

## 論文の内容の要旨

論文題目 Study on biological functions of Pentraxin 4 in liver

(肝臓における Pentraxin 4 の機能解析)

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Animals maintain the energy balance by shifting the source from carbohydrates to triglycerides during starvation like torpor. However, an initiator for this shift has not yet been identified. Here, we show that a newly defined lectin-like molecule, pentraxin 4 (Ptx4), is one of the candidates to take the oxidative balance between carbohydrates and triglycerides. Our previous study suggested that there might be a hepatic sensing system that can detect glycogen shortage, and this system can activate a live-brain-adipose neural network to shift the energy source from glycogen to triglycerides to supply glucose in mouse. To elucidate a key molecule which regulates the balance of use between glycogen and triglycerides, we sorted the Ptx4 gene as its candidate one, which might act like in a neuroactive ligand-receptor interaction, by conducting a microarray gene-annotation enrichment analysis. The Ptx is a superfamily of evolutionarily conserved molecules with multi-functional roles in innate immunity and inflammation such as regulation of complement activation and opsonization of pathogens, and in tumor progression through activating their unique receptors and pathways. C-Reactive protein and serum amyloid P-component belong to the short form of Ptxs. However, Ptx4 belongs to the long form of Ptxs and is a soluble and multifunctional pattern recognition protein with a cyclic multimeric structure due to the presence of an unique N-terminal domain. Here, we show that Ptx4 has a new crucial role on the energy metabolism of our bodies.

The present study showed that Ptx4 protein is detected mainly in liver, muscle, brain, spleen, and testes, but not in heart, pancreas, and thymus in mouse, as revealed by immunofluorescence microscopy. In mouse liver, Ptx4 appeared to be localized in cytoplasm and plasma membranes, and the

immunofluorescent signal was detected in the parenchymal cells but not in the blood vessel cells. Further analysis with use of a Nipkow disc confocal unit detecting for a single-molecule imaging showed that Ptx4 is clearly localized at the plasma membrane and at the same time in the cytoplasm. Ptx4 was expressed in the marginal zone of the red pulp of mouse spleen, and the immunofluorescent signal was strongly associated with the olfactory bulb in the central nervous system of mouse brain. Since Ptx4 was considered to be a secretory protein based on the UniProt database, proteins from the culture medium of Hepa1-6 cells with overexpressing the Ptx4 gene was subjected to Western blot analysis using an antibody against mouse Ptx4 as raised by ourselves. The results showed that the culture medium contains Ptx4 protein, suggesting that Ptx4 is indeed a soluble protein, and secreted into outside of the cells. The molecule was also presumed to have an activity of lectin that recognizes a specific glycan since Ptx3 has a carbohydrate-binding domain. Glycan array analysis with a series of carbohydrate structures revealed that Ptx4 prefers to bind to oligosaccharides with the tandem repeat structure, (Gal $\beta$ 1 $\rightarrow$ 4GlcNAc) $_n$ , while glycogen debranching enzyme and glycogen phosphorylase as controls showed different binding patterns toward glycans from that of Ptx4. This may indicate that Ptx4 has a new physiological function in which Ptx4 secreted acts like a lectin by binding to a glycan with the unique structure at the cell surface.

The functional analysis of Ptx4 was performed by creating the Ptx4-gene over-expression and knock-down *in vitro* and *in vivo* models. When the Ptx4 gene was over-expressed with use of an adenovirus vector in mouse, a total glycogen content in liver decreased while a total triglyceride content in liver increased. In fact, the expression of the genes involved in the glyconeogenesis such as glycogen phosphorylase (Pygl), glucose 6-phosphatase (G6pc), phosphoenolpyruvate carboxykinase 1 (Pck1), and peroxisome proliferator activated receptor  $\gamma$ -coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), the genes involved in the ketone body production such as D- $\beta$ -hydroxybutyrate dehydrogenase (Bdh1) and

pyruvate dehydrogenase E1-component subunit  $\alpha$  (Pdh $\alpha$ 1), the genes involved in the fatty acid oxidation such as carnitine palmitoyltransferase 1A (Cpt1a) and alternative oxidase (Aox), the gene involved in the fatty acid translocation, a cluster of differentiation 36 (CD36), and the genes involved in lipogenesis such as sterol regulatory element-binding transcription factor 1 (Srebf1), fatty acid synthase (Fasn), stearoyl-CoA desaturase 1 (Scd1), acetyl-CoA carboxylase 1 (Acc1) and carbohydrate-responsive element-binding protein (ChREBP), increased in mouse liver and Hepa1-6 mouse hepatoma cells. On the contrary, the expression of most of the genes as listed above decreased when the Ptx4-gene was knocked down in these model systems. These results indicate that the Ptx4-overexpression is associated with increased fatty acid oxidative activity and altered metabolic patterns, which appears to be a shift from the carbohydrate fuel to the lipid fuel. This metabolic shift is then enhanced by the increased  $\beta$ -oxidation of fatty acids, and induced the complete oxidation of glucose by pyruvate dehydrogenase kinase (PDK). In fact, the expression of the PDK4 gene increased significantly in the Ptx4 gene-overexpressed cells, and that its gene-expression tended to decrease in the Ptx4-knockdown cells. This mirror image showed that the PDK4-gene expression is coordinated with that of Ptx4, which suggests the existence of a new Ptx4-PDK4 pathway.

In summary, the present study shows that Ptx4 is expressed in mouse liver, skeletal muscle, brain, spleen, and testes, and that it may have a physiological function in liver regulating the biosynthesis and the degradation of carbohydrates and triglycerides as energy sources through the Ptx4-PDK4 pathway. It is also speculated that Ptx4 secreted extracellularly and located on the cell surface acts as a lectin that recognizes a glycan with a specific carbohydrate structures such as a tandem repeat, and induce a clustering of an unknown cell surface receptor to activate the intracellular signaling pathway(s), thus adjusting the energy balance in the organisms. Further study is required before reaching the conclusive roles of Ptx4 molecule.