Brain Tumour Initiating Cells: Why the Cancer Stem Cell Hypothesis matters to patients with brain tumours

Workshop on Mathematical Oncology 3, March 18-20 2010

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Scientist, SCC-RI,

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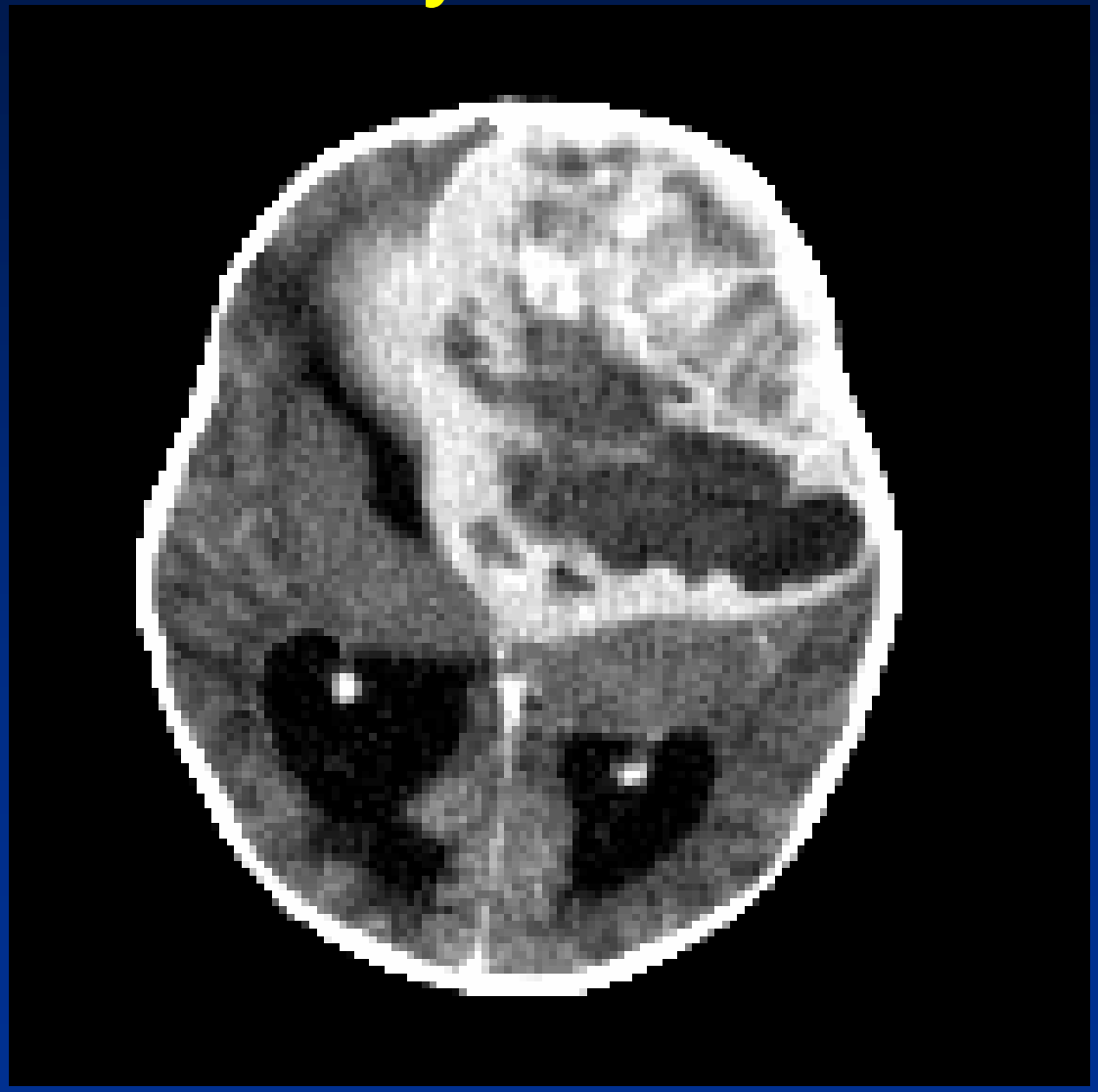
Brain Tumours: Aggressive and Refractory to Treatment

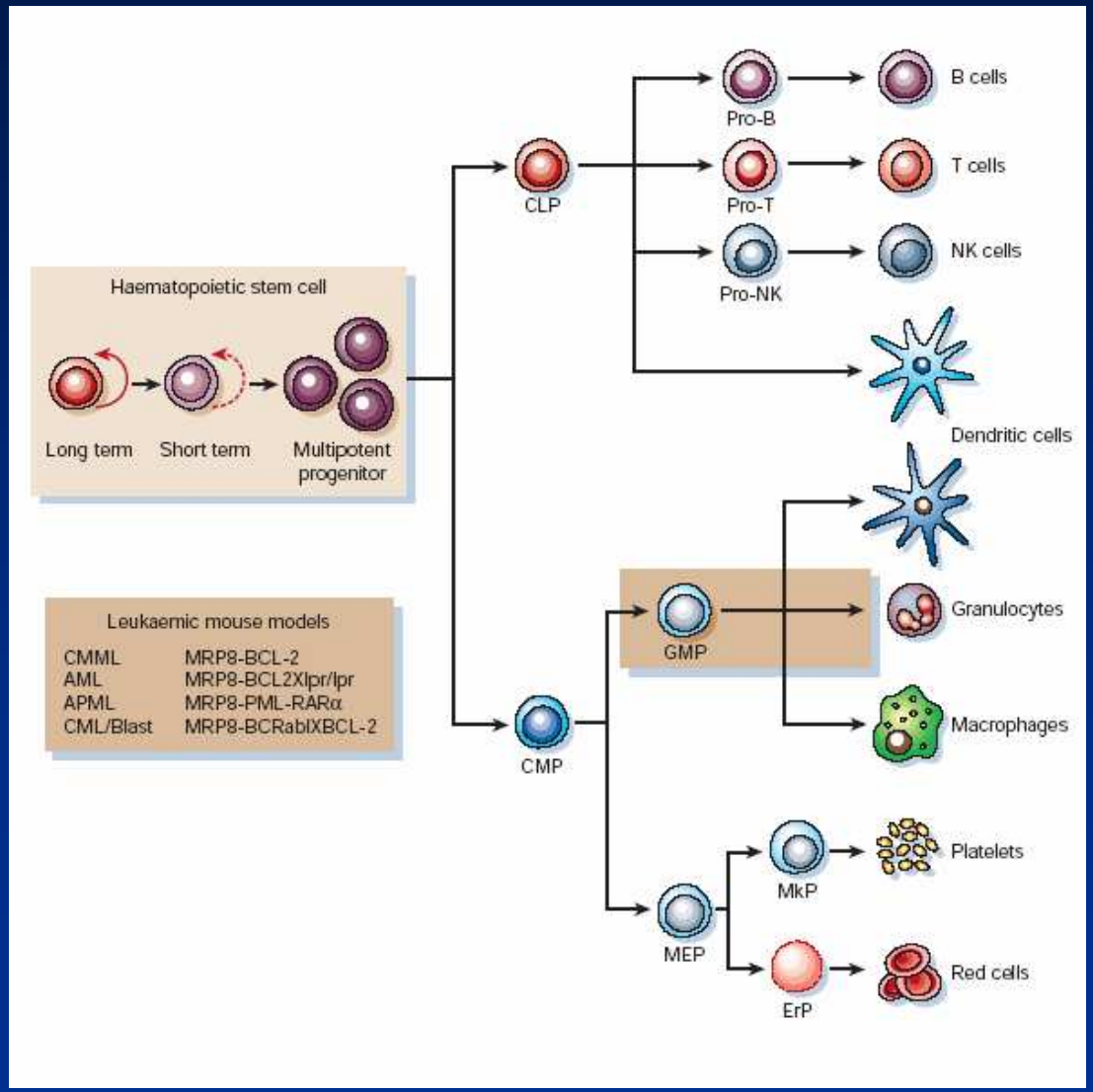


Properties of a Stem Cell

- Self-renewal
- Proliferation
- Multilineage differentiation
- Longevity

These features are reminiscent of cancer cell properties

Stem Cells Replenish Our Blood System for our Entire Lives



Humoral and cellular defense

Cellular defense

clotting

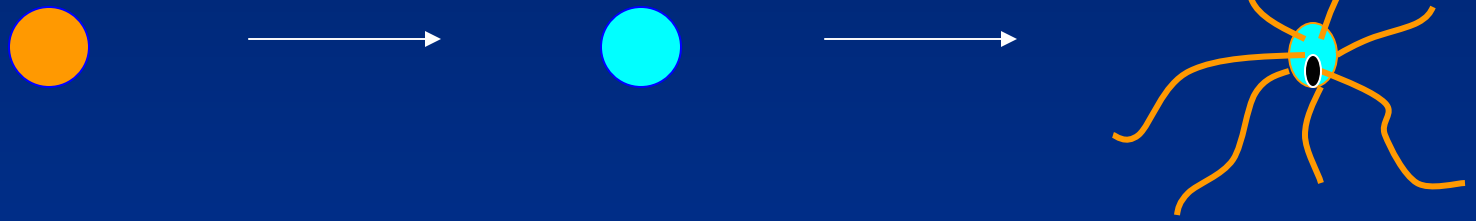
O2 carrying

Tissues are organized as a functional hierarchy derived from stem cells

Stem cell

Progenitor cell

Mature Cell (Differentiated)



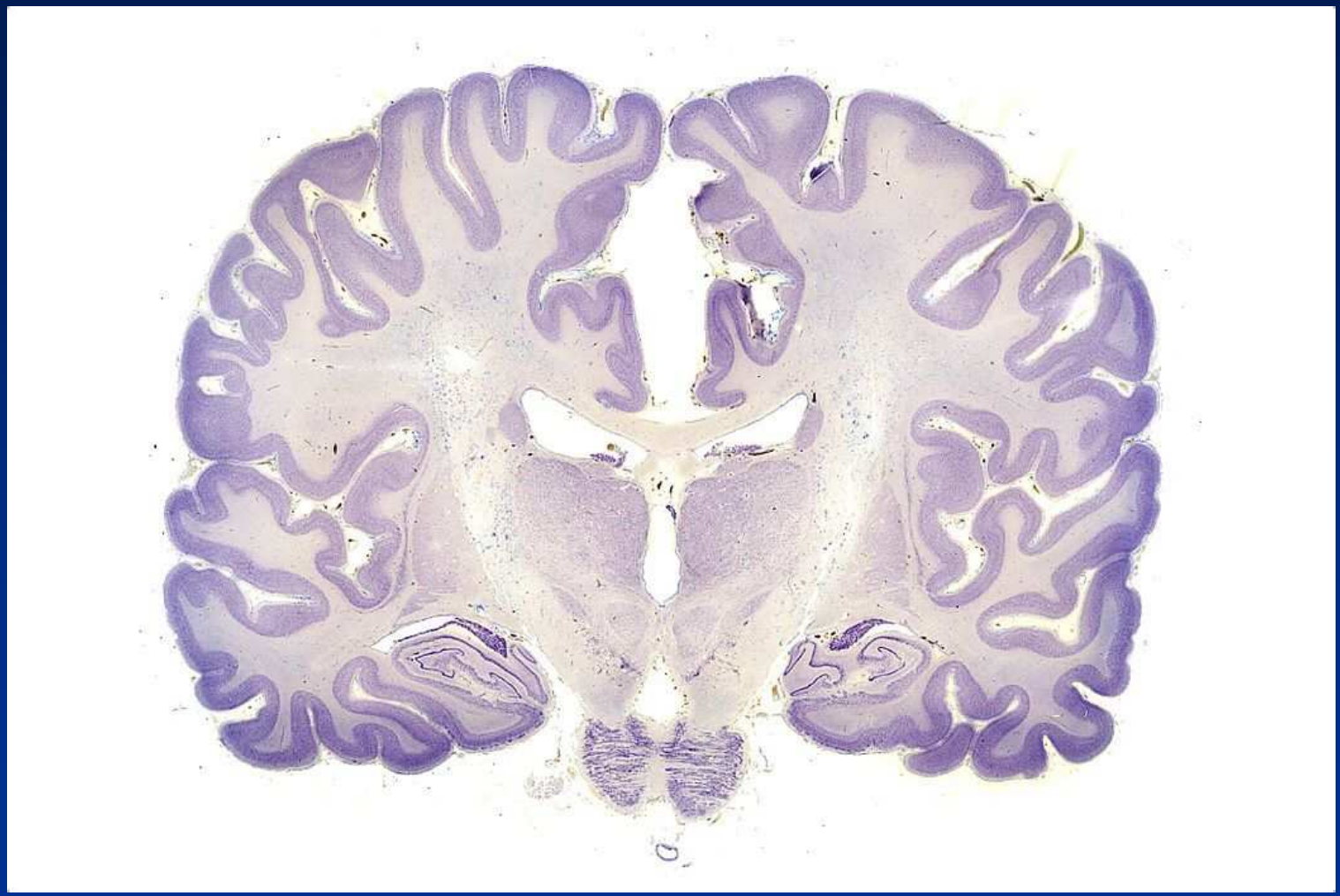
Self renewal

Divides Slowly

Divides rapidly, but then stops

Does not proliferate

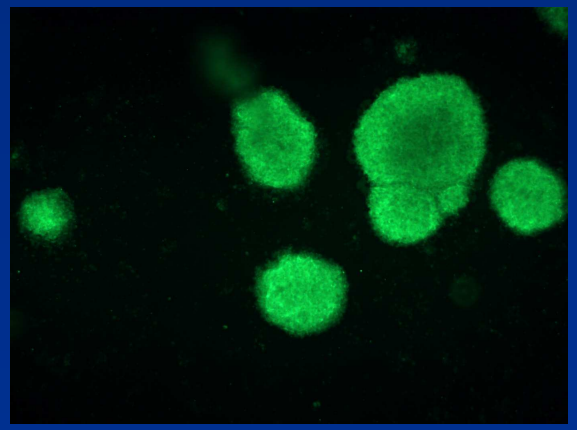
The Human Brain Has Stem Cells

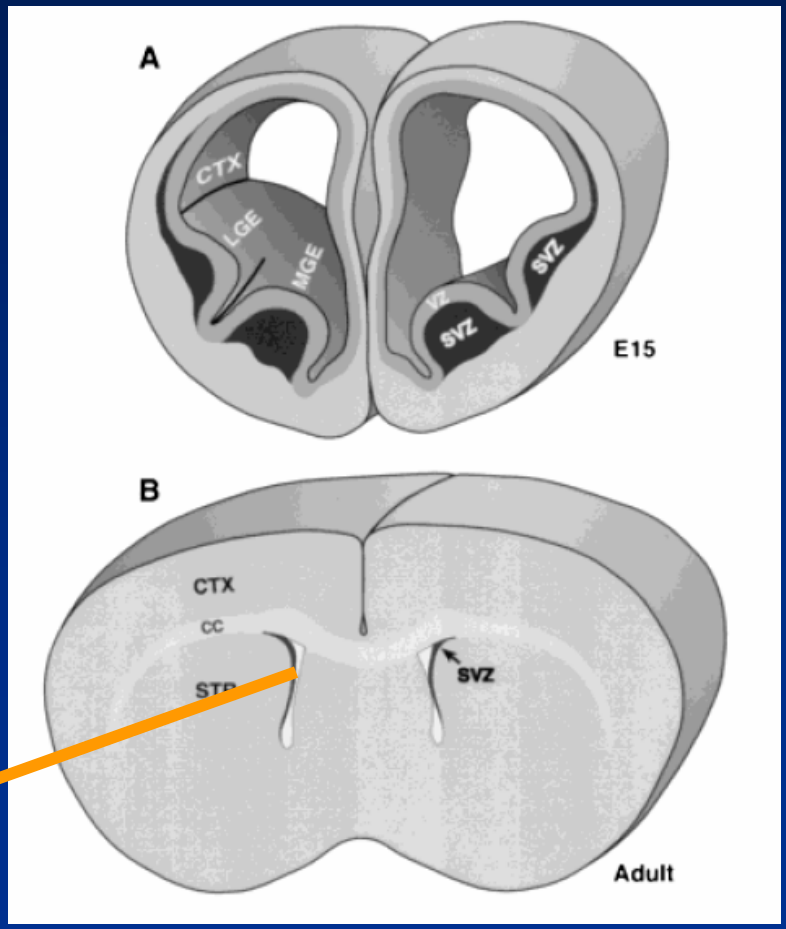


Neural Stem Cells and the Subventricular Zone (SVZ)

