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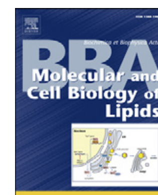

Critical role for cytosolic group IVA phospholipase A_2 in early adipocyte differentiation and obesity

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ABSTRACT

Adipogenesis is the process of differentiation of immature mesenchymal stem cells into adipocytes. Elucidation of the mechanisms that regulate adipocyte differentiation is key for the development of novel therapies for the control of obesity and related comorbidities. Cytosolic group IVA phospholipase A_2 ($cPLA_2\alpha$) is the pivotal enzyme in receptor-mediated arachidonic acid (AA) mobilization and attendant eicosanoid production. Using primary multipotent cells and cell lines predetermined to become adipocytes, we show here that $cPLA_2\alpha$ displays a proadipogenic function that occurs very early in the adipogenic process. Interestingly, $cPLA_2\alpha$ levels decrease during adipogenesis, but $cPLA_2\alpha$-deficient preadipocytes exhibit a reduced capacity to differentiate into adipocytes, which affects early and terminal adipogenic transcription factors. Additionally, the absence of the phospholipase alters proliferation and cell-cycle progression that takes place during adipogenesis. Preconditioning of preadipocytes with AA increases the adipogenic capacity of these cells. Moreover, animals deficient in $cPLA_2\alpha$ show resistance to obesity when fed a high fat diet that parallels changes in the expression of adipogenic transcription factors of the adipose tissue. Collectively, these results show that preadipocyte $cPLA_2\alpha$ activation is a hitherto unrecognized factor for adipogenesis *in vitro* and *in vivo*.

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1. Introduction

Obesity is a very significant health concern worldwide. Although obesity cannot be considered a disease by itself, it is strongly associated with the onset of insulin resistance, type II diabetes and cardiovascular disease [1]. All of these reduce life expectancy and quality, produce early death and collapse healthcare systems. Thus, investigating the mechanisms that support the development of obesity is important for the future discovery of treatments that prevent its occurrence and/or that of its associated disorders.

The expansion of adipose tissue in obesity is caused by an increase in adipocyte number (hyperplasia or adipogenesis) and/or adipocyte size (hypertrophy) [2]. Studies in humans suggest that adipose mass in adulthood is determined by the tight regulation of the number of adipocytes [3]. Adipogenesis is the complex process through which multipotent mesenchymal precursors commit to the adipocyte lineage and eventually mature into new adipocytes [2,4].

Different models of *in vitro* adipocyte differentiation, including 3T3-L1 and cultured embryonic fibroblasts from mice have provided useful information to help unravel the cascade of events that take place during adipogenesis [5]. Under *in vitro* conditions, adipogenesis requires a period of growth arrest by contact inhibition that ends by treatment with adipogenic factors which promote preadipocytes to re-enter the cell cycle and undergo one or two rounds of mitotic clonal expansion (MCE). Later, cells become permanently growth-arrested and undergo terminal adipocyte differentiation [6–8]. The morphological and genetic changes that occur during the process of adipogenic differentiation are regulated by a cascade of transcription factors. After adipogenic induction, cells immediately and transiently express C/EBPβ and C/EBPδ, implicated in the expression of C/EBPα, PPARγ and SREBP-1c. C/EBPα and PPARγ help each other to maintain their elevated expression throughout the life of the adipocyte and activate the expression of most genes that confer and support the adipocyte phenotype, such as FABP4/aP2,

Abbreviation: ADM, adipogenic differentiation medium; AA, arachidonic acid; $cPLA_2\alpha$, cytosolic group IVA phospholipase $A_2\alpha$; MCE, mitotic clonal expansion; ASCs, adipose stem cell; MEFs, mouse embryonic fibroblast; HFD, high fat diet; MTT, 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium-bromide.

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Table 1
Primers used for q-PCR.

