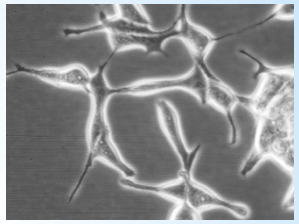
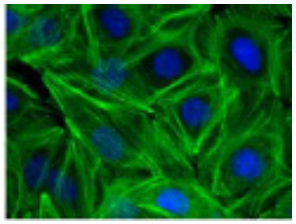
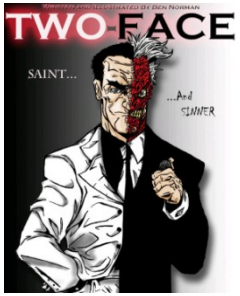
Growth factors in cancer cell signaling

- **pathological plasticity of epithelial cells**
  - neuroendocrine differentiation (NED)
    - senescence associated secretory phenotype
    - cell cycle
  - epithelial-mesenchymal transition (EMT)
    - cancer stem cells (CSCs)
    - role of MDM2

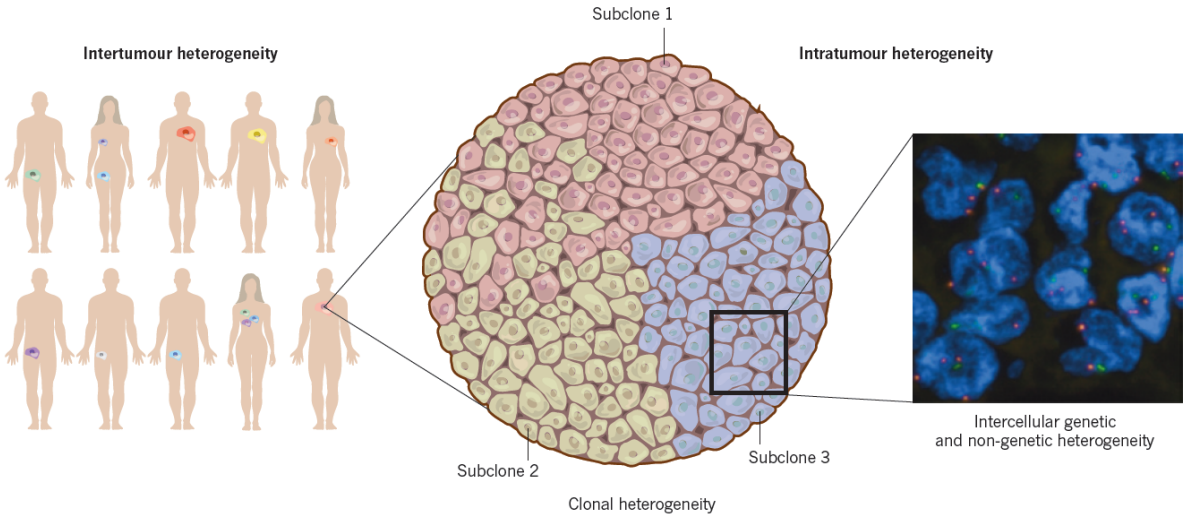


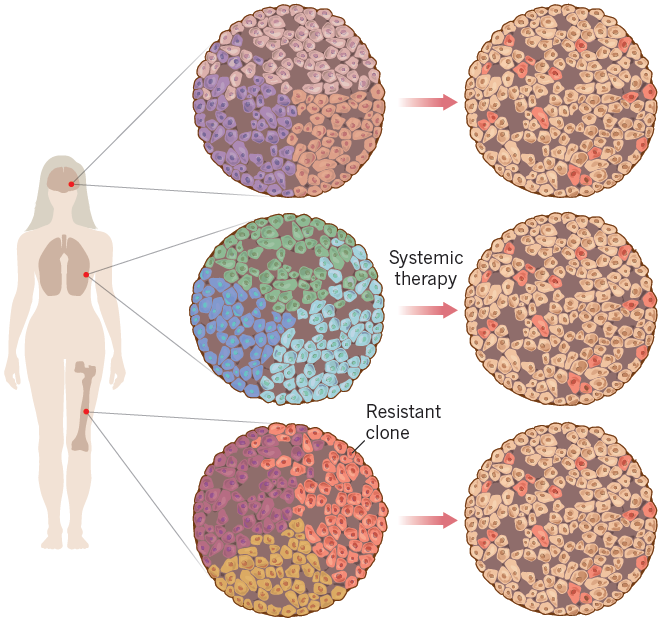


- **Transforming Growth Factor-β signal transduction**
  - Growth/differentiation factor – 15
    - signal transduction
    - role in cancer and damaged hematopoiesis

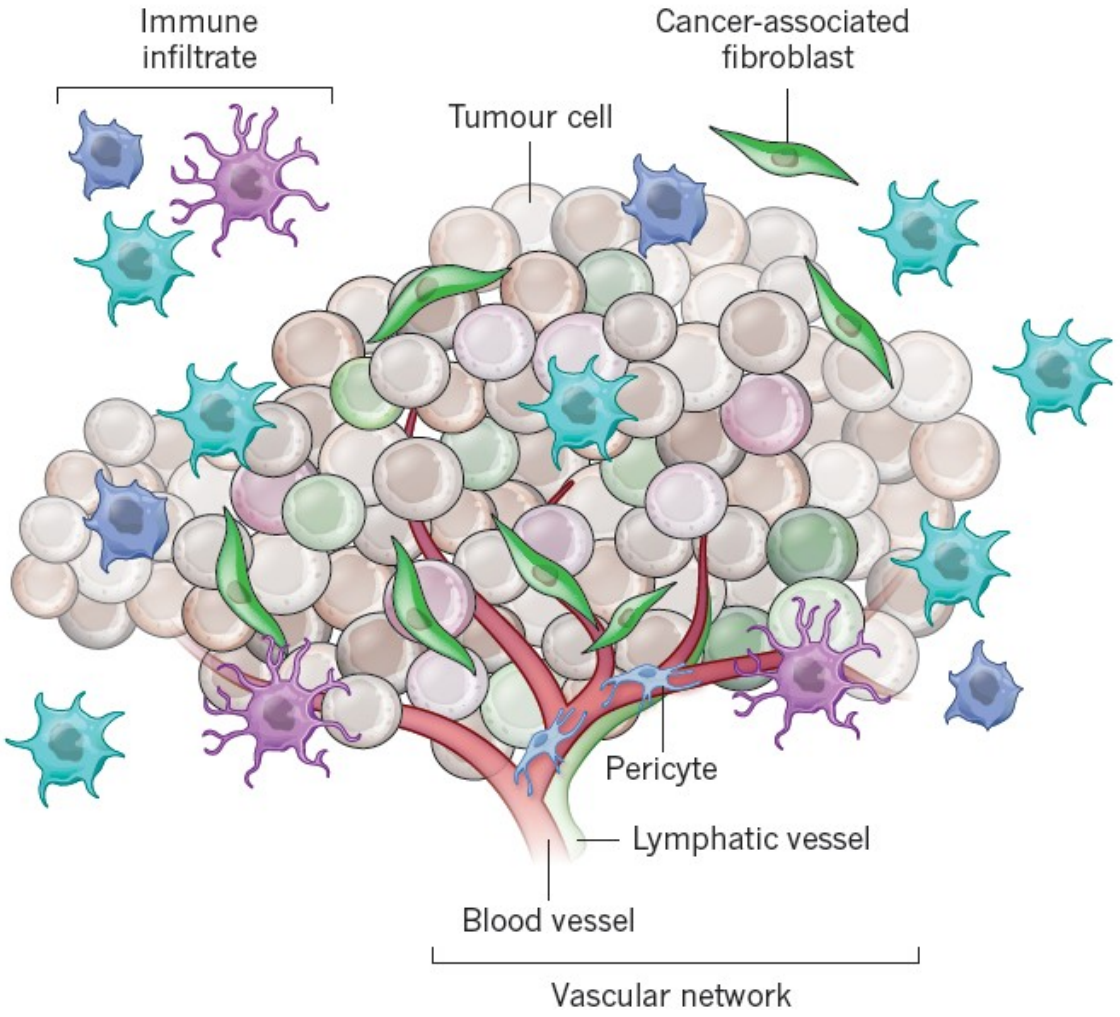


Cancer is heterogeneous …





…and **not** a single cell type disease.



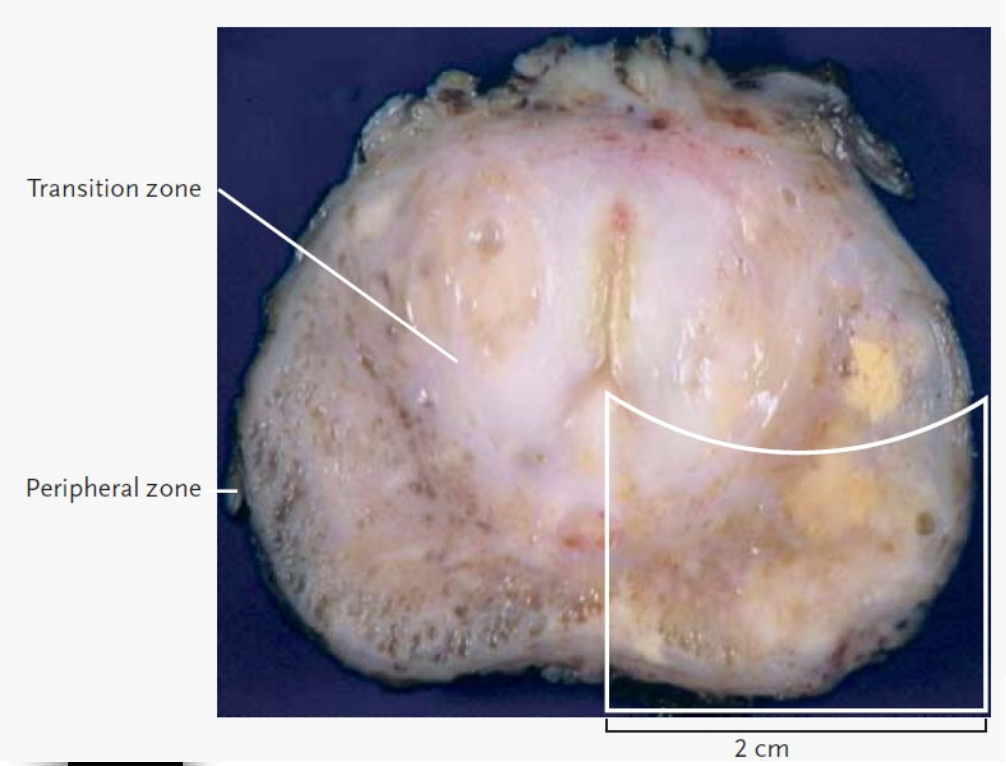
Pathological plasticity of prostate epithelial cells

