Title:**OPA1 in lipid metabolism: Function of OPA1 in lipolysis and thermogenesis of adipocytes****Dinh-Toi Chu^{1,2,5,6}, Yang Tao⁷ and Kjetil Taskén^{1,2,3,4,*}**¹ *Biotechnology Centre, University of Oslo, Oslo, Norway*² *Centre for Molecular Medicine Norway, Nordic European Molecular Biology Laboratory Partnership, University of Oslo and Oslo University Hospital, Oslo, Norway*³ *K.G. Jebsen Inflammation Research Centre; University of Oslo; Oslo, Norway*⁴ *Department of Infectious Diseases; Oslo University Hospital; Oslo, Norway*⁵ *Institute for Research and Development, Duy Tan University, K7/25 Quang Trung, Danang, Vietnam*⁶ *Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam*⁷ *College of Food Science and Technology, Nanjing Agricultural University, Nanjing 8 210095, China*^{*} *Correspondence to: Dr. Kjetil Taskén email: kjetil.tasken@ncmm.uio.no***Abstract**

OPA1 (Optic Atrophy 1) is a mitochondrial GTPase known to regulate fission of mitochondria. It was recently also shown to locate on lipid droplets in adipocytes where it functions as an A-kinase anchoring protein (AKAP) that mediates adrenergic control of lipolysis by facilitating PKA phosphorylation of perilipin (Plin1). In brown adipocytes indirect evidence support the notion that OPA1 regulation of fission serves to increase thermogenesis, which thereby contributes to dissipation of energy. In white adipocytes OPA1 located on lipid droplets serves as a gatekeeper to control lipolysis induced by adrenergic agonists. However, the function of OPA1 in lipolysis and thermogenesis in inducible brown adipocytes (brite/beige cells) remains elusive. Here we discuss the role of OPA1 in lipid metabolism.

Keywords: OPA1, AKAP, cAMP, lipid metabolism, brown adipocyte, white adipocyte

Running title: OPA1 in lipolysis and thermogenesis

Types of adipocytes and their function in fat metabolism

Both humans and rodents have two basic types of adipocytes, white and brown cells, the names of which refer to their presence in white adipose tissue (WAT) and brown adipose tissue (BAT), respectively (Figure 1). The white and brown cells have diametrically opposite roles in controlling the energy balance

of the organism and they can also co-exist in the same fat depots. In mice, a recent publication by Walden et al [1] identified 15 different types of fat depots with variable composition of adipocytes. Whereas some adipose tissues were found to be of only brown or white cells, other depots were composed of a mixture of brown and white adipocytes [1]. White adipocytes function to accumulate lipids for energy storage, while brown adipocytes burn lipids to serve the thermogenic requirements of the body [2]. Brown fat cells can be divided into classical brown and inducible brown adipocytes, two subtypes of brown cells that share several molecular characteristics and may serve the same function in lipid metabolism, but are developed from different precursors [3-7] (Figure 1).

Excess lipid storage in WAT resulting from the imbalance between food intake and energy expenditure is the major cause of obesity. The location of WAT depots varies among species, in mammals the most abundant sites of lipid storage are subcutaneous and visceral fat depots [8,9]. The anatomical location of WAT determines the types of obesity and its related metabolic disorders in the excess energy conditions [8,9]. In humans, the accumulation of extra fat under the skin results in subcutaneous or “pear-shape” obesity, whereas excess storage of lipids around the visceral organs causes visceral or “apple-shape” obesity [8]. Pear-shape obesity comes with lower risk of diabetes and metabolic disorders compared to visceral obesity [8]. With respect to molecular signatures, white adipocytes share the expression of *homeobox C8 (Hoxc8)* with inducible brown adipocytes [1], but they are distinguished from brown cells by expression of high levels of adipose expansion genes such as *specific transcript (Mest)*, *secreted frizzled related protein 5 (Sfrp5)*, *bone morphogenetic protein 3 (BMP3)* and *caveolin 1 (Cav1)* especially in a response to an obesogenic diet [10-15].

