Murine *in vitro* cellular models to better understand adipogenesis and its potential applications

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Abstract

Adipogenesis has been extensively studied using *in vitro* models of cellular differentiation, enabling long-term regulation of fat cell metabolism in human adipose tissue (AT) material. Many studies promote the idea that manipulation of this process could potentially reduce the prevalence of obesity and its related diseases. It is essential to understand the molecular basis of fat cell development if we are to tackle this pandemic disease, by identifying therapeutic targets and new biomarkers. This review explores murine cell models and their applications for study of the adipogenic differentiation process *in vitro*. We focus on the benefits and limitations of the different cell line models, with the aim of aiding data interpretation and selection of appropriate model cell lines model for future advances in adipose biology.

Keywords: Adipose tissue, Adipogenesis, Cellular models, Differentiation, Anti-obesity

1 Introduction

Obesity is one of the most widespread problematic health conditions, having tripled worldwide since 1980 (Chooi, Ding and Magkos, 2019). The condition is caused by accumulation of excess body fat in human body, to the extent that it is associated with several life-threatening diseases, predominantly hypertension, diabetes, heart disease, osteoarthritis and cancer (Włodarczyk and Nowicka, 2019,Lohmann, Goodwin, Chlebowski et al., 2016).

The alarming prevalence and severity of obesity has drastically increased both in children and adults, and corresponding morbidity and mortality has increased markedly over the past two decades (Abarca-Gómez, Abdeen, Hamid et al., 2017,Wolfenden, Ezzati, Larijani et al., 2019). According to NCD Risk Factor Collaboration, a network of health scientists around the world, suggested global occurrence of obesity could possibly reach 21% in women and 18% in men by 2025, if the trend continues (Collaboration, 2016). Therefore, understanding obesity and its intervention must become public health priorities worldwide.

This complex multifactorial disease is generally caused by a decrease in energy expenditure and/or increased energy intake for a prolonged period of time, resulting in an energy imbalance (Ross, Flynn and Pate, 2016). Environmental factors, like high caloric food consumption and sedentary lifestyle are strongly associated with the dysfunctionality of adipose tissue (AT) formation, which undergoes molecular and cellular alterations affecting metabolism, insulin sensitivity and promoting local and systemic inflammation (de Ferranti and Mozaffarian, 2008,van Meijel, Blaak and Goossens, 2019). AT increases through differentiation of pre-adipocytes to greater numbers of adipocytes, referred to as hyperplasia and/or by increase of size of existing adipocytes to accommodate further lipids, known as hypertrophy (Jo, Gavrilova, Pack et al., 2009).

AT is a complex organ, able to regulate whole-body energy supply through the accumulation of triglycerides. Besides adipocytes, these tissues also contain several other cell types, including fibroblasts, blood cells, endothelial cells, macrophages, and other immune cells. All these cells continuously interact to tune metabolic response and tissue expansion. Alongside its passive function, AT also plays an intricate role in whole-body homeostasis as an endocrine organ. AT produces adipokines and numerous other bioactive factors that communicate with other organs and moderate a variety of metabolic pathways (Booth, Magnuson, Fouts et al., 2016,PNandhini, Desai and Sahoo, 2019).

White, brown, and beige are names given to the three types of fat cells in AT. These cells have distinct locations and functions in the human body, differing in abundance of mitochondria and in thermogenic genes expression (Giralt and Villarroya, 2013). The majority of fats in adult humans are stored in white adipocytes of white adipose tissue (WAT) featuring a single large lipid droplet which serves as a storage depot for excess energy (Han, Zaretsky, Andrade-Oliveira et al., 2017). In contrast, brown adipose tissue (BAT) cells contain multiple lipid droplets and function by generating heat through mitochondrial uncoupling of lipid

oxidation that burns energy through thermogenesis (Villarroya, Cereijo, Villarroya et al., 2017). As for the third type of fat, beige adipocytes were recently discovered and showed similar functions to both white and brown adipocytes. Like BAT, beige adipocytes have enhanced thermogenic capacity, high uncoupling protein-1 (UCP1) expression, and energy expenditure when activated (Mottillo, Desjardins, Crane et al., 2016). Nevertheless, all these types of fat cells work together to maintain whole-body energy homeostasis.

All fat tissues are refined by a cell differentiation process, wherein preadipocytes differentiate into mature adipocytes and become fully functional. This is a complex process known as adipogenesis, that comprises of numerous stages extensively regulated by the specific expression of proteins and transcription factors leading to adipocyte development. Among them, Peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer-binding protein-α (C/EBPα) are considered the main regulators of adipogenesis (Rosen, Walkey, Puigserver et al., 2000). They induce expression of each other mutually and have their cooperation in activating a few other adipocyte genes has been previously reported (Munawar, Prakash and Vangalapati, 2018). However, newer studies on adipogenesis have revealed that several other transcription factors including C/EBPβ, C/EBPδ, as well as some of the Krupel-like factors (KLF), induce expression of PPARγ (Hammarstedt, Gogg, Hedjazifar et al., 2018). Nevertheless, transcriptional repressors including GATA2, KLF2, and CHOP have shown to reduce PPARγ expression in adipogenesis. To store or utilize energy, mature adipocytes respond to different metabolic stimuli. The various cell types are able to communicate with each other via adipokines, cytokines or lipid/glucose fluxes. Over time, adipocytes eventually lose their differentiation or thermogenic capability due to senescence (González-Casanova, Pertuz-Cruz, Caicedo-Ortega et al., 2020).

Our understanding of preadipocyte differentiation using *in vitro* culture models has advanced significantly in recent years (Ruiz-Ojeda, Rupérez, Gomez-Llorente et al., 2016). These cellular systems have become invaluable tools to determine the mechanisms involved in adipocyte proliferation, differentiation, adipokine secretion and gene/protein expression. An *in vitro* model which accurately recapitulates the properties of native human AT would greatly benefit therapy development and pathology studies. Genes, proteins, and signaling pathways involved in regulation of adipocytes are now rapidly identified using modern technologies like protein arrays, microarrays, and genetic manipulation. Yet, these techniques are only valuable if the most effective cell model is used in the research efforts. Moreover, the cell lines serve as useful systems to explore biochemical characteristic and functions of key adipogenic factors

and pathways. Additionally, murine derived cell culture systems have also assisted with several adipogenesis studies as they are easy to cultivate and translate to *in vivo*, which has further enhanced our knowledge on adipose biology (Wang, Scherer and Gupta, 2014).

The aim of this review is to provide a better knowledge and understanding of murine *in vitro* cellular models for the study of adipogenesis, focusing mainly on the cell differentiation and their applications for anti-adipogenic effects. This article discusses information relevant to the culture systems, highlighting benefits and limitations of the cell lines as well as their applications in adipocyte biology, and provides guidance for those seeking to select an appropriate model for their work. Our goal is to support a better understanding of the science of adipocytes and AT, as well as their mechanisms will assist with the development of novel therapeutic approaches and agents that can effectively treat these conditions.

2 Cellular models for study of adipogenesis

The availability of a vast range of cell models has enabled extensive study on differentiation of adipocyte using cell culture systems. These models represent the stages of adipocyte development, detailing the molecular and cellular events in transition from fibroblast-like preadipocytes into adipocyte cells (Ruiz-Ojeda et al., 2016). Hence, biologists and biochemists have been able to explore new and existing mechanisms using different sources of adipose cell models which have immensely facilitated research into the differentiation process and identification of regulatory elements that assist with coordinated expression during differentiation of adipocyte genes.

Unipotent preadipocyte and pluripotent cell lines are the two primary classes of *in vitro* cell models present for the study of adipogenesis (Figure 1) (Moreno-Navarrete and Fernández-Real, 2017). Preadipocyte models are unipotent cells which are useful in understanding the molecular events responsible for preadipocyte conversion, whereas, multipotent fibroblasts cells are pluripotent models committed to different lineages and used to study the cellular determination of the separate cell fates, including adipocytes.

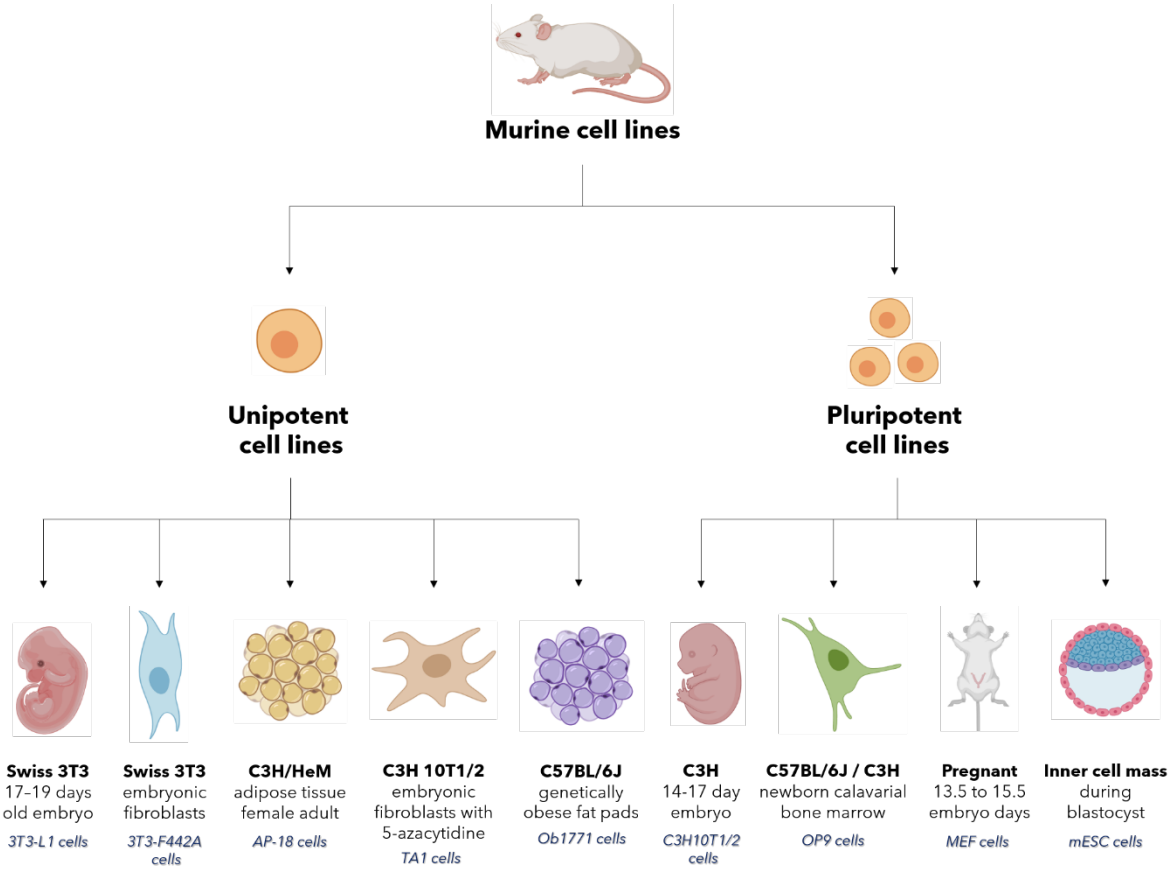

Figure 1: Murine cell line - Schematic diagram showing the source of each unipotent and pluripotent cell line from the *in vitro* model to study cellular differentiation for adipogenesis

Preadipocyte clonal cell lines (3T3-L1; 3T3-F442A; TA1; AP-18; Ob1771) (refer to Figure 1) present a homogenous cellular population with the same differentiation stages. The ability of these preadipocyte cells to passage indefinitely present them the lines as effective resources for study. Alternatively, pluripotent cell models can differentiate into various cell types other than adipocytes. These cell lines include C3H10T1/2, OP9 cells, mouse embryonic stem cells (mESCs) and mouse embryonic fibroblasts (MEFs) (see Figure 1). In particular, C3H10T1/2 have been beneficial for acknowledging the events responsible for lineage

determination. These cell lines have various practical features that make them suitable for adipocyte studies, especially OP9 cells which hold the capacity to rapidly differentiate. Likewise, mESCs provide an effectively infinite supply of cells when combined with retinoic acid (RA) and pro-adipogenic agents. More interestingly, MEFs are established and maintained easily, they proliferate rapidly, and can yield various cell types within several days, from just a single embryo (Yusuf, Gopurappilly, Dadheech et al., 2013). It should be noted that the development stage of each cell line has minute variations in their requirements for differentiation (Kassotis, Masse, Kim et al., 2017). Nonetheless, they also have similar functionalities to mesenchymal stem cells (MSCs) and maintain stable morphology for a long period in culture. Table 1 provides a summary of murine *in vitro* cell models and their applications for understanding adipogenesis.

Several researchers have thoroughly examined the adipogenic or anti-adipogenic potential of many pharmacological compounds including hormones and growth factors due to the availability of murine *in vitro* cell models. Identifying specific development markers allows us to align the development programs of each cell line. Comprehensive knowledge of the differentiation process could assist with manipulating adipocyte cell numbers to control specific diseases - study of adipocyte differentiation, expansion and endocrine function at a complex level will support the development of therapies against obesity and its metabolic complications.

Table 1: Adipogenic applications of murine *in vitro* cell line model

Cell line	Adipogenic agents	Differentiation time	Reported applications	References
3T3-L1	Dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX) and Insulin	7-10 days	• Understanding role of adipocyte-related proteins and genes • Used in co-culture and three-dimensional culture system for adipose tissue • Screening anti-adipogenic compounds, anti-adipogenic peptides, adipogenic agents in food products and anti-adipogenic crude extracts	(Kim, Lee, Kim et al., 2020,Zhao, Hu, Wang et al., 2019)
3T3-F442A	Insulin, Fetal bovine serum (FBS), Tri-iodothyronine (T3), IBMX and DEX	10-12 days	• Examination of adipogenic agents in differentiation processes • Screening anti-adipogenic compounds, effective adipogenic peptides, adipogenesis transcriptional factors and anti-adipogenic crude extracts	(Hemmeryckx, Vranckx, Bauters et al., 2019,Khalilpourfarshbafi, Murugan, Sattar et al., 2019)

TA1	DEX, Insulin and Indomethacin	6-10 days	• Understanding role and function of adipocyte-related proteins • Screening effective agents in adipose differentiation process • Estimating pre-existing and new genes involved in adipogenesis • Used in identification of early adipogenic markers	(Shinohara, Murata and Shimizu, 1992,Ninomiya-Tsuji, Torti and Ringold, 1993)
AP-18	Combination of DEX and IBMX or Insulin	7-21 days	• Potential in identification of mechanism for subcutaneous adipocytes biology	(Chen, Takahashi, Yoshida et al., 2010)
Ob1771	Insulin and T3	6-8 days	• Examination of different molecules in differentiation process of adipogenesis • Screening effective fatty acids in adipogenesis biology and agents involved in obesity and its related conditions	(Abderrahim-Ferkoune, Bezy, Astri-Roques et al., 2004)
C3H10T1/2	IBMX, DEX, Insulin, and Troglitazone/Rosiglitazone	12 days	• Screening natural compounds and crude extract for anti-adipogenic effects • Examination of the role and function of adipocyte-related proteins in adipogenesis • Estimation of the regulatory effects of non-coding RNA in adipogenic differentiation	(Schwind, Schetting and Montenarh, 2017,Hussain, Rehman, Luckett et al., 2020)
OP9	Serum replacement method (SRM), Insulin oleate method (IOM) and Adipogenic cocktail method (ACM)	2-3 days	• Identification of key regulators in adipocyte related disease conditions • Screening compounds on early and late differentiation of adipogenesis • Examination of natural crude extracts for anti-adipogenic effects • Used in high-throughput RNA screening and techniques	(Wolins, Quaynor, Skinner et al., 2006)
Mouse Embryonic Stem cells (mESCs)	Retinoic acid (RA), Insulin, T3 and Rosiglitazone	21 days	• Characterisation of pre-existing genes and new adipogenic regulatory genes • Used in advanced and high-throughput techniques • Identification of genetic and epigenetic mechanisms involved in adipogenesis • Potential in exploring developmental fate of	(Rosen and MacDougald, 2006,Ota, Tong, Goto et al., 2017)

			adipocytes origin and screening compounds on differentiation of adipogenesis	
Mouse Embryonic Fibroblasts (MEFs)	Insulin, DEX, IBMX, Troglitazone and FBS	14 days	• MEFs from genetically modified or knockout mice used for study the effects of genes in adipogenesis • Evaluation of the effects of proteins or genes in adipogenesis • Screening anti-adipogenic compounds and anti-adipogenic crude extracts	(Yusuf et al., 2013,Hou, Chen, Wang et al., 2020)

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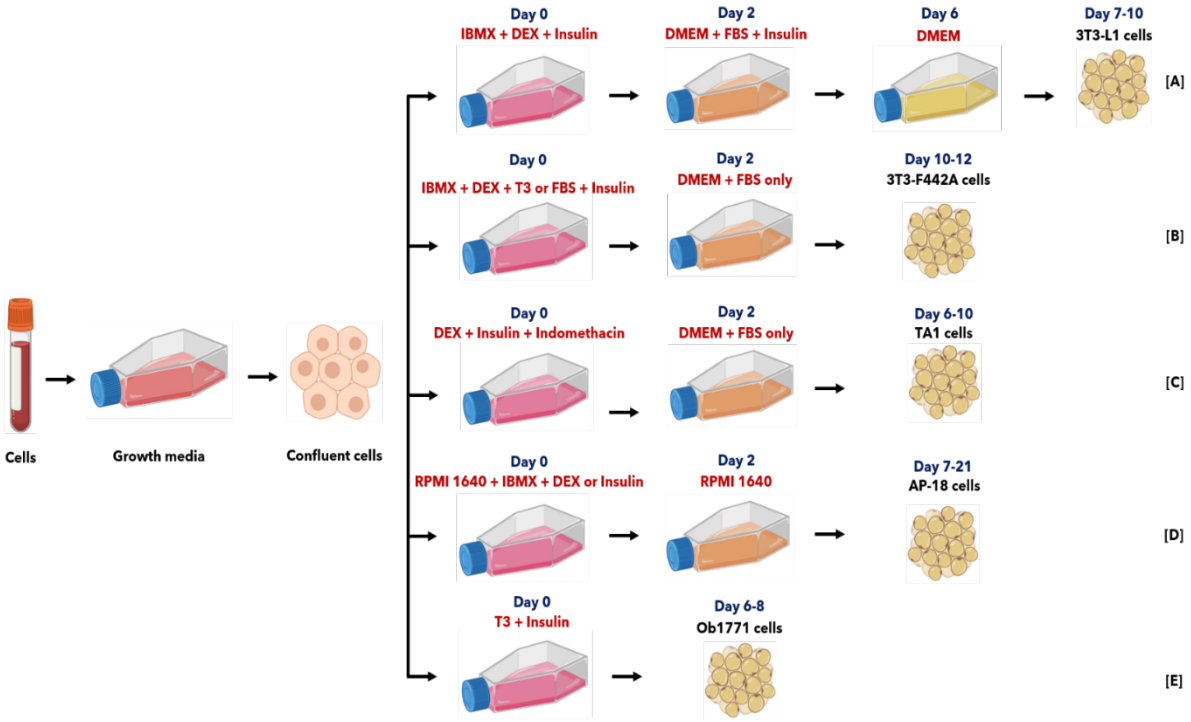

Figure 2 A-E: Differentiation process - Schematic diagram presents adipocytes differentiation process of unipotent murine cell line

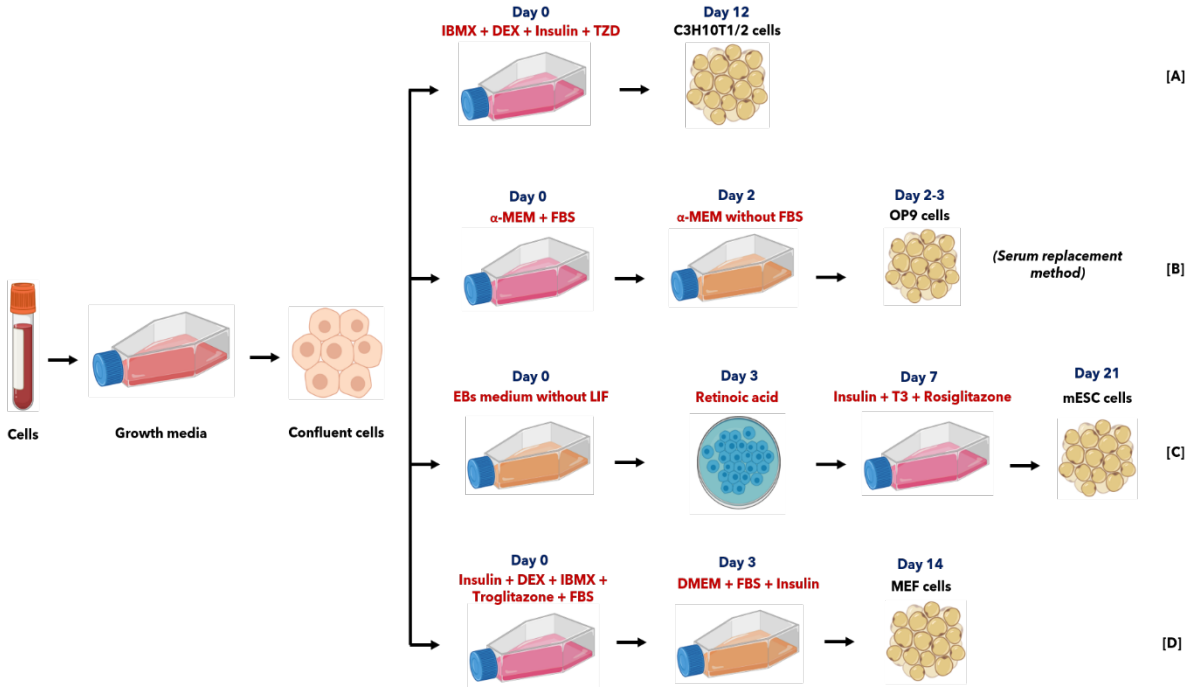

Figure 3 A-D: Differentiation process - Schematic diagram presents adipocytes differentiation process of pluripotent murine cell line

2.1 3T3-L1 Cell Line

In 1974, Green and Kehinde reported the discovery of the 3T3-L1 cell line (Green and Kehinde, 1974,Antony, Debroy, Manisha et al., 2019). 3T3-L1 is an established embryonic murine preadipocyte cell line with distinctive characteristics, extensively used for the study of adipocyte biology. The cell line was originally manufactured by selecting cells from the resting state of a disaggregated 17–19 days old Swiss 3T3 mouse embryo. Its significance was recognized when it was injected into mice, which formed fat pads that could not be differentiated from their normal AT (Green and Kehinde, 1979,Kuri-Harcuch, Velez-delValle, Vazquez-Sandoval et al., 2019). It is known that mature 3T3-L1 cells possess the majority of the ultrastructural properties of adipocytes in culture, i.e. same as that of an animal tissue (Novikoff, Novikoff, Rosen et al., 1980,Xiu, Xinong, Tianjia et al., 2017). Furthermore, 3T3-L1 culture displays spontaneous lipid accumulation when converted into its adipocyte-like phenotype. Adipogenic cocktails, also known as adipogenic agents, are defined prodifferentiative agents required for conversion of undifferentiated cells into differentiated adipocyte cells. Insulin, dexamethasone (DEX), and 3-isobutyl-1-methylxanthine (IBMX) are the most commonly used adipogenic cocktails in 3T3-L1 cell differentiation.

