Cellular mechanisms of MR regulation of adipose tissue physiology and pathophysiology

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Abstract

In addition to the well-documented expression and activity of the mineralocorticoid receptor (MR) in the kidney, in the last decade research on MR has also revealed its important role in regulating functions of extrarenal tissues, including adipose tissue, where MR is involved in adipocyte fundamental processes such as differentiation, autophagy and adipokine secretion. MR expression is increased in adipose tissue of murine models of obesity and in obese human subjects, suggesting that over-activation of the mineralocorticoid signaling leads to dysfunctional adipocyte and associated metabolic disorders. Notably, pharmacological blockade of MR prevents metabolic dysfunctions observed in obese mice and suggests a potential therapeutic use of MR antagonists in the treatment of obesity and metabolic syndrome. However, the molecular pathways affected by MR blockade have been poorly investigated. This review summarizes the functions of MR in the adipocyte, discusses potential signaling pathways mediating MR action, and describes post-translational modifications regulating its activity.

Key Words
- metabolism
- steroid hormones
- obesity
- insulin signaling

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Introduction

Mineralocorticoid receptor (MR) activation by its primary ligand aldosterone is a well-known mechanism acting at the renal level as a regulator of plasma volume, salt homeostasis and blood pressure (Funder 2005). Importantly, besides aldosterone, MR can bind glucocorticoids with a higher affinity (10-fold) than the glucocorticoid receptor (GR) (Reul & De Kloet 1985). In epithelial tissues, the enzyme 11beta hydroxysteroid dehydrogenase type II (11βHSD2), which converts active glucocorticoids into inactive metabolites, results in aldosterone selectivity of MR and excludes its activation by glucocorticoids (Funder 2009). On the other hand, in non-epithelial tissues such as the brain (De Kloet *et al.* 2000), cardiomyocytes (Farman & Bocchi 2000) and adipose tissue (Rondinone *et al.* 1993, Caprio *et al.* 2007) either low levels or lack of 11βHSD2 lead to prevailing occupancy of MR by glucocorticoids (Funder 2000). In the recent years, the identification of MR in different non-epithelial tissues has led to the development of a novel field of research whose aim is to investigate the extra-renal functions of MR. Indeed, distribution of MR in such a wide range of tissues suggested novel unexpected roles for MR, in diverse physiological and pathophysiological contexts, including metabolic syndrome, oxidative stress, stress adaptability, inflammation and more.

In this context, several research groups identified the adipocyte as a novel cellular model for investigating the

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function of MR in the pathophysiology of obesity and metabolic syndrome. Either knockdown or pharmacological blockade of MR inhibits adipogenesis in 3T3L1 cultures (Caprio *et al*. 2007, 2011), revealing an important role for this transcription factor in adipose tissue physiology and pathophysiology. Pharmacological MR antagonism has been shown to counteract dysfunctional metabolism of adipose tissue (Guo *et al*. 2008, Hirata *et al*. 2009, Wada *et al*. 2010). Remarkably, conversion of white into brown adipose tissue, a process induced by MR blockade, takes part in the anti-obesity effects of MR antagonism (Armani *et al*. 2014*a*).

However, most of the signaling pathways downstream of MR activation by aldosterone or glucocorticoids in the adipocyte still remain unclear.

This review highlights the state of the art in the field about the role of MR activation in adipose tissue, under physiologic and pathophysiologic conditions, and briefly describes potential signaling pathways of the adipocyte which could be targeted by MR. Finally, we discuss the potential modulation of MR activity in the adipocyte by post-translational modifications, which are known to influence MR function, hence potentially affecting the physiology of adipose tissue.

