Vasa 2013; 42: 8–16

Influence of glucose metabolism on vascular smooth muscle cell proliferation

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Summary

Differentiation of vascular smooth muscle cells (VSMC) is an essential process of vascular development. VSMC have biosynthetic, proliferative, and contractile roles in the vessel wall. Alterations in the differentiated state of the VSMC play a critical role in the pathogenesis of atherosclerosis and intimal hyperplasia, as well as in a variety of other human diseases, including hypertension, asthma, atherosclerosis and vascular aneurysm. This review provides an overview of the current state of knowledge of molecular mechanisms involved in controlling VSMC proliferation, with particular focus on glucose metabolism and its relationship with mitochondrial bioenergetics. Increased levels of glucose transporter 1 (GLUT1) are observed in VSMC after endothelial injury, suggesting a relationship between glucose uptake and VSMC proliferation. Mitochondrial dysfunction is a common feature in VSMC during atherosclerosis. Alterations in mitochondrial function can be produced by dysregulation of mitofusin-2, a small GTPase associated with mitochondrial fusion. Moreover, exacerbated proliferation was observed in VSMC from pulmonary arteries with hyperpolarized mitochondria and enhanced glycolysis/glucose oxidation ratio. Several lines of evidence highlight the relevance of glucose metabolism in the control of VSMC proliferation, indicating a new area to be explored in the control of vascular pathogenesis.

Key words: Vascular smooth muscle cells, proliferation, glucose metabolism, mitochondrial bioenergetics

Zusammenfassung

Einfluss des Glukosestoffwechsel auf die Proliferation vaskulärer glatter Muskelzellen

Die Differenzierung vaskulärer glatter Muskelzellen (VSMC) ist ein wesentlicher Prozess der vaskulären Entwicklung. VSMC haben biosynthetische, proliferative und kontraktile Eigenschaften in der Gefäßwand. Störungen des differenzierten Zustands der VSMC spielen eine entscheidende Rolle in der Pathogenese der Atherosklerose und Intimahyperplasie, sowie bei einer Vielzahl anderer Erkrankungen wie arterielle Hypertonie und vaskuläre Aneurysmata. Dieser Artikel gibt einen Überblick über den aktuellen Stand der Kenntnisse der molekulären Mechanismen, die die VSMC-Proliferation kontrollieren, mit Schwerpunkt auf den Glukosestoffwechsel und dessen Beziehung zu der mitochondrialen Bioenergetik. Eine Verletzung des Endothels erhöht den Glukosetransporter 1 in den VSMC, so dass von einer Beziehung zwischen Glukoseaufnahme und VSMC-Proliferation ausgegangen werden kann. Im Prozess der Atherosklerose ist die Mitochondrien-Dysfunktion ein gemeinsames Merkmal: es können zum Beispiel Änderungen in der mitochondrialen Funktion durch eine Fehlregulation des Mitofusin-2 (ein kleines GTPase mit einer wichtigen Rolle in der mitochondrialen Fusion) gefunden werden. Darüber hinaus wird eine erhöhte Proliferation von VSMC der Lungenarterien mit hyperpolarisierten Mitochondrien und einem verbesserten Verhältnis von Glykolyse und Glukoseoxidation beobachtet. Mehrere Befunde weisen auf die Bedeutung des Glukosestoffwechsels bei der Steuerung der VSMC-Proliferation hin, was einen neuen Forschungsschwerpunkt eröffnet.

Introduction

Vascular smooth muscle cells (VSMC) are the main component of the artery's medial layer. These cells undergo contraction and thereby regulate blood vessel tone and consequently blood flow and pressure. VSMC contraction depends on the interaction between smooth muscle (SM)-α-actin, β-myosin heavy chain, h-caldesmon and calponin [11, 45]. VSMC also possess important secretory properties that ensure synthesis and repair of extracellular matrix components and regulate the structure of the vascular wall [11]. Healthy VSMC are not terminally differentiated cells with very low rates of proliferation and secretion [11, 45]. However, changes in the VSMC phenotype have been extensively described in the development and progression of atherosclerosis, hypertension and neointimal formation [10, 11, 45]. This phenotypic switching includes

