

Cell clusters formation by ribosome is reproducible with various kinds of cell lines

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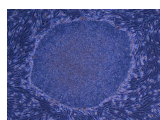
Previously, we have reported that incorporation of lactic acid bacteria (LAB) into the human dermal fibroblasts (HDFs) can generate cell clusters and they are similar to the embryoid bodies derived from embryonic stem cells (Ohta et al., 2012). After that, we found that the cellular transdifferentiation is caused by ribosomes (Ito et al., 2018). Our purpose in this study is to examine the transdifferentiation ability of ribosomes using other kind of cells. We used human hepatoma cells (Li-7), rabbit kidney cell line (CCD-IC), rabbit cornea cells (RC4), Chinese hamster lung cells (CHL), mink lung cells (NBL-7), medaka caudal fin cell line (OLHNI-2, J Comp Physiol B 2006) and ribosomes for the cell clusters formation assay. To perform it, we investigated several culture conditions by changing the amount of ribosome. We showed that the ribosomes incorporated Li-7, CCD-IC, RC4, CHL, NBL-7 and OLHNI-2 induced cell clusters. Then, we cultured cell clusters of RC4, Li-7 and CCD-IC in STEMPro Adipogenesis and Osteogenesis Differentiation Medium and conducted Oil Red O staining and Alizarin Red staining, respectively. The ribosomes incorporated RC4 and Li-7 were differentiated into adipocytes and osteoblasts. CCD-IC was differentiated into only adipocytes. These findings demonstrate that incorporation of ribosomes induces cellular transdifferentiation of not only HDFs but also other kind of animal cells.

0. Introduction

(1) iPS cell

induced pluripotent stem cell

(From Kyoto University's website)



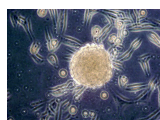
(2) cell clusters

Reprogramming of

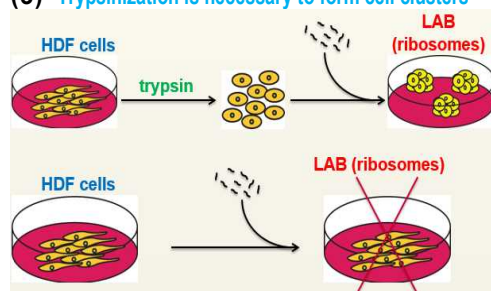
human dermal fibroblast cells(HDFs)

by lactic acid bacteria

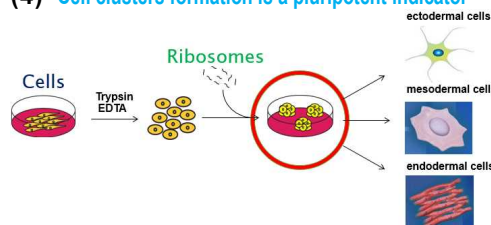
(Dr. Ohta's research team)



(3) Trypsinization is necessary to form cell clusters



(4) Cell clusters formation is a pluripotent indicator



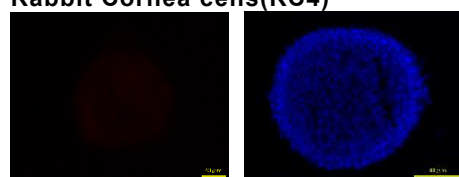
(5) Ribosomes refinement

E. coli Ribosome + His-tag

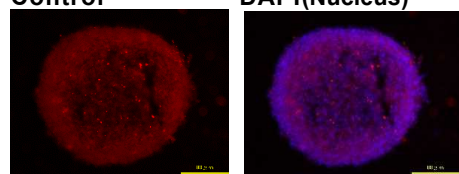


(6) The incorporated ribosomes localize in the nucleus as well as the cytoplasmic region

Rabbit Cornea cells(RC4)



Control DAPI(Nucleus)



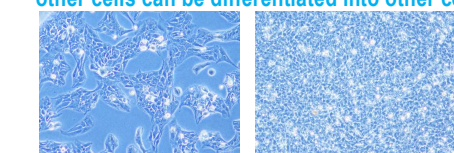
α-M-IgG-cy3(Ribosomes) Merge

1. Purposes

Cell cluster formation on Human hepatoma cells(Li-7) and rabbit kidney cells(CCD-IC)

⇒ Examine other cells incorporated ribosomes form cell clusters.

