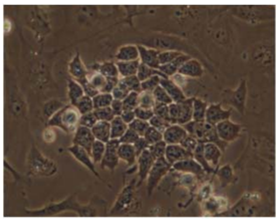
Einführung in die Stammzell- und Embryonenforschung II (ESF-II/9) WS2022/23

Zur Herstellen von Lebewesen aus einer Stammzelle

Biologische Grundlagen – Stand der Forschung – Gesellschaftliche Auswirkungen

Zentrale Frage:

Wie kann aus einer Stammzelle in autonomer Weise ein Säugetier-Embryo entstehen?



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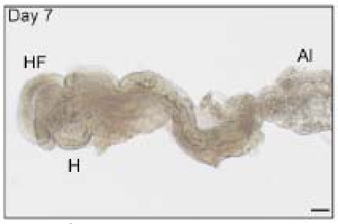
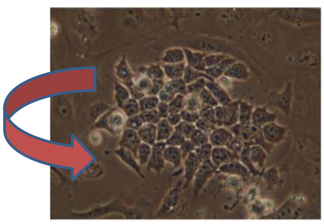
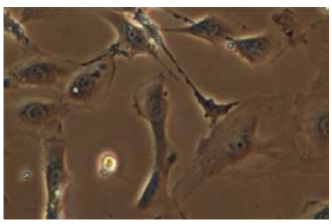


$5x\ 10^{-6}m$ → $8 \times 10^{-3}m$

https://doi.org/10.1016/j.stem.2022.08.013

1.1. Welche Eigenschaften haben Stammzellen und wie können daraus somatische Zellen entstehen?



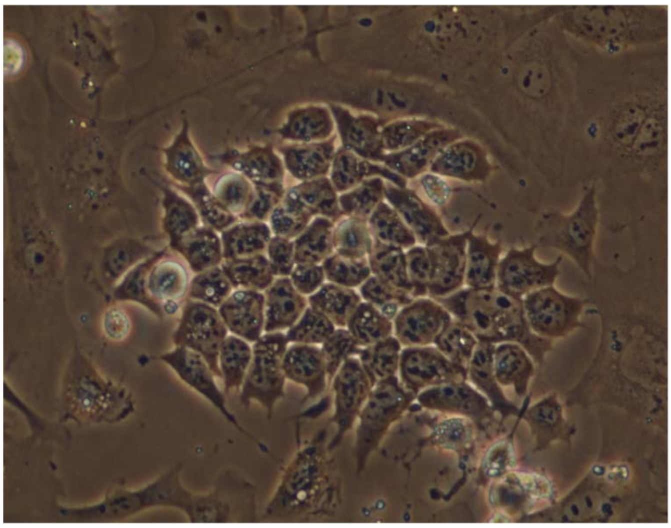


Teil 1 Biologische Grundlagen - Stammzellbiologie (1. bis 2. Doppelstunde)

1.1. Grundlagen der Stammzellbiologie

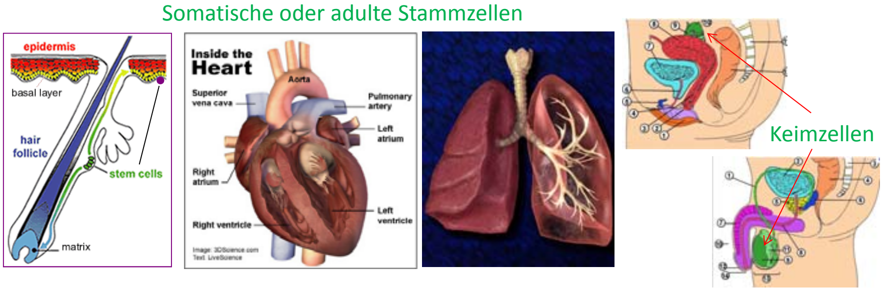
- 1.1.1. Was ist eine Stammzelle?
- 1.1.2. Welche Arten von pluripotenten Stammzelle gibt es?
- 1.1.3. Was ist eine adulte Stammzelle?
- 1.1.4. Entwicklung von somatischen Zellen in Stammzellaggregaten
  - Embryoid Bodies - Organoide - Autonome Morphogenese

1.1.1. Was ist eine Stammzelle?



Wo spielen somatische Stammzellen in unserem Körper eine Rolle?