Name	Gene	Forward	Reverse
Cyclophilin B	*Ppib*	5′-TGGAAGAGCCAAGACAGACA-3′	5′-TGCCGGAGTCGACAATGAT-3′
C/EBPα	*Cebpa*	5′-CAAGAACAGCAACGAGTACCG-3′	5′-TCACGCCTTTCATAACACATTCC-3′
PPARγ	*Pparg*	5′-TGCCAGTTTCGATCCGTAGA-3′	5′-AGGAGCTGTCATTAGGGACATC-3′
FABP4/aP2	*Fabp4/ap2*	5′-AAGGTGAAGAGCATCATAACCCT-3′	5′-TCACGCCTTTCATAACACATTCC-3′
Lipin-1α	*Lpin1a*	5′-GGTCCCCAGCCCCAGTCCTT-3′	5′-GCAGCCTGTGGCAATTCA-3′
Lipin-1β	*Lpin1b*	5′-CATGCTTCGGAAAGTCCTTCA-3′	5′-GGTTATTCTTTGGCGTCAACCT-3′
Adipophilin	*Plin2*	5′-GACCTTGTGTCCTCCGCTTAT-3′	5′-CAACCGCAATTTGTGGCTC-3'
GLUT4	*Glut4*	5′-TCGTCATTGGCATTCTGGTTG-3′	5′-AGCTCGTTCTACTAAGAGCACG-3′
SREBP-1c	*Srebp1c*	5′-GGAGCCATGGATTGCACATT-3′	5′-CTGAGTGTTTCCTGGAAGG-3′
PLA2 Group			
GIB PLA2	*Pla2g1b*	5'-GTGTGGCAGTTCCGCAATATG-3′	5'-CCTGTCTAAGTCGTCCACTGG-3′
GIIA PLA2	*Pla2g2a*	5′-TGGCTCAATACAGGACCAAGG-3′	5′-GTGGCATCCATAGAAGGCATAG-3′
GIIC PLA2	*Pla2g2c*	5′-GCTGCCAACCCATCTTGAATG-3′	5′-CACAGACTGTTTGTCACACTCA-3′
GIID PLA2	*Pla2g2d*	5′-TGCTGGCCGGTATAACTGC-3′	5′-CTGTGGCATCTTTGGGTTGC-3′
GIIE PLA2	*Pla2g2e*	5′-CCAGTGGACGAGACGGATTG-3′	5′-AGCAGCTCTCTTGTCACACTC-3′
GIIF PLA2	*Pla2g2f*	5′-ACTGGACGGAAGAGCCCAA-3′	5′-GGATGGAGTTTCTGTGTGTGAT-3′
GIII PLA2	*Pla2g3*	5′-AGAGACCACAGGGCCATTAAG-3′	5′-GCTGTAGAATGACATGGTGCT-3′
GIVA PLA2	*Pla2g4a*	5′-CAGCACATTATAGTGGAACACCA-3′	5′-AGTGTCCAGCATATCGCCAAA-3′
GIVB PLA2	*Pla2g4b*	5′-TGGCCCCTAGCCAACTTTG-3′	5′-GTTCTGGCCTCGACTCAGG-3′
GIVC PLA2	*Pla2g4c*	5′-AGGAGCTGAAACATCGGTATGA-3′	5′-CTGCAAAGATGGGATAGGGC-3′
GIVD PLA2	*Pla2g4d*	5′-CTCGAAGGACCCATCAGT-3′	5′-TTCCCGAAGCTTCAGTGTCT-3′
GIVE PLA2	*Pla2g4e*	5′-ATGGTGACAGACTCCTTCGAG-3′	5′-CCTCTGCGTAAAGCTGTGG-3′
GIVF PLA2	*Pla2g4f*	5′-AGCCATACTGCTACGGAAGAC-3′	5′-TTTGGACAACTTATCTGTGTGCT-3′
GV PLA2	*Pla2g5*	5′-CCAGGGGGCTTGCTAGAAC-3′	5′-AGCACCAATCAGTGCCATCC-3′
GVIA PLA2	*Pla2g6*	5′-GCCTCGTCAACACCCTCAG-3′	5′-CCTTCACCCGGAATGGTTC-3′
GVIB PLA2	*Pnpla8*	5′-GCAAGAAGTCTTTGTGGGAAACA-3′	5′-CTCACTTTTGTAAGTCCCTTGGG-3′
GVIC PLA2	*Pnpla6*	5′-CGGGTGCAGAAAACTCCAG-3′	5′-CGCATAATCTTCCGGCCATAGA-3′
GVID PLA2	*Pnpla3*	5′-TCACCTTCGTGTGCAGTCTC-3′	5′-CCTGGAGCCCGTCTCTGAT-3′
GVIE PLA2	*Ppnla2*	5′-CTGAGAATCACCATTCCCACATC-3′	5′-CACAGCATGTAAGGGGGAGA-3′
GX PLA2	*Pla2g10*	5′-GGATTCAGCGAAGCAACCAG-3′	5′-GGAAGTCACACGGCATAGGG-3′
GXIIA PLA2	*Pla2g12a*	5′-TGCCTTTCCTGTAGGCTGTTC-3′	5′-CGCAGGTCTCGTAGCATCTG-3′
GXIIB PLA2	*Pla2g12b*	5′-GAACCTGGCTCAGAGTGACC-3′	5′-GCAGCTCCATGAAGGAATCCA-3′
GXV PLA2	*Pla2g15*	5′-GGGTAACCAGTTGGAAGCAAA-3′	5′-TTGTCAATCCAGCAGTCAATGAT-3′
GXVI PLA2	*Pla2g16*	5′-CAGCCAGCATCAGTCTGCTT-3’	5′-AGGTACAGGGACGGTGACAC-3’