Classical brown adipocytes in mammals reside in depots such as the interscapular brown adipose tissue (iBAT) - the biggest brown adipose tissue. They are present in the newborn and small mammals as an adaptive organ to maintain body temperature in hypothermic conditions. These adipocytes function to transfer energy from food into heat [2,16] for warming the body in the early days of life and during hibernation in the winter. In humans, several recent reports confirm the existence of active classical brown adipocytes also in adults [17-23]. However, whereas the iBAT is most developed in infants, regresses in childhood and is absent in adults, humans may to a varying degree retain BAT in the neck region also as adults [23-25]. Classical brown adipocytes express genes for non-shivering thermogenesis such as *uncoupling protein 1 (Ucp1)*, *peroxisome proliferator-activated receptor- γ coactivator-1 α (Pgc1 α)*, and *peroxisome proliferator-activated receptor alpha (Ppara)* at high levels [2,7,21,22,26-32]. Furthermore, *cerebellum 1 (Zic1)* is identified as a specific marker for BAT [29].

Inducible brown adipocytes are generated in white fat depots in response to cold exposure or treatment with β -adrenergic receptor agonists *in vivo* [32-36]. These brown fat cells can be differentiated *in vitro* from preadipocyte cell lines and stem cells by adipogenic medium supplemented with one or more “browning” reagents such as norepinephrine, thiazolidinediones, natriuretic peptides, fibroblast growth factor 21 (Fgf21), Irisin and bone morphogenic protein 7 (Bmp7) [5,15,24,31,32,37-47]. This subtype of brown fat cells is called brite adipocytes, beige adipocytes, brown-like fat cells or induced-brown adipocytes. Induction of brown adipocytes in white fat depots of rodents was first investigated in the 1980s and 1990s by cold or β -adrenergic receptor agonist treatment [33-35]. Although inducible brown adipocytes have thermogenic function like classical brown adipocytes, this population of adipocytes has a distinct developmental origin from classical brown adipocytes [5,6,48]. Notably, the functional brite adipocytes are present and can be induced in both infant and adult humans, thus the induction of active brite adipocytes in humans may be a promising strategy for preventing and treating obesity-related disorders. As classical brown adipocytes, brite fat cells also have thermogenic genes e.g. *Ucp1*, *Ppara*, and *Pgc1 α* [26,28,32,49,50], but they share a common molecular signature named *Hoxc8* with white adipocytes [1]. However, inducible brown adipocytes also have their own molecular markers such as *homeobox C9 (Hoxc9)* and *tumor necrosis factor receptor superfamily member 9 (CD137)* [29,47,51].

Inducible brown adipocytes share the thermogenic function with classical brown fat cells, but have the same developmental origin as white adipocytes [3-7,52]. Classical brown adipocytes share their origin from myf5⁺ myotomal precursors with muscle cells [3,4], while brite and white adipocytes derive from myf5⁻ precursors [5-7]. In 2014, Jonathan et al showed that inducible brown adipocytes may come from a smooth muscle-like origin as the smooth muscle-like cells developed into brite adipocytes *in vivo* under cold stimulation and differentiated into brite cells in the presence of PR domain containing 16 (PRDM16) *in vitro* [52].

The expression of OPA1 in adipocytes

OPA1 is found to be expressed in both white and brown adipocytes at the mRNA and protein levels *in vivo* and *in vitro* [53-62] (Table 1). However, there are higher levels of OPA1 protein in brown versus white fat of mice with normal body weight [53]. It is known that OPA1 locates on the inner membrane of mitochondria facing the intermembranous space to regulate mitochondrial morphology. However, recently this protein has also been found on the lipid droplets of white adipocytes [53,63] (Fig. 2A). The pool of OPA1 located to lipid droplets was shown to be involved in the regulation of lipolytic function of adipocytes by Pidoux et al in 2011 [53]. Both OPA1 and the lipid droplet coat protein perilipin are increased during the differentiation of white adipocytes [53], which means that lipid accumulation and

expression of OPA1 occur concomitantly in white fat cells. This *in vitro* observation is supported by a dog model of diet-induced obesity *in vivo*, in which the expansion of white fat during obesity development is accompanied with the increased expression of OPA1 in adipose tissues [55]. There are at least two different forms of OPA1, long (L-OPA1, ~100 KDa) and short (S-OPA1, ~85 Kda) forms in mouse adipocytes in the basal condition [54,64].