Role of MR in adipocyte function

MR expression and function has been extensively studied in adipocyte cultures as well as in the fat of murine models of obesity, by our laboratory and other groups (Zennaro *et al*. 1998, Caprio *et al*. 2007, Guo *et al*. 2008, Hirata *et al*. 2009, Armani *et al*. 2014*a*). MR activation upregulates the expression of adipocyte markers and promotes adipogenesis in 3T3L1 adipose cells (Caprio *et al*. 2007). Conversely, pharmacological blockade of MR reduces adipocyte differentiation in 3T3L1 cells as well as in primary human adipocytes (Caprio *et al*. 2011). Consistent with these data, murine primary adipocyte cultures prepared from MR knockout mice show defective adipogenesis (Hoppmann *et al*. 2010) and knockdown of MR in human primary adipocyte cultures blocks adipogenesis, confirming that MR plays a relevant role both in mouse and human adipogenesis (Hoppmann *et al*. 2010, Armani *et al*. 2014*b*). Altogether, these studies *in vitro* suggest that impaired activity of MR inhibits adipocyte differentiation. *In vivo* studies in obese mouse models show increased levels of MR transcript in adipose tissue in comparison with lean mice, suggesting enhanced activation of this receptor (Hirata *et al*. 2009). Treatment of obese mice with MR antagonists counters fat mass gain (Wada *et al*. 2010, Armani *et al*. 2014*a*) as well as altered expression of adiponectin, PPARγ and leptin (Guo *et al*. 2008, Hirata *et al*. 2009), confirming that MR activity promotes expression of adipocyte genes and regulates adipose tissue function.

Notably, in obese mice, the increased transcript expression of cytokines tumor necrosis factor alpha (TNFα), monocyte chemoattractant protein-1 (MCP-1), interleukin-6 (IL-6) (Guo *et al*. 2008, Hirata *et al*. 2009), macrophage markers CD68 and CD11 as well as the increased presence of crown-like structures associated with enhanced inflammation and adipocyte apoptosis (Murano *et al*. 2008), are prevented by treatment with MR antagonist, indicating that adipocyte-specific MR activity affects the expression of pro-inflammatory adipokines and macrophage recruitment and plays a crucial role in generating the chronic inflammatory profile observed in obesity (Guo *et al*. 2008, Hirata *et al*. 2009). Production of reactive oxygen species (ROS) is higher in adipose tissue of obese mice and increased levels of ROS in adipose depots have been shown to cause metabolic dysfunction and accumulation of fat (Furukawa *et al*. 2004). Interestingly, altered expression of NADPH oxidase subunits, involved in ROS production, is prevented by MR antagonism, indicating an additional protective effect of MR blockade on adipocyte function (Hirata *et al*. 2009). However, H_2O_2 treatment of 3T3L1 adipocytes (Hirata *et al*. 2009) increases, by itself, MR transcript levels, suggesting that MR activity is amplified by oxidative stress and plays a role in reinforcing ROS production in the dysfunctional adipocyte.

Although these studies demonstrate that enhanced MR activity contributes to the dysregulation of adipocyte function, a recent study by Kuhn *et al*. (2014) found that transgenic mice globally overexpressing human MR, in comparison with WT mice, display resistance to high-fat-diet-induced weight gain, protection from fat mass expansion and impaired glucose tolerance, thus showing beneficial metabolic effects similar to those observed in obese mice treated with MR antagonists (Hirata *et al*. 2009, Wada *et al*. 2010, Armani *et al*. 2014*a*). These results may appear contradictory to the current understanding of adipocyte MR, but the study mentioned above is focused on a different model in which global overexpression of MR triggers an integrated adaptive response. Interestingly, adipose tissue of these transgenic mice showed decreased adipocyte size as compared to WT mice, suggesting that MR overactivation might inhibit adipocyte maturation, resulting in unexpected effects on adipogenesis. However, the authors showed that adipocyte cultures from mice

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overexpressing MR did not present defective adipogenesis, excluding the possibility that adipocyte MR overexpression may lead to intrinsic defects in adipocyte differentiation. On the other hand, conditioned media from cultures of MR overexpressing macrophages impaired *in vitro* adipogenesis, suggesting that secretory molecules released from macrophages with enhanced MR activity may impair adipogenesis also *in vivo*. Notably, together with the effects on adipocyte maturation, changes in M1 (pro-inflammatory)/M2 (anti-inflammatory) polarization of the MR-overexpressing macrophages, were observed in these transgenic mice, raising the possibility of a causal association between macrophage polarization and extent of adipocyte differentiation.

MR activity in the macrophage is known to influence its polarization (Usher *et al*. 2010). Interestingly, mice with macrophage-specific deletion of the MR gene display increased M2 polarization and cardio-protective effects; however, the metabolic profile and adipose tissue function of these mice has still not been investigated (Rickard *et al*. 2009, Usher *et al*. 2010). Indeed, as discussed by Marzolla *et al*. (2014), regulation of macrophage polarization by MR might have a role in regulating development, inflammatory state and insulin sensitivity of adipose tissue.

