Establishment of hepatocellular cancer stem cell line using reprogramming technique

Sangchul Yun[1], **Dongho Choi[1]** and Seung Bum Lee[2]

[1]Department of Surgery, Soonchunhyang University Hospital, College of Medicine,
[2]Laboratory of Experimental Pathology, Korea Institute of Radiological & Medical Science

Introduction

- **Hepatocellular carcinoma (HCC) is a highly malignant tumor with limited treatment options in its advanced state.**

- **The molecular mechanisms of HCC remain unclear because of the complexity of its multi-step development process.**

- **There have been two theories concerning the mechanism of carcinogenesis, the stochastic (clonal evolution) model and the hierarchical (cancer stem cell-driven) model.**

- **Cancer stem cells (CSCs) are defined as a small population of cells within a tumor that possess the capability for self-renewal and the generation of heterogeneous lineages of cancer cells.**

Introduction

- **The concept of the CSC has been established over the past decade, and the roles of CSCs in the carcinogenic processes of various cancers, including HCC, have been emphasized.**

- **Although definitive cell surface markers for liver CSCs have not yet been found, several putative markers have been identified, which allow the prospective isolation of CSCs from HCC.**

- **The identification and characterization of CSCs in HCC is essential for a better understanding of tumor initiation or progression in relation to signaling pathways.**

- **However, CSCs of HCC in humans are not fully elucidated.**

Introduction

Colon cancer stem cells

LETTERS

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]

Nature. 2007 445:111-5.

LETTERS

A human colon cancer cell capable of initiating tumour growth in immunodeficient mice

Catherine A. O'Brien[1], Aaron Pollett[2], Steven Gallinger[3] & John E. Dick[1,4]

Nature. 2007 445:106-10.

Introduction

Pancreatic, prostate, head &neck cancer stem cells

Identification of Pancreatic Cancer Stem Cells

Chenwei Li,[1] David G. Heidt,[1] Piero Dalerba,[4] Charles F. Burant,[2,3] Lanjing Zhang,[3] Volkan Adsay,[4] Max Wicha,[3] Michael F. Clarke,[5] and Diane M. Simeone[1,2]

Departments of [1]Surgery, [2]Molecular and Integrative Physiology, and [3]Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan; [4]Department of Pathology, Karmanos Cancer Center, Detroit, Michigan; and [5]Department of Internal Medicine, Stanford University School of Medicine, Palo Alto, California

Cancer Res 2007; 67: (3). February 1, 2007

Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma

M. E. Prince*, R. Sivanandan† A. Kaczorowski*, G. T. Wolf*, M. J. Kaplan†, P. Dalerba‡, I. L. Weissman‡, M. F. Clarke‡, and L. E. Ailles‡§

*Department of Otolaryngology–Head and Neck Surgery, University of Michigan, Ann Arbor, MI 48109; and †Department of Otolaryngology–Head and Neck Surgery and ‡Stanford Institute for Stem Cell Biology and Regenera

PNAS | January 16, 2007 | vol. 104 | no. 3 | 973–978

Identification of Putative Stem Cell Markers, CD133 and CXCR4, in hTERT–Immortalized Primary Nonmalignant and Malignant Tumor-Derived Human Prostate Epithelial Cell Lines and in Prostate Cancer Specimens

Jun Miki,[1] Bungo Furusato,[2] Hongzhen Li,[1] Yongpeng Gu,[1] Hiroyuki Takahashi,[4] Shin Egawa,[5] Isabell A. Sesterhenn,[2] David G. McLeod,[1,3] Shiv Srivastava,[1] and Johng S. Rhim[1]

[1]Center for Prostate Disease Research, Uniformed Services University of the Health Sciences, Bethesda, Maryland; [2]Department of Genitourinary Pathology, Armed Forces Institutes of Pathology; [3]Urology Service, Department of Surgery, Walter Reed Army Medical Center, Washington, District of Columbia; and Departments of [4]Pathology and [5]Urology, Jikei University School of Medicine, Tokyo, Japan

Cancer Res 2007; 67: (7). April 1, 2007

Introduction

HCC cancer stem cells

Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 351 (2006) 820–824
BBRC
www.elsevier.com/locate/ybbrc

