Embryonic stem cells differentiation into a functional thyroid gland

“Studying thyroid development using ESCs”

Francesco Antonica

44th BTC Meeting, Grimbergen 26th Apr 2014
Overview of the thyroid development

**Unknown signalling**

E8.5 (4th GW) pharynx (Endodermal layer) 

E10.5

E11.5 (5th GW)

E12.5 (7th GW)

E16.5 (11th GW)

**Overview**

- **E8.5 (4th GW)**
  - Pharynx (Endodermal layer)
  - ?

- **E10.5**
  - Nkx2.1, Pax8, Foxe1, Hhex
  - Specification

- **E11.5 (5th GW)**
  - Budding
  - Relocalization

- **E12.5 (7th GW)**
  - Tshr, Tpo, Tg
  - Proliferation

- **E16.5 (11th GW)**
  - Slc5a5
  - Folliculogenesis
  - T4
Overview of the thyroid development

**Which genes?**

In human, only 2% of cases of thyroid dysgenesis are due to mutations in NKX2-1, PAX8, HHEX, FOXE1
Tools available in our lab

mouse

ESCs

zebrafish
Our goal:
To model thyroid development *in vitro*

Pluripotent stem cells
(Embryonic stem cells)

Thyroid Follicular Cells
The origin of the pluripotency

Mouse Day 4
Human Day 4-5

Fertilization → Embryo → Blastocyst → Inner Cell Mass → EMBRYONIC STEM CELLS

- Ectoderm: Brain, Skin
- Mesoderm: Muscle, Blood, Bone, Cartilage
- Endoderm: Lung, Gut, Liver, Thyroid
In vitro plasticity of ESCs

...Your wishes in a Petri dish
How differentiation of pluripotent stem cells is achieved *in vitro*

1) Approaches using defined media

**ARTICLE**

doi:10.1038/nature10637

Self-formation of functional adeno-hypophysis in three-dimensional culture

Hidetaka Suga¹,²,³, Taisuke Kadoshima¹, Maki Minaguchi¹, Masatoshi Ohgushi², Mika Soen¹, Tokushige Nakano¹, Nozomu Takata¹, Takafumi Wataya¹, Keiko Muguruma¹, Hiroyuki Miyoshi¹, Shigenobu Yonemura², Yutaka Oiso² & Yoshiki Sasai³
How differentiation of pluripotent stem cells is achieved *in vitro*

1) Approaches using defined media
How differentiation of pluripotent stem cells is achieved \textit{in vitro}

2) Approaches using ectopic expression of transcription factors

Programming human pluripotent stem cells into white and brown adipocytes

Tim Ahfeldt\textsuperscript{1,2,3,14}, Robert T. Schinzel\textsuperscript{1,2,3,4,14}, Youn-Kyoung Lee\textsuperscript{1,2,3,14}, David Hendrickson\textsuperscript{1,5}, Adam Kaplan\textsuperscript{1,2,3}, David H. Lum\textsuperscript{6,7}, Raymond Camahort\textsuperscript{1,2,3}, Fang Xia\textsuperscript{1,2,3}, Jennifer Shay\textsuperscript{1,2,3}, Eugene P. Rhee\textsuperscript{3,5}, Clary B. Clish\textsuperscript{1}, Rahul C. Deo\textsuperscript{8,9,10}, Tony Shen\textsuperscript{1,2}, Frank H. Lau\textsuperscript{2,3}, Alicia Cowley\textsuperscript{1,2}, Greg Mowrer\textsuperscript{2,3}, Heba Al-Siddiqi\textsuperscript{11}, Matthias Nahrendorf\textsuperscript{12}, Kiran Musunuru\textsuperscript{1,5,13}, Robert E. Gerszten\textsuperscript{3,5}, John L. Rinn\textsuperscript{15} and Chad A. Cowan\textsuperscript{1,2,3,5,15}

3 transcription Factors:

