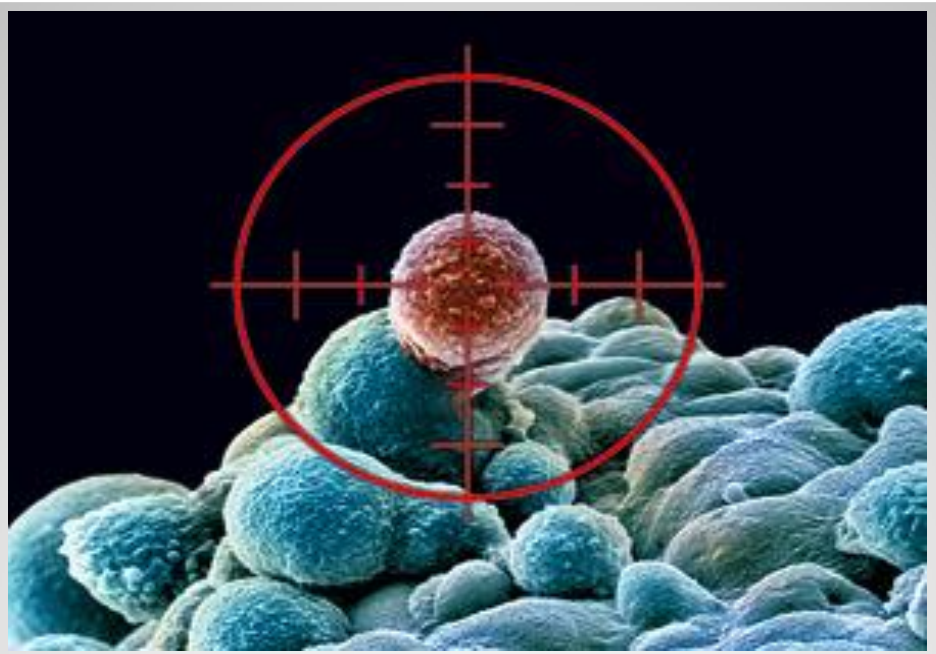


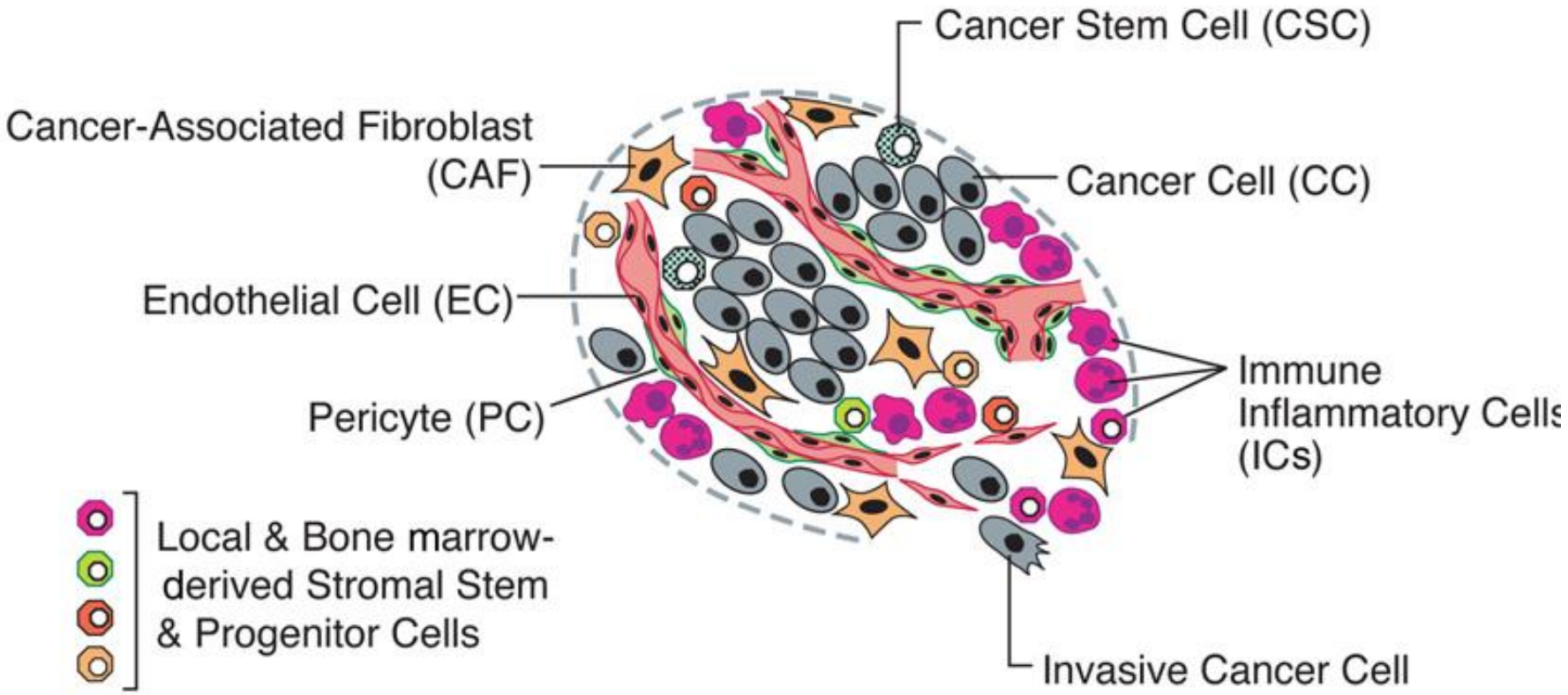
“Vēža cilmes šūnas” terminoloģija, hipotēzes, pierādījumi...

Latvijas Biomedicīnas
pētījumu un studiju centrs
biomedicīnas pētījumi un izglītība no gēniem līdz cilvēkam

Dr. Aija Linē

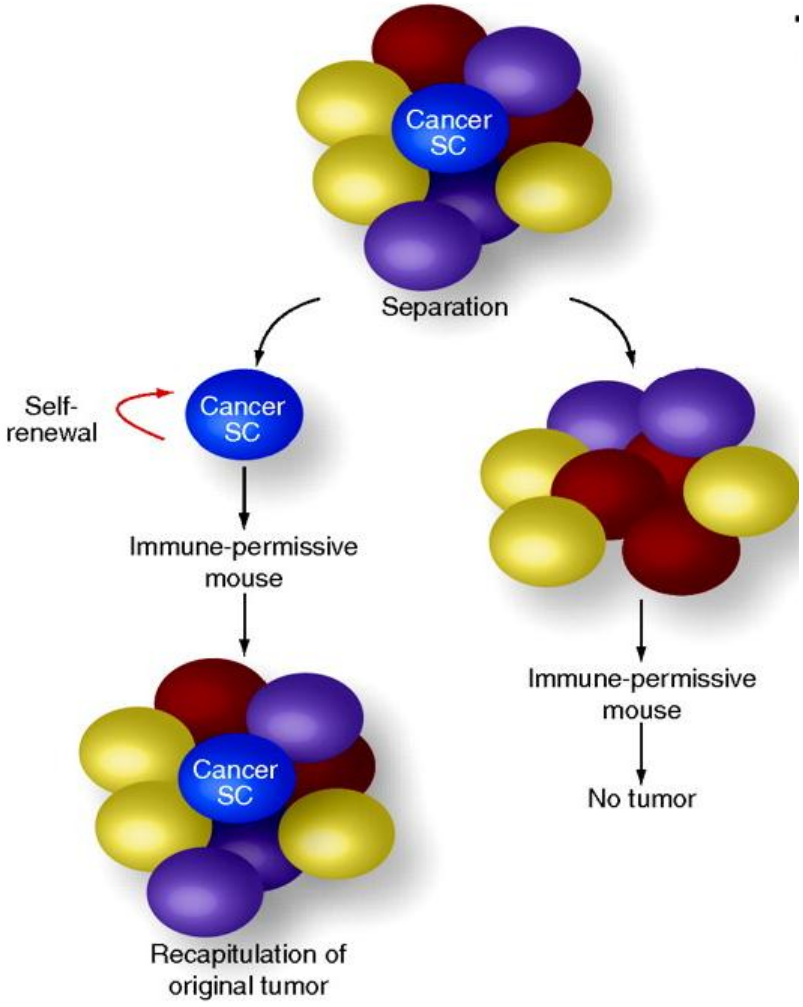
VPP 5.projekta pārskata seminārs

Vēzis – sarežģīts orgāns, kas sastāv no daudziem šūnu tipiem, nevis homogēna šūnu masa



Hanahan D & Weinberg RA, Cell 144, 2011

Vēža cilmes šūnu funkcionālā definīcija – audzēja šūnu populācija, kas efektīvi iniciē audzēja veidošanos eksperimenta dzīvniekos



Vēža cilmes šūnas

(Cancer stem cells, CSCs)

Audzēju iniciējošās šūnas

(Tumour initiating cells, TIC)

Cilmes šūnām līdzīgās šūnas

Stem cell-like cells (SCLC)

Vēža šūnu populācija, kam piemīt cilmes šūnu īpašības (pašatjaunošanās un spēja radīt daļēji diferencētas meitšūnas), spēj iniciēt audzēja veidošanos eksperimenta dzīvniekos un ir rezistentas pret staru un ķīmijterapiju

Vēža cilmes šūnu īpašības

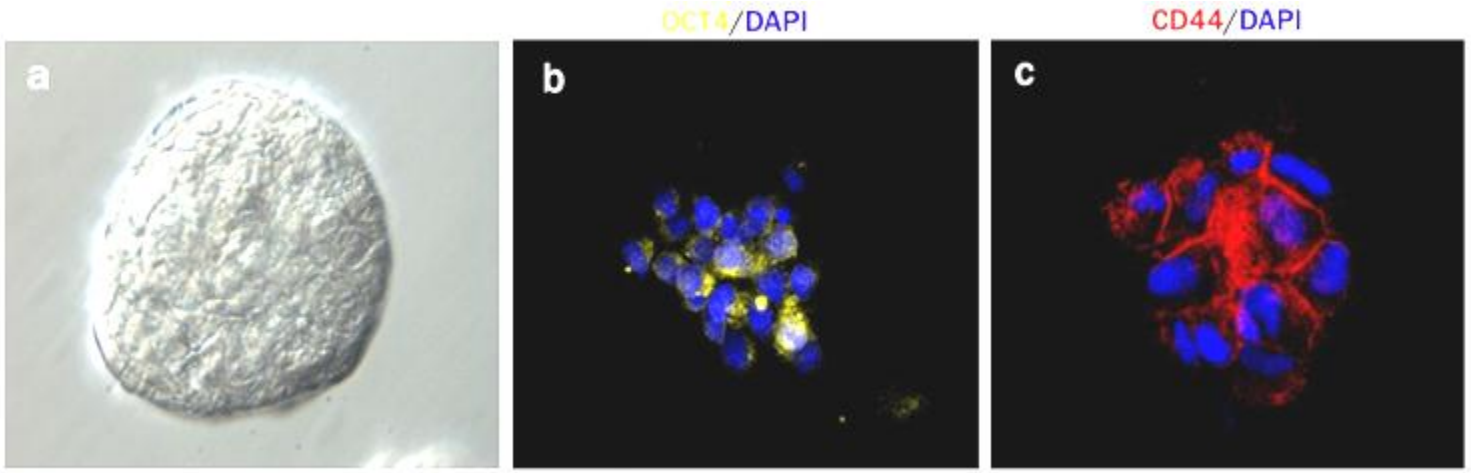
- ✓ Ir specifiski šūnas virsmas marķieri, piemēram CD133, CD44, ABCB5
- ✓ Ekspresē cilmes šūnām raksturīgus transkripcijas faktorus un gēnu ekspresijas regulatorus – Oct4, Lin28, Nanog, Sox2
- ✓ Rezistentas pret staru un ķīmijterapiju
- ✓ CSC biežums dažādos audzējos var būt dažāds – 0.1-30% no visas šūnu masas
- ✓ Neliels skaits (~100-500) CSCs spēj veidot audzēju imunodeficītās pelēs injicējot audzēju šūnas, kamēr ir vajadzīgas ~10^6 nešķirotas šūnas, lai iniciētu audzēja veidošanos

