Molecular connections of obesity and aging: a focus on adipose protein 53 and retinoblastoma protein

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Abstract

Obesity is an induced health problem that human being has been facing with non-optimal treatment so far. Humans are on average getting fatter with age, and obesity and aging interact each other to shorten lifetime and decrease life quality. Obesity also causes several aging related-disorders such as cancer, strokes, cardiovascular disease, high blood pressure and type 2 diabetes. So, the molecular connections between aging and obesity are promising targets for bio-medical researches and innovative therapies of many health problems in humans. In this review, we discuss the findings of adipose p53 and Rb - two central molecular linkages between aging and obesity - on lipid metabolism and obesity.

Keywords: p53 and Rb on obesity; p53 and Rb on adipogenesis; Aging and obesity

Running title: Adipose protein 53 and retinoblastoma protein on obesity

Introduction

Obesity is one of metabolic disorders related to aging and senescence. Obesity results in clinical consequences such as type 2 diabetes, cancer, hypertension, dyslipidemia and cardiovascular disease which also increase during aging, therefore obesity reduces life span (Ahima 2009; Tchkonia et al. 2010). Conversely, lifespan maybe expended by anti-obesity interventions (Tchkonia et al. 2010) such as caloric restriction (Barzilai and Gupta 1999; Masoro 2006), surgical removal of visceral fat (Muzumdar et al. 2008), knockout of some metabolic genes, e.g. insulin receptor substrate-1, growth hormone receptor and S6 kinase-1 (Berryman et al. 2008; Blüher et al. 2003; Selman et al. 2008; Um et al. 2004). The status of body mass index (BMI), bodyweight, fat accumulation, glucose metabolism and insulin sensitivity varies during aging (Horvath et al. 2014; Kozak et al. 2010; Tchkonia et al. 2010). High BMI is significantly correlated with epigenetic aging of liver in humans, and epigenetic age could be increased by 3.3 year for each 10 BMI units (Horvath et al. 2014). In a cross-sectional study conducted in 527 health subjects aged 20-87 years, overweight and obese were found to associate with the increase of brain age (around 10 years) in middle-age participants, this suggests that obesity increases risk for neurodegeneration in humans (Ronan et al. 2016). Health diet and physical activity are believed to be good ways to keep a healthy weight with lower risks for chronic diseases and the promotion of healthy aging (Fontana and Hu 2014).

White adipose tissues (WAT), especially visceral WATs tends to increase following aging, and the excess lipid storage in these fat tissues induces overweight and obesity. In humans, a predicted age for having a maximum fat mass ranges from 53 to 61 in 4 studied ethnic groups (Asians, blacks, Puerto Ricans, and whites) (Mott et al. 1999), and the types of white fat to store excess calories varies among genders (Power and Schulkin 2008). Accordingly, women seem to deposit more subcutaneous WAT, while men are more likely to have abdominal fat during obesity (Power and Schulkin 2008). The expression of white adipose expansion markers such as Mest, Sfrp5 and BMP3 in WATs also changes through the life (Chu et al. 2014; Dinh-Toi Chu et al. 2017a; Jura et al. 2016). Adiposity index, a ratio of fat mass and fat free mass, is transiently changed at pre-adulthood, but strikingly increased in adult (post 56 days of age) mice either on a standard (STD) or a high fat diet (HF) (Kozak et al. 2010). The developmental period, in turn, contributes to obesity formation through the effects on adipose biomarkers which play pivotal roles on lipid metabolism (Chu et al. 2014; Kozak et al. 2010). Being exposed to maternal overnutrition during pregnancy and lactation, only retroperitoneal white fat weight, not bodyweight, is increased on offspring phenotype (King et al. 2014). Whereas, early nutrition from birth to weaning modulates the genes encoding proteins of the caveolae and cytoskeleton (Cav1, Cav2, Cavin1, Ldlr, Vldlr and Mest) to determine white adipose expansion in adult animals exposed to a HF (Kozak et al. 2010). Post-weaning exposure to a high-fat high-sugar diet causes obesity, hyperinsulinemia and increase of cholesterol levels in both sexes at adulthood (King et al. 2014). But, the genomic fingerprints of adipose tissues in obese mice are different from those of aging mice (Miard and Picard 2008). It seems that obesity is somehow connected to aging, but underlying mechanisms are not fully understood.

The molecular connections between aging and obesity are so complex and regulated by several factors which include genetics, nutrition, and development period. Thus, controlling these factors could effect on the development of obesity and longevity. Several evidence indicates that caloric restriction (CR) can reduce bodyweight (Das et al. 2017; Gopalan et al. 2016) and enhance longevity (Holloszy and Fontana 2007; Lane et al. 1997; Martin-Montalvo et al. 2013; Mattison et al. 2017; Ravussin et al. 2015; Verdery et al. 1997). In monkeys, a 30% reduction of caloric intake leads to a significantly elevated level of a good cholesterol (HDL 2B) and a decreased level of triglyceride which further reduce risk for aging-related disorders such as cardiovascular disease (Verdery et al. 1997). CR was also found to reduce amounts of natural dehydroepiandrosterone (DHEA) - a biomarker of aging (Lane et al. 1997), and to increase the certain aspects of health in monkeys (Mattison et al. 2012). Thus, CR improves health and extends survival of these animals (Mattison et al. 2017).

