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SUMMARY OF DOCTORAL THESIS

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Title: **Novel roles of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and lipocalin-type prostaglandin D synthase in adipogenesis program of cultured preadipocytes**

(培養前駆脂肪細胞の脂肪形成プログラムにおける 15-デオキシ-$\Delta^{12,14}$-プロスタグランジン J_2 とリポカリン型プロスタグランジン D 合成酵素の新規の機能に関する研究)

Prostaglandins (PGs) and related compounds are called "local hormones" that are synthesized from polyunsaturated fatty acids including arachidonic acid as a major essential fatty acid, and provide specific effects on target cells close to their site of the biosynthesis. Almost all the mammalian cells except red blood cells produce prostaglandins and their related compounds such as prostacyclins, thromboxane, leukotrienes and other bioactive lipids that are collectively known as eicosanoids. Biosynthesis of prostaglandins through the cyclooxygenase (COX) pathway involves oxidation and subsequent isomerization of membrane-derived free arachidonic acid via three sequential enzymatic reactions. The process is initiated through the release of arachidonic acid from membrane phospholipids, a reaction catalysed by phospholipases A_2 that catalyzes the hydrolysis of arachidonic acid from *sn*-2 position of phospholipids. The released arachidonic acid is then subsequently metabolized to prostaglandin H_2 by the action of COX enzymes. There are two isoforms of COX. Of these, COX-1 is constitutively expressed in most of the cells and is mainly utilized in the immediate prostaglandin biosynthesis important for homeostasis and certain physiological functions. On the other hand, COX-2 is expressed inducibly and is utilized for delayed prostaglandin biosynthesis. The final step for the biosynthesis of prostaglandin is the conversion of prostaglandin H_2 to various bioactive derivatives such as PGD_2, PGE_2, $PGF_{2\alpha}$, and PGI_2 by their specific terminal synthases, such as PGD synthase (PGDS), PGE synthase, PGF synthase, and PGI synthase respectively. The J_2 series of PGs including PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2 and Δ^{12}-PGJ_2 are generated non-enzymatically from PGD_2 through the dehydration and isomerization. Alternatively, PGD_2 can be enzymatically converted to 11β-$PGF_{2\alpha}$ by the action of enzyme PGD 11-keto-reductase in some tissues. Among the prostaglandins, PGE_2 and $PGF_{2\alpha}$ have been shown to serve as anti-adipogenic prostanoids that exert their effects through the binding to the specific cell-surface receptors of EP receptors and FP receptor. Nevertheless, it remains still unclear regarding the role of these endogenous prostanoids synthesized by preadipocytes only during the growth phase in adipogenesis program leading to the differentiation and maturation of adipocytes. On the other hand, peroxisome proliferator-activated receptor γ (PPARγ), a member of nuclear hormone receptor and a ligand-dependent transcription factor, is a master regulator of adipocyte differentiation. The PGJ_2 derivatives are effective to be active ligands for PPARγ and to stimulate adipogenesis during the differentiation and maturation of cultured adipocytes. Cultured 3T3-L1 cells have been shown to express specifically lipocalin-type PGD synthase (L-PGDS) during the progress of adipogenesis associated with co-expression of PPARγ. However, the role of intracellular L-PGDS is still complicated because PGD_2 and the related metabolites or derivatives exert other different effects on the function of adipocytes. For example, 9α,11β-$PGF_{2\alpha}$, an enzymatic degraded product of PGD_2, acts as an anti-adipogenic factor in cultured adipocytes. In addition, 15d-PGJ_2 has been implicated in the inhibition of inflammatory response associated with inducible synthesis of COX-2. Moreover, PGD_2 also acts through the cell-surface receptors with different subtypes such as DP1 and CRTH2 receptors. Interestingly, active form of L-PGDS has been shown to inhibit the phosphorylation of Akt, a serie/threonine kinase. The phosphorylated Akt has been previously implicated in spontaneous

differentiation into adipocytes in a PG-independent manner. Thus, the functions of intracellular L-PGDS in adipocytes at different life stages still remain to be determined. Additionally, the effect of LPGDS product on the biosynthesis of other prostanoids in the adipocytes remains to be obscure.

Initially, I have focused the enhanced biosynthesis of PGE_2 and $PGF_{2\alpha}$ during the growth phase upon stimulation with a mixture of phorbol 12-myristate 13-acetate, a mitogenic factor, and calcium ionophore A23187. Here, I studied the interactive effect of 15d-PGJ_2 on the inducible synthesis of the endogenous prostaglandins in cultured preadipocytes and its implication in adipogenesis program.