Somatische oder adulte Stammzellen



Keimzellen

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1.1.4. Entwicklung von somatischen Zellen in Stammzellaggregaten Die in vitro Differenzierung von Stammzellen

- 1.1.4.1. Embryonale Stammzellen und iPSCs, scnt*-ESCs
  - Embryoid Bodies
- 1.1.4.2. Somatische Stammzellen
  - Herzstammzellen → Cardiac bodies / Cardiospheren
  - Hirnstammzellen → Neurospheren
  - Gezielte Transdifferenzierung durch Umprogrammieren von Fibroblasten etc. zu z.B. Kardiomyozyten-Vorläuferzellen
- 1.1.4.3. Organoide und Assembloide

* scnt…somatic cell nucelus transfer

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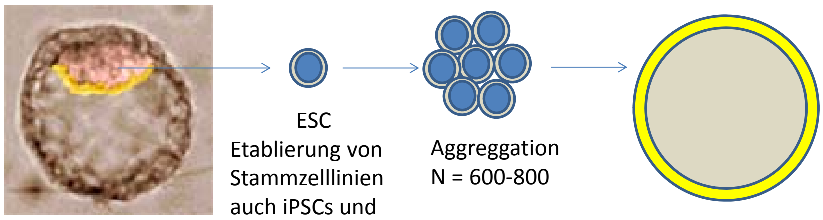
Herstellen von Embryoid Bodies und Organoiden

In vito

Durch Aggregation von wildtyp und genetisch veränderten embryonalen Stammzellen und spontaner autonomer aber regulierbarer Differenzierung dieser Zellen.



→ Erforschung der Funktionen der einzelnen Gene und des Entwicklungspotentials der verschiedenen Stammzellen wurde so möglich.

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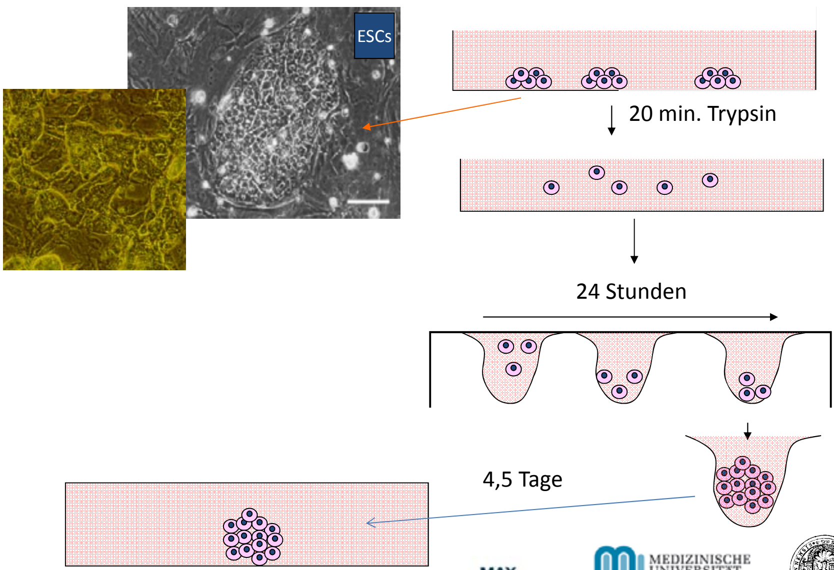
Herstellung von Embryoid Bodies

In vitro



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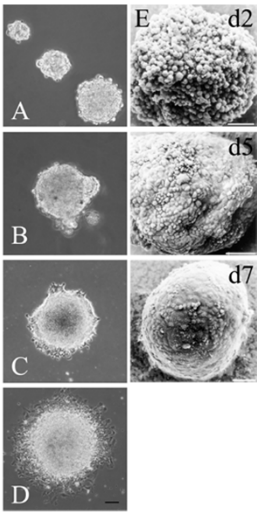
Embryoid Bodies

