

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

Karel Souček

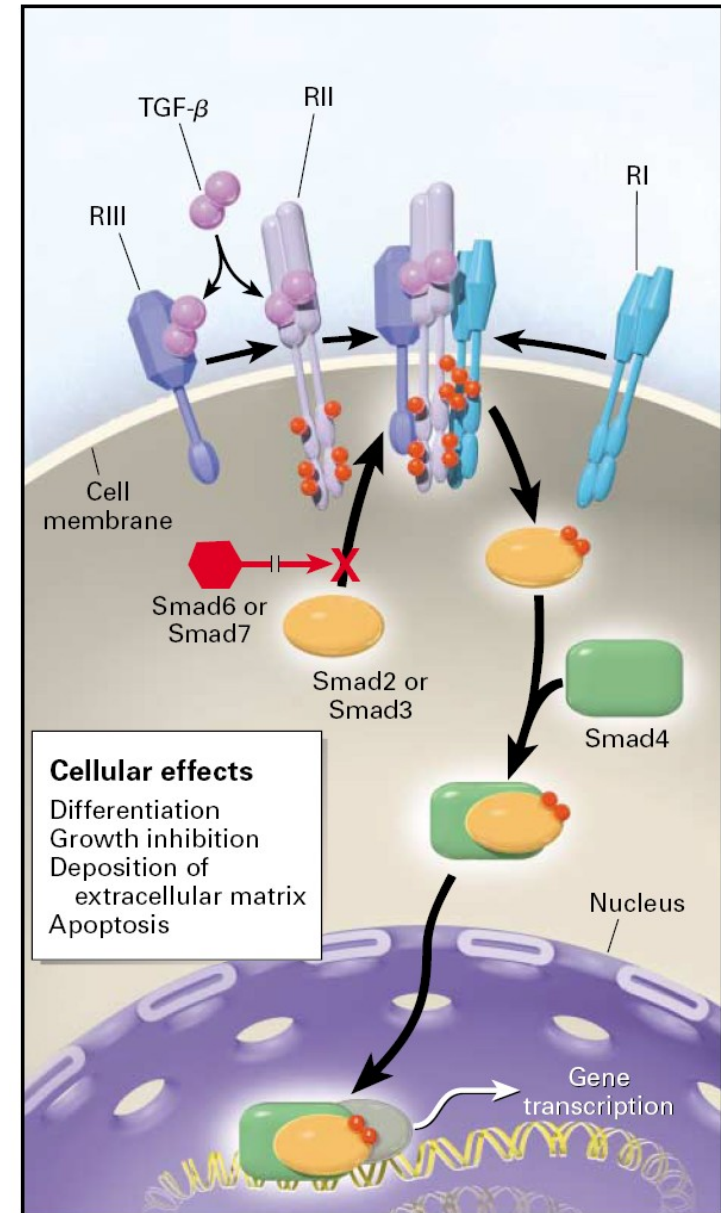
Bi6051 Molekulární fyziologie živočichů

Transforming growth factor - β (TGF- β)

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

TGF- β_1

- pleiotropní cytokin
- negativní regulátor





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?

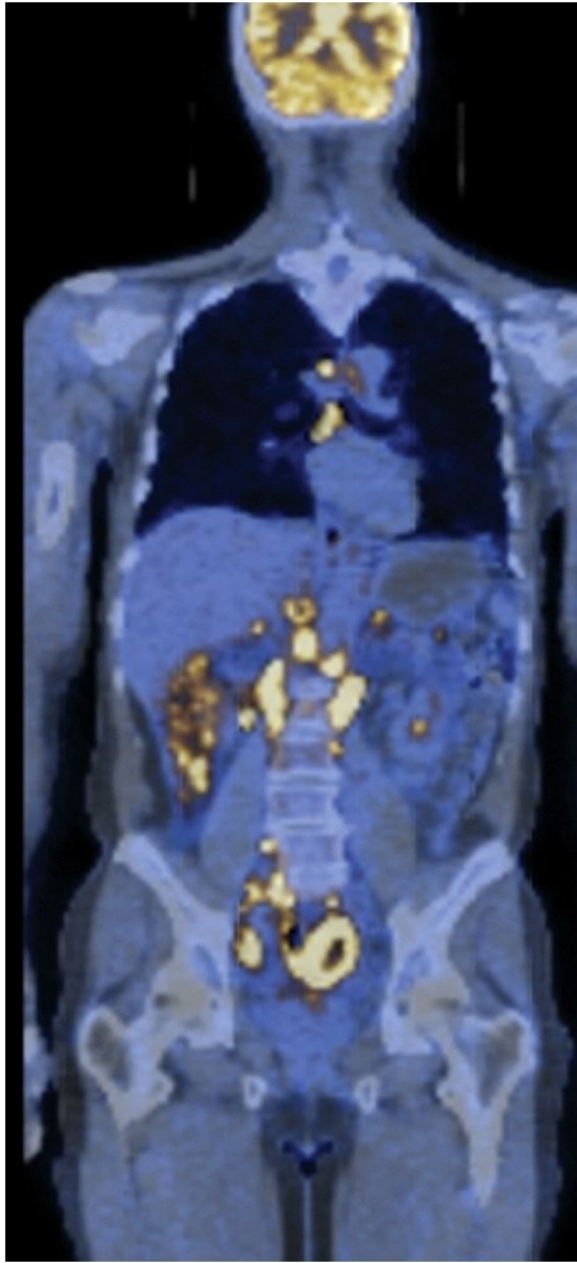


Figure 14.1 *The Biology of Cancer* (© Garland Science 2007)

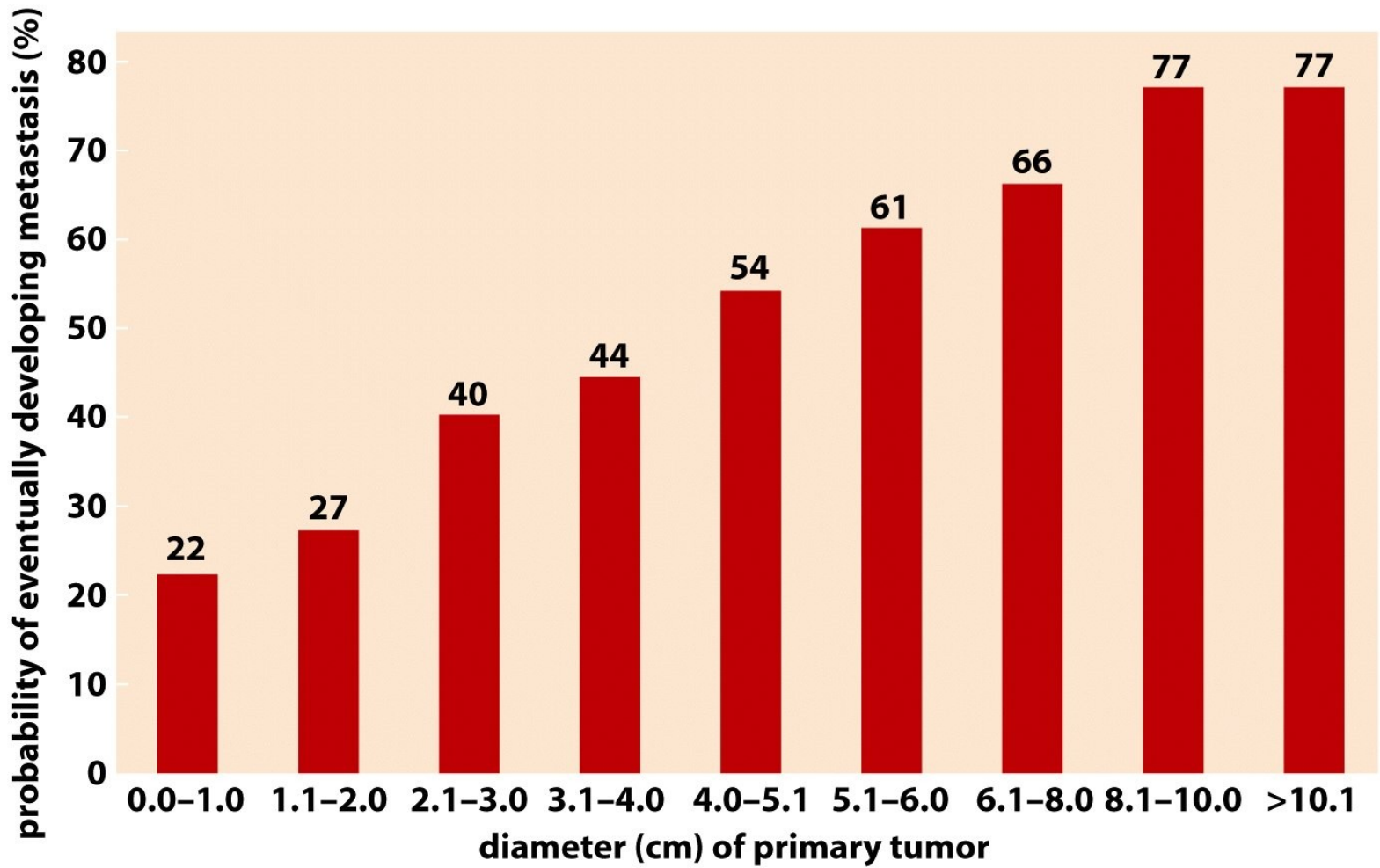


Figure 14.3 *The Biology of Cancer* (© Garland Science 2007)

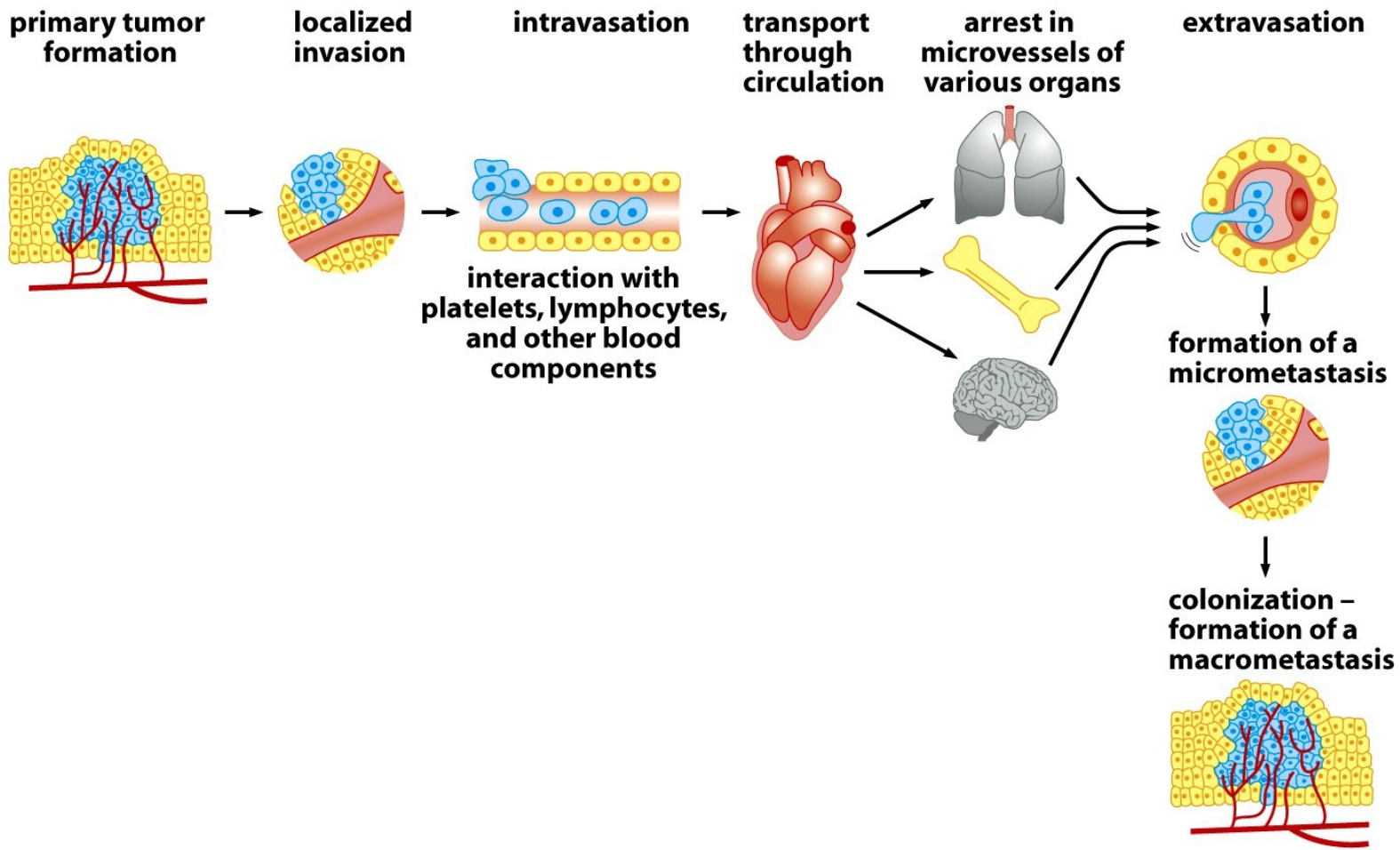
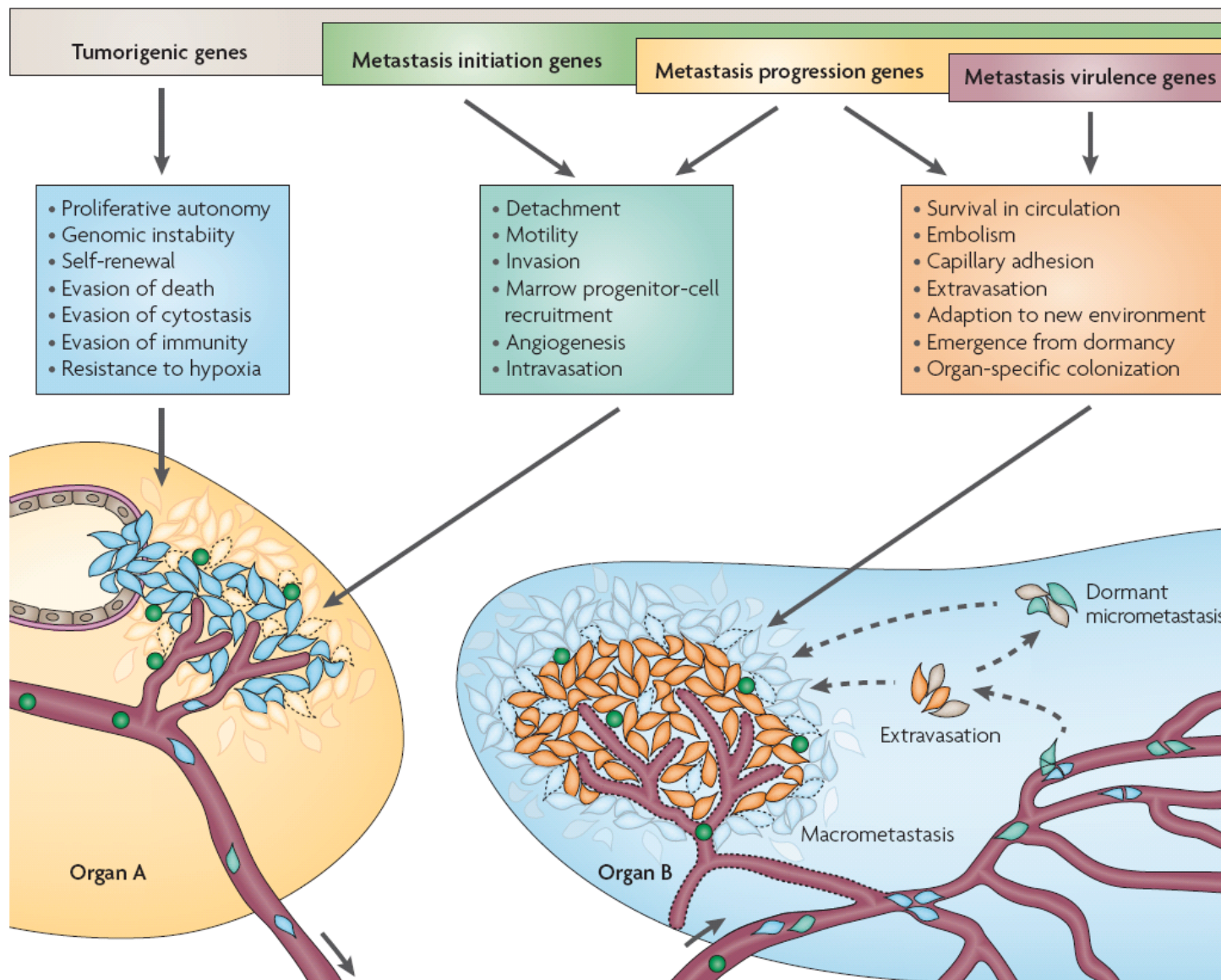


Figure 14.4 *The Biology of Cancer* (© Garland Science 2007)