15d-PGJ_2 interfered significantly the endogenous synthesis of those PGE_2 and $PGF_{2\alpha}$ in response to cell stimuli by suppressing the induction of COX-2 at both mRNA and protein level. In contrast, Δ^{12}-PGJ_2 and troglitazone, a PPARγ agonist, had almost no inhibitory effects, indicating a mechanism independent of the activation of peroxisome proliferator-activated receptor-γ for the action of 15-PGJ_2. Pyrrolidinedithiocarbamate (PDTC), an NF-κB inhibitor, effectively inhibited on the inducible synthesis of those both PGE_2 and $PGF_{2\alpha}$ in preadipocytes. To explore the molecular mechanism behind the reduced expression of COX-2, we have checked the activation of nuclear receptor NF-κB and degradation of IκB-α in presence or absence of 15d-PGJ_2 and PDTC under the stimulatory condition with a mixture of PMA and A23187. The stimulated nuclear translocation of NF-κB by PMA was significantly inhibited by both 15d-PGJ_2 and PDTC, which effects were further confirmed by the increase in the cytosolic levels of NF-κB. Moreover, 15d-PGJ_2 and PDTC were individually sufficient to inhibit the degradation of IκB-α, an inhibitory protein for NF-κB. Taken together, the findings indicate that 15d-PGJ_2 inhibits degradation of IκB-α as well as activation of NF-κB, resulting in the reduced expression of COX-2. Endogenous PGs generated by preadipocytes only during the growth phase in response to the cell stimuli autonomously attenuated the subsequent adipogenesis program leading to the differentiation and maturation of adipocytes. These effects were prevented by additional co-incubation of preadipocytes with either 15d-PGJ_2 or PDTC although 15d-PGJ_2 alone has no stimulatory effect. Moreover, while treating the cells with PGE_2 and $PGF_{2\alpha}$, 15d-PGJ_2 was unable to block the inhibitory effects of these anti-adipogenic PGs on the adipogenesis program in preadipocytes. The results suggest that 15d-PGJ_2 interferes at the biosynthetic pathway of those anti-adipogenic PGs but not at their receptors or subsequent pathways through which PGE_2 and $PGF_{2\alpha}$ provide anti-adipogenic actions. In short, the findings suggest that 15d-PGJ_2 inhibits the biosynthesis of PGE_2 and $PGF_{2\alpha}$ by inhibiting the activation of NF-κB followed by reduced expression of COX-2. This effect of 15d-PGJ_2 contributes to the up-regulation of adipogenesis during the maturation phase.

15d-PGJ_2 and Δ^{12}-PGJ_2 are nonenzymatic degraded product of PGD_2 which is formed by the action of terminal synthase enzyme called lipcalin-type prostaglandin D synthase. To evaluate the role of L-PGDS in cultured adipocytes and the precursor cells, I attempted to interfere the intracellular expression of L-PGDS in cultured 3T3-L1 preadipocytes by stable transfection with a mammalian expression vector having the full-length complementary DNA of L-PGDS oriented in the antisense direction. The neomycin-resistant gene in the vector was expressed in the cloned transfectants with the vector only or that with antisense L-PGDS, whereas it was not detectable in untransfected parent cells. As expected, the stable transfection of L-PGDS oriented in the antisense direction significantly suppressed the transcript levels and the protein levels of L-PGDS in the cloned transfectants. The cloned transfectants with antisense L-PGDS also exhibited the reduced biosynthesis of PGD_2 and the formation of its dehydration product of Δ^{12}-PGJ_2 from exogenous and endogenous arachidonic acid. By contrast, the synthesis of PGE_2 was not influenced appreciably, indicating that there were no interfering effects on cyclooxygenases and PGE synthases. The stable transfection of antisense L-PGDS induced markedly the stimulation of fat storage in cultured adipocytes compared to the wild type cells during the maturation phase. In addition, the spontaneous accumulation of fats occurred in the transfectants with antisense L-PGDS without undergoing the stimulation with inducing factors. Moreover, the transfectants with antisense L-PGDS expressed aP2, leptin, and GLUT-4 as typical adipogenesis markers at higher levels than other control cells although the expression levels of PPARγ, LPL, and adiponectin remained unchanged, indicating the up-regulation of adipogenesis program. The stimulated adipogenesis was significantly reversed by exogenous anti-adipogenic prostanoids such as PGE_2 and $PGF_{2\alpha}$, while the storage of fats was additionally enhanced by exogenous pro-adipogenic 15d-PGJ_2. These results suggest that the stably reduced expression levels of L-PGDS regulates positively adipogenesis program in a cellular mechanism independent of pro-adipogenic action of PGJ_2 series.