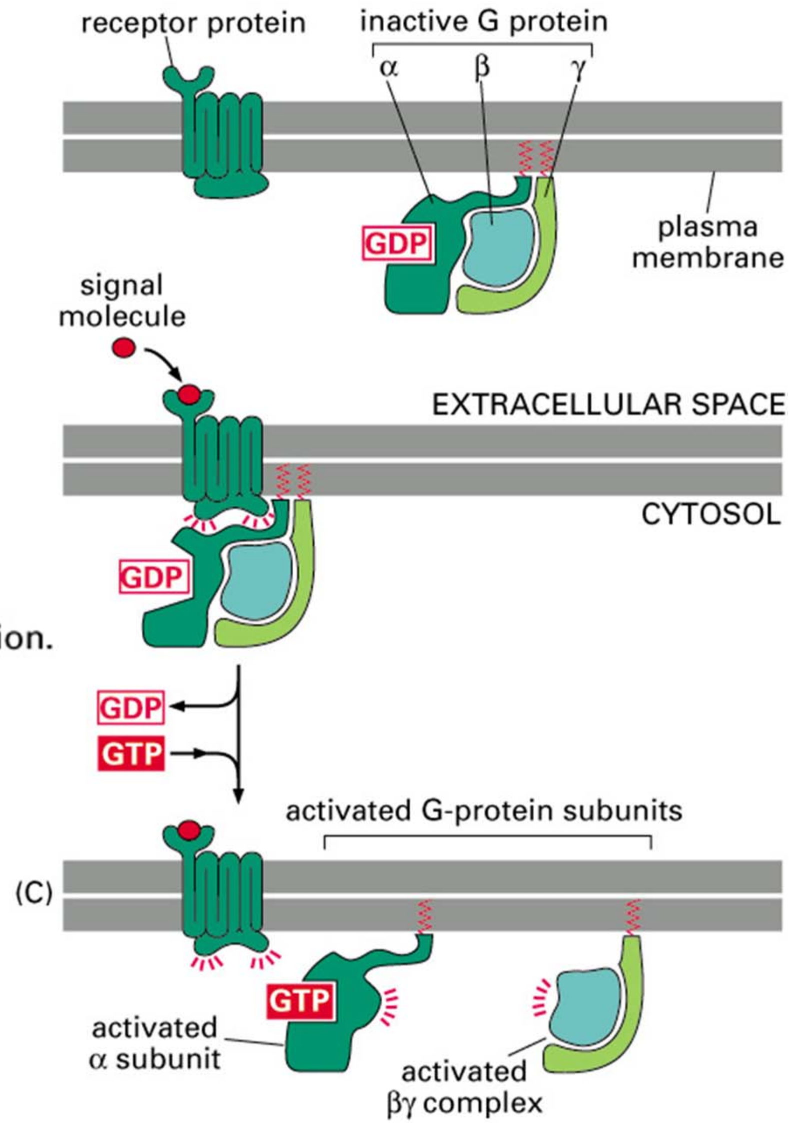
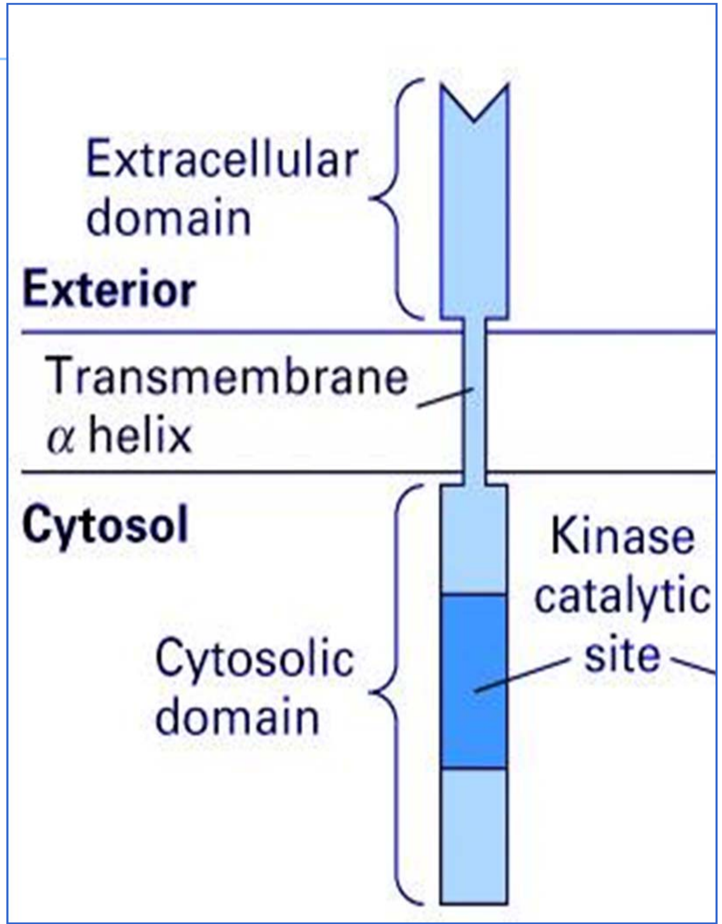


Figure 15–28. Molecular Biology of the Cell, 4th Edition.

Receptory s tyrosin kinázovou aktivitou

- **transmembránové proteiny z doménou vázající ligand na vnější straně a katalytickou doménou na vnitřní straně buněčné membrány**
- **Vazba růstových faktorů jako je insulin, epidermální růstový faktor na extracelulární část jejich receptoru vede k aktivaci kinázové aktivity katalytické domény receptoru a spouští fosforylaci substrátů přenášejících signál.**



Hlavní mechanismy přenosu signálů mají podobné principy

- **Jak u tyrosin kinázové signalizace tak u G-proteiny zprostředkované signalizace je signální protein aktivován fosforylací a deaktivován defosforylací.**

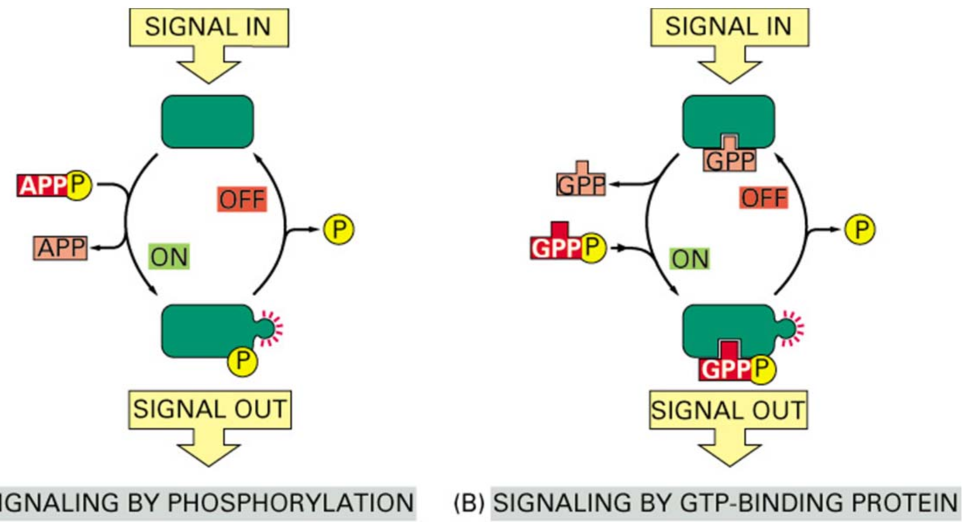
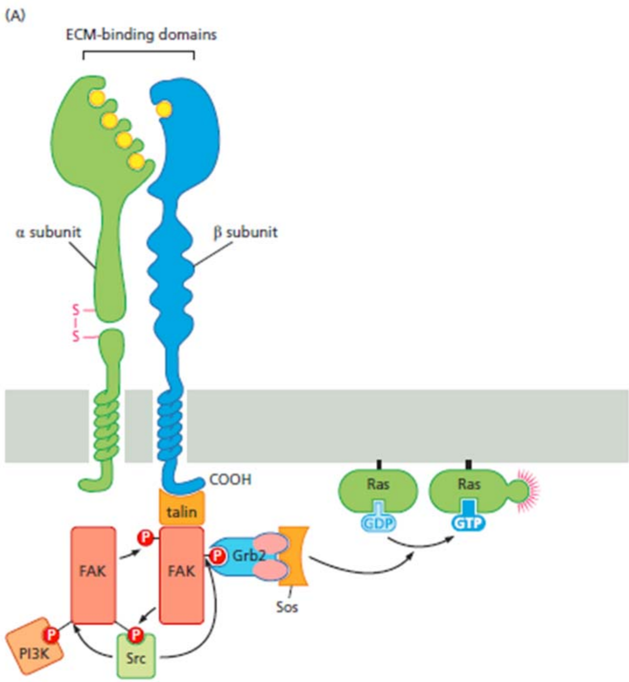
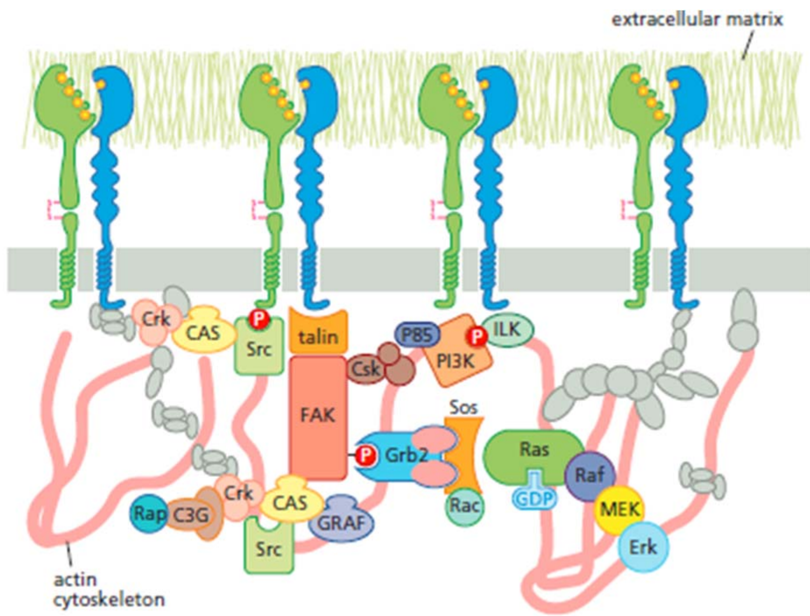


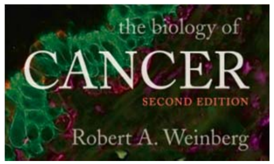
Figure 15–17. Molecular Biology of the Cell, 4th Edition.

Signaling by phosphorylation	**Signaling by GTP-binding protein**

Cell adhesion receptors emit signals





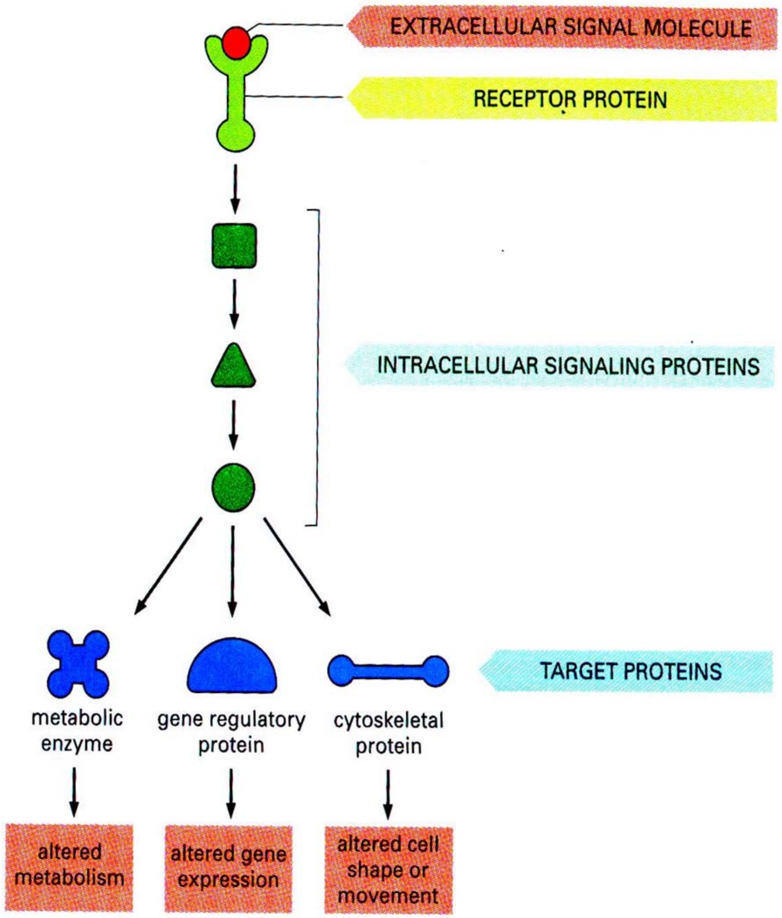


Signálová transdukce: shrnutí obecných principů

- **Buněčná signalizace vyžaduje jak extracelulární signální molekuly tak komplementární set buněčných receptorů a intracelulárních přenašečů**
- **Většina signálních molekul je hydrofilní a aktivuje povrchové buněčné receptory**

- **Existují tři hlavní skupiny povrchových receptorů.**
- **Precizně regulovaná kaskáda fosforylací stimulovaná aktivací receptoru vede k přenosu signálu skrz cytoplasmu do jádra.**

Signální molekuly - ligandy
-hydrofobní
-hydrofilní



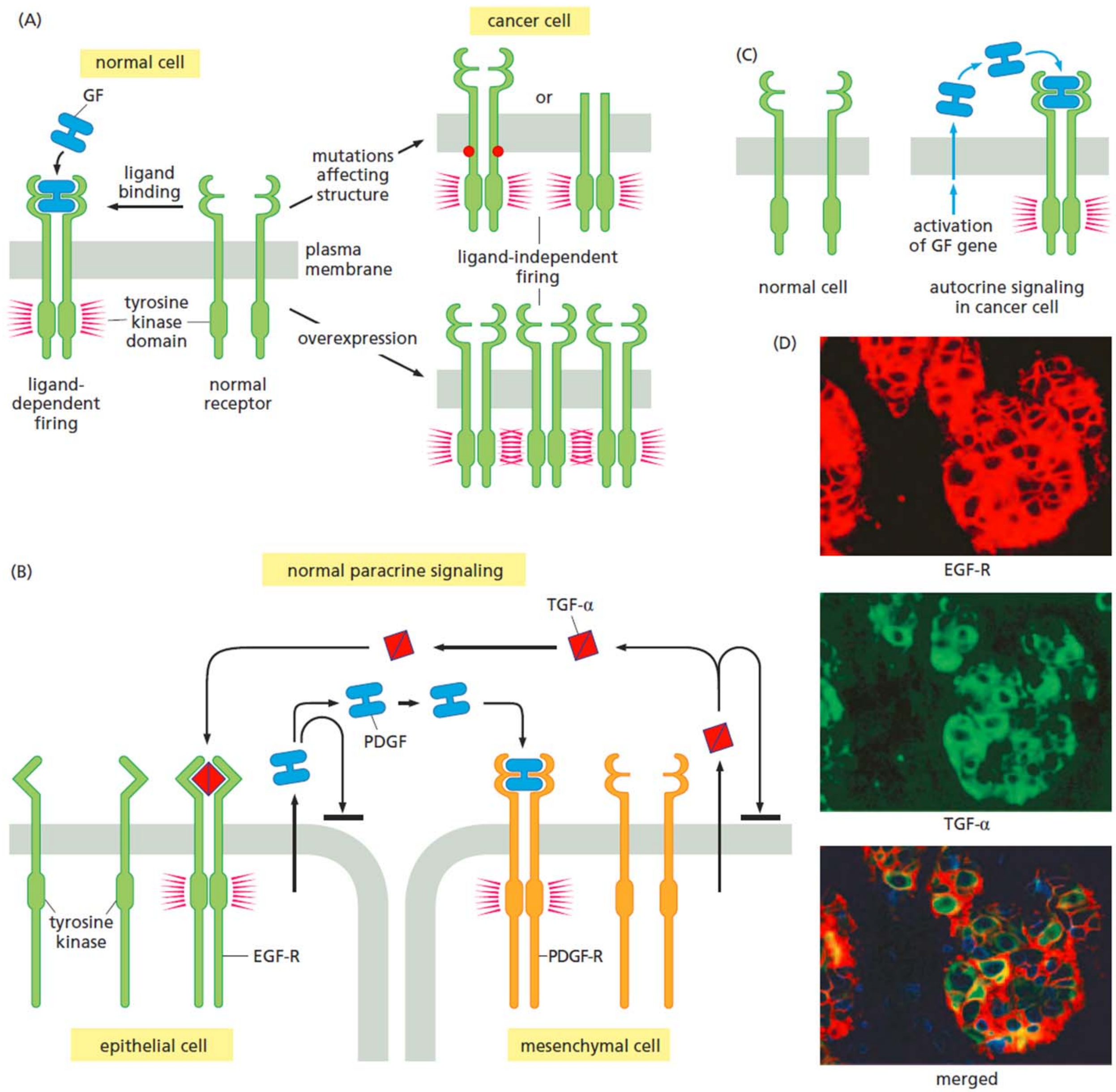
Receptory
- specifické
- intracelulární
- povrchové (extracelulární)

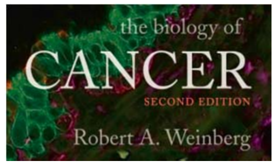
Receptory spřažené s iontovými kanály

Receptory spřažené s G-proteiny

Receptory spřažené s enzymatickou aktivitou

Deregulace signálové transdukce



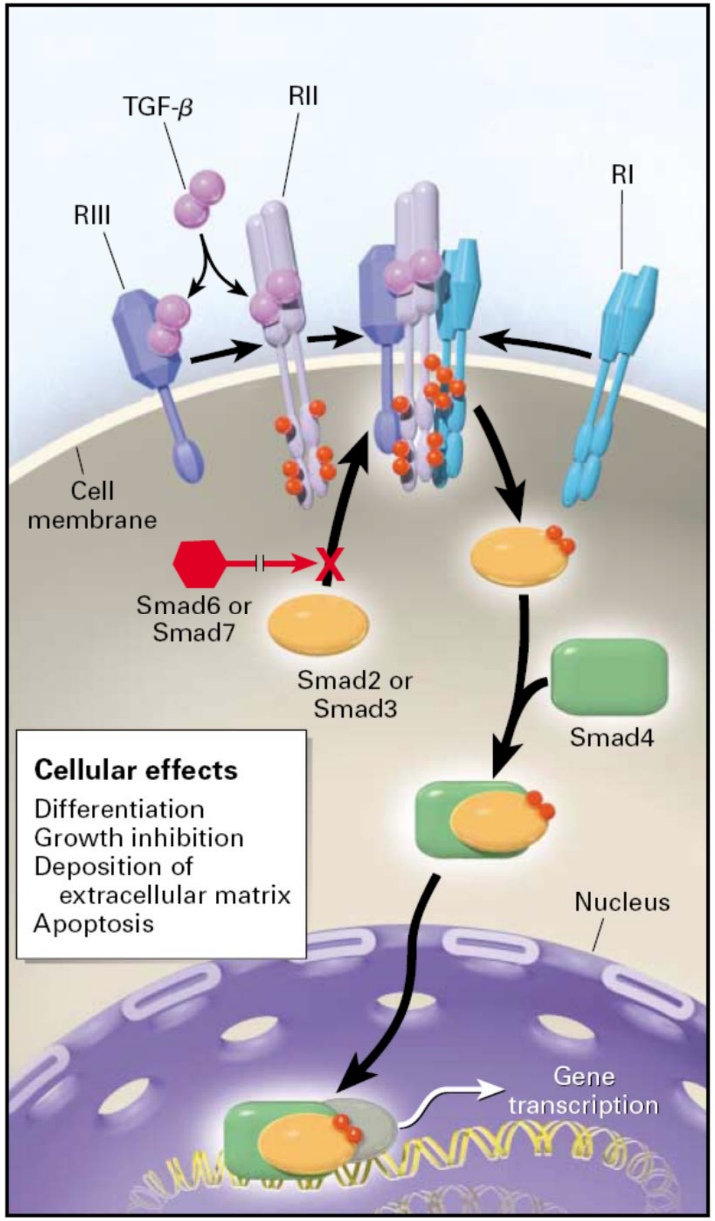


Transforming growth factor -β (TGF-β)

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

TGF-β_1

- pleiotropní cytokin
- negativní regulátor



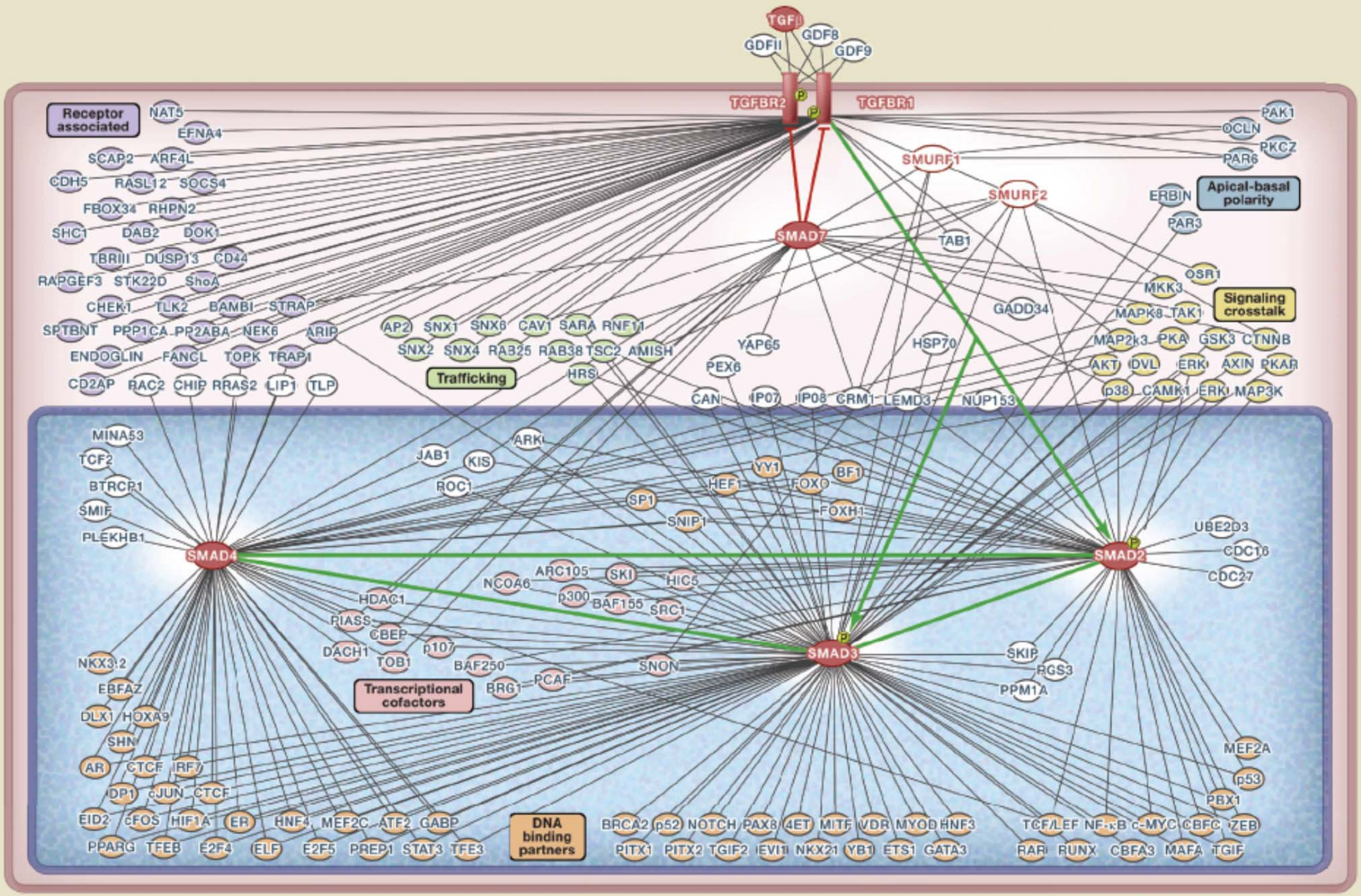
Epstein, F.H., N Engl J Med 2000; 342:1350-1358, 2000

SnapShot: The TGFβ Pathway Interactome

Ian W. Taylor and Jeffrey L. Wrana
Samuel Lunenfeld Research Institute and Department of Molecular Genetics, University of Toronto, Toronto, ON M5G 1X5, Canada

Cell

378 Cell 133, April 18, 2008 ©2008 Elsevier Inc. DOI 10.1016/j.cell.2008.04.007



See online version for legend and references.

Biologické funkce TGF-β

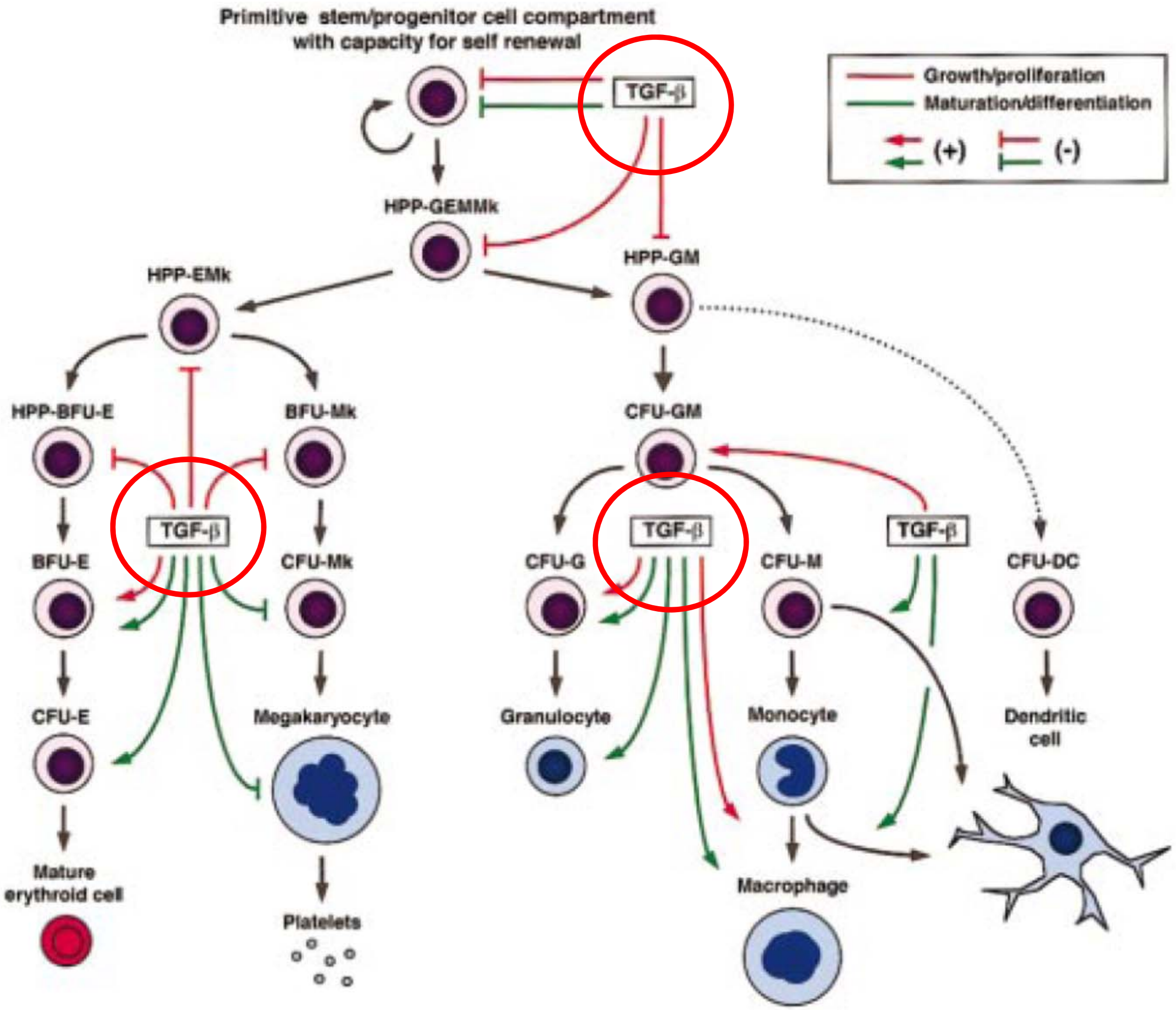
- Reguluje proliferaci, diferenciaci, buněčnou smrt, motilitu, adhezi (v závislosti na buněčném typu) = ovlivňuje homeostázu;
- reguluje expresi extracelulární matrix;
  - indukuje fibrilární kolagen a fibronectin;
  - inhibuje degradaci ECM (prostřednictvím inhibice MMPs a indukce TIMPs).

