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論文内容の要旨

Protein tyrosine phosphorylation plays a crucial role in regulation of cell proliferation, differentiation and transformation. The level of tyrosine phosphorylation is determined by the balance between the activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Molecular cloning of PTPs have revealed that PTPs comprise a diverse family of cytoplasmic and transmembrane enzymes. The receptor-like PTPs (RPTPs) are structurally composed of intracellular one or two phosphatase domains, a single transmembrane domain and extracellular segments. Extracellular segments of several RPTPs display structural motifs that are suggestive of a role in cell-cell or cell-extracellular matrix interactions, such as immunoglobulin (Ig)-like domains, fibronectin type Ⅲ (FN-Ⅲ)-like domains and carbonic anhydrase (CAH)-like domains.

RPTPγ belongs to RPTPs and shows a high degree of structural similarity to PTPζ/RPTPβ, a nervous system-specific RPTP. The extracellular regions of these molecules contain a CAH-like domain at the N-terminal, followed by a FN-Ⅲ-like domain. In the central nervous system, RPTPγ is up-regulated during cortical development and maintained in the regions containing postmitotic neurons, suggesting that it plays a role in neuronal differentiation. Despite these observations, RPTPγ gene products have not been fully characterized.

In this study, he identified four isoforms of RPTPγ from rat brain by cDNA cloning. They designated these molecules as RPTPγ-A, -B, -C and -S. RPTPγ-A was the longest form, contained an open reading frame of 4,326 bp encoding a protein of 1,442 amino acids, and had the same structure as human and mouse RPTPγ reported. RPTPγ-B lacked intracellular juxtamembrane 29 amino acids of RPTPγ-A. RPTPγ-C is a protein of 1,176 amino acids. The sequence of this isoform differed from that of RPTPγ-A from nucleotide residue 3,503, and terminated translation after adding 9 novel amino acids resulting in loss of the carboxy-terminal phosphatase domain. RPTPγ-S was a protein of 717 amino acids. The sequence of this isoform from nucleotide residue 2,147 was distinct from that of RPTPγ-A, where the translation terminated after adding 2 amino acids causing loss of the transmembrane segment and the intracellular region. Thus, RPTPγ-S is an extracellular variant of RPTPγ. mRNAs of the four isoforms were expressed in the brain, kidney, lung and heart. By Northern blot analysis, four RPTPγ transcripts of about 9.0, 7.0, 4.5 and 3.5 kb were detected in the rat brain. The former two transcripts were expressed at high levels and the latter two transcripts were expressed at low levels. The 9.0- and 7.0-kb transcripts likely encode RPTPγ-A and -B, and the 4.5-kb transcript likely encodes RPTPγ-S.

Transfection of RPTPγ-A and -S expression plasmids into COS7 cells

resulted in the expression of membrane-bound 190-kDa and secreted 120-kDa proteins, respectively. These proteins were highly glycosylated by sialic acid. However, RPTPγ isoforms were not expressed as proteoglycans like PTPζ/RPTPβ. In RPTPγ-A-expressing PC12D cell lines, a doublet RPTPγ-A protein bands with apparent molecular mass of 184 and 187 kDa were detected, suggesting that RPTPγ-A is proteolytically cleaved in PC12D cells.

To elucidate the functions of RPTPγ in neuronal cells, he first established PC12D cell clones expressing rat RPTPγ-A. PC12D cells, a subline of PC12 cells, extends neurites immediately in response to NGF, FGF or cAMP. In PC12 and PC12D cells, the 3.5-kb transcript was the sole transcript of RPTPγ, and enzymatically active RPTPγ isoforms were not expressed. RPTPγ-A-expressing PC12D cells showed remarkably reduced neurite outgrowth in response to NGF. Surprisingly, there were no obvious differences in tyrosine-phosphorylation of major cellular proteins between control and RPTPγ-A-expressing transfectants. Activation of MAP kinases was equally detected in the RPTPγ-A-expressing cells as well as in control cells. These results suggest that RPTPγ functions at the downstream of MAP kinases or through independent signal transduction pathways.

Rabin et al. (1993) reported that tyrosine phosphorylation of some proteins purified with $p13^{suc1}$ beads was augmented by NGF in PC12 cells. $p13^{suc1}$ was initially identified as a gene product which rescued $p34^{cdc2}$ mutations. Recently, it was also reported that complexes purified from squid axoplasm with $p13^{suc1}$ beads contained cdc2-like kinases, some other kinases and cytoskeletal proteins (Takahashi et al., 1995). These results suggested that $p13^{suc1}$ complexes are involved in phosphorylation/dephosphorylation processes underlying axonal cytoskeletal dynamics. Therefore, he next examined phosphorylation of the complexes purified with $p13^{suc1}$-agarose, and found that the pattern of protein phosphorylation was different between control and RPTPγ-A-expressing transfectants, which was not detected in the total cell lysates; tyrosine-phosphorylation of 140- and 117-kDa proteins were specifically reduced in the $p13^{suc1}$-complexes from RPTPγ-expressing cells. These 140- and 117-kDa proteins may be directly dephosphorylated by RPTPγ or indirectly through activation of other protein tyrosine phosphatases. $p13^{suc1}$-complexes from PC12D cells also contained cytoskeletal proteins such as actin, tubulin and neurofilaments, and kinases including cdk5 and MAP kinases. While the association of tubulin and actin with $p13^{suc1}$ beads-complexes did not change after NGF-treatment, the binding of MAP2 to the complexes and phosphorylation of neurofilaments in the complexes increased rapidly. MAP2 is a high molecular weight, neuron-specific phosphoprotein copurified with microtubules, and neurofilaments are neuron-specific proteins that constitute the major cytoskeleton in axons.

