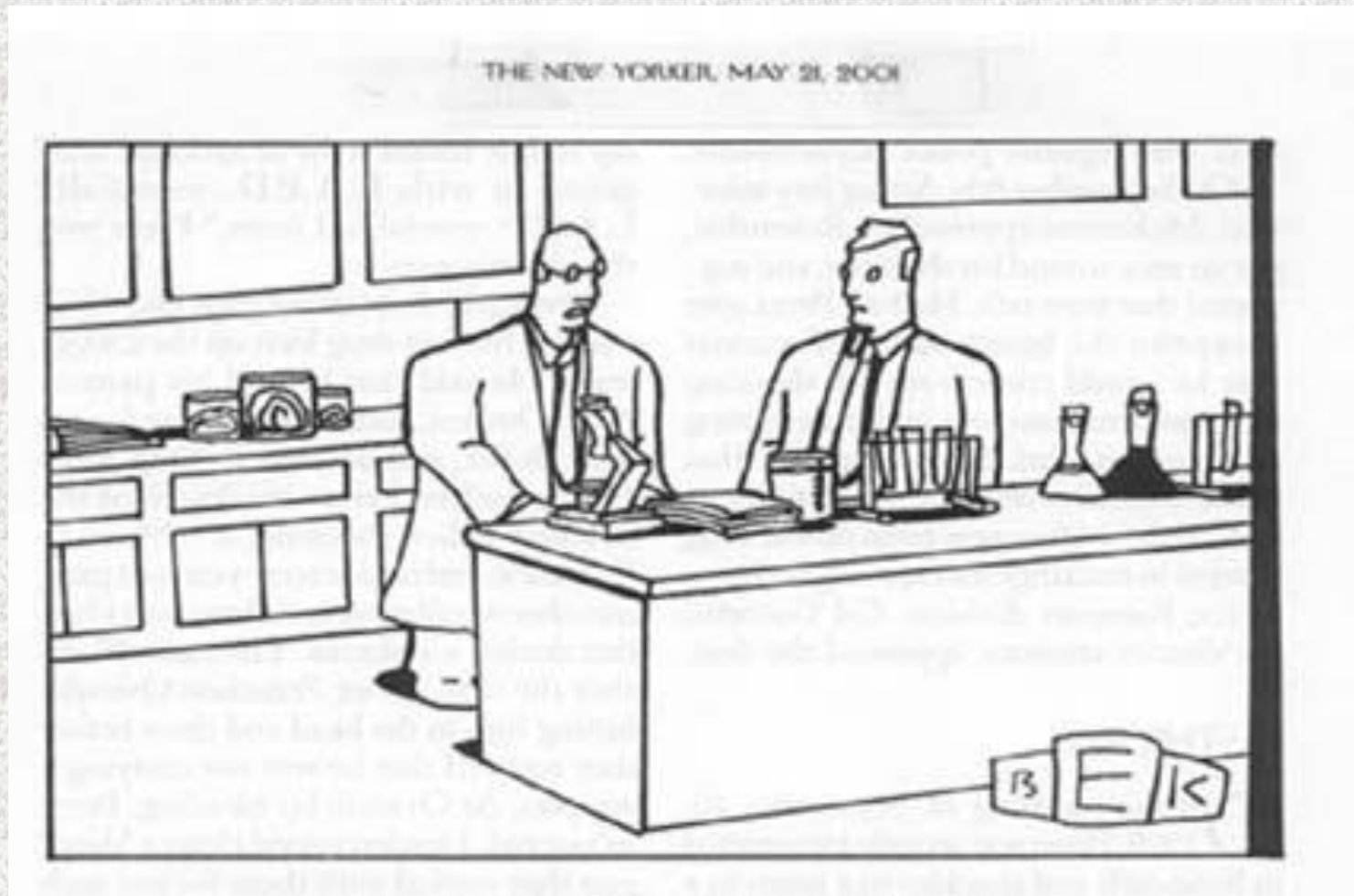


Stem Cell Research Community: 1998-?



Sometimes I wonder if there's more to life than unlocking the mysteries of the universe

Maryland Stem Cell Research Bill 2006

STOP *Forbids* human reproductive cloning

STOP Imposes criminal penalties

STOP Maryland now one of a handful of states, including California, where reproductive cloning is illegal

👉 *Funds* stem cell research

👉 Human adult and embryonic stem cell research

👉 SCNT (therapeutic cloning) and MSCRF, but remains prohibited

👉 Legislature must reauthorize law in 5 yrs

👉 No defined yearly budget but widely perceived as a priority

👉 In Maryland law, all funding requires yearly gubernatorial and legislative approval

“Reproductive Cloning”:
“I cloned a guy in Reno. How ‘bout yourself?”



SIPRESS

“I cloned a guy in Reno. How ‘bout yourself?”

New Yorker 10/8/01.

What *kind* of stem cell research can be supported by MSCRF?



Many advocates wanted to fund *only* human embryonic stem cell research. *Rationale:*



HESCs have greatest pluripotentiality and self-renewal capacity



Funding HESC research would precisely fill the current US Federal funding gap



Others, including Gov Ehrlich, wanted to fund *all* stem cell research. *Rationale:*



Fund the best stem cell science (All stem cell research is currently underfunded)



Adult or embryonic stem cells may be optimal for given applications

Compromise made to end a filibuster in the State Senate and secure Governor's support: Fund *all* kinds of *human* stem cell research

What if the State reduced or stopped funding the next year?



Legislators and governor became convinced that stable multi-year funding needed to propel research



Especially with no prospect of Federal support for stem cell research, NIH scale grants needed



State of MD agreed to play a new role to provide **sustained funding for stem cell research**

Administratively, how could the State of MD provide assured 2-3 yr funding?