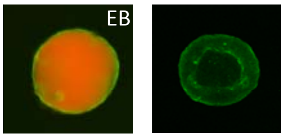
Tag 1-3

Tag 4,5

Tag 5.5

Tag 6.5



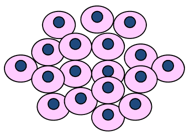


Quelle:CDvor2000

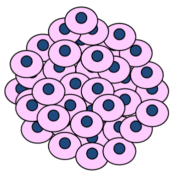
Anna Wobus, Gatersleben, D

Erfinderin der von ESC abstammenden Embryoid Bodies

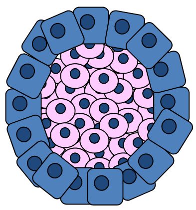
ESC Aggregat Tag 1



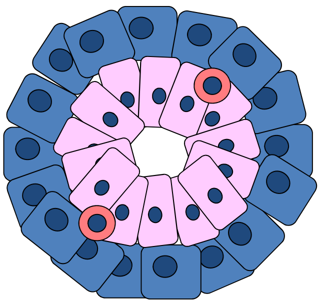
Kompaktierung Tag 1-2



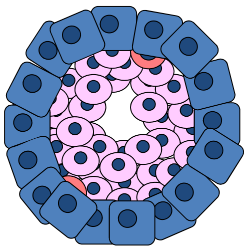
Embryoid Body Tag 3



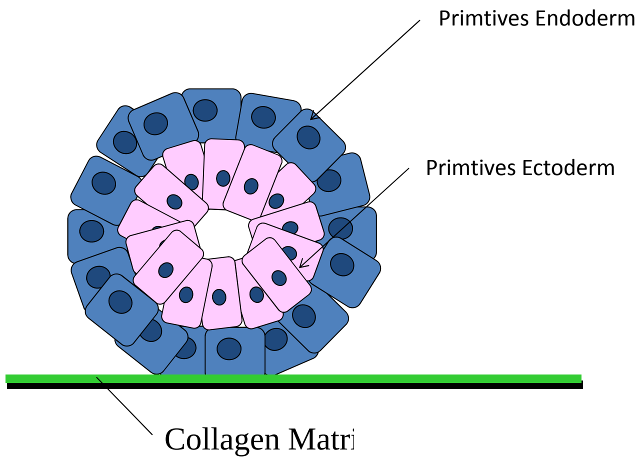
Embryoid Body Tag 4,5



Embryoid Body Tag 4



Pseudo-Implantation von Embryoid Bodies



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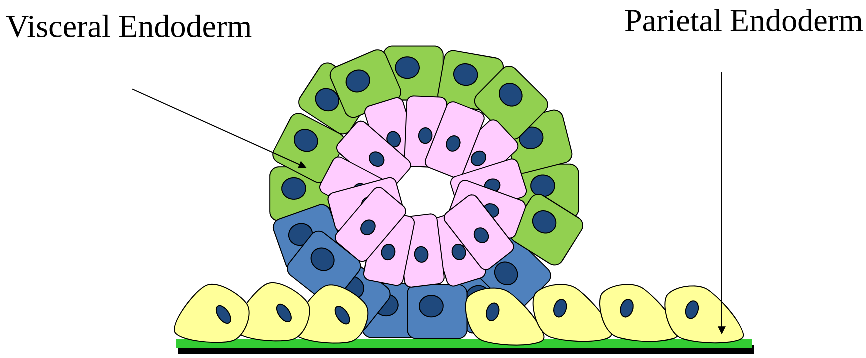

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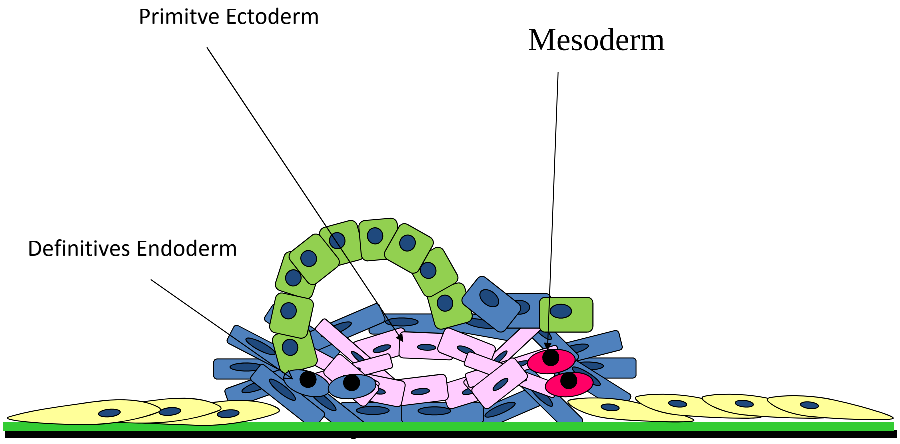
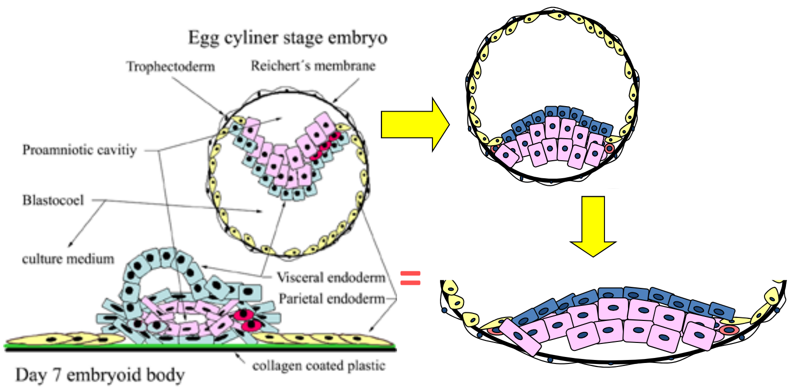
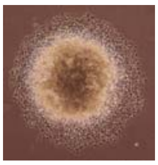
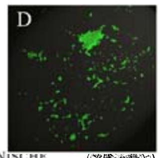