3T3-L1 cells are first cultured in a basal medium containing high glucose concentration, generally consisting of Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (FBS) and antibiotics. A humidified atmosphere of 37 °C containing 5% CO_2 is a standard protocol essential for cell culture. The medium is changed every 2–3 days, until the cells reach confluency. Once confluence is reached, 3T3-L1 differentiation is initiated by exchanging the growth media with the adipogenic cocktail medium, also known as induction medium. After 48 hours of exposure, the induction media is substituted with the growth medium containing insulin. Thereafter, the media is removed on day 6 and un-supplemented growth media is added. Subsequently, by day 7-10, the cells start to accumulate triglycerides in the form of lipid droplets identified as fully differentiated adipocyte-like cells. These mature fat cells grow in number and size over cultivation time and also express multiple metabolic characteristics (Figure 2-A) (Kim et al., 2020,Zhao et al., 2019).

Several researchers have tried to introduce numerous adipogenic agents to create more efficient methods of obtaining improved differentiation efficiency of 3T3-L1 cells (Subra, Fontana, Visentin et al., 2003,Katafuchi, Garbers and Albanesi, 2010). Thiazolidinediones (TZDs) are agonists of PPARγ that are frequently used as an additional component for 3T3-L1

differentiation. A further report by Zebish et al. suggested that the use of rosiglitazone (one of the TZDs) as an additional adipogenic agent resulted in differentiation within 2 weeks which persists for up to 10 cell passages (Zebisch, Voigt, Wabitsch et al., 2012). The experimental results indicated an increase of lipid accumulation and high glucose uptake with Troglitazone and DEX (Vishwanath, Srinivasan, Patil et al., 2013). Additionally, this combination was proven to generate better quality adipocytes over a shorter period of time in comparison to the combination of IBMX and DEX. Another study by Hua et al. on 3T3-L1 cells suggested that prolonged treatment of IBMX improved differentiation efficiency (Hua, Ke, Wang et al., 2016). A recent analysis of six different commonly used adipogenic cocktails and their protocols suggested that the concentration of 0.5 mM IBMX, 1 μM DEX, and 10 μg/mL insulin is most effective for 3T3-L1 cell differentiation (Zhao et al., 2019).

3T3-L1 cells are widely applicable for the study of adipocyte biology. Over the last decade, the 3T3-L1 *in vitro* model has been widely used to study molecular and cellular processes of adipogenesis. Studies using this cell line have focused on evaluating the role, function and possible mechanisms of adipocyte-related proteins to get a better insight of adipogenesis and its regulations. For example, Matrix Gla protein (MGP) has been identified in 3T3-L1 cells for involvement in fat metabolism and as a novel serum marker in central obesity (Li, Li, He et al., 2020). Likewise, the function of LIGHT/TNFSF14 was discovered in the diversion of energy in favour of immune activation that limited the adipogenic and thermogenic programs (Kou, Liu, Liu et al., 2019). Recently, liver kinase B1 (LKBI) was identified by up-regulation of brown adipocyte expression markers including UCP1, PGC-1α and PRDM16 in 3T3-L1 cells (Xi, Xue, Wu et al., 2019). Consequently, this work has helped us determine the use of these proteins as different biomarkers of obesity and their usefulness to acknowledge the development of obesity in adipocyte tissue.

This cell line has also enabled characterization of the proteins involved in obesity and its complications to identify its remedies. For example, W. Tang and Fan characterized Sirtuin 6 (SIRT6) as resulting in reduced insulin resistance and increased glucose metabolism in 3T3-L1 cells (Tang and Fan, 2019). Similarly, an investigation by Kobayashi et al. on WW domain containing E3 ubiquitin protein ligase 1 (WWP1) in 3T3-L1 cells and on an *in vivo* model revealed that it is an obesity-inducible E3 ubiquitin ligase that assists with protection against obesity-associated oxidative stress (Kobayashi, Hoshino, Abe et al., 2019). Several other proteins have also been explored in 3T3-L1 cells for their effects on the adipocyte differentiation process. For instance, Tyrphostin-AG17 was found effective in preventing

adipogenesis and lipid synthesis by activating caspase-3 mechanism which induced adipocyte apoptosis (Camacho, Segoviano-Ramírez, Sánchez-Garcia et al., 2018). In addition, the 3T3-L1 cell line was also used to understand the mechanisms underlying obesity and other metabolic disorders to investigate the regulators associated with adipose development. In this case, The role of pigment epithelium-derived factor (PEDF) has been established in lipid metabolism, which has been recognized for negatively regulating adipogenesis through various signaling intermediates (Huang, Hsu, Chen et al., 2018). Table 2 provides detailed information on the proteins examined for adipogenic properties in 3T3-L1 preadipocytes cells.

The large number of studies which use the 3T3-L1 cell line reflect its ability to rapidly screen and assess the inhibition of adipogenesis by measurement of intracellular triglyceride contents and lipid accumulation, making the cell line a valuable model for screening natural therapeutic agents. Some negatively regulated adipogenic compounds including bisphenol-A (BPA) and polychlorinated biphenyls 138 (PCBs) have been shown to contribute to the induction of obesity using 3T3-L1 cells (De Filippis, Li and Rosen, 2018,Kim, Kim, Oh et al., 2018). Further data on compounds investigated on 3T3-L1 cells and their possible adipogenic actions have been given in Table 3.

Co-culture is the growth of two different cell types together in the same environment. The study of co-culture systems assists with observing the interactions in functional structures, being somewhat closer to interactions *in vivo* (Marino, Bishop, de Ridder et al., 2019,Paschos, Brown, Eswaramoorthy et al., 2015,Hendriks, Riesle and van Blitterswijk, 2007). Dodson, and his coworkers (1997) discovered that co-culture systems are applicable for the study of obesity in humans. They developed a defined system from myogenic satellite cells and muscle-derived preadipocytes to examine soluble factors involved in their communication (Dodson, Vierck, Hossner et al., 1997). A recent investigation by Hao and her team, established a 3T3-L1 cell co-culture system with human prostate cancer cells to determine the inhibition ability of arctigenin as an effective agent that can co-target obesity in obese-related prostate cancer (Hao, Diaz, del Rio Verduzco et al., 2020). According to another study, a co-cultured system of differentiated 3T3-L1 and RAW264.7 cells was used to study the mechanism of macrophage-adipocytes interaction in innate and adaptive immunity (Lu, Ma, Zhao et al., 2020).

This 3T3-L1 co-culture system was also used to screen plant-derived components for biological activity. For example, Brassinin (BR) was recognized for inhibition of obesity-induced inflammation via Nrf2-HO-1 signaling pathway (Kang, Kim, Hwang et al., 2019). The

3T3-L1 cell line was also used to further evaluate the effects of saponin fraction from red ginseng on treatment of obesity through a co-culture system (Kim, Kang, Suh et al., 2018). Hence, evidence suggest that the use of 3T3-L1 cell line in a co-culture system has been proven effective for its effects on anti-obesity and obesity related inflammations.

Two-dimensional (2D) cell culture techniques usually require low-cost maintenance and are easily manipulated according to their conditions for cell growth. Yet, the tissues of the *in vivo* micro-environment are not fully duplicated through this approach. Therefore, three-dimensional (3D) cell culture systems were introduced to overcome the limitation of the 2D culture method (Marino et al., 2019). A scaffold-free method has now been used to generate 3D adipose spheroids from primary, immortal human and 3T3-L1 preadipocyte cells, developed by Turner, Tang, Weiss and Janorkar (Turner, Tang, Weiss et al., 2015)**.** This demonstrates that 3D culture of 3T3-L1 cells can effectively identify new biomarkers and effective therapeutics.

Many researchers have determined that co-culture systems better mimic the *in vivo* tissue microenvironment of cell morphology and structural complexity in a 3D platform, as well as biological processes and functions, such as proliferation, differentiation and gene or protein expression. A recent high-throughput proteomic analysis of 3D co-cultured system of 3T3-L1 cells was utilized to explore the differential protein expression between 2D and 3D co-cultured system using the iTRAQ-bases technique. This encouraged development of an insulin resistant model that produced an *in vitro* obesity model identical to the conditions of *in vivo*, considering the mechanisms underpinning metabolic syndromes (Lee, Park, Kim et al., 2019), consequently introducing new ways by utilizing 3T3-L1 cells to tackle obesity and its related-metabolic disorders.

In recent years, an integrated AT on-chip nano-plasmonic biosensing platform to investigate obesity-associated inflammation was developed using 3T3-L1 cells. The system was created for drug-efficacy screening and as a prognostic tool to create personalized treatment plans for risk prevention against obesity (Zhu, He, Verano et al., 2018). These cells were also served as a model system to develop a visual difference mapping (VDM) platform, a new method used to determine the program of adipogenesis. The system analyses the conversion process of fibroblast-like cells into a rounded shape with formation of lipid droplets (Lustig, Feng, Payan et al., 2019). These advanced techniques and high-throughput screening

could assist in finding effective and potent therapies to combat obesity and its related metabolic disorders.

In summary, the 3T3-L1 cell line has been used extensively in the last 50 years in lipogenesis and lipolysis research due to its abundant supply of homogeneous cells through culture, making it a good model to screen compounds for their potential antilipolytic effects (Pereira-Fernandes, Vanparys, Vergauwen et al., 2014). These preadipocyte cells are also suitable models to study molecular mechanisms and transcription factors in the adipogenesis process due to their adherent properties (Poulos, Dodson and Hausman, 2010). 3T3-L1 cells are ideal for the study of long-term regulation of adipocyte functions as they provide a monolayer culture of newly differentiated fat cells (Adler-Wailes, Guiney, Wolins et al., 2010). Though some research suggests that 3T3-L1 cells have low differentiation efficiency if they are repeatedly thawed from liquid nitrogen (Zhao et al., 2019). In addition, the cells are unable to differentiate robustly into adipocytes if they become confluent and are passaged extensively (Wolins et al., 2006,Hock, 2016,Hernández-Mosqueira, Velez-delValle and Kuri-Harcuch, 2015). Hence, culturing 3T3-L1 cells can become demanding and limit utility in a generation of stable cell lines. Nonetheless, extensive research demonstrates that 3T3-L1 cells are most effective, with significantly lower-costs than other mature adipocytes cell line models.

Table 2: List of proteins investigated for their adipogenic effects using 3T3-L1 *in vitro* cell model (↑ Increased; ↓ Decreased)

No.	Protein	Description	Mechanism	Comments	Reference
1.	Matrix Gla protein (MGP)	Vitamin K-dependent protein	↓ CEBPα ↓ FABP4	Involved in fat metabolism and novel serum marker	(Li et al., 2020)
2.	Sirtuin 6 (SIRT6)	Stress responsive protein deacetylase	↑ Glucose uptakes ↓ Insulin resistance	Controls insulin resistance and glucose update	(Tang and Fan, 2019)
3.	LIGHT/TNFSF14	Tumor necrosis factor superfamily protein 14	↓ UCP1 ↓ PPARγ ↓ PRDM16	Involves in beige fat biogenesis	(Kou et al., 2019)
4.	Liver kinase B1 (LKB1)	Serine/threonine protein kinase	↑ UCP1 ↑ PGC-1α ↑ PRDM16 ↑ PLIN	Browning of white adipocytes and increases lipid metabolism	(Xi et al., 2019)
5.	WW domain containing E3 ubiquitin protein ligase 1 (WWP1)	HECT E3 ubiquitin ligases	↓ Oxidative stress	Protective role in oxidative stress in WAT	(Kobayashi et al., 2019)
6.	Protein kinase D1 (PKD1)	G protein-coupled receptor	↓ C/EBPα ↓ C/EBPδ	Deletion of PKD1 improve insulin sensitivity and reduced liver steatosis	(Löffler, Mayer, Viera et al., 2018)
7.	Polymerase I and transcription release factor (PTRF)	Intracellular protein	↑ Hypertrophy ↑ Senescence	Behave as an adipokine and detrimental effects	(Perez-Diaz, Garcia-Sobreviela,

				in visceral fat accumulation	Gonzalez-Irazabal et al., 2018)
8.	Reticulon 3 (RTN_3)	Endoplasmic reticulum protein	↑ SREBP-1c ↑ AMPK activity	Induced obesity and increased hypertriglyceridemia	(Xiang, Fan, Huang et al., 2018)
9.	Erb-B2 Receptor Tyrosine Kinase 4 (ErbB4)	Epidermal growth factor (EGF) receptor family	↑ Inflammation ↑ Subcutaneous and visceral fat	ErbB4 deletion involved in metabolic syndrome	(Zeng, Wang, Kloepfer et al., 2018)
10.	Neuregulin-4 (Nrg4)	EGF family of proteins	↑ Angiogenesis	Disruption of Nrg4 decreased in obesity	(Nugroho, Ikeda, Barinda et al., 2018)
11.	S100A4	S100 calcium-binding protein family	↓ Inflammation ↑ Akt signaling	Inhibit adipogenesis and reduced inflammation factors	(Hou, Jiao, Yuan et al., 2018)
12.	Pentraxin 3 (PTX_3)	Long pentraxin protein family	↑ NPY/NPYR ↑ Oxidative stress	Involved in development of obesity	(Chang, Shin, Choi et al., 2018)
13.	Tyrphostin-AG17	Reversible Inhibitor of epidermal growth factor	↑ Adipocytes apoptosis by activating caspase-3	Anti-obesity effect	(Camacho et al., 2018)
14.	CD38	Type II transmembrane glycoprotein	↓ PPARγ ↓ aP2 ↓ C/EBPα ↓ SREBP1-c ↓ FAS	CD38 deficiency impairs adipogenesis and lipogenesis	(Wang, Miao, Wang et al., 2018)
15.	Pigment epithelium-derived factor (PEDF)	Serine protease inhibitor glycoprotein	↑ CD36	Negatively regulates the adipogenesis	(Huang et al., 2018)
16.	9-PAHSA	Endogenous mammalian lipid	↑ UCP1 ↑ PGC-1α ↑ PRDM16 ↑ C/EBPβ	Browning effects, anti-inflammatory and anti-obesity effects	(Wang, Liu and Fang, 2018)

Table 3: List of compounds, structure ID and possible mechanisms investigated in 3T3-L1 preadipocyte cell line (↑ Increased; ↓ Decreased)

No.	Agents	Structure ID	Description	Mechanism	Comments	Reference
1.	2,6-Dimethoxy-1,4-benzoquinone (DMBQ)	PubChem CID:68262	Present in fermented wheat germ	↓ CEBPα ↓ aP2 ↓ FAS ↓ PPARγ ↑ AMPK signaling ↓ SREBP-1c	Suppressed adipogenesis	(Son, Jang, Jung et al., 2019)
2.	Acrylamide (ACR)	PubChem CID:6579	Present in starch-rich food	↓ AMPK signaling ↑ PPARγ ↑ C/EBPα ↑ aP2 ↑ SREBP-1c ↑ FAS	Upregulated of adipogenesis	(Lee, Kim, Choi et al., 2019)
3.	Salt	PubChem CID:5234		↑ PPARγ ↑ C/EBPα	High salt increased adipogenesis and	(Lee, Sorn, Lee

				↑ SREBP-1c ↑ ACC ↑ FAS ↑ aP2	contribute in obesity	et al., 2019)
4.	Adenanthin	PubChem CID:15011073	A natural *ent*-kaurane diterpenoid extracted from the herb *Isodon adenantha*	↓ PPARγ ↓ FABP4 ↓ C/EBPβ	Anti-adipogenic effect	(Hu, Li, Tian et al., 2019)
5.	Vitexin	PubChem CID:5280441	Flavone obtained from *Crataegus pinnatifida* (hawthorn leaf)	↑ AMPK signaling ↓ C/EBPα ↓ FAS	Anti-adipogenic effect	(Peng, Sun, Xu et al., 2019)
6.	α, β-Amyrin	PubChem CID: 73170 and 73145	Triterpenoids isolated from *Protium heptaphyllum*	↓ PPARγ ↓ C/EBPα ↓ GLUT4	Anti-adipogenic effect	(de Melo, de Oliveira, Silva et al., 2019)
7.	Platycodin D (PD)	PubChem CID:162859	Active compound of *Platycodi radix*	↓ PPARγ ↓ C/EBPα ↑ UCP1 ↑ PGC-1α ↑ AMPK signaling	Anti-adipogenic effects and thermogenic actions	(Kim, Park, Jung et al., 2019)
8.	Oxyresveratrol	PubChem CID:5321884	Present in mulberry twigs and fruits (*Morus alba L.*)	↑ UCP1 ↑ Foxo3a	Increased energy expenditure through thermogenesis	(Choi, Song, Lee et al., 2019)
9.	7-Hydroxymatairesinol (7-HMR)	PubChem CID:45273284	7-HMR is Plant lignan	↓ PPARγ ↓ C/EBPα ↓ aP2	Inhibit adipogenesis and lipid uptake	(Biasiotto, Zanella, Predolini et al., 2018)
10.	2-bromo-4′-methoxychalcone (compound 5) and 2-iodo-4′-methoxychalcone (compound 6)	PubChem CID:11173046 (compound 6)	Synthetic halogen containing chalcone derivatives	↑ AMPK signaling ↑ ACC	Anti-obesity effect	(Hsieh, Chang, Tsai et al., 2018)
11.	Bisphenol-A (BPA)	PubChem CID:6623	Lipophilic compound, used in the manufacture of plastic items	↓ Adipocytes marker ↑ IL-6 ↑ TNFα	Increased inflammation and contribute in obesity	(De Filippis et al., 2018)
12.	Trans-1-methyoxy-4-propenyl-benzene (Trans-anethole)	PubChem CID:637563	Flavoring substance present in the essential oils of various plants	↑ PGC-1α ↑ PRDM16 ↑ UCP1 ↓ C/EBPα ↓ PPARα ↓ PPARγ ↓ FAS ↓ ACC ↑ HSL ↑ ATGL ↑ AMPK signaling	Induced white fat browning and anti-adipogenic effect	(Kang, Mukherjee, Min et al., 2018)
13.	Plumbagin	PubChem CID:10205	Naphthoquinone found in roots of *Plumbago zeylanica*	↓ Triglycerides content	Anti-adipogenic effect	(Pai, Martis, Joshi et al., 2018)
14.	Polychlorinated biphenyls 138 (PCBs)	PubChem CID:35823	Persistent organic pollutants (POPs) present in environment	↓ TNFα ↑ Survivin ↑ PLIN	Increased lipid droplets and induction of obesity	(Kim et al., 2018)

15.	Ginsenosides Rg1	PubChem CID:441923	Saponins present in leaves of *Panax quinquefoliu*	↓ PPARγ ↓ C/EBPα ↓ SERBP-1c ↓ FAS ↓ FABP4 ↑ AMPK/ACC signaling	Inhibiting lipogenesis and anti-adipogenic effect	(Liu, Wang, Liu et al., 2018)
16.	Epigallocatechin-3-gallate (EGCG)	PubChem CID:65064	Polyphenol catechin present in Green tea	↑ AMPK signaling ↑ UCP1 ↓ ACC ↓ PPARγ ↓ C/EBPα ↓ SERBP-1c ↓ FAS	Suppressed adipogenesis in white adipocytes	(Mi, Liu, Tian et al., 2018)

2.2 3T3-F442A Cell Line

In 1976, Green developed 3T3-F442A cells from Swiss 3T3 embryonic fibroblasts. The cells were isolated from the 3T3 clone-18 line that converted into fat cell clusters at a high frequency, and increased size as compared to the 3T3-L1 cell line (Green and Kehinde, 1976,Sadie-Van Gijsen, 2019). This demonstrates the cells' ability to develop morphological characteristics of mature adipocytes both *in vitro* and *in vivo* considering their spherical shape, increased lipogenic activity, accumulation of triglycerides, and adipocyte-specific marker expression. The reliability of this preadipocyte model was first tested on the nude mouse by subcutaneously injecting cells that produced ectopic fat which became histologically (Green and Kehinde, 1979) and biochemically (Mandrup, Loftus, MacDougald et al., 1997) indistinguishable from the host normal AT. Thereby, suggesting that the studies of the adipose conversion and its regulation on this model is supported hugely by the cell behavior in animals.

3T3-F442A cells generally require treatment of differentiating agents such as insulin and FBS to undergo adipose differentiation, which typically suggest high adipogenic activity (Kuri-Harcuch and Green, 1978). Though some researchers have also used triiodothyronine (T3), IBMX and DEX agents (Hemmeryckx et al., 2019). For differentiation, the preadipocyte cells are cultivated and maintained in a high basal medium containing DMEM supplemented with FBS and antibiotics. Evidence suggests that differentiation can be prevented by maintaining the cells at their pre-confluency stage. Once the cells reach confluency, the culture medium is exchanged for 48 hours with an induction medium. Thereafter, it is substituted with DMEM medium containing only FBS, for up to a week, replacing the media every 48 hours. Over an approximate period of 2 weeks, the cells will differentiate into adipocytes, which can be

confirmed by quantification of lipid containing cells and cell viability assay (Figure 2-B) (Hemmeryckx et al., 2019,Khalilpourfarshbafi, Murugan, Sattar et al., 2019).

Interestingly, defined small molecules can be used to induce commitment into adipocytes, thus this cell line has become a valuable tool to understand some of the mechanisms involved in the early stages of differentiation. For example, adipose differentiation of 3T3-F442A cells occurred rapidly when in the presence of low amounts of staurosporine, a selective serine–threonine kinase inhibitor, and absence of other adipogenic factors (Ayala-Sumuano, Velez-Del Valle, Beltrán-Langarica et al., 2008). Two stages were identified in early adipogenesis. In the first stage, staurosporine administration lasts for up to 4 hours in which GSK3β is activated. The second stabilization stage continues through from 4 to 48 hours after removal of staurosporine from the culture medium. The cells then enter into the clonal expansion stage and express the adipose specific phenotype (Ayala-Sumuano, Velez-delValle, Beltrán-Langarica et al., 2011,Diaz-Velasquez, Castro-Muñozledo and Kuri-Harcuch, 2008). Identifying these stages of early adipogenesis has assisted with study of the early molecular events that regulate the induction and stabilization stages of adipogenesis process in further detail, including the participating genes and the effect of different compounds on these processes.