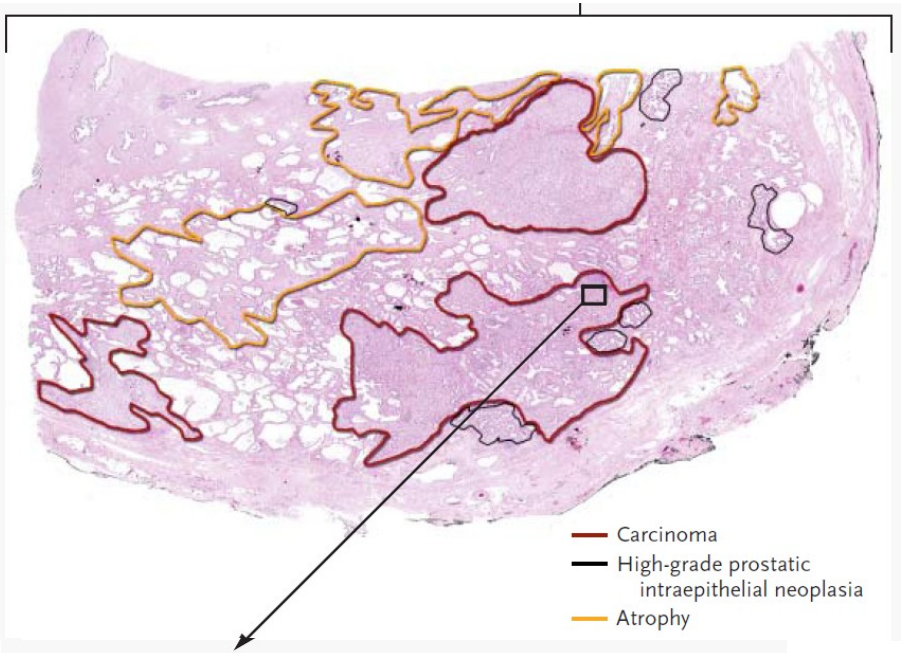
- Neuroendocrine transdiferentiation
- Epithelial to mesenchymal transition

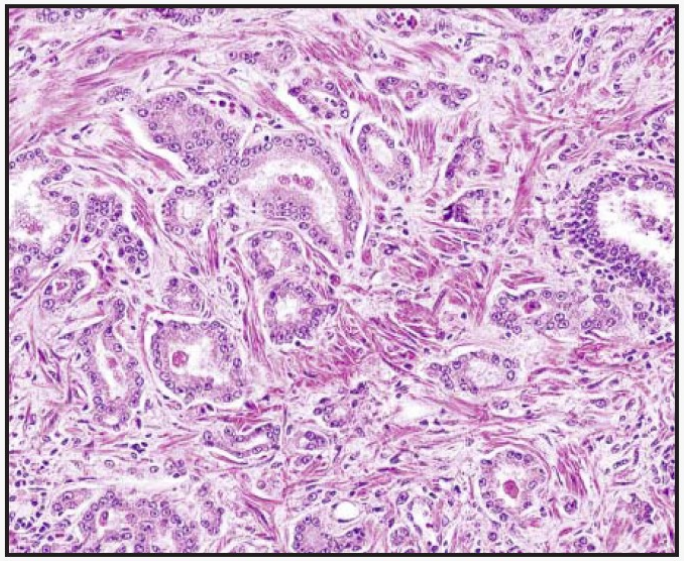
Prostate cancer

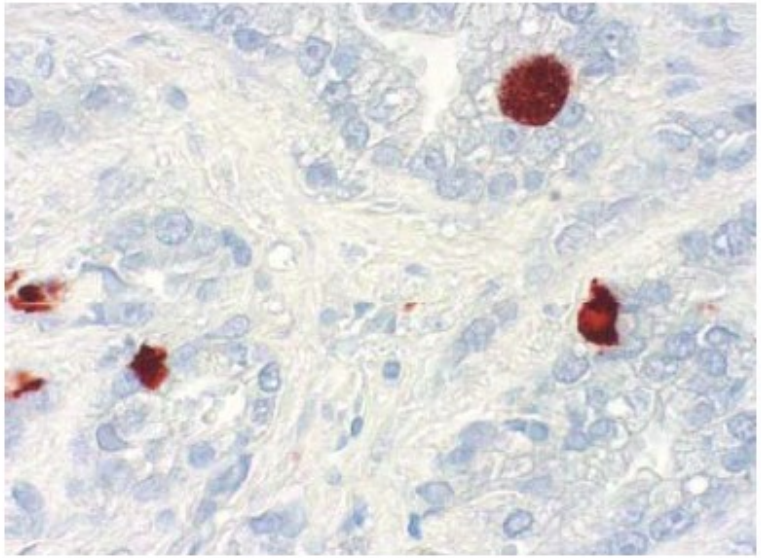
- one of the leading causes of cancer related death in men worldwide
- **conventional treatment** - hormonal therapy
  - luminal secretory cells are dependent on androgens
  - androgen deprivation therapy (hormonal therapy) – chirurgical castration or anti-androgen administration (Casodex (bicalutamide))

- **patients** - positive response at the beginning - tumor regression
  - after 1-2 years of treatment - tumor progression, metastasis - **development of androgen independence**



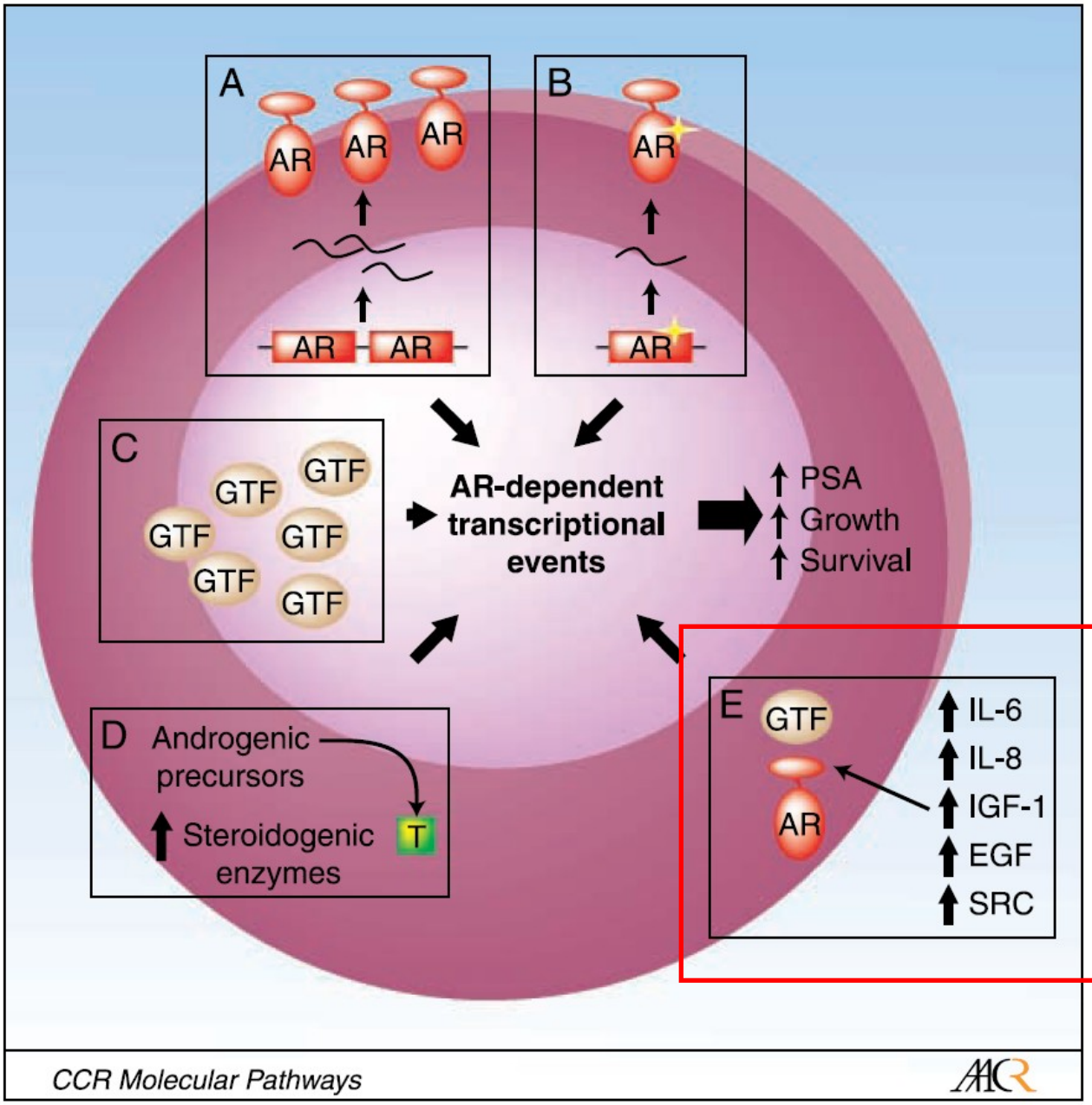






Sun et al., Am J Transl Res 2009, 1(2)

Nelson et al., NEJM 2003, 349(4)

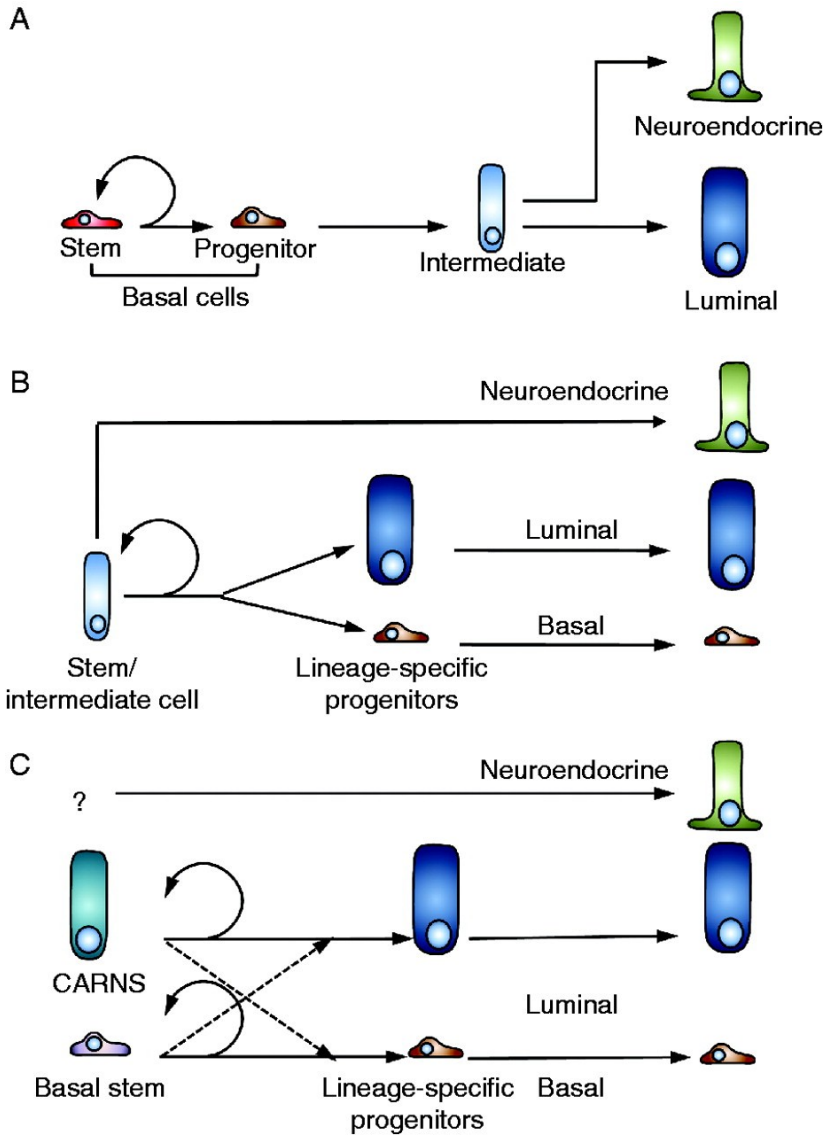


Produced by NE cells

Attar *et al.,* Clin Cancer Res 2009, 15(10)