AMPK: AMP activated protein kinase; cFLIP: c-FLICE inhibitory protein; GLUT: glucose transporter proteins; Grb-2: growth factor receptor-bound protein 2; GSK-3: glycogen synthase kinase-3; IGF-1: insulin-like growth factor-1; IGF-1R: IGF-1 receptor; IR: insulin receptor; IRS: insulin receptor substrate; JNK: c-Jun N-terminal kinase; MAPK: mitogen activated protein kinase; Mfn-2: mitofusin-2; mTOR: mammalian target of rapamycin; PCNA: proliferating cell nuclear antigen; PI3K: phosphoinositide 3-kinase; PKA: protein kinase A; PASMC: pulmonary artery smooth muscle cells; SASMC: systemic artery smooth muscle cells; Shc: Src homology and collagen domain protein; VSMC: vascular smooth muscle cells.

altered expression of contractile proteins, increased matrix production, expression of inflammatory cytokines and production of proteases [10]. The capacity for contraction, proliferation, migration and secretion in VSMC is affected by a wide range of factors, including mechanical forces, contractile agonists such as angiotensin II, extracellular matrix, reactive oxygen species (ROS), endothelial-VSMC interactions, platelet derived growth factor (PDGF), transforming growth factor-β-1 (TGF-β-1), and many other growth factors [10, 11]. As a result, VSMC constitute basic structural and functional elements in the artery wall and their malfunction leads to vascular disease. The importance of correct glucose metabolism on VSMC is depicted in diabetic patients: restenosis and atherosclerosis after balloon angioplasty, stroke, coronary heart disease, and peripheral arterial disease are more common in individuals with diabetes than in the general population [6, 24], suggesting a potential link between VSMC glucose metabolism and the progression of lesion formation. Recently, VSMC glucose metabolism, and particularly mitochondrial bioenergetics have been raised as part of novel mechanisms involved in the complex regulation of the VSMC phenotype, especially involving VSMC proliferation [48, 49]. Therefore, the focus of this review will be unifying current knowledge on glucose metabolism and related signaling pathways associated to VSMC proliferation with mitochondrial function. Unless otherwise indicated, the term VSMC will refer to conductance vessels-derived smooth muscle cells.

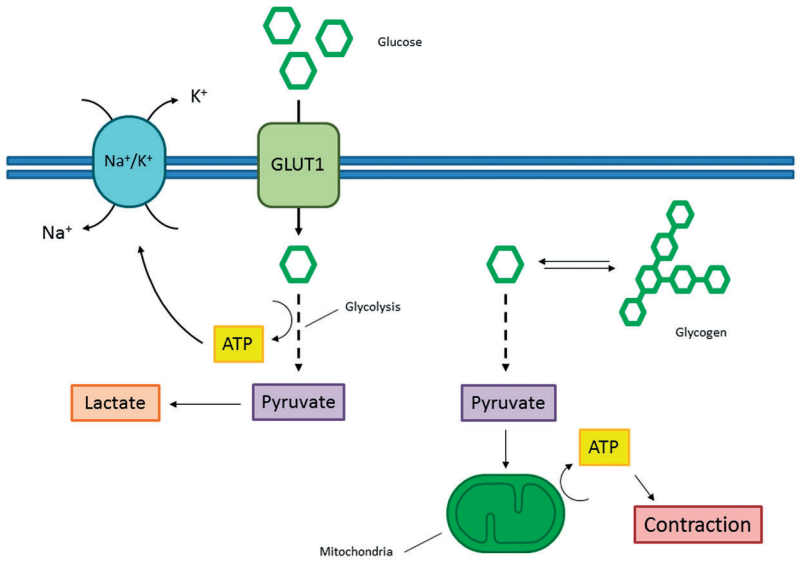

Figure 1: Compartmentalization of ATP generated by glycolysis and respiration. VSMC from porcine coronary artery show high rates of glycolysis with high lactate formation even under oxygenated conditions [9]. ATP derived from this process is required for Na^+ and K^+ transport and is obtained mainly from glucose taken from the medium. On the other hand, ATP derived from oxidative metabolism in the mitochondria is used for the contractile mechanism using glycogen as its main substrate [36].