Q. Q. Tang and Lane introduced an analog of staurosporine, stauprimide, also likely to activate the GSK3β to mediate adipose differentiation action in 3T3- F442A (Tang and Lane, 2012). A debate on the action of DEX found that it enhanced 3T3-F442A cell differentiation when it was induced by staurosporine, but caused impairment in lipid metabolism (Ayala-Sumuano, Velez-delValle, Beltrán-Langarica et al., 2013). As a result, it was concluded that DEX may have a complex dual role in the impairment of AT homeostasis because it stimulates the differentiation process of preadipocytes. However, it also alters the lipid metabolism and insulin sensitivity of differentiated fat cells (Ayala-Sumuano et al., 2013). Thus, it has been acknowledged through the 3T3-F442A model that the actions of DEX may impair lipid homeostasis, induce insulin resistance in the organism and cause obesity. These dual actions explain the effects of DEX *in vivo*, along with several other studies that suggest high glucocorticoid levels could cause metabolic syndrome (Masuzaki, Paterson, Shinyama et al., 2001).

Farnesol is another inducer that can cause differentiation in 3T3-F442A cells. It is a mevalonate-derived inducer of adipocyte differentiation that also plays the role of an insulin

sensitizer (Torabi and Mo, 2016). This cell line also has the capacity to differentiate into osteoblasts due to action of bone morphogenetic protein (BMP) and retinoic acid (RA) that stimulates cell proliferation, represses adipogenesis, and promotes osteoblast formation (Skillington, Choy and Derynck, 2002). In support of this notion, exposure to a specific inducer can cause these cells to oblige to either adipocytes or osteoblasts.

3T3-F442A cells are less commonly utilized for adipogenic study, compared to 3T3-L1 cells, considering they have minimal differences between their differentiation protocols. 3T3-F442A cells are used to test compounds to evaluate their potential effects on adipogenesis and their underlying mechanisms. Tocotrienols, specific components in vitamin E family, showed inhibition in differentiation of 3T3-F442A preadipocytes. The compounds reduced triglyceride contents by decreasing glucose uptake and lowering the amount of GLUT4 and HMG-CoA reductase proteins. Consequently, tocotrienols could inhibit adipocyte differentiation and enhance energy expenditure which can render this class of vitamins useful in creation of novel dietary approaches for prevention and treatment of obesity and diabetes (Torabi, Yeganehjoo, Shen et al., 2016). Alongside, the 3T3-F442A cell line and *in vivo* mice models were used to examine drug supplements such as OBEX or pterostilbene to evaluate their potential anti-adipogenic effects. Both drugs proved effective in reduction of adiposity in mice and in downregulation of key adipogenesis transcriptional factors (Carreira, Andrade, Gonzalez-Izquierdo et al., 2018,Gomez-Zorita, Belles, Briot et al., 2017).

Many studies have been conducted on 3T3-F442A preadipocytes clonal cells to examine the potential effects of peptides and their possible molecular mechanisms in adipogenesis. For example, Egg white hydrolysate (EWH) was explored for differentiation, insulin signaling and inflammatory effects, which indicated that EWH encouraged adipocyte differentiation by combining insulin mimetic and insulin sensitizing actions on 3T3-F442A cells. Treatment with EWH also resulted in increased expression of adiponectin and suppressed cytokine mediated inflammatory response in these cells (Jahandideh, Chakrabarti, Davidge et al., 2017).

Importantly, 3T3-F442A cells have facilitated the study of transcriptional factors involved in the differentiation process of adipocytes. For example, cytoglobin (Cygb) is a hexacoordinated haemoglobin protein, which when overexpressed in preadipocytes cells contributes to adipogenic differentiation as validated by higher lipid droplets and increased PPARγ, CEBPα and FABP4 expressions (Doğan, Demirci, Kıratlı et al., 2017). A few other proteins like Wnt-1 inducible signaling pathway protein-1 (WISP1) and intestinal

chemosensory signaling proteins when investigated found reduced adipocytes differentiation in 3T3-F442A cells and therapeutic ability in obesity related diseases (Ferrand, Béreziat, Moldes et al., 2017,Avau, Bauters, Steensels et al., 2015).

3T3-F442A cells can also be used to screen the effects of crude extracts from natural sources in aim of isolating and identifying active substances such as grape powder extract (GPE), which have been clarified for their risk/benefit in obesity and insulin resistance. Results revealed that polyphenolic extract induces browning of adipogenesis through increased glucose uptake and upregulation of AMPK signaling that upregulated energy expenditure and lipolysis in 3T3-F442A cells (Torabi and DiMarco, 2016). Hence, identifying the molecular mechanisms in adipogenic differentiation pathway could assist in developing new strategies to diagnose and prevent obesity and related diseases. Table 4 provides information on the compounds and peptides that have been explored for their adipogenic effects in 3T3-F442A cells.

As with 3T3-L1, the 3T3-F442A cell line has also been used in co-culture systems to examine the *in vitro* effects that influence the process of adipogenesis (Christiaens, Sujatha, Hellemans et al., 2010). 3D culture of mature adipocytes has been developed within a hydrogel scaffold using 3T3-L1 and 3T3-F442A cell lines with primary human white preadipocytes cells to create a robust adipose 3D model, that caused increase in adipocytes phenotypic and genotypic markers (Louis, Pannetier, Souguir et al., 2017). Although there is limited research related to co-culture and 3D culture of 3T3-F442A cells, this cell line has the capacity for exploration of adipogenesis through these advanced systems. It has further been acknowledged that these cells are also capable of accumulating higher fat than 3T3-L1 cells and develop morphological characteristics of mature white adipocytes both in *in vivo* and *in vitro*, representing 3T3-F442A cell line as a good model for the study of biology of adipogenesis.

Table 4: List of compounds and proteins investigated for adipogenic effects using 3T3-F442A cells (↑ Increased; ↓ Decreased)

No.	Compounds	Description	Mechanism	Comments	Reference
1.	Withaferin A (WFA)	A steroidal lactone derived from *Withania somnifera*	↓ PPARγ ↓ C/EBPα ↓ TNFα ↓ IL-6	Decreased adipogenesis and reduced inflammation	(Khalilpourfarshbafi et al., 2019)
2.	OBEX	Oral nutritional supplement contained many natural antioxidants	↓ PPARγ ↑ UCP1 ↑ PGC-1α ↓ GLUT4 ↓ Adiponectin	Decreased adiposity and increased browning	(Carreira et al., 2018)

3.	Pterostilbene	A naturally oral drug derived stilbenoid	↓ Triglycerides ↓ Glucose incorporation into lipids	Decreased adipocytes differentiation and increased glucose uptake	(Gomez-Zorita et al., 2017)
4.	d-δ-tocotrienol	Present in the vitamin E family	↓ GLUT4 ↓ HMG CoA reductase ↓ Akt protein	Decreased differentiation and enhanced energy expenditure	(Torabi et al., 2016)
5.	Farnesol	A mevalonate-derived sesquiterpene	↑ GLUT4 ↑ PPARγ ↑ FABP4 ↑ Adiponectin	Induced adipocytes differentiation and insulin sensitizer	(Torabi and Mo, 2016)
6.	Egg white hydrolysate (EWH)	Dietary protein	↑ Adiponectin ↑ PPARγ ↑ C/EBPα ↑ Akt phosphorylation ↓ COX-2	Promoted differentiation and reduced cytokine induced inflammation	(Jahandideh et al., 2017)
7.	Wnt1 inducible signaling pathway protein-1 (WISP1)	Member of CCN protein family	↓ PPARγ ↓ Adiponectin ↓ LPL ↓ FABP4	Novel regulator of adipogenesis	(Ferrand et al., 2017)
8.	Cytoglobin (Cygb)	New globin family member of hexacoordinated protein	↑ PPARγ ↑ C/EBPα ↑ FABP4	Cygb involved in adipogenesis and indicator for obesity	(Doğan et al., 2017)
9.	Gustatory G-protein, gustducin, and bitter taste receptors (TAS2R)	Involves in intestinal chemosensory signaling pathways	↓ Adiposity ↑ UCP1	Inhibited adipogenesis and increased browning	(Avau et al., 2015)
10.	Gelatinase A (MMP-2)	Type IV collagenase known as matrix metalloproteinase-2	↑ Differentiation ↑ Pro-adipogenic marker	Impaired adipogenesis	(Bauters, Scroyen, Van Hul et al., 2015)
11.	Grape powder extracted polyphenols (GPEP)	Grape products are rich in phenolic compounds	↑ FAS ↑ LPL ↑Adiponectin ↑ GLUT4 ↑ AMPK signaling	Increased energy expenditure and lipolysis effects	(Torabi and DiMarco, 2016)

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2.3 TA1 Cell Line

A new preadipocyte cell line was introduced in 1984 by Chapman and his colleagues. These TA1 cells were isolated and characterized as stable adipogenic cells extracted by treating a C3H 1OT1/2 mouse embryonic fibroblast with 5-azacytidine, a DNA methylation inhibitor (Chapman, Knight, Dieckmann et al., 1984). TA1 cells express the functional and morphological characteristics of mature adipocytes through the appearance of lipid droplets and adipocyte specific RNAs.

The differentiation of TA1 cells from preadipocytes to adipocytes is dramatically accelarated by the adipogenic agents DEX and insulin (Chapman, Knight and Ringold, 1985). However, in 1987, an anti-inflammatory drug known as indomethacin was recognized as a potent adipogenic inducer that stimulates differentiation in a shorter period of 3 days, with 90% adipocyte capacity in comparison to DEX-treated cells (Knight, Chapman, Navre et al., 1987). TA1 cells are maintained and cultured in 37°C with humidified air of 5% CO_2 with Eagle's basal DMEM medium, FBS and antibiotics, changing the medium every 2 days until the cells reached confluency. To induce differentiation, the cells must be exposed to the adipogenic cocktail for approximately 2 days. For the remaining days, the cells must be first washed with DMEM media and then cultured in DMEM with only fetal serum. Thereafter, the cells should begin to accumulate lipid droplets and reach differentiation approximately 6-10 days after becoming confluent (Figure 2-C) (Shinohara et al., 1992,Ninomiya-Tsuji et al., 1993).

There are very few studies as to the application of this cell line for adipogenesis, yet it holds great potential. Chapman and his colleagues first discovered fat-specific protein-27 (fsp-27) using TA1 cells in 1984. They cloned the protein using a screening approach to identify the cDNAs related to adipocytes differentiation (Chapman et al., 1984). However, it was later characterized by Danesch and his teams that fsp-27 plays a critical role in regulating the key adipogenic transcription of C/EBPs (Danesch, Hoeck and Ringold, 1992,Williams, Chang, Danesch et al., 1992). This cell line was also used to evaluate the mechanisms of lipogenic activity using cachectin factors which showed that cachectin reversely and specifically inhibits the expression of adipose specific genes, thereby immediately inhibiting the lipogenic activity in TA1 preadipocyte cells (Torti, Dieckmann, Beutler et al., 1985).

TA1 preadipocyte cells were treated with the tumor necrosis factors (TNF) to establish their molecular mechanisms of adipogenesis. TNF resulted in reduced expression of several adipose-inducible genes which inhibit and reverse the expression of adipose genes to fully

differentiated cells (Torti, Torti, Larrick et al., 1989). In later studies, TA1 cells were examined for the inhibitory role of the cell signaling protein, yielding genetic and pharmacologic evidence that TNF mediates its effects by two distinct or overlapping pathways (Reid, Torti and Ringold, 1989). Further research helped facilitate proto-oncogenes such as *c-fos* and *c-jun* that are transiently induced by TNF in TA1 cells (Haliday, Ramesha and Ringold, 1991). Ninomiya and her team provoked the idea that TNF induced *c-myc* expression in TA1 adipocyte cells. They concluded that TNF plays a central role in inhibition and reversal of adipocyte differentiation (Ninomiya-Tsuji et al., 1993).

Further studies have been conducted using TA1 cells to examine the cellular effects of chemical agents in the adipose differentiation process. For example, isoproterenol and ractopamine were investigated for lipid metabolism in TA1 preadipocytes. It was found that glycerol release increased and fatty acid synthase activity decreased by these agents in a dose-dependent manner (Weber, Merkel and Bergen, 1992). Finally, the functions of collagens were evaluated using the TA1 preadipocyte cells which demonstrated that the active synthesis of collagens are required in adipose conversion of preadipocytes into adipocytes (Ibrahimi, Bonino, Bardon et al., 1992).

TA1 cells have been reported to express more preexisting genes particularly involved in fatty acid and triglyceride synthesis as compared to other preadipocyte cell lines. TA1 cells also display dramatic changes in gene expression and create a large number of new gene products during adipocyte differentiation (Chapman et al., 1984). Subsequently, the qualities of the TA1 cell line make it a suitable model to evaluate the effects of preexisting and new genes that are specifically included in adipogenesis program. Evidence suggests that TA1 cells show adipocyte characteristics, like expressing early adipocyte specific genes within approximately 3 days of reaching its confluency (Torti et al., 1985). Due to this, the cell line may also be used for the identification of early adipogenic markers and their underlying mechanisms in adipogenesis. Additionally, these cells respond to physiological events in *in vivo* (RINGOLD, CHAPMAN, KNIGHT et al., 1988). TA1 cells may therefore be a plausible *in vitro* model for the study of lipid metabolism regulation. Furthermore, fully differentiated cells can be used to elucidate the effects of lipolytic and lipogenic agents in adipogenesis (Weber et al., 1992).

Interestingly, a recent study comparing 3T3-L1, 3T3-F442A and TA1 cells found that TA1 cells expressed higher levels of leptin than that of other cells in adipocyte differentiation

(Slieker, Sloop and Surface, 1998). Regardless of the limited studies in the last 20 years, numerous scientists have made insightful discoveries for the understanding of adipose biology using TA1 cells. In summary, this cell line requires further characterization to aid in the understanding of the adipogenesis process, and introduce new discoveries to tackle obesity and its related morbidities.

2.4 AP-18 Cell Line

AP-18 is a another preadipocyte cell line, discovered in 2005. It was developed from the normal AT of an adult female C3H/HeM mouse, specifically derived from the subcutaneous fat of the skin behind its ears (Doi, Masaki, Takahashi et al., 2005). These cells have the ability to accumulate lipids in the form of triglycerides, and they also express characteristics of preadipocyte and mature adipocyte genes.

This cell line requires differentiation agents DEX, IBMX and insulin, as with 3T3-L1 cells (Chen et al., 2010). The AP-18 cells are suspended in RPMI-1640 media supplemented with FBS, sodium pyruvate, L-glutamine and antibiotics at 37 ˚C and 10% CO_2. After growth, cells are treated with trypsin (0.5%) and diluted to 1:4 in a culture plate. Once the cells reach confluency, the medium is changed to high glucose RPMI-1640 medium with a combination of DEX and IBMX, otherwise insulin alone. Subsequently, the cells must be refreshed every 3 days with high glucose RPMI-1640 for the following 7–21 days, wherein they will begin revealing adipocyte characteristics (Figure 2-D) (Chen et al., 2010). Through this protocol, it was discovered that AP-18-cells developed lipid accumulation at a rapid rate when cells were cultured alone, or with a relatively low concentration of insulin. The result is 70 to 90% adipocyte development within 2 to 3 weeks.

Notably, when characterized for mRNA profile of adipogenesis, AP-18 cells induced expression of key transcriptional factors for adipocyte differentiation, including C/EBPβ, C/EBPα, PPARγ, aP2 and Adipisin (Doi et al., 2005). A study conducted by C. Chen and her team also confirmed that AP-18 cells differentiate into mature adipocytes by a decrease in expression of preadipocyte factor 1 (Pref-1) and increase in expression of lipoprotein lipase (LPL), retinoid X receptor-α (RXRα), PPARγ, GLUT4, adiponectin, resistin and leptin which have shown similar patterns to 3T3-L1 cells (Chen et al., 2010).

Through analysis of this cell line, two apparent advantages were identified from the somewhat minimal research available on AP-18 cells. They hold the ability to grow for many generations or passage in culture at a slow growth rate whilst differentiating into adipocytes without changing their morphology (Doi et al., 2005). Secondly, AP-18 cells have been recognized as a useful model for investigating the mechanism of subcutaneous adipocyte biology because they are one of the preadipocyte cell lines that are derived from a normal AT (Doi et al., 2005). Yet, genes are expressed at a slower and lower rate in comparison to 3T3-L1 cell line. It is also important to note that AP-18 cells have a doubling time of 50-60 hours,

in comparison to embryo-derived 3T3-L1 cells, that double in 22 hours (Doi et al., 2005). Nevertheless, Chen Chen and her team concluded that the AP-18 cells represent specific white adipocyte phenotypes under more physiological conditions than 3T3-L1 cells (Chen et al., 2010). Considering that this cell line has not yet been used for any research related to adipocyte differentiation, there is a need for further characterization to assist with developing new targets for obesity treatment through creating an understanding of its prevalence.

2.5 Ob1771 Cell Line

Ob1771 is another established preadipocyte cell line, obtained from subcloned Ob17 cells from the fat pads of a genetically obese C57BL6J mouse. This cell line exhibits a fibroblastic shaped appearance and has shown an exponential growth rate. It has also undergone 35 passages with no detectable changes and has doubling times of 12.5 and 19 hours in 10% and 1% FBS, respectively (Sadie-Van Gijsen, 2019,Negrel, Grimaldi and Ailhaud, 1978).

A standard growth medium supplemented with insulin and T3 is required for adipocyte differentiation of the Ob1771 strain (Abderrahim-Ferkoune et al., 2004). Interestingly, T3 (a thyroid growth hormone necessary for regulating the expression of differentiation-dependent genes) further stimulates the transcription of insulin-like growth factor-I (IGF-I) proteins in this cell line. A combination of IGF-I protein and T3 in medium is required for terminal differentiation of Ob17 preadipocyte cells (Kamai, Mikawa, Endo et al., 1996,Grimaldi, Djian, Negrel et al., 1982). Similar to other preadipocyte cells, Ob1771 cells are also grown and maintained in DMEM supplemented with FBS and antibiotics. After cells become confluent, they are shifted to the differentiation induction medium with insulin and T3, changing the media every other day (Abderrahim-Ferkoune et al., 2004). Within a short period of time, key adipocyte characteristics will appear, including the formation of triacylglycerol and lipid accumulation (Figure 2-E).

These characteristics are closely associated with the appearance of lipolytic and lipogenic enzymes. LPL is an early marker of adipocyte conversion and its expression is dependent upon the growth arrest stage (Amri, Dani, Doglio et al., 1986). Likewise, there is also a late differential marker whose expression is initiated by the accumulation of triacylglycerol (Ibrahimi, Abumrad, Maghareie et al., 1999,Ailhaud, Amri, Bertrand et al., 1990). Hence, expression of both early and late markers in this cell line can help track the process of adipogenesis.

The use of chemically defined molecules has been investigated with these cells, reflecting the line's reliability as a faithful *in vitro* model to investigate factors involved in the chronological events of adipogenesis. For example, a study on the role of spermidine concluded that it effects terminal differentiation of adipose cells and has a permissive effect on growth hormones (Amri, Barbaras, Doglio et al., 1986). Similarly, arachidonic acid is an adipogenic factor that plays a major role in controlling mitosis by increasing intracellular cyclic AMP

concentrations and promoting breakdown of inositol phospholipids, subsequently, causing terminal differentiation in Ob1771 cells (Gaillard, Negrel, Lagarde et al., 1989).

The Ob1771 cell line assisted in elucidating the effects of small molecules on the adipogenesis phenome. For example, fatty acids were investigated using Ob1771 cells, which revealed their role as signal transducing molecules, suggesting that they are involved in adipose cell differentiation (Amri, Ailhaud and Grimaldi, 1994,Ailhaud, Amri and Grimaldi, 1995). Later in 1997, another intrinsic adipogenic inducer for the Ob1771 cells was discovered, showing that calcitriol (1α, 25-$(OH)_2$ vitamin D3 or VD) was able to trigger the terminal differentiation of the cells when cultured in the presence of thyroid hormone-deprived medium (Dace, Martin-El Yazidi, Bonne et al., 1997).

Furthermore, the application of this cell line was used to discover the relationship between obesity and its related diseases. A potential link was recognized between insulin resistance and high blood pressure when investigated using Ob1771 cells, causing increased angiotensinogen secretion in AT, especially of obese subjects (Aubert, Safonova, Negrel et al., 1998). Additionally, extracellular and intracellular signaling pathways are involved in adipocyte differentiation; it was found that leukemia inhibitory factor (LIF) and its receptors are also responsible for early adipogenesis events (Aubert, Dessolin, Belmonte et al., 1999). In a recent investigation, the efficacy of pro-nucleotides (prodrugs) were tested in Ob1771 preadipocyte cells. This prodrug was considered useful for blocking obesity and related conditions due to its availability and characteristics, thus providing a potential new therapeutic approach (Laux, Pande, Shoshani et al., 2004).

The greatest advantage of Ob1771 is its ability to rapidly multiply its cells as compared to other established preadipocyte cell lines. In context, the cells have a doubling time of 12.5 and 19 hours in 10% and 1% FBS, respectively. On the other hand, 3T3-L1 and 3T3-F442A cells from the Swiss mouse have a doubling time of 24 hours in 10% FBS and 100 hours in 1% FBS. Furthermore, some studies suggested that low serum concentrations have a slight effect on Ob1771 cells (Doglio, Dani, Grimaldi et al., 1986); in the absence of added insulin, adipose conversion can occur significantly. Unlike other established preadipocyte cell lines, including 3T3-L1 and 3T3-F442A, Ob1771 cells strictly depend on the addition of insulin. Hence, it appears that this cell line is a useful system to study differentiation of adipocyte cells and their growth factor requirements involved in cell multiplication, in contrast to preadipocytes cell line from the non-genetically obese mouse.

2.6 C3H10T1/2 Cell Line

In 1973, Reznikoff et al. discovered C3H10T1/2 cells, a mesenchymal cell line derived from a C3H mouse embryo aged between 14 to 17 days (Reznikoff, Brankow and Heidelberger, 1973). In culture, these cells show a fibroblastic-like morphology and present similar functionalities to MSCs (Reznikoff, Bertram, Brankow et al., 1973). This multipotent fibroblast cell line can present several new types of cells such as adipose, muscles, bone and cartilage tissues when treated with an inhibitor of DNA methylation (Taylor and Jones, 1979).

The transformation of C3H10T1/2 cells into mature adipocytes requires adipogenic agents such as IBMX, DEX and insulin which have proven sufficient to induce differentiation within 12 days (Schwind et al., 2017). Troglitazone or rosiglitazone can also be used to cause differentiation (Hussain et al., 2020). In a culture containing DMEM media and heat-inactivated FBS, L-glutamate and antibiotics, C3H10T1/2 cells are cultivated at 37°C in a humidified atmosphere of 5% CO_2. Media must be replaced every 3 days until confluency is reached. The cells are then induced for adipocyte differentiation in DMEM supplemented with an adipogenic cocktail for 2-3 more days. Eventually they start revealing their adipocyte characteristics, but until then, they must be maintained in the same culture (Figure 3-A) (Haider and Larose, 2020,Moseti, Regassa, Chen et al., 2020). Additionally, BMP4, a member of the transforming growth factor type β superfamily is able to induce commitment of C3H10T1/2 cells to preadipocytes which develop into cells of the adipocyte phenotype when subjected to an adipocyte differentiation protocol (Tang, Otto and Lane, 2004).