Prostate neuroendocrine cells

- **Characteristic**
  - uncertain origin
  - scattered in prostatic epithelium
  - dendrite-like protrusions
  - markers: NSE, TUBB3, *CHGA*
  - quiescent
  - do not express AR
  - secretion of various factors (bombesin, adrenomedulin, VEGF, serotonin, IL-6, IL-8, etc.)
- **Function**
  - growth and differentiation regulation
  - modulates function of prostatic gland
  - regulation of homeostasis



Taylor R A et al. Endocr Relat Cancer 2010;17:R273-R285

1. androgen deprivation therapy induces secretory, tumor-promoting senescent cells in prostate tumors;
2. role of MDM2 in EMT of benign and transformed cells;
3. CHK1 inhibition & DNA damaging drugs in prostate epithelial cells – preliminary screen
4. new methods & approaches:
   * isolation of normal mouse prostate stem cells
   * multicolor protocol for characterization and separation of human prostate cancer stem cells
   * new automatic cell cloning assay (ACCA) for determination of clonogenic capacity of cancer stem-like cells

*Current research progress

1) androgen deprivation therapy induces secretory, tumor-promoting senescent cells in prostate tumors

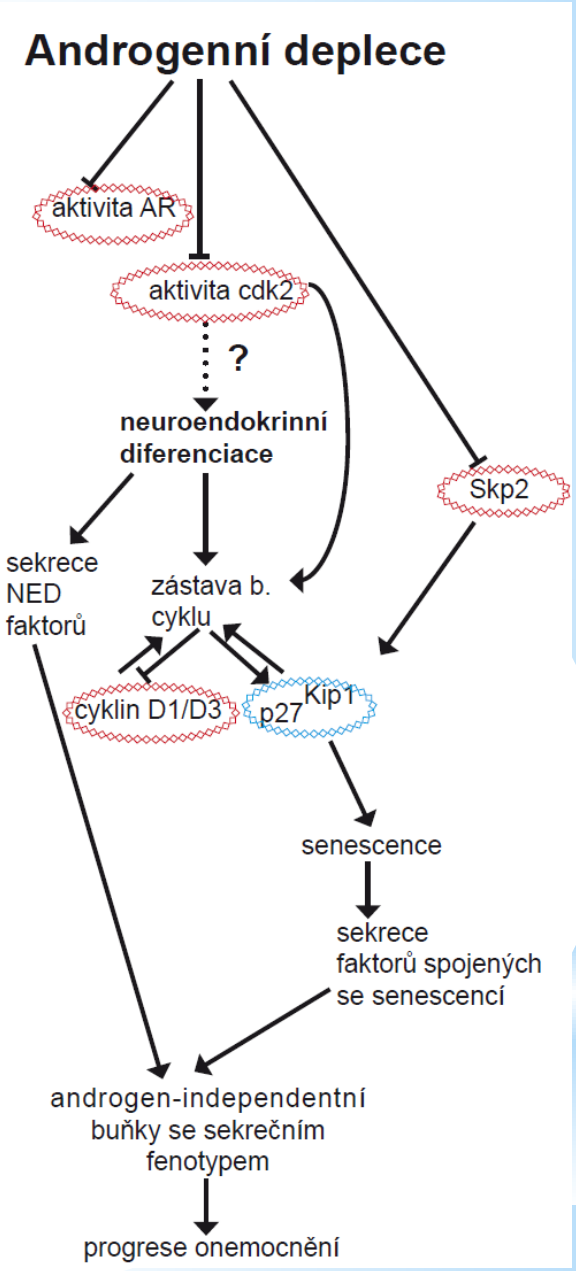
* We showed link between inhibition of androgen receptor signaling, down-regulation of S-phase kinase-associated protein 2, and the appearance of secretory, tumor-promoting **senescent cells** in prostate tumors.
* We propose that **androgen deprivation therapy** may contribute to the development of androgen-independent prostate cancer through **modulation of the tissue microenvironment by senescent cells.**

NEOPLASIA
www.neoplasia.com
Volume 13 Number 6 June 2011 pp. 526–536 526

Androgen Depletion Induces Senescence in Prostate Cancer Cells through Down-regulation of Skp2[1,2]

Zuzana Pernicová*, Eva Slabáková*, Gvantsa Kharaishvili†, Jan Bouchal†, Milan Král‡, Zuzana Kunická§, Miroslav Machala¶, Alois Kozubík*,§ and Karel Souček*

*Department of Cytokinetics, Institute of Biophysics, AS CR, Brno, Czech Republic; †Laboratory of Molecular Pathology and Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic; ‡Department of Urology, University Hospital, Olomouc, Czech Republic; §Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic; ¶Department of Chemistry and Toxicology, Veterinary Research Institute, Brno, Czech Republic



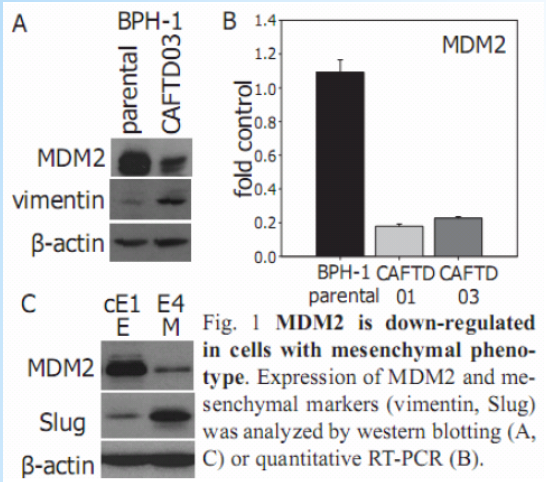


Fig. 1 **MDM2 is down-regulated in cells with mesenchymal phenotype.** Expression of MDM2 and mesenchymal markers (vimentin, Slug) was analyzed by western blotting (A, C) or quantitative RT-PCR (B).

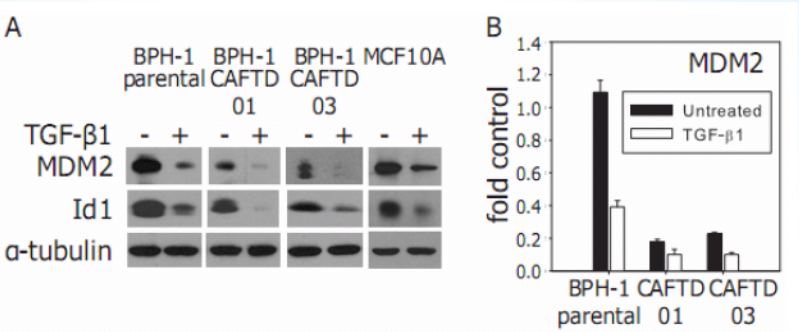
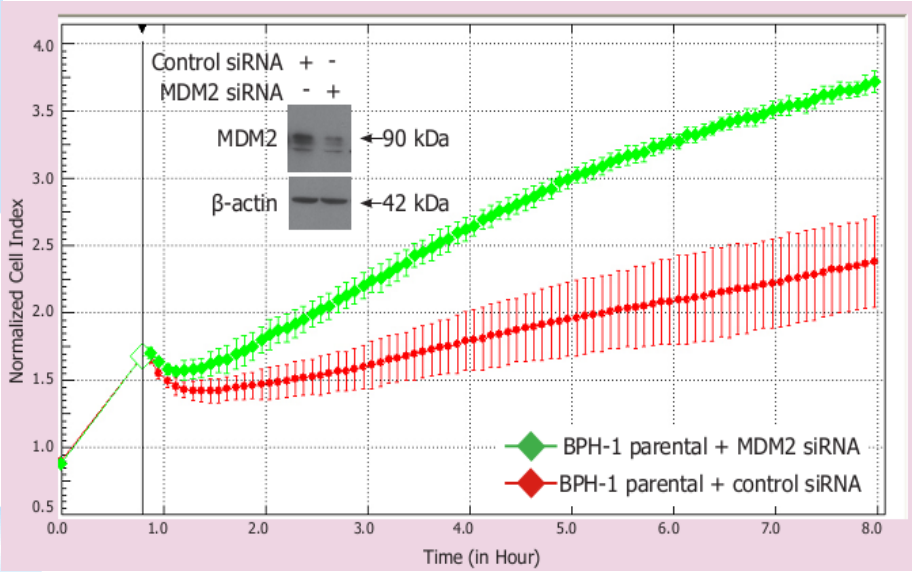


Fig. 3 **MDM2 is down-regulated during TGF-β1-induced EMT.** The cells were treated with TGF-β1 (10 ng/ml) for 96 hours; expression of proteins was analyzed by western blotting (A); expression of mRNA was analyzed by qRT-PCR (B).



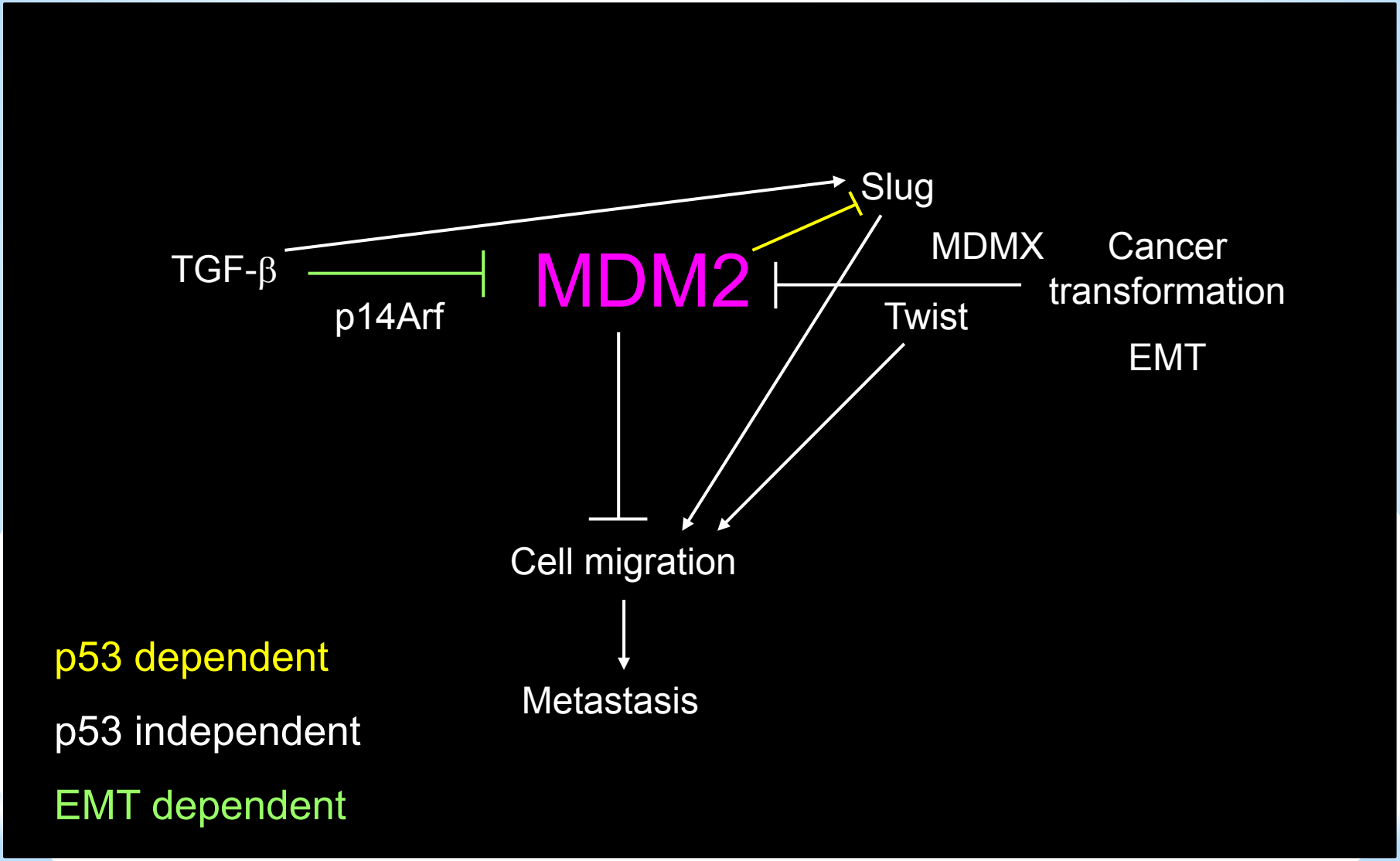
The Prostate 71:1332–1343 (2011)