In rodents, reduction of meal also improves heath, and reduces the risk for aging-related diseases (Anson et al. 2003; Duan et al. 2003). CR could protect neurons from the damage and increase insulin sensitivity; therefore CR delayed the onset of brain and metabolic diseases leading to prolong their lives (Anson et al. 2003; Duan et al. 2003). The treatment for metabolic disorders such as type 2 diabetes by a compound mimicking benefits of CR improved longevity of mice at the middle age (Martin-Montalvo et al. 2013). The effects of CR on the extension of lifespan were at least partially regulated by mTORC1, and inhibition of mTORC1 by rapamycin conferred longevity of mice (Lamming et al. 2012).

In overweight humans, reduction of 25% in caloric intake for six months could reduce the fasting insulin in blood, and core body temperature – two biomarkers of longevity (Heilbronn et al. 2006). Then a preliminary clinical study in cancer patients showed that fasting could support chemotherapy by reducing its side effects (Raffaghello et al. 2010). CR also reduced the risk factors (e.g. insulin resistance, blood

pressure and cholesterol) for aging and its-related diseases in humans, this was investigated in a two-year clinical trial in normal and moderately overweight subjects (Ravussin et al. 2015). Interestingly, CR was proved to improve exercise capacity and health, and to reduce symptoms in obese patients with heart failure in a clinical trial with 100 subjects (Kitzman et al. 2016). Then, the most recent clinical trial in nonobese humans showed that a 2 year CR regimen significantly reduced weight, waist circumference and fat mass in participants (Das et al. 2017). Therefore, CR shows as a promising method to reduce obesity and its related metabolic disorders as well as to prolong human lifespan. However, the molecular mechanisms of CR effects on the connection between obesity and aging are still in investigation.

P53 and Rb are two main players in cellular pathways of aging

Aging is characterized by reduced cell division and proliferation accompanied by increased senescence and apoptosis. There are two cellular pathways of aging which have the involvement of p53 tumor suppressor protein (p53) and the retinoblastoma protein (Rb) (Campisi 2003; Campisi and d'Adda di Fagagna 2007; Polager and Ginsberg 2009) (Fig 1). They are, maybe, independent or connect at some points such as via *Mdm2* (Yap et al. 1999) and CDKN2A (Polager and Ginsberg 2009).

The first cellular pathway of aging is triggered by the induction and activation of ARF under the stimulation of oncogenic stresses such as DNA damage (Campisi 2003; Carlos et al. 2013). This cellular way of aging involves a central role of p53, a transcription factor induces cell growth arrest and apoptosis, and modulates cellular senescence as wells as organismal aging (Rufini et al. 2013). In the nucleolus, ARF forms a stable complex with *Mdm2* and sequesters this molecule; *Mdm2* is a downstream factor of p53. The activation of p53 initiates cell cycle arrest and apoptosis by enhancing the transcription of target genes such as *WAF1* and *BAX*, which promotes cell death and senescence programs (Campisi 2003). WAF1 (CIP1/p21) protein regulates cell cycle progression at G1 and S phase by binding to and inhibiting the activity of CDK2/CycE and CDK4/CycD1 complexes (Rufini et al. 2013). On the other hand, up-regulation of p53 triggers the expression of another pro-senescence gene, E2F7 (Rufini et al. 2013), which is pivotal in repression of mitotic genes. Furthermore, deletion of BRCA2 gene (breast cancer 2, early onset) induced senescence in primary mouse and human cells, and this depended on the ARF activation (Carlos et al. 2013).

The second cellular pathway of aging is based on Rb, a master regulator of cell cycle progression (Burkhart and Sage 2008) or cell death (Polager and Ginsberg 2009), and this protein belongs to the pocket protein family. This pathway is initiated by induction of INK4A by oncogenic stresses. The increase of INK4A inhibits the expression and function of CDKs such as Cyclin D, CDK4 and CDK6. Down-regulation of CDKs enhances the expression and activity of Rb (Campisi 2003). Rb protein binds to E2F transcription factors and recruits them away from their target genes (Burkhart and Sage 2008) such as the genes regulating cell cycle progression (*CyclinE1* and *CyclinA2)* (Burkhart and Sage 2008), or apoptosis (*Apaf1*, *Puma*, and *SIVA*) (Polager and Ginsberg 2009). Interestingly, acute removals of Rb in postmitotic neurons results in apoptosis and loss of differentiated neurons both *in vivo* and in *vitro*, this is happened in the independent of the induction of classical E2f-mediated apoptotic genes, Apaf1 or Puma (Andrusiak et al. 2012). Therefore, Rb mediates cell death program by 2 different ways, directly by itself (at least in the case of postmitotic neurons (Andrusiak et al. 2012)) or indirectly through the interaction with E2f.

These two pathways have some common regulators such as *Mdm2* (Yap et al. 1999) and CDKN2A (Polager and Ginsberg 2009) (Fig 1). Mdm2 negatively regulates p53, but it has opposite roles on the Rb-E2f pathway. *Mdm2* promotes the G1/S cell cycle transition via its physical interaction with Rb, E2F1 and the heterodimeric partner of E2F1, DP1 (Polager and Ginsberg 2009), on the other hand, *Mdm2* induces the degradation of E2F1–DP1 heterodimers to inhibit E2F1 and DP1 subunits (Polager and Ginsberg 2009). Whereas, CDKN2A encodes INK4A and ARF to modulate aging via p53 or Rb. ARF is a positive regulator of p53 by inhibiting *Mdm2*, but it is a negative regulator of E2f (Polager and Ginsberg 2009). In the line with suppressive effect of ARF on E2f, INK4A reduces the RB phosphorylation to decrease the function of E2f (Polager and Ginsberg 2009).