Through investigation on *in vitro* cell models, several pharmacological studies were conducted which assisted with the development of anti-obesity drugs from natural resources that aim to induce weight loss and reduce fat accumulation. This suggests that use of C3H10T1/2 cells has mainly focused on evaluation of the anti-adipogenic effects of phytogenic compounds, and determination of their role and functions in the adipogenesis processes. For example, oxyresveratrol, a natural compound, and pyrvinium, an anthelminthic drug have showed anti-adipogenic properties in C3H10T1/2 cells (Choi et al., 2019,Wang, Dai, Luo et al., 2019). Similarly, the anti-adipogenic effects of hybrid molecules i.e. triazole and indole derivates were investigated in both *in vitro* C3H10T1/2 and *in vivo* Syrian golden hamster model (Rajan, Puri, Kumar et al., 2018). The cell line also allows measurement of negative effects, ultimately contributing to the development of obese conditions including bisphenol-A (BPA) and benzyl butyl phthalate (BBP) (De Filippis et al., 2018,Zhang and Choudhury, 2017).

C3H10T1/2 cells were also used to determine the effects of proteins and their related genes in adipocyte development to create an effective strategy to combat abnormal adipogenesis and related metabolic conditions. For instance, this cell line was used to understand the mechanism underlying the protective role of taurine, a non-proteinogenic amino acid proven useful in improving obesity by mediating the browning of WAT and activating the AMPK pathway (Guo, Li, Peng et al., 2019). An earlier study on adiponectin receptor agonist, AdipoRon in these cells was found to downregulate the expression of adipogenic transcription factors and adipocyte-specific genes by promoting the phosphorylation of AMPK (Wang, Lu and Liu, 2017).

It is vital to understand the importance of regular adipocyte proteins in the process of adipogenesis in order to find remedies for obesity related disorders. Hence, neprilysin (NEP), ahnak and CD38 were investigated in C3H10T1/2 cells to explore their role and functions in adipogenesis (Wang et al., 2018,Kim, Han, Byun et al., 2017,Shin, Seong and Bae, 2016). This cell line was also used to investigate the regulatory effects of long chain non-coding RNA (lncRNA) in obesity and adipogenic differentiation. According to another investigation, novel treatments for obesity found lncRNA *Plnc1* controls adipocyte differentiation by regulating PPARγ (Zhu, Zhang, Li et al., 2018). Screening crude extracts from medicinal plants is a good strategy to discover anti-obesity drugs as it can help derive potential anti-obesity compounds. Hence, C3H10T1/2 cells were used to uncover the effects of natural plant extracts, as detailed in the table provided below (Table 5). In conclusion, we believe that such discoveries will support the potential benefits of novel anti-adipogenic and anti-lipogenic agents in future clinical studies. Table 5 presents the detailed information of compounds, proteins and extracts investigated in C3H10T1/2 cells.

The C3H10T1/2 cell line is beneficial as it maintains a stable morphology even after long periods in culture. Another advantage of this mouse embryo cell line is the ability to examine the molecular genetic regulation of both the developmental determination of vertebrate stem cell lineages and their subsequent differentiation. Nonetheless, it acts as a good model to understand the events responsible for 10T1/2 lineage determination, a simple genetic control that mediates the formation of myogenic, chondrogenic and adipogenic lineages.

Table 5: List of compounds, proteins and extracts which is investigated in C3H10T1/2 cells (↑ Increased; ↓ Decreased)

No.	Compounds	Description	Mechanism	Comments	Reference

1.	25-Hydroxycholesterol	Specific oxysterol	↓ PPARγ ↓ C/EBPα ↓ FABP4	Inhibitory effect on adipogenesis	(Moseti et al., 2020)
2.	Pyrvinium	Classical anthelminthic drug	↓ C/EBPα ↓ PPARγ	Suppressed adipogenic differentiation	(Wang et al., 2019)
3.	Oxyresveratrol	Stilbenoid present in mulberry twigs and fruits (*Morus alba L.*)	↑ UCP1 ↑ Foxo3a	Increased energy expenditure through browning	(Choi et al., 2019)
4.	Bisphenol-A (BPA)	Lipophilic compound, used in the manufacture of plastic items	↓ PPARγ ↓ C/EBPα ↓ FABP4 ↓ FASN ↑ IL-6 ↑ TNFα	Increased inflammation and contributed in obesity	(De Filippis et al., 2018)
5.	Medicarpin (Med)	Natural pterocarpan involves in various beneficial biological roles	↑ PRDM16 ↑ PGC-1α ↑ UCP1 ↑ AMPK pathway	Promoted lipolysis activity	(Imran, Yoon, Lee et al., 2018)
6.	Licarin A (LA)	Obtained from Mexican medicinal plant *Aristolochia taliscana*	↑ PGC-1α ↑ PRDM16 ↑ UCP1	Induced browning and lipolytic effects	(Yoon, Imran and Kim, 2018)
7.	Cryptotanshinone (CT)	Natural compound from *Salvia miltiorrhiza* plant	↑ UCP1 ↑ PRDM16 ↑ PFC-1α ↑ AMPK	Activated browning of white adipocytes	(Imran, Rahman, Yoon et al., 2017)
8.	Triazole and Indole derivates (Hybrid molecules)		↓ PPARγ ↓ C/EBPα ↑ Wnt3a/β pathway	Ameliorated dyslipidemia and high anti-adipogenic effect	(Rajan et al., 2018)
9.	Protocatechuic acid (PCA)	A catechol-type *O*-diphenol phenolic acid (3,4-dihydroxybenzoic acid) present in plants	↑ RUNX2 ↓ PPARγ ↓ C/EBPα ↓ aP2 ↑ Wnt3a/β-catenin	Alleviated osteogenic differentiation and reduced adipocytes differentiation	(Rivera-Piza, An, Kim et al., 2017)
10.	Benzyl butyl phthalate (BBP)	An endocrine disrupting chemical (EDC)	↑ aP2 ↑ PPARγ ↓ Sirt1 ↓ PGC-1α	Contributed in obesity development	(Zhang and Choudhury, 2017)
11.	Xanthoangelol (XA) and 4-hydroxyderrcin (4-HD)	Chalcones obtained from *Angelica keiskei*	↓ Activator protein 1 (AP1) ↓ c-Jun N-terminal Kinase (JNK) ↓ NF-κB	Reduced inflammation induced by obesity	(Li, Goto, Ikutani et al., 2016)
12.	Artepillin C (ArtC)	Brazilian propolis (a resinous plant-based material)	↑ PPARγ ↑ UCP1 ↑ PRDM16	Induced browning in white adipocytes	(Nishikawa, Aoyama, Kamiya et al., 2016)
13.	Epigallocatechin gallate (EGCG)	Green tea polyphenol component	↓ Cells proliferation and migration ↓ Adipisin	Inhibited differentiation and anti-adipogenic effects	(Chani, Puri, Sobti et al., 2016)
14.	Kinsenoside	Obtained from *Anoectochilus formosanus* plant	↑ AMPK ↑ CPT1 ↑ PGC-1α	Increased browning and enhanced catabolic effects	(Cheng, Wang, Chou et al., 2015)

15.	Taurine	Non-protein amino acid	↑ PGC-1α ↑ UCP ↑ AMPK	Increased browning of white adipocytes	(Guo et al., 2019)
16.	Long noncoding RNA *Plnc1*	Type of non-codding RNA and regulates cell function	↑ PPARγ ↑C/EBPα ↑ aP2	Regulator in adipocyte differentiation	(Zhu et al., 2018)
17.	E1A-stimulated genes 1 (CREG1)	Secreted glycoprotein and involves control of cell growth and differentiation	↑ UCP1	Increased browning of adipogenesis	(Kusudo, Hashimoto, Kataoka et al., 2018)
18.	CD38	Type II transmembrane glycoprotein	↓ PPARγ ↓ AP2 ↓ C/EBPα ↓ SREBP1-c ↓ FASN ↑ Sirt1 signaling	CD38 deficiency impaired adipogenesis and lipogenesis	(Wang et al., 2018)
19.	AdipoRon	Adiponectin receptor agonist	↓ PPARγ ↓ C/EBPβ ↓ C/EBPα ↓ FABP4 ↓ FAS ↑ AMPK pathway ↑ ACC	Inhibitory effect on adipogenesis	(Wang et al., 2017)
20.	Enone fatty acids	Synthesized dietary polyunsaturated fatty acids (PUFAs)	↑ UCP1 ↓ Inflammatory cytokine	Decreased dysfunctions of adipocytes induced inflammation	(Yang, Li, Nishimura et al., 2017)
21.	Neprilysin (NEP)	Integral plasma membrane or zinc metallopeptidase protein	↑ PPARγ ↑ C/EBPα ↑ aP2 ↑ PI3K/Akt signaling	Accelerated adipogenesis	(Kim et al., 2017)
22.	Ahnak	Neuroblastomas or nucleoprotein protein	Smad1-dependent PPARγ expression	Regulated adipocyte differentiation	(Shin et al., 2016)
23.	Cytochrome P450 1B1 (CYP1B1)	Member of the cytochrome P450 superfamily of enzymes	CYP1B1 deficiency (-) ↓ PPARγ ↓ CD36 ↓ FAS ↓ SCD-1 ↑ UCP-2 ↑ CPT-1a ↑ AMPK pathway	CYP1B1 deficiency ameliorated obesity and glucose intolerance	(Liu, Huang, Li et al., 2015)
24.	Erucic acid	Natural extract from Rosemary	↓ PPARγ	Reduced adipogenesis and enhanced osteogenesis	(Takahashi, Dohi, Egashira et al., 2020)
25.	Phytanic acid (PA)	Branched-chain of fatty acid present in dietary food	↑ PGC-1α ↑ PRDM16 ↑ UCP1	Promoted beige adipogenic differentiation	(Wang, Mao and Du, 2019)
26.	Spirulina maxima 70% ethanol extract (SM70EE)	Microalga that is rich in essential nutrients and contains pigment proteins such as chlorophyll a and C-phycocyanin	↓ PPARγ ↓ SREBP1-c ↓ C/EBPα ↓ C/EBPβ ↓ aP2 ↓ FAS	Reduced adipogenesis and activated thermogenesis	(Seo, Kim, Choi et al., 2018)

			↓ ACC ↑ PRDM16 ↑ PGC-1α ↑ UCP1		
27.	Mulberry extract (ME) and Mulberry wine extract (MWE)	Extract of edible fruit of *Morus alba L.*	↑ UCP1 ↑ PGC-1α ↑ PRDM16 ↑ CPT-1	Increased mitochondrial biogenesis by browning	(You, Yuan, Lee et al., 2015)
28.	Peanut sprout extracts (PS)	Peanut extract from *Arachis hypogaea L.*	↑ AMPK ↓ aP2 ↑ PGC-1α ↑ CPT1	Increased fatty acid oxidation and enhanced beige adipogenesis	(Seo, Jo, Kim et al., 2019)

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2.7 OP9 Cell Line

OP9 is a stromal cell line taken from the calvarial bone marrow of a newborn C57BL/6J and C3H mouse, that is genetically deficient in functional macrophage colony-stimulating factor (M-CSF) (Nakano, Kodama and Honjo, 1994). This cell line is a tractable alternative model system for the study of adipogenesis that shows rapid accumulation of triglyceride droplets within 72 hours of differentiation (Gao, Yan, Li et al., 2010,Lane, Doyle, Fortin et al., 2014). The functionalities of these cells are similar to MSCs as they acquire the ability to support and facilitate the study of molecular mechanisms involved in the development and differentiation of hematopoietic cells. During co-culture with mouse embryonic stem cells (ES cells), OP9 cells are able to induce differentiation of the cells into blood cells of erythroid, myeloid, and B cell lineages.(Gao et al., 2010,Ueno, Sakita-Ishikawa, Morikawa et al., 2003).

Wolins and his colleagues described three different methodologies used for differentiation of OP9 cells; serum replacement, insulin oleate, and adipogenic cocktail methods (Wolins et al., 2006). The first step of the serum replacement method (SRM) is to grow the OP9 cells to confluence and then culture them for 2 additional days in propagation medium with α-minimum essential medium eagle (α-MEM), FBS, L-glutamine, and antibiotics. Then, the cells are cultured for a further 4 days in a serum replacement medium containing α-MEM media without FBS and antibiotics. Besides the purpose of studying insulin effects, it is important to change the medium to OP9 propagation medium after day 2 of differentiation, because the media contains high concentrations of insulin which may affect cell morphology (Figure 3-B). The second method is the insulin oleate method (IOM)**,** whereby the propagation medium of OP9 cells are replaced with the insulin oleate medium when the cells attach to the plate. They contain α-MEM, FBS, insulin, oleate to albumin (5.5:1 molar ratio), DEX, IBMX, L-glutamine, and antibiotics. As for the adipogenic cocktail method (ACM), the OP9 cells are grown to confluence and then cultured for a further 2 days in adipocyte differentiation medium including DMEM, FBS, $_{L}$-glutamine and antibiotics. The cells are cultured for 2 days in differentiation medium 1: DMEM, FBS, insulin, DEX, IBMX, L-glutamine and antibiotics. Then again, the cells are cultured for an additional 2 days in differentiation medium 2: DMEM, FBS, insulin, L-glutamine and antibiotics. All three treatment methods require OP9 cells to be maintained in propagation medium until they display the adipocyte morphology and the accumulation of triglycerides and abundant intracellular lipid droplets. This cell line expresses high levels of adipocyte specific markers like PPARγ,

C/EBPα, C/EBPβ and SREBP-1c. Moreover, at its pre-confluent stage, OP9 cells express detectable levels of PPARγ and C/EBPα (Wolins et al., 2006).

OP9 cells are used to identify key regulators and mechanistic in adipocyte related disease conditions for the development of a potential and effective treatment. An example is thymic adiposity, a condition which is characterized by deposition of adipocyte in age-related thymic involution causing detrimental effects on the thymic microenvironments, associated with obesity (Lamas, Lopez, Carrio et al., 2016). Some of the key regulators in thymic adipogenesis were investigated in OP9 cells using a label-free quantitative approach. This proteomic analysis revealed that transforming growth factor β (TGF-β) may have a role in thymic adiposity, indicated by inhibition in OP9-DL1 and primary thymic stromal cells. As a result, it has been acknowledged that activation of TGF-β serves as a useful tool for the prevention of thymic adiposity (Tan, Wang, Wang et al., 2019). A previous study on metabolomic alterations of thymic adipogenesis was tested in OP9 adipogenic differentiation using a liquid chromatography-mass spectrometry technique. The study suggested to address the underlying mechanism in thymic adipogenesis (Tan, Wang, Wang et al., 2017).

Other research on a Ca^{2+} permeable channel known as transient receptor potential mucolipin 1 (TRPML1) showed involvement of intracellular membrane trafficking including lysosomal degradation and lysosomal exocytosis, although its impairment was associated with several pathophysiological conditions including obesity (Dhakal and Lee, 2019). However, when the exact role of TRPML1 was investigated in OP9 cells it was recognized that it has diverse roles. Firstly, TRPML1 is crucial for the differentiation of adipocytes and secondly, it mediates the lipid metabolism by membrane trafficking, exosome formation, and exosomal release. Subsequently, results indicated that TRPML1 is a key factor for treatment of obesity-related diseases (Kim, Muallem, Kim et al., 2019).

This cell line has also been used to identify the underlying mechanism for alcohol-elicited dysfunction of WAT that causes visceral adipose tissue expansion resulting in hypoxia and low inflammation within the tissue, introducing a new mechanism to target in treatment of ethanol-related diabetes (He, Li, Zheng et al., 2015). It is essential to improve methods that detect the events in adipocyte differentiation in order to accelerate our understanding for treatment of metabolic and other disorders involving lipid accumulation. These treatments could potentially become powerful tools for future drug screening and mechanistic studies on adipocytic differentiation. Similarly, a new method was recently developed to study lipid

accumulation through refractive index. The robustness of a digital holographic microscopy (DHM) system was tested using OP9 cells. This label-free, non-perturbing method allows detection of lipid droplets in differentiating adipocytes, without the need for washing, staining, or other liquid manipulations (Campos, Rappaz, Kuttler et al., 2018).

OP9 mouse stromal cells have also been used to investigate the effects of different compounds and their underlying mechanisms on early and late differentiation of adipogenesis. With regards to drug screening, this cell line has proven a suitable model to evaluate the responses of novel drug therapies (Jiang, Di Wu, Weng et al., 2017). For example, quercetin showed anti-adipogenic and anti-lipolysis effects in OP9 stromal cells, indicating that the compound has potential anti-obesity therapeutic effect by upregulating ATGL and HSL expression and downregulating FAS, LPL and aP2 expression (Seo, Kang, Kim et al., 2015). OP9 cell lines were also used to confirm repression of lipid droplets formation. To which it was revealed that together with low cytotoxicity the extract suppresses adipogenesis associated lipid accumulation during differentiation of OP9 preadipocytes (Kato, Kato, Shibata et al., 2015).

OP9 cells in co-culture systems were found useful in evaluation of adipocyte biology. For example, a co-culture system using mouse bone marrow and OP9 cells indicated a novel function in control of hematopoiesis by identifying IL-1 as a therapeutic target for aged and obese individuals (Kennedy and Knight, 2015). Use of the OP9 cell line aims to generate a systemic model that identifies the genes essential for adipogenesis that can also become applicable to high-throughput RNA screening. Such models can help identify novel therapeutic targets and map disease pathways involved in obesity. Subsequently, Lane and her co-workers generated a new clonal population of OP9 cells, known as OP9-K. These cells were able to differentiate rapidly and revealed adipocyte morphology like rounded cell shape, lipid accumulation, and coalescence of lipids into a large droplet. This study contributes to the development of rapid screens that can deepen our understanding of adipose biology and test obesity therapeutics (Lane et al., 2014). Similarly, OP9-DL1 is another cell derived from the OP9 cell line characterized in order to study the commitment, differentiation, and proliferation of T-lineage *in vitro* that ectopically expresses Notch ligand Delta-like 1 (Dll1) by mimicking the thymic microenvironment to support T cell development *in vitro* (Schmitt and Zúñiga-Pflücker, 2002) (Holmes and Zúñiga-Pflücker, 2009).

These cells are derived non-clonally, which means that the heterogeneity of cells can be assessed for undergoing adipocytic differentiation (Campos et al., 2018). In addition, OP9 cells can be preserved at high density levels without loss of potential to differentiate into adipocytes, even at high passage numbers. Consequently, these features allow the cells to be maintained in culture and within a few days the OP9 adipocytes become available for experiment (Kassotis et al., 2017). OP9 cells are given any one of three adipogenic stimuli, while rapidly accumulating triglycerides, to form numerous large lipid droplets to express adipocyte marker proteins, and assume adipocyte morphology whilst expressing late adipocyte marker proteins. However, not every protocol of OP9 cells may be optimized for adipocyte differentiation and manipulation, and when maintained at low cell density, OP9 cells adopt a spindly morphology and differentiate into adipocytes poorly (Ruiz-Ojeda et al., 2016).

Accordingly, OP9 cells can differentiate into adipocytes within a period of 2 days and easily express detectable levels of transcription factors after confluency has been reached (Wolins et al., 2006). To conclude, the OP9 cell line represents a new model to understand the mechanisms of differentiation and investigate the effects of drugs on the biology of adipocytes. It is also useful for fast high-throughput studies such as non-perturbing quantification of lipid droplets and digital holographic microscopy. In summary, the capacity to rapidly differentiate and present several practical features, OP9 cell line is a suitable model for adipocyte studies.

2.8 Mouse embryonic stem cells (mESCs)

Martin, Evans and Kaufman described an alternative model for the study of adipogenesis biology known as mouse embryonic stem cells (mESCs). These proliferating, pluripotent stem cells deliver an unlimited supply of cells which can directly differentiate into adipocytes using a combination of RA and pro-adipogenic agents (Rosen and MacDougald, 2006). The mESCs are extracted from the inner cell mass during the developing blastocyst stage of murine embryo (Evans and Kaufman, 1981,Martin, 1981). They display numerous properties, including a stable and normal diploid karyotype, with the capacity to self-renew indefinitely and have the potential to reconstitute all embryonic lineages. Furthermore, mESCs also have the ability to integrate into an embryo and contribute to all cell lineage, whilst fully participating in fetal development when transplanted back into the mouse blastocyst (Nichols and Smith, 2009,Bradley, Evans, Kaufman et al., 1984). They promote proliferation and can be maintained in the presence of LIF to remain in an undifferentiated state (Stavridis and Smith, 2003). Yet, appropriate culture conditions without LIF tend to aggregate into embryoid bodies (EBs), causing differentiation *in vitro* into several derivatives of all ectodermal, mesodermal, and endodermal cells. Consequently, producing all the cell types in the body (Keller, 1995). Nevertheless, mESCs potentially offer a unique *in vitro* cell culture system to study the initial stages of mammalian development.

These highly efficient and reliable adipocytes can be differentiated with RA and pro-adipogenic agents like insulin, T_3 and rosiglitazone (PPARγ agonist) (Rosen and MacDougald, 2006). Two main phases were recognized in the adipogenesis process of mESCs. The permissive period is the first phase of adipogenesis which requires RA and begins after the formation of EBs, causing commitment of mESC cells. In the second phase, cells are treated with adipogenic agents that cause terminal differentiation of pre-adipocytes into adipocytes and lead to outgrowth with lipid droplet-containing adipose cells (Phillips, Vernochet and Dani, 2003). The mESCs are cultured and maintained at 37 °C with 5% to 10% CO_2 in DMEM media on gelatin-coated plates with FBS, LIF, L-glutamate, sodium pyruvate, antibiotics and β-Mercaptoethanol. To induce differentiation, mESCs cells will differentiate in up to 21 days forming a large cluster colony of mature adipocytes, confirmed by staining EBs with Oil Red O stain (refer to Figure 3-C) (Ota et al., 2017,Dani, 1999).

This embryonic stem cell line is an invaluable model for the characterization of genes expressed and identification of new adipogenic regulatory genes. In recent years, mESCs have

been utilized in understanding of the adipogenesis mechanisms associated with obesity. Chen and his colleagues examined RNA pol III transcripts in mESCs for their functional role, through which they discovered that Maf1 assists in promoting the mesoderm induction and adipocyte differentiation. Conversely, if Maf1 expression decreases in mESCs, then preadipocytes will display impaired adipogenesis; increased expression will enhance differentiation (Chen, Lanz, Walkey et al., 2018). The capacity for adipogenesis is determined using epigenetic regulators: the role of histone H3K27 demethylase encoded by Utx gene**,** has been investigated during the differentiation process of mESCs. Furthermore, results indicate that Utx mediates adipogenesis by regulating *c-myc* in a differentiation stage-specific manner. Targeting Utx signaling pathways is potentially valuable for the treatment of obesity, diabetes, and congenital Utx-deficiency disorders (Ota, Tong, Goto et al., 2017).