TGF-β1-Induced EMT of Non-Transformed Prostate Hyperplasia Cells Is Characterized by Early Induction of SNAI2/Slug

Eva Slabáková,[1] Zuzana Pernicová,[1] Eva Slavíčková,[1] Andrea Staršíchová,[1] Alois Kozubík,[1,2] and Karel Souček[1,2]*

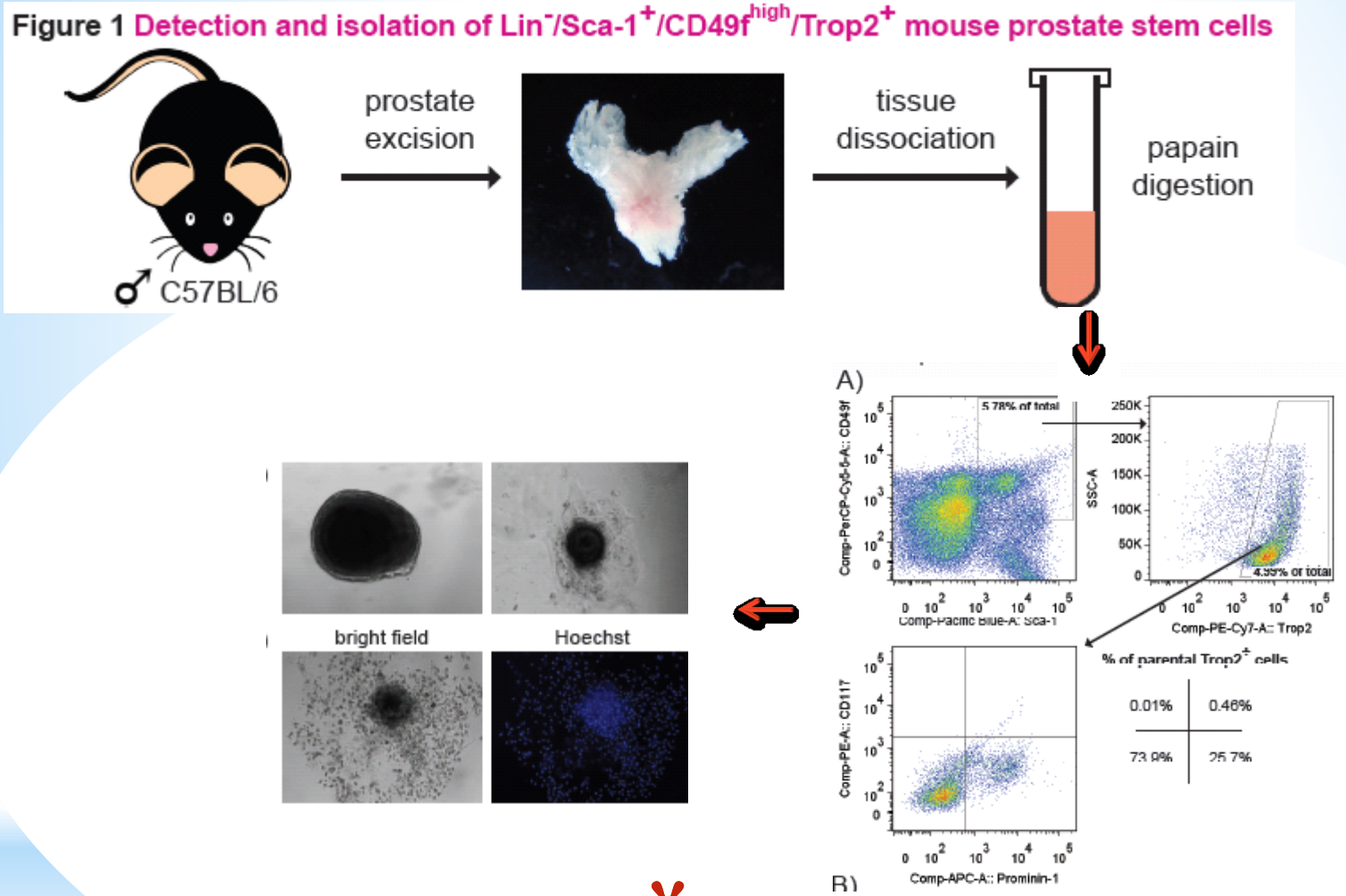
[1]Department of Cytokinetics, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic
[2]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

* 2) The role of MDM2 in epithelial-to-mesenchymal transition: implication for cancer progression


Slug
TGF-β
p14Arf
MDM2
MDMX
Cancer transformation
Twist
EMT
Cell migration
Metastasis
p53 dependent
p53 independent
EMT dependent

*Summary

a) isolation of normal mouse prostate stem cells

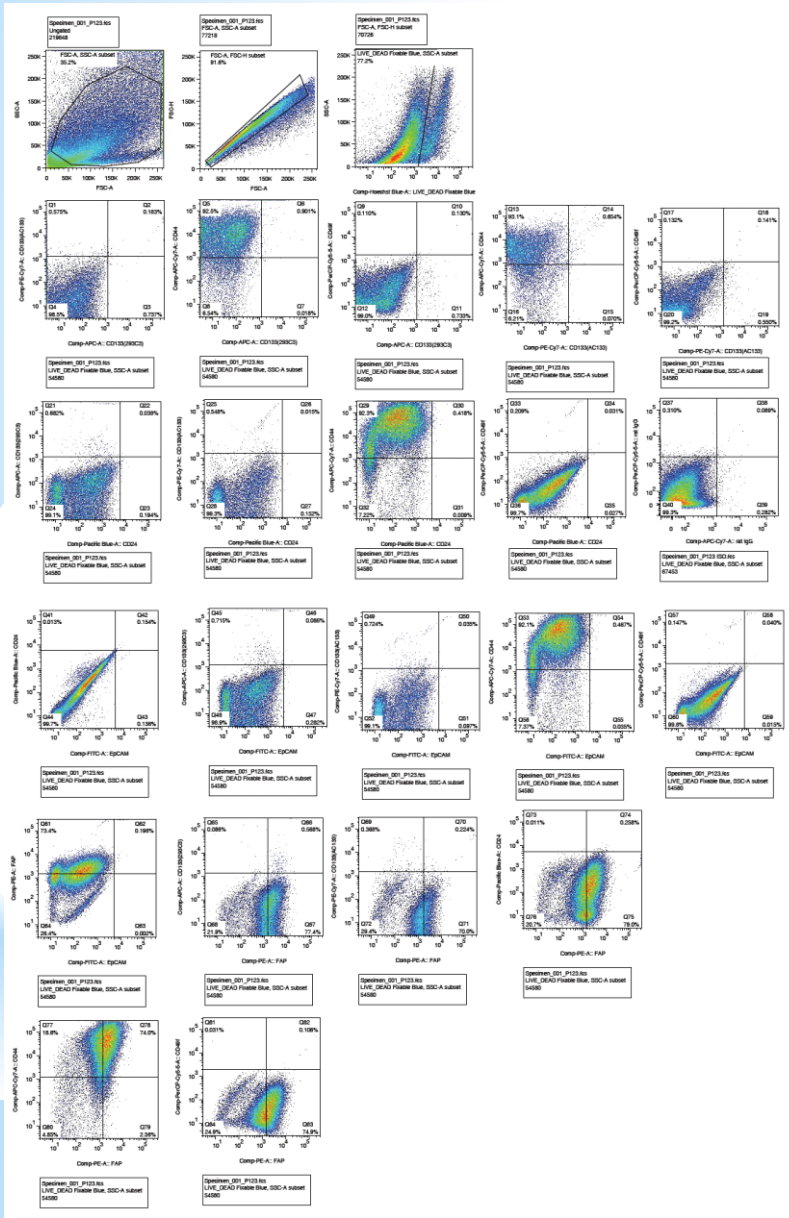


*4) new methods & approaches

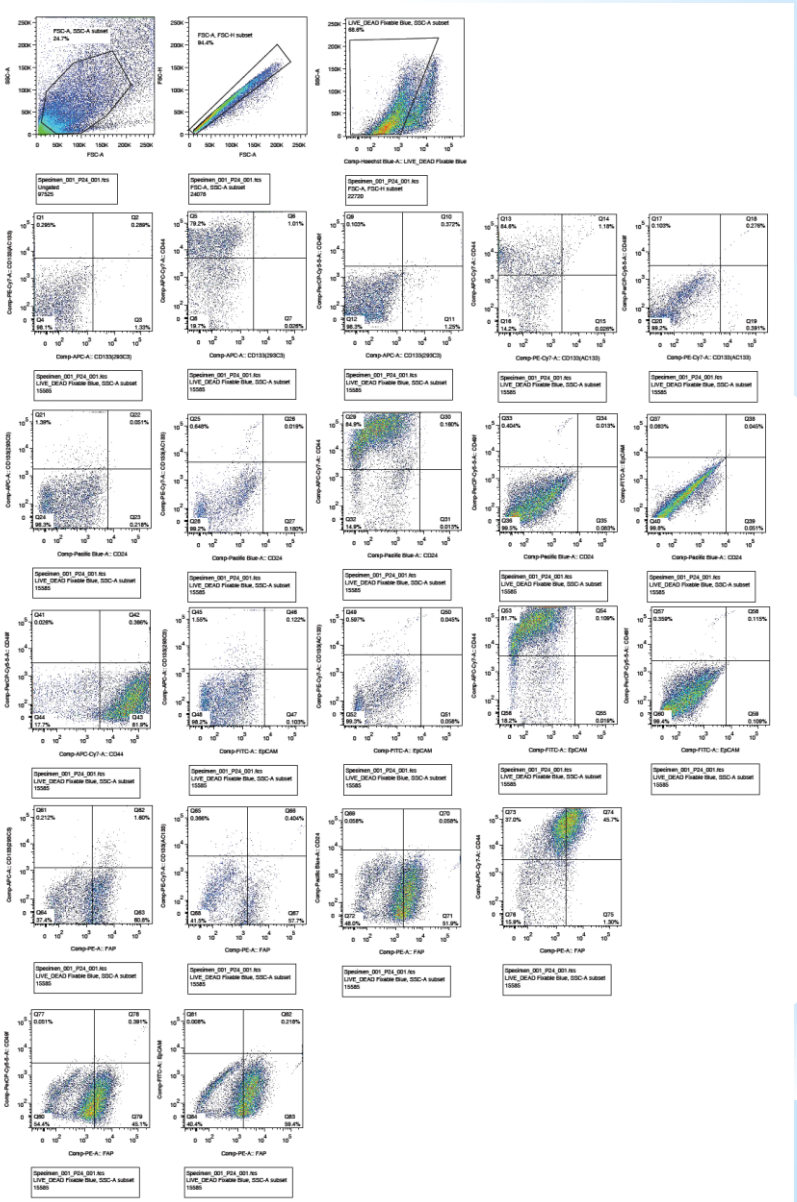
b) multicolor protocol for characterization and separation of human prostate cancer stem cells

