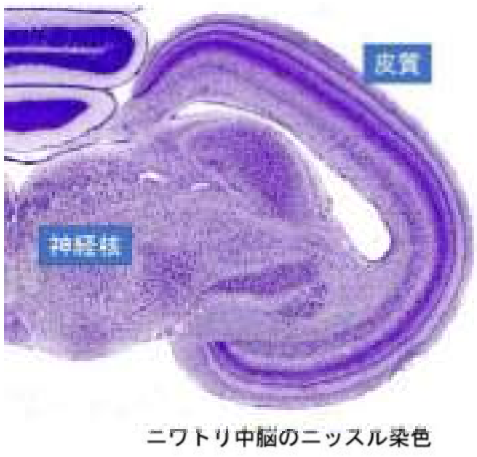
脳発生分野
Department of Brain Morphogenesis

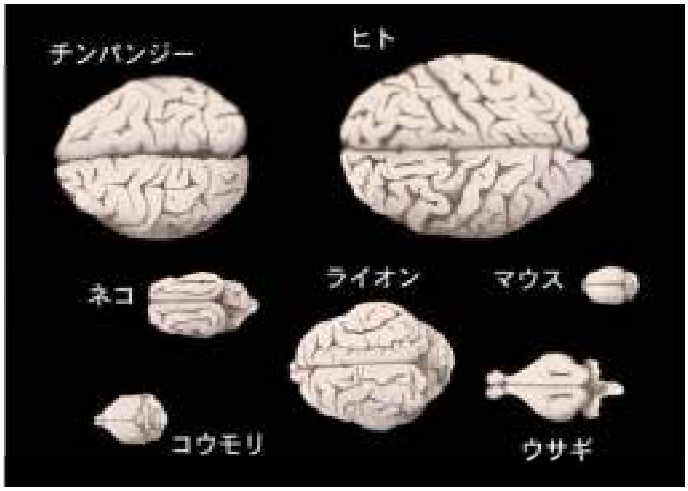
単純な上皮の管、または袋の形状をした脊椎動物の脳原基は、神経幹細胞が分裂によってその数を増やし、また多種多様なニューロンやグリアを産生し、様々な形態形成活動の結果複雑な脳が完成する。神経幹細胞は脳室帯と呼ばれる脳壁の内腔部で分裂し、産生されたニューロンはラジアルファイバーと呼ばれる神経幹細胞の突起に沿って、脳壁の辺縁部へと移動し蓄積する。これは、脳組織構築の基本原理であり、脳のほぼすべての領域、すべての脊椎動物で共通である。しかし、脳の領域によって組織形態は著しく異なっており、また種間できわめて多様化している。したがって、共通の基本原理からいかにしてこのような多様性を創出するかは、神経発生研究における重要な主題であり、当分野の中心命題である。当分野では、（１）脳の領域特異的な組織構築、（２）霊長類における脳の肥大化、（３）脳回・脳溝（脳のシワ）形成について集中的に研究を行い、脳の形態形成機構を明らかにしようとしている。得られる知見は、脳の正常発生、進化の理解のみならず、脳の形成異常、脳の高次機能・発達障害の病因、病態の解明につながるとともに、多能性幹細胞や神経幹細胞を用いた再生医療、疾患モデル、創薬等における細胞レベルから組織レベルの研究への発展に寄与する可能性がある。

Generation of distinct types of neurons which are allocated at the specific sites and construction of histologically divergent tissues, such as layers and nuclei, are fundamental processes during the brain development. Recent extensive studies have greatly advanced our understanding of brain development, establishing the basic principle of neurogenesis in which neural progenitors proliferate, self-renew, and produce neurons and glia to construct brain structure. Our laboratory has been focusing on 1) how regionally distinct brain structures within the same species are generated, and 2) how interspecific diversity in brain size, morphology, and structure is generated through this apparently common basic principle of neurogenesis. We are tackling these issues by means of molecular and cellular biology, experimental embryology, live imaging, and genetics. The expected outcomes may also contribute to understanding of human brain diseases and disorders as well as development of tissue level researches in regenerative medicine of brain.

図1. 領域特異的な組織構築（皮質、神経核）と、哺乳類における脳の多様性



ニワトリ中脳のニッスル染色



構成員　Staff (2018.3)

名前	職名	Name and Position
嶋村　健児	教授	Kenji Shimamura, Professor
畠山　淳	助教	Jun Hatakeyama, Assistant professor
佐藤　晴香	ポスドク（学振 PD）	Haruka Sato, Postdoctoral fellow (JSPS PD fellow)
サルカ　ピアリ	大学院生	Piyali Sarkar, Graduate Student
吉持　賛花	大学院生	Sanka Yoshimochi, Graduate student
楠浦　順子	技術補佐員	Junko Kusuura, Technical assistant, Lab manager

元在籍者　Staff in the past (2012.4～2018.3)

名前	Name	在籍期間	在籍時職名	転出先
松下　理香	Rika Matsushita	2013.4.1-2016.3.31	大学院生	島津製作所
武富　雄哉	Yuhya Taketomi	2013.4.1-2014.3.7	大学院生	所属変更
中野　貴仁	Takahito Nakano	2015.4.1-2015.8.31	大学院生	退学
工藤　三希子	Mikiko Kudo	2017.5.1-2017.11.30	実験補助員	大学院医学教育部
岡田　咲耶	Saya Okada	2017.5.1-2018.3.31	実験補助員	大学院自然科学教育部

研究概略　Projects

我々は、動物の形作りの基本原理を明らかにするために、脊椎動物胚の脳・神経組織を具体的な題材として研究を行っている。脳・神経系では、領域や部位によって異なる機能分担と形態的特徴が顕著であり、それが高次機能発揮のための構造的基盤となっている。このような脳の領域特異性は、発生初期に始まる一連の様々なパターン形成によって確立される。これまでの内外の研究で、中枢神経系の初期の領域化機構に関しては、大筋の理解、および基本概念の創出は既に一定の域に達し、位置情報を獲得した神経前駆細胞がいかに領域特異的な組織形態を生じるかが次なる重要な課題として残されている。脳の基本的な組織形態として、ニューロンが層状に配置する皮質と、ニューロンの集合塊が立体的に配置する神経核があり、脳の領域によって組織形態が異なっている。しかし、胚における中枢神経系の幹細胞である神経上皮細胞およびラジアルグリア細胞（以下、神経前駆細胞と称す）が増殖・自己複製し、ニューロン、グリア細胞を産生する原理は広く共通である。したがって、この共通原理のいかなるパラメーターの変化によって異なる組織形態がもたらされるかを解明し、神経前駆細胞の領域性を規定する因子との連携を明らかにすることが現在の目標である。

得られる知見は、発生生物学、神経生物学上の学術的価値に加え、再生医学において細胞レベルから組織レベルをめざす際の生物学的基盤となることが期待される。また、脳の形態、組織構造は、脊椎動物間できわめて高度に多様化している。領域による違いを生じるしくみと、種間の違いをもたらすしくみを比較検討しつつ、究極的には脊椎動物の脳の多様化・進化の一端を明らかにすることを目指している。

１．　新たな Notch シグナル調節機構の発見

Notch シグナルは隣接する細胞間の直接接触を介した細胞間相互作用であり、神経前駆細胞の分化調節においてもきわめて重要である。しかし、細長く伸びた偽重層上皮である神経前駆細胞のどの部位で相互作用しているか長らく不明であった。神経前駆細胞は動的な環境にあり、接着帯で強固に結合する脳室側（アピカル側）を除き、隣接する細胞との膜間距離は常に変動している。一般に、ニューロンは誕生後速やかに脳室帯から離脱するとされていたが、アピカル突起先端で前駆細胞と接着帯を一定時間保持することを見出し、この安定な接着を利用して Notch シグナルを活性化し、隣接する前駆細胞の分化を調節していることを明らかにした（Hatakeyama et al., 2014）。また、カドヘリン複合体による接着帯は、調節によって不安定化し、速やかな細胞の乖離をも担うダイナミックな接着機構である。幼若ニューロンのアピカル突起の保持時間を変化させる実験から、ニューロンの産生ペースの調節にこのアピカル突起の保持時間が重要であることを見出した。さらに、このペース調節は、大脳皮質形成において、各層の適切なプロポーションに重要であることがわかった。突起の保持時間、およびニューロン産生のペースは、脳の各領域によっても異なっており、このしくみが脳の領域特異的な組織構築の要因か検証を行っている。

これに関連し、韓国 KAIST の Kim 博士との共同研究で、網膜色素細胞と神経性網膜がアピカル面を介した Notch シグナルによって網膜幹細胞の分化調節を行っていることを明らかにした（Ha et al., 2017）。上皮細胞のアピカル面を介した細胞間相互作用についてはほとんど報告がなく、興味深い。

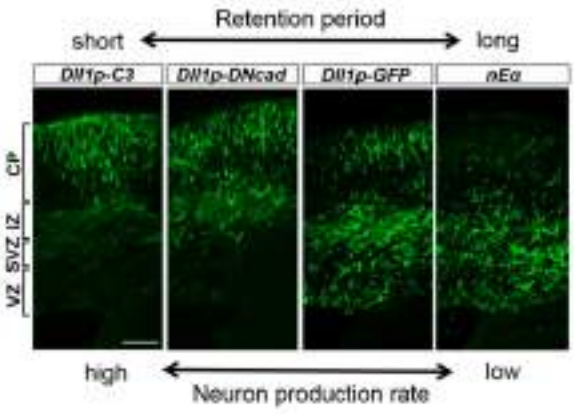


図２．アピカル突起の保持時間によるニューロン産生のペース調節

２．大脳皮質領野形成における視床線維の役割

哺乳類の大脳新皮質では、領野と呼ばれる領域性が形成される。それぞれの領野は、担う機能が異なるだけでなく、層の厚みが異なるなど構造的にも異なっており、大脳皮質内の形態的な不連続性として識別される。このような領野間の形態的、構造的差異をもたらすしくみについて、視床からの入力線維に注目して研究を行っている。これまでに、視床軸索が神経終末より分泌される因子（Sato et al., 2012)を介して、投射先の体性感覚野第４層ニューロンの量を調節していることを明らかにした。

