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Data Article

Data describing Rax positive optic-vesicle generation from mouse embryonic stem cells *in vitro*

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Taurin (PubChem CID: 211707)
Retinoic Acid (PubChem CID: 444795)

ABSTRACT

This article contains data related to the research article entitled "Specification of embryonic stem cell-derived tissues into eye fields by Wnt signaling using rostral diencephalic tissue-inducing culture" Sakakura (2016) [1]. Mouse embryonic stem cells (ESC) were used for the generation of optic vesicle-like tissues *in vitro*. In this article we described data in which a Rax::GFP knock-in ESC line was used to monitor the formation of optic tissues. In addition, we also described the data of regional marker expression of Rax, Sox2 and Pax6 *in vivo* around the forebrain and the eye tissues for comparative purposes. These data can be valuable to researchers interested in investigating forebrain and eye tissue development.

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Specifications Table

Subject area	Biology
More specific subject area	Stem cell biology, Developmental biology, Regenerative medicine
Types of data	Image, graph, schematic diagram
How data was acquired	Inverted fluorescent microscope (fluorescent, bright-field) and Fluorescence-activated cell sorting (FACS) analysis
Data format	Raw, analyzed
Experimental factors	Mouse embryonic stem cells (ESCs) were differentiated into optic tissues *in vitro*
Experimental features	A chemical inhibitor, CHIR99021 (CHIR), which inhibits GSK-3ß, was applied for the generation of optic tissues in a three-dimensional manner using a chemically defined medium (CDM) and matrigel (MG).
Data source location	Laboratory for *in vitro* Histogenesis, RIKEN Center for Developmental Biology. Center for Vascular and Developmental Biology, Feinberg Cardiovascular Research Institute, Northwestern University Feinberg School of Medicine.
Data accessibility	Supplementary data of the article

Value of the data

- The expression pattern of Sox2, Pax6 and Rax provides the characterization of ESC-derived tissues for regional identification of neural tissues.
- The data and diagram for the timed-addition of reagents into the differentiation media may assist the readers in readily using the *in vitro* system for inducing optic tissues from ESCs.
- A data of the ESC-derived Rax positive tissues from culture day 4 to day 24 may give better information of the ESC differentiation culture for researchers in the related fields.

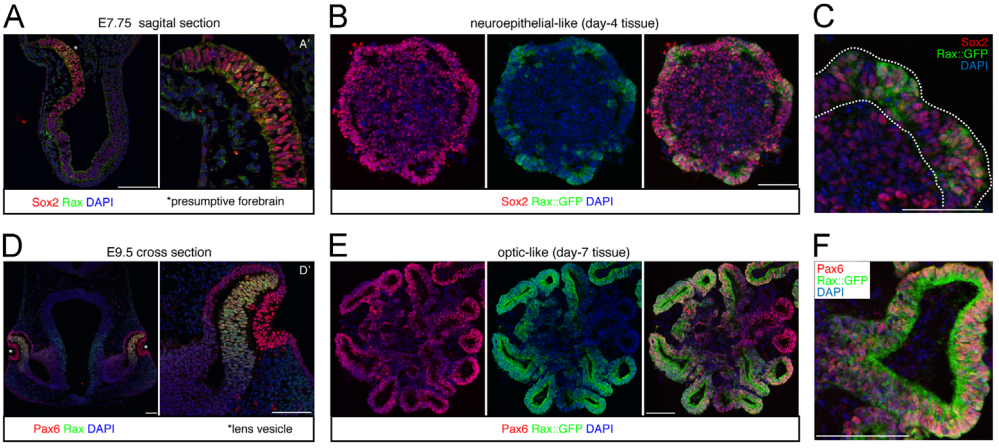

Fig. 1. Comparison between *in vivo* and *in vitro* marker gene expression in the neuroepithelium and optic tissues. (A, B, C) E7.75 embryo and day-4 ESC-derived tissues show Sox2, Rax and Rax::GFP signals via immunostaining. Image B and C are prior to CHIR addition. Image A′ is a high magnification of the forebrain region in Image A. Dotted lines in image C indicate epithelial-like structures. (D, E, F) E9.5 embryo and day-7 ESC-derived tissues in CDM/MG/CHIR condition show Pax6, Rax and Rax::GFP signals via immunostaining. Image D' is a high magnification of the eye region in image D. Scale bars; 100 μm.