Vēža cilmes šūnu izolēšanas metodes

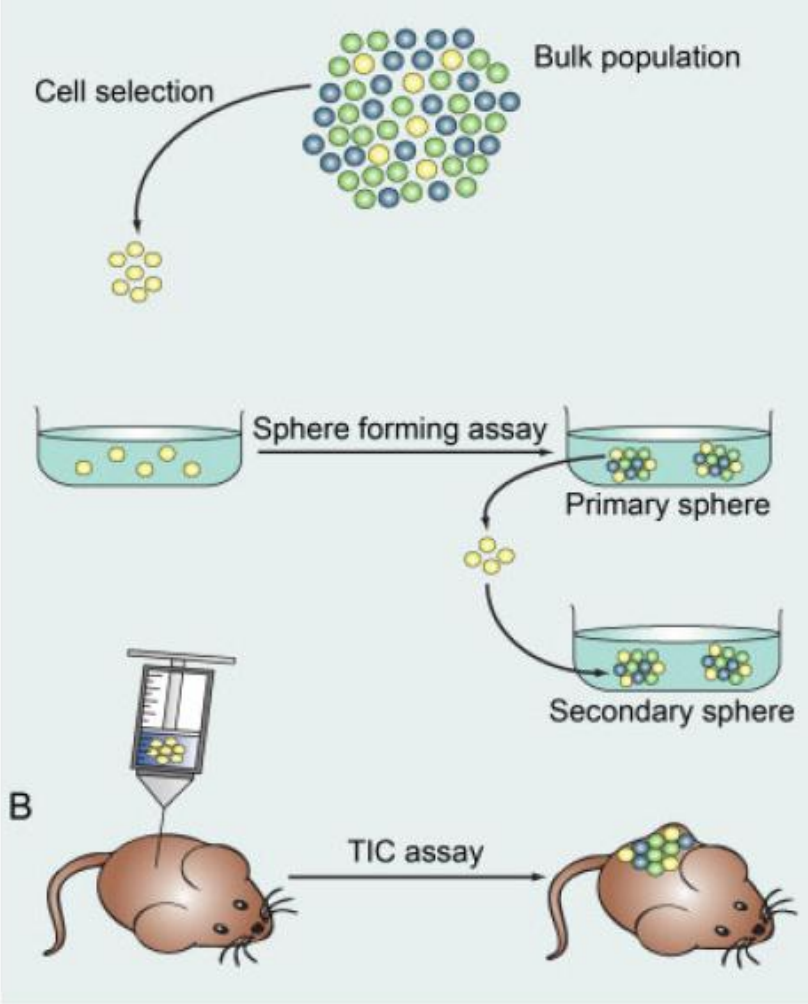
Cilmes šūnu īpašība, ko izmanto izolēšanai	Metode
Šūnas virsmas marķieri (CD133, CD44)	FACS, magnētiskās lodītes
Augsta zāļu transporta proteīnu aktivitāte	Hoechst side population: FACS
Augsta aldehīd-dehirogenāzes aktivitāte	Aldefluor tests: FACS
Spēja veidot sfēras bez-seruma vidē	Sfēru kultūra
Rezistence pret ķīmijterapiju	Rezistento populāciju selekcija

Sfēru kultūra

- ✓ **Bez-seruma vidē cilmes šūnas veido multicelulāras sfēras un saglabā nediferenciētu stāvokli**
- ✓ **Katra sfēra ir veidojusies no vienas šūnas (klons)**
- ✓ **Rezistentas pret zālēm**
- ✓ **Ekspresē cilmes šūnu marķierus (CD44, CD133) un embrionālo cilmes šūnu transkripcijas faktorus (OCT4, NANOG, SOX2)**
- ✓ **Fizioloģiski dabiskāks *in vitro* modelis kā 2D kultūras**
- ✓ **Veido audzējus *in vivo***

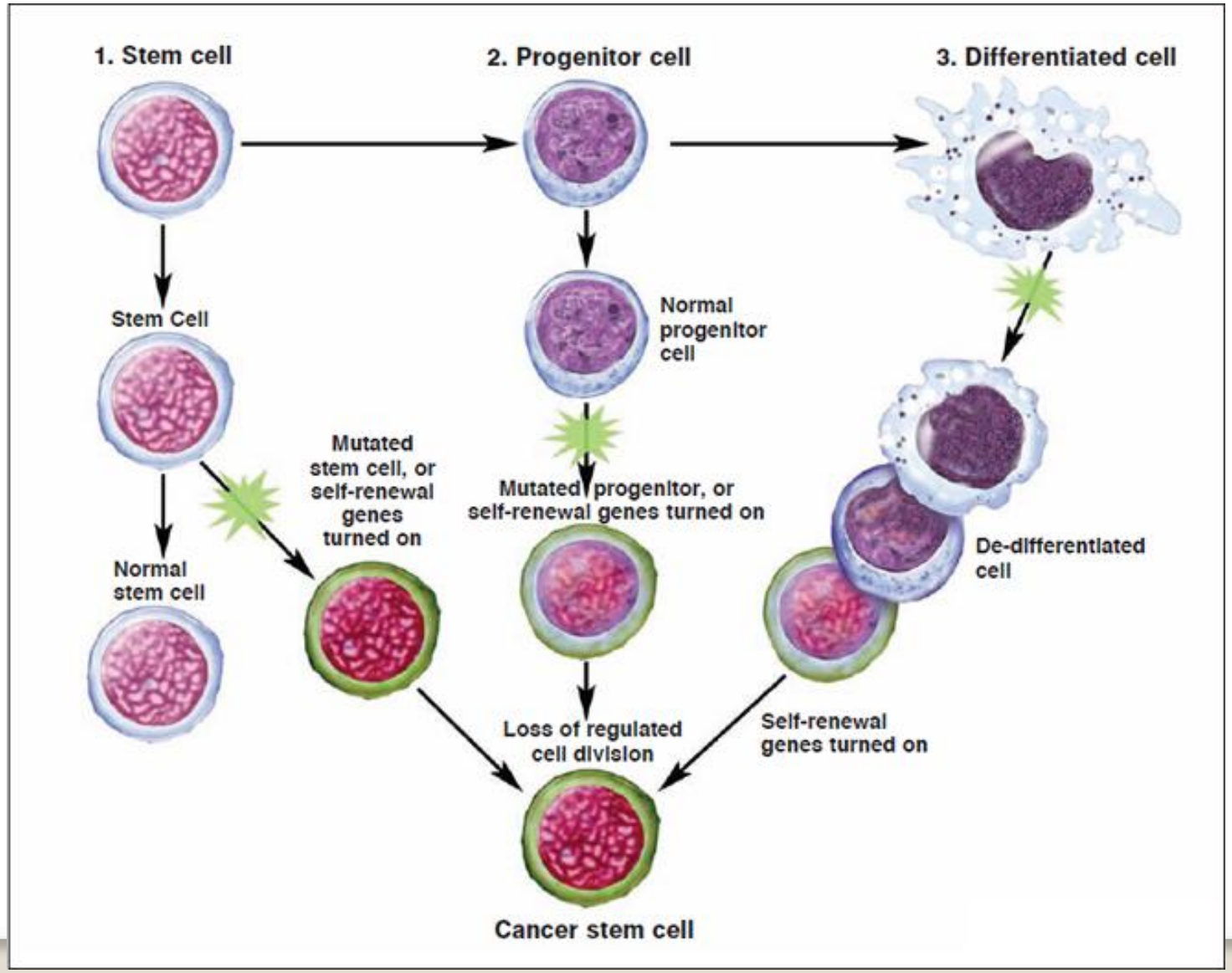


Vienīgais īstais pierādījums tam, ka izolētajām šūnām ir CSC īpašības, ir to spēja veidot histoloģiski identisku audzēju imunodeficītās pelēs



Alison MR et al, J Pathol, 2011

No kā rodas vēža cilmes šūnas?



letters to nature

Nature **367**, 645 - 648 (17 February 1994); doi:10.1038/367645a0

A cell initiating human acute myeloid leukaemia after transplantation into SCID mice

TSVEE LAPIDOT, CHRISTIAN SIRARD, JOSEF VORMOOR, BARBARA MURDOCH, TRANG HOANG*, JULIO CACERES-CORTES*, MARK MINDEN†, BRUCE PATERSON‡, MICHAEL A. CALIGIURI§ & JOHN E. DICK

Prospective identification of tumorigenic breast cancer cells

Muhammad Al-Hajj*, Max S. Wicha*, Adalberto Benito-Hernandez†, Sean J. Morrison*‡§, and Michael F. Clarke*‡¶

Departments of *Internal Medicine and †Pathology, Comprehensive Cancer Center, ‡Department of Developmental Biology, and §Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109

Communicated by Jack E. Dixon, University of Michigan Medical School, Ann Arbor, MI, January 16, 2003 (received for review December 18, 2002)

[CANCER RESEARCH 63, 5821–5828, September 15, 2003]

Identification of a Cancer Stem Cell in Human Brain Tumors

Sheila K. Singh, Ian D. Clarke, Mizuhiko Terasaki, Victoria E. Bonn, Cynthia Hawkins, Jeremy Squire, and Peter B. Dirks

The Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada [S. K. S., I. D. C., M. T., V. E. B., P. B. D.], and Program in Developmental Biology [S. K. S., I. D. C., M. T., V. E. B., P. B. D.], Division of Neurosurgery [S. K. S., P. B. D.], Department of Pediatric Laboratory Medicine [C. H.], and Department of Laboratory Medicine and Pathobiology [J. S.], University of Toronto, Toronto, Ontario M5G 1X8 Canada

Vol 445|4 January 2007|**doi:10.1038/nature05384**

Cell Death and Differentiation (2008) 15, 504–514

www.nature.com/cdd

Identification and expansion of the tumorigenic lung cancer stem cell population

A Eramo[1], F Lotti[2], G Sette[2], E Pilozzi[3], M Biffoni[1], A Di Virgilio[4], C Conticello[2], L Ruco[3], C Peschle[1] and R De Maria*[,1]

Identification and expansion of human colon-cancer-initiating cells

Lucia Ricci-Vitiani[1], Dario G. Lombardi[2], Emanuela Pilozzi[3], Mauro Biffoni[1], Matilde Todaro[4], Cesare Peschle[1] & Ruggero De Maria[1,2]

Related Commentary, page 2021 Research article

CD133 expression is not restricted to stem cells, and both CD133+ and CD133– metastatic colon cancer cells initiate tumors

Sergey V. Shmelkov,[1] Jason M. Butler,[1] Andrea T. Hooper,[1] Adilia Hormigo,[1] Jared Kushner,[1] Till Milde,[1] Ryan St. Clair,[1] Muhamed Baljevic,[1] Ian White,[1] David K. Jin,[1] Amy Chadburn,[1] Andrew J. Murphy,[2] David M. Valenzuela,[2] Nicholas W. Gale,[2] Gavin Thurston,[2] George D. Yancopoulos,[2] Michael D'Angelica,[3] Nancy Kemeny,[3] David Lyden,[1] and Shahin Rafii[1]

[1]Howard Hughes Medical Institute, Ansary Center for Stem Cell Therapeutics, and Department of Genetic Medicine, Weill Medical College of Cornell University, New York, New York, USA. [2]Regeneron Pharmaceuticals Inc., Tarrytown, New York, USA. [3]Sloan-Kettering Cancer Center, New York, New York, USA.

Int J Cancer. 2008 Feb 15;122(4):761-8.

CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells.

Wang J, Sakariassen PØ, Tsinkalovsky O, Immervoll H, Bøe SO, Svendsen A, Prestegarden L, Røsland G, Thorsen F, Stuhr L, Molven A, Bjerkvig R, Enger PØ.

Department of Biomedicine, University of Bergen, Bergen, Norway.