Ist die Entstehung der somatischen Zellen während der Gastrulation chaotisch?



1.1.4.3. Gerichtete in vitro Differenzierung von Stammzellen → Auf dem Weg zu Organoiden

Ohne Beeinflussung .... entstehen alle Zelltypen.

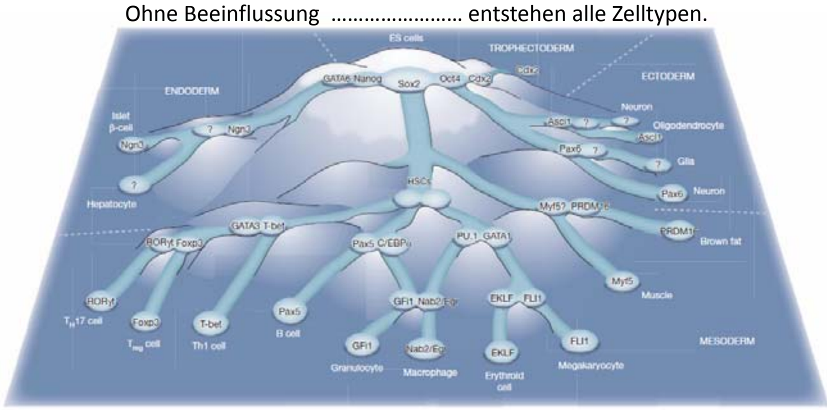


Figure 5 | Transcription factor cross-antagonisms in a cascading landscape of unstable and stable cell states. The territory, represented as a mountain range, depicts all possible solutions of a single regulatory network that specifies cell identity. Robust network states correspond to stably differentiated cell types (deep basins in the low-lying plains) whereas unstable solutions correspond to ridges and slopes in the landscape. The latter are only fleetingly occupied during development and thus unlikely to correspond to observable cell types. ES cells, embryonic stem cells; HSCs, haematopoietic stem cells.

Nach Konrad H. Waddington

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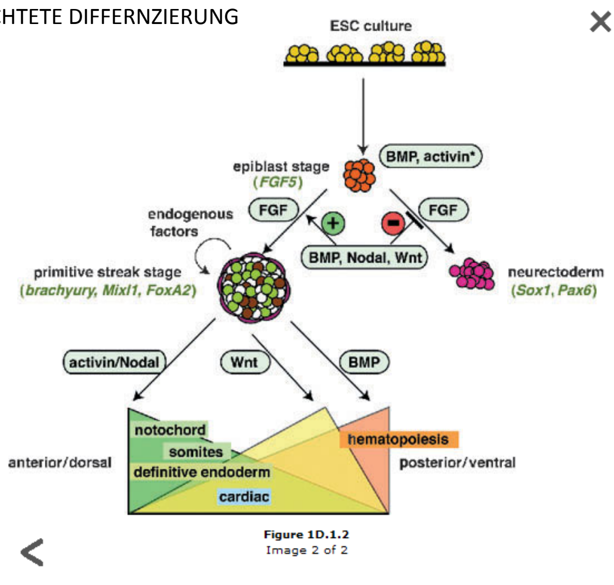
BEISPIELE FÜR GERICHTETE DIFFERNZIERUNG

1. Blutzellen



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Gezielte Differenzierung von ESCs in vitro

2. Herzmuskelzellen

Aus: Mapping the first stages of mesoderm commitment during differentiation of human embryonic stem cells
Denis Evseenkoa, Yuhua Zhua, Katja Schenke-Laylandb, Jeffrey Kuoa, Brooke Latoura, Shundi Gea, Jessica Scholesa,
Gautam Dravida, Xinmin Lia, W. Robb MacLellanb, and Gay M. Crooksa,1
13742–13747 | PNAS | August 3, 2010 | vol. 107 | no. 31