Genetic determinants of cancer metastasis

Don X. Nguyen and Joan Massagué



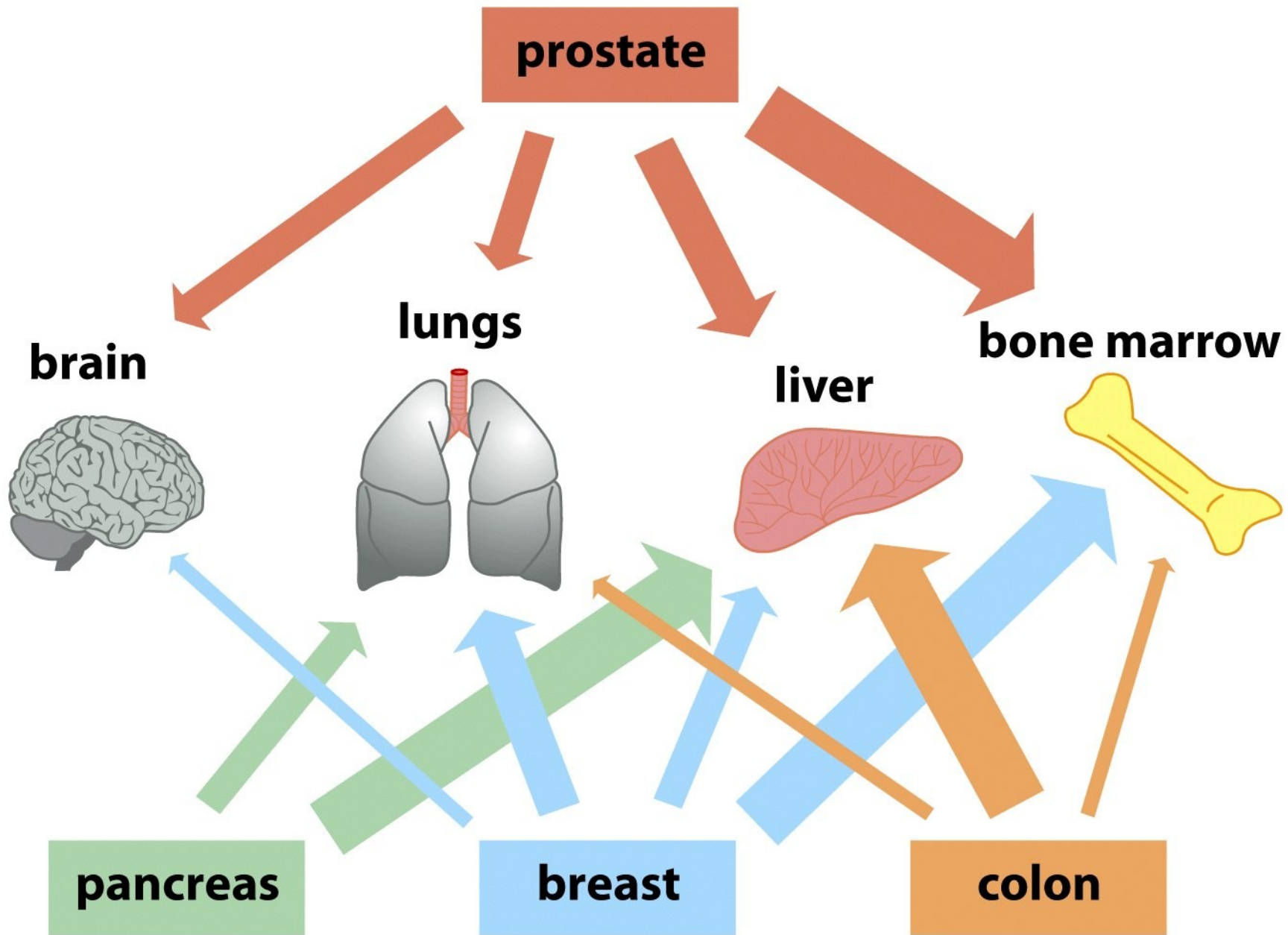
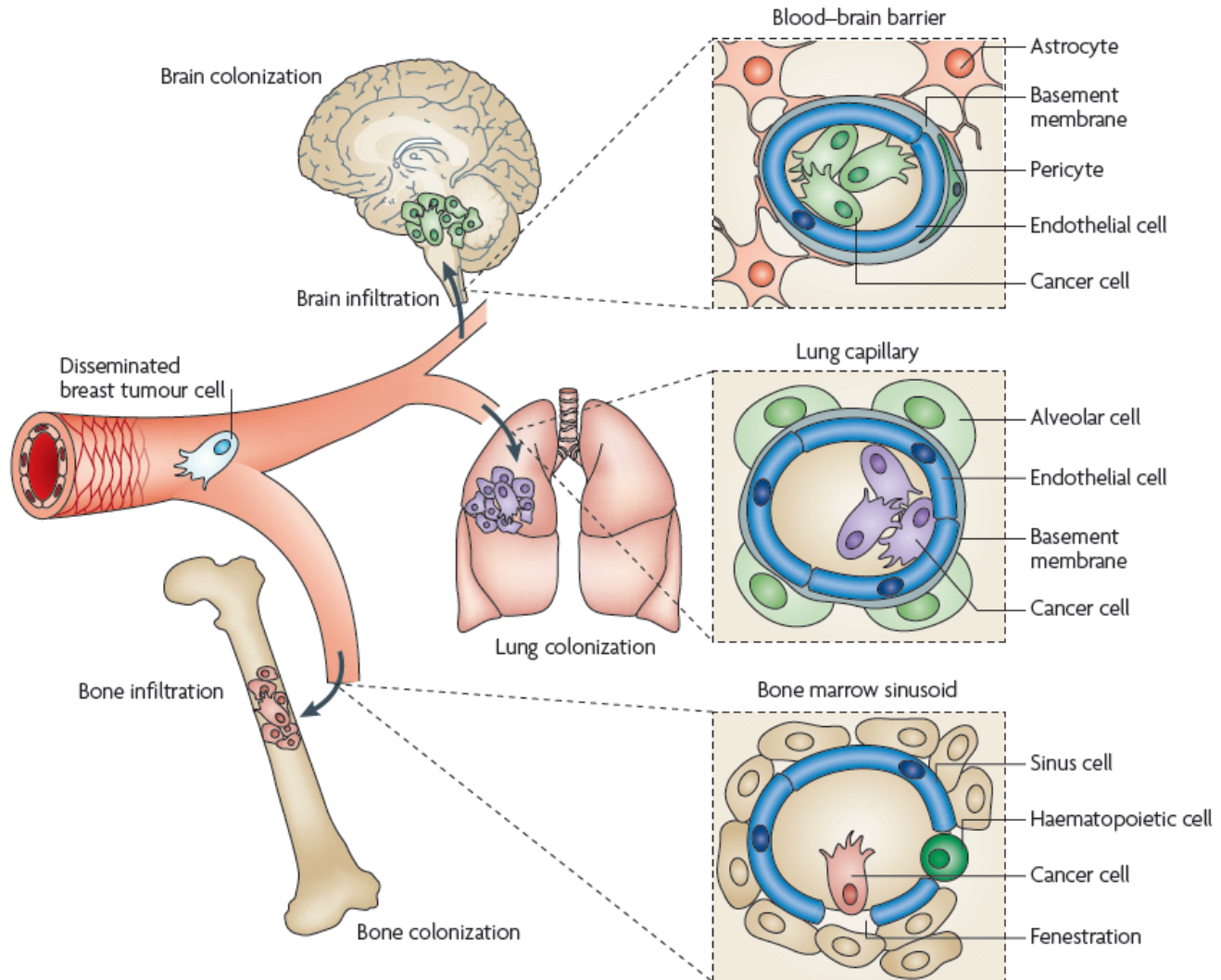


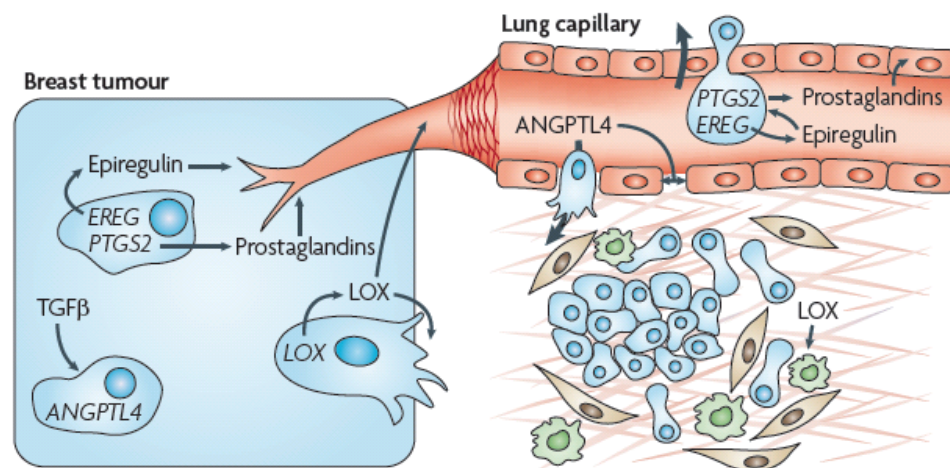
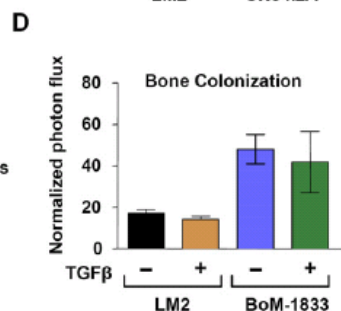
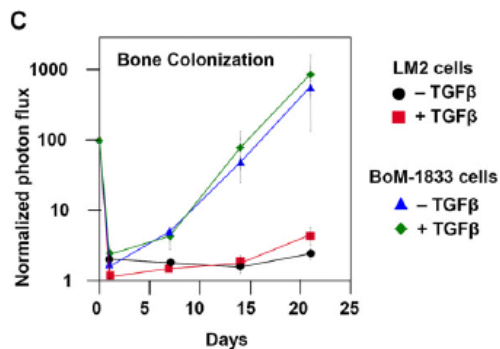
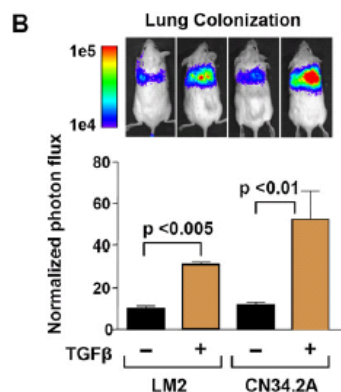
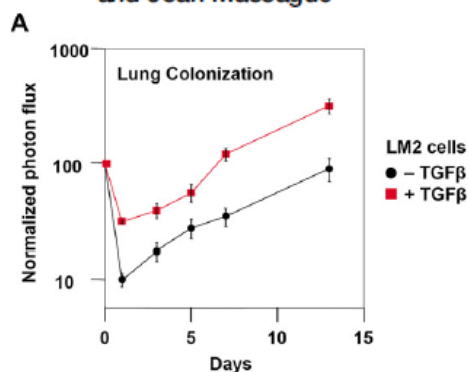
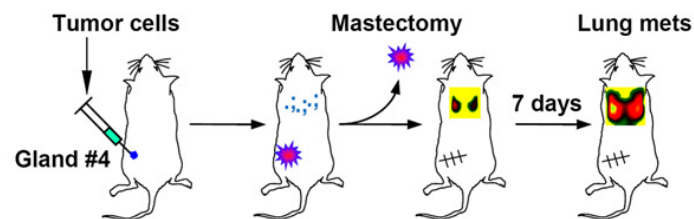
Figure 14.42 *The Biology of Cancer* (© Garland Science 2007)

Organ-specific barriers



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

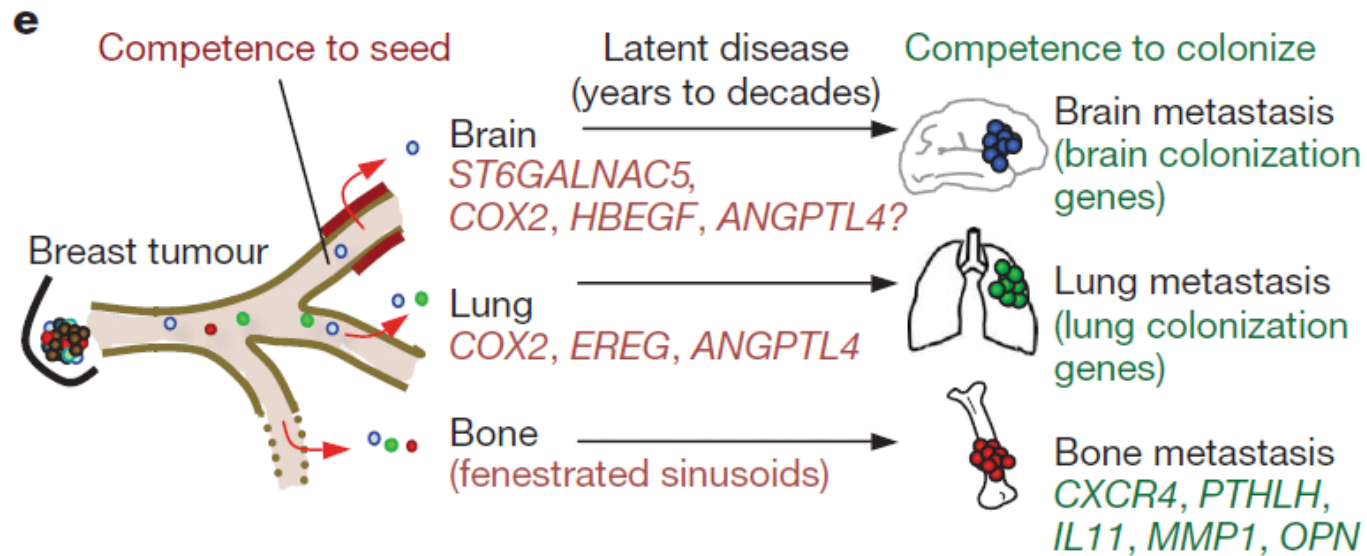
David Padua,¹ Xiang H.-F. Zhang,¹ Qionqing Wang,¹ Cristina Nadal,⁵ William L. Gerald,² Roger R. Gomis,⁴ and Joan Massagué^{1,3,*}



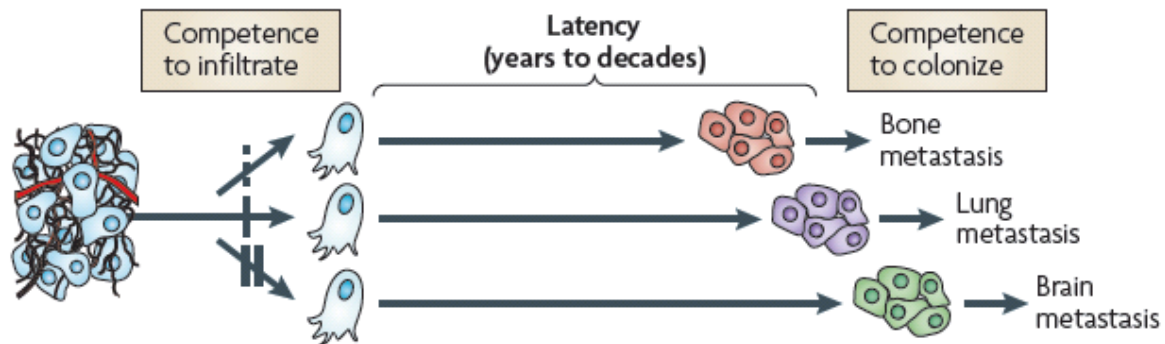
LETTERS

Genes that mediate breast cancer metastasis to the brain

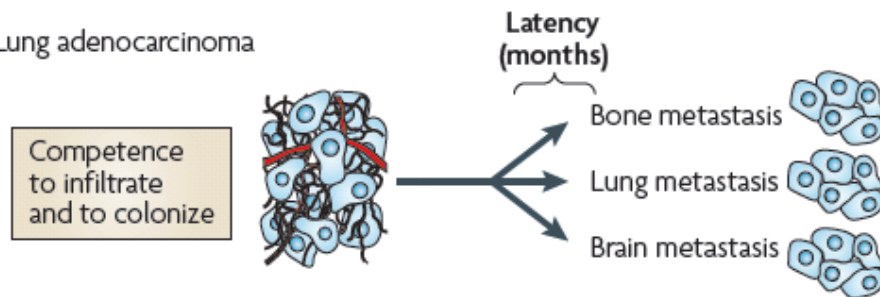
Paula D. Bos¹, Xiang H.-F. Zhang¹, Cristina Nadal^{1†}, Weiping Shu¹, Roger R. Gomis^{1†}, Don X. Nguyen¹, Andy J. Minn², Marc J. van de Vijver³, William L. Gerald⁴, John A. Foekens⁵ & Joan Massagué^{1,6}



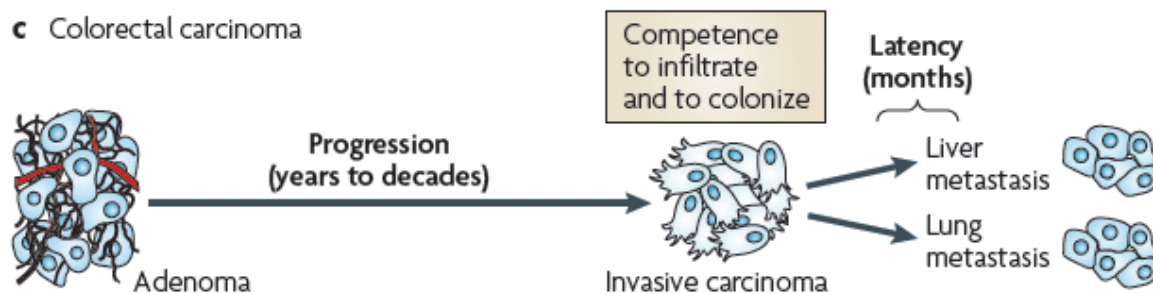
a Breast carcinoma



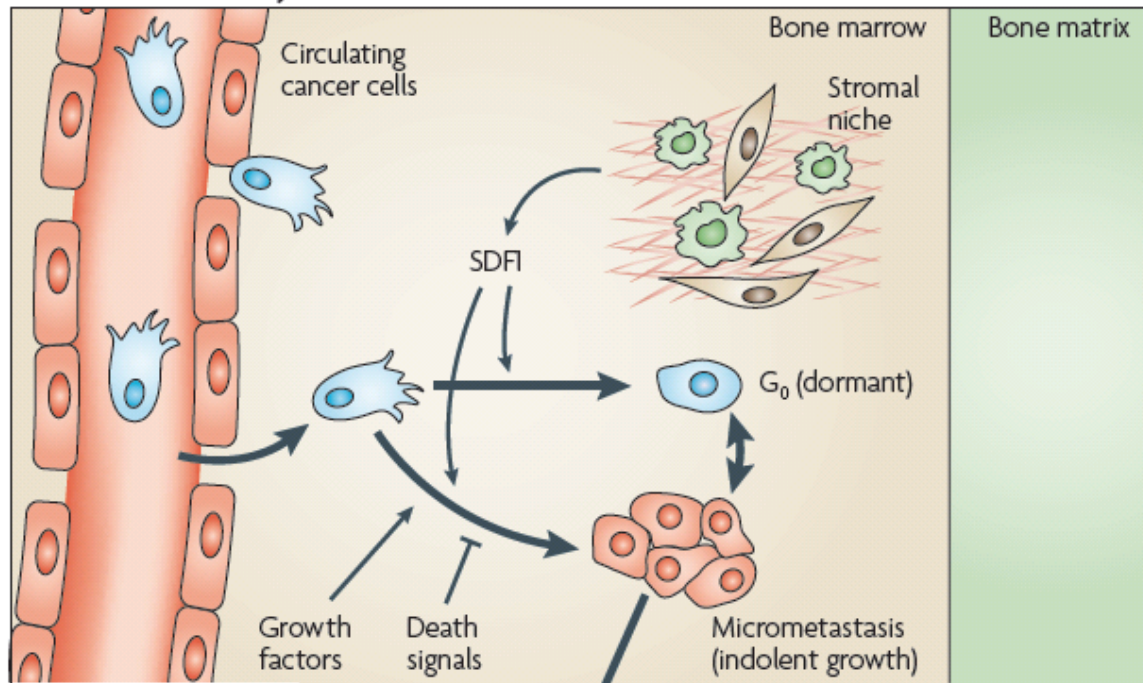
b Lung adenocarcinoma



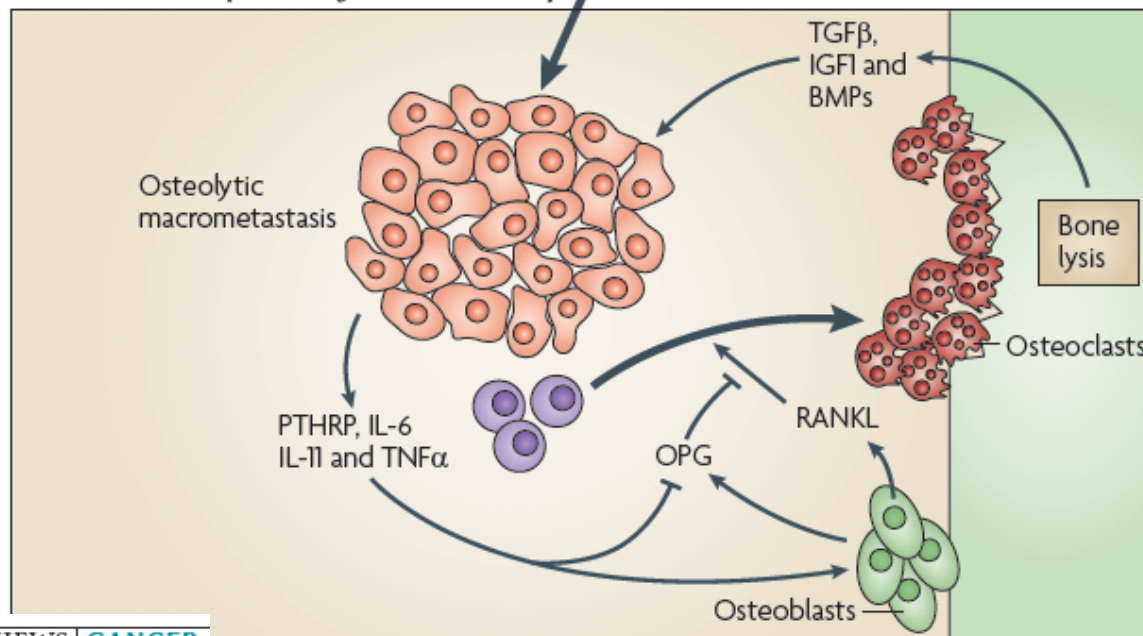
c Colorectal carcinoma



Infiltration and latency



Colonization competence (years to decades)





Transforming growth factor- β

- Role v rozvoji patologických stavů



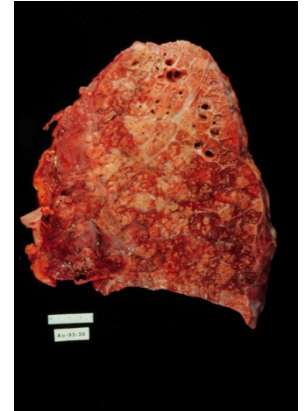
Biologické funkce TGF- β

- 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 působení TGF- β ;
- indukce angiogeneze.