Characterization of CD133$^+$ hepatocellular carcinoma cells as cancer stem/progenitor cells ☆

Atsushi Suetsugu [a], Masahito Nagaki [a,*], Hitomi Aoki [b], Tsutomu Motohashi [b], Takahiro Kunisada [b], Hisataka Moriwaki [a]

[a] *Department of Gastroenterology, Gifu University Graduate School of Medicine, Gifu 501 1194, Japan*
[b] *Department of Tissue and Organ Development, Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine,*

GASTROENTEROLOGY 2007;132:2542–2556

Identification and Characterization of Tumorigenic Liver Cancer Stem/Progenitor Cells

STEPHANIE MA,*,‡ KWOK–WAH CHAN,* LIANG HU,‡ TERENCE KIN–WAH LEE,§ JANA YIM–HUNG WO,§ IRENE OI–LIN NG,* BO–JIAN ZHENG,‖ and XIN–YUAN GUAN‡

**Department of Pathology, ‡Department of Clinical Oncology, §Department of Surgery, and ‖Department of Microbiology, The University of Hong Kong, Pokfulam, Hong Kong, China*

Cancer stem markers in solid cancers

Solid Cancer	Cancer stem cell markers	References
HCC	CD133, side population CD90, CD13	Hepatology 2006 44:240-251 Gastroenterology 2007 132:2542-2556
Colon cancer	CD133	Nature. 2007 445:106-110. Nature. 2007 445:111-115.
Breast cancer	CD44, CD24 low	PNAS 2003 100:3983-3988
Brain cancer	CD133	Nature 2004 432:396-401
Head and Neck	CD44, CD24	PNAS 2007 104:973-978
Pancreas	CD44, CD24, ESA	Cancer Research 2007 67:1030-1037
Prostate	CD133, CXCR4	Cancer Research 2007 67:3153-3161

Introduction

Cancer stem cell theory(HCC)

- **First, Side population (SP) cells.**

  Haraguchi N, et al. Stem Cells 2006;24:506-513.

- **Second, Various CD markers positive cells from HCC cell lines**

  Yang ZF, et al. Cancer Cell 2008;13:153-166.(CD90)

  Ma S, et al. Gastroenterology 2007;132:2542-2556. (CD133)

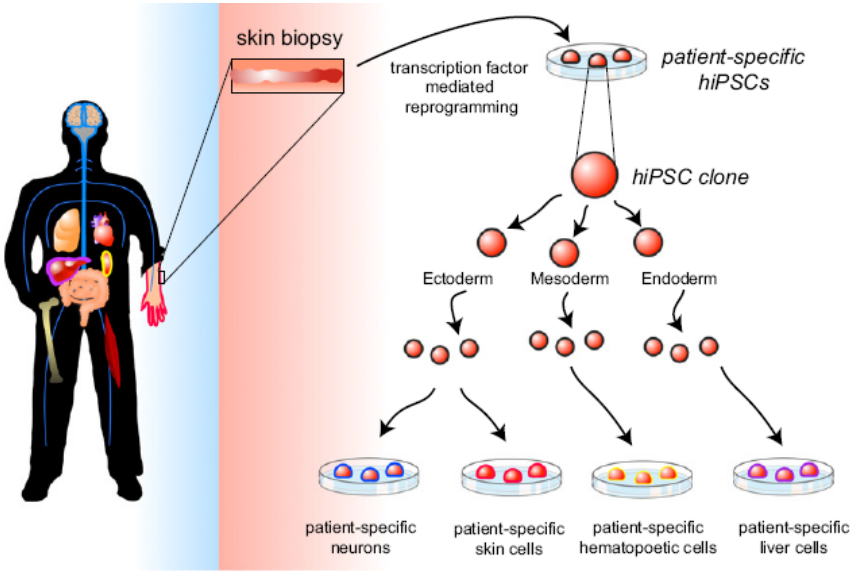
  Zhu Z, et al. Int J Cancer 2010;126:2067-2078. (CD44)