Problem: MD Constitution stipulates that MD can only appropriate a 1 yr budget



Solution (provided by Gov Ehrlich): TEDCO



TEDCO, a quasi public economic development agency with experience with (small) research grants programs, administers MSCRF funding



TEDCO encumbers funds to support a project's entire 2 or 3 yr award



Each year's funding appropriation (e.g. the \$15M in grants awarded in 2007) will provide the entire multiyear funds for a set of 2 or 3 yr research projects

Huge conceptual change, increasing funding



State of MD is successfully playing the new role of providing sustaining funding for stem cell research



\$15M in grant awards announced May 17, 2007



\$23M appropriated for 2008



We're advocating for \$23M again for 2009, as endorsed by the Fall MD State budget legislative session



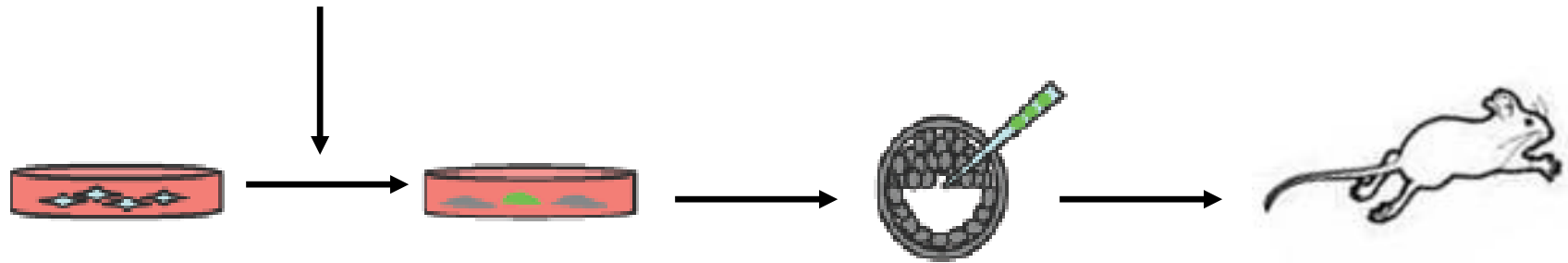
Governor O'Malley favors \$25M/yr for the remainder of the first 5 yrs, if the overall MD budget can handle it



Negative budgetary impact of induced pluripotent stem (iPS) cells (*aka* transduced adult cells, such as fibroblasts)??

Induced reprogramming of adult *mouse* fibroblasts to pluripotent ES like state

Retroviral infection with
Oct4/Sox2/Myc/Klf4



Mature fibroblasts
engineered with
Nanog-GFP reporter

Transduced pluripotent
stem cells are green

**Germ line
competent
mice**

Induction of Pluripotent Stem Cells
from Mouse Embryonic and Adult
Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi¹ and Shinya Yamanaka^{1,2*}

¹ Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

² CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

*Contact: yamanaka@frontier.kyoto-u.ac.jp

DOI: 10.1016/j.cell.2006.07.024

***In vitro* reprogramming of fibroblasts into a
pluripotent ES-cell-like state**

Marius Wernig^{1*}, Alexander Meissner^{1*}, Ruth Foreman^{1,2*}, Tobias Brambrink^{1*}, Manching Ku^{3*},
Konrad Hochedlinger^{1*}, Bradley E. Bernstein^{3,4,5} & Rudolf Jaenisch^{1,2}

Generation of germline-competent
induced pluripotent stem cells

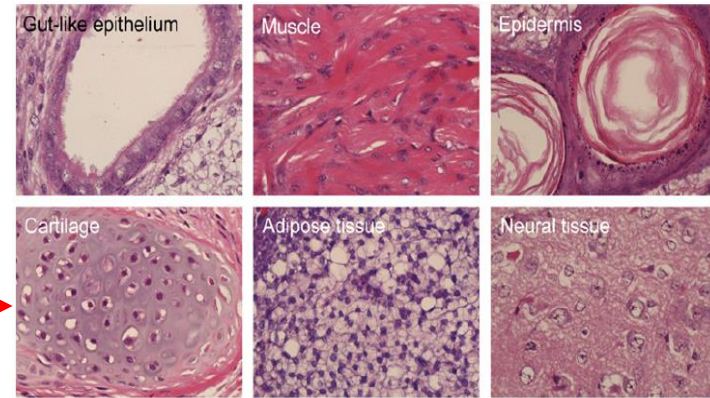
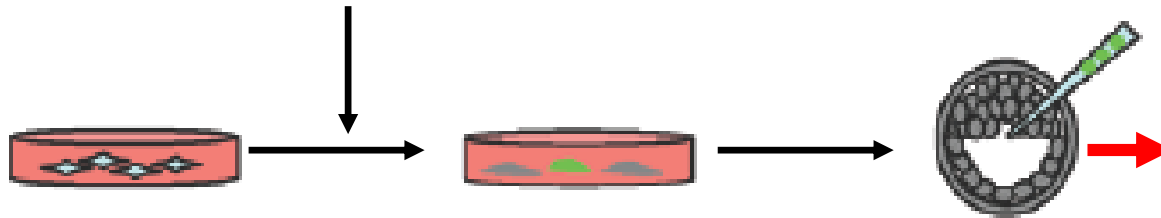
Keisuke Okita¹, Tomoko Ichisaka^{1,2} & Shinya Yamanaka^{1,2}

Directly Reprogrammed Fibroblasts
Show Global Epigenetic Remodeling
and Widespread Tissue Contribution

Nimet Maherali^{1,2,6}, Rupa Sridharan,^{3,6} Wei Xie,³ Jochen Utikal,¹ Sarah Eminli,¹ Katrin Arnold,¹
Matthias Stadtfeld,¹ Robin Yachechko,³ Jason Tchieu,³ Rudolf Jaenisch,⁵ Kathrin Plath,^{3,4,*}
and Konrad Hochedlinger^{1,*}

Induced reprogramming of adult *human* fibroblasts to pluripotent ES like state

Retroviral infection with
OCT4/SOX2/MYC/KLF4 or
OCT4, SOX2, NANOG, and LIN28



Mature **human fibroblasts** (from foreskin or adult skin) were engineered with Nanog-GFP or Oct4-Neo reporter retro- or lenti-vectors

Transduced pluripotent stem cells were green or Neo-resistant

Reprogrammed fibroblasts could be propagated (maintaining undifferentiated markers) for >20 passages or induced to undergo multilineage differentiation (to form all 3 germ layers both in embryoid body assays *in vitro* and in teratoma assays in immunodeficient NOD/SCID mice)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,^{1,2*} Maxim A. Vodyanik,² Kim Smuga-Otto,^{1,2} Jessica Antosiewicz-Bourget,^{1,2} Jennifer L. Frane,¹ Shulan Tian,³ Jeff Nie,³ Gudrun A. Jonsdottir,³ Victor Ruotti,³ Ron Stewart,³ Igor I. Slukvin,^{2,4} James A. Thomson^{1,2,5*}