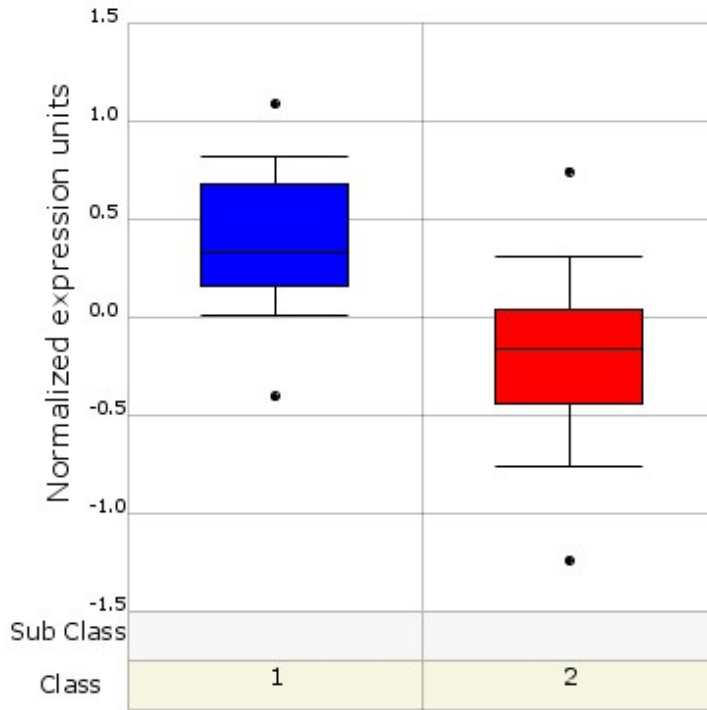
Role TGF- β v carcinogenezi

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)

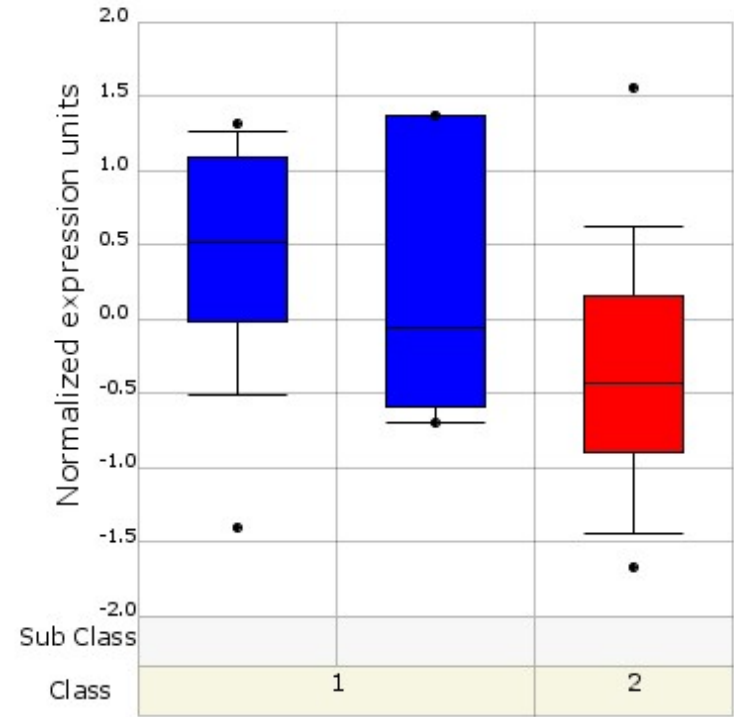


Box Plot - Description

Prostate – normal vs. cancer

TGFBR2

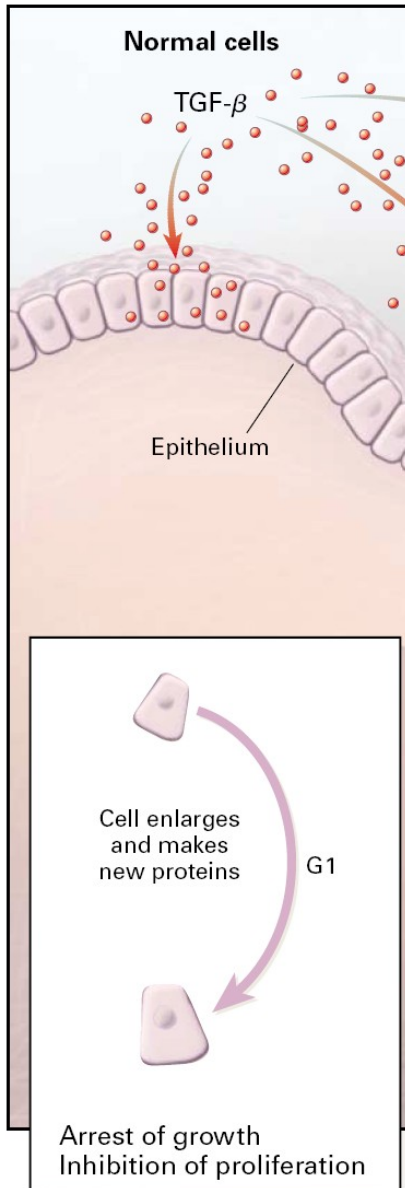
Transforming growth factor, beta receptor ii (70/80kda)



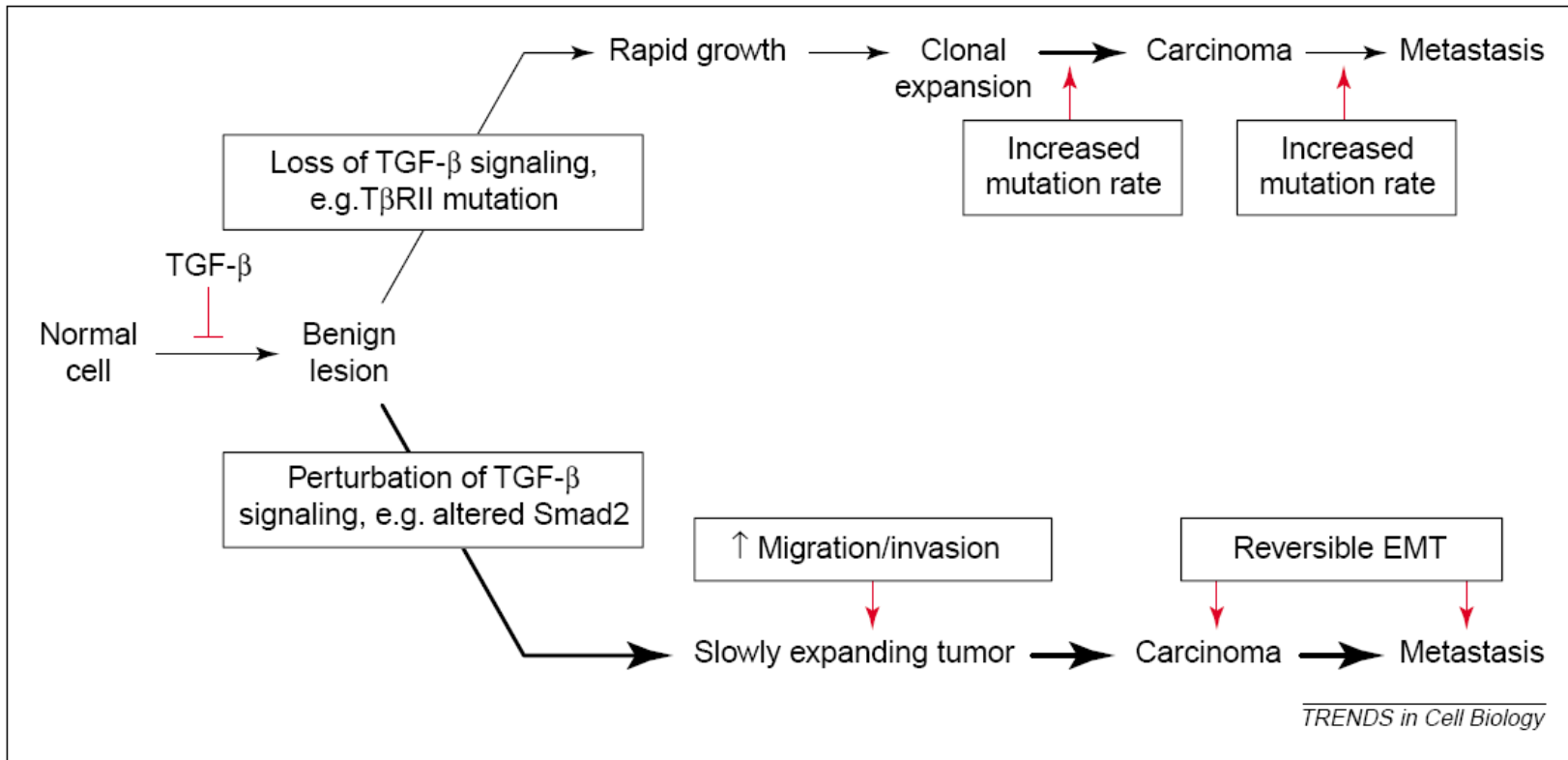
Box Plot - Description

normal, hyperplasia vs. cancer

Role TGF- β v carcinogenezi



Role TGF- β v carcinogenezi





Epithelial-Mesenchymal Transition (EMT)

- Změna buněčného fenotypu spojená se ztrátou adheze a zvýšením motility

EMT & Cancer

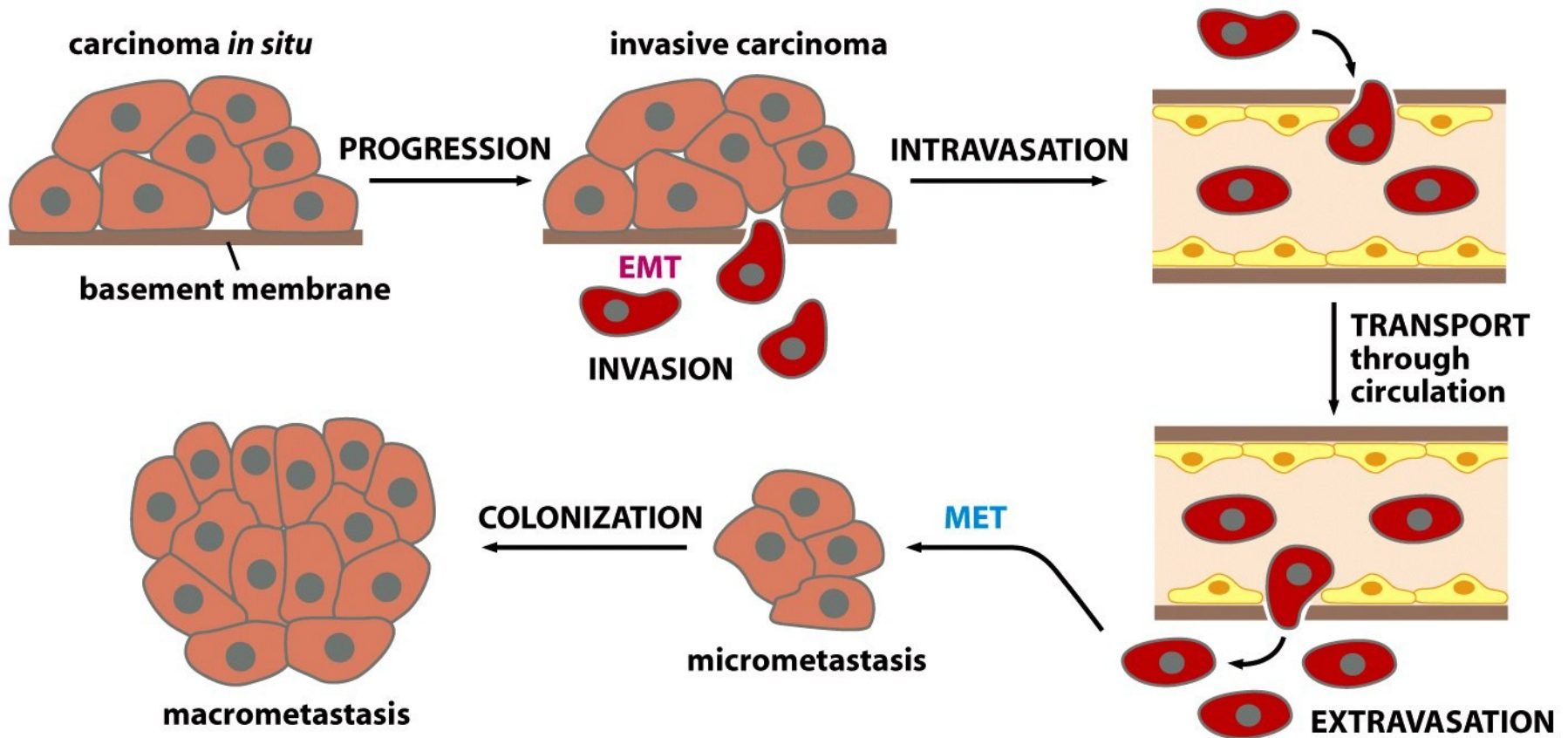
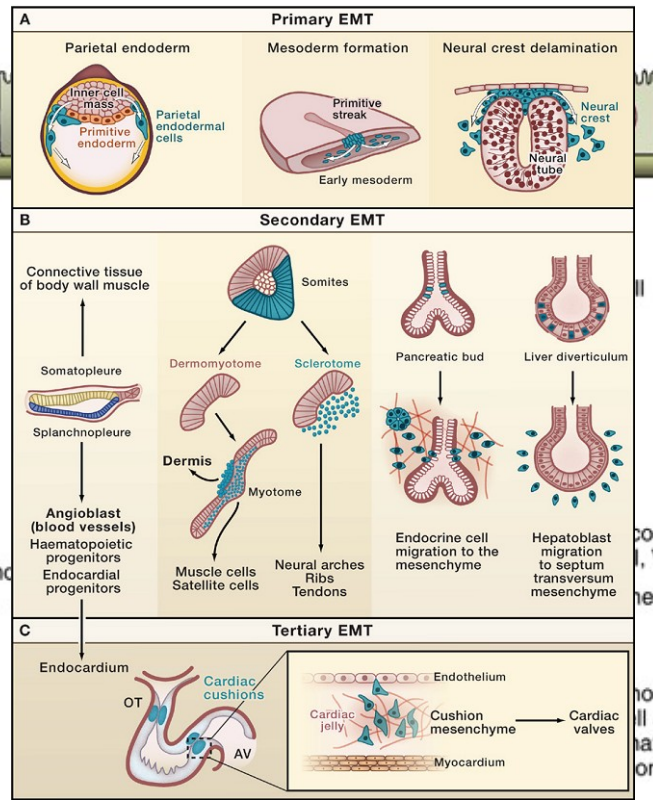


Figure 14.17b *The Biology of Cancer* (© Garland Science 2007)

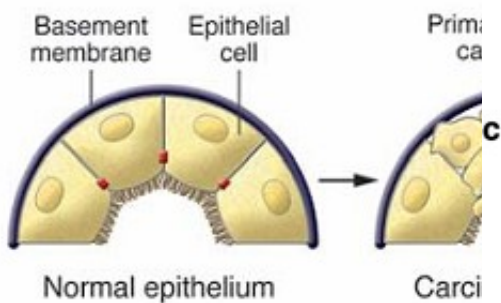
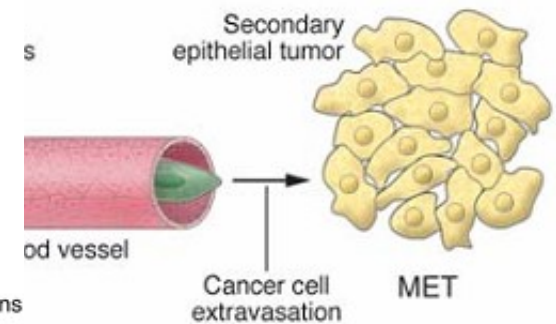
Epithelial-to-mesenchymal transition (EMT)

- Reversible acquisition of migratory and invasive properties by epithelial cells