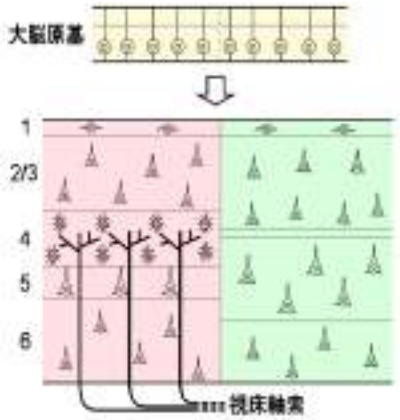


図３．視床軸索による領野特異的な層構造の制御

３．哺乳類における大脳の進化と多様化

我々ヒトを含む霊長類では、大脳、とりわけ大脳新皮質が著しく肥大化しており、認知、学習、行動、感情といった高次機能を支える基盤となっている。また、脳の形成異常は、自閉症、統合失調症といった精神疾患の原因となることが知られている。近年、主にマウスを用いた研究により、大脳皮質形成の基本原理について飛躍的に理解が進んだが、この基本原理は哺乳類で広く共通であり、この共通原理のいかなる変化によって、脳の多様性がもたらされるかは、次なる脳発生研究の大きな課題である。我々は、大脳の多様化に関し、次の２点に絞って研究を行っている。

１）霊長類における大脳皮質拡大のしくみ

この問題の現在の趨勢は、近年高等哺乳類で新たに発見された神経幹細胞に注目した研究であるが、我々は脈絡叢に注目して研究を行っている。脈絡層は脳脊髄液を産生する器官であり、霊長類など巨大な脳をもつ種では、マウスに比して脈絡叢が著しく発達している。我々は、脈絡叢の違いが脳脊髄液を介して大脳の肥大化をもたらしたという仮説を検証すべく、マウス、マーモセット、カニクイザルを用いて研究を行っている。これまでに、サル胚脈絡叢特異的に発現し、神経幹細胞の増殖を促進する因子を同定した。

２）脳のシワ（脳回・脳溝）の形成機構

マウス等の例外を除き、ほぼすべての哺乳類が大脳皮質にシワをもっており、そのパターンは種間で極めて多様化している。また、脳のシワは大脳拡大、高度な知能の獲得と密接に関係があると考えられており、その形成機構について関心が高まっている。我々は、シワの大部分がニューロン産生後に形成されることに着目し、脳形成後期のイベント（グリア産生、神経回路発達など）に注目して研究を行っている。さらに、独自の実験系として、極めて単純なシワをもつが、これまで脳発生の研究になじみのなかったモルモットを用いた実験系を確立した（Hatakeyama, Sato et al., 2017)。げっ歯類は分類上霊長類と同じグループに入り、また、モルモットに見られる脳回・脳溝は哺乳類の祖先型と類似している可能性があり進化的観点からも興味深いと考えている。

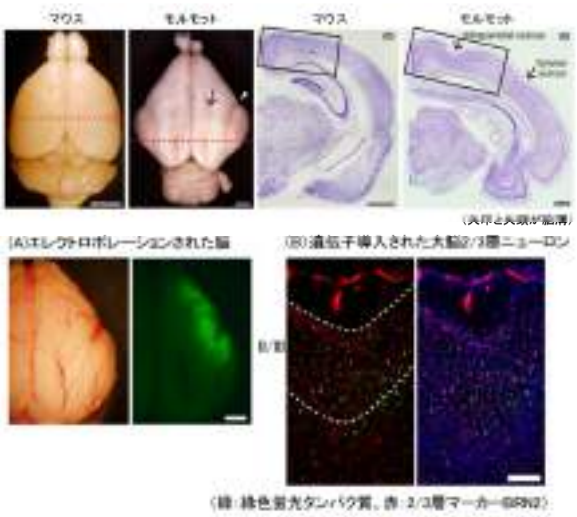


図４．モルモット脳に見られる脳回・脳溝とエレクトロポレーションによる遺伝子導入

４．脈絡叢形成における FGF シグナルの役割
脳脊髄液の産生器官である脈絡叢の発生に関し、細胞分化については知見が多いものの、その特徴的な形態形成のしくみについては不明である。我々は、脊髄において FGF シグナルが領域性を変化させることなく、脈絡叢原基に酷似した形態変化を蓋板にもたらすことを見出した。さらに、脈絡層が形成される後脳領域で FGF シグナルを阻害すると、劇的に委縮した脈絡叢が形成された。また、FGF シグナルは脈絡叢特異的な遺伝子発現は誘導しなかったことから、脈絡叢形成において、FGF シグナルが初期の形態形成を制御し、脈絡叢特異的な細胞形質の獲得は既に報告のある BMP シグナルによって制御されると考えられた。

Our ultimate goal in science is to understand how the animals shape their bodies both externally and internally during development, a process called morhphogenesis. We have been studying morphogenesis of the embryonic brain as a model system. In particular, we focus on the regional diversity explicitly exhibited in both developing and mature brain. Numerous studies, including ours, have established a principle scenario or outline for how the embryonic brain is patterned and each brain primordium acquires its regional identity during the early phase of development. However, it still remains to be understood how the neural progenitors at different locations generate distinct cytoachitecture and histological organization according to their assigned regional identities. Yet, the basic principle of neural histogenesis is apparently common throughout the brain; the neural progenitors undergo extensive cell division to produce themselves and constituents of the CNS, neurons and glia; neurons produced at the inner side of the ventricular wall then migrate outwards radially to populate marginal regions of the brain vesicles; in some cases they also migrate tangentially. Our current working hypothesis is that temporal and quantitative differences in kinetics of the basic principle would eventually lead to the distinct structures such as cortices and nuclei. These structural differences among the different regions within a single species are also found among different vertebrate species. We would like to know whether the same mechanisms operate to generate species' difference of the brain structures to gain an insight into how the vertebrates evolve their brain. We believe that our research outcomes will also help development of various technologies in regenerative medicine.

1. Novel mechanisms for the regulation of Notch signaling.

It is generally accepted that nascent neurons born at the luminal surface of the brain vesicles lose their anchoring at the ventricular lining to migrate basally. We found however that nascent neurons retain the apical engagement by maintaining the adherens junction for a limited period of time. We further revealed that these neuronal apical endfeet serve as a plug for neurogenesis through Notch signaling so that the retention period of the apical endfeet regulated by dynamics of the adherens junction is reflected by the pace of neuron production (Hatakeyama et al., 2014). We are currently investigating a role of differential pace control of neuron production in the regionally distinct histogenesis of the brain. Regarding another cellular mechanism for Notch signaling, we demonstrated in collaboration with Prof. Jin W. Kim at KAIST (Korea) that apical-to-apical interaction between the pigment retina and neural retina mediates Notch signaling to regulate retinal progenitor differentiation(Ha et al., 2017).

2. Role of the thalamic afferents in the areal differentiation of the neocortex.

The mammalian neocortex is organized into functionally and histologically distinct subregions called cortical areas. It has been reported that intrinsic mechanisms within the cortical primordium initially pattern the spatial organization of the neocortical areas. However, it was recently shown that secreted factors emanating from the thalamic afferents that innervate the specific areas could influence some cytoarchitectonic features of the neocortex in vitro (Sato et al., 2012). Since then, we have been investigating roles of the thalamic afferents in the development of the cortical areas in conjunction with the potential plasticity of the developing neocortices. We obtained evidence in vivo that thalamic afferents regulate the number of layer 4 neurons in the somatosensory area via secreted factors emanating from their nerve terminals.

3. Evolution and diversity of mammalian cerebral cortices

Extremely huge and extensively folded brain is a hallmark of human brain, on which higher cognitive, behavioral, emotional, and mental functions, as well as our intelligence are based. Moreover,

abnormalities in its construction often lead to mental diseases or disorder, such as autism and schizophrenia. As recent intensive studies on the development of cerebral cortex in mice have greatly advanced our understanding of brain development, next obvious question is how enormous diversity in brain morphology and structure among mammalian species arises through the apparently common principle. We have been focusing on two issues here: 1) Expansion of cerebral cortex in primates, and 2) Mechanism underlying the cortical folding. We have been studying a role of choroid plexus in expansion of the cerebral cortex in primates, and identified several secreting factors that expressed preferentially in monkey embryonic choroid plexus that promote proliferation of neural stem cells. We have also been studying how brain folds are formed, which is missing in mice and rats. We have been focusing on post-neurogenic developmental events unlike others based on the observation that the brain folds emerge after all the neurons are produced. Moreover we have established guinea pigs, which belong to rodents as mice but have very simply folded brain, as a new experimental model to study cortical folding or gyrification(Hatakeyama, Sato et al., 2017).

4. A novel role of FGF signaling in the choroid plexus formation

While the molecular cascade that leads to cellular differentiation of the choroid plexus, an organ that produces cerebrospinal fluid, has been studied, its morphogenetic aspects remain elusive. We found that activation of FGF signaling in the spinal cord resulted in broadly expanded roof plate, a feature characteristic to the choroid plexus rudiment of the third ventricle. Conversely, inhibition of FGF signaling in the hindbrain led to marked reduction in size of the choroid plexus. However, FGF signaling failed to induce the choroid plexus-specific gene expression (e.g., TTR), suggesting that early morphogenetic aspects of choroid plexus development are regulated by FGF signaling and its later differentiation aspects are regulated by other signaling(s), such as BMP signaling.

