Diabetes-Associated Kidney and Vascular Complications: Mechanisms of Disease Progression and Alternative Therapeutic Options

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Abstract

Vascular complications in diabetes are an emergent health care problem. Accelerated endothelial dysfunction in pathological settings connoted by hyperlipidaemia and hyperglycaemia is a crucial step for the development and the progression of atherosclerosis. Previous data support the central role of Advanced Glycated End-products (AGEs) and oxidation or glycation of Low Dense Lipoproteins (LDLs) in the impaired vascular remodelling associated with diabetes. Hyperglycemia, via NADPH Oxidase (NOX) enzymatic activity, upholds the production of Reactive Oxygen Species (ROS), which in turn mediate tissue damage and long-lasting “metabolic memory”. Nonetheless, in diabetic setting, ROS act as secondary messenger to strictly control stemness of visceral-derived adipose stem cells and to promote transcriptional and post-transcriptional events, also involving small non-coding microRNAs (miRs). In this article we provide an overview on the events elicited by acute and chronic hyperglycemia that account for vascular and kidney diseases. The deleterious effects of LDL and fatty acids on endothelial progenitor cells in condition connoted by hyperglycemia are also discussed. Moreover, as current therapeutic approaches failed to improve endothelial dysfunction/disease progression and consequently long-term outcomes in diabetics with vascular complications, particular attention has been devoted to describe efforts made to identify novel therapeutic options, for the management of one of the most relevant health care problems world wide. Finally, as targeting of epigenetic mechanisms is a future challenge, relevant data supporting their deep involvement in long-lasting “metabolic memory” have been also addressed.

Keywords: Diabetes; Vascular complications; Kidney disease; ROS; Epigenetics; miRNAs; UnAG

Introduction

Cardiovascular diseases are the major causes of morbidity and mortality in patients with type 2 diabetes [1]. Several lines of evidences indicate that the accelerated impairment of endothelial functions and the loss of an efficient blood vessel perfusion are crucial for the development of atherosclerosis in diabetes [2,3]. It is well established that atherosclerosis is a process, which results from interaction among plasma lipoproteins, cellular components (monocyte/macrophages, T lymphocytes, endothelial cells and smooth muscle cells, endothelial progenitor cells) and extracellular matrix [4].

Modified low density lipoproteins (particular the oxidized small dense LDL (sdLDL) act as crucial players in the initiation of the pro-inflammatory reaction which leads to vessel injury [4]. Besides abnormal LDL, the advanced glycated end-products (AGEs), resulting from long-term diabetes, are crucial determinants of diabetic vascular complications. Generation of oxidative stress and dysfunctional mitochondria, the lack of efficient antioxidant machinery, as well as the overproduction of growth factors and cytokines are mainly involved in disease progression [5-7]. In this review, we will focus on the molecular mechanisms leading to vascular damage and kidney disease associated with diabetes. Particular attention will be paid to the deleterious effects of LDL or protein oxidation or glycation and their downstream signals. The last part of the review will be focused on the mechanisms accounting for the long-lasting “metabolic memory” as well as on novel therapeutic approaches targeting oxidative stress.

Vascular Damage

Low Density Lipoproteins (LDL) and endothelial cells (ECs)