- Role in fibrosis



ment,



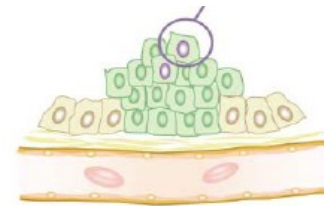
Kalluri, R. and R.A. Weinberg, *et al.*

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

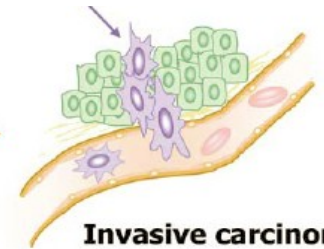
M S Simonson
Kidney International **71**,
846-854 (May (1) 2007)

Markers and regulators of EMT

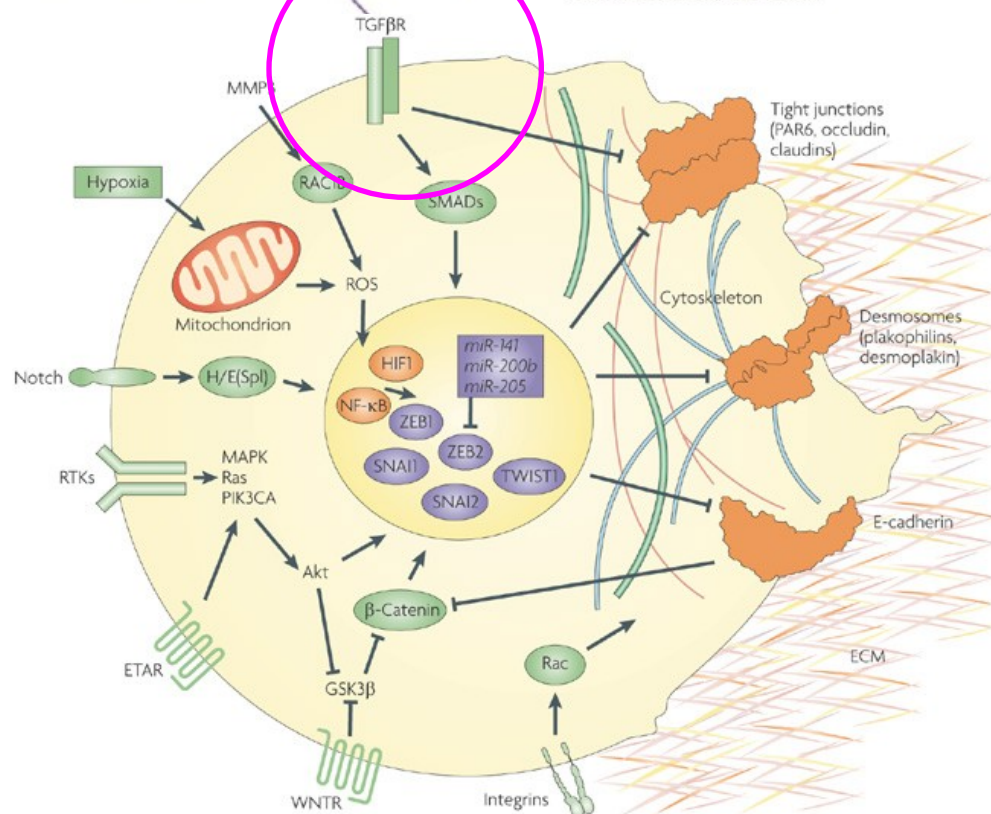
EMT Program	<i>E-cadherin</i>	Epithelial markers repressed
	<i>α-catenin</i> <i>γ-catenin</i>	
	<i>Vimentin</i> <i>Fibronectin</i> <i>N-cadherin</i>	Mesenchymal markers induced



Carcinoma in situ

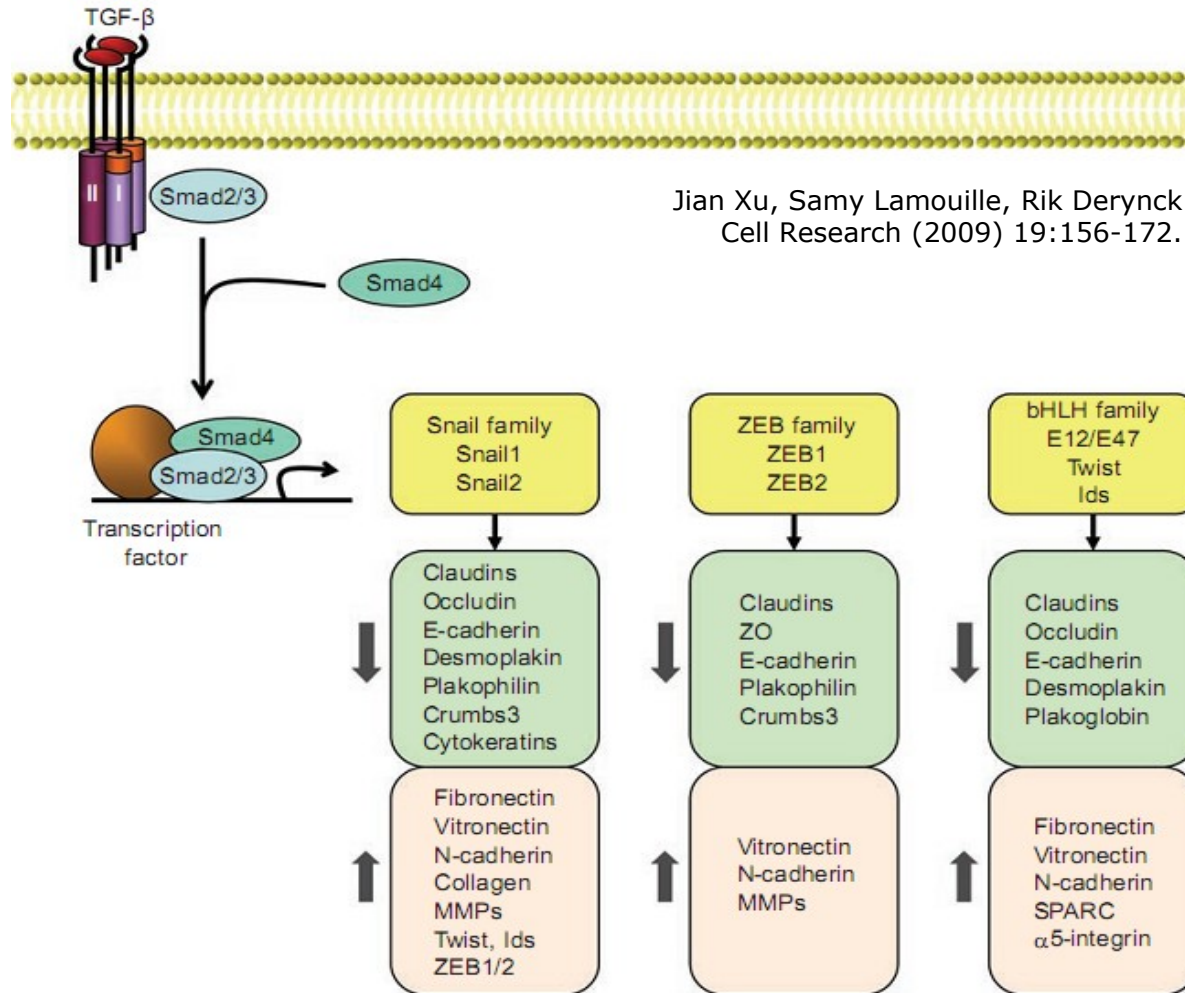


Invasive carcinoma



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

Transforming growth factor- β (TGF- β)

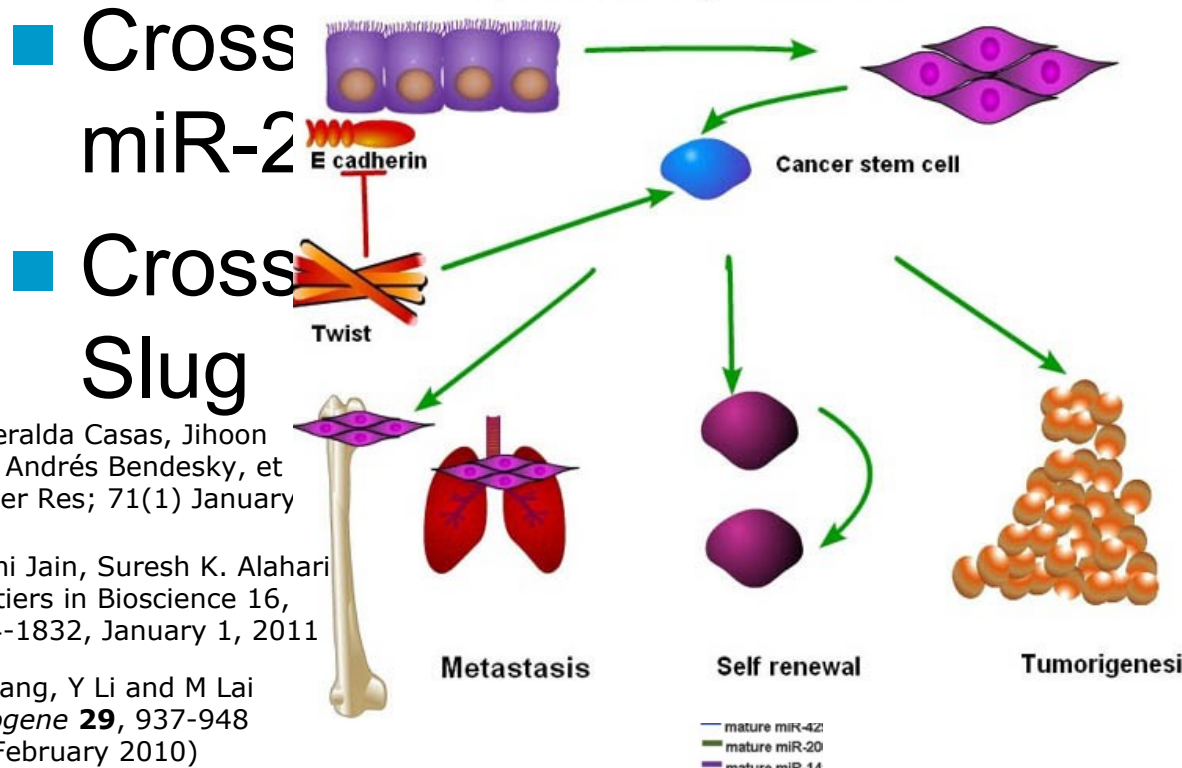


Jian Xu, Samy Lamouille, Rik Derynck
Cell Research (2009) 19:156-172.

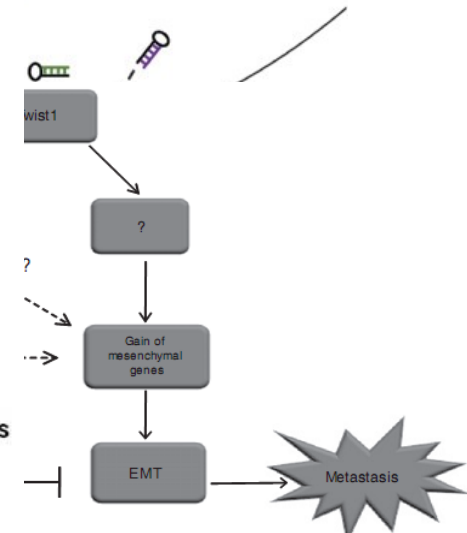
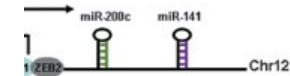
Recent discoveries in the EMT field

- EMT creates cells with cancer stem cell characteristics Mani SA, et al., Cell. 2008 May 16;133(4):704-15.

Epithelial Mesenchymal transition



31/2 and



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)

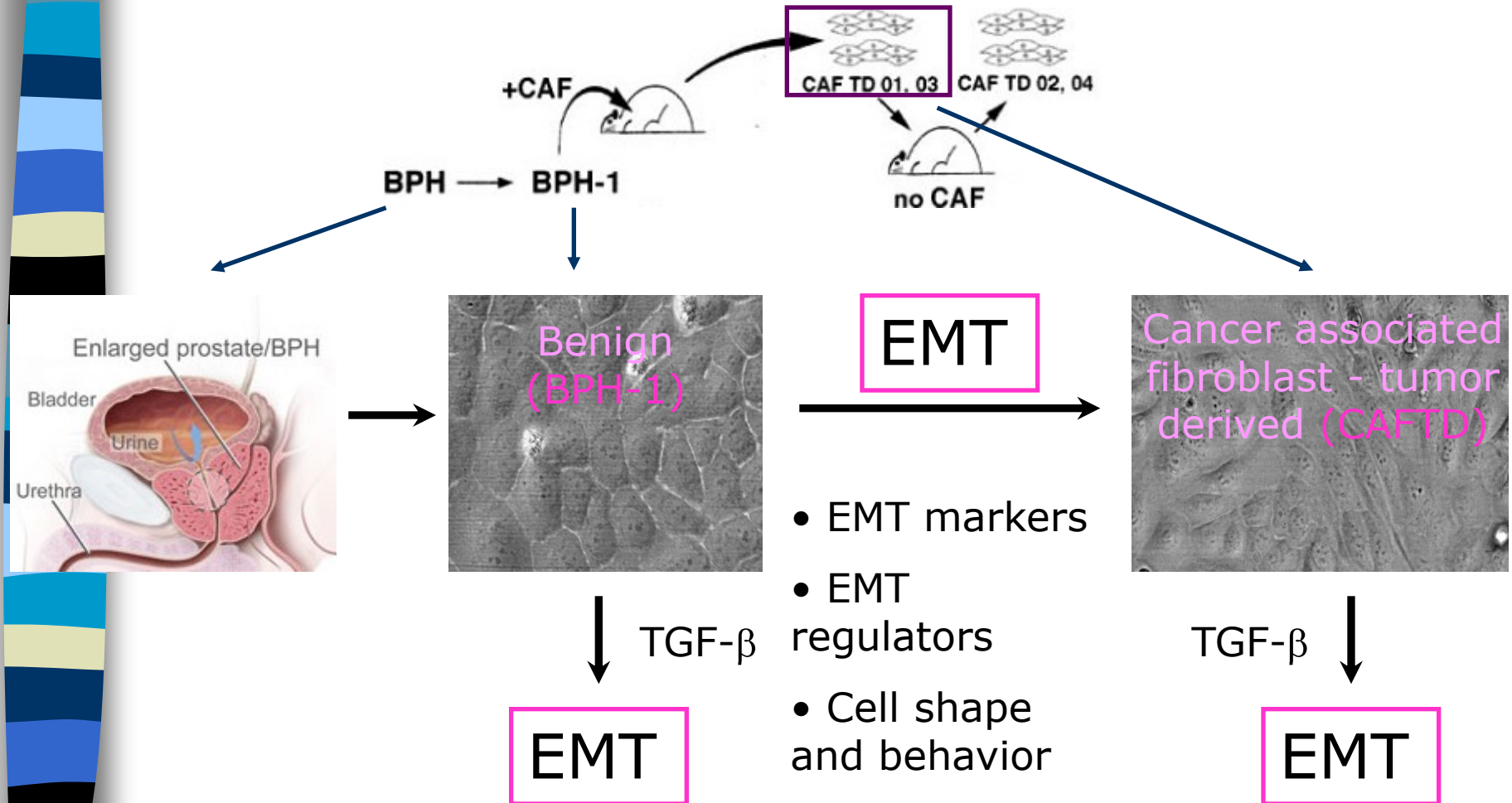
Experimental approach

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

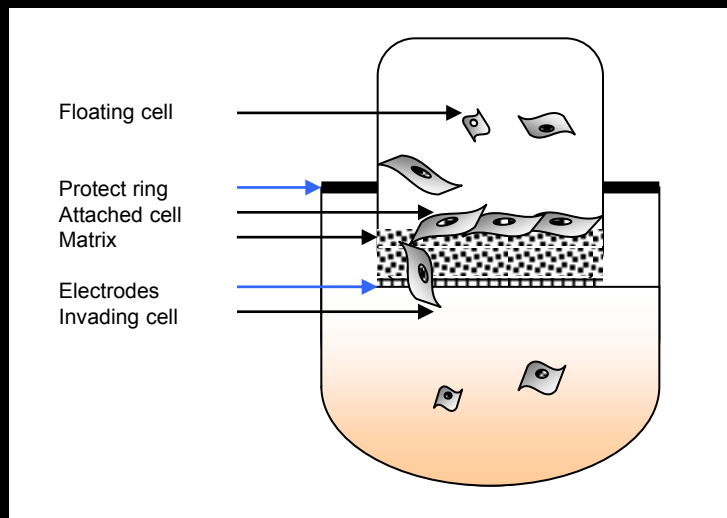
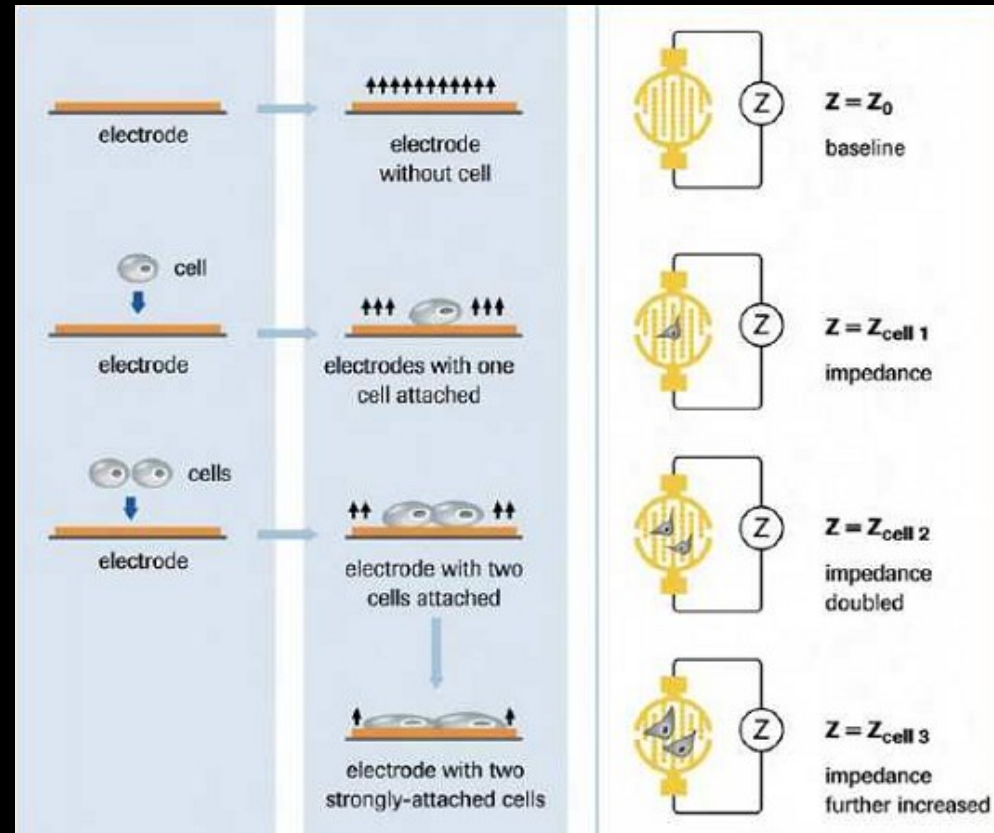
S. W. HAYWARD, R. DAHIYA, G. R. CUNHA, J. BARTEK, N. DESHPANDE, AND P. NARAYAN

Malignant Transformation in a Nontumorigenic Human Prostatic Epithelial Cell Line¹

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



xCELLigence – analýza migračního potenciálu





Dvojitá 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- β 1 je spojena s nádory tlustého střeva, prostaty a rozvojem fibrózy;
- polymorfismus genu pro TGF- β 1 vedoucí k jeho zvýšené produkci určuje predispozici k fibróze, hypertenzi a osteoporóze;
- 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?