Biologické funkce TGF-β

- **TGF-β1 knockout**
  - Multifocal inflammatory disease followed by a wasting syndrome
    - Multifocal infiltration of lymphocytes and macrophages into diverse organs. Increased adhesion of mononuclear leukocytes (MLN) to extracellular matrix and to endothelial cells in vitro. Blockage of MLN infiltration by synthetic fibronectin peptides
    - Decreased thymus size
    - Enlargement of lymph nodes
    - Elevated constitutive levels of IL-2 mRNA in the thymus
    - Elevated IL-2r mRNA in lymph nodes
  - Cachexia
    Death roughly 20 days after birth
- **TGF-β2 knockout**
  - Multi-organ defects (lung, heart, limb, craniofacial, spinal column, eye, inner ear, urinary tract, genital tract)
  - **Perinatal mortality**

http://www.bioscience.org/knockout/knochome.htm

Regulace růstu/proliferace a zrání/diferenciace hematopoetických buněk působením TGF-β



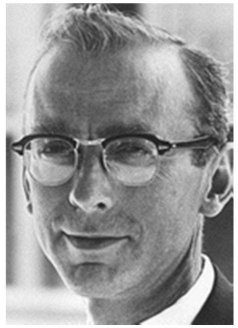
N.O. Fortunel, A. Hatzfeld, J.A. Hatzfeld, Transforming growth factor-beta: pleiotropic role in the regulation of hematopoiesis, Blood 96 (2000) 2022-2036.

Transforming growth factor-β family

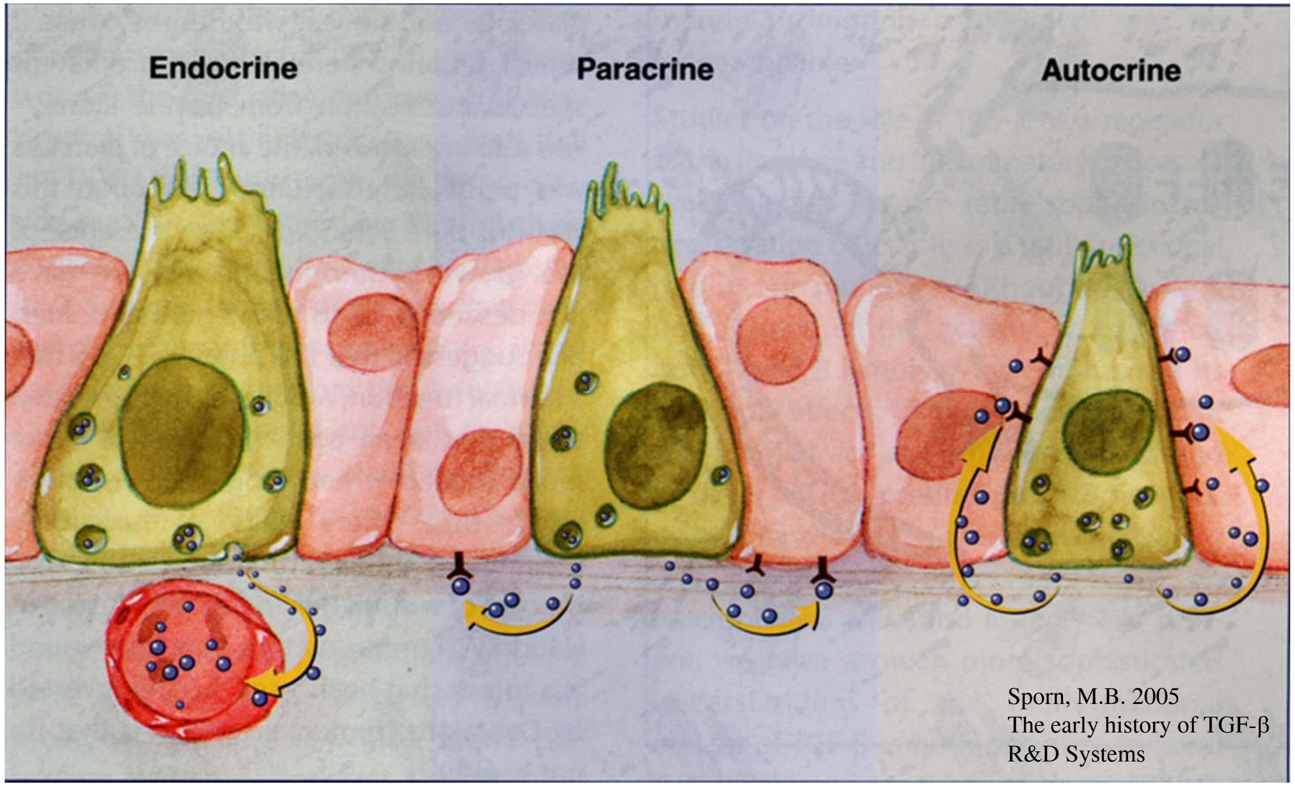
- Historie
- Zástupci rodiny a jejich nejvýznamnější funkce
- Syntéza, produkce a aktivace
- Přenos signálu a jeho regulace
  - receptory
  - sekundární přenašeči
  - „alternativní“ dráhy
  - regulace genové exprese
- Role v rozvoji patologických stavů

Historie TGF-β

- na konci 70. let byla identifikována celá řada růstových faktorů;
- bylo zřejmé, že nádorové buňky se liší od normálních buněk mmj. ve schopnosti reagovat na některé z nich;
- Robert Holley (1968) naznačil, že transformované nebo nádorové buňky unikají z normální růstové kontroly tím, že potřebují méně hormonů či růstových faktorů;
- Michael B. Sporn vyslovil v roce 1980 hypotézu o možném podílu autokrinních faktorů na buněčné transformaci



Endokrinní, autokrinní & parakrinní regulace



Historie TGF-β

- Todaro a De Larco (1978) popsali „faktor“ způsobující transformaci normálních buněk – pojmenovali ho *sarcoma growth factor (SGF)*

Proc. Natl. Acad. Sci. USA
Vol. 75, No. 8, pp. 4001–4005, August 1978
Microbiology

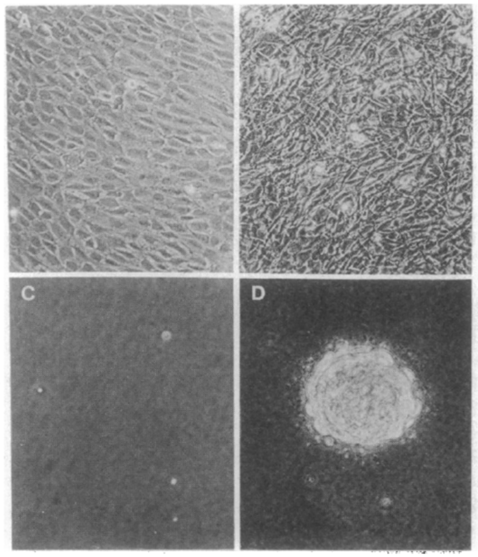
Growth factors from murine sarcoma virus-transformed cells

(epidermal growth factor/polypeptide hormones/cell transformation/radioreceptor assays)

JOSEPH E. DE LARCO AND GEORGE J. TODARO

Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Communicated by David Baltimore, May 11, 1978



Historie TGF-β

- A.B. Roberts a M.A. Anzano (1982) prokázali, že SGF není jen jeden faktor
  - Frakce indukující málo kolonií a silně kompetitivní s EGF= **TGF-α**
  - Frakce indukující mnoho velkých kolonií a bez kompetice s EGF = **TGF-β**

Cancer Res. 1982 Nov;42(11):4776-8.

Synergistic interaction of two classes of transforming growth factors from murine sarcoma cells.

Anzano MA, Roberts AB, Meyers CA, Komoriya A, Lamb LC, Smith JM, Sporn MB.

Historie TGF-β

- Na začátku 80. let byli vyvinuty metody purifikace TGF-β
- V té tobě již bylo jasné, že produkce TGF-β není nádorově specifická
- purifikace zejména z placenty, krevních destiček a prasečích ledvin

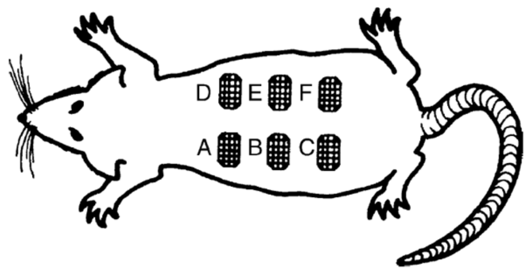
Historie TGF-β

- M.B. Sporn (1983) prokázal *in vivo* schopnost TGF-β podporovat tvorbu kolagenu a vaskularizaci normální tkáně během hojení

Science. 1983 Mar 18;219(4590):1329-31.

Polypeptide transforming growth factors isolated from bovine sources and used for wound healing in vivo.

Sporn MB, Roberts AB, Shull JH, Smith JM, Ward JM, Sodek J.



Historie TGF-β

- Rik Derynck et al. (1985) naklonovali TGF-β

NATURE VOL. 316 22 AUGUST 1985 ARTICLES 701

Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells

Rik Derynck, Julie A. Jarrett, Ellson Y. Chen, Dennis H. Eaton, John R. Bell*, Richard K. Assoian†, Anita B. Roberts†, Michael B. Sporn† & David V. Goeddel

Departments of Molecular Biology and * Protein Biochemistry, Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA
† Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20205, USA

Historie TGF-β

- Kathleen Flanders et al. (1988) vyvinula protilátky proti specifickým epitopům TGF-β a tím umožnil studium exprese *in vivo*

Transforming Growth Factor-β1: Histochemical Localization with Antibodies to Different Epitopes

Kathleen C. Flanders, Nancy L. Thompson, David S. Cissel, Ellen Van Obberghen-Schilling, Carl C. Baker,* Mary E. Kass,§ Larry R. Ellingsworth,‡ Anita B. Roberts, and Michael B. Sporn

Laboratory of Chemoprevention and *Laboratory of Tumor Virus Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; ‡Connective Tissue Research Laboratory, Collagen Corporation, Palo Alto, California 94303; and §Department of Pathology, Washington Hospital Center, Washington DC 20005

Historie TGF-β

- Joan Massagué (1985) detailněji charakterizoval receptory pro TGF-β

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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Vol. 260, No. 5, Issue of March 10, pp. 2636–2645, 1985
Printed in U.S.A.

Cellular Receptors for Type β Transforming Growth Factor

LIGAND BINDING AND AFFINITY LABELING IN HUMAN AND RODENT CELL LINES*

(Received for publication, August 6, 1984)

Joan Massagué and Betsy Like
From the Department of Biochemistry, University of Massachusetts Medical School, Worcester, Massachusetts 01605

Historie TGF-β

- V letech 1992 a 1993 byly naklonovány receptory pro TGF-β

Cell, Vol. 68, 775–785, February 21, 1992, Copyright © 1992 by Cell Press

Expression Cloning of the TGF-β Type II Receptor, a Functional Transmembrane Serine/Threonine Kinase

Herbert Y. Lin,*† Xiao-Fan Wang,*‡ Elinor Ng-Eaton,*
Robert A. Weinberg,*† and Harvey F. Lodish*†
*Whitehead Institute for Biomedical Research
Cambridge, Massachusetts 02142
†Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

TGF-β was originally described as a factor that induced normal rat kidney fibroblasts to proliferate in soft agar in the presen
1981). TG
number of
Roberts an
TGF-β can

Cell, Vol. 75, 681–692, November 19, 1993, Copyright © 1993 by Cell Press

Cloning of a TGFβ Type I Receptor That Forms a Heteromeric Complex with the TGFβ Type II Receptor

Petra Franzén, Peter ten Dijke, Hidenori Ichijo,*
Hidetoshi Yamashita, Peter Schulz,
Carl-Henrik Heldin, and Kohei Miyazono
Ludwig Institute for Cancer Research
Biomedical Center
S-751 24 Uppsala
Sweden

in morphogenesis, e.g., during different stages of development (Akhurst et al., 1991; Lyons et al., 1991).

TGFβs exert their effects through binding to specific cell surface receptors. By affinity labeling and cross-linking to radioiodinated TGFβs, a number of TGFβ receptors (or binding proteins) have been identified, including type I (53 kd), type II (75 kd), and type III receptors (or betaglycan [300 kd]), which are found in most cells (for reviews see

Historie TGF-β

- Objev intracelulárních přenašečů signálu TGF-β - v roce 1995 na *Drosophila melanogaster* a v roce 1996 na *Caenorhabditis elegans.*

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 790–794, January 1996
Developmental Biology

Caenorhabditis elegans genes _sma-2, sma-3, and sma-4_ define a conserved family of transforming growth factor β pathway components

(signal transduction/pattern formation/bone morphogenetic protein/multigene family)

CATHY SAVAGE*, PRADEEP DAS*, ALYCE L. FINELLI*, SCOTT R. TOWNSEND*, CHING-YU SUN†, SCOTT E. BAIRD‡, AND RICHARD W. PADGETT*§

*Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ 08855-0759; †Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; and ‡Department of Biological Sciences, Wright State University, Dayton, OH 45435

Communicated by Clyde A. Hutchison III, University of North Carolina, Chapel Hill, NC, September 15, 1995 (received for review August 11, 1995)

Genetic Characterization and Cloning of _Mothers against dpp_, a Gene Required for _decapentaplegic_ Function in _Drosophila melanogaster_

Jeff J. Sekelsky,[1] Stuart J. Newfeld, Laurel A. Raftery,[2] Elena H. Chartoff and William M. Gelbart

The Biological Laboratories, Harvard University
Cambridge, Massachusetts 02138

Manuscript received September 23, 1994
Accepted for publication December 7, 1994

Historie TGF-β

- Jeffrey Wrana, Lilana Attisano a Joan Massagué (1994) – popis aktivace receptoru

---

Mechanism of activation of the TGF-β receptor

Jeffrey L. Wrana, Liliana Attisano, Rotraud Wieser, Francesc Ventura & Joan Massagué*

Howard Hughes Medical Institute and Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA

NATURE · VOL 370 · 4 AUGUST 1994

Historie TGF-β

- *Mad* (*mothers against decapentaplegic*) + *Sma* = SMAD

Cell, Vol. 87, 173, October 18, 1996, Copyright ©1996 by Cell Press

Nomenclature: Vertebrate Mediators of TGFβ Family Signals

Rik Derynck,[1] William M. Gelbart,[2]
Richard M. Harland,[3] Carl-Henrik Heldin,[4]
Scott E. Kern,[5] Joan Massagué,[6,7] Douglas A. Melton,[2,7]
Marek Mlodzik,[8] Richard W. Padgett,[9]

Anita B. Roberts,[10] Jim Smith,[11] Gerald H. Thomsen,[14]
Bert Vogelstein,[7,12] and Xiao-Fan Wang,[13]

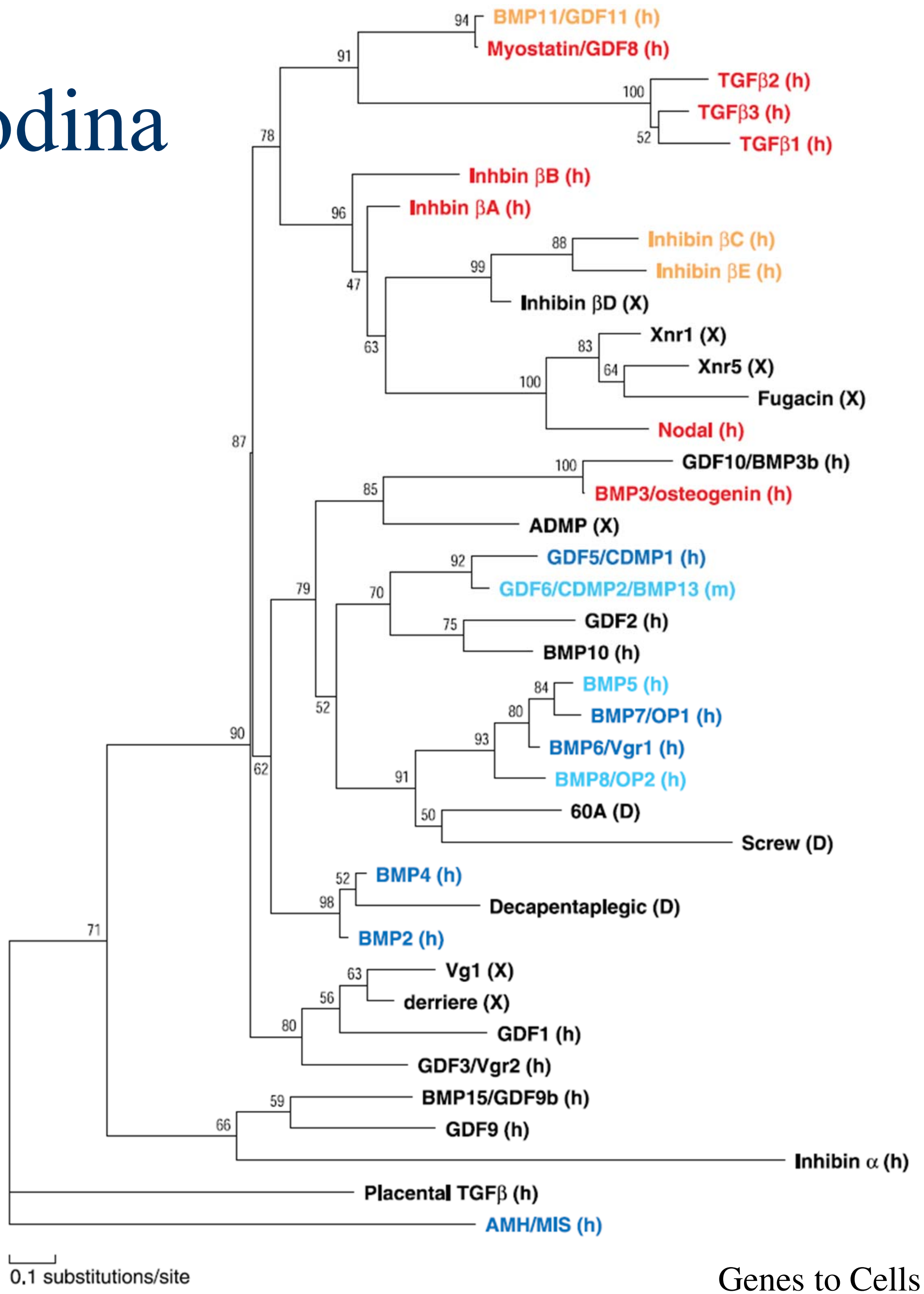
Transforming growth factor-β

- Zástupci rodiny a jejich nejvýznamnější funkce

TGF-β rodina

- více než 60 proteinů
  - Transformující růstové faktory – β (TGF-β)
  - Activin(y)
  - Bone Morphogenetic Proteins (BMP); Growth/Differentiation Factors (GDF)

TGF-β rodina



Genes to Cells (2002) 7, 1191-1204

TGF-β rodina

- Bone Morphogenetic Proteins (BMP)
  - klíčové faktory pro vývoj kostí a chrupavek, ale i další funkce.

BMP	Funkce	Genový lokus
BMP1	Nenáleží do TGF-β rodiny. Metaloproteinása.	8p21
BMP2	Indukuje vývoj kostí a chrupavek; klíčový regulátor diferenciace osteoblastů.	20p12
BMP3	Indukuje tvorbu kostí.	14p22
BMP4	Reguluje tvorbu zubů, končetin a kostí z mesodermu; ovlivňuje hojení zlomenin.	14q22-q23
BMP5	Úloha při tvorbě chrupavek.	6p12.1
BMP6	Důležitý pro funkci kloubů.	6p12.1
BMP7	Klíčový faktor diferenciace osteoblastů a vývoje ledvin.	20q13
BMP8a	Reguluje vývoj kostí a chrupavek.	1p35-p32
BMP8b	Exprimován v hipokampu.	1p35-p32
BMP10	? Vývoj srdce.	2p14
BMP15	?Vývoj oocytů a folykulů.	Xp11.2

Chen, Di, Zhao, Ming, and Mundy, Gregory R. (2004). "Bone Morphogenetic Proteins". *Growth Factors* **22** (4): 233–241

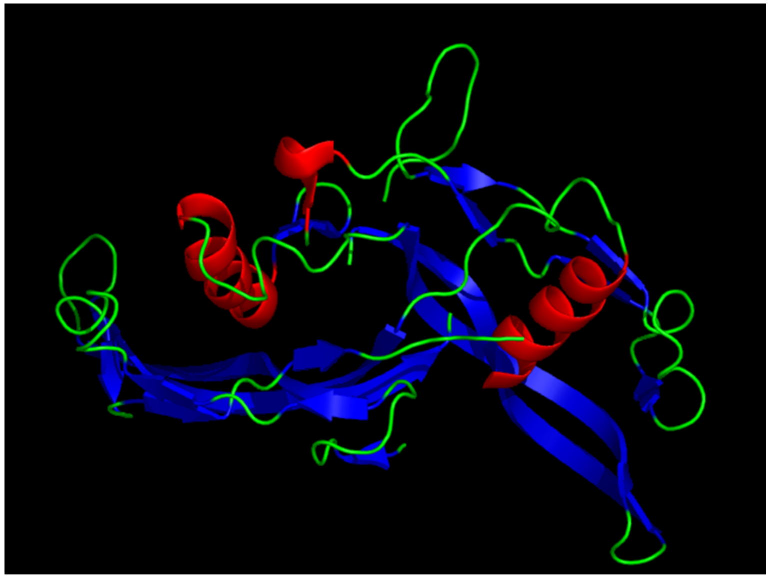
TGF-β rodina

- Growth/Differentiation Factors (GDF) – faktory podobné BMP

GDF	Funkce	Genový lokus
GDF1	Regulátor vývoje levo-pravé asymetrie během embryogeneze.	19p12
GDF2	Indukuje cholinergní fenotyp.	Chr.10
GDF3	?	12p13.1
GDF5	Důležitý pro vývoj kostí a kloubů.	20q11.2
GDF6	Důležitý pro vývoj kostí a kloubů.	Chr. 8
GDF7	Důležitý pro vývoj kostí a kloubů.	Chr. 2
GDF8	Myostatin; regulátor funkce kosterního svalstva. Negativní regulátor svalového růstu.	2q32.2
GDF9	Exprese v oocytech během folikulogeneze.	Chr. 5
GDF10	BMP3b; kontroluje endochondrální osifikaci	Chr. 28
GDF11	Důležitý při vývoji pátěře.	12q13.13
GDF15	Vysoká exprese v placentě; indukovaný NSAIDs; inhibitor karcinogeneze; induktor apoptózy ?	19p13.2-p13.1

TGF-β rodina

- Activin(y)
  - dimerické proteiny regulující syntézu a sekreci FSH a regulující menstruační cyklus, ale také imunitní a nervový systém; důležité faktory při tvorbě kůže a hojení ran;
  - dimer obsahuje dvě identické β podjednotky a dvě β podjednotky inhibin(u) (A nebo B);
  - Activin A (βAβA);
  - Activin B (βBβB);
  - Activin AB (βAβB).

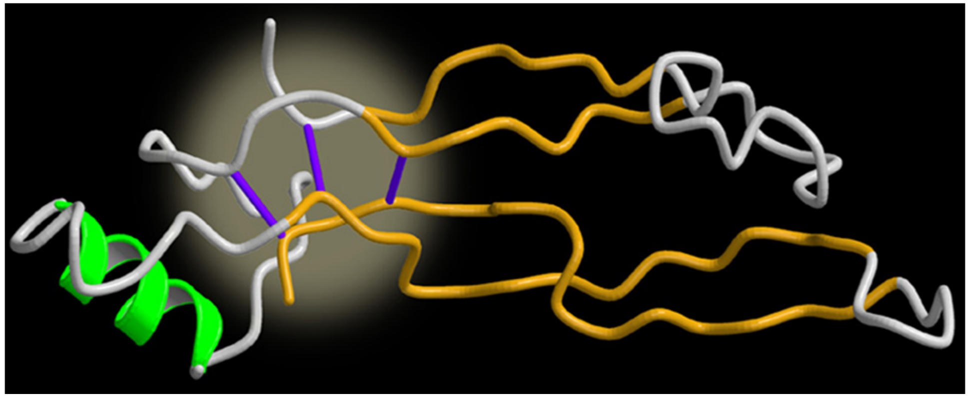


- Inhibin(y)
  - Inhibuje produkci FSH;
  - dimer α a β podjednotky (A nebo B)

Sulyok, S., Wankell, M., Alzheimer, C., and Werner, S. Activin: an important regulator of wound repair, fibrosis, and neuroprotection. Mol. Cell. Endocrinol., *225:* 127-132, 2004.

