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学位（専攻分野）	博士（理学）
学位記番号	総研大甲第1059号
学位授与の日付	平成１９年３月２３日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第６条第１項該当
学位論文題目	Molecular mechanisms of ventral tangential migration of lot cells, the guide post neurons in the lateral olfactory tract
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The brain functions are accomplished via communications between vast numbers of neurons. Thus, the construction of elaborated neural networks is indispensable for exerting a normal brain functions. Brain development is categorized into several steps such as neurogenesis, neuronal migration, axon projection, synaptogenesis, etc. Among them, neuronal migration is one of the most fundamental processes. If neurons do not migrate directionally, the consequences will be a chaotic brain because numerous subtypes of neurons will be randomly intermixed. Neuronal migration helps different neuronal populations to segregate into distinctive compartments, whereas it also contributes to the dispersion of one neuronal population to occupy a large domain. In this way, distinct neuronal populations are appropriately arranged in the brain, which enables the complicated brain functions.

Neurons migrate basically in two types of streams, radial and tangential. In the radial migration, neurons migrate vertically to the surface of a brain, whereas in the tangential migration, neurons migrate parallel to the surface. The radial migration is a main migratory mode for constructing the six-layer cerebral cortex, and thus this mode have been well studied by many laboratories for several decades. However, it has been only recently revealed that the tangential migration is also a critical migratory stream for the brain development. The most famous example of the tangential migration is provided by GABA interneurons, which migrate from the ventral telencephalon towards the dorsal neocortex thorough the so-called "dorsal tangential migration stream". However, there is also a stream in the opposite direction, "ventral tangential migration". Although this migration has been far less studied compared with the dorsal tangential migration, there is a good model system for analyzing the ventral tangential migration, which is lot cells.

Lot cells are a neuronal population recognized by monoclonal antibody (mAb) lot1. It has been revealed that these neurons are involved in the formation of the lateral olfactory tract (LOT), the fascicle of olfactory bulb axons extending on the surface of the telencephalon. Our group previously reproted that newborn lot cells migrate through the ventral tangential pathway. During the early developmental phase at E9-10, lot cells differentiate from the ventricular zone of the dorsal neocortex region, and migrate on the surface of the neocortex ventrally and tangentially. After finishing the migration, the cells accumulate at the presumptive LOT region and make a cellular array, which guides or allows olfactory bulb axons to form the accurate LOT. This migration pattern is quite unique from the viewpoint that the cell migration controls the following axon projection, but molecular mechanisms of the lot cell migration still remain unknown.

Our group previously performed combinational culture of early telencephalic explants, and suggested that the lot cell migration is non-cell autonomously

controlled by multiple guidance cues; the neocortex region contains gradually distributed guidance cues to orient the migrating cells into the ventral direction, whereas the ventral part of the telencephalon has some mechanisms to exclude lot cells, probably mediated by short-range repulsive cues. An axon guidance molecule, Netrin-1 has an attractive effect on the migration of lot cells in vitro. However, the expression of netrin-1 is only restricted in the ventral part of the telencephalon, thus Netrin-1 knockout mice exhibit only weak defects in the migration of lot cells. These results suggest that some other guidance molecules probably attract the lot cell migration. Also the repulsive cues for lot cells, which should be essential for the final arrangement of the cells, were virtually unidentified. In order to understand molecular mechanisms of the lot cell migration, I took two types of approaches; candidate screening and pharmacological perturbation.

First, I screened candidate guidance molecules. cDNAs for various guidance molecules were transfected into HEK293T line cells, and the cell aggregates expressing the candidate molecules were made. Subsequently, the effects of candidate molecules were investigated by co-culturing these cell aggregates with telencephalic slices, after labeling the cells in the ventral tangential migration stream with a fluorescent dye, DiI. Among many candidate molecules, I found that a repulsive axon guidance molecule, Semaphorin3F had a repulsive effect on the lot cell migration. Sema3F receptor, Neuropilin-2, was expressed in lot cells, and Sema3F was expressed in the region surrounding the presumptive LOT region. The cells in Neuropilin-2 knockout mice did not respond to Sema3F in the culture system. I examined the distribution pattern of lot cells in Nrp2 knockout mice and found that some lot cells were ectopically distributed in the medial region of the telencephalon. The majority of lot cells, however, normally aligned at the presumptive LOT region and they did not cross over the presumptive LOT region ventrally. These results indicate that Sema3F functions in confinement of lot cells on the surface of the neocortex, but not exclusion of cells from the ventral telencephalon