論文目録　Publications

1. Ha T, Moon KH, Dai L, Hatakeyama J, Yoon K, Park HS, Kong YY, Shimamura K, Kim JW. The retinal pigment epithelium is a Notch signaling niche in the mouse retina. ***Cell Rep*** 19: 351-363, 2017.

2. Hatakeyama J, Sato H, Shimamura K. Developing guinea pig brain as a model for cortical folding. ***Dev Growth Dffier*** 59: 286-301, 2017.

3. Abe S, Abe K, Zhang J, Harada T, Mizumoto G, Oshikawa H, Akiyama H, Shimamura K. Roles of CD34+ cells and ALK5 signaling in the reconstitution of seminiferous tuble-like structures in 3-D- re-aggregate culture of dissociated cells from mouse testes. ***PLoS One*** 12(11): e0188705, 2017.

4. Kameyama H, Kudoh S, Hatakeyama J, Matsuo A, Ito T. Significance of Stat3 signaling in epithelial cell differentiation of fetal mouse lungs. ***Acta Histochemica et Cytochemica*** 50: 1-9, 2017.

5. Alchini R, Sato H, Matsumoto N, Shimogori T, Sugo N, Yamamoto N. Nucleocytoplasmic shuttling of histone deacetylase 9 controls activity dependent thalamocortical axon branching. ***Sci Rep*** 7: 6024, 2017.

6. Zhang J, Hatakeyama J, Eto K and Abe SI. Reconstruction of a seminiferous tuble-like structure in a 3 dimensional culture system of re-aggregated mouse neonatal testicular cells within a collagen matrix. ***Gen Comp Endocrinol***, 205, 121-132, 2014

7. Ono K, Clayairoly A, Nomura T, Gotoh H, Uno A, Armant O, Takebayashi H, Zhang Q, Shimamura K, Itohara S, Parras CM Ikenaka K. Development of the prethalamus is crucial for thalamocortical projection formation and is regulated by Olig2. ***Development*** 141: 2075-2084, 2014

8. Hatakeyama J, Wakamatsu Y, Nagafuchi A, Kageyama R, Shigemoto R, Shimamura K. Cadherin-based adhesions in the apical endfoot are required for active Notch signaling to contorol neurogenesis in vertebrates. ***Development*** 141: 1671-1682, 2014.

9. Nagahara N, Nagano M, Ito T, Akimoto T, Shimamura K, Suzuki H. Antioxidant enzyme, 3-mercaptopyruvate sulfurtransferase-knockout mice exhibit increased anxiety-like behaviors: a model for human mercaptolactate-cysteine disulfiduria. ***Sci Rep*** 3: 1986, 2013.

10. Guiu J, Shimizu R, D'Altri T, Fraser ST, Hatakeyama J, Bresnick EH, Kageyama R, Dzierzak E, Yamamoto M, Espinosa L, Bigas A. Hes repressors are essential regulators of hematopoietic stem cell development downstream of Notch signaling ***J Exp Med***: 210: 71-84, 2013.

11. Sato H, Fukutani Y, Goto Y, Tatara E, Takemoto M, Shimamura K, Yamamoto N. Thalamus-derived molecules promote survival and dendritic growth of developing cortical neurons ***J Neurosci***. 44:15388-15402, 2012

著書・総説目録　Publications

1. Hatakeyama J, Shimamura K. Electroporation for the Chick Embryonic CNS ***Neuromethods*** 102: 115-125, 2015.

2. ギルバート発生生物学　Scott Gilbert 著, 日本語版, 第 10 章「外胚葉の出現：中枢神経系と表皮」翻訳 2015.

学会・研究集会 発表目録　Meeting Presentations

1. Hatakeyama J. How do humans have expanded cerebral cortex? The 3rd international symposium on stem cell traits and developmental systems, 11-12 January, 2018, Kumamoto.

2. Shimamura K, Hatakeyama J, Sato H, Yamamoto N. Development of mammalian cerebral cortex. The 6th Shandong University-Kumamoto University Medical Symposium, 14-16 December, 2017, Jinnan, China.

3. Hatakeyama J, Shimamura K. Extrinsic factors and the cortical expansion in primates. 第 40 回日本神経科学会, 5 月 10-13 日, 2017, 千葉.

4. Sato H, Hatakeyama J, Iwasato T, Yamamoto N, Shimamura K. The role of thalamic afferents in the formation of the area-specific laminar configuration in the developing neocortex. 第 40 回日本神経科学会, 5 月 10-13 日, 2017, 千葉.

5. Hatakeyama J, Sato H, Matsushita R, Tsuchiya H, Saitou M, Kageyama R, Shimamura K. Roles of extrinsic factors in the cortical expansion in primates. 第 50 回日本発生生物学会, 7 月 20-23 日, 2017, 東京

6. Shimamura K, Hatakeyama J, Sato H, Matsushita R, Tsuchiya H, Sasaki K, Saitou M, Isomura H, Shimojo H, Kageyama R. A role for the choroid plexus in the cortical expansion in primates. CDB Symposium 2017, 27-29 March, 2017, Kobe.

7. Shimamura K. A mechanism for the cerebral cortical expansion in primates. 東京医科歯科大難治研・熊大ジョイントシンポジウム, 2 月 24 日, 2017, 熊本.

8. 畠山淳、佐藤晴香、松下理香、影山龍一郎、斎藤通紀、土屋英明、嶋村健児. 霊長類の脳はいかにして大きくなったか？第 18 回日本進化学会大会, 8 月 25-28 日, 2016, 東京.

9. 畠山淳、松下理香、佐藤晴香、影山龍一郎、斎藤通紀、嶋村健児. 霊長類の脳はなぜ大きくなったのか？第 121 回日本解剖学会総会, 3 月 28-30 日, 2016, 郡山.

10. Hatakeyama J, Shimamura K. Novel roles of Fgf8 which is expressed in ventral region of the developing chick hindbrain. 第 48 回日本発生生物学会, 6 月 2-5 日, 2015, 筑波.

11. Shimamura K, Hatakeyama J. Pace control of neurogenesis regulated by transient retention of the apical endfoot of differentiating cells via Notch signaling. CDB Symposium 2015, 23-25 March, 2015, Kobe.

12. Hatakeyama J. 発生期の新生ニューロンによる Notch シグナルを介した「ニューロン産生ペース」の制御機構, 包括脳ネットワークシンポジウム. 12 月 11-13 日, 2014, 東京.

13. 畠山淳. 発生期の新生ニューロンによる Notch シグナルを介した「ニューロン産生ペース」の制御機構、遺伝研研究会「哺乳類脳の機能的神経回路の構築メカニズム」, 12 月 1-2 日, 2014, 三島,

14. Hatakeyama J, Shimamura K. A mechanism that controls “the pace of neurogenesis” through Notch signaling in the developing cerebral cortex. 第 37 回日本神経科学会大会, 9 月 9-11 日, 2014, 横浜.

15. Shimamura K, Hatakeyama J, Sato-Takemoto H. Pace control of neurogenesis regulated by transient retention of the apical endfoot of differentiating cells via Notch signaling. Cortical development, 23-25 May, 2014, Crete, Greece.

16. 畠山淳「脳の形作り」の解明を目指して、動物・植物・生態学会三学会合同熊本例会, 11 月 16 日, 2013, 熊本.

17. Shimamura K. Kinetic regulation of neurogenesis by transient retension of the apical endfoot of neuron via Notch signaling. International mini-Symposium Evo-Devo-Eco, 2 月 21 日, 2013, 京都.

18. Hatakeyama J, Lumsden A, Kageyama R, Shimamura K. A novel boundary in the developing central nervous system, Exciting Biologies, 17-19 October, 2013, Savudrija,

Croatia.

19. Sato H, Yamamoto N, Shimamura K. The role of thalamic afferent-derived extrinsic factors in cortical development. Neurogenesis 2013 in Matsushima, 10 月 16-18 日、2013, 宮城県松島.

20. 畠山淳「脳の形作り」の解明を目指して、熊本シンポジウム 2013, 6 月 25-26 日, 2013、熊本.

21. Hatakeyama J, Wakamatsu Y, Shigemoto R, Nagafuchi A, Shimamura K. The apical endfoot of nascent neuron controls the pace of neurogenesis through the regulation of Notch signaling. Neuro2013, 20-23 June, 2013, Kyoto.

22. Hatakeyama J, Wakamatsu Y, Shigemoto R, Nagafuchi A, Shimamura K. The apical endfoot of nascent neuron controls the pace of neurogenesis through the regulation of Notch signaling. CDB symposium 2013, 4-6 March, 2013, Kobe.

23. Hatakeyama J, Shimamura K. Kinetic regulation of neurogenesis by transient retention of the apical endfoot of neuron via Notch signaling. Notch 研究会, 2 月 15 日, 2013, 三島.

24. Sato-Takemoto H, Hatakeyama J, Yamamoto N, Shimamura K. Afferent-dependent formation of the cytoarchtectonic features in mouse neocortical areas. 第 35 回日本神経科学会, 9 月 18-21 日, 2012, 名古屋.

25. Ohta K, Shinmyo Y, Kaneko N, Hirata Y, Hatakeyama J, Yamaguchi M, Shimamura K, Sawamoto K, Tanaka H, Ito A. Tsukushi maintains the growth and undifferentiated properties of neuronal stem/progenitor cells as a niche molecule. 第 35 回日本神経科学会, 9 月 18-21 日, 2012, 名古屋.

26. Hatakeyama J, Wakamatsu Y, Shigemoto R, Shimamura K. Regulation of Notch signaling through the adherens junction in neurogenesis and somitogenesis。第 64 回日本細胞生物学会・第 45 回日本発生生物学会合同年会, 5 月 28-30 日, 2012, 神戸.

アウトリーチ活動　Outreach activity

1. 畠山淳　熊本県立天草高等学校スーパーサイエンスハイスクール　分子生物学実習、8 月 28 日、2017、熊本.