Role of MR in 'browning' of white adipose tissue

In rodents and newborn humans, white and brown adipose tissue (WAT and BAT, respectively) represent two distinct types of adipose tissue with different morphologies and functions (Cinti 2012). Whereas WAT is deputed to store energy in the form of triglycerides, the BAT function is to burn fat and convert chemical energy into heat for thermogenesis (Seale *et al*. 2009, Cinti 2012).

In addition to classical BAT, recent studies have discovered in mouse WAT the presence of inducible brown-like adipocytes termed 'beige' or 'brite' (brown in white) (Kajimura *et al*. 2010). These cells appear in WAT of mice exposed to cold or treated with β3-adrenergic agonists (Kajimura *et al*. 2010). The beige adipocytes have morphology with multilocular lipid-droplet and a high content of mitochondria and express a number of genes, including *UCP1, Cidea* and *PGC1α*, that are known to be expressed in BAT adipocytes (Harms & Seale 2013). Notably, although the beige adipocytes display thermogenic activity, these cells do not originate from myogenic factor 5 (myf5)-positive embryonic precursors, from which BAT and skeletal muscle derive, and should be considered as a distinct subtype of adipose cells (Seale *et al*. 2008). Interestingly, β-adrenergic stimulation has been shown to increase UCP1 levels and uncoupled respiration rate of beige adipocytes, leading to the acquisition of brown fat features by WAT (Wu *et al*. 2012), a process known as 'browning.' However, whether the beige cells represent a specific pool of adipose cells distinct from white adipocytes, or derive by conversion of mature white adipocytes to brown-like adipose cells, still remains unclear (Rosenwald *et al*. 2013). A number of studies shows that browning protects mice from weigh gain, fat mass expansion and associated dysregulation of glucose and lipid metabolism (Harms & Seale 2013). Importantly, recent data have suggested that brown adipocytes can be detected also in adult human subjects and its amount is inversely correlated with BMI, suggesting a potential protection against obesity (Wu *et al*. 2012, 2013). Therefore, induction of browning by appropriate pharmacological treatment may represent an innovative approach to treat obesity and metabolic syndrome. In this context, a recent study by our laboratory has shown that treatment of mice fed a high-fat-diet (HFD) with MR antagonists induces brown-typical features in WAT and confers protection against obesity and impaired glucose tolerance (Armani *et al*. 2014*a*). Increased expression of brown adipocyte-specific genes, including UCP1, was detected both in white fat depots from mice and in murine primary adipocyte cultures treated with MR antagonists. These data are consistent with findings from previous studies that showed a reduction of UCP1 transcripts in brown adipocyte cell line T37i treated with aldosterone (Viengchareun *et al*. 2001).

Importantly, our study shows that MR blockade promotes brown fat marker gene expression in white adipocyte precursor cells and concomitantly attenuates the levels of transcripts highly expressed in the white adipocyte, suggesting opposite effects of MR antagonism on white and brown adipogenesis (Armani *et al*. 2014*a*). However, there is still no evidence that MR may exert opposing effects on WAT- and BAT-specific gene expression through direct action on regulatory elements. Time-course analysis of gene expression might provide some hints about mechanisms of gene regulation mediated by MR. We speculate that MR signaling pathways in the adipocyte might modulate the expression of transcription factor(s) or transcriptional coregulators affecting adipocyte gene transcription profiles (Fig. 1).

Clearly, further studies are needed to investigate the molecular mechanisms by which MR affects the transcriptional program driving white and brown adipocyte differentiation.