The concept of LDL oxidative modification was first built up by Steinberg et al. in 1989 [8]. From then to now a number of preclinical and clinical studies have extensively documented that qualitative (glycation and/or oxidation) and quantitative LDL abnormalities in diabetes exert a pivotal role in the development and progression of atherosclerosis [9]. Modified LDL, no longer efficiently bound by the canonical LDL receptor, acquire the ability to bind a number of scavenger receptors expressed by endothelial cells (EC) and macrophages, such as LOX-1 (oxLDL lectin like Receptor 1), CD36, SR-PSOX [10] and the receptor for AGE (RAGE) [11]. LDL binding to these non canonical receptors brings on a vast array of proatherogenic responses intimately linked to lesion progression in diabetic setting [12-14]. Of interest oxLDL are much more vulnerable to glycoxidation [15] and, as suggested by the use of RAGE-deficient murine aortic ECs, for oxLDL-mediated proatherogenic signals RAGE is crucial [16]. This implies that ROS generation elicited by binding of “AGEs” to RAGE is central in the activation of the redox-sensitive nuclear transcription factor kB (NF-kB) and the transcription of a variety of atherosclerosis-related genes, including PAI-1, tissue factor, VCAM-1, ICAM-1, MCP-1, VEGF, and RAGE itself [17-19]. Alternatively we found that small and dense LDL recovered from diabetic patients (dm-LDL), via RAGE, induce ROS generation, activate the signal transducers and activators of transcription (STAT)5B and inhibit EC cell-cycle progression [12,13]. The observation that such deleterious cue was mediated by the STAT5B...
transcription activity on the cyclin dependent kinase inhibitor p21waf [12,13] delineates a new mechanism through which RAGE can stimulate intracellular events to drive changes in gene expression.

Essential to diabetes is the development of chronic hyperglycemia resulting in AGE formation [19]. As stated above this process also involves LDL which, by undergoing AGE-modification, acquire more relevant pro-atherogenic properties and accelerate atherosclerosis. As the result of such additional qualitative abnormality, LDL switch on a cascade of intracellular events, that besides STAT5 and PKC [20,21], also include the activation of the Erk1/Erk2 MAP and Src kinases [13,22]. More recent reports have highlighted novel mechanisms in RAGE signaling. In particular Shui et al. [23] have shown that the activation of the mTOR pathway, known to be associated with cardiac hypertrophy and atherosclerosis, is also crucial for LOX-1 expression in response to AGE-mediated signals. More interestingly, they provide evidences for a novel protective effect of metformin administration that open new insight to moving forward to human studies. Indeed, metformin by interfering with the mTOR pathway was found to reduce LOX-1 expression on ECs [23].

Accumulating evidences indicate that gly-LDL by oxidative stress generation can also induce thrombogenic reactions. At this regard, Sangle et al. [24] provide evidences that gly-LDL lead to heat shock factor 1 (HSF 1)-mediated PAI 1 production. By using both in vitro and in vivo models they elegantly showed that NOX, and H-Ras/Raf-1 signaling pathways are implicated in the up-regulation of HSF1 or PAI-1 in ECs [24].

Over the last few years a number of studies have demonstrated that T and B lymphocytes and dendritic cells express RAGE [25,26], suggesting a role of RAGE in adaptive immune responses as well. Although this novel RAGE role is still debated [25,27,28], it has been suggested that ligand-mediated RAGE activation could be relevant for effective T lymphocytepriming during acute inflammatory responses. Based on the notion that long-term diabetes recapitulates chronic diseases it is conceivable to hypothesize that, in diabetes, RAGE signaling could maintain a sustained inflammation andtissue damage also by acting on immune cells [19].

An immunological response to gly and oxLDL has been also reported and seems to predict, more efficiently than traditional risk factors, vascular disease progression in diabetes [29,30]. In keeping with this observation it has been reported that the progression of diabetic retinopathy positively correlates with the level of immune-complexes containing gly- and ox-LDL [31]. As gracefully shown by the authors immune-complex binding to macrophages leads to oxidative stress, mitochondrial dysfunction and retinal pericytes apoptosis [31]. More recently, Bernal Lopez et al. [32] also demonstrated a positive correlation between the levels of anti-ox-LDL IgM and the expression of genes involved in inflammation, apoptosis, plaque disruption, lipidic metabolism and cellular turnover. However, whether this correlation simply reflects plaque instability or the inflammatory milieu of diabetic patients is still open to debate [32].

Apart from glycation and oxidation, in diabetic patients LDL can get into homocysteinisation [33]. Owing to lipoproteinglycation, LDL appear to be more susceptible to bind homocysteine derivatives, and a direct correlation between homocystam ide LDL derivates and the Hba1C levels has been reported. Thus, additional EC oxidative damage can be induced by homocystamide LDL-mediated peroxynitrite production [33].