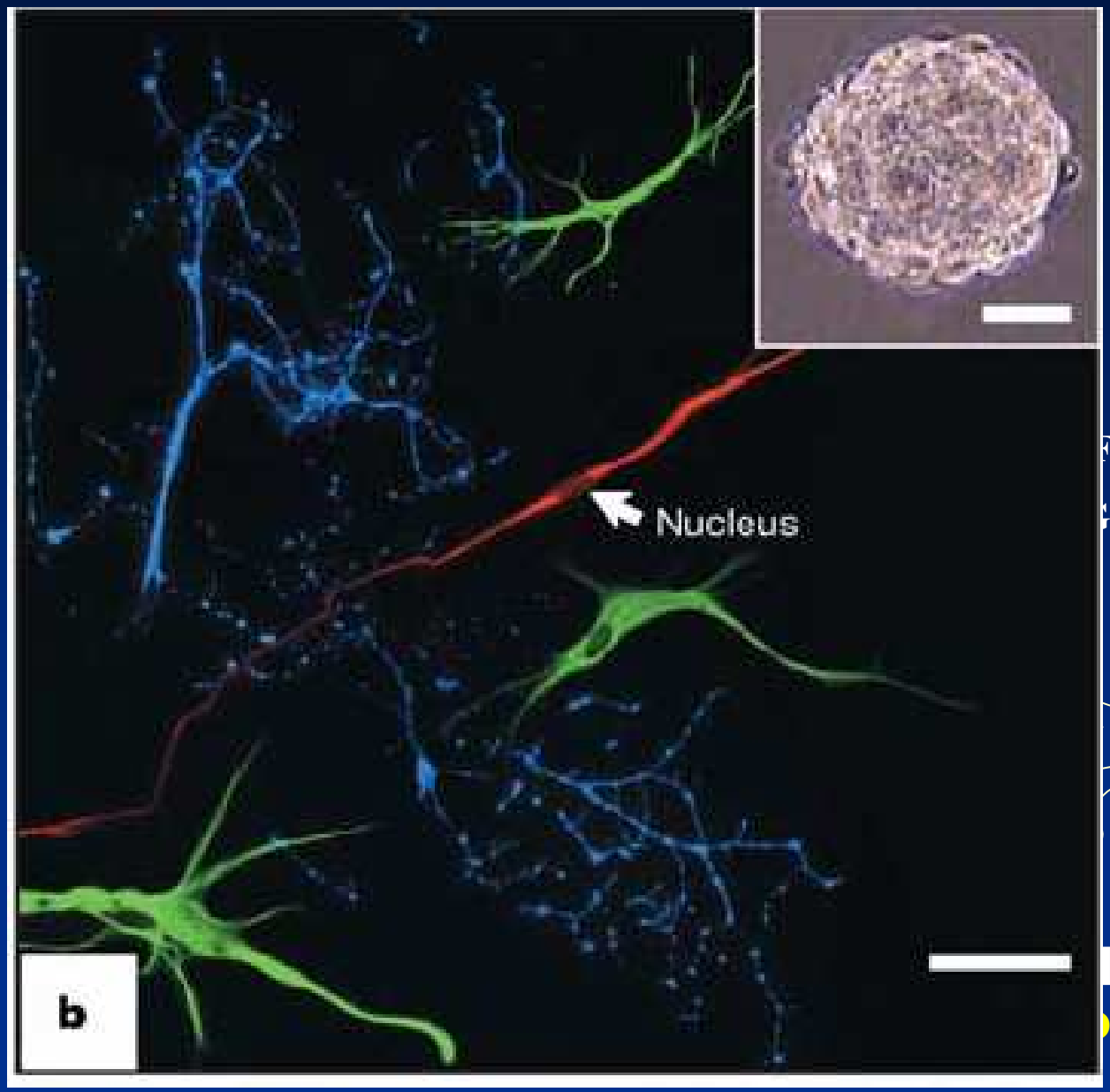
Mouse SVZ stained with Nestin-eGFP and CD133

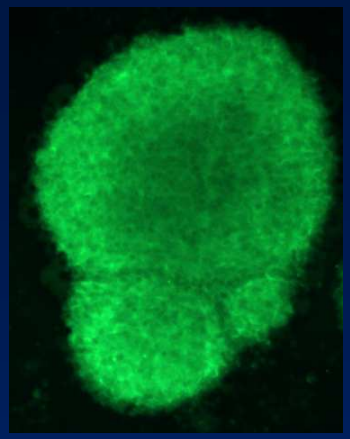
** From Sawamoto et al, J Neurosci 2001*





Neural Stem Cell





ligodendrocyte

GFRα+

From Nature 427 (2004): Sanai et al

Normal Stem Cell *vs* Cancer Stem Cell

- Normal stem cells are tightly regulated and differentiate into normal cells of a tissue

- Cancer stem cells, by virtue of mutations, are dysregulated and self renew and differentiate aberrantly, generating the abnormal cells that make up the cancer

A Stem Cell Origin for Brain Tumours?

JOURNAL OF NEUROPATHOLOGY & EXPERIMENTAL NEUROLOGY

VOLUME 3 JANUARY 1944 NUMBER 1

THE SUBEPENDYMAL CELL PLATE (MATRIX) AND ITS RELATIONSHIP TO BRAIN TUMORS OF THE EPENDYMAL TYPE*

JOSEPH H. GLOBUS, M.D.

[*New York*]

AND

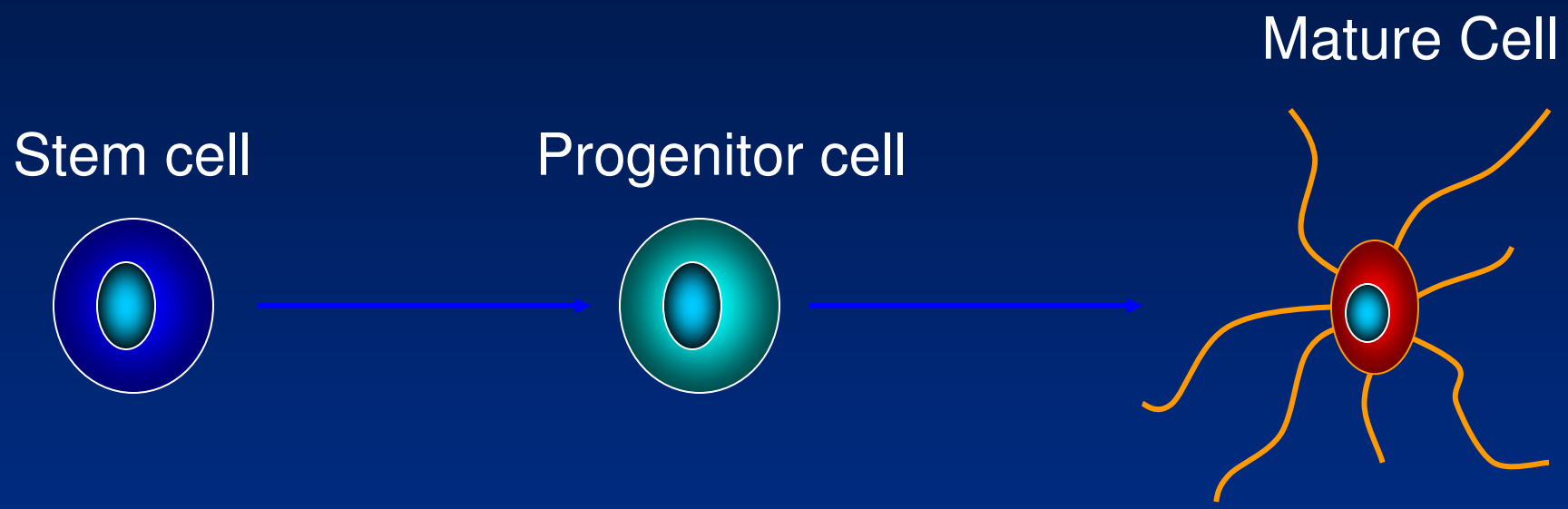
HARTWIG KUHLENBECK, M.D.

[*Philadelphia, Pa.*]

Stem Cell Origin for Brain Tumours?

- Brain tumours cells frequently resemble primitive normal brain cells, eg. Medulloblastomas resemble proliferating external granule cells of the cerebellum
- Animals exposed to carcinogens perinatally get tumours of different phenotypes arising from the SVZ
- Brain tumour cells express nestin
- Oncogene transfer to neural progenitors allows for more potent transformation effects

Stem Cell Hierarchy



Self renewal

Divides slowly

Divides rapidly but then stops

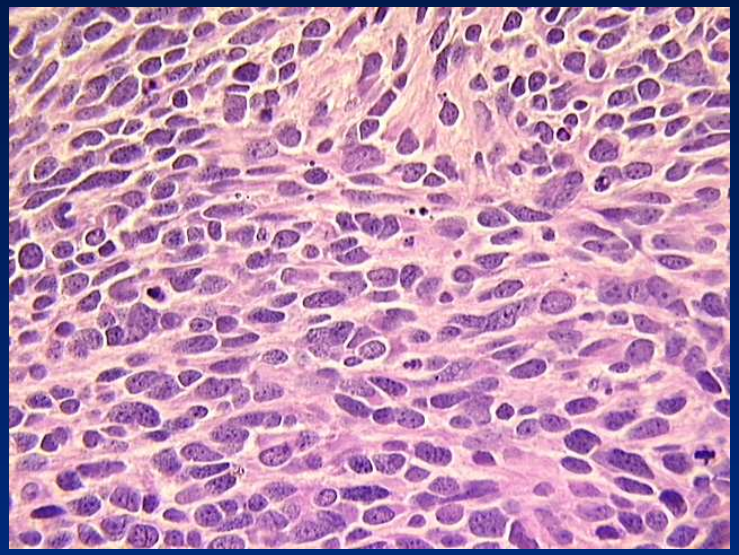
Does not divide

Why is a stem cell a more likely target of transformation?

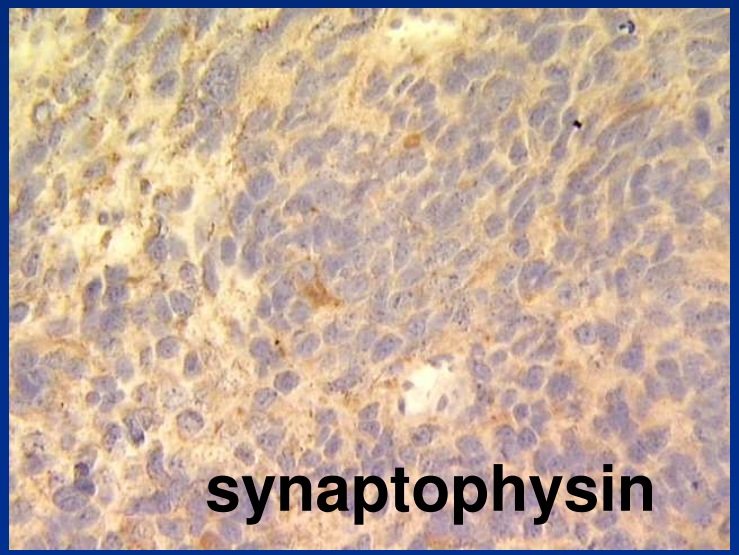
- **Longevity**: allows for accumulation of genetic mutations over the life span of the organism

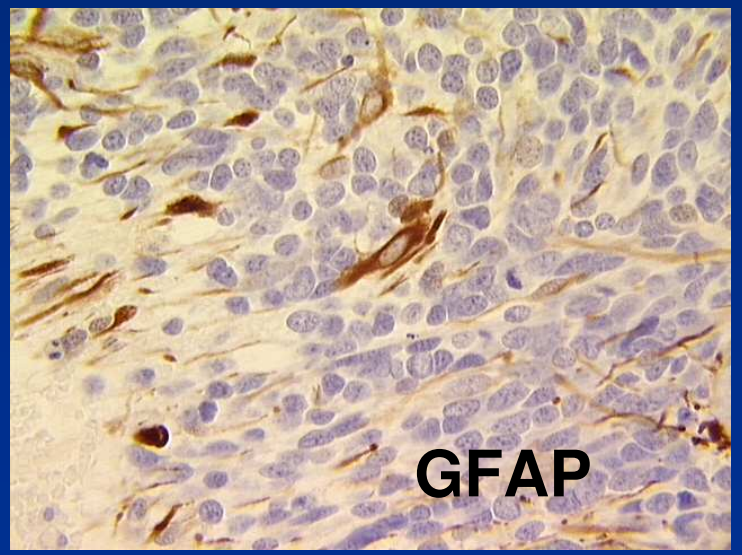
- **Self-renewal machinery**: already present, does not have to be generated *de novo*