In 2018, advanced tools for automated cell sorting in lineage analysis were established. Label-free quantitative imaging was used to classify the cell population on intermediate states during the differentiation of mESCs into adipocytes. The methodology was developed to distinguish undifferentiated cells from cells in other stages, and to estimate the optimal number of clusters in differentiated cells (Masia, Glen, Stephens et al., 2018). Other high-throughput techniques were developed by Guerrero-Robles and his team who introduced a new biosensor technique known as electrical bioimpedance spectroscopy (EBIS) using mESCs, MEFs and 3T3-L1 cells. This method helped to identify and measure the cell lineage population, cell differentiation and undifferentiation process of adipogenesis (Guerrero-Robles, Vazquez-Zapien, Mata-Miranda et al., 2017).

Earlier in 2011, ultrasound standing wave traps (USWT) were used to analyze the gene expression of mESCs. The technique promised safe cell manipulation techniques for a variety of applications, including tissue engineering and regenerative medicine (Bazou, Kearney, Mansergh et al., 2011). mESCs are also used in the bioengineered 3D culture system created by Unser and Mooney et al. to open up a new arena for studying the morphology of brown adipogenesis and its implications in obesity and metabolic disorders (Unser, Mooney, Corr et al., 2016). Previously, 3D culture of mESCs has been developed using electro-spun polymer scaffolds, this *in vitro* 3D model mimicking the *in vivo* environment required to effectively study adipogenesis (Kang, Xie, Powell et al., 2007). These studies promoted the idea that mESCs could be a potential method to understand the process of adipogenesis, especially when integrated with high through-put techniques.

Previous studies with immortalized mouse stromal cell line or other mesenchymal precursor cells isolated from adult tissues have been used to determine mesenchymal cell fate decisions. However, these systems were not found informative with respect to the developmental origin of mesenchymal stem cells and adipocytes (Billon, Kolde, Reimand et al., 2010). Thus, a more suitable source of embryonic cells should be used to address this issue and elucidate the exact pathways and intermediates between the embryonic stem cell and the mature adipocyte. Accordingly, mESCs have the capability to enable exploration of the developmental fate of adipocytes from their gene expression (Billon et al., 2010).

A major limitation of mESCs is the heterogeneity of the culture combined with the low efficiency of the adipocyte differentiation (Schaedlich, Knelangen, Navarrete Santos et al., 2010). It was later discovered that the addition of ascorbic acid (AA) in adipogenic cocktail causes robust and efficient differentiation of mESCs to mature adipocytes (Cuaranta-Monroy, Simandi and Nagy, 2015). In conclusion, this pluripotent stem cell provides a remarkable *in vitro* model to study the genetics and epigenetic mechanisms involved in adipogenesis and could also be promising in areas including anti-obesity drug screening and tissue engineering in order to understand the obesity-related complex diseases.

2.9 Mouse Embryonic Fibroblasts (MEFs)

Mouse Embryonic Fibroblasts (MEFs) are an actively used model in the study of adipose cells. This primary cell line is derived from a pregnant female mouse during 13.5 to 15.5 embryonic days by removing the head, limbs, tail and internal organs from the embryos. The remaining minced carcasses are then rinsed with phosphate buffer saline (PBS), and are cut into smaller pieces. These pieces are trypsinized and seeded into a culture medium for single-cell suspension forming them into a largely homogeneous population of cells after a few passages (Singhal, Sassi, Lan et al., 2016,Nagy, Gertsenstein, Vintersten et al., 2006). Primary MEFs have proven useful, but their lifespan is limited. Moreover, the isolation of these fibroblast cells is time-consuming and labour intensive, especially as they take a long time to be prepared for the experiment. After repeated transmissions, the fibroblasts will reach senescence and finally die off (Amand, Hanover and Shiloach, 2016).

Researchers have developed immortal MEFs with permanent growth features using two approaches. The first approach is using serial passages of MEF cells and the second approach is the transformation of the primary MEFs by overexpressing oncogenes using viral infections (Xu, 2005). These methods develop an immortalized MEF with the desired genetic manipulations, making MEF cell line maintenance time efficient, with indefinite growth. MEFs are well-known for their use as feeder layers during culture of mESCs as they provide factors that enhance proliferation and maintenance of undifferentiated states (Hogan, Costantini and Lacy, 1986). They also assist with the study of biological properties including cell cycle regulation, immortalization, transformation, senescence, apoptosis and differentiation (Yusuf et al., 2013). In addition, these fibroblast cells can differentiate into adipogenic, chondrogenic, and osteogenic lineages expressing typical differentiation markers (Dastagir, Reimers, Lazaridis et al., 2014).

As with other cell lines, MEFs are also cultured and maintained in a growth medium (DMEM media and FBS) at 37 °C and 5% CO_2. To induce differentiation in these cells, cells must reach confluence, after which media is changed to adipocyte induction medium. Once triglyceride concentration is quantified and lipid content is visualized, differentiation can be confirmed by Oil red O staining (refer to Figure 3-D) (Yusuf et al., 2013) (Hou et al., 2020).

The advancement of genomic manipulation has assisted with the creation of genetically engineered mice and knockout mice as efficient tools for human disease research, including the discovery, refinement, and utility of many currently available therapeutic

regimes. Likewise, the cells isolated from these mutant mice could become powerful tools to study the molecular and cellular mechanisms of mutated genes under well-defined culture conditions (Dobrowolski, Fischer and Naumann, 2018). Marian E Durkin, and her team established a protocol to obtain MEF cells from genetically manipulated mouse embryos. MEF cells obtained through this procedure are suitable for use in biochemical assays and further experiments of genetic manipulation (Durkin, Qian, Popescu et al., 2013). Thus, it was acknowledged that genetically modified MEF cells can be used to better understand the adipogenesis process. Recently, MEF cells were also investigated for the metabolic footprint of early adipocyte commitment. Data indicated that ceramide induced apoptosis is essential in initiating adipogenesis by providing lipophilic components that activate adipogenic transcription factor expression and facilitate lipid droplet formation. Therefore, Sirt1 may target treatment of obesity and other ceramide-associated metabolic syndromes (Hou et al., 2020). In another study, MEF cells derived from *Irx3*-knockout mice were developed to identify the role of Irx3 in beige preadipocyte functions and differentiation. Results suggested that complete loss of Irx3 in MEF cells could lead to reduced cell cycle progression, impaired mitochondrial respiration as well as loss of cell identity and an inability to undergo adipogenic differentiation (Bjune, Dyer, Røsland et al., 2020). Consequently, MEF cells developed from genetically modified or knockout mice are approved to study the effects of different genes in adipogenesis program.

Similarly, MEF cells can also assist in evaluating the effects of protein or its gene in adipogenesis in order to create new therapeutic targets to treat obesity and its associated diseases. The 14-3-3ζ scaffold protein was investigated in MEF cells which found the novel adipogenic factors that blocked the obesity-associated expansion of AT (Mugabo, Sadeghi, Fang et al., 2018). Another example is the loss of CD38 expression in MEF cells, which impairs adipogenesis and lipogenesis (Wang et al., 2018). Follistatin (Fst) is a glycoprotein which resulted in increased browning of WAT by increasing UCP1, PRDM16 and PGC-1α expression in MEF cells (Singh, Braga, Reddy et al., 2017), whilst, Pin1 is a peptidylprolyl cis/trans isomerase that was found in enhanced adipocytes differentiation in MEF cells by increased expression of PPARγ and ERK pathway (Han, Lee, Bahn et al., 2016). Meanwhile, Interferon-alpha (IFNα) was found to inhibit adipocyte differentiation at early stages of adipogenesis by decreasing the expression of PPARγ and C/EBPα (Lee, Um, Rhee et al., 2016). Additionally, ADAMTS5, a metalloproteinase superfamily protein has been identified as a promotor of angiogenesis effects in MEF cells as well as *in vivo* (Bauters, Scroyen, Deprez-Poulain et al.,

2016). Table 6 presents a list of proteins and their identified mechanism in MEFs cell in term of adipogenesis.

These cell lines were also integrated with high throughput techniques to identify and characterize diverse cells types and the cell differentiation process in adipogenesis. For example, EBIS was developed to identify populations of undifferentiated mESCs, MEFs and the differentiation process from preadipocytes (3T3-L1) to mature adipocytes (Guerrero-Robles et al., 2017). As with primary cultures, MEFs also have certain limitations due to their origin. Hence, the cellular heterogeneity of embryonic tissue and the culture of these cells often presents difficulties. However, a few steps could ensure a greater degree of homogeneity (Garfield, 2010).

Several studies revealed that fibroblast cells were identical to MSCs, and can differentiate into bone, fat and cartilage cells. In particular, MEFs are easily established and maintained, they proliferate rapidly and a can provide a large number of cells from a single embryo (Yusuf et al., 2013). Moreover, immortalized MEFs have the potential to expand through several passages. Accordingly, these properties make MEF an attractive cell culture model to further explore and create a better understanding of adipogenesis.

Table 6: List of compounds, protein and their mechanism investigated in MEF cells (↑ Increased; ↓ Decreased)

No.	Compound	Description	Mechanism	Comments	Reference
1.	RepSox	Inhibitor of transforming growth factor-beta receptor I (TGF-β-RI)	↑ UCP1 ↑ PRDM16 ↑ PGC-1α	Induced browning of adipogenesis	(Tu, Fu and Xie, 2019)
2.	2,6-Dimethoxy-1,4-benzoquinone (DMBQ)	Present in fermented wheat germ	↓ PPARγ ↓ C/EBPα ↓ FAS ↓ aP2 ↑ AMPK signaling	Decreased adipogenesis	(Son et al., 2019)
3.	Adenanthin	Natural *ent*-kaurane diterpenoid from *Isodon adenantha*	↓ PPARγ ↓ FABP4 ↓ C/EBPβ	Anti-obesity effects	(Hu et al., 2019)
4.	Di-2-ethylhexyl phthalate (DEHP)	A ubiquitous plasticizer and Endocrine disruptor chemical (EDC)	↑ PPARγ	Contributed in development of obesity	(Hunt, Wang, Chen et al., 2017)
5.	N-acetylcysteine (NAC)	A nutritional supplement from cysteine	↑ MAPK pathway ↓ aP2 ↓ PPARγ ↓ C/EBPβ	Inhibited lipid accumulation	(Pieralisi, Martini, Soto et al., 2016)
6.	Chrysin	An active flavonoid present in many herb	↓ PPARγ2 ↓ LPL ↓ aP2	Decreased adipogenic differentiation	(Gao, Ding, Shui et al., 2016)

7.	4-(1-(4-iso-propylbenzyl)-1H-1,2,3-triazol-4-yl) benzene-1,2-diol (2e)		↑ binding of 2e with Estrogen receptor (ERRγ)	Induced browning	(Xu, Mao, Ding et al., 2015)
8.	14-3-3ζ scaffolds protein	Regulator of cellular signaling cascades	PPARγ *Lpin1*	Novel adipogenic protein	(Mugabo et al., 2018)
9.	CD38	Transmembrane glycoprotein	CD38 deficiency (-) ↓ PPARγ ↓ AP2 ↓ C/EBPα ↓ SREBP1-c ↓ FASN	CD38 deficiency impaired adipogenesis and lipogenesis in AT	(Wang et al., 2018)
10.	$p16^{INK4a}$	Cell cycle regulator and tumor suppressor	$p16^{INK4a}$ deficiency (-) ↓ Adipogenesis	Involved in AT formation	(Wouters, Deleye, Hannou et al., 2017)
11.	Follistatin (Fst)	An autocrine glycoprotein and express in most tissues	↑ UCP1 ↑ PRDM16 ↑ PGC-1α ↑ GLUT4	Increased browning of WAT	(Singh et al., 2017)
12.	FK506-binding protein 51 (FKBP51)	Intracellular protein act as cochaperone in heat shock protein 90 (Hsp90) machinery		Involved in regulation of adipogenesis	(Zhang, Qiu, Wang et al., 2017)
13.	EP3 receptor	Gi protein-coupled prostaglandin receptor	↓ PPARγ ↑ HSL	Included in bodily lipid and glucose metabolism	(Xu, Fu, Miao et al., 2016)
14.	Pin1	Peptidylprolyl cis/trans isomerase and isomerizes Ser/Thr-Pro motifs	↑ PPARγ ↑ ERK pathway	Regulator of adipocyte differentiation	(Han et al., 2016)
15.	Interferon-alpha (IFN-α)	Key immunoregulatory cytokine	↓ PPARγ ↓ C/EBPα	Decreased adipocyte differentiation and high antiadipogenic effects	(Lee et al., 2016)
16.	A Disintegrin And Metalloproteinase with Thrombospondin type 1 motifs; member 5 (ADAMTS5)	Metalloproteinase superfamily protein		Increased adipogenesis and shows angiogenesis effects	(Bauters et al., 2016)
17.	BCL11B	Zinc finger-type transcription factor	↑ C/EBPβ ↓ Wnt/β-catenin signaling	Function as regulator of adipogenesis	(Inoue, Ihara, Tsukamoto et al., 2016)
18.	BACH1	BTB and CNC homology 1 (BACH1) repressor	↓ PPARγ	Decreased adipocyte differentiation	(Matsumoto, Kondo, Shiraki et al., 2016)
19.	Perilipin2 (Plin2)	Also known as adipose differentiation-related protein (ADRP)	Stabilized upon lipolytic stimuli ↑ lipolysis	Positive regulator of lipolysis	(Takahashi, Shinoda, Kamada et al., 2016)
20.	Transglutaminase 2 (TG2)	Multifunctional crosslinking enzyme	↓ PPARγ ↓ C/EBPα ↑ β-catenin	Negative regulator of adipogenesis	(Myneni, Melino and Kaartinen, 2015)
21.	Serine/threonine kinase 40 (Stk40)	A putative serine/threonine kinase protein	↓ C/EBP proteins	Decreased adipogenesis	(Yu, He, Wang et al., 2015)
22.	Gelatinase A (MMP-2)	Type IV collagenase known as matrix metalloproteinase-2	↑ PPARγ ↑ aP2 ↑ Adiponectin	Impaired adipogenesis	(Bauters et al., 2015)

23.	FTO gene	The fat mass and obesity-associated protein	↑ RUNX1T1-S ↑ FABP4 ↑ PPARγ ↑ C/EBPα ↑ PLIN1	Increased adipogenesis	(Merkestein, Laber, McMurray et al., 2015)
24.	Ewing sarcoma gene (EWS)	Putative RNA-binding protein	↑ C/EBPβ ↑ C/EBPδ	Essential during early differentiation	(Park and Lee, 2015)
25.	MEFs injection		Formed a single fat pad	Used as cell-based therapies for the treatment of leptin-deficient states	(Ferguson, Blenden, Hutson et al., 2018)
26.	Glyphosate-based herbicides (GF)	Active ingredient of herbicide	↓ PPARγ ↑ Oxidative stress	Reduced Prefiltration and differentiation	(Martini, Gabrielli, Brandani et al., 2016)

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3 Conclusion

Cellular differentiation is commonly used for adipogenesis studies. The process is used to transform preadipocytes into mature adipocytes via adipogenic cocktails. These cocktails are also known as adipogenic agents and are defined prodifferentiative agents required for conversion of undifferentiated cells into differentiated adipocyte cells (Moreno-Navarrete and Fernández-Real, 2017). Cultivating the cells in the growth media is a fundamental step to prepare the preadipocyte cells for induction. Of note, a humidified atmosphere of 37°C with 5-10% CO_2 is essential. Once the cells reach confluency, the cells are exposed to the adipogenic agents, which generally vary for each cell line. The three major inducers most commonly used for differentiation include insulin, DEX and IBMX (Zhao et al., 2019). The confluent cells are cultured in the differentiation agents, refreshing the medium periodically. Over time, the cells reveal adipocyte-like characteristics such as formation of lipid droplets which approve their differentiation (Moreno-Navarrete and Fernández-Real, 2017).

There are minor differences in the differentiation protocol for each cell line that has been discussed in Table 1. For example, additional adipogenic agents are usually necessary in FBS medium for 3T3-L1 cell conversion, however, 3T3-L1 cells can be differentiated with adipogenic serum without the addition of IBMX/DEX. The absence of bovine serum or growth hormone in the culture medium can prevent the 3T3-F442A cells from undergoing adipose differentiation, and hereby the cells can be maintained at their pre-confluency stage (Hemmeryckx et al., 2019). It was also identified in 1987, that an anti-inflammatory drug known as indomethacin was a potent adipogenic inducer that stimulates differentiation in a shorter period of 3 days, with 90% adipocyte capacity in comparison to DEX-treated cells (Knight et al., 1987). The greatest advantage of Ob1771 is its doubling time that allow its cells to rapidly multiply. Furthermore, some studies suggested that low serum concentrations have a slight effect on Ob1771 cells. Wherein, during the absence of added insulin, adipose conversion can occur significantly. We have also identified that OP9 cells can be differentiated by three different methods (Wolins et al., 2006). All three treatment methods require OP9 cells to be maintained in propagation medium until they differentiate into adipocytes morphology that accumulate triglycerides and abundant intracellular lipid droplets.

Applications of these cell lines are similar, as they all assist in understanding the role of adipocyte-related proteins and genes. However, 3T3-L1 cell line is majorly used in co-culture and three-dimensional culture systems for AT. The majority of these cell lines can be

used to screen anti-adipogenic compounds, anti-adipogenic peptides, adipogenic agents in food products and anti-adipogenesis crude extracts. TA1 cells also have the potential to identify early adipogenic markers. OP9 and mESCs are the more frequently used cell lines in advanced and high-throughput techniques, since the differentiation time for the cell lines are short, averaging around 10 days. Whilst some require the medium to be refreshed at short-time intervals, others require a change of medium. Studies indicate that Ob1771 cells are easy to differentiate within a short period, unlike AP-18 cells, which require RPMI 1640 medium and a rigorous differentiation process that can take up to 21 days for differentiation.

We note that, in addition to the heterogeneous mesenchymal cell populations discussed above, another important cellular model in the study of adipose biology is adipose-derived tissue from mouse stromal vascular fractions (SVF), comprising of adipose stromal/stem cells (ASC) (Cawthorn, Scheller and MacDougald, 2012,Bourin, Bunnell, Casteilla et al., 2013). ASCs provide cell renewal and repair functions, as well as maintenance of homeostasis in AT (Zhang, Liu, Yong et al., 2015). While these cells are significantly more heterogeneous than the examples we have focused upon, in common with MSCs, these cells can transform into adipogenic and other lineage *in vitro* (Kelly, Tanaka, Baron et al., 1998,Zheng, Cao, Li et al., 2006,Kilroy, Dietrich, Wu et al., 2018), and harvesting MSCs from AT can yield better accessibility and greater abundance of MSCs. Furthermore, ASC fractions are thought to be primarily composed of immune cells, which enables us to make links between the immune system and obesity-related health problems, taking into account several studies that have established that chronic inflammation of AT is characterized by the influx of immune cells into AT caused by obesity (Grant and Dixit, 2015). We refer readers to recent review papers (Sadie-Van Gijsen, 2019,Jankowski, Dompe, Sibiak et al., 2020,Chu, Nguyen Thi Phuong, Tien et al., 2019) for further detail upon the differentiation, characterization, and applications of ASCs.

An extensive number of studies have described that the development of obesity and related metabolic diseases are mainly instigated by dysregulation of AT. Thus, developing new strategies in this regard requires critical knowledge of molecular pathways regulating adipocyte development and metabolism. Importantly, employing cellular models has provided essential evidence of the contribution of AT to energy homeostasis. These cell lines have become suitable models for study of adipogenesis and its obesity-related metabolic alterations. Nevertheless, they have also been useful for studying adipocyte renewal, expansion and donor and depot-specific differences.

There are several benefits and limitations of different cell line models which must be acknowledged; hence, this review assists with interpreting data and selecting a good cell line model by creating a better understanding on the science of adipocytes and AT, as well as their mechanism. The review also provides a detailed insight of available *in vitro* cell models which enables the determination of the crucial factors and pathways that will assist in targeting new pharmacological interventions against obesity and diabetes. Whilst 3D cultures and co-cultures of adipocytes with other cell types have been used as crucial tools to elucidate the multiple metabolic connections between fat and other tissues.

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Conflicts of Interest

The authors report no conflict of interest.

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List of Abbreviation

PPARγ	Peroxisome Proliferator-Activated Receptor-γ
AT	Adipose Tissue
WAT	White Adipocytes Tissue
BAT	Brown Adipocytes Tissue
UCP1	Uncoupling protein-1
PPARα	Peroxisome Proliferator-Activated Receptor-α
mESCs	Mouse Embryonic Stem Cells
MEFs	Mouse Embryonic Fibroblasts
RA	Retinoic acid
T3	Triiodothyronine
MSCs	Mesenchymal Stem Cells
DEX	Dexamethasone
IBMX	3-isobutyl-1-methylxanthine
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
PGC-1α	Peroxisome Proliferator-Activated Receptor-gamma Coactivator-1α
PRDM16	PR domain containing 16
C/EBPα	CCAAT/enhancer-binding protein-α
C/EBPβ	CCAAT/enhancer-binding protein-β
C/EBPδ	CCAAT/enhancer-binding protein-δ
aP2	Adipocyte Protein 2
Pref-1	Preadipocyte factor 1
LPL	Lipoprotein lipase
SREBP-1c	Sterol regulatory element-binding protein-1c
FABP4	Fatty Acid-Binding Protein 4
FAS	Fatty acid synthase
HSL	Hormone-sensitive lipase
LPL	Lipoprotein lipase
CPT1	Carnitine palmitoyltransferase I
SVF	Stromal vascular fractions
ACS	Adipose stromal/stem cells
IL-6	Interleukin 6
IL-1β	Interleukin 1β
RUNX2	Runt-related transcription factor 2
DGATs	Diacylglycerol acyltransferases
SCD1	Stearoyl-CoA desaturase 1
ATGL	Adipose triglyceride lipase
AMPK	AMP-activated protein kinase
ACC	Acetyl-CoA carboxylase
Akt	Protein Kinase B
GLUT4	Glucose transporter type 4
FOXO3a	Forkhead box O3
PLIN	Perilipin 1
TNFα	Tumor necrosis factor-α
NPY	Neuropeptide Y
NPYR	Neuropeptide Y receptor
EBs	Embryoid bodies

LIF	Leukemia inhibitory factor
MAPK	Mitogen-activated protein kinase

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References

[1] Chooi, Y.C., Ding, C. and Magkos, F., 2019. The epidemiology of obesity, Metabolism. 92, 6-10.