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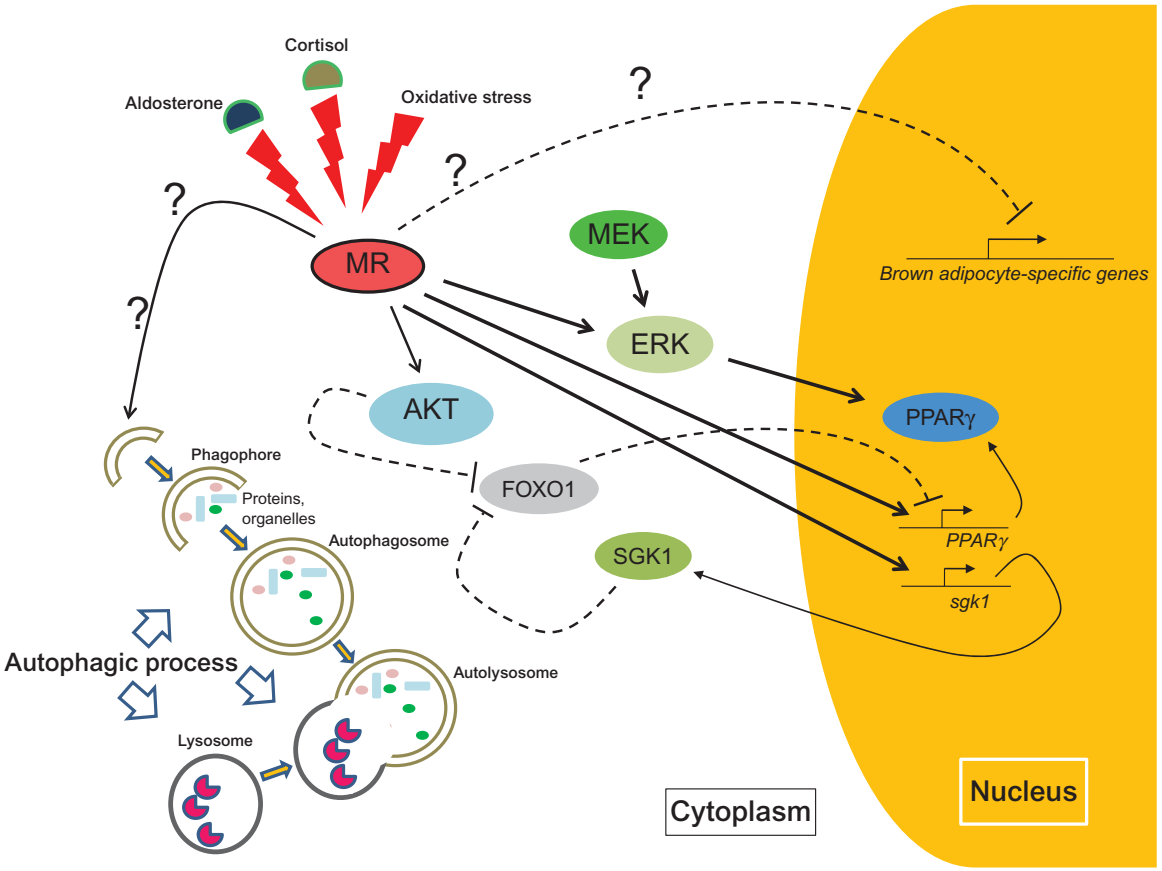

Figure 1

MR regulates adipogenesis-linked signaling pathways. In the adipocyte, MR activation by either aldosterone, cortisol or oxidative stress results in direct and non-direct transcriptional effects. MR activity promotes the autophagic flux and adipocyte differentiation. MR blockade has been shown to induce brown adipocyte-specific genes; MR induces transcription of PPARγ, the master regulator of adipocyte differentiation, and sgk1. SGK1 has been shown to phosphorylate and prevent nuclear translocation of FOXO1 and its anti-adipogenic function. Phosphorylation of FOXO1 by AKT also leads to block FOXO1 inhibitory effect on PPARγ expression. Indirect actions of MR include activation of AKT and ERK. Both of these protein kinases are required for adipocyte differentiation and may mediate the pro-adipogenic effects of MR.