One last word must be dedicated to the work of Pirillo et al. [34] which demonstrated how modified HDL (through the 15 lypoxigenasis) lose their protective effect and become pro atherogenic (Figure 1).

Figure 1: Schematic representation of mechanisms involved in diabetes-associated vascular damage (A) Circulating factors involved in diabetes-associated vascular damage are schematically described. Advanced glycation end-products (AGE), glycated LDL (gly-LDL), oxidized LDL (Ox-LDL), high glucose (HG) palmitic acid (PA) are crucial determinants of vascular damage. When AGE/gly-LDL interact with the receptors for AGE (RAGE) on EC, they stimulate generation of reactive oxygen species (ROS). This results in STAT5 activation and p21waf expression, leading to cell-cycle arrest, or in the activation of the nuclear factor κB (NF-κB). NF-κB in turn transcriptionally regulates its target genes: plasminogen activator inhibitor-1 (PAI-1)/vascular cell adhesion molecule-1 (VCAM-1)/intercellular adhesion molecule-1 (ICAM-1). In diabetic condition circulating endothelial progenitor cells (EPC) are reduced in number and impaired in function. AGE and Ox-LDL via RAGE down-regulate VEGF and eNOS activity. Moreover, while high palmitic acid (PA) concentrations activate STAT5/PPAR complexes leading to p21waf expression, HG up-regulates miR-221/222 expression to control p21waf, p27Kip1 and p57Kip2 content that results in EPC cell-cycle arrest. (B) Persistent hyperglycaemia induces epigenetic changes that determine the so called “metabolic memory”. The relevant mediators of epigenetic changes that sustain vascular damage are depicted: mitochondrial p66shc, sirtuin-1 (SIRT-1), p53, and Set7/9.

Hyperlipemia/hyperglycaemia and endothelial progenitor cell dysfunctions

The bone marrow pool of endothelial progenitor cells (EPCs) has attracted particular attention due to their potential clinical application [6,35]. However, EPCs are reduced in number in patients with type 1 or type 2 diabetes [36-38]. Moreover, their functional capabilities are impaired in these pathological conditions [36-38]. Of note, in diabetic patients with peripheral vascular complications a further and
progressive impairment of EPC number has been reported [37]. A number of studies indicate that besides hyperglycaemia different metabolic stress factors associated with diabetes contribute to such quantitative and qualitative abnormalities [39,40]. Among these, oxidized LDL (ox-LDL), altered fatty acids and AGE are included [36-38,41].

AGE, by binding to RAGE, induce apoptosis and impair EPC migration and tube-like structure formation by affecting VEGF-signalling pathway and eNOS activity [42]. Liang et al. [43] reported that such dysfunctional EPC phenotype could be reverted by administration of anti-AGE antibodies or resiglitazone, a well-known PPARgamma agonist [43,44]. That the inflammatory milieu, via the up-regulation of RAGE, is also crucial for AGE-mediated effects is supported by experiments performed on EPC exposed to C-reactive protein [45].

Along with ox-LDL, alteration of lipid and in particular of fatty acid (NEFA) metabolism is common in type 2 diabetes [46]. Indeed, metabolic profiling of plasma NEFAs in type 2 diabetes patients has discovered different biomarkers, including Palmitic Acid (PA) [47]. PA is a ligand for cell surface receptors and for transcription factor receptors, the PPARs. PPARs are crucial regulators of genes involved in lipid and glucose metabolism, vascular functions, and inflammation [48]. We have recently demonstrated that EPCs recovered from diabetic patients, displaying high concentrations of PA, or EPCs cultured in diabetic concentration of PA are unable to undergo cell-cycle progression [49]. We found that, as expected, diabetic concentrations of PA induce the expression of PPAR gamma. However, in this particular condition PPAR gamma binds to the promoter region of STAT5 and negatively influences its transcription. Despite the reduction of STAT5 content, the STAT5/PPAR gamma complex is still formed, but changes its target. It binds to the p21<sub>WAF</sub> promoter and induces its transcription. So that EPCs undergo cell-cycle arrest.