[2] Włodarczyk, M. and Nowicka, G., 2019. Obesity, DNA damage, and development of obesity-related diseases, International journal of molecular sciences. 20, 1146.

[3] Lohmann, A.E., Goodwin, P.J., Chlebowski, R.T., Pan, K., Stambolic, V. and Dowling, R.J., 2016. Association of obesity-related metabolic disruptions with cancer risk and outcome, Journal of Clinical Oncology. 34, 4249-4255.

[4] Abarca-Gómez, L., Abdeen, Z.A., Hamid, Z.A., Abu-Rmeileh, N.M., Acosta-Cazares, B., Acuin, C., Adams, R.J., Aekplakorn, W., Afsana, K. and Aguilar-Salinas, C.A., 2017. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128· 9 million children, adolescents, and adults, The Lancet. 390, 2627-2642.

[5] Wolfenden, L., Ezzati, M., Larijani, B. and Dietz, W., 2019. The challenge for global health systems in preventing and managing obesity, Obesity Reviews. 20, 185-193.

[6] Collaboration, N.R.F., 2016. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19· 2 million participants, The Lancet. 387, 1377-1396.

[7] Ross, S.E., Flynn, J.I. and Pate, R.R., 2016. What is really causing the obesity epidemic? A review of reviews in children and adults, Journal of sports sciences. 34, 1148-1153.

[8] de Ferranti, S. and Mozaffarian, D., 2008. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences, Clinical chemistry. 54, 945-955.

[9] van Meijel, R.L., Blaak, E.E. and Goossens, G.H., 2019. Adipose tissue metabolism and inflammation in obesity, Mechanisms and Manifestations of Obesity in Lung Disease. Elsevier, pp. 1-22.

[10] Jo, J., Gavrilova, O., Pack, S., Jou, W., Mullen, S., Sumner, A.E., Cushman, S.W. and Periwal, V., 2009. Hypertrophy and/or hyperplasia: dynamics of adipose tissue growth, PLoS computational biology. 5.

[11] Booth, A., Magnuson, A., Fouts, J. and Foster, M.T., 2016. Adipose tissue: an endocrine organ playing a role in metabolic regulation, Hormone molecular biology and clinical investigation. 26, 25-42.

[12] PNandhini, L., Desai, A. and Sahoo, J., 2019. Adipose Tissue as an Endocrine Organ, Lipocrinology: The Relationship between Lipids and Endocrine Function.

[13] Giralt, M. and Villarroya, F., 2013. White, brown, beige/brite: different adipose cells for different functions?, Endocrinology. 154, 2992-3000.

[14] Han, S.-J., Zaretsky, A.G., Andrade-Oliveira, V., Collins, N., Dzutsev, A., Shaik, J., da Fonseca, D.M., Harrison, O.J., Tamoutounour, S. and Byrd, A.L., 2017. White adipose tissue is a reservoir for memory T cells and promotes protective memory responses to infection, Immunity. 47, 1154-1168. e6.

[15] Villarroya, F., Cereijo, R., Villarroya, J. and Giralt, M., 2017. Brown adipose tissue as a secretory organ, Nature Reviews Endocrinology. 13, 26.

[16] Mottillo, E.P., Desjardins, E.M., Crane, J.D., Smith, B.K., Green, A.E., Ducommun, S., Henriksen, T.I., Rebalka, I.A., Razi, A. and Sakamoto, K., 2016. Lack of adipocyte AMPK exacerbates insulin resistance and hepatic steatosis through brown and beige adipose tissue function, Cell metabolism. 24, 118-129.

[17] Rosen, E.D., Walkey, C.J., Puigserver, P. and Spiegelman, B.M., 2000. Transcriptional regulation of adipogenesis, Genes & development. 14, 1293-1307.

[18] Munawar, T.M., Prakash, D.S. and Vangalapati, M., 2018. Development of response surface methodology for optimization of parameters and quantitative analysis of chebulinic acid from composition of medicinal herbs by HPLC, Saudi Journal of Biological Sciences.
[19] Hammarstedt, A., Gogg, S., Hedjazifar, S., Nerstedt, A. and Smith, U., 2018. Impaired adipogenesis and dysfunctional adipose tissue in human hypertrophic obesity, Physiological reviews. 98, 1911-1941.
[20] González-Casanova, J.E., Pertuz-Cruz, S.L., Caicedo-Ortega, N.H. and Rojas-Gomez, D.M., 2020. Adipogenesis Regulation and Endocrine Disruptors: Emerging Insights in Obesity, BioMed Research International. 2020.
[21] Ruiz-Ojeda, F.J., Rupérez, A.I., Gomez-Llorente, C., Gil, A. and Aguilera, C.M., 2016. Cell models and their application for studying adipogenic differentiation in relation to obesity: a review, International journal of molecular sciences. 17, 1040.
[22] Wang, Q.A., Scherer, P.E. and Gupta, R.K., 2014. Improved methodologies for the study of adipose biology: insights gained and opportunities ahead, Journal of lipid research. 55, 605-624.
[23] Moreno-Navarrete, J.M. and Fernández-Real, J.M., 2017. Adipocyte differentiation, Adipose tissue biology. Springer, pp. 69-90.
[24] Yusuf, B., Gopurappilly, R., Dadheech, N., Gupta, S., Bhonde, R. and Pal, R., 2013. Embryonic fibroblasts represent a connecting link between mesenchymal and embryonic stem cells, Development, growth & differentiation. 55, 330-340.
[25] Kassotis, C.D., Masse, L., Kim, S., Schlezinger, J.J., Webster, T.F. and Stapleton, H.M., 2017. Characterization of adipogenic chemicals in three different cell culture systems: implications for reproducibility based on cell source and handling, Scientific reports. 7, 42104.
[26] Kim, J.H., Lee, S., Kim, H.Y. and Cho, E.J., 2020. Acer okamotoanum inhibits adipocyte differentiation by the regulation of adipogenesis and lipolysis in 3T3-L1 cells, International Journal of Molecular Medicine. 45, 589-596.
[27] Zhao, X., Hu, H., Wang, C., Bai, L., Wang, Y., Wang, W. and Wang, J., 2019. A comparison of methods for effective differentiation of the frozen-thawed 3T3-L1 cells, Analytical biochemistry. 568, 57-64.
[28] Hemmeryckx, B., Vranckx, C., Bauters, D., Lijnen, H.R. and Scroyen, I., 2019. Does butein affect adipogenesis?, Adipocyte. 8, 209-222.
[29] Khalilpourfarshbafi, M., Murugan, D.D., Sattar, M.Z.A., Sucedaram, Y. and Abdullah, N.A., 2019. Withaferin A inhibits adipogenesis in 3T3-F442A cell line, improves insulin sensitivity and promotes weight loss in high fat diet-induced obese mice, PloS one. 14.
[30] Shinohara, O., Murata, Y.-I. and Shimizu, M., 1992. Enhancement of differentiation of cultured adipogenic cells (TA1) by pertussis toxin, Biochemistry and Cell Biology. 70, 650-655.
[31] Ninomiya-Tsuji, J., Torti, F.M. and Ringold, G.M., 1993. Tumor necrosis factor-induced c-myc expression in the absence of mitogenesis is associated with inhibition of adipocyte differentiation, Proceedings of the National Academy of Sciences. 90, 9611-9615.
[32] Chen, C., Takahashi, K., Yoshida, A., Takizawa, Y., Lee, Y., Nakui, M., Doi, H., Takebayashi, Y., Fukumoto, M. and Yamada, T., 2010. Characterization of a novel murine preadipocyte line, AP-18, isolated from subcutaneous tissue: Analysis of adipocyte-related gene expressions, Cell biology international. 34, 293-299.
[33] Abderrahim-Ferkoune, A., Bezy, O., Astri-Roques, S., Elabd, C., Ailhaud, G. and Amri, E.-Z., 2004. Transdifferentiation of preadipose cells into smooth muscle-like

cells: role of aortic carboxypeptidase-like protein, Experimental cell research. 293, 219-228.
[34] Schwind, L., Schetting, S. and Montenarh, M., 2017. Inhibition of protein kinase CK2 prevents adipogenic differentiation of mesenchymal stem cells like C3H/10T1/2 cells, Pharmaceuticals. 10, 22.
[35] Hussain, S., Rehman, A.U., Luckett, D.J., Blanchard, C.L., Obied, H.K. and Strappe, P., 2020. Phenolic Compounds with Antioxidant Properties from Canola Meal Extracts Inhibit Adipogenesis, International Journal of Molecular Sciences. 21, 1.
[36] Wolins, N.E., Quaynor, B.K., Skinner, J.R., Tzekov, A., Park, C., Choi, K. and Bickel, P.E., 2006. OP9 mouse stromal cells rapidly differentiate into adipocytes: characterization of a useful new model of adipogenesis, Journal of lipid research. 47, 450-460.
[37] Rosen, E.D. and MacDougald, O.A., 2006. Adipocyte differentiation from the inside out, Nature reviews Molecular cell biology. 7, 885.
[38] Ota, K., Tong, K.I., Goto, K., Tomida, S., Komuro, A., Wang, Z., Nishio, K. and Okada, H., 2017. The H3K27 demethylase, Utx, regulates adipogenesis in a differentiation stage-dependent manner, PloS one. 12.
[39] Hou, W., Chen, Q., Wang, H., Qiu, P., Lyu, X., Chen, W., Chua, M.L., Chinn, Y.E., Deng, C.-X. and Wang, R., 2020. The metabolic footprint during adipocyte commitment highlights ceramide modulation as an adequate approach for obesity treatment, EBioMedicine. 51, 102605.
[40] Green, H. and Kehinde, O., 1974. Sublines of mouse 3T3 cells that accumulate lipid, Cell. 1, 113-116.
[41] Antony, J., Debroy, S., Manisha, C., Thomas, P., Jeyarani, V. and Choephel, T., 2019. In-vitro cell line Models and Assay methods to study the Anti-diabetic Activity, Research Journal of Pharmacy and Technology. 12, 2200-2206.
[42] Green, H. and Kehinde, O., 1979. Formation of normally differentiated subcutaneous fat pads by an established preadipose cell line, Journal of cellular physiology. 101, 169-171.
[43] Kuri-Harcuch, W., Velez-delValle, C., Vazquez-Sandoval, A., Hernández-Mosqueira, C. and Fernandez-Sanchez, V., 2019. A cellular perspective of adipogenesis transcriptional regulation, Journal of cellular physiology. 234, 1111-1129.
[44] Novikoff, A.B., Novikoff, P.M., Rosen, O.M. and Rubin, C.S., 1980. Organelle relationships in cultured 3T3-L1 preadipocytes, The Journal of Cell Biology. 87, 180-196.
[45] Xiu, L., Xinong, L., Tianjia, L. and Bao, L., 2017. Research Progress of the Differentiation of 3T3-L1 Preadipocytes into Mature Adipocytes, Chinese Academy of Medical Sciences. 39, 727-731.
[46] Subra, C., Fontana, E., Visentin, V., Testar, X. and Carpéné, C., 2003. Tyramine and benzylamine partially but selectively mimic insulin action on adipose differentiation in 3T3-L1 cells, Journal of physiology and biochemistry. 59, 209-216.
[47] Katafuchi, T., Garbers, D.L. and Albanesi, J.P., 2010. CNP/GC-B system: A new regulator of adipogenesis, Peptides. 31, 1906-1911.
[48] Zebisch, K., Voigt, V., Wabitsch, M. and Brandsch, M., 2012. Protocol for effective differentiation of 3T3-L1 cells to adipocytes, Analytical biochemistry. 425, 88-90.
[49] Vishwanath, D., Srinivasan, H., Patil, M.S., Seetarama, S., Agrawal, S.K., Dixit, M. and Dhar, K., 2013. Novel method to differentiate 3T3 L1 cells in vitro to produce highly sensitive adipocytes for a GLUT4 mediated glucose uptake using fluorescent glucose analog, Journal of cell communication and signaling. 7, 129-140.

[50] Hua, Y., Ke, S., Wang, Y., Irwin, D.M., Zhang, S. and Wang, Z., 2016. Prolonged treatment with 3-isobutyl-1-methylxanthine improves the efficiency of differentiating 3T3-L1 cells into adipocytes, Analytical biochemistry. 507, 18-20.
[51] Li, C., Li, J., He, F., Li, K., Li, X. and Zhang, Y., 2020. Matrix Gla protein regulates adipogenesis and is serum marker of visceral adiposity, Adipocyte. 9, 68-76.
[52] Kou, Y., Liu, Q., Liu, W., Sun, H., Liang, M., Kong, F., Zhang, B., Wei, Y., Liu, Z. and Wang, Y., 2019. LIGHT/TNFSF14 signaling attenuates beige fat biogenesis, The FASEB Journal. 33, 1595-1604.
[53] Xi, P., Xue, J., Wu, Z., Wang, H., Han, J., Liang, H. and Tian, D., 2019. Liver kinase B1 induces browning phenotype in 3 T3-L1 adipocytes, Gene. 682, 33-41.
[54] Tang, W. and Fan, Y., 2019. SIRT6 as a potential target for treating insulin resistance, Life sciences. 116558.
[55] Kobayashi, M., Hoshino, S., Abe, T., Okita, N., Tagawa, R., Nagai, W., Konno, R., Suzuki, Y., Furuya, K. and Ishikawa, N., 2019. Identification of WWP1 as an obesity-associated E3 ubiquitin ligase with a protective role against oxidative stress in adipocytes, Biochemical and biophysical research communications. 508, 117-122.
[56] Camacho, A., Segoviano-Ramírez, J.C., Sánchez-Garcia, A., García-Juarez, J., Hernandez-Puente, C.A., Calvo-Anguiano, G., Maltos-Uro, S.R., Olguin, A., Gojon-Romanillos, G. and Gojon-Zorrilla, G., 2018. Tyrphostin AG17 inhibits adipocyte differentiation in vivo and in vitro, Lipids in health and disease. 17, 128.
[57] Huang, K.-T., Hsu, L.-W., Chen, K.-D., Kung, C.-P., Goto, S. and Chen, C.-L., 2018. Decreased PEDF Expression Promotes Adipogenic Differentiation through the Up-Regulation of CD36, International journal of molecular sciences. 19, 3992.
[58] De Filippis, E., Li, T. and Rosen, E.D., 2018. Exposure of adipocytes to bisphenol-A in vitro interferes with insulin action without enhancing adipogenesis, PloS one. 13, e0201122.
[59] Kim, Y.A., Kim, H.Y., Oh, Y.J., Kwon, W.Y., Lee, M.H., Bae, J.Y., Woo, M.S., Kim, J.-M. and Yoo, Y.H., 2018. Polychlorinated biphenyl 138 exposure-mediated lipid droplet enlargement endows adipocytes with resistance to TNF-α-induced cell death, Toxicology letters. 292, 55-62.
[60] Marino, S., Bishop, R.T., de Ridder, D., Delgado-Calle, J. and Reagan, M.R., 2019. 2D and 3D in vitro co-culture for cancer and bone cell interaction studies, Bone Research Protocols. Springer, pp. 71-98.
[61] Paschos, N.K., Brown, W.E., Eswaramoorthy, R., Hu, J.C. and Athanasiou, K.A., 2015. Advances in tissue engineering through stem cell-based co-culture, Journal of tissue engineering and regenerative medicine. 9, 488-503.
[62] Hendriks, J., Riesle, J. and van Blitterswijk, C.A., 2007. Co-culture in cartilage tissue engineering, Journal of tissue engineering and regenerative medicine. 1, 170-178.
[63] Dodson, M., Vierck, J., Hossner, K., Byrne, K. and McNamara, J., 1997. The development and utility of a defined muscle and fat co-culture system, Tissue and Cell. 29, 517-524.
[64] Hao, Q., Diaz, T., del Rio Verduzco, A., Magyar, C.E., Zhong, J., Elshimali, Y., Rettig, M.B., Henning, S.M., Vadgama, J.V. and Wang, P., 2020. Arctigenin inhibits prostate tumor growth in high-fat diet fed mice through dual actions on adipose tissue and tumor, Scientific Reports. 10, 1-11.
[65] Lu, Y., Ma, J., Zhao, J., Song, Z., Zhou, C., Liu, X., Teng, W., Wang, W., Zhang, Q. and Yan, W., 2020. The Role of MKP-5 in Adipocyte-Macrophage Interactions during Obesity, Obesity Facts. 1-16.
[66] Kang, B., Kim, C.Y., Hwang, J., Suh, H.J. and Choi, H.S., 2019. Brassinin, a phytoalexin in cruciferous vegetables, suppresses obesity-induced inflammatory

responses through the Nrf2-HO-1 signaling pathway in an adipocyte-macrophage co-culture system, Phytotherapy Research. 33, 1426-1437.
[67] Kim, C.Y., Kang, B., Suh, H.J. and Choi, H.-S., 2018. Red ginseng-derived saponin fraction suppresses the obesity-induced inflammatory responses via Nrf2-HO-1 pathway in adipocyte-macrophage co-culture system, Biomedicine & Pharmacotherapy. 108, 1507-1516.
[68] Turner, P.A., Tang, Y., Weiss, S.J. and Janorkar, A.V., 2015. Three-dimensional spheroid cell model of in vitro adipocyte inflammation, Tissue Engineering Part A. 21, 1837-1847.
[69] Lee, S.Y., Park, S.B., Kim, Y.E., Yoo, H.M., Hong, J., Choi, K.-J., Kim, K.Y. and Kang, D., 2019. iTRAQ-Based Quantitative Proteomic Comparison of 2D and 3D Adipocyte Cell Models Co-cultured with Macrophages Using Online 2D-nanoLC-ESI-MS/MS, Scientific reports. 9, 1-13.
[70] Zhu, J., He, J., Verano, M., Brimmo, A.T., Glia, A., Qasaimeh, M.A., Chen, P., Aleman, J.O. and Chen, W., 2018. An integrated adipose-tissue-on-chip nanoplasmonic biosensing platform for investigating obesity-associated inflammation, Lab on a Chip. 18, 3550-3560.
[71] Lustig, M., Feng, Q., Payan, Y., Gefen, A. and Benayahu, D., 2019. Noninvasive continuous monitoring of adipocyte differentiation: From macro to micro scales, Microscopy and Microanalysis. 25, 119-128.
[72] Pereira-Fernandes, A., Vanparys, C., Vergauwen, L., Knapen, D., Jorens, P.G. and Blust, R., 2014. Toxicogenomics in the 3T3-L1 cell line, a new approach for screening of obesogenic compounds, Toxicological Sciences. 140, 352-363.
[73] Poulos, S.P., Dodson, M.V. and Hausman, G.J., 2010. Cell line models for differentiation: preadipocytes and adipocytes, Experimental biology and medicine. 235, 1185-1193.
[74] Adler-Wailes, D.C., Guiney, E.L., Wolins, N.E. and Yanovski, J.A., 2010. Long-term ritonavir exposure increases fatty acid and glycerol recycling in 3T3-L1 adipocytes as compensatory mechanisms for increased triacylglycerol hydrolysis, Endocrinology. 151, 2097-2105.
[75] Hock, F.J., 2016. Drug discovery and evaluation: Pharmacological assays, Springer.
[76] Hernández-Mosqueira, C., Velez-delValle, C. and Kuri-Harcuch, W., 2015. Tissue alkaline phosphatase is involved in lipid metabolism and gene expression and secretion of adipokines in adipocytes, Biochimica et Biophysica Acta (BBA)-General Subjects. 1850, 2485-2496.
[77] Löffler, M.C., Mayer, A.E., Viera, J.T., Valdes, A.L., El-Merahbi, R., Ade, C.P., Karwen, T., Schmitz, W., Slotta, A. and Erk, M., 2018. Protein kinase D1 deletion in adipocytes enhances energy dissipation and protects against adiposity, The EMBO journal. 37.
[78] Perez-Diaz, S., Garcia-Sobreviela, M.P., Gonzalez-Irazabal, Y., Garcia-Rodriguez, B., Espina, S., Arenaz, I. and Arbones-Mainar, J.M., 2018. PTRF acts as an adipokine contributing to adipocyte dysfunctionality and ectopic lipid deposition, Journal of physiology and biochemistry. 74, 613-622.
[79] Xiang, R., Fan, L.-l., Huang, H., Chen, Y.-q., He, W., Guo, S., Li, J.-J., Jin, J.-y., Du, R. and Yan, R., 2018. Increased Reticulon 3 (RTN3) Leads to Obesity and Hypertriglyceridemia by Interacting With Heat Shock Protein Family A (Hsp70) Member 5 (HSPA5), Circulation. 138, 1828-1838.
[80] Zeng, F., Wang, Y., Kloepfer, L.A., Wang, S. and Harris, R.C., 2018. ErbB4 deletion predisposes to development of metabolic syndrome in mice, American Journal of Physiology-Endocrinology and Metabolism. 315, E583-E593.