Since neurite formation and extension are regulated by cytoskeletal proteins and their associated proteins, abnormality of tyrosine phosphorylation in this complex caused by RPTPγ expression may explain the inhibition of neurite extension in RPTPγ-A-expressing cells.

Neurite extension and axon guidance are regulated by neurite outgrowth -promoting and -inhibiting molecules distributed in the local environment. Recently, it was suggested that activities of RPTPs might be down-regulated by the ligand-mediated dimerization (Bilwes et al., 1996). Ligand molecules of RPTPγ may control neurite extension or axon guidance by regulating RPTPγ activity: in the absence of ligands, active RPTPγ on neuron may dephosphorylate the proteins such as 140- and 117-kDa proteins and inhibit the neurite outgrowth, while ligand binding to RPTPγ may lead to dimerization and inactivation of this receptor resulting in promotion of neurite outgrowth.

The RPTPγ-expressing PC12D transfectants suggested that RPTPγ plays an important role in neuronal differentiation. The findings obtained in this study will contribute to elucidating the regulation mechanism of neurite extension in neuronal cells. From this standpoint of views, it is important to characterize the tyrosine phosphorylated 140- and 117-kDa proteins. Moreover, identification of ligand molecules which regulate RPTPγ activity is a pre-requisite for revealing the functional roles of RPTPγ in the brain development. The RPTPγ-expressing PC12D transfectants which he prepared here will be useful for these investigations.

審査結果の要旨

本研究は受容体型プロテインチロシンホスファターゼγ(PTPγ)の分子多様性と神経分化における機能を明らかにすることを目的としている。

申請者はまずラット脳からPTPγ cDNAのクローニングを行い、数多くのcDNAクローンの一次構造解析の結果、RNAスプライシングの違いによって生ずる4つの分子種（PTPγ-A,B,C,Sと命名）を見い出した。この内PTPγ-Aと-Bについてはマウスにおいて既に報告されている分子種に相当したが、PTPγ-Cと-Dは新規の分子であった。PTPγ-Cは第二PTPaseドメインを欠く分子種であり、PTPγ-Sは細胞外へ分泌される分子種（膜貫通ドメインと2つのPTPaseドメインを欠く）であった。脳内でのRNA発現レベルを調べた結果、PTPγ-A及び-Bが主要な分子種であること、また4分子種ともに神経ネットワークが形成される出生期前後をピークにした発現を示すことが明らかとなった。また、培養細胞を用いてこれらのcDNAを発現させた結果、PTPγは一次構造が類似した同一ファミリー分子であるPTPζとは異なり、コンドロイチン硫酸等のグリコサミノグリカン鎖を含むプロテオグリカンとしては発現しないことが示された。

次に神経分化におけるPTPγの機能を見るため、NGF刺激に応答して神経細胞に分化することが知られているPC12細胞の亜株であるPC12D細胞を用いて、PTPγ-Aを構成的に発現する細胞株を樹立し、その神経分化に与える効果を解析した。PC12D細胞はNGFあるいはcAMP刺激に応答して、遺伝子発現を介さず直ちに神経突起を伸長する性質を有する。申請者はPTPγを発現したPC12D細胞がNGF及びcAMP応答性の神経突起伸展能を欠失することを発見した。この時、MAP kinase(ERK1,2)を始めとした主要な細胞内蛋白のチロシンリン酸化レベルに変化は見られなかった。しかしながら、$p13^{suc1}$ complex中の蛋白を調べた結果、117kDaと140kDaの分子のチロシンリン酸化レベルが、PTPγ発現細胞においてほとんど消失していることを見出した。また、PC12D細胞より単離した$p13^{suc1}$ complex中には種々のプロテインキナーゼ、ニューロフィラメント、チューブリン等の細胞骨格分子が集合しており、その集合体形成はNGF刺激に応じてダイナミックに変化することを示した。

以上、本研究は、PTPγの分子多様性を明らかにすると共に、脳形成の神経細胞の分化過程におけるPTPγの役割について有力な手掛かりを与えるものであり、審査委員は全員一致で博士論文として充分なものと結論した。

また、提出された学位申請論文の研究内容についての説明を30分行わせ、審査委員4人による口述審査をした。その後、判定会議を行い、1）研究内容の学問水準、2）英語力、3）国際誌での論文公表の三点について、申請者は、学位を受けるに十分足る水準に達していると判定した。