	primary antibody	conjugate	made in	isotype	source
LIN specific	EpCAM	FITC	mouse	IgG1	Miltenyi 130-080-301
	FAP	PE-TexasRed Light-Link	mouse	IgG1	R&D MAB3715
CSCs-specific	CD49f	PerCP/Cy5.5	rat	IgG2a kappa	BioLegend 313618
	CD44	APC/Cy7	rat	IgG2b kappa	BioLegend 103028
	CD24 (SN3)	PB	mouse	IgG1	Exbio PB-503-T025
	Trop-2	PerCP Light-Link	mouse	IgG2A	R&D MAB650
	CD133/1 (AC133)	Biotin+streptavidin PE/Cy7	mouse	IgG1	Miltenyi 130-090-664
	CD133/2 (AC141)	PE	mouse	IgG1	Miltenyi 130-080-901
	CD133/2 (293C3)	APC	mouse	IgG2b	Miltenyi 130-090-854

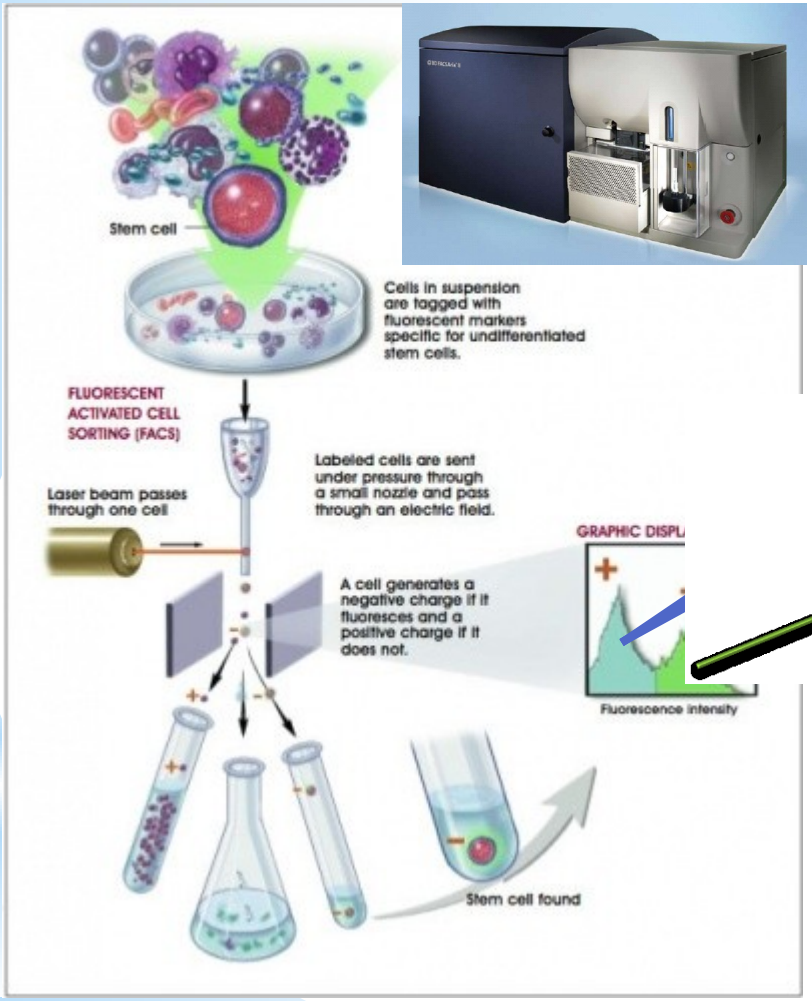
Patient #123



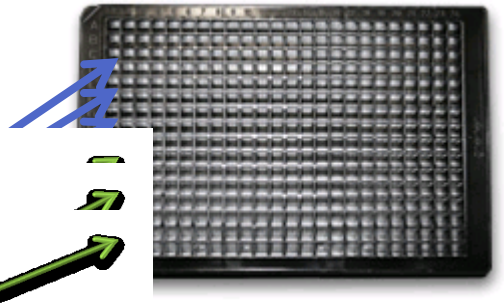
Patient #24



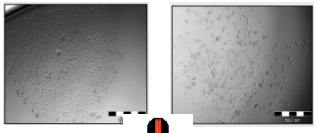
c) new automatic cell cloning assay (ACCA) for determination of clonogenic capacity of CSCs



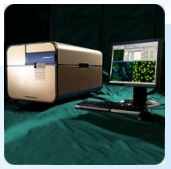
single cell/well
up to 384 well plate



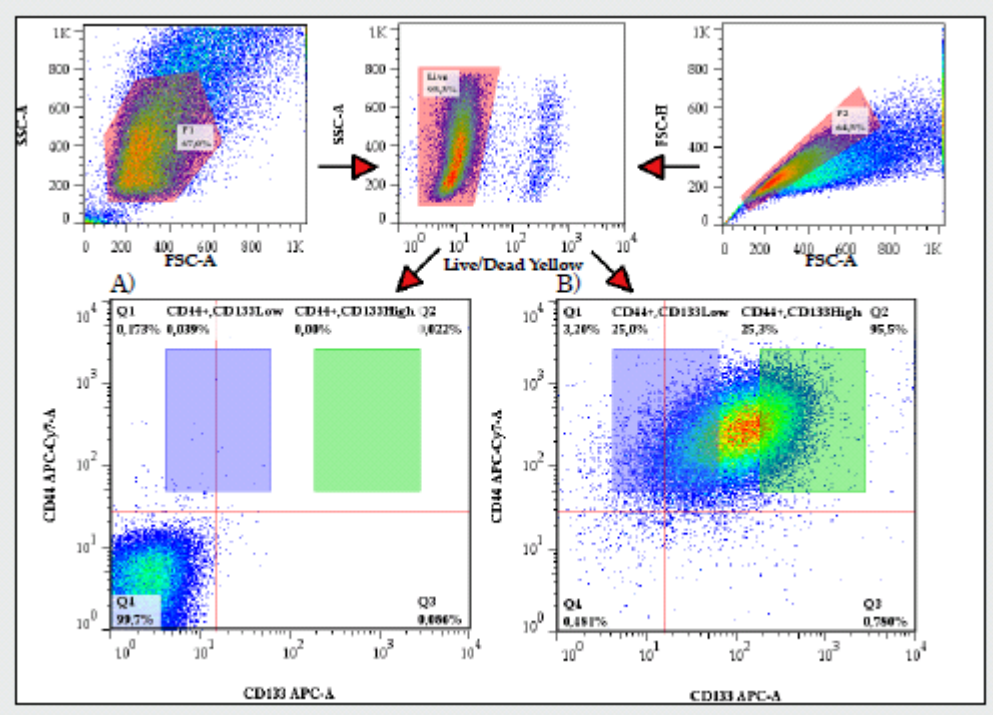
re-culture after sorting (2D, 3D)

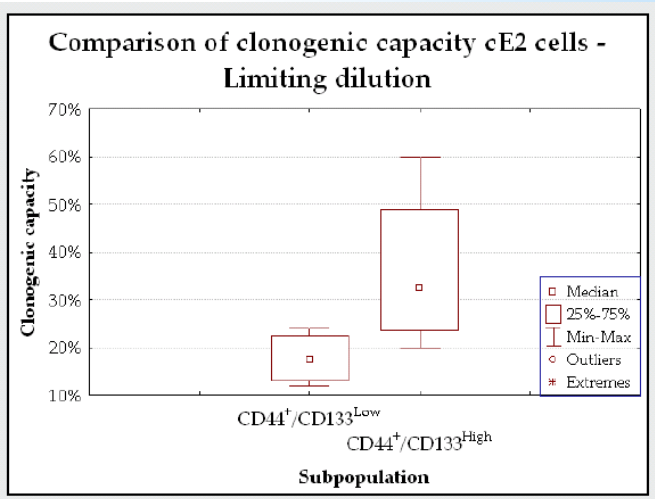


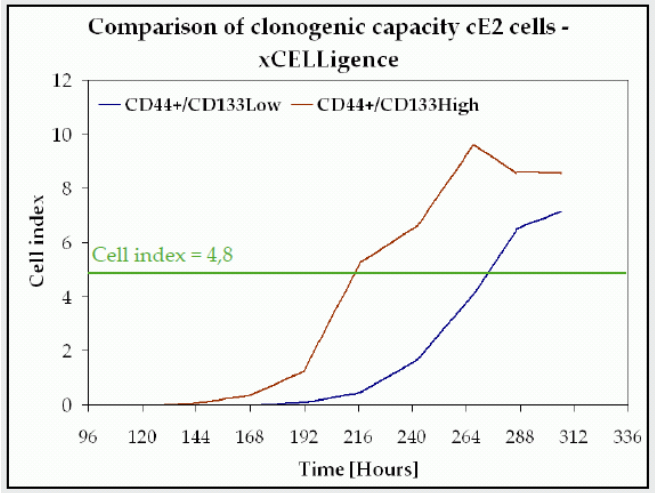
analysis: CyQuant, ATP, xCelligence, microscopy



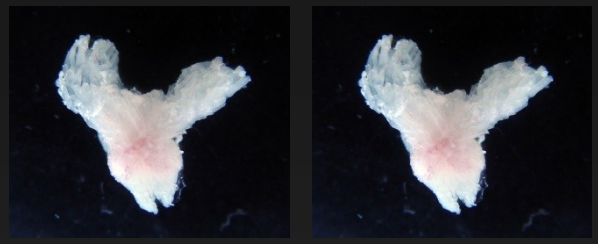
Clonogenic capacity of CD44/CD133^{low} vs. CD44/CD133^{high} cells