[81] Nugroho, D.B., Ikeda, K., Barinda, A.J., Wardhana, D.A., Yagi, K., Miyata, K., Oike, Y., Hirata, K.-i. and Emoto, N., 2018. Neuregulin-4 is an angiogenic factor that is critically involved in the maintenance of adipose tissue vasculature, Biochemical and biophysical research communications. 503, 378-384.
[82] Hou, S., Jiao, Y., Yuan, Q., Zhai, J., Tian, T., Sun, K., Chen, Z., Wu, Z. and Zhang, J., 2018. S100A4 protects mice from high-fat diet-induced obesity and inflammation, Laboratory Investigation. 98, 1025.
[83] Chang, E.-J., Shin, M.-K., Choi, B., Kim, E.-Y., Park, J.-E., Hwang, E.S., Lee, H.J., Kim, M.K., Kim, S.W. and Kim, J.-E., 2018. Elevated pentraxin 3 in obese adipose tissue promotes adipogenic differentiation by activating neuropeptide Y signaling, Frontiers in immunology. 9, 1790.
[84] Wang, L.F., Miao, L.J., Wang, X.N., Huang, C.C., Qian, Y.S., Huang, X., Wang, X.L., Jin, W.Z., Ji, G.J. and Fu, M., 2018. CD 38 deficiency suppresses adipogenesis and lipogenesis in adipose tissues through activating Sirt1/PPAR γ signaling pathway, Journal of cellular and molecular medicine. 22, 101-110.
[85] Wang, Y.-M., Liu, H.-X. and Fang, N.-Y., 2018. 9-PAHSA promotes browning of white fat via activating G-protein-coupled receptor 120 and inhibiting lipopolysaccharide/NF-kappa B pathway, Biochemical and biophysical research communications. 506, 153-160.
[86] Son, H.J., Jang, Y.J., Jung, C.H., Ahn, J. and Ha, T.Y., 2019. 2, 6-Dimethoxy-1, 4-benzoquinone Inhibits 3T3-L1 Adipocyte Differentiation via Regulation of AMPK and mTORC1, Planta medica. 85, 210-216.
[87] Lee, M.R., Kim, J.E., Choi, J.Y., Park, J.J., Kim, H.R., Song, B.R., Choi, Y.W., Kim, K.M., Song, H. and Hwang, D.Y., 2019. Anti-obesity effect in high-fat-diet-induced obese C57BL/6 mice: Study of a novel extract from mulberry (Morus alba) leaves fermented with Cordyceps militaris, Experimental and therapeutic medicine. 17, 2185-2193.
[88] Lee, M., Sorn, S., Lee, Y. and Kang, I., 2019. Salt induces adipogenesis/lipogenesis and inflammatory adipocytokines secretion in adipocytes, International journal of molecular sciences. 20, 160.
[89] Hu, J., Li, X., Tian, W., Lu, Y., Xu, Y., Wang, F., Qin, W., Ma, X., Puno, P.-T. and Xiong, W., 2019. Adenanthin, a Natural ent-Kaurane Diterpenoid Isolated from the Herb Isodon adenantha Inhibits Adipogenesis and the Development of Obesity by Regulation of ROS, Molecules. 24, 158.
[90] Peng, Y., Sun, Q., Xu, W., He, Y., Jin, W., Yuan, L. and Gao, R., 2019. Vitexin ameliorates high fat diet-induced obesity in male C57BL/6J mice via the AMPKα-mediated pathway, Food & function. 10, 1940-1947.
[91] de Melo, K.M., de Oliveira, F.T.B., Silva, R.A.C., Quinderé, A.L.G., Marinho Filho, J.D.B., Araújo, A.J., Pereira, E.D.B., Carvalho, A.A., Chaves, M.H. and Rao, V.S., 2019. α, β-Amyrin, a pentacyclic triterpenoid from Protium heptaphyllum suppresses adipocyte differentiation accompanied by down regulation of PPARγ and C/EBPα in 3T3-L1 cells, Biomedicine & Pharmacotherapy. 109, 1860-1866.
[92] Kim, H.-L., Park, J., Jung, Y., Ahn, K.S. and Um, J.-Y., 2019. Platycodin d, a novel activator of amp-activated protein kinase, attenuates obesity in db/db mice via regulation of adipogenesis and thermogenesis, Phytomedicine. 52, 254-263.
[93] Choi, J., Song, N.-J., Lee, A., Lee, D., Seo, M.-J., Kim, S., Chang, S.-H., Yang, D., Hwang, Y.-J. and Hwang, K.-A., 2019. Oxyresveratrol Increases Energy Expenditure through Foxo3a-Mediated Ucp1 Induction in High-Fat-Diet-Induced Obese Mice, International journal of molecular sciences. 20, 26.

[94] Biasiotto, G., Zanella, I., Predolini, F., Archetti, I., Cadei, M., Monti, E., Luzzani, M., Pacchetti, B., Mozzoni, P. and Andreoli, R., 2018. 7-Hydroxymatairesinol improves body weight, fat and sugar metabolism in C57BJ/6 mice on a high-fat diet, British Journal of Nutrition. 120, 751-762.
[95] Hsieh, C.-T., Chang, F.-R., Tsai, Y.-H., Wu, Y.-C. and Hsieh, T.-J., 2018. 2-Bromo-4′-methoxychalcone and 2-Iodo-4′-methoxychalcone Prevent Progression of Hyperglycemia and Obesity via 5′-Adenosine-Monophosphate-Activated Protein Kinase in Diet-Induced Obese Mice, International journal of molecular sciences. 19, 2763.
[96] Kang, N.H., Mukherjee, S., Min, T., Kang, S.C. and Yun, J.W., 2018. Trans-anethole ameliorates obesity via induction of browning in white adipocytes and activation of brown adipocytes, Biochimie. 151, 1-13.
[97] Pai, S., Martis, E., Joshi, S., Munshi, R. and Juvekar, A., 2018. Plumbagin exerts antiobesity effects through inhibition of pancreatic lipase and adipocyte differentiation, Phytotherapy research. 32, 1631-1635.
[98] Liu, H., Wang, J., Liu, M., Zhao, H., Yaqoob, S., Zheng, M., Cai, D. and Liu, J., 2018. Antiobesity effects of ginsenoside Rg1 on 3T3-L1 preadipocytes and high fat diet-induced obese mice mediated by AMPK, Nutrients. 10, 830.
[99] Mi, Y., Liu, X., Tian, H., Liu, H., Li, J., Qi, G. and Liu, X., 2018. EGCG stimulates the recruitment of brite adipocytes, suppresses adipogenesis and counteracts TNF-α-triggered insulin resistance in adipocytes, Food & function. 9, 3374-3386.
[100] Green, H. and Kehinde, O., 1976. Spontaneous heritable changes leading to increased adipose conversion in 3T3 cells, Cell. 7, 105-113.
[101] Sadie-Van Gijsen, H., 2019. Adipocyte biology: It is time to upgrade to a new model, Journal of cellular physiology. 234, 2399-2425.
[102] Mandrup, S., Loftus, T.M., MacDougald, O.A., Kuhajda, F.P. and Lane, M.D., 1997. Obese gene expression at in vivo levels by fat pads derived from sc implanted 3T3-F442A preadipocytes, Proceedings of the National Academy of Sciences. 94, 4300-4305.
[103] Kuri-Harcuch, W. and Green, H., 1978. Adipose conversion of 3T3 cells depends on a serum factor, Proceedings of the National Academy of Sciences. 75, 6107-6109.
[104] Khalilpourfarshbafi, M., Murugan, D.D., Sattar, M.Z.A., Sucedaram, Y. and Abdullah, N.A., 2019. Withaferin A inhibits adipogenesis in 3T3-F442A cell line, improves insulin sensitivity and promotes weight loss in high fat diet-induced obese mice, PloS one. 14, e0218792.
[105] Ayala-Sumuano, J.-T., Velez-Del Valle, C., Beltrán-Langarica, A., Hernández, J.M. and Kuri-Harcuch, W., 2008. Adipogenic genes on induction and stabilization of commitment to adipose conversion, Biochemical and biophysical research communications. 374, 720-724.
[106] Ayala-Sumuano, J.-T., Velez-delValle, C., Beltrán-Langarica, A., Marsch-Moreno, M., Cerbón-Solorzano, J. and Kuri-Harcuch, W., 2011. Srebf1a is a key regulator of transcriptional control for adipogenesis, Scientific reports. 1, 178.
[107] Diaz-Velasquez, C.E., Castro-Muñozledo, F. and Kuri-Harcuch, W., 2008. Staurosporine rapidly commits 3T3-F442A cells to the formation of adipocytes by activation of GSK-3β and mobilization of calcium, Journal of cellular biochemistry. 105, 147-157.
[108] Tang, Q.Q. and Lane, M.D., 2012. Adipogenesis: from stem cell to adipocyte, Annual review of biochemistry. 81, 715-736.
[109] Ayala-Sumuano, J.-T., Velez-delValle, C., Beltrán-Langarica, A., Marsch-Moreno, M., Hernandez-Mosqueira, C. and Kuri-Harcuch, W., 2013. Glucocorticoid

paradoxically recruits adipose progenitors and impairs lipid homeostasis and glucose transport in mature adipocytes, Scientific reports. 3, 2573.
[110] Masuzaki, H., Paterson, J., Shinyama, H., Morton, N.M., Mullins, J.J., Seckl, J.R. and Flier, J.S., 2001. A transgenic model of visceral obesity and the metabolic syndrome, Science. 294, 2166-2170.
[111] Torabi, S. and Mo, H., 2016. Trans, trans-farnesol as a mevalonate-derived inducer of murine 3T3-F442A pre-adipocyte differentiation, Experimental Biology and Medicine. 241, 493-500.
[112] Skillington, J., Choy, L. and Derynck, R., 2002. Bone morphogenetic protein and retinoic acid signaling cooperate to induce osteoblast differentiation of preadipocytes, The Journal of cell biology. 159, 135-146.
[113] Torabi, S., Yeganehjoo, H., Shen, C.-L. and Mo, H., 2016. Peroxisome proliferator–activated receptor γ down-regulation mediates the inhibitory effect of d-δ-tocotrienol on the differentiation of murine 3T3-F442A preadipocytes, Nutrition Research. 36, 1345-1352.
[114] Carreira, M.C., Andrade, S., Gonzalez-Izquierdo, A., Amil, M., Folgueira, C., Monteiro, M.P., Sanz, E., Crujeiras, A.B. and Casanueva, F.F., 2018. Anti-obesity activity of OBEX is regulated by activation of thermogenesis and decreasing adiposity gain, Scientific reports. 8, 17155.
[115] Gomez-Zorita, S., Belles, C., Briot, A., Fernández-Quintela, A., Portillo, M.P. and Carpéné, C., 2017. Pterostilbene inhibits lipogenic activity similar to resveratrol or caffeine but differently modulates lipolysis in adipocytes, Phytotherapy research. 31, 1273-1282.
[116] Jahandideh, F., Chakrabarti, S., Davidge, S.T. and Wu, J., 2017. Egg white hydrolysate shows insulin mimetic and sensitizing effects in 3T3-F442A pre-adipocytes, PloS one. 12, e0185653.
[117] Doğan, A., Demirci, S., Kıratlı, B. and Şahin, F., 2017. Cytoglobin: a potential marker for adipogenic differentiation in preadipocytes in vitro, Cytotechnology. 69, 157-165.
[118] Ferrand, N., Béreziat, V., Moldes, M., Zaoui, M., Larsen, A.K. and Sabbah, M., 2017. WISP1/CCN4 inhibits adipocyte differentiation through repression of PPARγ activity, Scientific reports. 7, 1749.
[119] Avau, B., Bauters, D., Steensels, S., Vancleef, L., Laermans, J., Lesuisse, J., Buyse, J., Lijnen, H.R., Tack, J. and Depoortere, I., 2015. The gustatory signaling pathway and bitter taste receptors affect the development of obesity and adipocyte metabolism in mice, PLoS One. 10, e0145538.
[120] Torabi, S. and DiMarco, N.M., 2016. Polyphenols extracted from grape powder induce lipogenesis and glucose uptake during differentiation of murine preadipocytes, Experimental Biology and Medicine. 241, 1776-1785.
[121] Christiaens, V., Sujatha, R., Hellemans, K.H., Pipeleers, D. and Lijnen, H.R., 2010. Functional interactions between pancreatic beta cells and (pre) adipocytes, Endocrine. 38, 118-126.
[122] Louis, F., Pannetier, P., Souguir, Z., Le Cerf, D., Valet, P., Vannier, J.P., Vidal, G. and Demange, E., 2017. A biomimetic hydrogel functionalized with adipose ECM components as a microenvironment for the 3D culture of human and murine adipocytes, Biotechnology and bioengineering. 114, 1813-1824.
[123] Bauters, D., Scroyen, I., Van Hul, M. and Lijnen, H.R., 2015. Gelatinase A (MMP-2) promotes murine adipogenesis, Biochimica et Biophysica Acta (BBA)-General Subjects. 1850, 1449-1456.

[124] Chapman, A.B., Knight, D., Dieckmann, B. and Ringold, G., 1984. Analysis of gene expression during differentiation of adipogenic cells in culture and hormonal control of the developmental program, Journal of Biological Chemistry. 259, 15548-15555.
[125] Chapman, A.B., Knight, D.M. and Ringold, G.M., 1985. Glucocorticoid regulation of adipocyte differentiation: hormonal triggering of the developmental program and induction of a differentiation-dependent gene, The Journal of cell biology. 101, 1227-1235.
[126] Knight, D.M., Chapman, A.B., Navre, M., Drinkwater, L., Bruno, J.J. and Ringold, G.M., 1987. Requirements for triggering of adipocyte differentiation by glucocorticoids and indomethacin, Molecular Endocrinology. 1, 36-43.
[127] Danesch, U., Hoeck, W. and Ringold, G., 1992. Cloning and transcriptional regulation of a novel adipocyte-specific gene, FSP27. CAAT-enhancer-binding protein (C/EBP) and C/EBP-like proteins interact with sequences required for differentiation-dependent expression, Journal of Biological Chemistry. 267, 7185-7193.
[128] Williams, P.M., Chang, D.J., Danesch, U., Ringold, G.M. and Heller, R.A., 1992. CCAAT/enhancer binding protein expression is rapidly extinguished in TA1 adipocyte cells treated with tumor necrosis factor, Molecular Endocrinology. 6, 1135-1141.
[129] Torti, F.M., Dieckmann, B., Beutler, B., Cerami, A. and Ringold, G.M., 1985. A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia, Science. 229, 867-869.
[130] Torti, F.M., Torti, S.V., Larrick, J.W. and Ringold, G.M., 1989. Modulation of adipocyte differentiation by tumor necrosis factor and transforming growth factor beta, The Journal of cell biology. 108, 1105-1113.
[131] Reid, T.R., Torti, F. and Ringold, G., 1989. Evidence for two mechanisms by which tumor necrosis factor kills cells, Journal of Biological Chemistry. 264, 4583-4589.
[132] Haliday, E.M., Ramesha, C.S. and Ringold, G., 1991. TNF induces c-fos via a novel pathway requiring conversion of arachidonic acid to a lipoxygenase metabolite, The EMBO journal. 10, 109-115.
[133] Weber, P.D., Merkel, R. and Bergen, W., 1992. Adipogenic cell line TA1: A suitable model to study the effect of β-adrenergic agonists on lipid metabolism, Proceedings of the Society for Experimental Biology and Medicine. 201, 47-53.
[134] Ibrahimi, A., Bonino, F., Bardon, S., Ailhaud, G. and Dani, C., 1992. Essential role of collagens for terminal differentiation of preadipocytes, Biochemical and biophysical research communications. 187, 1314-1322.
[135] RINGOLD, G.M., CHAPMAN, A.B., KNIGHT, D.M., NAVRE, M. and TORTI, F.M., 1988. Hormonal control of adipocyte differentiation and adipocyte gene expression, Proceedings of the 1987 Laurentian Hormone Conference. Elsevier, pp. 115-140.
[136] Slieker, L.J., Sloop, K.W. and Surface, P.L., 1998. Differentiation method-dependent expression of leptin in adipocyte cell lines, Biochemical and biophysical research communications. 251, 225-229.
[137] Doi, H., Masaki, N., Takahashi, H., Komatsu, H., Fujimori, K. and Satomi, S., 2005. A new preadipocyte cell line, AP-18, established from adult mouse adipose tissue, The Tohoku Journal of Experimental Medicine. 207, 209-216.
[138] Negrel, R., Grimaldi, P. and Ailhaud, G., 1978. Establishment of preadipocyte clonal line from epididymal fat pad of ob/ob mouse that responds to insulin and to lipolytic hormones, Proceedings of the National Academy of Sciences. 75, 6054-6058.

[139] Kamai, Y., Mikawa, S., Endo, K., Sakai, H. and Komano, T., 1996. Regulation of insulin-like growth factor-I expression in mouse preadipocyte Ob1771 cells, Journal of Biological Chemistry. 271, 9883-9886.
[140] Grimaldi, P., Djian, P., Negrel, R. and Ailhaud, G., 1982. Differentiation of Ob17 preadipocytes to adipocytes: requirement of adipose conversion factor (s) for fat cell cluster formation, The EMBO journal. 1, 687-692.
[141] Amri, E.-Z., Dani, C., Doglio, A., Grimaldi, P. and Ailhaud, G., 1986. Coupling of growth arrest and expression of early markers during adipose conversion of preadipocyte cell lines, Biochemical and biophysical research communications. 137, 903-910.
[142] Ibrahimi, A., Abumrad, N., Maghareie, H., Golia, M., Shoshani, I., Désaubry, L. and Johnson, R.A., 1999. Adenylyl cyclase P-site ligands accelerate differentiation in Ob1771 preadipocytes, American Journal of Physiology-Cell Physiology. 276, C487-C496.
[143] Ailhaud, G., Amri, E., Bertrand, B., Barcellini-Couget, S., Bardon, S., Catalioto, R., Dani, C., Deslex, S., Djian, P. and Doglio, A., 1990. Obesity: Towards a Molecular Approach, AR Liss, New York. 219-236.
[144] Amri, E.-Z., Barbaras, R., Doglio, A., Dani, C., Grimaldi, P. and Ailhaud, G., 1986. Role of spermidine in the expression of late markers of adipose conversion Effects of growth hormone, Biochemical Journal. 239, 363-370.
[145] Gaillard, D., Negrel, R., Lagarde, M. and Ailhaud, G., 1989. Requirement and role of arachidonic acid in the differentiation of pre-adipose cells, Biochemical Journal. 257, 389-397.
[146] Amri, E., Ailhaud, G. and Grimaldi, P., 1994. Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells, Journal of Lipid Research. 35, 930-937.
[147] Ailhaud, G., Amri, E. and Grimaldi, P., 1995. Fatty acids and adipose cell differentiation, Prostaglandins, leukotrienes and essential fatty acids. 52, 113-115.
[148] Dace, A., Martin-El Yazidi, C., Bonne, J., Planells, R. and Torresani, J., 1997. Calcitriol is a positive effector of adipose differentiation in the OB 17 cell line: relationship with the adipogenic action of triiodothyronine, Biochemical and Biophysical Research Communications. 232, 771-776.
[149] Aubert, J., Safonova, I., Negrel, R. and Ailhaud, G., 1998. Insulin down-regulates angiotensinogen gene expression and angiotensinogen secretion in cultured adipose cells, Biochemical and biophysical research communications. 250, 77-82.
[150] Aubert, J., Dessolin, S., Belmonte, N., Li, M., McKenzie, F.R., Staccini, L., Villageois, P., Barhanin, B., Vernallis, A. and Smith, A.G., 1999. Leukemia inhibitory factor and its receptor promote adipocyte differentiation via the mitogen-activated protein kinase cascade, Journal of Biological Chemistry. 274, 24965-24972.
[151] Laux, W.H., Pande, P., Shoshani, I., Gao, J., Boudou-Vivet, V., Gosselin, G. and Johnson, R.A., 2004. Pro-nucleotide inhibitors of adenylyl cyclases in intact cells, Journal of Biological Chemistry. 279, 13317-13332.
[152] Doglio, A., Dani, C., Grimaldi, P. and Ailhaud, G., 1986. Growth hormone regulation of the expression of differentiation-dependent genes in preadipocyte Ob1771 cells, Biochemical Journal. 238, 123-129.
[153] Reznikoff, C.A., Brankow, D.W. and Heidelberger, C., 1973. Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division, Cancer research. 33, 3231-3238.
[154] Reznikoff, C.A., Bertram, J.S., Brankow, D.W. and Heidelberger, C., 1973. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse

embryo cells sensitive to postconfluence inhibition of cell division, Cancer research. 33, 3239-3249.
[155] Taylor, S.M. and Jones, P.A., 1979. Multiple new phenotypes induced in 10T12 and 3T3 cells treated with 5-azacytidine, Cell. 17, 771-779.
[156] Haider, N. and Larose, L., 2020. Activation of the PDGFRα-Nrf2 pathway mediates impaired adipocyte differentiation in bone marrow mesenchymal stem cells lacking Nck1, Cell Communication and Signaling. 18, 1-12.
[157] Moseti, D., Regassa, A., Chen, C. and Kim, W.K., 2020. 25-Hydroxycholesterol Inhibits Adipogenic Differentiation of C3H10T1/2 Pluripotent Stromal Cells, International Journal of Molecular Sciences. 21, 412.
[158] Tang, Q.-Q., Otto, T.C. and Lane, M.D., 2004. Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage, Proceedings of the National Academy of Sciences. 101, 9607-9611.
[159] Wang, Z., Dai, Z., Luo, Z. and Zuo, C., 2019. Identification of Pyrvinium, an Anthelmintic Drug, as a Novel Anti-Adipogenic Compound Based on the Gene Expression Microarray and Connectivity Map, Molecules. 24, 2391.
[160] Rajan, S., Puri, S., Kumar, D., Babu, M.H., Shankar, K., Varshney, S., Srivastava, A., Gupta, A., Reddy, M.S. and Gaikwad, A.N., 2018. Novel indole and triazole based hybrid molecules exhibit potent anti-adipogenic and antidyslipidemic activity by activating Wnt3a/β-catenin pathway, European journal of medicinal chemistry. 143, 1345-1360.
[161] Zhang, J. and Choudhury, M., 2017. The plasticizer BBP selectively inhibits epigenetic regulator sirtuin during differentiation of C3H10T1/2 stem cell line, Toxicology in Vitro. 39, 75-83.
[162] Guo, Y.-Y., Li, B.-Y., Peng, W.-Q., Guo, L. and Tang, Q.-Q., 2019. Taurine-mediated browning of white adipose tissue is involved in its anti-obesity effect in mice, Journal of Biological Chemistry. 294, 15014-15024.
[163] Wang, S.J., Lu, W.Y. and Liu, K.Y., 2017. Adiponectin receptor agonist AdipoRon suppresses adipogenesis in C3H10T1/2 cells through the adenosine monophosphate-activated protein kinase signaling pathway, Molecular medicine reports. 16, 7163-7169.
[164] Kim, J., Han, D., Byun, S.-H., Kwon, M., Cho, S.-J., Koh, Y.H. and Yoon, K., 2017. Neprilysin facilitates adipogenesis through potentiation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway, Molecular and cellular biochemistry. 430, 1-9.
[165] Shin, S., Seong, J.K. and Bae, Y.S., 2016. A hnak stimulates BMP 2-mediated adipocyte differentiation through S mad1 activation, Obesity. 24, 398-407.
[166] Zhu, E., Zhang, J., Li, Y., Yuan, H., Zhou, J. and Wang, B., 2018. Long noncoding RNA Plnc1 controls adipocyte differentiation by regulating peroxisome proliferator–activated receptor γ, The FASEB Journal. 33, 2396-2408.
[167] Imran, K.M., Yoon, D., Lee, T.-J. and Kim, Y.-S., 2018. Medicarpin induces lipolysis via activation of protein kinase A in brown adipocytes, BMB reports. 51, 249.
[168] Yoon, D., Imran, K.M. and Kim, Y.-S., 2018. Distinctive effects of licarin A on lipolysis mediated by PKA and on formation of brown adipocytes from C3H10T1/2 mesenchymal stem cells, Toxicology and applied pharmacology. 340, 9-20.
[169] Imran, K.M., Rahman, N., Yoon, D., Jeon, M., Lee, B.-T. and Kim, Y.-S., 2017. Cryptotanshinone promotes commitment to the brown adipocyte lineage and mitochondrial biogenesis in C3H10T1/2 mesenchymal stem cells via AMPK and p38-MAPK signaling, Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 1862, 1110-1120.