Alteration of p53 homeostasis in adipose tissues contributes to the induction and development of obesity

In vitro, the roles of p53 on adipogenesis depend on the type of adipocytes, the cumulative evidence shows that p53 negatively regulates the differentiation into white adipocytes (white adipogenesis) (Armesilla-Diaz et al. 2009; Calo et al. 2010; Hallenborg et al. 2009; Huang et al. 2014; Molchadsky et al. 2013; Molchadsky et al. 2010; Molchadsky et al. 2008; Zhu and Prives 2009). It inhibits an adipogenic program in 3T3-L1 preadipocytes and mouse embryonic fibroblasts (MEFs)(Huang et al. 2014; Molchadsky et al. 2008). Knockdown of p53 by a specific sh-RNA enhances the adipogenic capacity of both mouse and human cell lines indicated by increased levels of adipogenic markers such as Pparγ, aP2 and Adiponectin (AdipoQ) (Molchadsky et al. 2013; Molchadsky et al. 2008), and p53 KO MEFs differentiate into adipocytes more robust compared to wild type cells in an adipogenic medium (Huang et al. 2014). Furthermore, sh-p53 RNA treated MEFs differentiate into white adipocytes *in vitro* even without hormonal induction as proved by lipid accumulation in oil red "O" staining, and p53 overexpression reduces the adipogenic differentiation of 3T3-L1 preadipocytes (Huang et al. 2014). In the opposite side, p53 is required for the development of brown adipocytes both *in vivo* and *in vitro*, there are abnormalities in brown adipogenesis of p53 knockdown C2 cells, and in the development of iBAT tissues in p53 KO embryos (Molchadsky et al. 2013). Deletion of p53 significantly reduces the levels of brown markers including *Prdm16*, *Pgc1α* and *CEBPβ* in embryonic iBAT (Molchadsky et al. 2013). Furthermore, the absence of p53 reduces the mRNA expression of thermogenic genes such as *Ucp1* and *Pgc1α* of rosiglitazone-induced-adipocytes from fibroblasts *in vitro* (Hallenborg et al. 2016).

In vivo, the expression and activation of adipose p53 is elevated during obesity in both diet and genetic murine models (Fausto Bogazzi et al. 2013; Homayounfar et al. 2014; Minamino et al. 2009; Vergoni et al. 2016; Yahagi et al. 2003; Zand et al. 2016) and in human obese subjects (Francisco José Ortega et al. 2014). Diet induced obesity (DIO) rat and mice have disturbances in insulin sensitivity and glucose tolerance with the selective increase of p53 expression and phosphorylation in white fat tissues (Fausto Bogazzi et al. 2013; Homayounfar et al. 2014; Zand et al. 2016), not in other tissues such as liver, skeletal muscle and kidney (Fausto Bogazzi et al. 2013). In genetic models of obesity (Ay and ob/ob mice), insulin resistance, glucose intolerance and induction of adipose inflammation is accompanied with significantly higher expression of p53 and *Cdkn1a* in adipose tissues (Minamino et al. 2009; Yahagi et al. 2003). The increase in activation of adipose p53 in obese animals was recently found due to an elevated level of DNA damage in fat deport of mice fed a HF (Vergoni et al. 2016). Notably, beta-adrenergic receptor agonists such isoproterenol and forskolin, which can induce browning of white fat and activate

thermogenic function of brown/brite adipocytes to burn lipid (Chu-Dinh and Chu 2014; Chu and Tao 2017; Dinh-Toi Chu et al. 2017b), can prevent obesity-induced p53 activation in rat (Zand et al. 2016).

Interestingly, most published articles show that deletion of p53 or inhibition of its activity leads to an obesogenic phenotype *in vivo* (Armata et al. 2010; Homayounfar et al. 2014; Minamino et al. 2009; Molchadsky et al. 2013) except a report done by Philip Hallenborg et al (Hallenborg et al. 2016). The increase in fat mas of p53KO animals is consistent with the negative regulation of p53 on white adipogenesis *in vitro* (Armesilla-Diaz et al. 2009; Molchadsky et al. 2013; Molchadsky et al. 2008; Zhu and Prives 2009). Knockout of p53 results in higher fat mass either on a STD or a HF, this elevation is associated to a higher expression level of adipogenic marker (Pparγ) in epididymal fat (Molchadsky et al. 2013).

In Ay mice, specific deletion of adipose p53 increases fat accumulation and expression of adiponectin in adipose depots, even adipose-p53–deficient mice (ad-p53KO mice) have a better insulin sensitivity in a HF (Minamino et al. 2009). This is in line with the effects of systemic inhibition of p53 by a selective inhibitor (Pifithrin-α, PFT) , PFT repairs the abnormal metabolism caused by a HF in both rat and mouse models (Fausto Bogazzi et al. 2013; Homayounfar et al. 2014). Furthermore, this p53 inhibitor reduces the expression and phosphorylation of p53 (Fausto Bogazzi et al. 2013; Homayounfar et al. 2014), but increases phosphorylation of *Mdm2* in white fat depots of DIO rat (Homayounfar et al. 2014). Inactivation of p53 by a mutation at the ATM site Ser18 (Ser15 in humans) induces obesity in STD fed mice with insulin resistance, higher bodyweight, inflammation in fat depots and higher levels of serum leptin as well as triglyceride versus WT mice (Armata et al. 2010). In a mouse model for a common polymorphism of p53 at codon 72 in human, Kung CP et al in 2016 showed that R72 variant of p53 increased risks for obesity and others metabolic dysfunctions induced by a HF (Kung et al. 2016).

