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学 位 論 文 要 旨
SUMMARY OF DOCTORAL THESIS

氏名　Name:　Li Xu

題目　Title:　Gene expression of arachidonate cyclooxygenase pathway and the regulation by metabolic factors during life cycle of adipocytes

脂肪細胞のライフサイクルにおけるアラキドン酸シクロオキシゲナーゼ経路の遺伝子発現と代謝因子による調節

Prostalandins (PGs) have been shown to play diverse roles in adipogenesis and other functions of adipocytes. To determine the role of endogenous PGs, we attempted to investigate the gene expression of isoformic enzymes in the arachidonate cyclooxygenase (COX) pathway and the regulation by metabolic factors during life cycle of adipocytes.

Adipocytes serve not only as a storage depot of fats but also as endocrine cells secreting adipocytokines including tumor necrosis factor α (TNFα). Using preadipogenic 3T3-L1 cells, we attempt to determine the response of adipocytes at different stages of the life cycle to TNFα with respect to the gene expression of the arachidonate COX pathway and the role of endogenous PGs. The gene expression analysis of the COX pathway revealed the marked increase in mRNA and protein levels of COX-2 in response to TNFα in preadipocytes, whereas COX-1 was expressed constitutively. Moreover, the cells at different cycle stages exhibited the specific gene expression of isoformic enzymes of PG synthases for PGs of the D_2, E_2, and $F_{2\alpha}$ series upon exposure to TNFα. The treatment of preadipocytes with TNFα along with calcium ionophore A23187 resulted in the stimulated formation of PGE_2 and $PGF_{2\alpha}$, attenuating the apoptotic cell death induced by TNFα alone. The response of adipocytes to synthesize these PGs declined during the differentiation and maturation phases. The cells during the differentiation phase were the most sensitive to TNFα in terms of the decrease in adipogenesis without the mediation of endogenous PGs. TNFα was also effective in suppressing adipogenesis during the maturation process. Taken together, TNFα can control cell number of preadipocytes as well as the size of fat storage in mature adipocytes. The action of TNFα on preadipocytes can be modulated by the production of endogenous PGs through the induction of COX-2.

Several types of PGs are synthesized in adipocytes and the precursor cells and involved differently in the control of adipogenesis. To elucidate how the PG synthesis is regulated at different stages in the life cycle of adipocytes, we examined the gene expression of arachidonate COX pathway leading to the delayed synthesis of PGE_2 and $PGF_{2\alpha}$ and their roles in adipogenesis after exposure of cultured cells to phorbol 12-myristate 13-acetate (PMA), which is a useful system for monitoring mitogen-induced changes. While the expression of COX-1 remained constitutive, mRNA and protein levels of COX-2 were up-regulated by treatment with PMA. Preadipocytes exhibited higher gene expression of cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) and PGF synthase. In contrast, three isoforms of PGE synthase were expressed constitutively during all phases. The delayed synthesis of PGE_2 and $PGF_{2\alpha}$ following the stimulation for 24 with a mixture of PMA and calcium ionophore A23187 was the highest in

preadipocytes, reflecting the increased expression levels of $cPLA_2\alpha$ and COX-2. Cultured cells treated with PMA during the differentiation phase and then exposed to the maturation medium, or cells treated with PMA in the maturation medium after the differentiation phase showed the suppression of adipogenesis in adipocytes. The attenuating effect of PMA was additionally enhanced when the cell were treated along with A23187 during the differentiation phase, suggesting the involvement of endogenous PGs. The cells at the stages of the differentiation and maturation phases were highly sensitive to exogenous PGE_2 and $PGF_{2\alpha}$, respectively, resulting in the marked suppression of the stored fats in adipocytes. Taken together, these results provided the evidence for the distinct gene expression of isoformic enzymes in the COX pathway leading to the synthesis of PGE_2 and $PGF_{2\alpha}$ and the specific action of these prostanoids at different cycle stages of adipocytes.