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

NCBI Resources How To

PubMed.gov
U.S. National Library of Medicine
National Institutes of Health

Search: PubMed

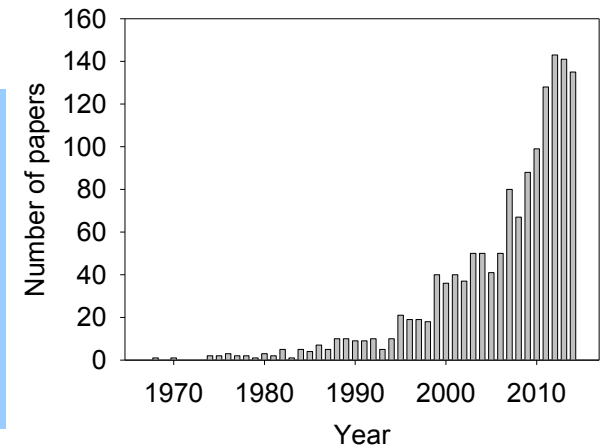
mic-1 OR Nag-1 OR GDF-15

RSS Save search Advanced search Help

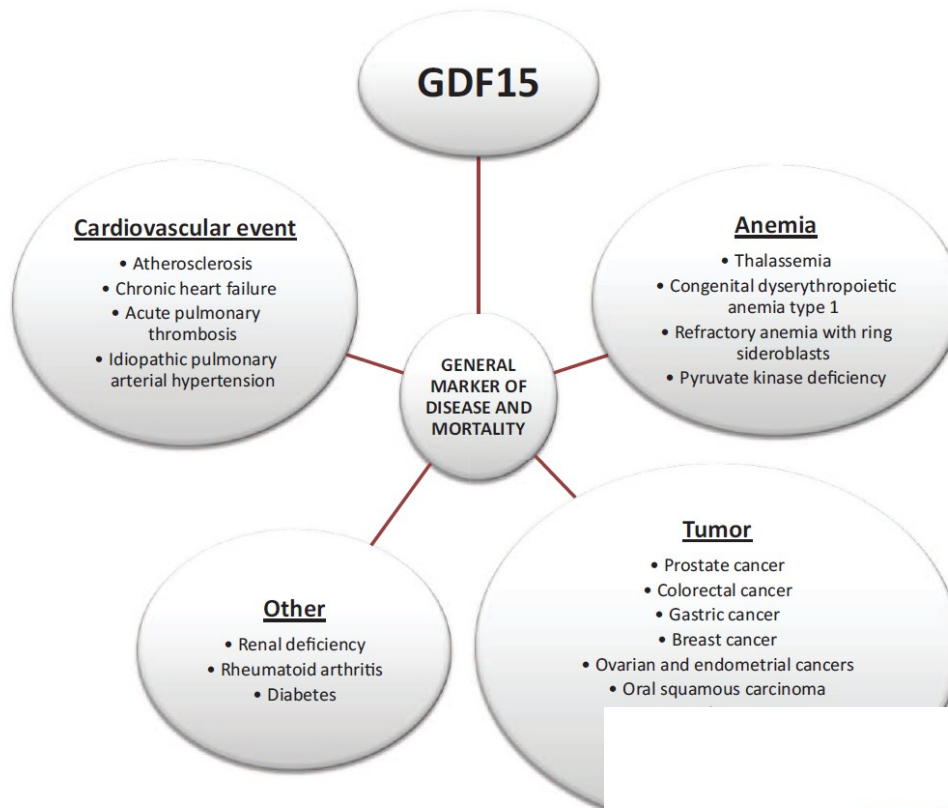
Search Clear

25/9/2014 ~ 1411 records

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



GDF-15 v patologických stavech



GDF 15 and Cancer

Pro-tumoral activity

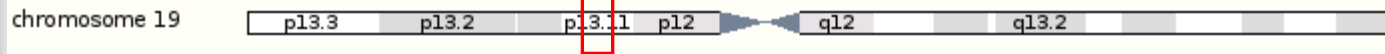
- proliferation and survival
- chemoprotection
- survival
- angiogenesis
- migration
- bone lysis
- immunomodulation

Anti-tumoral activity

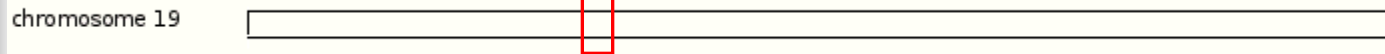
- growth arrest
- apoptosis

GDF15 gene

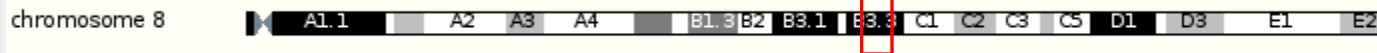
Homo sapiens



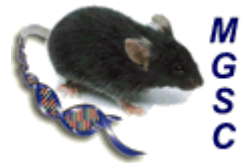
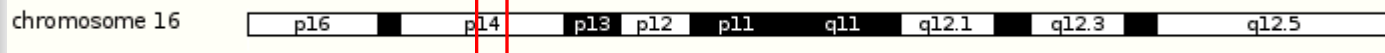
Pan troglodytes



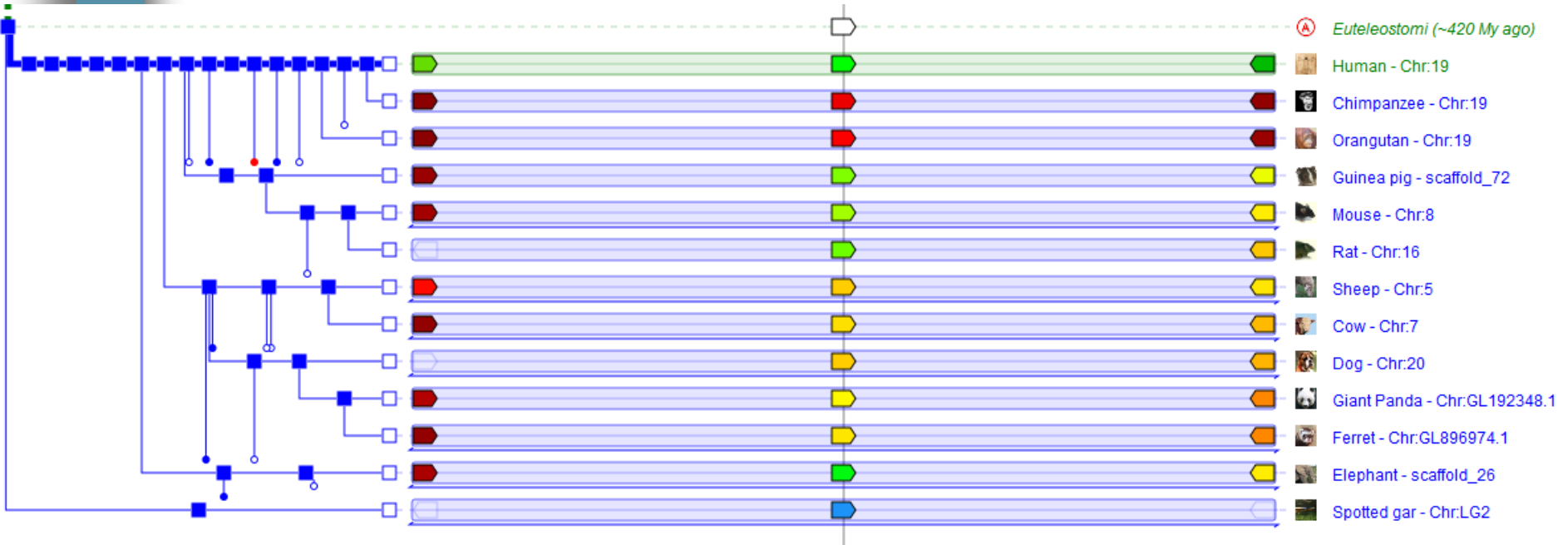
Mus musculus



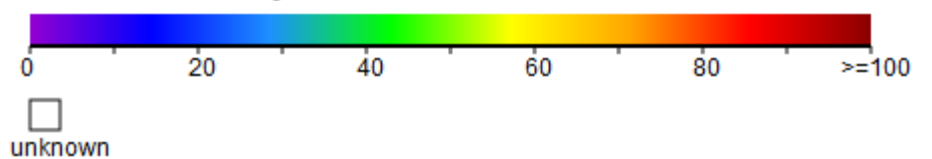
Rattus norvegicus



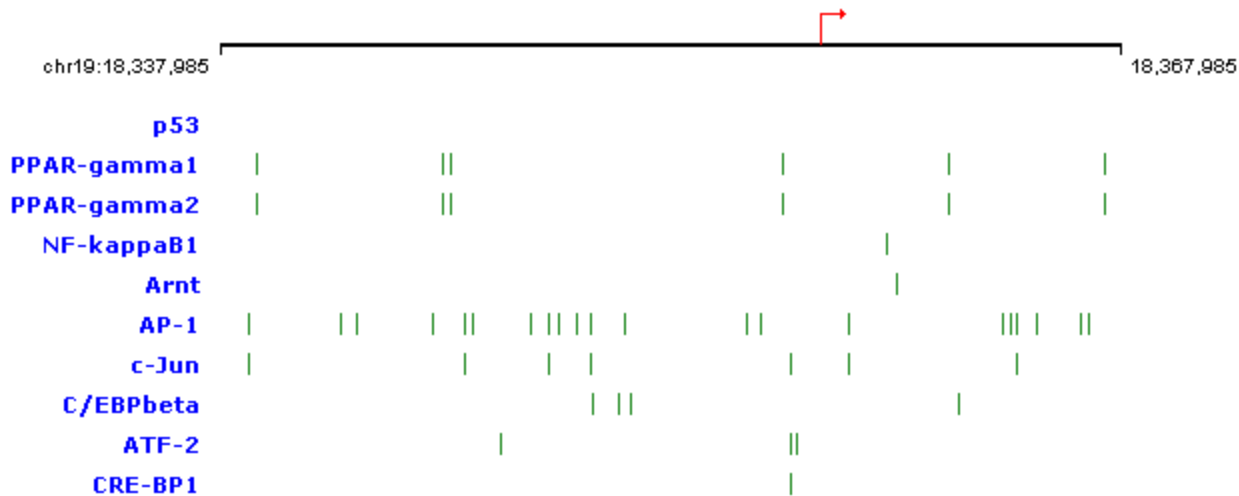
GDF-15: mezidruhová podobnost proteinu



Proteins Similarity - %

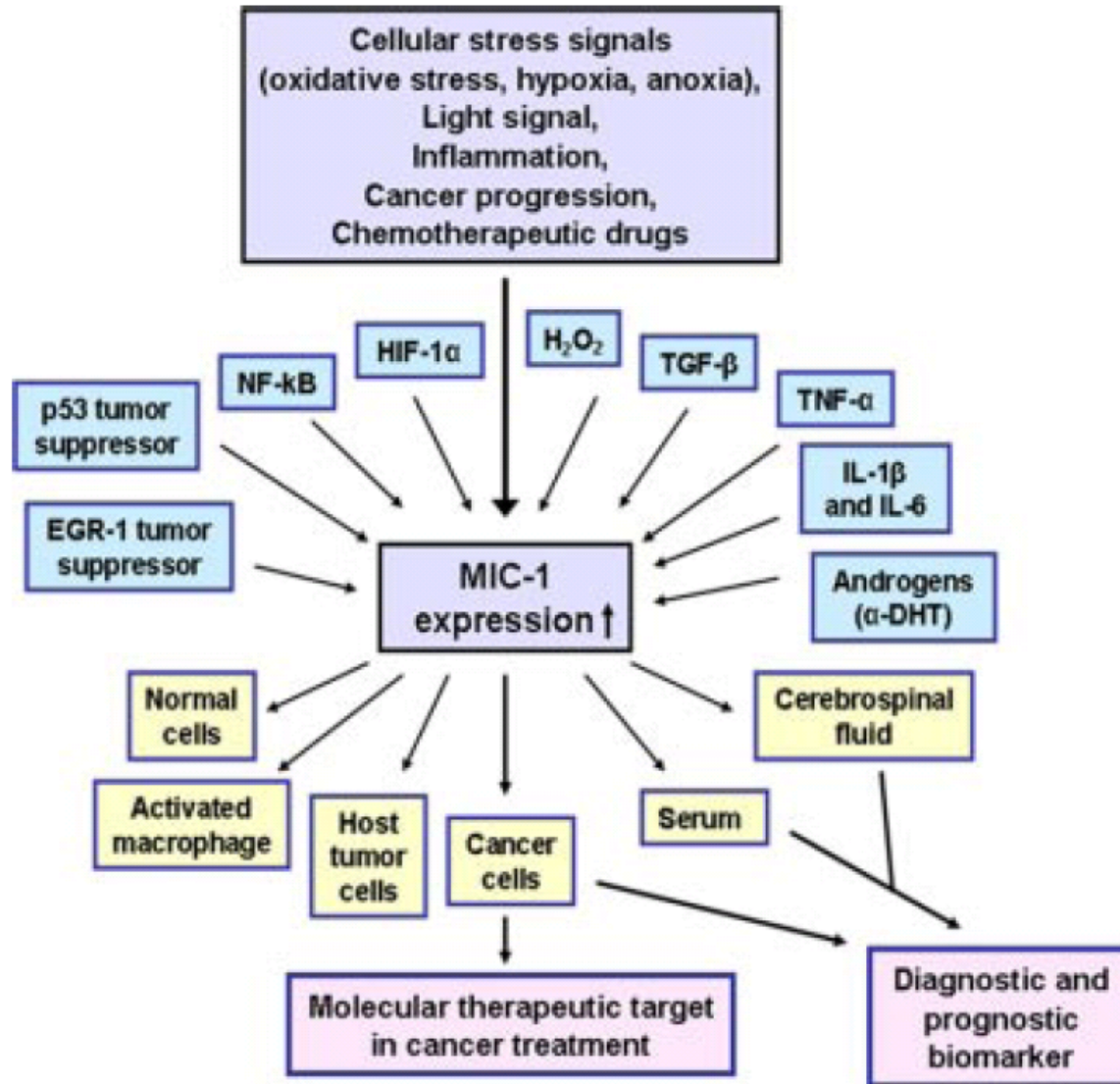


Control of *GDF15* expression

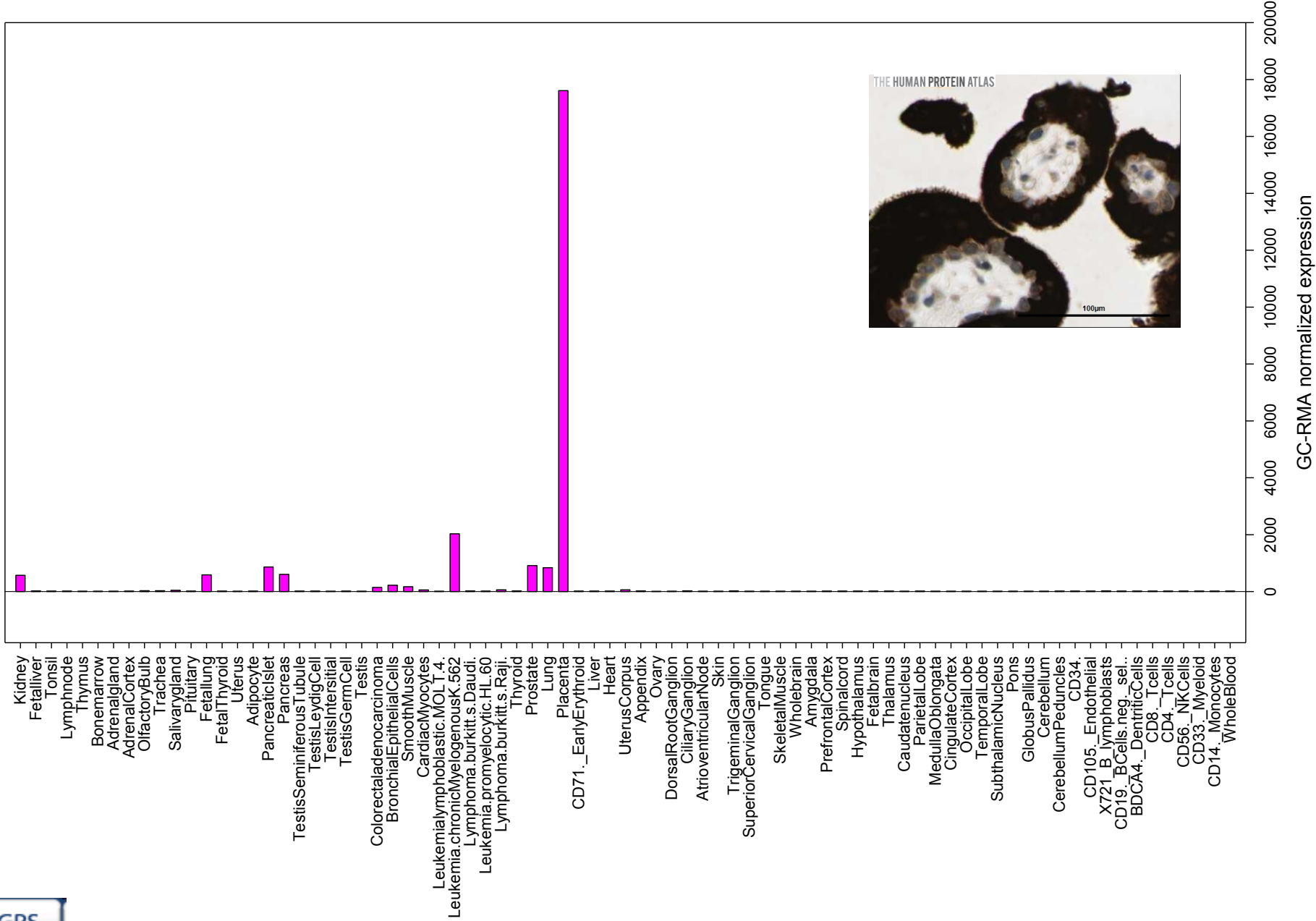


Legend: Transcription start site of *GDF15* Transcription factor binding site
Scale: 670 bp