The expression of OPA1 and mitochondrial genes in adipocytes are regulated by transcriptional factors, hormones, enzymes, tumor suppressor proteins, cytokines and even food constituents and medication [54,55,57-60,64-69] (Fig. 2B). We know that transcriptional factors such as the forkhead box protein C2 (FOXC2) and peroxisome proliferator-activated receptors (PPARs) control the genes of mitochondrial morphology and biogenesis in adipocytes [56-58]. In a primary culture of brown adipocytes, overexpression of FOXC2 increases the expression of both mitochondrial fusion genes (*Opa1*, *Mitofusin-1* and *2* (*Mfn1* and *Mfn2*)) and brown-fat-related-markers (*Prdm16* and *DIO2 deiodinase (Dio2)*) [57]. In adipocytes differentiated from 3T3-L1 or C3H/10T1/2 cells, the stimulation of PPAR γ by its agonist rosiglitazone elevates mRNA levels of *Opa1*, mitofilin and the transcription factors/cofactors of mitochondrial genes such as *Pgc-1 β* (*Ppargc1b*) and *estrogen related receptor alpha (Erra)* [58]. The regulatory effect of PPAR γ on adipose OPA1 was confirmed by Anusree et al in 2015 [62], as their findings showed that puniic acid (PA), a poly unsaturated fatty acid with PPAR γ agonist properties [70,71], blocked the inhibitory effect of tumor necrosis factor- α (TNF- α) on OPA1 expression in 3T3-L1 adipocytes [62].

Some hormones including sex hormones and catecholamines have effects on the expression of OPA1 in adipocytes [59,64]. For example, 17- β estradiol, increases adipogenesis and mitochondrial proliferation in 3T3-L1, and enhances the mRNA expression of *Opa1* and *dynammin related protein 1 (Drp1)* in differentiated adipocytes; while testosterone has the opposite effects [59]. Ghrelin is an orexigenic hormone, which has been known to increase lipid accumulation in white fat and appetite, but that decreases norepinephrine (NE) release from brown adipocytes and therefore promotes obesity [60,68,72-76]. Mice lacking Ghrelin (Ghsr^{-/-} mice) has increased mRNA expression of *Opa1* as well as the genes of thermogenesis and mitochondria in adipose tissues [68]. Additionally, a combined treatment of NE and palmitate (P) induces OPA1 cleavage resulting in decreasing L-OPA1 but elevating S-OPA1 in brown adipocytes [64].

Master regulators of OPA1 expression in adipocytes also include tumor suppressor proteins, proteolytic enzymes and cytokines [60,69]. Specifically, knockout of the retinoblastoma protein (pRb), a tumor suppressor, in adipose tissues results in higher expression of *Opa1* and brown genes such as *Ucp1* and

Pgc1 α in white fat depots [69]. In contrast, loss of the metalloendopeptidase OMA1 (OMA1), a proteolytic enzyme located in the inner membrane of mitochondria [60], leads to decreasing expression of OPA1 at both mRNA and protein levels in brown fat cells of mice [60]. This decrease in OPA1 expression in brown adipocytes is also found in mice lacking the non-receptor tyrosine-protein kinase TYK2 (Tyk2), an enzyme that belong to the Jak kinase family members [67]. In 3T3-L1 adipocytes, TNF- α suppresses the expression levels of total *Opa1* and *Pgc1 α* [62,65]. Moreover, some chemicals which have browning effects on white adipocytes such as fucoxanthin [77] and exenatide [78] can promote OPA1 expression in white fat depots of animals [66,79].

The function of OPA1 in lipolysis of adipocytes

Lipolysis, the hydrolysis of triglycerides into glycerol and free fatty acids, is a specific function of adipocytes including white, brite and brown cells [32,80,81]. Physiologically, this process is mainly induced by β -adrenoceptor (β -AR) stimulation of catecholamines such as epinephrine and norepinephrine; these hormones are released in response to cold exposure [32]. The catecholamine triggering of G protein-coupled receptors (7TM receptors) activates adenylate cyclase (adenylyl cyclase) followed by an increase in intracellular cAMP levels which activates protein kinase A (PKA) [80-82]. The phosphorylation of PKA subsequently activates lipases such as adipose triglyceride lipase (ATGL), lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) to stimulate lipolysis in adipocytes [32,80-83].