Regulation of autophagy by MR in the adipocyte

Autophagy is a process allowing eukaryotic cells to regulate organelle and protein turnover and maintain tissue homeostasis (Mizushima & Levine 2010). The autophagic process consists in a complex sequence of subsequent steps beginning with the formation of the 'phagophore,' which expands and sequesters organelles and proteins in a double-membrane autophagosome. Fusion of the autophagosome with the lysosome leads to degradation of the contents of the autophagosome; the resulting degradation products move out to the cytoplasm where they can be used to build proteins and macromolecules (Mizushima 2007, Glick *et al.* 2010). Increased autophagosome abundance has been observed during 3T3L1 preadipocyte differentiation and enhanced autophagic flux has been detected in primary mouse embryonic fibroblasts (MEFs) induced to differentiate into adipocytes (Novikoff *et al.* 1980, Baerga *et al.* 2009). Among the autophagy-related genes (atg) identified in mammals, atg7 and atg5 roles have been studied both *in vivo* and in cell culture (Singh *et al.* 2009, Baerga *et al.* 2009). atg7 functions as an E1 ubiquitin activating enzyme for atg12 conjugation to atg5; atg5-atg12 conjugate formation is required for autophagosome formation (Glick *et al.* 2010).

Notably, knockdown of atg7 in 3T3L1 adipocytes represses differentiation (Singh *et al.* 2009) and atg5−/− MEFs show defective adipogenesis, altogether indicating a pivotal role of autophagy in adipocyte differentiation (Baerga *et al.* 2009).

Adipocyte-specific atg7-knockout mice have been explored in two distinct studies, in order to investigate

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the effects of impaired autophagy in adipose tissue. These models show resistance against HFD-induced obesity and display improved insulin sensitivity (Singh *et al*. 2009, Zhang *et al*. 2009). Interestingly, Singh *et al*. show that these transgenic mice have reduced WAT size and an increased amount of interscapular BAT. Moreover, WAT in these knockout mice display histological features of BAT: smaller adipocyte size, adipocytes with multilocular lipid droplets and higher number of mitochondria. Moreover, WAT showed increased protein levels of uncoupling protein 1 (UCP1) and PPARγ coactivator 1alpha (PGC1α), a master regulator of mitochondrial biogenesis, and higher abundance of the mitochondrial enzymes cytochrome oxidase and cytochrome *c*. Singh *et al*. excluded the possibility that the reduced size of WAT could be due to a decreased number of adipocyte precursors whose levels were not changed in the transgenic mice, and suggested that the effects of impaired autophagy occurred after the development of white adipose cells through transdifferentiation of mature white into brown adipocytes.

Notably, although Zhang *et al*. observed that WAT of atg7 knockout mice acquires morphological features of BAT, no changes in brown adipocyte markers were detected, suggesting that autophagy inhibition in this study has beneficial effects on adipose tissue and glucose metabolism even in the absence of conversion of white adipocytes into thermogenic brown adipocytes.

Interestingly, autophagy not only affects WAT differentiation and metabolism, but also classical BAT development. BAT derives from myogenic factor 5-positive (myf5) progenitors. Transgenic mice lacking atg7 in myf5+ progenitors show loss of autophagy in BAT that displays impaired differentiation (Martinez-Lopez *et al*. 2013).

A recent study from our laboratory has shown that MR activity modulates autophagic flux in adipose cell cultures (Armani *et al*. 2014*a*). In 3T3L1 cells and primary murine adipocytes, MR activation by aldosterone treatment increases autophagy, whereas MR blockade reduces it (Fig. 1). In mice fed HFD, pharmacological blockade of MR reduces adipose tissue autophagic flux and, in turn, induces browning of WAT, confirming the effects of impaired autophagy observed in WAT of atg7 knockout mice (Armani *et al*. 2014*a*).

Importantly, autophagy is increased in adipose tissue of obese humans and correlates with the degree of visceral adiposity and adipocyte hypetrophy, and elevation of autophagic rate has been detected in adipose tissue of subjects with insulin resistance (Kovsan *et al*. 2011).

All these data suggest that dysregulation of autophagy may lead to dysfunctional adipocytes. Indeed, the ability of MR to modulate adipocyte autophagic flux confirms the key role of this transcription factor in regulating adipose tissue function.