Glucose metabolism and VSMC proliferation

Glucose metabolism is a key player in vascular reactivity [27]. VSMC exhibit unusually high rates of glucose utilization and lactate production under normal, well-oxygenated conditions [9]. In fact, under resting conditions, the rate of oxygen consumption and lactate production are often almost equal on a molar basis, resulting in approximately 30 % of the ATP supply coming from "aerobic glycolysis", but at least 90 % of the flux through glycolysis resulting in lactate production[36]. This enhanced lactate production conditions seems not to be associated with inadequate tissue oxygenation or a limitation in the oxidative capacity of the muscle [9]. Paul et al. postulated that, under fully oxygenated conditions, glycolysis with lactate formation provides the ATP required for Na^+ and K^+ transport across plasmatic membrane, while oxidative metabolism is the energy source for the contractile machinery on SMC from porcine coronary arteries, suggesting functional "metabolic destination" of glucose [36]. Furthermore, the same group has demonstrated compartmentalization of glycolysis and glycogenolysis in VSMC, such that glucose taken up from the medium appears as lactate, whereas glycogen is preferentially oxidized [28]. These data are consistent with the idea that ATP generated from glycolysis and from respiration could provide the energy supply for different processes. Such compartmentalization could result from an association of the glycolytic enzymes with the Na^+/K^+ pump (Figure 1) [36]. Another possibility is that creatine kinase might be localized near the contractile elements so that phosphocreatine generated by respiration through the phosphocreatine shuttle mechanism is preferentially used for actin-activated myosin ATPase activ-

ity [5]. The work of Hellstrand et al. [17] on the rat portal vein supports this hypothesis in general, but suggests that the compartmentalization is not absolute.

Glucose transport is an important step in glucose metabolism and is controlled through a family of glucose transporter proteins (GLUT1-GLUT14), with GLUT1 being the predominant isoform in VSMC [23]. These cells also express the insulin-responsive glucose transporter GLUT4, which exhibits a significant insulin-responsive glucose uptake similar to that of skeletal muscle and adipose tissue [2]. Besides the classical insulin receptor substrate 1 (IRS1)/phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, Bergandi et al. have shown that in human VSMC derived from microarterioles, insulin also elicites glucose transport and GLUT4 recruitment into the plasma membrane mediated by an increased synthesis of NO, which stimulates the production of cGMP and the subsequent activation of a cGMP-dependent protein kinase [4]. Few studies have examined the regulation of GLUT1-mediated glucose uptake in VSMC. Activation of the PI3K/Akt pathway by growth factors induced phosphorylation and inactivation of both glycogen synthase kinase 3-β (GSK-3) and tuberous sclerosis complex 2 (TSC2) [8]. Chronic inhibition of GSK-3-β resulted in increased GLUT1 levels and enhanced glucose uptake on the aortic SMC line A7r5 [8]. Conversely, when the PI3K/Akt pathway is inactivated, that is, on withdrawal of growth factors, GSK-3 and TSC2 are activated resulting in TSC2-mediated inhibition of Rheb, which, in turn, inactivates the mammalian target of rapamycin (mTOR), leading to decreased GLUT1 content as well as decreased glucose uptake [8].

VSMC, accumulated in the neointima after endothelial injury, have increased GLUT1 expression [16], increasing the contribution of glucose to lactate and acetyl-CoA formation, while glucose incorporation into glycogen did not change [16]. Moreover, increased GLUT1 expression in VSMC reduces apoptosis and induces proliferation by changing the expression of c-FLICE inhibitory protein (cFLIP) [53]. Vesely et al. showed that induction of cFLIP is essential for the antiapoptotic and cell proliferative effects in GLUT1-overexpressing VSMC subjected to serum withdrawal (Figure 2) [53].

Transgenic mice overexpressing GLUT1 in VSMC show a significant increase in glucose uptake in VSMC without altering circulating concentrations of glucose, insulin, or non-esterified fatty acids [1]. These animals showed impaired contractility of the vessel wall and, in the absence of vascular intervention, GLUT1 overexpression did not induce hypertrophy or proliferation of VSMC. In response to vascular injury, increased inflammation and medial hypertrophy was, however, detected [1].