Observation: Tumours are Heterogeneous

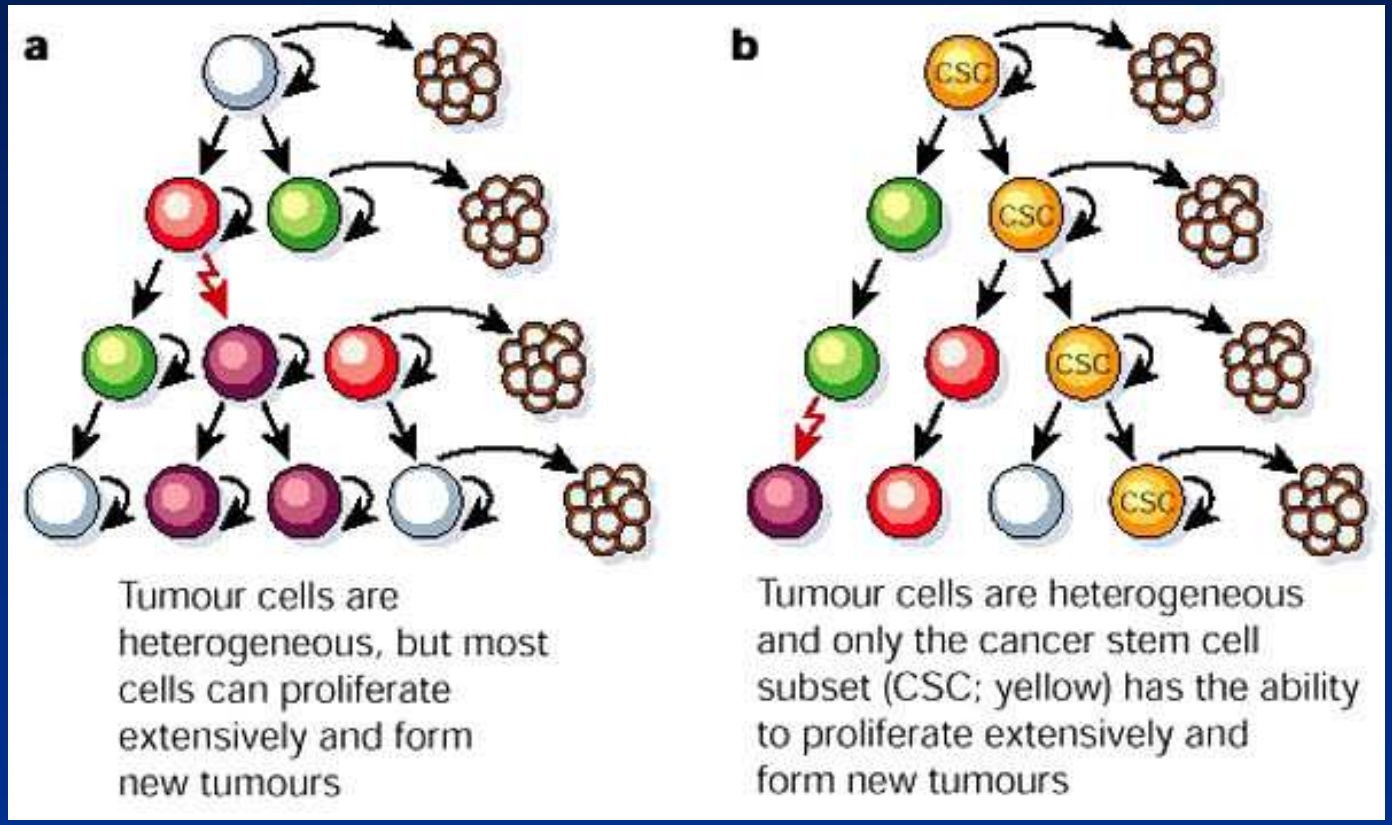


medulloblastoma





Heterogeneity and the Cancer Stem Cell



From Reya et al. Nature 414:105,2001

Cancer-Development Connection

- Do brain tumours originate from normal brain stem cells?
- Which cell in the heterogeneous brain tumour population is responsible for maintaining the tumour?
- Are brain tumours organized as a hierarchical stem cell system?

Lessons from Leukemia

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‖

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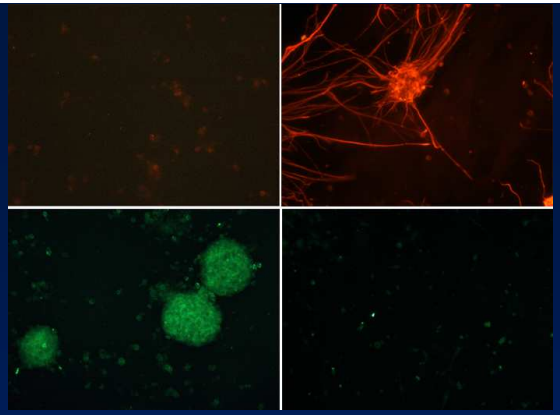
§ Department of Medicine, Roswell Park Cancer Institute, Buffalo, New York 14263-0001, USA

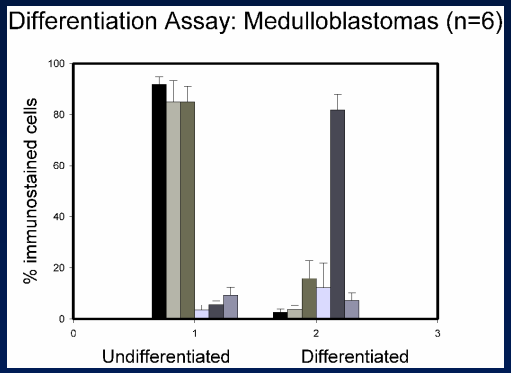
MOST human acute myeloid leukaemia (AML) cells have limited proliferative capacity, suggesting that the leukaemic clone may be maintained by a rare population of stem cells[1–5]. This putative leukaemic stem cell has not been characterized because the available *in vitro* assays can only detect progenitors with limited proliferative and replating potential[4–7]. We have now identified an AML-initiating cell by transplantation into severe combined immune-deficient (SCID) mice. These cells homed to the bone marrow and proliferated extensively in response to *in vivo* cytokine treatment, resulting in a pattern of dissemination and leukaemic cell morphology similar to that seen in the original patients. Limiting dilution analysis showed that the frequency of these leukaemia-initiating cells in the peripheral blood of AML patients was one engraftment unit in 250,000 cells. We fractionated AML cells on the basis of cell-surface-marker expression and found that the leukaemia-initiating cells that could engraft SCID mice to produce large numbers of colony-forming progenitors were $CD34^+CD38^-$; however, the $CD34^+CD38^+$ and $CD34^-$ fractions contained no cells with these properties. This *in vivo* model replicates many aspects of human AML and defines a new leukaemia-initiating cell which is less mature than colony-forming cells.

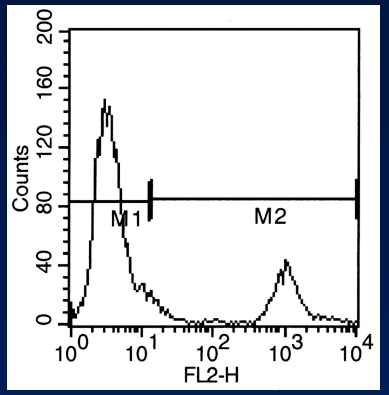
John Dick

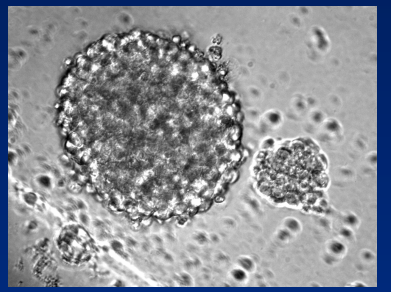
Rare human leukemic stem cells have the ability recreate the patient's leukemia following transplantation into immunodeficient mice

Nature 367:646, 1994
Nature Med 3:730,1997









[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,
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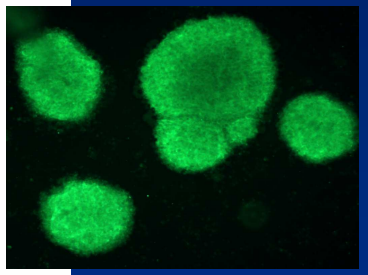
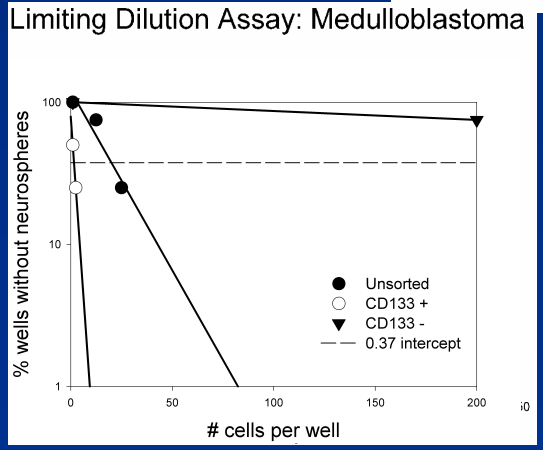
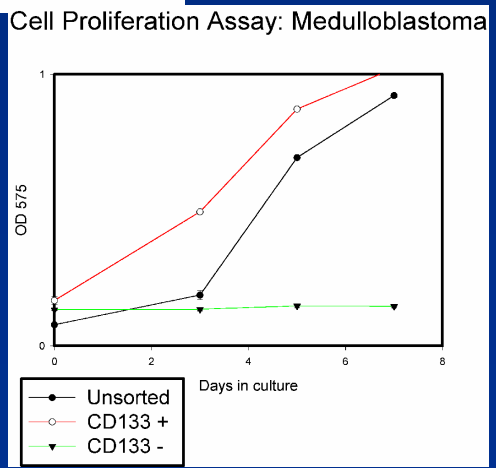
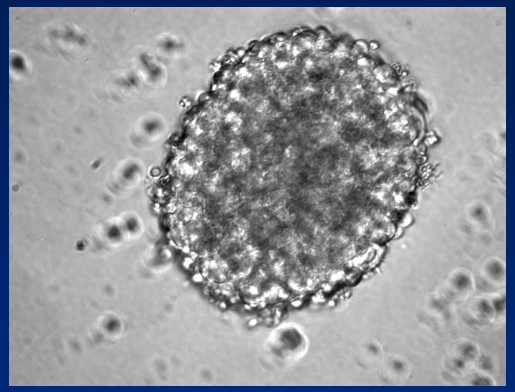
[S. K. S., I. D. C., M. T., V. E. B., P. B. D.],and Program in Developmental Biology
[S. K. S., I. D. C., M. T., V. E. B., P. B. D.], Division of Neurosurgery [S. K. S., P. B. D.],
Department of Pediatric Laboratory Medicine [C. H.], and
Department of Laboratory Medicine and Pathobiology [J. S.],
University of Toronto, Toronto, Ontario M5G 1X8 Canada



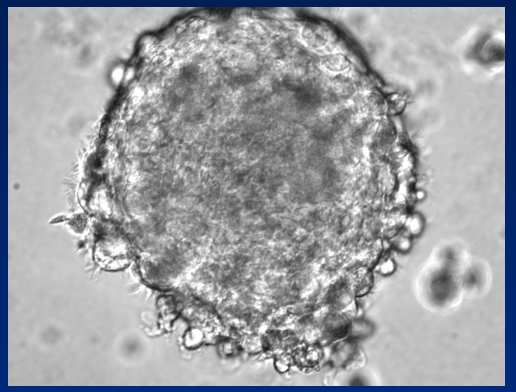




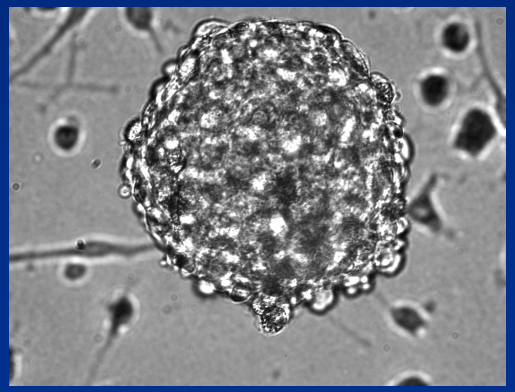
Brain tumours from 12 pathological subtypes form spheres in EGF/FGF



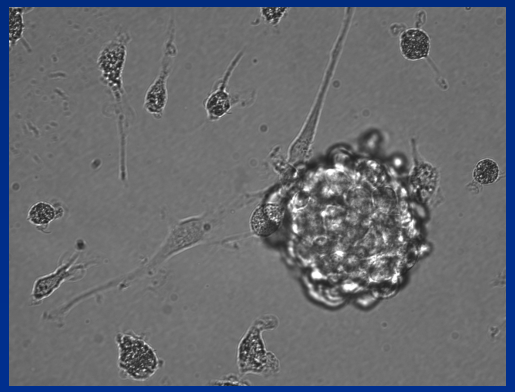
Medulloblastoma



Ependymoma



Pilocytic Astrocytoma



Ganglioglioma

Clonogenic Frequency (primary limiting dilution assay) n = 40

Glioblastoma	10-30%
Medulloblastoma	10-25%
Ependymona	5-10%
Pilocytic Astrocytoma	0.2-1.5%

Increasing biological aggressiveness

CD133: A Novel BTIC Marker

Direct isolation of human central nervous system stem cells

Nobuko Uchida*†, David W. Buck*, Dongping He*, Michael J. Reitsma*, Marilyn Masek*, Thinh V. Phan*, Ann S. Tsukamoto*, Fred H. Gage‡, and Irving L. Weissman§

*StemCells, Inc., 525 Del Rey Avenue, Suite C, Sunnyvale, CA 94085; ‡Laboratory of Genetics, The Salk Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037; and §Departments of Pathology and Developmental Biology, Stanford University, Stanford, CA 90305

Contributed by Irving L. Weissman, October 20, 2000

** CD133 may enrich for a stem cell population in brain tumours, and it is these cells that originate and regenerate the brain tumour**