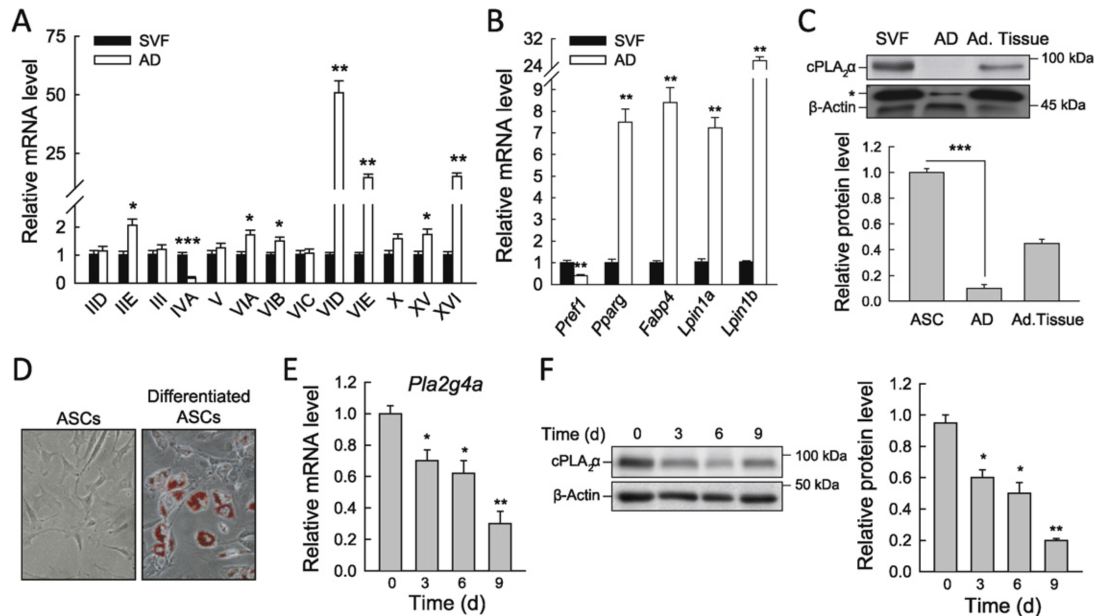

Fig. 1. $cPLA_2\alpha$ expression is reduced during ASCs differentiation. (A) SVF cells and adipocytes (AD) were isolated from mice adipose tissue and the levels of mRNA of the PLA_2 family genes were analyzed by qPCR. (B) qPCR analysis of adipogenesis related genes in samples shown in A. (C) Homogenates from SVF cells, AD and total adipose tissue were analyzed by immunoblot for $cPLA_2\alpha$ expression. β-Actin was used as loading control. Since AD express low levels of β-Actin, asterisk denotes a nonspecific band that shows similar signal for AD and SVF cells. Lower panel shows the relative quantification of $cPLA_2\alpha$ expression against β-Actin. (D) ASCs were isolated and differentiated *in vitro*. Pictures from day 0 and 9 after differentiation are shown. (E) ASCs mRNA levels for $cPLA_2\alpha$ (*Pla2g4a*) were analyzed by qPCR at different time points during adipogenesis. (F) ASCs homogenates were analyzed for $cPLA_2\alpha$ expression by immunoblot. Relative quantification against β-Actin is shown in the lower panel. Error bars represent the SEM of three independent determinations and statistical significance is indicated * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Experiments are representative of at least three different ones.

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