Exposure to high extracellular concentrations of glucose in vitro induces VSMC proliferation and increases growth rates [18, 35]. The enhanced cell proliferation is through increased DNA synthesis, which results in acceleration of the cell cycle by stimulating the progression of cells from G1 to S/M phases [18]. This effect could be due, at least in part, to activation of aldose reductase [51] which is induced by hyperglycaemia (Figure 2) [41]. In agreement with that, Suzuki et al. showed that the stimulatory effect of diabetes in vivo on VSMC proliferation and accumulation is also likely to be mediated by altered plasma lipid profiles or indirectly through other cell types present in the lesion, rather

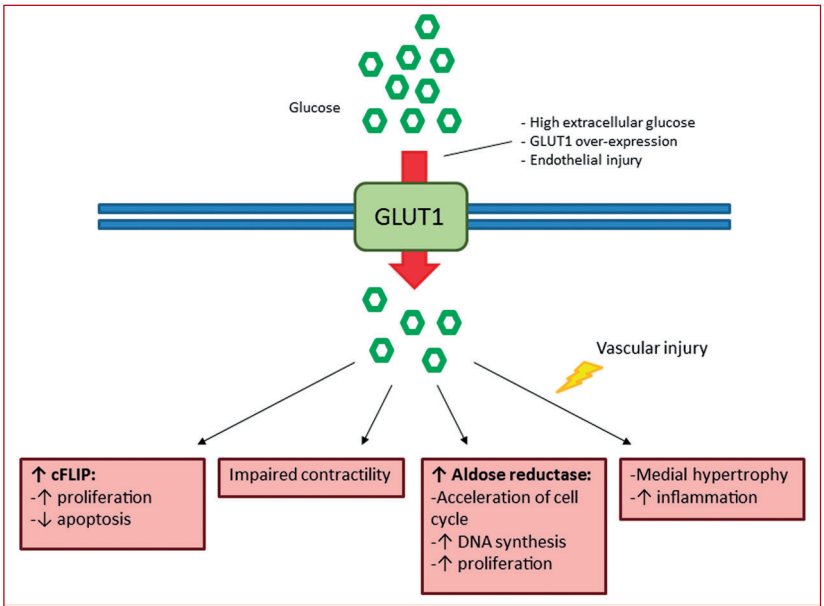

Figure 2: Effect of increased glucose availability in cell proliferation. An increase in glucose uptake due to augmented extracellular glucose or GLUT1 levels, leads to VSMC proliferation and impaired contractility [16]. Two molecular mechanisms that mediate this process have been identified: the induction of cFLIP [53] and the activation of aldose reductase [51]. However, in vivo studies showed that an initial vascular injury is required to trigger these effects [1].

than by a direct growth-promoting effect of hyperglycaemia [50], suggesting that high glucose does not directly stimulate proliferation in the absence of injury [50].

Hyperglycaemia and IGF-1/insulin signaling

Atherosclerosis is more common in individuals with type 2 diabetes than in the general population [6, 24]. VSMC proliferation, commonly observed in patients with type 2 diabetes, is probably triggered by a combined effect of hyperinsulinemia with hyperglycaemia. Due to hyperinsulinemia, the main effects of insulin on VSMC proliferation depend on the transactivation of insulin-like growth factor receptor (IGF-1R) rather than activation of insulin receptor [43, 54]. In fact, Avena et al. showed in infragenicular VSMC of diabetic patients, that insulin and glucose effect over proliferation and DNA synthesis rate are mediated through IGF-1R, but not insulin receptor [3]. This phenomena could be due to substantial structural and functional homology of IGF-1R with IR, and because IGF-1R is more abundant in VSMC than insulin receptor [20]. On the other hand, hyperglycaemia also alters the signal transduction of IGF-1R. When VSMC from porcine aorta were cultured in normal physiological glucose (5.6 mM), they respond to IGF-1 with increased protein synthesis, but they do not proliferate. In contrast, when these cells were cultured under hyperglycaemia (25 mM) the responsiveness to IGF-1 was altered, resulting in the induction of VSMC proliferation and migration [40]. Once activated, IGF-1 receptor (IGF-1R) phosphorylates downstream signaling molecules including IRS-1 and SHP substrate-1 [SHPS-1] promoting DNA synthesis and proliferation [39, 40]. IRS-1 is, however, down-regulated in response to hyperglycaemia in VSMC [39], leading to reduced tyrosine phosphorylation of IRS-1 and the subsequent Grb2 binding in response to IGF-1 [30]. In those conditions, phosphorylation of SHPS-1 predominates and the subsequent assembly of a signaling complex that includes SHP-2, Src, Shc, and Grb2 enhances the ability of IGF-1 to activate the MAPK pathway, leading to increased proliferation and migration [39].