In addition to precise transcriptional regulation, vascular remodeling requires post-transcriptional regulation, involving small non-coding microRNAs (miRs) [50]. miRs are highly conserved, non-coding small RNAs which regulate gene expression at the post-transcriptional level [51]. Recently, a miRNA signature in insulin target tissues and a plasma miRNA profile in type 2 diabetes have been reported [52]. Different families of miRs, including miR-221/222, have been shown to be involved in the control of endothelial cell fate and vascular remodeling [53]. In particular, miR-221/222-driven post-transcriptional regulation of cyclin-dependent kinase (Cdk) inhibitors (CKIs) p27<sub>Kip1</sub> and p57<sub>Kip2</sub> have been involved in mechanical vascular diseases [54]. Consistently, we demonstrated that acute or chronic exposure to high glucose concentrations prevented cell-cycle progression of both ECs and EPCs by modulating the expression of p27<sub>Kip1</sub> and p57<sub>Kip2</sub> [55]. Moreover, we found that high-glucose and AGES were also able to inhibit vessel formation in an <i>in vivo</i> model of angiogenesis by regulating the expression of miR221/222. Along with miR-221/222, miR-320 [56] and miR-503 [57] have been shown to contribute to the impaired vascular remodeling in preclinical models of diabetes. In particular, Caporali et al. [57] demonstrated that forced expression of miR-503 in ECs leads to inhibition of vascular growth in diabetic mice subjected to hind limb ischemia. This effect relies on miR-503-driven post-transcriptional regulation of the cell-cycle related CCNE1 and cdc25A genes [57]. The potential clinical implication of miR-503 in diabetes-associated vascular complications is sustained by the observation that diabetic patients display increased plasma miR-503 levels [57]. Thus, these data, besides confirming that deregulation of miR-221/222, miR-320 and miR-503 expression could be involved in high glucose-driven anti-angiogenic signals, identify miRNAs as potential targets for pharmacological intervention to improve vascular dysfunction in conditions connoted by altered glucose metabolism [55-57].

**Kidney Disease**

**AGE effects on mesangial cells**

Diabetic nephropathy consists of an early onset of glomerular and tubular hypertrophy and a late extracellular matrix accumulation leading to a progressive expansion of the mesangial cells (MCs) [58]. Mesangial cell hypertrophy mainly depends on intracellular signals elicited by transforming growth factor beta (TGF-β) [59]. Cell-cycle independent or dependent mechanisms are implicated in the control of cell hypertrophy [60]. TGFβ-induced cell-cycle dependent hypertrophy involves the synthesis of structural protein such as p21<sub>WAF</sub> and p27<sub>KIP1</sub> key regulators of G1 phase progression (TGFβ) [61]. At this regard, we demonstrated that AGE, but not high glucose (HG), were able to increase p21<sub>WAF</sub> while inhibit cyclin D1 expression in MCs [62]. We also showed that, in response to both TGF-β and AGE, cell cycle arrest of MCs is the result of STAT5 binding to the promoter region of p21<sub>WAF</sub> [62]. As stated above, along with MC hypertrophy, extracellular matrix accumulation contributes to kidney disease in diabetic setting. We provided evidences that while STAT5 controls TGF-β and AGE-mediated MC hypertrophy it was not involved in collagen production. Moreover, the increase of immunoreactivity for the activated STAT5 and p21<sub>WAF</sub> in kidney biopsies from early to advanced stage of diabetic nephropathy sustains the possibility that these molecular events could also be crucial for human disease [62].