2. 畠山淳　熊本県立人吉高校　学問ガイダンス“開講　人吉大学”、7 月 11 日、2014、熊本.

Cell Reports
Article

OPEN ACCESS
CellPress

The Retinal Pigment Epithelium Is a Notch Signaling Niche in the Mouse Retina

Taejeong Ha,[1] Kyeong Hwan Moon,[1] Le Dai,[1] Jun Hatakeyama,[2] Keejung Yoon,[3] Hee-Sae Park,[4] Young-Yoon Kong,[5] Kenji Shimamura,[2] and Jin Woo Kim[1,6,*]
[1]Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, South Korea
[2]Department of Brain Morphogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan
[3]School of Life Science and Biotechnology, Sungkyunkwan University, Suwon, Gyeonggi-do 16419, South Korea
[4]Hormone Research Center, School of Biological Sciences and Technology, Chonnam National University, Gwangju 61186, South Korea
[5]Department of Biological Sciences, Seoul National University, Seoul 08826, South Korea
[6]Lead Contact
*Correspondence: jinwookim@kaist.ac.kr
http://dx.doi.org/10.1016/j.celrep.2017.03.040

SUMMARY

Notch signaling in neural progenitor cell is triggered by ligands expressed in adjacent cells. To identify the sources of active Notch ligands in the mouse retina, we negatively regulated Notch ligand activity in various neighbors of retinal progenitor cells (RPCs) by eliminating mindbomb E3 ubiquitin protein ligase 1 (Mib1). Mib1-deficient retinal cells failed to induce Notch activation in intra-lineage RPCs, which prematurely differentiated into neurons; however, *Mib1* in post-mitotic retinal ganglion cells was not important. Interestingly, Mib1 in the retinal pigment epithelium (RPE) also contributed to Notch activation in adjacent RPCs by supporting the localization of active Notch ligands at RPE-RPC contacts. Combining this RPE-driven Notch signaling and intra-retinal Notch signaling, we propose a model in which one RPC daughter receives extra Notch signals from the RPE to become an RPC, whereas its sister cell receives only a subthreshold level of intra-retinal Notch signal and differentiates into a neuron.

INTRODUCTION

Neural progenitor cells (NPCs) not only produce a variety of neurons but also renew themselves, providing a mechanism for coupling neurogenesis with tissue growth. An NPC can produce two NPCs or two neurons after a division. Alternatively, an NPC can produce a neuron and an NPC through asymmetric cell division and is able to preserve the NPC population during neurogenesis. Cell-contact-dependent Notch signaling is known to play a key role in maintaining NPCs during development and in adulthood (Gaiano and Fishell, 2002; Louvi and Artavanis-Tsakonas, 2006). Moreover, this signaling can differentiate the fates of the two NPC daughter cells upon the asymmetric activation of Notch in these cells.

In *Drosophila*, the unequal segregation of the Notch signaling regulator Numb differentiates the fates of two sensory precursor (SOP) daughter cells (Frise et al., 1996; Guo et al., 1996). One daughter cell contains Numb, which prevents Notch signaling by triggering the endocytic downregulation of Notch (Berdnik et al., 2002; Santolini et al., 2000), and becomes a secondary precursor pIIb cell before differentiating into a neuron, whereas the other lacks Numb and carries active Notch to become a pIIa cell and then later shaft and socket cells. Similar asymmetric inheritance of Numb by neuroblast daughter cells also differentiates the fate of Numb-expressing ganglion mother cells (GMCs), which divide a few more times to produce neurons at their final division from a Numb-negative type-1 neuroblast (Karcavich and Doe, 2005; Shen et al., 2002; Skeath and Doe, 1998). In opposing to Numb inheritance to future neurons, a different set of proteins, including Inscuteable (Ins), is accumulated in neuroblast by means of Par3/6 complex at the apical side, which contacts to epidermis (Betschinger et al., 2003; Schober et al., 1999). Therefore, both the intrinsic asymmetry of Notch activity in two neuroblast daughters and positional cues provided by the epidermis appear to be critical for polarizing the intracellular distribution of Notch regulators and thus ensuring asymmetric Notch activation.

However, Numb is less critical for asymmetric Notch activation in vertebrate NPCs. Mouse homologs of Numb are equally segregated into mitotic NPCs (Cayouette and Raff, 2002; Huttner and Kosodo, 2005; Kechad et al., 2012). Moreover, the fates of NPCs in developing mouse retinas lacking both *Numb* and *Numb-like* (*Nbl*) are grossly indistinguishable from those in wild-type mice, except that more photoreceptors are produced at the final division of NPCs in the retina (Kechad et al., 2012). In the embryonic zebrafish forebrain, Numb is also dispensable for NPC maintenance, although Notch activity is still inherited asymmetrically by two NPC daughter cells (Dong et al., 2012). The daughter cell with higher Notch activity remains an NPC, whereas the other, which has higher-level expression of Notch ligands and the ubiquitin E3-ligase Mindbomb (Mib), exits the cell cycle and differentiates into a neuron. Mib not only increases the amount of active Notch ligands in the plasma membrane that contacts the neighboring cell membrane harboring Notch, it also

Development, Growth & Differentiation
The Japanese Society of Developmental Biologists
Develop. Growth Differ. (2017) **59**, 286–301
doi: 10.1111/dgd.12371

Original Article

Developing guinea pig brain as a model for cortical folding

Jun Hatakeyama,[†] Haruka Sato[†] and Kenji Shimamura*
Department of Brain Morphogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan

The cerebral cortex in mammals, the neocortex specifically, is highly diverse among species with respect to its size and morphology, likely reflecting the immense adaptiveness of this lineage. In particular, the pattern and number of convoluted ridges and fissures, called gyri and sulci, respectively, on the surface of the cortex are variable among species and even individuals. However, little is known about the mechanism of cortical folding, although there have been several hypotheses proposed. Recent studies on embryonic neurogenesis revealed the differences in cortical progenitors as a critical factor of the process of gyrification. Here, we investigated the gyrification processes using developing guinea pig brains that form a simple but fundamental pattern of gyri. In addition, we established an electroporation-mediated gene transfer method for guinea pig embryos. We introduce the guinea pig brain as a useful model system to understand the mechanisms and basic principle of cortical folding.

Key words: cerebral cortex, cortical folding, electroporation, guinea pig, gyrification.

Introduction

Animals on the earth at present and in the past have acquired huge diversity in their brains, which is in accordance with the animals' characteristic features. The neocortex in mammals, which exhibits a six-layered organization unique to this lineage, has dramatically evolved. Among mammals, the cerebral cortex has diversified extremely both in size and gross morphology, such that distinct patterns of convoluted ridges and grooves on the surface of cortex, called gyri and sulci, respectively, are visible (Welker 1990; Defelipe 2011; Zilles *et al.* 2013; Lewitus *et al.* 2014). For instance, the human cerebral cortex is extremely enlarged to cover up almost the entire brain surface, and presented with numerous gyri that are carved in an extremely complicated manner. These features of the human brain have likely led our species to acquire an outstanding intelligence over all mammals. However, the underlying mechanisms of gyrification remain largely elusive. Herein, we first review the hypotheses and notions for gyrification. In addition, we report on our attempt to address this question using the guinea pig as a model system. Our approach focuses on the characterization of basic features of the gyrus formation in guinea pigs, and on establishing a method to introduce exogenous genes into developing guinea pig embryos.

Difference in cortical neurogenesis between primates with large brains and mice with small brains

The vast majority of cellular constituents in the vertebrate brain originate from radial glia (RG), which are themselves derived from neuroepithelia of the early neural tube. The RG repeatedly undergo cell division over time, first to increase their numbers as the progenitor pool, and then to self-renew and produce neurons and glia (Fujita 2003; Kageyama *et al.* 2005; Rowitch & Kriegstein 2010). While this basic principle of the brain development is common in all mammals, the resulting brain is highly diverse such that mice have a small and lissencephalic brain, whereas primates including humans have a large and gyrencephalic brain. Thus, the obvious question here is how such diversity is generated through the apparently common basic mechanism. Around the middle of the neurogenic period, a progenitor population known as the intermediate progenitors (IPs) is generated from the original RG. In mice, the IPs divide once to produce two neuronal siblings in most cases (Haubensak

*Author to whom all correspondence should be addressed.
Email: simamura@kumamoto-u.ac.jp
[†]These authors contributed equally.
Received 31 January 2017; revised 5 May 2017; accepted 6 May 2017.

PLOS ONE

RESEARCH ARTICLE

Roles of CD34$^+$ cells and ALK5 signaling in the reconstruction of seminiferous tubule-like structures in 3-D re-aggregate culture of dissociated cells from neonatal mouse testes

Shin-ichi Abe[1]*, Kazuko Abe[1], Jidong Zhang[2], Tomoaki Harada[3], Go Mizumoto[1], Hiroki Oshikawa[3], Haruhiko Akiyama[4], Kenji Shimamura[5]

1 Center for General Education, Kumamoto Health Science University, Kumamoto, Japan, **2** School of Basic Medical Sciences, ZunYi Medical University, Zunyi, Guizhou Province, China, **3** Department of Biological Science, Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan, **4** Department of Orthopaedics, Graduate School of Medicine, Gifu University, Gifu, Japan, **5** Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan

* abe@kumamoto-hsu.ac.jp

OPEN ACCESS

Citation: Abe S-i, Abe K, Zhang J, Harada T, Mizumoto G, Oshikawa H, et al. (2017) Roles of CD34$^+$ cells and ALK5 signaling in the reconstruction of seminiferous tubule-like structures in 3-D re-aggregate culture of dissociated cells from neonatal mouse testes. PLoS ONE 12(11): e0188705. https://doi.org/10.1371/journal.pone.0188705

Editor: Stefan Schlatt, University Hospital of Münster, GERMANY

Received: September 3, 2017

Accepted: November 10, 2017

Published: November 30, 2017

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by Japan Society for The Promotion of Science KAKENHI Grant Number 15K07129, Kumamoto Health Science University special fellowship grant number 2015-C-12 and 2017-C-07 to SA; the National Natural Science Foundation of China grant No.