Scienceexpress / www.scienceexpress.org / 20 November 2007 / Page 1 / 10.1126/science.1151526

Kazutoshi Takahashi,¹ Koji Tanabe,¹ Mari Ohnuki,¹ Megumi Narita,^{1,2} Tomoko Ichisaka,^{1,2} Kiichiro Tomoda,³ and Shinya Yamanaka^{1,2,3,4,*}

Cell 131, 1–12, November 30, 2007

How to make grant awards?: Peer review, minimizing conflicts and politics



Scientific peer review by panels of *expert stem cell researchers from other states*



Since MSCRF monies must be spent in MD, this simple mechanism greatly reduced expert reviewer conflicts



Second tier oversight by an appointed **Commission of Marylanders**. *Considerations:*



Ethics



Geographical distribution



Breadth of research portfolio



All kinds of stem cells



Basic, translational, clinical research



New and established investigators



Not politics

86 applications were submitted by Jan 8 2007; total requests >\$80M

2007 MSCRF grant awards



Investigator-initiated (large) research grants:
≤\$500K/yr (+15% indirect costs to institution)
x3 yrs = up to \$1.725M each



7 large grants awarded to established stem cell researchers with extensive preliminary results



5 to JHU faculty



1 to Univ MD faculty



1 to a Gaithersburg company



Exploratory (small) research grants: \$100K/yr
(+15% indirect costs) x1 yrs = \$230K each



17 small grants awarded to young investigators and investigators from other fields



10 to JHU faculty



7 to Univ MD faculty

~\$10M to JHU

PRESS RELEASE: Maryland Stem Cell Research Commission

Recommends 24 Projects for Funding (May 17, 2007)

➤ **7 applications awarded for RFA-MD-06-1 (Investigator Initiated Research Grants) designed for investigators with preliminary data supporting the grant application:**

- ❖ **Dr. Angelo All, [JHU](#), Whitaker Biomedical Engineering Institute: Human Embryonic Stem Cell-Derived Oligodendrocytes and Electrophysiological Studies in a Contusion Model of Spinal Cord Injury in the Rat**
- ❖ **Dr. Jeff Bulte, [JHU](#), School of Medicine: Human Embryonic Stem Cell-Derived Neurospheres for Treatment of Multiple Sclerosis**
- ❖ **Dr. Curt Civin, [JHU](#), Kimmel Cancer Center: MicroRNA Regulation of Adult and Embryonic Human Hematopoietic Development**
- ❖ **Dr. William Lederer, University of Maryland, Biotechnology Institute, Medical Biotechnology Center: Human Mesenchymal Stem Cell Treatment for Heart Damage**
- ❖ **Dr. Lloyd Mitchell, RetroTherapy, LLC: Preservation of Potentiality in Genetically Altered Stem Cells**
- ❖ **Dr. Hongjun Song, [JHU](#), School of Medicine: Characterization of Neuronal Potentials of Human Embryonic Stem and Adult Neural Stem Cells**
- ❖ **Dr. Elias Zambidis, [JHU](#), Kimmel Cancer Center: Human Embryonic Stem Cell Models of Normal and Leukemic Human Stem Cells**

5 to JHU

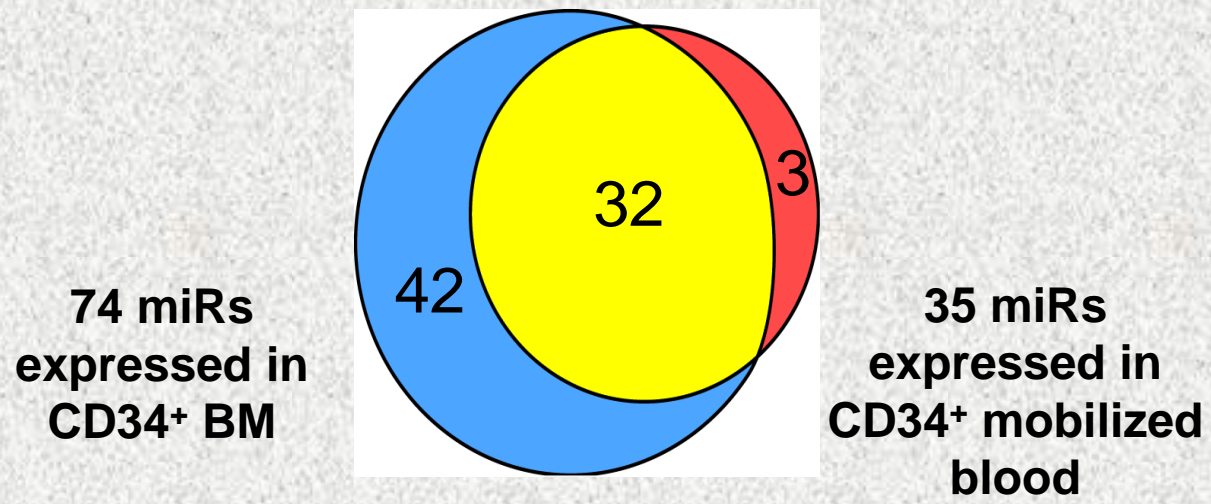
17 applications awarded for RFA-MD-06-2 (Exploratory Research Grants) designed for new investigators and investigators from other fields