However, inhibition of total p53 in Ay mice (Ay Trp53 $^{+/-}$mice) reduces fat weight and inflammatory cytokines (Tnf, Ccl2), improves insulin sensitivity and glucose tolerance, these seems to be not consistent with an increase in fat mass seen in ad-p53KO mice (Minamino et al. 2009). Adipose tissues of Ay Trp53 $^{+/-}$mice express lower levels of senescence markers, p53 and Cdkn1a compared to those of Ay and wild type (WT) mice (Minamino et al. 2009).

Furthermore, activation of p53 in endothelial cells contributes to development of metabolic disorders (Yokoyama et al. 2014). Specific deletion of endothelial p53 leads to significant anti-obesity outcomes in HF fed mice with reduction of bodyweight, visceral fat, subcutaneous fat, and adipose inflammatory cytokine (Ccl2), which are associated with improvement of insulin sensitivity and glucose tolerance (Yokoyama et al. 2014). This is due to a striking elevation of mitochondrial biogenesis, energy expenditure and glucose uptake caused by the disruption of endothelial p53 expression (Yokoyama et al. 2014). In contrast, overexpression of p53 in endothelial cells by knockdown of its negative regulator (Mdm4) induces an obese phenotype like in ob/ob mice. On a HF, endothelial overexpressing p53 mice (EC Mdm4 KO mice) are heavier, fatter than WT mice (Yokoyama et al. 2014). The EC Mdm4 KO mice also have abnormalities in glucose tolerance and insulin response with decreased expression levels of the genes responsible for energy expenditure and glucose uptake markers such as Pgc1α and Glut1 respectively, in skeletal muscle (Yokoyama et al. 2014).

These findings indicate that p53 of adipose tissues has a crucial role in the formation and development of obesity and type 2 diabetes. The homeostasis of adipose p53 contributes to maintenance of healthy

metabolism, both interruption (Armata et al. 2010; Fausto Bogazzi et al. 2013; Homayounfar et al. 2014; Minamino et al. 2009; Molchadsky et al. 2013) and over-induction (Homayounfar et al. 2014; Minamino et al. 2009; Yahagi et al. 2003) of p53 activity in fat depots result in metabolic disorders such as obesity. Oxygen species (ROS) is a link between p53 in adipose tissues and obesity and/or diabetes in the case of overreaction of p53 (Ahima 2009; Minamino et al. 2009). Increase of fat by excessive calorie intake seems to accumulate ROS in fat tissues resulting in high induction and activation of p53 which leads to an inflammation response in adipose tissues followed by an insulin resistance state (Minamino et al. 2009; Shimizu et al. 2012). Another connector of adipose p53 and obesity with insulin resistance is growth hormone (GF) (Fausto Bogazzi et al. 2013), GH blockage down-regulates p53 in fat depots and reverts the abnormalities in glucose metabolism of DIO mice through p38 pathway (Fausto Bogazzi et al. 2013).

Adipose retinoblastoma protein regulates obesity via its effects on white and brown/brite adipogenesis

In vitro, retinoblastoma protein (Rb) is required for adipogenic differentiation of pre-adipocytes and primary fibroblasts (Capasso et al. 2014; Chen et al. 1996; Classon et al. 2000; Hallenborg et al. 2009; Hu et al. 2015; Moreno-Navarrete et al. 2013). The expression and activity of Rb are significantly increased during adipogenesis of animal and human pre-adipocytes, 3T3-L1 cell lines (Moreno-Navarrete et al. 2013), and mouse primary fibroblasts (Chen et al. 1996; Hu et al. 2015). Permanent (50%) Rb knockdown by a specific siRNA decreases the expression of adipogenic and lipogenic markers (*Pparγ*, *AdipoQ*, and *Fas*), whereas this inhibition increases the expression of brown adipogenic genes (*Prdm16* and *Ucp1*) of 3T3-L1 cell lines in an adipogenic medium (Moreno-Navarrete et al. 2013). Syngeneic $Rb^{-/-}$ fibroblasts and $Rb^{-/-}$ 3T3 cell lines do not differentiate into adipocytes under stimulation of an identical adipogenic condition (Chen et al. 1996; Classon et al. 2000). The absence of Rb also induces the impairment of the terminal adipogenic differentiation and dysregulated adipocytes from bone marrow stromal cells (Capasso et al. 2014). Inducible expression of Rb by a transfection with plasmids containing wild-type or point-mutant restores the capacity of $Rb^{-/-}$ fibroblasts in differentiating into adipocytes (Chen et al. 1996), and this is also observed in $Rb^{-/-}$ 3T3 cell lines with an exogenous Rb expression (Classon et al. 2000). Mechanism investigations show that Rb activates CCAAT/enhancer-binding proteins (C/EBPs) to trigger the adipogenic program of murine fibroblasts and 3T3-L1 cell lines (Chen et al. 1996; Classon et al. 2000).

But, Rb has an opposite role on the adipogenesis of osteoblast [14]. Knockdown of Rb restores adipogenic ability of p53KO osteosarcoma cell lines (Calo et al. 2010). In an adipogenic medium, double knockout (DKO) of Rb and p53 in osteosarcoma cell lines induces a differentiation of these cells into adipocytes expressing high levels of adipose bio-functional markers such as *aP2*, *Pparγ*, *C/ebpα* and *Pgc1α*, while p53 KO cells fail to start an adipogenesis (Calo et al. 2010). Furthermore, a doxocycline-inducible expression of Rb drops an adipogenic differentiation state of DKO osteosarcoma cell lines (Calo et al. 2010). This result indicates that Rb suppresses fate choice and lineage commitment of pre-osteoblasts into adipocytes *in vitro*, and the same result is found *in vivo* (Calo et al. 2010).