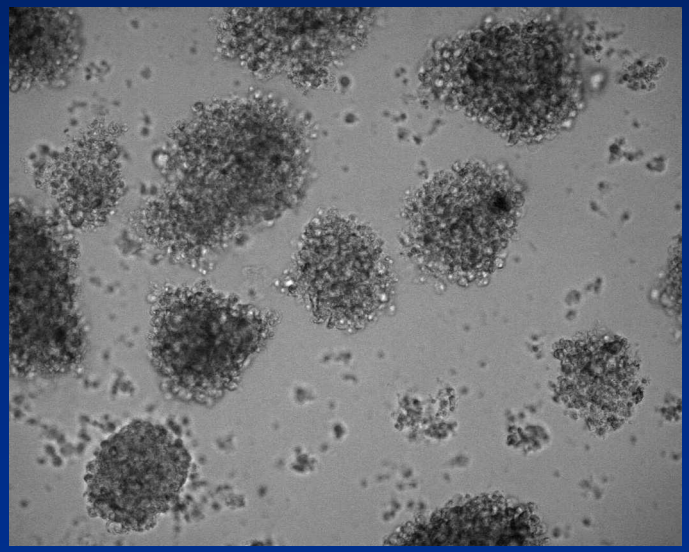
CD133 Index

Glioblastoma	15-30%
Medulloblastoma	5-25%
Ependymoma	5-10%
Ganglioglioma	3-5%
Astrocytoma	0.2-3%

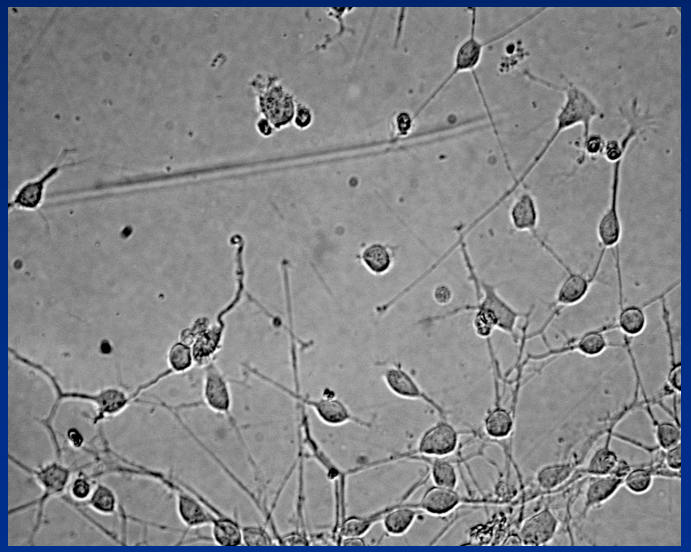
Increasing biological aggressiveness

CD133 identifies a stem cell population in brain tumours

- Magnetic bead sorting was used to purify a CD133+ cell population from a medulloblastoma



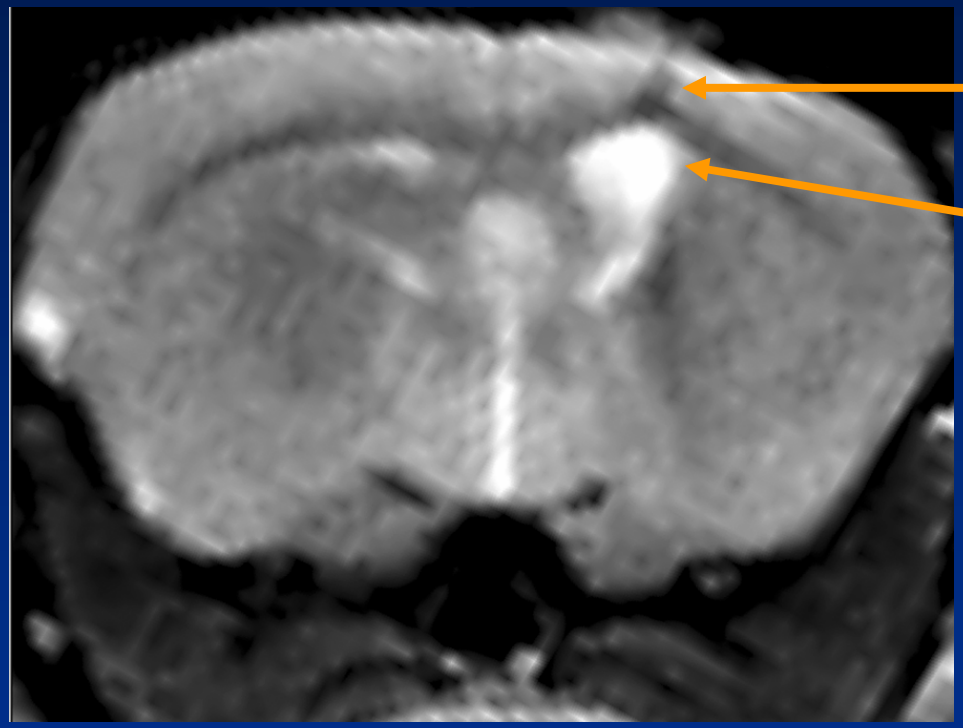
CD133 +



CD133 -

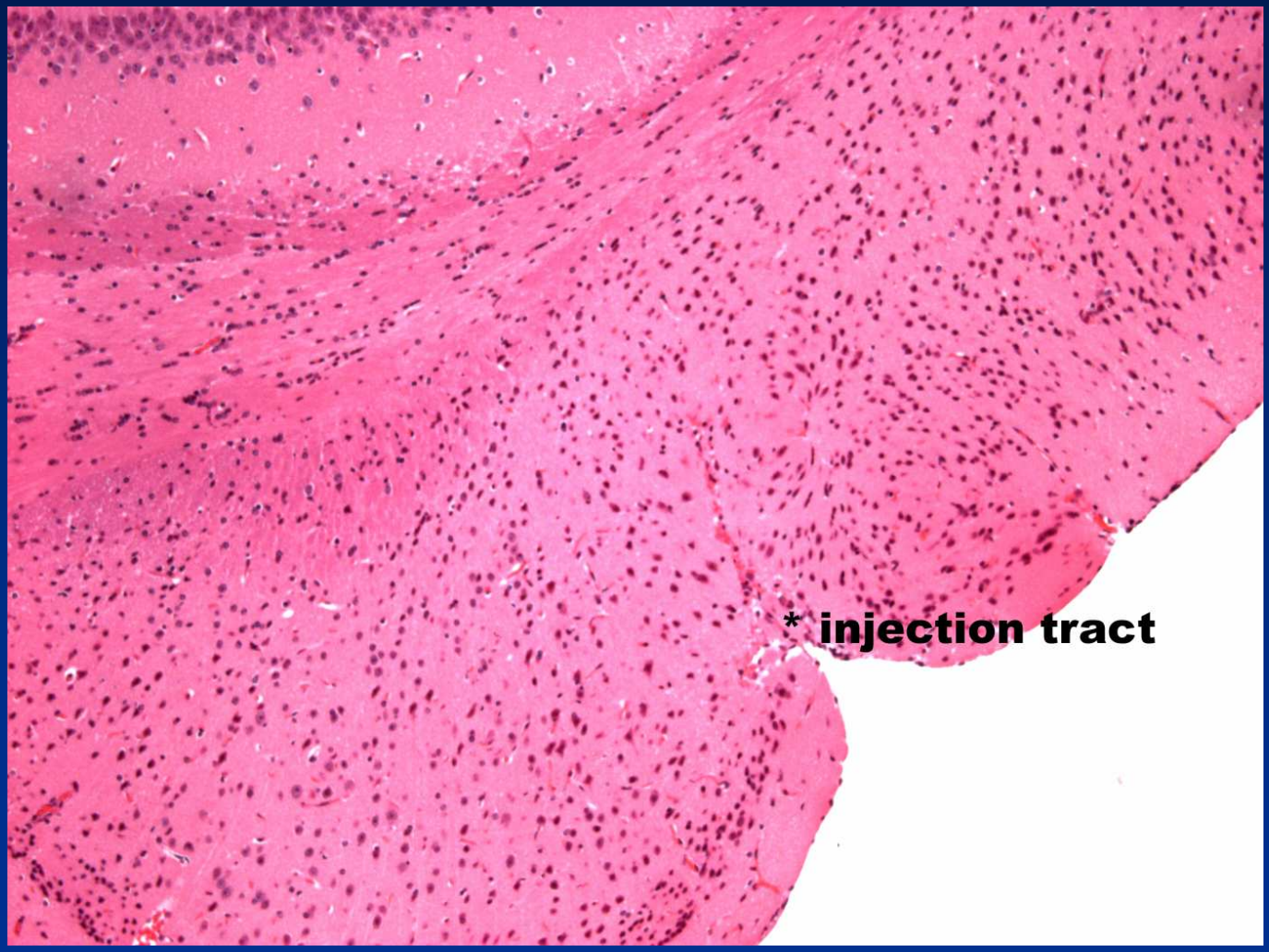
The Brain Tumour Stem Cell has undergone Transformation

1,000 CD133+ Cells form tumours upon Intracranial Xenograft



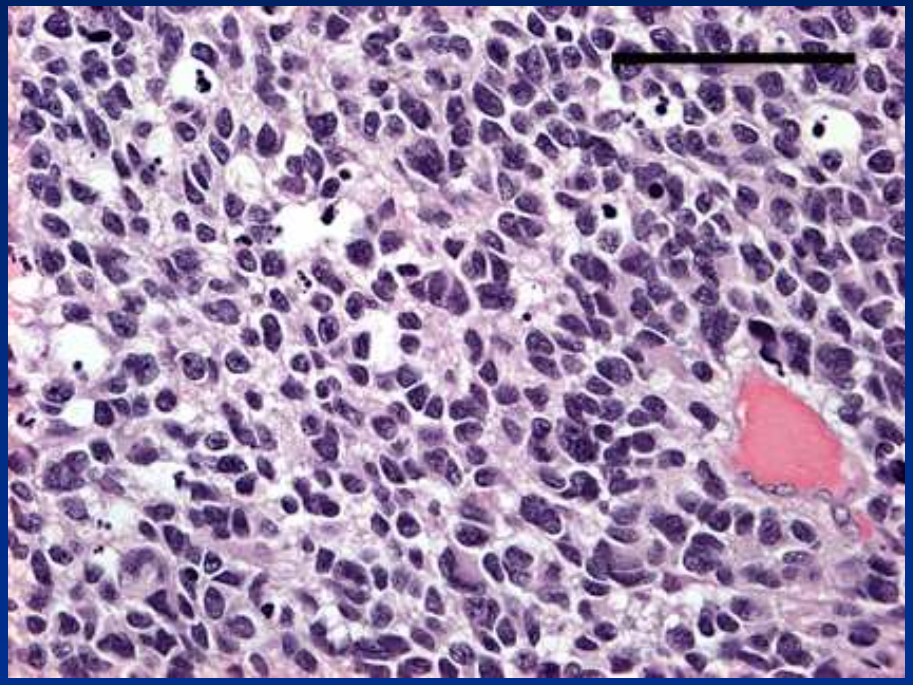
- MRI of mouse brain taken 14 weeks after injection

