Short communication

Expression of multiple Transient Receptor Potential channel genes in murine 3T3-L1 cell lines and adipose tissue

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Abstract:

Background: Calcium and its signaling have a role in adipogenesis. Transient Receptor Potential (TRP) channels are non-selective cation channels with a high permeability to calcium.

Methods: In the present study the expression of multiple TRP channels on mouse 3T3-L1 preadipocyte and adipocyte cells, white (WAT) and brown (BAT) adipose tissues was investigated using real time PCR (RT-PCR).

Results: TRPV1, TRPV3, TRPM8, TRPC4, TRPC6 were differentially expressed in preadipocytes and adipocytes suggesting their significance in adipogenesis. Genes for multiple TRP channels were also expressed in murine WAT and BAT, out of which TRPV4, TRPV6 and TRPC6 showed differential expression.

Conclusion: Present study demonstrates the expression of TRP channels in mouse cell lines and adipose tissues.

Key words:

adipogenesis, adipocyte, adipose tissue, TRP channel, calcium, 3T3L1 cells, arrays

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Introduction

Several studies have implicated the role of calcium in adipogenesis. An increase in cytosolic calcium in 3T3-L1 preadipocytes resulted in inhibition of the early phases of adipogenesis [6, 9, 11]. Continuous exposure to high extracellular calcium was shown to inhibit preadipocyte

differentiation to adipocytes *via* G-protein coupled mechanism mediated by either a novel calcium sensor or receptor [3]. Multiple calcium signaling pathways are present in human preadipocytes. A study from Hu and colleagues demonstrated the molecular identities of calcium signaling pathways in human preadipocytes [2]. Further, in human adipocytes, increases in cytosolic calcium exhibited a biphasic effect (increase in cytosolic

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calcium inhibited the early stages but enhanced the late stage of differentiation) in the regulation of adipocyte differentiation [1, 8]. Calcium activated downstream signaling in the cell includes both pro- and antiadipogenic factors.

Calcium transport in the cells was also reported to be governed by non-selective cation channels referred as “Transient Receptor Potential (TRP) channels”, which are highly permeable to calcium [7]. Till date, more than 30 mammalian TRP channels have been identified, cloned and characterized. These are grouped into six subfamilies on the basis of their amino acid sequence homology: TRPC (“canonical”), TRPM (“melastatin”), TRPV (“vanilloid”), TRPA (“ankyrin”), TRPML (“mucolipin”), and TRPP (or PKD) (“polycystin”) [7]. TRPs are known to be modulated by chemicals that include dietary factors such as capsaicin (TRPV1 activator), menthol (TRPM8 activator), allicin (TRPA1 activator), 6-gingerol (TRPV1 activator), and polyunsaturated fatty acids (PUFA, TRPV1 activators and TRPC1/5 inhibitors) [12]. Based on calcium permeability of these channels and much published role of calcium and its signaling in adipogenesis, these channels are potentially important in this area. There are multiple reports on TRP channel function in adipocyte biology. Zhang et al. suggested the functional presence of TRPV1 in preadipocytes and adipocytes [13]. Motter et al. suggested no function of TRPV1 in preadipocytes or adipocytes but a role in sensory nerves of adipose tissue [5]. TRPM8 channel was reported in brown adipose tissue (BAT) where its stimulation mediates thermogenesis [4]. Recent reports have suggested that TRPC channels are constitutively active in murine adipocytes and are also expressed in human preadipocytes [2, 10].

The molecular mechanisms of the regulation of adipocyte differentiation and obesity are of considerable scientific interest. In the present study, we studied the expression of calcium permeable TRP channel genes in preadipocytes/adipocytes using 3T3-L1 murine preadipocyte cell lines and mouse adipose tissues (white adipose tissue – WAT and brown adipose tissue – BAT). We have selected 12 TRP channel subtypes out of total 28 TRP channels based on their cumulative properties related to adipogenesis, energy metabolism and nutrition i.e., (i) the calcium permeability of these channels [7]; (ii) available literature on the modulation of these channels by dietary constituents [12]; (iii) temperature related activation of these channels. Also, we have hypothesized the sig-

nificance of these channels in adipogenesis and obesity. TRP channels, in the future, may be the potential targets for regulating adipogenesis, weight gain and obesity.