Therefore, the imbalance between the PI3K and the MAPK pathways of insulin/IGF-1 signaling induced by hyperglycaemia may be the basis of the predominant vasoconstrictor and pro-atherogenic roles of insulin in these pathological conditions [33].

AMPK, mTOR and VSMC proliferation

The signaling pathways by which changes in glucose metabolism modulate VSMC biology are poorly understood. Recently, AMP activated protein kinase (AMPK) has emerged as a major metabolic regulator, and has been the subject of intense research into obesity, diabetes and heart failure [46]. AMPK activation leads to the down-regulation of anabolic processes and the stimulation of catabolic processes [46].

AMPK is present in VSMC and the predominant isoforms described are $\alpha1/\beta1/\gamma$ and $\alpha1/\beta2/\gamma$ [13, 19, 46]. Metabolic stress of VSMC, such as increase of the AMP/ATP ratio, challenge with 2-deoxyglucose plus anoxia induces a rapid activation of AMPK [42]. AMPK activation inhibits VSMC proliferation and migration [19, 34, 37]. Suppression of AMPK activity with AMPKα siRNA or AMPK-dominant negative augments VSMC proliferation [37]. The mechanism of growth suppression induced by AMPK involves cell cycle arrest at G1 by increasing CDKI p21CIP. This action involves the upregulation of p53, which in turn inhibits the Rb phosphorylation required for cell cycle progression [19]. Therefore, AMPK inhibition is a link between hyperglycaemia and induction of VSMC proliferation under stress conditions. One possible link between AMPK activation and its growth arrest/antiproliferative properties is its downstream target, the mTOR complex. In simplistic words, AMPK activates TSC1/2 complex, which in turn inactivates mTOR [26]. The effect of this signaling pathway on VSMC have been extensively studied since rapamycin, a specific mTOR inhibitor, was first used on drug-eluting stents and was demonstrated to decrease the incidence of restenosis [33]. Rapamycin inhibits VSMC migration and proliferation in vitro and intimal hyperplasia in vivo and also induces differentiation in cultures of synthetic VSMC [34]. A critical step in VSMC proliferation and migration is the down-regulation of the cyclin-dependent kinase inhibitor, p27Kip1. Elevated levels of this protein arrest VSMC in the G1-phase, block proliferation, and inhibit cellular migration [35, 36]. Inhibition of mTOR by rapamycin induces inactivation of P70S6K and eIF4E, increasing the activity of p27Kip1 and RB, subsequently resulting in inhibition of VSMC proliferation [37, 38].

Mitochondria and VSMC proliferation

VSMC proliferation and apoptosis might be subject to mitochondrial control through the action of the large dynamin-related GTPase mitofusin-2 (Mfn-2, also called HSG), a mitochondrial fusion protein. Chen et al. showed that Mfn-2 diminishes in highly proliferative aorta VSMC from atherosclerosis-prone or bal-

loon-injured rats and that Mfn-2 overexpression blocks proliferation of neointimal VSMC after balloon injury [12]. Moreover, Mfn-2 overexpression also suppresses the proliferating effects of oxidized-LDL in rabbit VSMC cultures. The induction of Mfn-2 in vivo reduces proliferating cell nuclear antigen (PCNA) positive cells at the neointimal and medial layers from rabbit carotid arteries subjected to air-drying damage [15]. These data correlate with the fact that overexpression of Mfn-2 promotes mitochondrial-mediated apoptosis in VSMC cultures, and that Mfn-2 is up-regulated and necessary for proxide-induced apoptosis albeit in a mitochondrial fusion-independent mechanism [14]. This anti-proliferative activity of Mfn-2 can be negatively regulated by protein kinase A (PKA) as shown by the decreased PCNA positive cells and neointimal hyperplasia after balloon injury on rats with overexpression of a Mfn-2 S422A mutant form (a variant that cannot be phosphorylated on Ser422, the residue within the PKA-phosphorylation consensus site) [56]. The authors suggest that the anti-proliferative/pro-apoptotic activity of Mfn-2 might be because of down-regulation of the Raf/MAPK pathway or control over apoptotic-related proteins, given that cells overexpressing Mfn-2 exhibit lower levels of ERK-1/2 and Akt in response to certain hormones [12, 14, 15] and an increased Bax/Bcl-2 ratio (Figure 3) [14].