- Dr. Shyam Biswal, **JHU**, School of Public Health: Nrf2 as a Target for Cancer Stem Cell Chemoresistance
- Dr. Srinivasan Chandrasegaran, **JHU**, School of Public Health: Targeted Engineering of the Human Genome in Stem Cells
- Dr. Nancy Craig, **JHU**, School of Medicine: Genome Engineering of Human Stem Cells for Gene Therapy
- Dr. Shengyun Fang, University of Maryland, Biotechnology Institute, Medical Biotechnology Center: Ubiquitination-Dependent Regulation of Oct-4 Activity
- Dr. Ricardo Feldman, University of Maryland, Baltimore, School of Medicine: A Model for Generation of Gaucher-Specific Human Embryonic Stem Cells, and for Reconstitution of Glucocerebrosidase Expression in Human Embryonic Stem Cell-Derived Macrophages
- Dr. Paul Fishman, University of Maryland, Baltimore, School of Medicine: Transcription Factor Driven Differentiation of Neural Stem Cells
- Dr. Gary Fiskum, University of Maryland, Baltimore, School of Medicine: Stem Cell Antioxidant Gene Preconditioning for Improved Cell Survival and Neurologic Outcome after Traumatic Brain Injury
- Dr. John Fisher, University of Maryland College Park, School of Engineering: Human Mesenchymal Stem Cells for Craniofacial Bone Regeneration
- Dr. M. Jafri, University of Maryland, Baltimore, School of Medicine: A Delivery System for Stem Cells
- Dr. Candace Kerr, **JHU**, School of Medicine: Defining Genes Associated with Human Stem Cell Pluripotency and Self-Renewal
- Dr. Hai-Quan Mao, **JHU**, Whiting School of Engineering: Engineering an Artificial Neural Stem Cell Niche
- Dr. Andrew McCallion, **JHU**, School of Medicine: Illuminating Human Cardiac Development and Disease through Transcriptional Analysis in Differentiating Human Embryonic Stem Cells
- Dr. Guo-li Ming, **JHU**, Institute for Cell Engineering: Mechanisms Regulating Self Renewal of Human Embryonic Stem Cells
- Dr. Akhilesh Pandey, **JHU**, School of Medicine: Proteomic Characterization of Neural Differentiation in Human Embryonic Stem Cells
- Dr. Adam Puche, University of Maryland, Baltimore, School of Medicine: Regulating Stem Cell Migration in the Adult Brain
- Dr. Hamid Rabb, **JHU**, School of Medicine: Isolation, Expansion and Regenerative Potential of Human Adult Kidney Derived Stem Cells
- Dr. Karen Zeller, **JHU**, School of Medicine: Myc's Role in Maintenance and Tumorigenicity of Human Embryonic Stem Cells

10 to JHU

MicroRNAs expressed in HSPCs

- Measured 228 human microRNAs in CD34⁺ HSPCs using Calin/Croce microarray chips
- Intersection analysis found 32 microRNAs expressed in CD34⁺ HSPCs from both BM and PBSC harvests

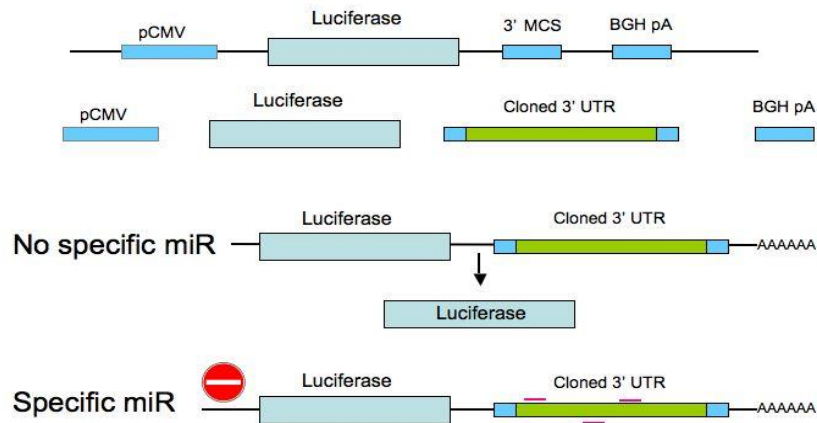


These “HE-microRNAs” are predicted to downregulate translation of >15% of HSC-expressed mRNAs

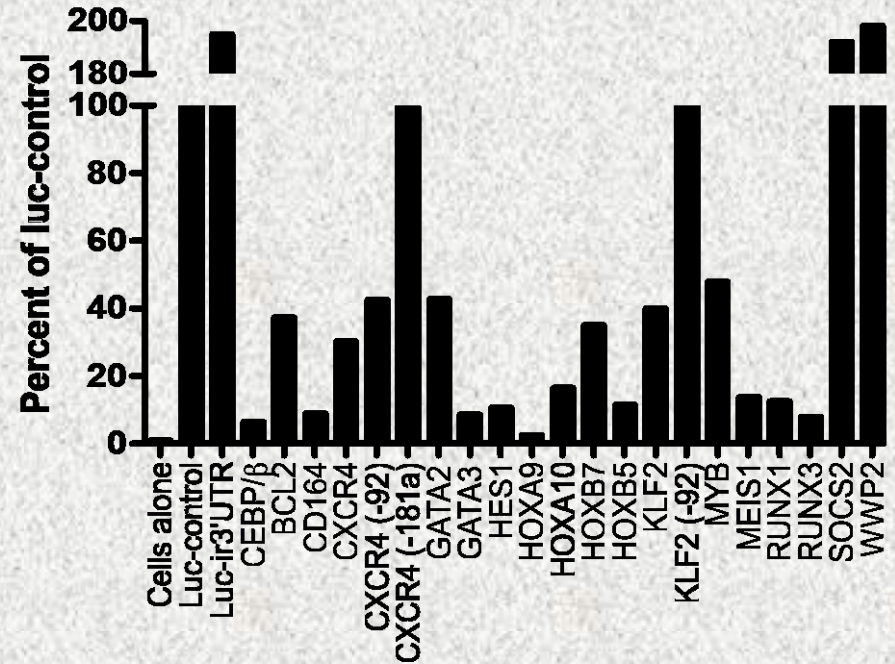
Georgantas
PNAS 2007

Several thermodynamically-predicted microRNA targets were verified by luciferase assays