In human, adipose tissues of obesity subjects with or without type 2 diabetes have significantly lower Rb expression (mRNA and protein) and activity compared to those of healthy subjects (Moreno-Navarrete et al. 2013). The expression of Rb is positively associated with the expression of adipogenic markers (Pparγ, IRS1) and lipogenic markers (Fas, Acc) in white adipose tissues (Moreno-Navarrete et al. 2013), but it is

negatively associated with BMI, serum glucose and insulin. . The similar observation is found in a rat model of DIO, 16 weeks on a cafeteria diet induces obesity in rat with decreased expression of Rb in white fat depots at both mRNA and protein levels (Moreno-Navarrete et al. 2013). But, the obesogenic phenotype increases phosphorylation of Rb in white adipose tissues during obesity in rat (Moreno-Navarrete et al. 2013).

Inactivation or deletion of Rb in adipose tissues provides anti-obesity outcomes in mouse model of diet induced obesity (Dali-Youcef et al. 2007;Mercader et al. 2009; Petrov et al. 2015). On a HF, $Rb^{+/-}$ mice are smaller, leaner than WT mice, because Rb haploinsufficiency improves insulin sensitivity, glucose tolerance as well as energy expenditure and a thermogenic program in inguinal white fat tissues (ingWAT) (Mercader et al. 2009; Petrov et al. 2015). However, the anti-obesity effect and reduction of white adipose tissues induced by Rb haploinsufficiency appear only in mature not young adult mice (Petrov et al. 2015). Recently (2016), Petar D. Petrov et al show that preadipocytes in WAT from $Rb^{+/-}$ mice have increased capacity for brown-like adipogenesis than beige adipogenesis (Petrov et al. 2016a). Specific inhibition of adipose Rb leads to a stronger protective effect against obesity, $Rb^{ad-/-}$ mice have significantly lower bodyweight, WATs, and serum leptin than control mice when they eat an obesogenic diet (Dali-Youcef et al. 2007). Adipose tissue-specific inactivation of Rb protects against DIO due to promoting energy expenditure by increasing mitochondrial biogenesis and the activity of thermogenic genes such as *Ucp1*, *Pgc1α* and *Pparα* in both WAT and iBAT tissues (Dali-Youcef et al. 2007). Moreover, cold exposure, an inducer and activator of brite and brown adipogenesis, reduces Rb in brown adipose tissues by phosphorylating this protein (Hansen et al. 2004).

Down-regulation of Rb is occurred with the reduction of white adipogenesis and obesity in hormone-sensitive lipase (HSL) null mice fed with a HF (Ström et al. 2008). The lack of HSL inhibits obesogenic effect of a HF as indicated by lower bodyweight, lower expression levels of adipogenesis markers (*Pparγ, AdipoQ* and *Scd1*) in white fat depots of HSL null mice versus WT mice (Ström et al. 2008). Notably, DIO mice also drop mRNA level of Rb in white fat tissues when they are absent of HSL. Like Rb KO mice (Dali-Youcef et al. 2007; Mercader et al. 2009), HSL null mice increases the brown adipogenesis in white fats as proved by higher mitochondrial size and *Ucp1* expression (Ström et al. 2008). As mentioned above, the expression and activity of Rb in white adipose tissues are increased by obesity, and the inactivation or deletion of adipose Rb reduces obesity, but expression of hypothalamus Rb was inhibited by high fat feeding on a model of DIO, therefore Rb in hypothalamus seems to be an obesity suppressor (Lu et al. 2013). Additionally, in muscle cells, the silencing of Rb by small interfering RNAs enhances mitochondrial oxidative metabolism, and fatty acid and glucose disposal as well as decreases intracellular lipid accumulation (Petrov et al. 2016b).

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These findings demonstrate that adipose retinoblastoma protein is an important player in white and brown and/or brite adipogenesis (Chen et al. 1996; Classon et al. 2000; Dali-Youcef et al. 2007; Hansen et al. 2004; Lizcano and Vargas 2016; Mercader et al. 2009; Moreno-Navarrete et al. 2013; Ström et al. 2008). Its expression and activity in fat tissues determine the development and function of white and brown/brite adipocytes leading to variation of obesity status (Dali-Youcef et al. 2007; Mercader et al. 2009; Moreno-Navarrete et al. 2013). Up-regulation of Rb increases the white adipogenesis by activating C/EBPs, the vital transcription factors of adipogenic and lipogenic markers (*Pparγ, IRS1, Scd1, Fas, Acc*) (Chen et al.

1996; Classon et al. 2000), which lead to fat expansion and obesity, but when the overweight reaches a limited status the activity of Rb will be decreased like adipogenic genes (*Pparγ*, *IRS1* and *Scd1)*(Moreno-Navarrete et al. 2013). In contrast, one of possible mechanisms underlying the inhibition of Rb on the formation and function of both brown and brite adipocytes (Dali-Youcef et al. 2007; Lizcano and Vargas 2016; Mercader et al. 2009) is the suppressive effect of this protein on the expression of Foxc2 and its target gene (RIα) resulting in decreased cAMP activity (Hansen et al. 2004). Thus, down-regulation of pRb expression promotes the brown adipogenesis in iBAT and brite adipogenesis in WATs in response to β3-adrenergic receptor agonist treatment and cold exposure (Hansen et al. 2004) which further enhance thermogenesis and energy expenditure against obesity (Dali-Youcef et al. 2007; Mercader et al. 2009).