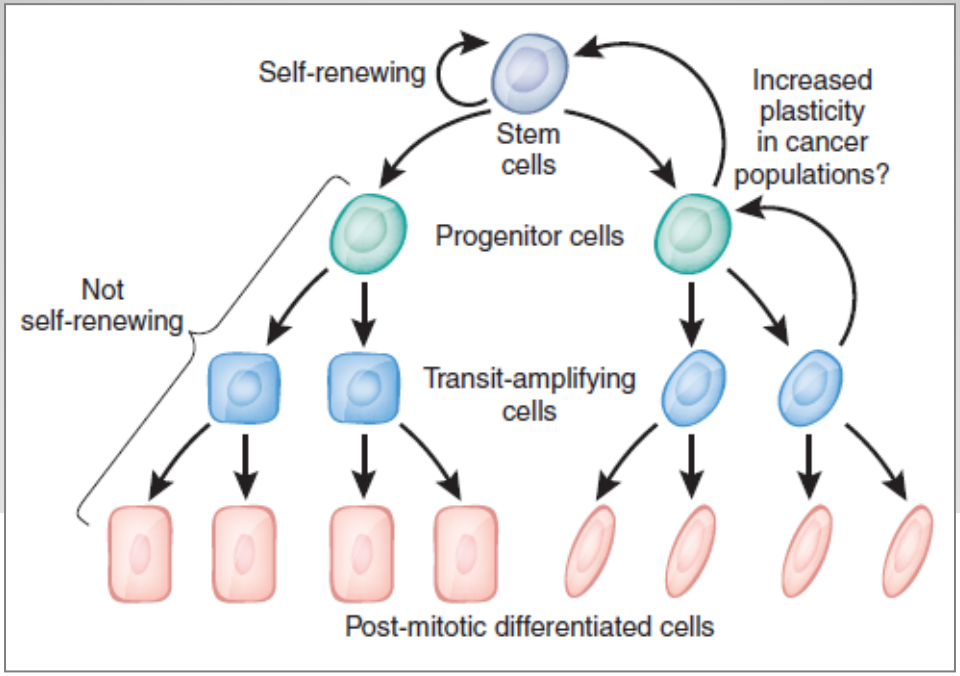
Vol 456|4 December 2008|doi:10.1038/nature07567 nature

ARTICLES

Efficient tumour formation by single human melanoma cells

Elsa Quintana[1]*, Mark Shackleton[1]*, Michael S. Sabel[2], Douglas R. Fullen[3], Timothy M. Johnson[4] & Sean J. Morrison[1]

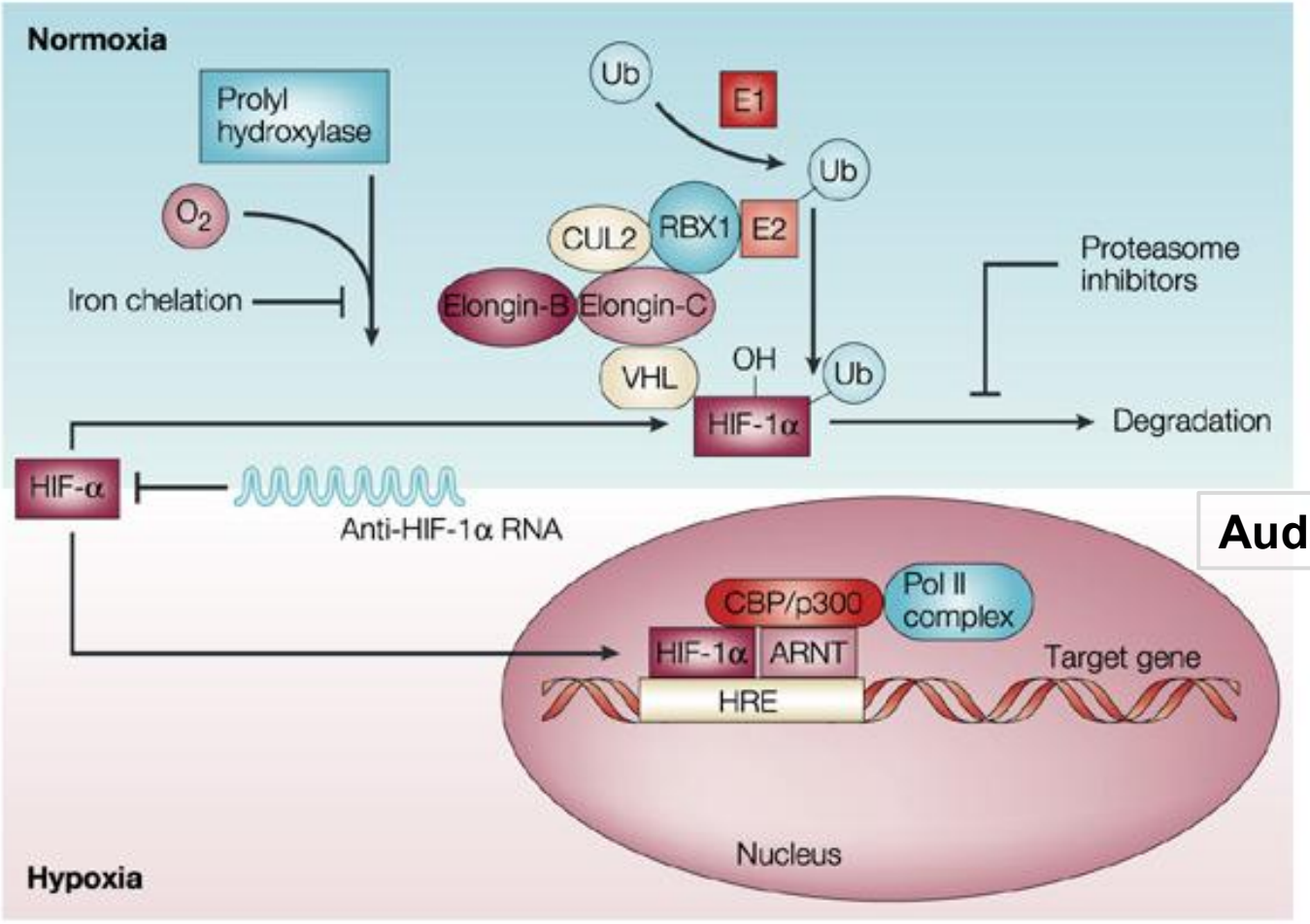
Vēža (cilmes) šūnu plasticitāte



- ✓ **Hipoksijas atbilde**
- ✓ **Epiteliāli-mezenhimāla tranzīcija**
- ✓ **Audzēja mikrovides ietekme**
- ✓ **Ķīmijterapijas ietekme?**

Hipoksijas atbilde

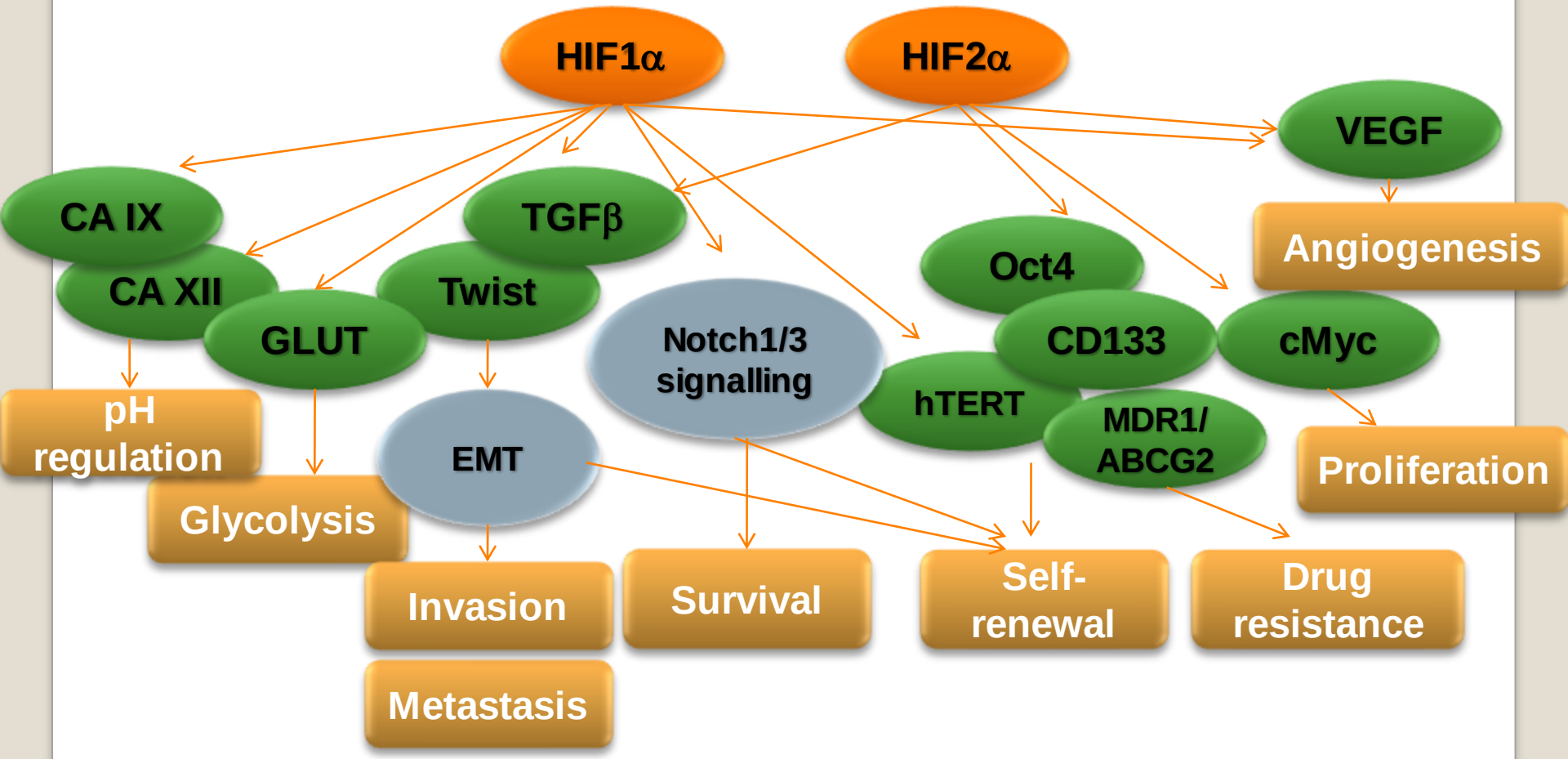
Veselos audos: ~7% O_2



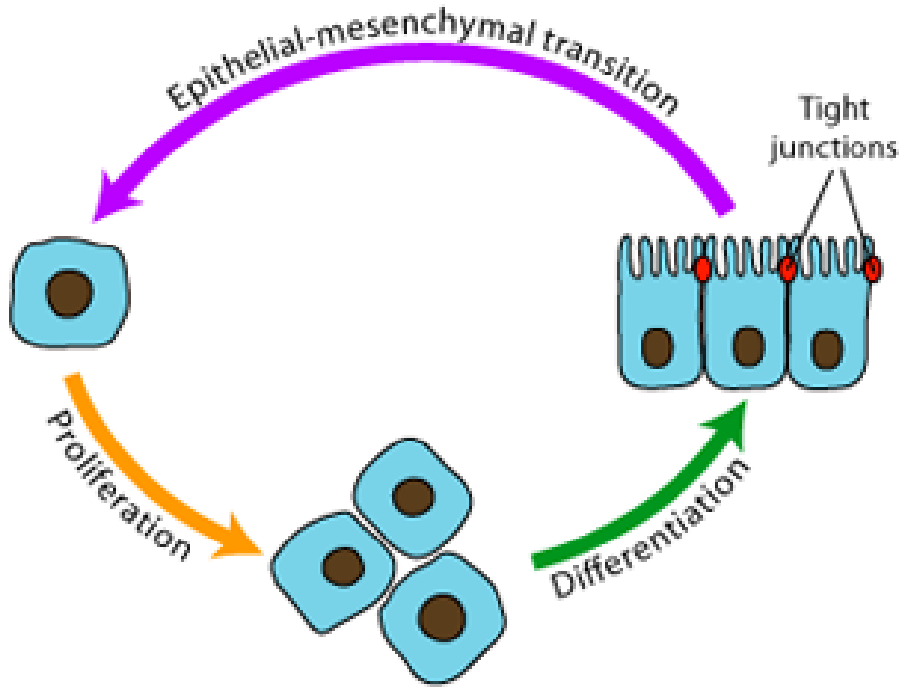
Audzējos: ~2-5% O_2

Nature Reviews | Cancer

HIF1α un HIF2α mērķgēni



Epiteliāli-mezenhimāla tranzīcija (EMT)



Programma, kuras rezultātā epiteliālās šūnas zaudē to diferenciācijas pazīmes un iegūst mezenhimālo šūnu īpašības, ieskaitot kustīgumu un rezistenci pret apoptozi; nepieciešama embrija attīstībai un brūču dzīšanai

Cell. 2008 May 16; 133(4): 704–715. doi:10.1016/j.cell.2008.03.027.