The abundant expression of OPA1 in functional adipocytes [53-60], especially the increase of its level during lipid accumulation in adipocytes both *in vitro* and *in vivo* [53,55], shows the potential involvement of OPA1 in lipid metabolism of adipocytes. The first report on OPA1 function in adipose lipolysis came from a new discovery of OPA1 location on lipid droplets by Pidoux and colleagues in 2011 [53,84] (Table 2). In white adipocytes differentiated from the 3T3-L1 cell line, a perfect overlap between OPA1 and a specific lipid-droplet-protein (perilipin) was found [53]. This analysis was triggered by the finding that perilipin phosphorylation depended on an anchored pool of PKA bound to an unidentified A-kinase anchoring protein (AKAP). By a combination of proteomic analysis of lipid droplets, a bioinformatic search for putative PKA binding sites in AKAPs and biochemical approaches to analyze PKA-AKAP interactions, OPA1 was identified as an AKAP on lipid droplets. Further investigation of OPA1 function in relation to lipolysis in adipocytes revealed that OPA1 is the AKAP responsible for perilipin phosphorylation to induce lipolysis in response to adrenergic stimuli (Fig. 3) [53,84].

The function of OPA1 in regulation of mitochondrial morphology and thermogenesis of adipocytes

OPA1 is known as a regulator of mitochondrial fusion [86-88]. In mitochondria, OPA1 and mitofusins (Mfns such as Mfn1 and Mfn2) work together to promote fusion, whereas mitochondrial fission 1 protein (Fis1) and Drp1 are essential in mitochondrial fission [89]. For this reason, efforts have focussed on investigating the role of OPA1 in mitochondria also in adipocytes [54,57,58,62,67,68] whereas work in the role of OPA1 on lipid droplets is much more limited [53] (Table 2). Among investigations of OPA1 in adipocytes, most studies have examined mitochondria of classical brown cells, probably because of the abundant mitochondria in brown fat cells connected to their thermogenic function [33,57,90-98].

The high expression of *Opal* and thermogenic genes (*Ucp1*, *Cidea*, *Pgc1a* and *Ppara*) in brown adipocytes or brown fat depots [53,54,57,58] suggests its involvement in thermogenic function. In fact, upregulation or activation of factors controlling OPA1 expression such as FOXO2 and PPAR γ results in alteration of brown adipocyte heat production [57,58]. Furthermore, it has been known that FOXO2 enhances thermogenic function by increasing PKA signaling in brown adipose tissues [56].

Non shivering-thermogenesis is a unique function of brown adipocytes, enabled by uncoupling protein 1 (Ucp1) in mitochondria [2,75,99,100]. The resulting heat production in Ucp1-expressing-adipocytes is activated by cold exposure, catecholamines and thyroid hormones [101]. Interestingly, NE and other catecholamines can induce lipolysis both in brown and white fat cells, but they preferentially stimulate thermogenesis in brown adipocytes [32]. In brown adipocyte tissues, either cold stress or the combined action of NE and palmitate (P) induce OPA1 cleavage [54,64] and a resulting increase in both mitochondrial content and thermogenesis [75]. Notably, the energy expenditure and S-OPA1 formation of brown adipocytes induced by the combined NE and P stimulation go together with Drp1-mediated fission and upstream of depolarization in mitochondria [64]. These findings indicate that in classical brown adipocytes the mitochondrial fission and OPA1 cleavage amplifies the thermogenic function [64] (Fig. 4A).

The relation between OPA1 and energy expenditure in brown adipocytes has been investigated [54,67,68]. Fatty acid oxidation is required for thermogenesis of brown fat depots [102], and the loss of OPA1 activities impairs the β -oxidation of brown adipocytes [54]. Primary brown adipocytes treated with OPA1 siRNA had around a 25% lower rate of palmitate oxidation compared to control adipocytes [54]. This result demonstrates the effect of OPA1 on the thermogenic function of brown adipocytes. In addition, some models have showed the coordinated expression and/or activities of OPA1 and thermogenic genes in brown adipocytes [54,67,68]. *Oma1*^{-/-} and *Tyk2*^{-/-} mice have suppressed expression of OPA1 and energy expenditure in brown fat depots [54,67], whereas *Ghr*^{-/-} mice have an increase in mRNA expression of both *Opal* and *Ucp1* [68]. OPA1 processing and mitochondrial fusion are regulated