Role of MR in regulating adipogenesis-linked signaling pathways

MR regulates adipocyte differentiation and metabolism mostly through regulating gene transcription (Yang & Young 2009). Such modulation may occur both via direct binding on regulatory regions of target genes or through regulation of protein kinase signaling pathways. During differentiation of 3T3L1 preadipocytes MR activation has been shown to upregulate transcription of adiponectin, leptin, resistin and PPARγ (Caprio *et al*. 2007). IL-6, TNFα and MCP-1 (Guo *et al*. 2008) transcripts have been also found increased by aldosterone in terminally differentiated 3T3L1 adipocytes. Inhibition of MR transcriptional activity by the MR antagonist drospirenone (Caprio *et al*. 2011) reduces transcript levels of the pro-adipogenic genes PPARγ and CEBPα in human adipocyte cultures (Caprio *et al*. 2011). However, *in vitro* studies never explored the molecular mechanisms by which MR activates the transcription of the above-mentioned adipogenic genes, and a direct binding of MR on regulatory regions of these genes has still not been described in the adipocyte.

A consensus glucocorticoid response element (GRE) is located at position −429/−414 in the human serum- and glucocorticoid-regulated kinase 1 (*Sgk1*) promoter; chromatin immunoprecipitation (ChiP) data have shown that MR directly binds this region of the *Sgk1* gene and stimulates its transcription in embryonic kidney 293 cells (HEK293) (Lee *et al*. 2013). SGK1 has been shown to mediate aldosterone-induced Na+ reabsorption by renal epithelia (McCormick *et al*. 2005), but a role for *Sgk1* has been also demonstrated in adipocyte differentiation (Di Pietro *et al*. 2010). In fact, *Sgk1* expression increases during adipose differentiation and promotes this process through direct phosphorylation of forkhead box protein O1 (FOXO1) and subsequent exclusion of this transcription factor from the nucleus (Di Pietro *et al*. 2010). FOXO1 inhibits adipogenesis via reduced expression and transcriptional activity of PPARγ; SGK1 prevents the anti-adipogenic activity of FOXO1, counteracting its nuclear localization (Nakae *et al*. 2003).

In 3T3L1 adipocytes, transcription of *Sgk1* is induced by glucocorticoids, which are able to stimulate *Sgk1* expression through MR activation (Di Pietro *et al*. 2010). Thus, as shown in kidney cells, MR might also directly bind and promote activity of the *Sgk1* gene promoter in adipose tissue (Fig. 1).

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The mitogen-activated protein kinase/MEK/ERK pathway has been recently confirmed as a fundamental regulator of adipocyte metabolism (Banks *et al*. 2015). MEK is a dual threonine and tyrosine recognition kinase that phosphorylates and activates ERKs, a family of serine/threonine kinase, which in turn affects cellular proliferation and differentiation (Boulton & Cobb 1991, Pearson *et al*. 2001, Ebisuya *et al*. 2005). Preadipocytes purified from ERK1−/− mice display defective adipogenesis, indicating that ERK1 activity is required for adipocyte differentiation (Bost *et al*. 2005). Notably, adipose tissue shows increased activity of ERK in mice fed HFD and in obese human subjects with type 2 diabetes (Carlson *et al*. 2003, Bost *et al*. 2005). At a molecular level, ERK phosphorylates PPARγ at serine 273 (Banks *et al*. 2015). Such specific phosphorylation of PPARγ has been detected in obese mice, with concomitant reduced expression of adiponectin and adipsin, which in turn favor insulin sensitivity (Banks *et al*. 2015). Blockade of the phosphorylation at this serine residue by MEK inhibitors results in beneficial effects on adipocyte metabolism. In fact, obese *ob/ob* mice treated with MEK inhibitor display improved glucose tolerance, increased expression of adiponectin and adipsin, and upregulation of genes involved in the induction of browning of WAT (Banks *et al*. 2015). In rat renal fibroblasts, activation of MR by aldosterone has been shown to activate ERK (Nagai *et al*. 2005) and treatment with the MR antagonist eplerenone blocks aldosterone-induced ERK activation, indicating specific involvement of MR in ERK modulation and suggesting potential regulation of a fundamental metabolic pathway in the adipocyte (Fig. 1).

Among the adipogenic factors commonly used in the induction of adipogenesis *in vitro*, insulin promotes the adipose conversion process through activation of AKT protein kinase (Cho *et al*. 2004, Armani *et al*. 2010). Notably, ectopic expression of activated AKT is able to induce differentiation of 3T3L1 preadipocytes (Gagnon *et al*. 1999) and, in mice, disruption of insulin signaling in adipose tissue leads to reduced fat mass (Bluher *et al*. 2002). In human macrophage cell lines, MR activation has been shown to increase AKT activity (Lin *et al*. 2014). It is tempting to speculate that, if such mechanism occurs also in differentiating preadipocytes, increased AKT activation by MR might display relevant effects on adipogenesis (Fig. 1).