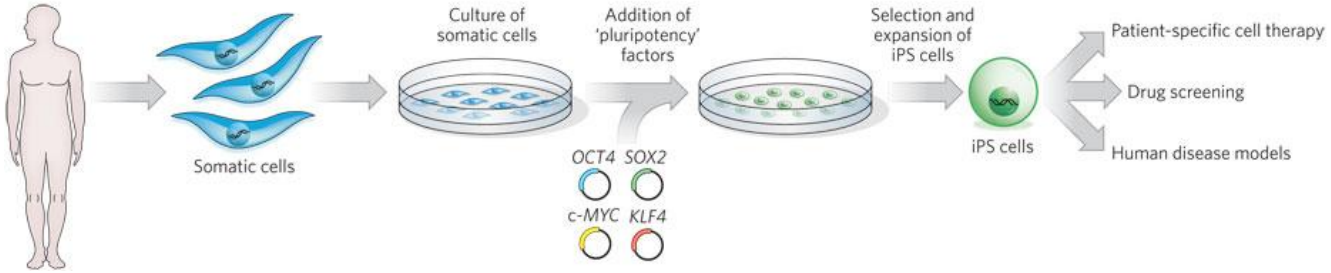
  Haraguchi N, et al. J Clin Invest 2010;120:33263339.(CD13)

- **Third, Induced cancer stem cells from HCC cell lines**

Introduction

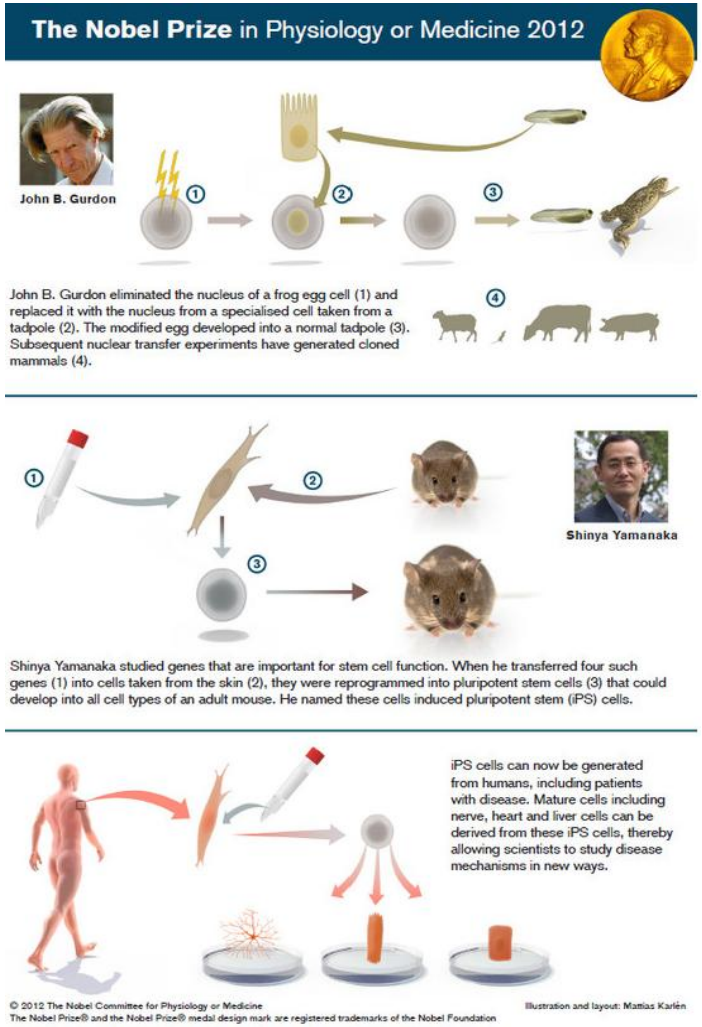
Induced pluripotent stem cells





Nature 2010 1465;704-712

Introduction



Introduction

•Recently, reprogramming is the fundamental part of stem cell biology understanding basic cellular mechanism of stem cells.

•In terms of reprogramming, induced pluripotent stem cells (iPSCs) are derived by introducing a combination of four transcription factors (KLF4, Oct4, Sox2 and Myc) into somatic cells.