The CXC chemochine ligand 16 (CXCL16) is a transmembrane molecule acting as an adhesion molecule for immune cells, smooth muscle cells and ECs or as a scavenger receptor for ox-LDL in different cell types [63-65]. CXCL16 consists of a surface-bound and a soluble form released by two disintegrin like metalloproteinases ADAM10 and ADAM17 [66,67]. Both CXCL16 and ADAMs are constitutively expressed in human podocytes where CXCL16 is crucial for ox-LDL uptake [68]. Proinflammatory stimuli such as interferon-gamma and tumor necrosis factor-alpha are known to induce the expression of CXCL16 and the release of the soluble form from human podocytes [68]. In addition it has been also reported that the soluble form of CXCL16 contribute to the recruitment of CXCR6-expressing immune cells to sites of active inflammation [69-71]. The crucial role of podocytes in kidney diseases along with the observation that soluble CXCL16 promotes pro-atherogenic signals and can predict long-term mortality in acute coronary syndrome [72] prompted Zhao et al. [73] to evaluate the serum levels of CXCL16 in diabetic patients with or without kidney disease. The results of this study clearly demonstrate that CXCL16 serum levels are higher in diabetic patients with nephropathy than in healthy subjects or diabetic patients without kidney disease. Although the authors concluded that serum CXCL16 levels might represent an indicator of renal injury in diabetic subjects, prospective studies with larger sample sizes are required to assess whether, indeed, CXCL16 could be usefully exploited as a diagnostic biomarker to identify the onset and/or the development of kidney disease in diabetic patients [73].
Current therapies for diabetic nephropathy are based on drugs that act on the renin-angiotensin converting enzyme used to control systemic and intraglomerular hypertension [74]. Although a good pressure control reduces the incidence of complications, mortality and progression of nephropathy [75] the achievement of therapeutic goals in diabetic patients is still a major unmet need. Likewise, alternative strategies based on anti-TGF-β antibody poorly control the early glomerular injury to progressive glomerulosclerosis [78]. Unlike TGF-β and AGE, lipid abnormalities and oxidative stress stimulate MC proliferation and contribute to the development of diabetes-associated renal disease by expanding MCs. However, the molecular mechanisms involved in this process are still undefined. We demonstrate that when cultured in presence of ox-LDL MCs proliferate by activating Akt and Erk1/2 MAPK pathways [79]. Moreover, we first demonstrated that this event is strictly controlled by ROS production and Rac-1 GTPase activation resulting in transactivation of the epidermal growth factor receptor (EGFR). In both physiological and pathological conditions proliferation is strictly controlled by extracellular matrix receptors, the integrins, and the cytokine/tyrosine kinase receptors [80-82]. We provide evidences that MCs challenged with ox-LDL joint β4 integrin/EGFR signaling to drive proliferative signals. Therefore the results of this study identify a novel molecular mechanism induced by ox-LDL that in the early stage of renal disease could account for MC expansion via β4 integrin activation. By microarray technology Kim et al. [83] provide an alternative mechanism involved in MC hypertrophy upon gly-LDL challenge. Actually, they showed that gly-LDL by activating the Axl/ growth arrest gene 6 axis induce TGF-βand its deleterious effects on glomerular MCs [83]. Overall these studies open new perspectives for future pharmacological approaches.