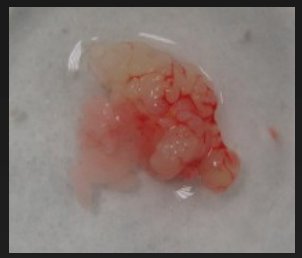


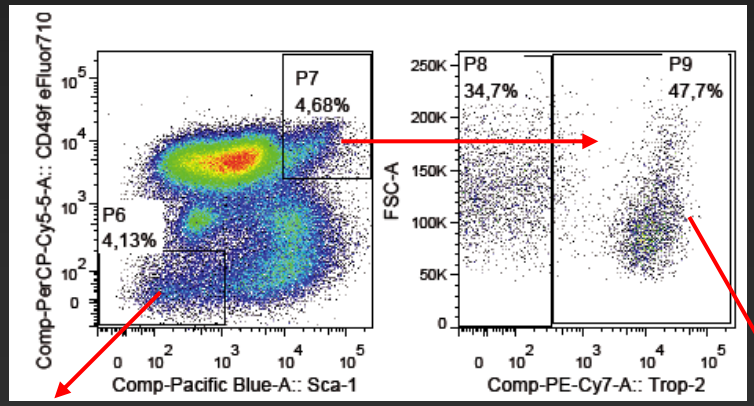




EMT in SCs/CSCs-like subpopulation







$Lin^-/Sca1^-CD49f^-$

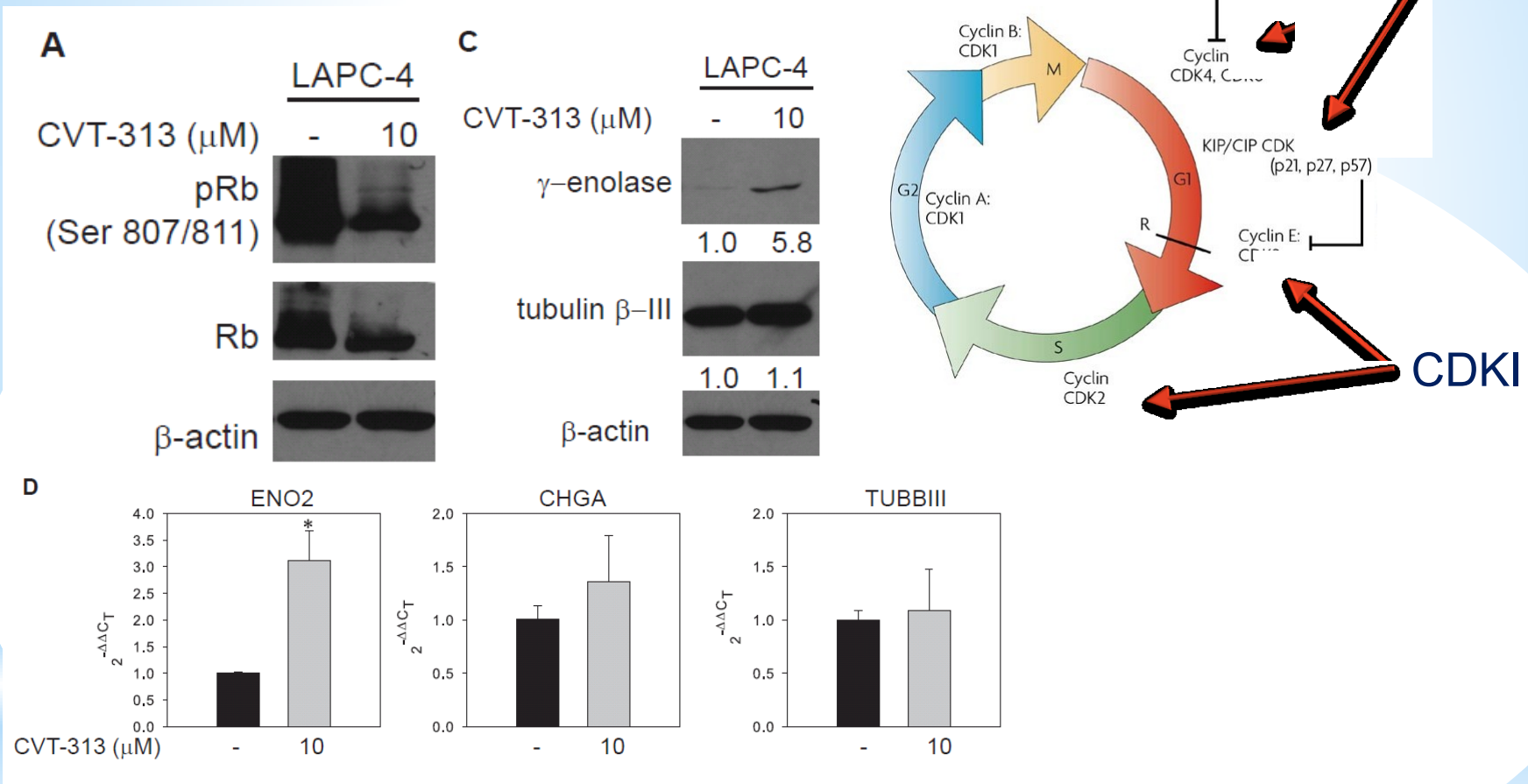
$Lin^-/Sca1^+CD49f^{high}/Trop2^+$

RNA isolation → cDNA synthesis → RealTime Ready Custom Panel RT-qPCR

RealTime ready Custom Panel 384

group	gene	product
EMT markers	CDH1	E-cadherin
	CDH2	N-cadherin
	VIM	Vimentin
	FN1	Fibronectin
	ACTA2	α-smooth muscle actin
EMT regulators	SNAI1	Snail
	SNAI2	Slug
	TWIST1	Twist1
	TWIST2	Twist2
	ZEB1	Zeb1
	ZEB2	Zeb2
	FOXC2	Forkhead box protein C2
	AXL	Axl
	Tcf3	E2A
housekeeping	GAPDH	glyceraldehyde-3-phosphate dehydrogenase
	ACTB	β-actin
	TBP	TATA-binding protein

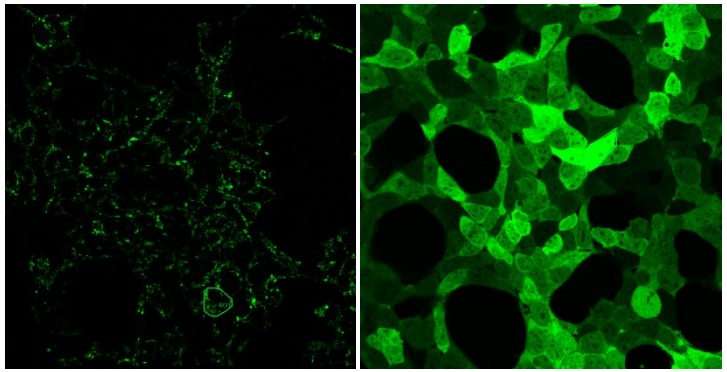
*Cdk inhibition induces neuroendocrine differentiation in prostate cancer cells

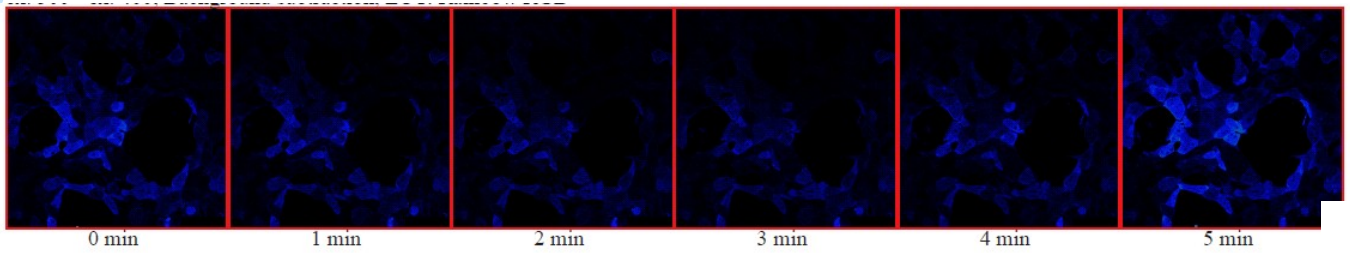


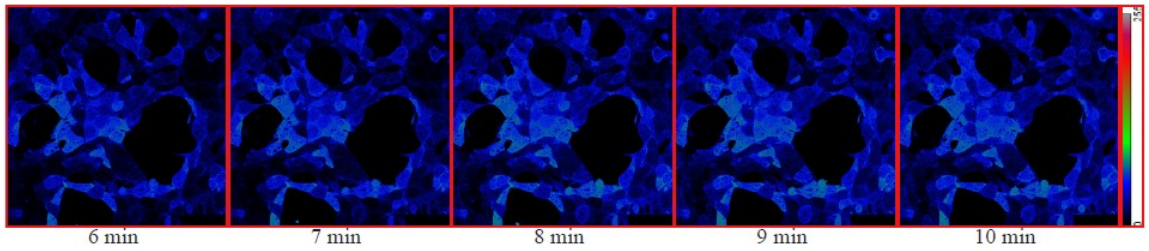
*tools for molecular imaging

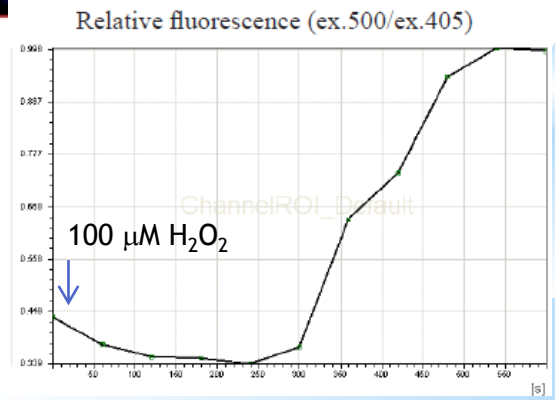
*hydrogen peroxide sensor HyPer (Evrogen) for ratiometric detection of intracellular H_2O_2 level changes

- *HEK293 HyPer-dMito
- *HEK293 HyPer-cyto





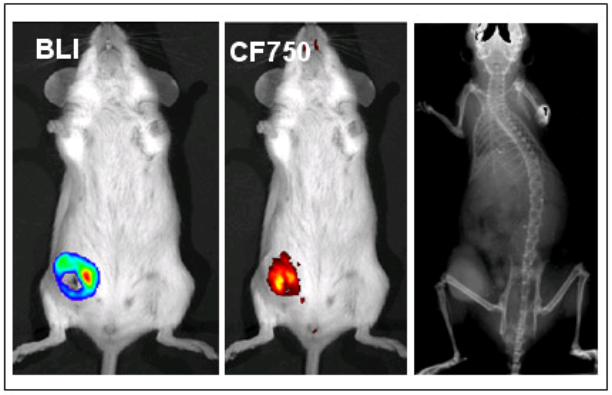




tools for *in vivo* molecular imaging

- **Prostate, Breast, Melanoma, and Colon Carcinoma Models**
  - for syngeneic immunocompetent strains C57Bl/6 or BALB/c
  - stable transfected with lentiviral *luc* vector
    - CT26 luc - mouse colon cancer
    - 4T1 luc - mouse breast cancer
    - B16 F10 luc – mouse melanoma
    - TRAMP-C1 – mouse prostate cancer
    - RM-1 – mouse prostate cancer

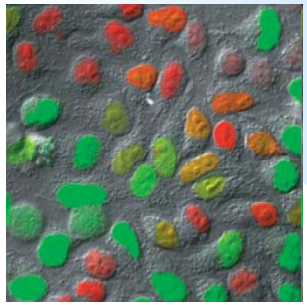
Visualizing Fluorescent Probe Targeted Tumor by Lumina-XR



A Nu/Nu mouse with an orthotopic 4T1-luc mammary tumor (3 weeks after injection of 1 million cells) was imaged with the Lumina XR for its bioluminescent signal. The tumor was targeted with the Avastin-750 probe and visualized with fluorescent imaging as described as above. The targeted tumor is clearly visible by X-ray imaging.