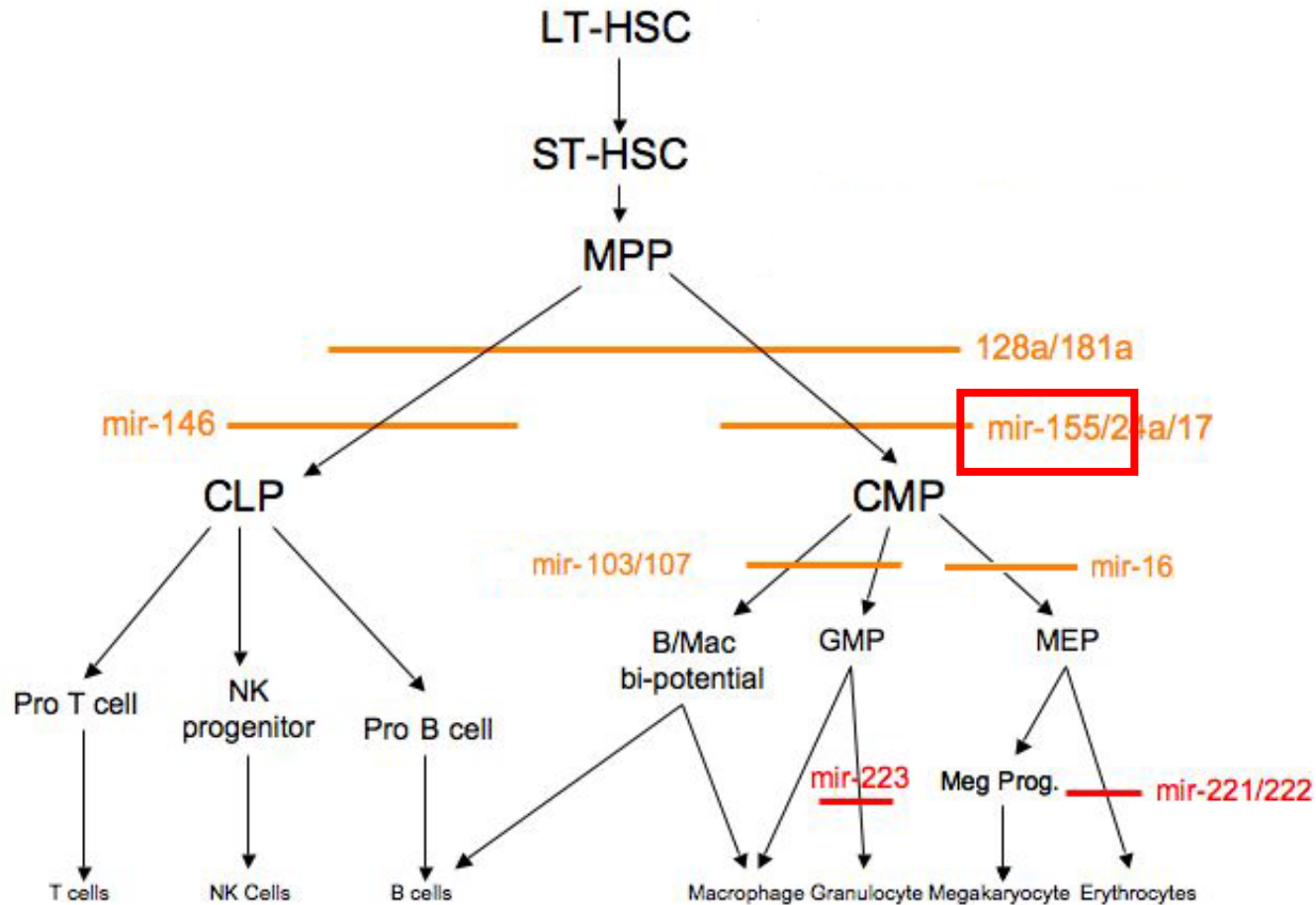
Assay for mRNA specific miR Function



3'UTR constructs were electroporated into K562 cells, which express most of the HE-microRNAs



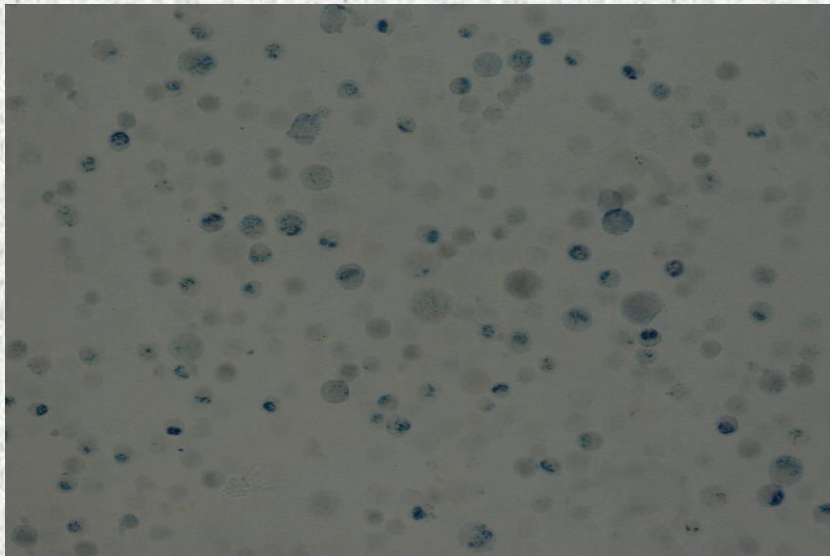
Mir-155 was predicted to control both myelo- and erythropoiesis



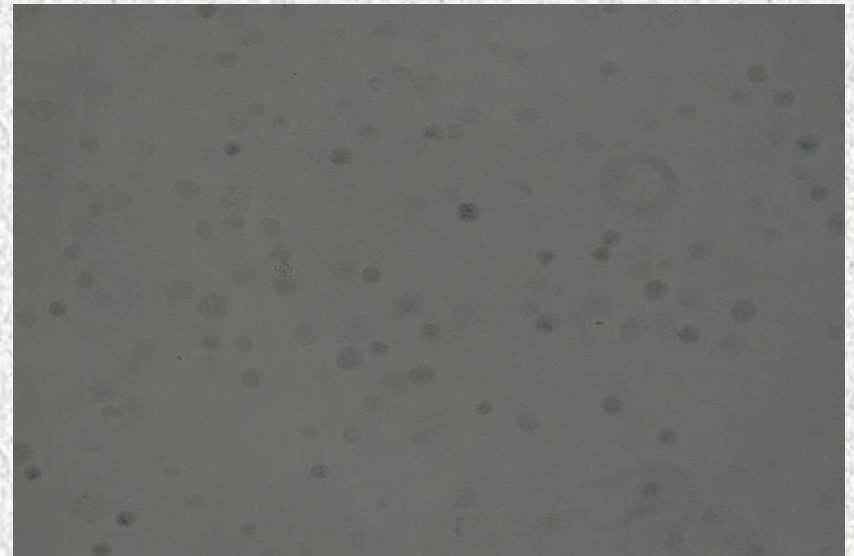
***Mir-155* blocked erythroid differentiation of the K562 leukemia cell line**