Epigenetic mechanisms and cardiovascular outcomes in diabetic patients

The signalling pathways involved in glucose-induced vascular damage in diabetes have been deeply characterized [19]. However, a successful therapeutic approach to improve the cardiovascular outcomes in patients with diabetes is still far from being achieved. That hyperglycaemia per se can contribute to diabetes-associated vascular complications has been recently reconsidered in the light of the results of clinical trials indicating that while in new onset diabetes glucose-lowering treatments preserve patients from vascular complications in long-term diabetes the intensive glycaemic control is not effective [84-86]. These observations led to the conclusion that glycaemic environment could be “better or worse remembered” by the vascular system and could contribute to the natural history of diabetic-associated vascular complications [84-86]. The persistence of glucose-mediated oxidative stress, AGE production and the positive feedback loop between AGEs and RAGE signals [19], despite a return to good metabolic control, provides the mechanistic justification of the so called “metabolic memory”. The “metabolic memory” mainly reflects epigenetic changes driven by hyperglycemia-mediated mitochondrial ROS production [87-91]. Epigenetic deposition on histone modifying enzymes, DNA methylation, and chromatin remodeling proteins [89]. These mechanisms are reviewed and consisting in small changes in the epigenome over time [87-91], seem to be also crucial determinants of the early “hyperglycemia memory” sustained by the DCCT/EDIC studies [84,85]. Moreover, it has been suggested that epigenetics and in particular DNA methylation, could also explain the awful “metabolic memory” of post-prandial hyperglycaemia episodes [92]. Current knowledge supports single mechanisms as relevant mediators of this process however Paneni et al. [91] proposed an intriguing molecular pathway that links together chromatin remodeling, ROS production and inflammation of the vessel wall. Over the last few years a number of studies have shown that epigenetic mechanisms, maintained even after glucose normalization, are involved in the transcription of the p65 subunit of NF-kB and on the expression of its target genes: monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) [93,94]. Moreover, by molecular approaches driving superoxide dismutase-2 (SOD2) overexpression, it has been clearly demonstrated that interfering with ROS generation of mitochondrial origin prevents sugar-induced histone metilation of p65 [95]. Similar results were obtained by using a quite selective mitochondrial antioxidant able to interfere with the recruitment of the Set7 histone methyltransferase to the “chromatinized p65 template” [91]. Moreover, the recent findings that the expression of the mitochondrial adaptor protein p66hsc is increased in response to

Figure 2: The mechanisms involved in kidney disease and some of the novel therapeutic strategies targeting ROS-mediated damage are schematically represented. Upper panel: The relevant signaling pathways involved in AGE/transforming growth factor beta (TGFβ)/Ox-LDL-induced mesangial cell (MC) hypertrophy are depicted. STAT5, Rac-1-mediated ROS generation and the CXC chemokine ligand 16 (CXCL-16) shedding are reported. Middle panel: visceral adipose tissue-derived stem cells (ASCs) in presence of HG de-differentiate into self-renewing cells. Oct-4: octamer-binding transcription factor 4; Nanog: homeobox protein Nanog. Lower panel: schematic representation of the anti-oxidant properties of UnAG in a pre-clinical model of PAD. UnAG administration rescues oxidative-stress-mediated skeletal muscle damage inducing satellite cell proliferation and muscle regeneration. SOD2: Superoxide dismutase 2.
Adipose tissue-derived stem cells (ASCs) populations in preclinical models of vascular diseases have spurred regeneration [6]. Results of cell-based therapy using diverse “stem/progenitor” cells involving promoter demethylation and acetylation of histone 3 [96]. In cut evidences that this event is mediated by epigenetic mechanisms involving promoter demethylation and acetylation of histone 3 [96]. In keeping with the possibility that, despite glucose normalization, vascular damaging signals could be self-maintained by epigenetics, recent studies have also discovered a tight connection among p66shc, the class III histone deacetylase SIRT1 and the tumor transcription factor p53 [96,98,99]. Indeed, SIRT1 overexpression in EPCs inhibits high glucose-mediated p66shc up-regulation, improves endothelial function and reduces oxidative stress markers [100].

In keeping with the crucial role of p66shc, p53 and SIRT1 cross-talk in this context are the following observations: i. p66 is under the transcriptional control of p53 [101]; ii. SIRT1 inhibition leads to p53 acetylation and to its increased transcription activity [102,103]. To close the circle, Liu et al. [104] demonstrated that Set7, along with Set9 histone methyltransferase, negatively regulate p53 via SIRT1. That epigenetic mechanisms involving SIRT1 are not endothelial specific is supported by the recent finding that SIRT1 over-expression leads to podocyte Claudin-1 “re-writing” resulting in the improvement of renal function in db/db mice [105]. Collectively these observations indicate that, despite glucose normalization, epigenetic mechanisms could drive a persistent activation of the intracellular signalling pathways that ultimately lead to endothelial dysfunction and apoptosis [91]. Thus, as an early good glycaemic control could be maintained despite the return to worse metabolic control [84-86], to identify the onset of post-transcriptional modification of histones that change gene expression pattern in diabetic patients should be a future challenge.