The epithelial-mesenchymal transition generates cells with properties of stem cells

Sendurai A. Mani[1,3,9,10], Wenjun Guo[1,9], Mai-Jing Liao[1,9], Elinor Ng. Eaton[1], Ayyakkannu Ayyanan[4], Alicia Y. Zhou[1,2], Mary Brooks[1], Ferenc Reinhard[1], Cheng Cheng Zhang[1], Michail Shipitsin[5,6], Lauren L. Campbell[5,7], Kornelia Polyak[5,6,7], Cathrin Brisken[4], Jing Yang[8], and Robert A. Weinberg[1,2,10]

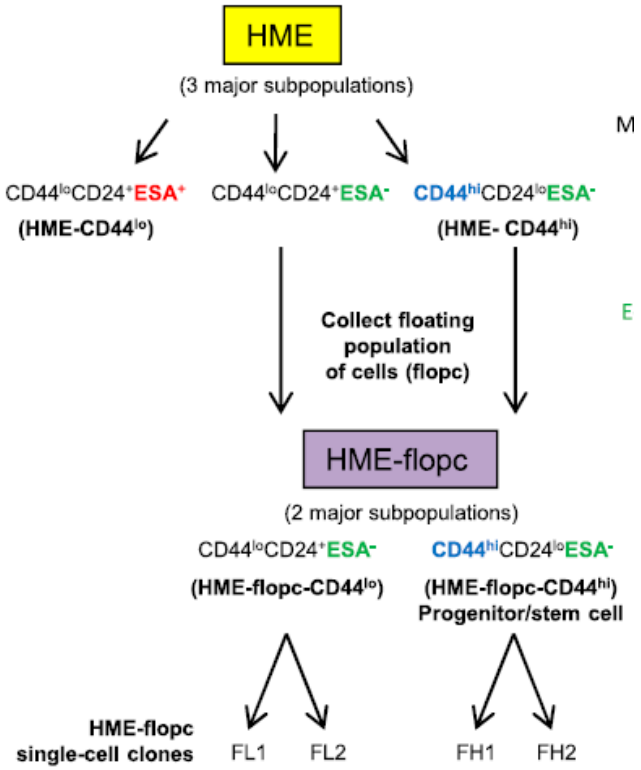
- **Imortlizētās krūts dziedzera epiteliālajās šūnas var inducēt EMT, tajās ekspresējot transkripcijas faktorus Twist un Snail;**
- **Šūnas, kas iegūst mezenhimālo šūnu īpašības, vienlaikus iegūst arī sekojošas īpašības:**
  - ✓ **Spēj diferencēties par luminālajām un mioepiteliālajām šūnām;**
  - ✓ **Veido sfēras;**
  - ✓ **Veido kolonijas pusmīkstajā agarā;**
  - ✓ **Veido audzēju bez-tīmusa (nu-/nu-) pelēs.**

Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state

Christine L. Chaffer[a,b], Ines Brueckmann[a], Christina Scheel[a,b], Alicia J. Kaestli[a], Paul A. Wiggins[a], Leonardo O. Rodrigues[a,b], Mary Brooks[a,b], Ferenc Reinhardt[a,b], Ying Su[c], Kornelia Polyak[c], Lisa M. Arendt[d,e], Charlotte Kuperwasser[d,e], Brian Bierie[a,b], and Robert A. Weinberg[a,b,f,1]

[a]Whitehead Institute for Biomedical Research, Cambridge, MA 02142; [b]Ludwig MIT Center for Molecular Oncology, Cambridge, MA 02139; [c]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115; [d]Department of Anatomy and Cellular Biology, Sackler School, Tufts University School of Medicine, Boston, MA 02111; [e]Molecular Oncology Research Institute, Tufts Medical Center, Boston, MA 02111; and [f]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Robert A. Weinberg, March 2, 2011 (sent for review December 8, 2010)

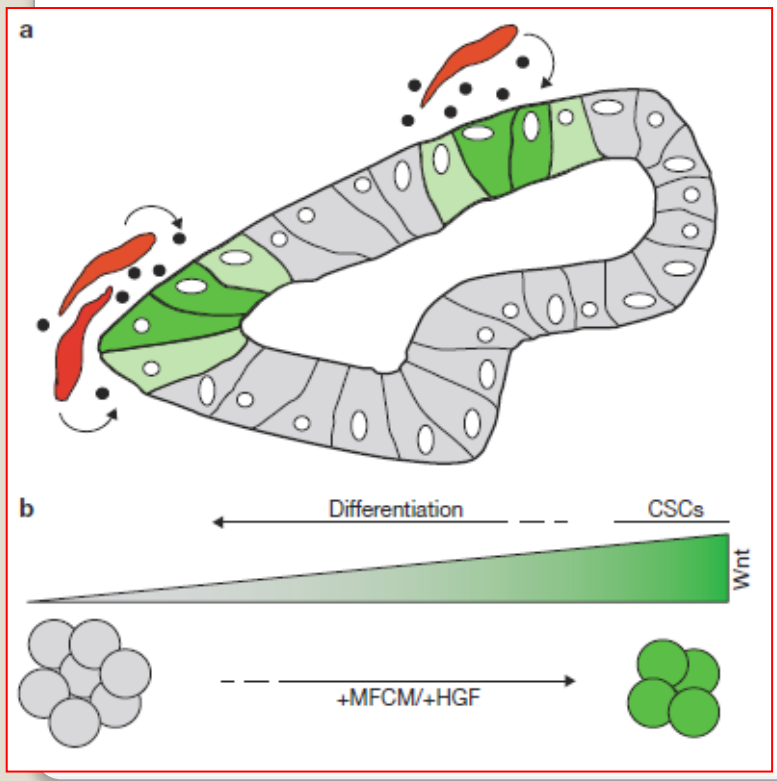


- ✓ **No CD44^{lo}CD24^{+}ESA^{-} dziedzerepitēlija šūnu kloniem spontāni veidojas CD44^{hi} šūnas;**
- ✓ **Gan pre-eksistējošām, gan *de novo* iegūtajām CD44^{hi} šūnām piemīt cilmes šūnu īpašības – spēja veidot mammosfēras un veidot vadiem līdzīgas struktūras;**
- ✓ **Šāda pat īpašība piemīt arī transformētām epitēlija šūnām**

nature cell biology

Wnt activity defines colon cancer stem cells and is regulated by the microenvironment

Louis Vermeulen[1,5], Felipe De Sousa E Melo[1,5], Maartje van der Heijden[1], Kate Cameron[1], Joan H. de Jong[1], Tijana Borovski[1], Jurriaan B. Tuynman[1], Matilde Todaro[2], Christian Merz[3], Hans Rodermond[1], Martin R. Sprick[1], Kristel Kemper[1], Dick J. Richel[1], Giorgio Stassi[2,4] and Jan Paul Medema[1,6].



- ✓**Wnt signālceļa aktivitāte ir nepieciešama zarnu normālo cilmes šūnu uzturēšanā;**
- ✓**CSCs ar augstu Wnt aktivitāti lokalizētas blakus mioepiteliālajām šūnām;**
- ✓**Mioepiteliālās šūnas producē hepatocītu augšanas faktoru (HRF), kas aktivē Wnt signālceļu;**
- ✓**HGF atjauno CSC fenotipu daļēji diferencētās vēža šūnās**

Cancer Res. 2009 Apr 1;69(7):2887-95. Epub 2009 Mar 10.

Immune-induced epithelial to mesenchymal transition in vivo generates breast cancer stem cells.

Santisteban M, Reiman JM, Asiedu MK, Behrens MD, Nassar A, Kalli KR, Haluska P, Ingle JN, Hartmann LC, Manjili MH, Radisky DC, Ferrone S, Knutson KL.

Department of Oncology, Mayo Clinic, Rochester, Minnesota 55905, USA.

Abstract

The breast cancer stem cell (BCSC) hypotheses suggest that breast cancer is derived from a single tumor-initiating cell with stem-like properties, but the source of these cells is unclear. We previously observed that induction of an immune response against an epithelial breast cancer led in vivo to the T-cell-dependent outgrowth of a tumor, the cells of which had undergone epithelial to mesenchymal transition (EMT). The resulting mesenchymal tumor cells had a CD24(-/lo)CD44(+) phenotype, consistent with BCSCs. In the present study, we found that EMT was induced by CD8 T cells and the resulting tumors had characteristics of BCSCs, including potent tumorigenicity, ability to reestablish an epithelial tumor, and enhanced resistance to drugs and radiation. In contrast to the hierarchal cancer stem cell hypothesis, which suggests that breast cancer arises from the transformation of a resident tissue stem cell, our results show that EMT can produce the BCSC phenotype. These findings have several important implications related to disease progression and relapse.

J Cell Biochem. 2011 May 26. doi: 10.1002/jcb.23199. [Epub ahead of print]

Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile.

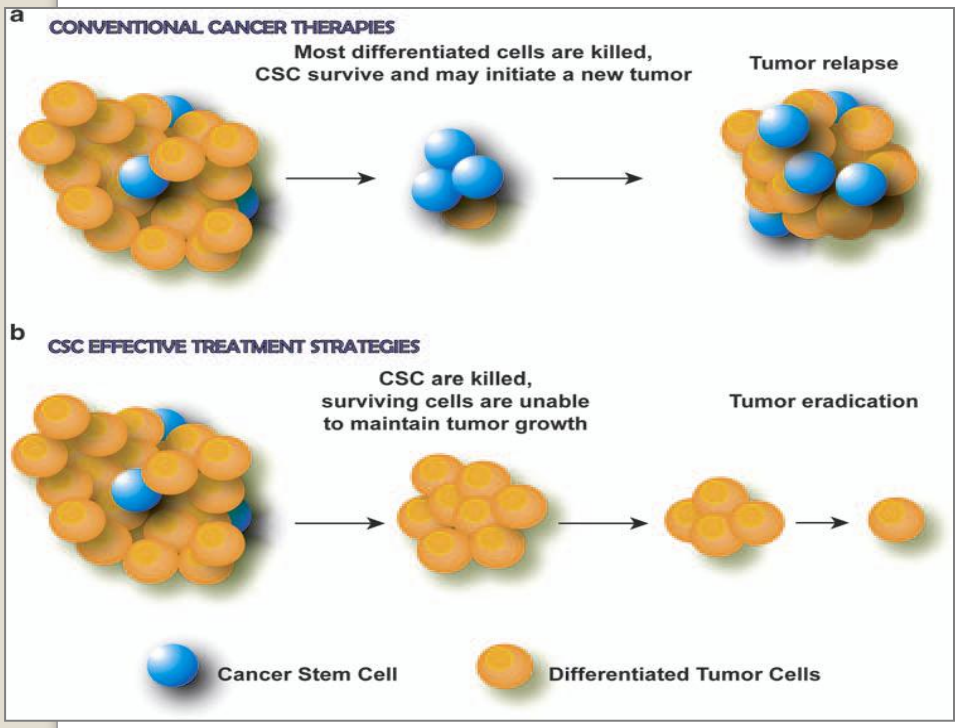
Latifi A, Abubaker K, Castrechini N, Ward AC, Liongue C, Dobill F, Kumar J, Thompson EW, Quinn MA, Findlay JK, Ahmed N.

Women's Cancer Research Centre, Royal Women's Hospital, Victoria 3052, Australia; University of Melbourne Department of Surgery, St Vincent Hospital, Victoria 3065, Australia.