TGF-β rodina

- Transformující růstové faktory – β
  - důležité faktory řídící embryogenezi, diferenciaci, tkáňovou regeneraci, ale i rozvoj řady onemocnění
  - TGF-β1 (Chr. 19)
  - TGF-β2 (Chr. 1)
  - TGF-β3 (Chr. 14)
  - 76-80% homologie



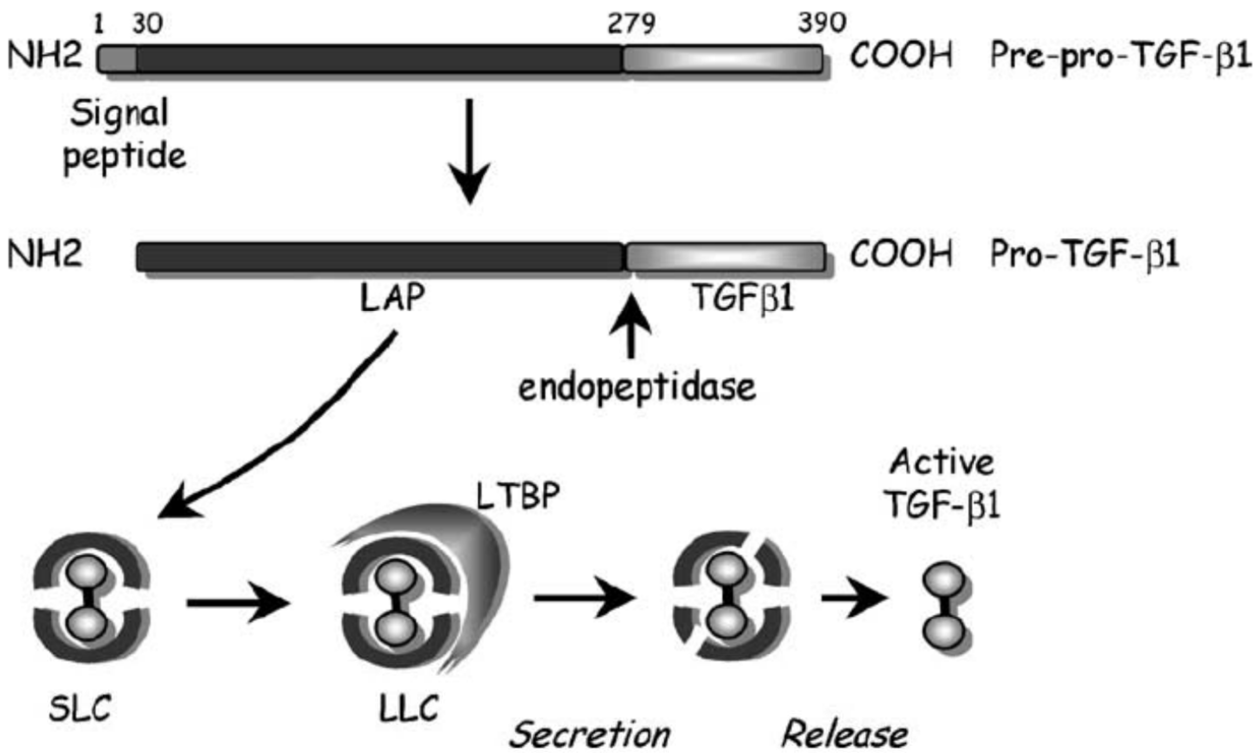
Transforming growth factor-β

- Syntéza, produkce a aktivace

Syntéza a sekrece TGF-β

- Syntetizován a sekretován v podobě latentního komplexu
- asociovaný s Latency Associated Peptide (LAP) = small latent complex;
- LAP dimer spolu s TGF-β dimerem je kovalentně vázán na Latent TGF-β Binding Protein (LTBP) = large latent complex

Syntéza a sekrece TGF-β

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The International Journal of Biochemistry & Cell Biology 36 (2004) 1161–1165
IJBCB
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Molecules in focus

Mammalian transforming growth factor-βs: Smad signaling and physio-pathological roles

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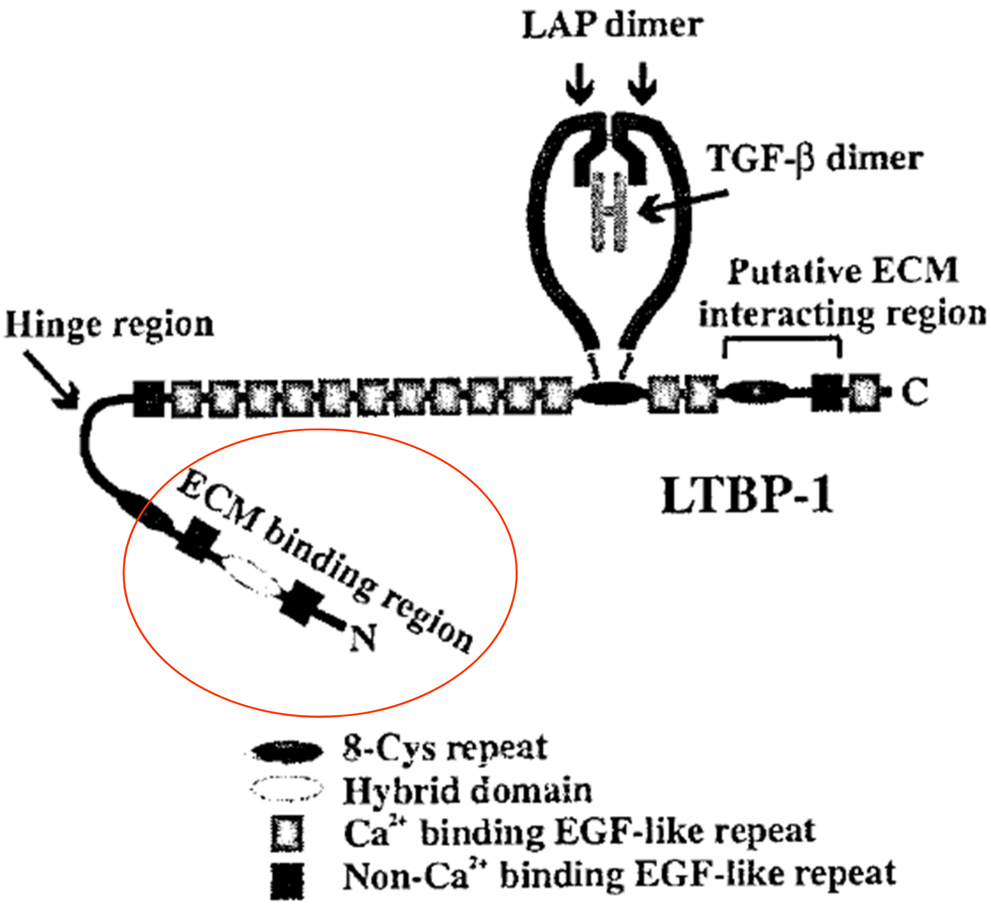
INSERM U532, Institut de Recherche sur la Peau, Université Paris VII, Hôpital Saint-Louis, Pavillon Bazin, 1, Avenue Claude Vellefaux, 75010 Paris, France

Received 15 May 2003; received in revised form 13 June 2003; accepted 13 June 2003

Syntéza a sekrece TGF-β

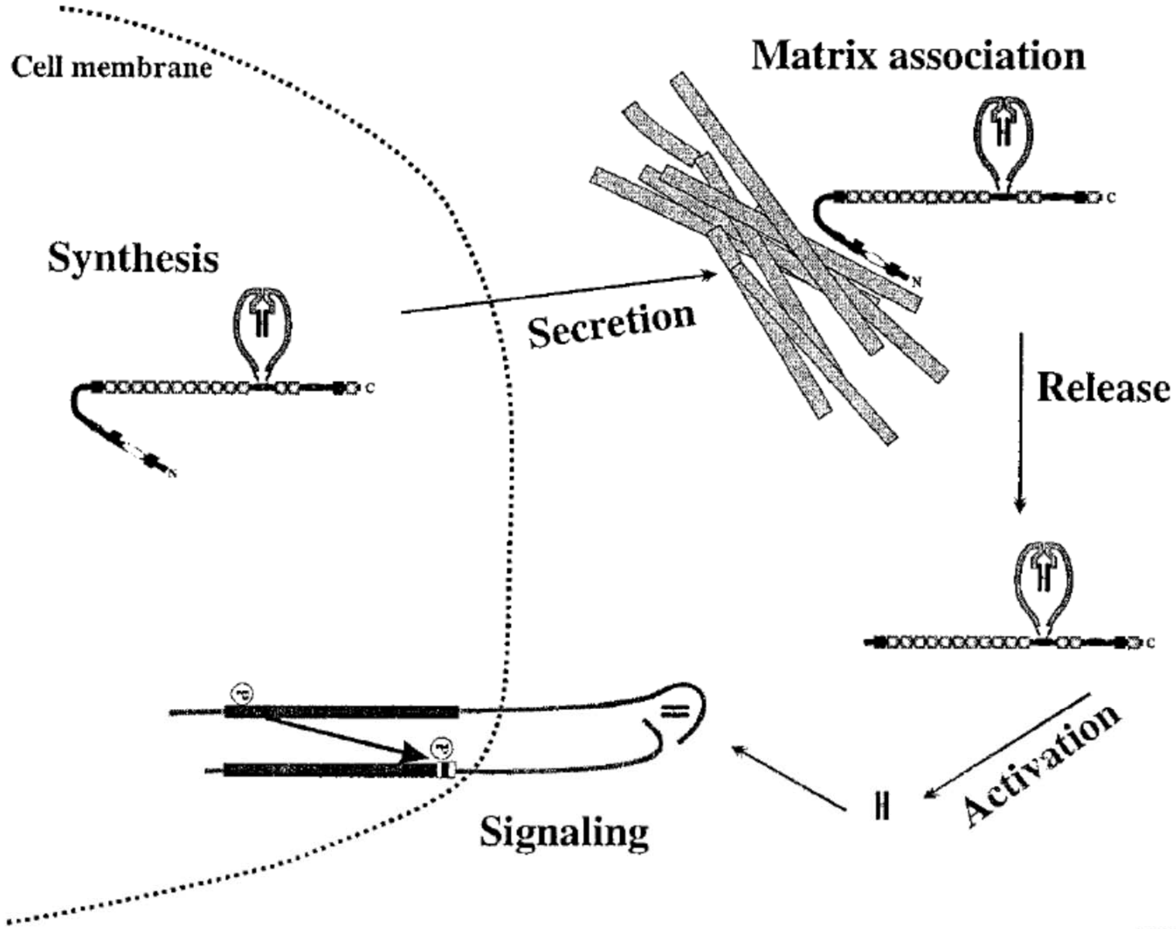


MICROSCOPY RESEARCH AND TECHNIQUE 52:354–362 (2001)

Latency, Activation, and Binding Proteins of TGF-β

KATRI KOLI,* JUHA SAHARINEN, MARKO HYYTIÄINEN, CARITA PENTTINEN, AND JORMA KESKI-OJA
Departments of Virology and Pathology, Haartman Institute, University of Helsinki, FIN-00014 Helsinki, Finland

Syntéza a sekrece a aktivace TGF-β



MICROSCOPY RESEARCH AND TECHNIQUE 52:354–362 (2001)

Latency, Activation, and Binding Proteins of TGF-β

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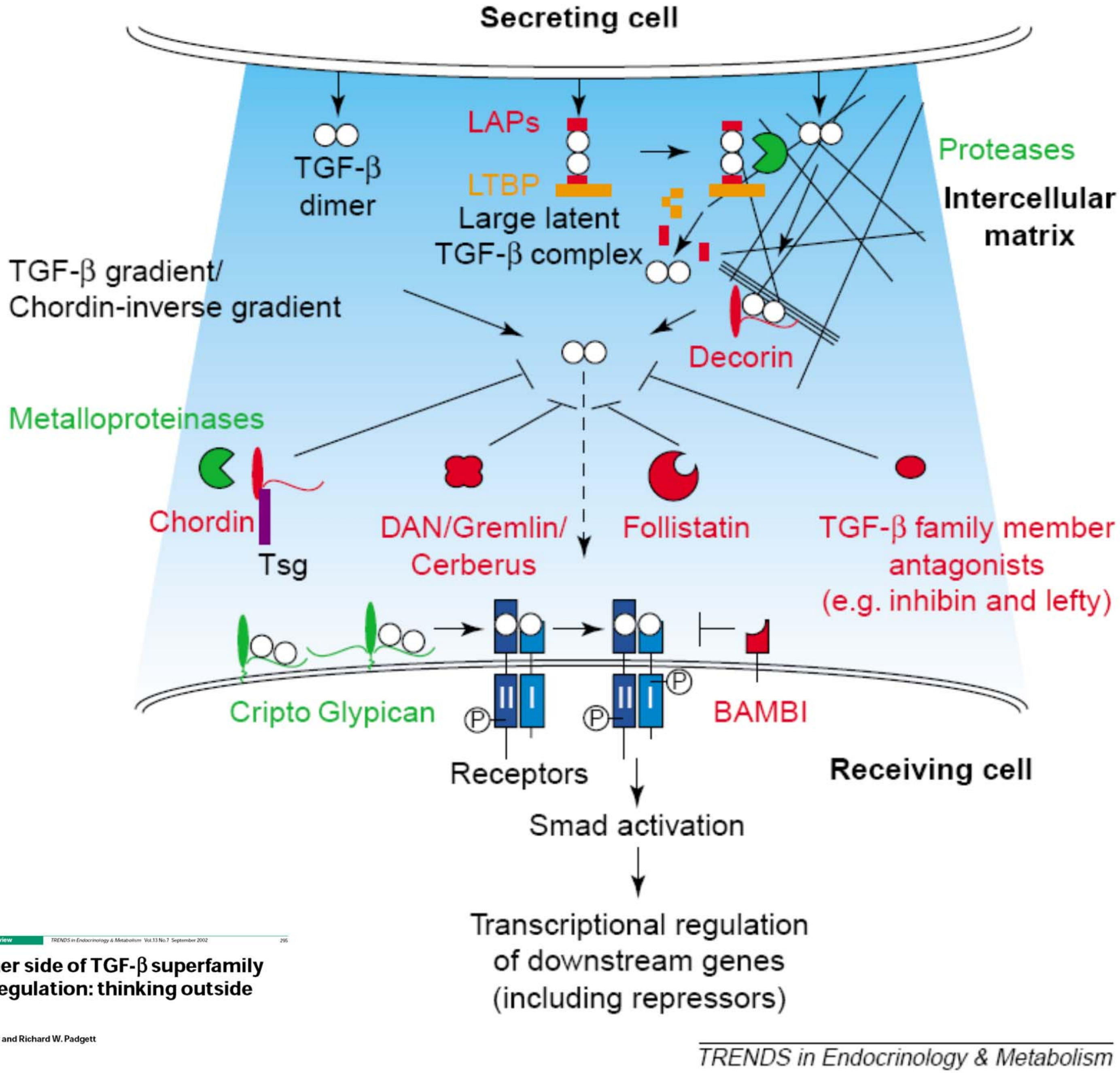
Departments of Virology and Pathology, Haartman Institute, University of Helsinki, FIN-00014 Helsinki, Finland



Review TRENDS in Endocrinology & Metabolism Vol.13 No.7 September 2002 295

The other side of TGF-β superfamily signal regulation: thinking outside the cell

Tina L. Gumienny and Richard W. Padgett

Aktivace TGF-β

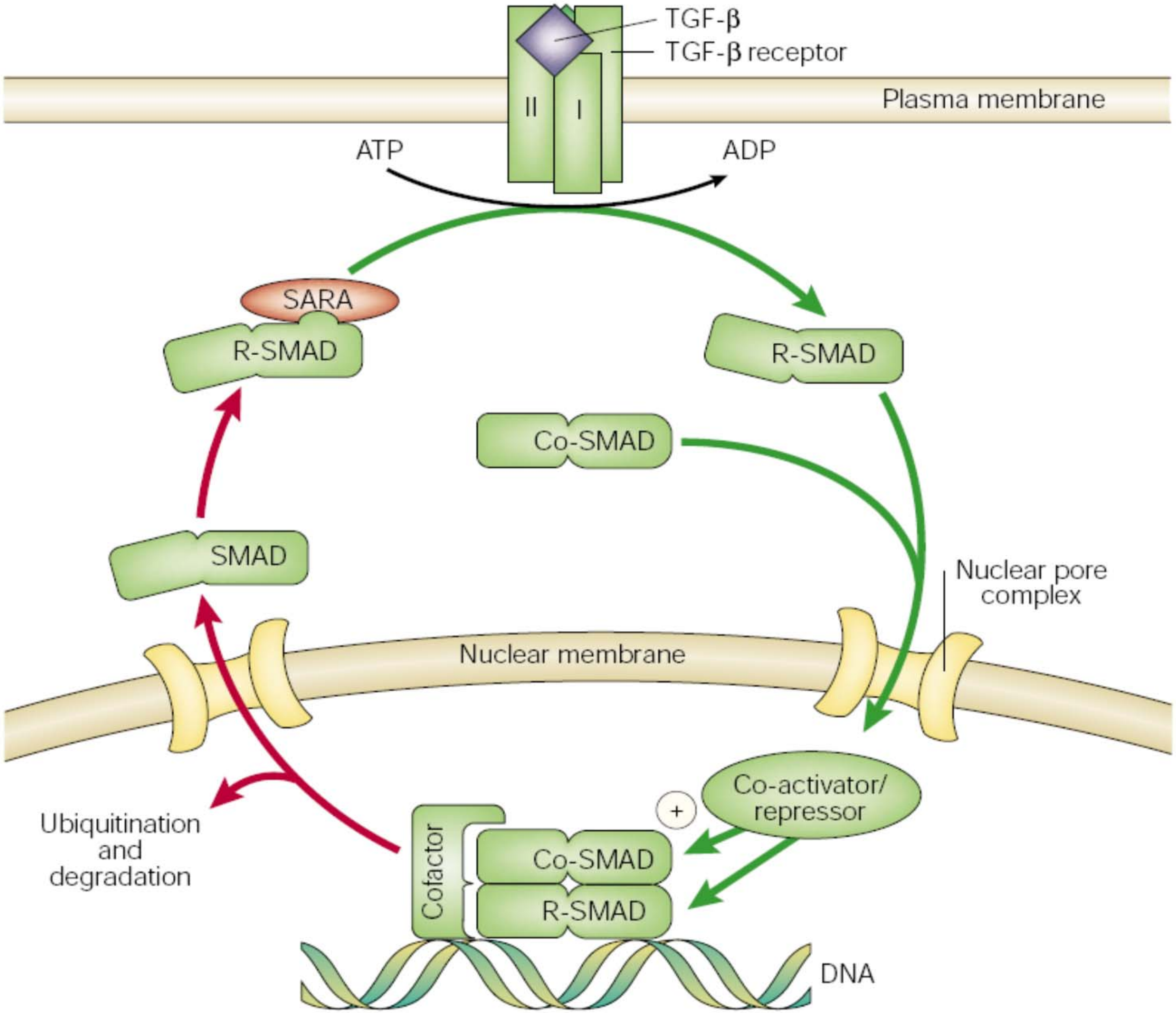
- Fyzikálně-chemicky
  - v kyselém mikroprostředí buněk
  - extrémními změnami pH
  - γ zářením
  - reaktivními skupinami kyslíku
  - zvýšenou teplotou

- Enzymaticky a prostřednictvím nespecifických proteinových interakcí
  - Proteázy
    - Plasmin, Catepsin G
    - Calpain
  - MMP-9 a MMP-2
  - Glykosidázy
  - Interakce s trombospondinem
  - Interakce s integrinem $\alpha_v\beta_6$

Transforming growth factor-β

- Přenos signálu a jeho regulace
  – receptory
  – sekundární přenašeči
  – „alternativní“ dráhy
  – regulace genové exprese

Přenos signálu TGF-β

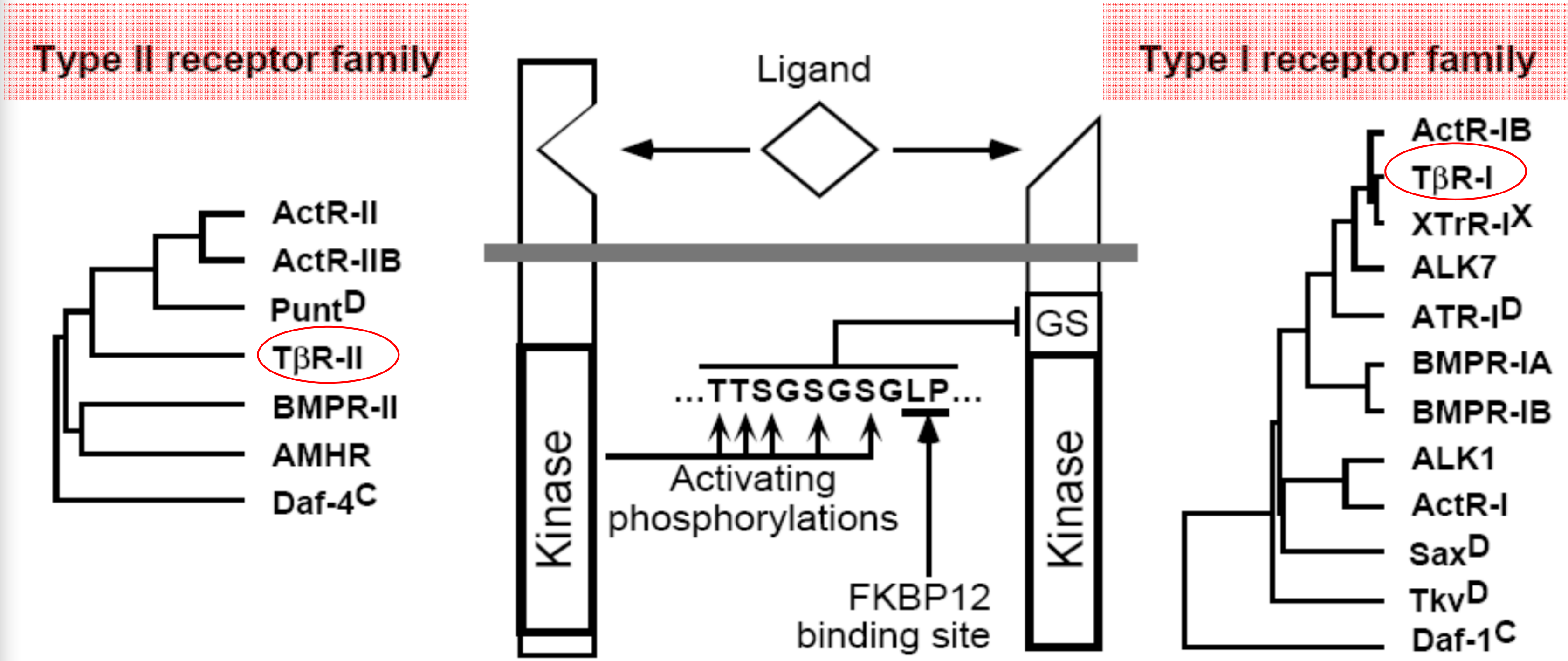


Massague J. Nat. Rev. Mol. Biol. 2000

Transforming growth factor-β

- Přenos signálu a jeho regulace
  - – receptory
  - – sekundární přenašeči
  - – „alternativní“ dráhy
  - – regulace genové exprese

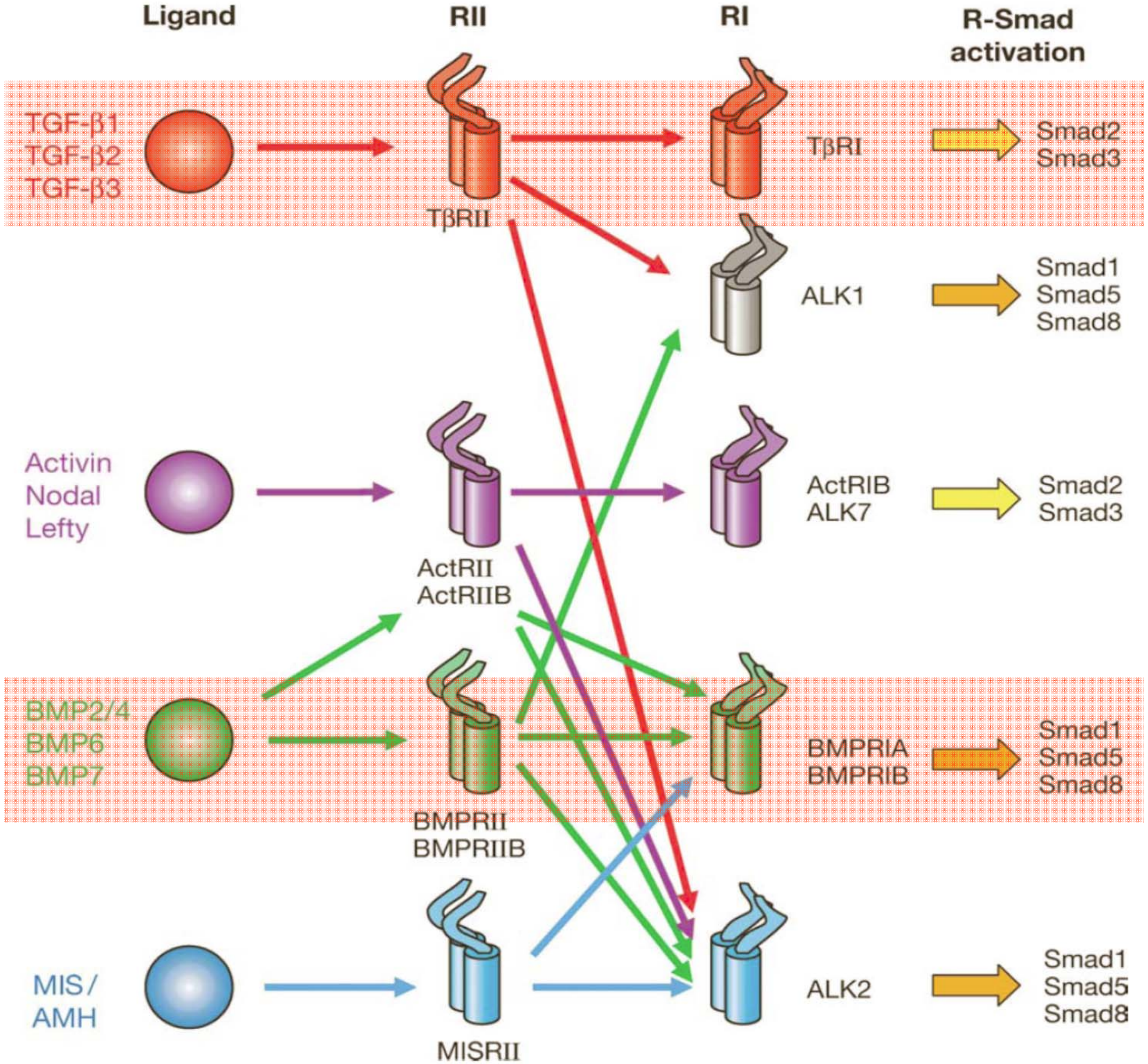
Receptory TGF-β rodiny



Accesory Receptors – Type III: betaglycan, endoglin

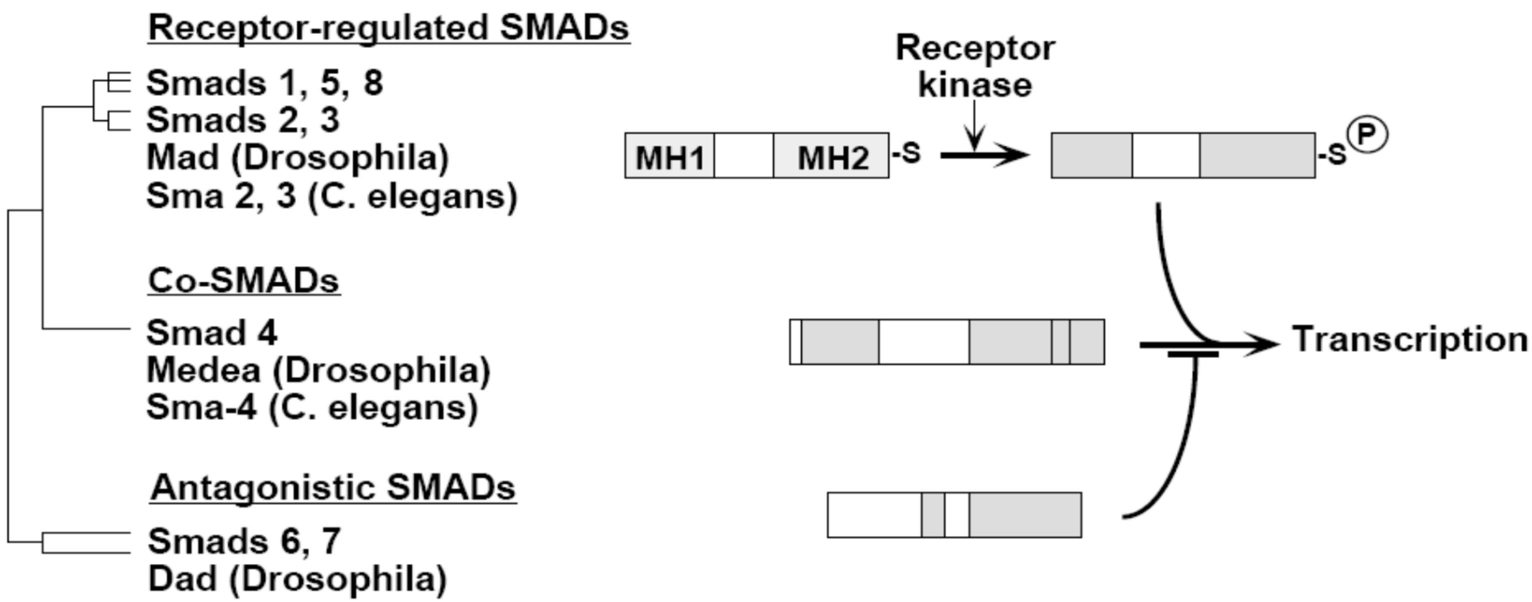
Massague J. Annu. Rev. Biochem. 1998. 67: 753-91