Abstract

Tissue reconstruction *in vitro* can provide, if successful, a refined and simple system to analyze the underlying mechanisms that drive the morphogenesis and maintain the ordered structure. We have recently succeeded in reconstruction of seminiferous cord-like and tubule-like structures using 3-D re-aggregate culture of dissociated testicular cells. In testis formation, endothelial cells that migrated from mesonephroi to embryonic gonads have been shown to be critical for development of testis cords, but how endothelial cells contribute to testis cord formation remains unknown. To decipher the roles of endothelial and peritubular cells in the reconstruction of cord-like and tubule-like structures, we investigated the behavior of CD34$^+$ endothelial and p75$^+$ cells, and peritubular myoid cells (PTMCs) in 3-D re-aggregate cultures of testicular cells. The results showed that these 3 types of cells had the capacity of re-aggregation on their own and with each other, and of segregation into 3 layers in a re-aggregate, which were very similar to interstitial and peritubular tissues *in vivo*. Observation of behaviors of fluorescent Sertoli cells and other non-fluorescent types of cells using testes from *Sox9-EGFP* transgenic mice showed dynamic cell movement and segregation in re-aggregate cultures. Cultures of testicular cells deprived of interstitial and peritubular cells resulted in dysmorphic structures, but re-addition of them restored tubule-like structures. Purified CD34$^+$ cells in culture differentiated into p75$^+$ cells and PTMCs. These results indicate that CD34$^+$ cells differentiate into p75$^+$ cells, which then differentiate into PTMCs. TGFβ signaling inhibitors, SB431542 and ALK5i, disturbed the reconstruction of cord-like and tubule-like structures, and the latter compromised re-construction of interstitial-like and peritubular-like structures, as well as the proliferation of CD34$^+$, p75$^+$, PTMCs, and Sertoli cells, and their movement and differentiation. These results indicate that CD34$^+$ cells and signaling through ALK5 play pivotal roles in the morphogenesis of interstitial-like, peritubular-like and cord-like structures.

JSHC Japan Society of Histochemistry and Cytochemistry Established 1959

Acta Histochem. Cytochem. 50 (1): 1–9, 2017
doi: 10.1267/ahc.16032

Significance of Stat3 Signaling in Epithelial Cell Differentiation of Fetal Mouse Lungs

Hiroki Kameyama[1,3], Shinji Kudoh[1], Jun Hatakeyama[2], Akira Matuo[1] and Takaaki Ito[1]

[1]Department of Pathology and Experimental Medicine, Kumamoto University Graduate School of Medical Sciences, [2]Department of Brain Morphogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University and [3]Division of Pathology, Kumamoto Health Science University, Kumamoto, Japan

Received September 23, 2016; accepted November 28, 2016; published online February 23, 2017

To study the significance of signal transducer and activator of transcription (Stat) 3 in lung epithelial development of fetal mice, we examined fetal mouse lungs, focusing on the expression of Clara cell secretory protein (CCSP), Forkhead box protein J1 (Foxj1), calcitonin gene-related peptide (CGRP), phosphorylated Stat3 (Tyr705), and hairy/enhancer of split (Hes) 1, and observed cultured fetal lungs upon treatment with IL-6, a Stat3 activator, or cucurbitacin I, a Stat3 inhibitor. Moreover, the interaction of Stat3 signaling and Hes1 was studied using Hes1 gene-deficient mice. Phosphorylated Stat3 was detected in fetal lungs and, immunohistochemically, phosphorylated Stat3 was found to be co-localized in developing Clara cells, but not in ciliated cells. In the organ culture studies, upon treatment with IL-6, quantitative RT-PCR revealed that CCSP mRNA increased with increasing Stat3 phosphorylation, while cucurbitacin I decreased Hes1, CCSP, Foxj1 and CGRP mRNAs with decreasing Stat3 phosphorylation. In the lungs of Hes1 gene-deficient mice, Stat3 phosphorylation was not markedly different from wild-type mice, the expression of CCSP and CGRP was enhanced, and the treatment of IL-6 or cucurbitacin I induced similar effects on mouse lung epithelial differentiation regardless of Hes1 expression status. Stat3 signaling acts in fetal mouse lung development, and seems to regulate Clara cell differentiation positively. Hes1 could regulate Clara cell differentiation in a manner independent from Stat3 signaling.

Key words: Stat3, Hes1, Clara cell, ciliated cell, neuroendocrine cell

I. Introduction

Signal transducer and activator of transcription (Stat) 3 is one of the main intracellular signaling molecules that mediate proinflammatory interleukin 6 (IL-6) [1, 5, 13, 26]; it plays critical roles in several biological functions, including proliferation, migration, survival and differentiation [13]. The significance of Stat3 signaling in development and differentiation has been reported in various organs and cells, including kidney [23], mammary gland [4], skin keratinocytes [15] and embryonic stem cells [27]. However, direct studies of Stat3 in lung development and lung epithelial cell differentiation remain limited. A high concentration of IL-6, an activator of Stat3, has been reported to promote lung branching morphogenesis [19], and leukemia inhibitory factor inhibits lung branching morphogenesis accompanied by the downregulation of Stat3 phosphorylation [20]. In addition, Stat3 signaling is reported to be important in the process of repair of injured bronchiolar epithelium as defect of Stat3 gene disturbs bronchiolar epithelial cell proliferation [11]. Important molecular pathways for lung development are recaptured during injury and regeneration [12], and, the pathways such as Stat3 for lung regeneration, vice versa, should be important during development.

Lung branching morphogenesis and epithelial cell differentiation during the fetal developmental period pro-

Correspondence to: Takaaki Ito, MD, Department of Pathology and Experimental Medicine, Kumamoto University Graduate School of Medical Sciences, Kumamoto 860–8556, Japan.
E-mail: takaito@kumamoto-u.ac.jp

SCIENTIFIC REPORTS

OPEN

Nucleocytoplasmic Shuttling of Histone Deacetylase 9 Controls Activity-Dependent Thalamocortical Axon Branching

Received: 17 February 2017
Accepted: 13 June 2017

Ricardo Alchini[1,3], Haruka Sato[1], Naoyuki Matsumoto[1], Tomomi Shimogori[2], Noriyuki Sugo[1] & Nobuhiko Yamamoto[1]

During development, thalamocortical (TC) axons form branches in an activity-dependent fashion. Here we investigated how neuronal activity is converted to molecular signals, focusing on an epigenetic mechanism involving histone deacetylases (HDACs). Immunohistochemistry demonstrated that HDAC9 was translocated from the nucleus to the cytoplasm of thalamic cells during the first postnatal week in rats. In organotypic co-cultures of the thalamus and cortex, fluorescent protein-tagged HDAC9 also exhibited nucleocytoplasmic translocation in thalamic cells during culturing, which was reversed by tetrodotoxin treatment. Transfection with a mutant HDAC9 that interferes with the translocation markedly decreased TC axon branching in the culture. Similarly, TC axon branching was significantly decreased by the mutant HDAC9 gene transfer *in vivo*. However, axonal branching was restored by disrupting the interaction between HDAC9 and myocyte-specific enhancer factor 2 (MEF2). Taken together, the present results demonstrate that the nucleocytoplasmic translocation of HDAC9 plays a critical role in activity-dependent TC axon branching by affecting transcriptional regulation and downstream signaling pathways.

During development, neurons initially project their axons following a genetically defined developmental program, while neural activity refines neuronal circuits at later developmental stages. Axon branching is a phenomenon which represents the refinement process in the developing brain. The thalamocortical (TC) projection is one of the best-characterized systems of activity-dependent axon branching, exemplified by eye-specific projections in the primary visual cortex of certain higher mammals and barrel-specific projections in the somatosensory cortex of rodents[1–8].

Our previous studies have shown that neuronal activity such as evoked and spontaneous activity acts as a positive regulator for TC axon branching[9,10]. Moreover, manipulation of spontaneous firing activity has demonstrated that both presynaptic (thalamic cells) and postsynaptic synaptic cell (cortical cells) activity is necessary[11,12]. As for the molecular mechanism, postsynaptic cell activity has been shown to release branch-promoting molecules from these cells themselves[13]. However, the presynaptic mechanism by which neural activity modifies axonal branching remains largely unknown, although several factors including cytoskeleton-regulatory molecules have been shown to contribute to branch emergence[14,15].