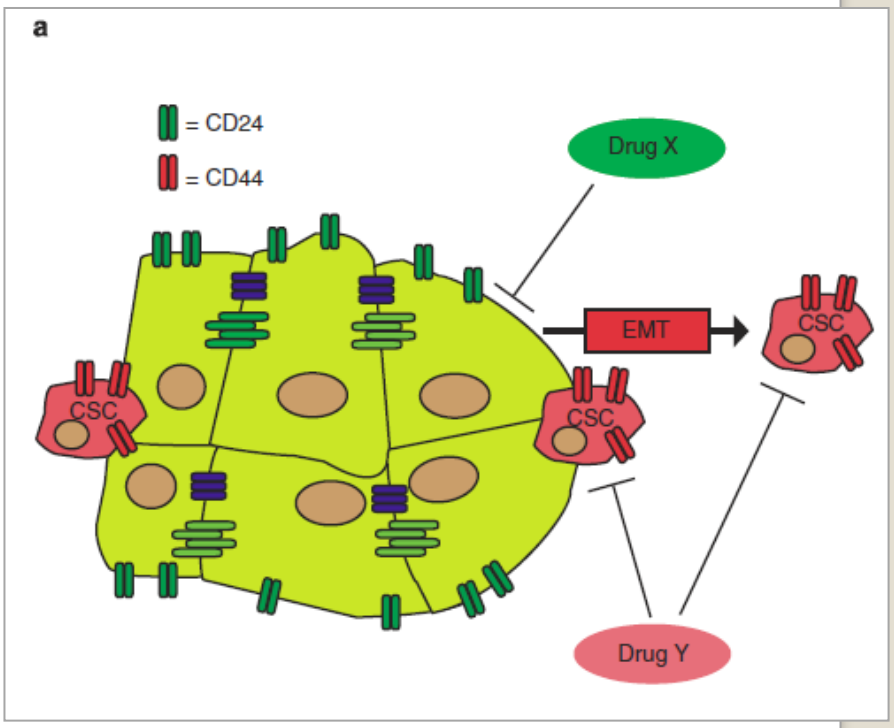
Abstract

Epithelial mesenchymal transition (EMT) and cancer stem cells (CSC) have been associated with resistance to chemotherapy. 80% of ovarian cancer patients initially respond to platinum based combination therapy but most return with recurrence and ultimate demise. To better understand such chemoresistance we have assessed the potential role of EMT in tumor cells collected from advanced-stage ovarian cancer patients and the ovarian cancer cell line OVCA 433 in response to cisplatin in vitro. We demonstrate that cisplatin-induced transition from epithelial to mesenchymal morphology in residual cancer cells correlated with reduced E-cadherin, and increased N-cadherin and vimentin expression. The mRNA expression of Snail, Slug, Twist and MMP-2 were significantly enhanced in response to cisplatin and correlated with increased migration. This coincided with increased cell surface expression of CSC-like markers such as CD44, α2 integrin subunit, CD117, CD133, EpCAM and the expression of stem cell factors Nanog and Oct-4. EMT and CSC-like changes in response to cisplatin correlated with enhanced activation of extracellular signal-regulated kinase (ERK)1/2. The selective MEK inhibitor U0126 inhibited ERK2 activation and partially suppressed cisplatin induced EMT and CSC markers. In vivo xenotransplantation of cisplatin treated OVCA 433 cells in zebra fish embryos demonstrated significantly enhanced migration of cells compared to control untreated cells. U0126 inhibited cisplatin induced migration of cells in vivo, suggesting that ERK2 signaling is critical to cisplatin-induced EMT and CSC phenotypes, and that targeting ERK2 in the presence of cisplatin may reduce the burden of residual tumor, the ultimate cause of recurrence in ovarian cancer patients. J. Cell. Biochem. © 2011 Wiley-Liss, Inc.

Vēža cilmes šūnas kā terapeitiskais mērķis



Eramo A et al, Oncogene, 2010



EMT, cancer stem cells and drug resistance
A Singh and J Settleman

Molekulārie mērķi vēža cilmes šūnās

Šūnas virsmas marķieri	
CD33+	AML, Gemtuzumab ozogamacin, FDA approved
CLL-1	AML, pre-clinical
Signālceļi	
PI3K/Akt/mTOR	Temsirolimus, everolimus, FDA approved
Hedgehog	GDC-0449, phase I (Genentech)
	PF04449913, phase I (Pfizer)
Notch	Phase II, Cancer Research UK
Jauni potenciāli mērķi	
Epiģenētiskās izmaiņas	HDACi iduce HIF1α degradation
Hipoksija	Bioreductive prodrugs that are activated in hypoxic tissue or moleculat targets involved in the survival of hypoxic cells
Mikrovide, EMT???	

Take-home messages:

✓ **Lai gan apšaubīta pašos pamatos, vēža cilmes šūnu hipotēze ir izturējusi laika pārbaudi vismaz 15 gadu garumā;**

✓ **Iespējams, vēža cilmes šūnas nav statiskas un fenotipiski nemainīgas, bet gan var veidoties no daļēji diferencētām šūnām un iegūt cilmes šūnu īpašības dažādu procesu rezultātā – hiposkijas, EMT, mikrovidē esošu signālu un dažādu stresa faktoru (staru vai ķīmijterapija, imūnreakcija) ietekmē;**

✓ **Nepietiek ar zāļu mērķēšanu uz esošajām cilmes šūnām, ja tās var veidoties arī no citām vēža šūnām – ir vajadzīga zāļu kombinācija;**

✓ **Signālceļi, kas nodrošina vēža cilmes šūnu pašatjaunošanos un izdzīvošanu, ir perspektīvi zāļu mērķi un tiek intensīvi pētīti;**

✓ **Procesi, kas inducē vēža cilmes šūnu veidošanos varētu būt jauni zāļu mērķi.**

Valsts pētījumu programma:

„Jaunu profilakses, ārstniecības, diagnostikas līdzekļu un metožu, biomedicīnas tehnoloģiju izstrāde sabiedrības veselības uzlabošanai”

Vadītājs: Prof. Valdis Pīrāgs

5. projekta mērķis:

izstrādāt jaunu pieeju individualizētu pretvēža ārstniecības līdzekļu radīšanai, kas balstīta uz vēža cilmes šūnām kā terapeitisko mērķi

Projekta uzdevumi:

Organiskās sintēzes institūts

- ✓ **Cinka atkarīgo enzīmu inhibitoru dizains un sintēze;**
- ✓ **Sintezēto savienojumu citotoksiskās aktivitātes (IC_{50}) un toksicitātes (LD_{50}) testēšana šūnu līnijās;**

Projekta uzdevumi:

Rīgas Austrumu Klīniskā Universitātes slimnīca

Paula Stradiņa Klīniskā Universitātes slimnīca

- ✓ **Biobankas veidošana un klīniskās un patoloģiskās informācijas apkopošana;**
- ✓ **Iegūto gēnu ekspresijas datu korelācija ar pacientu klīniskajiem un patoloģijas datiem**

Projekta uzdevumi:

Latvijas Biomedicīnas
pētījumu un studiju centrs
biomedicīnas pētījumi un izglītība no gēniem līdz cilvēkam

- ✓ **Metodikas izstrāde vēža cilmes šūnu un normālu cilmes šūnu izolēšanai no audu paraugiem;**
- ✓ **Iegūto cilmes šūnu populāciju fenotipa raksturošana;**
- ✓ **Terapeitiski nozīmīgāko cinka enzīmu ekspresijas analīze un funkcionālie pētījumi vēža un normālo cilmes šūnu populācijās;**
- ✓ **Audzēju šūnu pašatjaunošanās un rezistences attīstības molekulāro mehānismu padziļināta izpēte *in vitro* modeļos;**

Projekta uzdevumi:

- **Līdersavienojumu testēšana dažādās vēža cilmes šūnu populācijās, vērtējot to ietekmi uz šūnas ciklu, diferenciāciju, koloniju veidošanas spēju un invazivitāti.**

Krūts vēža cilmes šūnas

Prospective identification of tumorigenic breast cancer cells

Muhammad Al-Hajj*, Max S. Wicha*, Adalberto Benito-Hernandez†, Sean J. Morrison*‡§, and Michael F. Clarke*‡¶

Departments of *Internal Medicine and †Pathology, Comprehensive Cancer Center, ‡Department of Developmental Biology, and §Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109

Communicated by Jack E. Dixon, University of Michigan Medical School, Ann Arbor, MI, January 16, 2003 (received for review December 18, 2002)

Research article

Open Access

Brca1 breast tumors contain distinct CD44+/CD24- and CD133+ cells with cancer stem cell characteristics

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Research article

Open Access

Mammosphere culture of metastatic breast cancer cells enriches for tumorigenic breast cancer cells

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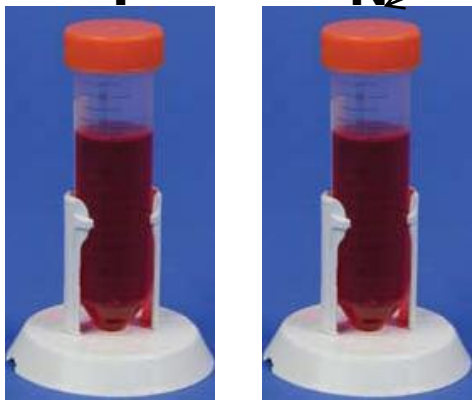
Krūts vēža klīniskā materiāla biobankas veidošana (RAKUS, Onkoloģijas centrs)