No tumours formed in xenografts injected with 100,000 CD133- cells

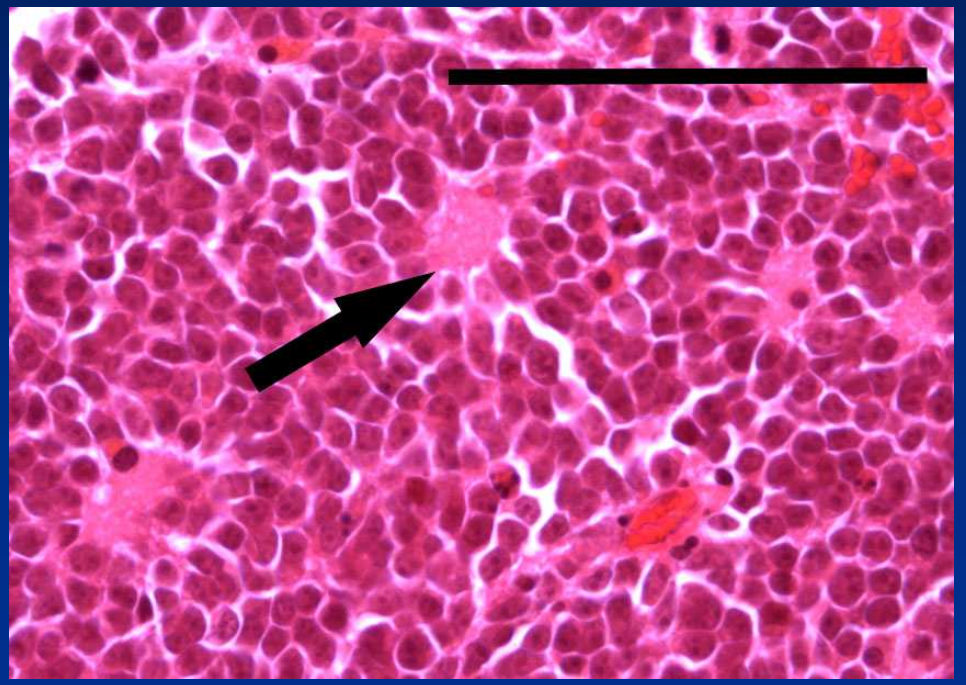


Medulloblastoma CD133+ xenograft is a phenocopy of the original patient tumour

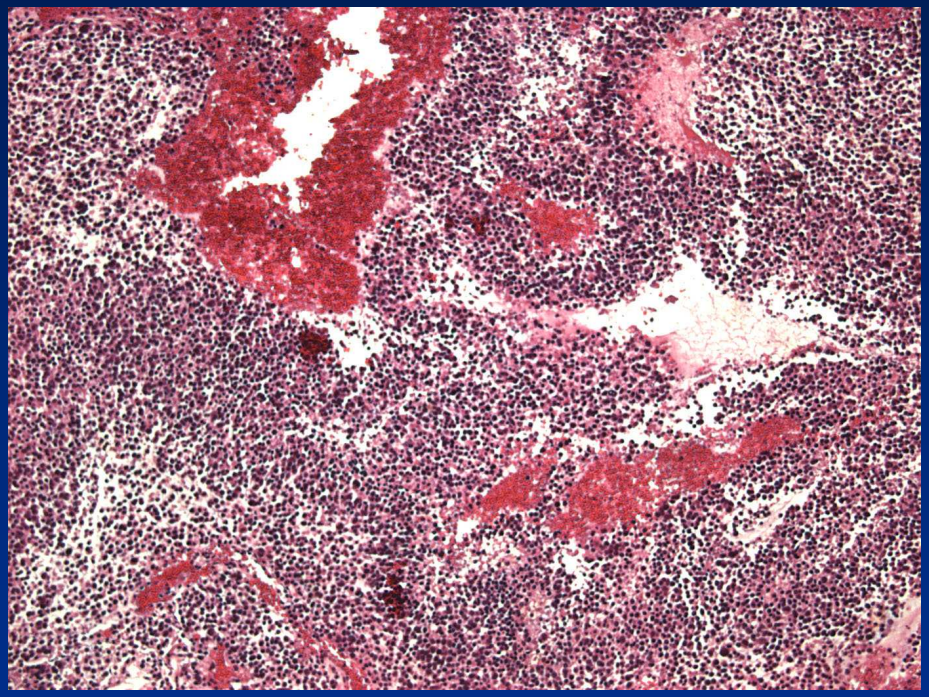
Patient



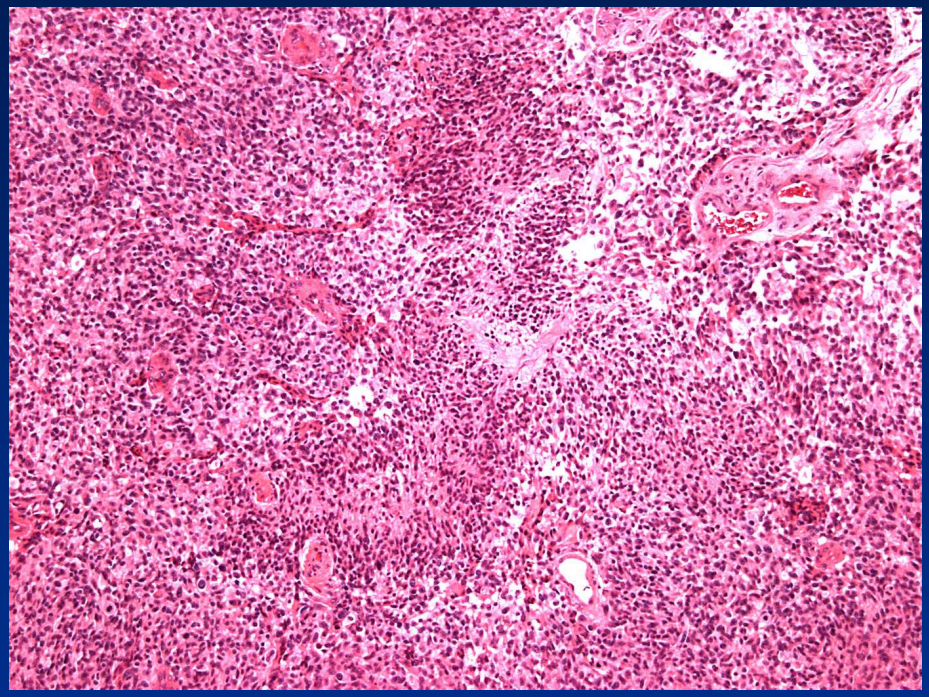
Primary Xenograft



Glioblastoma CD133+ xenograft is a phenocopy of the original patient tumour

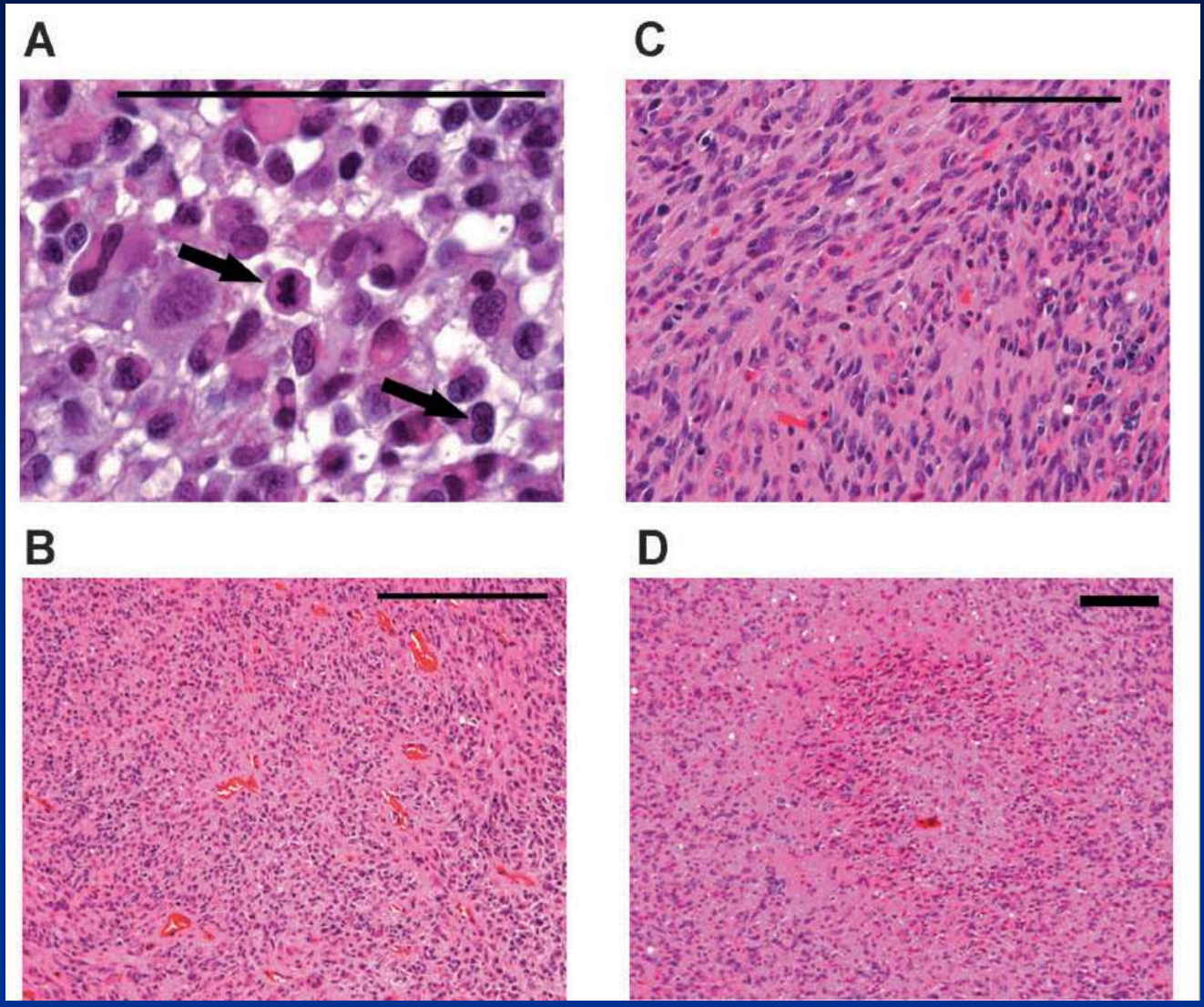


BTSC Xenograft

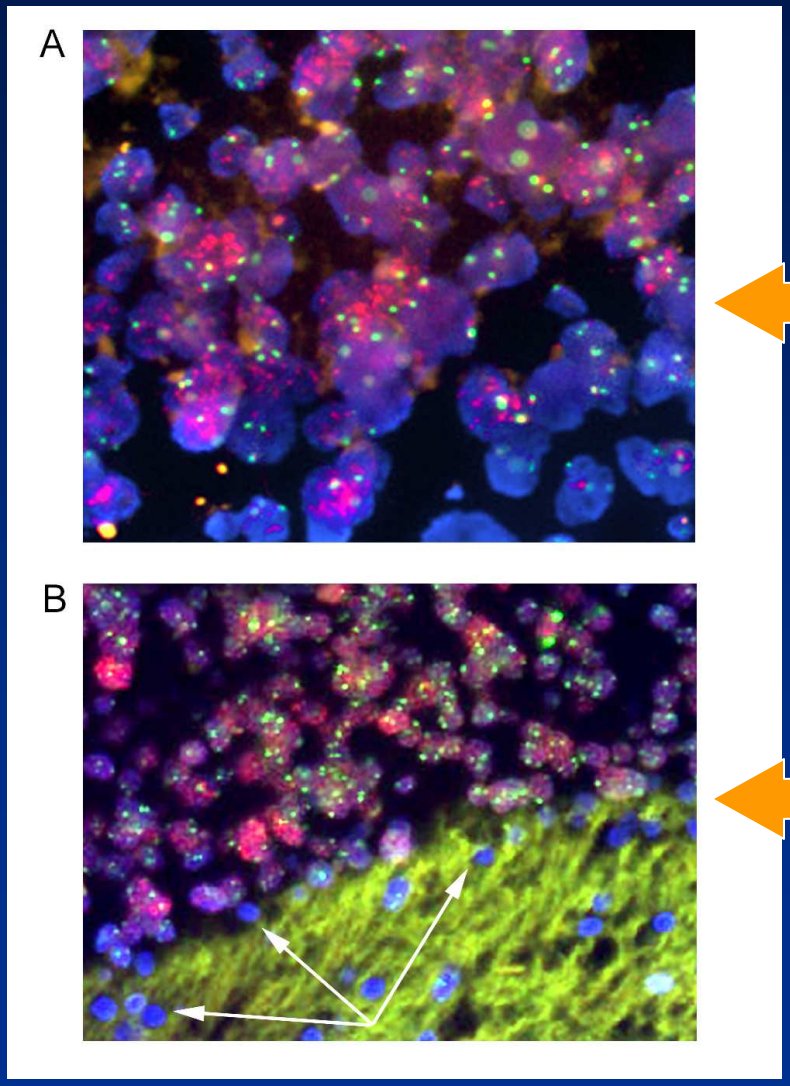


Original Tumour

GBM BTSC Xenograft: Diagnostic Histopathology



GBM CD133+ Xenograft bears EGFR Amplification identical to the Original Tumour



Original GBM shows gains of Chromosome 7 (green) and EGFR gene amplification (red)

…. As does the GBM BTSC xenograft.

Summary

- **The cell surface marker CD133 can be used to enrich for brain tumour cells that have stem cell properties**
- **The CD133+ cells show all the proliferative and self- renewal ability of the tumour *in vitro and* exclusively possesses tumorigenic ability *in vivo***
- **The tumour-initiating CD133+ cell has potent *in vivo* self-renewal and proliferative capacity, generating tumours from as few as 100 CD133+ cells**
- **The tumour generated by CD133 transplants is a phenocopy of the patient's tumour**

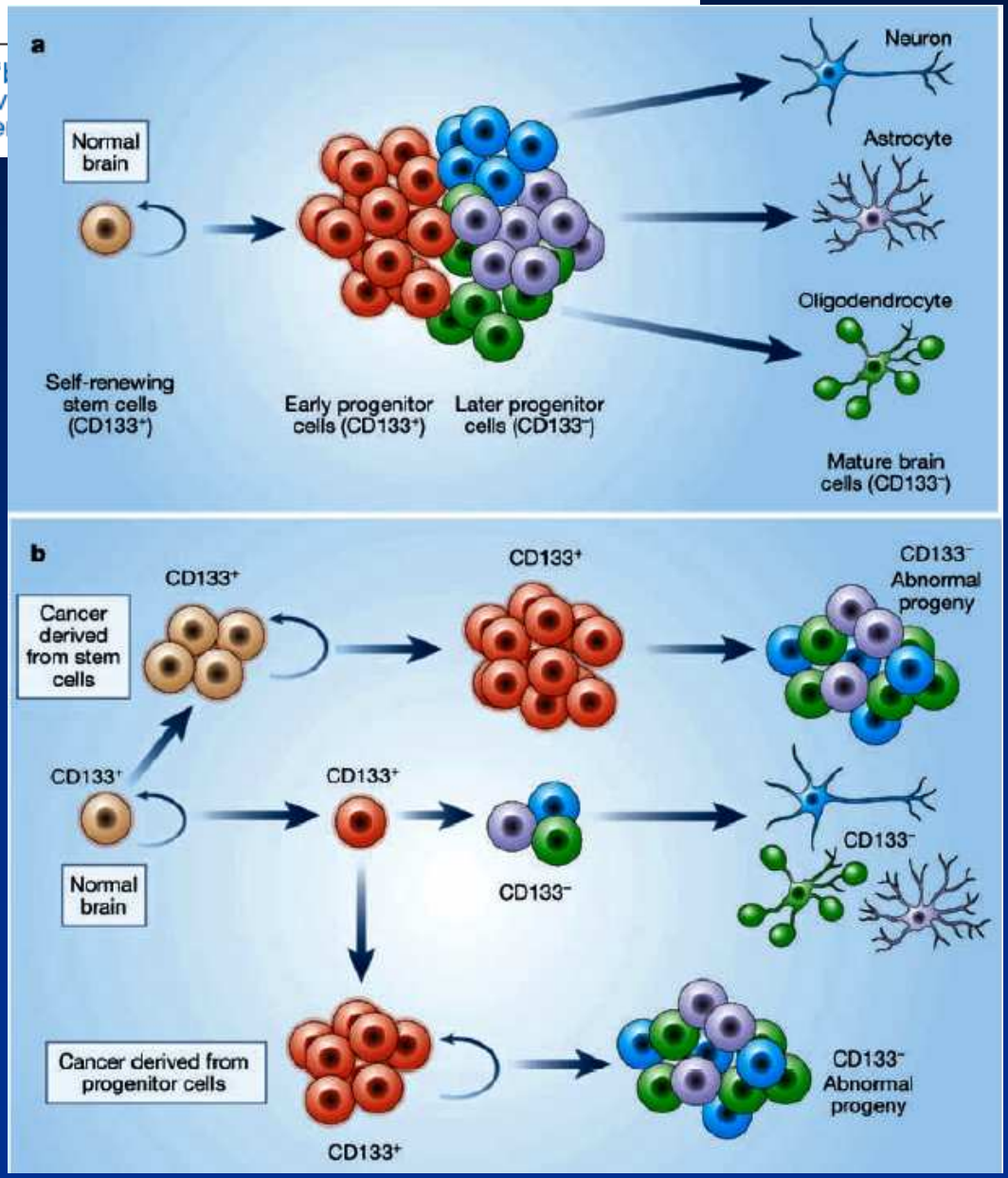
Clinical Implications

- Traditional bulk analysis of tumours may not detect key molecular alterations in a rare cancer stem cell
- Therapy directed against the bulk tumour may spare the cancer stem cell, allowing for continued tumour growth or relapse
- Therapy should be redirected to focus on killing the brain tumour stem cell

At the root of brain cancer

Michael F. Clarke

A small subpopulation of cells, '
humans. They have the exclusiv
prove an effective target for the



Corroboration: A New Perspective on Cancer?