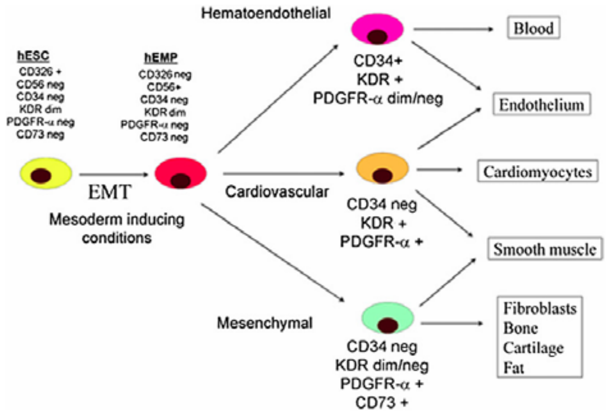

Fig. 4. Proposed model of cell surface marker expression during mesodermal specification from hESCs. The initial stage of mesoderm commitment is marked by the process of EMT during which the CD326−CD56+ population is generated. Subsequent commitment to mesoderm populations with more restricted potential is identified by day 7 of induction cultures by differential expression of the surface markers KDR, PDGFR-α, CD34, and CD73. The phenotype of precursors to the day 7 populations shown is yet to be delineated.

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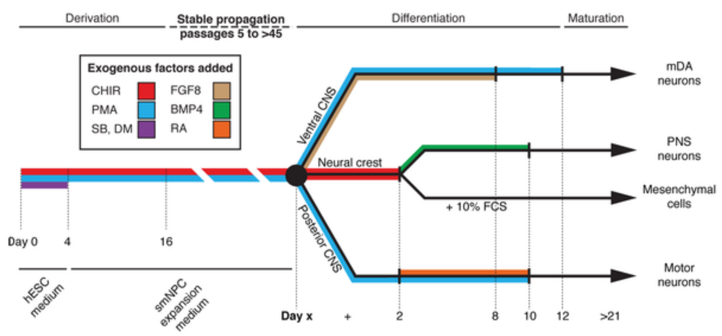
Gezielte Differenzierung von ESCs in vitro

3. Motorneuronen

Figure 8. Summary of smNPCs.

PLOS | ONE



Aus: Reinhardt P, Glatza M, Hemmer K, Tsytsyura Y, et al. (2013) Derivation and Expansion Using Only Small Molecules of **Human Neural Progenitors** for Neurodegenerative Disease Modeling. PLoS ONE 8(3): e59252. doi:10.1371/journal.pone.0059252
http://www.plosone.org/article/info:doi/10.1371/journal.pone.0059252

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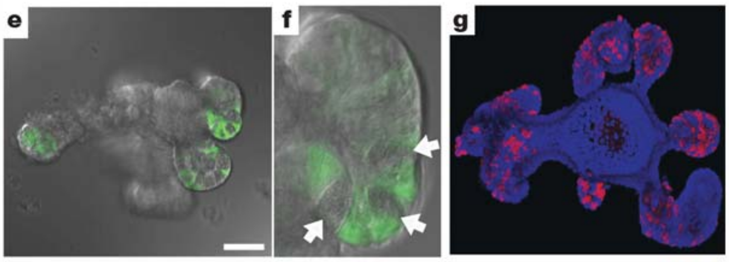
BEISPIELE FÜR Organoide in Suspensionskulturen

1. Mini Darm (Erstes Organoid) - Single Lgr5⁺ cells generate crypt–villus structures.

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We have recently demonstrated the presence of about six cycling Lgr5⁺ stem cells at the bottoms of small-intestinal crypts[1]. Here we describe the establishment of long-term culture conditions under which single crypts undergo multiple crypt fission events, while simultanously generating villus-like epithelial domains in which all differentiated cell types are present. Single sorted Lgr5⁺ stem cells can also initiate these crypt–villus organoids. T racing experiments indicate that the Lgr5⁺ stem-cell hierarchy is maintained in organoids. We conclude that intestinal crypt–villus units are self-organizing structures, which can be built from a single stem cell in the absence of a non-epithelial cellular niche.



e, **f**, Fourteen days after sorting, single GFP^{hi} cells form crypt organoids, with Lgr5–GFP⁺ cells and Paneth cells (white arrows) located at crypt bottoms. Scale bar, 50 m. **f**, Higher magnification of **e**. **g**, Organoids cultured with the thymidine analogue EdU (red) for 1 h. Note that only crypt domains incorporate EdU. Counterstain, 4,6-diamidino-2-phenylindole (DAPI; blue).