R-SMAD



Annu. Rev. Cell. Dev. Biol. 2005. 21: 659-93

SMAD

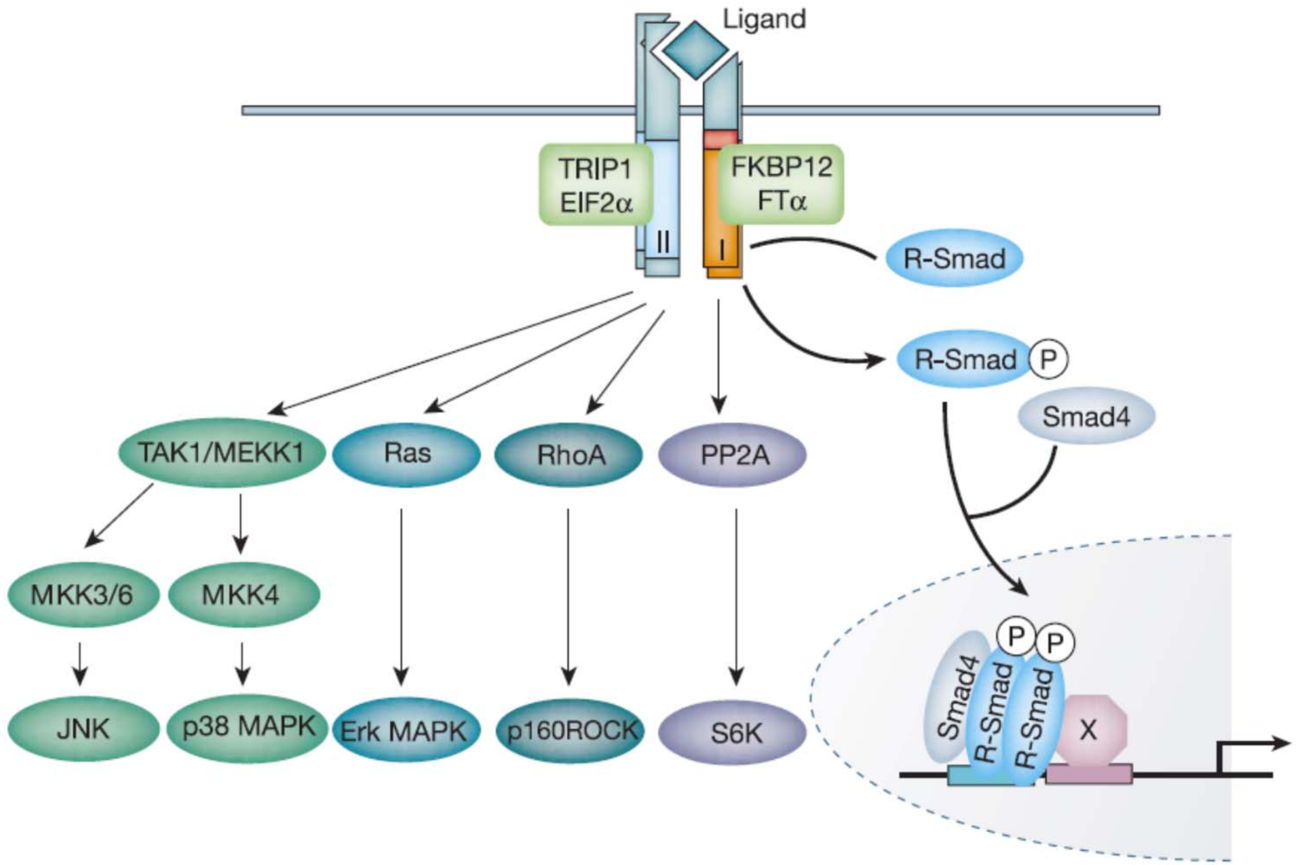


Massague J. Annu. Rev. Biochem. 1998. 67: 753-91

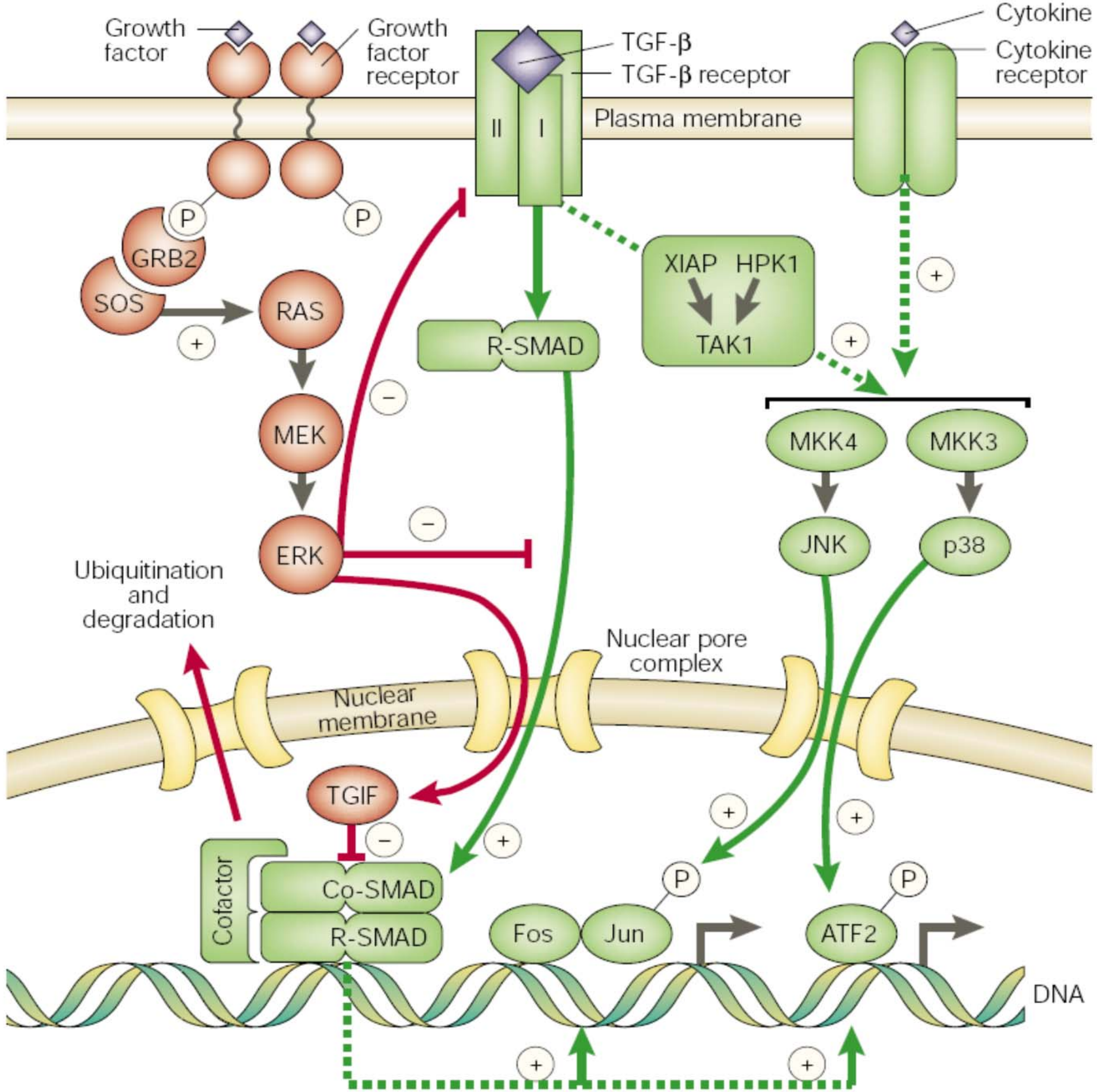
Transforming growth factor-β

- Přenos signálu a jeho regulace
  - receptory
  - sekundární přenašeči
  - „alternativní“ dráhy
  - regulace genové exprese

Přenos signálu nezávislý na SMAD

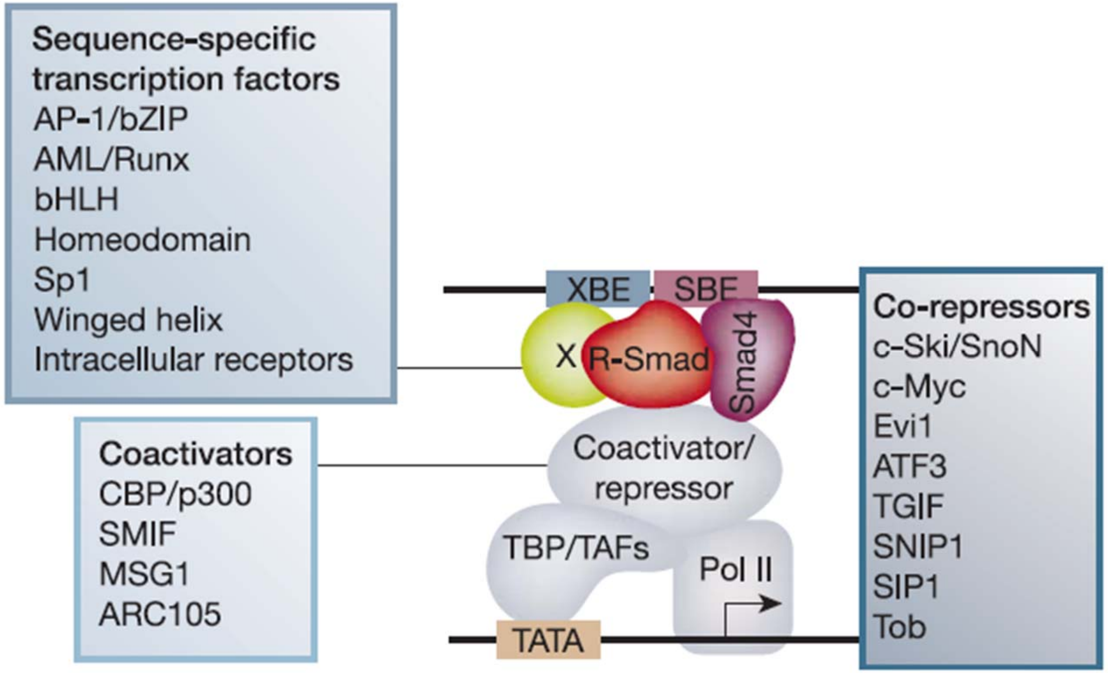


Nature 425 (2003) 577-584



Massague J. Nat. Rev. Mol. Biol. 2000

Regulace transkripce



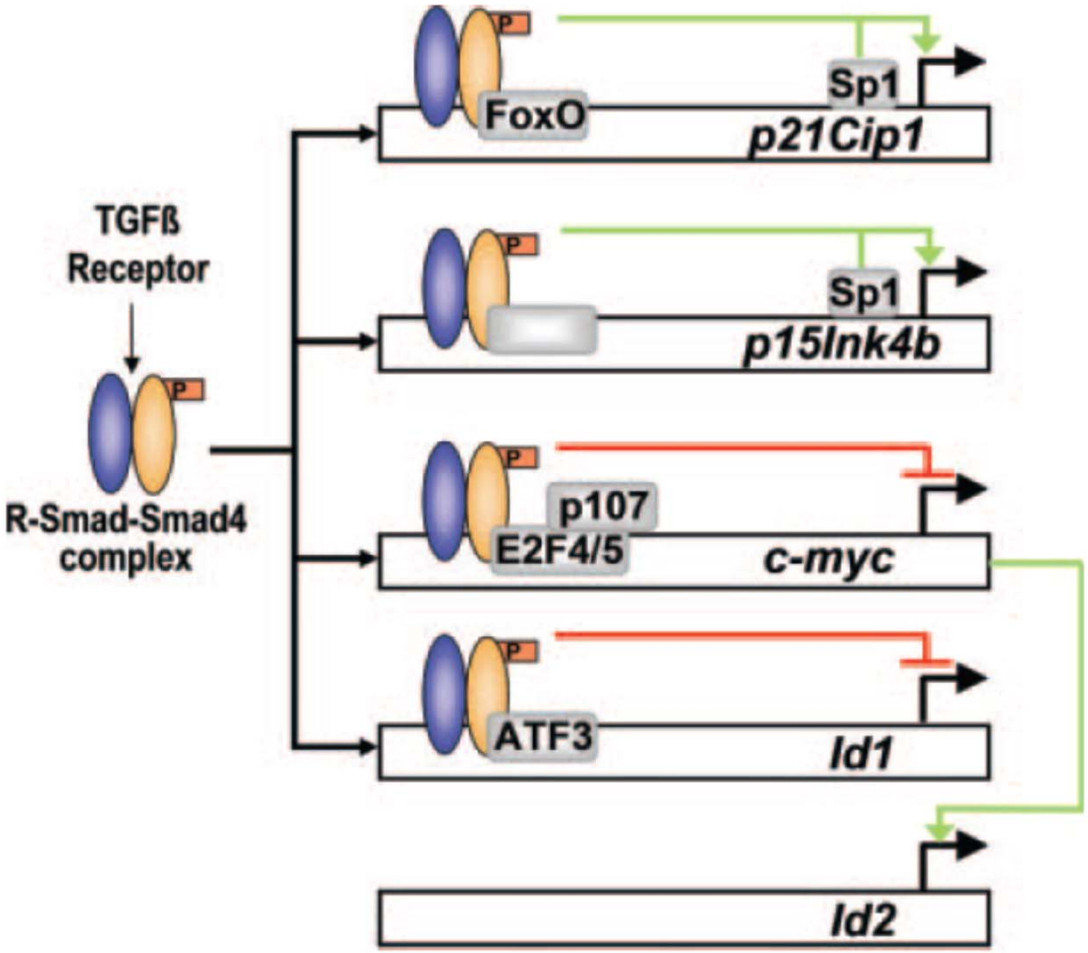
Nature 425 (2003) 577-584

Kofaktory

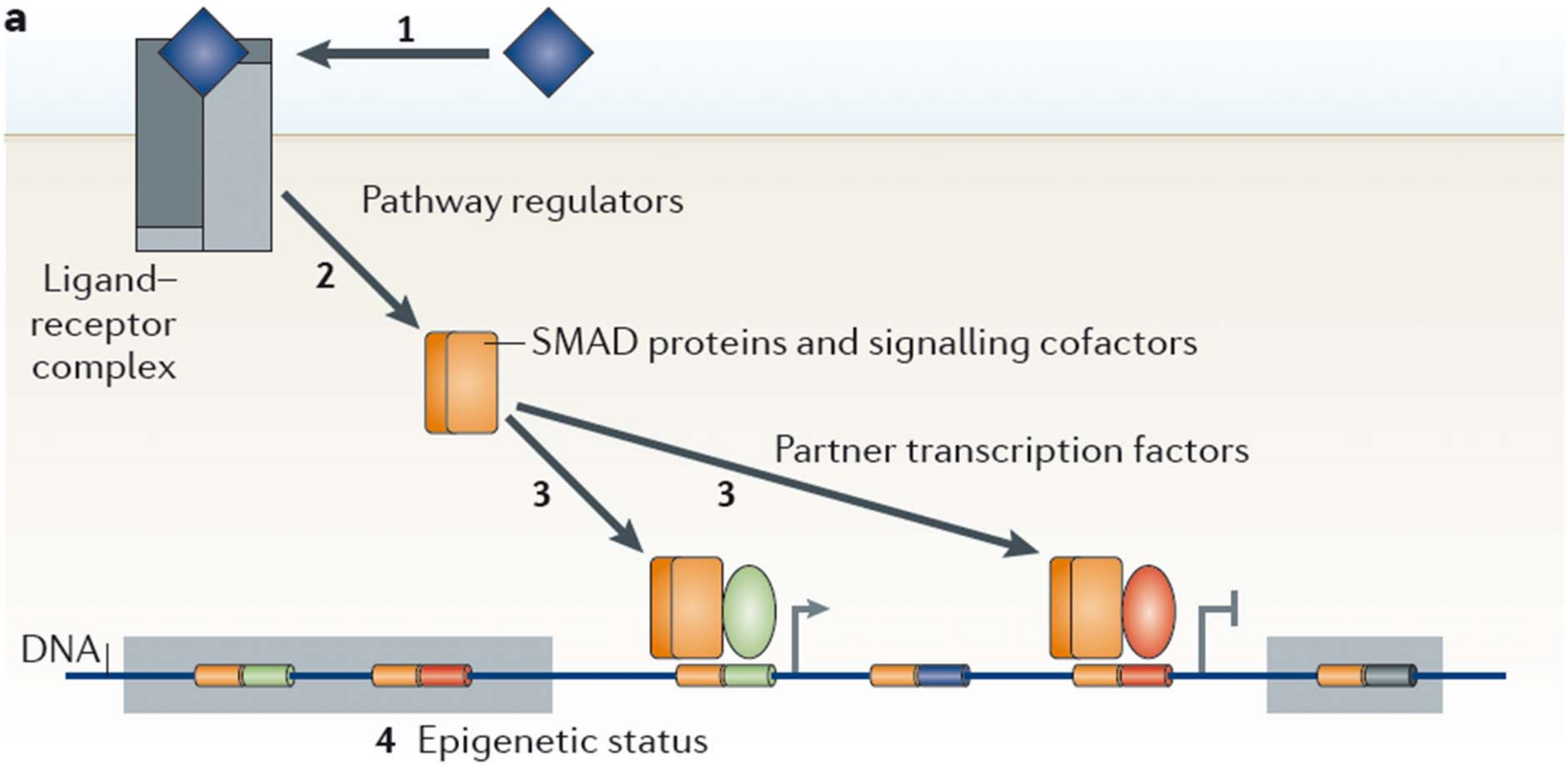
Inputs	SMAD	Cofactor	Target	Function
BMP, DPP inputs	SMAD1, MAD	OAZ	*Vent.2*	Ventral mesoderm specification by BMP in *Xenopus*
		CBFA1?	*Osteocalcin?*	Osteoblast differentiation by BMP in human, mouse
		Tinman	*Tinman*	Visceral mesoderm formation by Dpp in *Drosophila*
		CREB	*Ubx*	Endoderm formation by Dpp in *Drosophila*
TGF-β, Nodal, Activin inputs	SMAD2,3	FAST	*Mix.2*	Mesoderm specification by Nodal in *Xenopus*
			Nodal, Lefty2	Left plate mesoderm formation by Nodal in mouse
		Mixer	*Goosecoid*	Anterior mesoderm induction by Nodal in mouse
		TFE3	*PAI-1*	Plasminogen system control by TGF-β in human, mouse
		CBFA3	*IgA*	Immunoglobulin A class switching by TGF-β in human
		Jun	*c-Fos*	Diverse TGF-β responses
		Lef1/TCF	*Xtwn*	Mesoendoderm differentiation by Nodal in *Xenopus*

Massague J. Nat. Rev. Mol. Biol. 2000

Inhibice proliferace u epiteliálních buněk



Carcinogenesis vol.27 no.11 pp.2148–2156, 2006 a



b

Determinants

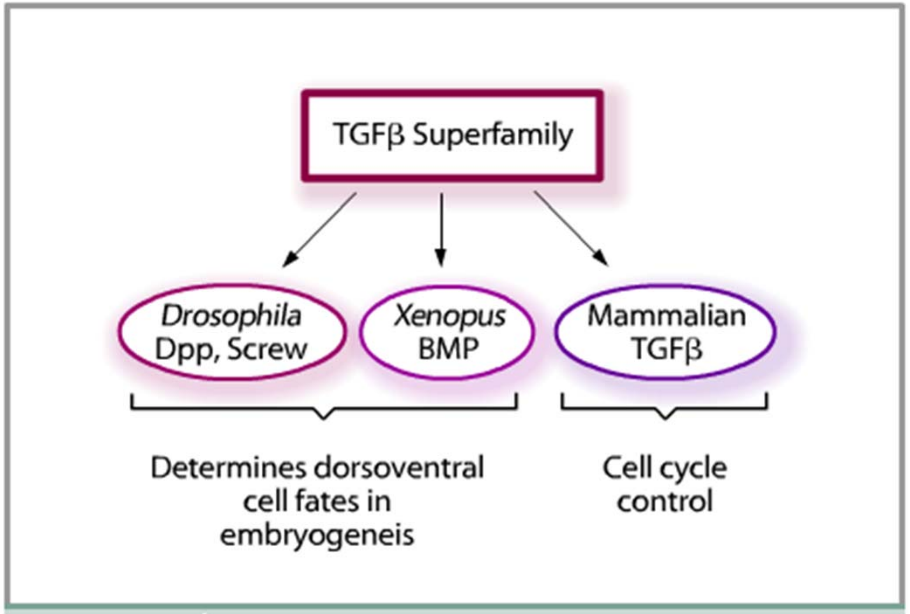
Signal transduction	Transcription	Epigenetic status
Ligand isoforms	Pluripotency factors	Heterochromatin
Ligand traps	Lineage regulators	Pluripotency marks
Co-receptors	DNA-binding cofactors	Lineage marks
Receptor subtypes	HATs and HDACs	EMT marks
Inhibitory SMAD proteins	SWI/SNF	iPS cell marks
Crosstalk inputs	Chromatin readers	Oncogenic marks

TGFβ signalling in context

Joan Massagué

OCTOBER 2012 | VOLUME 13 NATURE REVIEWS | MOLECULAR CELL BIOLOGY

Signálová transdukce TGF-β rodiny



Shrnutí přednášky I.

- TGF-β rodina zahrnuje řadu multifunkčních proteinů.
- Přenos signálu je intracelulárně přenášen SMAD proteiny, ale interaguje s řadou dalších signálních drah.
- Působení TGF-β je závislé na buněčném typu a také přítomnosti dalších faktorů.

Na konci dnešní přednášky byste měli:

1. rozumět základním principům přenosu signálu;
2. znát základní zástupce a funkce proteinů TGF-β rodiny;
3. umět popsat přenos signálu který závisí na SMAD.

Transformující růstový faktor – β: rozmanitost přenosu signálu a funkce část 2.

Karel Souček

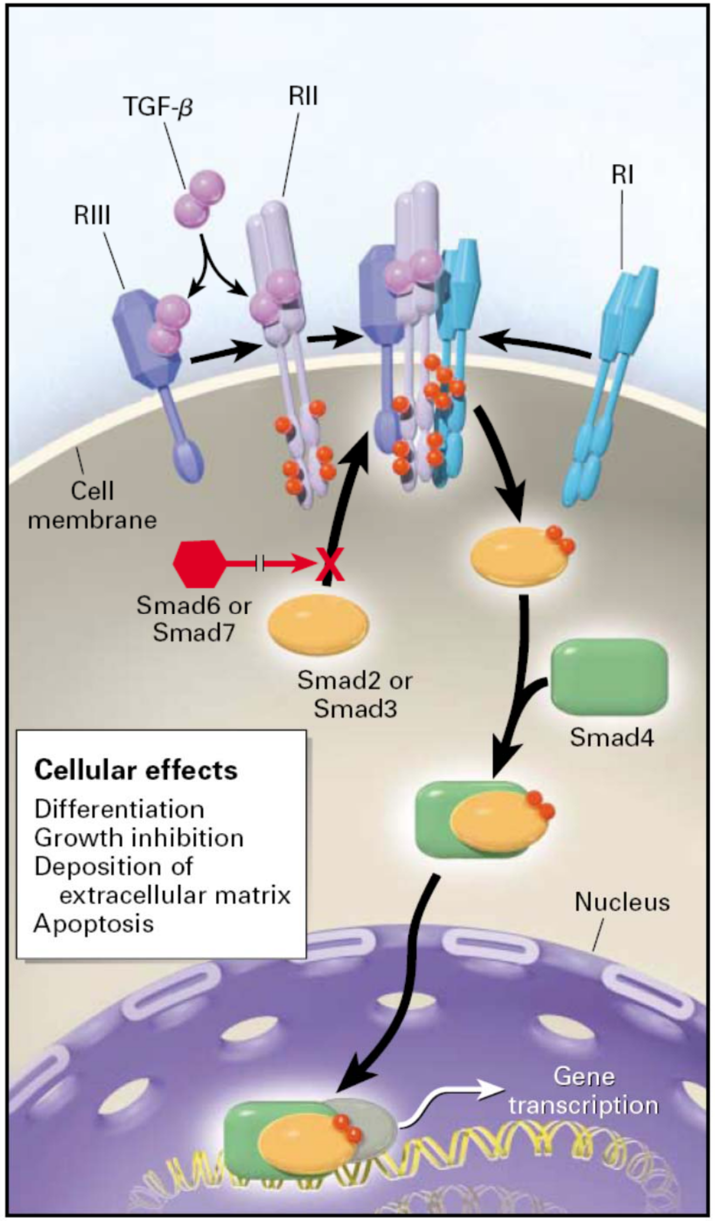
Bi6051 Molekulární fyziologie živočichů

Transforming growth factor -β (TGF-β)

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

TGF-β_1

- pleiotropní cytokin
- negativní regulátor



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

Growth factors in cancer cell signaling

- cancer **is not** single cell disease;
- **tissue microenvironment** plays an important role in tumor initiation and progression;
- **growth factors - cytokines** - play crucial role in cancer development and some of them belong to the **significant autocrine/paracrine factors** produced by various cell types in tumor microenvironment;
- modulation of their signal transduction represent potential target for therapy.

Growth factors in cancer cell signaling

- What is a role of TGF-β family cytokines in cancer progression?
- How we can effectively modify pathological plasticity of the cancer cells?