Klīniski-patoloģiskās info datubāze

DMEM, Ab

T N



Dzīvas šūnas sfēru un adherentajām kultūrām

RNALatter

T N

RNS, DNS un proteīnu kolekcija

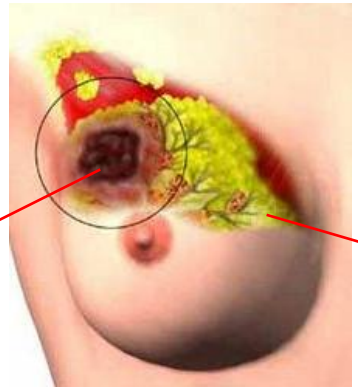
Ficoll

Asins šūnas funkcionāliem imunoloģijas pētījumiem

EDTA

DNS ģenētiskajai izpētei

Audu dezintegrēšana, šūnu suspensijas iegūšana un kultivēšana

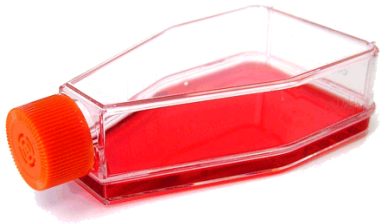


Audzējs

Veselie audi

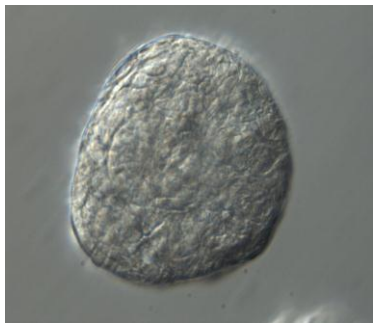
Bez seruma vide

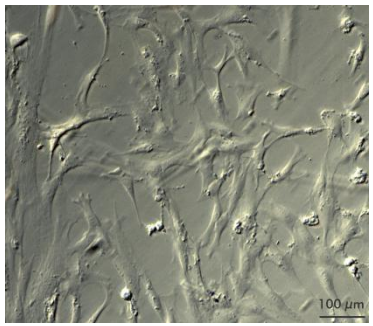
DMEM+10% FBS

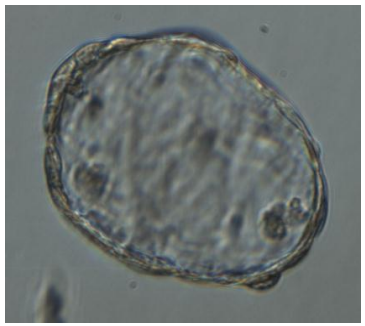


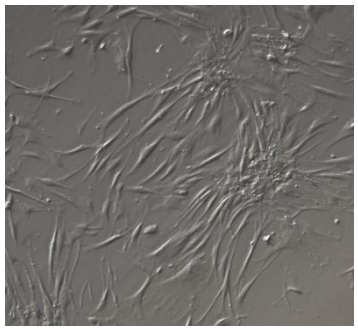
Bez seruma vide

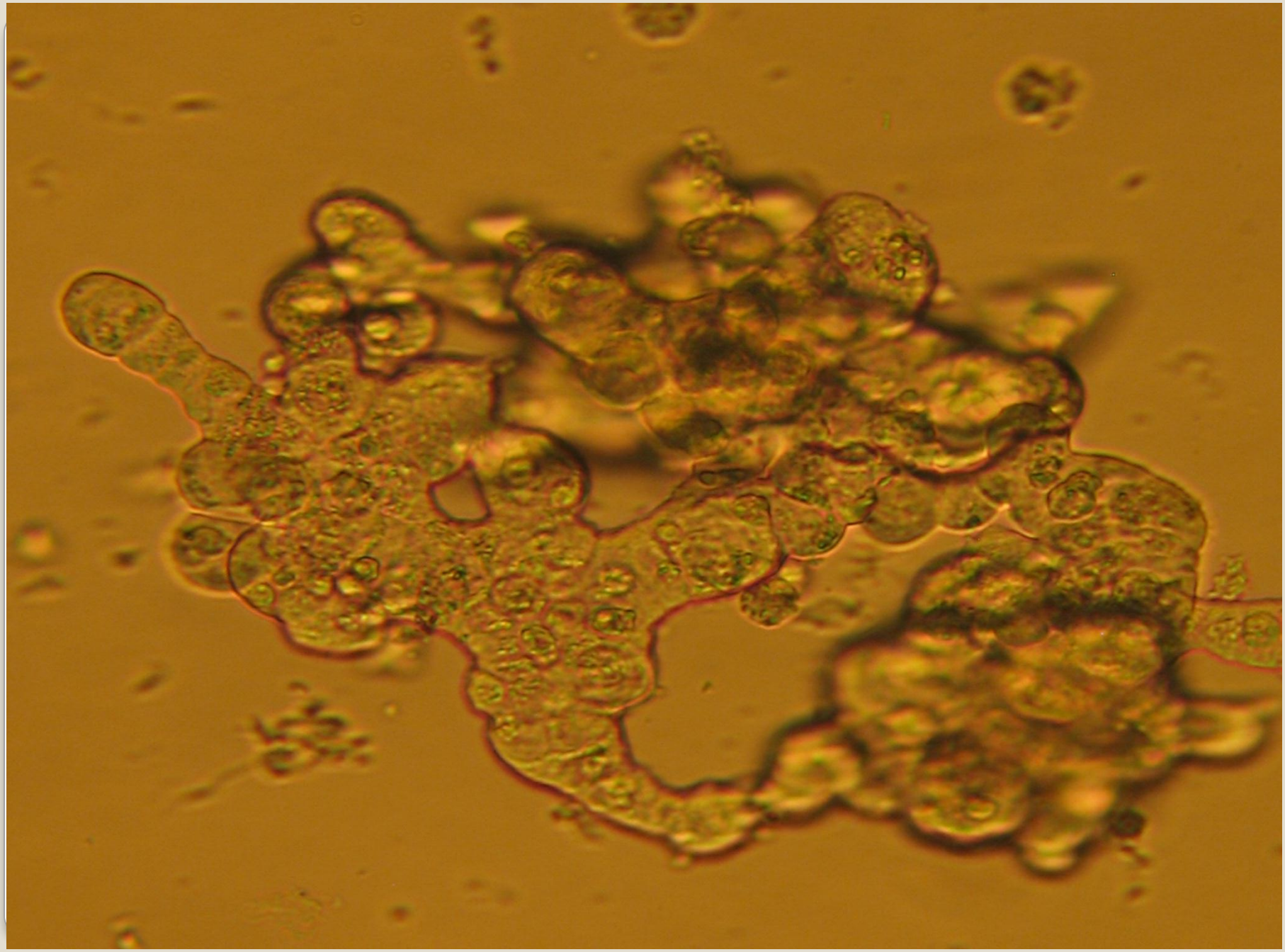
DMEM+10% FBS











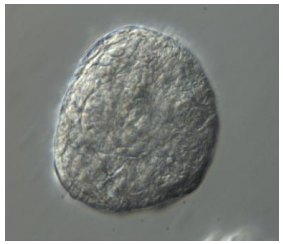
Adherenta kultūra

P3-P5 izmanto eksperimentiem

P2 stock

P3 stock

Sfēru kultūra



Primārā sfēru kultūra

dezintegrē

Sekundārā sfēru kultūra

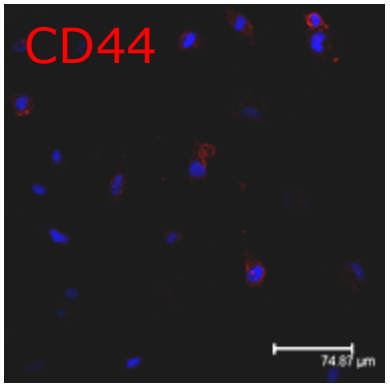
2. un 3. sfēru kultūru izmanto eksperimentiem

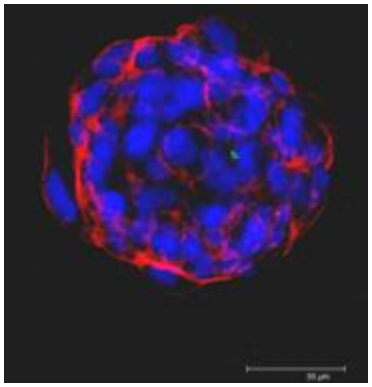
Iegūtās krūts vēža un normālo audu primārās kultūras

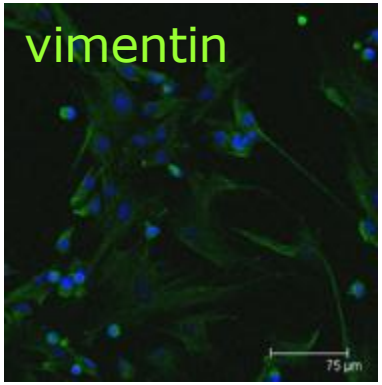
Parauga Nr		28	29 L	29 R	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
N	Sfēras	+	+								⊕	⊕									
T	Sfēras		+	+	+					⊕	⊕	⊕				+	+		+	+	+
LN	Sfēras																			+	
N	Adherenta										+	+						+			
T	Adherenta		+							+	+	+							+	+	+
LN	Adherenta																			+	

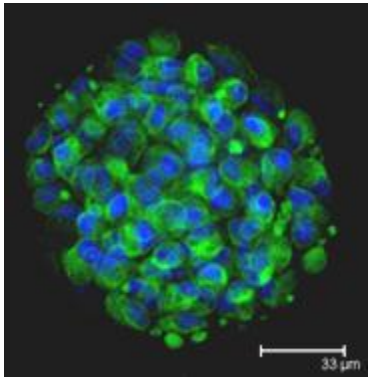
Imunocitoķīmiska primāro kultūru raksturošana

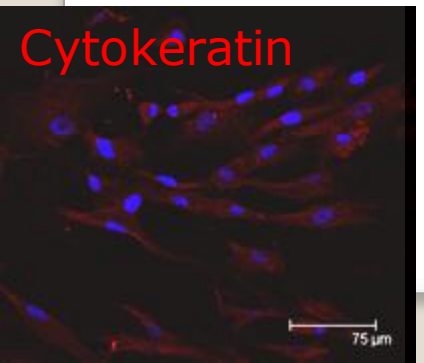
Citokeratīns
E-kadherīns
Vimentīns
ESA
CD44
CD24
Oct3/4
Nanog

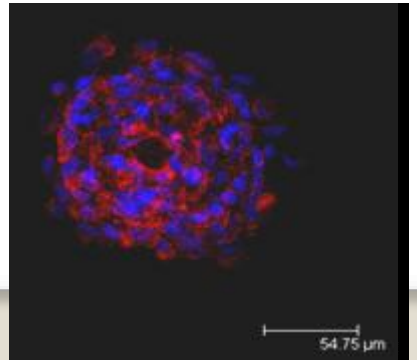


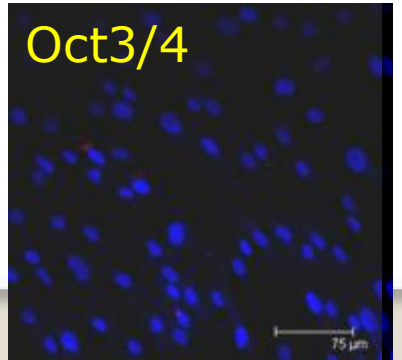


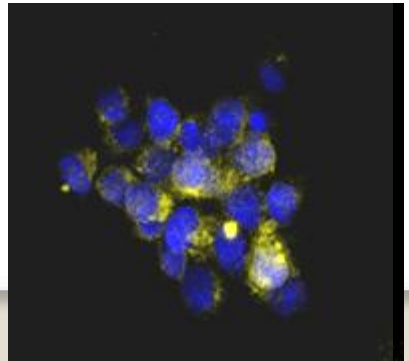








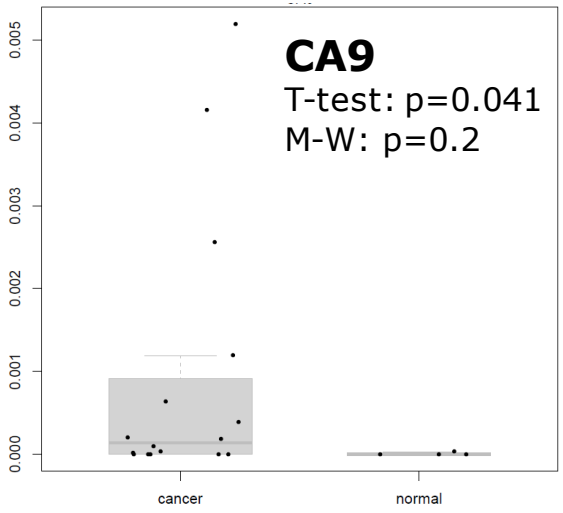


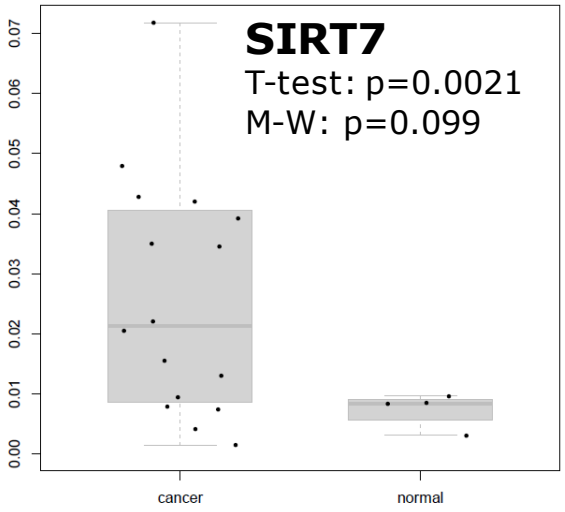


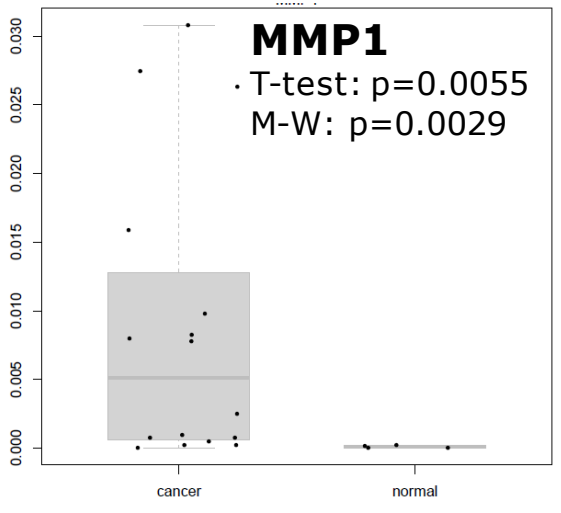
qPCR-array gēnu ekspresijas profilēšanai

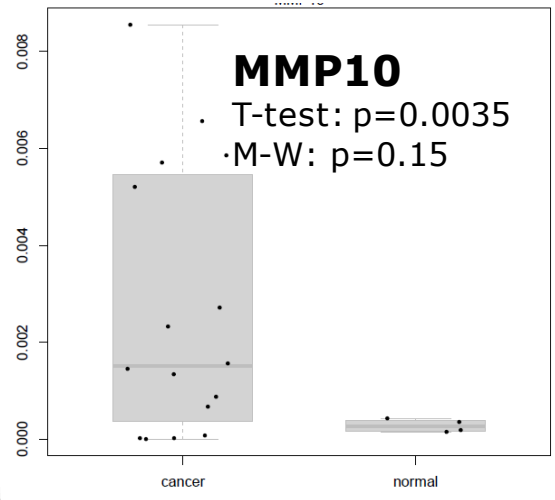
Cinka enzīmi	***CA9, CA12 HDAC2, HDAC3, HDAC4, HDAC6, HDAC8, SIRT1, SIRT7 MMP1, MMP2, MMP7, MMP9, MMP10,***
Wnt/β-katenīna signālceļš	***LEF1, MYC, WISP1, JUN , CCND1, (MMP7, CD44, TWIST1, VEGFA, TERT)***
Notch signālceļš	***HES1, (CD44, ERBB2, CCND1)***
Hedgehog signālceļš	***GLI1, MYCN, SHH***
Epiteliāli mezenhimālā tranzīcija	***SNAI1, TWIST1, CDH1, VIM, (MMP2, MMP9, SIRT1)***
Hipoksijas atbildes signālceļš	***LDHA, SLC2A1, VEGFA, (CA9, CA12, JUN, MMP2)***
Telomerāze	***TERT***
Krūts vēža cilmes šūnu marķieri	***CD44, CD24, FLOT2***
Krūts vēža subtipu marķieri	***ESR1, ESR2, KRT5, PGR, ERBB2***
Pluripotences marķieri	***NANOG, SOX2, LIN28***