T Sato *et al. Nature* **000**, 1-4 (2009) doi:10.1038/nature07935 Hans Clevers Lab

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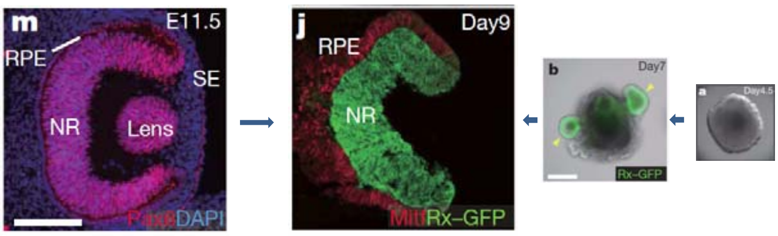
2. Augen

Self-organizing optic-cup morphogenesis in three-dimensional culture

Mototsugu Eiraku, Nozomu Takata, Hiroki Ishibashi, Masako Kawada, Eriko Sakakura, Satoru Okuda, Kiyotoshi Sekiguchi, Taiji Adachi & Yoshiki **Sasai**



Maus

Embryoid bodies

doi:10.1038/nature09941

7 APRIL **2011** | VOL 472 | NATURE | 51

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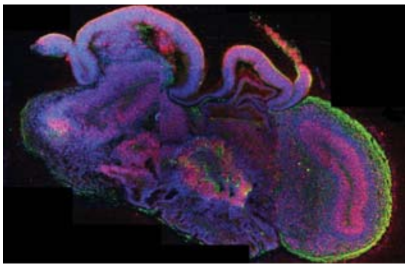
3. Hirn



Cerebral organoids model human brain development and microcephaly

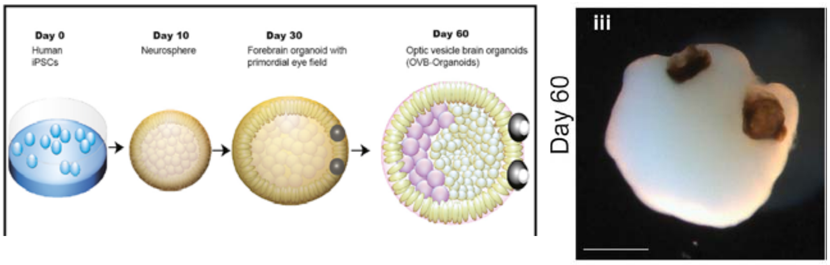
Madeline A. Lancaster,[1] Magdalena Renner,[1] Carol-Anne Martin,[2] Daniel Wenzel,[1] Louise S. Bicknell,[2] Matthew E. Hurles,[3] Tessa Homfray,[4] Josef M. Penninger,[1] Andrew P. Jackson[2] & Juergen A. Knoblich[1]

Nature Volume: 501, Pages: 373–379 Date published: (19 September 2013) DOI: doi:10.1038/nature12517
Published online 28 August **2013**

Cell Stem Cell

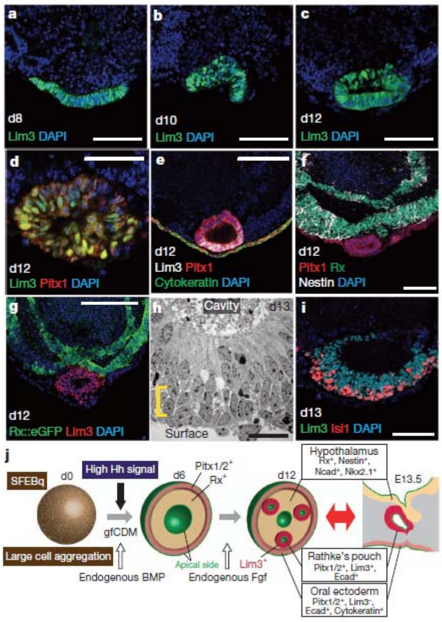
Human brain organoids assemble functionally integrated bilateral optic vesicles

