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論文内容の要旨

Monoclonal antibody (MAb) is a useful tool to study the molecular basis of development, construction and function of the nervous system. MAb technique can find unknown molecules which exhibit characteristic spatial or temporal expression patterns, possibly playing significant roles on the nervous system. A protein antigen recognized by a MAb, designated HPC-1, localized in the plasma membrane of the amacrine cell somas and the inner plexiform layer (IPL) in rat retinae. MAb HPC-1 recognized several proteins of about 35 kDa in SDS-PAGE.

In the chapter I of this thesis, an MAb HPC-1-positive cDNA clone, HPC-113, was isolated from a rat hippocampus cDNA library constructed in a lambda phage vector, λgt11, which expressed β-galactosidase/cDNA fusion protein. The rabbit antiserum raised against the β-galactosidase/HPC-113 fusion protein showed the almost same characteristics both in immunoblotting and immunohistochemical studies as those with MAb HPC-1 in the rat retina. Thus it was concluded that HPC-113 coded for the antigen molecule(s) recognized by MAb HPC-1. HPC-113 had 894-bp nucleotide sequence in the same open reading frame of *E. coli* β-galactosidase gene and followed by a 1326-bp possible 3' noncoding sequence, and the calculated molecular weight of the deduced amino acid (298 residues) was 33989 Da, implying that

HPC-113 contains almost the full-length coding region of HPC-1 mRNA. The hydrophathy profile of the deduced amino acid sequence showed the presence of an obvious hydrophobic region at the carboxyl-terminal end, suggesting that HPC-1 antigen is an integrated membrane protein. These results were comparable to the results of biochemical and immunohistochemical studies all of which indicated that HPC-1 should be tightly associated with plasma membrane. Although HPC-1 antigen sequence had no typical amino(N)-terminal signal peptide sequence which was required for secretory and membrane proteins, it was suggested that the large N-terminal side was in the extracellular domain, since MAb HPC-1, of which the epitope was in the N-terminal side, reacted with living cells. Thus, HPC-113 might not include the complete full-length of the coding region of HPC-1 antigen molecule. On the other hand, the *in vitro* transcription/translation product from methionine, the 11th residue of the 298-deduced amino acid sequence, co-migrated with the lowest band of HPC-1 antigens detected in the immunoblot analysis. The cDNA probe, the insert of λHPC-113, detected single 2.4-kb mRNA in the RNA blot analysis, and the S1 nuclease protection analysis probed with the coding region of cDNA also indicated that there was only single kind of mRNA for HPC-1 antigen. Therefore, it might be possible that HPC-1 antigen was translated from Met^{11} and its heterogeneity was generated by any posttranslational modifications. However, sufficient understanding about the HPC-1 antigen mRNA, its primary structure and heterogeneity are not attained at present. Secondary structure prediction analysis revealed that a part of HPC-1 antigen protein formed α-helical structure with the periodical heptad repeats by hydrophobic amino acids, which was usually seen in fibrous proteins with dimer or trimer coiled-coil

structures. These results implied that HPC-1 molecule might bind to other proteins by its intra- or inter-polypeptide chain association capacity. Although the whole amino acid sequence did not show significant homology to any known proteins so far, a few local sequences in the N-terminal side had notable homologies with some partial sequences in mouse laminin B1 chain, a subunit of laminin. Its well known that laminin, an extracellular matrix protein showed various biological function in the nervous system. Interestingly these homologous sequences in laminin B1 were involved in the fragments which revealed neurite-outgrowth and/or survival promoting activity.

cDNA clones for bovine HPC-1 antigen were also isolated. The longest cDNA clone, BHPC-109, revealed high nucleotide sequence homology in the coding region (91.3 %), whereas, homology of 3' noncoding regions was lower than that of coding region. The 5' end was similar to that of HPC-113, indicating that reverse transcriptions were stopped at this 5' portion of both of rat and bovine mRNAs of which sequences possessed quite high G-C contents. The comparison between the deduced amino acid sequences of rat and bovine represented remarkable conservation (98 %), suggesting a physiological significance of HPC-1 antigen through the mammalian evolution.