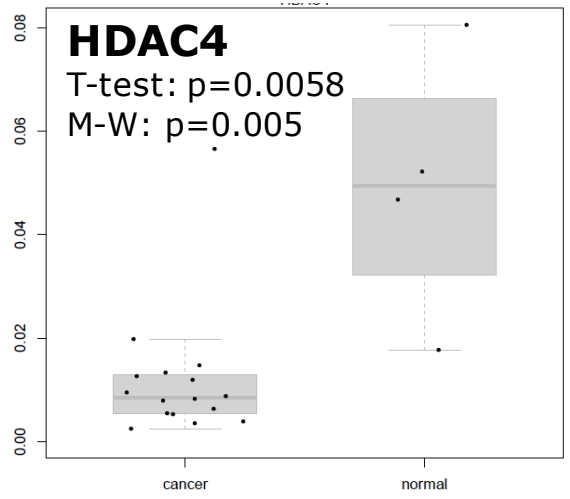
CA9, SIRT7, MMP1 un MMP10 ekspresija ir ievērojami paaugstināta, bet HDAC4 un SIRT1 - pazemināta krūts vēža audos, salīdzinot ar normāliem audiem

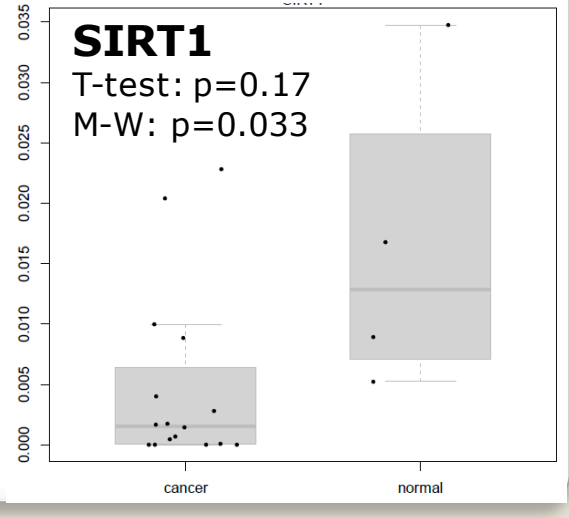




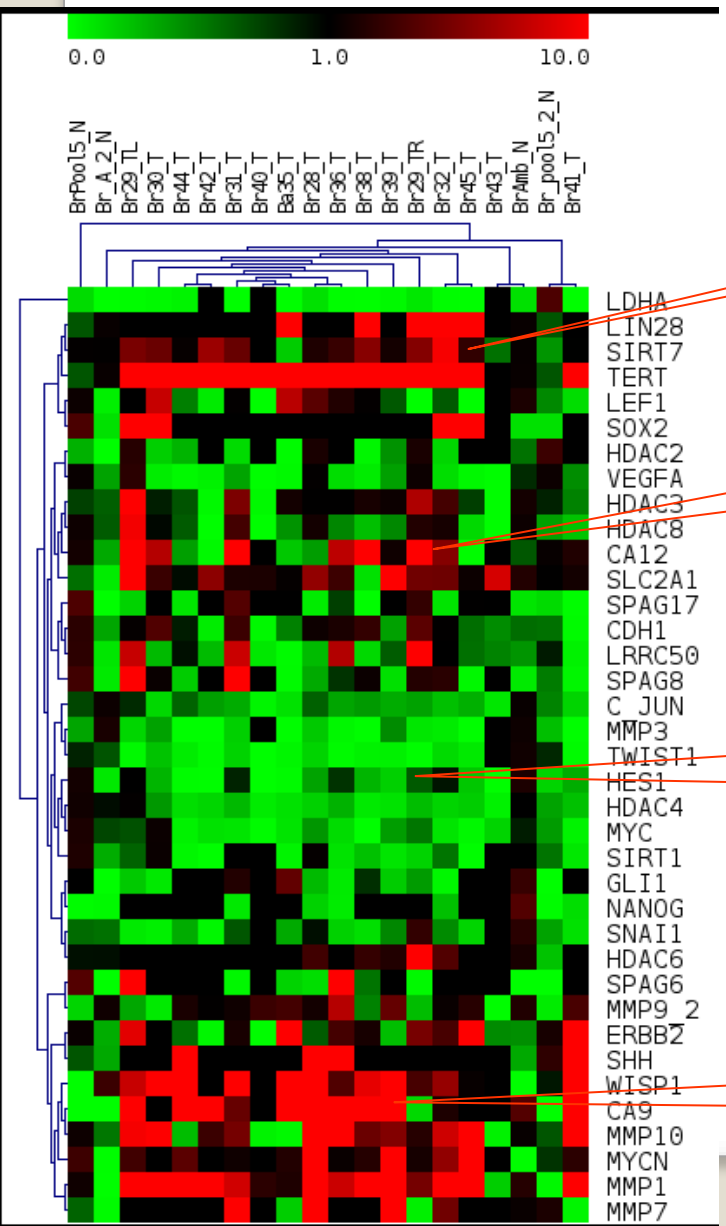








Gēnu ekspresijas izmaiņas audzējos, salīdzinot ar normāliem audiem



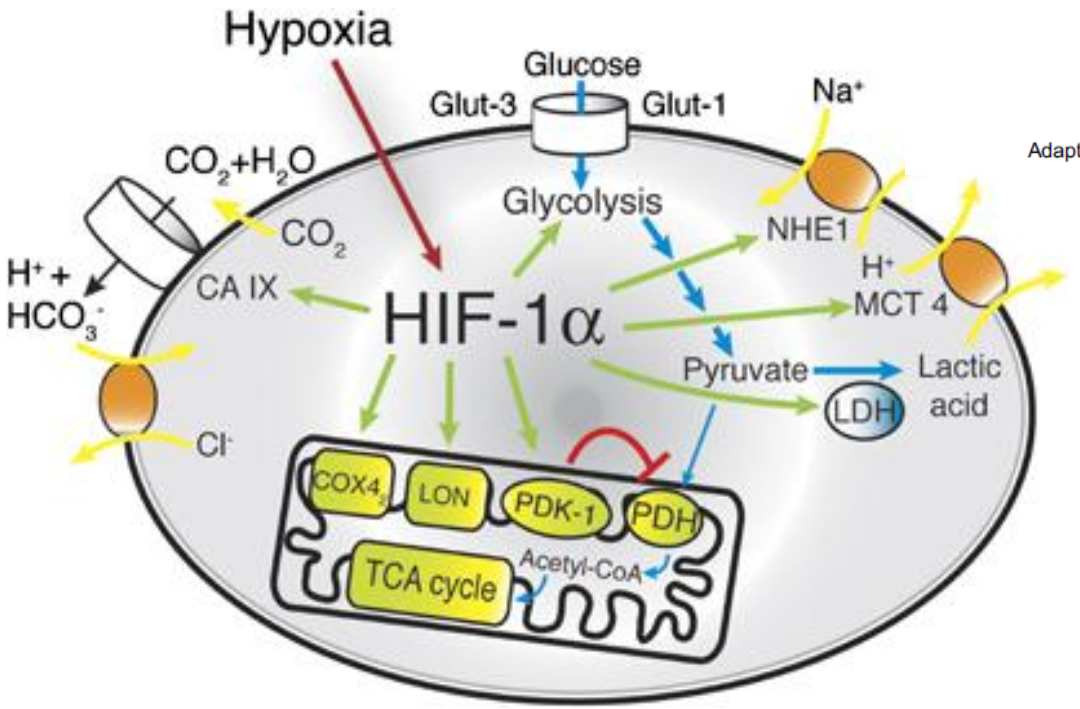
SIRT7
Lin28

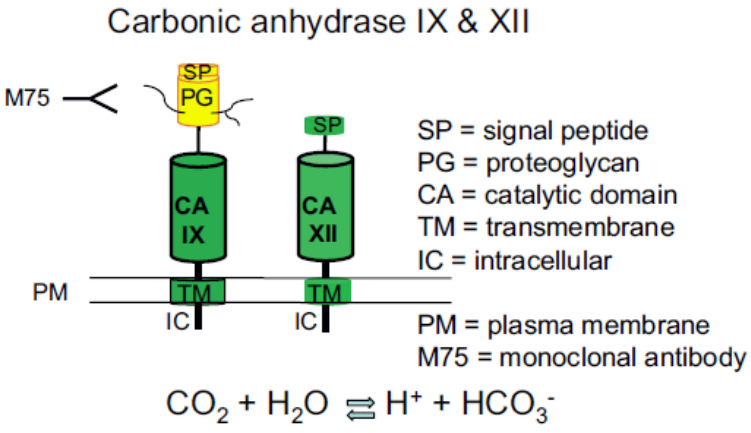
SLC2A1/Glut1
VEGFA
CA12
HDAC3
HDAC8

TWIST1
HES1
C-JUN
MYC
SIRT1
HDAC4

MMP1
MMP10
CA9
MYCN
WISP1

CAIX – nodrošina intracelulārā pH regulāciju





Biology of HIF-1
A Weidemann and R S Johnson

CAIX, MMP1 un MMP10

- ✓ CA IX stabilizē intracelulāro pH – padara bāziskāku intracelulāro vidi, bet paskābina ekstracelulāro vidi
- ✓ CA IX koordinē pHi regulāciju multicelulārās sfērās
- ✓ pHi regulācija ir svarīgs šūnu izdzīvošanas mehānisms
- ✓ CA IX un angioģenēzes inhibitoru kombinācija varētu būt perspektīva terapijas stratēģija
- ✓ MMP1 un MMP10 – pastiprināti ekspresētas endoteliālajās šūnās pēc VEGF stimulācijas un ir nepieciešamas angioģenēzei
- ✓ Daļā krūts vēžu CA IX, MMP1 un MMP10 tiek koordinēti ekspresēti – to inhibīcijai varētu būt terapeitisks efekts
- ✓ Vides skābums izraisa T šūnu anerģiju – tās nespēj sekretēt perforīnu
- ✓ CA IX inhibitoru kombinēšana ar imunoterapiju?!?

Kuņģa vēža cilmes šūnas

Published in final edited form as:
Stem Cells. 2009 May ; 27(5): 1006–1020. doi:10.1002/stem.30.

Identification of Gastric Cancer Stem Cells Using the Cell Surface Marker CD44

Shigeo Takaishi[a], Tomoyuki Okumura[a,b], Shuiping Tu[a], Sophie S.W. Wang[a,c], Wataru Shibata[a], Ramanathan Vigneshwaran[a], Shanisha A.K. Gordon[a], Yutaka Shimada[b], and Timothy C. Wang[a]

Cell. Mol. Life Sci.
DOI 10.1007/s00018-011-0672-z
Cellular and Molecular Life Sciences

RESEARCH ARTICLE

Cancer spheres from gastric cancer patients provide an ideal model system for cancer stem cell research

Myoung-Eun Han · Tae-Yong Jeon · Sun-Hwi Hwang · Young-Suk Lee · Hyun-Jung Kim · Hye-Eun Shim · Sik Yoon · Sun-Yong Baek · Bong-Seon Kim · Chi-Dug Kang · Sae-Ock Oh

EpCAM+/CD44+

Received: 10 December 2010 / Revised: 6 February 2011 / Accepted: 24 February 2011

Vai kuņģa vēža cilmes šūnas ir radušās no kaulu smadzeņu šūnām???