Cancerous stem cells can arise from pediatric brain tumors

Houman D. Hemmati*, Ichiro Nakano†, Jorge A. Lazareff‡§, Michael Masterman-Smith‡, Daniel H. Geschwind¶, Marianne Bronner-Fraser*, and Harley I. Kornblum†§‖

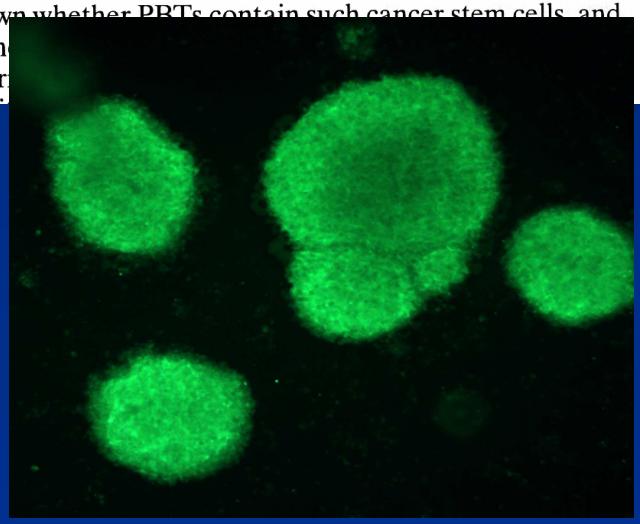
*Division of Biology 139-74, California Institute of Technology, Pasadena, CA 91125; and † Department of Molecular and Medical Pharmacology, ‡Division of Neurosurgery, and Departments of §Pediatrics and ¶Neurology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095

Communicated by Michael E. Phelps, University of California School of Medicine, Los Angeles, CA, October 8, 2003 (received for review June 20, 2003)

Pediatric brain tumors are significant causes of morbidity and mortality. It has been hypothesized that they derive from self-renewing multipotent neural stem cells. Here, we tested whether different pediatric brain tumors, including medulloblastomas and gliomas, contain cells with properties similar to neural stem cells.

Such individual cells are capable of self-renewal, proliferation, and differentiation to create the complex heterogeneous tumor. It is unknown whether PBTs contain such cancer stem cells, and if so, wheth

In the cur

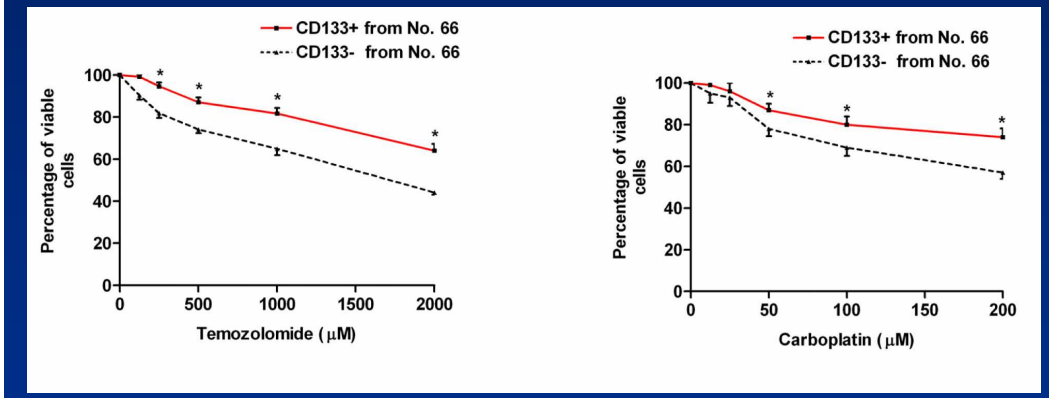


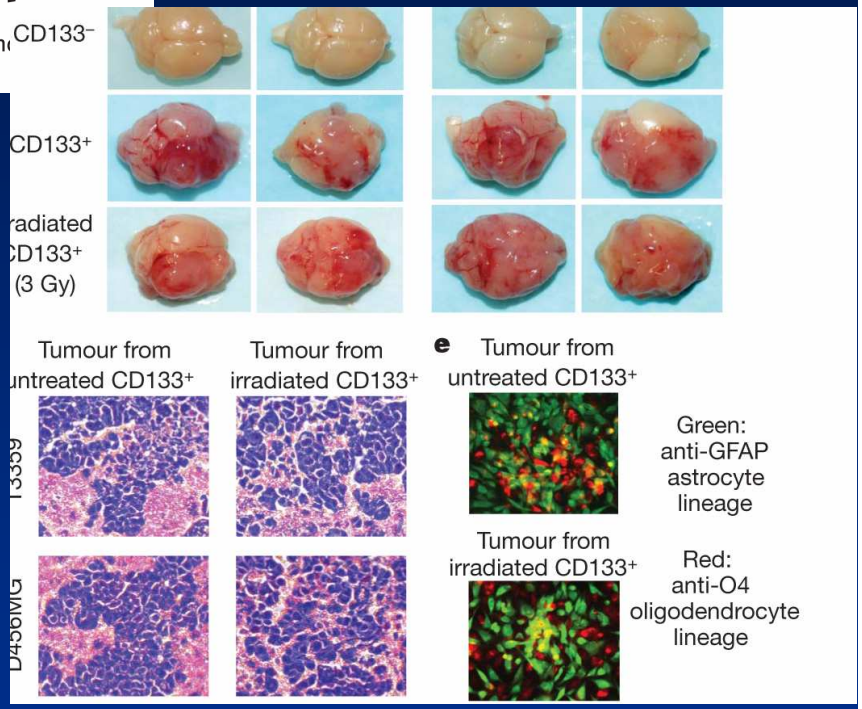
PNAS 100:15178,2003

…with new therapeutic implications

Glioma stem cells promote radioresistance by preferential activation of the DNA damage response

Shideng Bao[1,2], Qiulian Wu[1,2], Roger E. McLendon[2,3], Yueling Hao[1,2], Qing Shi[1,2], Anita B. Hjelmelan
Mark W. Dewhirst[4], Darell D. Bigner[2,3] & Jeremy N. Rich[1,2,5,6]



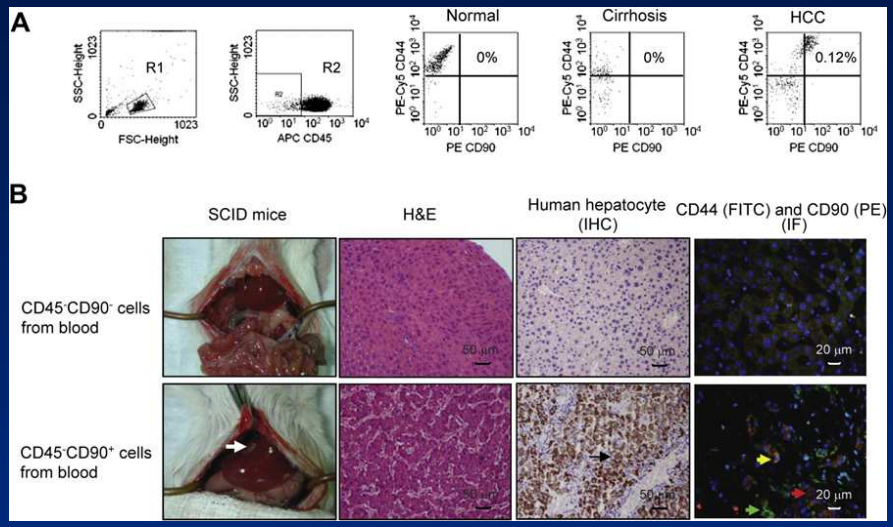


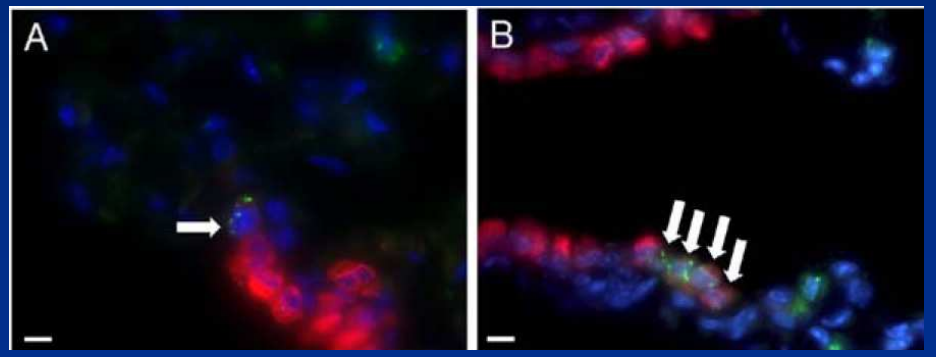
Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma

Gentao Liu[1,2], Xiangpeng Yuan[1], Zhaohui Zeng[1], Patrizia Tunici[1], Hiushan Ng[1], Iman R Abdulkadir[1], Lizhi Lu[1,3], Dwain Irvin[1], Keith L Black[1] and John S Yu*[1,4]

Cancer Stem Cells in Solid Tumours

- Breast Cancer (CD24-CD44+)
- Colon Cancer (CD133+)
- Pancreatic Cancer (CD44+ CD133+)
- Hepatic cancer (CD133+ CD90+)
- Neuroblastoma (SP+)
- Head and Neck (CD44+)
- Prostate cancer (CD44+ CD133+)
- Lung cancer (CD133+)
- Melanoma (CD133+)

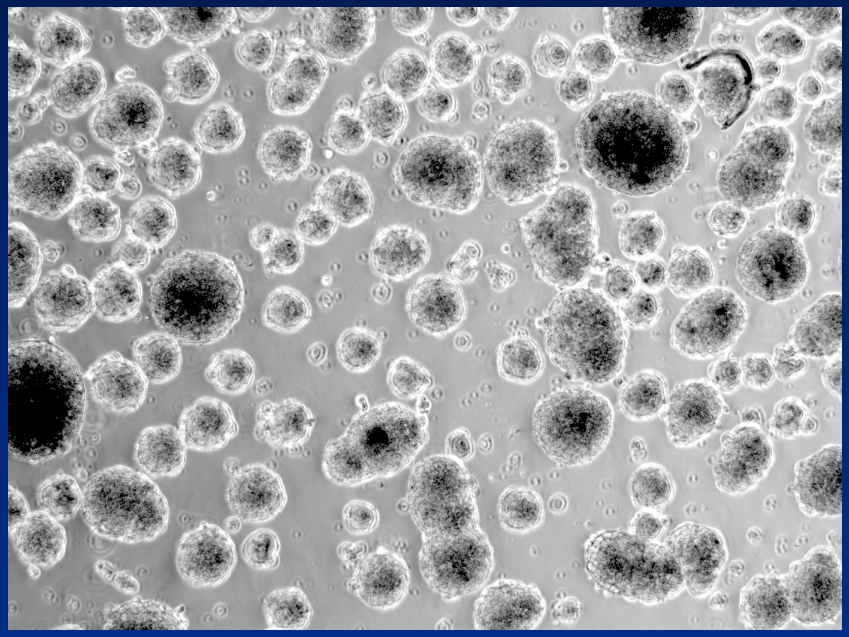


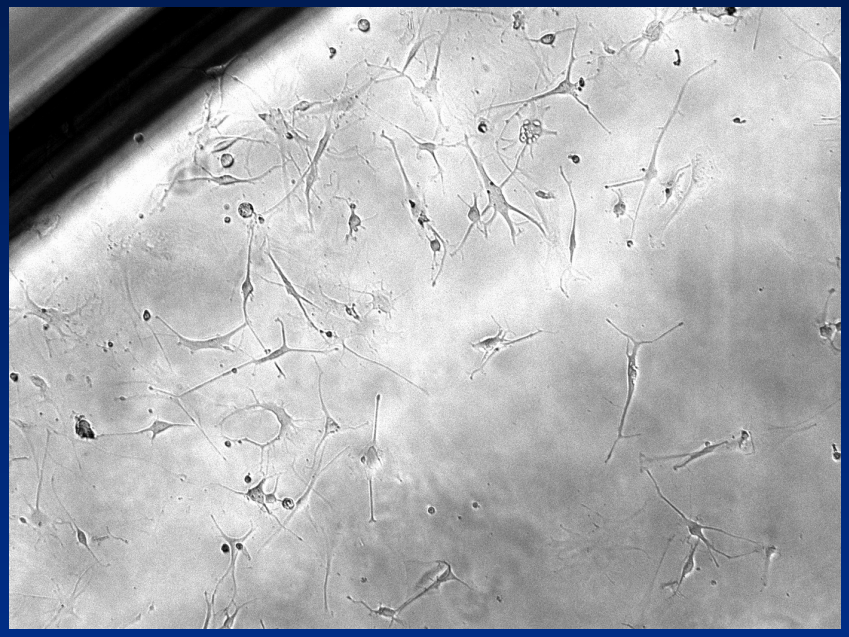


** From Yang, Fan et al, Cancer Cell, 2008 and Kim and Jacks, Cell 2005*

Controversies

1. BTIC Culture Conditions





Non-adherent vs Adherent…….

With growth factors? Without growth factors?