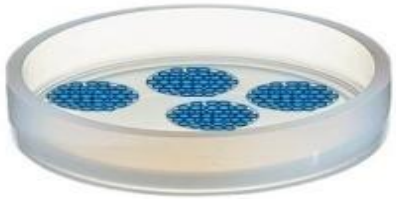
•Although cancer is a disease with genetic and epigenetic origins, the possible effects of reprogramming by defined factors remain to not be fully understood.

Introduction

Induced pluripotent cancer stem cells

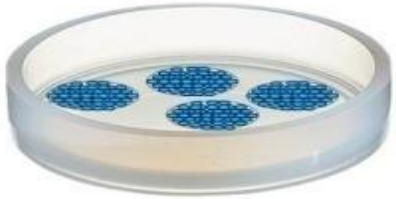
Human somatic cells

Retrovirus-KOSM vector



Human induced pluripotent stem cells

Human HCC cancer cells



Human HCC induced cancer stem cells

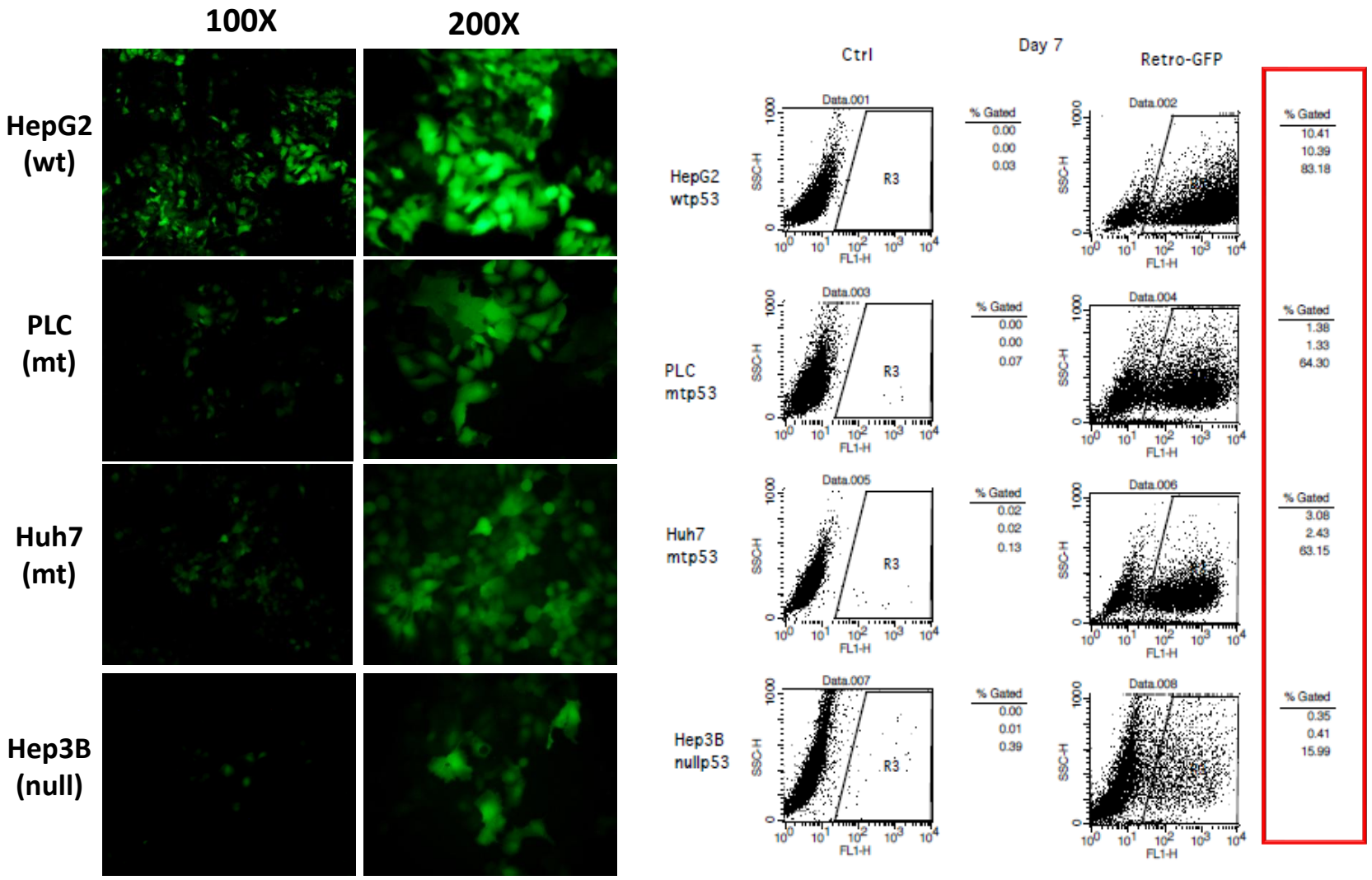
Aim

- To know the effects of the induction of pluripotent-related genes of HCC cancer cells and whether to get induced HCC stem cell lines from various HCC cell lines

Materials and methods

- To better understand cancer specific-iPSCs, We used 4 liver cancer cell lines (HepG2, Hep3B, Huh7 and PLC).