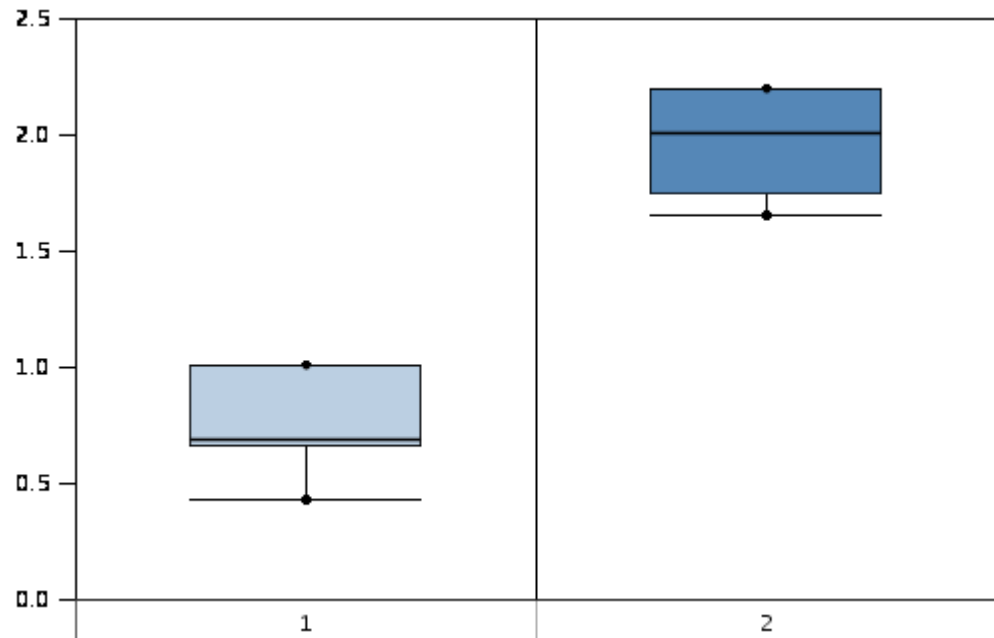
GDF-15 regulation



human GDF15 mRNA expression



GDF15 mRNA
normal vs. prostate adenocarcinoma



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

Various forms of GDF-15



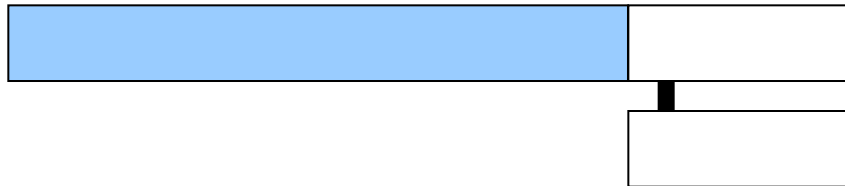
pro-GDF-15 monomer ~40kDa



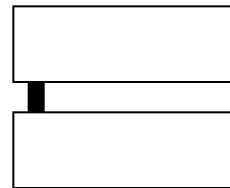
pro-GDF-15 dimer ~80kDa



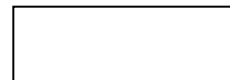
pro-GDF-15 hemidimer ~55kDa



propeptide ~28kDa



mature GDF-15 dimer ~30kDa



mature GDF-15 monomer ~15kDa

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

Adrenal gland

Appendix

Bone marrow

Breast

Bronchus

Cerebellum

Cerebral cortex

Cervix, uterine

Colon

Corpus, uterine 1

Corpus, uterine 2

Duodenum

Epididymis

Esophagus

Fallopian tube

Gall bladder

Heart muscle

Hippocampus

Kidney

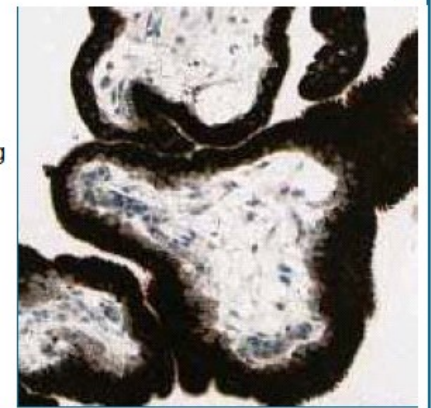
Lateral ventricle

Liver

Lung

Normal Tissues - IHC

cortical cells		<u>Lymph node</u>	lymphoid cells outside reaction centra	
glandular cells			reaction center cells	
lymphoid tissue		<u>Nasopharynx</u>	respiratory epithelial cells	
bone marrow poietic cells		<u>Oral mucosa</u>	squamous epithelial cells	
glandular cells		<u>Ovary</u>	follicle cells	
respiratory epithelial cells			ovarian stromal cells	
cells in granular layer		<u>Pancreas</u>	exocrine glandular cells	
cells in molecular layer			islet cells	
purkinje cells		<u>Parathyroid gland</u>	glandular cells	
glial cells		<u>Placenta</u>	decidual cells	
neuronal cells			trophoblastic cells	
glandular cells		<u>Prostate</u>	glandular cells	
squamous epithelial cells		<u>Rectum</u>	glandular cells	
glandular cells		<u>Salivary gland</u>	glandular cells	
cells in endometrial stroma		<u>Seminal vesicle</u>	glandular cells	
glandular cells		<u>Skeletal muscle</u>	myocytes	
cells in endometrial stroma		<u>Skin</u>	adnexal cells	
glandular cells			epidermal cells	
glandular cells		<u>Small intestine</u>	glandular cells	
glandular cells		<u>Smooth muscle</u>	smooth muscle cells	
squamous epithelial cells		<u>Soft tissue 1</u>	mesenchymal cells	
glandular cells		<u>Soft tissue 2</u>	mesenchymal cells	
glandular cells		<u>Spleen</u>	cells in red pulp	
myocytes			cells in white pulp	
glial cells		<u>Stomach 1</u>	glandular cells	
neuronal cells		<u>Stomach 2</u>	glandular cells	
cells in glomeruli		<u>Testis</u>	cells in seminiferus ducts	
cells in tubules			leydig cells	
glial cells		<u>Thyroid gland</u>	glandular cells	
neuronal cells		<u>Tonsil</u>	lymphoid cells outside reaction centra	
bile duct cells			reaction center cells	
hepatocytes		<u>Urinary bladder</u>	squamous epithelial cells	
alveolar cells		<u>Vagina</u>	urothelial cells	
macrophages		<u>Vulva/anal skin</u>	squamous epithelial cells	
			squamous epithelial cells	

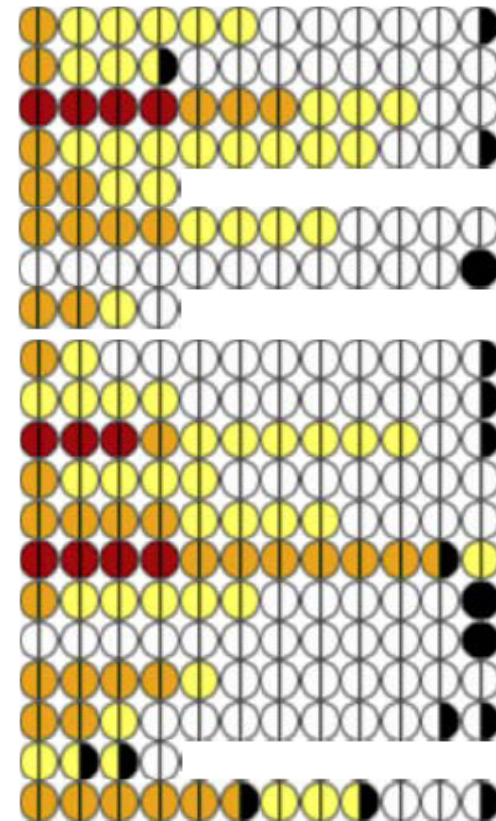


Protein expression

- 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

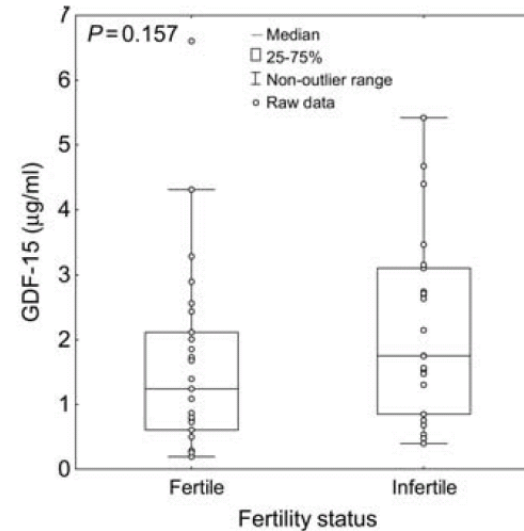
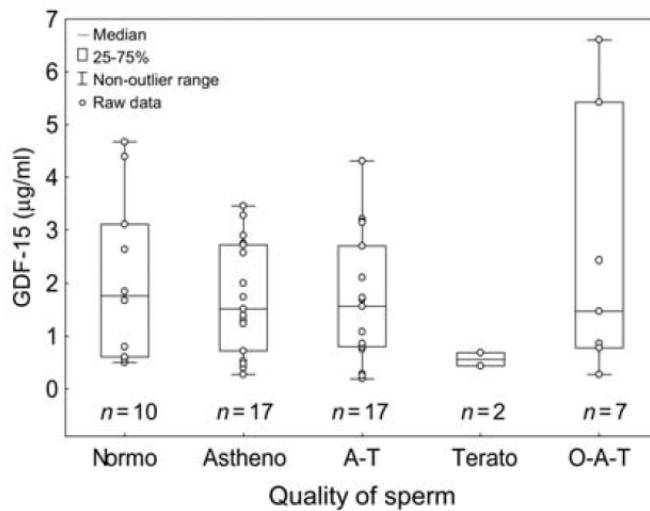
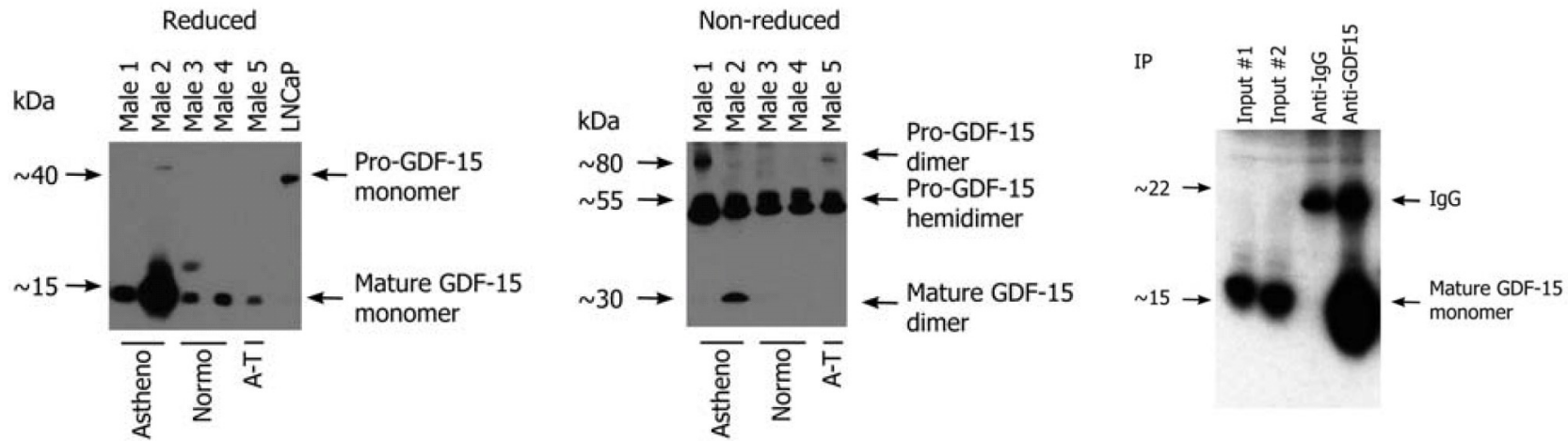


Protein expression

- Strong
- Moderate
- Weak
- Negative
- Not representative

GDF-15	Concentration	Reference
Normal	450 50 pg/ml	Tanno, T. et al., <i>Nat Med</i> 13 (9), 1096 (2007)
beta-thalassemia syndrome	66,000 9,600 pg/ml	
CSF - non-neoplastic	156 pg/m	Sophie Shnaper et al., <i>International Journal of Cancer</i> in press (2009).
CSF - glioblastoma	229 pg/ml	
normal	495 pg/ml	Brown, D. A. et al., <i>Clin Cancer Res</i> 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., <i>Blood</i> 112 (13), 5241 (2008).
Normal	16.1 23.4 pg/ml	Baek, K. E. et al., <i>Clinica Chimica Acta</i> 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., <i>Cancer Epidemiology Biomarkers & Prevention</i> 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., <i>Clin Cancer Res</i> 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., <i>The Lancet</i> 359 (9324), 2159 (2002).
Women w/o cardiovascular events	538 pg/mL	

Growth/differentiation factor-15 is an abundant cytokine in human seminal plasma



The Transforming Growth Factor- β Superfamily Cytokine Macrophage Inhibitory Cytokine-1 Is Present in High Concentrations in the Serum of Pregnant Women*

A. G. MOORE†, D. A. BROWN†, W. D. FAIRLIE, A. R. BAUSKIN, P. K. BROWN, M. L. C. MUNIER, P. K. RUSSELL, L. A. SALAMONSEN, E. M. WALLACE, AND S. N. BREIT

Centre for Immunology (A.G.M., D.A.B., W.D.F., A.R.B., P.K.B., M.L.C.M., P.K.R., S.N.B.), St. Vincent's Hospital and University of New South Wales, Sydney, New South Wales, Australia; Department of Obstetrics and Gynecology (E.M.W.), Monash University, Clayton, Victoria, Australia; and Prince Henry's Institute of Medical Research (L.A.S.), Clayton, Victoria, Australia

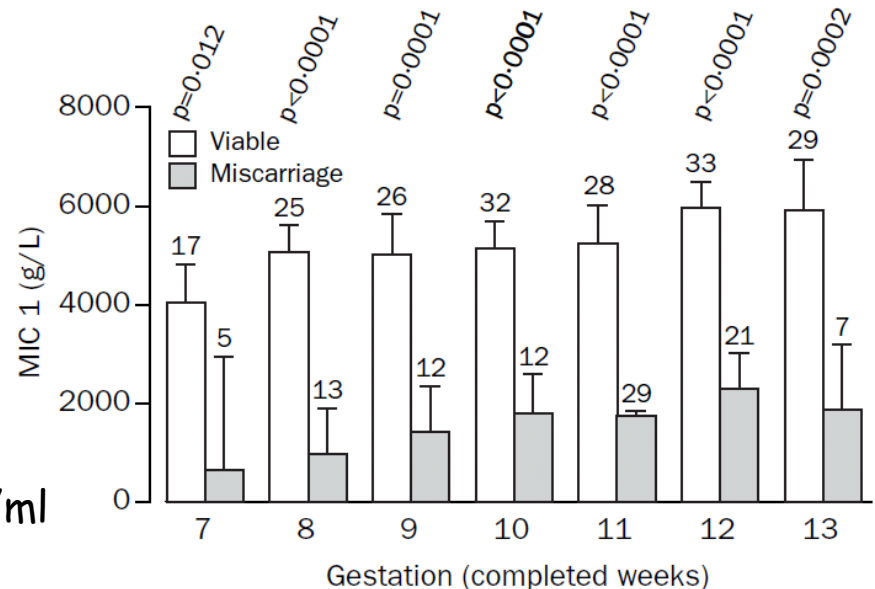
normal 0.36 0.04 ng/mL
first trimester 6.3 ± 0.02 ng/mL
second trimester 12.2 ± 0.5 ng/mL
third trimester 15.3 ± 1.3 ng/mL

Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage

Stephen Tong, Budi Marjono, David A Brown, Shella Mulvey, Samuel N Breit, Ursula Manueljilal, Euan M Wallace

Lancet 2004; 363: 129-30

? x E-02 mg/ml

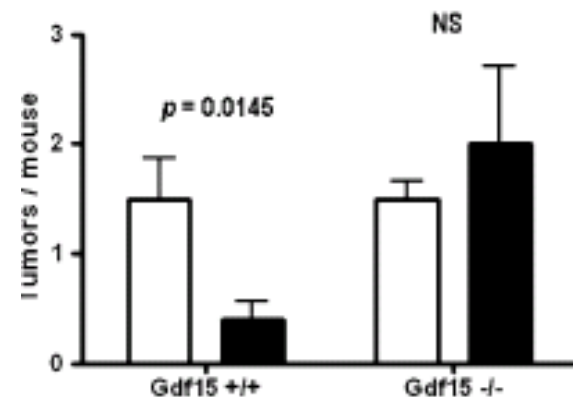


Loss of GDF-15 abolishes Sulindac chemoprevention in the $Apc^{Min/+}$ mouse model of intestinal cancer

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



Colon



Oncogene (2009), 1–10

© 2009 Macmillan Publishers Limited All rights reserved 0950-9232/09 \$32.00

www.nature.com/onc



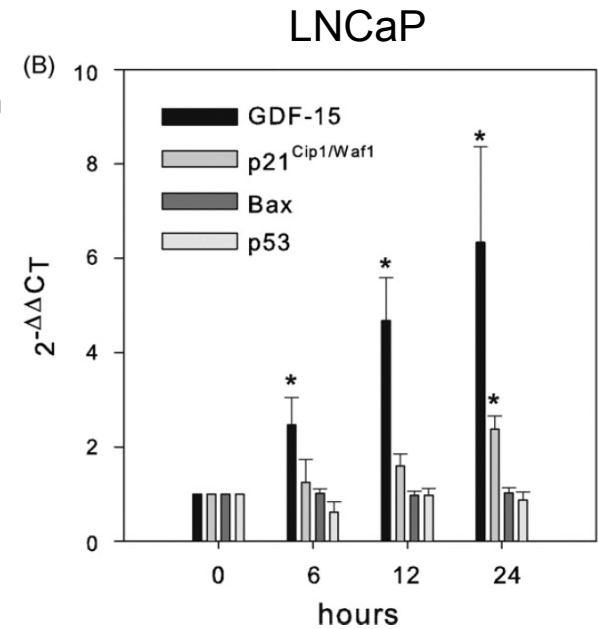
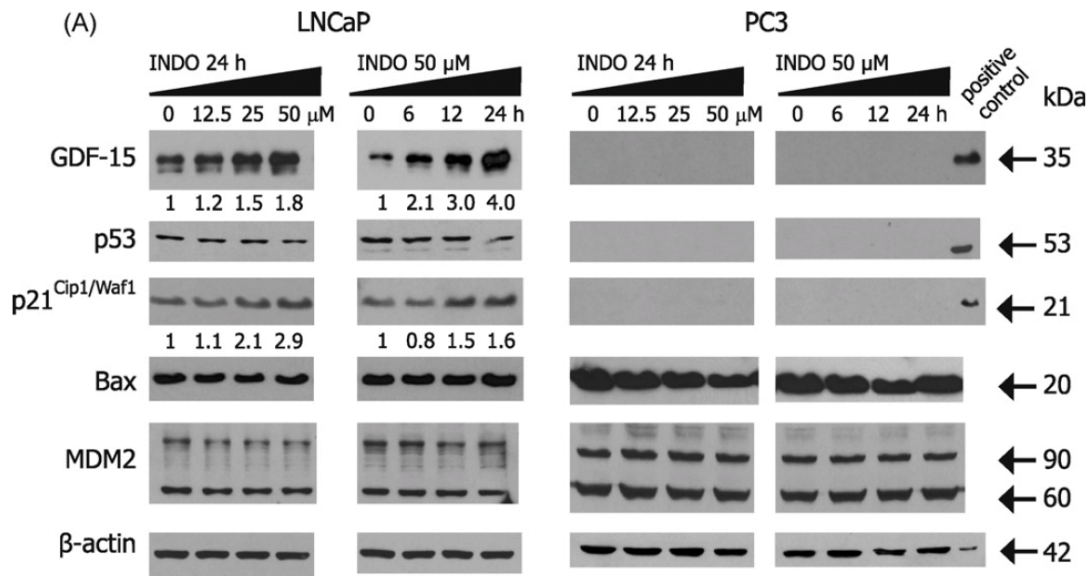
ORIGINAL ARTICLE