Materials and Methods

Mouse 3T3-L1 preadipocytes (obtained from National Centre for Cell Science (NCCS), Pune, India) were differentiated into adipocytes (quantified by total lipid content and Oil red O staining) as per American Type Culture Collection (ATCC) guidelines. RNA was extracted from preadipocytes (undifferentiated) and adipocytes (differentiated) using ribopure RNA extraction kit (Ambion, Invitrogen, USA) following the manufacturer’s instructions. Laca mice ($n = 5$) of weight 25 ± 3 g procured from Central Animal House Facility of Panjab University, Chandigarh (India) were used. The animals were housed under standard laboratory conditions (temperature $22 \pm 2^\circ\text{C}$; humidity $55 \pm 5\%$), maintained in a 12 h light and dark cycle and had free access to food (Hindustan Lever Products, Kolkata, India) and water. The experiments were carried out in accordance with the animal experimentation guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA). WAT (visceral region) and BAT (intrascapular) were dissected from the mice. RNA was extracted from WAT and BAT using ribopure RNA extraction kit (Ambion, Invitrogen, USA). The integrity of extracted RNA was verified and quantified using Tecan Nanoquant. One microgram of total RNA in a 20 μl reaction was reverse transcribed using single strand cDNA synthesis kit (Qiagen, USA) as per manufacturer’s instructions. Relative expression of different TRP channels was determined by quantitative PCR (qPCR) (Applied Biosystems 7500 Fast Real-Time PCR machine) using SYBR green based custom designed TRP channel PCR array (CAPM 11044, Custom Mouse RT² profile PCR array, SABiosciences, Qiagen, USA). The conditions for RT-PCR were: 95°C for 10 min, followed by 40 cycles of 95°C and 60°C for 1 min. Gene expression was normalized by GAPDH (for preadipocytes and adipocytes) and TRPP2 (PKD2, for murine adipose tissues). Since the expression of GAPDH showed significant variation in WAT and BAT, TRPP2 was chosen as housekeeping

gene (gene based selection) as its expression remain unchanged in BAT and WAT. Data were analyzed using web based software provided by SABiosciences, Qiagen, USA.

Results

Based on RT-PCR results, multiple TRP channel genes were found to be expressed in mouse 3T3-L1 preadipocytes (high to moderate expression: TRPP2 (PKD2), TRPC1, TRPV2, TRPM2, TRPV4, TRPV1, TRPV3, TRPV6, TRPC4; low expression: TRPA1, TRPC6, and TRPM8) and adipocytes (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC1, TRPV4, TRPM2; low expression: TRPA1, TRPV3, TRPV6, TRPC4, TRPV1, TRPC6, and TRPM8) (Tab. 1). TRPV1, TRPV3, TRPM8, TRPC4, TRPC6 were differentially expressed (multiple fold higher in preadipocytes than adipocytes) (Fig. 1). Further, these channels are also expressed in murine WAT (high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4;

low expression: TRPV1, TRPV6, TRPM8 and TRPA1] and BAT [high to moderate expression: TRPP2 (PKD2), TRPV2, TRPC6, TRPC1, TRPV4, TRPM2, TRPV3, TRPC4, TRPV6; low expression: TRPV1, TRPM8, and TRPA1] (Tab. 1). TRPV4, TRPV6 and TRPC6 were differentially expressed in murine WAT and BAT (Fig. 1).

Discussion

The present study gives insight on the existence of various TRPs in undifferentiated and differentiated mouse 3T3-L1 cell line and WAT and BAT. It was reported that elevated levels of calcium suppress adipogenesis *via* different mechanisms [3, 6, 11]. Elevated calcium levels caused a significant decrease in triglyceride accumulation in adipocytes as well as decrease the expression of adipogenic genes such as PPAR- γ [13]. In the present study, based on their calcium permeability, multiple TRP channels were targeted and quantified using qRT-PCR and for their existence on preadipocytes and adipocytes and WAT and BAT. Among the selected channels, TRPV1, TRPV3, TRPM8, TRPC4, TRPC6 showed significantly higher expression in preadipocytes as compared to adipocytes. Based on this, we hypothesize that activation of these channels during preadipocyte stage using either dietary [capsaicin (TRPV1), menthol (TRPM8), piperine (TRPV1), 6-gingerol (TRPV1), and polyunsaturated fatty acids (TRPV1)] or endogenous activators can cause calcium influx which may inhibit its differentiation into adipocytes. Previously, it has been suggested that capsaicin-induced increase in the calcium influx significantly blocked adipogenesis in stimulated 3T3-L1-preadipocytes as evidenced by a decrease in intracellular lipid droplets, reduced triglyceride levels, reduced expression of PPAR- γ and reduced fatty acid synthase [13, 14].