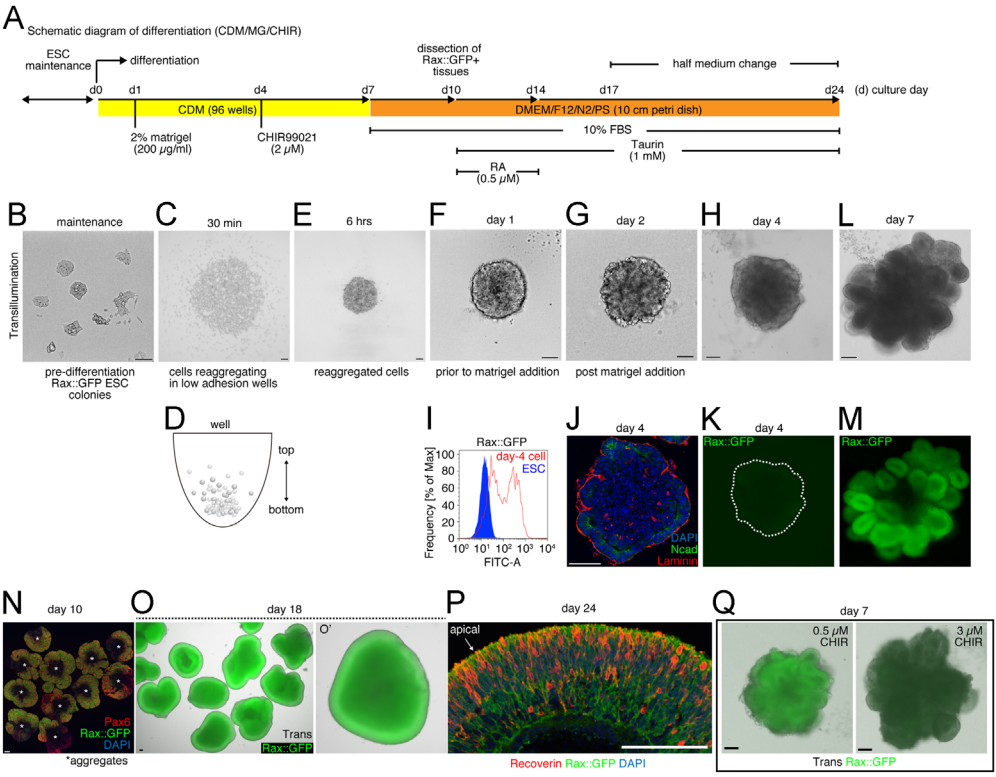

Fig. 2. Schematic diagrams and data for the generation of optic tissues from mouse ESCs *in vitro*. (A) A brief step by step instruction in the CDM/MG/CHIR condition. (B, C, E–H, K–M, O, O′, Q) Transillumination and fluorescent images of living cells and tissues. Trans, transillumination. Images B, C, E-G were acquired via EVOS microscope. (D) Schematic of the quick reaggregation of ESCs. (I) FACS analysis of ESC-derived day-4 cell. ESC serves as a negative control. (J, N, P). Immunostaining of ESC-derived tissues showing N-cadherin (Ncad), Laminin, Pax6, Rax::GFP and Recoverin. Day-4 data are prior to CHIR addition. CDM, chemically defined medium. MG, matrigel. CHIR, CHIR99021. PS, Penicillin-Streptomycin. RA, Retinoic acid. Scale bars; 100 μm.

1. Data

This data mainly focuses on describing the regional marker expression of neural tissues and the data of eye tissue-inducing culture (Figs. 1 and 2), and refers to our recently published [1]. We used mouse embryo and mouse ESC-derived tissue samples to analyze the regional marker expression via immunostaining and also showed the images of ESC-derived tissues in living condition. The data shown are microscopy images (Fluorescent and Bright-field), graphs (Population of GFP+cells) and schematic diagrams (Step-by-step processes).

2. Experimental design, materials and methods

2.1. Regional marker expressions of neuroepithelium in vivo and in vitro

Rax was expressed in the anterior neuroepithelium, which expressed Sox2 as well at embryonic day (E) 7.75 (Fig. 1A, A′) [2,3]. The optic tissues at E9.5 co-expressed Rax and Pax6 (Fig. 1D, D′) [4]. Using a Rax::GFP ESC line, Rax+ tissues were locally induced at culture day 4, at which point Sox2

was globally expressed in the neuroepithelial-like structure (Fig. 1B, C). Subsequently, by adding CHIR99021, Rax+/Pax6+ optic vesicle-like structures were formed at culture day 7, reminiscent of *in vivo* optic tissues (Fig. 1E, F).