- peroxisome proliferator-activated receptor (PPARG2)
- CCAAT/enhancer-binding protein beta (CEBPB)
- PR domain containing 16 (PRDM16)
Self-organizing optic-cup morphogenesis in three-dimensional culture

Mototsugu Etaka1,2, Nozomu Takeya1, Hiroki Ishitani3, Masako Kawada1, Eriko Sakakura1,2, Satoru Oyoda2, Kiyotoshi Sekiguchi4, Tatsuya Adachi5,6 & Yoshiki Sasai1,2

ESCs as model of morphogenesis
Adapted from Parlato et al., *Developmental biology*, 2004
Overview of the thyroid development

Unknown signalling

E8.5
- pharynx (Endodermal layer)
- TFCs

E10.5

E11.5

E12.5

E16.5

From E16-E17 on
- NKX2-1
- Tg
- TSHR
- TPO
- NIS
- PAX8
- Tg
- TSHR
- TPO
- NIS

TSHR, TPO, TG

NIS

T4

(Adapted from Christophe D., Molecular and cellular endocrinology, 2004)
NKX2-1 and PAX8 are involved in an auto-regulative loop

Unknown signalling

E8.5

pharynx
(Endodermal layer)

TFCs

Nkx2-1, Pax8, Hhex, FoxE1

Nakazato et al. 1997
Oguchi and Kimura, 1998
(Rat FRTL5 cells)

Pax8

Hobertus, Christophe, 2012
(Rat PCCL3 cells)

Hhex

Presta et al. 2005
(Human ARO cells)

D’Andrea et al. 2006
(Rat PCCL3 cells)

Di Gennaro et al. 2012
(Rat FRTL5 cells)

FoxE1
I: the ES cell line

Tet-On

\[ P_{EF-1\alpha} \rightarrow \text{rtTA} \]

\[ P_{\text{minCMV}} \rightarrow \text{TRE} \rightarrow \text{gene of interest} \]

\[ \text{rtTA} \]

\[ \text{ADD DOX} \rightarrow \text{rtTA} \]

\[ \text{REMOVE DOX} \]
Inducible cassette exchange: a rapid and efficient system enabling conditional gene expression in embryonic stem and primary cells.

Ectopic co-expression of *Nkx2-1* and *Pax8*
Embryonic bodies are three-dimensional aggregates of pluripotent stem cells.

ESCs within embryonic bodies undergo spontaneous differentiation and cell specification along the three germ lineages.
Ectopic co-expression of *Nkx2-1* and *Pax8*

Day 7 (→3 days after Dox addition)

Endogenous *Nkx2.1* and *Pax8* (with PCR primers targeting the 3’UTR regions)
Ectopic co-expression of Nkx2-1 and Pax8

Day 7 (3 days after Dox addition)

ESC\s Hanging drops Matrigel-supported 3D culture
Day 0 Day 4 Day 7

Dox

NIS=Slc5a5

mRNA relative expression

4500
3000
1500
45
15
0

Foxe1 Tshr Slc5a5 Tg Tpo

NKX2-1 PAX8

**
***

Tg ↔ TSHR

TPO

NIS
Protocol for thyroid differentiation

ESC → Hanging drops → Matrigel-supported 3D culture

Day 0 → Day 4 → Day 7

Nkx2-1-Pax8

Nkx2-1, Pax8

Dox, rhTSH

NKX2-1, PAX8

Tg, TSHR, TPO, NIS

TSH
**Nkx2-1-Pax8**

<table>
<thead>
<tr>
<th>Dox</th>
<th>rhTSH</th>
</tr>
</thead>
</table>

- ESCs: Hanging drops
- Matrigel-supported 3D culture

**Day 0**

- NKX2.1 PAX8 Hoechst
- NKX2.1 FOXE1 Hoechst
- NKX2.1 NIS Hoechst
- NKX2.1 TG Hoechst