Higher expression of some of the calcium permeable TRP (TRPP2 (PKD2), TRPV2, TRPC1, TRPV4, and TRPM2) channels in adipocytes may also have clinical significance. These may be involved in calcium entry mechanism of adipocytes. An increase in intracellular calcium levels in adipocytes may lead to inflammatory state which may further link to diabetes, insulin resistance and cardiovascular complications (hypertension, atherosclerosis and heart failure). Inhibition of these channels using their dietary and

Tab. 1. Summary of TRP channels expression (Ct values $\pm$ SEM) in undifferentiated (preadipocytes) and differentiated (adipocytes) 3T3-L1 cells and mouse white (WAT) and brown (BAT) adipose tissues

TRP Gene	Mouse 3T3-L1 cell line		Mouse adipose tissue	
	Preadipocyte	Adipocyte	WAT	BAT
V1	28.5 $\pm$ 0.6	31.8 $\pm$ 0.3*	29.5 $\pm$ 0.4	29.5 $\pm$ 0.3
V2	27.8 $\pm$ 0.4	28.0 $\pm$ 0.1	23.0 $\pm$ 0.6	24.8 $\pm$ 0.3
V3	29.8 $\pm$ 0.5	31.9 $\pm$ 0.2*	29.3 $\pm$ 0.4	28.6 $\pm$ 0.5
V4	28.5 $\pm$ 0.3	29.2 $\pm$ 0.2	25.0 $\pm$ 0.5	26.7 $\pm$ 0.2
V6	29.0 $\pm$ 0.9	31.7 $\pm$ 0.3*	23.8 $\pm$ 0.3	29.2 $\pm$ 0.4*
M2	27.9 $\pm$ 0.4	29.5 $\pm$ 0.1	27.3 $\pm$ 0.3	28.1 $\pm$ 0.3
M8	28.5 $\pm$ 0.5	32.1 $\pm$ 0.3*	31.7 $\pm$ 1.1	30.7 $\pm$ 0.3
A1	29.4 $\pm$ 0.6	30.6 $\pm$ 0.7	32.1 $\pm$ 1.0	31.2 $\pm$ 0.8
C1	24.8 $\pm$ 0.3	26.1 $\pm$ 0.3*	25.6 $\pm$ 0.7	25.5 $\pm$ 0.4
C4	29.5 $\pm$ 0.6	31.4 $\pm$ 0.3*	27.6 $\pm$ 0.9	28.1 $\pm$ 0.4
C6	30.4 $\pm$ 0.8	31.7 $\pm$ 0.5	26.7 $\pm$ 0.9	24.0 $\pm$ 0.3*
P2	24.8 $\pm$ 0.1	25.1 $\pm$ 0.1	23.1 $\pm$ 0.4	23.4 $\pm$ 0.3
GAPDH	16.2 $\pm$ 0.1	17.0 $\pm$ 0.1	17.8 $\pm$ 0.3	14.7 $\pm$ 0.3

* Significant with p value $\leq$ 0.05

Fig.1. Differential expression of TRP channels in undifferentiated (preadipocytes) and differentiated (adipocytes) 3T3-L1 cells relative to GAPDH (House Keeping Gene, HKG) **(A)** and mouse white (WAT) and brown adipose tissue (BAT) relative to TRPP2 (HKG) **(B)**. * Significant with p value ≤ 0.01 ; ** significant with p value ≤ 0.03

endogenous antagonists is expected to confer protection against development of insulin resistance and endothelial dysfunction. Recently, Sukumar et al. demonstrated the constitutive expression TRPC1/C5 in cell lines and mouse/human adipocytes [10]. Further, inhibition of these channels by unsaturated fatty acids can raise adiponectin levels suggesting a cardioprotective role for TRPC1/C5 inhibitors [10].

Further, expression of TRP channels in WAT and BAT of normal mice was also studied. Most of these channels were present in both WAT and BAT. TRPV4, TRPV6 were significantly higher in WAT whereas TRPC6 was significantly higher in BAT. TRPC1/C5 and TRPV1 are constitutively present in murine and human adipose tissues [10, 13] whereas TRPM8 has functional role in BAT [4]. Stimulation of TRPM8 mediates BAT induced thermogenesis through the modulation of uncoupling protein (UCP-1) [4]. Based on this, menthol and other dietary constituents, which activate these channels, could constitute a promising way to treat obesity. Based on the differential expression of

some of these channels we speculate that alteration (loss/gain of function) of these channels can be linked with conversion of WAT to BAT and *vice versa*.

In conclusion, our study provides evidence for the presence and differential expression of multiple TRP channels in mouse 3T3-L1 cell line and adipose tissues. Activation or inhibition of these channels by various dietary constituents makes them novel targets to design obesity preventing dietary regimens. Further studies are required to ensure that these channels are functional and their differential expression has clinical relevance in obesity and related complications.

Conflict of interest:

There is no conflict of interest among authors of this manuscript.

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