第 569 回 難 研 セ ミ ナ ー
第 142 回 難治疾患共同研究拠点セミナー

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記

演 題：Enhancing survival, maturation and axonal growth of stem cell-derived neurons

講 師：Iván Velasco　先生

Instituto de Fisiología Celular - Neurociencias, Universidad Nacional Autónoma de México

日 時：平成 29 年 11 月 9 日(木) 17 時 00 分～18 時 00 分

場 所：M&D タワー24 階・共用セミナー室 1

講義趣旨: Neuronal differentiation during Central Nervous System development has been studied to identify key factors involved in the generation of several neuronal populations. Such information allowed the *in vitro* differentiation of embryonic stem cells (ESC) to midbrain dopamine (DaN) and spinal motor neurons (MN). Our group described a negative effect of histamine applied at early stages of DaN development *in vivo*. I will present epigenetic changes on DNA methylation caused by histamine over important genes for DaN differentiation in the rat developing midbrain. After neuronal differentiation, neurotrophic factors promote maturation and survival. However, the role of neurotrophic factors on neuronal commitment is elusive. We developed lines of mouse ESC that constitutively release Glial-derived neurotrophic factor (GDNF) and observed that GDNF-ESC differentiated more efficiently than controls to DaN with mesencephalic identity. To investigate a broader role of GDNF in neuronal differentiation, MN were produced from mouse ESC. In GDNF-overexpressing cells, significant increases in proliferative MN precursors positive for Olig2, as well as in differentiated MN, were found. These MN express GFP under the control of the Hb9 promoter, allowing us to perform electrophysiological recordings. MN derived from GDNF-expressing cells exhibited a higher number of evoked action potentials and more mature phenotypes. We have described that Semaphorin 3C (Sema3C) can attract and enhance the growth of axons of mouse ESC-derived DaN, both *in vitro* and *in vivo*. We tested if conjugation of recombinant Sema3C with a biocompatible hydrogel releases this protein to the medium and if such release can enhance axonal growth of human ESC-derived DaN in microfluidic chambers. We observed a significant increase of dopaminergic axonal growth after exposure to the Sema3C hydrogel, compared to the effect of the hydrogel containing a control protein. Using organotypic cultures, we are studying if the Sema3C hydrogel can guide human DaN placed on top of the brain slice.

参考（講師発表論文）

Carballo-Molina et al, **Tissue Eng. Part A** 22: 850-861 (2016)
Cortés et al, **Front. Cell. Neurosci.** 10: 217 (2016)
Velasco et a, **Stem Cells**. 32: 2811 (2014)
Escobedo-Avila et al, **Mol. Brain** 7: 58 (2014)
Díaz-Martínez et al, **Mol. Ther.** 75: 4224-4234 (2013)

【連絡先】幹細胞制御分野　田賀 哲也（内線 5814）【共　催】発生再生生物分野　仁科 博史

The 569th Medical Research Institute Seminar
The 142th Joint Usage/Research Program of Medical Research Institute Seminar

Date: **Thursday, Nov. 9, 2017, from 17:00 to 18:00**

Venue: **M&D Tower 24F, Seminar Room 1**

Lecturer: **Dr. Iván Velasco** ・Instituto de Fisiología Celular - Neurociencias, Universidad Nacional Autónoma de México and Laboratorio de Reprogramación Celular, Instituto Nacional de Neurología y Neurocirugía "Manuel Velasco Suárez".

Title: **Enhancing survival, maturation and axonal growth of stem cell-derived neurons**

Summary: Neuronal differentiation during Central Nervous System development has been studied to identify key factors involved in the generation of several neuronal populations. Such information allowed the *in vitro* differentiation of embryonic stem cells (ESC) to midbrain dopamine (DaN) and spinal motor neurons (MN). Our group described a negative effect of histamine applied at early stages of DaN development *in vivo*. I will present epigenetic changes on DNA methylation caused by histamine over important genes for DaN differentiation in the rat developing midbrain. After neuronal differentiation, neurotrophic factors promote maturation and survival. However, the role of neurotrophic factors on neuronal commitment is elusive. We developed lines of mouse ESC that constitutively release Glial-derived neurotrophic factor (GDNF) and observed that GDNF-ESC differentiated more efficiently than controls to DaN with mesencephalic identity. To investigate a broader role of GDNF in neuronal differentiation, MN were produced from mouse ESC. In GDNF-overexpressing cells, significant increases in proliferative MN precursors positive for Olig2, as well as in differentiated MN, were found. These MN express GFP under the control of the Hb9 promoter, allowing us to perform electrophysiological recordings. MN derived from GDNF-expressing cells exhibited a higher number of evoked action potentials and more mature phenotypes. We have described that Semaphorin 3C (Sema3C) can attract and enhance the growth of axons of mouse ESC-derived DaN, both *in vitro* and *in vivo*. We tested if conjugation of recombinant Sema3C with a biocompatible hydrogel releases this protein to the medium and if such release can enhance axonal growth of human ESC-derived DaN in microfluidic chambers. We observed a significant increase of dopaminergic axonal growth after exposure to the Sema3C hydrogel, compared to the effect of the hydrogel containing a control protein. Using organotypic cultures, we are studying if the Sema3C hydrogel can guide human DaN placed on top of the brain slice.

Organizers: Stem Cell Regulation・Prof. Tetsuya Taga ・ex. 5816
Co-organizer: Developmental and Regenerative Biology・Prof. Hiroshi Nishina