**Novel options for Diabetes-Associated Complications**

**Adipose tissue-derived stem cells (ASCs)**

The negligible effects of current therapeutic strategies on both endothelial dysfunction and disease progression have highlighted the need for novel therapeutic approaches. Recently, the encouraging results of cell-based therapy using diverse “stem/progenitor” cell populations in preclinical models of vascular diseases have spurred clinicians to exploit these cells in regenerative medicine [6]. For many years bone marrow-derived MSCs (BM-MSCs) have been considered the most relevant sources of stem cells [106,107]. However, the low number of cells recovered and the invasive procedures to obtain them have led to several concerns on the feasibility of exploiting these cells in humans. Different tissue sources have been used, but because of the effortless access by minimally invasive procedures adipose subcutaneous tissue, is nowadays considered a promising alternative source of adipose-derived stem cells (ASCs) for regenerative medicine. This is particularly true for cardiovascular diseases as they can concomitantly stimulate neovascularization, cytoprotection and tissue regeneration [6].

Originally engraftment and terminal differentiation of stem/progenitor cells were the most often mechanisms studied [106,107]. However, up to now, there is no definitive evidence on their clinical efficacy. In fact up-and-coming data indicate that their therapeutic effectiveness mainly rely on their paracrine effects [108,109]. At this regard, Rehman et al. [110] have shown that ASC administration enhances endothelial cell survival in hypoxic conditions and improves ischemic limb perfusion by means of the vascular endothelial growth factor (VEGF) release [110]. Likewise, Nakagami et al. [111] reported that implantation of ASCs into the ischemic hindlimb improves both angiogenic score and capillary density. As stated above the majority of preclinical studies were performed by using ASCs derived from subcutaneous adipose tissue. However, based on the notion that visceral and subcutaneous adipose tissue possesses distinctive cell autonomous assets [112] it cannot be barred that functional differences in ASCs derived from different fat depots might also exist. Moreover, whether environmental cues, as hyperglycaemia or pathological conditions connoted drastic changes in visceral adipose mass can change their self-renewal capability or secretome is still debated. To address this issue we recently investigated whether visceral adipose tissue-derived ASCs could represent a valuable source of stem cells for regenerative purpose in diabetic condition. To this end ASCs recovered from diabetic patients or cultured in high glucose concentration were assayed for their self-renewal capability and cytokine production [113]. Stem cell pluripotency are under the control of a network of transcription factors [114], including octamer-binding transcription factor 4 (Oct4) and Nanog [115]. We demonstrated that the diabetic milieu promotes stem cell self-renewal potential by activating NOX and “nontoxic” levels of ROS [113]. Consistent with the crucial role of Oct4 and Nanog in stem cell self-renewing capabilities we demonstrated that ASCs derived from diabetic patients or cultured in the presence of HG express high level of Oct4 and Nanog [113]. By knocking-down NOX we also demonstrated that this pathway is relevant for HG-mediated ASC stemness, measured as “spheroid” formation. Moreover consistently with the ability of ASCs to secrete soluble mediators [116], the de-differentiation status induced by HG resulted in an increase of cytokine production. This effect was partially lost when de-differentiated ASCs were put in culture in the presence of low glucose concentrations. This implies that hyperglycemic condition can impact ASC stemness and secretion profile. Moreover, the results of this study suggest that onmectectomy, along with ameliorating the metabolic profile [117] can offer an additional benefit: the recovered ASCs could be exploited to repair deteriorating tissues. Finally, the results of this study indicate that HG pre-conditioning could be exploited to ex-vivo expand autologous ASCs.

