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| 学位論文名 | Hepatoma-Derived Growth Factor Is a Neurotrophic Factor Harbored in the Nucleus<br>（肝癌由来増殖因子 HDGF は核に局在する神経栄養因子である） |
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## 論文内容の要旨


**Objective**：Hepatoma-derived growth factor (HDGF) is a heparin-binding proliferating factor originally isolated from conditioned medium of the hepatoma-derived cell line HuH-7. HDGF represents a new family of growth factors called HDGF-related proteins (HRPs), which includes HRP1, HRP2, HRP3, HRP4 and lens epithelium-derived growth factor (LEDGF)/p75/p52. HDGF has greatest homology in amino acid sequence with high mobility group 1 (HMG1), which has been characterized as a DNA-binding, inflammatory, and potent neurite outgrowth molecule. HDGF was reported to be widely expressed and acted as a growth factor in many kinds of cells. Although intensively studied in many tissues and cells, it has not been investigated in the nervous system. Therefore, we carried out experiments to clarify the localization and functions of HDGF in the mouse nervous system.

**Methods and Results:**

1). HDGF is synthesized and localized in neurons in the nervous system.

To examine the expression of HDGF protein in the nervous system, a developmental study was carried out by Western blot analysis using the mice brains (C57/BL6). We found that HDGF protein was constantly expressed in the brain from embryonic day 13 until 2 years of age.

To identify cells producing HDGF in the nervous system, *in situ* hybridization was performed. Eight-week-old male mice brains were carefully removed, frozen, and 15 $\mu$ m-thick serial sections were used. HDGF mRNA was highly expressed in neurons, especially in hippocampal neurons and Purkinje cells. To examine the localization of HDGF protein in the nervous system, immunohistochemical experiments were carried out using 8-week-old male mouse brains. Brains were fixed in the 4% paraformaldehyde in PBS and embedded in paraffin, and the blocks were sectioned 6 $\mu$ m thick. HDGF was highly stained in the nuclei of most of the hippocampal neurons, and Purkinje cells. Thus, HDGF is produced in the neurons and retained in their nuclei.

2). HDGF and HMG1 are secreted in physiological conditions.

Using the mouse primary hippocampal neurons and mouse neuroblastoma cell line Neuro2a, we tested whether HDGF and HMG1 were secreted. Mouse primary hippocampal neurons were prepared from embryonic day 17 C57/BL6 mouse embryos and seeded onto dishes at a density of $1\times10^5/cm^2$. The medium conditioned by incubation with the hippocampal neurons was concentrated 100-fold. For analyses of Neuro2a, $1\times10^6$ cells were seeded onto a 10 cm tissue culture dish. The concentrated conditioned media and cell extracts were used for Western blot analysis with anti-HDGF polyclonal antibody (C-terminus) or anti-HMG1 polyclonal antibody. Both HDGF and HMG1 were detected in the conditioned media and cell extracts by Western blot analysis. The results indicated that both HDGF and HMG1 were secreted in neuronal cells.

3). Necrotic cells release HDGF.

We tested whether HDGF was released in pathological conditions. Neuro2a cells were either induced to undergo necrosis by treatment with 10 $\mu$M ionomycin and 20 $\mu$M carbonyl cyanide 3-chlorophenylhydrazone (CCCP) for 24 h, or induced to undergo apoptosis by treatment with 300 nM etoposide. Neuro2a cells without treatment served as a control. For Western blot analysis, the media from treated and untreated cells were collected and concentrated ten fold. For staining, Neuro2a cells were seeded onto chamber slides. Western blot analysis showed that HDGF was not detected in the conditioned medium from apoptotic cells, while it was detected in the conditioned medium from untreated cells and necrotic cells. Immunohistochemical analyses revealed that HDGF was localized in the nuclei of untreated cells and apoptotic cells, while HDGF was found neither in necrotic nuclei nor cytoplasm.

4). HDGF is a neurotrophic factor.

After the mouse primary hippocampal neurons were incubated with a serum-free defined medium supplemented with B27 for 6 h, the medium was replaced with serum-free defined medium lacking B27. Neurons were cultured for 5 days in the serum-free defined medium with HDGF (1-500 ng/ml) or BDNF (10 ng/ml) or medium alone. Cell viability and cell death were assessed using the MTT colorimetric assay and by measuring lactate dehydrogenase (LDH) activity, respectively. Although compared to BDNF, a ten-times higher concentration of HDGF was required for similar activity, we found that HDGF had a dose-dependent neurotrophic effect on hippocampal neurons.

Conclusion : In the present study, we found that HDGF had a neurotrophic effect. Peculiar to HDGF among neurotrophic factors is that it is localized in the nuclei in neurons, and it has the potential to function in physiological and pathological conditions from the mouse embryonic period through to adulthood.

## 論文審査の結果の要旨

Hepatoma-derived growth factor (HDGF) はヒト肝細胞癌由来培養株 Huh-7 の培地から精製された蛋白である。HDGF は多くの細胞で発現しており、増殖因子として働くことは、肝細胞、内皮細胞はじめ、多くの細胞で報告されている。しかし、中枢神経系においては、ノザンプロット法にて発現が報告されているものの、局在、機能は不明であった。本研究では、ウエスタンブロット法、*in situ* hybridization 法、免疫組織染色法を用いて HDGF の産生と蛋白の局在を明らかにした。HDGF 蛋白の発現は胎生期から老齢期まで観察された。HDGF は、成熟マウスでは、主に神経細胞で産生され、その核に局在していた。また、HDGF は、生理条件下で分泌され、壊死条件下では放出され

ることを示唆する実験結果を提示している。さらに海馬神経細胞培養系を使って、リコンビナント HDGF が神経栄養因子として働くことを示した。

これらの結果は、HDGF の増殖因子以外の神経系における機能を示唆するもので、今後生体内での生理的役割および神経変性疾患における関与などの解明が期待される。その基盤となる研究として、博士（医学）の学位論文として価値があるものと認める。