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3T3L1 Cell line as an *in vitro* model of Obesity

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ABSTRACT

The rising prevalence and increased incidence of health risks associated with the Obesity leads to severe threat to the whole world. This leads to intense research on understanding the Pathophysiology and molecular mechanisms underlying the progression of disease. One of the best promising ways is to understand the molecular events involved in adipocyte biology, adipocyte differentiation and adipogenesis. The study of these processes is possible only by using different in vitro models. Among them, the 3T3L1 cell line is one of the important cell lines for better understanding of adipogenesis and adipocyte dysfunction associated with the Obesity. Here, we will discuss the crucial events that take place during differentiation of pre-adipocytes into mature adipocytes, the transcriptional factors that regulate the process, the artificial media that brings about the differentiation process and the importance of this cell line over others.

Keywords –3T3L1 pre-adipocytic cell line, Adipogenesis, Differentiation media, Obesity.

I. INTRODUCTION

Obesity is associated with a number of risk factors primarily due to the overabundance of fat cells. In recent years, the understanding of adipocyte development and differentiation has become an area of intense research. It leads to the understanding of the developmental process and their comparison to other systems as well as the knowledge of physiology in general. The transition of adipocyte precursor cell into mature adipocytes involves series of events, thereby limiting the use of *in vivo* models for studying pre-adipocyte differentiation. Thus, the pre-adipocyte primary cultures were used, but they suffer significant drawbacks. It's hard to isolate pre-adipocytes from the other fibroblast-like cells, and a large amount of fat tissue is required due to small percentage of pre-adipocytes in total mass. Moreover, the primary cultures have a short life span in culture, and thus, the *in vitro* models for studying pre-adipocyte differentiation are used. The advantages of using cell line are that the population of cells used are homogeneous, thereby allowing a definitive response to treatments. The cells can be passaged and thus a constant source of cells is available for study. The adipocyte precursor cells are of two types, i.e., pluripotent fibroblasts and unipotent pre-adipocytes. The pluripotent fibroblasts (10T1/2, RCJ3.1 and CHEF/18) have the ability to be converted into several cell types upon treatment with different chemical agents [1]. The unipotent adipocyte precursor cells (3T3-L1, 3T3-F442A, Ob1771, TA1 and 30A5) have undergone determination and can remain either as pre-adipocytes or converted into mature adipocytes. The 3T3-L1 and 3T3-F442A cell lines are the derivatives of Swiss 3T3 mouse embryo fibroblasts and are widely used as *in vitro* obesity models [2].

The 3T3-L1 cell line is a well-established pre-adipose cell line that was developed from murine Swiss 3T3 cells [3].

II. MOLECULAR EVENTS THAT TAKE PLACE DURING ADIPOCYTE DIFFERENTIATION:

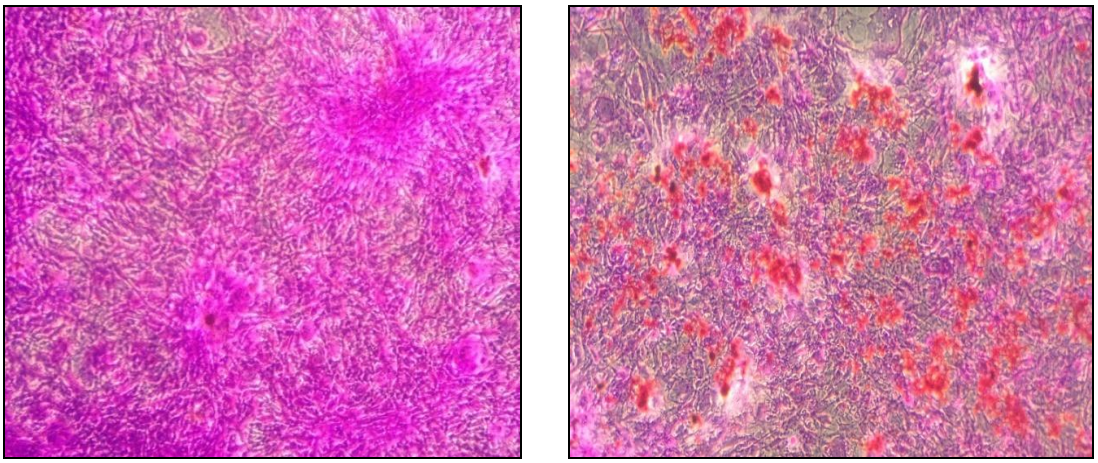
In culture systems, 3T3-L1 cells mimic the formation and appearance of developing fat cells. When the 3T3-L1 cells reach confluence, they are differentiated into adipocytes by using a cocktail mixture that includes insulin, dexamethasone and Isobutylmethylxanthine. Insulin elevates the intracellular cAMP levels and acts through IGF-1 (Insulin-Like Growth factor 1). Dexamethasone is a synthetic glucocorticoid agonist and IBMX is a cAMP phosphodiesterase inhibitor and stimulates cAMP-dependent protein kinase pathway. About 24hrs after induction by differentiation media, the preadipocytes undergo mitosis and subsequent growth arrest. The mitosis is believed to unwind DNA, allowing the transcriptional factors to access the regulatory elements present in the genes and cells are converted to become adipocytes.