During hypoxia most blood vessels relax but the pulmonary arteries constrict, ultimately becoming occluded by excessive SMC proliferation, a condition that causes pulmonary hypertension (PAH). This is in part because the mitochondria of the pulmonary artery smooth muscle cells (PASMC) are different from those of the systemic arterial smooth muscle cells (SASMC). SASMC and PASMC have different levels of electron transport chain proteins, antioxidant enzymes, respiration rates, and mitochondrial membrane potential [32]. During the incipient pulmonary hypertension, the mitochondria from PASMC become hyperpolarized and generate less reactive oxygen species, indices of a shift in metabolism. In wild-type mice, these changes are accompanied by an excessive PASMC proliferation, decrease in glucose oxidation and increased glycolysis [48]. When fatty acid oxidation is abolished by deletion of the gene for malonyl-coenzyme A decarboxylase, thereby shifting the metabolic balance back to glucose oxidation, the mice do not develop pulmonary hypertension and less or no PASMC proliferation is observed [48]. Sutendra et al. suggest that the shift from glucose oxidation towards glycolysis and fatty acid usage in the PASMC during hypertension is accompanied by alterations in mitochondrial function [48]. These changes could be responsible for the excessive proliferation of PASMC (Figure 3).

Recent work from our group indicates that the association between two organelles such as endoplasmic reticulum and mitochondria regulate mitochondrial energetic function, especially as a novel adaptative mechanism during ER stress [7]. Interestingly, when the association of both organelles is inhibited, by a reduced expression of the protein Mfn-2, the metabolic changes are abrogated [7]. When ER stress is induced by chronic normobaric hypoxia in mice PASMC, mitochondrial Ca^{2+} and 2-oxoglutarate content and pyruvate dehydrogenase activity were decreased, depicting mitochondrial malfunction under these conditions [49]. Sutendra et al. show that the Nogo-B protein is activated by hypoxia only in lung vessels, where it disrupts the contacts between the ER and the mitochondria [49]. This alteration disrupts essential mitochondrial functions, causing overgrowth of PASMC and ultimately PAH. The protein Nogo controls the shape of the ER, forming its tubes and tunnels, and inhibits apoptosis during vascular remodeling [49].

Conclusions

VSMC proliferation plays a key role in atherogenesis and restenosis. Insulin signaling, high glucose level, increased glucose oxidation, AMPK inactivation and mitochondrial dysfunction are metabolic features that control VSMC phenotype. Clinical therapy targeting of mTOR with rapamycin [25, 38, 47], mitochondrial function with dichloroacetate [31] or trimetazidine [48] has been successfully been used to avoid VSMC or PASMC proliferation, mostly in animal models. To our knowledge, there are few studies on human patients that elucidate VSMC proliferation mechanism. Even when clinical analyses suggest a clear association between diabetes/insulin resistance and VSMC phenotype, there are not cause-effect studies regarding this issue [3]. For instance, Creager's group showed in the 90's that diabetic patients had lower vasodilation responses to different agents, such as metacholine and the NO-donor nitroprusside, with some differences observed in insulin-dependent and non-insulin-dependent patients, suggesting a diminished response to endothelial-derived vasomodulators [21, 55]. Other works have shown that SMC derived from saphenous vein of type 2 diabetic patients had lower proliferative response to calf serum, although enhanced migratory properties [29], and that internal mammary arteries of diabetic patients present resistance to apoptosis [44]. However, only mTOR-dependent signaling has been proven to modulate VSMC phenotype in humans, as rapamycin has been tested in human specifically in