* 3) CHK1 inhibition & DNA damaging drugs

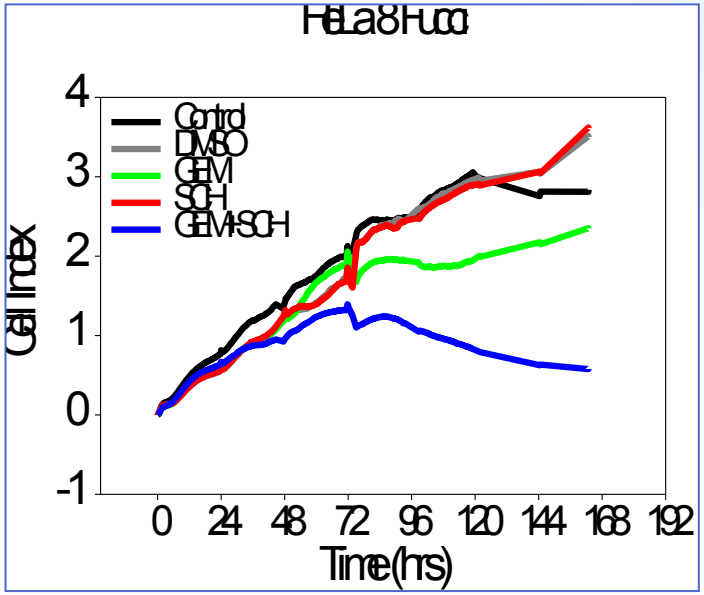
- tested compounds
  - CHK1 inhibitor SCH900779
  - Ara-C, gemcitabine, HU
- tested cell lines
  - non-tumorigenic – HPEpiC, BPH-1
  - primary cancer – CAFTD-01, -03, LAPC-4
  - metastatic cancer – LNCaP, PC3
  - other – HeLa Fucci 8
- design
  - treatment with gem, HU, Ara-C for 24h
  - 2h treatment with CHK inhibitor, than media exchange
  - harvest 48h after treatment
- readouts
  - CyQuant – concentration screen, synthetic lethality analysis
  - xCelligence – real-time analysis (selected concentrations)

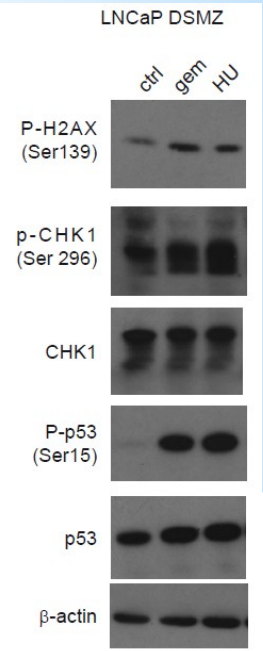


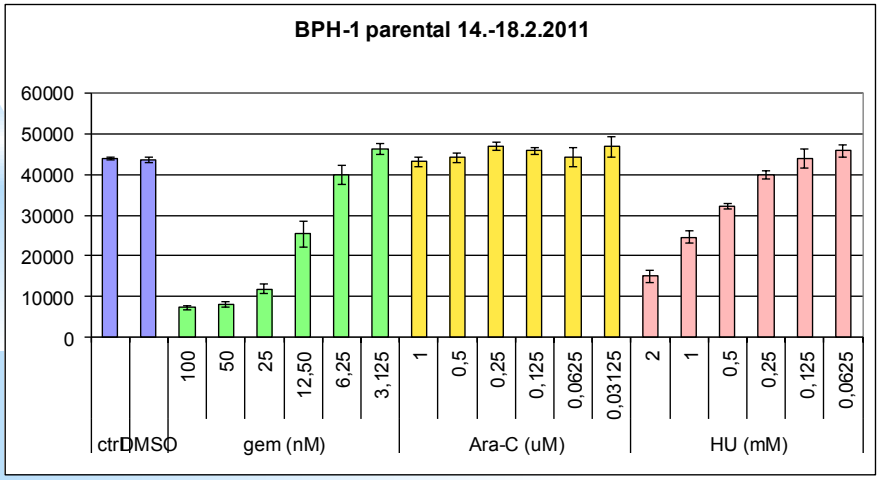
Nature Methods - **5, 283 (2008)**

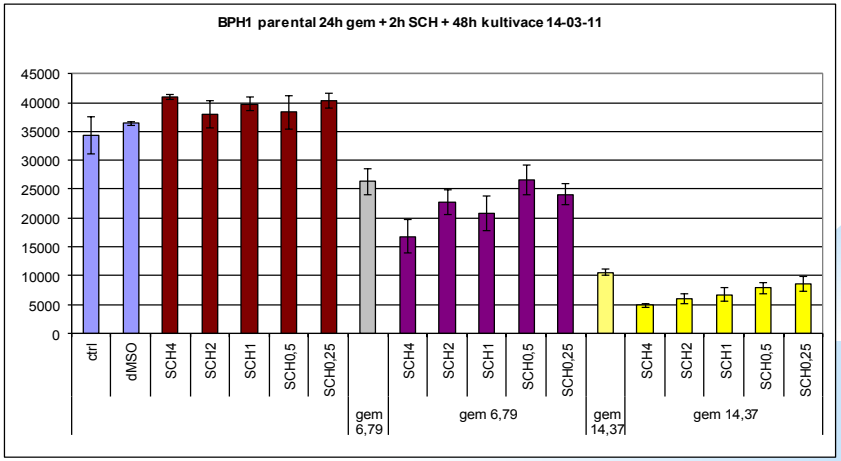
* pre-screen results

* Gemcitabine
  * in all tested cell lines very toxic – use lower concentrations
* Ara-C + CHKI
  * BPH-1 – 5 + 0.25 μM synthetic effect
  * LNCaP – 5 + 2 μM synthetic effect
  * PC-3 – 5 + 2 μM synthetic effect
* HU + CHKI
  * all tested cell lines 0.5 + 2 uM synthetic effect



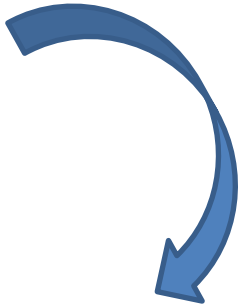


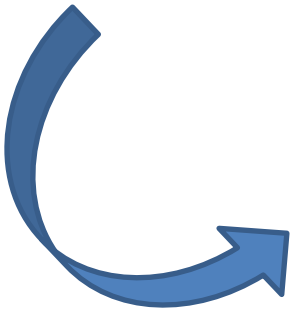




Experimental approaches and models

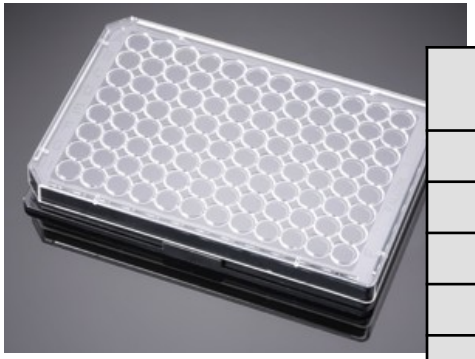
- screening of cytotoxic concentrations of DNA damaging agents and inhibitors
  - evaluation by CyQuant® proliferation assay
- monitoring of dynamics of cytotoxic effects of DNA damaging drugs
  - xCELLigence system
  - live microscopic imaging
- evaluation of treatment in 3D conditions
  - microscopic imaging
  - ATP bioluminiscence proliferation assay



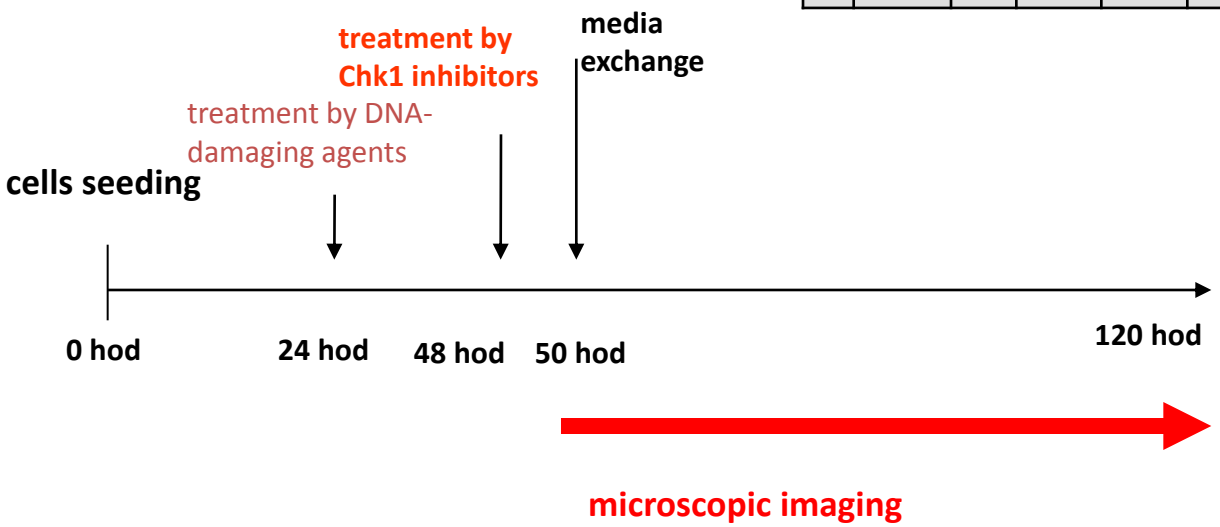


	TISSUE	ORIGIN	p53 STATUS
BPH1 parental	*Prostate non – tumorigenic*	human	inactivated
BPH1 CAFTD03	*Prostate tumorigenic*	human	inactivated
DU-145	*brain metastais of prostate carcinoma*	human	mt
PC3	*bone metastatis of prostatic adenocarcinoma*	human	null
HCT116 p53 +/+	*colorectal carcinoma*	human	+/+
HCT116 p53 -/-	*colorectal carcinoma*	human	-/-
HCT116 PTEN -/-	*colorectal carcinoma*	human	+/+
MDCK	*kidney tissue non – tumorigenic*	dog	wt
B16-F10	*skin melanoma*	mouse	wt
TRAMP C1	*prostate adenocarcionma*	mouse	wt