Importantly, aldosterone treatment in vascular smooth muscle cells (VSMCs) has been shown to cause proteosomal degradation of insulin receptor substrate1 (IRS1), a signaling adaptor for insulin (Taniguchi *et al*. 2006), with subsequent reduction in insulin-induced AKT activation and glucose uptake (Bender *et al*. 2013). Thus, MR activity might also trigger adverse effects on insulin signaling. In 3T3L1 adipocytes, aldosterone treatment reduces IRS1 protein levels with subsequent impaired insulin-induced glucose uptake. Co-treatment with eplerenone does not change IRS1 protein abundance, whereas the GR antagonist RU486 prevents such degradation, suggesting that MR activity is not responsible for changes in the IRS1 protein (Wada *et al*. 2009).

Clearly, a deeper understanding of the effects of MR activity on IRS1 protein levels and on insulin signaling in adipose tissue *in vivo* will require further investigations.

Modulation of MR activity by adipogenic signaling pathways and post-translational modifications

Modulation of MR activity can occur through post-translational modification by protein kinases and enzymes involved in adipogenic pathways (Faresse 2014). Among the transcription factors involved in adipogenesis, a relevant role for CREB has been demonstrated. Adipogenesis is promoted by CREB, whose phosphorylation/activation stimulates expression of adipocyte genes such as phosphoenolpyruvate carboxykinase (PEPCK), fatty acid binding protein (FABP), fatty acid synthase (FAS), CEBP-β and -δ (Reusch *et al*. 2000). cAMP-dependent protein kinase A (PKA) is able to phosphorylate CREB and, in turn, stimulate adipocyte differentiation (Zhang *et al*. 2004, Yang *et al*. 2008, Doorn *et al*. 2012), although other evidence suggests that PKA activation suppresses adipogenesis (Li *et al*. 2008).

In HepG2 hepatocytes, PKA has been shown to increase MR binding to promoters containing GRE elements and its transcriptional activity, hence showing synergistic effect with aldosterone (Massaad *et al*. 1999). Interestingly, phosphorylation of MR by PKA has not been detected in these cells; these data suggest an indirect effect of PKA on MR transactivation, probably through interaction with transcriptional repressors/co-activators of MR. (Massaad *et al*. 1999). We cannot exclude that similar regulatory mechanism involving these factors might promote MR transactivation and stimulate transcription of MR-induced genes in the adipocyte (Fig. 2).

However, phosphorylation of MR has been detected in renal cells, cardiomyocytes and adipocytes (Galigniana 1998, Desarzens *et al*. 2014). Aldosterone itself is able to induce phosphorylation of MR on serine residues located in the N-terminal domain with subsequent proteosomal

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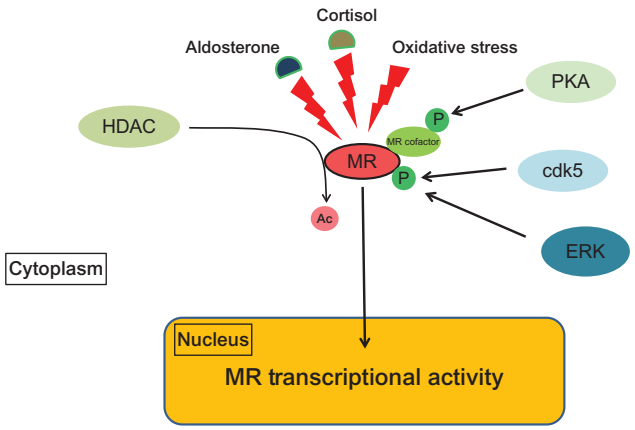

Figure 2
Potential modulation of adipocyte MR activity by post-translational modifications. The phosphorylation and acetylation state of MR affects its transcriptional activity. MR hyperacetylation leads to reduced transcriptional activity of MR. Direct phosphorylation of MR by ERK and cdk5 has been described, although the effects on the transcriptional activity are not still clear. PKA activity has also been shown to promote MR transactivation.

degradation of the receptor (Faresse *et al.* 2012). Such phosphorylation occurs in the ligand-bound form of MR and is prevented by ERK1/2 inhibition, suggesting that ERK can also regulate MR signaling through its direct phosphorylation (Fig. 2).