An intriguing view of activity-dependent axon branching is transcriptional regulation by neuronal activity[16]. The class IIa histone deacetylases (HDACs) attracted our attention as tentative mediators of neural activity in axonal remodeling because of their role in the interplay between neural activity and gene expression[17]. While HDACs primarily act in the nucleus by modifying histones, which results in suppression of gene expression, class IIa HDACs (HDAC4, 5, 7 and 9) form a distinct phylogenetic group, characterized by their ability to shuttle between the nucleus and the cytoplasm[18,19]. This nucleocytoplasmic translocation is evoked by calcium influx, such as that downstream of neuronal activity, resulting in phosphorylation of the amino-terminal extensions of HDACs and leading to their association with 14-3-3 adaptor proteins and the nuclear export of protein complexes

[1]Neuroscience Laboratories, Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka, 565-0871, Japan. [2]Riken Brain Science Institute, Wako, Saitama, 351-0198, Japan. [3]Present address: Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4, Canada. Correspondence and requests for materials should be addressed to N.Y. (email: nobuhiko@fbs.osaka-u.ac.jp)

General and Comparative Endocrinology 205 (2014) 121–132

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Reconstruction of a seminiferous tubule-like structure in a 3 dimensional culture system of re-aggregated mouse neonatal testicular cells within a collagen matrix

Jidong Zhang [a], Jun Hatakeyama [b], Ko Eto [a], Shin-ichi Abe [a,*]

[a] Department of Biological Science, Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto 860-8555, Japan
[b] Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan

ARTICLE INFO

Article history:
Available online 6 April 2014

Keywords:
Seminiferous tubule
3 Dimensional culture
Re-aggregated testicular cells
KnockOut Serum Replacement (KSR)
Collagen
Mouse

ABSTRACT

Male gonad development is initiated by the aggregation of pre-Sertoli cells (SCs), which surround germ cells to form cords. Several attempts to reconstruct testes from dissociated testicular cells have been made; however, only very limited morphogenesis beyond seminiferous cord formation has been achieved. Therefore, we aimed to reconstruct seminiferous tubules using a 3-dimensional (D) re-aggregate culture of testicular cells, which were dissociated from 6-dpp neonatal mice, inside a collagen matrix. We performed a short-term culture (for 3 days) and a long-term culture (up to 3 wks). The addition of KnockOut Serum Replacement (KSR) promoted (1) the enlargement of SC re-aggregates; (2) the attachment of peritubular myoid (PTM) cells around the SC re-aggregates; (3) the sorting of germ cells inside, and Leydig cells outside, seminiferous cord-like structures; (4) the alignment of SC polarity inside a seminiferous cord-like structure relative to the basement membrane; (5) the differentiation of SCs (the expression of the androgen receptor); (6) the formation of a blood–testis-barrier between the SCs; (7) SC elongation and lumen formation; and (8) the proliferation of SCs and spermatogonia, as well as the differentiation of spermatogonia into primary spermatocytes. Eventually, KSR promoted the formation of seminiferous tubule-like structures, which accompanied germ cell differentiation. However, these morphogenetic events did not occur in the absence of KSR. This in vitro system presents an excellent model with which to identify the possible factors that induce these events and to analyze the mechanisms that underlie cellular interactions during testicular morphogenesis and germ cell differentiation.

1. Introduction

Testicular development is initiated when the gene sex-determining region Y (Sry) is expressed in somatic cells in the mouse at 10.5–12.0 days post coitum (dpc). Primordial germ cells (PGCs) migrate into the gonads approximately 10.0–11.0-dpc, when germ cells are mixed with somatic cells; then, pre-SCs aggregate and surround germ cells to form cords (Pelliniemi et al., 1993). During the process of postnatal and pubertal development, SCs show remarkable structural changes and functional maturation (Gondos and Berndston, 1993; Sharpe et al., 2003). Immediately after birth, SCs become small and short columnar. By 1 wk after birth, SCs have increased in size with high columnar shape and are located along the basal lamina, and their long axis assumes a perpendicular orientation toward the basal lamina. By 2 wks, SCs have remarkably increased in size with extensive interdigitation of the apical parts. By 3 wks, most of the seminiferous tubules have acquired a lumen to which SCs secrete fluid for the transportation of sperm to the collecting ducts (Russell et al., 1989). Androgen receptor (AR) activity in SCs has been demonstrated to be vital for spermatogenesis (Wang et al., 2009). One of the characteristic features of the structure in mature testis is the blood–testis-barrier (BTB), which is formed of tight junctions between SCs, that restrict the flow of materials inside the seminiferous tubules. This barrier is not formed in the early postnatal period, and the formation of this barrier is completed between 10- and 16-dpp in the mouse (Gondos and Berndston, 1993).

To address the mechanisms of development into testis, various in vitro models have been adopted, such as organ culture, cell culture, and 3-dimensional (D) culture. The importance of the 3-D culture using an extracellular matrix (ECM) for the study of tissue development has become evident in recent years because ECM

* Corresponding author. Fax: +81 96 342 2020.
E-mail addresses: jidong@kumamoto-u.ac.jp (J. Zhang), jhatakey@kumamoto-u.ac.jp (J. Hatakeyama), etoko@gpo.kumamoto-u.ac.jp (K. Eto), abeshin@gpo.kumamoto-u.ac.jp (S.-i. Abe).

http://dx.doi.org/10.1016/j.ygcen.2014.03.030
0016-6480/

© 2014. Published by The Company of Biologists Ltd | Development (2014) 141, 2075-2084 doi:10.1242/dev.097790

RESEARCH ARTICLE

Development of the prethalamus is crucial for thalamocortical projection formation and is regulated by Olig2

Katsuhiko Ono[1,*,‡], Adrien Clavairoly[2,*], Tadashi Nomura[1,3], Hitoshi Gotoh[1], Aoi Uno[1], Olivier Armant[4], Hirohide Takebayashi[3,5], Qi Zhang[6], Kenji Shimamura[7], Shigeyoshi Itohara[6], Carlos M. Parras[2] and Kazuhiro Ikenaka[8]

ABSTRACT

Thalamocortical axons (TCAs) pass through the prethalamus in the first step of their neural circuit formation. Although it has been supposed that the prethalamus is an intermediate target for thalamocortical projection formation, much less is known about the molecular mechanisms of this targeting. Here, we demonstrated the functional implications of the prethalamus in the formation of this neural circuit. We show that Olig2 transcription factor, which is expressed in the ventricular zone (VZ) of prosomere 3, regulates prethalamus formation, and loss of Olig2 results in reduced prethalamus size in early development, which is accompanied by expansion of the thalamic eminence (TE). Extension of TCAs is disorganized in the *Olig2-KO* dorsal thalamus, and initial elongation of TCAs is retarded in the *Olig2-KO* forebrain. Microarray analysis demonstrated upregulation of several axon guidance molecules, including *Epha3* and *Epha5*, in the *Olig2-KO* basal forebrain. *In situ* hybridization showed that the prethalamus in the wild type excluded the expression of *Epha3* and *Epha5*, whereas loss of Olig2 resulted in reduction of this Ephas-negative area and the corresponding expansion of the Ephas-positive TE. Dissociated cultures of thalamic progenitor cells demonstrated that substrate-bound EphA3 suppresses neurite extension from dorsal thalamic neurons. These results indicate that Olig2 is involved in correct formation of the prethalamus, which leads to exclusion of the EphA3-expressing region and is crucial for proper TCA formation. Our observation is the first report showing the molecular mechanisms underlying how the prethalamus acts on initial thalamocortical projection formation.

KEY WORDS: Dorsal thalamus, Thalamic eminence, EphA3, Microarray, *In situ* hybridization, Mouse

INTRODUCTION

The cerebral cortex and dorsal thalamus have reciprocal connections, which are essential morphological bases for cortical functions. Thalamocortical axons (TCAs) send sensory information and feedback of motor programming from the caudal brain areas, and these connections are organized in a topographical manner (Vanderhaeghen and Polleux, 2004). Formation of the topographic connections is regulated by several axon guidance molecules (Braisted et al., 2000, 2009; Dufour et al., 2003; Vanderhaeghen and Polleux, 2004; Torii and Levitt, 2005; Uemura et al., 2007). Developing thalamic neurons send axons towards the ventral telencephalon through the prethalamus (or ventral thalamus). Special guidance cells named corridor cells in the ventral telencephalon guide TCAs to the pallium (López-Bendito et al., 2006). Thus, the ventral telencephalon is regarded as an important intermediate target for the formation of reciprocal connections.

TCAs need to pass through the prethalamus on exiting from the dorsal thalamus to the ventral telencephalon as the prethalamus occupies exiting points of TCAs. The prethalamus has been supposed to be an intermediate target of TCAs (Deng and Elberger, 2003; Molnár et al., 2012); however, evidence is scarce that identifies the molecular mechanisms underlying the axon guidance role of the prethalamus, and functions of the prethalamus in thalamocortical projection are not fully understood (Leyva-Diaz and Lopez-Bendito, 2013). Mouse lines showing defects in prethalamus formation would be a useful model for analyzing the functional role of the prethalamus in thalamocortical projection formation.

Olig2 is a bHLH transcription factor that is essential for oligodendrocyte and somatic motoneuron development (Lu et al., 2002; Takebayashi et al., 2002b; Zhou and Anderson, 2002), and is also involved in dorsoventral patterning of the spinal cord, which is required for pMN domain specification. In the diencephalon, Olig2 is expressed in the VZ of the prethalamus at early fetal stages, such as E9.5 in mice (Ono et al., 2008). These Olig2+ cells differentiate into GABAergic neurons in the thalamic reticular nucleus (TRN) as well as into macroglial cells in the diencephalon, whereas loss of Olig2 does not affect GABAergic neuron differentiation (Takebayashi et al., 2008). The function of Olig2 in this area has not been elucidated. Here, we report that loss of Olig2 results in hypoplasia of the prethalamus, which leads to defects of TCA extension. The prethalamus is devoid of *Epha3* and *Epha5* expression whereas ventrally adjacent thalamic eminence (TE) expresses *Epha3* and *Epha5* (referred to here as Ephas positive) and, in the E13.5 *Olig2-KO* diencephalon, Ephas-positive TE expanded dorsally. Furthermore, the substrate-bound Epha3 suppresses neurite extension in cultured thalamic neurons. These results together indicate that Olig2 regulates proper formation of the prethalamus, which leads to exclusion of the EphA3-expressing non-permissive region for TCA and is crucial for proper TCA formation.