Live microscopic imaging experiment set up

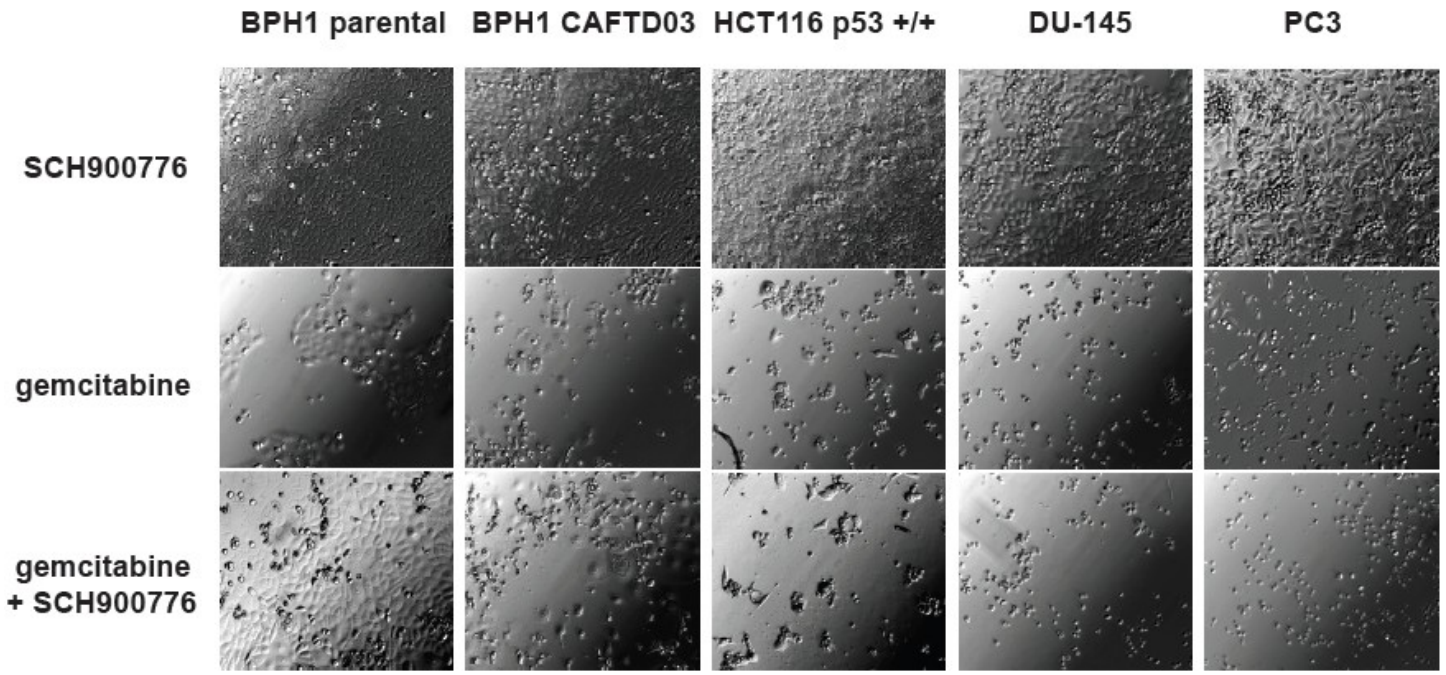


	BPH1par	BPH1 c.03	HCT116 p53+/+	PC-3	DU-145	
	dmso					
	OH209					
	SCH900776					
	GEMCITABINE					
	GEMCITABINE + OH209					
	GEMCITABINE + SCH900776					



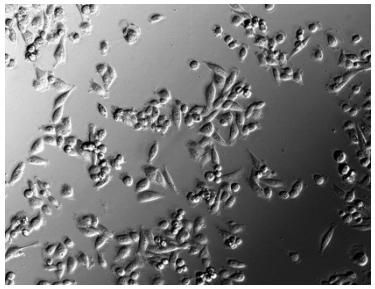
Results

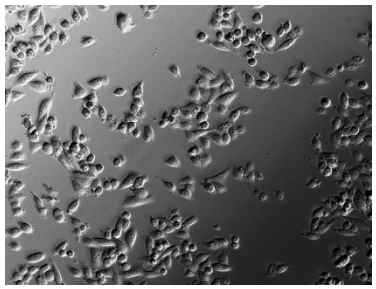
Live microscopic imaging



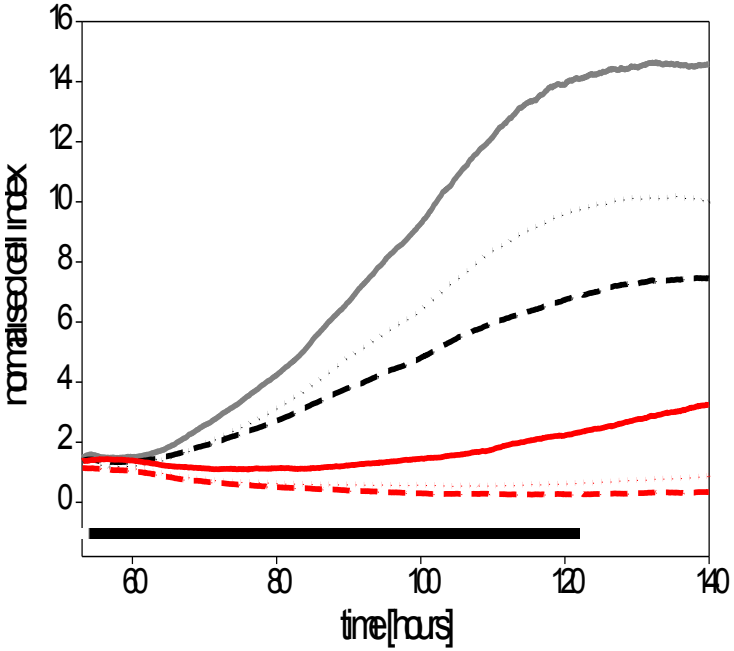
Cell lines indicated were seeded in microplate wells, treated and then monitored in real time using bright-field microscope and CCD camera. Images obtained at final time (120 hours) are compared for wells treated by gemcitabine (IC50 vaules for selected cell lines), by gemcitabine and SCH900776 (4 μM) and by SCH900776 (4 μM) only

PC3 cell line





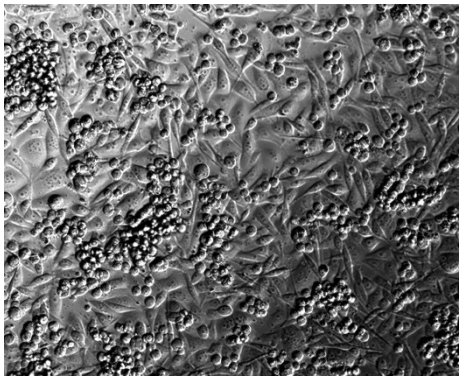
media exchange effect (inhibitor only)

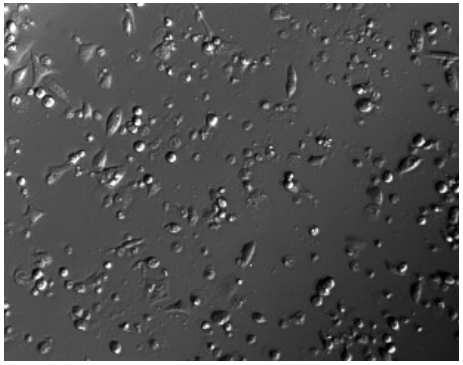


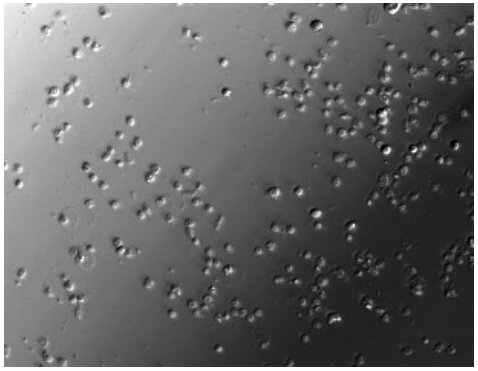
inhibitor only

gemcitabine

gemcitabine + inhibitor

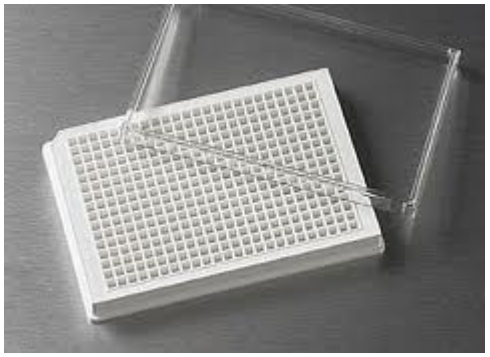






Running experiments - screening for synthetic lethality in panel of cell lines

TYPE	CELL LINE
Colon	SW480
	SW620
	HCT-116 p53+/+
	HCT-116 p53 -/-
	HT29
Breast	MCF10A
	MDA-MB-231
	Sk-Br-3
Lung	H441
	A549
Ovarian	A2780
	A2780cis
	SKOV-3
Prostate	BPH-1
	BPH-1 CAFTD04
	PC3
	DU145
	LNCaP
	LAPC-4
Pancreas	MiaPaCa2
	PANC-1
Kidney	MDCK



- 6 concentrations of DNA-damage drug (hydroxyurea)
- 6 concentrations of 2 inhibitors of CHK1 (SCH900776, OH209)
- all combinations in quadruplicate and two biological repetition

* Cooperations

* **Aleš Hampl (LF MU)** – mGDF15 ICC (cryosections), CSCs, tissue engineering, SCID
* **Petr Vaňhara (LF MU)** – GDF15 in dendritic cells, glioblastoma and ovarian cancer, lentiviral particles
* **Petr Beneš (PřF MU)** – mGDF15 inducible plasmids
* **Kamil Paruch (PřF MU)** – inhibitors
* **Stjepan Uldrijan (PřF MU)** – MDM2 story
* **Lukáš Kubala (BFÚ)** – EMT & ECM, tissue engineering

* **Jiří Kohoutek (VÚVeL)** – *gdf15* knock-out colony managment
* **Michal Hofer (BFÚ)**– hematopoiesis study
* **Jiří Pacherník (PřF MU)** – GDF-15 in hypoxia, mES
* **Pavel Matula, Petr Matula (FI MU)** – tube forming assay data analysis
* **Jiřina Procházková , Jan Vondráček (BFÚ) -** GDF15 in cardiomyocytes, interaction of AhR and TGF-β
* **Miroslav Machala (VÚVeL) -** interaction of AhR signaling with TGF-β
* **Jan Bouchal (UJP Olomouc)** – EMT & ECM, prostate cancer clinical samples, CSCs, IHC

* **Lukas Kenner (Ludwig Boltzman Institute, Vienna) -** prostate cancer – mouse model, CSCs,
* **Giuseppe Valacchi (University of Ferrara)** – redox signaling, autophagy

Současná témata studentských prací

- **Bakalářské**
  - Úloha SNX9 v epiteliálně mesenchymálním přechodu u epiteliálních buněk prostaty
- **Diplomové**
  - Změny v expresi proteinů MDM2 a MDMX v průběhu epiteliálně mesenchymálního přechodu
  - Epiteliálně mesenchymální přechod u normálních a nádorových kmenových buněk prostaty
- **Doktorské**
  - Úloha Skp2 v cytokinetice nádorových kmenových buněk
  - Úloha epitelialně mesenchymálního přechodu v regulaci fenotypu nadorových kmenových buněk