In differentiating preadipocytes, the phosphorylated form of MR is observed at day 9 of differentiation and its detection is prevented by treatment with geldanamycin, an inhibitor of Hsp90, which inhibits MR transcriptional activity (Bamberger *et al.* 1997, Desarzens *et al.* 2014). Importantly, treatment of preadipocytes with geldanamycin during differentiation impairs their conversion in mature adipose cells. These data suggest that loss of phosphorylation may alter MR transcriptional activation and, in turn, the adipogenic process. In intercalated cells of the distal nephron, phosphorylation of MR was detected at serine 843 in the ligand-binding domain and prevented ligand binding, nuclear translocation and transcriptional activity. Angiotensin II and with-no-lysine kinase 4 (WNK4) signaling inhibit dephosphorylation of this residue and promote MR activation (Shibata *et al.* 2013). However, the occurrence of phosphorylation at serine 843 and its effects on MR functions have never been investigated in adipose cells.

Interestingly, in cancer cells, phosphorylation of MR can also occur through cyclin-dependent kinase 5 (*Cdk5*), leading to reduced transcriptional activity of the receptor (Kino *et al.* 2010). In the adipocyte, *Cdk5* has been shown to phosphorylate the adipogenic master regulator PPARγ on serine 273, with subsequent altered expression of genes encoding adiponectin and insulin-sensitizing adipokines (Choi *et al.* 2010). However there is still no evidence that *Cdk5* is able to phosphorylate the MR directly in the adipocyte, and this aspect needs further studies.

Adipogenesis is a complex process regulated by the sequential cascade of transcription factors whose function can be modulated by the modification enzymes histone acetyltransferases and histone deacetylases (HDACs). HDACs do not only modulate the acetylation of histones but also of non-histone proteins such as DNA binding transcription factors, steroid receptors, transcription coregulators and chaperone proteins (Glozak & Seto 2007). Recent data by Kuzmochka *et al.* (2014) show that inactivation of HDAC1 promotes adipose conversion. However, other studies indicate that inhibitors of HDACs repress expression of the late markers of differentiation CEBPα (Catalioto *et al.* 2009) and aP2 (Haberland *et al.* 2010) and curb adipogenesis. Thus, there is still controversy about the effects of HDACs activity on adipogenesis. In HEK293 cells, MR acetylation is regulated by HDAC3, and treatment with valproic acid, a HDAC inhibitor, leads to an increase in acetylation and decreased recruitment of MR on the promoter of the target gene *Sgk1* (Lee *et al.* 2013). A similar modulation of MR activity by HDAC3 might occur in the adipocyte (Fig. 2), and affect MR ability in regulating *Sgk1* and other target genes that drive adipogenesis, finally regulating adipocyte metabolism.

Conclusion

Adipocyte MR activity has been shown to regulate the expression of genes involved in adipogenesis and inflammation, both *in vivo* and *in vitro*. However, it is still unclear if MR can directly activate the transcription of target genes or if its effects are mediated through recruitment and activation of other transcription factors on the promoters of MR-regulated genes. Indirect transcriptional regulation of MR activity may include protein kinases-controlling adipogenesis, insulin sensitivity and browning of WAT; notably, modulation of MR transcriptional activity by post-translational modification of the receptor could occur, adding a further level of complexity to MR function.

In obese mouse models, treatment with MR antagonists results in several beneficial effects on the metabolic profile. At a molecular level, in the adipocyte, MR antagonism inhibits transcription of adipogenic and inflammatory genes, downregulates transcript levels of enzymes producing ROS, promotes transcription of brown

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fat genes and reduces the autophagic flux. However, the molecular mechanisms downstream of the MR antagonism, which lead to the marked changes in the transcriptional profile of adipose tissue, are still largely unknown.

Indeed, a deeper understanding of the signaling pathways regulated by MR in the adipose tissue may lead to the discovery of novel molecular targets for therapies to treat obesity and its related metabolic dysfunctions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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