RESULTS

Reduced size of the prethalamus in *Olig2-KO* mice

We first explored early development of the prethalamus in the *Olig2-KO* mouse to examine whether *Olig2-KO* mice can be used to

[1]Department of Biology, Kyoto Prefectural University of Medicine, Kyoto 603-8334, Japan. [2]Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, and Inserm U 1127, and CNRS UMR 7225, and ICM, 75013, Paris, France. [3]PRESTO, JST, Kawaguchi 332-0012, Japan. [4]Institute of Toxicology and Genetics, KIT Campus Nord, Eggenstein-Leopoldshafen D-76344, Germany. [5]Division of Neurobiology and Anatomy, Graduate School of Medical and Dental Sciences, Niigata University, Niigata 951-8510, Japan. [6]Laboratory for Behavioral Genetics, RIKEN BSI, Wako 351-0198, Japan. [7]Department of Brain Morphogenesis, Institutes for Molecular and Embryological Genetics, Kumamoto University, Kumamoto 860-8556, Japan. [8]Division of Neurobiology and Bioinformatics, National Institute for Physiological Sciences, Okazaki 444-8787, Japan.
*These authors contributed equally to this work

‡Author for correspondence (katsono@koto.kpu-m.ac.jp)

Received 19 April 2013; Accepted 11 March 2014

© 2014. Published by The Company of Biologists Ltd | Development (2014) 141, 1671-1682 doi:10.1242/dev.102988

RESEARCH ARTICLE

STEM CELLS AND REGENERATION

Cadherin-based adhesions in the apical endfoot are required for active Notch signaling to control neurogenesis in vertebrates

Jun Hatakeyama[1,*], Yoshio Wakamatsu[2], Akira Nagafuchi[3], Ryoichiro Kageyama[4,5], Ryuichi Shigemoto[6,7] and Kenji Shimamura[1,*]

ABSTRACT

The development of the vertebrate brain requires an exquisite balance between proliferation and differentiation of neural progenitors. Notch signaling plays a pivotal role in regulating this balance, yet the interaction between signaling and receiving cells remains poorly understood. We have found that numerous nascent neurons and/or intermediate neurogenic progenitors expressing the ligand of Notch retain apical endfeet transiently at the ventricular lumen that form adherens junctions (AJs) with the endfeet of progenitors. Forced detachment of the apical endfeet of those differentiating cells by disrupting AJs resulted in precocious neurogenesis that was preceded by the downregulation of Notch signaling. Both Notch1 and its ligand Dll1 are distributed around AJs in the apical endfeet, and these proteins physically interact with ZO-1, a constituent of the AJ. Furthermore, live imaging of a fluorescently tagged Notch1 demonstrated its trafficking from the apical endfoot to the nucleus upon cleavage. Our results identified the apical endfoot as the central site of active Notch signaling to securely prohibit inappropriate differentiation of neural progenitors.

KEY WORDS: Notch signaling, Adherens junction, Neurogenesis, Neural stem/progenitor cell, Apical endfoot, Cadherin, Mouse, Chick

INTRODUCTION

During vertebrate development, specific progenitors in each organ primordium repeatedly undergo cell division with profound proliferative and differentiation potentials to produce an adequate number of cellular constituents for a given organ. Because the differentiated cells responsible for the physiological functions of the organ typically do not proliferate, excess temporal differentiation of progenitors often results in hypoplastic organ formation (e.g. Hatakeyama et al., 2004; Self et al., 2006; Zhu et al., 2006). Thus, proper preservation and differentiation of the progenitor cells is crucial for the growth and formation of organs, and how it is achieved cellularly and molecularly has been a major issue in developmental biology, as well as in regenerative medicine.

Differentiating cells participate in the regulation of progenitor differentiation by a feedback control through Notch signaling. Notch signaling is a juxtamembrane cell interaction system used in many contexts of metazoan development, which play a central role in lateral inhibition, a mechanism that coordinates the balance of differentiated and undifferentiated states within a progenitor pool (Louvi and Artavanis-Tsakonas, 2006). Although much is known about how Notch signaling is activated, transmitted and modulated (Fortini, 2009; Kopan and Ilagan, 2009), little is known about the regulation of Notch-ligand interactions between juxtaposed cells during massive cell rearrangements that dynamically occur in morphogenesis. In particular, how the signaling is turned off is poorly understood beyond the transcriptional control of the ligands and the post-transcriptional modulations by the auxiliary proteins of the pathway (Fortini, 2009). The termination of Notch signaling is crucial in order that progenitors are allowed to differentiate in the next round of cell division.

Cell-cell junctions act as physical connections between cells, and also play a role in juxtacrine communication between epithelial cells for controlling cell growth and differentiation (Fuchs et al., 2004). In *Drosophila*, Notch and its ligands are predominantly localized to the apical junction of epithelial cells (Fehon et al., 1991; Kooh et al., 1993). Notch forms a complex with E-cadherin, a transmembrane component of the adherens junction (AJ), depletion of which downregulates Notch signaling, indicating a functional link between Notch signaling and cadherin-mediated AJ in *Drosophila* (Sasaki et al., 2007). However, the details of this relationship remain elusive for the vertebrate central nervous system (CNS).

Neuroepithelial cells and radial glia residing in the ventricular zone (VZ) of the neural tube function as the stem/progenitor cells of the embryonic CNS. These cells are highly polarized epithelial cells connected through apically localized junctional complexes, including AJs and tight junctions (TJs). These pseudostratified epithelial cells span the ventricular wall with thin processes extending both apically and basally from the soma, and undergo interkinetic nuclear migration as the cell cycle progresses (Fujita, 2003; Rakic, 2007). In addition, these cells also serve as architectural scaffolds that physically support tissue integrity and morphogenesis (Hatakeyama et al., 2004). Neural progenitor cells (NPC) undergo extensive cell division to support the proliferation, self-renewal and production of neurons. These cells persist long enough to produce an adequate number of neurons with divergent properties. Thus, a quantitative balance between the proliferation and differentiation of NPCs must be exquisitely coordinated both spatially and temporally for the proper development of the CNS.

In the present study, we investigated the regulation of neurogenesis through interactions between differentiating cells and undifferentiated progenitors in the early phase of neurogenesis in the vertebrate CNS, when the preservation of NPC pools is crucial

[1]Department of Brain Morphogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan. [2]Division of Developmental Neuroscience, Tohoku University, Graduate School of Medicine, 2-1 Seiryo-Machi, Aoba-ku, Sendai 980-8575, Japan. [3]Department of Biology, Nara Medical University, 840 Shijo, Kashiwara, Nara 634-8521, Japan. [4]Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan. [5]World Premier International Research Initiative–Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto 606-8501, Japan. [6]Division of Cerebral Structure, National Institute for Physiological Sciences, 5-1 Higashiyama, Myodaiji, Okazaki 444-8787, Japan. [7]Institute of Science and Technology Austria, Am Campus 1, Klosterneuburg A-3400, Austria.

*Authors for correspondence (jhatakey@kumamoto-u.ac.jp; simamura@kumamoto-u.ac.jp)

Received 23 August 2013; Accepted 11 February 2014

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ANXIETY
PATHOGENESIS
GENETIC ENGINEERING
METABOLIC DISORDERS

Received
4 April 2013

Accepted
28 May 2013

Published
13 June 2013

Correspondence and requests for materials should be addressed to N.N. (noriyuki@nms.ac.jp)

* These authors contributed equally to this work.

Antioxidant enzyme, 3-mercaptopyruvate sulfurtransferase-knockout mice exhibit increased anxiety-like behaviors: a model for human mercaptolactate-cysteine disulfiduria

Noriyuki Nagahara[1]*, Masatoshi Nagano[2]*, Takaaki Ito[3], Kenji Shimamura[4], Toshio Akimoto[5] & Hidenori Suzuki[2]

[1]Isotope Research Center, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan, [2]Department of Pharmacology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan, [3]Department of Pathology and Experimental Medicine, Graduate School of Medical Science, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan, [4]Department of Brain Morphogenesis, Division of Organogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan, [5]Division of Laboratory Animal Science, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan.

Human mercaptolactate-cysteine disulfiduria (MCDU) was first recognized and reported in 1968. Most cases of MCDU are associated with mental retardation, while the pathogenesis remains unknown. To investigate it, we generated homozygous 3-mercaptopyruvate sulfurtransferase (MST: EC 2.8.1.2) knockout (KO) mice using C57BL/6 embryonic stem cells as an animal model. The MST-KO mice showed significantly increased anxiety-like behaviors with an increase in serotonin level in the prefrontal cortex (PFC), but not with abnormal morphological changes in the brain. MCDU can be caused by loss in the functional diversity of MST; first, MST functions as an antioxidant protein. MST possessing 2 redox-sensing molecular switches maintains cellular redox homeostasis. Second, MST can produce H_2S (or HS^-). Third, MST can also produce SO_x. It is concluded that behavioral abnormality in MST-KO mice is caused by MST function defects such as an antioxidant insufficiency or a new transducer, H_2S (or HS^-) and/or SO_x deficiency.

3-Mercaptopyruvate sulfurtransferase (MST) catalyzes the reaction from mercaptopyruvate ($SHCH_2C(=O)COOH$) to pyruvate ($CH_3C(=O)COOH$) in cysteine catabolism. MST is widely distributed in prokaryotes and eukaryotes[1]. Interestingly, MST is localized in the cytoplasm and mitochondria, but not all cells contain MST[2]. In our studies of the structure–function relationship, we found that a disulfide bond between the dimeric MST, which regulated a monomer (active form)-dimer (inactive form) equilibrium[3,4]. The dimer is formed via an intersubunit disulfide bond by oxidation of exposed cysteine residues on the surface of 2 subunits. This disulfide bond serves as a thioredoxin-specific molecular switch[3,4]. On the other hand, a catalytic-site cysteine is easily oxidized to form a low-redox-potential sulfenate, resulting in loss of activity[4,5]. Then, thioredoxin can uniquely restore the activity[4,5]. Thus, a catalytic site cysteine contributes to redox–dependent regulation of MST activity serving as a redox-sensing molecular switch. These findings suggest that MST serves as an antioxidant protein and partly maintain cellular redox homeostasis. Further, Shibuya et al proposed that MST can produce hydrogen sulfide (H_2S) by using a persulfurated acceptor substrate[6]. As an alternative functional diversity of MST, we recently demonstrated in vitro that MST can produce sulfur oxides (SO_x) in the redox cycle of persulfide ($-S-S^-$) formed at the catalytic-site cysteine of the reaction intermediate[7]. To elucidate these functional diversity of MST in living organism, we tried to generate homogynous (null)