by m-AAA protease isoenzymes and OMA1 [60], therefore brown adipose tissues of *Oma1*^{-/-} mice display lower expression of mitochondrial dynamic genes such as *Opa1*, *Mfn2* and *Drp1* together with a decrease in mRNA levels of β -oxidation genes including *very long-chain acyl-CoA dehydrogenase (Vlcad)* and *carnitine palmitoyltransferase 1 (Cpt1b)* [54]. Similar results were found in *Tyk2*^{-/-} mice [67]. Mice lacking *Tyk2* have defects in thermogenesis and OPA1 expression in brown adipose tissues [67]. The impaired function of brown fat cells in *Tyk2*^{-/-} mice is evident from the suppression of mRNA and protein levels of thermogenic markers such *Ucp1*, *Prdm16*, *Cidea* and *Ppara*, resulting in development of obesity [67]. Furthermore, brown fat tissues of *Tyk2* KO mice do not respond to cold exposure. However, the function of *Tyk2*^{-/-} brown adipocytes is restored upon reexpressing *Tyk2* [67]. In contrast, brown fat of mice lacking ghrelin has significantly higher mRNA expression of *Opa1*, *Mfns*, *Drp1* and *Fis1* compared to brown fat of wild type mice; this elevation of mitochondrial dynamic genes is coupled with an increase in the expression and phosphorylation of HSL and PKA protein in brown adipose tissues [68].

In inducible brown adipocytes mitochondrial fission events are not connected with thermogenesis. Thiazolidine-induced-adipocytes from the 3T3-L1 cell line have a decrease in the expression of total OPA1 and the thermogenic gene *Pgc1 α* , but show increased mitochondrial fragmentation upon stimulation with TNF- α [62,65]. TNF- α induction mitochondrial dysfunction in inducible brown adipocytes is also indicated by its suppression in the genes of mitochondrial biogenesis (*cytochrome c oxidase subunit I (Cox-1)*, *nuclear respiratory factor 1 (Nrf1)*, *transcription factor A mitochondrial (Tfam)* and *mitochondrial DNA (mtDNA)*) [62]. This is supported by experiments of PA treatment *in vitro* [62] and fucoxanthin supplementation *in vivo* [79]; PA increases the OPA1 levels, glucose uptake, mitochondrial biogenesis and energy expenditure in 3T3-L1 adipocytes *in vitro*, but decreases the expression of *Fis1* [62]. *In vivo* diet-induced obesity (DIO) mice receiving fucoxanthin-supplemented food for 5 weeks have higher levels of OPA1 and other fusion proteins together with higher expression of thermogenic genes such as *Ucp1* and *Pgc1 α* in white fat depots. Fucoxanthin, an edible seaweed carotenoid, has been previously reported to have browning effects on white fat depots of mice [77]. In rats on high fat diet, injection of exenatide – a glucagon-like peptide-1 (GLP-1) receptor agonist increases the expression of *Hsl*, *Opa1* and other mitochondrial dynamic genes in WAT [66]. Therefore, exenatide enhances lipid use in adipose tissue to improve hepatic steatosis [66]. Furthermore, as a mitochondrial protein, OPA1 may also be involved in the browning of white adipose tissues [69]. In a mouse model, specific knockout of the retinoblastoma (pRb) protein in adipose tissues results in significant expression of *Opa1* and brown genes such as *Ucp1* and *Pgc1 α* in white fat depots [69]. This deficiency of adipose pRb also increases energy expenditure to protect animals against diabetes [69]. Together these findings suggest that the thermogenesis of brown adipocytes induced in white fat depots is at least partially

regulated by OPA1, and the relationship between the thermogenic program and OPA1 seems to be linked to fusion rather than fission event of mitochondria (Fig. 4B).

Perspectives

The available data regarding OPA1 and adipocytes indicates that OPA1 is present in all three types of fat cells – white, inducible and classical brown adipocytes. OPA1 is present in the inner membrane of fat cell mitochondria, but there is also evidence showing its localization to lipid droplets of adipocytes. Functional studies of OPA1 effects on lipid metabolism reveal the connection of OPA1 location and its functions in thermogenesis and lipolysis of adipocytes.

In classical brown adipocytes, OPA1 in the mitochondrial inner-membrane contributes to regulation of mitochondrial morphology and thermogenic function. Consequently, the loss of OPA1 leads to dysregulation of mitochondrial morphology and impairment in heat production of brown adipocytes. It is clear that the thermogenesis of classical brown fat cells is increased by fission events and OPA1 cleavage in mitochondria induced by NE. Furthermore, free fatty acids (FFAs) released from lipid droplets in lipolysis stimulated by NE induces mitochondrial depolarization which is required to convert OPA1-L to OPA1-S form in classical brown adipocytes. However, in inducible brown adipocytes indirect data indicate that the increase of OPA1 activity is paralleled by elevation of thermogenic function, but these increases seems to be accompanied with fusion not fission events.