Benzidine Staining for Hemoglobin

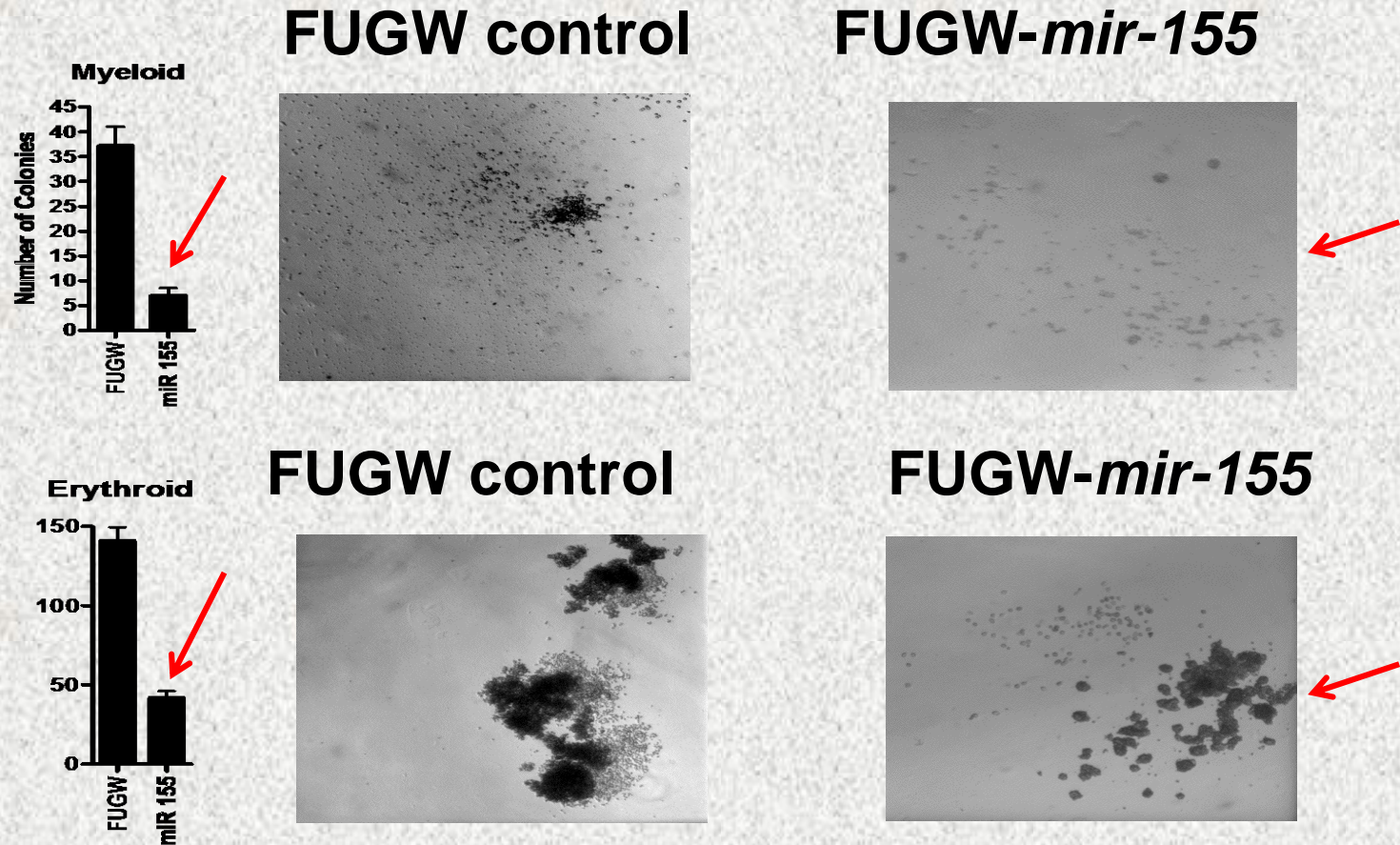
FUGW control
72% positive



FUGW-*mir-155*
<1% positive

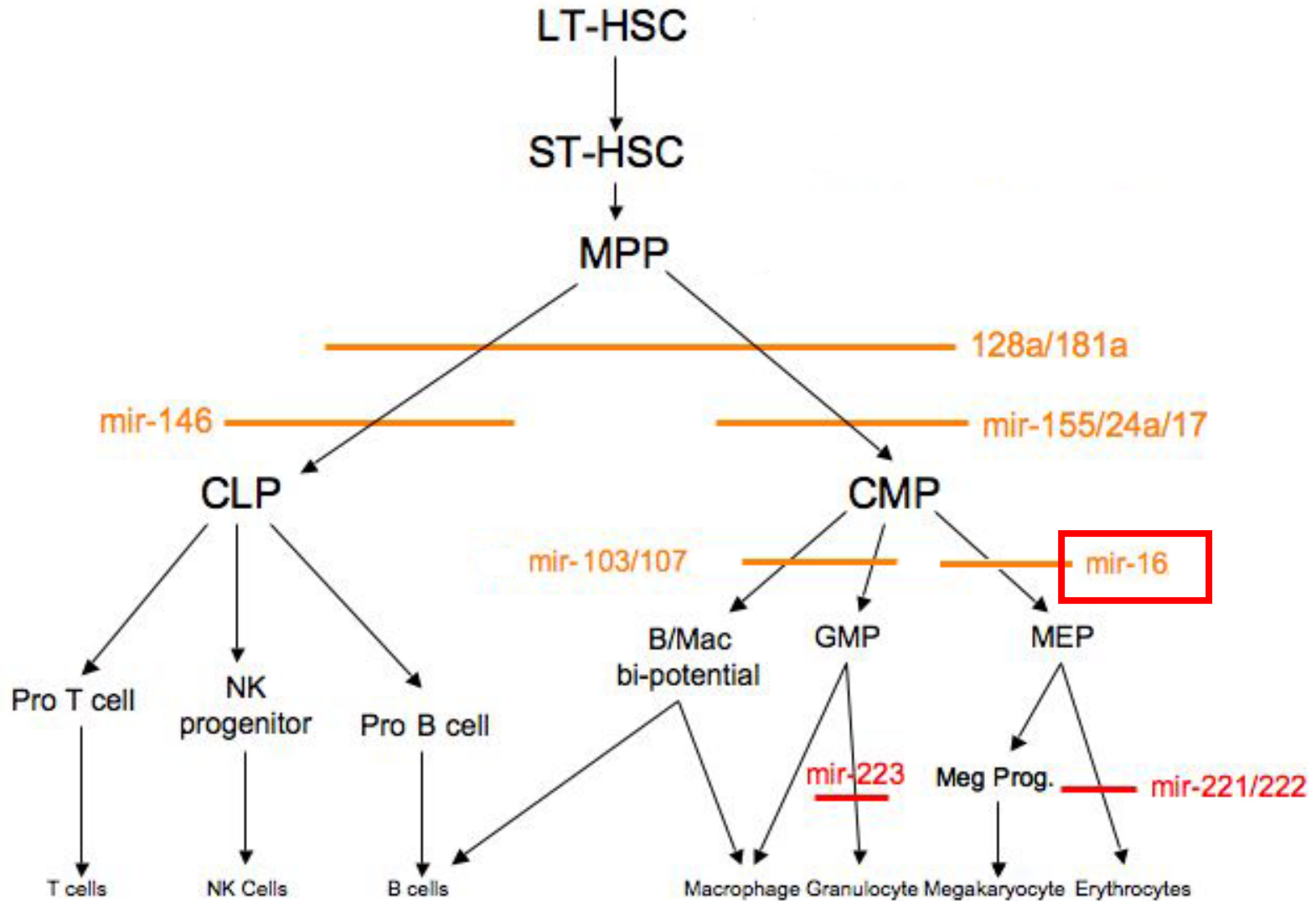


Mir-155 blocked both myeloid and erythroid colony formation from primary cells

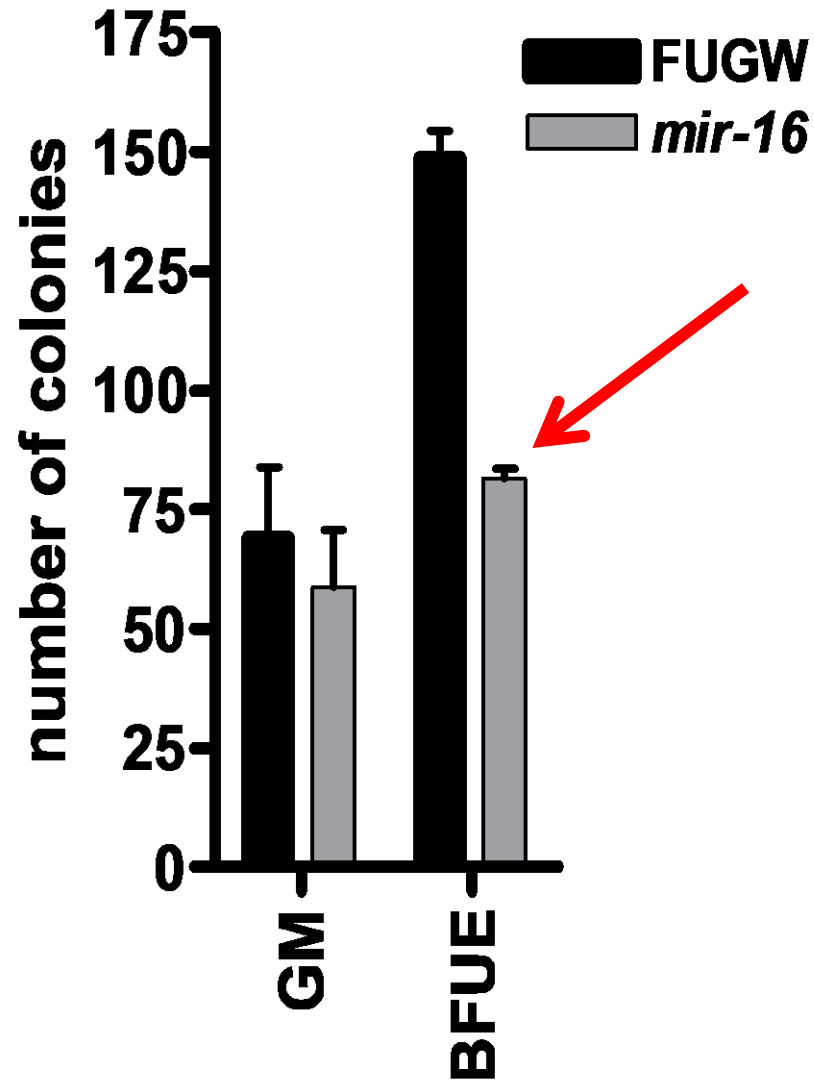


Normal donor CD34⁺ HSPC were lentivirally transduced with hsa-miR-155/GFP dual promoter expression constructs. Transduced cells were sorted based on GFP expression, and grown in standard CFC assays. Colonies were then counted to determine changes in colony formation, and photos taken by light microscopy to determine colony morphology.