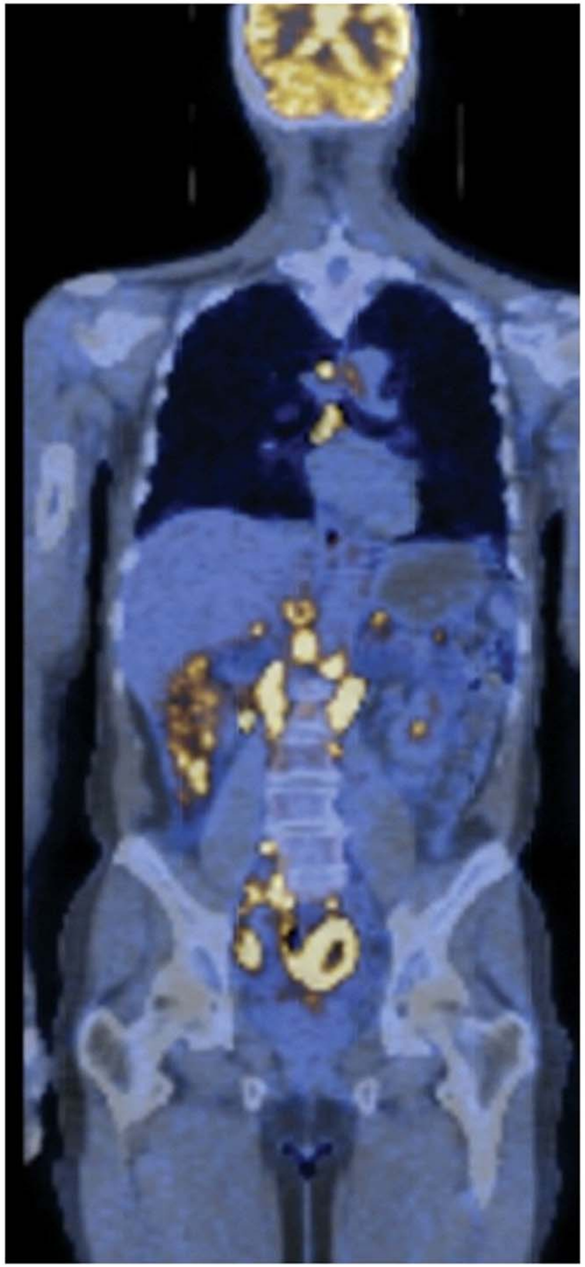


Figure 14.1 *The Biology of Cancer* (© Garland Science 2007)

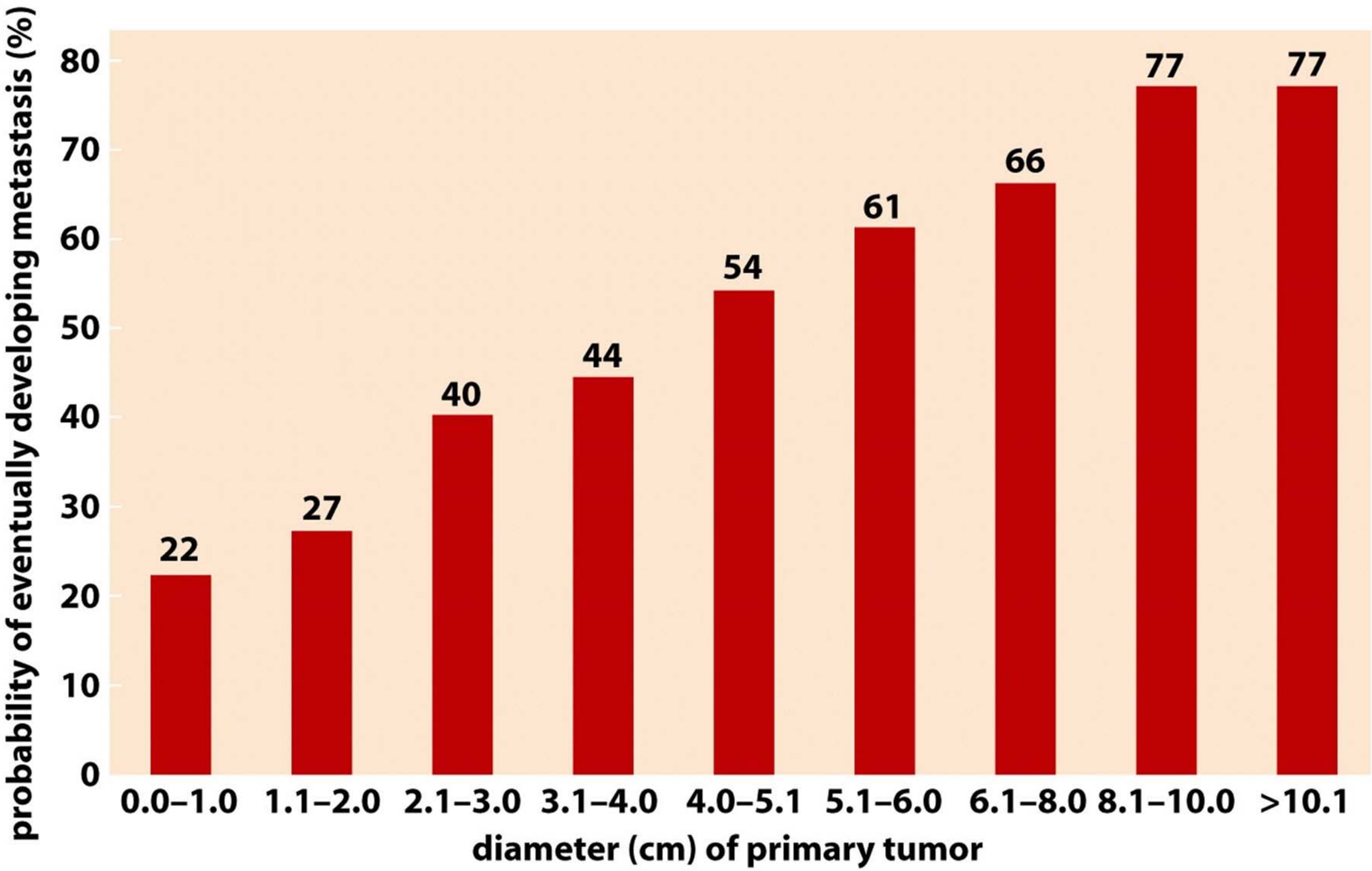


Figure 14.3 *The Biology of Cancer* (© Garland Science 2007)

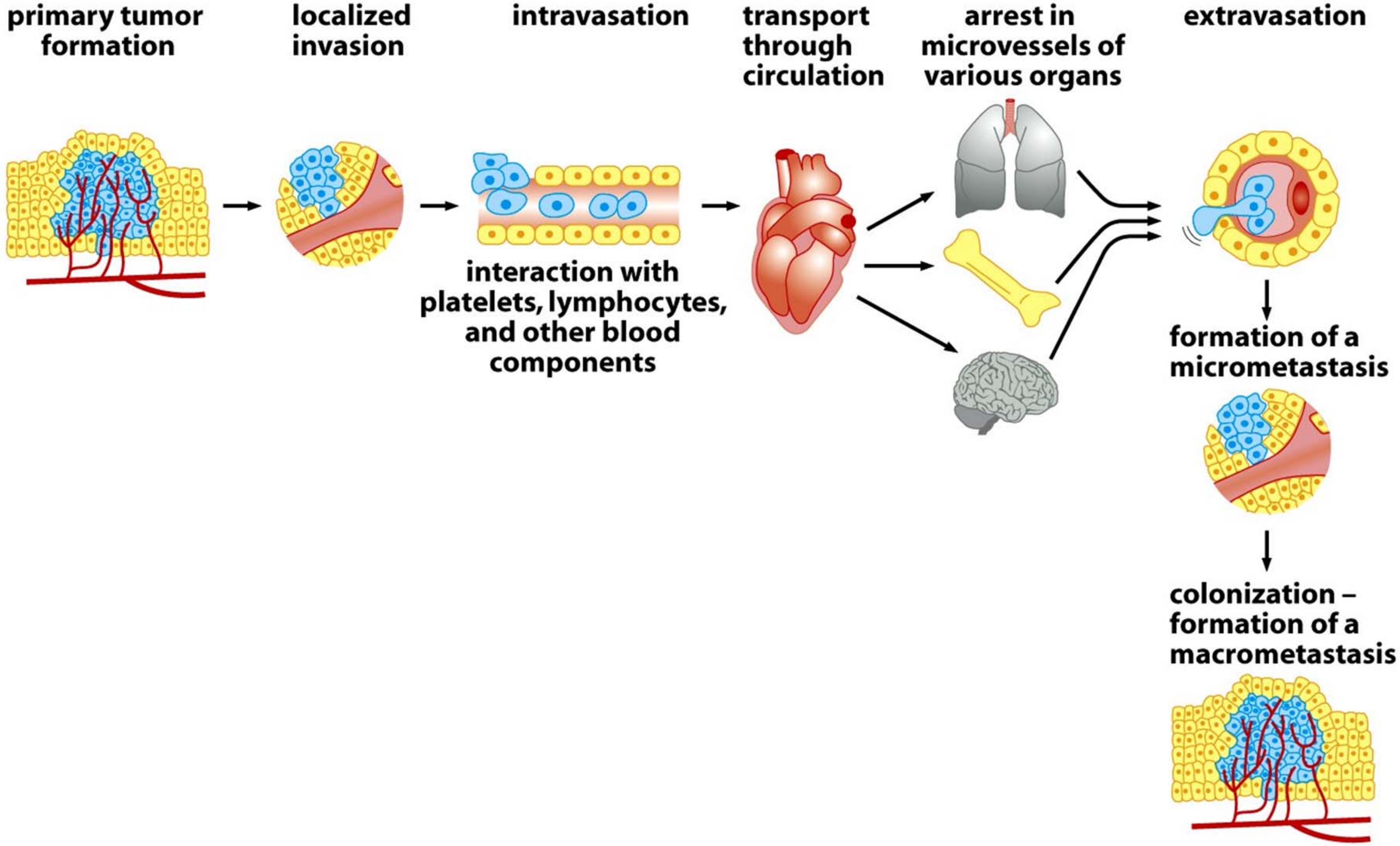
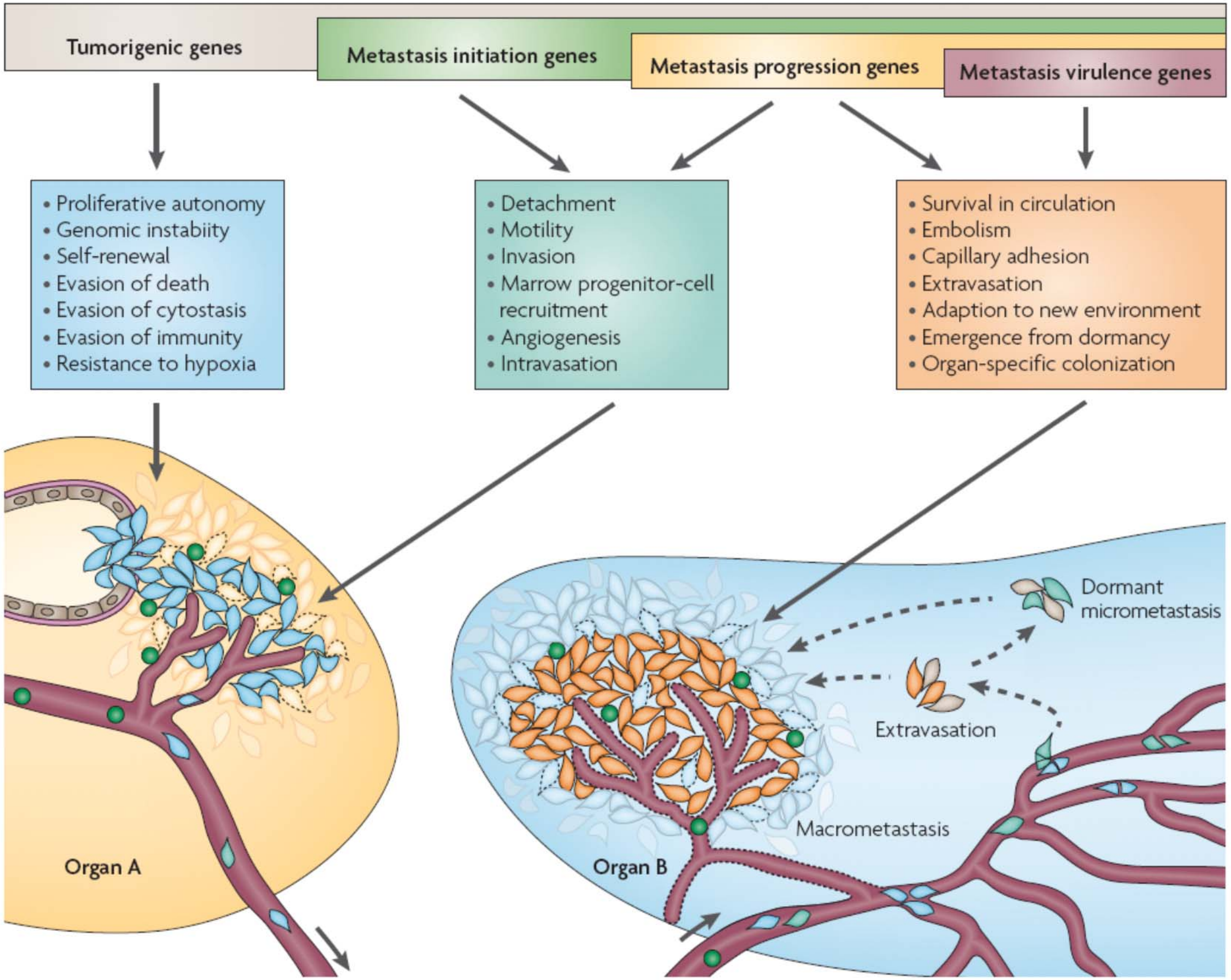


Figure 14.4 *The Biology of Cancer* (© Garland Science 2007)

Genetic determinants of cancer metastasis

Don X. Nguyen and Joan Massagué



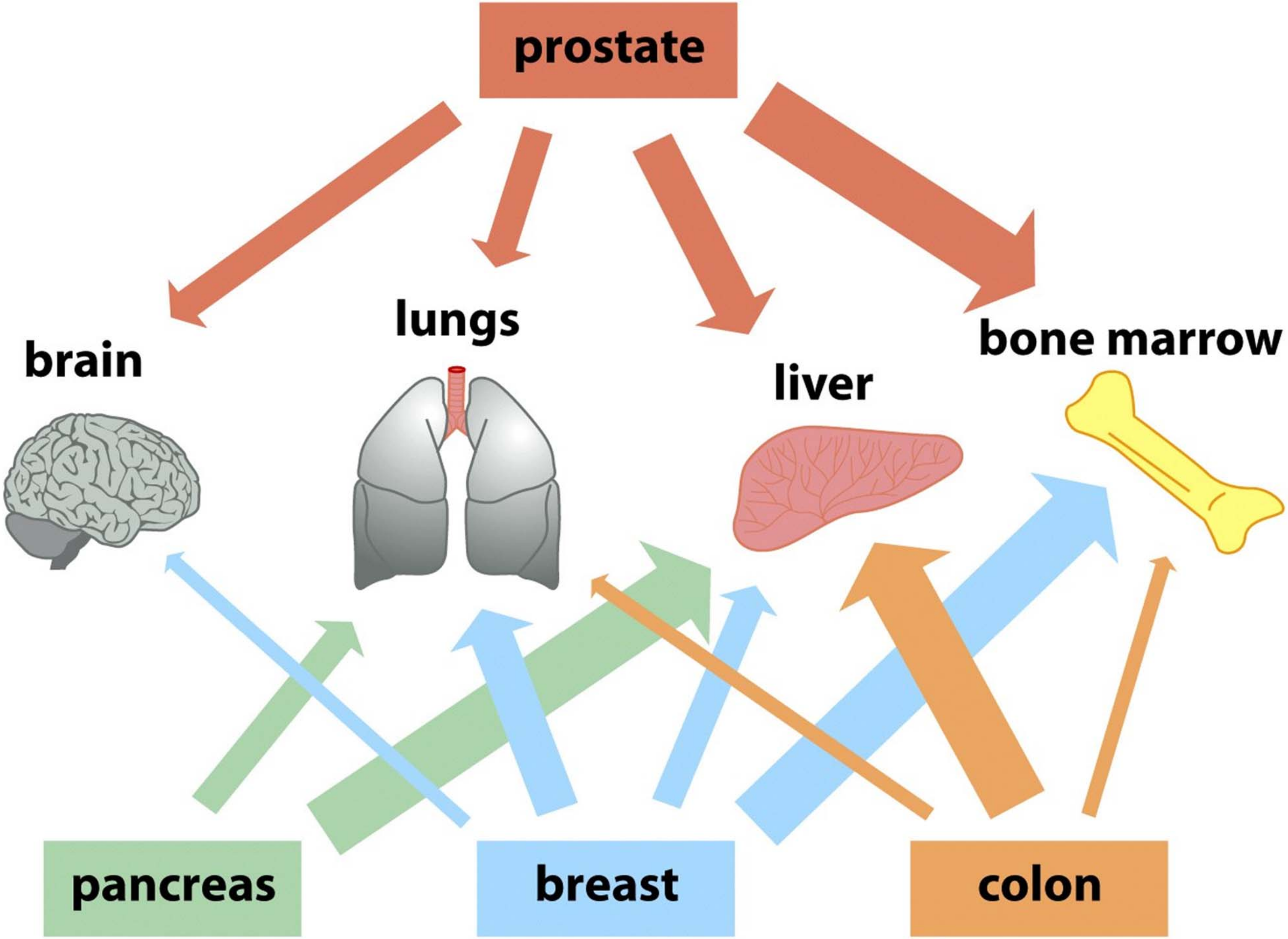
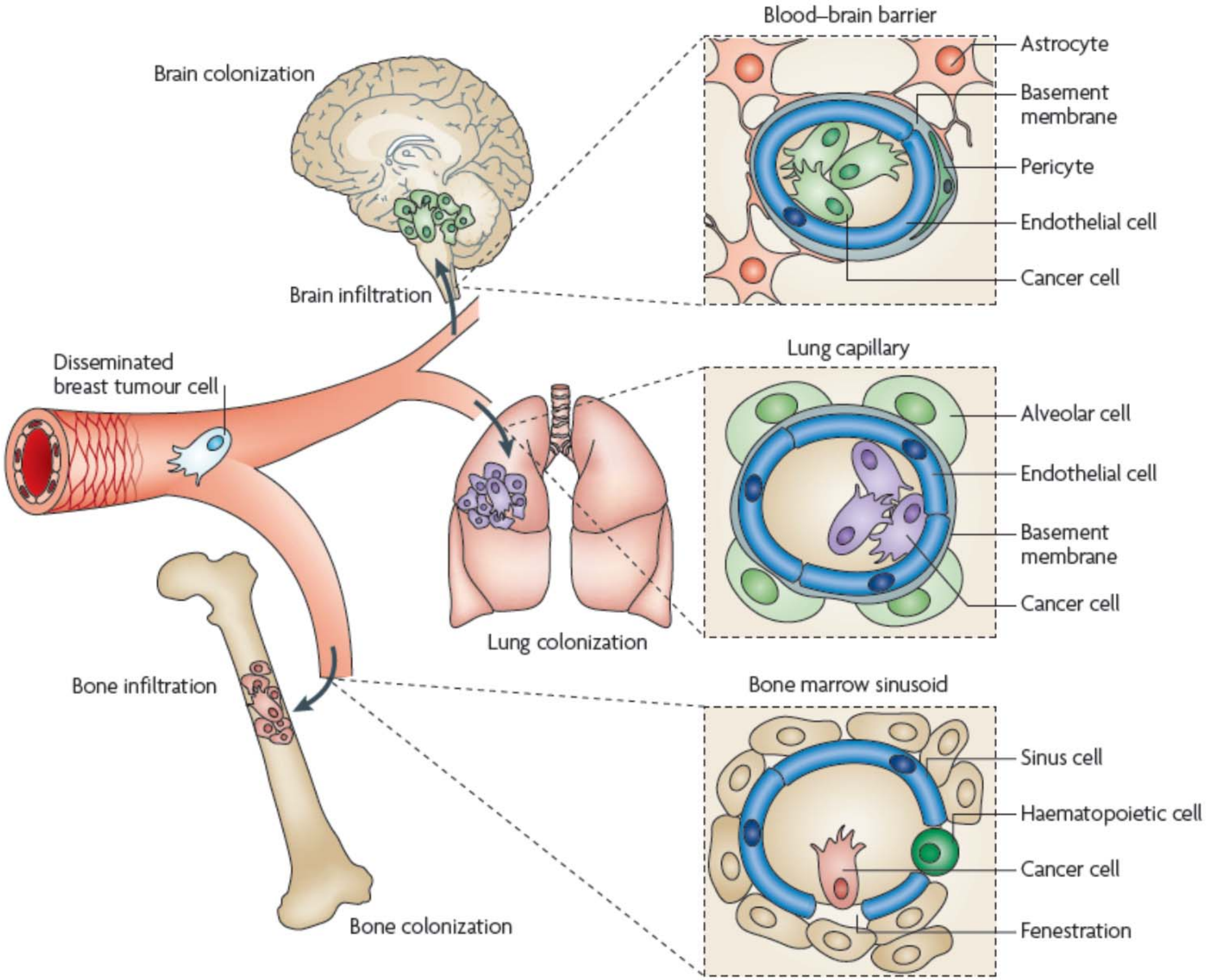


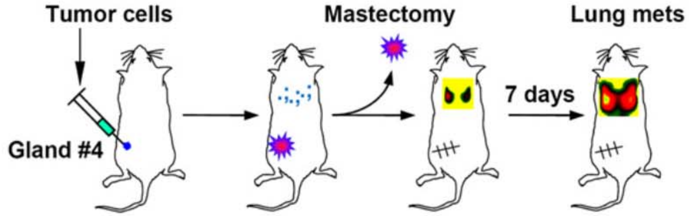
Figure 14.42 *The Biology of Cancer* (© Garland Science 2007)

Organ-specific barriers



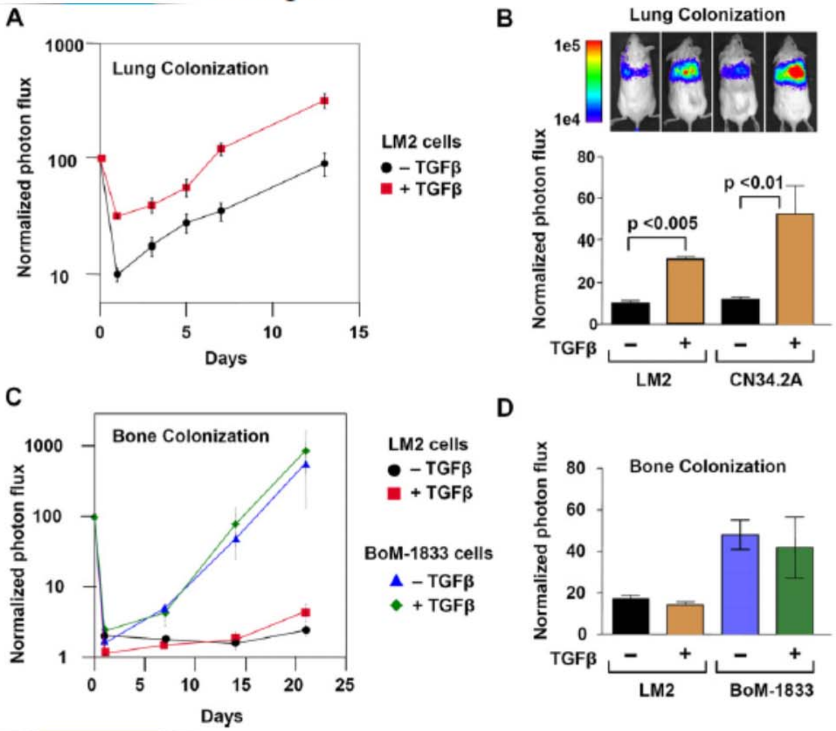
NATURE REVIEWS | CANCER

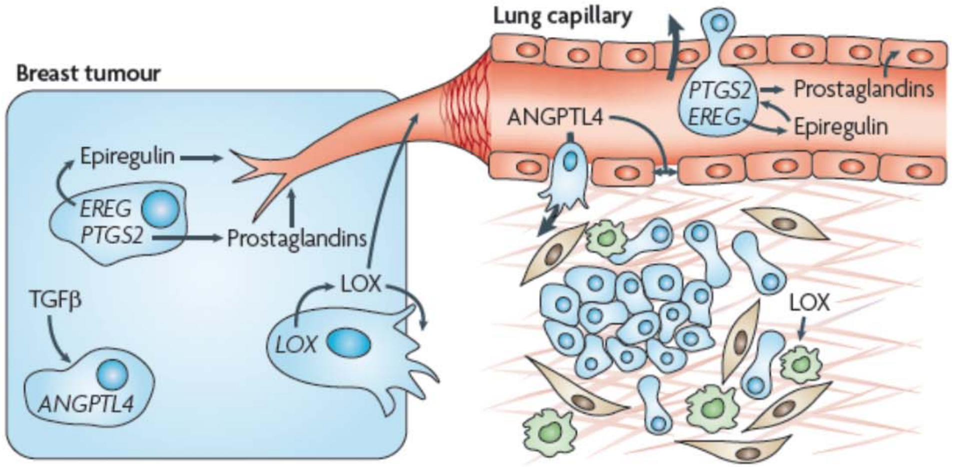
Cell 133, 66–77, April 4, 2008



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

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





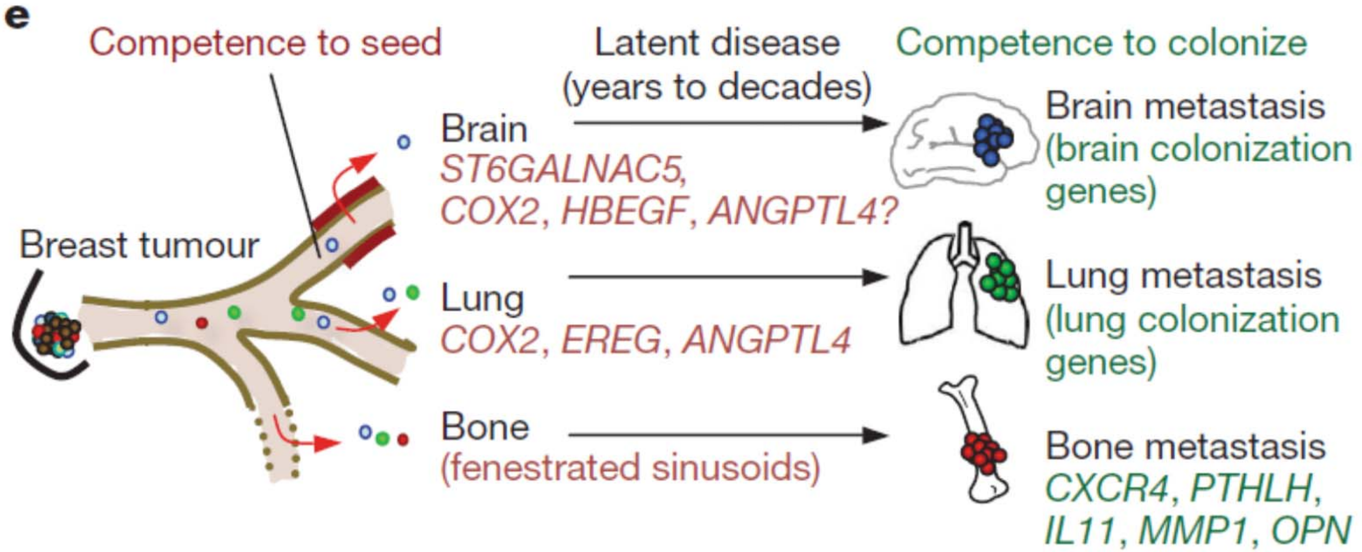
Vol 459 | 18 June 2009 | doi:10.1038/nature08021