The addition of differentiation media leads to changes in the adipocytic gene expression through the sequence of the transcriptional cascade. The early differentiation markers LPL (lipoprotein lipase) and type IV collagen genes are expressed when the 3T3-L1 pre-adipocytes reach confluence. Within a short period of one hour after the addition of differentiation media, the expression levels of CEBP β and δ, c-jun, c-fos, jun B, c-myc increases [4]. The C/EBP-β and δ are postulated to be involved in directing the adipocyte differentiation process as they are the first transcriptional factors that are expressed after induction of differentiation process. The CEBP β and δ are thought to mediate the expression of PPAR-γ and CEBP-α [5]. These two factors are induced two days after induction with differentiation media. These two transcriptional factors influence the transcription of many other genes involved in creating and maintaining the adipocyte phenotype. Both the factors are crucial for the late stages of differentiation in a cooperative manner, and neither of them is expressed at high levels in pre-adipocytes infers that they are not involved in early development.

III. CULTURE OF 3T3L1 CELLS BY USING DIFFERENTIATION MEDIA:

For culturing 3T3-L1 cells, normal media is used. However, for the differentiation of pre-adipocytes into mature adipocytes the differentiation media is used [6]. The normal media contains Dulbecco's modified Eagles medium (DMEM) containing 10% (v/v) Fetal Calf Serum (FCS) or Fetal Bovine Serum, 100 U/ml of penicillin and 100 µg/ml of streptomycin, until confluent. The differentiation media contains normal media along with insulin, dexamethasone and Isobutylmethylxanthine. Two days after confluence (Day 0), the cells were stimulated to differentiate with differentiation media (DI media) consisting of DMEM, 10% FCS, 167 ηM insulin, and 0.5 µM Isobutylmethylxanthine and 1 µM Dexamethasone for two days (Day 2). The differentiation media was replaced by DMEM+10% FCS+ 167 ηM insulin for next two days (Day 4), followed by culturing with DMEM+10% FCS for additional 4 days (Day 8), at which about 90% of cells were found to be mature adipocytes with fat droplets. The cells are cultured at 37°C in a humidified 5% CO2 atmosphere under controlled conditions. The maturation of pre-adipocytes into fully mature adipocytes is accompanied by the

presence of fat droplets inside the cells. This is visualised by staining the cells by using Oil Red O Stain. This stain binds to the neutral lipids and gives intense red colour when visualised under microscope (Fig 1).



(A) **(B)**

Figure 1: Differentiation of pre-adipocytes into adipocytes. The figure A represents 3T3L1 cells cultured in normal media. The figure B represents the culture of cells in presence of Differentiation media. The red dots represent the oil droplets stained by Oil Red O Staining which appear when the cells mature into adipocytes.

IV. IMPORTANCE OF 3T3L1 CELL LINE OVER OTHER CELL LINES:

The culture of 3T3L1 cell line is easier to carry out and less costly to use than freshly isolated cells, such as mature adipocytes. This cell line can be used for many passages and are homogeneous in terms of the cell population. Thus, homogenous response is provided by this cell line following treatments and changes in experimental conditions [7]. Due to this ability, the 3T3-L1 cells have been extensively used to elucidate the effects of several compounds or nutrients on adipogenesis, to unravel the underlying molecular mechanisms of adipogenesis and to screen the potential application of various compounds and nutrients for the treatment of obesity [8-10].

3T3-L1 cells have also been used to know the mechanisms of action underlying differentiation process of numerous compounds or nutrients that have previously been shown *in vivo* to inhibit obesity [11]. Further several obesogenic agents and endocrine disruptors and obesogenic compounds have also been checked during the differentiation of 3T3-L1 cells [12]. Different Transfection procedures and gene silencing techniques have been applied to study the role of different genes associated with adipogenesis in 3T3-L1 cells. The role of different microRNA's in adipocyte differentiation has also been detected in 3T3-L1 pre-adipocytic cell line. Moreover, this cell line is useful to study the co-culture as well as three dimensional cultures.

V. CONCLUSION

The present review discusses 3T3L1 as an *in vitro* cellular model for evaluating the adipogenesis process and adipocyte differentiation which is an important aspect of adipocyte biology and eventually Obesity. The protocols for culturing these cells are highly developed and standardized. They have been largely used for studying different aspects related to Obesity. In particular, they have become excellent models for studying adipogenesis and obesity-related metabolic alterations as well as for studying adipocyte renewal and expansion and donor and depot-specific differences.

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