Controversies
1. BTIC Culture Conditions

Brain Cancer Stem Cells: A Level Playing Field

Steven Pollard,[1] Ian D. Clarke,[2] Austin Smith,[1] and Peter Dirks,[2,*] (on behalf of all authors)
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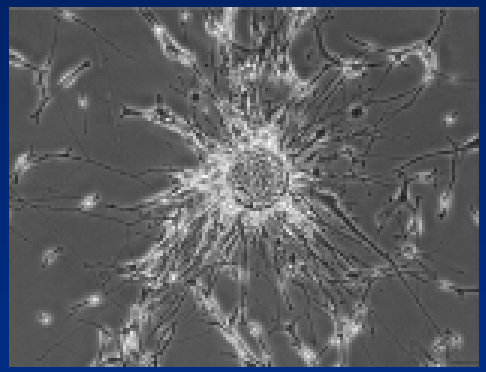
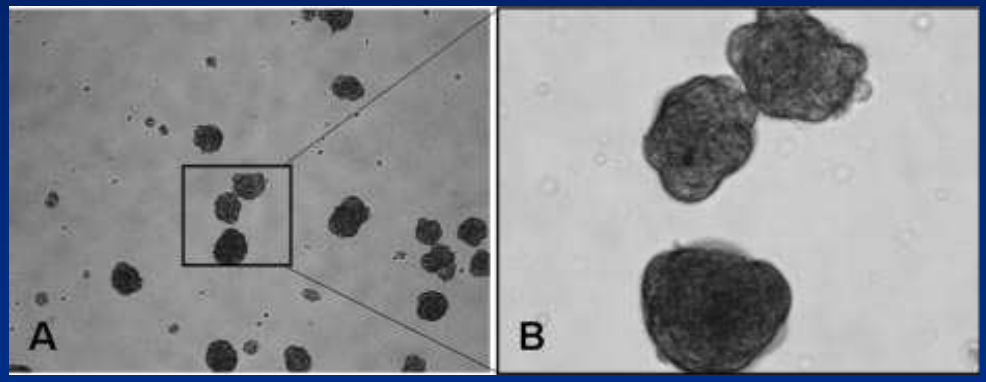
*Correspondence: peter.dirks@sickkids.ca
DOI 10.1016/j.stem.2009.10.016



From Pollard et al, Cell Stem Cell 2009



Brain Cancer Stem Cells: Think Twice before Going Flat

Brent A. Reynolds[1,*] and Angelo L. Vescovi[2,*]
[1]Department of Neurosurgery, McKnight Brain Institute, University of Florida, Gainesville, FL 32610, USA
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DOI 10.1016/j.stem.2009.10.017

Controversies
2. BTIC Markers

STEM CELLS
TECHNOLOGY DEVELOPMENT

Optimized Flow Cytometric Analysis of Central Nervous System Tissue Reveals Novel Functional Relationships Among Cells Expressing CD133, CD15, and CD24

DAVID M. PANCHISION,[a] HUI-LING CHEN,[a] FRANCESCA PISTOLLATO,[a] DANIELA PAPINI,[b] HSIAO-TZU NI,[c] TERESA S. HAWLEY[d]

[a]Center for Neuroscience Research, Children's Research Institute, Children's National Medical Center, Washington, DC, USA; [b]Stem Cell Core Facility, Children's Research Institute, Children's National Medical Center, Washington, DC, USA; [c]R&D Systems Inc., Minneapolis, Minnesota, USA; [d]Flow Cytometry Core Facility, George Washington University Medical Center, Washington, DC, USA

Key Words. Brain • Brain tumors • Flow cytometry • Cell viability • Stem cells • Progenitor cells

Tumor and Stem Cell Biology

Cancer Research

Transcriptional Profiles of CD133$^+$ and CD133$^-$ Glioblastoma-Derived Cancer Stem Cell Lines Suggest Different Cells of Origin

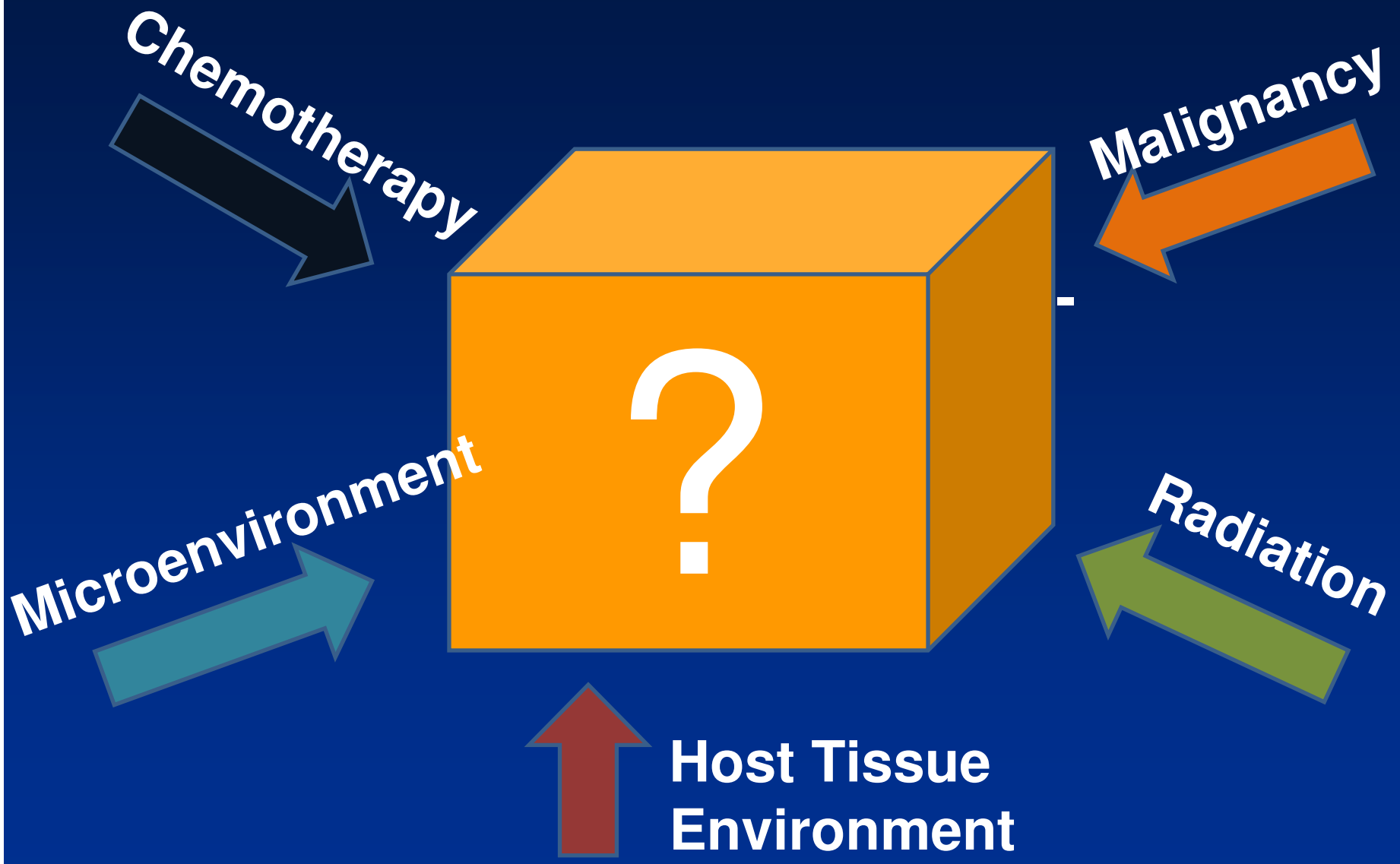
Claudio Lottaz[1], Dagmar Beier[2], Katharina Meyer[1], Praveen Kumar[2], Andreas Hermann[3], Johannes Schwarz[4], Markus Junker[5], Peter J. Oefner[1], Ulrich Bogdahn[2], Jörg Wischhusen[5], Rainer Spang[1], Alexander Storch[3], and Christoph P. Beier[2,6]

Brain Tumor Stem–Like Cells Identified by Neural Stem Cell Marker CD15

Xing-gang Mao*, Xiang Zhang*, Xiao-yan Xue†, Geng Guo*, Peng Wang*, Wei Zhang*, Zhou Fei*, Hai-ning Zhen*, Si-wei You‡ and Hao Yang‡

*Department of Neurosurgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi Province, China; †Department of Pharmacology, The Fourth Military Medical University, Xi'an, Shaanxi Province, China; ‡Institute of Neurosciences, The Fourth Military Medical University, Xi'an, Shaanxi Province, China

Cancer stem cell: Cell, or state of being?



Future Work

- Purification
- Developmental Signaling Pathways regulating self-renewal
- Gene Expression Profiling of Tumorigenic Stem Cells
- Epigenetics: role of Chromatin Remodelling in determining Tumorigenic Stem Cell Fate
- Prognosis Determination
- Cell of Origin

Epigenetics, Genetics and Cancer

CANCER

Cell acquires growth advantages through stepwise accumulation of mutations in

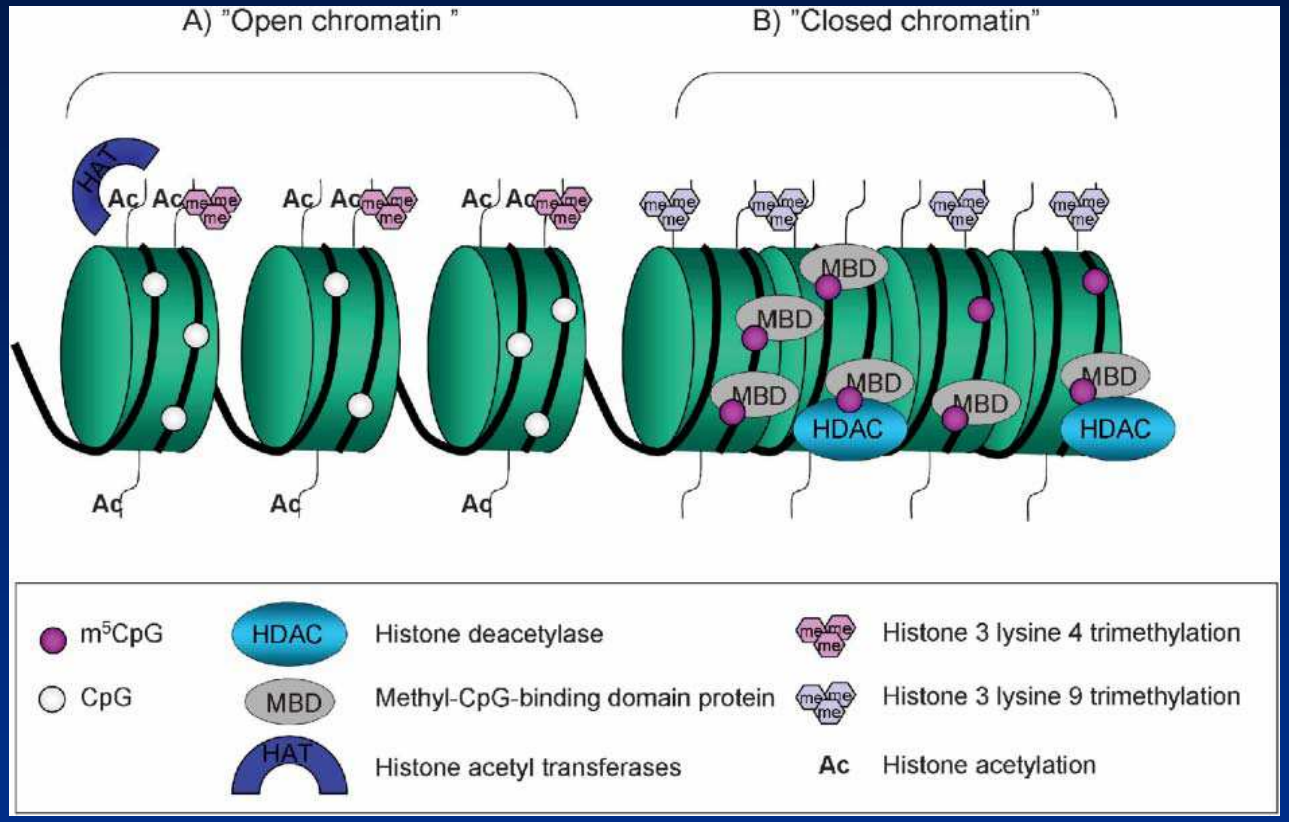
TUMOUR SUPPRESSOR GENES

ONCOGENES

EPIGENETICS: Heritable changes in gene expression NOT caused by change in DNA sequence: ie "GENE SILENCING"

- Modification of covalent bonds between amino acids in histones around which DNA is wrapped
- Methylation of cytosine bases (CpG Islands) in promoters of genes, causes HERITABLE GENE SILENCING (potentially reversible…)

Epigenetics and Cancer



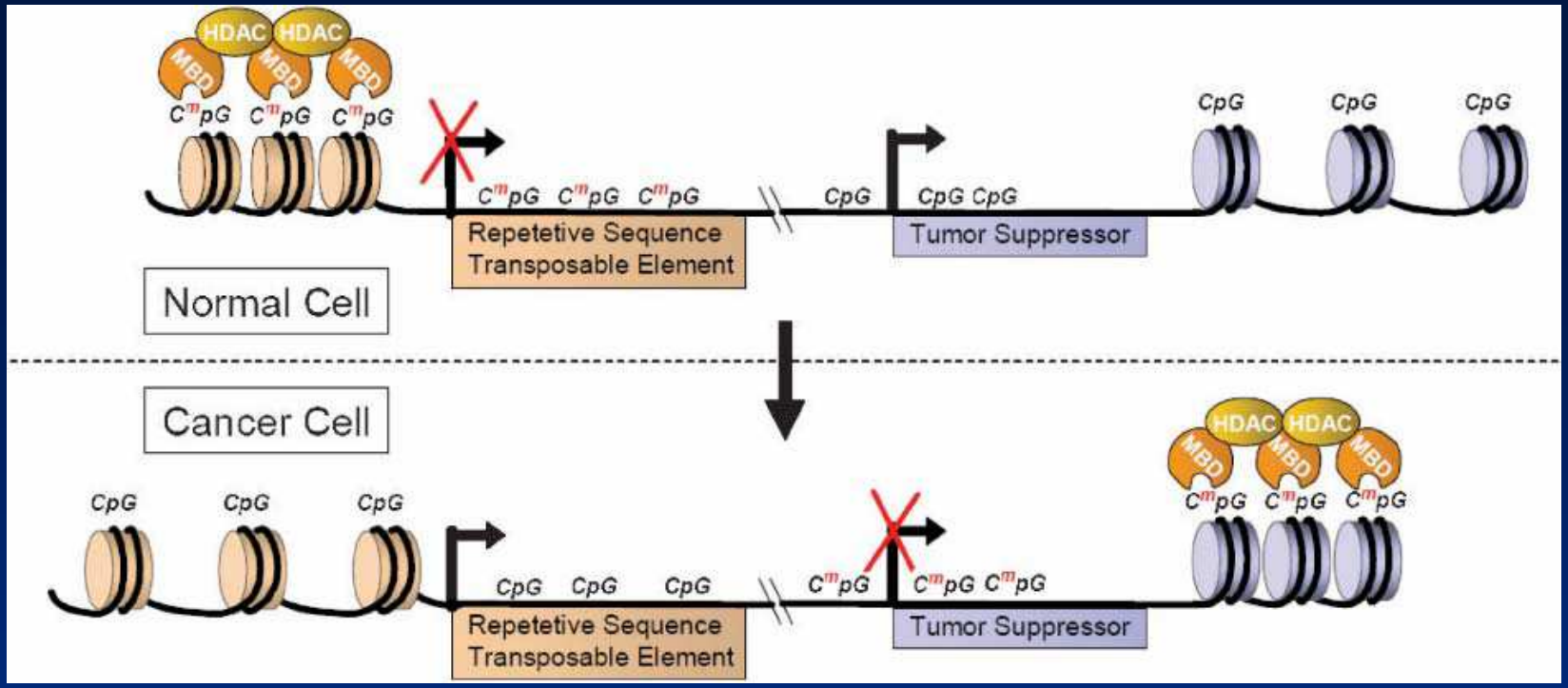
OPEN CHROMATIN: open to transcriptional machinery

CLOSED CHROMATIN: transcriptionally silent

- Post-translational covalent modifications "MARK" transcriptional state of chromatin