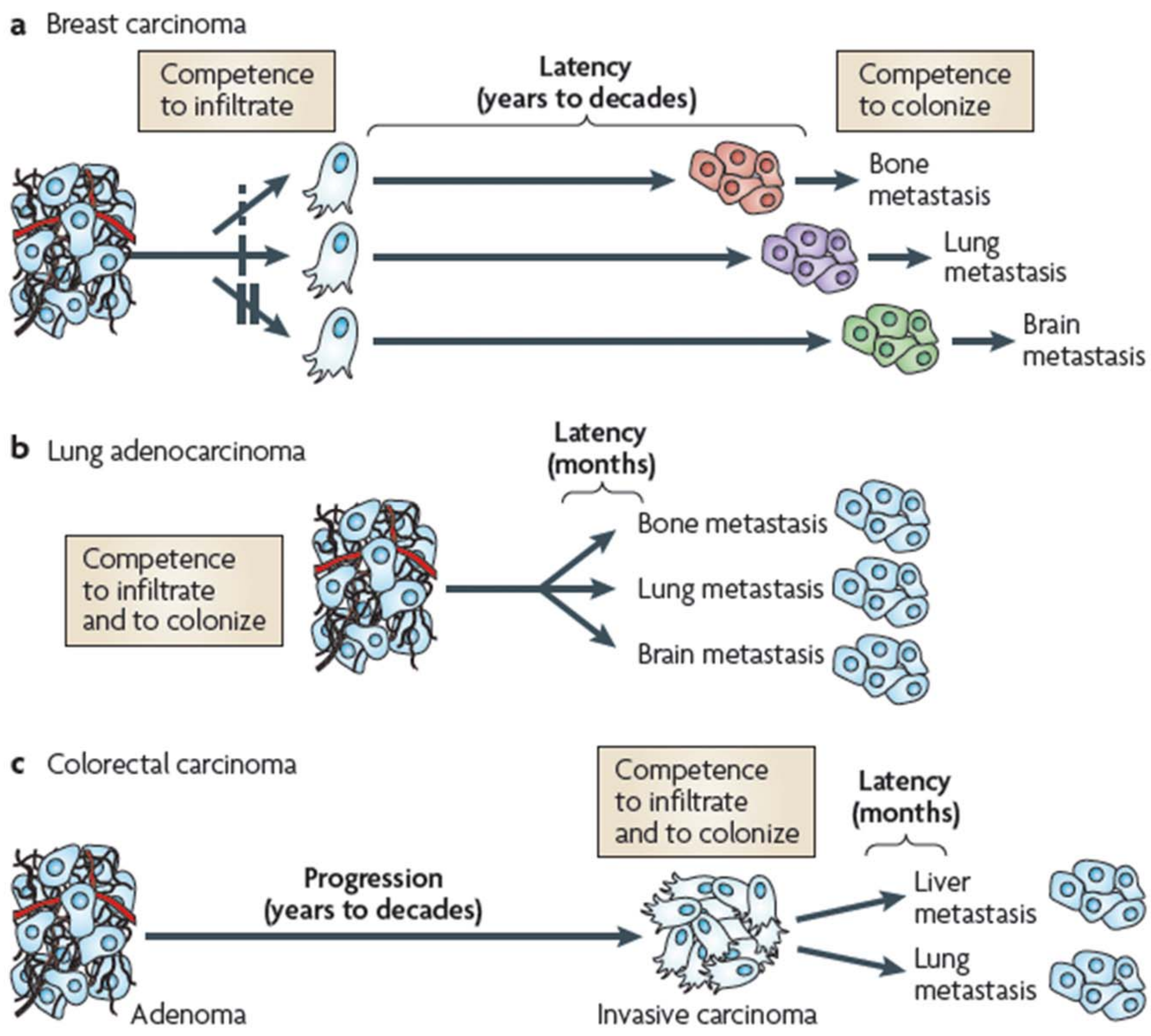
nature

LETTERS

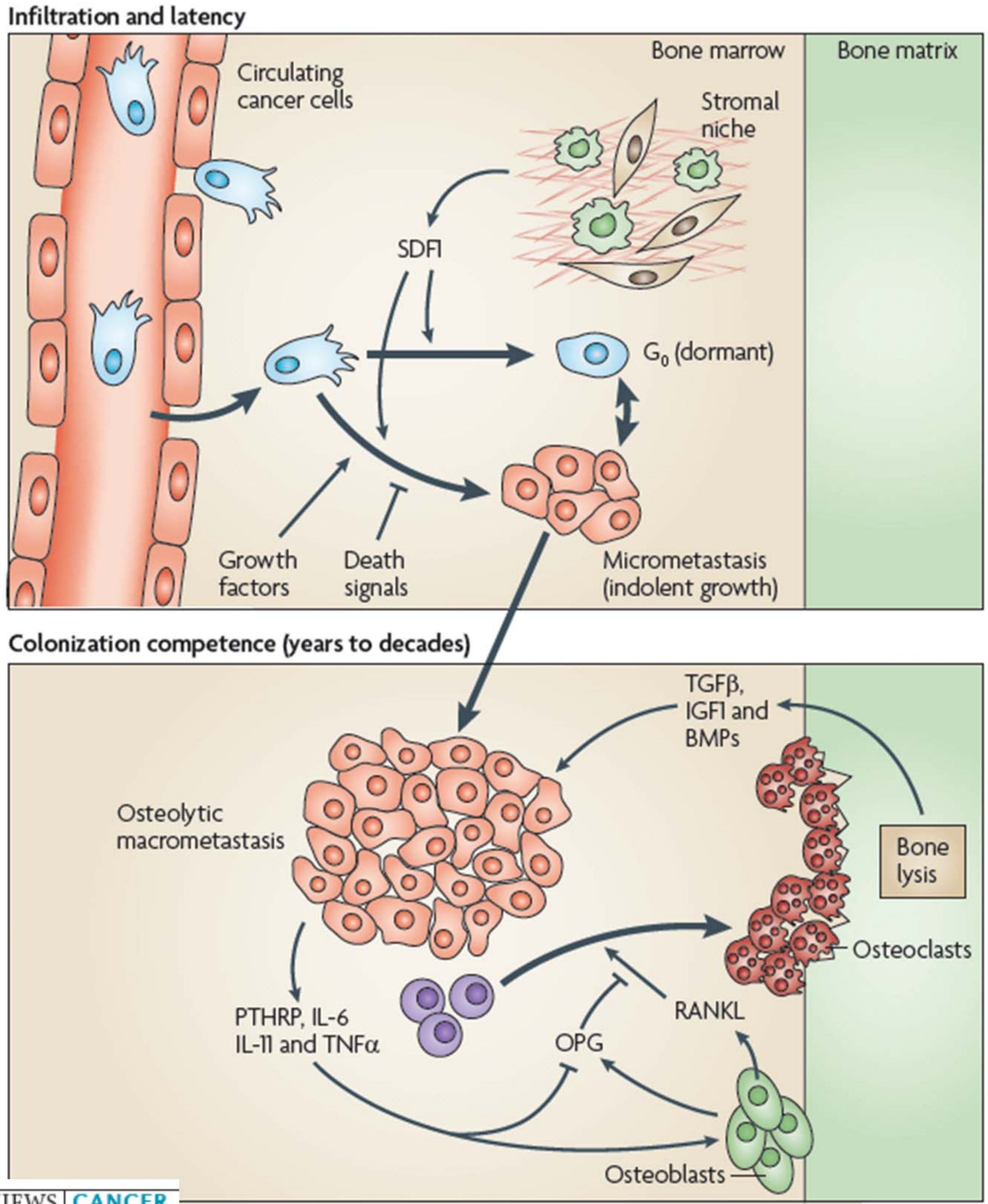
Genes that mediate breast cancer metastasis to the brain

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





NATURE REVIEWS | CANCER



Infiltration and latency
Bone marrow
Bone matrix
Circulating cancer cells
Stromal niche
SDF1
G0 (dormant)
Growth factors
Death signals
Micrometastasis (indolent growth)
Colonization competence (years to decades)
TGFβ, IGF1 and BMPs
Osteolytic macrometastasis
Bone lysis
Osteoclasts
PTHRP, IL-6 IL-11 and TNFα
RANKL
OPG
Osteoblasts

Transforming growth factor-β

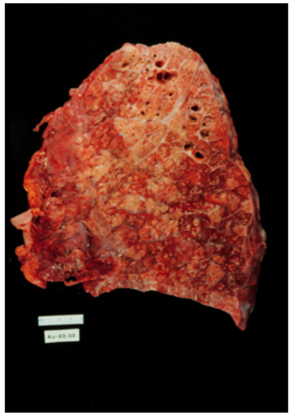
- Role v rozvoji patologických stavů

Biologické funkce TGF-β

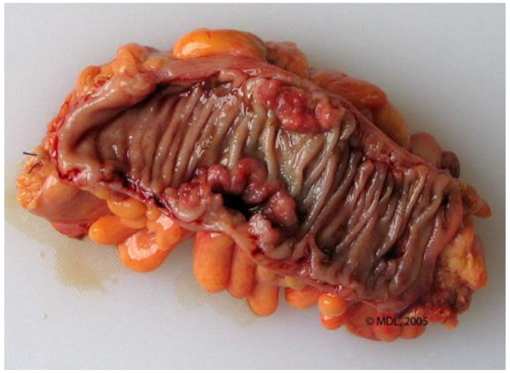
- Hraje klíčovou úlohu během embryogeneze;
- reguluje proliferaci, diferenciaci, buněčnou smrt, motilitu, adhezi (v závislosti na buněčném typu) = ovlivňuje homeostázu;
- reguluje expresi extracelulární matrix;
  - indukuje fibrilární kolagen a fibronectin;
  - inhibuje degradaci ECM (inhibicí MMPs a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

Role TGF-β v rozvoji patologických stavů

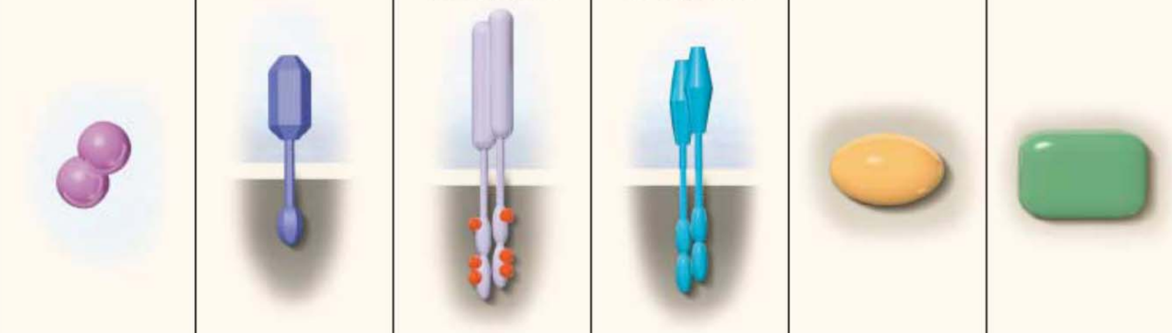
- Fibróza
  - deregulace exprese ECM prostřednictvím indukce proliferace fibroblastů a jejich myofibroblastového fenotypu.



- Nádorová onemocnění
  - ztráta citlivosti epiteliálních buněk k inhibičnímu působeni TGF-β;
  - indukce angiogeneze.



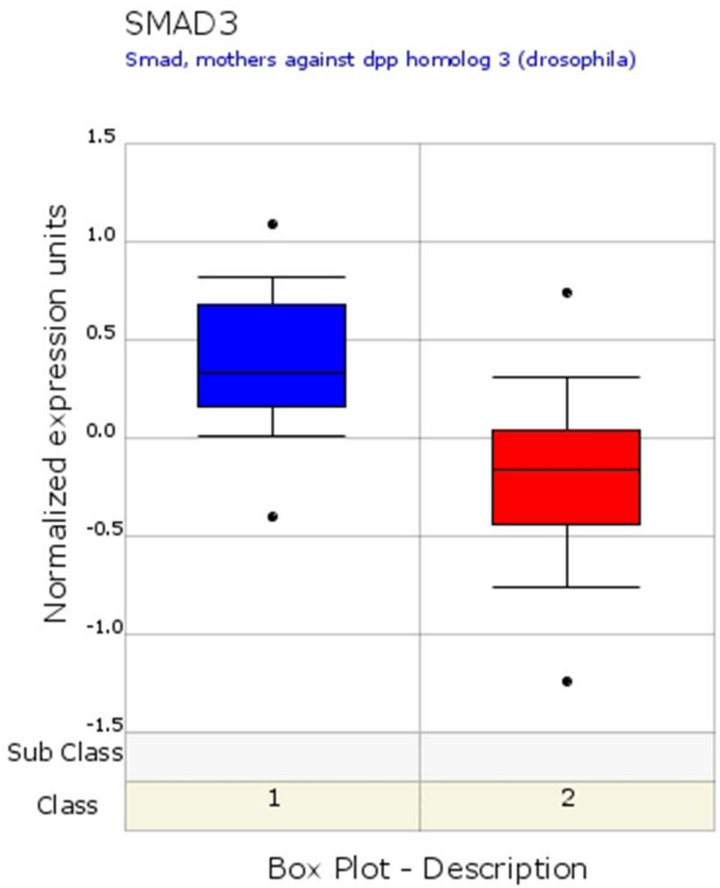
Role TGF-β v carcinogenezi

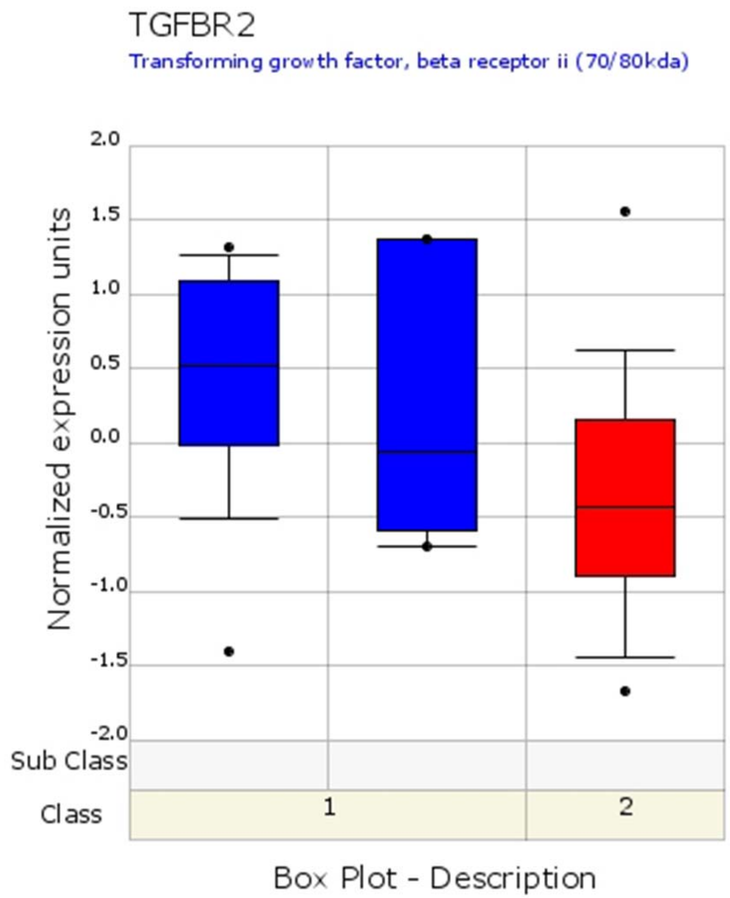


TGF-β signaling component	TGF-β	Endoglin	Type II receptors	Type I receptors	Smad2	Smad4
Cancers (somatic mutations)	Increased expression leads to enhanced invasion and metastasis		Colorectal (30%) Gastric (15%) Endometrial Prostate Breast Lung Hepatic Pancreatic Cervical Glioma Head and neck	Breast (16%)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

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

Role TGF-β v carcinogenezi

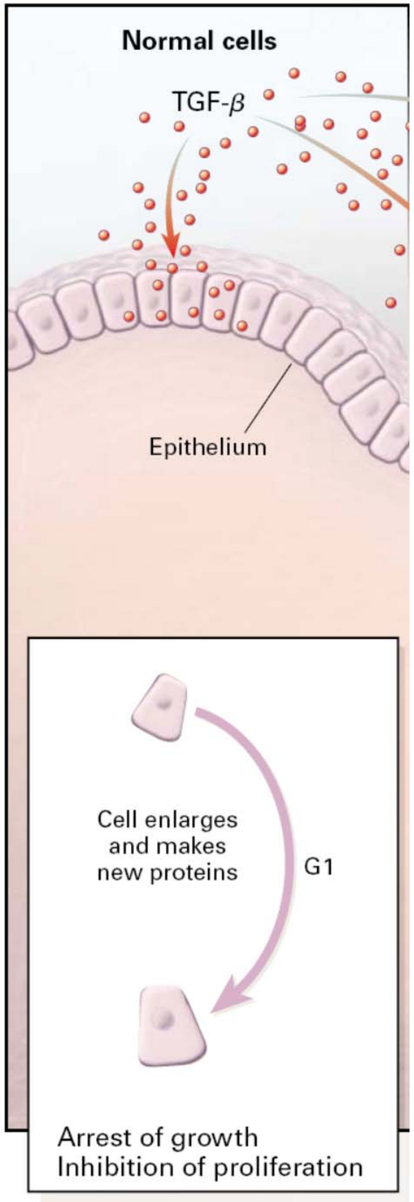




Prostate – normal vs. cancer normal, hyperplasia vs. cancer

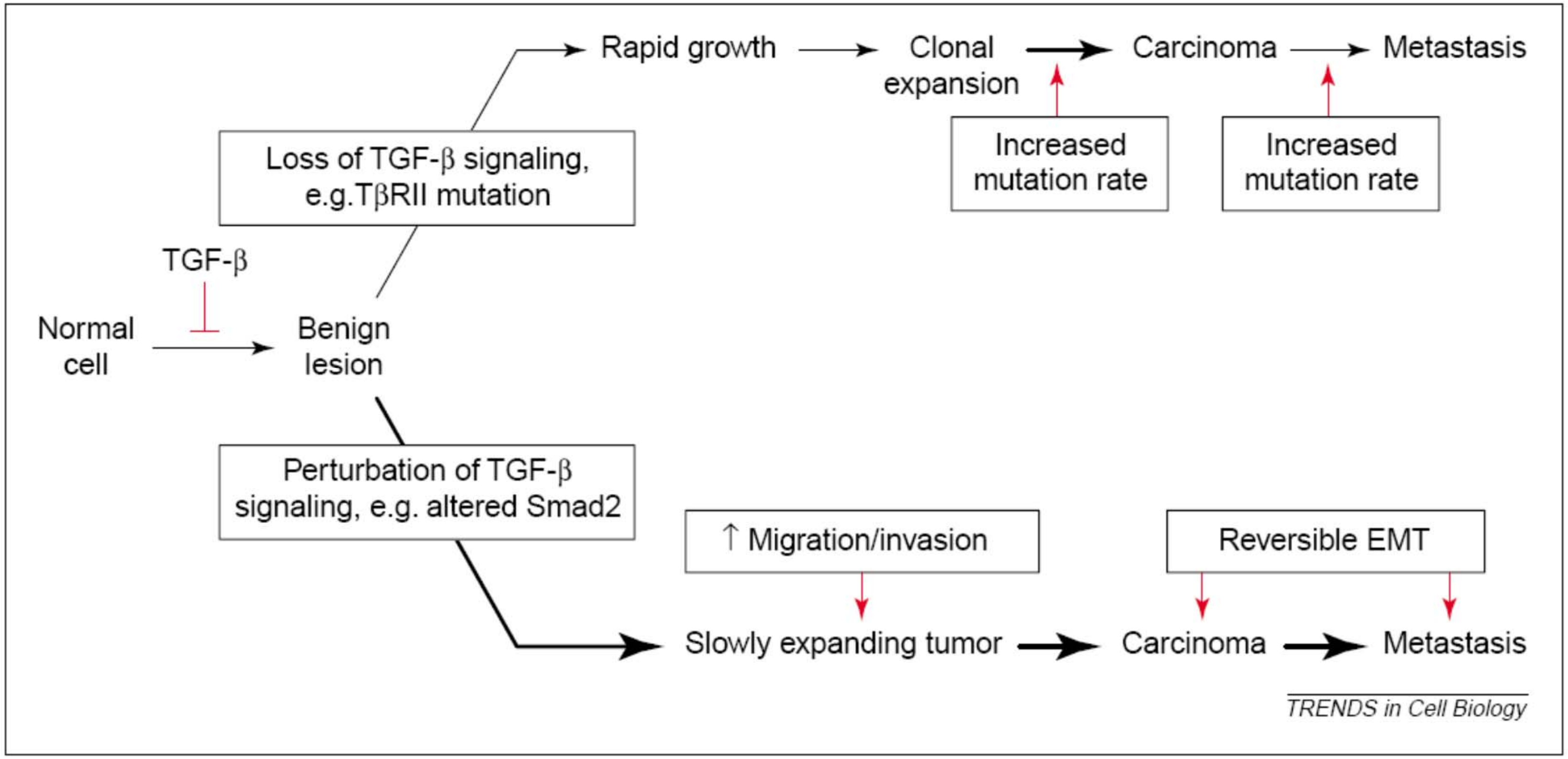
CANCER PROFILING DATABASE ONCOMINE™

Role TGF-β v carcinogenezi



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

Role TGF-β v carcinogenezi



TRENDS in Cell Biology Vol.11 No.11 2001

Epithelial-Mesenchymal Transition (EMT)

- Změna buněčného fenotypu spojená se ztrátou adheze a zvýšením motility

EMT & Cancer

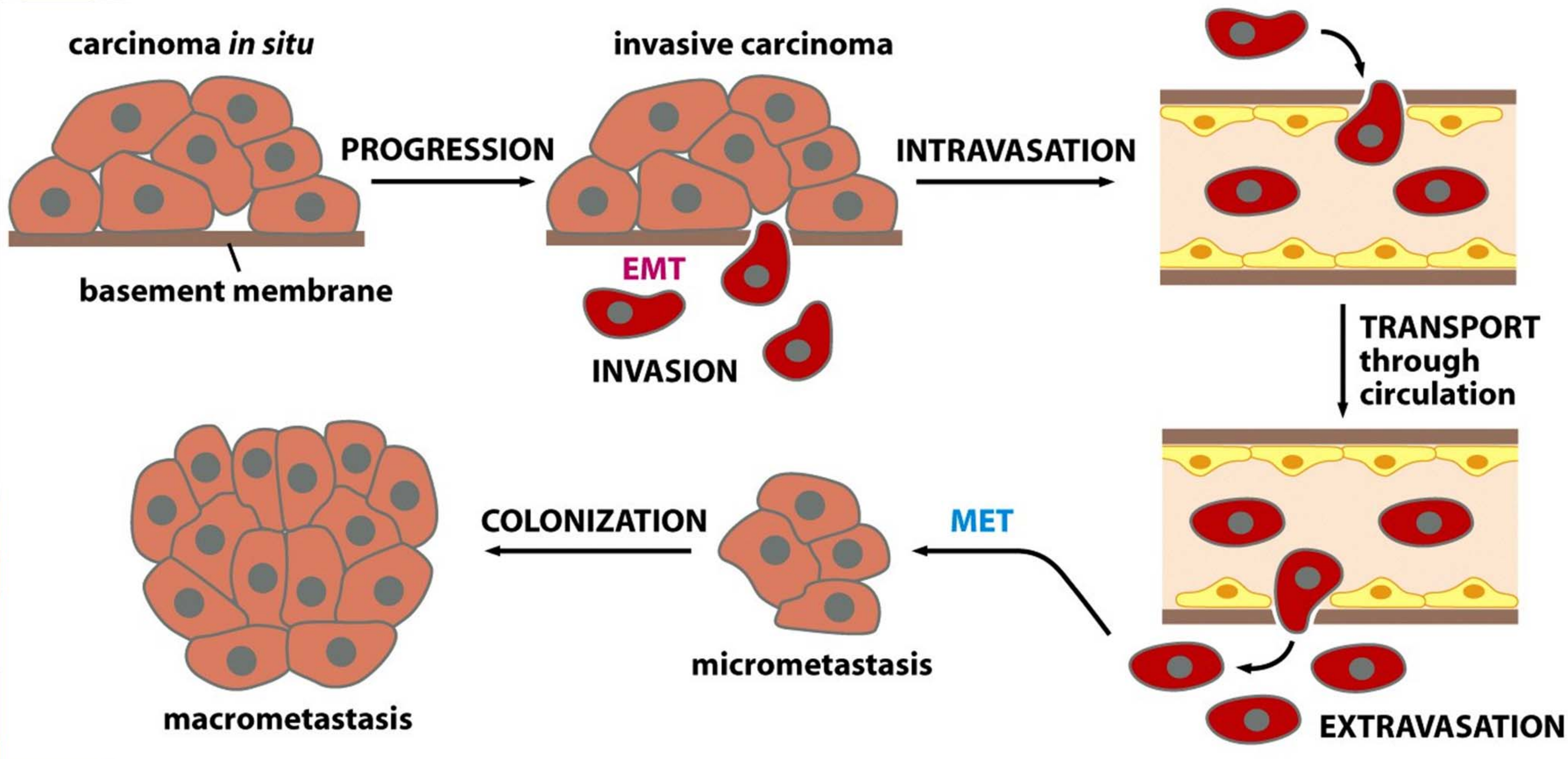
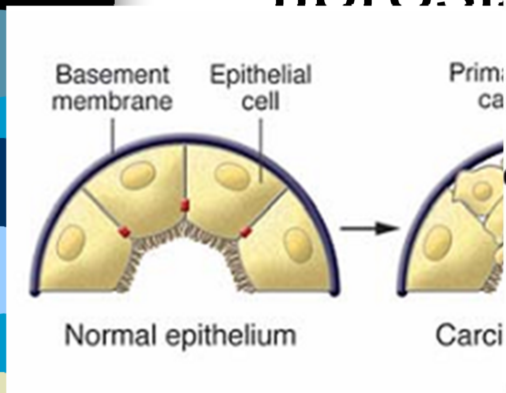
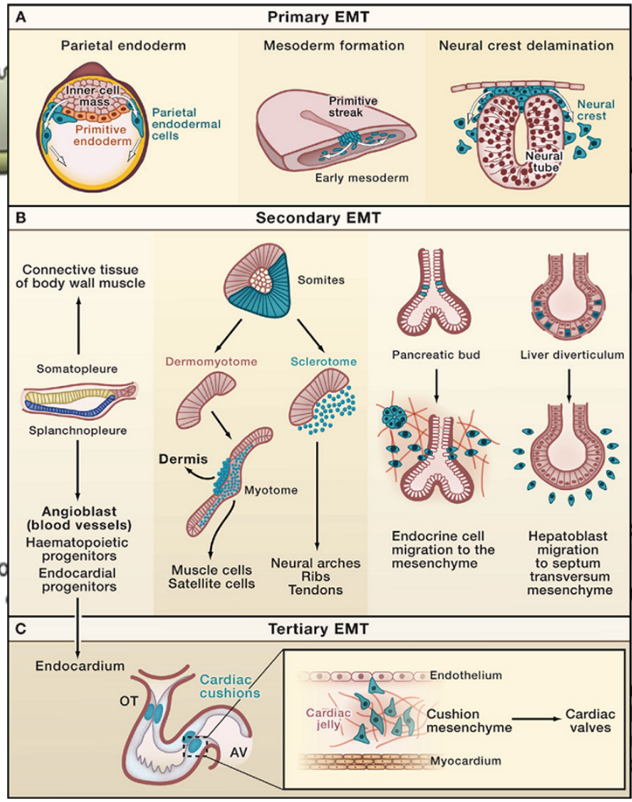


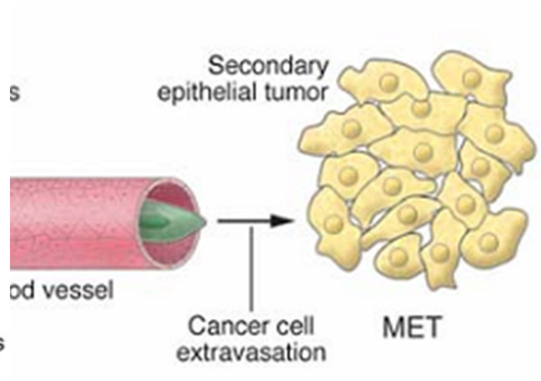
Figure 14.17b *The Biology of Cancer* (© Garland Science 2007)