Gastric Cancer Originating from Bone Marrow–Derived Cells

JeanMarie Houghton,[1*] Calin Stoicov,[1] Sachiyo Nomura,[2,3] Arlin B. Rogers,[4] Jane Carlson,[1] Hanchen Li,[1] Xun Cai,[1] James G. Fox,[4] James R. Goldenring,[2,5] Timothy C. Wang[1*†]

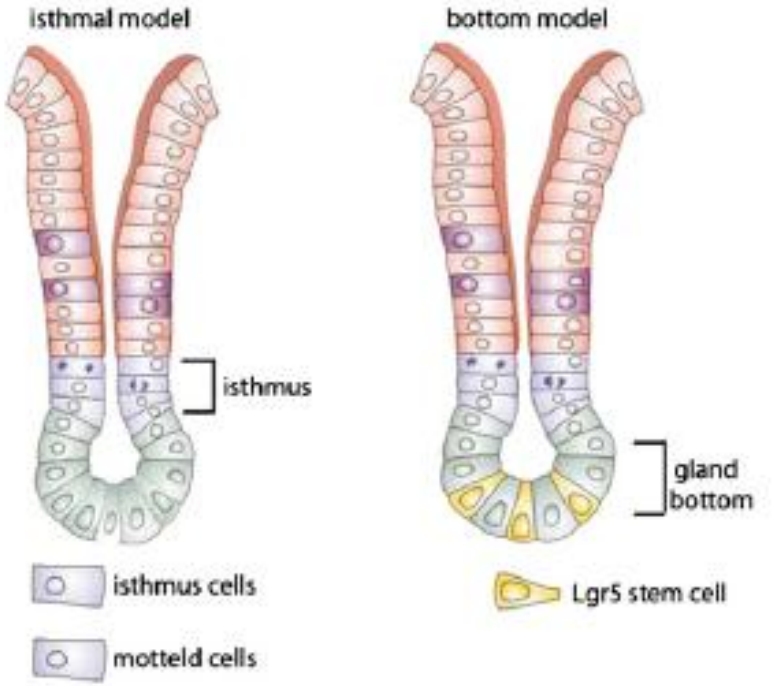
Epithelial cancers are believed to originate from transformation of tissue stem cells. However, bone marrow–derived cells (BMDCs), which are frequently recruited to sites of tissue injury and inflammation, might also represent a potential source of malignancy. We show that although acute injury, acute inflammation, or transient parietal cell loss within the stomach do not lead to BMDC recruitment, chronic infection of C57BL/6 mice with *Helicobacter*, a known carcinogen, induces repopulation of the stomach with BMDCs. Subsequently, these cells progress through metaplasia and dysplasia to intraepithelial cancer. These findings suggest that epithelial cancers can originate from marrow-derived sources and thus have broad implications for the multistep model of cancer progression.

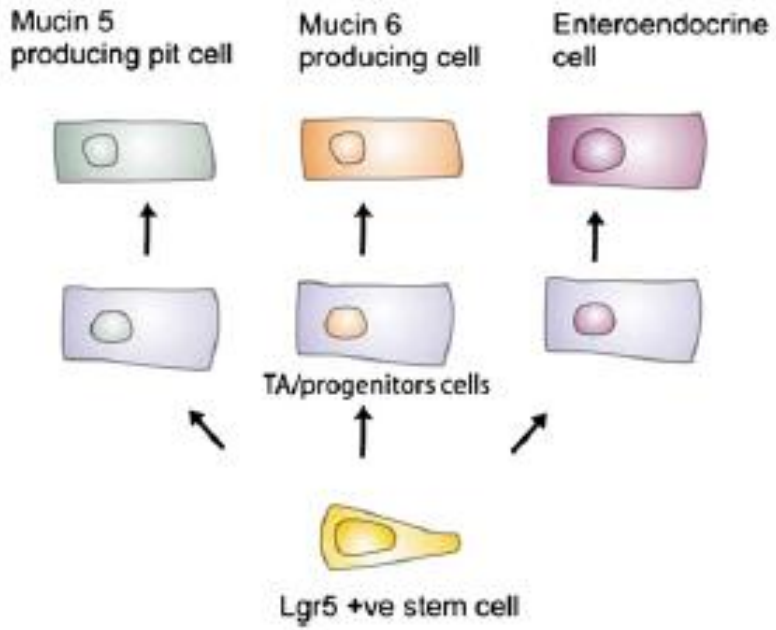
Vai tomēr dziedzerepitēlija cilmes šūnām???

Stem cells and cancer of the stomach and intestine

Robert G.J. Vries[1], *Meritxell Huch*[1], *Hans Clevers*[*]

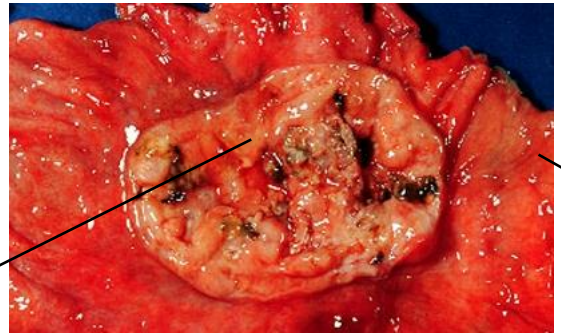
Hubrecht Institute for Developmental Biology and Stem Cell Research, Uppsalalaan 8, 3584CT Utrecht & University Medical Centre Utrecht, Netherlands





(GPR49)

Kuņģa vēža un normālo audu primāro kultūru iegūšana



Audzējs

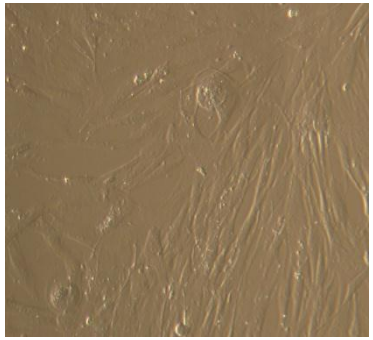
Veselie audi

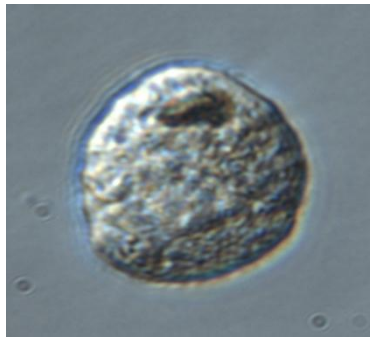
Bez seruma vide

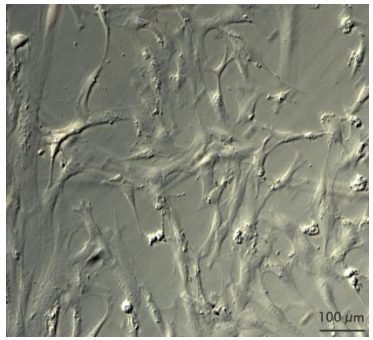
DMEM+10% FBS

DMEM+10% FBS

?







Izvirzītie jautājumi

- ✓ **Vai iespējams iegūt sfēru kultūru tieši no primārās audzēja šūnu suspensijas?**
- ✓ **Kāds ir sfēru veidojošo šūnu fenotips (t.i. epiteliālo vs mezenhimālo cilmes šūnu marķieri)?**
- ✓ **Vai no viena audu parauga iegūtās sfēras ir fenotipiski homogēnas vai heterogēnas?**
- ✓ **Cinka enzīmu ekspresijas profils audos un iegūtajās cilmes šūnu populācijās un tā korelācija ar cilmes šūnu bioloģijā svarīgu signālceļu aktivāciju.**
- ✓ **Ekspresijas datu korelācija ar klīniskajām un patoloģiskajām pazīmēm.**

Plaušu vēža cilmes šūnas

Cell Death and Differentiation (2008) 15, 504–514

www.nature.com/cdd

Identification and expansion of the tumorigenic lung cancer stem cell population

A Eramo[1], F Lotti[2], G Sette[2], E Pilozzi[3], M Biffoni[1], A Di Virgilio[4], C Conticello[2], L Ruco[3], C Peschle[1] and R De Maria*[,1]

OPEN ACCESS Freely available online

PLoS one

Oct-4 Expression Maintained Cancer Stem-Like Properties in Lung Cancer-Derived CD133-Positive Cells

Yu-Chih Chen[1☯], Han-Shui Hsu[1,3,5☯], Yi-Wei Chen[1,6☯], Tung-Hu Tsai[2,9], Chorng-Kuang How[1,3,7], Chien-Ying Wang[1,7], Shih-Chieh Hung[1,8], Yuh-Lih Chang[2,8], Ming-Long Tsai[8], Yi-Yen Lee[1,9], Hung-Hai Ku[4,9*], Shih-Hwa Chiou[1,8*]

PNAS

Highly tumorigenic lung cancer CD133^{+} cells display stem-like features and are spared by cisplatin treatment

Giulia Bertolini[a,1], Luca Roz[a,1], Paola Perego[b], Monica Tortoreto[b], Enrico Fontanella[c], Laura Gatti[b], Graziella Pratesi[b], Alessandra Fabbri[d], Francesca Andriani[a], Stella Tinelli[b], Elena Roz[e], Roberto Caserini[a], Salvatore Lo Vullo[f], Tiziana Camerini[f], Luigi Mariani[f], Domenico Delia[c], Elisa Calabrò[g], Ugo Pastorino[g], and Gabriella Sozzi[a,2]

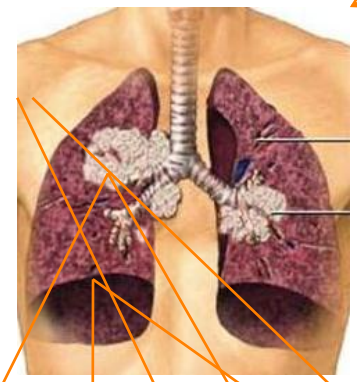
[a]Molecular Cytogenetics Unit, [b]Preclinical Chemotherapy and Pharmacology Unit, [c]Cell Cycle Control Unit, Department of Experimental Oncology, [d]Department of Pathology, [f]Unit of Medical Statistics and Biometry, and [g]Unit of Thoracic Surgery, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico, Istituto Nazionale dei Tumori, 20133 Milan, Italy; and [e]Pathology Unit, Casa di Cura "La Maddalena," 90136 Palermo, Italy

Edited by Carlo M. Croce, The Ohio State University, Columbus, Ohio, and approved August 10, 2009 (received for review May 29, 2009)

Plaušu audzēju biobankas veidošana

NSCLC pacienti

Operējami pacienti (~30%)

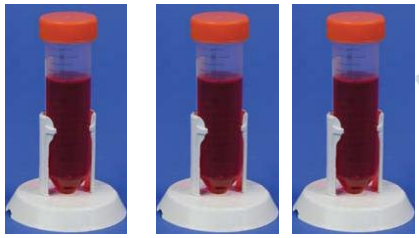


Neoperējami pacienti (~70%) Staru un/vai ķīmijterapija

Biopsija (diagnostiskā bronhoskopija)

RNALatter

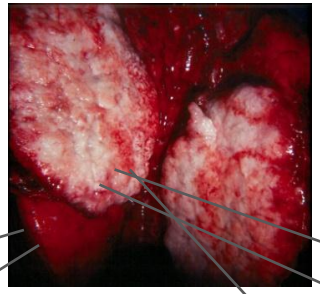
T N LN



T N LN

RNALatter

Plaušu audu primāro kultūru iegūšana un cisplatīna rezistento šūnu selekcija



T (LN)

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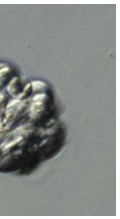
Sfēras



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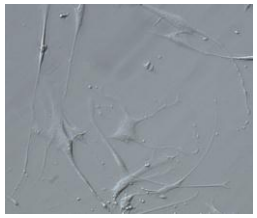
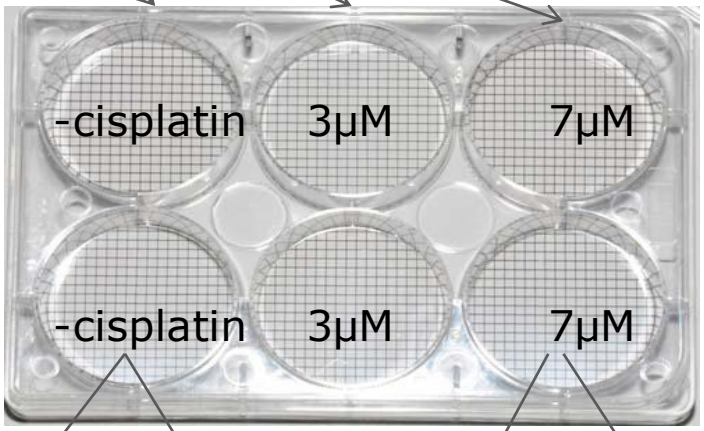
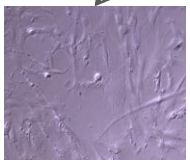
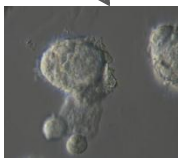
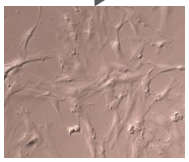
Adherenta kultūra

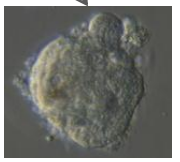












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