[170] Rivera-Piza, A., An, Y.J., Kim, D.K., Lee, S.-H., Kim, J.-B., Choi, J.-S. and Lee, S.-J., 2017. Protocatechuic acid enhances osteogenesis, but inhibits adipogenesis in C3H10T1/2 and 3T3-L1 cells, Journal of medicinal food. 20, 309-319.
[171] Li, Y., Goto, T., Ikutani, R., Lin, S., Takahashi, N., Takahashi, H., Jheng, H.F., Yu, R., Taniguchi, M. and Baba, K., 2016. Xanthoangelol and 4-hydroxyderrcin suppress obesity-induced inflammatory responses, Obesity. 24, 2351-2360.
[172] Nishikawa, S., Aoyama, H., Kamiya, M., Higuchi, J., Kato, A., Soga, M., Kawai, T., Yoshimura, K., Kumazawa, S. and Tsuda, T., 2016. Artepillin C, a typical Brazilian propolis-derived component, induces brown-like adipocyte formation in C3H10T1/2 cells, primary inguinal white adipose tissue-derived adipocytes, and mice, PloS one. 11, e0162512.
[173] Chani, B., Puri, V., Sobti, R.C. and Puri, S., 2016. Epigallocatechin gallate inhibits mouse mesenchymal stem cell differentiation to adipogenic lineage, Journal of stem cells & regenerative medicine. 12, 16.
[174] Cheng, K.-T., Wang, Y.-S., Chou, H.-C., Chang, C.-C., Lee, C.-K. and Juan, S.-H., 2015. Kinsenoside-mediated lipolysis through an AMPK-dependent pathway in C3H10T1/2 adipocytes: Roles of AMPK and PPARα in the lipolytic effect of kinsenoside, Phytomedicine. 22, 641-647.
[175] Kusudo, T., Hashimoto, M., Kataoka, N., Li, Y., Nozaki, A. and Yamashita, H., 2018. CREG1 promotes uncoupling protein 1 expression and brown adipogenesis in vitro, The Journal of Biochemistry. 165, 47-55.
[176] Yang, H.E., Li, Y., Nishimura, A., Jheng, H.F., Yuliana, A., Kitano-Ohue, R., Nomura, W., Takahashi, N., Kim, C.S. and Yu, R., 2017. Synthesized enone fatty acids resembling metabolites from gut microbiota suppress macrophage-mediated inflammation in adipocytes, Molecular nutrition & food research. 61, 1700064.
[177] Liu, X., Huang, T., Li, L., Tang, Y., Tian, Y., Wang, S. and Fan, C., 2015. CYP1B1 deficiency ameliorates obesity and glucose intolerance induced by high fat diet in adult C57BL/6J mice, American journal of translational research. 7, 761.
[178] Takahashi, A., Dohi, H., Egashira, Y. and Hirai, S., 2020. Erucic acid derived from rosemary regulates differentiation of mesenchymal stem cells into osteoblasts/adipocytes via suppression of peroxisome proliferator-activated receptor γ transcriptional activity, Phytotherapy Research.
[179] Wang, H., Mao, X. and Du, M., 2019. Phytanic acid activates PPARα to promote beige adipogenic differentiation of preadipocytes, The Journal of Nutritional Biochemistry. 67, 201-211.
[180] Seo, Y.-J., Kim, K.-J., Choi, J., Koh, E.-J. and Lee, B.-Y., 2018. Spirulina maxima extract reduces obesity through suppression of adipogenesis and activation of browning in 3T3-L1 cells and high-fat diet-induced obese mice, Nutrients. 10, 712.
[181] You, Y., Yuan, X., Lee, H.J., Huang, W., Jin, W. and Zhan, J., 2015. Mulberry and mulberry wine extract increase the number of mitochondria during brown adipogenesis, Food & function. 6, 401-408.
[182] Seo, S.H., Jo, S.-M., Kim, J., Lee, M., Lee, Y. and Kang, I., 2019. Peanut Sprout Extracts Attenuate Triglyceride Accumulation by Promoting Mitochondrial Fatty Acid Oxidation in Adipocytes, International journal of molecular sciences. 20, 1216.
[183] Nakano, T., Kodama, H. and Honjo, T., 1994. Generation of lymphohematopoietic cells from embryonic stem cells in culture, Science. 265, 1098-1101.
[184] Gao, J., Yan, X.-L., Li, R., Liu, Y., He, W., Sun, S., Zhang, Y., Liu, B., Xiong, J. and Mao, N., 2010. Characterization of OP9 as authentic mesenchymal stem cell line, Journal of Genetics and Genomics. 37, 475-482.

[185] Lane, J.M., Doyle, J.R., Fortin, J.-P., Kopin, A.S. and Ordovás, J.M., 2014. Development of an OP9 derived cell line as a robust model to rapidly study adipocyte differentiation, PloS one. 9, e112123.
[186] Ueno, H., Sakita-Ishikawa, M., Morikawa, Y., Nakano, T., Kitamura, T. and Saito, M., 2003. A stromal cell–derived membrane protein that supports hematopoietic stem cells, Nature immunology. 4, 457.
[187] Lamas, A., Lopez, E., Carrio, R. and Lopez, D.M., 2016. Adipocyte and leptin accumulation in tumor-induced thymic involution, International journal of molecular medicine. 37, 133-138.
[188] Tan, J., Wang, Y., Wang, S., Wu, S., Yuan, Z. and Zhu, X., 2019. Label-free quantitative proteomics identifies transforming growth factor β1 (TGF-β1) as an inhibitor of adipogenic transformation in OP9-DL1 cells and primary thymic stromal cells, Cell & bioscience. 9, 48.
[189] Tan, J., Wang, Y., Wang, S., Zhang, N., Wu, S., Yuan, Z. and Zhu, X., 2017. Untargeted metabolomics analysis of adipogenic transformation in OP9-DL1 cells using liquid chromatography-mass spectrometry: Implications for thymic adipogenesis, Cell biology international. 41, 447-456.
[190] Dhakal, S. and Lee, Y., 2019. Transient Receptor Potential Channels and Metabolism, Molecules and cells. 42, 569.
[191] Kim, M.S., Muallem, S., Kim, S.H., Kwon, K.B. and Kim, M.S., 2019. Exosomal release through TRPML1-mediated lysosomal exocytosis is required for adipogenesis, Biochemical and biophysical research communications. 510, 409-415.
[192] He, Z., Li, M., Zheng, D., Chen, Q., Liu, W. and Feng, L., 2015. Adipose tissue hypoxia and low-grade inflammation: a possible mechanism for ethanol-related glucose intolerance?, British Journal of Nutrition. 113, 1355-1364.
[193] Campos, V., Rappaz, B., Kuttler, F., Turcatti, G. and Naveiras, O., 2018. High-throughput, nonperturbing quantification of lipid droplets with digital holographic microscopy, Journal of lipid research. 59, 1301-1310.
[194] Jiang, Z., Di Wu, W.Y., Weng, J., Lai, P., Shi, P., Guo, X., Huang, G., Deng, Q., Tang, Y. and Zhao, H., 2017. Defined, serum/feeder-free conditions for expansion and drug screening of primary B-acute lymphoblastic leukemia, Oncotarget. 8, 106382.
[195] Seo, Y.-S., Kang, O.-H., Kim, S.-B., Mun, S.-H., Kang, D.-H., Yang, D.-W., Choi, J.-G., Lee, Y.-M., Kang, D.-K. and Lee, H.-S., 2015. Quercetin prevents adipogenesis by regulation of transcriptional factors and lipases in OP9 cells, International journal of molecular medicine. 35, 1779-1785.
[196] Kato, S., Kato, Y., Shibata, H., Saitoh, Y. and Miwa, N., 2015. Repressive effects of oat extracts on intracellular lipid-droplet formation in adipocytes and a three-dimensional subcutaneous adipose tissue model, Materials Science and Engineering: C. 49, 269-273.
[197] Kennedy, D.E. and Knight, K.L., 2015. Inhibition of b lymphopoiesis by adipocytes and il-1–producing myeloid-derived suppressor cells, The Journal of Immunology. 195, 2666-2674.
[198] Schmitt, T.M. and Zúñiga-Pflücker, J.C., 2002. Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro, Immunity. 17, 749-756.
[199] Holmes, R. and Zúñiga-Pflücker, J.C., 2009. The OP9-DL1 system: generation of T-lymphocytes from embryonic or hematopoietic stem cells in vitro, Cold Spring Harbor Protocols. 2009, pdb. prot5156.
[200] Evans, M.J. and Kaufman, M.H., 1981. Establishment in culture of pluripotential cells from mouse embryos, Nature. 292, 154-156.

[201] Martin, G.R., 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells, Proceedings of the National Academy of Sciences. 78, 7634-7638.
[202] Nichols, J. and Smith, A., 2009. Naive and primed pluripotent states, Cell stem cell. 4, 487-492.
[203] Bradley, A., Evans, M., Kaufman, M.H. and Robertson, E., 1984. Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines, Nature. 309, 255-256.
[204] Stavridis, M. and Smith, A., 2003. Neural differentiation of mouse embryonic stem cells, Biochemical Society Transactions. 31, 45-49.
[205] Keller, G.M., 1995. In vitro differentiation of embryonic stem cells, Current opinion in cell biology. 7, 862-869.
[206] Phillips, B.W., Vernochet, C. and Dani, C., 2003. Differentiation of embryonic stem cells for pharmacological studies on adipose cells, Pharmacological research. 47, 263-268.
[207] Dani, C., 1999. Embryonic stem cell-derived adipogenesis, Cells Tissues Organs. 165, 173-180.
[208] Chen, C.-Y., Lanz, R.B., Walkey, C.J., Chang, W.-H., Lu, W. and Johnson, D.L., 2018. Maf1 and Repression of RNA Polymerase III-Mediated Transcription Drive Adipocyte Differentiation, Cell reports. 24, 1852-1864.
[209] Ota, K., Tong, K.I., Goto, K., Tomida, S., Komuro, A., Wang, Z., Nishio, K. and Okada, H., 2017. The H3K27 demethylase, Utx, regulates adipogenesis in a differentiation stage-dependent manner, PloS one. 12, e0173713.
[210] Masia, F., Glen, A., Stephens, P., Langbein, W. and Borri, P., 2018. Label-free quantitative chemical imaging and classification analysis of adipogenesis using mouse embryonic stem cells, Journal of biophotonics. 11, e201700219.
[211] Guerrero-Robles, C., Vazquez-Zapien, G., Mata-Miranda, M., Noriega-Gonzalez, J. and Gonzalez-Diaz, C., 2017. Electrical bioimpedance spectroscopy as biosensor technique to identify cells lineages and cell differentiation process, 2017 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). IEEE, pp. 3568-3571.
[212] Bazou, D., Kearney, R., Mansergh, F., Bourdon, C., Farrar, J. and Wride, M., 2011. Gene expression analysis of mouse embryonic stem cells following levitation in an ultrasound standing wave trap, Ultrasound in medicine & biology. 37, 321-330.
[213] Unser, A.M., Mooney, B., Corr, D.T., Tseng, Y.-H. and Xie, Y., 2016. 3D brown adipogenesis to create “Brown-Fat-in-Microstrands”, Biomaterials. 75, 123-134.
[214] Kang, X., Xie, Y., Powell, H.M., Lee, L.J., Belury, M.A., Lannutti, J.J. and Kniss, D.A., 2007. Adipogenesis of murine embryonic stem cells in a three-dimensional culture system using electrospun polymer scaffolds, Biomaterials. 28, 450-458.
[215] Billon, N., Kolde, R., Reimand, J., Monteiro, M.C., Kull, M., Peterson, H., Tretyakov, K., Adler, P., Wdziekonski, B. and Vilo, J., 2010. Comprehensive transcriptome analysis of mouse embryonic stem cell adipogenesis unravels new processes of adipocyte development, Genome biology. 11, R80.
[216] Schaedlich, K., Knelangen, J.M., Navarrete Santos, A., Fischer, B. and Navarrete Santos, A., 2010. A simple method to sort ESC-derived adipocytes, Cytometry Part A. 77, 990-995.
[217] Cuaranta-Monroy, I., Simandi, Z. and Nagy, L., 2015. Differentiation of adipocytes in monolayer from mouse embryonic stem cells, Embryonic Stem Cell Protocols. Springer, pp. 407-415.
[218] Singhal, P.K., Sassi, S., Lan, L., Au, P., Halvorsen, S.C., Fukumura, D., Jain, R.K. and Seed, B., 2016. Mouse embryonic fibroblasts exhibit extensive developmental

and phenotypic diversity, Proceedings of the National Academy of Sciences. 113, 122-127.
[219] Nagy, A., Gertsenstein, M., Vintersten, K. and Behringer, R., 2006. Preparing mouse embryo fibroblasts, Cold Spring Harbor Protocols. 2006, pdb. prot4398.
[220] Amand, M.M.S., Hanover, J.A. and Shiloach, J., 2016. A comparison of strategies for immortalizing mouse embryonic fibroblasts, Journal of Biological Methods. 3.
[221] Xu, J., 2005. Preparation, culture, and immortalization of mouse embryonic fibroblasts, Current protocols in molecular biology. 70, 28.1. 1-28.1. 8.
[222] Hogan, B., Costantini, F. and Lacy, E., 1986. Manipulating the mouse embryo: a laboratory manual.
[223] Dastagir, K., Reimers, K., Lazaridis, A., Jahn, S., Maurer, V., Strauß, S., Dastagir, N., Radtke, C., Kampmann, A. and Bucan, V., 2014. Murine embryonic fibroblast cell lines differentiate into three mesenchymal lineages to different extents: new models to investigate differentiation processes, Cellular Reprogramming (Formerly" Cloning and Stem Cells"). 16, 241-252.
[224] Dobrowolski, P., Fischer, M. and Naumann, R., 2018. Novel insights into the genetic background of genetically modified mice, Transgenic research. 27, 265-275.
[225] Durkin, M.E., Qian, X., Popescu, N.C. and Lowy, D.R., 2013. Isolation of mouse embryo fibroblasts, Bio Protoc. 3, e908.
[226] Bjune, J.-I., Dyer, L., Røsland, G.V., Tronstad, K.J., Njølstad, P.R., Sagen, J.V., Dankel, S.N. and Mellgren, G., 2020. The homeobox factor Irx3 maintains adipogenic identity, Metabolism. 103, 154014.
[227] Mugabo, Y., Sadeghi, M., Fang, N.N., Mayor, T. and Lim, G.E., 2018. Elucidation of the 14-3-3ζ interactome reveals critical roles of RNA-splicing factors during adipogenesis, Journal of Biological Chemistry. 293, 6736-6750.
[228] Singh, R., Braga, M., Reddy, S.T., Lee, S.-J., Parveen, M., Grijalva, V., Vergnes, L. and Pervin, S., 2017. Follistatin targets distinct pathways to promote brown adipocyte characteristics in brown and white adipose tissues, Endocrinology. 158, 1217-1230.
[229] Han, Y., Lee, S.H., Bahn, M., Yeo, C.-Y. and Lee, K.Y., 2016. Pin1 enhances adipocyte differentiation by positively regulating the transcriptional activity of PPARγ, Molecular and cellular endocrinology. 436, 150-158.
[230] Lee, K., Um, S.H., Rhee, D.K. and Pyo, S., 2016. Interferon-alpha inhibits adipogenesis via regulation of JAK/STAT1 signaling, Biochimica et Biophysica Acta (BBA)-General Subjects. 1860, 2416-2427.
[231] Bauters, D., Scroyen, I., Deprez-Poulain, R. and Lijnen, H.R., 2016. ADAMTS5 promotes murine adipogenesis and visceral adipose tissue expansion, Thrombosis and haemostasis. 116, 694-704.
[232] Garfield, A.S., 2010. Derivation of primary mouse embryonic fibroblast (PMEF) cultures, Mouse Cell Culture. Springer, pp. 19-27.
[233] Tu, W.-z., Fu, Y.-b. and Xie, X., 2019. RepSox, a small molecule inhibitor of the TGFβ receptor, induces brown adipogenesis and browning of white adipocytes, Acta Pharmacologica Sinica. 1.
[234] Hunt, B.G., Wang, Y.-L., Chen, M.-S., Wang, S.-C. and Waltz, S.E., 2017. Maternal diethylhexyl phthalate exposure affects adiposity and insulin tolerance in offspring in a PCNA-dependent manner, Environmental research. 159, 588-594.
[235] Pieralisi, A., Martini, C., Soto, D., Vila, M., Calvo, J.C. and Guerra, L.N., 2016. N-acetylcysteine inhibits lipid accumulation in mouse embryonic adipocytes, Redox biology. 9, 39-44.

[236] Gao, M., Ding, Z., Shui, Y., Zhou, F. and Gao, J., 2016. Chrysin inhibits adipogenic differentiation of mouse embryonic fibroblasts, Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica. 41, 106-111.
[237] Xu, S., Mao, L., Ding, P., Zhuang, X., Zhou, Y., Yu, L., Liu, Y., Nie, T., Xu, T. and Xu, Y., 2015. 1-Benzyl-4-phenyl-1H-1, 2, 3-triazoles improve the transcriptional functions of estrogen-related receptor γ and promote the browning of white adipose, Bioorganic & medicinal chemistry. 23, 3751-3760.
[238] Wouters, K., Deleye, Y., Hannou, S.A., Vanhoutte, J., Maréchal, X., Coisne, A., Tagzirt, M., Derudas, B., Bouchaert, E. and Duhem, C., 2017. The tumour suppressor CDKN2A/p16INK4a regulates adipogenesis and bone marrow-dependent development of perivascular adipose tissue, Diabetes and Vascular Disease Research. 14, 516-524.
[239] Zhang, L., Qiu, B., Wang, T., Wang, J., Liu, M., Xu, Y., Wang, C., Deng, R., Williams, K. and Yang, Z., 2017. Loss of FKBP5 impedes adipocyte differentiation under both normoxia and hypoxic stress, Biochemical and biophysical research communications. 485, 761-767.
[240] Xu, H., Fu, J.-L., Miao, Y.-F., Wang, C.-J., Han, Q.-F., Li, S., Huang, S.-Z., Du, S.-N., Qiu, Y.-X. and Yang, J.-C., 2016. Prostaglandin E2 receptor EP3 regulates both adipogenesis and lipolysis in mouse white adipose tissue, Journal of molecular cell biology. 8, 518-529.
[241] Inoue, J., Ihara, Y., Tsukamoto, D., Yasumoto, K., Hashidume, T., Kamimura, K., Nakai, Y., Hirano, S., Shimizu, M. and Kominami, R., 2016. Identification of BCL11B as a regulator of adipogenesis, Scientific reports. 6, 32750.
[242] Matsumoto, M., Kondo, K., Shiraki, T., Brydun, A., Funayama, R., Nakayama, K., Yaegashi, N., Katagiri, H. and Igarashi, K., 2016. Genomewide approaches for BACH 1 target genes in mouse embryonic fibroblasts showed BACH 1-Pparg pathway in adipogenesis, Genes to Cells. 21, 553-567.
[243] Takahashi, Y., Shinoda, A., Kamada, H., Shimizu, M., Inoue, J. and Sato, R., 2016. Perilipin2 plays a positive role in adipocytes during lipolysis by escaping proteasomal degradation, Scientific reports. 6, 1-14.
[244] Myneni, V., Melino, G. and Kaartinen, M., 2015. Transglutaminase 2—a novel inhibitor of adipogenesis, Cell death & disease. 6, e1868.
[245] Yu, H., He, K., Wang, L., Hu, J., Gu, J., Zhou, C., Lu, R. and Jin, Y., 2015. Stk40 represses adipogenesis through translational control of CCAAT/enhancer-binding proteins, J Cell Sci. 128, 2881-2890.
[246] Merkestein, M., Laber, S., McMurray, F., Andrew, D., Sachse, G., Sanderson, J., Li, M., Usher, S., Sellayah, D. and Ashcroft, F.M., 2015. FTO influences adipogenesis by regulating mitotic clonal expansion, Nature communications. 6, 6792.
[247] Park, J.H. and Lee, S.B., 2015. An essential role for E wing sarcoma gene (EWS) in early white adipogenesis, Obesity. 23, 138-144.
[248] Ferguson, D., Blenden, M., Hutson, I., Du, Y. and Harris, C.A., 2018. Mouse Embryonic Fibroblasts Protect ob/ob Mice From Obesity and Metabolic Complications, Endocrinology. 159, 3275-3286.
[249] Martini, C.N., Gabrielli, M., Brandani, J.N. and Vila, M.d.C., 2016. Glyphosate inhibits PPAR gamma induction and differentiation of preadipocytes and is able to induce oxidative stress, Journal of biochemical and molecular toxicology. 30, 404-413.
[250] Cawthorn, W.P., Scheller, E.L. and MacDougald, O.A., 2012. Adipose tissue stem cells meet preadipocyte commitment: going back to the future, Journal of lipid research. 53, 227-246.

[251] Bourin, P., Bunnell, B.A., Casteilla, L., Dominici, M., Katz, A.J., March, K.L., Redl, H., Rubin, J.P., Yoshimura, K. and Gimble, J.M., 2013. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT), Cytotherapy. 15, 641-648.
[252] Zhang, Q., Liu, L.-N., Yong, Q., Deng, J.-C. and Cao, W.-G., 2015. Intralesional injection of adipose-derived stem cells reduces hypertrophic scarring in a rabbit ear model, Stem cell research & therapy. 6, 145.
[253] Kelly, K.A., Tanaka, S., Baron, R. and Gimble, J.M., 1998. Murine bone marrow stromally derived BMS2 adipocytes support differentiation and function of osteoclast-like cells in vitro, Endocrinology. 139, 2092-2101.
[254] Zheng, B., Cao, B., Li, G. and Huard, J., 2006. Mouse adipose-derived stem cells undergo multilineage differentiation in vitro but primarily osteogenic and chondrogenic differentiation in vivo, Tissue engineering. 12, 1891-1901.
[255] Kilroy, G., Dietrich, M., Wu, X., Gimble, J.M. and Floyd, Z.E., 2018. Isolation of murine adipose-derived stromal/stem cells for adipogenic differentiation or flow cytometry-based analysis, Adipose-Derived Stem Cells. Springer, pp. 137-146.
[256] Grant, R.W. and Dixit, V.D., 2015. Adipose tissue as an immunological organ, Obesity. 23, 512-518.
[257] Jankowski, M., Dompe, C., Sibiak, R., Wąsiatycz, G., Mozdziak, P., Jaśkowski, J.M., Antosik, P., Kempisty, B. and Dyszkiewicz-Konwińska, M., 2020. In Vitro Cultures of Adipose-Derived Stem Cells: An Overview of Methods, Molecular Analyses, and Clinical Applications, Cells. 9, 1783.
[258] Chu, D.-T., Nguyen Thi Phuong, T., Tien, N.L.B., Tran, D.K., Minh, L.B., Thanh, V.V., Gia Anh, P., Pham, V.H. and Thi Nga, V., 2019. Adipose tissue stem cells for therapy: An update on the progress of isolation, culture, storage, and clinical application, Journal of clinical medicine. 8, 917.