2.2. Step-by-step data during generating Rax+ optic vesicle-like tissues in the CDM/MG/CHIR condition

From here we describe data for generating Rax+ optic vesicle-like tissues using mouse ESCs (Fig. 2A). ESCs were maintained in maintenance medium before differentiation [5](Fig. 2A, B). At day 0, we performed SFEBq (Serum-free Floating culture of Embryoid Body-like aggregates with quick reaggregation) [6]. ESCs were dissociated and quickly aggregated in the non-adhesion coated 96 wells (Fig. 2D). 30 min later, we could observe the beginning of aggregation and, 6 h later, cells had mostly aggregated (Fig. 2C, E). At day 1, 2% matrigel was directly added into the medium (Fig. 2F). At day 2, the surface portion of the day-2 aggregate was changed morphologically (Fig. 2G). By day 4, neurorpithelial-like structures expressing apico-basal polarity marker, N-cadherin (inside), and Laminin (outside) were observed (Fig. 2H, J). Simultaneously, Rax::GFP cells started to faintly appear in the epithelial-like structure (Fig. 2I, K). This time (day 4), 2 μM CHIIR99021 was added, which inhibited GSK-3ß. At day 7, Rax+ optic vesicle-like structures were generated (Fig. 2L, M). Subsequently, at day 7, we replaced CDM with DMEM/F12/N2/10% FBS (fetal bovine serum) medium to grow tissues for long-term culture (Fig. 2A). At day 10, we chopped Rax+ tissue portions which also expressed Pax6 in order to separate them from other tissues (Fig. 2N). From day 10, we precisely followed the method reported previously [5]. We confirmed that day-18 tissues mostly expressed Rax::GFP (Fig. 2O, O′) and day-24 tissues expressed Rax::GFP and Recoverin, which is expressed in the photoreceptor cells of the eyes *in vivo* [7,8] (Fig. 2P). The addition of 0.5 or 3 μM CHIR99021 from day 4 to day 7 did not efficiently: induce the formation of Rax+ vesicle-like structure (Fig. 2Q).

2.3. ESC culture and reagents

ES cell maintenance; Using ESC-qualified Fetal calf serum (FCS) and KnockOut Serum Replacement, a Rax::GFP knock-in ESC line was maintained as described previously [5]. ES cell differentiation; Based on SFEBq using Nunclon Sphera 96U Bottom Plate (Thermo Scientific, 174925) [6], the differentiation method was performed in the CDM/MG/CHIR condition for inducing Rax+ optic vesicle-like tissues. CDM was prepared as follows: IMDM, GlutaMAX™ Supplement (Thermo Fisher Scientific, 31980030)/ Ham's F-12 Nutrient Mix, GlutaMAX™ Supplement (Thermo Fisher Scientific, 31765035) 1:1, 1x Chemically Defined Lipid Concentrate (Thermo Fisher Scientific, 11905031), 450 μM 1-Thioglycerol (SIGMA-ALDRICH, M6145), 5 mg/ml Bovine Serum Albumin (SIGMA-ALDRICH, A3156) and 15 μg/ml apo-Transferrin bovine (SIGMA-ALDRICH, T1428). At day 0, ESC were dissociated into single cells and the number of cells was counted via trypan-blue staining (SIGMA-ALDRICH, T8154 for excluding dead cells) to seed 3000 cells per well in CDM. At day 1, 2% (vol/vol) of matrigel, growth factor reduced (BD, 354230) were added. The final protein concentration of matrigel is typically 200 μg/ml (from around 10 mg/ml protein concentration) and several matrigel lots are routinely tested using a previous eye inducing protocol [5]. At day 4, 2 μM CHIR99021 (STEMGENT, 04-0004) was added. Infrequently, ESC-derived tissue at day 4 does not expressed Rax::GFP (typically due to ESC-passage number or incorrect ESC maintenance state). In this case, the addition of CHIR99021 led to the formation of Rax negative tissues at day 7 (data not shown). At day 7, ESC-derived tissues were transferred into DMEM/F12/N2/Penicillin-Streptomycin (PS), containing 10% FBS (vol/vol). At day 10, from here, ESC-derived tissue were grown exactly the same as previously published [5], and its process is also briefly shown in Fig. 2A.

2.4. Immunostaining and acquiring images

Slc:ICR mouse embryos were purchased from Japan SLC, Inc. to serve as a positive control. Immunostaining of cryosectioned samples was performed as previously described [9]. Primary antibodies were used as follows: GFP (rat, 1/500, NACALAI, 04404-84), Sox2 (goat, 1/250, santa cruz, sc-17320), Rax (guinea pig, 1/1000, TaKaRa custum, MS8407-3), Pax6 (mouse, 1/500, R&D, MAB1260),

N-cadherin (mouse, 1/1000, BD, 610920), Laminin (rat, 1/1000, CHEMICON, MAB1905) and Recoverin (rabbit, 1/1000, chemicon, AB5585). DAPI was used for counter staining. Immunofluorescent images were taken by LSM710 or 780 (Zeiss). For the visualization of living ESCs and ESC-derived tissues, KEYENCE (KEYENCE) and EVOS (Thermo Fisher) microscopes were used. Acquired images were merged using Photoshop and ImageJ softwares.

2.5. Fluorescence-activated cell sorting (FACS) analysis

FACS analysis was performed as previously described [9].

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Transparency document. Supplementary material

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2016.05.070.

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