Furthermore, beside Rb1 other proteins of the retinoblastoma gene family may also play a role in lipid metabolism and obesity such as Rb2/P130 and P107 (Alessio et al. 2013; Galderisi et al. 2006). Because they can possess overlapping functions and compensate for each other to control the cell cycle and related phenomena including proliferation, quiescence, apoptosis, senescence, and cell differentiation (Galderisi et al. 2006).

Conclusions

Among several molecular signatures of aging and senescence in mammals, p53 and Rb have been shown to be the extensively described regulators (Figure 1) taking main roles in aging or apoptosis pathways. Interestingly, accumulating evidences indicate that these molecules are also the molecular linkages between aging and obesity (Table 1 and 2). They however play their own ways in adipogenesis, fat metabolism and obesity. The balance of p53 expression and activity in white adipose tissues maintains healthy body fat and bodyweight; either under- or over-expression of this gene in fat tissues causes abnormalities of white adipogenesis and fat metabolism resulting in obesity. While adipose Rb regulates the bio-functions of white or brown adipose tissues to accumulate or burn energy that determines statuses of body fat and obesity, but obesity level at some limitative states inhibits the expression and activity of adipose Rb (Figure 2).

Further elucidation at the systemic level of the relationship between aging and obesity based on adipose p53 and Rb will give us more benefits to prevent obesity, aging and other related disorders. Therefore, we need to have more *in vivo* and *in vitro* experiments to confirm these linkages of aging and obesity as well as to investigate their other roles and other possible connections between aging and obesity. Perhaps, some new genetic models, poly genetic models of obesity should be developed to investigate deeply adipose p53 and Rb in aging, obesity and its related metabolic disorders.

Competing interests

None

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors

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Table 1. The relationship of p53 and obesity

Models	Outcome effects on obesity	Outcome on molecular aging	Ref
WT rat on HF (DIO rat)	↑↑bodyweight, ↑PTEN, insulin resistance, glucose intolerance, ↓pAKT	↑p53, ↑p-p53, ↓pMdm2	(Homayounfar et al. 2014)
PFT treated DIO rat	↑insulin sensitivity, ↑glucose tolerance, ↑pAKT, ↓PTEN	↓↓p53, ↑pMdm2	(Homayounfar et al. 2014)
WT mice on HF (DIO mice)	↑↑bodyweight, insulin resistance, glucose intolerance, ↑p-P38	↑↑p53, ↑p-p53	(Fausto Bogazzi et al. 2013)
PFT treated DIO mice	↑insulin sensitivity, ↑glucose tolerance, ↑glucose uptake	↓↓p53	(Fausto Bogazzi et al. 2013)
PEG treated DIO mice	↑insulin sensitivity, ↑glucose tolerance, ↑glucose uptake	↓↓p53, ↓p-p53	(Fausto Bogazzi et al. 2013)
Acro mice on HF	↑↑bodyweight, insulin resistance, glucose intolerance, ↓Glut4, ↓Srebp-1, ↑p-P38, ↓p-AKT	↑↑p53, ↑p-p53	(Fausto Bogazzi et al. 2013)
PET treated acro mice on HF	↑insulin sensitivity, ↑glucose tolerance, ↑glucose uptake	↓↓p53	(Fausto Bogazzi et al. 2013)
PEG treated acro mice on HF	↑insulin sensitivity, ↑glucose tolerance, ↑glucose uptake, ↓p-P38, ↑p-AKT	↓↓p53, ↓p-p53	(Fausto Bogazzi et al. 2013)
p53 KO mice on STD or HF	↑↑fat weight, ↑↑ bodyweight, ↑Pparγ, ↓iBAT	↓↓p53	(Molchadsky et al. 2013)
Ob/Ob mice on STD	↑↑fat weight, insulin resistance, glucose intolerance, ↑TNFα	↑p53, ↑WAF1	(Yahagi et al. 2003)
Ay mice on STD	↑↑fat weight, insulin resistance, glucose intolerance, ↑TNF, ↑Ccl2, ↓ AdipoQ	↑p53, ↑Cdkn1a	(Minamino et al. 2009)
Ay Trp53 $^{+/-}$mice on STD	↓fat weight, ↓TNF, ↓Ccl2, ↑insulin sensitivity, ↑glucose tolerance, ↑ AdipoQ	↓↓p53, ↓↓Cdkn1a	(Minamino et al. 2009)
Ad-p53 KO mice on HF&HS	↑fat weight , ↓TNF, ↓Ccl2, ↑insulin sensitivity, ↑glucose tolerance, ↑ AdipoQ	↓↓p53, ↓↓Cdkn1a	(Minamino et al. 2009)
p53^{S18A} mice on STD	↑ bodyweight, insulin resistance, glucose intolerance, ↑TNFα, ↑TNFγ, ↑IL6, ↑Leptin, ↑triglyceride	↓↓p53	(Armata et al. 2010)
Endothelial cell-specific p53 KO mice on HF	↓body weight, ↓fat weight , ↑insulin sensitivity, ↑glucose tolerance, ↑mitochondrial biogenesis, ↑ energy expenditure, ↑glucose uptake, ↓Ccl2	↓p53, ↓Cdkn1a	(Yokoyama et al. 2014)
Endothelial cell-specific Mdm4 KO mice on HF	↑ bodyweight, ↑fat weight, insulin resistance, glucose intolerance, ↓Pgc1α and Glut1 in muscle	↑↑p53	(Yokoyama et al. 2014)

STD: Standard diet, HF&HS: High fat & high sucrose diet; Pifithrin-a (PFT or PIF): selective inhibitor of p53 transcriptional; P-p53: phosphorylation of p53; Pegvisomant or Somavert (PEG): growth hormone receptor antagonist; Acro mice: Transgenic mice overexpressing bovine growth hormone; "↑" and "↑↑" indicates "a significant increase" and "a very significant increase", respectively; "↓" and "↓↓" indicates "a significant decrease" and "a very significant decrease", respectively.