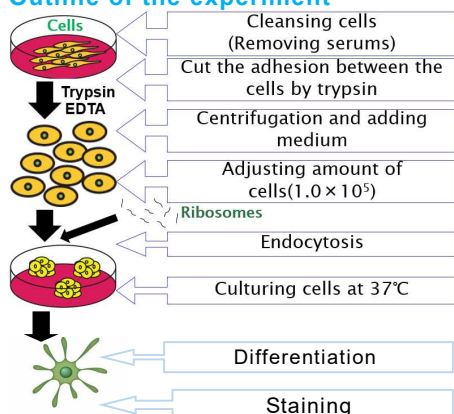
⇒ Examine generated cell clusters of other cells can be differentiated into other cells.



Li-7 CCD-IC

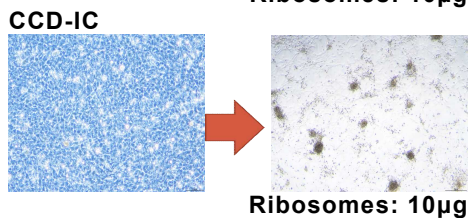
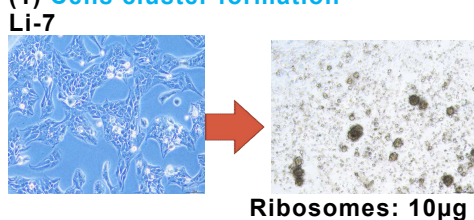
2. Method

Outline of the experiment



3. Results

(1) Cells cluster formation



(2) Compare Ribosomes and Protein X

	Protein 0µg	4 0 µg	6 0 µg
Li-7			
CCD-IC			

(3) Differentiation & Staining

RC4 and Li-7 were differentiated into adipocytes and osteoblasts.

CCD-IC was differentiated into only adipocytes.

	Osteoblasts	Adipocytes	Chondrocytes
RC4			
Li-7			
CCD-IC			
CHL			
NBL-4			

(4) Summary

Incorporation of ribosomes induces cellular transdifferentiation of not only HDFs but also other kind of animal cells.

kind of cells	cell adhesion	germ layer	medium	Ribosome amount	cell clusters formation
First term OLF-136 (medaka)	Yes	Ectoderm	M199	3µg	×
			L-15	3	○
			mFSF	3	○
			L-15 A+B	3	×
			L-15 SSR	3	×
second third term LAH1 (Frog)	Yes	Mesoderm	L-15	10,20,30,40	×
			m-FSF	3,6,10,15	×
				10,20,30,40	×
MDCK(Dog)	Yes	Mesoderm	MEM	3,6,10,15	×
				10,20,30,40	×
			mFSF	3,6,10,15	×
				10,20,30,40	×
CHL(Hamster)	Yes	Endoderm	MEM	3,6,10,15	×
				10,20,30,40	×
			SCM132	3,6,10,15	×
				10,20,30,40	×
NBL-7(Mink)	Yes	Endoderm	MEM	3,6,10,15	×
				10,20,30,40	×
			SCM132	3,6,10,15	×
				10,20,30,40	×
RC4(Rabbit)	Yes	Ectoderm	MEM	3,6,10,15	Δ
				10,20,30,40	○
			SCM132	3,6,10,15	○
				10,20,30,40	×
HL-60(Human)	No	Endoderm	RPMI1640	3,6,10,15	×
				10,20,30,40	×
			mFSF	3,6,10,15	×
				10,20,30,40	×
				3,6,10,15	×
fourth term(now) Li-7(Human)	Yes	Endoderm	RPMI1640	0,10,20,30	○
CCD-IC (Rabbit)	Yes	Mesoderm	DMEM	0,10,20,30	Δ
				15,20,25,30	○
RF/6A (Monkey)	Yes	Ectoderm	RPMI1640	—	○

4. References

·Kunimasa Ohta: Lactic Acid Bacteria Convert Human Fibroblasts to Multipotent Cells
Japanese Journal of Lactic Acid Bacteria
· <http://www.kikin.kyoto-u.ac.jp/contribution/ips/>

5. Acknowledgments

RIKEN, who provided us cell