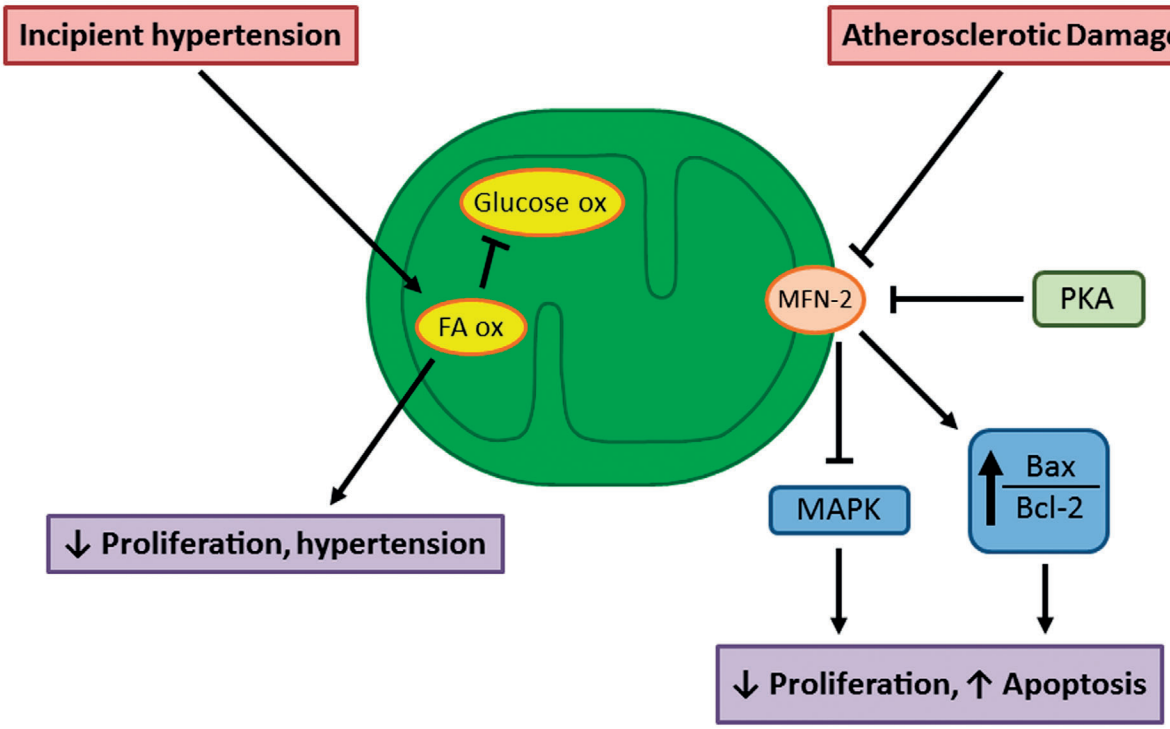

Figure 3: Mitochondrial control of VSMC proliferation. Mitochondria can control cell proliferation through their metabolic state. During incipient pulmonary hypertension a shift in metabolism occurs in the pulmonary artery smooth muscle cells, leading to an increase in fatty acid oxidation and glycolysis and a reduced glucose oxidation. This alteration in mitochondrial function causes an increase in proliferation and leads to development of hypertension [48]. Mitochondria can also control rat aorta VSMC proliferation and apoptosis through the mitochondrial protein Mfn-2. The anti-proliferative and pro-apoptotic effect is carried out by a downregulation of MAPK and a decrease in Bcl-2/Bax ratio. Atherosclerotic damage reduces Mfn-2 levels, causing an increase in VSMC proliferation. Similarly, Mfn-2 phosphorylation by PKA reduces its anti-proliferative activity [12, 56].

drug eluting stents to avoid restenosis [22, 52]. Interventions on other signaling pathways to control VSMC proliferation on humans have not yet been described.

Acknowledgments

This research was funded in part by Comision Nacional de Ciencia y Tecnologia (CONICYT), Chile (FONDECYT 1110180 to M.C.; ANILLO ACT1111 to M.C.). P.M. holds a CONICYT Master fellowship. G.T. holds CONICYT PhD fellowship.

Conflict of interest

There are no conflicts of interest existing.

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Submitted: 14.08.2012
Accepted after revision: 10.10.2012