Published Online: 24 December, 2012 | Supp Info: http://doi.org/10.1084/jem.20120993

The Journal of Experimental Medicine

Hes repressors are essential regulators of hematopoietic stem cell development downstream of Notch signaling

Jordi Guiu,[1] Ritsuko Shimizu,[2] Teresa D'Altri,[1] Stuart T. Fraser,[4,5,7] Jun Hatakeyama,[7] Emery H. Bresnick,[6] Ryoichiro Kageyama,[7] Elaine Dzierzak,[8] Masayuki Yamamoto,[3] Lluis Espinosa,[1] and Anna Bigas[1]

[1]Program in Cancer Research, Hospital del Mar Medical Research Institute (IMIM), Barcelona Biomedical Research Park, 08003 Barcelona, Spain
[2]Molecular Hematology and [3]Medical Biochemistry, Tohoku University School of Medicine, Sendai 980-8575, Japan
[4]Discipline of Physiology and [5]Discipline of Anatomy and Histology, School of Medical Sciences, University of Sydney, Sydney, New South Wales 2006, Australia
[6]Department of Cell and Regenerative Biology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53705
[7]Institute for Virus Research, Kyoto University, Kyoto 606-8507, Japan
[8]Department of Cell Biology and Genetics, Erasmus University, 3000 CA Rotterdam, Netherlands

Previous studies have identified Notch as a key regulator of hematopoietic stem cell (HSC) development, but the underlying downstream mechanisms remain unknown. The Notch target *Hes1* is widely expressed in the aortic endothelium and hematopoietic clusters, though *Hes1*–deficient mice show no overt hematopoietic abnormalities. We now demonstrate that *Hes* is required for the development of HSC in the mouse embryo, a function previously undetected as the result of functional compensation by de novo expression of *Hes5* in the aorta/gonad/mesonephros (AGM) region of *Hes1* mutants. Analysis of embryos deficient for *Hes1* and *Hes5* reveals an intact arterial program with overproduction of nonfunctional hematopoietic precursors and total absence of HSC activity. These alterations were associated with increased expression of the hematopoietic regulators *Runx1*, *c-myb*, and the previously identified Notch target *Gata2*. By analyzing the *Gata2* locus, we have identified functional RBPJ-binding sites, which mutation results in loss of *Gata2* reporter expression in transgenic embryos, and functional Hes-binding sites, which mutation leads to specific *Gata2* up-regulation in the hematopoietic precursors. Together, our findings show that Notch activation in the AGM triggers *Gata2* and *Hes1* transcription, and next HES-1 protein represses *Gata2*, creating an incoherent feed-forward loop required to restrict *Gata2* expression in the emerging HSCs.

CORRESPONDENCE
Anna Bigas:
abigas@imim.es

Abbreviations used: AGM, aorta/gonad/mesonephros; CFC, colony-forming cell; ChIP, chromatin immunoprecipitation; ee, embryo equivalent; HSC, hematopoietic stem cell; HSC/P, HSC and progenitor; I1-FFL, type I incoherent feed-forward loop; SCF, stem cell factor; YS, yolk sac.

Hematopoietic stem cells (HSCs) originate during embryonic life in association with arterial vessels including the aorta in the aorta/gonad/mesonephros (AGM) region and the umbilical and vitelline arteries (Dzierzak and Speck, 2008). The first HSCs are detected around embryonic day (E) 10 of mouse development in hematopoietic clusters emerging from the ventral wall of the aorta in the AGM and are defined by their unique capacity to reconstitute hematopoiesis of immunodepleted adult recipients in transplantation assays. The Notch pathway is required for HSC development in the vertebrate embryo, upstream of Runx1 (Burns et al., 2005) and GATA-2 (Robert-Moreno et al., 2005), but is dispensable for yolk sac (YS) primitive hematopoiesis (Robert-Moreno et al., 2007; Bertrand et al., 2010; Bigas et al., 2010). Importantly, *Gata2* but not *Runx1* expression was totally impaired in the aorta of *Jag1*$^{-/-}$ mutant mouse embryos, which fail to generate hematopoiesis (Robert-Moreno et al., 2005, 2008). Re-expression of

L. Espinosa and A. Bigas contributed equally to this paper.
S.T. Fraser's present address is University of Sydney, Sydney, New South Wales 2006, Australia.

The Rockefeller University Press $30.00
J. Exp. Med. 2013 Vol. 210 No. 1 71-84
www.jem.org/cgi/doi/10.1084/jem.20120993

Development/Plasticity/Repair

Thalamus-Derived Molecules Promote Survival and Dendritic Growth of Developing Cortical Neurons

Haruka Sato,[1] Yuma Fukutani,[1] Yuji Yamamoto,[1] Eiichi Tatara,[1] Makoto Takemoto,[1] Kenji Shimamura,[2] and Nobuhiko Yamamoto[1]

[1]Laboratory of Cellular and Molecular Neurobiology, Graduate School of Frontier Biosciences, Osaka University, Suita, Osaka 565-0871, and [2]Department of Brain Morphogenesis, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan

The mammalian neocortex is composed of various types of neurons that reflect its laminar and area structures. It has been suggested that not only intrinsic but also afferent-derived extrinsic factors are involved in neuronal differentiation during development. However, the role and molecular mechanism of such extrinsic factors are almost unknown. Here, we attempted to identify molecules that are expressed in the thalamus and affect cortical cell development. First, thalamus-specific molecules were sought by comparing gene expression profiles of the developing rat thalamus and cortex using microarrays, and by constructing a thalamus-enriched subtraction cDNA library. A systematic screening by *in situ* hybridization showed that several genes encoding extracellular molecules were strongly expressed in sensory thalamic nuclei. Exogenous and endogenous protein localization further demonstrated that two extracellular molecules, Neuritin-1 (NRN1) and VGF, were transported to thalamic axon terminals. Application of NRN1 and VGF to dissociated cell culture promoted the dendritic growth. An organotypic slice culture experiment further showed that the number of primary dendrites in multipolar stellate neurons increased in response to NRN1 and VGF, whereas dendritic growth of pyramidal neurons was not promoted. These molecules also increased neuronal survival of multipolar neurons. Taken together, these results suggest that the thalamus-specific molecules NRN1 and VGF play an important role in the dendritic growth and survival of cortical neurons in a cell type-specific manner.

Introduction

The neocortex is composed of various cell types having distinct morphology, connection patterns, and physiological properties (Ramón y Cajal, 1899; McConnell, 1989; Hevner, 2007; Molyneaux et al., 2007). How cortical neurons differentiate into specific cell types during development is one of the most interesting issues in neurobiology. Developmental studies in the mammalian neocortex have shown that intrinsic factors, including particular transcription factors, are necessary for cell type specification (Grove and Fukuchi-Shimogori, 2003; Molyneaux et al., 2007; O'Leary and Sahara, 2008). The transcription factors Tbr1, Ctip2, Satb2, and Cux2, which are expressed in specific layers of the developing cortex, contribute to laminar fate determination (Alcamo et al., 2008; Chen et al., 2008; Cubelos et al., 2008; Bedogni et al., 2010), while Emx2, Pax6, and Coup-tf1 are involved in area patterning by being expressed in a graded fashion (Bishop et al., 2000; Mallamaci et al., 2000; Armentano et al., 2007; O'Leary et al., 2007).

Extrinsic factors produced by the surrounding cells also affect cellular differentiation. For instance, environmental molecules such as growth factors, extracellular matrix proteins, and neurotrophic factors can regulate cell type specification and morphological differentiation of cortical neurons (Ferri and Levitt, 1995; McAllister et al., 1995). Afferent input is also an important source of extrinsic factors. It has been shown that afferent-derived molecules regulate neurogenesis and synapse formation in their target region (Huang and Kunes, 1996; Sanes and Lichtman, 2001). Thalamic fibers are the major input into the neocortex and may influence differentiation of cortical cells (O'Leary, 1989). In support of this view, the finding has been reported that grafted cortical neurons exhibit characteristics of the host cortical area regardless of their origins (Schlaggar and O'Leary, 1991). It has also been demonstrated that duplicate somatosensory areas are formed in association with additional thalamic inputs (Fukuchi-Shimogori and Grove, 2001). Thalamic fibers may also affect the composition of cell types. The thalamocortical recipient layer, layer 4, in the primary sensory areas receives extensive axonal projections from sensory thalamic nuclei. It is enriched with spiny stellate neurons with multipolar dendritic morphology, whereas other layers are mainly composed of pyramidal neurons. An intriguing hypothesis is that thalamus-derived factor(s) promote the survival and morphological differentiation of a subset of cortical neurons. However, such factor(s) have yet been identified.

To address this issue, we searched for extracellular molecules that are specifically expressed in the thalamus, but not in the

Received Jan. 10, 2012; revised July 18, 2012; accepted Sept. 3, 2012.

Author contributions: M.T. and N.Y. designed research; H.S., Y.F., Y.Y., E.T., M.T., and N.Y. performed research; H.S., Y.F., Y.Y., E.T., and K.S. analyzed data; H.S., K.S., and N.Y. wrote the paper.

This work was supported by Grants-in-Aid for Scientific Research on Innovative Areas "Mesoscopic Neurocircuitry" (No. 23115102) of the Japanese Ministry of Education, Culture, Sports, Science and Technology to N.Y., and for Scientific Research (B) (No. 20300110 and 23300118) of Japan Society for the Promotion of Science (JSPS) to N.Y. We thank Dr. Ian Smith for critical reading of this manuscript.

The authors declare no competing financial interests.

Correspondence should be addressed to Nobuhiko Yamamoto, Laboratory of Cellular and Molecular Neurobiology, Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: nobuhiko@fbs.osaka-u.ac.jp.

DOI:10.1523/JNEUROSCI.0293-12.2012

 0270-6474/12/3215388-15$15.00/0