Epithelial-to-mesenchymal transition (EMT)

- Reversible acquisition of migratory and invasive properties by epithelial cells
- Role ir ment, fibrosi







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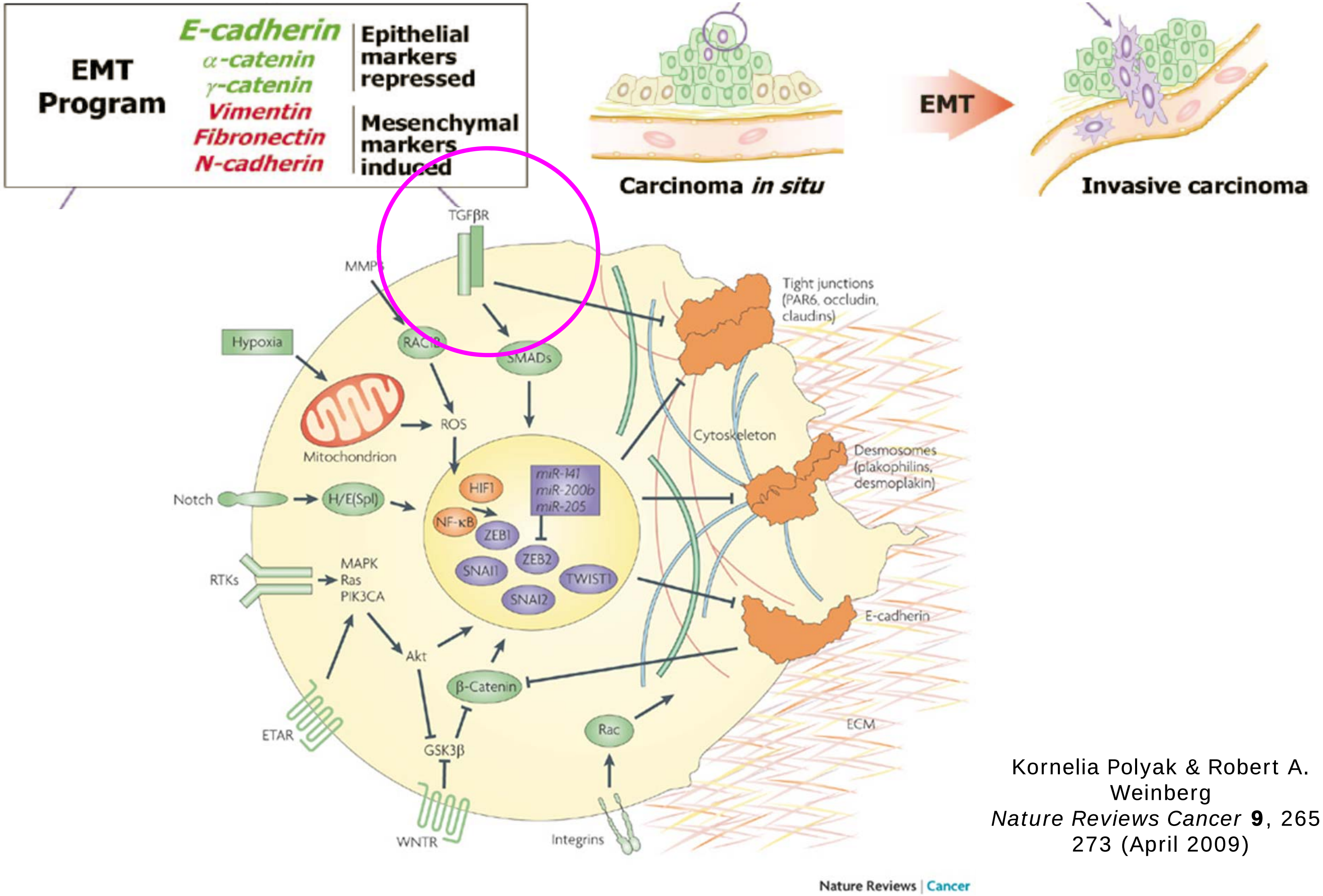
Kalluri, R. and R.A. Weinberg, ...est, 2009. 119(6): p. 1420-8.

...transitions in development and disease. Cell, 2009. 139(5): p. 871-90.

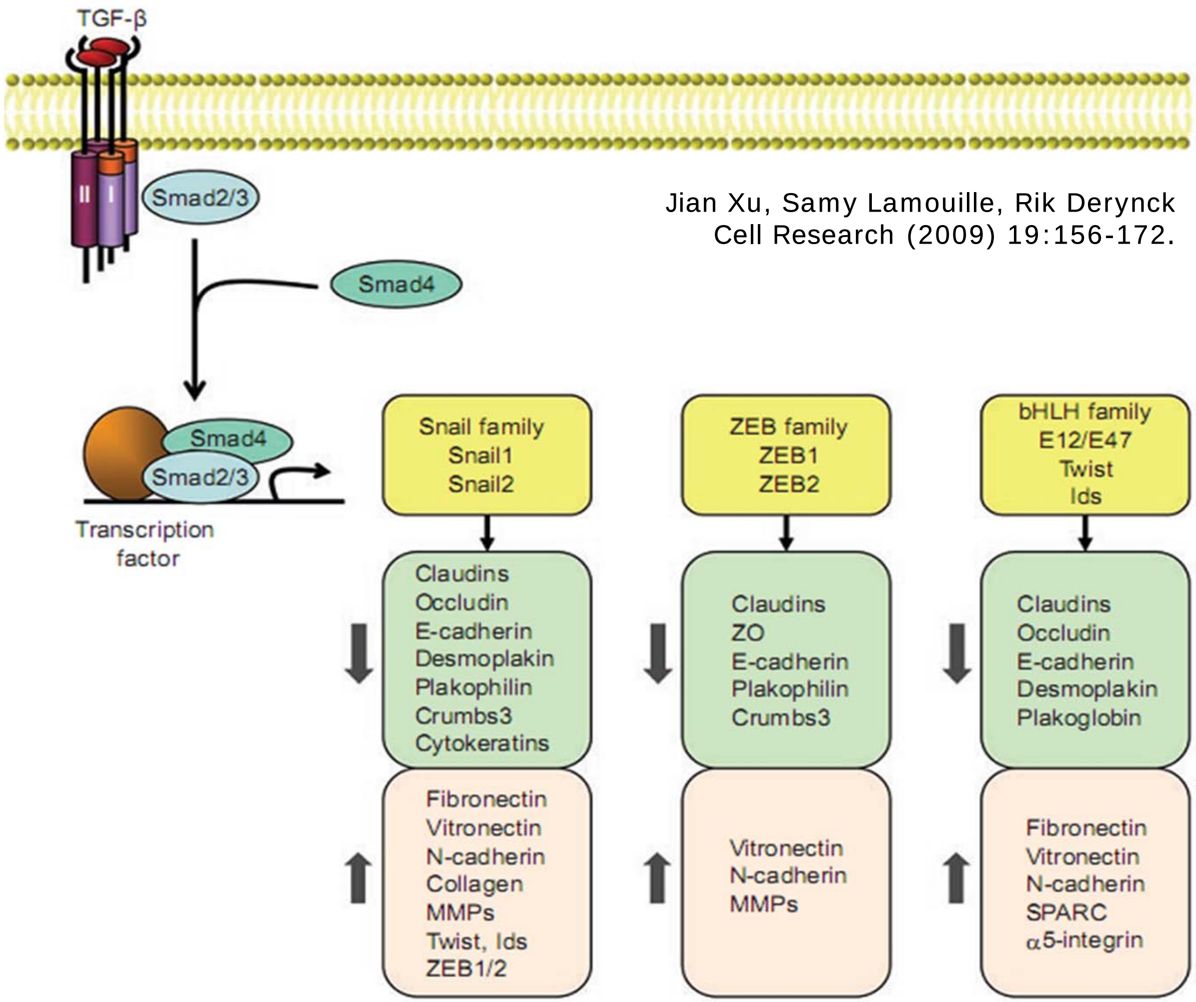
M S Simonson
Kidney International **71**, 846-854 (May (1) 2007)

Markers and regulators of EMT



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

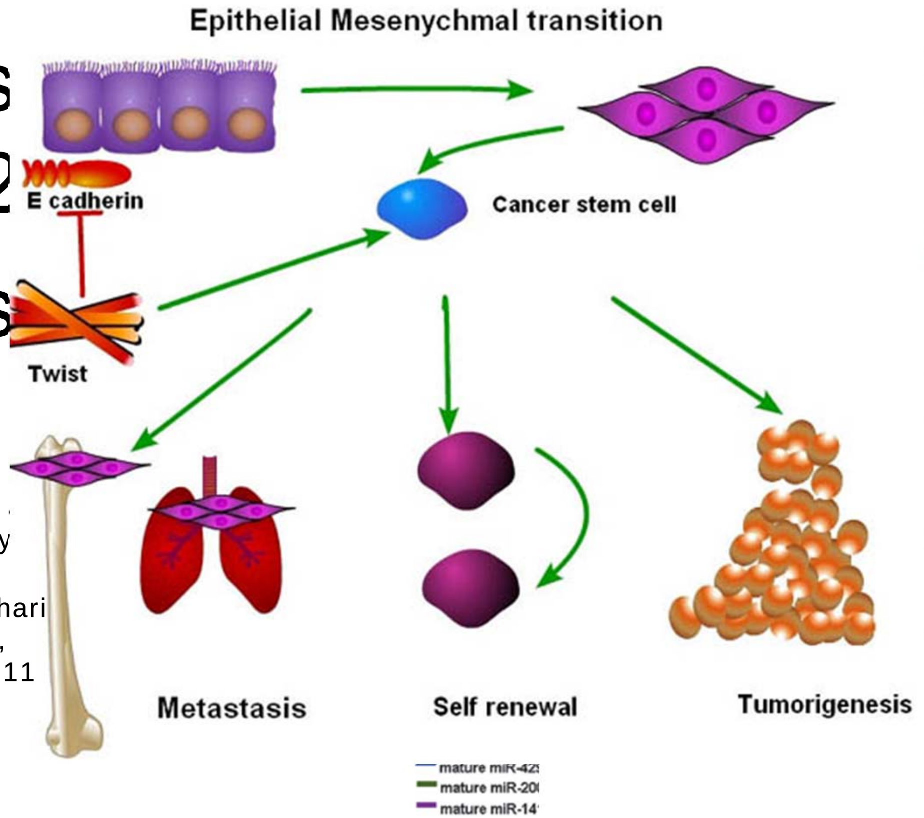
Transforming growth factor-β (TGF-β)

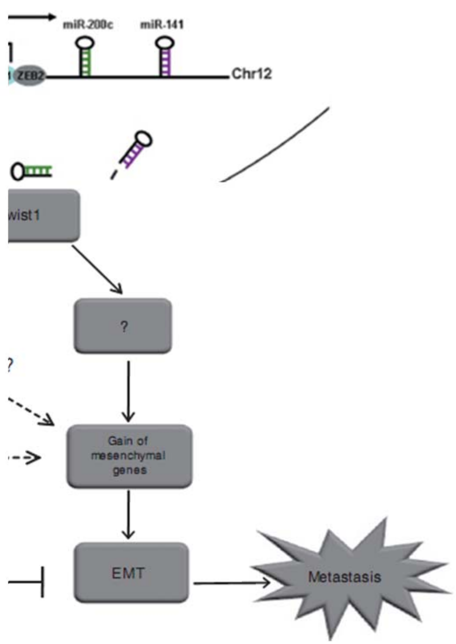


Jian Xu, Samy Lamouille, Rik Derynck
Cell Research (2009) 19:156-172.

Recent discoveries in the EMT field

- EMT creates cells with cancer stem cell characteristics **Mani** SA, et al., Cell. 2008 May 16;133(4):704-15.
- Cross
  miR-2
  31/2 and
- Cross
  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)

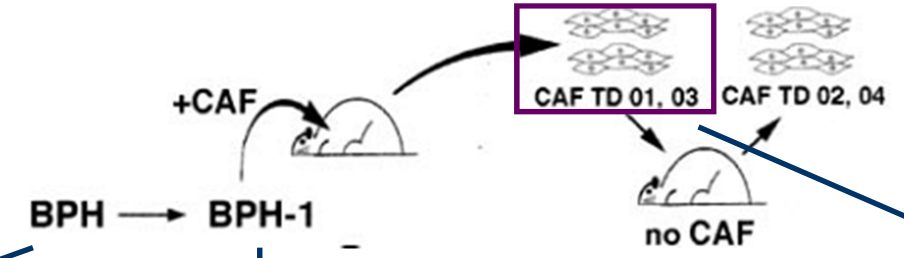
Experimental approach

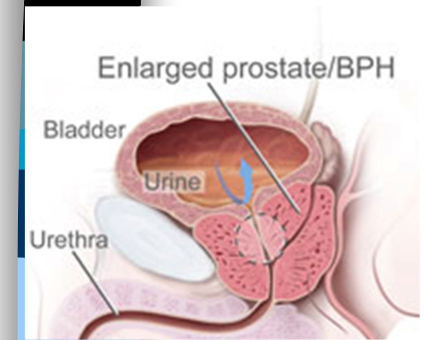
ESTABLISHMENT AND CHARACTERIZATION OF AN IMMORTALIZED BUT NON-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELL LINE: BPH-1

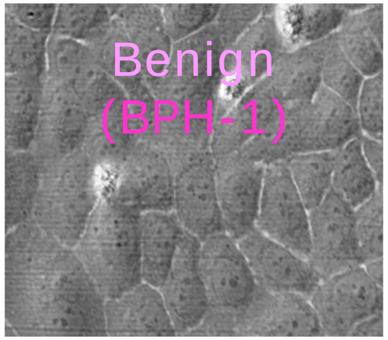
S. W. HAYWARD, R. DAHIYA, G. R. CUNHA, J. BARTEK, N. DESHPANDE, AND P. NARAYAN

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

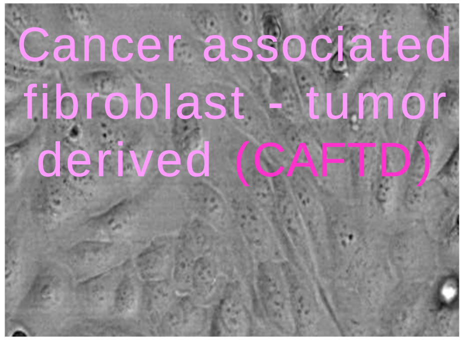
Simon W. Hayward,[2] Yuzhuo Wang, Mei Cao, Yun Kit Hom, Baohui Zhang, Gary D. Grossfeld, Daniel Sudilovsky, and Gerald R. Cunha







EMT

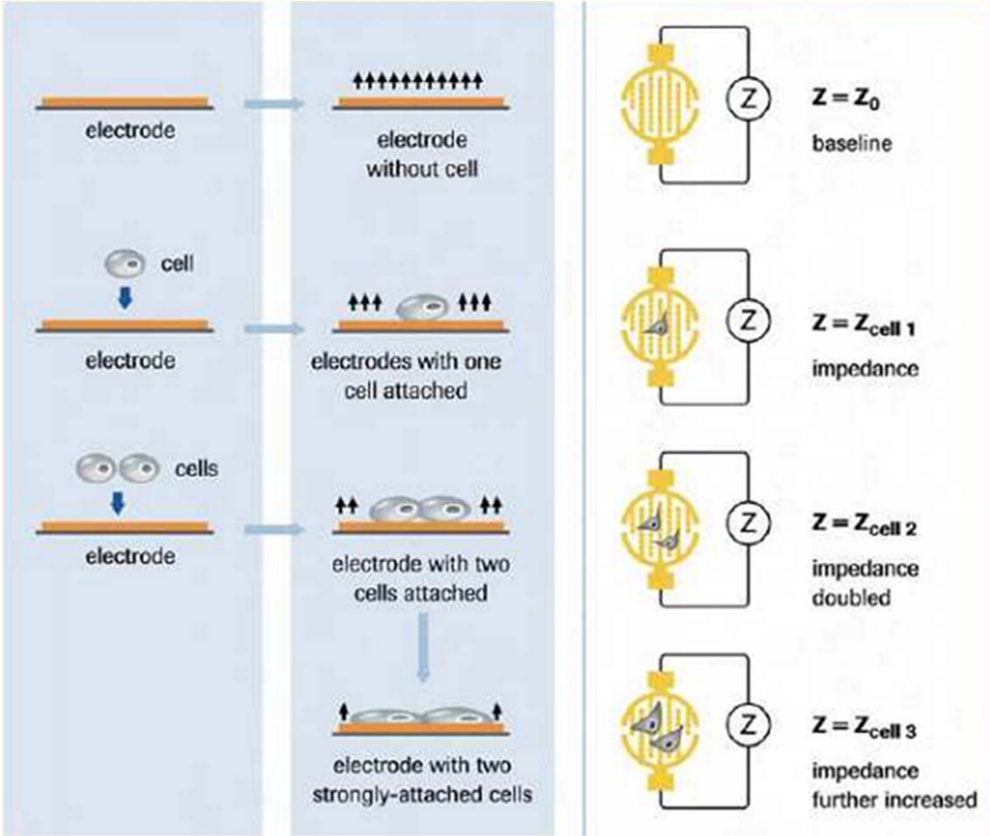


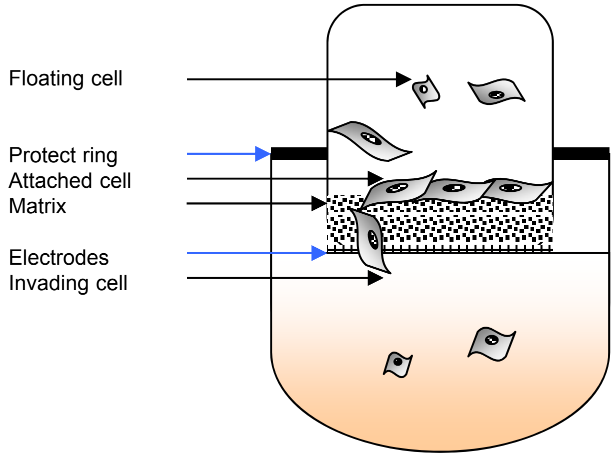
- EMT markers
- EMT regulators
- Cell shape and behavior

TGF-β → EMT

TGF-β → EMT


xCELLigence


electrode
electrode without cell
cell
electrode
electrodes with one cell attached
cells
electrode
electrode with two cells attached
electrode with two strongly-attached cells
Z
Z = Z0
baseline
Z = Zcell 1
impedance
Z = Zcell 2
impedance doubled
Z = Zcell 3
impedance further increased


Floating cell
Protect ring
Attached cell
Matrix
Electrodes
Invading cell

Dvojí úloha TGF-β v carcinogenezi

- Deregulace inhibice proliferace epiteliálních buněk;
- Epithelia-mesenchymal transition
- podpora migrace, metastázování a angiogeneze.

Role TGF-β v diagnóze, prognóze a léčbě

- Vysoká sérová hladina TGF-β1 je spojena s nádory tlustého střeva, prostaty a rozvojem fibrózy;
- polymorfismus genu pro TGF-β1 vedoucí k jeho zvýšené produkci určuje predispozici k fibróze, hypertenzi a osteoporéze;
- blokování produkce a aktivity TGF-β má velký potenciál pro léčbu fibrózy;
- protektivní účinek retinoidů a vitamínu D3 může být způsoben prostřednictvím TGF-β.

GDF-15: nádorový promotor nebo supresor?

Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 11514–11519, October 1997
Immunology, Cell Biology

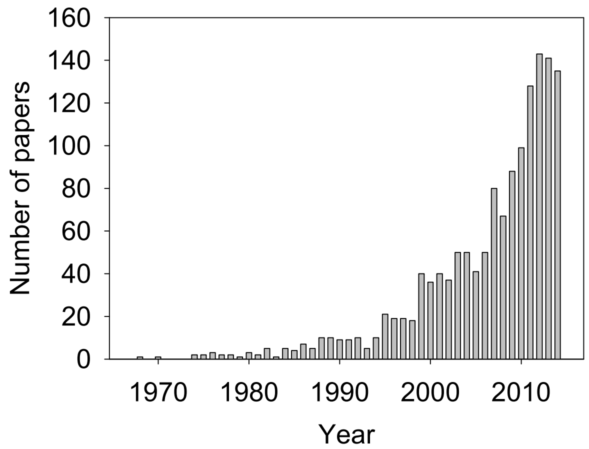
MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-β superfamily

Michelle R. Bootcov*†, Asne R. Bauskin*†, Stella M. Valenzuela*, Anthony G. Moore*, Mohinder Bansal*, Xiao Yan He*, Hong Ping Zhang*, Melissa Donnellan*, Stephen Mahler‡, Kimberley Pryor*, Bradley J. Walsh*, Richard C. Nicholson*, W. Douglas Fairlie*, Suzanne B. Por*, Joan M. Robbins*, and Samuel N. Breit*§

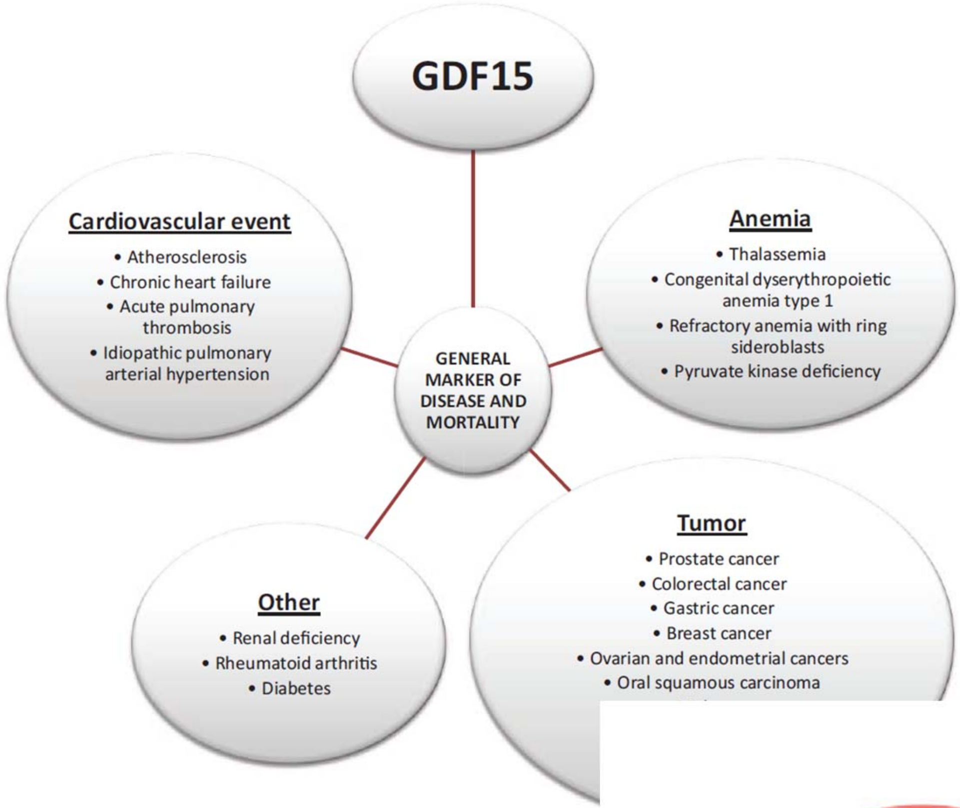
*Centre for Immunology, St. Vincent's Hospital, and University of New South Wales, Sydney, 2010, Australia; and ‡Department of Biotechnology, University of New South Wales, Sydney, 2010, Australia

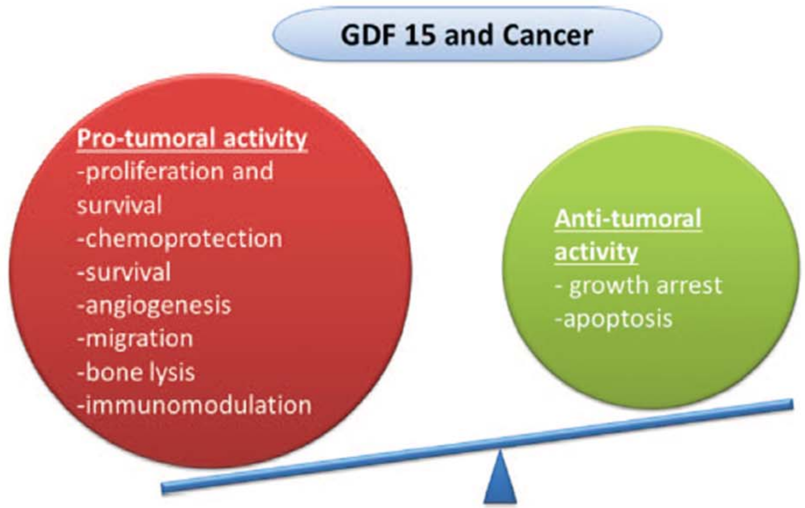
24/9/2015 ~ 1578 records

- Membrane receptor(s) – not identified
- Signal transduction – not full understood
- Target genes – not identified
- Function – not clear



GDF-15 v patologických stavech





STEM CELLS TRANSLATIONAL MEDICINE
TISSUE-SPECIFIC PROGENITOR AND STEM CELLS

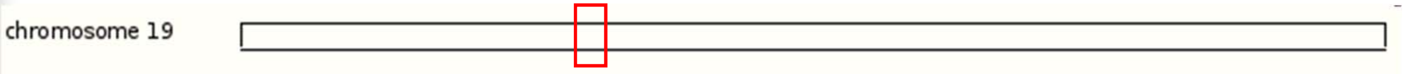
Concise Review: Growth Differentiation Factor 15 in Pathology: A Clinical Role?

