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学位論文の内容の要旨

専　攻	分子情報制御医学	部　門	分子細胞医学
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論文題目	**Comparative analyses of isoforms of the calcium-independent phosphatidylethanolamine *N*-acyltransferase PLAAT-1 in humans and mice**		

Introduction:

N-Acyl-phosphatidylethanolamine (NAPE), first isolated from infarcted myocardium of dogs, is a unique membrane phospholipid found in trace amounts in animals and plants under normal physiological conditions. Its unusual structure in terms of having three acyl chains is reported to stabilize cell membrane. More importantly, NAPE serves as the precursor of a lipid mediator *N*-acylethanolamine (NAE) including anti-inflammatory palmitoylethanolamide, anorectic oleoylethanolamide and the endocannabinoid arachidonoylethanolamide.

In mammals, NAPE is enzymatically synthesized by *N*-acyltransferase which transfers an acyl chain of glycerophospholipid to the amino group of phosphatidylethanolamine (PE). So far, it has been revealed that two groups of *N*-acyltransferase are present on the basis of Ca^{2+}-dependency. The phospholipase A/acyltransferase family consisting of five members (PLAAT-1–5) function as Ca^{2+}-independent *N*-acyltransferase, while cytosolic phospholipase $A_2\varepsilon$ ($cPLA_2\varepsilon$) was identified as Ca^{2+}-dependent *N*-acyltransferase. Among these enzymes, PLAAT-1 particularly drew attention since this *N*-acyltransferase is highly expressed in some organs like brain, heart, skeletal muscle and testis where NAPEs and NAEs rapidly accumulate under certain pathological conditions. Recent database search revealed the presence of an uncharacterized isoform of PLAAT-1 which has an extra polybasic sequence consisting of about 100 amino acids at the N terminus. In this study, I examined the occurrence, intracellular localization, and catalytic properties of this long isoform, as well as the original short isoform from humans and mice.

Methods:

Both the short and long isoforms of PLAAT-1 were cloned from human and mouse origins, and their recombinant proteins expressed in COS-7 cells were purified and used to examine their catalytic properties. The *N*-acyltransferase activity forming [^{14}C]NAPE was assayed using

[^{14}C]phosphatidylcholine and non-radioactive PE as acyl donor and acyl acceptor, respectively. Tissue distributions of PLAAT-1 isoforms were investigated by reverse transcription (RT)-PCR. The localization of enhanced green fluorescent protein-fused PLAAT-1 short and long isoforms in COS-7 cells were observed by confocal laser microscopy. Finally, [^{14}C]ethanolamine-labeled intact COS-7 cells were analyzed to measure cellular NAPE and NAE levels.

Results:

As assayed with the purified recombinant proteins, both of long and short isoforms of human and mouse PLAAT-1 produced NAPE Ca^{2+}-independently. RT-PCR experiments showed that human tissues express only the long isoform of PLAAT-1 but not the short isoform at all. In contrast, mice expressed both the isoforms with different tissue distribution. Unlike the exclusive cytoplasmic localization of the short isoform, the long isoform was found in both cytoplasm and nucleus, suggesting that the extra polybasic sequence contains a nuclear localization signal. Moreover, the overexpression of each isoform largely increased cellular NAPE and NAE levels in intact cells.

Discussion:

My study revealed that PLAAT-1 long isoform possesses *N*-acyltransferase activity almost as high as the short isoform and exists in both cytoplasm and nucleus. Besides, the expression of PLAAT-1 short isoform was utterly absent in humans while mice expressed both the isoforms. These results clarified that PLAAT-1 long isoform is a functional protein, showing the first evidence of a possible NAPE generation within the nucleus. This suggests that PLAAT-1 may utilize phospholipids of nuclear membrane to produce NAPE as a precursor of bioactive NAE.

The finding of this study is also significant for future research works on PLAAT-1, for example, the generation and analysis of PLAAT-1 knockout mice to elucidate its physiological significance and to develop PLAAT-1-specific therapeutic tools to manage clinical conditions related to its function.

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