In the chapter II, tissue distribution of HPC-1 antigenecity and its mRNA was studied by biochemical and histochemical methods. Immunoblot analysis showed that the antiserum against the fusion protein described above detected several proteins of about 35 kDa in the neuronal tissues (retina, cerebral cortex, hippocampus, cerebellum and spinal cord), but no proteins is detected in the non-neuronal tissues (liver, kidney, heart, muscle and adrenal). On the immuohistochemistry of rat nervous system, HPC-1 antigen was

also observed specifically in the nervous system: the matrices of cerebral cortex and hippocampus (particularly in stratum radiatum); molecular layer, membrane of granular cell soma and gromeruli in cerebellum; gray matter of spinal cord. However, little staining was detected in white matters of the central nervous tissues. The RNA blot analysis also indicated nervous system-specific expression of HPC-1 mRNA. In the nonneuronal tissue, however, the high sensitive RNA polymerase chain reaction assay revealed presence small amount of a HPC-1 gene transcript which appeared to be closely related to but distinguishable from the neuronal HPC-1 gene transcript. *In situ* hybridization was performed by the nonradioactive detection method to identify cellular localization. HPC-1 mRNA was present in most of neurons in the central and peripheral nervous systems except for retina. In the retina, signals were detected in amacrine cells, and also in ganglion cells which HPC-1 immunoreactivity was not present in the soma, suggesting selective localization of HPC-1 mRNA in the IPL or the axon terminal. Amount of HPC-1 antigen(s) gradually increased in accordance with development or the IPL formation in chick retina. Considering from accumulation of HPC-1 antigenecity in the hippocampal stratum radiatum, cerebellar gromeruli, and retinal IPL, HPC-1 antigen may associate to synaptic formation and/or maintenance of neurons.

In conclusion, it was proved that HPC-1 antigen(s) was a novel class of membrane protein(s) of 35 kDa with α-helical structure containing typical heptad repeats which related to association between other polypeptide. A few local sequences had notable homology to some partial laminin sequences that were included in the fragments baring neurite-outgrowth and/or survival promoting activity. HPC-1 antigen(s) was expressed predominantly

in the neuronal tissues with characteristic localization, such as accumulation into synapse-rich regions, but discrepancy between immunoreactivity and presence of mRNA in subpopulations of neurons should be solved in future.

論文の審査結果の要旨

本論文は神経細胞に特異的な膜蛋白であるHPC-1抗原につき、cDNAクローニングを行い、その分子構造と細胞・組織内分布を解析・考察したものである。HPC-1はそのモノクローナル抗体が網膜でアマクリン細胞の細胞膜にのみ結合することから、細胞識別、組織形成、シナプス伝達などでの役割が想定される分子である。この研究の目的はこの分子の構造と機能を明らかにすることである。発現ベクターによるラット脳cDNAライブラリーを抗体でスクリーニングすることによってcDNAクローニングを行い、塩基配列を決定した。ウシについてもクローニングし、ラットと一致することを示した。これがコードする蛋白質は分子量約3万5千で、C末端に膜結合領域を持ち、他の部は親水性の強いと考えられた。ラミニンに類似の部位がある他は既知の蛋白質とはまったく異なり、新しい蛋白質であることがわかった。またこの遺伝子を解析し、発現機構に考察を加えた。作成した特異抗体及びアンチセンスRNAプローブを用いて、HPC-1蛋白及びmRNAの組織分布と発生による変化を明らかにした。その生理的役割を明らかにするため、遺伝子導入実験、培養細胞による実験を行っている。

以上のごとく、本研究は神経科学領域において進展が期待されている神経特異分子の同定と解析につき、その一つであるHPC-1の研究を分子生物学、細胞生物学の技術を駆使して遂行したものである。その成果の一部は学会での発表や論文掲載(J. Biol. Chem. 267:10613, 1992)によっても評価を得ており、博士（学術）の学位に値するものと判定した。