JILL CORRE,[a,b,c] BENJAMIN HÉBRAUD,[a,d] PHILIPPE BOURIN[e]

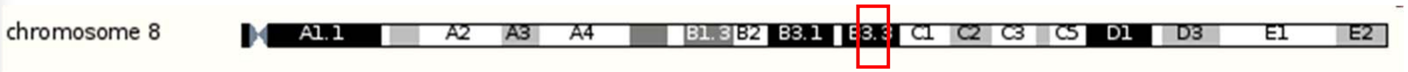
GDF15 gene

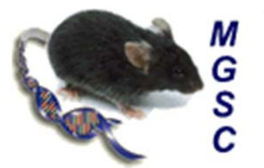
Homo sapiens

Pan troglodytes



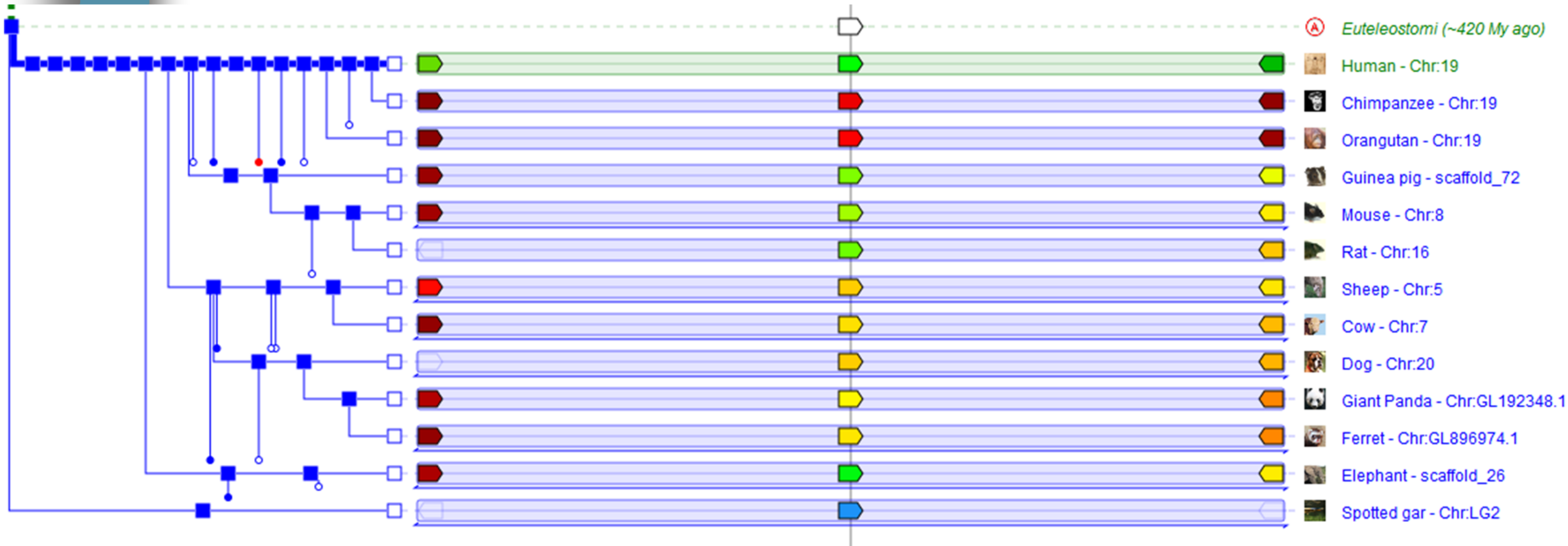
Mus musculus

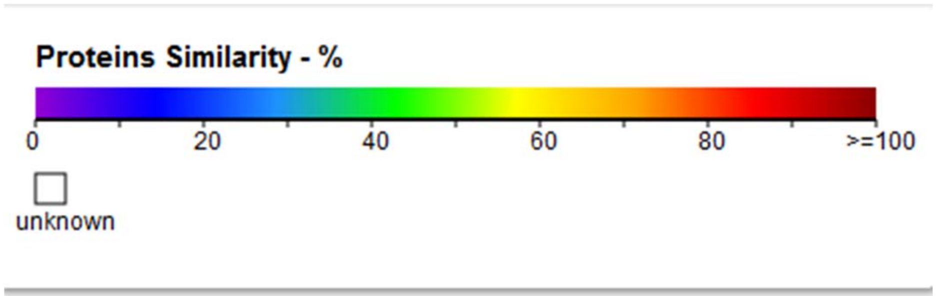




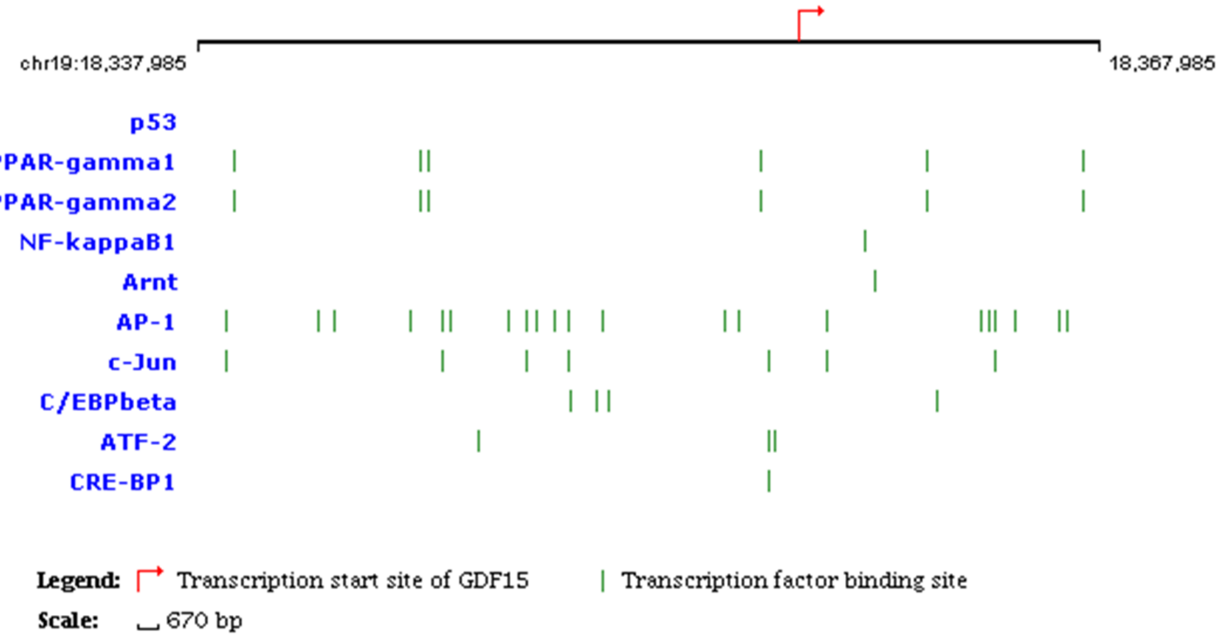
Rattus norvegicus

GDF-15: mezidruhová podobnost proteinu

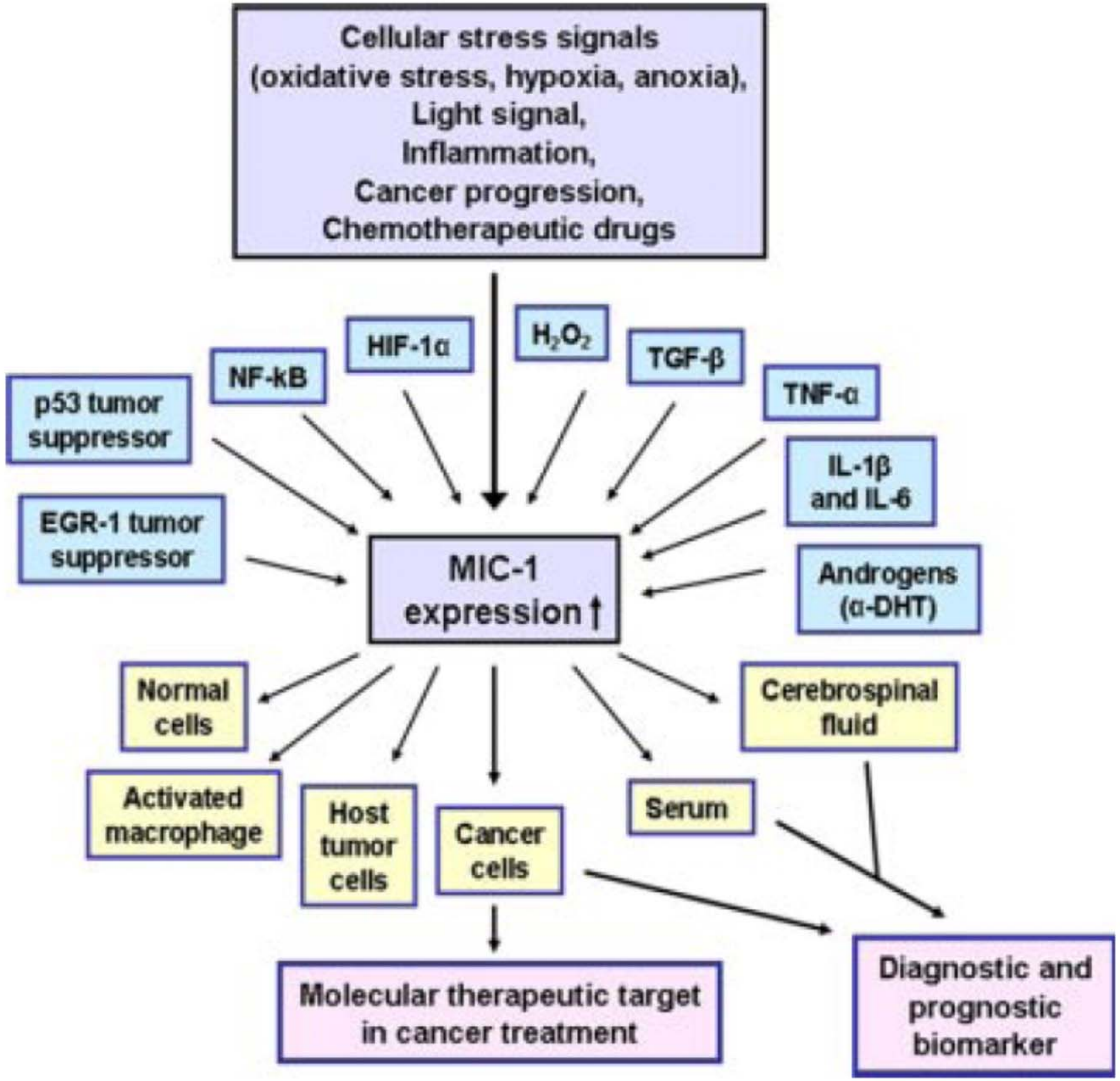




Control of *GDF15* expression

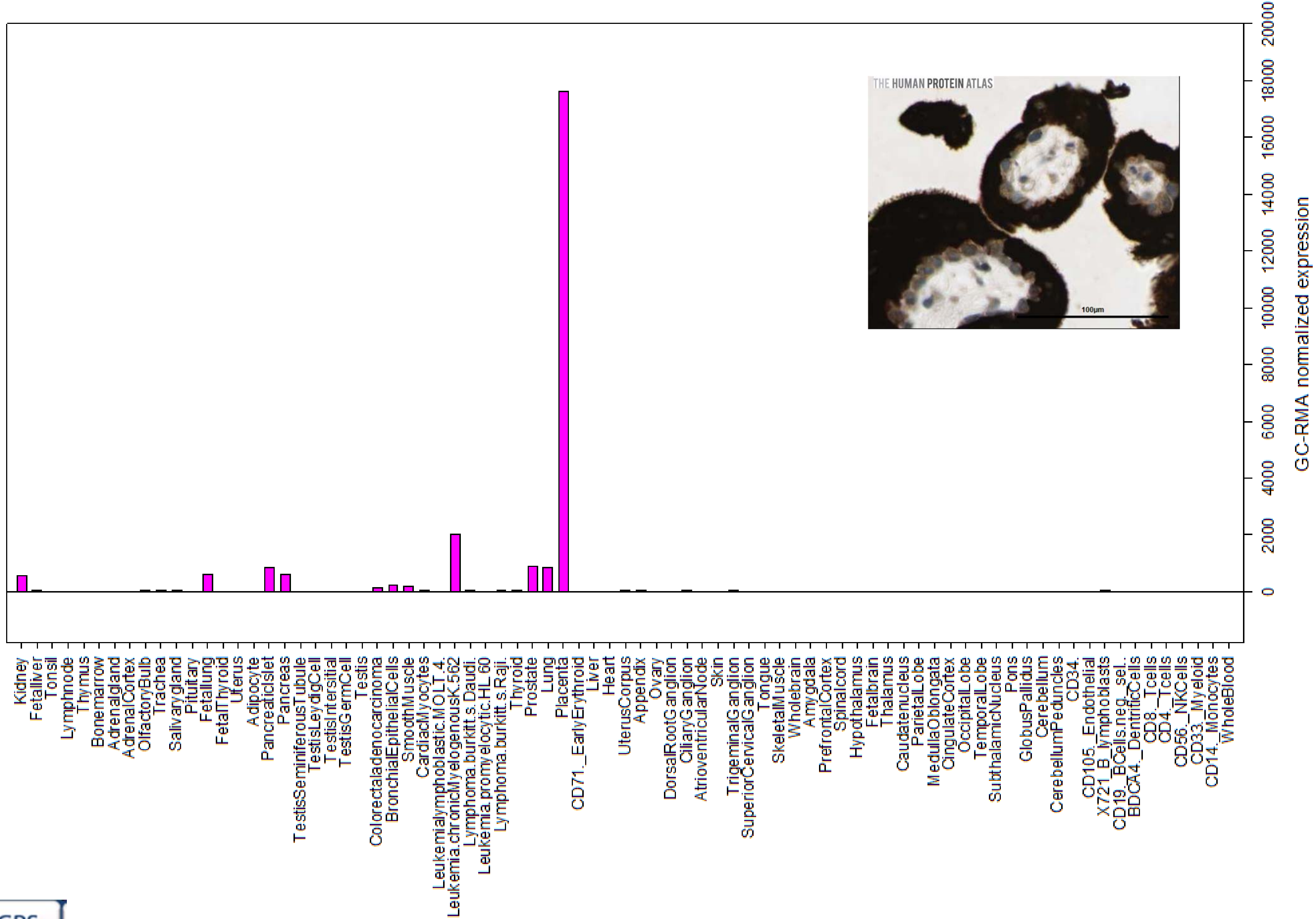


GDF-15 regulation



M, Mimeualt, JCP 2010

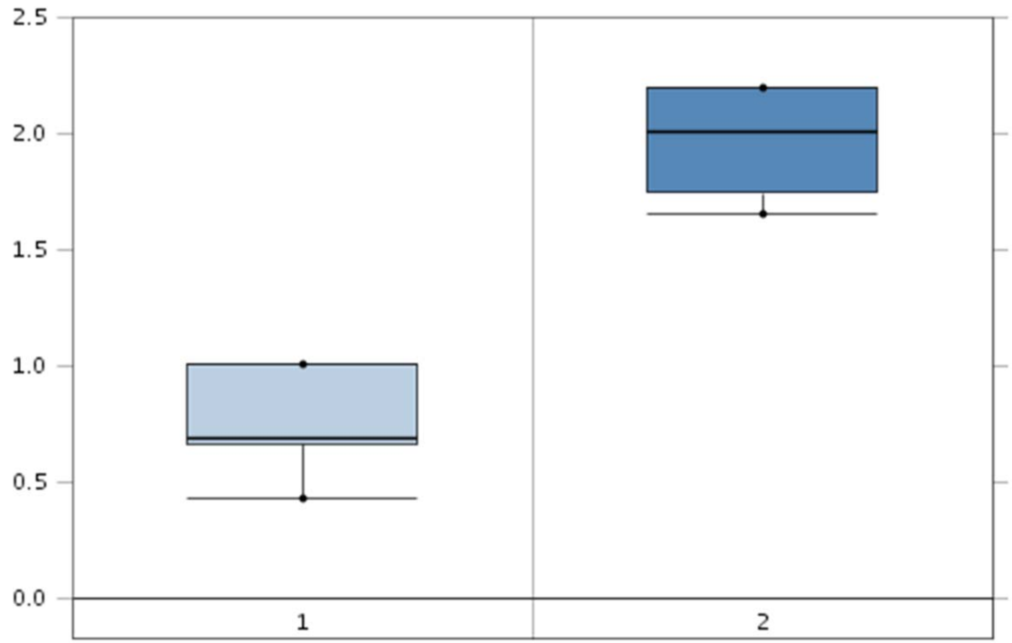
human GDF15 mRNA expression



BioGPS

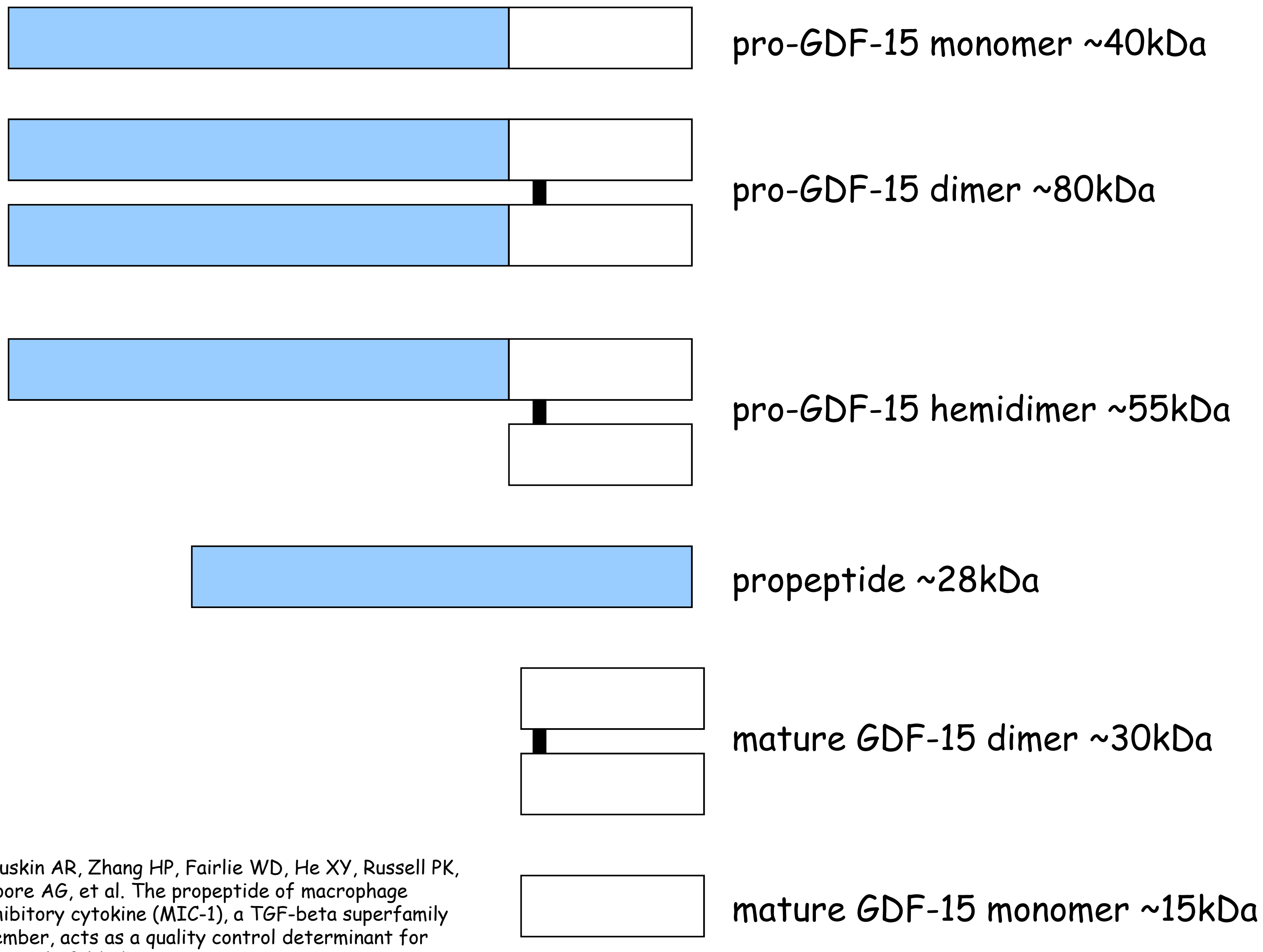
ONCOMINE™

GDF15 mRNA
normal vs. prostate adenocarcinoma



Varambally, S. et al., *Cancer Cell* **8** (5), 393 (2005).

Various forms of GDF-15



Bauskin AR, Zhang HP, Fairlie WD, He XY, Russell PK, Moore AG, et al. The propeptide of macrophage inhibitory cytokine (MIC-1), a TGF-beta superfamily member, acts as a quality control determinant for correctly folded MIC-1. **EMBO J** 2000;19:2212-20.

HUMAN PROTEIN ATLAS

alph. sort order

Normal Tissues - IHC

Tissue	Cell type
Adrenal gland	cortical cells
Appendix	glandular cells
	lymphoid tissue
Bone marrow	bone marrow poietic cells
Breast	glandular cells
Bronchus	respiratory epithelial cells
Cerebellum	cells in granular layer
	cells in molecular layer
	purkinje cells
Cerebral cortex	glial cells
	neuronal cells
Cervix, uterine	glandular cells
	squamous epithelial cells
Colon	glandular cells
Corpus, uterine 1	cells in endometrial stroma
	glandular cells
Corpus, uterine 2	cells in endometrial stroma
	glandular cells
Duodenum	glandular cells
Epididymis	glandular cells
Esophagus	squamous epithelial cells
Fallopian tube	glandular cells
Gall bladder	glandular cells
Heart muscle	myocytes
Hippocampus	glial cells
	neuronal cells
Kidney	cells in glomeruli
	cells in tubules
Lateral ventricle	glial cells
	neuronal cells
Liver	bile duct cells
	hepatocytes
Lung	alveolar cells
	macrophages

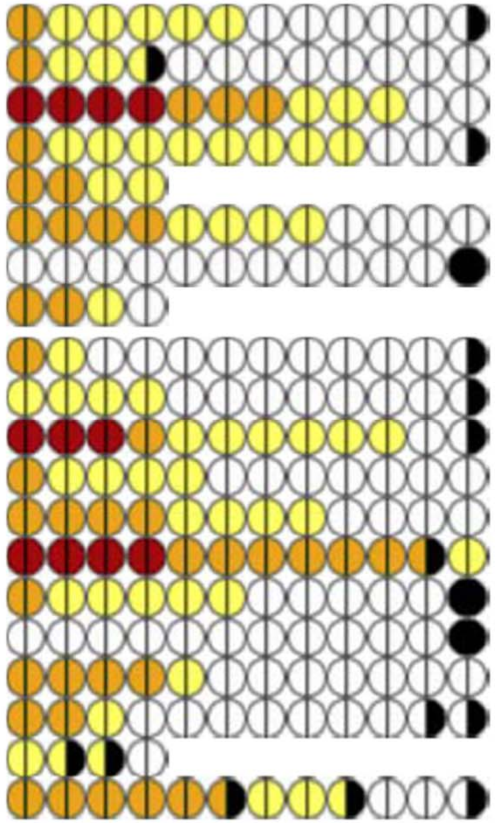
Tissue	Cell type
Lymph node	lymphoid cells outside reaction centra
	reaction center cells
Nasopharynx	respiratory epithelial cells
Oral mucosa	squamous epithelial cells
Ovary	follicle cells
	ovarian stromal cells
Pancreas	exocrine glandular cells
	islet cells
Parathyroid gland	glandular cells
Placenta	decidual cells
	trophoblastic cells
Prostate	glandular cells
Rectum	glandular cells
Salivary gland	glandular cells
Seminal vesicle	glandular cells
Skeletal muscle	myocytes
Skin	adnexal cells
	epidermal cells
Small intestine	glandular cells
Smooth muscle	smooth muscle cells
Soft tissue 1	mesenchymal cells
Soft tissue 2	mesenchymal cells
Spleen	cells in red pulp
	cells in white pulp
Stomach 1	glandular cells
Stomach 2	glandular cells
Testis	cells in seminiferus ducts
	leydig cells
Thyroid gland	glandular cells
Tonsil	lymphoid cells outside reaction centra
	reaction center cells
	squamous epithelial cells
Urinary bladder	urothelial cells
Vagina	squamous epithelial cells
Vulva/anal skin	squamous epithelial cells

Protein expression

- Strong
- Moderate
- Weak
- Negative
- Not representative

HUMAN PROTEIN ATLAS

- Breast cancer
- Cervical cancer
- Colorectal cancer
- Endometrial cancer
- Head & neck cancer
- Liver cancer
- Lung cancer
- Malignant carcinoid
- Malignant glioma
- Malignant lymphoma
- Malignant melanoma
- Ovarian cancer
- Pancreatic cancer
- Prostate cancer
- Renal cancer
- Skin cancer
- Stomach cancer
- Testis cancer
- Thyroid cancer
- Urothelial cancer



Protein expression

- Strong
- Moderate
- Weak
- Negative
- Not representative

GDF-15	Concentration	Reference
Normal	450 ± 50 pg/ml	Tanno, T. et al., Nat Med 13 (9), 1096 (2007)
beta-thalassemia syndrome	66,000 ± 9,600 pg/ml	
CSF - non-neoplastic	156 pg/m	Sophie Shnaper et al., *International Journal of Cancer* **in press** (2009).
CSF - gliosblastoma	229 pg/ml	
normal	495 pg/ml	Brown, D. A. et al., *Clin Cancer Res* **9** (7), 2642 (2003).
Adenomatous polyps	681 pg/ml	
High-grade dysplasia	1114 pg/ml	
colorectal carcinoma	783 pg/ml	
Congenital dyserythropoietic anemia	10 239 ± 3049 pg/ml	Tamary, H. et al., *Blood* **112** (13), 5241 (2008).
Normal	16.1 ± 23.4 pg/ml	Baek, K. E. et al., *Clinica Chimica Acta* **401** (1-2), 128 (2009).
Gastric cancer	164.5 ± 183.7 pg/ml	
Prostate cancer Grade 3	2,326.1 pg/ml	Selander, K. S. et al., *Cancer Epidemiology Biomarkers & Prevention* **16** (3), 532 (2007).
Prostate cancer Grade 2	2,054.1 pg/ml	
Prostate cancer Grade 1	761.5 pg/ml	
normal	859 ± 619 pg/ml	Brown, D. A. et al., *Clin Cancer Res* **12** (1), 89 (2006).
BPH	983 ± 850 pg/ml	
Prostate cancer	731 ± 500 pg/mL	
Women with cardiovascular events	618 pg/mL	Brown, D. A. et al., *The Lancet* **359** (9324), 2159 (2002).
Women w/o cardiovascular events	538 pg/mL	

J Cancer Res Clin Oncol
DOI 10.1007/s00432-009-0691-4

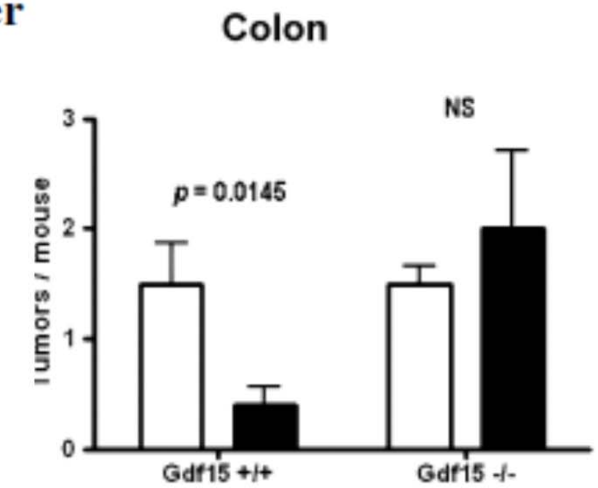
ORIGINAL PAPER

Received: 15 July 2009 / Accepted: 16 September 2009

Loss of GDF-15 abolishes Sulindac chemoprevention in the $Apc^{Min/+}$ mouse model of intestinal cancer

Teresa A. Zimmers · Juan C. Gutierrez · Leonidas G. Koniaris

?



Oncogene (2009), 1–10

www.nature.com/onc

ORIGINAL ARTICLE

Overexpression of macrophage inhibitory cytokine-1 induces metastasis of human prostate cancer cells through the FAK–RhoA signaling pathway

S Senapati[1], S Rachagani[1], K Chaudhary[1], SL Johansson[2,3], RK Singh[2,3] and SK Batra[1,3]

[1]Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA; [2]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA and [3]Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE, USA

Shrnutí přednášky II.

- TGF-β hraje významnou roli v rozvoji karcinogeneze a dalších patologických stavů.
- EMT je významný proces ovlivňující schopnost nádorových buněk diseminovat
- GDF-15 hraje důležitou úlohu v nádorové progresi

Na konci dnešní přednášky byste měli:

1. být schopni vysvětlit úlohu TGF-β v karcinogenezi;
2. charakterizovat proces EMT včetně hlavních znaků a regulátorů;
3. popsat známé vlastnosti GDF-15.