Second, I tested various pharmacological drugs in culture, and found that a protein kinase inhibitor, K252a inhibits the migration of lot cells but does not inhibit the extension of leading processes. This result is interesting because it may provide a new insight into the mechanisms of neuronal migration. Neurons usually migrate long distances by the locomotion mode in which the leading processes and the cell body migrate in a coordinated manner. However, K252a seemed to convert this migration mode into the neurite extension mode such as the axon projection or dendrite extension. Thus, I hoped that the effect of K252a would give an important clue for understanding the switch of the migratory modes, and further analyzed this

interesting phenomenon in the time-lapse video microscopy to detail the kinetics of the effect. This analysis showed that K252a robustly decreased the migration speed of cell bodies but not the extension of leading processes. K252a also converted the locomotion mode of the cerebellar granule cells into the neurite extension mode, suggesting that the switch of the migratory modes by K252a is rather a general phenomenon observable in various neuronal populations. I found one drug, roscovitine had a similar effect with K252a. Roscovitine is an inhibitor for cyclin dependent kinases (CDK), and thus I overexpressed a dominant negative form of neuronal CDK, CDK5 in migrating neurons. Overexpression of the dominant negative CDK5 induced the extension of leading processes and slowdowned the migration speed of cell bodies. Therefore, CDK5 activity may be one of the critical components for the switching of the migratory modes from the locomotion to the neurite extension.

論文の審査結果の要旨

脳の発生過程において、誕生した神経が特異的な場所に移動するメカニズムに関しては不明な点が多い。伊藤君は、嗅索(lateral olfactory tract; LOT)という神経回路の形成を規定している嗅索道標細胞(lot 細胞)の細胞移動の機構を明らかにすることを目的として研究を行った。この細胞群は発生初期に終脳の新皮質全体で産まれ、皮質表層を腹側に向かって移動し、将来嗅索が形成される領域に到達すると、90° 回転してそこに蓄積・配列していく。嗅索を作る軸索はこの lot 細胞の配列の上を伸びる事によって正しい道筋を歩む事が可能となる。しかしなぜ細胞が予定嗅索領域に向かって移動できるのか、なぜ予定嗅索領域より腹側には侵入できないのかといった、移動を制御する分子機構についてはほとんど分かっていなかった。そこで伊藤君は終脳の培養系を用いて、さまざまな候補分子の candidate screening を行い、lot 細胞の移動に影響を与える分子を探した。その結果、軸索ガイダンス分子・Sema3F が lot 細胞を脳の表面上に閉じ込める作用を持つことを明らかにした。さらに、Sema3F 以外にも FGF8、EphB1 など lot 細胞の移動を制御する分子が存在する事を示唆する結果も得た。

一方、伊藤君は Candidate screening と平行して阻害剤を用いた薬理実験も行なった。その結果 protein kinase 阻害剤である K252a が興味深い現象をもたらす事を発見した。通常の移動の際、lot 細胞は移動方向に先導突起を伸ばし、細胞体と先導突起は同調して移動する。ところが K252a を投与すると細胞体の動きが抑えられる一方で、先導突起の伸長は抑えられないという事を発見した。同様な現象が小脳顆粒細胞の移動過程で観察された。また CDK の阻害剤である roscovitine も同様な効果を示した。そこで神経細胞特異的に働く CDK である、CDK5 の dominant negative 体をニューロンに発現させた結果、突起の過伸長と細胞体の移動速度の減少という、K252a 投与の際に見られる現象とよく似た現象が導かれる事が分かった。

このように、伊藤君の学位論文は、lot 細胞の移動の位置情報に「表層 vs. 内側」という情報も含まれていることを示した点はこれまでにない観点として評価できる。さらに、阻害剤を用いた実験から導き出した現象を、細胞が移動した後、軸策伸張を開始する際の細胞内機構の解析につなげるなどオリジナリティの高い内容となっており遺伝学専攻の博士論文としての条件を満たしていると審査員全員が認めた。