In white adipocytes, OPA1 protein on lipid droplets (LD) functions as an AKAP to facilitate adrenergic stimulation of lipolysis. Catecholamines thus activate PKA bound to OPA1 located on the surface of lipid droplets and induce phosphorylation of perilipin, the gatekeeper that controls lipid hydrolysis. These findings provided an important new role of OPA1 in lipid metabolism which has led to several interesting questions that need to be addressed such as: (i) Does OPA1 also locate on LDs and stimulate lipolysis in inducible brown adipocytes which has characteristics of both white and classical brown adipocytes with both quite big lipid droplets and high amounts of mitochondria?; (ii) What are the roles of OPA1 in thermogenic function of inducible brown fat cells?; and (iii) Does OPA1 also function as an AKAP and mediate adrenergic control of thermogenesis in brown cells?

Interestingly, previous reports have showed that the phosphorylation of perilipin is required for both lipolysis and thermogenesis of brown adipose tissues [103] and that perilipin 5 via its C-terminal region contributes to recruiting mitochondria to a juxtaposition versus lipid droplets in cardiac myocytes [104]. Furthermore, the overexpression of perilipin in white adipocyte tissues induces brown fat cells [105] which contribute to protecting mice against diet-induced obesity [106]. Thus, another open question (iv)

is whether OPA1 can connect and coordinate lipolysis and thermogenesis in inducible brown adipocytes to control lipid metabolism? Addressing these questions will advance understanding of the interaction between lipid droplets and mitochondria.

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Table legends**Table 1. Expression of OPA1 in adipocytes****Table 2. Studies of OPA1 in lipolytic and thermogenic functions of adipocytes**

Figure legends

Figure 1. Classification of adipocytes and their effects on obesity

Figure 2. The expression of OPA1 in adipocytes and its regulators. **A.** In adipocytes, OPA1 locates to the inner membrane of mitochondria facing the intermembranous space or on the surface of lipid droplets. **B.** The expression of OPA1 in adipocytes is regulated by several factors including hormones, transcription factors, proteolytic enzymes, tumor suppressor proteins, and cytokines. Forkhead box protein C2 - FOXC2; Peroxisome proliferator-activated receptors - PPARs; Norepinephrine – NE; Metalloendopeptidase OMA1 - OMA1; Tumor necrosis factor- α – TNF- α .

Figure 3. Function of OPA1 in facilitating adrenergic regulation of lipolysis of adipocytes. In adipocytes stimulated by isoproterenol, an adrenergic agonist, PKA bound to OPA1 located on the surface of lipid droplets (LD) is activated by cAMP signaling to phosphorylate perilipin. Subsequently, the perilipin allows access of lipases, start a lipolytic process to break down neutral fat to free fatty acids and glycerol.

Figure 4. Function of OPA1 in the thermogenesis of adipocytes. **A.** In classical brown adipocytes stimulated by catecholamines such as norepinephrine, OPA1 located in the inner membrane of mitochondria is involved in thermogenesis. The cleavage of OPA1 and mitochondrial fission amply the thermogenic function of these adipocytes. **B.** The function of OPA1 in mitochondrial morphology and thermogenesis of inducible brown adipocytes has not been fully understood, some indirect data show that thermogenic function of this type of fat cells is increased together with mitochondrial fusion under the stimulation of some reagents such as punicic acid, fucoxanthin and exenatide.

Figure 1.

Figure 2.

A. The expression of OPA1 in adipocytes

B. Factors regulate OPA1 expression in adipocytes

Up-regulators:

Transcriptional factors:
e.g. *FOXC2* and *PPARs*

Hormones:
e.g. *17-β estradiol* and *NE*

Enzymes:
e.g. *OMA1* and *TYK2*

Other chemicals:
e.g. *Fucoxanthin* and *Exenatide*

OPA1

Down-regulators:

Hormone: e.g. *Ghrelin*

Tumor suppressor protein:
e.g. *Retinoblastoma*

Cytokine: e.g. *TNF-α*

Figure 3.

Figure 4.