**A naturally occurring hormone, the unacylated ghrelin (UnAG) to target ROS-mediated damage**

As stated above vascular remodelling relies on resident ECs and on circulating EPCs [6,36]. Thus, changes in EPC number and functional activities, as occurs in diabetes, impact on their delivery to sites of ischemia where new vessel formation might be crucial [36]. This implies that molecules able to revert EPC dysfunction might represent an alternative therapeutic strategy for diabetes-associated vascular complications. Recently it has been shown that gastric-released hormones, such as ghrelin, impact on glucose metabolism as well as on diabetes-associated vascular complications [118]. Indeed, circulating total ghrelin levels are negatively associated with body mass index [119] and obese [120] and in type 2 diabetic patients have a reduced ghrelin secretion [121]. Ghrelin is a 28 amino-acid peptide circulating in two different forms: the acylated (AG) and the unacylated (UnAG) ghrelin [122]. Of particular interest, it has been reported that in clinical settings associated with insulin resistance a relative excess of AG compared to UnAG is common [123]. We have shown that UnAG
and its cyclic analogue, AZP351 protect vascular cells from ROS-mediated damage in diabetic condition [124,125]. In particular we showed that UnAG, but not AG, systemic administration protects diabetic EPCs from oxidative stress and senescence and improves their vasculogenic potential [124]. Moreover, we provided evidences that only UnAG was able to rescue defective EPC mobilization in diabetic patients by restoring eNOS activity [124]. Consistently with the antioxidant properties of UnAG, Shimada et al. [126] recently demonstrated that UnAG protects human retinal microvascular endothelial cells from oxidative stress-induced apoptosis through the SIRT-1 signalling pathway. Moreover a prospective study has shown a correlation between circulating des-acyl ghrelin levels and cardiovascular events. In this study Yano et al. [127] demonstrated that a low circulating level of des-acyl ghrelin, is a useful cardiometabolic marker predicting atherosclerosis in elderly hypertensive patients. Based on evidences for the favorable cardiovascular effects of UnAG [124,125] we recently investigated its therapeutic effects on peripheral arterial disease (PAD). PAD is associated with high rate of myocardial infarction, stroke and amputation [128]. The incidence of PAD is expected to increase because of the increasing prevalence of diabetes and population ages [129]. Current surgical approaches are associated with increased perioperative morbidity and mortality [130], while alternative strategies including cell-based therapies or angiogenic growth factor delivery employed so far, were found ineffective [6]. Data obtained in a preclinical model of ischemia demonstrate that UnAG rescues oxidative stress-mediated skeletal muscle damage and induces satellite cell (SC) proliferation and muscle regeneration [131]. We also demonstrated that such effect relies on its ability to induce an efficient antioxidant response mediated by SOD2. By interfering with the expression of SOD2 we also showed that SOD2 strictly controls post-transcriptional mechanisms driven by miR-221/222 to induce skeletal muscle regeneration [131] (Figure 2).

Conclusions and Perspectives

Defective mitochondrial electron transfer chain along with the increased ROS generation is crucial determinants of cell damage [132,133]. This is particularly true in diabetes setting where unbalance of oxidative stress has causal role in its ongoing vascular complications. In the last decade different therapeutic strategies have been exploited to overcome these hurdles, however so far, many of them failed to show clinical benefits [6,36]. This implies that the development of a valuable approach is still a thorny challenge. Thus, to design novel therapeutic approaches able to prevent or ameliorate tissue damage, future research efforts should be focused on an in-depth understanding of the mechanisms involved in ROS production and mitochondrial defects. Pre-clinical models of vascular diseases indicate that a naturally occurring hormone, such as UnAG, can act as antioxidant molecules able to reverse vascular damage and to induce skeletal muscle regeneration after ischemia [124,125,131]. In coming years efforts should be directed to evaluate its potential clinical impact in humans. Finally, molecular and pharmacological interventions able to interfere with refractory hyperglycemia resulting in plastic modification of chromatin and the so called “metabolic memory” should be future therapeutic challenges.

Disclosures

All the authors declare no conflict of interest or duality of interest.

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