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| 論文題目 | *Ex vivo* lineage analysis of a single newborn cell in the slice culture of postnatal rat hippocampus.（生後ラット海馬スライス培養における生体外単一新生細胞の系譜解析） |
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論文内容の要旨

***Introduction***

New neurons are continuously generated in the hippocampus at the subgranular zone of the dentate granule cell layer (GCL) throughout life. In the postnatal hippocampus, neurogenesis is a multi-step and rate-limiting process consisting of the division of a putative multipotent stem/precursor cells (NSCs), the transient amplification of putative progenitor cells, the selection of surviving cells and their differentiation into functional neurons. Every step has been presumed to be under the regulation of various internal and external stimuli such as the local network activities, hippocampal dependent learning and exposure to the environmental enrichment. Under these circumstances, the most well known regulatory steps are the rate of proliferation in progenitor cells and the survivability of newborn neurons. However, the fundamental lineage of newly generated neurons is unknown in detail on the postnatal neurogenesis. To further investigate how the proliferation and the survival are regulated, the lineage has to be followed up for an individual progenitor cell since the onset of each key process of neurogenesis occurs asynchronously.

***Methods***

We had conceived of the combination paradigm with the retrovirus vector and the organotypic hippocampal slice culture. Using retrovirus vectors encoding EGFP, we inoculated the suprapyramidal blade of GCL with these vectors and investigated the lineage of individual progenitors for up to four weeks in the hippocampal slice culture. At 28 days post-inoculation (DPI), the phenotypes of the cells were immunohistochemically identified using specific antibodies to cell-type markers such as HuC/D (pan-neuronal marker), GFAP (astrocyte marker), Prox1 (dentate granule cell marker) or NeuN (mature neuronal marker).

***Results***

In the linage analysis, we found that the cells were mostly GFAP-negative in the HuC/D-positive lineages. As for the proliferation in both lineage groups, the frequency of cell division was higher during the early DPI period (1-7 and 8-14) than the later (15-21 and 22-28 DPI) with significant differences. The number of EGFP expressing cells in HuC/D-positive lineage was significantly less than the expected from the predicted value during the period, while that in GFAP-positive lineage was almost identical to the prediction. As for the survival, the EGFP-expressing cells were often untraceable shortly after cell division in the HuC/D-positive lineages. The survival probability in the HuC/D-positive lineages was significantly smaller than that in the GFAP-positive lineages. We

measured the postmitotic period (defined as the period from the last cell division to the time of interest) of the untraceable cells in the HuC/D-positive lineages from lineage tree. It distributed between 2 and 14 days. In addition, we also investigated the lineages of the neuron-like descendants by using the other markers, Prox1 and NeuN. The both Prox1- and NeuN-positive lineages were similar to HuC/D-positive lineages on the proliferation manner, and also showed that newborn cells became untraceable in the similar periods (2-10 days).

***Discussion***

In the present study, no evidence was found that the GFAP-positive cells arose in the HuC/D-positive lineage. Rather, when one of the descendants was HuC/D-positive, most of the other cells in the same lineage were also HuC/D-positive. These results suggest the presence of neuron-generating lineages which are destined to produce neurons, although we have no evidence if they are derived from multipotent stem/precursor cells or the progenitor cells. Since frequency of cell division was higher in the early DPI period (1-14 DPI) than in the late (15-28 DPI) in the HuC/D-positive lineages as well as Prox1- and NeuN-positive, many of the differentiated cells were produced early DPI period in the neuron-generating lineage. Moreover, the similar postomitotic period was observed in both neuron-generating lineages. Therefore, it is suggested that a neuron differentiates into its mature form once it survives this period.

We found that (i) the descendent cells of the neuron-generating lineages were mostly GFAP-negative, (ii) the newly generated cells became frequently untraceable in the neuron-generating lineages and (iii) this critical traceability period was 2-14 days. It is suggested that newly generated neurons differentiate into mature GCL neurons once they survive this period. Our lineage analysis methods using slice culture system would be advantageous to test several hypotheses concerning these regulatory mechanisms under experimental manipulations.

Reference:

Jun Yokose, Takeshi Yoshida, Jun Aoki, Toru Ishizuka, Yoshio Koyanagi and Hiromu Yawo.

"Lineage analysis of newly-generated neurons in organotypic culture of hippocampus."

Neurosci Res, 69(3):223-33 (2011).

論文審査結果の要旨

本研究は、海馬スライス培養とレトロウイルスを介した遺伝子導入法を併用することにより、生後ラット海馬において、単一のニューロン前駆細胞が分裂し、成熟ニューロンに分化する過程を追跡し、その系譜を解明したものである。このような研究は、本研究以前になされたことがなく、生後ニューロン新生の研究において、高いインパクトを与えるものと評価できる。また、生後ニューロン新生は、動物の運動、環境などによる制御を受けるとともに、記憶の形成やうつ病などの病態との関連が示唆されている。本研究の成果と、遺伝子操作、光遺伝学、薬理学などを組み合わせることにより、制御メカニズムの詳細が分子レベルで解明されることが期待される。

学位申請者は、本研究において、新生ニューロンのあるものは、分裂後まもなく消失するのに対し、他のものは生存し、成熟したニューロンに分化することを見出した。また、ニューロンの生死を決定する平均約１週間の臨界期が存在することを明らかにした。これらの知見は、先行研究のプログラム細胞死の考えを裏付けるものであるが、系譜として証明したことの意義は大きい。

学位申請者がこのような時間のかかる研究を粘り強く成し遂げたことは、高く評価される。また、生後ニューロン新生に関し、分子から行動にいたる幅広い知見を有していることが認められる。以上の所見は、学位申請者が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，横瀬淳提出の論文は，博士（生命科学）の博士論文として合格と認める。