Elke Gabriel,[1] Walid Albanna,[2,3] Giovanni Pasquini,[4] Anand Ramani,[1] Natasa Josipovic,[5,12] Aruljothi Mariappan,[1] Friedrich Schinzel,[1] Celeste M. Karch,[6] Guobin Bao,[7] Marco Gottardo,[1] Ata Alp Suren,[1] Jürgen Hescheler,[2] Kerstin Nagel-Wolfrum,[8] Veronica Persico,[9] Silvio O. Rizzoli,[7] Janine Altmüller,[10,12] Maria Giovanna Riparbelli,[9] Giuliano Callaini,[9] Olivier Goureau,[11] Argyris Papantonis,[5] Volker Busskamp,[4] Toni Schneider,[2] and Jay Gopalakrishnan[1,13,*]
[1]Institute of Human Genetics, University Hospital, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany



Cell Stem Cell **2021** Oct 7;28(10):1740-1757.e8. doi: 10.1016/j.stem.2021.07.010

4. Adenohypophyse



Self-formation of functional adenohypophysis in three-dimensional culture
Hidetaka Suga, Taisuke Kadoshima, Maki Minaguchi, Masatoshi Ohgushi, Mika Soen, Tokushige Nakano, Nozomu Takata, Takafumi Wataya, Keiko Muguruma, Hiroyuki Miyoshi, Shigenobu Yonemura, Yutaka Oiso & Yoshiki **Sasai**
Nature **volume 480**, pages 57–62 (01 December 2011)

Figure 2 **Spontaneous generation of Rathke's pouch-like vesicles in ES cell culture.** a–c, Morphogenesis of Lim31 epithelia. d–g, Immunostaining of day-12 pouch vesicles and surrounding tissues for Pitx1 (red, d–f), Lim3 (green,d; white, e; red, g), pancytokeratin (green, e), nestin (white, f) and Rx (green, f, g) in ES cell culture. h, Electron microscopy of the day-13 pouch. Delaminating cells on the basal side (bracket). i, Islet11 cells in the basal zone of the day-13 pouch. j, Schematic of in vitro generation of Rathke's pouches. Scale bars, 100 mm (a–c, e–g); 50 mm (d, i); 20 mm (h).

Self-formation of functional adenohypophysis in three-dimensional culture
Suga et al., **2 0 1 1** | VO L 4 8 0 | N AT U R E | 5 7 doi:10.1038/nature10637
The adenohypophysis (anterior pituitary) is a major centre for systemic hormones. At present, no efficient stem-cell culture for its generation is available, partly because of insufficient knowledge about how the pituitary primordium (Rathke's pouch) is induced in the embryonic head ectoderm. Here we report efficient self-formation of three-dimensional adenohypophysis tissues in an aggregate culture of mouse embryonic stem (ES) cells. ES cells were stimulated to differentiate into non-neural head ectoderm and hypothalamic neuroectoderm in adjacent layers within the aggregate, and treated with hedgehog signalling. Self-organization of Rathke's-pouch-like three-dimensional structures occurred at the interface of these two epithelia, as seen *in vivo*, and various endocrine cells including **corticotrophs** and **somatotrophs** were subsequently produced. The corticotrophs efficiently secreted adrenocorticotropic hormone in response to corticotrophin releasing hormone and, when grafted *in vivo*, these cells rescued the systemic glucocorticoid level in hypopituitary mice. Thus, functional anterior pituitary tissue self-forms in ES cell culture, recapitulating local tissue interactions.

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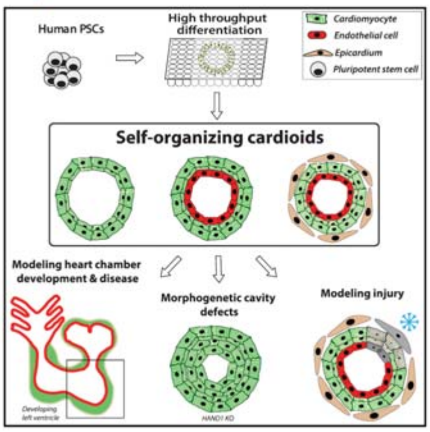
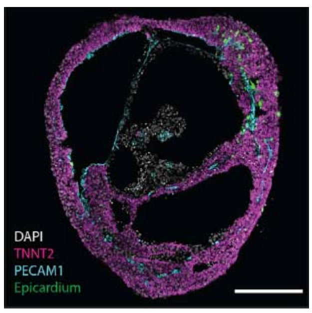
5. Herz-ähnliche Organoide - Cardioids reveal self-organizing principles of human cardiogenesis.