- Different mutant state of p53 from the liver cancer cells (HepG2:wild p53, Hep3B:null p53, Huh7:mutant p53 and PLC: mutant p53) were used.
- Retroviral mediated introduction of induced pluripotent stem (iPS) cell genes (KLF4, Oct4, Sox2 and Myc) were used for inducing various HCC cell lines
- Expression of pluripotent status related proteins, including Tra1-81 and Nanog were used for identification of pluripotent cells in cancer cells.

Result



Efficiency of infection on various liver cancer cells using Retrovirus-GFP

Result

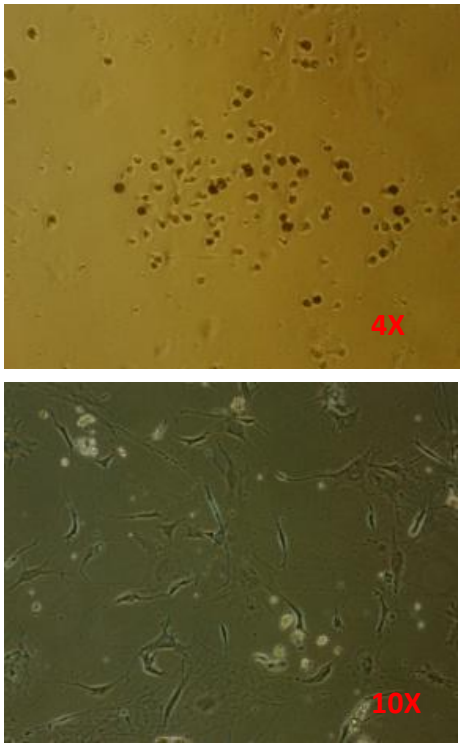
Colony number after 3-4 weeks on feeder
(1X104 /six well plate)

	3 weeks			4 weeks		
	Density	Number (about)	Tra1- 81	Density	Number	Tra1-81
HepG2 (wtp53)	40%	<100	X	50%	>100	X
PLC (mtp53)	80%	>200	X	>100%	Mess up	X
Huh7 (mtp53)	60%	100-200	X	80%	100-200	X
Hep3B nullp53)	60%	100-200	2	80%	100-200	2