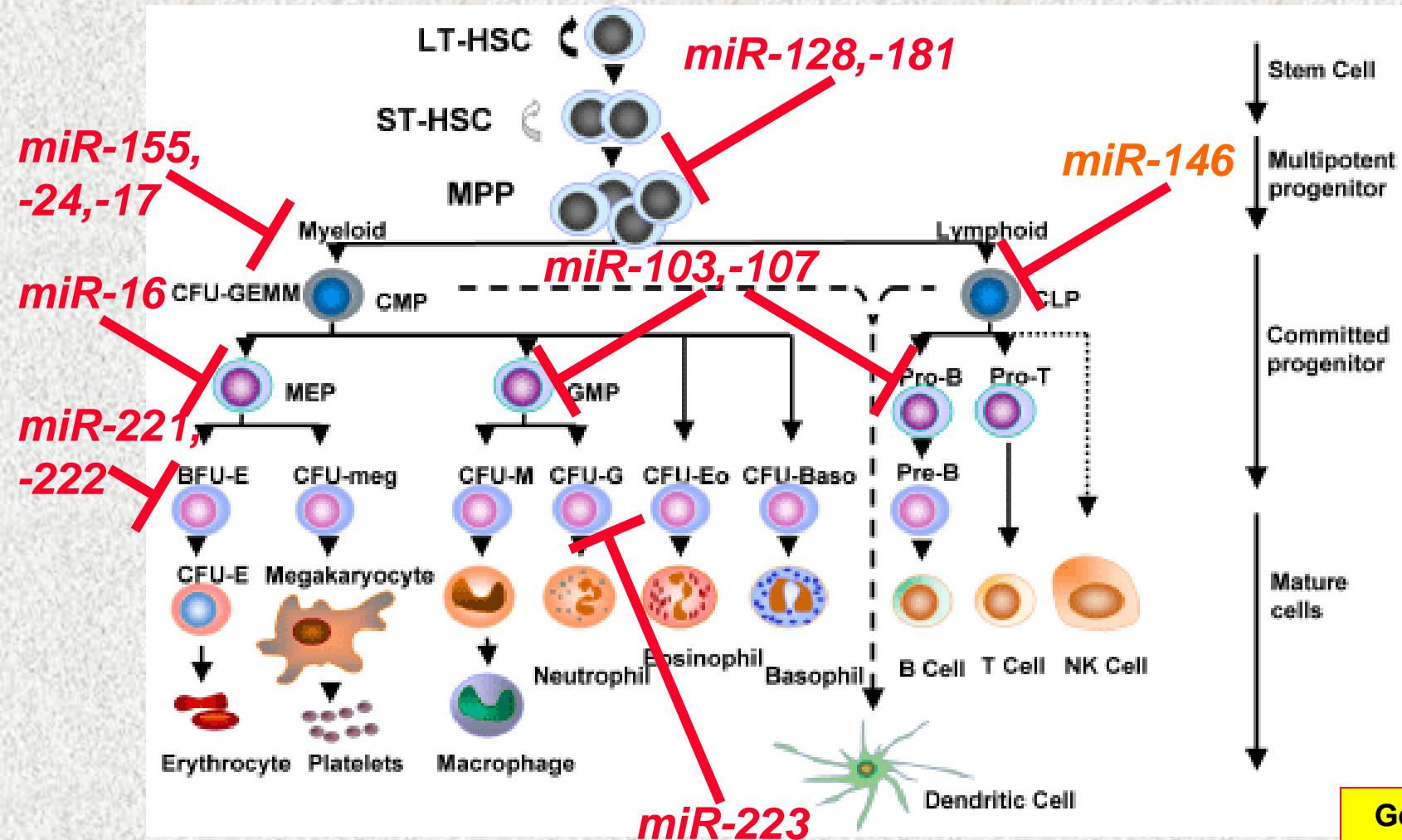
Mir-16 predicted to down-regulate erythropoiesis



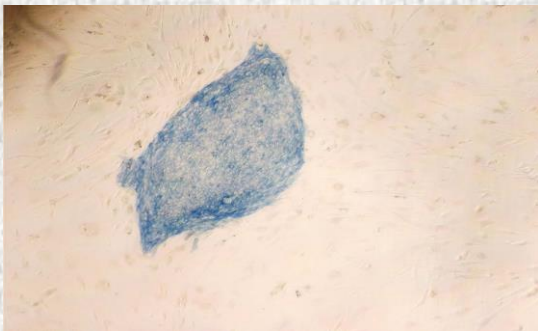
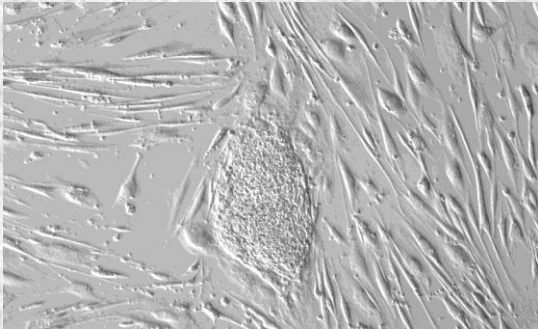
Mir-16 selectively blocked erythroid differentiation of primary HSPCs



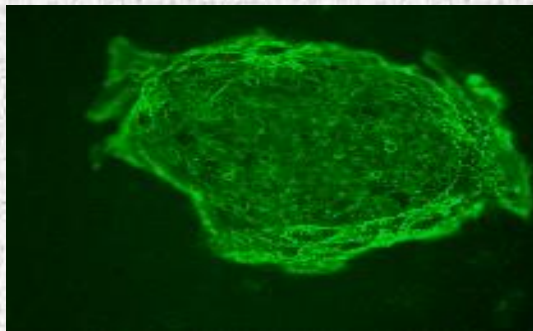
MicroRNAs regulate hematopoietic development: developmental regulatory “circuit breakers”



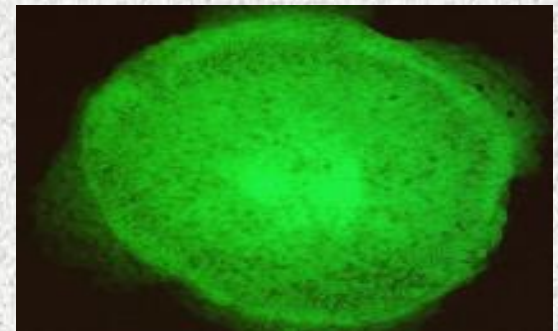
H1 and H9 human embryonic stem cell lines



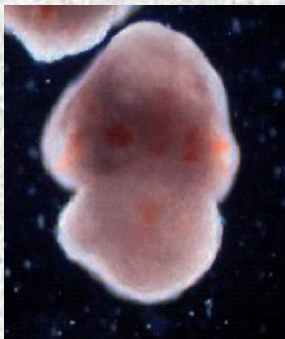
alk phosphatase⁺



SSEA-4⁺



**GFP⁺ colony after
lentiviral transduction**



Embryoid body containing hot pink primitive erythroid cells (embryonic & fetal hemoglobin)

Ongoing studies: Investigate microRNA expression and roles in early **embryonic stem cell** development and **leukemias**, as well as normal **mouse and human** hematopoiesis