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

S Senapati¹, S Rachagani¹, K Chaudhary¹, SL Johansson^{2,3}, RK Singh^{2,3} and SK Batra^{1,3}

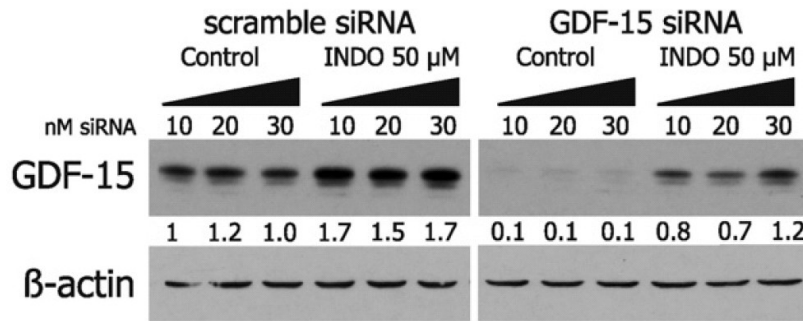
¹Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA; ²Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE, USA and ³Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha,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

GDF-15 & NSAIDs

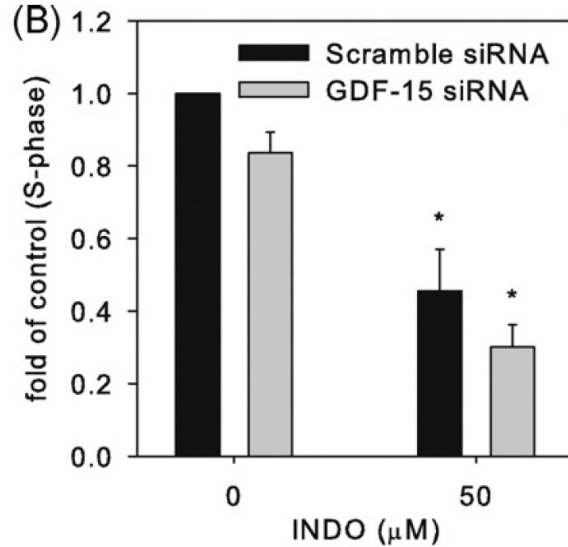


GDF-15 & NSAIDs

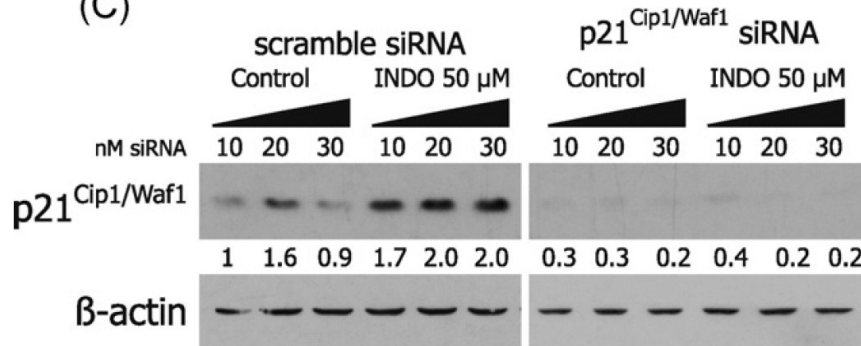
(A)



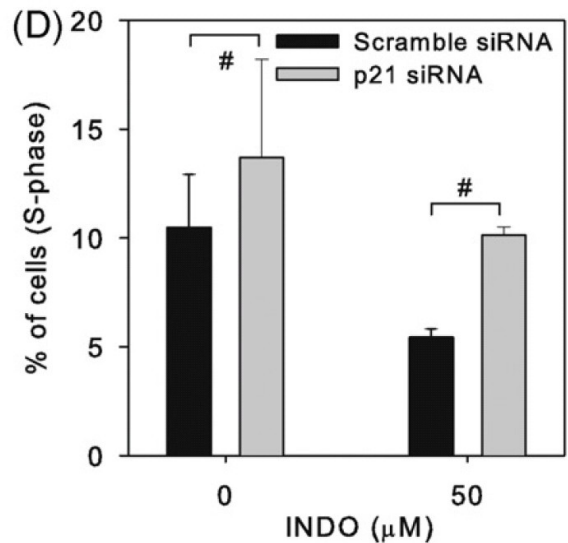
(B)



(C)

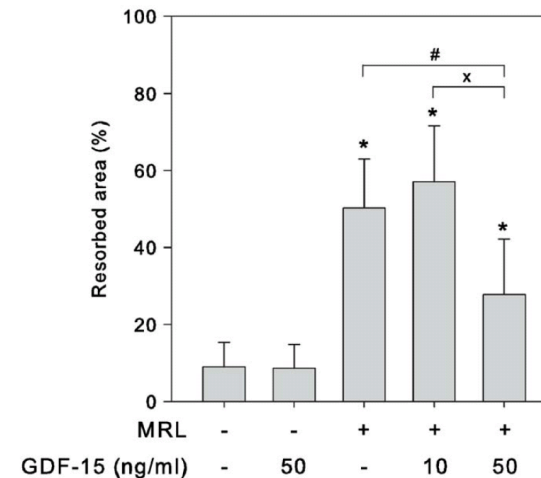
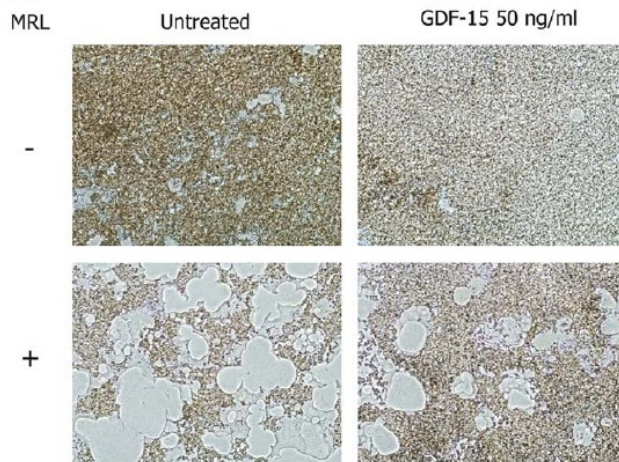
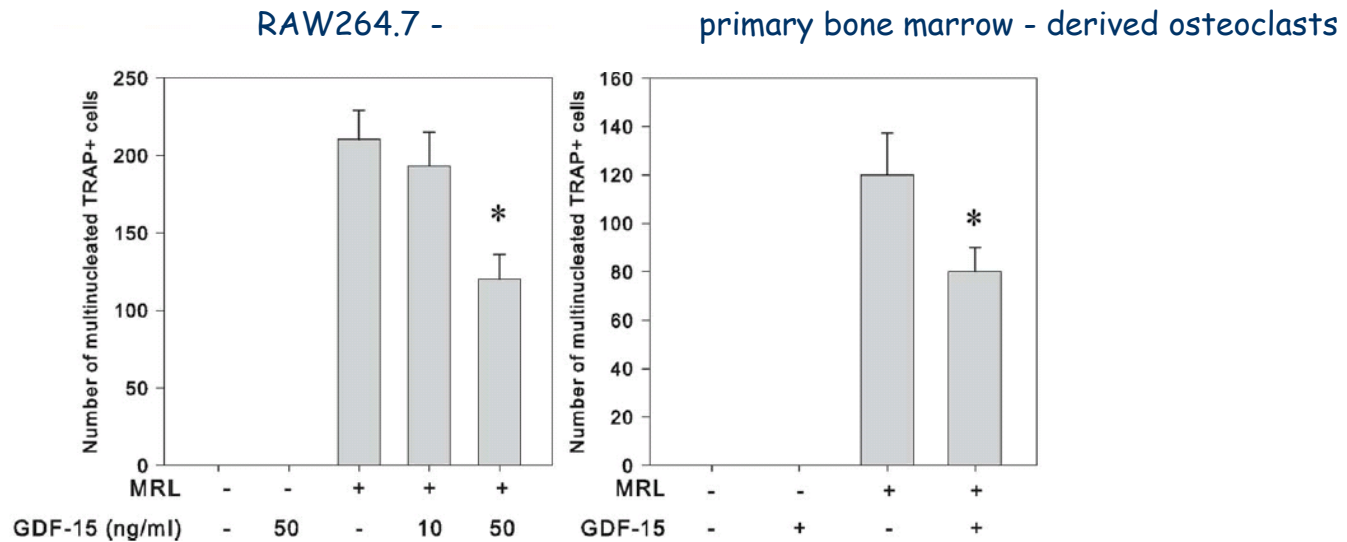


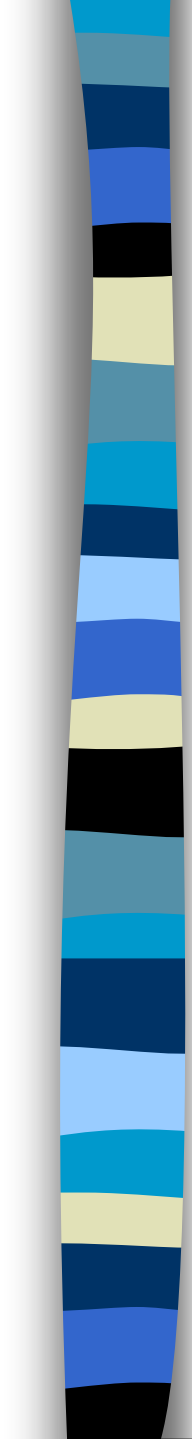
(D)



Growth-differentiation factor-15 **inhibits** differentiation into osteoclasts

- A novel factor involved in control of osteoclast differentiation





BIOLOGY OF REPRODUCTION 80, 1036–1045 (2009)
Published online before print 21 January 2009.
DOI 10.1095/biolreprod.108.074658

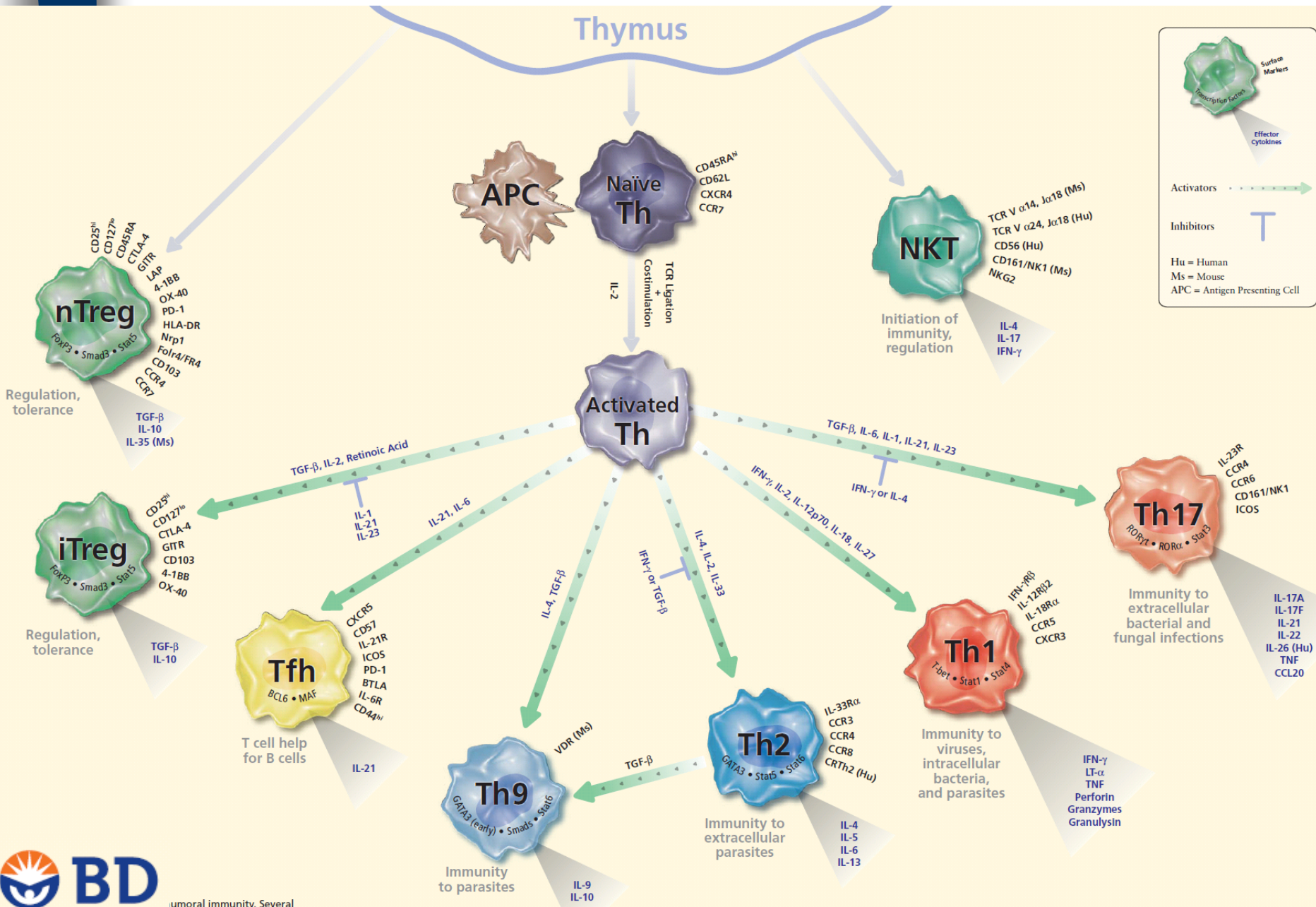
Seminal Fluid Drives Expansion of the CD4⁺CD25⁺ T Regulatory Cell Pool and Induces Tolerance to Paternal Alloantigens in Mice¹

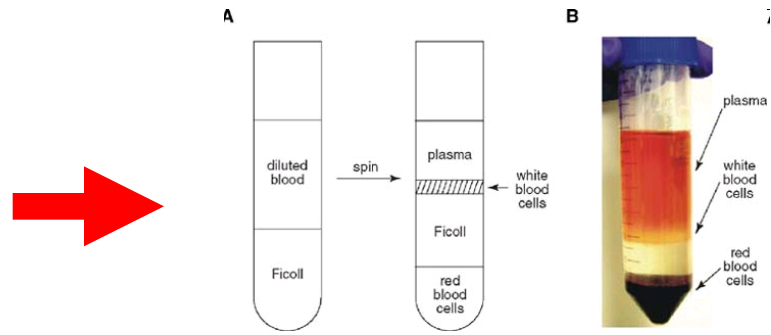
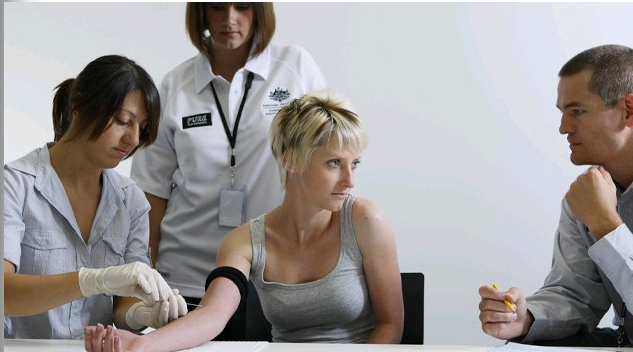
Sarah A. Robertson,² Leigh R. Guerin, John J. Bromfield, Kim M. Branson, Aisling C. Ahlström, and Alison S. Care

Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, South Australia, Australia

.....Both seminal plasma and sperm components of the seminal fluid are necessary to confer full tolerance and elicit the Treg cell response, potentially through provision of immunedeviating cytokines and antigens, respectively