- Ex. Trimethylation of lysine 9 at N-terminal of histone 3 = SILENCING SIGNATURE

** From APMIS 115, 2007: Groenback, Jones et al*



DNA METHYLATION = Marker of disease, pre-malignancy or malignancy

TARGET with hypo-methylating drugs

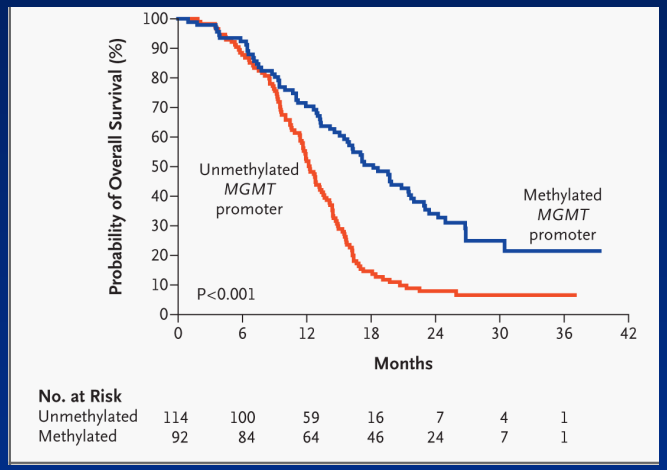
APC	Colorectal/ breast cancer
BRCA1	Breast, ovarian. lung
p16	Melanoma, glioma, lymphoma
PTEN	Breast, brain, thyroid, prostate

** From APMIS 115, 2007: Fog, Jensen and Lund*

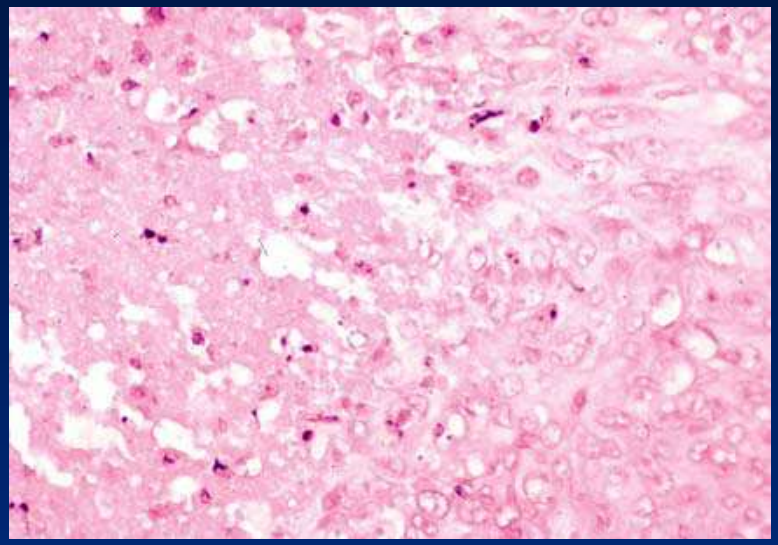
Epigenetics and Brain Tumours

MGMT Gene Silencing and Benefit from Temozolomide in Glioblastoma

Monika E. Hegi, Ph.D., Annie-Claire Diserens, M.Sc., Thierry Gorlia, M.Sc., Marie-France Hamou, Nicolas de Tribolet, M.D., Michael Weller, M.D., Johan M. Kros, M.D., Johannes A. Hainfellner, M.D., Warren Mason, M.D., Luigi Mariani, M.D., Jacoline E.C. Bromberg, M.D., Peter Hau, M.D., ené O. Mirimanoff, M.D., J. Gregory Cairncross, M.D., Robert C. Janzer, M.D., and Roger Stupp, M.D.



Improved outcome associated with epigenetic silencing of the MGMT gene by promoter methylation, thereby blocking its repair capability, thus rendering the alkylating agents (temozolamide) more effective.



ATRT = hSNF5-INI1 mutation (SWI-SNF chromatin remodelling complex) results in loss of DNA damage repair, and increased loss of p53.

The Role of Epigenetics in BTIC Fate Determination

Chromatin remodeling and histone modification in the conversion of oligodendrocyte precursors to neural stem cells

Toru Kondo[1,2,3] and Martin Raff[1]

[1]Medical Research Council Laboratory for Molecular Cell Biology, Cell Biology Unit, and the Biology Department, University College London, London WC1E 6BT, United Kingdom; [2]Centre for Brain Repair, University of Cambridge, Cambridge CB2 2PY, United Kingdom

Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells

Jenny Hsieh[1,4], Kinichi Nakashima[1,4,5], Tomoko Kuwabara[1], Eunice Mejia[1], and Fred H. Gage[1,3]

[1]Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037; and [4]Laboratory of Molecular Neuroscience, Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma 630-0101, Japan

articles

Role of the proto-oncogene *Pokemon* in cellular transformation and *ARF* repression

Takahiro Maeda[1,2], Robin M. Hobbs[1,2], Taha Merghoub[1,2], Ilhem Guernah[1,2], Arthur Zelent[3], Carlos Cordon-Cardo[2], Julie Teruya-Feldstein[2] & Pier Paolo Pandolfi[1,2]

Rejuvenation of aged progenitor cells by exposure to a young systemic environment

Irina M. Conboy[1]*†, Michael J. Conboy[1]*, Amy J. Wagers[2]†, Eric R. Girma[1], Irving L. Weissman[2] & Thomas A. Rando[1,3]

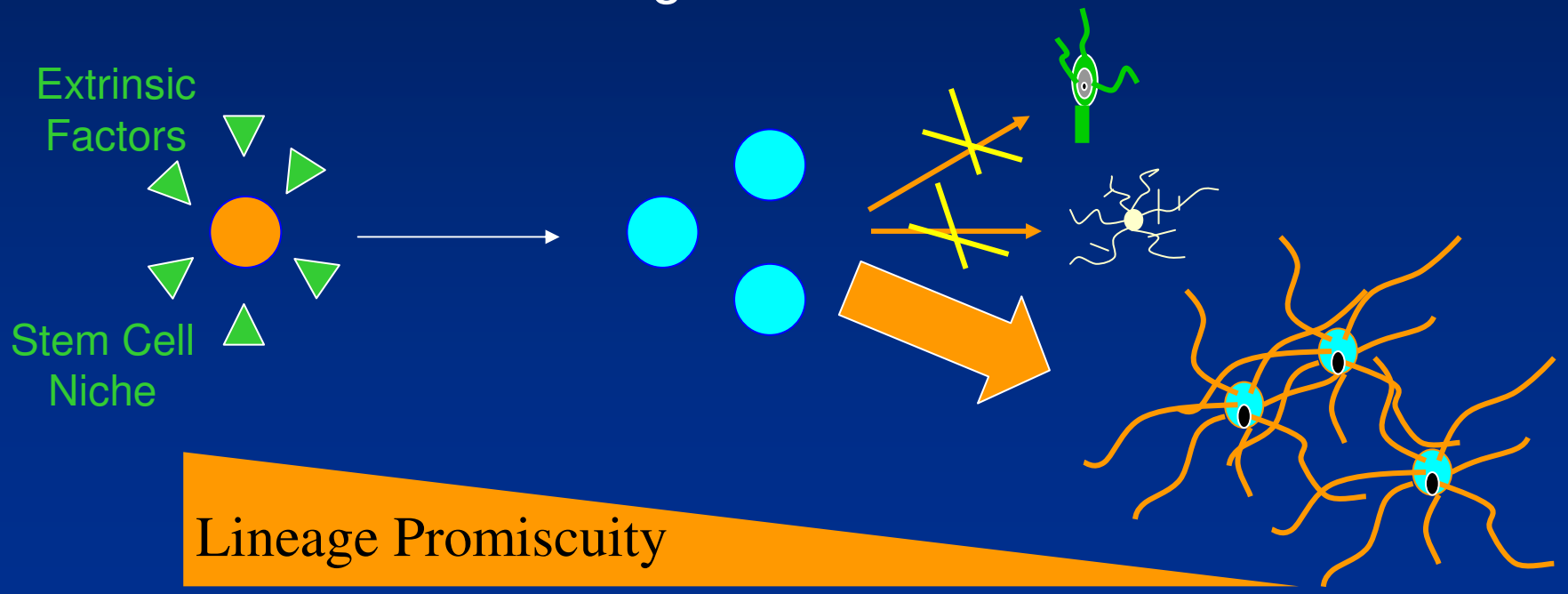
[1]*Department of Neurology and Neurological Sciences and* [2]*Department of Pathology, Stanford University School of Medicine, Stanford, California 94305, USA*
[3]*GRECC and Neurology Service, VA Palo Alto Health Care System, Palo Alto, California 94304, USA*

Tumorigenesis: Multilineage priming of the BTIC dictates tumour phenotype

Multipotential BTIC

Lineage-restricted Progenitor cells

Astrocytoma



McMaster Stem Cell and Cancer Research Institute (SCC-RI)

Researchers

Mick Bhatia
Human Stem Cell Biology

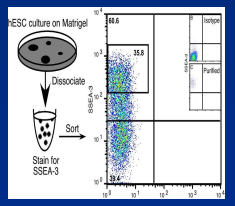
Brad Doble
Stem Cell Signaling

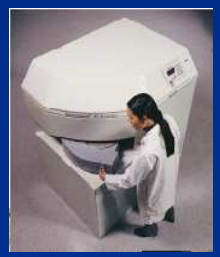
Jon Draper
Lineage Development of Human Stem Cells

Sheila Singh
Human Cancer Stem Cell Biology

Chris Wynder
Epigenetic Control of Stem Cells

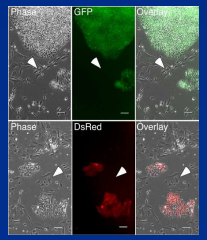
Dedicated Facilities





Experimental Animal Unit

Stem Cell Isolation



Tissue Culture

Strategic Initiatives

>IPS

>Cancer Stem Cells

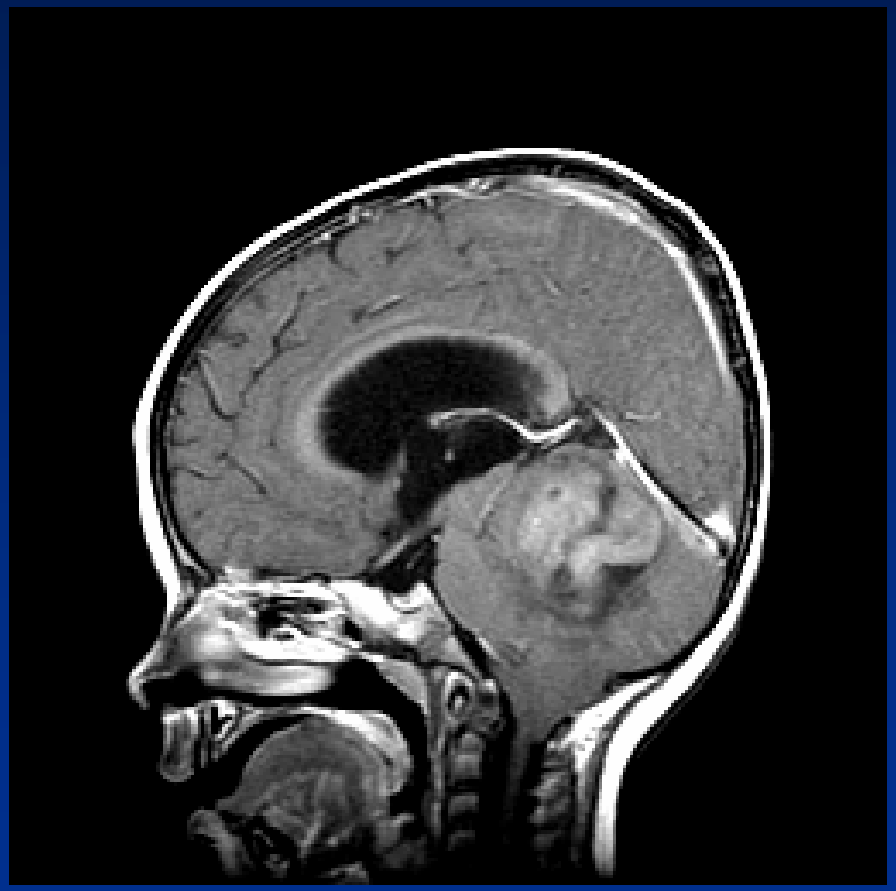
>Stem Cell Engineering

>Human Stem Cell Library/Screening

Learn more about us @

www.fhs.mcmaster.ca/SCCRI

Brain Tumour Stem Cells: The Future?



- Profile each individual patient's brain tumour stem cell population
- Tailor molecular therapy to target cancer stem cells by unique marker expression
- Provide differentiation therapy to render cancer stem cells impotent

Acknowledgements

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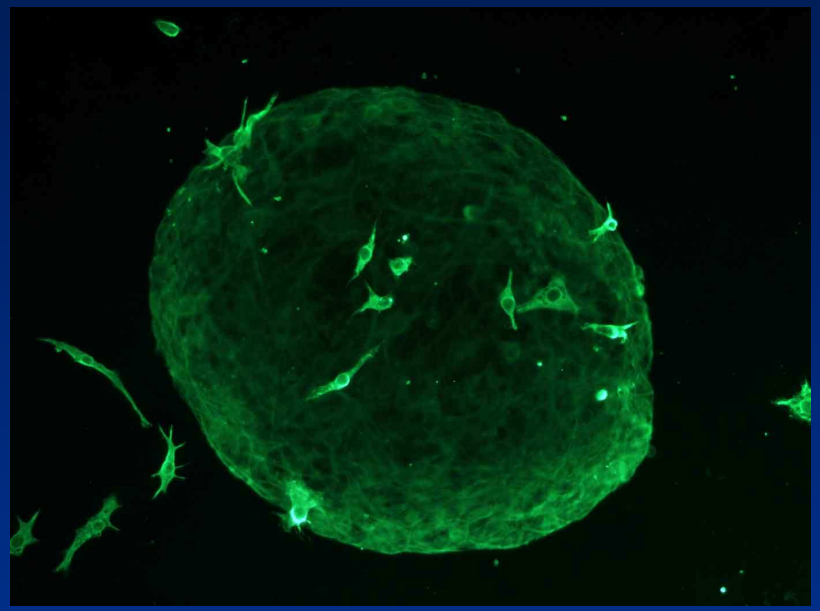
- Department of Surgery, HHSC
- Siv Sivaloganathanan
- Mohammed Kohandel



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- National Cancer Institute of Canada