- Untreated cells: 60%

**Day 22**
In vitro differentiation of thyroid follicles

NKX2.1  E-Cadh  Hoechst
NKX2.1  ZO-1  Hoechst
NKX2.1  TG  Hoechst
NKX2.1  TG-I  Hoechst
**In vitro** differentiation of functional thyroid follicles

**Nkx2.1-Pax8**

<table>
<thead>
<tr>
<th>% Iodide Organization (cpm PBI/ cpm Uptake)</th>
</tr>
</thead>
</table>
| ![Graph](image)

**Nkx2.1**

<table>
<thead>
<tr>
<th>% Iodide Organization (cpm PBI/ cpm Uptake)</th>
</tr>
</thead>
</table>
| ![Graph](image)

**Pax8**

<table>
<thead>
<tr>
<th>% Iodide Organization (cpm PBI/ cpm Uptake)</th>
</tr>
</thead>
</table>
| ![Graph](image)

- Dox d4-d7 - + +
- rhTSH d7-d22 - - +
In vivo validation of mESC-derived thyroid follicles
In vivo functionality of mESC-derived thyroid follicles

- Generation of hypothyroidism mouse model

[131-I] i.p. injection 4 weeks Hypothyroidism confirmed
In vivo functionality of mESC-derived thyroid follicles

- Generation of hypothyroidism mouse model
In vivo functionality of mESC-derived thyroid follicles

- Transplantation of mESC-derived thyroid follicles
In vivo functionality of mESC-derived thyroid follicles

- Integration of transplanted tissue in the host organ
In vivo functionality of mESC-derived thyroid follicles

- Exogenous follicular epithelium shows thyroid MOLECULAR signatures

<table>
<thead>
<tr>
<th>NKX2.1</th>
<th>PAX8</th>
<th>FOXE1</th>
</tr>
</thead>
</table>

[Images of immunohistochemical staining for NKX2.1, PAX8, and FOXE1]
In vivo functionality of mESC-derived thyroid follicles

- Exogenous follicular epithelium shows thyroid FUNCTIONAL signatures
Rescue of thyroid homeostasis

- Normalization of T4 plasma level

Before transplantation…

![Graph showing T4 (μg dL⁻¹) levels before and after 131-I injection.](image)
Rescue of thyroid homeostasis

- Normalization of T4 plasma level

Before transplantation…

…After transplantation

$^{131}$I treatment

4 weeks after $^{131}$-I Injection

Grafted cells

4 weeks after transplantation

$^{131}$I treatment

0 1 2 3 4
T4 (ug dL$^{-1}$)

n = 5

n = 13

n = 5

n = 4

n = 9

$^{131}$I treatment

- +

- +

- +

- Undiffer.

Thyroid follicles
Rescue of thyroid homeostasis

Transplanted tissue is responsible for plasma T4 normalization

whole-body scintigraphy ($^{99m}$Tc-pertechnate)

K. Peremans
Ghent University
Rescue of thyroid homeostasis

Pituitary-thyroid axis

4 weeks post-transplantation

Hypo mice

Grafted mice

untreated mice

TSH (mU mL⁻¹)

T4 (μg dL⁻¹)

S. Refetoff
University of Chicago
Rescue of thyroid homeostasis

Body temperature

- Control (no $^{131}$I, no graft)
- $^{131}$I-injected, undiff. cells
- $^{131}$I-injected, TFC-like cells

Body temperature (°C)

$^{131}$I treatment
- -  +  +

Grafted cells
- - Undiffer. Thyroid follicles

4 weeks after transplantation

n = 5  n = 4  n = 9
Recapitulate the model with hES cells

Work with iPSc derived from patients with thyroid agenesis or thyroid hypoplasia → Congenital hypothyroidism Modeling
Acknowledgements

Nika Figini Kasprzyk
Celine Ballot
Robert Opitz
Achim Truhora
Isabelle vandernoot
Benoit Haerlingen
Véronique Janssens

Sabine Costagliola
The Mentor

http://costalab.ulb.ac.be
Thank You