CD4+ T Cell Differentiation

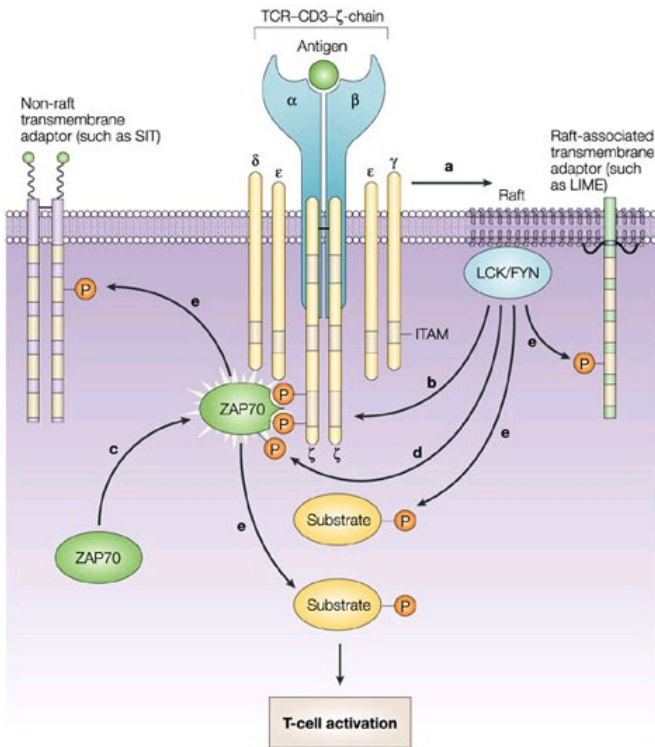




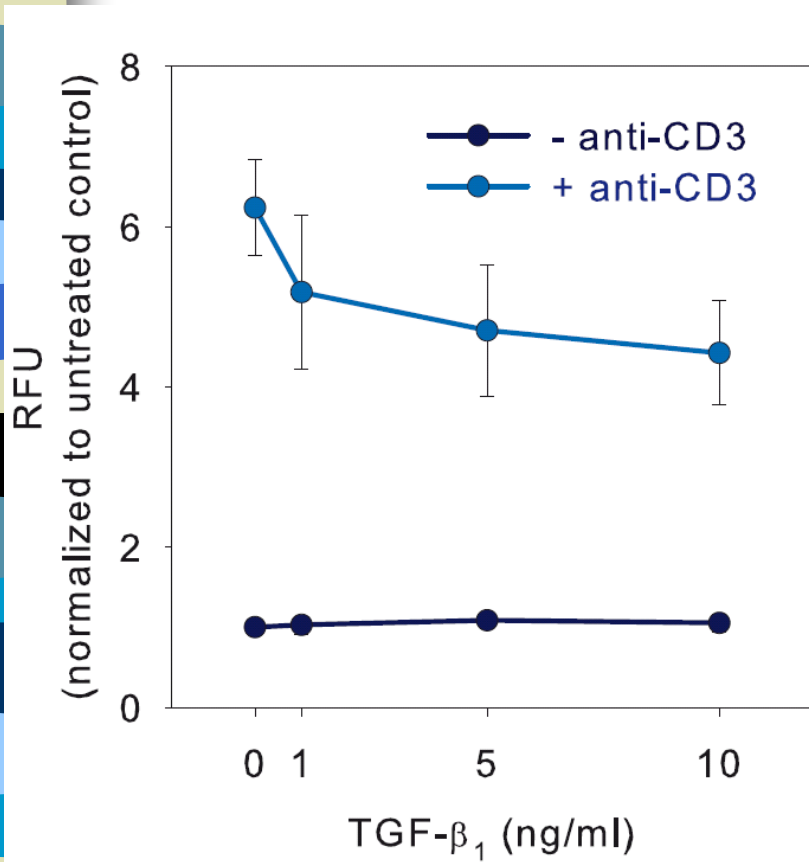
CD3-coated plates +
in vitro treatment



cells quantification - CyQuant



Naive T-cells activation



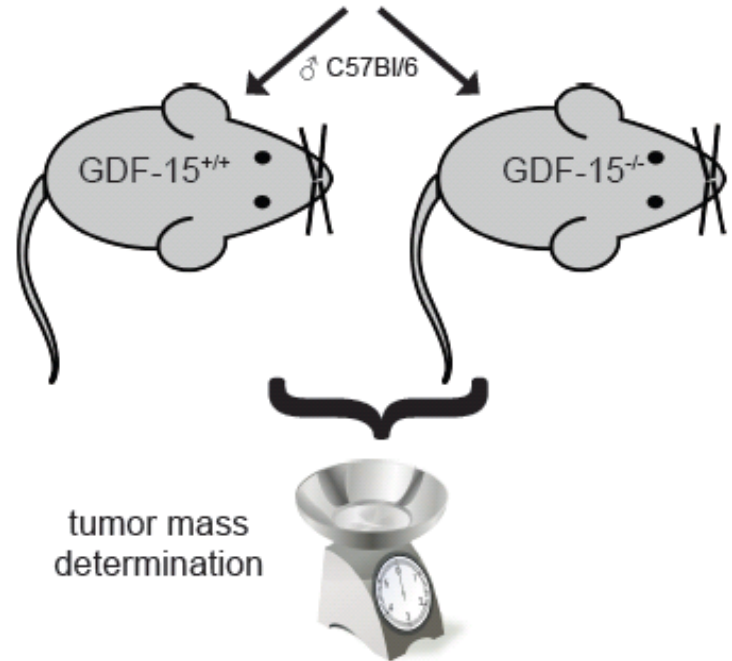
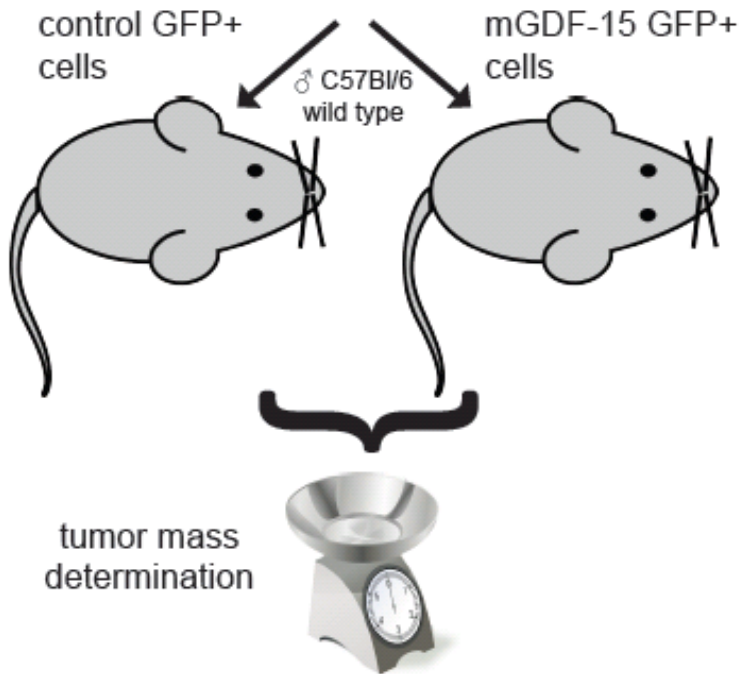
GDF-15 & mice prostate cancer

- The TRAMP-C1 (ATCC - CRL-2730) cell line was derived in 1996 by Norman Greenberg from a heterogeneous 32 week primary tumor in the prostate of a PB-Tag C57BL/6 (TRAMP) mouse.
- TRAMP is a transgenic line of C57BL/6 mice harboring a construct comprised of the minimal -426/+28 rat probasin promoter (426 base pairs of the rat probasin (PB) gene promoter and 28 base pairs of 5'-untranslated region) to target expression of the SV40 large T antigen to prostatic epithelium.
- TRAMP-C1 cells are tumorigenic when grafted into syngeneic C57BL/6 hosts.

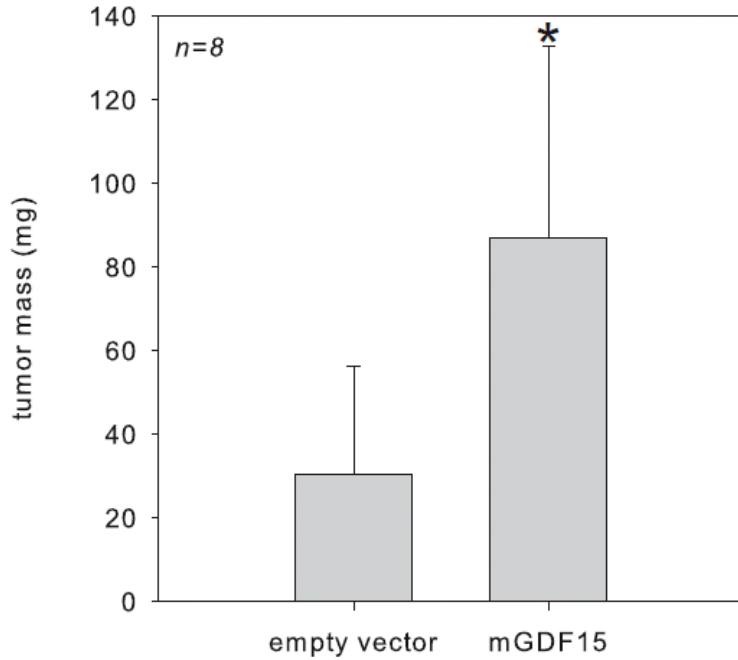


- control GFP
- mGDF-15 GFP
transfected TRAMP-C1 cells

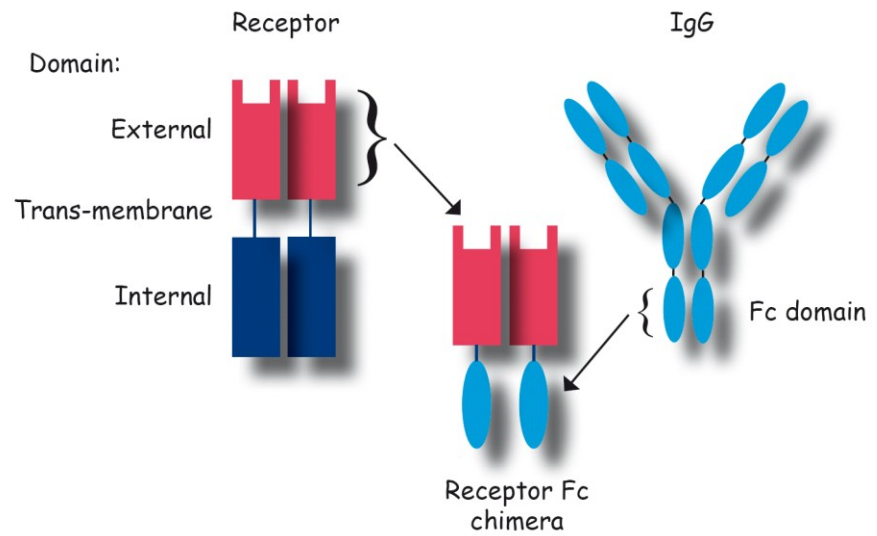
TRAMP-C1 cells



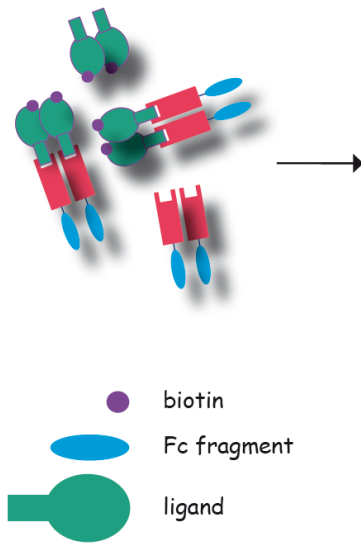
GDF-15 stimulates growth of syngeneic grafts of TRAMP-C1 cells



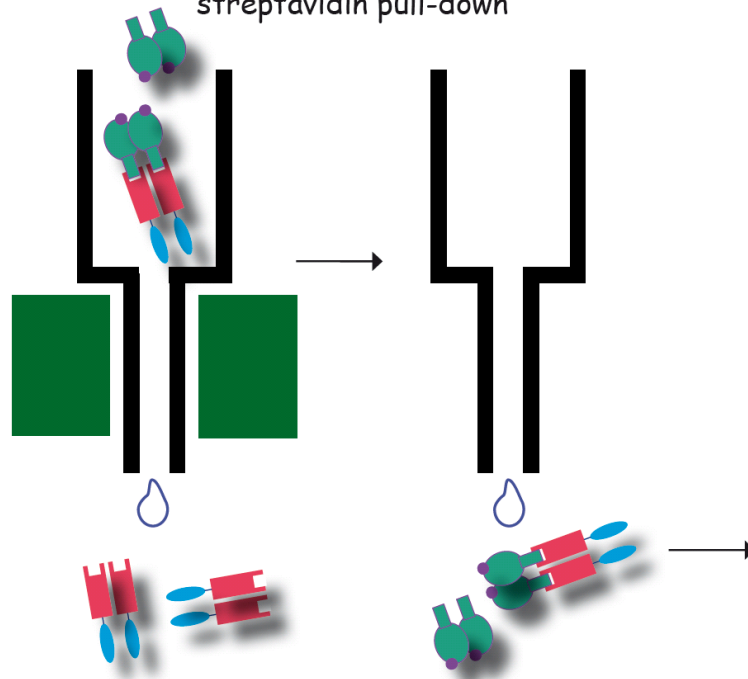
GDF-15 signaling



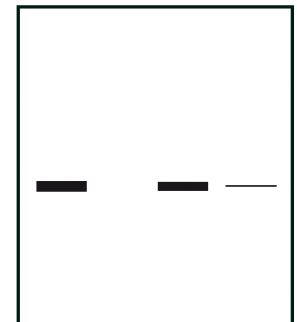
Incubation:
Biotinylated ligand +
Receptor Fc Chimera



μ MACS MicroBeads
streptavidin pull-down

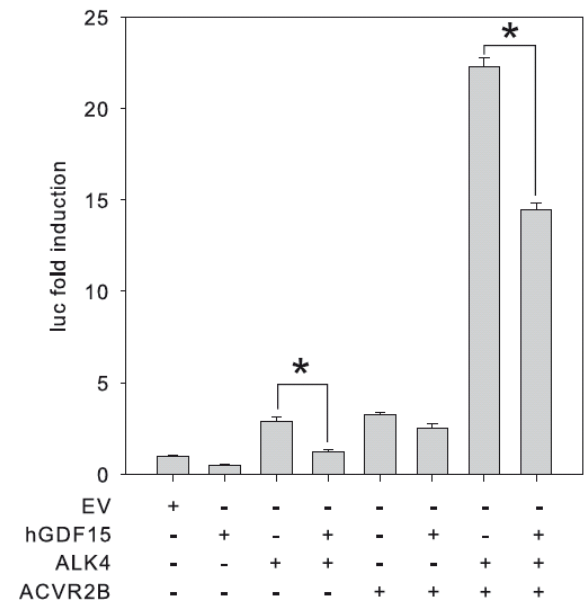
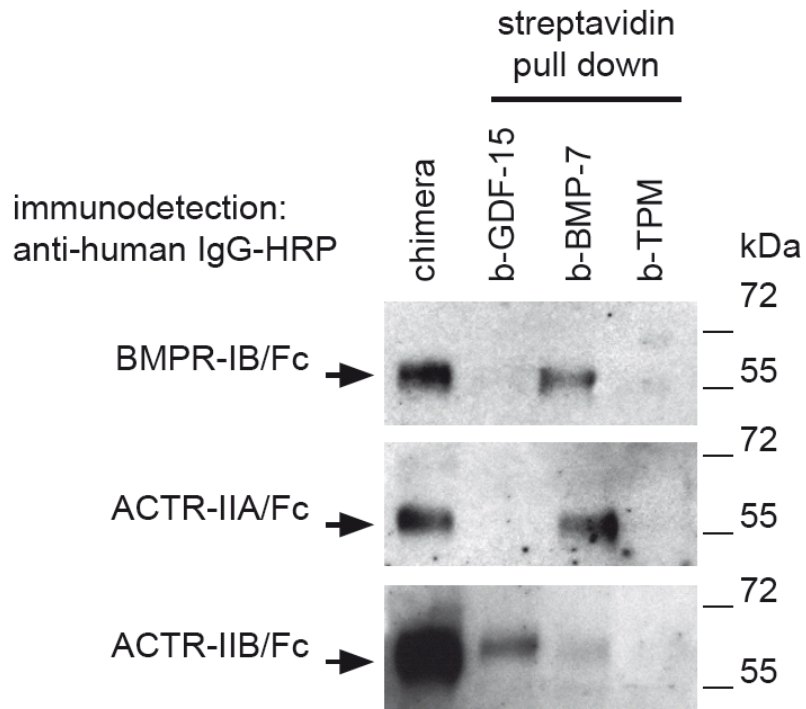


western blot
anti-IgG antibody



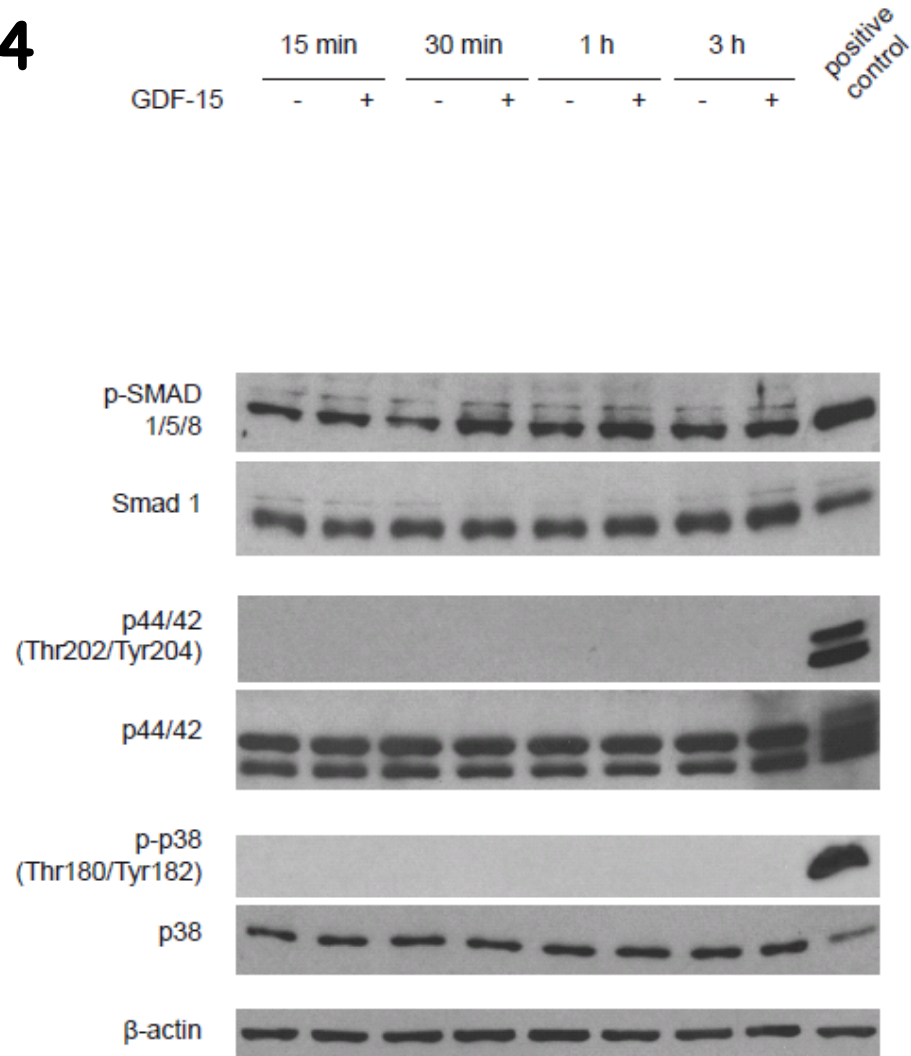
- biotin
- Fc fragment
- ligand

GDF-15 signaling



GDF-15 signaling

LAPC-4





Summary

GDF-15:

- inhibits naïve T-cells activation (proliferation) and induce Treg *in vitro*;
- its overexpression potentiate tumor graft progression in syngeneic hosts;
- tumor graft progression is significantly potentiate by ectopic overexpression of mGDF-15 and inhibited in GDF-15 knockout mice;
- GDF-15 binds ACVR2B receptors and activates SMAD2 in LAPC-4 cells



Shrnutí přednášky

- 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.