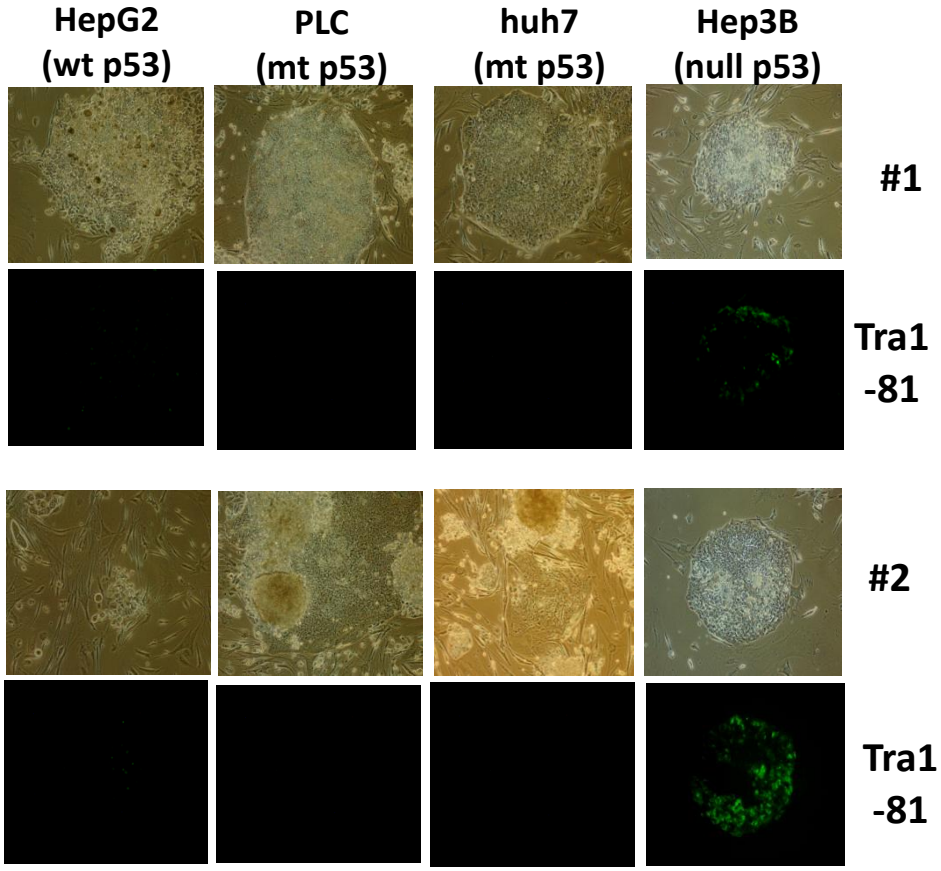
Staining Tra1-81 Ab (pluripotent surface mark) after reprogramming using Retrovirus-KOSM

Result

Non-transduced cells (control-HepG2)

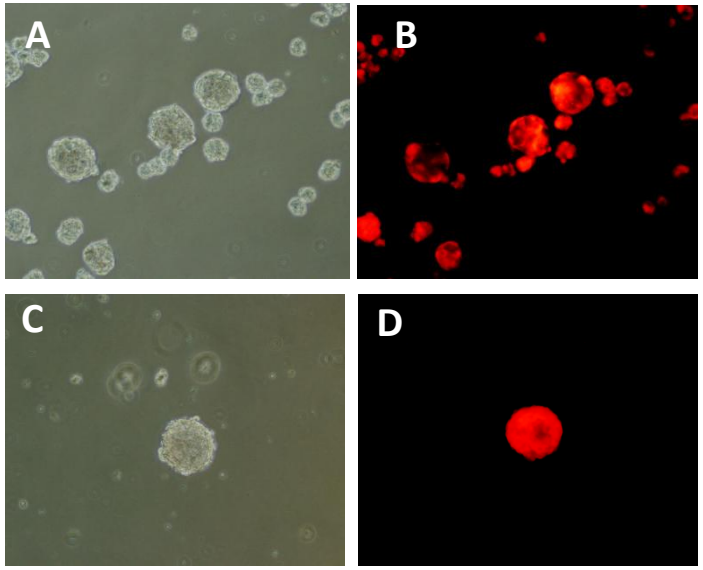


Transduced cells with KOSM 3 weeks on feeders



Staining Tra1-81 Ab (pluripotent surface mark) after reprogramming using Retrovirus-KOSM