Table 2. The relationship of Rb and obesity

Models	Outcome effects on obesity	Outcome on molecular aging	Ref
Obese subjects	↑↑BMI, ↑blood glucose and insulin, ↑leptin, ↓Glut4, Pparγ, IRS1, Fas, and Acc	↓Rb, ↑p-Rb	(Moreno-Navarrete et al. 2013)
Obese subjects with type 2 diabetes	↑↑BMI, ↑blood glucose and insulin, ↑leptin, ↓Glut4, Pparγ, IRS1, Fas, and Acc	↓Rb, ↑p-Rb	(Moreno-Navarrete et al. 2013)
WT rat on a cafeteria diet	↑↑bodyweight	↓Rb, ↑p-Rb	(Moreno-Navarrete et al. 2013)
$Rb^{ad-/-}$ mice on HF	↓bodyweight, ↓WATs, ↓ serum leptin, ↑energy expenditure, ↑mtDNA and brown genes in WAT and iBAT	↓↓Rb	(Dali-Youcef et al. 2007)
Mature adult $Rb^{+/-}$ mice on STD	↓bodyweight, ↓WATs, ↓adipocyte size, ↓ liver lipid, ↓bodyweight, ↓WATs, ↑energy expenditure, ↑insulin sensitivity, ↑glucose tolerance, ↑ Ucp1, Pgc1α in ingWAT	↓Rb	(Petrov et al. 2015)
$Rb^{+/-}$ mice on HF	↓bodyweight, ↓WATs, ↓adipocyte size, ↓ liver lipid, ↑energy expenditure, ↑insulin sensitivity, ↑glucose tolerance, ↑ Ucp1, Pgc1α in ingWAT	↓Rb	(Mercader et al. 2009)
HSL null mice on HF	↓bodyweight, ↓leptin, ↓Pparγ, AdipoQ and Scd1 in WAT, ↑energy expenditure, ↑Mitochondrial size and Ucp1 in WAT	↓Rb	(Ström et al. 2008)
WT mice at 4°C	↓bodyweight, ↑energy expenditure, ↑mtDNA and brown genes in WAT and iBAT	↓Rb, ↑p-Rb	(Hansen et al. 2004)

HF: high fat diet; P-Rb: phosphorylation of Rb; HSL: Hormone-Sensitive Lipase; "↑" and "↑↑" indicates "a significant increase" and "a very significant increase", respectively; "↓" and "↓↓" indicates "a significant decrease" and "a very significant decrease", respectively.

Figure legends

Figure 1. P53 and Rb play the central roles on molecular pathways of aging. In aging, there are two common pathways (Campisi 2003; Campisi and d'Adda di Fagagna 2007; Polager and Ginsberg 2009) which have the pivotal involvement of p53 and Rb, these pathways work independently or they connects at some points such as via *Mdm2* (Yap et al. 1999) and CDKN2A (Polager and Ginsberg 2009).

Figure 2. The relationship between adipose Rb and obesity. Adipose Rb enhances the formation and development of white adipocytes, but it suppresses the differentiation and function of brown/brite adipocytes therefore under particular conditions adipose Rb stimulates the development of obesity. However, when the obesity is severe, the adipose tissues will be severely dysfunctional, both activity of adipose Rb and adipogenesis of white adipocytes will be inhibited.

Figure 1.