Hofbauer P, Jahnel SM, Papai N, Giesshammer M, Deyett A, Schmidt C, Penc M, Tavernini K, Grdseloff N, Meledeth C, Ginistrelli LC, Ctortecka C, Šalic Š, Novatchkova M, **Mendjan S**. Cell. 2021 Jun 10;184(12):3299-3317.e22. doi: 10.1016/j.cell.2021.04.034. Epub 2021 May 20. PMID: 34019794

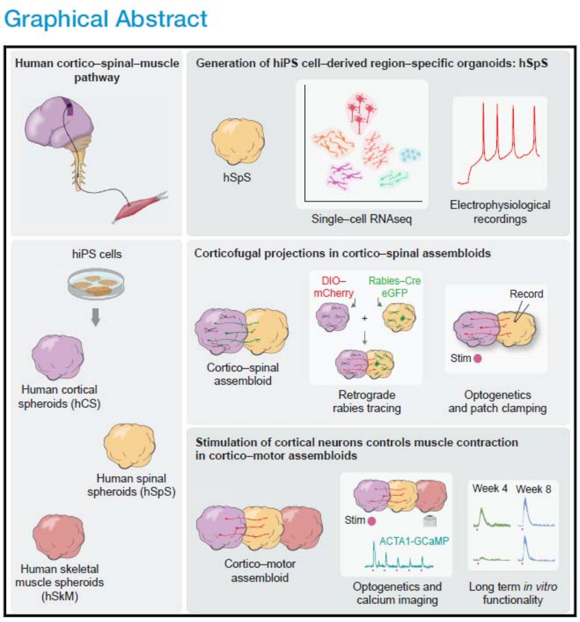
Graphical abstract



→ Eigentlich ein Assembloid



6. Assembloide

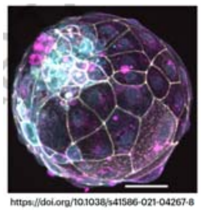


https://doi.org/10.1016/j.cell.2020.11.017

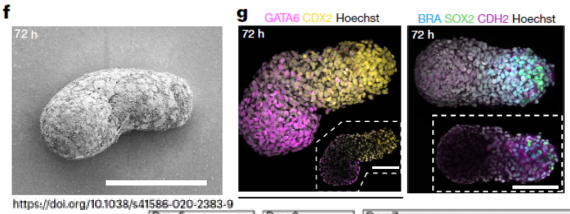
7. Blastoide

8. Gastruloide

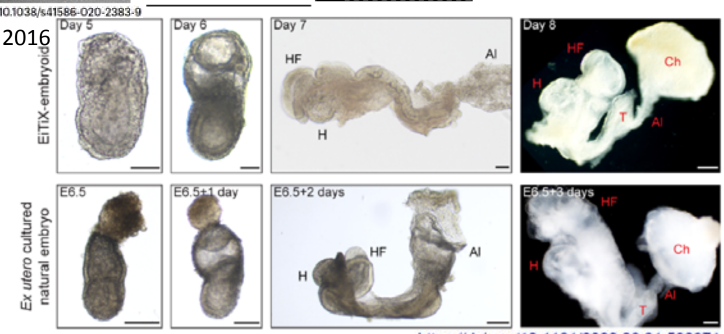
9. Embryoids: Synthetische Embryonen und EiTiX Embryoids



Rivron, 2018



Brivanlou, 2016



Zernicka-Götz und Hanna, August 2022

https://doi.org/10.1101/2022.08.01.502371

Pluripotent stem cells (PSCs)

In vitro Entwicklung zu somatischen Zellen	In vitro Entwicklung zu Keimzellen	In vitro Entwicklung zu Lebewesen
Embryoid bodies	Eier und Spermien	Ex vivo Blastoide Ex vivo Gastruloide Ex vivo Embryoids
Cardiac bodies	Zygotzen	
…	Blastozysten	Placenta Organoide
Organoide	Weitere Entwicklung in Leihmütter oder künstlichen Uteri	Komplettierung der Embryonalentwicklung & ex vivo Foetalentwicklung (Ausstehend)
Assembloide		
Gewebe- und Organ-Ersatztherapie	Lebewesen mit festgelegten Genotyp	Lebewesen aus einer Stammzelle

Teil 1 Biologische Grundlagen - Stammzellbiologie (1. bis 2. Doppelstunde)

1.1. Grundlagen der Stammzellbiologie

Antwort auf die Frage was eine Stammzelle ist und was für Eigenschaften sie hat:

Eine Stammzelle hat in geeigneter Umgebung das unbegrenzte Potenzial zur phänotypisch stabilen Selbsterneuerung, zum Ruhen, und zur Hervorbringung von somatischen Zellen.