Result



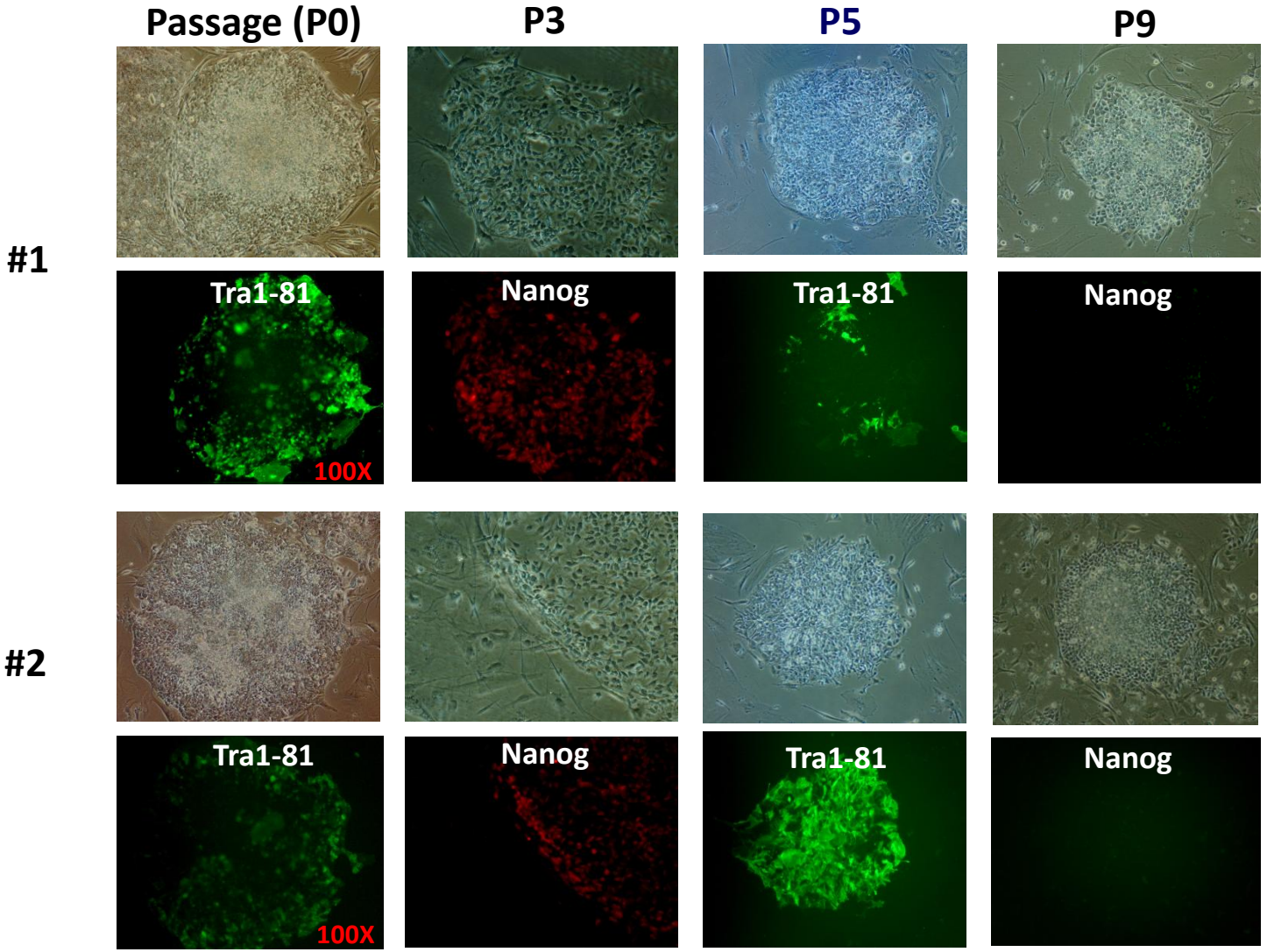
X100

mCherry (Red)

EB fromation at Day 3 from mCherry stable hep3B-iPC cells

Embryonic body (EB) at different wells (A-D)

Result



The stemness of Hep3B-iPC is disappeared upon continual passage

Staining of Tra1-81 Ab in Hep3B-iPC after continual passage

Summary

- Hep3B (null p53) showed the better efficiency of reprogramming compared to other liver cancer cell lines.
- Characterization of reprogrammed Hep3B-iPC expressed pluripotent markers such as Tra1-81 and Nanog.
- Hep3B-iPCs were able to form embryonic body (EB).
- Even though loss of stemness in Hep3B-iPC was detected during continual passage. Induced cells, but not parental cells, possessed the potential to express morphological patterns of iPSC and express plurpotent markers.
- Futher studies should be done for maintenance of HCC iPC with long term passages


Thank you for your attention !