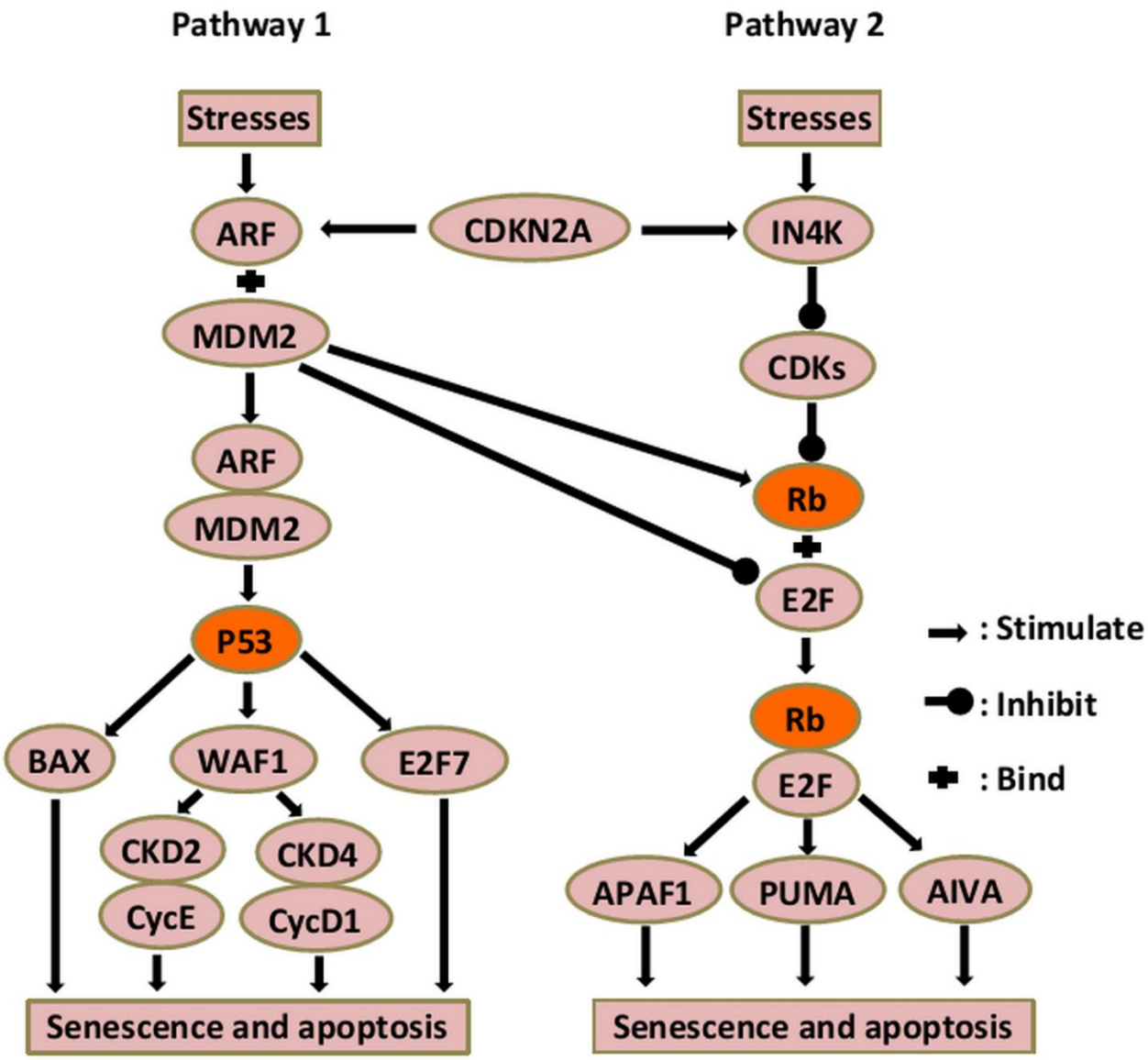
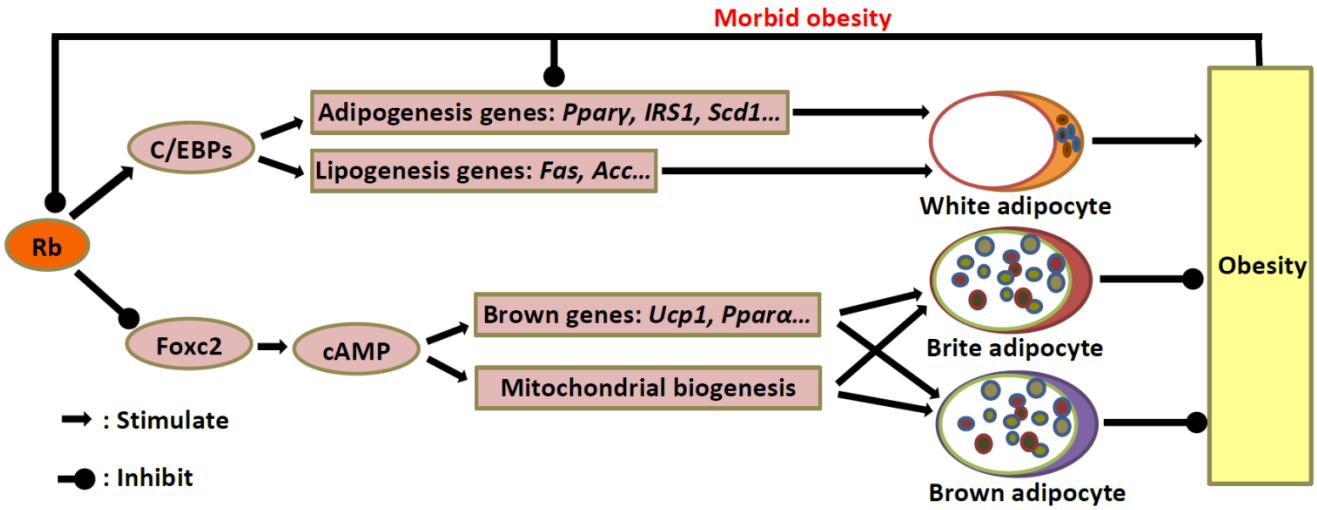

Pathway 1
Pathway 2
Stresses
Stresses
ARF
CDKN2A
IN4K
MDM2
CDKs
ARF
Rb
MDM2
E2F
P53
: Stimulate
: Inhibit
: Bind
Rb
BAX
WAF1
E2F7
E2F
CKD2
CKD4
APAF1
PUMA
AIVA
CycE
CycD1
Senescence and apoptosis
Senescence and apoptosis

Figure 2.


Morbid obesity
C/EBPs
Adipogenesis genes: Pparγ, IRS1, Scd1…
Lipogenesis genes: Fas, Acc…
White adipocyte
Rb
Foxc2
cAMP
Brown genes: Ucp1, Pparα…
Mitochondrial biogenesis
Brite adipocyte
Brown adipocyte
Obesity
: Stimulate
: Inhibit